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Synthesis of sphingomyelin and its analogs

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SYNTHESIS OF SPHINGOMYELIN AND ITS ANALOGS

by

ZHONG-SHI RUAN

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Abstract

Synthesis of Sphingomyelin and Its Analogs

by

Zhong-shi Ruan

Advisor: Professor Robert Bittman

Sphingomyelin and its analogs are synthesized for use in biophysical studies, especially for kinetic studies of the movement of cholesterol between membranes, and for analysis of sphingomyelin and cholesterol interactions. In this thesis sphingomyelin is prepared from DL-*erythro*-sphingosine, which is prepared in an aldol condensation of tris(trimethylsilyl)glycine with (*E*)-hexadec-2-enal followed by lithium aluminum hydride reduction. The diastereoselectivity of the aldol condensation is determined by chiral high-pressure liquid chromatography of the biphenylcarboxamido and (*R*)-(+)-α-methoxy-α-(trifluoromethyl)phenylacetic acid ester derivatives of sphingosine. The ratio of *erythro- to threo*-sphingosines is 98.0:2.0, and the ratio of D- to L-*erythro* isomer is 50:50, both based on integration of the peak areas. Three different phosphorylation or phosphitylation reagents are used for the conversion of ceramide to sphingomyelin; they are 2-chloro-2-oxo-1,3,2-dioxaphospholane, 2-bromoethylphosphoric acid dichloride, and *N,N*-diisopropylmethylphosphoramidic chloride. The syntheses of sphingomyelin and analogs such as 3-*O*-alkylsphingomyelins

(3-*O*-methyl and 3-*O*-ethyl-DL-*erythro*-sphingomyelins) and 3-deoxy-DL-sphingomyelin, isotopically labeled sphingomyelins ([4,5-$^{2}H_{2}$]-DL-*erythro*-sphingomyelin and ^{15}N-DL-*erythro*-sphingomyelin), and different *N*-acyl sphingomyelins (*N*-dodecanoyl-, *N*-tetradecanoyl-, *N*-octadecanoyl-, *N*-docosanoyl-, *N*-tetracosanoyl-, *N*-(*cis*-15-tetracosenoyl)-, and *N*-[(2'*R,S*)-hydroxyhexadecanoyl]-DL-*erythro*-sphingomyelins) are described in detail.

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I extend my thanks to all the colleagues who not only helped me in the research but provided such a nice atmosphere in the laboratory. Thanks to all the other members of the department who offered their assistance.

Very special thanks to my husband and best friend Shu-ping for his patience, understanding, and encouragement during these years.

Finally, I would like to acknowledge the financial support from the NIH.

Dedicated to those who made this thesis possible:

My parents, my husband, and my wonderful daughter Julia.

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Introduction

Sphingomyelin (SPM) is one of the most abundant components of biological membranes and blood plasma lipoproteins.[1] Although the specific function of SPM in membranes is not well understood, it has been suggested that it forms stable complexes with cholesterol[2] and strong intermolecular hydrogen bonds with other phospholipids.[3]

Many biophysical studies have concluded that the affinity of SPM for cholesterol is higher than that of other phospholipids.[1,3-5] Differential-scanning calorimetry (DSC) studies[2] and kinetic studies of cholesterol movement between membranes[6-8] indicate that cholesterol associates preferentially with SPM. In a binary mixture showing phase separation, DSC studies showed that SPM, either isolated from beef erythrocytes or containing a single N-acyl chain (*N*-palmitoyl), interacted with cholesterol to a greater degree than did various synthetic phosphatidylcholines. The rate of exchange of [4-^{14}C]cholesterol from *Mycoplasma gallisepticum* containing *N*-C16-SPM[6] and from small unilamellar vesicles (SUV) prepared with *N*-C16-SPM[7] was slower than that from mycoplasma and vesicle membranes containing dipalmitoyl-PC (DPPC). These authors concluded that *N*-C16-SPM has a higher affinity for cholesterol than does DPPC both in vesicles and in mycoplasma membranes. [^{3}H]Cholesterol transfer studies also indicated that bovine-brain SPM has a higher affinity for cholesterol than does dimyristoyl-PC and dioleoyl-PC.[8] Fluorescence polarization measurements on phospholipid-cholesterol bilayers also showed that cholesterol has a higher affinity for egg SPM than for glycerophospholipids (except for DPPC).[9] Recently, studies of the surface pressure-molecular area isotherms of mixed monolayers of cholesterol and SPM revealed that cholesterol has a higher affinity for SPM than for DPPC.[10]

The resistance of oxidation of cholesterol by cholesterol oxidase in monolayers is also related to strong SPM-cholesterol interactions.[11] Hence it is evident that the interaction of cholesterol with egg or synthetic SPM is greater than that with egg PC or DPPC.

Hydrogen bonding has reported to take place between the 3β-hydroxy group of cholesterol and the ester oxygens of glycerolipids and amide oxygen of sphingolipids.[12,13] The relationship between the hydrogen-donating ability of cholesterol and its role in the induction of a component with a broad phase transition at a higher temperature than that of pure SPM has been attempted to be established.[4] Although there is no direct evidence of such hydrogen bonding, a different approach may be necessary to determine whether such interactions between the 3β-hydroxy group of cholesterol and SPM occurs. In spite of the possibility that the amide oxygen or NH bond of SPM may form a hydrogen bond with the 3β-hydroxy group of cholesterol, the role of the free hydroxy group of SPM is an interesting feature in the study of the interaction between cholesterol and SPM. The hydroxy group and the amide bond of SPM afford an important intermolecular hydrogen-bond capability.[14] One of the goals of this thesis is to prepare SPM analogs for studies directed to understanding the role of the hydroxy group of SPM in its interaction with cholesterol. SPM analogs are prepared in which the hydroxy group is replaced but the amide linkage was maintained. Studies of [4-^{14}C]cholesterol exchange rates between vesicles containing these SPM analogs indicate that hydrogen bonding between cholesterol and the hydroxy group of SPM does not account for the selective affinity of SPM for cholesterol.[15]

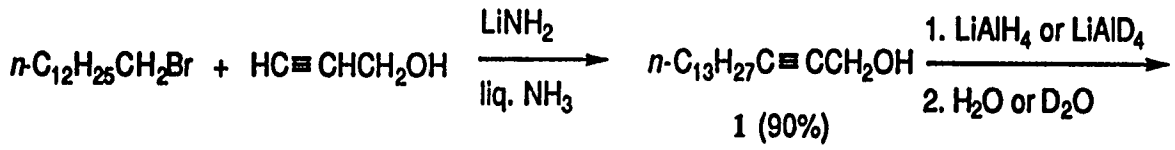
Up to now, three different types of synthetic SPM have been used for biophysical studies. Semisynthetic SPM is obtained by the acidic hydrolysis

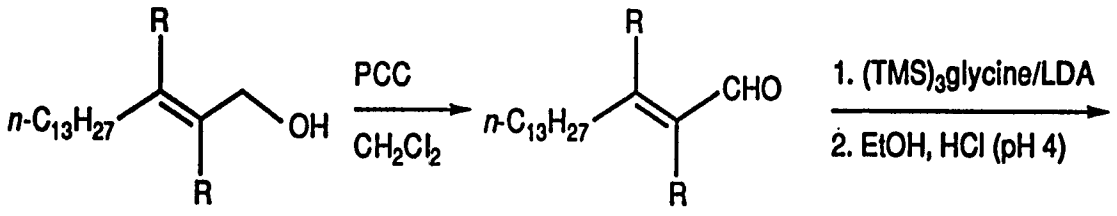
of natural SPM (typically from bovine brain), followed by the acylation of the resulting sphingosyl 1-phosphorylcholine with an appropriate acylating reagent obtained from the desired fatty acid.[16,17] Synthetic homogeneous SPM was reported by Shapiro.[18] Fully synthetic DL-*erythro*-SPM was used to examine the themal behavior of SPM bilayers.[19,20] Recently, Bruzik reported the synthesis of D-*erythro*-SPM and the phosphorothioyl analog of SPM with high chemical and stereochemically purity.[21] In order to help us understand the extent to which the hydroxy group of SPM contributes to the greater affinity of SPM to cholesterol, DL-*erythro*-SPM and analogs with high diastereoselectivity are synthesized as shown in Schemes 1-4.

D-*erythro*-Sphingosine 5 is the backbone of SPM and glycosphingolipids. It is not only an important intermediate in the biosynthesis of SPM, but also a potent inhibitor of protein kinase C in vitro.[22-25] Recently, many synthetic procedures for the synthesis of D- and DL-*erythro*-sphingosine have been published. A review of the enantioselective syntheses of sphingosine and other sphingolipids is in press.[26] The approach to SPM used in this thesis is via DL-*erythro*-sphingosine as an intermediate. The latter is prepared in an aldol condensation of (*E*)-hexadec-2-enal with tris(trimethylsilyl)glycine under kinetic conditions, followed by reduction with lithium aluminum deuteride or lithium aluminum hydride[27] (Scheme 1). In this synthetic procedure, an achiral starting material (glycine) is used to form sphingosine, which contains two chiral centers. In general, aldol condensation reactions give four diastereoisomeric products (a pair of *erythro* and a pair of *threo* isomers).[28] The ratio of the diastereoisomers depends on the reaction conditions, such as substituents, temperature, and solvent. In general, the principal method for the assignment of aldol stereochemistry is proton nuclear magnetic resonance (^{1}H-NMR) spectroscopy.[29] In many

instances *erythro-threo* stereochemical assignments are made from the magnitude of the vicinal coupling constant, J_{AB}. For α-amino alcohols, when intramolecular hydrogen bonding provides the dominant conformational bias, J_{AB} values for *threo* isomers fall in the approximate range of 3 to 6 Hz, and J_{AB} values for *erythro* isomers are about 7 to 9 Hz.[29] In 1982, Schmidt and Kläger reported the diastereoselectivity of this aldol condensation by using the ^1H NMR method.[27] Their results showed that the reaction is diastereoselective, and only *erythro* isomers are obtained. In order to study the stereochemistry of sphingosine **5** (Scheme 1), we converted sphingosine **5b** into biphenylcarboxamidosphingosine **19** (Scheme 6).[30] By comparing the HPLC retention times and integrated peak areas of **19**, D-*erythro*-biphenylcarboxamidosphingosine (**19'**), and a mixture of *erythro*- and *threo*-biphenylcarboxamidosphingosines (**19"**), we determined the diastereoselectivity of the aldol condensation and the ratio of *erythro*- to *threo*-sphingosine. In addition, we prepared the (*R*)-(+)-bis-Mosher esters of biphenylcarboxamidosphingosines (**21** and **21'**) and determined the ratio of D- and L-isomers by chiral HPLC analysis. *N*-Methylsphingosine (**18**) was prepared by aldol condensation using (*E*)-hexadec-2-enal and bis(trimethylsilyl)sarcosine (Scheme 5). The HPLC analysis of *N*-methyl-*N*-biphenylcarboxamidosphingosine (**20** and **20'**) showed a low diastereoselectivity in this reaction compared with the result obtained in the aldol condensation of (*E*)-hexadec-2-enal and tris(trimethylsilyl)glycine. The reactions have different diastereoselectivities because the methyl group in bis(trimethylsilyl)sarcosine is much smaller than the corresponding trimethylsilyl group in tris(trimethylsilyl)glycine.

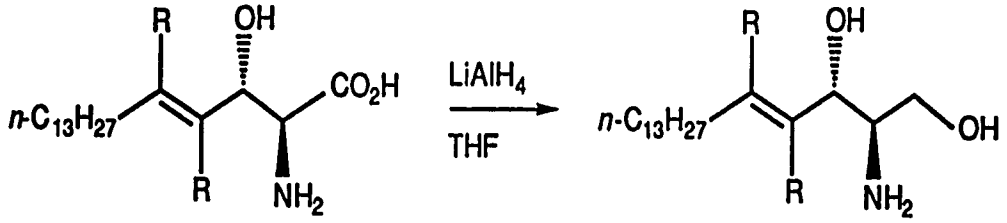
Scheme 1. Syntheses of isotopically labeled sphingomyelins





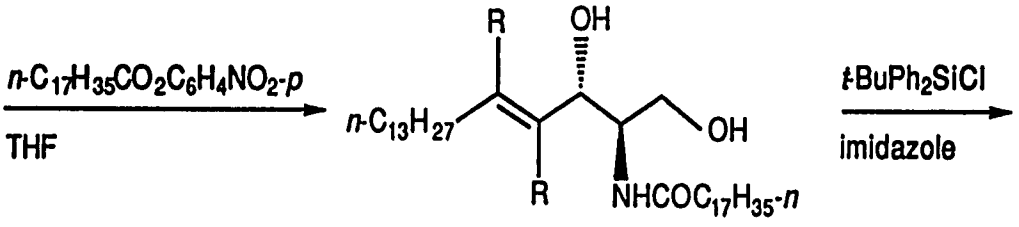
2a, R = D (94%)
2b, R = H (88%)

3a, R = D (80%)
3b, R = H (80%)



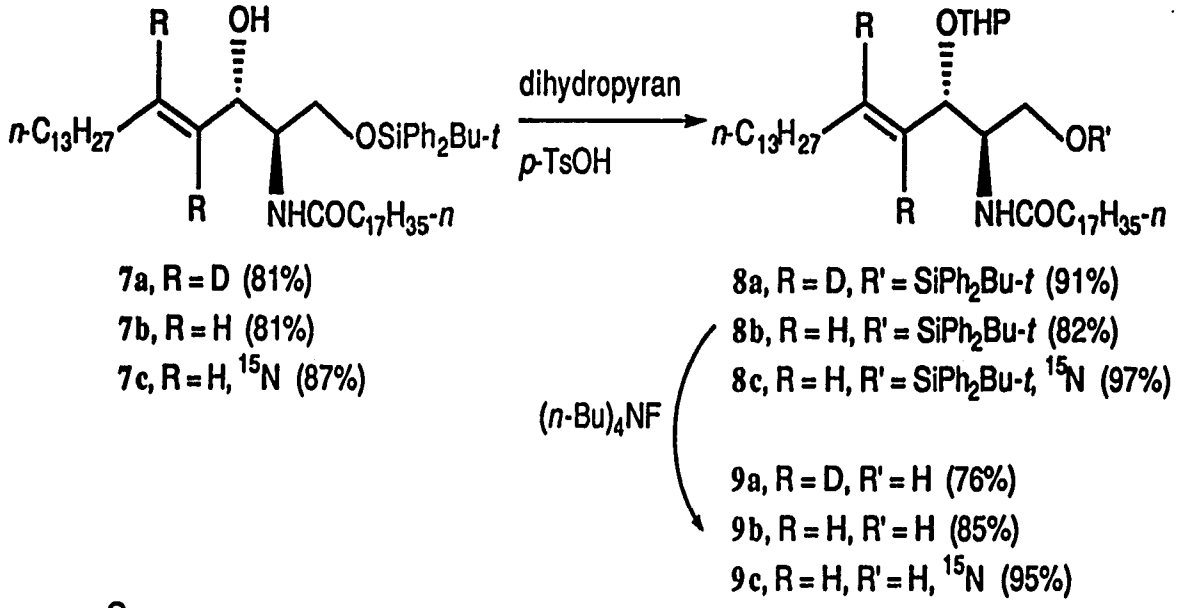
4a, R = D (90%)
4b, R = H (80%)

5a, R = D (72%)
5b, R = H (70%)
5c, R = H, ^{15}N (55%)



6a, R = D (74%)
6b, R = H (75%)
6c, R = H, ^{15}N (85%)

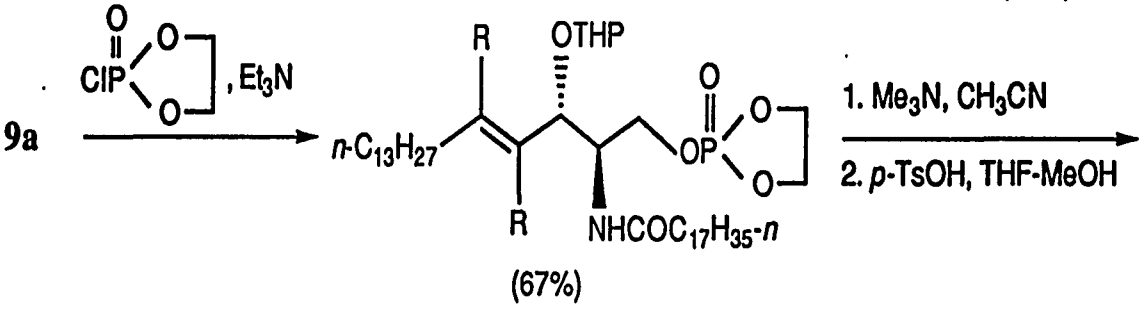
Scheme 1. (Continued)



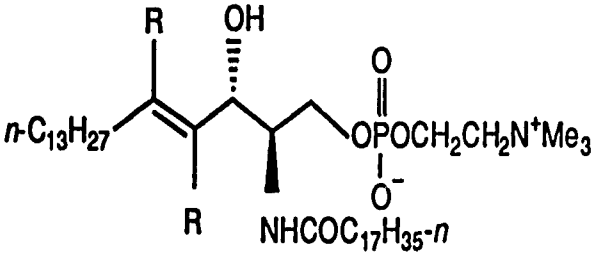
7a, R = D (81%)
7b, R = H (81%)
7c, R = H, ^{15}N (87%)

8a, R = D, R' = $SiPh_2Bu$-*t* (91%)
8b, R = H, R' = $SiPh_2Bu$-*t* (82%)
8c, R = H, R' = $SiPh_2Bu$-*t*, ^{15}N (97%)

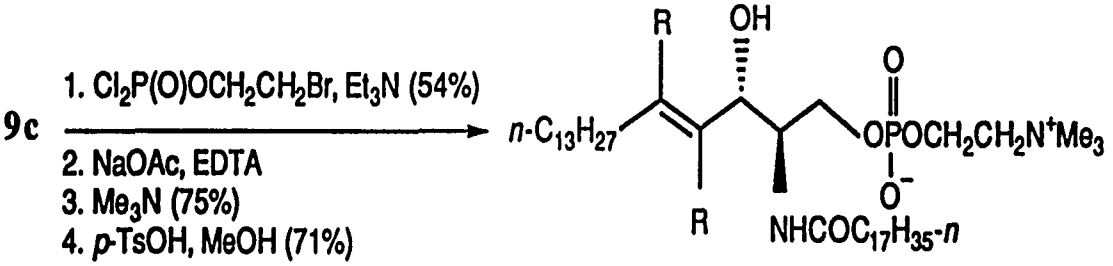
9a, R = D, R' = H (76%)
9b, R = H, R' = H (85%)
9c, R = H, R' = H, ^{15}N (95%)



(67%)

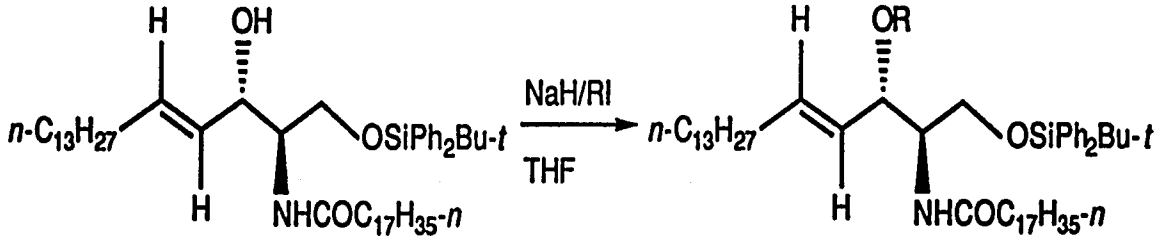


10a, R = D (66%)
10b, R = H (72%)



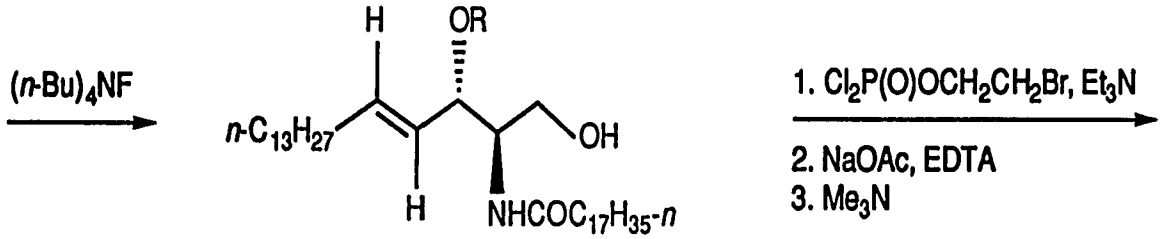
10c, R = H, ^{15}N

Scheme 2. Synthesis of 3-*O*-alkyl analogs of sphingomyelin

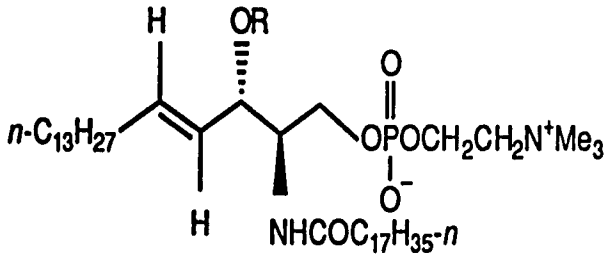


7b
7c (^{15}N)

11b, R = Me (64%); **11c**, R = Me, ^{15}N (63%)
12b, R = Et (54%); **12c**, R = Et, ^{15}N (57%)

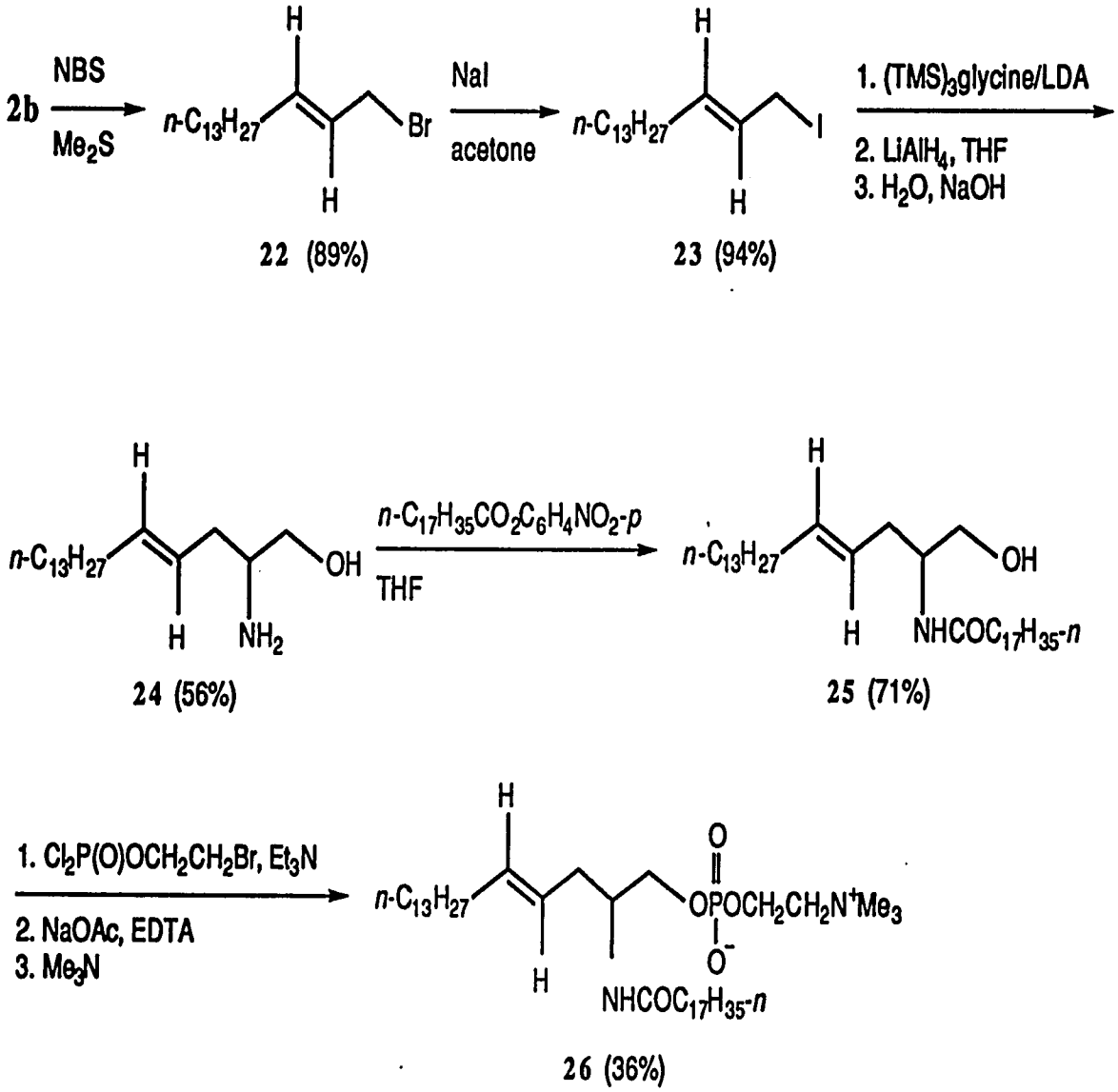


13b, R = Me (97%); **13c**, R = Me, ^{15}N (93%)
14b, R = Et (95%); **14c**, R = Et, ^{15}N (91%)

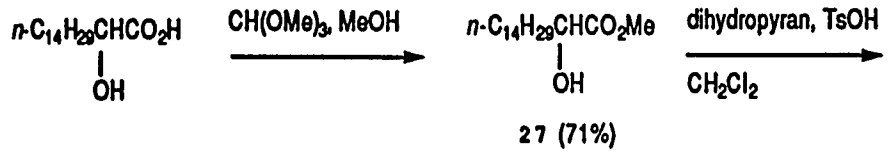


16b, R = Me (36%); **16c**, R = Me, ^{15}N (43%)
17b, R = Et (41%); **17c**, R = Et, ^{15}N (39%)

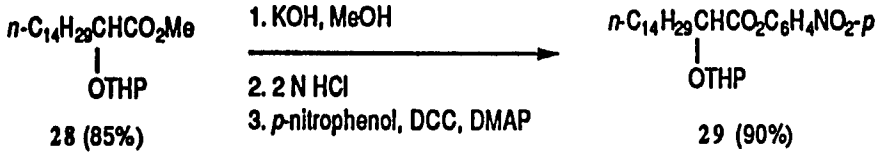
Scheme 3. Synthesis of 3-deoxysphingomyelin



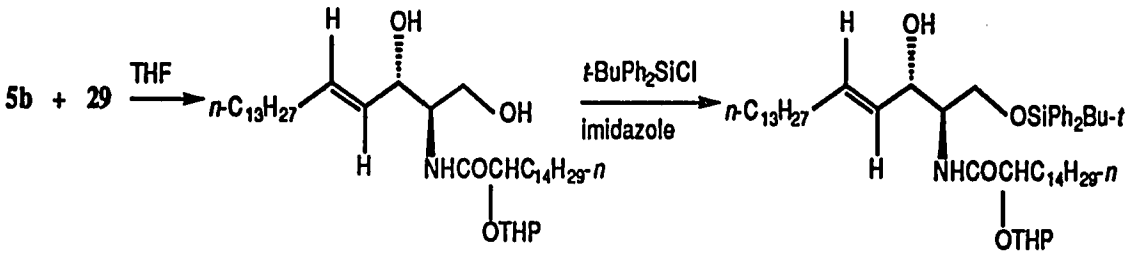
Scheme 4. Synthesis of α-hydroxy analog of sphingomyelin



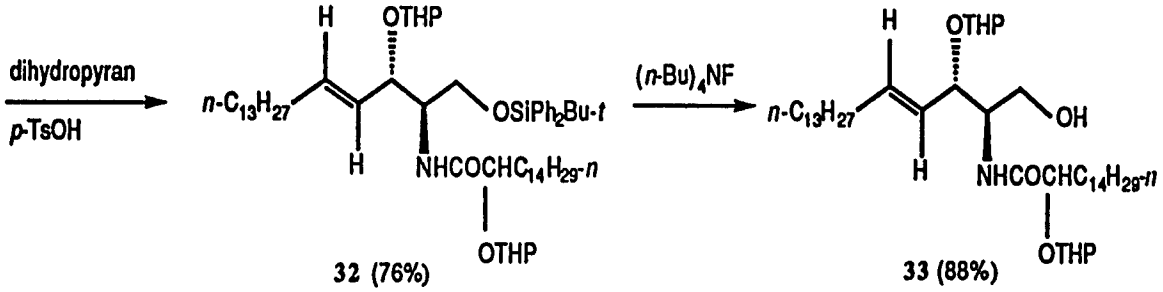
27 (71%)



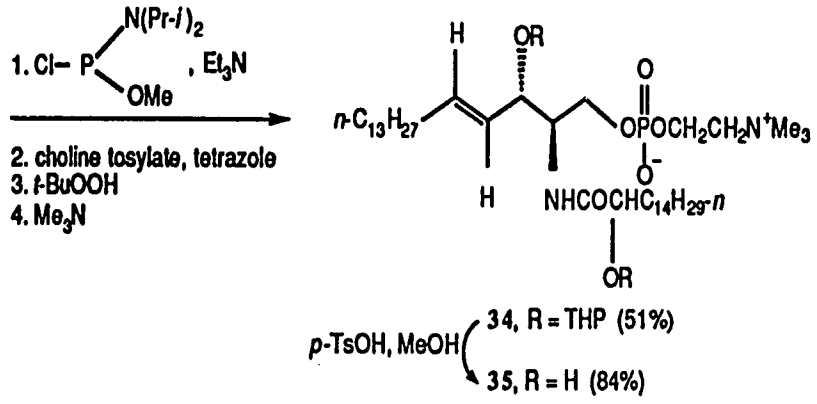
28 (85%) 29 (90%)



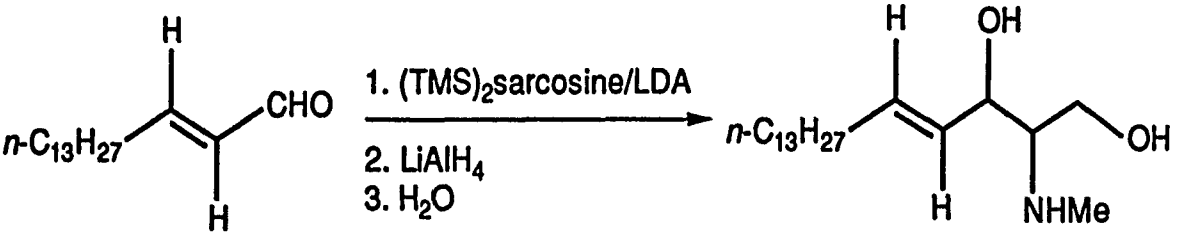
30 (86%) 31 (83%)



32 (76%) 33 (88%)

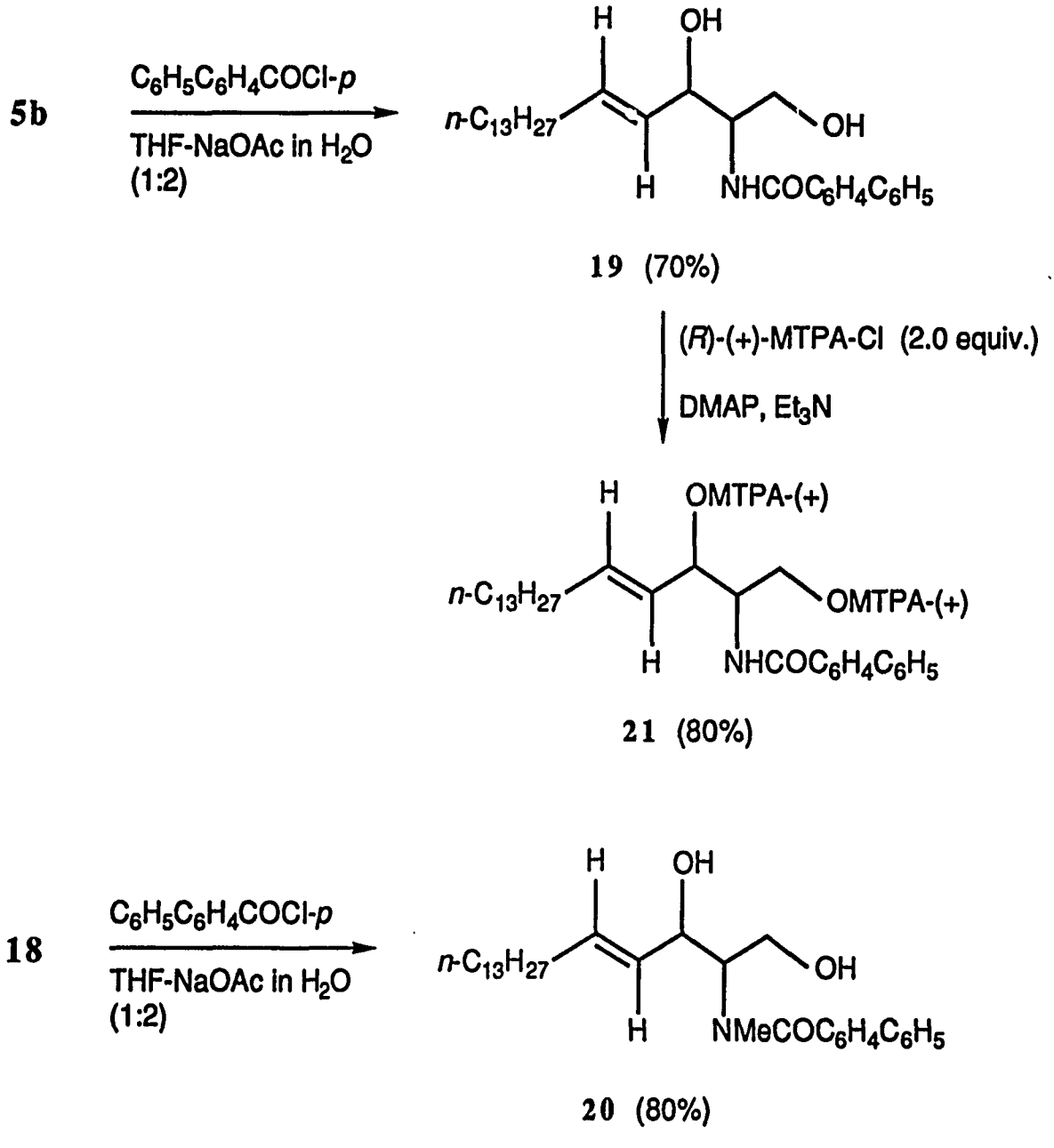


Scheme 5. Synthesis of *N*-methylsphingosine



3b **18** (38%)

Scheme 6. Preparation of sphingosine derivatives for HiPLC analyses



Experimental Section

1. Materials and Methods

General procedures. The solvents used were dried as follows: methylene chloride, ethanol-free chloroform, toluene, and acetonitrile were distilled from calcium hydride and stored over type 3 Å molecular sieves. Acetone was stored over calcium sulfate for at least one week. Triethylamine and diisopropylamine were dried and stored over calcium hydride. Tetrahydrofuran was refluxed over sodium benzophenone ketyl for several hours and then used immediately. DMF was dried over barium oxide and distilled under reduced pressure. Other chemicals were obtained from the following sources: glycine, ^{15}N-glycine, sarcosine, D-*erythro*-sphingosine (catalog number S6879), lauric acid, mystric acid, lignoceric acid (C24:0), behenic acid (C22:0), nervonic acid (C24:1), and *p*-nitrophenyl stearate were from Sigma Chemical Co. Propargyl alcohol, 1-bromotridecane, lithium aluminum hydride, lithium aluminum deuteride, pyridinium chlorochromate, 1,1,1,3,3,3-hexamethyldisilazane, *n*-butyllithium, *tert*-butyldiphenylsilyl chloride, imidazole, dihydropyran, *p*-toluenesulfonic acid, tetra-*n*-butylammonium fluoride, phosphorus oxychloride, 2-bromoethanol, 25% trimethylamine in water, sodium hydride, methyl iodide, ethyl iodide, 4-(dimethylamino)pyridine (DMAP), dicyclohexylcarbodiimide (DCC), 4-biphenylcarbonyl chloride, 3-(dimethylamino)propylamine, 1*H*-tetrazole, *tert*-butyl hydroperoxide, *N,N*-diisopropylmethylphosphoramidic chloride, and dimethyl sulfide were from Aldrich Chemical Co. Anhydrous trimethylamine, tetra-*n*-butylammonium iodide, and (R)-(+)-α-methoxy-α-(trifluoromethyl)-phenylacetic acid (MTPA) were from Fluka Chemical Corp. *N*-Bromosuccinimide was from J. T. Baker Chemical Co. (2*R,S*)-Hydroxyhexadecanoic acid and ethylene chlorophosphite were from

Lancaster Synthesis Ltd. The samples of *N*-methyl-D-*erythro*-sphingosine and a mixture of *erythro*- and *threo*-sphingosines used for HPLC analyses of stereoisomers were generously supplied by Dr. D. C. Liotta, Emory University.

2-Chloro-2-oxo-1,3,2-dioxaphospholane was prepared by bubbling dry oxygen through a benzene solution of ethylene chlorophosphite by P. N. Guivisdalsky in this laboratory according to a published procedure;[31] this compound was then distilled; bp 74 °C/0.3 mm Hg (lit.[31] bp 79 °C/0.4 mm Hg).

Reactions were monitored on 0.25-mm thick silica gel GF TLC plates purchased from Analtech, Newark, DE. Detection of the compounds on TLC plates was by short-wavelength ultraviolet light or by spraying with 10% sulfuric acid in ethanol followed by charring on a hot plate, or with ninhydrin for amino-containing compounds.[32] Phospholipids were visualized by spraying with phosphomolybdic acid solution,[33] followed by heating the TLC plates on a hot plate. Flash chromatography was carried out with Kieselgel (230-400 ASTM mesh purchased from Aldrich). Preparative TLC was carried out on 1-mm thick plates purchased from Analtech.

^{1}H NMR spectra were recorded on an IBM-Bruker WP 200-MHz spectrometer or on a GE QE 300-MHz spectrometer. Infrared spectra were recorded on a Perkin-Elmer FT-IR 1600 series spectrometer. Melting points are uncorrected. Elemental analyses (C, H, N) were performed by Desert Analytics, Tucson, AZ; phosphorus was analyzed by Desert Analytics and by Schwarzkopf Microanalytical Laboratories (Woodside, NY). HPLC was carried out on a Perkin-Elmer Model 410 system equipped with a LC-235 diode array detector and LCI-100 recorder-integrator.

2. Synthetic Procedures

Hexadec-2-yn-1-ol (1) (Scheme 1). Lithium metal (15 g, 2.1 mol) was added to a 3-L three-neck flask charged with 1.5 L of liquified ammonia at -78 °C. When the solution turned blue, $FeCl_3 \cdot 6H_2O$ (1.0 g) was added. The reaction mixture was stirred at -78 °C until the blue color disappeared. The dry ice bath was removed, and THF (400 mL) was added, followed by 56.6 g (1.01 mol) of prop-2-yn-1-ol. After the mixture had stirred for 1 h, 1-bromotridecane (50 g, 0.19 mol) was slowly added, and the suspension was refluxed for 4 h and left to evaporate. The residue was cooled to 0 °C, and concentrated HCl was slowly added until the solution reached pH 2. The organic solvent was removed with a rotary evaporator, and the water layer was washed with ether (2 x 200 mL). The ether phase was dried ($MgSO_4$) and the solvent was concentrated. The crude product was purified by flash chromatography (elution with hexane-ethyl acetate, 3:1) to give 41 g (90%) of hexadec-2-yn-1-ol (**1**); mp 53.5-54 °C (lit.[34] mp 54.2-54.5 °C); TLC (hexane-ethyl acetate, 3:1) R_f 0.49; ^1H NMR (200 MHz, $CDCl_3$) δ (ppm) 0.85-0.90 (t, J = 7.0 Hz, 3 H, CH_3), 1.26 (m, 22 H, $(CH_2)_{11}$), 1.91 (s, 1 H, OH), 2.17-2.44 (t, J = 6.8 Hz, 2 H, CH_2C C), 4.25 (s, 2 H, CH_2OH). Anal. Calcd for $C_{16}H_{30}O$: C, 80.61; H, 12.68. Found: C, 80.79; H, 12.94.

(*E*)-Hexadec-2-en-1-ol (2b). To a solution of hexadec-2-yn-1-ol (**1**) (12 g, 50.4 mmol) in 125 mL of dry THF at room temperature was slowly added 2.1 g (55 mmol) of lithium aluminum hydride. The reaction mixture was refluxed overnight with stirring, and then was cooled to 5 °C and hydrolyzed with caution by the sequential addition of 2.1 mL of water, 2.1 mL of 15% aqueous sodium hydroxide, and 6.3 mL of water. The aluminum hydroxide precipitate was filtered and washed with ether. The filtrate was

dried ($MgSO_4$) and concentrated under vacuum. The crude product was purified by flash chromatography (elution with hexane-ethyl acetate, 3:1) to give 10.6 g (88%) of (*E*)-hexadec-2-en-1-ol **(2b)** as a white solid; mp 35 °C (lit.[35] 33.5-34 °C); TLC (hexane-ethyl acetate, 3:1) R_f 0.45; ^1H NMR (200 MHz, $CDCl_3$) δ (ppm) 0.85-0.91 (t, J = 7.0 Hz, 3 H, CH_3), 1.25 (m, 22 H, $(CH_2)_{11}$), 2.01-2.06 (m, 2 H, CH_2CH=CH), 3.48 (s, 1 H, OH), 4.07-4.09 (d, J = 3.5, 2 H, CH_2OH), 5.55-5.77 (m, 2 H, *trans*-CH=CH). Anal. Calcd for $C_{16}H_{32}O$: C, 79.94; H, 13.42. Found: C, 80.55; H, 13.81.

[2, 3-$^2H_2$]-(*E*)-Hexadec-2-en-1-ol (2a). The synthetic route to **2a** was analogous to that used to prepare **2b**. A solution of hexadec-2-yn-1-ol **(1)** (2.0 g, 8.4 mmol) in 30 mL of THF was added to a 50-mL round-bottom flask that had been previously washed with D_2O and dried at 120 °C. Lithium aluminum deuteride (388 mg, 9.2 mmol) was added. The reaction mixture was refluxed overnight under nitrogen, then cooled to 5 °C and hydrolyzed with 388 μL of D_2O, 388 μL of 15% of NaOD solution, and 3 x 388 μL of D_2O. The solid was removed by filtration and washed with ether. The filtrate was dried ($MgSO_4$), and the solvents were removed with a rotary evaporator. The residue was purified by flash chromatography (elution with hexane-ethyl acetate, 3:1) to give 1.90 g (94%) of **2a** as a white solid; mp 35 °C; TLC (hexane-ethyl acetate, 3:1) R_f 0.45; IR ($CHCl_3$) 3600-3070, 2951, 2913, 2836, 2214, 1466, 1456 cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$) δ (ppm) 0.85-0.91 (t, J = 7.0 Hz, 3 H, CH_3), 1.25 (m, 23 H, $(CH_2)_{11}$, OH), 1.99-2.06 (t, J = 6.8 Hz, 2 H, CH_2CD=CD), 4.08 (s, 2 H, CH_2OH). Anal. Calcd for $C_{16}H_{30}D_2O$: C, 79.27; H, 13.30. Found: C, 79.41; H, 13.20.

[2, 3-$^2H_2$]-(*E*)-Hexadec-2-enal (3a). Pyridium chlorochromate (2.40 g, 11.7 mmol) was suspended in 20 mL of methylene chloride, and 1.90 g (7.85 mmol) of [2, 3-$^2H_2$]-(*E*)-hexadec-2-en-1-ol (**2a**) in 4 mL of methylene chloride was added rapidly at room temperature. The reaction mixture was stirred at this temperature for 2 h in the dark. The reaction mixture was diluted with ether (100 mL). The solid was removed by filtration through silica gel. The solvents were concentrated under vacuum, giving a residue that was purified by flash chromatography (elution with hexane-ethyl acetate, 5:1). There was obtained 1.50 g (80%) of product; TLC (hexane-ethyl acetate, 5:1) R_f 0.60; ^1H NMR (200 MHz, $CDCl_3$) δ (ppm) 0.85-0.91 (t, J = 6.6 Hz, 3 H, CH_3), 1.26 (m, 22 H, $(CH_2)_{11}$), 2.10-2.14 (t, J = 6.8 Hz, 2 H, $CH_2CD{=}CD$), 9.60 (s, 1 H, CHO).

(*E*)-Hexadec-2-enal (3b). This compound was prepared from **2b** in 80% yield by using the same procedure as described for **3a**; ^1H NMR (200 MHz, $CDCl_3$) δ (ppm) 0.86-0.92 (t, J = 6.7 Hz, 3 H, CH_3), 1.25 (m, 22 H, $(CH_2)_{11}$), 2.12-2.23 (m, 2 H, $CH_2CH{=}CH$), 5.93-6.03 (dd, J = 15.5 Hz, 6.4 Hz, 1 H, vinyl H), 6.48-6.62 (dt, J = 15.6 Hz, 6.8 Hz, 1 H, vinyl H), 9.35-9.39 (d, J = 7.6 Hz, 1 H, CHO).

Tris(trimethylsilyl)glycine. A mixture of 21.0 g (0.13 mol) of 1,1,1,3,3,3-hexamethyldisilazane, 3.9 g (0.052 mol) of glycine, and 200 mg (1.5 mmol) of ammonium sulfate was refluxed for 7 days at 140-150 °C; glycine went into solution after a few hours. Distillation of the mixture under vacuum afforded 9.2 g (60%) of tris(trimethylsilyl)glycine, bp 115-120 °C/13 mm Hg (lit.[36] bp 101 °C/12 mm Hg); ^1H NMR (200 MHz, $CDCl_3$) δ 0.02 (s, 18 H, $N(SiMe_3)_2$), 0.23 (s, 9 H, CO_2SiMe_3), 3.28 (s, 2 H, $CH_2N(SiMe_3)_2$).

Bis(trimethylsilyl)sarcosine. A mixture of 7.1 g (33.7 mmol) of 1,1,1,3,3,3-hexamethyldisilazane, 2.5 g (28.1 mmol) of sarcosine, and 100 mg (0.76 mmol) of ammonium sulfate was refluxed for 7 days (150 °C). Distillation of the mixture under vacuum afforded 3.9 g (60%) of bis(trimethylsilyl)sarcosine, bp 47-49 °C/1.2 mm Hg; ^{1}H NMR ($CDCl_3$) δ 0.01 (s, 9 H, $NSiMe_3$), 0.25 (s, 9 H, CO_2SiMe_3), 2.43 (s, 3 H, NCH_3), 3.36 (s, 2 H, CH_2N).

2-Amino-[4,5-$^2H_2$]-(*E*)-octadec-4-enoic acid (4a). Dry diisopropylamine (0.925 mL, 6.6 mmol) was added to a solution of *n*-butyllithium in hexane (4.3 mL, 6.6 mmol of a 1.55 M solution) at 0 °C in freshly distilled THF (30 mL). The colorless solution was then stirred for 30 min at 0 °C, cooled to -78 °C, and treated dropwise with tris(trimethylsilyl)glycine (1.98 g, 6.6 mmol). The resulting light-orange reaction mixture was stirred for 1 h at -78 °C, and subsequently treated with [2, 3-^{2}H]-(*E*)-hexadec-2-enal **(3a)** (1.33 g, 5.5 mmol) in 1.5 mL of THF. Stirring was continued for 1 h at -78 °C, for 1 h at 0 °C, then overnight at 5 °C. The solution was acidified to pH 4 with ethanolic hydrogen chloride (EtOH-conc. HCl, 95:5), and the volatile components were removed under vacuum. The residue was dissolved in 150 mL of chloroform, then washed with 75 mL of water; a small volume of methanol was added to achieve a better separation. The organic phase was dried ($MgSO_4$) and evaporated under vacuum. Hexane (40 mL) was added to the residue, and the mixture was filtered. The solid was washed with hexane and dried to give 1.57 g (90%) of **4a**.

(*E*)-2-Aminooctadec-4-enoic acid (4b). This compound was prepared from **3b** in 82% yield by using the same procedure as described for

4a.

[4,5-$^2H_2$]-DL-*erythro*-Sphingosine (5a). Lithium aluminum hydride (0.46 g, 12 mmol) was slowly added to a solution of 0.94 g (3.0 mmol) of amino-acid **4a** in 20 mL of THF. The reaction mixture was refluxed 16 h, and then left at room temperature overnight. The suspension was hydrolyzed with 0.46 mL of water, 0.46 mL of 15% aqueous NaOH, and 1.38 mL of water. The aluminum hydroxide precipitate was removed by filtration and washed with ether (10 mL). The filtrate was dried ($MgSO_4$) and the solvents were concentrated under vacuum. The residue was purified by flash chromatography (elution with $CHCl_3$-MeOH, 65:25) to give 0.65 g (72%) of [4,5-$^2H_2$]-sphingosine **5a**; TLC ($CHCl_3$-MeOH, 3:2) R_f 0.32; ^1H NMR (200 MHz, $CDCl_3$) δ (ppm) 0.85-0.91 (t, J = 6.9 Hz, 3 H, CH_3), 1.26 (m, 22 H, $(CH_2)_{11}$), 2.04-2.07 (t, J = 6.8 Hz, 2 H, $CH_2CD{=}CD$), 2.75-2.78 (m, 1 H, $CHNH_2$), 3.00 (s, 4 H, NH_2, OH, OH), 3.63-3.65 (d, J = 5.0 Hz, 2 H, CH_2OH), 3.97-4.04 (d, J = 6.6 Hz, 1 H, $CD{=}CDCHOH$).

DL-*erythro*-Sphingosine (5b). This compound was prepared from **4b** in 70% yield by using the same procedure as described for **5a**; ^1H NMR (200 MHz, $CDCl_3$) δ (ppm) 0.85-0.90 (t, J = 6.2 Hz, 3 H, CH_3), 1.26 (m, 22 H, $(CH_2)_{11}$), 2.00-2.07 (m, 2 H, $CH_2CH{=}CH$), 2.32 (s, 4 H, NH_2, OH, OH), 2.84-2.86 (m, 1 H, CHNH), 3.66 (m, 2 H, CH_2OH), 4.05-4.09 (m, 1 H, $CHOH$), 5.41-5.52 (dd, J = 15.4 Hz, 6.7 Hz, 1 H, vinyl H), 5.69-5.80 (dt, J = 15.4 Hz, 6.5 Hz, 1 H, vinyl H).

DL-*erythro*-^{15}N-Sphingosine (5c). This compound was prepared from ^{15}N-glycine in 55% yield by the same procedure as described above;

TLC ($CHCl_3$-MeOH, 3:2) R_f 0.33; IR ($CHCl_3$) 3624-3060, 3013, 2920, 2851, 1584, 1468, 1215, 1091, 1044, 1030, 972, 944, 868, 760, 668 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ (ppm) 0.85-0.91 (t, *J* = 6.6 Hz, 3 H, CH_3), 1.26 (m, 22 H, $(CH_2)_{11}$), 2.00-2.07 (m, 2 H, CH_2CH=CH), 2.32 (s, 4 H, NH_2, 2 OH), 2.84-2.86 (m, 1 H, $CHNH_2$), 3.66 (m, 2 H, CH_2OH), 4.05-4.09 (m, 1 H, CHOH), 5.41-5.52 (dd, *J* = 15.4 Hz, 6.7 Hz, 1 H, vinyl H), 5.69-5.80 (dt, *J* = 15.4 Hz, 6.7 Hz, 1 H, vinyl H). Anal. Calcd for $C_{18}H_{37}O_2N$: C, 71.59; H, 12.41; N, 4.99. Found: C, 72.09; H, 12.66; N, 4.91.

***N*-Octadecanoyl-[4,5-$^2H_2$]-DL-*erythro*-sphingosine (6a).** A solution of *p*-nitrophenyl stearate (1.04 g, 2.55 mmol) in 4 mL of THF was slowly added to a solution of [4, 5-$^2H_2$]-sphingosine **(5a)** (0.70 g, 2.32 mmol) in 15 mL of dry THF. After the mixture was stirred at room temperature for 22 h, the solvent was removed under vacuum. The residue was dissolved in 30 mL of chloroform and washed with 15% aqueous NaOH (2 x 20 mL) and water (3 x 20 mL) to remove *p*-nitrophenol. The organic layer was dried ($MgSO_4$), and the solvents were concentrated with a rotary evaporator. The crude product was purified by flash chromatography (elution with $CHCl_3$-MeOH, 95:5) to give 0.98 g (74%) of product as a white solid, mp 97-97.5 °C; TLC ($CHCl_3$-EtOH, 9:1) R_f 0.62; ^1H NMR (200 MHz, $CDCl_3$) δ 0.85-0.91 (t, *J* = 6.2 Hz, 6 H, $CH_3(CH_2)_{11}$, $CH_3(CH_2)_{15}$), 1.26 (m, 50 H, $(CH_2)_{11}$, $(CH_2)_{14}$), 1.61 (m, 2 H, CH_2CH_2CO), 1.99-2.05 (t, *J* = 6.5 Hz, 2 H, CH_2CD=CD), 2.17-2.24 (t, *J* = 7.5 Hz, 2 H, CH_2CO), 3.61-3.67 (m, 1 H, CHNH), 3.82-3.86 (m, 2 H, CH_2CH_2OH), 4.09-4.18 (d, *J* = 6.6 Hz, 1 H, CD=CDCHOH), 6.51 (d, *J* = 7.0 Hz, 1 H, NH). Anal. Calcd for $C_{36}H_{69}D_2O_3N$: C, 76.19; H, 12.17; N, 2.47. Found: C, 75.46; H, 12.12; N, 2.48.

***N*-Octadecanoyl-DL-*erythro*-sphingosine (6b).** This compound was prepared from **5b** in 75% yield by using the same procedure as described for **6a**; mp 97-97.5 °C (lit.[37,38] mp 97-98 °C for *N*-octadecanoyl-D-*erythro*-sphingosine); TLC ($CHCl_3$-EtOH, 100:7) R_f 0.45; ^1H NMR (300 MHz, $CDCl_3$) δ (ppm) 0.85-0.91 (t, J = 6.6 Hz, 6 H, $CH_3(CH_2)_{11}$, $CH_3(CH_2)_{14}$), 1.25 (m, 50 H, $(CH_2)_{11}$, $(CH_2)_{14}$), 1.60 (m, 2 H, CH_2CH_2CO), 2.01-2.05 (m, 2 H, CH_2CH=CH), 2.15-2.22 (t, J = 7.5 Hz, 2 H, CH_2CH_2CO), 3.61-3.67 (m, 1 H, CHNH), 3.85-3.90 (m, 2 H, CH_2OH), 4.10-4.18 (m, 1 H, CH=CHCHOH), 5.42-5.53 (dd, J = 15.4 Hz, 6.5 Hz, 1 H, vinyl H), 5.68-5.79 (dt, J = 15.4 Hz, 6.5 Hz, 1 H, vinyl H), 6.50 (d, J = 7.2 Hz, 1 H, NH). Anal. Calcd for $C_{36}H_{71}O_3N$: C, 76.40; H, 12.64; N, 2.47. Found: C, 76.46; H, 12.12; N, 2.48.

^{15}*N*-Octadecanoyl-DL-*erythro*-sphingosine (6c). This compound was prepared from **5c** in 85% yield by using the same procedure as described above; mp 97-97.5 °C ; TLC ($CHCl_3$-EtOH, 100:7) R_f 0.45; IR ($CHCl_3$) 3695-3072, 2916, 2848, 1635, 1603, 1541, 1466, 1249, 1149, 1096, 1067, 1046, 970 cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$) δ (ppm) 0.85-0.91 (t, J = 6.2 Hz, 6 H, $CH_3(CH_2)_{11}$, $CH_3(CH_2)_{15}$), 1.25 (m, 50 H, $CH_2)_{11}$, $(CH_2)_{14}$), 1.60 (m, 2 H, CH_2CH_2CO), 1.97-2.08 (m, 2 H, CH_2CH=CH), 2.15-2.22 (t, J = 7.5 Hz, 2 H, CH_2CO), 3.47-3.64 (m, 1 H, CHNH), 3.89-3.99 (m, 2 H, CH_2OH), 4.10-4.20 (m, 1 H, CHOH), 5.45-5.54 (dd, J = 15.4 Hz, 6.6 Hz, 1 H, vinyl H), 5.62-5.76 (dt, J = 15.4 Hz, 6.8 Hz, 1 H, vinyl H). Anal. Calcd for $C_{36}H_{71}O_3{}^{15}N$: C, 76.27; H, 12.62; N, 2.64. Found: C, 75.37; H, 12.96; N, 2.59.

1-(*O-tert*-Butyldiphenylsilyl)-2-(*N*-octadecanoyl)-[4,5-$^2H_2$]-DL-*erythro*-sphingosine (7a). A solution of ceramide **6a** (0.9 g, 1.59 mmol), *tert*-butyldiphenylsilyl chloride (0.44 g, 1.59 mmol), and imidazole (0.22 g, 3.18 mmol) in 25 mL of DMF-THF (1:1) was allowed to stir at room temperature for 3 h. TLC (chloroform-ethanol, 98:2) showed that the reaction was complete. Ether (25 mL) was added, and the organic phase was extracted three times with water. The water phase was extracted with ether twice. The combined ethereal solution was dried ($MgSO_4$), and the solvents were removed using a rotary evaporator. The residue was purified by flash chromatography (elution with $CHCl_3$-EtOH, 98:2) to give 1.04 g (81%) of the product; TLC ($CHCl_3$-EtOH, 98:2) R_f 0.43; (hexane-ethyl acetate, 4:1) R_f 0.27; ^1H NMR (200 MHz, $CDCl_3$) δ (ppm) 0.85-0.91 (t, J = 6.5 Hz, 6 H, $CH_3(CH_2)_{11}$, $CH_3(CH_2)_{15}$), 1.09 (s, 9 H, $C(CH_3)_3$), 1.25 (m, 50 H, $(CH_2)_{11}$, $(CH_2)_{14}$), 1.60 (m, 2 H, CH_2CH_2CO), 2.00-2.04 (t, J = 6.6 Hz, 2 H, $CH_2CD{=}CD$), 2.11-2.19 (t, J = 7.6 Hz, 2 H, CH_2CO), 3.70-3.74 (m, 1 H, CHNH), 3.90-3.96 (m, 2 H, CH_2OH), 4.17 (d, J = 6.3 Hz, 1 H, CHOH), 6.30-6.34 (d, J = 7.5 Hz, 1 H, NH), 7.39-7.46 (m, 10 H, Ar).

1-(*O-tert*-Butyldiphenylsilyl)-2-(*N*-octadecanoyl)-DL-*erythro*-sphingosine (7b). Imidazole (0.22 g, 3.18 mmol) was dissolved in 25 mL of dry methylene chloride, and *tert*-butyldiphenylsilyl chloride (0.44 g, 1.59 mmol) was added. Immediately some white precipitate was formed (imidazolium chloride). After the mixture has stirred for 1 hour at room temperature, a solution of alcohol **6b** (0.9 g, 1.59 mmol) in 5 mL of methylene chloride was added slowly. The reaction mixture was stirred at room temperature overnight. Additional methylene chloride (15 mL) was added to dilute the mixture, and the mixture was washed with saturated sodium chloride

solution (30 mL) and water (30 mL). The organic phase was dried ($MgSO_4$), and the solvent was removed by rotary evaporation. The residue was purified by flash chromatography (elution with hexane-ethyl acetate, 4:1) to give 1.04 g (81%) of the pure product; mp 33 °C; TLC (hexane-ethyl acetate, 4:1) R_f 0.26; IR ($CHCl_3$) 3600-3170, 3072, 3060, 2919, 2849, 1655, 1606, 1490, 1461, 1425, 1108, 903, 820, 732 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ (ppm) 0.85-0.91 (t, J = 6.3 Hz, 6 H, $CH_3(CH_2)_{11}$, $CH_3(CH_2)_{14}$), 1.07 (s, 9 H, $C(CH_3)_3$), 1.26 (m, 50 H, $(CH_2)_{11}$, $(CH_2)_{14}$), 1.60 (m, 2 H, CH_2CH_2CO), 2.01-2.05 (m, 2 H, CH_2CH=CH), 2.11-2.19 (t, J = 7.6 Hz, 2 H, CH_2CH_2CO), 3.74-3.78 (m, 1 H, CHNH), 3.92-3.98 (m, 2 H, CH_2O), 4.19 (m, 1 H, CH=CHCHOH), 5.42-5.52 (dd, J = 15.4 Hz, 5.5 Hz, 1 H, vinyl H), 5.70-5.89 (dt, J = 15.6 Hz, 6.4 Hz, 1 H, vinyl H), 6.30-6.34 (d, J = 7.5 Hz, 1 H, NH), 7.39-7.46 (m, 10 H, Ar). Anal. Calcd for $C_{52}H_{89}O_3NSi$: C, 77.65; H, 11.15; N, 1.74. Found: C, 77.81; H, 11.02; N, 1.80.

1-(*O-tert*-Butyldiphenylsilyl)-2-(^{15}N-octadecanoyl)-DL-*erythro*-sphingosine (7c). This compound was prepared from **6c** in 87% yield by using the same procedure as described for **7b**; mp 33.5 °C; TLC (hexane-ethyl acetate, 4:1) R_f 0.27; IR ($CHCl_3$) 3601-3248, 2924, 2853, 1656, 1490, 1466, 1428, 1216, 1113, 758, 709 cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$) δ (ppm) 0.85-0.91 (t, J = 6.3 Hz, 6 H, $CH_3(CH_2)_{11}$, $CH_3(CH_2)_{14}$), 1.07 (s, 9 H, $C(CH_3)_3$), 1.26 (m, 50 H, $(CH_2)_{11}$, $(CH_2)_{14}$), 1.60 (m, 2 H, CH_2CH_2CO), 2.01-2.05 (m, 2 H, CH_2CH=CH), 2.11-2.19 (t, J = 7.6 Hz, 2 H, CH_2CH_2CO), 3.74-3.78 (m, 1 H, CHNH), 3.92-3.99 (m, 2 H, CH_2O), 4.19 (m, 1 H, CH=CHCHOH), 5.42-5.52 (dd, J = 15.4 Hz, 5.5 Hz, 1 H, vinyl H), 5.70-5.89 (dt, J = 15.6 Hz, 6.4 Hz, 1 H, vinyl H), 6.30-6.34 (d, J = 7.5 Hz, 1 H, CHNH), 7.39-7.46 (m, 10 H, Ar). Anal. Calcd for $C_{52}H_{89}O_3{}^{15}NSi$: C, 77.55; H, 11.14;

N, 1.74. Found: C, 77.24; H, 11.19; N, 1.60.

1-(*O-tert*-Butyldiphenylsilyl)-2-(*N*-octadecanoyl)-3-(*O*-tetrahydropyranyl)-[4,5-$^2H_2$]-DL-*erythro*-sphingosine (8a). To a solution of allylic alcohol **7a** (0.94 g, 1.17 mmol) in 12 mL of methylene chloride was added 181.5 μL (1.99 mmol) of dihydropyran. A few crystals of *p*-toluenesulfonic acid were added, which were dissolved by swirling. The reaction mixture was stirred at room temperature for 6 h. The solution was diluted with ether, and washed with saturated sodium bicarbonate solution and water. The organic layer was dried ($MgSO_4$), and the solvents were removed with a rotary evaporator. The crude product was purified by flash chromatography (elution with hexane-ethyl acetate, 4:1) to give 0.96 g (91%) of pure product; TLC (hexane-ethyl acetate, 4:1) R_f 0.52.

1-(*O-tert*-Butyldiphenylsilyl)-2-(*N*-octadecanoyl)-3-(*O*-tetrahydropyranyl)-DL-*erythro*-sphingosine (8b). This compound was prepared from **7b** in 82% yield by using the same procedure as described for **8a**.

1-(*O-tert*-Butyldiphenylsilyl)-2-(^{15}*N*-octadecanoyl)-3-(*O*-tetrahydropyranyl)-DL-*erythro*-sphingosine (8c). This compound was prepared from **7c** in 97% yield by using the same procedure as described above; mp 53 °C; TLC (hexane-ethyl acetate, 4:1) R_f 0.52; IR ($CHCl_3$) 3436, 3284, 2920, 2851, 1666, 1643, 1537, 1489, 1466, 1428, 1378, 1260, 1216, 1200, 1183, 1112, 1022, 969 cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$) δ (ppm) 0.85-0.91 (t, J = 6.6 Hz, 6 H, $CH_3(CH_2)_{11}$, $CH_3(CH_2)_{14}$), 1.08 (s, 9 H, $C(CH_3)_3$), 1.26 (m, 50 H, $(CH_2)_{11}$, $(CH_2)_{14}$), 1.57-1.64 (m, 8 H, CH_2CH_2CO,

(CH_2)$_3$ of THP), 2.01-2.05 (m, 4 H, C*H*$_2$CH=CH, CH_2C*H*$_2$CO), 3.39-3.45 (m, 1 H, C*H*NH), 3.75-3.84 (m, 2 H, C*H*$_2$O of THP), 3.95-4.20 (m, 3 H, CH=CHC*H*O, CH_2O), 4.64-4.70 (m, 1 H, 1'-CHO of THP), 5.22-5.38 (dd, *J* = 15.4 Hz, 6.7 Hz, 1 H, vinyl H), 5.46-5.70 (dt, *J* = 15.6 Hz, 6.7 Hz, 1 H, vinyl H), 6.04 (d, *J* = 7.5 Hz, 1 H, CHN*H*), 7.40-7.70 (m, 10 H, Ar). Anal. Calcd for $C_{57}H_{97}O_4{}^{15}NSi$: C, 76.97; H, 10.99; N, 1.57. Found: C, 77.22; H, 11.20; N, 1.48.

2-(*N*-Octadecanoyl)-3-(*O*-tetrahydropyranyl)-[4,5-$^2H_2$]-DL-*erythro*-sphingosine (9a). To a solution of 960 mg (1.08 mmol) of protected sphingosine **8a** in 10 mL of THF was added 2.16 mL (2.16 mmol) of a 1 M solution of tetra-*n*-butylammonium fluoride in THF. After the mixture was allowed to stir for 30 min, water was added. The resulting mixture was extracted with ether, and the combined extracts were dried over $MgSO_4$. The solvents were removed under reduced pressure. The residue was purified by flash chromatography (elution with $CHCl_3$-EtOH, 98:2) to give 540 mg (76%) of the product **9a** as a white solid; TLC (hexane-ethyl acetate, 4:5) R_f 0.46; ^{1}H NMR (200 MHz, $CDCl_3$) δ (ppm) 0.85-0.91 (t, *J* = 6.4 Hz, 6 H, C*H*$_3$(CH_2)$_{11}$, C*H*$_3$(CH_2)$_{15}$), 1.25 (m, 50 H, (CH_2)$_{11}$, (CH_2)$_{14}$), 1.51-1.80 (m, 8 H, C*H*$_2$C*H*$_2$CO, (CH_2)$_3$ of THP), 1.99-2.06 (t, *J* = 6.8 Hz, 2 H, CH_2CD=CD), 2.15-2.23 (t, *J* = 7.8 Hz, 2 H, CH_2CO), 3.31-3.35 (m, 1 H, C*H*NH), 3.89-3.99 (m, 4 H, CH_2OH, CH_2O of THP), 4.16-4.22 (d, *J* = 6.6 Hz, 1 H, CD=CDC*H*OH), 4.45-4.48 (m, 1 H, 1'-CHO of THP), 6.37-6.41 (d, *J* = 7.5 Hz, 1 H, NH). Anal. Calcd for $C_{41}H_{67}D_2O_4N$: C, 75.64; H, 12.23; N, 2.30. Found: C, 74.85; H, 12.26; N, 2.02.

2-(*N*-Octadecanoyl)-3-(*O*-tetrahydropyranyl)-DL-*erythro*-sphingosine (9b). This compound was prepared from **8b** in 85% yield by using the same procedure as described for **9a**; ^{1}H NMR (200 MHz, $CDCl_3$) δ (ppm) 0.85-0.90 (t, J = 6.6 Hz, 6 H, $CH_3(CH_2)_{11}$, $CH_3(CH_2)_{14}$), 1.25 (m, 50 H, $(CH_2)_{11}$, $(CH_2)_{14}$), 1.61-1.80 (m, 8 H, CH_2CH_2CO, $(CH_2)_3$ of THP), 1.99-2.03 (m, 2 H, CH_2CH=CH), 2.12-2.19 (t, J = 7.2 Hz, 2 H, CH_2CH_2CO), 3.33-3.38 (m 1 H, CHNH), 3.87-3.99 (m, 4 H, CH_2OH, CH_2O of THP), 4.16-4.19 (m, 1 H, CHOTHP), 4.46-4.49 (m, 1 H, O'CHO of THP), 5.33-5.45 (dd, J = 15.4, Hz, 6.7 Hz, 1 H, vinyl H), 5.61-5.72 (dt, J = 15.4 Hz, 6.7 Hz, 1 H, vinyl H), 6.36-6.40 (d, J = 7.6 Hz, 1 H, CHNH).

2-(^{15}*N*-Octadecanoyl)-3-(*O*-tetrahydropyranyl)-DL-*erythro*-sphingosine (9c). This compound was prepared from **8c** in 95% yield by using the same procedure as described above; mp 69 °C; TLC (hexane-ethyl acetate, 4:5) R_f 0.46; IR ($CHCl_3$) 3670-3060, 2919, 2851, 1643, 1551, 1466, 1378, 1261, 1200, 1110, 1027, 968, 908, 758 cm^{-1}; ^{1}H NMR (200 MHz, $CDCl_3$) δ (ppm) 0.85-0.90 (t, J = 6.4 Hz, 6 H, $CH_3(CH_2)_{11}$, $CH_3(CH_2)_{15}$), 1.25 (m, 50 H, $(CH_2)_{11}$, $(CH_2)_{14}$), 1.53-1.78 (m, 8 H, CH_2CH_2CO, $(CH_2)_3$ of THP), 2.02-2.05 (m, 2 H, CH_2CH=CH), 2.15-2.22 (t, J = 7.2 Hz, 2 H, CH_2CH_2CO), 3.28-3.32 (m, 1 H, CHNH), 3.47-3.62 (m, 2 H, CH_2O of THP), 3.94-3.99 (m, 2 H, CH_2OH), 4.16-4.18 (m, 1 H, CHO), 4.46 (m, 1 H, 1'-CHO of THP), 5.31-5.42 (dd, J = 15.6 Hz, 6.9 Hz, 1 H, vinyl H), 5.63-5.74 (dt, J = 15.5 Hz, 6.9 Hz, 1 H, vinyl H), 6.13-6.17 (d, J = 7.7 Hz, 1 H, CHNH). Anal. Calcd for $C_{41}H_{79}O_4{}^{15}N$: C, 75.63; H, 12.23; N, 2.15. Found: C, 74.85; H, 12.26; N, 2.02.

[4,5-$^2H_2$]-DL-*erythro*-Sphingomyelin (10a). A solution of primary alcohol **9a** (50 mg, 0.08 mmol) and triethylamine (13.2 μL, 0.09 mmol) in 0.5 mL of THF was added slowly to a solution of 2-chloro-2-oxo-1,3,2-dioxaphospholane (0.5 M, 240 μL) in 2 mL of dry THF at 0 °C. The reaction mixture was kept at 5 °C for 30 min, then at room temperature for 2 days. The solid (Et_3N·HCl) was removed by filtration, and the filtrate was concentrated under vacuum. The crude product was purified by flash chromatography (elution with $CHCl_3$-MeOH, 4:1) to give 39 mg (67%) of the intermediate cyclic phosphate as a white solid; TLC ($CHCl_3$-MeOH-7 N NH_4OH, 65:25:4) R_f 0.73. The above solid was dissolved in a mininum volume of chloroform and transferred to a 25-mL pressure bottle, the solvent was evaporated under a stream of nitrogen. Acetonitrile (2 mL) was added, and the mixture was cooled to -78 °C. Liquid anhydrous trimethylamine (0.2 mL) was added to the pressure bottle, the reaction mixture was allowed to warm to room temperature, and then was heated with stirring at 60-70 °C on an oil bath for 44 h. After the reaction was complete as monitored by TLC ($CHCl_3$-MeOH-7 N NH_4OH, 65:25:5), the solvent was removed by rotary evaporation. The residue was dissolved in 4 mL of MeOH-THF (9:1). A trace of *p*-toluenesulfonic acid was added, and the reaction mixture was refluxed overnight. The solvents were concentrated under vacuum, leaving a residue that was purified by flash chromatography (elution with $CHCl_3$-MeOH-7 N NH_4OH, 65:25:4). Suspended silica gel was removed by passing a $CHCl_3$ solution of the product through a 0.45-μm Metricel filter (Gelman Sciences, Fisher Scientific) three times to give 25 mg (66%) of [4,5-$^2H_2$]-sphingomyelin (**10a**); TLC ($CHCl_3$-MeOH-7 N NH_4OH, 65:25:5) R_f 0.21; ^1H NMR (200 MHz, $CDCl_3$) δ (ppm) 0.85-0.91 (t, J = 6.3 Hz, 6 H, $CH_3(CH_2)_{11}$, $CH_3(CH_2)_{15}$), 1.25 (m, 50 H, $(CH_2)_{11}$, $(CH_2)_{14}$), 1.54 (m, 2 H, CH_2CH_2CO), 2.00-2.07 (t, J =

6.5 Hz, 2 H, $CH_2CD{=}CD$), 2.11-2.17 (t, J = 7.6 Hz, 2 H, CH_2CO), 3.35 (s, 9 H, $N(CH_3)_3$), 3.70 (m, 2 H, $CH_2N(CH_3)_3$), 3.93 (m, 1 H, CHNH), 4.15 (m, 4 H, $CH_2OP(O)OCH_2$), 4.39 (d, J = 6.6 Hz, 1 H, $CD{=}CDCHOH$). Anal. Calcd for $C_{41}H_{81}D_2O_6N_2P{\cdot}3H_2O$: C, 62.60; H, 11.32; N, 3.56; P, 3.94. Found: C, 62.52; H, 11.30; N, 3.65; P, 4.21.

DL-*erythro*-Sphingomyelin (10b). This compound was prepared from **9b** in 34% yield by using the same procedure as described for **10a**.

^{15}N-DL-*erythro*-Sphingomyelin (10c). 2-Bromoethylphosphoric acid dichloride[39] (0.5 M in THF, 800 µL, 0.40 mmol) was cooled in an ice bath. After the addition of dry triethylamine (167 µL, 1.20 mmol), the phosphorylation mixture was kept at 25 °C and a solution of alcohol **9c** (127 mg, 0.20 mmol) in 2 mL of dry THF was added dropwise with stirring. The reaction mixture was stirred for 1 day at room temperature. A precipitate (triethylammonium hydrochloride) formed, which was removed by filtration. The filtrate was evaporated to dryness. The residue was dissolved in 1.5 mL of THF. Sodium acetate (1.5 mL of a 0.5 M solution in water) and ethylenediaminetetraacetate disodium salt (0.1 mL of a 0.5 M solution in water) were added with stirring. After overnight hydrolysis, chloroform (10 mL) was added, and the mixture was extracted with water. The organic layer was dried ($MgSO_4$) and evaporated to dryness. The residue was purified by flash chromatography (first with hexane-ethyl acetate, 4:5, then with $CHCl_3$-MeOH-7 N NH_4OH, 65:25:4) to give 85 mg (54%) of the bromoethyl ester intermediate as a yellow oil; TLC ($CHCl_3$-MeOH-7 N NH_4OH, 65:25:4) R_f 0.65.

To a solution of the bromoethyl ester (85 mg, 0.10 mmol) in chloroform

(0.9 mL) were added 2-propanol (1.5 mL) and acetonitrile (1.5 mL), followed by trimethylamine in water (3 mL of a 25% aqueous solution). The reaction was completed after stirring for 48 h at room temperature. The solvent was removed using a rotary evaporator (azeotropic distillation with excess 2-propanol). The crude product was purified by flash chromatography (elution first with $CHCl_3$-MeOH, 9:1, then with $CHCl_3$-MeOH-7 N NH_4OH, 65:25:4); yield (contains silica gel), 60 mg (75%); TLC ($CHCl_3$-MeOH-7 N NH_4OH, 65:25:4) R_f 0.33.

The above compound (60 mg, 0.073 mmol) was dissolved in 4 mL of methanol. A trace of *p*-toluenesulfonic acid was added, and the reaction mixture was refluxed overnight. The solvents were concentrated under vacuum, leaving a residue that was purified by flash chromatography (elution with $CHCl_3$-MeOH-7 N NH_4OH, 65:25:4). Suspended silica gel was removed by passing a $CHCl_3$ solution of the product through a 0.45-μm Metricel filter three times to give 38 mg (71%) of ^{15}N-sphingomyelin (**10c**); TLC ($CHCl_3$-MeOH-7 N NH_4OH, 65:25:5) R_f 0.22; ^1H NMR (300 MHz, $CDCl_3$) δ (ppm) 0.85-0.91 (t, J = 6.5 Hz, 6 H, $CH_3(CH_2)_{11}$, $CH_3(CH_2)_{15}$), 1.26 (m, 50 H, $(CH_2)_{11}$, $(CH_2)_{14}$), 1.57 (m, 2 H, CH_2CH_2CO), 2.00-2.07 (m, 2 H, $CH_2CH{=}CH$), 2.11-2.17 (t, J = 7.4 Hz, 2 H, CH_2CO), 3.35 (s, 9 H, $N(CH_3)_3$), 3.70 (m, 2 H, $CH_2N(CH_3)_3$), 3.95 (m, 1 H, CHNH), 4.14 (m, 4 H, $CH_2OP(O)OCH_2$), 4.39 (m, 1 H, $CH{=}CHCHOH$), 5.55-5.64 (dd, J = 15.5 Hz, 6.9 Hz, 1 H, vinyl H), 5.81-5.90 (dt, J = 15.4 Hz, 7.0 Hz, 1 H, vinyl H). Anal Calcd for $C_{41}H_{38}O_6N_2P{\cdot}3H_2O$: C, 61.23; H, 11.40; N, 3.50; P, 3.85. Found: C, 61.00; H, 10.82; N, 3.01; P, 3.73.

1-(*O*-*tert*-Butyldiphenylsilyl)-2-(*N*-octadecanoyl)-3-*O*-methyl-DL-*erythro*-sphingosine (11b) (Scheme 2). Sodium

hydride (35 mg, 1.44 mmol) was added to a solution of 580 mg (0.72 mmol) of allylic alcohol **8b** in 15 mL of THF. After the reaction mixture was stirred at room temperature for 1 h, a trace of tetra-*n*-butylammonium iodide and 450 μL (7.2 mmol) of methyl iodide (10-fold molar excess) were added. The mixture was stirred at room temperature for 6 h. The solvent was removed under reduced pressure. The residue was dissolved in 25 mL of chloroform, and washed with 10% ammonium chloride solution (20 mL). The organic phase was dried ($MgSO_4$). The residue was purified by flash chromatography (elution with hexane-ethyl acetate, 5:1) to give 380 mg (64%) of the pure product as a white solid; mp 49-49.5 °C; TLC (hexane-ethyl acetate, 5:1) R_f 0.48; IR ($CHCl_3$) 3425, 3072, 3060, 2919, 2849, 1661, 1590, 1490, 1461, 1378, 1261, 1108, 903, 820, 732, 703 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ (ppm) 0.85-0.91 (t, J = 6.6 Hz, 6 H, $CH_3(CH_2)_{11}$, $CH_3(CH_2)_{14}$), 1.07 (s, 9 H, $C(CH_3)_3$), 1.26 (m, 50 H, $(CH_2)_{11}$, $(CH_2)_{14}$), 1.67 (m, 2 H, CH_2CH_2CO), 2.00-2.13 (m, 4 H, $CH_2CH{=}CH$, CH_2CH_2CO), 3.22 (s, 3 H, OCH_3), 3.65-3.72 (m, 2 H, $CHOCH_3$, CHNH), 3.95-4.09 (m, 2 H, CH_2O), 5.29-5.47 (m, 1 H, vinyl H), 5.61-5.91 (m, 1 H, vinyl H), 7.39-7.67 (m, 10 H, Ar). Anal. Calcd for $C_{53}H_{91}O_3NSi$: C, 77.78; H, 11.21; N, 1.71. Found: C, 77.90; H, 11.17; N, 1.84.

1-(*O*-*tert*-Butyldiphenylsilyl)-2-(^{15}*N*-octadecanoyl)-3-*O*-methyl-DL-*erythro*-sphingosine (11c). This compound was prepared in 63% yield by using the same procedure as described above; mp 49.5 °C; TLC (hexane-ethyl acetate, 5:1) R_f 0.48; IR ($CHCl_3$) 3284, 2919, 2849, 1663, 1637, 1525, 1467, 1425, 1378, 1361, 1261, 1102, 1067, 961 cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$) δ (ppm) 0.85-0.88 (t, J = 6.6 Hz, 6 H, $CH_3(CH_2)_{11}$, $CH_3(CH_2)_{14}$), 1.07 (s, 9 H, $C(CH_3)_3$), 1.25 (m, 50 H, $(CH_2)_{11}$, $(CH_2)_{14}$),

1.57-1.64 (m, 2 H, CH_2CH_2CO), 2.00-2.12 (m, 4 H, CH_2CH=CH, CH$_2$CH_2CO), 3.22 (s, 3 H, OCH$_3$), 3.65-3.72 (m, 1 H, C*H*NH), 3.94-4.09 (m, 3 H, CH$_2$O, C*H*OCH$_3$), 5.21-5.33 (dd, *J* = 15.4 Hz, 7.0 Hz, 1 H, vinyl H), 5.57-5.68 (dt, *J* = 15.4 Hz, 7.0 Hz, 1 H, vinyl H), 5.89-5.91 (d, *J* = 9.1 Hz, 1 H, CHN*H*), 7.39-7.67 (m, 10 H, Ar). Anal. Calcd for $C_{53}H_{91}O_3{}^{15}NSi$: C, 77.69; H, 11.19; N, 1.71. Found: C, 77.58; H, 11.43; N, 1.76.

1-(*O-tert*-Butyldiphenylsilyl)-2-(*N*-octadecanoyl)-3-*O*-ethyl-DL-*erythro*-sphingosine (12b). This compound was prepared from **7b** in 54% yield by using the same procedure as described for **11b**; TLC (hexane-ethyl acetate, 6:1) R_f 0.55; IR ($CHCl_3$) 3440, 3071, 3050, 2926, 2854, 1655, 1590, 1514, 1465, 1428, 1361, 1263, 1216, 1113, 1008, 938, 872, 823, 759, 741, 701 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ (ppm) 0.86-0.90 (t, *J* = 6.5 Hz, 6 H, CH_3(CH$_2$)$_{11}$, CH$_3$(CH$_2$)$_{14}$), 1.07 (s, 9 H, C(CH$_3$)$_3$), 1.10-1.13 (t, *J* = 7.0 Hz, 3 H, OCH$_2$CH_3), 1.25 (m, 50 H, (CH$_2$)$_{11}$, (CH$_2$)$_{14}$), 1.60 (m, 2 H, CH_2CH$_2$CO), 1.97-2.11 (m, 4 H, CH_2CH=CH, CH$_2$CH_2CO), 3.22-3.27 (q, *J* = 7.0 Hz, 2 H, OCH_2CH$_3$), 3.51-3.56 (m, 1 H, C*H*NH), 3.67-3.71 (dd, *J* = 9.9 Hz, 3.0 Hz, 1 H, C*H*OCH$_2$CH$_3$), 3.99-4.03 (m, 2 H, CH$_2$O), 5.26-5.34 (dd, *J* = 15.4 Hz, 7.0 Hz, 1 H, vinyl H), 5.57-5.69 (m, 2 H, vinyl H, NH), 7.37-7.64 (m, 10 H, Ar). Anal. Calcd for $C_{54}H_{93}O_3NSi$: C, 77.92; H, 11.26; N, 1.68. Found: C, 78.20; H, 11.52; N, 1.75.

1-(*O-tert*-Butyldiphenylsilyl)-2-(^{15}*N*-octadecanoyl)-3-*O*-ethyl-DL-*erythro*-sphingosine (12c). This compound was prepared in 57% yield by using the same procedure as described above; TLC (hexane-ethyl acetate, 5:1) R_f 0.50; IR ($CHCl_3$) 3464-3331, 3060, 3037, 2919, 2849, 1655, 1514, 1467, 1425, 1362, 1263, 1108, 1008, 872, 820 cm^{-1}; ^1H NMR (200

MHz, $CDCl_3$) δ 0.84-0.90 (t, J = 6.6 Hz, 6 H, $CH_3(CH_2)_{11}$, $CH_3(CH_2)_{14}$), 1.02-1.14 (m, 12 H, $C(CH_3)_3$, OCH_2CH_3), 1.24 (m, 50 H, $(CH_2)_{11}$, $(CH_2)_{14}$), 1.48-1.74 (m, 4 H, CH_2CH_2CO, $CH_2CH{=}CH$), 1.93-2.03 (m, 2 H, CH_2CH_2CO), 3.25-3.47 (q, J = 6.9 Hz, 2 H, OCH_2CH_3), 3.69-3.76 (m, 1 H, CHNH), 4.10-4.28 (m, 3 H, $CHOCH_2CH_3$, CH_2O), 5.20-5.34 (m, 2 H, $CH_2CH{=}CH$), 5.72-5.77 (d, J = 9.1 Hz, 1 H, CHNH), 7.29-7.68 (m, 10 H, Ar). Anal. Calcd for $C_{54}H_{93}O_3{}^{15}NSi$: C, 77.82; H, 11.25; N, 1.68. Found: C, 77.07; H, 10.92; N, 1.55.

2-(*N*-Octadecanoyl)-3-*O*-methyl-DL-*erythro*-sphingosine (13b). This compound was prepared from **11b** in 97% yield by using the same procedure as described for **9a**; mp 79.5°C; TLC (hexane-ethyl acetate, 4:5) R_f 0.40; ^1H NMR (300 MHz, $CDCl_3$) δ (ppm) 0.85-0.91 (t, J = 6.6 Hz, 6 H, $CH_3(CH_2)_{11}$, $CH_3(CH_2)_{14}$), 1.25 (m, 50 H, $(CH_2)_{11}$, $(CH_2)_{14}$), 1.64 (m, 2 H, CH_2CH_2CO), 2.03-2.09 (m, 2 H, $CH_2CH{=}CH$), 2.18-2.26 (t, J = 7.5 Hz, 2 H, CH_2CH_2CO), 3.26 (s, 3 H, OCH_3), 3.57-3.62 (m, 1 H, CHNH), 3.83-3.99 (m, 3 H, CH_2O, $CHOCH_3$), 5.29-5.41 (dd, J = 15.4 Hz, 7.6 Hz, 1 H, vinyl H), 5.70-5.81 (dt, J = 15.4 Hz, 6.9 Hz, 1 H, vinyl H), 6.01-6.04 (d, J = 7.6 Hz, 1 H, NH). Anal. Calcd for $C_{37}H_{73}O_3N$: C, 76.62; H, 12.64; N, 2.41. Found: C, 75.17; H, 12.61; N, 2.23.

2-(^{15}*N*-Octadecanoyl)-3-*O*-methyl-DL-*erythro*-sphingosine (13c). This compound was prepared in 93% yield by using the same procedure as described above; mp 79-79.5°C; TLC (hexane-ethyl acetate, 4:5) R_f 0.40; ^1H NMR (200 MHz,$CDCl_3$) δ (ppm) 0.85-0.91 (t, J = 6.6 Hz, 6 H, $CH_3(CH_2)_{11}$, $CH_3(CH_2)_{14}$), 1.25 (m, 50 H, $(CH_2)_{11}$, $(CH_2)_{14}$), 1.62 (m, 2 H, CH_2CH_2CO), 2.01-2.07 (m, 2 H, $CH_2CH{=}CH$), 2.15-2.22 (t, J = 7.5 Hz, 2 H,

CH_2CH_2CO), 3.26 (s, 3 H, OCH_3), 3.55-3.59 (m, 1 H, C*H*NH), 3.85-4.01 (m, 3 H, CH_2O, C*H*OCH_3), 5.29-5.43 (dd, J = 15.5 Hz, 7.4 Hz, 1 H, vinyl H), 5.68-5.80 (dt, J = 15.4 Hz, 7.0 Hz, 1 H, vinyl H), 6.05-6.10 (d, J = 7.6 Hz, 1 H, C*H*NH). Anal. Calcd for $C_{37}H_{73}O_3{}^{15}N$: C, 76.49; H, 12.66; N, 2.41. Found: C, 76.60; H, 12.83; N, 2.25.

2-(*N*-Octadecanoyl)-3-*O*-ethyl-DL-*erythro*-sphingosine (14b). This compound was prepared from **12b** in (95%) yield by using the same procedure as described for **9a**; TLC (hexane-ethyl acetate, 4:5), R_f 0.52; ^1H NMR (300 MHz, $CDCl_3$) δ (ppm) 0.85-0.90 (t, J = 6.9 Hz, 6 H, $CH_3(CH_2)_{11}$, $CH_3(CH_2)_{14}$), 1.14-1.19 (t, J = 7.1 Hz, 3 H, OCH_2CH_3), 1.25 (m, 50 H, $(CH_2)_{11}$, $(CH_2)_{14}$), 1.57-1.66 (m, 2 H, CH_2CH_2CO), 2.03-2.10 (m, 2 H, CH_2CH=CH), 2.19-2.22 (t, J = 7.6 Hz, 2 H, CH_2CH_2CO), 3.17-3.29 (m, 2 H, OCH_2CH_3), 3.53-3.61 (m, 2 H, C*H*OCH_2CH_3, C*H*NH), 3.96-4.00 (m, 2 H, CH_2O), 5.33-5.41 (dd, J = 15.4 Hz, 7.4 Hz, 1 H, vinyl H), 5.72-5.77 (dt, J = 15.5 Hz, 6.7 Hz, 1 H, vinyl H), 6.28-6.30 (d, J = 7.6 Hz, 1 H, NH). Anal. Calcd for $C_{38}H_{75}O_3N$: C, 76.84; H, 12.72; N, 2.36. Found: C, 76.64; H, 13.00; N, 2.26.

2-(^{15}*N*-Octadecanoyl)-3-*O*-ethyl-DL-*erythro*-sphingosine (14c). This compound was prepared in 91% yield by using the same procedure as described above; TLC (hexane-ethyl acetate, 4:5), R_f 0.52; ^1H NMR (200 MHz, $CDCl_3$) δ (ppm) 0.85-0.90 (t, J = 6.6 Hz, 6 H, $CH_3(CH_2)_{11}$, $CH_3(CH_2)_{14}$), 1.13-1.17 (t, J = 7.0 Hz, 3 H, OCH_2CH_3), 1.26 (m, 50 H, $(CH_2)_{11}$, $(CH_2)_{14}$), 1.55-1.64 (m, 2 H, CH_2CH_2CO), 2.03-2.10 (m, 2 H, CH_2CH=CH), 2.17-2.22 (t, J = 7.6 Hz, 2 H, CH_2CH_2CO), 3.17-3.25 (m, 2 H, OCH_2CH_3), 3.53-3.61 (m, 2 H, C*H*OCH_2CH_3, C*H*NH), 3.99-4.03 (m, 2 H, CH_2O), 5.25-5.35 (dd, J = 15.4 Hz, 7.0 Hz, 1 H, vinyl H), 5.60-5.75 (dt, J = 15.5

Hz, 7.0 Hz, 1 H, vinyl H), 6.30-6.33 (d, J = 7.6 Hz, 1 H, CHN*H*). Anal. Calcd for $C_{38}H_{75}O_3{}^{15}N$: C, 76.71; H, 12.70; N, 2.52. Found: C, 76.57; H, 12.87; N, 2.58.

3-*O*-Methyl-DL-*erythro*-sphingomyelin (16b). This compound was prepared from **13b** in 36% yield by using the same procedure as described for **10c** (page 27); TLC ($CHCl_3$-MeOH-7 N NH_4OH, 65:25:4) R_f 0.36; ^1H NMR (200 MHz, $CDCl_3$) δ (ppm) 0.85-0.91 (t, J = 6.6 Hz, 6 H, C*H*$_3(CH_2)_{11}$, C*H*$_3(CH_2)_{14}$), 1.25 (m, 50 H, $(CH_2)_{11}$, $(CH_2)_{14}$), 1.65 (m, 2 H, C*H*$_2CCH_2CO$), 2.03-2.10 (m, 2 H, C*H*$_2CH$=CH), 2.22-2.30 (t, J = 7.4 Hz, 2 H, CH_2CH_2CO), 3.30 (s, 12 H, $N(CH_3)_3$, OCH_3), 3.78 (m, 3 H, C*H*NH, C*H*$_2N(CH_3)_3$), 3.95-4.10 (m, 4 H, $CH_2OP(O)(O^-)OCH_2$), 5.26-5.40 (dd, J = 15.4, 6.8 Hz, 1 H, vinyl H), 5.62-5.74 (dt, J = 15.4, 7.0 Hz, 1 H, vinyl H). Anal. Calcd for $C_{42}H_{85}O_6N_2P{\cdot}3.5H_2O$: C, 62.42; H, 11.46; N, 3.46. Found: C, 62.34; H, 11.57; N, 3.09.

3-*O*-Methyl-^{15}*N*-DL-*erythro*-sphingomyelin (16c). This compound was prepared from **13c** in 43% yield by using the same procedure as described for **10c**; TLC ($CHCl_3$-MeOH-H_2O-Conc. NH_4OH, 60:30:2:2), R_f 0.30; ^1H NMR (300 MHz, $CDCl_3$) δ (ppm) 0.85-0.90 (t, J = 6.6 Hz, 6 H, C*H*$_3(CH_2)_{11}$, C*H*$_3(CH_2)_{14}$), 1.25 (m, 50 H, $(CH_2)_{11}$, $(CH_2)_{14}$), 1.64 (m, 2 H, C*H*$_2CH_2CO$), 2.00-2.07 (m, 2 H, C*H*$_2CH$=CH), 2.18-2.26 (t, J = 7.5 Hz, 2 H, CH_2CH_2CO), 3.29 (s, 12 H, $N(CH_3)_3$, OCH_3), 3.62-3.67 (m, 3 H, C*H*$_2N(CH_3)_3$), C*H*NH), 3.95-4.12 (m, 4 H, $CH_2OP(O)(O^-)OCH_2$), 5.29-5.41 (dd, J = 15.4 Hz, 6.8 Hz, 1 H, vinyl H), 5.65-5.77 (dt, J = 15.5 Hz, 6.8 Hz, 1 H, vinyl H), 6.30-6.33 (d, J = 7.6 Hz, 1 H, CHN*H*).

3-*O*-Ethyl-DL-*erythro*-sphingomyelin (17b). This compound was prepared from **14b** in 41% yield by using the same procedure as described above; TLC ($CHCl_3$-MeOH-7 N NH_4OH, 65:25:4) R_f 0.30; ^1H NMR (200 MHz, $CDCl_3$) δ (ppm) 0.85-0.89 (t, J = 6.8 Hz, 6 H, $CH_3(CH_2)_{11}$, $CH_3(CH_2)_{14}$), 1.10-1.13 (t, J = 7.0 Hz, 3 H, OCH_2CH_3), 1.25 (m, 50 H, $(CH_2)_{11}$, $(CH_2)_{14}$), 1.55-1.60 (m, 2 H, CH_2CH_2CO), 1.99-2.05 (m, 2 H, CH_2CH=CH), 2.12-2.17 (t, J = 7.6 Hz, 2 H, CH_2CH_2CO), 3.30-3.50 (m, 11 H, $N(CH_3)_3$, OCH_2CH_3), 3.70-3.85 (m, 4 H, CHNH, $CH_2N(CH_3)_3$, $CHOCH_2CH_3$), 4.23 (m, 4 H, $CH_2OP(O)(O^-)OCH_2$), 5.33-5.41 (dd, J = 15.4, 7.4 Hz, 1 H, vinyl H), 5.57-5.65 (dt, J = 15.4, 6.8 Hz, 1 H, vinyl H).

3-*O*-Ethyl-^{15}*N*-DL-*erythro*-sphingomyelin (17c). This compound was prepared from **14c** in 39% yield by using the same procedure as described above; TLC ($CHCl_3$-MeOH-H_2O-conc. NH_4OH, 60:30:2:2) R_f 0.30; ^1H NMR (300 MHz, $CDCl_3$) δ (ppm) 0.85-0.90 (t, J = 6.6 Hz, 6 H, $CH_3(CH_2)_{11}$, $CH_3(CH_2)_{14}$), 1.13-1.17 (t, J = 7.0 Hz, 3 H, OCH_2CH_3), 1.26 (m, 50 H, $(CH_2)_{11}$, $(CH_2)_{14}$), 1.55-1.60 (m, 2 H, CH_2CH_2CO), 2.00-2.07 (m, 2 H, CH_2CH=CH), 2.17-2.22 (t, J = 7.6 Hz, 2 H, CH_2CH_2CO), 3.29-3.50 (m, 11 H, $N(CH_3)_3$, OCH_2CH_3), 3.68-3.81 (m, 4 H, $CH_2N(CH_3)_3$), CHNH, $CHOCH_2CH_3$), 4.12-4.23 (m, 4 H, $CH_2OP(O)(O^-)OCH_2$), 5.26-5.34 (dd, J = 15.4 Hz, 7.0 Hz, 1 H, vinyl H), 5.59-5.75 (dt, J = 15.4 Hz, 7.0 Hz, 1 H, vinyl H).

***N*-Methyl-DL-*erythro*, *threo*-sphingosine (18) (Scheme 5).** Diisopropylamine (701 µL, 5.0 mmol) was added slowly to a 250-mL flask containing 2.0 mL (5.0 mmol) of *n*-butyllithium (2.5 M solution in hexane) at -78 °C. Some white precipitate was formed in a few minutes. After the

reaction mixture had stirred at -78 °C for 1 h, THF (30 mL) was added, and the solution was stirred for 10 min. A solution of 1.07 g (4.6 mmol) of bis(trimethylsilyl)sarcosine in 1.5 mL of THF was added slowly to the mixture. The reaction mixture was stirred for 1.5 h, then a solution of (*E*)-hexadec-2-enal (1.0 g, 4.2 mmol) in 2 mL of THF was added. The mixture was stirred for 1 h at -78 °C, then overnight at 0 °C. The mixture was warmed to room temperature, and an additional 10 mL of THF was added; then lithium aluminum hydride (633 mg, 16.7 mmol) was slowly added to the reaction mixture. The suspension was refluxed for 24 h, cooled to room temperature, and then quenched with water (2.5 mL). The reaction mixture was filtered and washed with THF (15 mL). The solvents were removed under vacuum. The residue was dissolved in 50 mL of chloroform, and washed with 10% aqueous ammonium chloride solution (2 x 30 mL). The organic phase was concentrated by rotary evaporation. The crude product was purified by flash chromatography (elution first with hexane-ethyl acetate, 4:1, then with $CHCl_3$-MeOH, 9:1, and $CHCl_3$-MeOH, 3:1) to give 500 mg (38%) of pure *N*-methylsphingosine (**18**); TLC ($CHCl_3$-MeOH, 3:2) R_f 0.33; ^1H NMR (200 MHz, $CDCl_3$) δ (ppm) 0.85-0.90 (t, J = 6.7 Hz, 3 H, $CH_3(CH_2)_{11}$), 1.26 (m, 22 H, $(CH_2)_{11}$), 2.03-2.05 (m, 2 H, $CH_2CH{=}CH$), 2.81 (d, J = 2.7 Hz, 3 H, $NHCH_3$), 3.01 (m, 1 H, C*H*NH), 3.69-3.88 (m, 1 H, C*H*OH), 3.90-3.96 (m, 2 H, CH_2OH), 5.20 (s, 3 H, CHN*H*, CHO*H*, CH_2OH; when D_2O was added, this peak was shifted to 4.70 ppm), 5.43-5.50 (dd, J = 14.3 Hz, 6.7 Hz, 1 H, vinyl H), 5.81-5.88 (dt, J = 14.4 Hz, 6.7 Hz, 1 H, vinyl H). Anal. Calcd for $C_{17}H_{39}O_2N$: C, 72.79; H, 12.54; N, 4.47. Found: C, 72.62; H, 12.50; N, 4.21.

***N*-Biphenylcarboxamido-DL-*erythro*-sphingosine (19) (Scheme 6).** 4-Biphenylcarbonyl chloride 50 mg (0.23 mmol) in 0.6 mL of

THF was added to sphingosine **5b** (15 mg, 0.05 mmol) in 0.9 mL of THF and 3 mL of a saturated solution of sodium acetate in water. The biphasic reaction mixture was vigorously agitated at room temperature overnight. After TLC ($CHCl_3$-EtOH, 10:1) showed that reaction was complete, chloroform (10 mL) and 0.1 N aqueous NaOH (5 mL) were added. The two phases were separated, and the organic phase was washed with water. After the solvents were removed under vacuum, the crude product was purified by flash chromatography (elution with $CHCl_3$-EtOH, 10:1) to give 17 mg (70%) of the pure product; TLC ($CHCl_3$-EtOH, 10:1) R_f 0.35; ^1H NMR (200 MHz, $CDCl_3$) δ (ppm) 0.85-0.91 (t, J = 6.4 Hz, 3 H, CH_3), 1.26 (m, 22 H, $(CH_2)_{11}$), 2.03-2.06 (m, 2 H, $CH_2CH{=}CH$), 3.80-3.85 (m, 1 H, C*H*NH), 4.10-4.85 (m, 2 H, CH_2OH), 4.48 (m, 1 H, CHOH), 5.54-5.60 (dd, J = 15.4 Hz, 6.6 Hz, 1 H, vinyl H), 5.80-5.92 (dt, J = 15.2 Hz, 6.4 Hz, 1 H, vinyl H), 7.04-7.09 (d, J = 8.2 Hz, 1 H, CHN*H*), 7.45-7.90 (m, 9 H, $C_6H_4C_6H_5$).

***N*-Biphenylcarboxamido-D-*erythro*-sphingosine (19').** This compound was prepared from D-*erythro*-sphingosine in 82% yield by using the same procedure as described for **19**.

***N*-Biphenylcarboxamidosphingosine-A mixture of *erythro*- and *threo*-isomer (19").** This compound was prepared from a mixture of *erythro*- and *threo*-sphingosine in 80% yield by using the same procedure as described for **19**.

***N*-Methyl-*N*-biphenylcarboxamido-DL-*erythro, threo*-sphingosine (20).** This compound was prepared from *N*-methylsphingosine (**18**) in 80% yield by using the same procedure as

described for **19**. ^{1}H NMR (200 MHz, $CDCl_3$) δ (ppm) 0.85-0.90 (t, *J* = 6.6 Hz, 3 H, C*H*$_3$(CH$_2$)$_{11}$), 1.25 (m, 22 H, (CH$_2$)$_{11}$), 2.01-2.06 (m, 2 H, C*H*$_2$CH=CH), 3.01 (s, 3 H, NCH$_3$), 3.90-3.97 (m, 1 H, C*H*NCH$_3$), 4.10 (m, 2 H, C*H*$_2$OH), 4.60-4.68 (m, 1 H, C*H*OH), 5.57-5.69 (dd, *J* = 15.4 Hz, 6.7 Hz, 1 H, vinyl H), 5.79-5.90 (dt, *J* = 15.4 Hz, 6.7 Hz, 1 H, vinyl H), 7.38-7.66 (m, 9 H, Ar).

***N*-Methyl-*N*-biphenylcarboxamido-D-*erythro*-sphingosine (20').** This compound was prepared from *N*-methyl-D-*erythro*-sphingosine in 81% yield by using the same procedure as described for **19**.^{1}H NMR (200 MHz, $CDCl_3$) δ (ppm) 0.85-0.90 (t, *J* = 6.6 Hz, 3 H, C*H*$_3$(CH$_2$)$_{11}$), 1.25 (m, 22 H, (CH$_2$)$_{11}$), 2.08 (m, 2 H, C*H*$_2$CH=CH), 3.02 (s, 3 H, NCH$_3$), 3.91-3.97 (m, 1 H, C*H*NCH$_3$), 4.10 (m, 2 H, C*H*$_2$OH), 4.47 (m, 1 H, C*H*OH), 5.57-5.69 (dd, *J* = 15.4 Hz, 6.7 Hz, 1 H, vinyl H), 5.80-5.94 (dt, *J* = 15.4 Hz, 6.7 Hz, 1 H, vinyl H), 7.38-7.65 (m, 9 H, Ar).

Bis-(*R*)-(+)-Mosher Ester of 19 (21). A mixture of 5.04 mg (0.021 mmol) of DMAP and 28 μL (0.20 mmol) of triethylamine in 1.0 mL of methylene chloride was added to a solution of *N*-biphenylcarboxamidosphingosine **19** (10 mg, 0.021 mmol) in 4.0 mL of methylene chloride. Immediately, 8.4 μL of neat (*R*)-(+)-MTPA chloride[40] was added. The reaction was completed in 1 h. The reaction mixture was quenched by adding 3-(dimethylamino)propylamine (30 μL). The mixture was concentrated, and the residue was passed through a short column of silica gel (elution with hexane-ethyl acetate, 4:1) to give 21 mg of crude **21**; ^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm) 0.84-0.91 (t, *J* = 6.3 Hz, 3 H, CH_3), 1.24 (m, 22 H, (CH$_2$)$_{11}$), 1.97-2.00 (m, 2 H, C*H*$_2$CH=CH), 3.49 (s, 3 H, OCH_3), 3.52 (s, 3 H, OCH_3), 4.44 (dd, J_{AB} = 11.5 Hz, J_{AC} = 3.65 Hz, 1 H, C*H*$_A$H$_B$OMTPA), 4.58 (dd, J_{AB} =

11.5 Hz, J_{BC} = 5.39 Hz, 1 H, CH_AH_BOMTPA), 4.77-4.79 (m, 1 H, $CH_CNHCH_AH_BOMTPA$), 5.38 (dd, J = 15.3 Hz, 7.7 Hz, 1 H, CH=CHCHOMTPA), 5.52-5.59 (m, 1 H, CH=CHCHOMTPA), 5.80-5.94 (dt, J = 14.9 Hz, 6.8 Hz, 1 H, $C_{13}H_{27}CH$=CH), 6.12 (d, J = 9.0 Hz, 1 H, CHCNH), 7.28-7.61 (m, 19 H, Ar).

Bis-(*R*)-(+)-Mosher ester of 19' (21'). This compound was prepared from **19'** in 81% yield by using the procedure described above.

1-Bromo-(*E*)-hexadec-2-ene (22) (Scheme 3). To a flame-dried, 100-mL, two-necked round-bottom flask equipped with a magnetic stirrer, dropping funnel with rubber septum, and nitrogen inlet adapter was added 1.22 g (6.88 mmol) of *N*-bromosuccinimide. Methylene chloride (35 mL) was added, and the resulting solution was cooled to -30 °C with a dry ice/acetonitrile bath. Freshly distilled dimethyl sulfide (550 µL, 7.5 mmol) was added dropwise. The mixture was warmed to 0 °C with an ice bath, maintained at that temperature for 5 min, and cooled to -30 °C. To the resulting milky yellow suspension a solution of 1.5 g (6.25 mmol) of (*E*)-hexadec-2-en-1-ol **(2)** in 5 mL of methylene chloride was added dropwise. The suspension was warmed to 0 °C with an ice bath, and stirred for 1.5 h. The ice bath was removed, the reaction mixture was allowed to warm to room temperature, and stirring was continued for an additional 15 min. The reaction mixture was poured into a 250-mL separatory funnel and washed with saturated sodium chloride solution (35 mL). The aqueous layer was washed with chloroform (2 x 20 mL). The combined organic phases were washed with saturated sodium chloride solution (25 mL), dried ($MgSO_4$), and concentrated with a rotary evaporator. The crude product was purified by

flash chromatography. Elution with hexane afforded 1.68 g (89%) of pure 1-bromo-(*E*)-hexadec-2-ene **(22)**; TLC (hexane) R_f 0.59; ^1H NMR (200 MHz, $CDCl_3$) δ (ppm) 0.80-0.92 (t, *J* = 7.0 Hz, 3 H, CH_3), 1.25 (m, 22 H, $(CH_2)_{11}$), 2.05 (m, 2 H, $CH_2CH{=}CH$), 3.98-4.08 (d, *J* = 3.6 Hz, 2 H, CH_2OH), 5.58-5.64 (dt, *J* = 15.5 Hz, 6.8 Hz, 1 H, vinyl H), 5.76-5.85 (dt, *J* = 15.4 Hz, 7.1 Hz, 1 H, vinyl H).

1-Iodo-(*E*)-hexadec-2-ene (23). To a solution of sodium iodide (619 mg, 4.13 mmol) in acetone (25 mL) in a 50-mL flask fitted with a reflux condenser protected by a calcium chloride guard-tube was added 1.0 g (3.30 mmol) of 1-bromo-(*E*)-hexadec-2-ene (**22**). A precipitate of sodium bromide soon began to form. After the reaction mixture was left at room temperature overnight, sodium bromide was removed by filtration and the residue was washed with acetone. The filtrate was concentrated on a rotary evaporator, leaving a residue that was dissolved in 30 mL of hexane and then washed with 20 mL of water. The organic phase was dried ($MgSO_4$), and the solvents were removed under vacuum. The crude product was purified by flash chromatography (elution with hexane) to give 1.10 g (94%) of **23** as a light yellow oil; TLC (hexane) R_f 0.59. The ^1H NMR spectrum was identical to that of **22**.

3-Deoxy-DL-sphingosine (24). Diisopropylamine (192 μL, 1.37 mmol) was slowly dropped into 0.85 mL (1.37 mmol) of *n*-butyllithium (1.6 M in hexane) at -78 °C. Some white precipitate was formed immediately. After the mixture had stirred for 1 h at -78 °C, THF (15 mL) was added and the solution was stirred for 10 min. A solution of 365 mg (1.25 mmol) of tris(trimethylsilyl)glycine in 1.25 mL of THF was added slowly to the mixture.

The reaction mixture was stirred for 1 h, then a solution of 1-iodo-(*E*)-hexadec-2-ene (400 mg, 1.14 mmol) in 1.5 mL of THF was added. The mixture was stirred for 2 h at -78 °C, 30 min at 0 °C, then warmed to room temperature. Lithium aluminum hydride (216 mg, 5.70 mmol) was added slowly to the reaction mixture. The suspension was refluxed for 24 h, and then was hydrolyzed with water (216 μL), 15% NaOH (216 μL), and H_2O (3 x 216 μL). The reaction mixture was filtered and washed with chloroform (15 mL). Some isopropyl alcohol was added to the filtrate, and the solvents were removed under vacuum. The crude product was purified by flash chromatography (elution with $CHCl_3$-MeOH, 7:3) to afford 180 mg (56%) of pure 3-deoxysphingosine (**24**); TLC ($CHCl_3$-MeOH, 7:3) R_f 0.51; ^1H NMR (200 MHz, $CDCl_3$) δ (ppm) 0.85-0.90 (t, J = 6.8 Hz, 3 H, CH_3), 1.25 (m, 22 H, $(CH_2)_{11}$), 2.01-2.08 (m, 2 H, CH_2CH=CH), 2.15-2.11 (m, 2 H, CH=CHCH_2), 2.33 (s, 3 H, NH_2, OH), 3.09 (m, 1 H, $CHNH_2$), 3.58-3.66 (m, 2 H, CH_2OH), 5.35-5.40 (dt, J = 15.5 Hz, 7.1 Hz, 1 H, vinyl H), 5.54-5.67 (dt, J = 15.4 Hz, 6.8 Hz, 1 H, vinyl H).

***N*-Octadecanoyl-3-deoxy-DL-sphingosine (25).** This compound was prepared from **24** in 71% yield by using the same procedure as described for **6a** (page 19); TLC (hexane-ethyl acetate, 4:5) R_f 0.32; ^1H NMR (200 MHz, $CDCl_3$) δ (ppm) 0.85-0.90 (t, J = 6.6 Hz, 6 H, $CH_3(CH_2)_{11}$, $CH_3(CH_2)_{14}$), 1.25 (m, 50 H, $CH_2)_{11}$, $(CH_2)_{14}$), 1.58-1.64 (m, 2 H, CH_2CH_2CO), 1.99-2.10 (m, 2 H, CH_2CH=CH), 2.15-2.21 (m, 4 H, CH=CHCH_2, CH_2CH_2CO), 3.10 (m, 1 H, CHNH), 3.58-3.66 (m, 2 H, CH_2OH), 5.32-5.37 (dt, J = 15.5 Hz, 7.2 Hz, 1 H, vinyl H), 5.49-5.63 (dt, J = 15.4 Hz, 6.8 Hz, 1 H, vinyl H). Anal. Calcd for $C_{36}H_{71}NO_2$: C, 78.62; H, 13.01; N, 2.55. Found: C, 78.12; H, 13.26; N, 2.29.

3-Deoxy-DL-sphingomyelin (26). This compound was prepared from **25** in 36% yield by using the same procedure as described for **10c**; TLC ($CHCl_3$-MeOH-7 N NH_4OH, 65:25:4) R_f 0.32; ^1H NMR (200 MHz, $CDCl_3$) δ (ppm) 0.85-0.91 (t, J = 7.0 Hz, 6 H, $CH_3(CH_2)_{11}$, $CH_3(CH_2)_{14}$), 1.26 (m, 50 H, $(CH_2)_{11}$, $(CH_2)_{14}$), 1.58 (m, 2 H, CH_2CCH_2CO), 1.96-1.99 (m, 2 H, $CH_2CH{=}CH$), 2.22-2.31 (m, 4 H, CH_2CH_2CO, $CH_2CH{=}CH$), 3.38 (s, 9 H, $N(CH_3)_3$), 3.86 (m, 3 H, $CHNH$, $CH_2N(CH_3)_3$), 4.25-4.36 (m, 4 H, $CH_2OP(O)(O^-)OCH_2$), 5.31-5.35 (dt, J = 15.5 Hz, 6.8 Hz, 1 H, vinyl H), 5.44-5.58 (dt, J = 15.4 Hz, 7.1 Hz, 1 H, vinyl H).

Methyl (2*R,S*)-Hydroxyhexadecanoate (27) (Scheme 4). (2*R,S*)-Hydroxyhexadecanoic acid (2.0 g, 7.34 mmol) was dissolved in 35 mL of dry THF. Methanol (327 μL, 8.07 mmol), trimethyl orthoformate (3.21 mL, 29.4 mmol), and a trace of *p*-toluenesulfonic acid (monohydrate) were added. After the reaction mixture was refluxed overnight, the solvents were removed on a rotary evaporator. The crude product was purified by flash chromatography (elution with hexane-ethyl acetate, 3:1) to give 1.50 g of pure product **27**; yield, 71%; TLC (hexane-ethyl acetate, 3:1) R_f 0.44; ^1H NMR (200 MHz, $CDCl_3$) δ (ppm) 0.85-0.90 (t, J = 6.3 Hz, 3 H, CH_3), 1.25 (m, 24 H, $(CH_2)_{12}$), 1.64 (m, 2 H, CH_2CHOH), 3.73 (t, J = 6.0 Hz, 1 H, CH_2CHOH), 3.79 (s, 3 H, CH_3).

Methyl (2*R,S*)-*O*-(Tetrahydropyranyloxy)hexadecanoate (28). Methyl (2*R,S*)-hydroxyhexadecanoate (**27**) (1.30 g, 4.54 mmol) was dissolved in 35 mL of methylene chloride. Dihydropyran (704 μL, 7.72 mmol) and a trace of *p*-toluenesulfonic acid were added. The reaction mixture was stirred at room temperature overnight. The solution was diluted with ether (15 mL),

and washed with saturated sodium bicarbonate solution (25 mL) and water (25 mL). The organic phase was dried ($MgSO_4$), the solvents were removed with a rotary evaporator, and the residue was purified by flash chromatography (elution with hexane-ethyl acetate, 5:1) to give 1.33 g (79%) of **28**; TLC (hexane-ethyl acetate, 5:1) R_f 0.52.

***p*-Nitrophenyl [(2'*R,S*)-Tetrahydropyranyloxy]hexadecanoate (29).** A solution of potassium hydroxide (180 mg, 3.10 mmol) in 10 mL of methanol was added dropwise to 1.0 g (2.80 mmol) of methyl ester **28** in a 100-mL flask at room temperature. The reaction mixture was stirred at this temperature overnight, then heated at 60-70 °C for 3 h. The solvent was removed under reduced pressure. The residue was dissolved in 30 mL of water, cooled to 0 °C, and 2 N HCl was added slowly until pH of the solution was 3. The product was isolated by extraction with methylene chloride (2 x 30 mL). The organic phases were combined and the solvent was removed by rotary evaporation. To a solution of crude product in 20 mL of methylene chloride were added *p*-nitrophenol (442 mg, 3.08 mmol), DCC (635 mg, 3.08 mmol), and DMAP (34.2 mg, 0.28 mmol). The reaction mixture was allowed to stir at room temperature for 3 h. The *N, N*-dicyclohexyl urea precipitate was removed by filtration and the filtrate was washed with 5 % aqueous NaOH solution (3 x 20 mL). The organic phase was dried ($MgSO_4$), and the solvent was removed by rotary evaporation. The crude product was purified by flash chromatography (elution with hexane-ethyl acetate, 7:1) to give 1.2 g (90%) of the product **29**; TLC (hexane-ethyl acetate, 7:1) R_f 0.68; ^1H NMR (300 MHz, $CDCl_3$) δ (ppm) 0.85-0.90 (t, J = 6.5 Hz, 3 H, CH_3), 1.20-1.33 (m, 24 H, $(CH_2)_{12}$), 1.55-2.00 (m, 8 H, $C_{13}H_{27}CH_2CHO$, 3 CH_2 of THP), 3.65-3.80 (m, 2 H, CH_2O), 4.56-4.61 (t, J = 6.1 Hz, 1 H, $CHOCO_2$), 4.78-4.82 (m, 1 H,

OCHO), 7.25-7.30 (m, 2 H, Ar), 8.25-8.30 (m, 2 H, Ar).

***N*-[(2'-Tetrahydropyranyloxy)hexadecanoyl]-DL-*erythro*-sphingosine (30).** This compound was prepared from the reaction of sphingosine (**5b**) with *p*-nitrophenyl ester **29** in 86% yield by using the same procedure as described for **6a**; TLC ($CHCl_3$-EtOH, 10:1) R_f 0.45; ^1H NMR (200 MHz, $CDCl_3$, primes refer to the THP group in the amide chain) δ (ppm) 0.85-0.90 (t, J = 6.6 Hz, 6 H, $CH_3(CH_2)_{11}$, $CH_3(CH_2)_{12}$), 1.29-1.37 (m, 46 H, $(CH_2)_{11}$, $(CH_2)_{12}$), 1.68-1.90 (m, 8 H, $(CH_2)_3$ of THP, CH_2CH_2CO), 2.05-2.12 (m, 2 H, $CH_2CH{=}CH$), 3.55-3.58 (m, 1 H, CHNH), 3.71-3.76 (m, 1 H, CHOH), 3.89-4.00 (m, 4 H, CH_2OH, CH_2O of THP), 4.22-4.26 (t, J = 7.2 Hz, 1 H, OCHCO), 4.63-4.66 (m, 1 H, O'-CHO of THP), 5.51-5.59 (m, 1 H, vinyl H), 5.80-5.85 (m, 1 H, vinyl H), 7.19-7.22 (d, J = 7.5 Hz, 1 H, CHNH).

1-(*O-tert*-Butyldiphenylsilyl)-*N*-[(2'-tetrahydropyranyloxy)-hexadecanoyl]-DL-*erythro*-sphingosine (31). This compound was prepared from **30** in 83% yield by using the same procedure as described for **7b**; TLC (hexane-ethyl acetate, 4:1), R_f 0.44; ^1H NMR (200 MHz, $CDCl_3$, primes refer to the THP group in the amide chain) δ 0.85-0.90 (t, J = 6.6 Hz, 6 H, $CH_3(CH_2)_{11}$, $CH_3(CH_2)_{12}$), 1.08 (s, 9 H, $C(CH_3)_3$), 1.25 (m, 46 H, $(CH_2)_{11}$, $(CH_2)_{12}$), 1.50-1.88 (m, 8 H, $(CH_2)_3$ of THP, $CH_2CHOTHP$), 1.97-2.03 (m, 2 H, $CH_2CH{=}CH$), 3.48-3.55 (m, 1 H, CHNH), 3.76-4.00 (m, 5 H, CHOH, CH_2O, CH_2O of THP), 4.20-4.24 (t, J = 7.2 Hz, 1 H, OCHCO), 4.60-4.63 (m, 1 H, O'CHO of THP), 5.40-5.52 (dd, J = 15.4 Hz, 6.7 Hz, 1 H, vinyl H), 5.70-5.82 (dt, J = 15.4 Hz, 6.7 Hz, 1 H, vinyl H), 7.10-7.13 (d, J = 7.5 Hz, 1 H, CHNH), 7.38-7.65 (m, 10 H, Ar). Anal. Calcd for $C_{55}H_{93}O_5NSi$: C, 75.38; H, 10.70; N, 1.60. Found: C, 75.55; H, 10.83; N, 1.57.

1-(*O-tert*-Butyldiphenylsilyl)-*N*-[(2'-tetrahydropyranyloxy)-hexadecanoyl]-3-(*O*-tetrahydropyranyl)-DL-*erythro*-sphingosine (32). This compound was prepared from **31** in 76% yield by using the same procedure as described for **8a** (page 23); TLC (hexane-ethyl acetate, 5:1) R_f 0.65.

***N*-[(2'-Tetrahydropyranyloxy)hexadecanoyl]-3-(*O*-tetrahydropyranyl)-DL-*erythro*-sphingosine (33).** This compound was prepared from **32** in 88% yield by using the same procedure as described for **9a** (page 24); TLC (hexane-ethyl acetate, 2:3) R_f 0.36; ^{1}H NMR (200 MHz, $CDCl_3$) δ (ppm) 0.85-0.90 (t, J = 6.3 Hz, 6 H, C*H*$_3$(CH$_2$)$_{11}$, C*H*$_3$(CH$_2$)$_{12}$), 1.26 (m, 46 H, (CH$_2$)$_{11}$, (CH$_2$)$_{12}$), 1.55-1.78 (m, 14 H, (CH$_2$)$_3$ of THP, ((CH$_2$)$_3$ of THP in amide chain, and O-'C*H*CH$_2$), 2.02-2.06 (m, 2 H, CH$_2$CH=CH), 3.21 (m, 1 H, C*H*NH), 3.51-3.60 (m, 1 H, CHOTHP), 3.71-4.02 (m, 6 H, CH$_2$OH, CH$_2$O of THP, and CH$_2$O of THP in amide chain), 4.13-4.18 (m, 1 H, OCHCO), 4.52 (m, 1 H, O'CHO of THP), 4.61 (m, 1 H, O'CHO of THP in amide chain), 5.30-5.42 (dd, J = 15.4 Hz, 6.7 Hz, 1 H, vinyl H), 5.65-5.76 (dt, J = 15.4 Hz, 6.7 Hz, 1 H, vinyl H), 7.15-7.19 (d, J = 7.7 Hz, 1 H, CHN*H*). Anal. Calcd for $C_{44}H_{83}O_6N$: C, 73.18; H, 11.58; N, 1.94. Found: C, 72.57; H, 11.49; N, 1.84.

***N*-[(2'-Tetrahydropyranyloxy)hexadecanoyl]-3-(*O*-tetrahydropyranyl)-DL-*erythro*-sphingomyelin (34).** To a solution of 160 mg (0.22 mmol) of ceramide **33** and 62 μL (0.44 mmol) of triethylamine in 5 mL of chloroform at 0 °C was added with syringe *N,N*-diisopropylmethylphosphoramidic chloride (54.3 μL, 0.26 mmol, 20% molar excess). After the mixture was allowed to react for 10 min at 0 °C, TLC

(hexane-ethyl acetate, 1:1) indicated that no starting ceramide remained. The mixture was concentrated to dryness under vacuum (without using a rotary evaporator in order to avoid water vapor) and 1*H*-tetrazole (55.5 mg, 0.79 mmol, 3.6-fold excess) and dry choline tosylate (182 mg, 0.66 mmol, 3.0-fold excess) were added to the reaction flask. Six milliliters of acetonitrile-THF (1:1) were added and the solution was stirred for 4 h at room temperature, after which time TLC analysis showed that the reaction was complete. The mixture was again evaporated to dryness under reduced pressure. The residue was dissolved in THF (5 mL) and added to *tert*-butyl hydroperoxide (81 μL of a 3 M solution in hexane, 0.25 mmol, 10% excess). The reaction mixture was stirred for 2 h at room temperature. Ethyl acetate (10 mL) was added, and the layers were separated. The organic layer was washed with 15 mL of 1 M triethylammonium hydrogen carbonate buffer, pH 7.5, to remove the excess of tetrazole and choline tosylate. The organic phase was concentrated to dryness and the residue was dried thoroughly by repeated evaporation with dry toluene. Finally, the toluene solution (5 mL) was treated with anhydrous trimethylamine (1 mL) in a 25-mL pressure bottle for 12 h at room temperature. After this period of time, the demethylation of the methyl ester of phosphocholine moiety was complete as judged by TLC ($CHCl_3$-MeOH-H_2O-conc. NH_4OH, 60:30:2:2). The solvent was removed using a rotary evaporator. The residue was purified by flash chromatography (elution with $CHCl_3$-MeOH-H_2O-conc. NH_4OH, 60:30:2:3). Suspended silica gel was removed by passing a chloroform solution of the product through a 0.45-μm Metricel filter three times to give 100 mg (51%) of product **34** (from ceramide **33**).

***N*-[(2'*R,S*)-Hydroxyhexadecanoyl]-DL-*erythro*-sphingomyelin (35).** To a solution of 100 mg (0.11 mmol) of **34** in 5 mL of dry methanol was added a trace of *p*-toluenesulfonic acid. After the reaction mixture was heated at 70 °C overnight, chloroform (15 mL) was added. The mixture was washed with saturated aqueous sodium bicarbonate solution (10 mL). The organic phase was dried by repeated evaporation with isopropyl alcohol. The crude product was purified by flash chromatography (elution first with $CHCl_3$-MeOH, 3:1, then with $CHCl_3$-MeOH-H_2O-conc. NH_4OH, 60:30:2:3). Suspended silica gel was removed by passing a chloroform solution of the product through a 0.45-μm Metricel filter three times to give 68 mg (84%) of the product; TLC ($CHCl_3$-MeOH-H_2O-conc. NH_4OH, 60:30:2:3) R_f 0.28; ^1H NMR (200 MHz, $CDCl_3$) δ (ppm) 0.85-0.90 (t, J = 6.6 Hz, 6 H, $CH_3(CH_2)_{11}$, $CH_3(CH_2)_{12}$), 1.25 (m, 46 H, $(CH_2)_{11}$, $(CH_2)_{12}$), 1.46 (m, 2 H, CH_2CHOH), 1.98 (m, 2 H, $CH_2CH{=}CH$), 3.29 (s, 9 H, $N(CH_3)_3$), 3.41 (m, 1 H, CHNH), 3.75-3.92 (m, 3 H, $CH_2N(CH_3)_3$, CHOH), 4.05-4.25 (m, 5 H, $CH_2OP(O)O^-OCH_2$, CHOHCO), 5.34-5.49 (dd, J = 15.4 Hz, 6.7 Hz, 1 H, vinyl H), 5.69-5.80 (dt, J = 15.4 Hz, 6.7 Hz, 1 H, vinyl H). Anal Calcd for $C_{39}H_{79}O_7N_2P{\cdot}2H_2O$: C, 62.04; H, 11.08; N, 3.71. Found: C, 61.87; H, 10.84; N, 3.44.

***N*-Tetradecanoyl-DL-*erythro*-sphingosine (36) (Scheme 7, page 56).** This compound was prepared from sphingosine (**5b**) and *p*-nitrophenyl myristate in 82% yield by using the same procedure as described for **6a**; TLC ($CHCl_3$-EtOH, 10:1) R_f 0.47; ^1H NMR (200 MHz, $CDCl_3$) δ (ppm) 0.85-0.90 (t, J = 6.5 Hz, 6 H, $CH_3(CH_2)_{11}$, $CH_3(CH_2)_{10}$), 1.25 (m, 42 H, $(CH_2)_{11}$, $(CH_2)_{10}$), 1.56 (m, 2 H, CH_2CH_2CO), 2.00-2.07 (m, 2 H, $CH_2CH{=}CH$), 2.19-2.26 (t, J = 7.5 Hz, 2 H, CH_2CH_2CO), 3.68-3.72 (m, 1

H, C*H*NH), 3.89-3.98 (m, 2 H, CH_2OH), 4.07-4.14 (m, 1 H, C*H*OH), 5.47-5.58 (dd, *J* = 15.4 Hz, 6.7 Hz, 1 H, vinyl H), 5.75-5.87 (dt, *J* = 15.4 Hz, 6.7 Hz, 1 H, vinyl H), 6.20-6.25 (d, *J* = 7.2 Hz, 1 H, CHN*H*). Anal. Calcd for $C_{32}H_{63}O_3N$: C, 75.38; H, 12.45; N, 2.75. Found: C, 75.29; H, 12.50; N, 2.69.

1-(*O-tert*-Butyldiphenylsilyl)-2-(*N*-tetradecanoyl)-DL-*erythro*-sphingosine (37). This compound was prepared from **36** in 89% yield by using the same procedure as described for **7a**; ^{1}H NMR (200 MHz, $CDCl_3$) δ (ppm) 0.85-0.90 (t, *J* = 6.6 Hz, 6 H, CH_3(CH_2)$_{11}$, CH_3(CH_2)$_{10}$), 1.07 (s, 9 H, C(CH_3)$_3$), 1.25 (m, 42 H, (CH_2)$_{11}$, (CH_2)$_{10}$), 1.62-1.68 (m, 2 H, CH_2CH_2CO), 2.01-2.08 (m, 2 H, CH_2CH=CH), 2.15-2.22 (t, *J* = 7.5 Hz, 2 H, CH_2CH_2CO), 3.80-3.84 (m, 1 H, C*H*NH), 3.99-4.06 (m, 2 H, CH_2O), 4.20-4.25 (m, 1 H, C*H*OH), 5.49-5.58 (dd, *J* = 15.4 Hz, 6.7 Hz, 1 H, vinyl H), 5.77-5.86 (dt, *J* = 15.4 Hz, 6.7 Hz, 1 H, vinyl H), 6.13-6.17 (d, *J* = 7.5 Hz, 1 H, CHN*H*), 7.40-7.69 (m, 10 H, Ar). Anal. Calcd for $C_{48}H_{81}O_3NSi$: C, 77.05; H, 10.91; N, 1.87. Found: C, 76.98; H, 11.06; N, 1.80.

2-(*N*-Tetradecanoyl)-3-(*O*-tetrahydropyranyl)-DL-*erythro*-sphingosine (38). This compound was prepared from **37** in 72% yield by using the same procedure as described for **8a** followed by desilylation with tetra-*n*-butylammonium fluoride in THF (see preparation of **9a**); ^{1} H NMR (200 MHz, $CDCl_3$) δ (ppm) 0.85-0.91 (t, *J* = 6.4 Hz, 6 H, CH_3(CH_2)$_{11}$, CH_3(CH_2)$_{10}$), 1.26 (m, 42 H, (CH_2)$_{11}$, (CH_2)$_{10}$), 1.51-1.78 (m, 8 H, CH_2CH_2CO, (CH_2)$_3$ of THP), 1.99-2.06 (m, 2 H, CH_2CH=CH), 2.15-2.22 (t, *J* = 8.0 Hz, 2 H, CH$_2$CH_2CO), 3.32 (m, 1 H, C*H*NH), 3.89-3.99 (m, 2 H, CH_2OH), 4.16-4.22 (m, 1 H C*H*OTHP), 4.45 (m, 1 H, O'CHO of THP), 5.31-5.43 (dd, *J* = 15.4 Hz, 6.7 Hz, 1 H, vinyl H), 5.64-5.78 (dt, *J* = 15.4 Hz, 6.7 Hz, 1 H, vinyl H),

6.36-6.40 (d, J = 7.6 Hz, 1 H, CHN*H*). Anal. Calcd for $C_{37}H_{71}O_4N$: C, 74.82; H, 12.05; N, 2.36. Found: C, 75.29; H, 11.80; N, 2.21.

***N*-Tetradecanoyl-DL-*erythro*-sphingomyelin (39).** This compound was prepared from **38** in 42% yield by using the same procedure as described for **34** followed by deprotection of the THP group with *p*-toluenesulfonic acid in methanol (see preparation of **35**; ^{1}H NMR (200 MHz, $CDCl_3$) δ (ppm) 0.85-0.90 (t, J = 6.6 Hz, 6 H, C*H*$_3$(CH$_2$)$_{11}$, C*H*$_3$(CH$_2$)$_{10}$), 1.25 (m, 42 H, (CH$_2$)$_{11}$, (CH$_2$)$_{10}$), 1.46 (m, 2 H, C*H*$_2$CH$_2$CO), 1.98-2.34 (m, 4 H, C*H*$_2$CH=CH, CH$_2$C*H*$_2$CO), 3.29 (s, 9 H, N(CH$_3$)$_3$), 3.77 (m, 3 H, C*H*$_2$N(CH$_3$)$_3$, C*H*NH), 4.04 (m, 4 H, CH$_2$OP(O)(O$^-$)OCH$_2$), 4.39 (m, 1 H, C*H*OH), 5.34-5.44 (dd, J = 15.4 Hz, 6.7 Hz, 1 H, vinyl H), 5.55-5.67 (dt, J = 15.4 Hz, 6.7 Hz, 1 H, vinyl H). Anal. Calcd for $C_{37}H_{75}O_6N_2P\cdot 3H_2O$: C, 60.95; H, 11.20; N, 3.84; P, 4.25. Found: C, 60.80; H, 11.16; N, 3.98; P, 4.28.

***N*-Dodecanoyl-DL-*erythro*-sphingosine (40) (Scheme 7).** This compound was prepared from sphingosine **5b** in 86% yield by using the same procedure as described for **6a** (page 19); ^{1}H NMR (200 MHz, $CDCl_3$) δ (ppm) 0.84-0.90 (t, J = 6.2 Hz, 6 H, C*H*$_3$(CH$_2$)$_{11}$, C*H*$_3$(CH$_2$)$_8$), 1.26 (m, 38 H, (CH$_2$)$_{11}$, (CH$_2$)$_8$), 1.49 (m, 2 H, C*H*$_2$CH$_2$CO), 1.93-1.97 (m, 2 H, C*H*$_2$CH=CH), 2.00-2.20 (t, J = 7.8 Hz, 2 H, CH$_2$C*H*$_2$CO), 3.42 (m, 1 H, C*H*NH), 3.81-3.91 (m, 2 H, C*H*$_2$OH), 4.03-4.11 (m, 1 H, C*H*OH), 5.40-5.51 (dd, J = 15.4 Hz, 6.7 Hz, 1 H, vinyl H), 5.68-5.80 (dt, J = 15.4 Hz, 6.7 Hz, 1 H, vinyl H), 6.13-6.18 (d, J = 7.5 Hz, 1 H, CHNH).

1-(*O*-*tert*-Butyldiphenylsilyl)-2-(*N*-dodecanoyl)-DL-*erythro*-sphingosine (41). This compound was prepared from **40** in 80% yield by

using the same procedure as described for **7a**.

1-(*O-tert*-Butyldiphenylsilyl)-2-(*N*-dodecanoyl)-3-(O-tetrahydropyranyl)-DL-*erythro*-sphingosine (42). This compound was prepared from **41** in 89% yield by using the same procedure as described for **8a**; ^{1}H NMR (200 MHz, $CDCl_3$) δ (ppm) 0.84-0.91 (t, J = 6.6 Hz, 6 H, C*H*$_3$(CH$_2$)$_{11}$, C*H*$_3$(CH$_2$)$_8$), 1.07 (s, 9 H, C(CH$_3$)$_3$), 1.70-1.79 (m, 6 H, (CH$_2$)$_3$ of THP), 1.25 (m, 38 H, (CH$_2$)$_{11}$, (CH$_2$)$_8$), 1.51 (m, 2 H, C*H*$_2$CH$_2$CO), 1.97-2.09 (m, 2 H, C*H*$_2$CH=CH), 2.15-2.28 (t, J = 7.3 Hz, 2 H, CH$_2$C*H*$_2$CO), 3.33-3.44 (m, 1 H, C*H*NH), 3.70-3.89 (m, 2 H, CH$_2$O of THP), 3.98-4.03 (m, 2 H, CH$_2$O), 4.19-4.23 (m, 1 H, CHOTHP), 4.64-4.69 (m, 1 H, O'CHO of THP), 5.46-5.53 (dd, J = 15.4 Hz, 6.6 Hz, 1 H, vinyl H), 5.62-5.77 (dt, J = 15.4 Hz, 6.6 Hz, 1 H, vinyl H), 7.37-7.74 (m, 10 H, Ar).

***N*-Dodecanoyl-3-(O-tetrahydropyranyl)-DL-*erythro*-sphingosine (43).** This compound was prepared from **42** in 83% yield by using the same procedure as described for **9a**; ^{1}H NMR (200 MHz, $CDCl_3$) δ (ppm) 0.85-0.90 (t, J = 6.4 Hz, 6 H, C*H*$_3$(CH$_2$)$_{11}$, C*H*$_3$(CH$_2$)$_8$), 1.26 (m, 38 H, (CH$_2$)$_{11}$, (CH$_2$)$_8$), 1.54-1.80 (m, 8 H, C*H*$_2$CH$_2$CO, (CH$_2$)$_3$ of THP), 2.02-2.04 (m, 2 H, C*H*$_2$CH=CH), 2.15-2.22 (t, J = 7.2 Hz, 2 H, CH$_2$C*H*$_2$CO), 3.34 (m, 1 H, C*H*NH), 3.47-3.63 (m, 2 H, CH$_2$O of THP), 3.89-4.00 (m, 2 H, CH$_2$O), 4.17-4.22 (m, 1 H, CHOTHP), 4.45 (m, 1 H, O'CHO of THP), 5.31-5.42 (dd, J = 15.5 Hz, 6.6 Hz, 1 H, vinyl H), 5.63-5.75 (dt, J = 15.5 Hz, 6.6 Hz, 1 H, vinyl H), 6.36-6.40 (d, J = 7.3 Hz, 1 H, CHN*H*).

***N*-Dodecanoyl-DL-*erythro*-sphingomyelin (44).** This compound was prepared from **43** in 41% yield by using the same procedure as

described for **34** followed by deprotection of the THP group with *p*-toluenesulfonic acid in methanol (see preparation of **35**); ^{1}H NMR (200 MHz, $CDCl_3$) δ (ppm) 0.85-0.90 (t, *J* = 6.3 Hz, 6 H, C*H*$_3$(CH$_2$)$_{11}$, C*H*$_3$(CH$_2$)$_8$), 1.25 (m, 38 H, (CH$_2$)$_{11}$, (CH$_2$)$_8$), 1.53 (m, 2 H, C*H*$_2$CH$_2$CO), 1.95 (m, 2 H, C*H*$_2$CH=CH), 2.12 (t, *J* = 7.2 Hz, 2 H, CH$_2$C*H*$_2$CO), 3.32 (s, 9 H, N(CH$_3$)$_3$), 3.66-3.79 (m, 3 H, C*H*NH, C*H*$_2$N(CH$_3$)$_3$), 3.97-4.19 (m, 4 H, CH$_2$OP(O)(O$^-$)OCH$_2$), 4.29 (m, 1 H C*H*OH), 5.39-5.46 (dd, *J* = 15.4 Hz, 6.6 Hz, 1 H, vinyl H), 5.61-5.72 (dt, *J* = 15.4 Hz, 6.6 Hz, 1 H, vinyl H), 7.04-7.08 (d, *J* = 7.5 Hz, 1 H, CHN*H*).

***N*-Docosanoyl-DL-*erythro*-sphingosine (45).** This compound was prepared from sphingosine (**5b**) and *p*-nitrophenyl behenate in 64% yield by using the same procedure as described for **6a**; TLC ($CHCl_3$-EtOH, 95:7) R_f 0.55; ^{1}H NMR (200 MHz, $CDCl_3$) δ (ppm) 0.85-0.90 (t, *J* = 6.5 Hz, 6 H, C*H*$_3$(CH$_2$)$_{11}$, C*H*$_3$(CH$_2$)$_{18}$), 1.25 (m, 58 H, (CH$_2$)$_{11}$, (CH$_2$)$_{18}$), 1.54-1.71 (m, 2 H, C*H*$_2$CH$_2$CO), 2.00-2.09 (m, 2 H, C*H*$_2$CH=CH), 2.20-2.28 (t, *J* = 7.1 Hz, 2 H, CH$_2$C*H*$_2$CO), 3.60-3.70 (m, 1 H, C*H*NH), 3.80-3.98 (m, 2 H, C*H*$_2$OH), 4.29-4.34 (m, 1 H, C*H*OH), 5.47-5.58 (dd, *J* = 15.4 Hz, 6.4 Hz, 1 H, vinyl H), 5.75-5.87 (dt, *J* = 15.4 Hz, 6.4 Hz, 1 H, vinyl H), 6.20-6.25 (d, *J* = 7.7 Hz, 1 H, CHN*H*).

1-(*O-tert*-Butyldiphenylsilyl)-2-(*N*-docosanoyl)-DL-*erythro*-sphingosine (46). This compound was prepared from **45** in 90% yield by using the same procedure as described for **7a**; TLC (hexane-ethyl acetate, 4:1) R_f 0.45; ^{1}H NMR (200 MHz, $CDCl_3$) δ (ppm) 0.85-0.90 (t, *J* = 6.4 Hz, 6 H, C*H*$_3$(CH$_2$)$_{11}$, C*H*$_3$(CH$_2$)$_{18}$), 1.17 (s, 9 H, C(CH$_3$)$_3$), 1.25 (m, 58 H, (CH$_2$)$_{11}$, (CH$_2$)$_{18}$), 1.75 (m, 2 H, C*H*$_2$CH$_2$CO), 2.04-2.10 (m, 2 H, C*H*$_2$CH=CH),

2.20-2.28 (t, J = 7.5 Hz, 2 H, CH_2CH_2CO), 3.81 (m, 1 H, C*H*NH), 4.02-4.10 (m, 2 H, CH_2OH), 4.25 (m, 1 H, C*H*OH), 5.50-5.60 (dd, J = 15.4 Hz, 6.7 Hz, 1 H, vinyl H), 5.75-5.83(dt, J = 15.4 Hz, 6.7 Hz, 1 H, vinyl H), 6.15-6.20 (d, J = 7.6 Hz, 1 H, CHN*H*), 7.42-7.70 (m, 10 H, Ar).

2-(*N*-Docosanoyl)-3-(*O*-tetrahydropyranyl)-DL-*erythro*-sphingosine (47). This compound was prepared from **46** in 70% yield by using the same procedure as described for **8a** followed by desilylation with tetra-*n*-butylammonium fluoride in THF (see preparation of **9a**); ^{1}H NMR (200 MHz, $CDCl_3$) δ (ppm) 0.85-0.91 (t, J = 7.0 Hz, 6 H, $CH_3(CH_2)_{11}$, $CH_3(CH_2)_{18}$), 1.26 (m, 58 H, $(CH_2)_{11}$, $(CH_2)_{18}$), 1.46-1.80 (m, 8 H, CH_2CH_2CO, $(CH_2)_3$ of THP), 1.98-2.05 (m, 2 H, CH_2CH=CH), 2.12-2.20 (t, J = 7.1 Hz, 2 H, CH_2CH_2CO), 3.45-3.50 (m, 2 H, CH_2O of THP), 3.62 (m, 1 H, C*H*NH), 3.84-3.95 (m, 2 H, CH_2OH), 4.12-4.18 (m, 1 H C*H*OTHP), 4.40 (m, 1 H, O'CHO of THP), 5.28-4.40 (dd, J = 15.4 Hz, 6.7 Hz, 1 H, vinyl H), 5.58-5.70 (dt, J = 15.4 Hz, 6.7 Hz, 1 H, vinyl H), 6.33-6.37 (d, J = 7.7 Hz, 1 H, CHN*H*). Anal. Calcd for $C_{45}H_{87}O_4N$: C, 76.54; H, 12.42; N, 1.98. Found: C, 76.37; H, 12.37; N, 1.89.

***N*-Docosanoyl-DL-*erythro*-sphingomyelin (48).** This compound was prepared from **47** in 43% yield by using the same procedure as described for **10c**; ^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm) 0.85-0.90 (t, J = 6.4 Hz, 6 H, $CH_3(CH_2)_{11}$, $CH_3(CH_2)_{18}$), 1.25 (m, 58 H, $(CH_2)_{11}$, $(CH_2)_{18}$), 1.57 (m, 2 H, CH_2CH_2CO), 1.88-1.98 (m, 2 H, CH_2CH=CH), 2.08-2.18 (t, J = 7.2 Hz, 2 H, CH_2CH_2CO), 3.27 (s, 9 H, $N(CH_3)_3$), 3.80 (m, 3 H, $CH_2N(CH_3)_3$, C*H*NH), 3.95-4.15 (m, 4 H, $CH_2OP(O)(O^-)OCH_2$), 4.38 (m, 1 H, C*H*OH), 5.40-5.50 (dd, J = 15.4 Hz, 6.7 Hz, 1 H, vinyl H), 5.59-5.71 (dt, J = 15.4 Hz, 6.7

Hz, 1 H, vinyl H). Anal. Calcd for $C_{45}H_{91}O_6N_2P \cdot 3H_2O$: C, 64.24; H, 11.62; N, 3.33. Found: C, 64.27; H, 11.51; N, 3.14.

***N*-Tetracosanoyl-DL-*erythro*-sphingosine (49).** This compound was prepared from sphingosine (**5b**) in 89% yield by using the same procedure as described for **6a**; TLC (chloroform-ethanol, 95:7) R_f 0.57; ^1H NMR (300 MHz, $CDCl_3$) δ (ppm) 0.85-0.90 (t, J = 6.6 Hz, $CH_3(CH_2)_{11}$, $CH_3(CH_2)_{20}$), 1.25 (m, 62 H, $(CH_2)_{11}$, $(CH_2)_{20}$), 1.62 (m, 2 H, CH_2CH_2CO), 1.85-1.92 (m, 2 H, $CH_2CH{=}CH$), 2.10-2.20 (t, J = 7.5 Hz, 2 H, CH_2CH_2CO), 3.72 (m, 1 H, CHNH), 3.95-4.01 (m, 2 H, CH_2OH), 4.34 (m, 1 H, CHOH), 5.45-5.59 (dd, J = 15.4 Hz, 6.6 Hz, 1 H, vinyl H), 5.66-5.78 (dt, J = 15.4 Hz, 6.6 Hz, 1 H, vinyl H), 6.26-6.30 (d, J = 7.5 Hz, 1 H, CHNH).

1-(*O*-*tert*-Butyldiphenylsilyl)-2-(*N*-tetracosanoyl)-DL-*erythro*-sphingosine (50). This compound was prepared from **49** in 97% yield by using the same procedure as described for **7a**; TLC (hexane-ethyl acetate, 2:1) R_f 0.75.

2-(*N*-Tetracosanoyl)-3-(*O*-tetrahydropyranyl)-DL-*erythro*-sphingosine (51). This compound was prepared from **50** in 59% yield by using the same procedure as described for **8a** followed by desilylation with tetra-*n*-butylammonium fluoride in THF (see preparation of **9a**); TLC (hexane-ethyl acetate, 1:1) R_f 0.45; ^1H NMR (200 MHz, $CDCl_3$) δ (ppm) 0.85-0.91 (t, J = 6.0 Hz, $CH_3(CH_2)_{11}$, $CH_3(CH_2)_{20}$), 1.25 (m, 62 H, $(CH_2)_{11}$, $(CH_2)_{20}$), 1.51-1.71 (m, 8 H, CH_2CH_2CO, $(CH_2)_3$ of THP), 1.99-2.06 (m, 2 H, $CH_2CH{=}CH$), 2.15-2.23 (t, J = 7.4 Hz, 2 H, CH_2CH_2CO), 3.67 (m, 1 H, CHNH), 3.89-3.99 (m, 4 H, CH_2OH, CH_2O of THP), 4.16-4.22 (m, 1 H,

C*H*OTHP), 4.45-4.48 (m, 1 H, O'CHO of THP), 5.31-5.44 (dd, J = 15.4 Hz, 6.7 Hz, 1 H, vinyl H), 5.63-5.75 (dt, J = 15.4 Hz, 6.7 Hz, 1 H, vinyl H), 6.37-6.41 (d, J = 7.6 Hz, 1 H, CHN*H*). Anal. Calcd for $C_{47}H_{91}O_4N$: C, 76.88; H, 12.49; N, 1.90. Found: C, 76.52; H, 12.55; N, 1.87.

***N*-Tetracosanoyl-DL-*erythro*-sphingomyelin (52).** This compound was prepared from **51** in 46% yield by using the same procedure as described for **10c**; ^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm) 0.85-0.90 (t, J = 6.6 Hz, 6 H, C*H*$_3$(CH$_2$)$_{11}$, C*H*$_3$(CH$_2$)$_{20}$), 1.25 (m, 62 H, (CH$_2$)$_{11}$, (CH$_2$)$_{20}$), 1.55 (m, 2 H, C*H*$_2$CH$_2$CO), 2.01-2.08 (m, 2 H, C*H*$_2$CH=CH), 2.13-2.19 (t, J = 7.2 Hz, 2 H, CH$_2$C*H*$_2$CO), 3.35 (s, 9 H, N(CH$_3$)$_3$), 3.70 (m, 2 H, C*H*$_2$N(CH$_3$)$_3$), 3.95 (m, 1 H, C*H*NH), 4.15-4.23 (m, 4 H, CH$_2$OP(O)(O$^-$)OCH$_2$), 4.40 (m, 1 H, C*H*OH), 5.40-5.52 (dd, J = 15.4 Hz, 6.7 Hz, 1 H, vinyl H), 5.60-5.72 (dt, J = 15.4 Hz, 6.7 Hz, 1 H, vinyl H).

***N*-(*cis*-15-Tetracosenoyl)-DL-*erythro*-sphingosine (53).** This compound was prepared from sphingosine (**5b**) and *p*-nitrophenyl nervonate in 70% yield by using the same procedure as described for **6a**; TLC (chloroform-ethanol, 95:7) R_f 0.57; ^{1}H NMR (200 MHz, $CDCl_3$) δ (ppm) 0.85-0.90 (t, J = 6.6 Hz, 6 H, C*H*$_3$(CH$_2$)$_{11}$, C*H*$_3$(CH$_2$)$_7$CH=CH(CH$_2$)$_{11}$), 1.26 (m, 54 H, (C*H*$_2$)$_{11}$, (C*H*$_2$)$_6$CH$_2$CH=CHCH$_2$(C*H*$_2$)$_{10}$), 1.58-1.66 (m, 2 H, C*H*$_2$CH$_2$CO), 2.00-2.06 (m, 6 H, C*H*$_2$CH=CH, C*H*$_2$CH=CHC*H*$_2$ of the amide chain), 2.25 (t, J = 7.5 Hz, 2 H, CH$_2$C*H*$_2$CO), 3.73 (m, 1 H, C*H*NH), 3.94-4.00 (m, 2 H, C*H*$_2$OH), 5.32-5.37 (m, 2 H, *cis*-vinyl H), 5.50-5.63 (dd, J = 15.4 Hz, 6.6 Hz, 1 H, vinyl H), 5.70-5.81 (dt, J = 15.4 Hz, 6.6 Hz, 1 H, vinyl H), 6.37-6.41 (d, J = 7.7 Hz, 1 H, vinyl H).

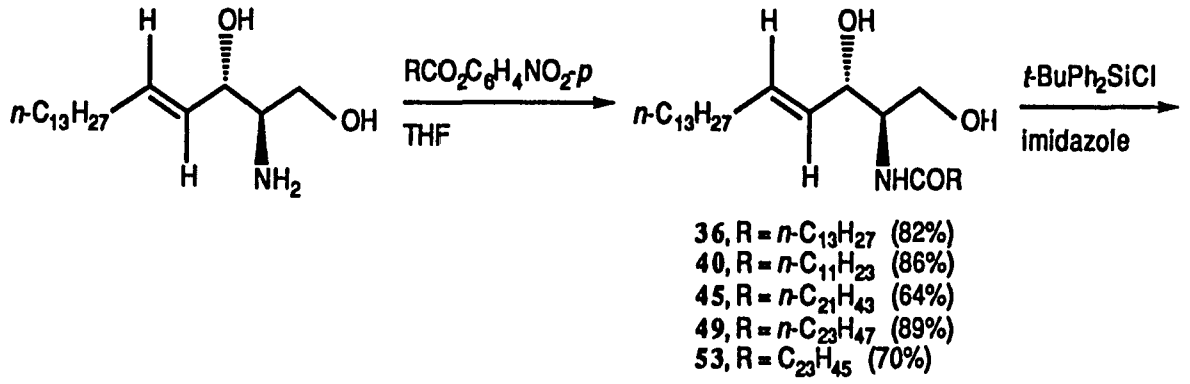
1-(*O-tert*-Butyldiphenylsilyl)-2-(*N-cis*-15-tetracosenoyl)-DL-*erythro*-sphingosine (54). This compound was prepared from **53** in 93% yield by using the same procedure as described for **7a**; TLC (hexane-ethyl acetate, 2:1) R_f 0.76.

2-(*N-cis*-15-Tetracosenoyl)-3-(*O*-tetrahydropyranyl)-DL-*erythro*-sphingosine (55). This compound was prepared from **54** in 65% yield by using the same procedure as described for **8a** followed by desilylation with tetra-*n*-butylammonium fluoride in THF (see preparation of **9a**); TLC (hexane-ethyl acetate, 1:1) R_f 0.47; ^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm) 0.85-0.91 (t, J = 6.6 Hz, 6 H, $CH_3(CH_2)_{11}$, $CH_3(CH_2)_7CH=CH(CH_2)_{11}$), 1.25 (m, 54 H, $(CH_2)_{11}$, $(CH_2)_6CH_2CH=CHCH_2(CH_2)_{10}$), 1.71-1.84 (m, 8 H, CH_2CH_2CO, $(CH_2)_3$ of THP), 2.02-2.08 (m, 6 H, $CH_2CH=CH$, $CH_2CH=CHCH_2$ of the amide chain), 2.20-2.25 (t, J = 7.4 Hz, 2 H, CH_2CH_2CO), 3.67 (m, 1 H, CHNH), 3.92-4.02 (m, 4 H, CH_2OH, CH_2O of THP), 4.20-4.26 (m, 1 H, CHOTHP), 4.48-4.51 (m, 1 H, O'CHO), 5.35-5.42(m, 2 H, *cis*-vinyl H), 5.48-5.61 (dd, J = 15.4 Hz, 6.6 Hz, 1 H, vinyl H), 5.68-5.80 (dt, J = 15.4 Hz, 6.6 Hz, 1 H, vinyl H), 6.39-6.43 (d, J = 7.6 Hz, 1 H, CHNH). Anal. Calcd for $C_{47}H_{89}O_4N$: C, 77.10; H, 12.25; N, 1.91: Found: C, 77.06; H, 12.49; N, 1.74.

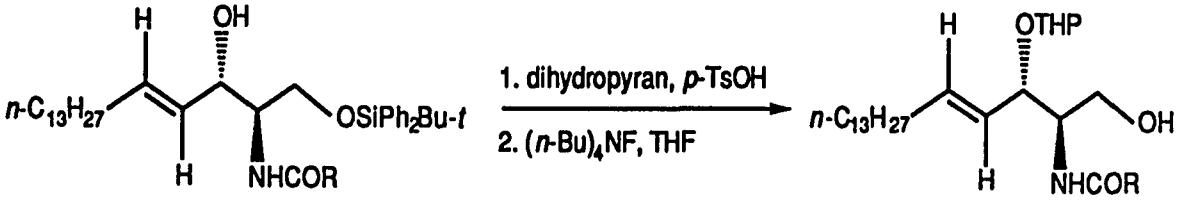
***N*-(*cis*-15-Tetracosenoyl)-DL-*erythro*-sphingomyelin (56).** This compound was prepared from **55** in 48% yield by using the same procedure as described for **10c**; TLC ($CHCl_3$-MeOH-H_2O-conc. NH_4OH, 65:25:2:2) R_f 0.28.

3-(*O*-Tetrahydropyranyl)-egg sphingomyelin (57). This compound was prepared from egg sphingomyelin in 62% yield by using a similar procedure as described for **8a** (page 23) with the following modification: The reaction was carried out at 45 °C; TLC ($CHCl_3$-MeOH-H_2O-conc. NH_4OH, 65:25:2:2) R_f 0.36. The R_f value of egg sphingomyelin in the same solvent system was 0.24.

Scheme 7. Syntheses of different *N*-acyl sphingomyelins

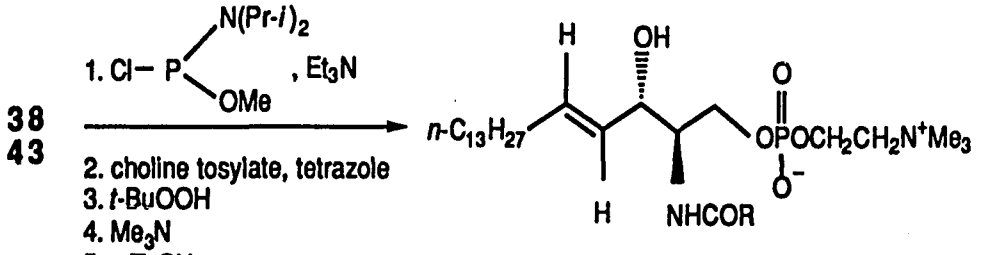


36, R = *n*-$C_{13}H_{27}$ (82%)
40, R = *n*-$C_{11}H_{23}$ (86%)
45, R = *n*-$C_{21}H_{43}$ (64%)
49, R = *n*-$C_{23}H_{47}$ (89%)
53, R = $C_{23}H_{45}$ (70%)

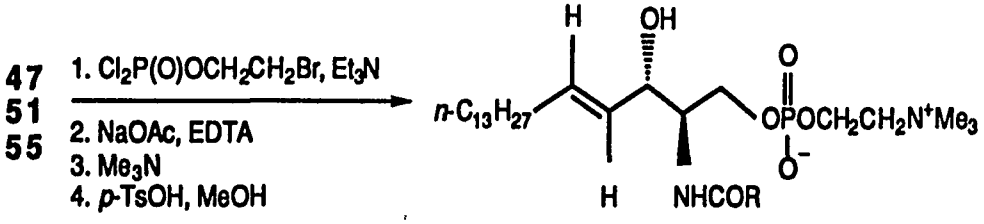


37, R = *n*-$C_{13}H_{27}$ (89%)
41, R = *n*-$C_{11}H_{23}$ (80%)
46, R = *n*-$C_{21}H_{43}$ (90%)
50, R = *n*-$C_{23}H_{47}$ (97%)
54, R = $C_{23}H_{45}$ (93%)

38, R = *n*-$C_{13}H_{27}$ (72%)
43, R = *n*-$C_{11}H_{23}$ (74%)
47, R = *n*-$C_{21}H_{43}$ (70%)
51, R = *n*-$C_{23}H_{47}$ (59%)
55, R = $C_{23}H_{45}$ (65%)



39, R = *n*-$C_{13}H_{27}$ (42%)
44, R = *n*-$C_{11}H_{23}$ (41%)



48, R = *n*-$C_{21}H_{43}$ (43%)
52, R = *n*-$C_{23}H_{47}$ (46%)
56, R = $C_{23}H_{45}$ (48%)

Results and Discussion

HPLC analysis of stereochemistry of the aldol condensation

Results

A. Analytical HPLC: Evaluation of erythro/threo ratio. The ratio of *erythro* to *threo* **19** was determined by HPLC on a chiral stationary column (Pirkle type 1 A, 4.6 x 250 mm, J. T. Baker). Baseline separation of the diastereomers of *erythro*- and *threo*-biphenylcarboxamidosphingosines **19** was achieved. As shown in Figure 1, D-*erythro*-biphenylcarboxamidosphingosine **19'** has a retention time of 20.49 min under these conditions (panel A), whereas a mixture of *erythro*- and *threo*-biphenylcarboxamidosphingosines **19"** gave two peaks, with retention times of 14.10 min and 20.38 min (panel B). The first peak corresponds to the *threo* isomer, since the second peak is identified as the *erythro* isomer by comparision with the derivative prepared from an authentic sample obtained from Sigma. Biphenylcarboxamidosphingosine **19** prepared according to Scheme 1 has two peaks. The peak with R_t of 14.20 min corresponds to the *threo*-sphingosine derivative, and the peak with R_t of 20.51 min corresponds to the *erythro*-sphingosine derivative (panel C). The ratio of the integrated areas of the *erythro* to *threo* peaks is 98.0:2.0.

B. Analytical HPLC: Evaluation of D/L ratio. The ratio of D- to L-isomers of sphingosine was estimated by chiral HPLC analysis of the (*R*)-(+)-bis-Mosher esters derived from the biphenylcarboxamidosphingosines **20**. Figure 2 shows the chromatograms of the bis-(*R*)-(+)-Mosher esters derived from the biphenylcarboxamidosphingosines **20** by the sequence outlined in Scheme 6. Panel A shows a peak with a retention time of 23.86 min for the D-*erythro* isomer obtained from Sigma; the peak with retention time of 21.86 min is considered to represent the L-*erythro* isomer, which is

present as an impurity of less than 5% in the commerical sample. Panel B shows a peak with R_t of 21.83 min, corresponding to the L-*erythro* isomer, and a peak with R_t of 23.9 min corresponding to the D-*erythro* isomer. The ratio of D- to L-isomers is 50:50, based on integration of the peak areas.

C. Evaluation of erythro/threo ratio of N-methylsphingosine (18). In general, the kinetic aldol condensation gives both *erythro* and *threo* stereoisomers, and there are many examples of low diastereoselectivity.[41-50] In an attempt to evaluate why the *erythro/threo* ratio obtained in Scheme 1 is unsually high, we carried out the aldol condensation using bis(trimethylsilyl)sarcosine and (*E*)-hexadec-2-enal (Scheme 5). The HPLC analysis of *N*-methyl-*N*-biphenylcarboxamidosphingosine (**20'**) is presented in Figure 3. *N*-Methyl-*N*-biphenylcarboxamido-D-*erythro*-sphingosine has a retention time of 14.24 min; the authentic sample of *N*-methyl-D-*erythro*-sphingosine was provided by Dr. D. C. Liotta. In contrast, the *N*-methyl-*N*-biphenylcarboxamidosphingosine we synthesized from bis(trimethylsilyl)sarcosine by the sequence shown in Scheme 5 gives a peak with R_t of 11.72 min (assumed to be the *threo* isomer) and a peak at 14.31 min (corresponding to the *N*-methyl-*erythro*-sphingosine derivative). The ratio of *erythro*- to *threo*-*N*-methylsphingosine is 62.5:37.5, based on the ratio of the integrated areas of the two peaks. The low selectivity seen in this reaction is about what is expected for an aldol condensation of an α-amino acid with a small group attached to nitrogen.[46,47]

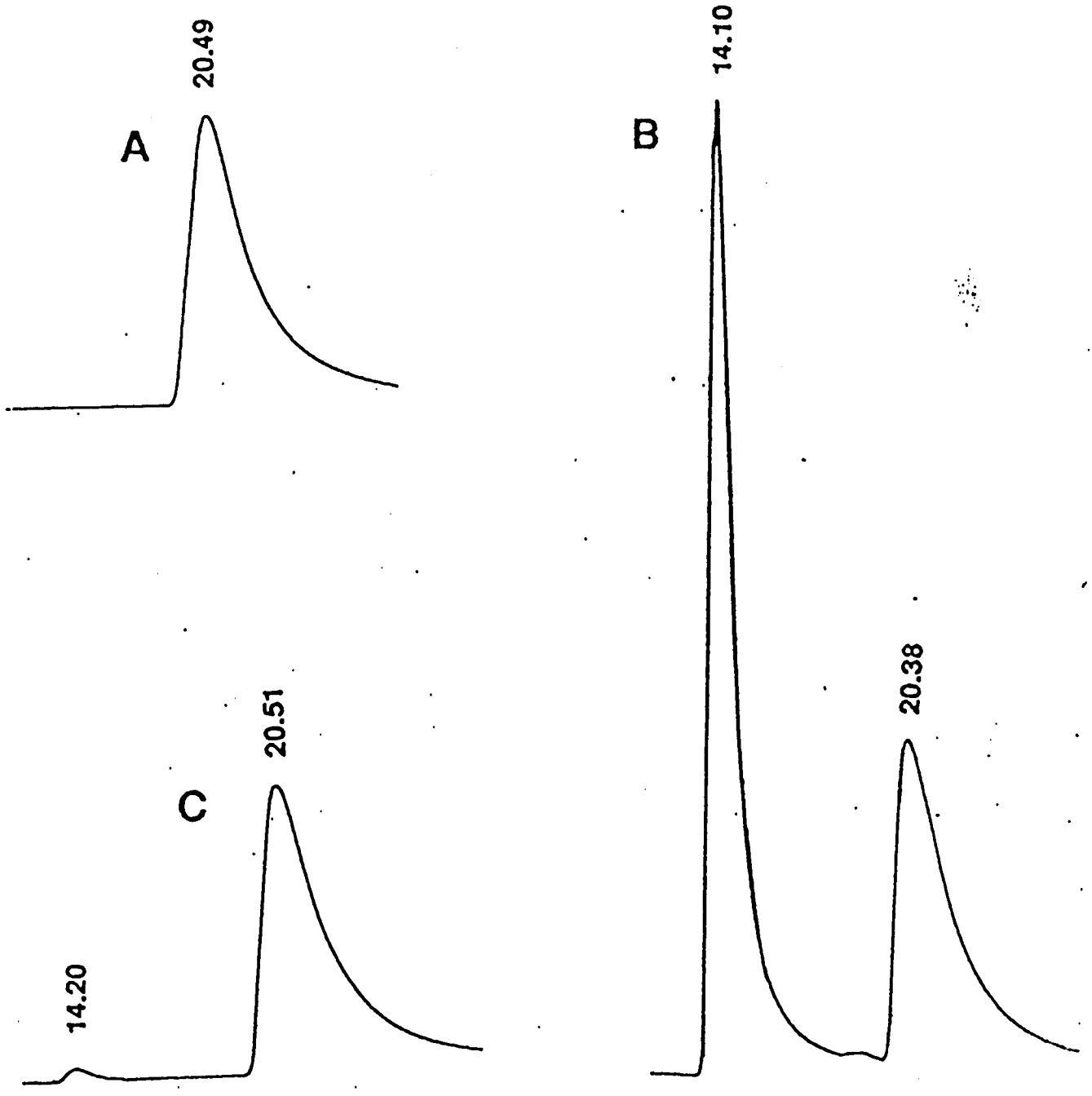


Figure 1. HPLC traces of biphenylcarboxamido derivatives of *erythro*- and *threo*-sphingosines. **A**, *erythro*-sphingosine from Sigma; **B**, a mixture of *erythro*- and *threo*-sphingosine from Dr. D. C. Liotta; **C**, sphingosine prepared from aldol condensation (see Scheme 1). Solvent: hexane-isopropyl alcohol, 80:20; flow rate, 0.5 mL/min. Retention times: *erythro*-sphingosine, 20.38-20.51min; *threo*-sphingosine, 14.10-14.20 min.

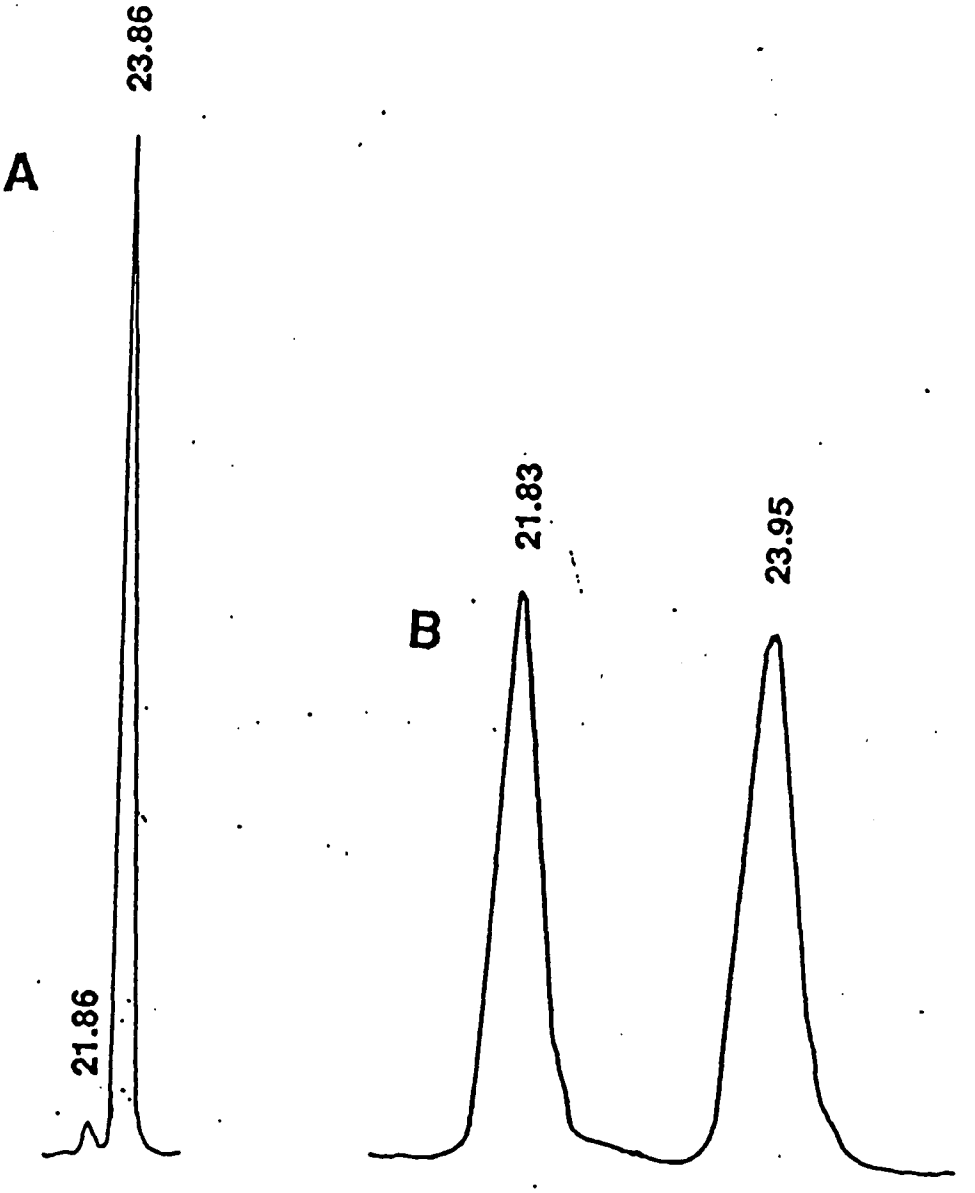


Figure 2. HPLC analysis of bis-(*R*)-(+)-MTPA esters of biphenylcarboxamido-D- and L-*erythro*- sphingosines. **A**, D-*erythro*-sphingosine from Sigma was used (an impurity of less than 5% is present in the commerical sample; this represents L-*erythro*-sphingosine); **B**, D- and L-*erythro*-sphingosine from the aldol condensation outlined in Scheme 1. Solvent: hexane-isopropyl alcohol, 95:5; flow rate, 0.55 mL/min. Retention times: L-*erythro*-sphingosine, 21.83-21.86 min; D-*erythro*-sphingosine, 23.86-23.95 min.

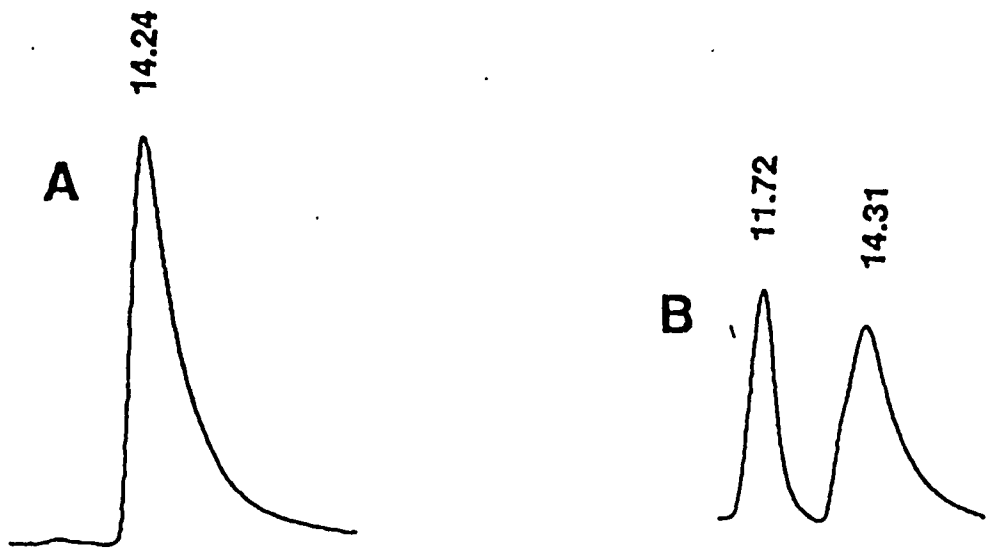
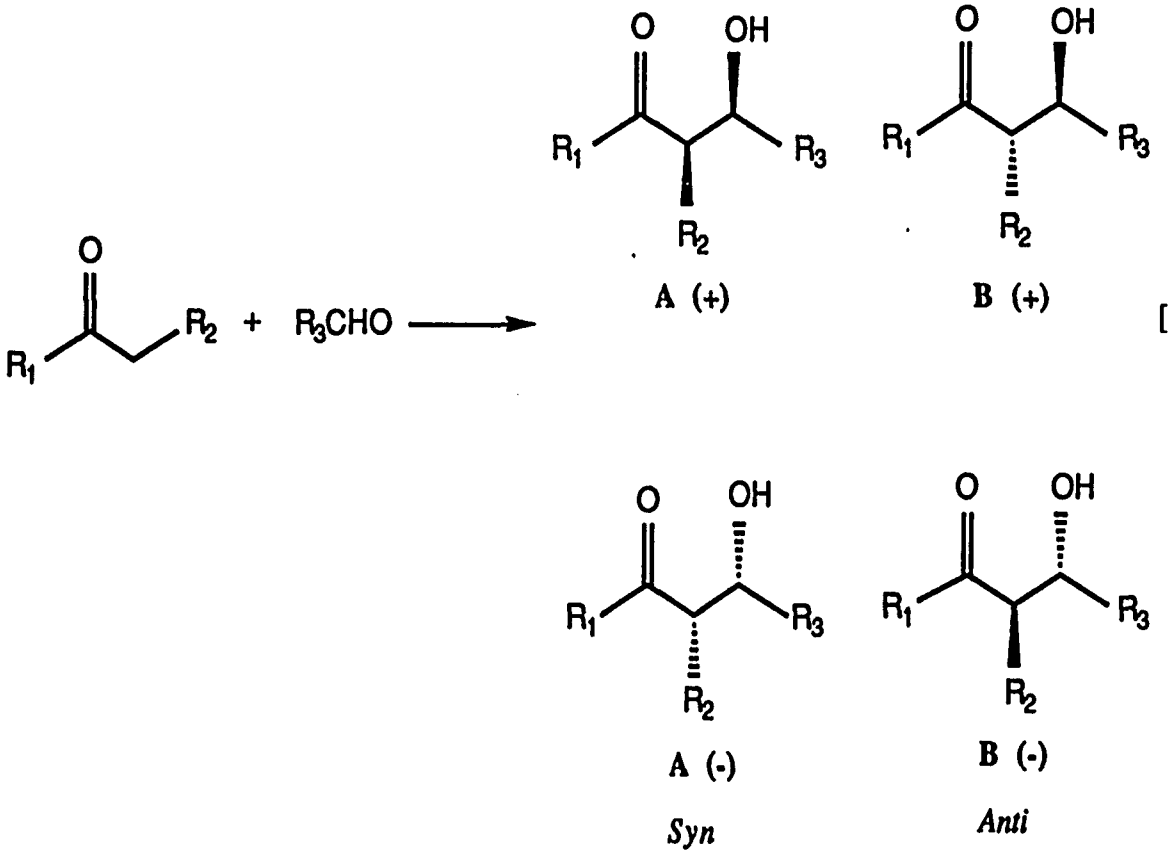


Figure 3. HPLC analysis of *N*-methyl-*N*-biphenylcarboxamido derivatives of *erythro*- and *threo*-sphingosines. A, *N*-methyl-*erythro*-sphingosine obtained from Dr. D. C. Liotta was derivatized as shown in Scheme 6; B, *N*-methyl-*erythro,threo*-sphingosine obtained from the aldol condensation of bis(trimethylsilyl)sarcosine and (*E*)-hexadec-2-enal (see Scheme 5) was derivatized. Solvent: hexane-isopropyl alcohol, 80:20; flow rate, 1.0 mL/min. Retention times: *N*-methyl-*erythro*-sphingosine, 14.24-14.31 min; *N*-methyl-*threo*-sphingosine, 11.72 min.

Discussion

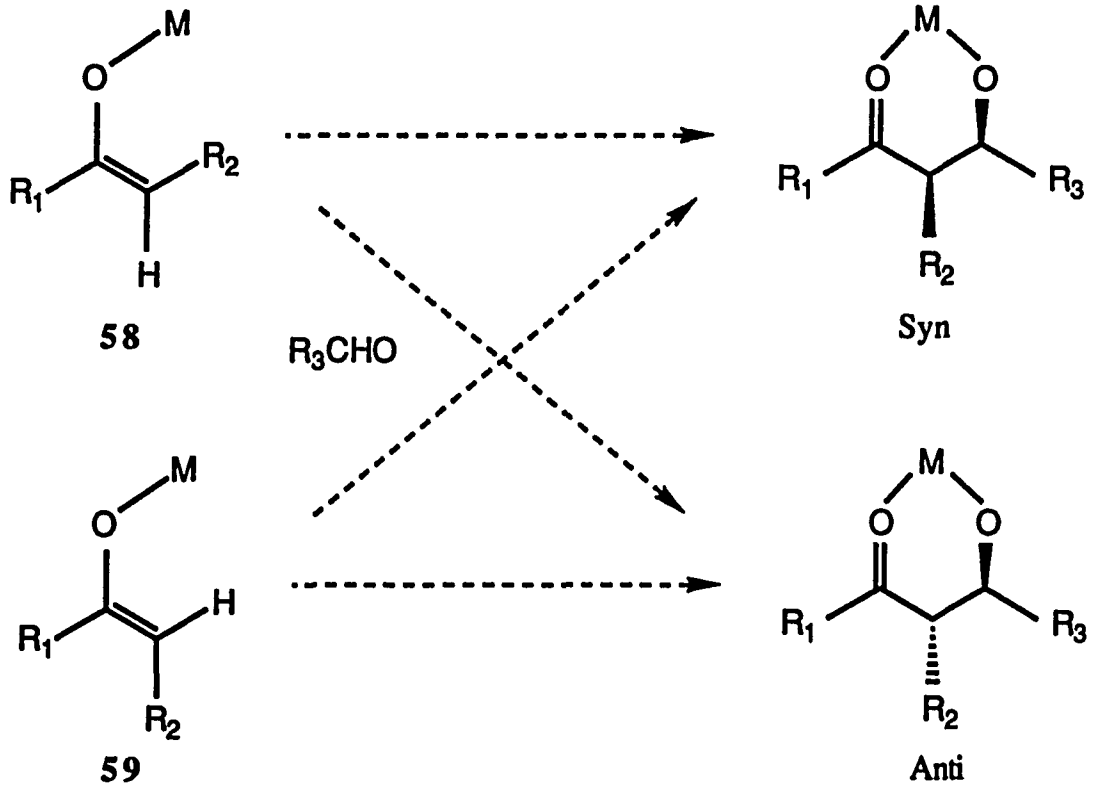
Stereochemistry of the kinetic aldol condensation. In an aldol condensation between carbonyl partners there are four possible product stereoisomers (eq. 1). Consequently, there are two stereochemical aspects associated with the reaction: one dealing with internal stereochemical control or diastereoselection [A (±) vs. B (±)], and the other dealing with absolute stereochemical control for a given diastereomer or enantioselection [A (+) vs. A (-) or B (+) vs. B (-)].[28]



[1]

There is an abundant body of data that correlates aldol product stereochemistry with enolate geometry for kinetically controlled condensations.[41,42,51,52] This aspect of the topic has been treated in detail.[41,42,51,52] With regard to enolate stereochemical nomenclature (eq. 2), a *cis* stereochemical relationship between the enolate ligand $\mathbf{R_2}$ and

oxygen substituent (OM, M = Li) as shown in structure **58** will be referred to as the *Z* enolate. In a similar case, the *trans* stereochemical relationship between R_2 and **OM** as in **59** will be designated as the *E* enolate. The normal correlation is found when lithium is used as a metal, i.e. *Z* enolates tend to give *syn* aldols and *E* enolates tend to give *anti* products.[51b] Evans *et al.* pointed out that for many metal enolates, kinetic aldol diastereoselection is strongly influenced by enolate geometry,[28b,51b] and the product ratio *anti*/*syn* will be no greater than the enolate ratio.[28a]



[2]

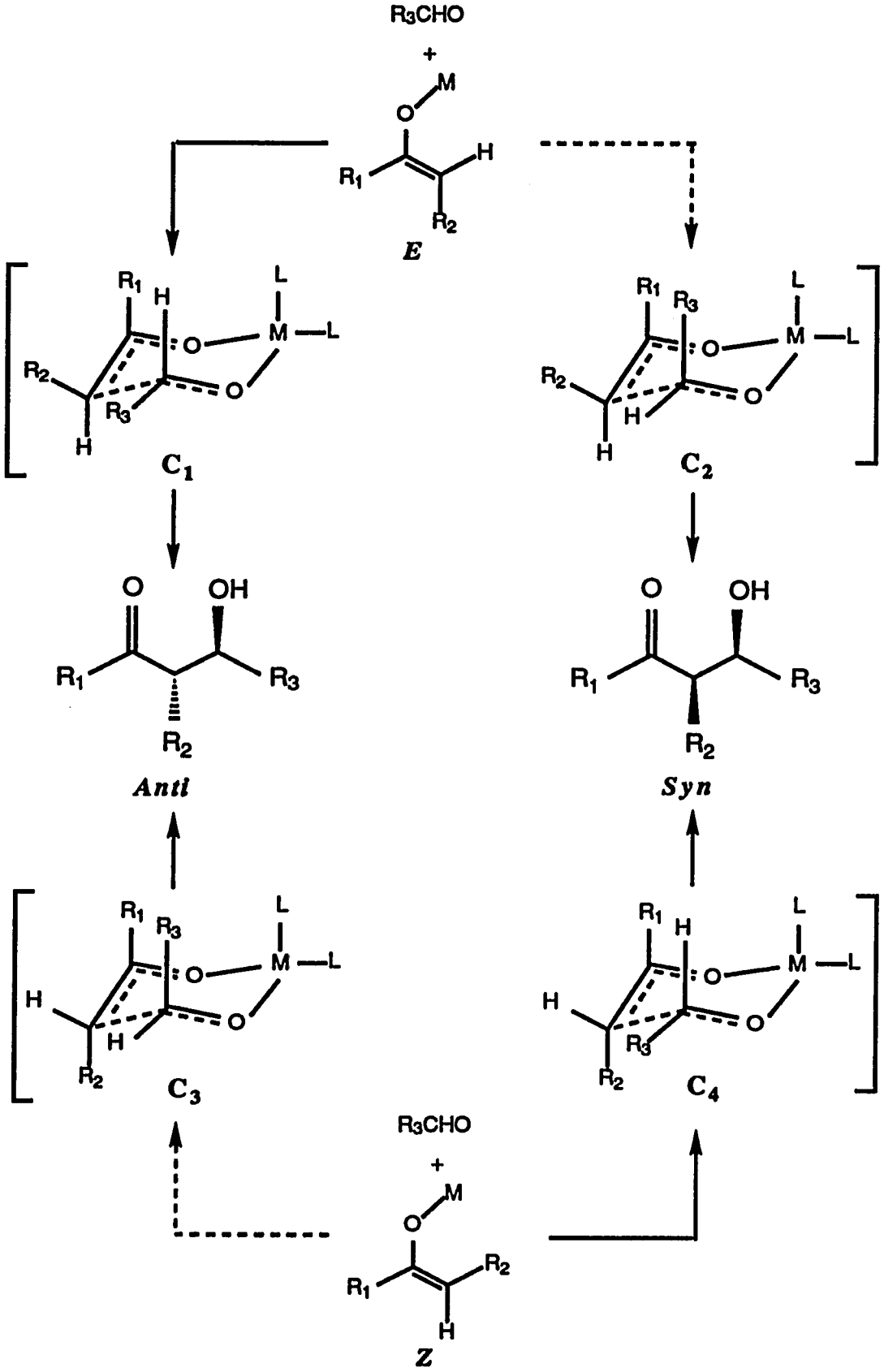
Under kinetically controlled conditions, the correlation of metal enolate geometry and aldol product stereochemistry via diastereomeric chair-preferred transition states has been widely accepted.[41,42,51-53] The observations that the steric bulk of the enolate ligand R_1 and attendant aldol diastereoselection are directly coupled are consistent with the Zimmerman

model illustrated in Scheme 8 for chair-preferred transition states.[54] For *E* enolates, transition state C_2 is predicted to be destabilized relative to C_1 because of the R_1‹—› R_3 variable steric parameter; therefore, *E* enolates lead to the *anti* aldols.[41,43,48,55] In a similar fashion, transition state C_3 is destabilized relative to C_4 for *Z* enolates; thus *Z* enolates lead predominately to *syn* aldols, and stereoselectivity increases with increasing steric bulk of R_1.[41,43,48,55,56]

An alternative interpretation of the closed transition state, which considers both chair and boat arrangements, has been proposed by Evans.[28b] In addition to the four idealized chair transition states depicted in Scheme 8 (page 65),[54] this model also considers the four boat transition states shown in Scheme 9 (page 66). The stereochemical data are explained by assuming that a given *Z* or *E* enolate can choose one of two chair transition states (Scheme 8) or one of two boat transition states (Scheme 9). Of the boat transition states, B_2 and B_4 are considered to be unimportant because of the R_2‹—›R_3 eclipsing interaction. However, when gauche R_2‹—›R_3 interaction becomes important (large R_2), a *Z* enolate might find the chair transition state C_4 in Scheme 8 less attractive than the boat transition states B_3 in Scheme 9. The "boat alternative" hypothesis predicts that increasing bulk of R_2 in an *E* enolate would either have no effect on stereoselectivity or would increase *anti* selectivity.[57]

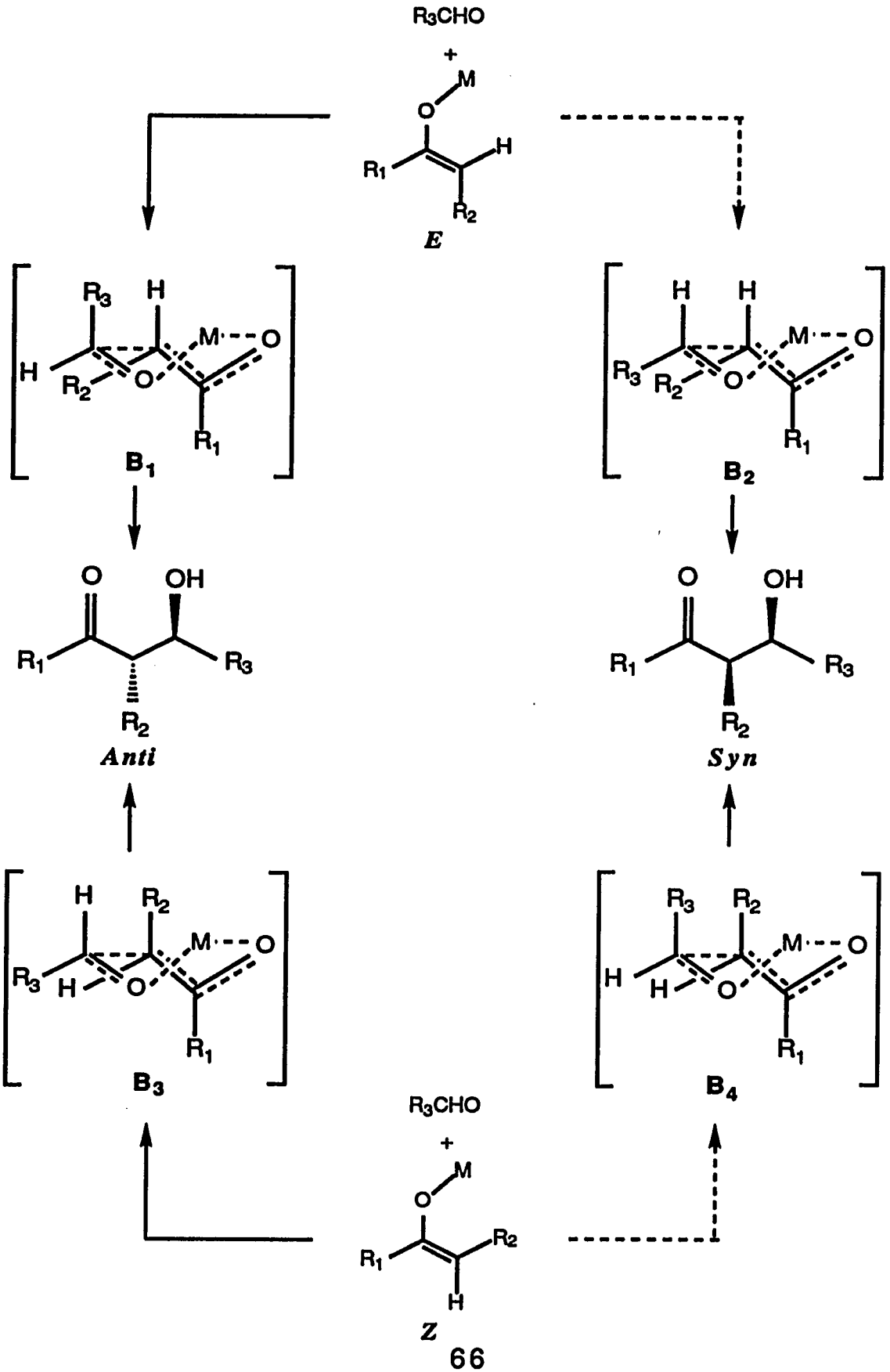
Kinetically selective enolization of esters with a dialkylamide base such as lithium diisopropylamide (LDA) has been investigated by several groups.[41,43,44] The stereochemical assignments of the enolates formed from the esters were determined by silylation followed by separation of the silyl enol ethers and NMR analysis.[45] The deprotonation process might be proceeding via either of the two metal-centered pericyclic chairlike transition

Scheme 8. Zimmerman-Traxler transition states



The dashed arrows show unfavorable pathways.

Scheme 9. Boat forms of the closed transition states

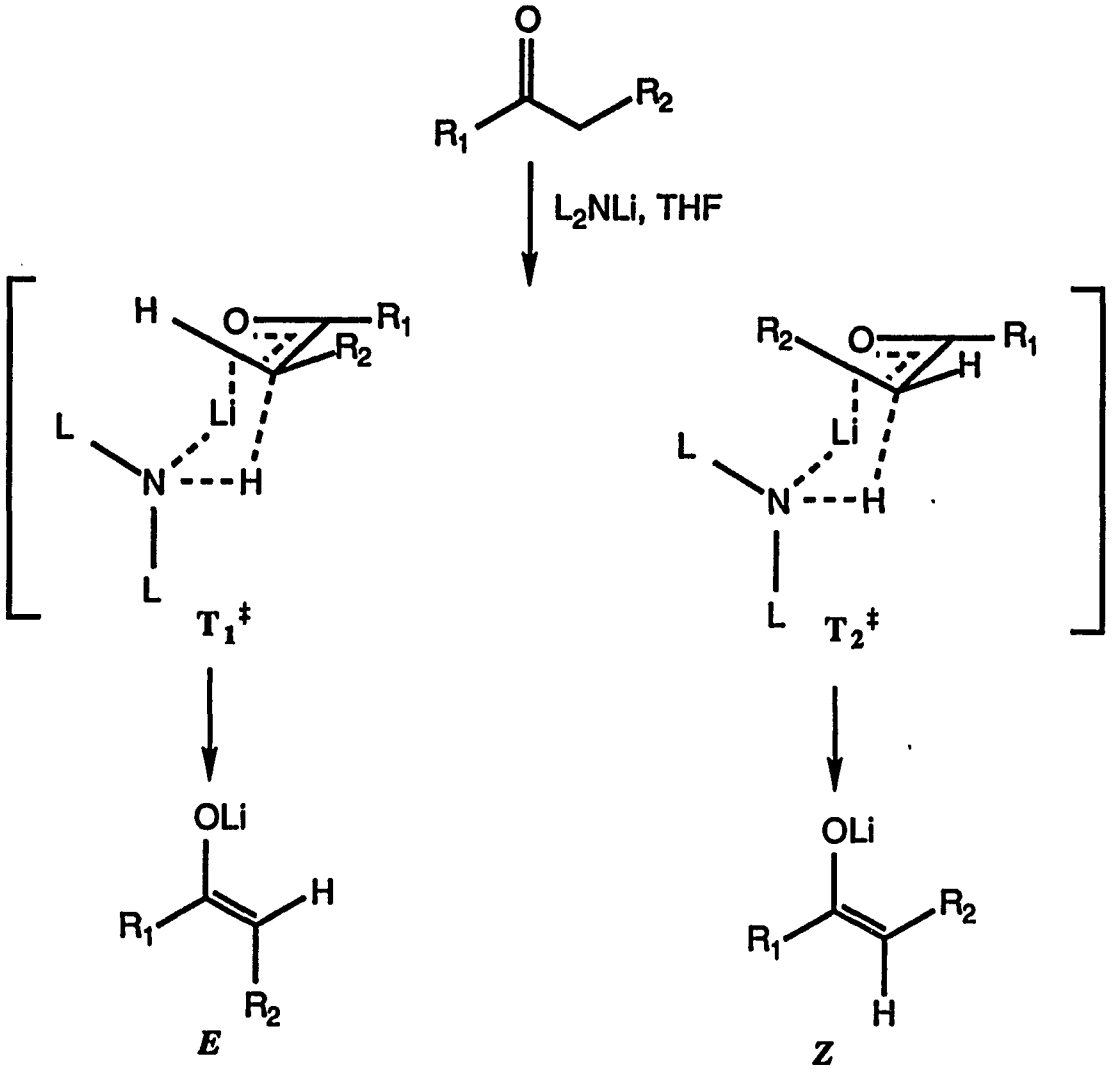


states, $T_1‡$ and $T_2‡$, to give the corresponding *E* and *Z* enolates, respectively (Scheme 10, page 68).[45] When the R_2 group is large, R_1 ⟷ R_2 nonbonded interactions should disfavor transition state $T_1‡$, and tend to give the *Z* geometry, whereas dominant R_2 ⟷ L nonbonded interactions should disfavor transition state $T_2‡$ and tend to afford the *E*-enolate geometry. The experimental results proved that under conditions of "apparent" kinetic control, esters afforded largely *E* enolates (transition state $T_1‡$).[41,45]

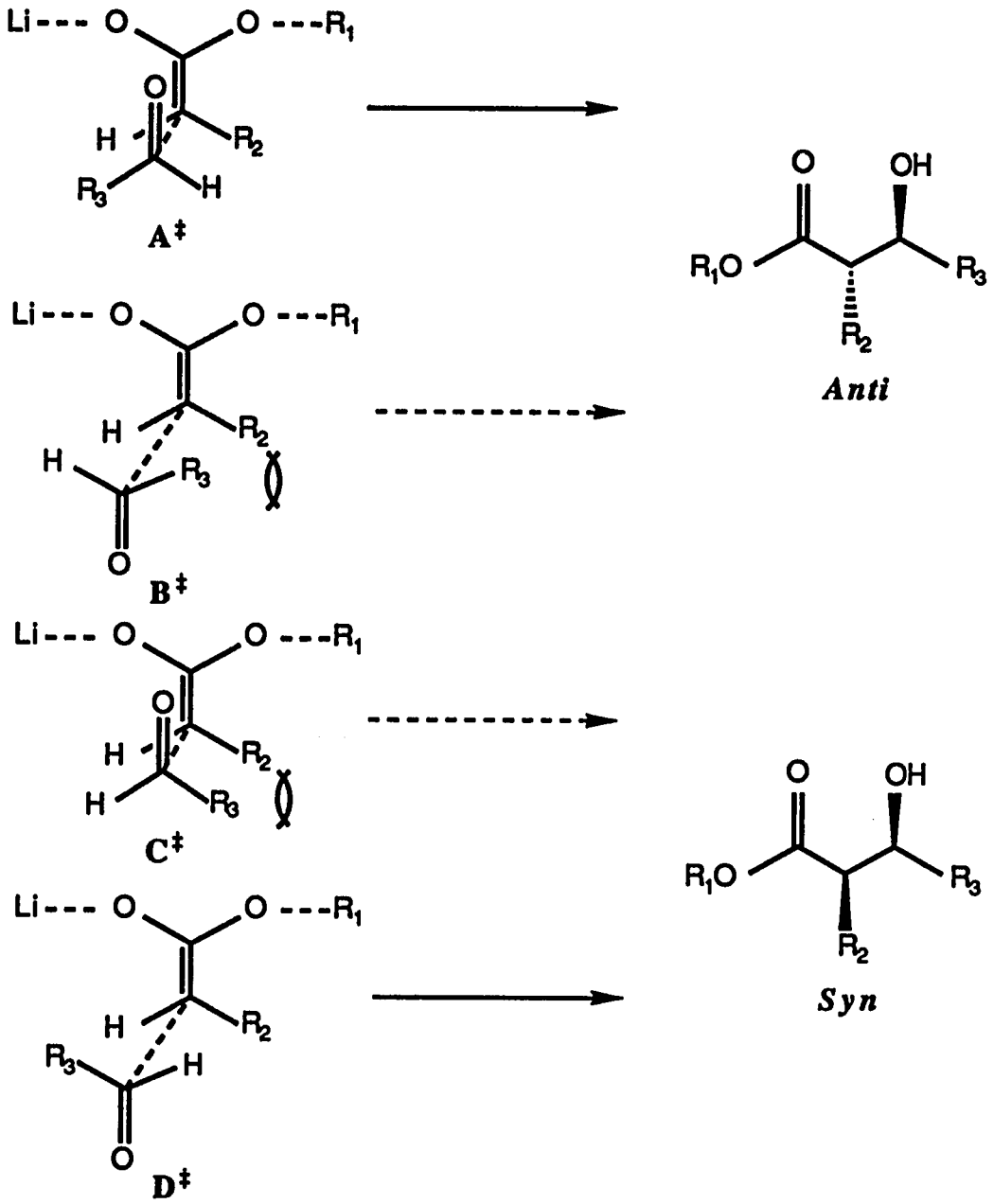
Several nonchelated, or "open," transition states have been considered. In a study of the addition of ketones, carboxylic acids and esters to aldehydes, the favored *E* enolate attacks the aldehyde to give the four transition state geometries illustrated in Scheme 11 (page 69).[58-60] Based on the steric requirements of enolate and aldehyde substituents chosen (for large R_3), transition states **B**‡ and **C**‡ which have gauche and eclipsed substituents were excluded. It was concluded by Mulzer et al. that the observed high levels of *anti* diastereoselection imply that there is an intrinsic preference for the *syn*-carbonyl-enolate transition state orientation (e.g., **A**‡ or **C**‡), with transition state **A** being preferred on steric grounds.[61] Mulzer *et al.* suggested that the highest occupied and the lowest unoccupied molecular orbital interactions may be responsible for this preference.[61]

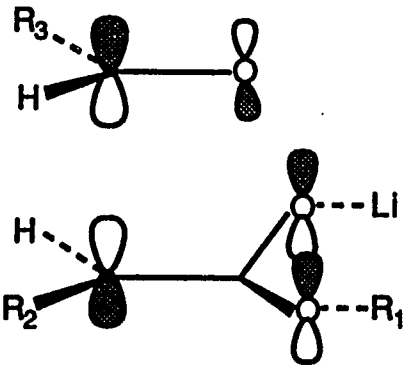
The enolization (LDA, THF) and condensation of α-amino ester **60** (eq. 3, page 70) under kinetic conditions (-78 °C, 5-10 min) afforded low levels of kinetic aldol diastereoselection.[42] From the preceding discussion it is probable that the major enolate derived from **60** possessed the *E* geometry.[62] The experimental result shows no selectivity with acetaldehyde and low *threo* selectivity with benzaldehyde (when $R_3 = CH_3$, the ratio of **61** to **62** is 50:50; when R = Ph, the ratio of **61** to **62** is 75:25). In complementary studies, the condensation of amide **63** with acetaldehyde and benzaldehyde

Scheme 10. Pericyclic transition states for deprotonation

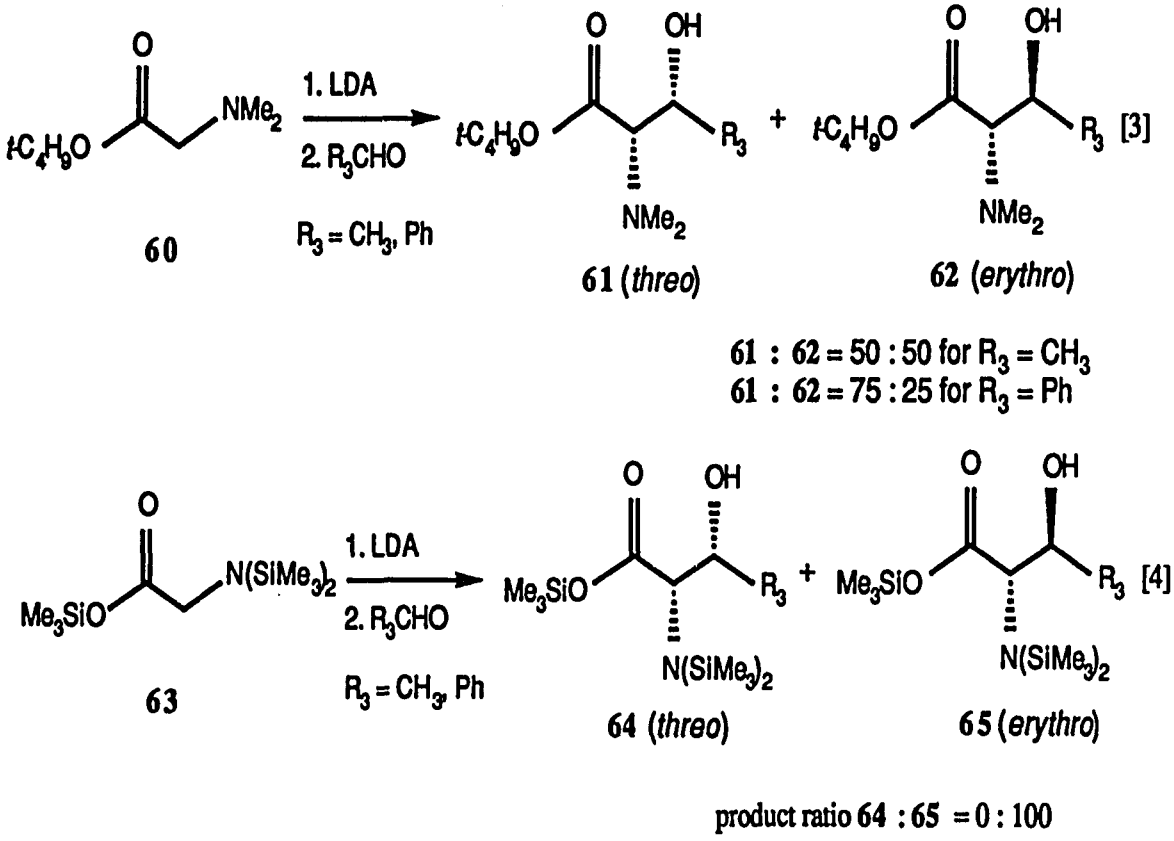


Scheme 11. Open transition states for the aldol addition reaction



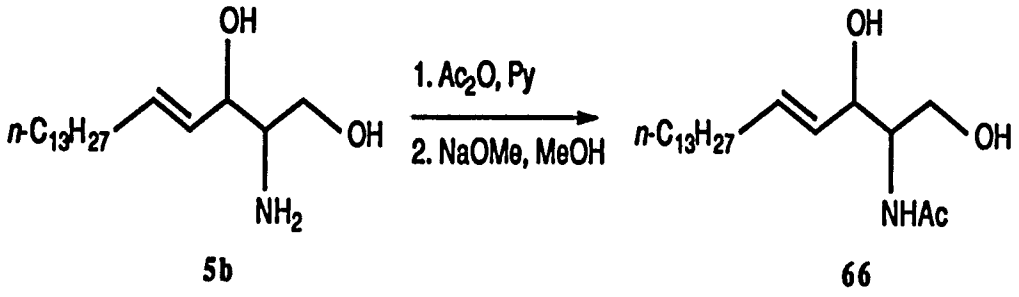


(eq. 4, page 70)[47] was carried out under conditions of *apparent* thermodynamic control (-78 °C, 1.5 h), and an excellent level of diastereoselectivity was noted, i.e. only *erythro* aldol product was obtained.[47] Related condensations have been reported by others.[63]

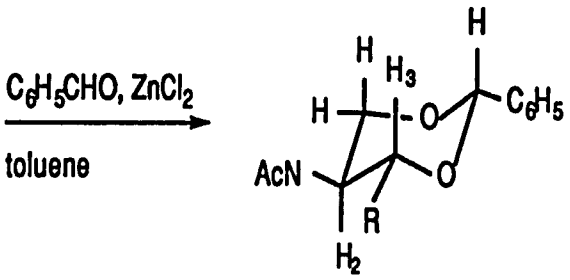


In 1982, Schmidt and Kläger reported that *erythro*-sphingosine was obtained in the aldol condensation of tris(trimethylsilyl)glycine with (*E*)-hexadec-2-enal.[27] Their method of analyzing the ratio of *erythro*- to *threo*-sphingosine involved formation of triacetyl derivatives, followed by base-catalyzed hydrolysis of the *O*-acetyl groups to give *N*-acetylsphingosine **66** and subsequent reaction with benzaldehyde to afford the 1,3-dioxalane derivative[56] **67** (Scheme 12, page 71). The *trans* arrangement of H^2 and H^3, which is expected for the *erythro* configuration, is demonstrated in the

Scheme 12. Preparation of *N*-acetylsphingosyl-1,3-dioxalane derivative 67



5b 66



67 $J_{H_2\text{-}H_3} = 8.9$ Hz

R = *n*-$C_{13}H_{27}CH{=}CH$

The *trans* arrangement of H_2 and H_3 as is expected for the *erythro* configuration is indicated in the ^1H NMR spectrum by the typical constant 8.9 Hz for such protons.

^1H NMR spectrum by the typical coupling constant of 8.9 Hz for such protons.[21] Our HPLC results (Figure 1) also show that the reaction of (*E*)-hexadec-2-enal with tris(trimethylsilyl)glycine in the presence of LDA/THF is highly diastereoselective; 98% of the sphingosine has the *erythro* configuration (Scheme 1). In addition, we found that the ratio of D- to L-*erythro*-sphingosine is 1:1 (Figure 2), as expected since glycine is achiral. However, when bis(trimethylsilyl)sarcosine reacted with (*E*)-hexadec-2-enal under the same conditions, the ratio of *erythro* to *threo* is 63:37 (Scheme 5). The reactions have different diastereoselectivity because the methyl group in bis(trimethylsilyl)sarcosine is much smaller than the corresponding trimethylsilyl group in tris(trimethylsilyl)glycine. According to the Schemes 8-10, a small $\mathbf{R_2}$ group would form both *E* and *Z* enolates, with the *E* enolate favored. When the $\mathbf{R_2}$ group is very bulky as is trimethylsilyl, formation of the *Z* enolate becomes impossible, and only the *E* enolate is formed, leading to *anti* product. Thus the aldol condensation with bis(trimethylsilyl)sarcosine leads to substantial amounts of both *erythro* and *threo* products, whereas the reaction with tris(trimethylsilyl)glycine affords 98% of the *erythro* isomer. NMR[27] and HPLC analyses of suitably derivatized sphingosines show that the aldol condensation outlined in Scheme 1 is highly diastereoselective and leads to a product that has the same *erythro* stereochemistry as in the naturally derived D-*erythro*-sphingosine.

Influence of the hydroxy group of sphingomyelin on the state of cholesterol exchange between vesicles. Kinetic studies of radiolabeled cholesterol exchange between membranes have shown that cholesterol molecules exchange more slowly in membranes containing SPM than in those lacking SPM.[1,3-5] This observation indicates that the lipid-water interfacial region of SPM may be more tightly packed in cholesterol-containing membranes than the corresponding region of glycerophospholipids. The region between the hydrophilic surface and the hydrocarbon interior of sphingomyelin consists of the allylic hydroxy group at C-3 and the N-H and the carbonyl group of the ceramide amide group attached to C-2 of sphingosine. In contrast, this region in PC consists of the oxygen atoms of the ester (or ether) linkages and of the glycerol backbone. Since the hydroxy group and amide N-H group of SPM can act as both donors and acceptors of hydrogen bonds, but no hydrogen-donating group is present in PC, it is apparent that the interfacial region of SPM has greater opportunity for hydrogen bonding. In a binary mixture showing phase separation, differential scanning calorimetry studies indicated that SPM, either isolated from erythrocytes or with a chemically defined (*N*-palmitoyl) chain, interacted with cholesterol to a greater degree than did various synthetic phosphatidylcholines.[2] Fluorescence polarization measurements of phospholipid-cholesterol bilayers also indicated a higher degree of structural order in membranes from egg SPM than from various synthetic phosphatidylcholines.[9] Studies of the surface pressure-molecular area isotherms of mixed monolayers of cholesterol and SPM revealed that cholesterol has the capacity to condense bovine brain SPM to a greater extent than phosphatidylcholines.[10,11] The resistance of oxidation of cholesterol by cholesterol oxidase in monolayers is also related to strong SPM/cholesterol

interactions.[11] Hence, there is evidence that the interaction of cholesterol with various naturally occurring (egg or bovine) or synthetic SPM is greater than that with egg PC or DPPC in bilayer membranes. In order to investigate whether the hydroxy group at C-3 of SPM plays an important role in the interaction of SPM with cholesterol, the SPM analogs in which the 3-hydroxy group of SPM was substituted by hydrogen (**26**), methoxy (**16b**), ethoxy (**17b**), and tetrahydropyranyloxy (**57**) were inserted into bilayers or monolayers and used in biophysical studies of SPM-cholesterol interactions.

To investigate the role of the hydroxy group at the 3 position of SPM in the interaction between SPM and cholesterol, the rate of [4-^{14}C]cholesterol exchange between unilamellar vesicles prepared with *N*-stearoyl-SPM was compared with the rate obtained using synthetic analogs in which the hydroxy group of *N*-stearoyl-SPM is replaced with an *O*-alkyl group or with hydrogen.[15,64] The half-times of cholesterol exchange from vesicles containing *O*-methyl- or deoxy-*N*-stearoyl-SPM and 10 mol % of cholesterol at 50 °C were only slightly faster (a factor of only 1.5) than that found using vesicles containing *N*-stearoyl-SPM and 10 mol % cholesterol. Our results show that the 3-hydroxy group of SPM is not an additional site involved in the interaction between these lipids, since vesicles from our deoxy (**26**) and *O*-methyl (**16b**) analogs of SPM gave the same rates of [4-^{14}C]cholesterol desorption. The rate of cholesterol desorption from vesicles could be accelerated by preparing vesicles from bulky *O*-alkyl analogs of SPM. Vesicles containing 3-*O*-ethyl-*N*-stearoyl-SPM (**17b**) and 3-*O*-THP-egg SPM (**57**) gave rate enhancements of ~14 and 35, compared with the rates observed in vesicles made from *N*-stearoyl- and egg SPM, respectively. These data suggest that insertion of sterically bulky groups at the 3 position of SPM (such as ethoxy and tetrahydropyranyloxy) in place of hydroxy interfere

markedly with the molecular packing of cholesterol and SPM in bilayer membranes, as estimated by the rate of cholesterol movement between membranes. However, the hydroxy group of SPM is not critical for the strong interaction of cholesterol with SPM.

The ability of cholesterol to condense monolayers of SPMs was measured in the laboratory of Dr. J. Peter Slotte, Åbo Akademi University, Turku, Finland. 3-*O*-Methyl-*N*-stearoyl-SPM (**16b**) is condensed by cholesterol to the same extent as *N*-stearoyl-SPM (**10b**); 3-deoxy-*N*-stearoyl-SPM (**26**) is also condensed by cholesterol to a similar extent as the methoxy and hydroxy derivatives. The activity of cholesterol oxidase was also measured in Dr. Slotte's laboratory with cholesterol/SPM monolayers at 0.5 mol fraction, 25 °C, and 15 mN/m. It was found that the enzyme activity is similar in monolayers containing hydroxy- and methoxy-SPM. The oxidation rate was also low in monolayers containing 3-deoxy-SPM (**26**) (0.08% cholesterol oxidized per s) compared with monolayers containing 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphocholine (0.25% cholesterol oxidized per s). These results indicate that replacing the hydroxy group at C3 of SPM by hydrogen or methoxy does not cause a dramatic difference in molecular packing with cholesterol.[65]

Comparison of reagents for conversion of ceramides to sphingomyelins. Three different methods for phosphorylation or phosphitylation of ceramides have been presented in this thesis for the synthesis of sphingomyelin analogs (Scheme 13). These methods are based on those used in nucleotide and glycerolipid chemistry. First, we used 2-chloro-2-oxo-1,3,2-dioxaphospholane.[31] This reagent gives good yields of the cyclic phosphate ester when it reacts with ceramide (Scheme 13A, page

79). When the ceramide has an amide chain length of less than 18 carbons (**47, 51, 55**), the ring-opening step with trimethylamine was very successful; but if the amide chain length is longer than 18 carbons, the ring-opening reaction did not take place. The reason for this is probably caused by the low solubility of the cyclic phosphate in acetonitrile. When the amide chain contains more than 18 carbons (**47, 51, 55**), the cyclic phosphate did not dissolve in refluxing acetonitrile (82 °C). The ring-opening reaction was carried out in a sealed pressure bottle in the presence of trimethylamine. Heterogeneous reactions involving gas and solid phases take place much more slowly than those involving gas and liquid phases. In order to increase the solubility of the intermediate cyclic phosphate, we used propionitrile instead of acetonitrile. Although the intermediate cyclic phosphate dissolved in this solvent before reaching its boiling point, again the reaction was not successful. Since the ring-opening reaction is a S_N2 reaction, a polar solvent is needed. Propionitrile is possibly a good solvent for S_N2 reactions (dielectric constant, 27); thus the low yields obtained with the long-chain ceramides may reflect steric hindrance caused by chain folding. Steric hindrance was proposed to account for the low yields obtained in the alkylation of 1-*O*-benzyl-*sn*-glycerol 3-tosylate by long-chain alkyl triflates.[66]

The conversion of ceramides (**9c, 13b, 13c, 14b, 14c, 47, 51, 55**) into SPMs (**10c, 16b,16c, 17b, 17c, 48, 52, 56**) was carried out by phosphorylation of the primary hydroxy group of ceramides with 2-bromoethylphosphoric acid dichloride in the presence of triethylamine followed by hydrolysis of the remaining chloride at the phosphorus atom and trimethylamine replacement of the bromoethyl phosphodiester derivatives. (Scheme 13B, page 80). After phosphorylation of ceramides and hydrolysis of the remaining P-Cl bond, the bromoethyl phosphodiester derivatives are

aminated with trimethylamine in the presence of a mixture of solvents ($CHCl_3$-*i*-PrOH-CH_3CN, 3:5:5). Bromoethyl phosphodiester derivatives dissolved well in this solvent system, and the S_N2 reaction with trimethylamine in water was complete after stirring for 48 h at room temperature.

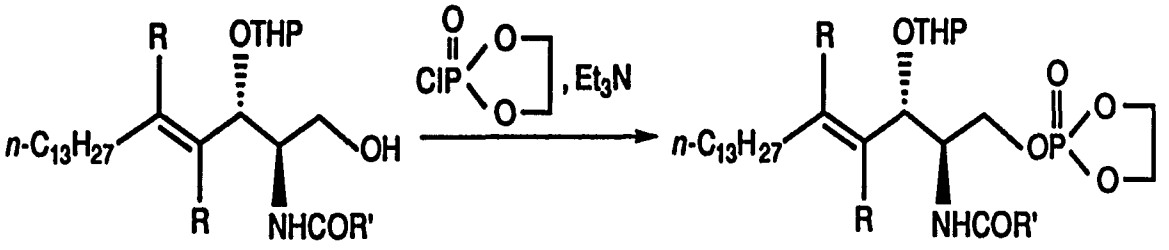
Third, we used *N,N*-diisopropylmethylphosphoramidic chloride (Scheme 13C, page 81). This reagent was used for SPM synthesis by Bruzik.[21] Ceramides (**33, 38, 43**) were treated with *N,N*-diisopropylmethylphosphoramidic chloride in the presence of triethylamine in chloroform. The resulting phosphoramidites were treated with a mixture of choline tosylate and 1*H*-tetrazole in acetonitrile-THF (1:1). The phosphites were oxidized with *tert*-butyl hydroperoxide in THF to give the corresponding phosphates. The desired phosphodiesters were obtained by demethylation of the triesters with anhydrous trimethylamine in toluene. The syntheses of SPMs (**34, 39, 44**) were carried out in an one-pot procedure without isolation of the intermediate compounds. Phosphitylation is a more reactive process than phosphorylation, and TLC analysis (hexane-ethyl acetate, 1:1) indicated that for all the ceramides shown in Scheme 13C, phosphitylation reactions were finished in 5-10 min.

A comparison of the yields obtained by using the three phosphorylation or phosphitylation reagents in conversion of ceramides to sphingomyelins indicates that all give about 40% yields of SPMs based on corresponding ceramides. 2-Chloro-2-oxo-1,3,2-dioxaphospholane gives poor yields when used in reactions with the longer chain ceramides (**47, 51, 55**). The mixed solvent system used in the 2-bromoethylphosphoric acid dichloride reaction overcomes the solubility problem encountered when acetonitrile is used as the solvent in the reaction involving 2-chloro-2-oxo-1,3,2-dioxaphospholane.

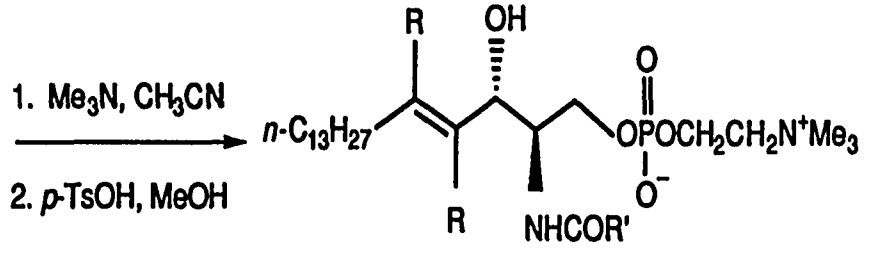
Phosphitylation by using *N,N*-diisopropylmethylphosphoramidic chloride is the best of the three reagents we used for conversion of ceramides to SPMs. Although *N,N*-diisopropylmethylphosphoramidic chloride gives SPMs in similar yields as did the two phosphorylation reagents, it has the advantage that the four steps can be carried out in an one-pot procedure without separation.

Scheme 13. Phosphorylation and phosphitylation reagents used in conversion of ceramides to sphingomyelins

A. 2-Chloro-2-oxo-1,3,2-dioxaphospholane:



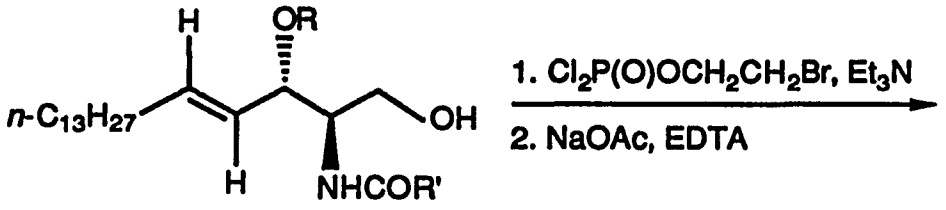
9a, R = D, R' = *n*-$C_{17}H_{35}$
9b, R = H, R' = *n*-$C_{17}H_{35}$
47, R = H, R' = *n*-$C_{21}H_{43}$
51, R = H, R' = *n*-$C_{23}H_{47}$
55, R = H, R' = $C_{23}H_{45}$



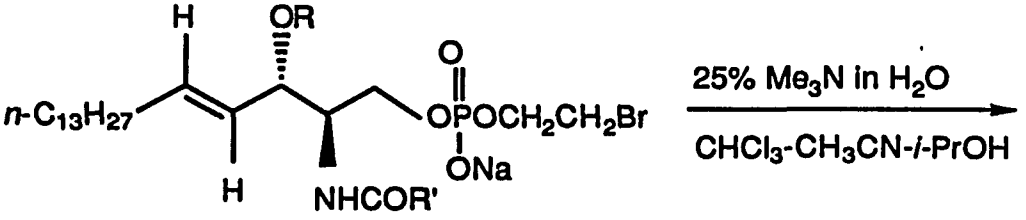
10a, R = D, R' = *n*-$C_{17}H_{35}$ (66%)
10b, R = H, R' = *n*-$C_{17}H_{35}$ (71%)
48, R = H, R' = *n*-$C_{21}H_{43}$ (no reaction)
52, R = H, R' = *n*-$C_{23}H_{47}$ (no reaction)
56, R = H, R' = $C_{23}H_{45}$ (no reaction)

Scheme 13. (Continued)

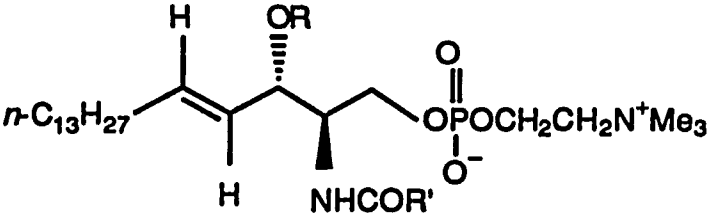
B. 2-Bromoethylphosphoric acid dichloride:



9c, ^{15}N, R = H, R' = *n*-$C_{17}H_{35}$
13b, ^{14}N, R = Me, R' = *n*-$C_{17}H_{35}$
13c, ^{15}N, R = Me, R' = *n*-$C_{17}H_{35}$
14b, ^{14}N, R = Et, R' = *n*-$C_{17}H_{35}$
14c, ^{15}N, R = Et, R' = *n*-$C_{17}H_{35}$
47, R = H, R' = *n*-$C_{21}H_{43}$
51, R = H, R' = *n*-$C_{23}H_{47}$
55, R = H, R' = $C_{23}H_{45}$



50-57%

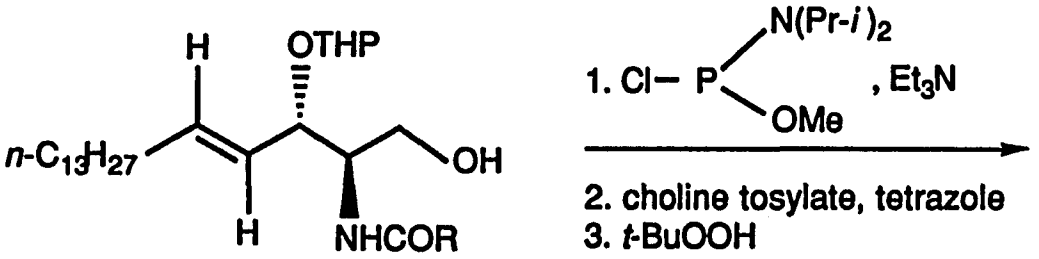


10c, ^{15}N, R = H, R' = *n*-$C_{17}H_{35}$ (71%, 41% based on **9c**)
16b, ^{14}N, R = Me, R' = *n*-$C_{17}H_{35}$ (36% based on **13b**)
16c, ^{15}N, R = Me, R' = *n*-$C_{17}H_{35}$ (43% based on **13c**)
17b, ^{14}N, R = Et, R' = *n*-$C_{17}H_{35}$ (41% based on **14b**)
17c, ^{15}N, R = Et, R' = *n*-$C_{17}H_{35}$ (39% based on **14c**)
48, R = H, R' = *n*-$C_{21}H_{43}$ (43% based on **47**)
52, R = H, R' = *n*-$C_{23}H_{47}$ (46% based on **51**)
56, R = H, R' = $C_{23}H_{45}$ (48% based on **55**)

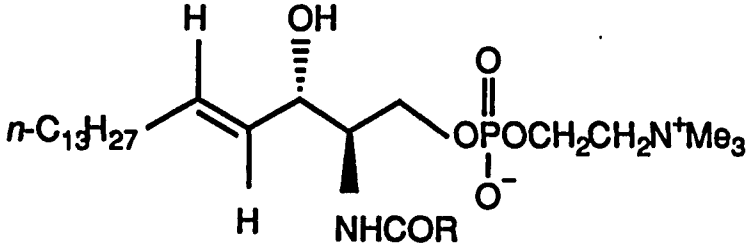
This phosphorylation and amination procedure gives DL-3-deoxysphingomyelin in 36% yield (**26**).

Scheme 13. (Continued)

C. *N,N*-Diisopropylmethylphosphoramidic chloride:



33, R = $CH(OTHP)C_{14}H_{29}$-*n*
38, R = *n*-$C_{13}H_{27}$
43, R = *n*-$C_{11}H_{23}$



35, R = $CH(OH)C_{14}H_{29}$-*n* (43%)
39, R = *n*-$C_{13}H_{27}$ (42%)
44, R = *n*-$C_{11}H_{23}$ (41%)

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