Pergamon

Tetrahedron: *Asymmetry* 10 (1999) 1697–1707

TETRAHEDRON: *ASYMMETRY*

A new approach to the stereoselective total synthesis of isotopically labeled D-*ribo*-phytosphingosine

Shengrong Li, Jihai Pang, William K. Wilson * and George J. Schroepfer Jr. †

Departments of Biochemistry and Cell Biology and of Chemistry, Rice University, 6100 Main Street, Houston, Texas 77005-1892, USA

Received 14 March 1999; accepted 19 April 1999

Abstract

We describe a novel stereoselective total synthesis of D-*ribo*-[1,1-^{2}H-1,2-^{13}C]phytosphingosine (**12**). Chirality at the incipient C-4 position was derived from asymmetric dihydroxylation of 1-hexadecene. The remaining chiral centers were formed by Sharpless epoxidation of an allylic alcohol, followed by benzoylcarbamate cyclization to furnish a 2-amino-1,3,4-triol derivative with the desired stereochemistry. The ^{2}H and ^{13}C labels were introduced by Horner–Emmons condensation of ^{13}C-labeled triethyl phosphonoacetate, followed by reduction of the resulting carboxylic ester with $AlCl_3$/$LiAlD_4$. Mass spectral results indicated the suitability of **12** as an internal standard for analyses by the isotope dilution method. © 1999 Elsevier Science Ltd. All rights reserved.

1. Introduction

Sphingolipids are important structural constituents of eukaryotic cell membranes. Complex sphingolipids and their metabolites also have diverse roles as receptors and as second messengers in the regulation of a broad range of cellular processes, including growth and apoptosis.[1–3] Among the metabolites showing potent biological activities are the simple long-chain bases, such as sphingosine, dihydrosphingosine, and phytosphingosine. Assessing the physiological relevance of various in vitro activities of the long-chain bases requires the reliable determination of their normal range of concentration in tissues of healthy and diseased subjects. However, the most common approaches[4–7] for measuring levels of the long-chain bases provide little or no structural evidence for the authenticity of the analyte and consequently are susceptible to significant errors. More dependable measurements can be expected from LC–MS[8] or GC–MS analysis in conjunction with mass fragmentography and the isotope-dilution technique,[9] which involves quantitation against an isotopically labeled internal standard.

* Corresponding author. Tel: +1 713-527-4865; fax: +1 713-285-5154; e-mail: billw@bioc.rice.edu

† Deceased 11 December 1998.

0957-4166/99/$ - see front matter © 1999 Elsevier Science Ltd. All rights reserved.
PII: S0957-4166(99)00161-5

We have recently described the synthesis of [1,1,3-^{2}H]D-*erythro*-sphingosine[10] as an internal standard for isotope-dilution GC–MS analysis, and minor synthetic modifications would also provide [1,1,3-^{2}H]D-*erythro*-dihydrosphingosine. In designing a similar isotopomer of phytosphingosine, we first considered the mass spectral fragmentation of its volatile tetra-TMS derivative, which is readily formed in a single step and produces distinctive fragment ions under electron-impact conditions. Apart from the $(CH_3)_3Si$ ion (*m/z* 73), the two most abundant ions (*m/z* 132 and 204) contain C-1, C-2, and the C-1 hydrogens. For isotope-dilution analyses, it would be desirable to place isotopic labels (^{13}C and ^{2}H) at each of these positions to furnish an isotopomer of 4 added mass units.

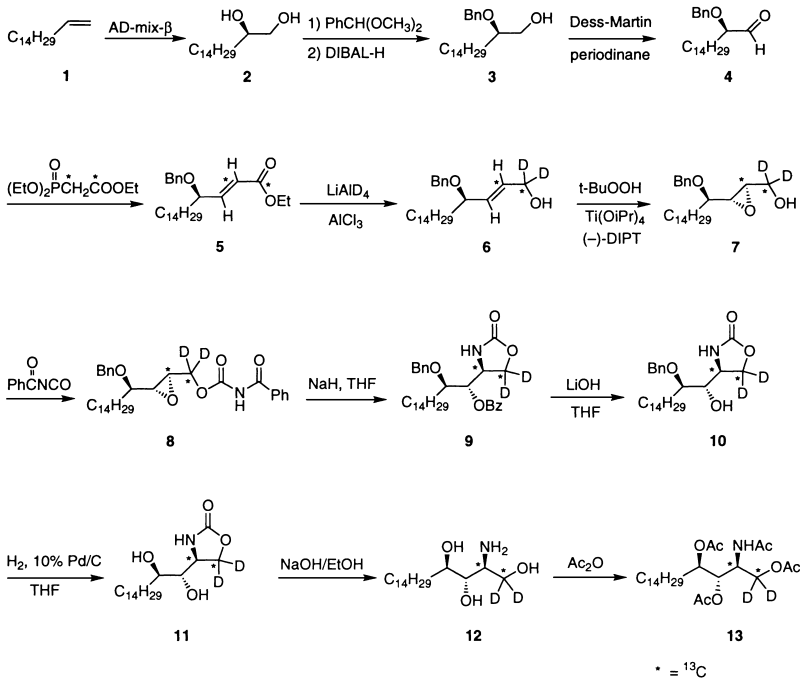
In designing a route to [1,1-^{2}H-1,2-^{13}C]-C_{18}-phytosphingosine, we investigated the possibility of adapting an existing synthesis for the introduction of the isotopic labels. In most syntheses of phytosphingosine, the stereochemistry at the critical C-2 position is derived from sugars,[11–16] L-serine,[17–20] sphingosine,[21,22] or other natural materials[23–25] that are either costly or unavailable with ^{13}C labels. Other reported syntheses[26–28] are also unsuitable for introducing the isotopic labels at the appropriate positions. This situation prompted us to develop a new total synthesis of D-*ribo*-phytosphingosine. Our synthesis uses relatively inexpensive ^{13}C and ^{2}H reagents to provide a facile introduction of ^{13}C labels at C-1 and C-2 and ^{2}H labels at C-1. Furthermore, the final product is obtained in good overall yield and shows high stereochemical homogeneity.

2. Results and discussion

Our synthesis of D-*ribo*-C_{18}-phytosphingosine began with the asymmetric dihydroxylation[29] of 1-hexadecene with AD-mix-β. A 9:1 mixture of 2*R* and 2*S* diols (**2**) was obtained, a respectable enantioselectivity for the dihydroxylation of a terminal olefin in view of similar results for 1-heptene.[30] Purification of the 9:1 mixture was delayed until the introduction of the remaining two chiral centers. By use of standard methodology,[31] the 1,2-diol **2** was selectively protected as the 2-benzyl ether **3**, followed by mild oxidation to aldehyde **4** with Dess–Martin periodinane (Scheme 1).[32] The remaining two carbons of the phytosphingosine chain were introduced by Horner–Emmons condensation of aldehyde **4** with labeled triethyl phosphonoacetate, a reagent that is readily available at reasonable cost with ^{13}C labels at the incipient C-1 and C-2 carbons. Reduction of the resulting α,β-unsaturated ester **5** with $AlCl_3/LiAlD_4$ gave the allylic alcohol **6** and completed the introduction of the isotopic labels.

The two remaining chiral centers were introduced by Sharpless epoxidation of **6**. Owing to the presence of the minor (10%) enantiomer of **6**, epoxide **7** was obtained as a 9:1 mixture of C-4 epimers, which were not readily separable on silica gel. Following an elegant strategy for the stereospecific transformation of epoxy alcohols into amino alcohols,[33] we converted **7** into its benzoylcarbamate derivative **8**, which was cyclized with NaH to furnish a 2:3:2 mixture of starting material (**8**), oxazolidinone **9**, and the debenzoylated oxazolidinone **10**. The benzoylcarbamate cyclization involved backside attack by the amido nitrogen anion on C-2 of the epoxide, accompanied in part by the anticipated[33] transfer of the benzoyl group to the C-3 oxygen, to produce the desired stereochemistry for D-*ribo*-phytosphingosine. MPLC purification furnished homogenous samples of **9** and **10**, free of the minor C-4 epimers derived ultimately from incomplete enantioselectivity in the dihydroxylation of **1**.

Stepwise removal of the protecting groups afforded D-*ribo*-phytosphingosine, which showed a single component by NMR as the free base **12** and its tetraacetate **13**. After consideration of the effects of the isotopic labels, NMR spectra of **12** and **13** were essentially superimposable with those[34] of the unlabeled analogs. Comparison of the ^{1}H and ^{13}C NMR spectra for **13** with detailed NMR data reported for the four



Scheme 1.

phytosphingosine tetraacetate diastereomers[35] also confirmed the stereochemistry as *ribo* and indicated the absence of the *lyxo*, *arabino*, and *xylo* isomers at a detection limit of 0.5%.

The locations of the isotopic labels were confirmed by NMR. The ^{2}H NMR spectrum of tetraacetate **13** showed two signals with chemical shifts matching those of the C-1 protons of unlabeled **13**.[34] In the ^{13}C NMR spectrum of **13**, intense signals for C-1 and C-2 and a weak doublet for C-3 showed the anticipated shieldings and coupling patterns for the 1,1-^{2}H-1,2-^{13}C isotopic substitution. The ^{1}H NMR spectrum showed signals for H-2, H-3, and H-4 with the expected chemical shifts and coupling constants (Fig. 1). Essentially no signal was observed for the C-1 protons, and a very minor signal corresponding to H-2 bonded to ^{12}C indicated 99% ^{13}C at C-2. These high isotopic purities were confirmed by the FAB mass spectrum of **12**, which showed *m/z* 322 (M+H) as the base peak and 0.1–0.9% relative abundances for *m/z* 318–321. The EI mass spectra of tetraacetate **13** and the tetra-TMS derivative of **12** were essentially identical to those of their unlabeled counterparts except for shifts in mass of certain ions due to isotopic substitution. These shifts supported the ion assignments shown in Fig. 2 for the tetra-TMS derivative of D-*ribo*-phytosphingosine and confirmed that the abundant ions at *m/z* 132 and 204 are suitable for isotope-dilution GC–MS analyses.

The present synthesis of phytosphingosine is unique in furnishing isotopic labels at C-1, C-2, and the C-1 protons. We have demonstrated that these labels produce the desired mass shifts in the EI mass spectrum. Consequently, the [1,1-^{2}H-1,2-^{13}C]phytosphingosine should be valuable as an internal standard for measuring levels of phytosphingosine in biological matrices and to compensate for losses of this labile sphingolipid base during sample processing. Another feature of our synthesis of **12** is its comparability with other total syntheses of unlabeled phytosphingosine[26–28] in terms of diastereo- and enantioselectivity, length, and overall yield (9% from 1-hexadecene).

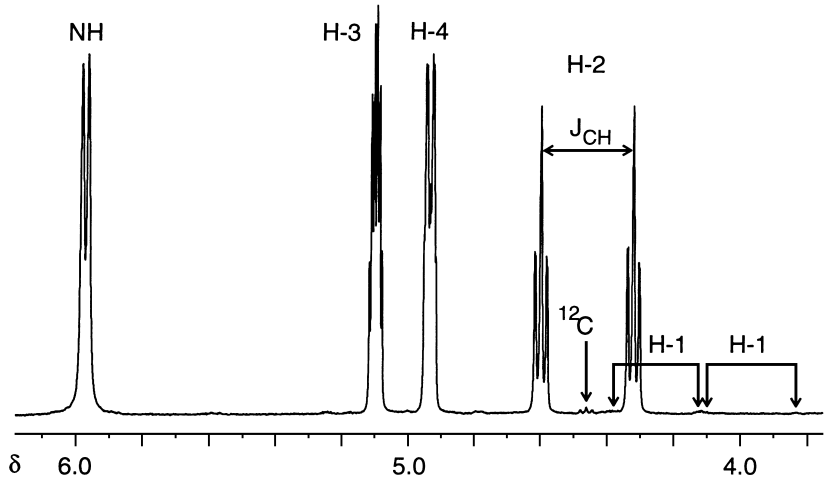


Figure 1. ^{1}H NMR spectrum of D-*ribo*-[1,1-^{2}H-1,2-^{13}C]phytosphingosine tetraacetate (**13**). Integration of the triplets at δ 4.32 and 4.60 relative to the low-intensity triplet at δ 4.46 indicated a 99:1 ratio of ^{13}C:^{12}C isotopes at C-2. A similarly high incorporation of ^{13}C and ^{2}H labels at C-1 is indicated by the absence of signals corresponding to the chemical shifts for the C-1 protons (δ 4.01 and 4.29) or their ^{13}C satellites (indicated by arrows in the figure)

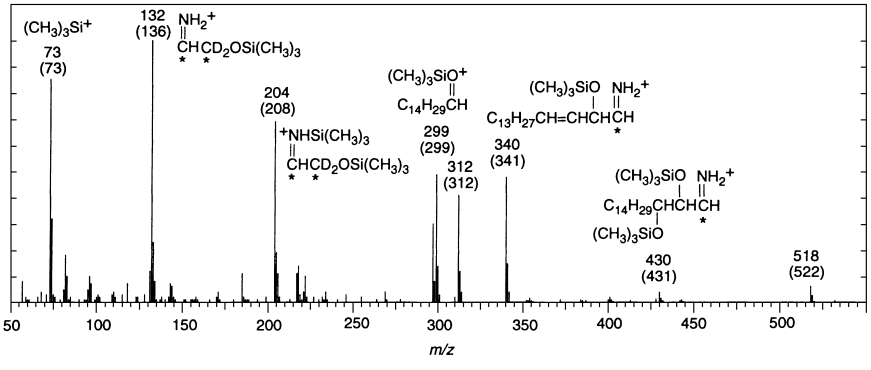


Figure 2. Electron-impact mass spectrum of the tetra-TMS derivative of D-*ribo*-phytosphingosine (FW 605). Masses of corresponding ions observed in the spectrum of the 1,1-^{2}H-1,2-^{13}C labeled derivative are given in parentheses. Suggested assignments are presented for selected ions; ^{13}C labeled atoms are indicated by an asterisk (*). No significant ions were observed above *m/z* 519

3. Experimental

3.1. Materials and methods

Melting points (mps) were measured with a Thomas–Hoover apparatus in sealed, evacuated capillary tubes. IR spectra were obtained from KBr pellets on a Mattson Galaxy 6020 Fourier-transform infrared spectrometer. Optical rotations were measured on a JASCO DIP-4 digital polarimeter. TLC was carried out on aluminum-backed, silica gel 60 F_{254} plates (EM Science, Gibbstown, NJ). Components of the plates were charred by spraying with 5% ammonium molybdate in 10% sulfuric acid followed by heating. MPLC was done on glass columns dry-packed with silica gel (230–400 mesh; EM Science); fraction volumes were 20 mL. Direct-inlet electron-impact (EI) mass spectra and fast atom bombardment (FAB, positive ion) mass spectra were acquired with a ZAB-HF reverse-geometry double-focusing instrument in the positive-ion mode and are reported as *m/z* (relative abundance, suggested assignment). NMR

spectra were measured on a Bruker AMX500 instrument (500 MHz for ^{1}H) at 25°C and referenced to tetramethylsilane or to CD_3OD at 3.30 ppm (^{1}H) or $CDCl_3$ at 77.0 ppm (^{13}C). Signal assignments were made from HSQC, COSY-DQF, and 1D spectra as described previously.[36] Identified ^{13}C–^{1}H couplings in ^{1}H spectra and ^{13}C signals corresponding to ^{13}C-labeled positions are denoted by ‡, and multiplets with line positions distorted by second-order effects at 500 MHz are designated with §. NMR data and stereochemistry of carbamates **8**–**11** are described using the usual carbon numbering for the phytosphingosine backbone.

1-Hexadecene, AD-mix-β [$K_3Fe(CN)_6$, K_2CO_3, $K_2OsO_4 \cdot 2H_2O$, hydroquinidine 1,4-phthalazinediyl diether ($(DHQ)_2PHAL$)], diisobutylaluminum hydride (DIBAL), (*S*)-α-methoxy-α-(trifluoromethyl)phenylacetyl chloride (98% ee), silver cyanate, (−)-diisopropyl-D-tartrate (DIPT), titanium tetraisopropoxide, *tert*-butyl hydroperoxide, 10% palladium on carbon, lithium hydroxide, and triethyl phosphonoacetate-$^{13}C_2$ ($(Et_2O)_2P(O)^{13}CH_2{}^{13}CO_2Et$) were purchased from Aldrich Chemical Co. (Milwaukee, WI), Dess–Martin periodinane and benzaldehyde dimethyl acetal from Lancaster Synthesis Inc. (Windham, NH), and lithium aluminum deuteride (99% D) from Isotech (Miamisburg, OH). α-Methoxy-α-(trifluoromethyl)phenylacetate (MTPA) derivatives were prepared by reaction of the alcohol (2 mg) with (*S*)-α-methoxy-α-(trifluoromethyl)phenylacetyl chloride (30 μL) in pyridine (100 μL) as described previously.[37]

3.2. (2R)-Hexadecane-1,2-diol (2)

To a solution of 1-hexadecene (2.0 g, 8.1 mmol, 92% purity) in *tert*-butanol:water (1:1, 72 mL) was added AD-mix-β (10 g) at 0°C. The resulting solution was stirred at 4°C for 40 h, followed by addition of solid sodium sulfite (9 g). The solution was warmed to room temperature, stirred for 1 h, and extracted with dichloromethane (3×100 mL). The combined extracts were washed with water (100 mL) and brine (40 mL), dried (Na_2SO_4), and evaporated to give a white solid, which was subjected to MPLC (500×25 mm i.d. column, ethyl acetate:hexane, 1:3). Evaporation of fractions 35–50 gave **2** as a white solid (1.6 g, 76% yield): mp, 78–79°C; single component by TLC (R_f 0.38, ethyl acetate:hexane, 1:1); $[\alpha]_D^{23}$ −1.6 (c 1.6, $CHCl_3$); IR, 3477, 1084, 989, 719 cm^{-1}; ^{1}H NMR, δ 3.71 (m, 1H), 3.66 (ddd, 11.0, 5.4, 3.1 Hz, 1H), 3.44 (ddd, 11.0, 7.7, 3.8 Hz, 1H), 2.08 (br s, 0.7H), 1.97 (br s, 0.7H), 1.63 (br s, 0.6H), 1.44 (m, 3H), 1.26 (m, ~23H), 0.88 (t, 7 Hz, 3H); ^{13}C NMR, δ 72.32 (C-2), 66.85 (C-1), 33.21, 31.91, 29.68, 29.67, 29.65, 29.64 (2C), 29.63, 29.58, 29.54, 29.35, 25.53, 22.68, 14.11; MS (EI), 241 (2), 227 (100), 208 (5), 153 (13), 139 (24), 125 (45), 111 (66), 97 (88), 83 (90), 71 (55); high-resolution MS (EI), calcd for $C_{15}H_{31}O$ ($M-CH_2OH$) 227.2375, found 227.2373. ^{1}H NMR of the MTPA derivative of **2** indicated a 9:1 ratio of 2*R* and 2*S* isomers.

3.3. (−)-(2R)-2-Benzyloxyhexadecan-1-ol (3)

To a solution of diol **2** (2.2 g, 8.5 mmol) in benzene (150 mL) was added benzaldehyde dimethyl acetal (2.6 g, 17 mmol) and *p*-toluenesulfonic acid monohydrate (0.1 g). The resulting solution was stirred at room temperature for 18 h, followed by concentration to dryness under vacuum. The residue was dissolved in dichloromethane (100 mL) and cooled to −78°C, followed by dropwise addition of diisobutylaluminum hydride (38 mL, 1.0 M in toluene). The reaction mixture was stirred at −78°C for 30 min, gradually warmed to room temperature, and quenched with methanol (4 mL). Diethyl ether (300 mL) and saturated Rochelle's salt (100 mL) were added, and the mixture was stirred until the two phases became clear. The separated organic phase was washed with water (100 mL) and brine (40 mL), dried (Na_2SO_4), and evaporated to a yellow oil, which was subjected to MPLC (500×25 mm i.d. column, ethyl

acetate:hexane, 15:85). Evaporation of fractions 71–98 gave **3** as a white solid (2.5 g, 84% yield): mp, 36–37°C; single component by TLC (R_f 0.70, ethyl acetate:hexane, 3:7); $[\alpha]_D^{23}$ −13.0 (c 1.9, $CHCl_3$); IR, 3447, 1057, 749, 696 cm^{-1}; ^1H NMR, δ 7.34 (m, 4H), 7.29 (m, 1H), 4.61 (d, 11.5 Hz, 1H), 4.54 (d, 11.5 Hz, 1H), 3.68 (ddd§, 11, 7, 2.5 Hz, 1H), 3.52 (ddd, 11.1, 6.3, 5.2 Hz, 1H), 3.49 (m, 1H), 1.62 (m, 1H), 1.49 (m, 1H), 1.27 (m, ~24H), 0.88 (t, 7 Hz, 3H); ^{13}C NMR, δ 138.49, 128.45 (2C), 127.77 (2C), 127.72, 79.82 (C-2), 71.48, 64.29 (C-1), 31.91, 30.78, 29.78, 29.68, 29.67, 29.65, 29.63 (2C), 29.57, 29.53, 29.34, 25.36, 22.67, 14.10; MS (FAB), 387 (3, M+K), 371 (5, M+Na), 347 (2, M−H), 107 (20), 91 (100); MS (EI), 348 (4, M^+), 317 (12, M−CH_2OH), 225 (18), 107 (13), 91 (100); high-resolution MS (EI), calcd for $C_{23}H_{40}O_2$ 348.3028, found 348.3022. ^1H NMR of the MTPA derivative of **3** showed a 9:1 ratio of 2*R* and 2*S* isomers.

*3.4. (+)-(2*R*)-2-Benzyloxyhexadecan-1-al (**4**)*

To a solution of Dess–Martin periodinane (3.4 g) in dichloromethane (40 mL) was added pyridine (1.3 mL). The resulting slurry was stirred until the reagents dissolved (~5 min), followed by dropwise addition of a solution of **3** (2.4 g, 6.9 mmol) in dichloromethane (15 mL). The reaction mixture was stirred at room temperature for 3 h, quenched with brine (20 mL) and saturated sodium sulfide solution (20 mL), and extracted with dichloromethane (3×50 mL). The extracts were washed with water (100 mL) and brine (40 mL), dried (Na_2SO_4), and evaporated to give a residue, which was subjected to flash chromatography (150×10 mm i.d. column, ethyl acetate:hexane, 15:85). Evaporation of fractions 3–7 gave **4** as an oil (2.2 g, 92% yield): single component by TLC (R_f 0.70, ethyl acetate:hexane, 15:85); $[\alpha]_D^{23}$ +9.6 (c 0.8, $CHCl_3$); IR, 1738, 1111, 737, 698 cm^{-1}; ^1H NMR, δ 9.65 (d, 2.2 Hz, 1H), 7.35 (m, 4H), 7.31 (m, 1H), 4.67 (d, 11.7 Hz, 1H), 4.53 (d, 11.7 Hz, 1H), 3.75 (ddd§, 6.5, 5.6, 2.2 Hz, 1H), 1.67 (m, 2H), 1.41 (m, 2H), 1.26 (m, ~22H), 0.88 (t, 7 Hz, 3H); ^{13}C NMR, δ 203.89 (C-1), 137.37, 128.49 (2C), 128.03, 127.98 (2C), 83.47, 72.48 (C-2), 31.90, 30.02, 29.67, 29.66, 29.63 (2C), 29.59, 29.51, 29.37 (2C), 29.34, 24.72, 22.67, 14.10; MS (FAB), 345 (2, M−H), 181 (7), 91 (100); MS (EI), 317 (11, M−CHO), 248 (7), 225 (25), 208 (12), 91 (100); high-resolution MS (EI), calcd for $C_{22}H_{37}O$ (M−CHO) 317.2827, found 317.2831.

*3.5. (2*E*,4*R*)-[1,2-^{13}C]Ethyl 4-benzyloxy-2-octadecenoate (**5**)*

Triethyl phosphonoacetate-$^{13}C_2$ (1.0 g, 4.4 mmol) was added dropwise at 0°C to a suspension of NaH (0.11 g, 4.6 mmol) in anhydrous THF (30 mL), followed by stirring at 0°C for 0.5 h and addition of **4** (1.7 g, 4.9 mmol) in one portion. The reaction mixture was allowed to warm to room temperature and extracted with dichloromethane (3×50 mL). The organic extracts were dried (Na_2SO_4) and evaporated to give a residue, which was purified by MPLC (500×25 mm i.d. column; ethyl acetate:hexane, 15:85). Evaporation of fractions 40–57 gave a colorless oil (1.65 g, 90% yield), which was characterized as an 8:1 mixture of **5** and **4** (as judged by ^1H NMR): single component by TLC (R_f 0.80; diethyl ether:hexane, 5:95); IR, 1680, 1149, 732, 698 cm^{-1}; ^1H NMR, δ 7.33 (m, 4H), 7.28 (m, 1H), 6.86 (dtd, 15.8, 6.6, 2.5 Hz, 1H, H-3), 6.00 (dddd, 163.2‡, 15.8, 3.4, 1.2 Hz, 1H, H-2), 4.59 (d, 11.9 Hz, 1H), 4.37 (d, 11.9 Hz, 1H), 4.22 (qd, 7.1, 3.0‡ Hz, 2H), 1.66 (m, 1H), 1.55 (m, 1H), 1.38 (m, 1H), 1.31 (t, 7.1 Hz, 3H), 1.25 (m, ~23H), 0.88 (t, 7 Hz, 3H); ^{13}C NMR, δ 166.30‡ (d, 74.5 Hz, C-1), 148.50 (dd, 70.7, 1.3 Hz, C-3), 138.19, 128.37 (2C), 127.69 (2C), 127.62, 121.93‡ (d, 74.8 Hz, C-2), 78.07 (dd, 6.5, 1.4 Hz, C-4), 70.94, 60.46 (dd, 2.3, 1.5 Hz), 34.91, 31.91, 29.68, 29.67, 29.65, 29.64, 29.62, 29.57, 29.49, 29.47, 29.35, 25.13, 22.68, 14.23 (d, 2.3 Hz), 14.10; MS (FAB), 419 (2, M+H), 373 (10, M−EtO), 311 (16, M−$PhCH_2O$), 91

(100); MS (EI), 418 (1, M^+), 373 (7, M−EtO), 327 (14, M−$PhCH_2$), 298 (9), 281 (6), 225 (12), 221 (16), 91 (100); high-resolution MS (EI), calcd for $C_{23}{}^{13}C_2H_{39}O_2$ (M−EtO) 373.3017, found 373.2988.

3.6. (2E,4R)-[1,1-^{2}H-1,2-^{13}C]4-Benzyloxy-2-octadecen-1-ol (6)

Alane-d_3 (AlD_3) was formed by stirring $AlCl_3$ (0.36 g, 2.7 mmol) and $LiAlD_4$ (0.27 g, 6.4 mmol) in dry diethyl ether (20 mL) for 15 min at 0°C. To this solution was added dropwise a solution of **5** (1.60 g, 3.8 mmol, containing 12% **4**) in dry diethyl ether (10 mL). After the addition was complete, the mixture was stirred at 0°C for 40 min. The reaction was quenched by addition of water (0.2 mL), followed by filtration of the resulting inorganic precipitate and evaporation of the filtrate to dryness. The residue was subjected to MPLC purification (500 mm×25 mm i.d. column; ethyl acetate:hexane, 5:95). Evaporation of fractions 63–83 gave **6** as a colorless oil (1.1 g, 76%); single component by TLC (R_f 0.62, diethyl ether:hexane, 3:7); $[\alpha]_D{}^{23}$ +22.6 (c 0.9, $CHCl_3$); IR, 3377, 2181, 2085, 1068, 748, 696 cm^{-1}; ^{1}H NMR, δ 7.33 (m, 4H), 7.27 (m, 1H), 5.79 (ddd[§], ~164[‡], 15.6, 4.5 Hz, 1H, H-2), 5.62 (m, 1H, H-3), 4.57 (d, 11.9 Hz, 1H), 4.37 (d, 11.9 Hz, 1H), 3.76 (m, 1H, H-4), 1.64 (m, 1H), 1.49 (m, 1H), 1.25 (m, ~24H), 0.88 (t, 7 Hz, 3H); ^{13}C NMR, δ 138.83, ~132 (C-3), 131.56[‡] (d, 45.8 Hz, C-2), 128.29 (2C), 127.70 (2C), 127.39, 79.46 (dd, 5.9, 1.5 Hz, C-4), 70.17, 62.36[‡] (d of quintet, 45.8, 21.7 Hz, C-1), 36.63 (d, 2.9 Hz, C-5), 31.91, 29.69, 29.68, 29.66, 29.65, 29.64, 29.61, 29.57, 29.55, 29.35, 25.38, 22.68, 14.10; MS (FAB), 417 (5, M+K), 401 (3, M+Na), 377 (1, M−H), 271 (3, M−$PhCH_2O$), 253 (4), 91 (100); MS (EI), 378 (1, M^+), 344 (3, M−$^{13}CD_2OH$), 287 (4, M−$PhCH_2$), 225 (6), 181 (49), 91 (100); high-resolution MS (EI), calcd for $C_{23}{}^{13}C_2H_{40}D_2O_2$ 378.3377, found 378.3377.

3.7. (2R,3S,4R)-[1,1-^{2}H-1,2-^{13}C]-2,3-Epoxy-4-benzyloxyoctadecan-1-ol (7)

To a solution of D-(−)-DIPT (0.72 mL, 3.4 mmol) containing oven-dried molecular sieve (2 g; 3 Å) in dichloromethane (50 mL) was added titanium tetraisopropoxide (0.87 mL, 2.9 mmol) at −30°C, followed by stirring for 15 min at −30°C and dropwise addition of *tert*-butyl hydroperoxide (2 mL, 5–6 M) in decane. After further stirring at −30°C for 15 min, a solution of **6** (1.1 g, 2.9 mmol) in dichloromethane (10 mL) was added dropwise. The reaction mixture was stirred for an additional 5 min at −30°C and stored in a −20°C freezer for 3 days. TLC analysis indicated conversion of nearly all the starting material to give a polar product. The reaction mixture was washed vigorously for 15 min with 30% sodium hydroxide solution (15 mL), and the aqueous washings were extracted with dichloromethane (2×100 mL). The combined organic phase was dried (Na_2SO_4) and evaporated to give an oil, which was purified by MPLC (500 mm×25 mm i.d. column, 15% ethyl acetate–hexane). Fractions 46–70 gave **7** as a white solid (0.8 g, 70% yield): mp, 37–40°C; single component by TLC (R_f 0.50, ethyl acetate:hexane, 3:7); IR, 3443, 2170, 2085, 1107, 729, 692 cm^{-1}; ^{1}H NMR, δ 7.33 (m, 4H), 7.28 (m, 1H), 4.61 (d, 11.9 Hz, 1H), 4.55 (d, 11.9 Hz, 1H), 3.32 (dtd, 7.1, 5.2, 3.7, 1H, H-4), 3.10 (ddd, 173.4[‡], 7.7[‡], 2.3 Hz, 1H, H-2), 2.94 (dddd, 5.3, 2.0, 2.0[‡], 2.0[‡] Hz, 1H, H-3), 1.62 (m, 2H), 1.49 (m, 1H), 1.38 (m, 1H), 1.26 (m, ~22H), 0.88 (t, 7 Hz, 3H); ^{13}C NMR, δ 138.62, 128.37 (2C), 127.66, 127.63 (2C), 77.58 (C-4), 72.46, 60.69 (d of quintet, 46.0, 21.7 Hz, C-1), ~57 (C-3), 56.71 (d, 46.0 Hz, C-2), 32.79 (d, 2.0 Hz, C-5), 31.91, 29.68, 29.67, 29.66, 29.64 (2C), 29.62, 29.59, 29.54, 29.34, 25.10, 22.67, 14.10; ^{2}H NMR, δ 3.83 (d, 21.7 Hz), 3.55 (d, 21.7 Hz); MS (FAB), 433 (1, M+K), 417 (1, M+Na), 393 (1, M−H), 287 (4, M−$PhCH_2O$), 91 (100); MS (EI), 394 (1, M^+), 329 (3), 317 (4), 287 (2, M−$PhCH_2O$), 254 (27), 225 (13), 107 (38), 91 (100); high-resolution MS (EI), calcd for $C_{23}{}^{13}C_2H_{40}D_2O_3$ 394.3327, found 394.3315. ^{1}H NMR analysis indicated a 85:15 mixture of 4*R* and 4*S* isomers; NMR data for minor (4*S*) epimer (originating from the minor enantiomer of **4**, which ultimately was derived from incomplete stereoselectivity in the asymmetric

dihydroxylation of **1**): δ_H 4.80 (d, 11.8 Hz), 4.58 (d, 11.8 Hz), 3.15 (dddd, 8.0, 6.8, 5.3, 1.3 Hz, H-4), 3.07 (dq, 7.0, 2.1 Hz, H-3), 2.94 (ddd, 172.0‡, 7.3‡, 2.4 Hz, H-2); δ_C 54.60‡ (d, 46.3 Hz).

*3.8. (2*R*,3*S*,4*R*)-[1,1-^{2}H-1,2-^{13}C]-Carbamate (**8**)*

Benzoyl chloride (0.46 mL, 4.0 mmol) was added to a suspension of silver cyanate (0.66 g, 4.4 mmol) in CCl_4 (10 mL) at room temperature, followed by refluxing for 12 h, cooling, and filtration under nitrogen. To the filtrate was added **7** (0.80 g, 2.0 mmol) in CCl_4 (10 mL), and the reaction mixture was stirred at room temperature for 4 h. Dichloromethane (100 mL) was added, and the solution was washed with saturated sodium bicarbonate and brine and evaporated to give a residue, which was subjected to MPLC (500 mm×25 mm i.d. column, ethyl acetate:hexane, 15:85). Evaporation of fractions 46–76 gave **8** as a white solid (0.94 g, 86% yield; 10:1 mixture of **8** and its 4*S* epimer by ^{1}H NMR): mp, 50–52°C; single component by TLC (R_f 0.47, ethyl acetate:hexane, 3:7); IR, 3288, 2158, 1772, 1753, 1207, 694 cm^{-1}; ^{1}H NMR, δ 8.22 (br s, 1H, NH), 7.82 (dd§, 8.4, 1.3 Hz, 2H), 7.59 (tt§, 7.5, 1.3 Hz, 1H), 7.48 (tt§, 8, 1.5 Hz, 2H), 7.33 (m, 4H), 7.28 (m, 1H), 4.61 (d, 11.9 Hz, 1H), 4.54 (d, 11.9 Hz, 1H), 3.23 (ddd, 178.0‡, 5.6, 2.2 Hz, 1H, H-2), 3.33 (dtd, 7.2, 5.1, 4.0 Hz, 1H, H-4), 2.91 (dq, 5.2, 1.9 Hz, 1H, H-3), 1.62 (m, 2H), 1.49 (m, 1H), 1.38 (m, 1H), 1.26 (m, ~22H), 0.88 (t, 7 Hz, 3H); ^{13}C NMR, δ 164.60, 150.48, 138.49, 133.11, 132.74, 128.90 (2C), 128.40 (2C), 127.71, 127.63 (2C), 127.60 (2C), 77.18 (C-4), 72.59, 65.28‡ (d of quintet, 49, 22 Hz, C-1), 53.34‡ (d, 49.7 Hz, C-2), 32.73 (d, 2.1 Hz), 31.90, 29.68, 29.67, 29.65, 29.63 (2C), 29.60, 29.59, 29.53, 29.34, 25.06, 22.67, 14.10; ^{2}H NMR, δ 4.49 (d, 22.4 Hz), 3.99 (d, 22.7 Hz); MS (FAB), 580 (13, M+K), 564 (16, M+Na), 542 (1, M+H), 105 (38), 91 (100); high-resolution MS (FAB), calcd for $C_{31}{}^{13}C_2H_{45}D_2NO_5Na$ (M+Na) 564.3545, found 564.3553. NMR data for the minor (4*S*) isomer: δ_H 4.79 (d, 11.8 Hz), 4.57 (d, 11.9 Hz), 3.14 (dddd, 8.0, 6.6, 5.1, 1.4 Hz, H-4), 3.08 (ddd, 176.6‡, 5.5, 2.2 Hz, H-2), 2.95 (dq§, 5.3, 1.9 Hz, H-3); δ_C 51.46‡ (d, 49.8 Hz, C-2).

*3.9. (2*S*,3*S*,4*R*)-[1,1-^{2}H-1,2-^{13}C]-Oxazolidinone-benzoate (**9**)*

Crude **8** (0.80 g, 1.5 mmol) was added to a suspension of NaH (14 mg, 0.6 mmol) in freshly distilled THF (30 mL), followed by refluxing for 6 h, cooling to room temperature, addition of water (100 mL), and extraction with dichloromethane (2×200 mL). The organic extracts were washed with brine, dried (Na_2SO_4), and evaporated to give a residue, which was subjected to MPLC (500 mm×25 mm i.d. column, 10–35% ethyl acetate in hexane). Fractions 35–48 gave **8** as a white solid (200 mg, 25% recovery). Evaporation of fractions 58–82 provided **9** as a colorless oil (300 mg, 38% yield, ≥98% purity by ^{1}H NMR): single component by TLC (R_f 0.40, ethyl acetate:hexane, 3:7); IR, 3420, 2240, 1759, 1722, 1271, 1111, 712 cm^{-1}; ^{1}H NMR, δ 7.99 (dd§, 8.4, 1.3 Hz, 2H), 7.59 (tt, 7.5, 1.3 Hz, 1H), 7.46 (ddt§, 8.3, 7.5, 1.6 Hz, 2H), 7.31 (m, 5H), 5.35 (dd§, 5.5, 4.0 Hz, 1H, NH), 5.25 (ddt, 5.4, 4.3, 3.6 Hz, 1H, H-3), 4.60 (11.4, 1H), 4.52 (11.5, 1H), 4.31 (dddd, 146.0‡, 3.6, 2.7, 1.0, 1H, H-2), 3.71 (tt, 5.9, 4.2 Hz, 1H, H-4), 1.61 (m, 2H), 1.43 (m, 1H), 1.38 (m, 1H), 1.25 (m, ~22H), 0.88 (t, 7 Hz, 3H); ^{13}C NMR, δ 165.85 (d, 1.5 Hz), 158.95, 137.40, 133.60, 129.75 (2C), 129.14, 128.61 (2C), 128.57 (2C), 128.08, 127.99 (2C), 78.81 (d, 1.1 Hz), 74.61 (d, 41.6 Hz), 72.79, 65.84‡ (d of quintet, 32, 24 Hz), 52.31‡ (d, 32.8 Hz), 31.90, 31.30, 29.67, 29.66, 29.63 (2C), 29.59, 29.57, 29.50, 29.41, 29.34, 24.95, 22.66, 14.10; ^{2}H NMR, δ 4.56 (d, 23 Hz), 4.40 (d, 23 Hz); MS (FAB), 580 (2, M+K), 564 (2, M+Na), 542 (7, M+H), 105 (47), 91 (100); high-resolution MS (FAB), calcd for $C_{31}{}^{13}C_2H_{46}D_2NO_5$ (M+H) 542.3725, found 542.3727. Evaporation of fractions 146–169 gave **10** (150 mg, 23% yield, 98% purity) as a white solid.

3.10. (2S,3S,4R)-[1,1-^{2}H-1,2-^{13}C]-Oxazolidinone-alcohol (**10**)

Lithium hydroxide (2 mL, 2.25 M in water) was added to a solution of **9** (300 mg, 0.55 mmol) in THF (20 mL), followed by stirring at room temperature for 12 h and extraction with ethyl acetate (2×30 mL). The organic extracts were washed with water (20 mL) and brine (20 mL), dried (Na_2SO_4), and evaporated to give a residue, which was subjected to MPLC (1 m×10 mm i.d. column; ethyl acetate:hexane, 3:7). Evaporation of fractions 32–55 gave **10** as a white solid (0.21 g, 87% yield): mp, 73–74°C; single component by TLC (R_f 0.40; ethyl acetate:hexane, 1:1); $[\alpha]_D^{23}$ −16.6 (c 0.7, $CHCl_3$); IR, 3487, 3360, 2246, 2168, 1786, 1099, 748, 698 cm^{-1}; ^{1}H NMR δ 7.36 (m, 2H), 7.31 (m, 3H), ~5.9 (ddd[§], 5.8, 4.0, 0.8 Hz, 1H, NH), 4.60 (d, 11.4 Hz, 1H), 4.47 (d, 11.4 Hz, 1H), 4.00 (dddd, 145.6[‡], 4.6, 2.2[‡], 1.0[‡] Hz, 1H, H-2), 3.72 (dqd, 5.6, 4.6, 3.1 Hz, 1H, H-3), 3.45 (dddd, 6.3, 5.4, 4.7, 3.1 Hz, 1H, H-4), ~3.0 (dd, 4.7, 2.9 Hz, 1H, 3-OH), 1.59 (m, 2H), 1.42 (m, 1H), 1.35 (m, 1H), 1.25 (m, ~22H), 0.88 (t, 7 Hz, 3H); ^{13}C NMR, δ 160.56, 137.72, 128.59 (2C), 128.04, 128.01 (2C), 79.73 (d, 2.0 Hz, C-4), 72.69 (d, 40.8 Hz, C-3), 72.26, 65.93[‡] (d of quintet, 32, 23 Hz, C-1), 53.75[‡] (d, 33.1 Hz, C-2), 31.91, 30.12, 29.82, 29.70, 29.69, 29.68, 29.66, 29.65, 29.61, 29.58, 29.35, 24.72, 22.68, 14.11; ^{2}H NMR, δ ~4.45 (d, 23 Hz), ~4.26 (d, 23 Hz); MS (FAB), 476 (11, M+K), 460 (18, M+Na), 438 (2, M+H), 394 (7, M−CO_2+H), 91 (100); high-resolution MS (FAB), calcd for $C_{24}{}^{13}C_2H_{42}D_2NO_4$ (M+H) 438.3463, found 438.3459.

3.11. (2S,3S,4R)-[1,1-^{2}H-1,2-^{13}C]-Oxazolidinone-diol (**11**)

To a solution of **10** (300 mg, 0.68 mmol) in THF (30 mL) was added 10% palladium on carbon (30 mg). The solution was stirred under a balloon of hydrogen at room temperature for 12 h. The catalyst was removed by filtration on filter paper, and the filtrate was evaporated to a residue, which was subjected to MPLC (1 m×10 mm i.d. column; methanol:chloroform, 5:95). Fractions 41–54 gave **11** as a white solid (160 mg, 67% yield): mp, 77–78°C; single component by TLC (R_f 0.35, methanol:chloroform, 1:9); IR, 3406, 2255, 2160, 1747, 1261, 1074 cm^{-1}; ^{1}H NMR ($CDCl_3$–CD_3OD; referenced to tetramethylsilane; the CD_3OD quintet appeared at δ 3.37) δ 3.96 (ddd, 145.3[‡], 4.9, 2.2 Hz, 1H, H-2), 3.74 (br s, 2H), 3.41 (ddt, 9.2, 6.3, 3.0 Hz, 1H, H-4), 3.35 (dtd, 6.5, 4.6, 2.9, 1H, H-3), 1.58 (m, 1H), 1.45 (m, 1H), 1.19 (m, ~24H), 0.81 (t, 7 Hz, 3H); ^{13}C NMR ($CDCl_3$–CD_3OD) δ 160.8, 74.1 (dd, 41.2, 1.2 Hz, C-3), 73.0 (br s, C-4), 66.0 (d of quintet, 33, 23 Hz, C-1), 54.2 (d, 33.1 Hz, C-2), 33.4, 31.7, 29.5–29.4 (8C), 29.1, 25.3, 22.4, 13.8; ^{2}H NMR ($CHCl_3$–CH_3OH), δ 4.24 (d, 24 Hz); MS (FAB), 733 (4, 2M+K), 717 (6, 2M+Na), 695 (8, 2M+H), 386 (93, M+K), 370 (52, M+Na), 348 (70, M+H), 304 (51, M−CO_2+H), 192 (45); high-resolution MS (FAB), calcd for $C_{17}{}^{13}C_2H_{36}D_2NO_4$ (M+H) 348.2993, found 348.2980.

3.12. (2S,3S,4R)-[1,1-^{2}H-1,2-^{13}C]-2-Aminooctadecane-1,3,4-triol (D-ribo-[1,1-^{2}H-1,2-^{13}C]phytosphingosine) (**12**)

To a solution of **11** (120 mg, 0.35 mmol) in ethanol (10 mL) was added 2.0 M sodium hydroxide (10 mL), followed by refluxing under nitrogen for 12 h. The product was extracted with chloroform:methanol (9:1; 2×30 mL) and washed with water (20 mL) and brine (20 mL). The organic extracts were dried (Na_2SO_4) and evaporated to give a residue, which was subjected to MPLC (1 m×10 mm i.d. column, ammonia:methanol:chloroform, 1.5:15:85). Evaporation of fractions 30–37 gave **12** as a white solid (78 mg, 70% yield): mp, 78–80°C; single component by TLC (R_f 0.40, ammonia:methanol:chloroform, 1.5:15:85); $[\alpha]_D^{23}$ +6.8 (c 1.1, pyridine); IR, 3373, 2199, 2097, 1093, 721 cm^{-1}; ^{1}H NMR (CD_3OD), δ 3.51 (tt[§], 8, 2.5[‡] Hz, 1H, H-4), 3.34 (dddd, 7.8, 5.5, 4.3[‡], 3.1[‡] Hz, 1H, H-3), 2.95 (ddd, 135.7[‡], 5.5, 3.2[‡] Hz, 1H, H-2), 1.73 (m, 1H), 1.55 (m, 1H), 1.28 (m, ~24H), 0.89 (t, 7 Hz, 3H); ^{13}C NMR (CD_3OD),

δ 76.4 (d, 40.4 Hz, C-3), 74.4 (d, 1.3 Hz, C-4), 63.3 (d of quintet, 39, 21 Hz, C-1), 55.7 (d, 38.8 Hz, C-2), 34.8, 33.1, 31.0, 30.8 (7C), 30.5, 26.6, 23.7, 14.4; ^{2}H NMR (CD_3OD), δ 3.72 (d, 22 Hz), 3.53 (d, 22 Hz); MS (FAB), 344 (5, M+Na), 322 (100, M+H), 304 (5, M−H_2O+H); high-resolution MS (FAB), calcd for $C_{16}{}^{13}C_2H_{38}D_2NO_3$ (M+H) 322.3201, found 322.3192. GC–MS of the tetra-TMS derivative of **12**, 522 (3), 431 (2), 341 (30), 312 (23), 299 (25), 297 (15), 222 (9), 208 (44), 136 (100), 73 (97). The overall yield of **12** from **1** was 9% (after compensation in the two-step conversion of **8** to **10** for recovered starting material and for the direct formation of **10** in 23% yield from **8**).

3.13. D-ribo-*[1,1-^{2}H-1,2-^{13}C]-Phytosphingosine tetraacetate (**13**)*

A solution of **12** (4 mg) in pyridine (100 μL), acetic anhydride (100 μL), and dichloromethane (5 mL) was stirred at room temperature for 12 h, followed by addition of water (2 mL) and extraction with dichloromethane (2×5 mL). The organic extracts were washed with water (2 mL) and brine (2 mL), dried (Na_2SO_4), and evaporated under nitrogen to give a residue, which was purified by HPLC (250×10 mm i.d. Sphereclone ODS-2 C_{18} column; Phenomenex, Torrance, CA; elution with acetonitrile). Evaporation of the eluate corresponding to the peak at 10.5 min gave **13** as a white solid (2.5 mg): single component by TLC (R_f 0.5, methanol:chloroform, 1:9); ^{1}H NMR, δ 5.97 (d, ∼9 Hz, 1H), 5.099 (dddd, 8.3, 4.5‡, 3.2‡, 3.2 Hz, 1H, H-3), 4.934 (dddd, 9.9, 3.3‡, 3.3, 1.9‡, 1H, H-4), 4.459 (dddd, 139.2‡, 9.5, 8.3, 1.6‡ Hz, 1H, H-2), 2.080 (s, 3H), 2.049 (s, 3H), 2.047 (s, 3H), 2.026 (s, 3H), 1.65 (m, 2H), 1.25 (m, ∼24H), 0.880 (t, 7 Hz, 3H); ^{13}C NMR, δ 171.17, 170.87, 170.09 (d, 1.8 Hz), 169.71, 72.97, 71.97 (d, 43 Hz), 62.23‡ (d of quintet, 44, 22 Hz), 47.49‡ (d, 40.8 Hz), 31.91, 29.69, 29.68, 29.67, 29.65, 29.62, 29.57, 29.49, 29.35, 29.29, 28.16, 25.50, 23.30, 22.68, 21.04, 20.77, 20.74, 14.11; ^{2}H NMR, 4.29 (d, 23 Hz), 4.00 (d, 23 Hz); MS (EI), 490 (1, M+H), 429 (2, M−AcOH), 413 (9, M−$^{13}CD_2OAc$), 387 (10), 370 (20), 353 (30), 327 (17), 311 (31), 309 (36), 293 (34), 284 (11), 269 (21), 222 (16), 205 (14), 190 (13), 149 (68), 148 (90), 106 (48), 88 (100); high-resolution MS (EI), calcd for $C_{24}{}^{13}C_2H_{46}D_2NO_7$ (M+H) 490.3530, found 490.3565.

Acknowledgements

We gratefully acknowledge the support of the National Institutes of Health (HL-49122) and the Robert A. Welch Foundation (C-583).

References

1. Hannun, Y. A.; Bell, R. M. *Science* **1989**, *243*, 500–507.
2. Merrill Jr., A. H.; Schmelz, E. M.; Dillehay, D. L.; Spiegel, S.; Shayman, J. A.; Schroeder, J. J.; Riley, R. T.; Voss, K. A.; Wang, E. *Toxicol. Appl. Pharmacol*. **1997**, *142*, 208–225.
3. Ariga, T.; Jarvis, W. D.; Yu, R. K. *J. Lipid Res*. **1998**, *39*, 1–16.
4. Kobayashi, T.; Mitsuo, K.; Goto, I. *Eur. J. Biochem*. **1988**, *172*, 747–752.
5. Merrill Jr., A. H.; Wang, E.; Mullins, R. E.; Jamison, W. C. L.; Nimkar, S.; Liotta, D. C. *Anal. Biochem*. **1988**, *171*, 373–381.
6. Van Veldhoven, P. P.; Bishop, W. R.; Bell, R. M. *Anal. Biochem*. **1989**, *183*, 177–189.
7. Alvarez, J. G.; Touchstone, J. C.; Storey, B. T.; Grob, R. L. *J. Liquid Chromatogr*. **1989**, *12*, 3115–3120.
8. Mano, N.; Oda, Y.; Yamada, K.; Asakawa, N.; Katayama, K. *Anal. Biochem*. **1997**, *244*, 291–300.
9. Johnstone, R. A. W.; Rose, M. E. *Mass Spectrometry for Chemists and Biochemists*; Cambridge University Press: Cambridge, 1996; pp. 205–231.
10. Li, S.; Pang, J.; Wilson, W. K.; Schroepfer Jr., G. J. *J. Labelled Compd. Radiopharm*., in press.

11. Wild, R.; Schmidt, R. R. *Tetrahedron: Asymmetry* **1994**, *5*, 2195–2208.
12. Li, Y.-L.; Mao, X.-H.; Wu, Y.-L. *J. Chem. Soc., Perkin Trans. 1* **1995**, 1559–1563.
13. Murakami, T.; Minamikawa, H.; Hato, M. *Tetrahedron Lett*. **1994**, *35*, 745–748.
14. Matsumoto, K.; Ebata, T.; Matsushita, H. *Carbohydr. Res*. **1995**, *279*, 93–106.
15. Wild, R.; Schmidt, R. R. *Liebigs Ann. Chem*. **1995**, 755–64.
16. Wee, A. G. H.; Tang, F. *Can. J. Chem*. **1998**, *76*, 1070–1081.
17. Dondoni, A.; Fantin, G.; Fogagnolo, M.; Pedrini, P. *J. Org. Chem*. **1990**, *55*, 1439–1446.
18. Imashiro, R.; Sakurai, O.; Yamashita, T.; Horikawa, H. *Tetrahedron* **1998**, *54*, 10657–10670.
19. Shimizu, M.; Wakioka, I.; Fujisawa, T. *Tetrahedron Lett*. **1997**, *38*, 6027–6030.
20. Kemp, S. J.; Bao, J.; Pedersen, S. F. *J. Org. Chem*. **1996**, *61*, 7162–7167.
21. Kulmacz, R. J.; Kisic, A.; Schroepfer Jr., G. J. *Chem. Phys. Lipids* **1979**, *23*, 291–319.
22. Takikawa, H.; Muto, S.; Mori, K. *Tetrahedron* **1998**, *54*, 3141–3150.
23. Mulzer, J.; Brand, C. *Tetrahedron* **1986**, *42*, 5961–5968.
24. Guanti, G.; Banfi, L.; Narisano, E. *Tetrahedron Lett*. **1989**, *30*, 5507–5510.
25. Yoda, H.; Oguchi, T.; Takabe, K. *Tetrahedron: Asymmetry* **1996**, *7*, 2113–2116.
26. Murakami, M.; Ito, H.; Ito, Y. *Chem. Lett*. **1996**, 185–186.
27. Lin, G.-Q.; Shi, Z.-C. *Tetrahedron* **1996**, *52*, 2187–2192.
28. Kobayashi, S.; Hayashi, T.; Kawasuji, T. *Tetrahedron Lett*. **1994**, *35*, 9573–9576.
29. Sharpless, K. B.; Amberg, W.; Bennani, Y. L.; Crispino, G. A.; Hartung, J.; Jeong, K.-S.; Kwong, H.-L.; Morikawa, K.; Wang, Z.-M.; Xu, D.; Zhang, X.-L. *J. Org. Chem*. **1992**, *57*, 2768–2771.
30. Smith, A. B.; Chen, S. S.-Y.; Nelson, F. C.; Reichert, J. M.; Salvatore, B. A. *J. Am. Chem. Soc.* **1997**, *119*, 10935–10946.
31. Curtis, N. R.; Holmes, A. B.; Looney, M. G. *Tetrahedron Lett.* **1992**, *33*, 671–671.
32. Dess, D. B.; Martin, J. C. *J. Am. Chem. Soc*. **1991**, *113*, 7277–7287.
33. Knapp, S.; Kukkola, P. J.; Sharma, S.; Dhar, T. G. M.; Naughton, A. B. J. *J. Org. Chem*. **1990**, *55*, 5700–5710.
34. Li, S.; Wilson, W. K.; Schroepfer Jr., G. J. *J. Lipid Res*. **1999**, *40*, 117–125.
35. Sugiyama, S.; Honda, M.; Komori, T. *Liebigs Ann. Chem*. **1990**, 1069–1078.
36. Kisic, A.; Tsuda, M.; Kulmacz, R. J.; Wilson, W. K.; Schroepfer Jr., G. J. *J. Lipid Res*. **1995**, *36*, 787–803.
37. Li, S.; Wilson, W. K.; Schroepfer Jr., G. J. *J. Lipid Res*. **1999**, *40*, 764–772.