Syntheses of Sphingolipid Analogs

by

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Abstract

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This dissertation presents the asymmetric syntheses of sphingolipid analogs, including (a) fluorescent lactosylceramide stereoisomers, (b) sphingosine 1-phosphate-d_8, (c) deuterated sphingomyelin analogs, and (d) (2*S*,3*S*)-3-fluoro-4,5-dihydrosphingosine and (2*S*,3*R*)-1-fluorosphingosine.

Chapter 1 presents the synthesis of three stereoisomers of BODIPY-LacCer: (2*R*,3*R*)-, (2*S*,3*S*)-, and (2*R*,3*S*)-. These analogs will be used to probe the role of stereochemistry in the long-chain base of LacCer in the mechanism of endocytic uptake in living cells.

Chapter 2 presents the synthesis of sphingosine 1-phosphate-d_8 which is used as an internal standard for quantitative analysis of serum sphingosine 1-phosphate.

Chapter 3 presents the first synthesis of four deuterated sphingomyelin analogs: *N*-palmitoyl-d_{31}-SM, 4,5-dihydro-*N*-palmitoyl-d_{31}-SM, *N*-palmitoyl-3,3-d_2-SM, and *N*-palmitoyl-2,2,3,3,4,4-d_6-SM. These analogs will be used to study the conformation of the sphingomyelin (SM)-water interface in bilayers by ^{2}H nuclear magnetic resonance (NMR) spectroscopy.

Chapter 4 presents the synthesis of (2*S*,3*S*)-3-fluoro-4,5-dihydrosphingosine and (2*S*,3*R*)-1-fluorosphingosine. The phosphorylated derivatives of these compounds would be of interest in a study of binding to sphingosine 1-phosphate receptors.

Dedicated to those who made this thesis possible:

My parents, my wife

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Abbreviations

Ac	acetyl
ATP	adenosine 5'-triphosphate
Boc	*tert*-butoxycarbonyl
BODIPY™	boron dipyrromethene difluoride
Bz	benzoyl
DCC	*N*,*N*'-dicyclohexylcarbodiimide
DHP	3,4-dihydro-2H-pyran
DIBAL-H	diisobutylaluminum hydride
DMAP	4-(dimethylamino)pyridine
DMF	*N*,*N*-dimethylformamide
DMP	2,2-dimethoxypropane
DMSO	dimethyl sulfoxide
DAST	diethylaminosulfur trifluoride
GC	gas chromatography
GSL	glycosphingolipid
HMPA	hexamethylphosphoramide
HR-MS	high-resolution mass spectrum
LacCer	lactosylceramide
LC	liquid chromatography
MS	mass spectrum

NBS	*N*-bromosuccinimide
NMR	nuclear magnetic resonance
NHS	*N*-hydroxysuccinimidoyl
Py	pyridine
S1P	sphingosine 1-phosphate
SM	sphingomyelin
TBAF	tetra-*n*-butylammonium fluoride
TBDPS	*tert*-butyldiphenylsilyl
Tf	trifluoromethanesulfonyl
THF	tetrahydrofuran
TMS	trimethylsilyl
TLC	thin-layer chromatography
Ts	*p*-toluenesulfonyl
Red-Al	sodium bis(2-methoxyethoxy)aluminum hydride
UV	ultraviolet

Chapter 1

Synthesis of Fluorescent Lactosylceramide Stereoisomers

Abstract

The intracellular distribution of synthetic glycosphingolipids bearing a fluorophore can be monitored in living cells by fluorescence microscopy (Pagano et al., 2000). The previous research showed that variation in the length of the long-chain base and in the structure of the carbohydrate-containing polar head group of (2*S*,3*R*)-(or D-*erythro*-)-β-lactosylceramide (LacCer) did not alter the mechanism of endocytic uptake from the plasma membrane of various mammalian cell types (Singh, R. D., Puri, V., Valiyaveettil, J. T., Marks, D. L., Bittman, R., Pagano, R. E., 2003. Mol. Biol. Cell 14, 3254-3265). To extend our examination of the molecular features in LacCer that are responsible for its uptake by the caveolar-requiring endocytic pathway, we have synthesized the three unnatural stereoisomers [(2*R*,3*R*)-, (2*S*,3*S*)-, and (2*R*,3*S*)] of BODIPY-LacCer. These analogs will be used to probe the role of stereochemistry in the long-chain base of LacCer in the mechanism of endocytic uptake.

1. Introduction

A boron dipyrromethene difluoride (BODIPY) (Johnson et al., 1991) fluorophore linked to the long-chain base of naturally occurring (2*S*,3*R*)-β-lactosylceramide (LacCer) via the ω end of a *N*-pentanoyl moiety (compound **a** in Fig. 1) has been used to examine the intracellular trafficking of this and other glycosphingolipids (GSLs) in normal and disease cell types. This GSL was localized in lysosomes of a diseased cell type, but was observed at the Golgi complex in normal fibroblasts (Chen et al., 1998). (2*S*,3*R*)-C_5-BODIPY-LacCer (which is available commercially) and a synthetic analog bearing a maltosyl polar head group, (2*S*,3*R*)-C_5-BODIPY-MalCer, utilized the same caveolar-dependent endocytic pathway for uptake from the plasma membrane of different cells (Singh et al., 2003; Bittman, 2004). In contrast, BODIPY-sphingomyelin utilizes both a clathrin- and a caveolar-dependent pathway in approximately equal extents for internalization (Puri et al., 2001). To examine the role of stereochemistry at C2 and C3 of the sphingosine chain of LacCer in determining the mechanism of endocytosis we have prepared the following unnatural stereoisomeric analogs: (2*R*,3*R*)-, (2*S*,3*S*)-, and (2*R*,3*S*)-BODIPY-LacCer (compounds **b**-**d** in Fig. 1).

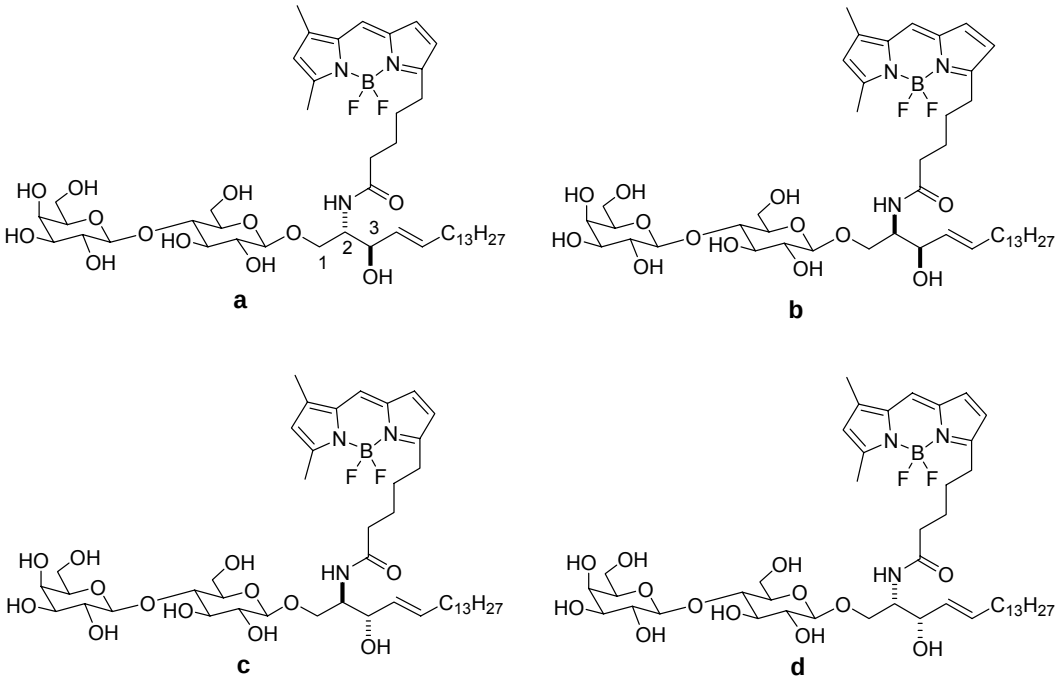


Fig. 1. Structures of (a) (2*S*,3*R*) (or D-*erythro*), (b) (2*R*,3*R*) (or D-*threo*), (c) (2*R*,3*S*) (or L-*erythro*), and (d) (2*S*,3*S*) (or L-*threo*)-BODIPY-LacCer.

2. Experimental

2.1. Materials and analytical procedures

2.1.1. Chemicals

The sources of the chemicals were as follows: BODIPY-C_5-*N*-hydroxysuccinimidoyl (NHS) ester, Invitrogen/Molecular Probes (Eugene, OR); *N*-Boc-D-serine and diisobutylaluminum hydride (DIBAL-H, a 20 weight % solution in toluene), Acros (Morris Plains, NJ); L-*threo*-sphingosine, Avanti Polar Lipids (Alabaster, AL); 1-pentadecyne, *p*-toluenesulfonic acid monohydrate (*p*-TsOH), and sodium bis(2-methoxyethoxy)aluminum hydride (Red-Al, a 70% w/w solution in toluene), Alfa Aesar/Lancaster (Pelham, NH); β-D-lactosyl octaacetate, triphenylphosphine, trichloroacetonitrile, *tert*-butyldiphenylsilyl chloride (TBDPSCl), hydrazine acetate, benzoic anhydride, $BF_3{\cdot}OEt_2$, imidazole, 4-(dimethylamino)pyridine (DMAP), and $(n\text{-}Bu)_4NF$ (TBAF), Sigma-Aldrich. Trifluoromethanesulfonyl azide (TfN_3) was prepared according to Vasella et al. (1991). Hepta-*O*-acetyllactosyl-1-trichloroacetimidate (compound **13**) was synthesized from per-*O*-acetyllactose as described (Amvam-Zollo and Sinay, 1986). Molecular sieves (300AW) were dried for 5 h at 150 °C and stored under vacuum over P_2O_5.

2.1.2. General methods

Air- and moisture-sensitive reactions were carried out under nitrogen in flame-dried glassware. THF and toluene were distilled from sodium/benzophenone and dichloromethane was distilled from calcium

hydride prior to use. DMF was dried over calcium hydride. TLC was performed using aluminum-backed or glass-backed silica gel 60 F254 plates (0.25-mm thick), and the compounds were visualized by charring with 10% H_2SO_4 in EtOH or by UV light. Column chromatography was carried out with silica gel 60 (230-400 mesh) using the elution solvents indicated in the text. Suspended silica gel was removed by filtration through an Osmonics Cameo filter (Fisher Scientific, Pittsburgh, PA). The ^1H and ^{13}C NMR spectra were recorded at 400 and 100 MHz, respectively, and were referenced to the residual $CHCl_3$ at δ 7.24 (^1H) and the central line of $CDCl_3$ at δ 77.0 ppm (^{13}C). Optical rotations were measured on a digital polarimeter at rt in the solvents stated. Mass spectra were recorded at the University of Waterloo and at Ohio State University.

2.2. Synthesis

*2.2.1. N-[(1,1-Dimethylethoxy)carbonyl]-D-serine Methyl Ester (**2**)*

To a cold solution of *N*-Boc-D-serine (compound **1** in Scheme 1, 3.0 g, 14.6 mmol) in DMF (20 mL) was added potassium carbonate (2.28 g, 16.5 mmol). After the mixture was stirred for 10 min in an ice-water bath, methyl iodide (1.88 mL, 4.26 g, 30 mmol) was added to the white suspension, and stirring was continued at 0 °C for 30 min, whereupon the mixture solidified. The reaction mixture was warmed to rt and stirred for an additional hour. The reaction mixture was filtered by suction and the filtrate was partitioned between EtOAc (30 mL) and water (30 mL). The organic phase was washed

with brine (2 x 30 mL), dried (Na_2SO_4), filtered, and concentrated to give 2.76 g (86%) of compound **2** as a pale amber oil, which was used without further purification.

*2.2.2. 3-(1,1-Dimethylethyl) 4-Methyl-(R)-2,2-dimethyl-3,4-oxazolidine-dicarboxylate (**3**)*

To a 250-mL round-bottomed flask were added a solution of compound **2** (2.76 g, 12.5 mmol) in benzene (75 mL), 2,2-dimethoxypropane (DMP, 2.61 g, 25 mmol), and *p*-TsOH (33 mg, 0.18 mmol). The colorless solution was heated under reflux for 1 h, then slowly distilled until a volume of 65 mL was collected over 30 min. The cooled, amber solution was partitioned between saturated $NaHCO_3$ solution (20 mL) and Et_2O (2 x 50 mL). The organic layer was washed with brine (20 mL), then dried (Na_2SO_4), filtered, and concentrated to give crude product **3** as an amber oil. The material was vacuum distilled to give 2.68 g (80%) of compound **3** as a pale yellow liquid, bp 101-102 °C (2 mm Hg); ^{1}H NMR ($CDCl_3$) δ 4.48-4.37 (m, 1H), 4.18-4.12 (m, 1H), 1.67-1.64 (m, 3H), 1.53-1.41 (m, 15H); ^{13}C NMR ($CDCl_3$) δ 171.4, 151.2, 95.1, 80.4, 66.3, 59.3, 52.4, 28.4, 28.3, 27.3, 26.0, 25.2, 25.0, 24.4.

*2.2.3. 1,1-Dimethylethyl (R)-4-Formyl-2,2-dimethyl-3-oxazolidinecarboxylate (**4**)*

A solution of compound **3** (2.68 g, 10 mmol) in toluene (25 mL) was cooled to -78 °C under nitrogen. To the cooled solution was slowly added a solution of 1.5 M DIBAL-H in toluene (12 mL, 18 mmol). The reaction mixture

was stirred for 2 h at -78 °C, and was then quenched by slowly adding 5 mL of cold MeOH. The resulting white emulsion was slowly poured into 50 mL of ice-cold 1 N HCl with swirling over 15 min, and the aqueous mixture was extracted with EtOAc (3 x 50 mL). The combined organic layers were washed with brine (50 mL), dried (Na_2SO_4), filtered, and concentrated to give the crude product as a colorless oil. The material was vacuum distilled to give 1.72 g (75%) of compound **4** as a colorless liquid, bp 83-88 °C (1.0 - 1.4 mm Hg).

*2.2.4. tert-Butyl (4R,1'R)-2,2-Dimethyl-4-(1'-hydroxyhexadec-2'-ynyl)-oxazolidione-3-carboxylate (**5**)*

n-Butyllithium (2.5 M in hexane, 2.0 mL, 5.0 mmol) was added dropwise to a solution of 1-pentadecyne (832 mg, 4.0 mmol) in dry Et_2O (20 mL) at -20 °C (see Scheme 2). After the white suspension was stirred at -20 °C for 1 h, anhydrous $ZnBr_2$ (1.2 g, 5.0 mmol) was added at 0 °C, with stirring for 1 h at 0 °C and 1 h at rt. A solution of compound **4** (690 mg, 3.0 mmol) in dry Et_2O (10 mL) was added dropwise at -78 °C. The reaction mixture was allowed to warm to rt overnight. The reaction was quenched by the addition of saturated aqueous NH_4Cl solution (20 mL) at -20 °C. After dilution with water (20 mL), the aqueous layer was separated and extracted with Et_2O (2 x 20 mL). The combined organic layers were washed with brine (10 mL), dried (Na_2SO_4), filtered, and concentrated. The residue was purified by chromatography (elution with hexane/EtOAc 4:1) to give 814 mg (62%) of

compound **5**; R_f 0.45 (hexane/EtOAc 4:1); ^{1}H NMR ($CDCl_3$) δ 4.37-4.36 (m, 1H), 2.46-2.45 (m, 1H), 1.89-1.88 (m, 1H), 1.74-1.68 (m, 2H), 1.47-1.45 (m, 2H), 1.40-1.26 (m, 37H), 0.88 (t, 3H, J = 7.2 Hz); ^{13}C NMR ($CDCl_3$) δ 82.7, 70.4, 60.0, 35.3, 29.5, 27.3, 27.2, 27.16, 27.12, 27.0, 26.9, 22.6, 20.3, 11.7.

*2.2.5. tert-Butyl (1R,2R)-N-[2-Hydroxy-1-(hydroxymethyl)-3-heptadecynyl]-carbamate (**6**)*

To a solution of 0.70 g (1.60 mmol) of compound **5** in 10 mL of MeOH was added 0.50 g of Amberlyst 15 resin. After the heterogeneous mixture was stirred at rt for 48 h, the mixture was filtered through a Celite pad, and the filtrate was concentrated. Purification by chromatography (elution with hexane/EtOAc 1:1) gave 480 mg (75%) of compound **6** as a white solid; R_f 0.52 (hexane/EtOAc 1:1); ^{1}H NMR ($CDCl_3$) δ 5.18 (m, 1H), 4.60 (s, 1H), 3.83-3.77 (m, 3H), 3.35 (s, 1H), 2.91 (s, 1H), 2.22-2.18 (m, 2H), 1.52-1.30 (m, 31H), 0.88 (t, 3H, J = 7.2 Hz); ^{13}C NMR ($CDCl_3$) δ 156.4, 87.4, 80.0, 78.1, 63.6, 62.9, 55.9, 31.9, 29.7, 29.6, 29.5, 29.3, 29.1, 28.9, 28.6, 28.3, 28.1, 22.7, 18.7, 14.1.

*2.2.6. tert-Butyl (3E,1R,2R)-N-[2-Hydroxy-1-(hydroxymethyl)-3-heptadecenyl]-carbamate (**7**)*

To a solution of 440 mg (1.0 mmol) of compound **6** in dry Et_2O (20 mL) was added dropwise 3.0 mL (10.5 mmol) of Red-Al (a 3.5 M solution in toluene) at 0 °C under nitrogen. After the reaction mixture was stirred at rt for 24 h, the reaction was quenched by the slow addition of 3 mL of MeOH at 0 °C. The product was extracted with EtOAc (3 x 20 mL), and the combined

organic layers were washed with brine (10 mL), dried (Na_2SO_4), filtered, and concentrated. The residue was purified by chromatography (elution with hexane/EtOAc 1:1) to give 264 mg (60%) of compound **7** as a white solid; R_f 0.38 (hexane/EtOAc 1:1); ^{1}H NMR ($CDCl_3$) δ 5.75 (m, 1H), 5.53 (m, 1H), 5.18 (m, 1H), 4.33 (s, 1H), 3.80-3.55 (m, 3H), 2.71 (s, 2H), 2.05 (m, 2H), 1.45-1.05 (m, 31H), 0.88 (t, 3H, J = 6.8 Hz); ^{13}C NMR ($CDCl_3$) δ 156.6, 134.0, 129.0, 79.8, 73.5, 64.4, 55.5, 32.3, 31.9, 29.69, 29.66, 29.6, 29.5, 29.4, 29.2, 29.1, 28.4, 22.7, 14.1.

*2.2.7. D-threo-Sphingosine (**8**)*

A solution of 240 mg (0.60 mmol) of compound **7** in 5 mL of 1 M HCl and 5 mL of THF was heated at 70 °C with stirring for 8 h under nitrogen. The reaction mixture was cooled to rt and neutralized with saturated aqueous $NaHCO_3$ solution (5 mL). The product was extracted with EtOAc (3 x 20 mL), and the combined organic layer was washed with brine, dried (Na_2SO_4), filtered, and concentrated to give 140 mg (78%) of compound **8** as a white powder, which was used without further purification.

*2.2.8. (2R,3R,4E)-2-Azido-octadec-4-ene-1,3-diol (**9**)*

Dichloromethane (10 mL) and DMAP (150 mg, 1.23 mmol) were added to compound **8** (120 mg, 0.40 mmol), followed by dropwise addition of TfN_3 in CH_2Cl_2 (0.4 M solution, 10 mL, 4.0 mmol) (see Scheme 3). The reaction mixture was stirred at rt for 24 h, and then concentrated under reduced pressure. The residue was purified by chromatography (elution with

hexane/EtOAc 1:1) to give 60 mg (46%) of azido diol **9**; R_f 0.60 (hexane/EtOAc 1:1); $[\alpha]^{25}_D$ -3.05° (*c* 2.59, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 5.78 (m, 1H), 5.52 (m, 1H), 4.21 (m, 1H), 3.80 (m, 1H), 3.72 (m, 1H), 2.42 (s, 2H), 2.07 (m, 2H), 1.45-1.18 (m, 22H), 0.88 (t, 3H, *J* = 6.8 Hz); ^{13}C NMR ($CDCl_3$) δ 135.5, 128.2, 73.5, 67.6, 62.9, 32.3, 31.9, 29.7, 29.6, 29.5, 29.47, 29.36, 29.2, 29.1, 28.9, 22.7, 14.1.

*2.2.9. (2R,3R,4E)-2-Azido-1-(tert-butyldiphenylsilanyloxy)-octadec-4-en-3-ol (**10**)*

A solution of TBDPSCl (50 mg, 0.18 mmol) and imidazole (25 mg, 0.36 mmol) in 10 mL of CH_2Cl_2 was stirred at rt for 1 h. A solution of compound **9** (55 mg, 0.167 mmol) in 5 mL of CH_2Cl_2 was added, and the reaction mixture was stirred overnight. The solvent was removed under reduced pressure, and the residue was purified by chromatography (elution with hexane/EtOAc 4:1) to give 68 mg (91%) of compound **10**; R_f 0.57 (hexane/EtOAc; 4:1); $[\alpha]^{25}_D$ -11.92° (*c* 2.50, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 7.68-7.66 (m, 4H), 7.45-7.34 (m, 6H), 5.70 (m, 1H), 5.40 (m, 1H), 4.12 (m, 1H), 3.80 (m, 2H), 3.41 (m, 1H), 2.16 (s, 1H), 1.99 (m, 2H), 1.40-1.01 (m, 31H), 0.88 (t, 3H, *J* = 6.8 Hz); ^{13}C NMR ($CDCl_3$) δ 136.1, 135.63, 135.59, 135.2, 132.9, 132.8, 129.9, 129.7, 129.5, 128.3, 127.8, 127.7, 127.6, 127.3, 72.3, 67.9, 64.6, 32.3, 31.9, 29.70, 29.68, 29.6, 29.5, 29.4, 29.2, 29.1, 28.9, 28.4, 26.8, 22.7, 19.2, 14.1.

*2.2.10. (1'R,1R,2E)-Benzoic Acid 1-[1'-Azido-2'-(tert-butyldiphenylsilyloxy)-ethyl]-hexadec-2-enyl Ester (**11**)*

To a solution of compound **10** (65 mg, 0.115 mmol) in 10 mL of dry CH_2Cl_2 was added DMAP (50 mg, 0.40 mmol), followed by the dropwise addition of a solution of benzoic anhydride (45 mg, 0.20 mmol) in 5 mL of CH_2Cl_2 at 0 °C. The reaction mixture was allowed to warm to rt and stirred overnight. The solvent was removed under reduced pressure, and the residue was purified by chromatography (elution with hexane/EtOAc 19:1) to give 70 mg (92%) of compound **11**; R_f 0.80 (hexane/EtOAc 4:1); $[\alpha]^{25}_D$ -4.82° (*c* 3.90, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 8.00 (m, 2H), 7.68-7.60 (m, 4H), 7.55 (m, 1H), 7.45-7.28 (m, 8H), 5.87 (m, 1H), 5.63 (m, 1H), 5.43 (m, 1H), 4.12 (m, 1H), 3.80 (m, 2H), 3.62 (m, 1H), 2.00 (m, 2H), 1.40-1.01 (m, 31H), 0.88 (t, 3H, *J* = 6.8 Hz); ^{13}C NMR ($CDCl_3$) δ 165.3, 137.7, 135.9, 133.3, 133.2, 133.1, 132.9, 132.8, 74.1, 65.9, 63.3, 32.3, 32.0, 29.7, 29.6, 29.5, 29.4, 29.2, 29.1, 28.9, 28.7, 26.9, 26.7, 22.7, 19.2, 14.1.

*2.2.11. (1'R,1R,2E)-Benzoic Acid 1-(1'-Azido-2'-hydroxyethyl)hexadec-2-enyl Ester (**12**)*

To a solution of compound **11** (68 mg, 0.10 mmol) and 50 mg (0.72 mmol) of imidazole in 5 mL of dry THF was added TBAF (0.2 mL, 0.20 mmol, a 1 M solution in THF) at -23 °C. The reaction mixture was stirred at -23 °C for 3 h, and was then quickly passed (to minimize benzoyl migration) through a silica gel column that was prewashed with cold elution solvent (elution with hexane/EtOAc 4:1) to give 26 mg (60%) of compound **12**; R_f 0.35 (hexane/EtOAc 4:1); $[\alpha]^{25}_D$ -8.34° (*c* 0.42, $CHCl_3$). ^{1}H NMR ($CDCl_3$) δ 8.08 (m,

2H), 7.72-7.37 (m, 3H), 5.98 (m, 1H), 5.65 (m, 1H), 5.56 (m, 1H), 3.70 (m, 3H), 2.17 (m, 1H), 2.06 (m, 2H), 1.53-1.24 (m, 22H), 0.88 (t, 3H, *J* = 6.8 Hz); ^{13}C NMR ($CDCl_3$) δ 165.8, 138.1, 134.8, 133.4, 129.9, 129.6, 128.5, 127.7, 124.0, 74.7, 66.2, 61.7, 32.3, 31.9, 29.7, 29.6, 29.42, 29.37, 29.2, 28.7, 26.6, 22.7, 14.1.

*2.2.12. (1'R,1R,2E)-Benzoic Acid 1-[1'-Azido-2'-(β-hepta-O-acetyllactosyl)-ethyl]-hexadec-2-enyl Ester (**14**)*

A mixture of 53 mg (0.068 mmol) of trichloroacetimidate **13** (see Scheme 4), 25 mg (0.058 mmol) of compound **12**, 200 mg of molecular sieves 300AW, and 5 mL of CH_2Cl_2 was stirred at rt for 1 h. A solution of $BF_3{\cdot}Et_2O$ (40 μL, 0.32 mmol) in 5 mL of CH_2Cl_2 was added, and the reaction mixture was stirred overnight. The solvent was removed under reduced pressure, and the residue was purified by chromatography (elution with hexane/EtOAc 1:1) to give 40 mg (66%) of compound **14**; R_f 0.55 (hexane/EtOAc 1:1); $[\alpha]^{25}_D$ -13.1° (*c* 2.0, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 8.07 (m, 2H), 7.62-7.41 (m, 3H), 5.88 (m, 1H), 5.60 (m, 1H), 5.48 (m, 1H), 5.35 (m, 1H), 5.24-5.08 (m, 2H), 4.93 (m, 2H), 4.50 (m, 3H), 4.11 (m, 4H), 3.86 (m, 3H), 3.62 (m, 2H), 2.30-1.90 (m, 21H), 1.80-1.00 (m, 24H), 0.89 (t, 3H, *J* = 6.8 Hz); ^{13}C NMR ($CDCl_3$) δ 170.4, 170.2, 170.1, 169.8, 169.7, 169.1, 135.0, 124.1, 101.1, 100.7, 72.8, 72.7, 71.5, 71.0, 70.7, 69.1, 66.6, 61.7, 60.8, 32.6, 31.9, 29.69, 29.66, 29.5, 29.4, 29.3, 29.2, 22.7, 20.9, 20.8, 20.7, 20.6, 20.5, 14.1.

*2.2.13. (2R,3R,4E)-2-Azido-1-(β-hepta-O-acetyllactosyl)-octadec-4-en-3-ol (**15**)*

A solution of 6 mg (0.26 mmol) of sodium in 1 mL of MeOH was added to 38 mg (0.036 mmol) of compound **14**. The reaction mixture was stirred for 6 h, the solvent was removed under reduced pressure, and the residue was purified by chromatography (elution with MeOH/$CHCl_3$ 3:7) to give 16 mg (68%) of compound **15**; R_f 0.40 (MeOH/$CHCl_3$ 3:7); $[\alpha]^{25}_D$ -10.6° (*c* 0.80, $CHCl_3$/MeOH 1:1); ^{1}H NMR ($CDCl_3$/CD_3OD) δ 5.58-5.30 (m, 2H), 5.03 (m, 1H), 4.15-2.95 (m, 17H), 1.00 (m, 24H), 0.89 (t, 3H, *J* = 6.8 Hz); HRMS (ESI) calcd for $C_{30}H_{55}N_3O_{12}Na$ $(M + Na)^+$ *m/z* 672.3683, found 672.3666.

*2.2.14. C_5-BODIPY-D-threo-LacCer (**16**)*

BODIPY-C_5-NHS (5 mg, 0.020 mmol), triphenylphosphine (6 mg, 0.023 mmol), 2.7 mL of THF, and 0.3 mL of water were added to 13 mg (0.020 mmol) of compound **15**. After the reaction mixture was stirred overnight at rt, the solvents were removed under reduced pressure, and the residue was purified by chromatography (elution with MeOH/$CHCl_3$ 1:4). After suspended silica gel was removed, 7 mg (38%) of compound **16** were obtained; R_f 0.35 (MeOH/$CHCl_3$ 1:4); ^{1}H NMR ($CDCl_3$/CD_3OD) δ 7.60 (m, 2H), 7.07 (s, 1H), 6.87 (s, 1H), 6.23 (m, 1H), 6.04 (m, 1H), 3.83-2.15 (m, 18H), 1.70-0.70 (m, 35H); LRMS (APCI, negative-ion mode) calcd for $C_{46}H_{74}BClF_2N_3O_{13}$ $(M + {}^{35}Cl)^-$ *m/z* 960.5, found 960.5; HRMS (EI) calcd for $C_{46}H_{75}N_3O_{13}F_2B$ (MH^+ of the boron-10 isotope) *m/z* 925.5397, found 925.5408.

*2.2.15. tert-Butyl (4R,1'S)-2,2-Dimethyl-4-(1'-hydroxyhexadec-2'-ynyl)-oxazolidione-3-carboxylate (**17**)*

n-Butyllithium (2.5 M in hexane, 2.0 mL, 5.0 mmol) was added dropwise to a solution of 1-pentadecyne (832 mg, 4.0 mmol) in dry THF (20 mL) at -20 °C (see Scheme 5). After the mixture was stirred at -20 °C for 2 h, HMPA (0.73 mL, 5.0 mmol) was added, followed by a solution of compound **4** (690 mg, 3.0 mmol) in dry THF (10 mL) at -78 °C. The reaction mixture was stirred for 1 h at -78 °C, allowed to warm to -20 °C within 2 h, and quenched by the addition of saturated aqueous NH_4Cl solution (20 mL). The mixture was diluted with water (20 mL), and the aqueous layer was separated and extracted with Et_2O (3 x 20 mL). The combined organic layers were washed with 0.5 N HCl (2 x 10 mL) and brine (10 mL), dried (Na_2SO_4), filtered, and concentrated. The residue was purified by chromatography (elution with hexane/EtOAc 4:1) to give 788 mg (60%) of compound **17**; R_f 0.48 (hexane/EtOAc 4:1); ^{1}H NMR ($CDCl_3$) δ 4.74 (m, 1H), 4.51 (m, 1H), 4.10 (m, 2H), 3.90 (s, 1H), 2.19 (m, 2H), 1.65-1.45 (m, 15H), 1.40-1.20 (m, 22H), 0.88 (t, 3H, J = 6.8 Hz); ^{13}C NMR ($CDCl_3$) δ 152.1, 92.9, 84.6, 79.2, 75.9, 63.1, 62.1, 60.9, 29.9, 27.7, 27.6, 27.5, 27.4, 27.1, 26.9, 26.6, 26.4, 26.0, 23.8, 23.4, 21.0, 20.7,16.8, 12.1.

*2.2.16. tert-Butyl (1R,2S)-N-[2-Hydroxy-1-(hydroxymethyl)-3-heptadecynyl]-carbamate (**18**)*

To a solution of 0.50 g (1.14 mmol) of compound **17** in 10 mL of MeOH was added 0.40 g of Amberlyst 15 resin, and the heterogeneous mixture was stirred at rt for 48 h. The mixture was filtered through a Celite pad, and the filtrate was concentrated. Purification by chromatography (elution with hexane/EtOAc 1:1) afforded 329 mg (73%) of compound **18** as a white solid, which was used without further purification; R_f 0.55 (hexane/EtOAc 1:1).

*2.2.17. tert-Butyl (3E,1R,2S)-N-[2-Hydroxy-1-(hydroxymethyl)-3-heptadecenyl]-carbamate (**19**)*

To a solution of 300 mg (0.76 mmol) of compound **18** in dry Et_2O (20 mL) was added dropwise 2.0 mL (7.0 mmol) of Red-Al (a 3.5 M solution in toluene) at 0 °C under nitrogen. After the reaction mixture was stirred at rt for 24 h, the reaction was quenched by the slow addition of 3 mL of MeOH at 0 °C. The product was extracted with EtOAc (3 x 20 mL), and the combined organic layers were washed with brine (10 mL), dried (Na_2SO_4), filtered, and concentrated. The residue was purified by chromatography (elution with hexane/EtOAc 1:1) to give 181 mg (60%) of compound **19** as a white solid; R_f 0.40 (hexane/EtOAc 1:1); ^{1}H NMR ($CDCl_3$) δ 5.78 (m, 1H), 5.52 (m, 1H), 5.33 (m, 1H), 4.28 (m, 1H), 3.90 (m, 1H), 3.70 (m, 1H), 3.60 (s, 1H), 3.05 (s, 2H), 2.05 (m, 2H), 1.50-1.30 (m, 31H), 0.88 (t, 3H, J = 6.8 Hz); ^{13}C NMR ($CDCl_3$) δ 156.3, 134.1, 129.0, 79.8, 74.7, 62.61, 55.47, 32.3, 31.9, 31.7, 29.70, 29.67, 29.6, 29.5, 29.4, 29.3, 29.2, 28.4, 22.7, 14.1.

*2.2.18. L-erythro-Sphingosine (**20**)*

A solution of 160 mg (0.40 mmol) of compound **19** in 5 mL of 1 M HCl and 5 mL of THF was heated at 70 °C with stirring for 8 h under nitrogen. The reaction mixture was cooled to rt and neutralized with saturated aqueous $NaHCO_3$ solution (5 mL). The product was extracted with EtOAc (3 x 20 mL), and the combined organic layer was washed with brine, dried (Na_2SO_4), filtered, and concentrated to give 90 mg (75%) compound **20** as a white powder, which was used without further purification.

*2.2.19. (2R,3S,4E)-2-Azido-octadec-4-ene-1,3-diol (**21**)*

The diazo transfer reaction was carried out as described for the synthesis of compound **9**. To compound **20** (80 mg, 0.27 mmol) were added CH_2Cl_2 (10 mL) and DMAP (100 mg, 0.82 mmol), followed by the dropwise addition of a solution of TfN_3 in CH_2Cl_2 (0.4 M solution, 7.0 mL, 2.8 mmol) (see Scheme 6), with stirring at rt for 24 h. Concentration gave a residue that was purified by chromatography (elution with hexane/EtOAc 1:1), affording 40 mg (46%) of azido diol **21**; R_f 0.50 (hexane/EtOAc 1:1); $[\alpha]^{25}_D$ +25.2° (*c* 0.80, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 5.80 (m, 1H), 5.53 (m, 1H), 4.25 (m, 1H), 3.78 (m, 2H), 3.50 (m, 1H), 2.23 (s, 2H), 2.08 (m, 2H), 1.40-1.20 (m, 22H), 0.88 (t, 3H, *J* = 7.2 Hz); ^{13}C NMR ($CDCl_3$) δ 136.0, 128.0, 73.8, 66.8, 62.6, 32.3, 31.9, 29.69, 29.66, 29.6, 29.5, 29.4, 29.2, 28.9, 22.7, 14.1.

*2.2.20. (2R,3S,4E)-2-Azido-1-(tert-butyldiphenylsilyloxy)-octadec-4-en-3-ol (**22**)*

The silylation reaction was carried out as described for the preparation of compound **10**. A solution of compound **21** (39 mg, 0.12 mmol) in 5 mL of

CH_2Cl_2 was added to TBDPSCl (35 mg, 0.13 mmol) and imidazole (18 mg, 0.26 mmol) in 10 mL of CH_2Cl_2. The reaction mixture was stirred overnight, the solvent was removed under reduced pressure, and the residue was purified by chromatography (elution with hexane/EtOAc 4:1) to give 56 mg (83%) of compound **22**; R_f 0.60 (hexane/EtOAc 4:1); $[\alpha]^{25}_D$ +10.7° (*c* 0.80, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 7.68-7.66 (m, 4H), 7.45-7.34 (m, 6H), 5.72 (m, 1H), 5.43 (m, 1H), 4.22 (m, 1H), 3.79 (m, 2H), 3.51 (m, 1H), 2.11 (s, 1H), 2.01 (m, 2H), 1.36-1.01 (m, 31H), 0.88 (t, 3H, *J* = 6.8 Hz); ^{13}C NMR ($CDCl_3$) δ 134.1, 134.0, 133.7, 133.6, 133.5, 131.9, 131.7, 131.2, 130.9, 128.0, 127.8, 127.6, 125.9, 125.8, 70.9, 65.0, 62.2, 50.3, 30.4, 30.0, 27.8, 27.7, 27.6, 27.5, 27.3, 27.1, 25.0, 24.9, 24.8, 20.8, 17.3, 17.2, 12.2.

*2.2.21. (1'R,1S,2E)-Benzoic Acid 1-[1'-Azido-2'-(tert-butyldiphenylsilanyloxy)-ethyl]-hexadec-2-enyl Ester (**23**)*

Compound **23** was prepared by the method used to synthesize compound **11**. DMAP (40 mg, 0.32 mmol) was added to a solution of compound **22** (50 mg, 0.089 mmol) in 10 mL of dry CH_2Cl_2, followed by the dropwise addition of a solution of benzoic anhydride (34 mg, 0.15 mmol) in 5 mL of CH_2Cl_2 at 0 °C. After the reaction mixture was stirred overnight rt, concentration gave a residue that was purified by chromatography (elution with hexane/EtOAc 19:1), affording 53 mg (89%) of compound **23**; R_f 0.85 (hexane/EtOAc 4:1); $[\alpha]^{25}_D$ +11.6° (*c* 0.71, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 8.01 (m, 2H), 7.68-7.50 (m, 5H), 7.48-7.28 (m, 8H), 5.90 (m, 1H), 5.68 (m, 1H), 5.50 (m,

1H), 3.90-3.70 (m, 4H), 2.00 (m, 2H), 1.50-1.01 (m, 31H), 0.88 (t, 3H, *J* = 6.8 Hz); ^{13}C NMR ($CDCl_3$) δ 165.2, 135.6, 135.4, 133.1, 132.9, 132.7, 130.1, 129.8, 129.7. 128.4, 127.8, 123.2, 74.1, 65.9, 63.3, 31.9, 29.7, 29.6, 29.4, 19.1, 28.7, 26.7, 22.7, 19.2, 14.1.

*2.2.22. (1'R,1S,2E)-Benzoic Acid 1-(1'-Azido-2'-hydroxyethyl)hexadec-2-enyl Ester (**24**)*

The desilylation reaction was carried out as described for the preparation of compound **11** (2 equiv of TBAF, ~7 equiv of imidazole, dry THF, -23 °C). The reaction mixture was stirred at -23 °C for 3 h, and then was quickly passed through a silica gel column that was prewashed with cold elution solvent (elution with hexane/EtOAc 4:1) to give 19 mg (59%) of compound **24**; R_f 0.30 (hexane/EtOAc 4:1); $[\alpha]^{25}_D$ +38.2° (*c* 0.40, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 8.06 (m, 2H), 7.60-7.44 (m, 3H), 5.94 (m, 1H), 5.61 (m, 2H), 3.80 (m, 2H), 3.64 (m, 1H), 2.10 (m, 3H), 1.50-1.24 (m, 22H), 0.88 (t, 3H, *J* = 6.8 Hz); ^{13}C NMR ($CDCl_3$) δ 165.5, 138.9, 133.4, 129.8, 129.7, 128.5, 123.3, 74.6, 66.2, 62.0, 32.4, 31.9, 29.7, 29.6, 29.43, 29.37, 29.2, 28.7, 22.7, 14.1.

*2.2.23. (1'R,1S,2E)-Benzoic Acid 1-[1'-Azido-2'-(β-heptaacetyllactosyl)-ethyl]-hexadec-2-enyl Ester (**25**)*

A mixture of 53 mg (0.068 mmol) of trichloroacetimidate **13**, 19 mg (0.044 mmol) of compound **24**, 100 mg of molecular sieves 300AW, and 5 mL of CH_2Cl_2 was stirred at rt for 1 h (see Scheme 7). Then a solution of $BF_3{\cdot}OEt_2$ (40 μL, 0.32 mmol) in 5 mL of CH_2Cl_2 was added, and the reaction mixture

was stirred overnight. The solvent was removed under reduced pressure, and the residue was purified by chromatography (elution with hexane/EtOAc 1:1) to give 25 mg (54%) of compound **25**; R_f 0.50 (hexane/EtOAc 1:1); ^{1}H NMR ($CDCl_3$) δ 7.97 (m, 2H), 7.56-7.38 (m, 3H), 5.80 (m, 1H), 5.63 (m, 1H), 5.50 (m, 1H), 5.40 (m, 1H), 5.28 (m, 1H), 5.10 (m, 2H), 4.89 (m, 2H), 4.45 (m, 3H), 4.05 (m, 4H), 3.80 (m, 3H), 3.55 (m, 2H), 2.10-1.86 (m, 21H), 1.35-1.12 (m, 24H), 0.80 (t, 3H, J = 6.8 Hz); ^{13}C NMR ($CDCl_3$) δ 170.4, 170.2, 170.1, 169.8, 169.7, 169.5, 135.0, 124.1, 101.1, 100.7, 72.8, 72.6, 71.5, 71.0, 70.7, 69.1, 66.8, 61.7, 60.8, 32.6, 31.9, 29.8, 29.7, 29.5, 29.4, 29.3, 29.2, 22.7, 20.9, 20.8, 20.7, 20.6, 20.5, 14.1.

*2.2.24. (2R,3R,4E)-2-Azido-1-(β-hepta-O-acetyllactosyl)-octadec-4-en-3-ol (**26**)*

A solution of 5.0 mg (0.22 mmol) of sodium in 1 mL of MeOH was added to 21 mg (0.020 mmol) of compound **25**. After the reaction mixture was stirred for 6 h, the solvent was removed to give 8 mg (62%) of compound **26**. HRMS (ESI) calcd for $C_{30}H_{55}N_3O_{12}Na$ $(M + Na)^+$ *m/z* 672.3683, found 672.3682.

*2.2.25. C_5-BODIPY-L-erythro-LacCer (**27**)*

A mixture of 8 mg (0.012 mmol) of compound **26**, BODIPY-C_5-NHS (5.0 mg, 0.020 mmol), triphenylphosphine (6.0 mg, 0.023 mmol), 2.7 mL of THF, and 0.3 mL of water was stirred overnight at rt. Removal of the solvents gave a residue that was purified by chromatography (elution with

MeOH/$CHCl_3$ 1:1), to afford 4 mg (36%) of compound **27** after removal of suspended silica gel; R_f 0.38 (MeOH/$CHCl_3$ 1:4); ^{1}H NMR ($CDCl_3$/CD_3OD) δ 7.78-6.04 (m, 4H), 6.23 (m, 1H), 6.04 (m, 1H), 3.83-2.05 (m, 18H), 1.70-0.70 (m, 35H); LRMS (APCI, negative-ion mode) calcd for $C_{46}H_{74}BClF_2N_3O_{13}$ (M + $^{35}Cl)^-$ *m/z* 960.5, found 960.5; HRMS (EI) calcd for $C_{46}H_{75}N_3O_{13}F_2B$ (MH^+ of the boron-10 isotope) *m/z* 925.5397, found 925.5409.

*2.2.26. 2-Azido-L-threo-sphingosine (**29**)*

TfN_3 (1 mL, 0.4 mmol, a 0.4 M solution in CH_2Cl_2) was added dropwise to L-*threo*-sphingosine (compound **28**, 25 mg, 0.084 mmol) and DMAP (20 mg, 0.164 mmol) in 5 mL of CH_2Cl_2 (see Scheme 8). The reaction mixture was stirred at rt for 24 h, and then concentrated to give a residue that was purified by chromatography (elution with hexane/EtOAc 3:1), affording 26 mg (95%) of compound **29**; R_f 0.82 (hexane/EtOAc 1:1); ^{1}H NMR ($CDCl_3$) δ 5.69 (dt, 1H, *J* = 10.8, 7.2 Hz), 5.48 (dd, 1H, *J* = 10.8, 8.8 Hz), 4.62 (m, 1H), 3.81 (m, 2H), 3.52 (m, 1H), 2.13 (m, 2H), 1.45-1.20 (m, 22H), 0.91 (t, 3H, *J* = 7.2 Hz); ^{13}C NMR ($CDCl_3$) δ 136.0, 127.5, 68.2, 66.9, 62.6, 31.9, 29.69, 29.66, 29.6, 29.5, 29.4, 29.3, 28.0, 22.7, 14.1.

*2.2.27. 2-Azido-1-(tert-butyldiphenylsilanyloxy)-L-threo-sphingosine (**30**)*

Compound **30** was prepared by the method used to synthesize compound **10**. A solution of compound **29** (26 mg, 0.080 mmol) in 5 mL of CH_2Cl_2 was added to TBDPSCl (23 mg, 0.084 mmol) and imidazole (12 mg, 0.17 mmol) in 5 mL of CH_2Cl_2. The reaction mixture was stirred overnight, the

solvent was removed, and the residue was purified by chromatography (elution with hexane/EtOAc from 9:1 to 4:1) to give 39 mg (91%) of compound **30**; R_f 0.77 (hexane/EtOAc 4:1); ^{1}H NMR ($CDCl_3$) δ 7.71 (m, 4H), 7.46 (m, 6H), 5.64 (dt, 1H, *J* = 10.8, 7.2 Hz), 5.42 (dd, 1H, *J* = 10.8, 8.8 Hz), 4.61 (m, 1H), 3.84 (m, 2H), 3.56 (m, 1H), 2.01 (m, 2H), 1.61 (s, 1H), 1.40-1.06 (m, 31H), 0.91 (t, 3H, *J* = 6.8 Hz); ^{13}C NMR ($CDCl_3$) δ 135.8, 135.6, 134.6, 129.9, 129.1, 127.9, 127.5, 67.5, 66.0, 64.1, 32.0, 29.7, 29.4, 28.0, 26.9, 26.8, 22.7, 19.1, 14.1.

*2.2.28. 2-Azido-3-benzoic acid-1-(tert-butyldiphenylsilanyloxy)-L-threo-sphingosine (**31**)*

To a solution of compound **30** (38 mg, 0.067 mmol) in 5 mL of dry CH_2Cl_2 was added DMAP (25 mg, 0.20 mmol), followed by the dropwise addition of a solution of benzoic anhydride (18 mg, 0.080 mmol) in 5 mL of CH_2Cl_2 at 0 °C. The reaction mixture was stirred overnight, the solvent was removed, and the residue was purified by chromatography (elution with hexane, then with hexane/EtOAc 19:1) to give 38 mg (85%) of compound **31**; R_f 0.90 (hexane/EtOAc 4:1); ^{1}H NMR ($CDCl_3$) δ 8.04 (m, 2H), 7.71 (m, 4H), 7.46 (m, 9H), 6.06 (m, 1H), 5.77 (dt, 1H, *J* = 10.8, 7.2 Hz), 5.50 (dd, 1H, *J* = 10.8, 8.8 Hz), 3.82 (m, 3H), 2.25 (m, 2H), 1.50-1.10 (m, 31H), 0.93 (t, 3H, *J* = 6.8 Hz); ^{13}C NMR ($CDCl_3$) δ 162.8, 135.8, 133.5, 133.23, 133.19, 133.0, 132.4, 132.0, 130.7, 130.5, 130.4, 128.1, 127.7, 127.52, 127.51, 127.4, 127.2, 127.1, 126.1, 125.5, 125.44, 125.35, 125.1, 120.5, 67.0, 63.72, 63.67, 61.1, 29.6,

27.4, 27.34, 27.28, 27.2, 27.09, 27.06, 27.0, 25.9, 24.7, 24.5, 24.4, 23.3, 20.4, 16.8, 11.8. HRMS (ESI) calcd for $C_{41}H_{57}N_3O_3SiNa$ $(M + Na)^+$ *m/z* 690.4067, found 690.4081.

*2.2.29. 2-Azido-3-benzoic acid-L-threo-sphingosine (**32**)*

TBAF (0.1 mL, 0.1 mmol, 1 M in THF) was added to a solution of compound **31** (35 mg, 0.051 mmol) and 25 mg (0.36 mmol) of imidazole in 5 mL of dry CH_2Cl_2 at -23 °C. After being stirred at -23 °C for 3 h, the reaction mixture was quickly passed through a silica gel column that had been prewashed with cold elution solvent. Elution with hexane/EtOAc 4:1) afforded 14 mg (63%) of compound **32**; R_f 0.65 (hexane/EtOAc 4:1); ^{1}H NMR ($CDCl_3$) δ 8.09 (m, 2H), 7.46 (m, 3H), 6.00 (m, 1H), 5.75 (dt, 1H, *J* = 10.8, 7.2 Hz), 5.52 (dd, 1H, *J* = 10.8, 8.8 Hz), 4.51 (m, 2H), 3.88 (m, 1H), 2.13 (m, 2H), 1.50-1.10 (m, 31H), 0.93 (t, 3H, *J* = 6.8 Hz); ^{13}C NMR ($CDCl_3$) δ 166.4, 165.6, 138.4, 136.5, 135.2, 134.8, 133.4, 129.9, 129.8, 129.7, 129.5, 128.53, 128.51, 127.7, 126.7, 122.8, 69.6, 67.3, 66.4, 65.1, 64.3, 61.9, 32.0, 29.71, 29.68, 29.6, 29.50, 29.48, 29.4, 29.3, 28.2, 28.1, 26.6, 22.7, 19.0, 14.2.

*2.2.30. L-threo-C_5-BODIPY-LacCer (**33**)*

A mixture of 21 mg (0.027 mmol) of compound **32**, 12 mg (0.027 mmol) of trichloroacetimidate **13**, and 100 mg of molecular sieves 300AW in 5 mL of CH_2Cl_2 was stirred at rt for 1 h. A solution of $BF_3{\cdot}OEt_2$ (20 μL, 0.16 mmol) in 2 mL of CH_2Cl_2 was added, and the reaction mixture was stirred overnight. The solvent was removed under reduced pressure, and the residue was purified

by chromatography (elution with $CHCl_3$, then with MeOH/$CHCl_3$ 1:9) to give 9 mg (30%) of the glycosylation product. Alkaline methanolysis of the acetate and benzoate ester functionalities was carried out by adding a solution of 2 mg (0.08 mmol) of sodium in 1 mL of dry MeOH, followed by stirring at rt for 6 h. Dowex 50W-X8 resin (prewashed with 50 mL of MeOH) was added to neutralize the reaction mixture. The reaction mixture was filtered and solvent was removed under vacuum. After BODIPY-C_5-NHS (3 mg, 0.012 mmol), triphenylphosphine (4 mg, 0.016 mmol), 2.7 mL of THF, and 0.3 mL of water were added, the reaction mixture was stirred overnight at rt. The solvents were removed, and the residue was purified by chromatography (elution with MeOH/$CHCl_3$ from 1:9 to 1:4) to give 1.5 mg (38%) of compound **33**; R_f 0.45 (MeOH/$CHCl_3$ 1:4); ^{1}H NMR ($CDCl_3$): same as for compound **27**; HRMS (EI) calcd for $C_{46}H_{75}N_3O_{13}F_2B$ (MH^+ of the boron-10 isotope) *m/z* 925.5397, found 925.5416.

3. Results

3.1. Retrosynthetic plan

As shown in the retrosynthetic plan (Fig. 2), the preparation of the BODIPY-LacCer stereoisomers consists of three building blocks: a 2-azido-3-benzoylsphingosine derivative composed of the desired configurations at C2 and C3, an activated BODIPY-linked fatty acid, and an activated and

protected lactosyl donor (hepta-*O*-acetyl-β-lactosyl-1-trichloroacetimidate) (Amvam-Zollo and Sinay, 1986).

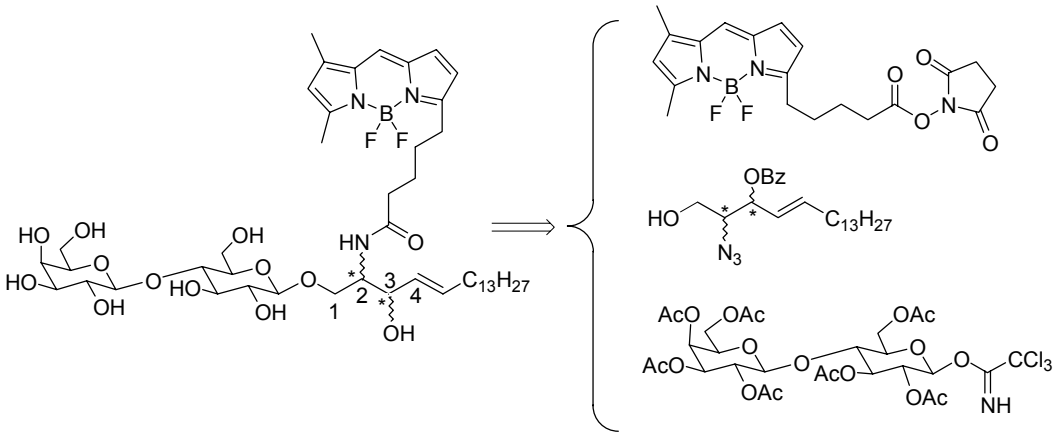
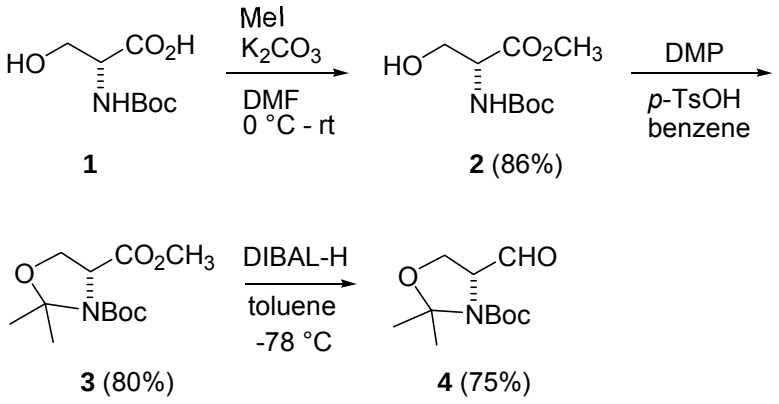
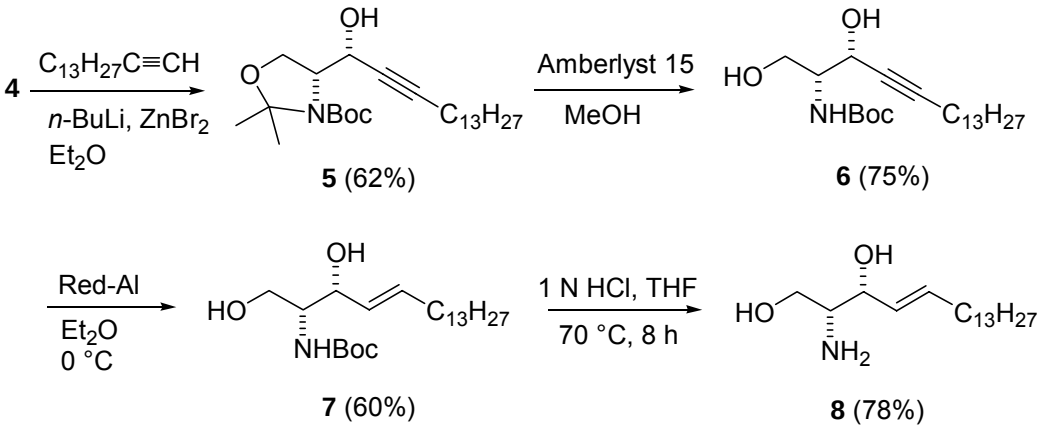


Fig. 2. Retrosynthetic plan for the preparation of D-*threo*- and L-*erythro*-C_5-BODIPY-Lac-Cer

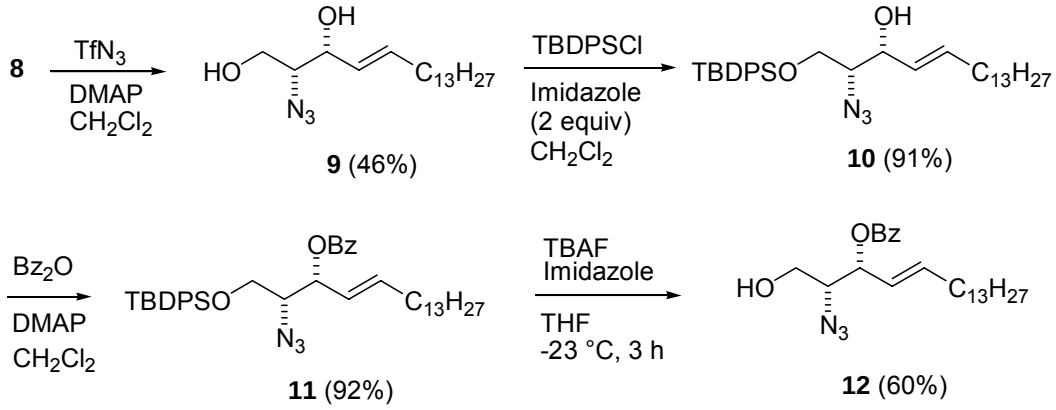
*3.2. Synthesis of D-threo C_5-BODIPY-Lac-Cer Analog (**16**)*



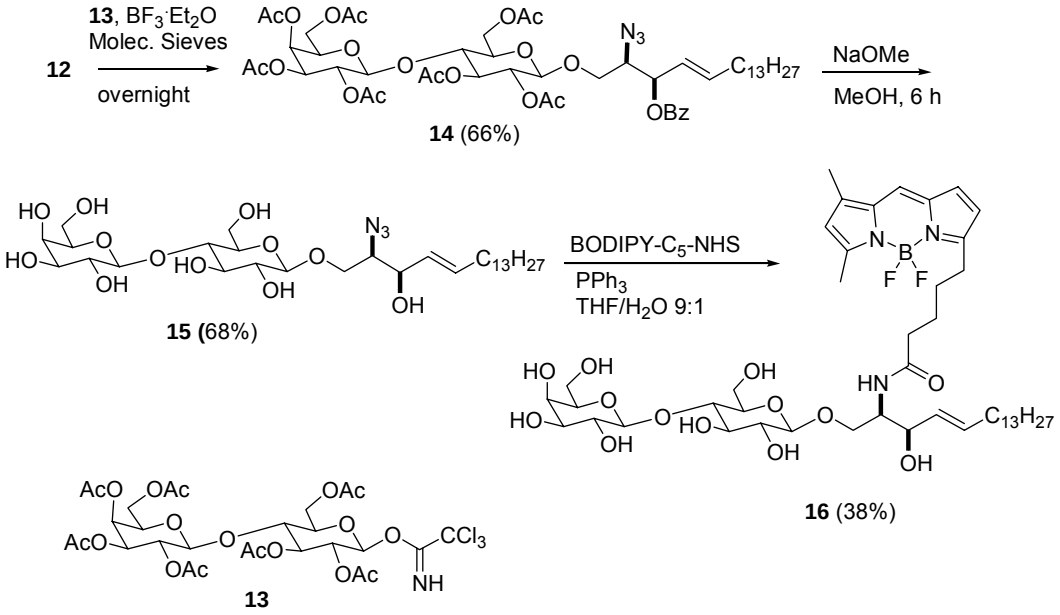
Scheme 1. Synthesis of (*R*)-Garner aldehyde (**4**)



Scheme 2. Synthesis of (2*R*,3*R*)-sphingosine (**8**)

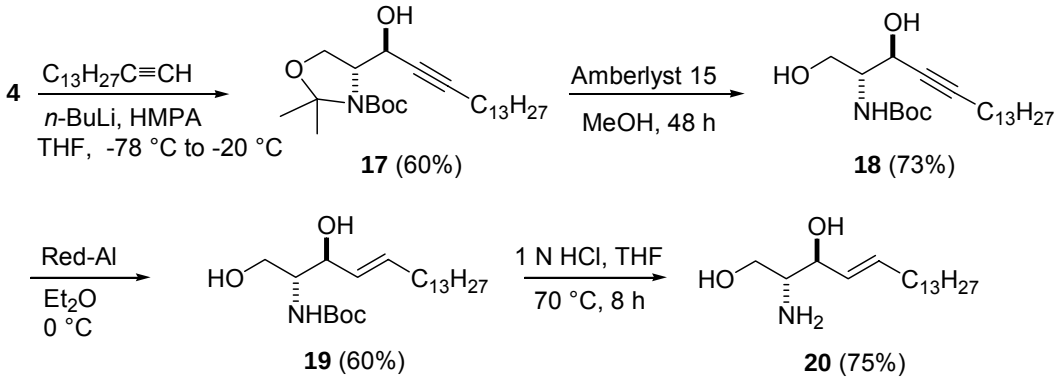


Scheme 3. Synthesis of (2*R*,3*R*)-2-azido-3-benzoylsphingosine (**12**)

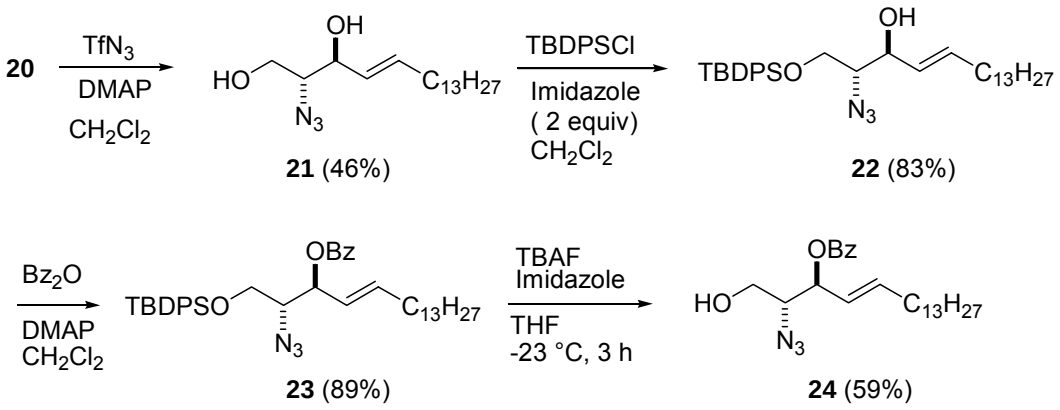


Scheme 4. Synthesis of (2*R*,3*R*)-C5-BODIPY-LacCer (**16**)

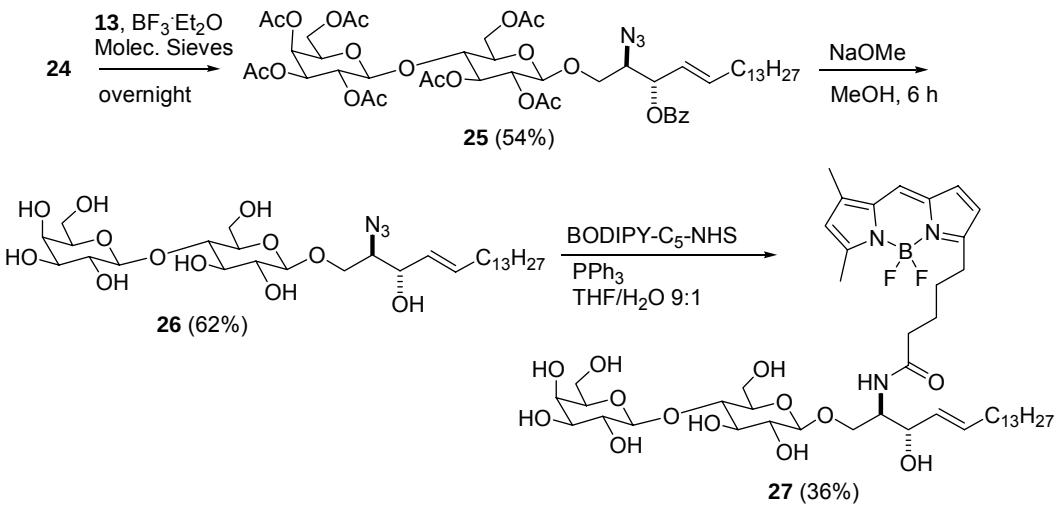
*3.3. Synthesis of L-erythro C_5-BODIPY-Lac-Cer Analog (**27**)*



Scheme 5. Synthesis of (2*R*,3*S*)-sphingosine (**20**)

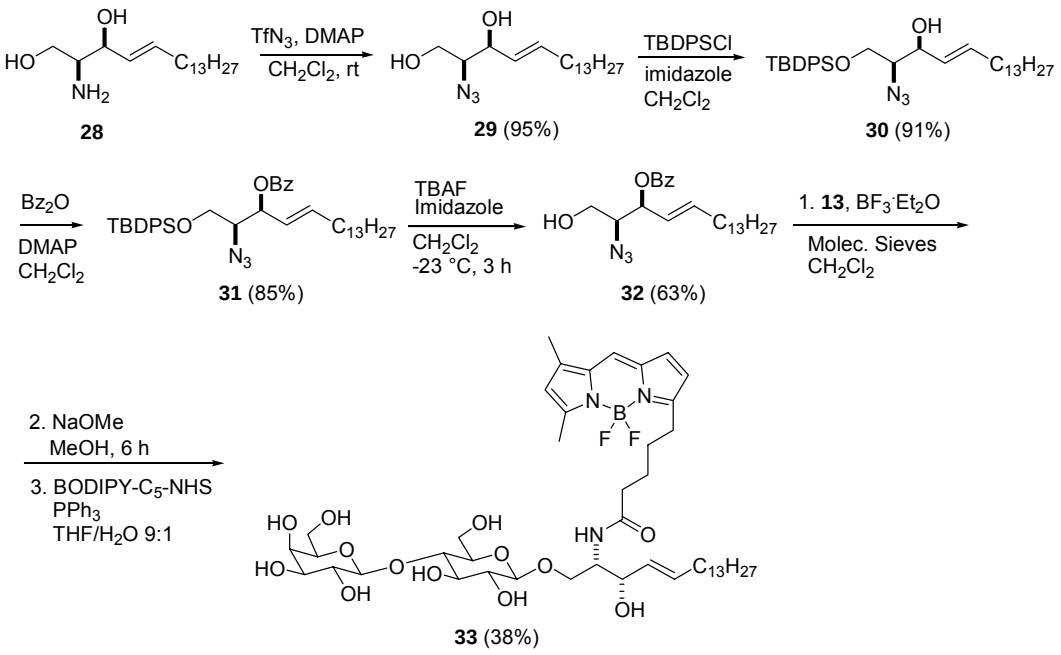


Scheme 6. Synthesis of (2*R*,3*S*)-2-azido-3-benzoylsphingosine (**24**)



Scheme 7. Synthesis of (2*R*,3*S*)-C_5-BODIPY-LacCer (**27**)

*3.4. Synthesis of L-threo C_5-BODIPY-Lac-Cer Analog (**33**)*



Scheme 8. Synthesis of L-*threo*-C_5-BODIPY-LacCer (**33**)

4. Summary

The (2*R*,3*R*) (or D-*threo*, compound **8**) and (2*R*,3*S*) (or L-*erythro*, compound **20**) sphingosines were synthesized as outlined in Schemes 2 and 5, respectively, from (*R*)-Garner aldehyde (compound **4**) (Garner et al., 1988; Garner and Park, 1987; Garner and Park, 1992). (*R*)-Garner aldehyde was prepared (see Scheme 1) from *N*-Boc-D-serine (**1**), which was converted to its methyl ester and then treated with 2,2-dimethoxypropane in the presence of *p*-toluenesulfonic acid in benzene, followed by DIBAL-H reduction at -78 °C. Compound **4** was reacted with lithium pentadecyne in the presence of zinc

bromide in Et_2O or HMPA in THF (Herold, 1988) to give the (2*R*,3*R*) product **5** or the (2*R*,3*S*) product **17**, respectively. The oxazolidine ring was opened with Amberlyst 15 resin, and Red-Al reduction (Van Overmeire et al., 1999) of the propargylic alcohol in Et_2O afforded (2*R*,3*R*)- and (2*R*,3*S*)-*N*-Boc-sphingosines (compounds **7** and **19**, respectively). The diazo transfer reaction afforded the stereoisomeric 2-azido sphingosine derivatives, compounds **9**, **21**, and **29**. Silylation of the primary hydroxy group was carried out in the presence of 2 equivalents of imidazole. After benzoylation of the secondary hydroxy group, the desilylation reaction was performed at -23 ºC (Mattjus et al., 2002), followed by rapid elution through a cold silica gel column, to minimize benzoyl migration (which was monitored by TLC, as there is a slight R_f difference between the primary and secondary alcohol), furnishing the three stereoisomers of 2-azido-3-benzoylsphingosines: compounds **12** (Scheme 3), **24** (Scheme 6), and **32** (Scheme 8). After $BF_3{\cdot}OEt_2$-mediated lactosylation of the 2-azido-3-benzoylsphingosine stereoisomers with hepta-*O*-acetyllactosyl trichloroacetimidate in CH_2Cl_2 in the presence of molecular sieves, base-catalyzed deprotection afforded the β-lactosyl-2-azidosphingosines (Zimmermann et al., 1988). Staudinger reduction of the azido group with triphenylphosphine in aqueous THF (Gololobov et al., 1981), followed by *N*-acylation with the *N*-hydroxysuccinimidoyl ester of BODIPY-C_5 and purification by column chromatography on silica gel (elution with $CHCl_3$/MeOH 4:1 vol/vol), furnished the target unnatural BODIPY-LacCer

stereoisomers: compounds **16** (Scheme 4), **27** (Scheme 7), and **33** (Scheme 8). NMR spectroscopy and mass spectrometry confirmed the structures of these analogs.

Chapter 2

Synthesis of Sphingosine 1-Phosphate-d_8 as an Internal Standard for Quantitative Analysis of Serum Sphingosine 1-Phosphate

Abstract

Isotopic analogs of sphingolipids are often used as internal standards for the quantitative analysis of natural molecules by GC/MS and LC/MS procedures. To satisfy the need for an internal standard of sphingosine 1-phosphate (S1P), we synthesized S1P-d_8 from THF-d_8.

1. Introduction

Stable isotope dilution mass spectrometry is used to quantify amounts of bioactive compounds in biological samples (Hayakawa et al., 2006). Sphingosine 1-phosphate (S1P, **1**) is currently considered to be a predictor of coronary artery disease, which is the leading cause of death in many countries (Deutschman et al., 2003). Although S1P is a constituent of normal serum, its level in the serum is elevated under conditions associated with coronary disease (such as hypoxia). In order to use stable isotope dilution mass spectrometry for quantification of **1** in biological samples, this chapter

describes the preparation of S1P-d8 (2) from THF-d_8. Compound **2** will be used as a stable isotope-labeled internal standard of **1.**

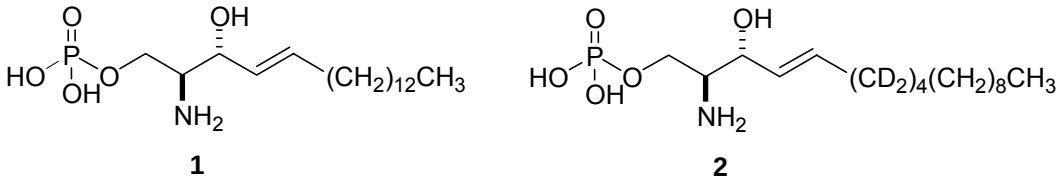


Fig. 1. S1P (**1**) and S1P-d_8 (**2**)

2. Experimental

2.1. Materials and analytical procedures

2.1.1. Chemicals

Tetrahydrofuran-d_8 (**3**) was purchased from Cambridge Isotope Laboratories (Andover, MA); hexamethylphosphorous triamide, *p*-toluenesulfonic acid monohydrate (*p*-TsOH), and sodium bis(2-methoxyethoxy) aluminum hydride (Red-Al, a 70% w/w solution in toluene) were from Alfa Aesar/Lancaster (Pelham, NH); bromotrimethylsilane, lithium tetrachlorocuprate(II) (a 0.1 M solution in THF), Amberlyst 15 ion-exchange resin, and lithium acetylide/ethylenediamine complex were from Sigma-Aldrich; triphenylphosphine and *N*-bromosuccinimide were from Acros; 1-bromononane was from TCI America (Portland, OR); 3,4-dihydropyran (DHP) was from Eastman Organic (Rochester, NY). Garner aldehyde was synthesized from *N*-Boc-L-serine as described by Garner et al. (1988).

2.1.2. General methods

See page 4-5.

2.2. Synthesis

*2.2.1. 4-Bromo-1-(tetrahydropyranyloxy)butane-d_8 (**5**)*

To a solution of TMSBr (15.3 g, 100 mmol) in 70 mL of CH_2Cl_2 was added THF-d_8 (compound **3** in Scheme 1, 3.33 g, 46 mmol) at 0 °C. The solution was heated to reflux for 18 h and then cooled to 0 °C. After DHP (12.6 g, 13.7 mL, 150 mmol), *p*-TsOH (12 mg, 6 mmol), and silica gel (1 g) were added, the reaction mixture was warmed to rt and stirred overnight. The solvent was evaporated under vacuum to give a brown residue that was purified by flash chromatography (elution with hexane/EtOAc 15:1) to give 8.03 g (32.8 mmol, 71%) of **5** as a light yellow oil; R_f 0.45 (hexane/EtOAc 9:1); ^{1}H NMR ($CDCl_3$) δ 4.58 (m, 1H), 3.84 (m, 1H), 3.50 (m, 1H), 1.84 (m, 1H), 1.70 (m, 1H), 1.51 (m, 4H); ^{13}C NMR ($CDCl_3$) δ 98.8, 65.6 (quintet, J = 21 Hz), 62.4, 33.1 (quintet, J = 23 Hz), 30.8, 28.8 (quintet, J = 20 Hz), 27.4 (quintet, J = 21 Hz), 25.5, 19.6.

*2.2.2. Tetrahydropyranyl Tridecyl-1,1,2,2,3,3,4,4-d_8 Ether (**6**)*

A mixture of 1-bromononane (1.24 g, 6.0 mmol) and Mg (200 mg, 8.0 mmol) in 15 mL of dry Et_2O was stirred for 1 h at rt, 1 h at reflux, and then 1 h at rt. The reaction mixture was filtered through a sand pad to remove unreacted Mg, and the filtrate was added to a solution of **5** (734 mg, 3.0 mmol) in 15 mL of dry Et_2O and 200 µL of 0.2 M Li_2CuCl_4 (0.04 mmol) in THF at -78

°C. The reaction mixture was slowly warmed to rt and stirred overnight. The reaction mixture was diluted with 50 mL of Et_2O, and the solution was washed with water and brine and dried over sodium sulfate. The solvent was removed under reduced pressure and the residue was purified by column chromatography (elution with hexane/EtOAc 19:1) to give 580 mg (66%) of **6** as a light yellow oil; R_f 0.70 (hexane/EtOAc 9:1); ^{1}H NMR ($CDCl_3$) δ 4.56 (m, 1H), 3.86 (m, 1H), 3.49 (m, 1H), 1.90-1.20 (m, 22H), 0.88 (t, 3H, J = 6.8 Hz); ^{13}C NMR ($CDCl_3$) δ 98.6, 66.7 (quintet, J = 19 Hz), 31.7, 30.7, 29.6, 29.3, 28.4 (quintet, J = 19 Hz), 25.7, 25.4 (quintet, J = 18 Hz), 22.6, 19.6, 14.0, 13.0 (quintet, J = 19 Hz).

*2.2.3. 1-Tridecanol-1,1,2,2,3,3,4,4-d_8 (**7**)*

A solution of **6** (500 mg, 1.71 mmol) and *p*-TsOH (28 mg, 0.15 mmol) in 20 mL of MeOH was stirred at rt for 4 h. The solvent was removed under reduced pressure and the residue was purified by column chromatography (elution with hexane/EtOAc 4:1) to give 343 mg (1.60 mmol, 94%) of **7** as a white powder; R_f 0.40 (hexane/EtOAc 4:1); ^{1}H NMR ($CDCl_3$) δ 1.92 (s, 1H), 1.40-1.20 (m, 16H), 0.88 (t, 3H, J = 7.2 Hz); ^{13}C NMR ($CDCl_3$) δ 62.0 (quintet, J = 22 Hz), 31.9, 31.5 (quintet, J = 20 Hz), 29.69, 29.66, 29.58, 29.4, 28.5 (quintet, J = 18 Hz), 24.6 (quintet, J = 18 Hz), 22.7, 14.1.

*2.2.4. 1-Bromotridecane-1,1,2,2,3,3,4,4-d_8 (**8**)*

A solution of **7** (208 mg, 1.0 mmol) and PPh_3 (288 mg, 1.1 mmol) in 10 mL of dry CH_2Cl_2 was cooled to 0 °C. NBS (216 mg, 1.2 mmol) was added,

and the reaction mixture was stirred for 1 h at 0 °C, and then allowed to warm to rt and stirred overnight. The solvent was removed under reduced pressure and the residue was purified by column chromatography (elution with hexane) to give 248 mg (91%) of **8** as a colorless oil; R_f 0.70 (hexane); ^{1}H NMR ($CDCl_3$) δ 1.37-1.20 (m, 16H), 0.88 (t, 3H, J = 6.8 Hz); ^{13}C NMR ($CDCl_3$) δ 33.4 (quintet, J = 22 Hz), 32.0, 31.5 (quintet, J = 20 Hz), 29.70, 29.68, 29.55, 29.4, 27.9 (quintet, J = 18 Hz), 24.6 (quintet, J = 18 Hz), 22.7, 14.1.

*2.2.5. 1-Pentadecyne-3,3,4,4,5,5,6,6-d_8 (**9**)*

A solution of 200 mg (2.0 mmol) of lithium acetylide-ethylenediamine complex (90% purity) in 3 mL of dry DMSO was cooled to 0 °C. A solution of **8** (240 mg, 0.88 mmol) in 3 mL of dry DMSO was added dropwise over 5 min. After the addition, the reaction mixture was stirred at rt for 2 h. Water (2 mL) was added slowly, followed by 1 N HCl (3 mL). The mixture was extracted with hexane. The combined extracts were washed with water and brine, dried over sodium sulfate, and concentrated. Purification by column chromatography (elution with hexane) afforded 168 mg (88%) of **9** as a colorless oil; R_f 0.70 (hexane); ^{1}H NMR ($CDCl_3$) δ 1.91 (s, 1H), 1.40-1.20 (m, 16H), 0.88 (t, 3H, J = 7.2 Hz); ^{13}C NMR ($CDCl_3$) δ 84.7, 68.0, 31.9, 29.70, 29.69, 29.68, 29.6, 29.4, 29.3, 28.2 (quintet, J = 19 Hz), 27.7 (quintet, J = 18 Hz), 27.1 (quintet, J = 18 Hz), 22.7, 17.8 (quintet, J = 19 Hz), 14.1.

*2.2.6. tert-Butyl (4S,1'R)-2,2-Dimethyl-4-(1'-hydroxyhexadec-2'-ynyl)-oxazolidione-3-carboxylate-4',4',5',5',6',6',7',7'-d_8 (**10**)*

n-BuLi (2.8 M in hexane, 0.27 mL, 0.75 mmol) was added dropwise to a solution of **9** (120 mg, 0.58 mmol) in dry THF (5 mL) at -20 °C. After the reaction mixture was stirred at -20 °C for 2 h, HMPA (0.20 mL, 1.13 mmol) was added, followed by a solution of Garner aldehyde (125 mg, 0.55 mmol) in dry THF (5 mL) at -78 °C. After 1 h at -78 °C, the mixture was allowed to warm to -20 °C within 2 h, quenched by adding saturated aqueous NH_4Cl solution (5 mL), and diluted with water (5 mL). The aqueous layer was separated and extracted with Et_2O (3 x 10 mL). The combined organic layers were washed with 0.5 N HCl (2 x 5 mL) and brine (5 mL), dried over sodium sulfate, filtered, and concentrated. The residue was purified by column chromatography (elution with hexane/EtOAc 4:1) to give 155 mg (60%) of **10** as a colorless oil; R_f 0.50 (hexane/EtOAc 4:1); ^{1}H NMR ($CDCl_3$) δ 4.77 (m, 1H), 4.51 (m, 1H), 4.10 (m, 2H), 3.90 (s, 1H), 1.70-1.45 (m, 15H), 1.40-1.20 (m, 16H), 0.88 (t, 3H, J = 6.8 Hz); ^{13}C NMR ($CDCl_3$) δ 154.1, 94.9, 86.6, 81.2, 77.9, 65.1, 64.2, 62.8, 31.9, 29.67, 29.65, 29.6, 29.4, 29.3, 28.4, 28.0, 27.6 (quintet, J = 18 Hz), 26.0, 25.8, 25.4, 22.7, 18.1, 14.1.

*2.2.7. tert-Butyl (1S,2R)-N-[2-Hydroxy-1-(hydroxymethyl)-3-heptadecynyl]-carbamate-5,5,6,6,7,7,8,8-d_8 (**11**)*

To a solution of 150 mg (0.336 mmol) of **10** in 10 mL of MeOH was added 0.12 g of Amberlyst 15 resin. After the reaction mixture had stirred at rt for 48 h, it was filtered through a Celite pad, and the filtrate was concentrated. Purification by flash chromatography (elution with hexane/EtOAc 1:1) afforded

102 mg (75%) of **11** as a white solid; R_f 0.52 (hexane/EtOAc 1:1); ^{1}H NMR ($CDCl_3$) δ 5.33 (m, 1H), 4.53 (s, 1H), 4.00 (m, 1H), 3.68 (m, 2H), 3.47 (s, 1H), 3.00 (s, 1H), 1.39 (s, 9H), 1.20 (m, 16H), 0.81 (t, 3H, J = 6.8 Hz); ^{13}C NMR ($CDCl_3$) δ 153.8, 85.5, 77.6, 75.4, 62.0, 60.3, 53.3, 29.4, 27.21, 27.20, 27.18, 27.14, 26.88, 26.81, 25.9, 25.4 (quintet, J = 19 Hz), 20.2, 15.4 (quintet, J = 17 Hz), 11.6.

*2.2.8. tert-Butyl (3E,1S,2R)-N-[2-Hydroxy-1-(hydroxymethyl)-3-heptadecenyl]-carbamate-5,5,6,6,7,7,8,8-d_8 (**12**)*

A solution of 100 mg (0.246 mmol) of **11** in dry Et_2O (10 mL) was treated dropwise with 0.6 mL (2.1 mmol) of Red-Al (a 3.5 M solution in toluene) at 0 °C under nitrogen. The reaction mixture was stirred at rt for 24 h. The reaction was quenched by slowly adding 1 mL of MeOH at 0 °C. The product was extracted with EtOAc (3 x 20 mL), and the combined organic layers were washed with brine (10 mL), dried over sodium sulfate, filtered, and concentrated. The residue was purified by column chromatography (elution with hexane/EtOAc 1:1) to give 68 mg (68%) of **12** as a white powder; R_f 0.42 (hexane/EtOAc 1:1); ^{1}H NMR ($CDCl_3$) δ 5.69 (m, 1H), 5.45 (m, 1H), 5.28 (m, 1H), 4.22 (m, 1H), 3.85 (m, 1H), 3.62 (m, 1H), 3.52 (s, 1H), 2.94 (s, 2H), 1.38 (s, 9H), 1.28-1.12 (m, 16H), 0.81 (t, 3H, J = 6.8 Hz); ^{13}C NMR ($CDCl_3$) δ 156.3, 134.1, 128.9, 79.8, 74.7, 62.6, 55.4, 31.9, 31.4, 29.70, 29.66, 29.4, 28.8, 28.4, 27.9, 27.7, 22.7, 14.1; MS calcd for $C_{23}H_{37}D_8NO_4Na$ $(M + Na)^+$ *m/z* 430.4, found 430.2.

*2.2.9. D-erythro-Sphingosine-6,6,7,7,8,8,9,9-d_8 (**13**)*

A solution of 68 mg (0.17 mmol) of **12** in 2 mL of 1 M HCl and 2 mL of THF was heated at 70 °C with stirring for 8 h under nitrogen. The reaction mixture was cooled to rt, neutralized with saturated aqueous $NaHCO_3$ solution (2 mL), and extracted with EtOAc (3 x 10 mL). The combined organic layers were washed with brine, dried over sodium sulfate, filtered, and concentrated to give 42 mg (82%) of **13** as a white powder; ^{1}H NMR ($CDCl_3/CD_3OD$) δ 5.46 (m, 1H), 5.19 (m, 1H), 3.71 (m, 1H), 3.40 (m, 1H), 3.32 (m, 1H), 2.50 (m, 1H), 1.10-0.93 (m, 16 H), 0.61 (t, 3H, *J* = 6.8 Hz); ^{13}C NMR ($CDCl_3/CD_3OD$) δ 130.5, 124.8, 69.9, 58.8, 52.1, 27.7, 27.2, 25.41, 25.37, 25.10, 25.08, 23.7, 18.4, 9.6.

*2.2.10. D-erythro-Sphingosine-6,6,7,7,8,8,9,9-d_8 1-phosphate (**2**)*

Compound 2 was prepared by Dr. Dan Baker according to Lu et al. (2003).

3. Results

3.1. Retrosynthetic plan

As shown in the retrosynthetic plan (Fig. 2), the preparation of D-*erythro*-sphingosine-6,6,7,7,8,8,9,9-d_8 (**13**) consists of two building blocks: 1-pentadecyne-3,3,4,4,5,5,6,6-d_8 (**9**) and (*S*)-Garner aldehyde.

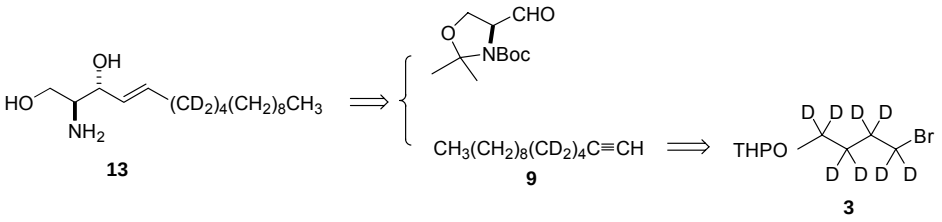
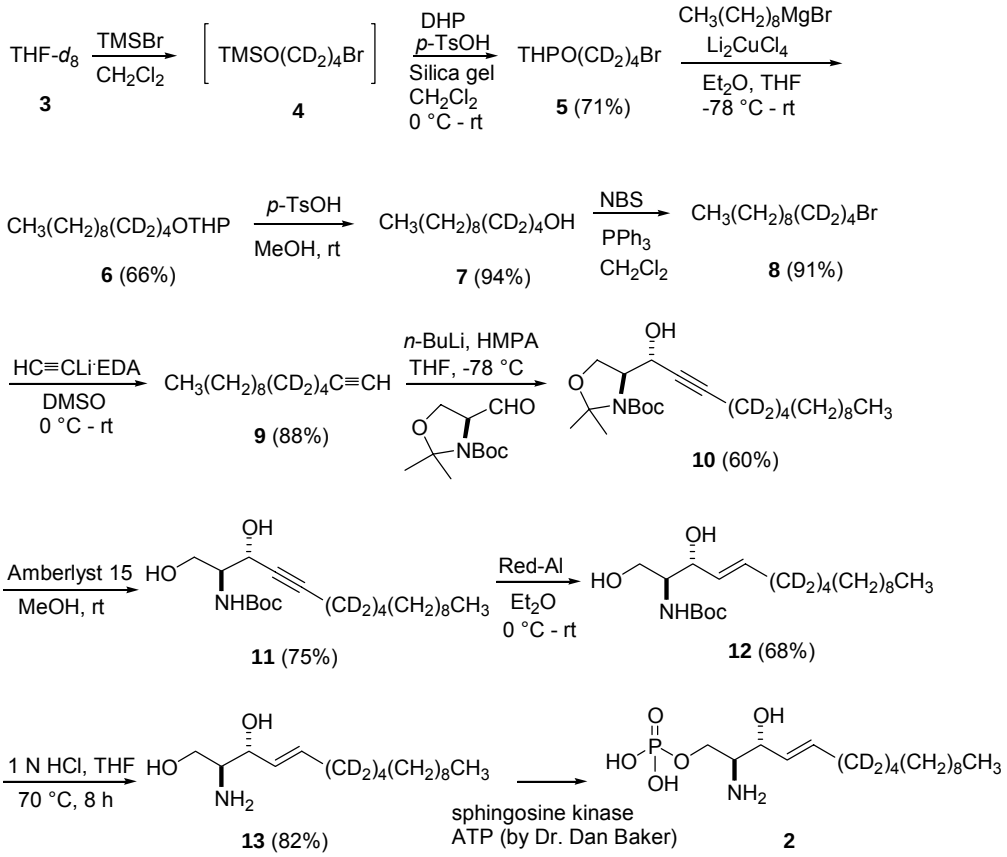


Fig. 2. Retrosynthetic plan for the preparation of D-*erythro*-sphingosine-6,6,7,7,8,8,9,9-d_8 (**13**)

3.2. Synthesis of D-erythro-sphingosine-6,6,7,7,8,8,9,9-d_8 and D-erythro-sphingosine-6,6,7,7,8,8,9,9-d_8 1-phosphate



Scheme 1. Synthesis of D-*erythro*-sphingosine-6,6,7,7,8,8,9,9-d_8 (**13**) and D-*erythro*-sphingosine-6,6,7,7,8,8,9,9-d_8 1-phosphate (**2**)

4. Summary

The isotope dilution mass spectrometry method involves the addition of a known amount of an enriched isotope of an element to the sample. By measuring the ratio of the sample to the compound containing the isotope, the sample concentration can be calculated.

4-Bromo-1-(tetrahydropyranyloxy)butane-d_8 (**5**) was synthesized by the ring-opening of THF-d_8 with TMSBr (Qin et al., 1996). A copper-catalyzed Grignard reaction was used to extend the length of carbon chain, providing compound **6**. After removal of the THP group, alcohol **7** was converted to bromide **8**. Reaction of **8** with the lithium acetylide-ethylenediamine complex gave alkyne **9**. HMPA competes effectively with the *N*-Boc group for the coordination to the RCHO/Li species, resulting in a high erythro selectivity (erythro/threo > 20/1) during the addition of lithium pentadecyne to the Garner aldehyde (Herold, 1988). The acetonide was removed by acid hydrolysis with Amberlyst 15 in MeOH. Red-Al reduction (Van Overmeire et al., 1999) of propargylic alcohol **11** afforded *N*-Boc sphingosine-d_8 **12**. Acid hydrolysis of **12** (1 M HCl in THF, 70 °C) provided sphingosine-d_8 **13**. Treatment of **13** with ATP in the presence of sphingosine kinase provided sphingosine-d_8 1-phosphate (**2**); this step was carried out by Dr. Dan Baker, University of Tennessee Health Science Center, Memphis.

Chapter 3

Synthesis of Deuterated Sphingomyelin Analogs

Abstract

To study the conformation of the sphingomyelin (SM)-water interface in bilayers by ^{2}H nuclear magnetic resonance (NMR) spectroscopy, four deuterated analogs have been prepared: *N*-palmitoyl-d_{31}-SM, 4,5-dihydro-*N*-palmitoyl-d_{31}-SM, *N*-palmitoyl-3,3-d_2-SM, and *N*-palmitoyl-2,2,3,3,4,4-d_6-SM.

1. Introduction

^{2}H-Nuclear magnetic resonance (NMR) spectroscopy is considered to be an effective method to study the conformation of the sphingolipid-water interface in bilayers, chain ordering, dynamics, and membrane elasticity of sphingolipid/phospholipids and sphingomyelin/cholesterol systems (Kurze et al., 2000; Mehnert et al., 2006; Sankaram and Thompson, 1990; Vist and Davis, 1990). Sphingomyelin (SM, **1**), a prevalent sphingophospholipid in mammalian membranes, together with glycosphingolipids and cholesterol, are essential components of transient membrane domains ("lipid rafts") that are implicated in signal transfuction and endocytosis (London, 2002). In order to study the properties of SM by ^{2}H-NMR spectroscopy in collaboration with

Professor Klaus Beyer (University of Munich), four deuterated SM analogs were synthesized: compounds **2**, **3**, **4** and **5**.

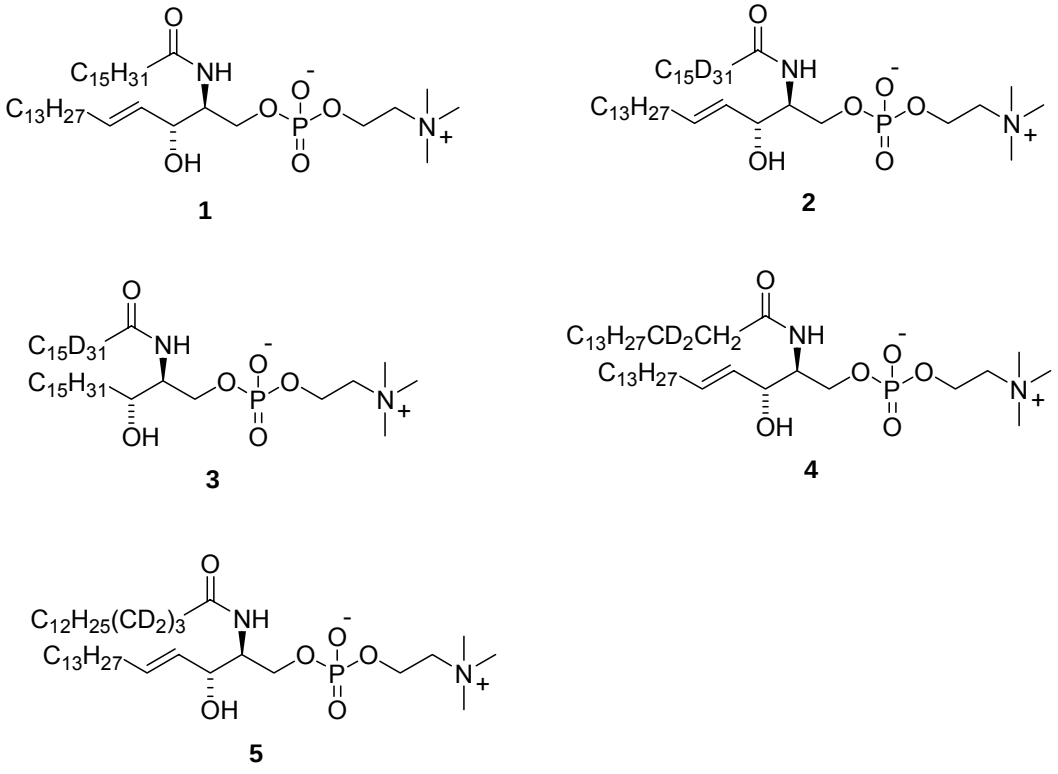


Fig 1. The predominant form of *N*-palmitoyl-SM found in egg SM (SM, **1**), *N*-palmitoyl-d_{31}-SM (SM-d_{31}, **2**), 4,5-dihydro-*N*-palmitoyl-d_{31}-SM (2HSM-d_{31}, **3**), *N*-palmitoyl-3,3-d_2-SM (**4**), and *N*-palmitoyl-2,2,3,3,4,4-d_6-SM (**5**)

2. Experimental

2.1. Materials and analytical procedures

2.1.1. Chemicals

SM from egg yolk was purchased from Avanti Polar Lipids (Alabaster, AL), and palmitic acid-d_{31} and -3,3-d_2 were from CDN Isotopes (Pointe-Claire, Quebec, Canada).

2.1.2. General methods

Anhydrous methanolic hydrogen chloride was prepared by dripping concentrated sulfuric acid onto solid sodium chloride and trapping the evolved gas in methanol, which had previously been dried by distillation from sodium methoxide. The concentration of hydrogen chloride was determined by potassium carbonate titration of an aqueous solution of the methanolic hydrogen chloride to an end point marked by phenolphthalein. Anhydrous DMF was prepared by distillation from P_2O_5 under nitrogen. Dichloromethane was stored over calcium hydride. Flash chromatography and TLC were carried out with silica gel 60 (230-400 ASTM mesh) and silica gel 60F_{254} (200-μm thick, precoated on aluminum), respectively. Amberlite IRA-400 ion-exchange resin and Cameo filters (0.45 μm) were from Fisher Scientific (Pittsburgh, PA). Phosphomolybdic acid spray was prepared by dissolving molybdenum metal (0.4 g) in concentrated sulfuric acid (150 mL), and diluting with a solution of molybdic acid anhydride (8 g) in water (200 mL). ^{1}H, ^{13}C and ^{31}P NMR spectra were recorded at 400, 100 and 162 MHz (Rana et al, 1991), respectively. The deuterated palmitic acids were converted to the corresponding *p*-nitrophenyl palmitates by reaction with *p*-nitrophenol, DCC,

and catalytic DMAP in CH_2Cl_2, followed by washing with water, drying over Na_2SO_4, and concentration.

2.2. Synthesis

*2.2.1. D-erythro-Lyso-SM (**6**)*

SM (**1**) (200 mg, ~0.24 mmol) was dissolved in anhydrous methanolic hydrogen chloride (20 mL, 0.5 M) in a screw-capped vial. The solution was heated to 50 °C with stirring for 3 days. The solvent was removed and the residue was purified by silica gel column chromatography (elution with $CHCl_3$/MeOH/H_2O 65:35:8). The product was dissolved in 5 mL of MeOH and passed through a column of Amberlite IRA-400 ion-exchange resin to remove HCl. The solution was evaporated, the residue was dissolved in $CHCl_3$ with a trace of MeOH, and the resulting solution was filtered through a Cameo filter to remove suspended silica gel. The filtrate was concentrated to give 74 mg (62%) of **6**; R_f 0.10 ($CHCl_3$/MeOH/H_2O 65:35:8); The ^1H and ^{13}C NMR spectra ($CDCl_3$/CD_3OD 1:1) are consistent with those reported by Sripada et al. (1987) and with Bittman and Verbicky (2000).

2.2.2. N-Palmitoyl-d_{31}-SM (**2**)

Compound **6** (70 mg, 0.14 mmol), *p*-nitrophenyl palmitate-d_{31} (122 mg, 0.30 mmol), and anhydrous potassium carbonate (29 mg, 0.21 mmol) were added to a 50-mL round-bottom flask equipped with a nitrogen inlet. The mixture was suspended in anhydrous DMF (5 mL) and CH_2Cl_2 (2 mL) and

stirred at rt. After 1 day, TLC analysis indicated that lyso-SM had been consumed. The reaction mixture was concentrated under reduced pressure. The residue was purified by column chromatography (elution with $CHCl_3$/MeOH/H_2O 65:35:8). The concentrated product was dissolved in $CHCl_3$ plus a trace of MeOH and passed through a Cameo filter. The filtrate was concentrated and the residue was lyophilized with benzene to give 66 mg (65%) of **2** as a fine white powder. R_f 0.50 ($CHCl_3$/MeOH/H_2O 65:35:8); ^{1}H NMR (CD_3OD) δ 5.72 (m, 1H), 5.48 (m, 1H), 4.30 (m, 2H), 4.14-3.94 (m, 3H), 3.67 (m, 3H), 3.24 (s, 9H), 2.05 (m, 2H), 1.52-1.30 (m, 22H), 0.92 (t, 3H, J = 6.8 Hz); ^{13}C NMR (CD_3OD) δ 176.0, 135.1, 131.3, 72.6, 70.9, 67.5, 66.0, 63.6, 60.5, 60.1, 55.6, 54.8, 53.7, 34.9, 33.5, 33.1, 30.9, 30.8, 29.9, 26.7, 23.8, 14.5; ^{31}P NMR (CD_3OD) δ 0.16; MS calcd for $C_{39}H_{49}D_{31}N_2O_6P$ $(M + H)^+$ *m/z* 734.8, found 734.8.

*2.2.3. 4,5-Dihydro-lyso-SM (**7**)*

A mixture of compound **6** (70 mg, 0.14 mmol) and platinum (5% on carbon, 20 mg) in 5 mL of MeOH was stirred overnight under a balloon filled with hydrogen. The reaction mixture was filtered through filter paper, concentrated, and lyophilized with benzene to give 63 mg (90%) of **7**; R_f 0.49 ($CHCl_3$/MeOH/H_2O 65:35:8); ^{1}H NMR (CD_3OD) δ 4.67 (s, 1H), 4.35 (m, 2H), 4.21-4.10 (m, 2H), 3.83 (m, 1H), 3.71 (m, 2H), 3.45-3.33 (m, 2H), 3.30 (s, 9H), 1.71-1.28 (m, 28H), 0.92 (t, 3H, J = 6.8 Hz); ^{13}C NMR (CD_3OD) δ 91.1, 70.0,

67.5, 64.3, 63.4, 60.8, 57.4, 54.8, 34.3, 33.1, 30.8, 30.7, 30.6, 30.5, 28.9, 27.7, 27.0, 23.8, 14.5; ^{31}P NMR (CD_3OD) δ -0.43.

*2.2.4. 4,5-Dihydro-N-palmitoyl-d_{31}-SM (2HSM-d_{31}, **3**)*

The same procedure used to prepare compound **2** from **6** was used to prepare compound **3** from **7**; R_f 0.50 ($CHCl_3$/MeOH/H_2O 65:35:8); ^{1}H NMR (CD_3OD) δ 4.65 (s, 1H), 4.34 (m, 2H), 4.08 (m, 2H), 3.80 (m, 1H), 3.59 (m, 2H), 3.31 (s, 9H), 1.77-1.32 (m, 28H), 0.91 (t, 3H, J = 6.8 Hz); ^{13}C NMR (CD_3OD) δ 176.0, 72.6, 70.9, 67.5, 66.0, 63.6, 60.5, 60.1, 55.6, 54.8, 53.7, 34.9, 33.5, 33.1, 30.9, 30.8, 29.9, 26.7, 23.8, 14.5; ^{31}P NMR (CD_3OD) δ 0.35; MS calcd for $C_{39}H_{51}D_{31}N_2O_6P$ $(M + H)^+$ *m/z* 736.8, found 736.8.

*2.2.5. N-Palmitoyl-3,3-d_2-SM (SM-d_2, **4**)*

The same procedure was used as in the preparation of compound **2** with *p*-nitrophenyl palmitate-C3-d_2 in place of *p*-nitrophenyl palmitate-d_{31}; R_f 0.50 ($CHCl_3$/MeOH/H_2O 65:35:8); ^{1}H NMR (CD_3OD) δ 5.60 (m, 1H), 5.35 (m, 1H), 4.20 (m, 2H), 4.08-3.80 (m, 3H), 3.57 (m, 3H), 3.21 (s, 9H), 2.00 (m, 2H), 1.47-1.15 (m, 48H), 0.80 (m, 6H); ^{13}C NMR (CD_3OD) δ 176.0, 135.1, 131.3, 72.6, 70.9, 67.5, 65.9, 60.5, 60.4, 55.6, 55.3, 54.7, 52.0, 40.3, 37.2, 37.1, 34.9, 34.7, 33.5, 33.2, 30.90, 30.85, 30.75, 30.64, 30.56, 30.5, 30.4, 30.3, 30.0, 29.2, 26.7, 26.1, 23.8, 14.5; ^{31}P NMR (CD_3OD) δ 0.17; MS calcd for $C_{39}H_{78}D_2N_2O_6P$ $(M + H)^+$ *m/z* 705.6, found 705.7.

*2.2.6. Tetrahydropyranyl Hexadecyl-1,1,2,2,3,3,4,4-d_8 Ether (**9**)*

The same procedure was used as in the preparation of compound **6** in Chapter 2 (p. 33), with 1-bromododecane instead of 1-bromononane; R_f 0.70 (hexane/EtOAc 9:1); ^{1}H NMR ($CDCl_3$) δ 4.50 (m, 1H), 3.81 (m, 1H), 3.45 (m, 1H), 1.70-1.10 (m, 28H), 0.88 (t, 3H, J = 7.2 Hz); ^{13}C NMR ($CDCl_3$) δ 98.8, 71.0, 67.7, 63.5, 62.4, 36.7, 31.9, 30.8, 30.3, 30.2, 29.80, 29.78, 29.71, 29.69, 29.4, 26.3, 25.6, 22.7, 19.8, 15.7, 14.3.

*2.2.7. Hexadecanol-1,1,2,2,3,3,4,4-d_8 (**10**)*

The procedure used for the preparation of compound **7** in Chapter 2 was used (p. 34). Thus, reaction of **9** with *p*-TsOH afforded **10**; R_f 0.35 (hexane/EtOAc 4:1); ^{1}H NMR ($CDCl_3$) δ 3.64 (m, 1H), 1.40-1.10 (m, 22H), 0.88 (t, 3H, J = 7.2 Hz); ^{13}C NMR ($CDCl_3$) δ 63.1m 32.8, 31.9, 29.71, 29.68, 29.63, 29.58, 29.4, 25.8, 22.7, 14.1.

*2.2.8. Palmitic acid-2,2,3,3,4,4-d_6 (**11**)*

A mixture of **10** (30 mg, 0.12 mmol) and PDC (376 mg, 1.0 mmol) in 2 mL of DMF was stirred at rt for 48 h. The reaction mixture was poured into 10 volumes of water, acidified with 3 N HCl, and extracted with Et_2O. Removal of solvents gave a residue that was purified by flash chromatography (elution with 500:100:5 hexane/EtOAc/85% formic acid) to yield 24 mg (80%) of **11** as a white solid; ^{1}H NMR ($CDCl_3$) δ 10.0 (br s, 1H), 1.70-1.20 (m, 22H), 0.88 (t, 3H, J = 6.4 Hz); ^{13}C NMR ($CDCl_3$) δ 171.4, 33.8, 31.9, 31.4, 30.2, 29.7, 29.6, 29.5, 29.4, 29.3, 29.2, 29.0, 24.9, 22.7, 14.1.

*2.2.9. p-Nitrophenyl palmitate-2,2,3,3,4,4-d_6 (**12**)*

A mixture of **11** (24 mg, 0.092 mmol), *p*-nitrophenyl (20 mg, 0.14 mmol), DCC (40 mg, 0.19 mmol) and DMAP (1 mg, 0.008 mmol) in 5 mL of dry CH_2Cl_2 was stirred at rt overnight. The solvent was removed under reduced pressure, and the residue was purified by chromatography (elution with hexane/EtOAc 19:1) to give 33 mg (93%) of compound **12**; ^{1}H NMR ($CDCl_3$) δ 8.27 (d, 2H, J = 8.8 Hz), 7.28 (d, 2H, J = 8.0 Hz), 1.49-1.12 (m, 22H), 0.88 (t, 3H, J = 6.6 Hz); ^{13}C NMR ($CDCl_3$) δ 171.3, 155.5, 145.2, 125.2, 122.5, 34.9, 31.9, 31.4, 30.2, 29.71, 29.68, 29.63, 29.60, 29.55, 29.3, 28.0, 25.1, 24.8, 22.7, 14.1; MS calcd for $C_{22}H_{33}D_6N_2O_4$ $(M + NH_4)^+$ *m/z* 401.3, found 401.3.

*2.2.10. N-Palmitoyl-2,2,3,3,4,4-d_6-SM (SM-d_6, **5**)*

The same procedure was used as in the preparation of compound **2**; R_f 0.50 ($CHCl_3$/MeOH/H_2O 65:35:8); ^{1}H NMR (CD_3OD) δ 5.65 (m, 1H), 5.45 (m, 1H), 4.20 (m, 2H), 4.28-3.98 (m, 3H), 3.71 (m, 3H), 3.31 (s, 9H), 1.97 (m, 2H), 1.53-1.15 (m, 44H), 0.88 (m, 6H); ^{13}C NMR (CD_3OD) δ 176.8, 136.1, 132.3, 72.6, 70.9, 67.5, 65.9, 60.5, 60.4, 55.6, 55.3, 54.7, 52.0, 40.3, 37.2, 37.1, 34.9, 34.7, 33.5, 33.2, 32.0, 30.5, 30.4, 30.3, 30.0, 29.4, 26.7, 26.1, 22.7, 14.1; ^{31}P NMR (CD_3OD) δ -0.18; MS calcd for $C_{39}H_{74}D_6N_2O_6P$ $(M + H)^+$ *m/z* 709.6, found 709.7.

3. Results

3.1. Retrosynthetic plan

As shown in the retrosynthetic plan (Fig. 2), the preparation of the deuterated sphingomyelin analogs consists of two building blocks: lyso-SM (**6**) and *p*-nitrophenyl palmitate derivatives containing deuteriums at the desired positions.

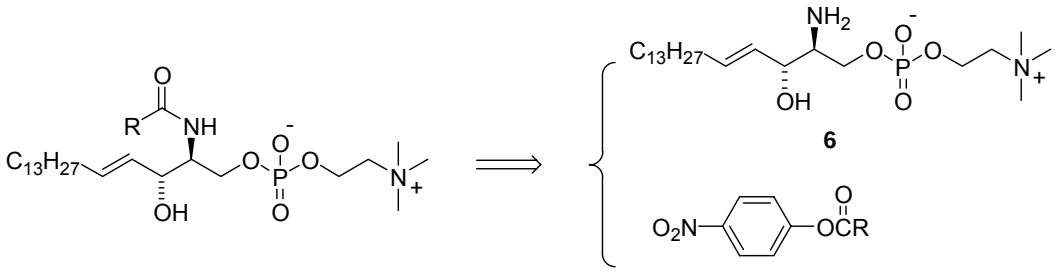
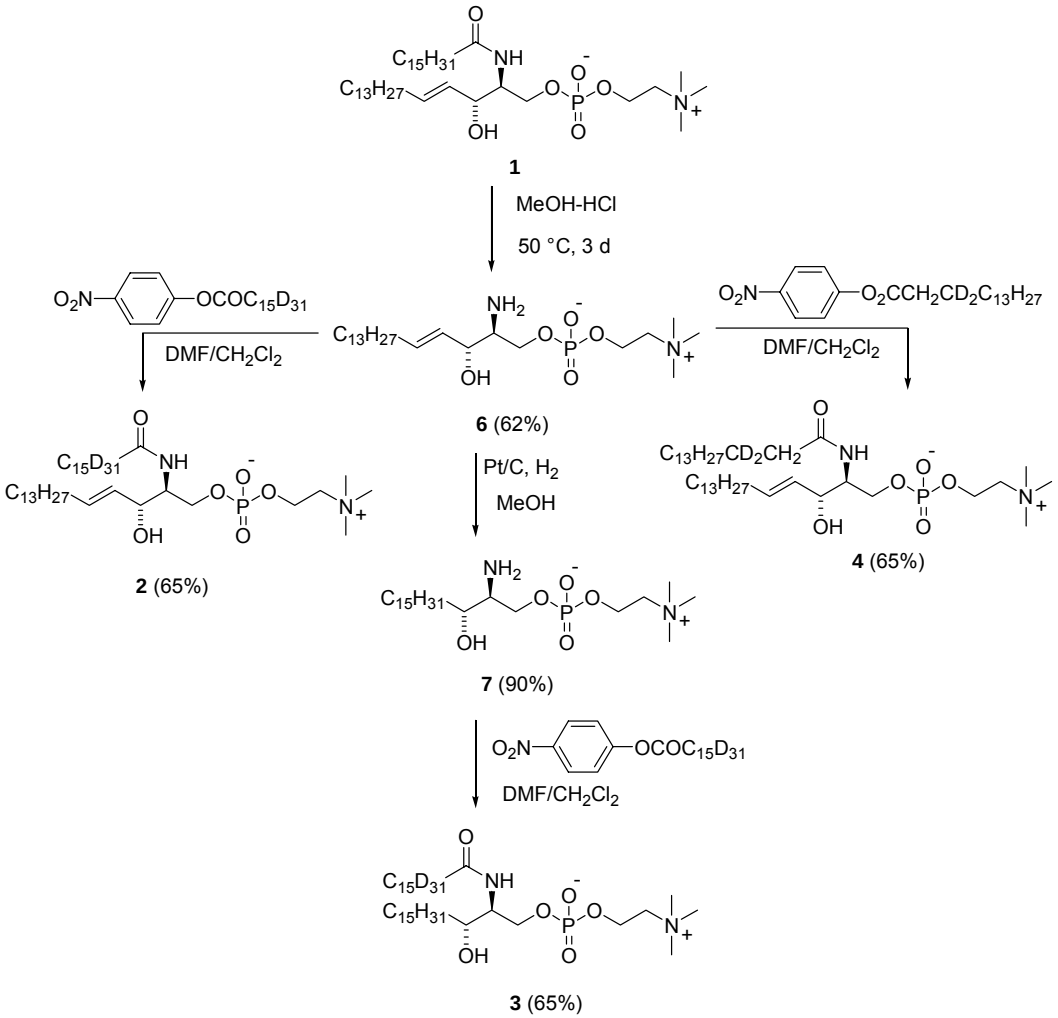


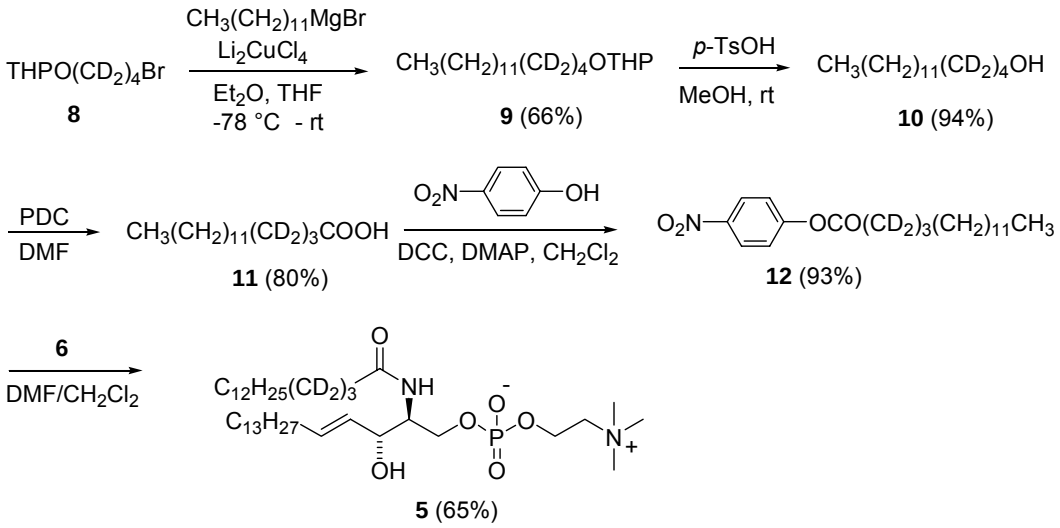
Fig. 2. Retrosynthetic plan for the preparation of deuterated sphingomyelin

*3.2. Synthesis of N-palmitoyl-d_{31}-SM (SM-d_{31}, **2**), 4,5-dihydro-N-palmitoyl-d_{31}-SM (2HSM-d_{31}, **3**), and N-palmitoyl-3,3-d_2-SM (SM-d_2, **4**)*



Scheme 1. Synthesis of SM-d_{31} (**2**), 2HSM-d_{31} (**3**), and SM-d_2 (**4**)

*3.3. Synthesis of N-palmitoyl-2,2,3,3,4,4-d$_6$-SM (SM-d$_6$, **5**)*



Scheme 2. Synthesis of SM-d$_6$ (**5**)

4. Discussion

Lyso-SM (**6**) was prepared from egg SM (**1**) by the acid hydrolysis method of Bittman and Verbicky (2000) because this method provides the product as a ~10:1 ratio of D-*erythro*/L-*threo* epimers. Egg SM was used because it consists predominantly of an 18-carbon sphingosine backbone [(2*S*,3*R*)-2-aminooctadec-4(*E*)-ene-1,3-diol]; homologs of this sphingoid base, such as 20-carbon species, are found in other natural SMs (e.g., milk and brain SM) (Ramstedt et al., 1999). Palmitic acid-2,2,3,3,4,4-d$_6$ (**11**) was synthesized by a copper-catalyzed Grignard reaction between compound **8** (from Chapter 2) and 1-bromododecane. After removal of the THP group, alcohol **10** was oxidized to acid **11**, and then converted to *p*-nitrophenyl

palmitate-d_6 **12**. The target SM analogs were synthesized by *N*-acylation of lyso-SM (**6**) and its hydrogenated derivative **7**.

Chapter 4

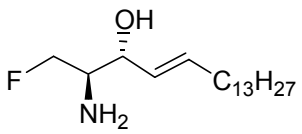
Synthesis of (2*S*,3*S*)-3-Fluoro-4,5-dihydrosphingosine and (2*S*,3*R*)-1-Fluorosphingosine

Abstract

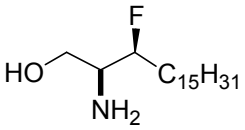
(2*S*,3*S*)-3-Fluoro-4,5-dihydrosphingosine and (2*S*,3*R*)-1-fluorosphingosine were synthesized from *N*-Boc-L-serine using diethylaminosulfur trifluoride (DAST) as a fluorinating agent.

1. Introduction

Sphingosine is the backbone of sphingosine 1-phosphate, ceramide, sphingomyelin, and other sphingolipids. Isosteric and isoelectronic substitution of a hydroxy group by a fluorine atom are made in order to compare the differences as potential inhibitors of sphingosine kinase (Bravo et al., 1999; Uneyama et al., 1998; Xu et al., 2003). The previous synthesis of (2*S*,3*R*)-1-fluorosphingosine (**1**) resulted in low stereoselectivity; the ratio of (2*S*,3*R*)/(2*S*,3*S*) was only 1/1.4 (Kozikowski and Wu, 1990). The 12-carbon analog of 3-fluoro-4,5-dihydrosphingosine was made by De Jonghe et al. (1999). Here we report the synthesis of C-1 fluorine-substituted sphingosine (**1**) and 18-carbon C-3 fluorine-substituted dihydrosphingosine (**2**).



1



2

Fig. 1. (2*S*,3*R*)-1-Fluorosphingosine (**1**) and (2*S*,3*S*)-3-fluoro-4,5-dihydrosphingosine (**2**)

2. Experimental

2.1. Materials and analytical procedures

2.1.1. Chemicals and general methods

N-Boc-L-serine methyl ester and diisobutylaluminum hydride (DIBAL-H, a 20 weight % solution in toluene) were purchased from Acros (Morris Plains, NJ); 1-pentadecyne and *p*-toluenesulfonic acid monohydrate (*p*-TsOH) were from Alfa Aesar/Lancaster (Pelham, NH); diethylaminosulfur trifluoride (DAST), *tert*-butyldiphenylsilyl chloride (TBDPSCl), and $(n\text{-Bu})_4NF$ (TBAF) in THF were from Sigma-Aldrich. Garner aldehyde (**3**) was synthesized from *N*-Boc-L-serine methyl ester as described by Garner et al. (1988). See pages 4-5 for general methods. The ^{19}F NMR spectra were recorded at 376 MHz .

2.2. Synthesis

*2.2.1. tert-Butyl (4S,1'R)-2,2-Dimethyl-4-(1'-hydroxyhexadec-2'-ynyl)-oxazolidione-3-carboxylate (**4**)*

The same procedure was used as in the preparation of compound **10** in Chapter 2 (p. 35); R_f 0.50 (hexane/EtOAc 4:1); the ^{1}H and ^{13}C NMR spectra (C_6D_6) are consistent with those reported by Garner et al. (1988).

*2.2.2. (2S,3R)-2-[(tert-Butoxycarbonyl)amino]-1,2-O,N-isopropylidene-octadecane-1,3-diol (**5**)*

A mixture of compound **4** (176 mg, 0.40 mmol) and platinum (5% on carbon, 80 mg) in 5 mL of MeOH was stirred overnight under a balloon filled with hydrogen. The reaction mixture was filtered through filter paper and concentrated to give 142 mg (81%) of **5** as a colorless oil; the ^{1}H and ^{13}C NMR spectra (C_6D_6) are consistent with those reported by Azuma et al. (2000).

*2.2.3. tert-Butyl (4S)-4-[(1S)-1-Fluorohexadecyl]-2,2-dimethyl-1,3-oxazolidine-3-carboxylate (**6**)*

A solution of **5** (132 mg, 0.30 mmol) in dry CH_2Cl_2 (5 mL) was added dropwise to a cooled solution (-78 °C) of DAST (200 μL, 1.5 mmol) in dry CH_2Cl_2 (5 mL). The mixture was allowed to warm to rt and stirred overnight. The organic layer was washed with water and brine, dried with $MgSO_4$, and concentrated in vacuo. Purification of the residue by flash chromatography (EtOAc/hexane 1:39) gave 74 mg (56%) of **6** as a colorless oil; ^{1}H NMR (C_6D_6) δ 4.93 (m, 1H), 4.17-3.68 (m, 3H), 1.95-1.23 (m, 43H), 1.03 (t, 3H, *J* = 6.8 Hz); ^{13}C NMR (C_6D_6) δ 152.6, 94.7, 91.5 (d, *J* = 81 Hz), 79.8, 64.1, 60.3, 32.7, 32.3, 30.2, 30.1, 30.0, 29.9, 29.8, 28.4, 28.3, 27.3, 26.9, 25.9, 25.1, 23.3, 23.1, 14.3; ^{19}F NMR (C_6D_6) δ -193.9.

*2.2.4. (2S,3S)-2-Amino-3-fluoro-1-octadecanol (**2**)*

A solution of **6** (70 mg, 0.16 mmol) in 2 mL of 1 M HCl and 2 mL of dioxane was stirred at 100 °C for 40 min. The reaction mixture was concentrated to give 42 mg (88%) of **2**; $[\alpha]^{25}_{D}$ -2.25° (*c* 1.24, $CHCl_3/CH_3OH$ 1:1); ^{1}H NMR ($CDCl_3$) δ 5.05 (m, 1H), 4.48 (m, 1H), 4.36 (m, 1H), 3.95 (m, 1H), 3.76 (m, 1H), 3.52 (m, 1H), 2.99 (m, 1H), 1.73-1.22 (m, 28H), 0.90 (t, 3H, *J* = 6.4 Hz); ^{13}C NMR (CD_3OD) δ 92.0 (d, *J* = 173 Hz), 58.3, 56.8, 33.2, 32.1, 31.9, 30.92, 30.87, 30.8, 30.7, 30.6, 30.4, 26.63, 26.60, 23.9, 15.0; ^{19}F NMR (CD_3OD) δ -195.6; MS calcd for $C_{18}H_{39}FNO$ $(M + H)^+$ *m/z* 304.3, found 304.4.

*2.2.5. (S)-tert-Butyl 4-((R,E)-1-Hydroxyhexadec-2-enyl)-2,2-dimethyl-oxazolidine-3-carboxylate (**7**)*

A solution of **4** (437 mg, 1.0 mmol) in 10 mL of anhydrous THF was added to a -78 °C blue solution of lithium (100 mg, 14.3 mmol) in $EtNH_2$ (20 mL) under N_2. After the reaction mixture was stirred at -78 °C for 2 h, TLC (hexane/EtOAc 4:1) showed that the starting material (R_f 0.50) had disappeared, and that the product had appeared (R_f 0.45). The reaction was quenched at -78 °C with 2 g of NH_4Cl, and the ethylamine was allowed to evaporate overnight at rt. The residue was partitioned between 100 mL of H_2O and Et_2O (3 x 100 mL). The combined ether layers were washed with brine, dried over Na_2SO_4, filtered, and concentrated to give 277 mg (63%) of **7** as a colorless oil; the ^{1}H and ^{13}C NMR spectra (C_6D_6) are consistent with those reported by Garner et al. (1988).

*2.2.6. tert-Butyl (E,2S,3R)-1,3-dihydroxyoctadec-4-en-ylcarbamate (**8**)*

To a solution of compound **7** (264 mg, 0.60 mmol) in MeOH (20 mL) was added Amberlyst 15 resin (500 mg), with stirring at rt for 48 h. Filtration through a Celite pad and concentration gave 184 mg (77%) of **8** as a white powder; The ^{1}H NMR spectrum (DMSO-d_6) is consistent with those reported by Herold (1988).

*2.2.7. Carbamic Acid [(1S,2R,3E)-1-[[[(1,1-Dimethylethyl)diphenylsilyl]-oxy]methyl]-2-hydroxy-3-heptadecenyl], 1,1-Dimethylethyl Ester (**9**)*

A mixture of TBDPSCl (50 mg, 0.18 mmol) and imidazole (25 mg, 0.36 mmol) in 10 mL of CH_2Cl_2 was stirred at rt for 1 h. A solution of compound **8** (67 mg, 0.167 mmol) in 5 mL of CH_2Cl_2 was added, and the reaction mixture was stirred overnight. The solvent was removed under reduced pressure, and the residue was purified by chromatography (elution with hexane/EtOAc 4:1) to give 97 mg (91%) of compound **9** as a colorless oil; R_f 0.47 (hexane/EtOAc 4:1); ^{1}H NMR (C_6D_6) δ 7.84 (m, 4H), 7.35 (m, 6H), 5.85 (m, 1H), 5.58 (m, 1H), 5.29 (m, 1H), 4.48 (m, 1H), 4.00 (m, 3H), 2.88 (m, 1H), 2.07 (m, 2H), 1.57 (s, 9H), 1.50-1.35 (m, 22H), 1.23 (s, 9H), 1.03 (t, 3H, J = 6.8 Hz); ^{13}C NMR ($CDCl_3$) δ 136.0, 135.3, 132.7, 130.3, 130.1, 129.7, 128.3, 128.2, 127.9, 73.9, 64.1, 56.2, 32.7, 32.3, 30.14, 30.09, 30.07, 30.0, 29.8, 29.7, 29.6, 29.5, 27.0, 26.8, 23.1, 14.3.

*2.2.8. (4S,5R)-3-Oxazolidinecarboxylic Acid, 4-[[[(1,1-Dimethylethyl)-diphenylsilyl]oxy]methyl]-2,2-dimethyl-5-(1E)-1-pentadecenyl, 1,1-Dimethyl-ethyl Ester (**10**)*

To a 50-mL round-bottomed flask were added a solution of compound **9** (191 mg, 0.30 mmol) in benzene (15 mL), 2,2-dimethoxypropane (DMP, 73 μL, 63 mg, 0.60 mmol), and *p*-TsOH (1.8 mg, 0.01 mmol). The colorless solution was heated at reflux for 1 h, then slowly distilled until a volume of 15 mL was collected over 30 min. The cooled, amber solution was partitioned between saturated aqueous $NaHCO_3$ solution (20 mL) and Et_2O (2 x 50 mL). The organic layer was washed with brine (20 mL), then dried (Na_2SO_4), filtered, and concentrated. The residue was purified by chromatography (elution with hexane/EtOAc 9:1) to give 175 mg (86%) of **10** as a colorless oil; R_f 0.87 (hexane/EtOAc 4:1).

*2.2.9. (4S,5R)-tert-Butyl 4-(Hydroxymethyl)-2,2-dimethyl-5-((E)-pentadec-1-enyl)oxazolidine-3-carboxylate (**11**)*

To a solution of compound **10** (170 mg, 0.25 mmol) and 50 mg (0.72 mmol) of imidazole in 5 mL of dry THF was added TBAF (0.5 mL, 0.50 mmol, a 1 M solution in THF). After the reaction mixture was stirred at rt for 3 h, concentration gave a residue that was purified by chromatography (elution with hexane/EtOAc 4:1) to give 102 mg (93%) of **11** as a white powder; R_f 0.37 (hexane/EtOAc 4:1); ^1H NMR ($CDCl_3$) δ 5.92 (m, 1H), 5.50 (m, 1H), 4.61 (m, 1H), 4.12 (m, 1H), 3.82 (m, 1H), 3.67 (m, 1H), 3.45 (m, 1H), 2.10 (m, 2H),

1.75-1.25 (m, 37H), 0.90 (t, 3H, *J* = 6.8 Hz); ^{13}C NMR ($CDCl_3$) δ 154.4, 137.3, 123.3, 92.9, 81.2, 63.7, 62.1, 61.4, 32.4, 31.9, 29.7, 29.6, 29.5, 29.4, 29.3, 29.2, 28.9, 28.4, 27.8, 26.8, 26.7, 24.7, 22.7, 14.1.

*2.2.10. (4S,5R)-tert-Butyl 4-(Fluoromethyl)-2,2-dimethyl-5-((E)-pentadec-1-enyl)oxazolidine-3-carboxylate (**12**)*

A solution of **11** (132 mg, 0.30 mmol) in dry CH_2Cl_2 (5 mL) was added dropwise to a cooled solution (-78 °C) of DAST (200 μL, 1.50 mmol) in dry CH_2Cl_2 (5 mL). The mixture was allowed to warm to rt and stirred overnight. The organic layer was washed with water and brine, dried over $MgSO_4$, and concentrated in vacuo. Purification of the residue by flash chromatography (EtOAc/hexane 2.5:97.5) gave 79 mg (60%) of **12** as a colorless oil; ^{1}H NMR ($CDCl_3$) δ 5.92 (m, 1H), 5.60 (m, 1H), 4.62-4.30 (m, 3H), 4.00 (m, 1H), 2.10 (m, 2H), 1.62-1.25 (m, 37H), 0.90 (t, 3H, *J* = 6.8 Hz); ^{13}C NMR ($CDCl_3$) δ 152.1, 137.8, 123.4, 93.2, 80.6 (d, *J* = 46 Hz), 59.8, 59.5, 32.4, 31.9, 30.4, 29.68, 29.66, 29.6, 29.5, 29.4, 29.2, 28.9, 28.4, 27.2, 26.6, 24.8, 23.6, 22.7, 14.1; ^{19}F NMR ($CDCl_3$) δ -182.5.

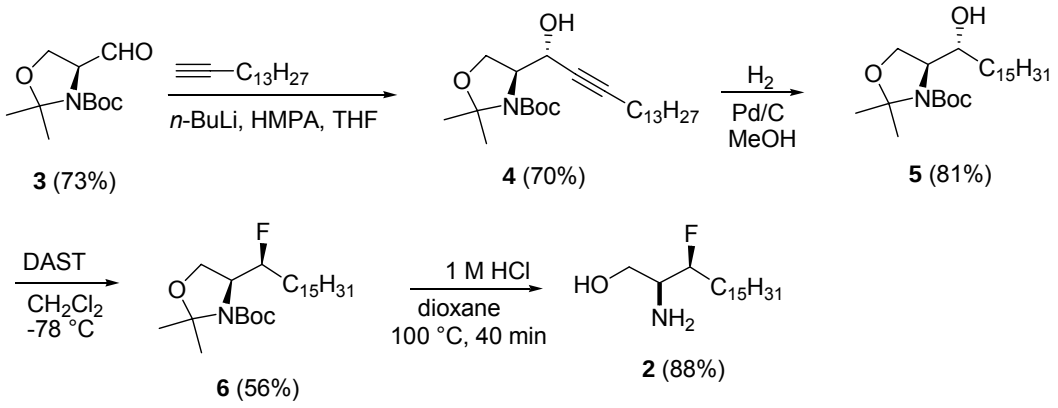
*2.2.11. (2R,3R,4E)-2-Amino-1-fluoro-octadec-4-en-3-ol (**1**)*

The same procedure was used as in the preparation of compound **2**; The ^{1}H NMR spectrum ($CDCl_3$) is consistent with that reported by Kozikowski and Wu (1990); ^{1}H NMR ($CDCl_3$) δ 5.80 (m, 1H), 5.48 (m, 1H), 4.60-4.37 (m, 2H), 4.10 (m, 1H), 3.12 (m, 1H), 2.07 (m, 2H), 1.87 (m, 3H), 1.42-1.23 (m, 22H), 0.90 (t, 3H, *J* = 7.2 Hz); ^{13}C NMR ($CDCl_3$) δ 135.2, 128.4, 86.0 (d, *J* =

186 Hz), 73.1, 55.3, 32.3, 31.9, 29.7, 29.6, 29.5, 29.4, 29.2, 29.1, 22.7, 14.2; $[\alpha]^{25}_{D}$ -6.5° (*c* 0.50, $CHCl_3/CH_3OH$ 1:1) ($[\alpha]^{22}_{D}$ -6.7° (*c* 0.15, CCl_4), Kozikowski and Wu,1990); MS calcd for $C_{18}H_{37}FNO$ $(M + H)^+$ *m/z* 302.3, found 302.3.

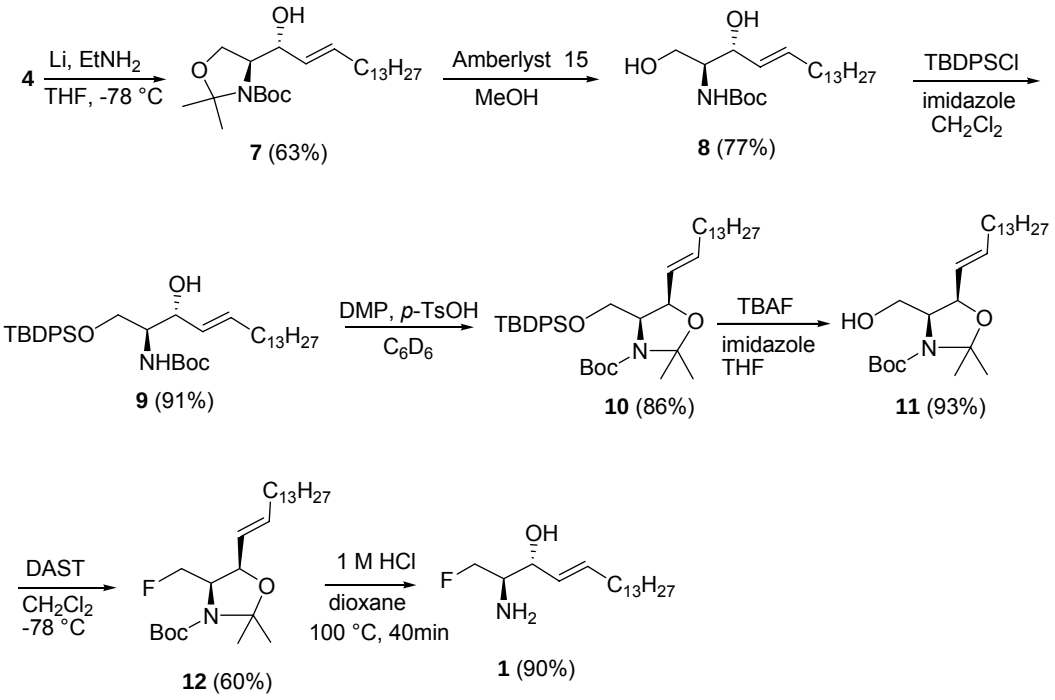
3. Results

*3.1. Synthesis of (2S,3S)-2-amino-3-fluoro-1-octadecanol (**2**)*



Scheme 1. Synthesis of (2*S*,3*S*)-2-amino-3-fluoro-1-octadecanol (**2**)

*3.2. Synthesis of (2R,3R,4E)-2-amino-1-fluoro-4-octadecen-3-ol (**1**)*



Scheme 2. Synthesis of (2*R*,3*R*,4*E*)-2-amino-1-fluoro-4-octadecen-3-ol (**1**)

4. Summary

3-Fluorodihydrosphingosine (**2**) was synthesized as outlined in Scheme 1 by the reaction of (*S*)-Garner aldehyde (compound **5**) (Garner et al., 1988) with lithium pentadecyne in the presence of HMPA in THF (Herold, 1988). (*S*)-Garner aldehyde was prepared from *N*-Boc-L-serine methyl ester (**3**), which was treated with 2,2-dimethoxypropane in the presence of *p*-toluenesulfonic acid in benzene, followed by DIBAL-H reduction at -78 °C. After catalytic hydrogenation, the hydroxy group was replaced with fluorine by

using DAST (Middleton, 1975). The oxazolidine ring was opened by heating at reflux in 1 M HCl/dioxane to give product **2**.

Compound **1** was synthesized as outlined in Scheme 2. Compound **6** was reduced with $Li/EtNH_2$ to form the E double bond. The oxazolidine ring was opened with Amberlyst 15 resin, and the primary hydroxy group was protected as a TBDPS ether. After desilylation, the free primary hydroxyl group was converted to fluorine with DAST, thereby completing the synthesis of **1** after acid reflux to open the ring.

References

Amvam-Zollo, P. H., Sinay, P., 1986. Streptococcus pneumoniae type XIV polysaccharide: synthesis of a repeating branched tetrasaccharide with dioxa-type spacer arms. Carbohydr. Res. 150, 199-212.

Azuma, H., Tamagaki, S., Ogino, K., 2000. Stereospecific total syntheses of sphingosine and its analogs from L-serine. J. Org. Chem. 65, 3538-3541.

Bittman, R., 2004. The 2003 ASBMB-Avanti award in lipids address: applications of novel synthetic lipids to biological problems. Chem. Phys. Lipids 129, 111-131.

Bittman, R., Verbicky, C. A., 2000. Methanolysis of sphingomyelin: toward an epimerization-free methodology for the preparation of D-*erythro*-sphingosylphosphocholine. J. Lipid Res. 41, 2089-2093.

Bravo, P., Crucianelli, M., Ono, T., Zanda, M., 1999. Synthesis and biological activity of β-fluoroalkyl β-amino alcohols. J. Fluorine Chem. 97, 27-49.

Chen, C., Bach, G., Pagano, R. E., 1998. Abnormal transport along the lysosomal pathway in mucolipidosis, type IV disease. Proc. Natl. Acad. Sci. USA 95, 6373-6378.

De Jonghe, S., Van Overmeire, I., Gunst, J., De Bruyn, A., Hendrix, C., Van Calenbergh, S., Busson, R., De Keukeleire, D., Philippe, J., Herdewijn, P., 1999. Synthesis and apoptogenic activity of fluorinated ceramide and dihydroceramide analogs. Bioorg. Med. Chem. Lett. 9, 3159-3164.

De Jonghe, S., Van Overmeire, I., Poulton, S., Hendrix, C., Busson, R., Van Calenbergh, S., De Keukeleire, D., Spiegel, S., Herdewijn, P., 1999. Structure-activity relationship of short-chain sphingoid bases as inhibitors of sphingosine kinase. Bioorg. Med. Chem. Lett. 9, 3175-3180.

Deutschman, D. H., Carstens, J. S., Klepper, R. L., Smith, W. S., Page, M. T., Young, T. R., Gleason, L. A., Nakajima, N., Sabbadini, R. A., 2003. Predicting obstructive coronary artery disease with serum sphingosine-1-phosphate. Am. Heart J. 146, 62-68.

Garner, P., Park, J. M., 1987. The synthesis and configurational stability of differentially protected β-hydroxy-α-amino aldehydes. J. Org. Chem. 52, 2361-2364.

Garner, P., Park, J. M., 1992. 1,1-Dimethylethyl (*S*)- or (*R*)-4-formyl-2,2-dimethyl-3-oxazolidinecarboxylate: a useful serinal derivative. Org. Synth. 70, 18-27.

Garner, P., Park, J. M., Malecki, E. J., 1988. A stereodivergent synthesis of D-*erythro*-sphingosine and D-*threo*-sphingosine from L-serine. J. Org. Chem. 53, 4395-4398.

Gololobov, Yu. G., Zhmurova, I. N., Kasukhin, L. F., 1981. Sixty years of Staudinger reaction. Tetrahedron 37, 437-472.

Hayakawa, J., Ono, T., Hanasaki, K., Okabayashi, Y., 2006. Simultaneous quantitative analysis for phospholipids in human HDL and LDL using two

internal standards by liquid chromatography/mass spectrometry. Anal. Lett. 39, 957-972.

Herold, P., 1988. Synthesis of D-*erythro*- and D-*threo*-sphingosine derivatives from L-serine. Helv. Chim. Acta 71, 354-362.

Johnson, I. D., Kang, H. C., Haugland, R. P., 1991. Fluorescent membrane probes incorporating dipyrrometheneboron difluoride fluorophores. Anal. Biochem. 198, 228-237.

Kozikowski, A. P., Wu, J.-P., 1990. Synthesis of fluorine-containing isosteres of sphingosine as inactivators of protein kinase C. Tetrahedron Lett. 31, 4309-4312.

Kricheldorf, H. R., Moerber, G., Regel, W., 1981. Syntheses of alkyl bromides from ethers and bromotrimethylsilane. Synthesis 1981, 383-384.

Kurze, V., Steinbauer, B., Huber, T., Beyer, K., 2000. A ^{2}H NMR study of macroscopically aligned bilayer membranes containing interfacial hydroxyl residues. Biophys. J. 78, 2441-2451.

London, E., 2002. Insights into lipid raft structure and formation from experiments in model membranes. Curr. Opin. Struct. Biol. 12, 480-486.

Lu, X., Cseh, S., Byun, H.-S., Tigyi, G., Bittman, R., 2003. Total synthesis of two photoactivatable analogues of the growth-factor-like mediator sphingosine 1-phosphate: differential interaction with protein targets. J. Org. Chem. 68, 7046-7050.

Mattjus, P., Malewicz, B., Valiyaveettil, J. T., Baumann, W. J., Bittman, R., Brown, R. E., 2002. Sphingomyelin modulates the transbilayer distribution of galactosylceramide in phospholipid membranes. J. Biol. Chem. 277, 19476-19481.

Mehnert, T., Jacob, K., Bittman, R., Beyer, K., 2006. Structure and lipid interaction of *N*-palmitoylsphingomyelin in bilayer membranes as revealed by ^{2}H-NMR spectroscopy. Biophys. J. 90, 939-946.

Middleton, W. J., 1975. New fluorinating reagents. Dialkylaminosulfur fluorides. J. Org. Chem. 40, 574-578.

Pagano, R. E., Watanabe, R., Wheatley, C., Dominguez, M., 2000. Applications of BODIPY-sphingolipid analogs to study lipid traffic and metabolism in cells. Methods Enzymol. 312, 523-534.

Puri, V., Watanabe, R., Singh, R. D., Dominguez, M., Brown, J. C., Wheatley, C. L., Marks, D. L., Pagano, R. E., 2001. Clathrin-dependent and -independent internalization of plasma membrane sphingolipids initiates two Golgi targeting pathways. J. Cell Biol. 154, 535-547.

Qin, D., Byun, H.-S., Bittman, R., 1996. Synthesis of half-deuterated palmitic and palmitoleic acids from THF-d_8. J. Org. Chem. 61, 8709-8711.

Ramstedt, B., Leppimäki, P., Axberg, M., Slotte, J. P., 1999. Analysis of natural and synthetic sphingomyelins using high-performance thin-layer chromatography. Eur. J. Biochem. 266, 997-1002.

Rana, F. R., Sultany, C. M., Blazyk, J., 1991. Determination of the lipid composition of Salmonella typhimurium outer membranes by phosphorus-31 NMR. J. Microbiol. Methods 14, 41-51.

Sankaram, M. B., Thompson, T. E., 1990. Modulation of phospholipids acyl chain order by cholesterol. A solid-state ^{2}H nuclear magnetic resonance study. Biochemistry 29, 10676-10684.

Singh, R. D., Puri, V., Valiyaveettil, J. T., Marks, D. L., Bittman, R., Pagano, R. E., 2003. Selective caveolin-1-dependent endocytosis of glycosphingolipids. Mol. Biol. Cell 14, 3254-3265.

Sripada, P. K., Maulik, P. R., Hamilton, J. A., Shipley, G. G., 1987. Partial synthesis and properties of a series of *N*-acyl sphingomyelins. J. Lipid Res. 28, 710-718.

Uneyama, K., Hao, J., Amii, H., 1998. A novel synthesis of β-trifluoromethyl-substituted isoserine via intramolecular rearrangement of imino ethers. Tetrahedron Lett. 39, 4079-4082.

Van Overmeire, I., Boldin, S. A., Dumont, F., Van Calenbergh, S., Slegers, G., De Keukeleire, D., Futerman, A. H., Herdewijn, P., 1999. Effect of aromatic short-chain analogs of ceramide on axonal growth in hippocampal neurons. J. Med. Chem. 42, 2697-2705.

Vasella, A., Witzig, C., Chiara, J., Martin-Lomas, M., 1991. Convenient synthesis of 2-azido-2-deoxy-aldoses by diazo transfer. Helv. Chim. Acta 74, 2073-2077.

Vist, M. R., Davis, J. H., 1990. Phase equilibria of cholesterol/dipalmitoyl-phosphatidylcholine mixtures: deuterium nuclear magnetic resonance and differential scanning calorimetry. Biochemistry 29, 451-464.

Xu, Y., Qian, L., Prestwich, G. D., 2003. Synthesis of monofluorinated analogs of lysophosphatidic acid. J. Org. Chem. 68, 5320-5330.

Zimmermann, P., Bommer, R., Bar, T., Schmidt, R. R., 1988. Glycosylimidates. Part 33. Azidosphinogosine glycosylation in glycosphingolipid synthesis. J. Carbohydr. Chem. 7, 435-452.