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Selective deuterium labeling of the sphingoid backbone: facile syntheses of 3,4, 5-trideuterio-*D*-erythro-sphingosine and 3-deuterio-*D*-erythro-sphingomyelin

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Abstract

Deuteration at C-4 and C-5 of sphingosine was achieved via a hydrogen–deuterium exchange reaction of a β -ketophosphonate intermediate catalyzed by ND_4Cl in D_2O /tetrahydrofuran. To install deuterium at C-3 of sphingosine and sphingomyelin, sodium borodeuteride reduction/cerium(III) chloride reduction of an α,β -enone in perdeuteromethanol was used.

Keywords

Lipid synthesis; Sphingolipid; ^2H -labeled

1. Introduction

Stable isotope-labeled natural products are widely used in the elucidation of the structures of metabolites, toxicity studies, and investigations of metabolism in normal and diseased states in vivo (Mutlib, 2008; Magkos and Mittendorfer, 2009; Postle and Hunt, 2009). There has been increased interest in lipids labeled with stable isotopes (principally deuterium) because of the dramatic success of lipidomics in identifying and quantifying individual molecular species present in lipid extracts by tandem mass spectrometry (Han, 2010; Shevchenko and Simons, 2010). Therefore, the development of efficient and facile synthetic methods to incorporate deuterium regioselectively into lipids is needed.

Deuterated sphingolipids have been used in deuterium NMR studies to determine the C–D bond order in the *N*-acyl chain of sphingomyelin bilayers (Mehnert et al., 2006; Bartels et al., 2008). However, selective deuteration of the interfacial region of the long-chain base of sphingomyelin has not yet been reported. In this communication we report a convenient methodology for inserting deuterium labels into the vinyl group and/or the C3 position of the sphingoid backbone, thereby enabling analysis of the conformational and order parameters in the lipid–water interfacial region of sphingolipid bilayers by solid-state ^2H NMR spectroscopy.

Many deuterium-labeled lipids can be prepared by employing the routes for the synthesis of their radiolabeled counterparts. For example, tritium was incorporated at the C3 positions of sphingosine, sphinganine, and ceramide by sodium [^3H]-borohydride or lithium aluminum [^3H]-hydride reduction of 3-keto analogues, which affords a mixture of the erythro and threo stereoisomers of the C3-labeled compound (Hoffman and Tao, 1998; reviewed by Bielawska

et al., 2000). Regiospecific oxidation of the primary hydroxy group was achieved by using C2- and C3-protected sphingolipids, giving a protected aldehyde that on reduction provided a C1-tritiated sphingolipid. Alternatively, an enantioselective aldol condensation of (*E*)-2-hexadecenal with a chiral iminoglycinate provided a 1-carboxylic ester intermediate (Solladié-Cavallo and Koessler, 1994); reduction of the 1-carboxylic ester with [³H]-NaBH₄ afforded [1,1-³H]-sphingosine (Li et al., 1999).

The structures of the deuterium-labeled sphingolipids we prepared are shown in Fig. 1. A key step in our synthesis of 3,4,5-trideuterio-*D*-*erythro*-sphingosine (**1**) is a Horner–Wadsworth–Emmons (HWE) olefination reaction of 1-deuteromyristaldehyde with a dideutero-β-ketophosphonate derived from *N*-Boc-*L*-serine methyl ester. 3-Deuterio-*D*-*erythro*-sphingomyelin (**2**) was prepared via a NaBD₄-mediated reduction of an unlabeled enone, which was synthesized in a HWE reaction of myristaldehyde with a β-ketophosphonate; in a subsequent step, the phosphocholine head group was installed.

2. Results and discussion

2.1. Synthesis of compound 1

1-Deuteriotetradecanal (**5**) was prepared from methyl myristate (**3**) via 2,2-dideuteromyristyl alcohol (**4**) in two steps, as shown in Scheme 1. Lithium aluminum deuteride reduction of **3** afforded α,α-dideuterated alcohol **4** in 91% yield after purification by column chromatography. Oxidation of alcohol **4** with PCC in CH₂Cl₂ gave deuterated aldehyde **5** in 82% yield, which was spectroscopically pure by ¹H NMR without the need for chromatography. As expected, the ¹H NMR spectrum of **5** showed no *CHO* peak at δ 10 ppm. The deuterium atom in **5** became incorporated into the 5 position of sphingosine (**1**) as discussed below.

The synthesis of 3,4,5-trideuteriosphingosine (**1**) is depicted in Scheme 2. Condensation of *L*-Garner methyl ester **6** (prepared from *N*-Boc-*L*-serine methyl ester as described by Garner and Park, 1987) with the lithium salt of dimethyl methylphosphonate in THF is known to afford β-ketophosphonate **7** (Koskinen and Koskinen, 2000) via enolate intermediate **8**. Since the H–D exchange reaction is a straightforward method to introduce C–D bonds vicinal to carbonyl and phosphonyl groups, we first attempted to carry out a hydrogen–deuterium exchange reaction with **7** by using D₂O in THF or MeCN and catalysis by either K₂CO₃ or ND₄Cl. This was unsuccessful. However, we succeeded in preparing the desired dideutero-β-ketophosphonate by the following sequence of reactions. First, lithium enolate **8** was converted to mono-deuterated β-ketophosphonate **9** with a catalytic amount of ND₄Cl in D₂O/THF (1:1) at room temperature. The hydrogen–deuterium exchange reaction was repeated three times, affording α,α-dideutero-β-ketophosphonate **10**. The product of the exchange reactions was confirmed by ¹H NMR (disappearance of the PCH₂CO multiplet peaks around δ 3.15 ppm). A precedent for hydrogen–deuterium exchange with ND₄Cl in D₂O/THF is the synthesis of 5-*epi*-[6-²H]-valiolone after reductive desulfurization of a bis(methylthio) moiety alpha to a carbonyl group (Mahmud et al., 2001).

In the next step, HWE reaction of α,α-dideutero-β-ketophosphonate **10** with 1-deuterotetradecanal (**5**) in D₂O/THF (1:1) containing 3 equiv. of K₂CO₃ at 0°C for 4 h and then at room temperature for 72 h provided α,β-enone **11**, which bears deuterium atoms in the vinyl group, in 61% yield. To incorporate deuterium at C-3, Luche reduction (Gemal and Luche, 1981) of the carbonyl group of **11** with sodium borodeuteride was employed. Thus, reaction of enone **11** with NaBD₄ in the presence of CeCl₃·7H₂O (to suppress 1,4-hydride addition) in THF/CD₃OD (10:1) for 4 h at room temperature gave alcohol **12** together with traces of its threo isomer (Chun et al., 2002a,b, 2003). In order to reduce amount of perdeuterated methanol, we used THF as the co-solvent. Column chromatography on silica gel (elution with hexane/EtOAc 10:1) gave *erythro*-enriched alcohol **12** in 89% yield. High-

resolution mass spectral (HRMS) analysis of the relative intensities in the isotope pattern of **12** indicated that the isotopic composition was approximately 16% dideuterio, 64% trideuterio, and 20% tetradeuterio. The tetradeuterio species may arise from 1,4-addition of deuteride ion to **11**, followed by protonation of the enolate ion intermediate with the traces of water present in the hydrated CeCl_3 rather than deuteration with CD_3OD . Therefore, the amount of tetradeuterio species may be enhanced by conversion of $\text{CeCl}_3 \cdot 7\text{H}_2\text{O}$ to $\text{CeCl}_3 \cdot 7\text{D}_2\text{O}$ via multiple treatments of the salt with D_2O .

Treatment of acetone **12** with 1M HCl in THF at room temperature provided 3,4,5-trideuterio-*N*-Boc-sphingosine **13** in 78% yield. At this stage, the traces of the threo isomer that had remained were completely removed by column chromatography on silica gel (elution with hexane/EtOAc 1:1). Finally, removal of the *N*-Boc group with 3M HCl in THF at 70 °C furnished deuterium-labeled sphingosine **1** as its hydrochloride salt in quantitative yield.

2.2. Synthesis of compound 2

Scheme 3 outlines the preparation of 3-deuterio-*N*-palmitoyl-*D*-*erythro*-sphingosylphosphocholine (**2**). Enone **14** was prepared by HWE reaction of **7** with unlabeled myristaldehyde. Reduction of **14** with NaBD_4 in the presence of $\text{CeCl}_3 \cdot 7\text{H}_2\text{O}$ in THF/ CD_3OD (10:1) for 4 h at room temperature gave alcohol **15** together with traces of its threo isomer. After removal of the isopropylidene group, the phosphocholine moiety was inserted into *N*-Boc-3-deuterio-sphingosine **16** via a one-pot reaction with ethylene chlorophosphite, oxidation with bromine, and quaternization with trimethylamine (Byun et al., 1994; Erukulla et al., 1994). After the *N*-Boc group was removed by heating with 3M HCl in methanol, the resulting protonated amine was treated with diisopropylethylamine. An *N*-acylation reaction of C3-deuteriosphingosylphosphocholine with *p*-nitrophenyl palmitate provided 3-deuterio-*N*-palmitoyl-*D*-*erythro*-sphingosylphosphocholine (**2**). The extent of deuteration was estimated to be approximately 95%, based on the relative intensities of the *m/z* peaks at 703.5752 ($[\text{M} + \text{H}^+]$ ion of nondeuterated **2**), 704.5812 ($[\text{M} + \text{H}^+]$ ion of mono-deuterated **2**), and 705.5844 (^{13}C isotope peak of the $[\text{M} + \text{H}^+]$ ion of mono-deuterated **2**).

In summary, we developed simple procedures for the preparation of deuterium-labeled sphingosine (**1**) and sphingomyelin (**2**) from *L*-Garner methyl ester **6**. The methodology described here can be extended to prepare a variety of other deuterated sphingolipids. For example, other deuterium-labeled derivatives of sphingosine are readily accessible by using multi-deuterated analogues of **5**, deuterated glyco- or phosphosphingolipids can be prepared from intermediate **13**, and deuterium-labeled ceramides can be conveniently obtained from **1**. Compounds containing deuterium at selected sites in the interfacial region of the sphingoid backbone may be useful as probes for ^2H NMR spectroscopic analysis and as internal standards for analysis of sphingosine and sphingomyelin in biological samples by electrospray ionization/tandem mass spectrometry.

3. Experimental procedures

3.1. General methods

HRMS were obtained by electrospray ionization (ESI) on an Agilent Technologies G1969A or G6520A Q-TOF mass spectrometer, which have mass accuracies of <3 and <2 ppm, respectively. Analyses were done using two reference ions. ^1H , ^{13}C , and ^{31}P NMR spectra were recorded at 400, 100, and 161.9 MHz, respectively, on a Bruker Avance I spectrometer, and were referenced to the residual chloroform at δ 7.24 (^1H) and δ 77.2 (^{13}C) and to external 85% H_3PO_4 at δ 0.00 ppm (^{31}P). TLC was carried out on aluminum-backed silica gel GF plates (250- μm thickness), and the compounds were visualized by charring with 10% sulfuric acid in ethanol and/or short wavelength UV light. For flash chromatography, silica gel 60 (230–

400 ASTM mesh) was used. THF was distilled from Na and benzophenone before use. CH₂Cl₂ and *i*-Pr₂NEt were dried over CaH₂.

3.2. 1,1-Dideuteriotetradecanol (4)

To a solution of methyl myristate (**3**, 2.50 g, 10.3 mmol) in THF was added LiAlD₄ (600 mg, 14.3 mmol) at −78 °C. After 2 h, the reaction mixture was gradually warmed to room temperature and stirred overnight. After the reaction was quenched with solid Na₂SO₄·10H₂O (2.0 g, 6.2 mmol), the mixture was passed through a pad of Celite, which was washed with 100mL of EtOAc. The filtrate was concentrated under reduced pressure, and the residue was purified by column chromatography on silica gel (elution with hexane/EtOAc 4:1) to give 2.03 g (91%) of alcohol **4** (which is available commercially from several vendors): ¹H NMR (CDCl₃) δ 0.88 (t, 3H, *J* = 6.4 Hz), 1.26 (m, 22H), 1.55 (t, 2H, *J* = 6.4 Hz); ¹³C NMR (CDCl₃) δ 14.1, 22.7, 25.7, 29.4, 29.4, 29.59, 29.60, 29.7, 31.9, 32.6, 62.3 (pentet, *J* = 21 Hz).

3.3. 1-Deuteriotetradecanal (5)

To a mixture of PCC (2.16 g, 10.0 mmol) and silica gel (3 g) in 50mL of CH₂Cl₂ was added a solution of alcohol **4** (984 mg, 4.55 mmol) in 10mL of CH₂Cl₂ at room temperature. After the mixture was stirred for 2 h, it was diluted with 300mL of Et₂O and passed through a pad of silica gel, which was washed 100mL of Et₂O. The filtrate was concentrated under reduced pressure. The residue was dissolved in a minimal volume of Et₂O and passed again through a pad of silica gel, which was washed with Et₂O. The filtrate was concentrated under reduced pressure to give 795mg (82%) of aldehyde **5**: ¹H NMR (CDCl₃) δ 0.88 (t, 3H, *J* = 6.8 Hz), 1.25 (s, 20H), 1.62 (m, 2H), 2.41 (t, 2H, *J* = 7.2 Hz); ¹³C NMR (CDCl₃) δ 14.1, 21.3, 22.5, 28.8, 28.9, 29.3, 29.60, 29.61, 29.7, 32.1, 43.73 (t, *J* = 4 Hz), 202.6 (t, *J* = 25 Hz).

3.4. N-tert-Butoxycarbonyl (4S)-[2',2'-dideuterio-2'-dimethylphosphonoacetyl]-2,2-dimethyl-1,3-oxazolidine (10)

To a solution of dimethyl methylphosphonate (1.65 g, 13.3 mmol) in 50mL of THF was added dropwise *n*-BuLi (5.2mL of a 2.5M solution in hexane, 13.0 mmol) at −78 °C. After 2 h, a solution of *L*-Garner methyl ester **6** (1.65 g, 6.36 mmol) in 10mL of THF was added slowly. The mixture was stirred at −78 °C for 2 h, then allowed to warm to room temperature, and stirred overnight. After the reaction was quenched by addition of solid ND₄Cl (770 mg, 13.4 mmol), it was concentrated under reduced pressure. The residue was dissolved in EtOAc (300 mL) and washed with brine. The organic layer was dried (MgSO₄) and concentrated. Purification by chromatography on silica gel (elution with hexane/EtOAc 1:1 and then EtOAc) gave 1.50 g (67%) of β-ketophosphonate **9** as a colorless oil. To a solution of **9** in 5mL of THF and 5mL of D₂O was added ND₄Cl (2.0 mg, 34.6 μmol) at room temperature. After the mixture was stirred overnight, it was concentrated under reduced pressure to give a residue. This exchange reaction was repeated three times. Dideuterated β-ketophosphonate **10** was used without further purification; ¹H NMR (CDCl₃) δ 1.45–1.75 (m, 15H), 3.65–3.95 (m, 6H), 4.04–4.17 (m, 2H), 4.49–4.60 (m, 1H).

3.5. N-tert-Butoxycarbonyl (4S)-[1'-oxo-(2'E)-2',3'-dideuteriohexadecenyl]-2,2-dimethyl-1,3-oxazolidine (11)

To a mixture of β-ketophosphonate **10** (1.50 g, 4.25 mmol) and K₂CO₃ (1.80 g, 13.0 mmol) in 10mL of D₂O was added a solution of aldehyde **5** (788 mg, 3.69 mmol) in 10mL of THF at 0 °C. After 4 h, the reaction mixture was gradually raised to room temperature and stirred for 72 h. After dilution with EtOAc (250 mL), the mixture was washed with brine, dried (Na₂SO₄), and concentrated under reduced pressure. Purification of the residue by column chromatography on silica gel (elution with a gradient of hexane, hexane/EtOAc 50:1 and 20:1) gave 991 mg (61%) of enone **11**: ¹H NMR (CDCl₃) δ 0.88 (t, 3H, *J* = 7.0 Hz), 1.09–1.31 (m,

20 H), 1.48 (s, 9H), 1.54 (s, 3H), 1.83 (s, 3H), 1.95 (q, 2H, $J = 6.5$ Hz), 3.82 (m, 2H), 5.85 (m, 1H), 5.98 (m, 1H), 6.23 (d, 1H, $J = 15.5$ Hz), 7.30 (m, 1H); ^{13}C NMR (CDCl_3) δ 14.1, 20.1, 28.4, 29.0, 29.1, 29.4, 29.5, 29.7, 32.2, 33.2, 33.3, (64.7), 65.0, (66.0), 66.3, (79.9), 80.0, (94.4), 95.3, 123.3, (124.4), (128.2), 129.2, (144.0), 144.2, (145.8), 146.4, 151.7, (152.2), (195.5), 196.0. The ^{13}C NMR chemical shifts in parentheses indicate the small peaks that arise from minor rotamers in the oxazolidine system, which undergoes a slow dynamic equilibrium at room temperature.

3.6. N-tert-Butoxycarbonyl (4S)-[(1'S)-hydroxy-1',2',3'-trideuterio-(2'E)-hexadecenyl]-2,2-dimethyl-1,3-oxazolidine (12)

To a mixture of enone **11** (985 mg, 2.24 mmol) and $\text{CeCl}_3 \cdot 7\text{H}_2\text{O}$ (186 mg, 0.50 mmol) in 40 mL of THF and 4 mL of CD_3OD was added NaBD_4 (123 mg, 2.94 mmol) at -78°C . The mixture was gradually raised to room temperature. After 4 h, the mixture was filtered through a pad of Celite, which was washed with 150 mL of EtOAc. The filtrate washed with brine, dried (Na_2SO_4), and concentrated under reduced pressure. The residue was purified by column chromatography on silica gel (elution with hexane/EtOAc 9:1) to give 882 mg (89%) of alcohol **12**: ^1H NMR (CDCl_3) δ 0.89 (t, 3H, $J = 6.6$ Hz), 1.27 (m, 20H), 1.48 (s, 9H), 1.53 (s, 3H), 1.57 (s, 3H), 1.98 (br s, 1H), 2.08 (q, 2H, $J = 6.8$ Hz), 4.03 (m, 1H), 4.08 (br s, 1H), 4.16 (br m, 1H), 4.31 (br s, 1H), 6.28 (m, 1H); ^{13}C NMR (CDCl_3) δ 14.1, 10.0, 22.9, 24.4, 26.8, 28.1, 28.4, 29.5, 29.67, 29.72, 29.89, 29.99, 30.0, 32.3, 32.9, 33.1, 62.0, 73.6, 80.2, 94.6, 128.4, 128.7, 133.8, 134.1, 161.1; HRMS $[\text{M}+\text{H}]^+$ m/z calcd. for $\text{C}_{26}\text{H}_{47}\text{D}_3\text{NO}_4$, 443.3923, found, 443.3924. Additional $[\text{M}+\text{H}]^+$ ions were also present: m/z calcd. for $\text{C}_{26}\text{H}_{46}\text{D}_4\text{NO}_4$, 444.3985, found, 443.3972; m/z calcd. for $\text{C}_{26}\text{H}_{48}\text{D}_2\text{NO}_4$, 442.3860, found, 442.3858. The relative intensities of these peaks, after correction for the natural abundance of ^{13}C , are 0.64, 0.20, and 0.16, respectively.

3.7. (2S,3R)-2-(N-tert-Butoxycarbonylamido)-4(E)-3,4,5-trideuteriooctadecene-1,3-diol (13)

A solution of acetamide **12** (874 mg, 1.97 mmol) in 1 M HCl (10 mL) and THF (20 mL) was stirred at room temperature until the reaction was completed. The mixture was neutralized with saturated aqueous NaHCO_3 solution, and the product was extracted with EtOAc (3×100 mL). The combined organic layers were washed with brine, dried (Na_2SO_4), and concentrated under reduced pressure. The residue was purified by column chromatography on silica gel (elution with hexane/EtOAc 2:1 then 1:1) to give 621 mg (78%) of *N*-Boc-sphingosine- d_3 **13** (containing minor amounts of the corresponding d_2 and d_4 species as described above): ^1H NMR (CDCl_3) δ 0.88 (t, 3H, $J = 6.8$ Hz), 1.26 (m, 20H), 1.37 (m, 2H), 1.45 (s, 9H), 2.04 (q, 2H, $J = 7.1$ Hz), 3.07 (br s, 2H), 3.68 (m, 1H), 3.77 (m, 1H), 3.91 (m, 1H), 5.32 (br s, 1H); ^{13}C NMR (CDCl_3) δ 14.1, 22.7, 28.4, 29.04, 29.07, 29.3, 29.47, 29.59, 29.62, 29.7, 31.9, 32.1, 55.4, 62.5, 64.1, 74.1 (t, $J = 18$ Hz), 79.8, 128.4 (t, $J = 16$ Hz), 133.6 (t, $J = 16$ Hz), 156.2; HRMS $[\text{M}+\text{Na}]^+$ m/z calcd for $\text{C}_{23}\text{H}_{42}\text{D}_3\text{NNaO}_4$, 425.3429, found, 425.3424.

3.8. (2S,3R)-2-Amino-(4E)-3,4,5-trideuteriooctadecene-1,3-diol hydrochloride salt (1)

A solution of *N*-Boc-sphingosine- d_3 (**13**) (417 mg, 1.03 mmol) in 2 mL of 3 M HCl and 10 mL of THF was heated at 70°C with stirring overnight under nitrogen. The reaction mixture was cooled to room temperature, concentrated, and lyophilized from C_6H_6 to give 351 mg (100%) of sphingosine- d_3 HCl salt **1**: ^1H NMR (CDCl_3) δ 0.85 (t, 3H, $J = 6.8$ Hz), 1.26 (m, 20H), 1.36 (m, 2H), 2.08 (q, 2H, $J = 7.1$ Hz), 3.07 (br s, 2H), 3.68 (m, 1H), 3.77 (m, 1H), 3.91 (m, 1H), 5.32 (br s, 1H); ^{13}C NMR ($\text{CDCl}_3/\text{CD}_3\text{OD}$) δ 14.2, 22.6, 28.4, 29.06, 29.08, 29.3, 29.48, 29.58, 29.63, 29.7, 31.9, 32.1, 55.4, 62.5, 64.1, 74.2 (t, $J = 18$ Hz), 128.3 (t, $J = 18$ Hz), 133.6 (t, $J = 18$ Hz); HRMS $[\text{M}+\text{H}]^+$ m/z calcd. for $\text{C}_{18}\text{H}_{35}\text{D}_3\text{NO}_2$, 303.3085, found, 303.3089; HRMS $[\text{M}+\text{Na}]^+$ m/z calcd for $\text{C}_{23}\text{H}_{42}\text{D}_3\text{NNaO}_2$, 325.2905, found, 325.2905.

3.9. N-tert-Butoxycarbonyl (4S)-4-[1'-hydroxy-1'-deutero-(2'E)-hexadecanyl]-2,2-dimethyl-1,3-oxazolidine (15)

To the mixture of enone **14** (298 mg, 0.68 mmol) and $\text{CeCl}_3 \cdot 7\text{H}_2\text{O}$ (56 mg, 0.15 mmol) in 20 mL of THF and 2 mL of CD_3OD was added NaBD_4 (37 mg, 0.89 mmol) at -78°C . The mixture was gradually raised to room temperature. After 10 h, the mixture was filtered through a pad of Celite, which was washed with 150 mL of EtOAc. The filtrate was washed with brine, dried (Na_2SO_4), and concentrated under reduced pressure. A residue was purified by column chromatography on silica gel (elution with hexane/EtOAc 9:1) to give 240 mg (80%) of 3-deuterio-alcohol **15** as a colorless oil: ^1H NMR δ 0.88 (t, 3H, $J = 6.6$ Hz), 1.25 (m, 20H), 1.49 (s, 9H), 1.30–1.70 (m, 10H), 2.05 (m, 2H), 3.75–4.25 (m, 4H), 5.44 (d, 1H, $J = 15.2$ Hz), 5.73 (m, 1H); ^{13}C NMR δ 14.1, 10.0, 22.9, 24.4, 26.8, 28.1, 28.4, 29.5, 29.67, 29.72, 29.89, 29.99, 30.0, 32.3, 32.9, 33.1, 59.4, 61.3, 62.3, 64.8, 65.2, 66.1, 66.4, 73.4, 81.0 (t, $J = 24$ Hz), 94.4, (95.0), 128.0, (129.5), 131.8, 133.4, (135.4), 171.3, (170.92).

3.10. (2S,3R)-2-(N-tert-Butoxycarbonylamido)-3-deutero-4(E)-octadecene-1,3-diol (16)

A solution of alcohol **15** (233 mg, 0.53 mmol) in 10 mL of 1M HCl and 50 mL of THF was stirred at room temperature until the reaction was completed. The mixture was neutralized with saturated aqueous NaHCO_3 solution, and the product was extracted with EtOAc (100 mL $\times$ 3). The combined organic layers were washed with brine, dried (Na_2SO_4), and concentrated under reduced pressure. The residue was purified by column chromatography on silica gel (elution with hexane/EtOAc 2:1 then 1:1) to give 180 mg (85%) of 3-deuterio-N-Boc-sphingosine **16**: ^1H NMR δ 0.88 (t, 3H, $J = 6.8$ Hz), 1.25 (m, 20H), 1.45 (s, 9H), 2.05 (q, 2H, $J = 7.1$ Hz), 2.45 (br s, 2H), 3.61 (br s, 1H), 3.71 (m, 1H), 3.95 (m, 1H), 5.81 (br s, 1H), 5.53 (d, 1H, $J = 15.3$ Hz), 5.78 (m, 1H); ^{13}C NMR δ 14.1, 22.6, 22.7, 25.8, 29.0, 29.2, 29.3, 29.59, 29.62, 29.65, 31.7, 31.9, 32.7, 36.8, 55.3, 62.6, 64.2, 74.1 (t, $J = 18$ Hz), 79.8, 134.0, 138.0, 154.6.

3.11. 3-Deuterio-N-palmitoyl-D-erythro-sphingosylphosphocholine (2)

To a solution of 2-chloro-1,3,2-dioxaphospholane (173 mg, 1.36 mmol) in 25 mL of CH_2Cl_2 was added 70 μL (1.36 mmol) of Br_2 at -78°C . After 10 min, a solution of **16** (175 mg, 0.437 mmol) and *N,N*-diisopropylethylamine (230 mg, 1.78 mmol) in 10 mL of CH_2Cl_2 was added. After the reaction mixture was stirred at room temperature for 48 h, it was concentrated under reduced pressure to give a residue, which was dried under high vacuum for 2 h. To a stirred solution of the residue in 9 mL of CHCl_3 were added 39 mL of aqueous NMe_3 (40%) solution, 15 mL of acetonitrile, and 15 mL of 2-propanol. After the mixture was stirred at room temperature for 72 h, it was concentrated under reduced pressure. The product was purified by column chromatography on silica gel (elution with $\text{CHCl}_3/\text{MeOH}/\text{H}_2\text{O}$ 65:25:4) to give 134 mg (54%) of 3-deuterio-N-Boc-sphingomyelin **17**; HRMS $[\text{M}+\text{H}^+]$ m/z calcd. for $\text{C}_{28}\text{H}_{57}\text{DN}_2\text{O}_7\text{P}$, 566.4044, found 566.4039. A solution of **17** (102 mg, 0.18 mmol) in 5 mL of 3M HCl and 50 mL of methanol was heated at reflux overnight. The mixture was concentrated under reduced pressure to give a residue. To the residue were added 20 mL of THF/ H_2O (9:1), *N,N*-diisopropylethylamine (100 μL , 0.57 mmol), and 4-nitrophenyl palmitate (300 mg, 0.79 mmol). After the mixture was stirred for 72 h at room temperature, it was concentrated under reduced pressure. The residue was purified by chromatography on silica gel (elution with $\text{CHCl}_3/\text{MeOH}/\text{H}_2\text{O}$, 65:25:4 and then 65:35:8) to afford 58 mg (35%) of **2** after filtration of a chloroform solution through a 0.45- μm Teflon syringe filter to remove suspended silica gel: ^1H NMR (CDCl_3) δ 0.88 (t, 6H, $J = 6.0$ Hz), 1.26 (m, 32H), 1.53 (m, 2H), 2.07 (m, 2H), 2.26 (m, 2H), 3.33 (s, 9H), 3.93–3.66 (m, 4H), 4.29 (m, 4H), 5.50 (br s, 1H), 5.52 (d, 1H, $J = 15.6$ Hz), 5.77 (m, 1H); ^{13}C NMR ($\text{CDCl}_3/\text{CD}_3\text{OD}$) δ 13.5, 25.1, 25.6, 28.91, 28.98, 29.0, 29.06, 29.1, 29.20, 29.21, 29.28, 29.31, 31.5, 32.0, 36.1, 36.6, 53.7, 58.6 (d, $J = 5.0$ Hz), 64.2 (d, $J = 5.0$ Hz), 66.1, 66.5 (d, $J = 5.0$ Hz), 74.9 (t, $J = 18$ Hz), 128.7, 134.1, 175.7; ^{31}P NMR

(CDCl₃/CD₃OD) δ 0.29; HRMS [M+H⁺] m/z calcd. for C₃₉H₇₉DN₂O₆P, 704.5811, found 704.5812.

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References

- Bartels T, Lankalapalli RS, Bittman R, Brown MF, Beyer K. Raftlike mixtures of sphingomyelin and cholesterol investigated by solid-state ²H NMR spectroscopy. *J Am Chem Soc* 2008;130:14521–14532. [PubMed: 18839945]
- Bielawska A, Hannum YA, Szulc Z. Radiolabeling of the sphingolipid backbone. *Methods Enzymol* 2000;311:480–498. [PubMed: 10563345]
- Byun H-S, Erukulla RK, Bittman R. Synthesis of sphingomyelin and ceramide 1-phosphate from ceramide without protection of the allylic hydroxyl group. *J Org Chem* 1994;59:6495–6498.
- Chun J, Li G, Byun H-S, Bittman R. Synthesis of new trans double-bond sphingolipid analogues: $\Delta^{4,6}$ and Δ^6 ceramides. *J Org Chem* 2002a;67:2600–2605. [PubMed: 11950306]
- Chun J, Li G, Byun H-S, Bittman R. A concise route to d-erythro-sphingosine from N-Boc-l-serine derivatives via sulfoxide or sulfone intermediates. *Tetrahedron Lett* 2002b;43:375–377.
- Chun J, Byun H-S, Arthur G, Bittman R. Synthesis and growth inhibitory activity of chiral 5-hydroxy-2-N-acyl-3E-sphingenines: ceramides with an unusual sphingoid backbone. *J Org Chem* 2003;68:355–359. [PubMed: 12530860]
- Erukulla RK, Byun H-S, Bittman R. Antitumor phospholipids: a one-pot introduction of a phosphocholine moiety into lipid hydroxy acceptors. *Tetrahedron Lett* 1994;35:5783–5784.
- Garner P, Park JM. The synthesis and configurational stability of differentially protected β -hydroxy- α -amino aldehydes. *J Org Chem* 1987;52:2361–2364.
- Gemal AL, Luche J-L. Lanthanoids in organic synthesis 6. The reduction of α -enones by sodium borohydride in the presence of lanthanoid chlorides: synthetic and mechanistic aspects. *J Am Chem Soc* 1981;100:5454–5459.
- Han X. Multi-dimensional mass spectrometry-based shotgun lipidomics and the altered lipids at the mild cognitive impairment stage of Alzheimer's disease. *Biochim Biophys Acta* 2010;1801:774–783. [PubMed: 20117236]
- Hoffman RV, Tao J. A synthesis of d-erythro- and l-threo-sphingosine and sphinganine diastereomers via the biomimetic precursor 3-ketosphinganine. *J Org Chem* 1998;63:3979–3985.
- Koskinen PM, Koskinen AMP. Total synthesis of sphingosine and its analogs. *Methods Enzymol* 2000;311:458–479. [PubMed: 10563344]
- Li S, Pang J, Wilson WK, Schroepfer GJ Jr. Efficient synthesis of deuterium or tritium-labeled d-erythro-sphingosine. *J Labeled Cpd Radiopharm* 1999;42:815–826.
- Magkos F, Mittendorfer B. Stable isotope-labeled tracers for the investigation of fatty acid and triglyceride metabolism in humans in vivo. *Clin Lipidol* 2009;4:215–230. [PubMed: 20161007]
- Mahmud T, Xu J, Choi YU. Synthesis of 5-*epi*-[6-²H₂]valiolone and stereo-specifically monodeuterated 5-*epi*-valiolones: exploring the steric course of 5-*epi*-valiolone dehydratase in validamycin A biosynthesis. *J Org Chem* 2001;66:5066–5073. [PubMed: 11463258]
- Mehnert T, Bittman R, Jacob R, Beyer K. Structure and lipid interaction of N-palmitoyl-d-erythro-sphingosylphosphocholine in bilayer membranes as revealed by ²H NMR spectroscopy. *Biophys J* 2006;90:939–946. [PubMed: 16284259]
- Mutlib AE. Application of stable isotope-labeled compounds in metabolism and in metabolism-based toxicity studies. *Chem Res Toxicol* 2008;21:1672–1689. [PubMed: 18702535]
- Postle AD, Hunt AN. Dynamic lipidomics with stable isotope labeling. *J Chromatogr B Analyt Technol Biomed Life Sci* 2009;877:2716–2721.
- Shevchenko A, Simons K. Lipidomics: coming to grips with lipid diversity. *Nat Rev Mol Cell Biol* 2010;11:593–598. [PubMed: 20606693]

Solladié-Cavallo A, Koessler JL. A four-step diastereoselective synthesis of d-*erythro*-sphingosine by an enantioselective aldol reaction using a titanium enolate derived from a chiral iminoglycinate. *J Org Chem* 1994;59:3240–3242.

Fig. 1. Structures of 3,4,5-trideuterio-D-erythro-sphingosine (**1**) and 3-deutero-D-erythro-N-palmitoylsphingosylphosphocholine (**2**).

Scheme 1.

Synthesis of 1-deuteriomyristaldehyde (**5**) from methyl myristate (**3**).

Scheme 2.
Synthesis of 3,4,5-trideuteriosphingosine (1).

Scheme 3.

Synthesis of 3-deuterio-*N*-palmitoyl-*D*-*erythro*-sphingosylphosphocholine (**2**).