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Practical multigram-scale synthesis of 4,6- and 4,8-sphingadienes, chemopreventive sphingoid bases

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Abstract

Sphingadienes are chemopreventive agents that act by blocking signaling pathways that are activated in cancer. A practical synthesis of 4,6- and 4,8-sphingadienes on a scale of gram quantities is reported here in order to allow evaluation of the biological properties of these sphingolipids. The key steps in the preparation of 4,6-sphingadiene (**1a**) are an intramolecular cyclization of *N*-Boc derivative **5a** to oxazolidinone derivative **6a**, followed by conversion to carbamate intermediate **7a** and base-mediated hydrolysis to afford the product without further purification. 4,8-Sphingadiene (**1b**) was prepared in a similar fashion; the requisite *trans*- γ,δ -unsaturated aldehyde **15** was prepared by an ester enolate Ireland-Claisen rearrangement.

Keywords

Lipid synthesis; Sphingadienine; Sphingolipid; Sphingoid base

1. Introduction

Sphingosine (*trans*-4-sphingenine) is the long-chain or sphingoid base that constitutes the backbone of sphingolipids. Sphingoid bases are structurally diverse, varying in the number of double bonds, alkyl chain length, and extent of hydroxylation or methyl branching. The predominant long-chain base in mammals is (2*S*,3*R*,4*E*)-2-aminooctadec-4-ene-1,3-diol (for reviews, see Pruett et al., 2008; Bittman, 2009). Sphingadienes, which are also called sphingadienines, contain two double bonds in the sphingoid base, and are commonly found in plants. The β -glucosylceramides (cerebrosides) of soybean, rice bran, and wheat grain often contain 4,8-sphingadiene (denoted as d18:2^{4,8} in lipidomics nomenclature) as the major sphingoid base (Lynch et al., 1992; Sullards et al., 2000). The geometry of the double bond is *E* at C4–C5 and an *E,Z* mixture at C8,C9. A branched methyl group may be present; for example, the ceramide monohexosides of fungal cells contain 9-methyl-4,8-sphingadiene as the long-chain base (Levery, 2005; Barreto-Bergter et al., 2011). In contrast to plant cerebrosides, the sphingadienes of insects such as *Drosophila* Sply mutants (Fyrst et al., 2008) and moths such as *Manduca sexta* (Abeytunga et al., 2004) have a shorter aliphatic chain and conjugated double bonds at C4,C5 and C6,C7; these 4,6-sphingadienes typically have 14 and 16 carbons.

Sphingadienes have attracted a great deal of interest because they have potential uses in colon cancer treatment (for a recent review, see Fyrst and Saba, 2010). Soy glucosylceramides, in which the predominant sphingoid base is 4,8-sphingadiene,

suppressed colon tumorigenesis in mice (Symolon et al., 2004). A monoglucosylceramide (β -glucosyl-N-2'-hydroxyarachidoyl-4,8-sphingadiene) from rice bran inhibited the induction of 1,2-dimethylhydrazine-induced aberrant crypt foci and β -catenin-accumulated crypt formation in F344 rats (Inamine et al., 2005). Moreover, aquatic plants containing sphingadienes have been used in oriental medicine (Tan et al., 2003; Sugawara et al., 2006). To assess whether the ability of soy sphingolipids to act as a chemopreventive agent is derived from the presence of the sphingadiene backbone, human colon cancer cells were treated with C14 (2*S*,3*R*,4*E*,6*E*)-4,6-sphingadiene and C18 (2*S*,3*R*,4*E*,8*E*)-4,8-sphingadiene (denoted as C14(4,6)- and C18(4,8)-sphingadiene, respectively). These sphingadienes prevented Akt translocation from the cytosol to the plasma membrane, and arrested the growth of human cancer cells in vitro more efficiently than soy glucosylceramides, suggesting that cerebroside's tumor-preventing activity in vivo results from metabolism to sphingadienes (Fyrst et al., 2009). This study also showed that C14(4,6)-sphingadiene inhibited intestinal tumorigenesis in *Apc*^{Min/+} mice. Sphingadienes may have a longer half-life in intestinal epithelial cells than sphingosine because of a slower conversion to the phosphorylated sphingoid base (Fyrst et al., 2009). 4,8-Sphingadienes induced apoptosis in Caco-2 human colon cancer cells (Aida et al., 2004) and were cytotoxic to HL-60, RAW 264.7, HUVEC, and HEK-293 cell lines in vitro (Rozema et al., 2012a). In addition to blocking the phosphoinositide 3-kinase (PI3K)/Akt pathway (Fyrst et al., 2009), sphingadienes inhibited colon carcinogenesis by downregulating Wnt signaling through a protein phosphatase 2A/Akt/GSK3 β -dependent mechanism (Kumar et al., 2012). 4,8-Sphingadiene also exerts an anti-inflammatory response by inhibiting the expression of pro-inflammatory agents (interleukin-8 and E-selectin) induced by tumor necrosis factor- α and lipopolysaccharide in human endothelial cells (Rozema et al., 2012b).

The studies described above implicate sphingadienes as potential therapeutic lipids. Thus sphingadiene is an important synthetic target. Indeed, many elegant syntheses of sphingadienes have been reported prior to the discovery that sphingadienes protect the intestine from inflammation and cancer (Murakami et al., 2000, 2005; Wang et al., 2000; Black and Kocienski, 2010; Moreno et al., 2011). Unfortunately, none of the reported syntheses can be readily scaled up. Furthermore, isolation of pure sphingadienes from natural sources is not practical on a large scale because they are obtained as a mixture with other sphingolipids, and thus the individual roles of each component in the mixture cannot be determined.

2. Experimental

2.1. Materials and analytical procedures

2.1.1. Chemicals—The sources of the chemicals were as follows: chlorotrimethyl-silane and potassium hydride (30 wt.% dispersion in mineral oil) from Acros (Morris Plains, NJ); pyridinium chlorochromate (PCC), *p*-toluenesulfonic acid monohydrate (*p*-TsOH), and decanal from Alfa Aesar (Pelham, NH); *N*-Boc-L-serine methyl ester, *n*-butyllithium (2.5 M solution in hexane), cerium(III) chloride, hexamethyldisilazane (TMS₂NH), 2,2-dimethoxypropane, dimethyl methylphosphonate, sodium borohydride, and vinylmagnesium bromide (1.0 M solution in THF) from Aldrich (Milwaukee, WI). 3-*tert*-Butyloxycarbonyl (*S*)-4-carbomethoxy-2,2-dimethyl-3,4-oxazolidine ester ((*S*)-Garner ester, **2**) was prepared from *N*-Boc-L-serine methyl ester as described previously (Garner and Park, 1987).

2.1.2. General methods—Air- and moisture-sensitive reactions were carried out under nitrogen in dried glassware. THF was distilled from sodium and benzophenone, and hexane was dried over sodium metal. TLC analyses was performed on precoated aluminum-backed or glass-backed silica gel 60 F254 plates (0.25-mm thick), and the compounds were visualized by charring with 10% H₂SO₄ in EtOH or by UV light (254 nm). 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 on a Bruker Avance I spectrometer 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). The high-resolution mass spectrometric data were recorded at the CUNY mass spectrometry facility on an Agilent Technologies G6520A Q-TOF mass spectrometer using electrospray ionization. Optical rotations were measured on an Autopol III digital polarimeter at room temperature in the solvents stated.

2.2. Synthesis

2.2.1. N-tert-Butoxycarbonyl (4S)-4-[1'-oxo-(2'E,4'E)-dodecadienyl]-2,2-dimethyl-1,3-oxazolidine (4a)—A solution of dimethyl methylphosphonate (31.1 g, 250 mmol) in 50 mL of hexane and 120 mL of THF was cooled in an Et_2O /dry ice bath (about -100°C) for 1 h. To this solution was added 100 mL (250 mmol) of *n*-BuLi (2.5 M in hexane). After the reaction mixture was stirred for an additional 6 h, a solution of **2** (26.0 g, 100 mmol) in 50 mL of THF was added, and the reaction mixture was kept at -78°C (acetone/dry ice bath) overnight. The reaction mixture was allowed to warm to 0°C with stirring for 1 h. After the reaction was quenched with acetic acid (15 mL, 260 mmol), removal of the solvents under reduced pressure gave a residue that contained β -ketophosphonate **3** and an excess amount of dimethyl methylphosphonate. To this residue was added a solution of K_2CO_3 (125 g, 904 mmol) in 150 mL of H_2O , followed by a solution of commercially available (*E*)-2-decenal (10.0 g, 64.8 mmol) in 150 mL of 2-propanol at 0°C . After the mixture had been stirred at 0°C for 4 h, the reaction mixture was gradually warmed to room temperature and stirred overnight. The reaction mixture was diluted with 100 mL of saturated aqueous NaCl solution, and the product was extracted with Et_2O (3×150 mL). The combined organic layers were washed with brine, dried (Na_2SO_4), and concentrated. Purification of the residue by column chromatography (elution with hexane/ EtOAc 20:1, 15:1, and 10:1) gave 17.1 g (45%) of **4a** (yield calculated from **2**): $[\alpha]_D^{25} -37.2^\circ$ (c 2.0, CHCl_3); ^1H NMR (400 MHz, CDCl_3) δ 0.88 (t, 3H, $J = 7.0$ Hz), 1.09–1.31 (m, 10 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), 4.43 (br s, 1H), 5.85 (m, 1H), 5.98 (m, 1H), 6.23 (d, 1H, $J = 15.5$ Hz), 7.30 (m, 1H); ^{13}C NMR (100 MHz, CDCl_3) δ 14.1, 20.1, 22.6, 23.6, 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, (94.3), 95.0, 122.2, (128.8), 128.8, (143.1), 144.8, (147.1), 147.5 151.4, (152.2), (196.2), 197.1; HRMS ($\text{M}+\text{Na}$) $^+$ calcd for m/z $\text{C}_{22}\text{H}_{37}\text{NNaO}_4$ 402.2620, found 402.2613. At ambient temperature, the ^{13}C NMR spectrum was complicated by the presence of several doublet signals, indicating that the oxazolidine system undergoes a dynamic equilibrium. The parentheses indicate the other peak sets generated by the dynamic equilibrium of the oxazolidine system.

2.2.2. N-tert-Butoxycarbonyl (4S)-4-[1'-oxo-(2'E,6'E)-hexadecadienyl]-2,2-dimethyl-1,3-oxazolidine (4b)—The compound was prepared by the procedure described for the synthesis of **4a**. The in situ preparation of β -ketophosphonate **3** (see Section 2.2.1) starting from 13.1 g (50.5 mmol) of **2** followed by reaction with 9.50 g (45.1 mmol) of aldehyde **15** gave 10.3 g (47%) of dienone **4b**: $[\alpha]_D^{25} -36.1^\circ$ (c 2.0, CHCl_3); ^1H NMR δ 0.88 (t, $J = 7.0$ Hz, 3H), 1.09–1.31 (m, 14H), 1.48 (s, 9H), 1.54 (s, 3H), 1.83 (s, 3H), 1.96 (m, 2H), 2.12–2.33 (m, 4H), 3.82 (m, 2H), 4.43 (br s, 1H), 5.35–5.51 (m, 2H), 6.23 (d, $J = 15.5$ Hz, 1H), 7.30 (m, 1H); ^{13}C NMR (100 MHz, CDCl_3) δ 14.1, 20.1, 22.7, 23.6, 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, (94.3), 95.0, 122.2, (128.8), 128.8, (143.1), 144.8, (147.1), 147.5 151.4, (152.2), (196.2), 197.1; HRMS ($\text{M}+\text{Na}$) $^+$ calcd for m/z $\text{C}_{26}\text{H}_{45}\text{NNaO}_4$ 458.3241, found 458.3234.

2.2.3. (4S,5R)-5-[(1'E,3'E)-Undecadienyl]-4-(hydroxymethyl)-2-oxazolidinone (7a)—To a mixture of **4a** (8.48 g, 22.3 mmol) and CeCl_3 (1.84 g, 7.47 mmol) in 250 mL of MeOH was added NaBH_4 (0.95 g, 25.1 mmol) at -78°C . The reaction mixture was gradually warmed to room temperature. Stirring was continued for 5 h, and the mixture was filtered through a pad of Celite, which was washed with 250 mL of Et_2O . The filtrate was washed with brine, dried (Na_2SO_4), and concentrated. The residue was dried further under high vacuum overnight to give crude **5a**. A solution of **5a** in 50 mL of THF was added to a suspension of KH (181 mg, 4.49 mmol) in 50 mL of THF at 0°C . After the reaction mixture was stirred overnight, the solvents were removed under reduced pressure in order to remove *tert*-butyl alcohol, which was formed by reaction of the C3-OH group with the N-Boc group. The residue was dissolved in dry THF and the mixture was stirred overnight. Because the R_f value of *N*-Boc derivative **5a** is the same as that of oxazolidinone derivative **6a**, the intramolecular base-catalyzed reaction (carbamate formation and loss of *tert*-butyl alcohol) was repeated three times to complete the cyclization reaction. In order to remove the isopropylidene group from **6a**, *p*-TsOH (853 mg, 4.49 mmol) was added to a solution of crude **6a** in 100 mL of THF/ H_2O (9:1). After the reaction mixture was stirred overnight, the solvents were removed under reduced pressure, and the resulting residue was diluted with Et_2O (250 mL) and washed with brine and water. The organic layer was dried (Na_2SO_4) and concentrated. The product was purified by column chromatography on silica gel (elution with hexane/ EtOAc 1:1, and then 1:2) to give 4.26 g (71%) of **7a**: $[\alpha]_{\text{D}}^{25} -38.1^\circ$ (c 2.2, CHCl_3); ^1H NMR (400 MHz, CD_2Cl_2) δ 0.88 (t, $J = 6.5$ Hz, 3H), 1.26 (m, 8H), 1.30–1.35 (m, 2H), 2.07 (q, $J = 6.9$ Hz, 2H), 3.62–3.77 (m, 2H), 3.91 (dd, $J = 3.6, 11.3$ Hz, 1H), 4.36 (t, $J = 4.7$ Hz, 1H), 5.14 (t, $J = 8.2$ Hz, 1H), 5.57–5.63 (m, 1H), 5.68–5.75 (m, 1H), 6.00–6.06 (m, 1H), 6.28 (dd, $J = 10.3, 15.3$ Hz, 1H); ^{13}C NMR (100 MHz, CD_2Cl_2) δ 14.1, 22.6, 28.3, 29.1, 31.8, 32.6, 55.4, 62.5, 74.4, 129.1, 132.5, 136.3, 156.2; HRMS ($\text{M}+\text{Na}$) $^+$ calcd for m/z $\text{C}_{19}\text{H}_{35}\text{NNaO}_4 + 364.2458$, found 364.2461. In order to confirm the structure of **7a**, (4*S*,5*R*)-*N*-acetyl-5-[(1'E,3'E)-undecadienyl]-4-(acetyloxymethyl)-2-oxazolidinone (**7a'**) was prepared from **7a** by using an excess of Ac_2O in pyridine: $[\alpha]_{\text{D}}^{25} -2.1^\circ$ (c 1.8, CHCl_3); ^1H NMR (400 MHz, CDCl_3) δ 0.88 (t, $J = 6.5$ Hz, 3H), 1.26 (m, 8H), 2.05 (s, 3H), 2.03–2.11 (m, 2H), 2.52 (s, 3H), 4.25 (dd, $J = 2.1, 11.3$ Hz, 1H), 4.36 (q, $J = 4.1$ Hz, 1H), 4.68 (dd, $J = 4.1, 11.3$ Hz, 1H), 4.82 (dd, $J = 3.1, 7.2$ Hz, 1H), 5.51 (dd, $J = 7.2, 15.1$ Hz, 1H), 5.85 (dt, $J = 6.7, 15.1$ Hz, 1H), 6.02 (dd, $J = 10.3, 15.1$ Hz, 1H), 6.32 (dd, $J = 10.3, 15.1$ Hz, 1H); ^{13}C NMR (100 MHz, CDCl_3) δ 14.1, 20.6, 22.6, 23.7, 28.9, 29.1, 31.7, 32.6, 58.3, 61.7, 76.8, 123.9, 126.2, 127.9, 135.5, 139.7, 153.0, 170.5; HRMS ($\text{M}+\text{Na}$) $^+$ calcd for m/z $\text{C}_{19}\text{H}_{29}\text{NNaO}_5 + 374.1938$, found 374.1940.

2.2.4. (4S,5R)-5-[(1'E,5'E)-Undecadienyl]-4-(hydroxymethyl)-2-oxazolidinone (7b)—This compound was prepared by the procedure described above by starting with 11.3 g (25.9 mmol) of **3b** to give 6.30 g (75%) of **7b**: $[\alpha]_{\text{D}}^{25} -37.0^\circ$ (c 2.3, CHCl_3); ^1H NMR (400 MHz, CD_2Cl_2) δ 0.88 (t, $J = 6.6$ Hz, 3H), 1.26 (m, 14H), 1.96 (q, $J = 6.7$ Hz, 2H), 2.07–2.12 (m, 2H), 2.12–2.20 (m, 2H), 3.64 (m, 3H), 3.80–3.96 (m, 1H), 5.07 (t, $J = 8.3$ Hz, 1H), 5.36 (dt, $J = 5.7, 15.2$ Hz), 5.43 (dt, $J = 6.3, 15.2$ Hz), 5.63 (dd, $J = 8.4, 15.4$ Hz), 5.89 (dt, $J = 6.2, 15.4$ Hz), 6.74 (br s, 1H); ^{13}C NMR (100 MHz, CD_2Cl_2) δ 14.2, 22.7, 29.2, 29.3, 29.50, 29.53, 29.58, 31.7, 31.9, 32.2, 32.6, 57.4, 61.6, 80.3, 122.4, 128.6, 131.5, 138.5, 160.5; HRMS ($\text{M}+\text{H}$) $^+$ calcd for m/z $\text{C}_{19}\text{H}_{34}\text{NO}_3 + 324.2533$, found 324.2530.

2.2.5. (2S,3R)-2-Amino-(4E,6E)-tetradecadiene-1,3-diol (1a)—A mixture of **7a** (4.66 g, 17.4 mmol) in 100 mL of 2 M NaOH solution and 100 mL of EtOH was heated at 80°C for 3 h. The reaction mixture was cooled to room temperature, concentrated under reduced pressure, and the product was extracted with Et_2O (3×20 mL). The organic layer was washed with brine, dried (K_2CO_3), and concentrated to give 4.20 g (100%) of **1a**; $[\alpha]_{\text{D}}^{25} -2.5^\circ$ (c 1.2, CHCl_3); ^1H NMR (400 MHz, 2 mg in 0.5 mL of CD_2Cl_2) δ 0.91 (t, $J =$

6.7 Hz, 3H), 1.20–1.43 (m, 8H), 2.79 (br s, 1H), 3.04 (br s, 3H), 3.48–3.66 (m, 2H), 4.09 (q, $J = 6.1$ Hz, 1H), 5.57 (dt, $J = 6.2, 15.2$ Hz, 1H), 5.71–5.80 (m, 1H), 6.08 (dd, $J = 10.5, 15.2$ Hz, 1H), 6.27 (dd, $J = 10.5, 15.2$ Hz, 1H); ^1H NMR (400 MHz, 20 mg in 0.5 mL of pyridine- d_5) δ 0.85 (t, $J = 6.7$ Hz, 3H), 1.26 (m, 8H), 1.30–1.35 (m, 2H), 2.05 (q, $J = 7.0$ Hz, 2H), 3.24 (q, $J = 5.3$ Hz, 0.55H), 3.38 (q, $J = 6.0$ Hz, 0.45 H), 3.99 (dd, $J = 10.4, 6.4$ Hz, 0.55H), 4.09–4.17 (m, 1H), 4.23 (dd, $J = 10.4, 4.7$ Hz, 0.45H), 4.64 (q, $J = 5.5$ Hz, 1H), 5.67–5.76 (m, 1H), 6.06–6.19 (m, 1H), 6.21–6.27 (m, 1H), 6.63 (dd, $J = 10.5, 15.3$ Hz, 1H); ^{13}C NMR ($\text{CD}_2\text{Cl}_2/\text{CD}_3\text{OD}$) δ 14.2, 22.9, 29.4, 29.6, 32.0, (58.83), 58.89, 64.69, (64.74), 73.2, 74.7, 130.7, 131.3, 131.7; HRMS ($\text{M}+\text{H}$) $^+$ calcd for m/z $\text{C}_{14}\text{H}_{27}\text{NNaO}_2 + 264.1934$, found 264.1932. In order to confirm the structure of **1a**, (2*S*,3*R*)-2-acetylamido-(4*E*,6*E*)-tetradecadiene-1,3-diol (**1c**) was prepared by the reaction of **1a** with 4-nitrophenyl acetate in THF/ H_2O (9:1): ^1H NMR (400 MHz, CDCl_3) δ 0.88 (t, $J = 6.6$ Hz, 3H), 1.26 (m, 8H), 1.30–1.44 (m, 2H), 1.82 (br s, 2H), 2.04–2.09 (m, 2H), 2.05 (s, 3H), 3.59 (dd, $J = 6.5, 11.6$ Hz, 1H), 3.65–3.69 (m, 1H), 3.74 (dd, $J = 2.9, 11.6$ Hz, 1H), 4.81 (t, $J = 6.5$ Hz, 1H), 5.59 (dd, $J = 7.9, 15.1$ Hz, 1H), 5.82 (dt, $J = 6.9, 15.1$ Hz, 1H), 6.04 (dd, $J = 10.5, 15.1$ Hz, 1H), 6.31 (dd, $J = 10.5, 15.1$ Hz, 1H), 6.38 (br s, 1H); ^{13}C NMR (100 MHz, CDCl_3) δ 14.1, 22.6, 29.0, 19.12, 29.13, 31.8, 32.7, 60.1, 62.7, 79.5, 125.2, 128.3, 135.5, 138.9, 159.8; HRMS ($\text{M}+\text{H}$) $^+$ calcd for m/z $\text{C}_{16}\text{H}_{31}\text{NO}_3 + 284.2220$, found 284.2225.

2.2.6. (2*S*,3*R*)-2-Amino-(4*E*,8*E*)-octadecadiene-1,3-diol (1b**)**—This compound was prepared by the procedure described above for **1a** by starting with 6.22 g (19.2 mmol) of **7b** to give 5.51 g (96%) of **1b**: $[\alpha]_D^{25} -2.7^\circ$ (c 1.3, CHCl_3); ^1H NMR (400 MHz, pyridine- d_5) δ 0.85 (t, $J = 6.7$ Hz, 3H), 1.26 (s, 8H), 1.30–1.35 (m, 2H), 2.05 (q, $J = 7.0$ Hz, 2H), 3.24 (q, $J = 5.3$ Hz, 0.55H), 3.38 (q, $J = 6.0$ Hz, 0.45 H), 3.99 (dd, $J = 10.4, 6.4$ Hz, 0.55H), 4.09–4.17 (m, 1H), 4.23 (dd, $J = 10.4, 4.7$ Hz, 0.45H), 4.64 (q, $J = 5.5$ Hz, 1H), 5.67–5.76 (m, 1H), 6.06–6.19 (m, 1H), 6.21–6.27 (m, 1H), 6.63 (dd, $J = 10.5, 15.3$ Hz); ^{13}C NMR (100 MHz, $\text{CDCl}_3/\text{CD}_3\text{OD}$) δ 14.2, 22.9, 29.4, 29.6, 32.0, (58.83), 58.89, 64.69, (64.74), 73.2, 74.7, 130.7, 131.3, 131.7; HRMS ($\text{M}+\text{H}$) $^+$ calcd for m/z $\text{C}_{18}\text{H}_{36}\text{NO}_2 + 298.2741$, found 298.2743.

2.2.7. rac-3-Hydroxy-1-dodecene (8**)**—To a mixture of decanal (15.7 g, 0.100 mol) in 100 mL of THF was added slowly vinylmagnesium bromide (110 mL, 0.11 mol, 1 M solution in THF) at -78°C . The reaction mixture was stirred -78°C for 3 h, and then was allowed to stir at 0°C for 1 h. After solid NH_4Cl (6.0 g, 112 mmol) and 5 mL of water were added slowly, the mixture was filtered. The filtrate was diluted with 250 mL of EtOAc and washed with brine and water. The organic layer was dried (MgSO_4) and concentrated. The product was purified by flash chromatography with 5% EtOAc/hexane as eluent to give 17.6 g (95%) of **8** as an oil: ^1H NMR (400 MHz, CDCl_3) δ 0.88 (t, $J = 6.6$ Hz, 3H), 1.26 (m, 14H), 1.50–1.54 (m, 2H), 4.08 (q, $J = 6.2$ Hz, 1H), 5.09 (dt, $J = 1.1, 10.4$ Hz, 1H), 5.21 (dt, $J = 1.4, 17.2$ Hz, 1H), 5.84 (ddd, $J = 6.2, 10.4, 17.2$ Hz, 1H); ^{13}C NMR (100 MHz, CDCl_3) δ 14.1, 22.7, 25.3, 29.3, 29.53, 29.56, 31.9, 37.0, 73.2, 114.5, 141.3. The ^1H and ^{13}C NMR spectra are in agreement with the published spectra of a mixture of **8** and **8a** (Cuzzupe et al., 2002; Itami et al., 2003).

2.2.8. rac-3-O-Acetyl-1-dodecene (10**)**—A solution of **8** (17.4 g, 94.4 mmol), acetic anhydride (10 mL, 105 mmol), and DMAP (600 mg, 4.9 mmol) in 25 mL of CH_2Cl_2 was stirred overnight at room temperature. After the mixture was concentrated under reduced pressure, the residue was dissolved in 250 mL of EtOAc and washed with water, aqueous saturated NaHCO_3 solution, and brine. The organic layer was dried (Na_2SO_4) and concentrated. The residue was dried under high vacuum overnight to give 20.4 g (95%) of **10**, which was used without further purification: ^1H NMR (400 MHz, CDCl_3) δ 0.88 (t, $J = 6.6$ Hz, 3H), 1.26 (m, 14H), 1.57–1.62 (m, 2H), 2.06 (s, 3H), 5.15 (d, $J = 10.4$ Hz, 1H), 5.20

(q, $J = 6.2$ Hz, 1H), 5.23 (d, $J = 7.4$ Hz, 1H), 5.77 (ddd, $J = 6.2, 7.4, 10.4$ Hz, 1H); ^{13}C NMR (100 MHz, CDCl_3) δ 14.1, 21.2, 22.7, 25.0, 29.27, 29.34, 29.5, 31.9, 34.2, 74.9, 116.5, 136.6, 170.4. The NMR spectra agree with the published spectra (Keinan and Greenspoon, 1983).

2.2.9. Methyl 4(E)-tetradecenoate (13)

To a solution of $(\text{TMS})_2\text{NH}$ (16.2 g, 100 mmol) in 40 mL of dry THF was added 40 mL (100 mmol) of *n*-BuLi (a 2.5 M solution in hexane) at -78°C . After the mixture had been stirred for 1 h, a solution of **10** (20.4 g, 90.1 mmol) in 100 mL of THF was added slowly. The resulting mixture was stirred at -78°C for 3 h, and then 13 mL (102 mmol) of TMSCl was added at -78°C with stirring for 1 h in order to form enol silyl ether **11**. The reaction mixture was allowed to warm to room temperature, and stirring was continued overnight. After concentration under reduced pressure, the residue was diluted with 250 mL of Et_2O and washed with aqueous 1 M HCl solution and brine. The organic layer was dried (Na_2SO_4) and concentrated to give carboxylic acid **12**. A mixture of crude **12**, trimethyl orthoformate (13 mL, 0.119 mol), and *p*-toluenesulfonic acid (1.0 g, 5.0 mmol) in 50 mL of MeOH was stirred overnight. The reaction mixture was concentrated, and the residue was purified by flash chromatography with 5% EtOAc/hexane as eluent to give 17.6 g (81%) of **13**: ^1H NMR (400 MHz, CDCl_3) δ 0.85 (t, $J = 6.6$ Hz, 3H), 1.26 (m, 14H), 1.91 (q, $J = 4.4$ Hz, 2H), 2.28 (t, $J = 6.1$ Hz, 2H), 2.32 (q, $J = 7.1$ Hz, 2H), 3.63 (s, 3H), 5.30–5.41 (m, 2H); ^{13}C NMR (100 MHz, CDCl_3) δ 14.1, 22.7, 27.9, 29.1, 29.3, 29.42, 29.49, 29.53, 29.6, 31.9, 32.5, 34.2, 51.4, 127.8, 131.9, 173.7; ^{13}C NMR (CDCl_3) δ 14.0, 14.2 (OCH_2CH_3), 22.8, 29.1, 29.3, 29.4, 29.5, 31.9, 32.4, 127.9, 131.8, 173.1. The NMR spectra agree with the published spectra (Baer and Schmidt, 1988).

2.2.10. 4(E)-Tetradecen-1-ol (14)—To a suspension of 3.80 g (100 mmol) of LiAlH_4 in 250 mL of THF was added a solution of 17.3 g (72.0 mmol) of **13** in 20 mL of THF at -78°C . After the reaction mixture was stirred overnight at room temperature, the reaction was quenched by addition of 4 mL of 1 M NaOH solution. The mixture was diluted with 150 mL of CH_2Cl_2 and passed through a pad of Celite, which was washed with 250 mL of EtOAc. The filtrate was concentrated under reduced pressure. The residue was purified by chromatography on silica gel (elution with hexane/EtOAc 6:1) to give 13.6 g (89%) of **14**: ^1H NMR (400 MHz, CDCl_3) δ 0.88 (t, $J = 6.6$ Hz, 3H), 1.26 (m, 14H), 1.45 (br s, 1H), 1.63 (q, $J = 6.7$ Hz, 2H), 2.07 (q, $J = 6.7$ Hz, 2H), 3.65 (t, $J = 6.5$ Hz, 2H), 5.40–5.46 (m, 2H); ^{13}C NMR (100 MHz, CDCl_3) δ 14.1, 28.9, 29.2, 29.3, 29.4, 29.50, 29.55, 29.57, 31.9, 32.4, 32.5, 62.6, 129.3, 131.3; ^{13}C NMR (CDCl_3) δ 14.0, 22.7, 28.9, 29.2, 29.3, 29.5, 29.6, 31.9, 32.5, 32.8, 62.6, 129.3, 131.3. The NMR spectra agree with the published spectra (Baer and Schmidt, 1988).

2.2.11. 4(E)-Tetradecenal (15)—To a mixture of pyridinium chlorochromate (26.0 g, 120 mmol) and silica gel (26 g) in 250 mL of CH_2Cl_2 was added a solution of **14** (12.8 g, 60.2 mmol) in 20 mL of CH_2Cl_2 . After the mixture had been stirred for 3 h, it was diluted with 250 mL of Et_2O and filtered through a pad of silica gel. The filtrate was concentrated and the residue was dissolved in Et_2O and passed through a pad of silica gel to remove a colored impurity. The filtrate was concentrated to give 9.51 g (75%) of **15**: ^1H NMR δ 0.88 (t, $J = 6.6$ Hz, 3H), 1.26 (m, 14H), 1.96 (q, $J = 6.7$ Hz, 2H), 2.33 (q, $J = 6.7$ Hz, 2H), 2.49 (dt, $J = 1.7, 7.0$ Hz, 2H), 5.35–5.51 (m, 2H), 9.76 (t, $J = 1.7$ Hz, 1H); ^{13}C NMR (100 MHz, CDCl_3) δ 14.1, 22.7, 25.2, 29.1, 29.2, 29.3, 29.5, 31.9, 32.4, 32.5, 43.5, 127.5, 132.1, 202.5. ^1H NMR (CDCl_3) δ 0.88 (t, 3H, $J = 7.4$ Hz), 1.26–1.32 (m, 14H), 2.31–2.34 (m, 2H), 2.47–2.51 (m, 2H), 5.37–5.47 (m, 2H), 9.77 (s, 1H); ^{13}C NMR (CDCl_3) δ 14.0, 22.7, 25.2, 29.1, 29.3, 29.4, 29.5, 29.7, 31.9, 32.5, 43.5, 127.6, 132.1, 202.4; HRMS ($\text{M}+\text{H}$) $^+$ calcd for

m/z C₁₄H₂₇O 211.2062, found 211.2052. The NMR spectra agree with the published spectra (Baer and Schmidt, 1988).

3. Results and discussion

In earlier work from our laboratory, we reported the synthesis of ceramides with a 4*E*,6*E*-sphingadiene backbone (Struckhoff et al., 2004) on a scale of several hundred milligrams. The source of chirality at C-2 was a derivative of L-serine. The key reaction was alkylation of a β -ketosulfoxide with a long-chain allylic bromide (Chun et al., 2002). Subsequently, we outlined the synthesis of 4*E*,6*E*-sphingadiene (**1a**), again on a small scale, via the olefination of the same β -ketophosphonate intermediate (Fyrst et al., 2009). In the present work, we report an improved multigram-scale synthesis of sphingadiene **1a** (Chart 1) from Garner ester **2** (Fig. 1). We found that the previous methods for the preparation of **1a** could not be scaled up because the acid-mediated stepwise removal of the acetonide and Boc protecting groups from the diene system **5** resulted in byproducts formed via allylic rearrangement and dehydration, requiring that the intermediate be isolated by careful column chromatography after each step. As a result, a large quantity of starting ester **2** is required. Moreover, during acid hydrolysis of the Boc group, the *erythro* form of **1a** partially undergoes conversion to its *threo* isomer, which cannot be removed from **1a** without making derivatives in which the stereoisomers are separable. To circumvent these problems, we have now developed a protocol for a scalable synthesis of enantiomerically pure C14(4,6)-sphingadiene (**1a**) and have applied a related methodology for the preparation of C18(4,8)-sphingadiene (**1b**).

3.1. Retrosynthetic plan

Fig. 1 outlines the retrosynthetic analysis we envisioned, in which **1a** and **1b** can be accessed from oxazolidinone **7**. The requisite cyclic carbamate can be obtained from the Boc-protected acetal **5**, which in turn is formed by a diastereoselective reduction of enone **4**. The enone can be prepared from L-serine derivative **2**, which is available commercially.

3.2. Synthesis of 4*E*,6*E*-sphingadiene (**1a**) from Garner ester **2**

Scheme 1 depicts the multigram synthesis of **1a** from (*S*)-Garner ester **2**. β -Ketophosphonate intermediate **3** was obtained by reaction of **2** (Garner and Park, 1987) with two equivalents of lithium dimethyl methylphosphonate (Koskinen and Krische, 1990; Byun and Bittman, 2010) in a dry ice/Et₂O bath, followed by standing overnight at -78°C . Dienone **4a** was prepared by the Horner–Wadsworth–Emmons (HWE) reaction of **3** with the commercially available aldehyde (*E*)-2-decenal. The HWE reaction was carried out in water/2-propanol (1:1) in the presence of K₂CO₃ because under these conditions the reaction was found to proceed with high *E*-selectivity (Liu et al., 2010). In the presence of DBU or Cs₂CO₃, under anhydrous conditions, a long-chain analog of dienone **4a** formed, but with a low optical rotation, suggesting that racemization may take place when strong bases are employed (Chun et al., 2002). In the presence of K₂CO₃ in aqueous alcohol, the reaction of CO₃²⁻ with the acidic proton of β -ketophosphonate **3** in water produces a CO₃²⁻/HCO₃⁻ buffered medium, and racemization of dienone **4a** may be avoided. The two-step sequence from the starting ester **2** gave **4a** in 45% overall yield based on **2**, and in 69% yield based on (*E*)-2-decenal. Diastereoselective reduction of ketone **4a** with NaBH₄/CeCl₃ in methanol, as reported previously (Chun et al., 2002), gave an unseparable 5:1 mixture of *erythro* alcohol **5a** together with the *threo* isomer (not shown). The *erythro*/*threo* selectivity was not improved when L-Selectride or DIBAL-H was used as the reducing agent; more bulky groups than 2,2-dimethyl-1,3-oxazolidine are apparently needed to improve the diastereoselectivity of the reduction (Kim et al., 2011). Since the minor amount of the *threo* diastereoisomer could not be removed by column chromatography (Chun et al., 2000, 2002), we decided to convert **5a** to the cyclic carbamate intermediate **7a**, where the minor

stereoisomer could be separated. The conversion of **5a** to **7a** was achieved in two steps via formation of the key intermediate **6a** (Triola et al., 2003). In the first step, the C3-hydroxyl group of crude **5a** reacted with a catalytic amount of KH in THF, resulting in cleavage of the Boc group. The intramolecular cyclization from *N*-Boc derivative **5a** to oxazolidinone derivative **6a** could not be monitored by TLC because the R_f -value of **5a** was the same as that of **6a** (R_f 0.6 in hexane/EtOAc 6:1); however, the conversion was confirmed by HRMS of the (M+H)⁺ ion of **6a**: calcd for m/z C₁₈H₃₀NO₃+ 308.2220, found 308.2216. The KH-catalyzed intramolecular carbamate formation reaction, with the loss of *tert*-butyl alcohol, was repeated three times, and each time *tert*-butanol was removed from the reaction mixture by rotary evaporation under reduced pressure, followed by addition of dry THF to the residue. In the second step, acid hydrolysis (*p*-TsOH in aqueous THF) of the acetonide group of **6a** provided cyclic carbamate **7a** in 71% overall yield from **4a**. As shown in Fig. 2A, the presence of diastereomers in cyclic carbamate **7a** was detected in the ¹H NMR spectrum. At this stage, the *threo* isomer was removed by column chromatography, and the stereochemical purity of **7a** was confirmed by its ¹H and ¹³C NMR spectra. The chemical shift of the *erythro* isomer at an allylic position of **7a** was δ 5.14 (t, J = 8.1 Hz), whereas that of *threo* isomer was δ 4.81 (t, J = 6.9 Hz) (see Fig. 2A). In the ¹³C NMR spectrum of **7a**, the C-3 signal of the *threo* isomer appears at δ 79.70 ppm, whereas the C-3 signal of the *erythro* isomer is at δ 80.27 ppm. Carbamate **7a** was fully characterized further by conversion to its diacetate derivative **7a'**. In the final step, hydrolysis of **7a** in ethanolic NaOH furnished the product **1a** in almost quantitative yield; further purification was not required because **1a** did not undergo elimination and epimerization reactions in basic media. In this procedure, only two column chromatographic purifications (one to obtain **4a** and the other to obtain **7a**) were needed to provide pure **1a** starting from **2**.

3.3. Synthesis of 4(E)-tetradecenal (15)

The multigram synthesis of C18(4,8)-sphingadiene (**1b**) employed a similar strategy as described for **1a**. Since the aldehyde required in the HWE reaction is not available commercially, it was first necessary to prepare a large quantity of 4(*E*)-tetradecenal (**15**). We initially attempted to prepare **15** via an orthoester Claisen rearrangement of allylic alcohol **8** (Baer and Schmidt, 1988). As shown in Scheme 2A, the reaction of decanal with vinylmagnesium bromide gave a mixture of **8** and **8a** (Cuzzupe et al., 2002; Itami et al., 2003). After byproduct **8a** was removed by column chromatography, pure allylic alcohol **8** was subjected to the orthoester Claisen rearrangement. The conversion to **9** proved difficult because byproduct **9a**, the rearrangement product of **8a**, could not be easily removed. Unfortunately, when the reaction was scaled up, the rearrangement gave a complex mixture, with multiple vinylic proton NMR signals. Therefore, an alternative approach became necessary in order to minimize the formation of products such as **9a**, and we decided to employ the intramolecular Ireland-Claisen rearrangement (Ireland et al., 1976; for a review, see Enders et al., 1996) to generate carboxylic acid **12** and ester **13**. As shown in Scheme 2B, the preparation of ester **13** commenced with the treatment of **8** with acetic anhydride in the presence of DMAP to furnish ester **10** (Keinan and Greenspoon, 1983) in 95% yield. In this step, the complete removal of the acetate derivative of **8a** could be confirmed by the ¹H NMR spectrum of **9**. The reaction of **9** with LHMDs led to a lithium enolate intermediate, which on *O*-silylation with TMSCl delivered silyl enol ether **11**. After the reaction mixture was brought to a neutral pH at room temperature, the Ireland-Claisen rearrangement provided γ,δ -alkenyl carboxylic acid **12**. Without purification, **12** was converted to methyl ester **13** by treatment with trimethyl orthoformate and a catalytic amount of *p*-tosic acid in MeOH, giving **13** in 81% overall yield from **10**. The *E* stereochemistry of **13** was confirmed by the ¹H NMR spectrum of the olefinic signals: δ 5.35 (dt, J = 6.0, 15.3 Hz), 5.45 (dt, J = 6.4, 15.3 Hz). Because the attempted reduction of **13** with DIBAL provided a mixture of **13**, **14**, and **15**, we employed the following two-step reaction sequence. Reduction of methyl

ester **13** with LiAlH₄ afforded primary alcohol **14** and PCC oxidation of **14** furnished the target aldehyde **15** in 63% overall yield. The *E* stereochemistry of aldehyde **15** was not changed during the reactions, as assessed by ¹H NMR: δ 5.39 (dt, *J* = 5.9, 15.3 Hz), 5.45 (dt, *J* = 6.4, 15.3 Hz). Thus the preparation of 4(*E*)-tetradecenal (**15**) was achieved on a multigram scale by an intramolecular ester enolate Ireland-Claisen rearrangement followed by subsequent conversion reactions via the intermediate. Although three column chromatographic purifications (for **13**, **14**, and **15**) were carried out in the conversion of **10**–**15**, the polarity of each intermediate was markedly different; therefore, the chromatography was simple and rapid.

3.4. Synthesis of 4,8-sphingadiene (**1b**) from Garner ester **2**

Similarly to the preparation of **1a**, the multigram synthesis of sphingadiene **1b** started with the reaction of (*S*)-Garner ester **2** with an excess of dimethyl methylphosphonate, as shown in Scheme 3. Deprotonation of β-ketophosphonate **3** with K₂CO₃ followed by the addition of γ,δ-unsaturated aldehyde **15** furnished the HWE product enone **4b** in 47% overall yield from ester **2**. Reduction of enone **4b**, cyclization, and removal of the acetonide group with *p*-tosic acid in aqueous THF provided cyclic carbamate **7b** in 75% yield. As shown in the partial ¹H spectrum of **7b** in Fig. 2B, the presence of the undesired *threo* enantiomer was readily detected by the ¹H NMR signal at δ 4.75 (dd, *J* = 6.4, 7.0 Hz), and the decrease in the area of this signal provides an indication of the enrichment in the *erythro* isomer. The *E* stereochemistry of **7b** was confirmed by the ¹H NMR spectrum of the olefinic signals: δ 5.36 (dt, *J* = 5.7, 15.2 Hz), 5.43 (dt, *J* = 6.3, 15.2 Hz), 5.63 (dd, *J* = 8.4, 15.4 Hz), 5.89 (dt, *J* = 6.2, 15.4 Hz). The *threo* isomer was removed by flash chromatography (elution with a gradient of hexane/EtOAc 2:1 to 1:1). Then, basic hydrolysis of the carbamate group in **7b** gave **1b** in quantitative yield.

4. Conclusions

We achieved a multigram preparation of C14(4,6)- and C18(4,8)-sphingadienes. A key reaction step is the introduction of a cyclic carbamate group into the sphingoid base to afford the intermediate **7a** or **7b**. In this intermediate, the unwanted *threo* isomer, which is generated during the reduction of the 3-keto group, can be readily removed from the mixture, and the absence of the *threo* byproduct can be confirmed by the ¹H and ¹³C NMR spectra of **7a** or **7b**. In addition, no purification of **1a** was needed after deprotection of the carbamate intermediate because **1a** did not undergo elimination and epimerization reactions in basic media (ethanolic NaOH). We also report the synthesis of γ,δ-unsaturated aldehyde **15**, which was required in the synthesis of C18(4,8)-sphingadiene (**1b**), by an Ireland-Claisen rearrangement.

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Abbreviations

C14(4,6)	sphingadiene
2 <i>S</i> , 3 <i>R</i> , 4 <i>E</i> , 6 <i>E</i>	2-aminotetradecadiene-1,3-diol
C(18)4,8	sphingadiene
2 <i>S</i> , 3 <i>R</i> , 4 <i>E</i> , 8 <i>E</i>	2-aminooctadecadiene-1,3-diol

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Fig. 1.
Retrosynthetic route to **1a** and **1b**.

Fig. 2. Partial ^1H NMR spectra of cyclic carbamate **7**. (A) *threo*-enriched portion of **7a** prior to further purification; (B) *erythro*-enriched portion of **7b** after purification by column chromatography (note the minor signal at δ 4.75 ppm, indicating that most of the *threo* byproduct was removed).

Scheme 1.
Synthesis of (4*E*,6*E*)-sphingadiene (**1a**).

A. Initial attempt to prepare ester 9 via an orthoester Claisen reaction⁷ **B. Preparation of aldehyde 15 via an Ireland-Claisen reaction**

Scheme 2.
Synthesis of 4(*E*)-tetradecenal (**15**).

Scheme 3.
Synthesis of (4*E*,8*E*)-sphingadiene (**1b**).

Chart 1.
Structures of sphingadienes **1a** and **1b**.