Chemical Synthesis of a Very Long-Chain Fatty Acid, Hexacosanoic Acid (C26:0), and the Ceramide Containing Hexacosanoic Acid

Yoshinori YAMAMOTO, Toshimasa ITOH and Keiko YAMAMOTO*

Laboratory of Drug Design and Medicinal Chemistry, Showa Pharmaceutical University,
3–3165 Higashi-Tamagawagakuen, Machida, Tokyo 194–8543, Japan

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Summary Hexacosanoic acid (C26:0) (**1**), a very long-chain fatty acid, is related to various diseases such as adrenoleukodystrophy (ALD), adrenomyeloneuropathy (AMN) and atherosclerosis. As the level of **1** higher than the normal is related to diseases above, hexacosanoic acid (**1**) and the ceramide **2**, which contains **1**, are thought to play an important role in various tissues. Hexacosanoic acid (**1**) is known to be a waxy solid and to be hard to dissolve in water as well as organic solvents. Due to this physical property, it is not easy to handle hexacosanoic acid (**1**) in a laboratory. Therefore, efficient chemical synthesis of the compounds **1** and **2** has not been reported. Here, we report a versatile synthetic method for hexacosanoic acid (**1**) and the ceramide **2** containing the fatty acid **1**. Synthesis of hexacosanoic acid (**1**) was achieved by applying the coupling of two alkyl units as a key step. Ceramide **2** was efficiently synthesized by applying the reported procedure together with hexacosanoic acid (**1**) synthesized here. This synthetic strategy has an advantage of getting various carbon chain length fatty acids and their ceramides by using a variety of carbon chain units. This method is also applicable for large-scale synthesis. In addition, these compounds **1** and **2** are useful for investigation of details of these compounds related to diseases such as ALD and AMN.

Key Words hexacosanoic acid, cerotic acid, ceramide, chemical synthesis, adrenoleukodystrophy

Long-chain fatty acids are biogenic substances. They are part of signaling pathways, sources of energy, and constituents of cell membranes. However, the physiological roles of some long-chain fatty acids, especially very long-chain fatty acids (VLCFA) such as hexacosanoic acid **1**, remain unclear.

Hexacosanoic acid (C26:0) (**1**), which is also called cerotic acid, is related to adrenoleukodystrophy (ALD) (**1**, **2**), adrenomyeloneuropathy (AMN) (**1**), atherosclerosis (**3**), coronary artery disease (**4**), aging (**3**, **5**) and metabolic syndrome (**6**). As the level of **1** higher than the normal is related to the diseases above, hexacosanoic acid (**1**) and the sphingolipid containing **1** are thought to play an important role in multiple tissues (**5**). Hexacosanoic acid is known to be a waxy solid and to be hard to dissolve in water as well as organic solvents. It has been reported that due to this physical property of hexacosanoic acid, its accumulation causes a decrease the fluidity of cell membranes (**3**). Ceramide, which is a kind of sphingolipid, has a function as a second messenger, so it is suggested that the ceramide is also related to those diseases. However, efficient chemical synthesis of these compounds has not been reported. In this article,

we report a useful synthetic method for hexacosanoic acid (**1**) and ceramide **2** containing a hexacosanoic acid (Fig. 1).

MATERIALS AND METHODS

General experimental procedures. All reagents were purchased from commercial sources and used without further purification. Organic solvents used were dried by standard methods. All reactions were performed under a nitrogen atmosphere. Silica gel (Kanto Chemical Silica gel 60, Spherical, 63–210 or 40–50 μm) was used for column chromatography, and pre-coated silica gel 60F₂₅₄ plates (0.25 mm, Merck) were used for TLC. ¹H NMR (300 MHz) and ¹³C NMR (75 MHz) were recorded using a Bruker AV300 instrument in CDCl₃ solution with TMS or CDCl₃ (δ 7.26) and CDCl₃ (δ 77.23) as an internal standard, respectively. The chemical shifts are given in δ values. Splitting patterns are indicated as follows: s, singlet; d, doublet; t, triplet; q, quartet; m, multiplet. Mass spectra were recorded on a JEOL AccuTOF LC-plus JMS-T100LP and JEOL MS700 spectrometer using NBA as the positive-ion FAB matrix.

1-Bromo-10-(methoxymethoxy)decane (3**):** To a solution of 10-bromo-1-decanol (2.01 g, 8.47 mmol) and *N,N*-diisopropylethylamine (3.08 g, 23.9 mmol) in CH₂Cl₂ (10 mL) at 0°C was added chloromethyl methyl ether (1.85 g, 22.9 mmol) and the mixture was stirred at 0°C for 3 h. The reaction was quenched with 1 N HCl and extracted with CH₂Cl₂. The organic layer was

E-mail: yamamoto@ac.shoyaku.ac.jp

Abbreviations: ALD, adrenoleukodystrophy; AMN, adrenomyeloneuropathy; EEDQ, *N*-ethoxycarbonyl-2-ethoxy-1,2-dihydroquinoline; TBAF, tetra-*n*-butylammonium fluoride; VLCFA, very long-chain fatty acids.

Fig. 1. Structures of hexacosanoic acid and the ceramide containing hexacosanoic acid.

washed with saturated NaHCO_3 aq. and brine, dried over MgSO_4 , and evaporated. The residue was flash-chromatographed on silica gel (50 g, hexane/AcOEt, and then 80 : 1) to give compound **3** (1.78 g, 75%). **3** ^1H NMR δ 1.02–1.51 (12 H, m, $-\text{CH}_2-$), 1.51–1.70 (2 H, m, $-\text{CH}_2-\text{CH}_2-\text{O}-\text{CH}_2-\text{O}-\text{CH}_3$), 1.70–1.98 (2 H, m, $\text{Br}-\text{CH}_2-\text{CH}_2-$), 3.36 (3 H, s, $-\text{O}-\text{CH}_2-\text{O}-\text{CH}_3$), 3.41 (2 H, t, $J=6.9$ Hz, $\text{Br}-\text{CH}_2-$), 3.52 (2 H, t, $J=6.6$ Hz, $-\text{CH}_2-\text{O}-\text{CH}_2-\text{O}-\text{CH}_3$), 4.62 (2 H, s, $-\text{O}-\text{CH}_2-\text{O}-\text{CH}_3$). ^{13}C NMR δ 26.3, 28.3, 28.9, 29.49, 29.50, 29.59, 29.9, 33.0, 34.1, 55.2, 67.9, 96.5. IR (neat): 2928, 2854, 1150, 1111, 1045, 644, 563 cm^{-1} .

1-(Methoxymethoxy)hexacosane (4): Hexadecan-1-yl magnesium bromide was prepared from Mg (0.954 g, 39.2 mmol) and 1-bromohexadecane (8.16 g, 26.7 mmol) in THF (22 mL). To a mixture of **3** (0.50 g, 1.79 mmol), Li_2CuCl_4 (100 mM solution in THF, 5.3 mL) and *N*-methylpyrrolidone (0.69 mL, 7.16 mmol) was slowly added hexadecan-1-yl magnesium bromide (0.476 mL, 7.6 mL, 3.62 mmol) at room temperature under nitrogen. The deep purple solution was stirred at room temperature for 2.5 h. The reaction was quenched with saturated NH_4Cl aq. and extracted with AcOEt. The organic layer was washed with water and brine, dried over MgSO_4 , and evaporated. The residue was flash-chromatographed on silica gel (50 g, hexane/AcOEt, 80 : 1) to give compound **4** (0.623 g, 81%). **4** ^1H NMR δ 0.88 (3 H, t, $J=6.5$ Hz, $-\text{CH}_2-\text{CH}_3$), 1.14–1.47 (46 H, m, $-\text{CH}_2-$), 1.57–1.65 (2 H, m, $-\text{CH}_2-\text{CH}_2-\text{O}-\text{CH}_2-\text{O}-\text{CH}_3$), 3.36 (3 H, s, $-\text{O}-\text{CH}_2-\text{O}-\text{CH}_3$), 3.52 (2 H, t, $J=6.6$ Hz, $-\text{CH}_2-\text{O}-\text{CH}_2-\text{O}-\text{CH}_3$), 4.62 (2 H, s, $-\text{O}-\text{CH}_2-\text{O}-\text{CH}_3$). ^{13}C NMR δ 14.3, 22.9, 26.5, 29.6, 29.7, 29.8 (2 carbons), 29.90, 29.93 (14 carbons), 30.0, 32.2, 55.3, 68.1, 96.6. MS (FAB $^+$) m/z (relative intensity): 426 (M^+ , 4), 395 (8), 307 (76), 289 (49), 154 (100), 136 (56). IR (CHCl_3): 2926, 2854, 1614, 1605, 947 cm^{-1} .

Hexacosan-1-ol (5): A solution of MOM ether **4** (0.201 g, 0.471 mmol) in 3% HCl-THF (20 mL) was stirred at 50°C for 4 h. The mixture was evaporated to give crude compound **5** (0.177 g, 98%). **5** ^1H NMR δ 0.88 (3 H, t, $J=6.8$ Hz, $-\text{CH}_2-\text{CH}_3$), 1.13–1.42 (46 H, m, $-\text{CH}_2-$), 1.52 (2 H, s, $-\text{CH}_2-\text{CH}_2-\text{OH}$), 3.56–3.68 (2 H, m, $-\text{CH}_2-\text{CH}_2-\text{OH}$). ^{13}C NMR δ 14.4, 22.9, 26.0, 29.6, 29.7, 29.8 (2 carbons), 29.93 (14 carbons), 29.98, 32.2, 33.1, 63.4.

Hexacosanal (6): A mixture of alcohol **5** (0.20 g, 0.523 mmol), Dess-Martin periodinane (15 wt% in CH_2Cl_2 solution, 2.7 mL, 0.785 mmol) and NaHCO_3 (0.836 g, 9.951 mmol) in CH_2Cl_2 (67 mL) was warmed to 30°C to dissolve compound **5** in the solution and then

the solution was stirred at 30°C for 2 h. After the addition of saturated NaHCO_3 aq. and saturated $\text{Na}_2\text{S}_2\text{O}_3$ aq., the mixture was extracted with CH_2Cl_2 . The organic layer was washed with water and brine, dried over MgSO_4 , and evaporated. The residue was chromatographed on silica gel (20 g, CH_2Cl_2 /hexane, 1 : 30 and then CH_2Cl_2) to give compound **6** (0.1679 g, 84%). **6** ^1H NMR δ 0.88 (3 H, t, $J=6.7$ Hz, $-\text{CH}_2-\text{CH}_3$), 1.16–1.38 (44 H, m, $-\text{CH}_2-$), 1.59–1.70 (2 H, m, $-\text{CH}_2-\text{CH}_2-\text{CHO}$), 2.42 (2 H, dt, $J=7.23, 1.85$ Hz, $-\text{CH}_2-\text{CHO}$), 9.77 (1 H, t, 1.86 Hz, $-\text{CHO}$). ^{13}C NMR δ 14.3, 22.3, 22.9, 29.4, 29.6, 29.7, 29.81, 29.89, 29.93 (15 carbons), 32.2, 44.1, 203.1. MS (ESI $^-$) m/z : 379 [$\text{M}-\text{H}$] $^-$. HRMS (ESI $^-$) calcd. for $^{12}\text{C}_{26}\text{H}_{51}\text{O}_1$ 379.3940, found 379.3947. IR (CHCl_3): 2926, 2854, 1724 cm^{-1} .

Hexacosanoic acid (1): To a solution of aldehyde **6** (0.0999 g, 0.262 mmol) and 2-methyl-2-butene (1.3 mL, 12.3 mmol) in *tert*-butyl alcohol (51 mL) was added a solution of sodium chlorite (0.22 g, 2.43 mmol) and sodium dihydrogenphosphate dihydrate (0.29 g, 1.82 mmol) in water (2.1 mL) dropwise over 4 min. The mixture was stirred at room temperature for 1 h. After volatile components were removed under vacuum, 2 N HCl was added. The mixture was extracted with CH_2Cl_2 . The organic layer was washed with water and brine, dried over MgSO_4 , and evaporated. The residue was chromatographed on silica gel (1 g, MeOH/ CH_2Cl_2 , 1 : 15) to give compound **1** (0.0772 g, 74%). **1** ^1H NMR δ 0.88 (3 H, t, $J=6.72$ Hz, $-\text{CH}_2-\text{CH}_3$), 1.14–1.40 (44 H, m, $-\text{CH}_2-$), 1.61–1.69 (2 H, m, $-\text{CH}_2-\text{CH}_2-\text{CHO}$), 2.36 (2 H, t, $J=7.5$ Hz, $-\text{CH}_2-\text{COOH}$). ^{13}C NMR δ 14.4, 22.9, 24.9, 29.3, 29.5, 29.6, 29.7, 29.8, 29.9 (15 carbons), 32.2, 33.8, 177.8. MS (ESI $^-$) m/z : 395 [$\text{M}-\text{H}$] $^-$. HRMS (ESI $^-$) calcd. for $^{12}\text{C}_{26}\text{H}_{51}\text{O}_2$ 395.3889, found 395.3903. IR (CHCl_3): 2926, 2854, 1724 cm^{-1} .

(E)-hexadec-2-enal (8): To a solution of oxalyl chloride (3.1 mL, 36.1 mmol) in CH_2Cl_2 (160 mL) was added a solution of DMSO (5.1 mL, 71.9 mmol) in CH_2Cl_2 (20 mL) at -78°C . After being stirred for 10 min, a solution of alcohol **7** (7.19 g, 29.9 mmol) in CH_2Cl_2 (83 mL) was added and the mixture was stirred at -78°C for 15 min. Et_3N (21 mL, 150.7 mmol) was added to the reaction mixture and stirred for 30 min at -78°C . Then the mixture was allowed to warm to room temperature. The reaction was quenched with water, and the mixture was extracted with CH_2Cl_2 . The organic layer was washed with brine, dried over MgSO_4 , and evaporated. The residue was chromatographed on silica gel (40 g, hexane/AcOEt, 20 : 1) to give compound **8** (6.93 g, 97%). **8** ^1H NMR δ 0.88 (3 H, t, $J=6.7$ Hz, $-\text{CH}_2-\text{CH}_3$), 1.12–1.41 (20 H, m, $-\text{CH}_2-$), 1.41–1.55 (2 H, m, $-\text{CH}=\text{CH}-\text{CH}_2-\text{CH}_2-$), 2.27–2.38 (2 H, m, $-\text{CH}=\text{CH}-\text{CH}_2-$), 6.11 (1 H, ddt, $J=15.6, 7.9, 1.5$ Hz, $\text{OHC}-\text{CH}=\text{CH}-$), 6.85 (1 H, dt, $J=15.6, 6.8$ Hz, $\text{OHC}-\text{CH}=\text{CH}-$), 9.50 (1 H, d, $J=7.9$ Hz, $\text{OHC}-$).

(S)-3-(2-Bromoacetyl)-4-isopropylloxazolidin-2-one (9): To a solution of (*S*)-4-isopropyl-2-oxazolidinone (1.29 g, 10.0 mmol) in THF (50 mL) at -78°C was added *n*-BuLi (1.65 M in hexanes, 6.1 mL, 10.1 mmol). The mixture was stirred for 30 min at -20°C and

then cooled to -78°C . Bromoacetyl bromide (0.9 mL, 10.4 mmol) in THF (15 mL) was added to the solution and the mixture was allowed to warm slowly to room temperature. The reaction was quenched with phosphate buffer (pH 7), and the mixture was extracted with AcOEt. The organic layer was washed with water and brine, dried over MgSO_4 , and evaporated. The residue was chromatographed on silica gel (40 g, hexane/AcOEt, 5 : 1) to give compound **9** (1.72 g, 69%). **9** ^1H NMR δ 0.91 (3 H, d, $J=6.9$ Hz, $-\text{CH}_3$), 0.94 (3 H, d, $J=7.5$ Hz, $-\text{CH}_3$), 2.34–2.51 (1 H, m, $-\text{CH}(\text{CH}_3)_2$), 4.24–4.64 (5 H, m, $-\text{C}-\text{CH}_2-\text{Br}$, $-\text{O}-\text{CH}_2-\text{CH}-$).

(S)-3-((2S,3R,E)-2-Bromo-3-hydroxyoctadec-4-enoyl)-4-isopropylloxazolidin-2-one (10): To a stirred solution of bromoacetyl **9** (8.41 g, 33.6 mmol) in ether (115 mL) at -78°C was added Et_3N (6.4 mL, 45.9 mmol). After 5 min, dibutylborontriflate (1.0 M in CH_2Cl_2 , 33.6 mL) was added slowly. After 15 min, the mixture was warmed to room temperature and stirred for 2 h. The mixture was slowly cooled to -78°C again and a solution of aldehyde **8** (5.03 g, 21.1 mmol) in Et_2O (100 mL) was added. The mixture was stirred for 45 min at -78°C , and then for 1.5 h at 0°C . The reaction mixture was diluted with ether and poured into saturated NaHSO_4 aq. The organic layer was washed with saturated NaHSO_4 aq. and brine and then evaporated. The residue was dissolved in Et_2O and cooled to 0°C . To the solution was slowly added a 1 : 1 mixture of MeOH and 30% aq. H_2O_2 (150 mL). The mixture was stirred for 1 h at 0°C and diluted with Et_2O . The mixture was poured into saturated NaHCO_3 aq. and extracted with Et_2O . The organic layer was washed with saturated NaHCO_3 aq. and brine, dried over MgSO_4 , and evaporated. The residue was flash-chromatographed on silica gel (200 g, hexane/AcOEt, 4 : 1) to give compound **10** (7.87 g, 76%). **10** ^1H NMR δ 0.88 (3 H, t, $J=6.6$ Hz, $-\text{CH}_3$), 0.94 (6 H, d, $J=7.2$ Hz, $-\text{CH}(\text{CH}_3)_2$), 1.11–1.43 (22 H, m, $-\text{CH}_2-$), 1.99–2.09 (2 H, m, $-\text{CH}=\text{CH}-\text{CH}_2-$), 2.30–2.50 (1 H, m, $-\text{CH}(\text{CH}_3)_2$), 3.11 (1 H, d, $J=2.9$ Hz, $-\text{OH}$), 4.19–4.39 (2 H, m, $-\text{O}-\text{CH}_2-$), 4.39–4.64 (2 H, m, $-\text{CH}(\text{OH})-$, $-\text{O}-\text{CH}_2-\text{CH}-\text{CH}(\text{CH}_3)_2$), 5.48 (1 H, ddt, $J=15.4$, 6.5, 1.36 Hz, $-\text{CH}=\text{CH}-$), 5.69 (1 H, d, $J=5.1$ Hz, $-\text{CO}-\text{CH}(\text{Br})-$), 5.87 (1 H, dt, $J=15.4$, 6.8 Hz, $-\text{CH}=\text{CH}-$).

(S)-3-((2R,3R,E)-2-Azido-3-hydroxyoctadec-4-enoyl)-4-isopropylloxazolidin-2-one (11): A solution of **10** (7.87 g, 16.1 mmol) and NaN_3 (2.11 g, 32.5 mmol) in DMSO (41 mL) was stirred at room temperature for 1.5 h. The reaction was quenched with water and extracted with AcOEt. The organic layer was washed with water and brine, dried over MgSO_4 , and evaporated. The residue was flash-chromatographed on silica gel (200 g, hexane/AcOEt, 4 : 1) to give compound **11** (6.22 g, 86%). **11** ^1H NMR δ 0.82–0.97 (9 H, m, $-\text{CH}_3$, $-\text{CH}(\text{CH}_3)_2$), 1.16–1.45 (22 H, m, $-\text{CH}_2-$), 2.08 (2 H, q, $J=6.86$ Hz, $-\text{CH}=\text{CH}-\text{CH}_2-$), 2.27–2.51 (2 H, m, $-\text{CH}(\text{CH}_3)_2$, $-\text{OH}$), 4.22–4.32 (1 H, m, $-\text{O}-\text{CH}_2-\text{CH}-\text{CH}(\text{CH}_3)_2$), 4.32–4.40 (1 H, m, $-\text{O}-\text{CH}_2-$), 4.40–4.55 (2 H, m, $-\text{CH}(\text{OH})-$, $-\text{O}-\text{CH}_2-$), 5.10 (1 H, d, $J=7.7$ Hz, $-\text{CH}(\text{N}_3)-$), 5.60 (1 H, ddt, $J=15.4$, 7.1, 1.3 Hz, $-\text{CH}=\text{CH}-$), 5.89 (1 H, dt, $J=15.4$, 6.6 Hz, $-\text{CH}=\text{CH}-$).

(S)-3-((2R,3R,E)-2-Azido-3-((tert-butyltrimethylsilyl)oxy)octadec-4-enoyl)-4-isopropylloxazolidin-2-one (12): To a solution of **11** (6.22 g, 13.8 mmol) in THF (69 mL) at 0°C was added 2,6-lutidine (3.2 mL, 27.6 mmol) followed by *tert*-butyltrimethylsilyltriflate (4.8 mL, 20.9 mmol). The mixture was stirred for 30 min at 0°C and then at room temperature for 1 h. The reaction was quenched with water and extracted with AcOEt. The organic layer was washed with water and brine, dried over MgSO_4 , and evaporated. The residue was flash-chromatographed on silica gel (200 g, hexane/AcOEt, 15 : 1) to give compound **12** (6.21 g, 80%). **12** ^1H NMR δ 0.05 (3 H, s, $-\text{Si}(\text{CH}_3)_2(\text{CH}_3)_3$), 0.08 (3 H, s, $-\text{Si}(\text{CH}_3)_2(\text{CH}_3)_3$), 0.82–0.90 (15 H, m, $-\text{Si}(\text{CH}_3)_2(\text{CH}_3)_3$, $-\text{CH}(\text{CH}_3)_2$), 0.91–0.96 (3 H, m, $-\text{CH}_3$), 1.16–1.43 (22 H, m, $-\text{CH}_2-$), 2.01–2.08 (2 H, m, $-\text{CH}=\text{CH}-\text{CH}_2-$), 2.25–2.35 (1 H, m, $-\text{CH}(\text{CH}_3)_2$), 4.22–4.26 (1 H, m, $-\text{O}-\text{CH}_2-$), 4.29–4.35 (1 H, m, $-\text{O}-\text{CH}_2-$), 4.49 (1 H, dt, $J=8.1$, 3.4 Hz, $-\text{O}-\text{CH}_2-\text{CH}-\text{CH}(\text{CH}_3)_2$), 4.64 (1 H, dd, $J=8.0$, 6.6 Hz, $-\text{CH}(\text{N}_3)-\text{CH}-$), 5.21 (1 H, d, $J=6.4$ Hz, $-\text{CH}(\text{N}_3)-$), 5.52 (1 H, dd, $J=15.3$, 8.1 Hz, $-\text{CH}=\text{CH}-$), 5.75 (1 H, dt, $J=15.2$, 6.8 Hz, $-\text{CH}=\text{CH}-$).

(2S,3R,E)-2-Azido-3-((tert-butyltrimethylsilyl)oxy)octadec-4-en-1-ol (13): To a solution of **12** (6.21 g, 11.0 mmol) in THF (55 mL) at 0°C was added LiBH_4 (0.722 g, 33.1 mmol). The mixture was stirred at 0°C for 1.5 h and then at room temperature for 30 min. The mixture was cooled to 0°C and then diluted with AcOEt. The reaction was quenched with saturated NH_4Cl aq. and extracted with AcOEt. The organic layer was washed with water and brine, dried over MgSO_4 , and evaporated. The residue was chromatographed on silica gel (100 g, hexane/AcOEt, 15 : 1) to give compound **13** (4.18 g, 86%). **13** ^1H NMR δ 0.05 (3 H, s, $-\text{Si}(\text{CH}_3)_2(\text{CH}_3)_3$), 0.09 (3 H, s, $-\text{Si}(\text{CH}_3)_2(\text{CH}_3)_3$), 0.82–0.97 (12 H, m, $-\text{Si}(\text{CH}_3)_2(\text{CH}_3)_3$, $-\text{CH}_3$), 1.15–1.44 (22 H, m, $-\text{CH}_2-$), 1.95–2.21 (3 H, m, $-\text{CH}=\text{CH}-\text{CH}_2-$, $-\text{OH}$), 3.36–3.45 (1 H, m, $-\text{CH}(\text{N}_3)-$), 3.62–3.83 (2 H, m, $\text{HO}-\text{CH}_2-$), 4.22 (1 H, dd, $J=7.2$, 5.3 Hz, $-\text{CH}-\text{O}-\text{Si}(\text{CH}_3)_2-$), 5.46 (1 H, ddt, $J=15.4$, 7.4, 1.3 Hz, $-\text{CH}=\text{CH}-$), 5.70 (1 H, dt, $J=15.4$, 6.7 Hz, $-\text{CH}=\text{CH}-$).

(2S,3R,E)-2-Amino-3-((tert-butyltrimethylsilyl)oxy)octadec-4-en-1-ol (14): A solution of **13** (4.18 g, 9.51 mmol) in THF (120 mL) was diluted with water (15 mL). To this solution, triphenylphosphine (4.99 g, 19.0 mmol) was added and the mixture was stirred at 60°C for 15 h. After evaporation, the residue was extracted with water and CH_2Cl_2 . The organic layer was washed with water and brine, dried over MgSO_4 , and evaporated. The residue was flash-chromatographed on silica gel (200 g, $\text{CH}_2\text{Cl}_2/\text{MeOH}$, 40 : 1 and then 1 : 1) to give compound **14** (3.24 g, 82%). **14** ^1H NMR δ 0.03 (3 H, s, $-\text{Si}(\text{CH}_3)_2(\text{CH}_3)_3$), 0.07 (3 H, s, $-\text{Si}(\text{CH}_3)_2(\text{CH}_3)_3$), 0.70–0.98 (12 H, m, $-\text{Si}(\text{CH}_3)_2(\text{CH}_3)_3$, $-\text{CH}_3$), 1.13–1.50 (22 H, m, $-\text{CH}_2-$), 1.76 (3H, broad s), 2.05 (2 H, q, $J=6.8$ Hz, $-\text{CH}=\text{CH}-\text{CH}_2-$), 2.75 (1 H, q, $J=5.3$ Hz, $-\text{CH}(\text{NH}_2)-$), 3.53–3.68 (2 H, m, $\text{HO}-\text{CH}_2-$), 4.01 (1 H, t, $J=6.3$ Hz, $-\text{CH}-\text{O}-\text{Si}(\text{CH}_3)_2-$), 5.38 (1 H, dd, $J=15.4$, 7.8 Hz, $-\text{CH}=\text{CH}-$), 5.65 (1 H, dt, $J=15.3$, 6.8 Hz, $-\text{CH}=\text{CH}-$).

Scheme 1. Synthetic scheme of hexacosanoic acid (**1**).

***N*-((2*S*,3*R*,*E*)-3-((*tert*-Butyldimethylsilyl)oxy)-1-hydroxyoctadec-4-en-2-yl)hexacosanamide (**15**):**

To a solution of **14** (0.207 g, 0.500 mmol) and **1** (0.196 g, 0.495 mmol) in CH_2Cl_2 (55 mL) was added EEDQ (0.183 g, 0.738 mmol). The mixture was stirred at 35°C for 10 h. After evaporation the residue was flash chromatographed on silica gel (80 g, hexane/ether, 3 : 1 and then CH_2Cl_2 /ether, 1 : 1) to give compound **15** (0.335 g, 84%). **15** ^1H NMR δ 0.03 (3 H, s, $-\text{Si}(\text{CH}_3)_2(\text{CH}_3)_3$), 0.06 (3 H, s, $-\text{Si}(\text{CH}_3)_2(\text{CH}_3)_3$), 0.63–0.98 (15 H, m, $-\text{Si}(\text{CH}_3)_2(\text{CH}_3)_3$, $-(\text{CH}_2)_{12}-\text{CH}_3$, $-(\text{CH}_2)_{24}-\text{CH}_3$), 0.98–1.15 (66 H, m, $-\text{CH}_2-$), 1.57–1.70 (2 H, m, $-\text{NH}-\text{CO}-\text{CH}_2-\text{CH}_2-$), 2.05 (2 H, q, $J=6.8$ Hz, $-\text{CH}=\text{CH}-\text{CH}_2-$), 2.21 (2 H, t, $J=7.5$ Hz, $-\text{NH}-\text{CO}-\text{CH}_2-$), 3.25 (1 H, dd, $J=10.0, 1.7$ Hz, $\text{HO}-$), 3.55 (1 H, td, $J=10.7, 3.1$ Hz, $\text{HO}-\text{CH}_2-$), 3.74–3.86 (1 H, m, $-\text{CH}-\text{O}-\text{Si}(\text{CH}_3)_2-$), 4.04 (1 H, dt, $J=11.9, 2.28$ Hz, $\text{HO}-\text{CH}_2-$), 4.38–4.49 (1 H, m, $-\text{CH}(\text{NHCO})-$), 5.46 (1 H, dd, $J=15.5, 6.2$ Hz, $-\text{CH}=\text{CH}-$), 5.72 (1 H, dt, $J=15.5, 6.7$ Hz, $-\text{CH}=\text{CH}-$), 6.25 (1 H, d, $J=7.5$ Hz, $-\text{NH}-\text{CO}-$). ^{13}C NMR δ $-4.9, -4.2, 14.3$ (2 carbons), 18.2, 22.9 (2 carbons), 25.8, 26.0 (4 carbons), 29.29, 29.35, 29.6 (3 carbons), 29.65, 29.67, 29.8 (3 carbons), 29.85 (5 carbons), 29.89 (13 carbons), 32.1 (2 carbons), 32.4, 37.0, 54.6, 62.5, 76.2, 130, 133.8, 173.3.

***N*-((2*S*,3*R*,*E*)-1,3-Dihydroxyoctadec-4-en-2-yl)hexacosanamide (**2**):** To a solution of **15** (0.137 g, 0.173 mmol) in THF (20 mL) was added acetic acid (15 μL , 0.262 mmol) and TBAF (1.0 M solution in THF, 260 μL , 0.260 mmol). The mixture was stirred at 35°C for 5 h. After evaporation the residue was diluted with water and extracted with CH_2Cl_2 . The organic layer was washed with water and brine, dried over MgSO_4 , and evaporated. The residue was flash-chromatographed on silica gel (50 g, $\text{CHCl}_3/\text{MeOH}$, 70 : 1) to give compound **2** (0.112 g, 96%). **2** ^1H NMR δ 0.88 (6 H, t, $J=6.6$ Hz, $-(\text{CH}_2)_{12}-\text{CH}_3$, $-(\text{CH}_2)_{24}-\text{CH}_3$), 0.99–1.52 (66 H, m, $-\text{CH}_2-$), 1.63–1.72 (2 H, m, $-\text{NH}-\text{CO}-\text{CH}_2-\text{CH}_2-$), 2.06 (2 H, q, $J=6.8$ Hz, $-\text{CH}=\text{CH}-\text{CH}_2-$), 2.23 (2 H, t, $J=7.3$ Hz, $-\text{NH}-\text{CO}-\text{CH}_2-$), 2.63–2.82 (1 H, m, $-\text{CH}(\text{NHCO})-\text{CH}(\text{OH})-$), 3.62–3.78 (1 H, m, $\text{HO}-\text{CH}_2-$), 3.86–4.01 (2 H, m, $\text{HO}-\text{CH}_2-$), 4.25–4.38 (1 H, m, $-\text{CH}(\text{NHCO})-$), 5.53 (1 H, dd, $J=15.5, 6.4$ Hz, $-\text{CH}=\text{CH}-$), 5.79 (1 H, dt, $J=14.9, 7.1$ Hz, $-\text{CH}=\text{CH}-$), 6.24 (1 H, d, $J=7.3$ Hz, $-\text{NH}-\text{CO}-$). ^{13}C NMR δ 14.3 (2 carbons), 22.9 (2 carbons), 26.0,

29.3, 29.45, 29.51, 29.6 (3 carbons), 29.71, 29.74, 29.85, 29.88 (4 carbons), 29.9 (16 carbons), 32.2 (2 carbons), 32.5, 37.1, 54.7, 62.7, 74.9, 129.0, 134.5, 174.2. HRMS (ESI $^-$) calcd. for $^{12}\text{C}_{44}\text{H}_{87}^{35}\text{Cl}_1^{14}\text{N}_1^{16}\text{O}_3$ 712.6375, found 712.6354.

RESULTS AND DISCUSSION

Synthesis of hexacosanoic acid

The synthetic scheme is shown in Scheme 1. To develop an efficient method applicable to the chemical synthesis of various carbon-chain fatty acids, we planned the synthesis of hexacosanoic acid (C26:0) (**1**) by applying the coupling of two alkyl units as a key step. As shown in Scheme 1, if C–C bond formation occurs between C10-unit compound **3** and C16-unit, it would be an efficient synthetic scheme for a variety of VLCFAs. 10-Bromo-1-decanol was protected by using chloromethyl methyl ether to provide methoxymethyl ether **3**. The cross coupling reaction between compound **3** and hexadecyl magnesium bromide catalyzed by Li_2CuCl_4 yielded hexacosanol derivative **4** in a moderate yield (58%) (7, 8). Cahiez et al. reported the addition of *N*-methylpyrrolidone improved Cu-catalyzed cross coupling (9). When we applied this method, the yield of compound **4** was improved to 81%. Compound **4** was deprotected to provide hexacosanol **5** in 98% yield.

Hexacosanol **5** has a very long chain of carbon hydride, which possesses specific physical properties in organic synthesis. It is a white waxy solid at room temperature and scarcely soluble in organic solvents as well as water. It seems that only CH_2Cl_2 and chloroform are slightly soluble solvents, indicating that the property makes organic reactions difficult. It was not exceptional for the oxidation reaction of primary alcohol **5** to carboxylic acid **1**. First, one-step oxidation of hexacosanol **5** to hexacosanoic acid (**1**) was tried by using several oxidation reagents such as bismuth (III) oxide (10), but the reaction did not proceed at all. Therefore, we changed one-step oxidation to two steps via aldehyde. First we tried Swern oxidation of alcohol **5** to aldehyde **6**, but we failed because alcohol **5** didn't dissolve in CH_2Cl_2 at -78°C . Next we tried oxidation using Dess-Martin periodinane (11), and we succeeded in obtaining **6** in 84% yield when the reaction in CH_2Cl_2 was carried out at 30°C . At that temperature alcohol **5** was some-

Scheme 2. Synthetic scheme of the ceramide **2** which contains hexacosanoic acid.

how soluble in CH_2Cl_2 . Finally, oxidation of **6** to hexacosanoic acid (**1**) was performed by Pinnick oxidation (**12**) in 74% yield. Aldehyde **6** was also hard to dissolve in organic solvents but it was a little more soluble than alcohol **5**. This is the first report of an efficient chemical synthesis of hexacosanoic acid (C26:0) (**1**) by carbon-carbon coupling reaction.

Synthesis of ceramide containing hexacosanoic acid

The synthetic scheme is shown in Scheme 2. According to the reported procedure, we obtained allylic alcohol **7** from 1-tetradecanol in three steps in yield of 64% (**13**, **14**). Alcohol **7** was converted to aldehyde **8** by Swern oxidation in 97% yield. To generate two desired stereocenters together with two-carbon elongation, we prepared (+)-(4*S*)-3-bromoacetyl-4-(1-methylethyl)-2-oxazolidinone **9** (**15**) which was then coupled with aldehyde **8** to give two-carbon elongated compound **10** in 76% yield (**16**). Oxazolidinone **9** was used as a chiral auxiliary for the diastereoselective aldol reaction. This reaction including the reaction mechanism and the stereochemistry of products has been reported in detail (**15**, **16**). Compound **10** was treated with NaN_3 to give azide **11**, whose secondary alcohol was then protected to give compound **12**. The amide group of **12** was reduced with LiBH_4 to give alcohol **13** (59% yield from **10**) (**16**). The azide group of **13** was reduced by the Staudinger reaction to give amine **14** in 82% yield (**17**, **18**). Spectral data of known compounds **9**–**14** were identical with those reported.

Compound **14** and hexacosanoic acid (C26:0) (**1**) were treated with *N*-ethoxycarbonyl-2-ethoxy-1,2-dihydroquinoline as a condensation reagent in CH_2Cl_2 at 35°C for 10 h to provide condensation product **15** in 85% yield (**19**–**21**). The reaction required 10 h because hexacosanoic acid (**1**) was scarcely soluble in the solvent. Compound **15** was deprotected by treatment with tetra-*n*-butylammonium fluoride to give ceramide **2**

in yield of only 7%, but an addition of one equivalent acetic acid to the reaction dramatically improved the yield of ceramide **2** to 96% (**22**). Ceramide **2** was hard to dissolve in organic solvents but it was a little soluble in ethanol and dimethylsulfoxide. This is the first report of the chemical synthesis of the ceramide containing hexacosanoic acid (**1**).

CONCLUSION

We synthesized hexacosanoic acid (C26:0) (**1**) and the ceramide **2** containing **1**. Combination of synthetic Schemes 1 and 2 allows obtaining various ceramides containing a motif of poorly soluble VLCFA. These compounds are useful for investigation of details of these compounds related to diseases.

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