Stereoselective total synthesis of sphingolipids

PARAMESH JANGILI, PERLA RAMESH and BISWANATH DAS*
Natural Products Chemistry Division, Indian Institute of Chemical Technology, Tarnaka, Hyderabad, Telangana 500 007, India
e-mail: biswanathdas@yahoo.com
MS received 13 July 2016; revised 9 September 2016; accepted 9 September 2016

Abstract. A novel sphingosine, 1,2-diacetyl D-erythro-sphinganine having a characteristic almond flavour was isolated from the edible mushroom *Grifola gargal*. We have synthesized this sphinganine along with the three other sphingolipids, such as 1,2-diacetyl L-threo-sphinganine, D-erythro-sphinganine triacetate and L-threo-sphinganine triacetate using Garner aldehyde as the starting material involving the Grignard reaction and Mitsunobu inversion. The sphingolipids 1,2-diacetyl D-erythro-sphinganine and 1,2-diacetyl L-threo-sphinganine have been synthesized for the first time.

Keywords. 1,2-Diacetyl D-erythro-sphinganine; 1,2-diacetyl L-threo-sphinganine; D-erythro-sphinganine triacetate; sphingolipids; total synthesis; Garner aldehyde.

1. Introduction

Sphingolipids are important structural and functional components of essentially all eukaryotic cells and are abundantly located in all plasma membranes as well as in some intracellular organelles.\(^1\) They exist as structural components of cell membranes in animals, plants and some microbial systems.\(^2\) Sphingosine 1 and sphinganine (dihydrosphingosine) 2 (Figure 1) are naturally occurring bioactive compounds (long-chain, aliphatic, 2-amino-1,3 diols). Dihydrosphingosines are biosynthetic precursors of sphingolipids (e.g., ceramides, sphingomyelin, cerebrosides and gangliosides), which play important roles in biological pathways such as cell regulation and signal transduction. Sphingoid bases contain two chiral centres, viz., at carbon atoms 2 and 3. Natural sphingoid bases occur in the D-erythro (2S, 3R) configuration, but other additional unnatural isomers have also been reported. Among the unnatural sphingoid bases L-threo-(2S, 3S) dihydrosphingosine (safingol) 3 (Figure 1) is of particular interest due to its medicinal importance. Safingol is an antineoplastic, antipsoriatic drug\(^4\) and a competitive inhibitor of protein kinase C.\(^5\)

Recently, Choi *et al.*, isolated\(^6\) a novel sphingosine, 1,2-diacetyl D-erythro-sphinganine (4) (Figure 2), from the edible mushroom *Grifola gargal*, having a characteristic almond flavour. This mushroom is collected and eaten by native people of southern Argentina and Chile. The compound 4 suppresses the formation of osteoclasts.

In continuation of our work carried out on the synthesis of natural bioactive compounds,\(^7\) herein we describe an efficient stereoselective total synthesis of naturally occurring sphingolipid 1,2-diacetyl D-erythro-sphinganine (4) along with three other sphingolipids, such as 1,2-diacetyl L-threo-sphinganine (5) (C-3 epimer of 4), D-erythro-sphinganine triacetate (6) (triacetyl derivative of compound 2) and L-threo-sphinganine triacetate (7) (triacetyl derivative of compound 3, safingol) (Figure 2). Our planned approach to the synthesis of the target molecules was initiated from Garner aldehyde (9) involving the Grignard reaction and Mitsunobu inversion. To our knowledge, there are a few reports towards the total synthesis of sphingolipids 6 and 7.\(^8\) However, synthesis of the sphingolipids 4 and 5 are reported here for the first time.

2. Experimental

2.1 General

Infrared spectra were recorded on Perkin-Elmer RX1 FT-IR spectrophotometer. NMR spectra were recorded on Inova 500 MHz and Bruker 300 MHz spectrometers using CDCl\(_3\) and CD\(_3\)OD as solvents and Me\(_4\)Si as internal standard. The chemical shifts are expressed as \(\delta\) values in parts per million (ppm) and the coupling constants (\(J\)) are given in hertz (Hz). ESIMS were recorded with VG-Autospec micromass. Optical rotations were

---

*For correspondence

Part 86 in the series “Synthetic studies on natural products”
measured with JASCO DIP 360 digit polarimeter. All reactions were monitored by thin-layer chromatography (TLC) using silica gel F\textsubscript{254} pre-coated plates.

2.2 tert-Butyl (S)-4-((S)-1-hydroxyhexadecyl)-2,2-dimethyloxazolidine-3-carboxylate (10)

To the solution of Garner aldehyde (9) (2.0 g, 8.72 mmol) in THF (10 mL), was added at −78 °C pentadecyl magnesium bromide in THF (10 mL) which was prepared from 1-bromopentadecane (7.58 mL, 26.16 mmol) and Mg (0.847 g, 34.88 mmol) in the usual manner. The mixture was stirred at the same temperature (−78 °C) for 1 hour and then gradually brought to r.t. The mixture was then stirred overnight at r.t. to produce the mixture of diastereomers 10 and 10a. The reaction was quenched by the addition of aqueous saturated NH\textsubscript{4}Cl (10 mL) and extracted with AcOEt. The organic layer was washed with 5% aqueous HCl (10 mL), water, brine and then dried over Na\textsubscript{2}SO\textsubscript{4}. The two diastereomers 10 and 10a were separated by careful CC (silica gel, 100–200 mesh, 0–5% increasing amount of AcOEt in hexane) to produce two diastereomeric alcohols 10 and 10a in the ratio 9:1 (syn:anti, 9:1) with 86% yield. The pure alcohol 10 (2.98 g) was obtained as a colorless oil. [α]\textsubscript{D}\textsuperscript{25} = −32.2 (c =2.0, CHCl\textsubscript{3}). IR δ\textsubscript{max} (KBr)/cm\textsuperscript{−1}: 3440, 2925, 2855, 1701, 1366, 1258, 1175, 1061. \textsuperscript{1}H NMR (500 MHz, CDCl\textsubscript{3}): 4.10–3.49 (m, 4H); 1.62–1.55 (m, 2H); 1.49 (s, 12H); 1.45 (s, 3H); 1.25 (br. s, 26H); 0.88 (t, J = 6.8 Hz, 3H). \textsuperscript{13}C NMR (125 MHz, CDCl\textsubscript{3}): 154.1; 94.2; 81.0; 72.9; 64.7; 62.3; 34.4; 32.7; 31.8; 29.6; 29.6; 29.5; 28.3; 26.4; 26.0; 24.2; 22.6; 14.0. ESIMS: m/z 442 [M+H]\textsuperscript{+}. Anal Calcd. for C\textsubscript{26}H\textsubscript{51}NO\textsubscript{4}: C, 70.70; H, 11.64%. Found: C, 70.60; H, 11.68%.

2.3 tert-Butyl ((2S,3S)-1,3-dihydroxyoctadecan-2-yl) carbamate (11)

Compound 10 (2.8 g, 6.34 mmol) and pyridinium \textit{p}-toluene sulfonate (0.159 g, 0.634 mmol) were dissolved in MeOH (10 mL) and stirred at r.t. for 2 h. The solvent was removed under reduced pressure. The residue was purified by flash CC (silica gel, hexane/AcOEt, 7:3) to give pure compound 11 (2.34 g, 92%) as a white solid. [α]\textsubscript{D}\textsuperscript{25} = + 16.2 (c = 2.5, CHCl\textsubscript{3}). IR δ\textsubscript{max} (KBr)/cm\textsuperscript{−1}: 3430, 2975, 2855, 1690, 1360, 1255, 1175, 1060. \textsuperscript{1}H NMR (500 MHz, CDCl\textsubscript{3}): 5.22 (d, J = 7.9 Hz, 1H); 4.01 (dd, J = 3.4, 11.4 Hz, 1H); 3.82 (d, J = 3.8 Hz, 1H); 3.76 (m, 1H); 3.53 (m, 1H); 2.54 (br. s, 2H); 1.56–1.48 (m, 2H); 1.46 (s, 9H); 1.25 (br. s, 26H); 0.88 (t, J = 6.7 Hz, 3H). \textsuperscript{13}C NMR (125 MHz, CDCl\textsubscript{3}): 156.1; 79.6; 73.7; 62.3; 54.9; 34.2; 31.8; 29.6; 29.6; 29.5; 28.3; 25.9; 22.6; 14.0. ESIMS: m/z 402 [M+H]\textsuperscript{+}. Anal Calcd. for C\textsubscript{23}H\textsubscript{47}NO\textsubscript{4}: C, 68.78; H, 11.80%. Found: C, 68.90; H, 11.75%.
2.4 (2S,3S)-2-((tert-Butoxycarbonyl)amino)-3-hydroxy octadecyl acetate (8)

To a solution of 1,3-diol 11 (2.2 g, 5.47 mmol) in dry CH2Cl2 (10 mL), Et3N (0.76 mL, 5.47 mmol) was added, followed by Ac2O (0.51 mL, 5.47 mmol) was added at 0°C and the reaction mixture was allowed to r.t. After completion of reaction (2 h), the reaction mixture was washed with brine (10 mL) and water (10 mL), dried over Na2SO4 and evaporated. The crude product was purified by silica gel CC (hexane/AcOEt, 9:1) to give pure monoacyl ester 8 (1.87 g, 77%) as a white solid. [α]D25 = +3.4 (c = 0.6, CHCl3). IR δmax (KBr/cm⁻¹): 3350, 2917, 2850, 1743, 1685, 1531, 1367, 1232, 1173, 1049. 1H NMR (300 MHz, CDCl3): 4.91 (d, J = 8.0 Hz, 1H); 4.28–4.17 (m, 1H); 4.06 (dd, J = 6.1, 11.0 Hz, 1H); 3.79 (m, 1H); 3.69–3.58 (m, 1H); 2.32 (br. s, 1H); 2.08 (s, 3H); 1.55–1.48 (m, 2H); 1.45 (s, 9H); 1.25 (s, 26H); 0.88 (t, J = 7.0 Hz, 3H). 13C NMR (75 MHz, CDCl3): 171.2; 156.0; 79.6; 70.1; 63.4; 52.7; 33.7; 31.9; 29.7; 29.6; 29.5; 29.3; 28.3; 25.6; 22.7; 20.9; 14.1. ESI MS: m/z 466 [M+Na]+.

2.6 L-threo-Sphinganine triacetate (7)

To a solution of compound 5 (0.1 g, 0.26 mmol) in dry CH2Cl2 (5 mL), Et3N (0.043 mL, 0.31 mmol) was added, followed by Ac2O (0.029 mL, 0.31 mmol) was added at 0°C and the reaction mixture was allowed to rise to r.t. After 2 h, the reaction mixture was washed with brine (5 mL) and water (5 mL), dried over Na2SO4 and evaporated. The crude product was purified by silica gel CC (hexane/AcOEt, 9:1) to give L-threo-sphinganine triacetate 7 (0.087 g, 79%) as a white solid. [α]D25 = −12.0 (c = 0.5, CHCl3). IR δmax (KBr/cm⁻¹): 2925, 2855, 1740, 1655, 1460, 1370, 1235, 1048. 1H NMR (500 MHz, CDCl3): 5.64 (d, J = 9.3 Hz, 1H); 5.06 (m, 1H); 4.39 (m, 1H); 4.07–4.01 (m, 2H); 2.05 (s, 3H); 2.02 (s, 3H); 1.99 (s, 3H); 1.62–1.54 (m, 2H); 1.24 (br. s, 26H); 0.87 (t, J = 6.7 Hz, 3H). 13C NMR (125 MHz, CDCl3): 170.7; 170.4; 170.0; 72.4; 63.3; 50.0; 31.9; 31.2; 29.6; 29.6; 29.5; 29.3; 29.2; 25.1; 23.2; 22.6; 20.9; 20.7; 14.1. ESI MS: m/z 428 [M+H]+. Anal. Calcd. for C24H34NO5: C, 67.41; H, 10.61%. Found: C, 67.25; H, 10.57%.

2.7 (2S,3R)-2-((tert-Butoxycarbonyl)amino)octadecane-1,3-diyi diacetate (12)

The monoacyl ester 8 (1.0 g, 2.25 mmol) was dissolved in anhydrous THF (20 mL), AcOH (0.257 mL, 4.50 mmol) and PPh3 (1.18 g, 4.50 mmol) were added at 0°C. To the reaction mixture, a solution of diisopropyl azodicarboxylate (0.88 mL, 4.50 mmol) in anhydrous THF (10 mL) was added. The solution was stirred at r.t. and after 2 h the reaction was quenched by the addition of water (10 mL), and extracted with EtO (30 mL). After phase separation the organic phase was dried (Na2SO4), concentrated and purified by CC to give pure 12 (0.843 g, 77%) as a colorless oil. [α]D25 = + 12.1 (c = 0.3, CHCl3). IR δmax (KBr/cm⁻¹): 2925, 2854, 1746, 1723, 1368, 1236, 1171. 1H NMR (300 MHz, CDCl3): 5.02 (m, 1H); 4.92 (dd, J = 6.2, 12.5 Hz, 1H); 4.09–3.99 (m, 2H); 2.06 (s, 6H); 1.67–1.57 (m, 2H); 1.45 (s, 9H); 1.25 (br. s, 26H); 0.88 (t, J = 6.8 Hz, 3H). 13C NMR (75 MHz, CDCl3): 170.8; 168.6; 151.3; 79.9; 72.3; 62.9; 51.2; 31.9; 31.2; 29.6; 29.6; 29.5; 29.4; 29.3; 28.3; 25.0; 22.6; 21.0; 20.8; 14.1. ESI MS: m/z 486 [M+H]+. Anal. Calcd. for C27H42NO5: C, 66.77; H, 10.58%. Found: C, 66.85; H, 10.54%.

2.8 D-erythro-Sphinganine triacetate (6)

To a solution of compound 12 (0.8 g, 1.64 mmol) in CH2Cl2 (5 mL), excess trifluoroacetic acid was added dropwise and stirred at r.t. for 1 h. The reaction mixture...
was dried on the rotary evaporator to remove the excess TFA. The resulting residue (unprotected amine) was dissolved in CH₂Cl₂ (8 mL) and basified to pH 8 with aq. NaHCO₃ followed by the addition of the acetyl chloride (0.128 mL, 1.80 mmol). The reaction was monitored by TLC. Upon completion (2.5 h), the reaction mixture was diluted with saturated aqueous NH₄Cl. The phases were separated and the aqueous layer was extracted with CH₂Cl₂. The combined organic extracts were washed with brine, dried (Na₂SO₄) and purified by CC to afford pure D-erythro-sphinganine triacetate 6 (0.507 g, 72%) as a white solid. [α]D²⁵ = +12.7 (c = 1.1, CHCl₃). IR δmax (KBr)/cm⁻¹: 3035, 2935, 2850, 1729, 1650, 1555, 1465, 1341, 1221. ¹H NMR (500 MHz, CDCl₃): 5.90 (d, J = 9.0 Hz, 1H); 4.90 (m, 1H); 4.38 (m, 1H); 4.25 (dd, J = 6.1, 11.5 Hz, 1H); 4.06 (d, J = 4.0, 11.7 Hz, 1H); 2.07 (s, 3H); 2.06 (s, 3H); 1.60 (m, 2H); 1.24 (br. s, 26H); 0.88 (s, J = 7.0 Hz, 3H). ¹³C NMR (125 MHz, CDCl₃): 170.9; 170.9; 169.7; 74.0; 62.5; 50.5; 31.9; 31.4; 29.6; 29.6; 29.5; 29.4; 29.3; 25.3; 22.6; 20.9; 20.8; 14.1. ESI MS: m/z 428 [M+H]⁺. Anal. Calcld. for C₂₀H₄₁NO₃: C, 69.92; H, 12.03%. Found: C, 69.92; H, 12.00%.

2.9 N-((25,3R)-1,3-Dihyroxyoctadecan-2-yl) acetamide (13).

To a solution of triacetate 6 (0.4 g, 0.935 mmol) in absolute MeOH (6 mL) was added anhydrous Na₂CO₃ (0.118 g, 1.12 mmol). The mixture was stirred at r.t. for 1 h. Then, the solution was filtered, the solvent evaporated and the solid residue was purified over silica gel (eluent, CH₂Cl₂ with increasing amount of MeOH) to give 1,3-diol 13 (0.263 g, 82%) as a white solid. [α]D⁻²⁵ = +6.6 (c = 0.2, CH₂OH). IR δmax (KBr)/cm⁻¹: 3400, 2940, 2835, 1650, 1548, 1430, 1362, 1061. ¹H NMR (500 MHz, CD₂OD): 3.86 (m, 1H); 3.72–3.64 (m, 2H); 3.59 (m, 1H); 1.97 (s, 3H); 1.52 (m, 2H); 1.24 (br. s, 26H); 0.89 (t, J = 7.0 Hz). ¹³C NMR (125 MHz, CD₂OD): 173.4; 72.3; 62.1; 57.0; 34.8; 33.1; 30.8; 30.5; 26.8; 23.7; 22.8; 14.4. ESI MS: m/z 344 [M+H]⁺. Anal. Calcld. for C₂₀H₄₁NO₃: C, 69.92; H, 12.03%. Found: C, 70.04; H, 12.00%.

2.10 1,2-Diacetyl D-erythro-sphinganine (4)

To a solution of 1,3-diol 13 (0.1 g, 0.29 mmol) in dry CH₂Cl₂ (4 mL), Et₃N (0.04 mL, 0.29 mmol) was added, followed by Ac₂O (0.027 mL, 0.29 mmol) was added at 0°C and the reaction mixture was allowed to rise to r.t. After completion of reaction (2 h), the reaction mixture was washed with brine (5 mL) and water (5 mL), dried over Na₂SO₄ and evaporated. The crude product was purified by silica gel CC using (AcOEt/hexane, 8:2) to give pure 1,2-diacetyl D-erythro-sphinganine 4 (0.088 g, 79%) as a white solid. [α]D²⁵ = +6.2 (c = 0.25, CH₂OH). IR δmax (KBr)/cm⁻¹: 3279, 2918, 2850, 1739, 1650, 1555, 1465, 1341, 1221. ¹H NMR (500 MHz, CDCl₃): 5.98 (d, J = 7.9 Hz, 1H); 4.33 (dd, J = 11.6, 6.4 Hz, 1H); 4.18 (dd, J = 11.6, 3.2 Hz, 1H); 4.11 (m, 1H); 3.63 (m, 1H); 2.06 (s, 3H); 2.00 (s, 3H); 1.47 (m, 2H); 1.24 (br. s, 26H); 0.86 (t, J = 6.8 Hz, 3H). ¹³C NMR (125 MHz, CDCl₃): 171.3; 170.3; 72.6; 63.1; 52.9; 34.0; 31.9; 29.6; 29.6; 29.5; 29.3; 25.9; 23.3; 22.7; 20.9; 14.1. ESI MS: m/z 386 [M+H]⁺. Anal. Calcld. for C₂₂H₄₃N₃O₃: C, 68.53; H, 11.24%. Found: C, 68.70; H, 11.21%.

3. Results and Discussion

The retrosynthetic analysis (Scheme 1) indicates that the 1,2-diacyt el D-erythro-sphinganine 4 can be synthesized from the D-erythro-sphinganine triacetate 6, which can be prepared from the monoacetyl ester 8. On the other hand, L-threo-sphinganine triacetate 7 can be prepared from its diacetyl compound 5, which can also be prepared from the same monoacetyl ester 8. The monoacetyl compound 8 can in turn, be prepared from the Garner aldehyde 9.

The present synthesis was initiated by treatment of the Garner aldehyde 9 with 1-bromo pentadecane and Mg in THF at −78°C and the mixture was brought to r.t. to produce the diastereomeric alcohols 10 and 10a (Scheme 2). The stereochemistry of the two diastereomers 10 and 10a were assigned by following Buono’s method. According to this method, syn alcohol is major product compared to anti-alcohol. This stereochemistry is further confirmed by subsequent conversion to the target molecules. The two diastereomers were separated by careful CC (silica gel 100–200 mesh, 0–5% increasing amount of AcOEt in hexane), which produced 10 and 10a in the ratio 9:1 (syn:anti; 9:1) with 86% yield.

Due to less amount of minor diastereomer 10a, we have synthesized 1,2-diacyt el D-erythro-sphinganine (4) and D-erythro-sphinganine triacetate (6) from major diastereomer 10.

The acetonide group of alcohol 10 was deprotected by treatment with PPTS in MeOH to form diol 11 with 92% yield (Scheme 3). The 1,3-diol 11 was mono protected of its primary hydroxyl group as acetyl ester 8 by treatment with Ac₂O and Et₃N in CH₂Cl₂ with 77% yield. The mono acetyl ester 8 was converted to 1,2-diacetyl L-threo-sphinganine 5 by following two steps: i) Boc group was deprotected from 8 by using excess TFA in CH₂Cl₂; ii) unprotected amine was converted to N-acetylated amine on treatment with acetyl chloride, aq.NaHCO₃ in CH₂Cl₂ with 72% yield.
Total synthesis of sphingolipids

Scheme 1. Retrosynthetic analysis of sphingolipids.

Scheme 2. Grignard reaction on Garner aldehyde.

Scheme 3. (a) PPTS/MeOH, r.t., 2 h, 92%. (b) Ac₂O, Et₃N, CH₂Cl₂, 0 °C-r.t., 2 h, 77%. (c) i) TFA/CH₂Cl₂, r.t., 1 h. ii) Acetyl chloride, NaHCO₃, CH₂Cl₂-H₂O, r.t., 2.5 h, 72% (Over two steps). (d) Ac₂O, Et₃N, CH₂Cl₂, 0 °C-r.t., 2 h, 79%.

Scheme 4. (a) PPh₃, AcOH, DIAD, THF, 0 °C-r.t., 4 h, 77%. (b) i) TFA/CH₂Cl₂, r.t., 1 h. ii) AcCl, NaHCO₃, CH₂Cl₂-H₂O, r.t., 2.5 h, 73% (Over two steps). (c) Na₂CO₃/MeOH, r.t., 1 h, 82%. (d) Ac₂O, Et₃N, CH₂Cl₂, 0 °C-r.t., 2 h, 79%.

1,2-Diacetyl L-threo-sphinganine 5 was converted to L-threo-sphinganine triacetate 7 by treatment with Ac₂O and Et₃N in CH₂Cl₂ with 79% yield.

The mono acetyl ester 8 was subjected to Mitsunobu inversion by using triphenyl phosphine (PPh₃), AcOH and diisopropyl azodicarboxylate (DIAD) in dry THF.
to obtain 1,3-diacetyl compound 12 with 77% yield\textsuperscript{13} (Scheme 4). 1,3-Diacetyl compound 12 was converted to D-erythro-sphinganine triacetate 6 by following two steps: i) Boc group was deprotected from 12 by using excess TFA in CH\(_2\)Cl\(_2\); ii) unprotected amine was converted to N-acylated amine on treatment with AcCl, aq. NaHCO\(_3\) in CH\(_2\)Cl\(_2\) with 73% yield. D-erythro-sphinganine triacetate 6 was converted to 1,3-diol 13 by treatment with Na\(_2\)CO\(_3\) in MeOH\textsuperscript{14} at r.t. with 82% yield. The 1,3-diol 13 was mono protected of its primary hydroxyl group as acetyl ester by treatment with Ac\(_2\)O and Et\(_3\)N in CH\(_2\)Cl\(_2\) to produce 1,2-diacetyl D-erythro-sphinganine 4 with 79% yield. The physical (optical rotation) and the spectral (\(\text{^1}H\) and \(\text{^13}C\) NMR and MS) properties of 4 were found to be identical to those reported for the naturally occurring compound.\textsuperscript{6}

4. Conclusions

In conclusion, we have described the stereoselective total synthesis of naturally occurring sphingolipid 1,2-diacetyl D-erythro-sphinganine (4) along with three other sphingolipids, namely, 1,2-diacetyl L-threo-sphinganine (5) (C-3 epimer of 4), D-erythro-sphinganine triacetate (6) (triacyl derivative of compound 2) and L-threo-sphinganine triacetate (7) (triacyl derivative of compound 3, Safingol). Synthesis of 1,2-Diacetyl D-erythro-sphinganine (4) and 1, 2-diacetyl L-threo-sphinganine (5) are reported here for the first time.

Supplementary information (SI)

All the copies of \(\text{^1}H\) NMR and \(\text{^13}C\) NMR are given in the supporting information. Supplementary Information is available at www.ias.ac.in/chemsci.

Acknowledgments

The authors thank CSIR and UGC, New Delhi for grant of fellowships and financial assistance.

References

1. Hakomori S 1990 J. Biol. Chem. 265 18713
4. USP Dictionary of USAN and International Drug Names (US Pharmacopeia: Rockville, MD, 2000636)