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Synthesis and stereochemistry of ceramide B, (2*S*,3*R*,4*E*,6*R*)-*N*-(30-hydroxytriacontanoyl)-6-hydroxy-4-sphingenine, a new ceramide in human epidermis

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Abstract—(2*S*,3*R*,4*E*,6*R*)-*N*-(30-Hydroxytriacontanoyl)-6-hydroxy-4-sphingenine (**1**) and its (6*S*)-isomer (**1′**) were synthesized by starting from pentadecan-15-olide, the enantiomers of 1-pentadecyn-3-ol, and (*S*)-Garner's aldehyde. Comparison of the ^{1}H NMR spectra of the tetraacetyl derivatives of **1** and **1′** with that of ceramide B, a new protein-bound ceramide in human stratum corneum, revealed it to be (2*S*,3*R*,4*E*,6*R*)-**1**.

Ceramides constitute predominant lipids of human epidermal stratum corneum, acting as the water barrier to prevent loss of body water.[1] There are two ceramides, ceramide A and ceramide B, which are chemically bound to the stratum corneum protein.[2] Ceramide A was shown to be *N*-(30-hydroxytriacontanoyl)-4-sphingenine, while ceramide B was identified as a new ceramide, *N*-(30-hydroxytriacontanoyl)-6-hydroxy-4-sphingenine, with an additional hydroxy group at C-6 of sphingosine.[2] Since all the known mammalian sphingosines possess (2*S*,3*R*,4*E*)-stereochemistry,[3] ceramide B almost certainly possesses the same stereochemistry. As to the absolute configuration of the hydroxy group at C-6, no assignment was suggested so far.

We became interested in synthesizing (2*S*,3*R*,4*E*,6*R*)-**1** (Fig. 1) and (2*S*,3*R*,4*E*,6*S*)-**1′** so as to firmly establish the stereostructure of this important ceramide of human epidermis. Because the detailed 600 MHz ^{1}H NMR data including the spectral chart itself of tetraacetylated ceramide B have been published by Downing and co-workers,[2] comparison of the ^{1}H NMR spectra of the tetraacetylated **1** and **1′** with that of the tetraacetyl derivative of the naturally occurring ceramide B will give us sufficient information to propose its stereochemistry. Prior to us Bittman synthesized **3** and its (6*S*)-isomer,[4] and Yadav reported the synthesis of **4** and its (6*S*)-isomer.[5] Neither of them, however, determined the absolute configuration at C-6 of ceramide B. As early as in 1991, we synthesized a human epidermal cerebroside (epidermoside) such as **5**,[6] and therefore had enough experience to synthesize **1** itself, instead of **3** and **4**.

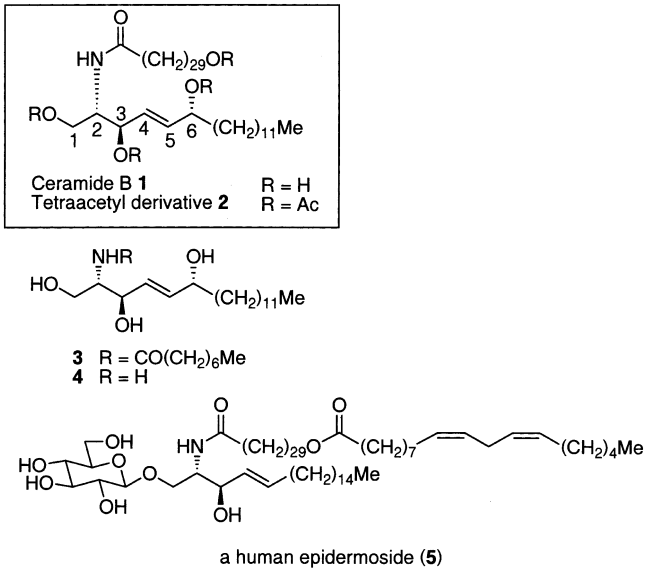

Figure 1. Structures of ceramide B and related compounds.

Keywords: alkynes; ceramides; configuration; lipase; lipids; sphingolipids.

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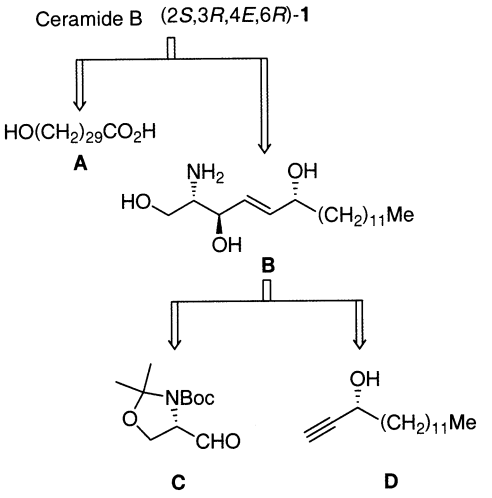


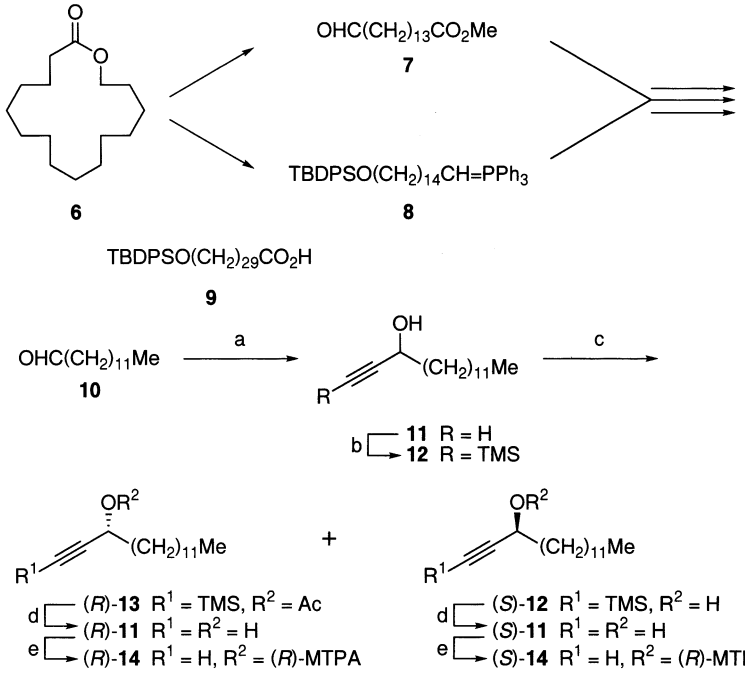
Scheme 1. Retrosynthetic analysis of ceramide B.

Scheme 1 shows our retrosynthetic analysis of ceramide B (**1**). Amide formation of 30-hydroxytriacontanoic acid (**A**)[6] with 6-hydroxy-4-sphingenine (**B**) gives ceramide B (**1**). 6-Hydroxy-4-sphingenine (**B**) can be prepared by coupling (*S*)-Garner's aldehyde (**C**)[7–9] with 1-pentadecyn-3-ol (**D**). Both Bittman[4] and Yadav[5] adopted the same strategy to prepare **B** by coupling **C** with **D**. The former prepared **D** by using Sharpless asymmetric dihydroxylation,[4] while the latter synthesized **D** by employing Sharpless asymmetric epoxidation.[5] We prepared **D** in a much simpler manner by using lipase.[10]

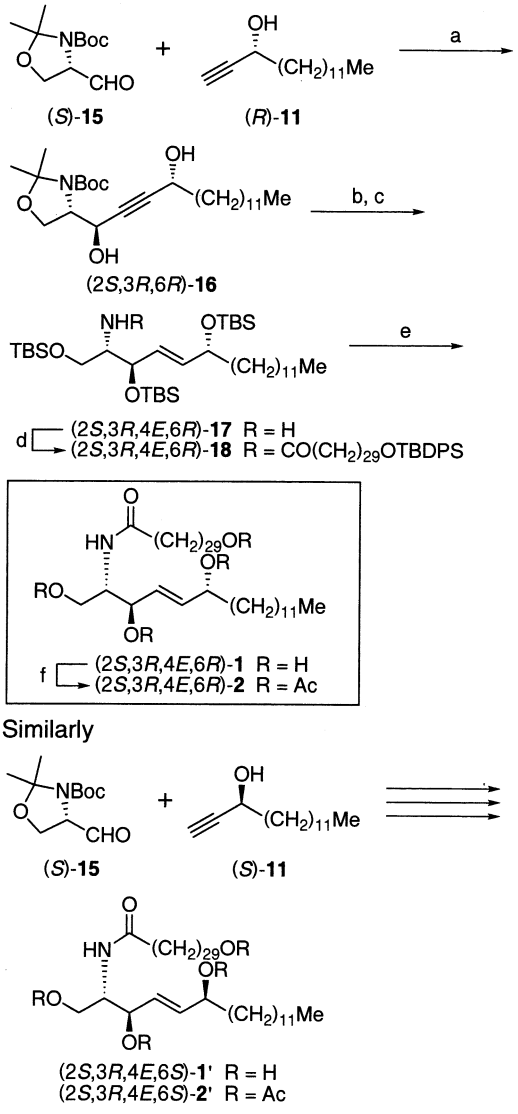
Synthesis of the two building blocks **9** and **11** is summarized in Scheme 2.

As to the preparation of 30-*t*-butyldiphenylsilyl(TBDPS)oxytriacontanoic acid (**9**), our previous method was adopted to synthesize **9** from commercially available pentadecan-15-olide (**6**) via the Wittig reaction of **7** with **8**.[6] Our synthetic route to the enantiomers of 1-pentadecyn-3-ol (**11**) was shorter and simpler than those reported previously.[4,5] Ethynylation of tridecanal (**10**) gave (±)-**11**, which was trimethylsilylated to give (±)-**12**.[11] Anastasia's method[12] for lipase-catalyzed enantiomer separation of 1-trimethylsilyl(TMS)-1-alkyn-3-ols was then applied to (±)-**12**. Treatment of (±)-**12** with vinyl acetate in the presence of lipase PS (Amano) on diatomite in diisopropyl ether for 10 days at room temperature was followed by chromatographic purification to give the acetylated (*R*)-**13** (49% yield, 98% *ee*) and the recovered (*S*)-**12** (48% yield, 95% *ee*). Both (*R*)-**13** and (*S*)-**12** were converted to the parent acetylenic alcohols, (*R*)-**11**, mp 40.5–42.0°C, $[\alpha]_D^{25}$ +2.49 ($c=1.20$, $CHCl_3$), and (*S*)-**11**, mp 42.0-43.0°C, $[\alpha]_D^{25}$ −2.30 ($c=1.00$, $CHCl_3$).[13] Their enantiomeric purities were determined by HPLC analysis after derivatizing them to the corresponding (*R*)-MTPA esters (**14**).

Scheme 3 illustrates further conversion of (*R*)-**11** to ceramide B (**1**). The acetylenic alcohol (*R*)-**11** was treated with 2.2 equiv. of *n*-butyllithium in THF to afford the corresponding dianion, to which was added (*S*)-Garner's aldehyde (**15**)[7–9] at −40°C under argon. The product was purified by silica gel chromatography to give predominantly the *anti*-product (2*S*,3*R*,6*R*)-**16**.[14] Reduction of the triple bond of **16** to (*E*)-double



Scheme 2. Synthesis of the two building blocks **9** and **11**. *Reagents and conditions*: (a) HC≡CMgBr, THF (89%); (b) *n*-BuLi, TMSCl, THF; then 1 M HCl aq. (80%); (c) Lipase PS (Amano) on diatomite, H_2C=CHOAc, $(i\text{-Pr})_2O$ [49% for (*R*)-**13** (98% *ee*); 48% for (*S*)-**12** (95% *ee*)]; (d) K_2CO_3, MeOH (quant.); (e) (*S*)-MTPACl, C_5H_5N.



Scheme 3. Synthesis of ceramide B (**1**) and its (6*S*)-isomer (**1′**). *Reagents and conditions*: (a) *n*-BuLi (2.2 equiv.), THF; (b) Li, $EtNH_2$, THF; (c) TBSOTf, 2,6-lutidine, CH_2Cl_2 (three steps, 18% after SiO_2 chromatog.); (d) EDC, HOBt, **9**, CH_2Cl_2 (76%). (e) TBAF, THF (64%). (f) Ac_2O, C_5H_5N (quant.).

bond was achieved with lithium in ethylamine, removing concomitantly the protective groups at C-1 and C-2. The generated 6-hydroxy-4-sphingenine was immediately silylated with *t*-butyldimethylsilyl (TBS) triflate to give (2*S*,3*R*,4*E*,6*R*)-**17** in 18% yield after purification by silica gel chromatography. This was acylated with 30-TBDPS-oxytriacontanoic acid (**9**) in the presence of 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (EDC) and 1-hydroxybenzotriazole (HOBt),[15] and the product **18** was treated with tetra(*n*-butyl)ammonium fluoride (TBAF) to afford (2*S*,3*R*,4*E*,6*R*)-**1** as colorless powder, mp 113.5-117.0°C.[16] The overall yield of **1** was 8.3% based on (*S*)-Garner's aldehyde (five steps). Similarly by employing (*S*)-acetylenic alcohol **11**, (2*S*,3*R*,4*E*,6*S*)-**1′** was also synthesized.

In order to compare the ^1H NMR spectra of our products with that of tetraacetylated ceramide B, both **1** and **1′** were acetylated to give (2*S*,3*R*,4*E*,6*R*)-**2** and (2*S*,3*R*,4*E*,6*S*)-**2′**, respectively. The low-field parts of their 500 MHz ^1H NMR spectra are shown in Figure 2. The two spectra were clearly different with regard to the δ values of the protons at C-4, 5 and 6, and the spectrum of (2*S*,3*R*,4*E*,6*R*)-**2** was identical to that of tetraacetylated ceramide B. The stereochemistry at C-6 of ceramide B must be *R*, assuming that it shares in common the (2*S*,3*R*,4*E*)-stereochemistry of the mammalian sphingolipids.

In conclusion the present synthesis of **1** and **1′** followed by the ^1H NMR analysis of **2** and **2′** allowed us to assign the structure (2*S*,3*R*,4*E*,6*R*)-**1** to ceramide B, the important ceramide of human epidermis.

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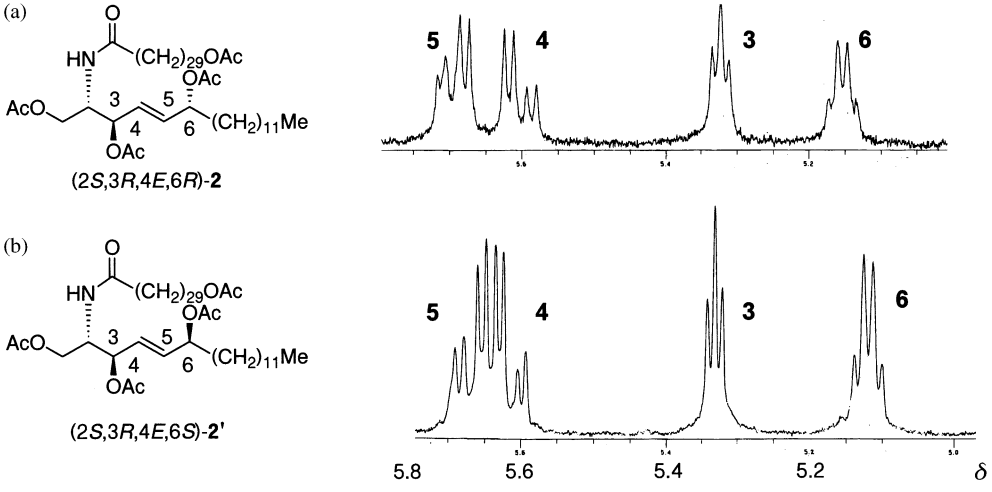

Figure 2. ^1H NMR spectra of (a) (2*S*,3*R*,4*E*,6*R*)-**2** and (b) (2*S*,3*R*,4*E*,6*S*)-**2′** (500 MHz, $CDCl_3/D_2O$). The spectrum of **2** was identical with that of tetraacetylated ceramide B.[2]

References

1. Hamanaka, S.; Suzuki, M.; Suzuki, A.; Yamakawa, T. *Proc. Japan Acad. Ser. B* **2001**, *77*, 51–56.
2. Robson, K. J.; Stewart, M. E.; Michelsen, S.; Lazo, N. D.; Downing, D. T. *J. Lipid Res.* **1994**, *35*, 2060–2068.
3. Kolter, T.; Sandhoff, K. *Angew. Chem., Int. Ed.* **1999**, *38*, 1532–1568.
4. Chung, J.; Byun, H.-S.; Bittman, R. *J. Org. Chem.* **2003**, *68*, 348–354.
5. Yadav, J. S.; Geetha, V.; Raju, A. K.; Gnaneshwar, D.; Chandrasekhar, S. *Tetrahedron Lett.* **2003**, *44*, 2983–2985.
6. Mori, K.; Matsuda, H. *Liebigs Ann. Chem.* **1991**, 529–535.
7. Garner, P.; Park, J. M. *J. Org. Chem.* **1987**, *52*, 2361–2364.
8. Garner, P.; Park, J. M.; Malecki, E. *J. Org. Chem.* **1988**, *53*, 4395–4398.
9. Review: Liang, X.; Andersch, J.; Bols, M. *J. Chem. Soc., Perkin Trans. 1* **2001**, 2136–2157.
10. Review on the use of lipases and other biocatalysis in synthesis: Mori, K. In *Stereoselective Biocatalysis*; Patel, R. N., Ed. Chemoenzymatic synthesis of pheromones, terpenes, and other bioregulators; Marcel Dekker: New York, 1999; pp. 59–85.
11. All the new compounds in this letter were characterized by consistent spectral (IR, ^{1}H, and ^{13}C NMR) and analytical (combustion or HRMS) data.
12. Allevi, P.; Ciuffreda, P.; Anastasia, M. *Tetrahedron: Asymmetry* **1997**, *8*, 93–99.
13. Literature data. (a) Bittmann[4] (*R*)-**11**, mp 40–41°C, $[\alpha]_D^{25}$ +2.60 ($c=1.0$, $CHCl_3$); (*S*)-**11**, mp 40–41°C, $[\alpha]_D^{25}$ −2.59 ($c=1.0$, $CHCl_3$); (b) Yadav.[5] (*R*)-**11**, mp not reported, $[\alpha]_D$ +1.3 ($c=1.05$, $CHCl_3$); (*S*)-**11**, mp 36–36.5°C, $[\alpha]_D$ −1.8 ($c=1.6$, $CHCl_3$).
14. The diastereoselectivity of this addition reaction was *anti*/*syn*=4.2:1. Bittman and co-workers reported that the addition of TBS-protected (*R*)-**11** to (*S*)-**15** in THF gave an 8:1 *anti*/*syn* mixture, and addition of HMPA further improved the ratio to >20:1.[4] cf. Gruza, H.; Kiciak, K.; Krasinski, A.; Jurczak, J. *Tetrahedron*: *Asymmetry* **1997**, *8*, 2627–2631.
15. Mori, K.; Uenishi, K. *Liebigs Ann. Chem.* **1994**, 41–48.
16. Physical data for **1**, **1′**, **2**, and **2′**. (a) (2*S*,3*R*,4*E*,6*R*)-**1**; mp 113.5–117.0°C. IR ν_{max} (Nujol) cm^{-1}: 3470 (w, N-H), 3310 (br.m, O-H), 1620 (m, C=O), 1545 (w), 1120 (w), 1060 (w), 960(w), 720 (w). HRFABMS m/z (M+H)$^+$: calcd for $C_{48}H_{96}NO_5$, 766.7210; found, 766.7269. (b) (2*S*,3*R*,4*E*,6*S*)-**1′**; mp 115.0–118.5°C. IR ν_{max} (Nujol) cm^{-1}: 3350 (br.m, N-H, O-H), 1660 (m, C=O), 1550 (w), 1140 (w), 1075 (w), 1020 (w), 975 (w), 720 (w). HRFABMS m/z (M+H)$^+$: calcd for $C_{48}H_{96}NO_5$, 766.7210; found, 766.7269. (c) (2*S*,3*R*,4*E*,6*R*)-**2**; $[\alpha]_D^{22}$ + 2.3 ($c=0.90$, $CHCl_3$). IR ν_{max} (Nujol) cm^{-1}: 3310 (br.w, N-H), 1740 (s, C=O), 1650 (m, NCO), 1540 (w), 1240 (m), 1040 (w), 975 (w), 720 (w). NMR δ_H (500 MHz, $CDCl_3$/D_2O): 0.86 (3H, t, $J=6.9$ Hz, 18-H_3), 1.21–1.40 (72H, m, 8∼17-H_2, 3′∼28′-H_2), 1.54–1.60 (4H, m, 7-, 29′-H_2), 2.02, 2.03, 2.04, 2.05 (12H, each s, OAc), 2.12–2.17 (2H, m, 2′-H_2), 3.98 (1H, dd, $J=11.6$, 4.6 Hz, 1-H_a), 4.02 (2H, t, $J=6.7$ Hz, 30′-H_2), 4.21 (1H, dd, $J=11.6$, 6.4 Hz, 1-H_b), 4.46 (1H, dd, $J=6.4$, 4.6 Hz, 2-H), 5.15 (1H, dt, $J=6.5$, 6.4 Hz, 6-H), 5.32 (1H, t-like, $J=5.8$ Hz, 3-H), 5.60 (1H, dd, $J=15.6$, 6.4 Hz, 4-H), 5.69 (1H, dd, $J=$ 15.6, 6.5 Hz, 5-H). These spectral data are in good agreement with the reported values at 600 MHz; 5.15 (1H, dt, $J=6.5$, 6.3 Hz, 6-H), 5.60 (1H, ddd, $J=15.6$, 6.6, 0.9 Hz, 4-H), 5.69 (1H, ddd, $J=15.6$, 6.6, 0.7 Hz, 5-H).[2] NMR δ_C (126 MHz, $CDCl_3$): 14.1, 21.0, 22.8, 25.9, 28.5, 29.21, 29.24, 29.31, 29.33, 29.36, 29.48, 29.50, 29.52, 29.55, 29.62, 29.65, 29.69, 31.89, 36.8, 50.3, 55.2, 62.4, 64.6, 72.7, 73.6, 126.5, 133.7, 169.8, 170.4, 170.8, 171.2, 172.9. HRFABMS m/z (M+H)$^+$: calcd for $C_{56}H_{104}NO_9$, 934.7711; found, 934.7709. (d) (2*S*,3*R*,4*E*,6*S*)-**2′**; $[\alpha]_D^{22}$ −11.6 ($c=0.90$, $CHCl_3$). IR ν_{max} (Nujol) cm^{-1}: 3305 (br.w, N-H), 1735 (s, C=O), 1650 (m, NCO), 1545 (w), 1245 (m), 1050 (w), 720 (w). NMR δ_H (500 MHz, $CDCl_3$/D_2O): 0.86 (3H, t, $J=7.0$ Hz, 18-H_3), 1.21–1.40 (72H, m, 8∼17-H_2, 3′∼28′-H_2), 1.53–1.60 (4H, m, 7-, 29′-H_2), 2.02, 2.03, 2.04, 2.05 (12H, each s, OAc), 2.10–2.14 (2H, m, 2′-H_2), 3.98–4.04 (3H, m, 1-H_a, 30′-H_2), 4.23 (1H, dd, $J=11.4$, 6.3 Hz, 1-H_b), 4.32–4.48 (1H, m, 2-H), 5.12 (1H, dt, $J=6.4$, 6.2 Hz, 6-H), 5.33 (1H, t-like, $J=5.3$ Hz, 3-H), 5.61 (1H, dd, $J=15.6$, 5.7 Hz, 4-H), 5.66 (1H, dd, $J=$ 15.6, 5.9 Hz, 5-H). NMR δ_C (126 MHz, $CDCl_3$): 14.1, 20.7, 22.6, 25.0, 25.6, 25.8, 29.2, 29.3, 29.37, 29.47, 29.50, 29.53, 29.55, 29.57, 29.63, 29.65, 29.69, 31.9, 34.1, 36.7, 50.2, 62.4, 64.6, 72.4, 74.0, 126.4, 133.5, 169.7, 170.5, 170.9, 171.2, 172.9. HRFABMS m/z (M+H)$^+$: calcd for $C_{56}H_{104}NO_9$, 934.7711; found, 934.7714.