



Tetrahedron Letters 44 (2003) 9197-9200

TETRAHEDRON LETTERS

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

Kenji Mori* and Yui Masuda

Glycosphingolipid Synthesis Group, Laboratory for Immune Regulation, RIKEN Research Center for Allergy and Immunology, c/o Seikagaku Corporation, Tateno 3-1253, Higashiyamato-shi, Tokyo 207-0021, Japan

Received 5 September 2003; revised 1 October 2003; accepted 3 October 2003

Abstract—(2S,3R,4E,6R)-N-(30-Hydroxytriacontanoyl)-6-hydroxy-4-sphingenine (1) and its (6S)-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 ¹H 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 (2S,3R,4E,6R)-1. © 2003 Elsevier Ltd. All rights reserved.

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-4sphingenine, with an additional hydroxy group at C-6 of sphingosine.² Since all the known mammalian sphingosines possess (2S,3R,4E)-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 (2S,3R,4E,6R)-1 (Fig. 1) and (2S,3R,4E,6S)-1' so as to firmly establish the stereostructure of this important ceramide of human epidermis. Because the detailed 600 MHz ¹H NMR data including the spectral chart itself of tetra-acetylated ceramide B have been published by Downing and co-workers,² comparison of the ¹H NMR spectra of the tetraacetylated 1 and 1' with that of the tetra-acetyl derivative of the naturally occurring ceramide B will give us sufficient information to propose its stereo-

and its (6S)-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.

chemistry. Prior to us Bittman synthesized 3 and its

(6S)-isomer,⁴ and Yadav reported the synthesis of 4



Figure 1. Structures of ceramide B and related compounds.

0040-4039/\$ - see front matter $\ensuremath{\mathbb{C}}$ 2003 Elsevier Ltd. All rights reserved. doi:10.1016/j.tetlet.2003.10.025

Keywords: alkynes; ceramides; configuration; lipase; lipids; sphin-golipids.

^{*} Corresponding author. Fax: +81-42-565-9906; e-mail: kjk-mori@ arion.ocn.ne.jp



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 Bittman⁴ and Yadav⁵ 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(TB-DPS)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 (\pm) -11, which was trimethylsilylated to give (\pm) -12.¹¹ Anastasia's method¹² for lipase-catalyzed enantiomer separation of 1-trimethylsilyl(TMS)-1alkyn-3-ols was then applied to (\pm) -12. Treatment of (\pm) -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, CHCl₃), and (*S*)-11, mp 42.0-43.0°C, $[\alpha]_D^{25}$ -2.30 (c=1.00, CHCl₃).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, $(i-Pr)_2O$ [49% for (*R*)-13 (98% *ee*); 48% for (*S*)-12 (95% *ee*)]; (d) K_2CO_3, MeOH (quant.); (e) (*S*)-MTPACl, C₅H₅N.



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, EtNH₂, THF; (c) TBSOTf, 2,6-lutidine, CH₂Cl₂ (three steps, 18% after SiO₂ chromatog.); (d) EDC, HOBt, 9, CH₂Cl₂ (76%). (e) TBAF, THF (64%). (f) Ac₂O, 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 (2S,3R,4E,6R)-17 in 18% yield after purification by silica gel chromatography. This was acylated with 30-TBDPSoxytriacontanoic 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 (2S,3R,4E,6R)-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, (2S,3R,4E,6S)-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 (2S,3R,4E,6R)-2 and (2S,3R,4E,6S)-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 (2S,3R,4E,6R)-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 (2S,3R,4E)-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 (2S, 3R, 4E, 6R)-1 to ceramide B, the important ceramide of human epidermis.

Acknowledgements

We thank Dr. S. Hamanaka (Hamanaka Dermatological Clinic, Saitama) for her suggestion and support to carry out the present synthesis. Our thanks are due to Dr. Y. Hirose (Amano Enzyme Inc., Gifu) for his generous gift of lipase PS. We are grateful to Dr. K. Sakai (Seikagaku Corporation, Tokyo) for his interest in this work.



Figure 2. ¹H NMR spectra of (a) $(2S_3R_4E_6R_7)$ and (b) $(2S_3R_4E_6S_7)$ (500 MHz, $CDCl_3/D_2O_7$). The spectrum of **2** was identical with that of tetraacetylated ceramide B.²

References

- Hamanaka, S.; Suzuki, M.; Suzuki, A.; Yamakawa, T. Proc. Japan Acad. Ser. B 2001, 77, 51–56.
- 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.
- Chung, J.; Byun, H.-S.; Bittman, R. J. Org. Chem. 2003, 68, 348–354.
- Yadav, J. S.; Geetha, V.; Raju, A. K.; Gnaneshwar, D.; Chandrasekhar, S. *Tetrahedron Lett.* 2003, 44, 2983– 2985.
- Mori, K.; Matsuda, H. Liebigs Ann. Chem. 1991, 529– 535.
- Garner, P.; Park, J. M. J. Org. Chem. 1987, 52, 2361– 2364.
- Garner, P.; Park, J. M.; Malecki, E. J. Org. Chem. 1988, 53, 4395–4398.
- Review: Liang, X.; Andersch, J.; Bols, M. J. Chem. Soc., Perkin Trans. 1 2001, 2136–2157.
- 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, ¹H, and ¹³C NMR) and analytical (combustion or HRMS) data.
- 12. Allevi, P.; Ciuffreda, P.; Anastasia, M. Tetrahedron: Asymmetry 1997, 8, 93–99.
- 13. Literature data. (a) Bittmann⁴ (*R*)-11, mp 40–41°C, [α]_D²⁵ +2.60 (*c*=1.0, CHCl₃); (*S*)-11, mp 40–41°C, [α]_D²⁵ -2.59 (*c*=1.0, CHCl₃); (b) Yadav.⁵ (*R*)-11, mp not reported, [α]_D +1.3 (*c*=1.05, CHCl₃); (*S*)-11, mp 36–36.5°C, [α]_D -1.8 (*c*=1.6, CHCl₃).
- 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.
- Physical data for 1, 1', 2, and 2'. (a) (2S,3R,4E,6R)-1; mp 113.5–117.0°C. IR v_{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 v_{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 C₄₈H₉₆NO₅, 766.7210; found, 766.7269. (c) (2S,3R,4E,6R)-2; $[\alpha]_{\rm D}^{22}$ + 2.3 (c = 0.90, CHCl₃). IR v_{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 $\delta_{\rm H}$ (500 MHz, CDCl₃/ D_2O : 0.86 (3H, t, J = 6.9 Hz, 18- H_3), 1.21–1.40 (72H, m, $8 \sim 17 \cdot H_2$, $3' \sim 28' \cdot H_2$), 1.54–1.60 (4H, m, 7-, 29'-H₂), 2.02, 2.03, 2.04, 2.05 (12H, each s, OAc), 2.12-2.17 (2H, m, 2'-H₂), 3.98 (1H, dd, J = 11.6, 4.6 Hz, 1-H₂), 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, 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, CDCl₃): 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 C₅₆H₁₀₄NO₉, 934.7711; found, 934.7709. (d) (2S,3R,4E,6S)-2'; $[\alpha]_D^{22}$ -11.6 (*c*=0.90, CHCl₃). IR *v*_{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 $\delta_{\rm H}$ (500 MHz, CDCl₃/ D_2O : 0.86 (3H, t, J = 7.0 Hz, 18-H₃), 1.21–1.40 (72H, m, $8 \sim 17 \cdot H_2$, $3' \sim 28' \cdot H_2$), 1.53–1.60 (4H, m, 7-, 29'-H₂), 2.02, 2.03, 2.04, 2.05 (12H, each s, OAc), 2.10-2.14 (2H, m, 2'-H₂), 3.98–4.04 (3H, m, 1-H_a, 30'-H₂), 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 $\delta_{\rm C}$ (126 MHz, CDCl₃): 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 C₅₆H₁₀₄NO₉, 934.7711; found, 934.7714.