Pergamon

SCIENCE DIRECT®

Bioorganic & Medicinal Chemistry Letters 13 (2003) 613–616

BIOORGANIC & MEDICINAL CHEMISTRY LETTERS

Synthesis of Non-Natural C2-*Homo*-ceramide and its Apoptotic Activity Against HL-60 Cells

Keiji Shikata, Hayato Niiro, Hideki Azuma, Taro Tachibana and Kenji Ogino*

Department of Applied & Bioapplied Chemistry, Graduate School of Engineering, Osaka City University, Sugimoto 3-3-138, Sumiyoshi-ku, Osaka 558-8585, Japan

Received 7 November 2002; revised 28 November 2002; accepted 28 November 2002

Abstract—Non-natural ceramide analogues, C2-*homo*-ceramide and C2-*homo*-dihydroceramide, were prepared from L-aspartic acid via L-*homo*-serine. The apoptotic activities of the synthesized ceramide analogues were examined in HL-60 human leukemia cells. C2-*homo*- and C2-*bishomo*-ceramide indicate low but considerable apoptotic activities in comparison with C2-ceramide.

Introduction

Sphingolipids are now well recognized as playing important roles in cell recognition, differentiation, cell-cell contact and cell growth.[1] Specifically, ceramide mediates apoptosis (cell death) through an intracellular action.[2] (Fig. 1) Consequently, in many studies, chemically prepared ceramide analogues have been used as a very powerful tool to investigate the mechanism of apoptosis. Previous studies demonstrated that sereral kinds of C2- and C6-ceramides, which are cell-permeable analogues of ceramide, have been employed for the investigation of structure–activity relationships in ceramide mediated apoptosis.[3] These results suggest that the apoptotic activity of dihydroceramide with the saturated sphingoid backbone is inactive whereas the introduction of a double bond at C4–C5 induced to apoptosis. However, the mechanism of apoptosis that ceramide induced has not been fully elucidated. Therefore, we prepared novel ceramide analogues to obtain more information on apoptotic phenomenon at the molecular level.

We have already established the method for preparation of (4*S*,5*R*,6*E*)-4-acylamino-6-eicosen-1,5-diol, a novel and non-natural ceramide analogue, having two methylene spacer between the primary hydroxyl and amino groups of sphingosine backbone from L-glutamic acid.[4] Although the experiments are preliminary, we found that this synthetic C2-*bishomo*-ceramide considerably induced apoptosis in HL60 human leukaemia cells in vitro.

In the present study, (3*S*,4*R*,5*E*)-3-acetylamino-5-nonadecen-1,4-diol and its dihydro-ceramide, which have one methylene spacer between the primary hydroxyl and amino group of the sphingosine backbone, were prepared. These analogues will allow us to elucidate structure–activity relationships in ceramide-mediated apoptosis.

Chemistry

The starting compound in the synthesis, *homo*-serine **3**, was obtained from L-aspartic acid according to a previously reported method.[5] In order to protect the primary hydroxyl and amino group of **3** using a cyclic system, such as Garner's aldehyde,[6] attempts to perform deprotection of diBoc at the amino group have not been successful. When a strong acid such as TFA was used to remove the Boc group, the major reactant was γ-lactone **4**. From these results, the treatment of compound **3** with *t*-butyldiphenylsilyl chloride in the presence of triethylamine gave **5**. One of the protecting groups in the amino group could be removed with a very dilute solution of TFA. When a concentrated solution of TFA was used, a product that removed both Boc groups was generated.

In the next step, treatment of **6** with the lithium anion of dimethyl methylphosphonate gave a Horner–Wadsworth–

*Corresponding author. Tel.: +81-6-6605-2799; fax: +81-6-6605-2167; e-mail: ogino@bioa.eng.osaka-cu.ac.jp

0960-894X/03/$ - see front matter © 2003 Elsevier Science Ltd. All rights reserved.
PII: S0960-894X(02)01026-0

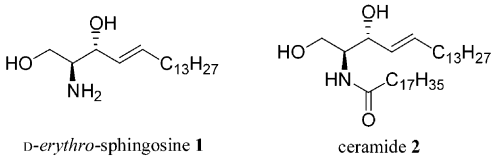
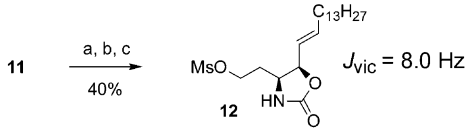


Figure 1. Structure of sphingolipids.



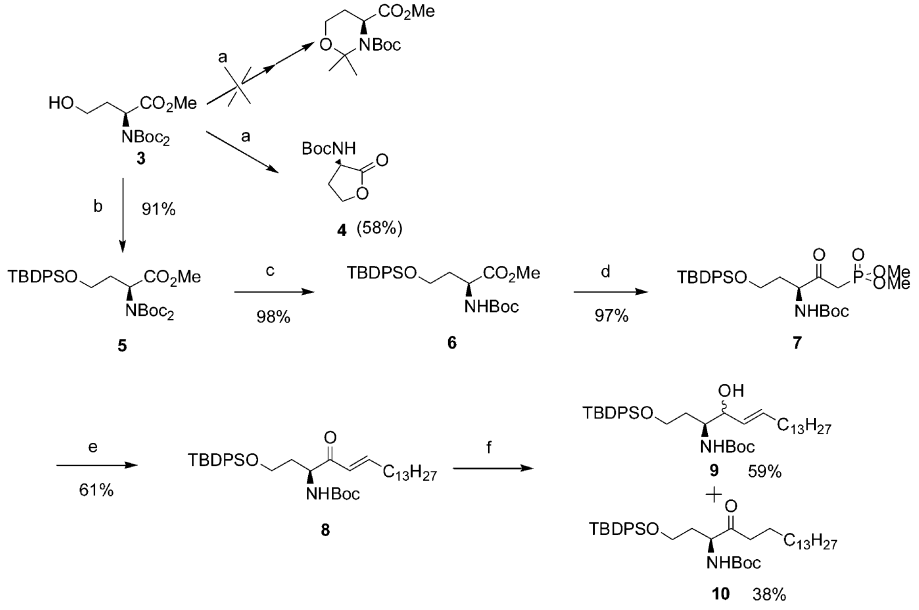
Scheme 3. Reagents and conditions: (a) MsCl, pyridine, room temperature, 30 min; (b) TFA Ch_2Cl_2, room temperature, 30 min; (c) CDI, THF, room temperature, 8 h.

Emmons (HWE) reagent **7**.[7] The HWE reaction with tetradecanal in the presence of diisopropylethylamine gave *E*-enone **8** exclusively. Based on our previous result, the reduction of **8** with DIBAL-H in toluene gave the aminoalchohol **9** (59%, *syn*/*anti* = 1/6) and the 1,4-reduction product **10** (38%).[4] (Scheme 1) The TBDPS group of **9** was removed with tetra-*n*-butylammonium fluoride. The stereochemistry of the newly induced chiral center in **11** was determined by spectral analysis after conversion to the corresponding oxazolidinone derivative **12** from **11**. (Scheme 3) Consequently, the stereochemical configuration of **11** is an *erythro* (8.0 Hz).[8] Further, the enantiomer excess of **11** was determined after conversion to the bis-Mosher ester by the ^{1}H NMR measurement (>90% e.e.).[9] Without further purification, the protecting group of **11** was removed using an acid catalyst, and then the treatment of the resulting product[10] with acetyl chloride in NaOAc gave the proposed compound, C2-*homo*-ceramide, **13**. (Scheme 2) The initial >90% e.e. could be easily raised to >99% of **13**[11] after single crystallization from AcOEt.

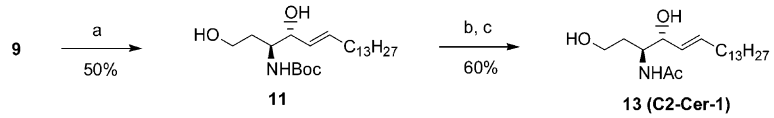
Furthermore, the C2-dihydro-*homo*-ceramide **15** was prepared from **10** by the same method used to convert **8** to **13**. (Scheme 4) The enantiomer excess of **14** was determined after conversion to the bis-Mosher ester by the ^{1}H NMR measurement (>90% e.e.). Compound **14**[12] (>99% e.e.) was obtained after purification by single recrystallization from AcOEt.

Biological Properties

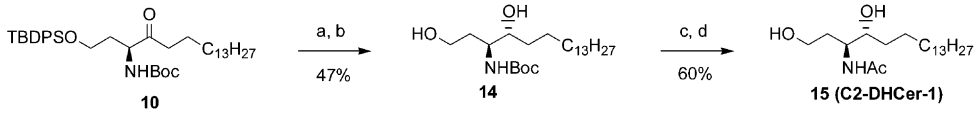
This study examined the effects of C2-ceramide, **C2-Cer**,[13] and its analogues, **C2-Cer-1** (**13**) and **C2-Cer-2**, [4] and their corresponding dihydroceramide analogues, **C2-DHCer**, **C2-DHCer-1** (**15**) and **C2-DHCer-2**. These analogues are structurally and stereochemically similar to C2-ceramide (Table 1). Structurally, both types of compounds have a secondary hydroxyl group in the α-position to the amino group, but have different methylene spacers between the primary hydroxyl group and amino group. To study structure–activity relationships of these compounds, the apoptotic activity



Scheme 1. Reagents and conditions: (a) TFA, CH_2Cl_2, room temperature, 10 min; (b) TBDPSCI, Et_3N, room temperature, 8 h; (c) dil.TFA, CH_2Cl_2, 0 °C, 1 h; (d) methylphosphonic acid dimethyl ester, *n*-BuLi, THF, −78 °C, 2 h; (e) tetradecanal, LiCl, diisopropylethylamine, THF, room temperature, 48 h; (f) DIBAL-H roluene, −78 °C, 1 h.

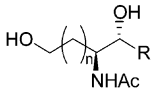


Scheme 2. Reagents and conditions: (a) tetra-*n*-butylammonium fluoride, THF, room temperature, 2 h; (b) 1M HCl aq, 1,4-dioxane 100 °C, 30 min; (c) AcCl, AcONa, THF, room temperature, 3 h.



Scheme 4. Reagents and conditions: (a) DIBAL-H, toluene, −78 °C, 1 h; (b) tetra-*n*-butylammonium fluoride, THF, room temperature, 2 h; (c) 1M HCl aq, 1,4-dioxane, 100 °C, 30 min; (d) AcCl, AcONa, THF, room temperature, 3 h.

Table 1. Structure of ceramide analogues and percentage of apoptosis in HL-60 cells after 6 h treatment with 10 μM



Compd		Apoptosis[a] (%)
Control		3
C2-Cer	($n=0$, R = –CH = $CHC_{13}H_{27}$)	62
C2-DHCer	($n=0$, R = $-CH_2CH_2C_{13}H_{27}$)	13
C2-Cer-1	($n=1$, R = –CH = $CHC_{13}H_{27}$)	48
C2-DHCer-1	($n=1$, R = $-CH_2CH_2C_{13}H_{27}$)	21
C2-Cer-2	($n=2$, R = –CH = $CHC_{13}H_{27}$)	52
C2-DHCer-2	($n=2$, R = $-CH_2CH_2C_{13}H_{27}$)	20

[a]Values are average of at least three separate experiments.

induced by them against HL-60 cells after 6 h of stimulation was measured by MTT assay.[14] (Table 1) In addition, to confirm that the cell death is apoptosis, the blebbing of cell membrane (data not shown) and the DNA fragmentation (Fig. 2), that were known as apoptotic characterization, were observed.

The apoptotic activities of the synthesized ceramide analogues were examined with 10 μM after 6 h. **C2-Cer** (a well-known inducer of apoptosis) was used as a positive control. As can be seen in Table 1, the apoptotic activity of these ceramide analogues is in the order **C2-Cer** > **C2-Cer-1** = **C2-Cer-2** > **C2-DHCer-1** = **C2-DHCer-2** > **C2-DHCer**. The DNA fragmentations of the synthesized ceramide analogues were observed with 10 μM after 8 h. Figure 2 shows that the stimulation of cell with **C2-Cer**, **C2-Cer-1** and **C2-Cer-2** resulted in the DNA fragmentations in quantity, whereas those of **C2-DHCer**, **C2-DHCer-1** and **C2-DHCer-2** resulted in small quantity. There was a good correlation between the DNA fragmentations and the apoptotic activities of these ceramide analogues. It is interesting that **C2-Cer-1** indicate a low but considerable apoptotic activity in comparison with **C2-Cer**, but comparable that of **C2-Cer-2**. Although the target molecule that induced apoptosis by direct interaction with C2-ceramide has not yet been identified, these data suggest that the location of the primary hydroxyl group does not significantly affect their apoptotic activities.

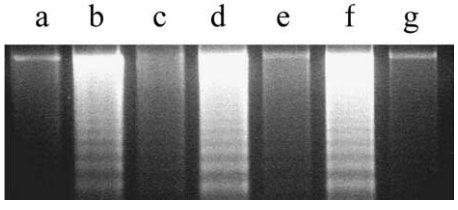


Figure 2. DNA fragmentations of HL-60 cells by treatment with (a) ethanol vehicle; (b) 10 μM **C2-Cer**; (c) 10 μM **C2-DHCer**; (d) 10 μM **C2-Cer-1**; (e) 10 μM **C2-DHCer-1**; (f) 10 μM **C2-Cer-2** and (g) 10 μM **C2-Cer-2** after 8 h.

Summary

Non-natural types of C2-*homo*-ceramide **13** and its C2-dihydro-analogue **15** were prepared from commercially available L-asparatic acid via L-*homo*-serine **3**. This practical method can be applied to the intermediate product of the novel sphingolipid analogue having the *homo*-sphingoid base. Biological observations suggest that our data could lead to the development of a new class of anti-cancer agents.

Further investigation related to this structure–activity relationships is in progress.

Acknowledgements

We thank Dr. N. Yasuhara (Osaka Univ.) for the generous gift of HL-60 cells.

References and Notes

1. (a) Hannun, Y.; Obeid, L. *J. Biol. Chem.* **2002**, *277*, 25847. (b) Adam, D.; Heinrich, M.; Kablitz, D.; Schutze, S. *Trends Immunol.* **2002**, *23*, 1. (c) Cuvillier, O.; Pirianov, G.; Kleuser, B.; Venek, P. G.; Coso, O. A.; Gutkind, J. S.; Spiegel, S. *Nature* **1996**, *381*, 800.
2. Hannun, Y. *Science* **1996**, *274*, 1855.
3. (a) Chang, Y. T.; Choi, J.; Ding, S.; Prieschl, E. E.; Baumruker, T.; Lee, J. A.; Chung, S. K.; Schultz, P. G. *J. Am. Chem. Soc.* **2002**, *124*, 1856. (b) Jonghe, S. D.; Overmeire, I. V.; Gunst, J.; Brunst, A. D.; Hendrik, C.; Calenbergh, S. V.; Busson, R.; Keukeleire, D. D.; Philippe, J.; Herdewijn, P. *Bioorg. Med. Chem. Lett.* **1999**, *9*, 3159. (c) Kishida, E.; Kasahara, M.; Takagi, Y.; Matsumura, M.; Hayashi, T.; Kobayashi, S.; Matsuzawa, Y. *J. Lipid Med. Cell Signal* **1997**, *16*, 127. (d) Karasavvas, N.; Erukulla, R. K.; Bittman, R.; Lockshin, R.; Zakeri, Z. *Eur. J. Biochem.* **1996**, *236*, 729. (e) Obeid, L. M.; Linardic, C. M.; Karolak, L. A.; Hannun, Y. A. *Science* **1993**, *259*, 1769. (f) Bielawska, A.; Crane, H. M.; Liotta, D.; Obeid, L. M.; Hannum, Y. A. *J. Biol. Chem.* **1993**, *268*, 26226.
4. Shikata, K.; Azuma, H.; Tachibana, T.; Ogino, K. *Tetrahedron* **2002**, *58*, 5803.
5. Sutherland, A.; Caplan, J. F.; Vederas, J. C. *Chem. Commun.* **1999**, 555.
6. (a) Garner, P. *Tetrahedron Lett.* **1984**, *25*, 5855. (b) Koskinen, P. M.; Koskinen, A. M. P. *Synlett* **1990**, 665. (c) Liang, X.; Andersch, J.; Bols, M. *J. Chem. Soc., Perkin Trans 1,* **2001**, *2136*.
7. Chung, S. K.; Lee, J. M. *Tetrahedron: Asymmetry* **1999**, *10*, 1441.

8. (a) Robert, V. H.; Najib, M.; Francisco, L. *J. Org. Chem.* **2002**, *67*, 1045. (b) Luly, J. R.; Dellaria, J. F.; Plattner, J. J.; Soderquist, J. L.; Yi, N. *J. Org. Chem.* **1987**, *52*, 1487.
9. Bakke, M.; Takizawa, M.; Sugai, T.; Ohta, H. *J. Org. Chem.* **1998**, *63*, 6929.
10. The structure of 3-amino-5-nonadecen-1,4-diol was appeared in Patent WO98/40349 from Takara Shuzo Co., Ltd, 1998, but its physical properties and stereochemistry were not described.
11. Compound **13**: Mp 107–10 °C; $[\alpha]_D^{25}$ −18.5 (c 0.508, $CHCl_3$); IR(KBr) 3294, 2922, 2851, 1663 and 1556 cm^{-1}; ^1H NMR ($CDCl_3$): δ 0.88 (3H, t, $J=6.8$ Hz), 1.26–1.89 (24H, m), 2.03 (3H, s), 2.05 (2H, dt, $J=6.8$, 6.8 Hz), 3.55–3.69 (2H, m), 4.10 (1H, m), 4.22 (1H, m), 5.47 (1H, dd, $J=15.4$, 6.1 Hz), 5.74 (1H, dt, $J=15.4$, 6.8 Hz) and 6.14 (1H, d, $J=7.8$ Hz); ^{13}C NMR ($CDCl_3$): δ 14.0, 22.6, 23.1, 29.2, 29.2, 29.3, 29.5, 29.6, 29.6, 31.7, 31.9, 32.3, 51.4, 58.7, 74.4, 128.6, 134.1 and 171.3. HRMS (FAB, positive), calcd for $C_{21}H_{42}NO_3$:$(M+H)^+$ 356.3165; found 356.3173. Anal. calcd for $C_{21}H_{41}NO_3$; C, 70.94; H, 11.62; N, 3.94. Found C, 70.63; H, 11.73; N, 3.95.
12. Compound **15**: Mp 125–126 °C; $[\alpha]_D^{25}$ −13.0 (c 0.500, $CHCl_3$); IR(KBr) 3296, 2916, 2851, 1649 and 1549 cm^{-1}; ^1H NMR ($CDCl_3$): δ 0.88 (3H, t, $J=6.8$ Hz), 1.21–1.87 (30H, m), 2.03 (3H, s), 3.55–3.70 (4H, m), 4.03–4.09 (1H, m) and 6.13 (1H, d, $J=7.8$ Hz); ^{13}C NMR ($CDCl_3$): δ 14.0, 22.7, 23.2, 25.9, 29.3, 29.5, 29.6, 29.7, 30.6, 31.9, 34.3, 50.5, 58.5, 74.0 and 171.0.HRMS (FAB, positive), calcd for $C_{21}H_{44}NO_3(M+H)^+$ 358.3321; found 358.3321.
13. Azuma, H.; Tamagaki, S.; Ogino, K. *J. Org. Chem.* **2000**, *65*, 3538.
14. Itakura, A.; Tanaka, A.; Aioi, A.; Tanogaito, H.; Matsuda, H. *Experimental Hematology* **2002**, *30*, 272.