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Synthesis of novel and non-natural ceramide analogues derived from L-glutamic acid

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

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

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Abstract—Novel and non-natural ceramide analogues, having a different methylene spacer between the primary hydroxymethyl group and aminomethane of sphingosine backbone, have been prepared from L-glutamic acid. The key step in the preparation is the diastereoselective reduction of an enone adjacent to a Boc protected amino group by reducing agents.

1. Introduction

Sphingolipids exist ubiquitously in the membranes of eukaryotic cells and may mediate diverse functions in the regulation of cell growth, differentiation, cell–cell contact and apoptosis.[1] These lipids consist of a long-chain aminoalcohol (sphingosine **1**, (2*S*,3*R*,4*E*)-2-aminooctadec-4-en-1,3-diol), an amide-linked fatty acid, and a polar phosphoric ester or glycosidic group. Ceramides **2**, fundamental backbones of sphingolipids, are composed of sphingosine connected via an amide bond with a fatty acid (Fig. 1). For example, ceramide generated by sphingomyelin hydrolysis rapidly occurs by stimulation of cell surface receptors including the tumor necrosis factor, anticancer drugs, oxidative stress, and Fas ligand, and then triggers caspase activation leading to apoptosis.[2] Treatment of cells exogenously with C2-ceramide (*N*-acetyl-sphingosine) has been reported to induce apoptosis in several cell systems, whereas a structural analog, C2-dihydroceramide, did not.[3] On the other hand, sphingosine has been a specific and strong inhibitor of protein kinase C, which is a principal regulatory enzyme in cell growth.[4]

Consequently, in many studies, sphingolipid analogues, which were chemically prepared, have been used as a very powerful tool in the elucidation of diverse physiological phenomena. For instance, HPA-12 has been used as an inhibitor of ceramide trafficking from the endoplasmic reticulum to the site of sphingomyelin synthesis.[5] Very recently, the synthesis of a ceramide library was reported and the apoptotic activity of those ceramide analogues depended on structural features.[6] However, since the diverse phenomena of the apoptotic cascades have not yet been fully elucidated, new ceramide analogues are required.

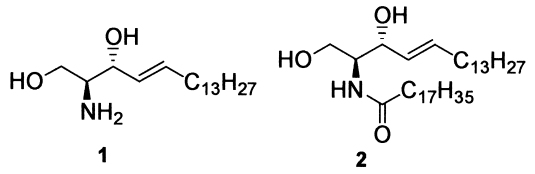

Figure 1. Structure of D-*erythro*-sphingosine **1** and ceramide **2**.

The wide varieties of physiological activities of ceramide and sphingosine derivatives have prompted reports of many synthetic procedures of these compounds,[7] including our method.[8] We are interested in the apoptotic activity of ceramide analogues against cancer cells and their inhibitory effects on the enzymes concerned in the biosynthetic and degradation pathways of sphingolipids.

This paper presents the preparation of novel and non-natural ceramide and C2-ceramide analogues, having a different methylene spacer between the primary hydroxyl and amino groups of sphingosine backbone, from L-glutamic acid. The starting material, protected ethyl 4-hydroxy-2-aminopentanoate, is available through a modified procedure,[9] and this makes it possible to obtain a *bishomo*-analogue of sphingosine. We would like to elucidate structure–activity relationships for ceramide-mediated apoptosis against cancer cells.

2. Results and discussion

The proposed compound is a novel and non-natural type of ceramide, C20 aliphatic 4-amino-1,5-diol with a C(6),C(7)-*trans* double bond, with two long carbon chains in comparison with natural ceramide. Our synthetic approach is

Keywords: ceramide; glutamic acid; sphingolipids.

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

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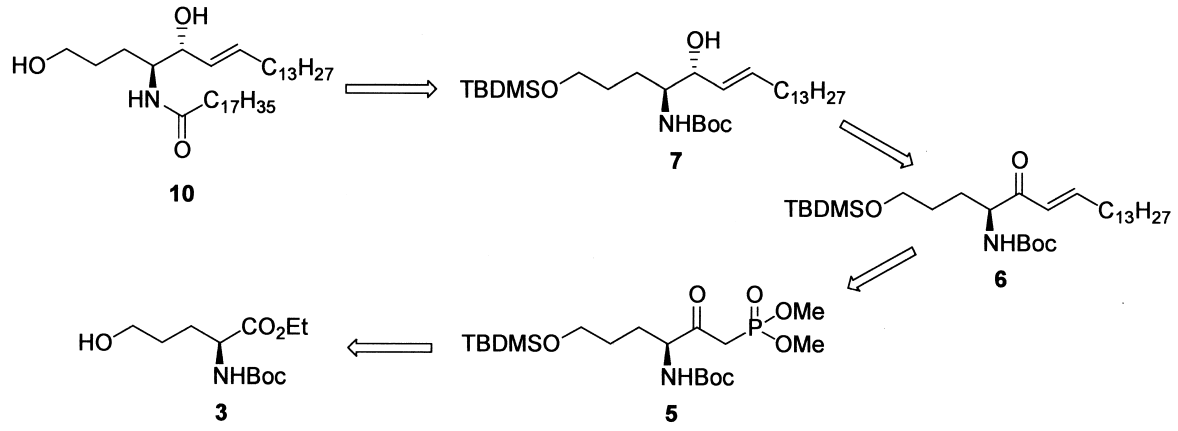
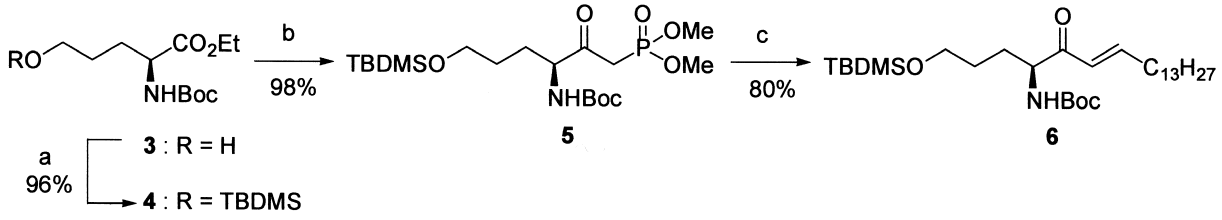


Figure 2. Retrosynthesis of D-*erythro*-ceramide analogue.

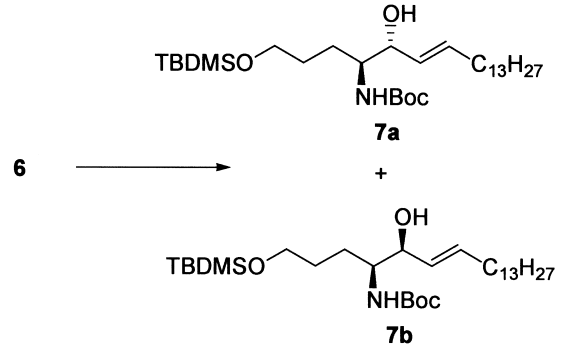


Scheme 1. *Reagents and conditions*: (a) TBDMSCl, Et_3N, room temperature, 8 h; (b) methylphosphonic acid dimethyl ester, *n*-BuLi, THF, −78°C, 2 h; (c) tetradecanal, LiCl, diisopropylethylamine, THF, room temperature, 48 h.

outlined in Fig. 2. Thus, the synthesis of enone **6** was performed by conversion to the chiral serine analogue **3** ($[\alpha]_D^{25}=+7.03$ [*c* 1.20 chloroform]) obtained from L-glutamic acid according to a previously reported method.[9] It was difficult to protect the primary hydroxyl and amino groups of **3** from a cyclic system by dimethoxypropane, such as Garner's aldehyde,[10] because of a 7-membered ring. The protected ethyl 4-hydroxy-2-aminopentanoate **3** was treated with *t*-butyldimethylsilyl chloride in the presence of triethylamine to give silylether **4**.

In the next step, the Horner–Wadsworth–Emmons (HWE) reaction was chosen to introduce a *trans* double bond and long aliphatic chain.[11] Thus, treatment of **4** with dimethyl methylphosphonate and *n*-butyllithium gave a high yield of β-ketophosphonate **5**. The HWE reaction of **5** with tetradecanal in the presence of diisopropylethylamine in THF gave *E*-enone **6** exclusively. The *E* isomer was indicated by a vinyl coupling constant $J=15.6$ Hz. Within the limit of NMR detection, the absence of the *Z* isomer of **6** was confirmed (Scheme 1).

Table 1. Hydride reduction of enone **6**



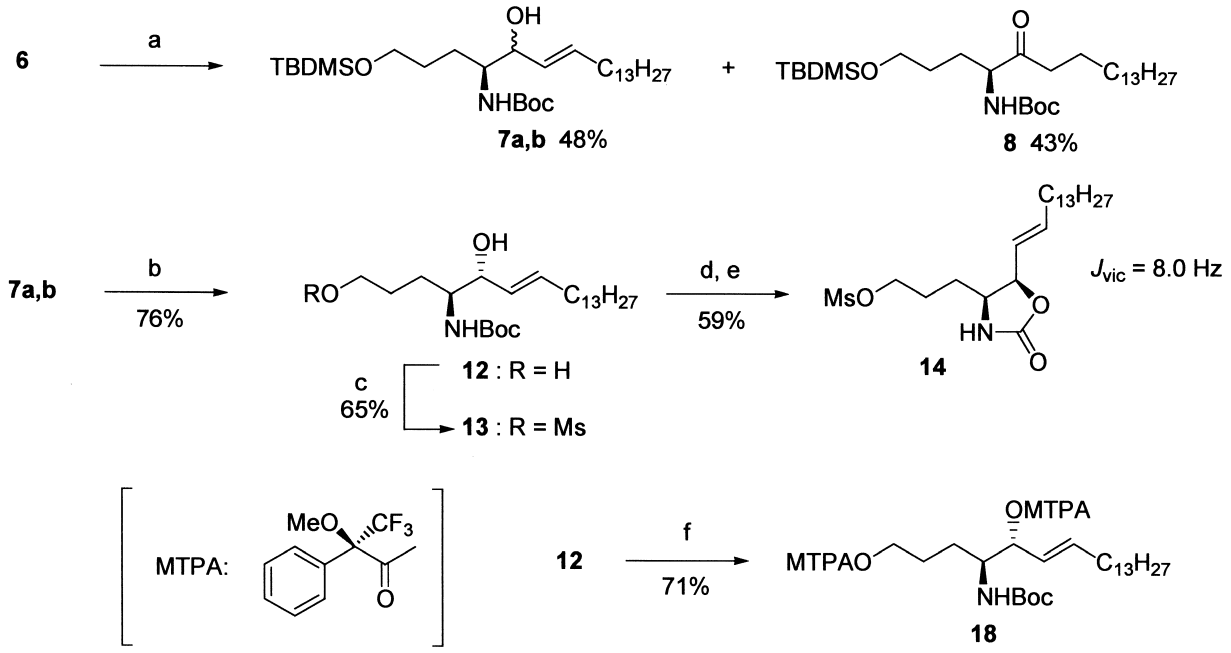
Entry	Conditions	Results (**7a**/**7b**)
1	DIBAL-H (1.2 equiv.)/toluene/−78°C	8.6:1.0
2	DIBAL-H (1.2 equiv.)/CH_2Cl_2/−78°C	4.9:1.0
3	Zn $(BH_4)_2$ (1.2 equiv.)/THF/0°C	2.0:1.0
4	$NaBH_4/CeCl_3$ (3 equiv.)/MeOH/r.t.	2.5:1.0
5	L-Selectride (1.2 equiv.)/THF/−78°C	0.8:1.0

The diastereomeric ratio was determined by ^{1}H NMR.

We investigated the hydride reduction employing various reducing agents and reaction conditions,[11,12] and the diastereoselectivity for the reduction of enone **6** is summarized in Table 1. The diastereomeric ratio of compound **7a**,**b** was determined by the integration of ^{1}H NMR spectra. The hydride reduction using DIBAL-H in toluene at −78°C gave the most diastereoselective result (lane 1) of an 8.6:1 mixture. Such a relatively high observed selectivity can probably be attributed to steric hindrance by the large *N*-Boc group in spite of the acyclic enone of **6**, thus it is likely that the reduction mechanism of enone **6** proceeded through a chelated transition state.[13]

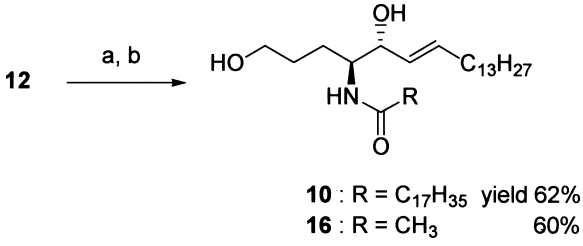
On the basis of Table 1, compound **6** was treated by the preceding reduction in spite of a relatively low chemoselectivity and converted to the aminoalchol **7** (47.6%; **7a**/**7b**=8.6:1) with the 1,4-reduction product **8** (43.4%) which was confirmed by ^{1}H NMR. Double reduction product **9** could not be observed by ^{1}H NMR and TLC. This saturated ketone **8** was used for the syntheses of dihydroceramide analogues.

The stereochemistry of the newly induced chiral center in **7** was determined in the following manner. First, the



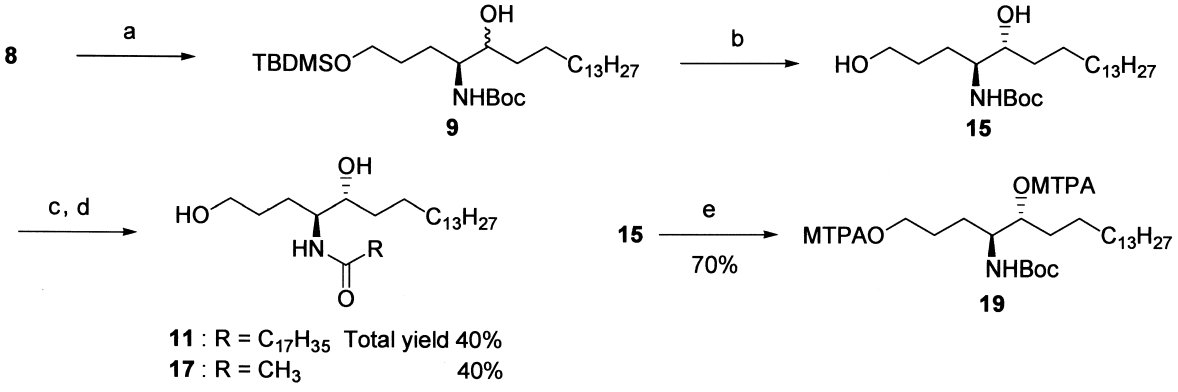
Scheme 2. *Reagents and conditions*: (a) DIBAL-H, toluene, −78°C, 1 h; (b) *p*-toluenesulfonate, EtOH, 50°C, 8 h; (c) MsCl, pyridine, room temperature, 30 min; (d) TFA, CH_2Cl_2, room temperature, 30 min; (e) CDI, THF, room temperature, 8 h; (f) (*R*)-MTPA acid, DCC, DMAP, CH_2Cl_2, room temperature, 2 h.

TBDMS group of **7** was removed with pyridinium *p*-toluenesulfonate in EtOH at 50°C, then the regioselective mesylation of resulting product **12** (partially purified after single recrystallization) with methanesulfonyl chloride in pyridine on the primary hydroxy group gave **13**. Second, the Boc group of **13** was removed with acid catalyst, and then treatment of the resulting product with carbonyl diimidazole in THF gave 2-oxazolidone **14**. The relative configuration at the 5 position of **14** was determined on the basis of the observed coupling constants for 4H and 5H (J=8.0 Hz), if the configuration is *anti*, the coupling constant should be observed J=7–8 Hz, whereas if it is *syn*, the coupling constant should be observed J=4–5 Hz.[11,13] Consequently, the major diastereomer **7** is an *erythro* configuration. The enantiomer excess of **12** was determined after conversion to the Mosher ester, bis-MTPA ester **18**, by the ^{1}H NMR measurement (90% e.e.)[14] (Scheme 2).



Scheme 3. *Reagents and conditions*: (a) 1 M HCl aq., 1,4-dioxane, 100°C, 30 min; (b) stearoyl chloride or AcCl, AcONa, THF, room temperature, 3 h.

Without further purification, the protecting group of **12** was removed using an acid catalyst, then the treatment of the resulting product with stearoyl chloride or acetyl chloride in the presence of NaOAc in THF gave ceramide analogue **10** or C2-ceramide analogue **16**, respectively, which were then purified by recrystallization. The initial 90% e.e. could be easily raised to >99% after single recrystallization from methanol (Scheme 3).



Scheme 4. *Reagents and conditions*: (a) DIBAL-H, toluene, −78°C, 1 h; (b) *p*-toluenesulfonate, EtOH, 50°C, 8 h; (c) 1 M HCl aq., 1,4-dioxane, 100°C, 30 min; (d) stearoyl chloride or AcCl, AcONa, THF, room temperature, 3 h; (e) (*R*)-MTPA acid, DCC, DMAP, CH_2Cl_2, room temperature, 2 h.

Furthermore, the dihydroceramide analogue **11** or **17** was prepared from ketone **8**, by the similar DIBAL-H reduction used to convert **6** to **10** or **16**. The enantiomer excess of **15** was determined after conversion to the bis-MTPA ester **19** by the ^{1}H NMR measurement (>90% e.e.).[14] The initial 90% e.e. could be easily raised to >99% after single recrystallization from methanol (Scheme 4).

Conclusively, non-natural types of ceramide **10** and C2-ceramide **16**, and the related dihydroceramide **11** and C2-dihydroceramide **17** were prepared from L-glutamic acid through the common intermediate **6**.

Though the experiments are preliminary, we found that the apoptotic activity of the synthesized C2-ceramide analogue **16** was more effective than the natural C2-ceramide against human leukaemia (HL-60) cells in vitro.

Further work is currently underway to accomplish the synthesis of another non-natural type of sphingosine and ceramide *homo*-analogues by a similar convergent assembly starting from L-aspartic acid.

3. Experimental

3.1. General

All the materials were obtained commercially (guaranteed reagent grade) and used without further purification. All solvents were freshly distilled under nitrogen before use; THF was distilled from $LiAlH_4$; CH_2Cl_2 was distilled from CaH_2; EtOH was distilled from CaO. Melting points were determined with a Yanaco MP-S3 micro melting point apparatus. Optical rotations were measured with a JASCO DIP-360 polarimeter. IR spectra were recorded on a Shimadzu FT-IR-8200A spectrometer. ^{1}H and ^{13}C NMR spectra were recorded on a JEOL JNM-A400 FT NMR spectrometer ($CDCl_3$ and CD_3OD solution with TMS as an internal standard). Column chromatography was performed on silica gel (Wakogel C-200).

3.1.1. Ethyl (2*S*)-[2-(*tert*-butoxycarbonyl)amino]-5-(*tert*-butyldimethylsiloxy)pentanoate (4). To a solution of 5-hydroxypentanoate **3** (1.93 g, 7.39 mmol) in CH_2Cl_2 (20 mL) was added 4-dimethylaminopyridine (DMAP) (0.012 g, 0.01 mmol), *tert*-butyldimethylchlorosilane (1.34 g, 8.87 mmol) and triethylamine (1.12 g, 11.1 mmol). After stirring for 8 h at room temperature, the mixture was quenched with 1 M aqueous HCl solution. The mixture was extracted with Et_2O three times. The organic layers were combined and washed with saturated aqueous $NaHCO_3$ solution and brine, dried over sodium sulfate, and concentrated under reduced pressure. The residue was purified by column chromatography on silica gel (diethylether/hexane=1:2) to give **4** (3.67 g, 96%), as a colorless oil. ^{1}H NMR ($CDCl_3$) δ 0.05 (6H, s×2), 0.89 (9H, s), 1.28 (3H, t, J= 7.3 Hz), 1.44 (9H, s), 1.57 (2H, m), 1.71, 1.88 (2H, m), 3.63 (2H, t, J=6.1 Hz), 4.19 (2H, m), 4.27 (1H, m) and 5.22 (1H, d, J=8.3 Hz). ^{13}C NMR ($CDCl_3$) δ −5.4, 14.1, 25.6, 25.9, 28.2, 28.3, 29.0, 53.2, 61.1, 62.2, 79.6, 155.4 and 172.8.

This compound was employed for the next step without further purification.

3.1.2. (*S*)-3-[(*tert*-Butoxycarbonyl)amino]-6-(*O*-*tert*-butyldimethylsilyloxy)-2-oxo-hexyl]-phosphonic acid dimethyl ester (5). To a solution of methylphosphonic acid dimethyl ester (13.1 g, 106 mmol) in dry THF was added *n*-BuLi (1.6 M in hexane, 64 mL, 102 mmol) under nitrogen at −78°C. After stirring for 30 min at −78°C, **4** (12.0 g, 32.0 mmol) in dry THF was added to the mixture. After stirring for 2 h, the mixture was poured into an ice-cooled saturated aqueous NH_4Cl solution and extracted with Et_2O three times. The organic layer was combined, dried over $MgSO_4$ and filtered. The filtrate was concentrated under reduced pressure. The residue was purified by silica gel column chromatography (hexane/Et_2O=2:1) to give **5** (13.3 g, 97.8%) as a colorless oil. $[\alpha]_D^{25}$=+2.76 [c 0.93, chloroform]. ^{1}H NMR ($CDCl_3$) δ 0.05 (6H, s), 0.89 (9H, s), 1.45 (9H, s), 1.45–1.72 (4H, m), 3.12 (1H, dd, $^2J_{PH}$= 22.0 Hz, $^3J_{HH}$=14.2 Hz), 3.33 (1H, dd, $^2J_{PH}$=22.0 Hz, $^3J_{HH}$=14.2 Hz), 3.63 (2H, t, J=5.4 Hz), 3.79 (3H, d, J=11.2 Hz), 3.80 (3H, d, J=11.2 Hz), 4.32 (1H, m) and 5.52 (1H, d, J=7.3 Hz). ^{13}C NMR ($CDCl_3$) δ −5.4, 18.3, 25.9, 27.1, 28.3, 28.3, 37.0, 38.4, 53.0, 53.1, 53.1, 60.1, 62.3, 79.9, 155.5, 201.6 and 201.6. IR (KBr) 3292, 2957, 1713, 1520 and 1254 cm^{-1}. HRMS (FAB, positive), calcd for $C_{19}H_{41}NO_7PSi$: $(M+H)^+$454.2390; found 454.2390.

3.1.3. (4*S*)-1-(*O*-*tert*-Butyldimethylsilyl)-4-*tert*-butoxycarbonyl-amino-5-oxo-eicos-6-en-1-ol (6). To a solution of **5** (5.00 g, 11.8 mmol) and lithium chloride (0.55 g, 13.0 mmol) in dry THF (100 mL) was added diisopropylethylamine (1.68 g, 13.0 mmol) in dry THF (10 mL) at room temperature. After stirring for 30 min, tetradecanal (2.51 g, 11.8 mmol) in dry THF (10 mL) was added at room temperature. After stirring for 48 h, 1N HCl solution (50 mL) was added and the mixture was extracted with Et_2O. The organic layer was washed with saturated aqueous $NaHCO_3$ and brine before being dried over $MgSO_4$ and filtered. The mixture was then concentrated under reduced pressure and the residue was purified by silica gel column chromatography to give **6** (4.90 g, 76.9%), as a yellow oil. $[\alpha]_D^{25}$=+28.3 [c 1.01, chloroform]. ^{1}H NMR ($CDCl_3$) δ 0.03, 0.04 (6H, s×2) 0.86–0.90 (12H, m) 1.21–1.97 (35H, m), 2.19–2.25 (2H, m), 3.61 (2H, t, J=5.4 Hz), 4.57 (1H, m), 5.41 (1H, d, J=7.8 Hz) 6.19 (1H, d, J=15.6 Hz) and 7.00 (1H, dt, J=15.6, 6.8 Hz). ^{13}C NMR ($CDCl_3$) δ −5.4, 14.0, 18.2, 22.6, 25.9, 27.9, 28.1, 28.3, 28.9, 29.0, 29.1, 31.7, 32.6, 57.0, 62.3, 79.4, 126.7, 149.7, 155.5 and 198.2. IR (neat) 3427, 2926, 1719, 1630 and 1101 cm^{-1} HRMS (FAB, positive), calcd for $C_{31}H_{62}NO_4Si$: $(M+H)^+$540.4448; found 540.4431.

3.1.4. (4*S*,5*R*,6*E*)-1-(*O*-*tert*-Butyldimethylsilyl)-4-(*tert*-butoxy-carbonylamino)-eicos-6-en-1,5-diol (7) and (4*S*,5*S*,6*E*)-1-(*O*-*tert*-butyldimethylsilyl)-4-(*tert*-butoxy-carbonylamino)-eicosane-1,5-diol (8). To a solution of **6** (11.0 g, 20.4 mmol) in toluene (100 mL) was added DIBAL-H (1.5 M solution in toluene, 20.4 mL, 30.6 mmol) under nitrogen at −78°C. After stirring for 1 h, the 1 M aqueous HCl solution was added and allowed to warm to room temperature. The mixture was extracted with Et_2O (3×100 mL). The organic layer was combined and washed

with saturated aqueous $NaHCO_3$ solution and brine, dried over sodium sulfate, and concentrated under reduced pressure. The residue was purified by column chromatography on silica gel (diethylether/hexane=1:2) to give **7** (5.26 g, 47.6%), as a colorless oil and **8** (4.80 g, 43.4%), as a colorless crystal. The diastereomeric ratio of **7** was estimated by the integrate ratio of the 5-OH signal. Major diastereomer **7**; ^{1}H NMR ($CDCl_3$) δ 0.05 (6H, s×2), 0.86–0.89 (12H, m), 1.26–1.63 (35H, m), 2.03 (2H, dt, J=6.83, 6.83 Hz), 3.60–3.66 (3H, m), 4.11 (1H, dd, J=6.6, 3.2 Hz), 4.74 (1H, brs), 5.43 (1H, dd, J=15.6, 6.6 Hz) and 5.71 (1H, dt, J=15.6, 7.1 Hz). ^{13}C NMR ($CDCl_3$) δ −5.4, 14.1, 18.3, 22.6, 25.9, 26.5, 26.7, 28.3, 29.1, 29.2, 29.3, 29.5, 29.6, 31.9, 32.4, 50.7, 55.5, 62.7, 75.7, 79.6, 128.1, 134.0 and 157.0. HRMS (FAB, positive), calcd for $C_{31}H_{64}NO_4Si$: $(M+H)^+$542.4605; found 542.4597.

Compound **8**. A colorless crystal. Mp 32–33°C. $[\alpha]_D^{25}$=+25.6 [c 0.93, chloroform]. ^{1}H NMR ($CDCl_3$) δ 0.04, 0.05 (6H, s×2), 0.86–0.90 (12H, m), 1.25–1.96 (39H, m), 2.41–2.55 (2H, m), 3.62 (2H, t, J=5.9 Hz), 4.29–4.31 (1H, m) and 5.35 (1H, d, J=7.32 Hz). ^{13}C NMR ($CDCl_3$) δ −5.37, 14.1, 18.3, 22.7, 23.5, 25.9, 28.0, 28.1, 28.3, 29.2, 29.4, 29.4, 29.6, 29.7, 31.9, 39.6, 59.0, 62.2, 79.5, 155.5 and 209.7. IR (KBr) 3651, 2924, 1719, 1684 and 1524 cm^{-1}. HRMS (FAB, positive), calcd for $C_{31}H_{64}NO_4Si$: $(M+H)^+$542.4605; found 542.4628.

3.1.5. (4*S*,5*R*,6*E*)-4-[(*tert*-Butoxycarbonyl)amino]-eicos-6-en-1,5-diol (12). To a solution of **7** (100 mg, 0.185 mmol) in EtOH (15 mL) was added pyridinium *p*-toluenesulfonate (10 mg, 0.040 mmol) at 50°C. After stirring for 8 h, the mixture was allowed to cool to room temperature and concentrated under reduced pressure and the residue was dissolved with Et_2O. The solution was washed with 1 M aqueous HCl solution, saturated aqueous $NaHCO_3$ solution and brine before being dried over $MgSO_4$ and concentrated under reduced pressure. The residue was purified by single recrystallization to give **12** (60 mg, 76%), as a colorless crystal. Mp 109–110°C. $[\alpha]_D^{25}$=−17.2 [c 0.97, methanol]. ^{1}H NMR ($CDCl_3$) δ 0.88 (3H, t, J=6.8 Hz), 1.26–1.73 (35H, m), 2.04 (2H, dt, J=7.1, 7.1 Hz), 2.59 (2H, brs) 3.66 (2H, t, J=5.6 Hz), 3.65–3.68 (1H, m), 4.12 (1H, dd, J=6.6, 2.7 Hz), 4.75 (1H, brs), 5.44 (1H, dd, J=15.4, 6.6 Hz) and 5.72 (1H, dt, J=15.4, 7.1 Hz). ^{13}C NMR ($CDCl_3$) δ 14.1, 22.7, 26.7, 28.3, 29.0, 29.2, 29.2, 29.3, 29.5, 29.6, 29.6, 31.9, 32.4, 55.2, 62.4, 75.3, 79.6, 128.2, 134.1 and 156.8. IR (KBr) 3342, 2920, 1684, 1531 and 1173 cm^{-1}. HRMS (FAB, positive), calcd for $C_{25}H_{50}NO_4$: $(M+H)^+$428.3740; found 428.3753.

A small portion of this compound **12** was converted to the corresponding bis (*R*)-MTPA ester **18**. To a solution of DCC (41.2 mg, 200 μmol), Mosher acid, (*R*)-MTPA, (32.8 mg, 140 μmol) and DMAP (8.6 mg, 35 μmol) in CH_2Cl_2 (0.5 mL) was added **10** (20 mg, 36 μmol) at room temperature. After stirring for 2 h, the reaction mixture was concentrated. The residue was purified by column chromatography on silica gel ($CHCl_3$) to give **12** (25 mg, 71%), as a colorless oil. ^{1}H NMR ($CDCl_3$) δ 0.88 (3H, t, J=6.8 Hz), 1.18–1.78 (35H, m), 1.96–2.03 (2H, m), 3.44, 3.55 (6H, s×2), 3.77–3.83 (1H, m), 4.22–4.37 (3H, m), 5.23 (1H, dd, J=15.1, 7.3 Hz), 5.42 (1H, d, J=3.9 Hz), 5.76 (1H, dt, J=15.1, 7.3 Hz) and 7.38–7.49 (10H, m). ^{13}C NMR ($CDCl_3$) δ 14.1, 22.7, 25.1, 26.3, 28.3, 28.7, 29.1, 29.3, 29.4, 29.6, 29.7, 31.9, 32.3, 52.7, 55.4, 65.7, 78.7, 79.8, 122.5, 124.7, 127.2, 127.5, 128.4, 129.6, 132.0, 132.2, 138.2, 155.3, 165.6 and 166.4.

By judging from the integrals of the C7 protons at δ 5.76 for **18a** (ca. 95%) and δ 5.87 for **18b** (ca. 5%), the e.e. of **12** was estimated at ca. 90%.

3.1.6. (4*S*,5*R*,6*E*)-4-Octadecanoylamino-eicos-6-en-1,5-diol (10). A solution of **7** (191 mg, 0.352 mmol) in 1,4-dioxane (3 mL) and 1 M aqueous HCl solution (2 mL) was stirred at 100°C for 30 min. After cooling to room temperature, 2 M aqueous NaOH solution (2 mL) was added and the mixture was extracted with Et_2O (2×50 mL). The combined Et_2O extract was washed with brine, dried over $MgSO_4$ and concentrated under reduced pressure. The residue was dissolved in THF (3 mL), to the solution was added sodium acetate (3 g) and subsequently under vigorous stirring, stearoyl chloride (110 mg, 0.363 mmol) in THF (1 mL) was added portionwise at room temperature. After stirring for 3 h, the mixture was dissolved in THF (100 mL). The solution was washed with brine, dried over $MgSO_4$ and concentrated under reduced pressure. The residue was purified by recrystallization to give **10** (130 mg, 62.2%), as a colorless crystal. Mp 120–122°C. $[\alpha]_D^{25}$=−13.1 [c 1.01, chloroform/methanol=9:1]. ^{1}H NMR ($CDCl_3$, 50°C) δ 0.88 (6H, t, J=6.8 Hz), 1.26–1.71 (56H, m), 2.04 (2H, dt, J=6.8, 6.8 Hz), 2.19 (2H, t, J=7.6 Hz), 2.49 (1H, brs) 3.66 (2H, t, J=6.0 Hz), 4.04 (1H, m), 4.14 (1H, m), 5.44 (1H, dd, J=15.2, 6.4 Hz), 5.73 (1H, dt, J=15.2, 6.8 Hz) and 5.75 (1H, brs). ^{13}C NMR ($CDCl_3$, 50°C) δ 14.0, 22.5, 25.8, 26.6, 29.0, 29.3, 29.5, 29.7, 29.9, 31.9, 32.4, 36.9, 54.3, 62.4, 75.3, 128.3, 134.0 and 174.4. IR: 3320, 2955, 1645, 1541 and 1466 cm^{-1}. HRMS (FAB, positive), calcd for $C_{38}H_{76}NO_3$: $(M+H)^+$594.5825; found 594.5833. Anal. calcd for $C_{38}H_{75}NO_3$; C, 76.84; H, 12.73; N, 2.36. Found; C, 76.51; H, 12.58; N, 2.44.

3.1.7. (4*S*,5*R*,6*E*)-4-Acetylamino-6-eicosen-1,5-diol (16). *N*-Acetylation was carried out according to the procedure described for **10**. A 60% yield of **16** was obtained.

Compound **16**. A colorless crystal. Mp 123–124°C, $[\alpha]_D^{25}$=−33.9 [c 1.04, chloroform/methanol=9:1]. ^{1}H NMR ($CDCl_3$, 50°C) δ 0.88 (3H, t, J=6.8 Hz), 1.26–1.71 (26H, m), 2.00 (3H, s), 2.04 (2H, dt, J=6.8, 6.8 Hz), 2.90 (2H, brs) 3.66 (2H, t, J=5.4 Hz), 3.99–4.05 (1H, m), 4.15 (1H, dd, J=6.4, 3.2 Hz), 5.45 (1H, dd, J=15.6, 6.4 Hz), 5.73 (1H, dt, J= 15.6, 7.4 Hz) and 5.86 (1H, d, J=7.8 Hz). ^{13}C NMR ($CDCl_3$, 50°C) δ 14.0, 22.6, 23.3, 26.4, 29.0, 29.2, 29.3, 29.5, 29.6, 29.7, 31.9, 32.3, 54.4, 62.5, 75.2, 128.3, 134.1 and 171.1. IR (KBr) 3277, 2918, 2851, 1647 and 1556 cm^{-1}. HRMS (FAB, positive), calcd for $C_{22}H_{44}NO_3$: $(M+H)^+$370.3321; found 370.3334. Anal. calcd for $C_{22}H_{43}NO_3$; C, 71.50; H, 11.73; N, 3.79. Found C, 71.26; H, 11.71; N, 3.60.

3.1.8. (4*S*,5*R*)-4-Octadecanoylamino-eicosan-1,5-diol (11) and (4*S*,5*R*)-4-acethylamino-eicosan-1,5-diol (17). *Compound* **15** was obtained from **8** by the DIBAL-H reduction and then the following deprotection of the silyl group of **9**.

Both **11** and **17** were prepared from **15** by the same procedure used to convert **10** to **12** with a 40 and 40% yield, respectively, after purification by recrystallization.

Compound **9**. ^{1}H NMR ($CDCl_3$) δ 0.05 (6H, s×2), 0.86–0.90 (12H, m), 1.26–1.63 (41H, m), 3.49–3.64 (3H, m), and 4.82 (1H, d, J=7.8 Hz). ^{13}C NMR ($CDCl_3$) δ −5.3, 14.1, 22.7, 25.7, 25.9, 26.1, 28.4, 29.2, 29.2, 29.3, 29.6, 29.7, 31.9, 62.9, 73.5, 74.7, 79.4 and 156.6. HRMS (FAB, positive), calcd for $C_{31}H_{66}NO_4Si$: $(M+H)^+$544.4761; found 544.4770.

Compound **15**. A colorless crystal. Mp 118–119°C. $[\alpha]_D^{25}$=−11.0 [*c* 0.955, chloroform]. ^{1}H NMR ($CDCl_3$) δ 0.88 (3H, t, J=6.8 Hz), 1.23–1.70 (41H, m), 2.12 (2H, brs), 3.60–3.68 (4H, m) and 4.78 (1H, brs). ^{13}C NMR ($CDCl_3$) δ 14.0, 22.6, 26.0, 26.1, 28.4, 29.2, 29.3, 29.7, 31.9, 33.5, 55.2, 62.6, 74.7, 79.6 and 156.5. IR (KBr) 3379, 3364, 2849, 1684 and 1529 cm^{-1}. HRMS (FAB, positive), calcd for $C_{25}H_{52}NO_4$: $(M+H)^+$430.3896; found 430.3902.

By judging from the integrals of the amide protons at δ 5.08 for **19a** (ca. >95%) and 5.16 for **19b** (ca. <5%), the e.e. of **15** was estimated at >90%. ^{1}H NMR ($CDCl_3$) δ 0.88 (3H, t, J=6.8 Hz), 1.11–1.66 (41H, m), 3.47, 3.53 (3H×2, s×2), 3.76–3.81 (1H, m), 4.21–4.36 (3H, m), 5.06–5.10 (1H, m) and 7.39–7.43, 7.49–7.53 (10H, m). ^{13}C NMR ($CDCl_3$) δ 14.1, 22.7, 25.1, 25.2, 25.6, 28.3, 29.3, 29.3, 29.4, 29.6, 29.6, 29.7, 30.1, 31.9, 51.9, 55.3, 55.4, 65.7, 78.7, 79.8, 127.2, 127.5, 128.4, 128.5, 129.6, 129.7, 132.0, 132.2, 155.3, 166.3 and 166.4.

Both **11** and **17** could not be measured by ^{1}H, ^{13}C NMR spectra, because the compounds were not easily soluble in solvents.

Compound **11**. colorless crystal. Mp 141–143°C. $[\alpha]_D^{25}$=−11.6 [*c* 0.50, chloroform/methanol=9:1]. IR (KBr) 3286, 2855, 2845, 1643 and 1547 cm^{-1}. HRMS (FAB, positive), calcd for $C_{38}H_{78}NO_3$: $(M+H)^+$596.5982; found 596.5974. Anal. calcd for $C_{38}H_{77}NO_3$; C, 76.58; H, 13.02; N, 2.35. Found C, 76.26; H, 12.77; N, 2.36.

Compound **17**. Colorless crystal. Mp 152–154°C. $[\alpha]_D^{25}$=−20.0 [*c* 1.00, chloroform/methanol=9:1]. IR (KBr) 3298, 2851, 1645, 1558 and 1468 cm^{-1}. HRMS (FAB, positive), calcd for $C_{22}H_{46}NO_3$: $(M+H)^+$372.3478; found 372.3490. Anal. calcd for $C_{22}H_{45}NO_3$; C, 71.11; H, 12.21; N, 3.77. Found C, 71.00; H, 12.32; N, 3.75.

3.1.9. (4*S*,5*R*,6*E*)-1-(*O*-Methanesulfonyl)-4-[(*tert*-butoxycarbonyl)amino]-eicos-6-en-1,5-diol (13). To a solution of **12** (400 mg, 0.940 mmol) in pyridine (10 mL) was added methanesulfonyl chloride (170 mg, 1.48 mmol) in pyridine (1 mL) at 0°C. After stirring for 30 min, the mixture was allowed to warm to room temperature and stirred for 30 min. The mixture was concentrated under reduced pressure at 40°C. The residue was dissolved with AcOEt. The solution was washed with 1 M aqueous HCl solution and brine before being dried over $MgSO_4$ and concentrated under reduced pressure. The residue was purified by column chromatography on silica gel (AcOEt/hexane=1:2) to give **13** (310 mg, 65.3%), as a colorless crystal. Mp 76–77°C. $[\alpha]_D^{25}$=−19.8 [*c* 0.79, chloroform]. ^{1}H NMR ($CDCl_3$) δ 0.88 (3H, t, J=6.8 Hz), 1.26–1.93 (35H, m), 2.04 (2H, dt, J=6.8, 6.8 Hz), 2.36 (1H, brs) 3.01 (3H, s), 3.66 (1H, m), 4.13 (1H, m), 4.25 (2H, t, J=6.3 Hz), 4.68 (1H, brs), 5.43 (1H, dd, J=15.6, 6.6 Hz) and 5.73 (1H, dt, J=15.6, 6.8 Hz). ^{13}C NMR ($CDCl_3$) δ 14.1, 22.7, 25.9, 28.3, 29.1, 29.2, 29.3, 29.4, 29.6, 31.9, 32.3, 37.3, 54.6, 69.8, 75.2, 79.7, 128.2, 134.4 and 156.4. IR (KBr) 3348, 2920, 1688, 1340 and 1171 cm^{-1}. HRMS (FAB, positive), calcd for $C_{26}H_{52}NO_6S$: $(M+H)^+$506.3515; found 506.3522.

3.1.10. (4*S*,5*R*,6*E*)-1-(*O*-Methanesulfonyl)-4,5-*O*,*N*-2-oxazolidone-eicos-6-en-1,5-diol (14). To a solution of **13** (200 mg, 0.395 mmol) in CH_2Cl_2 (5 mL) was added trifluoroacetic acid (1.00 mL, 13.0 mmol) at room temperature. After stirring for 30 min, the mixture was concentrated under reduced pressure. The residue was dissolved in THF (5 mL). The solution was cooled at 0°C. To the solution was added 1,1′-carbonyl-1*H*-diimidazole (192 mg, 0.988 mmol). After stirring for 5 min, the solution was allowed to warm to room temperature. After stirring for 8 h, the mixture was dissolved in Et_2O (30 mL). The solution was washed with 1 M aqueous HCl solution and brine before being dried over $MgSO_4$ and concentrated under reduced pressure. The residue was purified by column chromatography on silica gel (AcOEt/hexane=5:2) to give **14** (100 mg, 58.8%), as a colorless crystal. Mp 56–57°C. $[\alpha]_D^{25}$=−7.23 [*c* 1.03, chloroform]. ^{1}H NMR ($CDCl_3$) δ 0.88 (3H, t, J=6.6 Hz), 1.26–1.91 (26H, m), 2.10 (2H, dt, J=6.8, 6.8 Hz), 3.03 (3H, s), 3.86 (1H, m), 4.24 (2H, m), 5.03 (1H, dd, J=8.3, 8.3 Hz), 5.52 (1H, dd, J=15.6, 8.3 Hz), 5.88 (1H, dt, J=15.6, 6.8 Hz) and 7.18 (1H, brs). ^{13}C NMR ($CDCl_3$) δ 14.1, 22.6, 25.7, 27.4, 28.7, 29.1, 29.3, 29.4, 29.5, 29.6, 31.9, 32.2, 37.3, 55.5, 69.3, 81.0, 122.1, 138.8 and 159.9. IR (KBr) 3250, 2918, 1747, 1726 and 1356 cm^{-1}. HRMS (FAB, positive), calcd for $C_{22}H_{42}NO_5S$: $(M+H)^+$432.2784; found 432.2785.

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