Synthesis and Absolute Configuration of 6-Hydroxylated New Ceramides in Human Skin, Ceramides B, 4, 7 and 8[‡]

Yui Masuda[a] and Kenji Mori*[a]

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6*R*-Configuration was assigned to three new ceramides isolated from human skin such as ceramide B [**1**, (2*S*,3*R*,4*E*,6*R*)-6-hydroxy-*N*-(30′-hydroxytriacontanoyl)-4-sphingenine], ceramide 8 [**2**, (2*S*,3*R*,4*E*,6*R*)-6-hydroxy-*N*-(tetracosanoyl)-4-sphingenine] and ceramide 4 {**3**, (2*S*,3*R*,4*E*,6*R*)-6-hydroxy-*N*-[(30′-linoleoyloxy)triacontanoyl]-4-sphingenine}. (6*R*,2′*R*)-Configuration was given to another ceramide in human skin, ceramide 7 [**4**, (2*S*,3*R*,4*E*,6*R*,2′*R*)-6-hydroxy-*N*-(2′-hydroxytetracosanoyl)-4-sphingenine]. These assignments were made possible by enzymatic preparation of the enantiomers of 1-pentadecyn-3-ol and those of 2-hydroxytetracosanoic acid.

Introduction

Ceramides are predominant lipids of human epidermal stratum corneum, acting as the water barrier to prevent loss of body water.[1,2] They are classified into two groups, free ceramides and protein-bound ones.[3,4] In the former case, the acyl side-chain of ceramides do not possess an ω-hydroxy group, while in the latter it is present to enable the binding of ceramides with proteins. In 1994, Downing and co-workers reported the isolation and identification of several new ceramides with a hydroxy group at C-6 of its sphingosine part as shown in Figure 1.[3] Some of them are free ceramides such as ceramide 8 (**2**), ceramide 4 (**3**) and ceramide 7 (**4**), while ceramide B (**1**) is a protein-bound ceramide.[3,4] Downing assigned the structures **1**–**4** to these new 6-hydroxy-4-sphingenine-based ceramides by extensive ^{1}H NMR studies.

As to the stereochemistry of **1**–**4**, they almost certainly possess (2*S*,3*R*,4*E*)-configuration, since all the known mammalian sphingosines possess that configuration.[5] However, Downing was unable to determine the absolute configuration at C-6 of these 6-hydroxylated ceramides. This aroused interests among synthetic chemists, and three syntheses of 6-hydroxy-4-sphingenines were reported to date.[6–8] We were the first to determine the 6*R*-configuration of ceramide B as reported in our preliminary communication.[8] In the case of ceramide 7 (**4**), it possesses an additional hydroxy group at C-2′ of the acyl side-chain, whose absolute configuration must also be determined. Some marine sphingolipids are known to possess 2′*R*-hydroxy group in its acyl side-chain, as in the case of the sex pheromone [(2*S*,3*S*,4*R*,2′*R*)-2-(2′-hydroxy-21′-methyldocosanoylamino)-1,3,4-pentadecanetriol] of the female hair

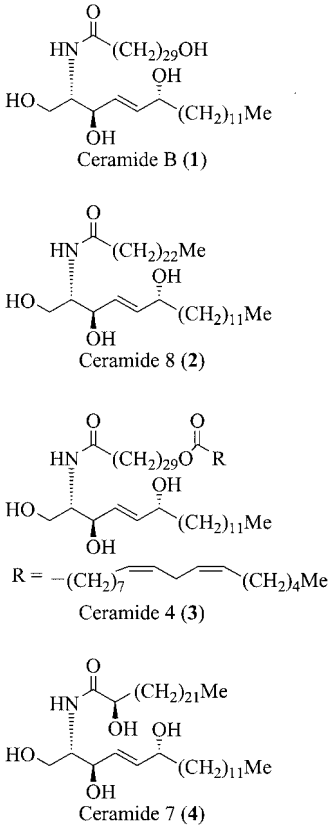

Figure 1. Structures of 6-hydroxylated new ceramides of human skin.

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[a] 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
Fax: +81-42-565-9906
E-mail: mori-riken@crocus.ocn.ne.jp

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crab, *Erimacrus isenbeckii*,[9,10] and also in the case of glycosphingolipids from marine sponge, *Agelas clathrodes*.[11] Nevertheless, the absolute configuration at C-2′ of ceramide 7 (**4**) must be elucidated beyond doubt. This paper describes our synthesis of **1**–**4**, which resulted in the determination of their stereochemistry as shown in Figure 1.

Results and Discussion

Retrosynthetic Analysis of the Ceramides 1–4

Because all of the target ceramides **1**–**4** are structurally related, our plan was to synthesize them according to the general retrosynthetic analysis as shown in Scheme 1. All the ceramides **1**–**4** can be prepared from the protected 6-hydroxy-4-sphingenine **A**, which is to be synthesized by treating Garner's aldehyde **B**[12–14] with either the dianion derived from acetylenic alcohol **C** or the anion derived from **D**. For the synthesis of ceramide 4 (**3**), the protected 6-hydroxy-4-sphingenine **A** is to be acylated with the activated ester **E**, which is a known compound prepared from **F**.[15] Acylation of **A** with **F** eventually gives ceramide B (**1**). For the synthesis of ceramide 7 (**4**), **A** must be acylated with (*R*)-2-acetoxy acid **G**, which is to be prepared from commercially available acid **H**. Tetracosanoic acid **H** and **A** yields ceramide 8 (**2**). It is therefore evident that the most important point in the present synthesis is how to prepare pure acetylenic alcohol **C** and its enantiomer.[8]

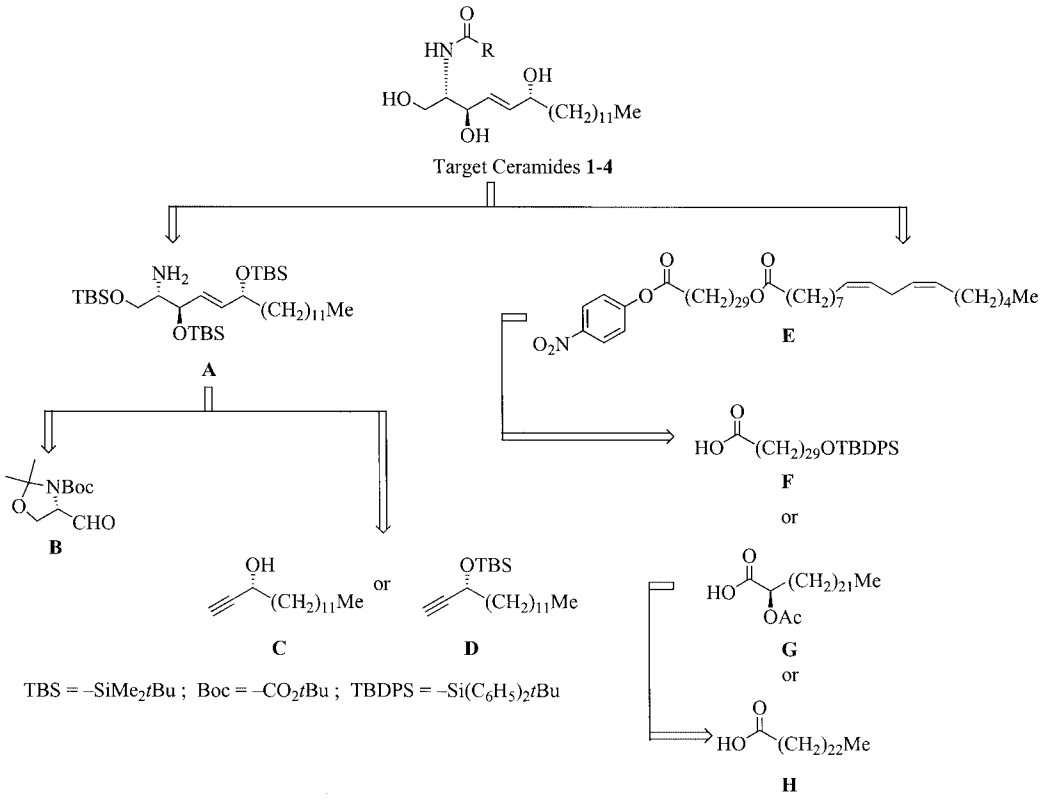
Synthesis of the Acid Parts E, F and G

As shown in Scheme 2, the known 30-*tert*-butyldiphenylsilyl(TBDPS)oxytriacontanoic acid (**8** = **F**) was synthesized from 15-pentadecanolide (**5**) by employing the Wittig reaction between **6** and **7** as the key step according to Mori and Matsuda.[15] Acid **8** was converted into *p*-nitrophenyl 30-linoleoyloxytriacontanoate (**9** = **E**) as reported previously.[15]

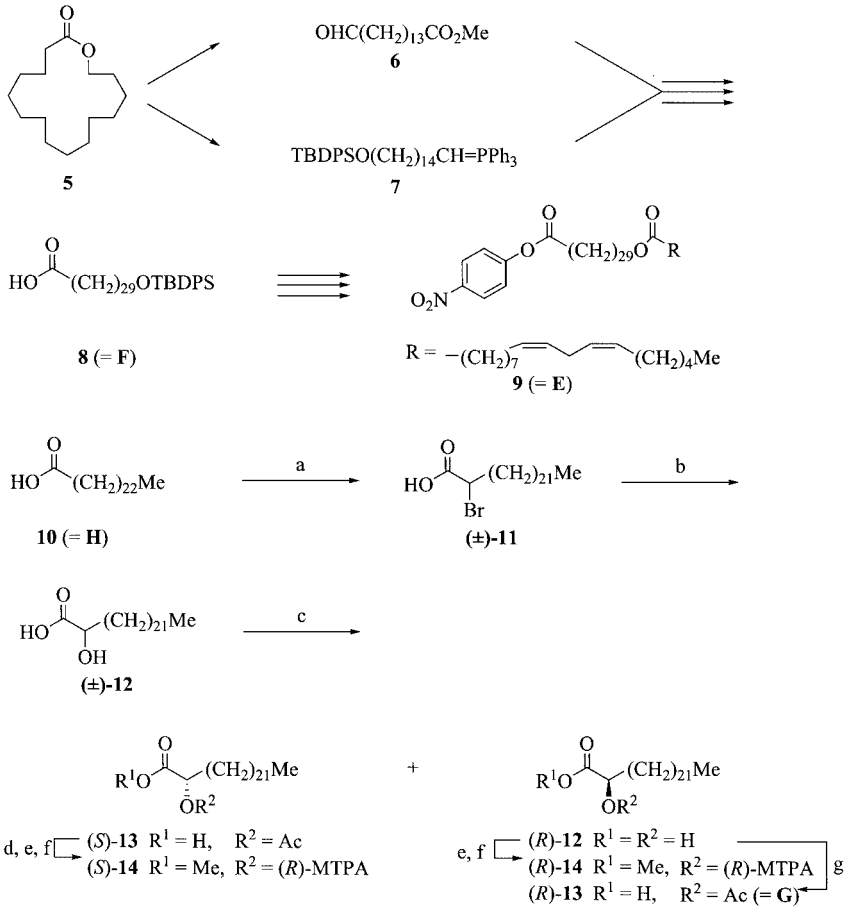
The first step of the synthesis of (*R*)-2-acetoxytetracosanoic acid (**13** = **G**) was bromination of tetracosanoic acid (**10**) with bromine in the presence of phosphorus. The resulting (±)-2-bromo acid **11** was treated with aqueous sodium hydroxide to give the (±)-2-hydroxy acid **12**. Its enzymatic kinetic resolution was achieved with lipase PS (Amano) and vinyl acetate at 65 °C in the presence of a small amount of butylated hydroxytoluene (BHT) according to Sugai and Ohta,[16][10] to give (*S*)-2-acetoxy acid **13** and the recovered (*R*)-2-hydroxy acid **12**. Their enantiomeric purities were determined by HPLC analysis of the corresponding (*R*)-2-methoxy-2-phenyl-2-(trifluoromethyl)acetates (Mosher esters), (*R*)- and (*S*)-**14**, and found to be 95% *ee* for both. The hydroxy acid (*R*)-**12**[17] was acetylated to give the (*R*)-2-acetoxy acid **13**.[17–19] Thus, lipase-catalyzed asymmetric acetylation proved to be a very practical method to make both the enantiomers of **13** available.

Synthesis of the Enantiomers of Acetylenic Alcohol 18 (= C)

Several asymmetric reactions were reported to be useful in preparing optically active 1-alkyn-3-ols. Asymmetric addition of a zinc acetylide to aldehydes in the presence of (+)-*N*-methylephedrine was recently reported by Carreira to



Scheme 1. Retrosynthetic analysis of the target ceramides **1**–**4**.

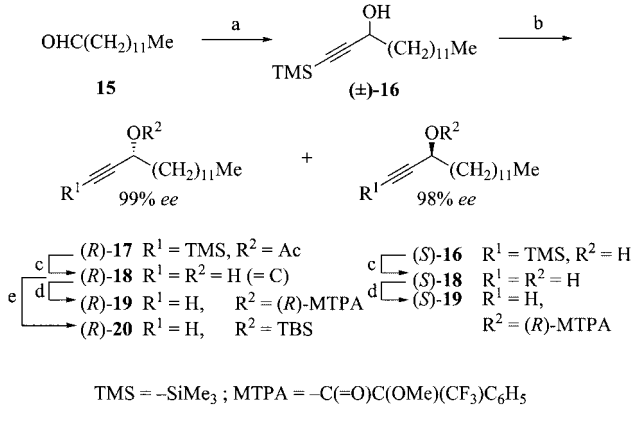


Scheme 2. Synthesis of the acid parts **8**, **9** and (*R*)-**13**; reagents (a) Br_2, P (77%); (b) NaOH, H_2O (97%); (c) lipase PS (Amano), CH_2=CHOAc, BHT, THF [38% for (*R*)-**12**, 38% for (*S*)-**13**]; (d) K_2CO_3, MeOH (quant.); (e) CH_2N_2, Et_2O; (f) (*S*)-MTPACl, C_5H_5N; (g) Ac_2O, C_5H_5N (99%).

give optically active 1-alkyn-3-ols (91-98% *ee*).[20] Simultaneously with our present work, asymmetric acetylation of (±)-1-alkyn-3-ols with vinyl acetate and lipases was found to give (*S*)-1-alkyn-3-ols and the corresponding (*R*)-acetates (ca. 90% *ee*).[21–23] It must be added that asymmetric hydrolysis of the acetates of (±)-1-alkyn-3-ols with *Bacillus subtilis* esterase gave optically active acetates and alcohols of only mediocre enantiomeric purity.[24]

Because substantical amounts of the enantiomers of 1-pentadecyn-3-ol (**C** = **18**) were necessary to synthesize **1**–**4**, we searched for a simple method which could afford both the enantiomers of **18** efficiently. Our choice was to adopt the method reported by Anastasia, that is, lipase-catalyzed enantiomer separtion of (±)-1-trimethylsilyl(TMS)-1-alkyn-3-ols.[25] By attaching a TMS group at C-1, the enantioselectivity of asymmetric acetylation with lipase could be improved to give long-chain 1-alkyn-3-ols of >90% *ee*. Indeed, lipase PS and vinyl acetate converted (±)-1-trimethylsilyl-1-alkyn-3-ols to (*R*)-acetates and (*S*)-alcohols in a single step without repeating the enzymatic kinetic resolution.[22]

Scheme 3 summarizes our results. Treatment of tridecanal (**15**) with lithium trimethylsilylacetylide in THF furnished the alkynol (±)-**16**. Asymmetric acetylation of (±)-**16** with vinyl acetate in the presence of lipase PS (Amano) on Celite in diisopropyl ether for 10 days at room temperature was followed by chromatographic purification to give the acetylated (*R*)-**17** [99% *ee* as determined by the HPLC analysis of the corresponding (*R*)-MTPA ester **19**] in 49%



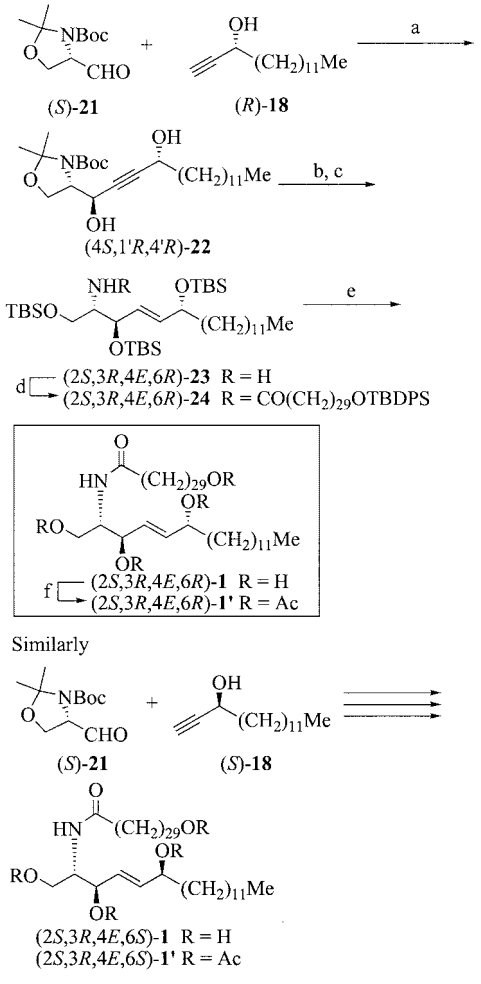
TMS = –$SiMe_3$; MTPA = –C(=O)C(OMe)(CF_3)C_6H_5

Scheme 3. Synthesis of the enantiomers of 1-pentadecyn-3-ol (**18**); reagents: (a) TMSC≡CH, *n*BuLi, THF (85%); (b) lipase PS-C, CH_2=CHOAc, $(iPr)_2O$ [49% for (*R*)-**17**, 48% for (*S*)-**16**]; (c) K_2CO_3, MeOH [92% for (*R*)-**18**, 95% for (*S*)-**18**]; (d) (*S*)-MTPACl, C_5H_5N; (e) TBSCl, imidazole, DMF (quant.).

yield based on (±)-**16**, and the recovered (*S*)-**16** [98% *ee* as determined by the HPLC analysis of the corresponding (*R*)-MTPA ester **19**] in 48% yield based on (±)-**16**. Both (*R*)-**17** and (*S*)-**16** were converted into the parent acetylenic alcohols, (*R*)-**18**, m.p. 40.5–42.0 °C, $[a]_D^{25}$ = +2.49 (*c* = 1.20, $CHCl_3$), and (*S*)-**18**, m.p. 41.0–42.0 °C, $[a]_D^{25}$ = –2.30 (*c* = 1.00, $CHCl_3$). These two enantiomers of **18** were silylated to the corresponding *tert*-butyldimethylsilyl (TBS) ethers (*R*)- and (*S*)-**20**. The overall yield of (*R*)- and (*S*)-**18** was ca. 38% based on **15** (3 steps).

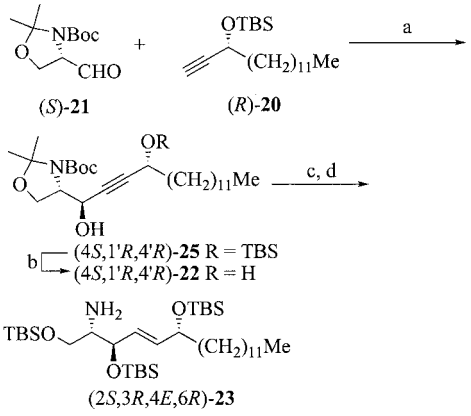
Synthesis of Ceramide B (1)

Scheme 4 illustrates further conversion of the acetylenic alcohol (*R*)-**18** to ceramide B (**1**). The alkynol (*R*)-**18** was treated with 2.2 equiv. of *n*-butyllithium in THF to afford the corresponding dianion, to which was added (*S*)-Garner's aldehyde (**21**) at –40 °C under argon. The product (4*S*,1′*R*,4′*R*)-**22** was rather unstable to preclude chromatographic purification on silica gel. It was therefore immediately treated with lithium in ethylamine to reduce the triple bond to an (*E*)-double bond, and also to remove the protective group, affording free 6-hydroxy-4-sphingenine. Protection of its hydroxy groups as TBS ethers gave (2*S*,3*R*,4*E*,6*R*)-**23**, which was acylated with carboxylic acid **8** in the presence of 3-(3-dimethylaminopropyl)-1-ethylcarbodiimide hydrochloride (EDC) and 1-hydroxybenzotriazole (HOBt) to give (2*S*,3*R*,4*E*,6*R*)-**24**. At this stage chromatographic purification was possible over silica gel to give pure protected ceramide (2*S*,3*R*,4*E*,6*R*)-**24** as the major product in 15% yield based on (*S*)-**21**. The diastereoisomeric ratio of the products was (2*S*,3*R*,4*E*,6*R*)-**24**/(2*S*,3*S*,4*E*,6*R*)-**24**, 4.2:1. Deprotection of the TBS protective groups of (2*S*,3*R*,4*E*,6*R*)-**24** with tetra(*n*-butyl)ammonium fluoride (TBAF) gave (2*S*,3*R*,4*E*,6*R*)-**1** as colorless powder, m.p. 113.5–117.0 °C. The overall yield of (2*S*,3*R*,4*E*,6*R*)-**1** was 8.3% based on (*S*)-Garner's aldehyde (**21**, 5 steps). Similarly, by employing (*S*)-alkynol **18**, (2*S*,3*R*,4*E*,6*S*)-**1** was also synthesized.



Scheme 4. Synthesis of ceramide B (**1**); ragents: (a) *n*BuLi, THF; (b) Li, $EtNH_2$, THF; (c) TBSOTf, 2,6-lutidine, CH_2Cl_2 (3 steps, 15%); (d) EDC, HOBt, **8**, CH_2Cl_2 (72%); (e) TBAF, THF (64%); (f) Ac_2O, C_5H_5N (85%).

In order to improve the yield and diastereoselectivity at the coupling step of (*S*)-**21** with alkynol (*R*)-**18**, coupling of (*S*)-**21** with the TBS-protected reactant (*R*)-**20** was attempted as shown in Scheme 5. The product (4*S*,1′*R*,4′*R*)-**25** was stable enough to be purified by silica gel chromatography, and obtained in 81% yield.



Scheme 5. Alternative synthesis of (2*S*,3*R*,4*E*,6*R*)-**23**; reagents: (a) *n*BuLi, THF, HMPA (81%); (b) TBAF, THF; (c) Li, $EtNH_2$, THF; (d) TBSOTf, 2,6-lutidine, CH_2Cl_2 (3 steps, 37%).

The diastereoselectivity at this coupling step was improved [(4*S*,1′*R*,4′*R*)-**25**/(4*S*,1′*S*,4′*R*)-**25**, 19:1]. The TBS protective group of (4*S*,1′*R*,4′*R*)-**25** was then removed with TBAF, and the resulting acetylenic diol (4*S*,1′*R*,4′*R*)-**22** was converted to (2*S*,3*R*,4*E*,6*R*)-**23** by reduction with lithium in ethylamine followed by silylation. The overall yield of **23** could not be improved so much, and remained as 23% in comparison to 15% in the previous case of employing the dianion (*R*)-**18**.

Fortunately, it was available to us the detailed 600 MHz ^{1}H NMR spectroscopic data including the spectral chart itself of ceramide B tetraacetate.[3] We measured the 500 MHz ^{1}H NMR spectra of our synthetic two diastereomers of ceramide B tetraacetate, (2*S*,3*R*,4*E*,6*R*)-**1′** and (2*S*,3*R*,4*E*,6*S*)-**1′**. As shown in Figure 2, the two spec-

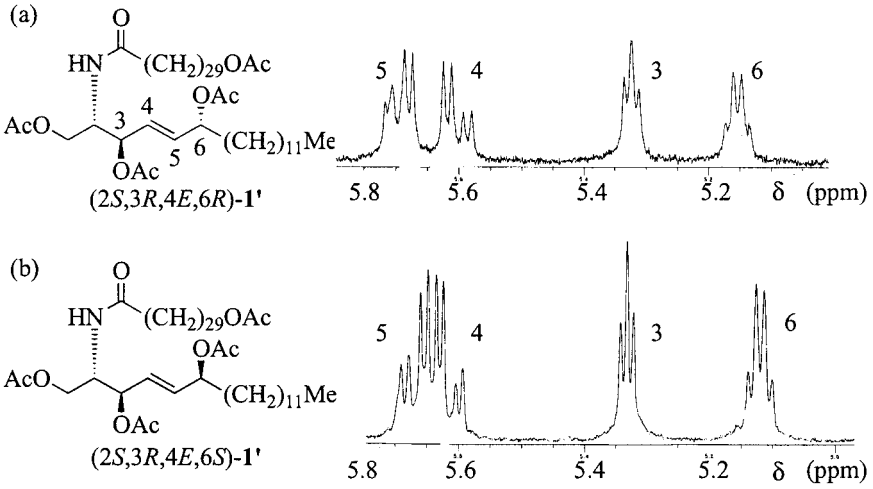


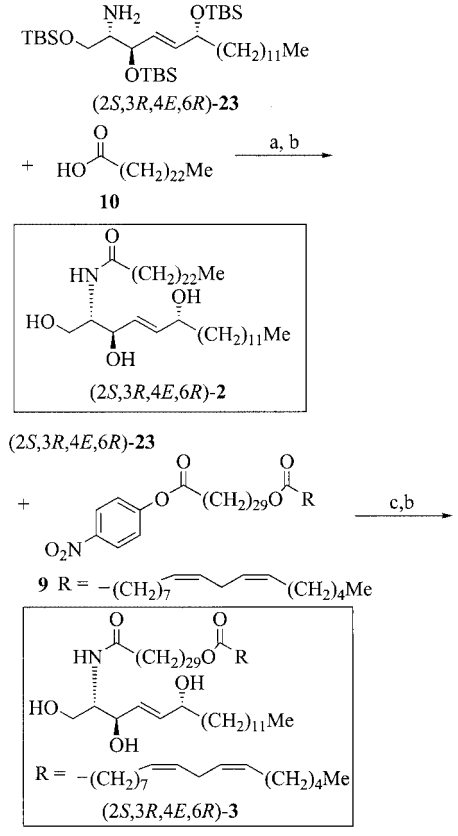
Figure 2. ^{1}H NMR spectra at 500 MHz of (a) (2*S*,3*R*,4*E*,6*R*)-**1′** and (b) (2*S*,3*R*,4*E*,6*S*)-**1′** as $CDCl_3$ solutions.

tra were clearly different with regard to the chemical shifts of the protons at C-4,5 and C-6, and the spectrum of (2*S*,3*R*,4*E*,6*R*)-**1′** was identical to that of ceramide B tetraacetate. The stereochemistry of ceramide B at C-6 must be *R*, assuming that it shares in common the (2*S*,3*R*,4*E*)-stereochemistry of the mammalian sphingolipids.

Synthesis of Ceramide 8 (2) and Ceramide 4 (3)

Acylation of the protected sphingosine (2*S*,3*R*,4*E*,6*R*)-**23** with tetracosanoic acid (**10**) under the conventional conditions was followed by deprotection to afford ceramide 8 [(2*S*,3*R*,4*E*,6*R*)-**2**] as shown in Scheme 6. The ^{1}H NMR spectroscopic data (500 MHz) of synthetic ceramide 8 triacetate was in good accord with those reported for the triacetate of the naturally occurring ceramide 8.[26]

For the synthesis of ceramide 4, the protected sphingosine (2*S*,3*R*,4*E*,6*R*)-**23** was acylated with the long-chain activated ester **9**. After deprotection, ceramide 4 [(2*S*,3*R*,4*E*,6*R*)-**3**] was obtained as a colorless solid. Its triacetate showed ^{1}H NMR spectroscopic data identical to those of the triacetate of the naturally occurring ceramide 4.[26] Accordingly, both ceramide 8 (**2**) and ceramide 4 (**3**) were shown to possess *R*-configuration at C-6, assuming their common (2*S*,3*R*,4*E*)-stereochemistry.



Scheme 6. Synthesis of ceramide 8 (**2**) and ceramide 4 (**3**): reagents: (a) EDC, HOBt, CH_2Cl_2 (96%); (b) TBAF, THF (71% for **2**; 73% for **3**); (c) C_5H_5N (76%).

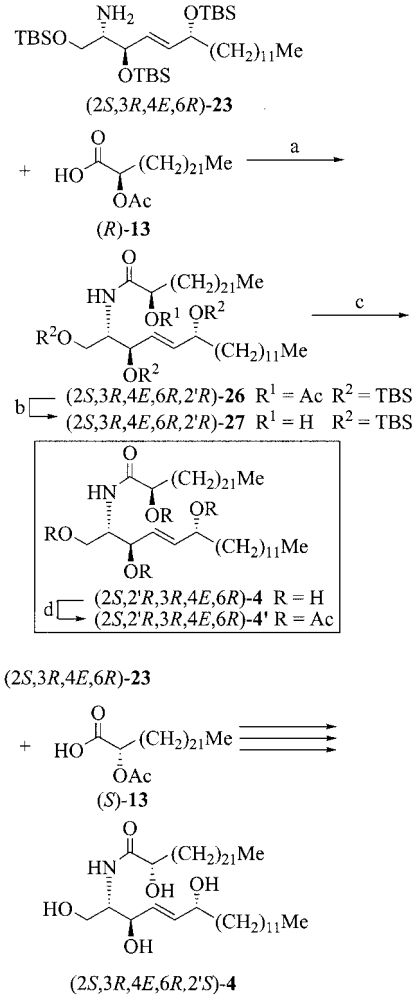
Synthesis of Ceramide 7 (4) and Its Diastereomer at C-2′

In the case of ceramide 7 (**4**), too, we assumed its common (2*S*,3*R*,4*E*,6*R*)-configuration, and planned to synthesize its two diastereomers at C-2′, (2*S*,3*R*,4*E*,6*R*,2′*R*)-**4** and (2*S*,3*R*,4*E*,6*R*,2′*S*)-**4**, one of which must be the natural product.

As shown in Scheme 7, acylation of the protected sphingosine (2*S*,3*R*,4*E*,6*R*)-**23** with (*R*)-2-acetoxy acid **13** yielded totally protected ceramide 7 (2*S*,3*R*,4*E*,6*R*,2′*R*)-**26**. Deacetylation followed by desilylation of **26** gave (2*S*,3*R*,4*E*,6*R*,2′*R*)-**4**, which was converted into the corresponding tetraacetate (2*S*,3*R*,4*E*,6*R*,2′*R*)-**4′**.

Similarly, acylation of **23** with (*S*)-**13** gave the protected ceramide 7 (2*S*,3*R*,4*E*,6*R*,2′*S*)-**26**, which was deprotected to give (2*S*,3*R*,4*E*,6*R*,2′*S*)-**4**. The corresponding tetraacetate (2*S*,3*R*,4*E*,6*R*,2′*S*)-**4′** was prepared, and the two tetraacetates were compared by ^{1}H NMR analysis.

Figure 3 shows ^{1}H NMR spectra (500 MHz) of the two diastereomers of **4′**. They are definitely different from each other, especially with regard to the signals due to two pro-



Scheme 7. Synthesis of ceramide 7 (**4**); reagents: (a) EDC, HOBt, CH_2Cl_2 (73%); (b) K_2CO_3, MeOH (78%); (c) TBAF, THF (75%); (d) Ac_2O, C_5H_5N (92%).

tons at C-1. The ^1H NMR spectrum (600 MHz) of naturally occurring ceramide tetraacetate[3] was identical with that of (2*S*,3*R*,4*E*,6*R*,2′*R*)-**4′**. Accordingly, the stereochemistry of ceramide 7 (**4**) was determined as 2*S*,3*R*,4*E*,6*R*,2′*R*.

It must be noted that the *R*-configuration at C-2′ of ceramide 7 is same as that of the known marine sphingolipids.[10,11]

Conclusions

Synthesis of all of the four 6-hydroxylated ceramides in human skin was accomplished, and their hitherto unknown stereochemistries at C-6 and C-2′ were determined as shown in Figure 1, with 6*R* [ceramide B (**1**), ceramide 8 (**2**), and ceramide 4 (**3**)] and 6*R*,2′*R* [ceramide 7 (**4**)] configuration.

The overall yield of ceramides based on tridecanal (**15**) were 5.4% (10 steps) for ceramide B (**1**), 33% (6 steps) for ceramide 8 (**2**), 27% (6 steps) for ceramide 4 (**3**) and 5.0% (11 steps) for ceramide 7 (**4**). The overall yield of ceramide 7 (**4**) was 12.1% (7 steps) based on tetracosanoic acid (**10**). Enzymatic asymmetric acetylation of 1-trimethylsilyl-1-alkyn-3-ol was demonstrated to be a useful method for the preparation of the enantiomers of 1-alkyn-3-ol.

Experimental Section

Melting point (Yanaco MP-S3) are uncorrected. IR spectra were measured with a Jasco FT/IR-460 spectrometer. ^1H NMR spectra were recorded at 90 MHz by a Jeol JNM-EX 90A spectrometer, at 400 MHz by a Jeol JNM-LA400 spectrometer, and at 500 MHz by a Jeol JNM-LA500 spectrometer. The peak for TMS, or $CHCl_3$ in $CDCl_3$ (at δ = 7.26 ppm), was used as the internal standard. ^{13}C NMR spectra were recorded at 100 MHz by a Jeol JNM-LA400 spectrometer and at 126 MHz by a Jeol JNM-LA500 spectrometer. The peak for $CDCl_3$ (at δ = 77.0 ppm) was used as the internal

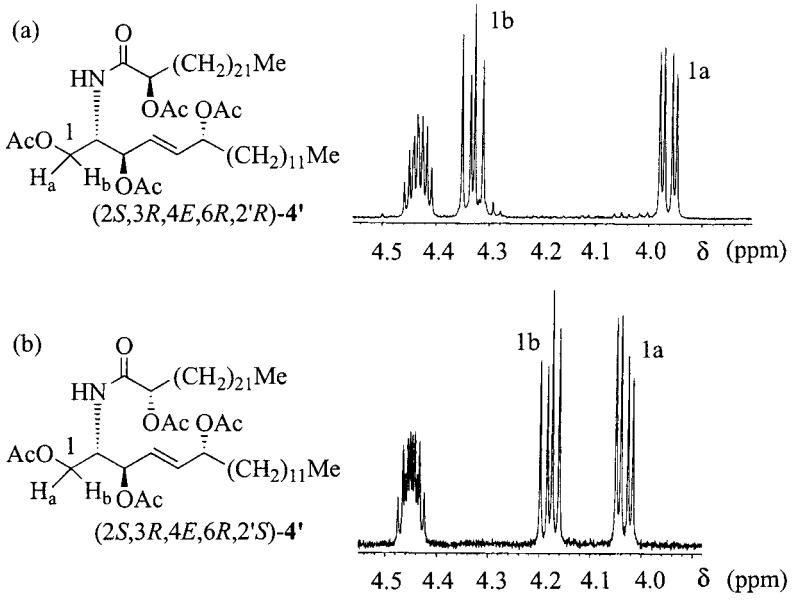


Figure 3. ^1H NMR spectra at 500 MHz of (a) (2*S*,3*R*,4*E*,6*R*,2′*R*)-**4′** and (b) (2*S*,3*R*,4*E*,6*R*,2′*S*)-**4′** as $CDCl_3$ solutions.

standard. Optical rotation values were measured with a Jasco P-1010 polarimeter, and mass spectra were measured with a Jeol JMS-SX102A spectrometer. Column chromatography was carried out on Merck Kieselgel 60 Art 1.07734, and TLC analyses were performed on Merck 60F–254 silica gel plates.

(±)-2-Bromotetracosanoic Acid [(±)-11]: Red phosphorus (1.24 g, 40 mmol) was added to tetracosanoic acid (**10**) (11.4 g, 31 mmol), and the mixture was heated at 95 °C. Bromine (19.7 g, 6.4 mL, 124 mmol) was added dropwise to the heated mixture, and heating and stirring were continued at 95 °C for 6 h. Water was then added, and the mixture was stirred for 20 min. The separated acid was extracted with diethyl ether, and the extract was successively washed with water and brine, dried with $MgSO_4$, and concentrated under reduced pressure. The residue was recrystallized from hexane to give (±)-**11** (10.7 g, 77%) as a colorless solid, m.p. 71.0–74.0 °C. IR (KBr): $\tilde{\nu}$ = 3000 cm^{-1} (br., O–H), 1700 (s, C=O), 900 (m), 660 (w) cm^{-1}. ^1H NMR (90 MHz, $CDCl_3$): δ = 0.68–1.10 (m, 3 H, 24-H_3), 1.11–1.70 (m, 41 H, 4–23-H_2, CO_2H), 1.81–2.21 (m, 2 H, 3-H_2), 4.24 (t, J = 7.3 Hz, 1 H, 2-H) ppm. $C_{24}H_{47}BrO_2$ (447.5): calcd. C 64.41, H 10.59; found C 64.18, H 10.69.

(±)-2-Hydroxytetracosanoic Acid [(±)-12]: 2 M NaOH aq. (200 mL) was added to (±)-**11** (19.0 g, 42.3 mmol), and the mixture was stirred and heated at 85 °C for 30 h. After cooling, the mixture was acidified with diluted aq. HCl and extracted with diethyl ether. The extract was successively washed with water and brine, dried with $MgSO_4$, and concentrated under reduced pressure. The residue was recrystallized from acetone to give (±)-**12** (15.7 g, 97%) as a colorless solid, m.p. 100.0–103.0 °C. IR (KBr): $\tilde{\nu}$ = 3445 cm^{-1} (br. m, O–H), 1745 (m, C=O) cm^{-1}. ^1H NMR (90 MHz, $CDCl_3/CD_3OD$, 1:1): δ = 0.58–0.75 (m, 3 H, 24-H_3), 1.04–1.24 (m, 42 H, 4–23-H_2, OH, CO_2H), 1.26–1.44 (m, 2 H, 3-H_2), 3.92 (t, J = 5.8 Hz, 1 H, 2-H) ppm. $C_{24}H_{48}O_3$ (384.6): calcd. C 74.94, H 12.58; found C 74.76, H 12.32.

(*R*)-2-Hydroxytetracosanoic Acid [(*R*)-12]: Lipase PS (Amano Enzyme, Inc.; 4.54 g) was suspended in a solution of (±)-**12** (5.00 g, 13.0 mmol), and BHT (butylated hydroxytoluene, 200 mg) in vinyl acetate (48 mL) and THF (48 mL), and the mixture was stirred at 65 °C for 30 h. After cooling, the mixture was filtered through Celite, and the Celite layer was washed with diethyl ether. The combined filtrate and washings were concentrated under reduced pressure, and the residue was dissolved in vinyl acetate (48 mL) and THF (48 mL) containing BHT (200 mg). Lipase PS (4.54 g) was added to the solution, and the mixture was stirred at 65 °C for 3 h. After filtration and concentration, the residue was recrystallized from acetone to give (*R*)-**12** (950 mg, 38%) as colorless powder, m.p. 101.0–102.0 °C. $[\alpha]_D^{23}$ = –2.7 (c = 0.1, $CHCl_3$/MeOH, 1:1). ref[17]: m.p. 104.5–105.5 °C, $[\alpha]_D^{25}$ = +3.4 (c = 1.6, pyridine). Its IR and ^1H NMR spectra were identical with those of (±)-**12**. $C_{24}H_{48}O_3$ (384.6): calcd. C 74.94, H 12.58; found C 74.74, H 12.65.

Determination of the Enantiomeric Purity of (*R*)-12: (*S*)-2-Methoxy-2-phenyl-2-(trifluoromethyl)acetyl chloride (MTPACl; 50 mg, 0.2 mmol) was added to a solution of the methyl ester derived from (*R*)-**12** [50 mg, 0.13 mmol; prepared by treating (*R*)-**12** with ethereal diazomethane] in dry pyridine (2 mL), and the mixture was stirred at 0 °C for 12 h. The reaction was quenched by adding water, and the mixture was diluted with diethyl ether. The extract was successively washed with saturated aq. $CuSO_4$, saturated aq. $NaHCO_3$, water and brine, dried with Na_2SO_4, and concentrated under reduced pressure. The residue was analyzed by HPLC (Pegasil-Senshu column, 25 cm × 4.6 mm; eluent *n*-hexane/ethyl acetate, 60:1; flow rate, 1 mL/min). (*R*)-MTPA ester of (*R*)-**12** methyl ester: t_R = 15.64 min (97.5%); (*R*)-MTPA ester of (*S*)-**12** methyl ester: t_R = 19.64 min (2.5%). The enantiomeric purity of (*R*)-**12** was therefore 95% *ee*.

(*S*)-2-Acetoxytetracosanoic Acid [(*S*)-13]: Lipase PS (960 mg) was suspended in a solution of (±)-**12** (1.00 g, 2.6 mmol), and BHT (9 mg) in vinyl acetate (10 mL) and THF (10 mL), and the mixture was stirred at 65 °C for 20 h. After filtration and concentration, the residue was chromatographed on silica gel (20 g, $CHCl_3$/MeOH, 30:1) to give (*S*)-**13** (422 mg, 38%) as colorless powder, m.p. 72.5–73.5 °C. $[\alpha]_D^{23}$ = –8.9 (c = 1.0, $CHCl_3$). ref.[18]: m.p. 81–82 °C, $[\alpha]_D^{20}$ = –7.65 (c = 0.98, $CHCl_3$). IR (KBr): $\tilde{\nu}$ = 3185 cm^{-1} (br. m, CO_2H), 1745 (m, C=O), 1690 (m, CO_2H), 1240 (m, C–OAc) cm^{-1}. ^1H NMR (500 MHz, $CDCl_3$): δ = 0.88 (t, J = 7.0 Hz, 3 H, 24-H_3), 1.10–1.31 (m, 41 H, 4–23-H_2, CO_2H), 1.34–1.45 (m, 2 H, 3-H_2), 2.17 (s, 3 H, OAc), 5.00–5.06 (m, 1 H, 2-H) ppm. HRMS: m/z $[M + H]^+$: calcd. for $C_{26}H_{51}O_4$, 427.3787; found, 427.3785.

Determination of the Enantiomeric Purity of (*S*)-13: (*S*)-2-Methoxy-2-phenyl-2-(trifluoromethyl)acetyl chloride (MTPACl; 50 mg, 0.2 mmol) was added to a solution of the hydroxylated methyl ester derived from (*S*)-**13** [50 mg, 0.13 mmol; prepared by treating (*S*)-**13** with diazomethane after deacetylation] in dry pyridine (2 mL), and the mixture was stirred at 0 °C for 12 h. The reaction was quenched by adding water, and the mixture was diluted with diethyl ether. The extract was successively washed with saturated aq. $CuSO_4$, saturated aq. $NaHCO_3$, water and brine, dried with Na_2SO_4, and concentrated under reduced pressure. The residue was analyzed by HPLC (Pegasil-Senshu column, 25 cm × 4.6 mm; eluent *n*-hexane/ethyl acetate, 60:1; flow rate, 1 mL /min). (*R*)-MTPA ester of the (*R*)-**13** methyl ester: t_R = 15.78 min (2.5%); (*R*)-MTPA ester of the (*S*)-**13** methyl ester: t_R = 19.82 min (97.5%). The enantiomeric purity of (*S*)-**13** was therefore 95% *ee*

(*R*)-2-Acetoxytetracosanoic Acid [(*R*)-13]: To a stirred solution of (*R*)-**12** (250 mg, 0.64 mmol) in dry pyridine (10 mL) was added Ac_2O (20 mL). The reaction mixture was stirred for 4 h at room temperature. It was then poured into water and extracted with ethyl acetate. The extract was successively washed with water and brine, dried with $MgSO_4$, and concentrated under reduced pressure. The residue was chromatographed on silica gel (5 g, $CHCl_3$/MeOH, 30:1) to give (*R*)-**13** as colorless powder, m.p. 72.5–75.5 °C. $[\alpha]_D^{23}$ = +8.8 (c = 1.0, $CHCl_3$). ref.[17]: m.p. 65–67 °C, $[\alpha]_D^{26}$ = +2.6 (c = 1.0, $CHCl_3$); ref.[18] m.p. 81.5–82.5 °C, $[\alpha]_D^{20}$ = –8.46 (c = 1.01, $CHCl_3$). This negative $[\alpha]_D$ value must be in error, because the same sample was reported to be: $[\alpha]_D^{20}$ = +8.5 (c = 1.0, $CHCl_3$).[19] Sugai and Ohta reported the specific rotation of (*S*)-**13** as: $[\alpha]_D^{20}$ = –7.65 (c = 0.98, $CHCl_3$).[18] Its IR and ^1H NMR spectra were identical with those of (*S*)-**13**. HRMS: m/z $[M + H]^+$: calcd. for $C_{26}H_{51}O_4$, 427.3787; found, 427.3790.

(±)-1-Trimethylsilyl-1-pentadecyn-3-ol [(±)-16]: To a stirred solution of trimethylsilylacetylene (14.9 g, 151 mmol) in dry THF (500 mL) was added *n*-butyllithium (1.59 M in hexane; 100 mL, 159 mmol) dropwise at –78 °C under Ar. After the reaction mixture had been stirred at –78 °C for 1 h, a solution of tridecanal (**15**) (20 g, 101 mmol) in dry THF (500 mL) was added dropwise at –78 °C. The mixture was then stirred for 5 h while being warmed to room temperature, before being quenched with NH_4Cl aq. and extracted with ethyl acetate. The extract was successively washed with water and brine, dried with $MgSO_4$, and concentrated under reduced pressure. The residue was chromatographed on silica gel (400 g, hexane/ethyl acetate, 30:1) to give (±)-**16** (25.4 g, 85%) as a colorless oil, n_D^{26} = 1.4546. IR (film): $\tilde{\nu}$ = 3315 cm^{-1} (w, OH), 2170 (w, C≡C), 1250 (s, Si–Me) cm^{-1}. ^1H NMR (90 MHz, $CDCl_3$): δ = 0.17 (m, 9 H, Si–Me), 0.88 (m, 3 H, 15-H_3), 1.26–1.41 (m, 22 H,

4–14-H_2), 1.72 (d, J = 5.5 Hz, 1 H, OH), 4.35 (dt, J = 5.5, 6.4 Hz, 1 H, 3-H) ppm. $C_{18}H_{36}OSi$ (296.6): calcd. C 72.90, H 12.24; found C 72.63, H 12.30.

(*S*)-1-Trimethylsilyl-1-pentadecyn-3-ol [(*S*)-16] and (*R*)-3-Acetoxy-1-trimethylsilyl-1-pentadecyne [(*R*)-17]: Lipase PS-C (Amano Enzyme, Inc.; 12.2 g) was suspended in a solution of (±)-**16** (14.5 g, 49.0 mmol) in vinyl acetate (290 mL) and (iPr)$_2$O (580 mL), and the mixture stirred at room temperature for 10 d. The mixture was filtered through Celite, and the Celite layer was washed with diethyl ether. The combined filtrate and washings were concentrated under reduced pressure. The residue was chromatographed on silica gel (900 g, hexane/ethyl acetate, 60:1) to give (*S*)-**16** (7.27 g, 48%) and (*R*)-**17** (8.12 g, 49%) as a colorless oil.

(*S*)-16: n_D^{23} = 1.4576. $[\alpha]_D^{22}$ = +1.1 (c = 1.10, $CHCl_3$). Its IR and ^{1}H NMR spectra were identical with those of (±)-**16**. $C_{18}H_{36}OSi$ (296.6): calcd. C 72.90, H 12.24; found C 73.15, H 12.52.

(*R*)-17: n_D^{25} = 1.4486. $[\alpha]_D^{25}$ = +65.4 (c = 1.05, $CHCl_3$). IR (film): $\tilde{\nu}$ = 2180 cm^{-1} (w, C≡C), 1750 (s, C=O) cm^{-1}. ^{1}H NMR (90 MHz, $CDCl_3$): δ = 0.17 (m, 9 H, Si–Me), 0.80 (m, 3 H, 15-H_3), 1.27–1.45 (m, 22 H, 4–14-H_2), 2.08 (s, 3 H, OAc), 5.37 (t, J = 6.7 Hz, 1 H, 3-H) ppm. $C_{20}H_{38}O_2Si$ (338.6): calcd. C 70.94, H 11.31; found C 70.99, H 11.56.

(*R*)-1-Pentadecyn-3-ol [(*R*)-18]: To a stirred solution of (*R*)-**17** (13.7 g, 40.4 mmol) in MeOH (175 mL) was added K_2CO_3 (16.4 g, 119 mmol) at room temperature. The mixture was stirred for 4 h at room temperature, before the solvent was removed under reduced pressure. The concentrate was diluted with water and extracted with diethyl ether. The resulting extract was successively washed with water, satd. $NaHCO_3$ aq. and brine, dried with $MgSO_4$, and concentrated under reduced pressure. The residue was chromatographed on silica gel (300 g, hexane/ethyl acetate, 30:1) to give (*R*)-**18** (8.33 g, 92%) as a colorless solid, m.p. 40.5–42.0 °C. $[\alpha]_D^{24}$ = +2.49 (c = 1.10, $CHCl_3$). ref.[6]: m.p. 40–41 °C, $[\alpha]_D^{25}$ = +2.60 (c = 1.0, $CHCl_3$); ref.[7]: m.p. not reported, $[\alpha]_D$ = +1.3 (c = 1.05, $CHCl_3$). IR (film): $\tilde{\nu}$ = 3335 cm^{-1} (m, O–H), 3280 (m, HC≡C–), 2115 (w, HC≡C–) cm^{-1}. ^{1}H NMR (90 MHz, $CDCl_3$): δ = 0.78–0.95 (m, 3 H, 15-H_3), 1.08–1.81 (m, 23 H, 4–14-H_2, OH), 2.46 (d, J = 2.2 Hz, 1 H, 1-H), 4.25–4.40 (dt, J = 2.2, 6.4 Hz, 1 H, 3-H) ppm. $C_{15}H_{28}O$ (224.4): calcd. C 80.29, H 12.58; found C 80.06, H 12.85.

(*S*)-1-Pentadecyn-3-ol [(*S*)-18]: In the same manner as that just described, (*S*)-**16** (13.2 g, 44.4 mmol) was converted into 9.46 g (95%) of (*S*)-**18** as a colorless solid, m.p. 42.0–43.0 °C. $[\alpha]_D^{25}$ = –2.30 (c = 1.00, $CHCl_3$). ref.[6]: m.p. 40–41 °C. $[\alpha]_D^{25}$ = –2.59 (c = 1.00, $CHCl_3$); ref.[7]: m.p. 36–36.5 °C. $[\alpha]_D$ = –1.8 (c = 1.6, $CHCl_3$) Its IR and ^{1}H NMR spectra were identical with those of (*R*)-**18**. $C_{15}H_{28}O$ (224.4): calcd. C 80.29, H 12.58; found C 80.11, H 12.86.

Determination of the Enantiomeric Purity of (*R*)-18 and (*S*)-18: (*S*)-2-Methoxy-2-phenyl-2-(trifluoromethyl)acetyl chloride (MTPACl; 25 mg, 0.1 mmol) was added to a solution of (*R*)-**18** (5 mg, 0.02 mmol) in dry pyridine (2 mL), and the mixture was stirred at 0 °C for 12 h. The reaction was quenched by adding water, and the mixture was diluted with diethyl ether. The extract was successively washed with saturated aq. $CuSO_4$, saturated aq. $NaHCO_3$, water and brine, dried with Na_2SO_4, and concentrated under reduced pressure. The residue was analyzed by HPLC (Pegasil-Senshu column, 25 cm × 4.6 mm; eluent n-hexane/ethyl acetate, 60:1; flow rate, 1 mL /min). (*R*)-MTPA ester of (*R*)-**18**: t_R = 9.78 min (99.5%); (*R*)-MTPA ester of (*S*)-**18**: t_R = 10.96 min (0.5%). The enantiomeric purity of (*R*)-**18** was therefore 99% *ee*. In the same manner as that just described, (*S*)-**18** was converted into its (*R*)-MTPA derivative, and analyzed under the same conditions. (*R*)-MTPA ester of (*R*)-**18**: t_R = 9.77 min (1.0%); (*R*)-MTPA ester of (*S*)-**18**: t_R = 10.94 min (99.0%). The enantiomeric purity of (*S*)-**18** was therefore 98% *ee*

(*R*)-3-*tert*-Butyldimethylsilyloxy-1-pentadecyne [(*R*)-20]: To a stirred solution of (*R*)-**18** (2.0 g, 8.91 mmol) in DMF (50 mL) was added imidazole (1.29 g, 18.7 mmol) and TBSCl (1.47 g, 9.74 mmol). The reaction mixture was stirred for 3 h at room temperature. It was then poured into water and extracted with diethyl ether. The extract was successively washed with water, diluted HCl aq. and brine, dried with $MgSO_4$, and concentrated under reduced pressure. The residue was chromatographed on silica gel (60 g, hexane) to give (*R*)-**20** (3.0 g, quant.) as a colorless oil, n_D^{22} = 1.4442. $[\alpha]_D^{23}$ = +32.3 (c = 1.0, $CHCl_3$). IR (film): $\tilde{\nu}$ 3310 (m, HC≡C–), 2115 (w, HC≡C–), 1250 (s, Si–Me) cm^{-1}. ^{1}H NMR (500 MHz, $CDCl_3$): δ = 0.11, 0.13 (each s, 6 H, Si–Me), 0.86 (t, J = 6.9 Hz, 3 H, 15-H_3), 0.91 (s, 9 H, tBu), 1.10–1.27 (m, 20 H, 5–14-H_2), 1.63–1.70 (m, 2 H, 4-H_2), 2.37 (d, J = 2.1 Hz, 1 H, 1-H), 4.33 (dt, J = 2.1, 6.6 Hz, 1 H, 3-H) ppm. $C_{21}H_{42}OSi$ (338.6): calcd. C 74.48, H 12.50; found C 74.48, H 12.78.

***tert*-Butyl (4*S*,1′*R*,4′*R*)-4-(1′,4′-Dihydroxy-2′-hexadecynyl)-2,2-dimethyl-3-oxazolidinecarboxylate [(4*S*,1′*R*,4′*R*)-22]:** To a stirred solution of (*R*)-**18** (1.00 g, 4.45 mmol) in dry THF (40 mL) was added n-butyllithium (1.56 M in hexane; 6.28 mL, 9.8 mmol) dropwise at –78 °C under Ar. After the reaction mixture had been stirred at –78 °C for 1.5 h, a solution of Garner's aldehyde [(*S*)-**21**, 1.53 g, 6.68 mmol] in dry THF (40 mL) was added dropwise at –78 °C. The mixture was then stirred overnight while being warmed to 0 °C, before being quenched with NH_4Cl aq. and extracted with diethyl ether. The extract was successively washed with water and brine, dried with $MgSO_4$, and concentrated under reduced pressure to give (4*S*,1′*R*,4′*R*)-**22** (2.52 g, crude) as a yellow oil. Because of its instability, this compound was used for the next reaction without purification.

***tert*-Butyl (4*S*,1′*R*,4′*S*)-4-(1′,4′-Dihydroxy-2′-hexadecynyl)-2,2-dimethyl-3-oxazolidinecarboxylate [(4*S*,1′*R*,4′*S*)-22]:** In the same manner as that just described, (*S*)-**18** (1.00 g, 4.45 mmol) and Garner's aldehyde [(*S*)-**21**, 1.53 g, 6.68 mmol] were converted into 2.01 g of (4*S*,1′*R*,4′*S*)-**22** (2.78 g) as a yellow oil. Because of its instability, this compound was used for the next reaction without purification.

(2*S*,3*R*,4*E*,6*R*)-2-Amino-1,3,6-tris(*tert*-butyldimethylsilyloxy)-4-octadecene [(2*S*,3*R*,4*E*,6*R*)-23]: A solution of crude (4*S*,1′*R*,4′*R*)-**22** (2.62 g) in dry THF (40 mL) was added dropwise to a blue solution of lithium (1.00 g, 148 mmol) in ethylamine (30 g) while stirring at –78 °C for 1 h. The mixture was then stirred overnight while being warmed to room temperature, before being quenched with NH_4Cl (ca. 16 g, 295 mmol). After removing ethylamine by evaporation, the mixture was diluted with saturated aq. NH_4Cl and extracted with $CHCl_3$. The extract was dried with Na_2SO_4, and concentrated under reduced pressure to give the reduction product (2.2 g) as a brown waxy solid. This reduction product was used for the next reaction without purification. To a stirred solution of the reduction product (2.2 g) in dry CH_2Cl_2 (30 mL) were added 2,6-lutidine (2,6-dimethylpyridine; 5.8 mL, 50 mmol) and TBSOTf (13.5 mL, 60 mmol) at 0 °C. After having been stirred at room temperature for 1 h, the resulting solution was quenched with MeOH. It was then poured into water and extracted with diethyl ether. The extract was successively washed with water, saturated aq. $NaHCO_3$ and brine, dried with $MgSO_4$, and concentrated under reduced pressure. The residue was chromatographed on silica gel (40 g, hexane/ethyl acetate, 50:1) to give (2*S*,3*R*,4*E*,6*R*)-**23** (420 mg, 3 steps, 15%) as a pale yellow oil, n_D^{24} = 1.4572. $[\alpha]_D^{24}$ = +0.8 (c = 1.0,

$CHCl_3$). IR (film): $\tilde{\nu}$ = 3395 cm^{-1} (w, N–H), 1255 (m, Si–Me) cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$): δ = 0.01, 0.02, 0.045, 0.051 (each s, 18 H, Si–Me), 0.86–0.91 (m, 30 H, *t*Bu, 18-H_3), 1.22–1.31 (m, 24 H, 7–17-H_2, N–H_2), 2.78 (dt, *J* = 5.6, 6.6 Hz, 1 H, 2-H), 3.53 (dd, *J* = 6.6, 9.6 Hz, 1 H, 1-H_a), 3.64 (dd, *J* = 4.8, 9.6 Hz, 1 H, 1-H_b), 4.04–4.16 (m, 2 H, 3-H, 6-H), 5.53 (dd, *J* = 6.8, 15.6 Hz, 1 H, 4-H), 5.65 (dd, *J* = 6.7, 15.6 Hz, 1 H, 5-H) ppm. $C_{36}H_{79}NO_3Si_3$ (658.3): calcd. C 65.68, H 12.10, N 2.13; found C 65.54, H 12.22, N 2.15.

(2*S*,3*R*,4*E*,6*S*)-2-Amino-1,3,6-tris(*tert*-butyldimethylsilyloxy)-4-octadecene [(2*S*,3*R*,4*E*,6*S*)-23]: In the same manner as that just described, (4*S*,1′*R*,4′*S*)-**22** (2.01 g) was converted into 350 mg (3 steps, 12%) of (2*S*,3*R*,4*E*,6*S*)-**23** as a pale yellow oil, n_D^{25} = 1.4616. $[\alpha]_D^{25}$ = –4.2 (*c* = 1.0, $CHCl_3$). IR (film): $\tilde{\nu}$ = 3385 cm^{-1} (w, N–H), 1670 (w, C=C), 1255 (m, Si–Me) cm^{-1}. ^1H NMR (500 MHz, $CDCl_3$): δ = 0.016–0.15 (m, 18 H, Si–Me), 0.87–0.90 (m, 30 H, *t*Bu, 18-H_3), 1.22–1.31 (m, 24 H, 7–17-H_2, N–H_2), 2.78 (q-like, *J* = 5.5 Hz, 1 H, 2-H), 3.53 (dd, *J* = 7.0, 9.8 Hz, 1 H, 1-H_a), 3.63 (dd, *J* = 5.2, 9.8 Hz, 1 H, 1-H_b), 4.02–4.20 (m, 2 H, 3-H, 6-H), 5.54 (dd, *J* = 6.7, 15.4 Hz, 1 H, 4-H), 5.64 (dd, *J* = 5.8, 15.4 Hz, 1 H, 5-H) ppm. $C_{36}H_{79}NO_3Si_3$ (658.3): calcd. C 65.68, H 12.10, N 2.13; found C 65.88, H 12.25, N 2.15.

(2*S*,3*R*,4*E*,6*R*)-1,3,6-Tris(*tert*-butyldimethylsilyloxy)-2-[30-(*tert*-butyldiphenylsilyloxy)triacontanoylamino]-4-octadecene [(2*S*,3*R*,4*E*,6*R*)-24]: To a stirred solution of **8** (415 mg, 0.59 mmol) and (2*S*,3*R*,4*E*,6*R*)-**23** (200 mg, 0.29 mmol) in dry CH_2Cl_2 (10 mL) were added EDC [3-(3-dimethylaminopropyl)-1-ethylcarbodiimide hydrochloride; 113 mg, 0.61 mmol] and HOBt (1-hydroxybenzotriazole; 163 mg, 1.26 mmol) at room temperature. The reaction mixture was stirred for 12 h at room temperature and then quenched with water. It was extracted with ethyl acetate, and the extract was successively washed with water and brine, dried with $MgSO_4$, and concentrated under reduced pressure. The residue was chromatographed (60 g, hexane/ethyl acetate, 60:1) on silica gel to give (2*S*,3*R*,4*E*,6*R*)-**24** (282 mg, 72%) as a colorless oil, n_D^{24} = 1.4877. $[\alpha]_D^{24}$ = –0.51 (*c* = 1.00, $CHCl_3$). IR (film): $\tilde{\nu}$ = 3445 cm^{-1} (w, N–H), 1685 (m, C=O), 1255 (m, Si–Me), 1110 (s, C–O) cm^{-1}. ^1H NMR (500 MHz, $CDCl_3$): δ = 0.01, 0.02, 0.04, 0.05 (each s, 18 H, Si–Me), 0.86–0.91 (m, 27 H, *t*Bu), 1.01–1.08 (m, 12 H, *t*Bu, 18-H_3), 1.22–1.28 (m, 76 H, 7–17-H_2, 3′–29′-H_2), 1.99–2.15 (m, 2 H, 2′-H_2), 3.54 (dd, *J* = 5.8, 10.4 Hz, 1 H, 1-H_a), 3.64 (t, *J* = 6.4 Hz, 2 H, 30′-H), 3.78 (dd, *J* = 5.5, 10.4 Hz, 1 H, 1-H_b), 3.97 (m, 1 H, 2-H), 4.12 (dt, *J* = 5.7, 6.4 Hz, 1 H, 6-H), 4.42 (t-like, *J* = 4.9 Hz, 1 H, 3-H), 5.46 (d, *J* = 8.6 Hz, 1 H, N–H), 5.60 (dd, *J* = 5.5, 15.3 Hz, 1 H, 4-H), 5.71 (dd, *J* = 5.7, 15.3 Hz, 1 H, 5-H), 7.34–7.45 (m, 6 H, aromatic), 7.64–7.68 (m, 4 H, aromatic) ppm. HRFABMS: *m*/*z* $[M + H]^+$: calcd. for $C_{82}H_{155}NO_5Si_4Na$, 1369.0880; found, 1369.0883.

(2*S*,3*R*,4*E*,6*S*)-1,3,6-Tris(*tert*-butyldimethylsilyloxy)-2-(30-*tert*-butyldiphenylsilyloxytriacontanoylamino)-4-octadecene [(2*S*,3*R*,4*E*,6*S*)-24]: In the same manner as that just described, (2*S*,3*R*,4*E*,6*S*)-**23** (49 mg, 0.07 mmol) and **8** (62 mg, 0.09 mmol) were converted into 66 mg (70%) of (2*S*,3*R*,4*E*,6*S*)-**24** as a colorless oil, n_D^{23} = 1.4869. $[\alpha]_D^{23}$ = –3.4 (*c*, 1.10, $CHCl_3$). IR (film): $\tilde{\nu}$ = 3445 cm^{-1} (w, N–H), 1685 (s, NCO), 1550 (m, NCO), 1255 (m, Si–Me), 1110 (s, C–O) cm^{-1}. ^1H NMR (500 MHz, $CDCl_3$): δ = 0.01–0.05 (m, 18 H, Si–Me), 0.85–0.90 (m, 27 H, *t*Bu), 1.01–1.06 (m, 12 H, *t*Bu, 18-H_3), 1.07–1.26 (m, 76 H, 7–17-H_2, 3′–29′-H_2), 2.07–2.15 (m, 2 H, 2′-H_2), 3.54 (dd, *J* = 5.8, 10.4 Hz, 1 H, 1-H_a), 3.65 (t, *J* = 6.4 Hz, 2 H, 30′-H), 3.81 (dd, *J* = 5.2, 10.4 Hz, 1 H, 1-H_b), 3.92–3.99 (m, 1 H, 2-H), 4.06–4.12 (m, 1 H, 6-H), 4.33–4.38 (m, 1 H, 3-H), 5.49 (d, *J* = 8.2 Hz, 1 H, N–H), 5.56 (dd, *J* = 6.1, 15.6 Hz, 1 H, 4-H), 5.66 (dd, *J* = 6.1, 15.6 Hz, 1 H, 5-H), 7.32–7.44 (m, 6 H, aromatic), 7.65–7.68 (m, 4 H, aromatic) ppm. HRFABMS: *m*/*z* $[M + H]^+$: calcd. for $C_{82}H_{155}NO_5Si_4Na$, 1369.0880; found, 1369.0880.

(2*S*,3*R*,4*E*,6*R*)-1,3,6-Trihydroxy-2-(30-hydroxytriacontanoylamino)-4-octadecene [(2*S*,3*R*,4*E*,6*R*)-1]: To a stirred solution of (2*S*,3*R*,4*E*,6*R*)-**24** (256 mg, 0.19 mmol) in dry THF (8 mL) was added TBAF in THF (1.01 M, 1.52 mL, 0.152 mmol) at 0 °C under Ar, and stirring was continued overnighat at room temperature. The reaction mixture was concentrated under reduced pressure. The residue was chromatographed on silica gel (50 g, $CHCl_3$/MeOH, 50:1) and the obtained **1** was recrystallized from MeOH to give (2*S*,3*R*,4*E*,6*R*)-**1** (90 mg, 62%) as a colorless solid, m.p. 113.5–117.0 °C. IR (KBr): $\tilde{\nu}$ = 3470 cm^{-1} (w, N–H), 3310 (m, O–H), 1620 (m, C=O), 1120 (w, C–O), 1060 (m, C–O) cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$/CD_3OD, 1:1): δ = 0.51 (t, *J* = 6.6 Hz, 3 H, 18-H_3), 0.80–1.22 (m, 81 H, 7–17-H_2, 3′–29′-H_2, N–H, OH), 1.84 (t, *J* = 7.9 Hz, 2 H, 2′-H_2), 3.17 (t, *J* = 6.7 Hz, 2 H, 30′-H_2), 3.27 (dd, *J* = 4.5, 11.3 Hz, 1 H, 1-H_a), 3.37 (dd, *J* = 5.3, 11.3 Hz, 1 H, 1-H_b), 3.51 (dt-like, *J* = 5.1, 5.3 Hz, 1 H, 2-H), 3.67 (dt-like, *J* = 5.7, 6.1 Hz, 1 H, 6-H), 3.83 (t-like, *J* = 5.8 Hz, 1 H, 3-H), 5.29 (dd, *J* = 5.8, 15.6 Hz, 1 H, 4-H), 5.37 (dd, *J* = 5.7, 15.6 Hz, 1 H, 5-H) ppm. HRFABMS: *m*/*z* $[M + H]^+$: calcd. for $C_{48}H_{96}NO_5$, 766.7210; found, 766.7269.

(2*S*,3*R*,4*E*,6*S*)-1,3,6-Trihydroxy-2-(30-hydroxytriacontanoylamino)-4-octadecene [(2*S*,3*R*,4*E*,6*S*)-1]: In the same manner as that just described, (2*S*,3*R*,4*E*,6*S*)-**24** (174 mg, 0.12 mmol) was converted into 53 mg (58%) of (2*S*,3*R*,4*E*,6*S*)-**1** as a colorless solid, m.p. 115.0–118.0 °C. IR (KBr): $\tilde{\nu}$ = 3335 cm^{-1} (br. m, N–H, O–H), 3310 (m, O–H), 1660 (s, NCO), 1550 (m, NCO), 1075 (w, C–O) cm^{-1}. HRFABMS: *m*/*z* $[M + H]^+$: calcd. for $C_{48}H_{96}NO_5$, 766.7210; found, 766.7269.

(2*S*,3*R*,4*E*,6*R*)-1,3,6-Triacetoxy-2-(30-acetoxytriacontanoylamino)-4-octadecene [(2*S*,3*R*,4*E*,6*R*)-1′]: To a stirred solution of (2*S*,3*R*,4*E*,6*R*)-**1** (21 mg, 0.028 mmol) in dry pyridine (2 mL) was added Ac_2O (3 mL). The reaction mixture was stirred overnight at room temperature. It was then poured into water and extracted with diethyl ether. The extract was successively washed with water, saturated aq. $CuSO_4$, saturated aq. $NaHCO_3$ and brine, dried with $MgSO_4$, and concentrated under reduced pressure. The residue was chromatographed on silica gel (2 g, hexane/ethyl acetate, 5:1) to give 22 mg (84%) of (2*S*,3*R*,4*E*,6*R*)-**1′** as a colorless powder, m.p. 76.0–78.0 °C. $[\alpha]_D^{22}$ = +2.3 (*c* = 0.90, $CHCl_3$). IR (KBr): $\tilde{\nu}$ = 3310 cm^{-1} (br.w, N–H), 1740 (s, C=O), 1650 (m, NCO), 1540 (m, NCO) cm^{-1}. ^1H NMR (500 MHz, $CDCl_3$/D_2O): δ = 0.86 (t, *J* = 6.9 Hz, 3 H, 18-H_3), 1.21–1.40 (m, 72 H, 8–17-H_2, 3′–28′-H_2), 1.54–1.60 (m, 4 H, 7-, 29′-H_2), 2.02, 2.03, 2.04, 2.05 (each s, 12 H, OAc), 2.12–2.17 (m, 2 H, 2′-H_2), 3.98 (dd, *J* = 4.6, 11.6 Hz, 1 H, 1-H_a), 4.02 (t, *J* = 6.7 Hz, 2 H, 30′-H_2), 4.21 (dd, *J* = 6.4, 11.6 Hz, 1 H, 1-H_b), 4.46 (dd, *J* = 4.6, 6.4 Hz, 1 H, 2-H), 5.15 (dt, *J* = 6.4, 6.5 Hz, 1 H, 6-H), 5.32 (t-like, *J* = 5.8 Hz, 1 H, 3-H), 5.60 (dd, *J* = 6.4, 15.6 Hz, 1 H, 4-H), 5.69 (dd, *J* = 6.5, 15.6 Hz, 1 H, 5-H) ppm. ^{13}C NMR (126 MHz, $CDCl_3$): δ = 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, 34.1, 36.8, 50.3, 62.4, 64.6, 72.7, 73.6, 126.5, 133.7, 169.8, 170.4, 170.8, 171.2, 172.9 ppm. HRFABMS: *m*/*z* $[M + H]^+$: calcd. for $C_{56}H_{104}NO_9$, 934.7711; found, 934.7709.

(2*S*,3*R*,4*E*,6*S*)-1,3,6-Triacetoxy-2-(30-acetoxytriacontanoylamino)-4-octadecene [(2*S*,3*R*,4*E*,6*S*)-1′]: In the same manner as that just described, (2*S*,3*R*,4*E*,6*S*)-**1** (21 mg, 0.028 mmol) was converted into 22 mg (84%) of (2*S*,3*R*,4*E*,6*S*)-**1′** as colorless powder, m.p. 71.5–73.0 °C. $[\alpha]_D^{22}$ = –11.6 (*c* = 0.8, $CHCl_3$). IR (KBr): $\tilde{\nu}$ = 3305 cm^{-1}

(br.w, N–H), 1735 (s, C=O), 1650 (m, NCO), 1545 (m, NCO) cm^{-1}. ^1H NMR (500 MHz, $CDCl_3/D_2O$): δ = 0.86 (t, J = 6.9 Hz, 3 H, 18-H_3), 1.21–1.40 (m, 72 H, 8–17-H_2, 3′–28′-H_2), 1.53–1.60 (m, 4 H, 7-, 29′-H_2), 2.02, 2.03, 2.04, 2.05 (each s, 12 H, OAc), 2.10–2.14 (m, 2 H, 2′-H_2), 3.98–4.04 (m, 3 H, 1-H_a, 30′-H_2), 4.23 (dd, J = 6.3, 11.4 Hz, 1 H, 1-H_b), 4.32–4.48 (m, 1 H, 2-H), 5.12 (dt, J = 6.2, 6.4 Hz, 1 H, 6-H), 5.33 (t-like, J = 5.3 Hz, 1 H, 3-H), 5.61 (dd, J = 5.7, 15.6 Hz, 1 H, 4-H), 5.66 (dd, J = 5.9, 15.6 Hz, 1 H, 5-H) ppm. ^{13}C NMR (126 MHz, $CDCl_3$): δ = 14.1, 20.7, 21.0, 21.2, 22.6, 25.0, 25.6, 25.8, 28.5, 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 ppm. HRFABMS: m/z $[M + H]^+$: calcd. for $C_{56}H_{104}NO_9$, 934.7711; found, 934.7714.

***tert*-Butyl (4*S*,1′*R*,4′*R*)-4-(4′-*tert*-Butyldimethylsilyloxy-1′-hydroxy-2′-hexadecynyl)-2,2-dimethyl-3-oxazolidinecarboxylate [(4*S*,1′*R*,4′*R*)-25]:** To a stirred solution of (*R*)-**20** (8.00 g, 23.6 mmol) in dry THF (200 mL) was added *n*-butyllithium (1.58 M in hexane; 15.7 mL, 25.0 mmol) dropwise at –78 °C under Ar. After the reaction mixture had been stirred at –78 °C for 1.5 h, a solution of Garner's aldehyde [(*S*)-**21**, 6.5 g, 23.6 mmol] in dry THF (200 mL) and HMPA (20 mL) was added dropwise at –78 °C. The mixture was then stirred overnight while being warmed to 0 °C, before being quenched with NH_4Cl aq. and extracted with diethyl ether. The extract was successively washed with water and brine, dried with $MgSO_4$, and concentrated under reduced pressure. The residue was chromatographed on silica gel (500 g, hexane/ethyl acetate, 20:1) to give 10.9 g (81%) of (4*S*,1′*R*,4′*R*)-**25** as a colorless oil, n_D^{23} = 1.4641. $[\alpha]_D^{23}$ = –4.9 (c = 1.0, $CHCl_3$). IR (film): $\tilde{\nu}$ = 3445 cm^{-1} (w, N–H), 1705 (w, C=C), 1255 (m, Si–Me) cm^{-1}. ^1H NMR (500 MHz, $CDCl_3$): δ = 0.08, 0.11 (each s, 6 H, Si–Me), 0.85–0.90 (m, 12 H, *t*Bu, 16′-H_3), 1.24–1.31 (m, 15 H, *t*Bu, 2–Me, 2–Me), 1.35–1.66 (m, 23 H, 5′–15′-H_2, OH), 3.90–4.18 (m, 3 H 1′-H, 3-H_2), 4.35 (t, J = 6.5 Hz, 1 H, 4′-H), 4.58–4.76 (m, 1 H, 4-H) ppm. $C_{32}H_{61}NO_5Si$ (567.9): calcd. C 67.68, H 10.83, N 2.47; found C 67.38, H 10.97, N 2.44.

(2*S*,3*R*,4*E*,6*R*)-2-Amino-1,3,6-tris(*tert*-butyldimethylsilyloxy)-4-octadecene [(2*S*,3*R*,4*E*,6*R*)-23]: To a stirred solution of (4*S*,1′*R*,4′*R*)-**25** (500 mg, 0.88 mmol) in dry THF (20 mL) was added TBAF in THF (1.01 M, 1.76 mL, 1.74 mmol) at 0 °C under Ar, and stirring was continued overnighat at room temperature and then the reaction was quenched with water. It was extracted with ethyl acetate. The extract was successively washed with water and brine, dried with $MgSO_4$, and concentrated under reduced pressure. Because of its instability, this product **22** was used for the next reaction without purification.

In the same manner as that just described, the crude product of (4*S*,1′*R*,4′*R*)-**22** was converted into 1.10 g (3 steps, 37%) of (2*S*,3*R*,4*E*,6*R*)-**23** as a pale yellow oil. Its physical data were identical with those of (2*S*,3*R*,4*E*,6*R*)-**23** which was derived from (*R*)-**18**.

(2*S*,3*R*,4*E*,6*R*)-1,3,6-Trihydroxy-2-tetracosanoylamino-4-octadecene [(2*S*,3*R*,4*E*,6*R*)-2]: To a stirred solution of tetracosanoic acid (**10**) (118 mg, 0.32 mmol) and (2*S*,3*R*,4*E*,6*R*)-**23** (200 mg, 0.29 mmol) in dry CH_2Cl_2 (15 mL) was added EDC (110 mg, 0.58 mmol) and HOBt (160 mg, 1.18 mmol) at room temperature. The reaction mixture was stirred for 12 h at room temperature and then quenched with water. It was extracted with ethyl acetate, and the extract was successively washed with water and brine, dried with $MgSO_4$, and concentrated under reduced pressure. The residue was chromatographed on silica gel (20 g, hexane/ethyl acetate, 50:1) to give the TBS-protected ceramide (280 mg, 96%) as a colorless solid, m.p. 32.0–34.5. $[\alpha]_D^{25}$ = –0.93 (c = 1.20, $CHCl_3$). IR (nujol): $\tilde{\nu}$ = 3445 cm^{-1} (w, N–H), 1685 (m, NCO), 1255 (s, Si–Me) cm^{-1}. ^1H NMR (500 MHz, $CDCl_3$): δ = 0.012, 0.013, 0.035, 0.042, 0.049 (each s, 18 H, Si–Me), 0.86–0.90 (m, 33 H, *t*Bu, 18-, 24′-H_3), 1.10–1.37 (m, 64 H, 7–17-H_2, 3′–23′-H_2), 2.09–2.14 (m, 2 H, 2′-H), 3.54 (dd, J = 6.0, 10.0 Hz, 1 H, 1-H_a), 3.78 (dd, J = 5.5, 10.5 Hz, 1 H, 1-H_b), 3.97 (ddt, J = 5.0, 6.0, 8.5 Hz, 1 H, 2-H), 4.12 (dt, J = 5.5, 6.0 Hz, 1 H, 6-H), 4.42 (t-like, J = 5.0 Hz, 1 H, 3-H), 5.47 (d, J = 8.5 Hz, 1 H, N–H) 5.60 (ddd, J = 1.5, 5.5, 15.5 Hz, 1 H, 4-H), 5.70 (ddd, J = 1.0, 5.5, 15.5 Hz, 1 H, 5-H) ppm. $C_{60}H_{125}NO_4Si_3$ (1008.9): calcd. C 71.43, H 12.49, N 1.39; found C 71.14, H 12.76, N 1.43.

To a stirred solution of protected ceramide (216 mg, 0.21 mmol) in dry THF (5 mL) was added TBAF in THF (1.01 M, 6.3 mL, 6.3 mmol) at 0 °C under Ar, and stirring was continued overnight at room temperature. The reaction mixture was concentrated under reduced pressure. The residue was chromatographed on silica gel (20 g, $CHCl_3$/MeOH, 50:1) and recrystallized from MeOH to give (2*S*,3*R*,4*E*,6*R*)-**2** (103 mg, 72%) as a colorless solid, m.p. 113.5–115.0 °C. IR (KBr): $\tilde{\nu}$ = 3470 cm^{-1} (br. m, N–H), 3310 (m, O–H), 1620 (s, NCO), 1545 (m, NCO) cm^{-1}. ^1H NMR (500 MHz, $CDCl_3$, 50 °C): δ = 0.89 (t, J = 7.0 Hz, 6 H, 18-, 24′-H_3), 1.24–1.38 (m, 61 H, 8–17-H_2, 4′–23′-H_2), 1.51–1.69 (m, 4 H, 7-, 3′-H_2), 2.23 (t, J = 7.5 Hz, 2 H, 2′-H), 2.34–2.41 (m, 1 H, OH), 2.75 (d, J = 5.5 Hz, 1 H, OH), 3.70–3.77 (m, 1 H, 1-H_a), 3.90–3.99 (m, 1 H, 1-H_b, 2-H), 4.12–4.19 (m, 1 H, 6-H), 4.39 (dt, J = 4.5, 5.0 Hz, 1 H, 3-H), 5.79 (dd, J = 5.5, 15.5 Hz, 1 H, 4-H) 5.85 (dd, J = 6.0, 15.5 Hz, 1 H, 5-H), 6.18 (d, J = 6.5 Hz, 1 H, N–H) ppm. HRFABMS: m/z $[M + H]^+$: calcd. for $C_{42}H_{83}NO_4Na$, 688.6946; found, 688.6226.

(2*S*,3*R*,4*E*,6*R*)-1,3,6-Trihydroxy-4*N*-[30′-(linoleoyloxy)triacontanoyl]octadecene [(2*S*,3*R*,4*E*,6*R*)-3]: A solution of **9** (267 mg, 0.36 mmol) and (2*S*,3*R*,4*E*,6*R*)-**23** (250 mg, 0.36 mmol) in pyridine (10 mL) was stirred at 45 °C for 21 h. The mixture was diluted with benzene (50 mL) and concentrated under reduced pressure. This operation was repeated three times to remove pyridine. The residue was chromatographed on silica gel (30 g, hexane/ethyl acetate, 100:1) to give the TBS-protected ceramide (377 mg 76%) as a colorless oil, n_D^{24} = 1.4712. $[\alpha]_D^{24}$ = –0.82 (c = 1.05, $CHCl_3$). IR (film): $\tilde{\nu}$ = 3445 cm^{-1} (w, N–H), 1740 (m, C=O), 1685 (m, NCO), 1255 (s, Si–Me) cm^{-1}. ^1H NMR (500 MHz, $CDCl_3$): δ = 0.01, 0.012, 0.03, 0.04, 0.047 (each s, 18 H, Si–Me), 0.86–0.91 (m, 33 H, *t*Bu, 18-, 18′′-H_3), 1.20–1.40 (m, 80 H, 8–14-H_2, 4′–28′-H_2, 3′′–6′′-H_2, 16′′–17′′-H_2), 1.58–1.68 (m, 12 H, 7-, 3′-, 29′-, 3′′-, 7′′-, 15′′-H_2), 2.01–2.08 (m, 4 H, 8′′-, 14′′-H_2), 2.10–2.15 (m, 2 H, 2′′-H_2), 2.28 (t, J = 7.5 Hz, 2 H, 2′-H_2), 2.77 (t, J = 6.7 Hz, 2 H, 11′′-H_2), 3.54 (dd, J = 6.0, 10.0 Hz, 1 H, 1-H_a), 3.77 (dd, J = 5.5, 10.0 Hz, 1 H, 1-H_b), 3.97 (ddt-like, J = 5.0, 5.5, 8.0 Hz, 1 H, 2-H), 4.05 (t, J = 6.8 Hz, 2 H, 30′-H_2), 4.11 (dt, J = 6.0, 12.0 Hz,1 H, 6-H), 4.41 (t-like, J = 5.0 Hz, 1 H, 3-H), 5.28–5.41 (m, 4 H, 9′′-, 10′′-, 12′′-, 13′′-H), 5.47 (d, J = 8.0 Hz, 1 H, N–H), 5.60 (ddd, J = 1.0, 5.5, 15.5 Hz, 1 H, 4-H), 5.70 (ddd, J = 1.0, 6.0, 15.5 Hz, 1 H, 5-H) ppm.

To a stirred mixture of the TBS-protected ceramide (337 mg, 0.25 mmol) in dry THF (5 mL) was added TBAF in THF (1.0 M, 1.1 mL, 1.1 mmol) at 0 °C under Ar, and stirring was continued overnight at room temperature. The mixture was then diluted with chloroform (10 mL) and concentrated under reduced pressure. This operation was repeated three times to remove THF. The residue was chromatographed on silica gel (20 g, $CHCl_3$/MeOH, 100:1) to give (2*S*,3*R*,4*E*,6*R*)-**3** (182 mg, 73%) as colorless powder, m.p. 111.5–112.5 °C. IR (KBr): $\tilde{\nu}$ = 3470 cm^{-1} (w, N–H), 3310 (m, O–H), 1740 (m, C=O), 1620 (m, NCO) 1545 (w, NCO) cm^{-1}. ^1H NMR

(500 MHz, $CDCl_3$): δ = 0.88, 0.89 (each t, each J = 7.0 Hz, 6 H, 18-, 18′′-H_3), 1.21–1.39 (m, 83 H, 8–14-H_2, 4′–28′-H_2, 3′′–6′′-H_2, 16′′–17′′-H_2, OH), 1.49–1.67 (m, 12 H, 7-, 3′-, 29′-, 3′′-, 7′′-, 15′′-H_2), 2.02–2.07 (m, 4 H, 8′-, 14′′-H_2), 2.23 (t, J = 7.5 Hz, 2 H, 2′-H_2), 2.29 (t, J = 7.5 Hz, 2 H, 2′′-H_2), 2.77 (t-like, J = 7.0 Hz, 1 H, 11′′-H_2), 3.67–3.75 (m, 1 H, 2-H), 3.90–3.98 (m, 2 H, 1-H_2), 4.05 (t, J = 6.5 Hz, 2 H, 30′-H_2), 4.15 (dt, J = 5.5, 6.0 Hz, 1 H, 6-H), 4.36–4.41 (m, 1 H, 3-H), 5.28–5.42 (m, 4 H, 9′′-, 10′′-, 12′′-, 13′′-H), 5.77 (ddd, J = 1.0, 5.5, 15.5 Hz, 1 H, 4-H), 5.84 (ddd, J = 1.0, 6.0, 15.5 Hz, 1 H, 5-H), 6.29 (d, J = 7.0 Hz, 1 H, N–H) ppm.

(2*S*,3*R*,4*E*,6*R*,2′*R*)-2-(2′-Acetoxytetracosanoylamino)-1,3,6-tris(*tert*-butyldimethylsilyloxy)-4-octadecene [(2*S*,3*R*,4*E*,6*R*,2′*R*)-26]: To a stirred solution of (*R*)-**13** (171 mg, 0.4 mmol) and (2*S*,3*R*,4*E*,6*R*)-**23** (250 mg, 0.36 mmol) in dry CH_2Cl_2 (15 mL) was added EDC (138 mg, 0.72 mmol) and HOBt (199 mg, 1.48 mmol) at room temperature. The reaction mixture was stirred for 12 h at room temperature and then quenched with water. It was extracted with ethyl acetate, and the extract was successively washed with water and brine, dried with $MgSO_4$, and concentrated under reduced pressure. The residue was chromatographed on silica gel (6 g, hexane/ethyl acetate, 100:1) to give (2*S*,3*R*,4*E*,6*R*,2′*R*)-**26** (280 mg, 73%) as a colorless oil, n_D^{24} = 1.4622. $[\alpha]_D^{24}$ = +8.9 (c = 1.00, $CHCl_3$). IR (film): $\tilde{\nu}$ = 3445 cm^{-1} (w, N–H), 1755 (m, C=O), 1690 (m, NCO), 1255 (m, Si–Me) cm^{-1}. ^1H NMR (500 MHz, $CDCl_3$): δ = 0.018, 0.022, 0.026, 0.034, 0.046, 0.060 (each s, 18 H, Si–Me), 0.85–0.91 (m, 33 H, *t*Bu, 18-H_3, 24′-H_3), 1.13–1.51 (m, 64 H, 7–17-H_2, 3′–23′-H_2), 2.10 (s, 3 H, OAc), 3.54 (dd, J = 6.0, 10.5 Hz, 1 H, 1-H_a), 3.74 (dd, J = 6.0, 10.5 Hz, 1 H, 1-H_b), 4.04 (ddt, J = 4.5, 6.0, 8.5 Hz, 1 H, 2-H), 4.13 (dt, J = 5.5, 6.0 Hz, 1 H, 6-H), 4.46 (t-like, J = 5.0 Hz, 1 H, 3-H), 5.21 (dt, J = 2.5, 4.5 Hz, 1 H, 2′-H), 5.62 (ddd, J = 1.0, 5.5, 15.5 Hz, 1 H, 4-H), 5.74 (ddd, J = 1.5, 5.0, 15.5 Hz, 1 H, 5-H), 6.16 (d, J = 8.5 Hz, 1 H, N–H) ppm. $C_{62}H_{127}NO_6Si_3$ (1066.93): calcd. C 69.79, H 12.00, N 1.31; found C 69.78, H 12.16, N 1.25.

(2*S*,3*R*,4*E*,6*R*,2′*S*)-2-(2′-Acetoxytetracosanoylamino)-1,3,6-tris(*tert*-butyldimethylsilyloxy)-4-octadecene [(2*S*,3*R*,4*E*,6*R*,2′*S*)-26]: In the same manner as that just described, (*S*)-**13** (137 mg, 0.32 mmol) and (2*S*,3*R*,4*E*,6*R*)-**23** (200 mg, 0.29 mmol) were converted into 291 mg (94%) of (2*S*,3*R*,4*E*,6*R*,2′*S*)-**26** as a colorless oil, n_D^{22} = 1.4631. $[\alpha]_D^{24}$ = –4.8 (c = 1.00, $CHCl_3$). IR (film): $\tilde{\nu}$ = 3445 cm^{-1} (w, N–H), 1755 (m, C=O), 1690 (m, NCO), 1255 (m, Si–Me) cm^{-1}. ^1H NMR (500 MHz, $CDCl_3$): δ = –0.02, –0.016, –0.014, 0.018, 0.03, 0.05 (each s, 18 H, Si–Me), 0.85–0.90 (m, 33 H, *t*Bu, 18-H_3, 24′-H_3), 1.10–1.52 (m, 64 H, 7–17-H_2, 3′–23′-H_2), 2.09 (s, 3 H, OAc), 3.52 (dd, J = 5.5, 10.5 Hz, 1 H, 1-H_a), 3.80 (dd, J = 6.0, 10.5 Hz, 1 H, 1-H_b), 3.95 (ddt, J = 5.0, 6.0, 8.0 Hz, 1 H, 2-H), 4.10 (dt, J = 5.5, 6.0 Hz, 1 H, 6-H), 4.44 (t-like, J = 5.0 Hz, 1 H, 3-H), 5.21 (dt, J = 2.0, 5.0 Hz, 1 H, 2′-H), 5.59 (ddd, J = 1.0, 5.5, 15.5 Hz, 1 H, 4-H), 5.69 (ddd, J = 1.5, 5.0, 15.5 Hz, 1 H, 5-H), 6.24 (d, J = 8.0 Hz, 1 H, N–H) ppm. $C_{62}H_{127}NO_6Si_3$ (1066.9): calcd. C 69.79, H 12.00, N 1.31; found C 69.83, H 12.25, N 1.30.

(2*S*,3*R*,4*E*,6*R*,2′*R*)-1,3,6-tris(*tert*-Butyldimethylsilyloxy)-2-(2′-hydroxytetracosanoylamino)-4-octadecene [(2*S*,3*R*,4*E*,6*R*,2′*R*)-27]: To a stirred solution of (2*S*,3*R*,4*E*,6*R*,2′*R*)-**26** (280 mg, 0.26 mmol) in MeOH (5 mL) was added K_2CO_3 (72 mg, 0.52 mmol) at room temperature. The mixture was stirred for 2 h at room temperature, before the solvent was removed under reduced pressure. The concentrate was diluted with water and extracted with diethyl ether. The resulting extract was successively washed with water and brine, dried with $MgSO_4$, and concentrated under reduced pressure. The residue was chromatographed on silica gel (6 g, hexane/ethyl acetate, 70:1) to give (2*S*,3*R*,4*E*,6*R*,2′*R*)-**27** (209 mg, 78%) as a colorless oil, n_D^{24} = 1.4669. $[\alpha]_D^{24}$ = +5.0 (c = 1.00, $CHCl_3$). IR (film): $\tilde{\nu}$ = 3395 cm^{-1} (br. m, N–H, O–H), 1655 (m, NCO), 1525 (m, NCO), 1255 (w, Si–Me) cm^{-1}. ^1H NMR (500 MHz, $CDCl_3$): δ = 0.02, 0.039, 0.042, 0.052 (each s, 18 H, Si–Me), 0.85–0.90 (m, 33 H, *t*Bu, 18-H_3, 24′-H_3), 1.13–1.60 (m, 65 H, 7–17-H_2, 3′–23′-H_2, O–H), 3.57 (dd, J = 5.5, 10.0 Hz, 1 H, 1-H_a), 3.77 (dd, J = 6.0, 10.0 Hz, 1 H, 1-H_b), 3.98–4.07 (m, 2 H, 2-, 2′-H), 4.12 (dt, J = 5.5, 6.0 Hz, 1 H, 6-H), 4.43 (t-like, J = 5.5 Hz, 1 H, 3-H), 5.61 (ddd, J = 1.0, 5.5, 15.5 Hz, 1 H, 4-H), 5.72 (ddd, J = 1.5, 5.5, 15.5 Hz, 1 H, 5-H), 6.36 (d, J = 8.5 Hz, 1 H, N–H) ppm. $C_{60}H_{125}NO_5Si_3$ (1024.9): calcd. C 70.31, H 12.29, N 1.37; found C 70.33, H 12.54, N 1.35.

(2*S*,3*R*,4*E*,6*R*,2′*S*)-1,3,6-Tris(*tert*-butyldimethylsilyloxy)-2-(2′-hydroxytetracosanoylamino)-4-octadecene [(2*S*,3*R*,4*E*,6*R*,2′*S*)-27]: In the same manner as that just described, (2*S*,3*R*,4*E*,6*R*,2′*S*)-**26** (271 mg, 0.25 mmol) was converted into 216 mg (83%) of (2*S*,3*R*,4*E*,6*R*,2′*S*)-**27** as a colorless oil, n_D^{24} = = 1.4661. $[\alpha]_D^{24}$ = –9.7 (c = 1.05, $CHCl_3$). IR (film): $\tilde{\nu}$ = 3395 cm^{-1} (br. m, N–H, O–H), 1650 (m, NCO), 1525 (m, NCO), 1255 (w, Si–Me) cm^{-1}. ^1H NMR (500 MHz, $CDCl_3$): δ = 0.02, 0.03, 0.04, 0.05 (each s, 18 H, Si–Me), 0.84–0.91 (m, 33 H, *t*Bu, 18-H_3, 24′-H_3), 1.14–1.63 (m, 65 H, 7–17-H_2, 3′–23′-H_2, O–H), 3.56 (dd, J = 5.5, 10.5 Hz, 1 H, 1-H_a), 3.78 (dd, J = 5.0, 10.5 Hz, 1 H, 1-H_b), 3.97–4.00 (m, 2 H, 2-, 2′-H), 4.11 (dt, J = 5.5, 6.0 Hz, 1 H, 6-H), 4.43 (t-like, J = 5.5 Hz, 1 H, 3-H), 5.60 (ddd, J = 1.0, 5.5, 15.5 Hz, 1 H, 4-H), 5.71 (ddd, J = 1.0, 5.5, 15.5 Hz, 1 H, 5-H), 6.43 (d, J = 9.0 Hz, 1 H, N–H) ppm. $C_{60}H_{125}NO_5Si_3$ (1024.9): calcd. C 70.31, H 12.29, N 1.37; found C 70.31, H 12.44, N 1.36.

(2*S*,3*R*,4*E*,6*R*,2′*R*)-1,3,6-Trihydroxy-2-(2′-hydroxytetracosanoylamino)-4-octadecene [(2*S*,3*R*,4*E*,6*R*,2′*R*)-4]: To a stirred solution of (2*S*,3*R*,4*E*,6*R*,2′*R*)-**27** (181 mg, 0.18 mmol) in dry THF (5 mL) was added TBAF in THF (1.01 M, 10 mL, 10.1 mmol) at 0 °C under Ar, and stirring was continued overnighat at room temperature. The reaction mixture was concentrated under reduced pressure. The residue was chromatographed on silica gel (15 g, $CHCl_3$/MeOH, 50:1) and the obtained **4** was recrystallized from MeOH to give (2*S*,3*R*,4*E*,6*R*,2′*R*)-**4** (90 mg, 75%) as a colorless solid, m.p. 115.5–117.0 °C. IR (KBr): $\tilde{\nu}$ = 3265 cm^{-1} (br. m, N–H), 3200 (m, O–H), 1650 (m, C=O), 1560 (m, NCO) cm^{-1}. ^1H NMR (500 MHz, $CDCl_3$, 50 °C): δ = 0.89 (t, J = 7.0 Hz, 6 H, 18-H_3, 24′-H_3), 1.20–1.38 (m, 60 H, 8–17-H_2, 4′–23′-H_2), 1.51–1.69 (m, 4 H, 7-, 3′-H_2), 1.82–1.89 (m, 1 H, OH), 2.50 (dd, J = 2.5, 5.0 Hz, 1 H, 1-H_a), 2.51–2.54 (m, 1 H, OH), 2.63 (dd, J = 2.0, 5.0 Hz, 1 H, 1-H_b), 3.75–3.80 (m, 1 H, OH), 3.87–3.92 (m, 1 H, 2-H), 3.99 (dt, J = 4.5, 11.5 Hz, 1 H, 2′-H), 4.10–4.19 (m, 2 H, 6-H, OH), 4.42 (q, J = 5.0 Hz, 1 H, 3-H), 5.77 (dd, J = 5.5, 15.5 Hz, 1 H, 4-H), 5.84 (dd, J = 5.5, 15.5 Hz, 1 H, 5-H), 7.05 (d, J = 5.5 Hz, 1 H, N–H) ppm. ^{13}C NMR (126 MHz, $CDCl_3$/CD_3OD, 1:1): δ = 13.2, 22.1, 24.8, 25.0, 28.75, 28.77, 29.0, 29.06, 29.09, 29.13, 31.4, 54.4, 60.7, 71.2, 71.60, 71.66, 128.8, 135.3, 175.7 ppm. $C_{42}H_{83}NO_5$ (682.1): calcd. C 73.95, H 12.26, N 2.05; found C 73.83, H 12.43, N 2.04.

(2*S*,3*R*,4*E*,6*R*,2′*S*)-1,3,6-Trihydroxy-2-(2′-hydroxytetracosanoylamino)-4-octadecene [(2*S*,3*R*,4*E*,6*R*,2′*S*)-4]: In the same manner as that just described, (2*S*,3*R*,4*E*,6*R*,2′*S*)-**27** (216 mg, 0.21 mmol) was converted into 103 mg (72%) of (2*S*,3*R*,4*E*,6*R*,2′*S*)-**4** as a colorless solid, m.p. 106.5–109.5 °C. IR (KBr): $\tilde{\nu}$ = 3365 cm^{-1} (br. m, N–H, O–H), 1655 (s, NCO), 1550 (m, NCO) cm^{-1}. ^{13}C NMR (126 MHz, $CDCl_3$:CD_3OD, 1:1): δ = 13.2, 22.2, 24.8, 25.1, 28.9, 29.0, 29.12, 29.18, 29.20, 31.5, 34.0, 36.7, 54.3, 60.9, 71.4, 71.60, 71.72, 129.0, 135.4, 175.9 ppm. $C_{42}H_{83}NO_5$ (682.1): calcd. C 73.95, H 12.26, N 2.05; found C 73.76, H 12.36, N 2.02.

(2*S*,3*R*,4*E*,6*R*,2′*R*)-1,3,6-Triacetoxy-2-(2′-acetoxytetracosanoylamino)-4-octadecene [(2*S*,3*R*,4*E*,6*R*,2′*R*)-4′]: To a stirred solution

of (2*S*,3*R*,4*E*,6*R*,2′*R*)-**4** (20 mg, 0.03 mmol) in dry pyridine (2 mL) was added Ac_2O (3 mL). The reaction mixture was stirred overnight at room temperature. It was then poured into water and extracted with diethyl ether. The extract was successively washed with water, saturated aq. $CuSO_4$, saturated aq. $NaHCO_3$ and brine, dried with $MgSO_4$, and concentrated under reduced pressure. The residue was chromatographed on silica gel (4 g, hexane/ethyl acetate, 5:1) to give (2*S*,3*R*,4*E*,6*R*,2′*R*)-**4′** (22 mg, 92%) as colorless powder, m.p. 91.0–93.0 °C. $[\alpha]_D^{25}$ = +12.1 (*c* = 0.6, $CHCl_3$). IR (KBr): $\tilde{\nu}$ = 3355 cm^{-1} (m, N–H), 1740 (s, OAc), 1660 (s, NCO), 1530 (m, NCO), 1235 (s, C–OAc) cm^{-1}. ^1H NMR (500 MHz, $CDCl_3$): δ = 0.88 (t, *J* = 7.0 Hz, 6 H, 18-, 24′-H_3), 1.21–1.32 (m, 6 H, 8–17-H_2, 4′–23′-H_2), 1.76–1.85 (m, 4 H, 7-, 3′-H), 2.02, 2.04, 2.06, 2.16 (each s, 12 H, OAc), 3.94 (dd, *J* = 4.3, 11.6 Hz, 1 H, 1-H_a), 4.31 (dd, *J* = 7.8, 11.6 Hz, 1 H, 1-H_b), 4.39–4.49 (m, 1 H, 2-H), 5.08 (dd, *J* = 4.5, 7.5 Hz, 1 H, 2′-H), 5.17 (dt, *J* = 6.5, 6.5 Hz, 1 H, 6-H), 5.36 (t-like, *J* = 5.5 Hz, 1 H, 3-H), 5.61 (ddd, *J* = 1.0, 6.4, 15.7 Hz, 1 H, 4-H), 5.70 (ddd, *J* = 1.0, 6.3, 15.7 Hz, 1 H, 5-H), 6.38 (d, *J* = 9.0 Hz, 1 H, N–H) ppm. ^{13}C NMR (126 MHz, $CDCl_3$): δ = 14.1, 20.7, 20.9, 21.0, 21.2, 22.7, 24.8, 25.1, 29.25, 29.34, 29.47, 29.49, 29.57, 29.59, 29.64, 29.66, 29.70, 31.8, 31.9, 34.1, 50.9, 61.7, 72.6, 73.6, 74.0, 126.3, 133.6, 169.7, 169.9, 170.1, 170.4, 171.4 ppm. HRFABMS: *m*/*z* $[M + H]^+$: calcd. for $C_{50}H_{91}NO_9Na$, 872.6592; found, 872.6592.

(2*S*,3*R*,4*E*,6*R*,2′*S*)-1,3,6-Triacetoxy-2-(2′-acetoxytetracosanoylamino)-4-octadecene [(2*S*,3*R*,4*E*,6*S*,2′*S*)-4′]: In the same manner as that just described, (2*S*,3*R*,4*E*,6*R*,2′*S*)-**4** (20 mg, 0.03 mmol) was converted into 20 mg (84%) of (2*S*,3*R*,4*E*,6*R*,2′*S*)-**4′** as colorless powder, m.p. 80.0–81.5 °C. $[\alpha]_D^{25}$ = –4.8 (*c* = 0.7, $CHCl_3$). IR (KBr): $\tilde{\nu}$ = 3340 cm^{-1} (m, N–H), 1735 (s, OAc), 1650 (s, NCO), 1540 (s, NCO), 1235 (s, C–OAc) cm^{-1}. ^1H NMR (500 MHz, $CDCl_3$): δ = 0.86 (t, *J* = 6.5 Hz, 6 H, 18-, 24′-H_3), 1.18–1.32 (m, 60 H, 8–17-H_2, 4′–23′-H_2), 1.71–1.82 (m, 4 H, 7-, 3′-H), 2.043, 2.047, 2.059, 2.16 (each s, 12 H, OAc), 4.03 (dd, *J* = 4.5, 11.5 Hz, 1 H, 1-H_a), 4.18 (dd, *J* = 7.0, 11.5 Hz, 1 H, 1-H_b), 4.40–4.48 (m, 1 H, 2-H), 5.08 (dd, *J* = 5.0, 7.5 Hz, 1 H, 2′-H), 5.15 (dt, *J* = 6.5, 6.5 Hz, 1 H, 6-H), 5.31 (t-like, *J* = 5.5 Hz, 1 H, 3-H), 5.58 (ddd, *J* = 0.5, 6.4, 15.5 Hz, 1 H, 4-H), 5.69 (ddd, *J* = 1.0, 6.5, 15.5 Hz, 1 H, 5-H), 6.37 (d, *J* = 9.0 Hz, 1 H, N–H) ppm. ^{13}C NMR (126 MHz, $CDCl_3$): δ = 14.1, 20.7, 20.8, 20.9, 21.2, 22.7, 24.7, 25.1, 29.27, 29.30, 29.34, 29.44, 29.47, 29.56, 29.63, 29.65, 29.69, 31.7, 31.9, 34.1, 50.5, 62.0, 72.9, 73.6, 74.0, 126.2, 133.6, 169.8, 169.9, 170.0, 170.4, 170.9 ppm. HRFABMS: *m*/*z* $[M + H]^+$: calcd. for $C_{50}H_{91}NO_9Na$, 872.6592; found, 872.6589.

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