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Synthesis of (2S,2′R,3S,4R)-2-(2′-Hydroxy-21′-methyldocosanoylamino)- 1,3,4-pentadecanetriol, the Ceramide Sex Pheromone of the Female Hair Crab,…

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Synthesis of (2*S*,2′*R*,3*S*,4*R*)-2-(2′-Hydroxy-21′-methyldocosanoylamino)-1,3,4-pentadecanetriol, the Ceramide Sex Pheromone of the Female Hair Crab, *Erimacrus isenbeckii*†

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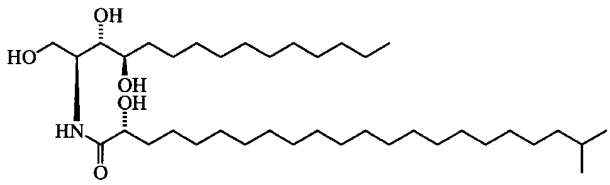
The ceramide sex pheromone [(2*S*,2′*R*,3*S*,4*R*)-2-(2′-hydroxy-21′-methyldocosanoylamino)-1,3,4-pentadecanetriol (1)] of the female hair crab (*Erimacrus isenbeckii*) was synthesized by starting from (*S*)-serine and 12-bromo-1-dodecanol.

Key words: ceramide; crab; *Erimacrus isenbeckii*; pheromone; sphingolipid, synthesis

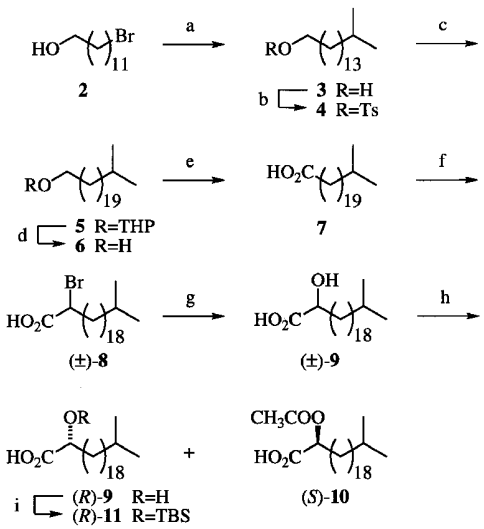
Sphingolipids possess diverse bioactivities such as fruiting body formation,[1,2] enzyme inhibition,[3] enzyme activation,[4,5] and immunosuppression,[6] in addition to their important role in cell membrane systems. Fusetani and his co-workers reported in 2000 that the ceramide, (2*S*,2′*R*,3*S*,4*R*)-2-(2′-hydroxy-21′-methyldocosanoylamino)-1,3,4-pentadecanetriol (**1**, Fig. 1), and its relatives functioned as the sex pheromone of the hair crab (*Erimacrus isenbeckii*) in the female urine, which elicited precopulatory behavior in males.[7] Since we are interested in both pheromones and sphingolipids, we immediately started the synthesis of **1**. This paper describes the method we used to prepare **1**. After our synthesis had been completed, Fusetani and his co-workers reported the synthesis of ceramide **1** by employing D-galactose as the chiral building block.[8]

A ceramide is usually synthesized by *N*-acylating its sphingosine part with its carboxylic acid part. Accordingly, in the present case, we planned to first synthesize the acid part and then the sphingosine part. Scheme 1 summarizes our synthesis of the acid part, (*R*)-**11**. Chain-elongation of commercially available 12-bromo-1-dodecanol (**2**) with 3-methylbutylmagnesium bromide was executed under Schlosser conditions in the presence of dilithium tetrachlorocuprate[9] to give **3**. Corresponding tosylate **4** was again chain-elongated by a treatment with 6-(2-tetra-



Hair crab pheromone (**1**)

Fig. 1. Structure of the Female Sex Pheromone of the Hair Crab, *Erimacrus isenbeckii*.



Scheme 1. Synthesis of Acid Part (*R*)-**11**.

Reagents: (a) $(CH_3)_2CH(CH_2)_2MgBr$, Li_2CuCl_4, THF (99%). (b) TsCl, C_5H_5N (89%). (c) $THPO(CH_2)_6MgBr$, Li_2CuCl_4, THF (79%). (d) TsOH, C_2H_5OH (97%). (e) Jones' CrO_3, $(CH_3)_2CO$ (74%). (f) Br_2, P, heat (84%). (g) NaOH, H_2O (90%). (h) $CH_2=CHOCOCH_3$, lipase PS, BHT, THF (23%). (i) TBSCl, imidazole, DMF (80%).

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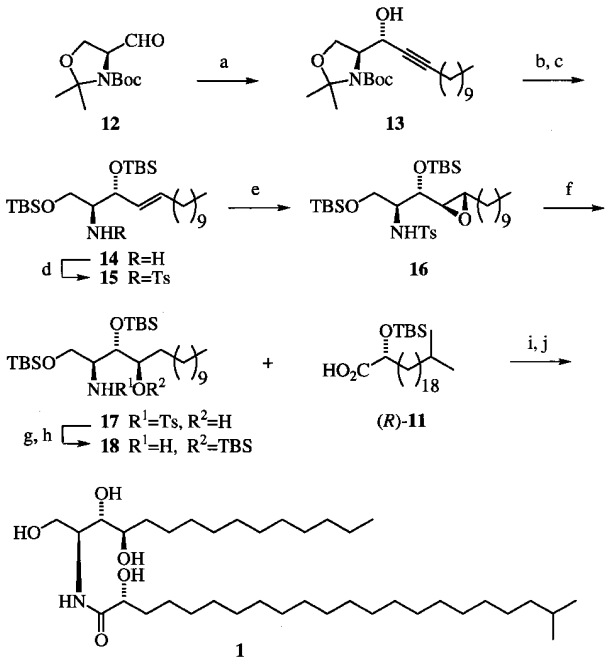
hydropyranyloxy)hexylmagnesium bromide under Schlosser conditions[9] to afford tetrahydropyranyl (THP) ether **5**. Removal of the THP protective group of **5** gave alcohol **6**. Oxidation of **6** with Jones chromic acid furnished acid **7**. To enable the introduction of a hydroxy group at C-2, **7** was first brominated to give bromo acid (±)-**8**. Alkaline hydrolysis of (±)-**8** yielded hydroxy acid (±)-**9**. Asymmetric acetylation of (±)-**9** with vinyl acetate and lipase PS at 65°C (twice) in the presence of butylated hydroxytoluene (BHT) as an antioxidant was carried out according to the method of Sugai and Ohta.[10] Desired (*R*)-hydroxy acid **9** (98.1% e.e. as estimated by an HPLC analysis) remained intact after the enzymatic acetylation, while its (*S*)-isomer was acetylated to give (*S*)-**10**. Acetoxy acid (*S*)-**10** was more soluble in acetone than (*R*)-**9**, and therefore the latter could be separated from the former. Protection of the hydroxy group of (*R*)-**9** as a *t*-butyldimethylsilyl (TBS) ether completed the synthesis of acid part (*R*)-**11** in a 7.0% overall yield based on **2** (9 steps).

Scheme 2 illustrates the synthesis of sphingosine part **18** and its conversion to target pheromone **1**. Garner's *t*-butoxycarbonyl(Boc)-protected aldehyde **12**[11,12] was prepared from (*S*)-serine, and alkynylated with 1-dodecynyllithium to give **13** stereoselectively.[1-6] Reduction of **13** with lithium in ethylamine proceeded with concomitant deprotection to give C_{15}-sphingosine, whose hydroxy groups were protected as TBS ethers to afford **14**. The amino group at C-2 of **14** was then protected as *p*-toluenesulfonamide to furnish **15**. Epoxidation of the double bond of **15** with dimethyldioxirane (DMD) was followed by chromatographic purification of the products to give later-eluted β-epoxide **16** (Rf = 0.66; silica gel plate; solvent, hexane:EtOAc = 5:1) in a 68% yield and earlier-eluted α-epoxide (Rf = 0.76; silica gel plate; solvent, hexane:EtOAc = 5:1) in a 17% yield. Epoxidation of the double bond of a sphingosine with DMD is known to give a (4*R*,5*S*)-epoxide as the major isomer.[13]

Reduction of **16** with diisobutylaluminum hydride cleaved the epoxy ring to give alcohol **17**, whose *p*-toluenesulfonyl protective group was then removed by a treatment with sodium naphthalenide in 1,2-dimethoxyethane to yield **18**, after TBS protection of the hydroxy group at C-4. Acylation of protected sphingosine **18** with acid (*R*)-**11** was achieved with 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (EDC) and 1-hydroxybenzotriazole (HOBt),[4] and the product was treated with tetrabutylammonium fluoride (TBAF) to afford sex pheromone **1** as a waxy solid. The ^{1}H- and ^{13}C-NMR spectral properties of synthetic **1** were in good agreement with those reported for the natural pheromone. The overall yield of **1** was 10.0% based on **12** (10 steps) or 2.7% based on **2** (11 steps).



Scheme 2. Synthesis of Ceramide Pheromone **1**.
Reagents: (a) $HC\equiv C(CH_2)_9CH_3$, $CH_3(CH_2)_3Li$, THF (85%). (b) Li, $C_2H_5NH_2$. (c) TBSOTf, 2,6-lutidine, CH_2Cl_2 (2 steps, 72%). (d) TsCl, C_5H_5N (90%). (e) DMD, acetone (68%). (f) $[(CH_3)_2CHCH_2]_2AlH$, toluene (84%). (g) Na, naphthalene, $CH_3O(CH_2)_2OCH_3$. (h) TBSOTf, 2,6-lutidine, CH_2Cl_2 (2 steps, 81%). (i) EDC, HOBt, CH_2Cl_2 (55%). (j) TBAF, THF (71%).

In conclusion, we achieved an efficient synthesis of the hair crab pheromone. Unfortunately, Professor Fusetani has informed us that its bioassay is quite difficult at present, and the biological evaluation of our synthetic **1** will therefore remain as a task for the future.

Experimental

Melting point (mp) data were measured with a Yanaco MP-S3 instrument and are uncorrected. IR spectra were measured with a Jasco FT/IR-460 spectrometer. ^{1}H-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), 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) was used as the internal 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.

15-Methyl-1-hexadecanol (3). A dry THF solution

of 3-methylbutylmagnesium bromide was prepared from 3-methylbutyl bromide (14.2 g, 94.2 mmol) and magnesium (2.75 g, 113 mmol) in dry THF (90 ml). To a stirred and cooled solution of **2** (4.60 g, 17.3 mmol) in dry THF (50 ml) was added the resulting Grignard reagent and then a solution of Li_2CuCl_4 (0.12 M in dry THF; 8.0 ml, 0.96 mmol) at −78°C under Ar. The resulting mixture was allowed to warm to room temperature while stirring overnight. After the reaction mixture had been quenched with saturated aq. NH_4Cl, it was extracted with ethyl acetate. 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 to give **3** (4.42 g, 99%) as a colorless solid, mp 39–40°C. IR ν_{max} (KBr) cm^{-1}: 3350 (s, O-H). NMR δ_H (400 MHz, $CDCl_3$): 0.86 (6 H, d, J=6.6 Hz, 15-Me, 16-H_3), 1.10–1.40 (28 H, m, 2∼14-H_2, 15-H, 1-OH), 3.64 (2H, t, J=6.4 Hz, 1-H). *Anal.* Found: C, 79.46; H, 14.15%. Calcd. for $C_{17}H_{36}O$: C, 79.61; H, 14.15%.

15-Methylhexadecyl Tosylate (4). To a solution of **3** (4.30 g, 16.8 mmol) in pyridine (40 ml) was added *p*-toluenesulfonyl chloride (4.79 g, 25.1 mmol) at 0°C. After having been stirred overnight at 4°C, the reaction mixture was poured into dil. aq. HCl and extracted with diethyl ether. The extract was successively washed with saturated aq. $CuSO_4$, water, saturated aq. $NaHCO_3$ and brine, dried with $MgSO_4$, and concentrated under reduced pressure to give **4** (6.11 g, 89%) as a colorless solid. This was employed in the next step without further purification. IR ν_{max} (KBr) cm^{-1}: 1595 (w, Ar), 1465 (s, Ar), 1355 (m, SO_2), 1175 (m, SO_2). NMR δ_H (90 MHz, $CDCl_3$): 0.86 (6 H, d, J=6.2 Hz, 15-Me, 16-H_3), 1.10–1.40 (27 H, m, 2∼14-H_2, 15-H), 2.45 (3H, s, Ar-CH_3), 4.02 (2H, t, J=6.4 Hz, 1-H_2), 7.33 (2H, d, J=8.3 Hz, Ar), 7.79 (2H, d, J=8.3 Hz, Ar).

21-Methyl-1-tetrahydropyranyloxydocosane (5). A dry THF solution of 6-(2-tetrahydropyranyloxy)hexylmagnesium bromide was prepared from 6-(2-tetrahydropyranyloxy)hexyl bromide (13.4 g, 50.4 mmol) and magnesium (1.47 g, 60.5 mmol) in dry THF (90 ml). The resulting Grignard reagent and Li_2CuCl_4 (0.12 M in dry THF; 4.2 ml, 0.50 mmol) were successively added to a solution of **4** (6.11 g, 14.9 mmol) in dry THF (200 ml) at −78°C under Ar. This mixture was allowed to warm to room temperature while stirring overnight. After the reaction mixture had been quenched with saturated aq. NH_4Cl, it was 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 to give **5** (5.01 g, 79%) as a colorless solid, mp 33.5–34.5°C. IR ν_{max} (KBr) cm^{-1}: 1120 (m, C-O-C), 1035 (s, C-O-C). NMR δ_H (90 MHz, $CDCl_3$): 0.86 (6 H, d, J=6.2 Hz, 21-Me, 22-H_3), 1.05–1.35 (39 H, m, 3∼20-H_2, 4′-H_2, 21-H,), 1.38–1.88 (6H, m, 2-, 3′-, 5-H_2), 3.20–4.01 (4H, m, 1-, 6′-H_2), 4.56 (1H, br.s, 2′-H). *Anal.* Found: C, 79.17; H, 13.20%. Calcd. for $C_{28}H_{50}O_2$: C, 79.18; H, 13.29%.

21-Methyl-1-docosanol (6). To a stirred solution of **5** (4.97 g, 11.7 mmol) in ethanol (100 ml) was added *p*-toluenesulfonic acid monohydrate (*ca.* 0.1 g) at room temperature. After having been refluxed while stirring for 3 h, the resulting solution was quenched with saturated aq. $NaHCO_3$ at room temperature 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 to give **6** (3.87 g, 97%) as a colorless solid, mp 62–63°C. IR ν_{max} (KBr) cm^{-1}: 3375 (w, O-H), 1055 (m, C-O). NMR δ_H (90 MHz, $CDCl_3$): 0.86 (6 H, d, J=6.1 Hz, 21-Me, 22-H_3), 1.05–1.40 (40 H, m, 2∼20-H_2, 21-H, 1-OH), 3.64 (2H, t, J=6.4 Hz, 1-H_2). *Anal.* Found: C, 80.87; H, 14.05%. Calcd. for $C_{17}H_{36}O$: C, 81.10; H, 14.20%.

21-Methyldocosanoic Acid (7). To a stirred and cooled suspension of **6** (2.21 g, 6.49 mmol) in acetone (40 ml) was added Jones' CrO_3 reagent (2.69 M; 4.8 ml, 13.0 mmol) dropwise at 0°C, and the reaction mixture was stirred at room temperature for 1.5 h. After the reaction mixture had been quenched with 2-propanol, it was 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 to give **7** (1.70 g, 74%) as a colorless solid, mp 71–72°C. IR ν_{max} (KBr) cm^{-1}: 1700 (m, C=O). NMR δ_H (90 MHz, $CDCl_3$): 0.86 (6 H, d, J=6.2 Hz, 21-Me, 22-H_3), 1.00–1.80 (38 H, m, 3∼20-H_2, 21-H, 1-OH), 2.36 (2H, t, J=6.4 Hz, 2-H_2). *Anal.* Found: C, 77.65; H, 12.86%. Calcd. for $C_{23}H_{46}O_2$: C, 77.90; H, 13.07%.

(±)-2-Bromo-21-methyldocosanoic Acid [(±)-8]. Red phosphorus (311 mg, 10.0 mmol) was added to **7** (2.74 g, 7.73 mmol), and the mixture was heated at 95°C. Bromine (4.9 g, 1.6 ml, 30.9 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 (±)-**8** (2.83 g, 84%) as a colorless solid, mp 75–76°C. IR ν_{max} (KBr) cm^{-1}: 3000 (br, O-H), 1700 (m, C=O), 660 (w, C-Br). NMR δ_H

(90 MHz, $CDCl_3$): 0.86 (6 H, d, $J=6.2$ Hz, 21-Me, 22-H_3), 1.00–1.60 (36 H, m, 4 ~ 20-H_2, 21-H, 1-OH), 1.90–2.10 (2H, m, 3-H_2), 4.25 (1H, t, $J=7.3$ Hz, 2-H). *Anal.* Found: C, 63.77; H, 10.40%. Calcd. for $C_{23}H_{45}BrO_2$: C, 63.72; H, 10.46%.

(±)-2-Hydroxy-21-methyldocosanoic Acid [(±)-9]. Sodium hydroxide (2.1 g, 51 mmol) in water (25 ml) was added to (±)-**8** (2.79 g, 6.44 mmol), and the mixture was stirred and heated at 80°C for 24 h. After cooling, the mixture was acidified with dil. 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 (±)-**9** (2.15 g, 90%) as a colorless solid, mp 93–96°C. IR ν_{max} (KBr) cm^{-1}: 3510 (m, O-H) 3000 (br, COO-H), 1740 (m, C=O). NMR δ_H (90 MHz, $CDCl_3$): 0.86 (6 H, d, $J=6.2$ Hz, 21-Me, 22-H_3), 1.00–1.65 (39 H, m, 3 ~ 20-H_2, 21-H, 2-OH, COO-H), 4.27 (1H, t, $J=6.6$ Hz, 2-H). *Anal.* Found: C, 74.70; H, 12.71%. Calcd. for $C_{23}H_{46}O_3$: C, 74.57; H, 12.51%.

(R)-2-Hydroxy-21-methyldocosanoic Acid [(R)-9]. Lipase PS (Amano Pharmaceutical Co.; 1.06 g) was suspended in a solution of (±)-**9** (1.13 g, 3.05 mmol), and BHT (butylated hydroxytoluene, 40 mg) in vinyl acetate (11 ml) and THF (11 ml), and the mixture 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 (11 ml) and THF (11 ml) containing BHT (40 mg). Lipase PS (1.06 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*)-**9** (270 mg 23%) as a powder, mp 91–93°C. $[\alpha]_D^{22}$ −2.2 (*c* 0.5, $CHCl_3$:MeOH = 1:1). Its IR and ^{1}H-NMR spectra were identical with those of (±)-**9**. *Anal.* Found: C, 74.64; H, 12.72%. Calcd. for $C_{23}H_{46}O_3$: C, 74.54; H, 12.51%.

Determination of the Enantiomeric Purity of (R)-9. (*S*)-α-Methoxy-α-trifluoromethylphenylacetyl chloride (MTPA Cl; 50 mg, 0.2 mmol) was added to a solution of the methyl ester derived from (*R*)-**9** (50 mg, 0.13 mmol; prepared by treating (*R*)-**9** with 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 ethereal solution was successively washed with sat. $CuSO_4$ aq., sat. $NaHCO_3$ aq., 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*)-**9** methyl ester: $t_R = 13.89$ min (99.05%); (*R*)-MTPA ester of the (*S*)-**9** methyl ester: $t_R = 17.53$ min (0.95%). The enantiomeric purity of (*R*)-**9** was therefore 98.1% e.e.

(R)-2-tert-Butyldimethylsilyloxy-21-methyldocosanoic Acid [(R)-11]. To a stirred solution of (*R*)-**9** (50 mg, 0.13 mmol) and imidazole (35 mg, 0.52 mmol) in DMF (5 ml) was added TBSCl (58 mg, 0.39 mmol) at 0°C. The reaction mixture was stirred for 4 h at room temperature and then quenched with water. It was extracted with diethyl ether, before the extract was succesively washed with water and brine, dried with $MgSO_4$, and concentrated under reduced pressure. The residue was diluted with THF (5 ml) and then acidified with 0.1 M HCl (1 ml). The reaction mixture was stirred for 4 h at room temperature. It was then poured into water 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 to give (*R*)-**11** (50.4 mg, 80%) as a colorless oil, n_D^{22} 1.4421. $[\alpha]_D^{25}$ + 1.63 (*c* 1.05, $CHCl_3$). IR ν_{max} (film) cm^{-1}: 1725 (C=O), 1250 (Si-O). NMR δ_H (500 MHz, $CDCl_3$): 0.13 (6H, s, Si-Me), 0.88 (6H, d, $J=6.1$ Hz, 21-Me, 22-H_3), 0.94 (9H, s, *t*-Bu), 1.16–1.38 (38H, m, 3 ~ 20-H_2, 21-H, O-H), 4.29 (1H, t, $J=5.4$ Hz, 2-H). *Anal.* Found: C, 71.79; H, 12.25%. Calcd. for $C_{29}H_{60}O_3Si$: C, 71.84; H, 12.47%.

tert-Butyl (4S,1′R)-4-(1′-hydroxy-2′-tridecynyl)-2,2-dimethyl-3-oxazolidinecarboxylate (13). To a stirred solution of 1-dodecyne (8.55 g, 51.4 mmol) in dry THF (90 ml) was added *n*-butyllithium (1.56 M in hexane; 28 ml, 43.8 mmol) dropwise at −40°C under Ar. After the reaction mixture had been stirred at −23°C for 30 min, a solution of Garner's aldehyde (**12**, 8.63 g, 37.6 mmol) in dry THF (90 ml) was added dropwise at −30°C. After having been stirred at −23°C for 1.5 h, the resulting solution was quenched with 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 to give **13** (12.7 g, 85%) as a colorless oil, n_D^{23} 1.4661. $[\alpha]_D^{25}$ −46.6 (*c* 1.00, $CHCl_3$). IR ν_{max} (film) cm^{-1}: 3450 (br.w, OH), 2230 (w, C≡C), 1700 (s, C=O). NMR δ_H (500 MHz, $CDCl_3$): 0.88 (3H, t, $J=7.1$ Hz, 13′-H), 1.20–1.41 (15H, m, *t*-Bu, 2-Me, 2-Me), 1.43–1.66 (17H, m, 5′ ~ 12′-H_2, 1′-OH), 2.19 (2H, t, $J=5.8$ Hz, 4′-H_2), 3.90 (1H, br.s, 1′-H), 4.05–4.12 (2H, m, 3-H), 4.66 (1H, br.s, 4-H). *Anal.* Found: C, 69.60; H, 10.27; N, 3.26%. Calcd. for $C_{23}H_{41}NO_4$: C, 69.83; H, 10.45; N, 3.54%.

(2S,3R,4E)-2-Amino-1,3-bis-tert-butyldimethylsilyloxy-4-pentadecene (14). A solution of **13** (11.8 g,

29.8 mmol) in dry THF (100 ml) was added dropwise to a blue solution of lithium (3.01 g, 447 mmol) in ethylamine (88 g) while stirring at −70°C for 1 h. The mixture was then stirred overnight while being allowed to warm to room temperature, before being quenched with NH_4Cl (*ca.* 48 g, 894 mmol). After removing the ethylamine by evaporation, the mixture was diluted with water and extracted with diethyl ether. The extract was successively washed with water and brine, dried with Na_2SO_4, and concentrated under reduced pressure to give the reduction product (8.14 g, quant.) as a brown waxy solid. This reduction product was used for the next reaction without purification.

To a stirred mixture of the reduction product (8.14 g, 31.6 mmol) in dry CH_2Cl_2 (100 ml) were added 2.6-lutidine (2,6-dimethylpyridine; 18.4 ml, 160 mmol) and TBSOTf (29 ml, 128 mmol) at 0°C. After having been stirred at room temperature for 1 h, the resulting solution was quenched with methanol. 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 to give **14** (10.4 g, 85%) as a pale yellow oil, n_D^{24} 1.4542. $[\alpha]_D^{24}$ −1.48 (*c* 1.01, $CHCl_3$). IR ν_{max} (film) cm^{-1}: 1255 (m, Si-Me). NMR δ_H (500 MHz, $CDCl_3$): 0.01, 0.04 (6H, each s, Si-Me), 0.06 (6H, s, Si-Me), 0.86–0.90 (21H, m, *t*-Bu, 15-H_3), 1.22–1.32 (16H, m, 7∼14-H_2), 1.34–1.41 (2H, m, N-H_2), 2.04 (2H, dd, J=13.6, 6.7 Hz, 6-H_2), 2.78 (1H, dt, J=6.7, 4.9 Hz, 2-H), 3.55 (1H, dd, J=9.8, 6.7 Hz, 1-H_aH_b), 3.70 (1H, dd, ϑ=9.8, 4.9 Hz, 1-H_aH_b), 4.04 (1H, t, J=6.7 Hz, 3-H), 5.38 (1H, dd-like, J=15.4, 6.7 Hz, 4-H), 5.70 (1H, dt, J=15.4, 6.7 Hz, 5-H). *Anal.* Found: C, 66.47; H, 12.28; N, 2.79%. Calcd. for $C_{27}H_{59}NO_2Si_2$: C, 66.74; H, 12.24; N, 2.88%.

(2S,3R,4E)-1,3-bis-tert-Butyldimethylsilyloxy-2-p-toluenesulfonylamino-4-pentadecene (15). To an ice-cooled solution of **14** (9.78 g, 20.1 mmol) in dry pyridine (90 ml), TsCl (5.76 g, 30.2 mmol) was added, and the mixture was stirred for 24 h at room temperature. The reaction mixture was then poured into dil. aq. HCl and extracted with diethyl ether. The extract was successively washed with saturated aq. $CuSO_4$, water, saturated aq. $NaHCO_3$ and brine, dried with $MgSO_4$, and concentrated under reduced pressure. The residue was chromatographed on silica gel to give **15** (11.6 g, 90%) as a colorless oil, n_D^{24} 1.4834. $[\alpha]_D^{26}$ −2.43 (*c* 1.04, $CHCl_3$). IR ν_{max} (film) cm^{-1}: 3290 (m, N–H), 1600 (w, Ar), 1335 (m, SO_2), 1255 (m, Si-Me), 1165 (s, SO_2). NMR δ_H (90 MHz, $CDCl_3$): −0.05 (6H, s, Si-Me), −0.01, 0.00 (6H, each s, Si-Me), 0.76–0.98 (21H, m, *t*-Bu, 15-H_3), 1.26 (16H, m, 7∼14-H_2), 1.96 (2H, m, 6-H_2), 2.41 (3H, s, Ar-Me), 3.15 (1H, m, 2-H), 3.45 (1H, dd, J=10.2, 6.1 Hz, 1-H_aH_b), 3.81 (1H, dd, J=10.2, 4.1 Hz, 1-H_aH_b), 4.24 (1H, dd-like, J=6.4, 5.9 Hz, 3-H), 4.63 (1H, d, J=6.8 Hz, N–H), 5.21 (1H, dd, J=15.8, 6.4 Hz, 4-H), 5.60 (1H, dt-like, J=15.8, 6.1 Hz, 5-H), 7.27 (2H, d, J=8.2 Hz, *m*-Ar), 7.74 (2H, d, J=8.2 Hz, *o*-Ar). *Anal.* Found: C, 64.07; H, 10.49; N, 1.93%. Calcd. for $C_{34}H_{65}NO_4SSi_2$: C, 63.79; H, 10.23; N, 2.19%.

(2S,3S,4R,5S)-1,3-bis-tert-Butyldimethylsilyloxy-4,5-epoxy-2-p-toluenesulfonylaminopentadecane (16). A solution of dimethyldioxirane in acetone (prepared from 250 g of Oxone®, 120 g of $NaHCO_3$, 200 ml of H_2O and 160 ml of acetone) was added to **15** (200 mg, 0.31 mmol) at 0°C, and the reaction mixture was stirred at 0°C for 4 d, before being concentrated under reduced pressure. The residue was chromatographed on silica gel to give later-eluted *β*-epoxide **16** (139 mg, 68%) as a colorless oil, n_D^{24} 1.4827. $[\alpha]_D^{25}$ −20.2 (*c* 1.05, $CHCl_3$). IR ν_{max} (film) cm^{-1}: 3290 (w, N–H), 1600 (w, Ar), 1335 (m, SO_2), 1255 (m, Si-Me), 1165 (s, SO_2), 1095 (m, Si–O). NMR δ_H (500 MHz, $CDCl_3$): −0.05, 0.02, 0.04, 0.05 (total 12H, each s, Si-Me), 0.82 (9H, s, *t*-Bu), 0.85 (9H, s, *t*-Bu), 0.88 (3H, t, J=6.9 Hz, 15-H_3), 1.26–1.43 (16H, br.s, 7∼14-H_2), 1.52–1.61 (2H, m, 6-H_2), 2.41 (3H, s, Ar-Me), 2.66 (1H, dd, J=5.2, 2.2 Hz, 4-H), 2.78 (1H, m, 5-H), 3.27 (1H, ddt, J=6.7, 5.2, 4.9 Hz, 2-H), 3.55 (1H, dd, J=10.4, 4.9 Hz, 1-H_aH_b), 3.71 (1H, dd, J=10.4, 4.9 Hz, 1-H_aH_b), 3.77 (1H, t, J=5.2 Hz, 3-H), 4.76 (1H, d, J=6.7 Hz, N–H), 7.27 (2H, d, J=8.1 Hz, *m*-Ar), 7.75 (2H, J=8.1 Hz, *o*-Ar). *Anal.* Found: C, 62.06; H, 9.79; N, 1.87%. Calcd. for $C_{34}H_{65}NO_5SSi_2$: C, 62.24; H, 9.99; N, 2.13%.

The earlier-eluted *α*-epoxy isomer was also obtained as an oil (36 mg, 17%).

(2S,3S,4R)-1,3-bis-tert-Butyldimethylsilyloxy-2-p-toluenesulfonylamino-4-pentadecanol (17). To a stirred and cooled solution of **16** (1.63 g, 2.48 mmol) in dry toluene (20 ml), a solution of diisobutylaluminum hydride (1.01 M in toluene; 8.8 ml, 8.9 mmol) was added dropwise at −78°C under Ar. This mixture was warmed gradually to 0°C while stirring for 3 h, quenched with saturated aq. Rochelle's salt and then diethyl ether was added. The resulting slurry was stirred at room temperature until two clear layers had formed. These layers were separated, and the aqueous layer was extracted with diethyl ether. The extract was successively washed with water and brine, dried with Na_2SO_4, and concentrated under reduced pressure. The residue was chromatographed on silica gel to give **17** (1.37 g, 84%) as a colorless oil, n_D^{25} 1.4841. $[\alpha]_D^{25}$ −1.51 (*c* 1.03, $CHCl_3$). IR ν_{max} (film) cm^{-1}: 3540 (m, O-H), 3320 (m, N–H), 1600 (m, Ar), 1335 (s, SO_2), 1255 (s, Si-Me), 1160 (s, SO_2). NMR δ_H (500 MHz, $CDCl_3$): −0.05, −0.02, 0.09, 0.11 (total 12H, each s, Si-Me), 0.82 (9H, s, *t*-Bu), 0.88

(9H, s, *t*-Bu), 0.88 (3H, t, $J=6.9$ Hz, 15-H_3), 1.18–1.38 (18H, m, 6～14-H_2), 1.40–1.51 (2H, m, 5-H_2), 2.42 (3H, s, Ar-Me), 2.57 (1H, br, 4-OH), 3.41 (1H, m, 4-H), 3.52–3.60 (2H, m, 1-H_aH_b, 2-H), 3.69 (1H, dd, $J=10.2$, 6.5 Hz, 1-H_aH_b), 3.81 (1H, dd, $J=4.7$, 3.2 Hz, 3-H), 4.81 (1H, d, $J=6.7$ Hz, N–H), 7.29 (2H, d, $J=8.3$ Hz, *m*-Ar), 7.73 (2H, d, $J=8.3$ Hz, *o*-Ar). *Anal.* Found: C, 61.88; H, 10.28; N, 2.13%. Calcd. for $C_{34}H_{67}NO_5SSi_2$: C, 62.05; H, 10.26; N, 2.13%.

*(2S,3S,4R)-2-Amino-1,3,4-tris-tert-butyldimethylsilyloxypentadecane (**18**).* Preparation of sodium naphthalenide: To a stirred solution of naphthalene (2.47 g, 19.3 mmol) in dry DME (20 ml), Na (400 mg, 17.4 mmol) was added under Ar. The mixture was stirred for 2 h at room temperature.

To a solution of **17** (1.26 g, 1.93 mmol) in dry DME (20 ml), the prepared sodium naphthalenide was added dropwise at −78°C under Ar. The mixture was then warmed gradually to −10°C while stirring for 4 h and diluted with water. The mixture was extracted with diethyl ether. The extract was successively washed with water and brine, dried with K_2CO_3, and concentrated under reduced pressure. The residue was chromatographed on silica gel to give recovered **17** (526 mg, 42%) and the detosylation product (490 mg, 87% based on consumed **17**) as a yellow oil. This detosylation product was used for the next reaction without further purification.

To a stirred mixture of the detosylation product (490 mg, 0.972 mmol) in dry CH_2Cl_2 (5 ml) were added 2,6-lutidine (2,6-dimethylpyridine; 340 μl, 2.92 mmol) and TBSOTf (450 μl, 1.94 mmol) at 0°C. After having been stirred at room temperature for 1 h, the resulting solution was quenched with methanol. 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 K_2CO_3, and concentrated under reduced pressure. The residue was chromatographed on silica gel to give **18** (556 mg, 2 steps, 81% based on consumed **17**) as a pale yellow oil, n_D^{24} 1.4551. $[\alpha]_D^{25}$ −6.30 (*c* 1.00, $CHCl_3$). IR ν_{max} (film) cm^{-1}: 1255 (s, Si-Me). NMR δ_H (500 MHz, $CDCl_3$): 0.05, 0.06, 0.063, 0.07, 0.09 (total 18H, each s, Si-Me), 0.85–0.93 (30H, m, *t*-Bu, 15-H_3), 1.18–1.34 (18H, m, 6～14-H_2), 1.35–1.44 (2H, m, 5-H_2), 1.45–1.59 (2H, m, NH_2), 2.92 (1H, m, 2-H), 3.47 (1H, dd, $J=9.5$, 8.1 Hz, 3-H), 3.56 (1H, br.d, $J=6.4$ Hz, 1-H_a), 3.81 (1H, ddd, $J=8.1$, 4.9, 1.5 Hz, 4-H), 3.84 (1H, dd, $J=9.8$, 3.7 Hz, 1-H_b). *Anal.* Found: C, 64.18; H, 12.23; N, 2.48%. Calcd. for $C_{33}H_{75}NO_3Si_3$: C, 64.11; H, 12.23; N, 2.27%.

*(2S,2′R,3S,4R)-2-(2′-Hydroxy-21′-methyldocosanoylamino)-1,3,4-pentadecanetriol (**1**).* To a stirred solution of (*R*)-**11** (31.5 mg, 0.065 mmol) and **18** (40.2 mg, 0.065 mmol) in dry CH_2Cl_2 (5 ml) was added EDC [1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide hydrochloride; 18.7 mg, 0.098 mmol] and HOBt (1-hydroxybenzotriazole; 25.6 mg, 0.20 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 to give the product (38 mg, 0.035 mmol) as a yellow oil. This product was used for the next reaction without further purification. To a stirred mixture of the product (38 mg, 0.035 mmol) in dry THF (1 ml) was added TBAF in THF (1.0 M, 0.21 ml, 0.21 mmol) at 0°C under Ar, and stirring was continued for 4 h at room temperature. The reaction mixture was poured into brine and extracted with $CHCl_3$. 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 to give **1** (15 mg, 2 steps, 40% based on consumed **18**) as a colorless solid, mp 129–133°C. $[\alpha]_D^{22}$ +14 (*c* 0.70, $CHCl_3$:MeOH = 1:1). IR ν_{max} (film) cm^{-1}: 3340 (br.m, O-H, and N–H), 2915 (s), 2850 (s), 1645 (m, C=O), 1560 (w), 1470 (m), 1080 (w), 720 (w). NMR δ_H (400 MHz, $CDCl_3$:CD_3OD = 1:1): 0.82–0.85 (9H, m, 15-, 22′-H_3, 21-Me), 1.10–1.35 (57H, m, 6～14-H_2, 4′～20′-H_2, 21′-H, 1-OH, 3-OH, 4-OH, 2′-OH), 1.35–1.50 (4H, m, 5-, 3′-H_2), 3.49–3.57 (2H, m, 2′-, 4-H), 3.71 (1H, dd, $J=11.1$, 4.7 Hz, 1-H_aH_b), 3.76 (1H, dd, $J=11.2$, 4.9 Hz, 1-H_aH_b), 4.01 (1H, dd, $J=8.0$, 3.9 Hz, 3-H), 4.09 (1H, m, 2-H), 4.43 (1H, s, N–H). NMR δ_C (100 MHz, $CDCl_3$:CD_3OD = 1:1): 13.7, 14.3, 22.8, 23.0, 25.6, 26.2, 27.7, 28.3, 29.70, 29.86, 29.94, 29.99, 30.01, 30.04, 30.09, 30.26, 32.27, 32.99, 34.8, 39.4, 51.89, 51.98, 61.3, 72.30, 72.65, 75.6, 176.3. These spectral data are in good agreement with the reported values.[7,8] HRFABMS *m/z* $(M+H)^+$: calcd. for $C_{38}H_{78}NO_5$, 628.5880; found, 628.5879.

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References

1) Abe, T. and Mori, K., Synthesis of sphingosine relatives, XV. Synthesis of (2*S*,2′*S*,3*R*,4*E*,8*E*)-*N*-2′-hydroxyoctadecanoyl-1-*O*-(β-D-glucopyranosyl)-9-methyl-4,8-sphingadienine (Pen III), a cerebroside isolated from *Penicillium funiculosum* as the fruiting-inducer against *Schizophyllum commune*. *Biosci. Biotechnol. Biochem.*, **58**, 1671–1674 (1994).
2) Mori, K. and Uenishi, K., Synthesis of sphingosine relatives, XVII. Synthesis of (2*S*,2′*R*,3*R*,3′*E*,4*Z*,8*E*)-1-*O*-(β-D-glucopyranosyl)-*N*-(2′-hydroxy-3′-

octadecanoyl)-9-methyl-4,8-sphingadienine (Pen II), the major cerebroside isolated from *Penicillium funiculosum* as the fruiting-inducer against *Schizophyllum commune*. *Liebigs Ann.*, 1–6 (1996).
3) Yajima, A., Takikawa, H., and Mori, K., Synthesis of sphingosine relatives, XVIII. Synthesis of penazetidine A, an alkaloid inhibitor of protein kinase C isolated from marine sponge *Penares sollasi*. *Liebigs Ann.*, 1083–1089 (1996).
4) Mori, K. and Uenishi, K., Synthesis of sphingosine relatives, XIV. A new synthesis of symbioramide, a Ca^{++}-ATPase activator from *Symbiodinium* sp. *Liebigs Ann. Chem.*, 41–48 (1994).
5) Takikawa, H., Maeda, T., Seki, M., Koshino, K., and Mori, K., Synthesis of sphingosine relatives, XIX. Synthesis of penaresidin A and B, azetidine alkaloids with actomyosin ATPase activating properties. *J. Chem. Soc., Perkin Trans. 1*, 97–111 (1997).
6) Seki, M. and Mori. K., Synthesis of a prenylated and immunosuppressive marine galactosphingolipid with cyclopropane-containing alkyl chains: (2*S*,3*R*,11*S*,12*R*,2‴*R*,5‴*Z*,11‴*S*,12‴*R*)-plakoside A, and its (2*S*,3*R*,11*R*,12*S*,2‴*R*,5‴*Z*,11‴*R*,12‴*S*) isomer. *Eur. J. Org. Chem.*, 3797–3809 (2001).
7) Asai, N., Fusetani, N., Matsunaga, S., and Sasaki, J., Sex pheromone of the hair crab *Erimacrus isenbeckii*, Part 1: Isolation and structure of novel ceramides. *Tetrahedron*, **56**, 9895–9899 (2000).
8) Asai, N., Fusetani, N., and Matsunaga, S., Sex pheromone of the hair crab *Erimacrus isenbeckii*. II. Synthesis of ceramides. *J. Nat. Prod.*, **64**, 1210–1215 (2001).
9) Fouquet, C. and Schlosser, M., Improved carbon-carbon linking by controlled copper catalysis. *Angew. Chem. Int. Ed. Engl.*, **13**, 82–83 (1974).
10) Sugai, T. and Ohta, H., Lipase-catalyzed kinetic resolution of 2-hydroxyhexadecanoic acid and its esters. *Agric. Biol. Chem.*, **54**, 3337–3338 (1990).
11) Garner, P. and Park, J. M., The synthesis and configurational stability of differentially protected β-hydroxy-α-amino aldehydes. *J. Org. Chem.*, **52**, 2361–2364 (1987).
12) Garner, P., Park, J. M., and Malecki, E., A stereodivergent synthesis of D-*erythro*-sphingosine and D-*threo*-sphingosine from L-serine. *J. Org. Chem.*, **53**, 4395–4398 (1988).
13) Takikawa, H., Muto, S., and Mori, K., Synthesis of sphingosine relatives, XX. Diastereoselective epoxidation of the double bond at C-4 of sphingosines to provide phytosphingosine relatives such as α-galactosylceramide KRN 7000. *Tetrahedron*, **54**, 3141–3150 (1998).