Sex Pheromones of the Hair Crab *Erimacrus isenbeckii*. II. Synthesis of Ceramides

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To confirm their structures and to assess the pheromonal activity, novel ceramides, possible sex pheromones of the hair crab *Erimacrus isenbeckii*, were synthesized from D-galactose. The synthetic ceramides were identical with the natural ceramides.

The hair crab *Erimacrus isenbeckii*, a commercially important species, exhibits a series of typical mating behaviors: precopulatory guarding (male grasps female), molting of female, copulation, and postcopulatory guard. The involvement of a pheromone released from pre- and postmolt females in these behaviors was shown by "sponge assay". A sexually competent male exhibits the typical mating behaviors toward a bath sponge containing water conditioned with pre- or postmolt females. Bioassay-guided isolation furnished a mixture of novel ceramides as possible sex pheromones.[1] It is well known that either pheromone structures or the combination of components are crucial for pheromonal activity in insect pheromones.[2] To confirm the structures proposed for the sex pheromones and to assess pheromonal activity of this ceramide mixture, we attempted to synthesize all ceramides. This paper deals with total synthesis of the ceramides.

Results and Discussion

There are many reports of syntheses of ceramides (cerebrosides);[3−7] syntheses of ceramides consisting of 2-hydroxy fatty acids and phytosphingosines have also been established. Usually, (2*S*,3*S*,4*R*)-phytosphingosines were synthesized from natural chiral pools such as D-galactose,[9,10] D-xylose,[11] L-ascorbic acid,[12] glyceraldehyde,[13] and L-serine.[14] The present work employed a protocol of Akiyama and Natori[15] starting from D-galactose, because of its advantage in large-scale synthesis. On the other hand, racemic synthesis of the fatty acid portion starting from 9-bromononanol was envisioned.[16] After the individual synthesis of two sphingosines (C_{15}, C_{16}) and three fatty acids (C_{23}, C_{24}, C_{25}), a combination of these components via mixed condensation was expected to afford a ceramide mixture. Acylation of the phytosphingosines with the racemic fatty acids was expected to give a diastereomeric mixture from which the desired 2′*R* isomer could be separated. Our synthetic strategy for ceramides is depicted in Scheme 1.

Syntheses of the C_{15} Phytosphingosine (12a) and C_{16} Phytosphingosine (12b). Starting from β-D-galactose pentaacetate (**2**), 3,4,6-tri-*O*-benzyl-D-galactopyranose (**6**) was prepared in four steps in 54% yield from **2**.[17] Oxidation of **6** with sodium metaperiodate provided an aldehyde **7**,[18] which was directly subjected to Wittig reaction without purification; **7** was treated with a C_{10} phosphonium salt prepared from bromodecane to afford a mixture of geometrically isomeric alcohols **8a**. Mesylation of **8a** followed by hydrogenation gave rise to a tetraol monomesylate **10a** in 73% yield from **6**. The mesylate **10a** was converted to an azide **11a** with sodium azide in 82% yield. Finally, catalytic hydrogenation of the azide afforded the C_{15} phytosphingosine **12a** in 32% overall yield.

Similarly, C_{16} phytosphingosine was synthesized. The aldehyde **7** and C_{11} phosphonium salt prepared from bromododecane were coupled to give alcohols **8b**. A four-step reaction sequence from **8b** afforded the C_{16} phytosphingosine **12b** in 23% overall yield from **7**.

Synthesis of C_{23} Fatty Acid 21a. The isopropyl terminus was initially introduced by treatment of 9-bromo-1-nonanal (**14**), which was obtained by PCC oxidation of 9-bromo-1-nonanol (**13**), with the ylide of isobutyltriphenylphosphonium bromide, which furnished a mixture of geometric isomers of branched bromoalkenes **15**. The mixture was heated with triphenylphosphine to give phosphonium salts **16**. Without purification, **16** was again subjected to a Wittig reaction with 8-bromo-1-octanal. The resulting diene **17a** showed complex ^{1}H NMR signals probably due to olefin migration, which could consequently be converted to a single bromoalkane **18a** by hydrogenation. Then, conventional acetamidomalonic ester synthesis was applied to **18a**. The resulting mixture was hydrolyzed and decarboxylated to afford racemic amino acid **19a**. Deamination of **19a** was effected with $NaNO_2$ in an acidic solution (H_2SO_4/H_2O/1,4-dioxane) to give the 2-hydroxy fatty acid **20a**. This was acetylated to afford **21a** with Ac_2O and pyridine for the following condensation step (5.7% overall yield from **13**).

Synthesis of C_{24} (21b) and C_{25} (21c) Fatty Acids. In a similar manner used for the preparation of the C_{23} fatty acid **21a**, C_{24} **21b** and C_{25} **21c** fatty acids were synthesized using 9-bromononanal or 10-bromodecanal in the second Wittig reaction. Acetates of the C_{24} and C_{25} fatty acids were synthesized in 4.9% and 14.5% overall yield, respectively.

Synthesis of the Hair Crab Ceramide Mixture (1a). Prior to the condensation step, each of the sphingosines and fatty acids was mixed according to the natural abundance [**12a**:**12b** (4:5); **21a**:**21b**:**21c** (5:5:3)], which had been roughly estimated from both HPLC and FABMS data of the natural ceramide mixture. Coupling of these components by using WSCI (water-soluble carbodiimide)/HOBt (1-hydroxybenzotriazole) and subsequent deacetylation with K_2CO_3 gave a diastereomeric mixture of ceramides **1a** and **1b**. Silica gel TLC ($CHCl_3$/MeOH, 95:5) clearly showed two well-separated spots, of which the slower-moving spot (**1a**) corresponded to the natural 2′*R* ceram-

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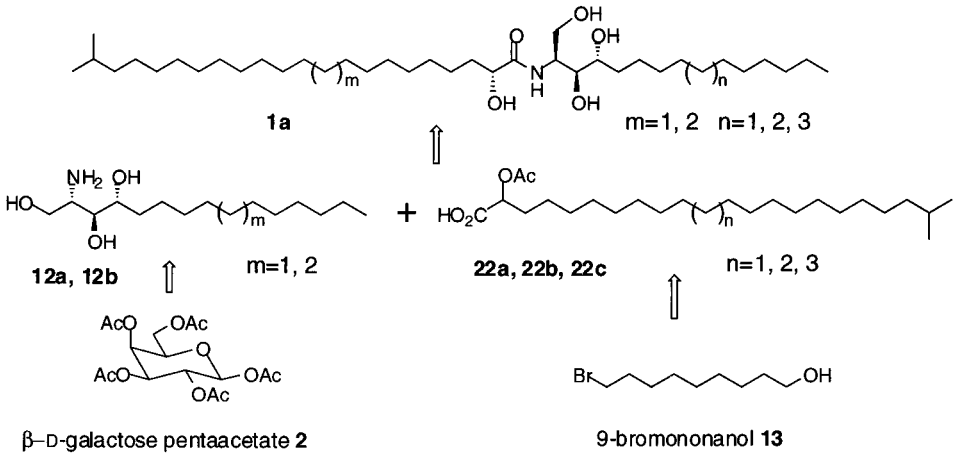
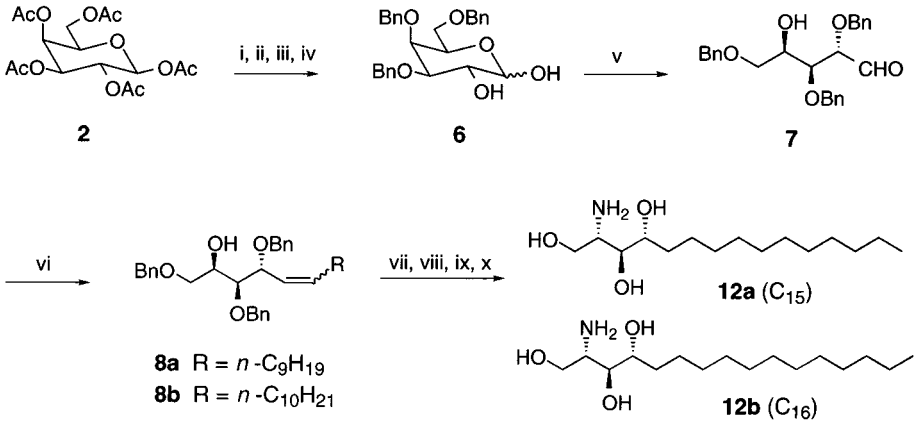
Scheme 1. Synthetic Plan for the Ceramide Mixture

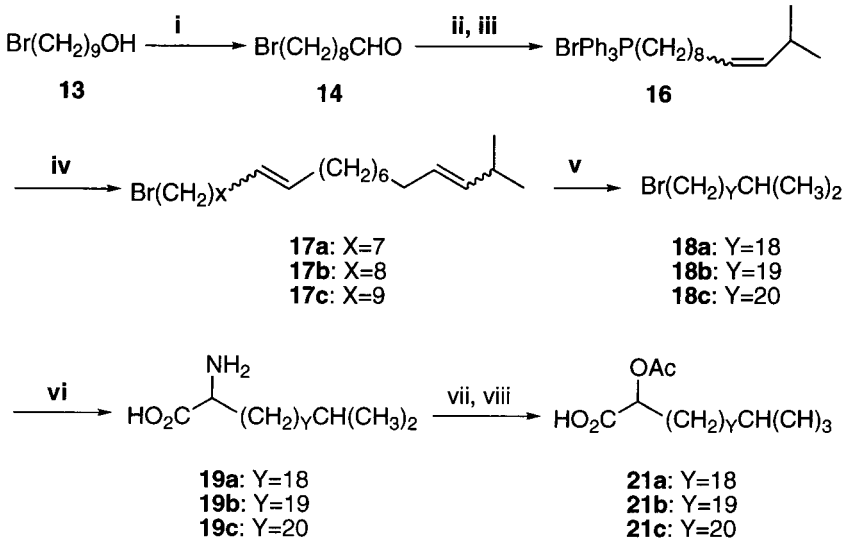


Scheme 2[a]



[a] Reagents and conditions: (i) HBr/AcOH; (ii) MeOH/TEA; (iii) BnCl/KOH; (iv) H_2SO_4; (v) $NaIO_4$; (vi) *n*-$C_{10}H_{21}PPh_3Br$ or *n*-$C_{11}H_{23}PPh_3Br$/*n*-BuLi; (vii) MsCl/pyridine; (viii) H_2/Pd−C; (ix) NaN_3; (x) H_2/Pd−C.

Scheme 3[a]



[a] Reagents and conditions: (i) PCC; (ii) *i*-$BuPPh_3Br$/n-BuLi; (iii) PPh_3; (iv) 8-bromooctanal or 9-bromononanal or 10-bromodecanal/*n*-BuLi; (v) H_2/Pd−C; (vi) $(EtO_2C)_2CHNHAc$/EtONa, then HCl; (vii) $NaNO_2$; (viii) Ac_2O/pyridine.

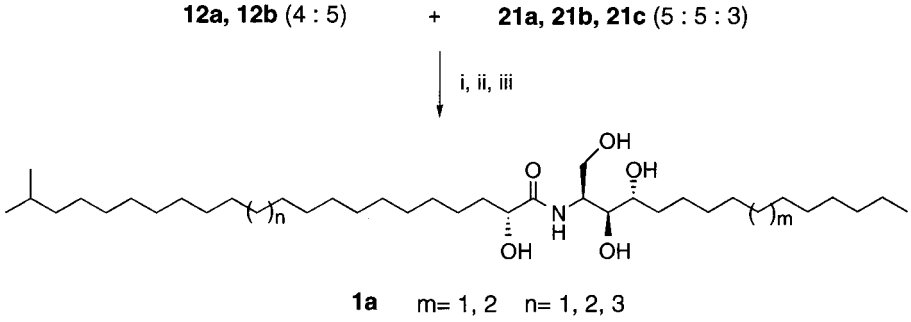
ides. This was then separated by silica gel column chromatography with $CHCl_3$/MeOH solvent systems.

The synthetic ceramide mixture **1a** was identical with the natural product in the ^1H and ^{13}C NMR spectra.[19] In addition, HPLC profiles of **1a** were almost superimposable on those of the natural ceramide mixture, except for the absence of minor components. However, no mating behavior of male crabs could be observed for the synthetic ceramide mixture, because no sexually competent males were available.

Experimental Section

General Experimental Procedures. NMR spectra were recorded on either a Bruker AC-300 (300 MHz for ^1H, 75 MHz

Scheme 4[a]



1a m= 1, 2 n= 1, 2, 3

[a] Reagents and conditions: (i) WSCI/HOBt; (ii) K_2CO_3; (iii) Si gel column separation.

for ^{13}C), a JEOL JMN-α600 (600 MHz for ^1H, 150 MHz for ^{13}C), or a JEOL JMN-α500 (500 MHz for ^1H, 125 MHz for ^{13}C) NMR spectrometer at 303 K. ^1H chemical shifts were referenced to residual solvent peaks: δ 7.24 for $CHCl_3$ in $CDCl_3$; δ 3.30 for CD_2HOD in CD_3OD; δ 3.30 for CD_2HOD in $CDCl_3$/CD_3OD (1:1). ^{13}C chemical shifts were referenced to ^{13}C signals of $CDCl_3$ (δ 77.0) or CD_3OD (δ 49.0). FAB mass spectra were measured on a JEOL JMX-SX102/SX102 tandem mass spectrometer. Glycerol or *m*-nitrobenzyl alcohol (NBA) was used as a matrix. Air- and moisture-sensitive reactions were carried out under an atmosphere of argon. When necessary, solvents were distilled from dehydrating agents (THF from sodium and benzophenone; EtOH from sodium and diethylphthalate; CH_2Cl_2, toluene, MeOH, and 1,2-dichloroethane from CaH_2). Routine monitoring of reactions and preparative TLC were performed using precoated Si gel TLC plates (Merck 60 F_{254}). Spots were visualized with either H_2SO_4, anisaldehyde-H_2SO_4, or ninhydrin reagent. Column chromatography was performed on Merck Si gel 60 (60−230 mesh).

3,4,6-Tri-*O*-acetyl-1,2-*O*-(1-methoxyethylidene)-α-D-galactopyranose (4). To penta-*O*-acetyl-*β*-D-galactose (**2**) (25.0 g, 64.1 mmol) was added 30% HBr/AcOH (50 mL), and the mixture was stirred for 3 h at room temperature. The reaction mixture was azeotropically evaporated with toluene under reduced pressure to afford a bromide **3**. Without purification, it was dissolved in 1,2-dichloroethane (200 mL) and triethylamine (18.0 mL, 129 mmol), MeOH (2.74 mL, 67.6 mmol), and *n*-tetrabutylammonium bromide (10.3 g, 31.9 mmol) were added. The mixture was stirred for 16 h at 45 °C, and the precipitated salt was removed by filtration. The filtrate was washed with brine and concentrated in vacuo. The residue was chromatographed over Si gel. Elution with *n*-hexane/EtOAc (9:1, 8:2, and 7:3) gave 22.1 g of **4** (61.0 mmol, 95% from **2**): ^1H NMR (300 MHz, $CDCl_3$) δ 5.78 (1H, d, J = 4.8 Hz), 5.40 (1H, dd, J = 2.9, 2.7 Hz), 5.03 (1H, dd, J = 6.8, 3.3 Hz), 4.29 (2H, m), 4.10 (2H, m), 3.27 (3H, s), 2.08 (3H, s), 2.04 (3H, s), 2.03 (3H, s), 1.64 (3H, s).

3,4,6-Tri-*O*-benzyl-1,2-*O*-(1-methoxyethylidene)-α-D-galactopyranose (5). To a stirred solution of **4** (22.0 g, 60.8 mmol) in toluene (60 mL) were added powdered KOH (35.0 g, 620 mmol) and benzyl chloride (48.0 mL, 417 mmol). The mixture was gradually heated over 1 h and refluxed for 1.5 h. It was then diluted with toluene and washed with water and brine. The toluene solution was concentrated in vacuo and chromatographed over Si gel. Elution with *n*-hexane/EtOAc (8:2) gave 19.1 g of **5** (37.5 mmol, 62%): ^1H NMR (300 MHz, $CDCl_3$) δ 7.40−7.20 (15H, m), 5.73 (1H, d, J = 4.5 Hz), 4.90 (1H, d, J = 11.5 Hz), 4.79 (1H, d, J = 12.2 Hz), 4.66 (1H, d, J = 12.2 Hz), 4.59 (1H, d, J = 11.9 Hz), 4.49 (1H, d, J = 11.9 Hz), 4.42 (1H, d, J = 11.9 Hz), 4.03−3.98 (2H, m), 3.63−3.58 (3H, m), 3.25 (3H, s), 1.56 (3H, s).

3,4,6-Tri-*O*-benzyl-D-galactopyranose (6). To a stirred solution of **5** (19.0 g, 37.5 mmol) in 1,4-dioxane (280 mL) was added 1 M H_2SO_4 (80 mL), and the mixture was refluxed (110 °C) for 2 h. The reaction mixture was cooled to room temperature, then solid $NaHCO_3$ was carefully added until the mixture was neutralized; stirring was continued for 30 min. The precipitated salts were removed by filtration, and the filtrate was evaporated to dryness. The resulting residue was partitioned between $CHCl_3$ and water; the organic layer was washed with brine and concentrated in vacuo to afford 15.5 g of **6** (34.4 mmol, 92%): ^1H NMR of the α-anomer (300 MHz, $CDCl_3$) δ 7.36−7.20 (15H, m), 5.27 (1H, d, J = 4.0 Hz), 4.84 (1H, d, J = 11.6 Hz), 4.69 (1H, d, J = 11.9 Hz), 4.62 (1H, d, J = 11.6 Hz), 4.52 (1H, d, J = 11.6 Hz), 4.46 (1H, d, J = 11.9 Hz), 4.38 (1H, d, J = 11.9 Hz), 4.10 (1H, dd, J = 6.1, 6.1 Hz), 4.10 (1H, dd, J = 10.0, 4.0 Hz), 3.88 (1H, m), 3.68 (1H, dd, J = 9.8, 2.8 Hz), 3.52 (1H, dd, J = 9.2, 6.7 Hz), 3.42 (1H, dd, J = 9.5, 6.1 Hz).

(2*S*,3*S*,4*R*)-2,3,5-Tribenzyloxy-4-hydroxypentanal (7). Sodium metaperiodate (7.2 g, 33.6 mmol) was added to a solution of diol **6** (7.6 g, 16.9 mmol) in EtOH/H_2O (4:1, 100 mL), and the mixture stirred for 17 h at room temperature. The reaction mixture was concentrated and partitioned between ether and water. The organic layer was washed with brine and dried (Na_2SO_4), and the solvent evaporated to give an oil **7** (7.3 g), which was used in the next step without further purification.

(2*R*,3*R*,4*R*)-1,3,4-Tribenzyloxy-5-pentadecene-2-ol (8a). A suspension of decyltriphenylphosphonium bromide (18.0 g, 37.2 mmol) in THF (80 mL) was stirred at 0 °C, while 16.0 mL of *n*-BuLi (1.6 M solution in *n*-hexane) was added dropwise. The resulting solution was stirred for 30 min, and then a solution of the aldehyde **7** (4.6 g, 10.7 mmol) in THF (20 mL) was added. The mixture was warmed to room temperature and stirred for 1 h. The reaction was quenched by adding water and extracted with ether. The ether layer was dried with Na_2SO_4 and concentrated in vacuo. SiO_2 chromatography with *n*-hexane/EtOAc (8:2) gave the alkene **8a** (4.41 g, 8.1 mmol, 76%) as a pale yellow oil: ^1H NMR of the 5*Z*-isomer (300 MHz, $CDCl_3$) δ 7.35−7.20 (15H, m), 5.72 (1H, m), 5.45 (1H, dd, J = 11.0, 10.9 Hz), 4.68 (1H, d, J = 11.3 Hz), 4.60 (1H, d, J = 11.7 Hz), 4.50−4.45 (3H, m), 4.40 (1H, dd, J = 9.5, 5.2 Hz), 4.33 (1H, d, J = 11.8 Hz), 4.07 (1H, td, J = 5.9, 2.9 Hz), 3.56 (1H, dd, J = 5.6, 2.9 Hz), 3.51 (2H, d, J = 5.9 Hz), 2.81 (1H, brs), 1.93 (2H, m), 1.30−1.20 (14H, m), 0.88 (3H, t, J = 7.0 Hz).

(2*R*,3*R*,4*R*)-1,3,4-Tribenzyloxy-5-hexadecene-2-ol (8b). A suspension of undecyltriphenylphosphonium bromide (16.5 g, 33.2 mmol) in THF (70 mL) was stirred at 0 °C, while 18.0 mL of *n*-BuLi (1.6 M solution in *n*-hexane) was added dropwise. The resulting solution was stirred for 10 min, and then a solution of the aldehyde **7** (4.6 g, 10.7 mmol) in THF (15 mL) was added. The mixture was warmed to room temperature and stirred for 30 min. The reaction was quenched by adding water and then extracted with ether. The ether layer was dried with Na_2SO_4 and concentrated in vacuo. SiO_2 chromatography with *n*-hexane/EtOAc (8:2) gave the alkene **8b** (4.15 g, 7.43 mmol, 69%) as a pale yellow oil: ^1H NMR of the 5*Z*-isomer (300 MHz, $CDCl_3$) δ 7.35−7.20 (15H, m), 5.71 (1H, dt, J = 11.0, 7.0 Hz), 5.44 (1H, dd, J = 11.0, 11.0 Hz), 4.66 (1H, d, J = 11.3 Hz), 4.61 (1H, d, J = 9.5 Hz), 4.50−4.45 (3H, m), 4.42 (1H, dd, J = 10.3, 5.0 Hz), 4.33 (1H, d, J = 11.8 Hz), 4.06 (1H, td, J = 5.9, 2.9 Hz), 3.54 (1H, dd, J = 5.5, 2.9 Hz), 3.50 (2H, d, J = 5.9

Hz), 1.94 (1H, ddt, J = 13.5, 7.0, 7.0 Hz), 1.87 (1H, ddt, J = 13.5, 7.0, 7.0 Hz), 1.30−1.20 (16H, m), 0.86 (3H, t, J = 7.0 Hz).

(2*R*,3*R*,4*R*)-1,3,4-Tribenzyloxy-2-methanesulfonyloxy-5-pentadecene (9a). A solution of **8a** (4.0 g, 7.17 mmol) in pyridine (10.0 mL) was ice-cooled, and then MsCl (1.20 mL, 15.5 mmol) was added. The mixture was stirred for 7 h at room temperature, then toluene was added, and the solution was concentrated in vacuo. The resulting residue was dissolved in ether, and the solution was washed successively with water and brine. The organic layer was dried over Na_2SO_4 and evaporated to dryness, and the residue was chromatographed over Si gel. Elution with *n*-hexane/EtOAc (9:1) gave the *O*-mesylate **9a** (4.37 g, 6.87 mmol, 96%): ^{1}H NMR of the 5*Z*-isomer (500 MHz, $CDCl_3$) δ 7.35−7.20 (15H, m), 5.77 (1H, dt, J = 11.2, 7.3 Hz), 5.46 (1H, t, J = 11.2 Hz), 5.03 (1H, ddd, J = 7.1, 4.0, 4.0 Hz), 4.73 (1H, d, J = 11.0 Hz), 4.51−4.35 (6H, m), 3.74 (1H, dd, J = 11.0, 3.7 Hz), 2.90 (3H, s), 2.04 (1H, ddt, J = 14.5, 7.3, 7.3 Hz), 1.94 (1H, ddt, J = 14.5, 7.3, 7.3 Hz), 1.35−1.20 (16H, m), 0.84 (3H, t, J = 7.0 Hz).

(2*R*,3*R*,4*R*)-2-Methanesulfonyloxy-1,3,4-pentadecanetriol (10a). To an ice-cooled solution of **20a** (4.0 g, 7.35 mmol) in pyridine (15.0 mL) was added MsCl (1.15 mL, 14.9 mmol). The mixture was stirred for 15 h at room temperature, then toluene was added, and the solution was concentrated in vacuo. The resulting residue was dissolved in ether, and the solution was washed successively with water and brine. The organic layer was dried over Na_2SO_4 and evaporated to dryness, and the residue was chromatographed over Si gel. Elution with *n*-hexane/EtOAc (9:1, 8:2) afforded the *O*-mesylate **9a** (4.66 g, quant.). Then the *O*-mesylate **9a** (2.26 g, 3.63 mmol) was dissolved in EtOH (30 mL), and 2.30 g of 10% palladium on carbon was added. Hydrogen gas was introduced to the reaction flask and the solution vigorously stirred for 20 h (room temperature, 1 atm). The reaction mixture was filtered through a pad of Celite (with $CHCl_3$ rinse), and the filtrate was concentrated to give 1.24 g (3.50 mmol, 96%) of **10a** as a white powder: ^{1}H NMR (300 MHz, $CDCl_3$/CD_3OD, 1:1) δ 4.89 (1H, ddd, J = 6.5, 5.3, 2.1 Hz), 3.90−3.75 (2H, m), 3.53−3.48 (1H, m), 3.46 (1H, dd, J = 8.5, 2.1 Hz), 3.14 (3H, s), 1.74 (2H, m), 1.52 (2H, m), 1.40−1.20 (16H, m), 0.84 (3H, t, J = 6.7 Hz).

(2*R*,3*R*,4*R*)-2-Methanesulfonyloxy-1,3,4-hexadecanetriol (10b). To a solution of **9b** (1.35 g, 2.12 mmol) in EtOH (30 mL) was added 10% palladium on carbon (1.36 g). The mixture was stirred for 22 h under a hydrogen atmosphere at room temperature (1 atm). The Pd/C was then removed by filtration through a pad of Celite, and the filtrate was concentrated to give the triol **10b** as a white powder (1.24 g, 3.50 mmol, 89%): ^{1}H NMR (300 MHz, $CDCl_3$/CD_3OD, 1:1) δ 4.90 (1H, ddd, J = 6.6, 5.3, 2.0 Hz), 3.86 (1H, dd, J = 12.2, 6.5 Hz), 3.80 (1H, dd, J = 12.1, 4.9 Hz), 3.52 (1H, ddd, J = 8.4, 8.4, 2.3 Hz), 3.46 (1H, dd, J = 8.4, 1.9 Hz), 3.15 (3H, s), 1.75 (2H, m), 1.52 (2H, m), 1.40−1.20 (18H, m), 0.84 (3H, t, J = 6.7 Hz).

(2*S*,3*S*,4*R*)-2-Azido-1,3,4-pentadecanetriol (11a). Sodium azide (600 mg, 9.2 mmol) was added to a solution of the mesylate **10a** (609 mg, 1.72 mmol) in DMF (20 mL) at room temperature. The mixture was slowly heated to 100 °C over 30 min and then stirred for 3 h. The mixture was diluted with EtOAc and washed with water and brine. The organic layer was dried (Na_2SO_4) and concentrated in vacuo, and remaining DMF was removed by lyophilization. The resulting residue was chromatographed over Si gel with $CHCl_3$/MeOH (97:3, 96:4) to yield 425 mg (1.41 mmol, 82%) of the azide **11a**: ^{1}H NMR (600 MHz, $CDCl_3$/CD_3OD, 1:1) δ 3.90 (1H, dd, J = 12.0, 3.6 Hz), 3.74 (2H, m), 3.54 (2H, m), 1.62 (2H, m), 1.56 (2H, m), 1.40−1.20 (16H, m), 0.85 (3H, t, J = 7.0 Hz).

(2*S*,3*S*,4*R*)-2-Azido-1,3,4-hexadecanetriol (11b). Sodium azide (200 mg, 3.08 mmol) was added to a solution of the mesylate **10b** (300 mg, 0.82 mmol) in DMF (12 mL) at room temperature. It was slowly heated to 100 °C over 30 min and then stirred for 3 h. The reaction mixture was partitioned between EtOAc and H_2O. The organic layer was dried (Na_2SO_4) and concentrated in vacuo. Chromatography (SiO_2) eluting with $CHCl_3$/MeOH (95:5) gave 191 mg (0.61 mmol, 74%) of the azide **11b**: ^{1}H NMR (300 MHz, $CDCl_3$/CD_3OD, 1:1) δ 3.90 (1H, dd, J = 11.9 and 3.4 Hz), 3.75 (2H, m), 3.55 (2H, m), 1.65−1.47 (4H, m), 1.40−1.20 (18H, m), 0.84 (3H, t, J = 6.9 Hz).

(2*S*,3*S*,4*R*)-2-Amino-1,3,4-pentadecanetriol (12a). To a solution of the azide **11a** (500 mg, 1.66 mmol) in EtOH (20 mL) was added 10% palladium on carbon (500 mg). The reaction flask was flushed with hydrogen gas and the solution vigorously stirred for 20 h (room temperature, 1 atm). The mixture was then filtered through a pad of Celite (with $CHCl_3$/MeOH rinse), the solvent concentrated, and the residue chromatographed over Si gel under basic conditions. The column was eluted with $CHCl_3$/MeOH/H_2O/25% NH_4OH (8:2:0.15:0.05), and the eluate was concentrated, diluted with water, and lyophilized to afford the C_{15} phytosphingosine **12a** (433 mg, 1.57 mmol, 95%): ^{1}H NMR (300 MHz, $CDCl_3$) δ 3.72 (1H, dd, J = 11.1, 4.3 Hz), 3.58 (1H, dd, J = 11.1, 6.2 Hz), 3.51 (1H, m), 3.32 (1H, dd, J = 7.7, 5.5 Hz), 2.94 (1H, dd, J = 10.1, 5.5 Hz), 1.70 (2H, m), 1.53 (2H, m), 1.40−1.20 (16H, m), 0.85 (3H, t, J = 6.9 Hz).

(2*S*,3*S*,4*R*)-2-Amino-1,3,4-hexadecanetriol (12b). To a solution of the azide **11b** (100 mg, 0.32 mmol) in EtOH (11 mL) was added 10% palladium on carbon (110 mg). The reaction flask was flushed with hydrogen gas and the solution vigorously stirred for 20 h (room temperature, 1 atm). The mixture was then filtered through a pad of Celite (with $CHCl_3$/MeOH rinse), the solvent concentrated, and the residue chromatographed over Si gel. Elution with $CHCl_3$/MeOH/H_2O (8:2:0.2, 7:3:0.5) gave the C_{16} phytosphingosine**12b** (51.0 mg, 0.18 mmol, 56%): ^{1}H NMR (300 MHz, $CDCl_3$/CD_3OD, 1:1) δ 3.87 (1H, dd, J = 11.8, 4.0 Hz), 3.75 (1H, dd, J = 11.8, 7.5 Hz), 3.62 (1H, m), 3.52 (1H, dd, J = 8.9, 4.4 Hz), 3.45 (1H, dd, J = 7.6, 3.7 Hz), 1.73 (2H, m), 1.49 (2H, m), 1.40−1.20 (18H, m), 0.83 (3H, t, J = 7.0 Hz).

9-Bromononanal (14). To a stirred solution of 9-bromononanol (**13**) (4.9 g, 22.0 mmol) in CH_2Cl_2 (30 mL) was added pyridinium chlorochromate (10.0 g, 46.4 mmol). Stirring was continued for 2 h at room temperature, and then ether (10 mL) and Na_2SO_4 were added to the solution. The suspension was filtered and rinsed with ether, and the solvent concentrated in vacuo. The residue was chromatographed over Si gel. Elution with *n*-hexane/EtOAc (96:4, 9:1) gave the aldehyde **14** (4.3 g, 19.5 mmol, 89%): ^{1}H NMR (300 MHz, $CDCl_3$) δ 9.74 (1H, t, J = 1.8 Hz), 3.38 (2H, t, J = 7.0 Hz), 2.40 (2H, brt, J = 7.0 Hz), 1.82 (2H, quint, J = 7.0 Hz), 1.60 (2H, quint, J = 7.3 Hz), 1.40 (2H, quint, J = 7.0 Hz), 1.35−1.25 (6H, m).

12-Bromo-2-methyl-3-dodecene (15). To an ice-cooled suspension of isobutyltriphenylphosphonium bromide (10.0 g, 25.0 mmol) in THF (50 mL) was added 14.0 mL of *n*-BuLi (1.6 M solution in *n*-hexane). The resulting solution was stirred for 30 min at 0 °C, and then a solution of 8-bromooctanal (4.1 g, 18.5 mmol) in THF (30 mL) was added. The mixture was warmed to room temperature and stirring was continued for 2 h. The reaction was quenched by addition of water, and the mixture extracted with ether. The combined ethereal fraction was washed with brine, dried over $MgSO_4$, and concentrated. Chromatography on Si gel with *n*-hexane gave the alkene **15** as a colorless oil (3.8 g, 14.4 mmol, 78%): ^{1}H NMR of the 3*Z*-isomer (300 MHz, $CDCl_3$) δ 5.20 (1H, dt, J = 11.0, 6.9 Hz), 5.16 (1H, ddt, J = 10.7, 10.7, 1.2 Hz), 3.38 (2H, t, J = 6.7 Hz), 2.56 (1H, m), 2.00 (2H, dt, J = 7.0, 7.0 Hz), 1.83 (2H, quint, J = 6.9 Hz), 1.42 (2H, m), 1.40−1.20 (8H, m), 0.92 (6H, d, J = 6.7 Hz).

11-Methyl-9-dodecenyltriphenylphosphonium Bromide (16). The mixture of the bromide **15** (3.6 g, 16.2 mmol) and triphenylphosphine (4.3 g, 16.4 mmol) was heated at 120 °C for 2 days to give **16**. In the same manner, this reaction was repeated twice for each of the next Wittig reactions. The resulting viscous materials were used in the next step without further purification.

1-Bromo-19-methyleicosane (18a). To a suspension of the phosphonium salt **16** (prepared from 3.6 g of **15**) in THF (20

mL) was added 8.5 mL of *n*-BuLi (1.6 M solution in *n*-hexane) at 0 °C. The resulting solution was stirred for 30 min at 0 °C, and then a solution of 8-bromoctanal (3.4 g, 16.4 mmol) in THF (15 mL) was added. The mixture was warmed to room temperature, and stirring was continued for 1 h. The reaction was quenched by the addition of water, and the mixture extracted with ether. The combined ethereal fraction was washed with brine, dried over $MgSO_4$, and concentrated. Chromatography over Si gel with *n*-hexane afforded the *E,Z*-mixture of the 1-bromo-19-methyl-8,17-eicosadiene **17a** as a colorless oil (2.7 g, 7.3 mmol, 45%). The ^{1}H NMR spectrum of **17a** exhibited additional signals for 1-bromo-19-methyl-8,16-eicosadiene and 1-bromo-19-methyl-8,18-eicosandiene probably produced through migration of the double bond, which were not separated, but were used as such in the next reaction. The mixture of **17a** (2.7 g, 7.3 mmol) and 10% palladium on carbon (2.7 g) in 30 mL of EtOH was stirred under a hydrogen atmosphere for 20 h (room temperature, 1 atm). The reaction mixture was filtered through a pad of Celite (with $CHCl_3$ rinse), and the filtrate was evaporated to dryness. Chromatography over Si gel, eluting with *n*-hexane, yielded the bromoalkane **18a** (2.0 g, 5.4 mmol, 74%) as a colorless oil: ^{1}H NMR (300 MHz, $CDCl_3$) δ 3.39 (2H, t, J = 6.8 Hz), 1.84 (2H, quint, J = 7.3 Hz), 1.50 (1H, m), 1.41 (2H, m), 1.35−1.20 (28H, m), 1.20−1.15 (2H, m), 0.85 (6H, d, J = 6.7 Hz).

1-Bromo-20-methylheneicosane (18b). Following the same procedure as described above for the preparation of **18a**, 9-bromononanal (2.0 g, 9.0 mmol) gave **18b** (1.1 g, 2.8 mmol, 31%): ^{1}H NMR (300 MHz, $CDCl_3$) δ 3.39 (2H, t, J = 7.1 Hz), 1.83 (2H, quint, J = 6.7 Hz), 1.49 (1H, m), 1.40 (2H, m), 1.35−1.20 (30H, m), 1.13 (2H, m), 0.84 (6H, d, J = 6.8 Hz).

1-Bromo-21-methyldocosane (18c). Following the same procedure as described above for the preparation of **18a**, 10-bromodecanal (3.7 g, 15.7 mmol) afforded **18c** (4.0 g, 10.0 mmol, 64%): ^{1}H NMR (300 MHz, $CDCl_3$) δ 3.40 (2H, t, J = 7.0 Hz), 1.83 (2H, quint, J = 6.8 Hz), 1.50 (1H, m), 1.40 (2H, m), 1.35−1.20 (32H, m), 1.15 (2H, m), 0.85 (6H, d, J = 6.8 Hz).

2-Hydroxy-21-methyldocosanoic acid (20a). To a suspension of diethyl acetamidomalonate (5.0 g, 23.0 mmol) in EtOH (10.0 mL) was added 9.0 mL of sodium ethoxide (2.4 M solution in EtOH, prepared from Na and EtOH). The mixture was stirred for 30 min at room temperature. Then, 7.0 mL (6.0 mmol) of the resulting solution was added to a solution of the bromide **18a** (935 mg, 2.49 mmol) in EtOH (5.0 mL), and the mixture was stirred and heated under reflux for 10 h. The reaction mixture was concentrated, diluted with water, and extracted with ether. The ether extract was concentrated in vacuo. The residue was dissolved in concentrated HCl (7.5 mL), water (2.5 mL), and 1,4-dioxane (5.0 mL), and the resulting mixture was stirred and heated under reflux for 12 h. The reaction mixture was lyophilized to give 500 mg (1.36 mmol) of amino acid **19a** as a white powder. Part of the resulting amino acid (91.0 mg, 0.26 mmol) was mixed with 2 N H_2SO_4 (5.0 mL) and 1,4-dioxane (5.0 mL). A solution of sodium nitrite (75.0 mg, 1.09 mmol) in water (1.0 mL) was added to the suspension, which was stirred for 1 h at 80 °C. The resulting solution was diluted with ether, washed with water, dried ($MgSO_4$), and concentrated. Chromatography on Si gel with *n*-hexane/EtOAc/AcOH (5:5:0.2) afforded the 2-hydroxy acid **20a** (56.0 mg, 0.15 mmol, 32% from **18a**): ^{1}H NMR (300 MHz, $CDCl_3$/CD_3OD, 1:1) δ 4.09 (1H, dd, J = 7.6, 4.5 Hz), 1.80−1.50 (2H, m), 1.50−1.20 (33H, m), 1.12 (2H, m), 0.82 (6H, d, J = 6.7 Hz).

2-Hydroxy-22-methyltricosanoic acid (20b). Following the same procedure as described above for the preparation of **20a**, the bromoalkane **18b** was converted to the 2-hydroxy acid **20b** (18%): ^{1}H NMR (300 MHz, $CDCl_3$/CD_3OD, 1:1) δ 4.09 (1H, dd, J = 7.6, 4.5 Hz), 1.80−1.50 (2H, m), 1.50−1.20 (35H, m), 1.12 (2H, m), 0.82 (6H, d, J = 6.5 Hz).

2-Hydroxy-23-methyltetracosanoic acid (20c). Following the same procedure as described above for the preparation of **20a**, the bromoalkane **18c** was converted to the 2-hydroxy acid **20c** (27%); ^{1}H NMR (300 MHz, $CDCl_3$/CD_3OD, 1:1) δ 4.09 (1H, dd, J = 7.6, 4.5 Hz), 1.80−1.50 (2H, m), 1.50−1.20 (37H, m), 1.12 (2H, m), 0.82 (6H, d, J = 6.5 Hz).

2-Acetoxy-21-methyldocosanoic acid (21a). Acetic anhydride (4.0 mL) was added to a solution of the alcohol **20a** (117 mg, 0.32 mmol) in pyridine (4.0 mL). The mixture was stirred for 13 h at room temperature. It was then diluted with ice water and extracted with ether. The ethereal extract was washed with brine, dried ($MgSO_4$), and concentrated. The residue was chromatographed over Si gel. Elution with *n*-hexane/EtOAc/AcOH (5:5:0.2) gave the acetate **21a** as a white solid (102 mg, 0.25 mmol, 77%): ^{1}H NMR (600 MHz, $CDCl_3$) δ 4.94 (1H, dd, J = 7.3, 5.0 Hz), 2.07 (3H, s), 1.79 (2H, m), 1.44 (2H, m), 1.36 (2H m), 1.40−1.20 (30H, m), 1.07 (2H, m), 0.79 (6H, d, J = 6.5 Hz).

2-Acetoxy-22-methyltricosanoic acid (21b). In the same manner as described above for the preparation of **21a**, the alcohol **20b** (109 mg, 0.28 mmol) was converted to 105 mg (0.25 mmol, 88%) of **21b**: ^{1}H NMR (600 MHz, $CDCl_3$) δ 4.99 (1H, t, J = 6.3 Hz), 2.12 (3H, s), 1.84 (2H, m), 1.49 (2H, m), 1.41 (2H m), 1.40−1.20 (30H, m), 1.13 (2H, m), 0.84 (6H, d, J = 6.9 Hz).

2-Acetoxy-23-methyltetracosanoic acid (21c). In the same manner as described above for the preparation of **21a**, the alcohol **20c** (180 mg, 0.45 mmol) was converted to 168 mg (0.38 mmol, 84%) of **21c**: ^{1}H NMR (600 MHz, $CDCl_3$) δ 4.98 (1H, dd, J = 7.3, 6.4 Hz), 2.12 (3H, s), 1.84 (2H, m), 1.49 (2H, m), 1.40 (2H, m), 1.40−1.20 (30H, m), 1.13 (2H, m), 0.83 (6H, d, J = 6.5 Hz).

Synthesis of the Ceramide Mixture (1a, 1b). The mixture of phytosphingosines **12a** (25.0 mg, 0.091 mmol) and **12b** (33.0 mg, 0.114 mmol), 2-acetoxy fatty acids **21a** (30.0 mg, 0.073 mmol), **21b** (30.0 mg, 0.070 mmol), and **21c** (20.0 mg, 0.045 mmol), and HOBt (1-hydroxybendotriazole) (40.0 mg, 0.30 mmol) was diluted with benzene, evaporated, and lyophilized to remove water. The residue was dissolved in CH_2Cl_2 (5.0 mL) and added to WSCI [1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride] (60.0 mg, 0.31 mmol). The mixture was stirred for 20 h at room temeperature, then diluted with $CHCl_3$, washed with water, dried ($NaSO_4$), and concentrated. To the residue were added MeOH (9.0 mL), THF (3.0 mL), and K_2CO_3 (90 mg), and the mixture was stirred for 2.5 h at room temperature. The reaction mixture was concentrated in vacuo and chromatographed over Si gel. Elution with $CHCl_3$/MeOH (97:3, 96:4) furnished 2′*S* (**1 b**, 45.0 mg) and 2′*R* (**1 a**, 36.0 mg) ceramide mixtures. **1a**: ^{1}H NMR (600 MHz, $CDCl_3$/CD_3OD, 1:1) δ 4.08 (1H, ddd, J = 4.8, 4.6, 3.8 Hz), 4.00 (1H, dd, J = 8.1, 3.8 Hz), 3.76 (1H, dd, J = 11.2, 4.6 Hz), 3.70 (1H, dd, J = 11.3, 4.8 Hz), 3.51 (2H, m), 1.74 (1H, m), 1.64 (1H, m), 1.60−1.15 (brm), 1.12 (2H, m), 0.84 (3H, t, J = 6.9 Hz), 0.82 (6H, d, J = 6.9 Hz); ^{13}C NMR (150 MHz, $CDCl_3$/CD_3OD, 1:1) δ 176.4s, 75.9d, 72.9d, 72.4d, 61.6t, 52.1d, 39.6t, 35.1t, 33.2t, 32.5t, 30.4t, 30.2 (18−21C), 29.9t, 28.5t, 27.9t, 26.4t, 25.6d, 23.2q (2C), 22.9t, 14.3q; HRFABMS for C_{15}/C_{23} *m/z* (M + H)$^+$ 628.5873 (calcd for $C_{38}H_{78}NO_5$ Δ −0.7 mmu); C_{16}/C_{23} *m/z* (M + H)$^+$ 642.6085 (calcd for $C_{39}H_{80}NO_5$ Δ +4.8 mmu); C_{15}/C_{25} *m/z* (M + H)$^+$ 656.6244 (calcd for $C_{40}H_{82}NO_5$ Δ +5.1 mmu); C_{16}/C_{25} *m/z* (M + H)$^+$ 670.6400 (calcd for $C_{41}H_{84}NO_5$ Δ +5.0 mmu). **1b**: ^{1}H NMR (600 MHz, $CDCl_3$/CD_3OD, 1:1) δ 4.09 (1H, ddd, J = 5.0, 4.6, 3.8 Hz), 4.00 (1H, dd, J = 8.1, 3.8 Hz), 3.76 (1H, dd, J = 11.2, 4.6 Hz), 3.70 (1H, dd, J = 11.2, 5.0 Hz), 3.52 (2H, m), 1.76 (1H, m), 1.64 (1H, m), 1.60−1.15 (brm), 1.12 (2H, m), 0.84 (3H, t, J = 6.9 Hz), 0.82 (6H, d, J = 6.6 Hz).

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Supporting Information Available: ^{1}H NMR spectra and HPLC profiles of the natural and synthetic ceramide mixtures. This material is available free of charge via the Internet at http://pubs.acs.org.

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(19) ^{13}C NMR data for the natural ceramide mixture (150 MHz, $CDCl_3$/CD_3OD, 1:1) δ 176.5s, 75.7d, 72.7d, 72.5d, 61.5t, 52.0d, 34.8t, 33.0t, 30.0t, 29.5d, 24.0t, 22.7q(2C), 14.2q.

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