Ceramide Constituents from Five Mushrooms[1)]

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Five mushrooms, *Panellus serotinus*, *Lyophyllum connatum*, *Amanita pantherina*, *Sarcodon aspratus* and *Lepista nuda*, have been investigated chemically. Two new ceramides, (2*S*,3*R*,4*E*,8*E*)-*N*-hexadecanoyl-2-amino-9-methyl-4,8-octadecadiene-1,3-diol (1) and (2*S*,3*R*,4*E*,8*E*,9′*Z*,12′*Z*)-*N*-9′,12′-octadecadienoyl-2-amino-9-methyl-4,8-octadecadiene-1,3-diol (2), have been isolated from *Panellus serotinus*. Compound 2 was also isolated from *Lyophyllum connatum*. Two new ceramides, (2*S*,2′*R*,3*R*,4*E*,8*E*)-*N*-2′-hydroxypentadecanoyl-2-amino-9-methyl-4,8-octadecadiene-1,3-diol (4) and (2*S*,2′*R*,3*R*,4*E*,8*E*)-*N*-2′-hydroxytetradecanoyl-2-amino-9-methyl-4,8-octadecadiene-1,3-diol (5), have been isolated from *Amanita pantherina* with (2*S*,2′*R*,3*R*,4*E*,8*E*)-*N*-2′-hydroxyhexadecanoyl-2-amino-9-methyl-4,8-octadecadiene-1,3-diol (3), a known synthetic compound. Compounds 3 and 4 were also isolated from *Sarcodon aspratus* and compound 3 was isolated from *Lepista nuda*. The structures of the new compounds were elucidated on the basis of their spectral data.

Key words ceramide; mushroom; structural elucidation; *Amanita pantherina*; *Panellus serotinus*

Recently we reported the isolation and structural elucidation of sterols,[2)] sesquiterpenoids,[3)] triterpenoids[2,4)] and ceramides[5)] from seventeen mushrooms. In a continuation of our investigation of chemical constituents from mushrooms, we describe here the isolation and structural elucidation of four new sphingosine-type ceramides, (2*S*,3*R*,4*E*,8*E*)-*N*-hexadecanoyl-2-amino-9-methyl-4,8-octadecadiene-1,3-diol (**1**), (2*S*,3*R*,4*E*,8*E*,9′*Z*,12′*Z*)-*N*-9′,12′-octadecadienoyl-2-amino-9-methyl-4,8-octadecadiene-1,3-diol (**2**), (2*S*,2′*R*,3*R*,4*E*,8*E*)-*N*-2′-hydroxypentadecanoyl-2-amino-9-methyl-4,8-octadecadiene-1,3-diol (**4**) and (2*S*,2′*R*,3*R*,4*E*,8*E*)-*N*-2′-hydroxytetradecanoyl-2-amino-9-methyl-4,8-octadecadiene-1,3-diol (**5**), as well as (2*S*,2′*R*,3*R*,4*E*,8*E*)-*N*-2′-hydroxyhexadecanoyl-2-amino-9-methyl-4,8-octadecadiene-1,3-diol (**3**), a known synthetic compound,[6)] from five mushrooms, *Panellus serotinus* (PERS.: FR.) KÜHN. (Mukitake in Japanese, Tricholomataceae, compounds **1** and **2**), *Lyophyllum connatum* (SCHUM.: FR.) SING. (Oshiroishimeji in Japanese, Tricholomataceae, compound **2**), *Amanita pantherina* (DC.: FR.) KROMBH. (Tengutake in Japanese, Amanitaceae, compounds **3**, **4** and **5**), *Sarcodon aspratus* (BERK.) S. ITO (Kotake in Japanese, Thelephoraceae, compounds **3** and **4**) and *Lepista nuda* (BULL.: FR.) COKE (Murasakishimeji in Japanese, Tricholomataceae, compound **3**).

Compound **1** was isolated as an amorphous powder. The molecular formula was determined to be $C_{35}H_{67}NO_3$ by high-resolution (HR)-electron ionization (EI)-MS. The IR spectrum showed absorption bands at 3605 cm^{-1} (hydroxyl), 3434, 1657, 1510 cm^{-1} (amide), 2928, 2855 and 1466 cm^{-1} (aliphatic), suggesting it to be a fatty acid amide. The ^{1}H-NMR spectrum (*vide* Experimental) showed signals due to two terminal methyl groups [δ_H 0.88 (6H, H_3-18, H_3-16′)], aliphatic methylenes [δ_H 1.25—1.33 (36H)], an olefinic methyl group [δ_H 1.58 (3H, H_3-19)], a methylene group [δ_H 3.70 (1H, H-1a), 3.96 (1H, H-1b)], two methine groups [δ_H 3.92 (1H, H-2), 4.33 (1H, H-3)], a trisubstituted olefinic proton [δ_H 5.09 (1H, H-8)], two disubstituted olefinic protons [δ_H 5.56 (1H, H-4), 5.80 (1H, H-5)] and an amide proton [δ_H 6.23 (1H)]. The ^{13}C-NMR spectrum (*vide* Experimental) showed characteristic signals appearing to be due to an amide carbonyl at δ_C 173.9 and a methine carbon linked to amide nitrogen at δ_C 54.4.[7)] These spectral data and molecular formula suggest that compound **1** is a ceramide. Detailed analysis of the ^{1}H–^{1}H shift correlation spectroscopy (^{1}H–^{1}H COSY) spectrum of **1** implied connectivities for H_2-1 to H_2-6; H_2-7 to H-8; H_2-10 to H_2-11; H-2 to an amide proton; and H_2-2′ to H_2-3′ (Fig. 1). Interpretation of the ^{1}H-detected heteronuclear multiple bond connectivity (HMBC) spectrum revealed correlations from H-5 to C-7; H_2-6 to C-7 and C-8; H_3-19 to C-8, C-9 and C-10; an amide proton to C-2 and C-1′; and H_2-2′ to C-1′ (Fig. 1). The lengths of the long chain base and the fatty acid were determined by EI-MS, which showed significant fragment ion peaks at *m*/*z* 298 (a) and 281 (b) (Fig. 2).[8)] Thus, the long chain base and fatty acid of **1** must be 2-amino-9-methyl-4,8-octadecadiene-1,3-diol and hexadecanoic acid, respectively. The geometry of the double bond at C-4 was deduced to be *E* from the ^{1}H–^{1}H coupling constant (J=15.8 Hz) between H-4 and H-5. The chemical shift value of the olefinic methyl group at C-9 (δ_C 16.0) suggests that the double bond at C-8 is *E* geometry.[9)] The same conclusion was derived from the nuclear Overhauser enhancement spectroscopy (NOESY) cross peak observed between H-8 and H_2-10. The relative stereochemistry at C-2 and C-3 was determined to be *erythro*, since the ^{1}H-NMR data of **1** was in good agreement with that of (2*S*,3*R*,4*E*,8*E*)-*N*-hexadecanoyl-2-amino-4,8-octadecadiene-1,3-diol (**6**)[8)] (Table 1). The absolute stereochemistry at C-2 and C-3 was determined to have the 2*S*, 3*R* configuration by comparing the optical rotation values of **1** ($[\alpha]_D$ −11.6°) and **6** ($[\alpha]_D$ −8.0°).[8)] On the basis of this evidence, the structure of **1** was determined to be (2*S*,3*R*,4*E*,8*E*)-*N*-hexadecanoyl-2-amino-9-methyl-4,8-octadecadiene-1,3-diol.

Compound **2** was isolated as an amorphous powder. The molecular formula was determined to be $C_{37}H_{67}NO_3$ by HR-EI-MS. The ^{1}H- and ^{13}C-NMR spectra of **2** closely resembled those of **1** except for the integration of the aliphatic methylene protons at δ_H 1.26—1.32 (26H) and the presence of two additional double bonds [δ_H 5.33 (2H, H-10′, H-12′), 5.39 (2H, H-9′, H-13′); δ_C 127.9 (C-12′), 128.1 (C-10′), 130.1 (C-9′), 130.2 (C-13′)]. The ^{1}H–^{1}H COSY spectrum implied connectivities for H_2-8′ and H_2-14′ to H_2-9′ and H_2-13′; and H_2-11′ to H_2-10′ and H_2-12′. The HMBC spectrum revealed

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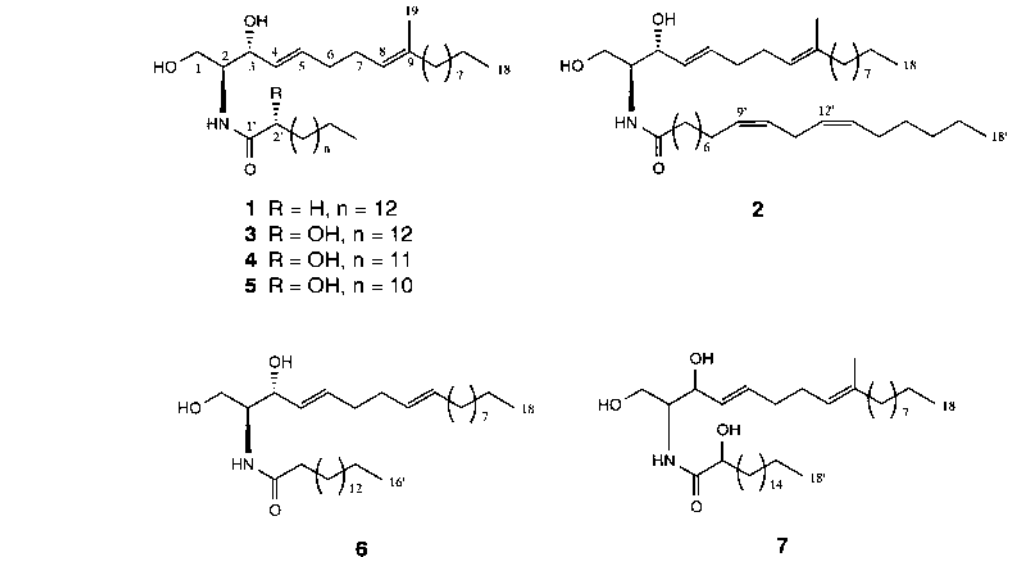

Chart 1

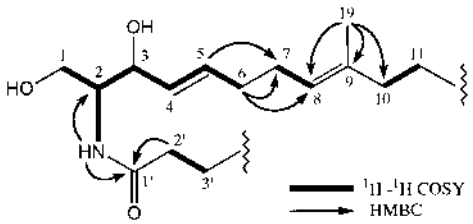

Fig. 1. ^{1}H–^{1}H COSY and HMBC Correlation for **1**

Table 1. Partial ^{1}H-NMR Spectral Data of **1** and **6** ($CDCl_3$)

Position	1	6
1	3.70 (dd, 11.4, 3.3)	3.69 (br dd, 11.2, 3.2)
	3.96 (dd, 11.4, 3.7)	3.95 (br dd, 11.2, 3.4)
2	3.92 (m)	3.90 (m)
3	4.33 (dd, 6.2, 4.8)	4.32 (br dd, 6.8, 4.4)
4	5.56 (dd, 15.8, 6.2)	5.53 (dd, 15.4, 6.8)
5	5.80 (dt, 15.8, 5.9)	5.77 (dt, 15.4, 6.1)
2′	2.23 (t, 7.7)	2.22 (t, 7.6)

Coupling constants (*J* in Hz) are given in parentheses.

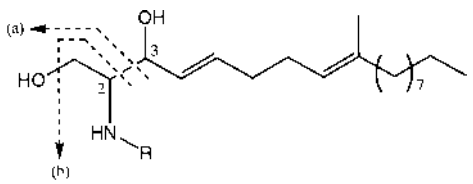

Fig. 2. EI-MS Fragmentation **1**—**5**

correlations from H_2-11′ to C-9′, C-10′, C-12′ and C-13′. Therefore, two double bonds were separated by a methylene. The lengths of the long chain base and the fatty acid were determined by EI-MS, which showed significant fragment ion peaks at *m*/*z* 322 (a) and 305 (b) (Fig. 2), indicating that the long chain base was the same as that of **1**, and the fatty acid must be octadecadienoic acid. The position of the double bonds of the fatty acid moiety was determined as follows. The EI-MS gave a fragment ion peak at *m*/*z* 444 ($M^+ - C_8H_{15} - H_2O$), resulting from cleavage of the C-10′—C-11′ bond and concomitant H_2O loss. In the ^{13}C-NMR spectrum, the signal for C-16′ appeared at δ_C 31.5, 0.4 ppm higher than that of (4*E*,8*E*)-*N*-2′-hydroxyoctadecanoyl-2-amino-9-methyl-4,8-octadecadiene-1,3-diol (**7**).[10] This is due to the γ-effect of the double bond between C-12′ and C-13′.[11] Thus, two double bonds should be between C-9′—C-10′ and C-12′—C-13′, respectively. Both of the double bonds were in the *Z* geometry, according to the chemical shifts of the allylic methylenes [δ_C 25.6 (C-11′), 27.2 (C-8′, C-14′)].[12] The optical rotation values of **2** ($[\alpha]_D$ −13.3°) and **1** ($[\alpha]_D$ −11.6°) suggested that **2** has the same absolute configuration as that of **1** for the C-2 and C-3 parts. Therefore, the structure of **2** was determined to be (2*S*,3*R*,4*E*,8*E*,9′*Z*,12′*Z*)-*N*-9′,12′-octadecadienoyl-2-amino-9-methyl-4,8-octadecadiene-1,3-diol.

Compound **3** was isolated as an amorphous powder, $[\alpha]_D$ +7.5°. The molecular formula was determined to be $C_{35}H_{67}NO_4$ by HR-EI-MS. The ^{1}H- and ^{13}C-NMR spectra, and the optical rotation value of **3** were in accord with those of (2*S*,2′*R*,3*R*,4*E*,8*E*)-*N*-2′-hydroxyhexadecanoyl-2-amino-9-methyl-4,8-octadecadiene-1,3-diol. Thus, compound **3** was as shown in Chart 1. Compound **3** was isolated from a natural source for the first time, although **3** has already been synthesized by Mori and Funaki.[6]

Compound **4** was isolated as an amorphous powder. The molecular formula was determined to be $C_{34}H_{65}NO_4$ by HR-EI-MS. The ^{1}H-NMR spectrum was virtually identical with that of **3** except for the integration of the aliphatic methylene protons at δ_H 1.25—1.33 (32H). The EI-MS gave fragment ion peaks at *m*/*z* 300 (a) and 283 (b), indicating that the long chain base was the same as that of **3**, and the fatty acid must be 2-hydroxypentadecanoic acid. The optical rotation values of **4** ($[\alpha]_D$ +7.0°) and **3** ($[\alpha]_D$ +7.5°) suggested that **4** has the same absolute configuration as that of **3** for the C-2, C-3 and C-2′ parts. Accordingly, the structure of **4** was determined to be (2*S*,2′*R*,3*R*,4*E*,8*E*)-*N*-2′-hydroxypentadecanoyl-2-amino-9-methyl-4,8-octadecadiene-1,3-diol.

Compound **5** was isolated as an amorphous powder. The molecular formula was determined to be $C_{33}H_{63}NO_4$ by HR-

EI-MS. The ^{1}H-NMR spectrum was virtually identical with that of **3** except for the integration of the aliphatic methylene protons at δ_H 1.25—1.33 (30H). The EI-MS gave fragment ion peaks at *m/z* 286 (a) and 269 (b), indicating that the long chain base was the same as that of **3**, and the fatty acid must be 2-hydroxytetradecanoic acid. The optical rotation values of **5** ($[\alpha]_D$ +6.3°) and **3** ($[\alpha]_D$ +7.5°) suggested that **5** has the same absolute configuration as that of **3** for the C-2, C-3 and C-2′ parts. Based on this evidence, the structure of **5** was determined to be (2*S*,2′*R*,3*R*,4*E*,8*E*)-*N*-2′-hydroxytetradecanoyl-2-amino-9-methyl-4,8-octadecadiene-1,3-diol.

Compounds **1**—**5** have the same long chain base, (2*S*,3*R*,4*E*,8*E*)-2-amino-9-methyl-4,8-octadecadien-1,3-diol, but differ in the structure of the fatty acids. The isolation of the ceramide with the 2-amino-9-methyl-4,8-octadecadiene-1,3-diol moiety from a natural source is rather unusual, and only one ceramide, *viz.*, (4*E*,8*E*)-*N*-2′-hydroxyoctadecanoyl-2-amino-9-methyl-4,8-octadecadiene-1,3-diol (**7**), has so far been reported from the fungus *Lactarium volemus*.[10] Compound **1** was the ceramide part of the glycosphingolipid named thraustochytroside B, which was recently isolated from the marine protist *Thraustochytrium globosum*.[13]

Experimental

General Procedures Optical rotations were determined using a JASCO DIP-360 digital polarimeter. IR spectra were recorded with a Perkin-Elmer FT-IR 1725X IR spectrophotometer. ^{1}H- and ^{13}C-NMR spectra were recorded using a JEOL JNM-LA 600 (600 and 150 MHz, respectively) spectrometer. Chemical shifts are given on a δ (ppm) scale, with tetramethylsilane as an internal standard (s, singlet; d, doublet; dd, double doublet; ddd, double double doublet; t, triplet; dt, double triplet; br, broad; m, multiplet). The EI-, FAB- and HR-EI-MS were recorded on a JEOL JMS-DX 303 mass spectrometer. Column chromatography was carried out on Kieselgel 60 (Merck; 230—400 mesh). Preparative HPLC was carried out on a Tosoh HPLC system (pump, CCPS; detector, RI-8020) using a TSK gel ODS-120T (7.8 mm i.d.×30 cm) column (Tosoh). HPLC conditions: mobile phase, MeOH; flow rate, 1.0 ml/min; column temperature, 40 °C.

Material *Panellus serotinus* (from Morioka City in Iwate Prefecture, Japan), *Lyophyllum connatum* (from Morioka City in Iwate Prefecture, Japan), *Sarcodon aspratus* (from Morioka City in Iwate Prefecture, Japan) and *Lepista nuda* (from Morioka City in Iwate Prefecture, Japan) were purchased in a food market. The fresh fruit bodies of *Amanita pantherina* were collected at Sendai City in Miyagi Prefecture, Japan, in September 1997.

Extraction and Isolation *P. serotinus*: The fresh fruit bodies of *P. serotinus* (1.1 kg) were extracted six times with Et_2O at room temperature for 2 weeks. The Et_2O extract (2.2 g) was chromatographed on a silica gel column using hexane–EtOAc (7 : 3—1 : 7), EtOAc and MeOH, to afford 40 fractions (frs. 1—40). Fraction 18 was purified by preparative HPLC to give **1** (0.9 mg) and **2** (2.0 mg).

L. connatum: The fresh fruit bodies of *L. connatum* (0.9 kg) were extracted four times with Et_2O at room temperature for 2 weeks. The Et_2O extract (1.9 g) was chromatographed on a silica gel column using hexane–EtOAc (7 : 3—1 : 7), EtOAc and MeOH, to afford 33 fractions (frs. 1—33). Fraction 17 was purified by preparative HPLC to give **2** (0.8 mg).

A. pantherina: The fresh fruit bodies of *A. pantherina* (0.6 kg) were extracted three times with Et_2O at room temperature for 2 weeks. The Et_2O extract (7.3 g) was chromatographed on a silica gel column using hexane–EtOAc (7 : 3—1 : 7), EtOAc and MeOH, to afford 21 fractions (frs. 1—21). Fraction 16 was purified by preparative HPLC to give **3** (6.9 mg), **4** (1.4 mg) and **5** (1.6 mg).

S. aspratus: The fresh fruit bodies of *S. aspratus* (1.1 kg) were extracted three times with Et_2O at room temperature for 2 weeks. The Et_2O extract (3.7 g) was chromatographed on a silica gel column using hexane–EtOAc (7 : 3—1 : 7), EtOAc and MeOH, to afford 20 fractions (frs. 1—20). Fraction 17 was purified by preparative HPLC to give **3** (1.9 mg) and **4** (1.8 mg).

L. nuda: The fresh fruit bodies of *L. nuda* (0.3 kg) were extracted five times with Et_2O at room temperature for 2 weeks. The Et_2O extract (1.4 g) was chromatographed on a silica gel column using hexane–EtOAc (7 : 3—1 : 7), EtOAc and MeOH, to afford 29 fractions (frs. 1—29). Fraction 25 was purified by preparative HPLC to give **3** (1.0 mg).

(2*S*,3*R*,4*E*,8*E*)-*N*-Hexadecanoyl-2-amino-9-methyl-4,8-octadecadiene-1,3-diol (**1**): Amorphous powder. $[\alpha]_D^{21}$ −11.6° (*c*=0.09, $CHCl_3$). IR ($CHCl_3$) cm^{-1}: 3605, 3434, 2928, 2855, 1657, 1510, 1466. HR-EI-MS *m/z*: 549.5113 (M^+, Calcd for $C_{35}H_{67}NO_3$: 549.5121). EI-MS *m/z* (rel. int. %): 549 (M^+, 2), 298 (a, 15), 281 (b, 43). FAB-MS (negative ion mode; matrix, triethanolamine) *m/z*: 548 $[M-H]^-$. ^{1}H-NMR (600 MHz, $CDCl_3$) δ: 0.88 (6H, t, *J*=7.3 Hz, H_3-18, H_3-16′), 1.25—1.33 (36H, br s, H_2-12—H_2-17, H_2-4′—H_2-15′), 1.36 (2H, m, H_2-11), 1.58 (3H, br s, H_3-19), 1.64 (2H, m, H_2-3′), 1.95 (2H, t, *J*=7.7 Hz, H_2-10), 2.08 (2H, m, H_2-7), 2.11 (2H, m, H_2-6), 2.23 (2H, t, *J*=7.7 Hz, H_2-2′), 3.70 (1H, dd, *J*=11.4, 3.3 Hz, Ha-1), 3.92 (1H, m, H-2), 3.96 (1H, dd, *J*=11.4, 3.7 Hz, Hb-1), 4.33 (1H, dd, *J*=6.2, 4.8 Hz, H-3), 5.09 (1H, t, *J*=6.6 Hz, H-8), 5.56 (1H, dd, *J*=15.8, 6.2 Hz, H-4), 5.80 (1H, dt, *J*=15.8, 5.9 Hz, H-5), 6.23 (1H, d, *J*=7.7 Hz, NH). ^{13}C-NMR (100 MHz, $CDCl_3$) δ: 14.1 (C-18, C-16′), 16.0 (C-19), 22.7 (C-17, C-15′), 25.8 (C-3′), 27.5 (C-7), 28.0—29.7 (C-11—C-15, C-4′—C-13′), 31.9 (C-16, C-14′), 32.5 (C-6), 36.8 (C-2′), 39.7 (C-10), 54.4 (C-2), 62.5 (C-1), 74.9 (C-3), 123.0 (C-8), 129.0 (C-4), 133.8 (C-5), 136.3 (C-9), 173.9 (C-1′).

(2*S*,3*R*,4*E*,8*E*,9′*Z*,12′*Z*)-*N*-9′,12′-Octadecadienoyl-2-amino-9-methyl-4,8-octadecadiene-1,3-diol (**2**): Amorphous powder. $[\alpha]_D^{30}$ −13.3° (*c*=0.08, $CHCl_3$). IR ($CHCl_3$) cm^{-1}: 3603, 3435, 2929, 2856, 1656, 1510, 1466. HR-EI-MS *m/z*: 573.5092 (M^+, Calcd for $C_{37}H_{67}NO_3$: 573.5121). EI-MS *m/z* (rel. int. %): 573 (M^+, 4), 444 ($M^+-C_8H_{15}-H_2O$, 3), 322 (a, 26), 305 (b, 100). FAB-MS (negative ion mode; matrix, triethanolamine) *m/z*: 572 $[M-H]^-$. ^{1}H-NMR (600 MHz, $CDCl_3$) δ: 0.88 (3H, t, *J*=7.0 Hz, H_3-18), 0.89 (3H, t, *J*=7.0 Hz, H_3-18′), 1.26—1.32 (26H, br s, H_2-12—H_2-17, H_2-4′—H_2-7′, H_2-15′—H_2-17′), 1.35 (2H, m, H_2-11), 1.58 (3H, d, *J*=0.7 Hz, H_3-19), 1.65 (2H, m, H_2-3′), 1.95 (2H, t, *J*=7.7 Hz, H_2-10), 2.05 (4H, m, H_2-8′, H_2-14′), 2.09 (2H, m, H_2-7), 2.11 (2H, m, H_2-6), 2.24 (2H, t, *J*=7.7 Hz, H_2-2′), 2.61 (2H, m, OH-1, OH-3), 2.77 (2H, t, *J*=7.0 Hz, H_2-11′), 3.70 (1H, m, Ha-1), 3.91 (1H, m, H-2), 3.96 (1H, ddd, *J*=11.4, 3.3, 3.3 Hz, Hb-1), 4.33 (1H, br s, H-3), 5.09 (1H, t, *J*=6.6 Hz, H-8), 5.33 (2H, m, H-10′, H-12′), 5.39 (2H, m, H-9′, H-13′), 5.56 (1H, dd, *J*=15.4, 6.6 Hz, H-4), 5.80 (1H, dt, *J*=15.4, 6.6 Hz, H-5), 6.25 (1H, d, *J*=7.3 Hz, NH). ^{13}C-NMR (100 MHz, $CDCl_3$) δ: 14.1 (C-18, C-18′), 16.0 (C-19), 22.6 (C-17′), 22.7 (C-17), 25.6 (C-11′), 25.7 (C-3′), 27.2 (C-8′, C-14′), 27.5 (C-7), 28.0—29.6 (C-11—C-15, C-4′—C-7′, C-15′), 31.5 (C-16′), 31.9 (C-16), 32.5 (C-6), 36.8 (C-2′), 39.7 (C-10), 54.3 (C-2), 62.5 (C-1), 74.9 (C-3), 123.0 (C-8), 127.9 (C-12′), 128.1 (C-10′), 129.0 (C-4), 130.1 (C-9′), 130.2 (C-13′), 133.8 (C-5), 136.3 (C-9), 173.8 (C-1′).

(2*S*,2′*R*,3*R*,4*E*,8*E*)-*N*-2′-Hydroxyhexadecanoyl-2-amino-9-methyl-4,8-octadecadiene-1,3-diol (**3**): Amorphous powder. $[\alpha]_D^{19}$ +7.5° (*c*=0.1, $CHCl_3$) [lit.,[6] $[\alpha]_D^{21}$ +6.4° (*c*=0.76, $CHCl_3$)]. IR ($CHCl_3$) cm^{-1}: 3601, 3409, 2927, 2855, 1662, 1523, 1467. HR-EI-MS *m/z*: 565.5071 (M^+, Calcd for $C_{35}H_{67}NO_4$: 565.5070). EI-MS *m/z* (rel. int. %): 565 (M^+, 2), 314 (a, 27), 297 (b, 83). FAB-MS (negative ion mode; matrix, triethanolamine) *m/z*: 564 $[M-H]^-$. ^{1}H-NMR (600 MHz, $CDCl_3$) δ: 0.88 (6H, t, *J*=7.0 Hz, H_3-18, H_3-16′), 1.25—1.34 (34H, br s, H_2-12—H_2-17, H_2-5′—H_2-15′), 1.37 (2H, m, H_2-11), 1.42 (2H, m, H_2-4′), 1.58 (3H, br s, H_3-19), 1.65 (1H, m, Ha-3′), 1.84 (1H, m, Hb-3′), 1.95 (2H, t, *J*=7.7 Hz, H_2-10), 2.08 (2H, m, H_2-7), 2.10 (2H, m, H_2-6), 2.62 (2H, br s, OH-3, OH-2′), 2.69 (1H, br s, OH-1), 3.74 (1H, br d, *J*=11.4 Hz, Ha-1), 3.92 (1H, m, H-2), 3.97 (1H, dd, *J*=11.4, 3.7 Hz, Hb-1), 4.15 (1H, dd, *J*=7.7, 3.3 Hz, H-2′), 4.33 (1H, br s, H-3), 5.09 (1H, t, *J*=6.6 Hz, H-8), 5.55 (1H, dd, *J*=15.4, 6.6 Hz, H-4), 5.81 (1H, dt, *J*=15.4, 6.6 Hz, H-5), 7.14 (1H, d, *J*=7.7 Hz, NH). ^{13}C-NMR (100 MHz, $CDCl_3$) δ: 14.1 (C-18, C-16′), 16.0 (C-19), 22.7 (C-17, C-15′), 25.0 (C-4′), 27.5 (C-7), 28.0—29.7 (C-11—C-15, C-5′—C-13′), 31.9 (C-16, C-14′), 32.5 (C-6), 35.0 (C-3′), 39.7 (C-10), 54.3 (C-2), 62.3 (C-1), 72.3 (C-2′), 74.6 (C-3), 123.1 (C-8), 128.9 (C-4), 134.1 (C-5), 136.3 (C-9), 174.4 (C-1′).

(2*S*,2′*R*,3*R*,4*E*,8*E*)-*N*-2′-Hydroxypentadecanoyl-2-amino-9-methyl-4,8-octadecadiene-1,3-diol (**4**): Amorphous powder. $[\alpha]_D^{21}$ +7.0° (*c*=0.1, $CHCl_3$). IR ($CHCl_3$) cm^{-1}: 3604, 3402, 2927, 2855, 1657, 1525, 1467. HR-EI-MS *m/z*: 551.4930 (M^+, Calcd for $C_{34}H_{65}NO_4$: 551.4913). EI-MS *m/z* (rel. int. %): 551 (M^+, 3), 300 (a, 26), 283 (b, 78). FAB-MS (negative ion mode; matrix, triethanolamine) *m/z*: 550 $[M-H]^-$. ^{1}H-NMR (600 MHz, $CDCl_3$) δ: 0.88 (6H, t, *J*=7.0 Hz, H_3-18, H_3-15′), 1.25—1.33 (32H, br s, H_2-12—H_2-17, H_2-5′—H_2-14′), 1.35 (2H, m, H_2-11), 1.44 (2H, m, H_2-4′), 1.58 (3H, br s, H_3-19), 1.65 (1H, m, Ha-3′), 1.84 (1H, m, Hb-3′), 1.95 (2H, t, *J*=8.1 Hz, H_2-10), 2.08 (2H, m, H_2-7), 2.10 (2H, m, H_2-6), 2.61 (2H, d, *J*=4.8 Hz, OH-3, OH-2′), 2.66 (1H, br s, OH-1), 3.74 (1H, br d, *J*=11.0 Hz, Ha-1), 3.92 (1H, m, H-2), 3.98 (1H, br d, *J*=11.0 Hz, Hb-1), 4.15 (1H, dd, *J*=7.7, 3.3 Hz, H-2′), 4.33 (1H, br s, H-3), 5.09 (1H, t, *J*=7.0 Hz, H-8), 5.55 (1H, dd, *J*=15.8, 6.6 Hz, H-4), 5.81 (1H, dt, *J*=15.8, 6.6 Hz, H-5), 7.15 (1H, d, *J*=7.7 Hz, NH).

(2*S*,2′*R*,3*R*,4*E*,8*E*)-*N*-2′-Hydroxytetradecanoyl-2-amino-9-methyl-4,8-octadecadiene-1,3-diol (**5**): Amorphous powder. $[\alpha]_D^{20}$ +6.3° (c=0.2, $CHCl_3$). IR ($CHCl_3$) cm^{-1}: 3604, 3401, 2928, 2855, 1657, 1526, 1467. HR-EI-MS *m/z*: 537.4728 (M^+, Calcd for $C_{33}H_{63}NO_4$: 537.4757). EI-MS *m/z* (rel. int. %): 537 (M^+, 2), 286 (a, 11), 269 (b, 72). FAB-MS (negative ion mode; matrix, triethanolamine) *m/z*: 536 $[M-H]^-$. ^{1}H-NMR (600 MHz, $CDCl_3$) δ: 0.88 (6H, t, J=7.0 Hz, H_3-18, H_3-14′), 1.25—1.33 (30H, br s, H_2-12—H_2-17, H_2-5′—H_2-13′), 1.35 (2H, m, H_2-11), 1.44 (2H, m, H_2-4′), 1.58 (3H, br s, H_3-19), 1.65 (1H, m, Ha-3′), 1.84 (1H, m, Hb-3′), 1.95 (2H, t, J=8.1 Hz, H_2-10), 2.08 (2H, m, H_2-7), 2.10 (2H, m, H_2-6), 2.61 (2H, d, J=4.4 Hz, OH-3, OH-2′), 2.67 (1H, br s, OH-1), 3.74 (1H, br d, J=11.0 Hz, Ha-1), 3.92 (1H, m, H-2), 3.97 (1H, br d, J=11.0 Hz, Hb-1), 4.16 (1H, dd, J=8.1, 3.3 Hz, H-2′), 4.33 (1H, br s, H-3), 5.09 (1H, t, J=6.6 Hz, H-8), 5.55 (1H, dd, J=15.4, 6.6 Hz, H-4), 5.81 (1H, dt, J=15.4, 6.6 Hz, H-5), 7.15 (1H, d, J=8.1 Hz, NH).

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References and Notes

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