New Cerebrosides from a Marine Sponge *Haliclona* (*Reniera*) sp.

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A chemical investigation of the MeOH extract of a marine sponge *Haliclona* (*Reniera*) sp., collected off the coast of Ulleung Island, Korea, led to the isolation of thirteen new cerebrosides (1—3, 5—14), along with a known analogue (4). Their structures were elucidated on the basis of 1D and 2D NMR spectroscopy, MS spectrometry, and chemical method. The major new features of these glucocerebrosides are C_{15} and C_{19} acyl chains, long (C_{24}–C_{28}) acyl chains, or the *S*-configuration of the acyl chains. It is noteworthy that both *R*- and *S*-configurations of the acyl chains were observed in the same specimen.

Key words *Haliclona* (*Reniera*) sp.; cerebroside; marine sponge

Sponges of the genus *Haliclona* have been extensively studied and afforded around 200 compounds belonging to different chemical classes. These compounds include the cytotoxic tertiary alkaloids haliclonacyclamines,[1] antifungal pentacyclic alkaloid papuamine,[2] antitumor alkaloid manzamine,[3] and anti-inflammatory cyclic depsipeptides halipeptins,[4] to name a few.

In our previous study on the marine sponge *Haliclona* (*Reniera*) sp., we reported the isolation and structure elucidation of eight new cerebrosides[5] and a new sphingosine[6] obtained from the MeOH extract. Cerebrosides and sphingosine derivatives have been isolated from diverse natural sources, including plants,[7] marine sources,[8] microorganisms,[9] and human.[10] Phytosphingosine type cerebrosides are reported to exhibit bioactivities such as the antitumor,[11] antiviral,[12] inhibition of histidine decarboxylase,[13] cytotoxicity,[14] and antifungal activity.[14]

In a continuing study on the same sponge, fourteen phytosphingosine type cerebrosides (**1**—**14**) were isolated from the *n*-hexane layer. Nine (**1**—**9**) of them contained saturated acyl chains, while five (**10**—**14**) possessed unsaturated acyl chains. Their structures were established on the basis of NMR and MS analyses, and chemical methods. Some of the cerebrosides (**1**, **3**, **5**, **7**, **11**, **12**) contained unusual acyl chains with *S*-configuration. It is interesting to isolate epimeric pairs (**1**:**2**, **3**:**4**, **5**:**6**, **7**:**8**) from the same specimen. Herein, we report isolation and structural characterization of cerebrosides from a marine sponge *Haliclona* (*Reniera*) sp.

The brine shrimp-active MeOH extract (LD_{50} 126 μg/ml) of the sponge was partitioned between CH_2Cl_2 and H_2O. The CH_2Cl_2 layer was further partitioned between aqueous MeOH (LD_{50} 27 μg/ml) and *n*-hexane (LD_{50} 45 μg/ml). Separation of the *n*-hexane layer by medium pressure liquid chromatography (MPLC) on a normal-phase flash column yielded 20 fractions. Fraction 16 was found to contain sugar moieties as observed in ^{1}H-NMR spectra, and was subjected to repeated HPLC chromatographic separation using ODS column to yield nine cerebrosides (**1**—**9**). And the mixture of fractions 10 to 15 afforded five analogues (**10**—**14**) through HPLC separation using the same condition as for fraction 16. Their structures were defined using NMR (^{1}H, ^{13}C, correlation spectroscopy (COSY), heteronuclear single quantum correlation (HSQC), heteronuclear multiple bond correlation (HMBC), and total correlation spectroscopy (TOCSY)) and MS analysis, optical rotation data, and chemical method.

Renieroside C_1 (**1**) was obtained as a white, amorphous solid. Its molecular formula was assigned as $C_{39}H_{78}NO_{10}$ on the basis of HR-FAB-MS ($[M+H]^+$ at *m*/*z*: 720.5600, Δ −2.6 mmu) and 1D and 2D NMR spectroscopic analyses. The characteristic signals of a sugar (an anomeric proton at δ_H 4.30), an amide linkage (a nitrogenated methine proton at δ_H 4.27, and a carbonyl carbon at δ_C 174.8), and a long acyl

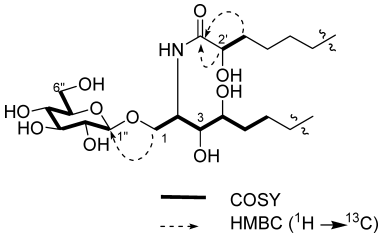
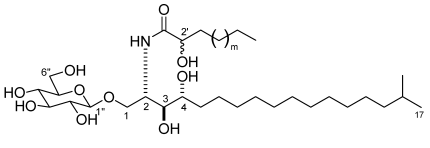
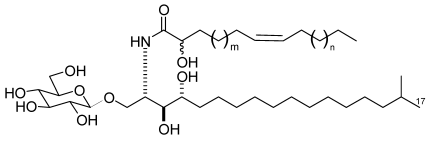


Fig. 1. Key COSY and HMBC Correlations of Compounds **1**—**14**





1 m = 10; 2′*S*
2 m = 10; 2′*R*
3 m = 11; 2′*S*
4 m = 11; 2′*R*
5 m = 12; 2′*S*
6 m = 12; 2′*R*
7 m = 13; 2′*S*
8 m = 13; 2′*R*
9 m = 14; 2′*R*

11 m = 11, n = 5, $\Delta^{16'}$; 2′*S*
12 m = 12, n = 5, $\Delta^{17'}$; 2′*S*
10 m = 10, n = 5, $\Delta^{15'}$; 2′*R*
13 m = 15, n = 3, $\Delta^{20'}$; 2′*R*
14 m = 16, n = 3, $\Delta^{21'}$; 2′*R*

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chain (terminal methyl protons at δ_H 0.90 and methylene protons at δ_H 1.55) were observed, indicating its nature as a glycosphingolipid. In the ^{13}C-NMR data, the carbon resonances appeared at 103.5 (C-1″), 73.8 (C-2″), 77.0 (C-3″), 70.5 (C-4″), 77.0 (C-5″), and 61.5 (C-6″), revealing the presence of a β-glucopyranoside.[15] The coupling constant of the anomeric proton at δ 4.30 (d, J_{HH}=8.0 Hz), and ^{13}C chemical shift value (δ_C 103.5) further confirmed the β configuration of the glucose unit (in the case of α-glucopyranoside: J_{HH}=3.7 Hz, δ_C 98.5).[16] Correlation spectroscopy (COSY) showed correlations between the H-2 signal (δ 4.27) and both the H-3 (δ 3.63) and the H-1 (δ 4.07, 3.82) signals. Similarly, COSY correlations were observed between H-3 signal (δ 3.63), H-4 (δ 3.53), and H-5 (δ 1.40) signals of the sphingosine moiety. The iso-form terminal of the sphingosine moiety was determined from the six-proton doublet at δ 0.87 (J=6.5 Hz) in the ^{1}H-NMR spectrum of the sphingosine base obtained by methanolysis of the mixture of **1**—**9** (*vide infra*). COSY spectrum also showed correlations from H-2′ (δ 4.04) to H-3′ (δ 1.58). HMBC spectrum showed correlations from H-2′ and H-3′ to carbonyl carbon C-1′ at δ 174.8. Furthermore, the HMBC spectrum revealed the correlation from H-1 (δ_H 4.07, 3.82) to anomeric carbon at δ 103.5 (C-1″) (Fig. 1). Methanolysis of **1** yielded fatty acid methyl ester (FAME), and its molecular formula was established as $C_{16}H_{32}O_3$ ([M+Na]$^+$ at *m/z*: 295, [M+H]$^+$ at *m/z*: 273). The optical rotation of the ester ($[\alpha]_D^{27}$ +3.2, MeOH) suggested it to be an *S* isomer.[17] Thus, the fatty acid moiety was deduced to be (2*S*)-2-hydroxypentadecanoic acid. On the basis of the above mentioned data the structure of **1** was defined as 1-*O*-β-D-glucopyranosyl-(2*S*,3*S*,4*R*)-2-[(2′*S*)-2′-hydroxypentadecanoylamino]-16-methyl-heptadeca-1,3,4-triol.

The molecular formula of renieroside C_2 (**2**) was found to be the same as compound **1** on the basis of HR-FAB-MS ([M+H]$^+$ at *m/z*: 720.5624, Δ −0.2 mmu), and ^{1}H- and ^{13}C-NMR data. The NMR data of **2** was found to be essentially identical to those of **1**. Methanolysis of **2** gave rise to a saturated FAME, which was analyzed to have the same molecular weight as that of **1** ($C_{16}H_{32}O_3$, [M+Na]$^+$ at *m/z*: 295) but with opposite optical rotation ($[\alpha]_D^{27}$ −3.7, MeOH). Hence, it was characterized as (2*R*)-2-hydroxypentadecanoic acid, and the structure of **2** was defined as 1-*O*-β-D-glucopyranosyl-(2*S*,3*S*,4*R*)-2-[(2′*R*)-2′-hydroxypentadecanoylamino]-16-methyl-heptadeca-1,3,4-triol.

Presence of both epimeric forms (**1**, **2**) in the same specimen is noteworthy. *R*-Configuration of α-hydroxyl group of the acyl chain is common in nature. However, the *S*-configuration is rare. Therefore, it was tried to confirm the configuration by Mosher's method[18] using compounds **10** and **11**. However, the adjacent multiplet methylene protons were crowded and not well resolved preventing accurate assignment.

The gross structures of compounds **3**—**9** were defined to be analogous to compound **1**, except differences in the length of acyl chains and the configuration of the hydroxyl group. The acyl moieties of compounds **3** and **4** were characterized as (2*S*)-2-hydroxyhexadecanoic acid and (2*R*)-2-hydroxyhexadecanoic acid, respectively, on the basis of analysis of the FAMEs thereof (FAME of **3**: [M+Na]$^+$ at *m/z* 309, $[\alpha]_D^{27}$ +3.8; FAME of **4**: [M+Na]$^+$ at *m/z* 309, $[\alpha]_D^{27}$ −6.0). The molecular structure of **4** (renieroside C_4) has been previously reported from the starfish *Linckia laevigata*.[19] Similarly, the structures of the acyl moieties of compounds **5**—**9** were defined as (2*S*)-2-hydroxyheptadecanoic acid, (2*R*)-2-hydroxyheptadecanoic acid, (2*S*)-2-hydroxyoctadecanoic acid, (2*R*)-2-hydroxyoctadecanoic acid, (2*R*)-2-hydroxynonadecanoic acid, respectively (see Experimental).

Due to the paucity of individual samples, stereochemistry of the sphingosine and glucose units was determined by using a mixture of **1**—**9**. Methanolysis of the mixture followed by silica-gel column chromatography afforded sphingosine base and methyl glucoside. ^{1}H- and ^{13}C-NMR data (Fig. 2) of the tetraacetyl derivative of the sphingosine base together with its specific rotation (Table 3) allowed the assignment of (2*S*,3*S*,4*R*) stereochemistry.[20] The specific rotation of the methyl glucoside ($[\alpha]_D^{27}$ +47.6, MeOH) indicated a D-configuration.[21]

Renieroside C_{10} (**10**) was obtained as a white amorphous solid. Its molecular formula was assigned as $C_{48}H_{94}NO_{10}$ on

Table 1. ^{1}H- and ^{13}C-NMR Data of **1** (CD_3OD, 500 MHz)

Position	δ_H [a)]	δ_C [b)]
Lipid base unit		
1	4.07 (dd, 11.0, 6.5)	68.5
	3.82 (dd, 10.5, 4.0)	
2	4.27 (m)	50.5
3	3.63 (m)	74.4
4	3.53 (m)	71.9
5	1.40 (m)	34.5
6—15	1.31 (m)	32—29
16	1.55 (m)	25.4
17, 18	0.88 (m)	21.5
N-Acyl unit		
1′		174.8
2′	4.04 (dd, 7.5, 4.0)	71.9
3′	1.58 (m)	32.0
4′—14′	1.31 (m)	32—29
15′	0.90 (m)	13.0
Glucose unit		
1″	4.30 (d, 8.0)	103.5
2″	3.19 (m)	73.8
3″	3.37 (m)	77.0
4″	3.26 (m)[c)]	70.5
5″	3.27 (m)[c)]	77.0
6″	3.87 (d, 12.0)	61.5
	3.69 (dd, 12.0, 5.5)	

a) Multiplicities and coupling constants are in parentheses. *b*) Assignments were based on HMBC and HSQC experiments. *c*) Overlapped with solvent peak (assignments were secured by HSQC experiment).

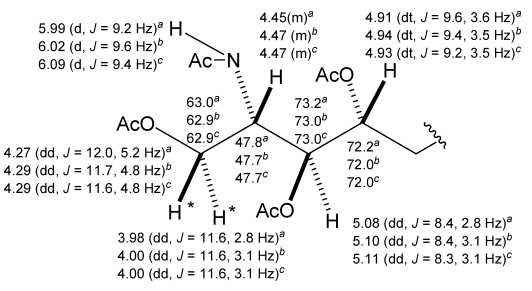

Fig. 2. Characteristic ^{1}H- and ^{13}C-NMR Data of the Sphingosine Base in $CDCl_3$ (δ Value)

[a] Renieroside C, [b] natural product isolated from *Asterina pectinifera*,[25] and [c] synthetic product.[26] (* Assignment may be reversed.)

Table 2. ^{1}H- and ^{13}C-NMR Data of **10** (C_5D_5N, 500 MHz)

Position	δ_H [a)]	δ_C [b)]
Lipid base unit		
1	4.74 (dd, 11.0, 6.5)	71.0
	4.55 (dd, 11.0, 4.5)	
2	5.29 (m)	52.1
3	4.30 (m)[c)]	76.0
4	4.22 (m)[c)]	71.9
5	1.93 (m)	34.6
6—15	1.29 (m)	32—29
16	1.47	28.5
17, 18	0.88 (m)	23.0
N-Acyl unit		
NH	8.60 (d, 9.5)	
1′		176.0
2′	4.60 (dd, 7.0, 3.0)	72.8
3′	2.01 (m)	32.0
4′—13′, 18′—23′	1.29 (m)	32.0—29.0
14′, 17′	2.12 (m)	28.0
15′, 16′	5.51 (m)	130.4
24′	0.88 (m)	14.7
Glucose unit		
1″	4.97 (d, 8.0)	106.2
2″	4.03 (t, J=8.5)	75.3
3″	4.22 (m)[c)]	78.9
4″	4.22 (m)[c)]	72.4
5″	3.89 (m)	79.0
6″	4.48 (d, 12.0, 2.5)	63.0
	4.35 (dd, 12.0, 5.5)	

a) Multiplicities and coupling constants are in parentheses. *b*) Assignments were based on HMBC and HSQC experiments. *c*) *J* values could not be observed because of overlapping with other signals.

Table 3. Comparison of Specific Rotations for Tetraacetyl Derivatives of the (2*S*,3*S*,4*R*)-Sphingosine Base

	Conc. (g/100 ml)	$[\alpha]_D^{27}$ ($CHCl_3$)
Renieroside C[a)]	0.1	+25.0
Natural product[b)]	1.5	+28.9
Synthetic product[c)]	1.5	+27.9

a) Renieroside C. *b*) Natural product isolated from *Asterina pectinifera*.[25] *c*) Synthetic product.[26]

the basis of HR-FAB-MS ($[M+H]^+$ at *m/z*: 844.6907, Δ +2.9 mmu) and 1D and 2D NMR spectroscopic analyses. The ^{1}H- and ^{13}C-NMR spectroscopic data were found to be nearly identical to those of **1**, except for the presence of a double bond (δ_H 5.51, δ_H 2.12). The methanolysis of **10** gave rise to a differed FAME. Its molecular formula was established as $C_{25}H_{48}O_3$ on the basis of FAB-MS data ($[M+Na]^+$ at *m/z*: 419). The location of the double bond was determined by FAB-CID-MS/MS analysis of the $[M+Na]^+$ ion at *m/z*: 419. Allylic cleavages were observed as enhanced peaks at *m/z*: 319 and 265, indicating the location of the double bond at C-15′ (Fig. 3). The geometry of $\Delta^{15'}$ was assigned as *Z* on the basis of chemical shifts of allylic carbons C-14′ and C-17′, which appeared at δ_C 28.0.[22] The optical rotation of the FAME ($[\alpha]_D^{27}$ −5.82, $CDCl_3$) indicated it as the *R* isomer.[21] On the basis of these data, the fatty acid moiety was deduced to be (2*R*,15*Z*)-2-hydroxytetracos-15-enoic acid. Therefore, the structure of compound **10** was defined as 1-*O*-*β*-D-glucopyranosyl-(2*S*,3*S*,4*R*)-2-[(2′*R*,15′*Z*)-2′-hydroxytetracos-15′-enoylamino]-16-methyl-heptadeca-1,3,4-triol.

The molecular formula of renieroside C_{11} (**11**) was found

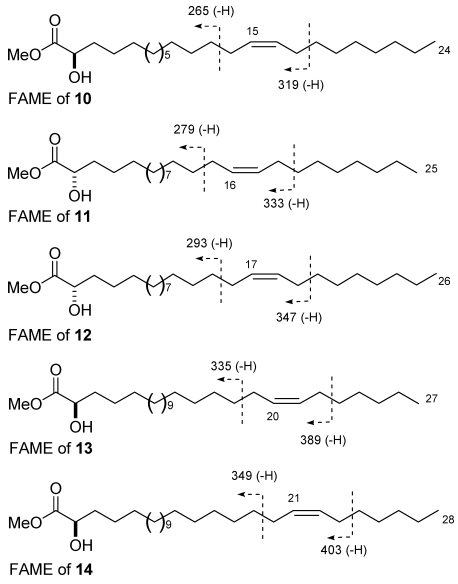

Fig. 3. Key FAB-CID Tandem Mass Fragmentations of the $[M+Na]^+$ Ions of the FAMEs Derived from **10**—**14**

to be $C_{49}H_{94}NO_{10}$ on the basis of HR-FAB-MS ($[M+H]^+$ at *m/z*: 858.7019, Δ −1.5 mmu), and ^{1}H- and ^{13}C-NMR spectroscopic results. Again, the ^{1}H- and ^{13}C-NMR spectroscopic data were found to be nearly identical to those of **10**. The methanolysis of **11** gave rise to a different FAME. Its molecular formula was established as $C_{26}H_{50}O_3$ on the basis of FAB-MS data ($[M+Na]^+$ at *m/z*: 433). The optical rotation of the FAME ($[\alpha]_D^{27}$ +22.3, $CDCl_3$) suggested it as the *S* isomer.[17] The acyl moiety was defined as (2*S*,16*Z*)-2-hydroxypentacos-16-enoic acid. The location of the double bond was determined by FAB-CID-MS/MS analysis of the [M+Na] ion at *m/z*: 433. Allylic cleavages were observed as enhanced peaks at *m/z*: 333 and 279, indicating the location of the double bond at C-16 (Fig. 3). All these data were in agreement with the proposed structure of **11** as 1-*O*-*β*-D-glucopyranosyl-(2*S*,3*S*,4*R*)-2-[(2′*S*,16′*Z*)-2′-hydroxypentacos-16′-enoylamino]-16-methyl-heptadeca-1,3,4-triol.

The gross structures of compounds **12**—**14** were defined to be analogous to compound **11**, with only differences in the length of acyl chain and the location of the double bond thereon. The acyl moieties of compounds **12**—**14** were characterized as (2*S*,17*Z*)-2-hydroxyhexacos-17-enoic acid, (2*R*,20*Z*)-2-hydroxyheptacos-20-enoic acid, and (2*R*,21*Z*)-2-hydroxyoctacos-21-enoic acid, respectively, on the basis of the analysis of FAMEs thereof (FAME of **12**: $[M+Na]^+$ *m/z* 447, $[\alpha]_D^{27}$ +10.0; FAME of **13**: $[M+Na]^+$ at *m/z* 461, $[\alpha]_D^{27}$ −6.38; FAME of **14**: $[M+Na]^+$ at *m/z* 475, $[\alpha]_D^{27}$ −22.0).

To the best of our knowledge, this is the first report on the isolation of glucocerebrosides containing saturated C_{15} and C_{19} acyl chains. This is also the first report on the isolation of isomeric pairs of cerebrosides. *α*-Hydroxy acyl chains with *S*-configuration were rather uncommon, and previously reported from bacterial genera *Salmonella* sp., *Klebsiella* sp., *Serratia* sp., *Escherichia coli*, and so on.[23,24]

Experimental

General Experimental Procedures Optical rotations were measured using a JASCO P-1020 polarimeter. 1D and 2D NMR spectra were recorded on Varian UNITY 400 and Varian INOVA 500 spectrometers. Chemical

shifts were reported with reference to the respective residual solvent or deuterated solvent peaks (δ_H 3.30 and δ_C 49.0 for CD_3OD). FAB-MS data were obtained on a JEOL JMS SX-102A. HR-FAB-MS data were obtained on a JEOL JMS SX-101A. HPLC was performed on a Gilson 370 pump with an YMC packed J'sphere ODS-H80 column (250×10 mm, 4 μm, 80 Å) using a Shodex RI-71 detector.

Animal Material The sponge, *Haliclona* (*Reniera*) sp., was collected off the coast of Ulleung Island, Korea in October, 2001, using SCUBA (20 m depth). The collected sample was frozen immediately. The specimen (sample No. J01U-6) was identified as *Haliclona* (*Reniera*) sp. by Prof. Sim, C. J. and has been described elsewhere.[5] A voucher specimen of the sponge (registry No. Spo. 45) was deposited in the Natural History Museum, Hannam University, Daejon, Korea.

Extraction and Isolation The frozen sponge (7 kg) was extracted with MeOH at room temperature. The MeOH extract (29.2 g) showed toxicity against brine shrimp larvae (LD_{50} 126 μg/ml). The MeOH extract was partitioned between CH_2Cl_2 and H_2O. The CH_2Cl_2 layer (6.6 g, LD_{50} 203 μg/ml) was further partitioned between aqueous MeOH (1.9 g, LD_{50} 27 μg/ml) and *n*-hexane layer (3.9 g, LD_{50} 45 μg/ml). A portion of the *n*-hexane layer (3.0 g) was subjected to Silica gel 60 (15—40 μm) column chromatography eluting with a gradient solvent system of 100% CH_2Cl_2 to 100% MeOH, to afford 20 fractions. Fraction 16 (64 mg) was subjected to RP-HPLC (YMC packed J'sphere ODS-H80 column, 250×10 mm, 4 μm, 80 Å), eluting with 96% aqueous MeOH to yield nine cerebrosides **1** (1.7 mg), **2** (0.5 mg), **3** (1.7 mg), **4** (2.3 mg), **5** (0.8 mg), **6** (0.5 mg), **7** (0.9 mg), **8** (1.0 mg), and **9** (0.5 mg). Compounds **10** (1.6 mg), **11** (2.4 mg), **12** (2.3 mg), **13** (3.2 mg), **14** (2.5 mg) were obtained by purification of combined fraction (10—15) by RP-HPLC (YMC packed J'sphere ODS-H80 column, 250×10 mm, 4 μm, 80 Å), eluting with 100% MeOH.

Renieroside C_1 (1), 1-*O*-β-D-Glucopyranosyl-(2*S*,3*S*,4*R*)-2-[(2′*S*)-2′-hydroxypentadecanoylamino]-16-methyl-heptadeca-1,3,4-triol White amorphous solid; $[\alpha]_D^{27}$ +11.9 (*c*=0.17, MeOH); ^{1}H- and ^{13}C-NMR see Table 1; FAB-MS (+ve mode) *m/z*: 742 $[M+Na]^+$, (−ve mode) *m/z* 718 $[M-H]^-$; HR-FAB-MS *m/z*: 720.5600 $[M+H]^+$ (Calcd for $C_{39}H_{78}NO_{10}$: 720.5626).

Methanolysis of 1 Cerebroside (1.6 mg) was dissolved in methanolic HCl (1.5 ml, 5% 1 N HCl in MeOH), and the mixture was refluxed on a magnetic stirrer for 18 h at 80 °C. It was cooled and extracted with *n*-hexane (3 ml×3). The *n*-hexane layer was evaporated under N_2 to yield the FAME (0.7 mg). $[\alpha]_D^{27}$ +3.2 (*c*=0.07, MeOH); ^{1}H-NMR (500 MHz, CD_3OD) δ: 4.12 (1H, dd, *J*=8.0, 4.8 Hz, H-2), 3.71 (3H, s, OCH_3), 1.61 (2H, m, H-3), 1.28 (22H, m, H-4—H-14), 0.89 (3H, t, *J*=7.0 Hz, H-15); ^{13}C-NMR (assignments based on HMBC and HSQC experiments, 500 MHz, CD_3OD) δ: 175.0 (C-1), 71.0 (C-2), 51.0 (OCH_3), 34.5 (C-3), 28.0—31.0 (C-4—C-14), 13.0 (C-15); FAB-MS *m/z*: 295 $[M+Na]^+$, 273 $[M+H]^+$.

Renieroside C_2 (2), 1-*O*-β-D-Glucopyranosyl-[(2*S*,3*S*,4*R*)-2-(2′*R*)-2′-hydroxypentadecanoylamino]-16-methyl-heptadeca-1,3,4-triol White amorphous solid; $[\alpha]_D^{27}$ +5.0 (*c*=0.05, MeOH); ^{1}H-NMR (500 MHz, CD_3OD) δ: 4.28 (1H, d, *J*=7.5 Hz), 4.25 (1H, m), 4.05 (1H, dd, *J*=11.0, 7.0 Hz), 4.03 (1H, dd, *J*=7.5, 4.0 Hz), 3.87 (1H, br d, *J*=11.5 Hz), 3.80 (1H, dd, *J*=10.5, 4.0 Hz), 3.67 (1H, dd, *J*=12.0, 6.0 Hz), 3.61 (1H, m), 3.52 (1H, m), 3.37 (1H, m), 3.26 (2H, m), 3.17 (1H, m), 1.80—1.10 (47H, m), 0.92—0.84 (9H, m); FAB-MS (+ve mode) *m/z*: 742 $[M+Na]^+$, (−ve mode) *m/z*: 718 $[M-H]^-$; HR-FAB-MS *m/z*: 720.5624 $[M+H]^+$ (Calcd for $C_{39}H_{78}NO_{10}$: 720.5626).

Methanolysis of 2 Cerebroside (0.5 mg) was subjected to methanolysis similarly to **1** to yield a FAME (0.3 mg). $[\alpha]_D^{27}$ −3.7 (*c*=0.03, MeOH); ^{1}H-NMR (500 MHz, CD_3OD) δ: 4.12 (1H, dd, *J*=8.0, 4.8 Hz, H-2), 3.71 (3H, s, OCH_3), 1.61 (2H, m, H-3), 1.28 (22H, m, H-4—H-14), 0.89 (3H, t, *J*=7.0 Hz, H-15); ^{13}C-NMR (assignments based on HMBC and HSQC experiments, 500 MHz, CD_3OD) δ: 175.0 (C-1), 71.0 (C-2), 51.0 (OCH_3), 34.5 (C-3), 28.0—31.0 (C-4—C-14), 13.0 (C-15); FAB-MS *m/z*: 295 $[M+Na]^+$.

Renieroside C_3 (3), 1-*O*-β-D-Glucopyranosyl-[(2*S*,3*S*,4*R*)-2-(2′*S*)-2′-hydroxyhexadecanoylamino]-16-methyl-heptadeca-1,3,4-triol White amorphous solid; $[\alpha]_D^{27}$ +4.2 (*c*=0.17, MeOH); ^{1}H-NMR (500 MHz, CD_3OD) δ: 4.28 (1H, d, *J*=8.0 Hz), 4.25 (1H, m), 4.05 (1H, dd, *J*=10.0, 6.0 Hz), 4.01 (1H, dd, *J*=8.0, 4.0 Hz), 3.86 (1H, br d, *J*=11.5 Hz), 3.80 (1H, dd, *J*=10.5, 4.0 Hz), 3.66 (1H, dd, *J*=11.5, 5.0 Hz), 3.61 (1H, m), 3.52 (1H, m), 3.37 (1H, m), 3.26 (2H, m), 3.17 (1H, m), 1.80—1.10 (49H, m), 0.92—0.84 (9H, m); FAB-MS (+ve mode) *m/z*: 756 $[M+Na]^+$, (−ve mode) *m/z*: 732 $[M-H]^-$; HR-FAB-MS *m/z*: 734.5771 $[M+H]^+$ (Calcd for $C_{40}H_{80}NO_{10}$: 734.5782).

Methanolysis of 3 Cerebroside (1.5 mg) was subjected to methanolysis similarly to **1** to yield the FAME (0.4 mg). $[\alpha]_D^{27}$ +3.8 (*c*=0.04, MeOH); ^{1}H-NMR (500 MHz, CD_3OD) δ: 4.12 (1H, dd, *J*=8.0, 4.8 Hz, H-2), 3.71 (3H, s, OCH_3), 1.61 (2H, m, H-3), 1.28 (24H, m, H-4—H-15), 0.89 (3H, t, *J*=7.0 Hz, H-16); ^{13}C-NMR (assignments based on HMBC and HSQC experiments, 500 MHz, CD_3OD) δ: 175.0 (C-1), 71.0 (C-2), 51.0 (OCH_3), 34.5 (C-3), 28.0—31.0 (C-4—C-15), 13.0 (C-16); FAB-MS *m/z*: 309 $[M+Na]^+$.

Renieroside C_4 (4), 1-*O*-β-D-Glucopyranosyl-[(2*S*,3*S*,4*R*)-2-(2′*R*)-2′-hydroxyhexadecanoylamino]-16-methyl-heptadeca-1,3,4-triol White amorphous solid; $[\alpha]_D^{27}$ +5.8 (*c*=0.23, MeOH); FAB-MS (+ve mode) *m/z*: 756 $[M+Na]^+$, (−ve mode) *m/z*: 732 $[M-H]^-$; HR-FAB-MS *m/z*: 734.5803 $[M+H]^+$ (Calcd for $C_{40}H_{80}NO_{10}$: 734.5782).

Methanolysis of 4 Cerebroside (2.0 mg) was subjected to methanolysis similarly to **1** to yield a FAME (0.3 mg). $[\alpha]_D^{27}$ −6.0 (*c*=0.03, MeOH); ^{1}H-NMR (500 MHz, CD_3OD) δ: 4.12 (1H, dd, *J*=8.0, 4.8 Hz, H-2), 3.71 (3H, s, OCH_3), 1.61 (2H, m, H-3), 1.28 (24H, m, H-4—H-15), 0.89 (3H, t, *J*=7.0 Hz, H-16); ^{13}C-NMR (assignments based on HMBC and HSQC experiments, 500 MHz, CD_3OD) δ: 175.0 (C-1), 71.0 (C-2), 51.0 (OCH_3), 34.5 (C-3), 28.0—31.0 (C-4—C-15), 13.0 (C-16); FAB-MS *m/z*: 309 $[M+Na]^+$.

Renieroside C_5 (5), 1-*O*-β-D-Glucopyranosyl-[(2*S*,3*S*,4*R*)-2-(2′*S*)-2′-hydroxyheptadecanoylamino]-16-methyl-heptadeca-1,3,4-triol White amorphous solid; $[\alpha]_D^{27}$ +1.6 (*c*=0.08, MeOH); ^{1}H-NMR (500 MHz, CD_3OD) δ: 4.28 (1H, d, *J*=7.0 Hz), 4.25 (1H, m), 4.05 (1H, dd, *J*=10.5, 6.0 Hz), 4.02 (1H, dd, *J*=8.0, 4.5 Hz), 3.87 (1H, br d, *J*=12.0 Hz), 3.80 (1H, dd, *J*=10.5, 4.0 Hz), 3.66 (1H, dd, *J*=12.0, 6.0 Hz), 3.61 (1H, m), 3.52 (1H, m), 3.34 (1H, m), 3.26 (2H, m) 3.17 (1H, m), 1.80—1.10 (51H, m), 0.92—0.85 (9H, m); FAB-MS (+ve mode) *m/z*: 748 $[M+H]^+$, (−ve mode) *m/z*: 746 $[M-H]^-$; HR-FAB-MS *m/z*: 748.5951 $[M+H]^+$ (Calcd for $C_{41}H_{82}NO_{10}$: 748.5939).

Methanolysis of 5 Cerebroside (0.8 mg) was subjected to methanolysis similarly to **1** to yield a FAME (0.3 mg). $[\alpha]_D^{27}$ +3.7 (*c*=0.03, MeOH); ^{1}H-NMR (500 MHz, CD_3OD) δ: 4.12 (1H, dd, *J*=8.0, 4.8 Hz, H-2), 3.71 (3H, s, OCH_3), 1.61 (2H, m, H-3), 1.28 (26H, m, H-4—H-16), 0.89 (3H, t, *J*=7.0 Hz, H-17); ^{13}C-NMR (assignments based on HMBC and HSQC experiments, 500 MHz, CD_3OD) δ: 175.0 (C-1), 71.0 (C-2), 51.0 (OCH_3), 34.5 (C-3), 28.0—31.0 (C-4–C-16), 13.0 (C-17); FAB-MS *m/z*: 323 $[M+Na]^+$.

Renieroside C_6 (6), 1-*O*-β-D-Glucopyranosyl-[(2*S*,3*S*,4*R*)-2-(2′*R*)-2′-hydroxyheptadecanoylamino]-16-methyl-heptadeca-1,3,4-triol White amorphous solid; $[\alpha]_D^{27}$ +27.6 (*c*=0.05, MeOH); ^{1}H-NMR (500 MHz, CD_3OD) δ: 4.28 (1H, d, *J*=7.5 Hz), 4.25 (1H, m), 4.05 (1H, dd, *J*=11.0, 6.5 Hz), 4.02 (1H, dd, *J*=7.5, 4.0 Hz), 3.87 (1H, br d, *J*=12.0 Hz), 3.80 (1H, dd, *J*=10.5, 4.5 Hz), 3.67 (1H, dd, *J*=11.5, 4.5 Hz), 3.61 (1H, m), 3.51 (1H, m), 3.34 (1H, m), 3.26 (2H, m) 3.17 (1H, m), 1.80—1.10 (51H, m), 0.92—0.85 (9H, m); FAB-MS (+ve mode) *m/z*: 770 $[M+Na]^+$, (−ve mode) *m/z*: 746 $[M-H]^-$; HR-FAB-MS *m/z*: 748.5944 $[M+H]^+$ (Calcd for $C_{41}H_{82}NO_{10}$: 748.5939).

Methanolysis of 6 Cerebroside (0.5 mg) was subjected to methanolysis similarly to **1** to yield a FAME (0.1 mg). $[\alpha]_D^{27}$ −3.6 (*c*=0.01, MeOH); ^{1}H-NMR (500 MHz, CD_3OD) δ: 4.12 (1H, dd, *J*=8.0, 4.8 Hz, H-2), 3.71 (3H, s, OCH_3), 1.61 (2H, m, H-3), 1.28 (26H, m, H-4—H-16), 0.89 (3H, t, *J*=7.0 Hz, H-17); ^{13}C-NMR (assignments based on HMBC and HSQC experiments, 500 MHz, CD_3OD) δ: 175.0 (C-1), 71.0 (C-2), 51.0 (OCH_3), 34.5 (C-3), 28.0—31.0 (C-4—C-16), 13.0 (C-17); FAB-MS *m/z*: 323 $[M+Na]^+$.

Renieroside C_7 (7), 1-*O*-β-D-Glucopyranosyl-[(2*S*,3*S*,4*R*)-2-(2′*S*)-2′-hydroxyoctadecanoylamino]-16-methyl-heptadeca-1,3,4-triol White amorphous solid; $[\alpha]_D^{27}$ +9.0 (*c*=0.09, MeOH); ^{1}H-NMR (500 MHz, CD_3OD) δ: 4.28 (1H, d, *J*=7.5 Hz), 4.24 (1H, m), 4.05 (1H, dd, *J*=10.0, 6.0 Hz), 4.01 (1H, dd, *J*=7.5, 3.5 Hz), 3.86 (1H, dd, *J*=12.0, 1.5 Hz), 3.80 (1H, dd, *J*=10.5, 4.5 Hz), 3.67 (1H, dd, *J*=11.5, 4.5 Hz), 3.61 (1H, m), 3.51 (1H, m), 3.34 (1H, m), 3.26 (2H, m) 3.18 (1H, m), 1.80—1.10 (53H, m), 0.90—0.84 (9H, m); FAB-MS (+ve mode) *m/z*: 784 $[M+Na]^+$, (−ve mode) *m/z*: 760 $[M-H]^-$; HR-FAB-MS *m/z*: 762.6071 $[M+H]^+$ (Calcd for $C_{42}H_{84}NO_{10}$: 762.6095).

Methanolysis of 7 Cerebroside (0.8 mg) was subjected to methanolysis similarly to **1** to yield a FAME (0.1 mg). $[\alpha]_D^{27}$ +3.8 (*c*=0.01, MeOH); ^{1}H-NMR (500 MHz, CD_3OD) δ: 4.12 (1H, dd, *J*=8.0, 4.8 Hz, H-2), 3.71 (3H, s, OCH_3), 1.61 (2H, m, H-3), 1.28 (28H, m, H-4—H-17), 0.89 (3H, t, *J*=7.0 Hz, H-18); ^{13}C-NMR (assignments based on HMBC and HSQC experiments, 500 MHz, CD_3OD) δ: 175.0 (C-1), 71.0 (C-2), 51.0 (OCH_3), 34.5 (C-3), 28.0—31.0 (C-4—C-17), 13.0 (C-18); FAB-MS *m/z*: 337 $[M+Na]^+$.

Renieroside C_8 (8), 1-*O*-β-D-Glucopyranosyl-[(2*S*,3*S*,4*R*)-2-(2′*R*)-2′-hydroxyoctadecanoylamino]-16-methyl-heptadeca-1,3,4-triol White amorphous solid; $[\alpha]_D^{27}$ +14.4 (*c*=0.1, MeOH); ^{1}H-NMR (500 MHz, CD_3OD) δ: 4.27 (1H, d, *J*=7.5 Hz), 4.24 (1H, m), 4.05 (1H, dd, *J*=10.5, 6.0 Hz), 4.01 (1H, dd, *J*=7.0, 3.5 Hz), 3.86 (1H, dd, *J*=11.5, 2.0 Hz), 3.79 (1H, dd, *J*=10.5, 3.5 Hz), 3.66 (1H, dd, *J*=11.5, 5.0 Hz), 3.61 (1H, m), 3.51

(1H, m), 3.34 (1H, m), 3.26 (2H, m) 3.17 (1H, m), 1.80—1.10 (53H, m), 0.91—0.84 (9H, m); FAB-MS (+ve mode) *m/z*: 784 $[M+Na]^+$, (−ve mode) *m/z*: 760 $[M-H]^-$; HR-FAB-MS *m/z*: 762.6110 $[M+H]^+$ (Calcd for $C_{41}H_{82}NO_{10}$: 762.6095).

Methanolysis of 8 Cerebroside (0.8 mg) was subjected to methanolysis similarly to **1** to yield a FAME (0.3 mg). $[\alpha]_D^{27}$ −3.6 (*c*=0.04, MeOH); ^{1}H-NMR (500 MHz, CD_3OD) δ: 4.12 (1H, dd, *J*=8.0, 4.8 Hz, H-2), 3.71 (3H, s, OCH_3), 1.61 (2H, m, H-3), 1.28 (28H, m, H-4—H-17), 0.89 (3H, t, *J*=7.0 Hz, H-18); ^{13}C-NMR (assignments based on HMBC and HSQC experiments, 500 MHz, CD_3OD) δ: 175.0 (C-1), 71.0 (C-2), 51.0 (OCH_3), 34.5 (C-3), 28.0—31.0 (C-4—C-17), 13.0 (C-18); FAB-MS *m/z*: 351 $[M+Na]^+$.

Renieroside C_9 (9), 1-*O*-*β*-D-Glucopyranosyl-[(2*S*,3*S*,4*R*)-2-(2′*R*)-2′-hydroxynonadecanoylamino]-16-methyl-heptadeca-1,3,4-triol White amorphous solid; $[\alpha]_D^{27}$ +10.2 (*c*=0.05, MeOH); ^{1}H-NMR (500 MHz, CD_3OD) δ: 4.28 (1H, d, *J*=8.0 Hz), 4.24 (1H, m), 4.05 (1H, dd, *J*=10.5, 6.0 Hz), 4.01 (1H, dd, *J*=7.5, 4.0 Hz), 3.86 (1H, dd, *J*=11.0, 1.5 Hz), 3.79 (1H, dd, *J*=10.5, 3.5 Hz), 3.66 (1H, dd, *J*=12.0, 5.0 Hz), 3.61 (1H, m), 3.52 (1H, m), 3.34 (1H, m), 3.26 (2H, m) 3.17 (1H, m), 1.80—1.10 (55H, m), 0.91—0.84 (9H, m); FAB-MS (+ve mode) *m/z*: 798 $[M+Na]^+$, (−ve mode) *m/z*: 774 $[M-H]^-$; HR-FAB-MS *m/z*: 776.6243 $[M+H]^+$ (Calcd for $C_{41}H_{82}NO_{10}$: 776.6252).

Methanolysis of 9 Cerebroside (0.5 mg) was subjected to methanolysis similarly to **1** to yield a FAME (0.13 mg). $[\alpha]_D^{27}$ −20.0 (*c*=0.03, MeOH); ^{1}H-NMR (500 MHz, CD_3OD) δ: 4.12 (1H, dd, *J*=8.0, 4.8 Hz, H-2), 3.71 (3H, s, OCH_3), 1.61 (2H, m, H-3), 1.28 (30H, m, H-4—H-18), 0.89 (3H, t, *J*=7.0 Hz, H-19); ^{13}C-NMR (assignments based on HMBC and HSQC experiments, 500 MHz, CD_3OD) δ: 175.0 (C-1), 71.0 (C-2), 51.0 (OCH_3), 34.5 (C-3), 28.0—31.0 (C-4—C-18), 13.0 (C-19); FAB-MS *m/z*: 337 $[M+Na]^+$.

Renieroside C_{10} (10), 1-*O*-*β*-D-Glucopyranosyl-[(2*S*,3*S*,4*R*)-2-(2′*R*,15′*Z*)-2′-hydroxytetracos-15′-enoylamino]-16-methyl-heptadeca-1,3,4-triol White amorphous solid; $[\alpha]_D^{27}$ +6.6 (*c*=0.16, MeOH); ^{1}H- and ^{13}C-NMR see Table 2; FAB-MS *m/z*: 866 $[M+Na]^+$, 682 $[(M+H)-162]^+$; HR-FAB-MS *m/z*: 844.6907 $[M+H]^+$ (Calcd for $C_{48}H_{94}NO_{10}$: 844.6878).

Methanolysis of 10 Cerebroside (1.5 mg) was subjected to methanolysis similarly to **1** to yield a FAME (1.1 mg). $[\alpha]_D^{27}$ −5.8 (*c*=0.11, $CHCl_3$); ^{1}H-NMR (500M Hz, CD_3OD) δ: 5.35 (2H, m, H-15, H-16) 4.21 (1H, dd, *J*=9.0, 6.0 Hz, H-2), 3.79 (3H, s, OCH_3), 2.01 (4H, m, H-14, 17), 1.62 (2H, m, H-3), 1.25 (32H, m, H-4—H-13, H-18—H-23), 0.87 (3H, t, *J*=5.5 Hz, H-24); FAB-MS *m/z*: 419 $[M+Na]^+$; FAB-CID-MS/MS *m/z*: 419 $[M+Na]^+$, 265, 319.

Renieroside C_{11} (11), 1-*O*-*β*-D-Glucopyranosyl-[(2*S*,3*S*,4*R*)-2-(2′*S*,16′*Z*)-2′-hydroxypentacos-16′-enoylamino]-16-methyl-heptadeca-1,3,4-triol White amorphous solid; $[\alpha]_D^{27}$ +7.7 (*c*=0.24, MeOH); ^{1}H-NMR (500 MHz, C_5D_5N) δ: 8.59 (1H, d, *J*=9.0 Hz), 5.51 (2H, m), 5.30 (1H, m), 4.97 (1H, d, *J*=8.0 Hz), 4.74 (1H, dd, *J*=10.5, 6.5 Hz), 4.60 (1H, m), 4.55 (1H, dd, *J*=11.0, 4.5 Hz), 4.50 (1H, d, *J*=12.0 Hz), 4.34 (1H, m), 4.30 (1H, m), 4.22 (3H, m), 4.03 (1H, t, *J*=7.5 Hz), 3.89 (1H, m), 2.13 (4H, m), 2.00—1.10 (59H, m), 0.90—0.84 (9H, m); FAB-MS: *m/z*: 880 $[M+Na]^+$, 696 $[(M+H)-162]^+$; HR-FAB-MS: *m/z*: 858.7019 $[M+H]^+$ (Calcd for $C_{49}H_{96}NO_{10}$: 8587034).

Methanolysis of 11 Cerebroside (2.2 mg) was subjected to methanolysis similarly to **1** to yield a FAME (0.15 mg). $[\alpha]_D^{27}$ +22.3 (*c*=0.15, $CHCl_3$); ^{1}H-NMR (500 MHz, CD_3OD) δ: 5.35 (2H, m, H-16, H-17), 4.22 (1H, dd, *J*=8.5, 5.5 Hz, H-2), 3.79 (3H, s, OCH_3), 2.02 (4H, m , H-15, H-18), 1.62 (2H, m, H-3), 1.26 (34H, m, H-4—H-14, H-19—H-24), 0.88 (3H, t, *J*=7.5 Hz, H-25); FAB-MS *m/z*: 433 $[M+Na]^+$; FAB-CID-MS/MS *m/z*: 433 $[M+Na]^+$, 279, 333.

Renieroside C_{12} (12), 1-*O*-*β*-D-Glucopyranosyl-[(2*S*,3*S*,4*R*)-2-(2′*S*,17′*Z*)-2′-hydroxyhexacos-17′-enoylamino]-16-methyl-heptadeca-1,3,4-triol White amorphous solid; $[\alpha]_D^{27}$ +8.5 (*c*=0.23, MeOH); ^{1}H-NMR (500 MHz, C_5D_5N) δ: 8.60 (1H, d, *J*=9.5 Hz), 5.51 (2H, m), 5.31 (1H, m), 4.97 (1H, d, *J*=8.0 Hz), 4.75 (1H, m), 4.60 (1H, m), 4.56 (1H, *J*=11.5, 3.0 Hz), 4.51 (1H, d, *J*=12.0 Hz), 4.36 (1H, m), 4.30 (1H, m), 4.22 (3H, m), 4.04 (1H, t, *J*=8.0 Hz), 3.89 (1H, m), 2.13 (4H, m), 2.00—1.10 (61H, m), 0.90—0.84 (9H, m); FAB-MS *m/z*: 894 $[M+Na]^+$, 710 $[(M+H)-162]^+$; HR-FAB-MS *m/z*: 872.7209 $[M+H]^+$ (Calcd for $C_{50}H_{98}NO_{10}$: 872.7191).

Methanolysis of 12 Cerebroside (2.2 mg) was subjected to methanolysis similarly to **1** to yield a FAME (1.3 mg). $[\alpha]_D^{27}$ +10.0 (*c*=0.13, $CHCl_3$); ^{1}H-NMR (500 MHz, CD_3OD) δ: 5.35 (2H, m, H-17, H-18), 4.21 (1H, m, H-2), 3.79 (3H, s, OCH_3), 2.01 (4H, m , H-16, H-19), 1.62 (2H, m, H-3), 1.26 (34H, m, H-4—H-15, H-20—H-25), 0.89 (3H, t, *J*=7.0 Hz, H-26); FAB-MS *m/z*: 447 $[M+Na]^+$; FAB-CID-MS/MS *m/z*: 447 $[M+Na]^+$, 293, 347.

Renieroside C_{13} (13), 1-*O*-*β*-D-Glucopyranosyl-[(2*S*,3*S*,4*R*)-2-(2′*R*,20′*Z*)-2′-hydroxyheptacos-20′-enoylamino]-16-methyl-heptadeca-1,3,4-triol White amorphous solid; $[\alpha]_D^{27}$ +6.6 (*c*=0.28, MeOH); ^{1}H-NMR (500 MHz, C_5D_5N) δ: 8.60 (1H, d, *J*=9.5 Hz), 5.50 (2H, m), 5.30 (1H, m), 4.97 (1H, d, *J*=8.0 Hz), 4.75 (1H, dd, *J*=10.5, 7.0 Hz), 4.61 (1H, dd, *J*=7.5, 4.0 Hz), 4.55 (1H, *J*=11.0, 4.5 Hz), 4.51 (1H, d, *J*=11.5 Hz), 4.35 (1H, dd, *J*=11.5, 5.5 Hz), 4.30 (1H, m), 4.22 (3H, m), 4.04 (1H, t, *J*=8.5 Hz), 3.89 (1H, m), 2.12 (4H, m), 2.00—1.10 (63H, m), 0.91—0.84 (9H, m); FAB-MS *m/z*: 908 $[M+Na]^+$, 724 $[(M+H)-162]^+$; HR-FAB-MS *m/z*: 886.7357 $[M+H]^+$ (Calcd for $C_{51}H_{100}NO_{10}$: 886.7347).

Methanolysis of 13 Cerebroside (2.5 mg) was subjected to methanolysis similarly to **1** to yield a FAME (0.8 mg). $[\alpha]_D^{27}$ −6.4 (*c*=0.08, $CHCl_3$); ^{1}H-NMR (500 MHz, CD_3OD) δ: 5.35 (2H, m, H-20, H-21), 4.24 (1H, dd, *J*=8.5, 5.5 Hz, H-2), 3.79 (3H, s, OCH_3), 2.01 (4H, m , H-19, H-22), 1.62 (2H, m, H-3), 1.26 (34H, m, H-4—H-18, H-23—H-26), 0.88 (3H, t, *J*=7.0 Hz, H-27); FAB-MS *m/z*: 461 $[M+Na]^+$; FAB-CID-MS/MS (+ve mode) *m/z*: 461 $[M+Na]^+$, 307, 361, (−ve mode) *m/z*: 437 $[M-H]^-$, 311, 365.

Renieroside C_{14} (14), 1-*O*-*β*-D-Glucopyranosyl-[(2*S*,3*S*,4*R*)-2-(2′*R*,21′*Z*)-2′-hydroxyoctacos-21′-enoylamino]-16-methyl-heptadeca-1,3,4-triol White amorphous solid; $[\alpha]_D^{27}$ +11.9 (*c*=0.1, MeOH); ^{1}H-NMR (500 MHz, C_5D_5N) δ: 8.60 (1H, d, *J*=9.5 Hz), 5.51 (2H, m), 5.31 (1H, m), 4.97 (1H, d, *J*=8.0 Hz), 4.75 (1H, m), 4.60 (1H, m), 4.56 (1H, *J*=11.5, 3.0 Hz), 4.51 (1H, d, *J*=12.0 Hz), 4.36 (1H, m), 4.30 (1H, m), 4.22 (3H, m), 4.04 (1H, t, *J*=8.0 Hz), 3.89 (1H, m), 2.13 (4H, m), 2.00—1.10 (65H, m), 0.90—0.84 (9H, m); FAB-MS *m/z*: 922 $[M+Na]^+$, 738 $[(M+H)-162]^+$; HR-FAB-MS *m/z*: 900.7510 $[M+H]^+$ (Calcd for $C_{52}H_{102}NO_{10}$: 900.7504).

Methanolysis of 14 Cerebroside (1.0 mg) was subjected to methanolysis similarly to **1** to yield a FAME (0.5 mg). $[\alpha]_D^{27}$ −22.0 (*c*=0.05, $CHCl_3$); ^{1}H-NMR (500 MHz, CD_3OD) δ: 5.34 (2H, M, H-21, H-22), 4.22 (1H, dd, *J*=8.5, 6.0 Hz, H-2), 3.79 (3H, s, OCH_3), 2.01 (4H, m, H-20, H-23), 1.62 (2H, m, H-3), 1.25 (34H, m, H-4—H-19, H-24—H-27), 0.87 (3H, t, *J*=5.5 Hz, H-28); FAB-MS *m/z*: 475 $[M+Na]^+$; FAB-CID-MS/MS *m/z*: 475 $[M+Na]^+$, 321, 375.

Methanolysis of the Glycoside Mixture of 1—9 The mixture of **1**—**9** was heated at 80 °C in 4.0 ml of 1 N HCl/MeOH (20 : 80) overnight. The reaction mixture was concentrated by evaporation. The residue was subjected to silica gel column chromatography with a MeOH/$CHCl_3$ solvent system. MeOH/$CHCl_3$ (1 : 4) fraction afforded a mixture of sphingosine bases (1.5 mg). ^{1}H-NMR (400 MHz, C_5D_5N) δ: 6.37 (–NH, br s), 5.01, 4.83, 4.48, 2.18, 1.81, 1.26, 0.87 (6H, d, *J*=6.5 Hz); ^{13}C-NMR (400 MHz, C_5D_5N) δ: 73.7, 72.7, 59.3, 57.4, 39.2, 35.4, 34.6, 32.1, 30.2, 29.9, 28.1, 27.7, 26.0, 22.8. The MeOH elute yielded an anomeric mixture of the methyl glucoside (1.8 mg); $[\alpha]_D^{27}$ +47.6 (*c*=0.18, MeOH); ^{1}H-NMR (MeOH) δ 5.11 (d, *J*=3.2 Hz, H-1, *α*-anomer), 4.47 (d, *J*=7.6 Hz, H-1, *β*-anomer).

Acetylation of the Mixture of the Sphingosine Bases A mixture of the sphingosine bases (1.5 mg) isolated from the methanolysis of a mixture of glucosides of **1**—**9** was treated with Ac_2O/pyridine (1 : 1, 0.5 ml) overnight at room temperature. The reaction mixture was diluted with 2 ml of H_2O and extracted with $CHCl_3$ (2 ml×3). The $CHCl_3$ layer was evaporated under N_2 to yield the mixture of peracetylated sphingosine bases (4.0 mg). $[\alpha]_D^{27}$ +25.0 (*c*=0.12, $CHCl_3$); ^{1}H-NMR (500 MHz, $CDCl_3$) δ: 5.99 (1H, d, *J*=9.2 Hz, –NH), 5.08 (1H, dd, *J*=8.4, 2.8 Hz, H-3), 4.91 (1H, dt, *J*=9.6, 3.6 Hz, H-4), 4.45 (1H, m, H-2), 4.27 (1H, dd, *J*=12.0, 5.2 Hz, H-1), 3.98 (1H, dd, *J*=11.6, 2.8 Hz, H-1), 2.06 (s), 2.03 (s), 2.01 (s), 1.63 (m), 1.49 (m), 1.20—1.42 (m), 0.85 (6H, d, *J*=6.4 Hz); ^{13}C-NMR (400 MHz, $CDCl_3$) δ: 171.3, 171.1, 170.3, 169.9, 73.2 (C-3), 72.2 (C-4), 63.0 (C-1), 47.8 (C-2), 39.3, 32.1, 29.8, 29.7, 28.3, 25.7, 23.5, 22.9, 21.3, 21.0; FAB-MS *m/z*: 486 $[M+H]^+$; HR-FAB-MS *m/z*: 486.3444 $[M+H]^+$ (Calcd for $C_{26}H_{48}NO_7$: 486.3431).

Preparation of *S*- and *R*-MTPA Derivatives of the Fatty Acid Methyl Esters (*S*)-*α*-Methoxy-*α*-(trifluoromethyl) phenylacetyl chloride (1 *μ*l) was individually added to solution of FAME (0.5 mg/$CDCl_3$, 0.4 mg/$CDCl_3$) of **10** in pyridine-d_5 (10 *μ*l). The mixture was then stirred at room temperature for 24 h, followed by evaporation under N_2 to yield the *R*-MTPA derivative. The progress of reaction was monitored by TLC. The same procedure was employed for *S*-MTPA derivative of FAME of **10**. The FAME (0.4 mg/$CDCl_3$) of **11** also subjected to Mosher method similarly to FAME of **10** to yield the *S*- and *R*-MTPA derivatives.

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