Biologically Active Glycosides from Asteroidea, 41.[1] Isolation and Structure Determination of Glucocerebrosides from the Starfish *Linckia laevigata*

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A new glucocerebroside, linckiacerebroside A (1) and a known glucocerebroside S-2a-3 (2), have been isolated from the cerebroside molecular species obtained from the less polar fraction of the $CHCl_3$/MeOH extract of the starfish *Linckia laevigata*, together with three pseudo homogeneous glucocerebroside, 3, 4, and 5. The structures of these cerebrosides were determined on the basis of chemical and spectroscopic evidence.

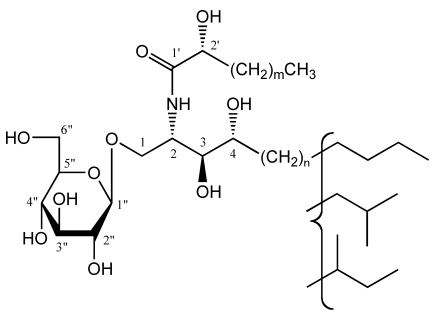
Key words glycosphingolipid; starfish; *Linckia laevigata*; cerebroside

In our ongoing search for biologically active glycosphingolipids from starfish, we have isolated numerous cerebrosides, ceramide-lactosides, sulfatides, and gangliosides with biological activities.[2—4] As for the starfish *Linckia laevigata* (Aohitode in Japanese), we reported the isolation and structure elucidation of a ganglioside molecular species obtained from the water-soluble lipid fraction of the $CHCl_3$/MeOH extracts of the starfish.[5] In a continuation of the study, the isolation and characterization of the cerebroside obtained from the less polar fraction were conducted in the hope of discovering new medicinal resources from marine natural products. We report here isolation and structure determination of a new cerebroside from the whole bodies of *L. laevigata*.

The AcOEt-insoluble part, which was obtained from the less polar fraction of the $CHCl_3$/MeOH extract of the whole bodies of *L. laevigata*, was separated by normal-phase column chromatography followed by Sephadex LH-20 column chromatography to give two cerebroside molecular species, LLC-1 and LLC-2, each showing a single spot on normal-phase silica gel TLC. In this time the major one, LLC-2, was examined.

Structure of Cerebroside Molecular Species LLC-2 The positive-ion fast-atom bombardment mass spectrometry (FAB-MS) spectrum of LLC-2 exhibits a series of $[M+Na]^+$ ion peaks at *m*/*z* 756, 770, 784, 812, 826, 840, 854, 868, and 882. LLC-2 shows the characteristic signals of a phytosphingosine-type cerebroside possessing 2-hydroxy fatty acid and β-glucopyranose moieties in its ^{1}H- and ^{13}C-NMR spectra (Fig. 1, Tables 1, 2). Furthermore, LLC-2 is thought to possess the normal, iso and *ante*-iso types[6] of side chains on the basis of the carbon atom signals due to the terminal methyl group (Table 2),[7] and the chemical degradation described below. The ^{1}H- and ^{13}C-NMR spectra of LLC-2 are in good agreement with that of known glucocerebrosides, which is composed of (2*S*,3*S*,4*R*)-phytosphingosine, (2*R*)-2-hydroxy fatty acid, and β-D-glucopyranose, except the side-chain moieties (Tables 1, 2).[8] Therefore LLC-2 is suggested to be a molecular species of phytosphingosine-type cerebroside possessing 2-hydroxy fatty acid and β-glucopyranose.

When LLC-2 was methanolyzed with methanolic hydrochloric acid, mixture of fatty acid methyl ester (FAM), long-chain base (LCB), and methyl glucopyranoside were obtained. Gas chromatography-mass spectrometry (GC-MS) analysis of the FAM mixture showed the existence of nine components, which were characterized as methyl 2-hydroxypentadecanoate (FAM-1), methyl 2-hydroxyhexadecanoate (FAM-2), methyl 2-hydroxyheptadecanoate (FAM-3), methyl 2-hydroxyoctadecanoate (FAM-4), methyl 2-hydroxyheneicosanoate (FAM-5), methyl 2-hydroxydocosanoate (FAM-6), methyl 2-hydroxytricosanoates (FAM-7′ and FAM-7), and methyl 2-hydroxytetracosanoate (FAM-8). The major FAM was methyl 2-hydroxydocosanoate (FAM-6). The retention time of the FAMs, except FAM-7′, was identical with that of the normal-type FAMs from the starfish ceramide AC-1.[9] A pair of FAMs (FAM-7′ and FAM-7), showed the same molecular ion peaks but the different retention time. It was supposed to be caused by the difference of the terminal methyl group. The retention time was nearly proportional to the hydrocarbon chain length, and moreover retention time of FAM possessing the iso and *ante*-iso moieties are shorter than that of normal-type (iso<*ante*-iso<normal).[10,11] Therefore, FAM-7′ must be iso or *ante*-iso-type of methyl 2-hydroxytricosanoate. On the other hand, GC-MS analysis of the trimethylsilyl (TMS) derivative of the LCB mixture suggested that the LCB components were 2-amino-1,3,4-hexadecanetriol (LCB-1), 2-amino-1,3,4-heptadecanetriols (LCB-2′, LCB-2″, and LCB-2), 2-amino-1,3,4-octadecanetriols (LCB-3′, LCB-3″, and LCB-3), and 2-amino-1,3,4-nonadecanetriol (LCB-4). The major LCB was 2-amino-1,3,4-heptadecanetriol (LCB-2′). By comparing the retention time, LCB-2′, -3′ were suggested to be iso-type, and LCB-2″, -3″ *ante*-iso-type.[10,11]



LLC-2 : m = 12, 13, 14, 15, 18, **19(major)**, 20, 21
n = 8, **9(major)**, 10, 11

Synthetic Cerebroside : m = 21
n = 8 (normal)

Fig. 1

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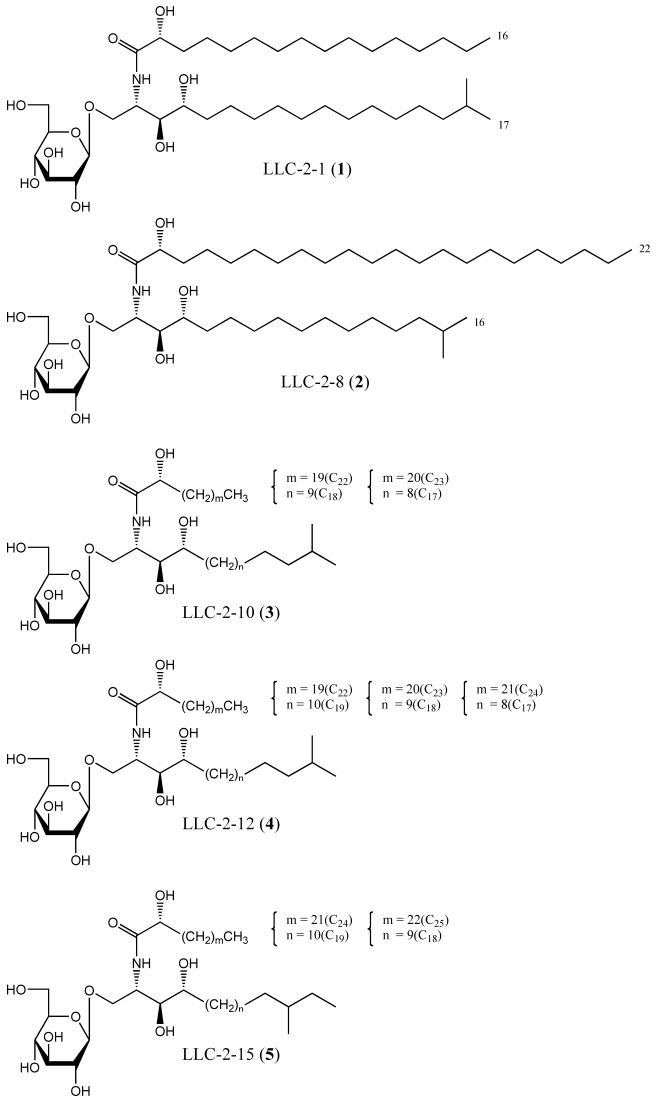

Fig. 2. Structure of LLC-2-1 (**1**), -8 (**2**), -10 (**3**), -12 (**4**), and -15 (**5**)

Accordingly, LLC-2 was a phytosphingosine-type glucocerebroside molecular species composed of the aforementioned fatty acids and long chain bases (Fig. 1).

Isolation and Structure of Cerebrosides from LLC-2

By means of reverse-phase HPLC, LLC-2 was separated into 15 peaks, which were recovered to give fractions LLC-2-1 to LLC-2-15. Five of the 15 fractions, LLC-2-1 (**1**), LLC-2-8 (**2**), LLC-2-10 (**3**), LLC-2-12 (**4**), and LLC-2-15 (**5**), showed a single quasi-molecular ion peak $[M+Na]^+$ in the positive-ion FAB-MS (*m*/*z* 756, 826, 840, 854, 882, respectively). Furthermore, two of the five compounds, **1** and **2**, gave single FAM upon methanolysis. Therefore these two compounds were regarded as homogeneous cerebrosides.

The ^{1}H- and ^{13}C-NMR spectra of these homogeneous and pseudo homogeneous glucocerebrosides, **1**—**5** are in good agreement with that of the synthetic glucocerebroside, which is composed of (2*S*,3*S*,4*R*)-phytosphingosine, (2*R*)-2-hydroxy fatty acid, and β-D-glucopyranose (Fig. 2, Tables 1, 2).[8] The above fact and the optical rotations of **1**—**5** (+9.4° to +18.9°) and the synthetic glucocerebrosides (+12.2°)[8] suggested that **1**—**5** has the same absolute configuration as that of the synthetic one for the core structure. Therefore,

Table 1. ^{1}H-NMR Spectral Data of LLC-2, **1**—**5**, and Synthetic Cerebroside (δ Values in C_5D_5N)

	LLC-2	**1**	**2**	**3**	**4**	**5**	Synthetic cerebroside[8]
Ceramide							
1	4.52[a]	4.52[a]	4.52[a]	4.52[a]	4.52[a]	4.52[a]	4.55 (dd, *J*=10.2, 3.6 Hz)
	4.72 (dd, *J*=10.6, 6.7 Hz)	4.71 (triplet-like)	4.71 (triplet-like)	4.71 (triplet-like)	4.72 (dd, *J*=10.3, 6.4 Hz)	4.71 (triplet-like)	4.74 (dd, *J*=10.6, 6.6 Hz)
2	5.29 (m)	5.27 (m)	5.27 (m)	5.27 (m)	5.26 (m)	5.27 (m)	5.30 (m)
3	4.34[a]	4.34[a]	4.33[a]	4.33[a]	4.33[a]	4.33[a]	4.32 (dd, *J*=4.6, 4.6 Hz)
4	4.20[a]	4.19[a]	4.19[a]	4.18[a]	4.18[a]	4.19[a]	4.21 (m)
2′	4.59[a]	4.58[a]	4.58[a]	4.57[a]	4.57[a]	4.56[a]	4.60 (dd, *J*=7.9, 3.6 Hz)
NH	8.57 (d, *J*=9.2Hz)	8.55 (d, *J*=9.4 Hz)	8.55 (d, *J*=9.0 Hz)	8.55 (d, *J*=8.5 Hz)	8.55 (d, *J*=9.4 Hz)	8.55 (d, *J*=9.9 Hz)	8.59 (d, *J*=9.2 Hz)
$-CH_3$	0.87 (m)	0.87 (m)	0.87 (m)	0.87 (m)	0.86 (m)	0.86 (m)	0.87 (m)
Glucose							
1″	4.97 (d, *J*=7.7 Hz)	4.96 (d, *J*=8.0 Hz)	4.96 (d, *J*=7.7 Hz)	4.96 (d, *J*=7.8 Hz)	4.96 (d, *J*=7.7 Hz)	4.96 (d, *J*=7.8 Hz)	4.98 (d, *J*=7.8 Hz)
2″	4.02 (m)	4.00 (m)	4.01 (m)	4.00 (m)	4.00 (m)	4.01 (m)	4.03 (dd, *J*=8.6, 7.9 Hz)
3″	4.20[a]	4.19[a]	4.19[a]	4.18[a]	4.18[a]	4.19[a]	4.21 (m)
4″	4.20[a]	4.19[a]	4.19[a]	4.19[a]	4.19[a]	4.19[a]	4.21 (m)
5″	3.87 (m)	3.87 (m)	3.87 (m)	3.86 (m)	3.86 (m)	3.87 (m)	3.88 (m)
6″	4.34[a]	4.35[a]	4.33[a]	4.32[a]	4.33[a]	4.34[a]	4.35 (dd, *J*=11.9, 5.3 Hz)
	4.47 (m)	4.47 (m)	4.47 (m)	4.47 (m)	4.47 (m)	4.47 (m)	4.50 (dd, *J*=10.9, 1.7 Hz)

a) *J* values could not be observed because of overlapping with another signals.

Table 2. ^{13}C-NMR Spectral Data of LLC-2, **1**—**5**, and Synthetic Cerebroside (δ Values in C_5D_5N)

	LLC-2	**1**	**2**	**3**	**4**	**5**	Synthetic cerebroside[8)]
Ceramide							
1	70.5	70.5	70.5	70.5	70.5	70.5	70.6
2	51.8	51.8	51.8	51.8	51.8	51.8	51.8
3	75.8	75.9	75.8	75.8	75.8	75.9	75.9
4	72.6	72.6	72.6	72.6	72.6	72.6	72.6
1′	175.6	175.6	175.6	175.6	175.6	175.6	175.7
2′	72.5	72.5	72.5	72.5	72.5	72.5	72.5
$-CH_2\underline{C}H_3$	14.3	14.3	14.3	14.3	14.3	14.3	14.3
$-CH_2(\underline{C}H_3)_2$	22.8	22.8	22.8	22.8	22.8		
$-CH(\underline{C}H_3)CH_2\underline{C}H_3$	11.7, 19.4					11.7, 19.4	
Glucose							
1″	105.6	105.6	105.6	105.6	105.6	105.6	105.6
2″	75.1	75.2	75.2	75.2	75.1	75.2	75.2
3″	78.4	78.5	78.5	78.5	78.4	78.5	78.6
4″	71.5	71.5	71.5	71.5	71.5	71.5	71.5
5″	78.5	78.5	78.5	78.5	78.5	78.5	78.5
6″	62.7	62.7	62.7	62.7	62.7	62.7	62.6

they were composed of (2*S*,3*S*,4*R*)-phytosphingosine, (2*R*)-2-hydroxy fatty acid, and β-D-glucopyranose.

Since their terminal methyl protons were estimated to nine protons (9H) by the ^{1}H-NMR spectrum (Table 1), one of chain of fatty acyl (FA) and LCB is normal-type, and the other is iso- or *ante*-iso-type. The GC-MS measurement of the FAMs from the cerebroside (**1**—**5**) indicate their normal-type. Therefore, the terminal groups in LCB of them must be iso-type or *ante*-iso-type. The ^{13}C-NMR spectra of **1**—**5** show the existence of iso-type (**1**—**4**) and *ante*-iso-type (**5**) terminal methyl groups (iso δ_C: 22.8, *ante*-iso δ_C: 11.7, 19.4).

On the basis of the above data and FAMs obtained by methanolysis, the structures of **1**—**5** were determined as follows. **1**: 1-*O*-(β-D-glucopyranosyl)-(2*S*,3*S*,4*R*)-2-[(2*R*)-2-hydroxyhexadecanoylamino]-16-methyl-heptadecane-1,3,4-triol. **2**: 1-*O*-(β-D-glucopyranosyl)-(2*S*,3*S*,4*R*)-2-[(2*R*)-2-hydroxydocosanoylamino]-15-methyl-hexadecane-1,3,4-triol. **3**: a mixture of 1-*O*-β-D-glucopyranoside of (2*S*,3*S*,4*R*)-2-[(2*R*)-2-hydroxydocosanoylamino]-16-methyl-heptadecane-1,3,4-triol and (2*S*,3*S*,4*R*)-2-[(2*R*)-2-hydroxytricosanoylamino]-15-methyl-hexadecane-1,3,4-triol. **4**: a mixture of 1-*O*-β-D-glucopyranoside of (2*S*,3*S*,4*R*)-2-[(2*R*)-2-hydroxydocosanoylamino]-17-methyl-octadecane-1,3,4-triol, (2*S*,3*S*,4*R*)-2-[(2*R*)-2-hydroxytricosanoylamino]-16-methyl-heptadecane-1,3,4-triol, and (2*S*,3*S*,4*R*)-2-[(2R)-2-hydroxytetracosanoylamino]-15-methyl-hexadecane-1,3,4-triol. **5**: a mixture of 1-*O*-β-D-glucopyranoside of (2*S*,3*S*,4*R*)-2-[(2*R*)-2-hydroxytetracosanoylamino]-16-methyl-octadecane-1,3,4-triol and (2*S*,3*S*,4*R*)-2-[(2*R*)-2-hydroxypentacosanoylamino]-15-methyl-heptadecane-1,3,4-triol (Fig. 2).

Compound **1**, named linckiacerebroside A, is, to the best of our knowledge, new cerebroside. Compound **2** have been found to be identical to S-2a-3, isolated from the starfish *Stellaster equesris*.[12)] The biological activities of these compounds will be examined in the future studies.

Experimental

Melting points were determined on a micro melting point apparatus (Yanako MP-3) without correction. Optical rotations were measured with a Jasco Dip-370 digital polarimeter at 25 °C. ^{1}H-NMR spectra were recorded on a Varian Unity-400 spectrometer (400 MHz), and ^{13}C-NMR spectra on a Varian Unity-500 spectrometer (125 MHz) with the internal standard (pyridine-d_5 or chloroform-*d*). FAB-MS spectra were acquired with a Jeol SX102A mass spectrometer [xenon atom beam; matrix, *m*-nitrobenzyl alcohol]. GC-MS were taken with a Shimadzu QP-5050A [EI mode; ionization potential, 70 eV; separator and ion-source temperature 300 °C; column, GL Science NEUTRA BOND-5 (ϕ 0.25 mm×30 m); carrier gas, He]. HPLC was performed with L-6200 and L-3350 (HITACHI) as a pump and RI detector, respectively.

Separation of LLC-2 Whole bodies of the starfish *L. laevigata* (wet weight 15 kg), which was collected in the Okinawa Prefecture Motobu town in 2000, were chopped and extracted successively with $CHCl_3$/MeOH (1 : 2) 18 l and 12 l. The combined extracts were concentrated *in vacuo* to 1 l. The extract was diluted with H_2O (1 l) and extracted with AcOEt/*n*-BuOH (2 : 1, 1 l, three times) in order to separate less polar lipids. The organic layer was concentrated *in vacuo* and the residue was washed with AcOEt (100 ml) to give an AcOEt insoluble fraction (25.4 g).

The AcOEt insoluble portion (5.5 g) was chromatographed on silica gel repeatedly using the solvents, $CHCl_3$/MeOH (9 : 1), $CHCl_3$/MeOH/H_2O (9 : 1 : 0.05, and 10 : 1 : 0.05), and to give two cerebroside molecular species LLC-1 (0.03 g)[13)] and LLC-2 (1.1 g) each showing single spot on silica gel TLC [solvent $CHCl_3$/MeOH/H_2O (9 : 1.5 : 0.05); *Rf* value of LLC-1 (0.29), LLC-2 (0.23)].

LLC-2: Amorphous powder. Positive ion FAB-MS: *m/z* 756, 770, 784, 812, 826, 840, 854, 868, 882 $[M+Na]^+$ series. ^{1}H- and ^{13}C-NMR: see Tables 1, 2.

Methanolysis of LLC-2 LLC-2 (1.0 mg) was heated with 5% HCl in MeOH (1 ml) at 70 °C for 20 h in a sealed small-volume vial. The reaction mixture was extracted with *n*-hexane, and the hexane layer was concentrated to give a mixture of fatty acid methyl ester (FAM) for GC-MS analysis. The MeOH layer was neutralized with Ag_2CO_3, filtrated, and the filtrate was concentrated *in vacuo* to give a mixture of long chain base (LCB) and methyl glycoside.

GC-MS Analysis of FAM from LLC-2 The FAM mixture from LLC-2 was subjected to GC-MS [column temperature 180—320 °C (rate of temperature increases 4 °C/min)]. The results were as follows: FAM-1 (methyl 2-hydroxypentadecanoate), t_R [min] (ratio of peak areas)=10.7 (2), *m/z* 272 (M^+), 213 $(M-59)^+$; FAM-2 (methyl 2-hydroxyhexadecanoate), t_R=12.6 (9), *m/z* 286 (M^+), 227 $(M-59)^+$; FAM-3 (methyl 2-hydroxyheptadecanoate), t_R=14.8 (6), *m/z* 300 (M^+), 241 $(M-59)^+$; FAM-4 (methyl 2-hydroxyoctadecanoate), t_R=17.0 (2), *m/z* 314 (M^+), 255 $(M-59)^+$; FAM-5 (methyl 2-hydroxyheneicosanoate), t_R=23.1 (5), *m/z* 356 (M^+), 297 $(M-59)^+$; FAM-6 (methyl 2-hydroxydocosanoate), t_R=25.0 (39), *m/z* 370 (M^+), 311 $(M-59)^+$; FAM-7′ (methyl 2-hydroxytricosanoate), t_R=26.3 (5), *m/z* 384 (M^+), 325 $(M-59)^+$; FAM-7 (methyl 2-hydroxytricosanoate), t_R=26.9 (24), *m/z* 384 (M^+), 325 $(M-59)^+$; FAM-8 (methyl 2-hydroxytetracosanoate), t_R=28.8 (9), *m/z* 398 (M^+), 339 $(M-59)^+$.

GC-MS Analysis of TMS Ethers of LCB from LLC-2 The LCB mixture from LLC-2 was heated with 1-(trimethylsilyl) imidazole/pyridine (1 : 1) for 15 min at 70 °C and the reaction mixture TMS ethers were ana-

lyzed by GC-MS [column temperature 180—320 °C (rate of temperature increases 4 °C/min)]. The results were as follow: LCB-1 (2-amino-1,3,4-hexadecanetriol), t_R [min] (ratio of peak areas)=13.9 (18), *m/z* 312 $(M-193)^+$, 271 $(M-234)^+$; LCB-2′ (2-amino-1,3,4-heptadecanetriol), t_R=14.5 (32), *m/z* 326 $(M-193)^+$, 285 $(M-234)^+$; LCB-2″ (2-amino-1,3,4-heptadecanetriol), t_R=14.6 (6), *m/z* 326 $(M-193)^+$, 285 $(M-234)^+$; LCB-2 (2-amino-1,3,4-heptadecanetriol), t_R=14.8 (6), *m/z* 326 $(M-193)^+$, 285 $(M-234)^+$; LCB-3′ (2-amino-1,3,4-octadecanetriol), t_R=15.5 (13), *m/z* 340 $(M-193)^+$, 299 $(M-234)^+$; LCB-3″ (2-amino-1,3,4-octadecanetriol), t_R=15.6 (13), *m/z* 340 $(M-193)^+$, 299 $(M-234)^+$; LCB-3 (2-amino-1,3,4-octadecanetriol), t_R=15.8 (3), *m/z* 340 $(M-193)^+$, 299 $(M-234)^+$; LCB-4 (2-amino-1,3,4-nonadecanetriol), t_R=16.5 (13), *m/z* 354 $(M-193)^+$, 313 $(M-234)^+$.

Isolation of Cerebrosides from LLC-2 The glucocerebroside molecular species LLC-2 showed 15 peaks in the reversed phase HPLC [column, Cosmosil $5C_{18}$ AR-II (10 mm×250 mm, nacalai tesque); solvent, MeOH; flow rate, 3 ml/min)]. Using these conditions, 200 mg of LLC-2 was separated to give 15 compounds: LLC-2-1 (**1**) (2.7 mg, t_R=13min), LLC-2-2 (5.1 mg, t_R=14.3 min), LLC-2-3 (5.9 mg, t_R=16.8 min), LLC-2-4 (8.7 mg, t_R=17.8 min), LLC-2-5 (3.1 mg, t_R=18.8 min), LLC-2-6 (6.2 mg, t_R=20 min), LLC-2-7 (10.1 mg, t_R=21.3 min), LLC-2-8 (**2**) (25.8 mg, t_R=22.8 min), LLC-2-9 (17.8 mg, t_R=24.3 min), LLC-2-10 (**3**) (33.6 mg, t_R=25.8 min), LLC-2-11 (8.8 mg, t_R=27.3 min), LLC-2-12 (**4**) (20.6 mg, t_R=29 min), LLC-2-13 (3.5 mg, t_R=31 min), LLC-2-14 (6.9 mg, t_R=33.3 min), LLC-2-15 (**5**) (1.9 mg, t_R=38 min).

LLC-2-1 (**1**) (Linckiacerebroside A): Amorphous powder, mp 214 °C. $[\alpha]_D$ +18.9° (*c*=0.18, 1-PrOH). Positive-ion FAB-MS: *m/z* 756 $[M+Na]^+$. ^{1}H- and ^{13}C-NMR: see Tables 1, 2. Compound **1** was methanolyzed using the same method as described for LLC-2 to yield FAM-2 (methyl 2-hydroxyhexadecanoate).

LLC-2-8 (**2**) (S-2a-3)[12]: Amorphous powder, mp 216 °C. $[\alpha]_D$ +9.4° (*c*=0.46, 1-PrOH). Positive-ion FAB-MS: *m/z* 826 $[M+Na]^+$. ^{1}H- and ^{13}C-NMR: see Tables 1, 2. Compound **2** was methanolyzed as above to yield FAM-6 (methyl 2-hydroxydocosanoate).

LLC-2-10 (**3**): Amorphous powder, mp 219 °C. $[\alpha]_D$ +12.0° (*c*=0.65, 1-PrOH). Positive-ion FAB-MS: *m/z* 840 $[M+Na]^+$. ^{1}H- and ^{13}C-NMR: see Tables 1, 2. Compound **3** was methanolyzed as above to yield FAM-6 (methyl 2-hydroxydocosanoate) and FAM-7 (methyl 2-hydroxytricosanoate). Ratio of FAM-6 and FAM-7, 1 : 1.

LLC-2-12 (**4**): Amorphous powder, mp 233 °C. $[\alpha]_D$ +11.4° (*c*=0.37, 1-PrOH). Positive-ion FAB-MS: *m/z* 854 $[M+Na]^+$. ^{1}H- and ^{13}C-NMR: see Tables 1, 2. Compound **4** was methanolyzed as above to yield FAM-6 (methyl 2-hydroxydocosanoate), FAM-7 (methyl 2-hydroxytricosanoate), and FAM-8 (methyl 2-hydroxytetracosanoate). Ratio of FAM-6, FAM-7, and FAM-8, 1 : 2 : 1.

LLC-2-15 (**5**): Amorphous powder, mp 235 °C. $[\alpha]_D$ +12.9° (*c*=0.17, 1-PrOH). Positive-ion FAB-MS: *m/z* 882 $[M+Na]^+$. ^{1}H- and ^{13}C-NMR: see Tables 1, 2. Compound **5** was methanolyzed as above to yield FAM-8 (methyl 2-hydroxytetracosanoate) and FAM-9 (methyl 2-hydroxypentacosanoate), t_R= [min]=30.4, *m/z* 412 (M^+), 353 $(M-59)^+$. Ratio of FAM-8, FAM-9, 4 : 1.

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6) Normal means the straight chain [–$CH_2CH_2CH_3$], iso means the branched chain possessing a methyl group on the second carbon atom of the terminal methyl group [–$CH_2CH(CH_3)_2$], and ante-iso means the branched chain possessing a methyl group on the third carbon atom of the terminal methyl group [–$CH(CH_3)CH_2CH_3$].
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13) Although LLC-1 was suggested to be the glucocerebroside molecular species from the behavior on TLC, further study was not conducted.