Isolation and Structure Determination of Six Glucocerebrosides from the Starfish *Luidia maculata*

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Two new glucocerebrosides, luidiacerebroside A (2) and B (6), were isolated from the cerebroside molecular species obtained from the less polar fraction of the $CHCl_3$/MeOH extract of the starfish *Luidia maculata* using HPLC. Four known cerebrosides, CE-2b (1), astrocerebroside B (3), acanthacerebroside B (4), and CE-3-2 (5) have also been isolated and characterized. The structures of these cerebrosides were determined on the basis of chemical and spectroscopic evidence. Mass spectrometry of dimethyl disulfide derivatives was useful for the determination of the double-bond position in the long-chain base.

Key words glycosphingolipid; starfish; *Luidia maculata*; cerebroside

In our previous studies, we reported the isolation and structure elucidation of the biologically active glycosphingolipids obtained from the water-soluble lipid fraction of the $CHCl_3$/MeOH extract of the starfish *Luidia maculata* (yatsudesunahitode in Japanese).[1,2] In a continuation of those studies, 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.

The acetone-insoluble part, which was obtained from the less polar fraction of the $CHCl_3$/MeOH extract of the whole bodies of *L. maculata*, was separated by column chromatography to give two cerebroside molecular species, LMC-1 and LMC-2, each showing a single spot on normal-phase silica gel TLC.

Structure of Cerebroside Molecular Species LMC-1 LMC-1 showed strong hydroxy (3413 cm^{-1}) and amide (1646, 1540 cm^{-1}) absorptions in the IR spectrum. The positive FAB mass spectrum of LMC-1 exhibited a series of $[M+Na]^+$ ion peaks at *m/z* 794, 808, 822, 836, 850, and 864. The ^1H- and ^{13}C-NMR spectra of LMC-1 exhibited the characteristic signals of a sphingosine-type cerebroside possessing 2-hydroxy fatty acid and β-glucopyranose moieties (Chart 1, Table 1). Furthermore, LMC-1 was thought to have *normal* and *ante-iso* types[3] of side chains, because the carbon atom signals due to terminal methyl groups were observed at δ=14.2 (*normal* form), 11.5 and 19.4 (*ante-iso* form) in the ^{13}C-NMR spectrum (Table 1). The ^1H-NMR spectrum of LMC-1 was in good agreement with that of known glucocerebrosides, which is composed of (2*S*,3*R*,4*E*)-sphingosine, (2*R*)-2-hydroxy fatty acid, and β-glucopyranose, expect for the side-chain moieties. Therefore LMC-1 was suggested to be a molecular species of sphingosine-type cerebroside possessing 2-hydroxy fatty acid and β-glucopyranose. Constituents and the absolute configuration of the ceramide and sugar moieties of LMC-1 were determined as follows.

The structure of the ceramide moiety was examined first. When LMC-1 was methanolyzed with methanolic hydrochloric acid, a mixture of fatty acid methyl esters (FAM) was obtained together with a mixture of long-chain base (LCB) and methyl glucopyranoside. Gas chromatography-mass spectrometry (GC-MS) analysis of the FAM mixture showed the existence of eight components, which were characterized as methyl 2-hydroxyhexadecanoate (FAM-1), methyl 2-hydroxyheptadecanoate (FAM-2), methyl 2-hydroxyoctadecanoate (FAM-3), methyl 2-hydroxyicosanoate (FAM-4), methyl 2-hydroxyheneicosanoate (FAM-5), methyl 2-hydroxydocosanoate (FAM-6), methyl 2-hydroxytricosanoate (FAM-7), and methyl 2-hydroxytetracosanoate (FAM-8). The major FAM was methyl 2-hydroxydocosanoate (FAM-6). On the other hand, in GC-MS analysis of the trimethylsilyl (TMS) derivative of the LCB mixture, the LCB components were suggested to be 2-amino-1,3-dihydroxy-4-hexadecene (LCB-1), 2-amino-1,3-dihydroxy-4-heptadecene (LCB-2), 2-amino-1,3-dihydroxy-4-octadecene (LCB-3), and 2-amino-1,3-dihydroxy-4-nonadecene (LCB-4). LCB-4 was the major sphingoid.

The stereochemistry of the ceramide moiety was deter-

Table 1. ^1H- and ^{13}C-NMR Spectral Data of LMC-1 and LMC-2 (δ Value in Pyridine-d_5)

Position	LMC-1		LMC-2	
	^1H	^{13}C	^1H	^{13}C
Ceramide				
NH	8.35 (d, *J*=7.9 Hz)		8.57 (d, *J*=8.9 Hz)	
1a	4.20*	70.1 (t)	4.52*	70.4 (t)
1b	4.70*		4.71 (dd, *J*=10.9, 6.6 Hz)	
2	4.77*	54.6 (d)	5.28 (m)	51.7 (d)
3	4.75*	72.3 (d)	4.32*	75.8 (d)
4	5.86*	131.8 (d)	4.18*	72.5 (d)
5	5.97*	132.7 (d)		
1′		175.6 (s)		175.7 (s)
2′	4.57 (dd, *J*=7.9, 3.6 Hz)	72.5 (d)	4.54*	72.4 (d)
CH_3	0.88 (m)	14.2 (q)[a]	0.88 (m)	14.2 (q)[a]
		11.5 (q)[b]		11.5 (q)[b]
		19.4 (q)[c]		19.4 (q)[c]
Glucose				
1″	4.90 (d, *J*=7.6 Hz)	105.6 (d)	4.93 (d, *J*=7.6 Hz)	105.4 (d)
2″	4.02 (m)	75.0 (d)	4.00 (t, *J*=7.6 Hz)	75.1 (d)
3″	4.20*	78.4 (d)	4.18*	78.4 (d)
4″	4.20*	71.5 (d)	4.18*	71.5 (d)
5″	3.89 (m)	78.4 (d)	3.87 (m)	78.4 (d)
6″a	4.34 (dd, *J*=11.9, 5.4 Hz)	62.6 (t)	4.32*	62.6 (t)
6″b	4.49 (dd, *J*=11.9, 2.0 Hz)		4.47 (m)	

*: The *J* values could not be observed due overlapping with another signals. *a*) Terminal methyl group in *normal* type of side chain (see ref. 3). *b*, *c*) Terminal methyl group in *ante-iso* type of side chain (see ref. 3).

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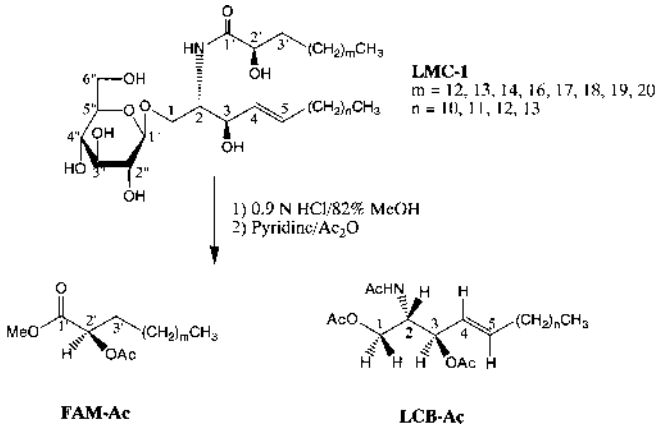


Chart 1

mined as follows. When LMC-1 was heated with 0.9 M HCl in 82% MeOH, FAM and LCB mixtures were obtained. The FAM mixture was acetylated to afford its acetate (FAM-Ac, Chart 1). The optical rotation of FAM-Ac (+12.9) showed its *R*-configuration by comparing with those of two synthetic diastereomers of methyl (2*R*)-2-*O*-acetyl hexadecanoate (+14.5)[4] and methyl (2*S*)-2-*O*-acetyl hexadecanoate (−13.8).[4] The LCB mixture was acetylated to LCB acetate mixture (LCB-Ac, Chart 1). The ^{1}H-NMR spectrum of LCB-Ac was in good agreement with that of synthetic (2*S*,3*R*,4*E*)-2-acetamido-1,3-diacetoxyoctadec-4-ene (par-acetyl sphingosine)[5] in terms of the signals due to 1-H to 5-H. Furthermore the optical rotation of LCB-Ac (−7.6) and the synthetic par-acetyl sphingosine (−7.7)[6] suggests that LCB-Ac has the (2*S*,3*R*,4*E*) configuration.

The absolute configuration of the glucopyranose moiety was determined to be the D-form using the Hara method.[7] Accordingly, LMC-1 is designated as a sphingosine-type glucocerebroside molecular species (Chart 1), which is composed of the aforementioned fatty acids and long-chain bases.

Isolation and Structure of Cerebrosides from LMC-1 LMC-1 was separated by reverse-phase HPLC into 11 peaks, which were recovered to give the fractions LMC-1-1 to LMC-1-11. Six of the 11 fractions, LMC-1-2, LMC-1-6, LMC-1-7, LMC-1-8, LMC-1-9, and LMC-1-10, revealed a single quasi-molecular ion peak $[M+Na]^+$ in the positive-ion FAB mass spectrum, but the other five fractions exhibited plural molecular ion peaks. Upon methanolysis, in the six fractions that were thought to be pure compounds, only LMC-1-6 (**1**) afforded homogeneous fatty acid and long-chain base.

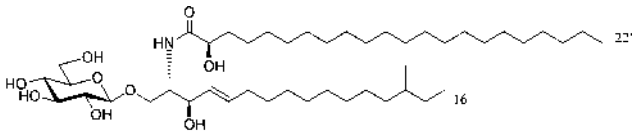
The ^{1}H- and ^{13}C-NMR spectra of **1** were essentially identical to those of LMC-1 (Table 2), and the methanolysis products of **1** were methyl 2-hydroxydocosanoate (FAM) and 2-amino-1,3-dihydroxy-14-methyl-4-hexadecene (LCB). On the basis of the above data and the molecular mass of **1** (*m/z* 808 $[M+Na]^+$), the structure of **1** was determined to be 1-*O*-(β-D-glucopyranosyl)-(2*S*,3*R*,4*E*)-2-[(2*R*)-2-hydroxydocosanoylamino]-14-methyl-4-hexadecene-1,3-diol (Chart 2).

Structure of Cerebroside Molecular Species LMC-2 LMC-2 revealed strong hydroxy (3366 cm^{-1}) and amide (1629, 1540 cm^{-1}) absorptions in the IR spectrum. The positive FAB mass spectrum of LMC-2 exhibited a series of

Table 2. ^{13}C-NMR Chemical Shifts (δ Value) of Cerebrosides in Pyridine-d_5

Position	1	2	3	4	5	6
Ceramide						
1 (t)	70.1	70.4	70.4	70.4	70.4	70.4
2 (d)	54.6	51.8	51.7	51.8	51.7	51.8
3 (d)	72.3	75.9	75.8	75.9	75.8	75.9
4 (d)	131.6	72.6	72.5	72.6	72.6	72.6
5 (d)	132.8					
8 (t)			27.5*			
9 (d)			130.1**			
10 (d)			130.3**			
11 (t)			27.8*			
1′ (s)	175.7	175.7	175.7	175.7	175.7	175.7
2′ (d)	72.5	72.5	72.5	72.5	72.4	72.5
CH_3 (q)	14.2[a]	14.2[a]	14.2[a]	14.2[a]	14.2[a]	14.2[a]
CH_3 (q)	11.5[b]	11.5[b]			11.5[b]	11.5[b]
CH_3 (q)	19.3[c]	19.3[c]			19.3[c]	19.4[c]
Glucose						
1″ (d)	105.6	105.5	105.4	105.5	105.4	105.5
2″ (d)	75.0	75.1	75.1	75.1	75.1	75.1
3″ (d)	78.4	78.4	78.4	78.4	78.4	78.4
4″ (d)	71.5	71.6	71.5	71.6	71.5	71.6
5″ (d)	78.5	78.4	78.4	78.5	78.4	78.5
6″ (t)	62.6	62.9	62.7	62.7	62.7	62.7

*,** Assignments may be interchanged in each vertical column. *a*) Terminal methyl group in *normal* type of side chain (see ref. 3). *b*) Terminal methyl group in *ante-iso* type of side chain (see ref. 3).



1

Chart 2

$[M+Na]^+$ ion peaks at *m/z* 770, 784, 810, 812, 826, 840, 854, 868, and 882. LMC-2 showed 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 (Chart 3, Table 1). Furthermore, LMC-2 was thought to possess the *normal* and *ante-iso*[3] types of side chains like LMC-1 on the basis of the carbon atom signals due to the terminal methyl (Table 1). The ^{1}H-NMR spectrum of LMC-2 was 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-glucopyranose, expect for the side-chain moieties. Therefore LMC-2 is suggested to be a molecular species of phytosphingosine-type cerebroside possessing 2-hydroxy fatty acid and β-glucopyranose. Constituents and the absolute configuration of the ceramide and sugar moieties of LMC-2 were determined as follows.

The structure of the ceramide moiety was examined first. LMC-2 was methanolyzed with methanolic hydrochloric acid to give a mixture of FAM, LCB, and methyl glucopyranoside. GC-MS analysis of the FAM mixture showed the existence of seven components, which were characterized as methyl 2-hydroxyhexadecanoate (FAM-1), methyl 2-hydroxyheptadecanoate (FAM-2), methyl 2-hydroxyheneicosanoate (FAM-3), methyl 2-hydroxydocosanoate (FAM-4), methyl 2-hydroxytricosanoate (FAM-5), and methyl 2-hydroxytetracosanoate

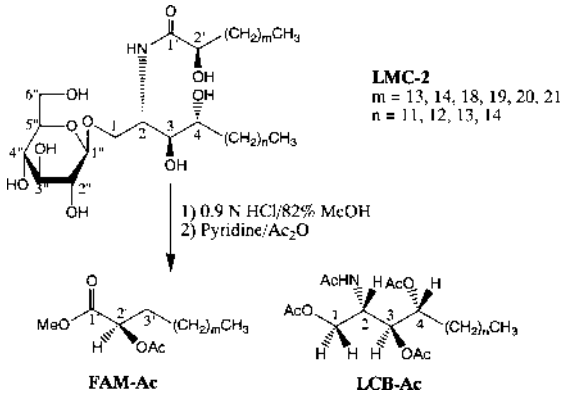


Chart 3

(FAM-6). The major FAM was methyl 2-hydroxydocosanoate. On the other hand, GC-MS analysis of the 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-heptadecanetriol (LCB-2′), 2-amino-1,3,4-octadecanetriol (LCB-3′), and 2-amino-1,3,4-nonadecanetriol (LCB-4′). LCB-4 was the major sphingoid.

The stereochemistry of the ceramide moiety was determined as follows. When LMC-2 was heated with 0.9 M HCl in 82% MeOH, FAM and LCB mixtures were obtained. The FAM mixture was acetylated to give the mixture of FAM acetate (FAM-Ac, Chart 3). The optical rotation of FAM-Ac (+13.0) indicated its *R*-configuration (*vide supra*). The LCB mixture was acetylated to yield a mixture of LCB acetate (LCB-Ac, Chart 3). The ^{1}H-NMR spectrum of LCB-Ac was in good agreement with that of synthetic (2*S*,3*S*,4*R*)-2-acetamido-1,3,4-triacetoxyoctadecane[8] in terms of signals of 1-H to 4-H. The optical rotation of LCB-Ac (+22.9) and synthetic (2*S*,3*S*,4*R*)-2-acetamido-1,3,4-triacetoxyoctadecane (+26.8) suggested that LCB-Ac had the (2*S*,3*S*,4*R*) configuration.

The absolute configuration of the glucopyranose moiety was determined to be the D-form as before. Accordingly, LMC-2 is a phytosphingosine-type glucocerebroside molecular species, as shown in Chart 3.

Isolation and Structure of Cerebrosides from LMC-2 By means of reverse-phase HPLC, LMC-2 was separated into 12 peaks, which were recovered to give fractions LMC-2-1 to LMC-2-12. Ten of the 12 fractions, LMC-2-1, LMC-2-3, and LMC-2-5 to LMC-2-12, showed a single quasi-molecular ion peak $[M+Na]^+$ in the positive-ion FAB mass spectrum, but the other two fractions exhibited plural molecular ion peaks. Furthermore, five of the 10 compounds, LMC-2-1 (**2**), LMC-2-3 (**3**), LMC-2-5 (**4**), LMC-2-6 (**5**), and LMC-2-12 (**6**) gave a single fatty acid upon methanolysis. Therefore these five compounds were regarded as homogeneous cerebrosides.

The positive-ion FAB mass spectrum of **3** showed the peak $[M+Na]^+$ ion at *m*/*z* 810. The ^{13}C-NMR spectrum of **3** was essentially identical to that of LMC-2, which confirms that **3** is a cerebroside component of LMC-2, and shows signals due to *normal*-type[3] terminal methyl groups. Furthermore, **3** was suggested to contain one olefinic group in the LCB side chain, since two olefinic carbon signals (δ=130.1, 130.3) were observed. Compound **3** yielded methyl 2-hydroxyhexadecanoate as the fatty acid component upon methanolysis. The location and geometry of the double-bonds in the LCB side chains of **3** were determined as follows.

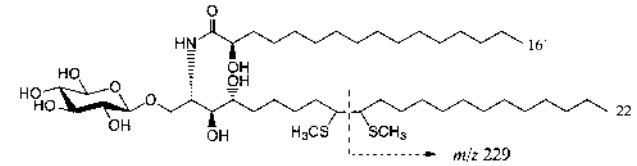


Chart 4

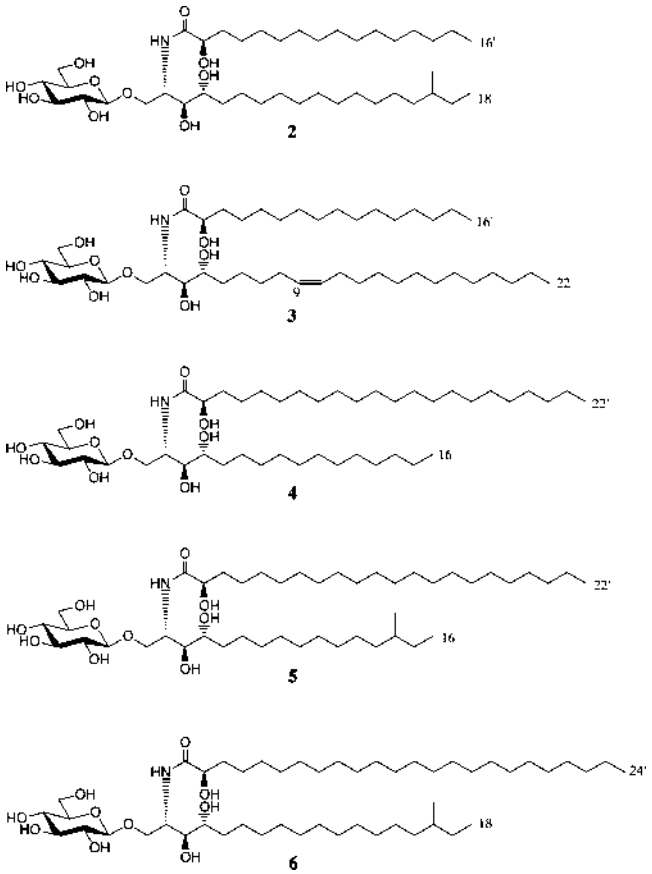


Chart 5

The LCB of **3** was regarded as 2-amino-1,3,4-docosenetriol on the basis of the above facts. The positive-ion FAB mass spectrum of the dimethyl disulfide (DMDS) derivatives[9,10] of **3** showed a remarkable fragment ion peak at *m*/*z* 229 due to cleavage of the bond between the carbons bearing a methylthio group (Chart 4). These data indicate that the double-bond in the LCB residue of **3** is located at C-9, as shown in Chart 5.

Furthermore, it is known[11] that the geometry of the double-bond in the long-chain alkene can be determined on the basis of the ^{13}C-NMR chemical shift of the methylene carbon adjacent to the olefinic carbon, which is observed at $\delta\approx27$ in (*Z*) isomers and at $\delta\approx32$ in (*E*) isomers. The proton signal at δ=5.43 was assigned to the olefin groups based on the ^{1}H–^{1}H correlation spectroscopy (COSY) spectrum of **3**. When the heteronuclear multiple bond connectivity (HMBC) spectrum of **3** was measured, significant correlations were observed between the signal of the olefin proton at δ=5.43

and the methylene carbon atoms at δ=27.5 and 27.8, as shown in Table 2. Accordingly, these methylene carbon atoms must be the carbon atoms adjacent to the double-bond and were thus assigned to C-8 and C-11 (δ=27.5, 27.8). Thus the olefin group in the LCB of **3** was determined to have *cis* (*Z*) geometry.

Therefore the structure of **3** was proposed to be 1-*O*-(β-D-glucopyranosyl)-(2*S*,3*S*,4*R*,9*Z*)-2-[(2*R*)-2-hydroxyhexadecanosylamino]-9-docosene-1,3,4-triol, as shown in Chart 5.

Four other cerebrosides, **2**, **4**, **5**, and **6** had molecular masses of 747 (**2**), 789 (**4**), 831 (**5**), and 859 (**6**), and their ^{13}C-NMR spectra were found to be essentially identical to that of LMC-2 (Tables 1, 2), which confirms that these compounds were also cerebroside components of LMC-2.

Compound **4** showed signals of the *normal* type[3] terminal methyl groups in its ^{13}C-NMR spectrum and afforded methyl 2-hydroxydocosanoate upon methanolysis. On the basis of the above data, the structure of **4** was proposed to be 1-*O*-(β-D-glucopyranosyl)-(2*S*,3*S*,4*R*)-2-[(2*R*)-2-hydroxydocosanosylamino]-hexadecane-1,3,4-triol, as shown in Chart 5.

Compounds **2**, **5**, and **6** possess the *normal* and *ante-iso* type[3] of terminal methyl groups, and upon methanolysis **2**, **5**, and **6** afforded methyl 2-hydroxyhexadecanoate, methyl 2-hydroxydocosanoate and methyl 2-hydroxytetracosanoate, respectively. Therefore the structures of **2**, **5**, and **6** were determined to be 1-*O*-(β-D-glucopyranosyl)-(2*S*,3*S*,4*R*)-2-[(2*R*)-2-hydroxyhexadecanoylamino]-16-methyl-octadecane-1,3,4-triol, 1-*O*-(β-D-glucopyranosyl)-(2*S*,3*S*,4*R*)-2-[(2*R*)-2-hydroxydocosanoylamino]-14-methyl-hexadecane-1,3,4-triol and 1-*O*-(β-D-glucopyranosyl)-(2*S*,3*S*,4*R*)-2-[(2*R*)-2-hydroxytetracosanoylamino]-16-methyl-octadecane-1,3,4-triol, respectively (Chart 5).

In conclusion, compounds **2** and **6**, called luidiacerebroside A and B, are, to the best of our knowledge, new cerebrosides. Compounds **1** and **5** have been found to be identical to CE-2b[12] and CE-3-2,[13] isolated from the sea cucumber *Cucumaria echinata*. Compounds **3** and **4** have been found to be identical to astrocerebroside B[14] and acanthacerebroside B,[15] isolated from the starfish *Astropecten latespinosus* and *Acanthaster planci*, respectively. The biological activities of these compounds will be examined in future studies.

Experimental

General Melting points: Micromelting point apparatus (Yanaco MP-3), uncorrected values. Optical rotations: Jasco Dip-307 digital polarimeter. IR spectra: Jasco FT-IR-410 Fourier transform infrared spectrophotometer. ^{1}H- and ^{13}C-NMR spectra: Jeol GX-270 spectrometer (270 MHz and 67.8 MHz), Varian Unity-500 (500 MHz and 125 MHz, 2D-NMR spectrum). Positive-ion FAB mass spectra: Jeol SX102A mass spectrometer (xenon atom beam, 5 kV; ion-source accelerating potential, 10 kV; matrix, *m*-nitrobenzyl alcohol saturated with NaCl). GLC: Shimadzu GC-14B by employing FID [capillary column, J & W SCIENTIFIC Fused Silica Capillary Column DB-17 (ϕ 0.317 mm×30 m)]. GC-MS: Shimadzu GC-17A/QP-5050A employing the EI mode [ionizing potential of 70 eV; separator and ion-source temperature of 250 °C; capillary column, TC-1701 (0.25 mm×30 m, GL Science Inc.); carrier gas, He. HPLC: Jasco PU-980; RI detector; column, COSMOSIL 5C18-AR-II (Nacalai Tesque).

Separation of LMC-1 and LMC-2 Whole bodies of the starfish *L. maculata* (wet weight 57 kg, collected in Hakata Bay in Fukuoka, Japan, in May 1995) were homogenized and extracted with $CHCl_3$/MeOH (1 : 3, 80 l) followed by further extraction with $CHCl_3$/MeOH (1 : 2, 24 l, twice). The combined extracts were concentrated *in vacuo* to give a condensed extract (2 l). The extract was added to H_2O (43 l) and this aqueous suspension extracted with AcOEt/*n*-BuOH (2 : 1, 40 l) for separation of less polar lipids. The organic layer was concentrated *in vacuo*, and residue was washed with cold acetone (600 ml, four times) to give an acetone-insoluble fraction (337 g). A portion of the acetone-insoluble material (230 g) was partitioned between MeOH and *n*-hexane (1 : 1, 2 l, two times). The MeOH layer was concentrated *in vacuo* to give a MeOH extract (191.5 g) and chromatographed on silica gel ($CHCl_3$/MeOH/H_2O, 1 : 0 : 0 to 9 : 1 : 0.05) to afford LMC-1 (927 mg) and LMC-2 (1069 mg). LMC-1 and LMC-2 each showed a single spot on silica gel TLC ($CHCl_3$/MeOH/H_2O, 9 : 1 : 0.05); *Rf*=0.29 (LMC-1), 0.23 (LMC-2).

LMC-1 Amorphous powder. IR (KBr) cm^{-1}: 3413 (hydroxyl), 1646, 1540 (amide). Positive-ion FAB-MS *m/z*: 794, 808, 822, 836, 859, 864 $[M+Na]^+$ series. ^{1}H- and ^{13}C-NMR: See Table 1.

Methanolysis of LMC-1 LMC-1 (1.0 mg) was heated with 5% HCl in MeOH (1 ml) at 70 °C for 12 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 FAM for GC-MS analysis. The MeOH layer was concentrated *in vacuo* to give a mixture of LCB and methyl glycoside.

GC-MS Analysis of FAM from LMC-1 The FAM mixture from LMC-1 was subjected to GC-MS [column temp. 100—250 °C (rate of temp. increases 5 °C/min)]. The results were as follows: FAM-1 (methyl 2-hydroxyhexadecanoate), t_R=25.9 (7.7), EI-MS *m/z*: 286 $[M]^+$, 227 $[M-59]^+$. FAM-2 (methyl 2-hydroxyheptadecanoate), t_R=27.8 (1.1), EI-MS *m/z*: 300 $[M]^+$, 241 $[M-59]^+$. FAM-3 (methyl 2-hydroxyoctadecanoate), t_R=29.5 (1.3), EI-MS *m/z*: 314 $[M]^+$, 255 $[M-59]^+$. FAM-4 (methyl 2-hydroxyicosanoate), t_R=33.0 (1.6), EI-MS *m/z*: 342 $[M]^+$, 283 $[M-59]^+$. FAM-5 (methyl 2-hydroxyheneicosanoate), t_R=35.0 (3.6), EI-MS *m/z*: 356 $[M]^+$, 297 $[M-59]^+$. FAM-6 (methyl 2-hydroxydocosanoate), t_R=37.5 (42.3), EI-MS *m/z*: 370 $[M]^+$, 311 $[M-59]^+$. FAM-7 (methyl 2-hydroxytricosanoate), t_R=40.6 (22.3), EI-MS *m/z*: 384 $[M]^+$, 325 $[M-59]^+$. FAM-8 (methyl 2-hydroxytetracosanoate), t_R=44.5 (5.1), EI-MS *m/z*: 398 $[M]^+$, 339 $[M-59]^+$.

GC-MS Analysis of TMS Ethers of LCB from LMC-1 The LCB mixture from LMC-1 was heated with 1-(trimethylsilyl)imidazole/pyridine (1 : 1) for 10 min at 70 °C and then the reaction mixture TMS ethers was analyzed by GC-MS [column temp. 180—250 °C (rate of temp. increases 5 °C/min)]. The results were as follows: LCB-1 (1,3-di-*O*-trimethylsilyl-2-amino-1,3-dihydroxy-hexadeca-4-ene), t_R [min]=12.6, EI-MS *m/z*: 312 $[M-103]^+$, 283 $[M-132]^+$. LCB-2 (1,3-di-*O*-trimethylsilyl-2-amino-1,3-dihydroxy-heptadeca-4-ene), t_R [min]=13.7, EI-MS *m/z*: 326 $[M-103]^+$, 297 $[M-132]^+$. LCB-3 (1,3-di-*O*-trimethylsilyl-2-amino-1,3-dihydroxy-octadeca-4-ene), t_R [min]=15.2, EI-MS *m/z*: 340 $[M-103]^+$, 311 $[M-132]^+$. LCB-4 (1,3-di-*O*-trimethylsilyl-2-amino-1,3-dihydroxy-nonadeca-4-ene), t_R [min]=16.8, EI-MS *m/z*: 354 $[M-103]^+$, 325 $[M-132]^+$.

GLC Analysis of TMS Ethers of Methyl Glycoside from LMC-1 The TMS ether of methyl glycoside was analyzed by GLC [column temp. 100—250 °C (rate of temp. increases 5 °C/min)]; t_R [min]=21.6, 21.9 (methyl-2,3,4,6-tetra-*O*-trimethylsilyl glucose from LMC-1), t_R [min]=21.5, 21.9 (standard methyl-2,3,4,6-tetra-*O*-trimethylsilyl glucose).

Determination of Absolute Configuration of the Glucose Moiety of LMC-1 LMC-1 (1.0 mg) was heated with 4 M H_2SO_4 aq. (0.2 ml) at 100 °C for 18 h. The reaction mixture was then extracted with *n*-hexane, and the acidic aqueous layer was neutralized with $Ba(OH)_2$, centrifuged, and the supernatant was concentrated. The residue (sugar fraction) was heated with L-cysteine methyl ester hydrochloride (0.25 mg) and pyridine (0.1 ml) at 60 °C for 1 h. Then 0.1 ml of 1-(TMS)imidazole was added and the mixture was heated at 60 °C for 20 min to yield the TMS ether derivative of the methyl thiazolidine-4(*R*)-carboxylate. The derivative was analyzed by GC [column temp. 200—250 °C (rate of temp. increases 2.5 °C/min)]; t_R=11.56 min (derivative of D-glucose, 11.58 min; L-glucose 12.11 min).

Determination of Absolute Configuration of the Fatty Acid Moiety of LMC-1 LMC-1 (60 mg) was heated with 0.9 M HCl in 82% MeOH at 80 °C for 18 h. The reaction mixture was extracted with *n*-hexane, and the *n*-hexane layer was concentrated *in vacuo*. The residue was purified by column chromatography on a silica gel column (eluent: $CHCl_3$) to afford the FAM mixture. The FAM mixture was acetylated using the standard procedure (Ac_2O, pyridine) at 70 °C for 2 h. The reaction mixture was diluted with $CHCl_3$ and successively washed with 2 M HCl. The organic layer was dried over Na_2SO_4, filtrated, and the filtrate was concentrated. The residue was fatty acid methylester acetate (FAM-Ac). $[\alpha]_D^{28}$ +12.9° (*c*=1.0, $CHCl_3$). ^{1}H-NMR ($CDCl_3$) δ: 4.98 (1H, t, *J*=6.4 Hz, 2-H), 3.74 (3H, s, $-COOCH_3$), 2.14 (3H, s, $-COCH_3$), 1.80 (2H, m, 3-H), 0.88 (3H, t, *J*=6.6 Hz, $-CH_3$).

Synthetic Methyl (2*R* or 2*S*)-2-*O*-Acetyl Hexadecanoate[4]: $[\alpha]_D^{24}$ +14.5° (*c*=1.75, $CHCl_3$, 2*R*), $[\alpha]_D^{24}$ −13.8° (*c*=2.5, $CHCl_3$, 2*S*). ^{1}H-NMR ($CDCl_3$) δ: 4.98 (1H, t, *J*=6.4 Hz, 2-H), 3.74 (3H, s, $-COOCH_3$), 2.14 (3H, s, $-COCH_3$), 1.82 (2H, m, 3-H), 0.88 (3H, t, *J*=6.6 Hz, $-CH_3$).

Determination of Absolute Configuration of the LCB Moiety of LMC-1 LMC-1 (60 mg) was heated with 0.9 M HCl in 82% MeOH at 80 °C for 18 h. The reaction mixture was extracted with *n*-hexane, and the MeOH layer was alkalinized with 6 M NaOH. The mixture was diluted with diethyl ether and successively washed with water. The organic layer was dried over Na_2SO_4, filtrated, and the filtrate was concentrated. The residue was acetylated using the standard procedure (Ac_2O, pyridine) at 70 °C for 2 h. The reaction mixture was diluted with $CHCl_3$ and successively washed with 2 M HCl. The organic layer was dried over Na_2SO_4, filtrated, and the filtrate was concentrated. The residue was purified by column chromatography on a silica gel column (eluent: *n*-hexane/AcOEt, 3 : 2) to afford long-chain base acetate (LCB-Ac). $[\alpha]_D^{28}$ −7.6° (c=1.0, $CHCl_3$). ^{1}H-NMR ($CDCl_3$) δ: 5.79 (1H, dt, J=15.3, 6.5, 6.5 Hz, 5-H), 5.64 (1H, d, J=8.9 Hz, –NH), 5.39 (1H, dd, J=15.3, 7.6 Hz, 4-H), 5.28 (1H, t-like, 3-H), 4.43 (1H, m, 2-H), 4.30 (1H, dd, J=11.6, 6.1 Hz, 1-Ha), 4.05 (1H, dd, J=11.7, 3.9 Hz, 1-Hb).

Isolation of Cerebrosides LMC-1 Series HPLC of LMC-1 (solvent 98% MeOH aq., flow rate 3.0 ml/min) showed 11 peaks. Using these conditions, 130 mg of LMC-1 was separated by HPLC to give 11 compounds: LMC-1-1 (12.3 mg, t_R=13.8 min), LMC-1-2 (2.1 mg, t_R=21.3 min), LMC-1-3 (2.5 mg, t_R=24.3 min), LMC-1-4 (2.4 mg, t_R=29.3 min), LMC-1-5 (4.7 mg, t_R=38.6 min), LMC-1-6 (**1**) (7.9 mg, t_R=41.9 min), LMC-1-7 (5.7 mg, t_R=45.6 min), LMC-1-8 (9.9 mg, t_R=49.8 min), LMC-1-9 (3.6 mg, t_R=53.8 min), LMC-1-10 (1.9 mg, t_R=59.6 min), LMC-1-11 (4.4 mg, t_R=70.6 min).

Compound 1 Amorphous powder, mp 114—115 °C. $[\alpha]_D^{27}$ +7.0° (c=0.45, 1-PrOH). Positive-ion FAB-MS *m/z*: 808 $[M+Na]^+$, 606 $[(M+H)-H_2O-\text{hexose}]^+$. ^{1}H-NMR (pyridine-$d_5$) δ: 8.32 (1H, d, J=8.9 Hz, –NH), 5.95 (1H, dt, J=15.3, 6.2 Hz, 5-Hb), 5.88 (1H, dd, J=15.6, 6.2 Hz, 5-Ha), 4.89 (1H, d, J=7.8 Hz, glc-1), 4.69 (1H, dd, J=10.5, 5.7 Hz, 1-Hb), 4.48 (1H, d, J=11.4 Hz, glc-6b), 4.22 (1H, dd, J=10.5, 3.8 Hz, 1-Ha), 4.01 (1H, t, J=7.3 Hz, glc-2), 3.88 (1H, m, glc-5). ^{13}C-NMR: See Table 2. HR positive-ion FAB-MS; $C_{45}H_{87}NO_9Na$ $[M+Na]^+$: Calcd 808.6278, Found 808.6256. Compound **1** was methanolyzed in the same method as described for LMC-1 to yield methyl 2-hydroxydocosanoate as FAM.

LMC-2 Amorphous powder. IR (KBr) cm^{-1}: 3413 (hydroxyl), 1646, 1540 (amide). Positive-ion FAB-MS *m/z*: 770, 784, 812, 826, 840, 854, 868, 882 $[M+Na]^+$ series. ^{1}H- and ^{13}C-NMR: See Table 1.

Methanolysis of LMC-2 LMC-2 (1.0 mg) was heated with 5% HCl in MeOH (1 ml) at 70 °C for 12 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 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 LCB and methyl glycoside.

GC-MS Analysis of FAM from LMC-2 The FAM mixture from LMC-2 was subjected to GC-MS [column temp. 100—250 °C (rate of temp. increases 5 °C/min)]. The results were as follows: FAM-1 (methyl 2-hydroxyhexadecanoate), t_R [min] (ratio of peak areas)=25.9 (7.3), EI-MS *m/z*: 286 $[M]^+$, 227 $[M-59]^+$; FAM-2 (methyl 2-hydroxyheptadecanoate), t_R [min]=27.8 (1.1), EI-MS *m/z*: 300 $[M]^+$, 241 $[M-59]^+$; FAM-3 (methyl 2-hydroxyheneicosanoate), t_R [min]=35.0 (2.6), EI-MS *m/z*: 356 $[M]^+$, 297 $[M-59]^+$; FAM-4 (methyl 2-hydroxydocosanoate), t_R [min]=37.5 (61.0), EI-MS *m/z*: 370 $[M]^+$, 311 $[M-59]^+$; FAM-5 (methyl 2-hydroxytricosanoate), t_R [min]=40.6 (25.5), EI-MS *m/z*: 384 $[M]^+$, 325 $[M-59]^+$; FAM-6 (methyl 2-hydroxytetracosanoate), t_R [min]=44.6 (1.3), EI-MS *m/z*: 398 $[M]^+$, 339 $[M-59]^+$.

GC-MS Analysis of TMS Ethers of LCB from LMC-2 The LCB mixture from LMC-2 was heated with 1-(TMS)imidazole/pyridine (1 : 1) for 10 min at 70 °C and then the reaction mixture (TMS ethers) was analyzed by GC-MS [column temp. 180—250 °C (rate of temp. increases 5 °C/min)]. The results were as follows: LCB-1′ (1,3,4-tri-*O*-trimethylsilyl-2-amino-1,3,4-hexadecanetriol), t_R [min]=14.9, EI-MS *m/z*: 312 $[M-193]^+$, 271 $[M-234]^+$. LCB-2′ (1,3,4-tri-*O*-trimethylsilyl-2-amino-1,3,4-heptadecanetriol), t_R [min]=16.0, EI-MS *m/z*: 326 $[M+193]^+$, 285 $[M-234]^+$. LCB-3′ (1,3,4-tri-*O*-trimethylsilyl-2-amino-1,3,4-octadecanetriol), t_R [min]=17.6, EI-MS *m/z*: 340 $[M-193]^+$, 299 $[M-234]^+$. LCB-4′ (1,3,4-tri-*O*-trimethylsilyl-2-amino-1,3,4-nonadecanetriol), t_R [min]=19.5, EI-MS *m/z*: 354 $[M-193]^+$, 313 $[M-234]^+$.

GCL Analysis of TMS Ethers of Methyl Glycoside from LMC-2 The TMS ether of methyl glycoside was analyzed by GLC [column temp. 100—250 °C (rate of temp. increases 5 °C/min)]; t_R [min]=21.6, 21.9 (methyl 2,3,4,6-tetra-*O*-trimethylsilyl glucose from LMC-2), t_R [min]=21.5, 21.9 (standard methyl 2,3,4,6-tetra-*O*-trimethylsilyl glucose).

Determination of Absolute Configuration of the Glucose Moiety of LMC-2 LMC-2 (1.0 mg) was heated with 2 M H_2SO_4 aq. (0.2 ml) at 100 °C for 18 h. The reaction mixture was then extracted with *n*-hexane, and the acidic aqueous layer was neutralized with $Ba(OH)_2$, centrifuged, and the supernatant was concentrated. The residue (sugar fraction) was heated with L-cysteine methyl ester hydrochloride (0.25 mg) and pyridine (100 μl) at 60 °C for 1 h. Then 0.1 ml of 1-(TMS)imidazole was added and the mixture was heated at 60 °C for 20 min to yield the TMS ether derivative of the methyl thiazolidine-4(*R*)-carboxylate. The derivative was analyzed by GC [column temp. 200—250 °C (rate of temp. increases 2.5 °C/min)]; t_R=11.56 min (derivative of D-glucose, 11.58 min; L-glucose 12.11 min).

Determination of Absolute Configuration of the Fatty Acid Moiety of LMC-2 LMC-2 (300 mg) was heated with 0.9 M HCl in 82% MeOH at 80 °C for 18 h. The reaction mixture was extracted with *n*-hexane, and the *n*-hexane layer was concentrated *in vacuo*. The residue was purified by column chromatography on a silica gel column (eluent: $CHCl_3$) to afford the FAM mixture. The FAM mixture was acetylated using the standard procedure (Ac_2O, pyridine) at 70 °C for 2 h. The reaction mixture was diluted with $CHCl_3$ and successively washed with 2 M HCl. The organic layer was dried over Na_2SO_4, filtrated, and the filtrate was concentrated. The residue was fatty acid methylester acetate (FAM-Ac). $[\alpha]_D^{28}$ +12.9° (c=1.0, $CHCl_3$). ^{1}H-NMR ($CDCl_3$) δ: 4.98 (1H, t, J=6.4 Hz, 2-H), 3.74 (3H, s, $-COOCH_3$), 2.14 (3H, s, $-COCH_3$), 1.80 (2H, m, 3-H), 0.88 (3H, t, J=6.6 Hz, $-CH_3$).

Determination of Absolute Configuration of the LCB Moiety of LMC-2 LMC-2 (300 mg) was heated with 0.9 M HCl in 82% MeOH at 80 °C for 18 h. The reaction mixture was extracted with *n*-hexane, and the MeOH layer was alkalinized with 6 M NaOH. The mixture was diluted with diethyl ether and successively washed with water. The organic layer was dried over Na_2SO_4, filtrated, and the filtrate was concentrated. The residue was acetylated using the standard procedure (Ac_2O, pyridine) at 70 °C for 2 h. The reaction mixture was diluted with $CHCl_3$ and successively washed with 2 M HCl. The organic layer was dried over Na_2SO_4, filtrated, and the filtrate was concentrated. The residue was purified by column chromatography on a silica gel column (eluent: *n*-hexane/AcOEt, 3 : 2) to afford LCB-Ac. $[\alpha]_D^{28}$ +22.9° (c=1.0, $CHCl_3$). ^{1}H-NMR ($CDCl_3$) δ: 6.09 (1H, d, J=9.2 Hz, –NH), 5.08 (1H, dd, J=8.3, 3.0 Hz, 3-H), 4.91 (1H, dt, J=9.2, 3.3, 3.3 Hz, 4-H), 4.47 (1H, m, 2-H), 4.26 (1H, dd, J=11.7, 4.8 Hz, 1-Ha), 3.98 (1H, dd, J=11.7, 3.1 Hz, 1-Hb).

Isolation of Cerebroside LMC-2 Series HPLC of LMC-2 (solvent MeOH, flow rate 2.5 ml/min) showed 12 peaks. Using these conditions, 130 mg of LMC-2 was separated by HPLC to give 12 compounds: LMC-2-1 (**2**) (3.2 mg, t_R=15.5 min), LMC-2-2 (1.7 mg, t_R=17.5 min), LMC-2-3 (**3**) (5.4 mg, t_R=20.5 min), LMC-2-4 (2.3 mg, t_R=24.3 min), LMC-2-5 (**4**) (7.5 mg, t_R=26.5 min), LMC-2-6 (**5**) (15.1 mg, t_R=28.0 min), LMC-2-7 (5.2 mg, t_R=30.5 min), LMC-2-8 (19.9 mg, t_R=32.8 min), LMC-2-9 (3.5 mg, t_R=35.5 min), LMC-2-10 (26.9 mg, t_R=38.5 min), LMC-2-11 (16.9 mg, t_R=44.8 min), LMC-2-12 (**6**) (4.7 mg, t_R=51.5 min).

Compound 2 (Luidiacerebroside A) Amorphous powder, mp 180—181 °C. $[\alpha]_D^{29}$ +7.7° (c=0.22, 1-PrOH). Positive-ion FAB-MS *m/z*: 770 $[M+Na]^+$, 568 $[(M+H)-H_2O-\text{hexose}]^+$. ^{1}H-NMR (pyridine-$d_5$) δ: 8.58 (1H, d, J=9.2 Hz, –NH), 5.25 (1H, m, 2-H), 4.96 (1H, d, J=7.9 Hz, glc-1), 4.70 (1H, d, J=10.6, 6.6 Hz, 1-Hb), 4.51 (1H, m, 1-Ha), 4.01 (1H, m, glc-2), 3.88 (1H, m, glc-5). ^{13}C-NMR: See Table 2. HR positive-ion FAB-MS; $C_{41}H_{81}NO_{10}Na$ $[M+Na]^+$: Calcd 770.5758, Found 770.5771. Compound **2** was methanolyzed using the same method to yield methyl 2-hydroxyhexadecanoate as FAM.

Compound 3 Amorphous powder, mp 190—191 °C. $[\alpha]_D^{29}$ +8.9° (c=0.55, 1-PrOH). Positive-ion FAB-MS *m/z*: 810 $[M+Na]^+$, 608 $[(M+H)-H_2O-\text{hexose}]^+$. ^{1}H-NMR (pyridine-$d_5$) δ: 8.56 (1H, d, J=8.9 Hz, –NH), 5.43 (2H, m, olefin), 5.26 (1H, m, 2-H), 4.93 (1H, d, J=7.9 Hz, glc-1), 4.70 (1H, d, J=10.6, 6.6 Hz, 1-Hb), 4.52 (1H, m, 1-Ha), 4.00 (1H, m, glc-2), 3.84 (1H, m, glc-5). ^{13}C-NMR: See Table 2. HR positive-ion FAB-MS; $C_{44}H_{85}NO_{10}Na$ $[M+Na]^+$: Calcd 810.6071, Found 810.6056. HMBC spectrum: Correlations were observed between the signals δ=5.43 (olefinic protons) and δ=27.5 (methylene carbon atoms). Compound **3** was methanolyzed using the same method as described for LMC-2 to yield methyl 2-hydroxyhexadecanoate as FAM.

DMDS Derivative of 3 Compound **3** (1.0 mg) was dissolved in carbon disulfide (DMDS, 0.2 ml) and iodine (1 mg) was added to the solution. The resulting mixture was stored at 60 °C for 40 h in a small-volume sealed vial. The reaction was subsequently quenched with aqueous $Na_2S_2O_3$ (5%), and mixture was extracted with *n*-hexane (0.3 ml). The extract was concentrated to give the **3** DMDS derivative. Positive-ion FAB-MS *m/z*: 229.

Compound 4 Amorphous powder, mp 203—205 °C. $[\alpha]_D^{29}$ +9.5° (c=0.49, 1-PrOH). Positive-ion FAB-MS *m/z*: 812 $[M+Na]^+$, 610 $[(M+H)-H_2O-\text{hexose}]^+$. ^{1}H-NMR (pyridine-$d_5$) δ: 8.58 (1H, d, J=8.9 Hz,

–NH), 5.26 (1H, m, 2-H), 4.94 (1H, d, J=7.9 Hz, glc-1), 4.70 (1H, d, J=10.6, 6.6 Hz, 1-Hb), 4.53 (1H, m, 1-Ha), 4.01 (1H, m, glc-2), 3.85 (1H, m, glc-5). ^{13}C-NMR: See Table 2. HR positive-ion FAB-MS; $C_{44}H_{87}NO_{10}Na$ $[M+Na]^+$: Calcd 812.6228, Found 812.6231. Compound **4** was methanolyzed using the same method to yield methyl 2-hydroxydocosanoate as FAM.

Compound 5 Amorphous powder, mp 218—220 °C. $[\alpha]_D^{29}$ +9.7° (c=1.00, 1-PrOH). Positive-ion FAB-MS m/z: 826 $[M+Na]^+$, 624 $[(M+H)-H_2O-\text{hexose}]^+$. ^{1}H-NMR (pyridine-$d_5$) δ: 8.56 (1H, d, J=9.2 Hz, –NH), 5.26 (1H, m, 2-H), 4.93 (1H, d, J=7.9 Hz, glc-1), 4.70 (1H, d, J=10.6, 6.6 Hz, 1-Hb), 4.52 (1H, m, 1-Ha), 4.00 (1H, m, glc-2), 3.84 (1H, m, glc-5). ^{13}C-NMR: See Table 2. HR positive-ion FAB-MS; $C_{45}H_{89}NO_{10}Na$ $[M+Na]^+$: Calcd 826.6384, Found 826.6397. Compound **5** was methanolyzed using the same method as described for LMC-2 to yield methyl 2-hydroxydocosanoate as FAM.

Compound 6 (Luidiacerebroside B) Amorphous powder, mp 216—218 °C. $[\alpha]_D^{29}$ +4.3° (c=0.22, 1-PrOH). Positive-ion FAB-MS m/z: 882 $[M+Na]^+$, 680 $[(M+H)-H_2O-\text{hexose}]^+$. ^{1}H-NMR (pyridine-$d_5$) δ: 8.58 (1H, d, J=8.9 Hz, –NH), 5.27 (1H, m, 2-H), 4.96 (1H, d, J=7.6 Hz, glc-1), 4.70 (1H, d, J=10.6, 6.6 Hz, 1-Hb), 4.53 (1H, m, 1-Ha), 4.03 (1H, m, glc-2), 3.84 (1H, m, glc-5). ^{13}C-NMR: See Table 2. HR positive-ion FAB-MS; $C_{49}H_{97}NO_{10}Na$ $[M+Na]^+$: Calcd 882.7010, Found 882.7001. Compound **6** was methanolyzed using the same method as described for LMC-2 to yield methyl 2-hydroxytetracosanoate as FAM.

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3) *normal* means a straight chain (. . . $CH_2CH_2CH_2CH_3$); *ante-iso* means a branched chain possessing a methyl group on the third carbon from the terminal methyl group [$CH_2CH(CH_3)CH_2CH_3$].
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