Isolation and Structural Determination of Four New Ceramide Lactosides from the Starfish *Luidia maculata*

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Four new ceramide lactosides, luidialactoside A (**1**), B (**3**), C (**4**), and D (**5**) were isolated from the water-insoluble lipid fraction of the $CHCl_3$/MeOH extract of the starfish *Luidia maculata*. The structures of these ceramide lactosides were determined on the basis of chemical and spectroscopic evidence.
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Introduction

During the course of our search for biologically active glycosphingolipids from starfish, the ganglioside molecular species GP-2, obtained from the starfish *Asterina pectinifera*, was found to support the survival of cultured neuronal cells.[1] GAA-7, obtained from *Asterias amurensis versicolor*, showed neuritogenic and growth-inhibitory activities towards the mouse neuroblastoma cell line (Neuro 2a).[2]

Continuing these previous studies, we have carried out the isolation and structural elucidation of a sulfatide molecular species LMG-1 and a ganglioside molecular species LMG-2, obtained from the starfish *Luidia maculata* (Yatsudesunahitode in Japanese).[3] In the present paper, we report on the isolation and characterization of the newly isolated ceramide lactosides from the starfish *L. maculata*.

Results and Discussion

The aqueous saturated *n*BuOH 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 ceramide lactoside molecular species, tentatively named LMCDH-1 and LMCDH-2, each showing a single spot on normal-phase silica gel TLC.

The IR and negative-ion FAB mass spectra of LMCDH-1 and LMCDH-2 exhibit strong hydroxy and amide absorptions and a series of $[M - H]^-$ ion peaks. Their ^{13}C NMR spectra also exhibit the characteristic signals due to sphingosine- and phytosphingosine-type ceramide moieties possessing 2-hydroxy fatty acids and two anomeric carbons (Table 1). Furthermore, LMCDH-1 and LMCDH-2 were thought to possess *normal* and *ante-iso* type[4] side chains, because the carbon atom signals due to terminal methyl groups were observed at $\delta = 14.2$ ppm (*normal* form) and 11.5 and 19.4 (*ante-iso* form) in the ^{13}C NMR spectra (Table 1). Therefore, LMCDH-1 and LMCDH-2 must be molecular species of sphingosine- and phytosphingosine-type ceramide dihexosides, respectively, composed of a 2-hydroxy fatty acid and two hexoses.

Structure of LMCDH-1

The structure of the ceramide moiety of LMCDH-1 was examined first. When LMCDH-1 was methanolyzed with 5% HCl/MeOH, a mixture of fatty acid methyl esters (FAMs) was obtained together with a mixture of methyl glycosides and long-chain bases (LCBs). A GC-MS analysis of the FAM mixture showed the existence of five components (FAM-1−5) which were characterized as methyl 2-hydroxyhexadecanoate (FAM-1), methyl 2-hydroxyheneicosanoate (FAM-2), methyl 2-hydroxydocosanoate (FAM-3), methyl 2-hydroxytricosanoate (FAM-4), and methyl 2-hydroxytetracosanoate (FAM-5). The major FAM was FAM-3. On the other hand, the GC-MS analysis of the TMS derivative of the LCB mixture indicated that the LCB components were 2-amino-4-heptadecene-1,3-diol (LCB-1), 2-amino-4-octadecene-1,3-diol (LCB-2) and 2-amino-4-nonadecene-1,3-diol (LCB-3). LCB-3 was the major long-chain base. The stereochemistry of the ceramide moiety was determined as follows. When LMCDH-1 was heated with 0.9 N HCl in 82% MeOH, FAM and LCB mixtures were obtained. The FAM mixture was acetylated to afford its acetate (FAM-Ac). The optical rotation of FAM-Ac (+13.0°) showed its *R* configuration upon comparison with those of two synthetic diastereomers of methyl (2*R*)-2-*O*-acetyl hexadecanoate (+14.5°) and methyl (2*S*)-2-*O*-acetyl hexadecanoate (−13.8°).[5] The LCB mixture was acetylated to form an LCB acetate mixture (LCBA). The ^1H NMR spec-

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Table 1. ^{13}C NMR chemical shifts of ceramide lactosides (δ values, in [D_5]pyridine); the asterisks indicate that the assignments may be interchanged within each vertical column

Position	LMCDH-1	**1**	LMCDH-2	**2**	**3**	**4**	**5**
1(t)	70.1	70.1	70.3	70.3	70.2	70.2	70.1
2(d)	54.5	54.5	51.6	51.6	51.7	51.6	51.6
3(d)	72.4*	72.4*	75.9	75.8	75.9	75.8	75.9
4(d)	131.8	131.8	72.6*	72.6	72.6*	72.6*	72.5*
5(d)	132.7	132.8					
8(t)						27.5**	
9(d)						130.1***	
10(d)						130.3***	
11(t)						27.8**	
1′ (s)	175.6	175.6	175.6	175.6	175.6	175.6	175.6
2′ (d)	72.6*	72.6*	72.5*	72.6	72.5*	72.5*	72.4*
CH_3 [a] (q)	14.3	14.3	14.3	14.3	14.2	14.3	14.3
CH_3 [b] (q)	11.6	11.6	11.5		11.5		
CH_3 [c] (q)	19.4	19.4	19.4		19.4		
1"(d)	105.2	105.2	105.0	105.0	105.0	105.0	105.0
2"(d)	74.6	74.6	74.6	74.6	74.6	74.6	74.6
3"(d)	76.5**	76.5**	76.6	76.5	76.5	76.5	76.6**
4"(d)	81.8	81.8	81.7	81.7	81.7	81.7	81.7
5"(d)	77.3	77.3	77.3	77.2	77.2	77.2	77.2
6"(t)	62.1	62.1	62.1	62.1*	62.1	62.0	62.1***
1′"(d)	105.7	105.7	105.7	105.8	105.7	105.7	105.7
2′"(d)	72.4*	72.4*	72.4	72.4	72.4	72.4	72.4
3′"(d)	75.2	75.2	75.2	75.2	75.2	75.2	75.2
4′"(d)	70.1	70.1	70.1	70.1	70.1	70.1	70.1
5′"(d)	76.6**	76.6**	76.6	76.5	76.5	76.5	76.5**
6′"(t)	62.1	62.1	62.1	62.0*	62.1	62.0	62.0***

[a] Terminal methyl group in *normal* type of side chain (see ref.[4a]). [b] Terminal methyl group in *ante-iso* type of side chain (see ref.[4b]). [c] Terminal methyl group in *ante-iso* type of side chain (see ref.[4b]).

trum of LCBA is in good agreement with that of synthetic (2*S*,3*R*,4*E*)-2-acetamido-1,3-diacetoxyoctadec-4-ene (par-acetyl sphingosine)[6] with respect to the signals due to 1-H to 5-H. Furthermore, the optical rotation of LCBA (−7.62°) and the synthetic par-acetyl sphingosine (−7.7°)[7] suggests that LCBA has the (2*S*,3*R*,4*E*) configuration.

Next, the structure of the sugar moiety of LMCDH-1 was examined. The GLC analysis of hexitol acetate derivatives of the saccharide, which were obtained by hydrolysis, reduction, and acetylation of LMCDH-1, showed the existence of glucose (Glc) and galactose (Gal). The sites of the linkage of the sugar moiety were determined by means of two-dimensional (2D) NMR spectroscopy. When the heteronuclear multiple bond coherence (HMBC) spectrum of LMCDH-1 was measured, $^3J_{CH}$ correlations from H-1 of Gal (δ_H = 5.04) to C-4 of Glc (δ_C = 81.8) and H-1 of Glc (δ_H = 4.81) to C-1 of ceramide (δ_C = 70.1) were observed. The configurations of each monosaccharide were considered to be β on the basis of the anomeric carbon signals (δ_C = 105.2, 105.7 ppm). The absolute configurations of glucose and galactose were determined as the D-form by means of Hara's method.[8]

Accordingly, LMCDH-1 was designated as a sphingosine-type ceramide lactoside molecular species, composed of the aforementioned fatty acids and long-chain bases, as depicted in Scheme 1.

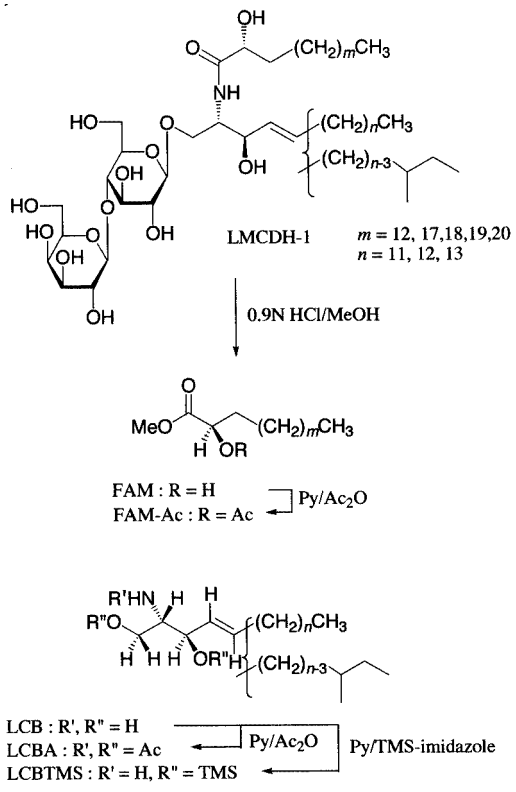
Isolation and Structure of the Ceramide Lactoside from LMCDH-1

LMCDH-1 was separated by reversed-phase HPLC into ten components, which were recovered to give the ten fractions LMCDH-1-1 to LMCDH-1-10. Two of the ten fractions, LMCDH-1-9 and LMCDH-1-10, show a single quasi-molecular ion peak $[M - H]^-$ in the negative-ion FAB mass spectrum, but the other eight fractions exhibit multiple molecular ion peaks. Upon methanolysis of the two fractions that were thought to be pure compounds, only LMCDH-1-10 (**1**) afforded a homogeneous fatty acid and a long-chain base.

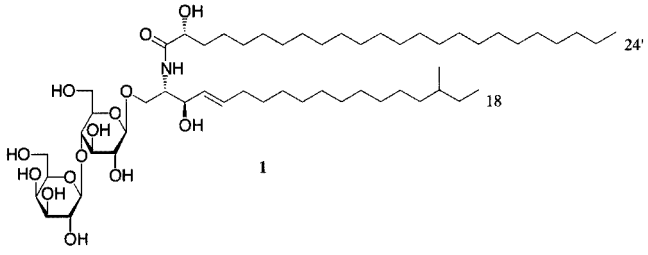
The ^{13}C NMR spectrum of **1** is essentially identical to that of the LMCDH-1 mixture (Table 1). Compound **1** also shows the signals of the *normal* and *ante-iso* type[4] of terminal methyl groups in its ^{13}C NMR spectrum and, upon methanolysis, **1** affords methyl 2-hydroxytetracosanoate. On the basis of the above data and the molecular mass of **1** (*m*/*z* = 1002 $[M - H]^-$), the structure of **1** was determined to be (2*S*,3*R*,4*E*)-1-*O*-[β-D-galactopyranosyl-(1→4)-β-D-glucopyranosyl]-2-[(2*R*)-2-hydroxytetra-cosanoylamino]-16-methyl-4-octadecene-1,3-diol (Scheme 2).

Structure of LMCDH-2

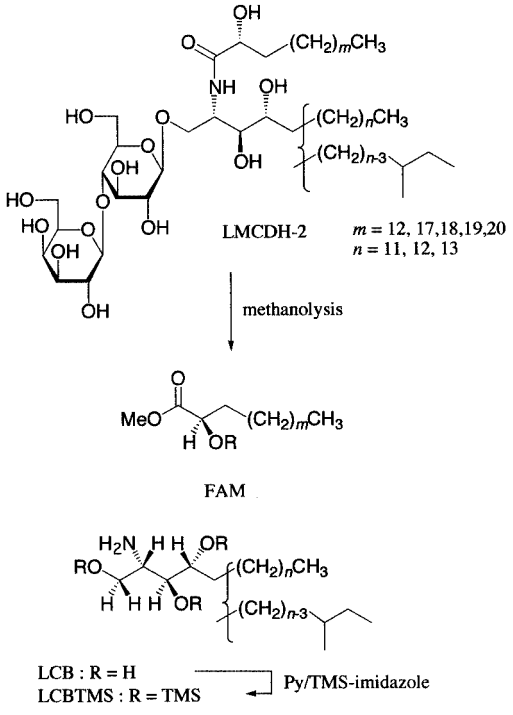
The structure of the ceramide moiety of LMCDH-2 was examined first. LMCDH-2 was methanolyzed and worked



Scheme 1



Scheme 2



Scheme 3

up in the same manner as described for LMCDH-1 to give a mixture of FAM, methyl glycosides, and LCB. A GC-MS analysis of the FAM mixture showed the existence of five components (FAM-1−5) which were identified as being the same as the FAMs obtained from LMCDH-1. The major FAM was methyl 2-hydroxydocosanoate (FAM-3). On the other hand, the 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-2′ was the major long-chain base.

The structure of the sugar moiety of LMCDH-2 was determined as β-D-Gal-(1 → 4)-β-D-Glc, by the same method as described for LMCDH-1.

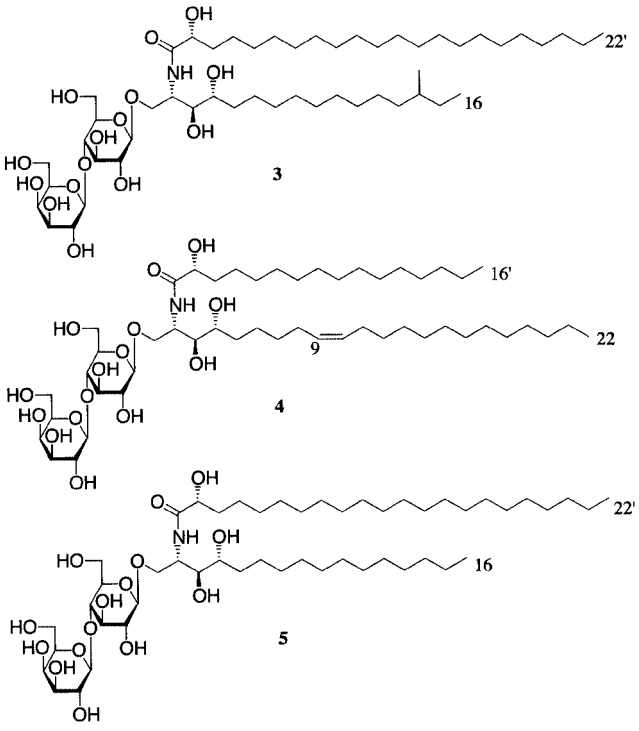
The stereochemistry of the ceramide moiety was determined as follows. The ^{13}C NMR spectrum of LMCDH-2 was compared with those of four synthetic diastereomers of lactosyl ceramide, (2*S*,3*S*,4*R*)-, (2*S*,3*S*,4*S*)-, (2*S*,3*R*,4*S*)-, and (2*S*,3*R*,4*R*)-1-*O*-[*O*-β-D-galactopyranosyl-(1→4)-β-D-glucopyranosyl]-2-[(2*R*)-2-hydroxytetracosanoylamino]-1,3,4-hexadecanetriol.[9] The spectrum of LMCDH-2 is in good agreement with that of the (2*S*,3*S*,4*R*)-isomer (**2**) (Table 1). This fact and the optical rotations of **2** (+12.9°)[9] and **3**−**5** (+7.56°, +8.62°, +7.45°, respectively) — the components of LMCDH-2, vide infra — suggest that LMCDH-2 has the same absolute configuration as **2** for the core structure (C-2, C-3, C-4, C-2′, and lactose). Accordingly, LMCDH-2 was designated as a phytosphingosine-type ceramide lactoside molecular species, composed of the aforementioned fatty acids and long-chain bases, as depicted in Scheme 3.

Isolation and Structure of the Ceramide Lactoside from LMCDH-2

LMCDH-2 was separated by reversed-phase HPLC into twelve components, which were recovered to give the twelve fractions LMCDH-2-1 to LMCDH-2-12. Eight of the twelve fractions, LMCDH-2-1 − LMCDH-2-3, LMCDH-2-7 − LMCDH-2-9, LMCDH-2-11, and LMCDH-2-12, show a single quasi-molecular ion peak $[M - H]^-$ in the negative-ion FAB mass spectrum, but the other four fractions exhibit multiple molecular ion peaks. Upon methanolysis of the eight fractions that were thought to be pure compounds, LMCDH-2-1 (**3**), LMCDH-2-9 (**4**), and LMCDH-2-11 (**5**) afforded a homogeneous fatty acid and a long-chain base.

The ^{13}C NMR spectra of **3**−**5** were essentially identical to that of the LMCDH-2 mixture (Table 1). Compound **3**

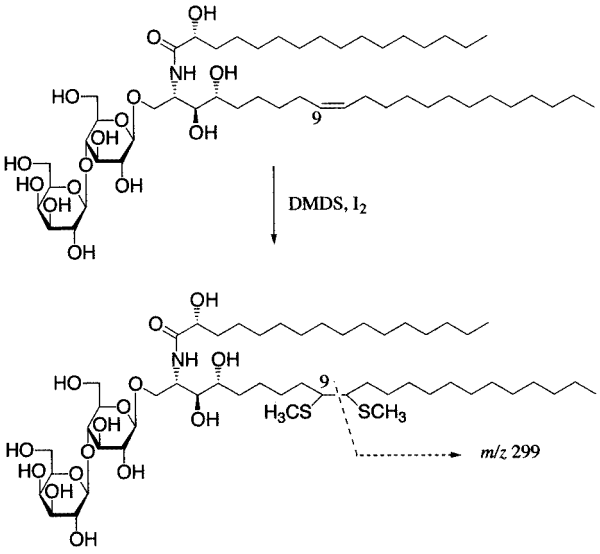
shows the signals of the *normal* and *ante-iso* type[4] of terminal methyl groups in its ^{13}C NMR spectrum and, upon methanolysis, **3** afforded methyl 2-hydroxydocosanoate. On the basis of the above data and the molecular mass of **3** (m/z = 964 $[M - H]^-$), the structure of **3** was determined to be (2*S*,3*S*,4*R*)-1-*O*-[β-D-galactopyranosyl-(1→4)-β-D-glucopyranosyl]-2-[(2*R*)-2-hydroxydocosanoylamino]-14-methyl-1,3,4-hexadecanetriol (Scheme 4). Compound **5** shows the signals of *normal* type[4] terminal methyl groups in its ^{13}C NMR spectrum and afforded methyl 2-hydroxydocosanoate upon methanolysis. Taking the molecular mass of **5** (m/z = 950 $[M - H]^-$) into account, the structure of **5** was proposed to be (2*S*,3*S*,4*R*)-1-*O*-[β-D-galactopyranosyl-(1→4)-β-D-glucopyranosyl]-2-[(2*R*)-2-hydroxydocosanoylamino]-1,3,4-hexadecanetriol (Scheme 4).



Scheme 4

On the other hand, **4** was thought to possess one olefinic group in the LCB residue, since two olefinic carbon signals (δ = 130.1 and 130.3) were observed in the ^{13}C NMR spectrum. In fact, **4** gave methyl 2-hydroxyhexadecanoate as the fatty acid component upon methanolysis. The location and geometry of the double bonds were determined as follows. The positive-ion FAB mass spectrum of the dimethyl disulfide (DMDS) derivative[10] of **4** shows a remarkable fragment ion peak at m/z = 229, due to cleavage of the bond between the carbons bearing a methylthio group (Scheme 5). This indicates that the double bond in the LCB residue of **4** is located at C-9, as shown in Scheme 4.

Furthermore, it is known[11] that the geometry of the double bond in a 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



Scheme 5

at δ ≈ 27 ppm in (*Z*) isomers and δ ≈ 32 ppm in (*E*) isomers. The proton signal at δ = 5.43 ppm was assigned to the olefin groups by means of a ^1H-^1H COSY spectrum of **4**. When the HMBC spectrum of **4** was measured, significant correlations were observed between the signal of this olefinic proton and the methylene carbon atoms at δ = 27.5 and 27.8 ppm, as shown in Table 1. Thus, the olefin group in the LCB of **4** was shown to have *cis* (*Z*) geometry. In addition, **4** shows the signals of *normal* type[4] terminal methyl groups in its ^{13}C NMR spectrum. Therefore, the structure of **4** was proposed to be (2*S*,3*S*,4*R*,9*Z*)-1-*O*-[β-D-galactopyranosyl-(1→4)-β-D-glucopyranosyl]-2-[(2*R*)-2-hydroxyhexadecanoylamino]-9-docosene-1,3,4-triol, as shown in Scheme 4.

The isolation and characterization of ceramide lactosides, including the determination of their, absolute configuration is noteworthy. The existence of ceramide lactosides in echinodermata is not well known. Furthermore, this is the first report about sphingosine-type ceramide lactosides from echinodermata, and luidialactoside A (**1**), B (**3**), C (**4**), and D (**5**) are new ceramide lactosides. The biological activities of these compounds will be examined.

Experimental Section

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. ^1H 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 FAB mass spectra: Jeol SX102A mass spectrometer [xenon atom beam, 5 kV; ion-source accelerating potential, 10 kV; matrix, triethylene glycol (negative ion mode), *m*-nitrobenzyl alcohol (positive ion mode)]. GLC: Shimadzu GC-14B by employing FID [capillary column, J & W Scientific Fused Silica Capillary Column DB-17 (φ0.317 mm × 30 m)]. GC-MS: Shimadzu GC-17A/QP-5050A by employing the

EI mode [ionizing potential, 70 eV; separator and ion-source temperature, 250 °C; capillary column, TC-1701 (φ0.25 mm × 30 m, GL Science Inc.); carrier gas, He. HPLC: Jasco PU-980; RI detector; column, COSMOSIL 5C18-AR-II (Nacalai Tesque).

Separation of LMCDH-1 and LMCDH-2: Whole bodies of the starfish *Luidia maculata* (wet weight 57 kg, collected at 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 EtOAc/*n*BuOH (2:1, 40 L) for separation of the less-polar lipids. The aqueous layer was further extracted with *n*BuOH saturated with H_2O (300 mL × 4/L), and the organic layer was concentrated in vacuo to give a brown syrup (308 g). This fraction was extracted with $CHCl_3$/MeOH (1:1, 2 L, twice), and the $CHCl_3$/MeOH soluble portion (205.9 g) was chromatographed on Cosmosil 140C_{18}-PREP (80% MeOH → 90% MeOH → $CHCl_3$/MeOH, 1:1), silica gel ($CHCl_3$/MeOH/H_2O, 9:1.5:0.05 → 8:2:0.2), and sephadex LH-20 ($CHCl_3$/MeOH, 1:1) to afford LMCDH-1 (160 mg) and LMCDH-2 (128 mg). LMCDH-1 and LMCDH-2 each showed a single spot on silica gel TLC ($CHCl_3$/MeOH/H_2O, 8:2:0.2); R_f = 0.32 (LMCDH-1), 0.28 (LMCDH-2).

LMCDH-1: Amorphous powder, m.p. 195−200 °C. IR (KBr): $\tilde{\nu}$ = 3389 cm^{-1} (hydroxyl), 1651, 1536 (amide). Negative ion FABMS: *m*/*z* = 1002, 992, 978, 964 $[M - H]^-$ series. ^{13}C NMR: Table 1.

LMCDH-2: Amorphous powder, m.p. 205−210 °C. IR (KBr): $\tilde{\nu}$ = 3375 cm^{-1} (hydroxyl), 1626, 1537 (amide). Negative ion FABMS: *m*/*z* = 1006, 998, 992, 978, 964 $[M - H]^-$ series. ^{13}C NMR: Table 1.

Methanolysis of LMCDH-1: LMCDH-1 (1.0 mg) was heated with 5% HCl in methanol (1 mL) at 80 °C for 18 h in a sealed small-volume vial. The reaction mixture was extracted with *n*-hexane, and the *n*-hexane layer was concentrated to give a mixture of fatty acid methyl esters (FAM) for GC-MS analysis. The methanol layer was neutralized with Ag_2CO_3 and filtered. The filtrate was concentrated in vacuo to give a mixture of long-chain bases (LCBs) and methyl glycosides.

GC-MS Analysis of FAM from LMCDH-1: The FAM mixture from LMCDH-1 was subjected to GC-MS [column temp. 180−250 °C (rate of temp. increases 5 °C/min)]. The results were as follows: FAM-1 (methyl 2-hydroxyhexadecanoate), t_R [min] (ratio of peak area) = 16.1 (2.8), *m*/*z* = 286 $[M]^+$, 227 $[M - 59]^+$; FAM-2 (methyl 2-hydroxyheneicosanoate), t_R = 25.2 (3.0), *m*/*z* = 356 $[M]^+$, 297 $[M - 59]^+$; FAM-3 (methyl 2-hydroxydocosanoate), t_R = 27.7 (53.9), *m*/*z* = 370 $[M]^+$, 311 $[M - 59]^+$; FAM-4 (methyl 2-hydroxytricosanoate), t_R = 30.9 (32.5), *m*/*z* = 384 $[M]^+$, 325 $[M - 59]^+$: FAM-5 (methyl 2-hydroxytetracosanoate), t_R = 34.8 (7.8), *m*/*z* = 398 $[M]^+$, 339 $[M - 59]^+$.

GC-MS Analysis of TMS Ethers of LCBs from LMCDH-1: The LCB mixture from LMCDH-1 was heated with 1-(trimethylsilyl)imidazole/pyridine (1:1, 0.2 mL) at 70 °C for 20 min, and the reaction mixture [trimethylsilyl (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 TMS (1,3-di-*O*-trimethylsilyl-2-amino-4-heptadecene-1,3-diol), t_R [min] (ratio of peak area) = 13.7 (11.9), *m*/*z* = 326 $[M - 103]^+$, 324 $[M - 105]^+$, 297 $[M - 132]^+$; LCB-2 TMS (1,3-di-*O*-trimethylsilyl-2-amino-4-octadecene-1,3-diol), t_R = 15.2 (15.2), *m*/*z* = 340 $[M - 103]^+$, 338 $[M - 105]^+$, 311 $[M - 132]^+$; LCB-3 TMS (1,3-di-*O*-trimethylsilyl-2-amino-4-nonadecene-1,3-diol), t_R = 16.8 (65.6), *m*/*z* = 354 $[M - 103]^+$, 352 $[M - 105]^+$, 325 $[M - 132]^+$.

GLC Analysis of Alditol Acetates from LMCDH-1: LMCDH-1 (1 mg) was heated with 2 N HCl (1 mL) at 100 °C for 30 h in a sealed small-volume vial. The reaction mixture was extracted with *n*-hexane and the aqueous layer was concentrated in vacuo. The residue was dissolved in H_2O, then 7% NH_3 aq. (1 mL) and $NaBH_4$ (40 mg) were added to the solution. After standing at room temperature for 4 h, the reaction mixture was acidified with AcOH to pH 3.5 and concentrated in vacuo. The H_3BO_3 contained in the residue was removed by distillation with MeOH (three times). The residue was heated with Ac_2O/pyridine (1:1, 2 mL) at 70 °C for 2 h, diluted with H_2O, extracted with $CHCl_3$, and the $CHCl_3$ layer was extracted with H_2O, dried over Na_2SO_4 and concentrated in vacuo to give alditol acetates. These acetates were subjected to GLC [column temp. 150−250 °C (rate of temp. increase 5 °C/min)]. The results were as follows: t_R [min] (ratio of peak area) = 15.2 (60) [1,2,3,4,5,6-hexa-*O*-acetyl galactohexitol]; t_R = 15.3 (40) [1,2,3,4,5,6-hexa-*O*-acetyl glucohexitol].

Determination of the Absolute Configuration of the Sugar Moiety of LMCDH-1: LMCDH-1 (1.0 mg) was heated with 2 N HCl (1 mL) at 100 °C for 18 h. The reaction mixture was extracted with *n*-hexane and the acidic aqueous layer was concentrated in vacuo. The residue was heated with L-cysteine methyl ester hydrochloride (1.0 mg) and pyridine (0.1 mL) at 60 °C for 1 h. Then, 0.2 mL of 1-(trimethylsilyl)imidazole was added and the mixture was heated at 60 °C for 15 min to yield the trimethylsilyl ether derivative of the methyl 2-(polyhydroxyalkyl)thiazolidine-4(*R*)-carboxylate. The derivative was analyzed by GLC [column temp. 180−250 °C (rate of temp. increase 2.5 °C/min)]; t_R [min] = 23.98 (standard derivative of D-glucose 23.91; L-glucose 24.59), 24.79 (standard derivative of D-galactose 24.78; L-galactose 25.56).

Determination of the Absolute Configuration of the Fatty Acid Moiety of LMCDH-1: LMCDH-1 (30 mg) was heated under reflux with 0.9 N HCl in 82% MeOH (10 mL) for 18 h. The reaction mixture was extracted with *n*-hexane, and the *n*-hexane layer was concentrated in vacuo. The residue was chromatographed on silica gel ($CHCl_3$) to yield a mixture of fatty acid methyl esters (FAMs) (10.1 mg). The FAM mixture was heated with Ac_2O/pyridine (1:1) at 70 °C for 2 h, diluted with H_2O, extracted with $CHCl_3$, and the $CHCl_3$ layer was extracted with 2 N HCl, dried over Na_2SO_4 and concentrated in vacuo. The residue was purified by silica gel chromatography ($CHCl_3$) to give a mixture of FAM acetate (FAM-Ac) (8.6 mg) as a colorless syrup, $[\alpha]_D^{28}$ = +13.0 (*c* = 0.78 in $CHCl_3$). ^1H NMR ($CDCl_3$): δ = 0.88 (t, *J* = 6.6 Hz, 3 H, $-CH_3$), 1.80 (m, 2 H, 3-H_2), 2.14 (s, 3 H, $-CO\mathit{CH_3}$), 3.74 (s, 3 H, $-COO\mathit{CH_3}$),4.98 (t, *J* = 6.4 Hz, 1 H, 2-H) ppm.

Synthetic methyl (2*R* or 2*S*)-2-*O*-acetyl hexadecanoate:[3] $[\alpha]_D^{24}$ = +14.5 (*c* = 1.75 in $CHCl_3$, 2*R*), $[\alpha]_D^{24}$ = −13.8 (*c* = 2.5 in $CHCl_3$, 2*S*). ^1H NMR ($CDCl_3$): δ = 0.88 (t, *J* = 6.6 Hz, 3 H, $-CH_3$), 1.26 (s-like, 24 H, 12 × CH_2), 1.82 (m, 2 H, 3-H_2), 2.14 (s, 3 H, $-CO\mathit{CH_3}$), 3.74 (s, 3 H, $-COO\mathit{CH_3}$), 4.98 (t, *J* = 6.4 Hz, 1 H, 2-H) ppm.

Determination of the Absolute Configuration of the LCB moiety of LMCDH-1: LMCDH-1 (30 mg) was heated under reflux with 0.9 N HCl in 82% MeOH (10 mL) for 18 h. The reaction mixture was extracted with *n*-hexane, and the aqueous MeOH layer was diluted with H_2O (70 mL), basified with 6 N NaOH, and extracted with Et_2O (50 mL × 3). The Et_2O layer was concentrated in vacuo to give a long-chain base (LCB) mixture (10.3 mg). The residue was heated with Ac_2O/pyridine (1:1) at 70 °C for 2 h, diluted with H_2O,

extracted with $CHCl_3$, and the $CHCl_3$ layer was extracted with 2 N HCl, dried over Na_2SO_4 and concentrated in vacuo. The residue was purified by column chromatography on a silica gel column (*n*-hexane/EtOAc, 3:2) to give a mixture of LCBA (4.5 mg) as a colorless syrup, $[\alpha]_D^{28} = -7.62$ ($c = 0.41$ in $CHCl_3$). ^1H NMR ($CDCl_3$): $\delta = 4.05$ (dd, $J = 11.6$, 4.0 Hz, 1-Hb), 4.30 (dd, $J = 11.6$, 5.9 Hz, 1-Ha), 4.42 (m, 1 H, 2-H), 5.30 (t-like, 1 H, 3-H), 5.38 (dd, $J = 15.2$, 7.6 Hz, 1 H, 4-H), 5.64 (d, $J = 9.6$ Hz, 1 H, −NH), 5.79 (dt, $J = 15.2$, 6.8, 6.8 Hz, 1 H, 5-H) ppm.

Isolation of the LMCDH-1 Series: HPLC of LMCDH-1 (solvent 100% MeOH, flow rate 2.5 mL/min) showed ten peaks. Using these conditions, LMCDH-1 (115 mg) was separated by HPLC to give ten components: LMCDH-1−1 (4.3 mg, $t_R = 16.0$ min), LMCDH-1−2 (6.3 mg, $t_R = 18.8$ min), LMCDH-1−3 (9.3 mg, $t_R = 23.8$ min), LMCDH-1−4 (7.6 mg, $t_R = 25.0$ min), LMCDH-1−5 (6.0 mg, $t_R = 26.2$ min), LMCDH-1−6 (11.6 mg, $t_R = 28.2$ min), LMCDH-1−7 (2.6 mg, $t_R = 30.0$ min), LMCDH-1−8 (12.6 mg, $t_R = 33.0$ min), LMCDH-1−9 (6.0 mg, $t_R = 37.7$ min), LMCDH-1−10 (**1**) (2.2 mg, $t_R = 42.2$ min).

Compound 1 (luidialactoside A): Amorphous powder, m.p. 234−236 °C, $[\alpha]_D^{27} = +8.23$ ($c = 0.17$ in $CHCl_3$/MeOH, 1:1); negative ion FABMS: $m/z = 1002$ $[M - H]^-$, 840 $[M - H -$ hexose$]^-$, 678 $[M - H -$ hexose $\times 2]^-$. ^{13}C NMR: see Table 1. HR positive FABMS: $C_{55}H_{105}NNaO_{14}$ $[M + Na]^+$: calcd. 1026.7432, found. 1026.7452. Compound **1** was methanolyzed by the same method as described for LMCDH-1 to yield methyl 2-hydroxytetracosanoate as FAM.

Methanolysis of LMCDH-2: In the same way as described for LMCDH-1, LMCDH-2 (1 mg) was methanolyzed and worked up to give a mixture of fatty acid methyl esters (FAMs) for GC-MS analysis, a mixture of long-chain bases (LCBs), and methyl glycosides.

GC-MS Analysis of FAM from LMCDH-2: The FAM mixture from LMCDH-2 was subjected to GC-MS [column temp. 180−250 °C (rate of temp. increases 5 °/min)]. The results were: FAM-1 (methyl 2-hydroxyhexadecanoate), t_R [min] (ratio of peak area) = 10.4 (3.4), $m/z = 286$ $[M]^+$, 227 $[M - 59]^+$; FAM-2 (methyl 2-hydroxyheneicosanoate), $t_R = 19.0$ (3.5), $m/z = 356$ $[M]^+$, 297 $[M - 59]^+$; FAM-3 (methyl 2-hydroxydocosanoate), $t_R = 21.6$ (52.2), $m/z = 370$ $[M]^+$, 311 $[M - 59]^+$; FAM-4 (methyl 2-hydroxytricosanoate), $t_R = 24.7$ (33.1), $m/z = 384$ $[M]^+$, 325 $[M - 59]^+$: FAM-5 (methyl 2-hydroxytetracosanoate), $t_R = 28.5$ (7.8), $m/z = 398$ $[M]^+$, 339 $[M - 59]^+$.

GC-MS Analysis of TMS Ethers of LCB from LMCDH-2: The LCB mixture from LMCDH-2 was trimethylsilylated and the TMS ethers were analyzed by GC-MS as described for the LCB mixture from LMCDH-1. The results were: LCB-1′ TMS (1,3,4-tri-*O*-trimethylsilyl-2-amino-1,3,4-hexadecanetriol), t_R [min] (ratio of peak area) = 14.8 (3.5), $m/z = 312$ $[M - 193]^+$, 271 $[M - 234]^+$; LCB-2′ TMS (1,3,4-tri-*O*-trimethylsilyl-2-amino-1,3,4-heptadecanetriol), $t_R = 15.8$ (38.2), $m/z = 326$ $[M - 193]^+$, 285 $[M - 234]^+$; LCB-3′ TMS (1,3,4-tri-*O*-trimethylsilyl-2-amino-1,3,4-octadecanetriol), $t_R = 17.5$ (22.9), $m/z = 340$ $[M - 193]^+$, 299 $[M - 234]^+$; LCB-4′ TMS (1,3,4-tri-*O*-trimethylsilyl-2-amino-1,3,4-nonadecanetriol), $t_R = 19.5$ (35.4), $m/z = 354$ $[M - 193]^+$, 313 $[M - 234]^+$.

GLC Analysis of Alditol Acetates from LMCDH-2: In the same way as described for LMCDH-1, LMCDH-2 (1 mg) was hydrolyzed, reduced, and acetylated to give alditol acetates. These acetates were subjected to GLC [column temp. 150−250 °C (rate of temp. increase 5 °C/min)]. The results were: t_R[min] (ratio of peak area) = 15.2 (60) [1,2,3,4,5,6-hexa-*O*-acetyl galactohexitol]; $t_R = 15.3$ (40) [1,2,3,4,5,6-hexa-*O*-acetyl glucohexitol].

Determination of the Absolute Configuration of the Sugar Moiety of LMCDH-2: LMCDH-2 (1 mg) was converted to the trimethylsilyl ether derivative of methyl 2-(polyhydroxyalkyl)-thiazolidine-4(*R*)-carboxylate in the same way as described for LMCDH-1. The derivative was analyzed by GLC [column temp. 180−250 °C (rate of temp. increase 2.5 °C/min)]; t_R [min] = 23.86 (standard derivative of D-glucose 23.91; L-glucose 24.57), 24.74 (standard derivative of D-galactose 24.78; L-galactose 25.65).

Isolation of LMCDH-2 Series: HPLC of LMCDH-2 (solvent 100% MeOH, flow rate 2.5 mL min^{-1}) showed nine peaks. Using these conditions, LMCDH-2 (114 mg) was separated by HPLC to give six components: fraction 1 (36.9 mg, $t_R = 7-20$ min), LMCDH-2−1 (**3**) (13.8 mg, $t_R = 21.2$ min), LMCDH-2−2 (19.1 mg, $t_R = 24.1$ min), LMCDH-2−3 (8.7 mg, $t_R = 28.0$ min), LMCDH-2−4 (9.1 mg, $t_R = 32.0$ min), LMCDH-2−5 (2.3 mg, $t_R = 36.0$ min). Fraction 1 was separated under different conditions (solvent 98% MeOH, flow rate 2.5 mL min^{-1}) to afford seven components: LMCDH-2−6 (2.6 mg, $t_R = 15.2$ min), LMCDH-2−7 (4.2 mg, $t_R = 20.0$ min), LMCDH-2−8 (2.5 mg, $t_R = 23.8$ min), LMCDH-2−9 (**4**) (2.5 mg, $t_R = 27.8$ min), LMCDH-2−10 (2.8 mg, $t_R = 33.0$ min), LMCDH-2−11 (**5**) (2.0 mg, $t_R = 36.0$ min), LMCDH-2−12 (2.0 mg, $t_R = 42.0$ min).

Compound 3 (luidialactoside B): Amorphous powder, m.p. 235−237 °C, $[\alpha]_D^{27} = +7.56$ ($c = 0.56$ in $CHCl_3$/MeOH, 1:1); negative ion FABMS: $m/z = 964$ $[M - H]^-$, 802 $[M - H -$ hexose$]^-$, 640 $[M - H -$ hexose $\times 2]^-$. ^{13}C NMR: see Table 1. HR positive FABMS: $C_{51}H_{99}NNaO_{15}$ $[M + Na]^+$; calcd. 988.6912, found. 988.6917. Compound **3** was methanolyzed as described for LMCDH-1 to yield methyl 2-hydroxydocosanoate as FAM.

Compound 4 (luidialactoside C): Amorphous powder, m.p. 217−220 °C, $[\alpha]_D^{27} = +8.62$ ($c = 0.17$ in $CHCl_3$/MeOH, 1:1); negative ion FABMS: $m/z = 948$ $[M - H]^-$, 786 $[M - H -$ hexose$]^-$, 624 $[M - H -$ hexose $\times 2]^-$. ^{13}C NMR: Table 1. HR positive FABMS: $C_{50}H_{95}NNaO_{15}$ $[M + Na]^+$; calcd. 972.6599, found. 972.6609. HMBC spectrum: Correlations were observed between the signals $\delta_H = 5.43$ ppm (olefinic protons) and $\delta_C = 27.5$, 27.8 ppm (methylene carbon atoms). Compound **4** was methanolyzed as described for LMCDH-1 to yield methyl 2-hydroxyhexadecanoate as FAM. The corresponding LCB could not be observed on GC-MS analysis of the LCB derived from LMCDH-2 because the content of **4** in the LMCDH-2 was too little to detect.

DMDS Derivative of 4: Compound **4** (1.0 mg) was dissolved in a mixture of carbon disulfide (0.1 mL) and dimethyl disulfide (DMDS; 0.1 mL), and iodine (1 mg) was added to the solution. The resulting mixture was kept 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 the mixture was extracted with *n*-hexane (0.3 mL). The extract was concentrated to give the DMDS derivative of **4**. Positive ion FABMS: Fragment ion peak $m/z = 229$ was observed.

Compound 5 (luidialactoside D): Amorphous powder, m.p. 223−225 °C, $[\alpha]_D^{27} = +7.45$ ($c = 0.17$ in $CHCl_3$/MeOH, 1:1); negative ion FABMS: $m/z = 950$ $[M - H]^-$, 788 $[M - H -$ hexose$]^-$, 626 $[M - H -$ hexose $\times 2]^-$. ^{13}C NMR: see Table 1. HR positive FABMS: $C_{50}H_{97}NNaO_{15}$ $[M + Na]^+$; calcd. 974.6756, found. 974.6773. Compound **5** was methanolyzed as described for LMCDH-1 to yield methyl 2-hydroxydocosanoate as FAM.

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