Biologically Active Glycosides from Asteroidea, 38[+] Glycosphingolipids from the Starfish *Luidia maculata*, 2[+]

Isolation and Structure of a GM_3-Type Ganglioside Molecular Species

Satoshi Kawatake,[a] Masanori Inagaki,[a] Tomofumi Miyamoto,[a] Ryuichi Isobe,[b] and Ryuichi Higuchi*[a]

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A ganglioside molecular species, LMG-2 (**1**), has been obtained from the water-soluble lipid fraction of the chloroform/methanol extract of the starfish *Luidia maculata*. On the basis of chemical and spectroscopic findings, the structure of **1** has been elucidated. **1** is a ganglioside molecular species of starfish resembling the mammalian ganglioside GM_3. In addition, **1** shows neuritogenic activity toward the rat pheochromocytoma cell line PC-12 cell.

In our previous studies, the ganglioside molecular species LG-2, which has been obtained from the starfish *Astropecten latespinosus*, has been found to show antitumor activity against murine lymphoma L1210 cells,[1] and another ganglioside molecular species GAA-7, obtained from the starfish *Asterias amurensis versicolor* showed neuritogenic and growth-inhibitory activities towards the mouse neuroblastoma cell line (Neuro 2a).[2] Continuing the previous studies, we have conducted the isolation and structure elucidation of the biologically active glycosphingolipids from the starfish *Luidia maculata* (Yatsudesunahitode in Japanese) with the object of searching for lead compounds for new medicines. In the preceding paper, we reported isolation and characterization of the new sulfatide molecular species LMG-1,[3] obtained from the starfish *L. maculata.* In this paper the isolation, structure determination and biological activity of a new ganglioside molecular species from the starfish are described.

A water-soluble lipid fraction, obtained from the chloroform/methanol extract of the whole bodies of *L. maculata*, was subjected to reversed-phase, followed by normal-phase column chromatography to give a ganglioside molecular species LMG-2 (**1**) showing a single spot in normal-phase thin-layer chromatography (TLC).

Compound **1** shows strong hydroxy (3389 cm^{-1}) and amide (1637 cm^{-1}) absorptions in the IR spectrum. The ^{13}C-NMR spectrum of **1** shows the characteristic signals of a phytosphingosine-type ceramide possessing a 2-hydroxy fatty acid and a sugar moiety at C-1 (Table 1) [δ_C = 70.0 (C-1), 51.4 (C-2), 75.6 (C-3), 72.5 (C-4), 175.8 (C-1′), 72.3 (C-2′)]. The ^{13}C-NMR spectrum of **1** also reveals signals due to three anomeric carbon atoms at δ_C = 105.0, 105.3, 101.0, one of which (δ_C = 101.0) is a quaternary carbon signal suggesting the existence of sialic acid. Furthermore, **1** is presumed to have *normal* type (major) and *ante-iso*[4] types (minor) of terminal methyl groups, because the carbon signals are observed at δ_C = 14.5 (*normal* form), δ_C = 11.7 and 19.5 (*ante-iso* form) in the ^{13}C-NMR spectrum of **1**. The negative-ion fast atom bombardment mass spectrum (FAB MS) exhibits a series of quasi-molecular ion peaks which are different by 14 mass units ($[M - H]^-$ at *m/z* 1241, 1255, 1269, 1283, 1297, 1311). Therefore, **1** is suggested to be a molecular species of phytosphingosine-type ganglioside possessing 2-hydroxy fatty acid and three monosaccharides. The fatty acid and long-chain base (phytosphingosine) constituents and the structure of sialosyl oligosaccharide moiety of **1** are determined as follows.

The structure of the ceramide moiety was examined first. When **1** was methanolyzed with 5% HCl/MeOH, a mixture of fatty acid methyl ester (FAM) was obtained together with a mixture of methyl glycosides and long-chain base (LCB). A gas chromatography−mass spectrometry (GC-MS) analysis of the FAM mixture shows the existence of three components (FAM 1−3) which are characterized as methyl 2-hydroxydocosanoate, methyl 2-hydroxytricosanoate, methyl 2-hydroxytetracosanoate. The major FAM is methyl 2-hydroxydocosanoate. On the other hand, by means of the GC−MS analysis of the TMS derivative of the LCB mixture, the LCB components are suggested to be 2-amino-1,3,4-pentadecantriol (LCB-1), 2-amino-1,3,4-hexadecantriol (LCB-2), 2-amino-1,3,4-heptadecanetriol (LCB-3), 2-amino-1,3,4-octadecanetriol (LCB-4) and 2-amino-1,3,4-nonadecanetriol (LCB-5). LCB-5 is the major long-chain base.

The stereochemistry of the ceramide moiety was determined as follows. When **1** was heated in water, compound **3** was obtained as the major product (Scheme 1).[5] The

[+] Parts 37 and 1: Ref.[3]

[a] Faculty of Pharmaceutical Sciences, Kyushu University, Maidashi 3-1-1, Higashi-ku, Fukuoka, 812-8582, Japan
Fax: (internat.) + 81-92/642-6639
E-mail: higuchi@shokka.phar.kyushu-u.ac.jp
[b] Department of Industrial Chemistry, Faculty of Engineering, Towa University, Chikushigaoka 1-1-1, Minami-ku, Fukuoka, 815-0036, Japan

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Table 1. ^{13}C-NMR chemical shifts (δ values) of **1**, **3** and synthetic lactosyl ceramide **4**

	C	**1**[a]	**3**[b]	**4**[c]
ceramide	1	70.0 (t)	69.9 (t)	70.3 (t)
	2	51.4 (d)	-[f]	51.6 (d)
	3	75.6 (d)	74.9 (d)	75.8 (d)
	4	72.5 (d)	72.3 (d)	72.6 (d)
	1'	175.8 (s)	175.8 (s)	175.6 (s)
	2'	72.3 (d)	72.3 (d)	72.6 (d)
	$-CH_3$[d]	14.5 (q)	14.3 (q)	14.3 (q)
	$-CH_3$[e]	11.7 (q)		
	$-CH_3$[e]	19.5 (q)		
Glc	1	105.0 (d)	104.7 (d)	105.0 (d)
	2	74.3 (d)	74.3 (d)	74.6 (d)
	3	76.3 (d)	76.4 (d)	76.5 (d)*
	4	81.3 (d)	81.3 (d)	81.7 (d)
	5	76.6 (d)	77.2 (d)	77.2 (d)*
	6	61.5 (t)*	61.9 (t)	62.1 (t)**
Gal	1	105.3 (d)	105.5 (d)	105.8 (d)
	2	70.2 (d)	72.2 (d)	72.4 (d)
	3	77.5 (d)	75.1 (d)	75.2 (d)
	4	68.7 (d)	69.9 (d)	70.1 (d)
	5	76.1 (d)	76.2 (d)	76.6 (d)
	6	61.9 (t)*	61.9 (t)	62.0 (t)**
NeuAc	1	174.6 (s)**		
	2	101.0 (s)		
	3	42.4 (t)		
	4	68.4 (d)		
	5	54.2 (d)		
	6	75.1 (d)		
	7	69.7 (d)		
	8	72.8 (d)		
	9	63.9 (t)		
	10	174.0 (s)**		
	11	22.7 (q)		

[a,b] Measured in C_5D_5N/D_2O (6:1)[a] or (98:2)[b]. Signal assignments are based on the 2D-NMR (HSQC) spectrum. – [c] Data from ref.[6] – [d] Terminal methyl group in *normal* type of side chain. – [e] Terminal methyl group in *ante-iso* type of side chain (minor component). – [f] The signals were not obtained. – *,** Assignments may be interchanged in each vertical column.

^{13}C-NMR spectrum of **3** 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*)-, (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.[6] It was found that the signals in the ^{13}C-NMR spectrum of **3** due to the sugar moiety and the core part of the ceramide moiety (C-1, C-2, C-3, C-4, C-1′ and C-2′) correspond well with those of (2*S*,3*S*,4*R*,2′*R*) isomer **4** (Table 1). The above fact and the optical rotations of **3** (+8.4) and **4** (+8.0) suggest that **3** has the same absolute configuration as **4** for the core structure (C-2, C-3, C-4, C-2′ and lactose). Therefore, the absolute configuration of the ceramide part of **1** must be 2*S*,3*S*,4*R*,2′*R* (Scheme 1).

The structure of the sugar moieties of **1** was determined as follows. The GLC analysis of hexitol acetate derivatives of the neutral sugars, which may be obtained by hydrolysis, reduction and acetylation of **1**, shows the existence of one mole each of glucose (Glc) and galactose (Gal). A detailed analysis of the ^{13}C-NMR spectrum of **1** reveals characteristic signals [δ_C = 174.6 (s) (C-1), 101.0 (s) (C-2), 42.4 (t) (C-3), 54.2 (d) (C-5), 63.9 (t) (C-9), 174.0 (s) (C-10) and 22.7 (q) (C-11)] due to an *N*-acetylneuraminic acid (NeuAc) derivative residue (Table 1). In the negative FAB MS of **1**, the molecular ion and fragment ion peaks arising from cleavage of the glycosidic linkage of the major component are observed at *m/z* 1269, 978, 816 and 654. These peaks are typical for the linear trisaccharide moiety NeuAc-*O*-Hexose-*O*-Hexose, as shown in Scheme 2.

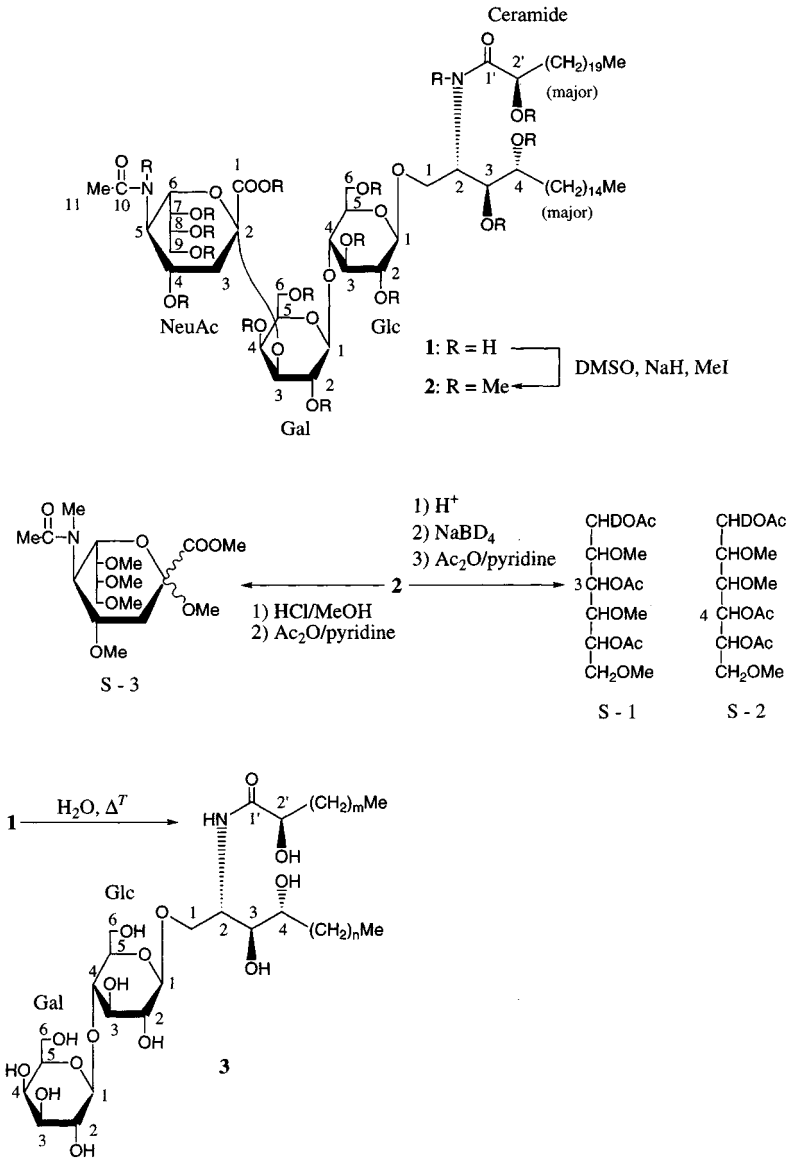
Methylation of **1** according to the Hakomori method[7] afforded the permethylated product **2**. Partially methylated alditol acetates (S-1 and S-2, Scheme 1) prepared from **2** were analyzed by GC-MS and identified as the alditol derived from 3-linked hexopyranose (1 mol) and 4-linked hexopyranose (1 mol). Compound **2** was subsequently methanolyzed and acetylated. Only the permethylated *N*-acetylneuraminate methyl glycoside (S-3, Scheme 1), which is derived from terminal NeuAc, was detected as a sialic acid derivative by means of its GC-MS that exhibits characteristic fragment ion peaks at *m/z* 129, 201, 274 and 348. The above data and the lactosyl ceramide **3** suggest that the sugar moiety of **1** is linear NeuAc [2→3]-galactopyranose-[1→4]-glucopyranose (Scheme 1).

The assignment of the structure of the sugar moiety is also supported by the following facts. A comparison of the proton and carbon signals of the sugar moiety of **1** and lactosyl ceramide (**3**) shows that the signals of the two compounds are the same except for those of NeuAc and C-3 in Gal, which are shifted (δ_C = 75.1 → 77.5) by glycosylation[8] (Table 1). The configurations of both hexopyranose in **1** are β on the basis of the large coupling constant (7.8 Hz) of anomeric proton signal in the ^{1}H-NMR spectrum of **3**. The configuration of NeuAc is considered to be α on the basis of the chemical shift value of its anomeric carbon (δ_C = 101.0) and equatorial 3-H signal (δ_H = 3.45).[9]

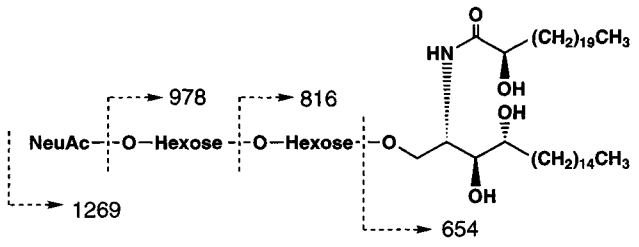
Therefore, if NeuAc is assumed to belong to the most commonly found D series, then LMG-2 (**1**) is the *N*-acetyl-α-D-neurosaminosyl-(2→3)-*O*-β-D-galactopyranosyl-(1→4)-β-D-glucopyranoside of a ceramide composed of heterogeneous phytosphingosine and 2-hydroxy fatty acid units. The major components of the fatty acid and long-chain base moiety of **1** are (2*R*)-2-hydroxydocosanoic acid and (2*S*, 3*S*, 4*R*)-2-amino-1,3,4-nonadecanetriol, respectively (Scheme 1).

LMG-2 (**1**) is the second GM_3-type ganglioside, after a ganglioside of *Lethasterias fuska*[10] as starfish ganglioside. However, **1** differs from the ganglioside in the components of the ceramide part.

The effects of the ganglioside molecular species **1** on the neuritogenesis of a rat pheochromocytoma cell line (PC-12 cell) have been investigated qualitatively. The results show that **1** displays neuritogenic activity relative to H_2O (control) in a concentration of 10 μg/mL.



Scheme 1



Scheme 2. Negative FAB mass fragmentation of the major component of **1**

Experimental Section

General: Melting points: Micromelting point apparatus (Yanaco MP-3), uncorrected values. − Optical rotations: Jasco Dip-307 digital polarimeter at 27°C. − 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), Varian Unity-600 (600 MHz and 150 MHz, 2D-NMR spectrum). − Negative FAB mass spectra: Jeol SX/SX102A tandem mass spectrometer (xenon atom beam, 5 kV; ion-source accelerating potential, 10 kV; matrix, HMPA/TEG). − GLC: Shimadzu GC-14B by employing FID [capillary column, J & W SCIENTIFIC Fused Silica Capillary Column DB-17 (∅ 0.317 mm × 30 m)]. − GC-MS: Shimadzu QP-1000 by employing the EI mode [ionizing potential of 70 eV; separator and ion-source temperature of 250°C; capillary column, Shimadzu CBP-10-W12−100 (∅ 0.53 mm × 12 m); carrier gas, He (30 mL /min).]

Separation of LMG-2 (1): Whole bodies of the starfish *Luidia maculata* (wet weight 40 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 AcOEt/*n*BuOH (2:1,

40 L) for separation of less polar lipids. The aqueous layer was further extracted with aqueous saturated *n*BuOH (54 L) to remove saponins, and the aqueous layer was concentrated in vacuo to give an aqueous solution (20 L). The aqueous solution was added to MeOH (9 L) and separated by chromatography on Cosmosil 140C18-PREP (reversed-phase) (solvent 60% and 90% and 100% MeOH) to give three fractions. The crude glycosphingolipid fraction (5.19 g), the 90% MeOH elute, were separated by chromatography on silica gel [$CHCl_3$/MeOH/H_2O (6:4:0.5)] to afford compound **1** (40.0 mg) which showed a single spot on silica-gel TLC [$CHCl_3$/MeOH/H_2O (6:4:1)], R_f = 0.36.

LMG-2 (1): Amorphous powder, m.p. 155−158°C. − IR (KBr): $\tilde{\nu}$ = 3389 cm^{-1} (−OH), 1637 cm^{-1} (amide). − Negative FAB MS; *m/z*: 1255, 1269, 1283, 1297, 1311 $[M - H]^-$, 978, 816, 657, 630, 468, 308, 290 (fragment ions of major component). − ^1H NMR [C_5D_5N/D_2O (6:1)]: δ_H = 0.83 (m, terminal methyl), 2.05 (s, 3 H, CH_3CO of NeuAc), 2.23 (t, *J* = 11.9, 1 H, 3-H_{ax} of NeuAc), 3.45 (m, 1 H, 3-H_{eq} of NeuAc), 3.64 (m, 1 H, 5-H of Glc), 3.76 (t, 1 H, *J* = 9.0, 2-H of Glc), 4.30 (t, *J* = 9.9, 1 H, 5-H of NeuAc), 4.36 (m, 1 H, 4-H of NeuAc), 4.56 (s, 1 H, 4-H of Gal). − ^{13}C NMR: See Table 1.

Methanolysis of 1: Compound **1** (2 mg) was heated with 5% HCl in MeOH (1 mL) at 70°C for 4 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.

GC-MS Analysis of FAM from 1: The FAM mixture from **1** was subjected to GC-MS [column temp. 200−250°C (rate of temp. increase 3°C/min)]. The results were as follows: FAM-1 (methyl 2-hydroxydocosanoate), t_R [min] (ratio of peak areas) = 12.3 (52), *m/z*: 370 $[M]^+$, 311 $[M - 59]^+$; FAM-2 (methyl 2-hydroxytricosanoate), t_R [min] = 13.7 (38), *m/z*: 384 $[M]^+$, 325 $[M - 59]^+$; FAM-3 (methyl 2-hydroxytetracosanoate), t_R [min] = 15.8 (10), *m/z*: 398 $[M]^+$, 339 $[M - 59]^+$.

GLC Analysis of Alditol Acetates from 1: Compound **1** (1 mg) was heated with 2 N HCl (1 mL) at 90°C for 22 h in a sealed small-volume vial. The reaction mixture was extracted with *n*-hexane and the aqueous layer concentrated in vacuo. The residue was dissolved in H_2O, then 28% NH_3 (two drops) and $NaBH_4$ (40 mg) were added to the solution. After standing at room temp. for 5 h, the reaction mixture was acidified with AcOH to pH = 3.5 and concentrated in vacuo. H_3BO_3 contained in the residue was removed by distillation with MeOH (three times). The residue was heated with Ac_2O/C_5H_5N (1:1, 0.5 mL) at 70°C for 2 h, and the mixture was concentrated in vacuo. The residue was extracted with $CHCl_3$ (1 mL), the $CHCl_3$ solution was washed with H_2O, dried ($NaSO_4$), and the solvent evaporated to give alditol acetates. These acetates were subjected to GLC [column temp. 200°C constant]. The results were as follows: t_R [min] = 20.6 (1 mol) [1,2,3,4,5,6-hexa-*O*-acetyl galactitol]; t_R [min] = 21.3 (1 mol) [1,2,3,4,5,6-hexa-*O*-acetyl glucitol].

GC-MS Analysis of TMS Ethers of LCB from 1: Compound **1** (1 mg) was heated with 5% HCl/MeOH (1 mL) at 70°C for 24 h in a sealed small-volume vial. The reaction mixture was washed with *n*-hexane to remove FAM, the MeOH layer neutralized with Ag_2CO_3 and filtered, and the filtrate was concentrated in vacuo. The residue (mixture of LCB and methyl glycosides) was heated with 1-(trimethylsilyl)imidazol/pyridine (1:1, 0.1 mL) for 7 min at 70°C and the reaction mixture (trimethylsilyl ethers) analyzed by GC-MS [column temp. 180−250°C (rate of temp. increase 4°C/min)]. The results were as follows: 1,3,4-tri-*O*-trimethylsilyl-2-amino-1,3,4-pentadecanetriol (LCB-1), t_R [min] (ratio of peak area) = 4.5 (12), *m/z*: 298 $[M - 193]^+$, 257 $[M - 234]^+$, 132; 1,3,4-tri-*O*-trimethylsilyl-2-amino-1,3,4-hexadecanetriol (LCB-2), t_R [min] = 5.6 (14), *m/z*: 312 $[M - 193]^+$, 271 $[M - 234]^+$, 132; 1,3,4-tri-*O*-trimethylsilyl-2-amino-1,3,4-heptadecanetriol (LCB-3), t_R [min] = 6.5 (23), *m/z*: 326 $[M - 193]^+$, 285 $[M - 234]^+$, 132; 1,3,4-tri-*O*-trimethylsilyl-2-amino-1,3,4-octadecanetriol (LCB-4), t_R [min] = 7.8 (14), *m/z*: 340 $[M - 193]^+$, 299 $[M - 234]^+$, 132; 1,3,4-tri-*O*-trimethylsilyl-2-amino-1,3,4-nonadecanetriol (LCB-5), t_R [min] = 9.3 (28), *m/z*: 354 $[M - 193]^+$, 313 $[M - 234]^+$, 132.

Methylation of 1 (Hakomori Method): Compound **1** (5 mg) was treated with NaH (40 mg) and MeI (1 mL) in DMSO (2 mL) according to the Hakomori method. The reaction mixture was diluted with H_2O, extracted with $CHCl_3$, and the $CHCl_3$ layer was washed with H_2O, dried (Na_2SO_4), and the solvent evaporated in vacuo. The residue was separated by chromatography on silica gel [*n*-hexane/acetone (2:1)] to give **2** (2 mg).

Preparation and GC-MS Analysis of Partially Methylated Alditol Acetates from 2: Compound **2** (1 mg) was heated with 90% HCOOH/10% CF_3COOH (1:1) (1 mL) at 90°C for 22 h in a sealed small-volume vial, and the mixture was concentrated in vacuo. The residue was dissolved in H_2O (5 mL), then 28% NH_3 aq. (two drops) and $NaBD_4$ (20 mg) were added to the solution. After standing at room temp. for a few hours, the reaction mixture was acidified with AcOH to pH = 3.5 and concentrated in vacuo. H_3BO_3 contained in the residue was removed by distillation with MeOH (four times). The residue was heated with Ac_2O/C_5H_5N (1:1) (0.5 mL) at 70°C for 2 h, and the mixture was concentrated in vacuo. The residue was extracted with $CHCl_3$ (1 mL), and the $CHCl_3$ solution was washed with H_2O, dried (Na_2SO_4), and the solvent evaporated to give partially methylated alditol acetates. These acetates were subjected to GC-MS [column temp. 150°C constant]. The results were as follows: S-1, t_R [min] = 18.5 (1 mol), *m/z*: 45, 118, 161, 234 [1,3,5-tri-*O*-acetyl-2,4,6-tri-*O*-methylhexitol (derived from 3-linked hexopyranose)]; S-2, t_R [min] = 19.5 (1 mol), *m/z*: 45, 118, 233 [1,4,5-tri-*O*-acetyl-2,3,6-tri-*O*-methylhexitol (derived from 4-linked hexopyranose)].

Preparation and GC-MS Analysis of S-3 from 2: Compound **2** (1 mg) was heated with 5% HCl/MeOH (1 mL) at 70°C for 4 h in a sealed small-volume vial. The reaction mixture was concentrated in vacuo, and the residue (methanolysate) was heated with Ac_2O/C_5H_5N (1:1) (1 mL) at 70°C for 2 h, then the mixture was concentrated in vacuo. The residue was subjected to GC-MS [column temp. 180−250°C (rate of temp. increase 8°C/min)]: S-3 t_R [min] = 4.6, *m/z*: 129, 201, 254, 274, 298, 318, 348, 362, 376 [methyl *N*-acetyl-*N*-methyl-2,4,7,8,9-tetra-*O*-methyl neuraminate (from terminal NeuAc)].

Partial Hydrolysis of 1: Compound **1** (10 mg) was heated with H_2O (1.5 mL) at 100°C for 8 h in a sealed small-volume vial. The reaction mixture was extracted with AcOEt/BuOH (2:1) (1 mL, three times), the combined organic layers were concentrated in vacuo, and the residue was separated by chromatography on silica gel with $CHCl_3$/MeOH/H_2O (8:2:0.2) as solvent to give **3** (1.4 mg), R_f = 0.23 [$CHCl_3$/MeOH/H_2O (8:2:0.2)].

Compound 3: Amorphous powder. − $[\alpha]_D$ = +8.4 [*c* = 0.13 in $CHCl_3$/MeOH (1:1)]; compound **3** was identified as synthetic ceramide lactoside **4**[6] {ref.[6]: $[\alpha]_D$ = +8.0 [*c* = 0.2 in $CHCl_3$/MeOH (1:1)]} by direct comparison. − ^1H NMR [C_5D_5N/D_2O (98:2)]: δ_H = 0.81 (m, 6 H, terminal methyl), 3.72 (m, 1 H, 5-H of Glc), 3.92 (t, *J* = 8.0, 1 H, 2-H of Glc), 4.06 (m, 1 H, 5-H of Gal), 4.08 (m, 1 H, 3-H of Gal), 4.13 (t, *J* = 8.7, 1 H, 3-H of Glc), 4.15 (m, 1 H, 4-H of ceramide), 4.17 (t, *J* = 8.7, 1 H, 4-H of Glc), 4.27 (dd, *J* = 4.6, 10.4, 1 H, 3-H of ceramide), 4.28 (d, *J* = 4.6, 2 H, 6-H of

Gal), 4.34 (d, J = 12.0, 1 H, 6-H_a of Glc), 4.37 (m, 1 H, 1-H_a of ceramide), 4.39 (d, J = 12.0, 1 H, 6-H_b of Glc), 4.40 (s, 1 H, 4-H of Gal), 4.43 (d, J = 9.3, 1 H, 2-H of Gal), 4.54 (dd, J = 4.2, 7.8, 1 H, 2′-H of ceramide), 4.65 (dd, J = 6.0, 10.2, 1 H, 1-H_a of ceramide), 4.81 (d, J = 7.8, 1 H, 1-H of Glc), 4.99 (d, J = 7.8, 1 H, 1-H of Gal), 5.17 (m, 1 H, 2-H of ceramide), 8.50 (d, J = 9.0, 1 H, NH of ceramide). − ^{13}C NMR: See Table 1.

Observation of Neuritogenic Activity on PC-12 Cells: PC-12 cells (RIKEN CELL BANK) were cultured at a density of 1×10^5 cells in 1 mL of medium in Dulbecco′s Modified Eagle′s Medium (DMEM) supplemented with 10% fetal calf serum and 10% horse serum in a collagen-coated 96-well plate (IWAKI) under a humidified atmosphere of 5% CO_2 in air at 37°C. After 24 h of culture, the growth medium was replaced by serum-free DMEM/Ham′s F12 (1:1) medium supplemented with N-2 Supplement (GIBCO). LMG-2 (**1**) was added to the medium at densities of 100, 10, 1, and 0.1 μg mL^{-1}, and the cells were further cultured at 37°C. After 2 d, the morphological change in cells were observed with microscope. Cells treated with 10 μg mL^{-1} of **1** showed neurite outgrowth whereas those with H_2O (control) did not.

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[3] S. Kawatake, M. Inagaki, R. Isobe, T. Miyamoto, R. Higuchi, *Liebigs Ann.* **1997**, 1797−1800.
[4] *Normal* means straight chain (−$CH_2CH_2CH_2CH_3$), *ante-iso* means branched chain possessing a methyl group on the third carbon atom from the terminal methyl group [−$CH_2CH(CH_3)CH_2CH_3$].
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