Constituents of Ophiuroidea. 1. Isolation and Structure of Three Ganglioside Molecular Species from the Brittle Star *Ophiocoma scolopendrina*

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Three ganglioside molecular species, OSG-0 (1), OSG-1 (2), and OSG-2 (3) have been obtained from the polar lipid fraction of the chloroform/methanol extract of the brittle star *Ophiocoma scolopendrina*. The structures of these gangliosides have been determined on the basis of chemical and spectroscopic evidence as 1-*O*-[(*N*-glycolyl-α-D-neuraminosyl)-(2→6)-β-D-glucopyranosyl]-ceramide (1), 1-*O*-[8-*O*-sulfo-(*N*-acetyl-α-D-neuraminosyl)-(2→6)-β-D-glucopyranosyl]-ceramide (2) and 1-*O*-[(*N*-glycolyl-α-D-neuraminosyl)-(2→8)-(*N*-acetyl- and *N*-glycolyl-α-D-neuraminosyl)-(2→6)-β-D-glucopyranosyl]-ceramide (3). The ceramide moieties were composed of heterogeneous unsubstituted fatty acid, 2-hydroxy fatty acid and phytosphingosine units. Compounds 2 and 3 represent new ganglioside molecular species.

Key words glycosphingolipid; ganglioside; brittle star; *Ophiocoma scolopendrina*

In the course of our continuing research on biologically active glycosphingolipids (GSLs) from echinoderms, a series of studies on the isolation and structure elucidation of the GSLs from starfish, sea cucumber and feather star species have been performed in our laboratory.[1)] In continuation of the previous studies, the isolation and characterization of the biologically active GSLs from the brittle star *Ophiocoma scolopendrina* (Udefurikumohitode in Japanese) has now been carried out in order to develop the novel medicinal resources from natural marine products. In this paper, we report the isolation and characterization of three ganglioside molecular species from the whole bodies of *O. scolopendrina.*

The polar lipid fraction, which was obtained from the chloroform/methanol extract of the whole bodies of *O. scolopendrina*, was subjected to repeated column chromatography to give three ganglioside molecular species, OSG-0 (**1**), OSG-1 (**2**), and OSG-2 (**3**), each showing a single spot on silica gel thin-layer chromatography (TLC).

In its ^{13}C-NMR spectrum (Chart 1, Table 1), **1** exhibits the characteristic signals of a phytosphingosine-type ceramide, possessing an unsubstituted fatty acid and a sugar moiety at C-1 [δ: 70.5 (C-1), 53.8 (C-2), 76.0 (C-3), 72.3 (C-4), 176.2 (C-1′), 36.8 (C-2′)]. The ^{13}C-NMR spectrum of **1** also features signals due to two anomeric carbons at δ: 105.4 and 100.7, one of which (δ: 100.7) is a quaternary carbon signal, indicating the presence of a sialic acid residue. The negative FAB-MS exhibits a series of quasi-molecular ion peaks $[M-H]^-$ at *m/z*: 1000—1100. Therefore, **1** is suggested to be a molecular species of a phytosphingosine-type ganglioside, possessing unsubstituted fatty acid groups and two monosaccharide units. Furthermore, **1** is presumed to have mainly normal-type fatty acids and normal and iso-type long-chain bases (LCB), since the carbon signals for the terminal methyl groups are observed at δ: 14.2 (normal form) and δ: 22.9 (iso form)[1a)] in the ^{13}C-NMR spectrum (Chart 1, Table 1).

The structure of the ceramide moiety was examined first. When **1** was methanolyzed with methanolic hydrochloric acid, a mixture of fatty acid methyl esters (FAM) and LCB was obtained, together with methyl glucopyranoside. The FAM mixture was analyzed by GC-MS, which revealed the presence of five components. These were characterized as methyl octadecanoate (major), methyl eicosanoate, methyl docosanoate, methyl tricosanoate, and methyl tetracosenoate. The LCB mixture was found to be composed of 2-amino-1,3,4-trihydroxy-heptadecane and -octadecane (major), based on GC-MS analysis of its trimethylsilyl (TMS) derivative (Chart 1).

The relative stereochemistry of the phytosphingosine of ceramide moiety is presumed to be (2*S*,3*S*,4*R*), since the aforementioned ^{13}C-NMR signals assignable to C-1, 2, 3, and 4 of **1** are in good agreement with those of the phytosphingosine-type ganglioside molecular species possessing (2*S*,3*S*,4*R*) configurations.[1e)]

The structure of the disaccharide moiety of **1** was established as follows. The presence of glucose (Glc) was obvious from the results of the methanolysis of **1** (*vide supra*). A detailed analysis of the ^{13}C-NMR spectrum of **1** revealed the characteristic signals [δ: 175.6 (C-1), 100.7 (C-2), 42.6 (C-3), 53.8 (C-5), 64.4 (C-9), 176.2 (C-10), 62.4 (C-11)] of an *N*-glycolylneuraminic acid (NeuGc) derivative residue coupled with a β-glucopyranose derivative residue (Table 1). In the negative FAB-MS of **1**, molecular ion and fragment ion peaks arising from cleavage of the glycosidic linkages are observed at *m/z*: 1000—1100, 720—750, and 550—650, indicating the presence of the disaccharide moiety, NeuGc→Hexose(β-glucopyranose), as shown in Fig. 1.

Methylation of **1**, according to Ciucanu–Kerek method,[2)] afforded the permethylated product **4**. Partially methylated alditol acetate (S-1), prepared from **4**, was analyzed by GC-MS and identified as the alditol derived from 6-linked hexopyranose. On the other hand, upon methanolysis followed by acetylation, the permethylated NeuGc (S-2) derived from the terminal NeuGc was detected by GC-MS analysis. On the basis of the above evidence, the disaccharide moiety of **1** must be NeuGc-(2→6)-β-glucopyranose. The configuration of NeuGc is believed to be α on the basis of its anomeric

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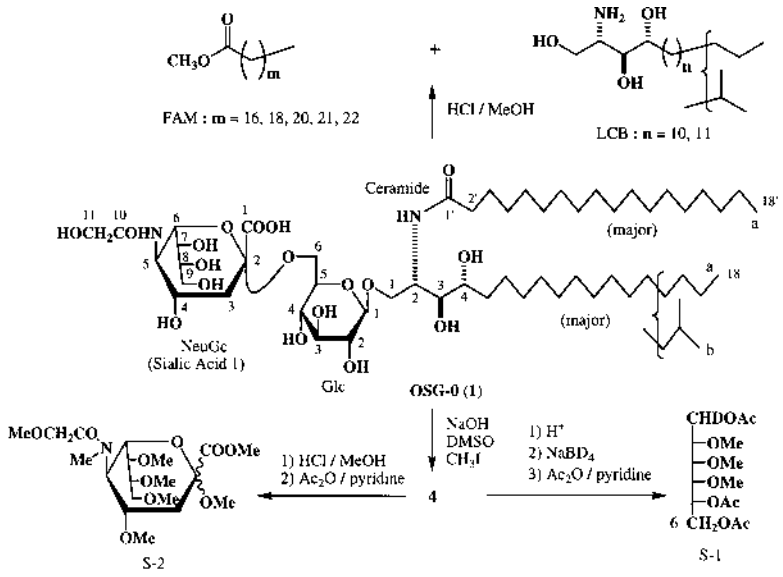

Chart 1

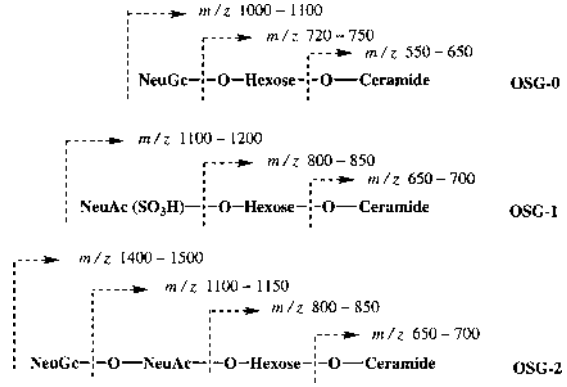

Fig. 1. The Negative FAB-MS Fragmentation of OSG-0, OSG-1, and OSG-2

carbon signal (δ: 100.7)[1c,3] in the ^{13}C-NMR spectrum of **1**. In addition, the absolute configuration of the glucose unit was verified as being of D-form by the Hara method.[4]

Consequently, if NeuGc and phytosphingosine are assumed to belong to the most commonly found D-series and 2*S*,3*S*,4*R* type, respectively, then **1** is the (*N*-glycolyl-α-D-neuraminosyl)-(2→6)-β-D-glucopyranoside of a ceramide, composed of heterogeneous (2*S*,3*S*,4*R*)-phytosphingosine and unsubstituted fatty acid units. The major components of the fatty acid and phytosphingosine moiety of **1** are octadecanoic acid and 2-amino-1,3,4-trihydroxyoctadecane, respectively (Chart 1).

Compound **2** exhibits the characteristic signals of a phytosphingosine-type ceramide, possessing a 2-hydroxy fatty acid and a sugar moiety at C-1 [δ: 70.3 (C-1), 51.3 (C-2), 76.0 (C-3), 72.2 (C-4), 175.7 (C-1′), 72.2 (C-2′)] in its ^{13}C-NMR spectrum (Chart 2, Table 1). The ^{13}C-NMR spectrum of **2** also shows signals due to two anomeric carbons at δ: 105.0 and 101.0, one of which (δ: 101.0) is a quaternary carbon signal, indicating the presence of a sialic acid residue. The negative FAB-MS exhibits a series of quasi-molecular ion peaks $[M-H]^-$ at *m*/*z*: 1100—1200, and the fragment ion peaks due to $[SO_4H]^-$ and $[SO_3]^-$ at *m*/*z*: 97 and 80.

Table 1. ^{13}C-NMR Spectral Data (δ Values) of the Gangliosides in C_5D_5N

C		OSG-0	OSG-1	OSG-2	C		OSG-0	OSG-1	OSG-2
Ceramide					Sialic acid 2				
1	(t)	70.5	70.3	70.5	1	(s)			176.1[d]
2	(d)	53.8	51.3	51.3	2	(s)			101.2
3	(d)	76.0	76.0	75.7	3	(t)			42.0
4	(d)	72.3	72.2	72.0	4	(d)			68.5
1′	(s)	176.2	175.7	176.1	5	(d)			53.2
2′	(t, d)	36.8	72.2	72.4	6	(d)			75.7
CH_3[a]	(q)	14.2	14.1	14.0	7	(d)			70.6
CH_3[b]	(q)	22.9	22.8[e]	22.6	8	(d)			73.0
Glc					9	(t)			64.0
1	(d)	105.4	105.0	104.6	10	(s)			176.0[d]
2	(d)	74.9	74.5	74.3	11	(t)			62.0[f]
3	(d)	76.6	77.1	77.2					
4	(d)	70.5	70.6	70.6					
5	(d)	75.5	75.5	75.7					
6	(t)	70.5	69.0	70.0					
Sialic acid 1									
1	(s)	175.6	175.7	176.1[d]					
2	(s)	100.7	101.0	101.2					
3	(t)	42.6	42.0	42.0					
4	(d)	68.5	68.3	68.5[e]					
5	(d)	53.8	54.3	53.2					
6	(d)	75.0	73.4	75.7					
7	(d)	70.5	69.3	68.4[e]					
8	(d)	73.0	80.4	83.3					
9	(t)	64.4	62.0	61.9[f]					
10	(s)	176.2	175.7	176.0[d]					
11	(t, q)	62.4	22.6[c]	22.6					
11	(t)			62.0[f]					

a, *b*) Terminal methyl groups in the normal and iso type of side chain (see Chart 1). *c*—*f*) Assignments may be interchanged in each vertical column.

Therefore, **2** is suggested to be a molecular species of a sulfated phytosphingosine-type ganglioside, possessing 2-hydroxy fatty acid groups and two monosaccharide units. The terminal methyl groups of the ceramide moiety of **2** must be the same as that of **1** from their carbon atom signals (Table 1).

When **2** was methanolyzed with methanolic hydrochloric acid, a mixture of FAM and LCB was obtained, together with

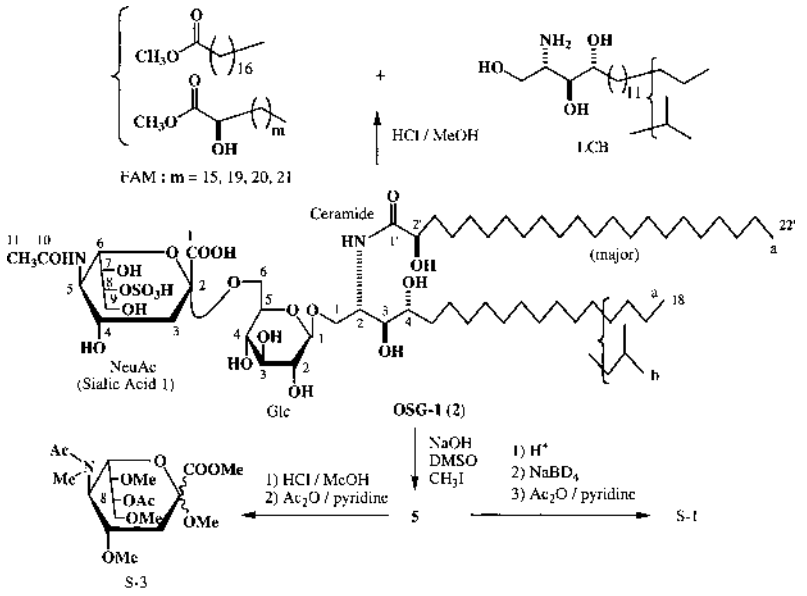

Chart 2

methyl glucopyranoside. The FAM mixture was analyzed by GC-MS, which revealed the presence of main five components. These were characterized as methyl octadecanoate, methyl 2-hydroxyoctadecanoate, methyl 2-hydroxydocosanoate (major), methyl 2-hydroxytricosanoate, and methyl 2-hydroxytetracosanoate. The LCB mixture was found to be composed of only 2-amino-1,3,4-trihydroxy-octadecane, based on GC-MS analysis of its TMS derivative (Chart 2). Furthermore, the relative stereochemistry of the ceramide moiety is presumed to be (2*S*,3*S*,4*R*,2′*R*), since the aforementioned ^{13}C-NMR signals ascribable to C-1, 2, 3, 4, 1′ and 2′ of **2** are in good agreement with those of the phytosphingosine-type ganglioside molecular species possessing (2*S*,3*S*,4*R*,2′*R*) configurations.[1c)]

The structure of the disaccharide moiety of **2** was elucidated as outlined below. The presence of Glc was obvious from the results of the methanolysis of this species and the absolute configuration (D-form) of the glucose unit was verified as before. In its ^{13}C-NMR spectrum (Table 1), **2** shows characteristic signals due to one mol each of *N*-acetylneuraminic acid (NeuAc) derivative and β-glucopyranose derivative residue. The negative FAB-MS of **2** shows the molecular and fragment ion peaks at *m/z*: 1100—1200, 800—850, and 650—700, corresponding to cleavage of the glycosidic linkages of **2**, thus indicating the disaccharide moiety, NeuAc-(SO_3H)→Hexose(β-glucopyranose), as shown in Fig. 1.

Partially methylated alditol acetate prepared from **5**, the permethylated **2**, was characterized as the alditol derived from 6-linked hexopyranose (S-1) by means of GC-MS. The acetate of partially methylated NeuAc (S-3) derived from 8-linked NeuAc was detected in the acetate of methanolysate prepared from **5**. These facts establish the structure of the disaccharide moiety as 8-*O*-sulfo-NeuAc-(2→6)-β-D-Glc (p).

The configuration of C-2 in the sialic acid (NeuAc) is also thought to be α, as in the case of **1**, based on its anomeric carbon signals (δ: 101.0) in the ^{13}C-NMR spectrum of **2**.

Consequently, if NeuAc and LCB are assumed to belong to the D-series and 2*S*,3*S*,4*R* type, **2** is the 8-*O*-sulfo-(*N*-acetyl-α-D-neuraminosyl)-(2→6)-β-D-glucopyranoside of a ceramide composed of (2*S*,3*S*,4*R*)-C_{18}-phytosphingosine and heterogeneous fatty acid units. The major component of the fatty acid is (2*R*)-2-hydroxydocosanoic acid units as shown in Chart 2.

In its ^{13}C-NMR spectrum, **3** exhibits characteristic signals attributable to the ceramide moiety, which correspond to those of **2** (Table 1). The ^{13}C-NMR spectrum of **3** also features signals due to three anomeric carbon atoms at δ: 104.6, 101.2 and 101.2, two of which (δ: 101.2) are quaternary carbon atom signals, indicating the presence of two sialic acid residues. The negative FAB-MS exhibits a series of quasi-molecular ion peaks $[M-H]^-$ at *m/z*: 1400—1500. Therefore, **3** is suggested to be a molecular species of ganglioside, like **2**, having three monosaccharide units. Since **3** gave the same FAM and LCB mixture as **2**, the major fatty acid and LCB of **3** must be (2*R*)-2-hydroxydocosanoic acid and (2*S*,3*S*,4*R*)-C_{18}-phytosphingosine, respectively.

The methanolysis and acidic hydrolysis of **3**, indicating the existence of D-Glc, together with the signals due to sugar moiety in the ^{13}C-NMR spectrum of **3** (Table 1), suggest that the sialosyl trisaccharide moiety of **3** is composed of one mol each of β-D-glucopyranose, α-NeuAc, and α-NeuGc. In its negative FAB-MS, **3** shows molecular (*m/z*: 1400—1500) and fragment ion peaks (*m/z*: 1100—1150, 800—850, 650—700) arising from cleavage of the glycosidic linkages of **3**, which are indicative of the linear trisaccharide moiety, NeuGc→NeuAc→Hexose, as shown in Fig. 1.

The GC-MS analysis of the partially methylated alditol acetates of the neutral sugars and of the acetates of partially methylated sialic acids, which were synthesized from **6**, the permethylated **3**, indicated the presence of 6-linked hexopyranose (S-1), terminal NeuGc (S-2), and 8-linked NeuAc (S-3) together with 8-linked NeuGc (S-4) as a minor component in the sugar moiety. On the basis of the above evidence, the sugar moiety of **3** is still heterogeneous for its inner sialic acid residue and therefore, the sialosyl trisaccharide moiety of **3** must be α-NeuGc-(2→8)-α-NeuAc and NeuGc-(2→6)-β-D-Glc (p). The major component of inner sialic acid is NeuAc.

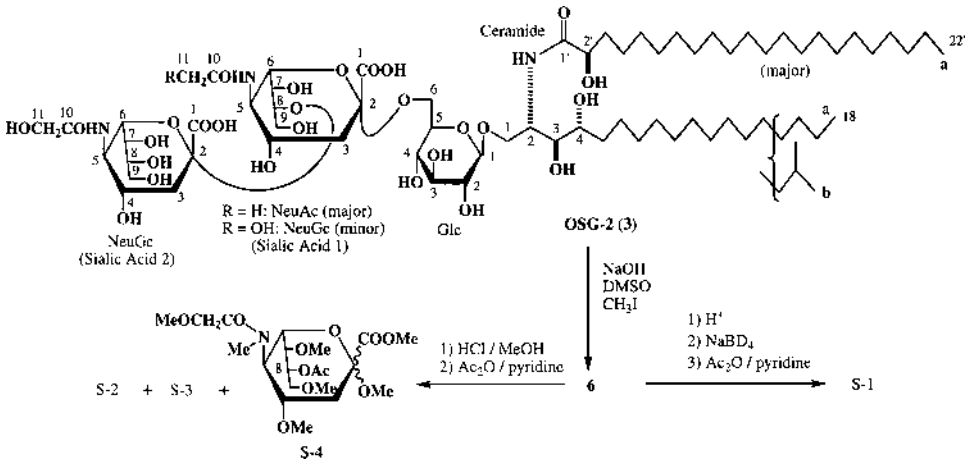

Chart 3

Accordingly, if NeuGc and NeuAc are assumed to belong to the D-series, **3** must be (*N*-glycolyl-α-D-neuraminosyl)-(2→8)-(*N*-acetyl- and *N*-glycolyl-α-D-neuraminosyl)-(2→6)-β-D-glucopyranoside of a ceramide composed of the same fatty acid and LCB units as **2**.

From the brittle star *Ophiura sarsi*,[5] *Ophiocoma echinata*[6] and *Ophiomastrix annulosa*,[6] five kinds of ganglioside molecular species have been obtained and characterized. However, the ganglioside molecular species isolated in this study, OSG-1 and OSG-2, are, to the best of our knowledge, new ganglioside molecular species. Although ganglioside molecular species possessing the same sugar and core of ceramide moieties as those of OSG-0 have been obtained from *Ophiocoma echinata*[6] and the sea cucumber *Stichopus japonicus*,[1e] OSG-0 slightly differs from them in the fatty acid and LCB components. The biological activity of these gangliosides will be examined.

Experimental

Melting points were determined on a micro melting point apparatus (Yanako MP-3) without correction. IR spectra were obtained on a Jasco FT/IR-410 IR spectrophotometer. NMR spectra were recorded on a Varian Unity-500 spectrometer (500 MHz). Negative-ion FAB-MS spectra were acquired with a JEOL SX-102 mass spectrometer (xenon atom beam; matrix, HMPA-TEG). GC-MS were taken with a Shimadzu QP-5050A [EI mode; ionizing potential, 70 eV; separator and ion-source temperature 250 °C; column, TC-1701 (0.25 mm×30 m, GL Science Inc.); carrier gas, He].

Separation of OSG-0 (1), OSG-1 (2) and OSG-2 (3) Whole bodies of the brittle star *Ophiocoma scolopendrina* (13 kg), which was collected at Cape Zanpa, Okinawa Prefecture, Japan in 1998, were chopped and extracted three times with $CHCl_3$/MeOH (1 : 2, 18 l). The combined extracts were concentrated *in vacuo* to give an aqueous solution (0.5 l). The suspension was diluted with H_2O (3 l) and extracted with AcOEt/*n*-BuOH (3 : 1, 3.4 l) for separation of less polar lipids. The aqueous phase was washed with *n*-BuOH saturated with H_2O (0.9 l), dialyzed, and freeze-dried to give the polar lipid fraction (35 g). This fraction was then chromatographed on Cosmosil 140 C_{18}-PREP (Nacalai Tesque) [reversed phase, eluent: 50%, 80%, 90% MeOH and $CHCl_3$/MeOH (1 : 1)] to give four fractions. The crude ganglioside fraction (6.7 g), the $CHCl_3$/MeOH eluate, was chromatographed on silica gel (solvent $CHCl_3$–MeOH–H_2O, 7 : 3 : 0.3) to give three fractions. Successive column chromatography of fraction 2 (silica gel, solvent $CHCl_3$–MeOH–H_2O, 7 : 3 : 0.2) afforded OSG-0 (**1**) (38 mg) (*Rf*=0.40) and OSG-1 (**2**) (49 mg) (*Rf*=0.33). Fraction 3 was further chromatographed on silica gel (solvent $CHCl_3$–MeOH–H_2O, 7 : 3 : 0.2 to 7 : 3 : 0.5) to afford OSG-2 (**3**) (57 mg) (*Rf*=0.28) [silica gel TLC, solvent $CHCl_3$–MeOH–H_2O (6 : 4 : 1)].

OSG-0 (**1**): Amorphous powder, mp 181—183 °C. IR (KBr) cm^{-1}: 3372 (OH), 1647, 1542 (amide). Negative-ion FAB-MS *m/z*: 1000—1100 $[M-H]^-$ series (see Fig. 1). ^{13}C-NMR: See Table 1.

OSG-1 (**2**): Amorphous powder, mp 223—228 °C. IR (KBr) cm^{-1}: 3378 (OH), 1648, 1541 (amide), 1230 (sulfate). Negative-ion FAB-MS *m/z*: 1100—1200 $[M-H]^-$ series (see Fig. 1). ^{13}C-NMR: See Table 1.

OSG-2 (**3**): Amorphous powder, mp 210—215 °C. IR (KBr) cm^{-1}: 3367 (OH), 1636, 1547 (amide). Negative-ion FAB-MS *m/z*: 1400—1500 $[M-H]^-$ series (see Fig. 1). ^{13}C-NMR: See Table 1.

Methanolysis of 1 Compound **1** (1 mg) was heated with 5% HCl in MeOH (1 ml) at 70 °C for 18 h. The reaction mixture was then extracted with *n*-hexane, and the extract was concentrated *in vacuo* to yield a mixture of FAM. The MeOH layer was neutralized with Ag_2CO_3, filtered, and the filtrate was concentrated *in vacuo* to give a mixture of LCB and methyl glycoside.

GC-MS Analysis of FAM from 1 A FAM mixture from **1** was subjected to GC-MS [column temp. 150—250 °C (rate of temp. increase 5 °C/min)]. The results were as follows: methyl octadecanoate (major), t_R [min]=16.6, *m/z*: 298 (M^+), 255 $(M-43)^+$; methyl eicosanoate, t_R=20.0, *m/z*: 326 (M^+), 283 $(M-43)^+$; methyl docosanoate, t_R=23.4, *m/z*: 354 (M^+), 311 $(M-43)^+$; methyl tricosanoate, t_R=25.5, *m/z*: 368 (M^+), 325 $(M-43)^+$; methyl tetracosanoate, t_R=28.0, *m/z*: 382 (M^+), 339 $(M-43)^+$.

GC-MS Analysis of TMS Ethers of LCB from 1 The mixture of LCB and methyl glycoside from **1** was heated with 1-(trimethylsilyl) imidazole–pyridine (1 : 1) for 20 min at 70 °C and the reaction mixture (TMS ethers) was analyzed by GC-MS [column temp. 180—250 °C (rate of temp. increase 5 °C/min)]. The results were as follows: 2-amino-1,3,4-trihydroxyheptadecane, t_R [min]=16.4, *m/z*: 326 $(M-193)^+$, 285 $(M-234)^+$, 132; 2-amino-1,3,4-trihydroxy-octadecane (major), t_R=18.0, *m/z*: 340 $(M-193)^+$, 299 $(M-234)^+$, 132.

GC-MS Analysis of TMS Ethers of Methyl Glycoside from 1 The mixture of TMS ethers of LCB and methyl glycoside was analyzed by GC-MS [column temp.: 150—200 °C (rate of temp. increase 2.5 °C/min), 200—250 °C (rate of temp. increase 10 °C/min)]: t_R [min]=15.5 and 16.1 (methyl glucopyranoside).

Determination of Absolute Configuration of Glucose Moiety of 1 (Hara Method[4]) Compound **1** (1 mg) was heated with 2 N HCl (1 ml) at 90 °C for 24 h. The reaction mixture was then extracted with *n*-hexane, and the acidic aqueous phase was concentrated. The residue (sugar fraction) was heated with L-cysteine methyl ester hydrochloride (0.3 mg) and pyridine (0.3 ml) at 70 °C for 1 h. Then, 0.1 ml of 1-(trimethylsilyl) imidazole was added and the mixture was heated at 60 °C for a further 0.5 h to yield trimethylsilyl ether of the methyl (4*R*)-thiazolidine-4-carboxylate derivative. The derivative was analyzed by GC-MS [column temp.: 180—250 °C (rate of temp. increase 2.5 °C/min)]; t_R [min]=23.7 (derivative of D-glucose, 23.7; L-glucose, 24.2).

Methylation of 1 (Ciucanu-Kerek Method)[2] NaOH–dimethylsulfoxide (DMSO) solution, which was prepared from powdered NaOH (40 mg) and DMSO (1 ml), and MeI (0.2 ml) were added to **1** (2 mg), and the mixture was stirred for 30 min. The reaction mixture was then diluted with H_2O (15 ml), extracted with $CHCl_3$ (10 ml×3), the $CHCl_3$ phases were washed with H_2O, and the solvent was evaporated *in vacuo* to give permethylated **1**, denoted **4** (1.8 mg).

Preparation and GC-MS Analysis of Partially Methylated Alditol Ac-

etate from 4 Compound **4** (0.8 mg) was heated with 90% HCOOH–10% CF_3COOH (1 : 1) (1 ml) at 70 °C for 18 h in a small-volume sealed vial, and then the solvents were evaporated *in vacuo.* The residue was dissolved in H_2O (5 ml), and 28% NH_3 (2 drops) and $NaBD_4$ (10 mg) were added. After allowing the mixture to stand at room temp. for 7 h, it was acidified with AcOH to pH=3.5 and concentrated *in vacuo.* H_3BO_3 present in the residue was removed by distillation with MeOH (three times). The residue was heated with Ac_2O–C_5H_5N (1 : 1, 0.3 ml) at 70 °C for 2 h. After dilution with H_2O, the mixture was extracted with $CHCl_3$ (0.2 ml×3). The combined $CHCl_3$ extracts were washed with H_2O, and the solvent was evaporated to give partially methylated alditol acetate. The acetate was subjected to GC-MS [column temp. 150—250 °C (rate of temp. increase 5 °C/min)]. The results were as follows: S-1, t_R [min]=15.2, *m/z*: 118, 162, 189, 233 [1,5,6-tri-*O*-acetyl-2,3,4-tri-*O*-methylhexitol (derived from 6-linked hexopyranose)].

Preparation and GC-MS Analysis of Acetate of Partially Methylated Sialic Acid from 4 Compound **4** (0.7 mg) was heated with 5% HCl in MeOH (0.5 ml) at 75 °C for 6 h in a small-volume sealed vial. The reaction mixture was then neutralized with Ag_2CO_3, filtered, and the filtrate was concentrated *in vacuo.* The residue (methanolysate) was heated with Ac_2O–C_5H_5N (1 : 1, 0.2 ml) at 70 °C for 2 h. The resulting mixture was diluted with H_2O and extracted with $CHCl_3$ (0.2 ml×3), the combined $CHCl_3$ extracts were washed with H_2O, and the solvent was evaporated *in vacuo.* The residue was subjected to GC-MS [column temp.: 200—250 °C (rate of temp. increase 2.5 °C/min)]: S-2, t_R [min]=23.2, *m/z*: 159, 348, 378 [methyl *N*-glycolyl-*N*-methyl-2,4,7,8,9,11-hexa-*O*-methylneuraminate (derived from terminal NeuGc)].

Methanolysis of 2 In the same manner as described for **1**, **2** was methanolyzed and the reaction mixture was worked up to give a mixture of FAM and a residue composed of LCB and methyl glycoside.

GC-MS Analysis of FAM from 2 A FAM mixture from **2** was subjected to GC-MS under the same conditions as described for the FAM mixture obtained from **1**. The results were as follows: methyl octadecanoate, t_R [min]=16.6, *m/z*: 298 (M^+), 255 $(M-43)^+$; methyl 2-hydroxyoctadecanoate, t_R=19.7, *m/z*: 314 (M^+), 255 $(M-59)^+$; methyl 2-hydroxydocosanoate (major), t_R=27.5, *m/z*: 370 (M^+), 311 $(M-59)^+$; methyl 2-hydroxytricosanoate, t_R=30.7, *m/z*: 384 (M^+), 325 $(M-59)^+$; methyl 2-hydroxytetracosanoate, t_R=34.5, *m/z*: 398 (M^+), 339 $(M-59)^+$.

GC-MS Analyses of TMS Ethers of LCB and Methyl Glycoside from 2 The residue (mixture of LCB and methyl glycoside) from **2** was trimethylsilylated and the reaction mixture was analyzed by GC-MS in the same manner as described for **1**. 2-Amino-1,3,4-trihydroxy-octadecane and methyl glucopyranoside were detected.

Determination of Absolute Configuration of Glucose Moiety of 2 Compound **2** (1 mg) was subjected to acid hydrolysis and the sugar fraction was treated in the same manner as described for **1**, thereby affording the TMS ethers of the methyl thiazolidine-4(*R*)-carboxylate derivatives. The derivative was analyzed by GC-MS under the same conditions as before, and D-glucose was detected.

Preparation of 5 and Partially Methylated Alditol Acetates from 5 Compound **2** (2 mg) was methylated according to the Ciucanu–Kerek method and the reaction mixture was worked up in the same manner as described for **1**, thereby yielding permethylated **2**, denoted **5** (1.7 mg). Compound **5** (0.5 mg) was hydrolyzed, reduced, and then acetylated, and the partially methylated alditol acetate was analyzed by GC-MS in the same manner as described for **4**, whereupon S-1 derived from 6-linked hexopyranose was detected.

Preparation and GC-MS Analysis of Acetate of Partially Methylated Sialic Acid from 5 Compound **5** (0.5 mg) was methanolyzed and then acetylated in the same manner as described for **4**. The acetates were subjected to GC-MS under the same conditions as mentioned above, and S-3, t_R [min]=22.6, *m/z*: 129, 201, 254, 318, 376 [methyl *N*-acetyl-8-*O*-acetyl-*N*-methyl-2,4,7,9-tetra-*O*-methylneuraminate (derived from 8-linked NeuAc)], was detected.

Analyses of FAM, LCB and Methyl Glycosides from 3 Experiments were conducted in the same manner as in the case of **1**, leading to a mixture of FAM and a residue composed of LCB and methyl glycosides derived from **3**. The FAM mixture was subjected to GC-MS under the same conditions as described for **1**, and methyl octadecanoate, methyl 2-hydroxyoctadecanoate, methyl 2-hydroxydocosanoate (major), methyl 2-hydroxytricosanoate, and methyl 2-hydroxytetracosanoate were detected. The mixture of LCB and methyl glycoside was trimethylsilylated and analyzed by GC-MS as in the case of **1**. 2-Amino-1,3,4-trihydroxy-octadecane and methyl glucopyranoside were detected.

Determination of Absolute Configuration of the Glucose Moieties of 3 In the same manner as described for **1**, **3** was hydrolyzed, the sugar derivatives were analyzed by GC-MS, and D-glucose was detected.

Preparation of 6 and Partially Methylated Alditol Acetates from 6 The partially methylated alditol acetate was obtained from **6** (prepared from **3** as above) and analyzed by GC-MS in the same way as for those from **4**. S-1 (derived from 6-linked hexopyranose) was detected.

Preparation and GC-MS Analysis of Acetates of Partially Methylated Sialic Acids from 6 The acetates were prepared from **6** and subjected to GC-MS as described for **4**. S-2 (derived from terminal NeuGc), S-3 (derived from 8-linked NeuAc) and a minor component S-4, t_R [min]=27.2, *m/z*: 159, 348, 356, 406 [methyl *N*-glycolyl-8-*O*-acetyl-*N*-methyl-2,4,7,9,11-penta-*O*-methylneuraminate (derived from 8-linked NeuGc)], were detected.

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