Constituents of Holothuroidea. 10.1) Isolation and Structure of a Biologically Active Ganglioside Molecular Species from the Sea Cucumber *Holothuria leucospilota*

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Three ganglioside molecular species, HLG-1 (1), HLG-2 (2), and HLG-3 (3) have been obtained from the lipid fraction of the chloroform/methanol extract of the sea cucumber *Holothuria leucospilota*. The structures of these gangliosides have been determined, on the basis of chemical and spectroscopic evidence, as 1-O-[(\(N\)-acyetyl-\(\alpha\)-D-neuraminosyl)-(2\(\rightarrow\)6)-\(\beta\)-D-glucopyranosyl]-ceramide (1), 1-O-[(\(N\)-glycolyl-\(\alpha\)-D-neuraminosyl)-(2\(\rightarrow\)4)-(\(N\)-acetyl-\(\alpha\)-D-neuraminosyl)]-(2\(\rightarrow\)6)-\(\beta\)-D-glucopyranosyl]-ceramide (2) and 1-O-[(\(\alpha\)-l-fucopyranosyl)-(1\(\rightarrow\)11)-(\(N\)-glycolyl-\(\alpha\)-D-neuraminosyl)-(2\(\rightarrow\)4)-(\(N\)-acetyl-\(\alpha\)-D-neuraminosyl)]-(2\(\rightarrow\)6)-\(\beta\)-D-glucopyranosyl]-ceramide (3). The ceramide moieties were composed of heterogeneous phytosphingosine, sphingosine and 2-hydroxy fatty acid units. Compounds 2 and 3 represent new ganglioside molecular species. These three ganglioside molecular species showed neuritogenic activity toward the rat pheochromocytoma cell line, PC-12 cell, in the presence of NGF (nerve growth factor).

Key words glycosphingolipid; ganglioside; sea cucumber; *Holothuria leucospilota*; neuritogenic activity

In our continuing research on biologically active glycosphingolipids (GSLs) from echinoderms, a series of studies on the isolation and structure elucidation of the GSLs from sea cucumber species have been performed in our laboratory. In continuation of the preceding studies on the sea cucumber *Holothuria pervicax*, the isolation and characterization of the biologically active GSLs from the sea cucumber *Holothuria leucospilota* (Nisekuronamako in Japanese) has now been carried out in order to develop the novel medicinal resources from natural marine products. In this paper, we report on the isolation and characterization of three ganglioside molecular species from the whole bodies of *H. leucospilota*. The biological activities of the gangliosides are also reported.

The polar lipid fraction, which was obtained from the chloroform/methanol extract of the whole bodies of *H. leucospilota*, was subjected to repeated silica gel column chromatography to give three ganglioside molecular species, HLG-1 (1), HLG-2 (2), and HLG-3 (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 a 2-hydroxy fatty acid and a sugar moiety at C-1 \([\delta: 70.1 (C-1), 51.0 (C-2), 75.8 (C-3), 72.0 (C-4), 175.8 (C-1′), 72.0 (C-2′)]\). The \(^{13}\)C-NMR spectrum of 1 also features signals due to two anomeric carbons at \(\delta: 104.4\) and 101.2, one of which (\(\delta: 101.2\)) 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 \(m/z: 1080–1180, 800–850, and 630–700\), in agreement with those of the phytosphingosine-type glucocerebroside molecular species possessing \((2,3,5,4,2'R)\) configurations. 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 \([\delta: 173.5 (C-1), 101.2 (C-2), 42.4 (C-3), 53.4 (C-5), 63.7 (C-9), 176.2 (C-10), 61.9 (C-11)]\) of an N-glycolylneuraminic acid (NeuGc) derivative residue coupled with a \(\beta\)-glucopyranosyl derivative residue (Table 1). In the negative FAB-MS of 1, the molecular ion and fragment ions arise from cleavage of the glycosidic linkages are observed at \(m/z: 1080–1180, 800–850, and 630–700\), indicating the presence of the disaccharide moiety, NeuGc\(\rightarrow\)Hexose(\(\beta\)-glucopyranose), as shown in Fig 1.

Methylation of 1, according to Ciucanu–Kerek method, 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, 4 was methanolyzed, the methanolsate was acetylated, and the permethylated NeuGc (S-2) derived from the terminal NeuGc was detected by means of GC-MS analysis. On the basis of the above evi-

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The disaccharide moiety of 1 must be NeuGc-(2→6)-\(\beta\)-glucopyranose. The configuration of NeuGc is believed to be \(\alpha\) on the basis of its anomeric carbon signal (\(\delta\): 101.2)\(^4\) in the \(^{13}\)C-NMR spectrum of 1. In addition, the absolute configuration of the glucose unit was verified as being of \(\alpha\)-form by means of the Hara method.\(^5\)

Consequently, if NeuGc is assumed to belong to the most commonly found \(\alpha\)-series, then 1 is the (N-glycolyl-\(\alpha\)-D-neuraminosyl)-(2→6)-\(\beta\)-D-glucopyranoside of a ceramide, composed of heterogeneous (2S,3S,4R)-phytosphingosine and (2R)-2-hydroxy fatty acid units, as shown in Chart 1.

Compound 2 exhibits the characteristic signals due to the sphingosine-type ceramide, possessing a 2-hydroxy fatty acid and a sugar moiety at C-1 [\(\delta\): 70.2 (C-1), 54.3 (C-2), 72.5 (C-3), 131.6 (C-4), 133.0 (C-5), 175.8 (C-1') in its \(^{13}\)C-NMR spectrum (Chart 2, Table 1). The \(^{13}\)C-NMR spectrum of 2 also shows three anomeric carbon signals at \(\delta\): 105.3, 101.0, and 99.7, two of which (\(\delta\): 101.0, 99.7) are quaternary carbon signals derived from two sialic acid moieties (Table 1). In the negative FAB-MS, 2 shows a series of quasi-molecular ion peaks [M–H]\(^+\) at \(m/z\): 1380—1480. Accordingly, 2 is suggested to be a molecular species of a sphingosine-type ganglioside, possessing 2-hydroxy fatty acid groups and three 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).
from 6-linked hexopyranose (S-1) by means of GC-MS. The permethylated species possessing (2 of ages of 600—700, corresponding to cleavage of the glycosidic link-
NeuGc together with those of a and shows characteristic signals due to one mole each of NeuGc and the absolute configuration (D-form) of the glucose unit obvious from the results of the methanolysis of this species peaks at negative FAB-MS of posed of one mole each of Glc, NeuGc, and NeuAc. The FAM mixture was analyzed by means of GC-MS and four components corresponding with those ob-
methyl glucopyranoside. The FAM mixture was analyzed by GC-MS analysis of its TMS derivative

These data suggest that the trisaccharide moiety of Ceramide NeuGc a—d): Terminal methyl groups in the normal, iso, and ante-iso type of side chain (see Chart 1). e, f): Assignments may be interchanged in each vertical column.

Table 1. 13C-NMR Spectral Data (δ values) of the Gangliosides in C12D2N–D2O (98:2)

<table>
<thead>
<tr>
<th>Ceramide</th>
<th>HLG-1</th>
<th>HLG-2</th>
<th>HLG-3</th>
<th>C</th>
<th>HLG-1</th>
<th>HLG-2</th>
<th>HLG-3</th>
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</thead>
<tbody>
<tr>
<td>1 (t)</td>
<td>70.1</td>
<td>70.2</td>
<td>70.3</td>
<td>1 (s)</td>
<td>173.5</td>
<td>173.8</td>
<td>174.0</td>
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<tr>
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<td>51.0</td>
<td>54.3</td>
<td>50.9</td>
<td>2 (s)</td>
<td>101.2</td>
<td>99.7</td>
<td>100.5</td>
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<tr>
<td>3 (d)</td>
<td>75.8</td>
<td>72.5</td>
<td>75.6</td>
<td>3 (t)</td>
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<td>4 (d)</td>
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<td>72.0</td>
<td>4 (d)</td>
<td>68.4</td>
<td>68.7</td>
<td>68.5</td>
</tr>
<tr>
<td>5 (d)</td>
<td></td>
<td>133.0</td>
<td></td>
<td>5 (d)</td>
<td>53.4</td>
<td>53.9</td>
<td>53.0</td>
</tr>
<tr>
<td>1' (s)</td>
<td>175.8</td>
<td>175.8</td>
<td>176.0</td>
<td>6 (d)</td>
<td>73.8</td>
<td>74.8</td>
<td>74.0</td>
</tr>
<tr>
<td>2' (d)</td>
<td>72.0</td>
<td>72.5</td>
<td>72.6</td>
<td>7 (d)</td>
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<td>14.3</td>
<td>14.3</td>
<td>8 (d)</td>
<td>72.4</td>
<td>72.5</td>
<td>72.0</td>
</tr>
<tr>
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<tr>
<td>CH₃(endo) (q)</td>
<td>11.3</td>
<td>11.6</td>
<td>11.6</td>
<td>10 (s)</td>
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<td>174.0</td>
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<tr>
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<td>19.4</td>
<td>11 (t)</td>
<td>61.9</td>
<td>62.6</td>
<td>64.0</td>
</tr>
</tbody>
</table>

Glc
| 1 (d)    | 104.4 | 105.3 | 105.3 | 1 (d) | 101.9 |
| 2 (d)    | 74.2  | 74.8  | 74.8  | 2 (d) | 69.5  |
| 3 (d)    | 76.9  | 77.7  | 77.4  | 3 (d) | 70.8  |
| 4 (d)    | 70.4  | 72.5  | 70.7  | 4 (d) | 72.4  |
| 5 (d)    | 75.8  | 76.4  | 76.3  | 5 (d) | 67.5  |
| 6 (t)    | 70.1  | 70.6  | 70.0  | 6 (q) | 17.1  |

NeuAc
| 1 (s)    | 173.8 |       |       |       |       |
| 2 (s)    | 101.0 |       |       |       |       |
| 3 (t)    | 39.3  |       |       |       |       |
| 4 (d)    | 74.3  |       |       |       |       |
| 5 (d)    | 51.8  |       |       |       |       |
| 6 (d)    | 74.8  | 75.3  |       |       |       |
| 7 (d)    | 70.6  |       |       |       |       |
| 8 (d)    | 73.0  |       |       |       |       |
| 9 (t)    | 64.0  |       |       |       |       |
| 10 (s)   | 176.2 |       |       |       |       |
| 11 (q)   | 22.8  |       |       |       |       |

Methanolysis of 2 afforded a mixture of FAM, LCB and methyl glucopyranoside. The FAM mixture was analyzed by GC-MS and four components corresponding with those obtained from 1 were detected. On the other hand, the LCB component was found to be 2-amino-1,3-dihydroxy-4-heptadecene by means of GC-MS analysis of its TMS derivative (Chart 2). Furthermore, the relative stereochemistry of the ceramide moiety is presumed to be (2S, 3R, 4E, 2'R) by comparison of the 13C-NMR signals due to C-1, 2, 3, 4, 5 and 2' of 2 and a sphingosine-type glucocerebrosidase molecular species possessing (2S, 3R, 4E, 2'R) configurations.

The structure of the trisaccharide moiety of 2 was elucidated as outlined below. The presence of glucose (Glc) was obvious from the results of the methanolysis of this species and the absolute configuration (α-form) of the glucose unit was verified as before. In its 13C-NMR spectrum (Table 1), 2 shows characteristic signals due to one mole each of NeuGc and N-acetylenuraminic acid (NeuAc) derivative residues, together with those of a β-glucopyranose derivative residue. These data suggest that the trisaccharide moiety of 2 is composed of one mole each of Glc, NeuGc, and NeuAc. The negative FAB-MS of 2 shows the molecular and fragment ion peaks at m/z: 1380—1480, 1050—1150, 750—850, and 600—700, corresponding to cleavage of the glycosidic linkages of 2, thus indicating the linear trisaccharide moiety, NeuGc→NeuAc→Hexose, 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 4-linked NeuAc and of permethylated NeuGc (S-2), derived from terminal NeuGc, were detected in the acetate of methanolysate prepared from 5. These facts establish the structure of the trisaccharide moiety as NeuGc-(2→4)-NeuAc-(2→6)-β-D-Glc (p).

The configurations of the sialic acids (NeuGc, NeuAc) are also thought to be α, as in the case of 1, based on their anomeric carbon signals (δ: 99.7, 101.0) in the 13C-NMR spectrum of 2.

Consequently, if NeuGc and NeuAc are assumed to belong to the α-series, then 2 is the (N-glycolyl-α-D-neuraminosyl)-(2→4)-(N-acetyl-α-D-neuraminosyl)-(2→6)-β-D-glucopyranoside of a ceramide composed of (2S,3R,4E)-C17-sphingosine and heterogeneous (2R)-2-hydroxy fatty acid units as shown in Chart 2.

In its 13C-NMR spectrum, 3 exhibits characteristic signals attributable to the ceramide moiety, which correspond to those of 1 (Table 1). The 13C-NMR spectrum of 3 also features signals due to four anomeric carbon atoms at δ: 105.3, 101.9, 100.5, and 100.5, two of which (δ: 100.5) 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: 1500—1600. Therefore, 3 is suggested to be a molecular species of ganglioside, like 1, having four monosaccharide units. Since 3 gave the same FAM and LCB mixture as those derived from 1, the components of the fatty acid and long-chain base moieties of 3 must be (2R)-2-hydroxy fatty acids and (2S,3S,4R)-
phytosphingosines as for 1.

The methanolysis and acidic hydrolysis of 3, indicating the existence of D-Glc and L-fucose (Fuc), together with the signals due to sugar moiety in the $^{13}$C-NMR spectrum of 3 (Table 1), suggest that the sialosyl tetrasaccharide moiety of 3 is composed of one mole each of $\beta$-D-glucopyranose, $\alpha$-L-fucopyranose, $\alpha$-NeuAc, and $\alpha$-NeuGc. In its negative FAB-MS, 3 shows molecular ($m/z$: 1500—1600) and fragment ion peaks ($m/z$: 1400—1480, 1050—1180, 800—850, 630—700) arising from cleavage of the glycosidic linkages of 3, which are indicative of the linear tetrasaccharide moiety, deoxyhexose$\rightarrow$NeuGc$\rightarrow$NeuAc$\rightarrow$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 terminal 6-deoxyhexopyranosyl (S-4), 6-linked hexopyranosyl (S-1), 4-linked NeuAc (S-3), and 11-linked NeuGc (S-5) in the sugar moiety. On the basis of the above evidence, the sialyl tetrasaccharide moiety of 3 must be $\alpha L$-Fuc (p)-$(1 \rightarrow 11)-\alpha$-NeuGc-$(2 \rightarrow 4)-\alpha$-NeuAc-$(2 \rightarrow 6)-\beta$-D-Glc (p).

Accordingly, if NeuGc and NeuAc are assumed to belong to the $d$-series, 3 must be $\alpha L$-lucopyranosyl-$l$-$(1 \rightarrow 11)$-$l$-glucopyranosyl-$l$-$(2 \rightarrow 4)$-$l$-acetyl-$d$-neuraminosyl-$l$-$(2 \rightarrow 6)$-$\beta$-D-glucopyranoside of a ceramide composed of the same fatty acid and long-chain base units as 1.

The effects of the isolated ganglioside molecular species on the neuritogenes of the rat pheochromocytoma cell line (PC-12 cells) have been investigated. The results show that the three ganglioside molecular species, 1, 2, and 3, display neuritogenic activity, compared with $H_2O$ (control), at a concentration of 10 $\mu$g in the presence of NGF (5 ng/ml).

From the sea cucumber *Cucumaria japonica*, Holothuria *atra*, *Telenota ananas*, *Cucumaria echinata*, and *Stichopus japonicus*, nine kinds of ganglioside molecular species have been obtained and characterized. However, the ganglioside molecular species isolated in this study, HLG-2 and HLG-3, are, to the best of our knowledge, new gangliosides. Although a ganglioside possessing the same sugar and core of ceramide moieties as that of HLG-1 has been obtained from the eggs of the sea urchin *Anthocidaris crassispina*, HLG-1 slightly differs from the ganglioside in the structure of its fatty acyl and LCB components. The isolation and characterization of such neuritogenically active gangliosides is attracting considerable attention with regard to the manufacturing of new medicines from natural marine products.

**Experimental**

Melting points were determined on a micro melting point apparatus (Yanako MP-3) without correction. NMR spectra were recorded on a Varian Unity-500 spectrometer (500 MHz). Negative-iron 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-1000 (EI mode; ionizing potential, 70 eV; separator and ion-source temperature 250°C; column, CBP10-W12-100 (0.53 mm × 12 m, Shimadzu); carrier gas, He). GC were run on a Shimadzu GC-14B (FID mode; column, Fused Silica Capillary Column DB-17 (0.317 mm × 30 m, J & W Scientific); carrier, N2).

**Separation of HLG-1 (1), HLG-2 (2) and HLG-3 (3)**

**Whole bodies of the sea cucumber Holothuria leucospilota** (170 kg), which was collected at Ushibuka, Kumamoto Prefecture, Japan, in 1997, were chopped and extracted three times with CHC3–MeOH (1 : 2, 55 l). The combined extracts were concentrated in vacuo to give a residue (352 g). The residue was dissolved in ace- tolene and concentrated to give a residue (22 mg) (2). The residue was then neutralized with Ag2CO3, filtered, and the filtrate was concentrated in vacuo to give a mixture of LCB and methyl glyco-side. The combined mixture was then neutralized with Ag2CO3, filtered, and the filtrate was concentrated in vacuo to give a mixture of LCB and methyl glycoside.

**GC-MS Analysis of TMS Ethers of LCB from 1**

The mixture of LCB and methyl glycoside from 1 was heated with 3-(trimethylsilyl)imidazole-pyridine (1 : 1) for 10 min at 60°C and the reaction mixture (TMS ethers) was analyzed by GC-MS (column temp. 180–250°C (rate of temp. increase 4°C/min)). The results were as follows: methyl 2-hydroxycosoatane, $t_R$ [min]= 7.5, $m/z$: 370 (M+), 311 (M−59); methyl 2-hydroxytricosanoate, $t_R$ = 9.0, $m/z$: 384 (M+), 325 (M−59); methyl 2-hydroxytricosanoate, $t_R$ = 9.9, $m/z$: 396 (M+), 337 (M−59); methyl 2-hydroxytricosanoate, $t_R$ = 10.6, $m/z$: 398 (M+), 339 (M−59).

**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 (column temp.: 100–250°C (rate of temp. increase 5°C/min)); $t_R$ [min]=17.9 and 18.1 (methyl $\alpha$- and $\beta$-glucopyranoside).

**Determination of Absolute Configuration of Glucose Moiety of 1 (Hara Method)**

(1) (0.5 mg) was heated with 4 $\times$ H2SO4 (0.3 ml) at 100°C for 8 h. The reaction mixture was then extracted with 3-n-hexane, and the acid aqueous phase was neutralized with Ba(OH)2, centrifuged, and the clear supernatant solution was concentrated. The residue (sugar fraction) was heated with L-cysteine methyl ester hydrochloride (0.3 mg) and pyridine (0.3 ml) at 60°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 (4R)-thiazolidine-4-carboxylate derivative. The derivative was analyzed by GC (column temp.: 200–250°C (rate of temp. increase 2.5°C/min); $t_R$ = 13.3 min (derivative of L-glucose, 13.3 min; L-galactose, 14.0 min).

**Methylation of 1 (Ciucanu–Kerek Method)**

NaOH–dimethylsulfoxide (DMSO) solution, which was prepared from powdered NaOH (40 mg) and DMSO (1 ml), and MeCl (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 CHCl3 (10 ml × 3), and the CHCl3 phases were washed with $H_2O$ and the solvent was evaporated in vacuo to give perme-porphicated 1, denoted 4 (4.9 mg).

**Preparation and GC-MS Analysis of Partially Methylated Alditol Acetate from 4**

Compound 4 (0.7 mg) was heated with 90% HCOOH–10% CF3COOH (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% NH3 (2 drops), and NaBD4 (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. The residue was removed by distillation with MeOH (three times). The residue was heated with $AcO$–C2H4 (1 : 1, 0.3 ml) at 70°C for 2 h. After dilution with $H_2O$, the mixture was extracted with CHCl3 (0.2 ml × 3). The combined CHCl3 extracts were washed with $H_2O$, and the solvent was evaporated in vacuo to give perme-porphicated 1, denoted 4 (4.9 mg).

**Preparation and GC-MS Analysis of Acetate of Partially Methylated Sialic Acid from 6**

Compound 6 (0.4 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 Ag2CO3, filtered, and the filtrate was concentrated in vacuo. The residue (methylaryl) was heated with $AcO$–C2H4 (1 : 1, 0.2 ml) at 70°C for 2 h. The resulting mixture was diluted with $H_2O$ and extracted with CHCl3 (0.2 ml × 3), the combined CHCl3 extracts were washed with $H_2O$, and the solvent was evaporated in vacuo. The residue was subjected to GC-MS (column temp.: 180–250°C (rate of temp. increase 4°C/min)); $S_2$, $t_R$ = 7.2 min, $m/z$: 159, 348, 378 (methyl N-glycolyl-N-methyl-2,4,7,8,9,11-hexa-O-methylneuraminic acid (derived from terminal NeuGc)).

**Methylation 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. Methyl 2-hydroxydocosanoate, methyl 2-hydroxytricosanoate, methyl 2-hydroxytetraicosanoate, and methyl 2-hydroxytetraicosanoate were detected.

**GC-MS and GC 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.

**Preparation of 5 and Partially Methylated Alditol Acetates from 5** Compound 2 (0.5 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 under the same conditions as before, and R-glucose was detected.

**Preparation of 6 and Partially Methylated Alditol Acetates from 6** Compound 2 (1.1 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 (1.2 mg). Compound 5 (0.4 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 hexopyranosate, was detected.

**Preparation and GC-MS Analysis of Acetates of Partially Methylated Sialic Acids from 3** Compound 5 (0.5 mg) was methanolyzed and then acetylated in the same manner as described for 3. The acetates were subjected to GC-MS under the same conditions as mentioned above, and S-2 (derived from terminal NeuGc) and S-3, 1, m/z: 326 (M−103)+, 297 (M−132)+, 236 (M−193)+, 132. Methyl glycoside (GC): methyl α- and β-glucopyranoside were detected.

**Determination of Absolute Configuration of Glucose Moiety of 2** Compound 2 (0.5 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 under the same conditions as before, and R-glucose was detected.

**Preparation of 6 and Partially Methylated Alditol Acetates from 6** The partially methylated alditol acetates were 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 hexopyranosate) and S-4, 16.9 min, m/z: 187, 201, 376, 406 [methyl N-glucopyranosyl-11-O-acetyl-N-methyl-2,4,7,8,9-penta-O-methyluronic acid (derived from 11-linked NeuGc)], were detected.

**Biological Assay** Neuritogenic activity of 1, 2, and 3 on PC-12 cells was observed according to the method previously reported. Cells treated with 10 μM of each of three gangliosides with NGF (5 ng/ml) showed neurite outgrowth compared with those treated with H2O (control).

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**References**