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Preliminary communication

A facile, systematic synthesis of ganglio-series gangliosides: Total synthesis of gangliosides GM_1 and GD_{1a} *

Akira Hasegawa, Takao Nagahama and Makoto Kiso
Department of Applied Bioorganic Chemistry, Gifu University, Gifu 501-11 (Japan)

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Mammals, birds, amphibians, and teleost fish all have similar brain gangliosides. These gangliosides are of the ganglio-series with predominantly, gangliotetraose, β-D-Gal-(1 → 3)-β-D-GalNAc-(1 → 4)-β-D-Gal-(1 → 4)-D-Glc, as the neutral oligosaccharide, though with a varying degree of sialylation. Since their discovery, the gangliosides have for several reasons elicited much interest. The concentration of complex gangliosides in membrane elements of the brain is suggestive of a functional role in the nervous system. Recently, the biological importance of gangliosides in the nervous system has been well documented[2–7]. As biologically derived gangliosides are polymolphous molecules and available in only limited quantity, the elucidation of their functions at the molecular level still remains to the carried out. We have reported[8] the successful syntheses of several series of gangliosides and their analogues, which are based on a facile dimethyl(methylthio)-sulfonium triflate[8,9] (DMTST) or *N*-iodosuccinimide (NIS)–trifluoromethane-sulfonic acid[9,10]. (TfOH) promoted, stereoselective α-glycosylation of sialic acid with suitably protected sugar residues in an acetonitrile medium. We describe herein a facile, total synthesis of gangliosides GM_1 and GD_{1a} in connection with development of the systematic synthesis of the ganglio-series of gangliosides. Ganglioside GM_1 was first synthesized by Ogawa and co-workers[11] after multiple steps in very low yield. For the synthesis of the both gangliosides, the core oligosaccharide **4** was selected as the glycosyl acceptor. Compound **4** has a sialyl α-(2 → 3) unit already linked and provides free hydroxyl groups at C-3 and C-4 of the GalNAc residue for further, regioselective glycosylation at C-3, with either methyl 2,4,6-tri-*O*-benzoyl-3-*O*-benzyl-1-thio-β-D-galactopyranoside (**5**) or methyl *O*-(methyl 5-acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosylonate)-(2 → 3)-2,4,6-tri-*O*-benzoyl-1-thio-β-D-galactopyranoside[12]

Correspondence to: Professor A. Hasegawa, Department of Applied Bioorganic Chemistry, Gifu University, Gifu 501-11, Japan.

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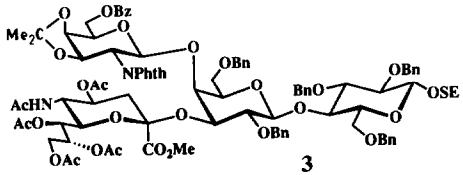

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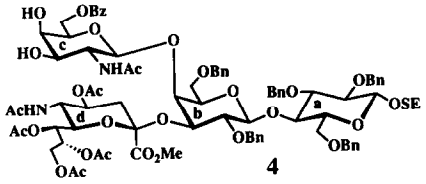

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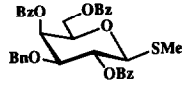

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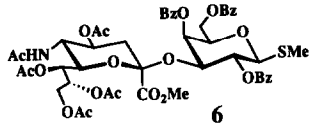

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(**6**) as the glycosyl donor. The glycosyl acceptor **4** was prepared from a known tetrasaccharide derivative **3** (ref. 13), an intermediate prepared in the total synthesis of ganglioside GM_2.

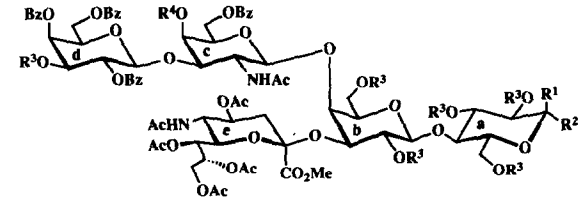
β-D-Gal-(1 → 3)-β-D-GalNAc-(1 → 4)-β-D-Gal-(1 → 4)-β-D-Glc-(1 → 1)-Cer
3
↑
3
α-D-Neu5Ac

1: GM_1

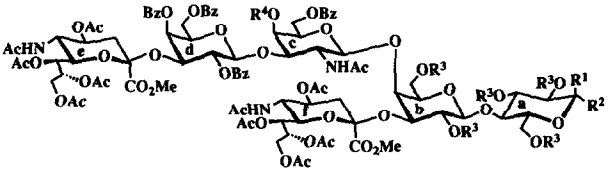
α-D-Neu5Ac-(2 → 3)-β-D-Gal-(1 → 3)-β-D-GalNAc-(1 → 4)-β-D-Gal-(1 → 4)-β-D-Glc-(1 → 1)Cer
3
↑
2
α-D-Neu5Ac

2: GD_{1a}

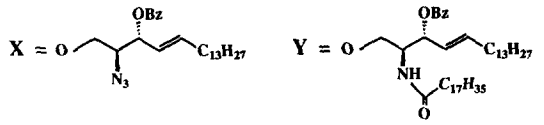
Treatment[11,14] of **3** with lithium iodide in pyridine for 6 h under reflux to remove the methyl ester group gave the free carboxyl derivative of **3** in 95% yield, which was converted via sequential treatment with hydrazine monohydrate, *N*-acetylation, and *O*-deisopropylidenation with aqueous acetic acid into 2-(trimethylsilyl)ethyl *O*-(2-acetamido-6-*O*-benzoyl-2-deoxy-β-D-galactopyranosyl)-(1 → 4)-*O*-[(methyl 5-acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosylonate)-(2 → 3)]-*O*-(2,6-di-*O*-benzyl-β-D-galactopyranosyl)-(1 → 4)-2,3,6-tri-*O*-benzyl-β-D-glucopyranoside (**4**) {$[\alpha]_D$ −1.8°, ($CHCl_3$)} in 88% yield. The glycosyl donor **5** was prepared from 2-(trimethylsilyl)ethyl 3-*O*-benzyl-β-D-galactopyranoside[15] via the sequence of *O*-benzoylation, conversion[16] of the 2-(trimethylsilyl)ethyl group with the acetoxy group, and replacement of the anomeric



	R^1	R^2	R^3	R^4
7	H	OSE	Bn	H
8	H	OSE	Ac	Ac
9	H, OH		Ac	Ac
10	$OC(=NH)CCl_3$	H	Ac	Ac
11	H	X	Ac	Ac
12	H	Y	Ac	Ac



	R^1	R^2	R^3	R^4
13	H	OSE	Bn	H
14	H	OSE	Ac	Ac
15	H, OH		Ac	Ac
16	$OC(=NH)CCl_3$	H	Ac	Ac
17	H	X	Ac	Ac
18	H	Y	Ac	Ac



acetoxy group with the methylthio group by use of (methylthio)trimethylsilane in the presence of trimethylsilyl trifluoromethanesulfonate. Glycosylation[10] of **4** with **5** in dichloromethane for 26 h at room temperature in the presence of NIS–TfOH and powdered 4A molecular sieves (MS-4A) gave a 66% yield of the desired β-glycoside **7** {$[\alpha]_D$ +37.5° ($CHCl_3$)}. Catalytic hydrogenolysis (10% Pd–C) in ethanol–acetic acid of the benzyl groups in **7** and subsequent *O*-acetylation gave **8** in 78% yield. Significant signals in the ^1H NMR spectrum of **8** were at δ 5.04 (d, $J_{1,2}$ 7, 9 Hz, H-1d) and at δ 5.70 (d, $J_{3,4}$ 3.3 Hz, H-4c), which showed the newly formed glycosidic configuration to be β and the linkage position of **5** to be C-3 of GalNAc residue in **4**.

On the other hand, glycosylation of **4** with **6** in dichloromethane for 25 h at room temperature in the presence of DMTST (4.0 equiv relative to the donor) and MS-4A, gave the expected hexasaccharide **13** {$[\alpha]_D$ +29.2° ($CHCl_3$)} in 62% yield, which was converted via catalytic hydrogenolysis of the benzyl groups and subse-

quent *O*-acetylation into the oligosaccharide **14** {$[\alpha]_D$ +8.1° ($CHCl_3$)} of GD_{1a} in 90% yield. NIS–TfOH-promoted glycosylation of **4** and **6**, however, did not give any product. The ^1H NMR spectrum of **14** showed the presence of seventeen, three-proton singlets at δ 1.37–2.20 (3 $NCOCH_3$ and 14 $OCOCH_3$), two three-proton singlets at 3.63 and 3.80 (2 OCH_3), a one-proton doublet at δ 5.64 ($J_{3,4}$ 3.1 Hz) due to H-4c, and multiples at δ 7.26–8.21 due to twenty aromatic protons, indicating the assigned structure.

Treatment[8a,17] of compounds **8** or **14** with trifluoroacetic acid in dichloromethane for 2 h at room temperature gave the corresponding 1-hydroxy compounds **9** and **15** in quantitative and 90% yields, respectively. Treatment[18] of **9** or **15** with trichloroacetonitrile in dichloromethane in the presence of 1,8-diazabicyclo[5.4.0]-undec-7-ene (DBU) for 4 h at 0° gave the corresponding α-trichloroacetimidates **10** {$[\alpha]_D$ +19.5° ($CHCl_3$)} and **16** {$[\alpha]_D$ +23.5° ($CHCl_3$)} in quantitative and 85% yields, respectively. Significant signals in the ^1H NMR spectra were at δ 6.55 ($J_{1,2}$ 3.5 Hz, H-1a) and 8.73 (C=NH) for **10**, and at δ 6.47 ($J_{1,2}$ 4.0 Hz, H-1a) and 8.64 (C=NH) for **16**, which showed the imidate to be α.

Glycosylation of (2*S*,3*R*,4*E*)-2-azido-3-*O*-benzoyl-4-octadecene-1,3-diol[19] was carried out in the presence of trimethylsilyl trifluoromethanesulfonate (TMSOTf) and MS-4A (AW300) at 0°, affording the corresponding β-glycosides **11** {$[\alpha]_D$ +1.0° ($CHCl_3$)} and **17** {$[\alpha]_D$ +8.1° ($CHCl_3$)} in 48 and 63% yields, respectively. The observed chemical shifts and coupling constants due to the newly formed glycosidic linkages in the ^1H NMR spectra were at δ 4.58 ($J_{1,2}$ 7.5 Hz, H-1a) for **11** and at δ 4.59 ($J_{1,2}$ 8.2 Hz, H-1a) for **17**, showing the both of the anomeric configurations to be β. Other ^1H NMR data are consistent with the structures assigned. Selective reduction[20] of the azido group in **11** or **17** with H_2S in 5:1 pyridine–water gave the amine, which on condensation with octadecanoic acid using 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (WSC) in dichloromethane, gave gangliosides GM_1 and GD_{1a} derivatives **12** and **18** in 72 and 76% yields, respectively. Finally, *O*-deacylation of **12** or **18** with sodium methoxide in methanol, and subsequent saponification of the methyl ester group yielded GM_1 (**1**) and GD_{1a} (**2**) in quantitative yield after chromatography on a column of Sephadex LH-20. Physicochemical data for GM_1: $[\alpha]_D$ +7.2° (5:5:1 $CHCl_3$–CH_3OH–H_2O); ^1H NMR [49:1 $(CD_3)_2SO$–D_2O] δ 0.85 (t, 6 H, 2 $MeCH_2$), 1.23 (s, 52 H, 26 CH_2), 1.80, 1.85 (2 s, 6 H, 2 AcN), 2.65 (dd, 1 H, J_{gem} 12.6, $J_{3eq,4}$ 4.6 Hz, H-3e-*eq*), 4.17 (d, $J_{1,2}$ 8.0 Hz, H-1b), 4.25 (d, $J_{1,2}$ 7.8 Hz, H-1d), 4.29 (d, $J_{1,2}$ 7.6 Hz, H-1c), 4.85 (d, $J_{1,2}$ 7.9 Hz, H-1a), and 5.43–5.65 (m, 2 H, H-4, 5 Cer unit). Physicochemical data for GD_{1a} (**2**): $[\alpha]_D$ +4.8° (5:5:1 $CHCl_3$–CH_3OH–H_2O); ^1H NMR [49:1 $(CD_3)_2SO$–D_2O] δ 0.85 (t, 6 H, 2 $MeCH_f2$), 1.23 (s, 52 H, 26 CH_2), 1.76, 1.88, 1.89 (3 s, 9 H, 3 AcN), 2.60, 2.68 (2 dd, 2 H, J_{gem} 12.5, $J_{3eq,4}$ 4.5 Hz, H-3e, f-*eq*), 4.18 (d, $J_{1,2}$ 8.0 Hz, H-1b), 4.28, 4.30 (2 d, 2 H, $J_{1,2}$ 7.7 Hz, H-1c, d), 4.80 (d, $J_{1,2}$ 7.8 Hz, H-1a), and 5.36 5.58 (2 m, 2 H, H-4,5 Cer unit).

In conclusion, a facile and stereocontrolled total synthesis of GM_1 and GD_{1a} was achieved by use of the key glycosyl acceptor **4** and the thioglycoside donors **5**

and **6**, promising a further development of the systematic synthesis of the ganglioseries of ganglioside. Elemental analyses, as well as the IR and ^{1}H NMR data (270 and 400 MHz) of all the new compounds reported here were quite satisfactory with the assigned structures.

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