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CARBOHYDRATE RESEARCH

Preliminary communication

Total synthesis of ganglioside GQ1b ☆

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Recently, glycoconjugates, especially gangliosides which contain sialic acid as an essential constituent, have received much attention owing to their important biological functions [2–11]. We have had a program in this area, and our efforts resulted in the development of a facile procedure for the α-stereoselective coupling [12–14] of sialic acid, α-sialyl-(2 → 8)-sialic acid, and α-sialyl-(2 → 8)-α-sialyl-(2 → 8)-sialic acid using their protected methyl or phenyl 2-thioglycoside as the glycosyl donor and the suitably protected sugar acceptors, with dimethyl(methylthio)sulfonium triflate (DMTST) or *N*-iodosuccinimide (NIS)–trifluoromethanesulfonic acid (TfOH) as the promoter in acetonitrile solution. There are several gangliosides containing the α-sialyl-(2 → 8)-sialic acid unit in their molecules. Ganglioside GQ1b, which contains two α-sialyl-(2 → 8)-sialyl residues attached to a gangliotetraosyl ceramide backbone, was first isolated [15] from human brain and characterized [16]. Many biological functions of GQ1b, such as nerve growth factor (NGF)-like activity [17] in some neuroblastoma cell lines, modulation [18] of protein phosphorylation, and terminal differentiation [19] in cultured mouse keratinocytes, have been demonstrated. As a continuation of our synthetic efforts toward the goal of elucidating the functions of sialoglycoconjugates at the molecular level, we describe herein the first, total synthesis of ganglioside GQ1b, which has one of the most complex structures among gangliosides. For the synthesis of the core tetrasialyl-oligosaccharide of GQ1b, trisaccharide **7** was selected as the glycosyl acceptor. Compound **7** provides one free hydroxyl group at C-3 of the Gal residue and a 3,4-*O*-isopropylidene group at the GalNAc residue, which is selectively removable for further glycosylations with methyl [phenyl 5-acetamido-8-*O*-

☆ Synthetic studies on sialoglycoconjugates, Part 63. For Part 62, see ref 1.

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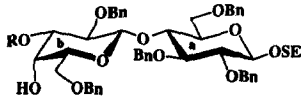
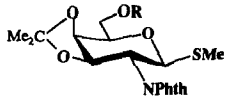


1 R = H
2 R = Bz



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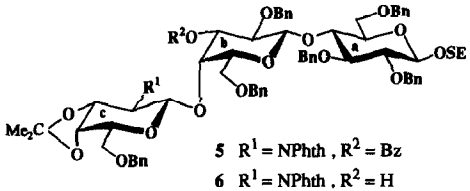
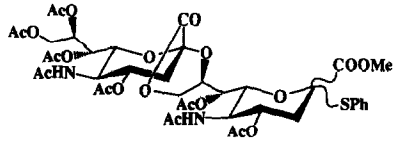
4 R = Bn



5 R^1 = NPhth , R^2 = Bz
6 R^1 = NPhth , R^2 = H
7 R^1 = NHAc , R^2 = H



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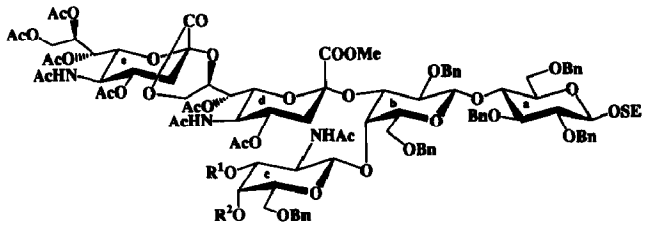
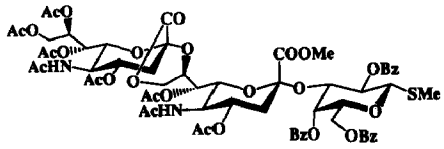
SE = 2-(trimethylsilyl)ethyl
Bn = benzyl
Bz = benzoyl
Phth = phthaloyl

(5-acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosylono-1′,9-lactone)-4,7-di-*O*-acetyl-3,5-dideoxy-2-thio-D-*glycero*-D-*galacto*-2-nonulopyranosid]onate (**8**) [14a,14b] and methyl *O*-[methyl 5-acetamido-8-*O*-(5-acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosylono-1′,9-lactone)-4,7-di-*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 (**11**) [14b] as the glycosyl donors. The octasaccharide **12** thus obtained could then, by coupling to the ceramide moiety, be transformed into target ganglioside GQ1b. The glycosylation of 2-(trimethylsilyl)ethyl 3′-*O*-benzoyl-2,3,6,2′,6′-penta-*O*-benzyl-β-lactoside (**2**), prepared from **1** [20] by selective benzoylation, was effected with the 6-*O*-benzyl derivative **4** derived by benzylation of methyl 2-deoxy-3,4-*O*-isopropylidene-2-phthaloylamino-1-thio-β-D-galactopyranoside (**3**) [20] with benzyl bromide using sodium hydride in *N*,*N*-dimethylformamide.

Reaction of **2** with **4** in the presence of NIS–TfOH and powdered 4A molecular sieves (MS-4A) in dichloromethane for 3 h at −30°C gave a 53% yield of the desired β-glycoside **5** {$[\alpha]_D$ + 30.0° (*c* 1.0, $CHCl_3$)}, showing in its ^1H NMR spectrum a one-proton doublet at δ 5.31 ($J_{1,2}$ 8.3 Hz, H-1c), characteristic of the



9 R^1, R^2 = isopropylidene
10 $R^1 = R^2 = H$



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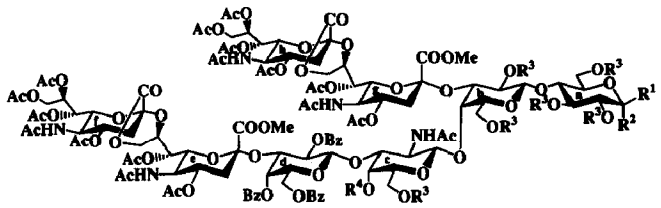
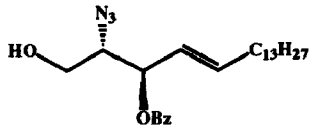


	R^1	R^2	R^3	R^4
12	OSE	H	Bn	H
13	OSE	H	Ac	Ac
14	OH, H		Ac	Ac
15	H	$OC(=NH)CCl_3$	Ac	Ac

2-phthalimido-β-D-galactopyranosyl unit. *O*-Debenzoylation of **5**, followed by heating with hydrazine hydrate in aq 95% ethanol, and *N*-acetylation of the product afforded the trisaccharide acceptor **7** {amorphous mass, $[\alpha]_D$ + 15.2° (*c* 0.8, $CHCl_3$)} in 95% yield. The glycosytaion of **7** with **8** in acetonitrile for 24 h at −25°C, in the presence of NIS, TfOH, and MS, afforded the desired pentasaccharide **9** {amorphous mass, $[\alpha]_D$ − 31.4° (*c* 0.7, $CHCl_3$)} in 50% yield, showing in its ^1H NMR spectrum a one-proton doublet of doublets at δ 2.71 ($J_{3ax,3eq}$ 13.5, $J_{3eq,4}$ 4.4 Hz, H-3d*eq*), characteristic of the α-sialyl linkage [12a,21]. Removal of the isopropylidene group from **9** with aq 80% acetic acid for 3 h at 60°C gave **10** {amorphous mass, $[\alpha]_D$ − 31° (*c* 1.2, $CHCl_3$)} in 91% yield.

Glycosylation of product **10** thus obtained with **11**, carried out in dichloromethane for 48 h at 0°C in the presence of DMTST and MS-4A, gave the protected GQ1b oligosaccharide **12** {amorphous mass, $[\alpha]_D$ − 17.2° (*c* 0.9, $CHCl_3$)} in 53% yield. Hydrogenolytic removal of the benzyl groups in **12** over 10% Pd–C in

3:1 ethanol–acetic acid for 3 days at 40°C, followed by acetylation of the free hydroxyls with acetic anhydride and pyridine for 24 h at 40°C, afforded the fully acylated oligosaccharide **13** {amorphous mass, $[\alpha]_D$ − 18° (*c* 0.7, $CHCl_3$)} in 53% yield. The ^1H NMR spectrum of **13** showed the presence of 24 three-proton singlets at δ 1.88–2.13 (5 AcN and 19 AcO), four one-proton multiplets at 2.45–2.74 (H-3e–h-*eq*), two three-proton singlets at 3.24 and 3.27 (2 OMe), a one-proton doublet at 5.44 ($J_{3,4}$ 3.4 Hz, H-4c), a one-proton doublet at 5.72 ($J_{3,4}$



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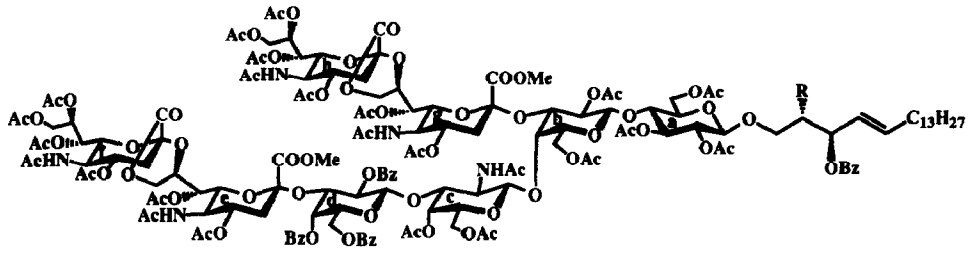
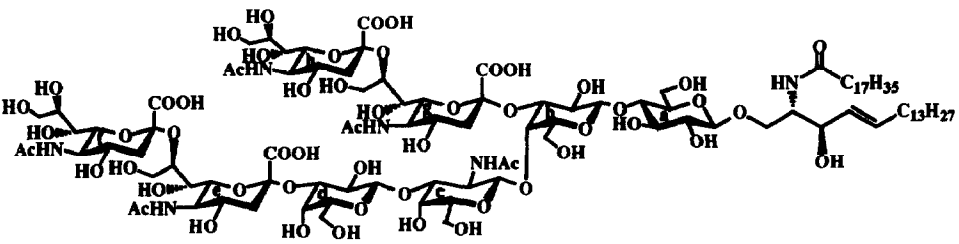


17 R = N_3
18 R = $NHCOC_{17}H_{35}$



19 ganglioside GQ1b

3.4 Hz, H-4d), and multiplets at 7.38–8.12 due to 15 aromatic protons, indicating the assigned structure.

Treatment [22] of **13** with trifluoroacetic acid in dichloromethane for 1.5 h at room temperature gave the 1-hydroxy compound **14** in 94% yield, which was subsequently treated [23] with trichloroacetonitrile in dichloromethane for 1 h at 0°C to give the α-trichloroacetimidate **15** {amorphous mass, $[\alpha]_D$ − 6.5° (*c* 1.1, $CHCl_3$)} in 95% yield. Significant signals in the ^{1}H NMR spectrum were at δ 6.47 ($J_{1,2}$ 3.7 Hz, H-1a) and 8.68 (C=NH), 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 (**16**) [24] was carried out in the presence of trimethylsilyl trifluoromethanesulfonate (TMSOTf) and MS-4A (AW-300) for 3 h at 10°C, affording the desired β-glycoside **17** {amorphous mass, $[\alpha]_D$ − 39.4° (*c* 0.3, $CHCl_3$)} in 46% yield. Selective reduction [24b,25] of the azido group in **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 the ganglioside GQ1b derivative **18** {amorphous mass, $[\alpha]_D$ − 9.4° (*c* 0.5, $CHCl_3$)} in 43% yield. Finally, *O*-deacylation of **18** with sodium methoxide in methanol, and subsequent saponification of the methyl ester and lactone functions, yielded ganglioside GQ1b (**19**) as an amorphous mass (quantitative), after chromatography on a column of Sephadex LH-20. Physicochemical data for GQ1b: $[\alpha]_D$ − 22.0° (*c* 0.2, 5:5:1 $CHCl_3$–CH_3OH–H_2O); ^{1}H NMR [49:1 $(CD_3)_2SO$–D_2O] δ 0.85 (t, 6 H, J_{Me,CH_2} 7.1 Hz, 2 *Me*CH_2), 1.23 (s, 52 H, 26 CH_2), 1.84, 1.88 (2 s, 15 H, 5 AcN), 2.03 (t, 2 H, J_{CH_2,CH_2} 7.0 Hz, COC$H_2$$CH_2$), 2.34–2.75 (4 m, 4 H, 4 H-3e–h-*eq*), 4.16 (d, 1 H, $J_{1,2}$ 7.5 Hz, H-1a), 4.31 (m, 2 H, H-1b, H-1d), 4.76 (d, 1 H, $J_{1,2}$ 8.8 Hz, H-1c), 5.33 (dd, 1 H, $J_{3,4}$ 7.5, $J_{4,5}$ 14.0 Hz, H-4 Cer unit), and 5.53 (m, 1 H, H-5 Cer unit).

In conclusion, the first, stereocontrolled total synthesis of ganglioside GQ1b was achieved by use of the phenyl 2-thioglycoside derivative **8** of α-sialyl-(2 → 8)-sialic acid as the key glycosyl donor and the trisaccharide acceptor **7**, promising a further development of the systematic synthesis of polysialogangliosides. Elemental analyses, as well as the IR and ^{1}H NMR data (270 MHz with Jeol JNM-GX-270 and 500 MHz with Varian VXR-500 spectrometers), of all the new compounds reported herein were quite satisfactory with the assigned structures.

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