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Preliminary communication

Total synthesis of a disialoganglioside GD1α*

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The first report on the isolation of ganglioside GD1α was by Taki *et al.*[2], who isolated the glycolipid from rat ascites hepatoma cells. Structural analysis revealed that it was a disialoganglioside having the terminal (2→3)-α-Neu5Ac (sialyl) unit linked to a (1→3)-β-D-Gal unit and the second (2→6)-α-Neu5Ac residue linked in an unusual manner to the GalNAc unit. In a recent report[3], the finding that this ganglioside is associated in extremely minor quantities with another ganglioside GM1b, isolated from adult bovine brain, has culminated in speculation that it may be synthesized *in vivo* from a synthetic sequence involving asialo GM1 and GM1b.

The biological importance of gangliosides in various aminal cells is now well documented[4–6]. However, the current scenario of gangliosides is not completely clear as their structure–function relationships at the molecular level remain largely unelucidated. As biologically derived gangliosides are amorphous and available in limited quantity, we have initiated efforts to provide chemically synthesized pure gangliosides and their analogs to be used in biological studies. We have reported[7,8] the successful syntheses of several gangliosides and their analogs, which are based on the facile dimethyl(methylthio)sulfonium triflate[9] (DMTST) promoted, stereoselective α-glycosidation[10] of sialic acid with various sugar residues in acetonitrile medium. We have successfully extended the application of this versatile method for the stereoselective α-glycosidation of sialic acid with even pentasaccharide acceptors. Herein we report the first total synthesis of ganglioside GD1α.

The synthesis of the core oligosaccharide of GD1α was designed starting from a pentasaccharide glycosyl acceptor **3**, having a sialyl α(2→3) unit already linked and providing a free primary hydroxyl group at C-6 of the GalNAc unit for further glycosidation with the methyl 2-thioglycoside of neuraminic acid[7b,10], to give upon reaction a disialyl hexasaccharide. The glycosyl acceptor was prepared from a known pentasaccharide derivative **2**, an intermediate prepared in the total synthesis of ganglioside of GM1b[8]. Thus treatment of intermediate **2** with aq. acetic acid (80%) for 24 h at 55° to remove the benzylidene group, afforded the glycosyl acceptor **3** {$[\alpha]_D$ +38.7° (CCl_3)} in 74% yield.

* Synthetic Studies on Sialoglycoconjugates Part 33. For Part 32 see ref. 1.
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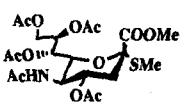
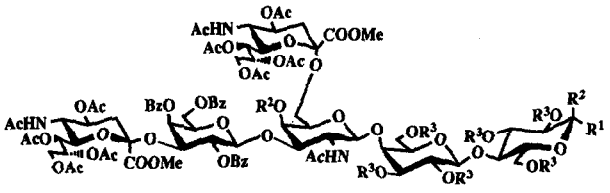


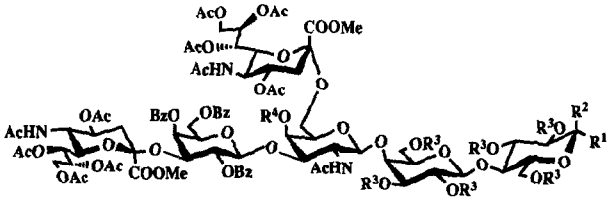
1 (α:β = 1:1)

2 R^1= OSE, R^2= H, R^3= Bn, R^4: R^5= *benzylidene*
3 R^1= OSE, R^2= R^4= R^5=H, R^3= Bn,

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

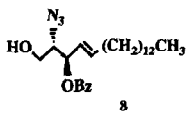


4a R^1= OSE, R^2= H, R^3= Bn,

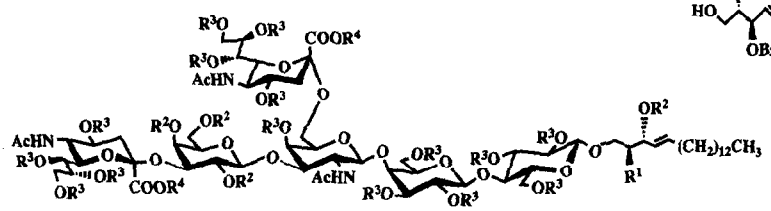


4b R^1= OSE, R^2= R^4= H, R^3= Bn,
5 R^1= OSE, R^2= H, R^3= R^4= OAc

6 R^1= OH, R^2= H, R^3= R^4= OAc
7 R^1= H, R^2= OC(=NH)CCl_3, R^3= R^4= OAc



8



9 R^1= N_3, R^2= Bz, R^3= Ac, R^4= Me
10 R^1= NH_2, R^2= Bz, R^3= Ac, R^4= Me

11 R^1= $NHCO(CH_2)_{16}CH_3$, R^2= Bz, R^3= Ac, R^4= Me
12 R^1= $NHCO(CH_2)_{16}CH_3$, R^2= R^3= R^4= H

Glycosidation of **3** with methyl (methyl 5-acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-2-thio-D-*glycero*-D-*galacto*-2-nonulopyranosid)onate[7b,10] (**1**, α:β = 1:1) in the presence of DMTST in acetonitrile for 9 h at 0° gave a 53% yield of an anomeric mixture of hexasaccharides. The moderate yield (41%) of the desired α anomer **4b** is quite appreciable, considering the bulkiness of the acceptor and the steric hindrance to glycosidation at C-6 of the GalNAc unit. The ^{1}H-n.m.r. spectrum of the chromatographically purified major fraction **4b** {$[\alpha]_D$ +17.3° ($CHCl_3$)} proved that it was an α anomer, based on its showing eleven, three-proton singlets at δ 1.55–2.15 (3 $NCOCH_3$

and 8 OCOCH_3), a one-proton doublet of doublets at δ 2.41 (refs. 7e, 10b) ($J_{3eq,4}$ 4.4 Hz) due to H-3*eq* of the sialyl $\alpha(2\rightarrow3)$ residue, a one-proton doublet of doublets at δ 2.54 ($J_{3eq,4}$ 5.1 Hz) due to H-3*eq* of the sialyl $\alpha(2\rightarrow6)$ residue, two three-proton singlets at δ 3.74 and 3.80 (2 OCH_3), and multiplets at δ 7.1–8.1 due to 45 aromatic protons.

Furthermore, the ^{1}H-n.m.r. spectrum of **4a** (12%) was more or less similar to that of the α anomer, except that it contained a one-proton doublet of doublets at δ 2.42 due to H-3*eq* of the sialyl $\alpha(2\rightarrow3)$ residue and a one-proton doublet of doublets at δ 2.37 due to H-3*eq* of the sialyl $\beta(2\rightarrow6)$ residue, indicating that **4a** was a β anomer. These ^{1}H-n.m.r. data of H-3*eq* in **4a** and **4b** are consistent with data from the earlier literature[11]. Attempts to synthesize the hexasaccharide using *N*-iodosuccinimide–trifluoromethanesulfonic acid (NIS–TfOH)[12] as a promoter in acetonitrile resulted in an anomeric mixture of hexasaccharides in which **4a** predominated (59%, α:β = 1:5).

Hydrogenolytic removal of the benzyl groups in **4b** over 10% Pd–C in 6:1 ethanol–acetic acid for 3 days at 43°, followed by acetylation of the free hydroxyls with acetic anhydride and pyridine for 3 days at 40°, afforded the fully acylated core oligosaccharide **5** {$[\alpha]_D$ +6.0° ($CHCl_3$)} in 76% yield. For the selective removal of the 2-(trimethylsilyl)ethyl group, the fully acylated oligosaccharide **5** was treated[13] with trifluoroacetic acid in dichloromethane for 2 h at room temperature to give a 91% yield of the 1-hydroxy compound **6** {$[\alpha]_D$ +19.3° ($CHCl_3$)}, which, on further treatment[14] with trichloroacetonitrile in the presence of 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) in dichloromethane for 3 h at 0°, gave the trichloroacetimidate **7** {$[\alpha]_D$ +23.0° ($CHCl_3$)} in 89% yield. The ^{1}H-n.m.r. spectrum of the trichloroacetimidate contained a one-proton doublet at δ 6.51 ($J_{1,2}$ 3.67 Hz, H-1) and a one-proton singlet at δ 8.7 (C=N*H*), which served to prove the anomeric configuration of **7** as α.

Glycosidation of the trichloroacetimidate **7** thus obtained with (2*S*,3*R*,4*E*)-2-azido-3-*O*-benzoyl-4-octadecene-1,3-diol[15] (**8**) was carried out in the presence of boron trifluoride etherate[14a,15b] and 4A molecular sieves (AW-300) for 12 h at 0° to give a 63% yield of the β glycoside **9** {$[\alpha]_D$ +2.9° ($CHCl_3$)}. For the selective reduction[15a,16] of the azide to the amine function for subsequent conversion to an *N*-acyl group, hydrogen sulfide was bubbled into a solution of compound **9** in aqueous pyridine for 2 days at 0–15°. The resulting amine **10**, without any purification, was condensed with octadecanoic acid in the presence of 1-(3-dimethylaminopropyl)-3-ethyl-carbodiimide hydrochloride (WSC) in dichloromethane for 24 h at room temperature to give the fully protected target compound **11** {$[\alpha]_D$ +44.8° ($CHCl_3$)} in 82.5% yield.

Finally, *O*-deacylation of **11** with sodium methoxide in methanol for 2 days at 45° and subsequent saponification of the methyl ester group afforded an 82% yield of the title compound **12** {$[\alpha]_D$ +3.4° (5:5:1 $CHCl_3$–CH_3OH–H_2O)}.

In conclusion, the introduction of a second sialyl unit into a pentasaccharide acceptor was accomplished in moderate yields using the methyl 2-thioglycoside of Neu5Ac in acetonitrile medium to give the product having the sialyl residue in the α configuration. In this study, DMTST was found to be far more efficient as a glycosidation promoter in the stereoselective α-glycosidation of sialic acid, when compared to NIS–TfOH. However, the latter promoter is being effectively used in our work in the stereoselective synthesis of sialyl-(2→3)-α-D-glycosides[9b].

Elemental analyses, as well as the i.r., and ^{1}H-n.m.r. data (270 and 400 MHz) of all the new intermediate compounds reported here were quite satisfactory with the assigned structures. The ^{1}H-n.m.r. data (400 MHz) of the ganglioside was identical with that of the earlier report by Hirabayashi *et al.*[3]

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