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Preliminary communication

A facile total synthesis of ganglioside GM1b and its positional analog*

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Gangliosides, sialic acid containing glycosphingolipids, are a class of structurally diverse molecules commonly present in vertebrate plasma membranes and especially enriched in nerve tissues[2]. These membrane components are thought to be responsible for important physiological activities, and it is hypothesized that the oligosaccharide moiety of these molecules is exposed as a ligand to the external environment, capable of expressing biological functions congruent to the chemical structure of the ganglioside[2,3]. An approach toward a systematic understanding of the structural and functional intricacies of the gangliosides necessitates efficient regio- and stereo-selective synthetic routes, affording various gangliosides and their analogs.

The focal point in the synthesis of gangliosides has been the stereoselective α-glycosidation of sialic acid with various sugar residues. Recently, we reported an abridged version[4a] of a versatile procedure for α-glycosidation with thioglycosides of sialic acid, using dimethyl(methylthio)sulfonium triflate (DMTST)[5] as the promoter for reactions with suitably protected galactose and lactose acceptors in acetonitrile[4b]. From the application of this procedure we have reported[6] the total synthesis of several gangliosides and their analogs, to be used in pursuit of our objective of elucidating the function of sialoglycoconjugates.

Ganglioside GM1b was first isolated by Yip[7] from rat brain and, in a recent report[8], it is described to be associated with GM1a in extremely minor quantities. A total synthesis of this ganglioside was first achieved by Ogawa *et al.*[9]. Here we describe a facile total synthesis of ganglioside GM1b (**18**) and its positional analog **22**, involving high-yielding glycosidation reactions performed according to our methods[4,6].

Compounds **4** and **5** were used as the glycosyl donors while intermediate **3** was selected as the glycosyl acceptor for the synthesis of the core oligosaccharide chains of GM1b and its analog. The stereoselective synthesis of the glycosyl donors has been reported elsewhere[1,6e].

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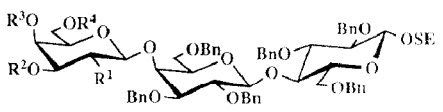

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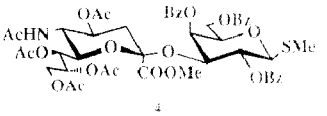
3 R^1= NHAc, R^2= H, R^3-R^4= benzylidene



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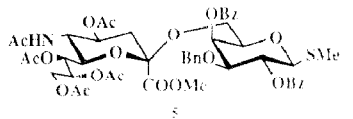

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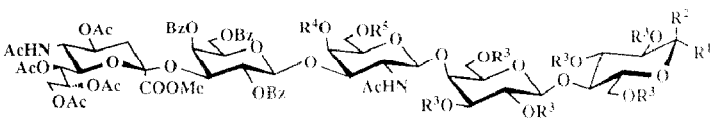


6 R^1= OSE, R^2= H, R^3= Bn, R^4-R^5= benzylidene
7 R^1= OSE, R^2= H, R^3= R^4= R^5= Ac
8 R^1, R^2 =H,OH, R^3= R^4= R^5= Ac
9 R^1= H, R^2= OC(=NH)CCl$_3$, R^3= R^4= R^5= Ac

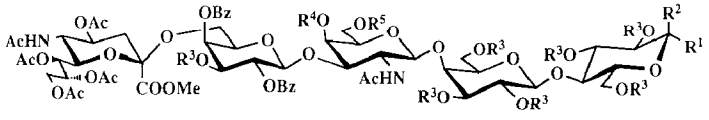
SE= 2-(trimethylsilyl)ethyl
Bn= benzyl
Bz= benzoyl

Compound **3** was prepared as follows. Glycosidation of 3,4,6-tri-*O*-acetyl-2-deoxy-2-phthalimido-α-D-galactopyranosyl bromide[10] with 2-(trimethylsilyl)ethyl *O*-(2,3,6-tri-*O*-benzyl-β-D-galactopyranosyl)-(1→4)-2,3,6-tri-*O*-benzyl-β-D-glucopyranoside[11] by the classical Koenigs–Knorr method, at room temperature in dichloromethane, gave the desired trisaccharide **1** ($[\alpha]_D$ +4.1° in CH_2Cl_2) in 81% yield. The ^{1}H-n.m.r. spectrum of the trisaccharide showed 3 three-proton singlets at δ 1.86, 2.01, and 2.19 (O–COCH_3), multiplets for 34 aromatic protons at δ 6.9–7.34, and a doublet at δ 5.34 ($J_{1,2}$ 8.4 Hz) due to H-1 at the newly formed β-glycosidic linkage. An ethanolic solution of compound **1** was treated with hydrazine monohydrate for 4 h at 80°, to remove *O*-acetyl and phthaloyl groups, and this was followed by *N*-acetylation to afford **2** ($[\alpha]_D$ +58.3° in CH_2Cl_2) in 69% yield. This intermediate was converted into glycosyl acceptor **3** ($[\alpha]_D$ +24° in CH_2Cl_2) in 71% yield by reaction with benzaldehyde dimethylacetal, in *N*,*N*-dimethylformamide (DMF) with a catalytic amount of *p*-TsOH, for 8 h at room temperature. The ^{1}H-n.m.r. spectrum of **3** showed a three-proton singlet at δ 1.59 (N–COCH_3), a one-proton singlet at δ 5.57 due to the benzylidene methine proton, and multiplets for 35 aromatic protons at δ 7.19–7.56, in agreement with the structure assigned.

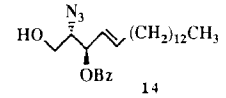
The glycosidation of **3** with methyl *O*-(methyl 5-acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosylonate)-(2→3)-2,4,6-tri-*O*-benzoyl-1-thio-β-D-galactopyranoside (**4**) was performed in the presence of DMTST in acetonitrile for 16 h at 5°, yielding 73% of the desired pentasaccharide derivative **6** ($[\alpha]_D$ +31° in CH_2Cl_2). The stereochemistry at the newly formed glycosidic center was β. The identity of the product was unambiguously proved by its ^{1}H-n.m.r. spectrum, which showed 6 three-proton singlets at δ 1.59, 1.74 (2 N–COCH_3), 1.81, 1.88, 2.07, and 2.16 (4

O–COCH_3), a doublet at δ 5.04 ($J_{1,2}$ 7.7 Hz, H-1) and a doublet of doublets at δ 5.46 ($J_{2,3}$ 7.7 Hz, H-2), both for the benzoylated galactose unit, and multiplets at δ 7.1–8.0 accounting for 50 aromatic protons. Catalytic hydrogenolysis of the benzyl groups in **6** over 10% Pd–C in 6:1 ethanol–acetic acid for 2 days at 45°, followed by acetylation with acetic anhydride and pyridine, afforded compound **7** ($[\alpha]_D$ +14.3° in CH_2Cl_2) in 55% yield. This on treatment with boron trifluoride etherate in dichloromethane for 18 h at 5° gave 89% of **8** ($[\alpha]_D$ +33° in CH_2Cl_2). Treatment[12] of **8** with trichloroacetonitrile in the presence of 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) in dichloromethane for 4 h at 0°, gave the trichloroacetimidate **9** ($[\alpha]_D$ +41.7° in CH_2Cl_2) in 89% yield. A one-proton doublet at δ 6.6 ($J_{1,2}$ 3.7 Hz, H-1) and a one-proton singlet at δ 8.6 (C=N*H*) in its ^{1}H-n.m.r. spectrum suggested that **9** was the α anomer.

The glycosidation reaction of **3** with **5**, conducted as just described for **6**, gave the analogous pentasaccharide derivative **10** ($[\alpha]_D$ +34° in CH_2Cl_2) in 70% yield. The ^{1}H-n.m.r. spectrum included 6 three-proton singlets at δ 1.85, 1.91, 1.95, 1.99, 2.01, and 2.13 (2 N–COCH_3 and 4 O–COCH_3), a doublet at δ 4.8 ($J_{1,2}$ 8.1 Hz, H-1) and a doublet of doublets at δ 5.47 ($J_{2,3}$ 9.9 Hz, H-2), both for the di-*O*-benzoylated galactose unit, and multiplets at δ 7.1–8.0 in support of the assigned structure. Further, a sequence of reactions, similar to those described for compounds **7**–**9**, respectively afforded compounds **11** ($[\alpha]_D$ +2.7° in CH_2Cl_2), **12** ($[\alpha]_D$ +22° in CH_2Cl_2) and **13** ($[\alpha]_D$ +26° in CH_2Cl_2). The ^{1}H-n.m.r. of trichloroacetimidate **13** showed a one-proton doublet at δ 6.48 ($J_{1,2}$ 3.7 Hz, H-1) and a one-proton singlet at δ 8.68 (C=C*H*), confirming its α-anomeric configuration.



10 R^1= OSE, R^2= H, R^3= Bn, R^4: R^5=benzylidene
11 R^1= OSE, R^2= H, R^3= R^4= R^5= Ac
12 R^1, R^2 =H,OH, R^3= R^4= R^5= Ac
13 R^1= H, R^2= OC(=NH)CCl_3, R^3= R^4= R^5= Ac



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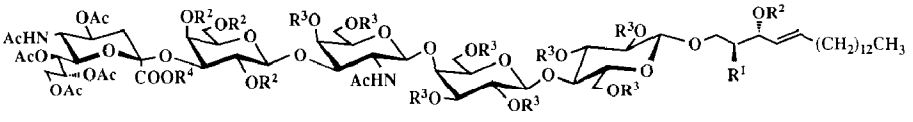
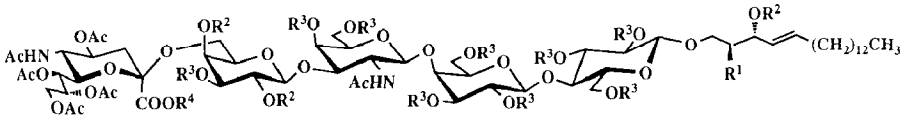


15 R^1= N_3, R^2= Bz, R^3= Ac, R^4= Me
16 R^1= NH_2, R^2= Bz, R^3= Ac, R^4= Me
17 R^1= $NHCO(CH_2)_{16}CH_3$, R^2= Bz, R^3= Ac, R^4= Me
18 R^1= $NHCO(CH_2)_{16}CH_3$, R^2= R^3= R^4= H



19 R^1= N_3, R^2= Bz, R^3= Ac, R^4= Me
20 R^1= NH_2, R^2= Bz, R^3= Ac, R^4= Me
21 R^1= $NHCO(CH_2)_{16}CH_3$, R^2= Bz, R^3= Ac, R^4= Me
22 R^1= $NHCO(CH_2)_{16}CH_3$, R^2= R^3= R^4= H

The thus obtained trichloroacetimidates **9** and **13** were separately coupled with two molar equivalents each of (2*S*,3*R*,4*E*)-2-azido-3-*O*-benzoyl-4-octadecene-1,3-diol[13] (**14**) in the presence of boron trifluoride etherate and 4Å molecular sieves (AW-300) for 12 h at 0° to give the respective azidosphingosine compounds **15** ($[\alpha]_D$ +5.9° in CH_2Cl_2) and **19** ($[\alpha]_D$ +3.9° in CH_2Cl_2). The ^{1}H-n.m.r. spectra of these intermediates showed signals for H-5 of the sphingosine unit as one-proton multiplets at δ 5.80 and δ 5.82, respectively. Solutions of **15** and **19** in aqueous 83% pyridine were treated with hydrogen sulfide (continuous bubbling) for 2 days at 0–15°, for the selective reduction[14] of the azido group to give the corresponding amino compounds **16** and **20**. These were subsequently condensed with octadecanoic acid in the presence of 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide (WSC) in dichloromethane for 24 h at room temperature to give the fully protected target compounds **17** ($[\alpha]_D$ +3.4° in CH_2Cl_2) and **21** ($[\alpha]_D$ +8.5° in CH_2Cl_2) in 79% and 82% yield, respectively.

Finally, *O*-deacylation of **17** and **21** with sodium methoxide in methanol for 2 days at 45°, and subsequent saponification of the methyl ester group, afforded the title compounds **18** ($[\alpha]_D$ +5.7° in 5:4:1 $CHCl_3$–CH_3OH–H_2O), 84%, and **22** ($[\alpha]_D$ −4.2° in 5:4:1 $CHCl_3$–CH_3OH–H_2O), 88.6%, respectively.

In conclusion, a facile synthesis of ganglioside GM1b and its positional analog has been accomplished by employing our versatile methods, *viz.* α-glycosidation of sialic acid with suitably protected galactose acceptors in the presence of DMTST, protection of the reducing terminal sugar residue by a 2-(trimethylsilyl)ethyl group, stable to various reaction conditions, and final functionalization of the ceramide moiety after attachment of the oligosaccharide chains. These synthetic routes have been successfully extended to the preparation of a few more analogs of GM1b, and the details of the syntheses will be reported shortly.

The elemental analysis and i.r. and ^{1}H-n.m.r. data of all the new compounds reported here were in conformity with the assigned structures.

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