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Preliminary communication

Synthesis of α-Neu5Ac*p*-(2→3)-D-Gal and α-Neu5Ac*p*-(2→3)-β-D-Gal*p*-(1→4)-D-Glc*

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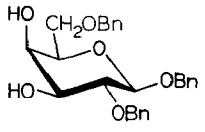
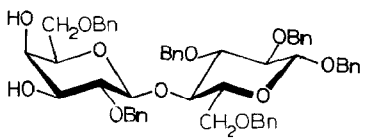
Sialosylcerebroside **1**, the ganglioside with the simplest molecular structure, has been isolated as a minor component of ganglioside mixtures[2]. Ozonolysis of **1** and subsequent base treatment has been reported to give sialosylgalactose **3** (ref. 3). Sialosyllactosylceramide **2**, first isolated from horse erythrocytes by Yamakawa and Suzuki[4] and termed hematoside, is identical with GM_3, one of the brain gangliosides[5]. The trisaccharide **5**, the glycan part of **2**, has been found to occur in bovine colostrum[6], and also in human urine[7].

Recently, several couplings of sialosyl units to primary hydroxyl groups have been reported[8]. As for sialic glycosides involving secondary hydroxyl groups, Shapiro[9] in 1973 claimed a total synthesis of hematoside (**2**), on the basis of t.l.c. evidence only. Therefore, the stereo- and regiochemistry of glycosylation by a Neu5Ac donor at a secondary hydroxyl group remained to be clarified.

α-Neu5Ac*p*-(2→3)-β-D-Gal*p*-(1→1)-Cer **1**
α-Neu5Ac*p*-(2→3)-β-D-Gal*p*-(1→4)-D-Glc*p*-(1→1)-Cer **2**
α-Neu5Ac*p*-(2→3)-D-Gal **3**
β-Neu5Ac*p*-(2→3)-D-Gal **4**
α-Neu5Ac*p*-(2→3)-β-D-Gal*p*-(1→4)-D-Glc **5**
β-Neu5Ac*p*-(2→3)-β-D-Gal*p*-(1→4)-D-Glc **6**



7 Bn = CH_2Ph



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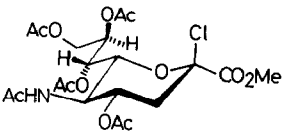

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*Part 31 in the series "Synthetic Studies on Cell-surface Glycans". For Part 30, see ref. 1.
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As part of our project on the synthesis of cell-surface glycoconjugates, we describe here a synthetic approach to sialosylgalactose (**3**), and sialosyllactose (**5**), along with their stereoisomers **4** and **6**. Our synthesis employs the glycosyl acceptors **7** and **8**, and the glycosyl donor **9** (ref. 10).

Isopropylidenation of benzyl β-D-galactopyranoside (**10**) in 2,2-dimethoxypropane–acetone–TsOH afforded **11** (ref. 11) in 67% yield, $[\alpha]_D$ −2.38° (*c* 1.08*); R_F 0.58 in ethyl acetate. Benzylation of **11** with BnBr–NaH in *N*,*N*-dimethyl formamide gave 71% of **12**, $[\alpha]_D$ +7.2° (*c* 1.02); R_F 0.55 in 1:1 toluene–ethyl acetate, and the hydrolysis of **12** in 80% aq. acetic acid at 60° afforded an 83% yield of **7**, crystals from ether, m.p. 107–108°, $[\alpha]_D$ −15.1° (*c* 1.00); R_F 0.17 in 10:1 toluene–ethyl acetate. The glycosylation of **7** with **9** in the presence of $Hg(CN)_2$–$HgBr_2$–powdered molecular sieves 4 Å (ref. 12) in $Cl(CH_2)_2Cl$ afforded a 15% yield (based on **9**) of a 2:3 mixture of **13** and **16**, which was separated by chromatography on a Lobar column (LiChroprep Si-60) in 9:1 toluene–methanol**. Compound **13** had $[\alpha]_D$ −21.7° (*c* 1.15); R_F 0.20 in 10:1 toluene–methanol, δ_H ($CDCl_3$): 2.53 (q, 1 H, *J* 4.6, 13.2 Hz, H-3b$_{eq}$), and δ_C ($CDCl_3$): 102.78 (C-1a) and 98.39 (C-2b). For **16** we found $[\alpha]_D$ −25.4° (*c* 1.40); R_F 0.24 in 10:1 toluene–methanol, δ_H ($CDCl_3$): 2.50 (q, 1H, *J* 4.6, 13.9 Hz, H-3b$_{eq}$); and δ_C ($CDCl_3$): 102.84 (C-1a) and 99.48 (C-2b).

Glycosylation was expected to occur at the C-3-OH, owing to the higher reactivity of this OH toward alkyl halides[13], and this regioselectivity was proven as follows. Compounds **13** and **16** were converted into the acetates **14** and **17**, respectively, which exhibited in their ^{1}H-n.m.r. spectra deshielded signals for H-4a at δ 5.06 (d, *J* 2.93 Hz) and δ 5.38 (partly overlapped with a signal for H-7b), respectively. The anomeric configuration could also be assigned from ^{1}H-n.m.r. data. In the case of the α anomer **13**, a signal for H-4b appeared at δ ~4.85, partly overlapping the signals of the benzyl-methylene protons, while in the case of the β anomer **16**, the signal was observed for H-4b at lower field (δ 5.11, td), in accordance with the reported trend[8e].

Deacetylation (NaOMe–MeOH) and saponification (NaOH–MeOH) of **13** and **16** afforded the acids **15** and **18** in 58 and 71% yields, respectively. Compound **15** had $[\alpha]_D$ −25.9° (*c* 1.62, MeOH); R_F 0.64 in solvent A (2:1:1 *n*-butanol–ethanol–water), and **18** had R_F 0.63 in the same solvent mixture. Catalytic hydrogenolysis of **15** and **18** over 10% Pd–C in methanol afforded quantitative yields of **3** and **4**, respectively. For **3** we observed $[\alpha]_D$ +23.1° (*c* 1.18, H_2O); R_F 0.37 in solvent A; δ_H (D_2O at 60°): 5.29 (d, *J* 3.9 Hz, H-1aα), 4.64 (d, *J* 8.1 Hz, H-1aβ), 2.76 (q, *J* 4.9, 12.4 Hz, H-3b$_{eq}$β), 2.74 (q, *J* 5.0, 12.0 Hz, H-3b$_{eq}$α), 2.03 (s, 3 H, NHAc), 1.79 (t, *J* 12.0 Hz, H-3b$_{ax}$α), and 1.81 (t, *J* 12.0 Hz, H-3b$_{ax}$β); and δ_C*** (D_2O): 100.81 (C-2bα), 100.68 (C-2bβ), 97.11 (C-1aβ, $^1J_{CH}$,

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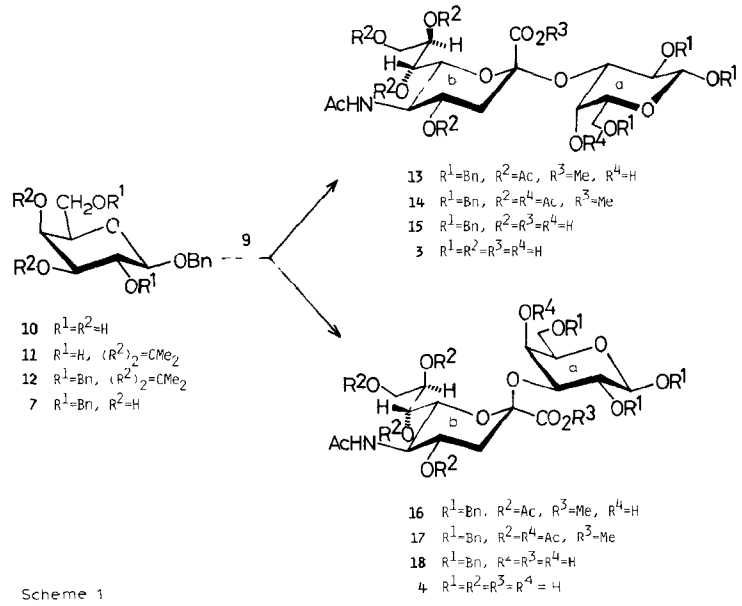
*Values of $[\alpha]_D$ were measured for $CHCl_3$ solutions at 25°, unless noted otherwise. Compounds having $[\alpha]_D$ recorded gave satisfactory elemental analyses.

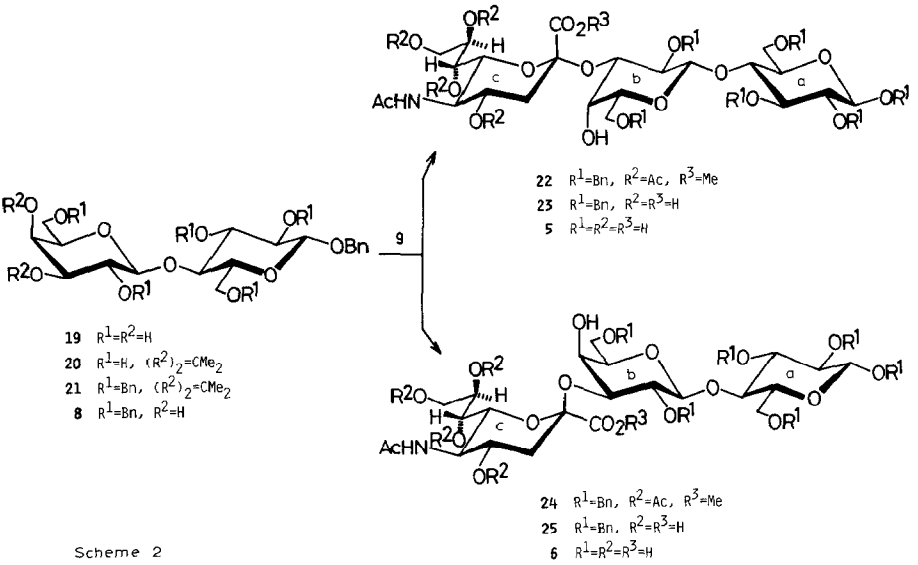
**A minor product of glycosylation at the C-4-OH was isolated as a lactone in 2.2% yield.

***Values of δ_C (in D_2O) are expressed in p.p.m. downward from Me_4Si, by reference to an internal standard of 1,4-dioxane (67.40).



Scheme 1

161.1 Hz), and 93.09 (C-1aα, $^1J_{CH}$ 169.7 Hz). Compound **4** showed $[\alpha]_D$ +11.0° (*c* 0.30, H_2O); R_F 0.26 in solvent A; δ_H (D_2O): 5.26 (d, *J* 3.2 Hz, H-1aα), 4.60 (d, *J* 7.6 Hz, H-1aβ), 2.47 (q, *J* 4.6, 12.9 Hz, H-3b$_{eq}$β), 2.42 (q, *J* 5.1, 13.4 Hz, H-3b$_{eq}$α), 2.05 (s, 3 H, NHAc), and 1.69 (t, *J* 12.7 Hz, H-3b$_{ax}$); δ_C (D_2O): 103.68 (C-2bβ), 103.62 (C-2bα), 97.52 (C-1aβ, $^1J_{CH}$ 161.1 Hz), and 93.23 (C-1aα, $^1J_{CH}$ 169.7 Hz). The ratio of anomers at C-1a was α:β = 1:2.



Scheme 2

Having prepared the target sialosylgalactose **3**, we now turned to the synthesis of sialosyllactose **5**. Benzyl 3′,4′-*O*-isopropylidene-β-D-lactoside (**20**), readily obtainable[14] from benzyl lactoside (**19**), was benzylated to give a 63% yield of **21**, $[\alpha]_D$ +9.4° (*c* 1.49);

R_F 0.64 in 4:1 toluene–ethyl acetate; δ_C ($CDCl_3$): 102.54 and 101.86. Acid hydrolysis of **21** then gave the glycosyl acceptor **8** in 99% yield, m.p. 86–88°, $[\alpha]_D$ +19.5° (*c* 1.20); R_F 0.35 in 4:1 toluene–ethyl acetate; δ_C ($CDCl_3$): 102.62. The glycosylation of **8** with the donor **9** in the presence of $HgBr_2$–$Hg(CN)_2$–powdered molecular sieves 4 Å in $Cl(CH_2)_2Cl$ afforded an 18% yield of a 1:2.1 mixture of the α anomer **22** and the β anomer **24**, which were separated by chromatography on a Lobar column in 9:1 toluene–methanol as described above*. For compound **22** we recorded $[\alpha]_D$ +5.8° (*c* 0.925); R_F 0.26 in 10:1 toluene–methanol, δ_H ($CDCl_3$): 4.86 (td, H-4c) and 2.51 (q, *J* 4.7, 13.3 Hz, H-3c_{eq}); and for **24**, $[\alpha]_D$ +3.1° (*c* 1.065); R_F 0.31 in 10:1 toluene–methanol; δ_H ($CDCl_3$): 5.15 (td, H-4c) and 2.53 (q, *J* 4.1, 13.4 Hz, H-3c_{eq}). The stereochemistry of **22** and **24** was assignable from the respective values of δ_H for H-4c (ref. 8e). Deacetylation, saponification, and hydrogenolysis of **22** and **24** afforded the target compound **5** and its stereoisomer **6** in 82 and 76% respective overall yields, via **23**, which had $[\alpha]_D$ +6.4° (*c* 0.87, MeOH); R_F 0.65 in solvent A; and **25**, which had $[\alpha]_D$ +6.6° (*c* 0.71, MeOH); R_F 0.70 in solvent A. Compound **5** showed $[\alpha]_D$ +19.2° (*c* 1.53, H_2O); R_F 0.34 in solvent A; δ_H (D_2O, pD 7.0): 5.21 (d, *J* 3.9 Hz, H-1aα), 4.65 (d, *J* 7.9 Hz, H-1aβ), 4.52 (d, *J* 7.8 Hz, H-1b), 3.27 (t, *J* 8.8 Hz, H-2aβ), 2.74 (q, *J* 4.4, 12.2 Hz, H-3c_{eq}), 2.02 (s, 3 H, NHAc), and 1.79 (t, *J* 12.2 Hz, H-3c_{ax}). For **6** the corresponding values were $[\alpha]_D$ +11.6° (*c* 1.08, H_2O); R_F 0.19 in solvent A; δ_H (D_2O, pD 7.0): 5.20 (d, *J* 3.9 Hz, H-1aα), 4.65 (d, *J* 7.8 Hz, H-1aβ), 4.48 (d, *J* 7.3 Hz, H-1b), 3.26 (t, *J* 8.3 Hz, H-2aβ), 2.46 (q, *J* 4.2, 12.7 Hz, H-3c_{eq}), 2.04 (s, 3 H, NHAc), and 1.68 (t, *J* 12.4 Hz, H-3c_{ax}). For both **5** and **6** the ratio of anomers at C-1a was α:β = 1:2.

In conclusion, the target molecules **3** and **5** were synthesized by employing the glycosyl acceptors **7** and **8**, and the glycosyl donor **9**. The stereochemistry of the synthetic product **5** was identified by comparison of its ^{1}H-n.m.r. data with those for a natural sample[15].

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*In this case also a regioisomeric product of the glycosylation was isolated in 3.8% yield.

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