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Preliminary communication

Synthesis of a linear tetrasaccharide unit of a complex type of glycan chain of a glycoprotein*

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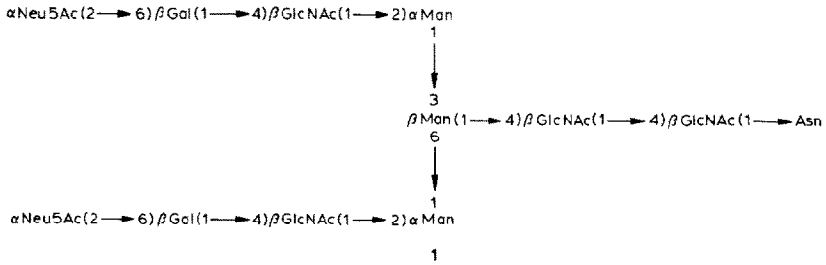
As part of a project on the synthesis of a complex type of glycan of a glycoprotein[2] such as **1**, we report here a regiocontrolled synthesis of the linear glycan unit **2** together with its stereoisomer **3**, which corresponds to the nonreducing end tetrasaccharide structure of **1**. In close connection with this report, it is to be noted that an elegant synthesis of the trisaccharide α-Neu5Ac-(2→6)-β-Gal-(1→4)-GlcNAc was recently reported by Paulsen and Tietz[3].



We designed the trihexosyl acceptor **8** as the key intermediate which should be glycosylated with the readily available donor[4] **10**. The key intermediate diol **8** was prepared as follows. The trihexosyl derivative **4** (ref. 5) was converted, *via* **5** (R_F 0.48 in 3:1 $CHCl_3$–MeOH), in 3 steps, into the isopropylidene derivatives **6** and **7**, in 69 and 11% overall yields, respectively; (*i*) NaOMe–MeOH, (*ii*) $Me_2C(OMe)_2$–TsOH in DMF for 15 h at 20°, and (*iii*) Ac_2O–pyridine. Compound **6**: $[\alpha]_D$*** +23.5° (*c* 1.33); R_F 0.40 in 1:3 toluene–EtOAc; δ_H ($CDCl_3$): 1.42 and 1.36 (s, two 3 H, CMe_2); δ_C ($CDCl_3$): 100.84 (C-1c, $^1J_{CH}$ 158.7 Hz), 99.08 (C-1b, $^1J_{CH}$ 158.7 Hz), 98.94 (=CMe_2), and 96.74 (C-1a, $^1J_{CH}$ 167.2 Hz). Compound **7**: R_F 0.53 in 1:3 toluene–EtOAc; δ_H ($CDCl_3$) 1.52 and 1.31 (s, two 3 H, CMe_2).

*Synthetic Studies on Cell-surface Glycans, Part XXVII. For Part XXVI, see ref. 1.
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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 data for elemental analyses.

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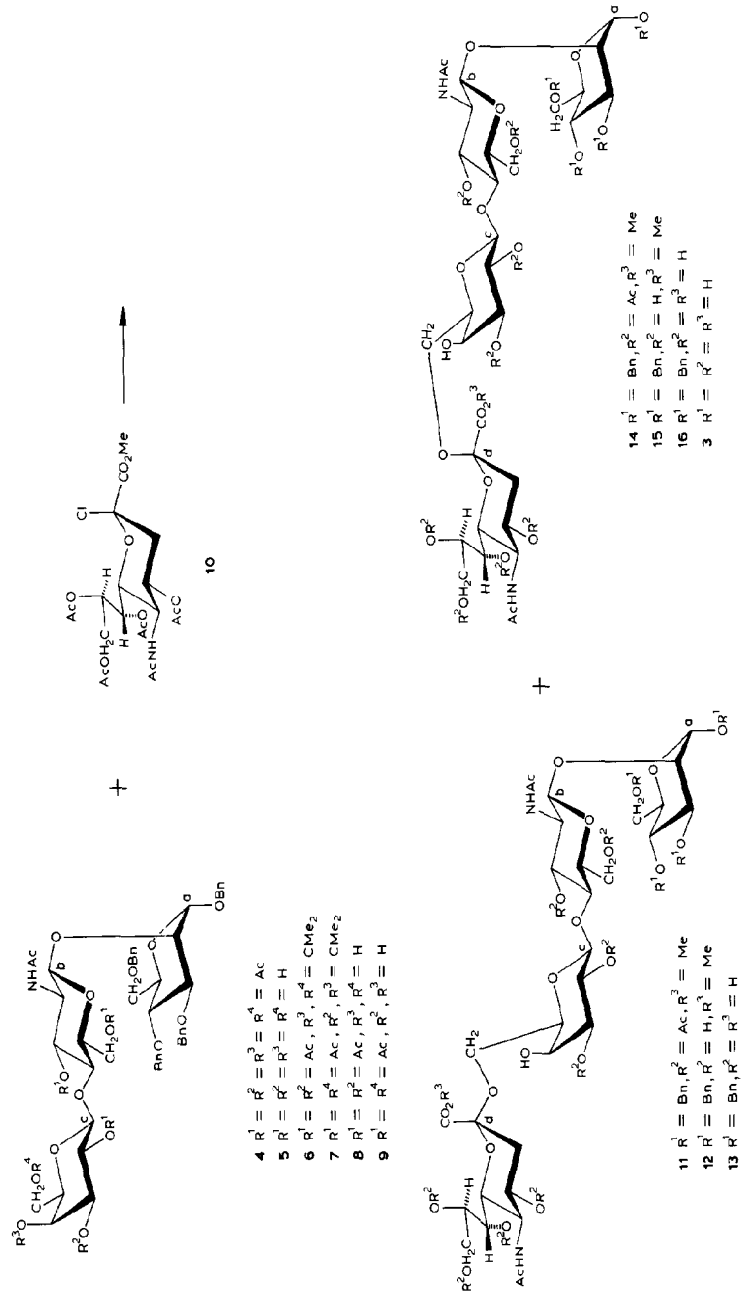

4 $R^1 = R^2 = R^3 = R^4 = Ac$
5 $R^1 = R^2 = R^3 = R^4 = H$
6 $R^1 = R^2 = Ac$, $R^3, R^4 = CMe_2$
7 $R^1 = R^4 = Ac$, $R^2, R^3 = CMe_2$
8 $R^1 = R^2 = Ac$, $R^3, R^4 = H$
9 $R^1 = R^4 = Ac$, $R^2, R^3 = H$

10

11 $R^1 = Bn, R^2 = Ac, R^3 = Me$
12 $R^1 = Bn, R^2 = H, R^3 = Me$
13 $R^1 = Bn, R^2 = R^3 = H$
2 $R^1 = R^2 = R^3 = H$

14 $R^1 = Bn, R^2 = Ac, R^3 = Me$
15 $R^1 = Bn, R^2 = H, R^3 = Me$
16 $R^1 = Bn, R^2 = R^3 = H$
3 $R^1 = R^2 = R^3 = H$

Scheme 1

O-De-isopropylidenation of the major product **6** in 1:1 AcOH–MeOH for 2 h at 80° afforded an 81% yield of the diol **8**, $[\alpha]_D$ +12.5° (*c* 1.18); R_F 0.54 in 10:1 $CHCl_3$–MeOH; δ_H ($CDCl_3$): 2.08 (s, 3 H), 2.04 (s, 6 H), 1.98 (s, 3 H), and 1.80 (s, 3 H) for five Ac groups; δ_C ($CDCl_3$): 101.76 (C-1c, $^1J_{CH}$ 159.9 Hz), 97.62 (C-1b, $^1J_{CH}$ 159.9 Hz), and 96.50 (C-1a, $^1J_{CH}$ 167.2 Hz).

The isomeric diol **9** (R_F 0.44 in 10:1 $CHCl_3$–MeOH) was obtained in a similar way from **7**. The structures of **8** and **9** were assigned according to the observation of the different reactivity of the hydroxyl groups; thus, treatment of **8** with a large excess of trityl chloride in pyridine for 21 h at 20° afforded the 6-*O*-trityl derivative (R_F 0.62 in 1:2 toluene–EtOAc), whereas, under the same conditions, **9** gave no tritylation product.

Glycosidation of the trihexosyl acceptor **8** with the *N*-acetylneuraminic acid donor **10** in the presence of 1:1:4 $HgBr_2$–$Hg(CN)_2$–powdered molecular sieves 4A in $Cl(CH_2)_2Cl$ for 4 days at 20° afforded a mixture of the anomers, in agreement with the low stereoselectivity reported previously[6] for glycosidation using the donor **10** (see Scheme 1). Separation by flash chromatography[7] over silica gel C-300 in 40:1 $CHCl_3$–MeOH gave **11** and **14** in 34 and 30% yield, respectively. Compound **11**: $[\alpha]_D$ +0.5° (*c* 0.94); R_F 0.19 in 20:1 $CHCl_3$–MeOH; δ_H ($CDCl_3$): 3.821 (s, 3 H, OMe), and 2.591 (q, 1 H, *J* 4.39 and 12.69 Hz, H-3d-*e*). Compound **14**: $[\alpha]_D$ +4.0° (*c* 0.50); R_F 0.24 in 20:1 $CHCl_3$–MeOH; δ_H ($CDCl_3$): 3.830 (3 H, s, OMe), and 2.464 (1 H, q, *J* 4.88 and 12.94 Hz, H-3d-*e*). Compounds **11** and **14** were separately subjected to the following deprotection steps: (*i*)NaOMe–MeOH, (*ii*) NaOH in 1:1 MeOH–THF, (*iii*) H_2–10% Pd–C in 9:1 EtOH–H_2O at 60°, and (*iv*) Sephadex G-25, to give the target tetrasaccharides **2** (80%) and the stereoisomer **3** (89%), *via* compounds **12** (R_F 0.55 in 2:1 $CHCl_3$–MeOH) and **13** (R_F 0.51 in 2:1:1 1-BuOH–EtOH–H_2O), and *via* compounds **15** (R_F 0.56 in 2:1 $CHCl_3$–MeOH) and **16** (R_F 0.53 in 2:1:1 1-BuOH–EtOH–H_2O). Compound **2**: $[\alpha]_D$ −20.0° (*c* 0.30, H_2O); R_F 0.20 in 2:1:1 1-BuOH–EtOH–H_2O, δ_C* (D_2O): 104.34 (C-1c, $^1J_{CH}$ 161.1 Hz), 100.97 (C-2d), 100.24 (C-1b, $^1J_{CH}$ 161.1 Hz), 91.94 (C-1a, $^1J_{CH}$ 169.7 Hz), 81.47 (C-4b), 78.07 (C-2a), and 64.17 (C-6c). Compound **3**: $[\alpha]_D$ −21.4° (*c* 0.36, H_2O); R_F 0.17 in 2:1:1 1-BuOH–EtOH–H_2O; δ_C* (D_2O): 104.01 (C-1c), 101.08 (C-2d), 100.29 (C-1b), 91.69 (C-1a), 80.69 (C-4b), 78.33 (C-2a), and 64.23 (C-6c).

The anomeric configurations at C-2d of the tetrasaccharides **2** and **3** were assigned to be 2α and 2β, respectively, by comparing the following ^{1}H-n.m.r. data with the data reported[8]. δ_H** (**2**, D_2O): 5.223 (d, *J* 0.5 Hz, H-1a), 4.634 (d, *J* 8.31 Hz, H-1b), 4.458 (d, *J* 7.82 Hz, H-1c), 2.679 (q, *J* 4.63 and 12.69 Hz, H-3d-*e*), 2.073 (3 H, s, Ac), 2.037 (3 H, s, Ac), and 1.731 (t, *J* 11.74 Hz, H-3d-*a*); δ_H (**3**, D_2O): 5.218 (d, *J* 0.5 Hz, H-1a), 4.623 (d, *J* 8.05 Hz, H-1b), 4.474 (d, *J* 7.81 Hz, H-1c), 2.395 (q, *J* 4.88 and 12.70 Hz, H-3d-*e*), 2.055 (6 H, s, 2 Ac), and 1.635 (t, *J* 12.70 Hz, H-3d-*a*).

In conclusion, the target tetrasaccharide **2** was synthesized in a regioselective way by employing the key trihexosyl acceptor **8** and the glycosyl donor **10**.

*The values of δ_C are expressed in p.p.m. downward from tetramethylsilane, referenced indirectly with an internal standard of 1,4-dioxane (δ_C 67.40).

**The values of δ_H are expressed in p.p.m. downward from the internal standard: sodium 2,2,3,3-tetradeuterio-4,4-dimethyl-4-silapentanoate.

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