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Preliminary communication

Synthesis of a hexasaccharide unit of a complex type of glycan chain of a glycoprotein*

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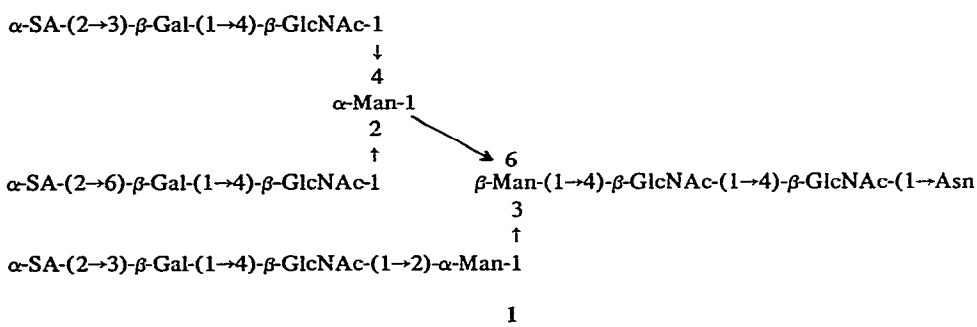
In 1979, Baenziger and Fiete[2] reported that the complete structure of the glycan chain of fetuin, the major glycoprotein in fetal-calf serum, is **1**. However, Svensson *et al.*[3] proposed an isomeric structure concerning the branching mode for this glycan chain. Similar, complex structures have recently been reported for the glycan part of such glycoproteins as human α_1-protease inhibitor[4], membrane glycoprotein of vesicular-stomatitis virus[5], α_1-acid glycoprotein of human plasma[6], and membrane glycoprotein of calf-thymocyte plasma[7].

The presence in several glycoproteins of biological importance of unique, branching glycans having three such antennae as **1** has stimulated our efforts directed towards their chemical synthesis, and we now report a regio- and stereo-controlled synthesis of the hexasaccharide unit **2**, a part of the structure of **1**. Retrosynthesis of **2** revealed the necessary monosaccharide synthons **13**, **8**, **7**, and **10** for the construction of **2** *via* the protected trisaccharide **12**. As the preparation of synthons **13** (ref. 8), **8** (ref. 9), and **10** (ref. 10) has already been reported, an efficient route for the synthesis of glycosyl donor **7** should first be developed.



*Part 8 in the series "Synthetic Studies on Cell-surface Glycans". For Part 7, see ref. 1.
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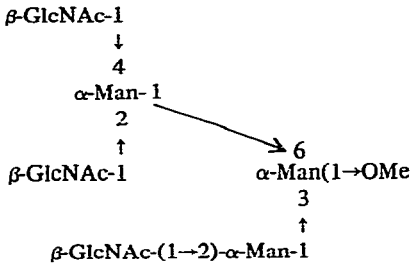


2

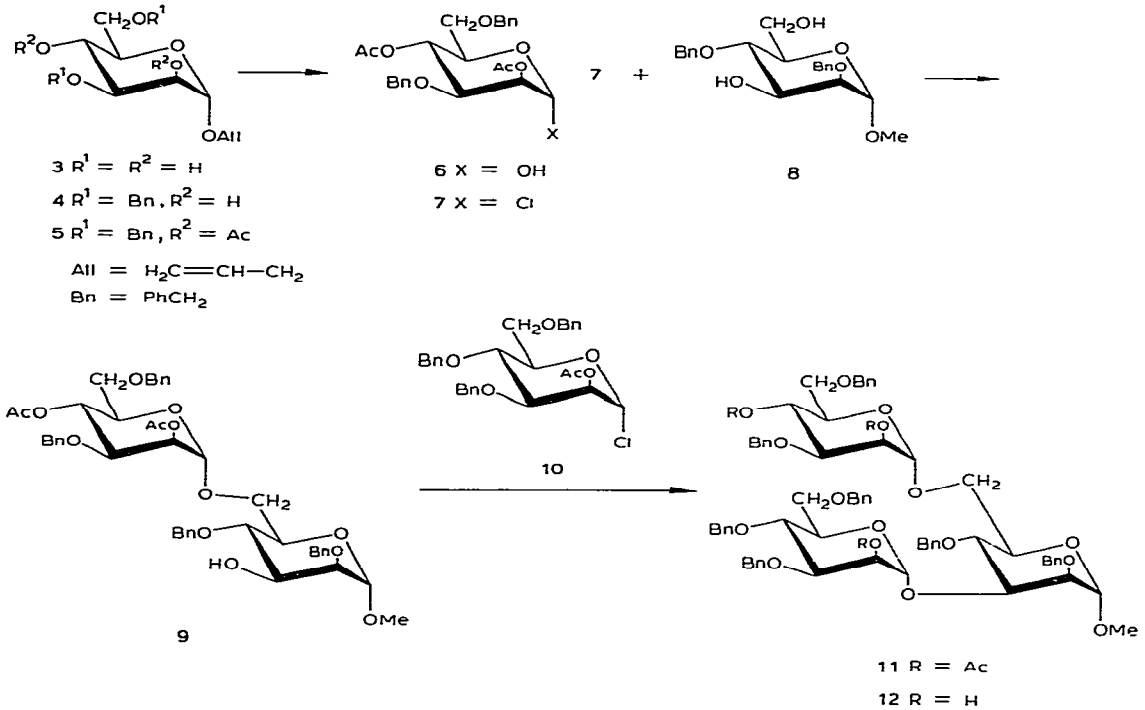
Allyl α-D-mannopyranoside **3** was regioselectively benzylated by the stannyl method[9,11], to give the 3,6-dibenzyl ether **4** in 54% yield†; $[\alpha]_D$ + 30.8° (*c* 0.39)††; R_F 0.44 (1 : 1 toluene—EtOAc). Acetylation of **4** give diacetate **5** and deallylation of **5** in the presence of $PdCl_2$—NaOAc in 20 : 1 AcOH—H_2O for 14 h at 25° afforded hemiacetal **6** in 64% yield; $[\alpha]_D$ -18.9° (*c* 0.37); R_F 0.30 in 3 : 1 toluene—EtOAc; n.m.r. ($CDCl_3$): δ_H 5.31 (t, *J* 3 Hz, H-2), 5.14 (d, *J* 2 Hz, H-1), 5.10 (t, *J* 10 Hz, H-4), 3.90 (dd, *J* 3, 10 Hz, H-3), and 2.10 and 1.90 (2 s, 2 Ac). Transformation of **6** into the α-chloride **7** was achieved quantitatively by treatment of **6** with $SOCl_2$ in the presence of a trace of $HCONMe_2$ in $Cl(CH_2)_2Cl$ for 14 h at 20°; **7**: $[\alpha]_D$ +40.0° (*c* 0.27); R_F 0.52 in 3.:1 toluene—EtOAc; δ_H ($CDCl_3$): 6.02 (d, *J* 2 Hz, H-1), 5.41 (dd, *J* 2, 4 Hz, H-2), 5.30 (t, *J* 10 Hz, H-4), 4.12 (dd, *J* 4, 10 Hz, H-3), and 2.10 and 1.89 (2 s, 2 Ac).

Glycosyl donor **7** having been synthesized, the route to the key intermediate, D-mannotrioside **12**, became evident. Selective D-mannosylation at the primary hydroxyl group of **8** with **7** was achieved under the Hanessian—Banoub conditions[12], to afford a 50% yield of **9**; $[\alpha]_D$ +29.7° (*c* 0.58); R_F 0.38 (3 : 1 toluene—EtOAc). Subsequent glycosylation of **9** with another D-mannosyl donor (**10**) afforded a 62% yield of the protected D-mannotrioside **11**; $[\alpha]_D$ +35.2° (*c* 0.29); R_F 0.49 in 3 : 1 toluene—EtOAc. Zemplén deacetylation of **11** afforded triol **12**; $[\alpha]_D$ +39.5° (*c* 0.39); R_F 0.30 in 1 : 1 toluene—EtOAc.

Glycosylation of **12** with donor **13** according to Lemieux *et al.*[13] gave a 35% yield of hexasaccharide **14**, $[\alpha]_D$ +22.4° (*c* 0.38); R_F 0.70 in 25 : 2 CH_2Cl_2—acetone. Deacylation of **14** with $BuNH_2$ according to Durette *et al.*[14]; and subsequent *N*-acetylation, led to the isolation of **15** in 20% yield; **15**: $[\alpha]_D$ +19.4° (*c* 0.18, MeOH); R_F 0.21 in 80 : 24 : 1 $CHCl_3$—MeOH—H_2O. Hydrogenolysis of **15** in the presence of 10% Pd—C in aq. EtOH afforded the target hexasaccharide **2**, $[\alpha]_D$ +14.7° (*c* 0.34, H_2O); R_F 0.20 in 10 : 30 : 3 $CHCl_3$—MeOH—H_2O. The ^{1}H-n.m.r. spectrum of **2** in D_2O at 80° showed signals for six anomeric protons: at δ 5.21 (d, 1 H, *J* 2 Hz), 5.00 (d, 1 H, *J* 2 Hz), and 4.80 (d, 1 H, *J* 1.5 Hz) for three anomeric protons for three α-D-mannopyranosyl

† Yields are not optimized.

†† Unless otherwise noted, optical rotations were determined at 25° for solutions in $CHCl_3$.



residues, and 4.68 (d, 1 H, *J* 6 Hz), 4.66 (d, 1 H, *J* 5 Hz), and 4.63 (d, 1 H, *J* 5 Hz) for three anomeric protons for three 2-acetamido-2-deoxy-β-D-glucopyranosyl residues. The stereochemistry at the six anomeric carbon atoms was confirmed by ^{13}C-n.m.r. spectroscopy (D_2O at 80°), which showed three signals for anomeric carbon atoms of α-D-mannopyranosyl residues at δ 101.5 ($^1J_{CH}$ 172.0 Hz, C-1a), 100.0 ($^1J_{CH}$ 172.0 Hz, C-1c), and 97.5 ($^1J_{CH}$ 170.8 Hz, C-1b), and two signals for anomeric carbon atoms of 2-acetamido-2-deoxy-β-D-glucopyranosyl residues, at 101.9 ($^1J_{CH}$ 156.0 Hz, C-1d) and 100.4 ($^1J_{CH}$ 156.0 Hz, C-1e, 1f), in good agreement with the observation of Bock and Pedersen[15] for the value of $^1J_{CH}$ at anomeric carbon atoms.

In conclusion, a regio- and stereo-controlled synthesis of the hexasaccharide unit 2 could be achieved by employing properly protected mannotrioside **12** as a key intermediate, which, in turn, was prepared from regioselectively benzylated monosaccharide synthons **8** and **7**. In close connection with our approach to the synthesis of **2**, it may be noted that an approach towards complex types of glycans having two antennae was recently reported by Anarp and Lönngren[16].

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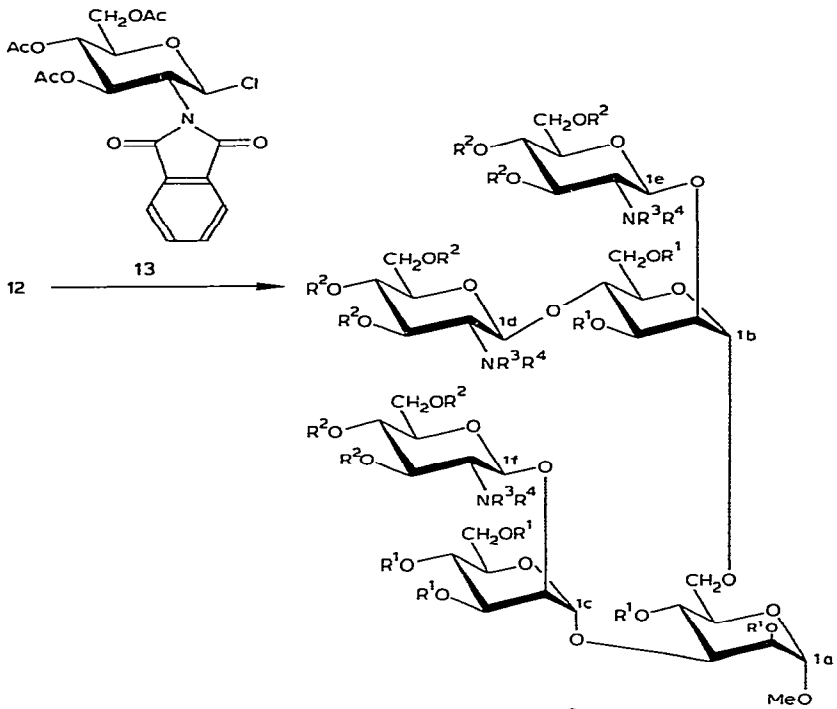

14 R^1 = Bn, R^2 = Ac, R^3, R^4 = phthaloyl

15 R^1 = Bn, R^2 = R^3 = H, R^4 = Ac

2 R^1 = R^2 = R^3 = H, R^4 = Ac

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