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Short Communication

Synthesis of a Haptasaccharide Hapten Related to an Anomalous Biantennary Glycan Chain of Human Chorionic Gonadotropin of a Patient with a Choriocarcinoma†

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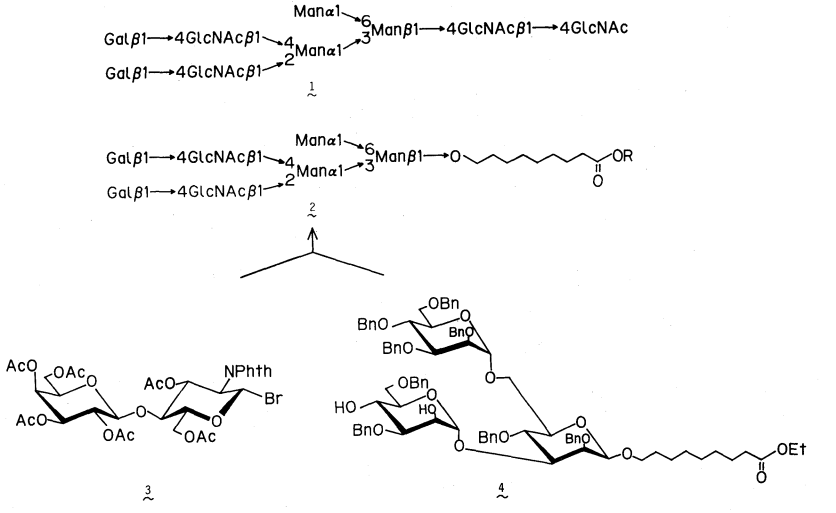
In 1983, anomalous structure **1** was proposed[2] for the biantennary glycan chain isolated from an α-subunit of hCG of a choriocarcinoma patient. As part of our project on the synthesis of artificial carbohydrate antigens, we describe here a synthetic approach for heptasaccharide hapten **2**, which may be of use for the preparation of a glycan chain specific antibody toward hCG of choriocarcinoma patients.

In order to develop a convergent type approach, target stucture **2** was retrosynthesised into readily available glycosyl donor **3** and regioselectively protected mannotriosyl C-9 ester **4** (Sheme 1). According to this synthetic plan, a synthetic route to key intermediate **4** and subsequent transformation of **4** into target structure **2** were developed as follows.

Regioselectively protected mannopyranosyl donor **6** was readily prepared from allyl α-D-mannopyranoside **5** according to the reported route[3] with slight modifications in a 44% overall yield. Glycosylation of mannobiosyl glycosyl acceptor **7**[1] with glycosyl donor **6** in the presence of silver triflate and powdered molecular sieves 4A in dichloroethane gave a 78% yield of mannotriosyl derivative **8**, $[\alpha]_D$ $-3.1°$ ($c=0.58$),*1 *Rf* 0.38 in 2:1 EtOAc-*n*-hexane. The stereochemistry of **8** was confirmed by the ^{13}C-NMR data, which showed three signals for anomeric carbon atoms at δ_C



SCHEME 1.

† Part 38 in the series "Synthetic Studies on Cell-surface Glycans." For Part 37, see ref. 1.

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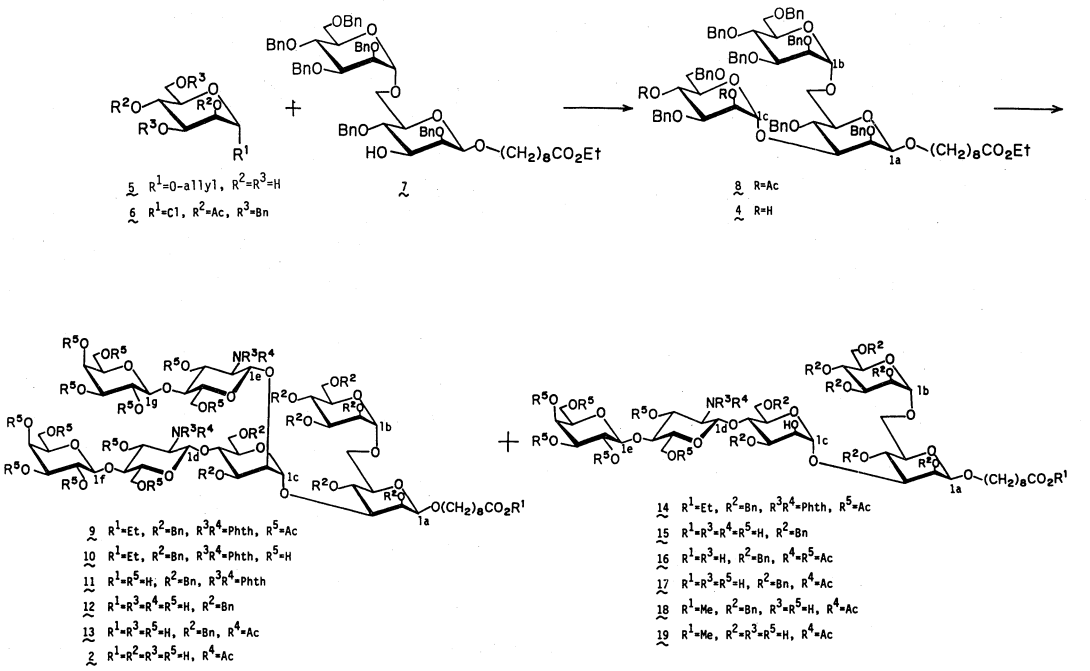
*1 $[\alpha]_D$ values were measured for $CHCl_3$ solutions at 25°, unless noted otherwise. Compounds having recordable $[\alpha]_D$ showed satisfactory elemental analyses results.



SCHEME 2.

(CDCl$_3$): 101.6 ($^1J_{CH}$ 153 Hz, C-1a), 99.7 ($^1J_{CH}$ 172 Hz, C-1c) and 98.6 ($^1J_{CH}$ 167 Hz, C-1b). Zemplén deacetylation of **8** with NaOEt in EtOH gave a quantitative yield of diol **4**, $[\alpha]_D$ +0.8° (c=1.07), *Rf* 0.49 in 1:1 EtOAc-*n*-hexane, δ_C (CDCl$_3$): 101.6 ($^1J_{CH}$ 154 Hz, C-1a and $^1J_{CH}$ 169 Hz, C-1c) and 98.6 ($^1J_{CH}$ 170 Hz, C-1b). Having prepared key glycosyl acceptor **4**, the crucial glycosylation of **4** with known lactosaminyl donor **3**[4] was performed in the presence of silver triflate and s-collidine[5] in dichloroethane. Separation of the products by flash chromatography over Wako gel C-300 in 3:2 toluene–EtOAc and subsequent purification by gel permeation chromatography over Bio-Beads SX 8 in benzene gave a 33% yield of monoglycosylated product **14**, $[\alpha]_D$ +14.4° (c=1.05), *Rf* 0.44 in 1:1 toluene–EtOAc, and a 38% yield of the desired biantennary product, **9**, $[\alpha]_D$ +2.5° (c=1.05), *Rf* 0.34 in 1:1 toluene–EtOAc. The structure of the glycosylation product was assignable from the reaction sequence and confirmed by the transformation into heptasaccharide hapten **2**. In the case of monoglycosylated product **14**, (1→4) linked structure **14** was expected from our previous observations[6] and was confirmed by the conversion to pentasaccharide hapten **19**, in the following way.

Successive treatment of **14** with (i) LiOH and aq. 31% H_2O_2 in THF,[7] (ii) $NH_2NH_2 \cdot H_2O$ in EtOH, and (iii) Ac_2O and pyridine gave a 40% overall yield of **16**, *Rf* 0.26 in 1:1 toluene–EtOAc, *via* **15**, *Rf* 0.23 in 6:1 $CHCl_3$–MeOH. Deacetylation of **16** with NaOMe in MeOH gave an 89% yield of **17**, *Rf* 0.21 in 6:1 $CHCl_3$–MeOH. Esterification of **17** with diazomethane in MeOH–Et_2O gave a 96% yield of methyl ester **18**, *Rf* 0.21 in 6:1 $CHCl_3$–MeOH. Hydrogenolysis of **18** in the presence of Pd–C in MeOH and purification on Sephadex G-25 (H_2O) finally afforded pentasaccharide hapten **19**, *Rf* 0.44 in 2:1:1 BuOH–EtOH–H_2O, δ_H (D_2O, at 20°)*[2]: 5.111 (1H, s, H-1c), 4.902 (1H, s, H-1b), 4.661 (1H, s, H-1a), 4.585 (1H, d, J=8.2 Hz, H-1d), 4.463

*[2] δ_H (D_2O) values are expressed as ppm downward from Me_4Si, with reference to an internal standard of Me_2CO (2.225).

(1H, d, J=7.6 Hz, H-1e) and 2.050 (3H, s, NAc).

These ^{1}H-NMR data for **19** were found to be different from those for the authentic pentasaccharide hapten[1] having a $\beta(1\rightarrow2)$ linkage between the GlcNAc and Man residues. Hence the structure of **19** was confirmed to contain a $\beta(1\rightarrow4)$ linkage between the GlcNAc and Man residues. In a similar way, diglycosylated product **9** was transformed into heptasaccharide hapten **2** as follows. Successive treatment of **9** with (i) LiOH, aq. 31% H_2O_2 in THF and then NaOH in MeOH, (ii) $NH_2NH_2 \cdot H_2O$ in EtOH, and (iii) Ac_2O in MeOH gave a 71% overall yield of **13**, *Rf* 0.58 in 2:1:1 BuOH–EtOH–H_2O, *via* **10**, **11** and **12** (*Rf* 0.65, 0.57 and 0.43, respectively, in 2:1:1 BuOH–EtOH–H_2O). Catalytic hydrogenolysis of **13** in the presence of Pd–C in MeOH–AcOH and purification on Sephadex G-25 (H_2O) gave a 44% yield of target heptasascharide hapten **2** (R=H) $[\alpha]_D$ +4.3° (c=0.50), *Rf* 0.17 in 2:1:1 BuOH–EtOH–H_2O. The structure of **2** was confirmed by the following ^{1}H-NMR data which were in good agreement with the reported data[8] for related complex type glycan chains: δ_H (D_2O, at 20°): 5.122 (1H, s, H-1c), 4.903 (1H, s, H-1b), 4.659 (1H, s, H-1a), 4.561 (1H, d, J= 7.0 Hz) and 4.542 (1H, d, J=8.2 Hz) for H-1d and H-1e, 4.462 (2H, d, J=7.6 Hz, H-1f and H-1g), 2.064 (3H, s, NAc) and 2.038 (3H, s, NAc).

In conclusion, heptasaccharide hapten **2** carrying an anomalous biantennary glycan chain of hCG of patients with choriocarcinomas was synthesized in a stereo- and regio-controlled manner by employing key intermediates **3** and **4**.

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