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Preliminary communication

Synthesis of glycopeptides having clusters of *O*-glycosylic disaccharide chains [β-D-Gal-(1→3)-α-D-GalNAc] located at vicinal amino acid residues of the peptide chain*

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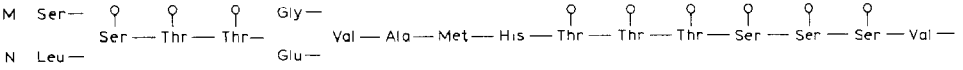
The Thomsen–Friedenreich (T) receptor is defined as the cryptic structure that is uncovered after neuraminidase treatment of vertebrate erythrocytes[1]. This enzyme removes sialic acid residues from the oligosaccharide chains which are mainly located at the amino-terminal region of glycophorin A (PAS-1), the major glycoprotein of the human red-cell membrane. This region, which is known to carry MN blood-group specificities, has the following primary structure[2].



One characteristic feature of this structure is a genetically determined polymorphism at the first and fifth positions which, in part, may account for the observed MN-specificities[3]. On the other hand, the amino-terminal segment uniquely displays two typical clusters of *O*-glycosylic oligosaccharide chains (♀) located at vicinal amino acid residues (Ser or Thr) of the polypeptide chain. After removal of sialic acid residues, the oligosaccharide chain is the disaccharide β-D-Gal-(1→3)-α-D-GalNAc, which is considered to be the antigenic determinant of the Thomsen–Friedenreich (T) antigen[1].

The chemical synthesis of such heavily clustered glycopeptides is important, in order to define the influence of a high density of carbohydrate antigenic-determinants on the expression of the T-antigenicity. All glycopeptides active toward *Vicia graminea* lectin have such heavy clusters[4]. Furthermore, knowledge of the precise chemical structure of the Thomsen–Friedenreich receptor, a tumour-associated antigen that is not oncofetal in origin[5], could be of importance in cancer research. We now describe a synthesis of such heavy clusters.

We recently reported[6] the synthesis of *O*-(2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl)-(1→3)-*O*-(2-acetamido-4,6-di-*O*-acetyl-2-deoxy-α-D-galactopyranosyl)-(1→3)-*N*-(benzyloxycarbonyl)-L-threonine *tert*-butyl ester (**1**) and the L-serine analogue **2**.

*Dedicated to Professor Edgar Lederer on the occasion of his 75th birthday.
**To whom enquiries should be sent.

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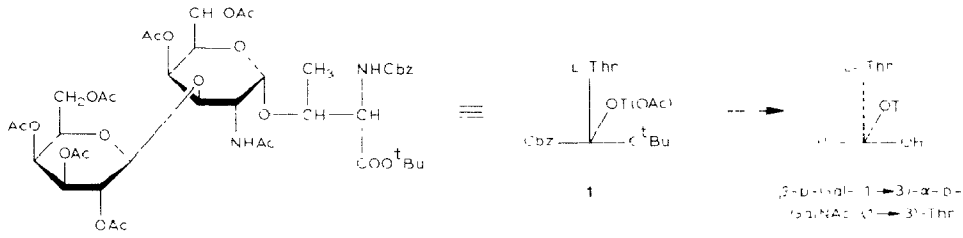
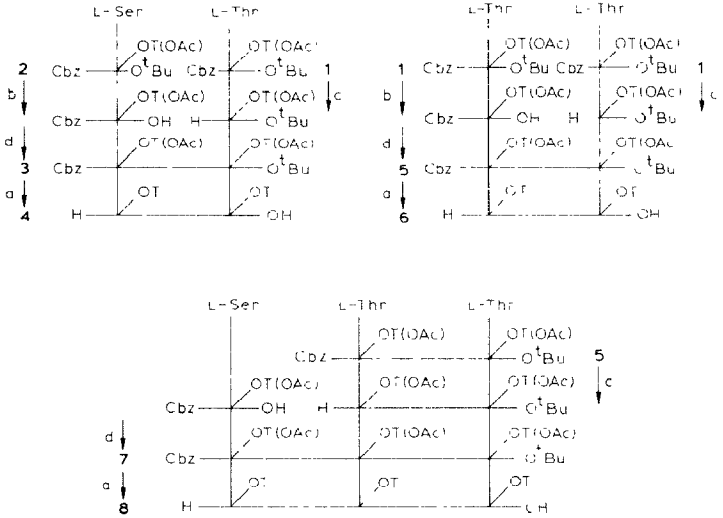


Scheme 1 Reagents (1) MeOH–Et_3N, room temperature, 48 h, (2) CF_3COOH, room temperature, 1 h, (3) H_2, 10% Pd/C, MeOH–AcOH (10 : 1), room temperature, 24 h

Application to **1** of the sequence shown in Scheme 1 gave, after purification on Sephadex G-10, *O*-β-D-galactopyranosyl-(1→3)-*O*-(2-acetamido-2-deoxy-α-D-galactopyranosyl)-(1→3)-L-threonine [β-D-Gal-(1→3)-α-D-GalNAc-(1→3)-Thr] (80%), $[\alpha]_D$ +83° (*c* 0.5, water); lit.[7] $[\alpha]_D$ +90° (*c* 1.3, water). *O*-Deacetylation of **1** with Et_3N–MeOH did not promote β-elimination of the disaccharide[8], and the *O*-glycosylic linkage was resistant to brief treatment with trifluoroacetic acid[9]. The homogeneity and identity of this disaccharide–amino acid were confirmed by p.c. and ^{1}H-n.m.r. spectroscopy (400 MHz, D_2O).

The selective deblocking of the *N*- and *C*-terminals of **1** and **2**, and of the tetrasaccharide-dipeptide **5**, was performed as shown in Scheme 2. The peptide linkage was formed in the presence of 2-ethoxy-*N*-(ethoxycarbonyl)-1,2-dihydroquinoline[11] (EEDQ), to give the protected clusters **3** (63%), **5** (65%), and **7** (62%).



Scheme 2 Reagents (a) see Scheme 1, (b) CF_3COOH, room temperature, 1 h, (c) 10% Pd/C, cyclohexene–EtOH (1 : 2), reflux, 1 h[10], (d) EEDQ, dichloromethane, −10°→room temperature, 15 h

Compound **3** had $[\alpha]_D$ +73° (*c* 1.5, chloroform). ^{1}H-N.m.r. data (400 MHz, $CDCl_3$): δ 7.34 (s, 5 H, Ph), 6.53 (d, 1 H, *J* 9 Hz, NH), 6.21 (d, 1 H, *J* 8 Hz, NH), 5.06 (d, 1 H, $J_{1,2}$ 3.8 Hz, H-1), 4.63 (d, 1 H, $J_{1',2'}$ 8 Hz, H-1′), 4.58 (d, 1 H, $J_{1',2'}$ 8 Hz, H-1′), 2.23–1.93 (14 s, 42 H, 14 Ac), 1.52 (s, 9 H, ^{t}Bu), and 1.29 (d, 3 H, $J_{CH,Me}$ 7 Hz, Thr Me).

Anal. Calc. for $C_{71}H_{98}N_4O_{39}$: C, 52.27; H, 6.05; N, 3.43. Found: C, 52.06; H, 6.09; N, 3.32.

Compound **5** had $[\alpha]_D$ +75° (*c* 1, chloroform). ^{1}H-N.m.r. data (400 MHz, $CDCl_3$): δ 7.35 (s, 5 H, Ph), 6.55, 6.11, and 5.83 (3 d, 3 H, *J* 9 Hz, NH), 5.15 (d, 1 H, $J_{1,2}$ 3.4 Hz, H-1), 5.02 (d, 1 H, $J_{1,2}$ 3.5 Hz, H-1), 4.66 (d, 1 H, $J_{1',2'}$ 7.6 Hz, H-1'), 4.64 (d, 1 H, $J_{1',2'}$ 7.8 Hz, H-1'), 2.28–2.00 (14 s, 42 H, 14 Ac), 1.57 (s, 9 H, ^{t}Bu), 1.48 and 1.40 (2 d, 6 H, $J_{CH,Me}$ 6.4 Hz, Thr Me).

Anal. Calc. for $C_{72}H_{100}N_4O_{39}\cdot H_2O$: C, 51.98; H, 6.18; N, 3.36. Found: C, 51.94; H, 6.07; N, 3.44.

Compound **7** had $[\alpha]_D$ +78° (*c* 0.9, chloroform).

Anal. Calc. for $C_{101}H_{140}N_6O_{57}\cdot 3H_2O$: C, 50.45; H, 6.12; N, 3.49. Found: C, 50.41; H, 6.17; N, 3.43.

Deprotection of the clusters was routinely performed, using the sequence described in Scheme 1, and purification was achieved on Sephadex G-10 (clusters **4** and **6**) or Sephadex G-25 (cluster **8**). Elution with water gave a single peak at 206 nm. Amino acid analyses were in agreement with the structures.

Compound **4** had $[\alpha]_D$ +67° (*c* 0.7, water). ^{1}H-N.m.r. data (400 MHz, D_2O): δ 4.91 (d, 1 H, $J_{1,2}$ 3 Hz, H-1), 4.89 (d, 1 H, $J_{1,2}$ 3 Hz, H-1), 1.93 and 1.87 (2 s, 6 H, 2 Ac), and 1.14 (d, 3 H, $J_{CH,Me}$ 6.3 Hz, Thr Me).

Compound **6** had $[\alpha]_D$ +97° (*c* 1.5, water). ^{1}H-N.m.r. data (400 MHz, D_2O): δ 5.03 (d, 1 H, $J_{1,2}$ 3.4 Hz, H-1), 4.92 (d, 1 H, $J_{1,2}$ 3.2 Hz, H-1), 1.97 and 1.95 (2 s, 6 H, 2 Ac), 1.25 and 1.22 (2 d, 6 H, $J_{CH,Me}$ 6.5 Hz, Thr Me).

Compound **8** had $[\alpha]_D$ +109° (*c* 0.5, water). ^{1}H-N.m.r. data (400 MHz, D_2O): δ 2.04, 2.01, and 1.99 (3 s, 9 H, 3 Ac), 1.33 and 1.18 (2 d, 6 H, $J_{CH,Me}$ 6.5 Hz, Thr Me).

Using the same methodology, analogues were prepared with either L-serine or L-leucine as the *N*-terminal amino acid. These compounds are useful for studies of the molecular basis of the sialic acid-independent MN-antigenicity[12] and to define the receptor site of *Vicia graminea* lectin[4]. Such studies will be reported elsewhere.

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