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Synthesis of histo blood-group antigens A and B (type 2), xenoantigen Galα1-3Galβ1-4GlcNAc and related type 2 backbone oligosaccharides as haptens in spacered form

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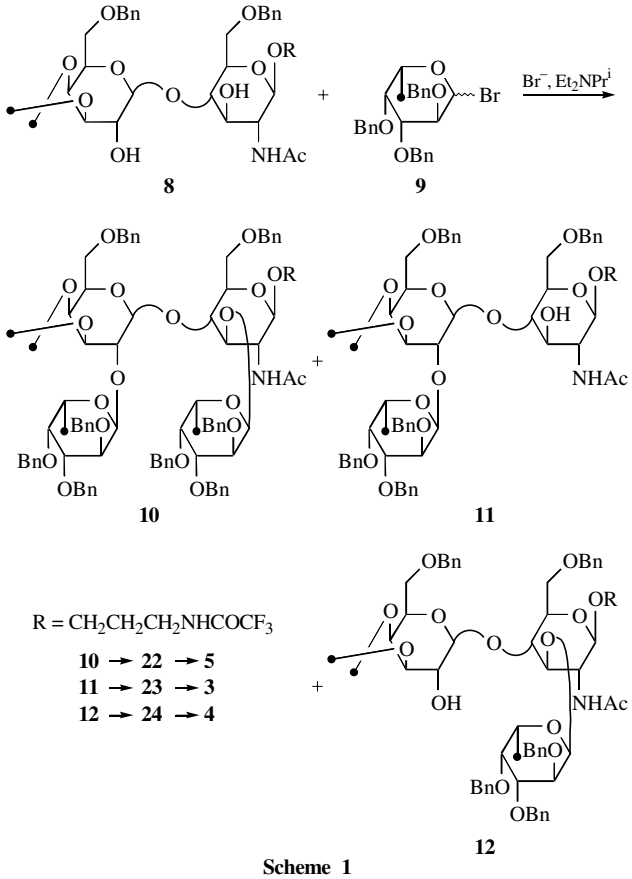
The partial α-fucosylation of spacered (sp = $CH_2CH_2CH_2NH_2$) *N*-acetyl β-lactosamine derivative having free hydroxy groups at C-3 and C-2′ gives rise to oligosaccharides H (type 2), Le^x and Le^y, the first one was further elongated giving rise to A (type 2) or B (type 2) tetrasaccharides; spacered trisaccharides Galα1-3Galβ1-4GlcNAc and Galα1-4Galβ1-4GlcNAc were synthesised by the partial α-galactosylation of *N*-acetyl β-lactosamine derivative, having free hydroxy groups at C-4′ and C-3′.

Type 2 blood group oligosaccharides, *e.g.*, the Galβ1-4GlcNAc backbone-based structures in the composition of glycoproteins and glycolipids, are widespread antigens; namely, A, B, H and P_1 are major human erythrocyte and histo blood-group antigens,[1] trisaccharide B (type 2) is the main xenoantigen causing hyperacute rejection during pig-to-human transplantation,[2] Le^x (Lewis X) is CD15 antigen[3] and Le^y (Lewis Y) is one of the most promising cancer-associated antigen for oncovaccination.[4] Here we present a practical convergent synthesis of oligosaccharides **1**–**7** in spacered form (R = $CH_2CH_2CH_2NH_2$) suitable for the further synthesis of various glycoprobes.[5] It should be noted that the methods of glycosylation, as well as a protecting–deprotecting strategy, are based on the approaches of H. Paulsen and R. Lemieux with co-workers.[6,7] In this work, we used a very convenient aminopropyl spacer arm, as well as a really practical strategy, which allowed us to synthesise several oligosaccharides by a single glycosylation procedure.

GalNAcα1-3(Fucα1-2)Galβ1-4GlcNAcβ-OR	A (type 2)	**1**
Galα1-3(Fucα1-2)Galβ1-4GlcNAcβ-OR	B (type 2)	**2**
Fucα1-2Galβ1-4GlcNAcβ-OR	H (type 2)	**3**
Galβ1-4(Fucα1-3)GlcNAcβ-OR	Le^x	**4**
Fucα1-2Galβ1-4(Fucα1-3)GlcNAcβ-OR	Le^y	**5**
Galα1-3Galβ1-4GlcNAcβ-OR	B_{tri}(type 2)	**6**
Galα1-4Galβ1-4GlcNAcβ-OR	P_1	**7**
(R = $CH_2CH_2CH_2NH_2$)		

The synthesis of oligosaccharides **3**–**5** was accomplished as a one-pot process based on a regio-nonspecific fucosylation of lactosamine derivative **8** (the syntheses of this compound and other starting compounds are described below) bearing two hydroxyls, at C-3 of a glucosamine moiety and at C-2 of a galactose moiety. The fucosylation of **8**[7] with a sixfold excess of fucosyl donor **9** (see Scheme 1) yields tetrasaccharide **10** (70–80%), whereas glycosylation with a smaller excess (2 mol) of **9** provides an easy-to-separate (chromatography on silica gel) mixture of tetrasaccharide **10** (19%) with trisaccharides **11** (43%) and **12** (9%) and starting diol **8** (17%). Thus, limited glycosylation leads to a moderate yield of trisaccharide H derivative **11**, which is a building block for the synthesis of tetrasaccharides A and B, together with 'side' oligosaccharides Le^x **12** and Le^y **10**.

As shown in Scheme 2, derivative **11** of trisaccharide H (type 2) was converted into diol **13** (84–93% yield) by O-acetylation followed by acid removal of benzylidene protection. Compound **13** was directly regioselectively α-galactosylated by bromide **14** at the 3-position giving rise to protected blood group B tetrasaccharide **15** (50%) and side 1-4 tetrasaccharide (28%). Corresponding A tetrasaccharide **16** was synthesised from alcohol **17** obtained from **13** by an orthoester procedure[8] (89% yield, see Scheme 2), using benzylated azido donor **18**. The yield of tetrasaccharide **16** was 40%; starting acceptor **17** (40%) was also isolated from the reaction mixture. Alternatively, a derivative of A tetrasaccharide **16a** was obtained using diol **13** as a glycosyl acceptor and bromide **18** as a glycosyl donor; in this case, the yield was as low as 25%.



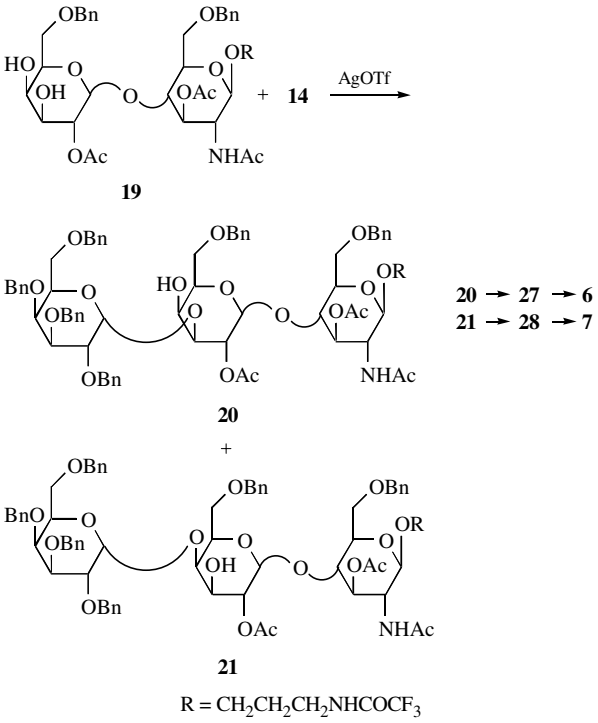
Scheme 1

Isomeric trisaccharides **20** and **21** were obtained in a one-pot α-galactosylation procedure starting from another lactosamine acceptor, diol **19**, bearing hydroxyls at C-3 and C-4 positions of a galactose moiety, and donor **14**. The yields of major product 1-3 isomer **20** and minor 1-4 isomer **21** were 50 and 10%, respectively (Scheme 3).

Fucosylation with donor **9** and galactosylation with donors **14** and **18**, performed under standard conditions,[6,7] were α-stereoselective; the corresponding β-anomeric products were isolated by low pressure chromatography in minor (5–10%) amounts, if any.

Standard deprotection[6,7] of oligosaccharides **10**–**12**, **15**, **16**, **20** and **21** gave spacered (sp = $CH_2CH_2CH_2NHCOCF_3$) oligosaccharides, which were purified and characterised by ^{1}H NMR spectra as per-O-acetates **22**–**24**[9] and **25**–**28**.† Acetylation was performed using Ac_2O/Py.

Starting disaccharide **8** was obtained by the glycosylation of acceptor **29**[10] with 6-O-Bn galactosyl donor **30**[11] (68% yield) followed by the Zemplen (catalytic amount of MeONa in MeOH) deacetylation and acetonation at 3′,4′-positions (Scheme 4), 78% yield. Diol **19** (62%) was obtained from diol **8** by sequential



Scheme 3

O-acetylation and removal of isopropylidene protection,[10] as shown in Scheme 4.

† *^{1}H NMR spectra of oligosaccharide peracetates.*

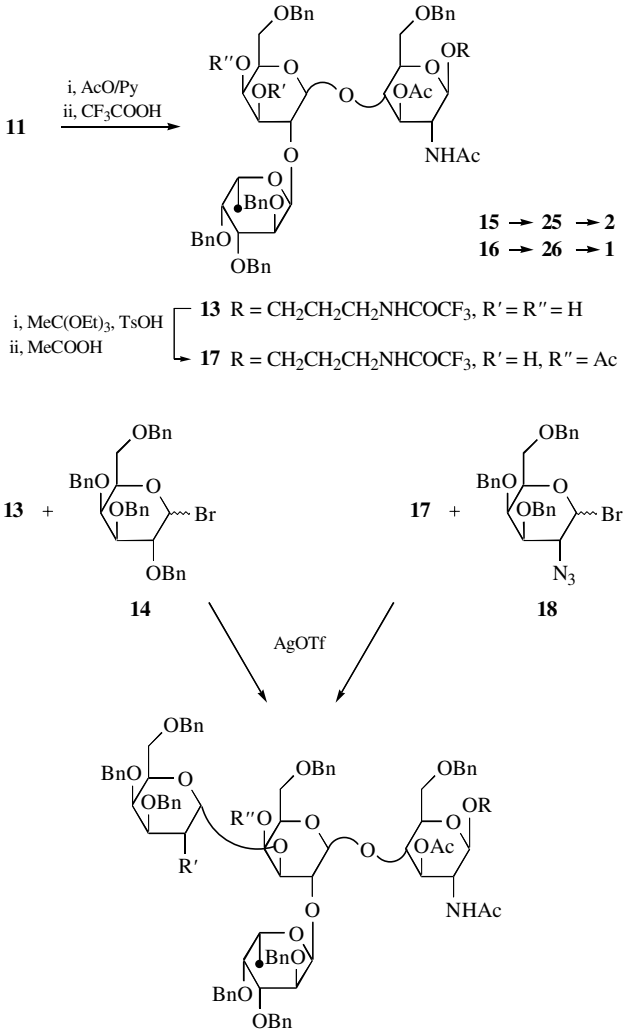
25: Ac: 2.155 (s), 2.140 (2s), 2.106 (s), 2.086 (s), 2.068 (s), 2.065 (s), 2.061 (s), 1.981 (s), 1.953 (s); Galα1-3-: 5.322 (d, H-1, $J_{1,2}$ 3.5 Hz), 5.335 (dd, H-2), 5.40–5.45 (m, H-3), 5.598 (dd, H-4, $J_{4,5}$ < 1.0 Hz), 4.446 (m, H-5), 7.429 (m, $NHCOCF_3$*); Fucα1-2-: 5.422 (d, H-1, $J_{1,2}$ 3.7 Hz), 5.207 (dd, H-2, $J_{2,3}$ 11.0 Hz), 5.040 (dd, H-3, $J_{3,4}$ 3.2 Hz), 5.248 (dd, H-4, $J_{4,5}$ 1.0 Hz), 4.459 (m, H-5); >Galβ1-4-: 4.386 (d, H-1, $J_{1,2}$ 7.4 Hz), 3.553 (dd, H-2, $J_{2,3}$ 10.0 Hz), 3.880 (dd, H-3, $J_{3,4}$ 2.7 Hz), 5.406 (dd, H-4, $J_{4,5}$ < 1.0 Hz), 3.785 (m, H-5); -GlcNAcβ1-Osp: 4.494 (d, H-1, $J_{1,2}$ 7.6 Hz), 4.018 (ddd, H-2, $J_{2,3}$ 8.8 Hz), 5.066 (dd, H-3, $J_{3,4}$ 9.1 Hz), 3.911 (dd, H-4, $J_{4,5}$ 8.3 Hz), 3.772 (m, H-5), 5.949 (d, N*H*Ac, $J_{NH,2}$ 8.3 Hz).

26: Ac: 1.906 (s), 1.965 (s), 1.982 (s), 1.993 (s), 2.066 (3s), 2.076 (s), 2.101 (s), 2.105 (s), 2.146 (s), 2.157 (s); Fuc Me: 1.15 (d, 3H, $J_{5,6}$ 6.6 Hz); GalNAcα1-3-: 5.212 (d, H-1, $J_{1,2}$ 3.20 Hz), 4.450 (m, H-2), 5.003 (dd, H-3, $J_{3,4}$ 3.0 Hz), 5.420 (dd, H-4, $J_{4,5}$ < 1.0), 6.316 (d, N*H*Ac, $J_{NH,2}$ 9.4 Hz), 7.444 (m, $NHCOCF_3$*); Fucα1-2-: 5.508 (d, H-1, $J_{1,2}$ 3.5 Hz), 5.274 (dd, H-2, $J_{2,3}$ 11.0 Hz), 5.104 (dd, H-3, $J_{3,4}$ 3.0 Hz), 5.300 (dd, H-4, $J_{4,5}$ < 1.0 Hz); >Galβ1-4-: 4.403 (d, H-1, $J_{1,2}$ 7.3 Hz), 3.748 (dd, H-2, $J_{2,3}$ 9.5 Hz), 3.868 (dd, H-3, $J_{3,4}$ 3.5 Hz), 5.372 (dd, H-4, $J_{4,5}$ < 1.0 Hz); –GlcNAcβ1-Osp: 4.487 (d, H-1, $J_{1,2}$ 8.0 Hz), 3.990 (ddd, H-2, $J_{2,3}$ 9.0 Hz), 5.072 (dd, H-3, $J_{3,4}$ 10.0 Hz), 3.860 (dd, H-4, $J_{4,5}$ 8.5 Hz), 5.886 (d, N*H*Ac, $J_{NH,2}$ 8.9 Hz).

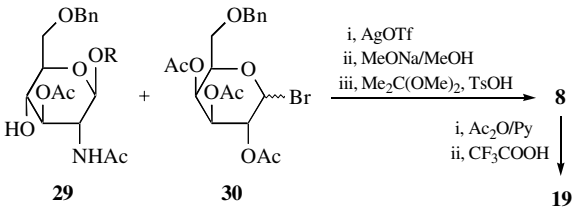
27 (300 MHz): Ac: 1.951 (s), 1.978 (2s), 1.998 (s), 2.062 (s), 2.079 (s), 2.085 (s), 2.118 (s), 2.125 (s), 2.139 (2s), 2.159 (s); Fuc Me: 1.168 (d, 3-H, $J_{5,6}$ 7.0 Hz); Galα1-3-: 5.254 (d, H-1, $J_{1,2}$ 3.5 Hz), 5.269 (dd, H-2, $J_{2,3}$ 10.7 Hz), 5.112 (dd, H-3, $J_{3,4}$ 3.1 Hz), 5.453 (dd, H-4, $J_{4,5}$ < 1.0 Hz), 4.223 (m, H-5), 7.399 (m, $NHCOCF_3$*); –Galβ1-4-: 4.454 (d, H-1, $J_{1,2}$ 8.0 Hz), 5.164 (dd, H-2, $J_{2,3}$ 10.2 Hz), 3.863 (dd, H-3, $J_{3,4}$ 3.0 Hz), 5.347 (dd, H-4, $J_{4,5}$ < 1.0 Hz), 3.821 (m, H-5); –GlcNAcβ1-Osp: 4.418 (d, H-1, $J_{1,2}$ 7.06 Hz), 4.029 (ddd, H-2, $J_{2,3}$ 9.0 Hz), 5.061 (dd, H-3, $J_{3,4}$ 8.6 Hz), 3.775 (dd, H-4, $J_{4,5}$ 8.1 Hz), 3.60–3.68 (m, H-5), 5.909 (d, N*H*Ac, $J_{NH,2}$ 8.9 Hz).

28 (300 MHz): Ac: 1.942 (s), 1.957 (s), 2.017 (s), 2.022 (s), 2.044 (2s), 2.054 (s), 2.079 (2s), 2.101 (s); Galα1-4-: 4.968 (d, H-1, $J_{1,2}$ 3.5 Hz); 5.167 (dd, H-2, $J_{2,3}$ 11.0 Hz), 5.347 (dd, H-3, $J_{3,4}$ 3.3 Hz), 5.542 (dd, H-4, $J_{4,5}$ < 1.0 Hz), 4.450 (m, H-5), 7.553 (m, $NHCOCF_3$*); –Galβ1-4-: 4.534 (d, H-1, $J_{1,2}$ 7.5 Hz), 5.083 (dd, H-2, $J_{2,3}$ 10.5 Hz), 4.740 (dd, H-3, $J_{3,4}$ 2.4 Hz), 4.011 (dd, H-4, $J_{4,5}$ < 1.0 Hz), 3.756 (m, H-5); –GlcNAcβ1-Osp: 4.411 (d, H-1, $J_{1,2}$ 7.3 Hz), 4.000 (ddd, H-2, $J_{2,3}$ 9.0 Hz), 5.049 (dd, H-3, $J_{3,4}$ 9.4 Hz), 3.756 (dd, H-4, $J_{4,5}$ 8.6 Hz), 3.620 (m, H-5), 6.118 (d, N*H*Ac, $J_{NH,2}$ 8.8 Hz).

* Signals of $O(CH_2)_3N$: 1.75–1.95, 3.20–3.35, 3.55–3.70, 3.85–3.095, H-5, Hb-6a; H-6b: 3.50–4.50.



Scheme 2



Scheme 4

The Zemplen de-O-acetylation of **22**–**28** followed by removal of the N-trifluoroacetyl group with Amberlyst (in OH^- form) gave oligosaccharides **5**,[9] **3**, **4**,[9] **2**, **1**, **6** and **7**, respectively, bearing $(CH_2)_3NH_2$ spacer groups. The NMR data of compounds **1**–**7** are consistent with the assumed structures: $J_{1,2}$ values correspond to expected anomeric configurations; characteristic groups such as Me–C and MeCONH are unambiguously identified.‡

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‡ **1**, A_{tetr} (type 2): ^{1}H NMR (500 MHz, D_2O) δ: 5.280 (d, 1H, H-1, $J_{1,2}$ 3.9 Hz), 5.108 (d, 1H, H-1, $J_{1,2}$ 3.7 Hz), 4.526 (d, 1H, H-1, $J_{1,2}$ 7.6 Hz), 4.432 (d, 1H, H-1, $J_{1,2}$ 8.6 Hz), 1.982 (s, 3H, NHCO*Me*), 1.970 (s, 3H, NHCO*Me*), 1.175 (d, 3H, Me fucose, $J_{5,6}$ 6.6 Hz). MS, *m*/*z*: 812 (789 + 23, $M^+ + Na^+$), $[\alpha]_D$ +17 (*c*1, H_2O).

2, B_{tetr} (type 2): ^{1}H NMR (500 MHz, D_2O) δ: 5.303 (d, 1H, H-1, $J_{1,2}$ 4.2 Hz), 5.216 (s, 1H, H-1), 4.587 (d, 1H, H-1, $J_{1,2}$ 7.6 Hz), 4.464 (d, 1H, H-1, $J_{1,2}$ 8.3 Hz), 2.027 (s, 3H, NHCO*Me*), 1.208 (d, 3H, Me fucose, $J_{5,6}$ 6.6 Hz). ^{13}C NMR (500 MHz, D_2O) δ: 102.54 (C-1), 101.54 (C-1), 100.08 (C-1), 94.39 (C-1), 56.60 (C-2, CNHAc). MS, *m*/*z*: 772 (749 + 23, $M^+ + Na^+$). $[\alpha]_D$ –5 (*c*1, H_2O).

6, B_{tri} (type 2): ^{1}H NMR (500 MHz, D_2O) δ: 5.127 (d, 1H, H-1, $J_{1,2}$ 3.7 Hz), 4.522 (d, 1H, H-1, $J_{1,2}$ 7.9 Hz), 4.501 (d, 1H, H-1, $J_{1,2}$ 7.9 Hz), 2.031 (d, 3H, NHCO*Me*). ^{13}C NMR (300 MHz, D_2O) δ: 104.03 (C-1), 102.37 (C-1), 96.67 (C-1), 56.25 (C-2, CNHAc). MS, *m*/*z*: 626 (603 + 23, $M^+ + Na^+$). $[\alpha]_D$ +54 (*c*1, H_2O).

7, P_1: ^{1}H NMR (500 MHz, D_2O) δ: 4.918 (d, 1H, H-1, $J_{1,2}$ 3.9 Hz), 4.497 (d, 1H, H-1, $J_{1,2}$ 7.6 Hz), 4.485 (d, 1H, H-1, $J_{1,2}$ 7.6 Hz), 2.019 (s, 3H, NHCO*Me*). MS, *m*/*z*: 626 (603 + 23, $M^+ + Na^+$). $[\alpha]_D$ +30 (*c*1, H_2O).

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