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Synthesis of Sug1-4GalNAcα disaccharides and their interaction with human blood antibodies

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^{1}H, ^{13}C NMR spectra were registered on a Bruker AVANCE spectrometer (Bruker BioSpin MRI GmbH, Germany) at 303K. Chemical shifts δ for characteristic protons are given in ppm using HOD (δ 4.750), $CHCl_3$ (δ 7.270) as references. Coupling constants *J* are given in Hz. The signals in ^{1}H NMR spectra were assigned using a technique of spin–spin decoupling (double resonance) and 2D-^{1}H,^{1}H- COSY experiments. The values of optical rotation were measured on a Perkin Elmer 341 digital polarimeter at 25°C. Mass spectra were registered on a MALDI-TOF Vision-2000 spectrometer using dihydroxybenzoic acid as a matrix. TLC was carried out on silica gel 60 precoated aluminum sheets (Merk, Germany).

6-OBn-3-OAc-GalNAcα-O(CH$_2$)$_3$NHC(O)CF$_3$ (2): ^{1}H-NMR (700 MHz, $CDCl_3$): 1.83–1.96 (m, 2H, CH_2 sp); 1.99 (s, 3H, COC*H*$_3$); 2.11 (s, 3H, NCOC*H*$_3$); 2.70 (br. s, 1H, O*H*); 3.36–3.42 (m, 1H, NC*H* sp); 3.47–3.52 (m, 1H, OC*H* sp); 3.63–3.68 (m, 1H, NC*H* sp); 3.71–3.77 (m, 2H, H6', H6"); 3.77–3.82 (m, 1H, OC*H* sp); 3.85 (dd, 1H, H-6"a, $J_{5,6"}$ 5.0, $J_{6',6"}$ 12.0); 3.93 (dd, 1H, H-6"b, $J_{5,6"}$ 2.2, $J_{6',6"}$ 12.3); 3.97 (br. t, 1H, H-5, *J* 5.2); 4.10 (br. d, 1H, H-4, *J* 2.5); 4.59 (br. s, 2H, C*H*$_2$Ph (Bn)); 4.68 (ddd, 1H, H-2, $J_{1,2}$ 3.7, $J_{2,3}$ 11.2, $J_{2,NH}$ 9.4); 4.89 (d, 1H, H-1, $J_{1,2}$ 3.7); 5.09 (dd, 1H, H-3, $J_{2,3}$ 11.2, $J_{3,4}$ 2.9); 5.93 (d, 1H, N*H*Ac, $J_{2,NH}$ 9.4); 6.60–6.67 (m, 1H, N*H*C(O)CF_3); 7.28–7.38 (m, 5H, Ar*H*). ^{13}C NMR (DEPT-135, 176 MHz, $CDCl_3$): δ 128.56, 127.97, 127.78 (Ph); 98.07 (C-1); 73.86 (Ph*C*H$_2$); 71.26, 69.98, 69.36, 68.37 (C-3, C-4, C-5, C-6); 64.85 (*C*H$_2$O sp); 47.83 (C-2); 36.83 (N*C*H$_2$ sp); 28.81 (*C*H$_2$ sp); 23.16 (NC(O)*C*H$_3$); 21.02 (OC(O)*C*H$_3$). R_f 0.36 (ethylacetate–isopropanol, 20:1). MS, *m/z* calculated for [$C_{22}H_{29}N_2O_8F_3$]Na^+: 529.18; found 529. $[\alpha]^{25}_{546\ nm}$ 110 (c 0.3; $CHCl_3$).

GalNAcβ1-4GalNAcα-O(CH$_2$)$_3$NH$_2$ (8β): ^{1}H-NMR (700 MHz, D_2O): 1.95–2.03 (m, 2H, CH_2 sp); 2.04, 2.05 (2s, 2×3H, NCOC*H*$_3$); 3.12 (br. t, 2H, NC*H*$_2$ sp, *J* 7.6); 3.55–3.60 (m, 1H, OC*H* sp); 3.67 (br. dd, 1H, H-5b, $J_{4,5}$ ≈1.0, $J_{5,6'}$ 8.2, $J_{5,6"}$ 4.3); 3.74–3.86 (m, 6H, H-6'a, H-6"a, H-6'b, H-6"b, OC*H* sp, H-3b); 3.90–3.97 (m, 3H, H-4b, H-5a, H-2b); 4.05 (dd, 1H, H-3a, $J_{3,4}$ 2.9, $J_{2,3}$ 11.1); 4.13 (dd, 1H, H-2a, $J_{1,2}$ 3.8, $J_{2,3}$ 11.1); 4.18 (br. d, 1H, H-4a, *J* 3.2); 4.67 (d, 1H, H-1b, $J_{1,2}$

8.5); 4.90 (d, 1H, H-1a, $J_{1,2}$ 3.8). R_f 0.43 (MeOH–1M aq. Py·AcOH, 5:1). MS, *m/z* calculated for $[C_{19}H_{35}N_3O_{11}]H^+$: 482.23; found 482. $[\alpha]_{546\ nm}$ 127 (c 0.23; MeCN-H_2O, 1:1).

GalNAcα1-4GalNAcα-O(CH₂)₃NH₂ (8α): ^{1}H-NMR (700 MHz, D_2O): 1.96–2.05 (m, 2H, CH_2 sp); 2.06, 2.10 (2s, 2×3H, NCOC*H*$_3$); 3.13 (br. t, 2H, NC*H*$_2$ sp, *J* 7.6); 3.58–3.61 (m, 1H, OC*H* sp); 3.68–3.79 (m, 4H, H-6'a, H-6"a, H-6'b, H-6"b); 3.81–3.86 (m, 1H, OC*H* sp); 4.01 (br. dd, 1H, H-5b, $J_{4,5}$ ≈1.0, $J_{5,6'}$ 8.0, $J_{5,6"}$ 4.6); 4.02–4.07 (m, 4H, H-4b, H-4a, H-3a, H-3b); 4.21 (dd, 1H, H-2b, $J_{1,2}$ 3.9, $J_{2,3}$ 11.3); 4.27 (dd, 1H, H-2a, $J_{1,2}$ 3.8, $J_{2,3}$ 11.3); 4.37 (br. t, 1H, H-5a, *J* 6.4); 4.96 (d, 1H, H-1b, $J_{1,2}$ 3.9); 4.98 (d, 1H, H-1a, $J_{1,2}$ 3.8). ^{13}C NMR (DEPT-135, 176 MHz, D_2O): δ 98.31, 97.16 (C-1a, C-1b); 77.09, 76.02, 71.97, 70.84, 68.26, 67.16, 67.09 (C-3a, C-4a, C-5a, C-3b, C-4b, C-5b); 65.20 (*C*H_2O sp); 60.79, 60.62 (C-6a, C-6b); 50.23, 50.13 (C-2a, C-2b); 37.21 (N*C*H_2 sp); 26.80 (*C*H_2 sp); 21.96, 21.92 (2×NCO*C*H_3). R_f 0.47 (MeOH–1M aq. Py·AcOH, 5:1). MS, *m/z* calculated for $[C_{19}H_{35}N_3O_{11}]H^+$: 482.23; found 482. $[\alpha]_{546\ nm}$ 207 (c 0.5; MeCN-H_2O, 1:1).

Galβ1-4GalNAcα-O(CH₂)₃NH₂ (9β): ^{1}H-NMR (700 MHz, D_2O): 1.96–2.05 (m, 2H, CH_2 sp); 2.07 (s, 3H, NCOC*H*$_3$); 3.14 (br. t, 2H, NC*H*$_2$ sp, *J* 7.6); 3.57–3.62 (m, 1H, OC*H* sp); 3.64 (dd, 1H, H-6'b, $J_{5,6'}$ 7.5, $J_{6',6"}$ 9.9); 3.67 (dd, 1H, H-6"b, $J_{5,6"}$ 3.3, $J_{6',6"}$ 9.9); 3.70 (ddd, 1H, H-5b, $J_{4,5}$ 1.2, $J_{5,6'}$ 7.5, $J_{5,6"}$ 3.3); 3.77–3.85 (m, 4H, H-2b, H-3b, H-6'a, OC*H* sp); 3.87 (dd, 1H, H-6"a, $J_{5,6"}$ 5.4, $J_{6',6"}$ 12.0); 3.93 (dd, 1H, H-4b, $J_{3,4}$ 3.3, $J_{4,5}$ 1.0); 3.99 (br. t, 1H, H-5a, *J* 6.3); 4.03 (dd, 1H, H-3a, $J_{3,4}$ 3.1, $J_{2,3}$ 11.1); 4.26 (br. d, 1H, H-4a, *J* 2.8); 4.28 (dd, 1H, H-2a, $J_{1,2}$ 3.8, $J_{2,3}$ 11.1); 4.60 (d, 1H, H-1b, $J_{1,2}$ 7.5); 4.95 (d, 1H, H-1a, $J_{1,2}$ 3.8).^{13}C NMR (DEPT-135, 176 MHz, D_2O): δ 104.48, 97.25 (C-1a, C-1b); 77.50, 75.22, 72.83, 71.43, 70.48, 68.66, 68.18 (C-3a, C-4a, C-5a, C-2b, C-3b, C-4b, C-5b); 65.19 (*C*H_2O sp); 61.07(2) (C-6a, C-6b); 50.48 (C-2a); 37.18 (N*C*H_2 sp); 26.78 (*C*H_2 sp); 20.26 (NCO*C*H_3). R_f 0.47 (MeOH–1M aq. Py·AcOH, 5:1). MS, *m/z* calculated for $[C_{17}H_{32}N_2O_{11}]H^+$: 441.21; found 441. $[\alpha]_{546\ nm}$ 125 (c 0.4; MeCN-H_2O, 1:1).

Galα1-4GalNAcα-O(CH₂)₃NH₂ (9α): ^{1}H-NMR (700 MHz, D_2O): 1.97–2.05 (m, 2H, CH_2 sp); 2.06 (s, 3H, NCOC*H*$_3$); 3.14 (br. t, 2H, NC*H*$_2$ sp, *J* 7.6); 3.59–3.63 (m, 1H, OC*H* sp); 3.69 (dd, 1H, H-6'a, $J_{5,6'}$ 7.2, $J_{6',6"}$ 11.5); 3.73 (dd, 1H, H-6"a, $J_{5,6"}$ 5.6, $J_{6',6"}$ 11.5); 3.82–3.90 (m, 3H, H-2b, H-6'b, OC*H* sp); 3.94 (dd, 1H, H-6"b, $J_{5,6"}$ 7.3, $J_{6',6"}$ 11.7); 3.98 (dd, 1H, H-3b, $J_{3,4}$ 3.4, $J_{2,3}$ 10.5); 4.01–4.05 (m, 2H, H-3a, H-5b); 4.06 (dd, 1H, H-4b, $J_{3,4}$ 3.4, $J_{4,5}$ 1.2); 4.12 (br. d, 1H, H-4a, *J* 2.9); 4.26 (dd, 1H, H-2a, $J_{1,2}$ 3.7, $J_{2,3}$ 11.3); 4.33 (ddd, 1H, H-5a, $J_{4,5}$ 1.2, $J_{5,6'}$ 7.2, $J_{5,6"}$ 5.6); 4.99 (d, 1H, H-1a, $J_{1,2}$ 3.8); 5.01 (d, 1H, H-1b, $J_{1,2}$ 4.0). ^{13}C NMR (DEPT-135, 176 MHz, D_2O): δ 100.64, 97.21 (C-1a, C-1b); 78.54, 71.52, 71.09, 69.14, 69.04, 68.66, 67.30 (C-3a, C-4a, C-5a, C-2b, C-3b, C-4b, C-5b); 65.22 (*C*H_2O sp); 60.81, 60.66 (C-6a, C-6b); 50.27 (C-2a); 37.19

(N*C*H_2 sp); 26.78 (*C*H_2 sp); 22.29 (NCO*C*H_3). R_f 0.45 (MeOH–1M aq. Py·AcOH, 5:1). MS, *m/z* calculated for $[C_{17}H_{32}N_2O_{11}]H^+$: 441.21; found 441. $[\alpha]_{546\ nm}$ 224 (c 0.3; MeCN-H_2O, 1:1).

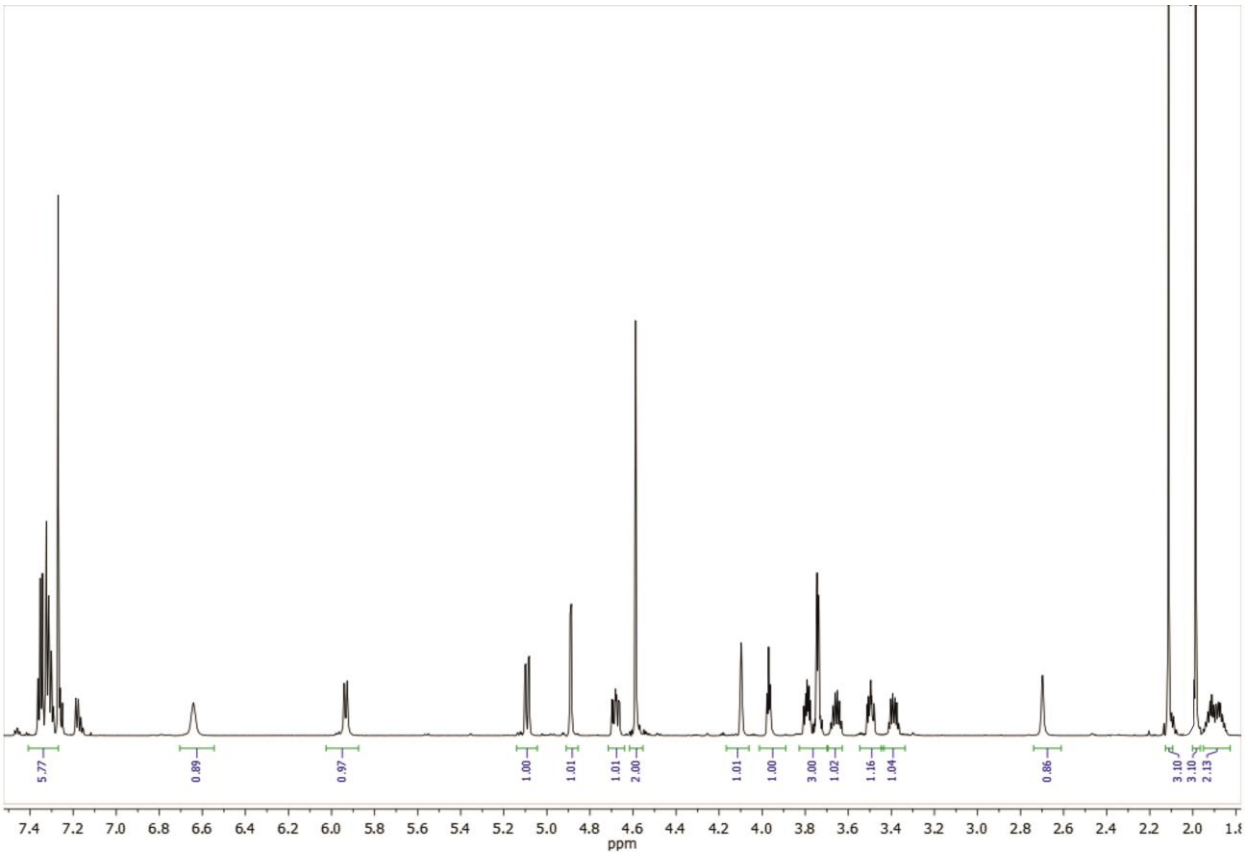
Glcβ1-4GalNAcα-O(CH$_2$)$_3$NH$_2$ (10β): ^{1}H-NMR (700 MHz, D_2O): 1.97–2.05 (m, 2H, CH_2 sp); 2.06 (s, 3H, NCOC*H*$_3$); 3.13 (br. t, 2H, NC*H*$_2$ sp, *J* 7.6); 3.39–3.44 (m, 2H, H2b, H4b); 3.46 (ddd, 1H, H-5b, $J_{4,5}$ 10.0, $J_{5,6'}$ 5.8, $J_{5,6''}$ 2.2); 3.51 (t, 1H, H-3b, *J* 9.1); 3.57–3.61 (m, 1H, OC*H* sp); 3.75 (dd, 1H, H-6'b, $J_{5,6'}$ 5.8, $J_{6',6''}$ 12.3); 3.79 (dd, 1H, H-6'a, $J_{5,6'}$ 7.5, $J_{6',6''}$ 12.0); 3.81–3.83 (m, 1H, OC*H* sp); 3.85 (dd, 1H, H-6''a, $J_{5,6''}$ 5.0, $J_{6',6''}$ 12.0); 3.93 (dd, 1H, H-6''b, $J_{5,6''}$ 2.2, $J_{6',6''}$ 12.3); 3.99 (br. dd, 1H, H-5a, $J_{4,5}$ ≈1.0, $J_{5,6'}$ 7.5, $J_{5,6''}$ 5.0); 4.03 (dd, 1H, H-3a, $J_{3,4}$ 3.0, $J_{2,3}$ 11.1); 4.25 (br. d, 1H, H-4a, $J_{3,4}$ 3.0, $J_{4,5}$ ≈1.0); 4.27 (dd, 1H, H-2a, $J_{1,2}$ 3.8, $J_{2,3}$ 11.1); 4.66 (d, 1H, H-1b, $J_{1,2}$ 7.9); 4.94 (d, 1H, H-1a, $J_{1,2}$ 3.8).^{13}C NMR (DEPT-135, 176 MHz, D_2O): δ 103.90, 97.23 (C-1a, C-1b); 77.70, 75.93, 75.80, 73.67, 70.55, 69.63, 68.15 (C-3a, C-4a, C-5a, C-2b, C-3b, C-4b, C-5b); 65.17 (*C*H_2O sp); 61.14, 60.81 (C-6a, C-6b); 50.47 (C-2a); 37.18 (N*C*H_2 sp); 26.77 (*C*H_2 sp); 21.94 (NCO*C*H_3). R_f 0.55 (MeOH–1M aq. Py·AcOH, 5:1). MS, *m/z* calculated for $[C_{17}H_{32}N_2O_{11}]H^+$: 441.21; found 441. $[\alpha]^{25}_{546\ nm}$ 134 (c 0.5; MeCN-H_2O, 1:1).

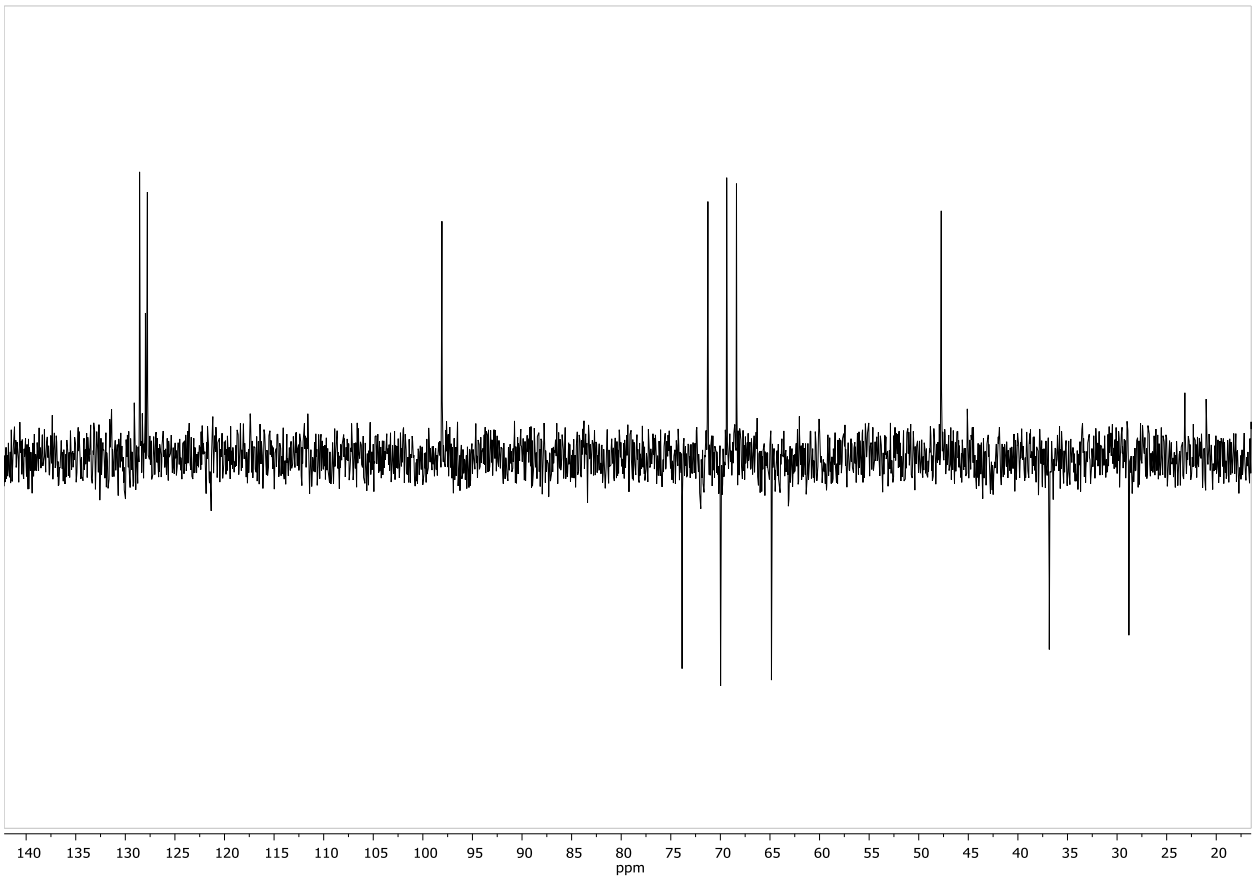
Glcα1-4GalNAcα-O(CH$_2$)$_3$NH$_2$ (10α): ^{1}H-NMR (700 MHz, D_2O): 1.96–2.04 (m, 2H, CH_2 sp); 2.05 (s, 3H, NCOC*H*$_3$); 3.13 (br. t, 2H, NC*H*$_2$ sp, *J* 7.6); 3.48 (dd ≈ t, 1H, H-4b, *J* 9.7); 3.58 (dd, 1H, H-2b, $J_{1,2}$ 3.8, $J_{2,3}$ 10.1); 3.59–3.63 (m, 1H, OC*H* sp); 3.75 (dd, 1H, H-6'b, $J_{5,6'}$ 2.4, $J_{6',6''}$ 12.4); 3.79–3.84 (m, 3H, H-3b, H-6''b, OC*H* sp); 3.85 (dd, 1H, H-6'a, $J_{5,6'}$ 4.6, $J_{6',6''}$ 11.7); 3.93 (dd, 1H, H-6''a, $J_{5,6''}$ 7.4, $J_{6',6''}$ 11.7); 4.00–4.02 (m, 1H, H-5a); 4.03 (dd, 1H, H-3a, $J_{3,4}$ 2.8, $J_{2,3}$ 11.3); 4.07 (ddd, 1H, H-5b, $J_{4,5}$ 10.2, $J_{5,6'}$ 2.4, $J_{5,6''}$ 4.2); 4.09 (br. d, 1H, H-4a, *J* 2.9); 4.25 (dd, 1H, H-2a, $J_{1,2}$ 3.6, $J_{2,3}$ 11.3); 4.98 (m ≈ t, 2H, H-1a, H1b, *J* 4.5). R_f 0.48 (MeOH–1M aq. Py·AcOH, 5:1). MS, *m/z* calculated for $[C_{17}H_{32}N_2O_{11}]H^+$: 441.21; found 441.

GlcNAcβ1-4GalNAcα-O(CH$_2$)$_3$NH$_2$ (11): ^{1}H-NMR (700 MHz, D_2O): 1.94–2.03 (m, 2H, CH_2 sp); 2.03, 2.04 (2s, 2×3H, NCOC*H*$_3$); 3.11 (br. t, 2H, NC*H*$_2$ sp, *J* 7.6); 3.41–3.47 (m, 2H, H-2b, H-5b); 3.54–3.59 (m, 2H, OC*H* sp, H-4b); 3.72–3.83 (m, 5H, H-6'a, H-6''a, H-6'b, OC*H* sp, H-3b); 3.91–3.96 (m, 2H, H-5a, H-6''b); 4.04 (dd, 1H, H-3a, $J_{3,4}$ 2.9, $J_{2,3}$ 11.1); 4.11 (dd, 1H, H-2a, $J_{1,2}$ 3.7, $J_{2,3}$ 11.1); 4.16 (br. d, 1H, H-4a, *J* 2.9); 4.72 (d, 1H, H-1b, $J_{1,2}$ 8.5); 4.88 (d, 1H, H-1a, $J_{1,2}$ 3.7). ^{13}C NMR (DEPT-135, 176 MHz, D_2O): δ 101.89, 97.16 (C-1a, C-1b); 75.64, 75.59, 73.84, 70.49, 70.12, 67.73 (C-3a, C-4a, C-5a, C-3b, C-4b, C-5b); 65.12 (*C*H_2O sp); 61.06, 60.90 (C-6a, C-6b); 55.74, 49.99 (C-2a, C-2b); 37.14 (N*C*H_2 sp); 26.73 (*C*H_2 sp); 22.26, 21.87 (2×NCO*C*H_3). R_f 0.42 (MeOH–1M aq. Py·AcOH, 5:1 MS, *m/z* calculated for $[C_{19}H_{35}N_3O_{11}]H^+$: 482.23; found 482. $[\alpha]_{546\ nm}$ 80 (c 0.5; MeCN-H_2O, 1:1).

6-OBn-3-OAc-GalNAcα-O$(CH_2)_3$NHC(O)CF_3 (2):

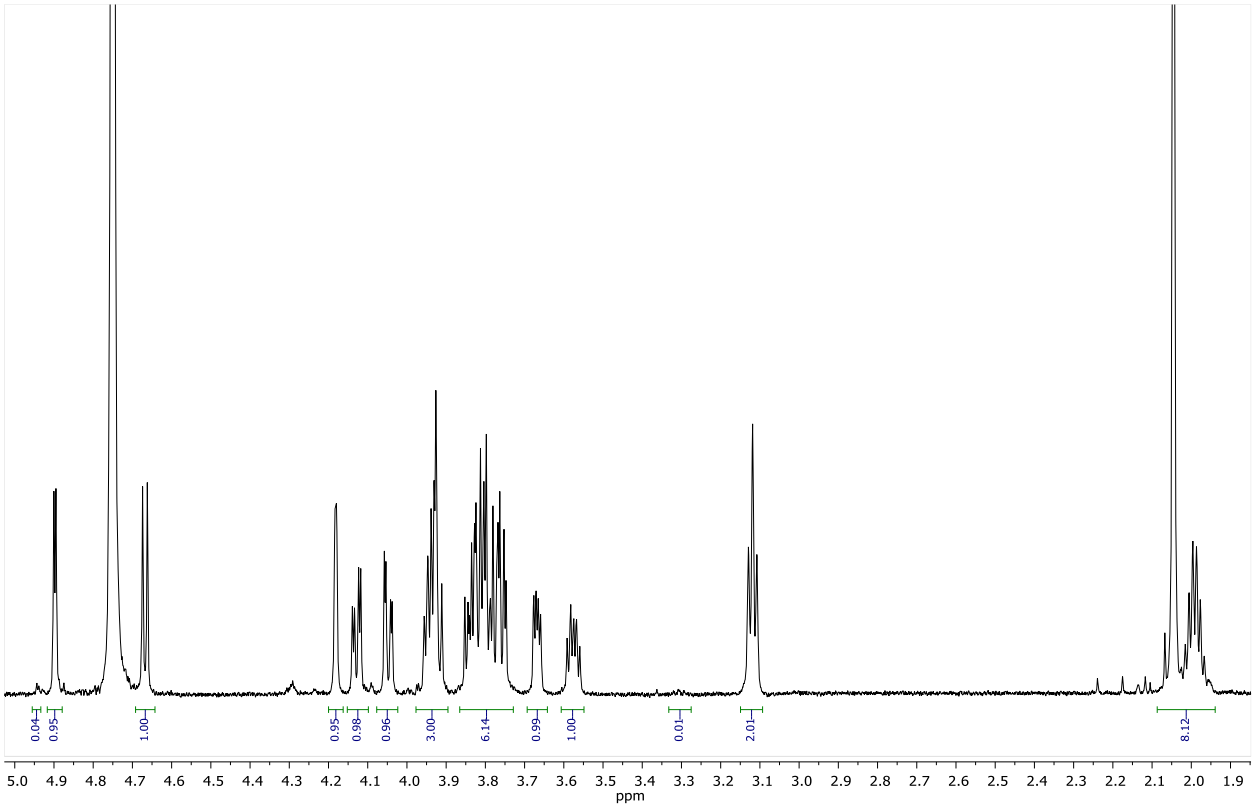
3-Trifluoroacetamidopropyl 6-*O*-benzyl-3-*O*-acetyl-2-acetamido-2-deoxy-α-D-galactopyranoside





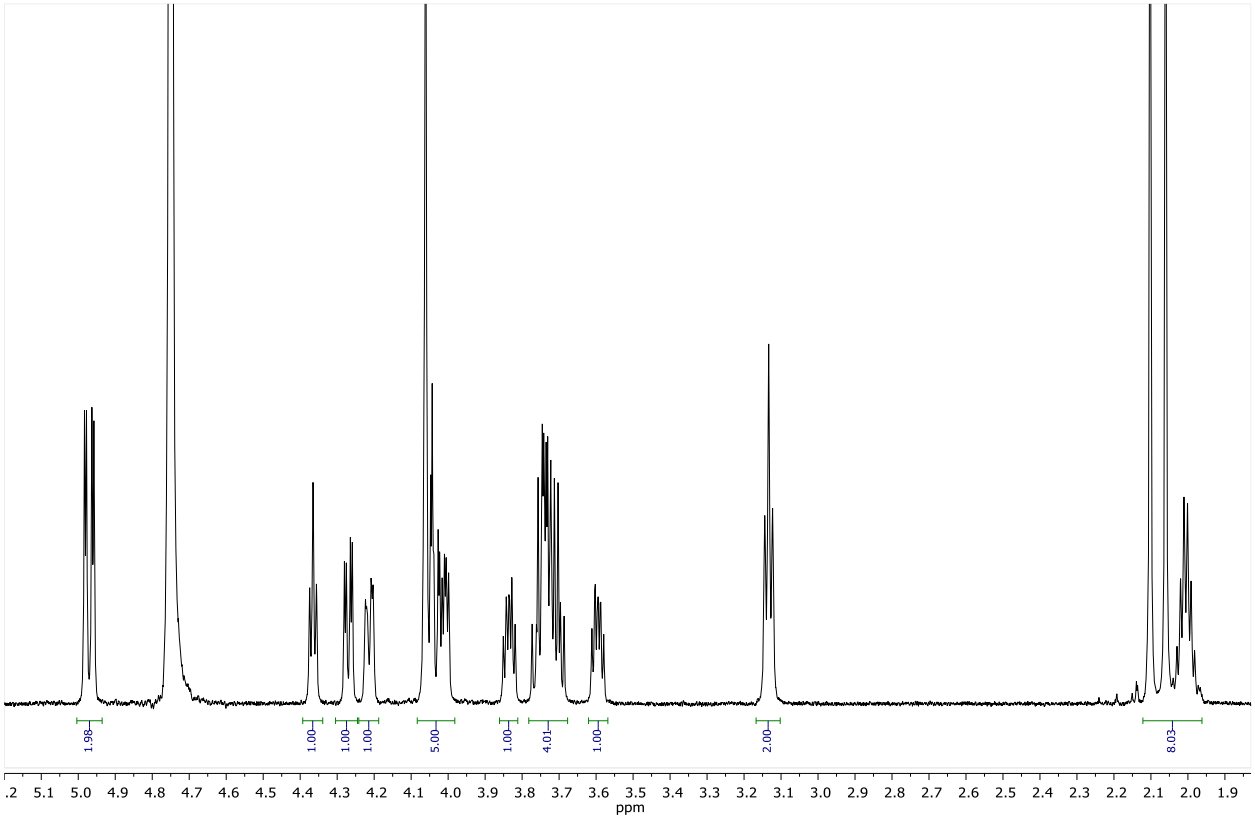
GalNAcβ1-4GalNAcα-O$(CH_2)_3NH_2$ (8β)

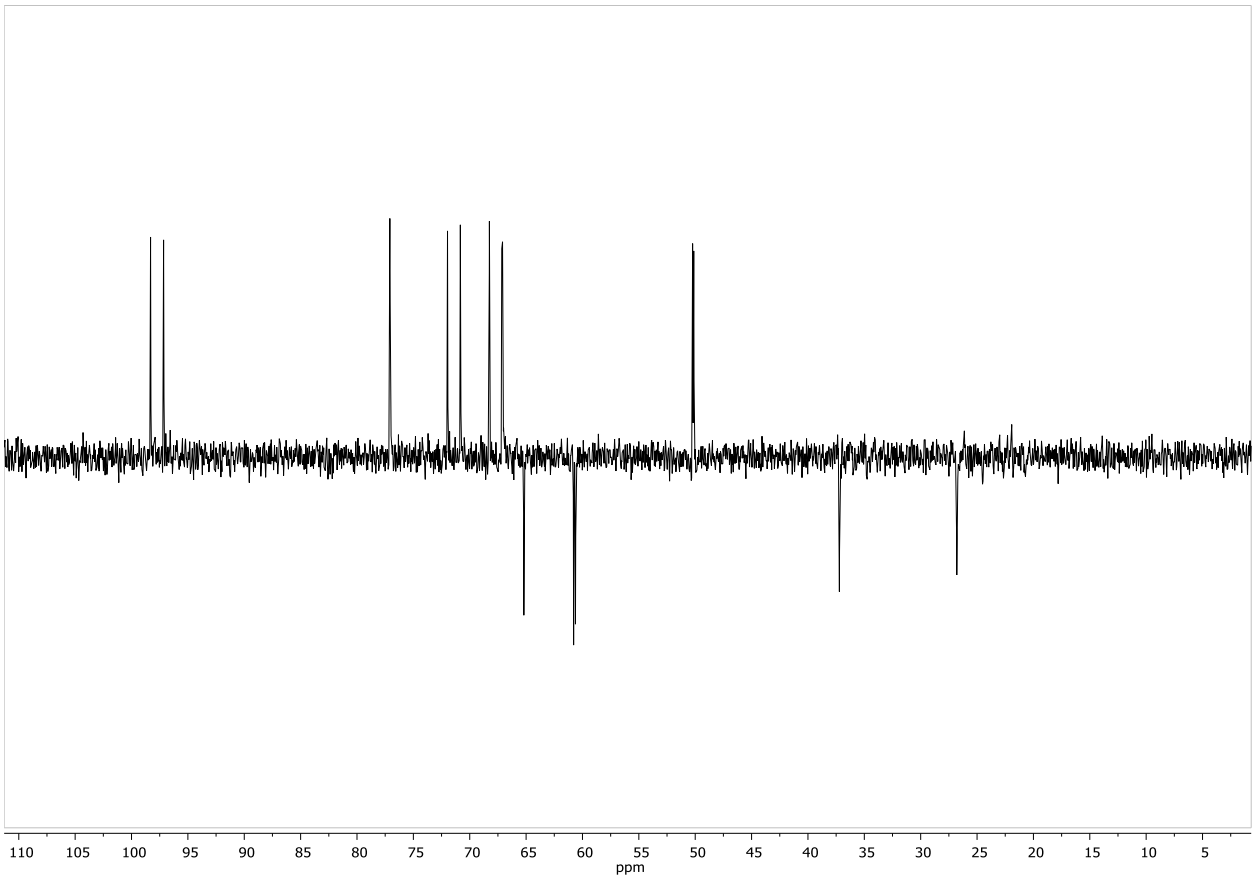
3-Aminopropyl 2-acetamido-2-deoxy-β-D-galactopyranosyl-(1→ 4)-2-acetamido-2-deoxy-α-D-galactopyranoside



GalNAcα1-4GalNAcα-O$(CH_2)_3NH_2$ (8α)

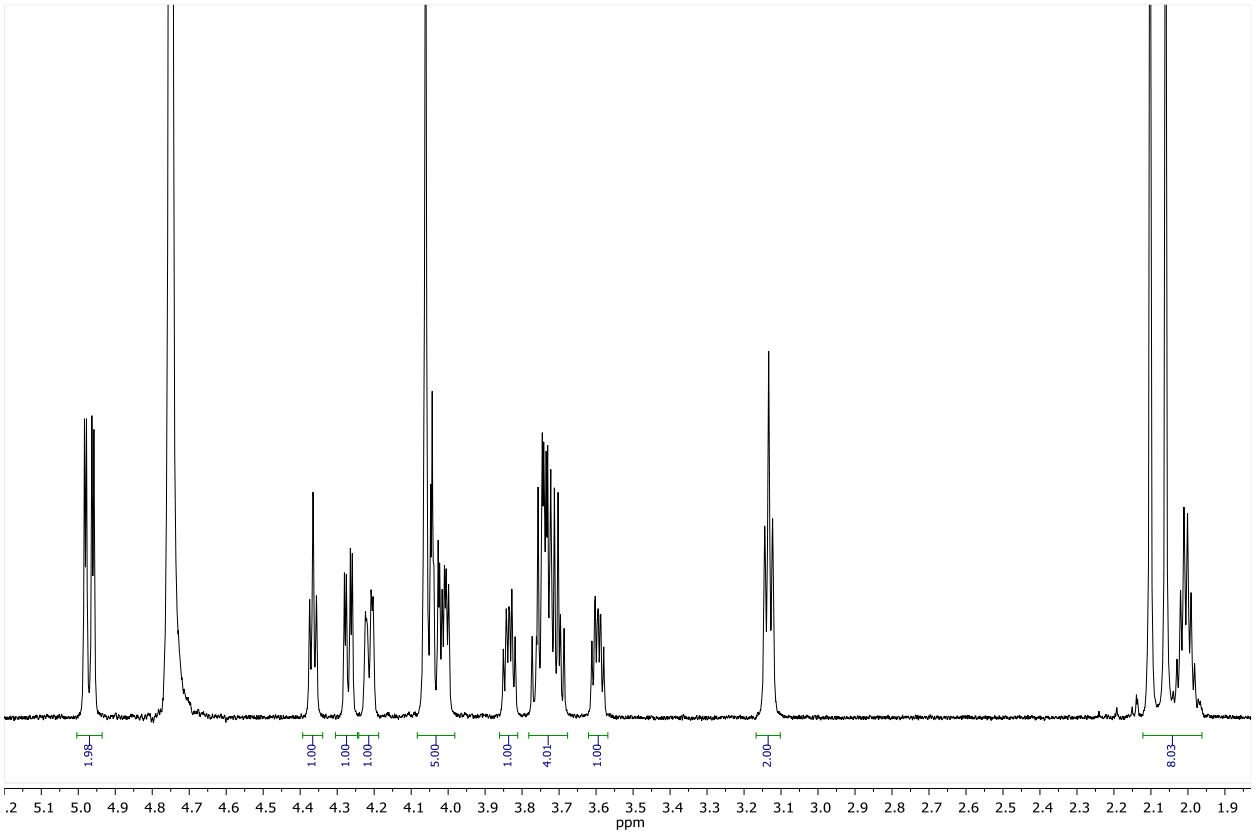
3-Aminopropyl 2-acetamido-2-deoxy-α-D-galactopyranosyl-(1→ 4)-2-acetamido-2-deoxy-α-D-galactopyranoside

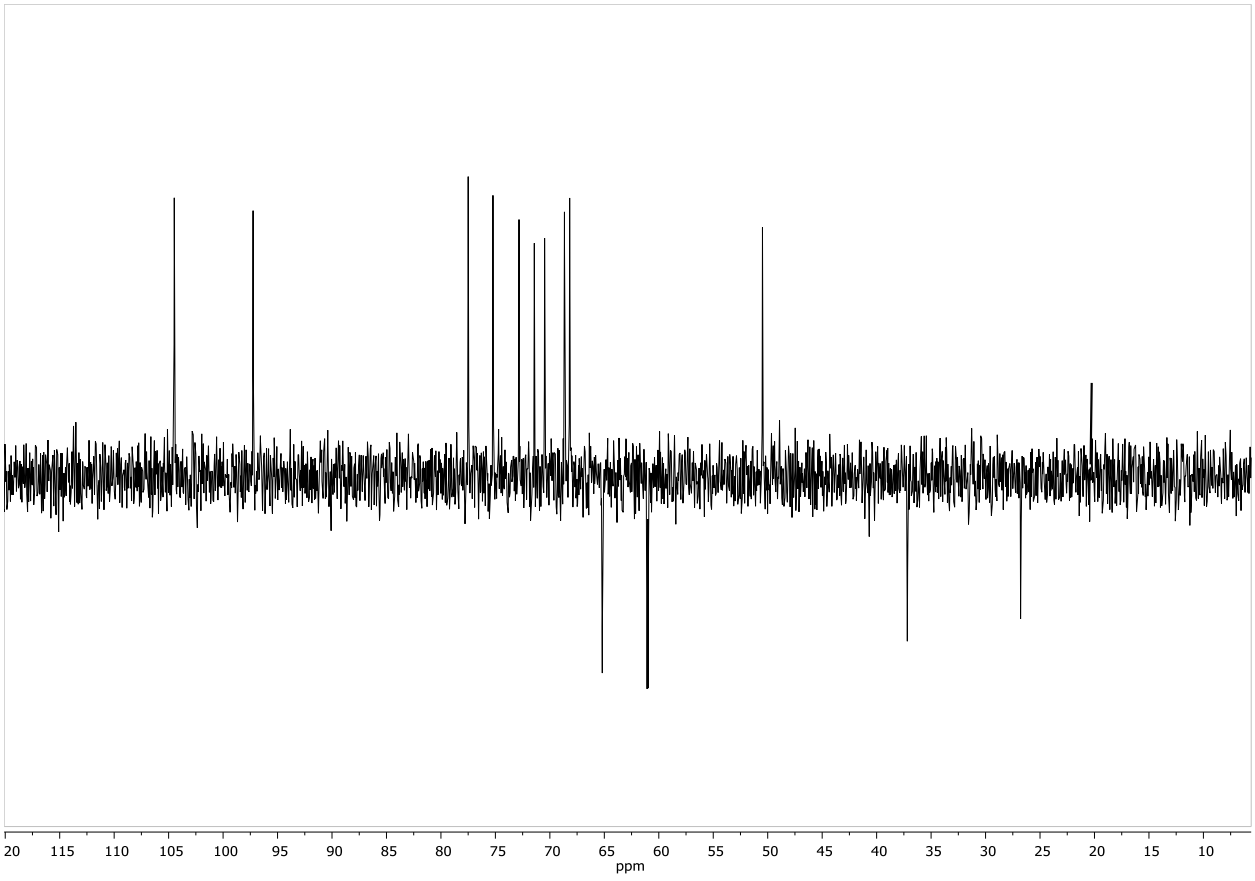




Galβ1-4GalNAcα-O(CH_2)$_3$$NH_2$ (9β)

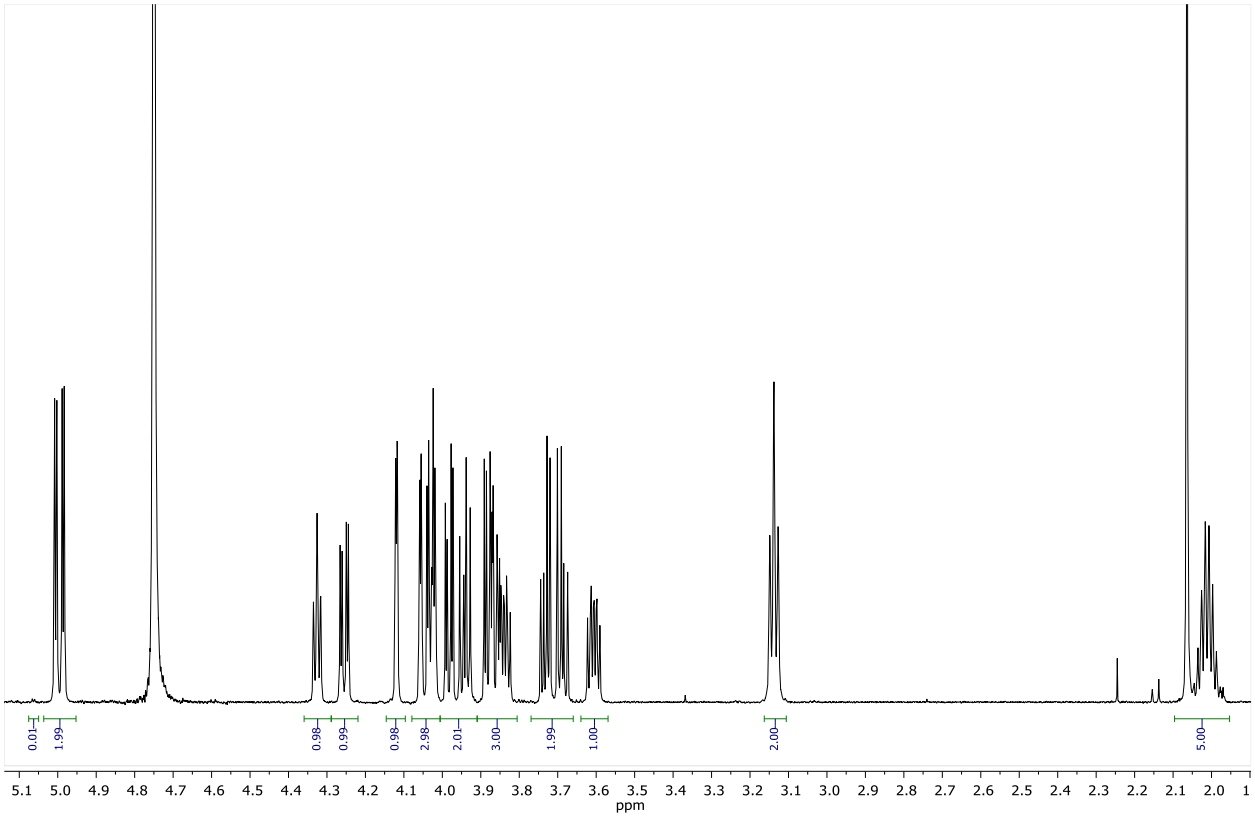
3-Aminopropyl β-D-galactopyranosyl-(1→ 4)-2-acetamido-2-deoxy-α-D-galactopyranoside

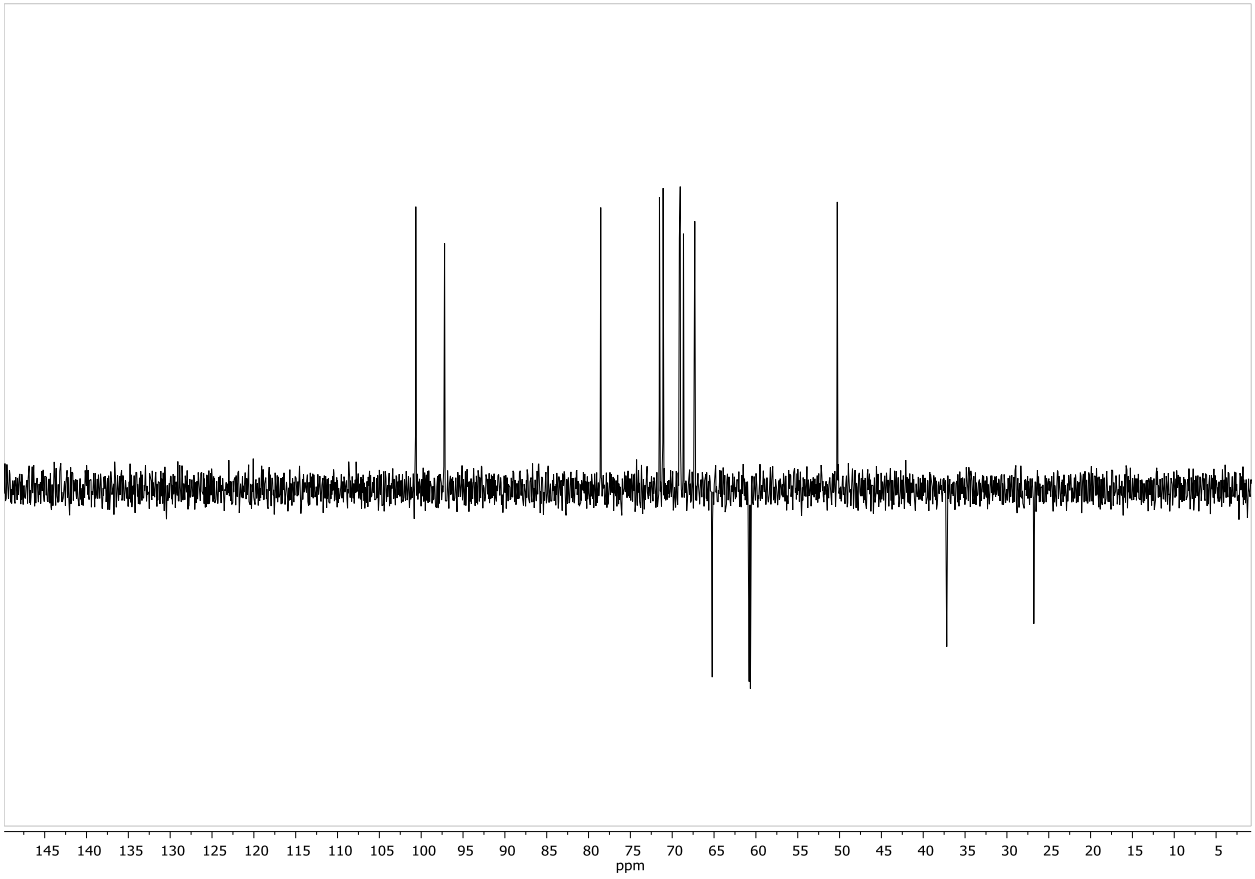




Galα1-4GalNAcα-O$(CH_2)_3NH_2$ (9α)

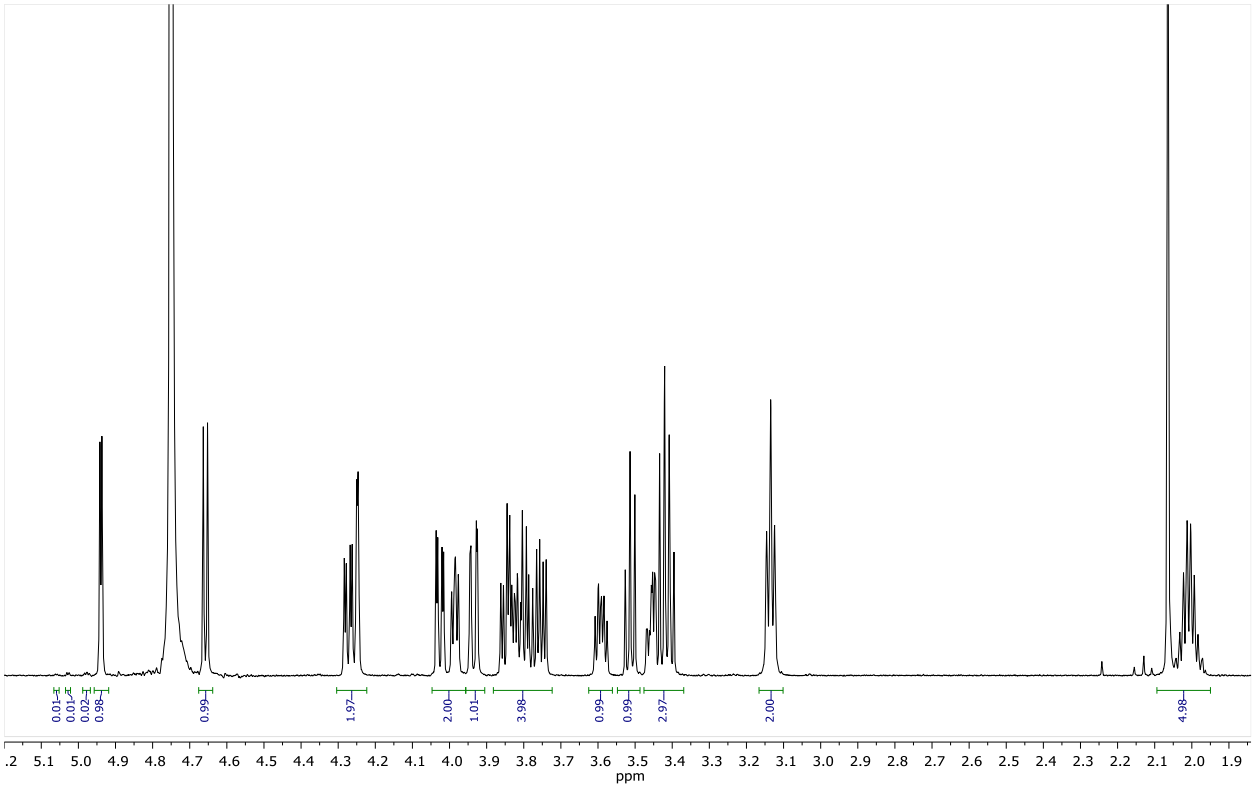
3-Aminopropyl α-D-galactopyranosyl-(1→ 4)-2-acetamido-2-deoxy-α-D-galactopyranoside

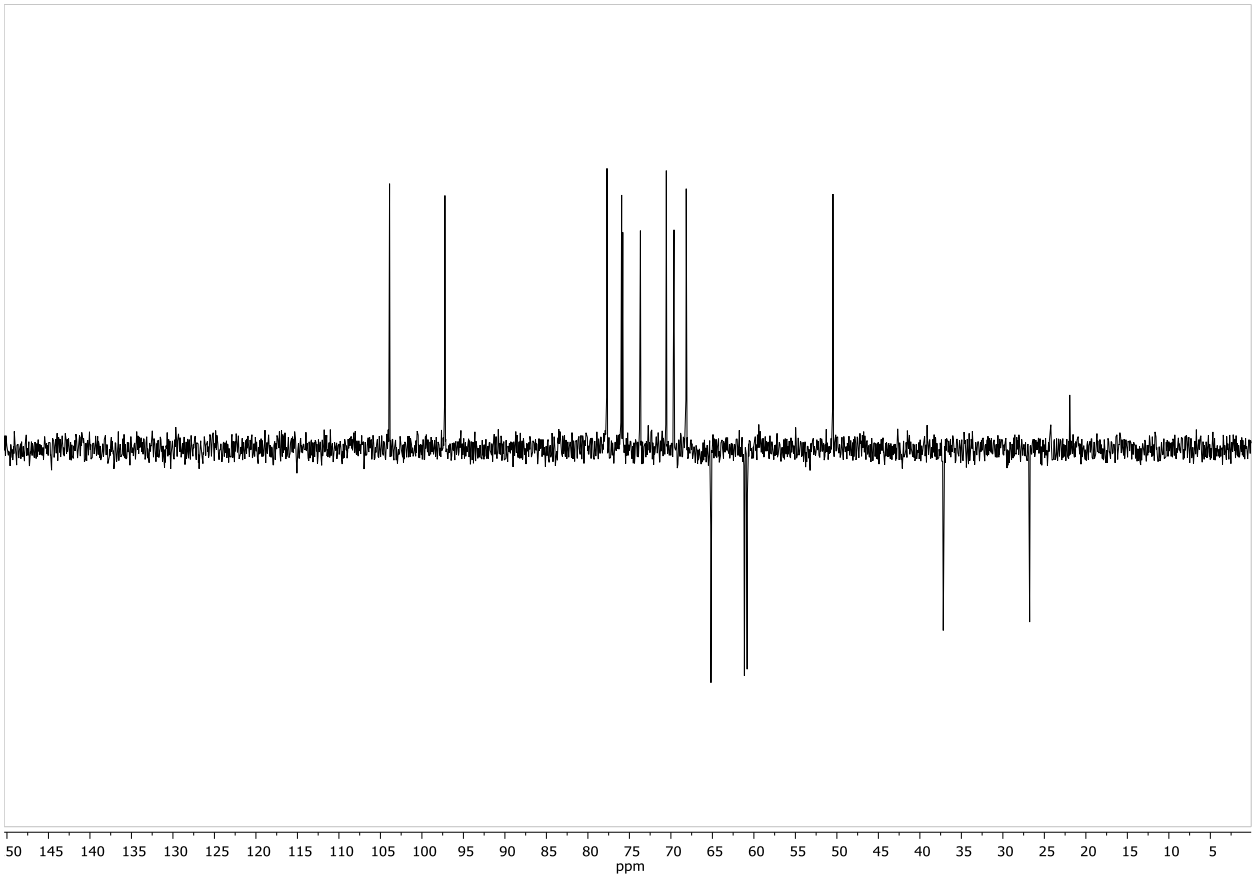




Glcβ1-4GalNAcα-O(CH_2)$_3$$NH_2$ (10β)

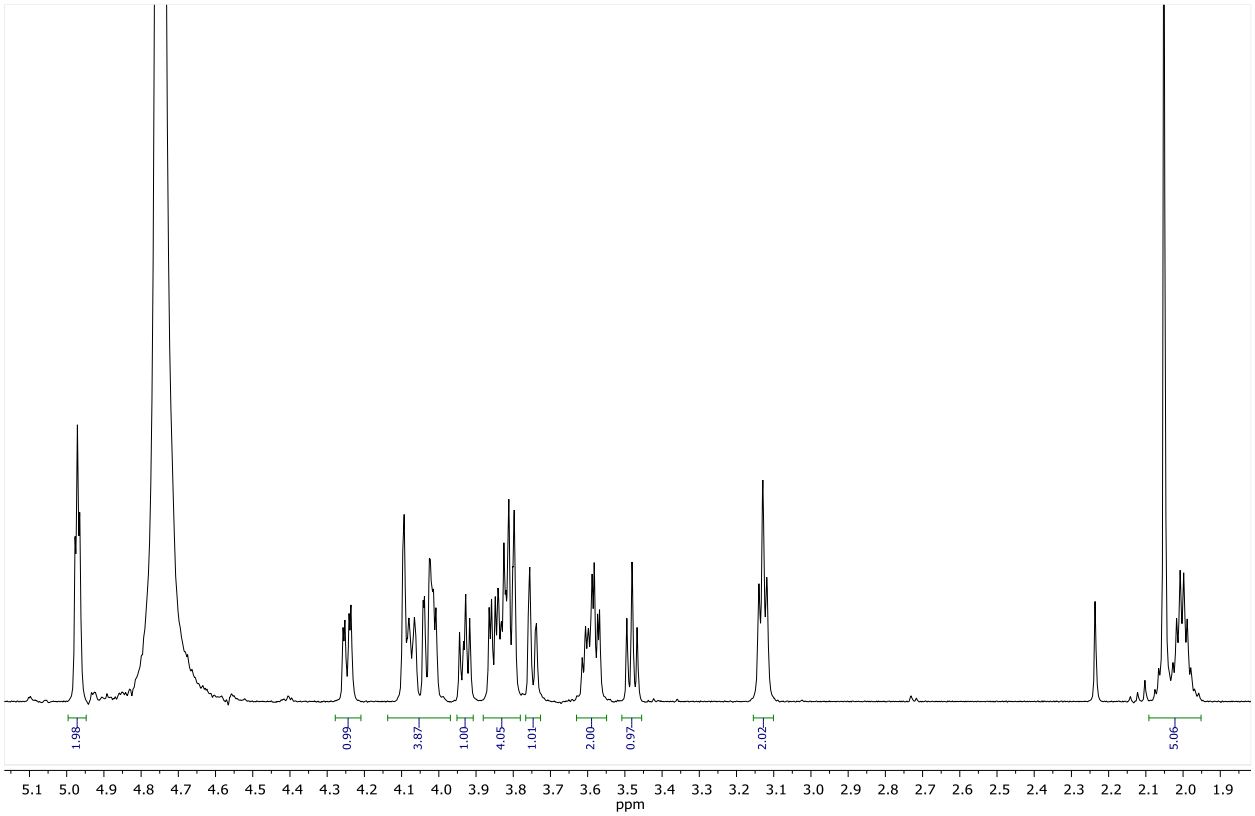
3-Aminopropyl β-D-glucopyranosyl-(1→ 4)-2-acetamido-2-deoxy-α-D-galactopyranoside





Glcα1-4GalNAcα-O(CH_2)$_3$$NH_2$ (10α)

3-Aminopropyl α-D-glucopyranosyl-(1→ 4)-2-acetamido-2-deoxy-α-D-galactopyranoside



GlcNAcβ1-4GalNAcα-O$(CH_2)_3NH_2$ (11)

3-Aminopropyl 2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→ 4)-2-acetamido-2-deoxy-α-D-galactopyranoside