

Synthesis of GalNGc α 1-3GalNAc α disaccharide and its interaction with human blood antibodies

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

Compound 4. ^1H NMR (700 MHz, CDCl_3) δ : 1.874–1.955 (m, 2H, CH_2 sp), 1.981, 2.034, 2.044 (2), 2.156, 2.179, 2.203 (7s, 7 \times 3H, COMe), 3.361–3.430 (m, 1H, NCHH sp), 3.472–3.527 (m, 1H, OCHH sp), 3.681–3.748 (m, 1H, NCHH sp), 3.754–3.801 (m, 1H, OCHH sp), 3.941 (dd, 1H, H-3a, $J_{3,4}$ 3.0 Hz, $J_{2,3}$ 11.0 Hz), 4.019–4.157 (m, 5H, H-5a, H-6'a, H-6''a, H-6'b, H-6''b), 4.235 (ddd \approx dd, 1H, H-5b, $J_{5,6''}$ 5.8 Hz, $J_{5,6'}$ 8.3 Hz, $J_{5,4} \leq 1$ Hz), 4.434 (d, 1H, CHHOAc, J_{gem} 15.1 Hz), 4.566–4.626 (m, 2H, H-2a, H-2b), 4.670 (d, 1H, CHHOAc, J_{gem} 15.1 Hz), 4.855 (d, 1H, H-1a, $J_{1,2}$ 3.6 Hz), 5.009 (dd, 1H, H-3b, $J_{3,4}$ 3.2 Hz, $J_{2,3}$ 11.5 Hz), 5.085 (d, 1H, H-1b, $J_{1,2}$ 3.4 Hz), 5.371 (br. d, 1H, H-4b, J 2.2 Hz), 5.401 (br. d, 1H, H-4a, J 2.7 Hz), 6.304 (d, 1H, NHAc a, $J_{2,\text{NH}}$ 9.5 Hz), 6.737 (d, 1H, NHAc b, $J_{2,\text{NH}}$ 9.5 Hz), 6.866–6.928 (m, 1H, NHCOCF_3 sp). R_f 0.50 (EtOAc–Pr i OH, 10 : 1).

Compound 5. ^1H NMR (700 MHz, CDCl_3) δ : 1.872–1.938 (m, 2H, CH_2 sp), 1.972, 1.984, 2.048 (3), 2.161, 2.211 (7s, 7 \times 3H, COMe), 3.364–3.435 (m, 1H, NCHH sp), 3.475–3.527 (m, 1H, OCHH sp), 3.667–3.738 (m, 1H, NCHH sp), 3.747–3.797 (m, 1H, OCHH sp), 3.947 (dd, 1H, H-3a, $J_{3,4}$ 3.0 Hz, $J_{2,3}$ 11.1 Hz), 4.046 (dd, 1H, H-6''b, $J_{6',6''}$ 10.9 Hz, $J_{5,6''}$ 9.0 Hz), 4.078–4.126 (m, 3H, H-5a, H-6'a, H-6''a), 4.141 (dd, 1H, H-6''b, $J_{6',6''}$ 10.9 Hz, $J_{5,6''}$ 5.2 Hz), 4.233 (ddd \approx dd, 1H, H-5b, $J_{5,6''}$ 5.2 Hz, $J_{5,6'}$ 9.0 Hz, $J_{5,4} \leq 1$ Hz), 4.580–4.662 (m, 2H, H-2a, H-2b), 4.874 (d, 1H, H-1a, $J_{1,2}$ 3.6 Hz), 4.952 (dd, 1H, H-3b, $J_{3,4}$ 3.1 Hz, $J_{2,3}$ 11.5 Hz), 5.055 (d, 1H, H-1b, $J_{1,2}$ 3.4 Hz),

5.375 (br. s, 2H, H-4b, H-4a), 6.349 (d, 1H, *NHAc* a, $J_{2,\text{NH}}$ 9.5 Hz), 6.378 (d, 1H, *NHAc* b, $J_{2,\text{NH}}$ 9.8 Hz), 6.884–6.940 (m, 1H, NHCOCF_3 sp). R_f 0.30 (EtOAc–PrⁱOH, 10 : 1).

Compound 5b. ^1H NMR (700 MHz, CDCl_3) δ : 1.863–1.910 (m, 2H, CH_2 sp), 1.999, 2.002, 2.054 (2), 2.068, 2.128, 2.150 (7s, 7 \times 3H, COMe), 3.431–3.495 (m, 2H, *NCHH*, *OCHH* sp), 3.557–3.618 (m, 1H, *NCHH* sp), 3.724–3.775 (m, 1H, *OCHH* sp), 3.885–3.949 (m, 2H, H-2b, H-5b), 3.981 (dd, 1H, H-3a, $J_{3,4}$ 3.2 Hz, $J_{2,3}$ 10.9 Hz), 4.017 (dd, 1H, H-6'a, $J_{6',6''}$ 11.0 Hz, $J_{5,6'}$ 6.8 Hz), 4.083 (br. t, 1H, H-5a, J 6.08 Hz), 4.100–4.154 (m, 3H, H-6''a, H-6''b, H-6''b), 4.367 (ddd, 1H, H-2a, $J_{1,2}$ 3.5 Hz, $J_{2,\text{NH}}$ 7.0 Hz, $J_{2,3}$ 10.9 Hz), 4.823 (d, 1H, H-1b, $J_{1,2}$ 8.3 Hz), 5.086 (d, 1H, H-1a, $J_{1,2}$ 3.5 Hz), 5.202 (dd, 1H, H-3b, $J_{3,4}$ 3.3 Hz, $J_{2,3}$ 11.2 Hz), 5.346 (br. d, 1H, H-4b, J 3.0 Hz), 5.404 (br. d, 1H, H-4a, J 2.8 Hz), 5.906 (d, 1H, *NHAc* b, $J_{2,\text{NH}}$ 8.1 Hz), 6.517 (d, 1H, *NHAc* a, $J_{2,\text{NH}}$ 7.0 Hz), 6.987–7.035 (m, 1H, NHCOCF_3 sp). R_f 0.16 (EtOAc–PrⁱOH, 10 : 1).

Compound 6. ^1H NMR (700 MHz, D_2O) δ : 1.965–2.069 (m, 2H, CH_2 sp), 2.081 (s, 3H, NCOMe), 3.094–3.176 (m, 2H, NCH_2 sp), 3.605–3.640 (m, 1H, *OCHH* sp), 3.742–3.851 (m, 5H, *OCHH* sp, H-6'a, H-6''a, H-6'b, H-6''b), 3.868 (dd, 1H, H-3b, $J_{3,4}$ 3.2 Hz, $J_{2,3}$ 11.0 Hz), 3.886–3.924 (m, 2H, H-5a, H-5b), 3.994 (dd, 1H, H-3a, $J_{3,4}$ 3.1 Hz, $J_{2,3}$ 11.2 Hz), 4.033 (br. d, 1H, H-4a, J 3.0 Hz), 4.152 (br. s, 3H, H-4b, CH_2OH Gc), 4.299 (dd, 1H, H-2b, $J_{1,2}$ 3.8 Hz, $J_{2,3}$ 11.0 Hz), 4.410 (dd, 1H, H-2a, $J_{1,2}$ 3.8 Hz, $J_{2,3}$ 11.2 Hz), 4.932 (d, 1H, H-1a, $J_{1,2}$ 3.8 Hz), 5.110 (d, 1H, H-1b, $J_{1,2}$ 3.8 Hz). ^{13}C NMR (176 MHz, D_2O) δ : 97.25 (C-1a), 93.93 (C-1b), 72.70, 71.50, 71.05, 68.45, 67.69, 65.04, 64.80 (C-3a, C-4a, C-5a, C-3b, C-4b, C-5b, CH_2O sp), 61.34, 61.06, 61.01 (C-6a, C-6b, CH_2OH Gc), 49.25, 47.98 (C-2a, C-2b), 37.17 (NCH_2 sp), 26.81 (CH_2 sp), 22.29 (NCOMe). R_f 0.53 (MeOH–1 M aq. Py·AcOH, 5 : 1). MS, m/z : 498 (calc. for $[\text{C}_{19}\text{H}_{35}\text{N}_3\text{O}_{12}]\text{H}^+$, m/z : 498.22). $[\alpha]_{546}^{25} +166$ (c 0.4, MeCN– H_2O , 1 : 1).

Compound 7. ^1H NMR (700 MHz, D_2O) δ : 1.973–2.065 (m, 2H, CH_2 sp), 2.053, 2.079 (2s, 2 \times 3H, NCOMe), 3.098–3.172 (m, 2H, NCH_2 sp), 3.600–3.649 (m, 1H, *OCHH* sp), 3.741–3.854 (m, 6H, H-3b, *OCHH* sp, H-6'a, H-6''a, H-6'b, H-6''b), 3.875 (br. t, 1H, H-5b, J 6.1 Hz), 3.909 (br. dd, 1H, H-5a, $J_{5,6'}$ 4.0 Hz, $J_{5,6''}$ 7.9 Hz, $J_{5,4} \leq 1$ Hz), 3.993 (dd, 1H, H-3a, $J_{3,4}$ 3.0 Hz, $J_{2,3}$ 11.2 Hz), 4.014 (br. d, 1H, H-4a, J 2.7 Hz), 4.168 (br. d, 1H, H-4b, J 2.7 Hz), 4.225 (dd, 1H, H-2b, $J_{1,2}$ 3.7 Hz, $J_{2,3}$ 11.0 Hz), 4.409 (dd, 1H, H-2a, $J_{1,2}$ 3.8 Hz, $J_{2,3}$ 11.2 Hz), 4.934 (d, 1H, H-1a, $J_{1,2}$ 3.8 Hz), 5.091 (d, 1H, H-1b, $J_{1,2}$ 3.7 Hz). ^{13}C NMR (176 MHz, D_2O) δ : 97.23 (C-1a), 93.47 (C-1b), 72.20, 71.43, 71.08, 68.42, 67.76, 65.03, 64.61 (C-3a, C-4a, C-5a, C-3b, C-4b, C-5b, CH_2O sp), 61.39, 61.12 (C-6a, C-6b), 49.48, 47.92 (C-2a, C-2b), 37.17 (NCH_2 sp), 26.81 (CH_2 sp), 22.23, 22.16 (2 \times NCOMe). R_f 0.54 (MeOH–1 M aq. Py·AcOH, 5:1). MS, m/z : 482 (calc. for $[\text{C}_{19}\text{H}_{35}\text{N}_3\text{O}_{11}]\text{H}^+$, m/z : 482.22). $[\alpha]_{546}^{25} +205$ (c 0.6, MeCN– H_2O , 1 : 1).

Compound 7β. ¹H NMR (700 MHz, D₂O) δ: 1.955–2.047 (m, 2H, CH₂ sp), 2.029, 2.061 (2s, 2×3H, NCOMe), 3.133 (m ≈ t, 2H, NCH₂ sp, *J* 7.4 Hz), 3.554–3.597 (m, 1H, OCHH sp), 3.649 (br. dd, 1H, H-5b, *J*_{5,6'} 4.4 Hz, *J*_{5,6''} 7.6 Hz, *J*_{5,4} ≤ 1 Hz), 3.726–3.834 (m, 6H, H-3b, OCHH sp, H-6'a, H-6''a, H-6'b, H-6''b), 3.872 (dd, 1H, H-2b, *J*_{1,2} 8.3 Hz, *J*_{2,3} 11.0 Hz), 3.938 (br. d, 1H, H-4b, *J* 2.5 Hz), 3.971 (br. dd, 1H, H-5a, *J*_{5,6'} 4.7 Hz, *J*_{5,6''} 7.4 Hz, *J*_{5,4} ≤ 1 Hz), 3.997 (dd, 1H, H-3a, *J*_{3,4} 2.6 Hz, *J*_{2,3} 11.0 Hz), 4.229 (br. s, 1H, H-4a), 4.271 (dd, 1H, H-2a, *J*_{1,2} 3.5 Hz, *J*_{2,3} 11.0 Hz), 4.539 (d, 1H, H-1b, *J*_{1,2} 8.3 Hz), 4.869 (d, 1H, H-1a, *J*_{1,2} 3.5 Hz). ¹³C NMR (176 MHz, D₂O) δ: 102.80 (C-1b), 97.23 (C-1a), 75.98, 75.00, 70.64, 70.52, 68.93, 67.86 (C-3a, C-4a, C-5a, C-3b, C-4b, C-5b), 64.92 (CH₂O sp), 61.31, 61.10 (C-6a, C-6b), 52.60, 48.54 (C-2a, C-2b), 37.14 (NCH₂ sp), 26.80 (CH₂ sp), 22.33, 22.06 (2×NCOMe). *R*_f 0.42 (MeOH–1 M aq. Py·AcOH, 5 : 1). MS, *m/z*: 482 (calc. for [C₁₉H₃₅N₃O₁₁]⁺H⁺, *m/z*: 482.22). [α]₅₄₆²⁵ +96 (*c* 0.4, MeCN–H₂O, 1 : 1).

Compound 8. ¹H NMR (700 MHz, D₂O) δ: 1.906–2.000 (m, 2H, CH₂ sp), 3.101 (m ≈ t, 2H, NCH₂ sp, *J* 6.8 Hz), 3.706 (br. dd, 1H, H-5, *J*_{5,6'} 7.9 Hz, *J*_{5,6''} 4.4 Hz, *J*_{5,4} ≤ 1 Hz), 3.719–3.759 (m, 1H, OCHH sp), 3.793 (dd, 1H, H-6', *J*_{6',6''} 11.7 Hz, *J*_{5,6'} 4.4 Hz), 3.824 (dd, 1H, H-6'', *J*_{6',6''} 11.7 Hz, *J*_{5,6''} 7.9 Hz), 3.847 (dd, 1H, H-3, *J*_{3,4} 3.3 Hz, *J*_{2,3} 10.8 Hz), 3.966 (br. d, 1H, H-4, *J* 3.3 Hz), 3.984 (dd, 1H, H-2, *J*_{1,2} 8.5 Hz, *J*_{2,3} 10.8 Hz), 4.014–4.060 (m, 1H, OCHH sp); 4.125 (d, 1H, CHHOH Gc, *J*_{gem} 16.5 Hz), 4.162 (d, 1H, CHHOH Gc, *J*_{gem} 16.5 Hz), 4.522 (d, 1H, H-1, *J*_{1,2} 8.5 Hz). ¹³C NMR (176 MHz, D₂O) δ: 101.53 (C-1), 75.18, 70.56, 67.94, 67.80 (C-3, C-4, C-5, CH₂O sp), 61.06 (2) (C-6, CH₂OH Gc), 52.16 (C-2), 37.77 (NCH₂ sp), 26.66 (CH₂ sp). *R*_f 0.51 (MeOH–1 M aq. Py·AcOH, 5 : 1). MS, *m/z*: 295 (calc. for [C₁₁H₂₂N₂O₇]⁺H⁺, *m/z*: 295.14). [α]₅₄₆²⁵ nm +1 (*c* 0.6, MeCN–H₂O, 1 : 1).

Compound 9. ¹H NMR (700 MHz, D₂O) δ: 1.246 (d, 3H, H-6c, *J*_{5,6} 6.6 Hz), 2.000–2.054 (m, 2H, CH₂ sp), 3.123–3.198 (m, 2H, NCH₂ sp), 3.692 (br. dd, 1H, H-5a, *J*_{5,6'} 7.9, *J*_{5,6''} 4.3, *J*_{5,4} ≤ 1), 3.748–3.859 (m, 8H), 3.873–3.919 (m, 1H, OCHH sp), 3.962–4.052 (m, 2H), 4.144 (d, 1H, CHHOH Gc, *J*_{gem} 16.5 Hz), 4.168 (d, 1H, CHHOH Gc, *J*_{gem} 16.5 Hz), 4.240 (br. d, 1H, H-4, *J* 3.2 Hz), 4.277–4.335 (m, 2H, H-2b, H-5b), 4.371 (q, 1H, H-5c, *J*_{5,6} 6.6 Hz), 4.587 (d, 1H, H-1a, *J*_{1,2} 7.9 Hz), 5.227 (d, 1H, H-1b, *J*_{1,2} 3.8 Hz), 5.281 (d, H-1c, *J*_{1,2} 3.6 Hz). ¹³C NMR (176 MHz, D₂O) δ: 101.67 (C-1a), 99.08 (C-1c), 91.48 (C-1b), 75.56, 74.91, 73.61, 71.90, 71.22, 69.83, 68.61, 67.85, 66.94, 63.02 (C-2a, C-3a, C-4a, C-5a, C-3b, C-4b, C-5b, C-2c, C-3c, C-4c, C-5c), 67.40 (CH₂O sp), 61.39, 61.07, 60.96 (C-6a, C-6b, CH₂OH Gc), 49.30 (C-2b), 37.23 (NCH₂ sp), 27.00 (CH₂ sp), 15.38 (C-6c). *R*_f 0.36 (EtOH–BuOH–Py–H₂O–AcOH, 100 : 10 : 10 : 10 : 2), MS, *m/z*: 625.25 (calc. for [C₂₃O₁₆H₄₂N₂]⁺Na⁺, *m/z*: 625.24). [α]₅₄₆²⁵ +42 (*c* 0.4, MeCN–H₂O, 1 : 1).

Compound 10. ^1H NMR (700 MHz, D_2O) δ : 1.979–2.063 (m, 2H, CH_2 sp), 3.134–3.168 (m \approx t, 2H, NCH_2 sp, J 7.2 Hz), 3.538 (dd, 1H, H-2b, $J_{1,2}$ 7.8 Hz, $J_{2,3}$ 9.9 Hz), 3.575–3.617 (m, 1H, OCHH sp), 3.630 (dd, 1H, H-3b, $J_{3,4}$ 3.0 Hz, $J_{2,3}$ 9.9 Hz), 3.672 (br. dd, 1H, H-5b, $J_{5,6'}$ 7.8 Hz, $J_{5,6'}$ 4.3 Hz, $J_{5,4} \leq 1$ Hz), 3.754 (dd, 1H, H-6'a, $J_{6',6''}$ 11.5 Hz, $J_{5,6'}$ 4.3 Hz), 3.764–3.796 (m, 2H, H-6'b, H-6''b), 3.805 (dd, 1H, H-6''a, $J_{6',6''}$ 11.5 Hz, $J_{5,6''}$ 8.0 Hz), 3.843–3.884 (m, 1H, OCHH sp), 3.923 (br. d, 1H, H-4b, J 3.3 Hz), 4.004 (br. dd, 1H, H-5a, $J_{5,6'}$ 8.0 Hz, $J_{5,6'}$ 4.3 Hz, $J_{5,4} \leq 1$ Hz), 4.095–4.170 (m, 3H, H-3a, CH_2OH Gc), 4.273 (br. d, 1H, H-4a, J 2.6 Hz), 4.416 (dd, 1H, H-2a, $J_{1,2}$ 3.8 Hz, $J_{2,3}$ 11.0 Hz), 4.499 (d, 1H, H-1b, $J_{1,2}$ 7.8 Hz), 4.959 (d, 1H, H-1a, $J_{1,2}$ 3.8 Hz). ^{13}C NMR (176 MHz, D_2O) δ : 104.55 (C-1b), 97.23 (C-1a), 77.11, 75.09, 72.60, 70.86, 70.66, 68.70, 68.65 (C-3a, C-4a, C-5a, C-2b, C-3b, C-4b, C-5b), 65.04 (CH_2O sp), 61.31, 61.08, 60.97 (C-6a, C-6b, CH_2OH Gc), 48.27 (C-2a), 37.27 (NCH_2 sp), 26.74 (CH_2 sp). R_f 0.46 (MeOH–1 M aq. Py·AcOH, 5 : 1). MS, m/z : 457 (calc. for $[\text{C}_{17}\text{H}_{32}\text{N}_2\text{O}_{12}]\text{H}^+$, m/z : 457.20). $[\alpha]_D^{25}$ $+0.5$ (c 0.5, MeCN– H_2O , 1 : 1).