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Synthesis of disaccharides for the study of human blood antibodies capable of recognizing the inner Glcβ1-3GalNAc disaccharide fragment of bacterial polysaccharides

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^{1}H, ^{13}C NMR (DEPT-135) spectra were registered on a Bruker AVANCE spectrometer (Bruker BioSpin MRI GmbH, Germany) at 303 K in D_2O with operating frequences of 700 MHz and 176 Hz, respectivly. Chemical shifts δ for characteristic protons are given in ppm using HOD (δ 4.750), as references. Coupling constants *J* are given in Hz. The signals in ^{1}H NMR spectra were assigned using 2D-^{1}H,^{1}H- COSY experiments. The values of optical rotation were measured on a Perkin Elmer 341 digital polarimeter at 25°C in solution MeCN-H_2O (1 : 1). Mass spectra were registered on a MALDI-TOF Vision-2000 spectrometer using dihydroxybenzoic acid as a matrix. Thin-layer chromatography (TLC) was carried out on silica gel 60 precoated aluminum sheets (Merck, Germany), substances were detected with a 5% aqueous solution of phosphoric acid at 150°C (for carbohydrates) or ninhydrin solution (5 g dm^{-3} in acetone–acetic acid–water, 95:1:4, for amines). Column chromatography was performed on Kieselgel 60 silica gel (0.004–0.063 mm; Merck, Germany). TLC of free 3-aminopropyl glycosides was carried out in system (methanol – 1M aqueous solution of Py – AcOH, 5:1).

Glcβ1-3GalNAcα-O(CH$_2$)$_3$NH$_2$ (**6**): ^{1}H-NMR: 1.97–2.04 (m, 2H, C*H*$_2$ sp); 2.05 (s, 3H, NCOC*H*$_3$); 3.15 (br. t, 2H, NC*H*$_2$ sp, *J* 7.5); 3.32 (dd≈t, 1H, H-2b, *J* 8.6); 3.39–3.45 (m, 2H, H-4b, H-5b); 3.48 (dd≈t, 1H, H-3b, *J* 8.8); 3.57–3.63 (m, 1H, OC*H* sp); 3.75–3.78 (m, 2H, H-6'b, H-6'a); 3.80 (dd, 1H, H-6"a, $J_{5,6"}$ 7.8, $J_{6',6"}$ 11.7); 3.82–3.86 (m, 1H, OC*H* sp); 3.91 (dd, 1H, H-6"b, $J_{5,6"}$ 1.4, $J_{6',6"}$ 12.1); 3.98 (br. dd, 1H, H-5a, $J_{4,5}$ <1, $J_{5,6'}$ 4.3, $J_{5,6"}$ 7.8); 4.03 (dd, 1H, H-3a, $J_{2,3}$ 11.1, $J_{3,4}$ 3.0); 4.25 (br. d, 1H, H-4a, *J* 3.0); 4.36 (dd, 1H, H-2a, $J_{1,2}$ 3.8, $J_{2,3}$ 11.1); 4.54 (d, 1H, H-1b, $J_{1,2}$ 7.9); 4.93 (d, 1H, H-1a, $J_{1,2}$ 3.8). ^{13}C NMR: 104.20 (C-1b); 97.29 (C-1a); 77.34, 75.80, 75.62, 72.94, 70.84, 69.50, 68.68 (C-3a, C-4a, C-5a, C-2b, C-3b, C-4b, C-5b); 64.99 (*C*H$_2$O sp); 61.31, 60.60 (C-6a, C-6b); 48.59 (C-2a); 37.17 (NC*H*$_2$ sp); 26.81 (*C*H$_2$ sp); 22.02 (NCOC*H*$_3$). R_f 0.58. MS, *m/z* calculated for [$C_{17}H_{32}N_2O_{11}$]H$^+$: 441.21; found 441. $[\alpha]_{589\ nm}$ +83 (c 0.7).

Glcβ1-3GalNAcβ-O(CH$_2$)$_3$NH$_2$ (**7**): ^{1}H-NMR: 1.93–2.03 (m, 2H, C*H*$_2$ sp); 2.04 (s, 3H, NCOC*H*$_3$); 3.11 (br. t, 2H, NC*H*$_2$ sp, *J* 6.8); 3.31 (dd, 1H, H-2b, $J_{1,2}$ 7.9, $J_{2,3}$ 9.2); 3.39–3.45 (m, 2H, H-4b, H-5b); 3.47 (dd≈t, 1H, H-3b, *J* 9.0); 3.71 (br. dd, 1H, H-5a, $J_{5,6'}$ 4.4, $J_{5,6"}$ 7.8, $J_{4,5}$ <1); 3.72–3.77 (m, 2H, OC*H* sp, H-6'b); 3.78 (dd, 1H, H-6'a, $J_{5,6'}$ 4.4, $J_{6',6"}$ 11.7); 3.82 (dd, 1H, H-6"a, $J_{5,6"}$ 7.8, $J_{6',6"}$ 11.7); 3.85–3.91 (m, 2H, H-6"b, H-3a, $J_{2,3}$ 10.9, $J_{3,4}$ 3.1); 4.02–4.06 (m, 2H, OC*H* sp, H-2a, $J_{2,3}$ 10.9, $J_{1,2}$ 8.5); 4.19 (br. d, 1H, H-4a, *J* 3.1); 4.50 (d, 1H, H-1a, $J_{1,2}$ 8.5); 4.52 (d, 1H, H-1b, $J_{1,2}$ 7.9). ^{13}C NMR: 104.31, 101.51 (C-1a, C-1b); 80.01, 75.77, 75.59, 74.82, 72.92, 69.44, 67.84 (C-3a, C-4a, C-5a, C-2b, C-3b, C-4b, C-5b); 67.96 (*C*H$_2$O sp); 61.01, 60.52 (C-6a, C-6b); 51.24 (C-2a); 37.76 (N*C*H$_2$ sp); 26.68 (*C*H$_2$ sp); 22.06 (NCO*C*H$_3$). R$_f$ 0.48. MS, *m/z* calculated for [C$_{17}$H$_{32}$N$_2$O$_{11}$]H$^+$: 441.21; found 441. [α]$_{589\ nm}$ –17 (c 0.6).

Glcβ1-3GlcNAcβ-O(CH$_2$)$_3$NH$_2$ (**8**): ^{1}H-NMR: 1.93–2.02 (m, 2H, C*H*$_2$ sp); 2.05 (s, 3H, NCOC*H*$_3$); 3.10 (br. t, 2H, NC*H*$_2$ sp, *J* 6.9); 3.31 (dd≈t, 1H, H-2b, *J* 8.6); 3.42 (dd≈t, 1H, H-4b, *J* 9.4); 3.45–3.53 (m, 3H, H-3b, H-5b, H-5a); 3.55 (dd≈t, 1H, H-4a, *J* 9.2); 3.71–3.76 (m, 2H, OC*H* sp, H-6'b); 3.76–3.80 (m, 2H, H-6'a, H-3a); 3.87 (dd, 1H, H-2a, $J_{1,2}$ 8.5, $J_{2,3}$ 10.3); 3.91 (dd, 1H, H-6"b, $J_{5,6"}$ 1.6, $J_{6',6"}$ 12.3); 3.95 (dd, 1H, H-6"a, $J_{5,6"}$ 1.6, $J_{6',6"}$ 12.3); 4.01–4.08 (m, 1H, OC*H* sp); 4.51 (d, 1H, H-1b, $J_{1,2}$ 7.9); 4.55 (d, 1H, H-1a, $J_{1,2}$ 8.5). ^{13}C NMR: 103.11, 101.07 (C-1a, C-1b); 82.66, 76.02, 75.55, 75.45, 72.95, 69.50, 68.63 (C-3a, C-4a, C-5a, C-2b, C-3b, C-4b, C-5b); 67.95 (*C*H$_2$O sp); 60.72, 60.66 (C-6a, C-6b); 54.55 (C-2a); 37.63 (N*C*H$_2$ sp); 26.71 (*C*H$_2$ sp); 22.24 (NCO*C*H$_3$). R$_f$ 0.54. MS, *m/z* calculated for [C$_{17}$H$_{32}$N$_2$O$_{11}$]H$^+$: 441.21; found 441. [α]$_{589\ nm}$ –32 (c 0.6).

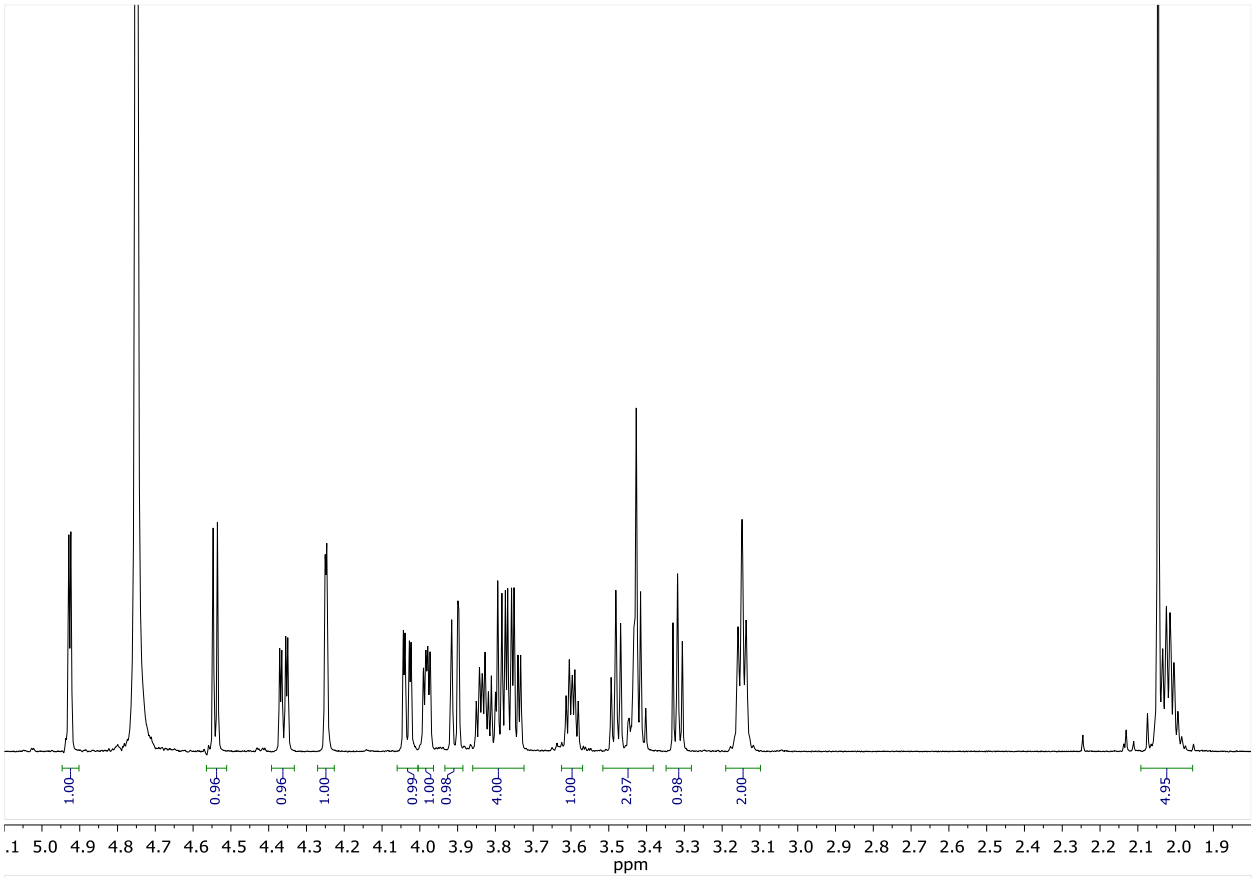
Glcβ1-3Galβ-O(CH$_2$)$_3$NH$_2$ (**9**): ^{1}H-NMR: 1.98–2.07 (m, 2H, C*H*$_2$ sp); 3.19 (br. t, 2H, NC*H*$_2$ sp, *J* 6.9); 3.39 (dd, 1H, H-2b, $J_{1,2}$ 7.9, $J_{2,3}$ 9.4); 3.44 (dd, 1H, H-4b, $J_{4,5}$ 9.8, $J_{3,4}$ 8.6); 3.46 (ddd, 1H, H-5b, $J_{4,5}$ 9.8, $J_{5,6'}$ 2.1, $J_{5,6"}$ 4.6); 3.52 (dd≈t, 1H, H-3b, *J* 8.9); 3.70 (dd, 1H, H-2a $J_{1,2}$ 8.0, $J_{2,3}$ 9.8); 3.72–3.78 (m, 3H, H-5a, H-6"b, H-6'a); 3.80 (dd, 1H, H-6"a, $J_{5,6"}$ 7.9, $J_{6',6"}$ 11.7); 3.82–3.87 (m, 2H, OC*H* sp, H-3a, $J_{2,3}$ 9.8, $J_{3,4}$ 3.4); 3.90 (dd, 1H, H-6'b, $J_{5,6'}$ 2.1, $J_{6',6"}$ 12.4); 4.05–4.10 (m, 1H, OC*H* sp); 4.21 (br. d, 1H, H-4a, *J* 3.1); 4.48 (d, 1H, H-1a, $J_{1,2}$ 8.0); 4.68 (d, 1H, H-1b, $J_{1,2}$ 7.9). ^{13}C NMR: 103.79, 102.55 (C-1a, C-1b); 82.48, 75.83, 75.59, 74.89, 73.34, 69.85, 69.43, 68.33 (C-2a, C-3a, C-4a, C-5a, C-2b, C-3b, C-4b, C-5b); 67.93 (*C*H$_2$O sp); 60.99, 60.53 (C-6a, C-6b); 35.82 (N*C*H$_2$ sp); 26.68 (*C*H$_2$ sp). R$_f$ 0.51. MS, m/z calculated for [C$_{15}$H$_{29}$NO$_{11}$]H$^+$: 400.18; found 400. [α]$_{589\ nm}$ +0.1 (c 0.7).

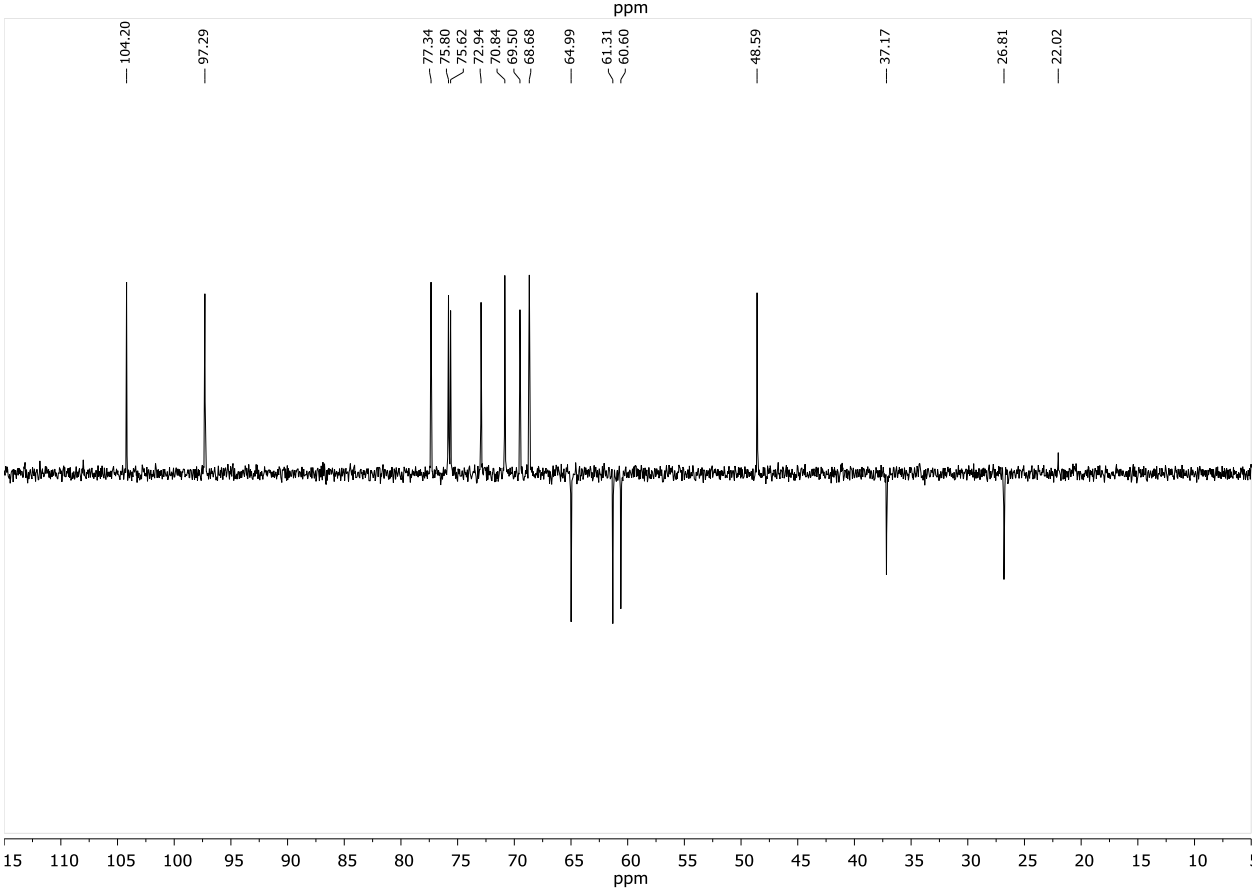
Glcα1-3Galβ-O(CH$_2$)$_3$NH$_2$ (**10**): ^{1}H-NMR: 1.98–2.09 (m, 2H, C*H*$_2$ sp); 3.20 (br. t, 2H, NC*H*$_2$ sp, *J* 6.9); 3.49 (dd, 1H, H-4b, $J_{3,4}$ 9.3, $J_{4,5}$ 10.0); 3.62 (dd, 1H, H-2b, $J_{1,2}$ 3.8, $J_{2,3}$ 9.9); 3.68 (dd, 1H, H-2a, $J_{1,2}$ 7.9, $J_{2,3}$ 9.9); 3.72 (br. dd, 1H, H-5a, $J_{5,6'}$ 4.5, $J_{5,6"}$ 7.7, $J_{4,5}$ <1); 3.75–3.87 (m, 7H, H-6"a, H-6'a, H-6"b, H-6'b, H-3b, OC*H* sp, H-3a, $J_{2,3}$ 9.9, $J_{3,4}$ 3.1); 3.98 (ddd, 1H, H-5b, $J_{4,5}$ 10.0, $J_{5,6'}$ 2.4, $J_{5,6"}$ 4.7); 4.06–4.11 (m, 1H, OC*H* sp); 4.20 (br. d, 1H, H-4a, *J* 3.1); 4.49 (d, 1H, H-1a, $J_{1,2}$ 7.9); 5.14 (d, 1H, H-1b, $J_{1,2}$ 3.8).^{13}C NMR: 102.79 (C-1a), 95.41 (C-1b); 77.53, 74.97, 72.87, 71.87, 71.32, 69.43, 69.22, 65.01 (C-2a, C-3a, C-4a, C-5a, C-2b, C-3b, C-4b, C-5b); 67.96 (*C*H$_2$O sp); 61.03, 60.41 (C-6a, C-6b); 37.76 (N*C*H$_2$ sp); 26.68 (*C*H$_2$ sp). R$_f$ 0.51. MS, m/z calculated for [C$_{15}$H$_{29}$NO$_{11}$]H$^+$: 400.18; found 400. [α]$_{546\ nm}$ +116 (c 0.3).

Glcβ1-6Galβ-O(CH$_2$)$_3$NH$_2$ (**11**): ^{1}H-NMR: 1.98–2.07 (m, 2H, C*H*$_2$ sp); 3.19 (br. t, 2H, NC*H*$_2$ sp, *J* 7.0); 3.31 (dd, 1H, H-2b, $J_{1,2}$ 7.9, $J_{2,3}$ 9.3); 3.40 (dd, 1H, H-4b, $J_{4,3}$ 9.2, $J_{4,5}$ 9.9); 3.48 (ddd, 1H, H-5b, $J_{4,5}$ 9.9, $J_{5,6'}$ 2.0, $J_{5,6"}$ 6.0); 3.51 (dd≈t, 1H, H-4b, *J* 9.3); 3.54 (dd, 1H, H-2a $J_{1,2}$ 8.0, $J_{2,3}$ 9.9); 3.68 (dd, 1H, H-3a, $J_{2,3}$ 9.9, $J_{3,4}$ 3.5); 3.74 (dd, 1H, H-6"b, $J_{5,6"}$ 6.0, $J_{6',6"}$ 12.3); 3.83–3.88 (m, 1H, OC*H* sp); 3.89–3.95 (m, 3H, H-5a, H-6'b, H-6'a); 3.97 (br. d, 1H, H-4a, *J* 3.5); 4.03–4.09 (m, 2H, OC*H* sp, H-6b"); 4.45 (d, 1H, H-1a, $J_{1,2}$ 8.0); 4.53 (d, 1H, H-1b, $J_{1,2}$ 7.9). ^{13}C NMR: 102.87, 102.82 (C-1a, C-1b); 76.00, 75.78, 73.82, 73.12, 72.64, 70.70, 69.67, 68.71 (C-2a, C-3a, C-4a, C-5a, C-2b, C-3b, C-4b, C-5b); 69.32 (C-6a); 67.99 (*C*H$_2$O sp); 60.77 (C-6b); 37.69 (N*C*H$_2$ sp); 26.71 (*C*H$_2$ sp). R$_f$ 0.54. MS, m/z calculated for [C$_{15}$H$_{29}$NO$_{11}$]H$^+$: 400.18; found 400. [α]$_{589\ nm}$ –21 (c 0.4).

Glcβ1-3GalNAcα-O(CH_2)$_3$$NH_2$ (6)

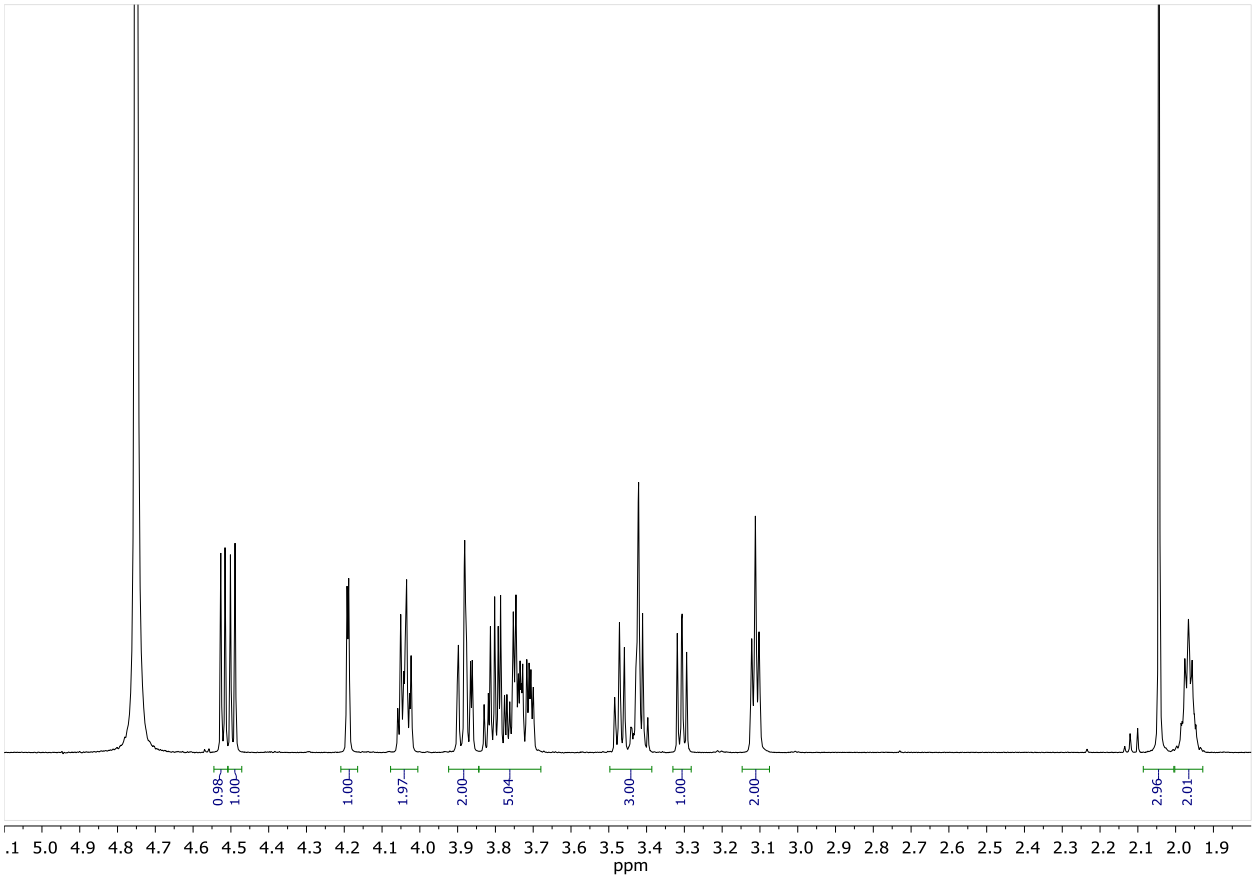
3-Aminopropyl β-D-glucopyranosyl-(1→3)-2-acetamido-2-deoxy-α-D-galactopyranoside

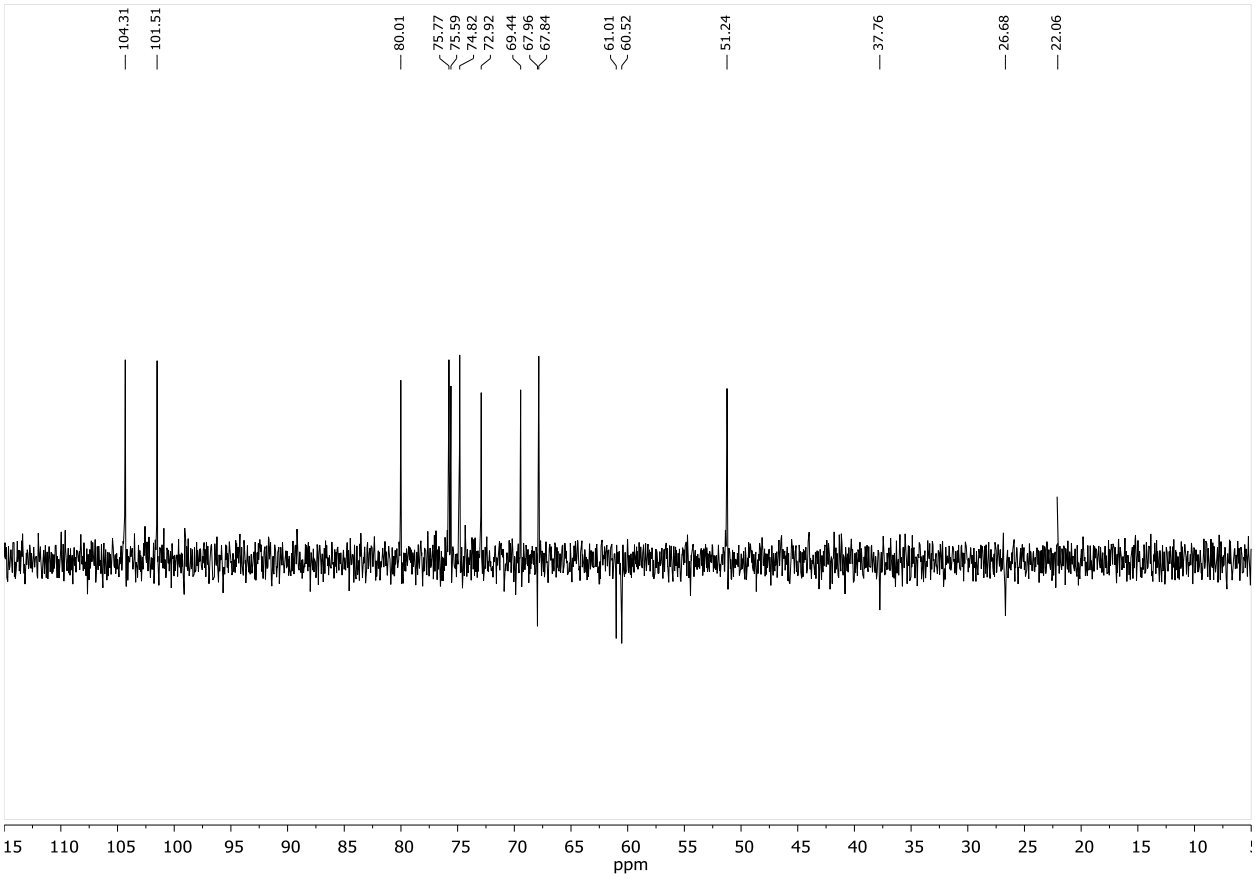




Glcβ1-3GalNAcβ-O(CH_2)$_3$$NH_2$ (7)

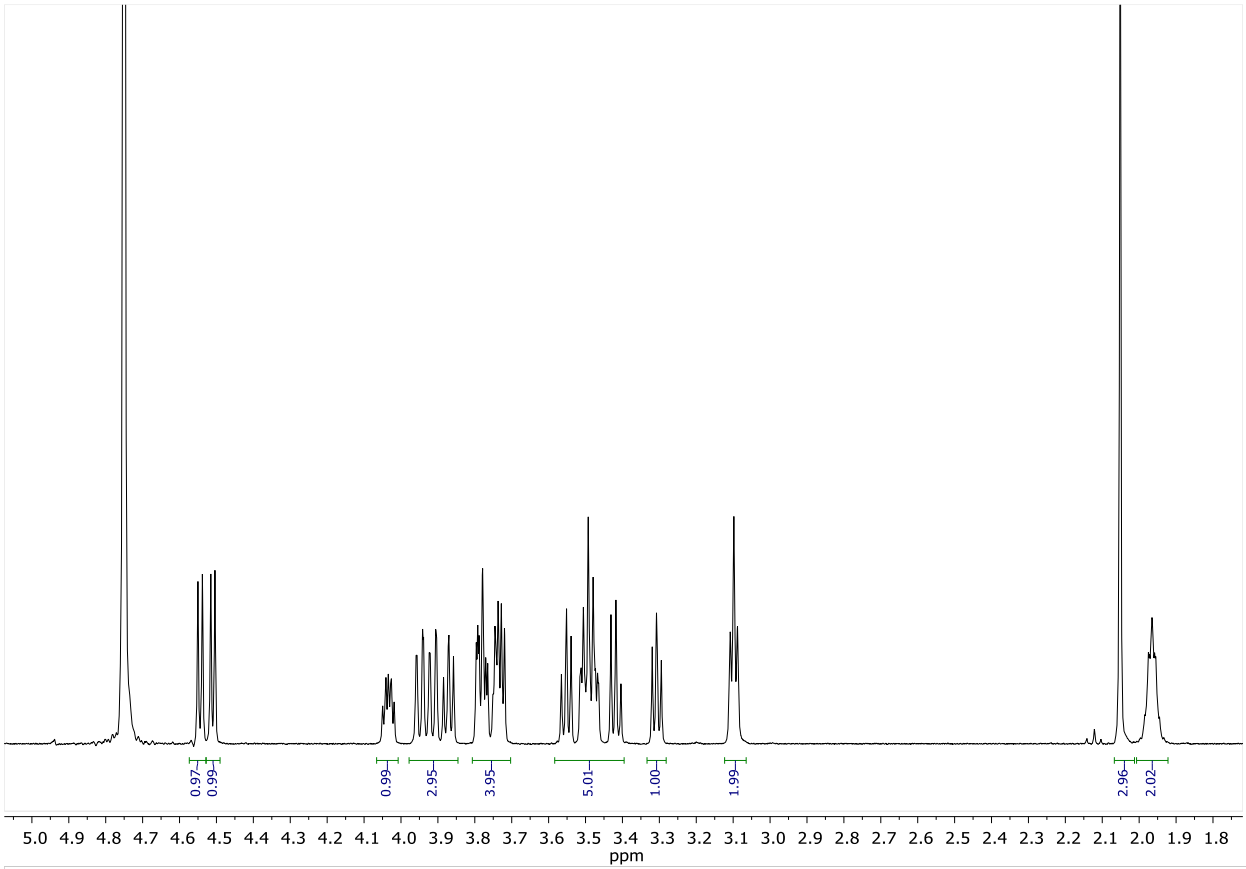
3-aminopropyl β-D-glucopyranosyl-(1→3)-2-acetamido-2-deoxy-β-D-galactopyranoside

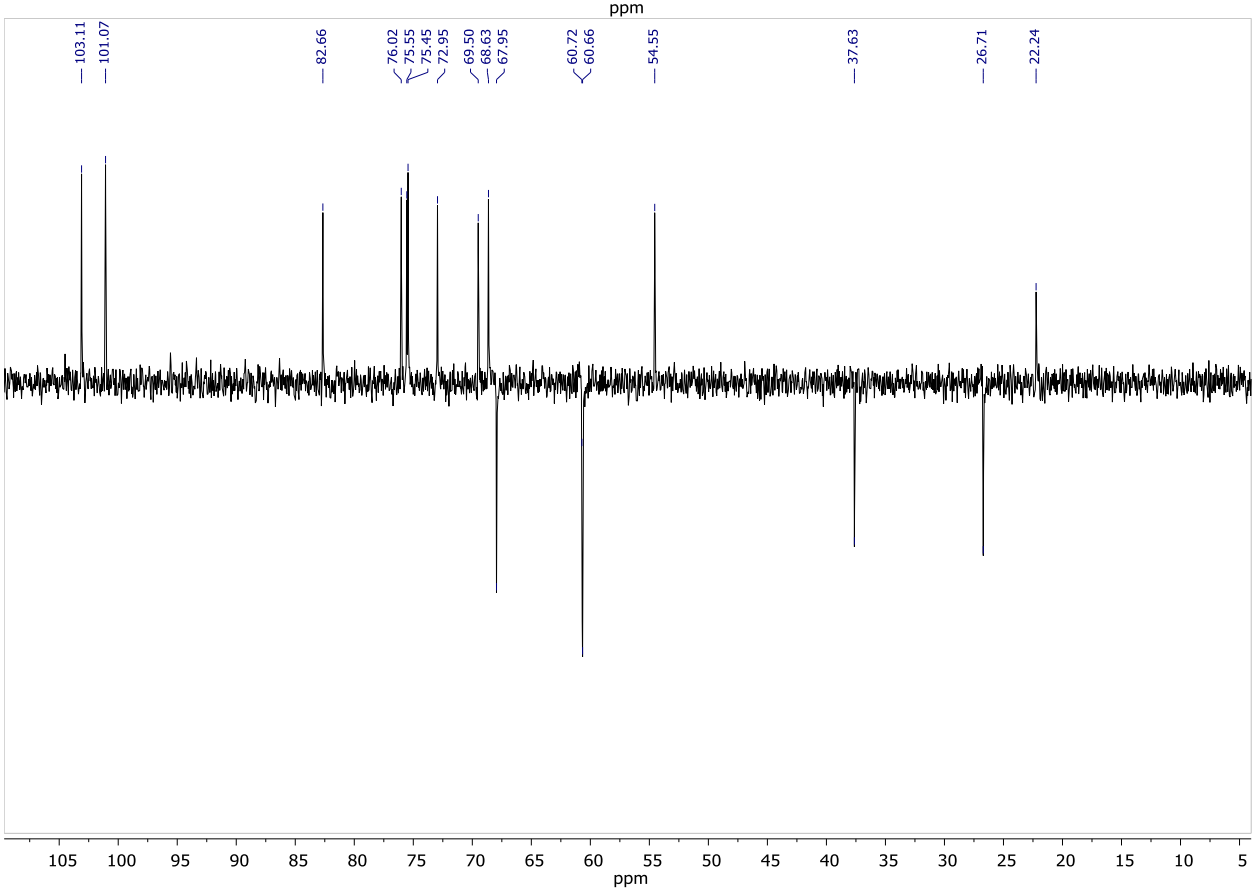




Glcβ1-3GlcNAcβ-O(CH$_2$)$_3$NH$_2$ (8)

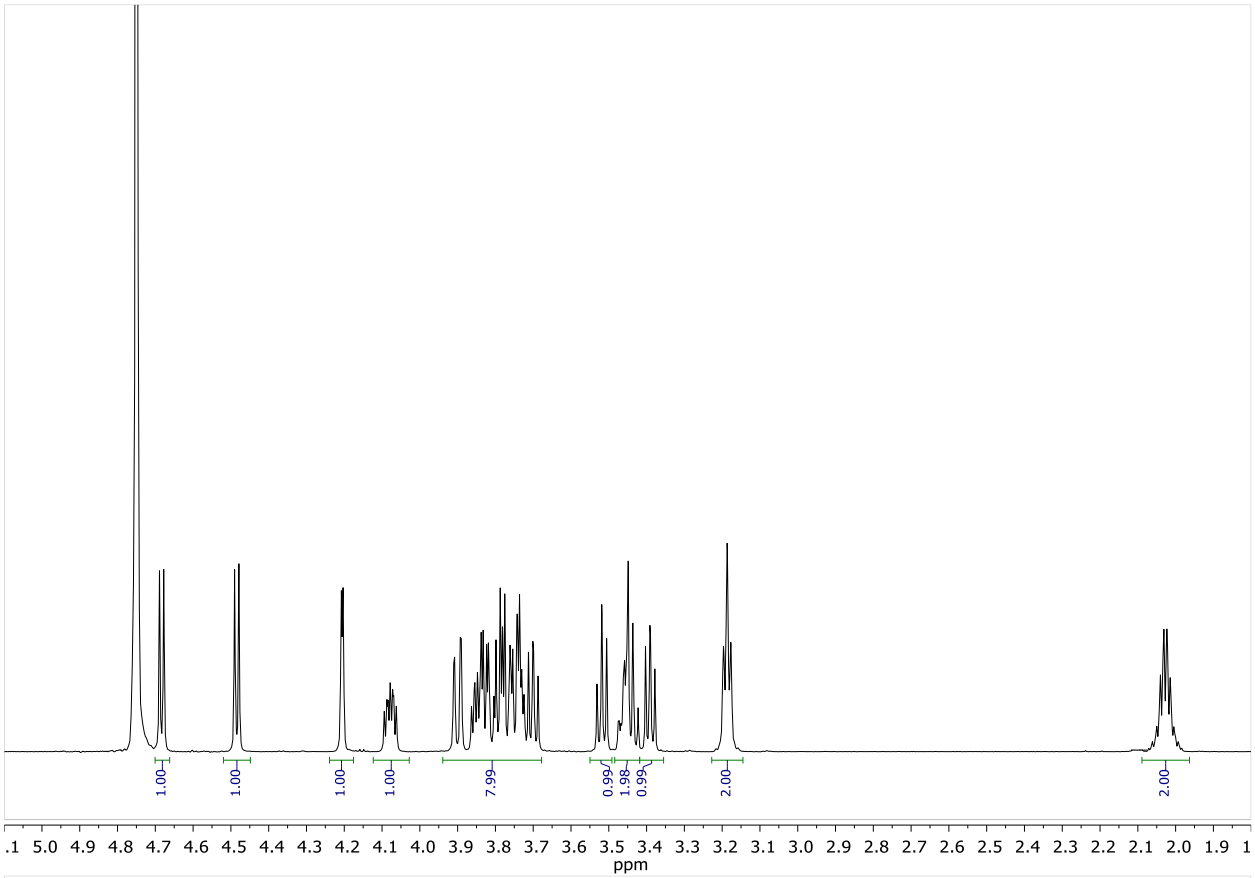
3-aminopropyl β-D-glucopyranosyl-(1→3)-2-acetamido-2-deoxy-β-D-glucopyranoside

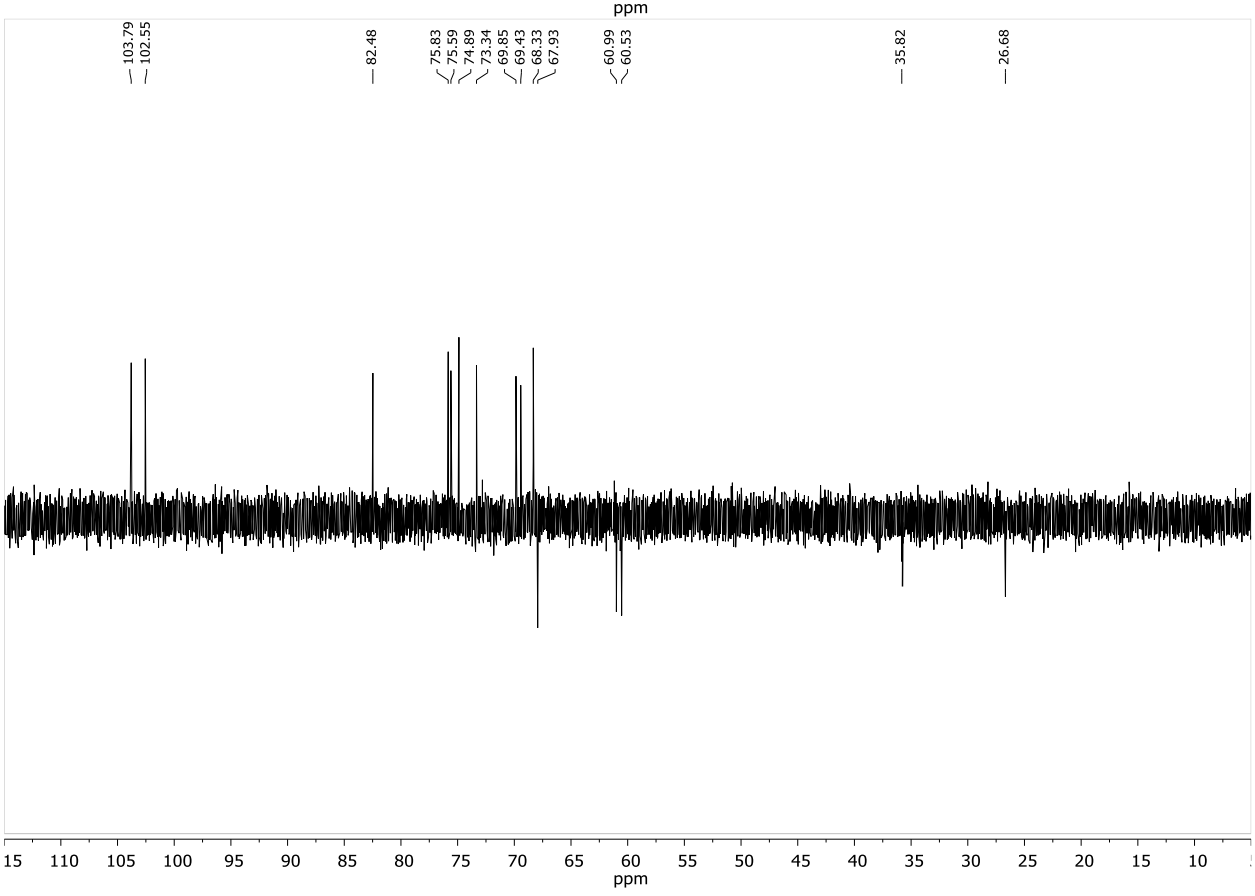




Glcβ1-3Galβ-O(CH_2)$_3$$NH_2$ (9)

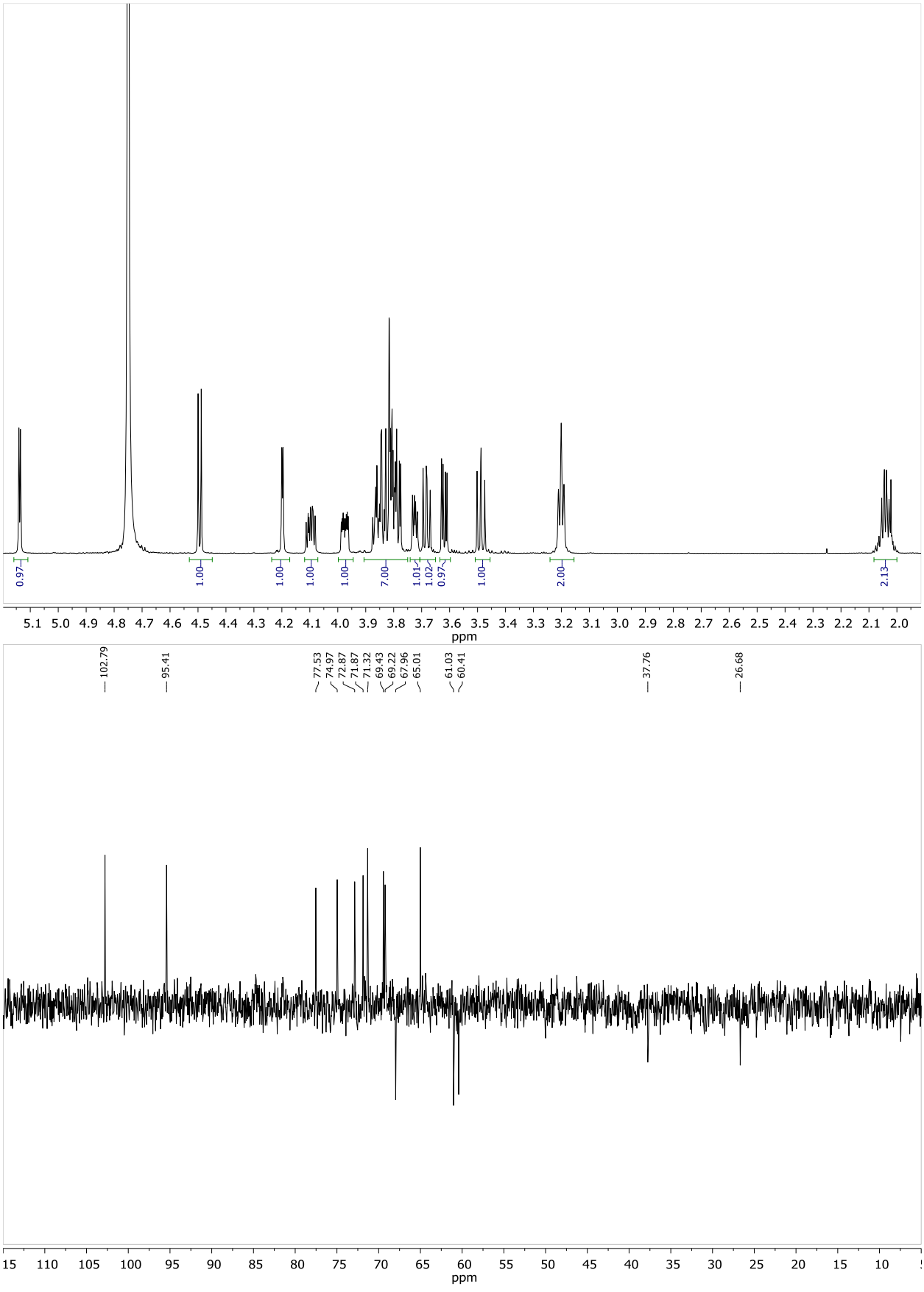
3-aminopropyl β-D-glucopyranosyl-(1→3)-β-D-galactopyranoside





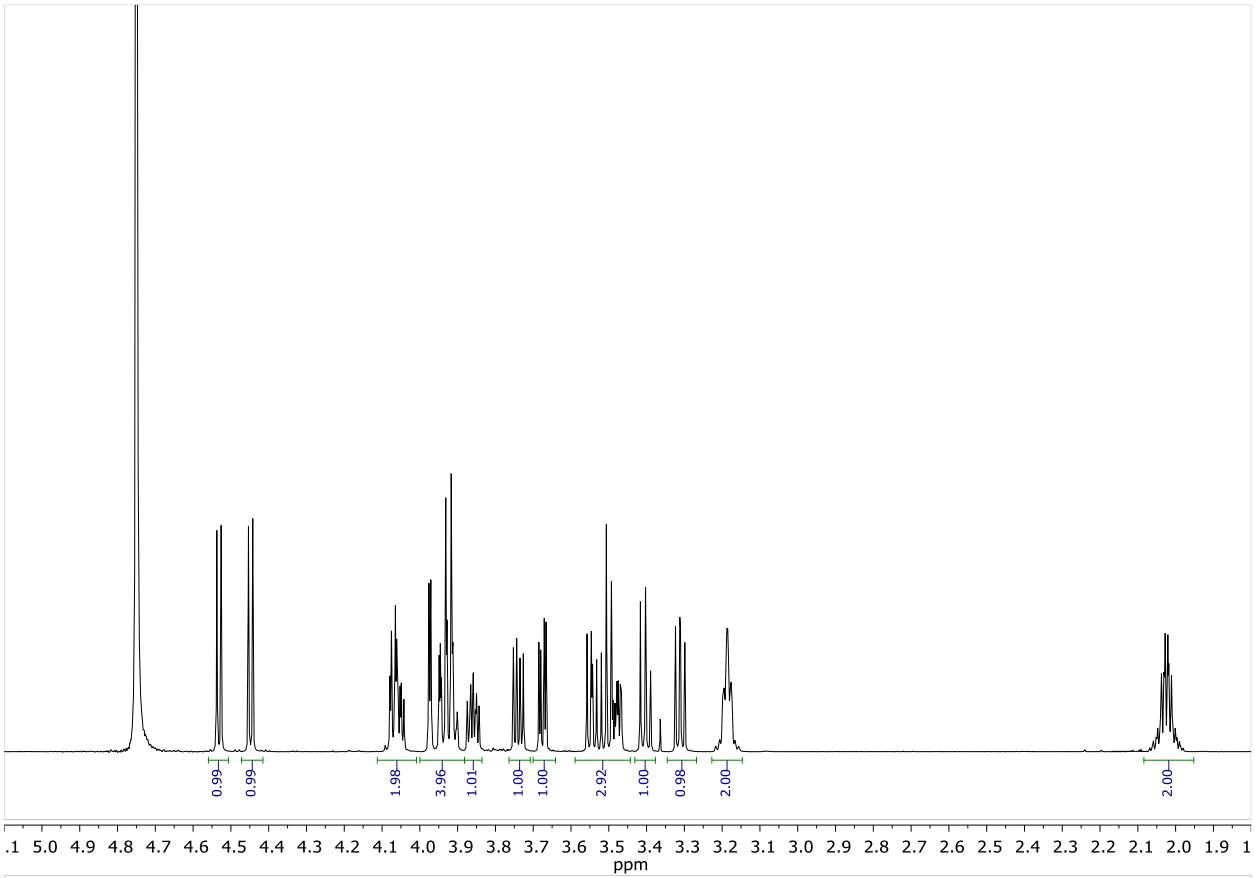
Glcα1-3Galβ-O(CH_2)$_3$$NH_2$ (10)

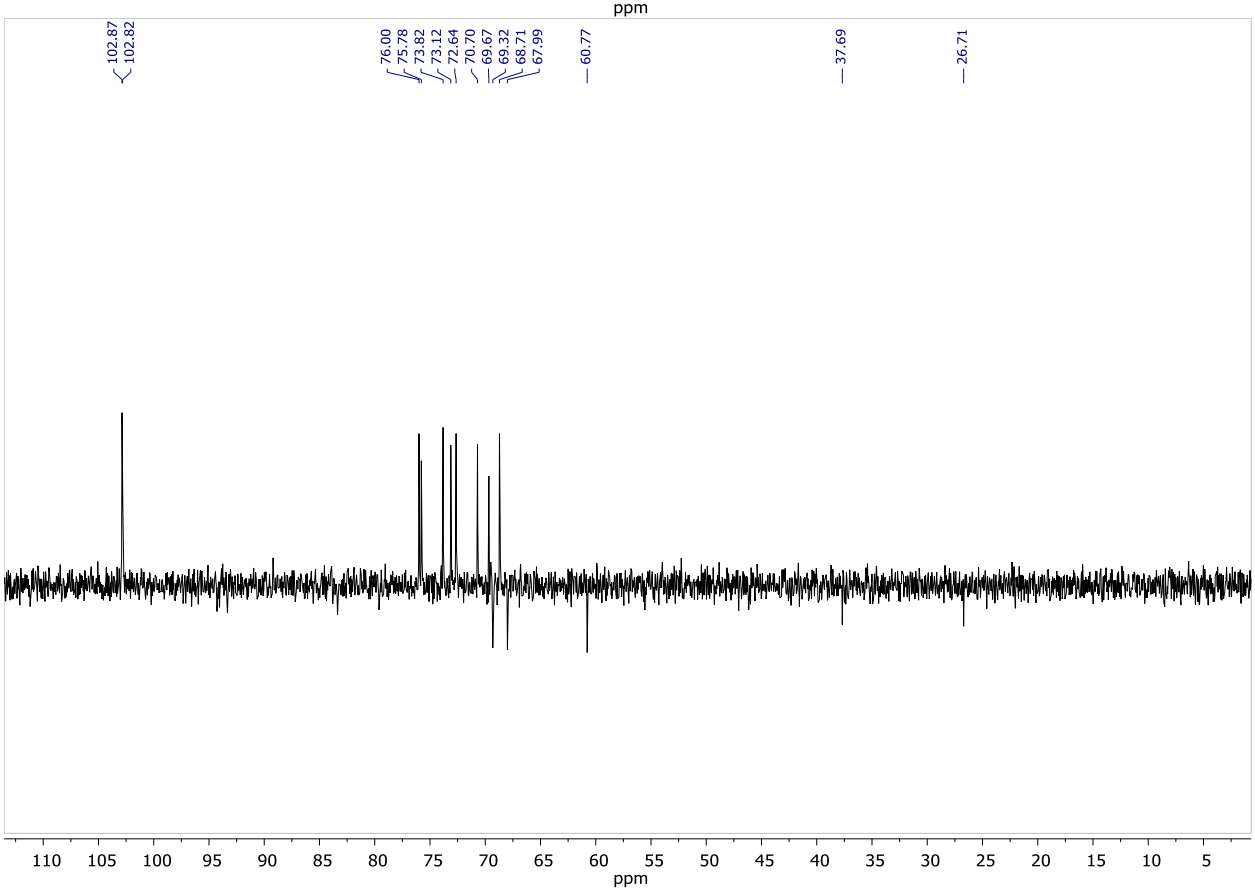
3-aminopropyl α-D-glucopyranosyl-(1→3)-β-D-galactopyranoside



Glcβ1-6Galβ-O(CH_2)$_3$$NH_2$ (11)

3-aminopropyl β-D-glucopyranosyl-(1→6)-β-D-galactopyranoside





Inhibition of binding of affinity-isolated human anti-Glcβ1-3GalNAcα antibodies to Glcβ1-3GalNAcβ-PAA

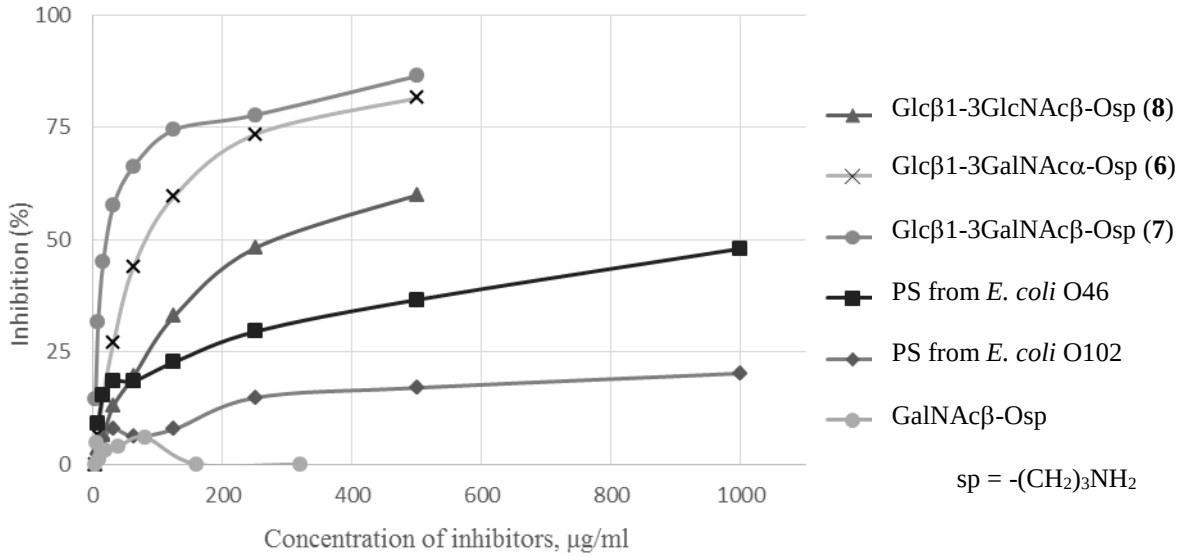


Figure S1 Inhibition* of anti-Glcβ1-3GalNAcα antibodies (IgG+M+A, 82 ng ml^{-1}) binding to Glcβ1-3GalNAcβ-PAA coated ELISA plates by unconjugated mono-, di- and polysaccarides.

Table S1 Values of 50% inhibition of anti-Glcβ1-3GalNAcα antibodies (IgG+M+A, 82 ng ml^{-1}) binding to Glcβ1-3GalNAcβ-PAA on the ELISA plates by unconjugated mono-, di- and polysaccarides.

Inhibitors	$I_{50\%}$ (μM)	$I_{50\%}$ (μg ml^{-1})
GalNAcβ-Osp	–	–
Glcβ1-3GlcNAcβ-Osp (**8**)	667	290
Glcβ1-3GalNAcα-Osp (**6**)	200	88
Glcβ1-3GalNAcβ-Osp (**7**)	50	22
PS from *E. coli* O46	~40	1100

The best inhibitors found to be disaccharide Glcβ1-3GalNAcβ (**7**) and the bacterial polysaccharide from *E. coli O46* -4(L-Thr3(70%)Ac2-6)GlcAβ1-6Galβ1-6**Glcβ1-3GalNAc**6(15%)Ac**β**1-, containing Glcβ1-3GalNAcβ-motif in its structure, $I_{50\%}$ were 50 and ~40 μM, correspondingly. The disaccharide Glcβ1-3GalNAcα (**6**), used as a ligand for the antibodies isolation, was weaker inhibitor ($I_{50\%}$ = 200 μM). The disaccharide Glcβ1-3GlcNAcβ (**8**) was more than 10 times weaker ($I_{50\%}$ = 667 μM) than the strongest inhibitor Glcβ1-3GalNAcβ (**7**); GalNAcβ monosaccharide was not an inhibitor.

* P. Obukhova, R. Rieben and N. Bovin, *Xenotransplantation*, 2007, **14**, 627.