Supplementary information

Mannosylated hemagglutinin peptides bind Cyanovirin-N independent of disulfide-bonds in complementary binding sites

Philipp E. Schilling, Georg Kontaxis, Martin Dragosits, Robert H. Schiestl, Christian F. W. Becker and Irene Maier

Table of contents

1. Carbohydrate synthesis

a) General information

Unless otherwise stated, all solvents and reagents were obtained from Acros Organics (Geel, Belgium), Carbosynth (Compton, UK), Sigma-Aldrich (Darmstadt, Germany), Synthose (Concord, Canada) or TCI-Europe (Zwijndrecht, Belgium) and used as received, without further purification. Under standard Schlenk conditions, flame-dried glassware, inert gas (argon), dry solvents and reagents were used. Column chromatography was performed on silica gel 60 (230–400 mesh, 0.04-0.063 nm; Macherey-Nagel, Düren, Germany). Reaction progress was monitored using thin-layer chromatography (TLC) on aluminum sheets, coated with silica gel 60 with 0.2 mm thickness (Pre-coated TLC-sheets ALUGRAM® Xtra SIL G/UV_{254}; Macherey-Nagel). Compounds were visualized using ceric ammonium molybdate (CAM) staining and heating. HRMS spectra were recorded on a BRUKER maXis ESI-Qq-oaTOF system in positive or negative ion mode. IR spectra were recorded on a FT-IR-BRUKER Vertex 70-spectrometer at room temperature (r.t.) and neat conditions. Identified peaks are reported as wavenumber $\tilde{\nu}$ [1/λ]. ^{1}H- and ^{13}C-NMR spectra were either recorded on BRUKER AVIII400 (400 MHz for ^{1}H and 101 MHz for ^{13}C) or BRUKER AVIII600 (600 MHz for ^{1}H and 151 MHz for ^{13}C), using $CDCl_3$ or D_2O as solvents. Temperature was set to 298.2 K. Chemical shift (δ) values are reported in ppm ('parts per million'). Spectra recorded in $CDCl_3$ as solvent were referenced to 7.26 (^{1}H) or 77.16 (^{13}C) ppm and in D_2O to 4.79 (^{1}H), respectively. NMR-spectroscopy splitting patterns were designated as singlet (s), doublet (d), doublet-doublet (dd), doublet of doublet of doublets (ddd) and triplet (t). Splitting patterns that could not be interpreted or easily visualized were designated as multiplet (m). Coupling constants (*J*) are reported in Hertz (Hz).

b) Synthetic scheme for the preparation of Manα(1→2)Manα azide (**8**)

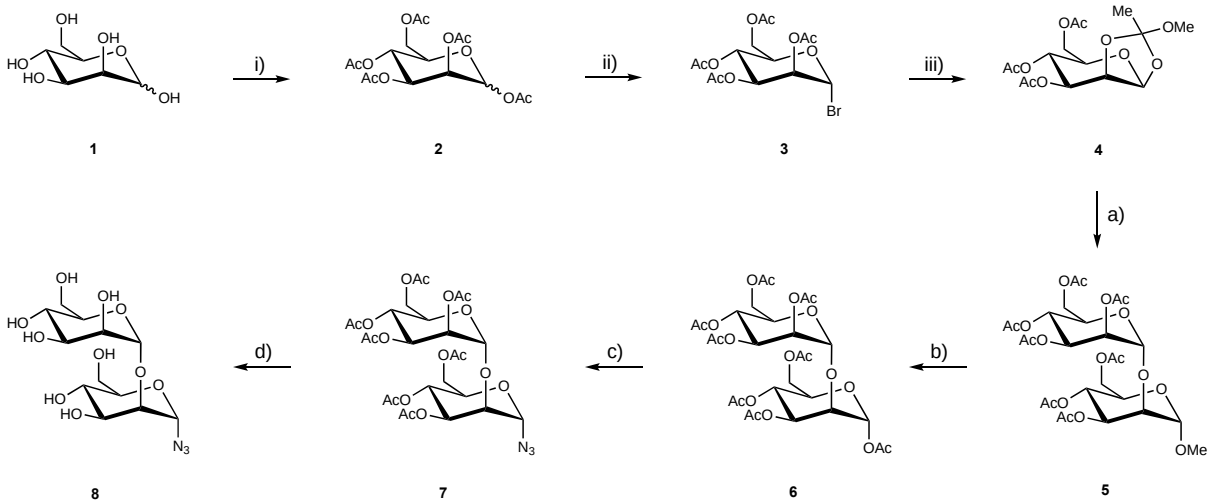
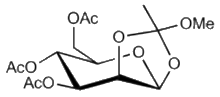


Figure S1. Synthesis route for the preparation of Manα(1–2)Manα azide (**8**). i) Ac_2O/pyridine, r.t., 16 h. ii) HBr/AcOH, r.t., 3 h. iii) 2,6-Lutidine, MeOH, CH_2Cl_2, r.t., 16 h. 64 % (over 3 steps). a) TMSOTf, CH_2Cl_2, - 30°C, 5 min, 46 %. b) H_2SO_4, Ac_2O, 0°C, 4 h, 86 %. c) $FeCl_3$, $TMSN_3$, CH_2Cl_2, r.t., 20 h, 82 %. d) NaOMe/MeOH, 24 h, r.t., 93 %. Overall yield: 20 % (over 7 steps).

c) Synthesis of 3,4,6-Tri-*O*-acetyl-1,2-*O*-(1-methoxyethylidene)-β-D-mannopyranose (**4**)[1,2]



4

Figure S2. Synthesis of 3,4,6-Tri-*O*-acetyl-1,2-*O*-(1-methoxyethylidene)-β-D-mannopyranose (**4**).

D-Mannose (**1**, 141 mg, 0.78 mmol) was dissolved in pyridine (1.5 mL) and acetic anhydride (0.5 mL) in an oven-dried round-bottom flask. The reaction mixture was stirred for 16 hours at room temperature. The solution was concentrated under vacuum; the residue was dissolved in ethyl acetate (20 mL) and washed with aqueous 10% $CuSO_4$ solution (2 x 5 mL) and water (5 mL). The aqueous phase was extracted with DCM (2 x 10 mL) and the combined organic phases were washed with brine (5 mL), followed by drying over $MgSO_4$. After filtration and concentration under vacuum, the crude product **2** (302 mg, 0.77 mmol, 99 %, mixture of both anomers) was directly used in the next step without purification. Analytical data in accordance with literature[2].

The crude product **2** (302 mg, 0.77 mmol) was mixed with 33% HBr/AcOH solution (3 mL) and stirred for 3 hours at room temperature. The solution was then diluted with DCM (12 mL) and washed with ice-water (4 x 5 mL). The combined aqueous phases were extracted with DCM (3 x 10 mL) and the combined organic phases dried with Na_2SO_4, filtered and concentrated in vacuum to give the crude product **3**, which was directly used in the next step.

The crude product **3** was dissolved in dry DCM (2.5 mL) with stirring. In a separate flask under argon atmosphere, dry MeOH (3 mL) and 2,6-Lutidine (0.3 mL) were mixed. 2.75 mL of this mixture (2.8 equiv. of 2,6-Lutidine) were then transferred to the first stirring solution. The mixture was stirred overnight at r.t., then diluted with DCM (15 mL) and washed with aqueous 10% $CuSO_4$ solution (4 mL). The organic phase was washed with water (2 x 4 mL) and the combined aqueous phases extracted with DCM (3 x 10 mL). The combined organic phases were dried over Na_2SO_4, filtered and concentrated under vacuum. The crude product was purified by column chromatography (heptanes/EtOAc = 3:1 → 2:1) to give 182 mg of pure compound **4** (181 mg, 0.50 mmol, 64 % over 3 steps) as colorless needles [1,3].

^{1}H NMR (600 MHz, $CDCl_3$) δ 5.50 (d, *J* = 2.5 Hz, 1H, H_1), 5.30 (t, *J* = 9.8 Hz, 1H, H_4), 5.15 (dd, *J* = 9.9, 4.0 Hz, 1H, H_3), 4.62 (dd, *J* = 4.0, 2.6 Hz, 1H, H_2), 4.24 (dd, *J* = 12.2, 4.9 Hz, 1H, H_{6b}), 4.14 (dd, *J* = 12.2, 2.6 Hz, 1H, H_{6a}), 3.68 (ddd, *J* = 9.6, 4.9, 2.6 Hz, 1H, H_5), 3.28 (s, 3H, OCH_3), 2.13 (s, 3H, OC(O)CH_3), 2.08 (s, 3H, OC(O)CH_3), 2.06 (s, 3H, OC(O)CH_3), 1.75 (s, 3H, C-CH_3) ppm.

^{13}C NMR (151 MHz, $CDCl_3$) δ 170.9 (O*C*(O)CH_3), 170.6 (O*C*(O)CH_3), 169.6 (O*C*(O)CH_3), 124.7 (quat. *C*, *C*-CH_3), 97.5 (C_1), 76.7 (C_2), 71.4 (C_5), 70.8 (C_3), 65.5 (C_4), 62.4 (C_6), 50.1 (O*C*H_3), 24.6 (C-*C*H_3), 20.9 (OC(O)*C*H_3), 20.9 (OC(O)*C*H_3), 20.9 (OC(O)*C*H_3) ppm.

ESI$^+$-HRMS (m/z) for $C_{15}H_{22}O_{10}Na$ = 385.1105 $[M+Na]^+$; found: 385.1108 Da.

d) Synthesis of 2,3,4,6-Tetra-*O*-acetyl-α-D-mannopyranosyl-(1→2)-3,4,6-tri-*O*-acetyl-α-D-mannopyranoside methylether (**5**)

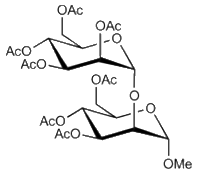


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Figure S3. Synthesis of 2,3,4,6-Tetra-*O*-acetyl-α-D-mannopyranosyl-(1→2)-3,4,6-tri-*O*-acetyl-α-D-mannopyranoside methylether (**5**).

Compound **4** (181 mg, 0.5 mmol, 1 equiv.) was dissolved in dry DCM (6 mL) and cooled to -30°C, followed by the dropwise addition of trimethylsilyl trifluoromethanesulfonate (275 µL, 1.5 mmol, 3 equiv.) with stirring. After 5 minutes, the solution was diluted with DCM (7 mL) and quenched with sat. aq. $NaHCO_3$ solution (7 mL). The phases were separated and the aqueous layer was extracted with DCM (3 x 7 mL). After combining organic layers, the solution was washed with brine (5 mL) and dried over Na_2SO_4, followed by filtration and concentration under vacuum. The crude product was purified by column chromatography (heptanes/EtOAc = 2/1 → 1/1), obtaining methylether-dimannose **5** as a white foam (75 mg, 0.12 mmol, 46 % yield), in accordance with literature[1,4,5].

^{1}H NMR (400 MHz, $CDCl_3$) δ 5.44-5.22 (m, 5H, *H*$_3$, *H*$_4$, *H*$_{2'}$, *H*$_{3'}$, *H*$_{4'}$), 4.91 and 4.84 (2 d, each 1H, *J* = 1.8 Hz, *H*$_1$, *H*$_{1'}$), 4.26-4.09 (m, 5H, *H*$_{5'}$, *H6*$_{a,b}$, *H*$_{6a',b'}$), 4.03 (dd, *J* = 3.0, 2.0 Hz, 1H, *H*$_2$), 3.90 (ddd, *J* = 9.5, 4.4, 2.5 Hz, 1H, *H*$_5$), 3.41 (s, 3H, OC*H*$_3$), 2.15 (s, 3H, OC(O)*CH*$_3$), 2.14 (s, 3H, OC(O)*CH*$_3$), 2.09 (s, 3H, OC(O)*CH*$_3$), 2.08 (s, 3H, OC(O)*CH*$_3$), 2.04 (s, 3H, OC(O)*CH*$_3$), 2.03 (s, 3H, OC(O)*CH*$_3$), 2.01 (s, 3H, OC(O)*CH*$_3$).

^{13}C NMR (151 MHz, $CDCl_3$) δ 171.1 (*C*OCH_3), 170.7 (*C*OCH_3), 170.6 (*C*OCH_3), 170.1 (*C*OCH_3), 169.9 (*C*OCH_3), 169.7 (*C*OCH_3), 169.6 (*C*OCH_3), 99.4 and 99.3 (*C*$_1$, *C*$_{1'}$), 77.0 (*C*$_2$), 70.4, 69.9, 69.2, 68.6 and 68.5 (*C*$_{2'}$, *C*$_3$, *C*$_{3'}$, *C*$_5$, *C*$_{5'}$), 66.5 and 66.2 (*C*$_4$, *C*$_{4'}$), 62.7 and 62.3 (*C*$_6$, *C*$_{6'}$), 55.4 (*OC*H_3), 20.9, 20.9, 20.9 and 20.8 (7 x CO*C*H_3) ppm.

ESI$^+$-HRMS (m/z) for $C_{27}H_{38}O_{18}Na$ = 673.1950 $[M+Na]^+$; found: 673.1964 Da.

e) Synthesis of 2,3,4,6-Tetra-*O*-acetyl-α-D-mannopyranosyl-(1→2)-1,3,4,6-tetra-*O*-acetyl-α-D-mannopyranose (**6**)[6]

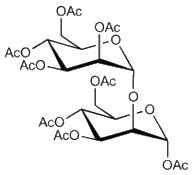

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Figure S4. Synthesis of 2,3,4,6-Tetra-*O*-acetyl-α-D-mannopyranosyl-(1→2)-1,3,4,6-tetra-*O*-acetyl-α-D-mannopyranose (**6**).

In a round-bottom flask, compound **5** (75 mg, 0.12 mmol) was cooled in an ice bath. After the addition of ice-cold acetic anhydride (1 mL), sulfuric acid (4 % in acetic anhydride (v/v), 1 mL) was added dropwise. The reaction mixture was stirred for 4 hours at 0°C. The solution was diluted with DCM (10 mL), neutralized with ice-cold sat. aq. $NaHCO_3$ solution (5 mL) and washed with water (10 mL). The combined aqueous phases were extracted with DCM (3 x 10 mL). After drying of the combined organic phases over Na_2SO_4 and filtration,

the solvent was removed under vacuum. The crude product was purified by column chromatography (heptanes/EtOAc = 1:1). The product **6** was obtained as a white foam (70 mg, 0.10 mmol, 86 % yield), in accordance with literature[7].

^{1}H NMR (600 MHz, $CDCl_3$) δ 6.24 (d, *J* = 2.0 Hz, 1H, H_1), 5.46-5.38 (m, 2H, $H_{3'}$, $H_{4'}$), 5.31-5.22 (m, 3H, $H_{2'}$, H_3, H_4), 4.94 (d, *J* = 1.6 Hz, 1H, $H_{1'}$), 4.25-4.09 (m, 5H, $H_{5'}$, $H6_{a,b}$, $H_{6a',b'}$), 4.05-4.03 (m, 1H, H_2), 4.01 (ddd, *J* = 10.1, 3.5, 2.6 Hz, 1H, H_5), 2.15 (s, 6H, COCH_3), 2.14 (s, 3H, COCH_3), 2.10 (s, 3H, COCH_3), 2.09 (s, 3H, COCH_3), 2.04 (s, 6H, COCH_3), 2.01 (s, 3H, COCH_3) ppm.

^{13}C NMR (151 MHz, $CDCl_3$) δ 171.1 (*C*$COCH_3$), 170.9 (*C*OCH_3), 170.7 (*C*OCH_3), 170.1 (*C*OCH_3), 169.9 (*C*OCH_3), 169.6 (*C*OCH_3), 169.3 (*C*OCH_3), 168.4 (*C*OCH_3), 99.4 ($C_{1'}$), 91.5 (C_1), 76.0 (C_2), 70.8, 69.9, 69.8, 69.7, 68.4 ($C_{2'}$, C_3, $C_{3'}$, C_5, $C_{5'}$), 66.3 and 65.6 (C_4, $C_{4'}$), 62.5 and 61.8 (C_6, $C_{6'}$), 21.1 (CO*C*H_3), 21.0 (CO*C*H_3), 20.9 (2x CO*C*H_3), 20.8 (4x CO*C*H_3).

ESI$^+$-HRMS (m/z) for $C_{28}H_{38}O_{19}Na$ = 701.1900 $[M+Na]^+$; found: 701.1888 Da.

f) Synthesis of 2,3,4,6-Tetra-*O*-acetyl-α-D-mannopyranosyl-(1→2)-3,4,6-tetra-*O*-acetyl-α-D-mannopyranosyl azide (**7**)

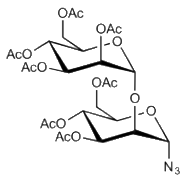

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Figure S5. Synthesis of 2,3,4,6-Tetra-*O*-acetyl-α-D-mannopyranosyl-(1→2)-3,4,6-tetra-*O*-acetyl-α-D-mannopyranosyl azide (**7**).

Compound **6** (64 mg, 94 µmol, 1 equiv.) was added to anhydrous iron(III) chloride (1.6 mg, 10 µmol, 0.1 equiv.), followed by the addition of dry DCM (0.3 mL). After stirring for 5 min at r.t., $TMSN_3$ (0.15 mmol, 1.5 equiv., 20 µL) in DCM (0.1 mL) was added dropwise [8]. The reaction mixture was stirred for 20 hours, until TLC indicated complete consumption of the starting material **6**. The solution was diluted with DCM (9 mL), washed with sat. aq. $NaHCO_3$ solution (5 mL) and the aqueous layer was extracted with DCM (3 x 5 mL). The organic layers were combined, washed with brine (5 mL), dried over Na_2SO_4, filtered and the solvent was removed under vacuum. The crude product was purified by column chromatography (heptanes/EtOAc = 1:1) to give product **7** as a white foam (51 mg, 77 µmol, 82 % yield).

^{1}H NMR (600 MHz, $CDCl_3$) δ 5.57 (d, *J* = 2.0 Hz, 1H, H_1), 5.39-5.30 (m, 2H), 5.26-5.21 (m, 2H), 5.17 (dd, *J* = 9.9, 3.2 Hz, 1H), 4.89 (d, *J* = 1.1 Hz, 1H, $H_{1'}$), 4.25 (dd, *J* = 12.4, 4.7 Hz, 1H), 4.20-4.13 (m, 4H), 4.12-4.06 (m, 1H), 3.96 (t, *J* = 3.1 Hz, 1H), 2.14 (s, 6H, CH_3), 2.09 (s, 3H, CH_3), 2.07 (s, 3H, CH_3), 2.04 (s, 3H, CH_3), 2.03 (s, 3H, CH_3), 2.00 (s, 3H, CH_3).

^{13}C NMR (151 MHz, $CDCl_3$) δ 171.0 (*C*OCH_3), 170.6 (*C*OCH_3), 170.4 (*C*OCH_3), 170.0 (*C*OCH_3), 169.9 (*C*OCH_3), 169.6 (*C*OCH_3), 169.4 (*C*OCH_3), 99.4 ($C_{1'}$), 88.3 (C_1), 76.8 (C_2), 70.7, 69.7, 69.6, 69.3 and 68.4 (5C, $C_{2'}$, C_3, $C_{3'}$, C_5, $C_{5'}$), 66.4 and 65.6 (2C, C_4, $C_{4'}$), 62.8 and 61.9 (2C, C_6, $C_{6'}$), 21.0 (2 x CO*C*H_3), 20.8 (3 x CO*C*H_3), 20.8 (2 x CO*C*H_3).

ESI$^+$-HRMS (m/z) for $C_{26}H_{35}N_3O_{17}Na$ = 684.1859 $[M+Na]^+$; found: 684.1854 Da.

g) Synthesis of α-D-mannopyranosyl-(1→2)-α-D-mannopyranosyl azide (**8**)

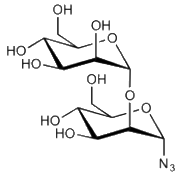

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Figure S6. Synthesis of α-D-mannopyranosyl-(1→2)-α-D-mannopyranosyl azide (**8**).

Compound **7** (51 mg, 77 µmol) was dissolved in dry methanol (1 mL), followed by the addition of sodium methoxide (0.5 M in MeOH, 50 µL) with stirring. After 24 hours, Dowex 50 H^+ was added until a neutral pH was reached. The solution was diluted with water, filtered and lyophilized to give product **8** as a white solid (26.4 mg, 72 µmol, 93 % yield).

^{1}H NMR (400 MHz, D_2O) δ 5.79-5.77 (m, 1H, H_1), 5.08 (d, J = 1.5 Hz, 1H, $H_{1'}$), 4.12-4.09 (m, 1H, skeleton H), 3.99-3.65 (m, 10H, skeleton protons).

^{13}C NMR (151 MHz, D_2O) δ 102.2 ($C_{1'}$, $J_{C1'\text{-}H1'}$ = 172 Hz (characteristic for α-anomer)), 88.2 (C_1, $J_{C1\text{-}H1}$ = 172 Hz (characteristic for α-anomer)), 78.4 (C_2), 74.5, 73.3, 70.2, 69.8 and 69.4 ($C_{2'}$, C_3, $C_{3'}$, C_5, $C_{5'}$), 66.7 and 66.4 (C_4, $C_{4'}$), 61.0 and 60.7 (C_6, $C_{6'}$) [6,9]

IR spectroscopy: $\tilde{\nu}$ = 3273 (-O-H), 2930 (-C-H), 2117 (N_3), 1412, 1243, 1063, 932, 820 cm^{-1}.

ESI$^+$-HRMS (m/z) for $C_{12}H_{21}N_3O_{10}Na$ = 390.1119 $[M+Na]^+$; found: 390.1104 Da.

h) Synthesis of α-D-mannopyranosyl azide (**9**)

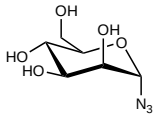

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Figure S7. Synthesis of α-D-mannopyranosyl azide (**9**).

For the introduction of the azide, as reported by Salunke et al.[8], TMSN3 and catalytic amounts of anhydrous iron(III) chloride were used (see above).

Tetraacetylated mannopyranosyl azide (56 mg, 0.15 mmol) was dissolved in dry methanol (1 mL) and sodium methoxide (0.5 M in MeOH, 60 µL) was added. The solution was stirred at r.t. for 24 hours. The reaction mixture was neutralized by the addition of Dowex 50 H^+ until neutral pH was reached, filtered and lyophilized, yielding deprotected azido-mannose **9** (28.2 mg, 0.14 mmol, 92%) as a white solid.

^{1}H-NMR (600 MHz, D_2O) δ 5.41 (d, J = 1.8 Hz, 1H, H_1), 3.94-3.51 (m, 6H, H_2, H_3, H_4, H_5, H_{6a}, H_{6b}) ppm.

^{13}C NMR (151 MHz, D_2O) δ 89.7 (C_1), 74.5 (C_5), 69.7 (C_3), 69.7 (C_2), 66.3 (C_4), 60.7 (C_6) ppm.

IR spectroscopy: $\tilde{\nu}$ = 3337 (-O-H), 2934 (-C-H), 2122 (N_3), 1248, 1051, 939, 783, 650 cm^{-1}.

ESI$^+$-HRMS (m/z) for $C_6H_{11}N_3O_5Na$ = 228.0591 $[M+Na]^+$; found: 228.0594 Da.

2. Synthesis of peptides P1-P3

a) Synthesis of peptide **P1**

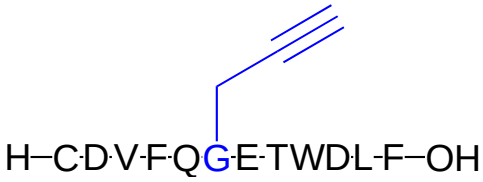

Figure S8. Propargylglycine-substituted peptide **P1.**

Peptide **P1** was synthesized using Fmoc-based, automated and microwave-assisted solid-phase peptide synthesis (Liberty Blue^{TM}, CEM, Kamp-Lintfort, Germany), under nitrogen atmosphere. Deprotection and coupling cycles were performed at 90°C. All stock solutions were freshly prepared prior to usage. *N*,*N*-Dimethylformamide (DMF) was used as main solvent, 20% piperidine (v/v, in DMF) as deprotection solution and DCM/DMF (1:1, v/v) as resin transfer solution. Fmoc-Phe-TentaGel® R PHB resin with loading of 0.2 mmol/g was used as solid phase and swelled for 1 hour (in DMF) prior to the start of the synthesis. Oxyma (1 M in DMF) and DIC (0.5 M in DMF) were used as coupling reagents. Side chain and *N*-Fmoc protected amino acids were prepared as 0.2 M stock solutions in DMF. TentaGel® resin was purchased from Rapp Polymere (Tübingen, Germany), side chain and *N*-Fmoc-protected amino acids as well as oxyma from Merck (Darmstadt, Germany), DIC from Sigma-Aldrich (Darmstadt, Germany), DCM, DMF and MeOH from VWR (Vienna, Austria). After complete synthesis and final deprotection of the *N*-terminal Fmoc group, the resin was washed with DMF, DCM, MeOH and dried under vacuum. Global deprotection of side chain protecting groups and cleavage of the peptide from the solid support were performed using 'cleavage cocktail K' (3.5 h, under rotation, r.t.), referring to a mixture of TFA/phenol/water/triisopropylsilyl ether/EDT (82.5/5/5/5/2.5, v/m/v/v/v). The obtained solution was treated with ice-cold diethyl ether to precipitate the crude peptide while removing TFA (washing with diethyl ether was repeated three times), followed by resolubilization, lyophilization and purification. Peptide **P1** was purified yielding 27 mg (18 µmol, 26% yield from initial loading). The product was analyzed using mass spectrometry as well as on a C18 column RP-HPLC with UV detection (Figure S9). For the incorporation of carbohydrates **8** and **9,** and the synthesis of glycopeptides **P2** and **P3**, however, the resin-bound peptide with protected side chains was used, then cleaved and purified (Figure 1 and chapter 2.b).

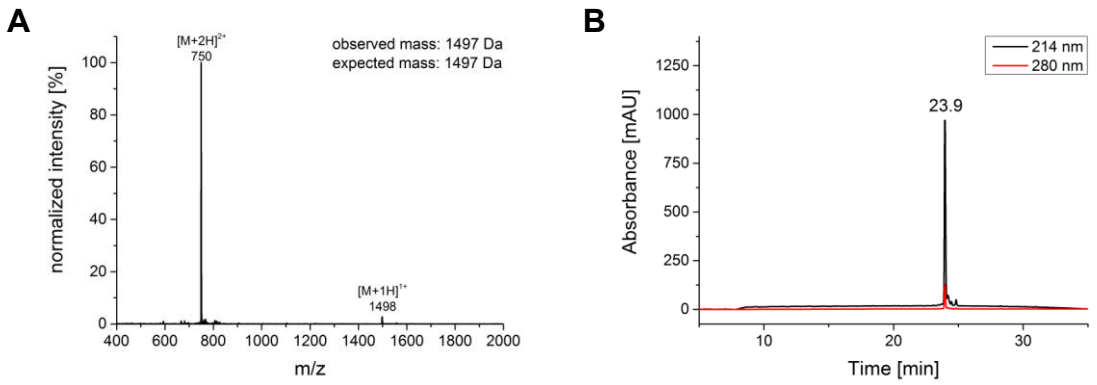

Figure S9. (A) Mass spectrum and (B) RP-HPLC chromatogram for **P1**; $[M+H]^{+}_{calc}$ = 1497.6 Da, found 1497.7 Da; $[M+2H]^{2+}_{calc}$ = 749.3 Da, found: 749.6 Da; Retention time (C18 column, 5-65% ACN over 30 min): 23.8 min.

b) Synthesis of glycopeptides **P2** and **P3** *via* copper(I)-catalyzed azide-alkyne cyclodaddition (CuAAC)[10,11]

Resin-bound and side chain protected peptide **P1** was dried after automated solid-phase peptide synthesis and stored in a desiccator. Standard CuAAC reaction conditions were carried out as described below. First, Eppendorf tubes were flushed with argon. In the next step, all solvents (H_2O, DMF) were degassed. Resin-bound peptide **P1** was weighed out and swelled in DMF for 1 hour. Stock solutions of $CuSO_4$ (400 mM in ddH_2O), sodium ascorbate (100 mM in ddH_2O) and Tris[(1-benzyl-1H-1,2,3-triazol-4-yl)methyl]amine (TBTA, 400 mM in anhydrous DMF) were freshly prepared. Aliquots of these stock solutions were combined in a separate Eppendorf tube in the order: $CuSO_4$ solution first (blue solution), followed by addition of the ligand TBTA (dark blue solution) and sodium ascorbate, to reduce Cu(II) to Cu(I) (yellow solution). The obtained mixture was homogenized using a pipette. The solution was combined with the mixture of resin-bound peptide **P1** (1 equiv.) and azide-functionalized carbohydrate **8** or **9** (4-5 equiv.) and the reaction vessel was flushed with argon. The reaction mixture (final concentrations: 2-4 mM peptide, 8-20 mM azide-functionalized sugar, 10-30 mM $CuSO_4$, 65-130 mM sodium ascorbate, 14-30 mM TBTA and DMF/H_2O = 4:1 (v/v)) was shaken on an Eppendorf thermomixer comfort. Reaction progress was monitored *via* standard washing and cleavage conditions (see 2.c). Hence, small resin aliquots were washed extensively with DMF, DMF/water (90/10, v/v), DCM and MeOH, followed by cleavage from the solid support, precipitation with ice-cold diethyl ether, resolubilization and analysis *via* LC-MS.

After purification, peptide **P2** (dimannosylated peptide, **DM**) and **P3** (monomannosylated peptide, **MM**) were isolated to yield - **P2**: 2 mg, 1.1 µmol, 10% yield; **P3**: 3.5 mg, 2.1 µmol, 20% yield from initial loading. The products were analyzed using mass spectrometry and C18 column RP-HPLC equipped with an UV detector (Figure S11 and S13).

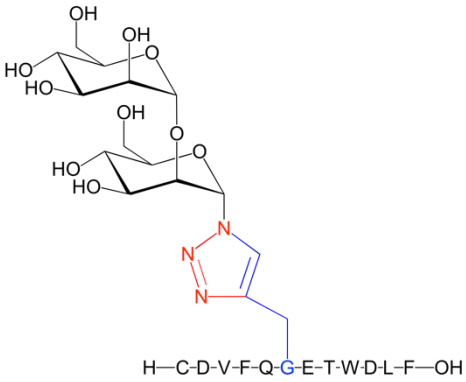

Figure S10. Representation of dimannosylated peptide **P2** (DM)

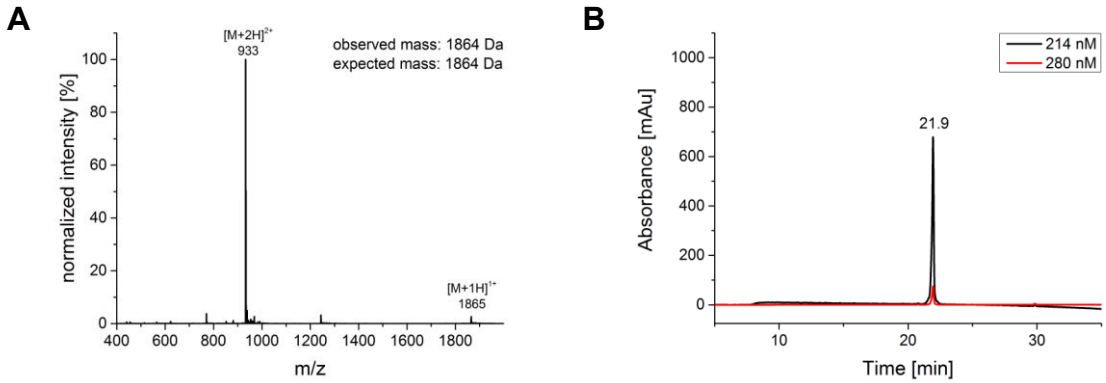

Figure S11. (A) Mass spectrum and (B) HPLC chromatogram of P2 (DM). $[M+H]^{+}_{calc}$ = 1864.8 Da, found 1865.8 Da; $[M+2H]^{2+}_{calc}$ = 932.9 Da, found 933.1 Da; Retention time (C18 column, 5-65% ACN over 30 min): 21.9 min.

ESI^{+}-HRMS (m/z) for $C_{82}H_{114}N_{17}O_{31}S$ = 1864.7582 $[M+H]^{+}$; found: 1864.7628 Da.
ESI^{+}-HRMS (m/z) for $C_{82}H_{115}N_{17}O_{31}S$ = 932.8830 $[M+2H]^{2+}$; found: 932.8820 Da.

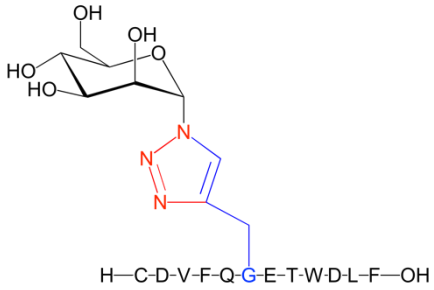

Figure S12. Representation of monomannosylated peptide **P3** (MM)

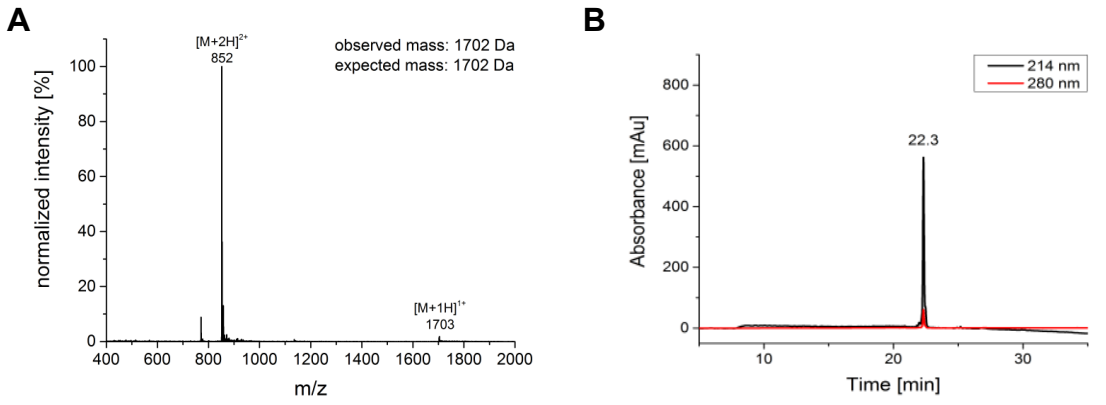

Figure S13. (A) Mass spectrum and (B) HPLC chromatogram of P3 (MM). $[M+H]^{+}_{calc}$ = 1702.7 Da, found 1703.4 Da; $[M+2H]^{2+}_{calc}$ = 851.9 Da, found 852.1 Da. Retention time (C18 column, 5-65% ACN over 30 min).

ESI$^+$-HRMS (m/z) for $C_{76}H_{104}N_{17}O_{26}S$ = 1702.7054 $[M+H]^+$; found: 1702.7060 Da.
ESI$^+$-HRMS (m/z) for $C_{76}H_{105}N_{17}O_{26}S$ = 851.8566 $[M+2H]^{2+}$; found: 851.8564 Da.

c) General procedure for the purification and analysis of peptides

After cleavage from the resin, global deprotection and precipitation, peptides were dissolved in 30/70 or 50/50 (v/v) ddH_2O/ACN containing 0.1% TFA and lyophilized. Crude peptides were analyzed using liquid chromatography-mass spectrometry (LC-MS) on a Waters AutoPurification HPLC/MS system (Milford, USA). For analytical runs, sample separation was accomplished either on an analytical 300-5-C4 or -C18 Kromasil column (4.6 x 50 mm, Sigma-Aldrich) at a flow rate of 1 mL/min and a linear gradient from 5-65% ACN + 0.05% TFA (‚buffer B') in ddH_2O with 0.05% TFA (‚buffer A') over 20 min. Mass spectra were obtained by electrospray ionization mass spectrometry (ESI-MS), operating in positive ion mode. A Dionex UltiMate 3000 (Thermo Fisher Scientific, Waltham, USA) HPLC system with UV-detection was used for monitoring reactions as well as for analysis of crude and final products. The separation was accomplished with a Kromasil 300-5-C4 or -C18 column (4.6 x 150 mm) at a flow rate of 1 mL/min using a linear gradient from 5-65% ACN (with 0.08% TFA) in ddH_2O (with 0.1% TFA) over 30 min.

Purification was performed on the Waters system or on a Varian Pro Star system (Palo Alto, USA) using a Prep (250 x 21.2 mm) or Semiprep (250 x 10 mm) Kromasil 300-10-C18 column at a flow rate of 10 mL/min or 3 mL/min, respectively. The gradient was set to increase from 5-20% of buffer B over 5 min and 20-50% of buffer B over 30 min. Crude peptides were dissolved in 10 mL 6 M GuaHCl (pH 4.7), filtered (0.2 µm pore size) and injected for purification. Fractions were automatically collected corresponding to the selected masses when using the Waters AutoPurification HPLC/MS system. Regarding the Varian Pro Star system, peptides were purified using a gradient from 5-25% ACN (5 min) to 25-55% ACN (or 25-65% ACN) over 60 min. Buffer systems of A (water) and B (ACN), both containing 0.08% TFA were used. Fractions were collected as 1 mL, at a flow rate of 3 mL/min and subsequently analyzed using direct injection mass spectrometric analysis (Waters ESI-MS). Product containing fractions were combined, lyophilized and used in further steps after final analysis.

HRMS spectra were recorded using a BRUKER maXis ESI-Qq-oaTOF system in positive and negative ion mode, respectively. Lyophilization of peptide solutions was performed on a Christ Alpha 2-4 plus system (Martin Christ GmbH, Osterode am Harz, Germany), using a cold trap (-85°C) operating at a pressure of < 0.1 mbar.

3. Recombinant expression of CVN2L0 and variants

a) Sequences

```
CVN2L0          MAHHHHHHIEGRLGKFSQTCYNSAIQGSVLTSTCERTNGGYNTSSIDLNSVIENVDGSLK   60
CVN2L0 V1       MAHHHHHHIEGRLGKFSQTCYNSAIQGSVLTSTCERTNGGYNTSSIDLNSVIENVDGSLK   60
CVN2L0 V2       MAHHHHHHIEGRLGKFSQTCYNSAIQGSVLTSTCERTNGGYNTSSIDLNSVIENVDGSLK   60
CVN2L0 V3       MAHHHHHHIEGRLGKFSQTCYNSAIQGSVLTSTCERTNGGYNTSSIDLNSVIENVDGSLK   60
CVN2L0 V4       MAHHHHHHIEGRLGKFSQTCYNSAIQGSVLTSTCERTNGGYNTSSIDLNSVIENVDGSLK   60
CVN2L0 V5       MAHHHHHHIEGRLGKFSQTCYNSAIQGSVLTSTCERTNGGYNTSSIDLNSVIENVDGSLK   60
CVN2L0 V6       MAHHHHHHIEGRLGKFSQTCYNSAIQGSVLTSTCERTNGGYNTSSIDLNSVIENVDGSLK   60
                ************************************************************

                WQPSNFIETWRNTQLAGSSELAAEMKTRAQQFVSTKINLDDHIANIDGTLKYELGKFSQT  120
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                WQPSNFIETERNTQLAGSSELAAERKTRAQQFVSTKINLDDHIANIDGTLKYELGKFSQT  120
                WQPSNFIETWRNTQLAGSSELAAEMKTRAQQFVSTKINLDDHIANIDGTLKYELGKFSQT  120
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                WQPSNFIETERNTQLAGSSELAAERKTRAQQFVSTKINLDDHIANIDGTLKYELGKFSQT  120
                WQPSNFIETMRNTQLAGSSELAAEWKTRAQQFVSTKINLDDHIANIDGTLKYELGKFSQT  120
                ********* ************** *********************************

                CYNSAIQGSVLTSTCERTNGGYNTSSIDLNSVIENVDGSLKWQPSNFIETCRNTQLAGSS  180
                CYNSAIQGSVLTSTCERTNGGYNTSSIDLNSVIENVDGSLKWQPSNFIETCRNTQLAGSS  180
                CYNSAIQGSVLTSTCERTNGGYNTSSIDLNSVIENVDGSLKWQPSNFIETCRNTQLAGSS  180
                CYNSAIQGSVLTSTCERTNGGYNTSSIDLNSVIENVDGSLKWQPSNFIETWRNTQLAGSS  180
                CYNSAIQGSVLTSTCERTNGGYNTSSIDLNSVIENVDGSLKWQPSNFIETERNTQLAGSS  180
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                CYNSAIQGSVLTSTCERTNGGYNTSSIDLNSVIENVDGSLKWQPSNFIETWRNTQLAGSS  180
                ************************************************* *********

                ELAAECKTRAQQFVSTKINLDDHIANIDGTLKYE  214
                ELAAECKTRAQQFVSTKINLDDHIANIDGTLKYE  214
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                ELAAERKTRAQQFVSTKINLDDHIANIDGTLKYE  214
                ELAAEYKTRAQQFVSTKINLDDHIANIDGTLKYE  214
                ***** ****************************
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Figure S14. Multiple sequence alignment of CVN2L0 [12] and the investigated variants

b) Expression

For each of the CVN2L0 variants and CVN2L0 'wild type' protein with an N-terminal pelB leader sequence and His-tag, lyophilized plasmid DNA was obtained from GenScript® (pET-27b(+) vector; Piscataway, USA) and dissolved in sterile ddH_2O to a final concentration of 100 ng/µL. Culture medium LB-Lennox was prepared as follows: 10 g/L peptone, 5 g/L yeast extract and 5 g/L NaCl in ddH_2O, pH was adjusted to 7.4.

Transformation into competent *E. coli* BL21 (DE3) was performed for each variant (V1-V6) by chemical transformation. An aliquot of competent cells was taken from -80°C and thawed on ice for 30 min. In a sterile Eppendorf tube, 2 µL of the plasmid DNA solution (100 ng/µL) were mixed with 80 µL of competent cell suspension and stored on ice for 30 min. Heat shock was performed at 42°C for 90s on a shaker, followed by a cooling step on ice for 3 min. Preheated SOC medium (37°C) was added to a final volume of 1 mL, followed by incubation at 37°C for 45 min. The solution was split (900 and 100 µL), transferred on LB-agar plates (50 µg/mL kanamycin) and gently plated using a sterile cell spreader. The plates were incubated overnight at 37°C.

Autoclaved LB medium (containing 50 µg/mL kanamycin) was used for all cultivations. Medium (50 mL) was transferred into a sterile Erlenmeyer flask, inoculated with the corresponding *E. coli* strain and incubated overnight at 180 RPM and 37°C. After determination of OD_{600}, the culture was transferred into a 3 L Erlenmeyer flask and diluted with LB broth to an OD_{600} of 0.05-0.1 (1 L total culture volume). The culture was incubated at 180 RPM and 37°C for 3 hours, until an OD_{600} of ~ 0.6 was reached. For induction, the culture was cooled to 18°C and isopropyl β-D-1-thiogalactopyranoside (IPTG) was added to a final concentration of 500 µM. The culture was incubated overnight at 180 RPM and 18°C.

For cell lysis, two approaches were performed. For test expressions, cell lysis and following protein purification from the soluble fraction were carried out as follows: Culture was centrifuged at 4,000 x g and 4°C for 15 min, the supernatant was discarded. The obtained pellet was resuspended in phosphate buffered saline (PBS) buffer and re-centrifuged. The supernatant was discarded and the remaining pellet was resuspended in 10 mL lysis buffer (50 mM Tris-HCl, 200 mM NaCl, 5 % glycerol, 500 ng/mL lysozyme, 1 mM PMSF, 1 mM DTT, 1 mM $MgCl_2$) and incubated for 1 hour at 37°C. The mixture was subjected to two freeze-thaw cycles (-80ºC) and sonicated. Soluble and insoluble fractions were separated by centrifugation at 4,000 x g and 4°C for 15 min and analyzed using SDS-PAGE and anti-His Western Blot. Variants V1 and V6 were not expressed. Variants were further purified from inclusion bodies. Proteins purified from the soluble fraction yielded much lower protein (< 1 mg/L culture) than if purified from inclusion bodies (2-15 mg/L culture).

For big scale protein production, cell lysis and CVN2L0 purification from the insoluble fraction was performed. Cell pellet was resuspended in 40 mL lysis buffer (50 mM Tris-HCl, 200 mM NaCl, 5 % glycerol, 1 mM PMSF, 1 mM DTT, 1 mM $MgCl_2$ and 1 % Triton™ X-100), followed by mechanical cell lysis using a IKA® T18 basic ULTRA-TURRAX® and Constant Systems LTD cell disrupter (1.8 kbar) attached to a recirculating chiller (VWR RC-10 Digital Chiller). The obtained mixture was centrifuged for 30 min at 4°C and 16,000 x g in order to separate the supernatant (soluble) from insoluble (inclusion bodies) fractions. The insoluble fraction was resuspended in 40 mL 8 M GuaHCl, 50 mM Tris, pH 8, by stirring the suspension for 24 hours before a second centrifugation step (30 min, 16,000 x g, 4°C); expressed proteins were purified from the supernatant by Ni-NTA chromatography.

c) Purification

Proteins were purified using Ni-NTA column chromatography (ÄKTA prime plus). The soluble fraction was loaded onto a regenerated Ni-NTA bead column (1 mL/min). The system washed with TBS buffer (50 mM Tris, 150 mM NaCl, pH 7.5), before a gradient (0-100% 500 mM imidazole in TBS buffer over 60 min) was started and fractions were collected (1 mL/min). Also, the insoluble fraction in 8 M GuaHCl, 50 mM Tris, pH 8, was purified using Ni-NTA column chromatography. Samples were loaded onto the column (1 mL/min), followed by washing with denaturizing running buffer (6 M GuaHCl, 150 mM NaCl, 50 mM Tris, pH 8) until the baseline level for protein content was reached (detected via UV measurement). Elution was performed applying a gradient from 0%-100% 6 M GuaHCl, 150 mM NaCl, 50 mM Tris, pH 8 and 500 mM imidazole over 60 min. Fractions were collected automatically (1 mL/min tube). Aliquots (20 µL) of the GuaHCl containing fractions were precipitated two times with 90% EtOH to exchange buffer solution with ddH2O containing 10% SDS. Samples were denatured for 5 min at 95°C and analyzed by SDS-PAGE (Figure S15). Purification from the insoluble fraction resulted in both, the desired CVN2L0 protein and protein with N-terminal pelB leader sequence. Both proteins were separated and the uncleaved one used for ITC and NMR measurements.

For NMR analysis, proteins were dialyzed against 100 mM PBS (pH 7.0). Protein solution (5-10 mL) was transferred into a dialysis tube with a 6 kDa pore filter. The casket was then placed in a beaker (3 L) and 1 L of PBS was added. The solution was slightly

stirred overnight at 4°C, followed by replacing the PBS and further dialysis for 5-10 hours. The protein solution was transferred to Amicon filters with 10 kDa cut-off and concentrated using centrifugation (Beckmann Coulter Cooler Allegra X-30R centrifuge was used at 4,500 x g, 4°C). For SPR measurements, the protein solution was buffer exchanged to 10 mM HEPES, 150 mM NaCl, 3 mM EDTA and 0.05 % Tween at pH 7.4 using Amicon 10 kDa cut-off filters (diluting 1:10 with SPR buffer and four times centrifugation to initial volume).

4. Protein analysis

Protein concentration was determined at 280 nm using a NanoDrop UV-Vis2000c spectrophotometer, based on the calculated extinction coefficient (23,474 Da for CVN2L0 and 20,440 M^{-1} cm^{-1}). PBS (100 mM, pH 7.0) or SPR buffer (10 mM HEPES, 150 mM NaCl, 3 mM EDTA and 0.05 % Tween at pH 7.4) were used as a blank and protein concentration was determined using three measurements of each dilution (1, 1:10 and 1:100). In-gel tryptic digest was performed from a silver-stained 15% polyacrylamide gel as described in [13] with modification.

Protein bands were cut in small gel pieces, washed two times with 25 mM NH_4HCO_3, dried with acetonitrile, and 200 µL of reduction buffer (20 mM DTT, 25 mM NH_4HCO_3) was added and incubated for 30 min at 54°C. For alkylation, 200 µL of freshly prepared 100 mM iodoacetamide in 25 mM NH_4HCO_3 solution was added to the gel pieces and incubated for 30 min at 37°C in the dark. Again, the gel pieces were washed and dried with 25 mM NH_4HCO_3 and ACN. After 10 min of additional vacuum drying, 1 µg trypsin was added per 20 µg of protein from a frozen stock solution (1 µg Trypsin, Lys-C / 10 µL 50 mM acetic acid, Promega Lot: 0000336820). The sample was incubated on ice for 15 min until the mixture was completely absorbed from the gel. Finally, 20 µL of 25 mM NH_4HCO_3 was added and the tube was incubated overnight at 37°C. Next, the cleaved peptide fragments were extracted from the gel with 3x40 µL of 25 mM NH_4HCO_3/50 % ACN. The supernatants of all three extractions were combined, vacuum dried and used for LC-MS-MS analysis.

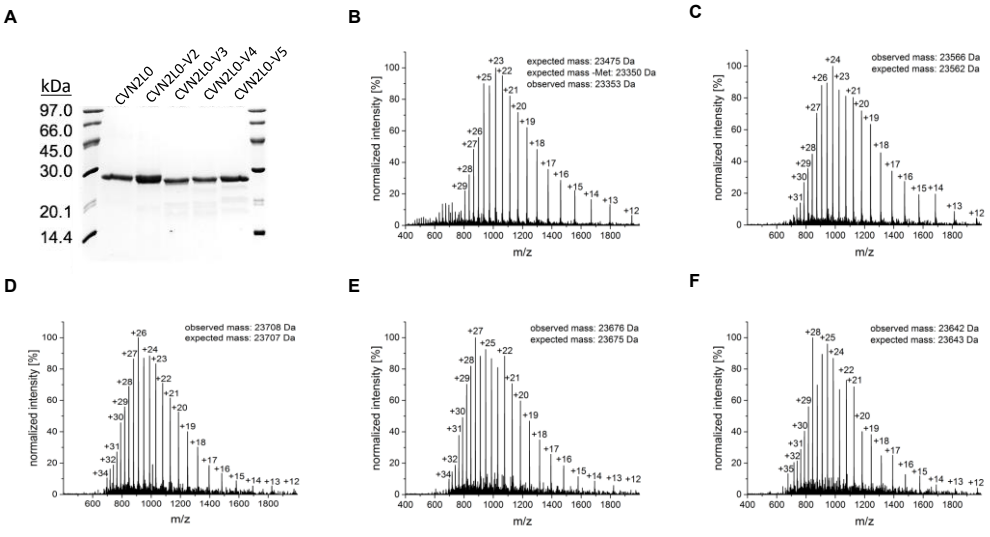


Figure S15. (A) SDS-PAGE. GE HC Amersham low molecular weight marker (6 µL) was used as a reference. Electrophoresis was performed at 250 V (const.) for 35 min. **(B-F) LC-MS mass spectra of purified proteins analyzed on a Waters HPLC/MS system.** B) CVN2L0, C) CVN2L0-V2, D) CVN2L0-V3, E) CVN2L0-V4, F) CVN2L0-V5. Sample separation was accomplished on an analytical 300-5-C4 Kromasil column (4.6 x 50 mm) at a flow rate of 1 mL/min and a linear gradient from 5-65 % ACN with 0.1 % TFA in ddH_2O with 0.1 % TFA over 20 min. Mass spectra were obtained by ESI-MS, operating in positive ion mode.

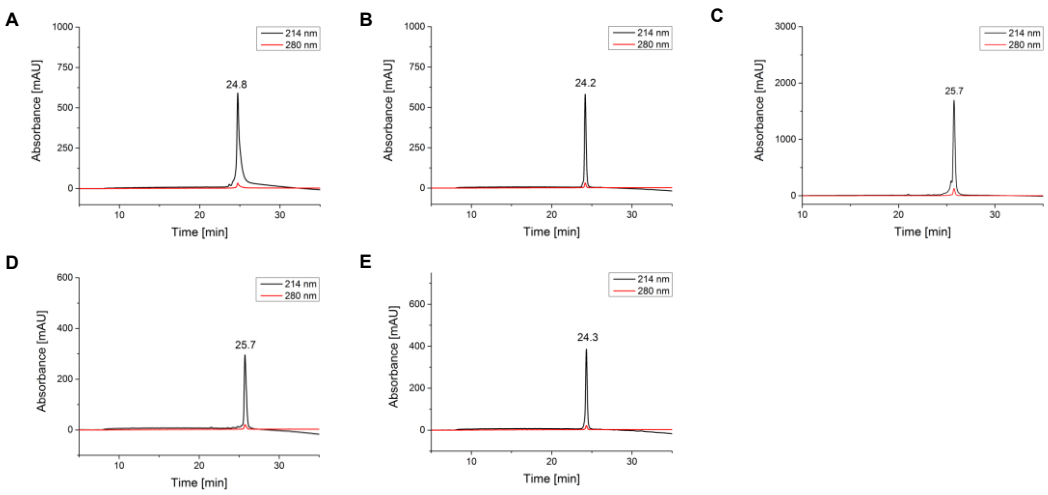


Figure S16. HPLC chromatograms of purified proteins. A) CVN2L0, B) CVN2L0-V2, C) CVN2L0-V3, D) CVN2L0-V4, E) CVN2L0-V5, as derived from Dionex UltiMate 3000 instrument at a flow rate of 1 mL/min with a linear gradient from 5 to 65 % buffer B in buffer A over 30 min. Buffer A was: 0.1 % (v/v) TFA in ddH_2O, buffer B was: 0.08 % (v/v) TFA in ACN. Proteins were analyzed on a Kromasil 300-5-C4 (150 x 4.6 mm) column at 214 and 280 nm.

5. Circular dichroism (CD) measurements

CD spectra were recorded using a Chirascan Plus CD-spectrophotometer (Applied Photophysics, UK) at 25°C from 195 to 260 nm in 1 nm steps. For each spectrum, 10 measurements were averaged and the background was subtracted manually. Protein concentration of 10 µM in a micro cuvette (1.0 mm path length, Hellma Analytics, Germany) was used for each measurement. CD spectra were recorded in 100 mM sodium phosphate buffer, pH 7.0. Samples and buffer were degassed before measurements. Obtained data (mdeg) were converted into theta in molar ellipticity (Figure S17A).

Thermal denaturation of proteins was monitored at 235 nm from 10-85°C in 0.2°C steps. Heating rate was set to 1°C/min and tolerance to 0.1°C. Obtained data (mdeg) were plotted against temperature and a sigmoidal fit was obtained from the Boltzmann function using the Levenberg Marquardt iteration algorithm (Figure S17B), of which melting temperatures T_m of CVN2L0 and variants V2, V4 and V5 were determined (Table S1).

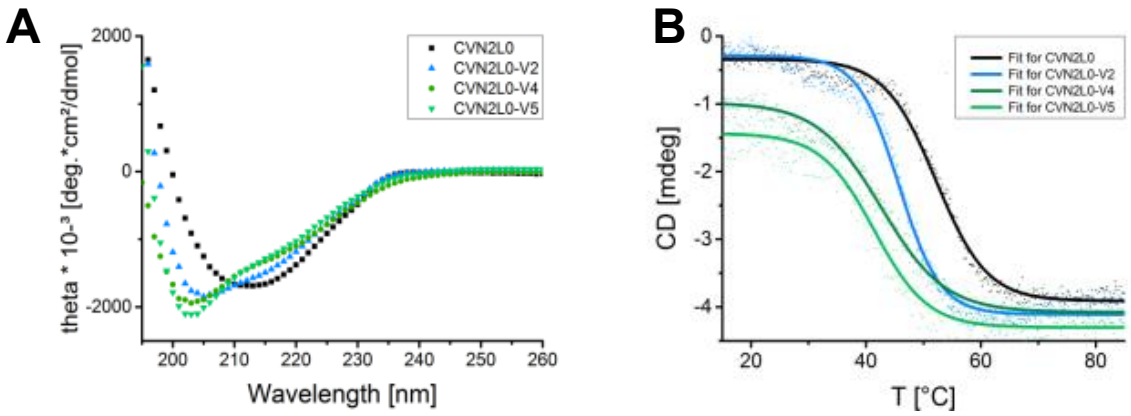


Figure S17. (A) CD Spectra of CVN2L0 and Cys-Cys variants with either 3 disulfide bonds (V2) or 2 disulfide bonds (V4 and V5) and **(B) melting curves.**

Table S1. Melting temperatures T_m for CVN2L0 and variants

	T_m [°C]
CVN2L0	52
CVN2L0 V2	46
CVN2L0 V4	43
CVN2L0 V5	42

Table S2. Kinetic data obtained from SPR sensorgrams for the binding of CVN2L0 and Cys-Cys bond variants to HA and DM using a Langmuir 1:1 binding model. K_D [M] = k_{off}/k_{on}.

Analyte	Ligand: HA			Ligand: Dimann. peptide **P2**		
	k_{on} [$M^{-1}*s^{-1}$]	k_{off} [s^{-1}]	K_D	k_{on} [$M^{-1}*s^{-1}$]	k_{off} [s^{-1}]	K_D
CVN2L0	5.1e3	1.3e-3	255 nM	9.0e2	9.2e-3	10 µM
V2	4.0e3	1.1e-3	275 nM	1.2e3	1.2e-2	10 µM
V4	1.2e3	1.3e-2	11 µM	not analyzable		
V5	1.2e3	5.8e-2	5 µM	not analyzable		

Data for monomannosylated peptide P3 and V3 (n.a.).

All data was generated at 25°C in HBS-EP(+) buffer on a Reichert SR7500DC instrument. Kinetic data was set from nanomolar to half of the micromolar range for binding of the analytes in correlation with a decreasing number of disulfide bonds.

K_{on}: $\frac{dR}{dt} = k_{a*}C\ (R_{max}-R) - k_{d*}R$ k_a=10^5 $M^{-1}s^{-1}$ k_d=10^-3 s^{-1}

K_D is related to the equilibrium data that is fit to the Langmuir Binding Isotherm where the equilibrium binding response is plotted as a function of analyte concentration.

K_{on} expresses good relative response values for all binding measurements of CVN2L0 and variants to HA and DM, while the dissociation rate constant K_{off} is showing faster off-rates for V4 and V5, two variants bearing two functional low-affinity carbohydrate binding sites. Concerning CVN2L0 and CVN2L0-V2, k_{off} was to the maximum 10-fold higher concerning CVN2L0-V2, if compared to the dissociation off-rate in binding curves of both molecules to HA.

6. SPR spectroscopy

Kinetic SPR binding studies were performed on Reichert's SR7500DC (Reichert, Buffalo, USA), a two channel surface plasmon resonance instrument. CMD500D SPR sensorchips were purchased from Xantec biosensors (Düsseldorf, Germany). Measurements were performed at 25°C and at a flow rate of 30 µL/min. Sensorchips were activated using single-channel amine coupling, where 0.4 M EDC/HCl and 0.1 M NHS were used to activate the carboxymethyldextran hydrogel chip surface. The ligand (HA or glycopeptides) was diluted to 20 µg/mL in 10 mM sodium acetate buffer (pH 5) and then injected to the activated chip surface. Recombinant influenza A virus hemagglutinin H3 protein (H3N2 A/Wisconsin/67/05) was obtained from abcam (Cambridge, UK). Its source of origin were baculovirus infected insect cells, whereas glycopeptides **P2** and **P3** were synthesized using

SPPS, and functionalized *via* CuAAC (see 2.b). Following the immobilization of the corresponding ligand, all remaining reactive groups on the SPR chip were capped using 1 M ethanolamine/HCl at pH 8.5.

Chip surface ligand density was estimated to be 400 µM for HA and 4 mM for DM, whereas MW_{HA} : MW_{DM} was determined to be 1:30. In order to reach R_{max} (Ligand:DM) level to be a third of R_{max} (Ligand:HA) injection time and concentration for R-ligand was inversely adjusted to elevate baseline at 3570 µRIU (micro Refractive Index Units) for the MM sensorchip, 4300 µRIU for the DM chip, and 2540 µRIU for the HA chip. The second channel, not coated with the ligand, served as a reference channel. Running buffer (HBS-EP (+)) contained 10 mM HEPES, 150 mM NaCl, 3 mM EDTA and 0.05% Tween at a pH of 7.4. All solutions were degassed and filtered (0.2 µm) before injection into the system. Kinetic studies were performed using various analyte concentrations in the range of 10^{-5}-10^{-8} M, with a regeneration step after each injection and blank measurements after different analytes. Response curves were obtained by aligning and subtracting signals of the second reference channel from those of the ligand channel. Blank measurements that were obtained by injecting buffer solution were subtracted to achieve measurable sensorgrams for different protein concentrations (double reference). Primary data were analyzed according to the integrated rate of association and rate of dissociation equations which describe the kinetics of the interaction of soluble cyanovirin with immobilized ligand and the dissociation of the formed complex from the chip surface. Two independent measurements were performed, and sensorgrams which were equivalent to those obtained from replicates were analyzed.
K_D was calculated as the quotient of k_{off}/k_{on} (k_{off}: Rate of dissociation, k_{on}: Rate of association) and curves were fit to a 1:1 binding model [(14)] using Scrubber (Biological Software). In a concentration-dependent manner, dissociation rate constant k_d was determined post-analyses and incorporated into association phase fitting (k_{on}) to estimate the association rate constant k_a. Channels and needles of the Reichert system were washed with iso-propanol/ACN/MeOH/H2O (1:1:1:1, v/v/v/v) + 1% formic acid and 20% EtOH.

7. NMR spectroscopy

^{1}H-NMR experiments were acquired on Bruker NEO 500 or 600 spectrometers equipped with either a 5 mm BBFO (broadband observe probe at 500 MHz) or a 5 mm TXI (H/N/C triple resonance probe at 600 MHz) each equipped with shielded z-gradients. An NMR sample of CVN2L0 and each variant was prepared in PBS (100 mM, pH 7.0) added to a peptide solution in phosphate buffer for a final peptide concentration of 100 µM and a ratio of peptide/CVN=10:1. The Saturation Transfer Difference (STD)-NMR experiments were recorded at 298K with 500-1000 scans for MM (and up to 4000 scans for DM). Selective saturation of protein resonances was performed at -1 ppm (40 ppm for off resonance spectra) using a series of Gaussian-shaped pulses (1.4 ms, $\gamma B1/2\pi$ = 200 Hz), for total saturation times ranging from 2 s to 4 s. The total saturation time was adjusted by the number of shaped pulses. Thus, irradiation at -1 ppm was expected to result in saturation of protein resonances across the shift range. Water suppression was achieved by a double Watergate element. The saturated and reference spectra were acquired in an interleaved manner switching the saturation offset and accumulating the difference spectrum automatically.

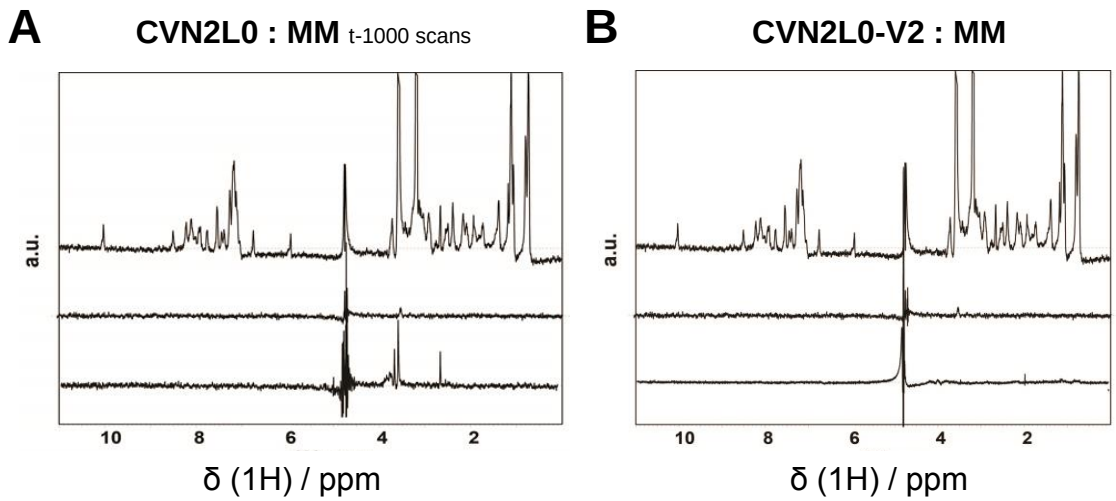


Figure S18. (A) STD-NMR of CVN2L0 vs. MM and (B) CVN2L0-V2 vs. MM: Upper trace: ^{1}H NMR spectrum of the native glycopeptide MM; middle trace: STD-NMR spectrum of the free peptide; lower trace: STD-NMR spectrum of MM in the presence of the respective CVN2L0. a.u.= arbitrary units. 100 µM peptide was measured over 10 µM protein for CVN2L0 showing a saturation transfer effect due to hydroxyl groups in the mannose modification of the peptide. The number of disulfide bridges was 4 in CVN2L0. CVN2L0-V2 (C58E and C73R in a single monomer).

8. Isothermal titration

Calorimetric titrations were performed using a PEAQ-ITC isothermal titration calorimeter (Malvern Pananalytical, UK). CVN2L0 protein solution (35-50 µM) was placed in the calorimeter cell (1.5 mL active volume), and 2 µL aliquots of the MM and DM solutions (300-600 µM) were added at 2 min intervals from a stirring syringe (operated at 750 rpm). A total of 20 injections were performed. Titrations were carried out at 298K and all solutions contained 100 mM PBS (pH 7.0). The isotherm was fit using the Malvern MicroCal Analysis software with the standard One Site or Two Site model. The heat of sugar binding capacity of CVN2L0 was depicted as values for enthalpy of binding; the apparent number of binding sites (*N*) on the protein, and the binding affinity separately derived from each fit. Other thermodynamic quantities were calculated using the standard expressions: $\Delta G = -RT\ ln\ K_a$; $\Delta G = \Delta H - T\Delta S$.

Abbreviations:

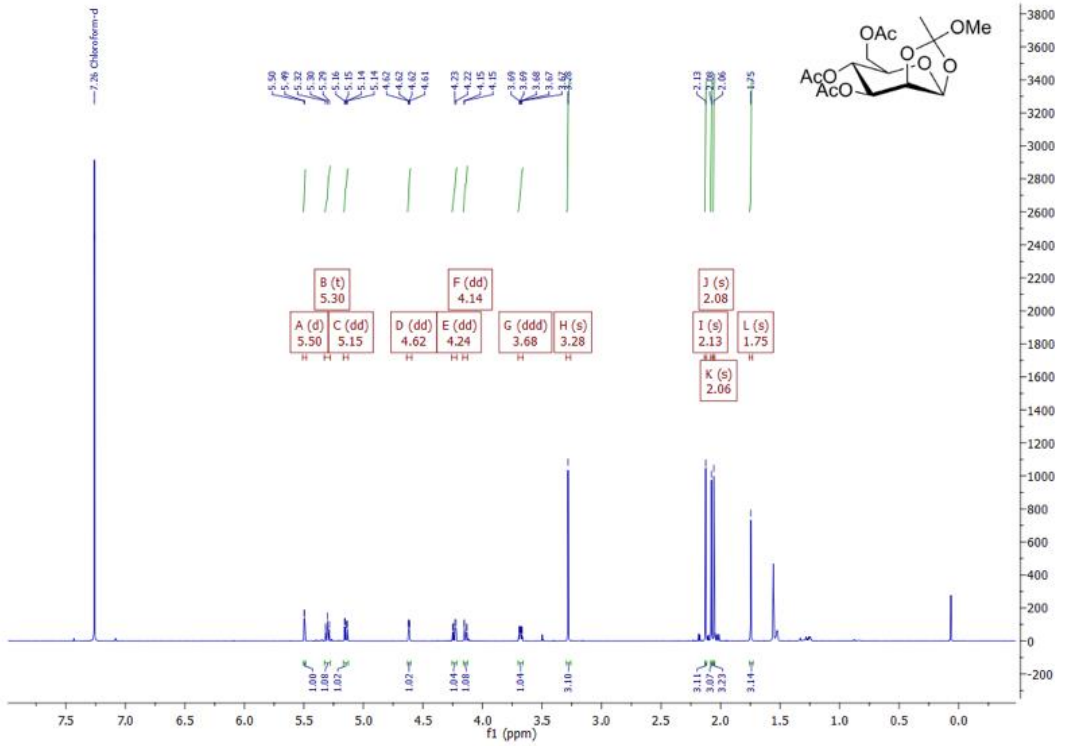
aq.	aqueous
ACN	acetonitrile
CD	circular dichroism
conc.	concentrated
CuAAC	copper(I)-catalyzed azide alkyne cycloaddition
CV-N	cyanovirin-N
DCM	dichloromethane
DIC	*N,N′*-Diisopropylcarbodiimide
DM	dimannosylated peptide (**P2**)
DMF	*N*,*N*-Dimethylformamide

DTT	dithiothreitol
E. coli	*Escherichia coli*
EDC	*N*-(3-Dimethylaminopropyl)-N′-ethylcarbodiimide
EDT	1,2-ethanedithiol
EDTA	ethylenediaminetetraacetic acid
ESI	electrospray ionization
EtOAc	ethyl acetate
EtOH	ethanol
equiv.	equivalent
FA	formic acid
Fmoc	fluorenylmethyloxycarbonyl
GuaHCl	guanidine hydrochloride
HA	hemagglutinin
HEPES	4-(2-Hydroxyethyl)piperazine-1-ethanesulfonic acid
HPLC	high performance liquid chromatography
HRMS	high resolution mass spectrometry
IPTG	isopropyl β-D-1-thiogalactopyranoside
KAN	kanamycin (A)
LB	lysogeny broth
LC-MS	liquid chromatography mass spectrometry
MeOH	methanol
MM	monomannosylated peptide (**P3**)
MW	molecular weight
NHS	*N*-Hydroxysuccinimide
NTA	nitrilotriacetic acid
OD_{600}	optical density at a wavelength of 600 nm
PAGE	polyacrylamide gel electrophoresis
PBS	phosphate-buffered saline
PE	petroleum ether
pET27b(+)	vector with kanamycin resistance for protein expression
PMSF	phenylmethylsulfonyl fluoride
RPM	rounds per minute
r.t.	room temperature
SDS	sodium dodecyl sulfate
SN	supernatant
SOC	super optimal broth with catabolite repression
SPPS	solid phase peptide synthesis
SPR	surface plasmon resonance (spectroscopy)
TBS	tris-buffered saline
TBST	tris-buffered saline with Tween 20
TBTA	tris[(1-benzyl-1H-1,2,3-triazol-4-yl)methyl]amine
TCEP	tris(2-carboxyethyl)phosphine
TFA	trifluoroacetic acid
TLC	thin layer chromatography
$TMSN_3$	trimethylsilyl azide
TMSOTf	trimethylsilyl trifluoromethanesulfonate
Tris	tris(hydroxymethyl)aminomethane
Vx	variant x (of $(CV\text{-}N)_2L_0$)
Xaa	any amino acid

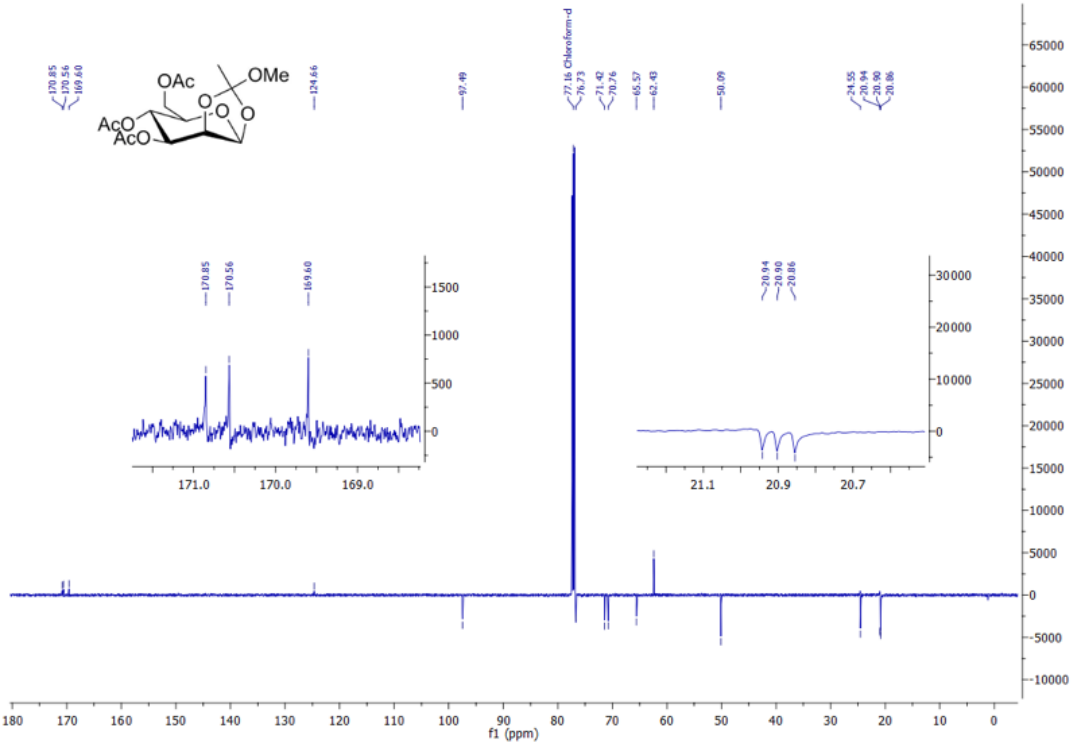
Asn (N) asparagine; Asp (D) aspartic acid; Arg (R) arginine; Cys (C) cysteine; Gln (Q) glutamine; Glu (E) glutamic acid; Phe (F) phenylalanine; Gly (G) glycine; Leu (L) leucine; Met (M) methionine; Ser (S) serine; Thr (T) threonine; Val (V) valine; Trp (W) tryptophan

9. NMR spectra

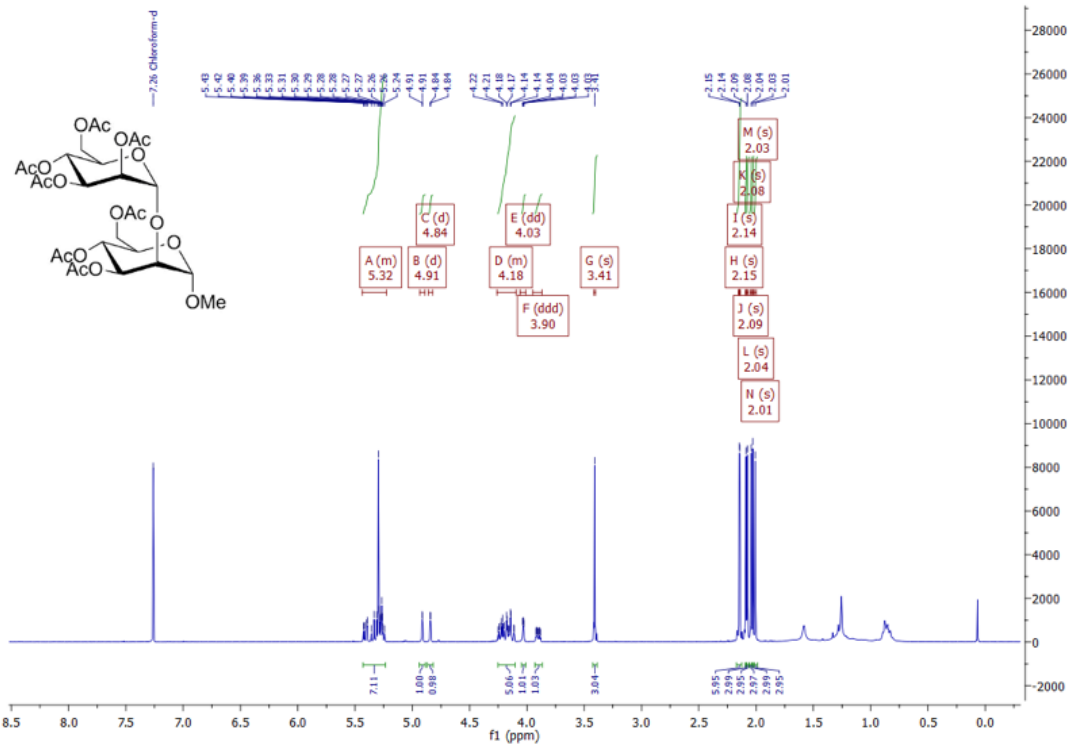
a) ^{1}H NMR spectrum of carbohydrate **4**



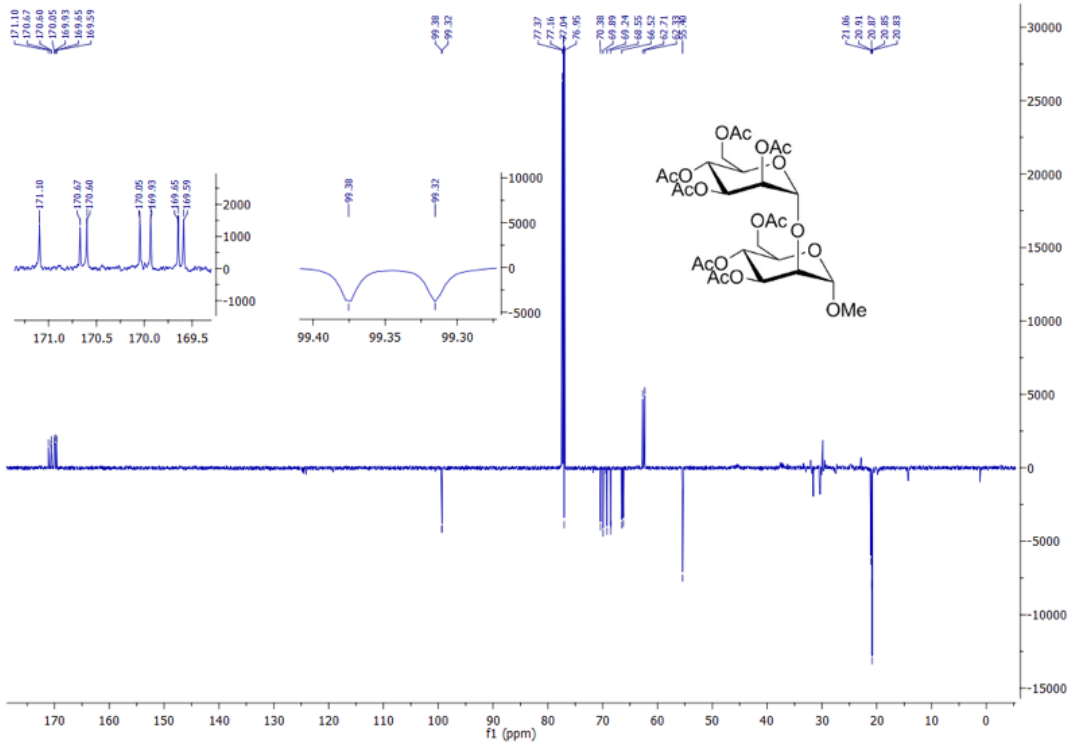
b) ^{13}C NMR spectrum of carbohydrate **4**



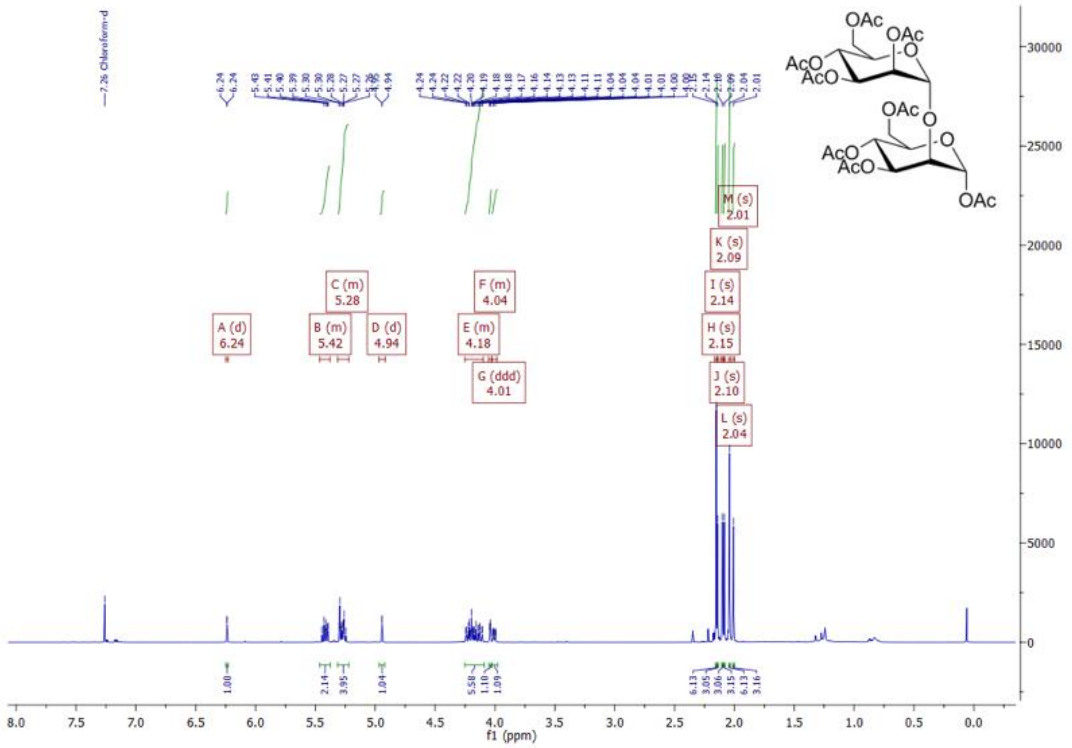
c) ^{1}H NMR spectrum of carbohydrate **5**



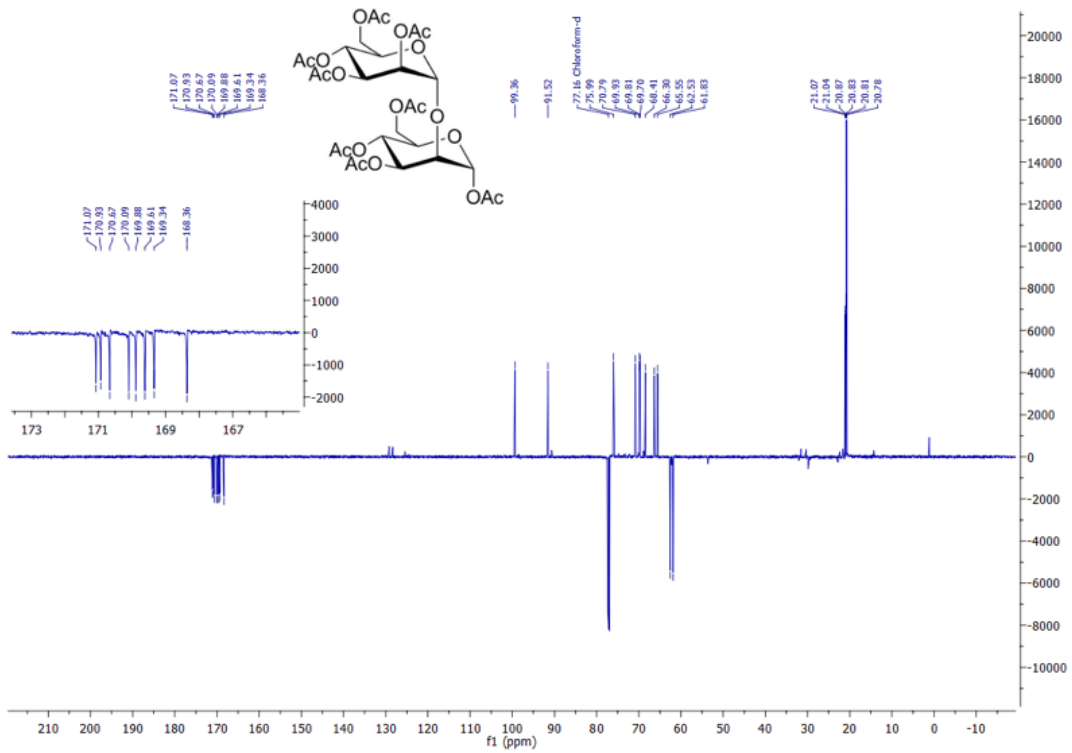
d) ^{13}C NMR spectrum of carbohydrate **5**



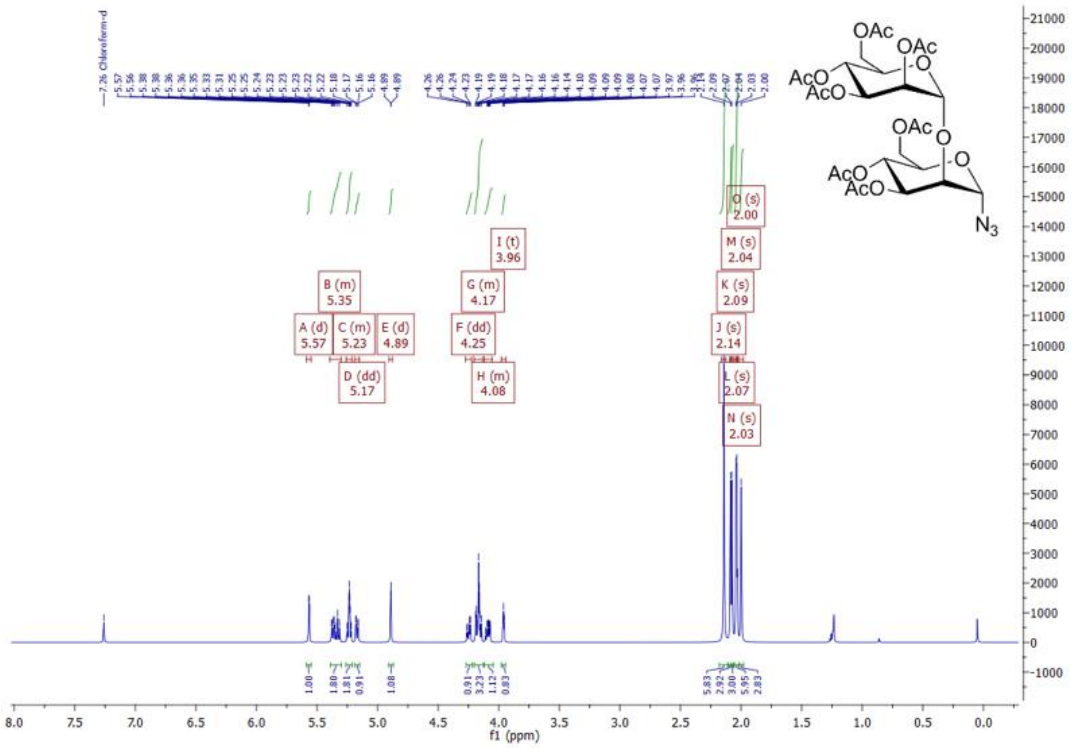
e) ^{1}H NMR spectrum of carbohydrate **6**



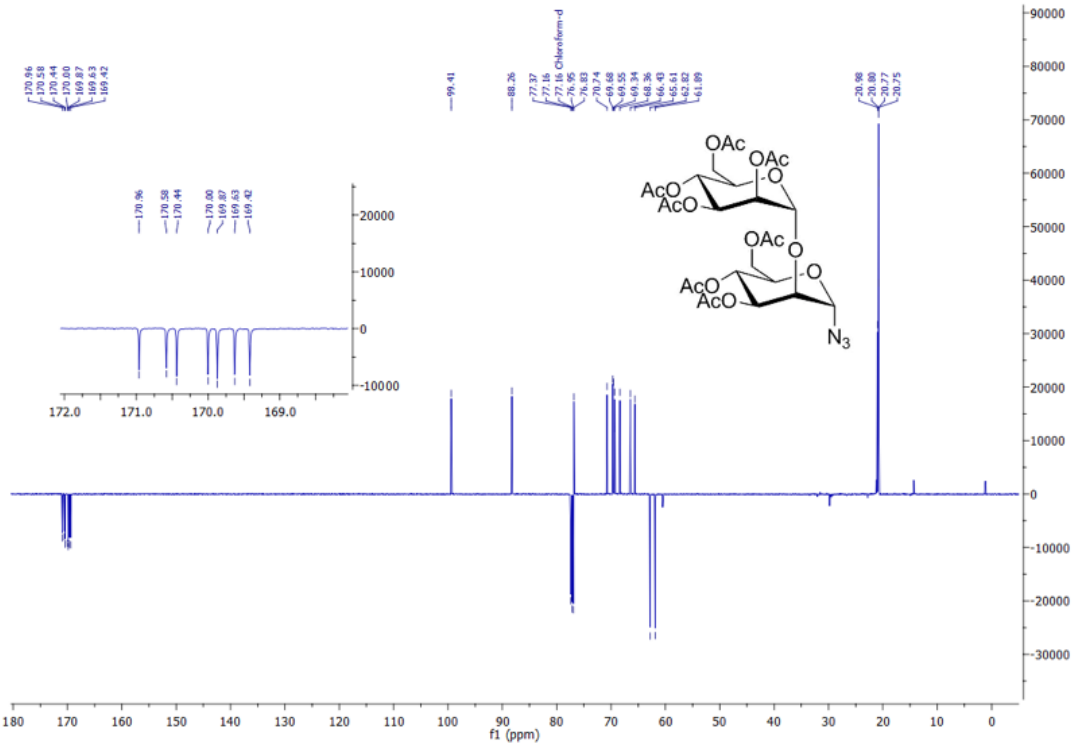
f) ^{13}C NMR spectrum of carbohydrate **6**



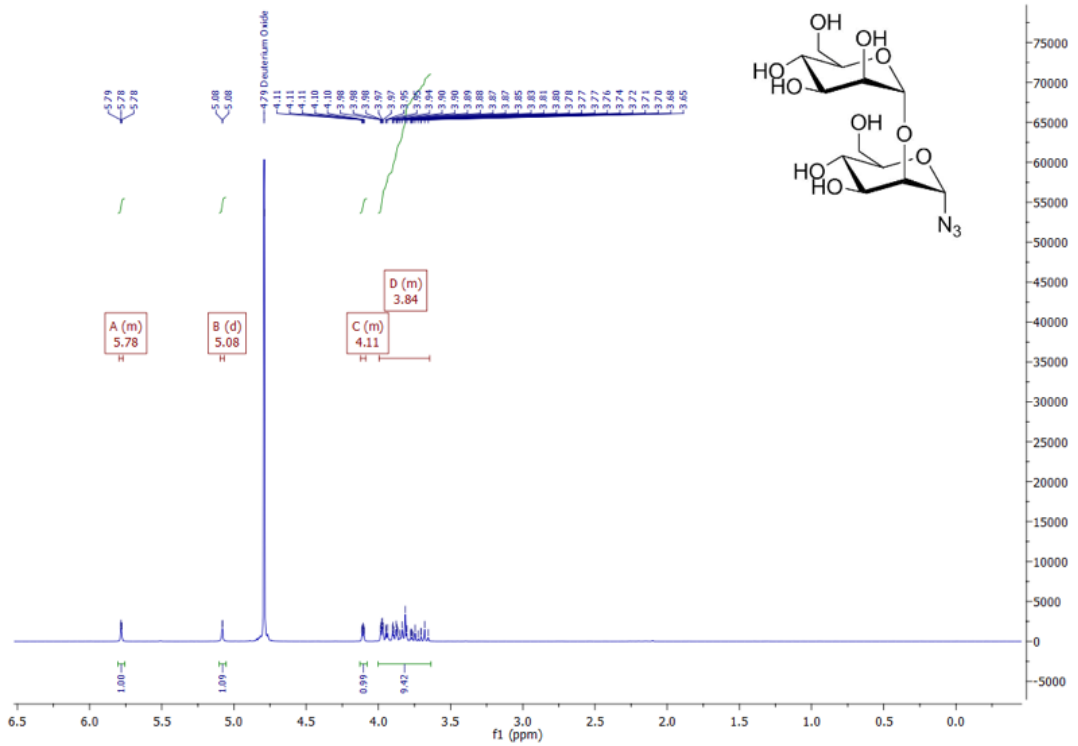
g) ^{1}H NMR spectrum of carbohydrate **7**



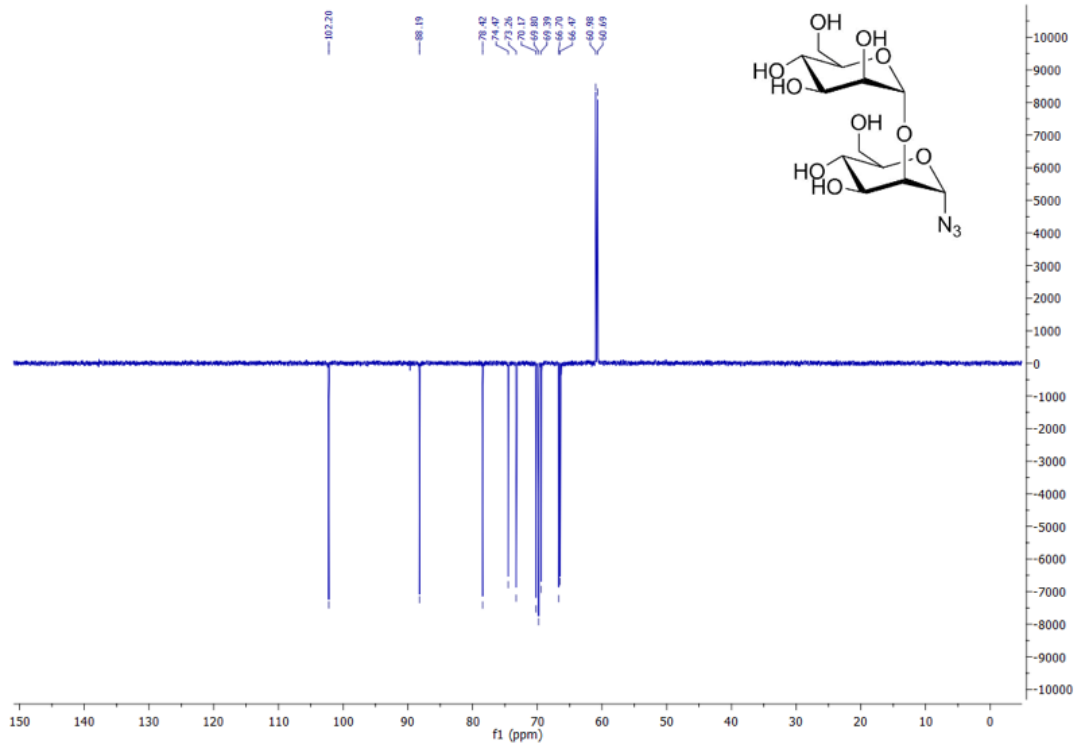
h) ^{13}C NMR spectrum of carbohydrate **7**



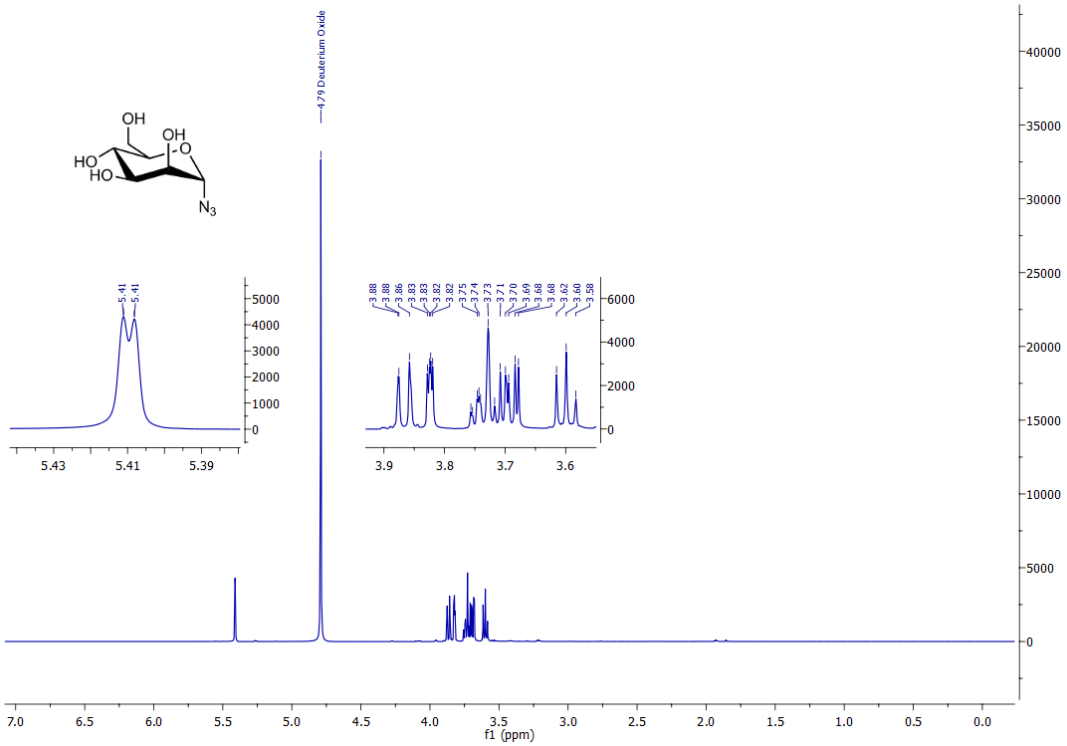
i) ^{1}H NMR spectrum of carbohydrate **8**



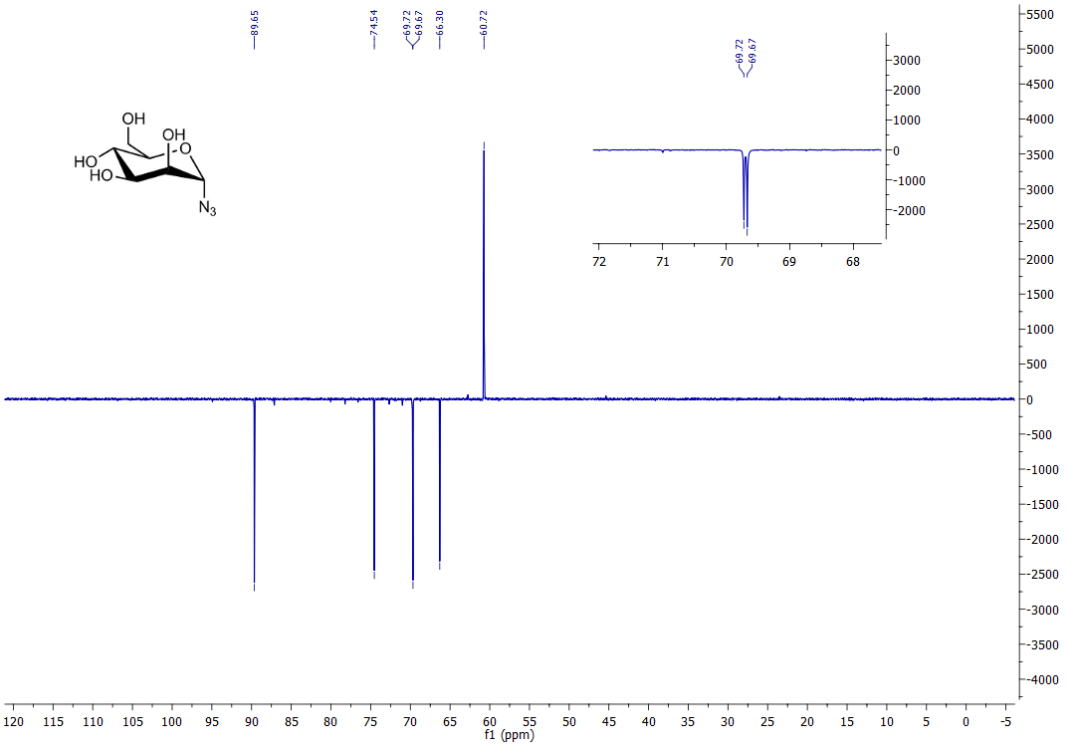
j) ^{13}C NMR spectrum of carbohydrate **8**



k) ^{1}H NMR spectrum of carbohydrate **9**



l) ^{13}C NMR spectrum of carbohydrate **9**



10. List of supporting figures

11. List of supporting tables

12. References

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