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Supplementary Information for:

A Glycan FRET Assay for Detection and Characterization of Catalytic Antibodies to the *Cryptococcus neoformans* Capsule

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Supporting Information

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General methods

Synthetic methods

Unless otherwise noted all reactions containing air- and moisture-sensitive reagents were carried out under an inert atmosphere of nitrogen in oven-dried glassware with magnetic stirring. N_2-flushed stainless cannulas or plastic syringes were used to transfer air- and moisture-sensitive reagents. All reactions were monitored by thin-layer chromatography (TLC) on Merck DC-Alufolien plates precoated with silica gel 60 F254. Visualization was performed with UV-light (254 nm) fluorescence quenching. Evaporation in vacuo/under vacuum refers to the removal at 40 °C, unless otherwise stated, of volatiles on a Buchi rotary evaporator with an integrated vacuum pump.

Chromatography

Silica gel flash chromatography was carried out using Davisil LC60A (40-63 µm) silica gel or with automated flash chromatography systems, Buchi Reveleris® X2 (UV 200-500 nm and ELSD detection, Reveleris® silica cartridges 40 µm, BÜCHI Labortechnik AG) and Biotage® SP4 HPFC (UV 200-500 nm, Biotage® SNAP KP-Sil 50 µm irregular silica, Biotage AB).

Synthetic Materials

All chemicals for the synthesis were purchased from commercial suppliers (Acros, Carbosynth Ltd, Fisher Scientific Ltd, Glycom A/S, Merck, Sigma-Aldrich, VWR, STREM Chemicals) and used without purification. Dry DCM and THF were obtained from a PureSolv-ENTM solvent purification system (Innovative Technology Inc.). All other anhydrous solvents were used as purchased from Sigma-Aldrich in AcroSeal® bottles.

Instrumentation

^{1}H NMR (400, 500 or 600 MHz), 13C NMR (101 MHZ or 125 MHz) spectra were recorded on Varian-inova at 25 °C in chloroform-d1 ($CDCl_3$), methanol-d4 (CD_3OD), water-d2 (D_2O), ^{1}H NMR spectra were standardized against the residual solvent peak ($CDCl_3$, δ = 7.26 ppm; CD_3OD, δ = 3.31 ppm; D_2O, δ = 4.79 ppm; d6-DSS δ = 0.0 ppm or internal tetramethylsilane, δ = 0.00 ppm). Bruker instrumentation spectrometers were equipped with Avance II console and triple resonance, TCI cryogenic probe with z-axis pulsed field. ^{13}C NMR spectra were standardized against the residual solvent peak ($CDCl_3$, δ = 77.16 ppm; CD_3OD, δ = 49.00 ppm. All ^{13}C NMR are ^{1}H decoupled. All NMR data is represented as follows: chemical shift (δ ppm), multiplicity (s = singlet, d = doublet, t = triplet, q = quartet, dd = doublet of doublets, ddd = doublet of doublets of doublets, dt = doublet of triplets, m = multiplet, br = broad signal, ad = apparent doublet, at = apparent triplet), coupling constant in Hz, integration. Assignments were aided by homonuclear ^{1}H-^{1}H (COSY, TOCSY), and ^{1}H-^{13}C heteronuclear (HSQC, HMBC) two-dimensional correlation spectroscopies. ^{13}C chemical shifts were reported with one digit after the decimal point, unless an additional digit was reported to distinguish overlapping peaks. Software for data processing: MestReNova, version 11.0.0-17609 (MestReLab Research S.L.). High-resolution mass spectrometry (HRMS) data were recorded on a Waters micromass LCT LC-Tof instrument using electrospray ionization (ESI) in either positive or negative mode. Low-resolution mass spectrometry (LRMS) experiments were recorded on a Waters micromass Quattro Micro LC-MS/MS instrument using electrospray ionization (ESI) in either positive or negative mode.

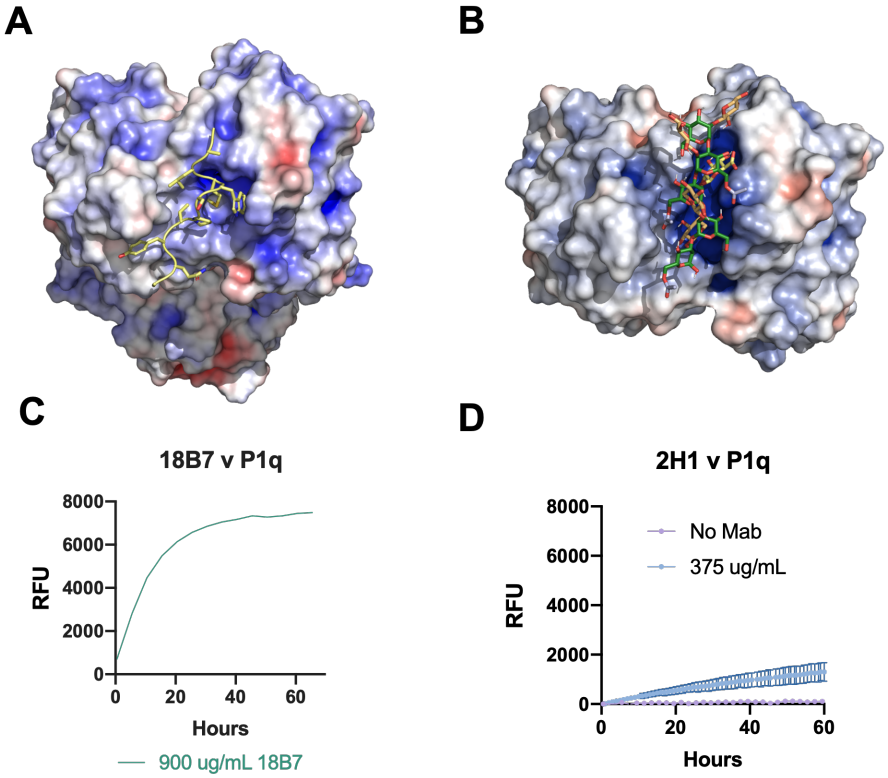
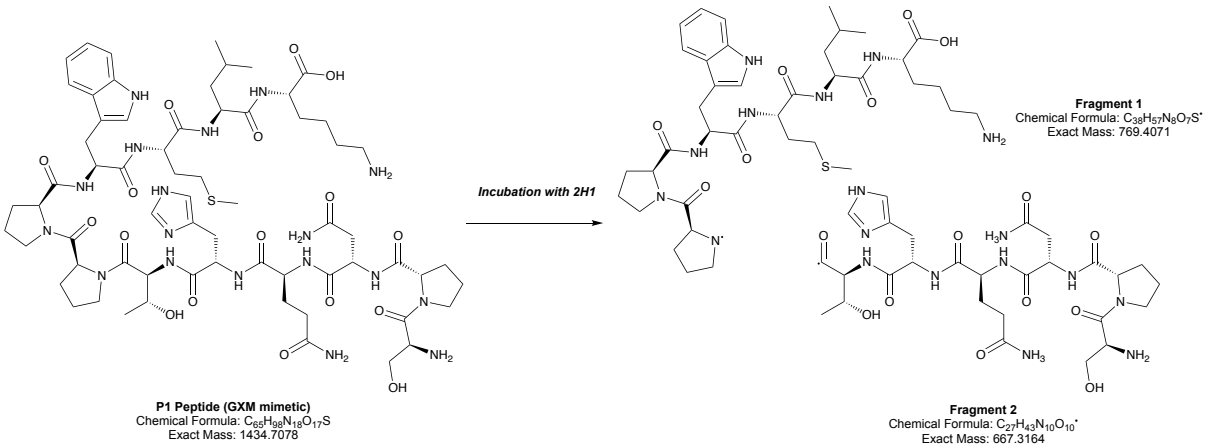


Figure S1. 2H1 mAb and P1 Peptide. A Known crystal structure of 2H1 mAb with P1 peptide (shown in green) in binding site. Calculated electrostatic surface potential of 2H1 mAb was complete using adaptive Poisson-Boltzmann solve (APBS) in PyMOL (+10 kT to -10 kT). Electrostatic surface potentials are colored red for negative charges, blue for positive charges, and white color represents neutral residues. **B** Modelling interactions between 2H1 and its decasaccharide substrate. Molecular model of known crystal structure of anti-GXM Mab 2H1. **C** Monoclonal antibody 18B7's catalytic activity against the P1q FRET peptide. **D** Monoclonal antibody 2H1 has catalytic activity against the P1q FRET peptide. The P1q FRET probe (200 uM) was incubated with 375 ug/mL of 2H1 or alone (No Mab). All measurements were performed at 37 °C with an excitation wavelength of 320 nm, an emission wavelength of 405 nm, and an emission cut-off filter of 325 nm. Change in fluorescence at 405 nm indicated hydrolysis of the FRET probe. Incubations were set up in triplicate, with error bars shown.



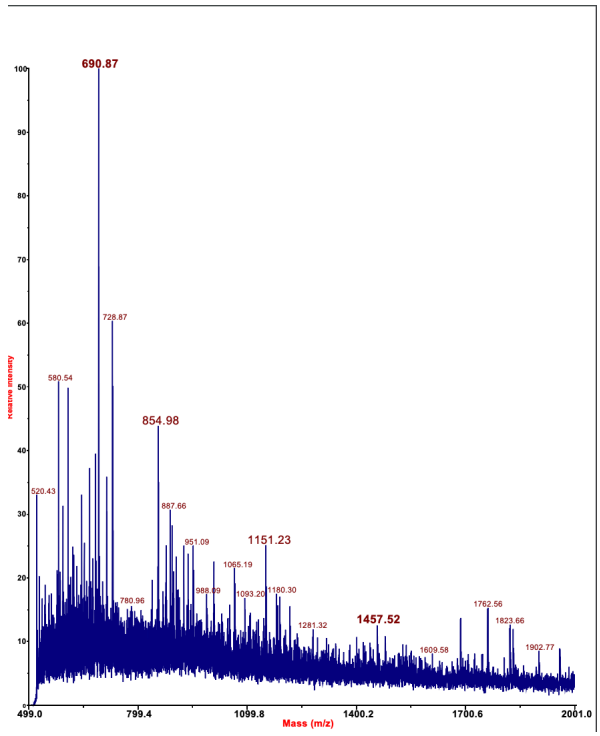


Figure S2. Mass Spectrometry Spectra of P1 Peptide Post Incubation with mAb 2H1. Fragment 1 769 *m/z* is in noise, while fragment 2 [+Na] is seen as 690 *m/z*.

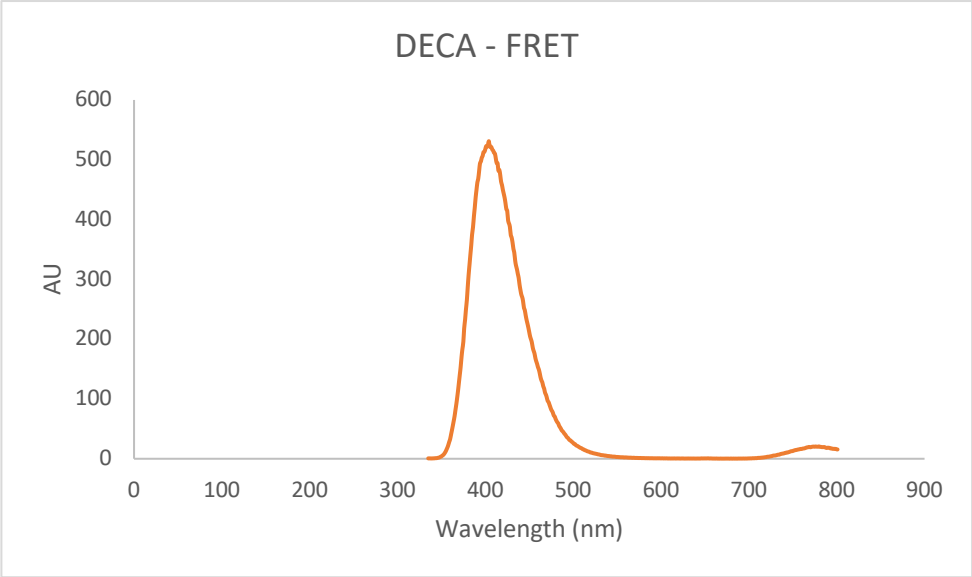


Figure S3. Fluorescence Spectrum of Oigosaccharide FRET Probe. Excitation at 325 nm, Fluorescence at 410 nm.

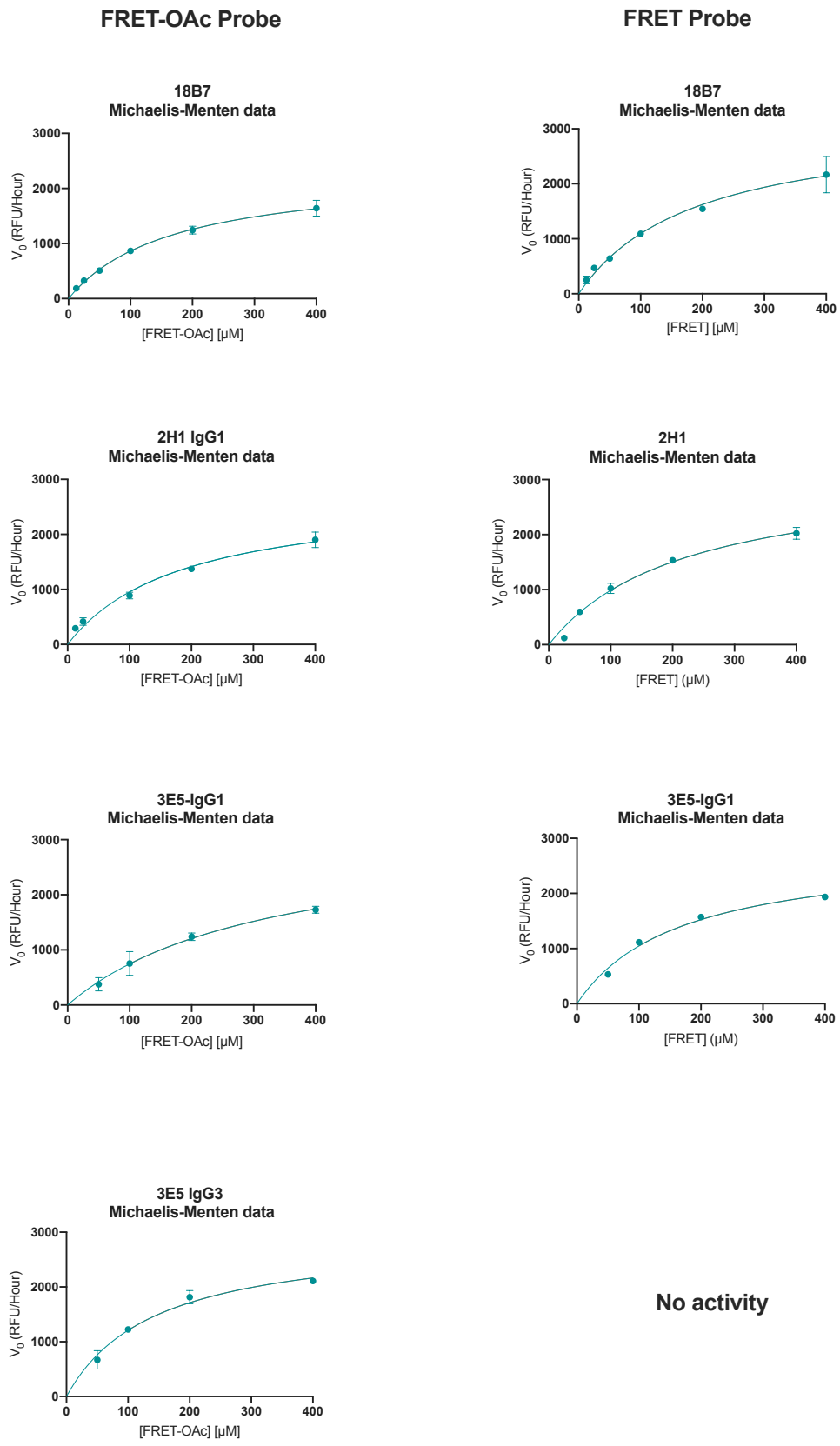


Figure S4. The FRET and FRET-OAc Probes are Hydrolyzed Following Michaelis-Menten Kinetics. FRET probes **17** and **16** was incubated at varying micromolar concentrations. It was not possible to determine the catalytic activity of 3E5-IgG$_3$ towards FRET probe **17**. The initial velocity (V_0) of each reaction was

determined and plotted as a function of substrate concentration, the data was fit to the Michaelis-Menten equation for a single-step bimolecular reaction using nonlinear regression. K_m and k_{cat} were calculated using Prism 8. Each point represents n = 3 and bars represent the mean ±SD.

Table S1. Supplemental Michaelis-Menten Kinetics Data.[a]

Entry	Antibody	FRET Probe	K_m [μM] 95% CI	k_{cat} [s^{-1}] 95% CI
1	18B7	**FRET**	30.0 - 102.4 x10^{-3}	26.0 - 45.5 x10^{-3}
2	2H1	**FRET**	36.1 - 113.1 x10^{-3}	35.9 - 62.5 x10^{-3}
3	3E5-IgG_1	**FRET**	16.0 - 158.5 x10^{-3}	19.5 -56.1 x10^{-3}
4	3E5-IgG_3	**FRET**	ND	ND
5	18B7	**FRET-OAc**	36.4 - 61.1 x10^{-3}	21.6 - 27.4 x10^{-3}
6	2H1	**FRET-OAc**	30.8 - 91.5 x10^{-3}	31.7 - 51.9 x10^{-3}
7	3E5-IgG_1	**FRET-OAc**	48.2 - 196.4 x10^{-3}	19.5 -56.1 x10^{-3}
8	3E5-IgG_3	**FRET-OAc**	25.7 - 62.6 x10^{-3}	25.8 - 37.5 x10^{-3}

[a] The initial velocity (V_0) of each reaction was determined and plotted as a function of substrate concentration, the data was fit to the Michaelis-Menten equation for a single-step bimolecular reaction using nonlinear regression. K_m and k_{cat} were calculated using Prism 8. Each experiment was repeated in triplicate.

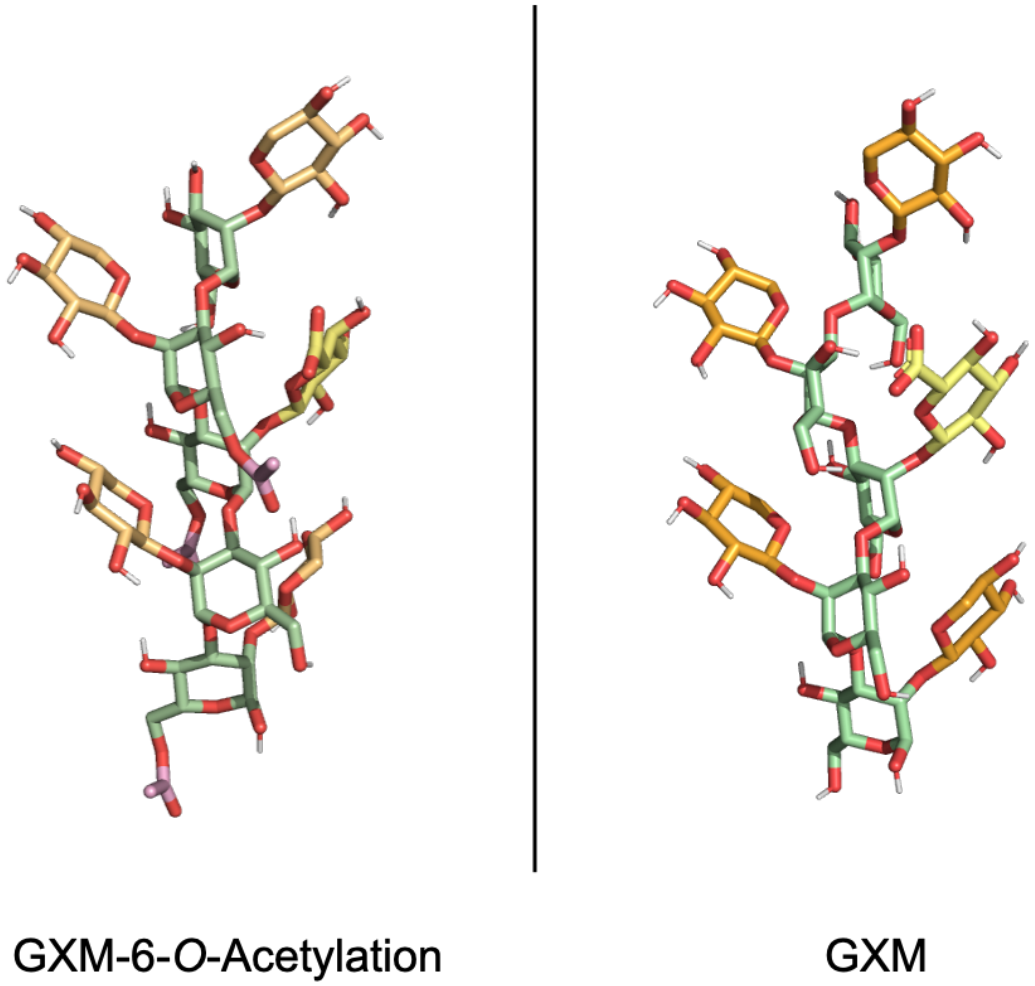


Figure S5. Effect of Acetylation on GXM Conformation. GLYCAM carbohydrate builder was used to predict conformation of acetylated and deacetylated GXM.(37–39) Mannan backbone is decorated with xylose and glucuronic substituents acid facing outwards from helical conformation. Acetylation does not cause change in conformation but creates new epitopes.

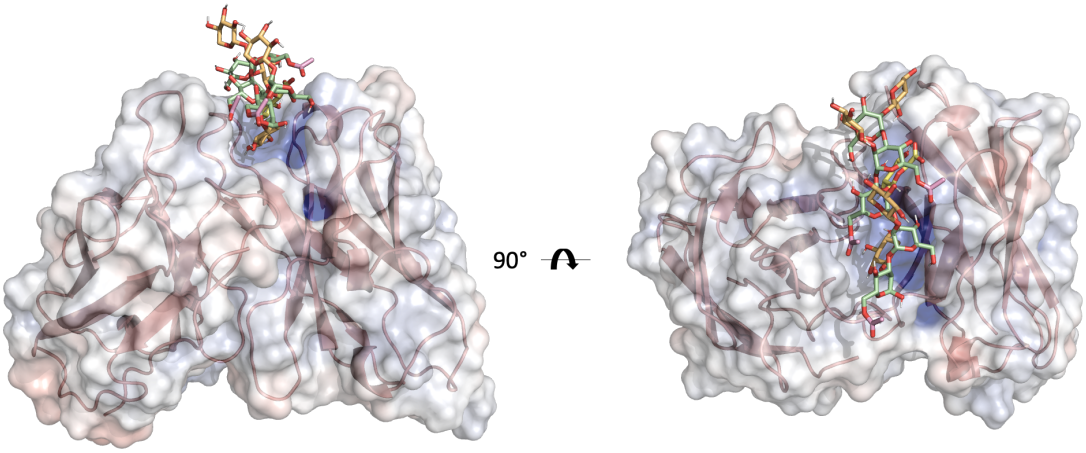


Figure S6. Docking Results of Acetylated GXM Decasaccharide with 2H1.

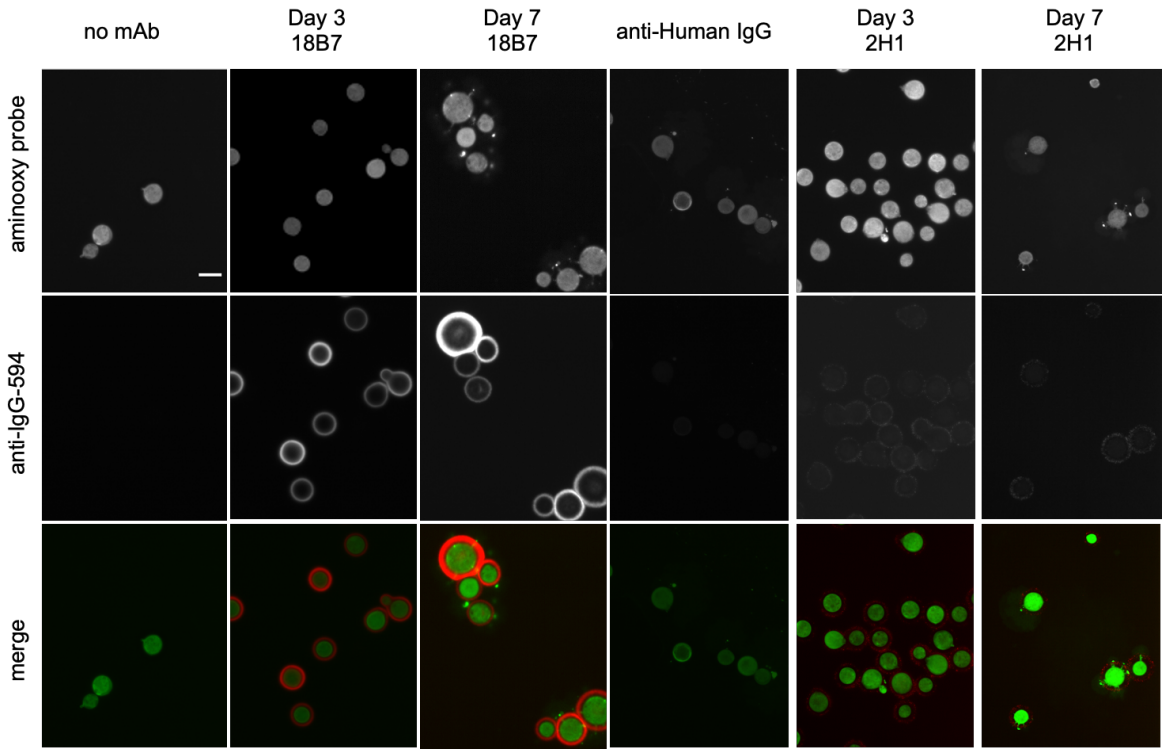


Figure S7. Fluorescent Microscopy of Heat-Killed *C. neoformans* cells Incubated Catalytic Antibodies Over Time. Heat-killed cells were incubated with 18B7, 2H1, anti-human IgG or no antibody (no mAb) and imaged on day 3 and 7. An aminooxy probe was used to visualize the change in capsule architecture after exposure to catalytic antibodies over time. Scale bar 5 μm.

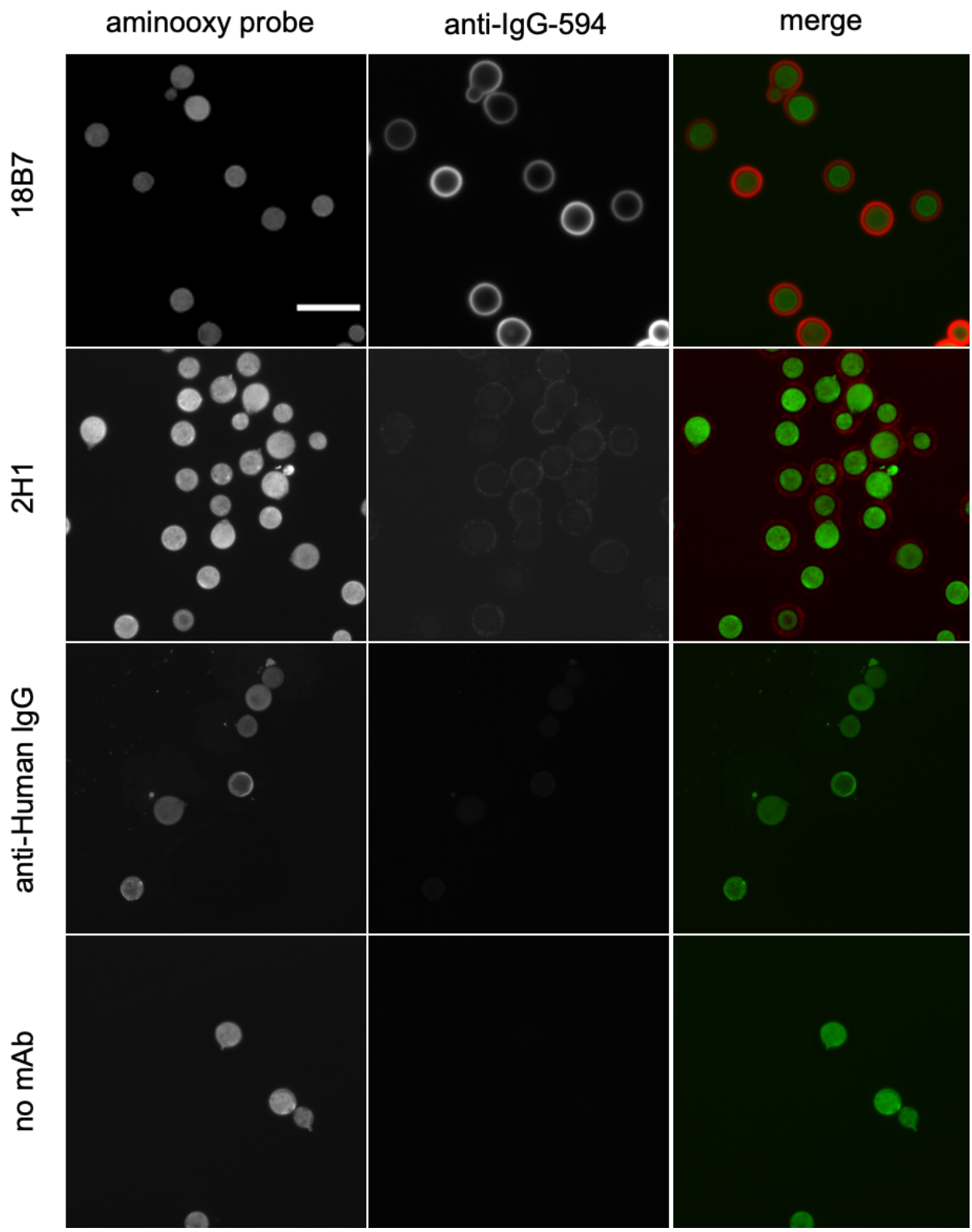


Figure S8. Fluorescent Microscopy of Heat-Killed *C. neoformans* Cells Incubated Catalytic Antibodies on Day 3. Scale 5 μm.

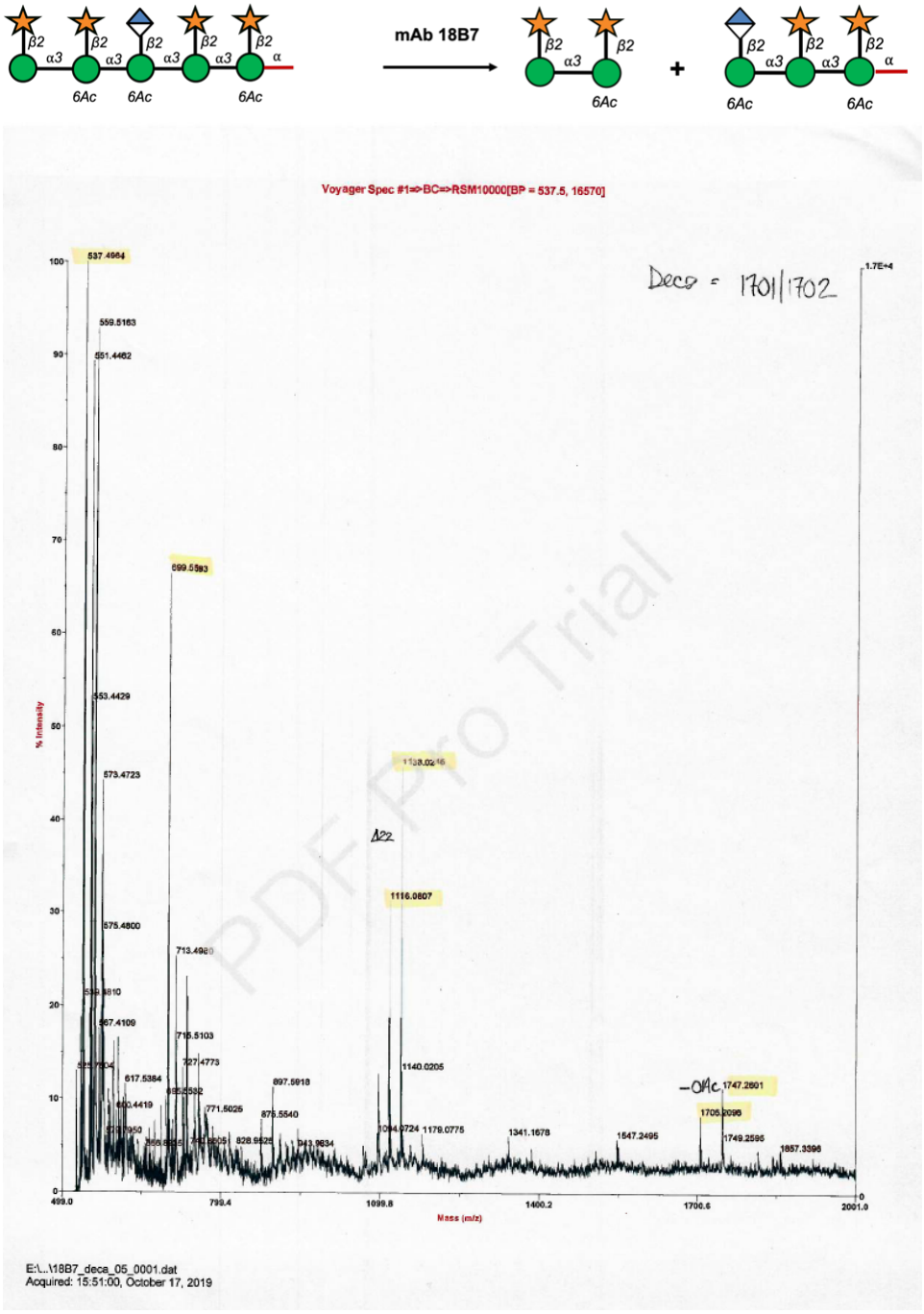
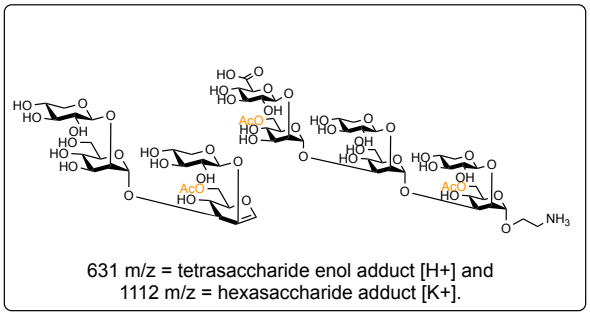


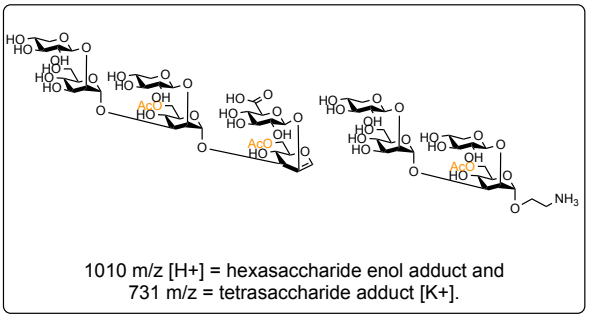
Figure S9. Mass Spectrum of 18B7 and Decasaccharide Incubation. Ion 1116.08 *m/z* corresponds to hexasaccharide fragment + [NMe_2 + 45], dimethylamine is a commonly observed adduct when using preconditioning deprecation strategy as described by Crawford *et al.* .(28)

Breakdown Products 1



631 m/z = tetrasaccharide enol adduct [H+] and
1112 m/z = hexasaccharide adduct [K+].

Breakdown Products 2



1010 m/z [H+] = hexasaccharide enol adduct and
731 m/z = tetrasaccharide adduct [K+].

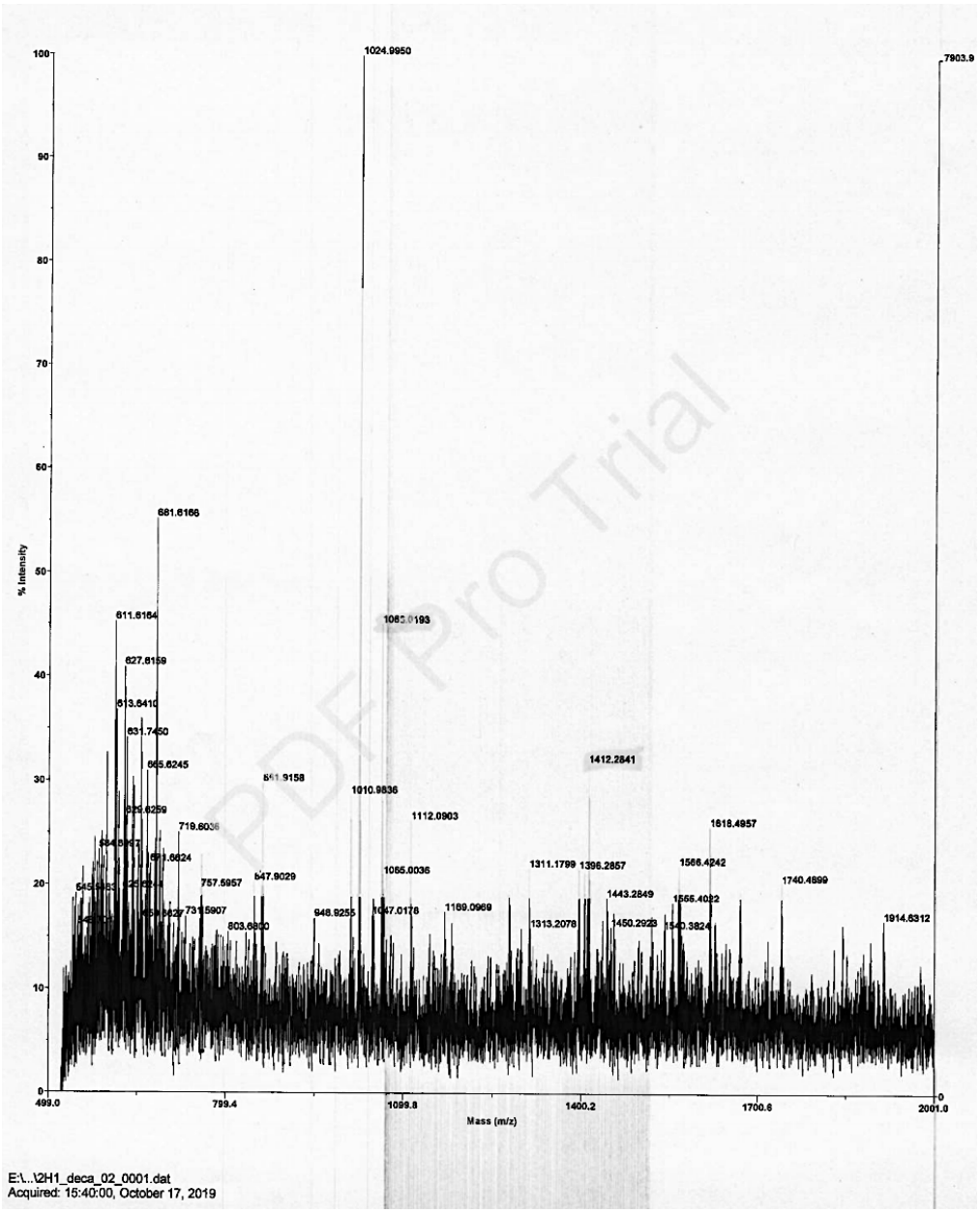
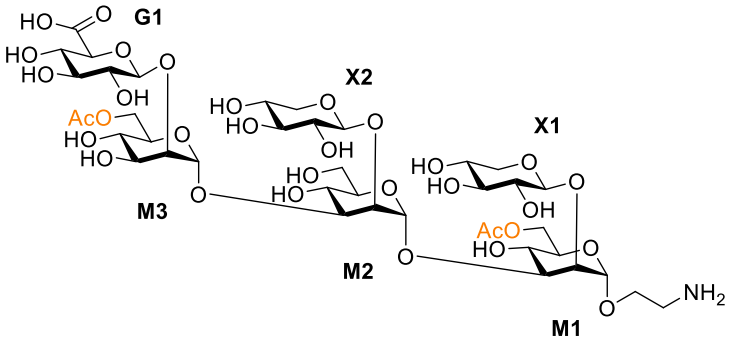


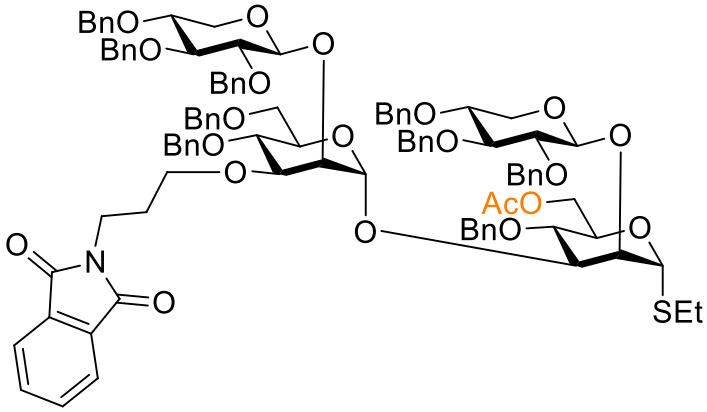
Figure S10. Mass Spectrum of 2H1 mAb and Decasaccharide Suggests Lyase Activity.

NMR Nomenclature



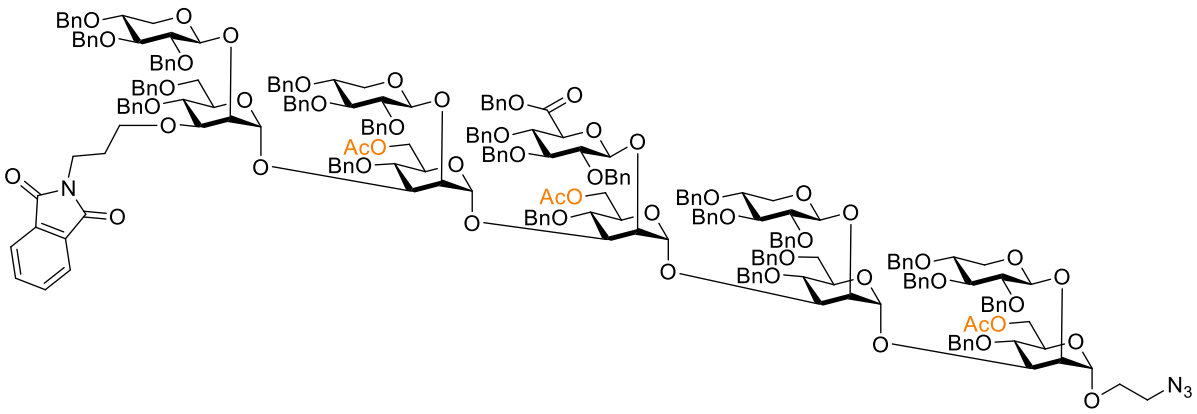
Compound Characterization

Ethyl 2,3,4-tri-*O*-benzyl-β-D-xylopyranosyl-(1→2)-4,6-di-*O*-benzyl-3-*O*-(3-phthalimdo-propyl)-α-D-mannopyranosyl-(1→3)-[2,3,4-tri-*O*-benzyl-β-D-xylo-pyranosyl-(1→2)]-6-*O*-acetyl-4-*O*-benzyl-1-thio-α-D-mannopyranoside (9)



Tetrasaccharide **8** (100 mg, 66.57 µmole, 1 eq) was dissolved in dry DMF (0.6 mL, 0.1M) and stirred with 4 Å MS for 1 h at room temperature, under nitrogen a atmosphere. The reaction was cooled to 0 °C, and 60% dispersion in mineral oil NaH was added (5.5 mg, 133.15 µmole, 2 eq). The reaction was left to stir for 5 minutes and *N*-(3-Bromopropyl)phthalimide (90 mg, 332.88 µmole, 5 eq) was added and the reaction was allowed to raise to room temperature. Once complete, the reaction was quenched with acetic acid dissolved in 1 mL ethyl acetate at 0 °C and concentrated *in vacuo* and subsequently purified *via* flash chromatography (cyclohexane:acetone, gradient) to yield **9** (88 mg, 78%) as a colorless syrup. **R*f*** (cyclohexane:acetone, 80:20) = 0.2 **^{1}H NMR** (500 MHz, Chloroform-d) δ 7.78 – 7.69 (m, 2H, H_{ar}), 7.69 – 7.60 (m, 2H, H_{ar}), 7.50 – 7.03 (m, 45H, H_{ar}), 5.29 (s, 1H, , H-1_{M2}), 5.26 (s, 1H, H-1_{M2}), 5.13 – 4.91 (m, 4H), 4.91 – 4.74 (m, 4H), 4.72 – 4.46 (m, 9H), 4.45 – 4.18 (m, 10H, H-1_{X1}), 4.02 – 3.69 (m, 10H, H-1_{X2}), 3.69 – 3.30 (m, 8H), 3.14 – 2.98 (m, 1H, H-$5_{axial\ X1}$), 2.82 (t, J = 10.8 Hz, 1H, H-$5_{axial\ X2}$), 2.63 – 2.43 (m, 2H, S$CH_2$$CH_3$), 2.11 – 1.91 (m, 2H, (Phth)N$CH_2$$CH_2$$CH_2$O), 1.84 (s, 3H, C(=O)$CH_3$), 1.22 (t, J = 7.4 Hz, 3H, S$CH_2$$CH_3$). **^{13}C NMR** (126 MHz, Chloroform-*d*) δ 170.6, 139.2, 138.8, 138.6, 138.4, 138.4, 138.3, 138.1, 138.1, 133.8, 132.1, 128.8, 128.5, 128.5, 128.3, 128.2, 128.2, 128.2, 128.1, 127.9, 127.8, 127.6, 127.6, 127.5, 127.4, 127.4, 127.3, 127.1, 123.1, 103.9 ($C1_{X1}$), 103.8 ($C1_{X2}$), 100.7, 83.8, 83.5, 82.8, 81.3, 80.9, 78.9, 77.5, 77.3, 75.8, 75.5, 75.3, 75.0, 74.9, 74.9, 74.7, 73.4, 73.0, 72.5, 72.1, 70.1, 70.0, 67.2, 63.7, 63.4, 63.2, 35.3, 29.2 (PhthN$CH_2$$CH_2$$CH_2$O), 25.5 (S$CH_2$$CH_3$), 20.6 (C(=O)$CH_3$), 15.0 (S$CH_2$$CH_3$). **HRMS (ESI)** $[M + Na]^+$ m/z Calc. for $C_{100}H_{107}NO_{21}NaS$ 1712.6954 Found: 1712.6954.

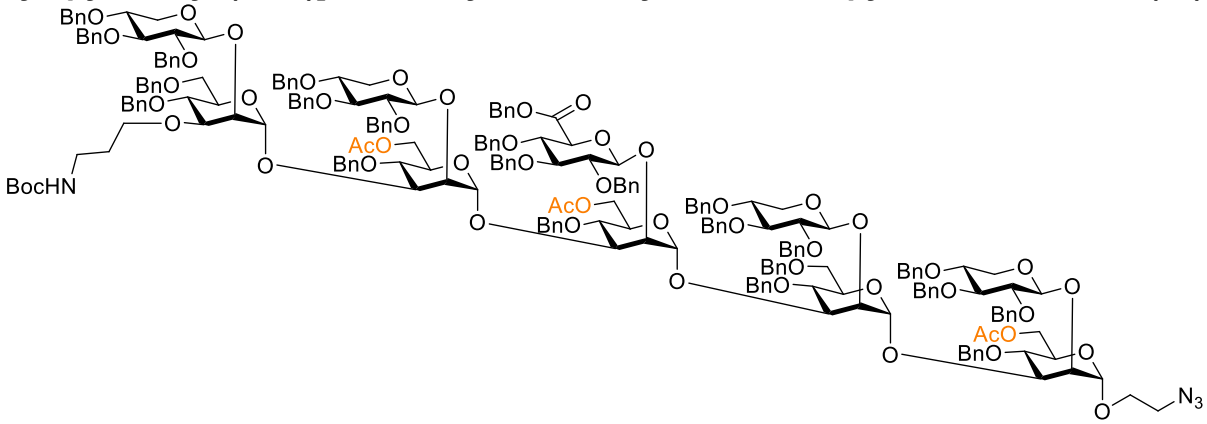
2-Azidoethyl 2,3,4-tri-*O*-benzyl-β-D-xylopyranosyl-(1→2)-4,6-di-*O*-benzyl-3-*O*-(3-phthalimdo-propyl)-α-D-mannopyranosyl-(1→3)-[2,3,4-tri-*O*-benzyl-β-D-xylo-pyranosyl-(1→2)]-6-*O*-acetyl-4-*O*-benzyl-α-D-mannopyranosyl-(1→3)-[benzyl 2,3,4-tri-*O*-benzyl-β-D-glucopyranosyluronate-(1→2)]-6-*O*-acetyl-4-*O*-benzyl-α-D-mannopyranosyl-(1→3)-[2,3,4-tri-*O*-benzyl-β-D-xylopyranosyl-(1→2)]-4,6-di-*O*-benzyl-α-D-mannopyranosyl-(1→3)-[2,3,4-tri-*O*-benzyl-β-D-xylopyranosyl-(1→2)]-6-*O*-acetyl-4-*O*-benzyl-α-D-mannopyranoside (10)



Tetrasaccharide donor **9** (50 mg, 30.5 µmole, 1.5 eq) and Hexasaccharide acceptor **7** (48 mg, 20 µmole, 1 eq) were dissolved in dry diethylether (2 mL, 0.01M) at room temperature, under nitrogen. 4 Å MS were added, and the reaction was stirred for 1 h. The reaction was cooled to 0 °C, and DMTST (15 mg, 61 µmole , 3 eq) was added, the reaction was monitored *via* TLC (cyclohexane:acetone, 80:20, v/v) once complete ca. 4 h, the reaction was quenched with triethylamine, and left stir for 5 minutes. The reaction was filtered through a bed of Ceilte® and concentrated *in vacuo,* the resulting residue was purified by flash chromatography (cyclohexane:acetone, gradient) to yield **10 (**57 mg, 85%) as a colourless oil. **R*f*** (cyclohexane-acetone, 80:20) = 0.3. **^{1}H NMR** (600 MHz, Chloroform-*d*) δ 7.73 (dd, *J* = 5.4, 2.9 Hz, 1H), 7.71 – 7.67 (m, 2H), 7.67 – 7.49 (m, 3H), 7.47 – 7.34 (m, 12H, H_{ar}), 7.34 – 7.02 (m, 116H, H_{ar}), 5.45 (s, 1H, H-1_{M5}), 5.33 (s, 1H, H-1_{M3}), 5.23 – 5.19 (m, 3H, H-1_{M2}, H-1_{M4}), 5.14 – 4.98 (m, 6H), 4.94 (d, *J* = 11.6 Hz, 4H), 4.89 – 4.72 (m, 11H), 4.73 – 4.47 (m, 27H), 4.45 – 4.23 (m, 17H), 4.23 – 4.08 (m, 16H), 4.08 – 3.82 (m, 14H), 3.82 – 3.61 (m, 11H), 3.62 – 3.46 (m, 7H), 3.44 (td, *J* = 9.5, 9.0, 3.8 Hz, 3H), 3.42 – 3.26 (m, 11H), 3.26 – 3.15 (m, 2H), 3.05 (t, *J* = 10.7 Hz, 1H, H-5 $_{axial\ X}$), 2.85 (t, *J* = 10.8 Hz, 1H, H-5 $_{axial\ X}$), 2.63 – 2.56 (m, 2H, PhthNCH_2CH_2CH_2O), 2.03 – 1.88 (m, 3H), 1.85 (s, 3H, (C=O)C$H_{3\ M1}$), 1.54 (d, *J* = 3.4 Hz, 6H, (C=O)C$H_{3\ M3}$, (C=O)C$H_{3\ M4}$). **^{13}C NMR (**151 MHz, Chloroform-d) δ 170.6, 170.4, 168.2, 168.1, 167.7, 139.3, 139.1, 138.9, 138.8, 138.8, 138.6, 138.6, 138.5, 138.4, 138.3, 138.3, 138.2, 138.1, 138.0, 137.9, 135.3, 133.8, 133.7, 133.7, 132.1, 132.1, 129.3, 128.8, 128.8, 128.8, 128.7, 128.7, 128.6, 128.6, 128.5, 128.4, 128.3, 128.3, 128.3, 128.27, 128.23, 128.19, 128.1, 128.1, 128.0, 127.9, 127.9, 127.8, 127.8, 127.7, 127.7, 127.6, 127.6, 127.5, 127.5, 127.5, 127.4, 127.4, 127.4, 127.3, 127.3, 127.3, 127.2, 127.2, 127.1, 127.0, 126.8, 126.6, 126.0, 123.1, 123.0, 104.3 (C-1_{X1}), 103.3 (C-1_{X3}), 103.2 (C-1_{X4}), 102.8 (C-1_{X2}), 102.3 (C-1_{G1}), 101.9 (C-1_{M4}), 101.0 (C-1_{M2}), 100.1 (C-1_{M3}), 99.8 (C-1_{M5}), 98.2 (C-1_{M1}), 83.8, 83.4, 83.3, 83.3, 83.2, 81.5, 81.4, 81.3, 81.1, 80.7, 80.3, 79.5, 79.2, 78.8, 78.5, 78.2, 78.0, 77.5, 75.4, 75.4, 75.3, 75.2, 75.2, 75.2, 75.1, 75.0, 74.9, 74.8, 74.6, 74.5, 74.4, 74.4, 74.3, 74.0, 73.6, 73.5, 73.1,

72.9, 72.9, 72.7, 72.2, 72.0, 71.6, 70.4, 70.0, 69.7, 69.5, 68.9, 67.1, 67.1, 66.6, 63.7, 63.5, 63.2, 63.1, 63.0, 62.9, 50.2 ($OCH_2CH_2N_3$), 35.3 ($PhthNCH_2CH_2CH_2O$), 29.2 ($PhthNCH_2CH_2CH_2O$), 20.6 ($C(=O)CH_3$ M1), 20.5 ($C(=O)CH_3$) 20.4 ($C(=O)CH_3$). **Mass (ESI)** $[M + H]^+$ m/z Calc. for $C_{236}H_{249}N_4O_{53}$ 3986.69 Found: 3986.68.

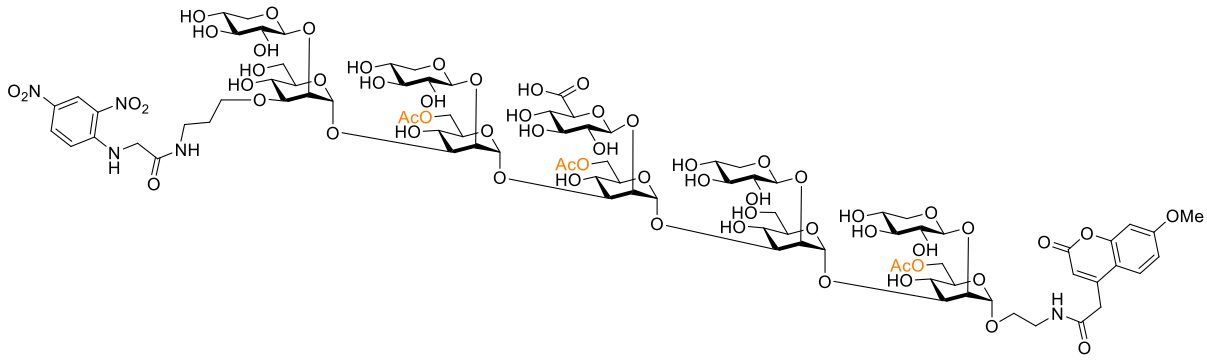
2-Azidoethyl 2,3,4-tri-*O*-benzyl-β-D-xylopyranosyl-(1→2)-4,6-di-*O*-benzyl-3-*O*-(3-*tert*-butyloxycarbonylpropyl)-α-D-mannopyranosyl-(1→3)-[2,3,4-tri-*O*-benzyl-β-D-xylopyranosyl-(1→2)]-6-*O*-acetyl-4-*O*-benzyl-α-D-mannopyranosyl-(1→3)-[benzyl 2,3,4-tri-*O*-benzyl-β-D-glucopyranosyluronate-(1→2)]-6-*O*-acetyl-4-*O*-benzyl-α-D-mannopyranosyl-(1→3)-[2,3,4-tri-*O*-benzyl-β-D-xylopyranosyl-(1→2)]-4,6-di-*O*-benzyl-α-D-mannopyranosyl-(1→3)-[2,3,4-tri-*O*-benzyl-β-D-xylopyranosyl-(1→2)]-6-*O*-acetyl-4-*O*-benzyl-α-D-mannopyranoside (12)



Compound **10** (50 mg, 12.54 µmole, 1 eq) was dissolved in *n*-BuOH (0.6 mL, 0.2M) and ethylene diamine (41 µL, 627 µmole, 100 eq) was added, the reaction was heated to 90°C in a sealed microwave vial under nitrogen, and left overnight (**R*f*** 0.65, toluene:acetone, 80:20 + drop of AcOH, v/v). The reaction was then concentrated *in vacuo* and co-evaporated with toluene (1 mL X3). The residue was then dissolved in THF:H_2O (2 mL, 70:30, v/v). Then $NaHCO_3$ (10 mg, 119 µmole, 9.5 eq) and Boc_2O (2.8 µL, 45.87 µmole, 3.65 eq) were added. Once complete the reaction was diluted with DCM and the organic layer was extracted and washed with MilliQ water, and then concentrated *in vacuo*. The resulting residue was purified by flash column chromatography (cyclohexane:ethyl acetate, gradient) to yield **12** (28 mg, 57%) as a colorless oil. **R*f*** (toluene:ethyl acetate, 80:20) = 0.8. **^{1}H NMR** (600 MHz, Chloroform-*d*) δ 7.47 – 7.32 (m, 12H H_{ar}), 7.39 – 6.76 (m, 108H, H_{ar}), 5.45 (s, 1H, H-1_{M5}), 5.33 (s, 1H, H-1_{M5}), 5.20 (dd, *J* = 11.9, 7.4 Hz, 3H, H-1_{M5}), 5.14 – 5.06 (m, 2H, H-1_{M5}), 5.07 – 5.01 (m, 2H), 5.01 – 4.94 (m, 1H), 4.91 – 4.80 (m, 7H), 4.80 – 4.73 (m, 4H), 4.73 – 4.67 (m, 2H), 4.68 – 4.57 (m, 10H), 4.57 – 4.44 (m, 10H), 4.44 – 4.23 (m, 17H), 4.23 – 4.01 (m, 19H), 4.00 – 3.86 (m, 9H), 3.87 – 3.78 (m, 3H), 3.79 – 3.57 (m, 8H), 3.57 – 3.48 (m, 3H), 3.48 – 3.40 (m, 4H), 3.42 – 3.26 (m, 8H), 3.21 – 3.10 (m, 1H), 3.05 (s, 1H), 2.78 (s, 1H), 2.61 (t, *J* = 10.7 Hz, 1H, H-$5_{axial\ X}$), 2.60 – 2.49 (m, 1H), 1.84 (s, 3H, $(C=O)CH_3$), 1.81 – 1.65 (m, 2H, $PhthNCH_2CH_2CH_2O$), 1.59 (s, 3H, $(C=O)CH_3$), 1.54 (s, 3H, $(C=O)CH_3$), 1.40 1.46 – 1.34 (m, 12H, 3 X CH_3 Boc). **^{13}C NMR** (151 MHz, Chloroform-d) δ 170.6, 170.4, 139.2, 138.9, 138.8, 138.4, 138.3, 138.3, 128.9, 128.8, 128.7, 128.6, 128.5, 128.5, 128.3, 128.3, 128.2, 128.2, 128.2, 128.2, 128.1, 128.1, 128.0, 127.8, 127.7, 127.7, 127.6, 127.6, 127.5, 127.5, 127.5, 127.4, 127.4, 127.3, 127.3, 127.2, 127.2, 126.7, 126.6, 126.0, 104.3, 102.5, 83.7, 83.4, 83.3, 83.2, 81.5,

81.4, 81.1, 80.1, 79.2, 78.2, 75.3, 75.2, 75.2, 75.0, 74.6, 74.4, 74.3, 74.1, 73.6, 73.5, 73.1, 72.9, 72.7, 72.1, 71.6, 70.4, 69.7, 68.9, 67.1, 66.5, 63.6, 63.5, 63.2, 63.1, 63.0, 50.2, 38.0, 29.9, 28.5, 26.9, 20.6. **HRMS (ESI)** [M + Na]$^+$ m/z Calc. for $C_{233}H_{254}N_4O_{53}Na$ 3978.7201 Found: 3978.7369.

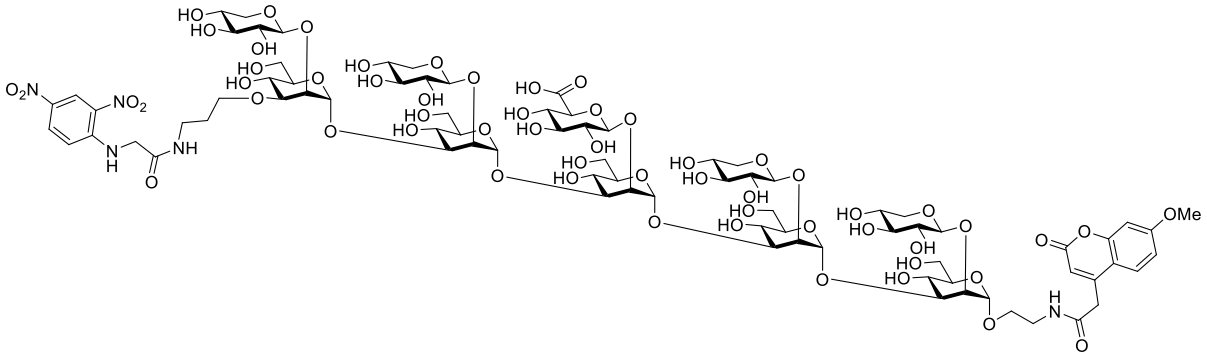
7-methoxycoumarin-4-acetic acid-2-aminoethyl-β-D-xylopyranosyl-(1→2))-6-*O*-acetyl-3-*O*-(aminopropyl-*N*-(2,4-dinitrophenyl)glycine)-α-D-mannopyranosyl-(1→3)-β-D-xylopyranosyl-(1→2)-α-D-mannopyranosyl-(1→3)-[β-D-glucopyranosyluronic acid-(1→2)]-6-*O*-acetyl-α-D-mannopyranosyl-(1→3)-[β-D-xylopyranosyl-(1→2)]-α-D-mannopyranosyl-(1→3)-[β-D-xylopyranosyl-(1→2)]-6-*O*-acetyl-α-D-mannopyranoside (16)



Compound **12** was dissolved in THF:^{t}BuOH:PBS (100 mM, pH 5.5) (2 mL,60:10:30, v/v/v) and "pre-treated 5% Pd/C catalyst" (370 mg, 0.5 eq per benzyl) was added. The reaction was put under an atmosphere of hydrogen (10 bar) and vigorously stirred for 2 days. The reaction was then filtered through a bed of Celite® and concentrated *in vacuo*. The residue was then dissolved in MilliQ water and purified *via* an automated size-exclusion chromatography system (P2 Biogel, flow rate 0.4 mL/min, eluent H_2O:^{t}BuOH, 99:1, v/v), then desired fractions were then collected and lyophilized to yield 8.1 mg (66%) of the desired deprotected intermediate. The intermediate was then dissolved in dry DMSO (0.5 mL) and 7-Methoxycoumarin-4-acetic Acid N-Succinimidyl Ester (7.2 mg, 5 eq) was added. The reaction was carried out in the dark and monitored *via* MALDITOF spectrometer (Super-DHB Matrix, Reflectron mode). Once complete, the unreacted NHS-ester was hydrolysed by the addition of water and stirred for 1 hour and concentrated. The resulting residue was purified *via* a Sep-Pak C18 cartridge (MeCN:H_2O, 95:5, v/v), then desired fractions were then collected and lyophilized to yield 7.7 mg of the fluorescently labelled intermediate. The sugar was dissolved in 3M HCl MeOH:H_2O (1 mL, 50:50, v/v) and stirred for ca. 1.5 hours, once complete removal of the *tert*-Butyloxycarbonyl was confirmed *via* MALDITOF (Super-DHB Matrix, Reflectron mode), the reaction was concentrated *in vacuo.* The resulting residue was dissolved in dry DMSO (500 µL) and NEt_3 was added adjust the pH to 8. Then NHS activated *N*-(2,4-Dinitrophenyl) glycine (7.3 mg, 5 eq) was added and the reaction was stirred for 4 hours. The reaction mixture was then purified *via* an automated size-exclusion chromatography system (P2 Biogel, flow rate 0.4 mL/min, eluent H_2O:^{t}BuOH, 99:1, v/v), then desired fractions were then collected and lyophilized to yield 5.4 mg (56%) of the oligosaccharide FRET probe **16**. **R*f*** (acetonitrile:water, 60:40) = 0.3. **^{1}H NMR** (400 MHz, Deuterium Oxide) δ 9.16 (d, *J* = 2.7 Hz, 1H, $H_{ar\ DNP}$), 8.42 – 8.28 (m, 1H, $H_{ar\ DNP}$), 7.84 – 7.62 (m, 1H, $H_{ar\ DNP}$), 7.09 – 7.02 (m, 2H, $H_{ar\ MCA}$), 6.96 (d, *J* = 9.6 Hz, 1H, $H_{ar\ MCA}$), 6.40 – 6.33 (m, 1H, $H_{ar\ MCA}$) 5.33 (s, 1H, H-1_{M5}), 5.25 (s, 1H, H-1_{M3}), 5.22 (s, 1H, H-1_{M2}), 5.16 (s, 1H, H-1_{M4}), 4.95 (s, 1H, H-1_{M1}), 4.54 (d, *J* = 8.1 Hz, 1H), 4.48 – 4.36 (m, 7H), 4.34 – 4.25 (m, 3H), 4.21

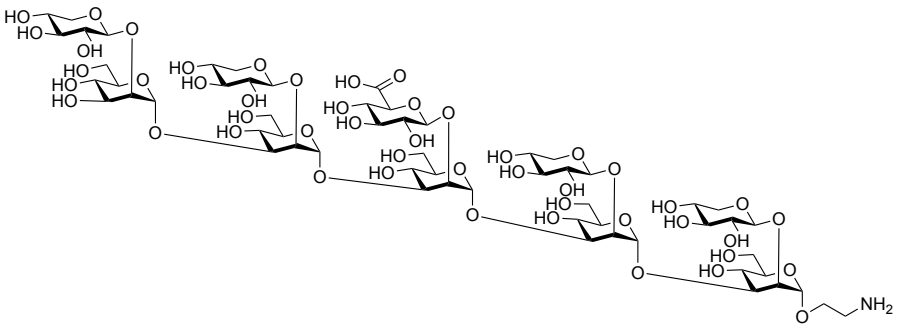
(s, 1H), 4.17 – 3.96 (m, 8H), 3.96 – 3.72 (m, 15H), 3.72 – 3.55 (m, 7H), 3.56 – 3.47 (m, 1H), 3.47 – 3.36 (m, 7H), 3.36 – 3.18 (m, 9H), 2.28 – 2.13 (m, 5H), 1.88 – 1.78 (m, 2H). **^{13}C NMR** (126 MHz, Deuterium Oxide) δ 126.5, 111.4, 122.0, 128.2, 103.2, 115.4, 114.4, 102.4, 102.2, 103.9, 99.9, 104.9, 76.0, 76.2, 105.6, 105.7, 48.1, 79.5, 80.8, 81.6, 80.5, 80.3, 75.2, 78.0, 75.5, 80.3, 77.3, 67.2, 68.2, 68.9, 58.0, 62.5, 75.7, 68.2, 57.6, 67.2, 62.4, 62.4, 67.9, 79.7, 71.7, 38.6, 69.3, 79.3, 64.7, 38.4, 72.2, 64.5, 71.3, 68.0, 69.2, 73.7, 75.2, 64.5, 41.1, 77.5, 74.5, 74.7, 68.2, 67.2, 30.6, 23.8. **HRMS (ESI)** $[M + Na]^+$ m/z Calc. for $C_{87}H_{123}N_5O_{60}Na$ 2220.6625 Found: 2220.6735.

7-methoxycoumarin-4-acetic acid-2-aminoethyl-β-D-xylopyranosyl-(1→2)-3-*O*-(aminopropyl-*N*-(2,4-dinitrophenyl)glycine)-α-D-mannopyranosyl-(1→3)-[β-D-xylopyranosyl-(1→2)]-α-D-mannopyranosyl-(1→3)-[β-D-glucopyranosyluronic acid-(1→2)]-α-D-mannopyranosyl-(1→3)-[β-D-xylopyranosyl-(1→2)]-α-D-mannopyranosyl-(1→3)-[β-D-xylopyranosyl-(1→2)]-α-D-mannopyranoside (17)



Compound **6** (3 mg) was dissolved in H_2O – 5% trimethylamine and left for 6 hours. The reaction was then concentrated and purified *via* an automated size-exclusion chromatography system (P2 Biogel, flow rate 0.4 mL/min), and lyophilized to yield 2 mg of **17**. **R*f*** (acetonitrile:water, 60:40) = 0.22. **^{1}H NMR** (500 MHz, Deuterium Oxide) δ 8.63 – 8.59 (m, 1H, $H_{ar\ DNP}$), 8.20 – 8.14 (m, 2H, $H_{ar\ DNP}$), 7.78 (d, *J* = 8.5, 1.6 Hz, 1H, $H_{ar\ DNP}$), 7.70 (d, *J* = 8.8 Hz, 1H, $H_{ar\ MCA}$), 7.10 – 7.03 (m, 2H, $H_{ar\ MCA}$), 6.35 (s, 1H, $H_{ar\ MCA}$), 5.34 (s, 1H, H-1_{M5}), 5.22 (s, 2H, H-$1_{M3,}$ H-1_{M2}), 5.12 (s, 1H, H-1_{M4}), 4.95 (s, 1H, H-1_{M1}), 4.51 – 4.46 (m, 9H), 4.44 – 4.33 (m, 5H), 4.31 – 4.24 (m, 4H), 4.23 – 4.19 (m, 2H), 4.14 – 4.05 (m, 2H), 4.01 (d, *J* = 16.3 Hz, 3H), 4.02 – 3.96 (m, 1H), 3.95 (s, 3H), 3.91 – 3.82 (m, 7H), 3.82 – 3.78 (m, 2H), 3.70 – 3.63 (m, 3H), 3.60 – 3.50 (m, 2H), 3.50 – 3.41 (m, 6H), 3.26 (s, 1H), 3.26 – 3.16 (m, 2H), 2.11 – 1.97 (m, 2H), 1.92 (s, 8H). **^{13}C NMR** (126 MHz, Deuterium Oxide) δ 109.1, 118.1, 119.7, 125.9, 101.0, 113.1, 112.1, 100.1, 100.0, 101.6, 97.6, 102.6, 73.7, 73.9, 103.3, 103.4, 77.2, 79.4, 78.5, 78.2, 78.0, 72.9, 75.7, 73.2, 78.0, 75.0, 65.9, 64.9, 66.6, 55.8, 60.2, 59.2, 73.4, 65.9, 55.3, 64.9, 60.1, 60.1, 59.1, 65.6, 77.4, 69.4, 36.3, 77.0, 67.1, 62.6, 62.4, 69.9, 68.1, 69.1, 62.2, 65.7, 66.9, 71.4, 72.9, 62.2, 38.8, 75.2, 72.2, 72.4, 64.9 28.3. **HRMS (ESI)** $[M + Na]^+$ m/z Calc. for $C_{81}H_{117}N_5O_{57}Na$ 2094.6308 Found: 2094.6204.

2-aminoethyl-β-D-xylopyranosyl-(1→2)-α-D-mannopyranosyl-(1→3)-[β-D-xylopyranosyl-(1→2)]-α-D-mannopyranosyl-(1→3)-[β-D-glucopyranosyluronic acid-(1→2)]-α-D-mannopyranosyl-(1→3)-[β-D-xylopyranosyl-(1→2)]-α-D-mannopyranosyl-(1→3)-[β-D-xylopyranosyl-(1→2)])]-α-D-mannopyranoside (18)



Acetylated decasaccharide(20) (4 mg) was dissolved in H_2O (0.5 mL), and sodium methoxide (0.5 mg, 9.40 µmole, 4 eq) was added to the reaction and it was left overnight. The reaction was then concentrated and purified *via* an automated size-exclusion chromatography system (P2 Biogel, flow rate 0.4 mL/min), and lyophilized to yield 3.7 mg of **18**. **^{1}H NMR** (600 MHz, Deuterium Oxide) δ 5.17 (s, 1H), 5.08 (s, 3H), 4.99 (s, 1H), 4.85 (s, 1H), 4.37 (d, *J* = 7.8 Hz, 1H), 4.32 (s, 1H), 4.30 (d, *J* = 7.8 Hz, 1H), 4.27 (s, 1H), 4.23 (s, 1H), 4.15 – 4.01 (m, 7H), 3.99 – 3.80 (m, 11H), 3.79 – 3.57 (m, 16H), 3.52 (s, 3H), 3.34 (s, 1H), 3.28 (s, 3H), 3.24 – 3.08 (m, 8H). **^{13}C NMR** (151 MHz, Deuterium Oxide) δ 100.2, 100.2, 101.6, 98.0, 102.4, 101.9, 103.3, 103.4, 78.4, 77.4, 78.3, 77.6, 77.9, 77.3, 72.9, 75.9, 77.8, 63.5, 63.4, 65.0, 65.1, 73.3, 69.4, 66.2, 60.2, 63.4, 77.1, 67.0, 69.0, 69.1, 75.4, 72.2, 72.5, 38.9, 65.1. **HRMS (ESI)** $[M + H]^+$ m/z Calc. for $C_{58}H_{98}NO_{48}Na$ 1576.5230 Found: 2094.6204.

Modeling Data

Highest scoring docking output

```
MODEL 22
REMARK VINA RESULT:     -10.2      2.598      3.173
REMARK  0 active torsions:
REMARK  status: ('A' for Active; 'I' for Inactive)
REMARK       I    between atoms: O1_2  and  C1_3
REMARK       I    between atoms: C2_4  and  O2_15
REMARK       I    between atoms: C3_5  and  O3_14
REMARK       I    between atoms: C4_6  and  O4_12
REMARK       I    between atoms: C5_7  and  C6_8
REMARK       I    between atoms: C6_8  and  O6_9
REMARK       I    between atoms: O3_14  and  C1_16
REMARK       I    between atoms: O2_15  and  C1_120
REMARK       I    between atoms: C2_17  and  O2_28
REMARK       I    between atoms: C3_18  and  O3_27
REMARK       I    between atoms: C4_19  and  O4_25
REMARK       I    between atoms: C5_20  and  C6_21
REMARK       I    between atoms: C6_21  and  O6_22
REMARK       I    between atoms: O3_27  and  C1_29
REMARK       I    between atoms: O2_28  and  C1_108
REMARK       I    between atoms: C2_30  and  O2_41
REMARK       I    between atoms: C3_31  and  O3_40
REMARK       I    between atoms: C4_32  and  O4_38
REMARK       I    between atoms: C5_33  and  C6_34
REMARK       I    between atoms: C6_34  and  O6_35
REMARK       I    between atoms: O3_40  and  C1_42
REMARK       I    between atoms: O2_41  and  C1_93
REMARK       I    between atoms: C2_43  and  O2_54
REMARK       I    between atoms: C3_44  and  O3_53
REMARK       I    between atoms: C4_45  and  O4_51
REMARK       I    between atoms: C5_46  and  C6_47
REMARK       I    between atoms: C6_47  and  O6_48
REMARK       I    between atoms: O3_53  and  C1_55
REMARK       I    between atoms: O2_54  and  C1_81
REMARK       I    between atoms: C2_56  and  O2_68
REMARK       I    between atoms: C3_57  and  O3_66
REMARK       I    between atoms: C4_58  and  O4_64
REMARK       I    between atoms: C5_59  and  C6_60
REMARK       I    between atoms: C6_60  and  O6_61
REMARK       I    between atoms: O2_68  and  C1_69
REMARK       I    between atoms: C4_72  and  O4_73
REMARK       I    between atoms: C3_75  and  O3_76
REMARK       I    between atoms: C2_78  and  O2_79
REMARK       I    between atoms: C4_84  and  O4_85
REMARK       I    between atoms: C3_87  and  O3_88
REMARK       I    between atoms: C2_90  and  O2_91
REMARK       I    between atoms: C5_95  and  C6_96
REMARK       I    between atoms: C4_99  and  O4_100
REMARK       I    between atoms: C3_102  and  O3_103
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REMARK       I    between atoms: C2_105  and  O2_106
REMARK       I    between atoms: C4_111  and  O4_112
REMARK       I    between atoms: C3_114  and  O3_115
REMARK       I    between atoms: C2_117  and  O2_118
REMARK       I    between atoms: C4_123  and  O4_124
REMARK       I    between atoms: C3_126  and  O3_127
REMARK       I    between atoms: C2_129  and  O2_130
ROOT
HETATM    1  HO1 ROH     1      -7.397  15.200   8.251  1.00
0.00     0.212 HD
HETATM    2  O1  ROH     1      -6.853  14.411   8.189  1.00
0.00    -0.369 OA
HETATM    3  C1  ZMA     2      -7.311  13.438   9.139  1.00
0.00     0.289 C
HETATM    4  C2  ZMA     2      -6.422  12.187   9.027  1.00
0.00     0.211 C
HETATM    5  C3  ZMA     2      -6.768  11.323   7.801  1.00
0.00     0.190 C
HETATM    6  C4  ZMA     2      -8.268  10.999   7.731  1.00
0.00     0.181 C
HETATM    7  C5  ZMA     2      -9.108  12.290   7.736  1.00
0.00     0.179 C
HETATM    8  C6  ZMA     2     -10.624  12.054   7.784  1.00
0.00     0.198 C
HETATM    9  O6  ZMA     2     -11.290  13.309   7.590  1.00
0.00    -0.398 OA
HETATM   10  H6O ZMA     2     -11.054  13.665   6.730  1.00
0.00     0.209 HD
HETATM   11  O5  ZMA     2      -8.736  13.089   8.932  1.00
0.00    -0.349 OA
HETATM   12  O4  ZMA     2      -8.516  10.298   6.500  1.00
0.00    -0.390 OA
HETATM   13  H4O ZMA     2      -8.020   9.477   6.496  1.00
0.00     0.210 HD
HETATM   14  O3  ZMA     2      -5.988  10.075   7.878  1.00
0.00    -0.348 OA
HETATM   15  O2  ZMA     2      -6.619  11.391  10.261  1.00
0.00    -0.346 OA
HETATM   16  C1  ZMA     3      -5.242   9.667   6.676  1.00
0.00     0.292 C
HETATM   17  C2  ZMA     3      -4.660   8.273   6.962  1.00
0.00     0.211 C
HETATM   18  C3  ZMA     3      -3.487   8.359   7.950  1.00
0.00     0.190 C
HETATM   19  C4  ZMA     3      -2.395   9.315   7.446  1.00
0.00     0.181 C
HETATM   20  C5  ZMA     3      -2.996  10.723   7.268  1.00
0.00     0.179 C
HETATM   21  C6  ZMA     3      -2.029  11.776   6.716  1.00
0.00     0.198 C
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HETATM   22  O6  ZMA     3      -2.666  13.058   6.788  1.00
0.00    -0.398 OA
HETATM   23  H6O ZMA     3      -2.888  13.254   7.701  1.00
0.00     0.209 HD
HETATM   24  O5  ZMA     3      -4.152  10.620   6.341  1.00
0.00    -0.348 OA
HETATM   25  O4  ZMA     3      -1.345   9.373   8.424  1.00
0.00    -0.390 OA
HETATM   26  H4O ZMA     3      -0.985   8.493   8.556  1.00
0.00     0.210 HD
HETATM   27  O3  ZMA     3      -2.951   7.011   8.179  1.00
0.00    -0.348 OA
HETATM   28  O2  ZMA     3      -4.187   7.680   5.692  1.00
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HETATM   29  C1  ZMA     4      -2.904   6.567   9.585  1.00
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HETATM   30  C2  ZMA     4      -2.237   5.187   9.589  1.00
0.00     0.211 C
HETATM   31  C3  ZMA     4      -3.186   4.132   9.010  1.00
0.00     0.190 C
HETATM   32  C4  ZMA     4      -4.509   4.074   9.789  1.00
0.00     0.181 C
HETATM   33  C5  ZMA     4      -5.204   5.446   9.707  1.00
0.00     0.179 C
HETATM   34  C6  ZMA     4      -6.503   5.562  10.512  1.00
0.00     0.198 C
HETATM   35  O6  ZMA     4      -7.128   6.811  10.187  1.00
0.00    -0.398 OA
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0.00     0.209 HD
HETATM   37  O5  ZMA     4      -4.257   6.482  10.201  1.00
0.00    -0.348 OA
HETATM   38  O4  ZMA     4      -5.356   3.081   9.190  1.00
0.00    -0.390 OA
HETATM   39  H4O ZMA     4      -4.916   2.228   9.220  1.00
0.00     0.210 HD
HETATM   40  O3  ZMA     4      -2.498   2.836   9.015  1.00
0.00    -0.348 OA
HETATM   41  O2  ZMA     4      -1.910   4.799  10.975  1.00
0.00    -0.346 OA
HETATM   42  C1  ZMA     5      -2.395   2.180   7.701  1.00
0.00     0.292 C
HETATM   43  C2  ZMA     5      -1.740   0.817   7.943  1.00
0.00     0.211 C
HETATM   44  C3  ZMA     5      -0.237   0.975   8.213  1.00
0.00     0.190 C
HETATM   45  C4  ZMA     5       0.444   1.665   7.019  1.00
0.00     0.181 C
HETATM   46  C5  ZMA     5      -0.127   3.094   6.921  1.00
0.00     0.179 C
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HETATM   47  C6  ZMA     5       0.433   3.964   5.790  1.00
0.00     0.198 C
HETATM   48  O6  ZMA     5      -0.082   5.295   5.944  1.00
0.00    -0.398 OA
HETATM   49  H6O ZMA     5       0.200   5.650   6.792  1.00
0.00     0.209 HD
HETATM   50  O5  ZMA     5      -1.598   2.976   6.725  1.00
0.00    -0.348 OA
HETATM   51  O4  ZMA     5       1.860   1.709   7.238  1.00
0.00    -0.390 OA
HETATM   52  H4O ZMA     5       2.047   2.212   8.035  1.00
0.00     0.210 HD
HETATM   53  O3  ZMA     5       0.330  -0.352   8.500  1.00
0.00    -0.348 OA
HETATM   54  O2  ZMA     5      -1.924  -0.012   6.732  1.00
0.00    -0.346 OA
HETATM   55  C1  2MA     6       0.926  -0.496   9.841  1.00
0.00     0.292 C
HETATM   56  C2  2MA     6       1.515  -1.909   9.951  1.00
0.00     0.211 C
HETATM   57  C3  2MA     6       0.448  -2.994  10.190  1.00
0.00     0.183 C
HETATM   58  C4  2MA     6      -0.494  -2.639  11.354  1.00
0.00     0.180 C
HETATM   59  C5  2MA     6      -1.136  -1.256  11.141  1.00
0.00     0.179 C
HETATM   60  C6  2MA     6      -2.006  -0.766  12.305  1.00
0.00     0.198 C
HETATM   61  O6  2MA     6      -2.682   0.433  11.899  1.00
0.00    -0.398 OA
HETATM   62  H6O 2MA     6      -3.271   0.235  11.166  1.00
0.00     0.209 HD
HETATM   63  O5  2MA     6      -0.053  -0.265  10.934  1.00
0.00    -0.348 OA
HETATM   64  O4  2MA     6      -1.548  -3.616  11.398  1.00
0.00    -0.390 OA
HETATM   65  H4O 2MA     6      -1.166  -4.492  11.491  1.00
0.00     0.210 HD
HETATM   66  O3  2MA     6       1.106  -4.243  10.474  1.00
0.00    -0.390 OA
HETATM   67  H3O 2MA     6       1.715  -4.451   9.761  1.00
0.00     0.210 HD
HETATM   68  O2  2MA     6       2.479  -1.913  11.071  1.00
0.00    -0.346 OA
HETATM   69  C1  0XB     7       3.886  -2.142  10.704  1.00
0.00     0.291 C
HETATM   70  O5  0XB     7       4.047  -3.421   9.978  1.00
0.00    -0.356 OA
HETATM   71  C5  0XB     7       5.424  -3.762   9.586  1.00
0.00     0.205 C
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HETATM   72  C4  0XB     7       6.258  -3.906  10.862  1.00
0.00     0.173 C
HETATM   73  O4  0XB     7       7.621  -4.099  10.455  1.00
0.00    -0.391 OA
HETATM   74  H4O 0XB     7       8.170  -4.232  11.232  1.00
0.00     0.210 HD
HETATM   75  C3  0XB     7       6.173  -2.650  11.750  1.00
0.00     0.182 C
HETATM   76  O3  0XB     7       6.770  -2.965  13.021  1.00
0.00    -0.390 OA
HETATM   77  H3O 0XB     7       6.723  -2.198  13.596  1.00
0.00     0.210 HD
HETATM   78  C2  0XB     7       4.715  -2.216  12.001  1.00
0.00     0.204 C
HETATM   79  O2  0XB     7       4.727  -0.904  12.588  1.00
0.00    -0.388 OA
HETATM   80  H2O 0XB     7       3.828  -0.638  12.796  1.00
0.00     0.210 HD
HETATM   81  C1  0XB     8      -2.681  -1.262   6.882  1.00
0.00     0.291 C
HETATM   82  O5  0XB     8      -2.078  -2.137   7.908  1.00
0.00    -0.356 OA
HETATM   83  C5  0XB     8      -2.735  -3.438   8.105  1.00
0.00     0.205 C
HETATM   84  C4  0XB     8      -2.625  -4.224   6.797  1.00
0.00     0.173 C
HETATM   85  O4  0XB     8      -3.373  -5.438   6.971  1.00
0.00    -0.391 OA
HETATM   86  H4O 0XB     8      -3.268  -5.991   6.193  1.00
0.00     0.210 HD
HETATM   87  C3  0XB     8      -3.207  -3.436   5.611  1.00
0.00     0.182 C
HETATM   88  O3  0XB     8      -2.851  -4.129   4.402  1.00
0.00    -0.390 OA
HETATM   89  H3O 0XB     8      -3.206  -3.656   3.645  1.00
0.00     0.210 HD
HETATM   90  C2  0XB     8      -2.634  -2.006   5.533  1.00
0.00     0.204 C
HETATM   91  O2  0XB     8      -3.418  -1.254   4.591  1.00
0.00    -0.388 OA
HETATM   92  H2O 0XB     8      -3.039  -0.378   4.488  1.00
0.00     0.210 HD
HETATM   93  C1  0ZB     9      -0.503   4.842  11.404  1.00
0.00     0.293 C
HETATM   94  O5  0ZB     9       0.369   4.068  10.493  1.00
0.00    -0.340 OA
HETATM   95  C5  0ZB     9       1.779   3.927  10.909  1.00
0.00     0.256 C
HETATM   96  C6  0ZB     9       2.657   3.410   9.734  1.00
0.00     0.206 C
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HETATM   97  O6B 0ZB     9       3.336   2.368   9.896  1.00
0.00    -0.646 OA
HETATM   98  O6A 0ZB     9       2.625   4.105   8.697  1.00
0.00    -0.646 OA
HETATM   99  C4  0ZB     9       1.787   3.107  12.214  1.00
0.00     0.189 C
HETATM  100  O4  0ZB     9       3.143   2.995  12.683  1.00
0.00    -0.390 OA
HETATM  101  H4O 0ZB     9       3.670   2.543  12.019  1.00
0.00     0.210 HD
HETATM  102  C3  0ZB     9       0.963   3.831  13.294  1.00
0.00     0.183 C
HETATM  103  O3  0ZB     9       0.829   2.949  14.423  1.00
0.00    -0.390 OA
HETATM  104  H3O 0ZB     9       1.701   2.713  14.746  1.00
0.00     0.210 HD
HETATM  105  C2  0ZB     9      -0.453   4.201  12.806  1.00
0.00     0.204 C
HETATM  106  O2  0ZB     9      -1.005   5.156  13.730  1.00
0.00    -0.388 OA
HETATM  107  H2O 0ZB     9      -1.886   5.406  13.443  1.00
0.00     0.210 HD
HETATM  108  C1  0XB    10      -4.875   6.459   5.233  1.00
0.00     0.291 C
HETATM  109  O5  0XB    10      -4.767   5.377   6.233  1.00
0.00    -0.356 OA
HETATM  110  C5  0XB    10      -5.351   4.078   5.862  1.00
0.00     0.205 C
HETATM  111  C4  0XB    10      -4.618   3.560   4.625  1.00
0.00     0.173 C
HETATM  112  O4  0XB    10      -5.279   2.351   4.220  1.00
0.00    -0.391 OA
HETATM  113  H4O 0XB    10      -4.811   1.964   3.476  1.00
0.00     0.210 HD
HETATM  114  C3  0XB    10      -4.673   4.583   3.477  1.00
0.00     0.182 C
HETATM  115  O3  0XB    10      -3.794   4.125   2.434  1.00
0.00    -0.390 OA
HETATM  116  H3O 0XB    10      -3.846   4.724   1.687  1.00
0.00     0.210 HD
HETATM  117  C2  0XB    10      -4.199   5.980   3.932  1.00
0.00     0.204 C
HETATM  118  O2  0XB    10      -4.530   6.923   2.900  1.00
0.00    -0.388 OA
HETATM  119  H2O 0XB    10      -4.214   7.794   3.150  1.00
0.00     0.210 HD
HETATM  120  C1  0XB    11      -5.448  11.249  11.148  1.00
0.00     0.291 C
HETATM  121  O5  0XB    11      -4.368  10.497  10.480  1.00
0.00    -0.356 OA
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HETATM  122  C5  0XB    11      -3.168  10.206  11.283  1.00
0.00     0.205 C
HETATM  123  C4  0XB    11      -3.587   9.355  12.482  1.00
0.00     0.173 C
HETATM  124  O4  0XB    11      -2.422   9.181  13.302  1.00
0.00    -0.391 OA
HETATM  125  H4O 0XB    11      -2.631   8.603  14.038  1.00
0.00     0.210 HD
HETATM  126  C3  0XB    11      -4.688  10.053  13.298  1.00
0.00     0.182 C
HETATM  127  O3  0XB    11      -5.168   9.121  14.284  1.00
0.00    -0.390 OA
HETATM  128  H3O 0XB    11      -5.808   9.557  14.850  1.00
0.00     0.210 HD
HETATM  129  C2  0XB    11      -5.878  10.473  12.408  1.00
0.00     0.204 C
HETATM  130  O2  0XB    11      -6.728  11.340  13.179  1.00
0.00    -0.388 OA
HETATM  131  H2O 0XB    11      -7.485  11.598  12.648  1.00
0.00     0.210 HD
ENDROOT
TORSDOF 51
ENDMDL
```

Ligand

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REMARK  0 active torsions:
REMARK  status: ('A' for Active; 'I' for Inactive)
REMARK       I    between atoms: O1_2  and  C1_3
```

```
REMARK       I    between atoms: C2_4  and  O2_15
REMARK       I    between atoms: C3_5  and  O3_14
REMARK       I    between atoms: C4_6  and  O4_12
REMARK       I    between atoms: C5_7  and  C6_8
REMARK       I    between atoms: C6_8  and  O6_9
REMARK       I    between atoms: O3_14  and  C1_16
REMARK       I    between atoms: O2_15  and  C1_120
REMARK       I    between atoms: C2_17  and  O2_28
REMARK       I    between atoms: C3_18  and  O3_27
REMARK       I    between atoms: C4_19  and  O4_25
REMARK       I    between atoms: C5_20  and  C6_21
REMARK       I    between atoms: C6_21  and  O6_22
REMARK       I    between atoms: O3_27  and  C1_29
REMARK       I    between atoms: O2_28  and  C1_108
REMARK       I    between atoms: C2_30  and  O2_41
REMARK       I    between atoms: C3_31  and  O3_40
REMARK       I    between atoms: C4_32  and  O4_38
REMARK       I    between atoms: C5_33  and  C6_34
REMARK       I    between atoms: C6_34  and  O6_35
REMARK       I    between atoms: O3_40  and  C1_42
REMARK       I    between atoms: O2_41  and  C1_93
REMARK       I    between atoms: C2_43  and  O2_54
REMARK       I    between atoms: C3_44  and  O3_53
REMARK       I    between atoms: C4_45  and  O4_51
REMARK       I    between atoms: C5_46  and  C6_47
REMARK       I    between atoms: C6_47  and  O6_48
REMARK       I    between atoms: O3_53  and  C1_55
REMARK       I    between atoms: O2_54  and  C1_81
REMARK       I    between atoms: C2_56  and  O2_68
REMARK       I    between atoms: C3_57  and  O3_66
REMARK       I    between atoms: C4_58  and  O4_64
REMARK       I    between atoms: C5_59  and  C6_60
REMARK       I    between atoms: C6_60  and  O6_61
REMARK       I    between atoms: O2_68  and  C1_69
REMARK       I    between atoms: C4_72  and  O4_73
REMARK       I    between atoms: C3_75  and  O3_76
REMARK       I    between atoms: C2_78  and  O2_79
REMARK       I    between atoms: C4_84  and  O4_85
REMARK       I    between atoms: C3_87  and  O3_88
REMARK       I    between atoms: C2_90  and  O2_91
REMARK       I    between atoms: C5_95  and  C6_96
REMARK       I    between atoms: C4_99  and  O4_100
REMARK       I    between atoms: C3_102  and  O3_103
REMARK       I    between atoms: C2_105  and  O2_106
REMARK       I    between atoms: C4_111  and  O4_112
REMARK       I    between atoms: C3_114  and  O3_115
REMARK       I    between atoms: C2_117  and  O2_118
REMARK       I    between atoms: C4_123  and  O4_124
REMARK       I    between atoms: C3_126  and  O3_127
REMARK       I    between atoms: C2_129  and  O2_130
ROOT
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HETATM    1  HO1 ROH     1       5.637   7.938  -1.582  1.00
0.00     0.212 HD
HETATM    2  O1  ROH     1       5.997   8.616  -2.158  1.00
0.00    -0.369 OA
HETATM    3  C1  ZMA     2       5.340   8.559  -3.433  1.00
0.00     0.289 C
HETATM    4  C2  ZMA     2       5.936   9.648  -4.342  1.00
0.00     0.211 C
HETATM    5  C3  ZMA     2       5.409  11.054  -4.004  1.00
0.00     0.190 C
HETATM    6  C4  ZMA     2       3.874  11.095  -3.948  1.00
0.00     0.181 C
HETATM    7  C5  ZMA     2       3.335  10.060  -2.943  1.00
0.00     0.179 C
HETATM    8  C6  ZMA     2       1.804   9.954  -2.910  1.00
0.00     0.198 C
HETATM    9  O6  ZMA     2       1.426   9.111  -1.814  1.00
0.00    -0.398 OA
HETATM   10  H6O ZMA     2       1.732   9.501  -0.991  1.00
0.00     0.209 HD
HETATM   11  O5  ZMA     2       3.873   8.724  -3.308  1.00
0.00    -0.349 OA
HETATM   12  O4  ZMA     2       3.478  12.406  -3.510  1.00
0.00    -0.390 OA
HETATM   13  H4O ZMA     2       3.784  13.059  -4.143  1.00
0.00     0.210 HD
HETATM   14  O3  ZMA     2       5.899  11.992  -5.029  1.00
0.00    -0.348 OA
HETATM   15  O2  ZMA     2       5.573   9.309  -5.738  1.00
0.00    -0.346 OA
HETATM   16  C1  ZMA     3       6.537  13.234  -4.561  1.00
0.00     0.292 C
HETATM   17  C2  ZMA     3       6.803  14.097  -5.805  1.00
0.00     0.211 C
HETATM   18  C3  ZMA     3       7.968  13.529  -6.629  1.00
0.00     0.190 C
HETATM   19  C4  ZMA     3       9.241  13.385  -5.781  1.00
0.00     0.181 C
HETATM   20  C5  ZMA     3       8.960  12.434  -4.602  1.00
0.00     0.179 C
HETATM   21  C6  ZMA     3      10.132  12.237  -3.634  1.00
0.00     0.198 C
HETATM   22  O6  ZMA     3       9.788  11.193  -2.713  1.00
0.00    -0.398 OA
HETATM   23  H6O ZMA     3       9.615  10.383  -3.199  1.00
0.00     0.209 HD
HETATM   24  O5  ZMA     3       7.808  12.974  -3.835  1.00
0.00    -0.348 OA
HETATM   25  O4  ZMA     3      10.280  12.825  -6.599  1.00
0.00    -0.390 OA
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HETATM   26  H4O ZMA     3      10.441  13.402  -7.349  1.00
0.00     0.210 HD
HETATM   27  O3  ZMA     3       8.199  14.392  -7.795  1.00
0.00    -0.348 OA
HETATM   28  O2  ZMA     3       7.134  15.473  -5.374  1.00
0.00    -0.346 OA
HETATM   29  C1  ZMA     4       8.151  13.726  -9.110  1.00
0.00     0.292 C
HETATM   30  C2  ZMA     4       8.502  14.790 -10.156  1.00
0.00     0.211 C
HETATM   31  C3  ZMA     4       7.346  15.782 -10.320  1.00
0.00     0.190 C
HETATM   32  C4  ZMA     4       6.043  15.071 -10.717  1.00
0.00     0.181 C
HETATM   33  C5  ZMA     4       5.662  14.063  -9.616  1.00
0.00     0.179 C
HETATM   34  C6  ZMA     4       4.421  13.216  -9.916  1.00
0.00     0.198 C
HETATM   35  O6  ZMA     4       4.081  12.475  -8.736  1.00
0.00    -0.398 OA
HETATM   36  H6O ZMA     4       3.871  13.086  -8.026  1.00
0.00     0.209 HD
HETATM   37  O5  ZMA     4       6.812  13.143  -9.404  1.00
0.00    -0.348 OA
HETATM   38  O4  ZMA     4       5.000  16.051 -10.839  1.00
0.00    -0.390 OA
HETATM   39  H4O ZMA     4       5.244  16.693 -11.510  1.00
0.00     0.210 HD
HETATM   40  O3  ZMA     4       7.736  16.790 -11.312  1.00
0.00    -0.348 OA
HETATM   41  O2  ZMA     4       8.739  14.141 -11.461  1.00
0.00    -0.346 OA
HETATM   42  C1  ZMA     5       7.692  18.183 -10.837  1.00
0.00     0.292 C
HETATM   43  C2  ZMA     5       8.036  19.066 -12.040  1.00
0.00     0.211 C
HETATM   44  C3  ZMA     5       9.538  19.002 -12.352  1.00
0.00     0.190 C
HETATM   45  C4  ZMA     5      10.351  19.462 -11.130  1.00
0.00     0.181 C
HETATM   46  C5  ZMA     5      10.103  18.445  -9.998  1.00
0.00     0.179 C
HETATM   47  C6  ZMA     5      10.837  18.717  -8.680  1.00
0.00     0.198 C
HETATM   48  O6  ZMA     5      10.624  17.600  -7.803  1.00
0.00    -0.398 OA
HETATM   49  H6O ZMA     5      10.977  16.804  -8.209  1.00
0.00     0.209 HD
HETATM   50  O5  ZMA     5       8.642  18.435  -9.716  1.00
0.00    -0.348 OA
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HETATM   51  O4  ZMA     5      11.743  19.499 -11.470  1.00
0.00    -0.390 OA
HETATM   52  H4O ZMA     5      12.036  18.620 -11.725  1.00
0.00     0.210 HD
HETATM   53  O3  ZMA     5       9.804  19.815 -13.549  1.00
0.00    -0.348 OA
HETATM   54  O2  ZMA     5       7.675  20.462 -11.713  1.00
0.00    -0.346 OA
HETATM   55  C1  2MA     6      10.356  19.070 -14.696  1.00
0.00     0.292 C
HETATM   56  C2  2MA     6      10.624  20.070 -15.828  1.00
0.00     0.211 C
HETATM   57  C3  2MA     6       9.348  20.493 -16.579  1.00
0.00     0.183 C
HETATM   58  C4  2MA     6       8.507  19.284 -17.026  1.00
0.00     0.180 C
HETATM   59  C5  2MA     6       8.180  18.369 -15.832  1.00
0.00     0.179 C
HETATM   60  C6  2MA     6       7.439  17.077 -16.198  1.00
0.00     0.198 C
HETATM   61  O6  2MA     6       7.039  16.419 -14.987  1.00
0.00    -0.398 OA
HETATM   62  H6O 2MA     6       6.420  16.978 -14.510  1.00
0.00     0.209 HD
HETATM   63  O5  2MA     6       9.452  17.991 -15.171  1.00
0.00    -0.348 OA
HETATM   64  O4  2MA     6       7.266  19.771 -17.566  1.00
0.00    -0.390 OA
HETATM   65  H4O 2MA     6       7.449  20.376 -18.289  1.00
0.00     0.210 HD
HETATM   66  O3  2MA     6       9.719  21.268 -17.734  1.00
0.00    -0.390 OA
HETATM   67  H3O 2MA     6      10.267  22.007 -17.459  1.00
0.00     0.210 HD
HETATM   68  O2  2MA     6      11.566  19.440 -16.777  1.00
0.00    -0.346 OA
HETATM   69  C1  0XB     7      12.889  20.076 -16.883  1.00
0.00     0.291 C
HETATM   70  O5  0XB     7      12.768  21.501 -17.262  1.00
0.00    -0.356 OA
HETATM   71  C5  0XB     7      14.037  22.228 -17.423  1.00
0.00     0.205 C
HETATM   72  C4  0XB     7      14.822  21.566 -18.559  1.00
0.00     0.173 C
HETATM   73  O4  0XB     7      16.110  22.198 -18.606  1.00
0.00    -0.391 OA
HETATM   74  H4O 0XB     7      16.618  21.833 -19.334  1.00
0.00     0.210 HD
HETATM   75  C3  0XB     7      15.013  20.056 -18.323  1.00
0.00     0.182 C
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HETATM   76  O3  0XB     7      15.529  19.480 -19.536  1.00
0.00    -0.390 OA
HETATM   77  H3O 0XB     7      15.650  18.535 -19.416  1.00
0.00     0.210 HD
HETATM   78  C2  0XB     7      13.684  19.350 -17.985  1.00
0.00     0.204 C
HETATM   79  O2  0XB     7      13.982  18.026 -17.511  1.00
0.00    -0.388 OA
HETATM   80  H2O 0XB     7      13.162  17.555 -17.342  1.00
0.00     0.210 HD
HETATM   81  C1  0XB     8       6.665  21.111 -12.560  1.00
0.00     0.291 C
HETATM   82  O5  0XB     8       7.065  21.098 -13.982  1.00
0.00    -0.356 OA
HETATM   83  C5  0XB     8       6.141  21.765 -14.912  1.00
0.00     0.205 C
HETATM   84  C4  0XB     8       6.076  23.245 -14.531  1.00
0.00     0.173 C
HETATM   85  O4  0XB     8       5.083  23.853 -15.371  1.00
0.00    -0.391 OA
HETATM   86  H4O 0XB     8       5.064  24.799 -15.209  1.00
0.00     0.210 HD
HETATM   87  C3  0XB     8       5.677  23.434 -13.057  1.00
0.00     0.182 C
HETATM   88  O3  0XB     8       5.873  24.819 -12.720  1.00
0.00    -0.390 OA
HETATM   89  H3O 0XB     8       5.628  24.963 -11.803  1.00
0.00     0.210 HD
HETATM   90  C2  0XB     8       6.547  22.581 -12.111  1.00
0.00     0.204 C
HETATM   91  O2  0XB     8       5.944  22.593 -10.806  1.00
0.00    -0.388 OA
HETATM   92  H2O 0XB     8       6.504  22.113 -10.191  1.00
0.00     0.210 HD
HETATM   93  C1  0ZB     9      10.122  14.031 -11.950  1.00
0.00     0.293 C
HETATM   94  O5  0ZB     9      10.804  15.344 -11.960  1.00
0.00    -0.340 OA
HETATM   95  C5  0ZB     9      12.150  15.372 -12.566  1.00
0.00     0.256 C
HETATM   96  C6  0ZB     9      12.894  16.691 -12.212  1.00
0.00     0.206 C
HETATM   97  O6B 0ZB     9      13.330  17.411 -13.142  1.00
0.00    -0.646 OA
HETATM   98  O6A 0ZB     9      13.012  16.927 -10.991  1.00
0.00    -0.646 OA
HETATM   99  C4  0ZB     9      11.982  15.032 -14.060  1.00
0.00     0.189 C
HETATM  100  O4  0ZB     9      13.282  14.994 -14.676  1.00
0.00    -0.390 OA
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HETATM  101  H4O 0ZB     9      13.697  15.856 -14.590  1.00
0.00     0.210 HD
HETATM  102  C3  0ZB     9      11.336  13.643 -14.213  1.00
0.00     0.183 C
HETATM  103  O3  0ZB     9      11.016  13.447 -15.602  1.00
0.00    -0.390 OA
HETATM  104  H3O 0ZB     9      11.816  13.522 -16.126  1.00
0.00     0.210 HD
HETATM  105  C2  0ZB     9      10.034  13.505 -13.398  1.00
0.00     0.204 C
HETATM  106  O2  0ZB     9       9.704  12.107 -13.323  1.00
0.00    -0.388 OA
HETATM  107  H2O 0ZB     9       8.898  11.996 -12.814  1.00
0.00     0.210 HD
HETATM  108  C1  0XB    10       6.197  16.538  -5.777  1.00
0.00     0.291 C
HETATM  109  O5  0XB    10       6.069  16.610  -7.246  1.00
0.00    -0.356 OA
HETATM  110  C5  0XB    10       5.216  17.684  -7.780  1.00
0.00     0.205 C
HETATM  111  C4  0XB    10       5.818  19.025  -7.361  1.00
0.00     0.173 C
HETATM  112  O4  0XB    10       4.909  20.048  -7.797  1.00
0.00    -0.391 OA
HETATM  113  H4O 0XB    10       5.281  20.911  -7.601  1.00
0.00     0.210 HD
HETATM  114  C3  0XB    10       5.984  19.106  -5.834  1.00
0.00     0.182 C
HETATM  115  O3  0XB    10       6.742  20.292  -5.534  1.00
0.00    -0.390 OA
HETATM  116  H3O 0XB    10       6.820  20.389  -4.583  1.00
0.00     0.210 HD
HETATM  117  C2  0XB    10       6.751  17.888  -5.276  1.00
0.00     0.204 C
HETATM  118  O2  0XB    10       6.631  17.900  -3.844  1.00
0.00    -0.388 OA
HETATM  119  H2O 0XB    10       7.129  17.167  -3.476  1.00
0.00     0.210 HD
HETATM  120  C1  0XB    11       6.686   8.971  -6.645  1.00
0.00     0.291 C
HETATM  121  O5  0XB    11       7.576  10.131  -6.844  1.00
0.00    -0.356 OA
HETATM  122  C5  0XB    11       8.685   9.959  -7.798  1.00
0.00     0.205 C
HETATM  123  C4  0XB    11       8.093   9.649  -9.173  1.00
0.00     0.173 C
HETATM  124  O4  0XB    11       9.194   9.378 -10.053  1.00
0.00    -0.391 OA
HETATM  125  H4O 0XB    11       8.865   9.234 -10.943  1.00
0.00     0.210 HD
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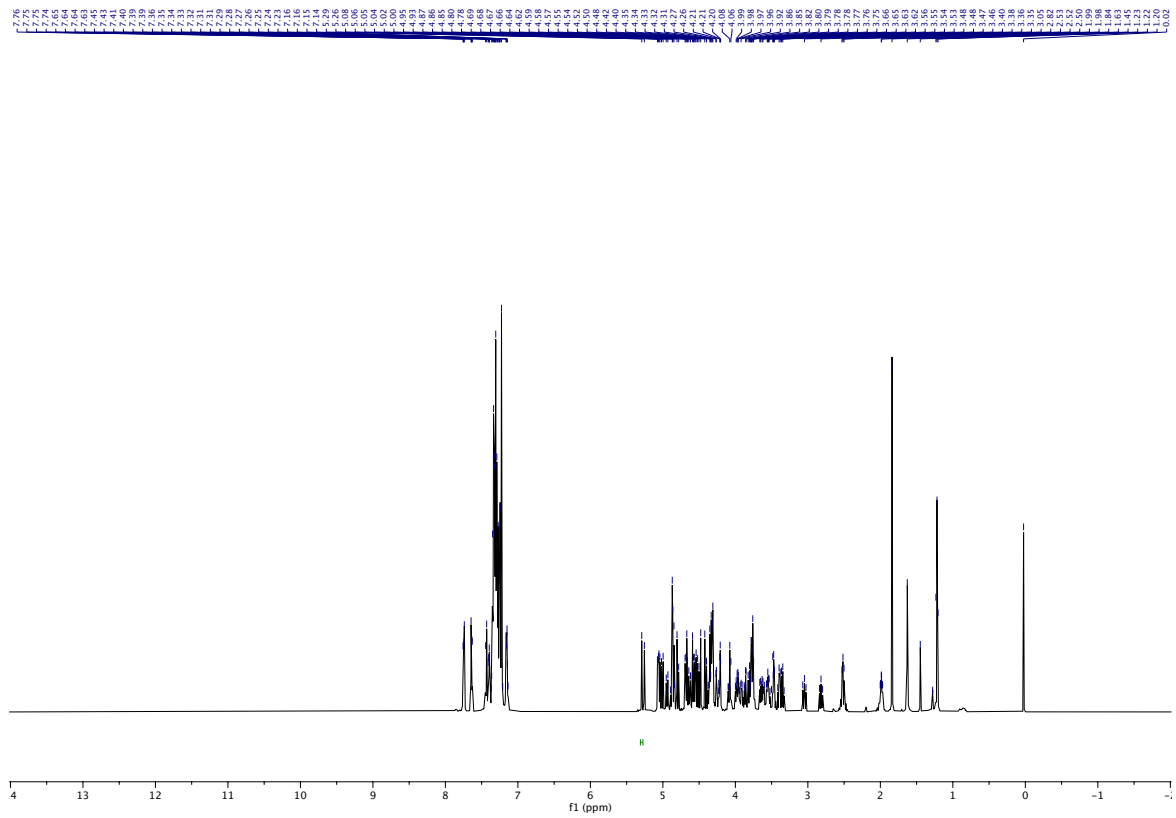
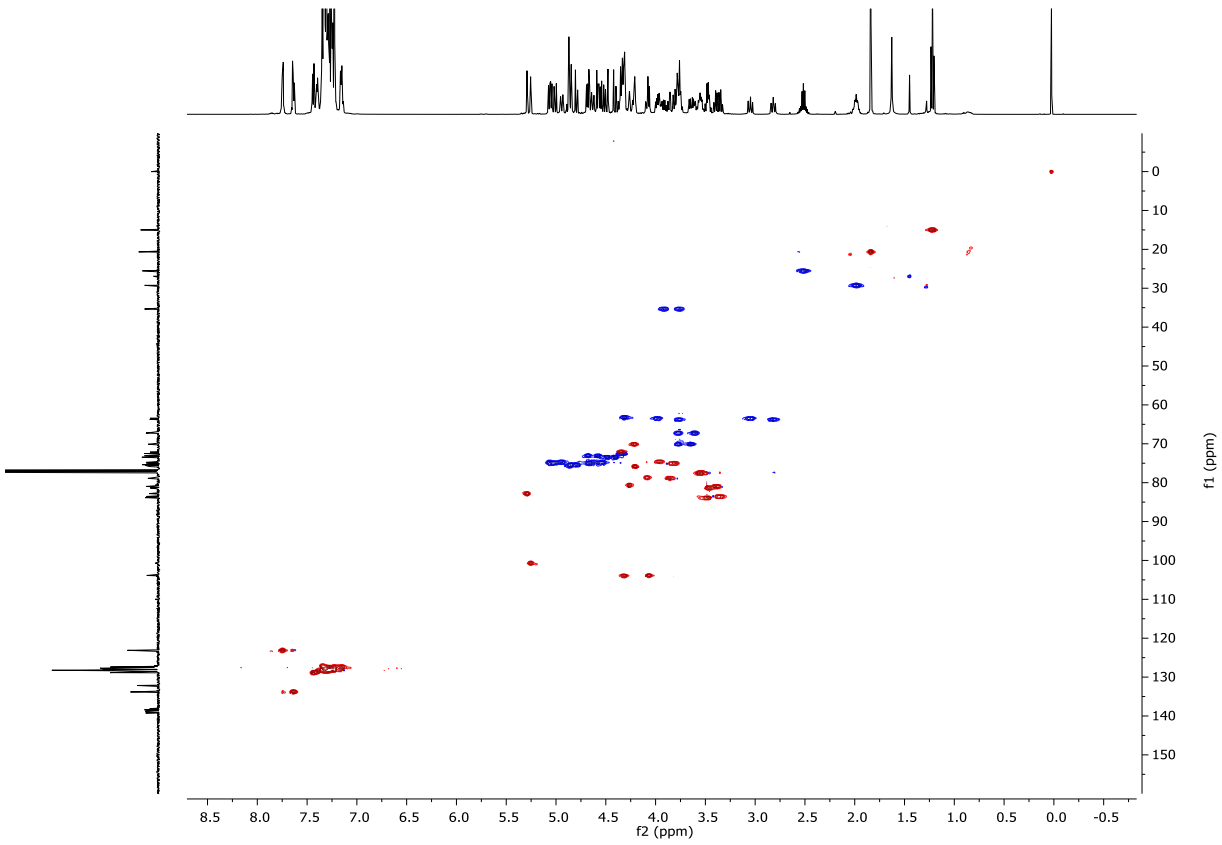
HETATM  126  C3  0XB    11       7.171   8.419  -9.113  1.00
0.00     0.182 C
HETATM  127  O3  0XB    11       6.502   8.304 -10.382  1.00
0.00    -0.390 OA
HETATM  128  H3O 0XB    11       5.972   7.504 -10.392  1.00
0.00     0.210 HD
HETATM  129  C2  0XB    11       6.099   8.563  -8.011  1.00
0.00     0.204 C
HETATM  130  O2  0XB    11       5.459   7.287  -7.841  1.00
0.00    -0.388 OA
HETATM  131  H2O 0XB    11       4.775   7.360  -7.171  1.00
0.00     0.210 HD
ENDROOT
TORSDOF 51
```

NMR Spectra

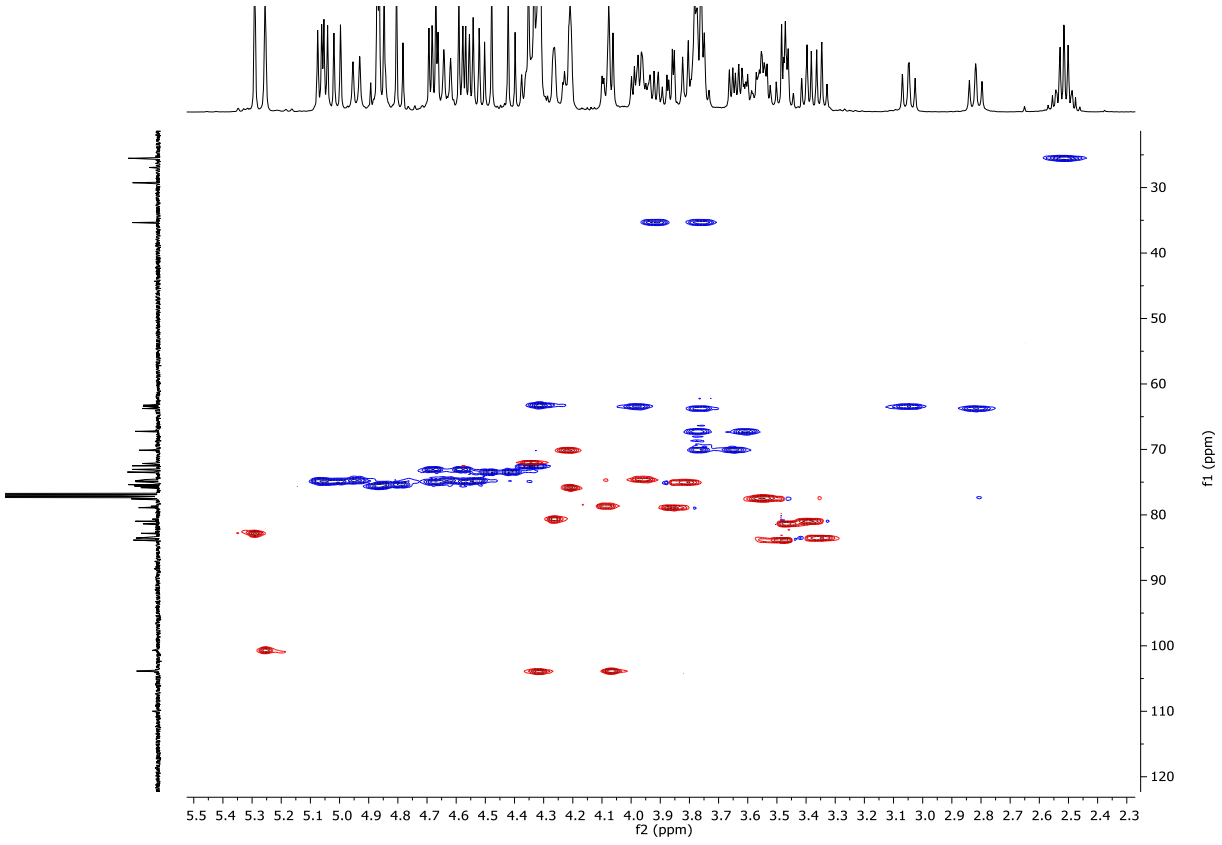
Compound 9



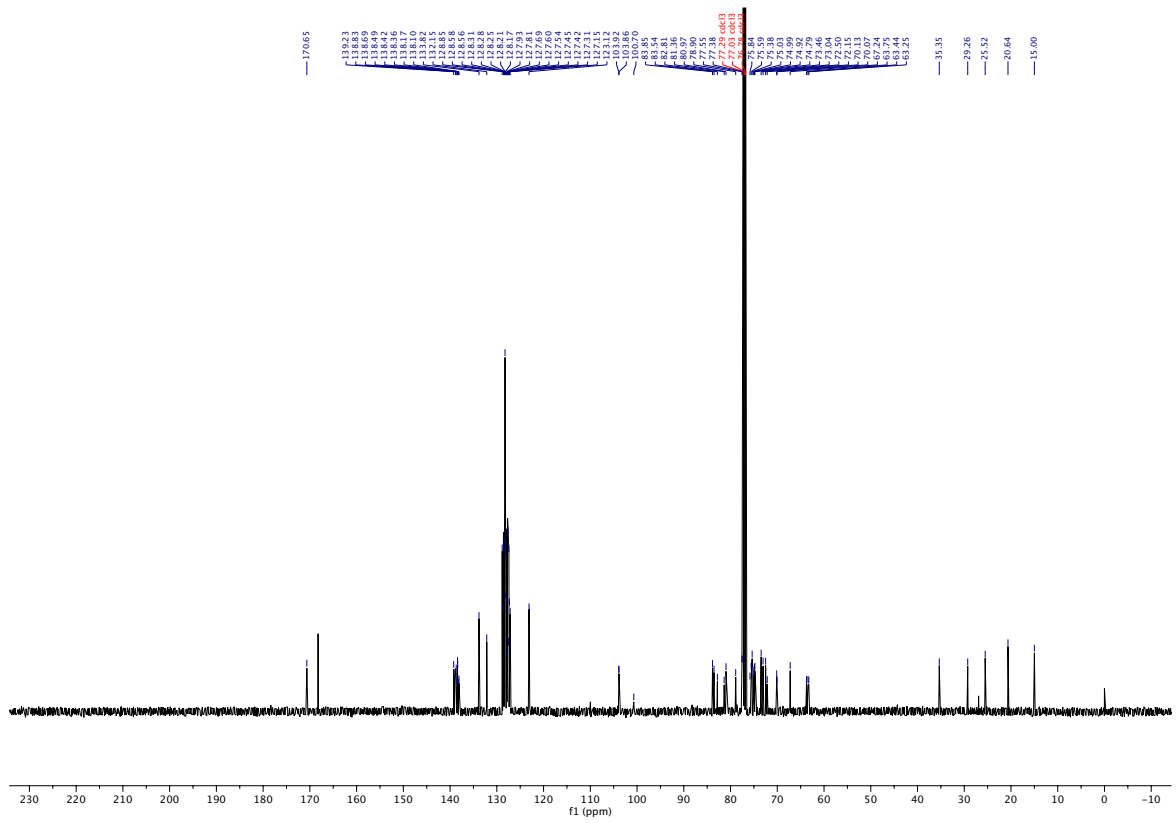
^{1}H NMR δ [ppm]



^{1}H-^{13}C-HSQC-NMR

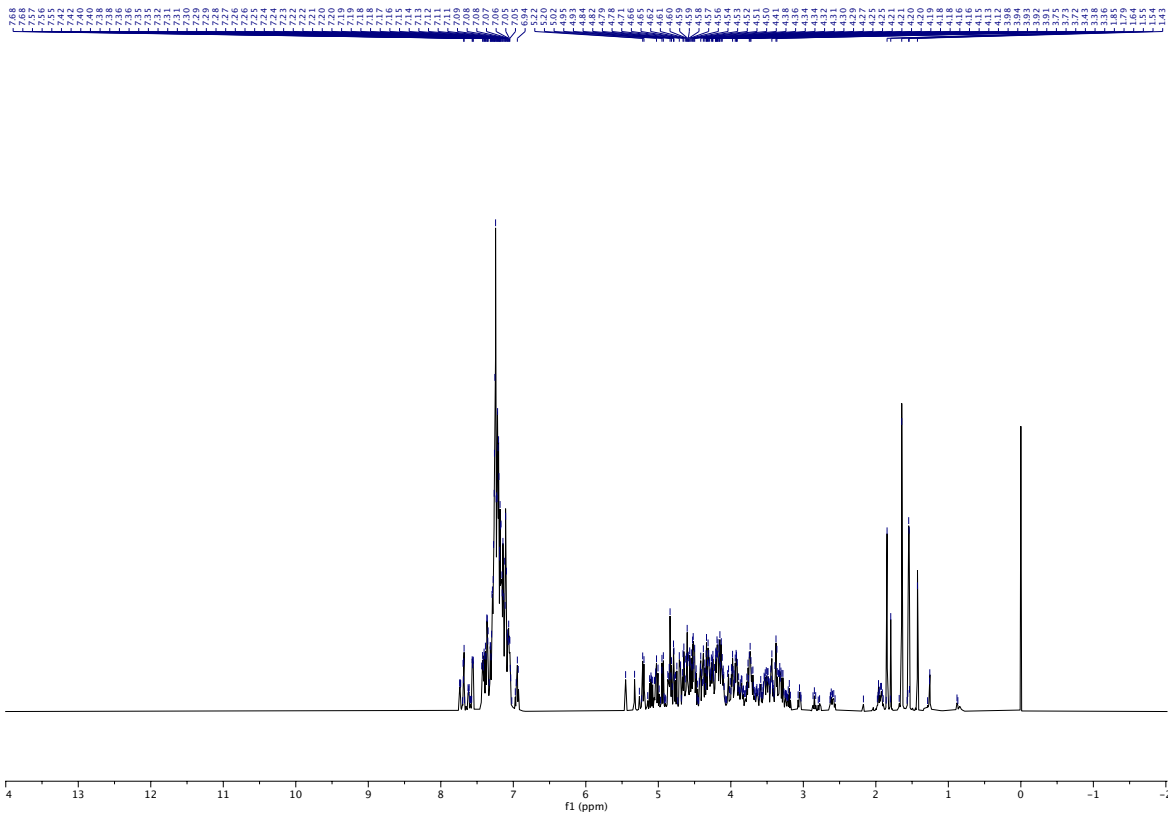


^{1}H-^{13}C-HSQC-NMR: zoomed

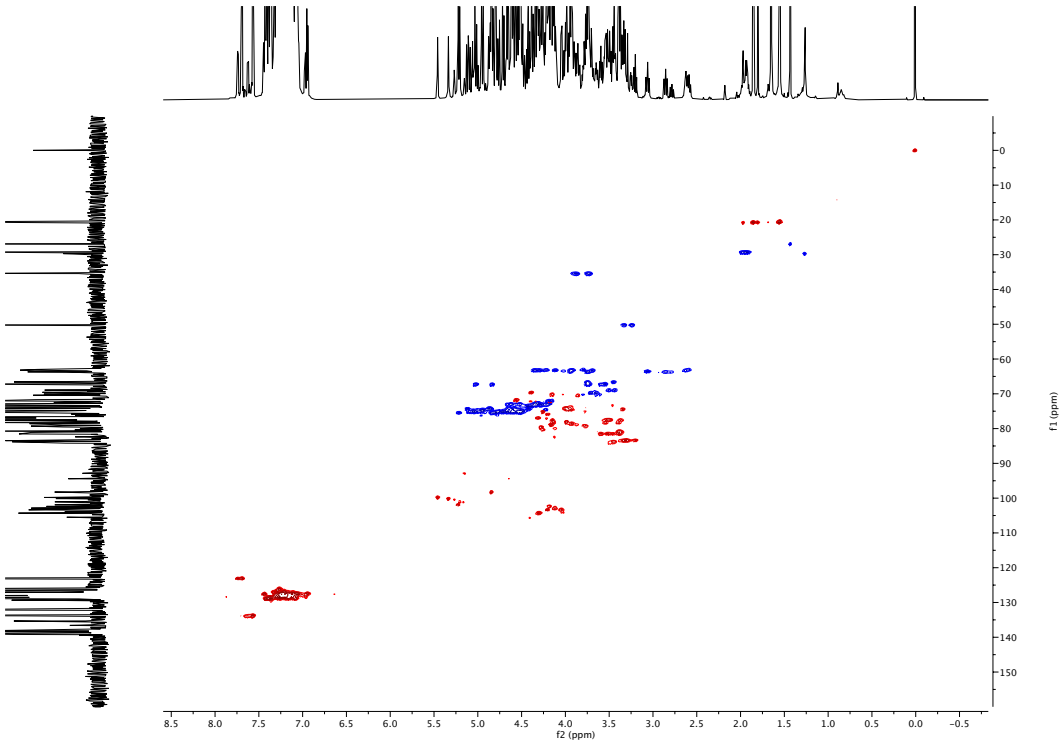

170.65
139.23
138.58
138.49
138.42
138.36
138.17
138.10
133.82
132.15
128.85
128.58
128.56
128.31
128.28
128.25
128.21
128.17
127.93
127.81
127.69
127.60
127.54
127.45
127.42
127.31
127.15
127.12
103.92
100.00
83.85
83.54
82.81
81.36
80.97
78.90
77.55
77.38
77.13 ddd:3
77.03 ddd:3
76.92 ddd:2
75.84
75.59
75.38
75.03
74.99
74.92
74.79
73.46
73.04
72.50
70.15
70.13
70.07
67.24
65.75
63.44
63.25
35.35
29.26
25.52
20.64
15.00
230 220 210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 -10
f1 (ppm)

^{13}C NMR δ [ppm]

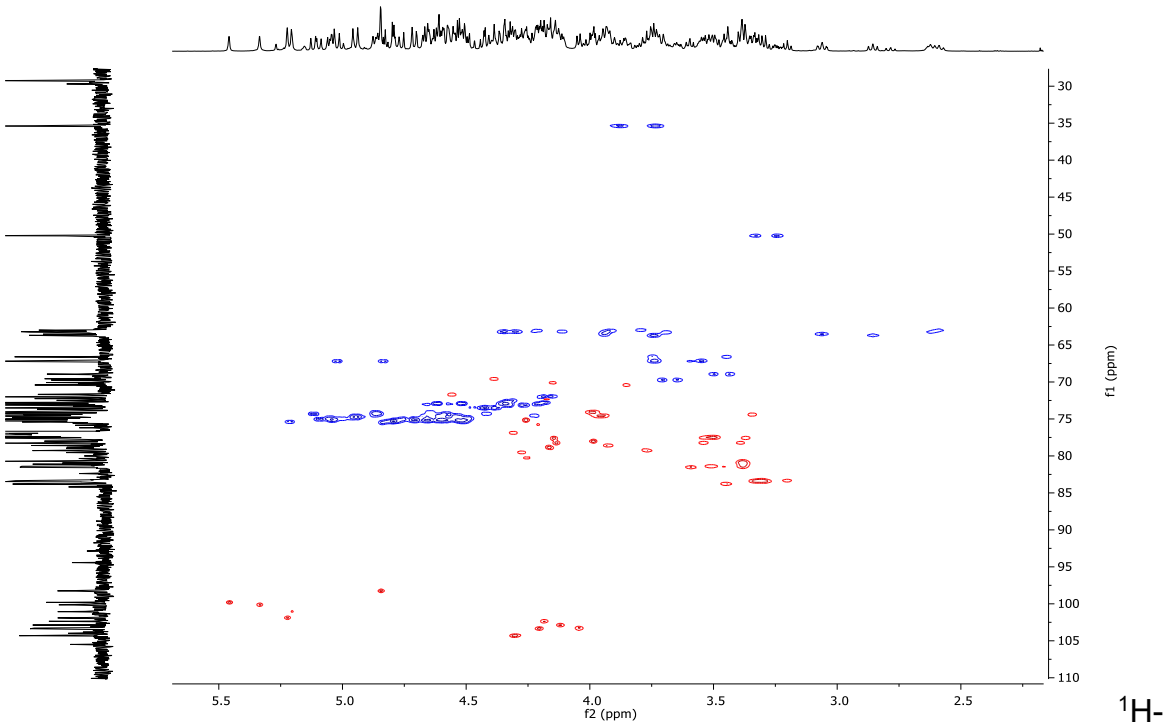
Compound 10



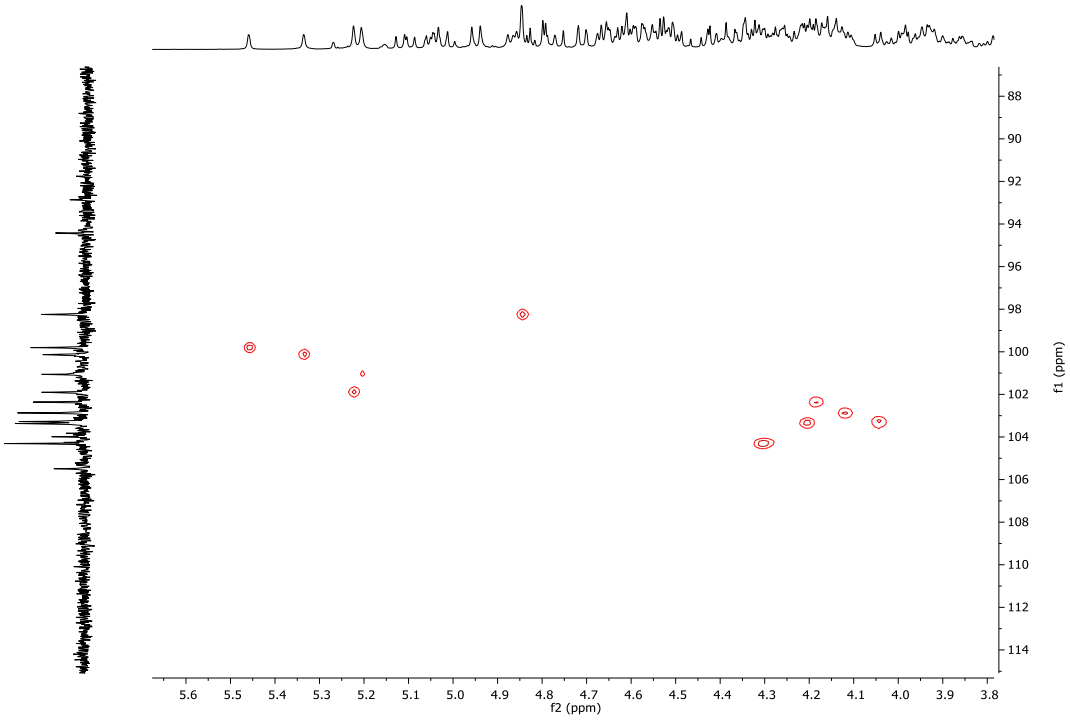
^{1}H NMR δ [ppm]



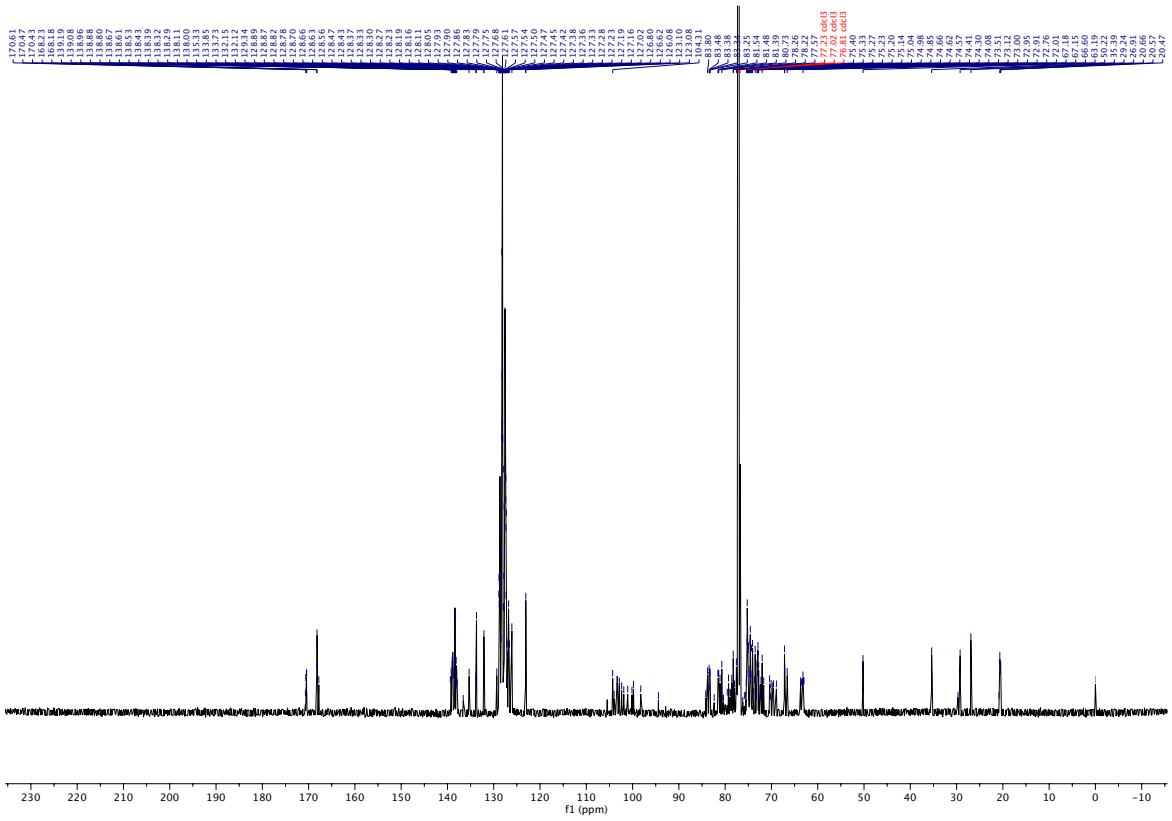
^{1}H-^{13}C-HSQC-NMR



^{1}H-^{13}C-HSQC-NMR: zoomed

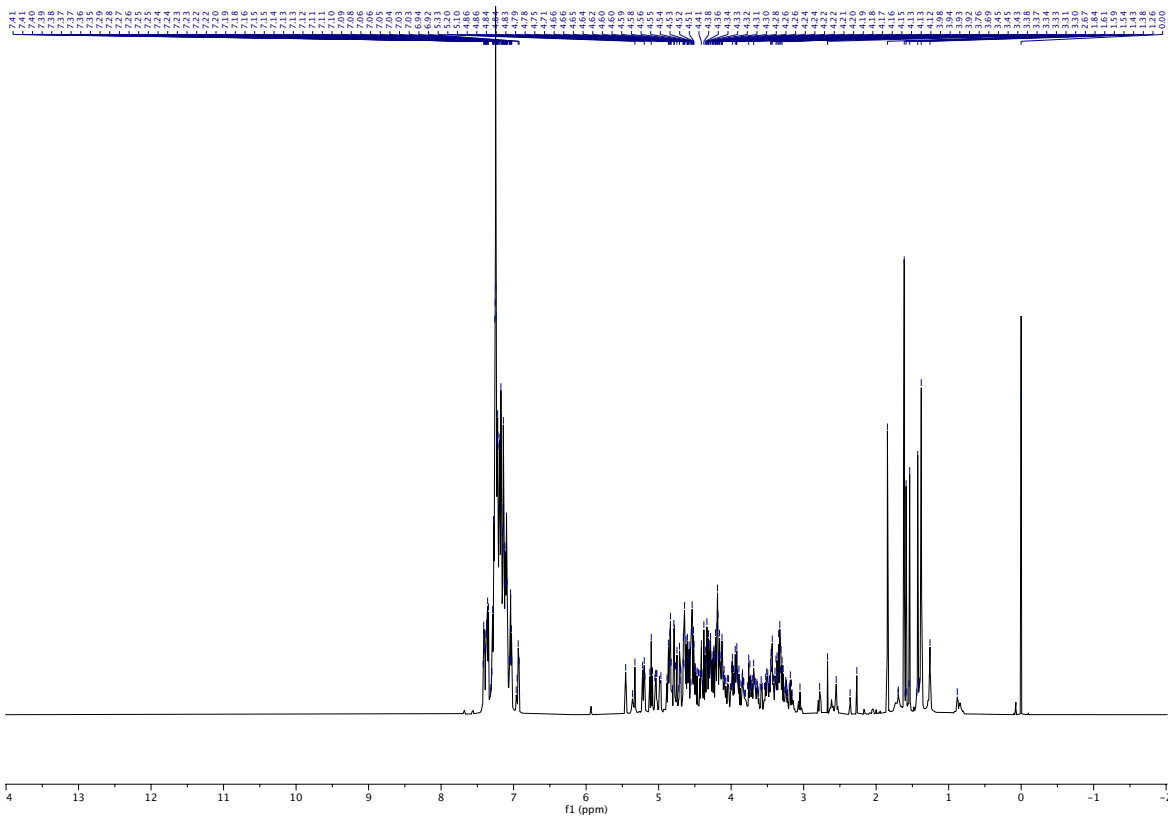


^{1}H-^{13}C-HSQC-NMR: zoomed anomeric

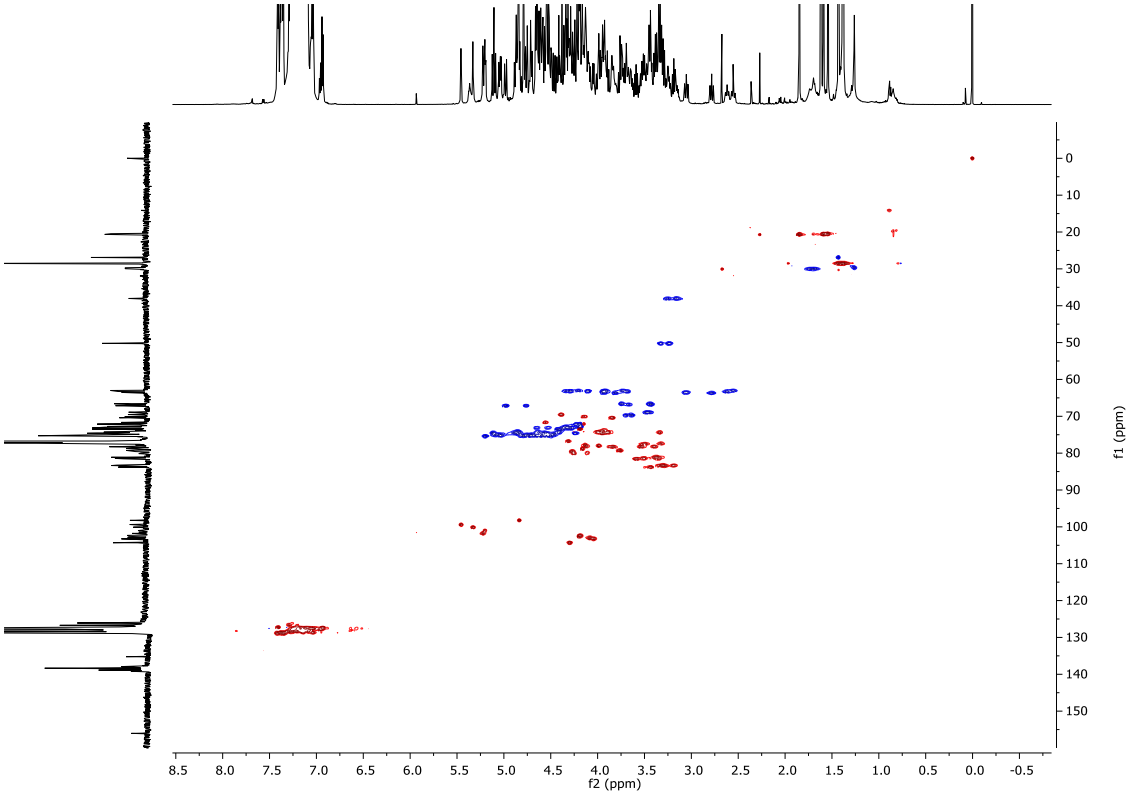


^{13}C NMR δ [ppm]

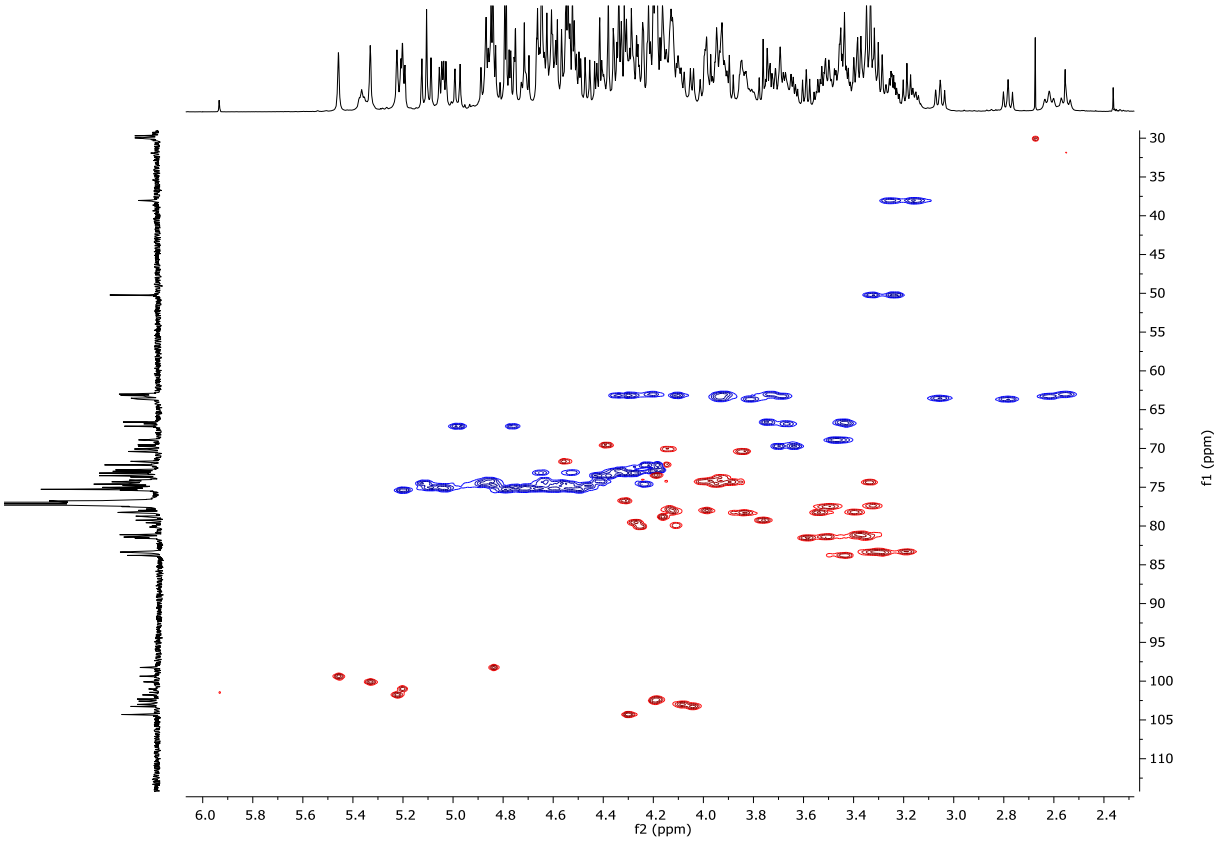
Compound 12



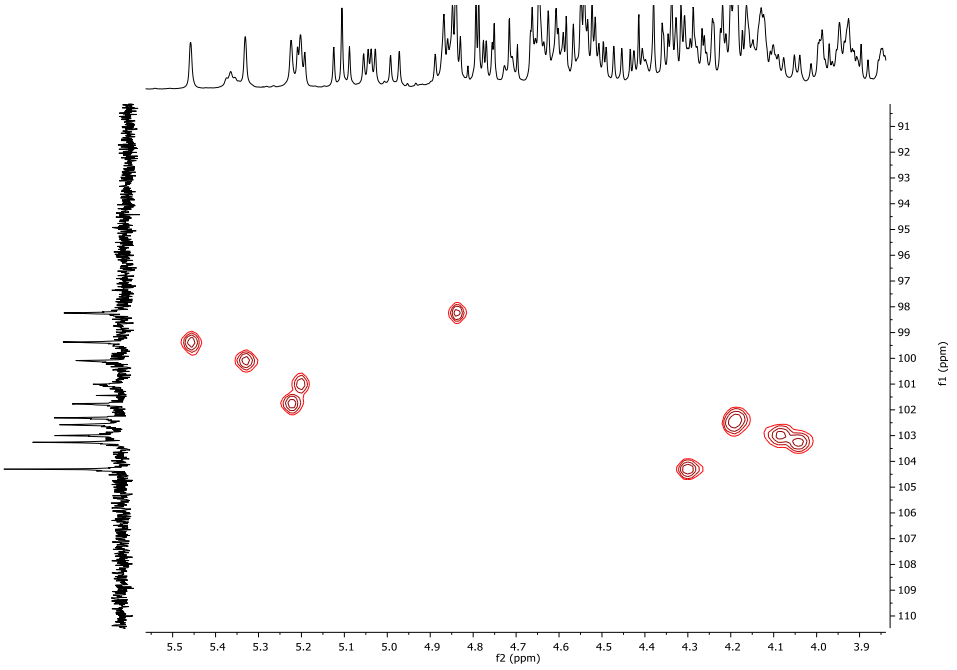
^{1}H NMR δ [ppm]



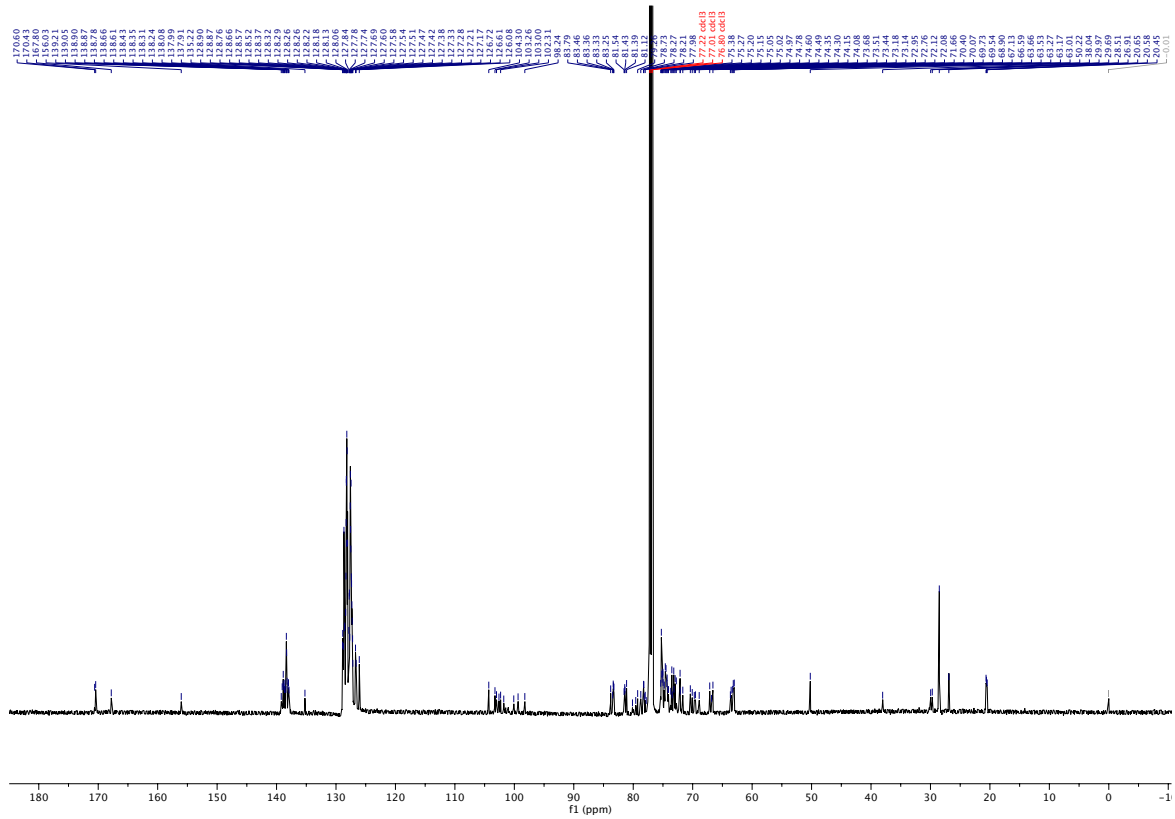
^{1}H-^{13}C-HSQC-NMR



^{1}H-^{13}C-HSQC-NMR: zoomed

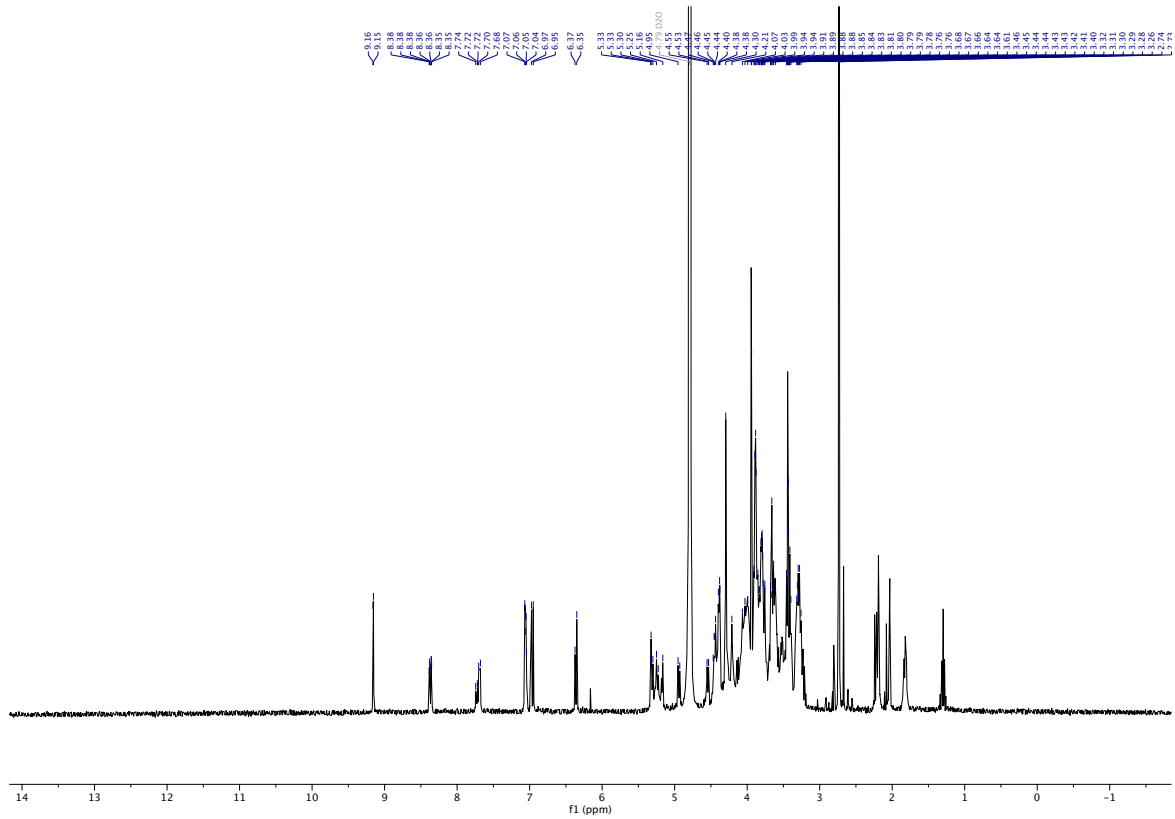


^{1}H-^{13}C-HSQC-NMR: zoomed anomeric

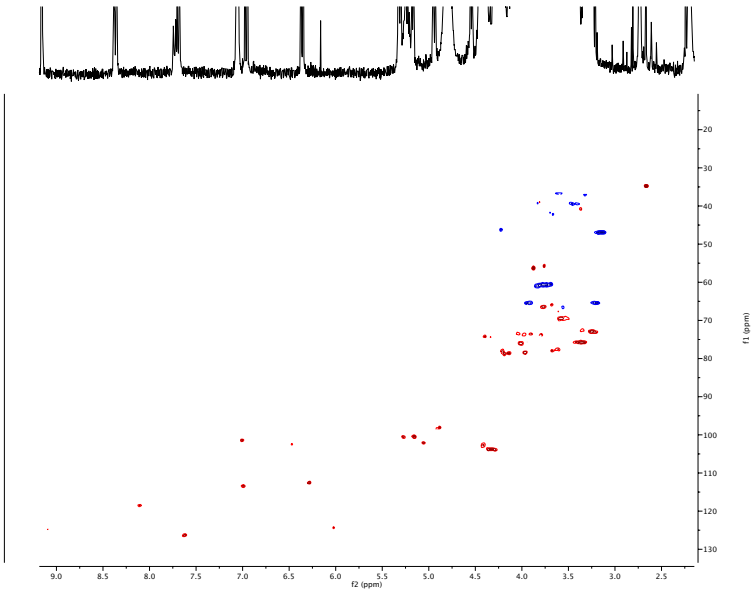


^{13}C NMR δ [ppm]

Compound 16

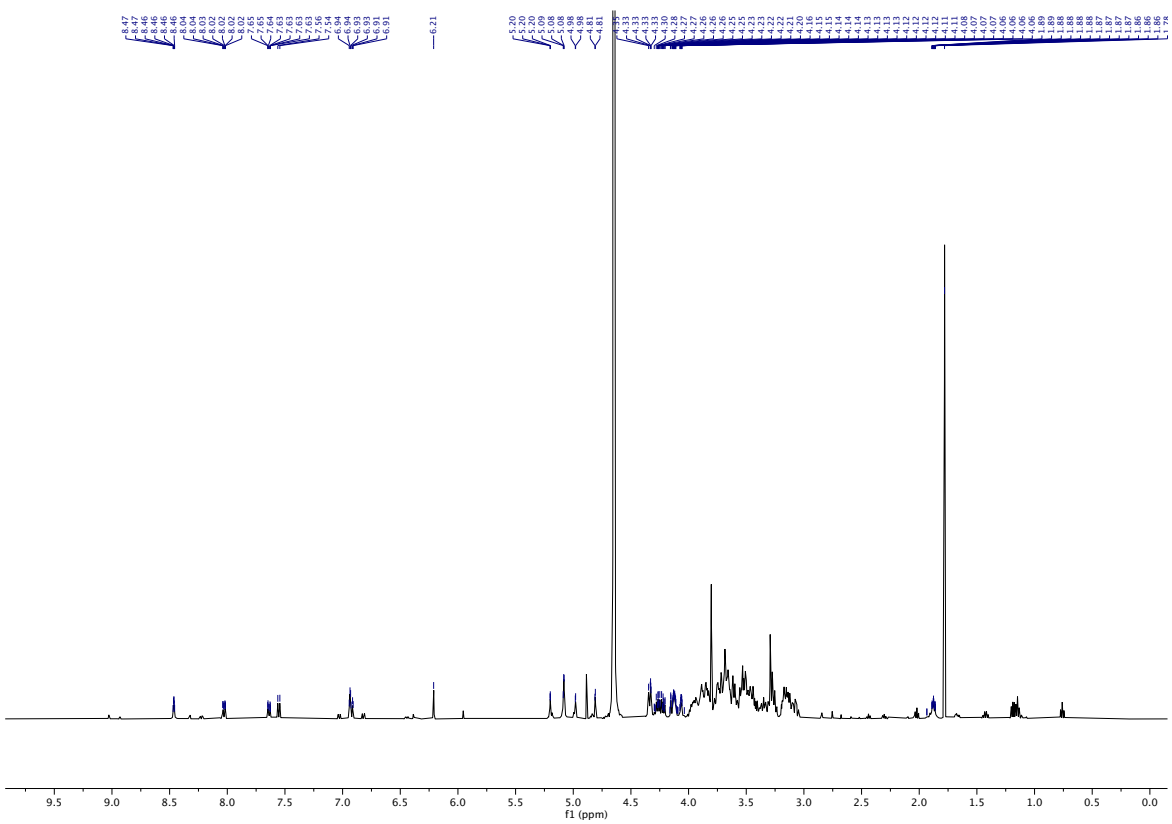


^{1}H NMR δ [ppm]

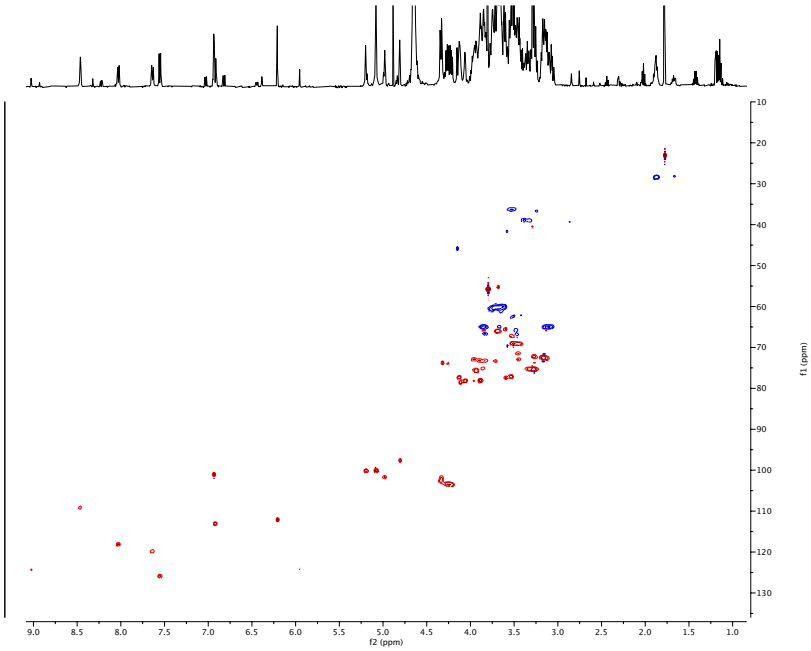


^{1}H-^{13}C-HSQC-NMR

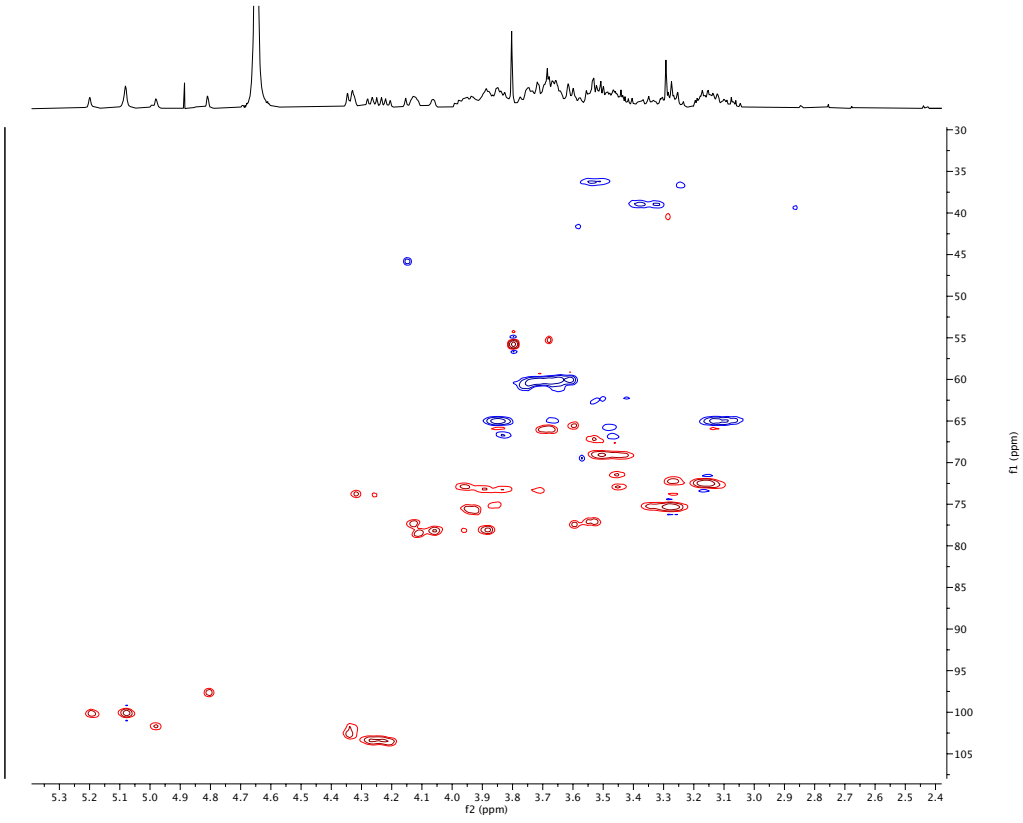
Compound 17



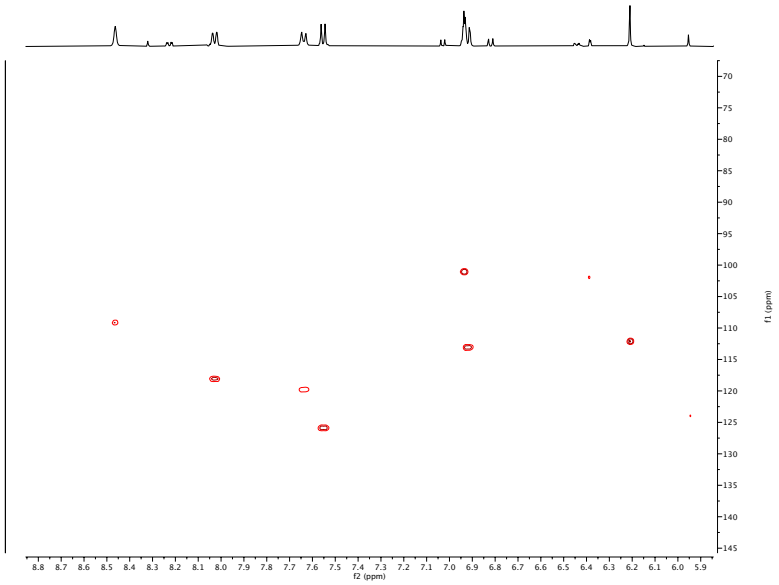
^{1}H NMR δ [ppm]



^{1}H-^{13}C-HSQC-NMR

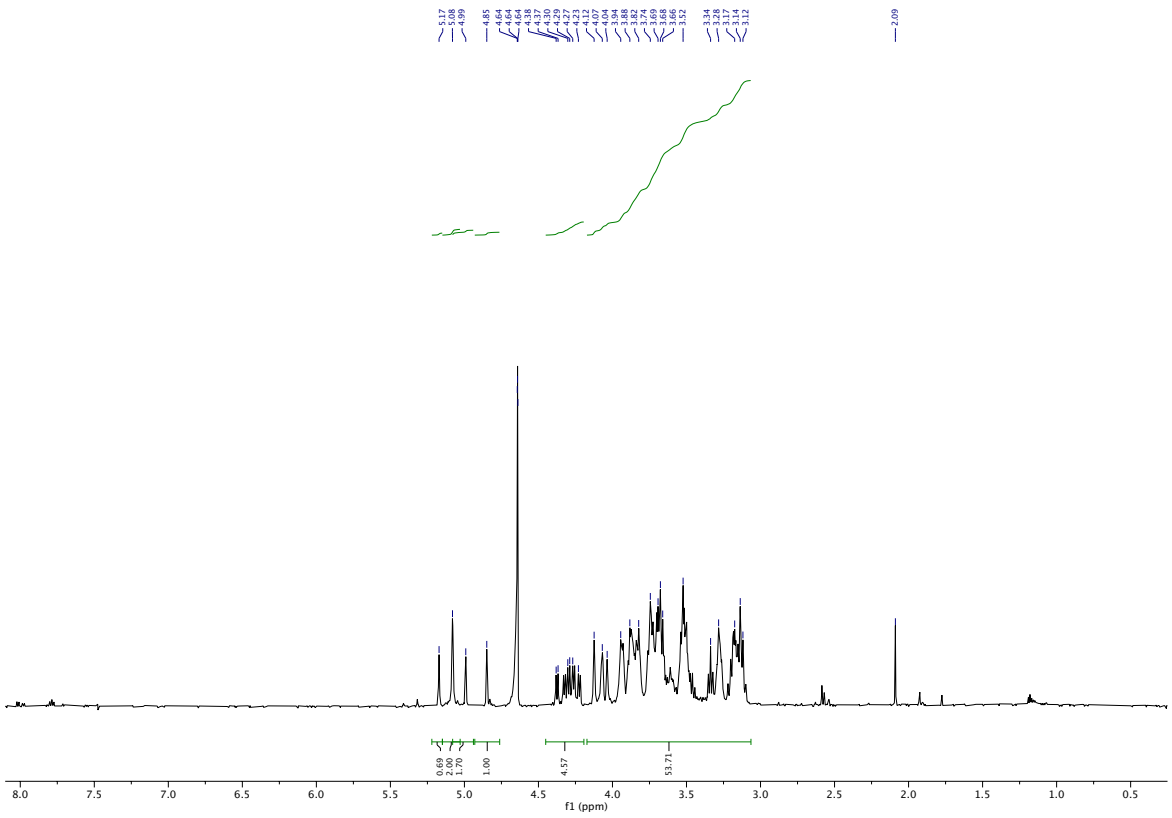


Zoomed ‘Glycan Region’ ^{1}H-^{13}C-HSQC-NMR

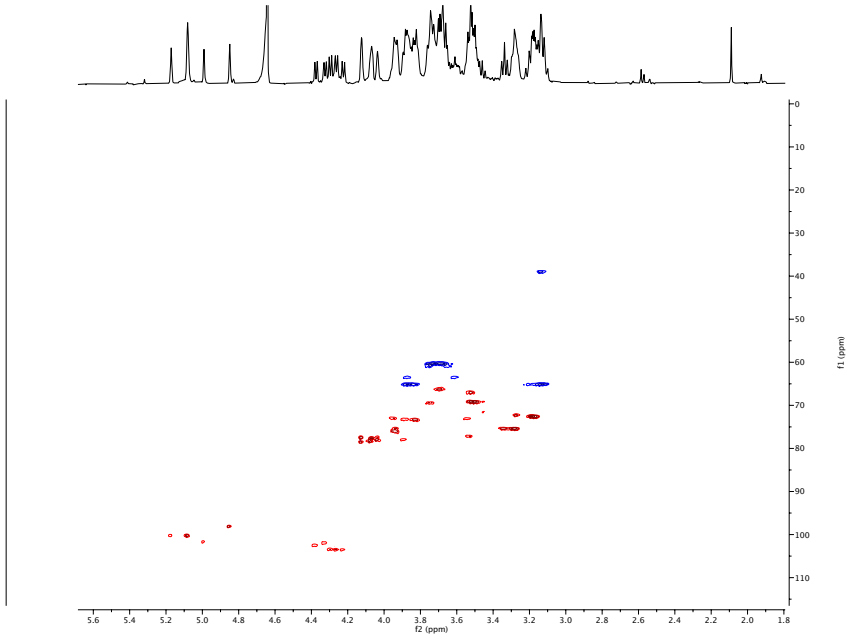


Zoomed ‘aromatic’ ^{1}H-^{13}C-HSQC-NMR

Compound 18



^{1}H NMR δ [ppm]



^{1}H-^{13}C-HSQC-NMR