

Making the longest sugars: A chemical synthesis of heparin-related [4]_n oligosaccharides from 16-mer to 40-mer

Steen U. Hansen,^{†1} Gavin J. Miller,^{†1} Matthew J. Cliff,² Gordon C. Jayson³ and John M. Gardiner^{1*}

Electronic Supplementary Information

1. Experimental procedures: Synthesis

General methods

All the chemicals used were purchased from commercial sources without further purification. All reactions were monitored by TLC on Merck silica gel plates 60 F254. Silica gel 60 (particle size 0.035-0.070 mm) was used for column chromatography. ¹H NMR spectra were recorded at 400 or 800 MHz and ¹³C spectra at 100 or 200 MHz respectively on Bruker DPX spectrometers. Mass spectra (MS) were recorded using a Micromass Platform II spectrometer using an electro spray ionization source or via the EPSRC National Mass Spectrometry Service (Swansea). Isotope patterns for compounds with mass > 1000 are included in the SI. Infrared spectra were obtained by using a Bruker Alpha instrument. Melting points were determined using Stuart Scientific SMP10 apparatus and are uncorrected. Optical rotations were obtained using an AA-1000 polarimeter. Elemental analyses were performed by Micro Analytical Laboratory, School of Chemistry, The University of Manchester. NMR Data were reprocessed using iNMR 4 from Nucleomatica or Topspin. Mass spectra report monoisotopic values where indicated, and most probable mass otherwise. Microwave reactor was a CEM Microwave Organic Synthesis Reactor. LCMS data were recorded using a LC-MSD-Trap-SL using a Supelco Ascentis C8, 5 micron column (4.6 x 30mm).

Methyl (2-azido-3,6-di-*O*-benzyl-2-deoxy- α -D-glucopyranosyl)-(1 $\rightarrow$ 4)-(methyl 2-*O*-benzoyl-3-*O*-benzyl- α -L-idopyranosyl uronate)-(1 $\rightarrow$ 4)-2-azido-3,6-di-*O*-benzyl-2-deoxy- α -D-glucopyranosyl)-(1 $\rightarrow$ 4)-(methyl 2-*O*-benzoyl-3-*O*-benzyl- α -L-idopyranosyl uronate)-(1 $\rightarrow$ 4)-2-azido-3,6-di-*O*-benzyl-2-deoxy- α -D-glucopyranosyl-(1 $\rightarrow$ 4)-(methyl 2-*O*-benzoyl-3-*O*-benzyl- α -L-idopyranosyl uronate)-(1 $\rightarrow$ 4)-2-azido-3,6-di-*O*-benzyl-2-deoxy- α -D-glucopyranosyl-(1 $\rightarrow$ 4)-(methyl 2-*O*-benzoyl-3-*O*-benzyl- α -L-idopyranosyl uronate)-(1 $\rightarrow$ 4)-2-azido-3,6-di-*O*-benzyl-2-deoxy- α -D-glucopyranosyl-(1 $\rightarrow$ 4)-(methyl 2-*O*-benzoyl-3-*O*-benzyl- α -L-idopyranoside) uronate, **3**

The dodecasaccharide **2** (452 mg, 0.096 mmol) was dissolved in a mixture of MeOH/pyridine (5 mL/2 mL) and heated to 50 °C for 7 h. The solvents were evaporated and co-evaporated with toluene (2x10 mL). The crude product was purified using flash column chromatography (EtOAc/hexane gradient 3:5 to 2:3). This yielded **3** (416 mg, 95%) as a white foam. *R*_f 0.19 (toluene/

¹Manchester Institute of Biotechnology, School of Chemistry, Faculty of EPS, The University of Manchester 131 Princess Street, Manchester M1 7DN, UK. ²Manchester Institute of Biotechnology, The University of Manchester 131 Princess Street, Manchester M1 7DN, UK. ³Institute of Cancer Sciences, The University of Manchester, Wilmslow Road, Manchester M20 4BX, UK. [†]Equal contributions.

acetone 10:1). $[\alpha]_{\text{D}}^{20} = +21.5$ ($c = 0.26$, CH_2Cl_2). ^1H NMR (400 MHz; CDCl_3): δ 8.10-8.08 (m, 2H, Bz), 7.98-7.90 (m, 10H, Bz), 7.53-6.98 (m, 108H, Ph), 5.60-5.52 (m, 5H, $\text{H}^{\text{CEGIK-1}}$), 5.22-5.15 (m, 5H, $\text{H}^{\text{CEGIK-2}}$), 5.07-5.06 (m, 1H, $\text{H}^{\text{A-2}}$), 5.04-5.03 (m, 1H, $\text{H}^{\text{A-1}}$), 4.99-4.87 (m, 6H, $\text{H}^{\text{BDFHJL-1}}$), 4.83-4.30 (m, 42H, $\text{H}^{\text{ACEGIK-5}}$, $18\times\text{CH}_2\text{Ph}$), 4.25-4.15 (m, 5H, $\text{H}^{\text{CEGIK-3}}$), 4.06-3.16 (m, 43H, $\text{H}^{\text{A-3}}$, $\text{H}^{\text{ACEGIK-4}}$, $\text{H}^{\text{BDFHJL-2}}$, $\text{H}^{\text{BDFHJL-3}}$, $\text{H}^{\text{BDFHJL-4}}$, $\text{H}^{\text{BDFHJL-5}}$, $\text{H}^{\text{BDFHJL-6ab}}$), 3.47 (s, 3H, COOCH_3), 3.46 (s, 3H, COOCH_3), 3.44 (s, 3H, COOCH_3), 3.29 (s, 3H, COOCH_3), 3.27 (s, 3H, COOCH_3), 3.26 (s, 3H, COOCH_3), 3.22 (s, 3H, OCH_3). ^{13}C NMR (101 MHz; CDCl_3): δ 169.6, 169.3, 169.3, 169.2, 165.6, 165.2, 165.1, 138.0, 137.9, 137.9, 137.8, 137.8, 137.7, 137.6, 137.4, 137.4, 137.3, 133.6, 133.5, 129.9, 129.9, 129.8, 129.5, 129.3, 129.3, 129.2, 128.8, 128.7, 128.6, 128.6, 128.4, 128.3, 128.2, 128.2, 128.2, 128.1, 128.0, 128.0, 127.9, 127.8, 127.8, 127.7, 127.6, 127.5, 127.4, 127.3, 127.3, 100.3, 99.3, 99.2, 99.2, 98.1, 98.0, 98.0, 98.0, 79.2, 78.3, 78.2, 78.1, 77.4, 77.3, 77.0, 76.7, 75.9, 75.8, 75.8, 75.6, 75.5, 75.5, 75.4, 75.3, 74.8, 74.8, 74.5, 74.4, 74.2, 74.1, 74.1, 73.8, 73.7, 73.6, 72.4, 72.4, 72.3, 71.3, 71.2, 71.1, 70.1, 70.6, 70.5, 70.5, 70.4, 70.4, 69.4, 67.9, 67.3, 67.3, 67.3, 67.3, 67.2, 67.1, 67.1, 63.4, 63.1, 63.0, 62.7, 56.2, 52.1, 52.0, 51.7, 51.6. MALDI TOF MS: monoisotopic m/z : calcd for $\text{C}_{247}\text{H}_{250}\text{N}_{18}\text{NaO}_{67}$ $[M+\text{Na}]^+$: 4562.7; found: 4562.6.

Methyl (2-azido-3,6-di-*O*-benzyl-2-deoxy-4-*O*-trichloroacetyl- α -D-glucopyranosyl)-(1 $\rightarrow$ 4)-(methyl 2-*O*-benzoyl-3-*O*-benzyl- α -L-idopyranosyl uronate)-(1 $\rightarrow$ 4)-2-azido-3,6-di-*O*-benzyl-2-deoxy- α -D-glucopyranosyl-(1 $\rightarrow$ 4)-(methyl 2-*O*-benzoyl-3-*O*-benzyl- α -L-idopyranosyl uronate)-(1 $\rightarrow$ 4)-2-azido-3,6-di-*O*-benzyl-2-deoxy- α -D-glucopyranosyl-(1 $\rightarrow$ 4)-(methyl 2-*O*-benzoyl-3-*O*-benzyl- α -L-idopyranosyl uronate)-(1 $\rightarrow$ 4)-2-azido-3,6-di-*O*-benzyl-2-deoxy- α -D-glucopyranosyl-(1 $\rightarrow$ 4)-(methyl 2-*O*-benzoyl-3-*O*-benzyl- α -L-idopyranosyl uronate)-(1 $\rightarrow$ 4)-2-azido-3,6-di-*O*-benzyl-2-deoxy- α -D-glucopyranosyl-(1 $\rightarrow$ 4)-(methyl 2-*O*-benzoyl-3-*O*-benzyl- α -L-idopyranosyl uronate)-(1 $\rightarrow$ 4)-2-azido-3,6-di-*O*-benzyl-2-deoxy- α -D-glucopyranosyl-(1 $\rightarrow$ 4)-(methyl 2-*O*-benzoyl-3-*O*-benzyl- α -L-idopyranosyl uronate)-(1 $\rightarrow$ 4)-2-azido-3,6-di-*O*-benzyl-2-deoxy- α -D-glucopyranosyl-(1 $\rightarrow$ 4)-(methyl 2-*O*-benzoyl-3-*O*-benzyl- α -L-idopyranoside) uronate, 4

Acceptor decasaccharide **3** (114 mg, 0.025 mmol) and thioglycoside tetrasaccharide donor **1** (53 mg, 0.030 mmol) was dissolved in dry DCM (1 mL) under N_2 . Freshly activated 4Å powdered molecular sieves (130 mg) was added and the solution cooled to 0 °C in an icebath. After 10 min. NIS (19 mg, 0.084 mmol) was added, and after another 10 min. AgOTf (catalytic amount) was added. The suspension changed colour from pale yellow to deep red, was stirred for 30 min. and the reaction was quenched into a separating funnel containing a mixture of DCM (30 mL), saturated aqueous NaHCO_3 (20 mL) and $\text{Na}_2\text{S}_2\text{O}_3$ (1 mL, 10% aqueous). After shaking until the iodine colour was removed the suspension was filtered through a short pad of Celite® washing with water and DCM. The layers were separated and the aqueous extracted with DCM (10 mL). The organic layers were combined, dried (MgSO_4) and solvent removed *in vacuo*. The crude was purified by silica gel flash column chromatography (toluene/acetone 20:1) to give **4** (118 mg, 76%) as a white foam. R_f 0.32 (toluene/acetone 10:1). $[\alpha]_{\text{D}}^{20} = +31.9$ ($c = 0.45$, CH_2Cl_2). ^1H NMR (400 MHz; CDCl_3): δ 8.15-8.13 (m, 2H, Bz), 8.07-8.05 (m, 2H, Bz), 8.00-7.96 (m, 12H, Bz), 7.57-7.02 (m, 144H, Ph), 5.64-5.56 (m, 7H, $\text{H}^{\text{CEGIKMO-1}}$), 5.35 (t, $J = 9.6$ Hz, 1H, $\text{H}^{\text{P-4}}$), 5.25-5.18 (m, 7H, $\text{H}^{\text{CEGIKMO-2}}$), 5.12-5.11 (m, 1H, $\text{H}^{\text{A-2}}$), 5.08-5.07 (m, 1H, $\text{H}^{\text{A-1}}$), 4.99-4.91 (m, 8H, $\text{H}^{\text{BDFHJLNP-1}}$), 4.86-4.35 (m, 56H, $\text{H}^{\text{ACEGIKMO-5}}$, $24\times\text{CH}_2\text{Ph}$), 4.29-4.19 (m, 7H, $\text{H}^{\text{CEGIKMO-3}}$), 4.12-3.20 (m, 80H, $\text{H}^{\text{A-3}}$, $\text{H}^{\text{ACEGIKMO-4}}$, $\text{H}^{\text{BDFHJLNP-2}}$, $\text{H}^{\text{BDFHJLNP-3}}$, $\text{H}^{\text{BDFHJLNP-4}}$, $\text{H}^{\text{BDFHJLNP-5}}$, $\text{H}^{\text{BDFHJLNP-6ab}}$, $8\times\text{COOCH}_3$), 3.27 (s, 3H, OCH_3). ^{13}C NMR (101 MHz; CDCl_3): δ 169.5, 169.3, 169.2, 165.5, 165.2, 165.1, 160.2,

idopyranosyl uronate)-(1→4)-2-azido-3,6-di-*O*-benzyl-2-deoxy- α -D-glucopyranosyl-(1→4)-(methyl 2-*O*-benzoyl-3-*O*-benzyl- α -L-idopyranosyl uronate)-(1→4)-2-azido-3,6-di-*O*-benzyl-2-deoxy- α -D-glucopyranosyl-(1→4)-(methyl 2-*O*-benzoyl-3-*O*-benzyl- α -L-idopyranoside) uronate, 6

Acceptor hexadecasaccharide **5** (286 mg, 0.047 mmol) and thioglycoside tetrasaccharide donor **1** (108 mg, 0.061 mmol) was dissolved in dry DCM (2 mL) under N₂. Freshly activated 4Å powdered molecular sieves (171 mg) was added and the solution cooled to 0 °C in an icebath. After 10 min. NIS (33 mg, 0.147 mmol) was added, and after another 10 min. AgOTf (catalytic amount) was added. The suspension changed colour from pale yellow to deep red, was stirred for 30 min. and the reaction was quenched into a separating funnel containing a mixture of DCM (30 mL), saturated aqueous NaHCO₃ (20 mL) and Na₂S₂O₃ (1 mL, 10% aqueous). After shaking until the iodine colour was removed the suspension was filtered through a short pad of Celite® washing with water and DCM. The layers were separated and the aqueous extracted with DCM (10 mL). The organic layers were combined, dried (MgSO₄) and solvent removed *in vacuo*. The crude was purified by silica gel flash column chromatography (toluene/acetone 20:1 to 15:1 to 10:1) followed by precipitation (dissolved in EtOAc (5 mL) and hexane (5 mL) added) and filtration to give **6** (295 mg, 81%) as a white solid. *R*_f 0.31 (toluene/acetone 10:1). [α]_D²⁰ = +52.5 (*c* = 0.33, CH₂Cl₂). ¹H NMR (800 MHz; CDCl₃) δ 8.10-8.09 (m, 2H, Bz), 8.02-8.01 (m, 2H, Bz), 7.94-7.91 (m, 16H, Bz), 7.55-7.02 (m, 180H, Ph), 5.58-5.53 (m, 9H, H^{CEGIKMOQS}-1), 5.31 (t, *J* = 9.6 Hz, 1H, H^T-4), 5.19-5.14 (m, 9H, H^{CEGIKMOQS}-2), 5.09-5.08 (m, 1H, H^A-2), 5.05-5.04 (m, 1H, H^A-1), 4.95-4.88 (m, 10H, H^{BDFHJLNPR}-1), 4.83-4.34 (m, 70H, H^{ACEGIKMOQS}-5, 30xCH₂Ph), 4.26-4.16 (m, 9H, H^{CEGIKMOQS}-3), 4.06-3.18 (m, 100H, H^A-3, H^{ACEGIKMOQS}-4, H^{BDFHJLNPR}-2, H^{BDFHJLNPR}-3, H^{BDFHJLNPR}-4, H^{BDFHJLNPR}-5, H^{BDFHJLNPR}-6_{ab}, 10xCOOCH₃), 3.24 (s, 3H, OCH₃). ¹³C NMR (201 MHz; CDCl₃): δ 169.6, 169.4, 165.7, 165.2, 160.3, 138.0, 137.9, 137.5, 137.3, 133.7, 133.5, 130.0, 129.9, 129.4, 128.8, 128.5, 128.1, 128.0, 127.9, 127.7, 127.6, 127.4, 127.2, 99.3, 98.4, 98.1, 78.1, 77.0, 76.1, 76.0, 75.6, 75.5, 75.0, 74.8, 74.5, 74.3, 73.9, 73.8, 72.7, 72.6, 71.4, 70.8, 70.7, 70.6, 69.8, 69.3, 67.4, 63.5, 63.1, 52.1, 52.0, 51.7. MALDI TOF MS: monoisotopic *m/z*: calcd for C₄₁₃H₄₁₃Cl₃N₃₀NaO₁₁₂ [*M*+Na]⁺: 7711.7; found: 7711.8.

Methyl (2-azido-3,6-di-*O*-benzyl-2-deoxy- α -D-glucopyranosyl)-(1→4)-(methyl 2-*O*-benzoyl-3-*O*-benzyl- α -L-idopyranosyl uronate)-(1→4)-2-azido-3,6-di-*O*-benzyl-2-deoxy- α -D-glucopyranosyl-(1→4)-(methyl 2-*O*-benzoyl-3-*O*-benzyl- α -L-idopyranosyl uronate)-(1→4)-2-azido-3,6-di-*O*-benzyl-2-deoxy- α -D-glucopyranosyl-(1→4)-(methyl 2-*O*-benzoyl-3-*O*-benzyl- α -L-idopyranosyl uronate)-(1→4)-2-azido-3,6-di-*O*-benzyl-2-deoxy- α -D-glucopyranosyl-(1→4)-(methyl 2-*O*-benzoyl-3-*O*-benzyl- α -L-idopyranosyl uronate)-(1→4)-2-azido-3,6-di-*O*-benzyl-2-deoxy- α -D-glucopyranosyl-(1→4)-(methyl 2-*O*-benzoyl-3-*O*-benzyl- α -L-idopyranosyl uronate)-(1→4)-2-azido-3,6-di-*O*-benzyl-2-deoxy- α -D-glucopyranosyl-(1→4)-(methyl 2-*O*-benzoyl-3-*O*-benzyl- α -L-idopyranosyl uronate)-(1→4)-2-azido-3,6-di-*O*-benzyl-2-deoxy- α -D-glucopyranosyl-(1→4)-(methyl 2-*O*-benzoyl-3-*O*-benzyl- α -L-idopyranoside) uronate, 7

The icosasaccharide **6** (278 mg, 0.036 mmol) was dissolved in a mixture of MeOH/pyridine (5 mL/2.5 mL) and heated to 60°C for 5 h. The solvents were evaporated and co-evaporated with toluene (2x10 mL). The crude product was purified using flash column chromatography (toluene/acetone

15:1) followed by precipitation (dissolved in EtOAc (5 mL) and hexane (5 mL) added) and filtration to yield **7** (264 mg, 97%) as a white solid. R_f 0.18 (toluene/acetone 10:1). $[\alpha]_D^{20} = +59.7$ ($c = 0.29$, CH_2Cl_2). ^1H NMR (400 MHz; CDCl_3) δ 8.11-8.09 (m, 2H, Bz), 7.99-7.92 (m, 18H, Bz), 7.55-7.01 (m, 180H, Ph), 5.60-5.55 (m, 9H, $\text{H}^{\text{CEGIKMOQS-1}}$), 5.23-5.16 (m, 9H, $\text{H}^{\text{CEGIKMOQS-2}}$), 5.08-5.07 (m, 1H, $\text{H}^{\text{A-2}}$), 5.04-5.03 (m, 1H, $\text{H}^{\text{A-1}}$), 4.99-4.87 (m, 10H, $\text{H}^{\text{BDFHJLNPT-1}}$), 4.83-4.34 (m, 70H, $\text{H}^{\text{ACEGIKMOQS-5}}$, 30x CH_2Ph), 4.24-4.15 (m, 9H, $\text{H}^{\text{CEGIKMOQS-3}}$), 4.06-3.18 (m, 101H, $\text{H}^{\text{A-3}}$, $\text{H}^{\text{ACEGIKMOQS-4}}$, $\text{H}^{\text{BDFHJLNPT-2}}$, $\text{H}^{\text{BDFHJLNPT-3}}$, $\text{H}^{\text{BDFHJLNPT-4}}$, $\text{H}^{\text{BDFHJLNPT-5}}$, $\text{H}^{\text{BDFHJLNPT-6ab}}$, 10x COOCH_3), 3.24 (s, 3H, OCH_3), 2.66 (broad s, 1H, OH). MALDI TOF MS: monoisotopic m/z : calcd for $\text{C}_{411}\text{H}_{414}\text{N}_{30}\text{NaO}_{111}$ [$M+\text{Na}$] $^+$: 7567.8; found: 7567.9.

Acceptor icosasaccharide **7** (254 mg, 0.034 mmol) and thioglycoside tetrasaccharide donor **1** (77 mg, 0.044 mmol) was dissolved in dry DCM (2 mL) under N₂. Freshly activated 4Å powdered molecular sieves (185 mg) was added and the solution cooled to 0 °C in an icebath. After 10 min. NIS (19 mg, 0.084 mmol) was added, and after another 10 min. AgOTf (catalytic amount) was added. The suspension changed colour from pale yellow to deep red, was stirred for 30 min. and the reaction was quenched into a separating funnel containing a mixture of DCM (30 mL), saturated aqueous NaHCO₃ (20 mL) and Na₂S₂O₃ (1 mL, 10% aqueous). After shaking until the iodine colour was removed the suspension was filtered through a short pad of Celite® washing with water and DCM. The layers were separated and the aqueous extracted with DCM (10 mL). The organic layers were combined, dried (MgSO₄) and solvent removed *in vacuo*. The crude was purified by silica gel flash column chromatography (toluene/acetone 20:1 to 15:1 to 10:1) followed by precipitation (dissolved in warm EtOAc (5 mL) and hexane (5 mL) added) and filtration to give **8** (239 mg, 77%) as a white solid. Also acceptor starting material (42 mg, 16%) was recovered. *R*_f 0.29 (toluene/acetone 10:1). [α]_D²⁰ = +37.9 (*c* = 0.45, CH₂Cl₂). ¹H NMR (400 MHz; CDCl₃) δ 8.17-8.15 (m, 2H, Bz), 8.09-8.07 (m, 2H, Bz), 8.02-7.97 (m, 20H, Bz), 7.59-7.05 (m, 216H, Ph), 5.62-5.58 (m, 11H, H^{CEGIKMOQS}UW-1), 5.31 (t, *J* = 9.6 Hz, 1H, H^X-4), 5.26-5.22 (m, 9H, H^{CEGIKMOQS}UW-2), 5.14-5.13 (m, 1H, H^A-2), 5.10-5.09 (m, 1H, H^A-1), 4.99-4.92 (m, 12H, H^{BDFHJLN}PRVTX-1), 4.88-4.36 (m, 84H, H^{ACEGIKMOQS}UW-5, 36xCH₂Ph), 4.30-4.19 (m, 11H, H^{CEGIKMOQS}UW-3), 4.12-3.20 (m, 120H,

HA-3, H^{ACEGIKMOQS}UW-4, H^{BDFHJLNPR}TVX-2, H^{BDFHJLNPR}TVX-3, H^{BDFHJLNPR}TV-4, H^{BDFHJLNPR}TVX-5, H^{BDFHJLNPR}TVX-6_{ab}, 12xCOOCH₃), 3.24 (s, 3H, OCH₃). MALDI TOF MS: m/z: calcd for C₄₉₅H₄₉₅Cl₃N₃₆NaO₁₃₄ [M+Na]⁺: 9221; found: 9221.

Methyl (2-azido-3,6-di-*O*-benzyl-2-deoxy- α -D-glucopyranosyl)-(1 $\rightarrow$ 4)-(methyl 2-*O*-benzoyl-3-*O*-benzyl- α -L-idopyranosyl uronate)-(1 $\rightarrow$ 4)-2-azido-3,6-di-*O*-benzyl-2-deoxy- α -D-glucopyranosyl-(1 $\rightarrow$ 4)-(methyl 2-*O*-benzoyl-3-*O*-benzyl- α -L-idopyranosyl uronate)-(1 $\rightarrow$ 4)-2-azido-3,6-di-*O*-benzyl-2-deoxy- α -D-glucopyranosyl-(1 $\rightarrow$ 4)-(methyl 2-*O*-benzoyl-3-*O*-benzyl- α -L-idopyranosyl uronate)-(1 $\rightarrow$ 4)-2-azido-3,6-di-*O*-benzyl-2-deoxy- α -D-glucopyranosyl-(1 $\rightarrow$ 4)-(methyl 2-*O*-benzoyl-3-*O*-benzyl- α -L-idopyranosyl uronate)-(1 $\rightarrow$ 4)-2-azido-3,6-di-*O*-benzyl-2-deoxy- α -D-glucopyranosyl-(1 $\rightarrow$ 4)-(methyl 2-*O*-benzoyl-3-*O*-benzyl- α -L-idopyranosyl uronate)-(1 $\rightarrow$ 4)-2-azido-3,6-di-*O*-benzyl-2-deoxy- α -D-glucopyranosyl-(1 $\rightarrow$ 4)-(methyl 2-*O*-benzoyl-3-*O*-benzyl- α -L-idopyranosyl uronate)-(1 $\rightarrow$ 4)-2-azido-3,6-di-*O*-benzyl-2-deoxy- α -D-glucopyranosyl-(1 $\rightarrow$ 4)-(methyl 2-*O*-benzoyl-3-*O*-benzyl- α -L-idopyranosyl uronate)-(1 $\rightarrow$ 4)-2-azido-3,6-di-*O*-benzyl-2-deoxy- α -D-glucopyranosyl-(1 $\rightarrow$ 4)-(methyl 2-*O*-benzoyl-3-*O*-benzyl- α -L-idopyranosyl uronate)-(1 $\rightarrow$ 4)-2-azido-3,6-di-*O*-benzyl-2-deoxy- α -D-glucopyranosyl-(1 $\rightarrow$ 4)-(methyl 2-*O*-benzoyl-3-*O*-benzyl- α -L-idopyranosyl uronate)-(1 $\rightarrow$ 4)-2-azido-3,6-di-*O*-benzyl-2-deoxy- α -D-glucopyranosyl-(1 $\rightarrow$ 4)-(methyl 2-*O*-benzoyl-3-*O*-benzyl- α -L-idopyranosyl uronate)-(1 $\rightarrow$ 4)-2-azido-3,6-di-*O*-benzyl-2-deoxy- α -D-glucopyranosyl-(1 $\rightarrow$ 4)-(methyl 2-*O*-benzoyl-3-*O*-benzyl- α -L-idopyranoside) uronate, 9

The tetracosasaccharide **8** (186 mg, 0.020 mmol) was dissolved in a mixture of MeOH/pyridine (3 mL/2 mL) and heated to 60 °C for 6 h. The solvents were evaporated and co-evaporated with toluene (2x10 mL). The crude product was purified using flash column chromatography (toluene/acetone 15:1 to 10:1) followed by precipitation (dissolved in warm EtOAc (5 mL) and hexane (5 mL) added) and filtration to yield **9** (173 mg, 95%) as a white solid. *R*_f 0.18 (toluene/acetone 10:1). [α]_D²⁰ = +44.0 (*c* = 0.36, CH₂Cl₂). ¹H NMR (400 MHz; CDCl₃) δ 8.10-8.08 (m, 2H, Bz), 7.99-7.90 (m, 22H, Bz), 7.56-7.01 (m, 216H, Ph), 5.60-5.54 (m, 11H, H^{CEGIKMOQS}UW-1), 5.24-5.16 (m, 11H, H^{CEGIKMOQS}UW-2), 5.08-5.07 (m, 1H, H^A-2), 5.04-5.03 (m, 1H, H^A-1), 4.99-4.87 (m, 12H, H^{BDFHJLNPR}TVX-1), 4.84-4.34 (m, 84H, H^{ACEGIKMOQS}UW-5, 36xCH₂Ph), 4.26-4.16 (m, 11H, H^{CEGIKMOQS}UW-3), 4.06-3.18 (m, 121H, H^A-3, H^{ACEGIKMOQS}UW-4, H^{BDFHJLNPR}TVX-2, H^{BDFHJLNPR}TVX-3, H^{BDFHJLNPR}TVX-4, H^{BDFHJLNPR}TVX-5, H^{BDFHJLNPR}TVX-6_{ab}, 12xCOOCH₃), 3.23 (s, 3H, OCH₃), 2.56 (d, *J* = 2.5 Hz, 1H, OH). MALDI TOF MS: m/z: calcd for C₄₉₃H₄₉₆N₃₆NaO₁₃₃ [M+Na]⁺: 9076; found: 9076.

Methyl (2-azido-3,6-di-*O*-benzyl-2-deoxy-4-*O*-trichloroacetyl- α -D-glucopyranosyl)-(1 $\rightarrow$ 4)-(methyl 2-*O*-benzoyl-3-*O*-benzyl- α -L-idopyranosyl uronate)-(1 $\rightarrow$ 4)-2-azido-3,6-di-*O*-benzyl-2-deoxy- α -D-glucopyranosyl-(1 $\rightarrow$ 4)-(methyl 2-*O*-benzoyl-3-*O*-benzyl- α -L-idopyranosyl uronate)-(1 $\rightarrow$ 4)-2-azido-3,6-di-*O*-benzyl-2-deoxy- α -D-glucopyranosyl-(1 $\rightarrow$ 4)-(methyl 2-*O*-benzoyl-3-*O*-benzyl- α -L-idopyranosyl uronate)-(1 $\rightarrow$ 4)-2-azido-3,6-di-*O*-benzyl-2-deoxy- α -D-glucopyranosyl-(1 $\rightarrow$ 4)-(methyl 2-*O*-benzoyl-3-*O*-benzyl- α -L-idopyranosyl uronate)-(1 $\rightarrow$ 4)-2-azido-3,6-di-*O*-benzyl-2-deoxy- α -D-glucopyranosyl-(1 $\rightarrow$ 4)-(methyl 2-*O*-benzoyl-3-*O*-benzyl- α -L-idopyranosyl uronate)-(1 $\rightarrow$ 4)-2-azido-3,6-di-*O*-benzyl-2-deoxy- α -D-glucopyranosyl-(1 $\rightarrow$ 4)-(methyl 2-*O*-benzoyl-3-*O*-benzyl- α -L-idopyranosyl uronate)-(1 $\rightarrow$ 4)-2-azido-3,6-di-*O*-benzyl-2-deoxy- α -D-glucopyranosyl-(1 $\rightarrow$ 4)-

(methyl 2-*O*-benzoyl-3-*O*-benzyl- α -L-idopyranosyl uronate)-(1 $\rightarrow$ 4)-2-azido-3,6-di-*O*-benzyl-2-deoxy- α -D-glucopyranosyl-(1 $\rightarrow$ 4)-(methyl 2-*O*-benzoyl-3-*O*-benzyl- α -L-idopyranosyl uronate)-(1 $\rightarrow$ 4)-2-azido-3,6-di-*O*-benzyl-2-deoxy- α -D-glucopyranosyl-(1 $\rightarrow$ 4)-(methyl 2-*O*-benzoyl-3-*O*-benzyl- α -L-idopyranosyl uronate)-(1 $\rightarrow$ 4)-2-azido-3,6-di-*O*-benzyl-2-deoxy- α -D-glucopyranosyl-(1 $\rightarrow$ 4)-(methyl 2-*O*-benzoyl-3-*O*-benzyl- α -L-idopyranosyl uronate)-(1 $\rightarrow$ 4)-2-azido-3,6-di-*O*-benzyl-2-deoxy- α -D-glucopyranosyl-(1 $\rightarrow$ 4)-(methyl 2-*O*-benzoyl-3-*O*-benzyl- α -L-idopyranosyl uronate)-(1 $\rightarrow$ 4)-2-azido-3,6-di-*O*-benzyl-2-deoxy- α -D-glucopyranosyl-(1 $\rightarrow$ 4)-(methyl 2-*O*-benzoyl-3-*O*-benzyl- α -L-idopyranosyl uronate)-(1 $\rightarrow$ 4)-2-azido-3,6-di-*O*-benzyl-2-deoxy- α -D-glucopyranosyl-(1 $\rightarrow$ 4)-(methyl 2-*O*-benzoyl-3-*O*-benzyl- α -L-idopyranoside) uronate, **10**

Acceptor tetracosasaccharide **9** (161 mg, 0.018 mmol) and thioglycoside tetrasaccharide donor **1** (41 mg, 0.023 mmol) was dissolved in dry DCM (1.5 mL) under N₂. Freshly activated 4Å powdered molecular sieves (174 mg) was added and the solution cooled to 0 °C in an icebath. After 10 min. NIS (15 mg, 0.067 mmol) was added, and after another 10 min. AgOTf (catalytic amount) was added. The suspension changed colour from pale yellow to deep red, was stirred for 30 min. and the reaction was quenched into a separating funnel containing a mixture of DCM (20 mL), saturated aqueous NaHCO₃ (10 mL) and Na₂S₂O₃ (1 mL, 10% aqueous). After shaking until the iodine colour was removed the suspension was filtered through a short pad of Celite® washing with water and DCM. The layers were separated and the aqueous extracted with DCM (10 mL). The organic layers were combined, dried (MgSO₄) and solvent removed *in vacuo*. The crude was purified by silica gel flash column chromatography (toluene/acetone 20:1 to 15:1 to 10:1) followed by precipitation (dissolved in warm EtOAc (5 mL) and hexane (5 mL) added) and filtration to give **10** (143 mg, 75%) as a white solid. Also acceptor starting material (35 mg, 21%) was recovered. *R*_f 0.29 (toluene/acetone 10:1). $[\alpha]_D^{20} = +40.7$ (*c* = 0.36, CH₂Cl₂). ¹H NMR (400 MHz; CDCl₃) δ 8.13-8.11 (m, 2H, Bz), 8.05-8.03 (m, 2H, Bz), 7.98-7.93 (m, 24H, Bz), 7.57-7.05 (m, 252H, Ph), 5.59-5.55 (m, 13H, H^(BCDEFGHIJKLMN)ido-1), 5.34 (t, *J* = 9.6 Hz, 1H, H^(N)gln-4), 5.26-5.22 (m, 13H, H^(BCDEFGHIJKLMN)ido-2), 5.10-5.09 (m, 1H, H^(A)ido-2), 5.07-5.06 (m, 1H, H^(A)ido-1), 4.99-4.88 (m, 14H, H^(ABCDEFGHIJKLMN)gln-1), 4.86-4.33 (m, 98H, H^(ABCDEFGHIJKLMN)ido-5, 42xCH₂Ph), 4.27-4.18 (m, 13H, H^(BCDEFGHIJKLMN)ido-3), 4.12-3.20 (m, 140H, H^(A)ido-3, H^(ABCDEFGHIJKLMN)ido-4, H^(ABCDEFGHIJKLMN)gln-2, H^(ABCDEFGHIJKLMN)gln-3, H^(ABCDEFGHIJKLMN)gln-4, H^(ABCDEFGHIJKLMN)gln-5, H^(ABCDEFGHIJKLMN)gln-6_{ab}, 14xCOOCH₃), 3.25 (s, 3H, OCH₃). MALDI TOF MS: *m/z*: calcd for C₅₇₇H₅₇₇Cl₃N₄₂NaO₁₅₆ [*M* + Na]⁺: 10725; found: 10726.

Methyl (2-azido-3,6-di-*O*-benzyl-2-deoxy- α -D-glucopyranosyl)-(1 $\rightarrow$ 4)-(methyl 2-*O*-benzoyl-3-*O*-benzyl- α -L-idopyranosyl uronate)-(1 $\rightarrow$ 4)-2-azido-3,6-di-*O*-benzyl-2-deoxy- α -D-glucopyranosyl-(1 $\rightarrow$ 4)-(methyl 2-*O*-benzoyl-3-*O*-benzyl- α -L-idopyranosyl uronate)-(1 $\rightarrow$ 4)-2-azido-3,6-di-*O*-benzyl-2-deoxy- α -D-glucopyranosyl-(1 $\rightarrow$ 4)-(methyl 2-*O*-benzoyl-3-*O*-benzyl- α -L-idopyranosyl uronate)-(1 $\rightarrow$ 4)-2-azido-3,6-di-*O*-benzyl-2-deoxy- α -D-glucopyranosyl-(1 $\rightarrow$ 4)-(methyl 2-*O*-benzoyl-3-*O*-benzyl- α -L-idopyranosyl uronate)-(1 $\rightarrow$ 4)-2-azido-3,6-di-*O*-benzyl-2-deoxy- α -D-glucopyranosyl-(1 $\rightarrow$ 4)-(methyl 2-*O*-benzoyl-3-*O*-benzyl- α -L-idopyranosyl uronate)-(1 $\rightarrow$ 4)-2-azido-3,6-di-*O*-benzyl-2-deoxy- α -D-glucopyranosyl-(1 $\rightarrow$ 4)-(methyl 2-*O*-benzoyl-3-*O*-benzyl- α -L-idopyranosyl uronate)-(1 $\rightarrow$ 4)-2-azido-3,6-di-*O*-benzyl-2-deoxy- α -D-glucopyranosyl-(1 $\rightarrow$ 4)-

idopyranosyl uronate)-(1→4)-2-azido-3,6-di-*O*-benzyl-2-deoxy- α -D-glucopyranosyl-(1→4)-(methyl 2-*O*-benzoyl-3-*O*-benzyl- α -L-idopyranosyl uronate)-(1→4)-2-azido-3,6-di-*O*-benzyl-2-deoxy- α -D-glucopyranosyl-(1→4)-(methyl 2-*O*-benzoyl-3-*O*-benzyl- α -L-idopyranoside) uronate, 12

Acceptor octacosasaccharide **11** (147 mg, 0.014 mmol) and thioglycoside tetrasaccharide donor **1** (32 mg, 0.018 mmol) was dissolved in dry DCM (1.5 mL) under N₂. Freshly activated 4Å powdered molecular sieves (125 mg) was added and the solution cooled to 0 °C in an icebath. After 10 min. NIS (10 mg, 0.044 mmol) was added, and after another 10 min. AgOTf (catalytic amount) was added. The suspension changed colour from pale yellow to deep red, was stirred for 30 min. and the reaction was quenched into a separating funnel containing a mixture of DCM (20 mL), saturated aqueous NaHCO₃ (10 mL) and Na₂S₂O₃ (1 mL, 10% aqueous). After shaking until the iodine colour was removed the suspension was filtered through a short pad of Celite® washing with water and DCM. The layers were separated and the aqueous extracted with DCM (5 mL). The organic layers were combined, dried (MgSO₄) and solvent removed *in vacuo*. The crude was purified by silica gel flash column chromatography (toluene/acetone 20:1 to 15:1 to 10:1) followed by precipitation (dissolved in warm EtOAc (4 mL) and hexane (4 mL) added) and filtration to give **12** (132 mg, 78%) as a white solid. Also acceptor starting material (28 mg, 19%) was recovered. *R*_f 0.28 (toluene/acetone 10:1). [α]_D²⁰ = +41.9 (*c* = 0.33, CH₂Cl₂). ¹H NMR (400 MHz; CDCl₃) δ 8.18-8.16 (m, 2H, Bz), 8.10-8.08 (m, 2H, Bz), 8.03-7.97 (m, 28H, Bz), 7.63-7.10 (m, 288H, Ph), 5.65-5.60 (m, 15H, H^(BCDEFGHIJKLMNOP)ido-1), 5.39 (t, *J* = 9.6 Hz, 1H, H^{Pglcn}-4), 5.27-5.23 (m, 15H, H^(BCDEFGHIJKLMNOP)ido-2), 5.15-5.14 (m, 1H, H^{Aido}-2), 5.12-5.11 (m, 1H, H^{Aido}-1), 5.02-4.94 (m, 16H, H^(ABCDEFGHIJKLMNPO)gln-1), 4.92-4.38 (m, 112H, H^(ABCDEFGHIJKLMNPO)ido-5, 48xCH₂Ph), 4.32-4.22 (m, 15H, H^(BCDEFGHIJKLMNOP)ido-3), 4.14-3.22 (m, 160H, H^{Aido}-3, H^(ABCDEFGHIJKLMNPO)ido -4, H^(ABCDEFGHIJKLMNPO)gln-2, H^(ABCDEFGHIJKLMNPO)gln-3, H^(ABCDEFGHIJKLMO)gln -4, H^(ABCDEFGHIJKLMNPO)gln-5, H^(ABCDEFGHIJKLMNPO)gln-6_{ab}, 16xCOOCH₃), 3.28 (s, 3H, OCH₃). MALDI TOF MS: *m/z*: calcd for C₆₅₉H₆₅₉Cl₃N₄₈NaO₁₇₈ [*M*+Na]⁺: 12229; found: 12230.

[illegible]

(methyl 2-*O*-benzoyl-3-*O*-benzyl- α -L-idopyranosyl uronate)-(1 $\rightarrow$ 4)-2-azido-3,6-di-*O*-benzyl-2-deoxy- α -D-glucopyranosyl-(1 $\rightarrow$ 4)-(methyl 2-*O*-benzoyl-3-*O*-benzyl- α -L-idopyranoside) uronate, 14

Acceptor dotriacontasaccharide **13** (93 mg, 0.008 mmol) and thioglycoside tetrasaccharide donor **1** (20 mg, 0.011 mmol) was dissolved in dry DCM (1 mL) under N₂. Freshly activated 4Å powdered molecular sieves (74 mg) was added and the solution cooled to 0 °C in an icebath. After 10 min. NIS (5 mg, 0.022 mmol) was added, and after another 10 min. AgOTf (catalytic amount) was added. The suspension changed colour from pale yellow to deep red, was stirred for 30 min. and the reaction was quenched into a separating funnel containing a mixture of DCM (20 mL), saturated aqueous NaHCO₃ (10 mL) and Na₂S₂O₃ (1 mL, 10% aqueous). After shaking until the iodine colour was removed the suspension was filtered through a short pad of Celite® washing with water and DCM. The layers were separated and the aqueous extracted with DCM (5 mL). The organic layers were combined, dried (MgSO₄) and solvent removed *in vacuo*. The crude was purified by preparative TLC (toluene/acetone 12:1) followed by precipitation (dissolved in warm EtOAc (4 mL) and hexane (4 mL) added) and filtration to give **14** (76 mg, 72%) as a white solid. Also acceptor starting material (22mg, 23%) was recovered. *R_f* 0.28 (toluene/acetone 10:1). [α]_D²⁰ = +38.8 (*c* = 0.45, CH₂Cl₂). ¹H NMR (800 MHz; CDCl₃) δ 8.09-8.08 (m, 2H, Bz), 8.01-8.00 (m, 2H, Bz), 7.95-7.91 (m, 32H, Bz), 7.54-7.02 (m, 324H, Ph), 5.57-5.52 (m, 17H, H(BCDEFGHIJKLMNOPQR)ido-1), 5.30 (t, *J* = 9.6 Hz, 1H, H^Rglcn-4), 5.18-5.14 (m, 17H, H(BCDEFGHIJKLMNOPQR)ido-2), 5.08-5.07 (m, 1H, H^Aido-2), 5.04-5.03 (m, 1H, H^Aido-1), 4.94-4.87 (m, 18H, H(ABCDEFGHIJKLMNPOQR)glcn-1), 4.81-4.32 (m, 126H, H(ABCDEFGHIJKLMNPOQR)ido-5, 54xCH₂Ph), 4.26-4.15 (m, 17H, H(BCDEFGHIJKLMNOPQR)ido-3), 4.05-3.18 (m, 176H, H^Aido-3, H(ABCDEFGHIJKLMNPOQR)ido -4, H(ABCDEFGHIJKLMNPOQR)glcn-2, H(ABCDEFGHIJKLMNPOQR)glcn-3, H(ABCDEFGHIJKLMOPQ)glcn -4, H(ABCDEFGHIJKLMNPOQR)glcn-5, H(ABCDEFGHIJKLMNPOQR)glcn-6_{ab}, 18xCOOCH₃), 3.24 (s, 3H, OCH₃). ¹³C NMR (201 MHz; CDCl₃): δ 169.6, 169.4, 165.7, 165.2, 138.0, 137.9, 137.5, 137.3, 136.9, 133.7, 133.5, 130.0, 129.9, 129.4, 129.0, 128.8, 128.5, 128.4, 128.1, 128.0, 127.9, 127.7, 127.6, 127.4, 99.3, 98.4, 98.1, 78.2, 77.0, 77.0, 76.1, 75.9, 75.6, 75.4, 75.1, 74.9, 74.5, 74.3, 73.9, 73.75, 73.5, 72.7, 72.6, 71.4, 70.8, 70.7, 69.8, 69.3, 67.4, 63.5, 63.1, 52.0, 51.7. MALDI TOF MS: *m/z*: calcd for C₇₄₁H₇₄₁Cl₃N₅₄NaO₂₀₀ [*M*+Na]⁺: 13732; found: 13729.

[illegible]

idopyranosyl uronate)-(1→4)-2-azido-3,6-di-*O*-benzyl-2-deoxy- α -D-glucopyranosyl-(1→4)-(methyl 2-*O*-benzoyl-3-*O*-benzyl- α -L-idopyranosyl uronate)-(1→4)-2-azido-3,6-di-*O*-benzyl-2-deoxy- α -D-glucopyranosyl-(1→4)-(methyl 2-*O*-benzoyl-3-*O*-benzyl- α -L-idopyranosyl uronate)-(1→4)-2-azido-3,6-di-*O*-benzyl-2-deoxy- α -D-glucopyranosyl-(1→4)-(methyl 2-*O*-benzoyl-3-*O*-benzyl- α -L-idopyranosyl uronate)-(1→4)-2-azido-3,6-di-*O*-benzyl-2-deoxy- α -D-glucopyranosyl-(1→4)-(methyl 2-*O*-benzoyl-3-*O*-benzyl- α -L-idopyranosyl uronate)-(1→4)-2-azido-3,6-di-*O*-benzyl-2-deoxy- α -D-glucopyranosyl-(1→4)-(methyl 2-*O*-benzoyl-3-*O*-benzyl- α -L-idopyranoside) uronate, **16**

Acceptor hexatriacontasaccharide **15** (42 mg, 0.0031 mmol) and thioglycoside tetrasaccharide donor **1** (8 mg, 0.0046 mmol) was dissolved in dry DCM (0.5 mL) under N₂. Freshly activated 4Å powdered molecular sieves (66 mg) was added and the solution cooled to 0 °C in an icebath. After 10 min. NIS (5 mg, 0.022 mmol) was added, and after another 10 min. AgOTf (catalytic amount) was added. The suspension changed colour from pale yellow to deep red, was stirred for 30 min. and the reaction was quenched into a separating funnel containing a mixture of DCM (20 mL), saturated aqueous NaHCO₃ (10 mL) and Na₂S₂O₃ (1 mL, 10% aqueous). After shaking until the iodine colour was removed the suspension was filtered through a short pad of Celite® washing with water and DCM. The layers were separated and the aqueous extracted with DCM (5 mL). The organic layers were combined, dried (MgSO₄) and solvent removed *in vacuo*. The crude product was purified using flash column chromatography (toluene/DCM/acetone 24:6:1 to 16:4:1 to 12:3:1) followed by precipitation (dissolved in warm EtOAc (2 mL) and hexane (1.5 mL) added) and filtration to give tetracontasaccharide **16** (30 mg, 64%) as a white solid. Also acceptor starting material (11 mg, 26%) was recovered. *R_f* 0.26 (toluene/acetone 10:1). ¹H NMR (800 MHz; CDCl₃) δ 8.10-8.09 (m, 2H, Bz), 8.02-8.01 (m, 2H, Bz), 7.95-7.91 (m, 36H, Bz), 7.55-7.02 (m, 360H, Ph), 5.58-5.53 (m, 19H, H^(BCDEFGHIJKLMNOPQRST)ido-1), 5.32 (t, *J* = 9.6 Hz, 1H, H^{Tg}lcn-4), 5.19-5.15 (m, 19H, H^(BCDEFGHIJKLMNOPQRST)ido-2), 5.08-5.07 (m, 1H, H^{Aido}-2), 5.05-5.04 (m, 1H, H^{Aido}-1), 4.95-4.89 (m, 20H, H^(ABCDEFGHIJKLMNQRST)glen-1), 4.81-4.33 (m, 140H, H^(ABCDEFGHIJKLMNQRST)ido-5, 60xCH₂Ph), 4.25-4.15 (m, 19H, H^(BCDEFGHIJKLMNOPQRST)ido-3), 4.06-3.16 (m, 200H, H^{Aido}-3, H^(ABCDEFGHIJKLMNQRST)ido-4, H^(ABCDEFGHIJKLMNQRST)glen-2, H^(ABCDEFGHIJKLMNQRST)glen-3, H^(ABCDEFGHIJKLMNQRST)glen-4, H^(ABCDEFGHIJKLMNQRST)glen-5, H^(ABCDEFGHIJKLMNQRST)glen-6_{ab}, 20xCOOCH₃), 3.23 (s, 3H, OCH₃). ¹³C NMR (201 MHz; CDCl₃): δ 169.4, 165.2, 138.0, 137.9, 137.5, 133.7, 129.9, 129.4, 128.8, 128.5, 128.1, 128.0, 127.9, 127.7, 127.6, 127.4, 99.3, 98.1, 78.1, 76.1, 75.6, 75.4, 74.5, 74.3, 73.8, 72.7, 72.6, 71.5, 70.8, 70.7, 67.7, 67.4, 63.5, 63.3, 63.1, 52.1, 52.0, 51.8. MALDI TOF MS: *m/z*: calcd for C₈₂₃H₈₂₃Cl₃N₆₀NaO₂₂₂ [*M*+Na]⁺: 15236; found: 15248.

Synthesis of icosasaccharide **18**

a) The icosasaccharide **6** (40 mg, 0.0052 mmol) was dissolved in THF (2 mL) and MeOH (0.5 mL) and then cooled to 0 °C in an icebath. Then LiOH·H₂O (14 mg, 0.33 mmol) dissolved in 0.5 mL water was added in portions over 20 min. The solution was stirred 12 h slowly warming to room temperature. The solution was then extracted with EtOAc (30 mL) and HCl (0.1 M, 20 mL). The organic phase was washed with water (2x10 mL), dried (MgSO₄), filtered and evaporated. The crude was purified using flash column chromatography (DCM/MeOH gradient 40:1 to 30:1). This

yielded the intermediate carboxylic acid icosasaccharide (16.0 mg) as solid. R_f 0.21 (DCM/MeOH 15:1).

b) The icosasaccharide acid (7.0 mg, 1.1 μ mol) was dissolved in dry DMF (0.5 mL) under N_2 in a microwave compatible tube. $SO_3 \cdot NMe_3$ (8.5 mg, 60.5 μ mol) was added and the solution heated in a microwave reactor for 1 h at 100 °C. Tlc analysis (DCM:MeOH, 5:1) showed one product spot, a few drops of Et_3N were added (to neutralize any H_2SO_4 formed) and the reaction loaded onto a column of Sephadex LH20. The column was eluted with DCM/MeOH (9:1) and the fractions containing the oligosaccharide pooled and then passed through an additional column containing Amberlite IRC 86 Na^+ resin to convert the triethylammonium salts to sodium salt. This yielded 2-*O*-sulfated icosasaccharide **17** (7.5 mg) as a glassy solid. R_f = 0.21 (DCM:MeOH 5:1).

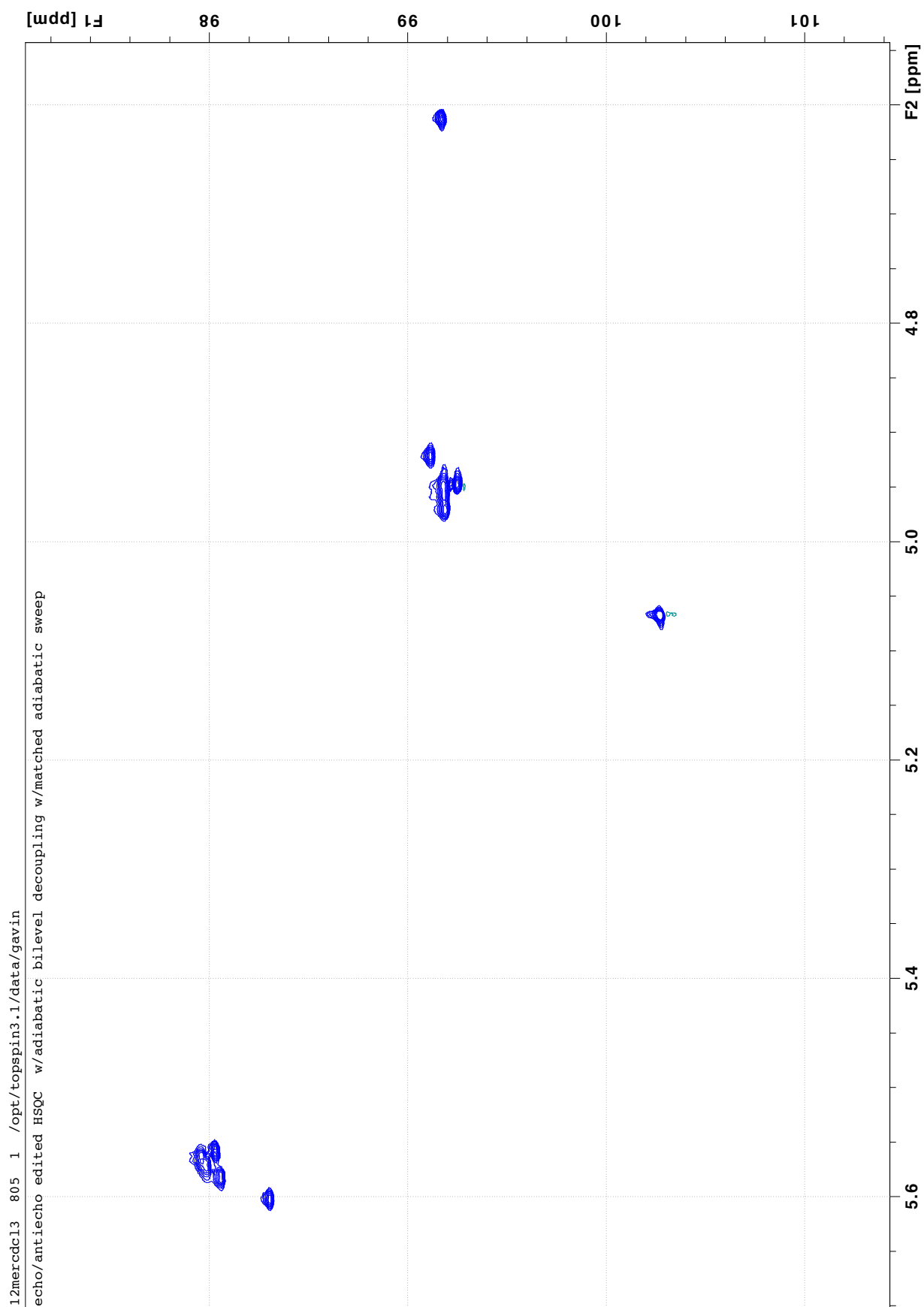
c) The icosasaccharide **17** (8 mg, 0.0010 mmol) was dissolved in a mixture of EtOH/ H_2O (1 mL / 1 mL). Then a three way tap was attached to the flask and fitted with a nitrogen balloon and the other tap was attached to a water aspirator vacuum. Switching between vacuum and nitrogen balloon 5 times ensured removal of all oxygen from flask and solvent. Then was added $Pd(OH)_2/C$ (32 mg, 10-20%) and again flushed with nitrogen. The nitrogen balloon was replaced with a hydrogen balloon and the flask again subjected to vacuum and hydrogen 5 times to ensure all the nitrogen was replaced with hydrogen. The reaction was heated to 50 °C for 36 h with vigorous stirring. The product mixture was filtered through Celite and washed with EtOH/water 1:2 (3x2 mL). The combined filtrate was then evaporated and purified by a short Sephadex G-25 column to give icosasaccharide amine **18** (4.1 mg, 38% 3 steps) as a glassy solid. $[\alpha]_D^{20} = +57.3$ ($c = 0.18$, H_2O). NMR data ring assignments coded from reducing terminal L-ido as ring A. 1H NMR (400 MHz; D_2O) δ 5.35-5.29 (m, 9H, $H^{CEGIKMOQS-1}$), 5.22-5.16 (m, 10H, $H^{BDFHJLNPRT-1}$), 5.01-4.99 (m, 1H, H^A-1), 4.89-4.85 (m, 9H, $H^{CEGIKMOQS-5}$), 4.50-4.49 (m, 1H, H^A-5), 4.35-4.17 (m, 21H, $H^{ACEGIKMOQS-2}$, $H^{ACEGIKMOQS-3}$, H^T-4), 4.13-4.07 (m, 10H, $H^{ACEGIKMOQS-4}$), 3.90-3.66 (m, 49H, $H^{BDFHJLNPRT-3}$, $H^{BDFHJLNPRT-4}$, $H^{BDFHJLNPRT-5}$, $H^{BDFHJLNPRT-6_{ab}}$), 3.39 (s, 3H, OMe), 3.26-3.18 (m, 10H, $H^{BDFHJLNPRT-2}$). ^{13}C NMR (201 MHz; D_2O): δ 175.3, 99.6, 99.0, 98.8, 91.7, 76.2, 76.0, 72.5, 71.4, 70.3, 70.1, 68.6, 66.9, 66.8, 66.2, 62.8, 62.6, 59.7, 59.3, 54.6, 54.1. NSI MS: m/z : calcd for $C_{121}H_{185}N_{10}O_{134}S_{11}$ [$M-9H$] $^{9+}$: 474.8327; found: 474.8324 (Na^+ ion exchanged with NH_4^+ before submitting for MS).[See **Figure 8**]

Synthesis of icosasaccharide 19

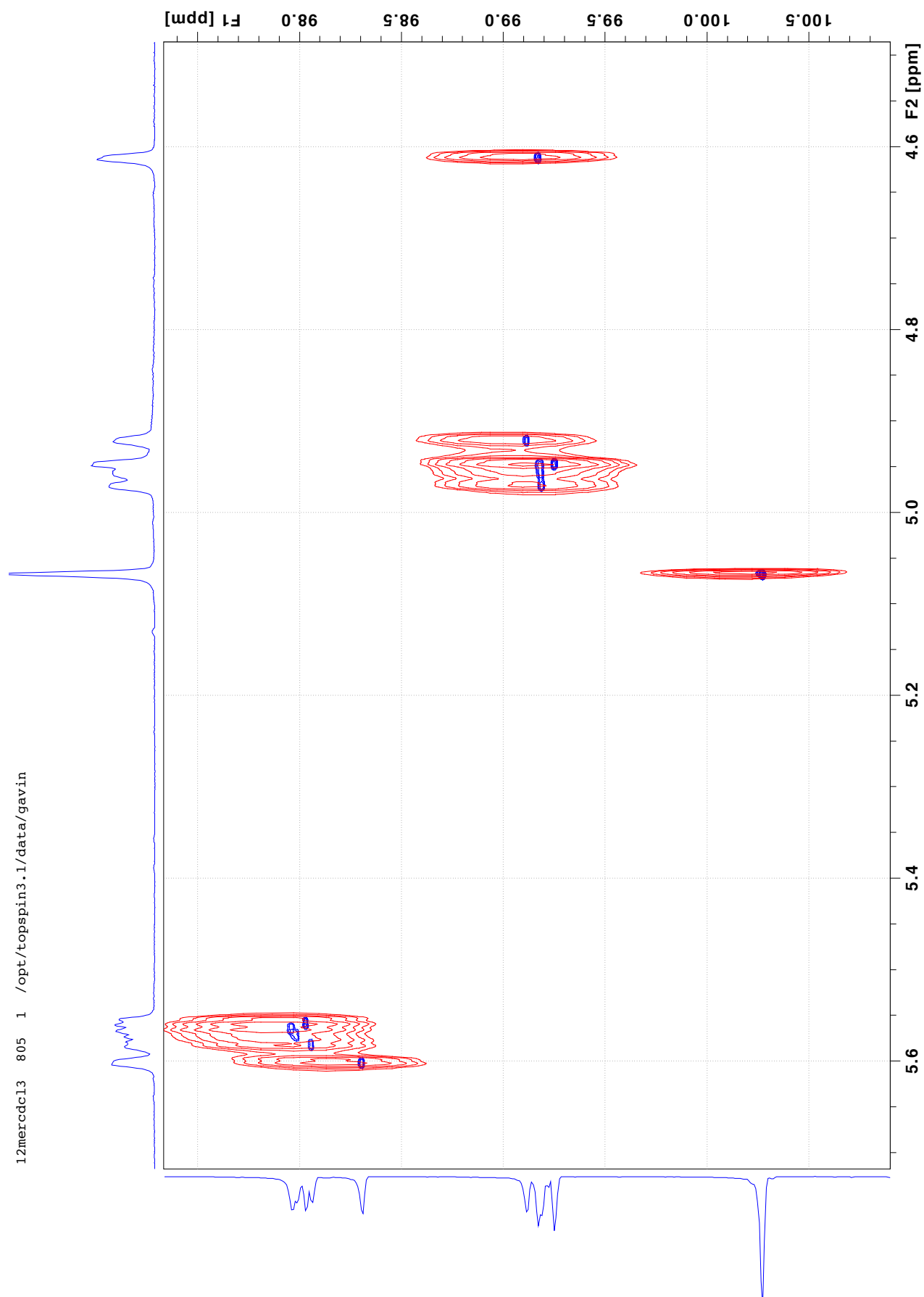
The icosasaccharide **18** (2.3 mg, 0.51 μ mol) was dissolved in water (0.5 mL), $NaHCO_3$ (2.8 mg, 0.0335 mmol) and pyridine sulfur trioxide complex (2.4 mg, 0.0152 mmol) was added with stirring. This procedure was repeated after 1 h, 2 h, 4 h, 5 h, 7 h, 10 h, 12 h and 16 h ($NaHCO_3$: 6.7 mg, 9 mg, 8.5 mg, 10 mg, 7.3 mg, 13 mg, 9 mg. $Py \cdot SO_3$: 6.5 mg, 7 mg, 5.7 mg, 6 mg, 5 mg, 10 mg, 5 mg.). After 20 h the mixture was evaporated. The crude containing Na_2SO_4 salts was redissolved in minimum amount of water and purified by passage through a Sephadex G-25 column (18x2.5 cm) by eluting with water. The fractions containing oligosaccharide were pooled and evaporated to yield **19** (2.7 mg, 93%) as a glassy solid. $[\alpha]_D^{20} = +64.8$ ($c = 0.13$, H_2O). 1H NMR (800 MHz; D_2O) δ 5.36-5.25 (m, 19H, $H^{BCDEFGHIJKLMNOPQRST-1}$), 5.04-5.03 (m, 1H, H^A-1), 4.89-4.85 (m, 9H, $H^{CEGIKMOQS-5}$), 4.45 (d, $J = 2.4$ Hz, 1H, H^A-5), 4.37-4.29 (m, 9H, $H^{CEGIKMOQS-2}$), 4.27-4.21 (m, 11H, H^A-2 , $H^{ACEGIKMOQS-3}$, H^T-4), 4.06-4.02 (m, 10H, $H^{ACEGIKMOQS-4}$), 3.93-3.81 (m, 30H, $H^{BDFHJLNPRT-5}$, $H^{BDFHJLNPRT-6_{ab}}$), 3.75-3.68 (m, 20H, $H^{BDFHJLNPRT-3}$, $H^{BDFHJLNPRT-4}$), 3.43 (s, 3H, OMe), 3.33-3.21 (m, 10H, $H^{BDFHJLNPRT-2}$). ^{13}C NMR (200 MHz; D_2O): δ 175.7, 99.8, 99.2, 97.3, 96.9, 77.3, 77.1, 75.8, 75.6, 75.0, 74.5, 71.8, 71.5, 71.3, 71.1, 69.5, 68.3, 68.2, 67.6, 59.7, 58.4, 58.3, 57.9, 55.3.

2. Spectral Data

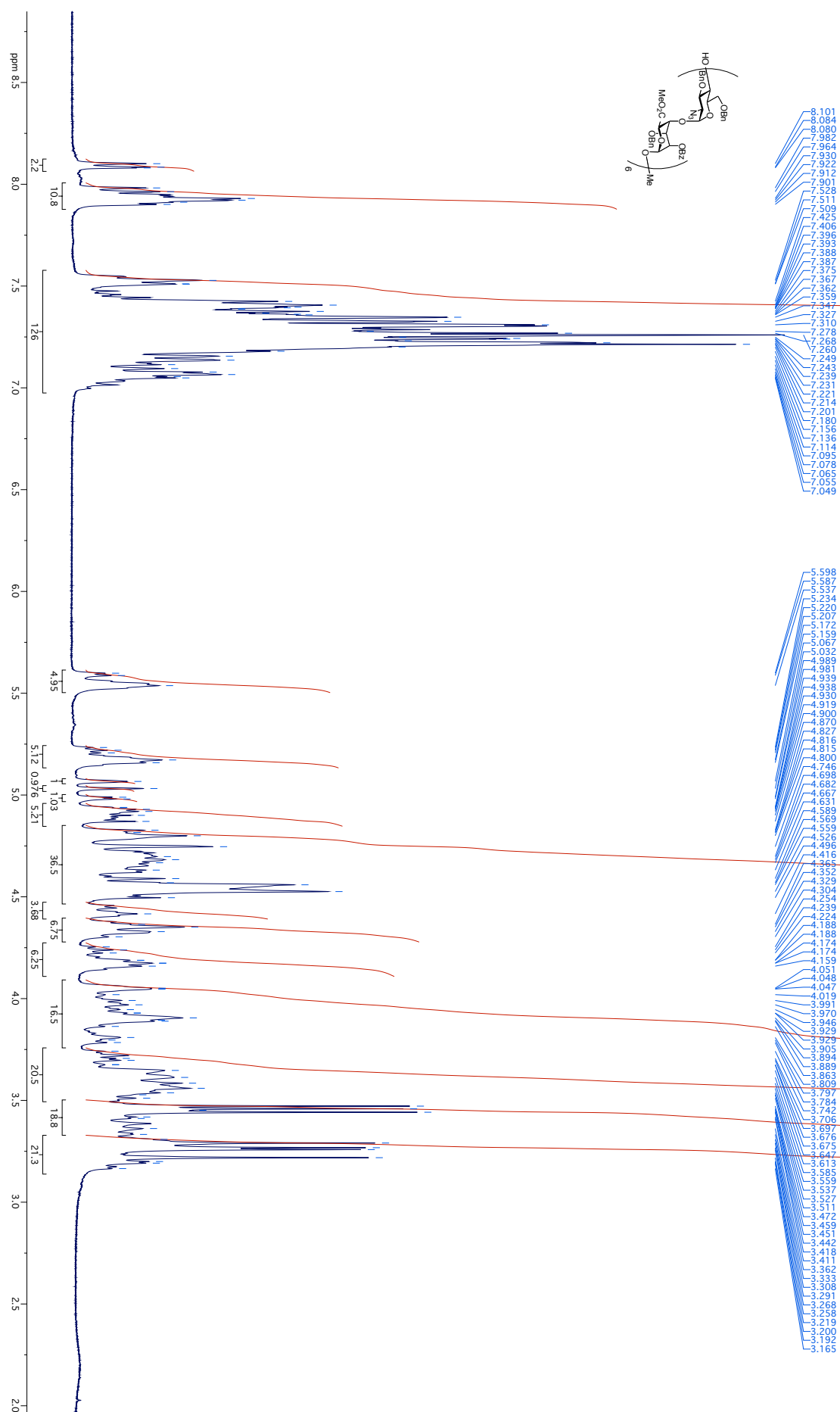
Supplementary Figure 1: NUS Pure Shift HSQC (800 MHz, CDCl₃) for **2** (2centres region)



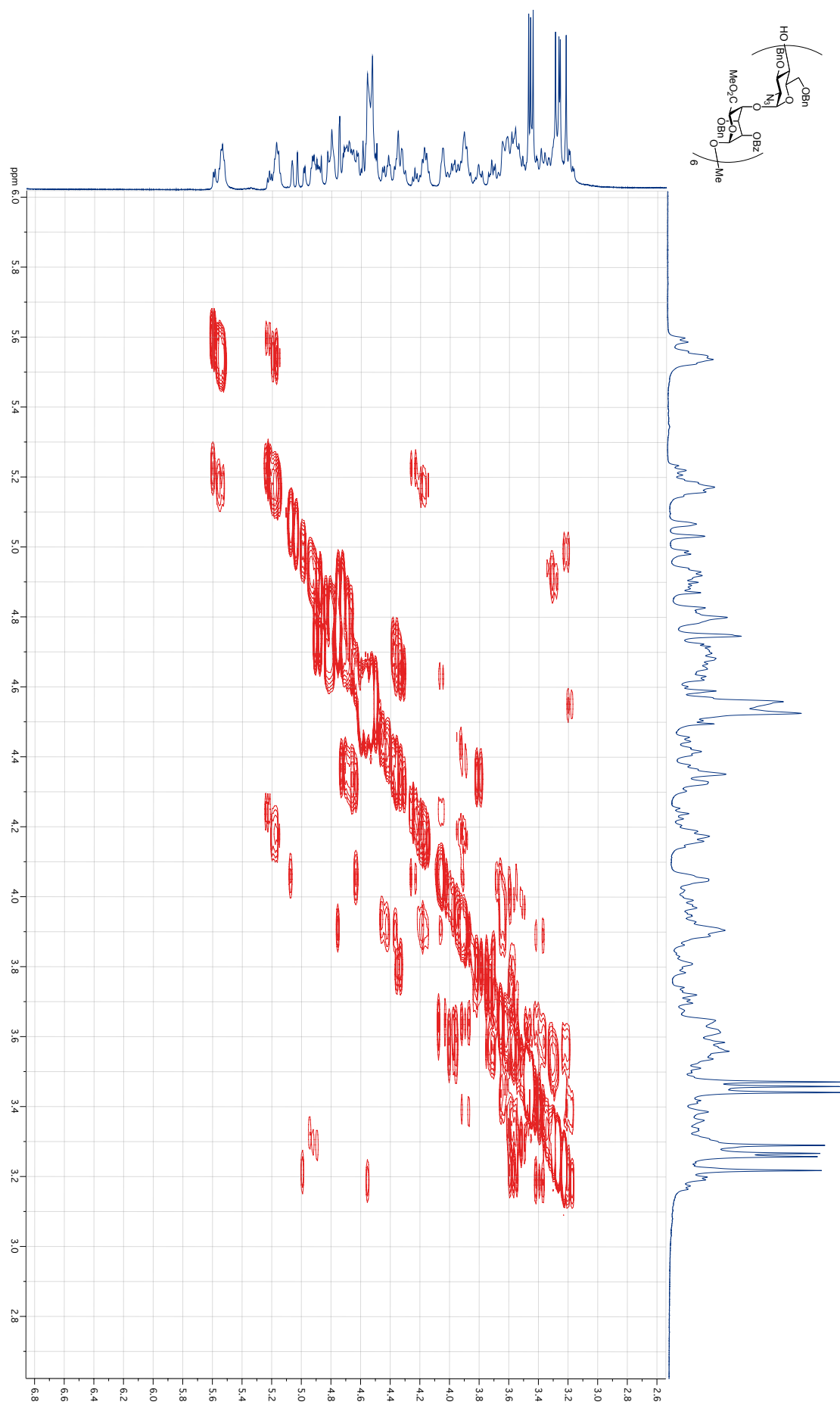
Supplementary Figure 2: NUS Pure Shift HSQC (800 MHz, CDCl_3) for **2** (anomeric centres region, comparison to standard HSQC (red))



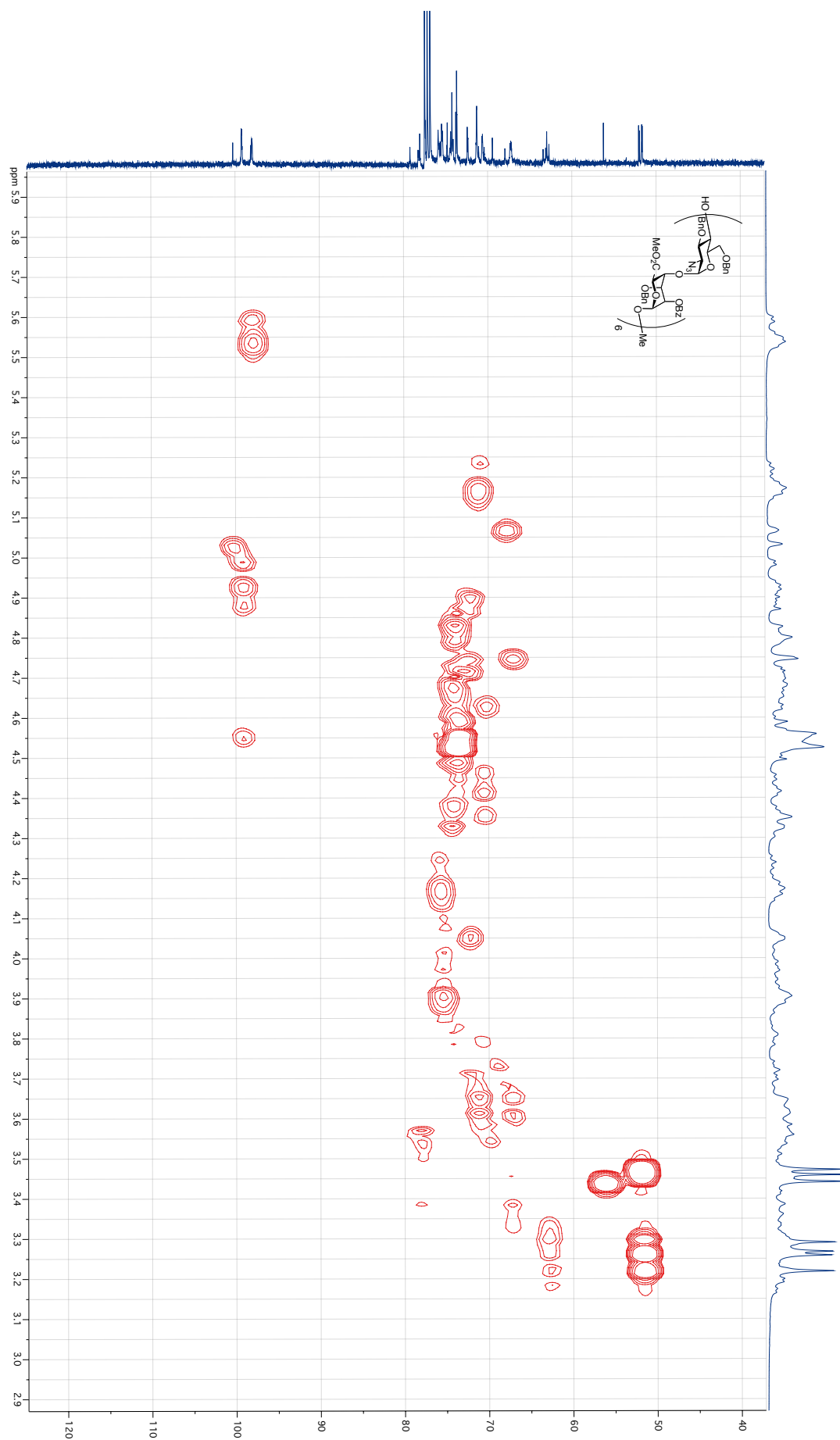
Supplementary Figure 3: ^1H NMR (400 MHz; CDCl_3) spectrum for **3**



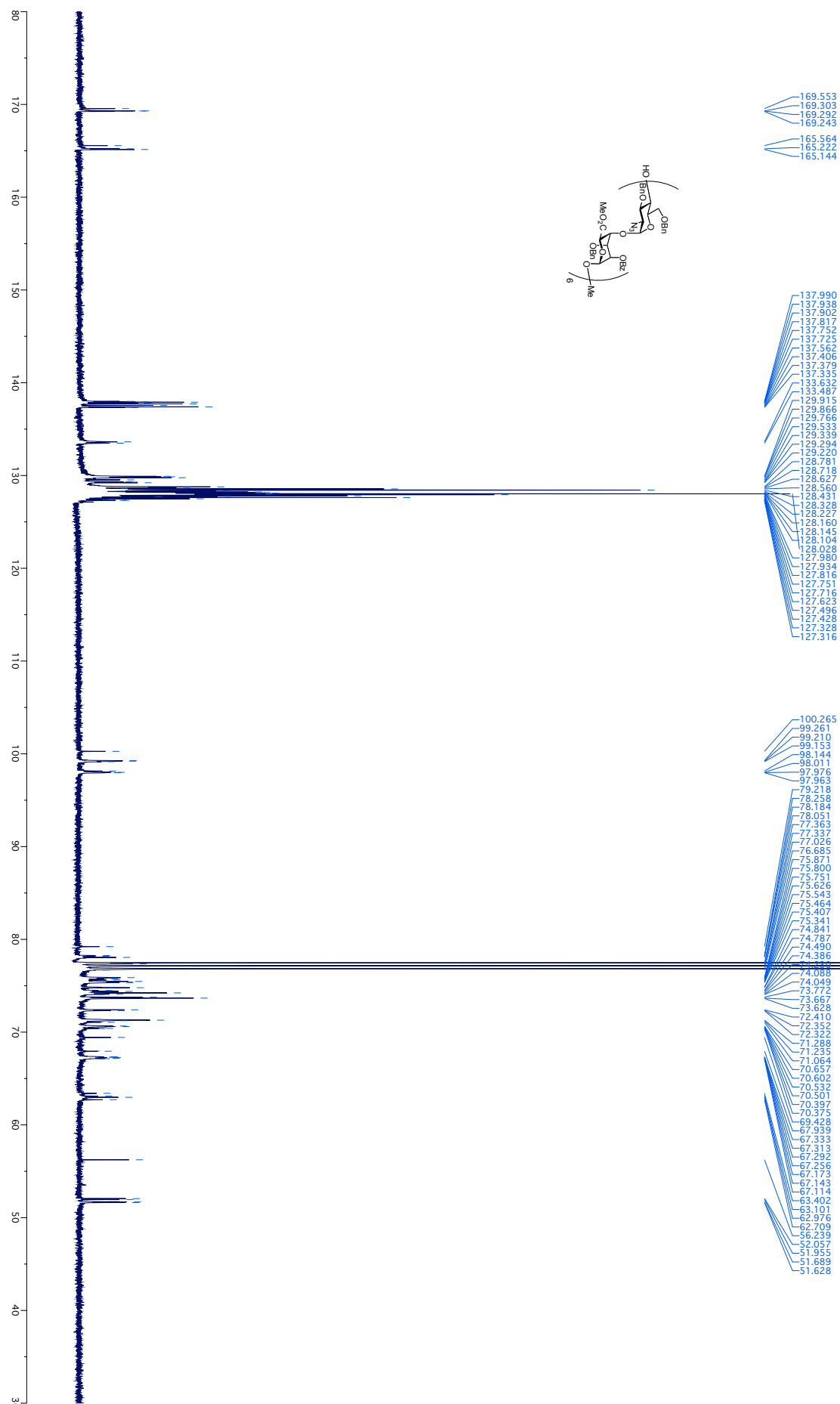
Supplementary Figure 4: COSY NMR (400 MHz; CDCl₃) spectrum for **3** **S1512**



Supplementary Figure 5: HMQC NMR (400 MHz; CDCl_3) spectrum for **3**



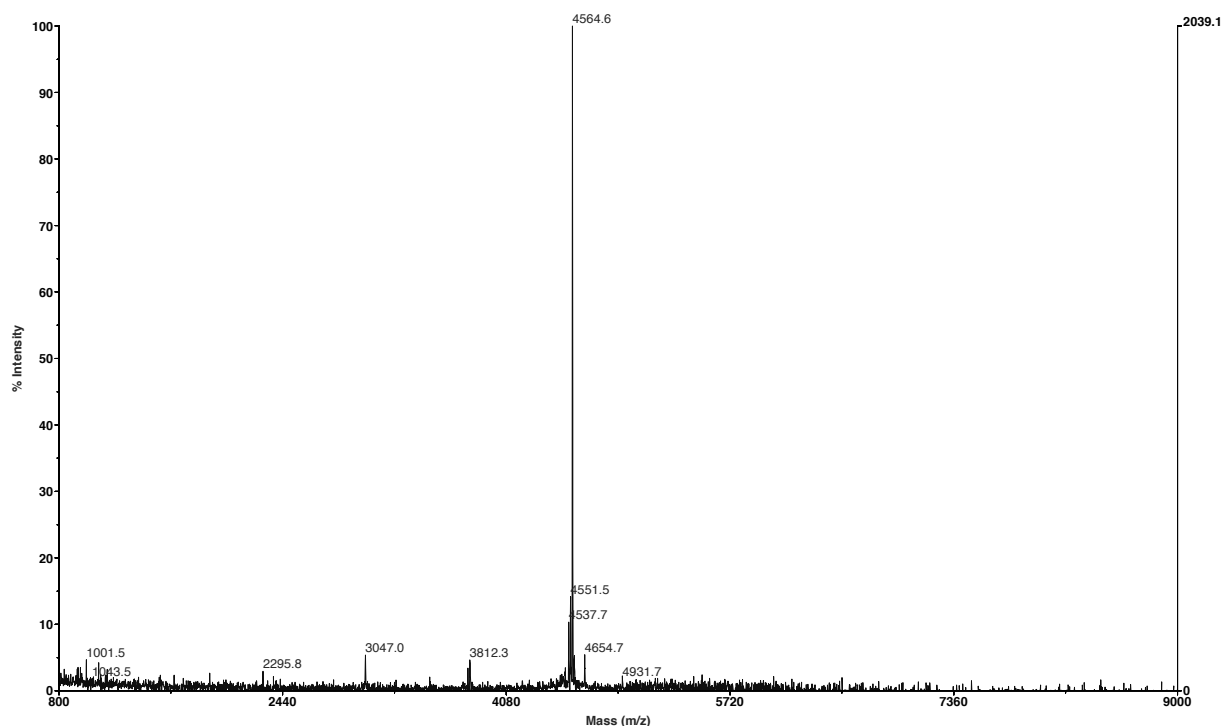
Supplementary Figure 6: ^{13}C NMR (100 MHz; CDCl_3) spectrum for **3**



Supplementary Figure 7: MALDI MS and isotope pattern for 3

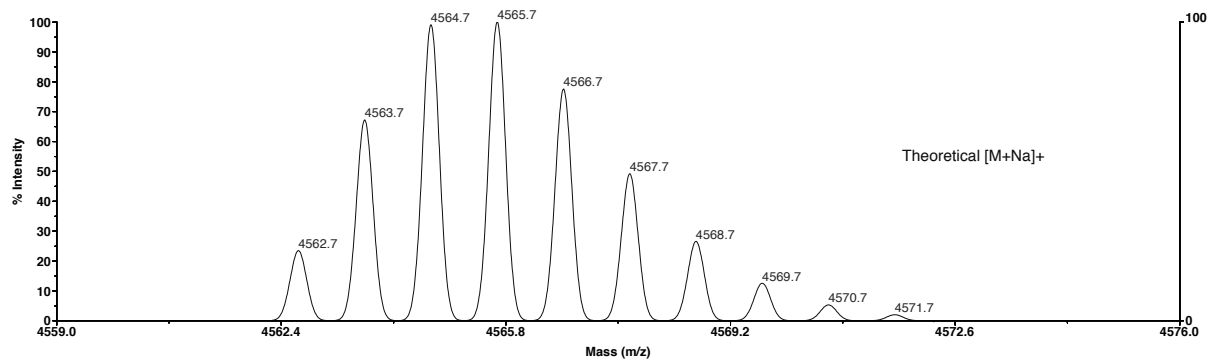
EPSRC National Mass Spectrometry Service Centre (NMSSC), Swansea

<<MANGAR173-VM-MAP_0001>> Voyager Spec #1=>AdvBC(64,0.5,0.1)=>SM5[BP = 4564.5, 2039]

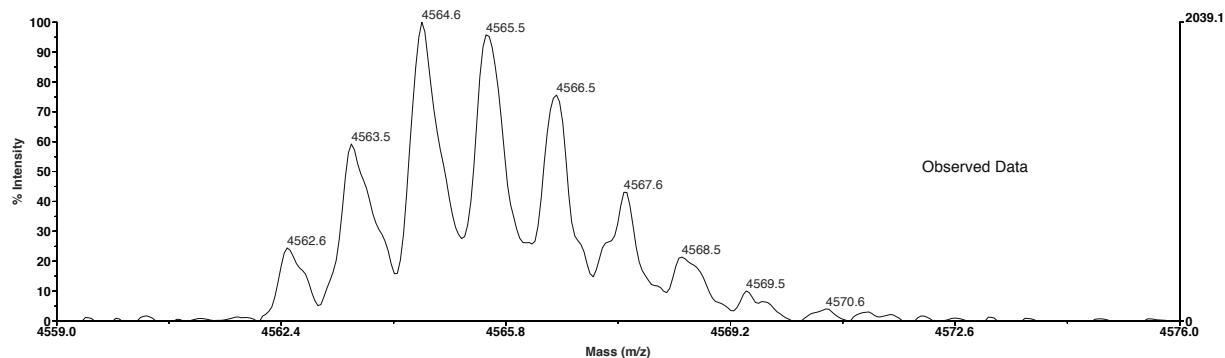


EPSRC National Mass Spectrometry Service Centre (NMSSC), Swansea

ISO:C247H250N18O67 + (Na)1



<<MANGAR173-VM-MAP_0001>> Voyager Spec #1=>AdvBC(64,0.5,0.1)=>SM5[BP = 4564.5, 2039]



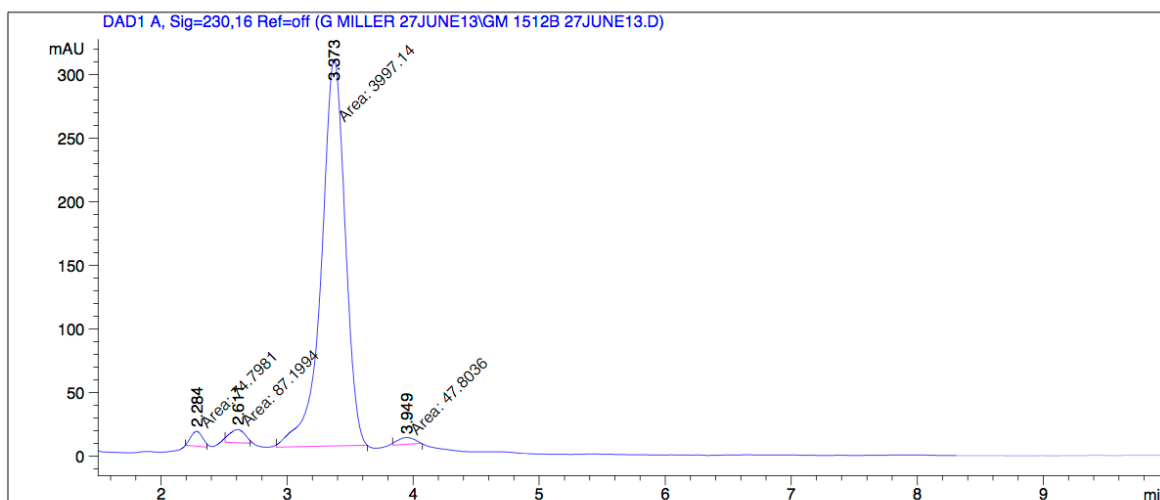
Acquired: 16:06:00, July 19, 2012
Hansen SU1421 MW=4539?? DCM PosRef [1:49] (DCB;DCM) +NaOAc
D:\2012Jul12\MANGAR173-VM-MAP_0001.dat

Printed: 10:24, July 20, 2012

Supplementary Figure 8: LCMS for 3

Data File C:\HPCHEM\1\DATA\G MILLER 27JUNE13\GM 1512B 27JUNE13.D
Sample Name: GM 1512B 27June13

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Acq. Instrument : Instrument 1                 Location  : Vial 72
Injection Date  : 28/06/2013 12:57:52         Inj       :    1
                                           Inj Volume: 20 µl
                                           Actual Inj Volume: 5 µl
Different Inj Volume from Sequence !
Acq. Method     : C:\HPCHEM\1\METHODS\GAVIN MILLER 3CM COL 100ACN.M
Last changed    : 28/06/2013 12:56:57 by Rehana
Analysis Method : C:\HPCHEM\1\METHODS\REHANA 60MEOH WITH COL.m
Last changed    : 16/07/2013 12:16:45 by Rehana
Sample Info     : Gavin Miller   GM 1512B      27th June 2013
                  Supelco Ascentis C8 5u  4.6x30mm
                  ESI source +ve ES 230nm 0.5ml/min
                  100ACN only  5ul inj
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Area Percent Report

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Sorted By      :      Signal
Multiplier     :      1.0000
Dilution       :      1.0000
Use Multiplier & Dilution Factor with ISTDs
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Signal 1: DAD1 A, Sig=230,16 Ref=off

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	2.284	MM	0.1062	74.79813	11.73921	1.7780
2	2.611	MM	0.1417	87.19936	10.25990	2.0728
3	3.373	MM	0.2189	3997.13647	304.35858	95.0130
4	3.949	MM	0.1502	47.80359	5.30468	1.1363

Totals : 4206.93755 331.66238

*** End of Report ***

Supplementary Figure 9: LCMS for 3

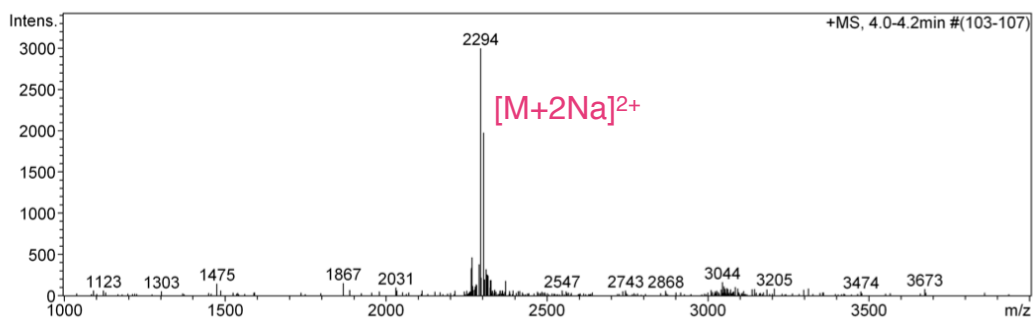
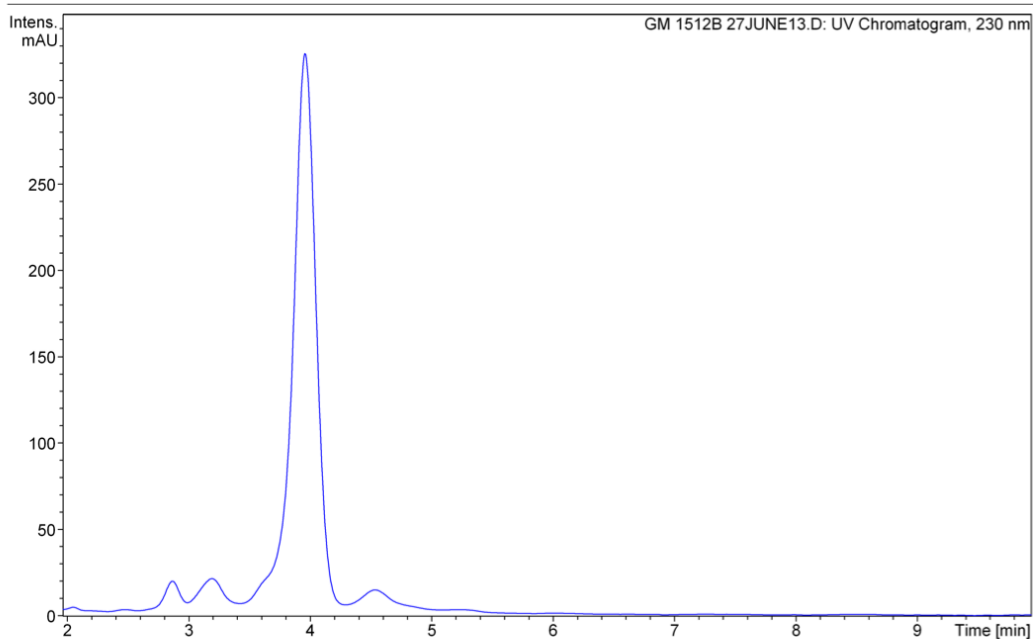
Display Report

Analysis Info

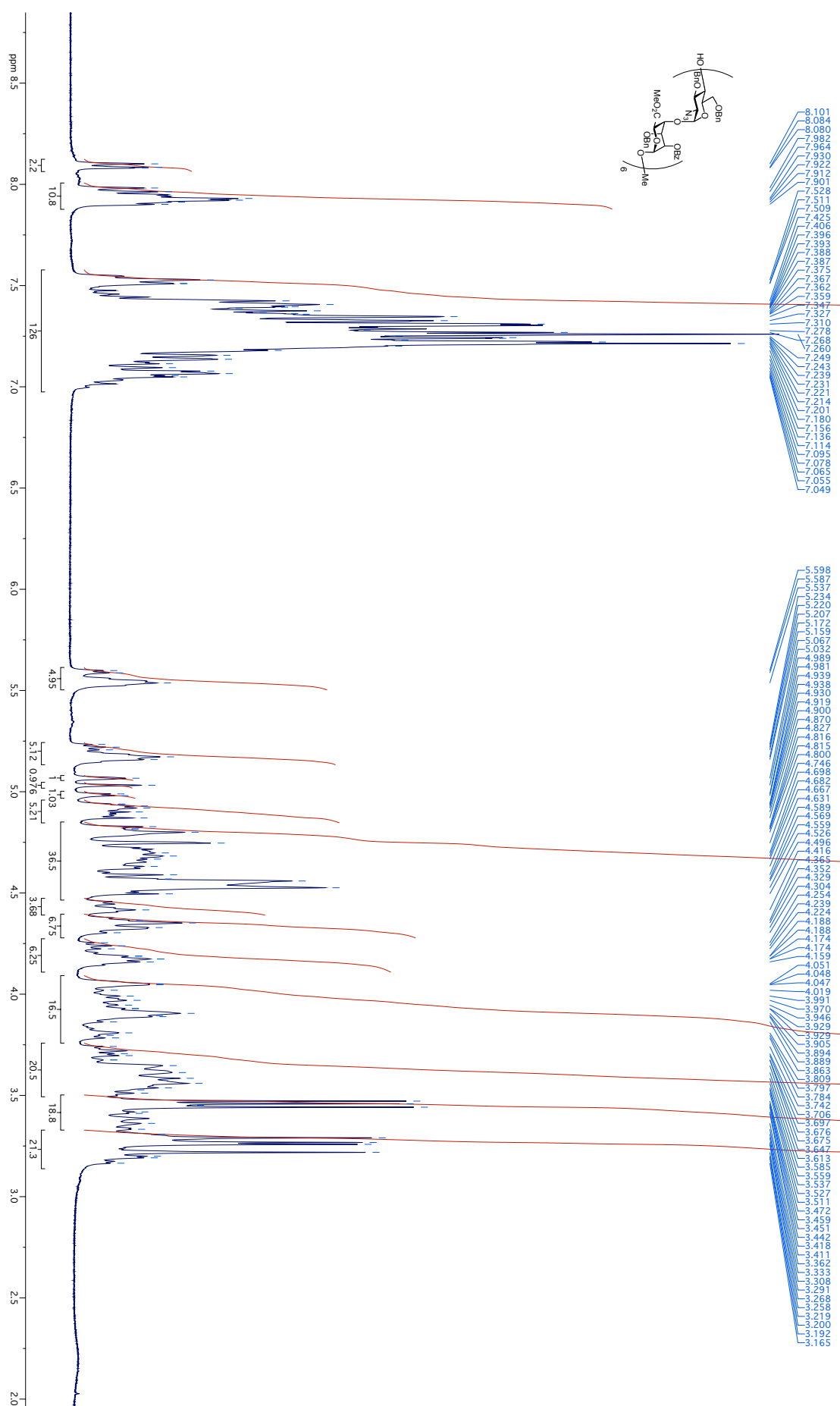
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Method	GAVIN MILLER 3CM COL 100ACN.M	Operator	mib
Sample Name	GM 1512B 27June13	Instrument	LC-MSD-Trap-SL
Comment	Gavin Miller GM 1512B 27th June 2013 Supelco Ascentis C8 5u 4.6x30mm ESI source +ve ES 230nm 0.5ml/min 100ACN only 5ul inj		

Acquisition Parameter

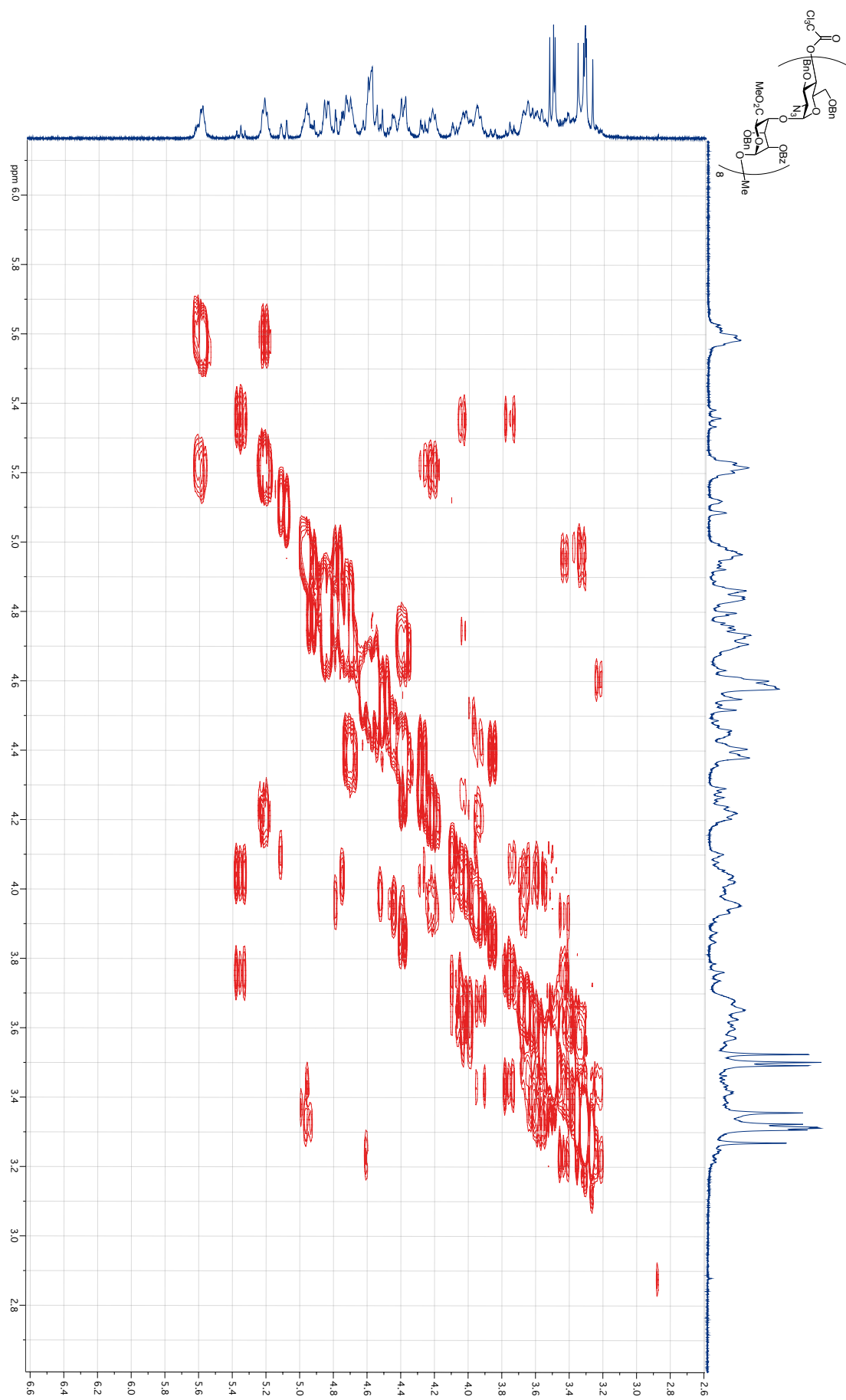
Ion Source Type	ESI	Ion Polarity	Positive	Alternating Ion Polarity	off
Mass Range Mode	Extended	Scan Begin	1000 m/z	Scan End	4000 m/z
Capillary Exit	280.0 Volt	Skim 1	40.0 Volt	Trap Drive	218.9
Accumulation Time	198516 μ s	Averages	7 Spectra	Auto MS/MS	off



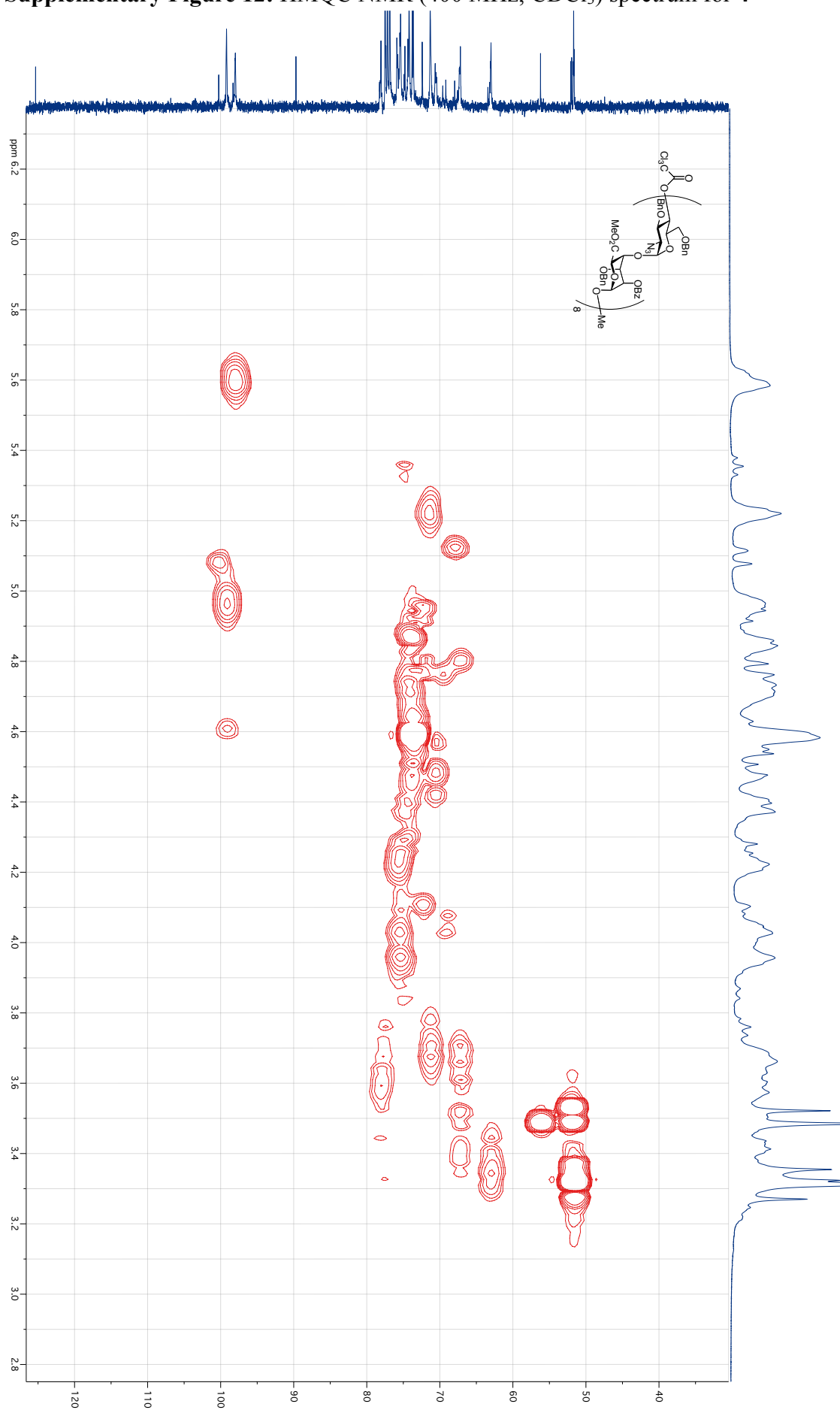
Supplementary Figure 10: ^1H NMR (400 MHz; CDCl_3) spectrum for **4**



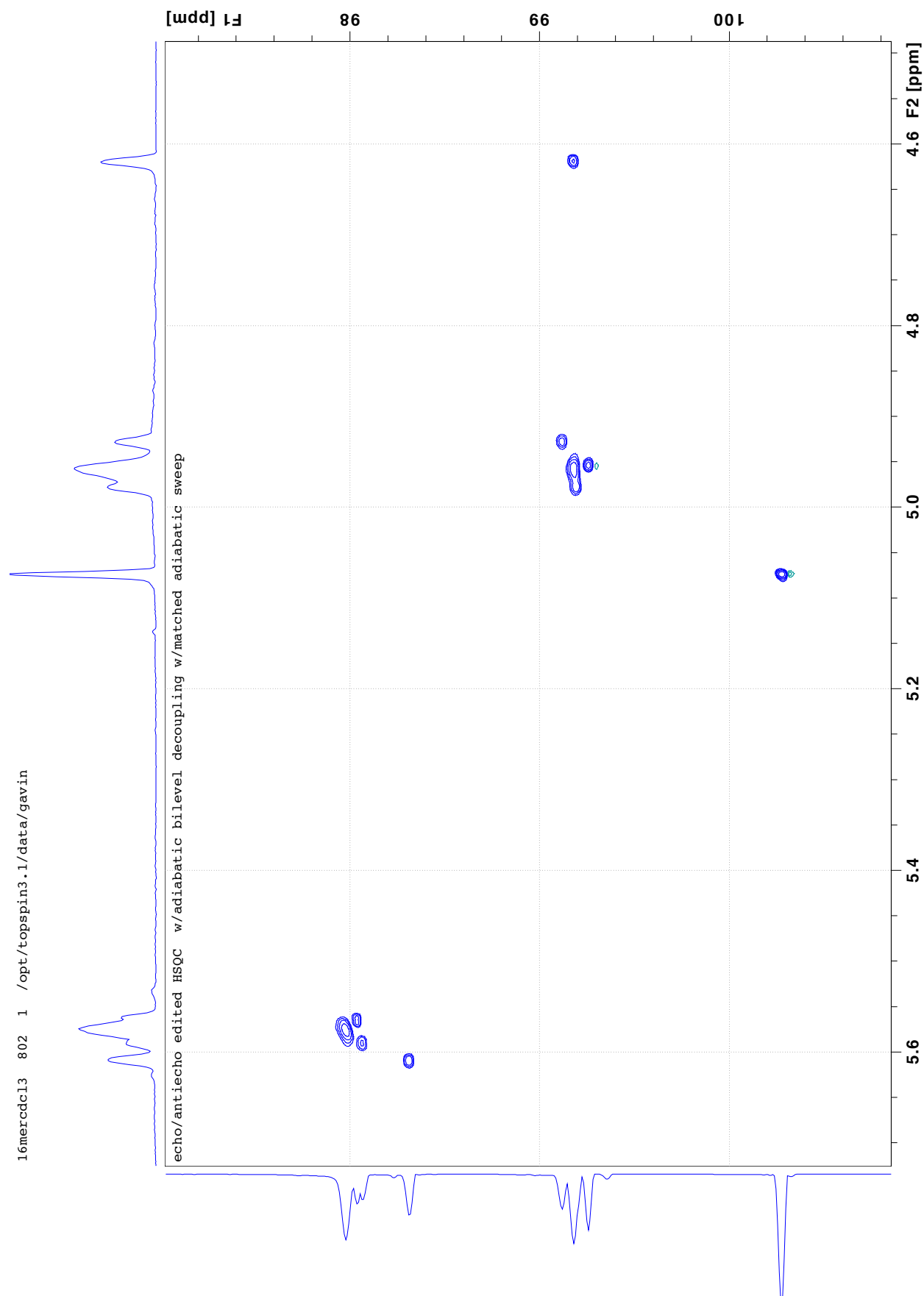
Supplementary Figure 11: COSY NMR (400 MHz, CDCl₃) spectrum for **4**



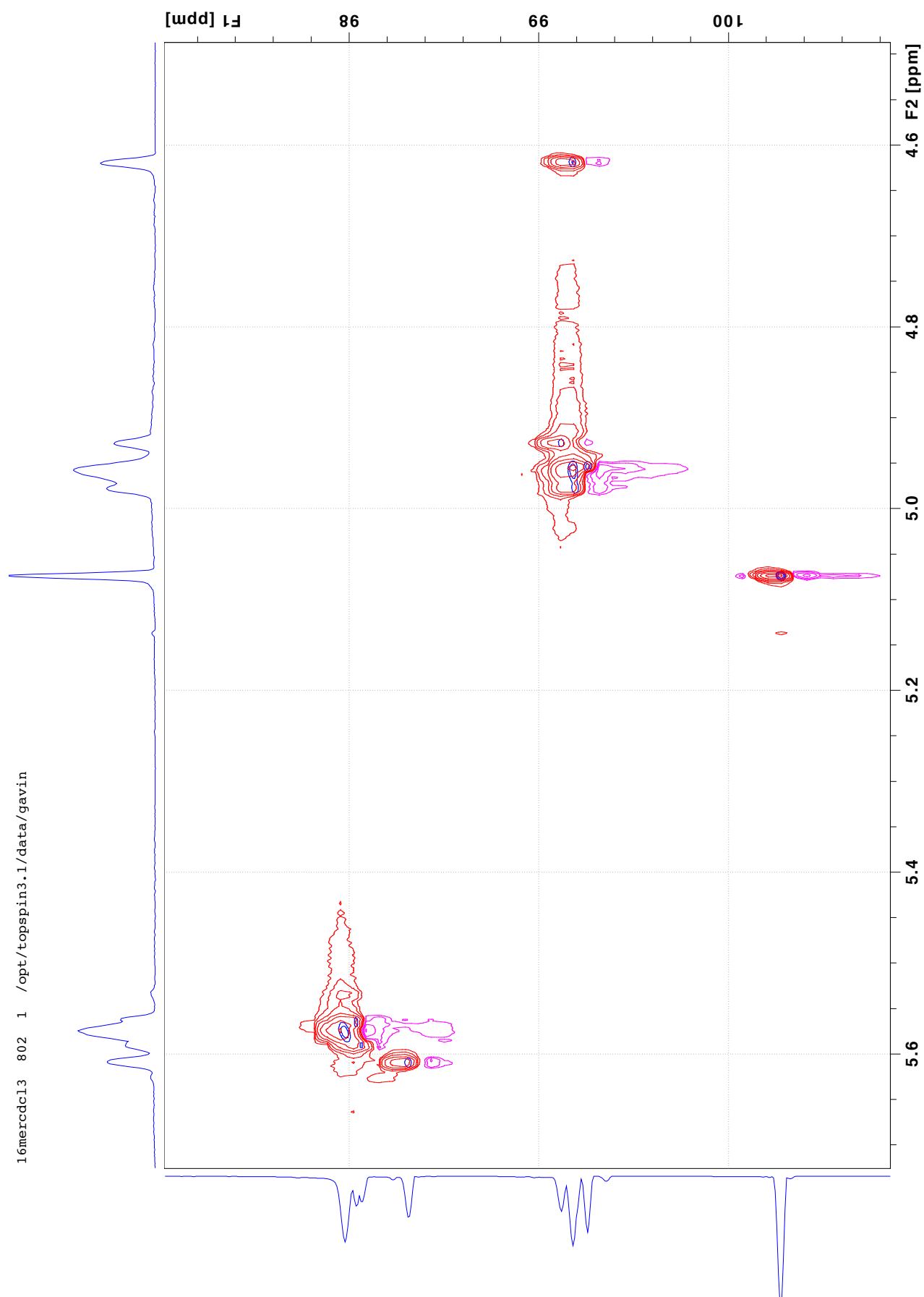
Supplementary Figure 12: HMQC NMR (400 MHz; CDCl₃) spectrum for **4**



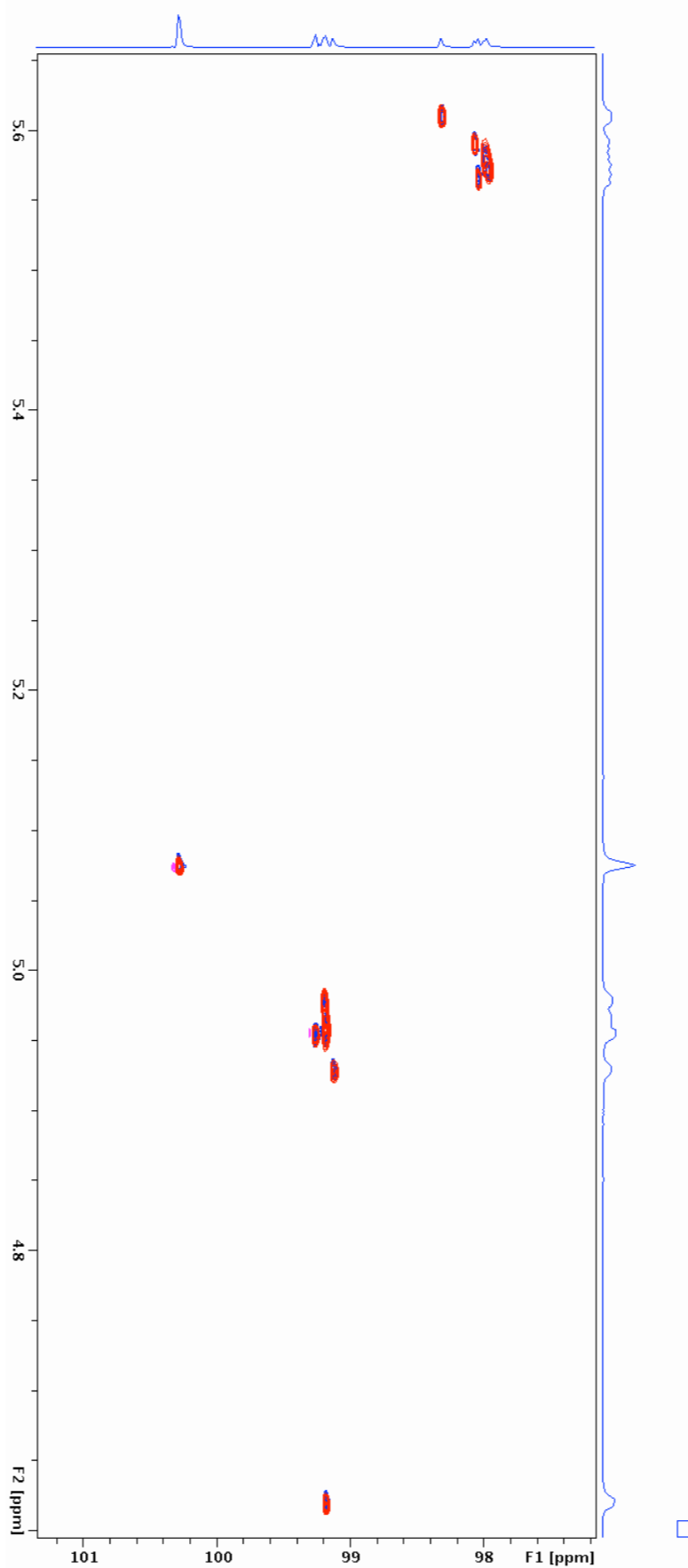
Supplementary Figure 13: NUS Pure Shift HSQC NMR (800 MHz; CDCl₃) spectrum for **4**



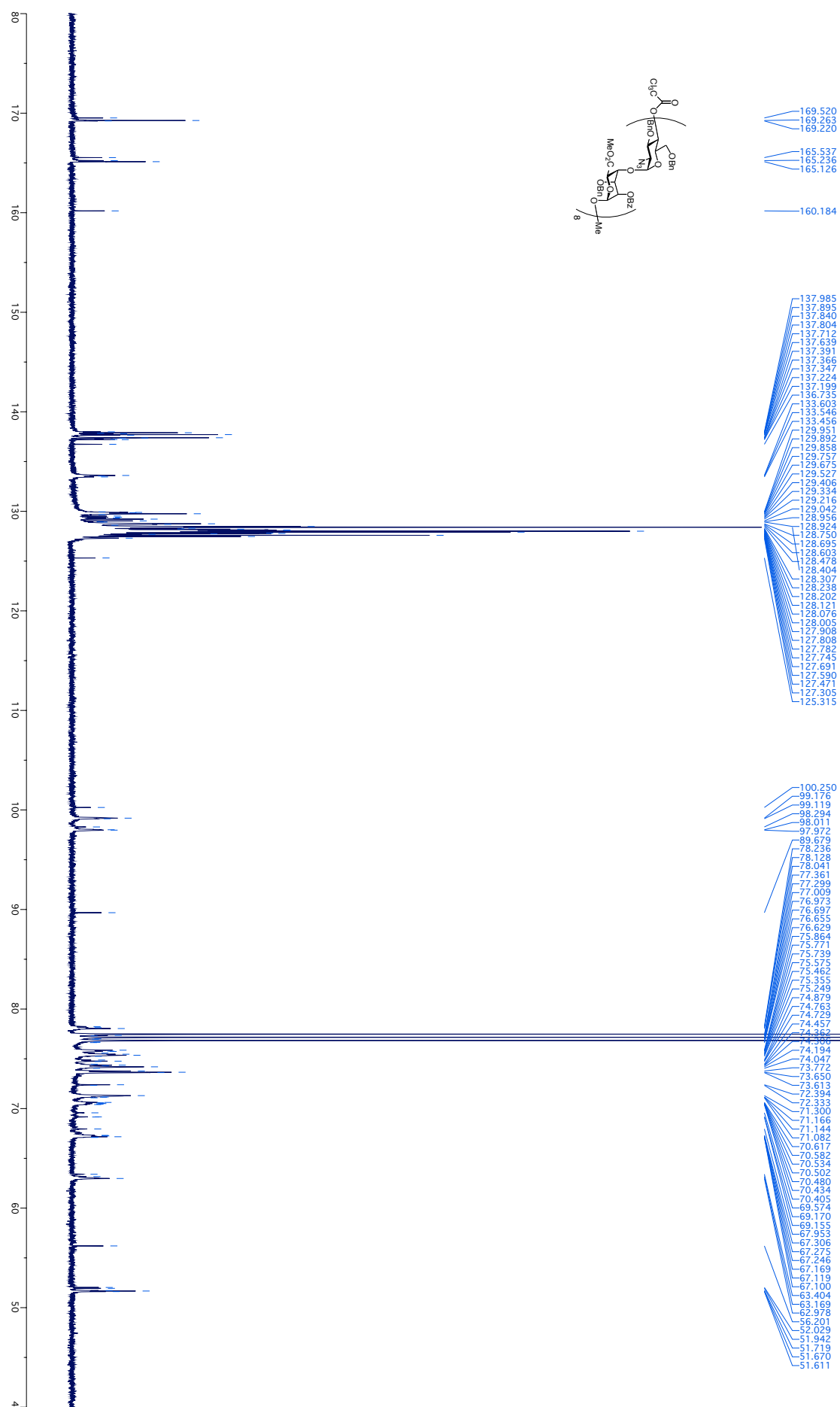
Supplementary Figure 14: NUS Pure Shift HSQC NMR (800 MHz; CDCl₃) spectrum for 4 - comparison to standard 800MHz HSQC NMR



Supplementary Figure 15: NUS Pure Shift HSQC NMR (800 MHz; CDCl₃) spectrum for **4** (red) overlaid with **2** (12mer) (blue) - anomeric centres region



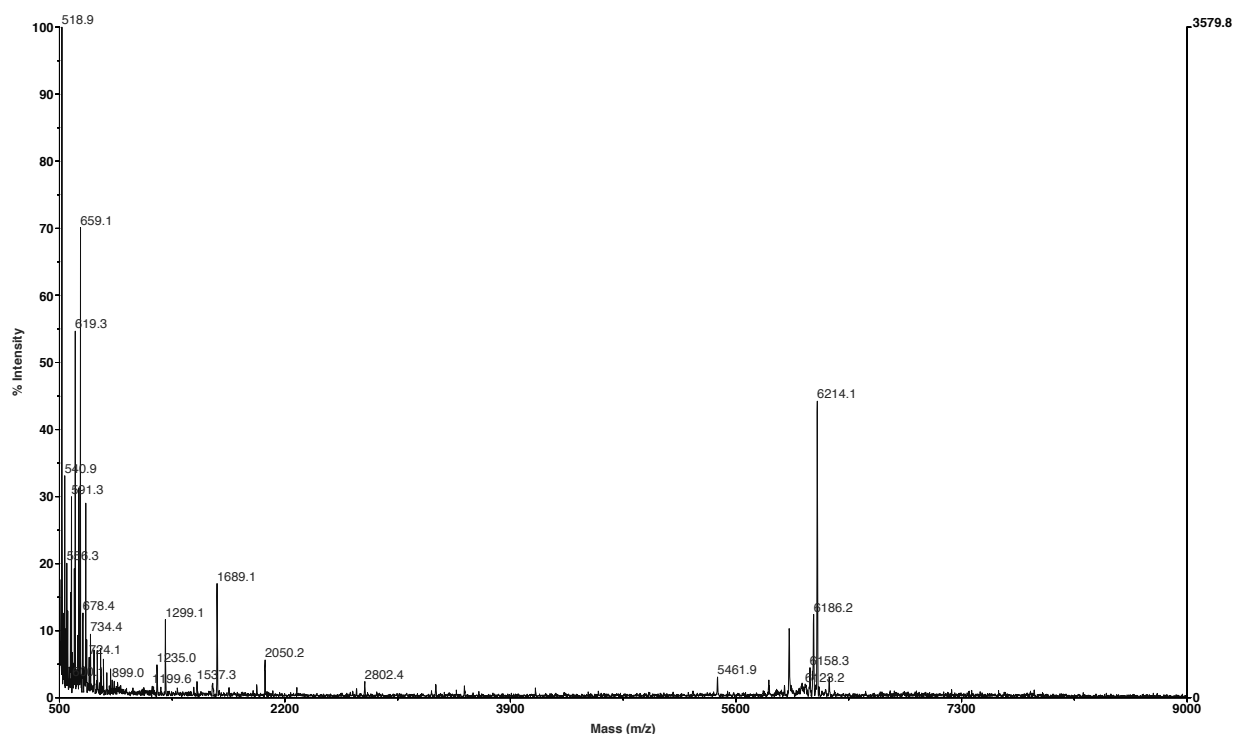
Supplementary Figure 16: ^{13}C NMR (100 MHz; CDCl_3) spectrum for 4



Supplementary Figure 17: MALDI MS for 4

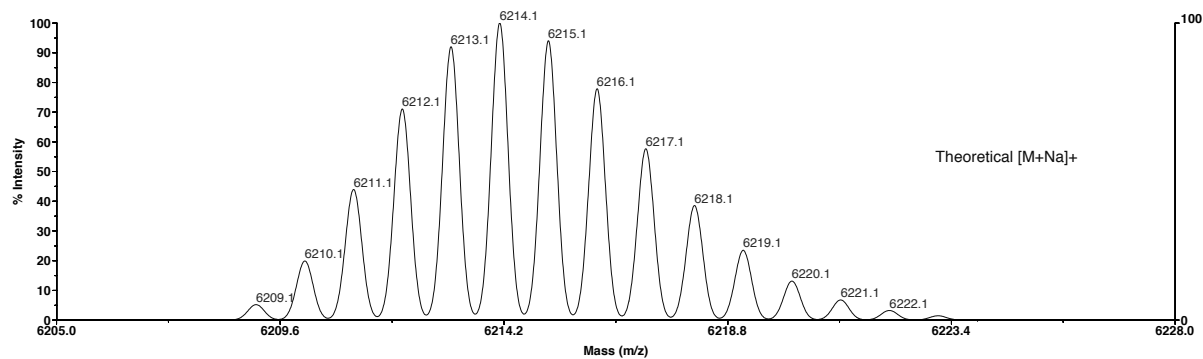
EPSRC National Mass Spectrometry Service Centre (NMSSC), Swansea

<<MANGAR186-VM-MAP_0001>> Voyager Spec #1=>AdvBC(64,0.5,0.1)=>SM5[BP = 518.9, 3580]

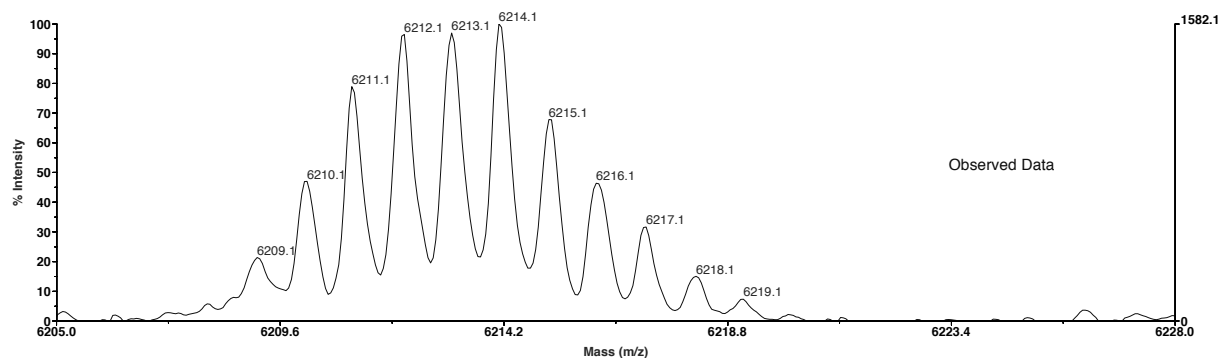


EPSRC National Mass Spectrometry Service Centre (NMSSC), Swansea

ISO:C331H331Cl3N24O90 + (Na)¹



<<MANGAR186-VM-MAP_0001>> Voyager Spec #1=>AdvBC(64,0.5,0.1)=>SM5[BP = 518.9, 3580]



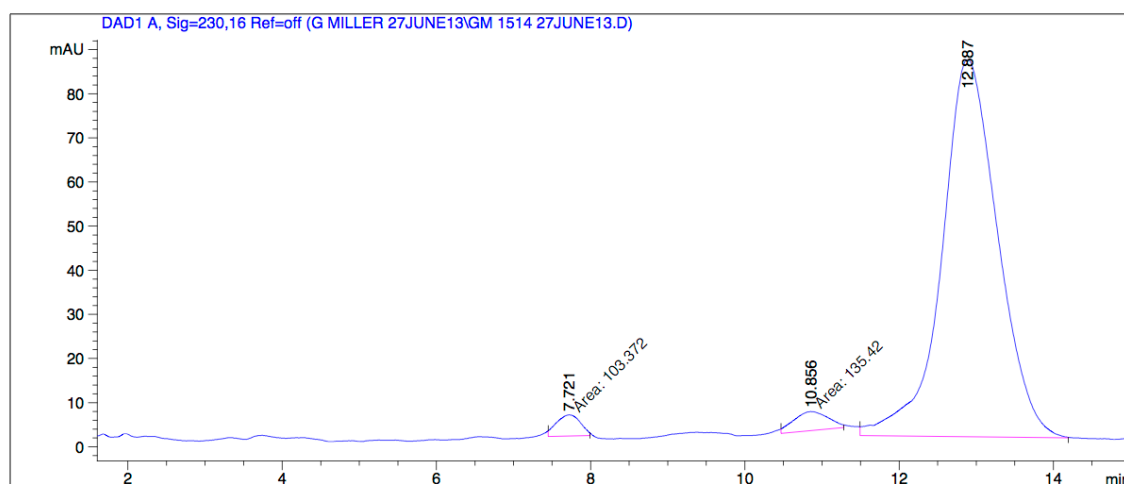
Acquired: 12:18:00, August 20, 2012
Hansen SU1514 MW=6186?? DCM PosRef [1:49] (Dith;DCM) +NaOAc
D:\2012Aug12\MANGAR186-VM-MAP_0001.dat

Printed: 15:59, August 20, 2012

Supplementary Figure 18: LCMS for 4

Data File C:\HPCHEM\1\DATA\G MILLER 27JUNE13\GM 1514 27JUNE13.D
Sample Name: GM 1514 27June13

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Acq. Operator   : Rehana                      Seq. Line :    1
Acq. Instrument : Instrument 1                 Location  : Vial 73
Injection Date  : 28/06/2013 13:20:10         Inj       :    1
                                           Inj Volume: 20 µl
Different Inj Volume from Sequence !      Actual Inj Volume: 5 µl
Acq. Method     : C:\HPCHEM\1\METHODS\GAVIN MILLER 3CM COL 100ACN.m
Last changed    : 28/06/2013 13:19:15 by Rehana
Analysis Method : C:\HPCHEM\1\METHODS\REHANA 60MEOH WITH COL.m
Last changed    : 16/07/2013 12:20:54 by Rehana
Sample Info     : Gavin Miller      GM 1514      27th June 2013
                  Supelco Ascentis C8 5u 4.6x30mm
                  ESI source +ve ES 230nm 0.5ml/min
                  100ACN only 5ul inj
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Area Percent Report

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Sorted By      : Signal
Multiplier     : 1.0000
Dilution       : 1.0000
Use Multiplier & Dilution Factor with ISTDs
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Signal 1: DAD1 A, Sig=230,16 Ref=off

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2	10.856	MM	0.5198	135.41997	4.34194	2.9726
3	12.887	VB	0.7506	4316.81592	85.56736	94.7583

Totals : 4555.60770 94.69706

*** End of Report ***

Supplementary Figure 19: LCMS for 4

Display Report

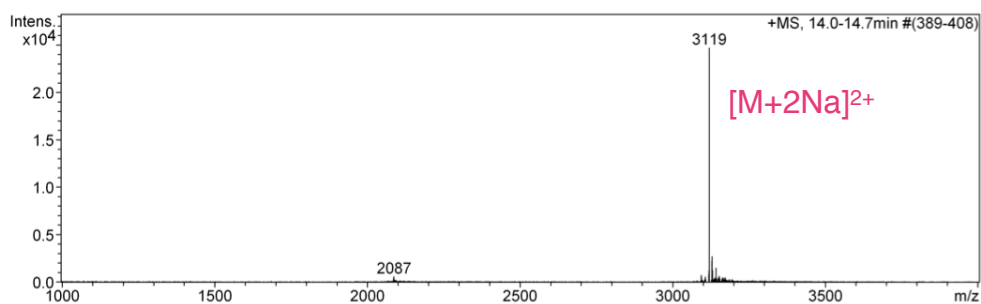
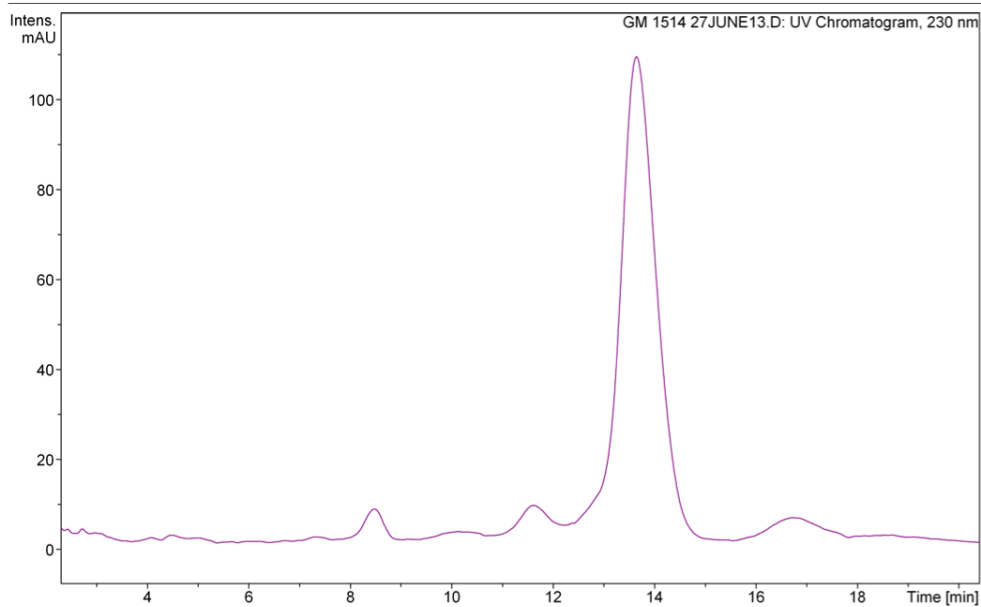
Analysis Info

Analysis Name GM 1514 27JUNE13.D
Method GAVIN MILLER 3CM COL 100ACN.M
Sample Name GM 1514 27June13
Comment Gavin Miller GM 1514 27th June 2013
Supelco Ascentis C8 5u 4.6x30mm
ESI source +ve ES 230nm 0.5ml/min
100ACN only 5ul inj

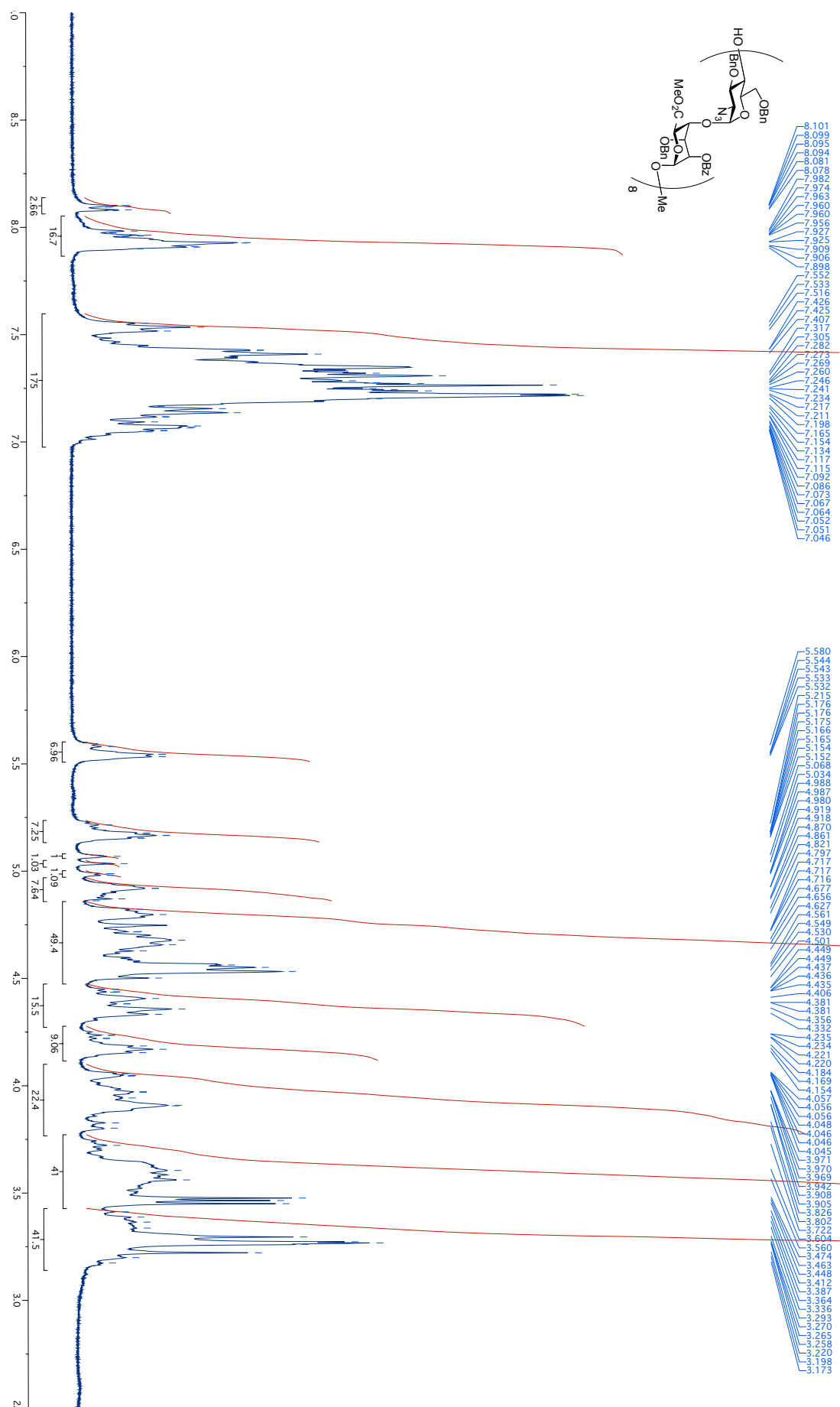
Acquisition Date 28/06/2013 13:20:24
Operator mib
Instrument LC-MSD-Trap-SL

Acquisition Parameter

Ion Source Type	ESI	Ion Polarity	Positive	Alternating Ion Polarity	off
Mass Range Mode	Extended	Scan Begin	1000 m/z	Scan End	4000 m/z
Capillary Exit	280.0 Volt	Skim 1	40.0 Volt	Trap Drive	218.9
Accumulation Time	199856 μ s	Averages	7 Spectra	Auto MS/MS	off

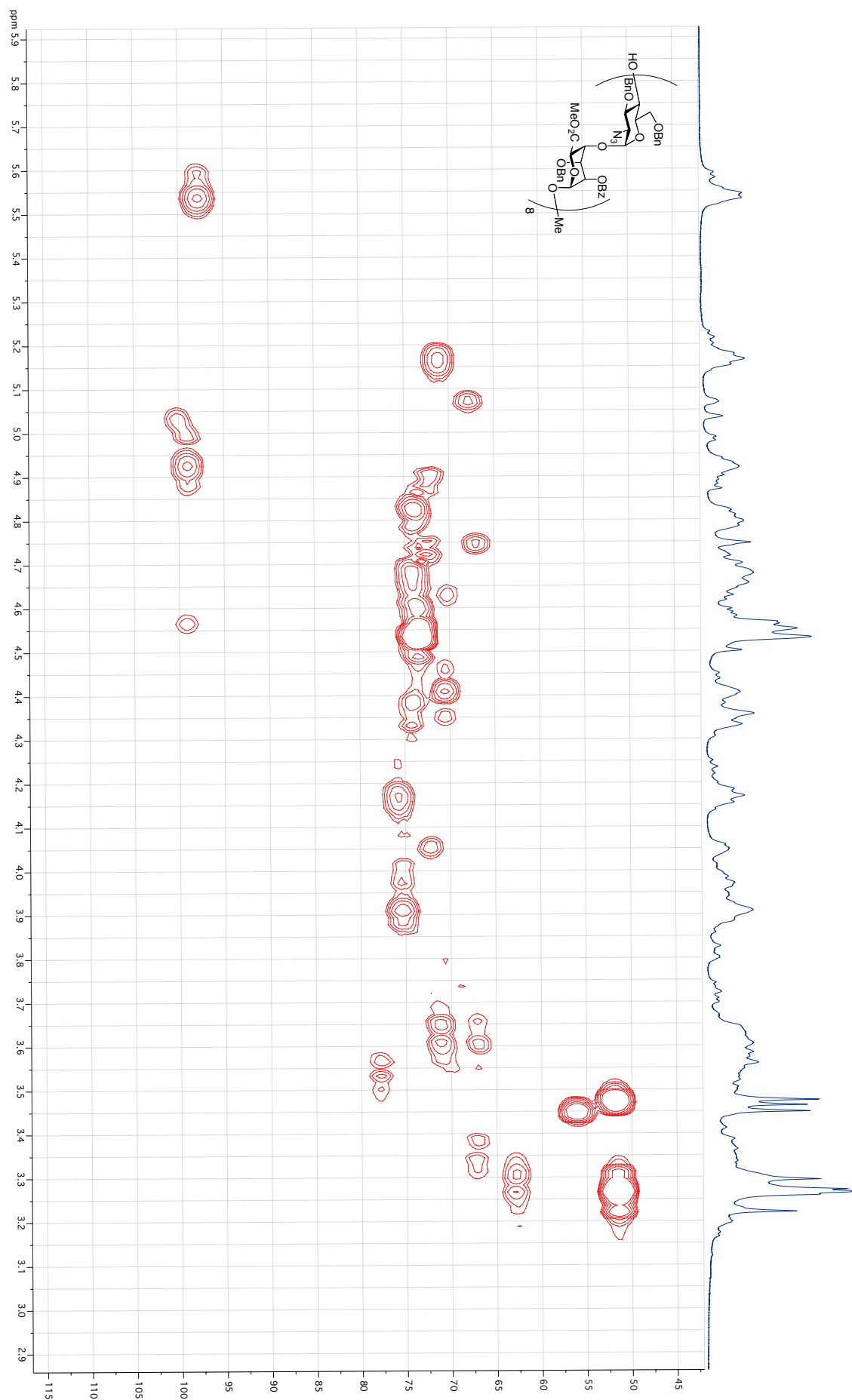


Supplementary Figure 20: ^1H NMR (400 MHz; CDCl_3) spectrum for **5**



The figure displays a 2D NMR spectrum, likely a COSY or HSQC experiment, with the chemical structure of the polymer repeat unit shown in the top right corner. The x-axis represents the chemical shift in ppm, ranging from 2.7 to 6.0. The y-axis represents the chemical shift in ppm, ranging from 2.6 to 6.2. The spectrum shows numerous cross-peaks, indicating correlations between protons in the polymer structure. The chemical structure is a polymer repeat unit with a backbone containing a hydroxyl group (HO), a benzyl group (Bn), a nitrogen atom (N), and a carbonyl group (C=O). The side chain includes a methoxy group (MeO), a benzyl group (Bn), and a carbonyl group (C=O). The structure is labeled with '8' and 'Me'.

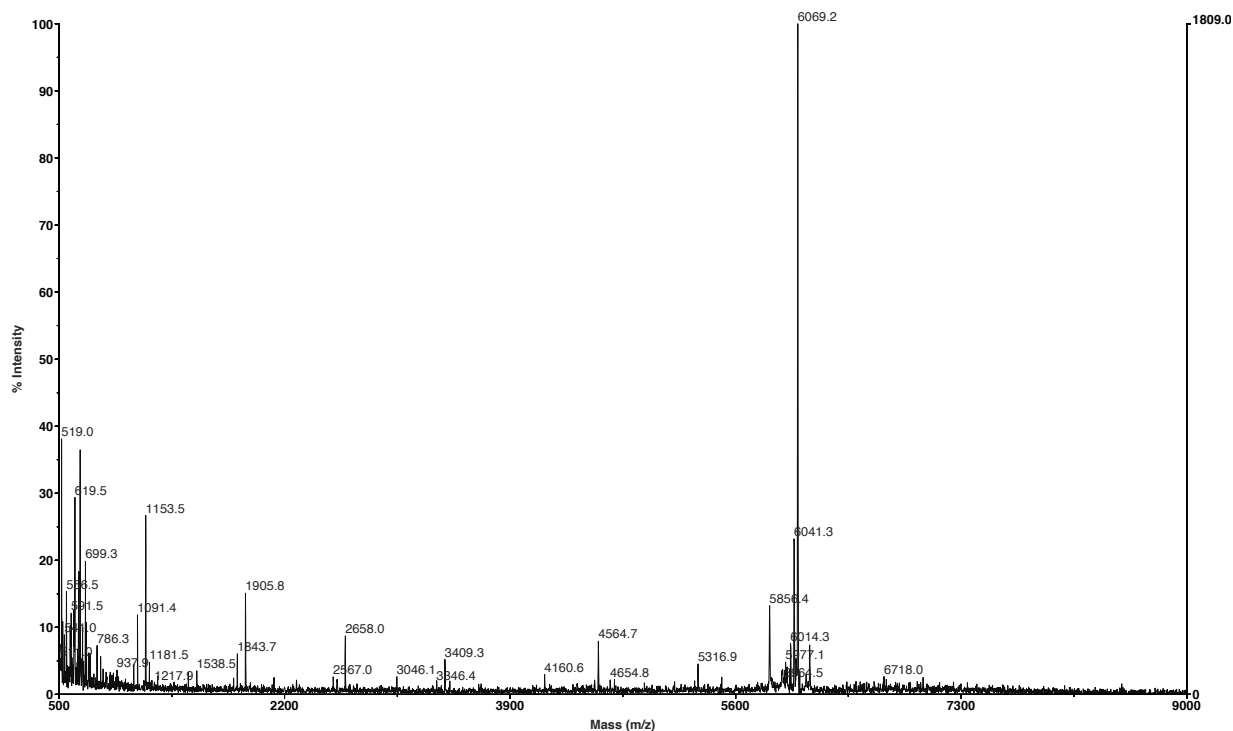
Supplementary Figure 22: HMQC NMR (400 MHz; CDCl₃) spectrum for **5**



Supplementary Figure 23: MALDI MS and isotope pattern for 5

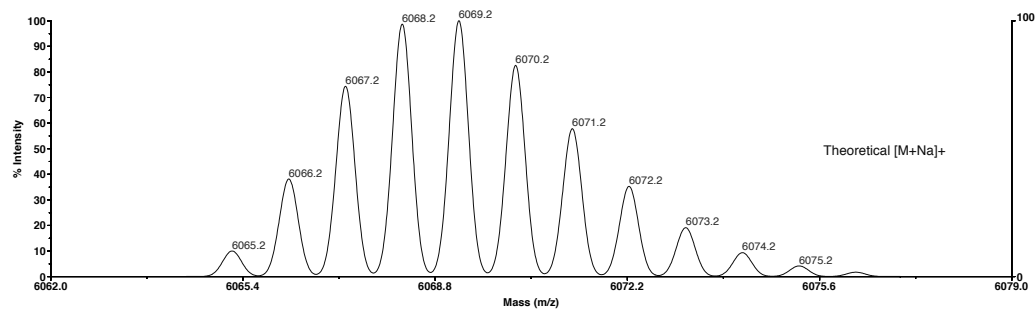
EPSRC National Mass Spectrometry Service Centre (NMSSC), Swansea

<<MANGAR187-VM-MAP_0001>> Voyager Spec #1=>AdvBC(64,0.5,0.1)=>SM5[BP = 6069.2, 1809]

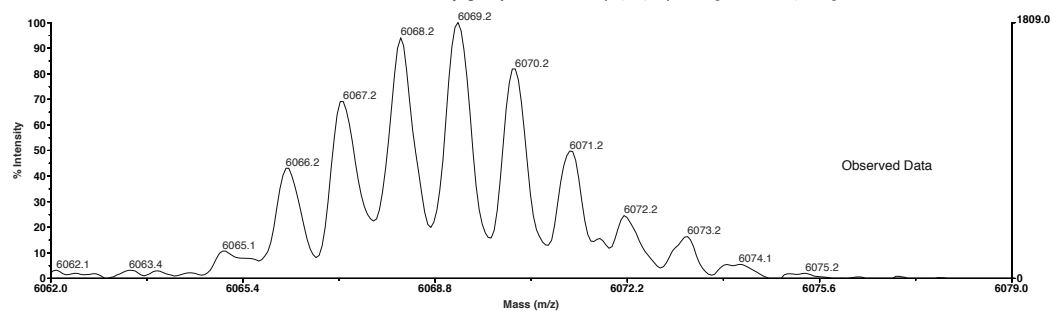


EPSRC National Mass Spectrometry Service Centre (NMSSC), Swansea

ISO:C329H332N24O89 + (Na)1



<<MANGAR187-VM-MAP_0001>> Voyager Spec #1=>AdvBC(64,0.5,0.1)=>SM5[BP = 6069.2, 1809]



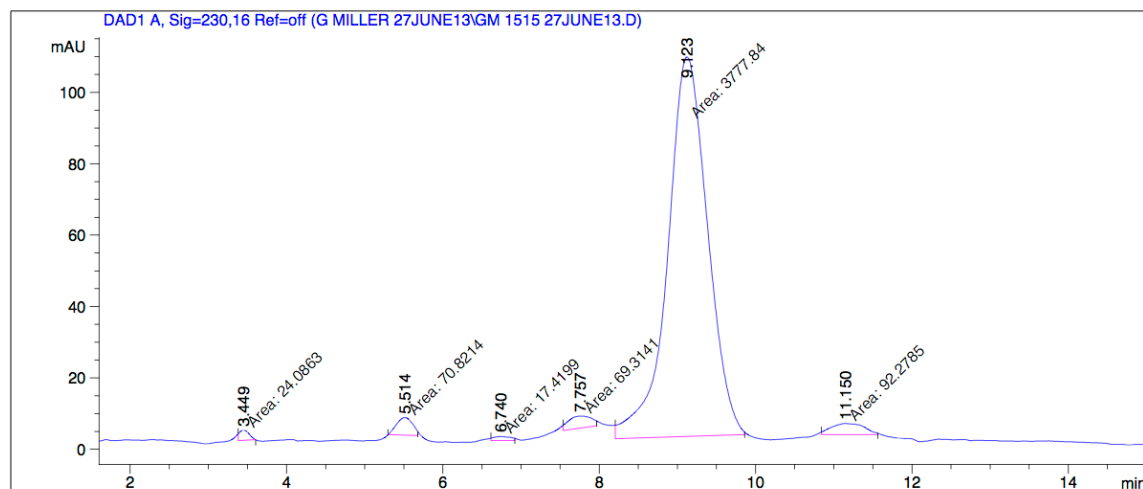
Acquired: 12:22:00, August 20, 2012
Hansen SU1515 MW=6042?? DCM PosRef [1:49] (Dith;DCM) +NaOAc
D:\2012\Aug12\MANGAR187-VM-MAP_0001.dat

Printed: 16:07, August 20, 2012

Supplementary Figure 24: LCMS for 5

Data File C:\HPCHEM\1\DATA\G MILLER 27JUNE13\GM 1515 27JUNE13.D
Sample Name: GM 1515 27June13

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Acq. Instrument : Instrument 1                 Location  : Vial 74
Injection Date  : 28/06/2013 14:56:16         Inj       :    1
                                           Inj Volume: 20 µl
Different Inj Volume from Sequence !      Actual Inj Volume: 5 µl
Acq. Method     : C:\HPCHEM\1\METHODS\GAVIN MILLER 3CM COL 100ACN.M
Last changed    : 28/06/2013 14:55:15 by Rehana
Analysis Method : C:\HPCHEM\1\METHODS\REHANA 60MEOH WITH COL.M
Last changed    : 16/07/2013 12:19:21 by Rehana
Sample Info     : Gavin Miller      GM 1515      27th June 2013
                  Supelco Ascentis C8 5u 4.6x30mm
                  ESI source +ve ES 230nm 0.5ml/min
                  100ACN only 5ul inj
=====
```



Area Percent Report

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Sorted By      : Signal
Multiplier     : 1.0000
Dilution       : 1.0000
Use Multiplier & Dilution Factor with ISTDs
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Signal 1: DAD1 A, Sig=230,16 Ref=off

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	3.449	MM	0.1394	24.08633	2.87877	0.5945
2	5.514	MM	0.2394	70.82142	4.93068	1.7479
3	6.740	MM	0.2571	17.41991	1.12947	0.4299
4	7.757	MM	0.3419	69.31406	3.37922	1.7107
5	9.123	MM	0.5920	3777.84351	106.36275	93.2395
6	11.150	MM	0.4915	92.27850	3.12892	2.2775

Totals : 4051.76371 121.80981

*** End of Report ***

Supplementary Figure 25: LCMS for 5

Display Report

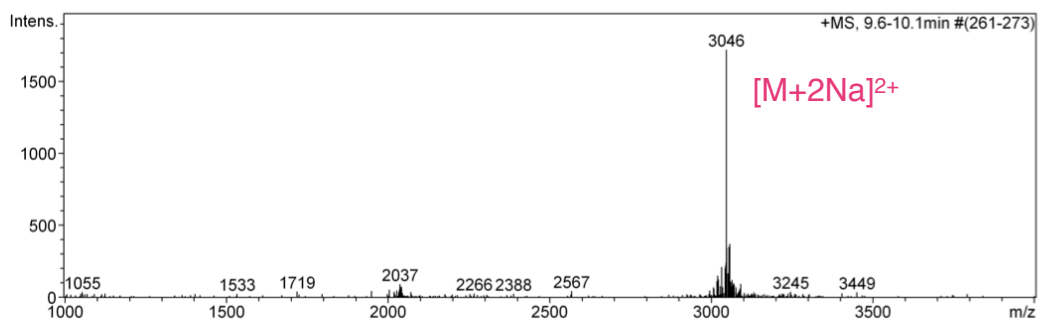
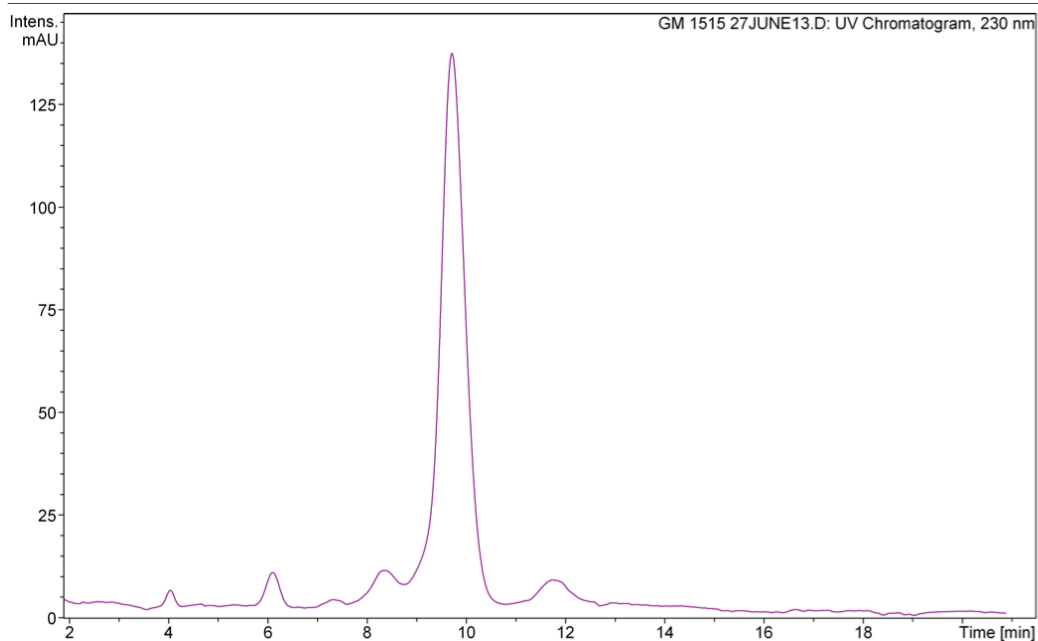
Analysis Info

Analysis Name GM 1515 27JUNE13.D
Method GAVIN MILLER 3CM COL 100ACN.M
Sample Name GM 1515 27June13
Comment Gavin Miller GM 1515 27th June 2013
Supelco Ascentis C8 5u 4.6x30mm
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100ACN only 5ul inj

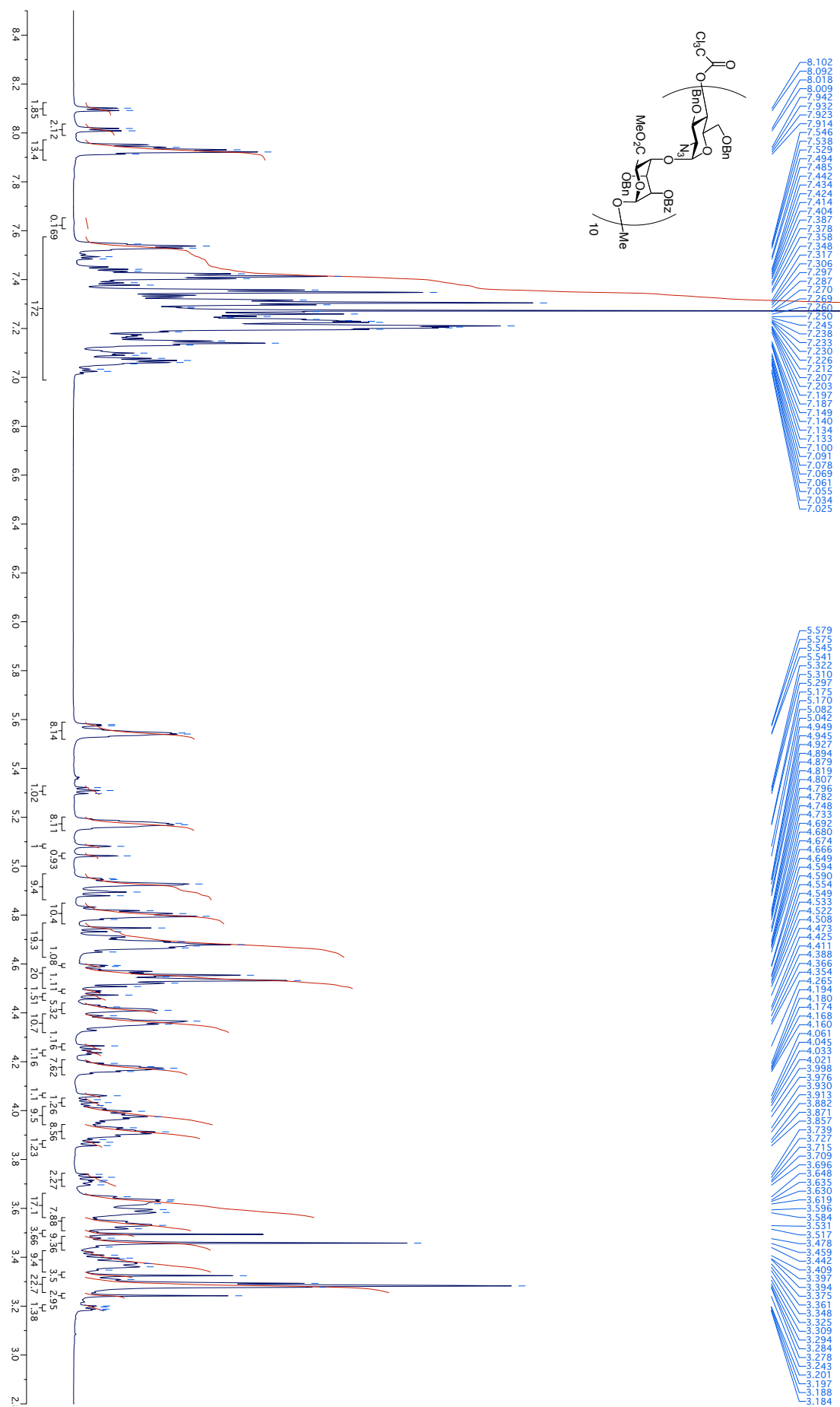
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Operator mib
Instrument LC-MSD-Trap-SL

Acquisition Parameter

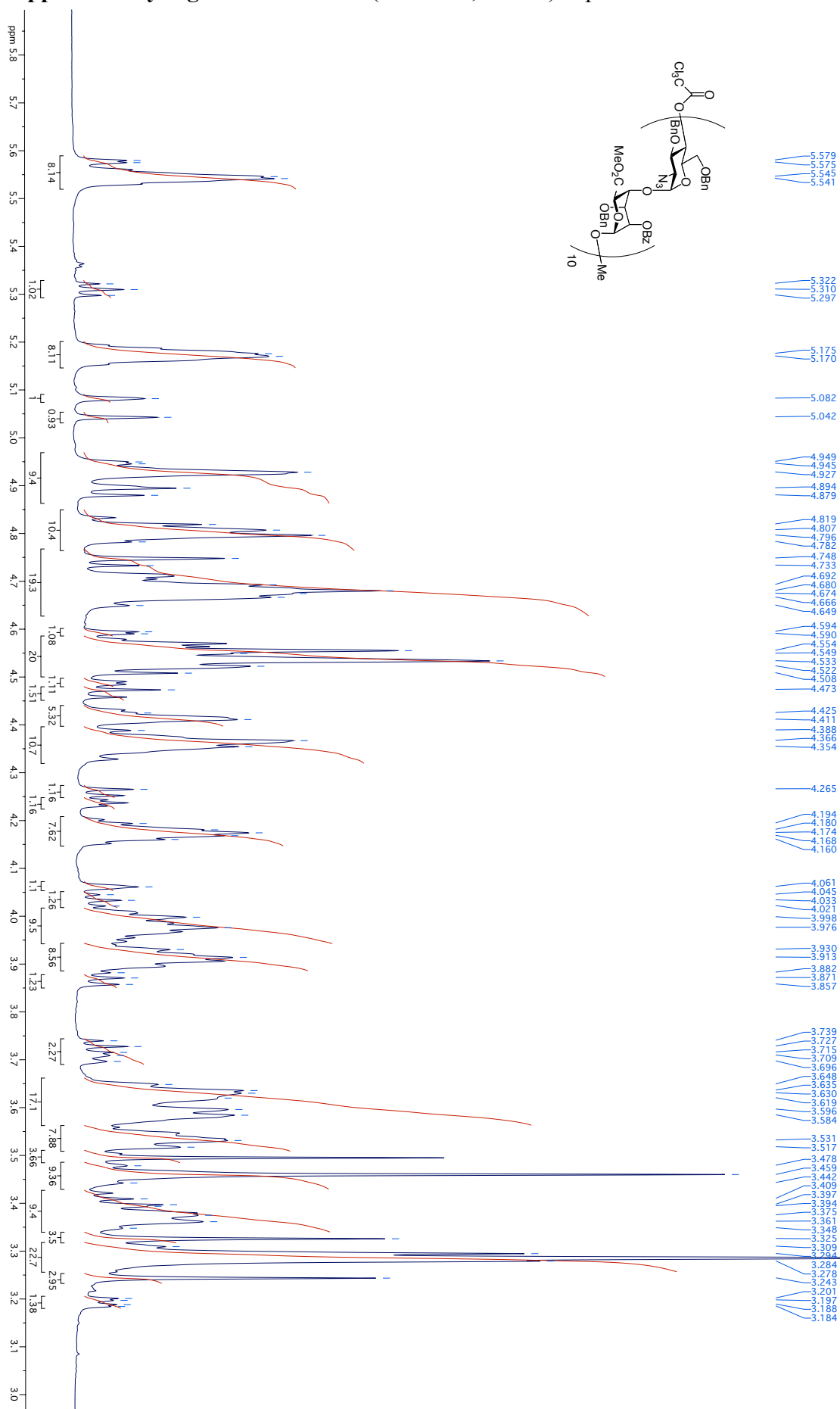
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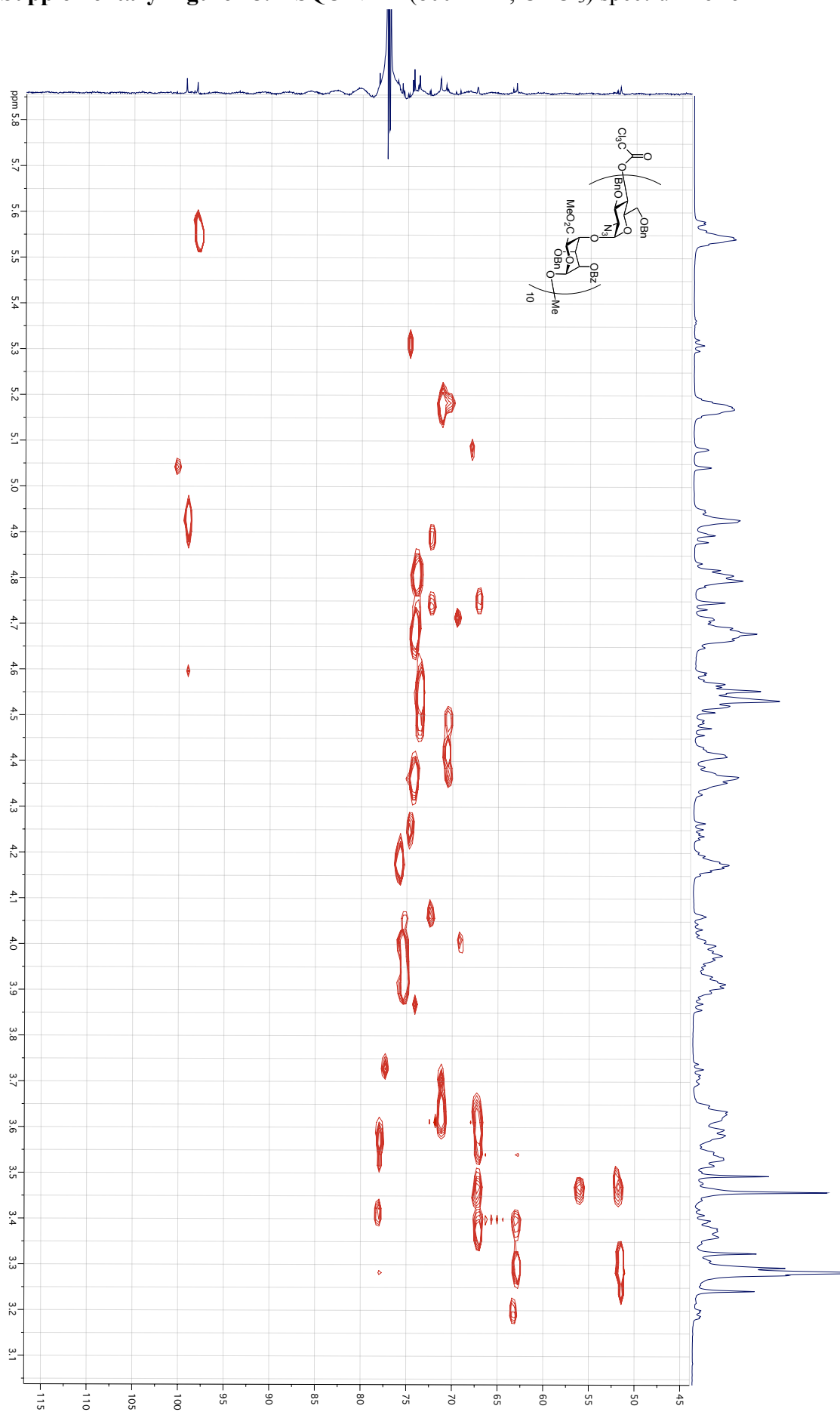
Supplementary Figure 26: ^1H NMR (800 MHz; CDCl_3) spectrum form **6**



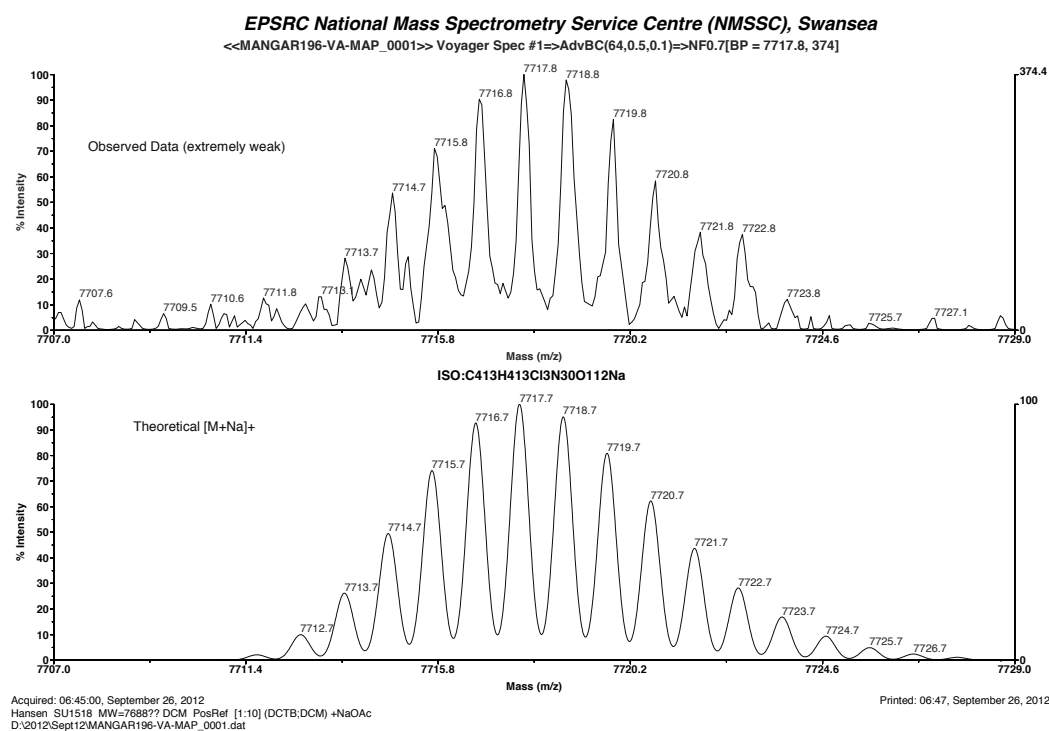
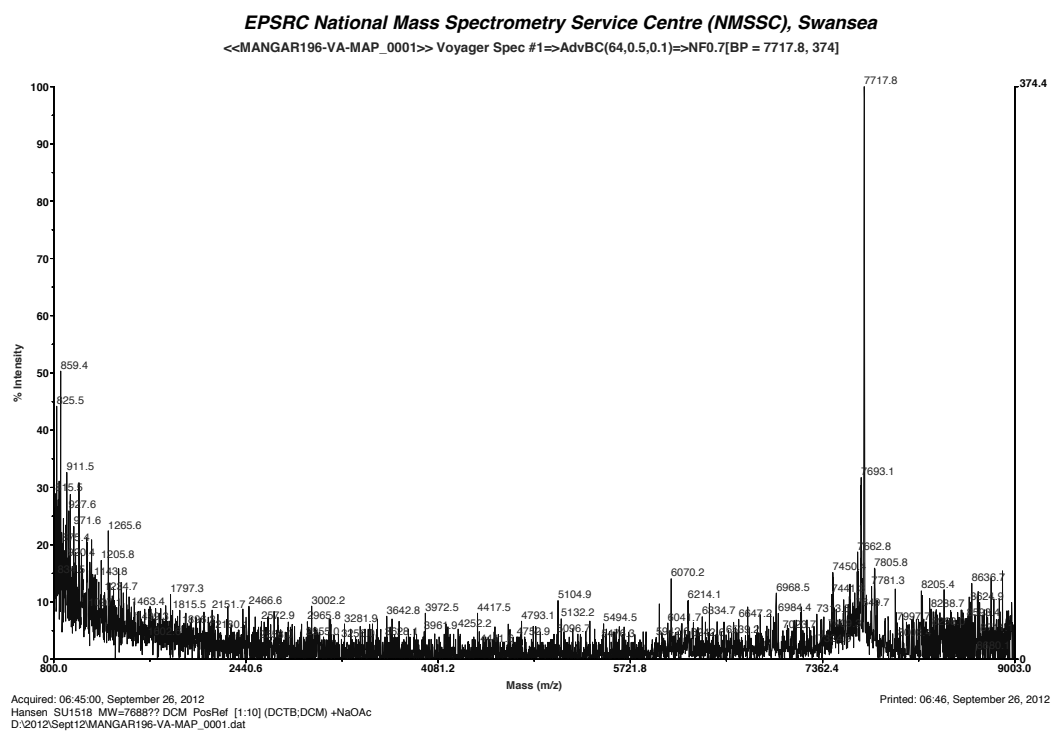
Supplementary Figure 27: ^1H NMR (800 MHz, CDCl_3) expansion for **6**



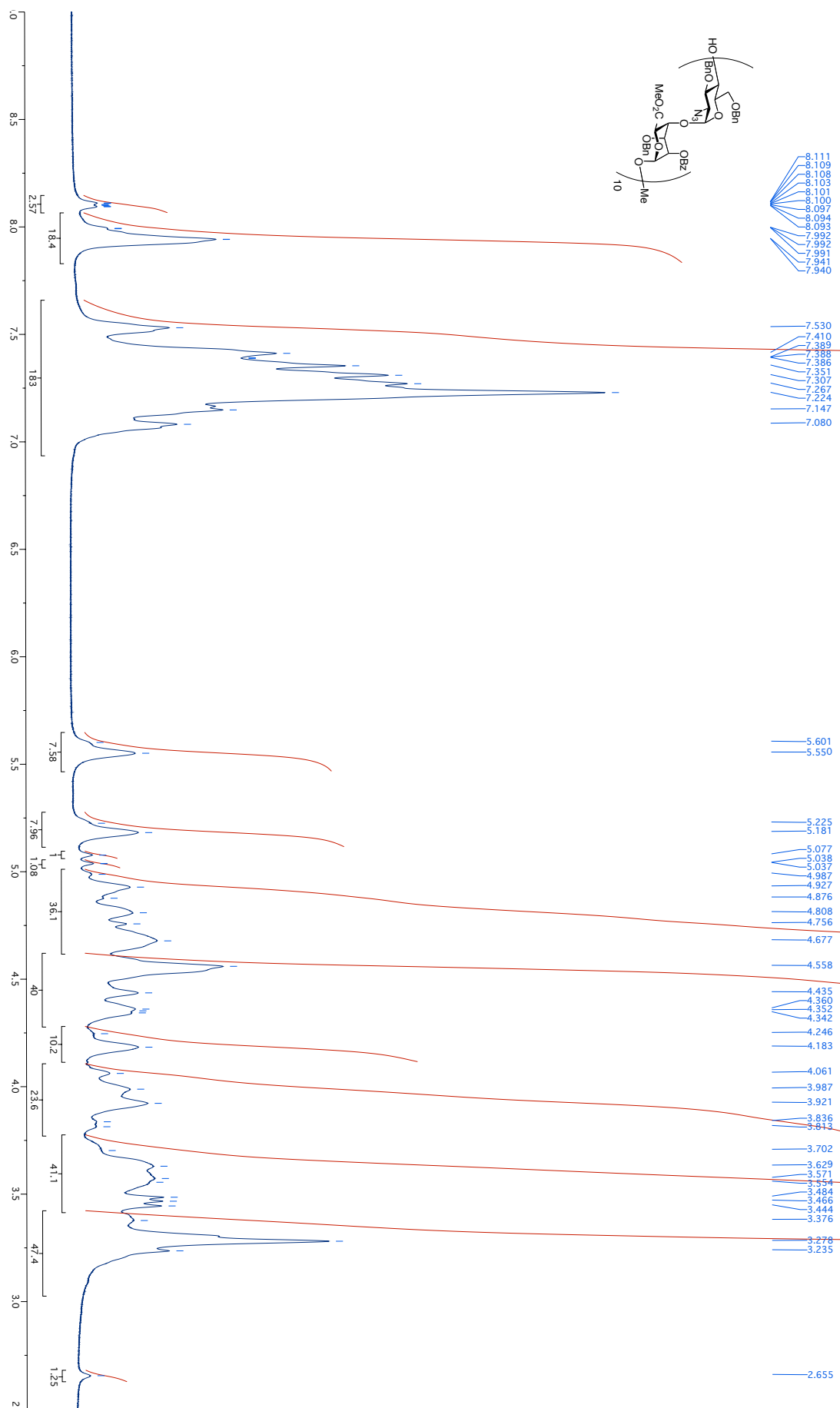
Supplementary Figure 28: HSQC NMR (800 MHz; CDCl₃) spectrum for **6**



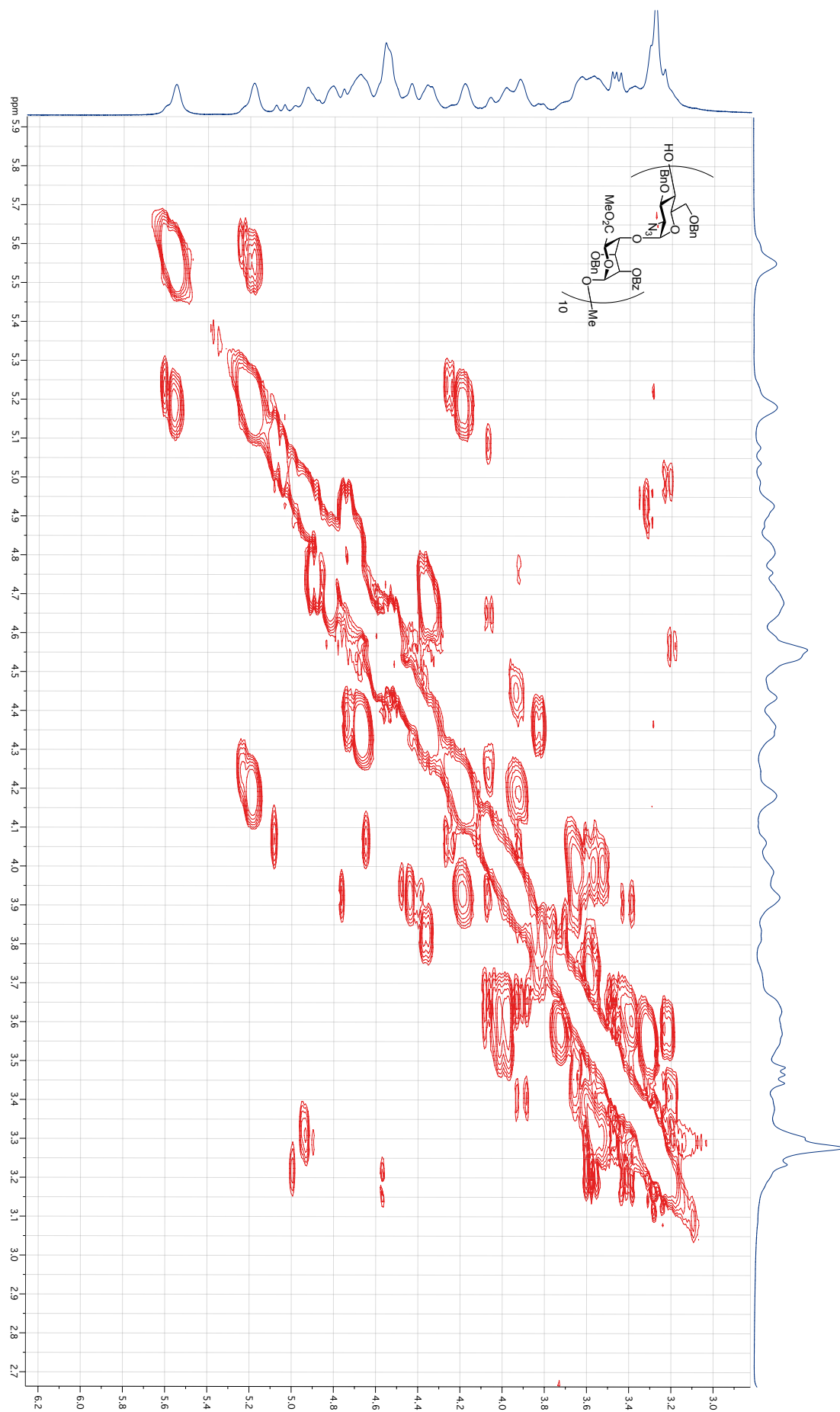
Supplementary Figure 29: MALDI MS and isotope pattern for 6



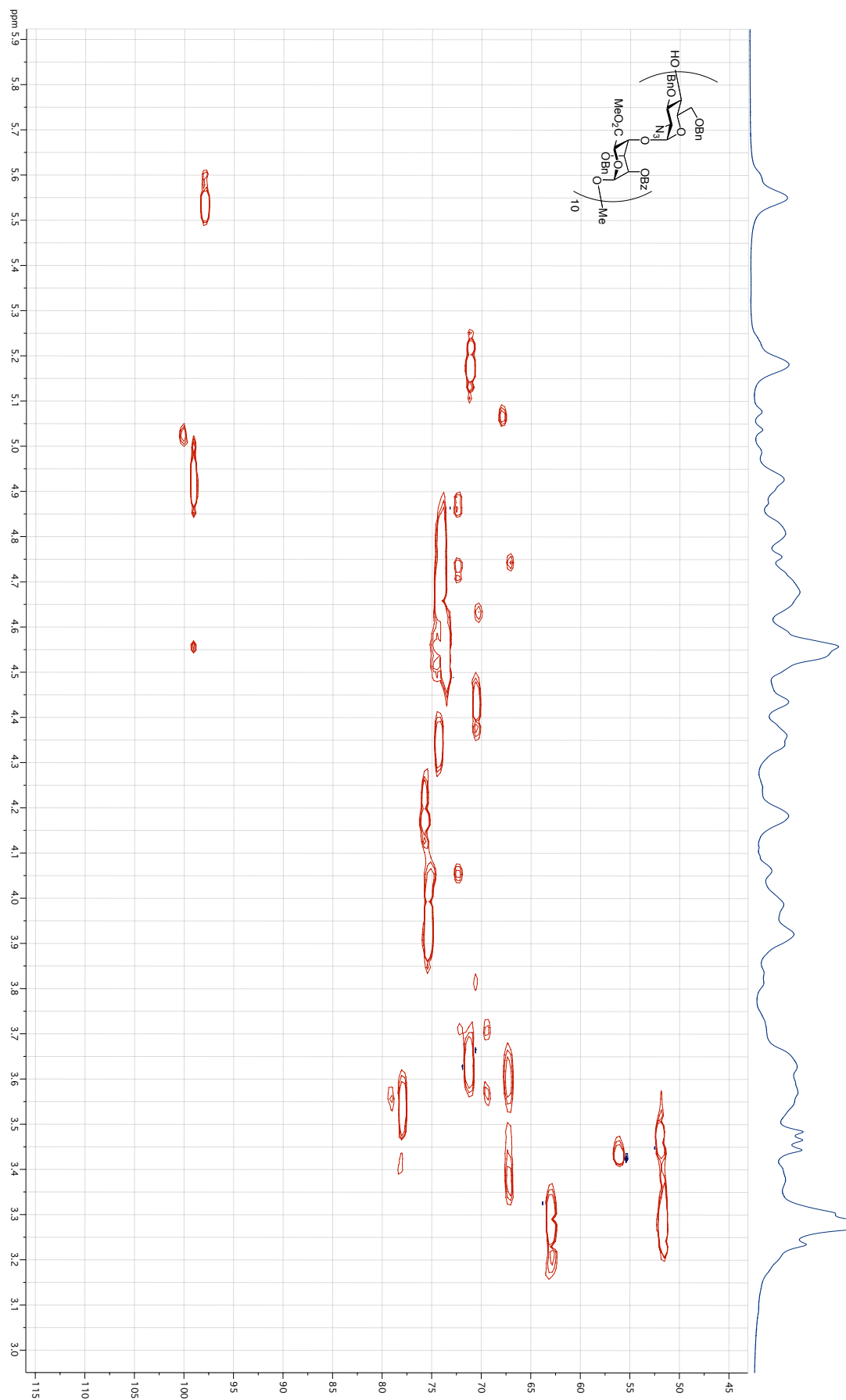
Supplementary Figure 30: ^1H NMR (400 MHz; CDCl_3) spectrum for **7**



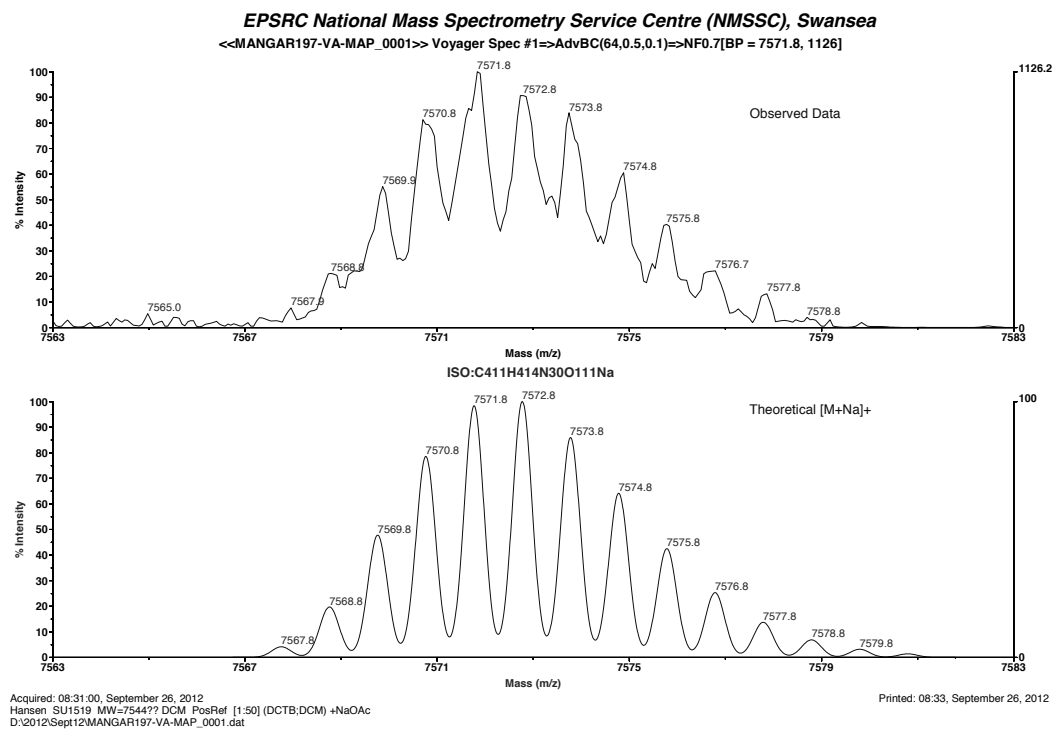
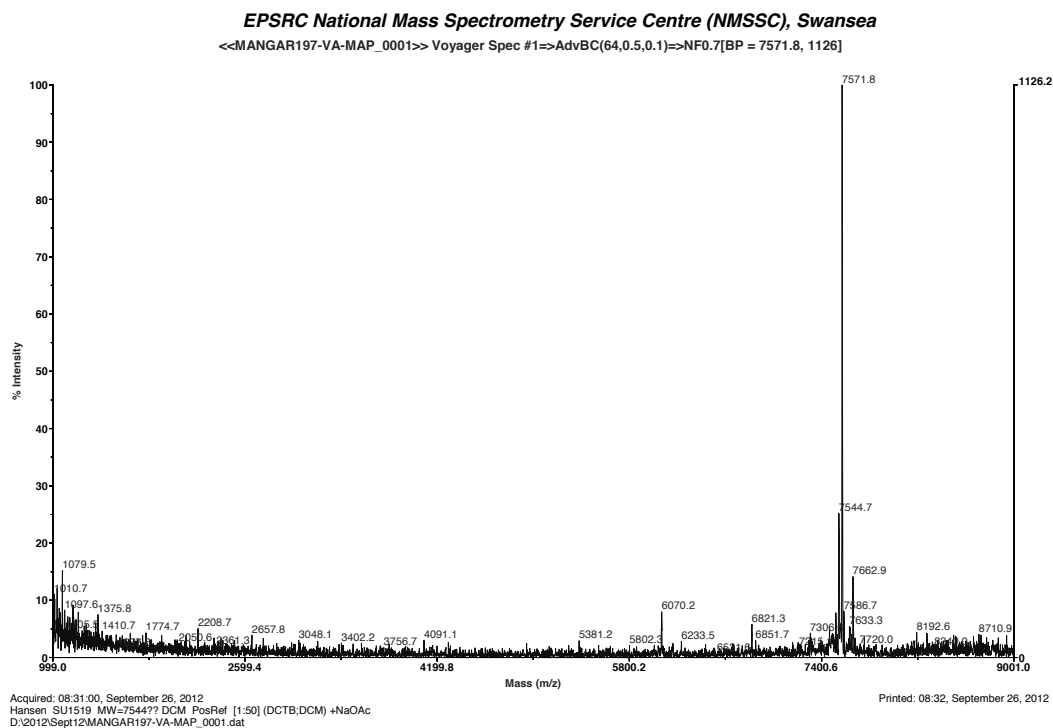
Supplementary Figure 31: COSY NMR (400 MHz; CDCl₃) spectrum for **7**



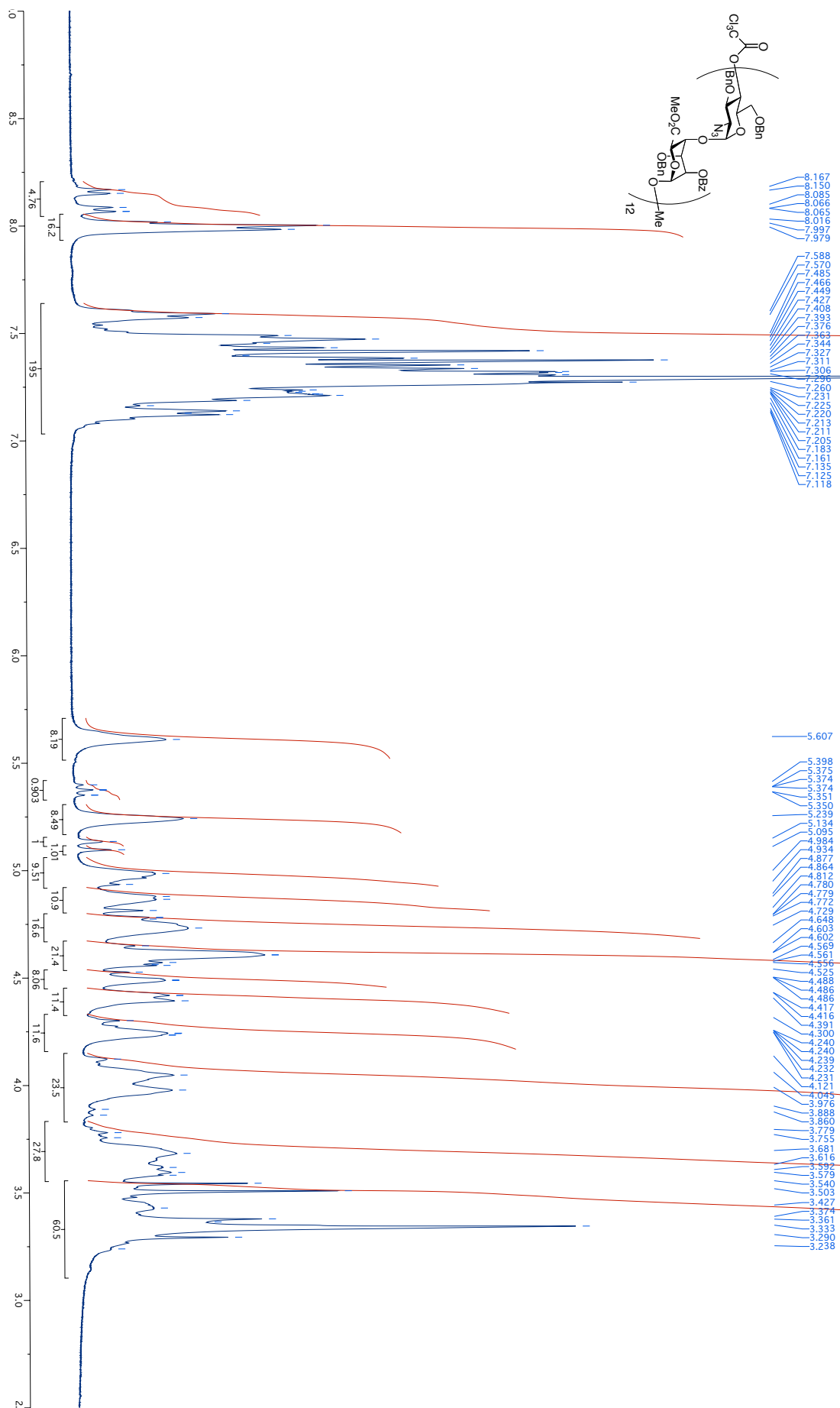
Supplementary Figure 31: HSQC NMR (400 MHz; CDCl₃) spectrum for **7**



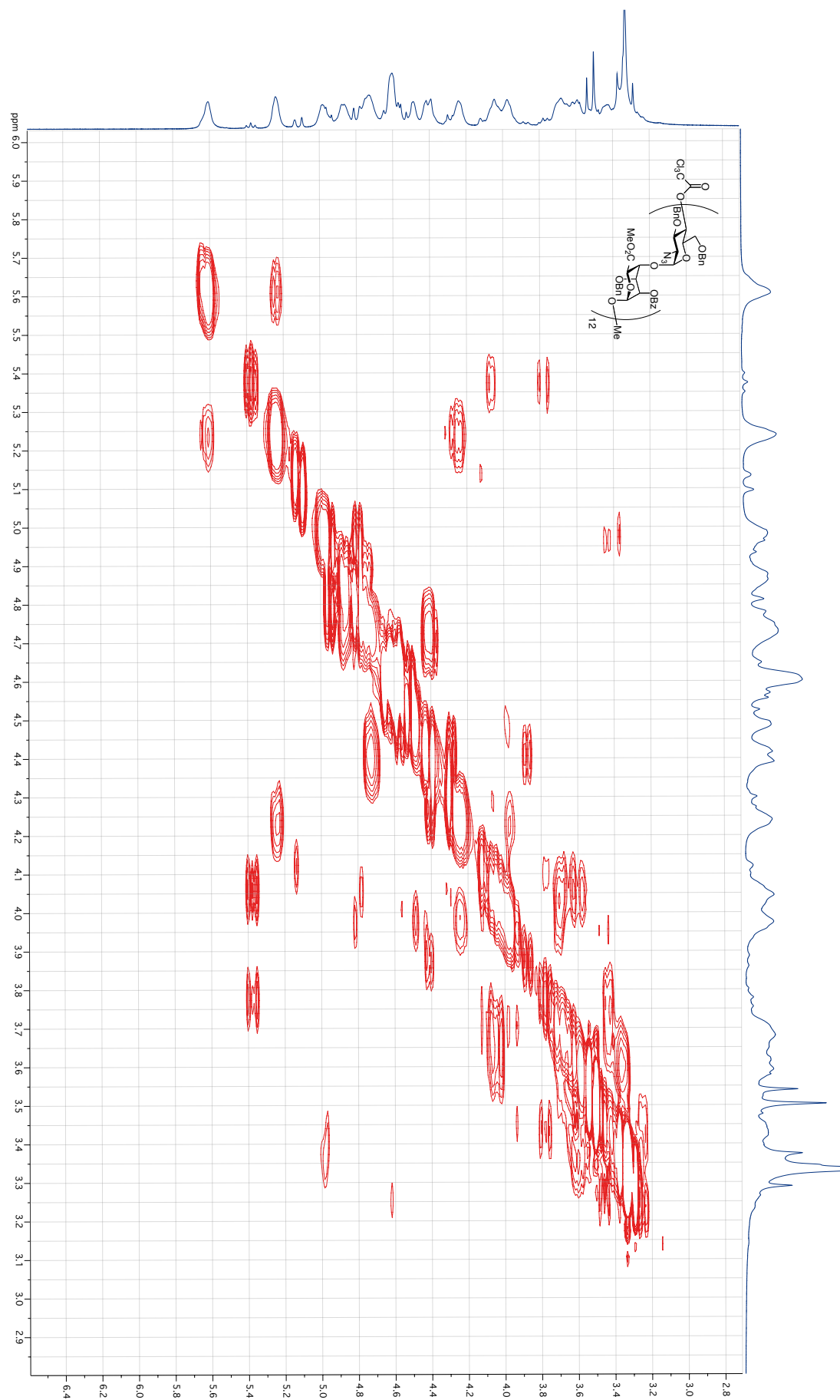
Supplementary Figure 32: MALDI MS and isotope pattern for 7



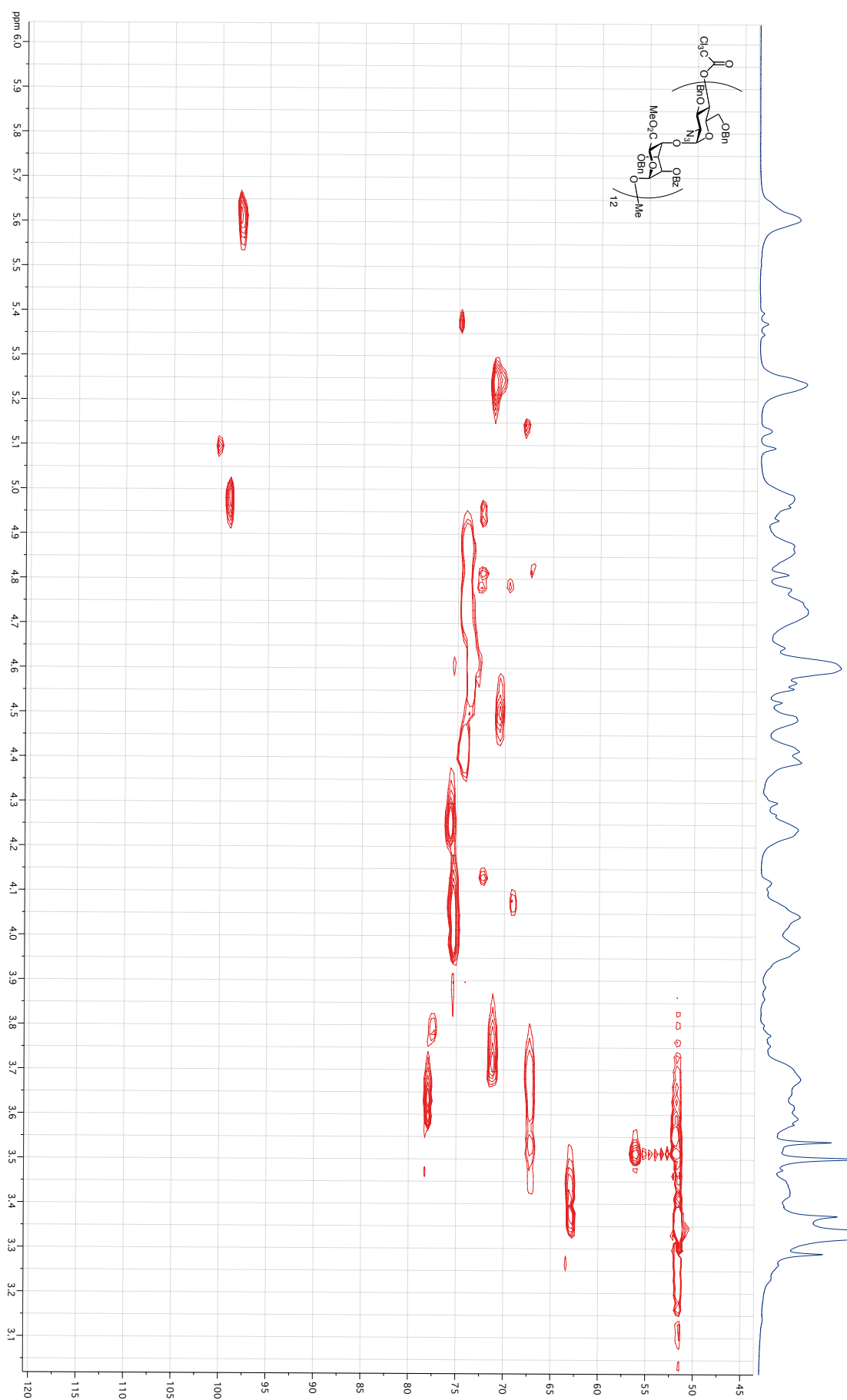
Supplementary Figure 33: ^1H NMR (400 MHz; CDCl_3) spectrum for **8**



Supplementary Figure 34: COSY NMR (400 MHz; CDCl₃) spectrum for **8**

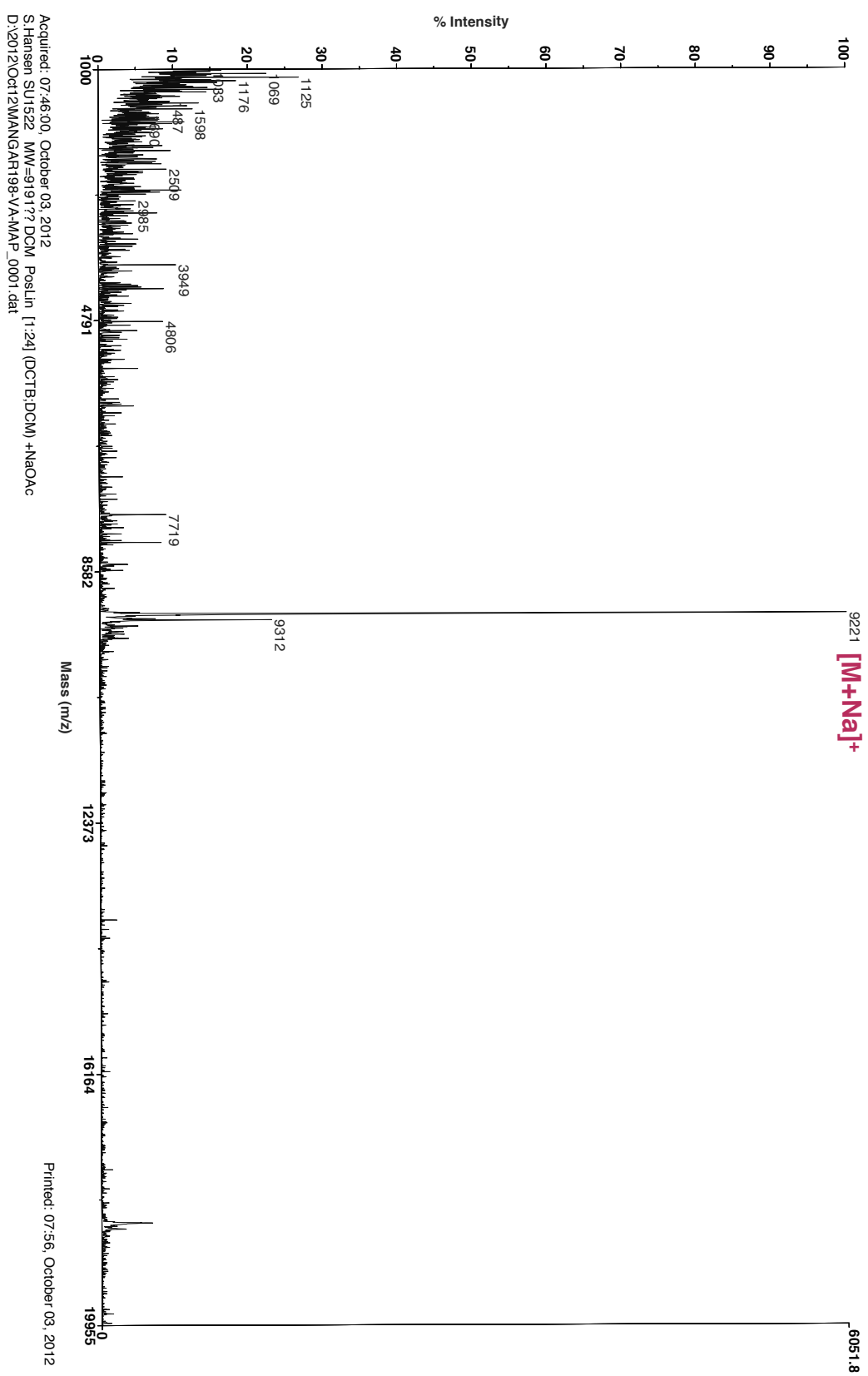


Supplementary Figure 35: HSQC NMR (400 MHz; CDCl₃) spectrum for **8**



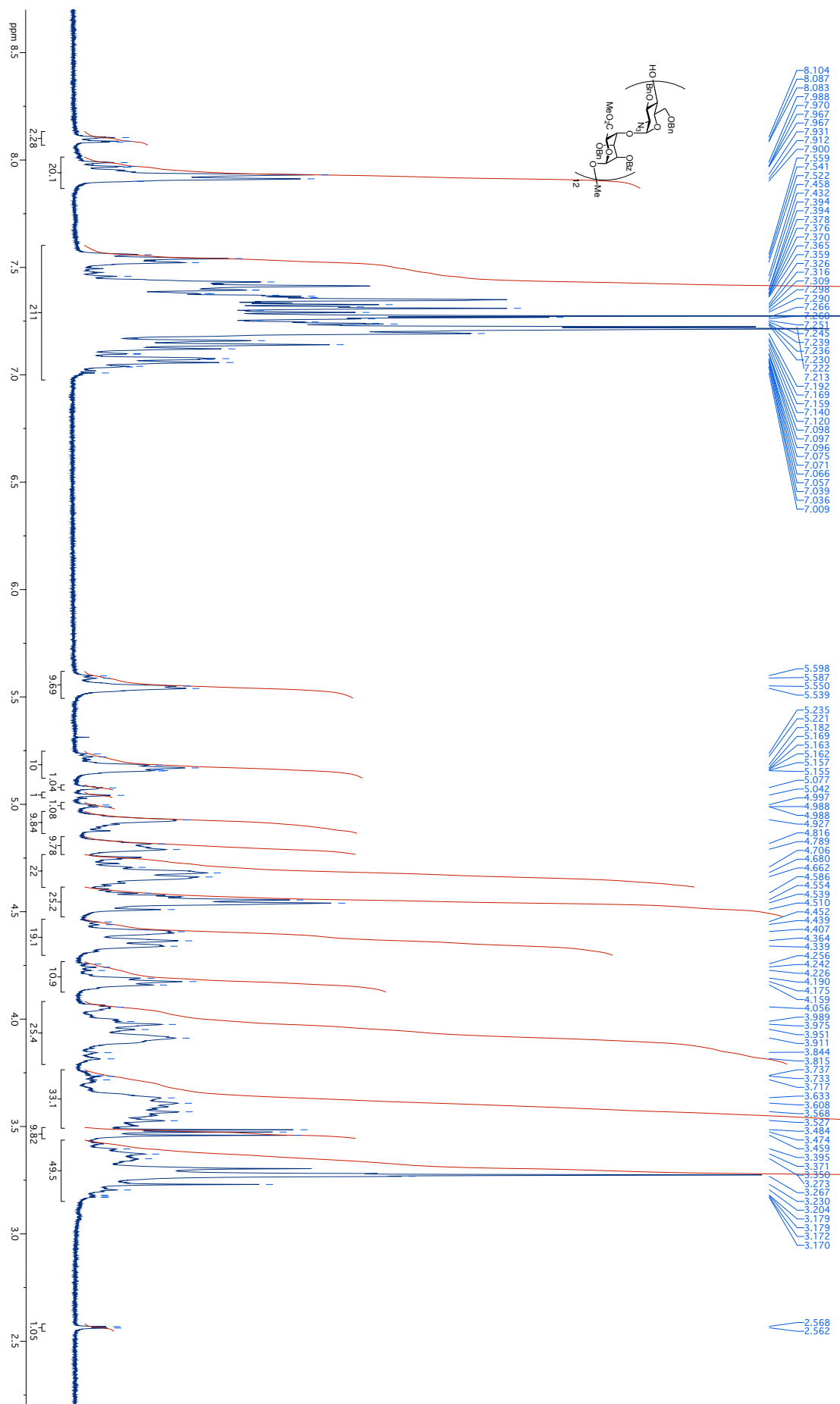
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<<MANGAR198-VA-MAP_0001>> Voyager Spec #1=>MC[BP = 9221.2, 6052]

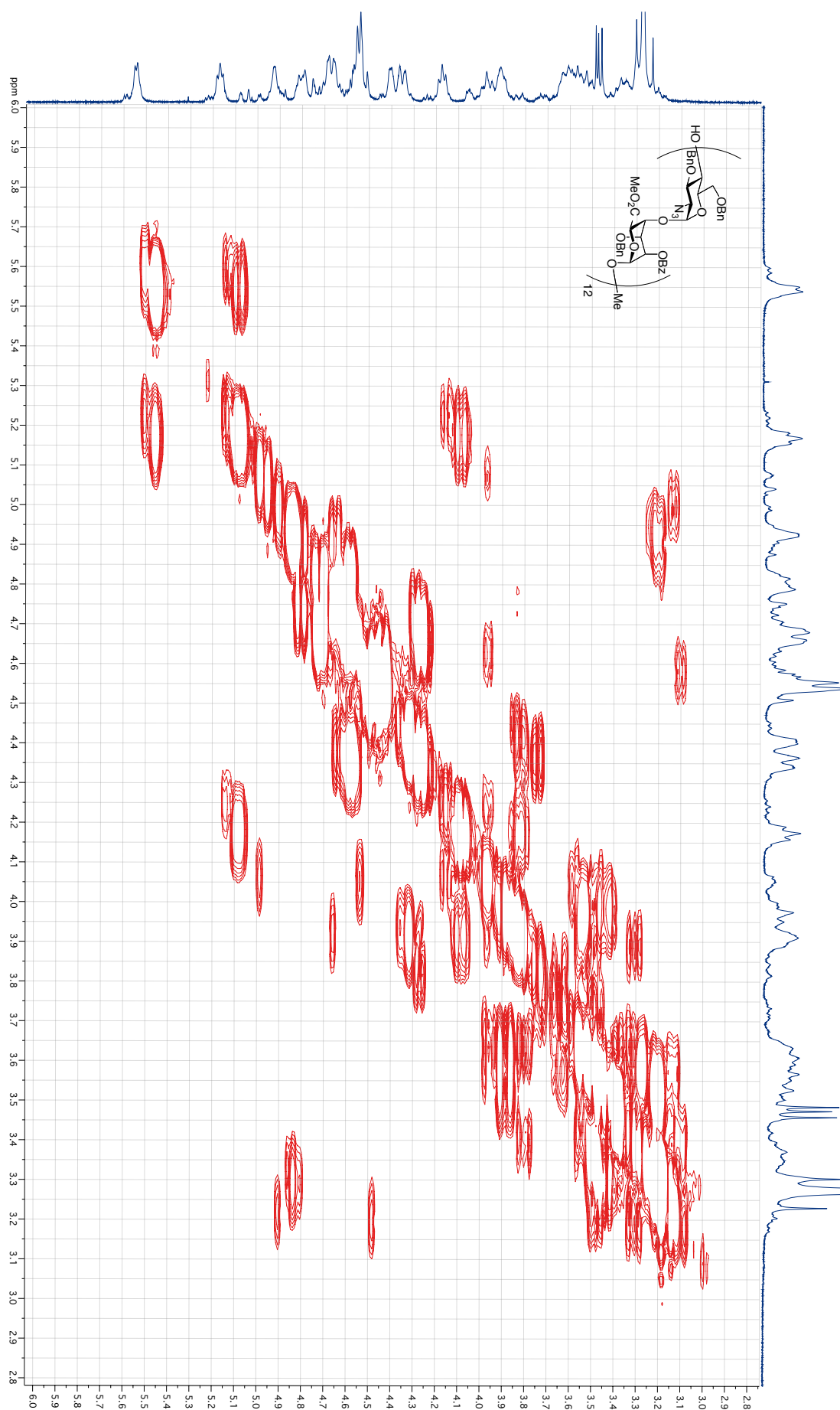


Supplementary Figure 36: MALDI MS spectrum for 8

Supplementary Figure 37: ^1H NMR (400 MHz; CDCl_3) spectrum for **9**

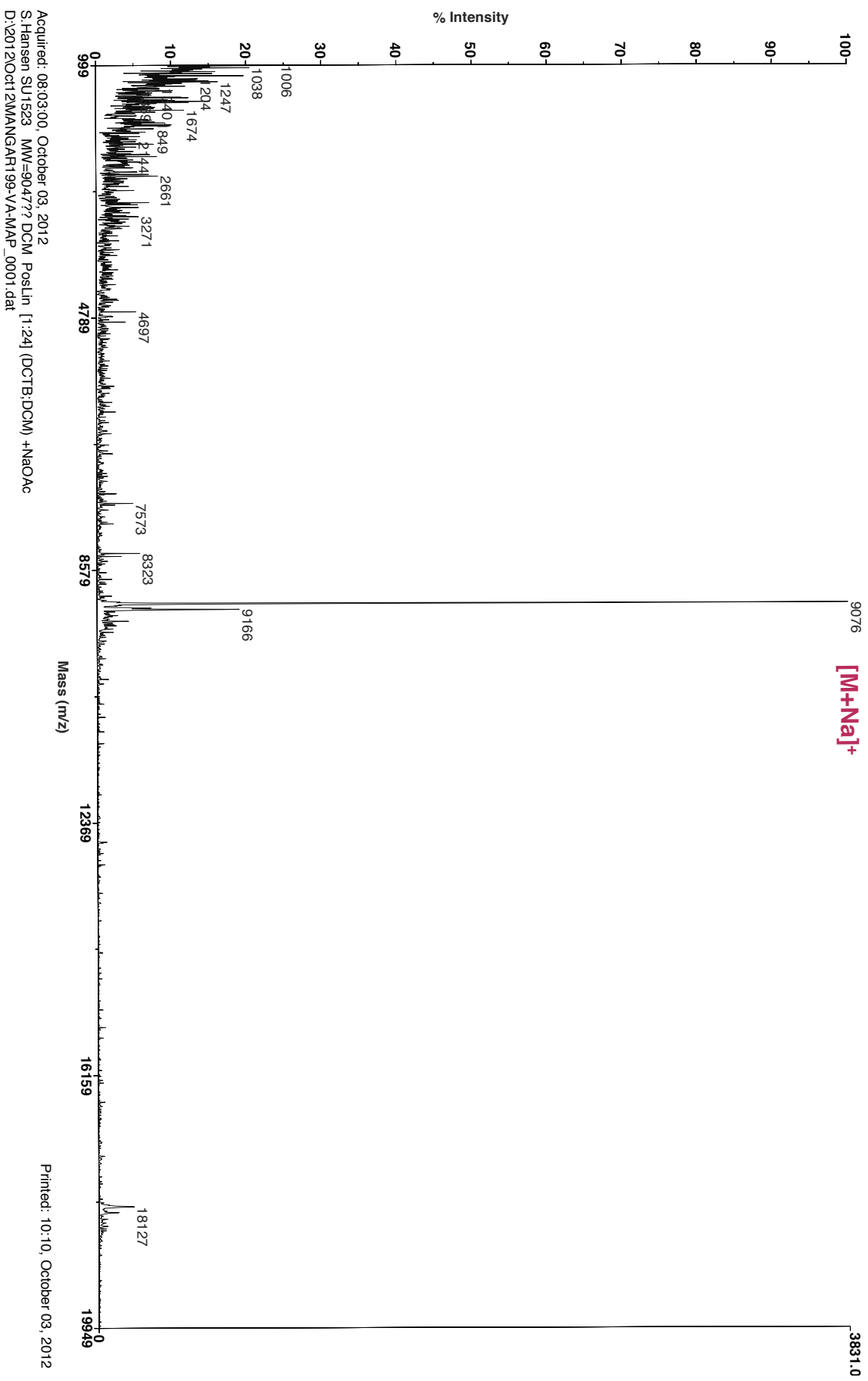


Supplementary Figure 38: COSY NMR (400 MHz; CDCl₃) spectrum for **9**

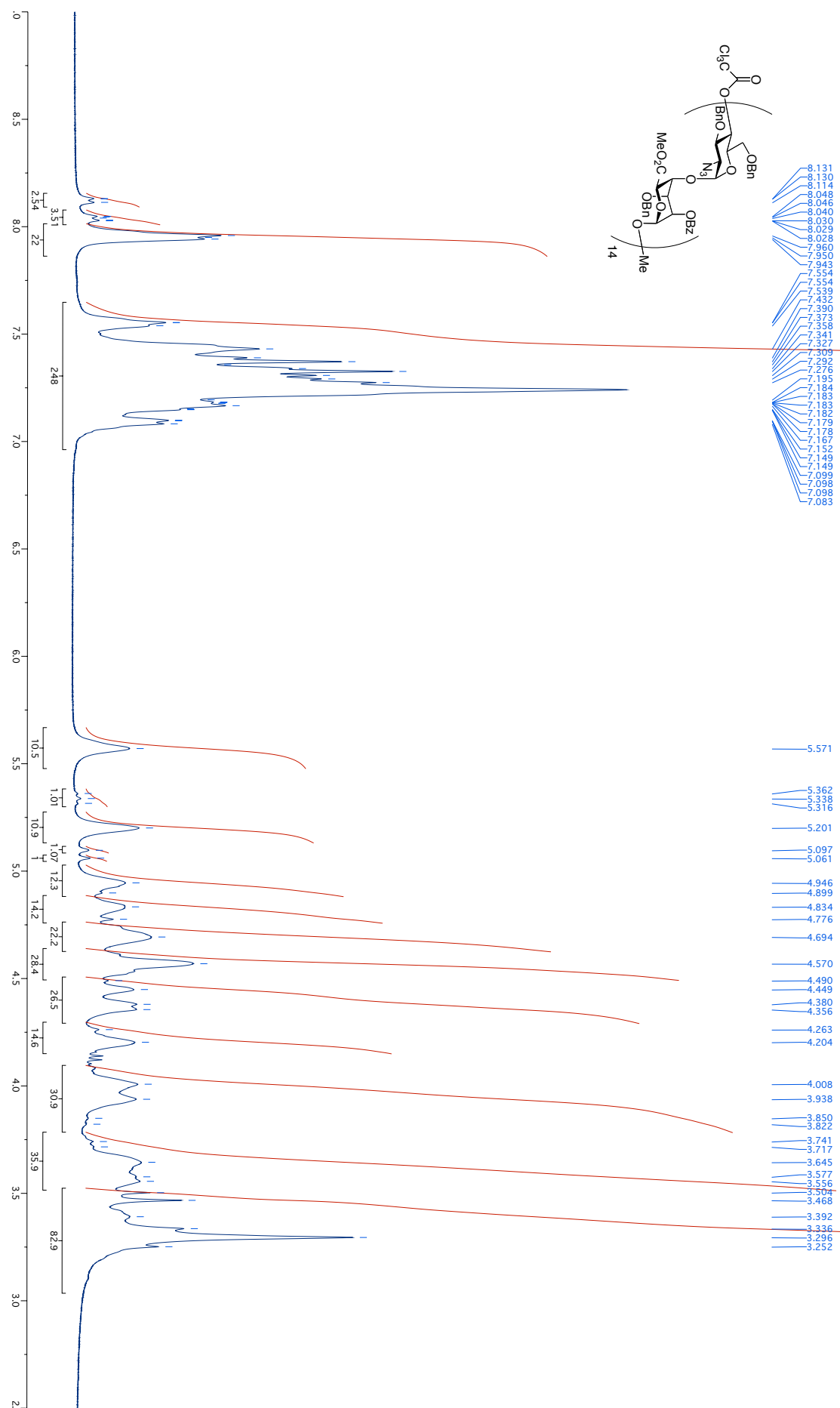


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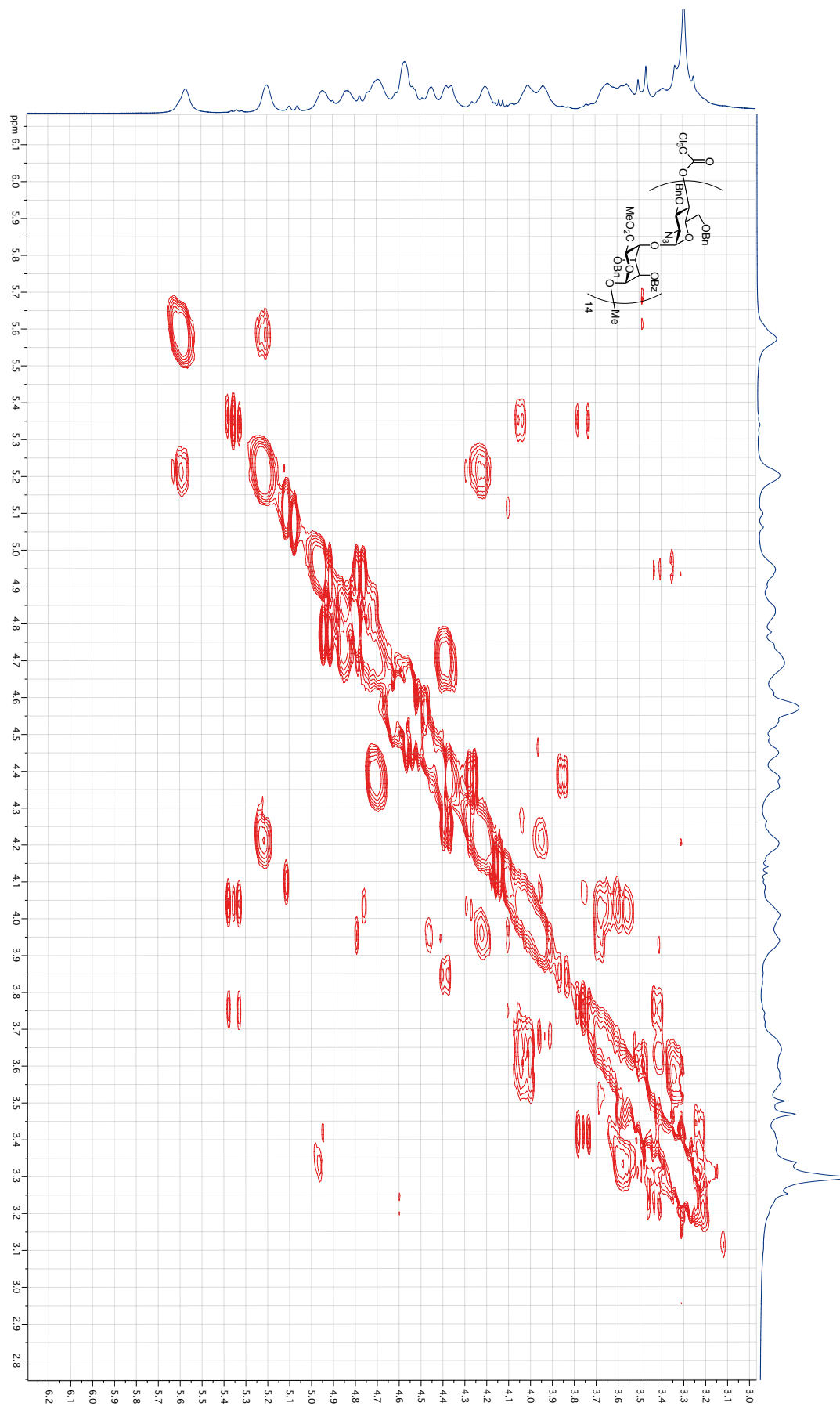
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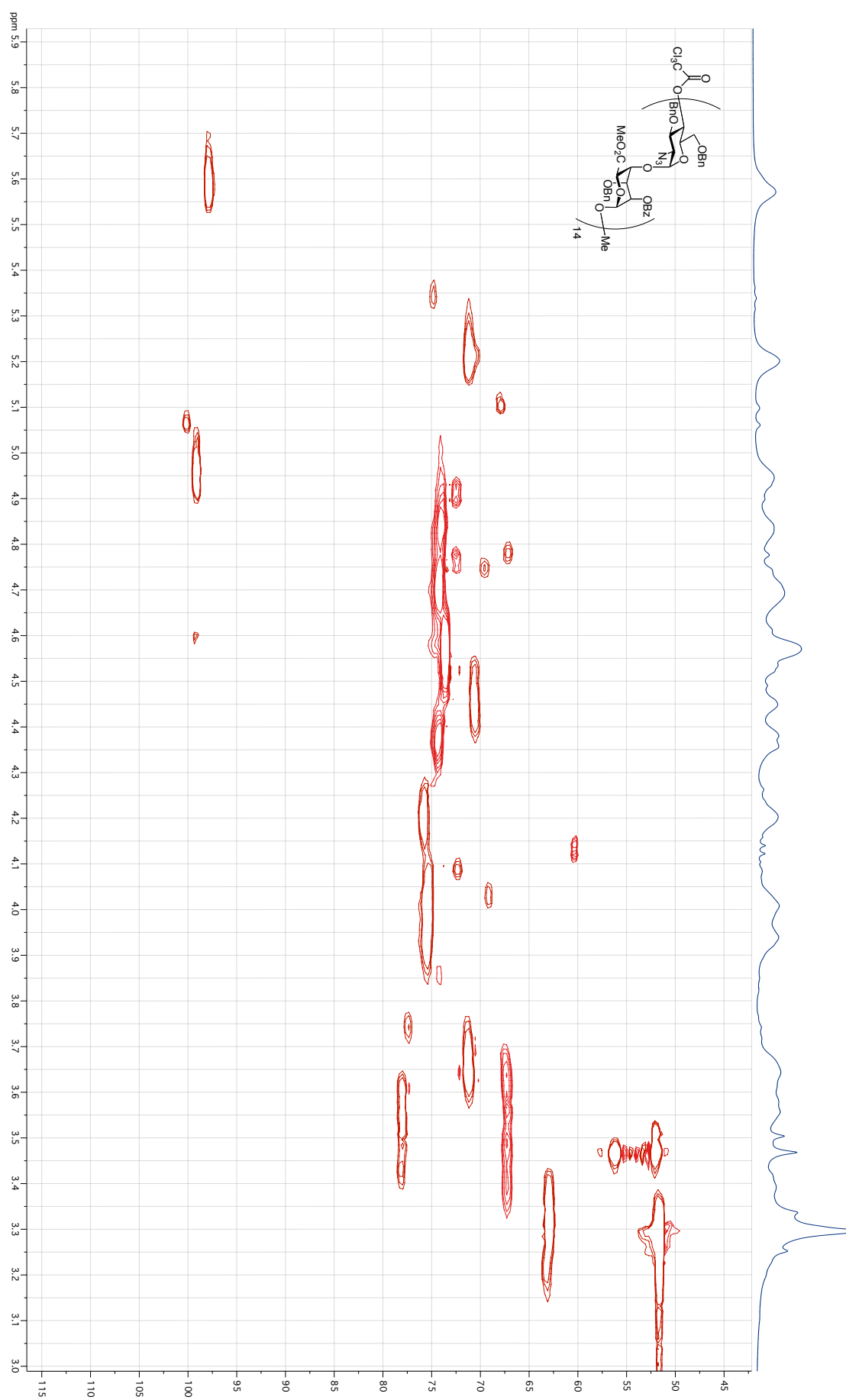
Supplementary Figure 40: ^1H NMR (400 MHz; CDCl_3) spectrum for **10**



Supplementary Figure 41: COSY NMR (400 MHz; CDCl₃) spectrum for **9**

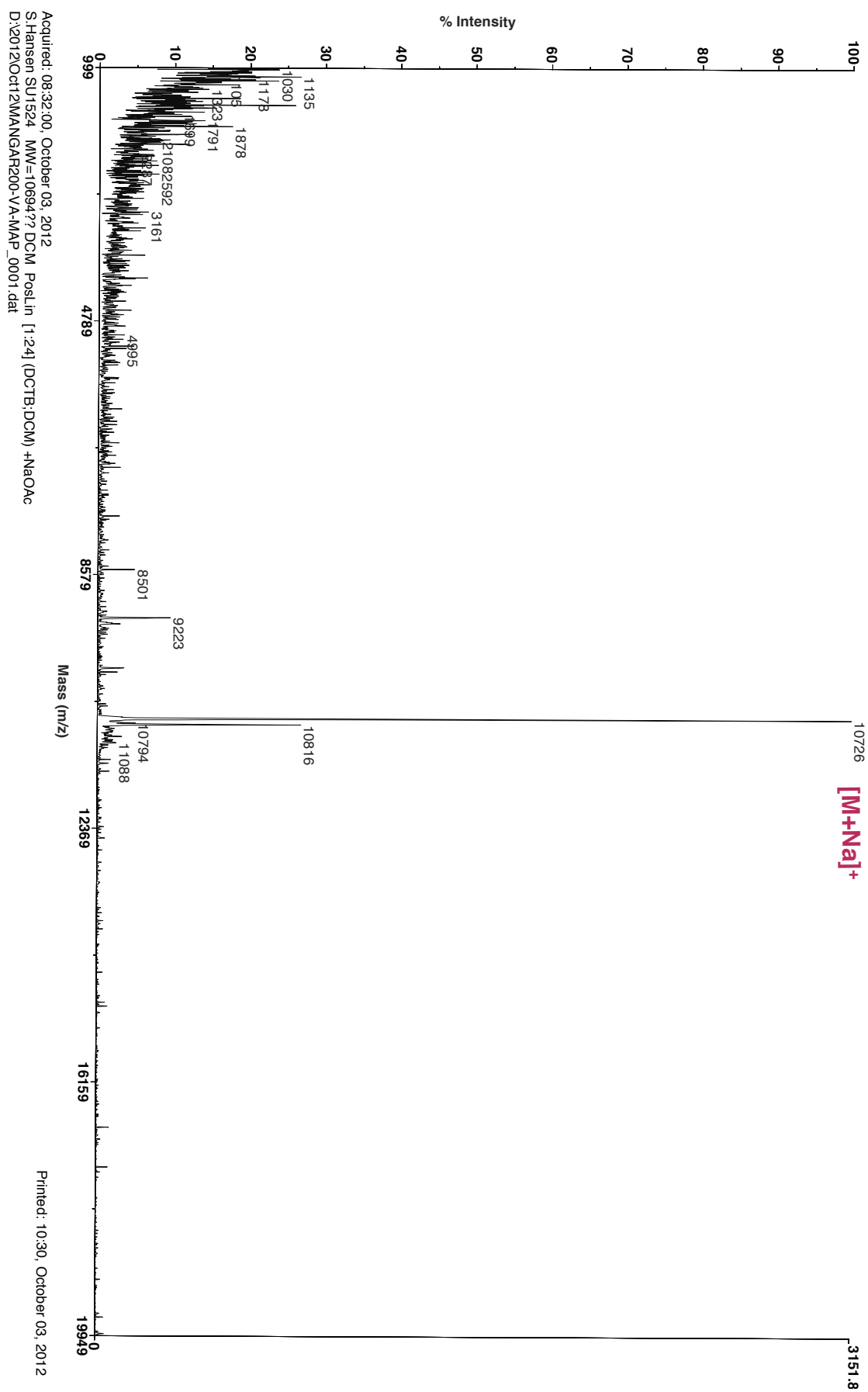


Supplementary Figure 42: HSQC NMR (400 MHz; CDCl₃) spectrum for **10**

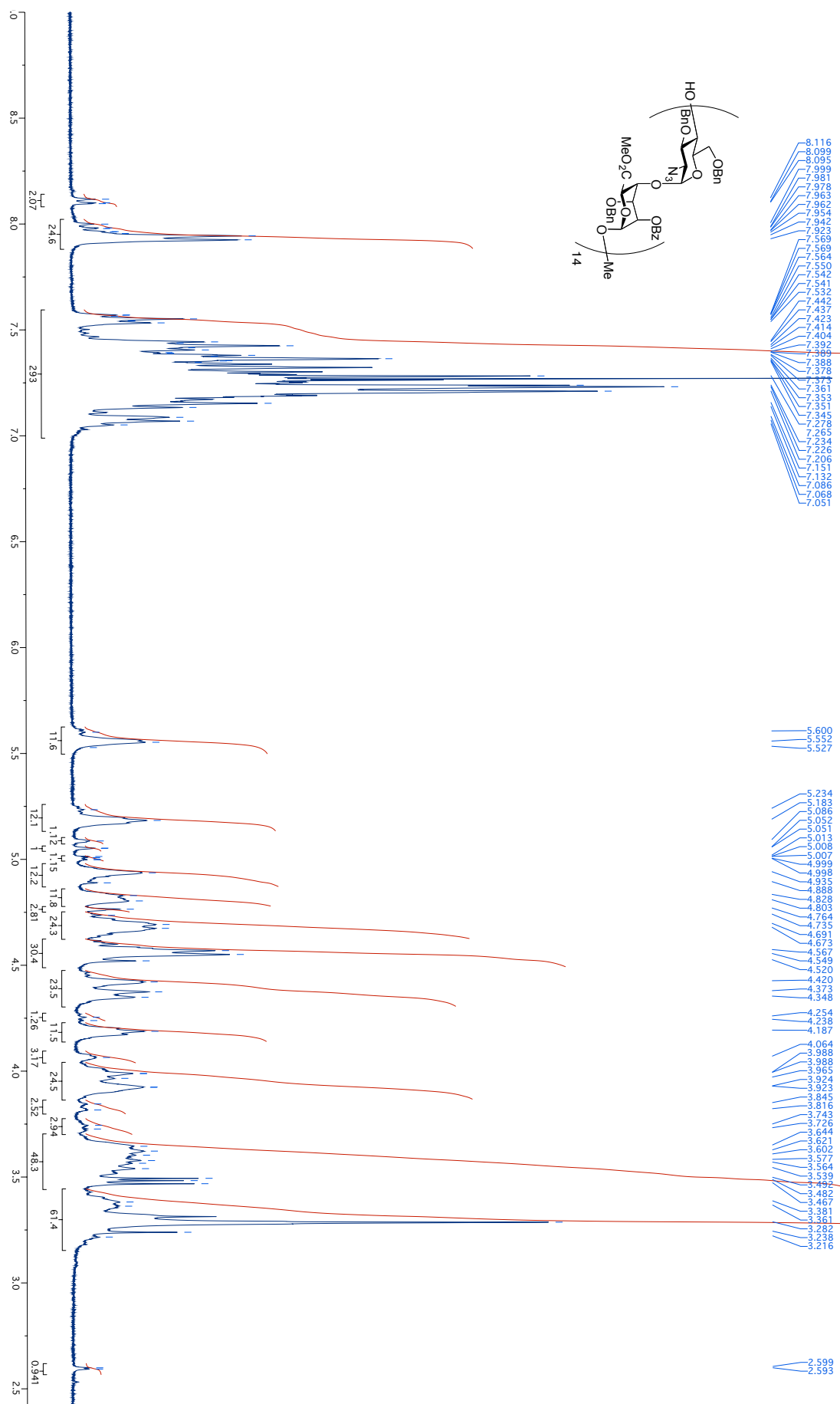


EPSRC National Mass Spectrometry Service Centre (NMSSC), Swansea

<<MANGAR200-VA-MAP_0001>> Voyager Spec #1=>MC=>NF0.7=>SM5[BP = 10725.0, 3152]

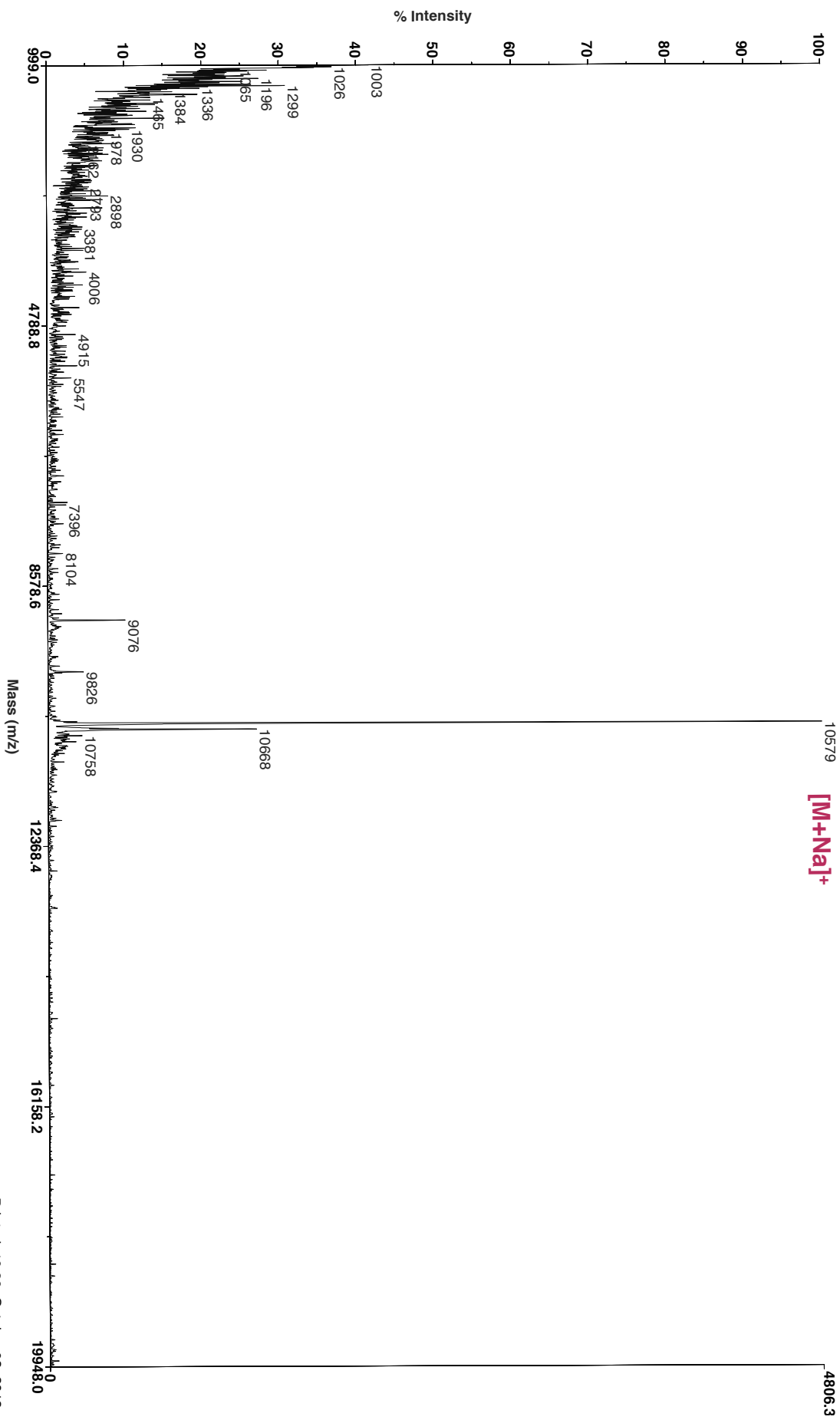
**Supplementary Figure 43: MALDI MS spectrum for 10**

Supplementary Figure 44: ^1H NMR (400 MHz, CDCl_3) spectrum for **11**



The figure displays a 2D NMR spectrum, likely a COSY or HSQC, with the chemical structure of the polymer repeat unit shown in the top right corner. The x-axis represents the chemical shift in ppm, ranging from 2.8 to 5.9. The y-axis represents the chemical shift in ppm, ranging from 2.8 to 6.2. The spectrum shows a complex pattern of peaks, with a prominent diagonal band of peaks indicating correlations between protons in the same repeat unit. The chemical structure of the polymer repeat unit is shown in the top right corner, featuring a backbone with a hydroxyl group (HO), a benzyl group (Bn), a methoxy group (MeO), and a methyl group (Me). The structure is labeled with '14' and '15' indicating specific protons. The spectrum shows a complex pattern of peaks, with a prominent diagonal band of peaks indicating correlations between protons in the same repeat unit. The peaks are distributed across the chemical shift range, with a dense cluster between 3.0 and 4.5 ppm on both axes, and several distinct peaks in the 5.0 to 5.8 ppm region.

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 <<MANGAR201-VA-MAP_0001>> Voyager Spec #1=>MC=>NF0.7=>SM5[BP = 10578.9, 4806]



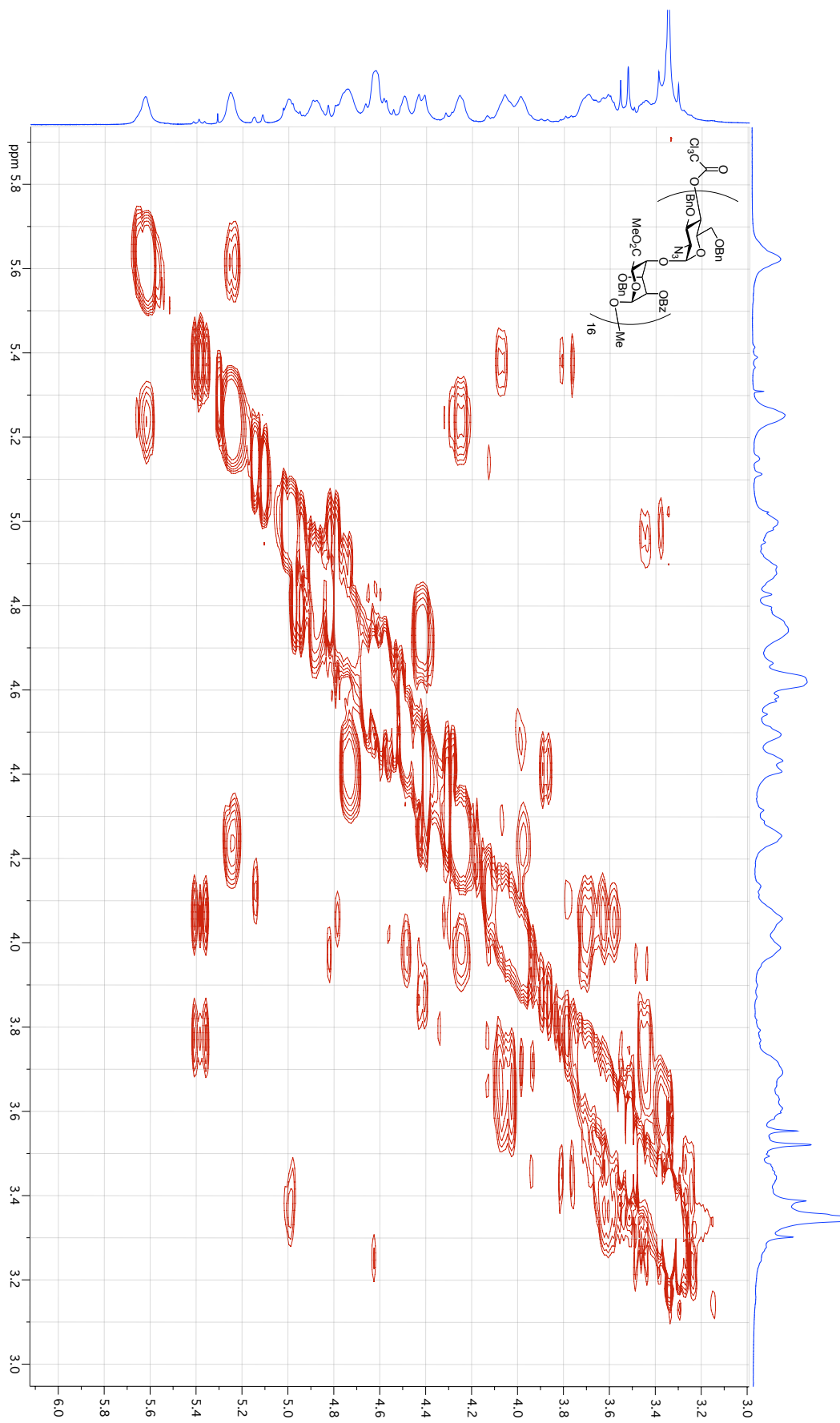
Supplementary Figure 46: MALDI MS spectrum for 11

Acquired: 08:35:00, October 03, 2012
 S.Hansen SU1527 MW=10549?? DCM PosLin [1:24] (DCTB:DCM) +NaOAc
 D:\2012Oct12\MANGAR201-VA-MAP_0001.dat

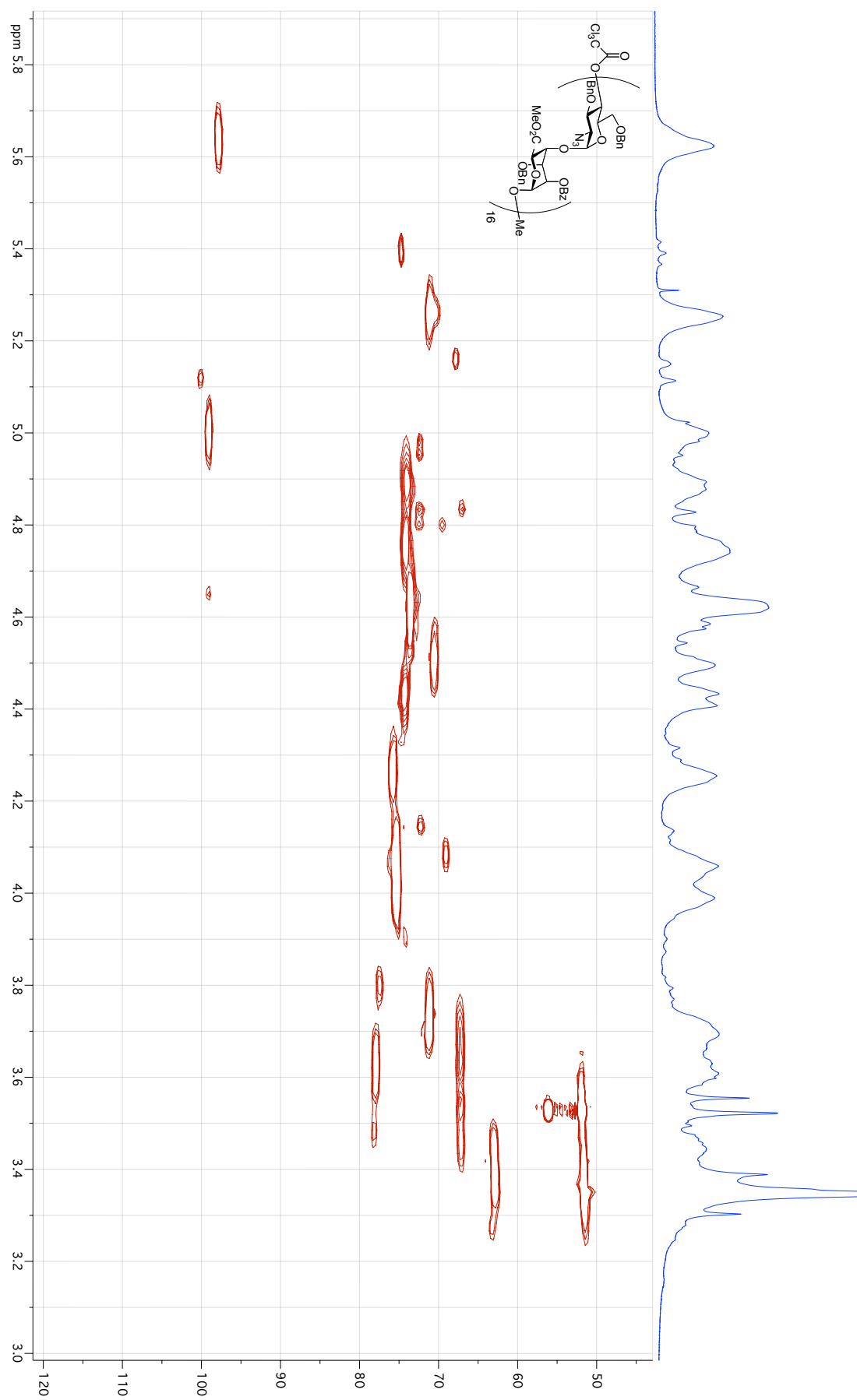
Printed: 10:36, October 03, 2012

Chemical structure of the polymer: 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Supplementary Figure 48: COSY NMR (400 MHz; CDCl₃) spectrum for **12**

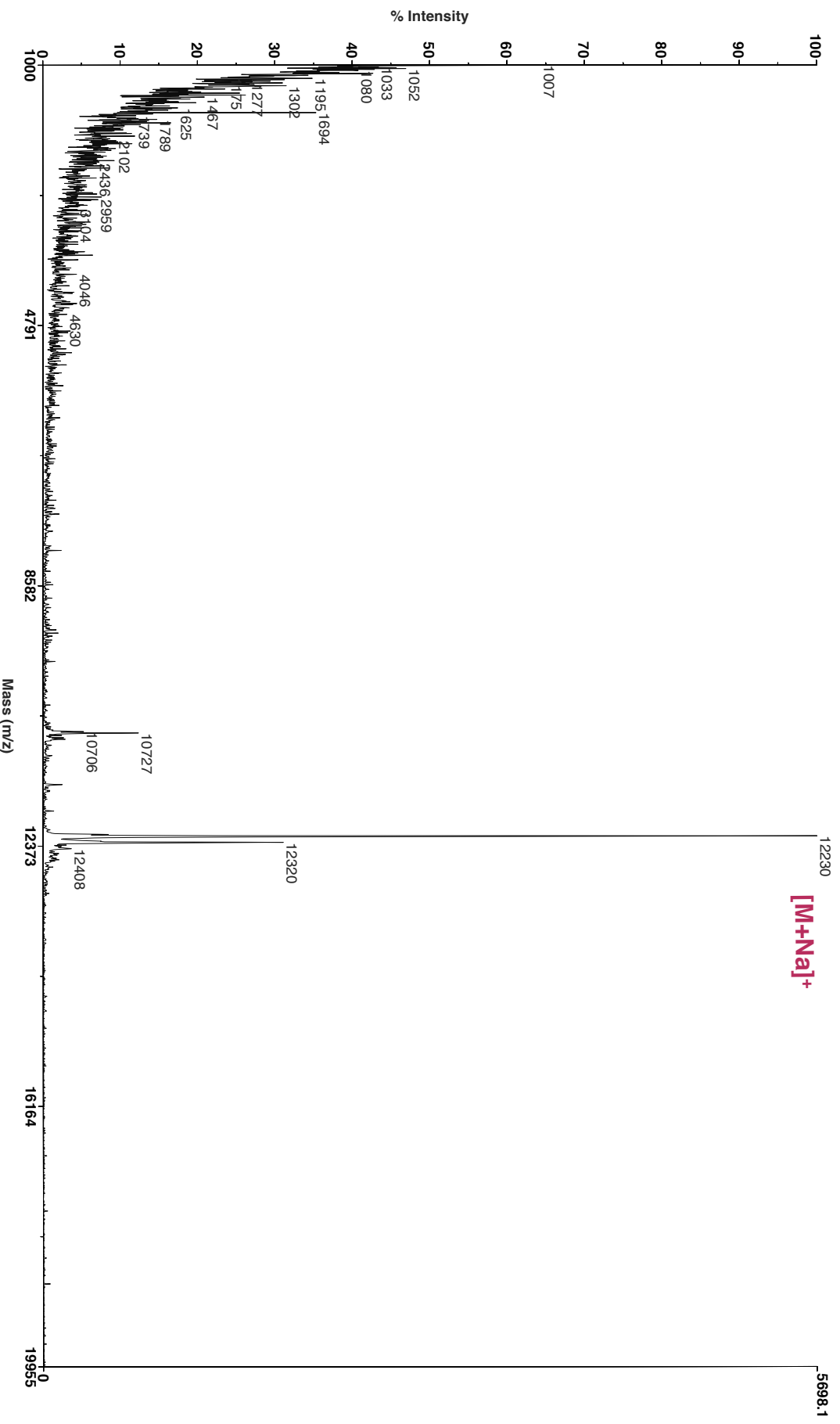


Supplementary Figure 49: HSQC NMR (400 MHz; CDCl₃) spectrum for **12**



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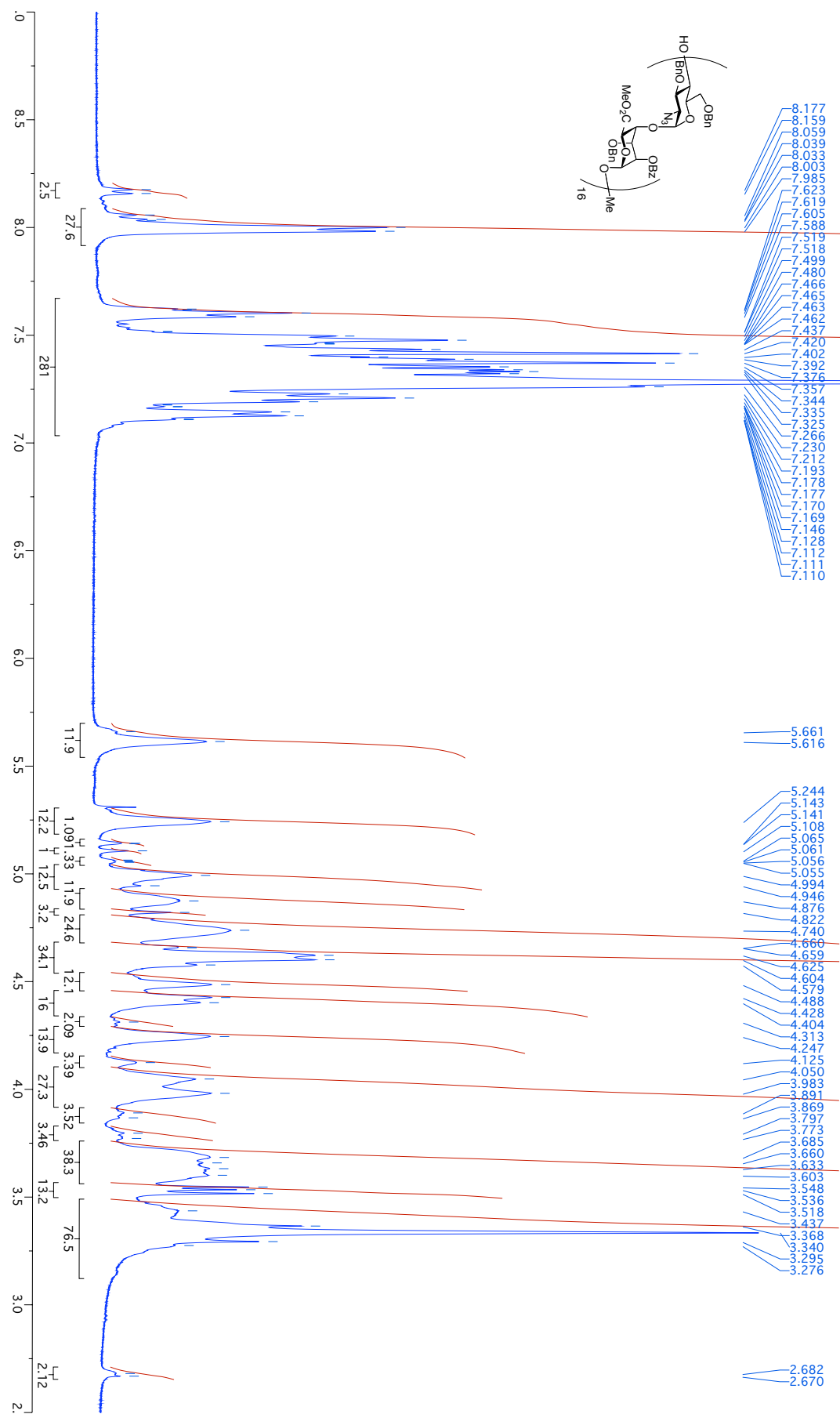
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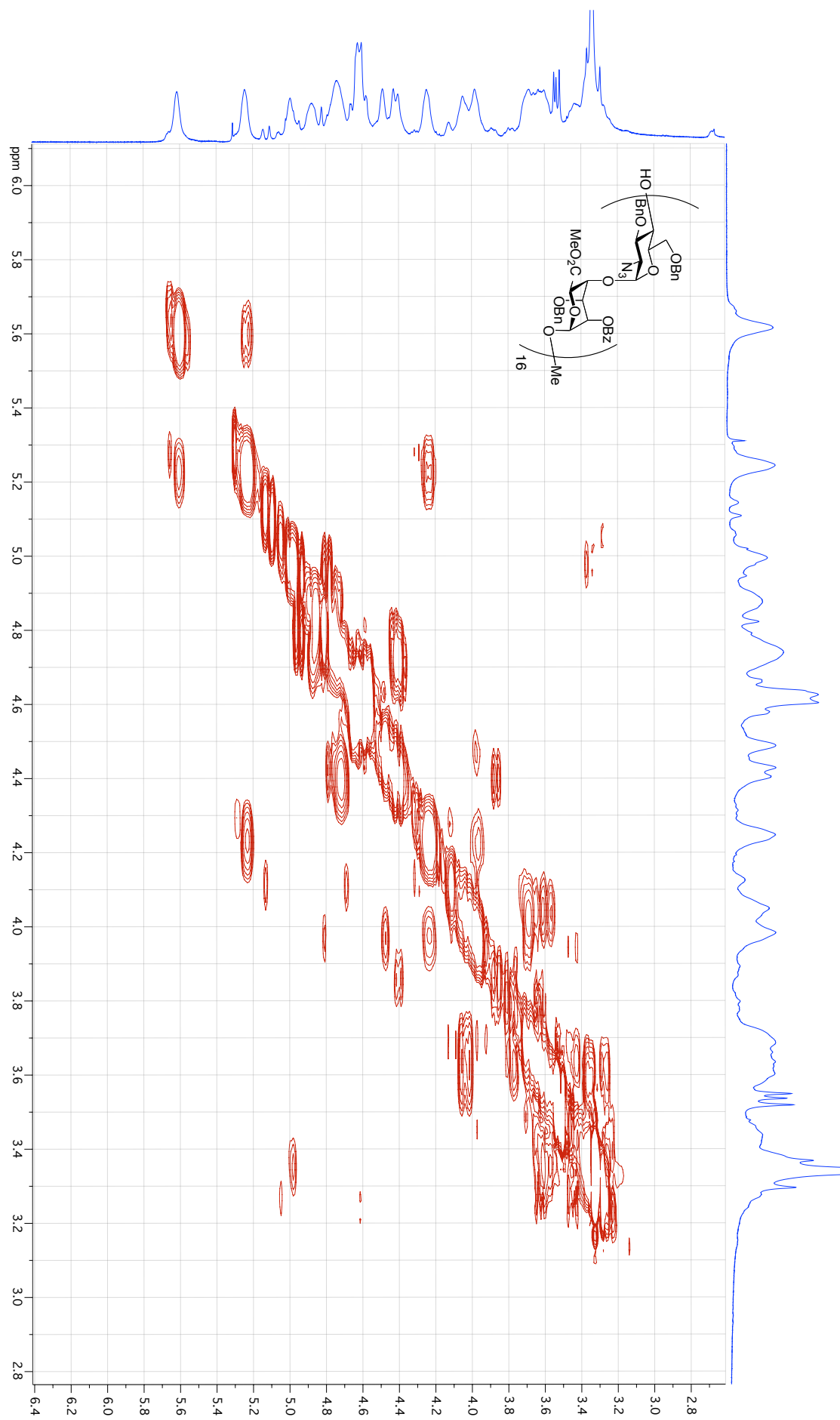
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S.Hansen.SU1528 MW=121967? DCM Poslin [1:24] (DCTB:DCM) +NaOAc
D:\2012\Oct12\MANGAR202-VA-MAP_0001.dat

Printed: 11:00, October 03, 2012

Supplementary Figure 51: ^1H NMR (400 MHz; CDCl_3) spectrum for **12**

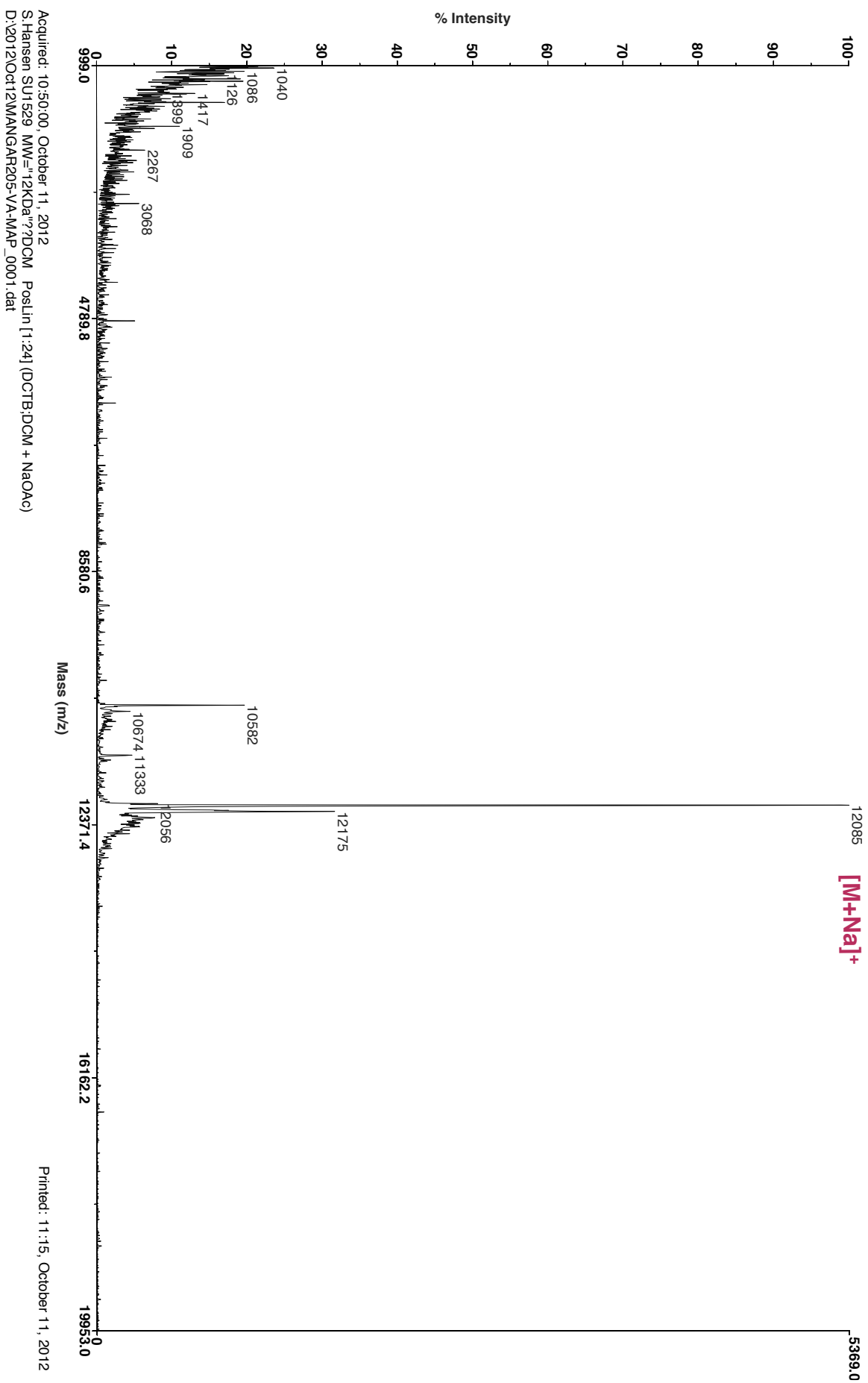


Supplementary Figure 52: COSY NMR (400 MHz; CDCl₃) spectrum for **13**

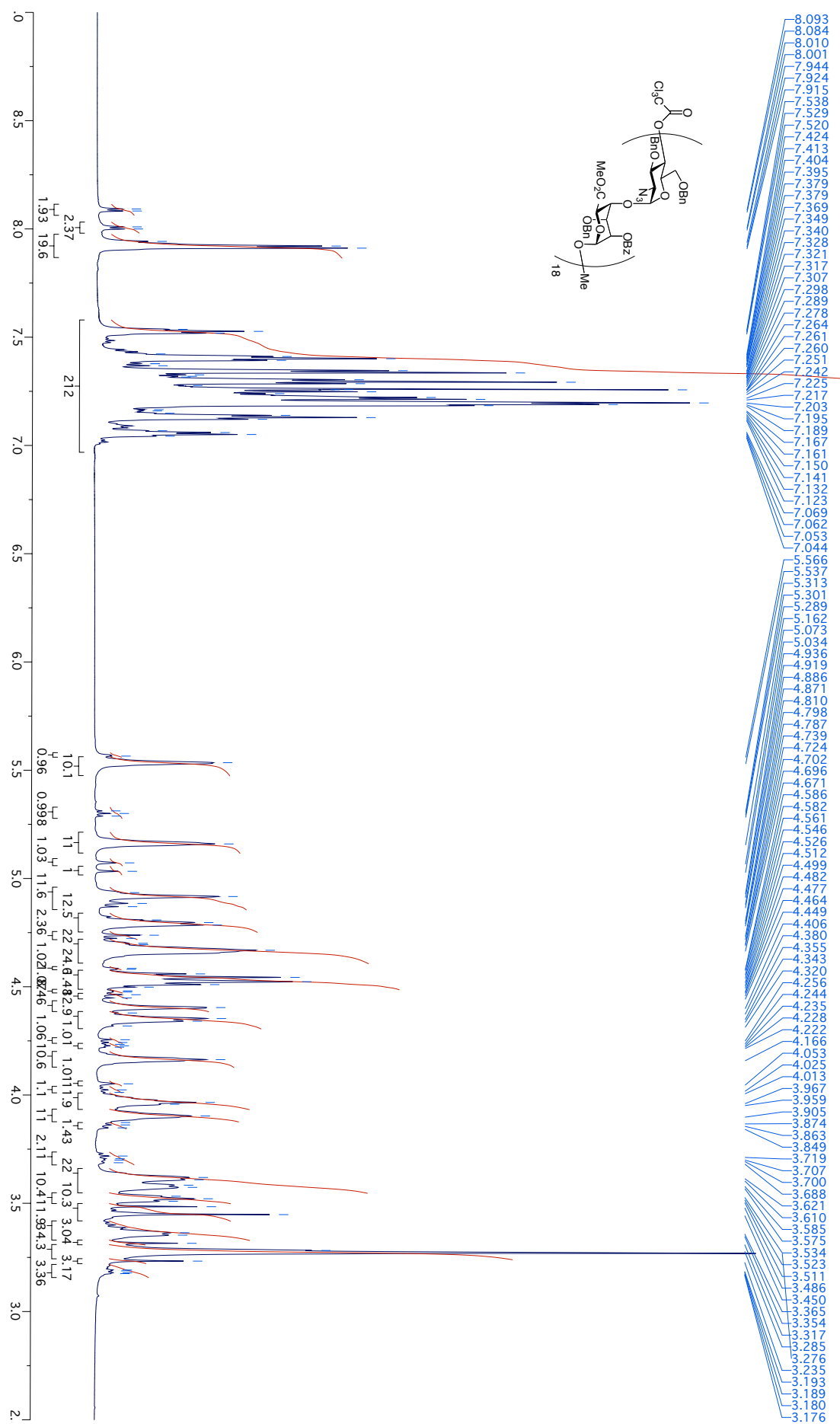


EPSRC National Mass Spectrometry Service Centre (NMSSC), Swansea

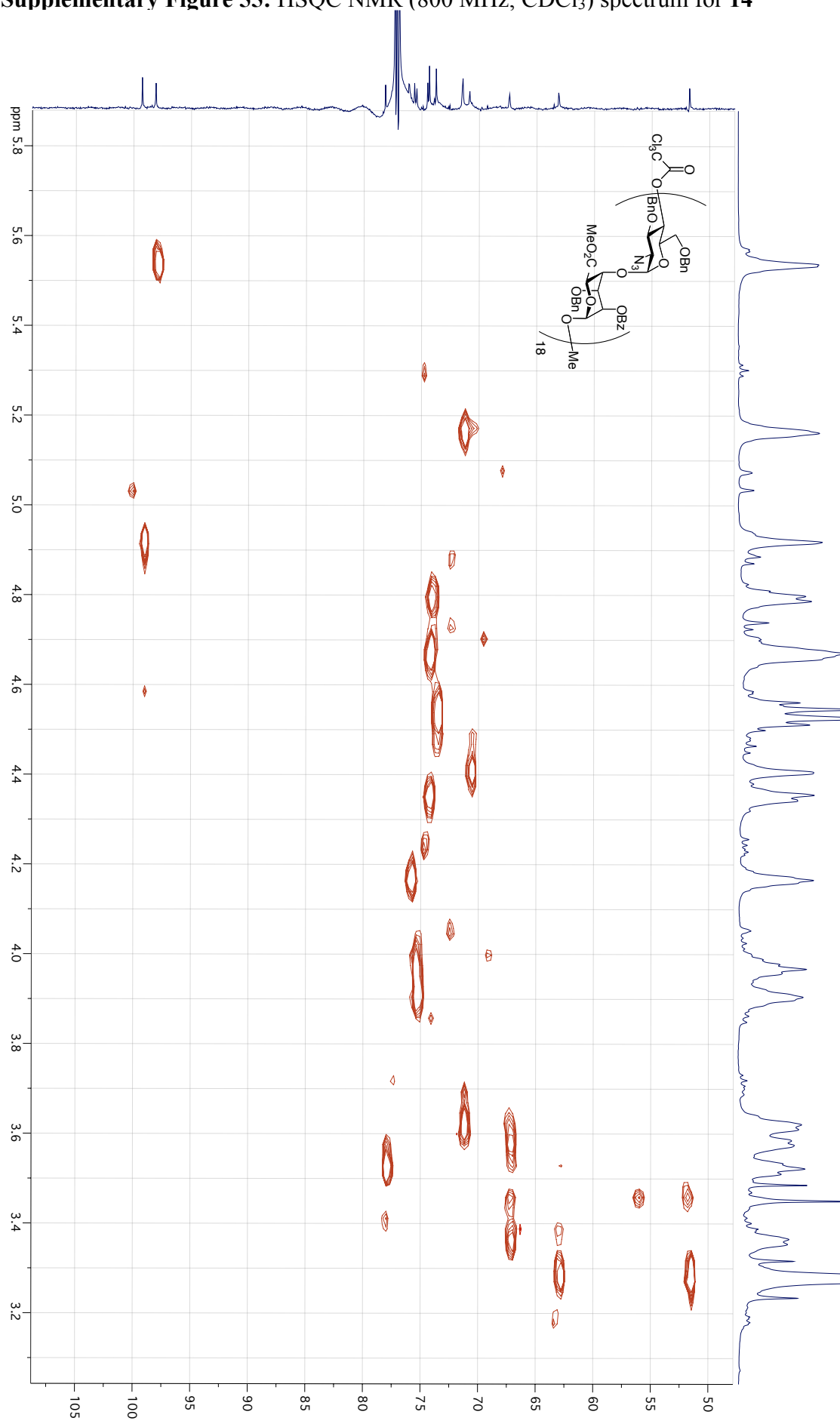
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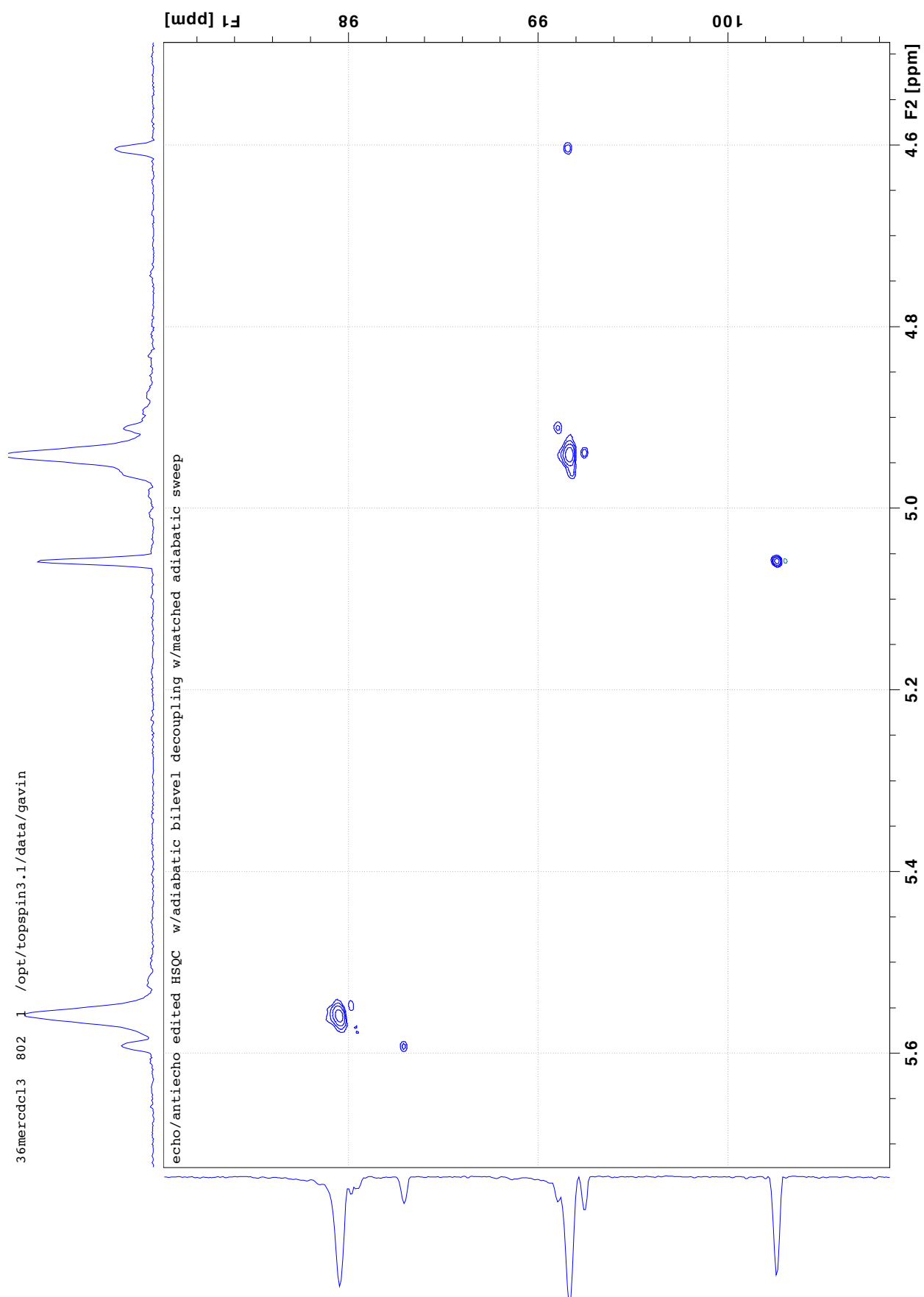
Supplementary Figure 54: ^1H NMR (800 MHz; CDCl_3) spectrum for **14**



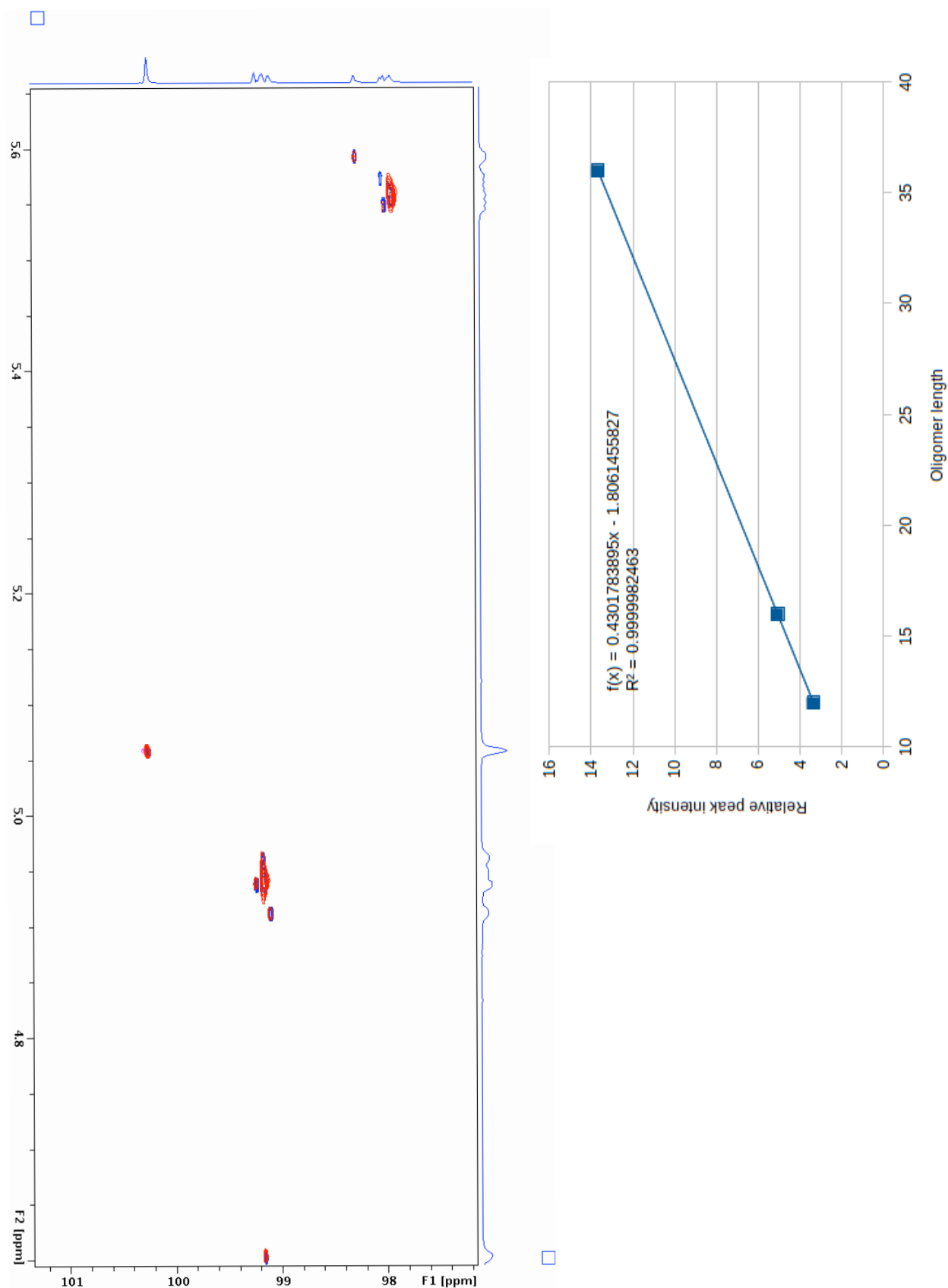
Supplementary Figure 55: HSQC NMR (800 MHz; CDCl₃) spectrum for **14**



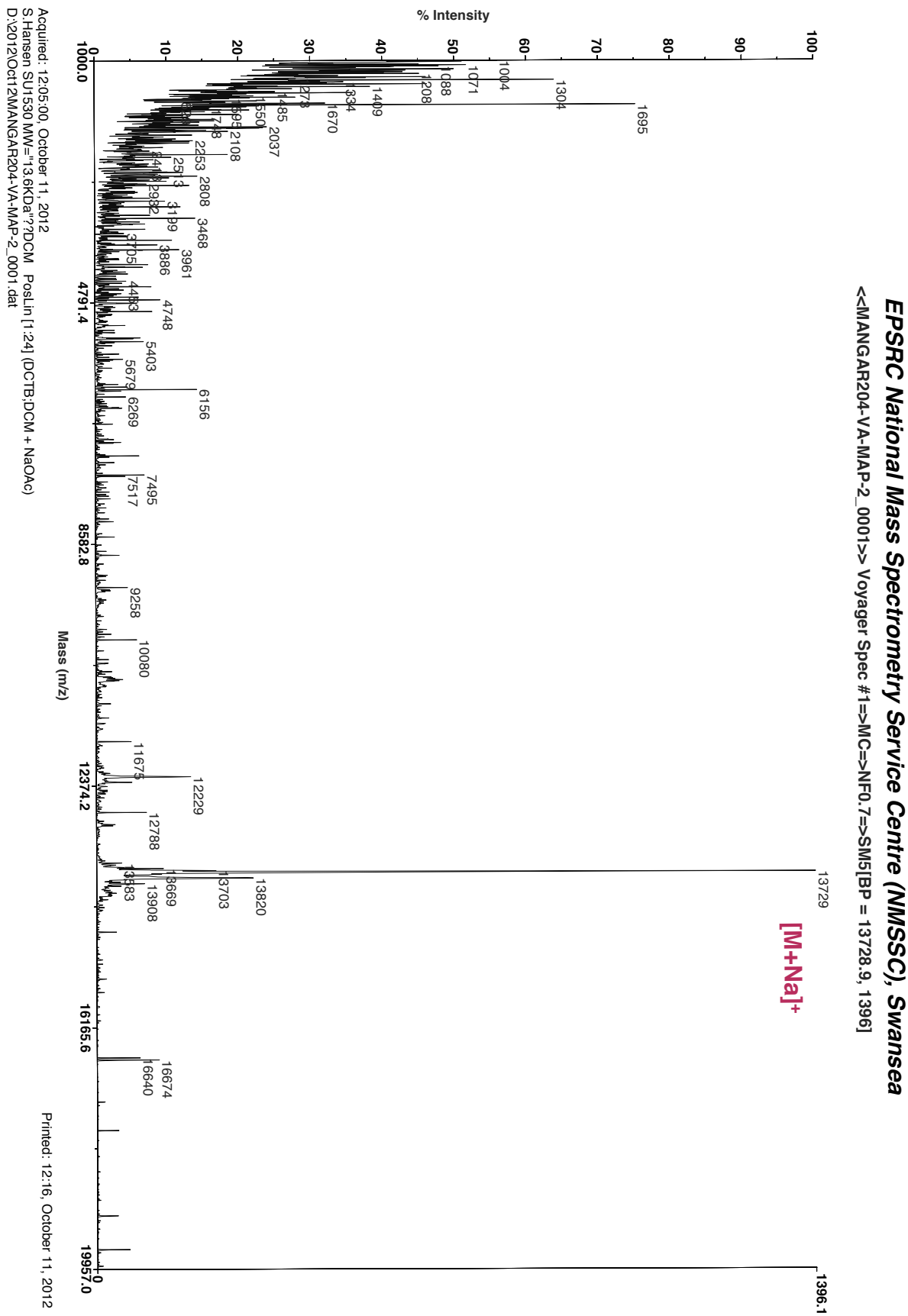
Supplementary Figure 56: NUS Pure Shift HSQC NMR (800 MHz; CDCl₃) spectrum for **14**



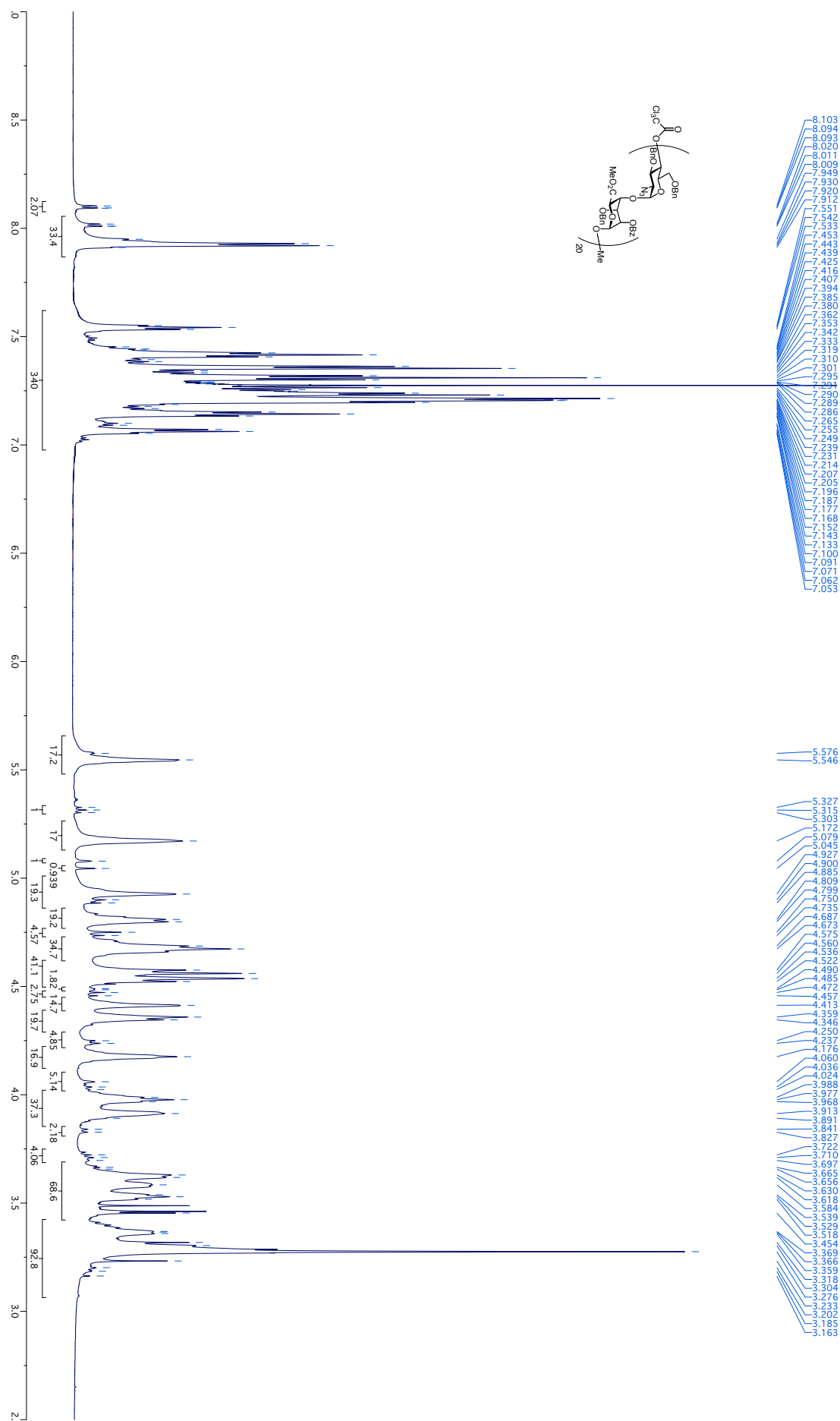
Supplementary Figure 57: (a) NUS Pure Shift HSQC NMR (800 MHz; CDCl₃) spectrum for **14** (red) overlaid with **12mer 2** (blue) - anomeric centres region. **(b)** sum of the crosspeak intensities of the 3 peaks at 4.94 H 99.15 C, relative to that at 4.91 H 99.1 C, as a function of oligomer length.



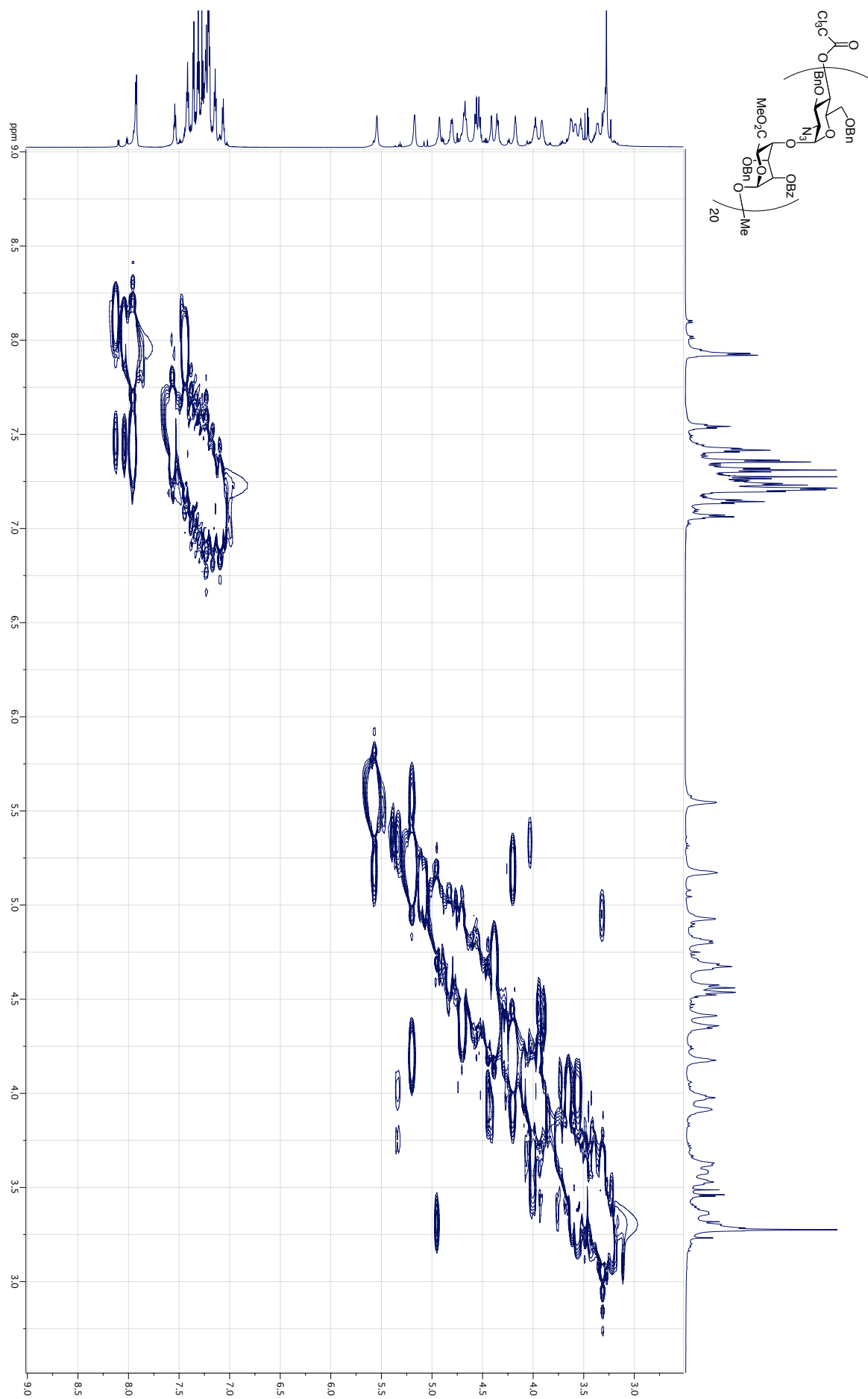
Supplementary Figure 58: MALDI MS spectrum for 14



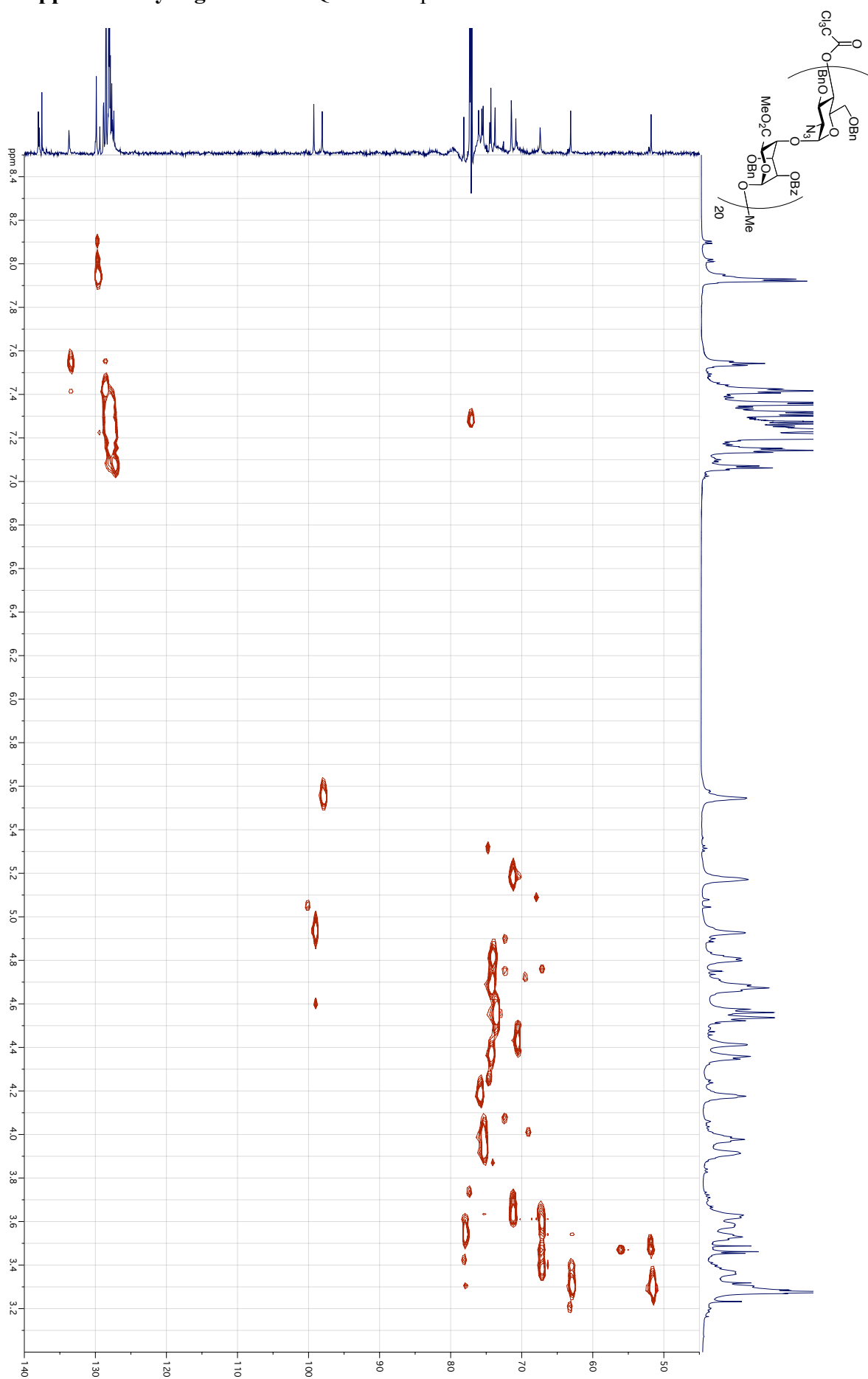
Supplementary Figure 59: ^1H NMR (800 MHz, CDCl_3) spectrum for **16**



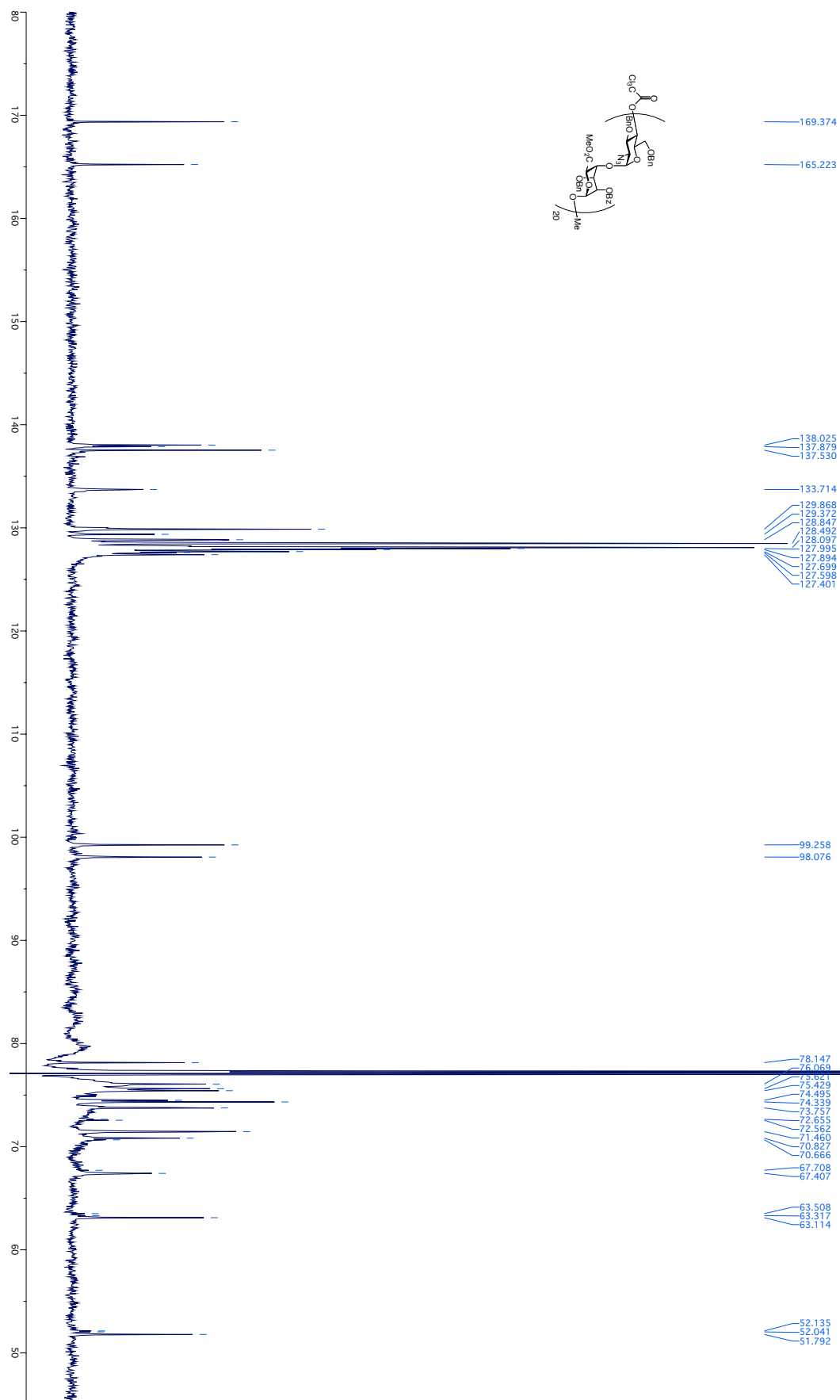
Supplementary Figure 60: COSY NMR spectrum for 16



Supplementary Figure 61: HSQC NMR spectrum for 16



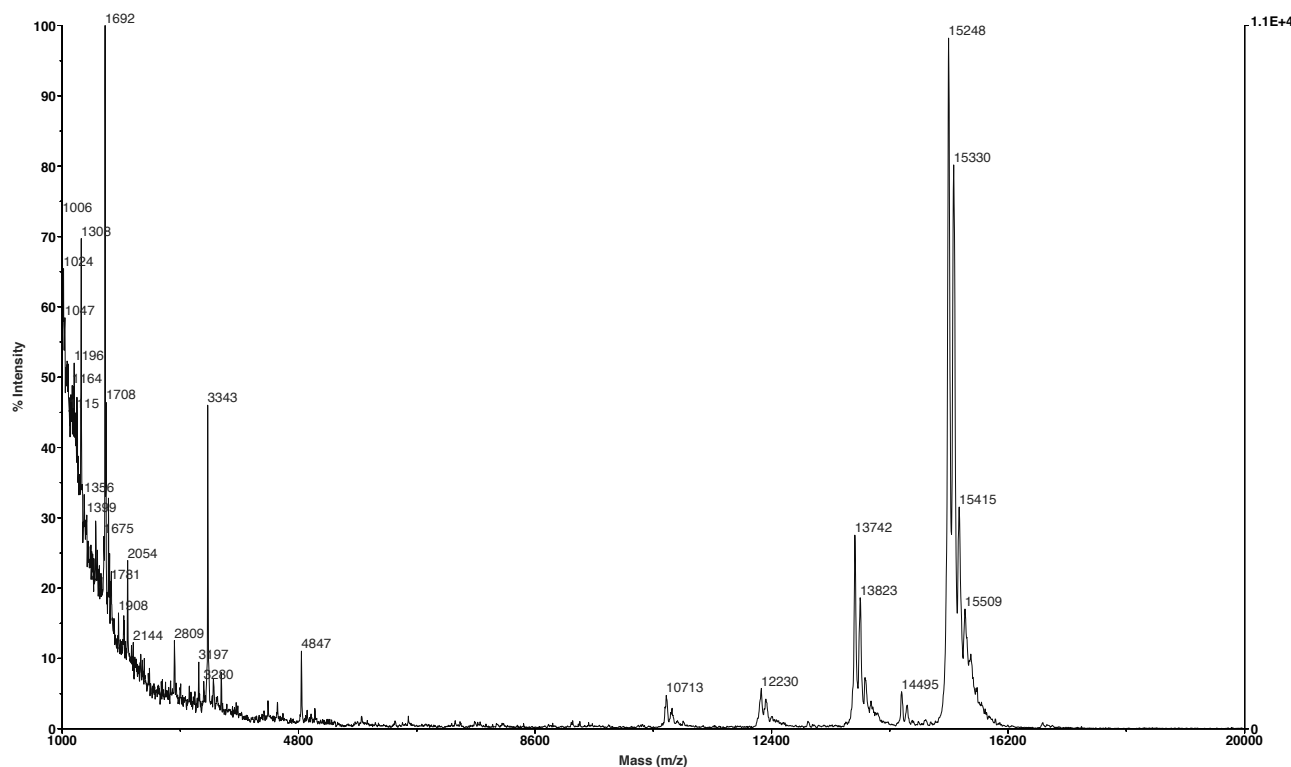
Supplementary Figure 62: ^{13}C NMR (201 MHz; CDCl_3) spectrum for **16**



Supplementary Figure 63: MALDI MS spectrum for 16

EPSRC National Mass Spectrometry Service Centre (NMSSC), Swansea

<<MANGAR230-VM-MAP_0001>> Voyager Spec #1=>NF0.7=>SM9=>MC[BP = 1691.6, 10581]

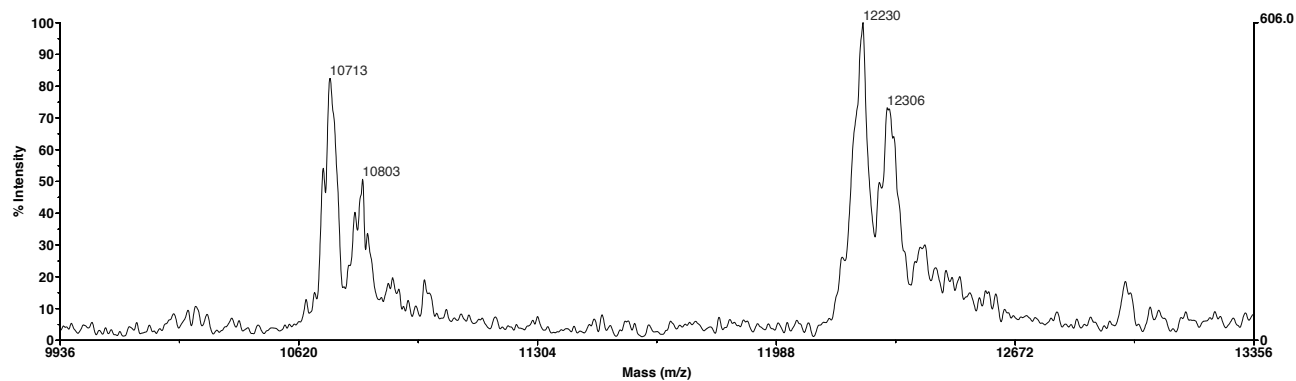


Acquired: 16:40:00, December 11, 2012
Hansen SU1555 MW=15201?? DCM PosLin [1:49] (DCTB;DCM) +NaOAc
D:\2012\Dec12\MANGAR230-VM-MAP_0001.dat

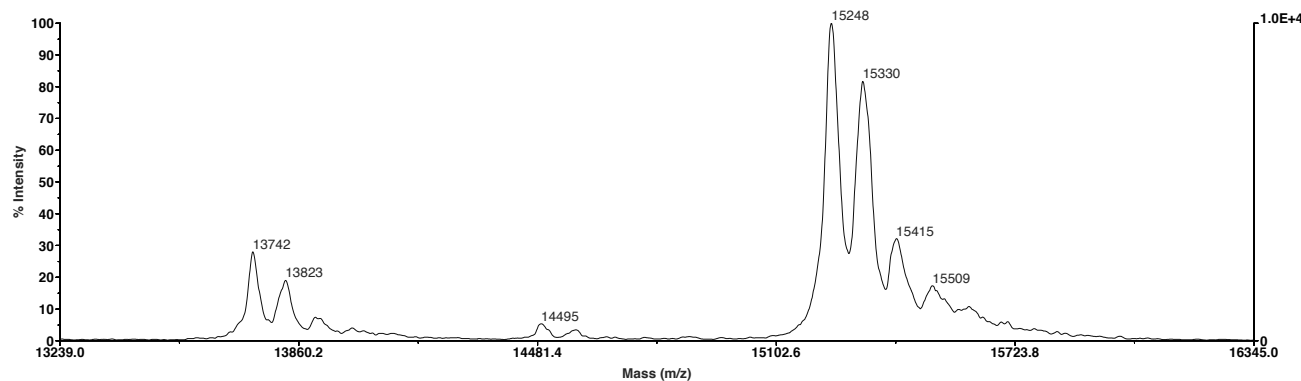
Printed: 17:08, December 11, 2012

EPSRC National Mass Spectrometry Service Centre (NMSSC), Swansea

<<MANGAR230-VM-MAP_0001>> Voyager Spec #1=>NF0.7=>SM9=>MC[BP = 1691.6, 10581]



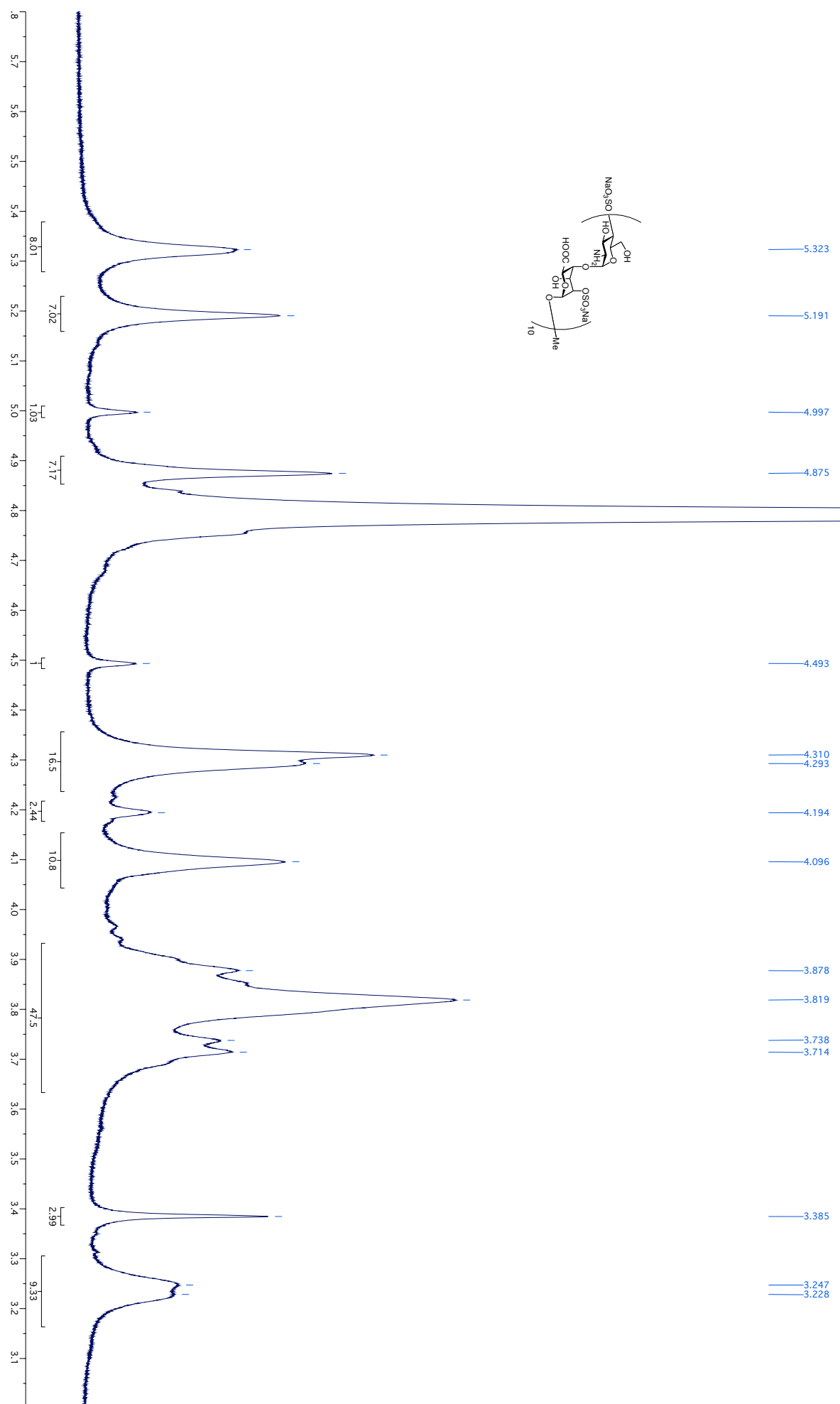
<<MANGAR230-VM-MAP_0001>> Voyager Spec #1=>NF0.7=>SM9=>MC[BP = 1691.6, 10581]



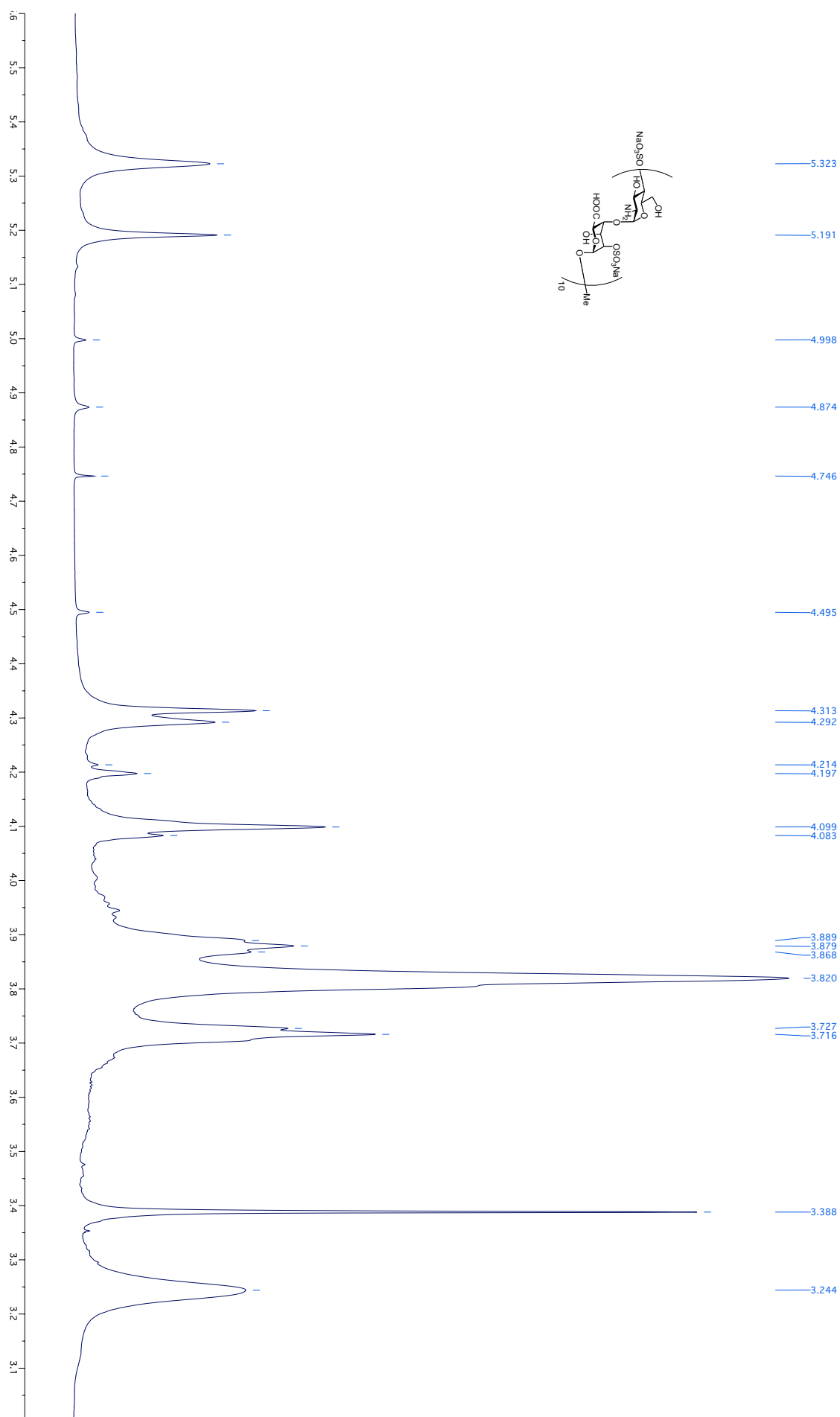
Acquired: 16:40:00, December 11, 2012
Hansen SU1555 MW=15201?? DCM PosLin [1:49] (DCTB;DCM) +NaOAc
D:\2012\Dec12\MANGAR230-VM-MAP_0001.dat

Printed: 17:10, December 11, 2012

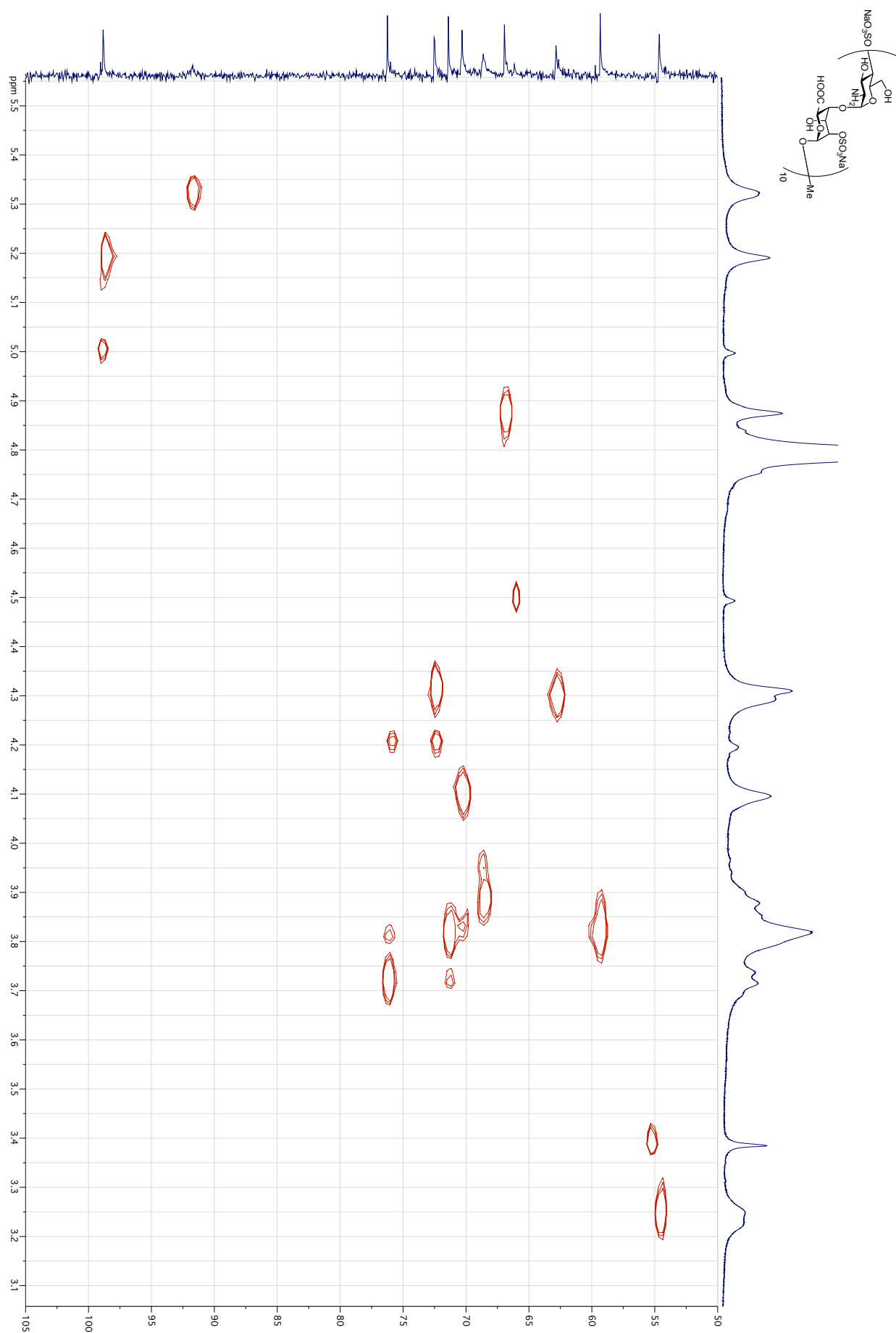
Supplementary Figure 64: ^1H NMR (800 MHz; D_2O) spectrum for **18**



Supplementary Figure 65: ^1H water suppression NMR (800 MHz; D_2O) spectrum for **18**



Supplementary Figure 66: HSQC NMR (800 MHz; D₂O) spectrum for **18**



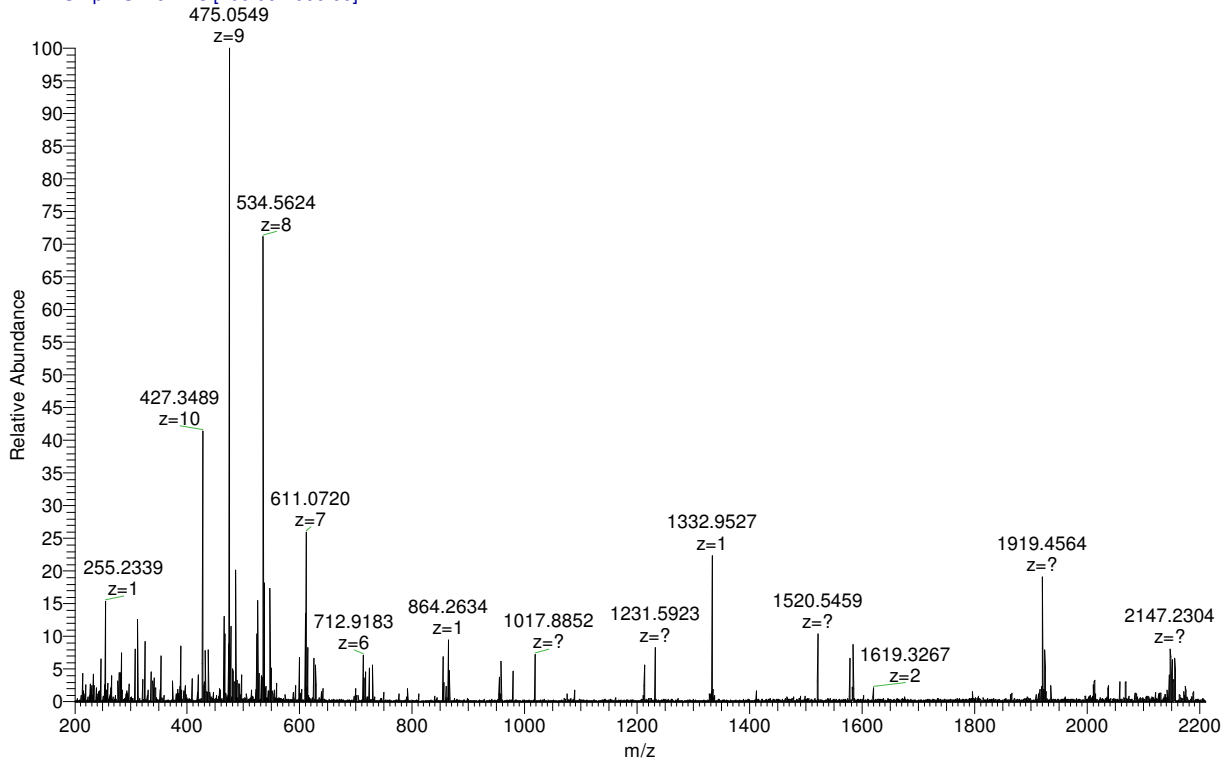
Supplementary Figure 67: FT NSI MS spectrum for 18

SU_1553 MW=4285?
(H₂O)/MeOH + DEA

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LTQ Orbitrap XL

Gavin Miller
18/06/2013 12:42:11

MANGAR291-OJ-HNESN-2 #62-70 RT: 2.31-2.94 AV: 9 SM: 7G NL: 1.81E6
T: FTMS - p NSI Full ms [200.00-4000.00]

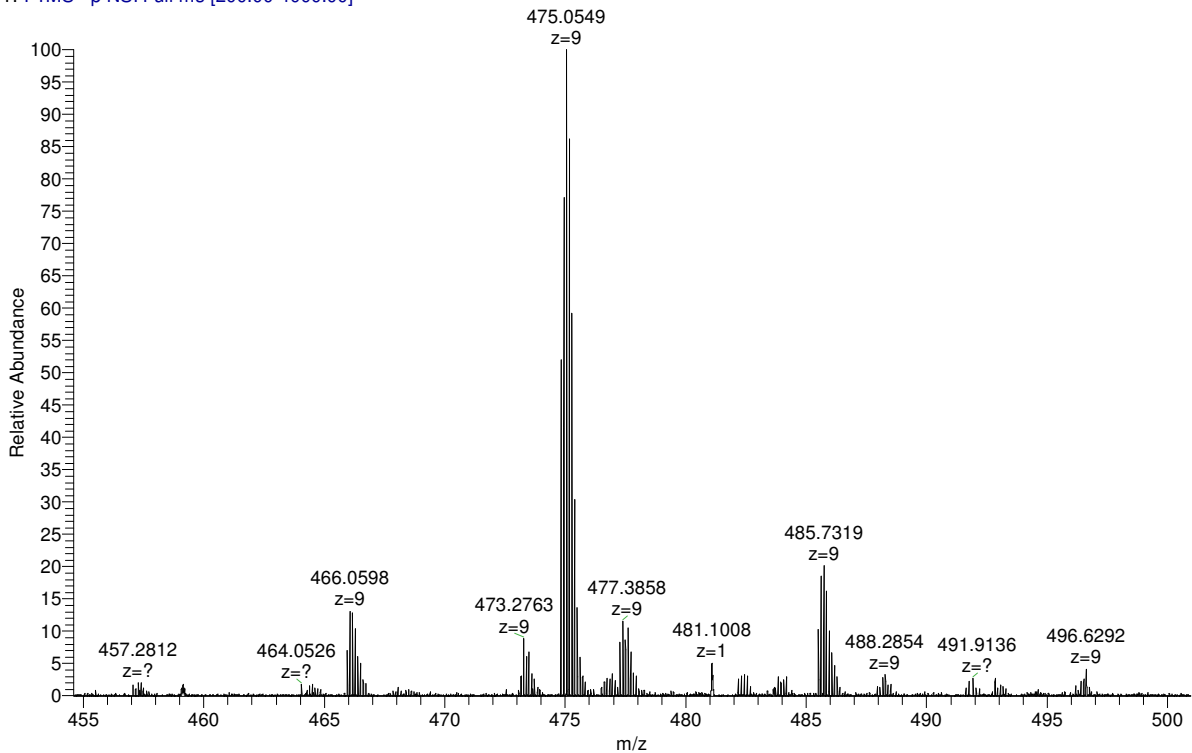


SU_1553 MW=4285?
(H₂O)/MeOH + DEA

EPSRC National Facility Swansea
LTQ Orbitrap XL

Gavin Miller
18/06/2013 12:42:11

MANGAR291-OJ-HNESN-2 #62-70 RT: 2.31-2.94 AV: 9 SM: 7G NL: 1.81E6
T: FTMS - p NSI Full ms [200.00-4000.00]



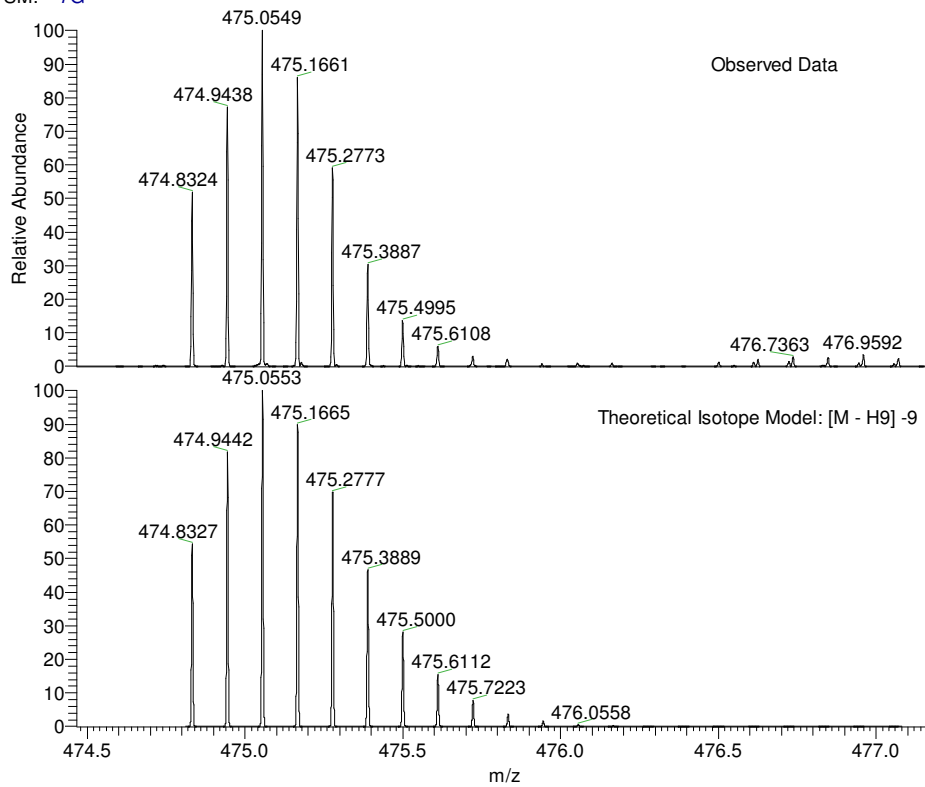
Supplementary Figure 68: Isotope Pattern for 18

SU_1553 MW=4285?
(H₂O)/MeOH + DEA

EPSRC National Facility Swansea
LTQ Orbitrap XL

Gavin Miller
18/06/2013 12:42:11

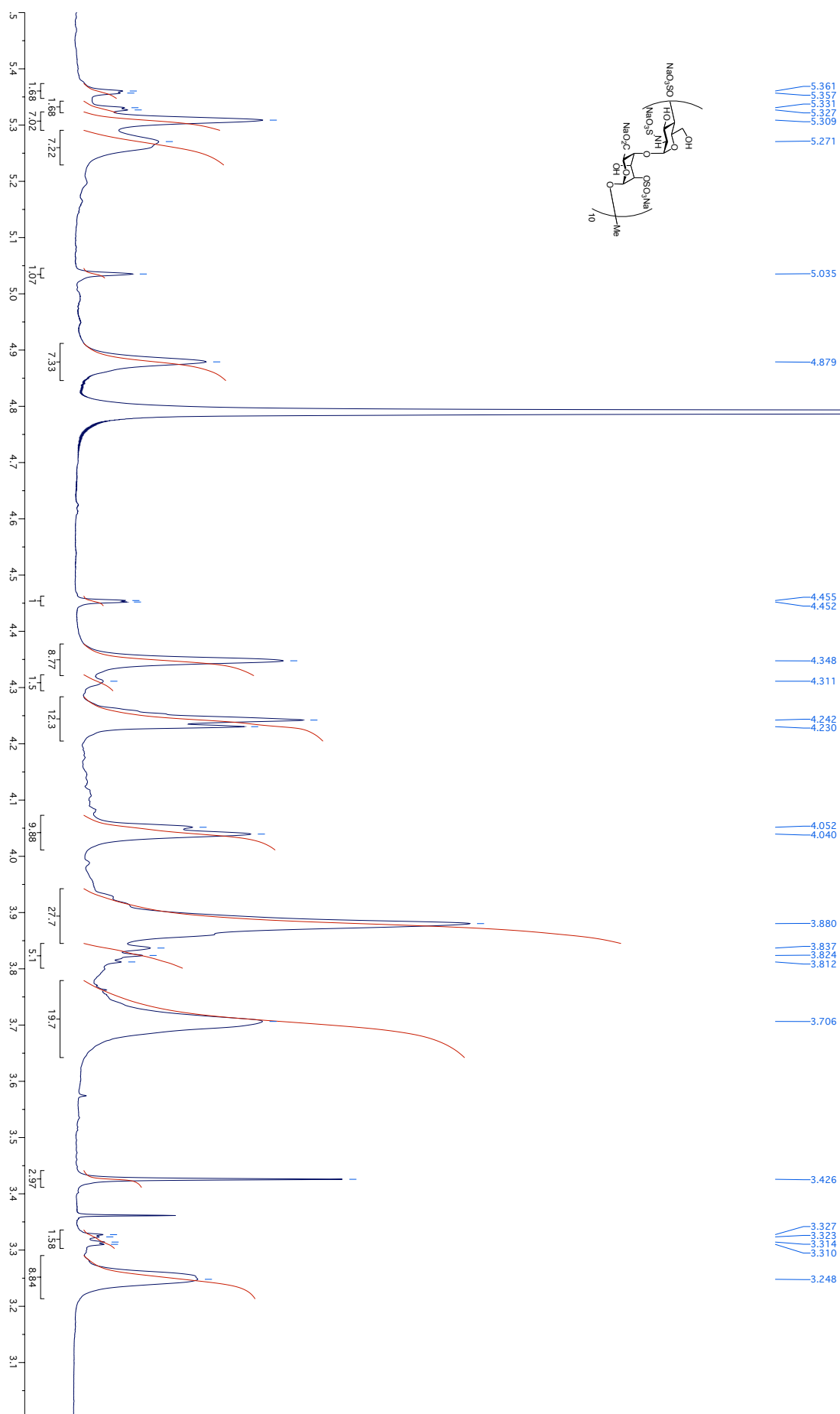
SM: 7G



NL:
1.81E6
MANGAR291-OJ-HNESN-2#62-
70 RT: 2.31-2.94 AV: 9 T:
FTMS - p NSI Full ms
[200.00-4000.00]

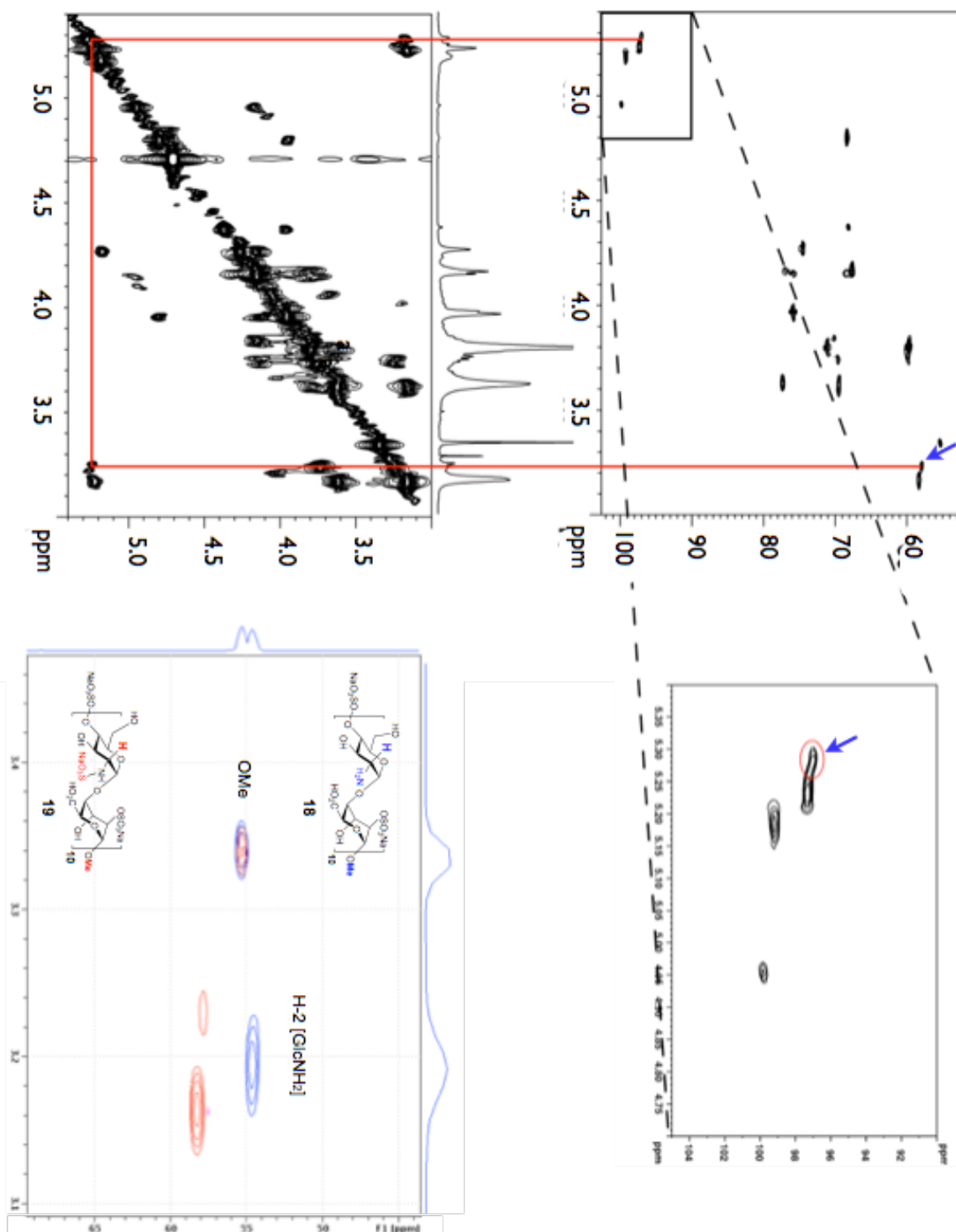
NL:
4.51E3
C₁₂₁ H₁₈₅ N₁₀ O₁₃₄ S₁₁:
C₁₂₁ H₁₈₅ N₁₀ O₁₃₄ S₁₁:
p (gss, s /p:40) Chrg -9
R: 100000 Res .Pwr . @FWHM

Supplementary Figure 69: ^1H NMR (800 MHz; D_2O) spectrum for **19**

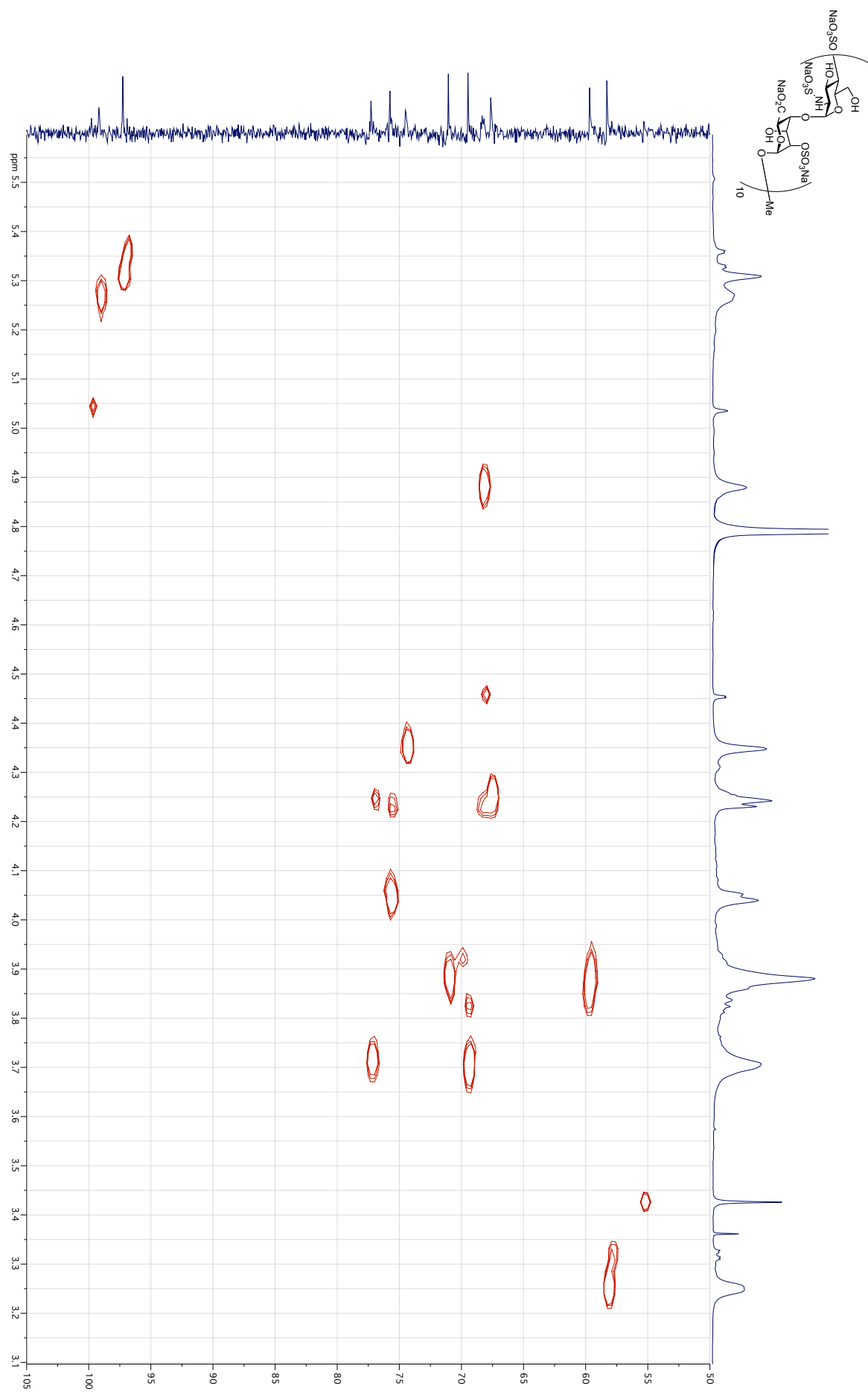


Supplementary Figure 70: NMR analysis of GlcN *N*-sulfation of icosasaccharide (A4I2-[A0I2]9)-OMe **18 to **19**.**

COSY and HSQC correlation of icosasaccharide (S4I2-[S0I2]9)-OMe **19** confirms peak (blue arrow) is additional H-2 NS correlated to H-1. See overlay spectra for **18** (blue) and **19** (red), showing coincidence of terminal methoxy methyl resonances, and definitive shift of H-2 signals due to *N*-2-sulfation in **19**.

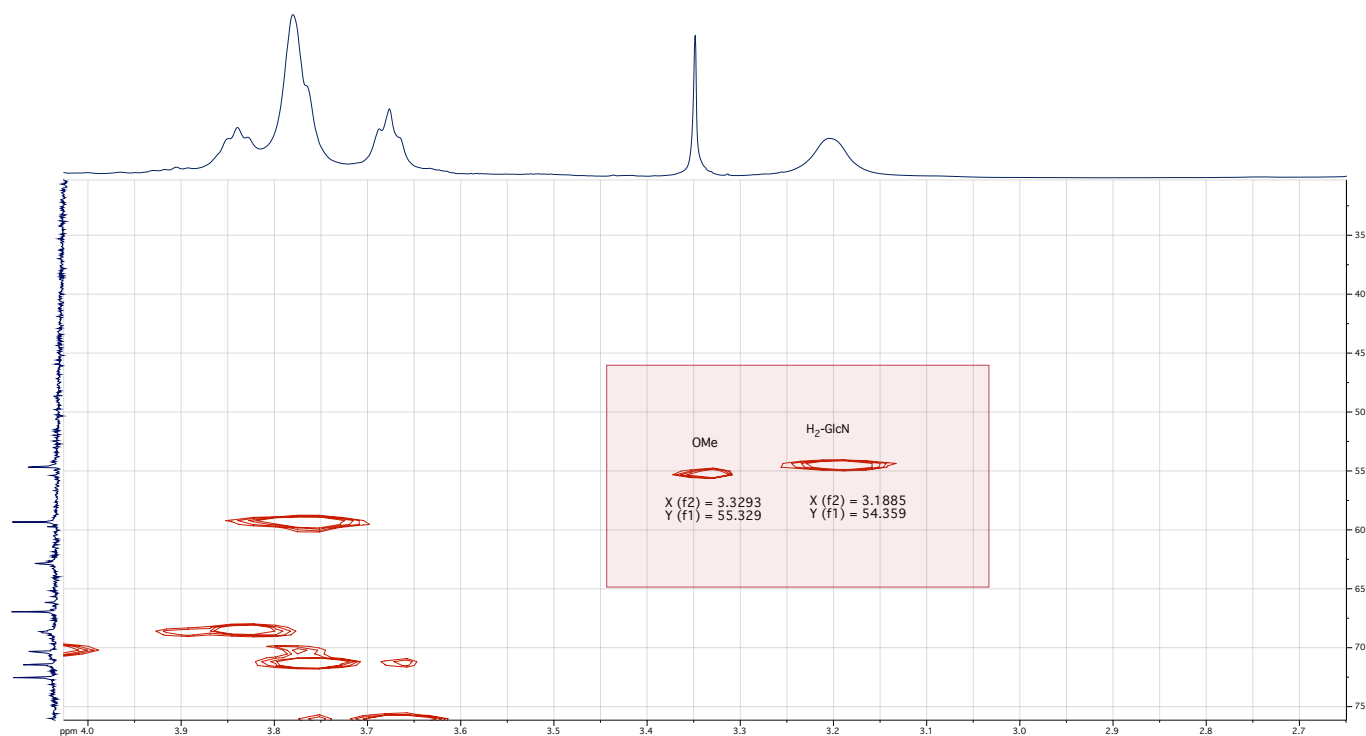


Supplementary Figure 71: HSQC NMR spectrum for **19**

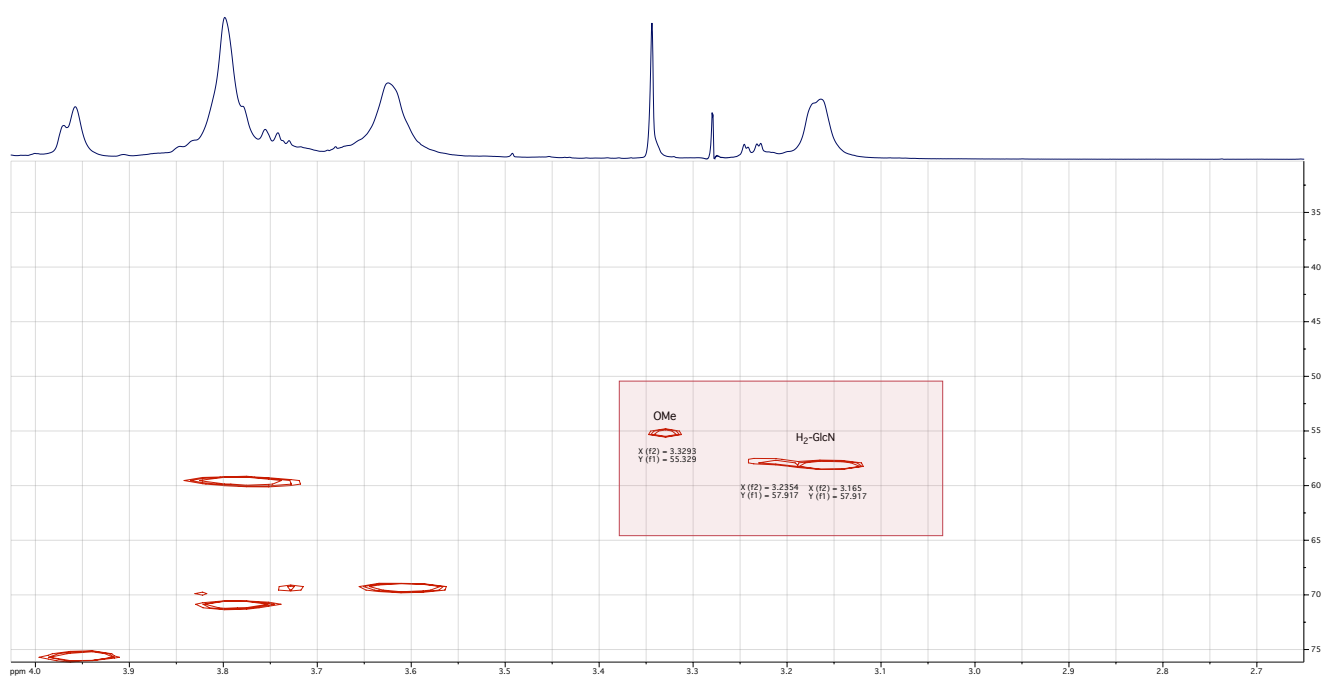


Supplementary Figure 72: HSQC (800 MHz, D₂O) spectra expansions for 18 (a) and 19 (b) to confirm ¹³C chemical shift changes upon icosaccharide N-sulfation

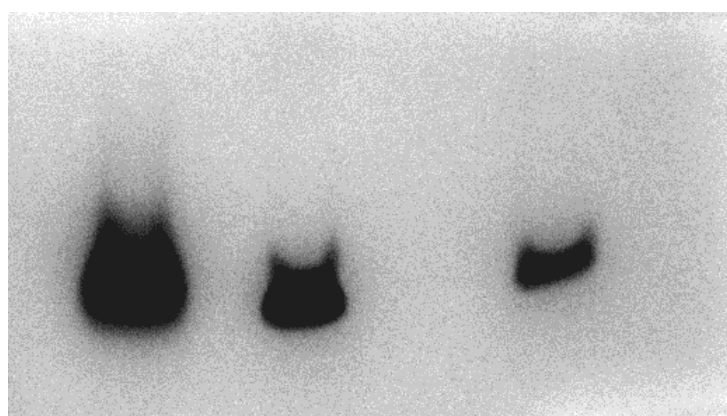
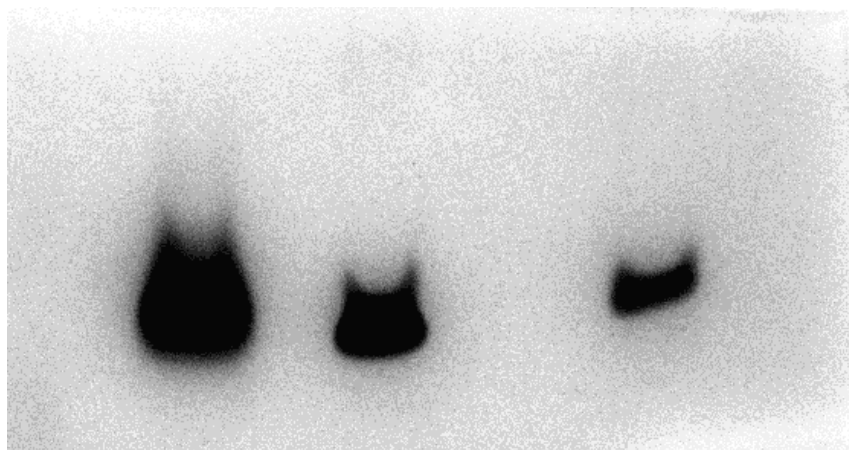
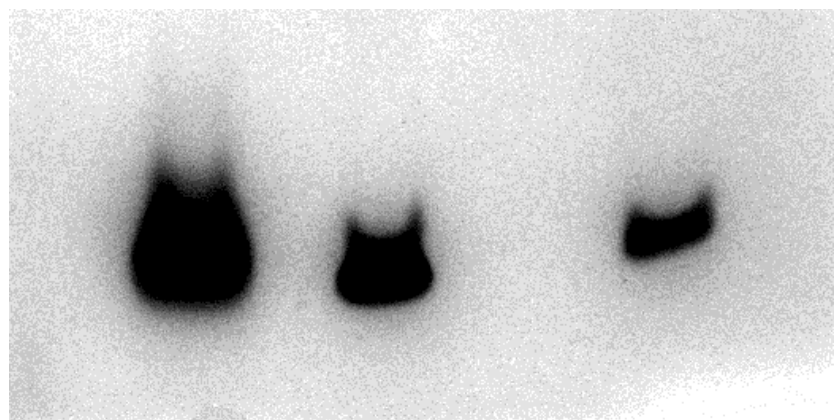
a)



b)



Supplementary Figure 73: PAGE analysis of 19



LHS is mixture of 18-22mers
middle and RHS are 20mer at
different concs..

Supplementary Figure 74: TLC system for differentiation and separation of long oligosaccharides

