

Supporting Information

Efficient chemical synthesis of heparin-like octa-, deca- and dodecasaccharides and inhibition of FGF2- and VEGF165-mediated endothelial cell functions

Gavin J. Miller,^a Steen Uldall Hansen,^a Graham Rushton,^b Claire Cole,^b Egle Avizyiente,^b Gordon C. Jayson^b and John M. Gardiner^a

^aManchester Institute of Biotechnology, School of Chemistry, The University of Manchester, 131 Princess Street, Manchester, M1 7DN, UK.

^bSchool of Cancer and Enabling Sciences, The University of Manchester, Wilmslow Road, Manchester M20 4BX, United Kingdom.

E-mail: gardiner@manchester.ac.uk

The following pages contains representative experimental protocols, data and spectra.

1. Synthetic procedures for compounds 3-28

2. Biological methods

3. Spectral Data: ¹H, ¹³C, COSY, HMQC/HSQC NMR and MS data for compounds 3-28

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 MHz and ¹³C spectra at 100 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). ESI spectra and isotope patterns for compounds with mass > 1000 are included with the NMR data. High resolution data are for the monoisotopic mass. 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. ESI MS conditions: prior to MS, the sample for analysis was salt counterion switched from Na⁺ to NH₄⁺ using Amberlite IRC86-H⁺ resin (pretreated with 5% aqueous NH₄OH solution). For ¹H analysis of oligosaccharide species **8-28**, signals for glucosamine and iduronate residues are assigned H_{1ido}, H_{1glu}. This applies to H₂, H₃, H₄, H_{5/6}

analogously. For ring assignment, H₁ is deemed the non-reducing end iduronate proton and subsequent rings proceeding towards the non-reducing end, are labelled H₁', H₁''' etc. This applies to H₂, H₃ analogously. Glucosamine C₆ is denoted with H_{6A} and H_{6B} (where assignable).

1. Synthetic procedures for compounds 3-28

Methyl (2,4-di-*O*-acetyl-3-*O*-benzyl-1- α/β -methoxy-*L*-idopyranoside) uronate **3** and methyl (2,5-di-*O*-acetyl-3-*O*-benzyl-1- α/β -methoxy-*L*-idofuranoside) uronate **4**

To the cyanohydrin **1** (33.8 g, 0.11 mol) was added MeOH (160 mL) and this was cooled in an ice bath. Acetyl chloride (16.0 mL, 0.22 mol) was then added drop-wise while cooled in an icebath (**NOTE:** exothermic reaction). The reaction mixture was heated to 60 °C for 3 h, more acetyl chloride (4.0 mL, 0.055 mol) was added and after 10 h a final addition of acetyl chloride (4.0 mL, 0.055 mol) was made and stirring continued for another 13 h. The solvents were evaporated *in vacuo* and the crude material partitioned between EtOAc (300 mL), water (300 mL) and shaken vigorously for a few minutes (**NOTE:** This converts imidate present to the methyl ester). The organic phase was then washed with saturated aqueous NaHCO₃ (500 mL), brine (500 mL), dried (MgSO₄) and evaporated. The crude material was purified by flash column chromatography (EtOAc/hexane 1:1, 2:1) yielding a mixture of methyl pyranoside and furanoside *L*-ido glycosides (26.6 g, 77%) as a pale yellow syrup (exact ratios not determined at this stage). See pp. 19 for ¹H NMR spectrum of this mixture.

To a mixture of the crude (above) methyl *L*-ido glycosides (482 mg, 1.54 mmol) was added dry dichloromethane (5 mL), pyridine (0.3 mL, 3.71 mmol), acetic anhydride (0.32 mL, 3.40 mmol) and DMAP (2 mg) at room temperature. The mixture was then stirred for 12 h. The solvents were evaporated *in vacuo* and co-evaporated twice with toluene (2 x 200 mL). The crude material was then purified by silica gel flash chromatography (EtOAc/hexane 2:3) yielding diacetylated *L*-ido furanoside **4** (247 mg, 0.62 mmol, 40%) and diacetylated *L*-ido pyranoside **3** (322 mg, 0.81 mmol, 53%) as syrups. (ratio of **3/4** 57:43). For **3**: ¹H NMR (400 MHz; CDCl₃) For assignment a and b descriptors constitute the different anomeric forms δ 7.40-7.28 (m, 10H, ArH), 5.19-5.17 (m, 1H, H_{2a}), 5.15 (ddd, *J* = 3.1, 2.2, 0.9 Hz, 1H, H_{4b}), 5.01 (ddd, *J* = 3.3, 1.7, 0.9 Hz, 1H, H_{2b}), 4.90-4.87 (m, 3H, H_{5a}, H_{4a}, H_{1a}), 4.80 (d, *J* = 1.9 Hz, 1H, H_{1b}), 4.80-4.71 (m, 4H, CH₂Ph), 4.65 (d, *J* = 2.2 Hz, 1H, H_{5b}), 3.97 (dd, *J* = 4.2, 2.3 Hz, 1H, H_{3b}), 3.76 (s, 6H, CO₂CH₃), 3.74-3.73 (m, 1H, H_{3a}), 3.47 (s, 3H, OCH₃), 2.10 (s, 3H, OCH₃), 2.06-2.02 (m, 12H, C(O)CH₃), ratio of anomers determined as 55:45, b/a; ¹³C NMR (100 MHz; CDCl₃) δ 170.2, 169.9, 169.8, 169.6, 169.0, 168.1, 137.3, 136.9, 128.8, 128.4, 128.2, 127.9, 127.8, 127.7, 99.6, 98.9, 73.1, 73.0, 72.3, 72.1, 71.9, 67.2, 67.5, 67.0, 67.0, 65.8, 57.4, 56.3, 52.5, 52.4, 20.9, 20.9, 20.7, 20.7; MS-ESI: *m/z*

= 419 [M+Na]⁺; HRMS (FTMS-NSI⁺) *m/z* calcd for C₁₉H₂₄O₉Na [M+Na]⁺ 419.1314, found 419.1313.

For **4**: See pages 24-26 for copies of ¹H, COSY and ¹³C NMR for this mixture of anomers; MS-ESI: *m/z* = 419 [M+Na]⁺; HRMS (FTMS-NSI⁺) *m/z* calcd for C₁₉H₂₄O₉Na [M+Na]⁺ 419.1314, found 419.1311.

Methyl (3-*O*-benzyl-1- α/β -methoxy-L-idopyranoside) uronate **5**

To the diacetylated L-ido pyranoside **3** (18.7 g, 0.047 mol) was added dry methanol (200 mL) and sodium (catalytic amount) under N₂. The mixture was stirred for 1 h, amberlite H⁺ resin (300 mg) was added and the mixture stirred for another 10 minutes. The resin was filtered off and solvent evaporated *in vacuo*. The crude material was purified by silica gel flash chromatography using EtOAc/hexane (1:1) as eluent, yielding an anomeric mixture of **5** (13.1 g, 42 mmol, 89%) as a syrup. See pages 27-30 for copies of ¹H, COSY and ¹³C NMR; MS-ESI: *m/z* = 313 [M+H]⁺; HRMS (FTMS-NSI⁺) *m/z* calcd. for C₁₅H₂₁O₇ [M+H]⁺ 313.1282, found 313.1287.

Methyl (2-*O*-benzoyl-4-hydroxy-3-*O*-benzyl-1- α/β -methoxy-L-idopyranoside) uronate **6**

To the methyl L-ido pyranoside **5** (5.30 g, 17.0 mmol) was added dry MeOH (120 mL) and dibutyltin oxide (4.44 g, 18 mmol). The reaction mixture was heated to 60 °C for 3 h under N₂. The solvent was removed *in vacuo* and dried on a high vacuum line for 2 h. This intermediate material was then dissolved in 1,4-dioxane (100 mL) under N₂, benzoyl chloride (2.17 mL, 19.0 mmol) added *via* syringe over 30 minutes and the mixture stirred for 14 h at room temperature. The solvent was removed *in vacuo* and the crude product purified by column chromatography using toluene/acetone (20:1) as the eluent. A second column was necessary to remove remaining tin reagent. This yielded **α -6** (3.00 g, 7.2 mmol, 44%) and **β -6** (1.74 g, 4.2 mmol, 26%) as syrups. Starting material was also recovered (1.54 g). For **α -6**: ¹H NMR (400 MHz; CDCl₃) δ 8.02-7.99 (m, 2H, ArH), 7.63-7.59 (m, 1H, ArH), 7.49-7.19 (m, 7H, ArH), 5.23 (dt, *J* = 2.4, 1.2 Hz, 1H, H₂), 5.04 (s, 1H, H₁), 4.93 (d, *J* = 1.6 Hz, 1H, H₅), 4.87 (d, *J* = 12.1 Hz, 1H, CH₂Ph), 4.71 (d, *J* = 12.1 Hz, 1H, CH₂Ph), 4.12-4.09 (m, 1H, H₄), 3.88-3.86 (m, 4H, H₃+C(O)OCH₃), 3.54 (s, 3H, OCH₃), 2.78 (d, *J* = 11.8 Hz, 1H, OH), matching that previously reported.² For **β -6**: [α]_D (c = 1.08, DCM) +55.5; ¹H NMR (400 MHz; CDCl₃) δ 8.05 (dt, *J* = 8.3, 1.5 Hz, 2H, ArH), 7.60 (tt, *J* = 7.4, 1.5 Hz, 1H, ArH), 7.48-7.44 (m, 2H, ArH), 7.38-7.19 (m, 5H, ArH), 5.32-5.30 (m, 1H, H₂), 4.94 (d, *J* = 2.0 Hz, 1H, H₁), 4.83-4.76 (m, 2H, CH₂Ph), 4.63 (d, *J* = 2.1 Hz, 1H, H₅), 4.08-4.06 (m, 2H, H₃, H₄), 3.87 (s, 3H, C(O)OCH₃), 3.58 (s, 3H, OCH₃), 3.14 (d, *J* = 11.6 Hz, 1H, OH); ¹³C NMR (100 MHz; CDCl₃): δ 169.8, 165.5, 137.4, 133.5, 129.9, 129.3, 129.1, 128.6, 128.3, 128.1, 127.9, 125.4, 99.4, 75.9, 73.7, 73.1, 69.3, 68.8, 57.4, 52.3; MS-ESI (+ve) *m/z* 439 [M+Na]⁺, 855 [2M

+Na]⁺; HRMS (TOF-ES⁺) *m/z* calcd. for C₂₂H₂₄O₈ [M+Na]⁺ 439.1364, found 439.1363, matching that previously reported.³

Methyl [Methyl-4-*O*-(2-azido-3-*O*-benzyl-6-*O*-benzoyl-4-*O*-trichloroacetyl- α -D-glucopyranosyl)-2-*O*-benzoyl-3-*O*-benzyl-1-*O*-methoxy- α -L-ido-pyranoside] uronate 8

Iduronate acceptor **6** (944 mg, 3.0 mmol) was dissolved in dry DCM (15 mL) under N₂. The reaction solution was cooled to -30 °C and TMSOTf (24 μ L, 0.15 mmol, 0.06 equiv.) added. The solution colour changed to pale yellow (pH = 3). Trichloroacetimidate **7**⁴ (5.46 g, 7.9 mmol, 1.2 equiv) was dissolved in dry DCM (50 mL) and added drop-wise to the acceptor/TMSOTf solution. Stirring was continued for 2 h at -30 °C and the reaction then allowed to warm to room temperature. Et₃N (50 μ L) was added and the solvents removed *in vacuo* to give the crude as a brown gum. This material was then purified, firstly by silica gel flash chromatography eluting with hexane/EtOAc, 4/1 (this also removed the unwanted β -linked glycoside) and then by passage through a Sephadex LH-20 column (eluting with DCM, to remove trichloroacetamide) to give **8** (1.67 g, 1.8 mmol, 78%) as a white foam. [α]_D (c = 0.04, DCM) +11.7; ¹H NMR (400MHz; CDCl₃) δ 8.35-8.33 (m, 2H, ArH), 8.20-8.16 (m, 2H, ArH), 7.72-7.39 (m, 14H, ArH), 7.19-7.17 (m, 2H, ArH), 5.40 (t, *J* = 9.9 Hz, 1H, H_{4'}), 5.31 (s, 1H, H₁), 5.23 (t, *J* = 1.0 Hz, 1H, H₂), 5.12 (d, *J* = 11.9 Hz, 1H, CH₂Ph), 5.03 (d, *J* = 1.8 Hz, 1H, H₅), 4.98-4.95 (m, 1H, H_{6''} or H_{6'}), 4.92 (d, *J* = 12.0 Hz, 1H, CH₂Ph), 4.71 (d, *J* = 3.7 Hz, 1H, H_{1'}), 4.41-4.34 (m, 2H, H_{5'} + H_{6''} or H_{6'}), 4.33 (s, 1H, H₄), 4.13 (d, *J* = 10.1 Hz, 1H, CH₂Ph), 4.07 (s, 1H, H₃), 4.01 (s, 3H, OCH₃), 3.86 (d, *J* = 10.1 Hz, 1H, CH₂Ph), 3.74 (t, *J* = 9.7 Hz, 1H, H_{3'}), 3.68 (s, 3H, C(O)OCH₃), 3.46 (dd, *J* = 10.0, 3.6 Hz, 1H, H_{2'}); ¹³C NMR (100 MHz; CDCl₃) δ 169.8, 165.9, 165.5, 160.5, 137.3, 136.6, 133.5, 133.3, 129.9, 129.8, 129.5, 128.8, 128.5, 128.45, 128.4, 128.2, 128.1, 127.9, 100.5, 100.0, 89.6, 77.7, 74.9, 74.5, 72.5, 71.9, 68.4, 68.0, 66.7, 63.6, 61.3, 56.3, 52.7; MS-ESI (+ve) *m/z* 961.10 [M+NH₄]⁺; HRMS (TOF-ES⁺) *m/z* calcd for C₄₄H₄₆N₄O₁₄Cl₃ [M+NH₄]⁺ 959.2099, found 959.2071; IR (cm⁻¹): 1024 (C-O), 1105 (C-O), 1221 (C-O), 1265 (C-O), 1715 (C=O), 1765 (C=O), 2108 (N₃).

Methyl [Methyl-4-*O*-(2-azido-3-*O*-benzyl-6-*O*-benzoyl-4-hydroxy- α -D-glucopyranosyl)-2-*O*-benzoyl-3-*O*-benzyl-1-*O*-methoxy- α -L-ido-pyranoside] uronate 9

Disaccharide **8** (1.67 g, 1.77 mmol) was dissolved in a mixture of dry pyridine (6 mL) and dry MeOH (15 mL) at room temperature under N₂. The clear solution was heated at 40 °C for 5h. Solvents were removed *in vacuo* and the residue azeotroped with toluene (3 x 10 mL). This material was then purified by silica gel flash chromatography eluting with hexane/EtOAc, 2/1 to give **9** (1.41 g, 1.8 mmol, 95%) as a clear foam. [α]_D (c = 1.0, DCM) +19.2; ¹H NMR (400 MHz; CDCl₃) δ 8.19 (d, *J* = 9.8 Hz, 2H, ArH),

8.04 (d, $J = 9.7$ Hz, 2H, ArH), 7.60-7.28 (m, 14H, ArH), 7.20 (d, $J = 9.5$ Hz, 2H, ArH), 5.16 (s, 1H, H₁), 5.13 (s, 1H, H₂), 4.95 (d, $J = 11.9$ Hz, 1H, CH₂Ph), 4.90 (dd, $J = 12.4, 2.4$ Hz, 1H, H_{6A}), 4.89 (d, $J = 2.0, 1H, H_5$), 4.79 (d, $J = 11.9$ Hz, 1H, CH₂Ph), 4.72 (d, $J = 3.6$ Hz, 1H, H₁), 4.46 (dd, $J = 12.5, 2.2$ Hz, 1H, H_{6B}), 4.33 (d, $J = 10.7$ Hz, 1H, CH₂Ph), 4.15 (s, 1H, H₄), 4.08 (d, $J = 11.0$ Hz, 1H, CH₂Ph), 4.07-4.06 (m, 1H, H₃), 4.04-4.01 (m, 1H, H₅), 3.87 (s, 3H, OCH₃), 3.54 (s, 3H, C(O)OCH₃), 3.52 (dd, $J = 8.8, 9.8$ Hz, 1H, H₃), 3.45 (dd, $J = 8.8, 3.8$ Hz, 1H, H₄), 3.13 (dd, $J = 9.8, 3.6$ Hz, 1H, H₂), 3.00 (d, $J = 3.8$ Hz, 1H, OH); ¹³C NMR (100 MHz; CDCl₃) δ 169.8, 167.5, 165.6, 137.8, 137.4, 133.5, 130.1, 129.9, 129.7, 129.5, 128.8, 128.5, 128.2, 128.1, 128.0, 127.9, 100.4, 99.4, 79.3, 75.5, 75.0, 72.7, 72.5, 71.4, 70.5, 68.0, 67.1, 63.2, 63.1, 56.3, 52.5; MS-ESI (+ve) m/z 815.20 [M+NH₄]⁺; HRMS (TOF-ES⁺) m/z calcd. for C₄₂H₄₇N₄O₁₃ [M+NH₄]⁺ 815.3135, found 815.3145; IR (cm⁻¹): 1024 (C-O), 1107 (C-O), 1266 (C-O), 1718 (C=O), 1764 (C=O), 2106 (N₃).

Tetrasaccharide 11

Disaccharide acceptor **9** (1.30 g, 1.62 mmol, 1.0 equiv.) and disaccharide donor **10** (1.98 g, 1.94 mmol, 1.2 equiv.) were combined in dry toluene (10 mL). Solvent was removed *in vacuo* and the residue dried under high vacuum for 1 h. The solid was then dissolved in dry DCM (40 mL) and cooled to 0 °C. 4Å molecular sieves (400 mg) were added followed by NIS (473 mg, 2.1 mmol, 1.3 equiv.) and AgOTf (1 mg, 0.16 mmol, 0.1 equiv.). The resultant suspension was stirred at this temperature for 0.5 h whereupon a deep red colour persisted. The reaction was quenched with NaHCO₃ (500 mg) and Na₂S₂O₃ (500 mg) in H₂O (20 mL) and the mixture filtered through a Celite™ plug, washing with DCM. The layers were separated and the organics dried (MgSO₄) and solvent removed *in vacuo* to reveal crude **11** as a yellow gum. This material was then purified by silica gel flash chromatography eluting with toluene/acetone, 20/1 to separate the product and unreacted disaccharide acceptor **9** (500 mg recovered). The product was then purified again by silica gel flash chromatography eluting with hexane/EtOAc, 3/1 to give **11** (1.79 g, 1.04 mmol, 66%) as a white foam. [α]_D (*c* = 0.2, DCM) +28.7; ¹H NMR (400 MHz; CDCl₃) δ 8.16 (dd, *J* = 7.9, 1.7 Hz, 2H, Ar*H*), 8.10 (dd, *J* = 8.4, 1.4 Hz, 2H, Ar*H*), 8.05-8.02 (m, 4H, Ar*H*), 7.59-7.26 (m, 28H, Ar*H*), 7.21-7.19 (m, 2H, Ar*H*), 7.15-7.13 (m, 2H, Ar*H*), 5.55 (d, *J* = 3.9 Hz, 1H, H_{1''}), 5.30 (d, *J* = 9.5 Hz, 1H, H_{4'''}), 5.19 (t, *J* = 4.2 Hz, 1H, H_{2'''}), 5.12-5.10 (m, 2H, H₁, H₂), 4.94 (d, *J* = 12.0 Hz, 1H, CH₂Ph), 4.87-4.81 (m, 3H, H₅, H_{1'''}, H_{5''}), 4.77 (d, *J* = 12.0 Hz, 1H, CH₂Ph), 4.70-4.65 (m, 3H, H_{6A''}, H_{6A'}, CH₂Ph), 4.58 (d, *J* = 3.7 Hz, 1H, H_{1'}), 4.49 (d, *J* = 10.7 Hz, 1H, CH₂Ph), 4.40 (d, *J* = 10.5 Hz, 1H, CH₂Ph), 4.24-4.09 (m, 7H, H₄, H_{4''}, H_{5'''}, H_{6B'''}, H_{6B'}, 2xCH₂Ph), 4.01-3.95 (m, 4H, H₃, H_{3''}, H_{5'}, H_{4'}), 3.87 (d, *J* = 10.5 Hz, 1H, CH₂Ph), 3.69 (s, 3H, OCH₃), 3.65 (t, *J* = 9.7 Hz, 1H, H_{3'''}), 3.49-3.51 (m, 1H, H_{3'}), 3.51 (s, 3H, C(O)OCH₃), 3.49 (s, 3H, C(O)OCH₃), 3.38 (dd, *J* = 10.1, 3.6 Hz, 1H, H_{2'''}), 3.25 (dd, *J*

= 10.2, 3.6 Hz, 1H, H_{2'}); ¹³C NMR (100 MHz; CDCl₃) δ 169.7, 169.2, 166.1, 165.9, 165.6, 165.2, 160.4, 137.7, 137.4, 137.2, 136.5, 133.5, 133.4, 133.1, 129.9, 129.8, 129.7, 129.4, 129.4, 128.8, 128.5, 128.4, 128.3, 128.2, 128.1, 127.9, 127.8, 127.6, 100.4, 99.1, 98.9, 98.6, 89.5, 78.6, 77.6, 75.9, 75.9, 75.8, 75.1, 74.5, 74.4, 74.3, 73.8, 72.5, 72.1, 70.5, 70.0, 69.7, 68.2, 68.0, 67.0, 63.8, 63.3, 62.4, 61.2, 56.3, 52.3, 52.0; HRMS (FTMS-NSI⁺) *m/z* calcd. for C₈₅H₈₅Cl₃N₇O₂₆ [M+NH₄]⁺ 1726.4607 found 1726.4574; IR (cm⁻¹): 1024 (C-O), 1104 (C-O), 1264 (C-O), 1719 (C=O), 1765 (C=O), 2107 (N₃).

Tetrasaccharide 12

Tetrasaccharide **11** (1.79 g, 1.04 mmol) was dissolved in a mixture of dry pyridine (6 mL) and dry MeOH (15 mL) at room temperature under N₂. The clear solution was then heated at 40 °C for 5 h. Solvents were removed *in vacuo* and the residue azeotroped with toluene (3 x 10 mL). This material was then purified by silica gel flash chromatography eluting with hexane/EtOAc, 2/1 to give **12** (1.48 g, 0.95 mmol, 91%) as a clear foam. [α]_D (c = 0.7, DCM) +24.4; ¹H NMR (400 MHz; CDCl₃) δ 8.18-8.01 (m, 9H, ArH), 7.61-7.21 (m, 31H, ArH), 5.60 (d, *J* = 4.8 Hz, 1H, H_{1''}), 5.24 (t, *J* = 4.9 Hz, 1H, H_{2''}), 5.10 (s, 2H, H₁, H₂), 4.96-4.90 (m, 2H, H_{1'}, CH₂Ph), 4.85-4.75 (m, 5H, H₅, 3xCH₂Ph, H_{6A'}), 4.70 (dd, *J* = 12.5, 1.7 Hz, 1H, H_{6A''}), 4.63 (d, *J* = 4.3 Hz, 1H, H_{5''}), 4.58 (d, *J* = 3.7 Hz, 1H, H_{1'''}), 4.52 (d, *J* = 10.8 Hz, 1H, CH₂Ph), 4.49-4.44 (m, 2H, H_{6B'}, CH₂Ph), 4.37 (d, *J* = 10.8 Hz, 1H, CH₂Ph), 4.32 (dd, *J* = 12.6, 2.1 Hz, 1H, H_{6B''}), 4.17 (t, *J* = 5.4 Hz, 1H, H_{3''}), 4.12-4.08 (m, 2H, H_{4''}, H₃), 4.00-3.89 (m, 4H, H₄, H_{5'}, H_{5'''}, H_{4'}), 3.86 (d, *J* = 10.3 Hz, 1H, CH₂Ph), 3.66 (s, 3H, OCH₃), 3.56-3.51 (m, 3H, H_{3'}, H_{3'''}, H_{4'''}), 3.50 (s, 3H, C(O)CH₃), 3.49 (s, 3H, C(O)CH₃), 3.24 (dd, *J* = 10.2, 3.7 Hz, 1H, H_{2'''}), 3.18 (dd, *J* = 10.0, 3.6 Hz, 1H, H_{2'}), 3.07 (d, *J* = 3.8 Hz, 1H, OH); ¹³C NMR (100 MHz; CDCl₃) δ 169.6, 169.3, 167.4, 166.2, 165.7, 165.3, 137.8, 137.4, 137.3, 133.6, 133.5, 133.1, 130.1, 129.9, 129.8, 129.7, 129.4, 129.3, 128.8, 128.6, 128.5, 128.4, 128.3, 128.2, 128.1, 128.0, 127.6, 100.4, 99.1, 99.0, 98.5, 79.1, 78.6, 75.9, 75.8, 75.5, 75.1, 74.7, 74.5, 74.0, 72.4, 72.1, 71.3, 71.2, 70.6, 70.4, 70.0, 68.0, 67.0, 63.6, 63.0, 62.7, 62.2, 56.3, 52.3, 51.9; HRMS (FTMS-NSI⁺) *m/z* calcd for C₈₃H₈₆N₇O₂₅ [M+NH₄]⁺ 1580.5671, found 1580.5611; IR (cm⁻¹): 1024 (C-O), 1040 (C-O), 1264 (C-O), 1718 (C=O), 2106 (N₃).

Hexaasaccharide 13

Tetrasaccharide acceptor **12** (1.34 g, 0.86 mmol, 1.0 equiv.) and disaccharide donor **10** (1.05 g, 1.03 mmol, 1.2 equiv.) were combined in dry toluene (10 mL). Solvent was removed *in vacuo* and the residue dried under high vacuum for 1 h. The solid was then dissolved in dry DCM (20 mL) and cooled to 0 °C. 4Å molecular sieves (200 mg) were added followed by NIS (252 mg, 1.1 mmol, 1.3 equiv.) and AgOTf (22 mg, 0.09 mmol, 0.1 equiv.). The resultant suspension was stirred at this temperature for 0.5 h whereupon a deep red colour persisted. The reaction was quenched with NaHCO₃ (500 mg) and

$\text{Na}_2\text{S}_2\text{O}_3$ (500 mg) in H_2O (20 mL) and the mixture filtered through a Celite™ plug, washing with DCM. The layers were separated and the organics dried (MgSO_4) and solvent removed *in vacuo* to reveal crude **13** as a yellow gum. This material was then purified by silica gel flash chromatography eluting with toluene/acetone, 20/1 to separate the product and unreacted tetrasaccharide acceptor **12** (250 mg recovered). The product was then purified again by silica gel flash chromatography eluting with hexane/EtOAc, 2/1 to give **13** (1.20 g, 0.48 mmol, 57%) as a white foam. $[\alpha]_{\text{D}}$ ($c = 0.2$, DCM) +33.4; ^1H NMR (400 MHz; CDCl_3) δ 8.17-8.01 (m, 13H, ArH), 7.59-7.13 (m, 47H, ArH), 5.58 (d, $J = 5.3$ Hz, 1H, $\text{H}_{1''''}$), 5.55 (d, $J = 4.1$ Hz, 1H, $\text{H}_{1''}$), 5.29 (t, $J = 9.7$ Hz, 1H, $\text{H}_{4''''''}$), 5.23 (t, $J = 5.4$ Hz, 1H, $\text{H}_{2''''''}$), 5.18 (t, $J = 4.5$ Hz, 1H, $\text{H}_{2''}$), 5.11-5.07 (m, 2H, H_1 , H_2), 4.92 (d, $J = 11.9$ Hz, 1H, CH_2Ph), 4.88 (m, 2H, $\text{H}_{1''''}$, $\text{H}_{1''''''}$), 4.81-4.78 (m, 5H, H_5'''' , $\text{H}_{6\text{A}}''''$, 2 x CH_2Ph), 4.71-4.66 (m, 3H, H_5'' , $\text{H}_{6\text{A}}''$, CH_2Ph), 4.63 (d, $J = 10.6$ Hz, 1H, CH_2Ph), 4.58 (d, $J = 3.6$ Hz, 1H, $\text{H}_{1'}$), 4.54 (dd, $J = 12.5, 2.1$ Hz, 1H, $\text{H}_{6\text{A}}'$), 4.49 (d, $J = 4.9$ Hz, 1H, H_5), 4.45-4.39 (m, 2H, CH_2Ph), 4.38 (d, $J = 10.5$ Hz, 1H, CH_2Ph), 4.30-4.25 (m, 2H, CH_2Ph), 4.20-4.14 (m, 4H, H_3'' , H_5'''''' , $\text{H}_{6\text{B}}'$, $\text{H}_{6\text{B}}''''$), 4.14-4.06 (m, 3H, H_3 , H_3'''' , $\text{H}_{6\text{B}}''''$), 3.92-3.99 (m, 7H, H_4 , H_4' , H_4'' , H_4''' , H_4'''' , H_5' , H_5''''), 3.84 (d, $J = 10.5$ Hz, 1H, CH_2Ph), 3.64-3.67 (m, 1H, H_3''''''), 3.64 (s, 3H, OCH_3), 3.56-3.50 (m, 2H, H_3' , H_3''''), 3.49 (s, 3H, $\text{C}(\text{O})\text{OCH}_3$), 3.47 (s, 3H, $\text{C}(\text{O})\text{OCH}_3$), 3.39 (dd, $J = 10.1, 3.5$ Hz, 1H, $\text{H}_{2''''''}$), 3.35-3.33 (m, 1H, $\text{H}_{2''''}$), 3.32 (s, 3H, $\text{C}(\text{O})\text{OCH}_3$), 3.22 (dd, $J = 10.3, 3.6$ Hz, 1H, H_2); ^{13}C NMR (100 MHz; CDCl_3) δ 169.6, 169.3, 169.2, 166.0, 165.9, 165.8, 165.6, 165.3, 165.2, 160.4, 137.9, 137.7, 137.5, 137.4, 137.3, 137.1, 136.5, 133.7, 133.6, 133.5, 133.4, 133.2, 133.1, 130.0, 129.9, 129.8, 129.7, 129.6, 129.4, 129.3, 129.2, 129.1, 128.9, 128.8, 128.7, 128.6, 128.5, 128.4, 128.3, 128.2, 128.1, 128.0, 127.9, 127.7, 127.6, 100.4, 99.1, 99.0, 98.4, 98.3, 89.5, 78.4, 77.6, 77.3, 76.2, 75.9, 75.8, 75.6, 75.3, 75.1, 74.8, 74.6, 74.5, 74.1, 73.9, 72.4, 72.2, 71.4, 70.8, 70.6, 70.0, 69.8, 68.3, 68.0, 67.0, 63.6, 63.3, 62.2, 62.0, 61.2, 56.3, 52.3, 52.0, 51.7; HRMS (FTMS NSI^+) m/z calcd for $\text{C}_{126}\text{H}_{124}\text{N}_{10}\text{O}_{38} [\text{M}+\text{NH}_4]^+$ 2492.7072, found 2492.7036; IR (cm^{-1}): 1024 (C-O), 1264 (C-O), 1718 (C=O), 2107 (N_3).

Hexasaccharide 14

Hexasaccharide **13** (1.20 g, 0.49 mmol) was dissolved in a mixture of dry pyridine (4 mL) and dry MeOH (10 mL) at room temperature under N_2 . The clear solution was then heated at 40 °C for 5h. Solvents were removed *in vacuo* and the residue azeotroped with toluene (3 x 10 mL). This material was then purified by silica gel flash chromatography eluting with hexane/EtOAc, 2/1 to give **14** (977 mg, 0.42 mmol, 86%) as a clear foam. $[\alpha]_{\text{D}}$ ($c = 0.5$, DCM) +36.6; ^1H NMR (400 MHz; CDCl_3) δ 8.16-8.01 (m, 13H, ArH), 7.61-7.13 (m, 47H, ArH), 5.60-5.58 (m, 2H, $\text{H}_{1''}$, $\text{H}_{1''''}$), 5.24-5.21 (m, 2H, $\text{H}_{2''}$, $\text{H}_{2''''}$), 5.10 (brs, 1H, H_1), 5.07 (brs, 1H, H_2), 4.97 (d, $J = 3.6$ Hz, 1H, $\text{H}_{1'}$), 4.92 (d, $J = 11.9$ Hz, 1H, CH_2Ph),

4.88 (d, $J = 3.7$ Hz, 1H, $H_{1''}$), 4.82-4.74 (m, 7H, H_5 , 4 x CH_2Ph , 2 x H_{6glu}), 4.71-4.66 (m, 2H, CH_2Ph , H_{6glu}), 4.62 (d, $J = 4.7$ Hz, 1H, $H_{5''}$), 4.59-4.53 (m, 3H, CH_2Ph , H_{6glu} , $H_{1''''}$), 4.47-4.40 (m, 3H, $H_{5''''}$, CH_2Ph , H_{6glu}), 4.40-4.35 (m, 2H, CH_2Ph , H_{6glu}), 4.33-4.27 (m, 2H, 2 x CH_2Ph), 4.19-4.16 (m, 1H, $H_{3''}$), 4.15-4.05 (m, 3H, $H_{4''}$, H_3 , $H_{3''''}$), 4.04-3.83 (m, 8H, H_4 , $H_{4'}$, $H_{4''}$, $H_{4''''}$, $H_{5'}$, $H_{5''}$, $H_{5''''}$, CH_2Ph), 3.95-3.89 (m, 3H), 3.88-3.83 (m, 2H), 3.64 (s, 3H, OCH_3), 3.47-3.57 (m, 4H, $H_{4''''}$, $H_{3'}$, $H_{3''''}$, $H_{3''''''}$), 3.49 (s, 3H, $C(O)CH_3$), 3.46 (s, 3H, $C(O)CH_3$), 3.33 (dd, $J = 10.2, 3.6$ Hz, 1H, H_{2glu}), 3.30 (s, 3H, $C(O)CH_3$), 3.24-3.17 (m, 2H, H_{2glu}), 3.04 (d, $J = 3.8$ Hz, 1H, OH); ^{13}C NMR (100 MHz; $CDCl_3$) δ 169.6, 169.3, 169.2, 167.4, 166.1, 166.0, 165.6, 165.2, 137.7, 137.6, 137.4, 137.3, 133.7, 133.5, 133.2, 133.0, 129.9, 129.8, 129.7, 129.6, 129.3, 129.2, 129.1, 128.9, 128.8, 128.7, 128.5, 128.4, 128.3, 128.2, 128.1, 128.0, 127.9, 127.7, 127.5, 100.4, 99.2, 99.1, 98.3, 98.2, 98.1, 79.0, 78.4, 78.3, 77.4, 77.3, 76.3, 75.8, 75.7, 75.5, 75.4, 75.1, 75.0, 74.6, 74.4, 74.1, 74.0, 72.4, 72.2, 71.5, 71.3, 70.9, 70.7, 70.4, 70.0, 69.8, 68.0, 67.0, 63.5, 63.2, 62.9, 62.7, 62.0, 56.2, 52.3, 51.9, 51.6; HRMS (FTMS NSI^+) m/z calcd for $C_{124}H_{125}N_{10}O_{37} [M+NH_4]^+$ 2346.809, found 2346.820; CHN analysis: calcd for $C_{124}H_{121}N_9O_{37}$ 63.94 H 5.24 N 5.41, found C 63.51 H 4.99 N 5.33; IR (cm^{-1}): 1023 (C-O), 1097 (C-O), 1264 (C-O), 1717 (C=O), 2106 (N_3), 2924 (O-H).

Octaasaccharide 15

Hexasaccharide acceptor **14** (950 mg, 0.41 mmol, 1.0 equiv.) and disaccharide donor **10** (502 mg, 0.49 mmol, 1.2 equiv.) were combined in dry toluene (10 mL). Solvent was removed *in vacuo* and the residue dried under high vacuum for 1 h. The solid was then dissolved in dry DCM (20 mL) and cooled to 0 °C. 4Å molecular sieves (200 mg) were added followed by NIS (120 mg, 0.53 mmol, 1.3 equiv.) and AgOTf (11 mg, 0.04 mmol, 0.1 equiv.). The resultant suspension was stirred at this temperature for 0.5 h whereupon a deep red colour persisted. The reaction was quenched with $NaHCO_3$ (500 mg) and $Na_2S_2O_3$ (500 mg) in H_2O (20 mL) and the mixture filtered through a Celite® plug, washing with DCM. The layers were separated and the organics dried ($MgSO_4$) and solvent removed *in vacuo* to reveal crude **15** as a yellow gum. This material was then purified by silica gel flash chromatography eluting with toluene/acetone, 25/1, 10/1 to separate the product and unreacted hexasaccharide acceptor **14** (321 mg recovered). The product was then purified again by silica gel flash chromatography eluting with hexane/EtOAc, 4/1, 1/1 to give **15** (842 mg, 0.26 mmol, 64%) as a white foam. $[\alpha]_D$ ($c = 0.1$, DCM) +33.3; 1H NMR (800 MHz; $CDCl_3$) δ 8.16-8.04 (m, 14H, ArH), 7.57-7.14 (m, 66H, ArH), 5.60-5.58 (m, 2H, H_{1Ido}), 5.57 (d, $J = 4.1$ Hz, 1H, H_{1Ido}), 5.30 (t, $J = 9.7$ Hz, 1H, H_{4Glu}), 5.24-5.19 (m, 3H, H_{2Ido}), 5.10 (s, 1H, H_{1Ido}), 5.08 (s, 1H, H_{2Ido}), 4.93-4.89 (m, 3H, H_{1Glu}), 4.81-4.65 (m, 12H, H_{5Ido} x 3, H_{6Glu} x 2, CH_2Ph x

7), 4.59 (d, $J = 3.5$ Hz, 1H, H_{1Glu}), 4.57-4.53 (m, 2H, H_{6Glu} x 2), 4.50 (d, $J = 5.0$ Hz, 1H, H_{5Ido}), 4.45-4.42 (m, 3H, CH₂Ph), 4.38-4.36 (m, 2H, CH₂Ph), 4.33-4.27 (m, 3H, CH₂Ph x 2, H_{6Glu}), 4.20-4.10 (m, 8H, H_{6Glu} x 3, H_{3Ido} x 4, H_{5Glu}), 4.05-3.91 (m, 10H, H_{4Glu} x 3, H_{4Ido} x 4, H_{5Glu} x 3), 3.86-3.84 (m, 2H, CH₂Ph), 3.67 (t, $J = 9.7$ Hz, 1H, H_{3Glu}), 3.64 (s, 3H, OCH₃), 3.56 (t, $J = 9.5$ Hz, 1H, H_{3Glu}), 3.53-3.49 (m, 8H, H_{3Glu} x 2, CO₂CH₃ x 2), 3.41 (dd, $J = 10.1, 3.4$ Hz, 1H, H_{2Glu}), 3.37-3.35 (m, 2H, H_{2Glu}), 3.33 (s, 3H, CO₂CH₃), 3.30 (s, 3H, CO₂CH₃), 3.23 (dd, $J = 10.2, 3.6$ Hz, 1H, H_{2Glu}); ¹³C NMR (200 MHz; CDCl₃) δ 169.7, 169.3, 169.3, 166.1, 166.0, 165.9, 165.6, 165.3, 160.5, 137.7, 137.6, 137.5, 137.4, 137.2, 136.5, 133.8, 133.6, 133.4, 133.3, 133.2, 133.1, 130.0, 129.9, 129.8, 129.7, 129.6, 129.4, 129.3, 128.6, 128.5, 128.4, 128.3, 128.2, 128.1, 128.02, 127.9, 100.4, 99.3, 99.1, 98.4, 98.3, 98.2, 89.5, 78.4, 78.3, 76.3, 76.0, 75.8, 75.7, 75.6, 75.4, 75.1, 75.0, 74.9, 74.6, 74.5, 74.4, 74.2, 73.9, 72.4, 72.2, 71.6, 71.0, 70.7, 70.08, 69.9, 68.3, 67.9, 67.8, 67.0, 63.6, 63.3, 63.2, 62.3, 62.0, 61.9, 61.3, 60.5, 56.3, 52.4, 52.1, 51.7, 51.6; FTMS (ESI⁺) m/z calcd for C₁₆₇H₁₅₉Cl₃N₁₂O₅₀Na [M+Na]⁺ 3262.93, found 3262.90; CHN: calcd for C₁₆₇H₁₅₉Cl₃N₁₂O₅₀ C 61.90 H 4.95 N 5.19, found C 61.54 H 4.73 N 5.23; IR (cm⁻¹): 1024 (C-O), 1098 (C-O), 1263 (C-O), 1719 (C=O), 1763 (C=O), 2106 (N₃).

Octaasaccharide 16

Octaasaccharide **15** (280 mg, 86.0 μ mol) was dissolved in a mixture of dry pyridine (0.5 mL) and dry MeOH (2 mL) at room temperature under N₂. The clear solution was heated at 40 °C for 5 h. Solvents were removed *in vacuo* and the residue azeotroped with toluene (3 x 5 mL). This material was then purified by silica gel flash chromatography eluting with hexane/EtOAc, 2/1 to give **16** (237 mg, 77.0 μ mol, 89%) as a clear foam. $[\alpha]_D$ ($c = 0.1$, DCM) +24.8; ¹H NMR (400 MHz; CDCl₃) δ 8.15-8.02 (m, 20H, ArH), 7.58-7.51 (m, 10H, ArH), 7.50-7.12 (m, 50H, ArH), 5.51-5.48 (m, 3H, H_{1Ido}), 5.15-5.09 (m, 3H, H_{2Ido}) 4.99-4.98 (m, 1H, H₁), 4.97 (brs, 1H, H₂), 4.98 (d, $J = 3.5$ Hz, 1H, H_{1Glu}), 4.84-4.77 (m, 3H, CH₂Ph, 2 x H_{1Glu}), 4.71-4.18 (m, 27H, H_{1Glu}, H_{6Glu} x 8, H_{5Ido} x 4, CH₂Ph x 14), 4.09-4.06 (m, 1H, H_{3Ido}), 4.04-3.72 (m, 15H, H_{3Ido} x 3, H_{4Ido} x 4, H_{5Glu} x 4, H_{4Glu} x 3, CH₂Ph), 3.53 (s, 3H, OCH₃), 3.47-3.35 (m, 5H, H_{4Glu}, H_{3Glu} x 4), 3.39 (s, 3H, C(O)CH₃), 3.75 (s, 3H, C(O)CH₃), 3.26-3.25 (m, 2H, H_{2Glu} x 2), 3.20 (s, 3H, C(O)CH₃), 3.18 (s, 3H, C(O)CH₃), 3.13-3.09 (m, 2H, H_{2Glu} x 2), 3.04 (d, $J = 3.8$ Hz, 1H, OH); ¹³C NMR (100 MHz; CDCl₃) δ 169.6, 169.3, 169.3, 167.4, 166.1, 166.0, 165.6, 165.3, 165.2, 137.7, 137.6, 137.5, 137.4, 137.3, 133.7, 133.5, 133.2, 133.1, 133.0, 130.0, 129.9, 129.8, 129.7, 129.6, 129.3, 129.1, 128.9, 128.8, 128.5, 128.4, 128.3, 128.2, 128.1, 128.0, 127.9, 127.8, 127.6, 100.4, 99.2, 99.0, 98.3, 98.2, 98.1, 79.1, 78.4, 78.3, 77.4, 76.3, 75.9, 75.8, 75.6, 75.5, 75.1, 75.0, 74.6, 74.5, 74.4,

74.1, 74.0, 72.4, 72.2, 71.7, 71.6, 71.4, 71.3, 71.1, 71.0, 70.8, 70.4, 70.0, 69.9, 67.9, 67.0, 63.5, 63.1, 62.9, 62.8, 62.0, 61.9, 56.3, 52.3, 52.0, 51.6, 51.6; FTMS (ESI⁺) *m/z* calcd for C₁₆₅H₁₆₈N₁₄O₄₉ [M + 2NH₄]²⁺ 1564.55, found 1564.55; IR (cm⁻¹): 1024 (C-O), 1263 (C-O), 1718 (C=O), 1763 (C=O), 2106 (N₃), 2939 (O-H).

Decasaccharide **17**

Octasaccharide acceptor **16** (230 mg, 74.0 μmol, 1.0 equiv.) and disaccharide donor **10** (91 mg, 89.0 μmol, 1.2 equiv.) were combined in dry toluene (10 mL). Solvent was removed *in vacuo* and the residue dried under high vacuum for 1 h. The foam was then dissolved in dry DCM (5 mL) and cooled to 0 °C. 4Å molecular sieves (100 mg) were added followed by NIS (22 mg, 96.0 μmol, 1.3 equiv.) and AgOTf (2.0 mg, 7.4 μmol, 0.1 equiv.). The resultant suspension was stirred at this temperature for 0.5 h whereupon a deep red colour persisted. The reaction was quenched with NaHCO₃ (20 mg) and Na₂S₂O₃ (20 mg) in H₂O (2 mL) and the mixture filtered through a Celite™ plug, washing with DCM. The layers were separated and the organics dried (MgSO₄) and solvent removed *in vacuo* to reveal crude **17** as a yellow gum. This material was then purified by silica gel flash chromatography eluting with toluene/acetone, 20/1, 10/1 to separate the product and unreacted octasaccharide acceptor **16** (60 mg recovered). The product was then purified again by silica gel flash chromatography eluting with hexane/EtOAc, 2/1 to give **17** (170 mg, 43.0 μmol, 57%) as a white foam. [α]_D (c = 0.2, DCM) +18.2; ¹H NMR (800 MHz; CDCl₃): δ 8.10-8.05 (m, 19H, ArH), 7.55-7.19 (m, 81H, ArH), 5.60-5.57 (m, 4H, H_{1Ido}), 5.30 (t, *J* = 9.6 Hz, 1H, H_{4Glu}), 5.23-5.19 (m, 4H, H_{2Ido}), 5.10 (s, 1H, H_{1Ido}), 5.07 (s, 1H, H_{2Ido}), 4.92-4.88 (m, 4H, H_{1Glu}), 4.81-4.63 (m, 15H, H_{5Ido} x 4, H_{6Glu} x 2, CH₂Ph x 9), 4.60 (s, 1H, H_{1Glu}), 4.57-4.52 (m, 3H, H_{6Glu} x 3), 4.51 (d, *J* = 4.6 Hz, 1H, H_{5Ido}), 4.43-4.35 (m, 7H, CH₂Ph), 4.33-4.28 (m, 4H, CH₂Ph x 2, H_{6Glu} x 2), 4.19-4.09 (m, 9H, H_{6Glu} x 3, H_{3Ido} x 5, H_{5Glu}), 4.05-3.81 (m, 15H, H_{4Glu} x 4, H_{4Ido} x 5, H_{5Glu} x 4, CH₂Ph x 2), 3.68 (t, *J* = 9.8 Hz, 1H, H_{3Glu}), 3.64 (s, 3H, OCH₃), 3.56-3.49 (m, 10H, H_{3Glu} x 4, CO₂CH₃ x 2), 3.40 (dd, *J* = 10.7, 3.2 Hz, 1H, H_{2Glu}), 3.34-3.29 (m, 12H, CO₂CH₃ x 3, H_{2Glu} x 3), 3.23-3.22 (m, 1H, H_{2Glu}); ¹³C NMR (200 MHz; CDCl₃) δ 169.6, 169.3, 169.2, 166.0, 165.9, 165.6, 165.2, 160.4, 137.7, 137.6, 137.5, 137.4, 137.3, 137.1, 136.5, 133.8, 133.7, 133.5, 133.3, 133.2, 133.1, 133.0, 129.9, 129.8, 129.7, 129.6, 129.4, 129.3, 129.2, 129.0, 128.8, 128.6, 128.5, 128.4, 128.3, 128.2, 128.1, 128.0, 127.9, 100.4, 99.2, 99.0, 98.4, 98.3, 98.2, 98.1, 89.5, 78.4, 78.3, 77.5, 76.3, 75.9, 75.7, 75.6, 75.1, 75.0, 74.8, 74.6, 74.5, 74.4, 74.2, 74.1, 73.9, 72.4, 72.2, 71.8, 71.6, 71.1, 71.0, 70.7, 70.0, 69.9, 69.8, 68.3, 67.9, 67.0,

63.5, 63.3, 62.3, 62.0, 61.9, 61.8, 61.2, 56.2, 52.3, 52.0, 51.7, 51.6; FTMS (ESI⁺) *m/z* calcd for C₂₀₈H₂₀₆N₁₇O₆₂Cl₃ [M+2NH₄]²⁺ 2020.63, found 2020.62.

Decasaccharide 18

Decasaccharide **17** (72 mg, 18.0 μmol) was dissolved in a mixture of dry pyridine (0.25 mL) and dry MeOH (1 mL) at room temperature under N₂. The clear solution was heated at 40 °C for 5 h. Solvents were removed *in vacuo* and the residue azeotroped with toluene (3 x 5 mL). This material was then purified by silica gel flash chromatography eluting with hexane/EtOAc, 1/1 to give **18** (63 mg, 16.0 μmol, 91%) as a white solid. [α]_D (c = 0.1, DCM) +20.7; ¹H NMR (400 MHz; CDCl₃) δ 8.05-7.93 (m, 30H, ArH), 7.49-7.03 (m, 70H, ArH), 5.52-5.48 (m, 4H, H_{1ido}), 5.15-5.08 (m, 4H, H_{2ido}), 4.99 (brs, 1H, H₁), 4.97 (brs, 1H, H₂), 4.88 (d, *J* = 3.2 Hz, 1H, H_{1glu}), 4.84-4.77 (m, 4H, CH₂Ph, 2 x H_{1glu}), 4.71-4.17 (m, 34H, H_{1glu}, H_{6glu} x 10, H_{5ido} x 5, CH₂Ph x 18), 4.08 (t, *J* = 6.0 Hz, 1H, H_{3ido}), 4.03-3.70 (m, 19H, H_{3ido} x 4, H_{4ido} x 5, H_{5glu} x 5, H_{4glu} x 4, CH₂Ph), 3.53 (s, 3H, OCH₃), 3.47-3.41 (m, 6H, H_{4ido}, H_{3glu} x 5), 3.39 (s, 3H, C(O)CH₃), 3.38 (s, 3H, C(O)CH₃), 3.27-3.21 (m, 2H, H_{2glu} x 2), 3.21 (s, 3H, C(O)CH₃), 3.12 (s, 6H, 2 x C(O)CH₃), 3.13-3.08 (m, 3H, H_{2glu} x 3), 2.95 (d, *J* = 3.2 Hz, 1H, OH); ¹³C NMR (100 MHz; CDCl₃) δ 169.6, 169.3, 167.4, 166.1, 166.0, 165.9, 165.6, 165.2, 165.1, 137.7, 137.6, 137.5, 137.3, 137.2, 133.7, 133.5, 133.2, 133.1, 133.0, 129.9, 129.3, 129.7, 129.5, 129.3, 129.2, 129.1, 129.0, 128.8, 128.7, 128.5, 128.4, 128.3, 128.2, 128.1, 128.0, 127.8, 127.7, 127.5, 100.3, 99.1, 98.2, 98.1, 98.0, 79.0, 78.4, 78.3, 78.2, 78.22, 76.3, 75.8, 75.5, 75.1, 75.0, 74.7, 74.4, 74.2, 74.1, 74.0, 72.4, 72.3, 71.8, 71.6, 71.4, 71.3, 71.2, 71.1, 71.0, 70.8, 70.4, 70.0, 69.9, 69.8, 68.0, 67.1, 63.5, 63.2, 63.0, 62.3, 62.7, 61.9, 61.8, 56.2, 52.3, 51.9, 51.6, 51.6, 51.5; HRMS (FTMS NSI⁺) *m/z* calcd for C₂₀₆H₂₀₇N₁₇O₆₁ [M+2NH₄]²⁺ 1947.1804, found 1947.1752.

Dodecasaccharide 19

Decasaccharide acceptor **18** (65 mg, 16.3 μmol, 1.0 equiv.) and disaccharide donor **10** (20 mg, 19.5 μmol, 1.2 equiv.) were combined in dry toluene (10 mL). Solvent was removed *in vacuo* and the residue dried under high vacuum for 1 h. The foam was then dissolved in dry DCM (5 mL) and cooled to 0 °C. 4 Å molecular sieves (100 mg) were added followed by NIS (5.0 mg, 21.2 μmol, 1.3 equiv.) and AgOTf (0.5 mg, 0.16 μmol, 0.1 equiv.). The resultant suspension was stirred at this temperature for 0.5 h whereupon a deep red colour persisted. The reaction was quenched with NaHCO₃ (5 mg) and Na₂S₂O₃ (5 mg) in H₂O (2 mL) and the mixture filtered through a Celite™ plug, washing with DCM. The layers were separated and the organics dried (MgSO₄) and solvent removed *in vacuo* to reveal crude **19** as a yellow gum. This material was then purified by silica gel flash chromatography eluting with toluene/

acetone, 20/1, 10/1 to isolate the product **19** (61.3 mg, 12.8 μmol , 79%) as a white foam and unreacted deca-saccharide acceptor **18** (6 mg recovered). $[\alpha]_D$ ($c = 0.1$, DCM) +24.1; ^1H NMR (800 MHz; CDCl_3): δ 8.15-8.03 (m, 23H, ArH), 7.68-7.13 (m, 97H, ArH), 5.59-5.57 (m, 4H, H_{1Ido}), 5.57 (d, $J = 4.2$ Hz, 1H, H_{1Ido}), 5.30 (t, $J = 9.6$ Hz, 1H, H_{4Glu}), 5.23-5.18 (m, 5H, H_{2Ido}), 5.10 (s, 1H, H_{1Ido}), 5.07 (s, 1H, H_{1Ido}), 4.92-4.88 (m, 5H, H_{1Glu}), 4.81-4.64 (m, 21H, H_{5Ido} x 5, H_{6Glu} x 2, CH₂Ph x 14), 4.60 (d, $J = 3.6$ Hz, 1H, H_{1Glu}), 4.56-4.53 (m, 4H, H_{6Glu}), 4.51 (d, $J = 5.1$ Hz, 1H, H_{5Ido}), 4.43-4.29 (m, 14H, CH₂Ph x 8, H_{6Glu} x 6), 4.20-4.09 (m, 7H, H_{3Ido} x 6, H_{5Glu}), 4.04-3.84 (m, 18H, H_{4Glu} x 5, H_{4Ido} x 6, H_{5Glu} x 5, CH₂Ph x 2), 3.68 (t, $J = 9.6$ Hz, 1H, H_{3Glu}), 3.64 (s, 3H, OCH₃), 3.56 (t, $J = 9.6$ Hz, 1H, H_{3Glu}), 3.52-3.48 (m, 10H, H_{3Glu} x 4, CO₂CH₃ x 2), 3.40 (dd, $J = 10.1, 3.5$ Hz, 1H, H_{2Glu}), 3.34-3.28 (m, 16H, H_{2Glu} x 4, CO₂CH₃ x 3), 3.22 (dd, $J = 10.2, 3.5$ Hz, 1H, H_{2Glu}); ^{13}C NMR (200MHz; CDCl_3) δ 167.3, 167.0, 166.9, 163.7, 163.6, 163.3, 162.9, 158.2, 135.6, 135.2, 135.1, 135.0, 134.9, 134.2, 131.4, 131.2, 131.1, 130.8, 130.7, 127.9, 127.6, 127.5, 127.4, 127.3, 127.1, 127.0, 126.9, 126.8, 126.6, 126.5, 126.4, 126.3, 126.2, 126.1, 126.0, 125.9, 125.8, 125.7, 125.6, 125.5, 98.1, 96.8, 96.7, 96.1, 96.0, 95.1, 95.8, 87.2, 76.0, 75.0, 74.0, 73.6, 73.5, 73.3, 72.8, 72.5, 72.4, 72.2, 72.1, 71.9, 71.6, 70.1, 70.0, 69.6, 69.4, 68.9, 68.7, 68.5, 67.7, 67.6, 66.0, 65.7, 64.8, 61.2, 61.0, 60.8, 59.9, 59.6, 59.5, 59.0, 58.9, 54.0, 50.0, 49.7, 49.4, 49.3, 49.3; MS MALDI-TOF m/z calcd for C₂₄₉H₂₃₇N₁₈O₇₄Cl₃Na [M+Na]⁺ 4794.4, found 4794.5.

Octasaccharide Saponification **20**

Octasaccharide **15** (100 mg, 31 μmol) was dissolved in THF (1 mL) and MeOH (0.5 mL). The solution was cooled to 0 °C and LiOH (25mg, 605 μmol , 24.0 equiv.) in H₂O (0.5 mL) added dropwise, whereupon the solution turned a faint yellow colour. Stirring was continued for 1 h at this temperature and quenched with 1M HCl (600 μL). Solvents were removed *in vacuo* and the crude white solid purified by silica gel flash chromatography, eluting with DCM/MeOH/NH₄OH, 14/6/2 to give **20** (63 mg, 29 μmol , 89%) as a white solid. ^1H NMR (400 MHz; Acetic acid, d₅) δ 7.52-7.12 (m, 40H, ArH), 5.38 (s, 1H, H_{1''}), 5.37 (s, 1H, H_{1'''}), 5.35 (s, 1H, H_{1''''}), 5.37-5.33 (m, 2H, H_{1'}, H_{1''}), 5.33-5.29 (m, 2H, H_{1'''}, H_{1''''}), 5.04 (s, 1H, H₁), 5.02-4.93 (m, 4H, H_{5''}, H_{5'''}, H_{5''''}, CH₂Ph), 4.92-4.89 (d, $J = 2.0$ Hz, 1H, H₅), 4.86-4.76 (m, 5H, CH₂Ph), 4.74-4.67 (m, 4H, CH₂Ph), 4.65-4.55 (m, 3H, CH₂Ph), 4.48-4.39 (m, 3H, CH₂Ph), 4.29-4.27 (s, 1H, H₄), 4.23-4.15 (m, 3H, H_{4''}, H_{4'''}, H_{4''''}), 4.15-3.97 (m, 12H, H₂, H_{2''}, H_{2'''}, H_{2''''}, H_{2''''''}, H₃, H_{3''}, H_{3'''}, H_{3''''}, H_{6B'}, H_{6B''}, H_{6B'''}, H_{6B''''}), 3.95-3.60 (m, 20H, H_{2'}, H_{2''}, H_{2'''}, H_{2''''}, H_{2''''''}, H_{3'}, H_{3''}, H_{3'''}, H_{3''''}, H_{4'}, H_{4''}, H_{4'''}, H_{4''''}, H_{5'}, H_{5''}, H_{5'''}, H_{5''''}, H_{6A'}, H_{6A''}, H_{6A'''}, H_{6A''''}), 3.51-3.46 (s, 3H,

OCH₃); FTMS (ESI⁺) *m/z* calcd for C₁₀₅H₁₂₀N₁₂O₄₁ [M+Na]⁺ 2228.76, found 2228.75, [M+2Na]²⁺ 1125.87, found 1125.87.

Octasaccharide 23

Octasaccharide **20** (57 mg, 26 μmol) was dissolved in dry pyridine (2 mL) under N₂ at RT. SO₃.Py complex (112 mg, 702 μmol, 27.0 equiv.) was added and the suspension stirred 50 °C for 5 h then at RT overnight. A white precipitate formed from the pale yellow solution after 2 h. TLC analysis (DCM/MeOH/NH₄OH, 14/6/2) showed no starting material remained and one product spot at lower R_f. The reaction was quenched by addition of NaHCO₃ (60 mg) in H₂O (0.5 mL) and the solvents removed *in vacuo* (co-evaporation with toluene). The crude white paste was then purified by silica gel flash chromatography, eluting with DCM/MeOH/NH₄OH, 14/6/2. The residue obtained was dissolved in MeOH/DCM (2 mL, v/v), stirred with Amberlite IRC86-Na⁺ resin for 2 h, filtered and solvent removed *in vacuo* to give the per-*O*-sulfated product (65 mg, 20 μmol, 78%) as a white solid. HRMS (FTMS-NSI⁻) *m/z* calcd for C₁₀₅H₁₁₄N₁₂O₆₈S₉Na [M-6H+Na]⁵⁻ 588.2637, found 588.2649. This material (64 mg, 19 μmol) was then dissolved in MeOH (2 mL) and THF (0.5 mL) at RT. NaHCO₃ (12 mg, 140 μmol, 7.0 equiv.) in H₂O (1 mL) was added along with Pd(OH)₂/C (40 mg) and the system purged with H₂. Stirring was continued at 50 °C for 4 d. The suspension was then filtered through Celite® and solvents removed *in vacuo*. The residue was then dissolved in water and desalted by passage through a Sephadex G-25 resin using water as eluent to reveal **23** (46 mg, 19 μmol, 96%) as a clear glass. ¹H NMR (400 MHz; D₂O) δ 5.24-5.20 (m, 4H, H_{1glu}), 5.13-5.06 (m, 4H, H_{1ido}), 4.41 (s, 1H, H_{5ido}), 4.23-3.88 (m, 23H, H_{6Aglu}, H_{6Bglu}, H_{5ido} x3, H_{3ido}, H_{2ido}, H_{4ido}), 3.81-3.62 (m, 12H, H_{5glu}, H_{3glu}, H_{4glu}), 3.29 (s, 3H, OCH₃), 3.19-2.86 (m, 4H, H_{2glu}); HRMS (FTMS-NSI⁻) *m/z* calcd for C₄₉H₇₆N₄O₆₈S₉ [M-4H]⁴⁻ 524.0030, found 524.0032 and for C₄₉H₇₅N₄O₆₈S₉ [M-5H]⁵⁻ 419.0009, found 419.0014.

Octasaccharide 26

Octasaccharide **23** (15 mg, 6.0 μmol) was dissolved in H₂O (1 mL) at RT. NaHCO₃ (10 mg, 120 μmol, 20.0 equiv.) and SO₃.pyr (10 mg, 60 μmol, 10.0 equiv) were then added and the suspension stirred at room temperature. Further additions of both these reagents were made at 1 h, 3 h, 8 h, 20 h and 24 h. After 28 h solvent was removed *in vacuo* to reveal a crude white solid which was purified by passage through a Sephadex G-20 resin using water as the eluent. The target material **26** (14 mg, 5.0 μmol, 78%) was isolated as a clear glass. ¹H NMR (800 MHz; D₂O) δ 5.40 (d, *J* = 3.7 Hz, 1H, H_{1glu}), 5.38-5.34 (m, 3H, H_{1glu}), 5.20-5.16 (m, 4H, H_{1ido}), 4.99-4.96 (m, 4H, H_{5ido}), 4.29-4.09 (m, 12H, H_{6Aglu}, H_{2ido}, H_{3ido}),

4.00-3.71 (m, 20H, H_{4ido}, H_{5glu}, H_{3glu}, H_{4glu}, H_{6Bglu}), 3.42 (dd, $J = 12.4, 1.6$ Hz, 1H, H_{2glu}), 3.43-3.41 (m, 7H, H_{2glu}, OCH₃); ¹³C NMR (200 MHz; D₂O) chemical shifts for HSQC anomeric carbon crosspeaks δ 101.3, 98.7, 98.5, 92.1, 91.7, 90.7, 90.6; HRMS (FTMS-NSI⁻) m/z calcd for C₄₉H₇₄N₄O₈₀S₁₃ [M-6H]⁶⁻, 402.3041 found 402.3043, calcd for C₄₉H₇₃N₄O₈₀S₁₃Na [M-7H+Na]⁶⁻, 405.9678 found 405.9679.

Decasaccharide 21

Decasaccharide **17** (75 mg, 19 μ mol) was dissolved in THF (1 mL) and MeOH (0.5 mL). The solution was cooled to 0 °C and LiOH (19 mg, 456 μ mol, 24.0 equiv.) in H₂O (0.5 mL) added dropwise, whereupon the solution turned a faint yellow colour. Stirring was continued for 5 h at this temperature and quenched with 1M HCl (450 μ L). Solvents were removed *in vacuo* and the crude white solid purified by silica gel flash chromatography, eluting with DCM/MeOH/NH₄OH, 14/6/2 to give **21** (49 mg, 17 μ mol, 90%) as an off-white solid. ¹H NMR (400 MHz; Acetic acid, d₄) δ 7.51-7.14 (m, 50H, ArH), 5.37 (s, 1H, H_{1'}), 5.35 (brs, 2H, H_{1''}, H_{1'''}), 5.30 (s, 1H, H_{1''''}), 5.21-5.13 (m, 5H, H_{1'}, H_{1''}, H_{1'''}, H_{1''''}, H_{1'''''}), 5.04-5.03 (m, 1H, H₁), 5.00-4.94 (m, 6H, H_{5'}, H_{5''}, H_{5'''}, H_{5''''}, 2 x CH₂Ph), 4.90 (d, $J = 2.0$ Hz, 1H, H₅), 4.86-4.76 (m, 6H, CH₂Ph), 4.73-4.65 (m, 5H, CH₂Ph), 4.63-4.55 (m, 4H, CH₂Ph), 4.47-4.39 (m, 3H, CH₂Ph), 4.30-4.28 (m, 1H, H₄), 4.21-4.17 (m, 4H, H_{4'}, H_{4''}, H_{4'''}, H_{4''''}), 4.13-3.97 (m, 15H, H₂, H_{2'}, H_{2''}, H_{2'''}, H_{2''''}, H_{2'''''}, H₃, H_{3'}, H_{3''}, H_{3'''}, H_{3''''}, H_{6B'}, H_{6B''}, H_{6B'''}, H_{6B''''}, H_{6B'''''}), 3.94-3.63 (m, 25H, H_{2'}, H_{2''}, H_{2'''}, H_{2''''}, H_{2'''''}, H₃, H_{3'}, H_{3''}, H_{3'''}, H_{3''''}, H_{3'''''}, H_{4'}, H_{4''}, H_{4'''}, H_{4''''}, H_{4'''''}, H₅, H_{5'}, H_{5''}, H_{5'''}, H_{5''''}, H_{6A'}, H_{6A''}, H_{6A'''}, H_{6A''''}, H_{6A'''''}), 3.50-3.47 (s, 3H, OCH₃); HRMS (FTMS ESI⁺) m/z calcd for C₁₃₁H₁₅₇N₁₇O₅₁ [M+2NH₄]²⁺ 1392.0102, found 1392.0077.

Decasaccharide 24

Decasaccharide **21** (19 mg, 6.9 μ mol) was dissolved in dry DMF (1 mL) under N₂ at RT. SO₃.NMe₃ complex (53 mg, 380 μ mol, 55.0 equiv.) was added and the clear solution heated in a microwave reactor at 100°C for 1.5 h. TLC analysis (DCM/MeOH/NH₄OH, 14/6/2) showed no starting material remained and one product spot at lower R_f. The reaction was quenched by addition of Et₃N (200 μ L) and the reaction solution loaded onto a Sephadex LH20 column and eluted with DCM/MeOH, 9/1. The fractions containing the oligosaccharide were pooled and passed through a short column of Amberlite IRC86⁺-Na⁺ resin with DCM/MeOH, 9/1 and solvent then removed *in vacuo* to give the per-*O*-sulfated product (27 mg, 6.7 μ mol, 95%) as a clear glass. HRMS (FTMS NSI⁻) m/z calcd for C₁₃₁H₁₄₃N₁₅O₈₄S₁₁ [M-6H]⁶⁻ 603.9062, found 603.9050. This material (25 mg, 6.3 μ mol) was then dissolved in MeOH (2 mL) and at RT. NaHCO₃ (4 mg, 44 μ mol, 7.0 equiv.) in H₂O (1.0 mL) was added along with Pd(OH)₂/C (30 mg) and the system purged with H₂. Stirring was continued at 40 °C for 4 d. The suspension was then filtered

through an Ultrafree-MC® centrifugal filter and solvents removed *in vacuo* to reveal **24** (17 mg, 5.8 μ mol, 92%) as a clear glass. ^1H NMR (400 MHz; D_2O) δ 5.07 (s, 2H, $\text{H}_{1\text{glu}}$), 5.00-4.80 (m, 8H, $\text{H}_{1\text{glu}}$, $\text{H}_{1\text{ido}}$), 4.37 (s, 1H, $\text{H}_{5\text{ido}}$), 4.21-4.09 (m, 19H, $\text{H}_{6\text{Aglu}}$, 4 x $\text{H}_{5\text{ido}}$, $\text{H}_{2\text{ido}}$, $\text{H}_{3\text{ido}}$), 3.96-3.75 (m, 14H, 4 x $\text{H}_{5\text{glu}}$, $\text{H}_{4\text{ido}}$, $\text{H}_{6\text{Bglu}}$), 3.68-3.52 (m, 11H, $\text{H}_{3\text{glu}}$, $\text{H}_{4\text{glu}}$, $\text{H}_{5\text{glu}}$ x 1), 3.29 (s, 3H, OCH_3), 2.71-2.59 (m, 5H, $\text{H}_{2\text{glu}}$); HRMS (FTMS NSI^-) m/z calcd for $\text{C}_{61}\text{H}_{93}\text{N}_5\text{O}_{84}\text{S}_{11}$ $[\text{M}-6\text{H}]^{6-}$ 431.8353, found 431.8351 and for $\text{C}_{61}\text{H}_{94}\text{N}_5\text{O}_{84}\text{S}_{11}$ $[\text{M}-5\text{H}]^{5-}$ 518.4038, found 518.4042.

Decasaccharide 27

Decasaccharide **24** (15 mg, 5.1 μ mol) was dissolved in H_2O (0.5 mL) at RT. The pH was adjusted to 9.0-9.5 using 1M NaOH with a pH meter. Successive additions of $\text{SO}_3\cdot\text{pyr}$ (20.0 mg, 128 μ mol, 25.0 equiv.) were made every 30 minutes up to a total of 5 additions. The pH was maintained at 9.0-9.5 throughout with 1M NaOH. After 4 h solvent was removed *in vacuo* to reveal a crude white solid which was purified by passage through a Sephadex G-20 column and then an Amberlite IRC86⁺-Na column using water as eluent. The target material **27** (12.4 mg, 3.6 μ mol, 73%) was isolated as a clear glass. ^1H NMR (800 MHz; D_2O): $\text{H}_{5\text{ido}}$ signal suppressed in H_2O region δ 5.39-5.12 (m, 10H, $\text{H}_{1\text{glu}}$, $\text{H}_{1\text{ido}}$), 4.37-3.90 (m, 25H, $\text{H}_{6\text{glu}}$, $\text{H}_{4\text{ido}}$, $\text{H}_{2\text{ido}}$, $\text{H}_{3\text{ido}}$), 3.81-3.56 (m, 15H, $\text{H}_{5\text{glu}}$, $\text{H}_{3\text{glu}}$, $\text{H}_{4\text{glu}}$), 3.33 (s, 3H, OCH_3), 3.26-3.15 (m, 5H, $\text{H}_{2\text{glu}}$); δ ^{13}C NMR (200 MHz; D_2O) chemical shifts for HSQC anomeric carbon crosspeaks δ 99.6, 99.0, 98.9, 96.7, 96.4, 95.9; HRMS (FTMS NSI^-) m/z calcd. for $\text{C}_{61}\text{H}_{90}\text{N}_5\text{Na}_2\text{O}_{99}\text{S}_{16}$ $[\text{M}-9\text{H}+2\text{Na}]^{7-}$ 433.3932, found 433.3935, calcd. for $\text{C}_{61}\text{H}_{92}\text{N}_5\text{O}_{99}\text{S}_{16}$ $[\text{M}-7\text{H}]^{7-}$ 427.1127, found 427.1128, calcd. for $\text{C}_{61}\text{H}_{92}\text{N}_5\text{NaO}_{99}\text{S}_{16}$ $[\text{M}-7\text{H}+\text{Na}]^{7-}$ 502.2969, found 502.2970 and for $\text{C}_{61}\text{H}_{93}\text{N}_5\text{O}_{99}\text{S}_{16}$ $[\text{M}-6\text{H}]^{7-}$ calcd. 498.4660, found 498.4667.

Dodecasaccharide 22

Dodecasaccharide **19** (60 mg, 13 μ mol) was dissolved in THF (0.5 mL). The solution was cooled to 0 $^\circ\text{C}$ and LiOH (15 mg, 365 μ mol, 29.0 equiv.) in H_2O (1 mL) added dropwise, whereupon the solution turned a faint yellow colour. Stirring was continued for 5 h at this temperature, room temperature overnight and quenched with 1M HCl (20 μ L). Solvents were removed *in vacuo* and the crude white solid purified by silica gel flash chromatography, eluting firstly with DCM/MeOH, 9/1 then with DCM/MeOH/ NH_4OH , 14/6/2 to give **22** (28 mg, 8.5 μ mol, 68%) as a pale white solid. ^1H NMR (400 MHz; acetic- d_5): δ 7.34-6.99 (m, 60H, ArH), 5.21-5.13 (m, 5H, $\text{H}_{1\text{ido}}$), 5.06-4.98 (m, 6H, $\text{H}_{1\text{glu}}$), 4.87 (s, 1H, $\text{H}_{1\text{ido}}$), 4.83-4.78 (m, 6H, $\text{H}_{5\text{ido}}$, CH_2Ph), 4.73 (s, 1H, $\text{H}_{5\text{ido}}$), 4.69-4.61 (m, 8H, CH_2Ph), 4.56-4.50 (m,

6H, CH_2Ph), 4.45-4.38 (m, 4H, CH_2Ph), 4.30-4.23 (m, 5H, CH_2Ph), 4.13 (s, 1H, H_{4ido}), 4.07-4.01 (m, 5H, H_{4ido}), 3.97-3.81 (m, 18H, H_{3ido} , H_{2ido} , H_{6Bglu}), 3.77-3.46 (m, 30H, H_{6Aglu} , H_{5glu} , H_{4glu} , H_{3glu} , H_{2glu}), 3.33 (s, 3H, OCH_3); ESI MS m/z 1645.5700 $[M-2H]^{2-}$, 1096.3717 $[M-3H]^{3-}$, 822.0268 $[M-4H]^{4-}$, 657.6195 $[M-5H]^{5-}$; HRMS (FTMS NSF) m/z calcd for $C_{157}H_{176}N_{18}O_{61}$ $[M-2H]^{2-}$ 1644.5617, found 1644.5686.

Dodecasaccharide 25

Dodecasaccharide **22** (23 mg, 7.0 μ mol) was dissolved in dry DMF (1 mL) under N_2 at RT. $SO_3 \cdot NMe_3$ complex (64 mg, 455 μ mol, 65.0 equiv.) was added and the clear solution heated in a microwave reactor at 100°C for 1.5 h. TLC analysis (DCM/MeOH/ NH_4OH , 14/6/2) showed no starting material remained and one product spot at lower R_f . The reaction was quenched by addition of Et_3N (200 μ L) and the reaction solution loaded onto a Sephadex LH20 column and eluted with DCM/MeOH, 9/1. The fractions containing the oligosaccharide were pooled and passed through a short column of Amberlite IRC86⁺-Na⁺ resin with DCM/MeOH, 9/1 and solvent then removed *in vacuo* to give the per-*O*-sulfated product (30 mg, 6.3 μ mol, 91%) as a pale yellow glass. FTMS (ESI⁻) m/z calcd for $C_{157}H_{171}N_{18}O_{100}S_{13}$ for $[M-7H]^{7-}$ 617.9332, found 617.9339. This material (27 mg, 6.0 μ mol) was then dissolved in MeOH (1 mL) and at RT. $NaHCO_3$ (4 mg, 44 μ mol, 7.0 equiv.) in H_2O (0.5 mL) was added along with $Pd(OH)_2/C$ (30 mg) and the system purged with H_2 . Stirring was continued at 50 °C for 4 d. The suspension was then filtered through Celite[®] and solvents removed *in vacuo*. The residue was dissolved in water and desalted by passage through a Sephadex G-20 resin, using water as eluent, to reveal **25** (18 mg, 5 μ mol, 78%) as a clear glass. 1H NMR (400 MHz; D_2O) δ 5.19-5.09 (m, 8H, H_{1glu} , H_{1ido}), 4.89-4.80 (m, 4H, H_{1ido}), 4.39 (d, J = 1.6 Hz, 1H, H_{5ido}), 4.22-4.09 (m, 23H, H_{6Aglu} , 5 x H_{5ido} , H_{2ido} , H_{3ido}), 4.02-3.86 (m, 14H, 2 x H_{5glu} , H_{4ido} , H_{6Bglu}), 3.77-3.62 (m, 16H, H_{3glu} , H_{4glu} , H_{5glu} x 4), 3.29 (s, 3H, OCH_3), 3.08-2.99 (m, 6H, H_{2glu}); FTMS (ESI⁻) m/z calcd for $C_{73}H_{111}N_6O_{100}S_{13}$ $[M-7H]^{7-}$ 441.0027, found 441.0031, calcd for $C_{73}H_{112}N_6O_{100}S_{13}$ $[M-6H]^{6-}$ 514.6711, found 514.6716.

Dodecasaccharide 28

Dodecasaccharide **25** (5.2 mg, 1.4 μ mol) was dissolved in H_2O (1.0 mL) at RT. The pH was adjusted to 9.0-9.5 using 1M NaOH with a pH meter. Successive additions of $SO_3 \cdot pyr$ (6.8 mg, 43 μ mol, 30.0 equiv.) were made every 30 minutes up to a total of 5 additions. The pH was maintained at 9.0-9.5 throughout with 1M NaOH. After 4 h solvent was removed *in vacuo* to reveal a crude white solid which was purified by passage through a Sephadex G-20 column and then an Amberlite IRC86⁺-Na column

using water as eluent. The target material **27** (5.0 mg, 1.2 μ mol, 82%) was isolated as a clear glass. ^1H NMR (800 MHz; D_2O): $\text{H}_{5\text{Ido}}$ signal suppressed in H_2O region δ 5.38-5.10 (m, 12H, $\text{H}_{1\text{glu}}$, $\text{H}_{1\text{ido}}$), 4.27-3.89 (m, 30H, $\text{H}_{6\text{glu}}$, $\text{H}_{4\text{ido}}$, $\text{H}_{2\text{ido}}$, $\text{H}_{3\text{ido}}$), 3.79-3.57 (m, 18H, $\text{H}_{5\text{glu}}$, $\text{H}_{3\text{glu}}$, $\text{H}_{4\text{glu}}$), 3.34 (s, 3H, OCH_3), 3.27-3.16 (m, 6H, $\text{H}_{2\text{glu}}$); ^{13}C NMR (200 MHz; D_2O) chemical shifts for HSQC anomeric carbon crosspeaks δ 99.3, 99.2, 98.7, 96.9, 96.4, 96.0, 95.7; FTMS (ESI^-) m/z calcd. for $\text{C}_{73}\text{H}_{107}\text{N}_6\text{Na}_5\text{O}_{109}\text{S}_{16}$ $[\text{M}-6\text{H}]^{6-}$ 573.3004, found 573.3013, calcd. for $\text{C}_{73}\text{H}_{106}\text{N}_6\text{Na}_5\text{O}_{112}\text{S}_{17}$ $[\text{M}-7\text{H}]^{7-}$ 502.6789, found 502.6794.

References:

1. (a) Gardiner, J. M.; Jayson, G. J.; Hansen, S. U.; Barath, M. "Production of L-iduronate-containing polysaccharides." 08/2009, WO2009/098449. (b) Gardiner, J. M.; Jayson, G. J.; Hansen, S. U. "Production of L-iduronate-containing polysaccharides from D-glucose via cyanation reaction." 06/2006. WO 2006129075.
2. Barroca, N.; Jacquinet, J. C. *Carbohydr. Res.* **2000**, 329, 667–679.
3. Rochepeau-Jobron, L.; Jacquinet, J. C.; *Carbohydr. Res.* **1997**, 303, 395-40
4. Hansen S. U.; Miller, G. J.; Baráth, M.; Broberg, K. R.; Avizienyte, E.; Jayson, G. C.; Gardiner, J. M. *J. Org. Chem.* **2012**, 77, 7823-7843.

2. Biological methods

Endothelial Cell Proliferation Assay

HUVECs were plated at 1250 cells/well in 24 well plates in EBM-2 medium without SingleQuot growth supplements (Lonza) plus 1% FBS and placed in the cell culture incubator for 6 hours before adding the growth factors. FGF2 and VEGF₁₆₅ (Lonza, SingleQuot supplements) were used at 5 ng/mL and 2.5 ng/mL, respectively. HUVECs were incubated with the growth factors for 96 hours. Cells were fixed with 5% trichloroacetic acid (TCA) for 1 hour at 4 °C, washed with water and allowed to dry. Cells were stained with 4% sulforhodamine B in 1% acetic acid for 30 min, washed in 1% acetic acid and allowed to dry. Stain was solubilised in 200 μ L 10 mM Tris pH 8.8 and transferred to 96-well plate for reading on a microplate reader at 540 nm.

Endothelial Cell Migration Assay

HUVECs were seeded in a 96-well plate at 1.7×10^4 cells per well. Confluent monolayers were serum-starved in EBM-2 medium lacking SingleQuot growth supplements (Lonza) and containing 1% FBS for 18 hours. The wound was created by manually scraping cell monolayer with a pipette tip. Cells were washed and incubated with either EBM-2 medium without SingleQuot growth supplements plus 0.1% FBS or medium supplemented with FGF2 (5 ng/ml) or VEGF₁₆₅ (2.5 ng/mL). Phase contrast images were taken immediately after adding growth factors and after 24 hours of incubation using Zeiss Axiovert 200M microscope (Zeiss) enclosed in a full environmental chamber. The unpopulated areas were analysed using MetaMorph image analysis software (Molecular Devices) by measuring unpopulated area at 0 and 24 hours and cell advancement area was derived for each treatment.

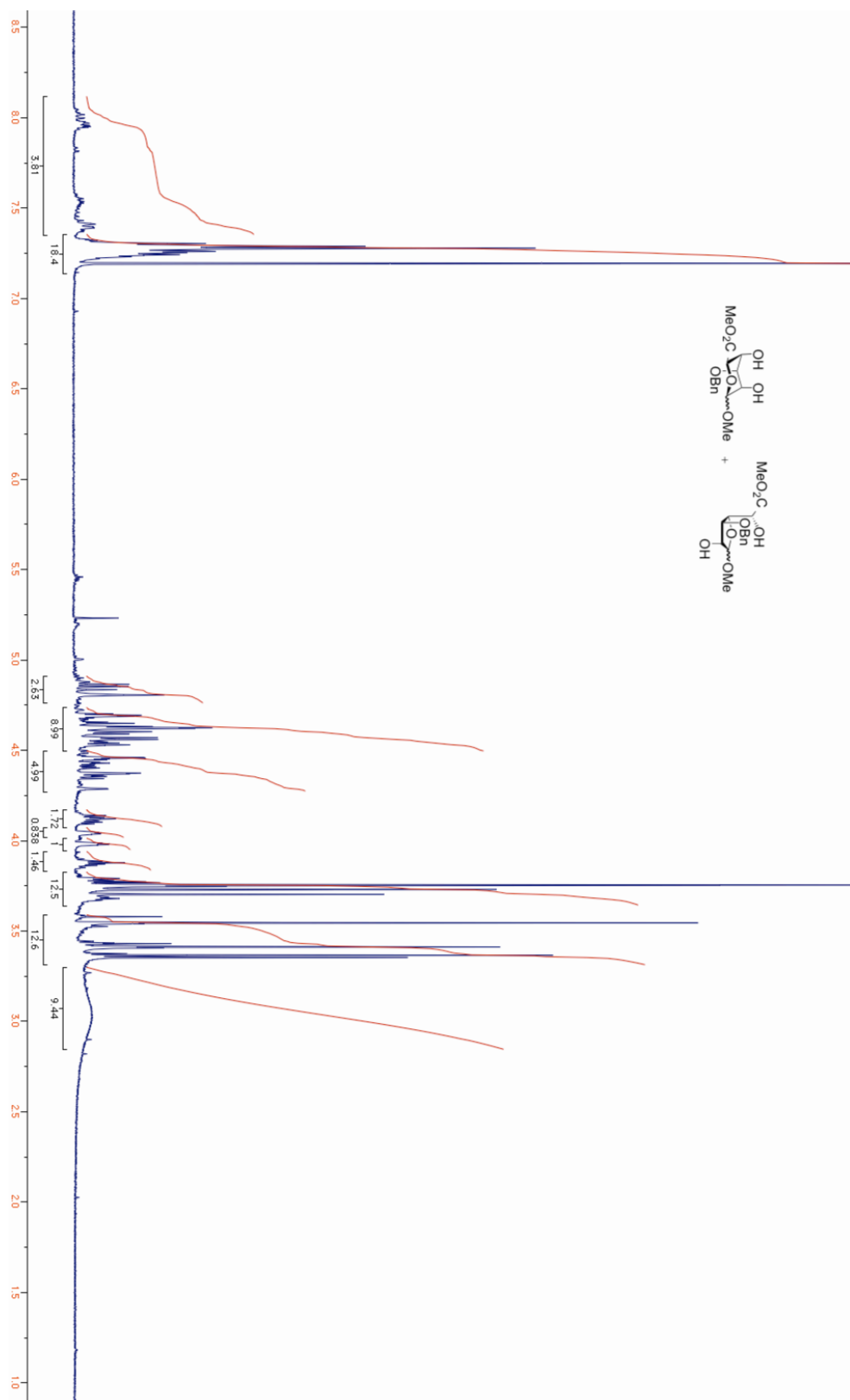
PAGE Analysis

Polyacrylamide gel electrophoresis of oligosaccharides was performed on Novex TBE (Tris-Borate – EDTA) 4-20% polyacrylamide gradient gels (Life technologies). Samples were loaded in 20% glycerol and run at a constant voltage of 150V in 45mM Tris Borate 1mM EDTA (pH 8.0) running buffer. Phenol red was run in a separate lane as a low Mr marker. After electrophoresis, oligosaccharide bands were visualised by staining with 0.1% aqueous Azure A in 50mM sodium formate /10mM Magnesium Chloride pH 3.5 for 5 minutes and destained with 20 minutes 200ml 10mM sodium acetate pH5.5, followed by frequent changes with distilled water.

Spectral Data: ^1H , ^{13}C , COSY, HMQC NMR and MS data for compounds 3-28

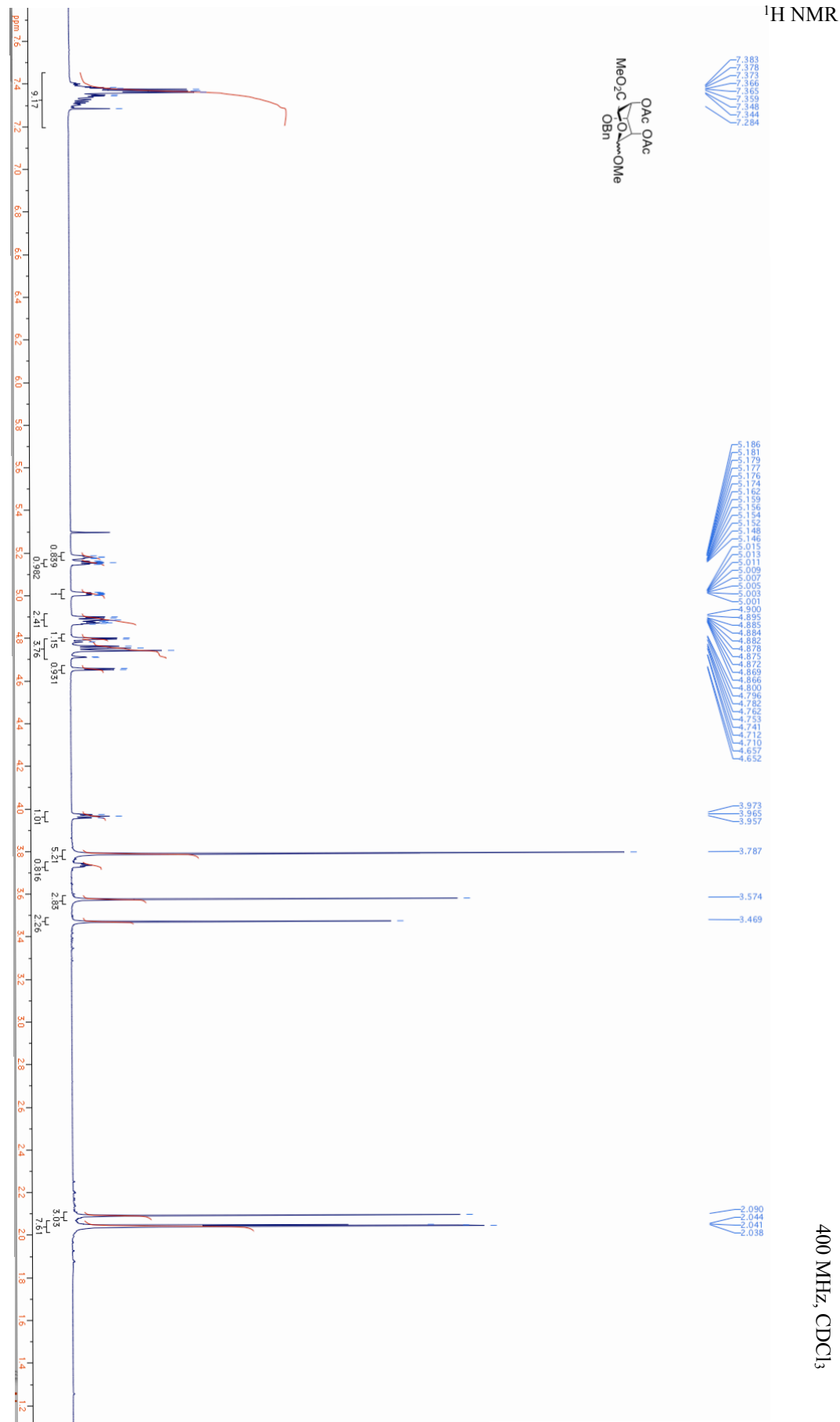
Precursor to 3 and 4 (mixture of anomers)

^1H NMR

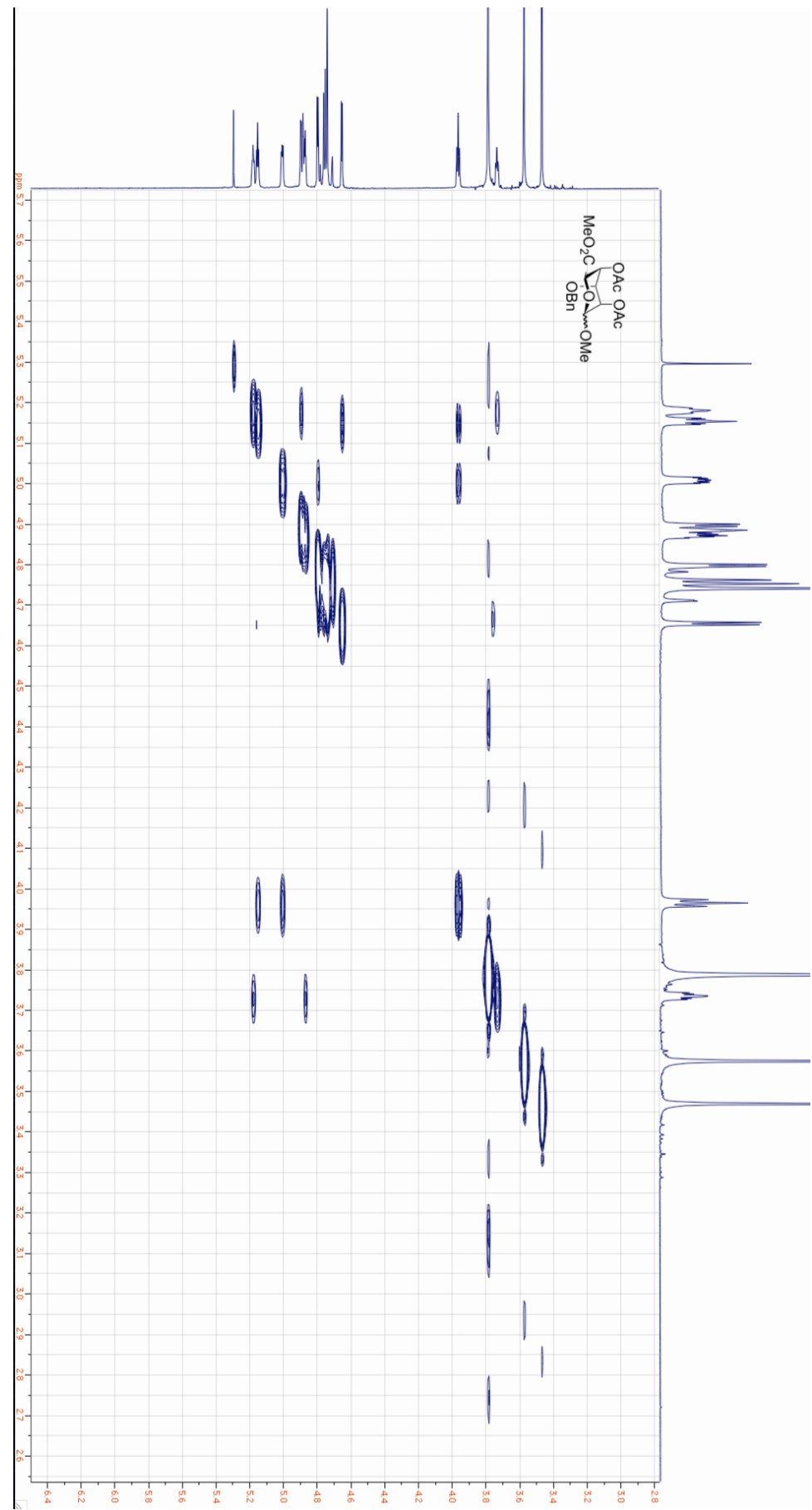


400 MHz, CDCl_3

Methyl (2,4-di-*O*-acetyl-3-*O*-benzyl-1- α/β -methoxy-*L*-idopyranoside) uronate 3



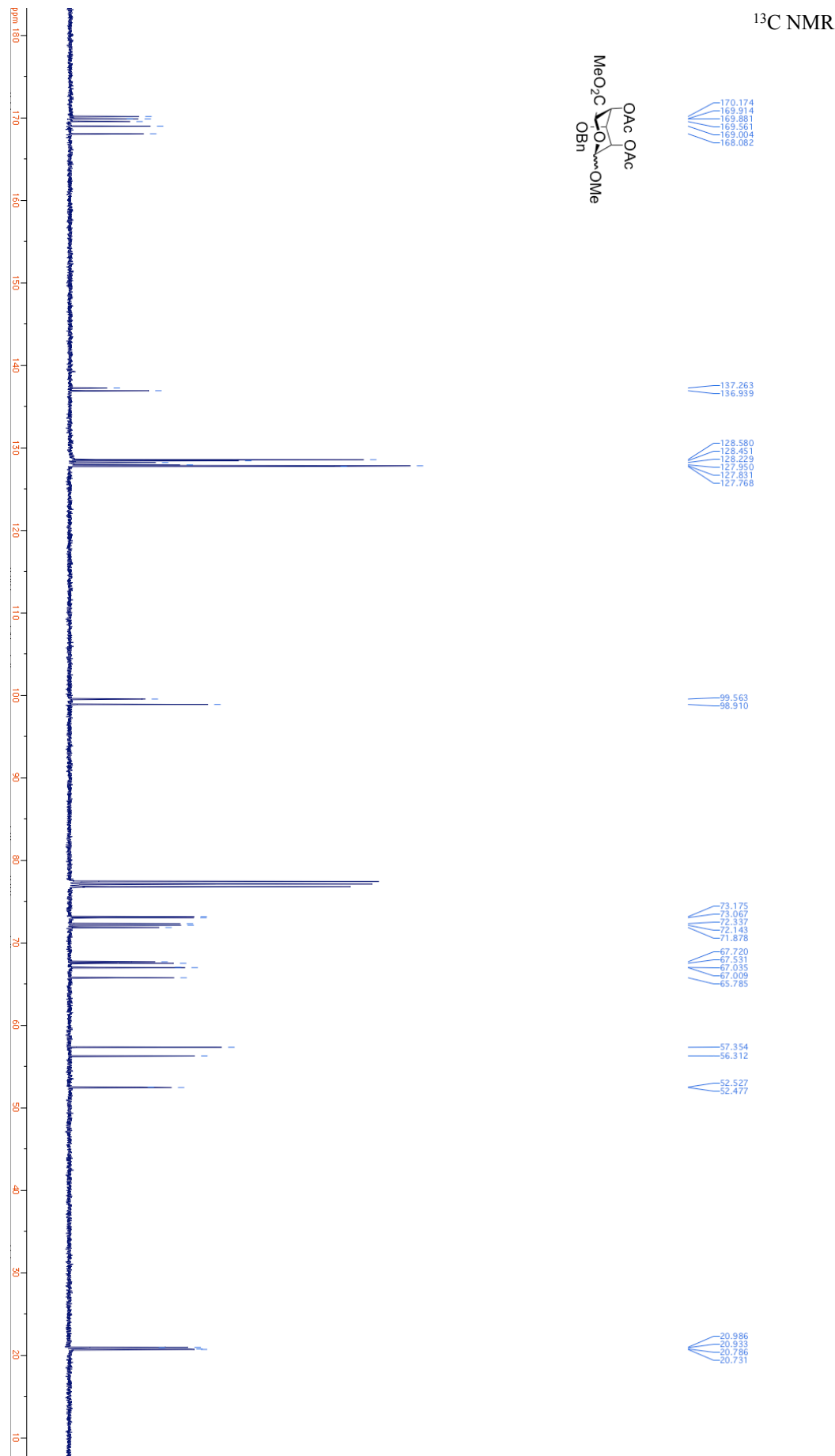
Methyl (2,4-di-*O*-acetyl-3-*O*-benzyl-1- α/β -methoxy-*L*-idopyranoside) uronate 3



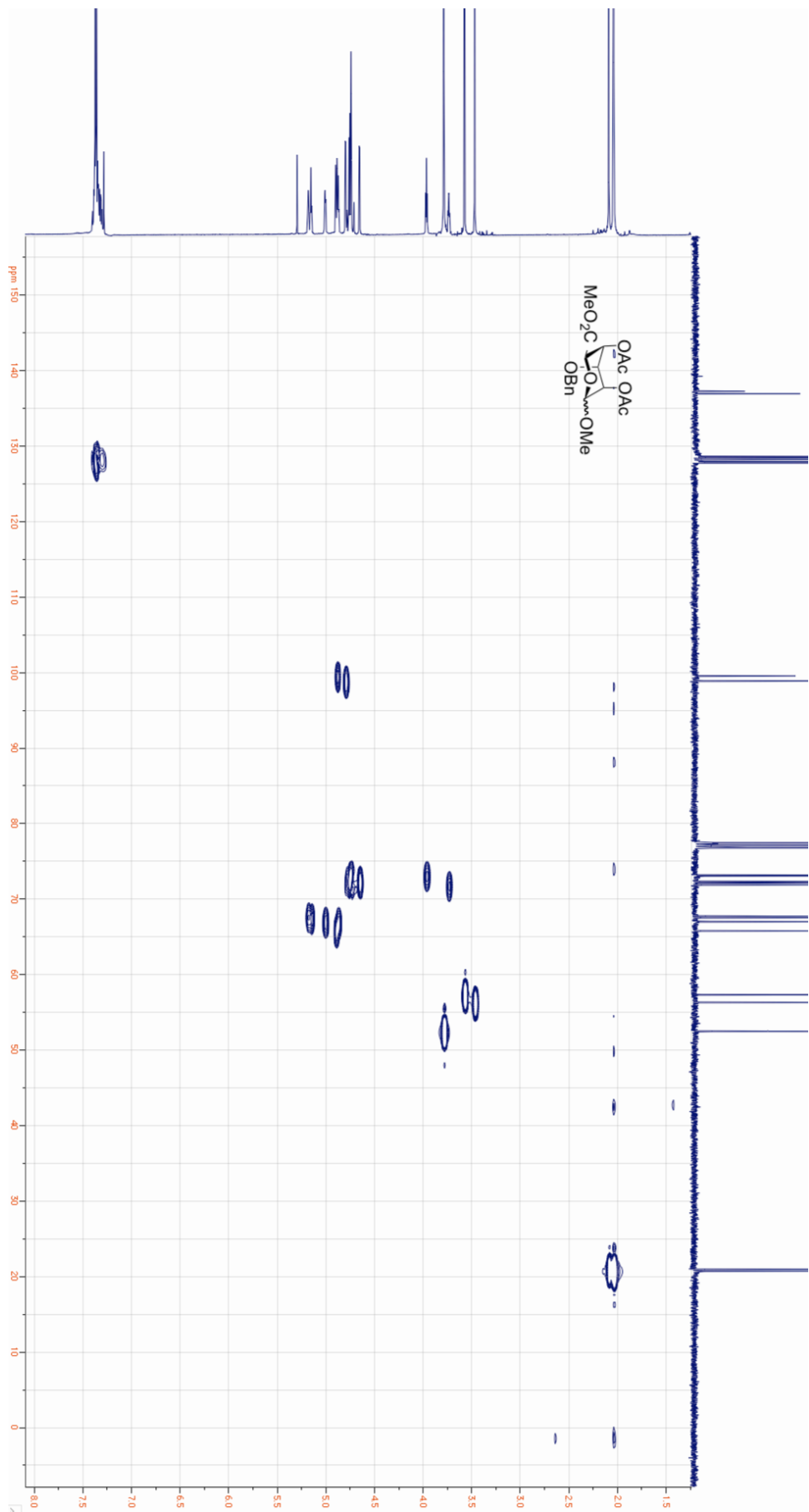
COSY

400 MHz, CDCl₃

Methyl (2,4-di-*O*-acetyl-3-*O*-benzyl-1- α/β -methoxy-*L*-idopyranoside) uronate 3



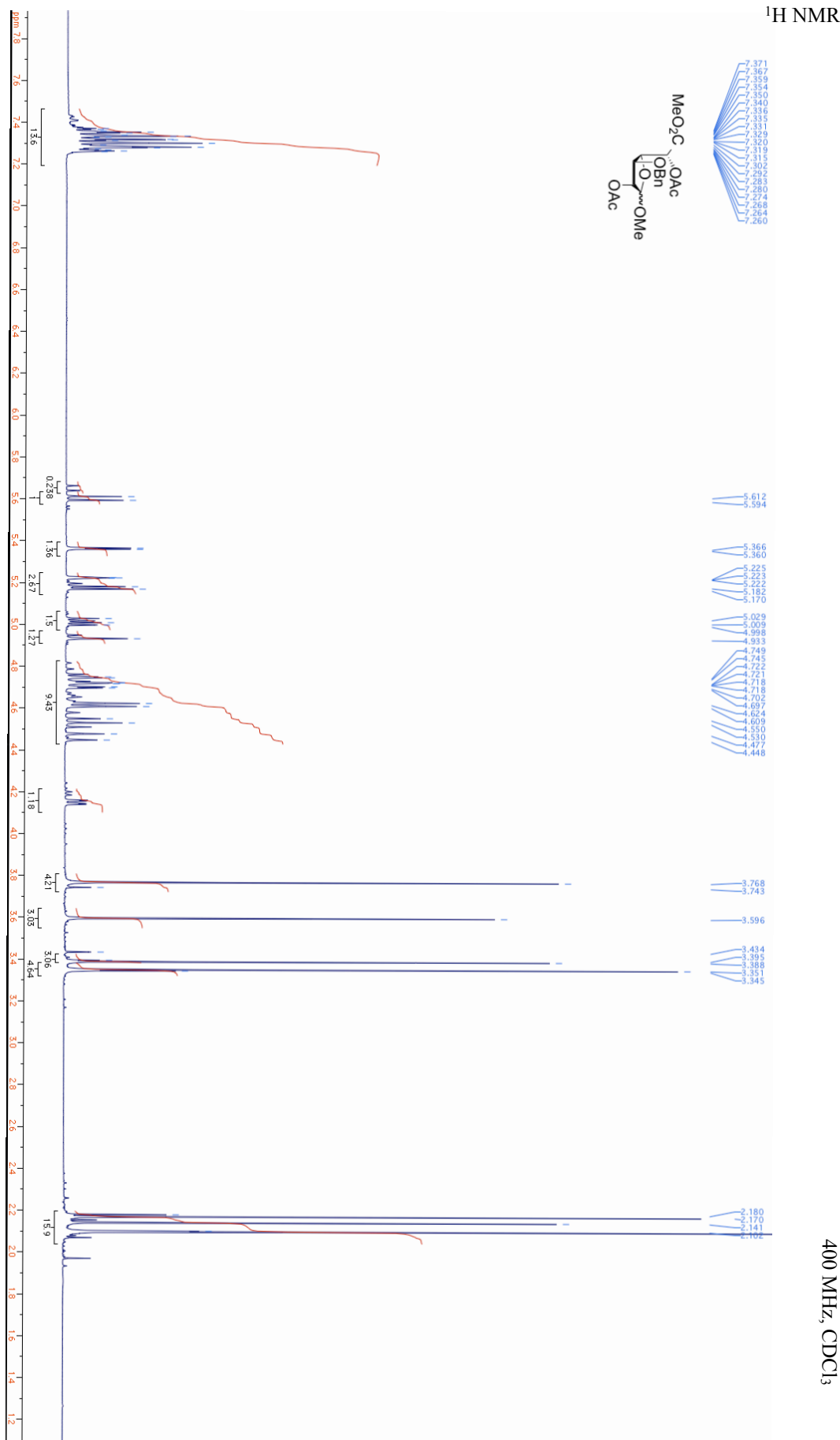
Methyl (2,4-di-*O*-acetyl-3-*O*-benzyl-1- α/β -methoxy-*L*-idopyranoside) uronate 3



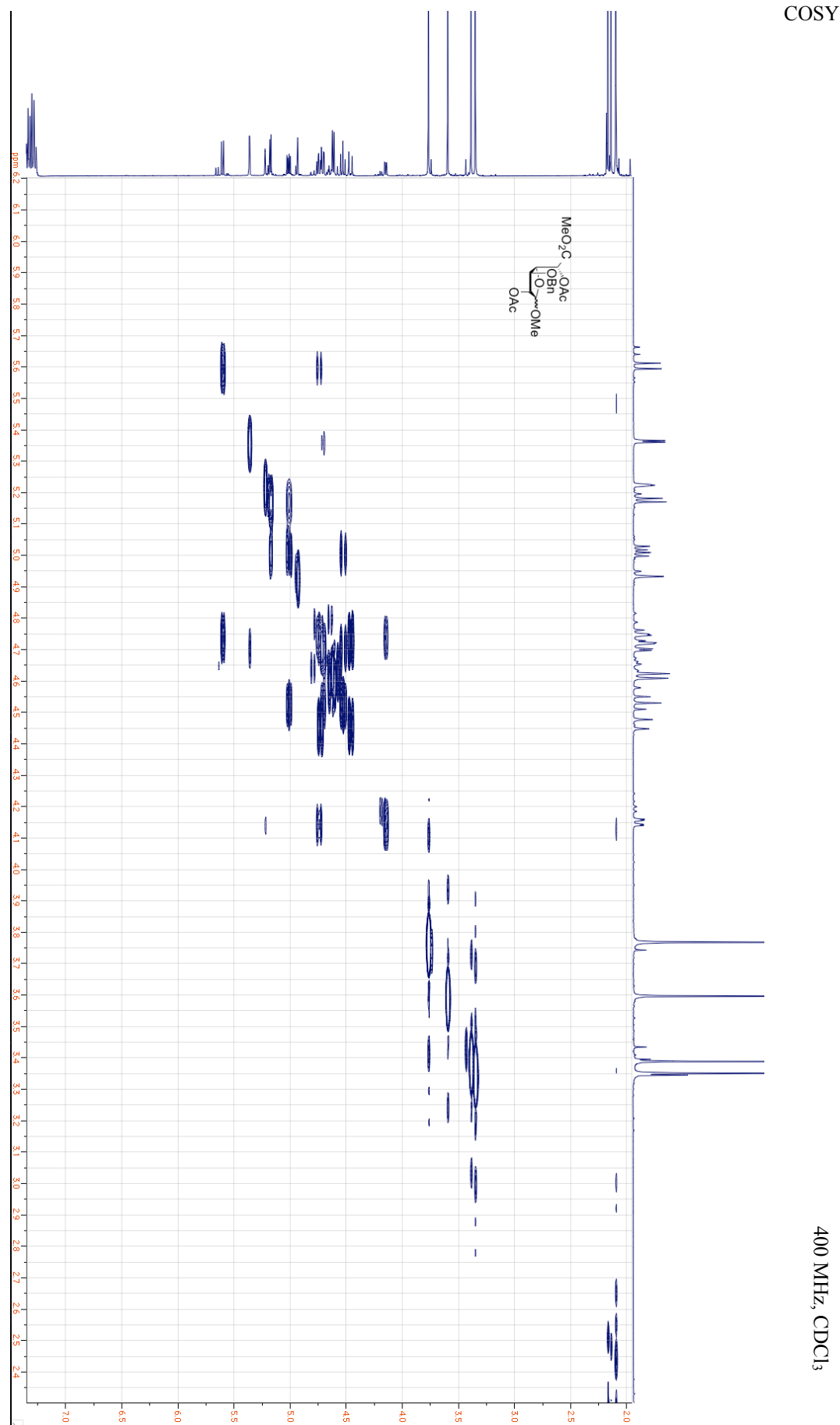
HMQC

400 MHz, CDCl₃

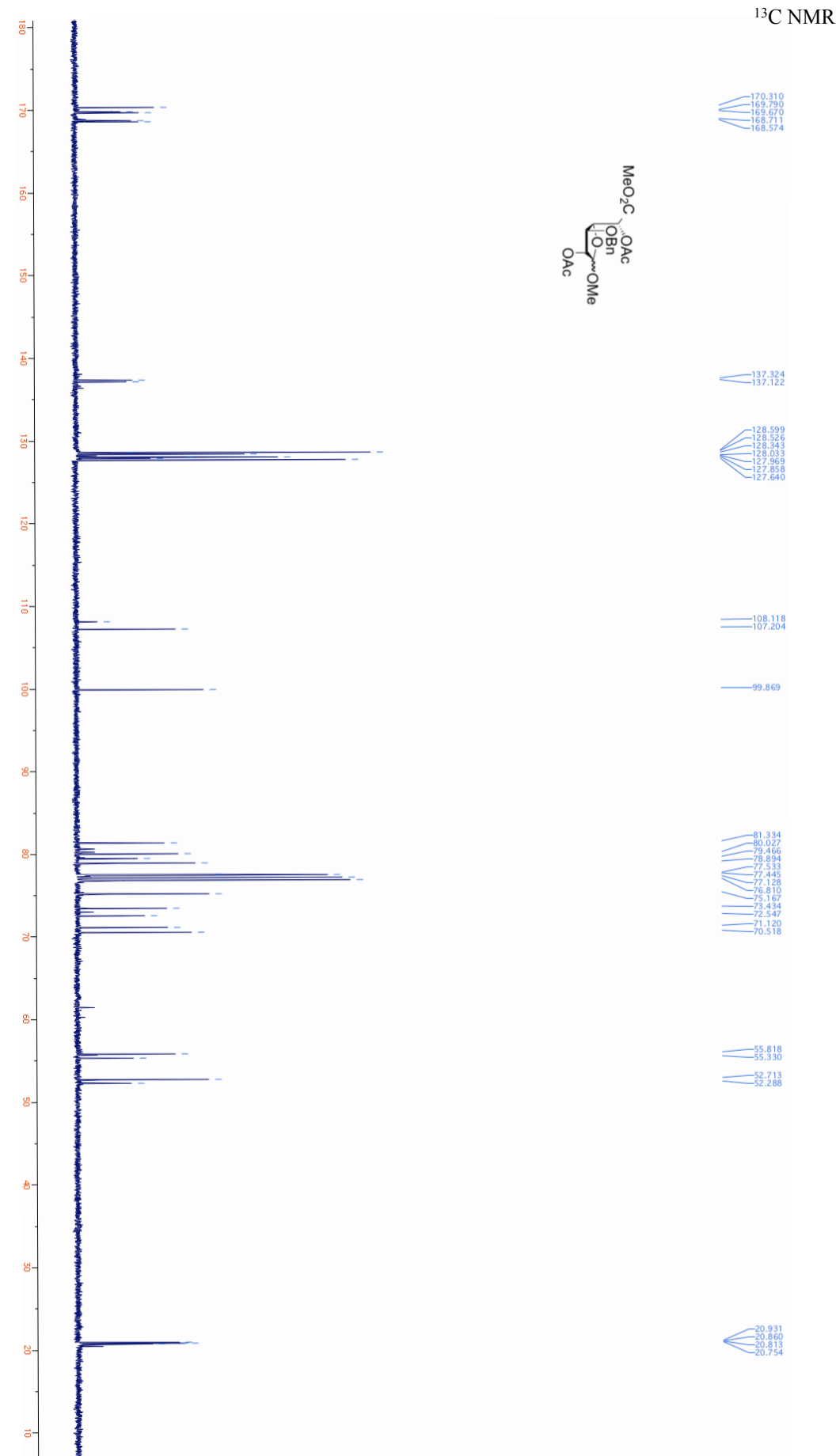
Methyl (2,5-di-*O*-acetyl-3-*O*-benzyl-1- α/β -methoxy-*L*-idofuranoside) uronate 4



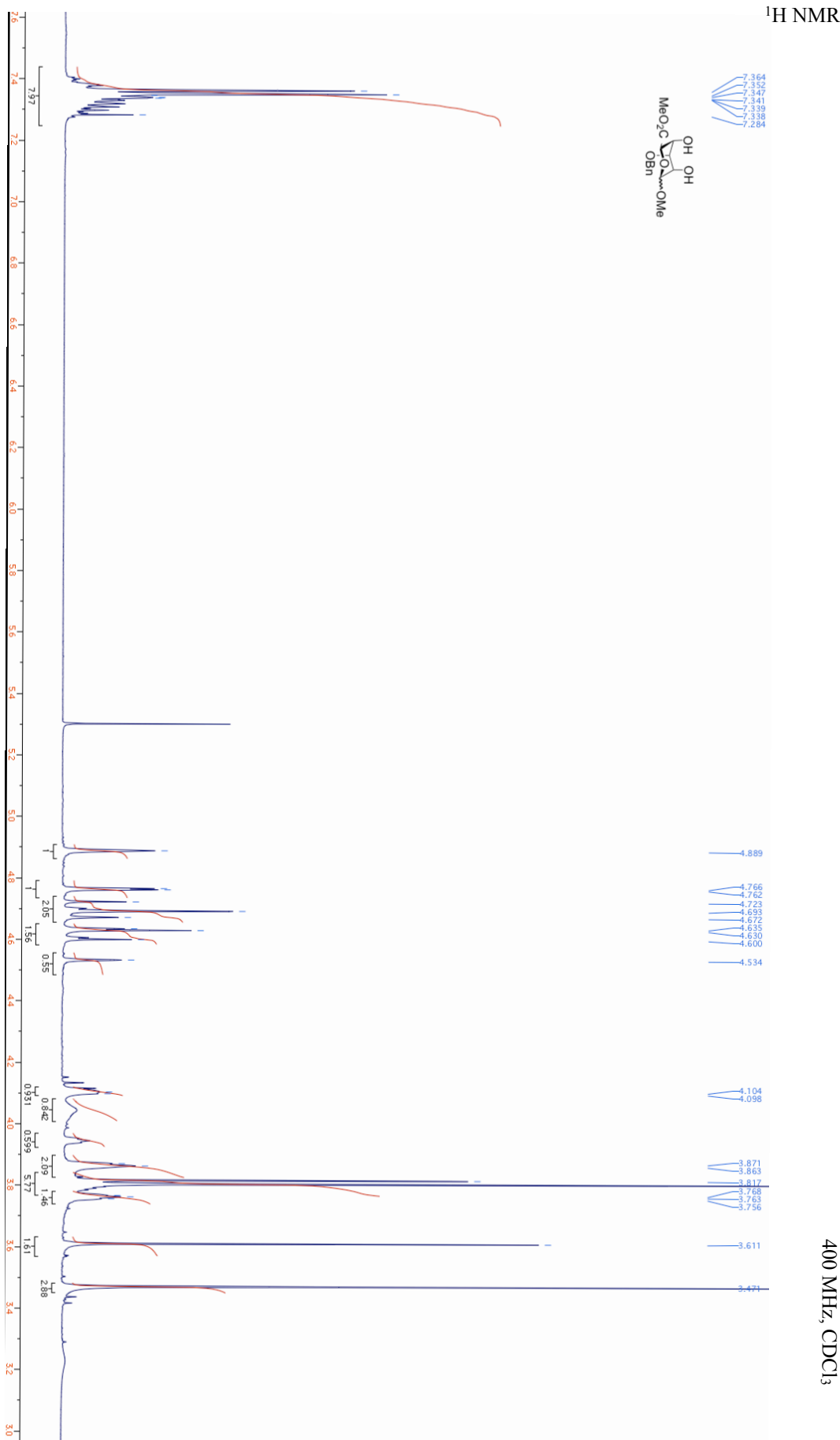
Methyl (2,5-di-*O*-acetyl-3-*O*-benzyl-1- α/β -methoxy-*L*-idofuranoside) uronate 4



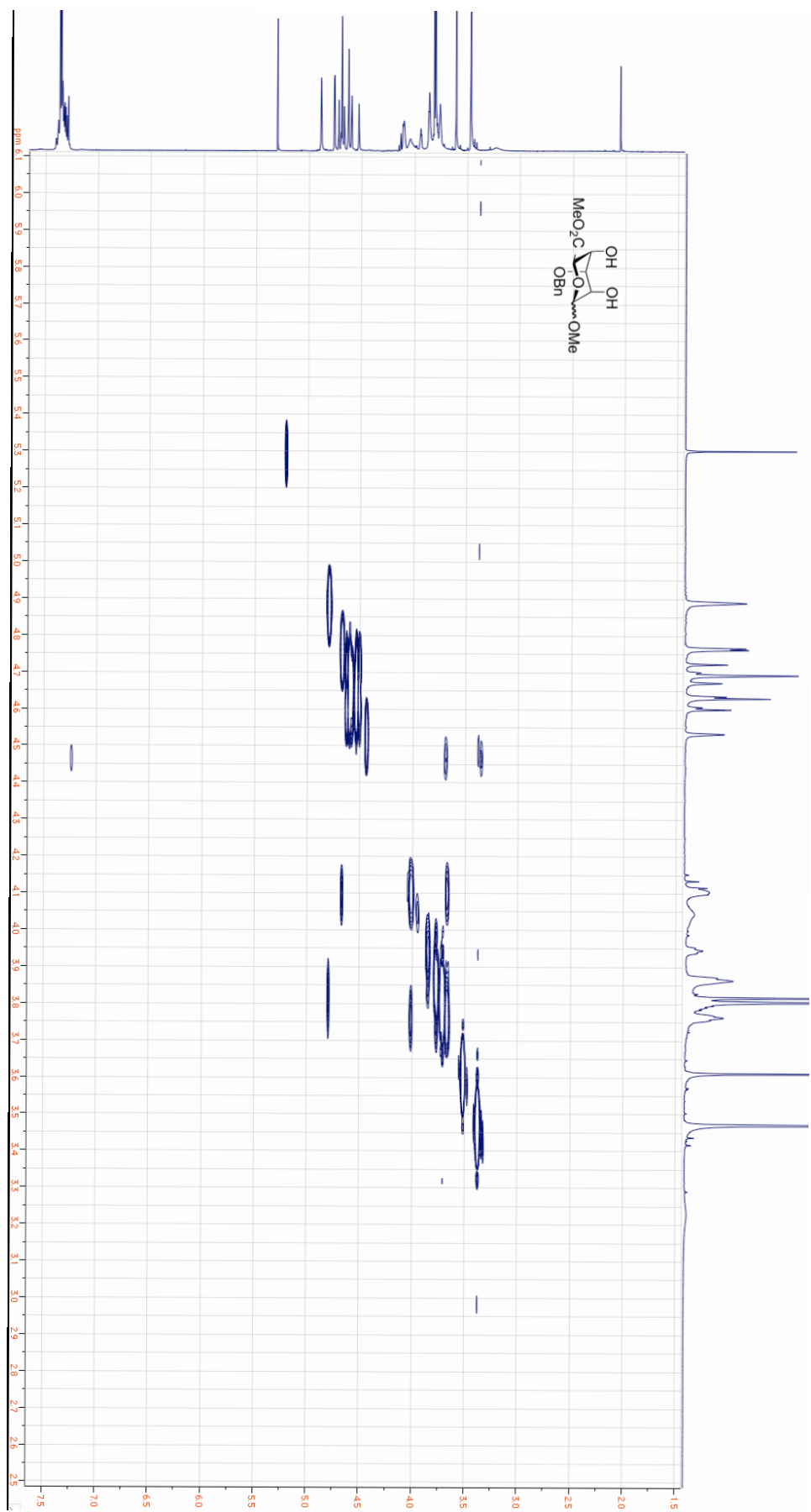
Methyl (2,5-di-*O*-acetyl-3-*O*-benzyl-1- α/β -methoxy-*L*-idofuranoside) uronate **4**



Methyl (2,4-dihydroxy-3-*O*-benzyl-1- α/β -methoxy-*L*-idopyranoside) uronate 5



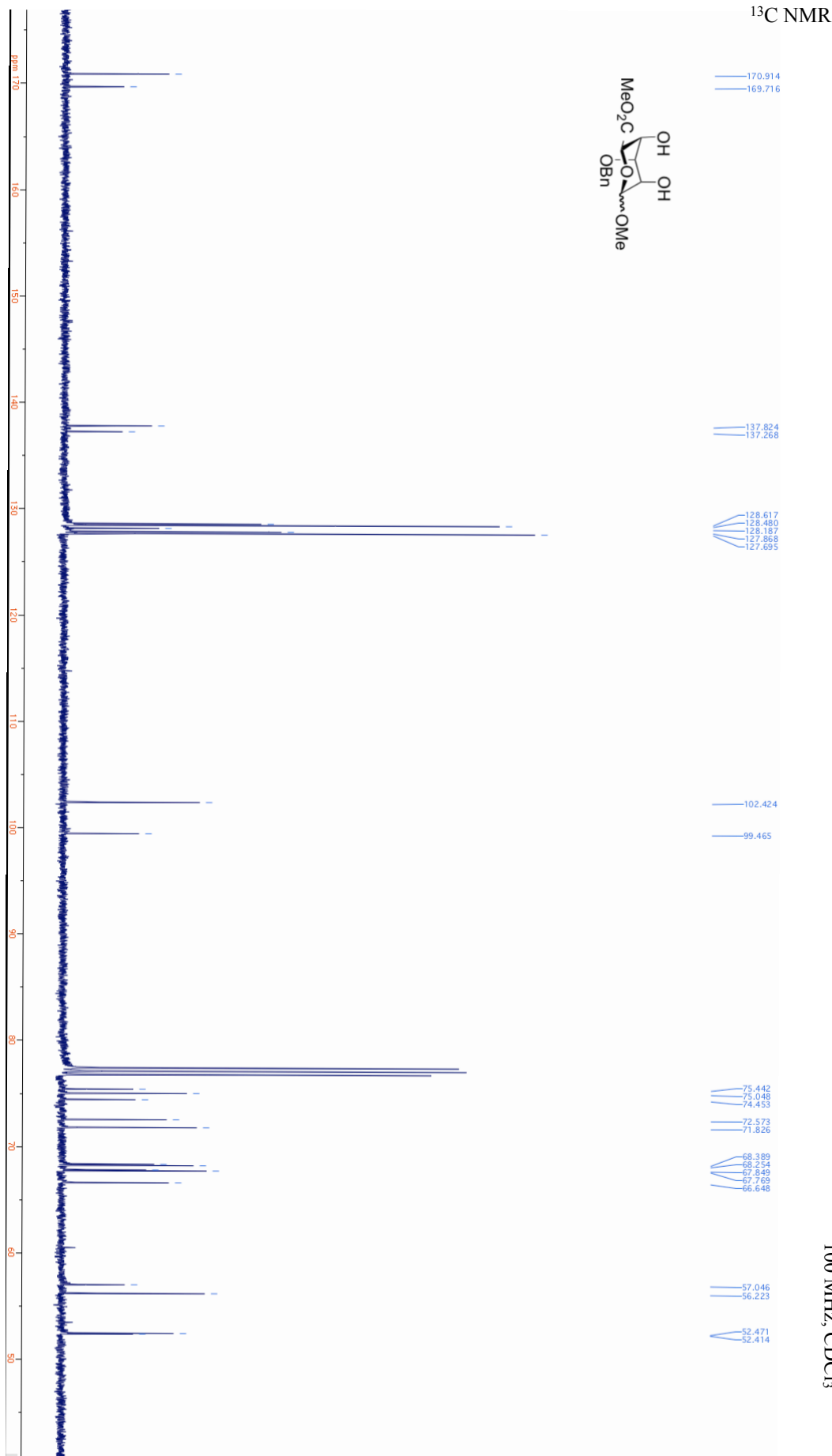
Methyl (2,4-dihydroxy-3-*O*-benzyl-1- α/β -methoxy-*L*-idopyranoside) uronate 5



COSY

400 MHz, CDCl₃

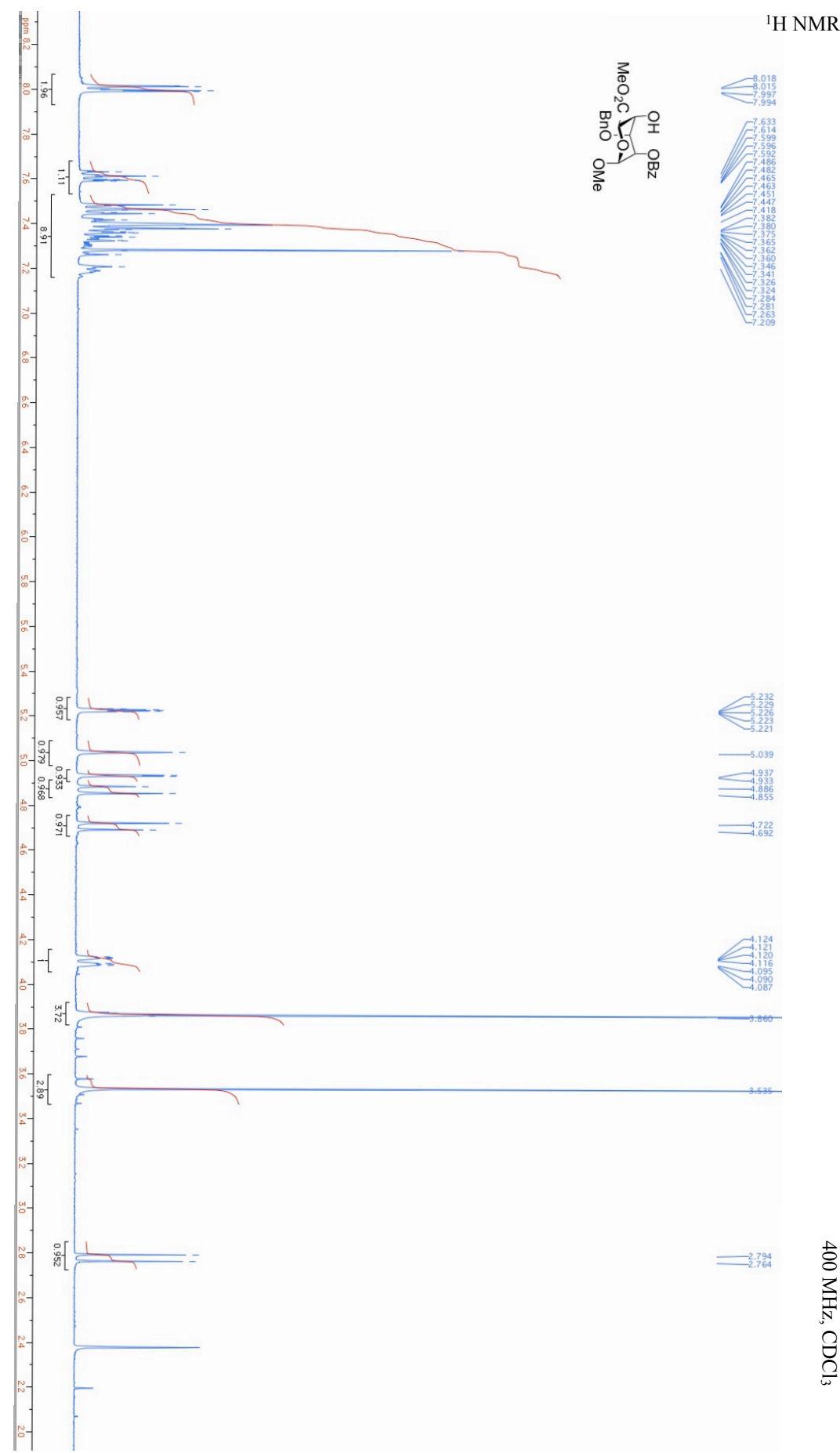
Methyl (2,4-dihydroxy-3-*O*-benzyl-1- α / β -methoxy-*L*-idopyranoside) uronate 5



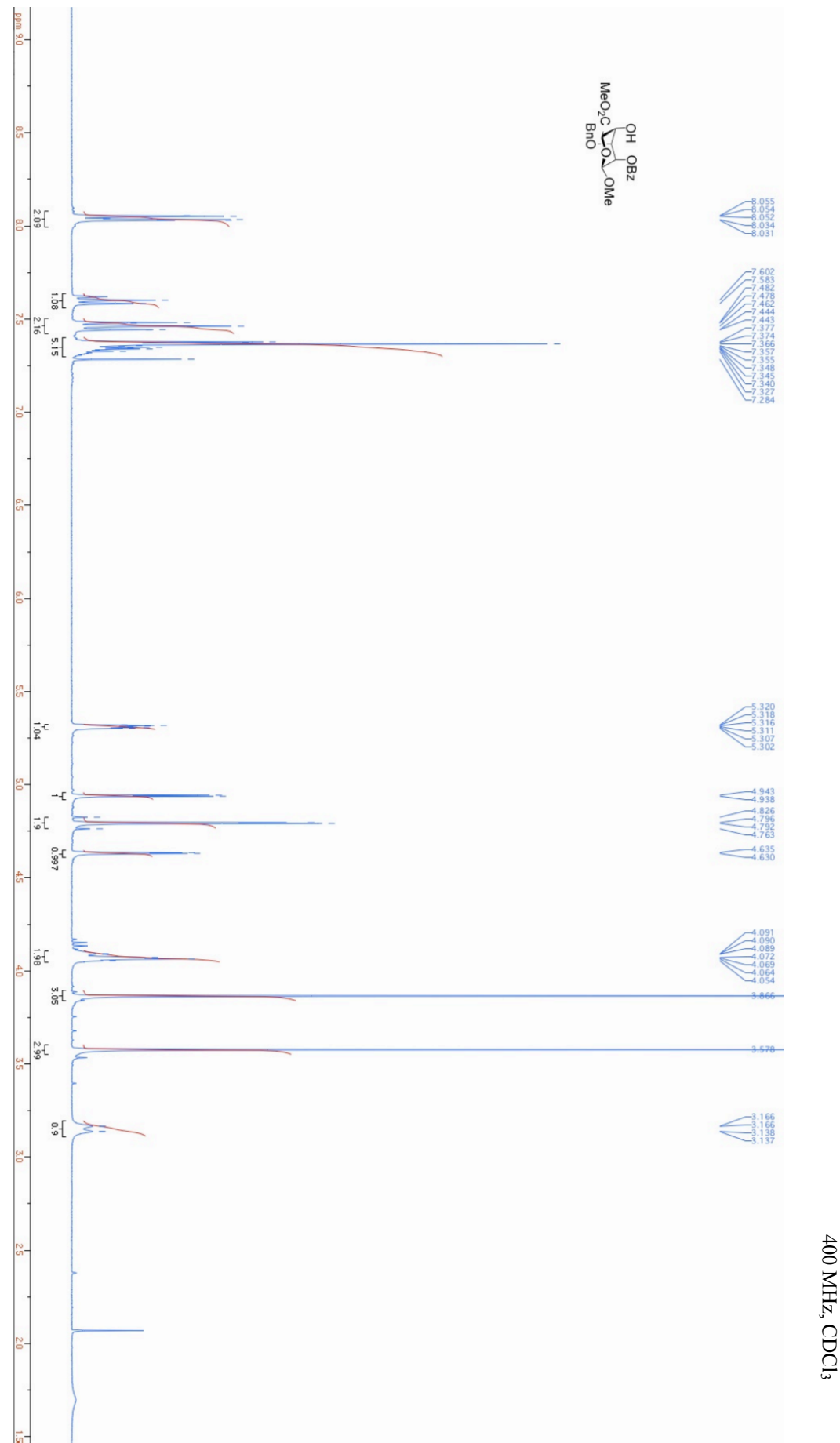
Methyl (2,4-dihydroxy-3-*O*-benzyl-1- α/β -methoxy-*L*-idopyranoside) uronate **5**



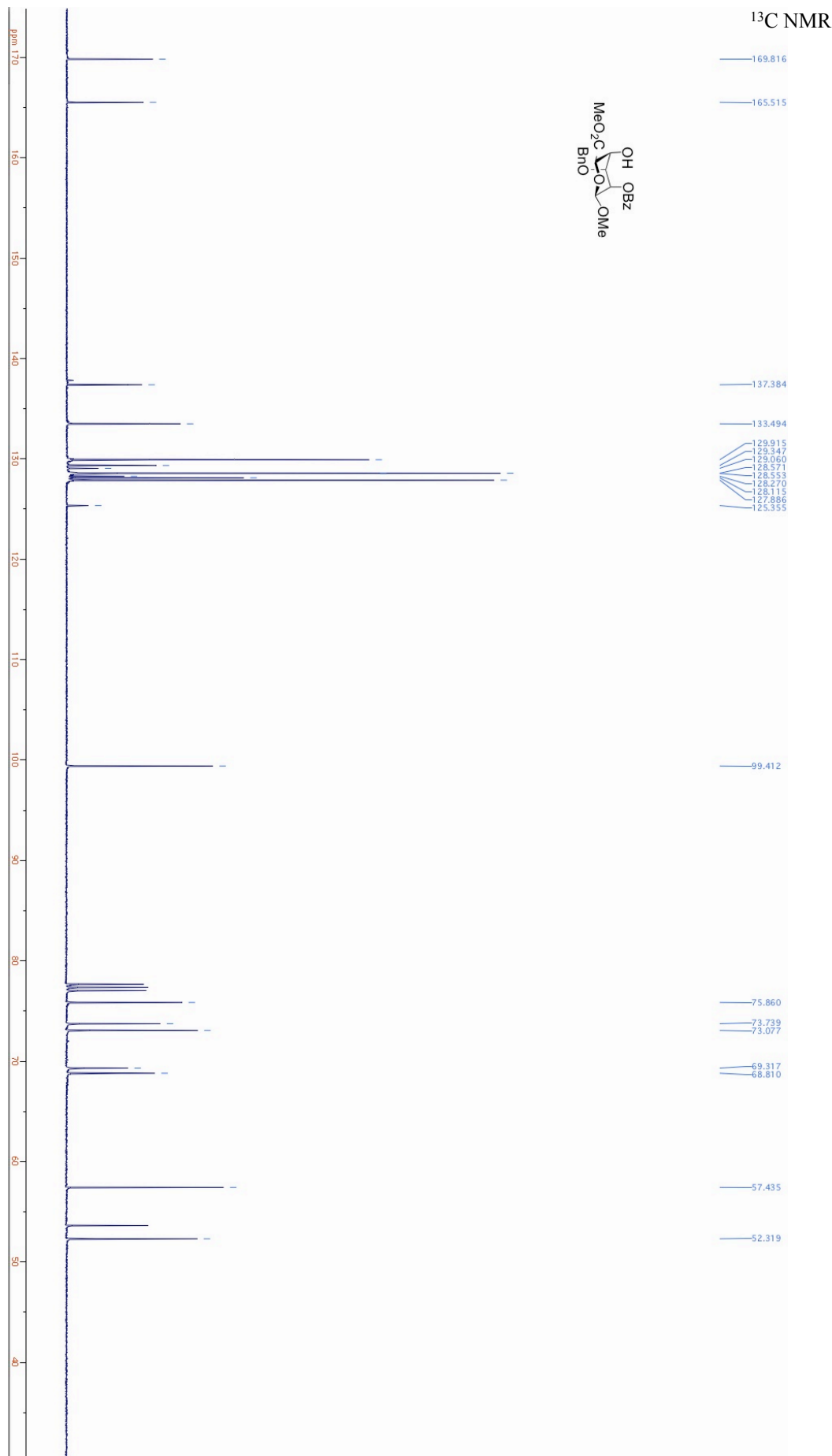
Methyl (2-*O*-benzoyl-4-hydroxy-3-*O*-benzyl-1- α -methoxy-*L*-idopyranoside) uronate 6



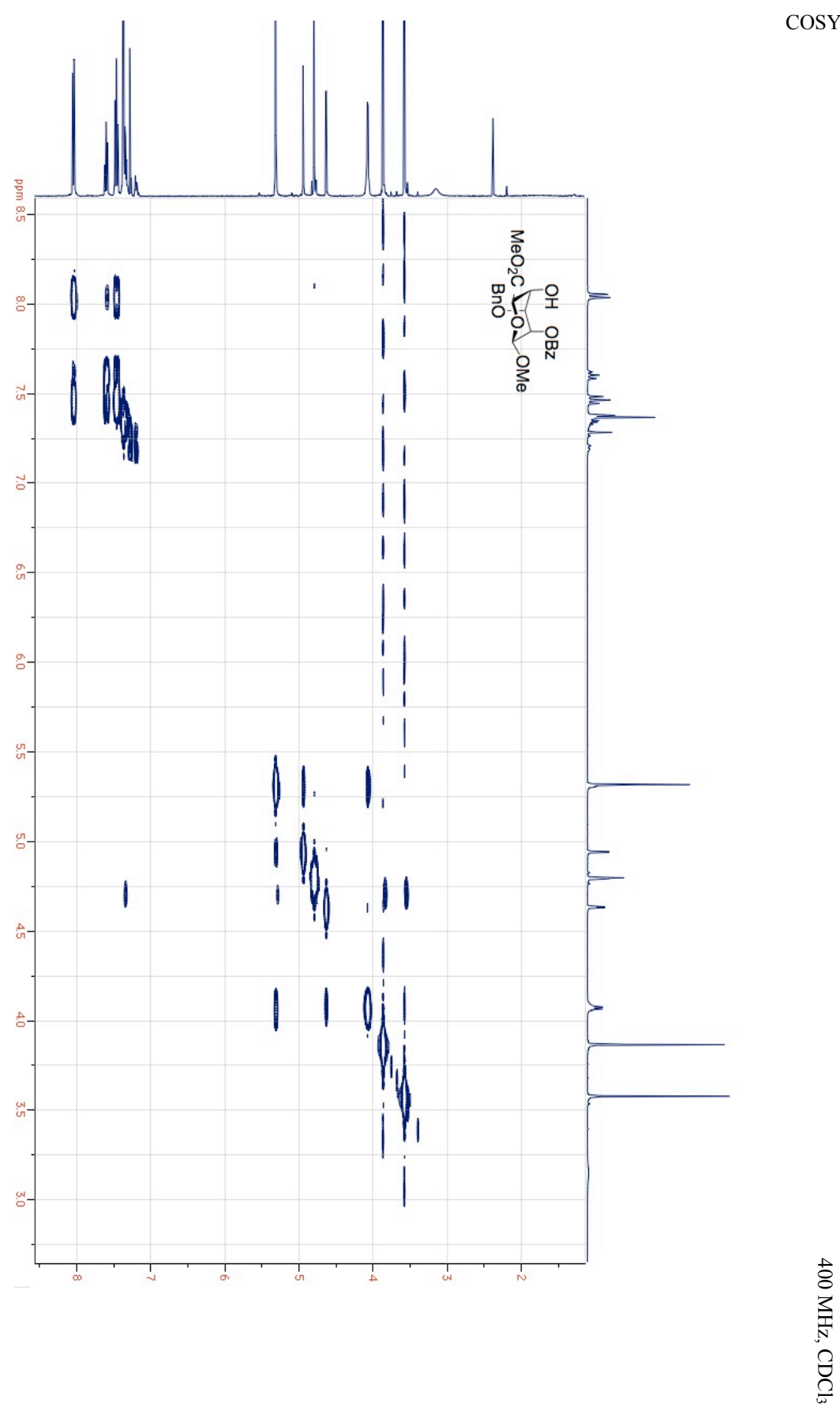
Methyl (2-*O*-benzoyl-4-hydroxy-3-*O*-benzyl-1-β-methoxy-*L*-idopyranoside) uronate 6



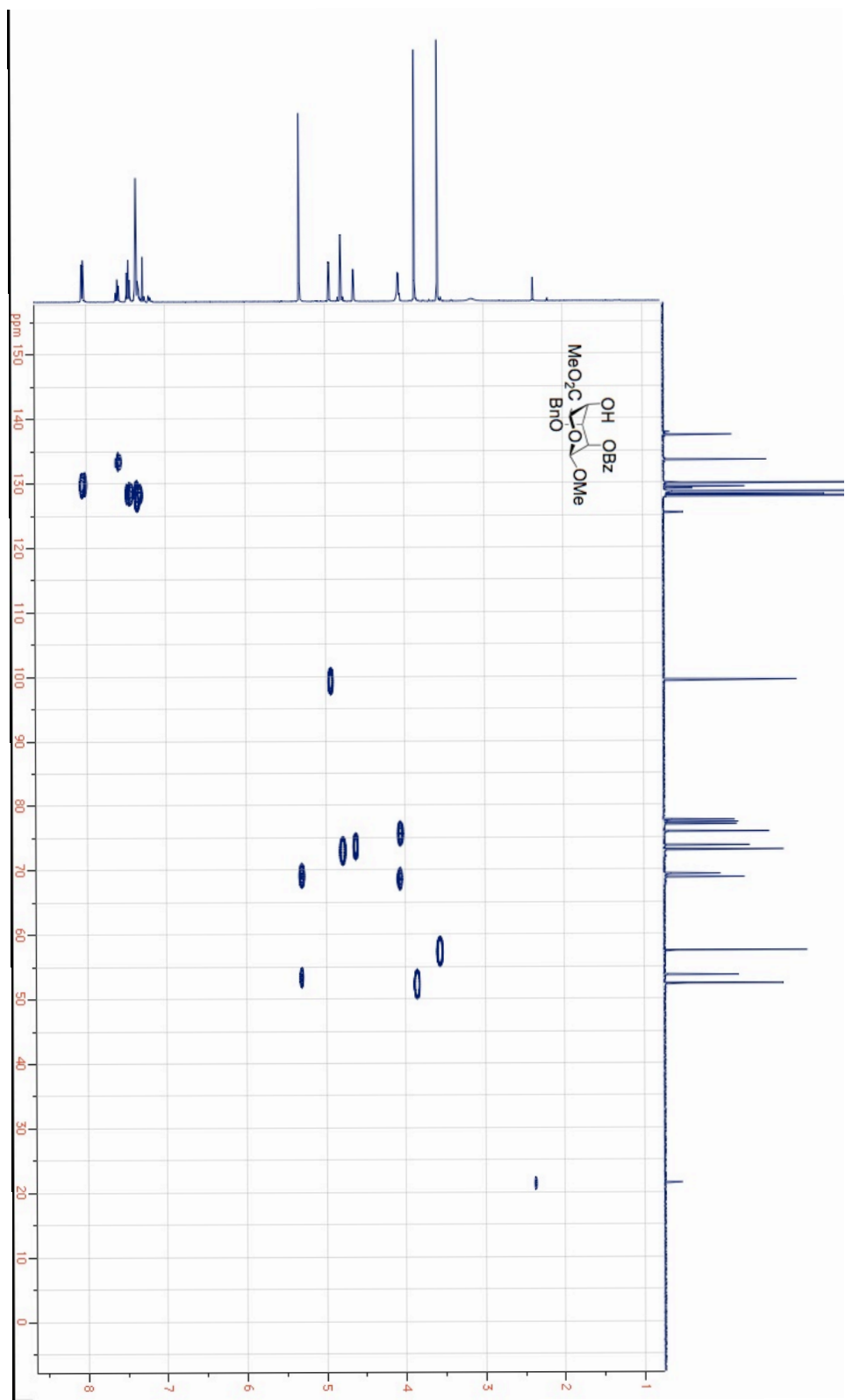
Methyl (2-*O*-benzoyl-4-hydroxy-3-*O*-benzyl-1-β-methoxy-*L*-idopyranoside) uronate 6



Methyl (2-*O*-benzoyl-4-hydroxy-3-*O*-benzyl-1-β-methoxy-*L*-idopyranoside) uronate 6



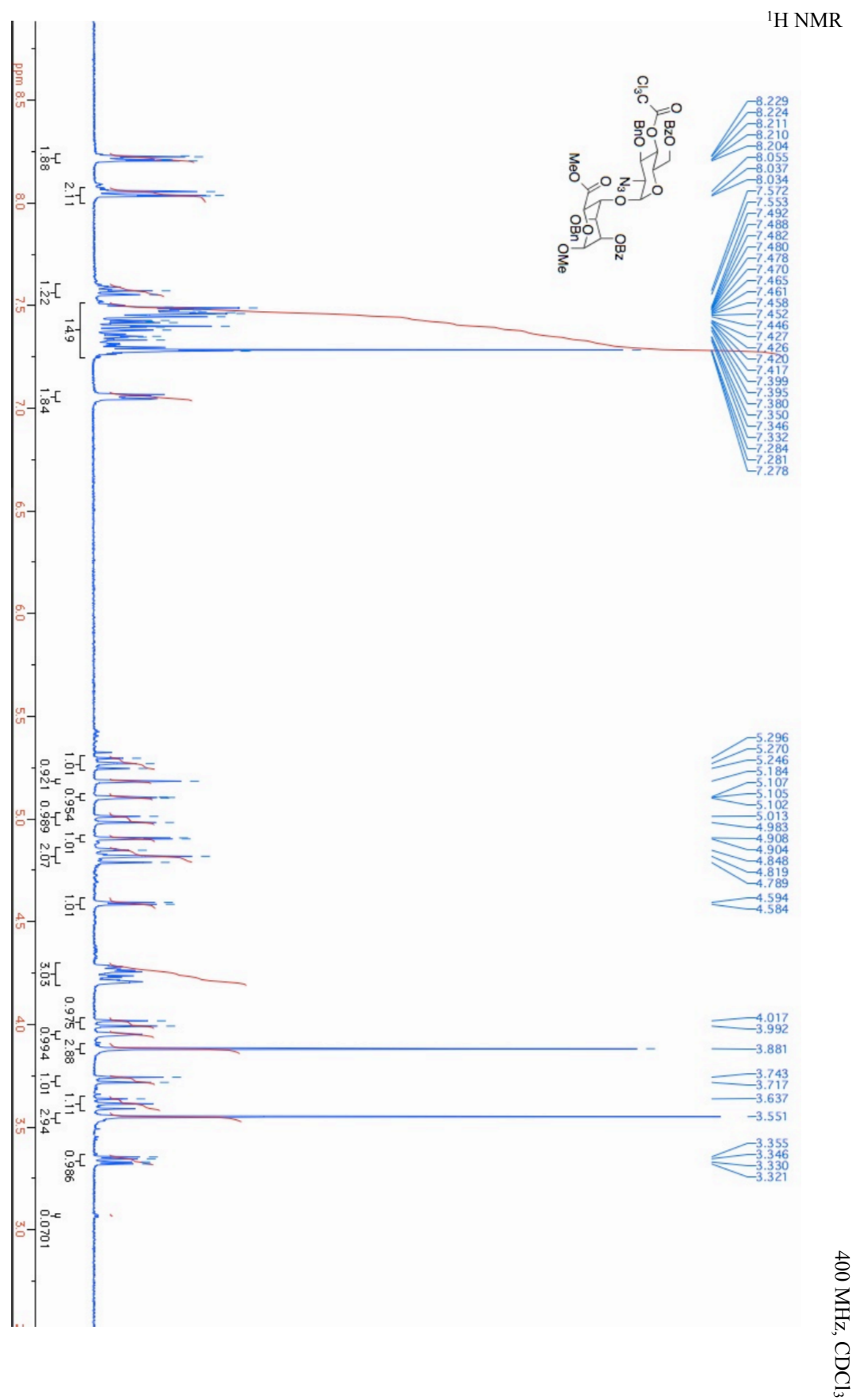
Methyl (2-*O*-benzoyl-4-hydroxy-3-*O*-benzyl-1-β-methoxy-*L*-idopyranoside) uronate 6



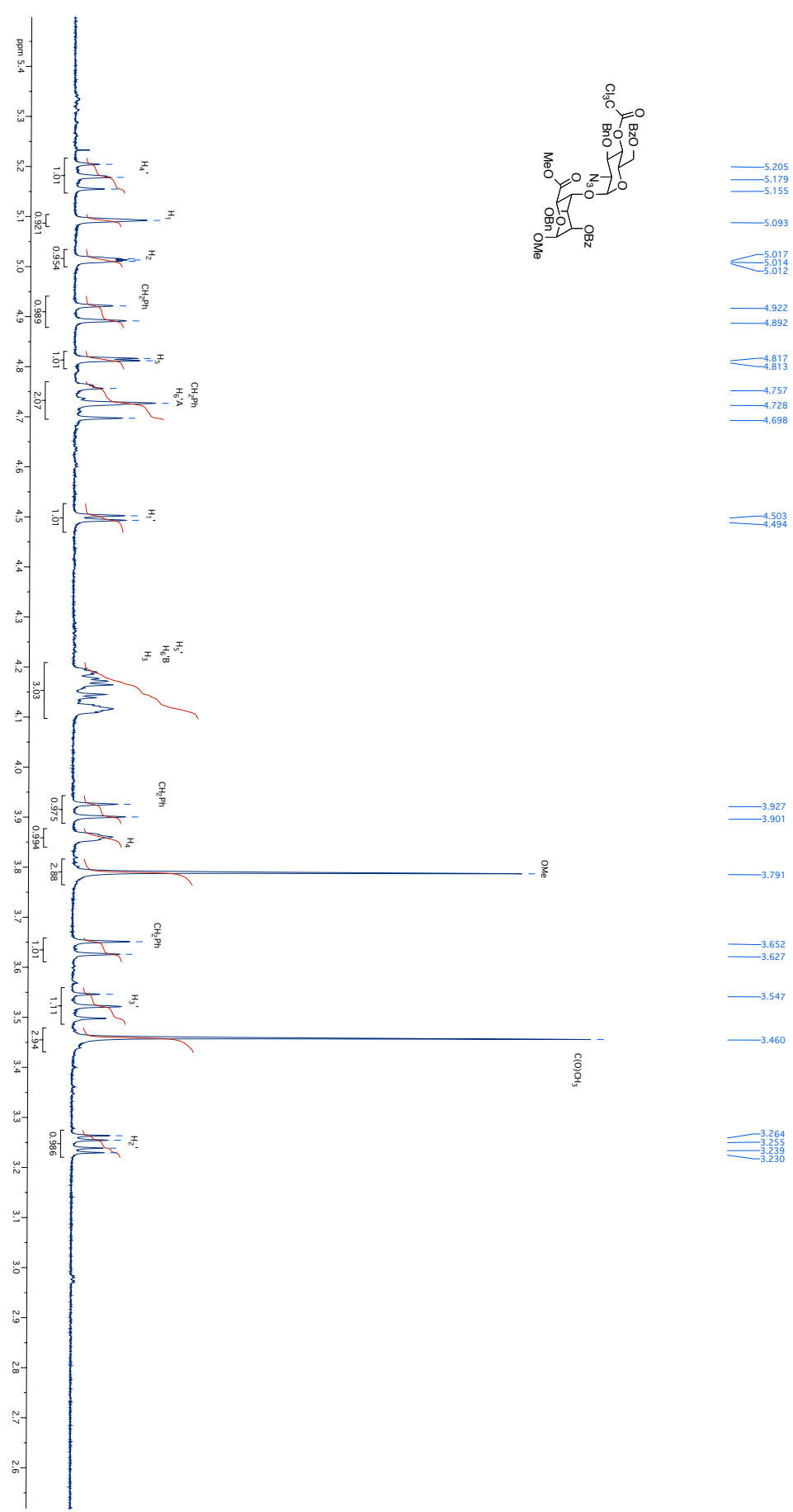
HMQC

400 MHz, CDCl₃

Methyl [Methyl-4-*O*-(2-azido-3-*O*-benzyl-6-*O*-benzoyl-4-*O*-trichloroacetyl- α -D-glucopyranosyl)-2-*O*-benzoyl-3-*O*-benzyl-1-*O*-methoxy- α -L-ido-pyranoside] uronate 8

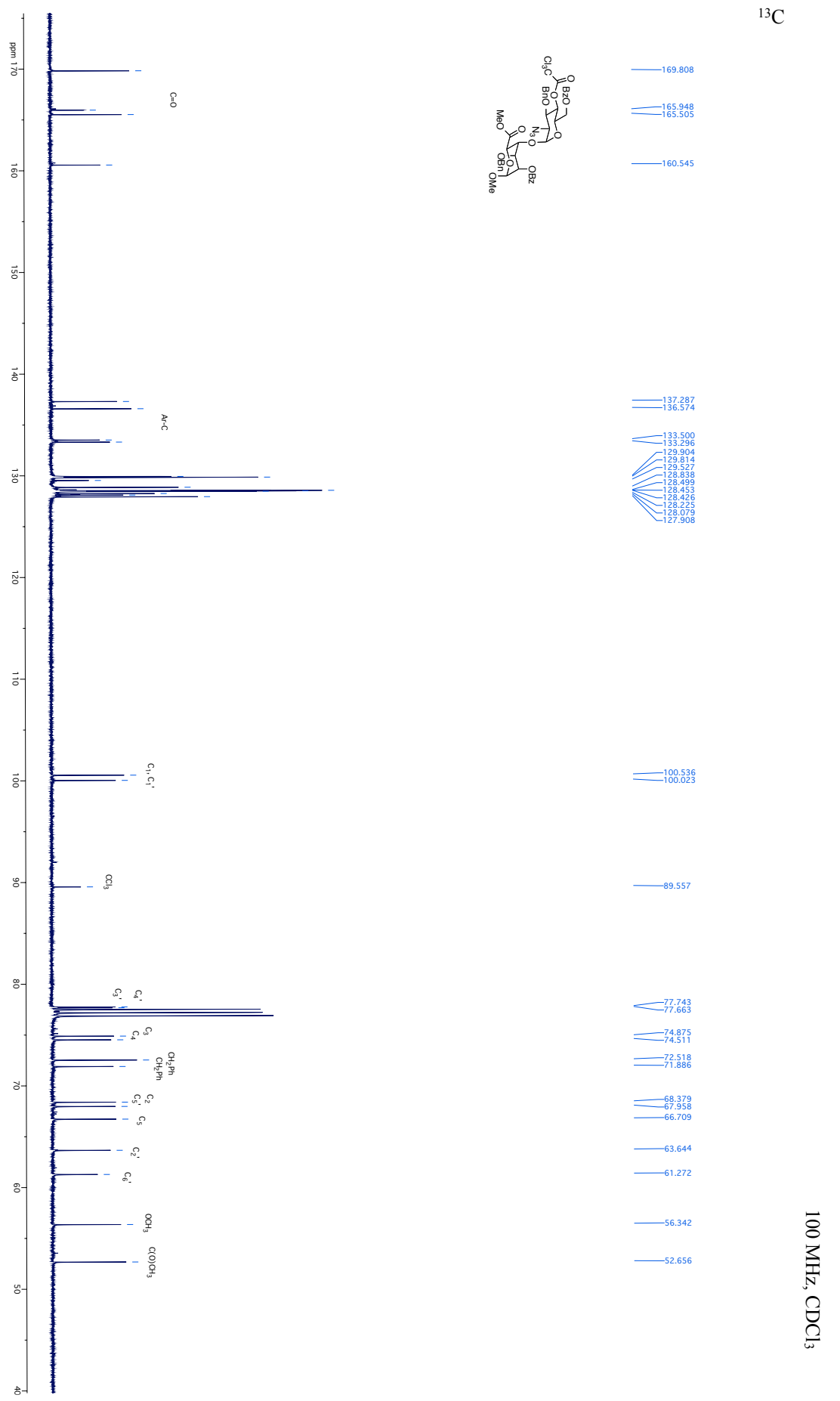


¹H expansion of 8

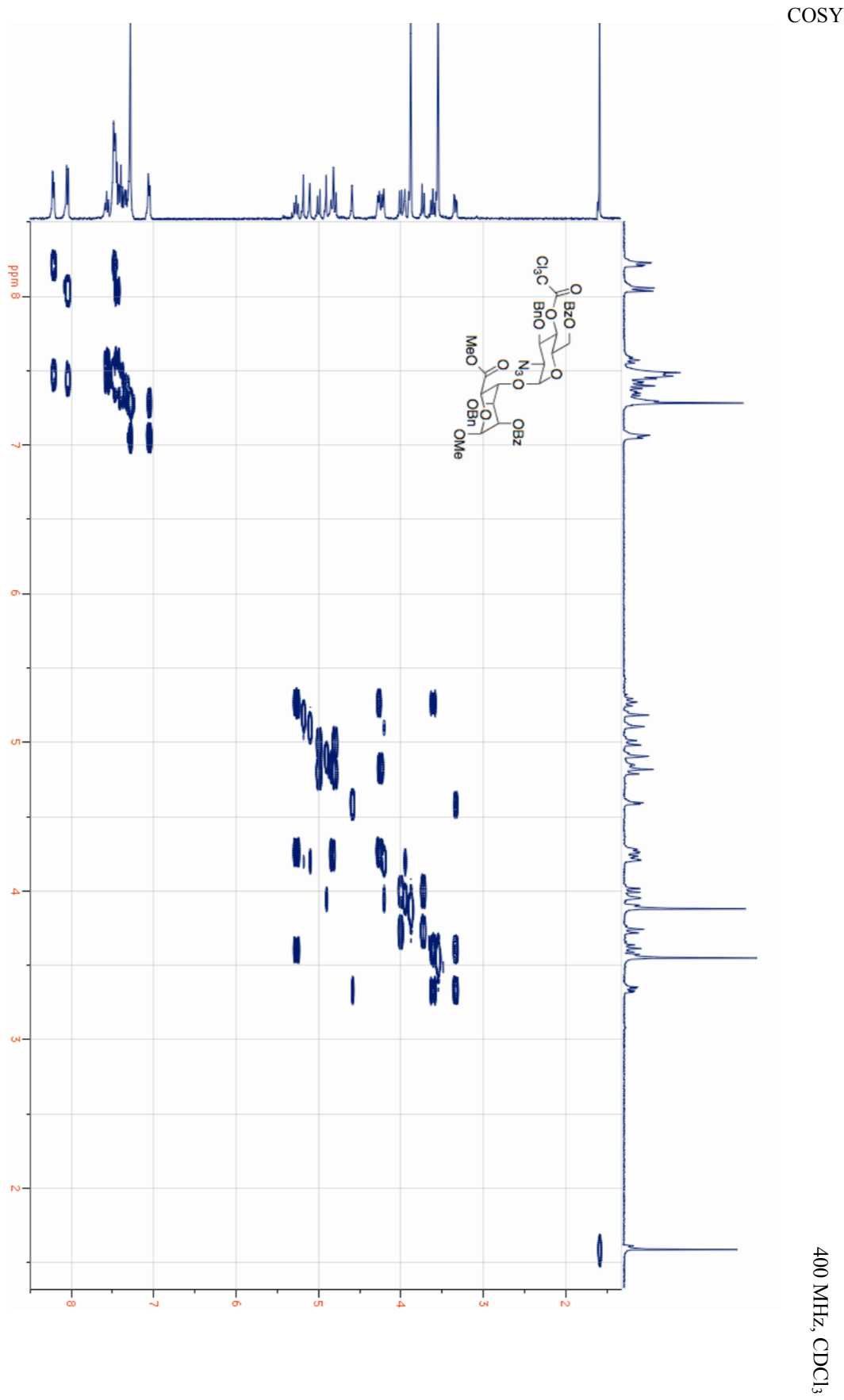


400 MHz, CDCl₃

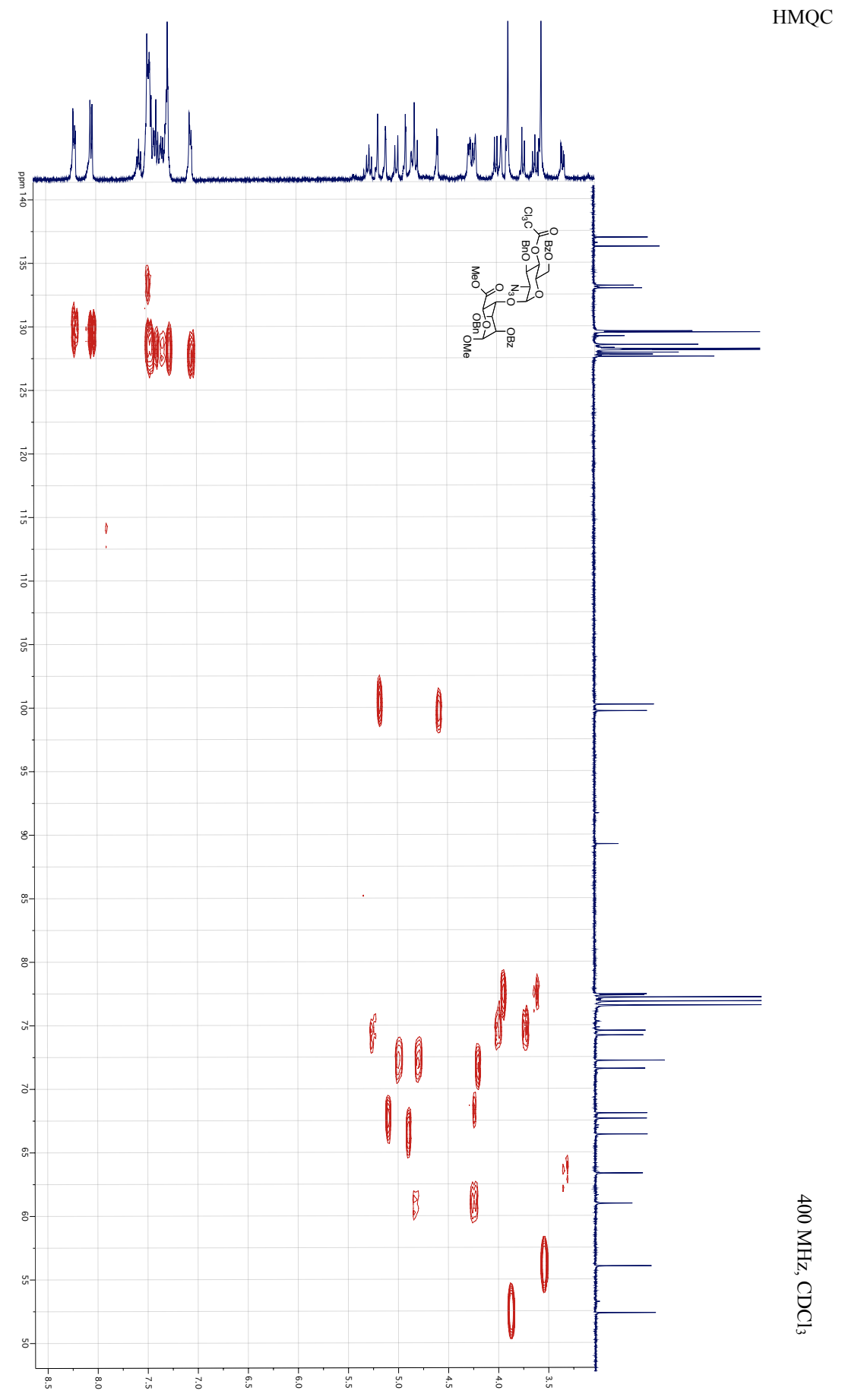
Methyl [Methyl-4-*O*-(2-azido-3-*O*-benzyl-6-*O*-benzoyl-4-*O*-trichloroacetyl- α -D-glucopyranosyl)-2-*O*-benzoyl-3-*O*-benzyl-1-*O*-methoxy- α -L-ido-pyranoside] uronate 8



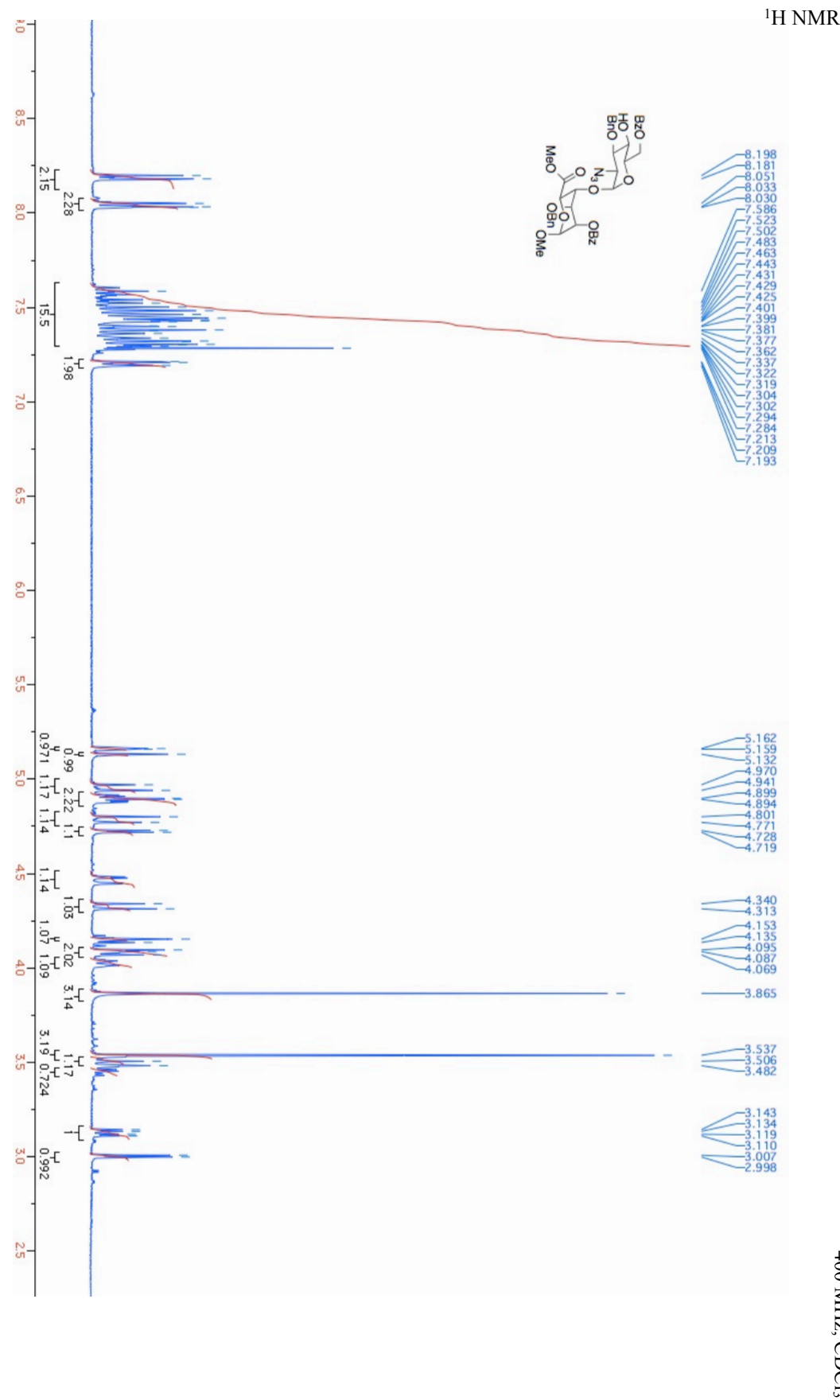
Methyl [Methyl-4-*O*-(2-azido-3-*O*-benzyl-6-*O*-benzoyl-4-*O*-trichloroacetyl- α -D-glucopyranosyl)-2-*O*-benzoyl-3-*O*-benzyl-1-*O*-methoxy- α -L-ido-pyranoside] uronate 8



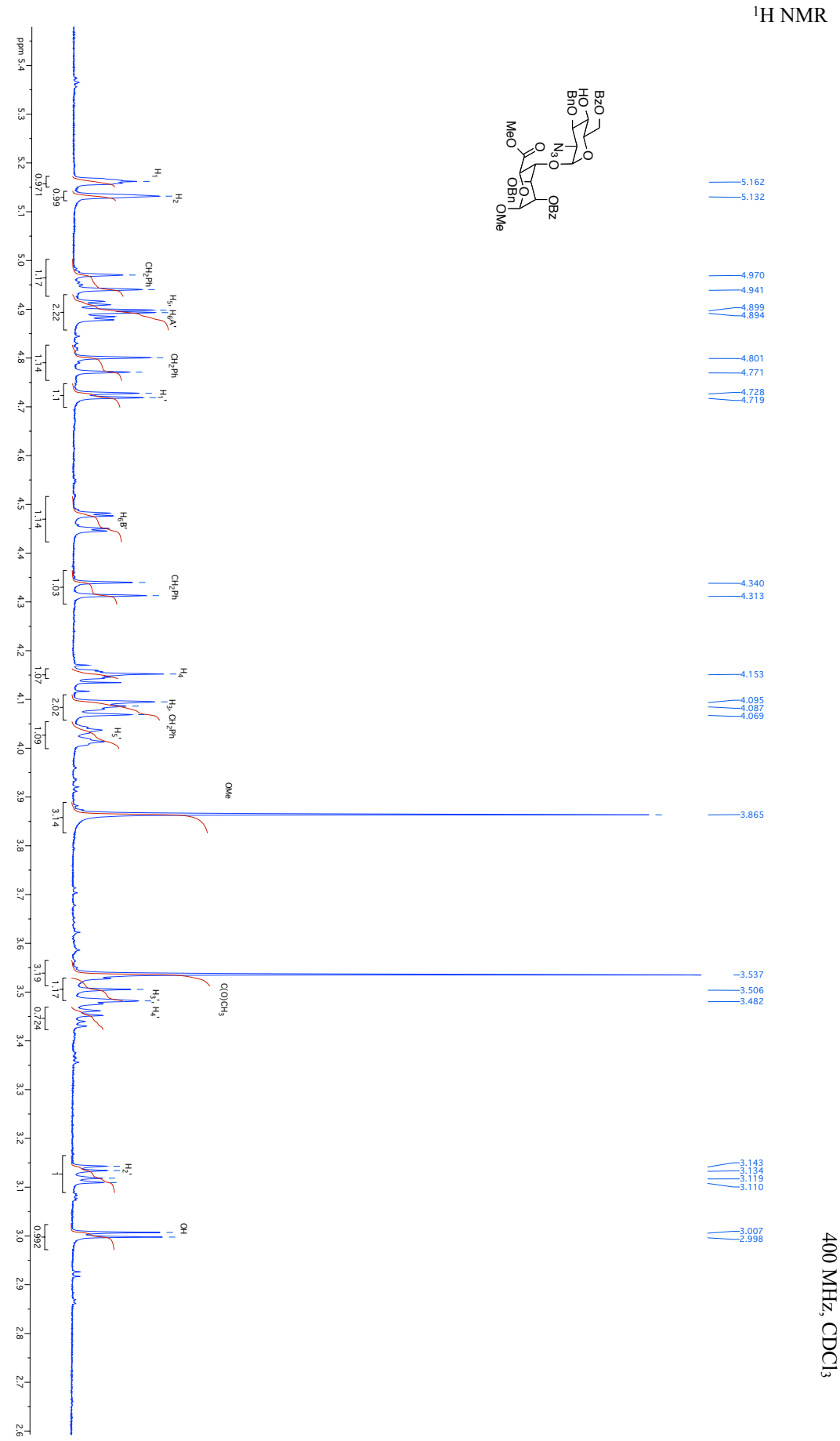
Methyl [Methyl-4-*O*-(2-azido-3-*O*-benzyl-6-*O*-benzoyl-4-*O*-trichloroacetyl- α -D-glucopyranosyl)-2-*O*-benzoyl-3-*O*-benzyl-1-*O*-methoxy- α -L-ido-pyranoside] uronate 8



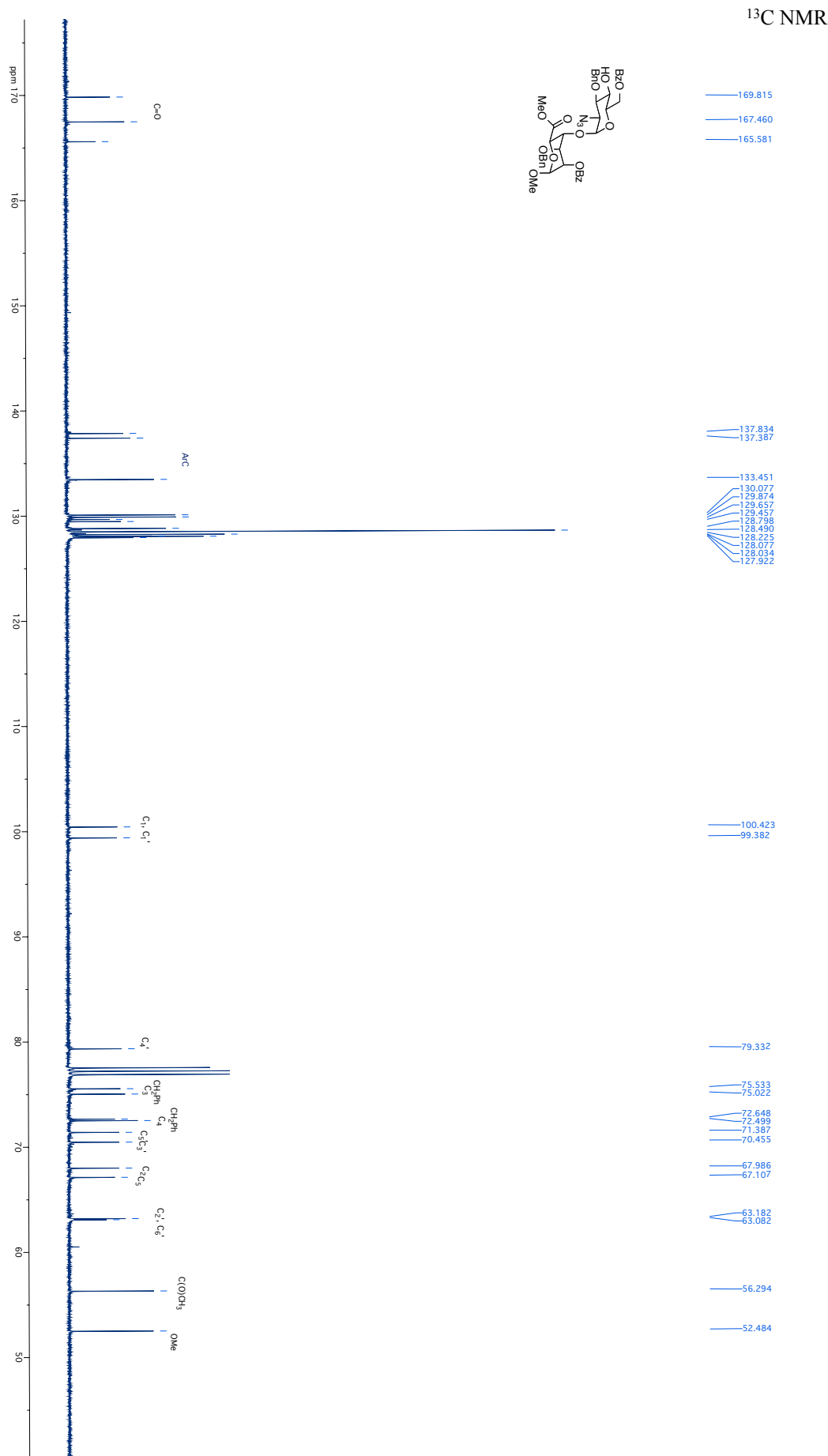
Methyl [Methyl-4-*O*-(2-azido-3-*O*-benzyl-6-*O*-benzoyl-4-hydroxy- α -D-glucopyranosyl)-2-*O*-benzoyl-3-*O*-benzyl-1-*O*-methoxy- α -L-ido-pyranoside] uronate 9



¹H expansion of 9



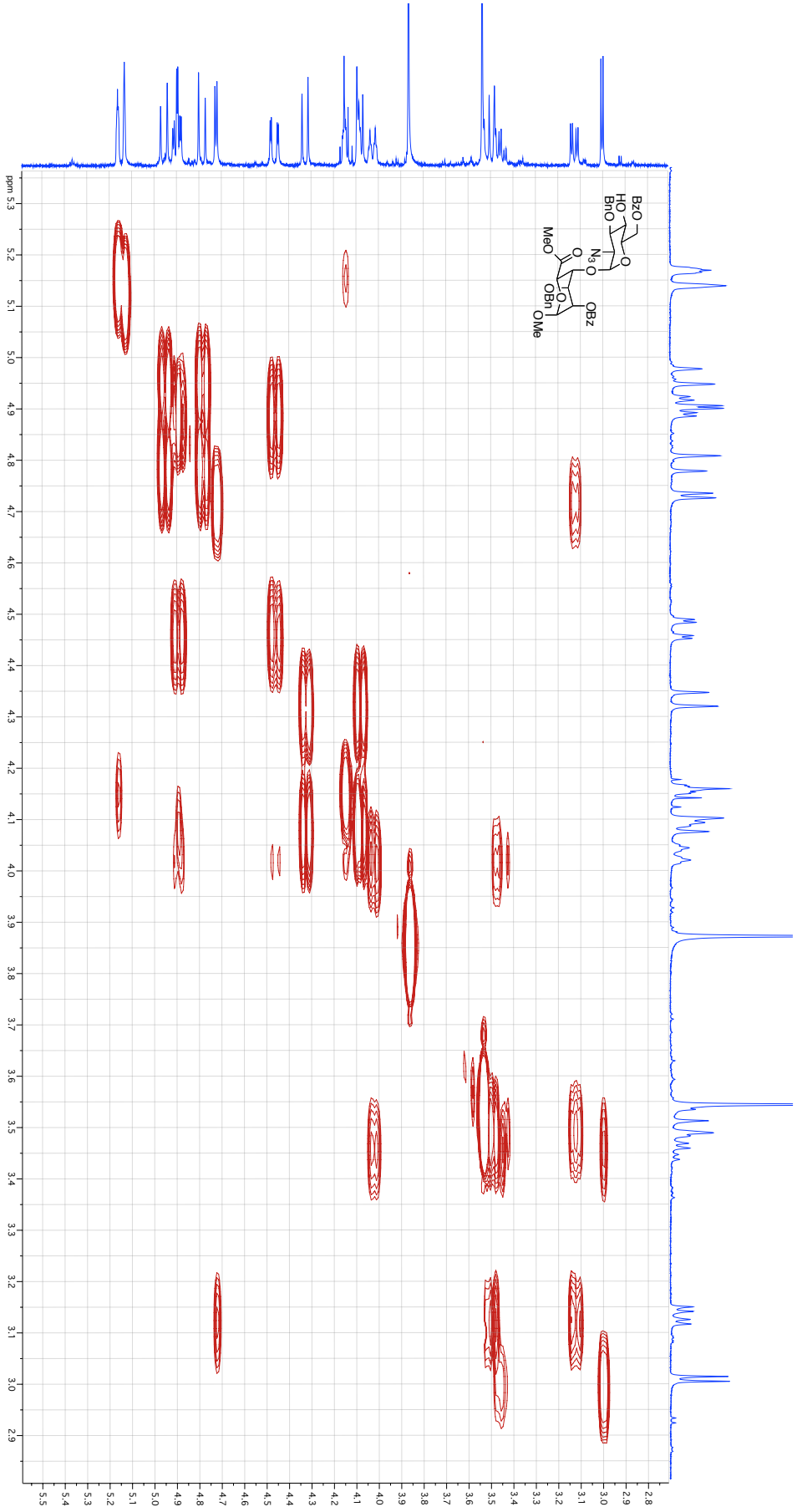
Methyl [Methyl-4-*O*-(2-azido-3-*O*-benzyl-6-*O*-benzoyl-4-hydroxy- α -D-glucopyranosyl)-2-*O*-benzoyl-3-*O*-benzyl-1-*O*-methoxy- α -L-ido-pyranoside] uronate 9



100 MHz, CDCl₃

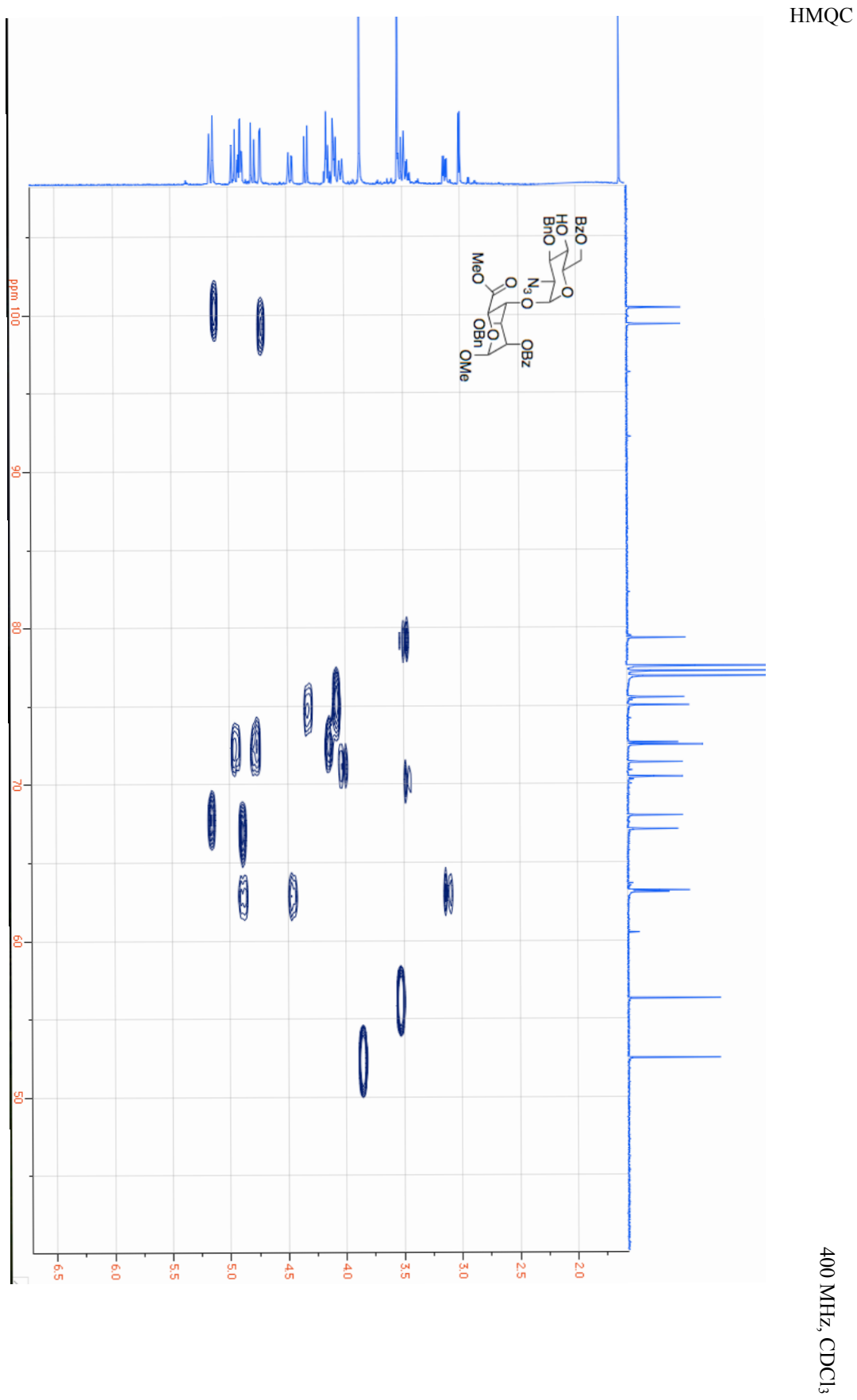
Methyl [Methyl-4-*O*-(2-azido-3-*O*-benzyl-6-*O*-benzoyl-4-hydroxy- α -D-glucopyranosyl)-2-*O*-benzoyl-3-*O*-benzyl-1-*O*-methoxy- α -L-ido-pyranoside] uronate 9

COSY

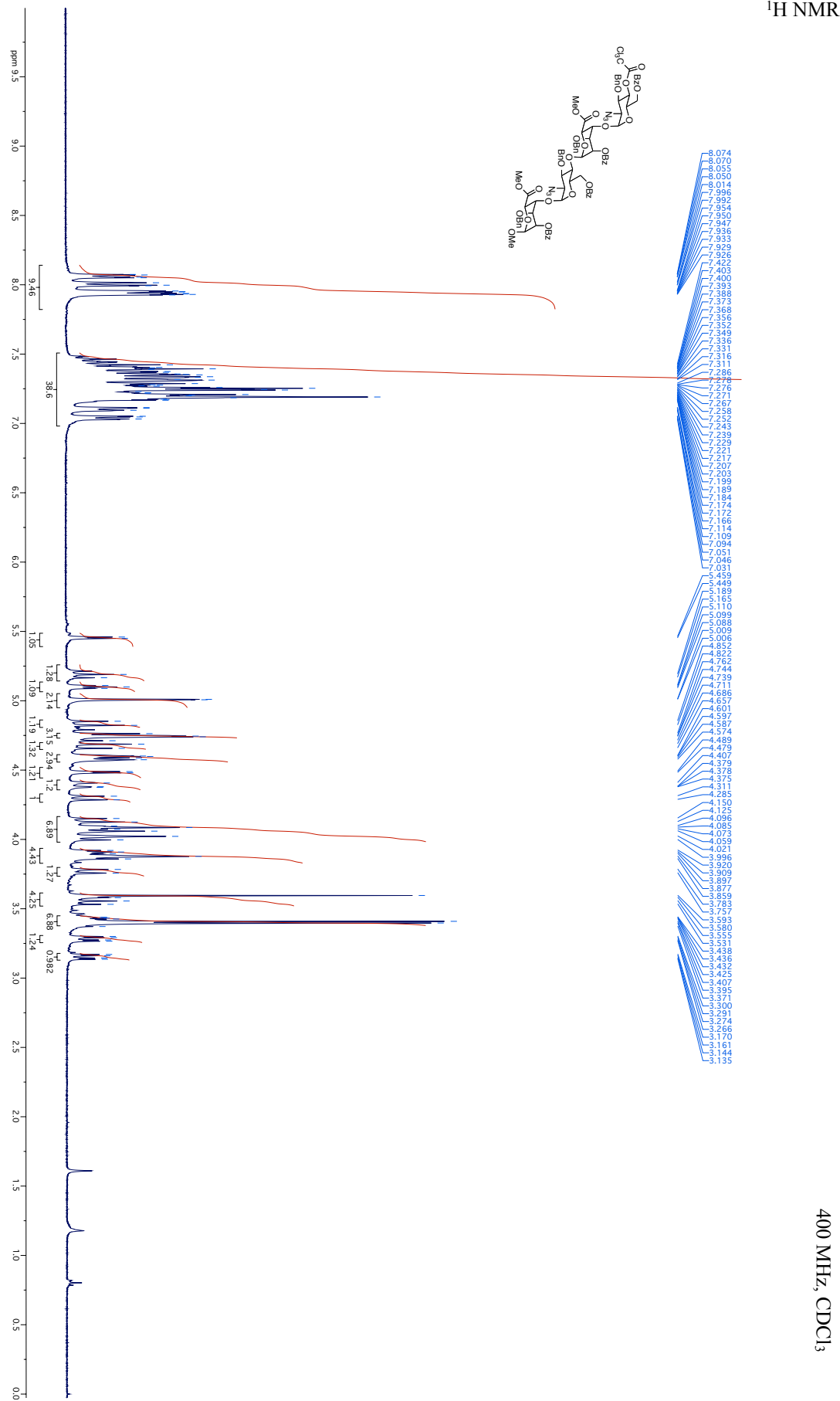


400 MHz, CDCl₃

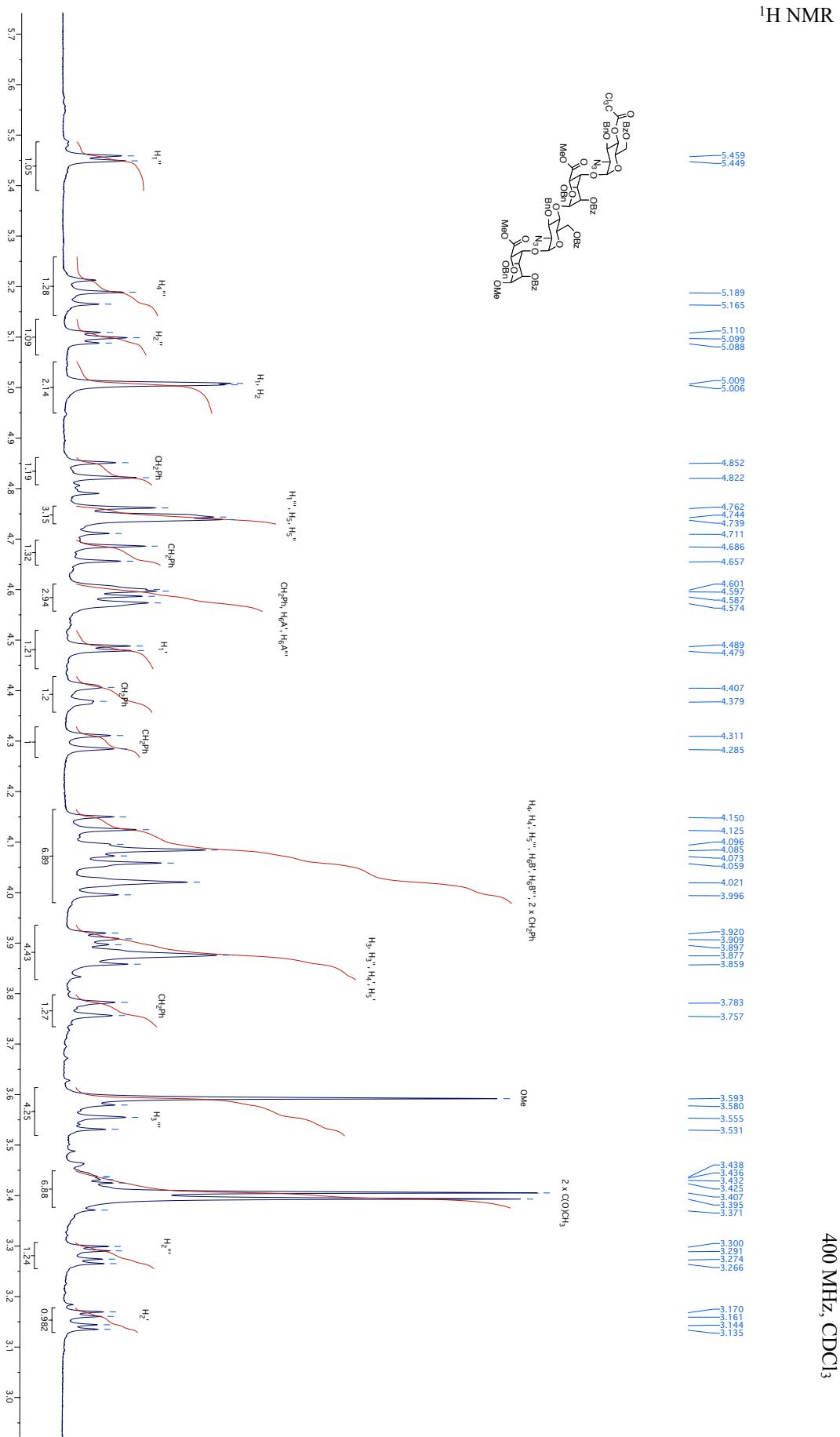
Methyl [Methyl-4-*O*-(2-azido-3-*O*-benzyl-6-*O*-benzoyl-4-hydroxy- α -D-glucopyranosyl)-2-*O*-benzoyl-3-*O*-benzyl-1-*O*-methoxy- α -L-ido-pyranoside] uronate 9



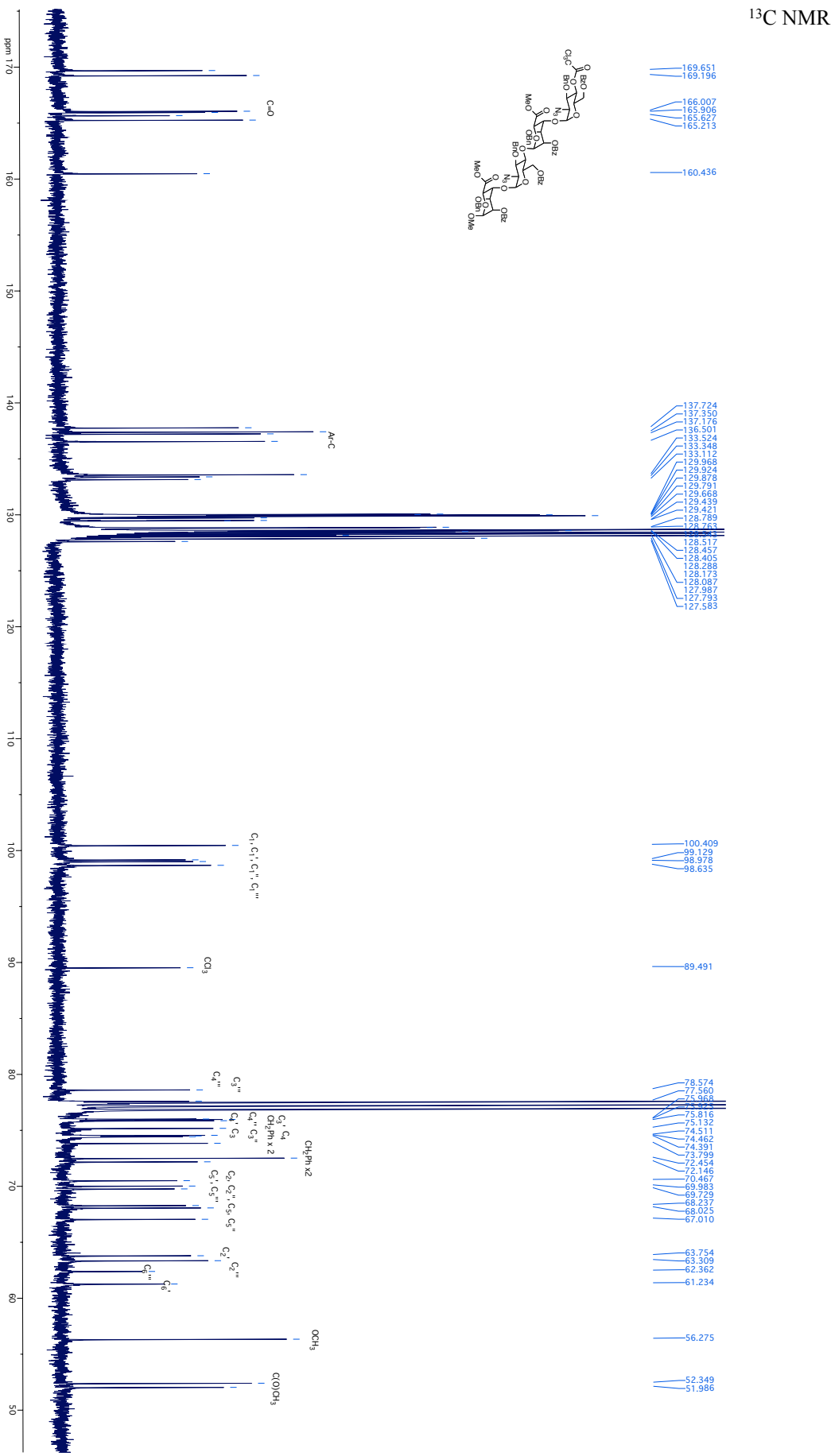
Tetrasaccharide 11



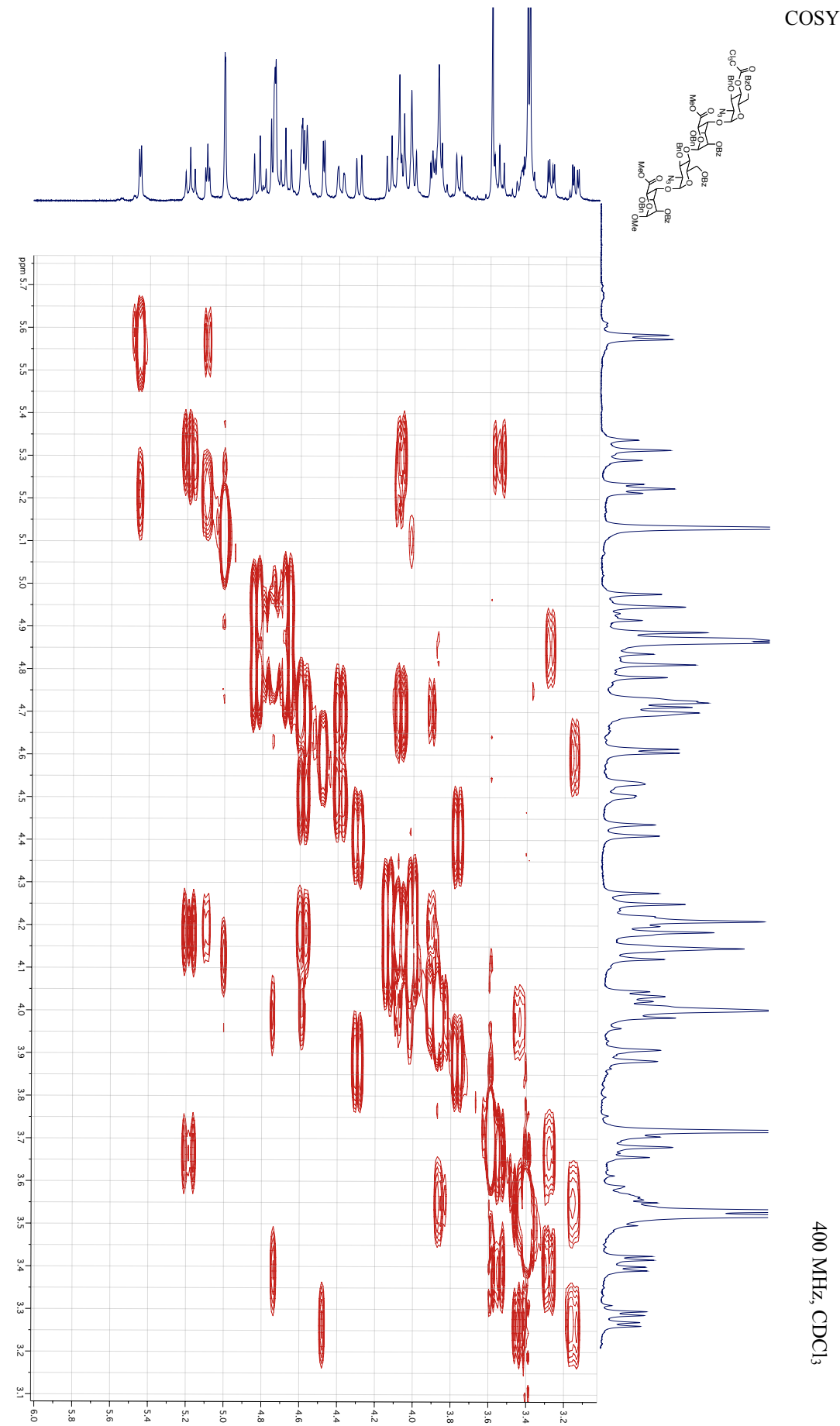
Tetrasaccharide 11 - ¹H Expansion



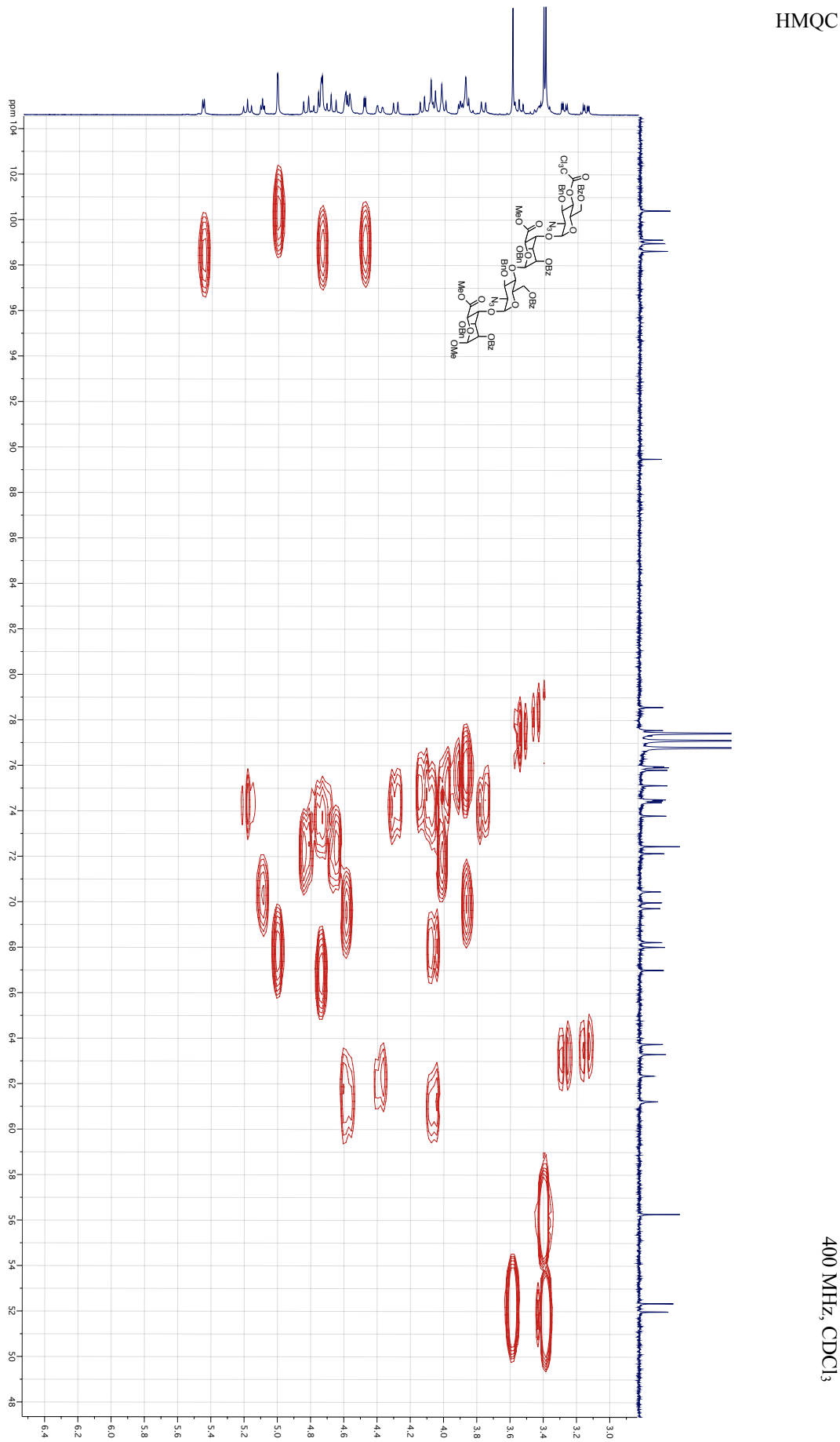
Tetrasaccharide 11



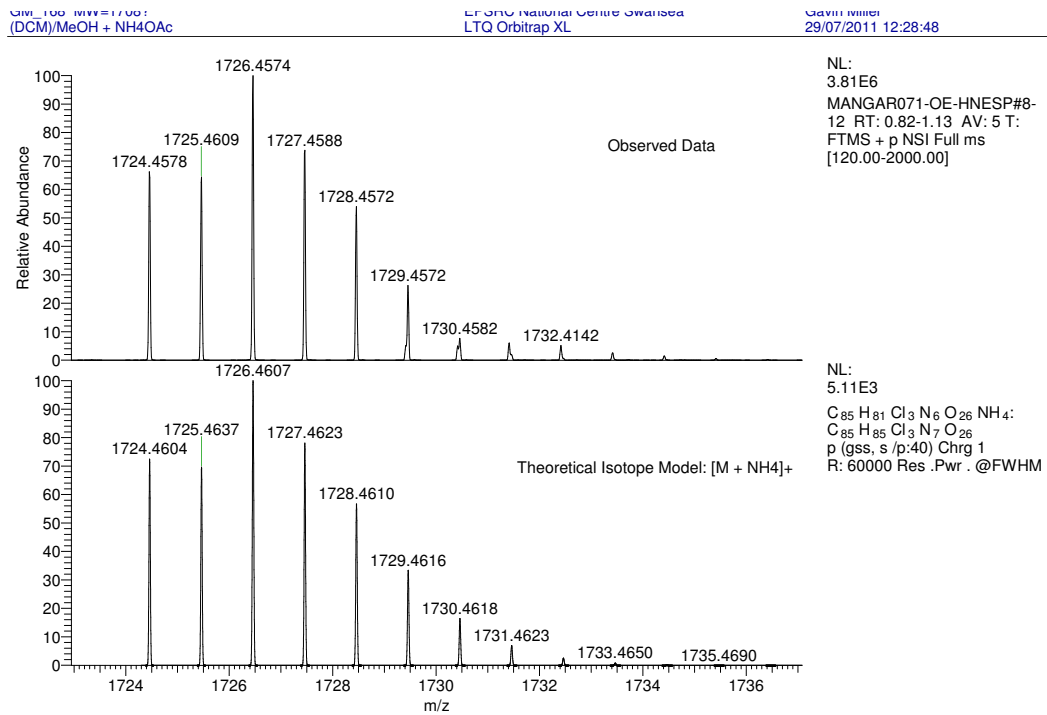
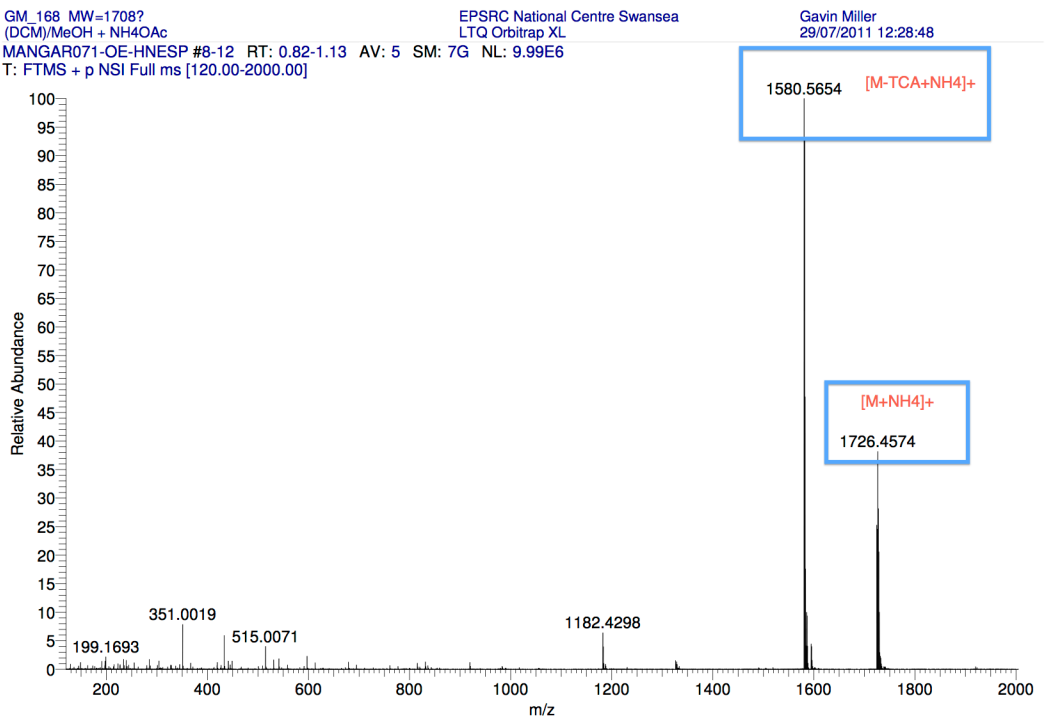
Tetrasaccharide 11



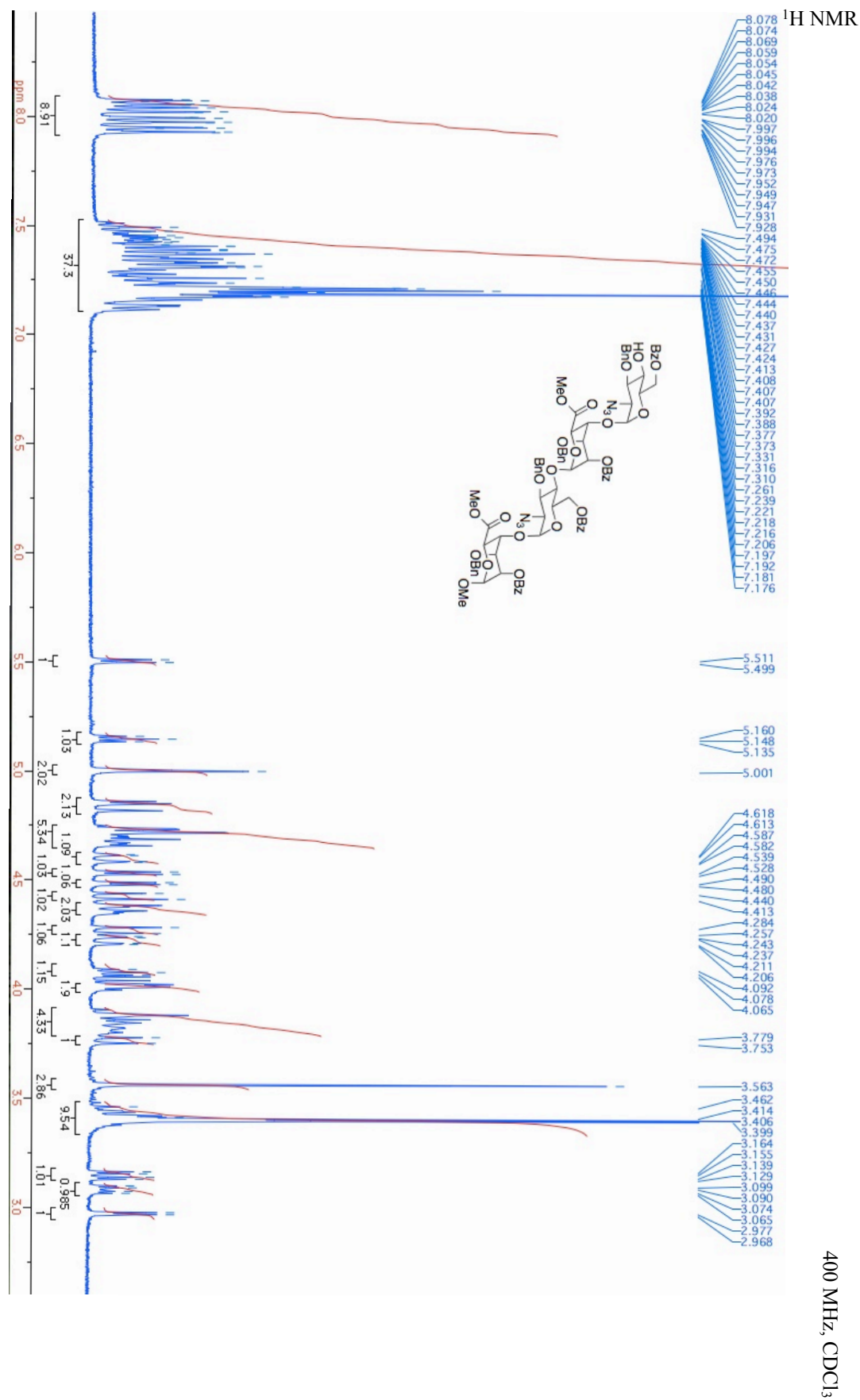
Tetrasaccharide 11



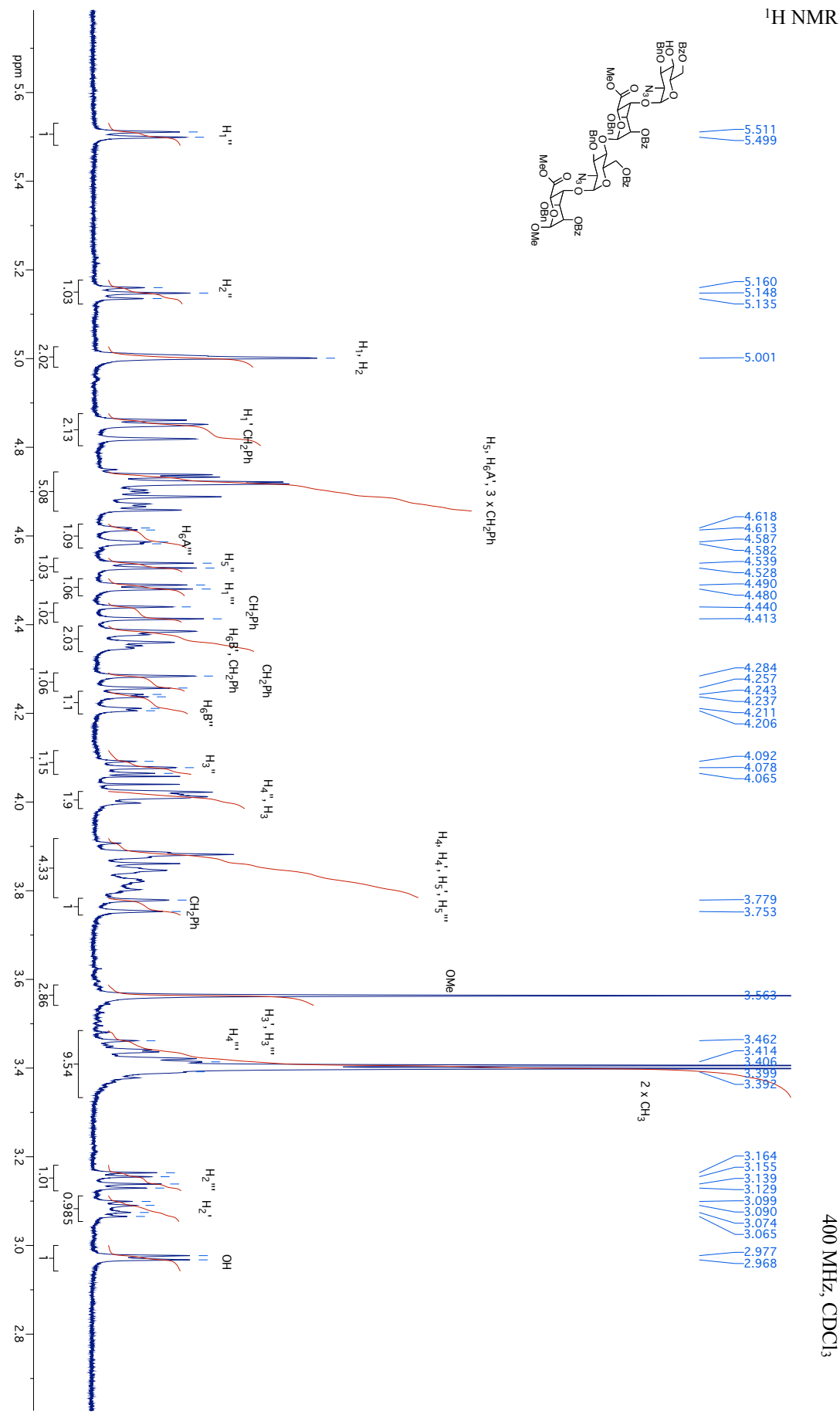
Tetrasaccharide 11 FTMS and Isotope Pattern



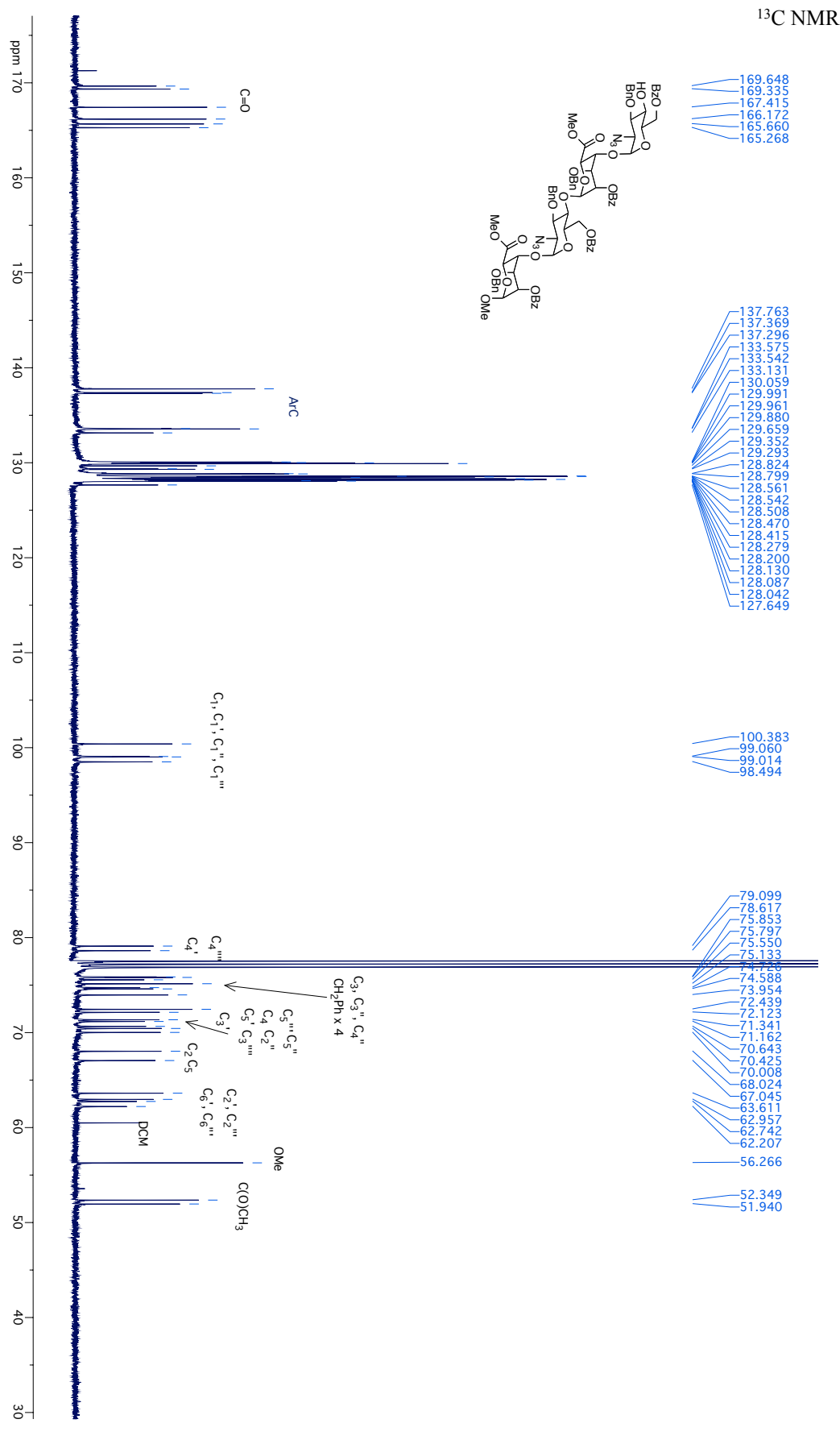
Tetrasaccharide 12



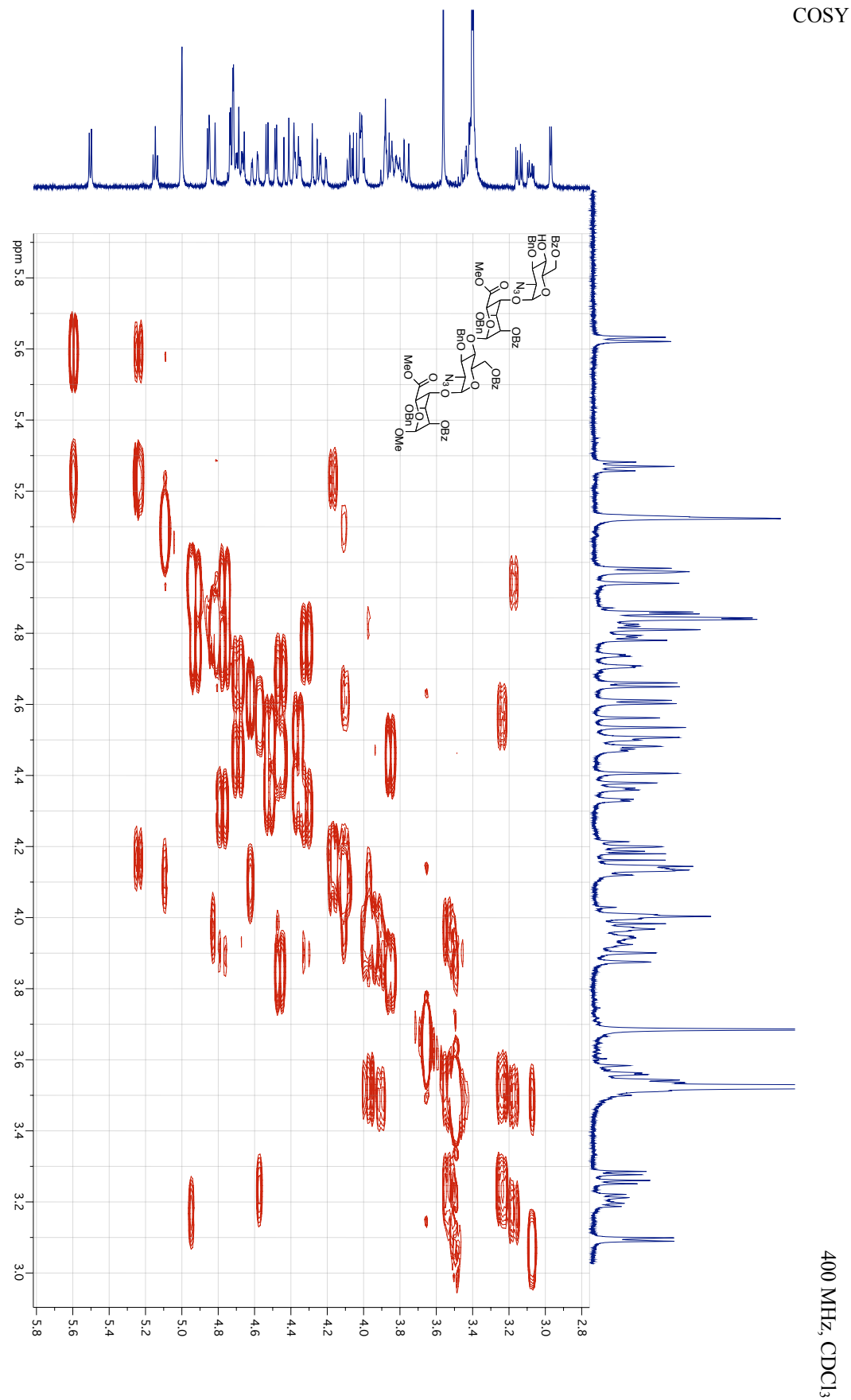
Tetrasaccharide 12 - ¹H Expansion



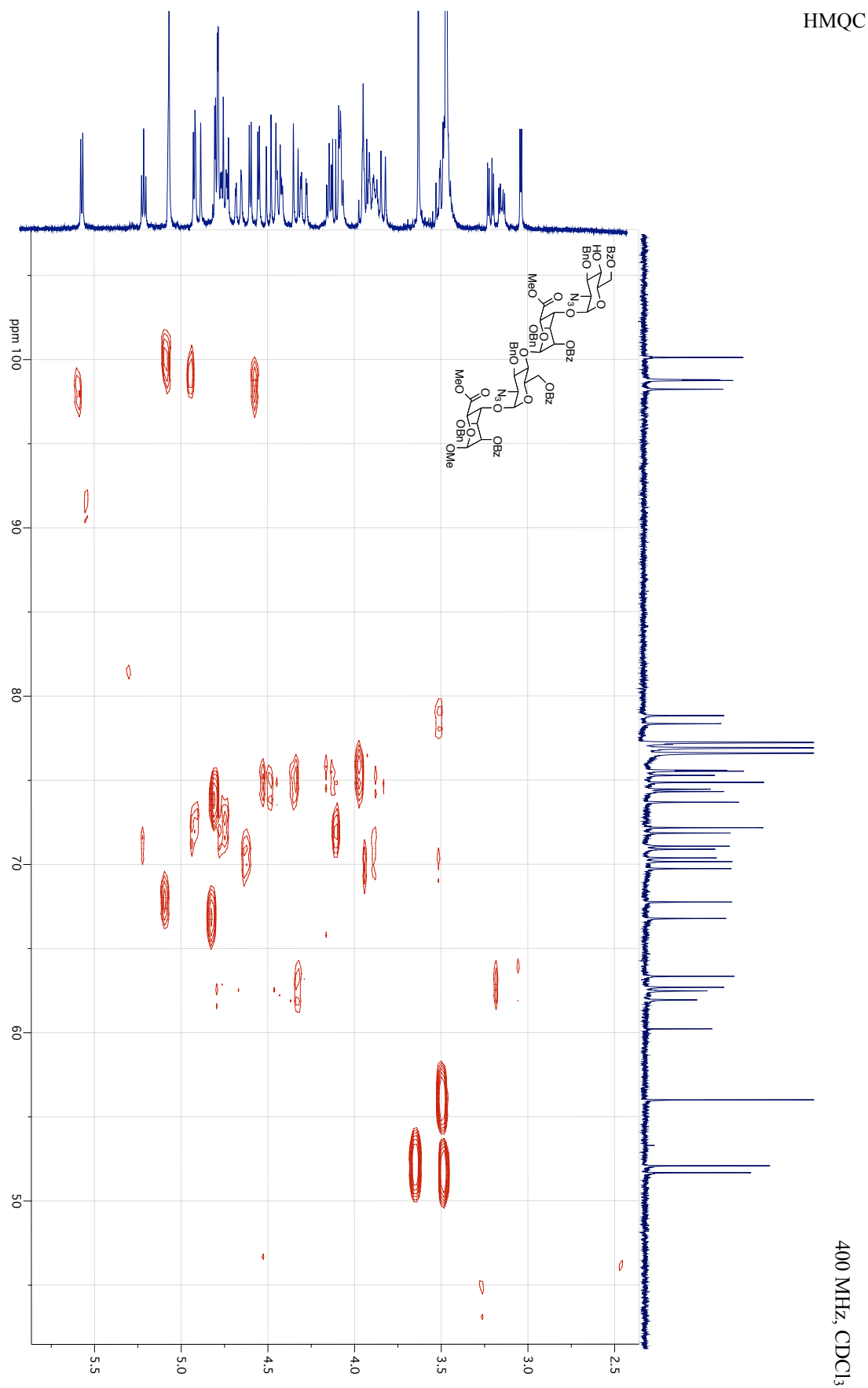
Tetrasaccharide 12



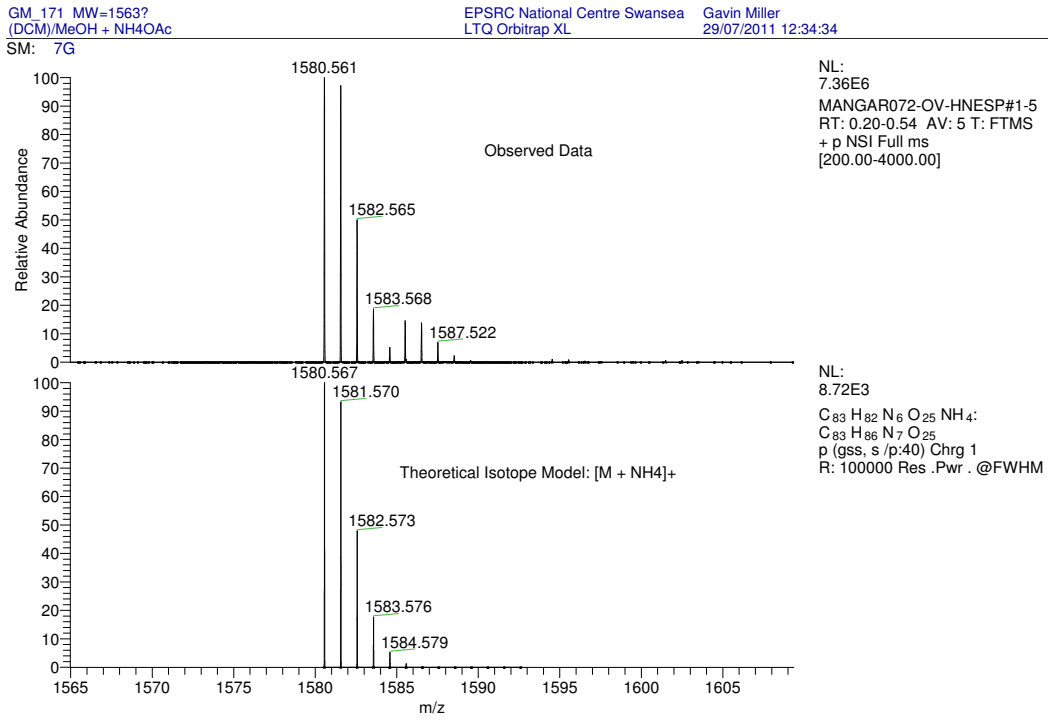
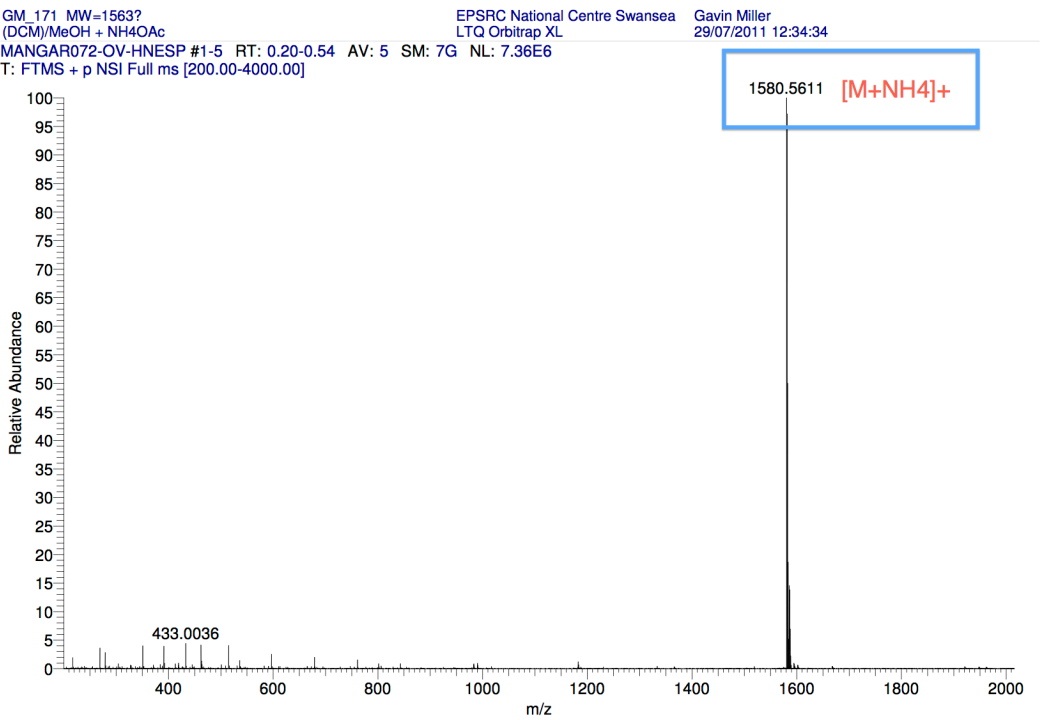
Tetrasaccharide 12



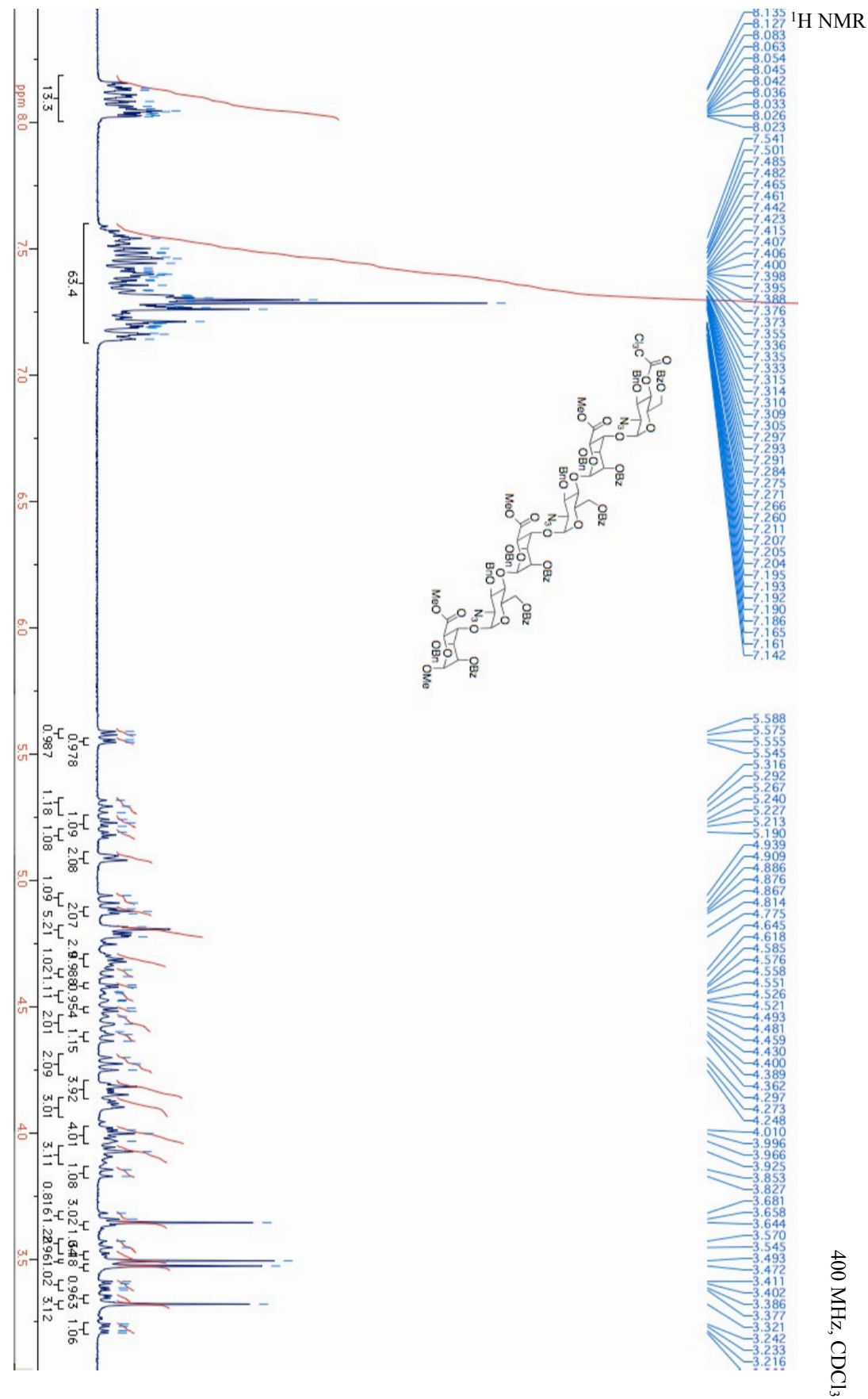
Tetrasaccharide 12



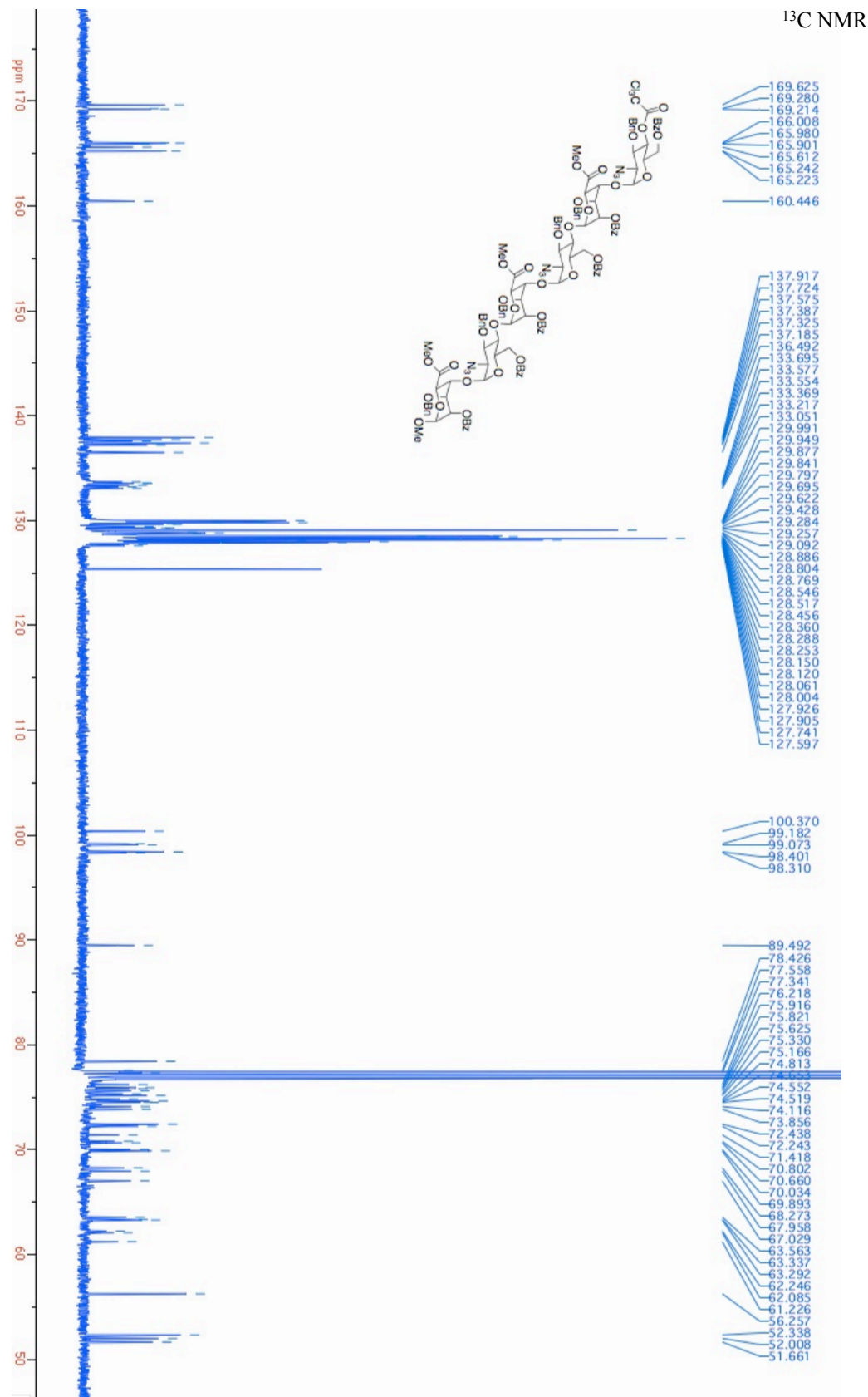
Tetrasaccharide 12 FTMS and Isotope Pattern



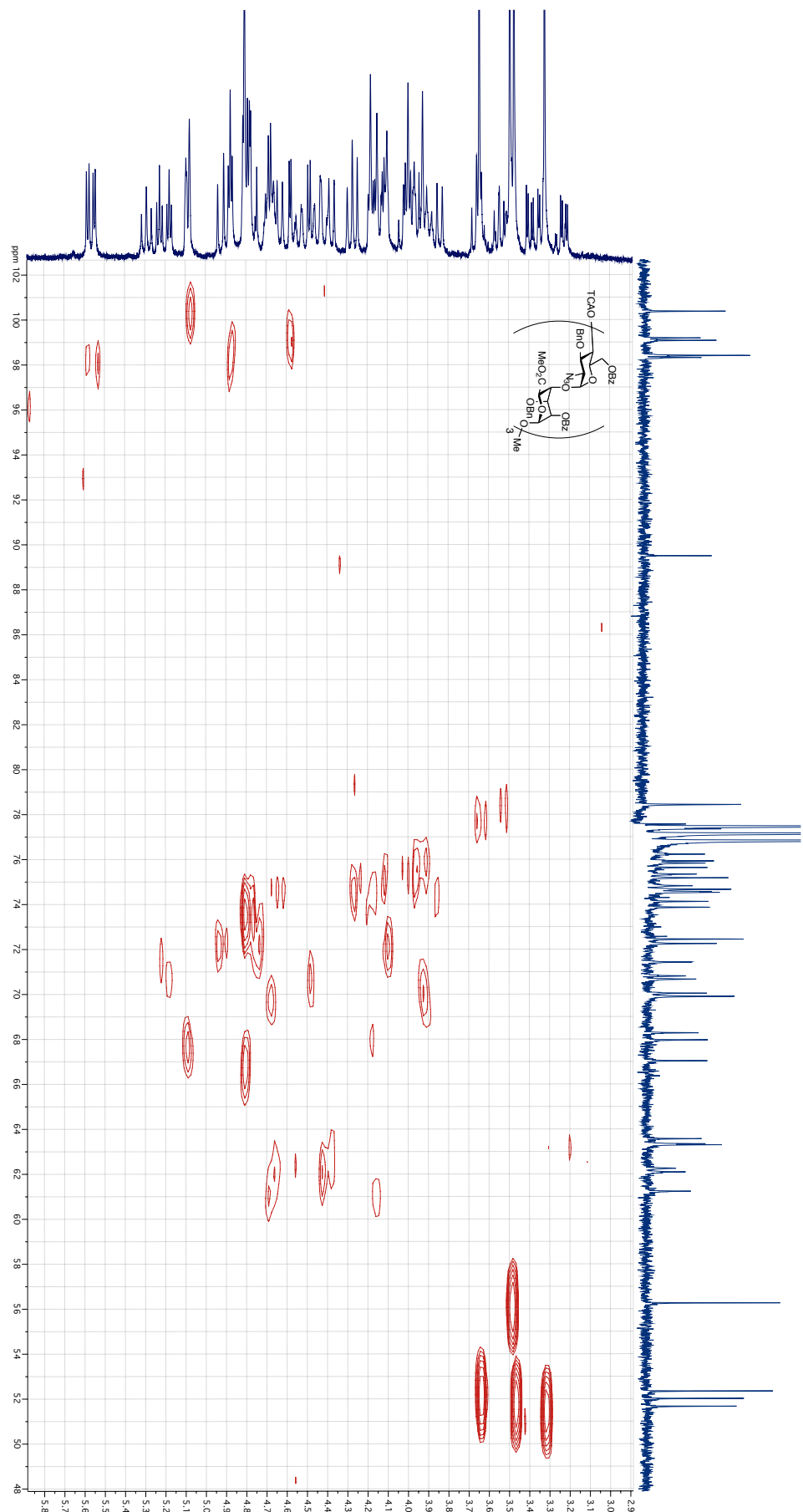
Hexaasaccharide 13



Hexaasaccharide 13



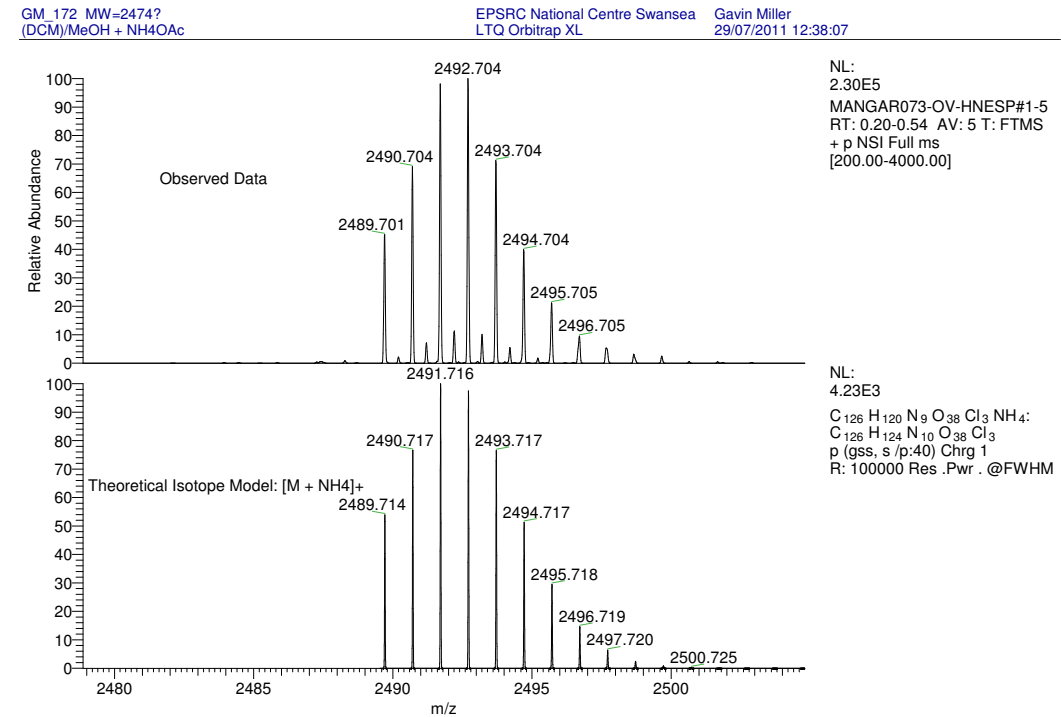
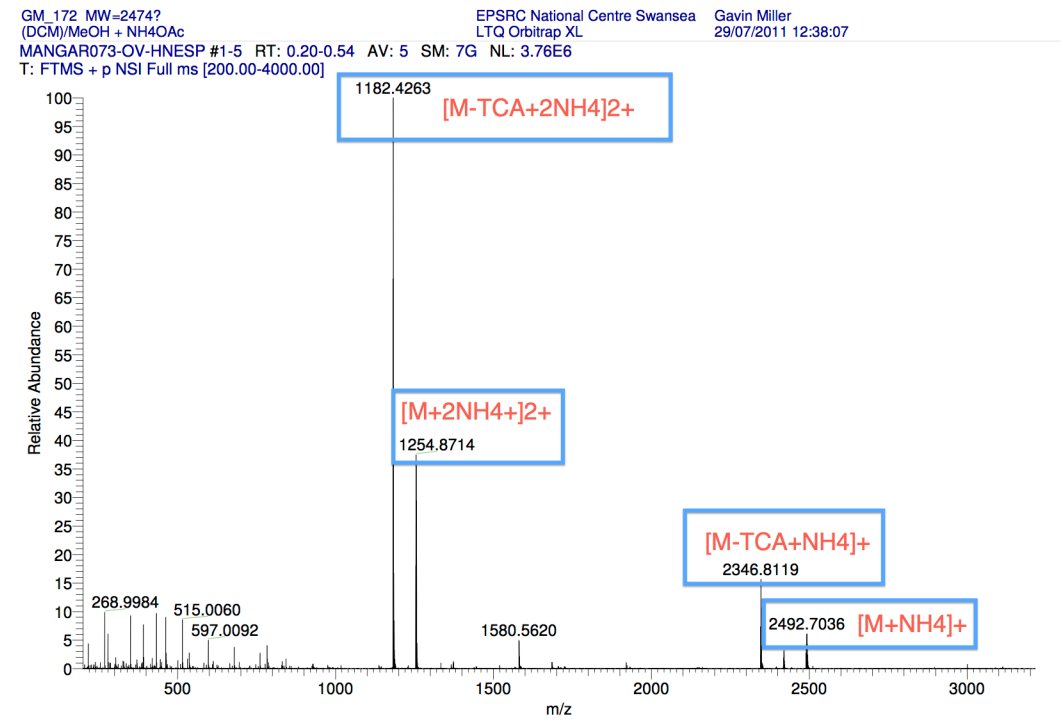
Hexaasaccharide 13



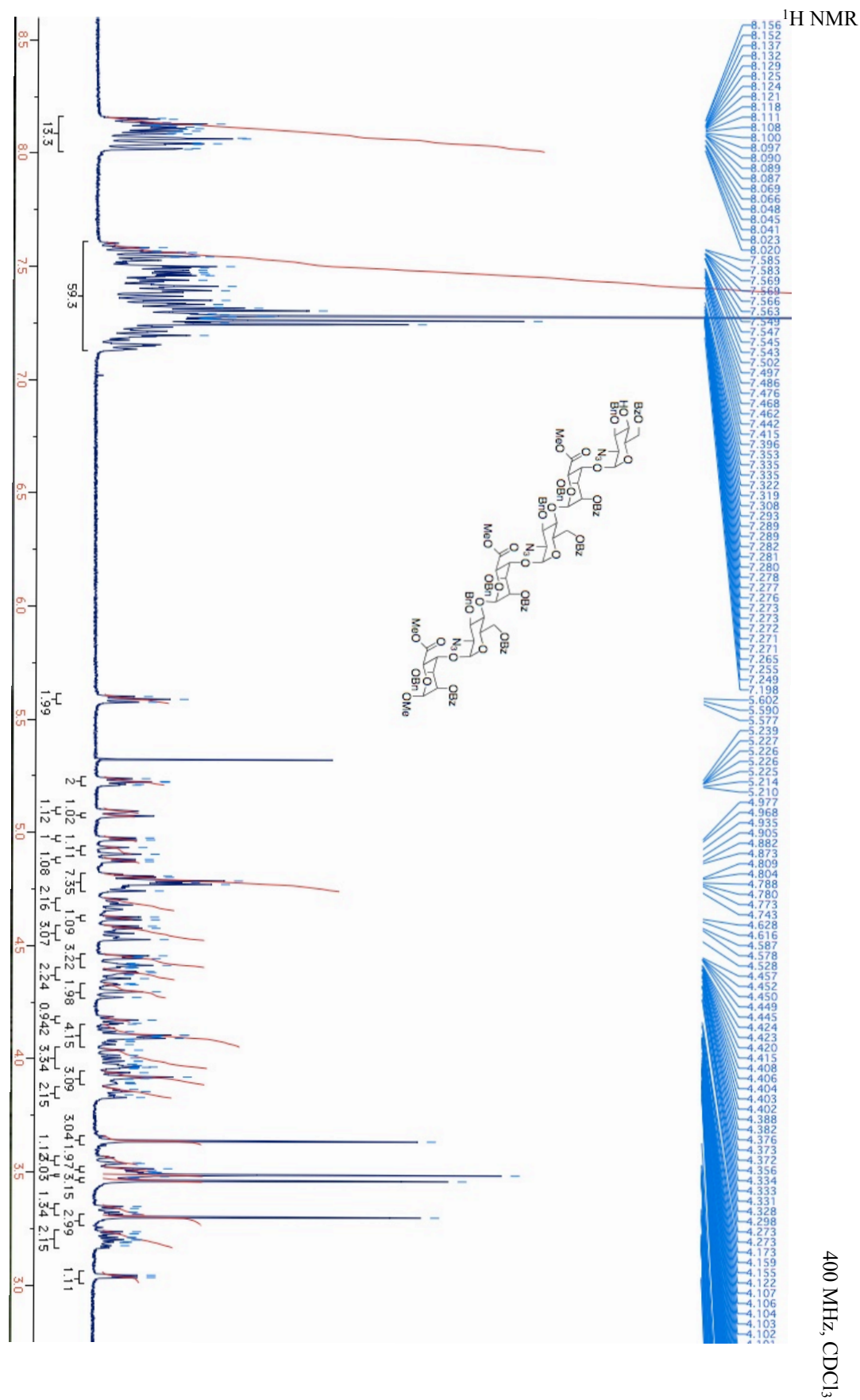
HMQC

400 MHz, CDCl₃

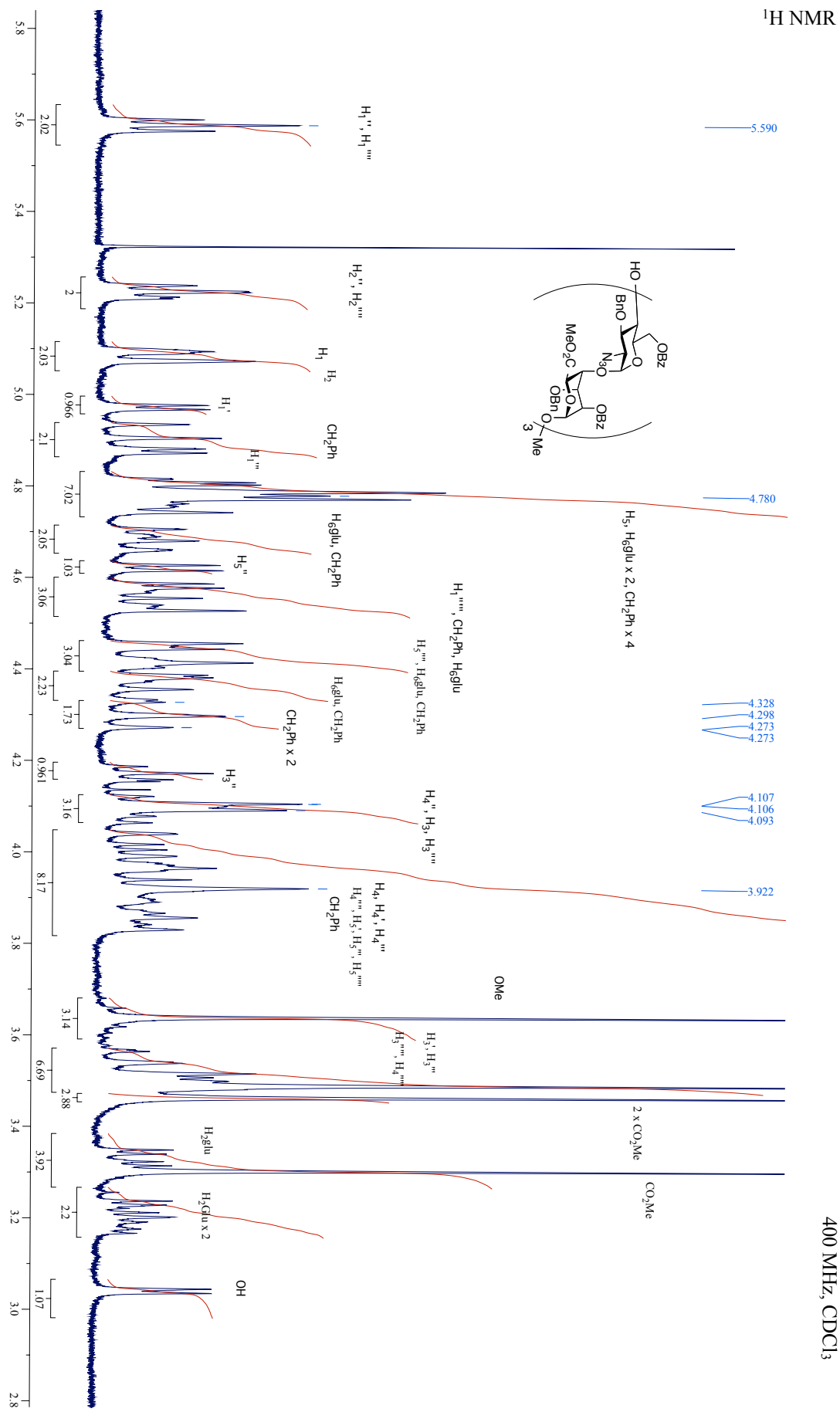
Hexaasaccharide 13 FTMS and isotope pattern



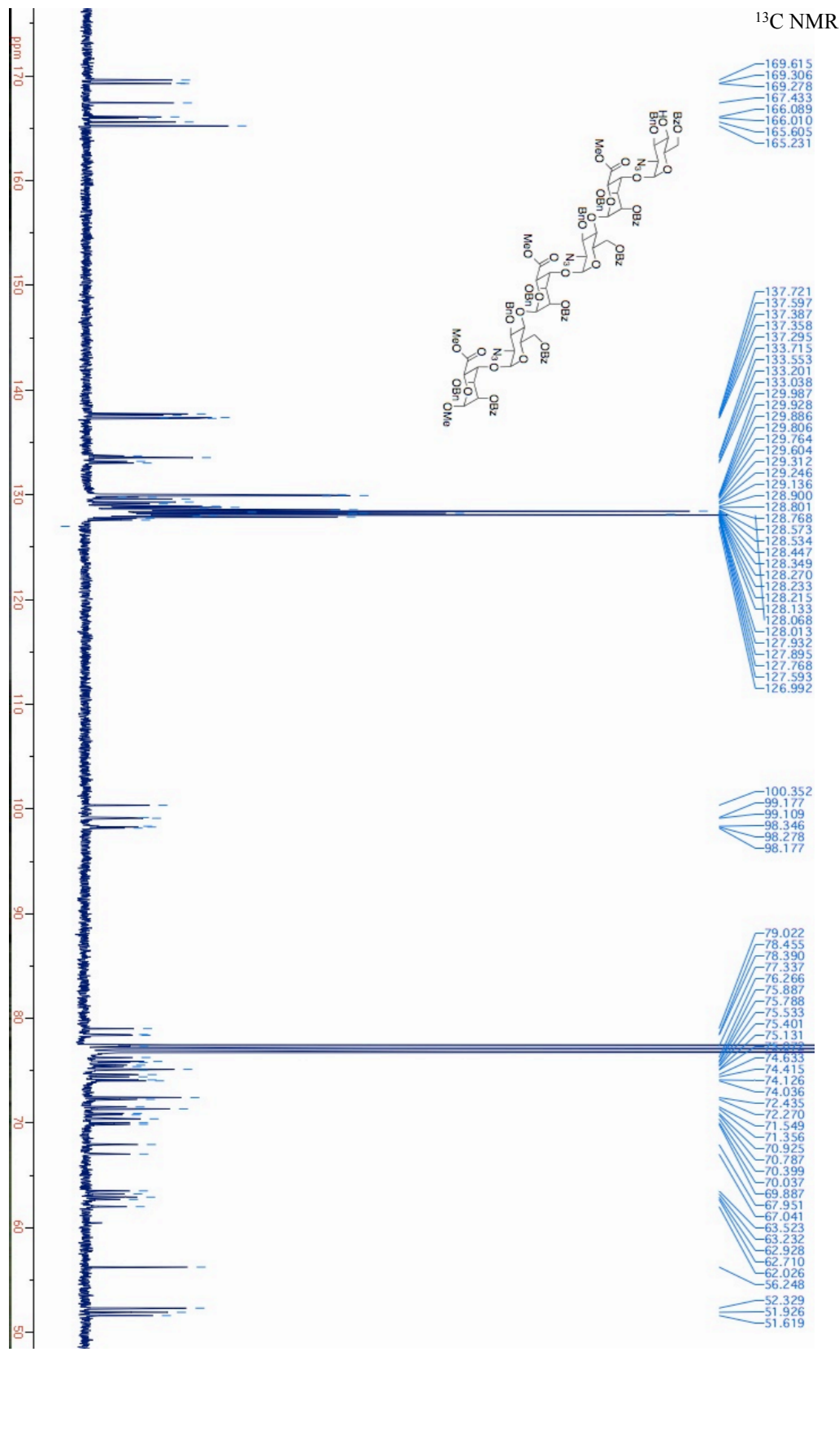
Hexaasaccharide 14



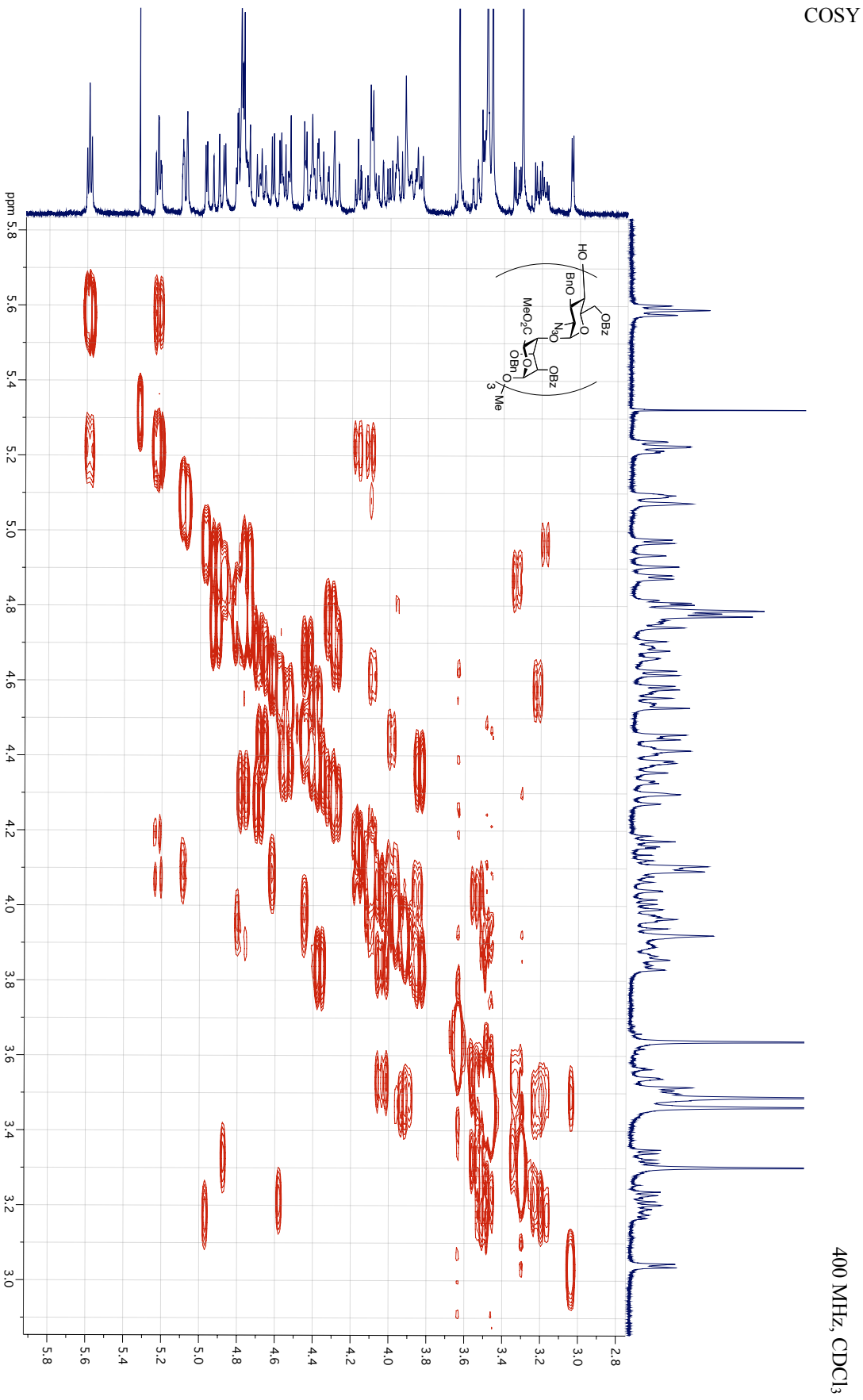
Hexaasaccharide 14 - ¹H NMR expansion



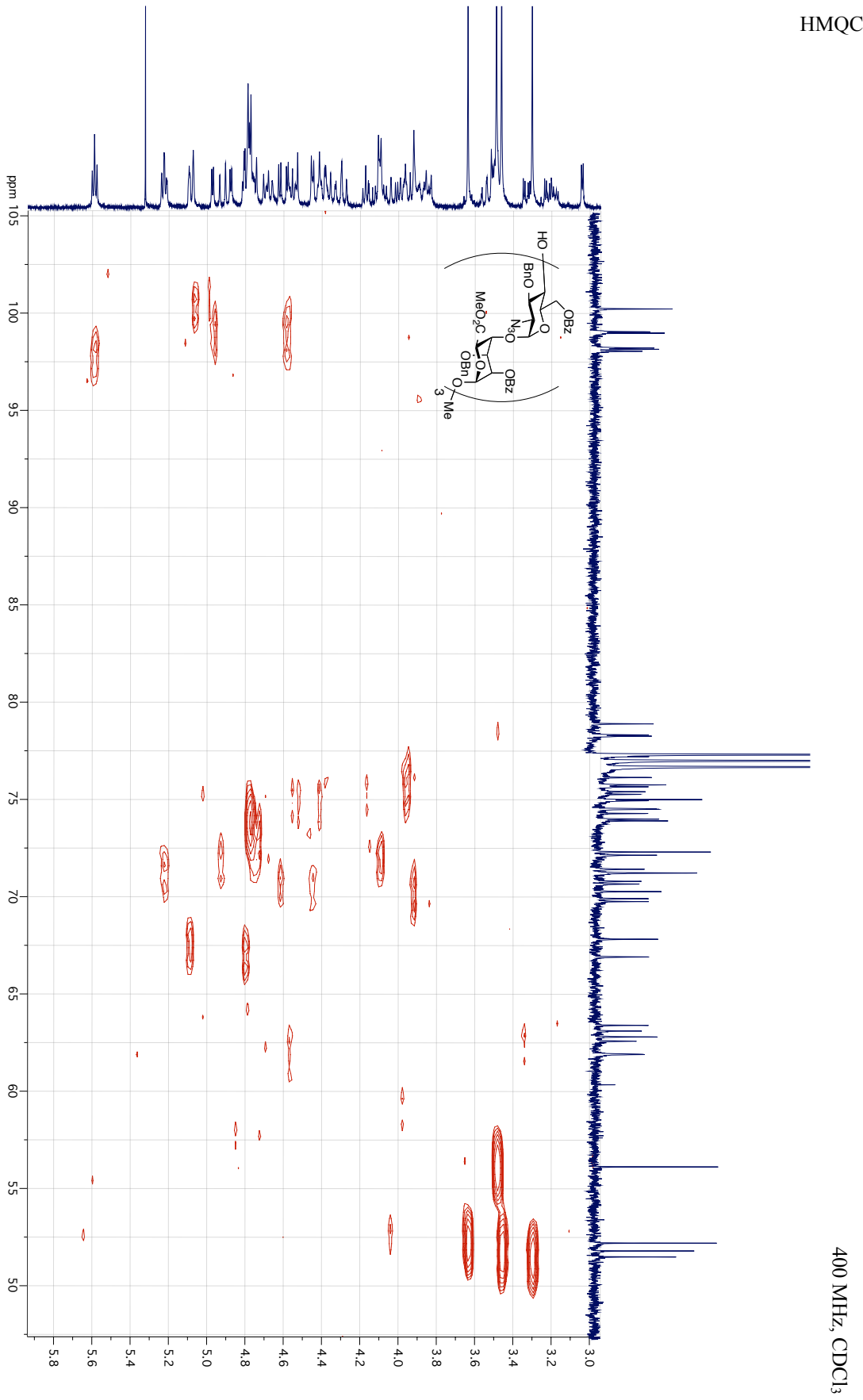
Hexaasaccharide 14



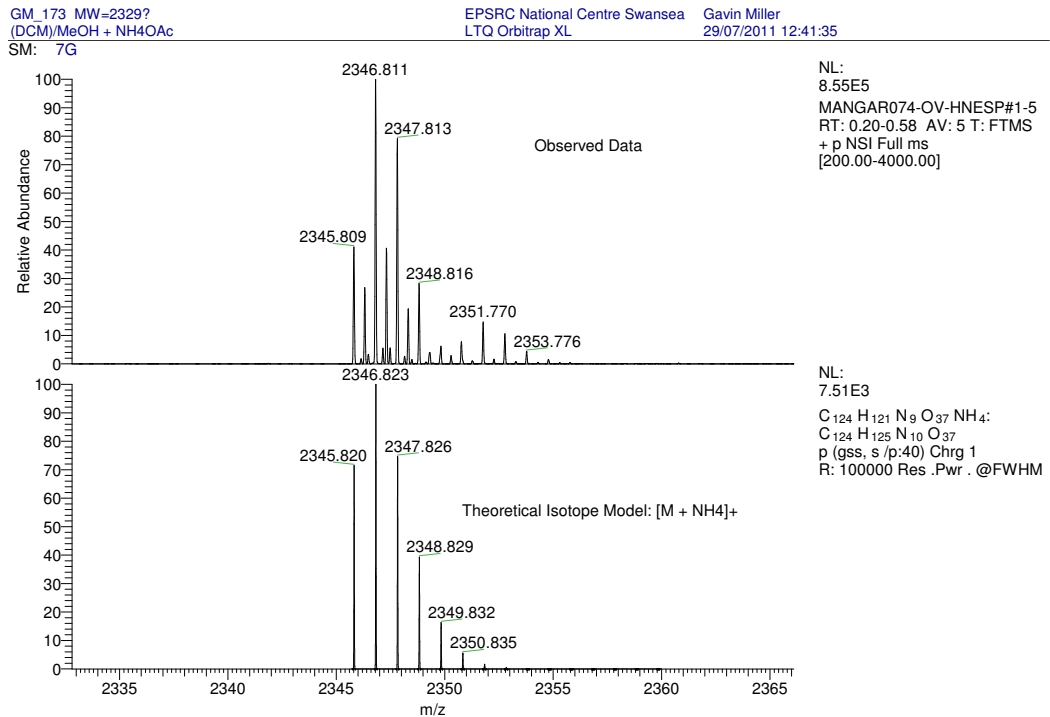
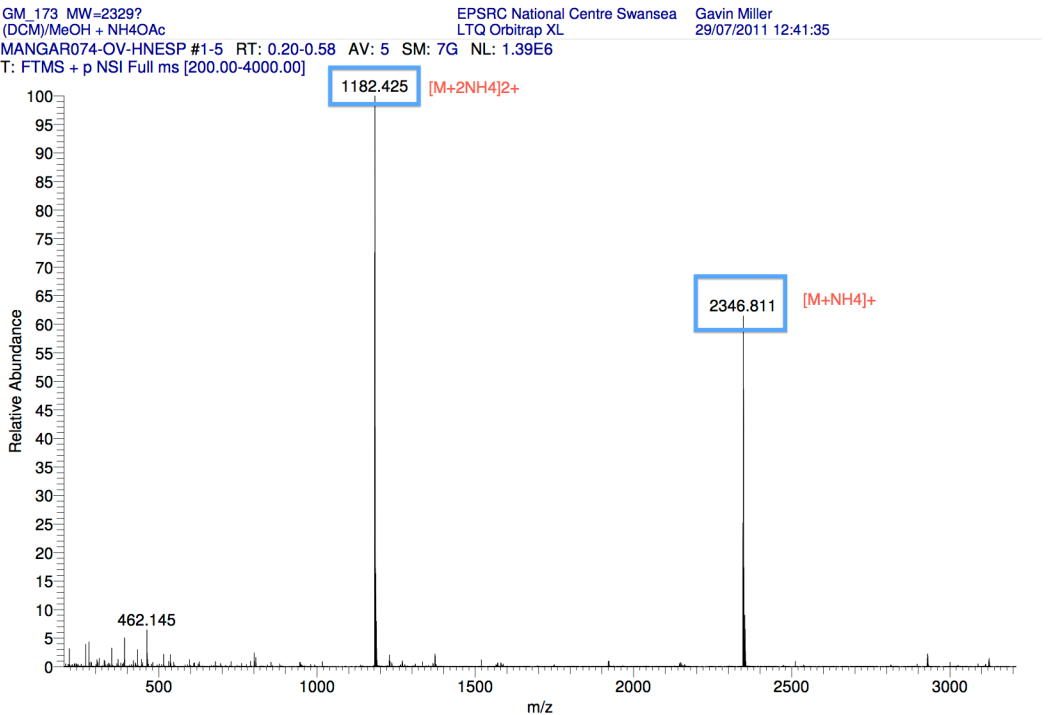
Hexaasaccharide 14

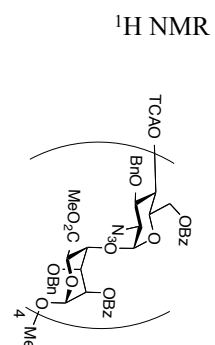


Hexaasaccharide 14

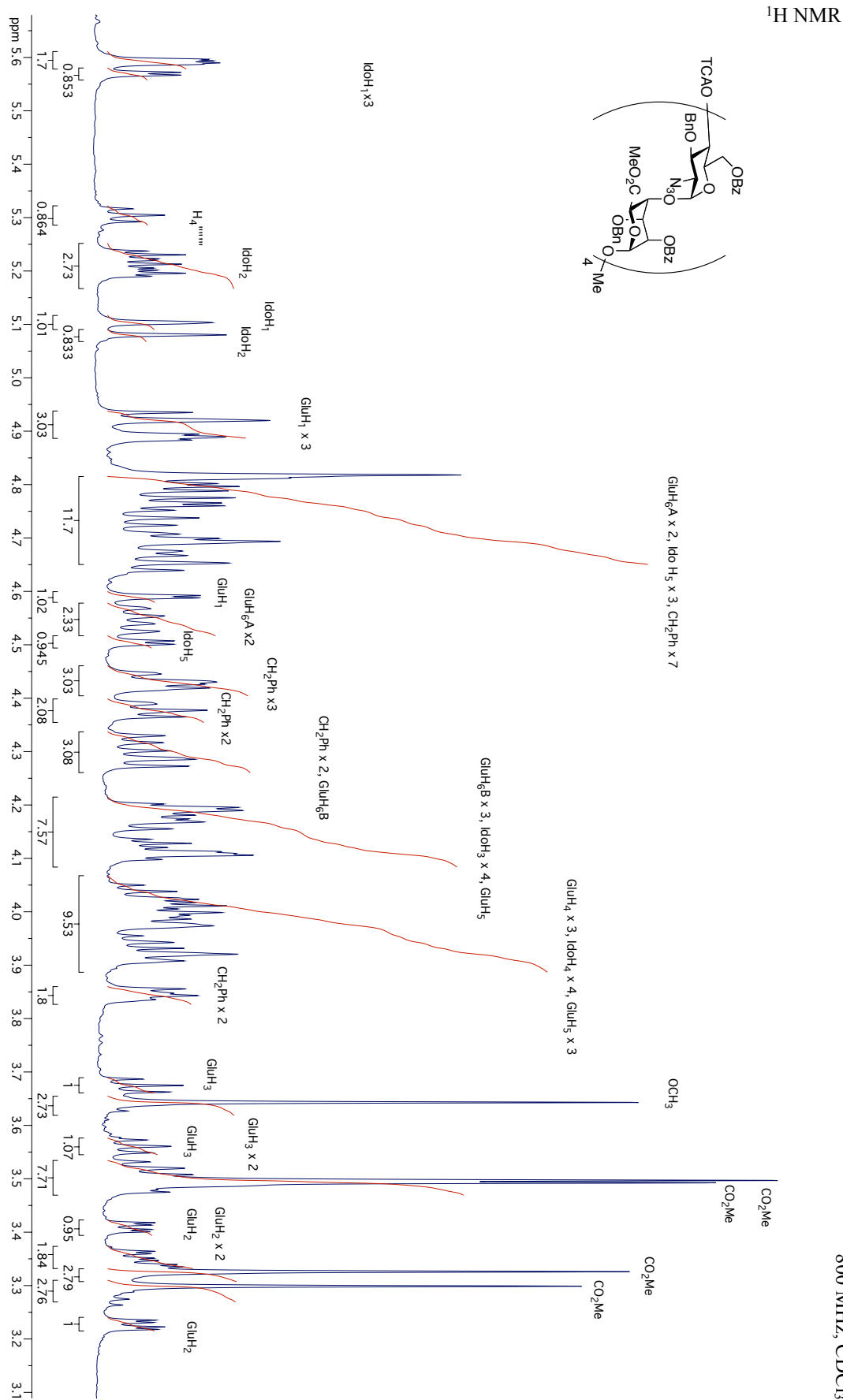


Hexaasaccharide 14 - FTMS and isotope pattern



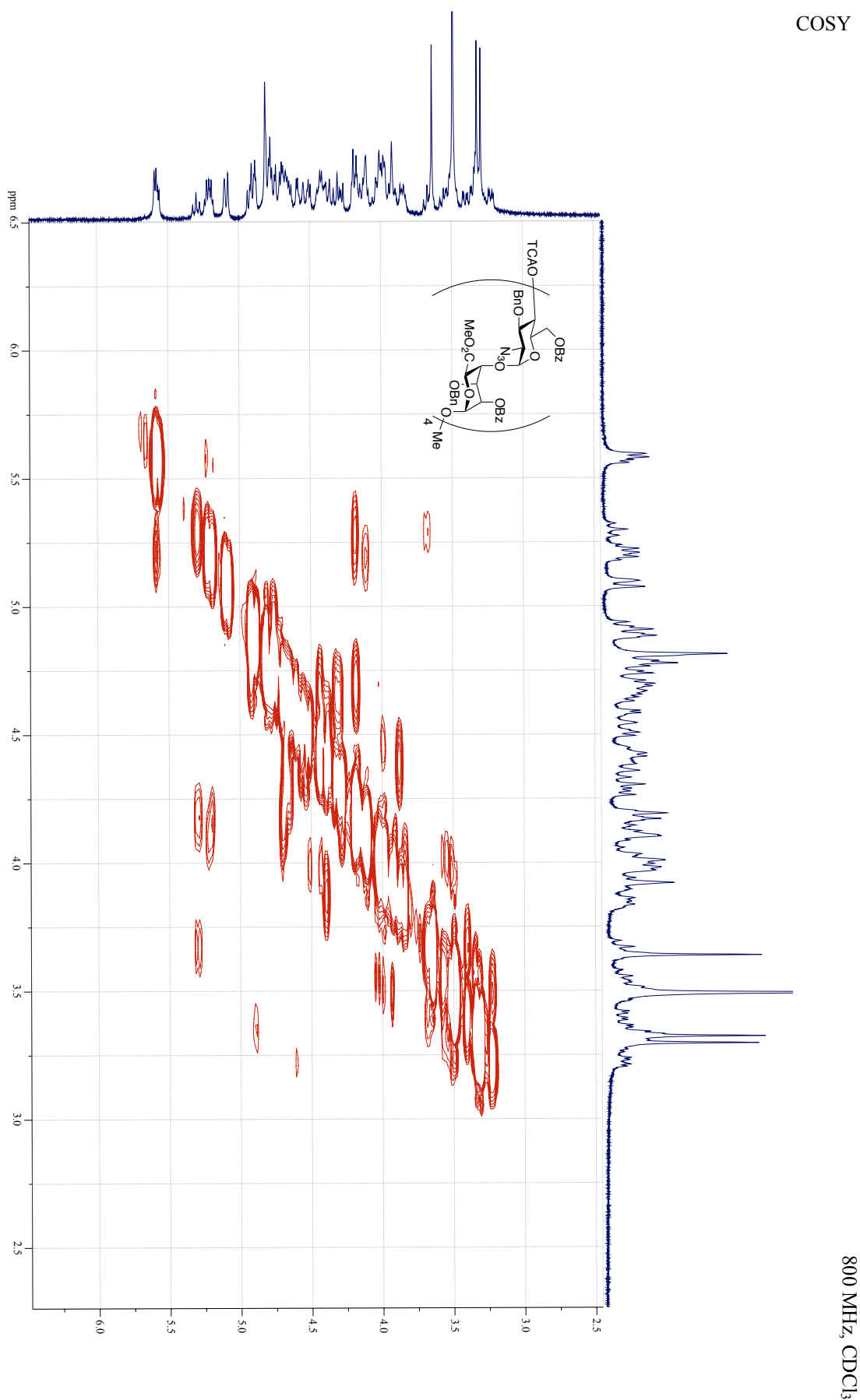
800 MHz, CDCl₃

Octaasaccharide 15 - ¹H NMR Expansion

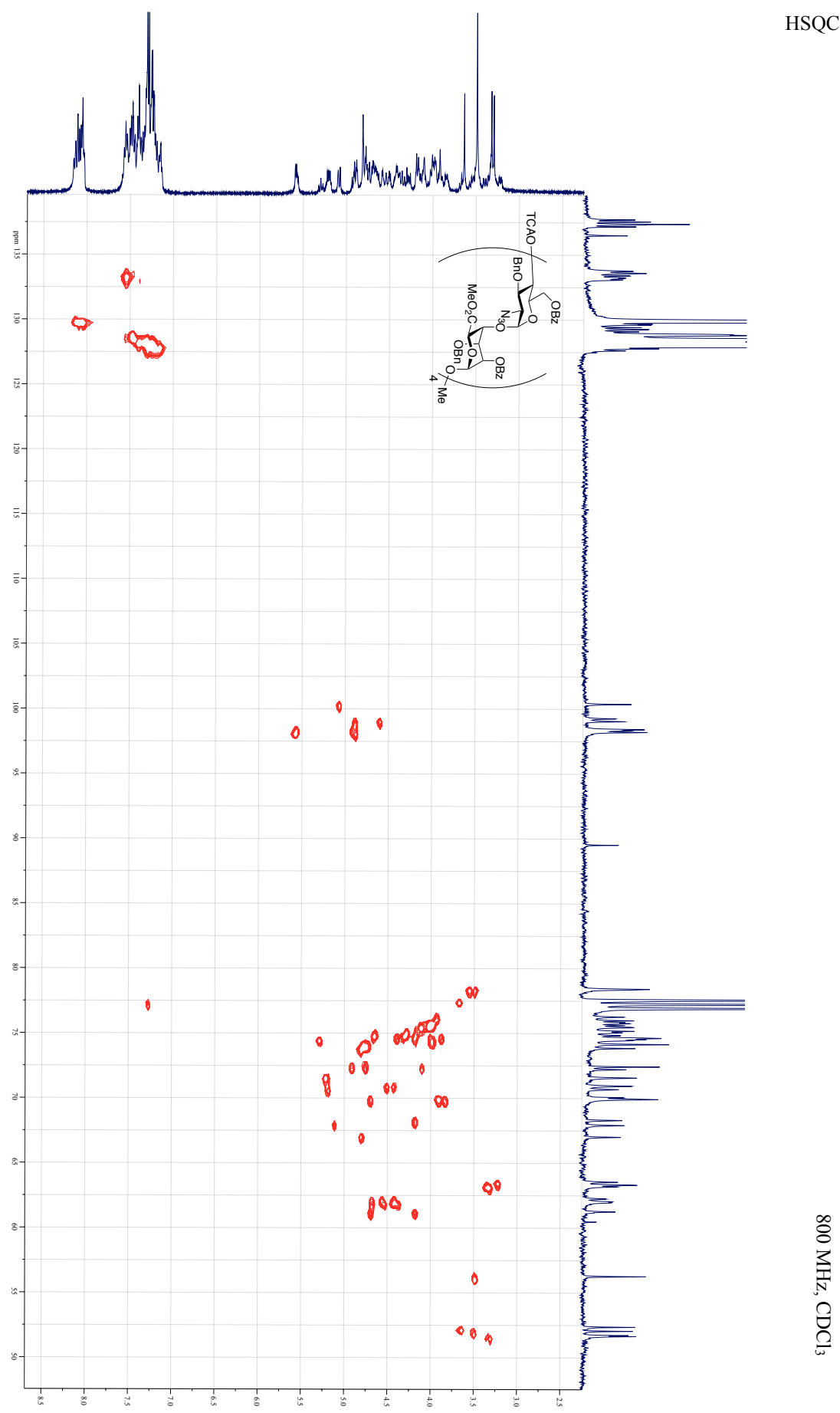




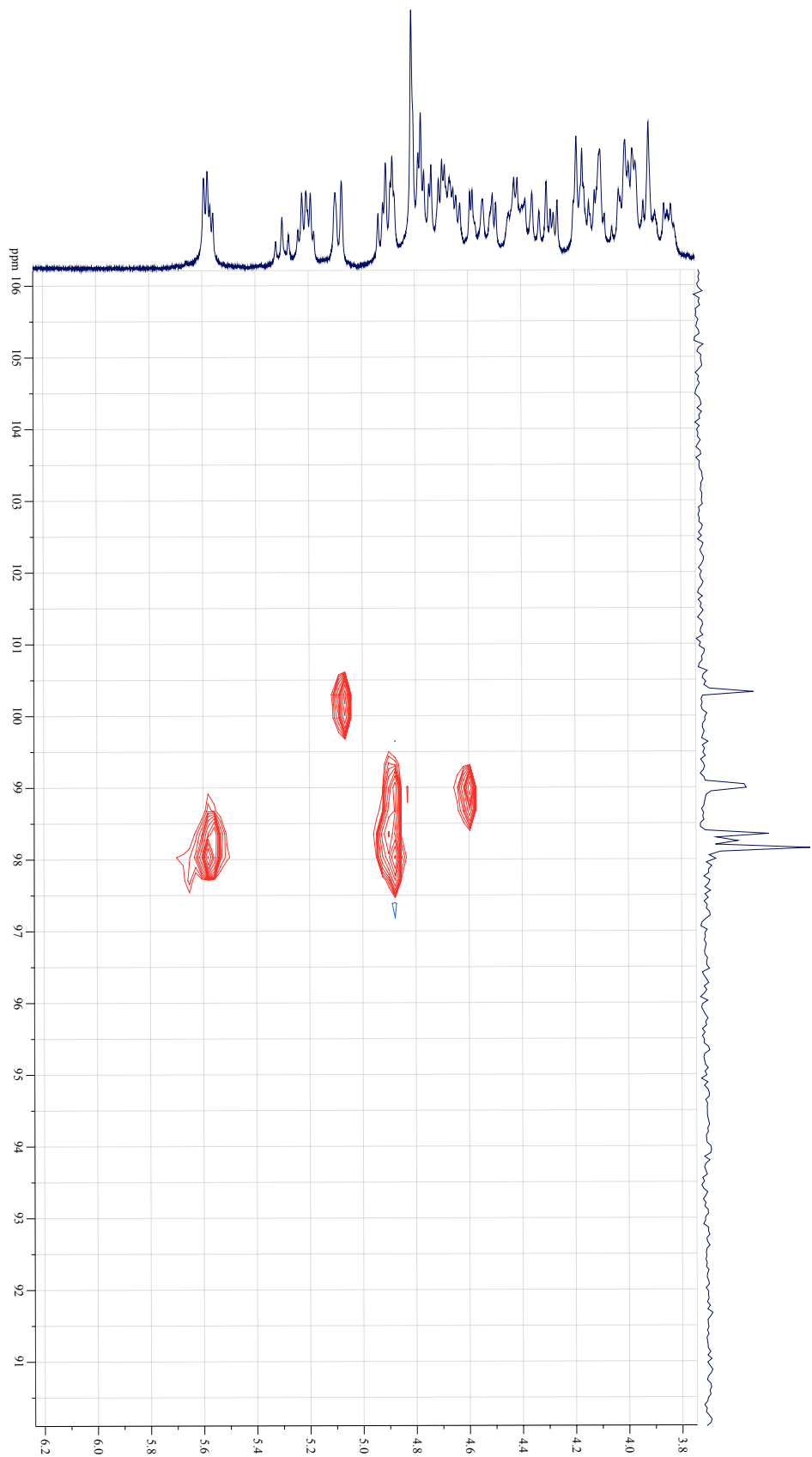
Octaasaccharide 15



Octasaccharide 15



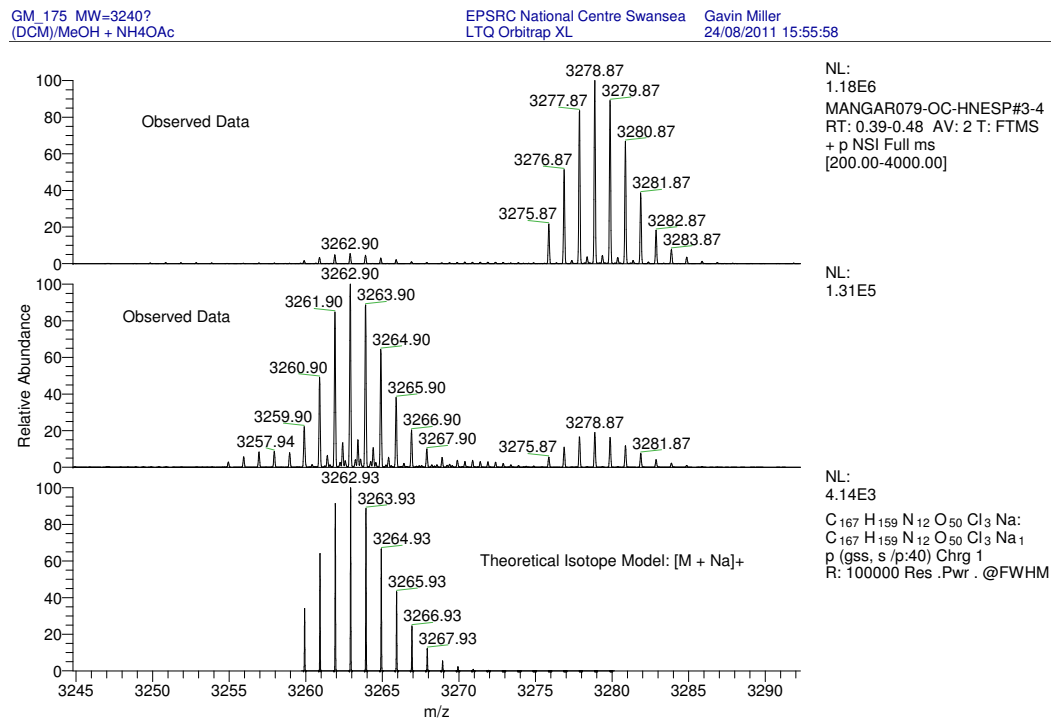
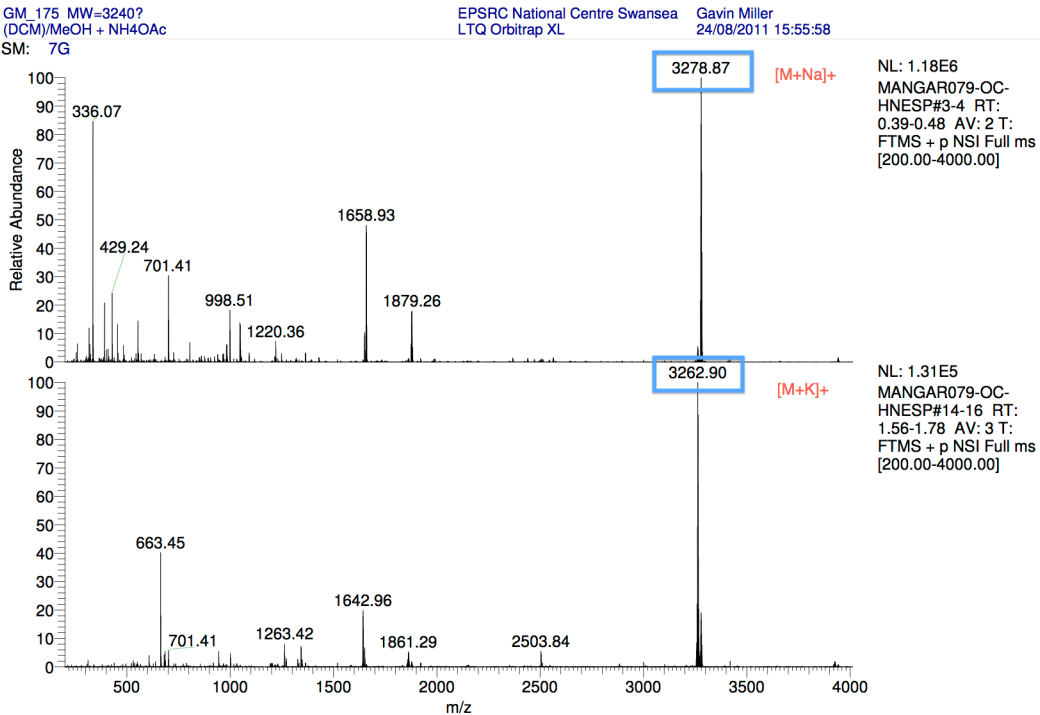
Octasaccharide 15 - Expanded anomeric centres region



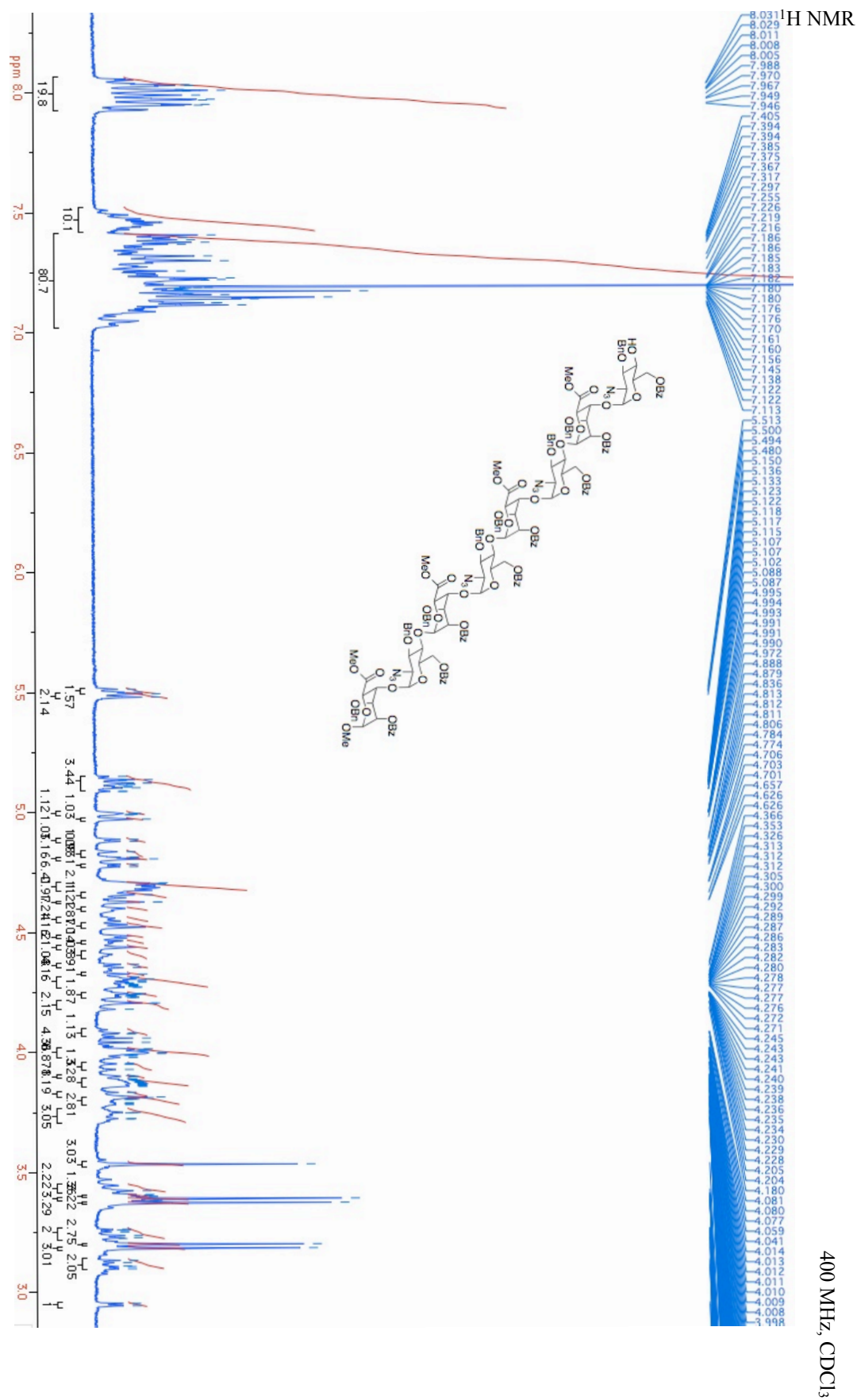
HSQC

800 MHz, CDCl₃

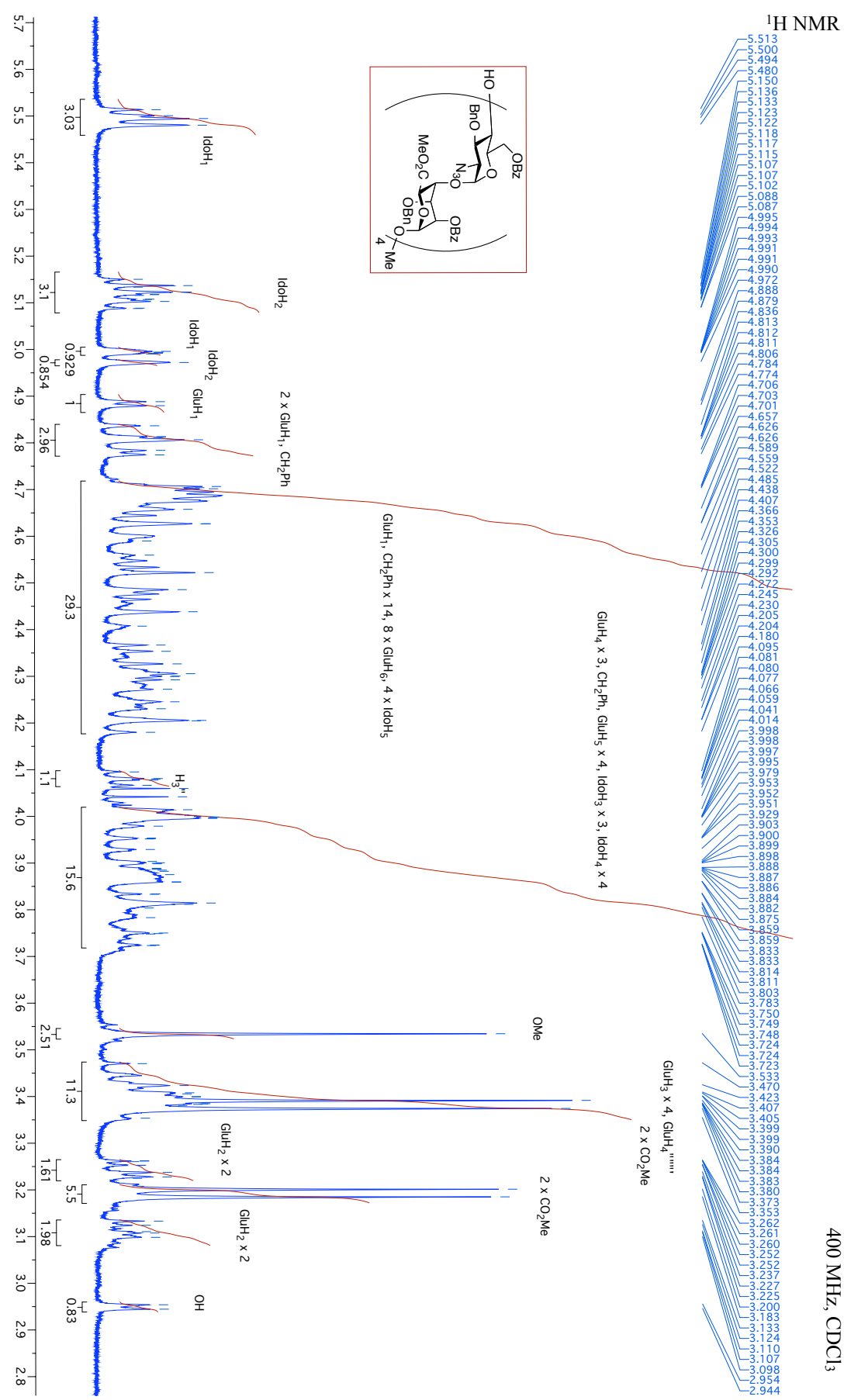
Octaasaccharide 15 MS and isotope patterns



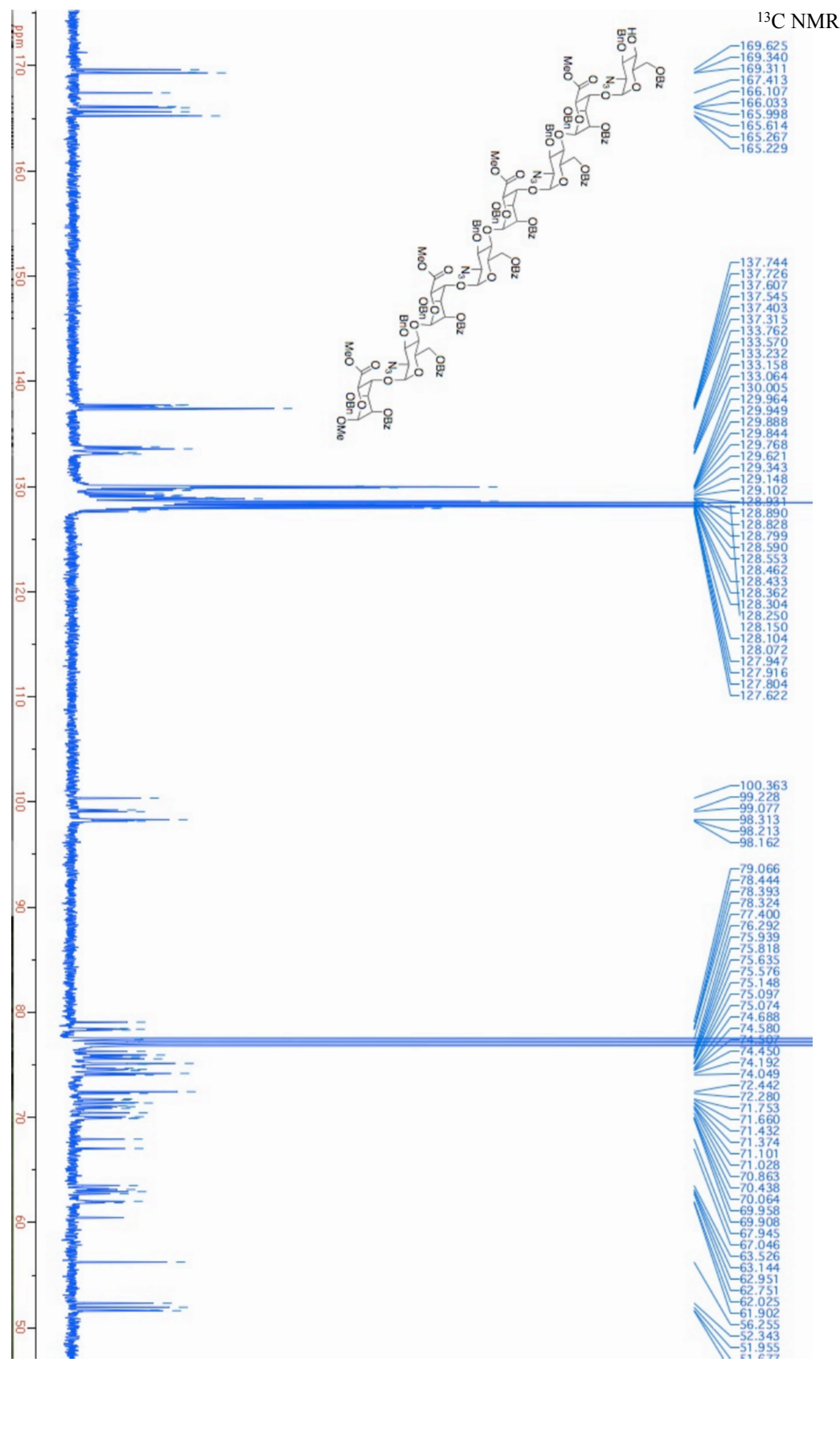
Octaasaccharide 16



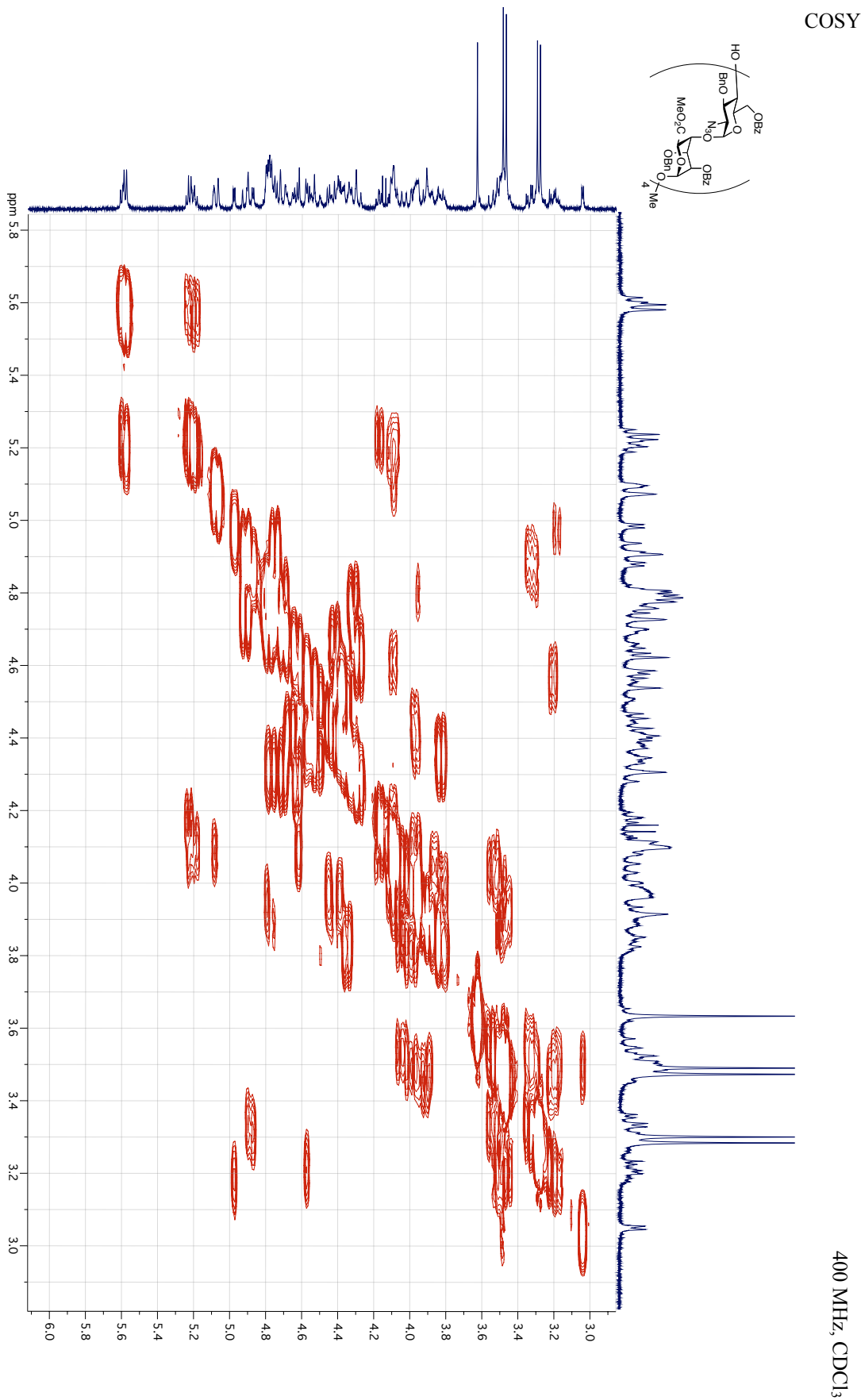
Octaasaccharide 16 - ¹H NMR expansion



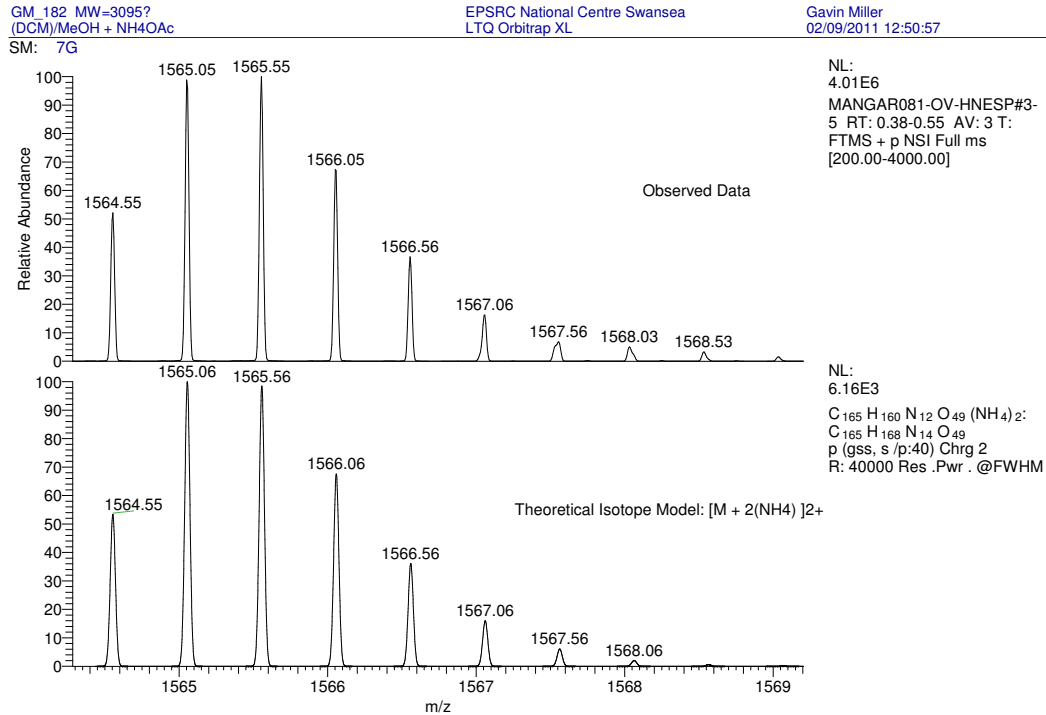
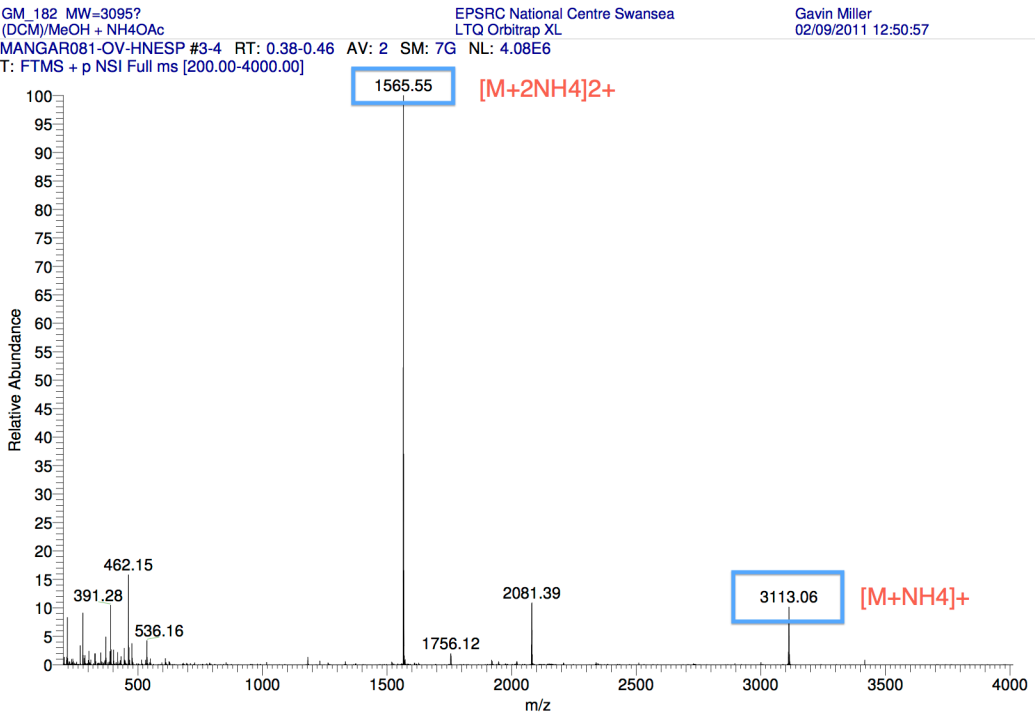
Octaasaccharide 16



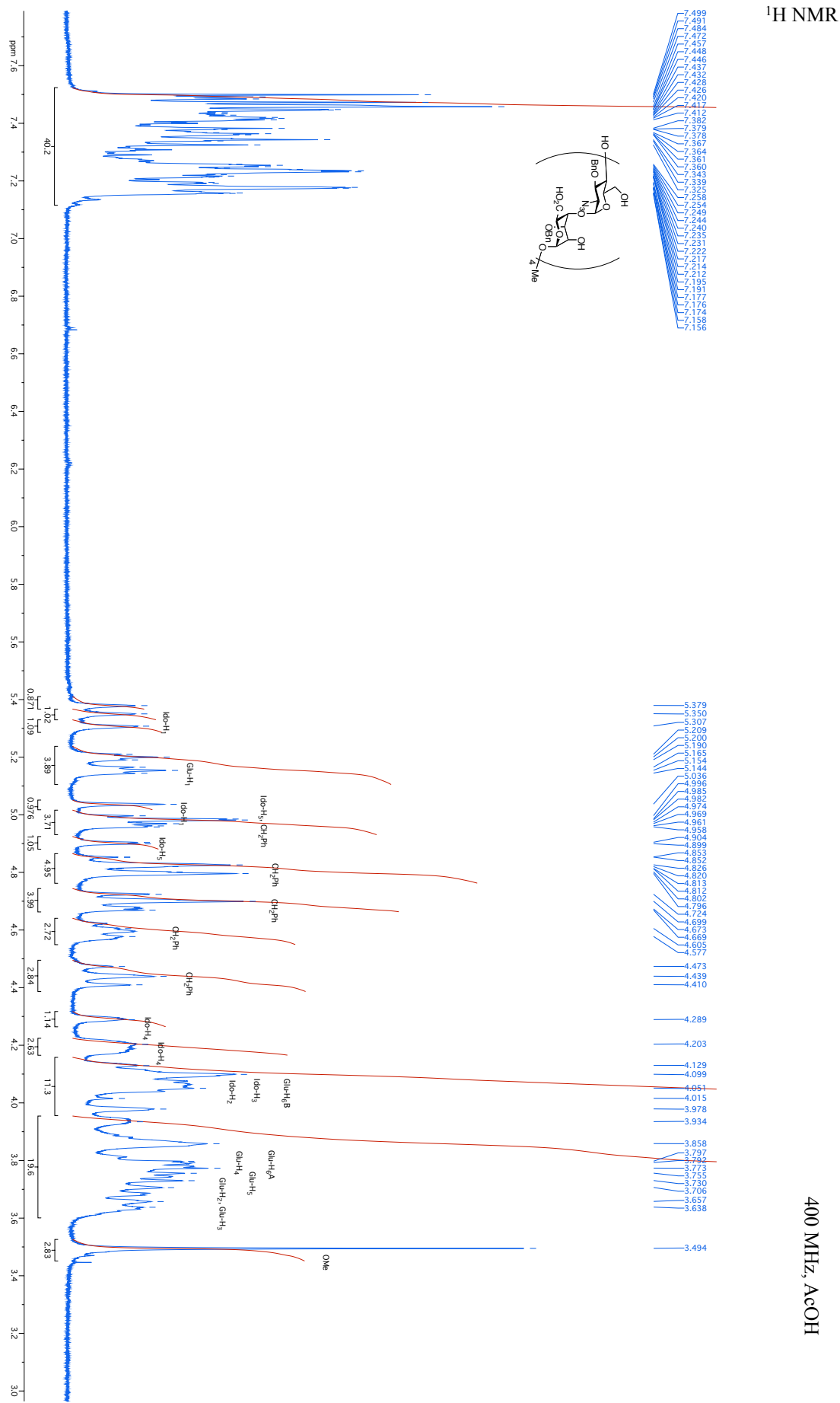
Octaasaccharide 16



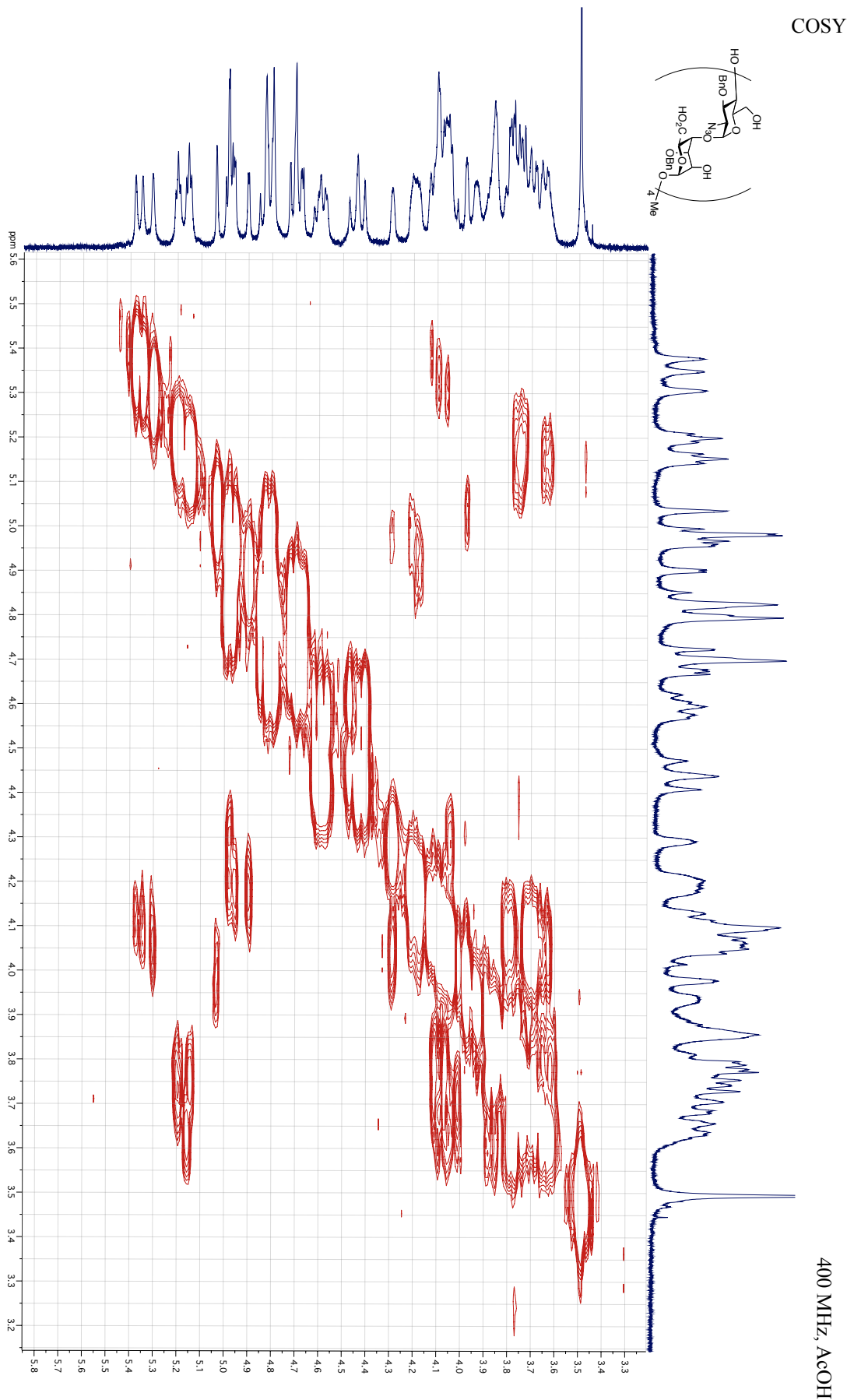
Octaasaccharide 16 - ESI MS and Isotope Pattern



Octasaccharide Saponification 20



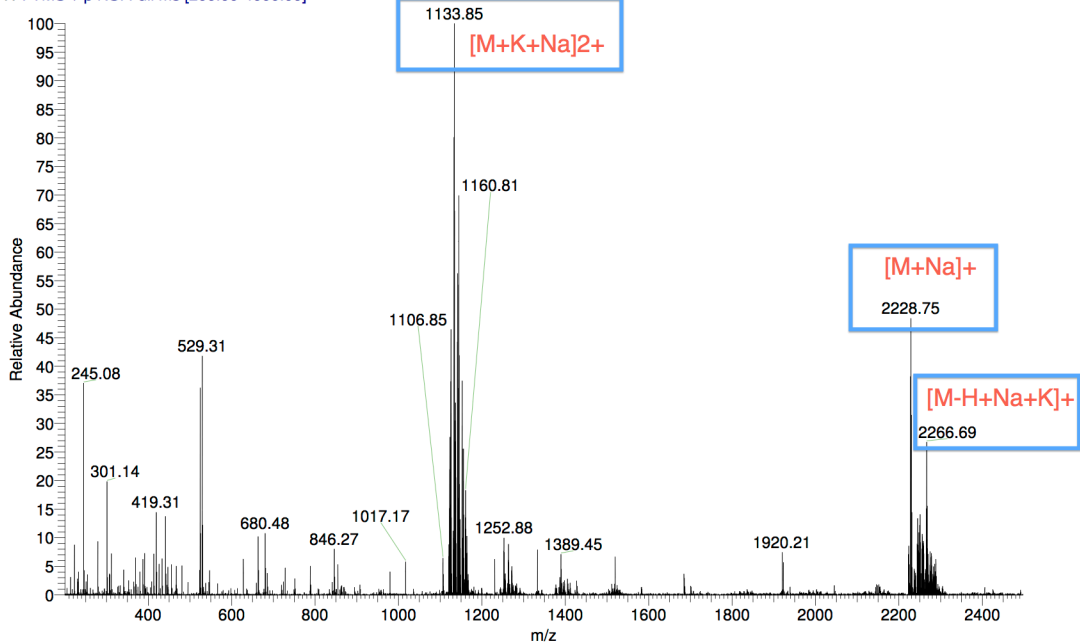
Octasaccharide Saponification 20



Octasaccharide Saponification 20 ESI MS

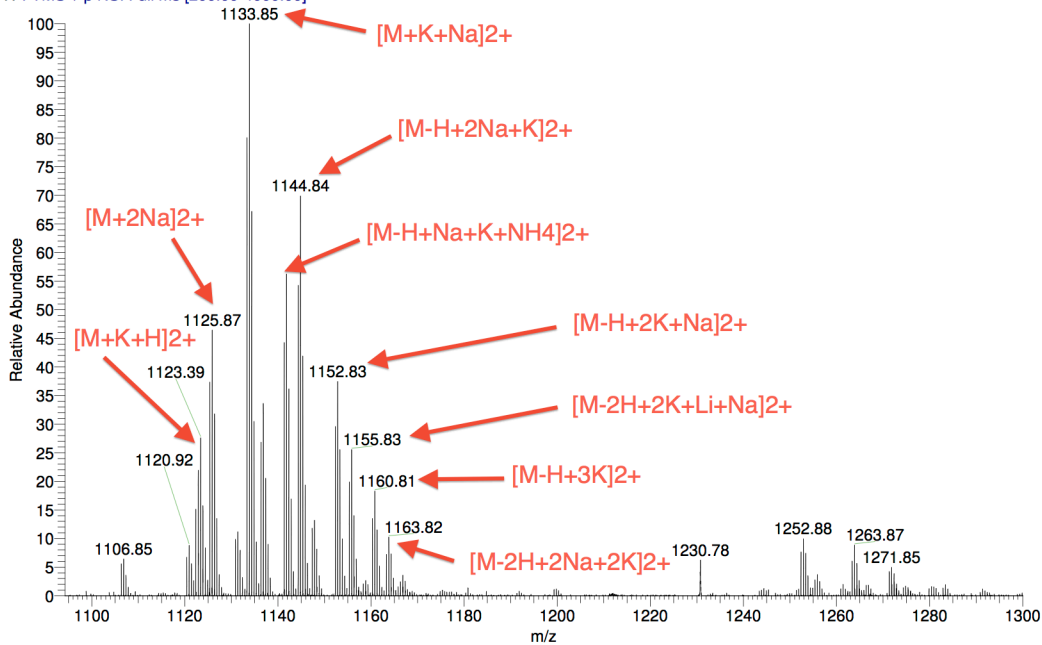
GM_179 MW=2206?
(DCM)/MeOH + NH4OAc
MANGAR080-OC-HNESP #13-16 RT: 1.25-1.50 AV: 4 SM: 7G NL: 7.55E5
T: FTMS + p NSI Full ms [200.00-4000.00]

EPSRC National Centre Swansea
LTQ Orbitrap XL
Gavin Miller
24/08/2011 15:59:34

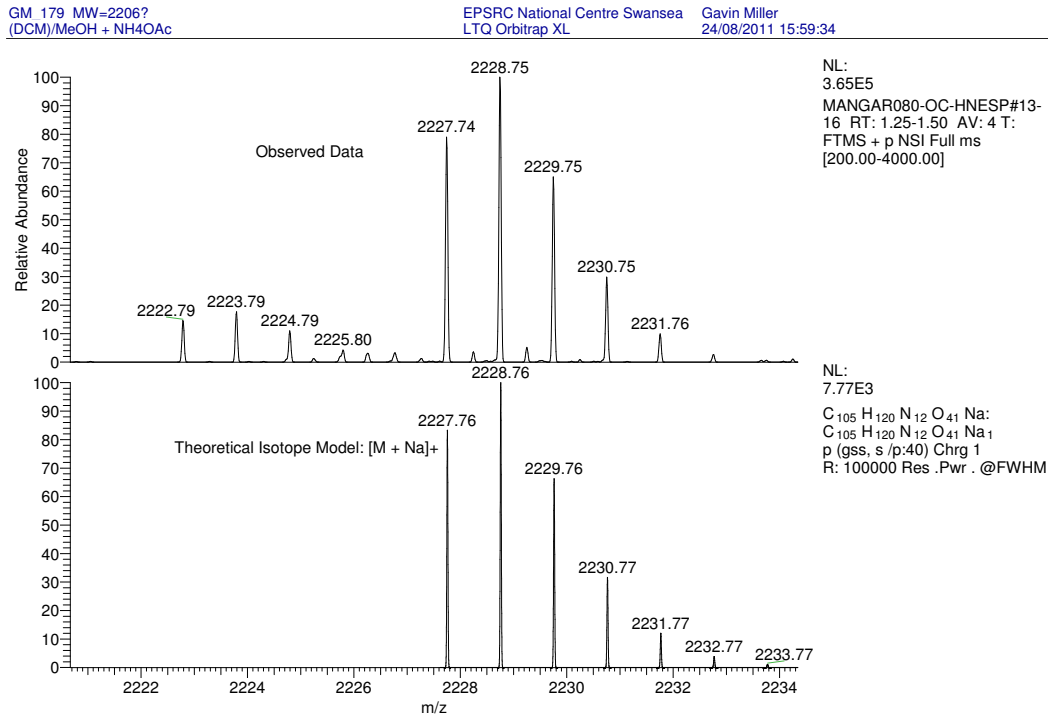
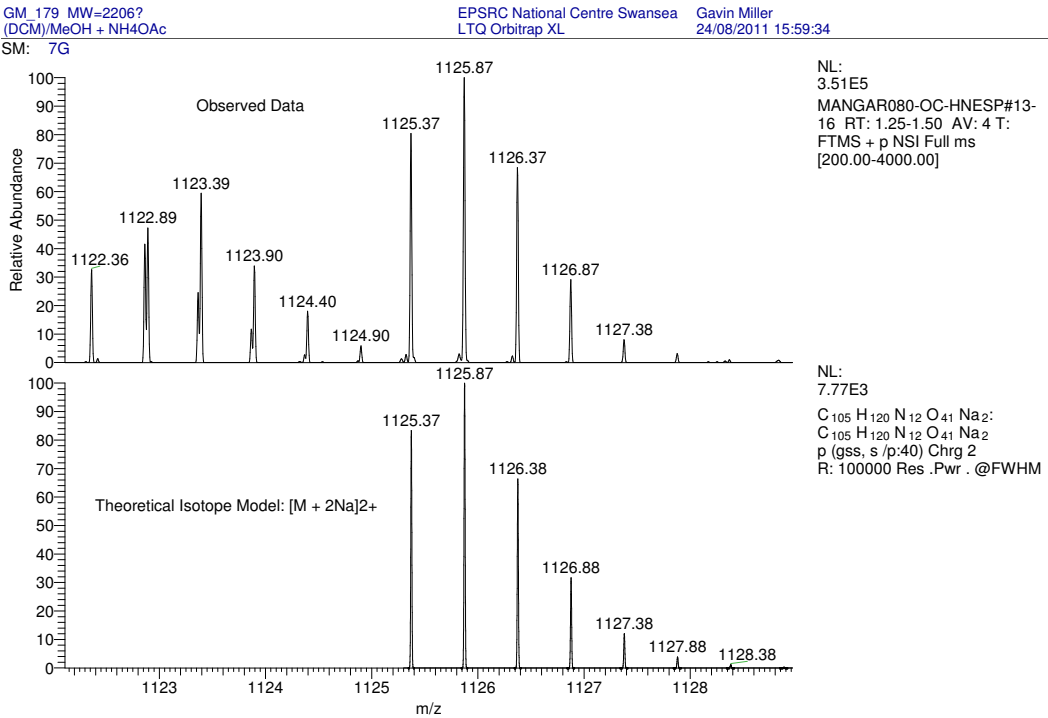


GM_179 MW=2206?
(DCM)/MeOH + NH4OAc
MANGAR080-OC-HNESP #13-16 RT: 1.25-1.50 AV: 4 SM: 7G NL: 7.55E5
T: FTMS + p NSI Full ms [200.00-4000.00]

EPSRC National Centre Swansea
LTQ Orbitrap XL
Gavin Miller
24/08/2011 15:59:34



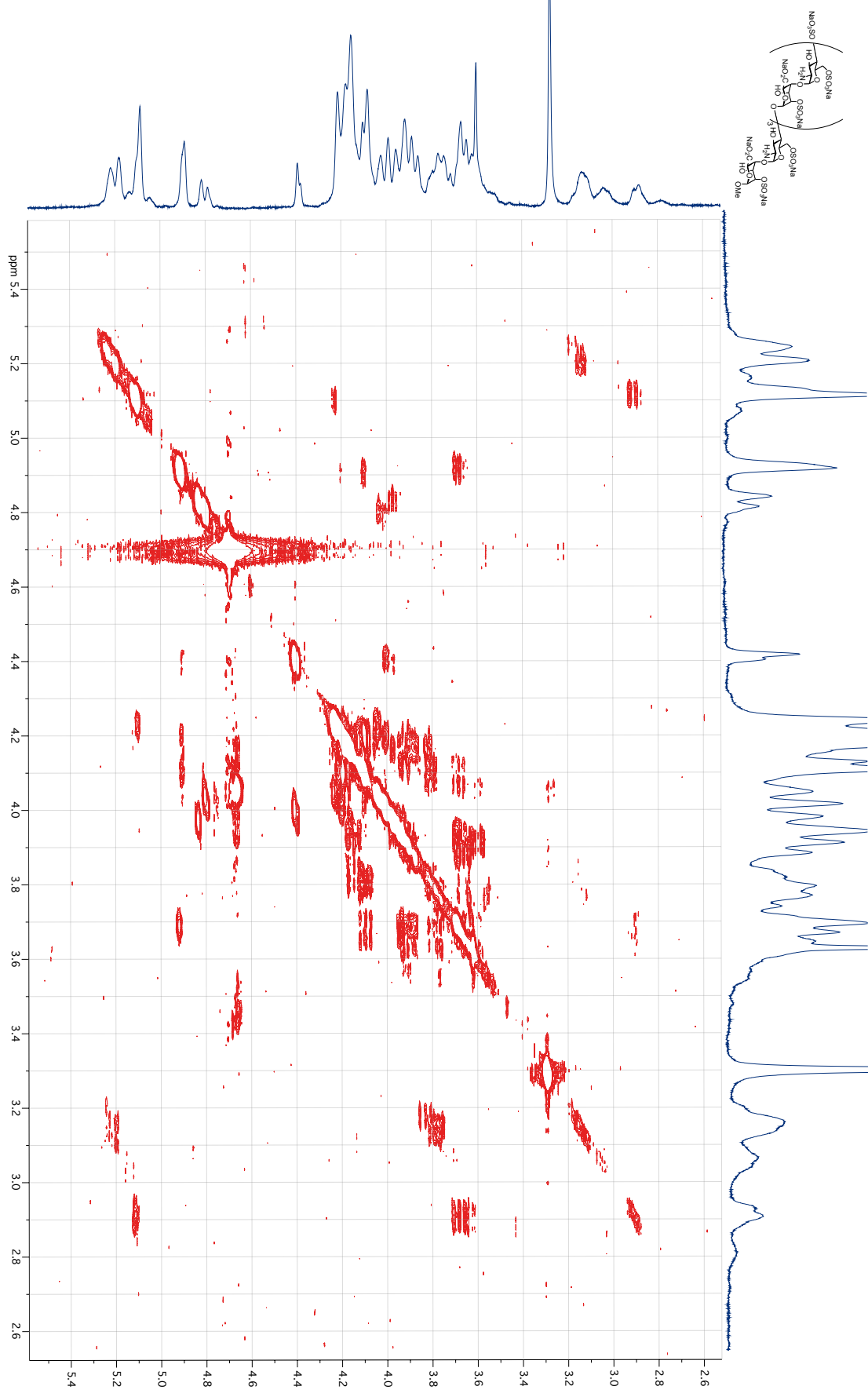
Octasaccharide Saponification 20 ESI MS Isotope Patterns



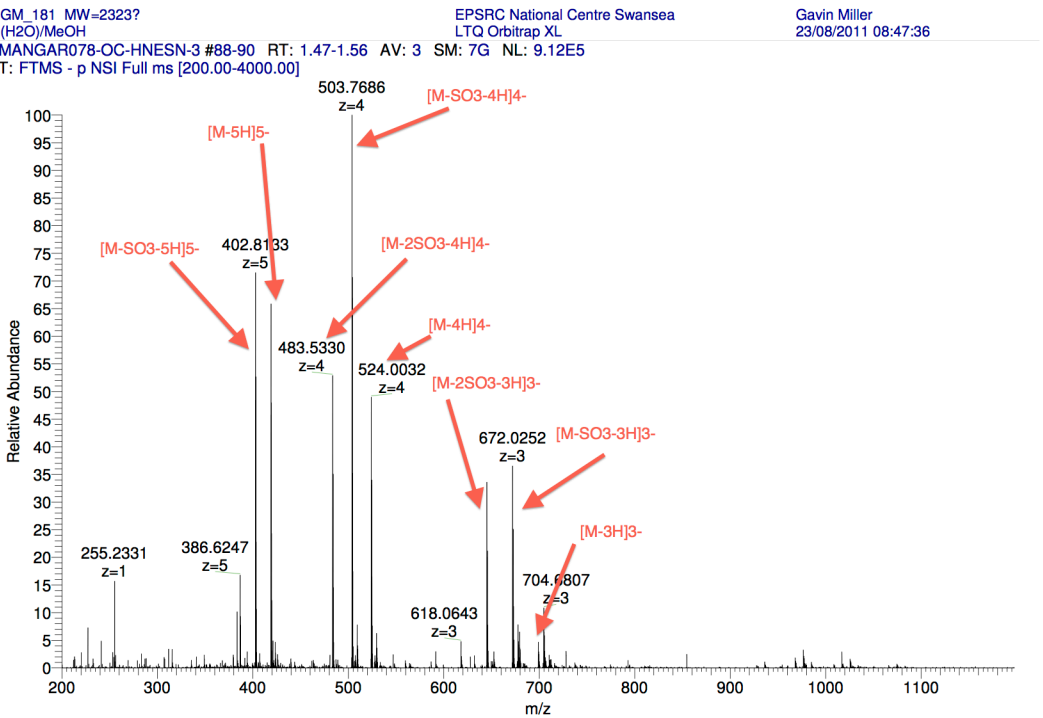
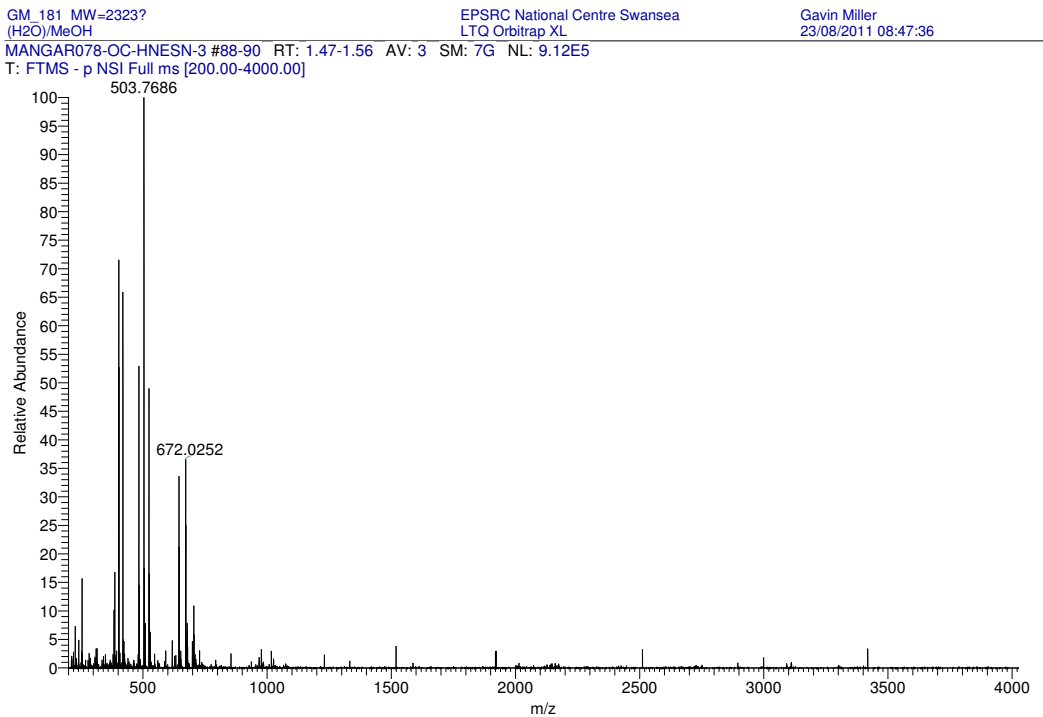
Octasaccharide Hydrogenation 23

COSY

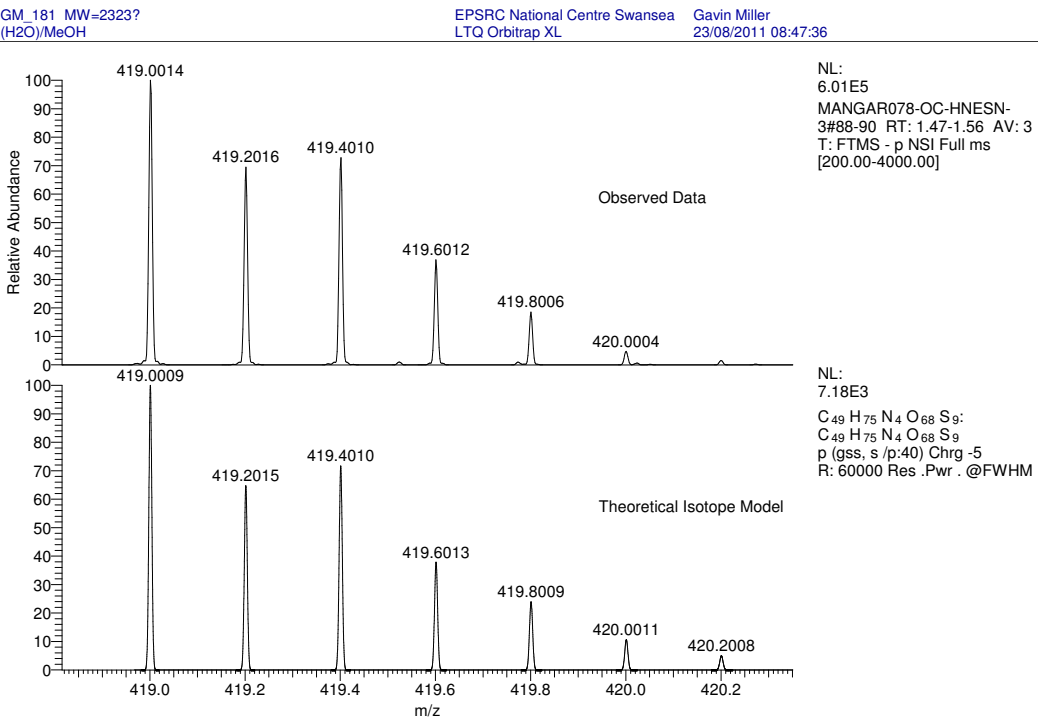
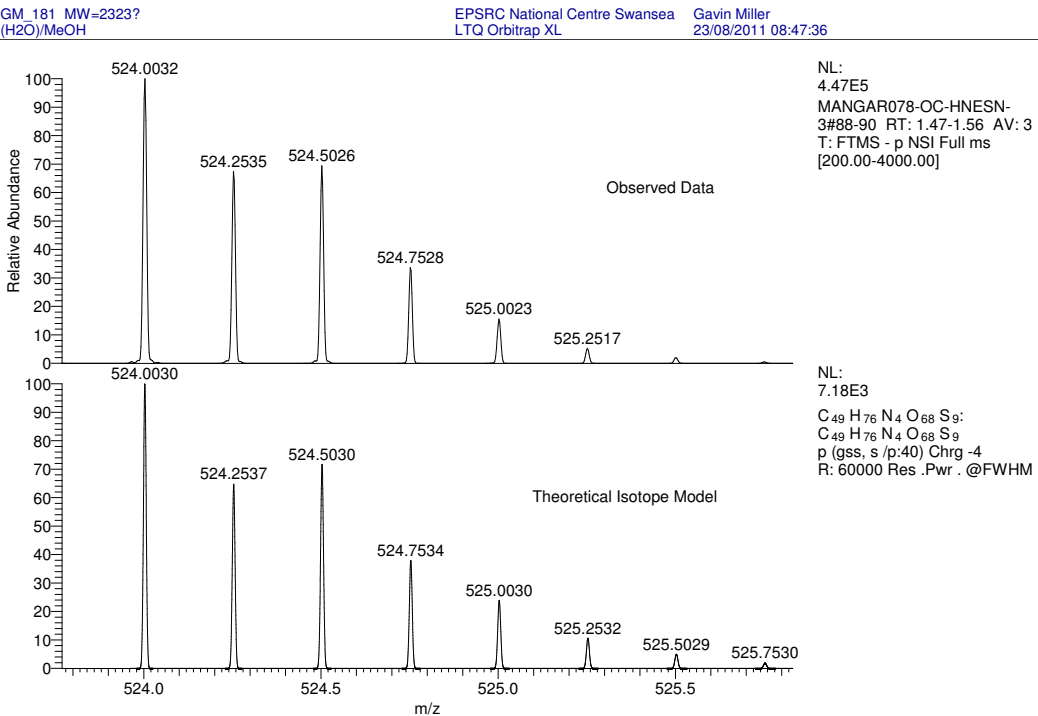
400 MHz, AcOH

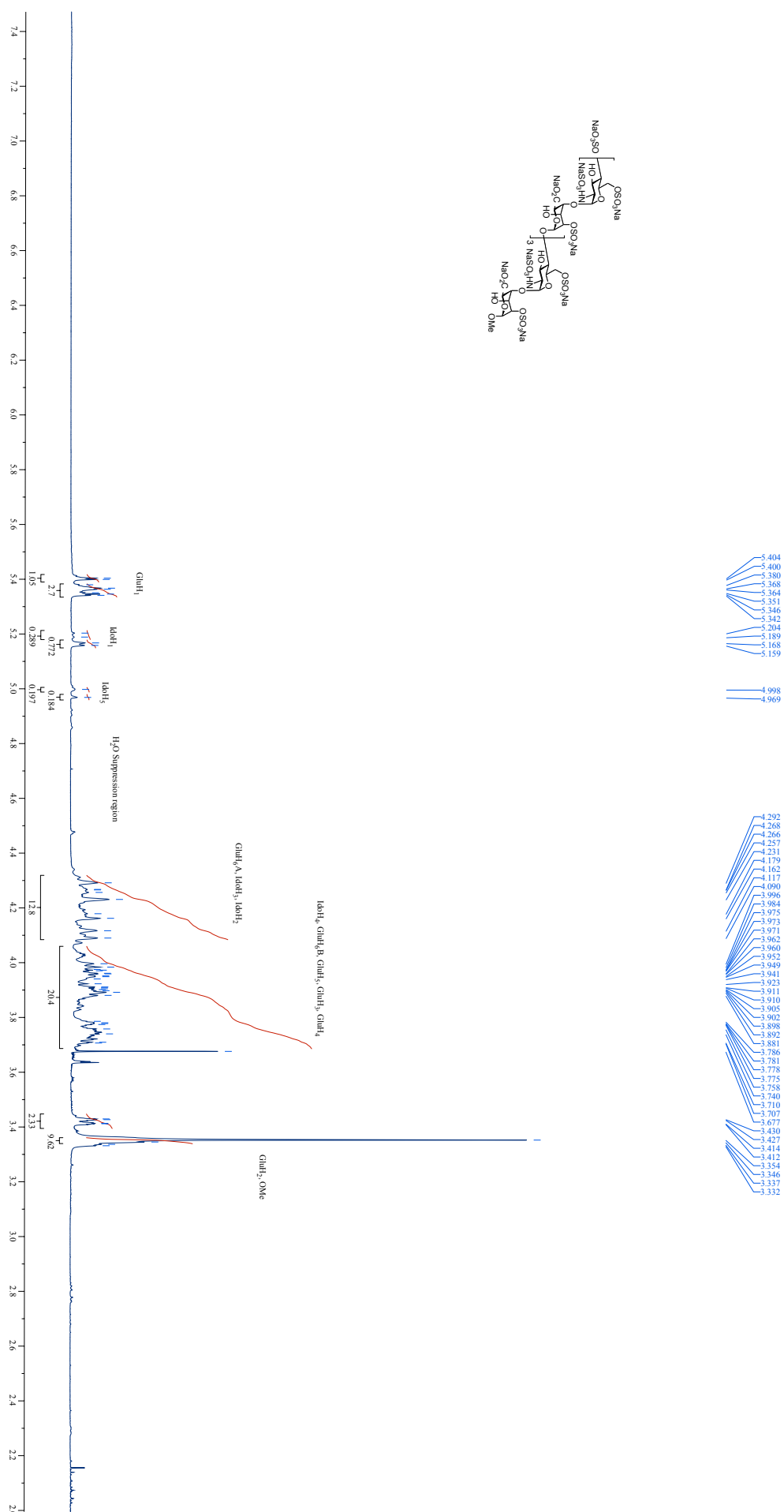


Octasaccharide Hydrogenation 23 ESI MS

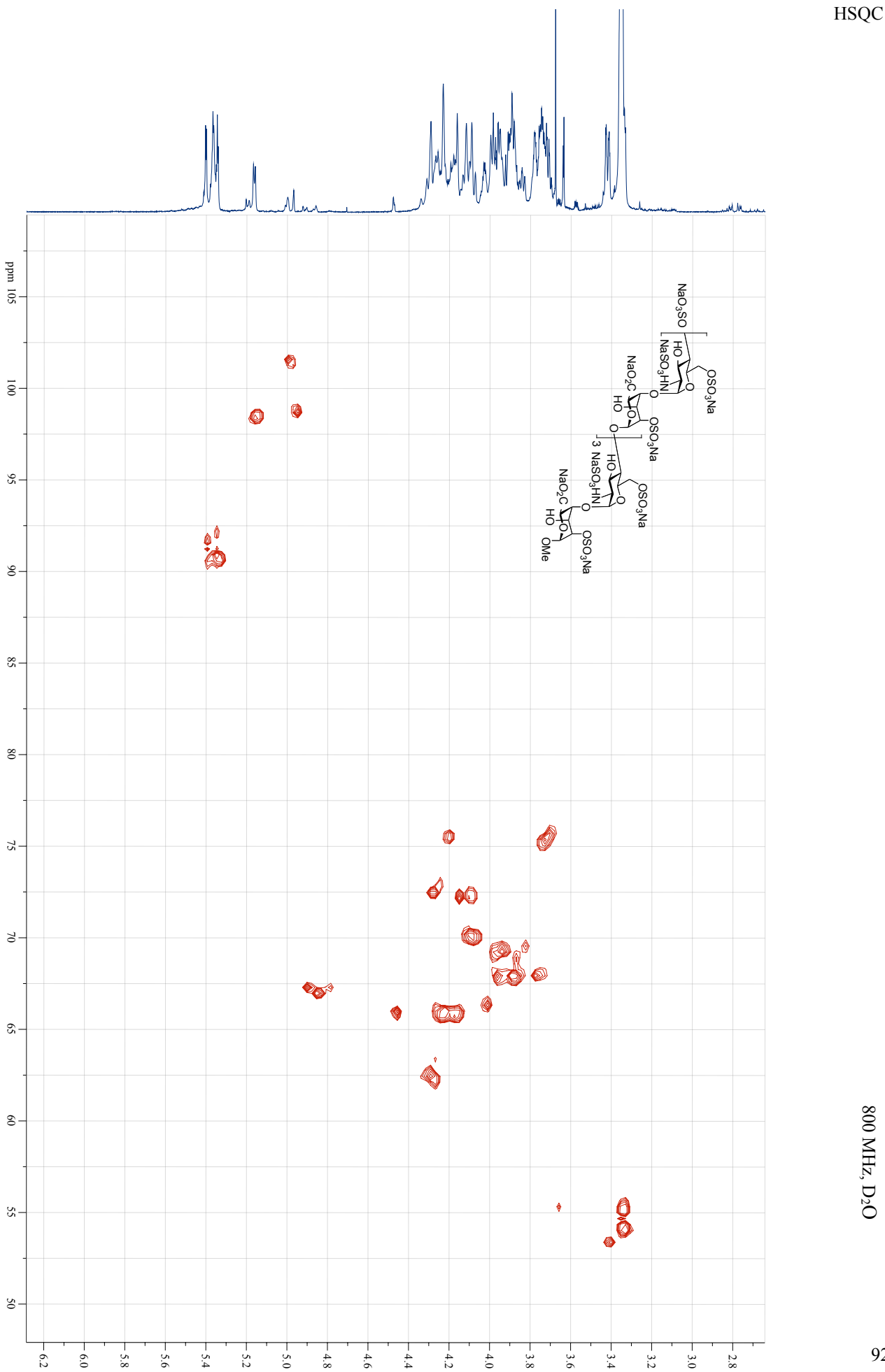


Octasaccharide Hydrogenation 23 ESI MS Isotope patterns

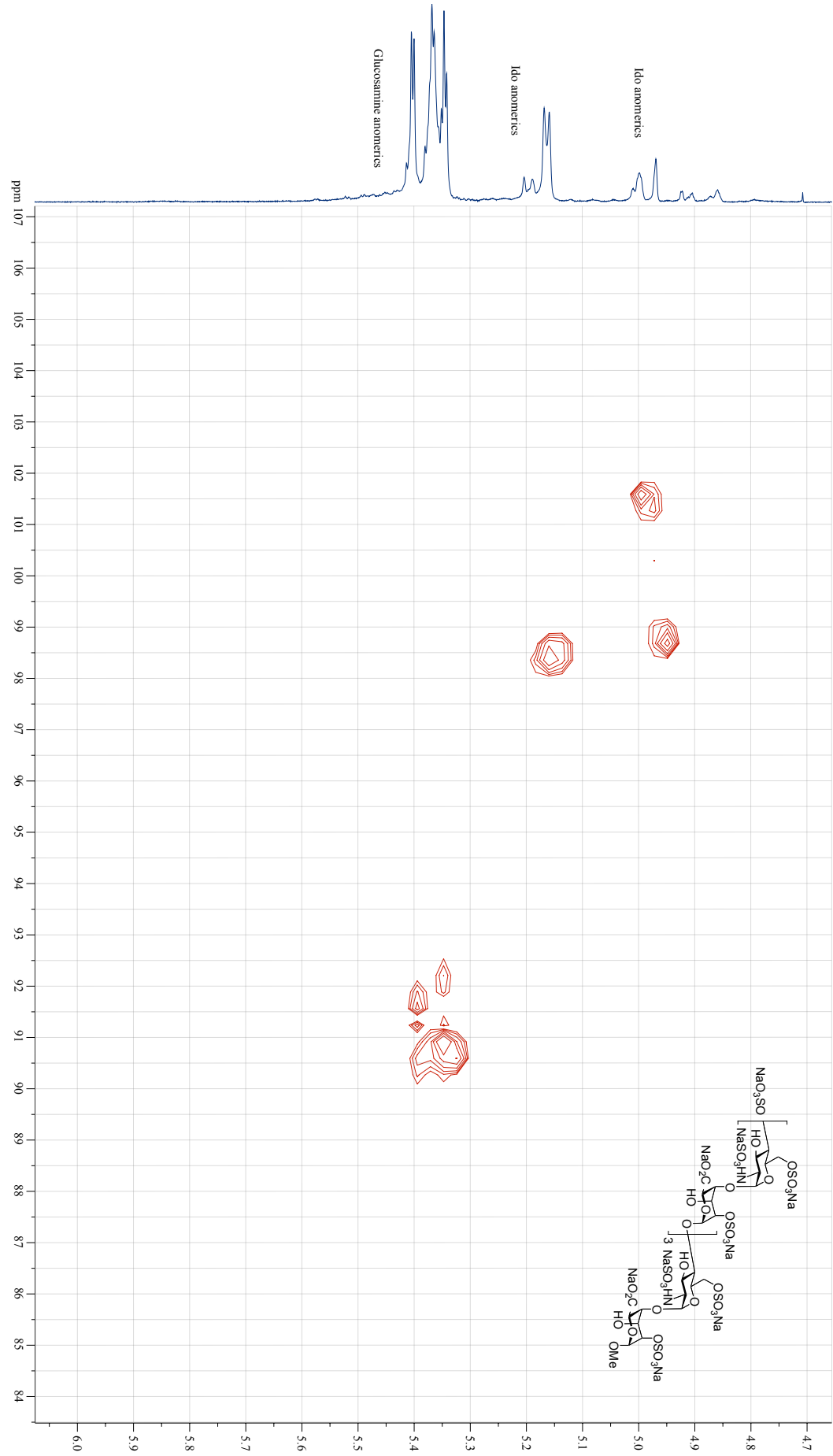




Octasaccharide *N*-sulfation 26

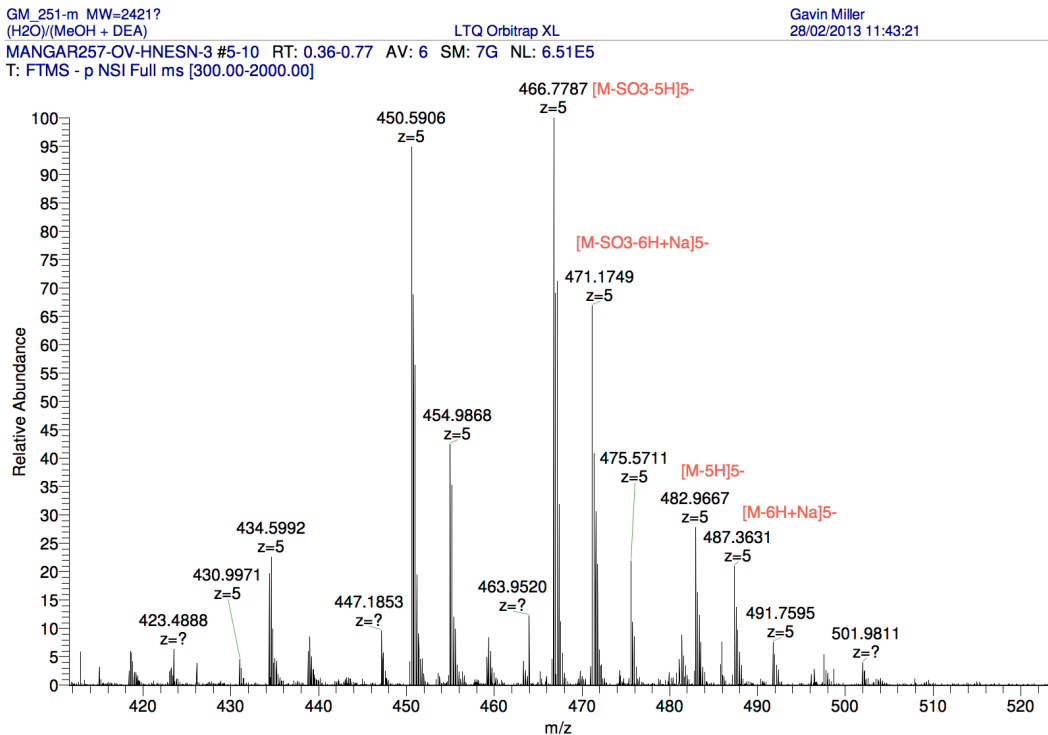
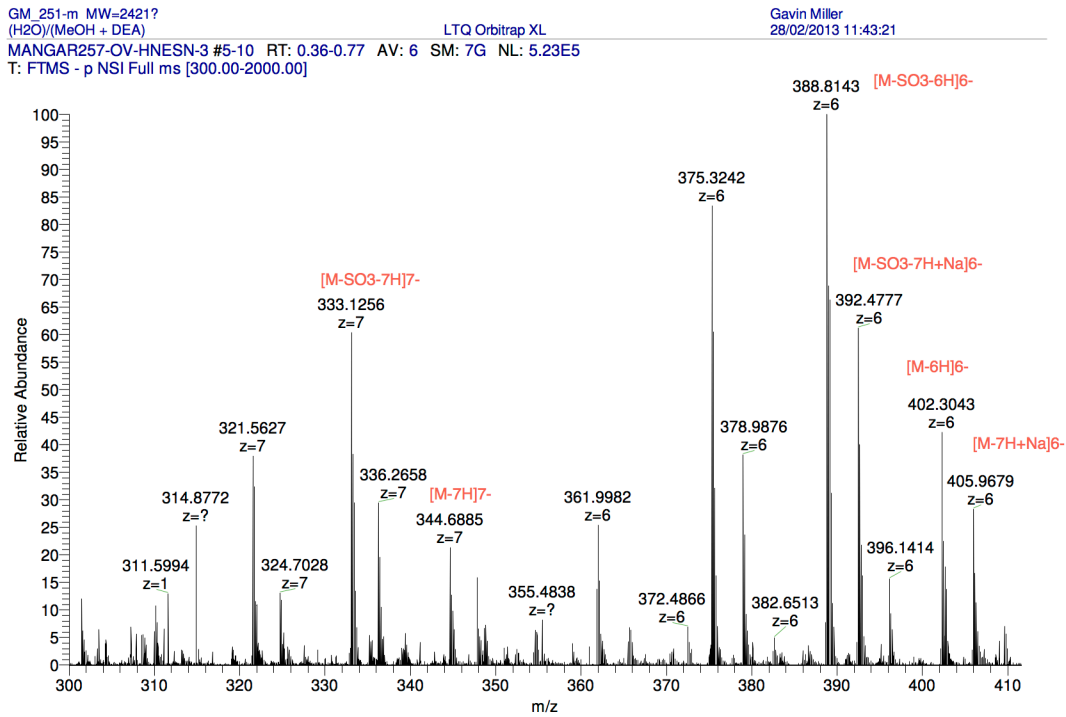


Octasaccharide *N*-sulfation 26 - HSQC expanded anomeric centres region

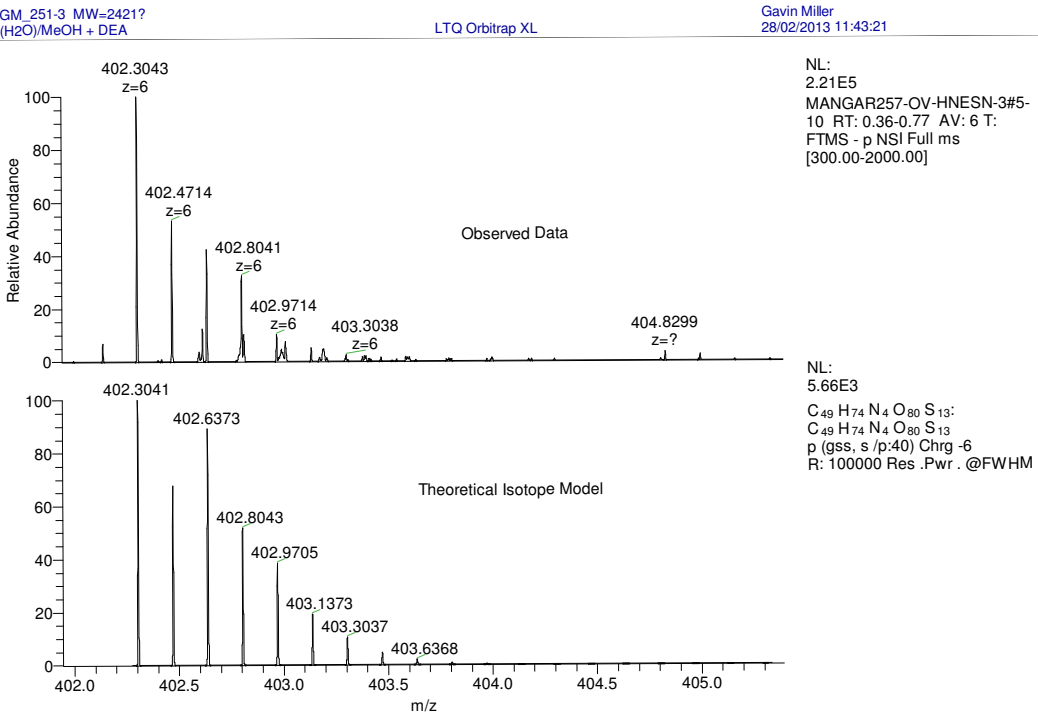
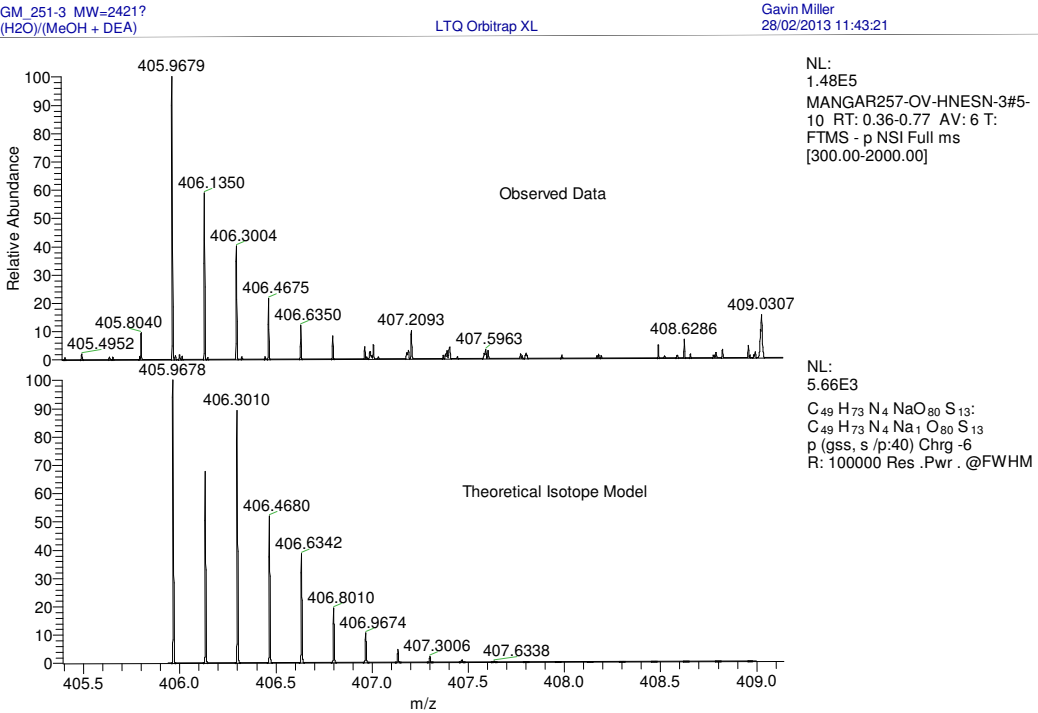


800 MHz, D₂O

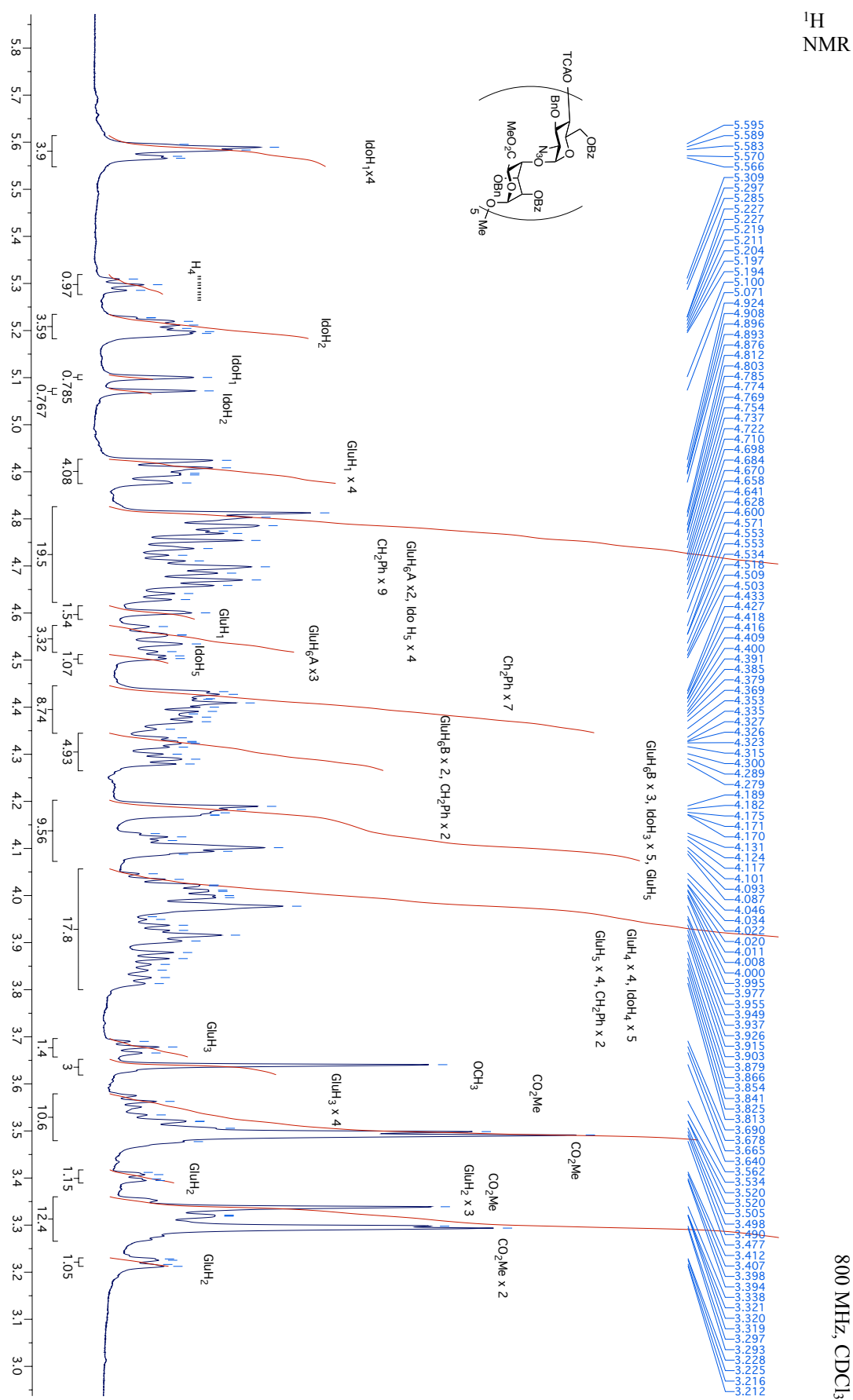
Octasaccharide *N*-sulfation 26 ESI MS



Octasaccharide N-sulfation 26 ESI MS Isotope patterns



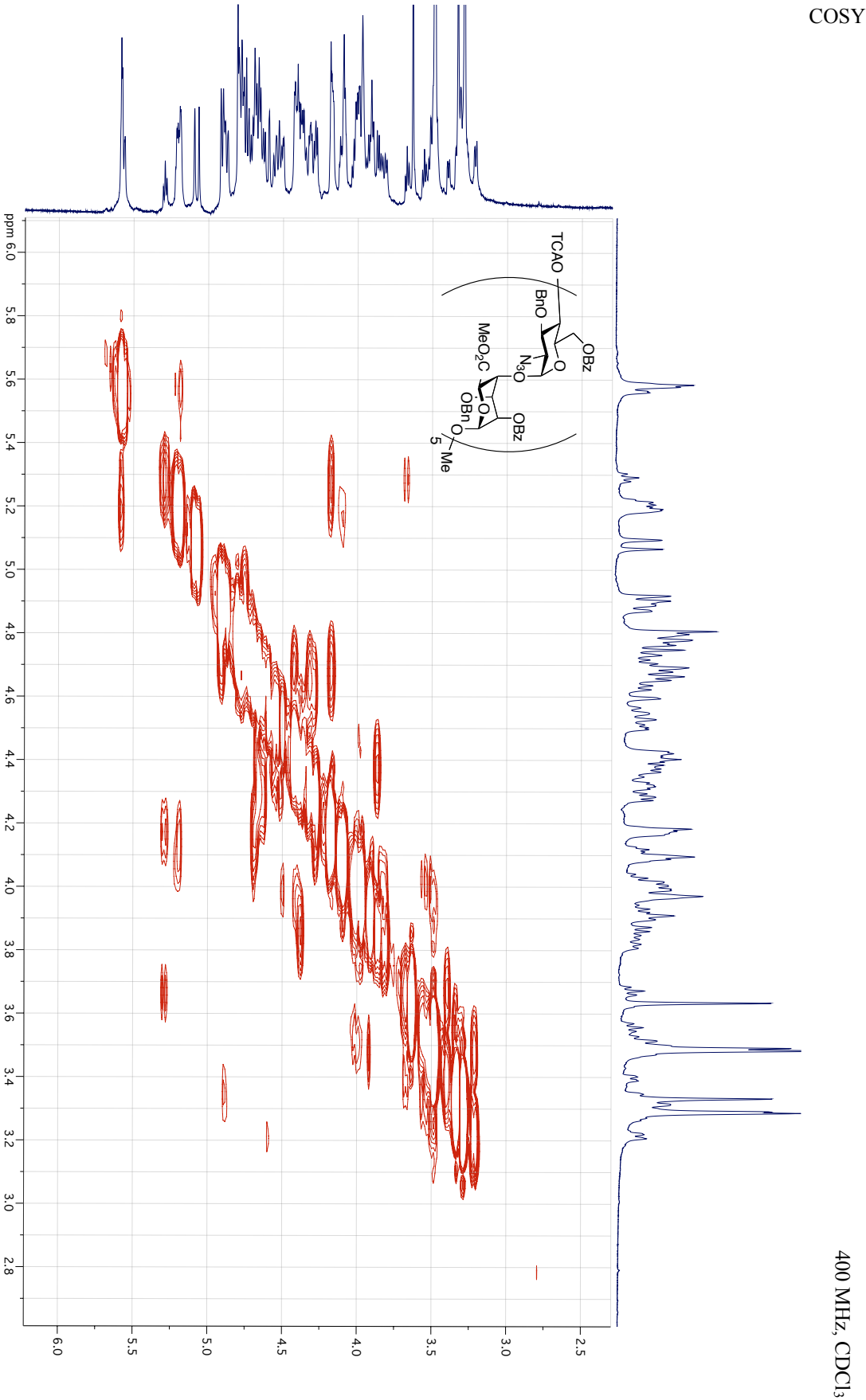
Decaasaccharide 17 - ¹H NMR Expansion



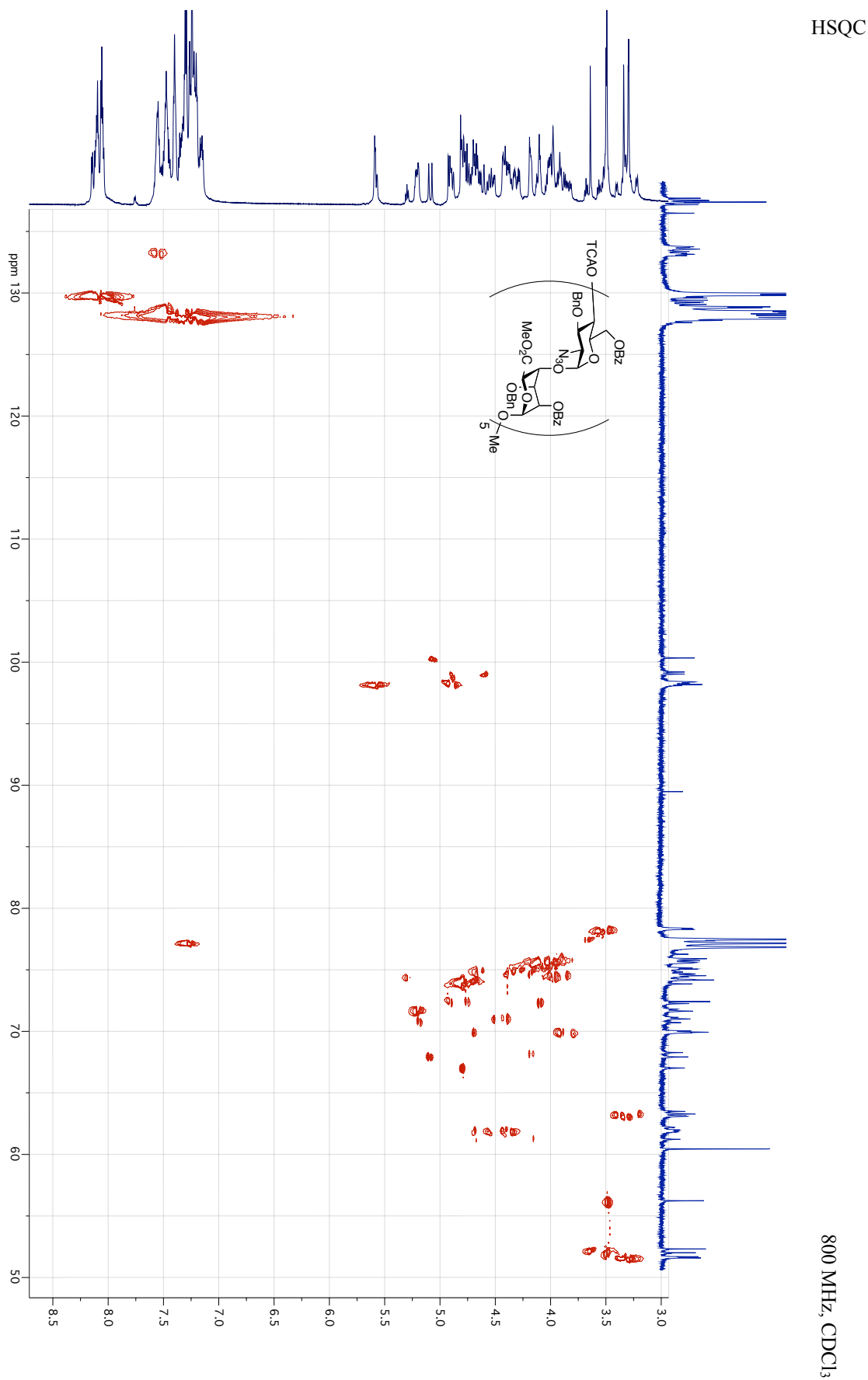
200 MHz, CDCl₃



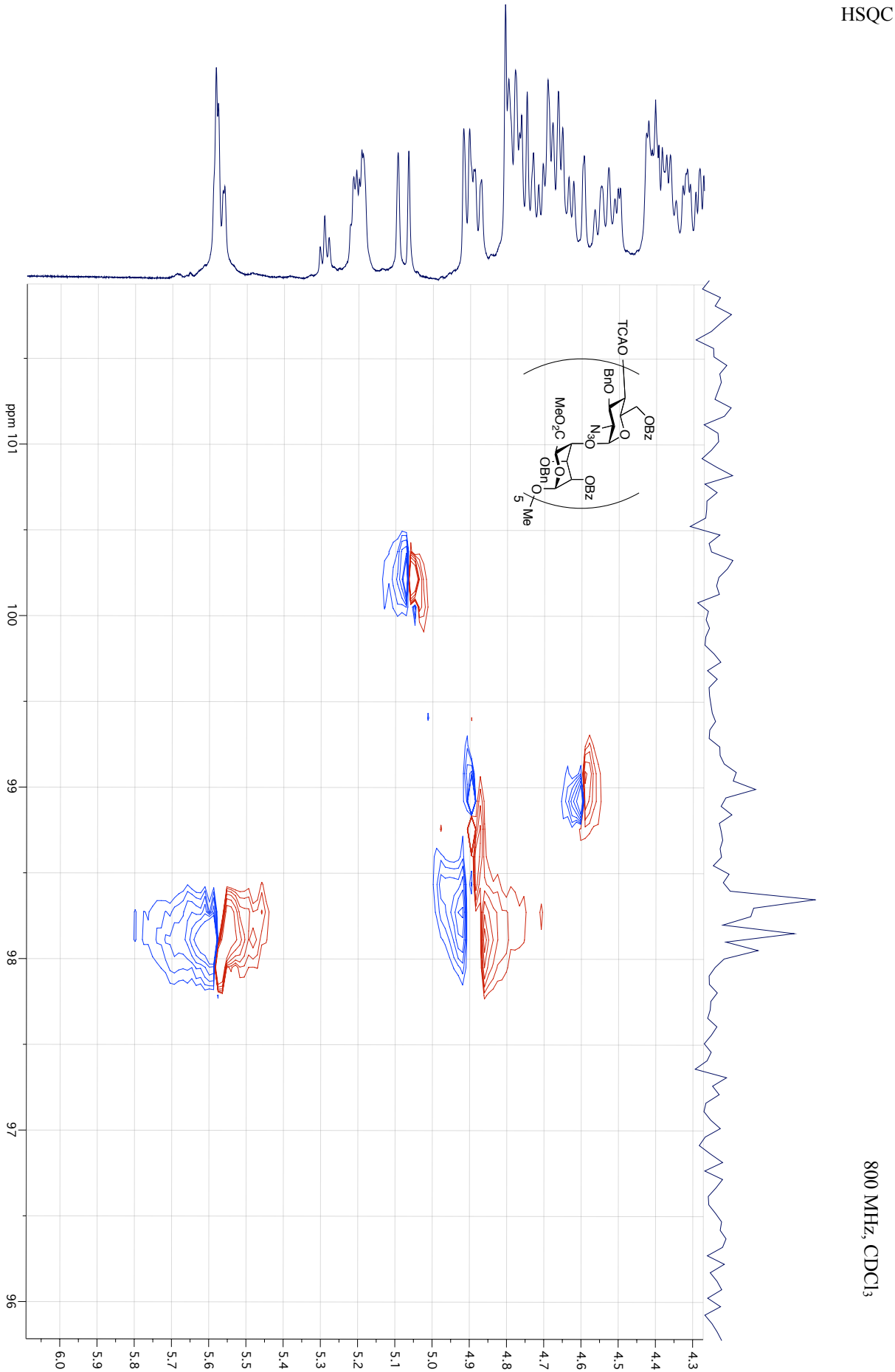
Decasaccharide 17



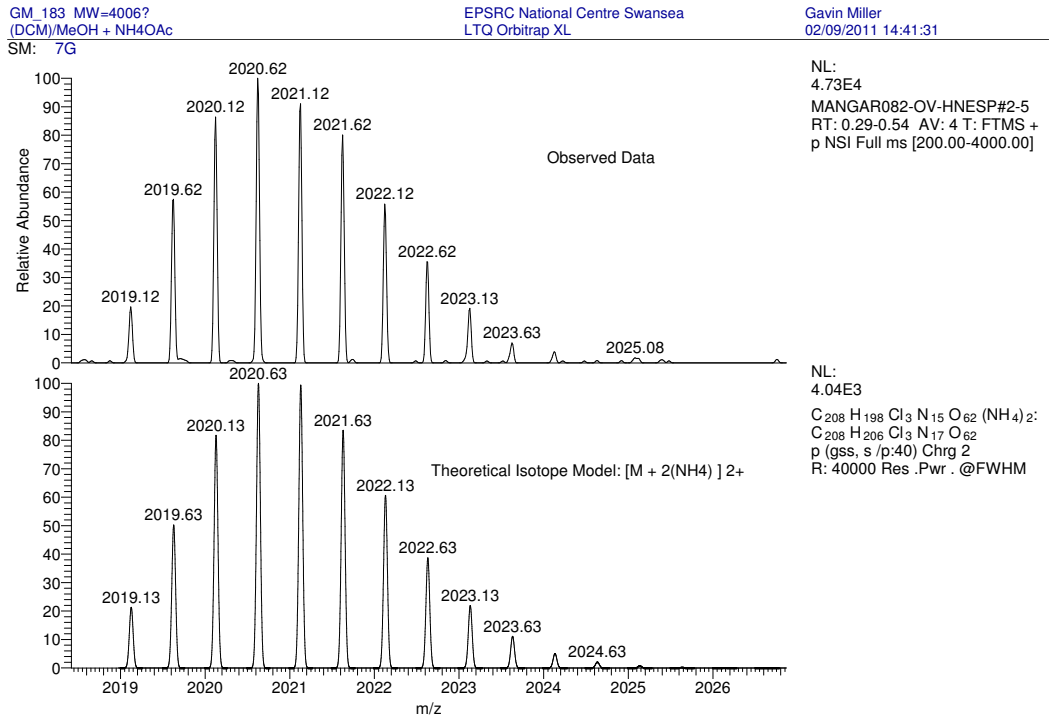
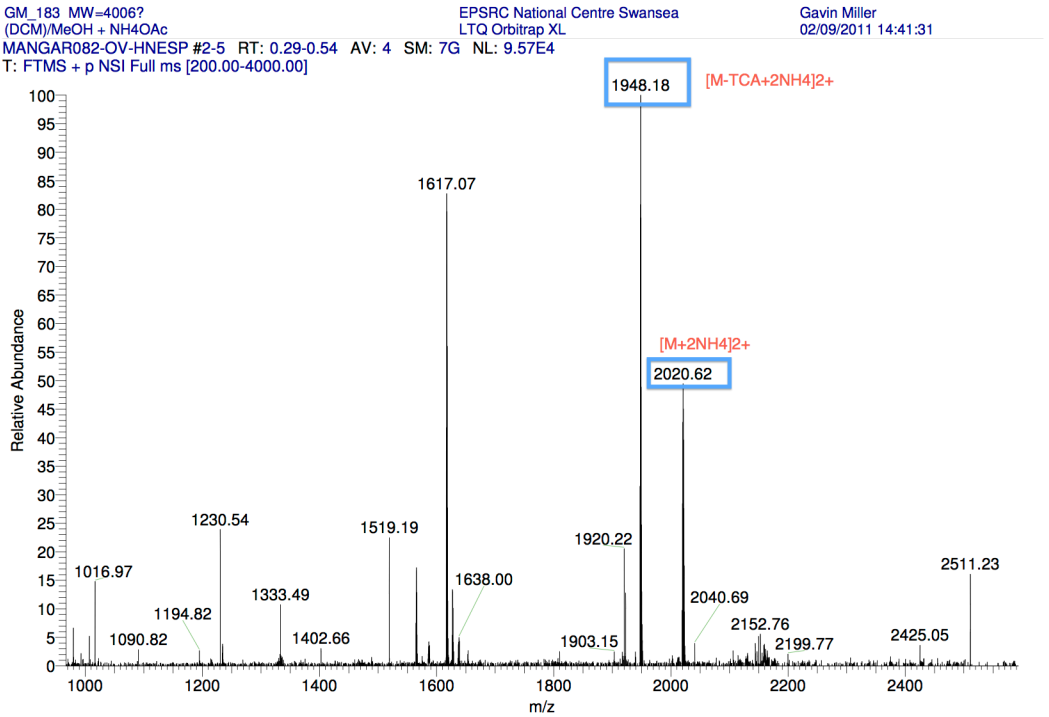
Decasaccharide 17



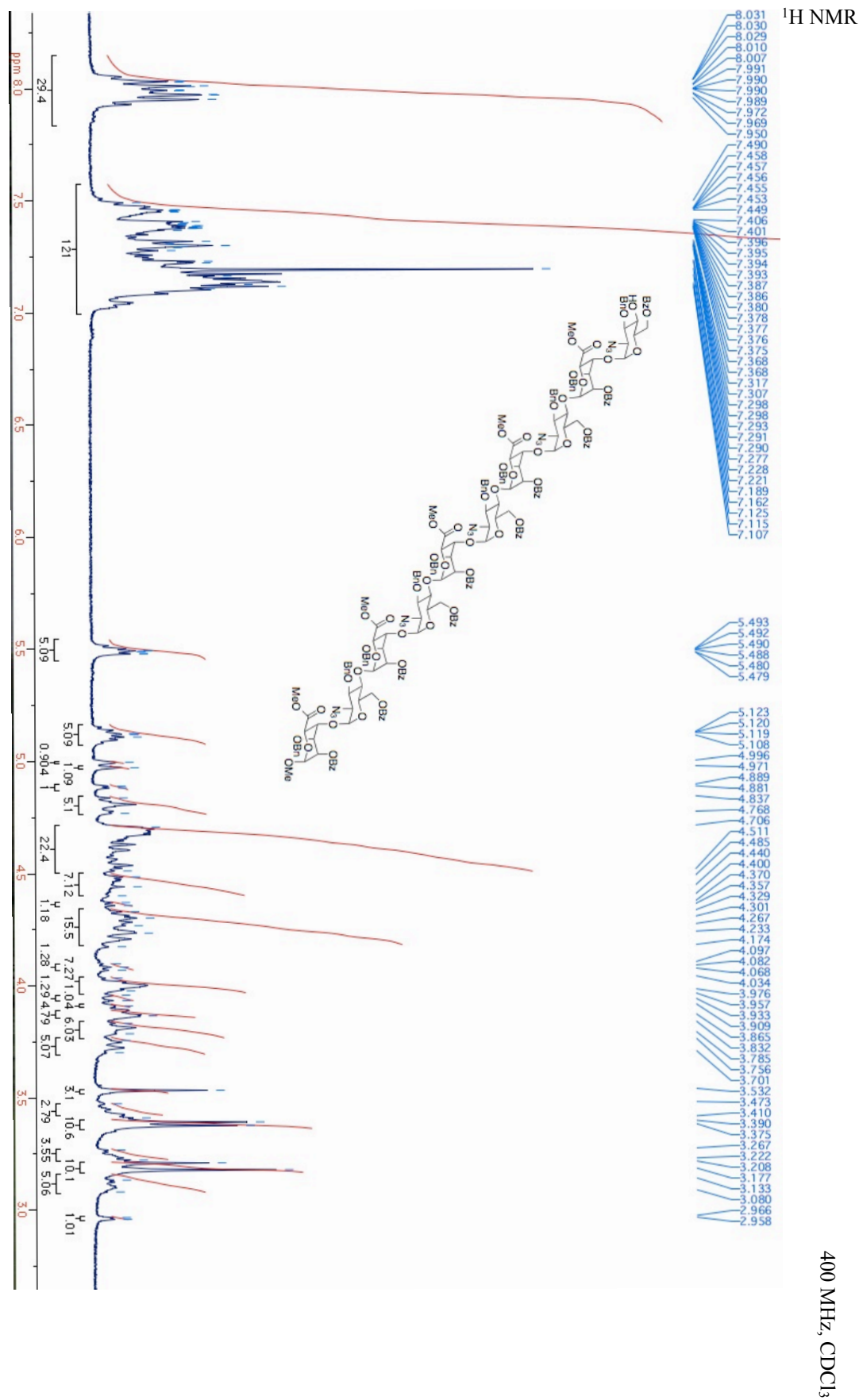
Decasaccharide 17 HSQC Expanded Anomeric Centres Region



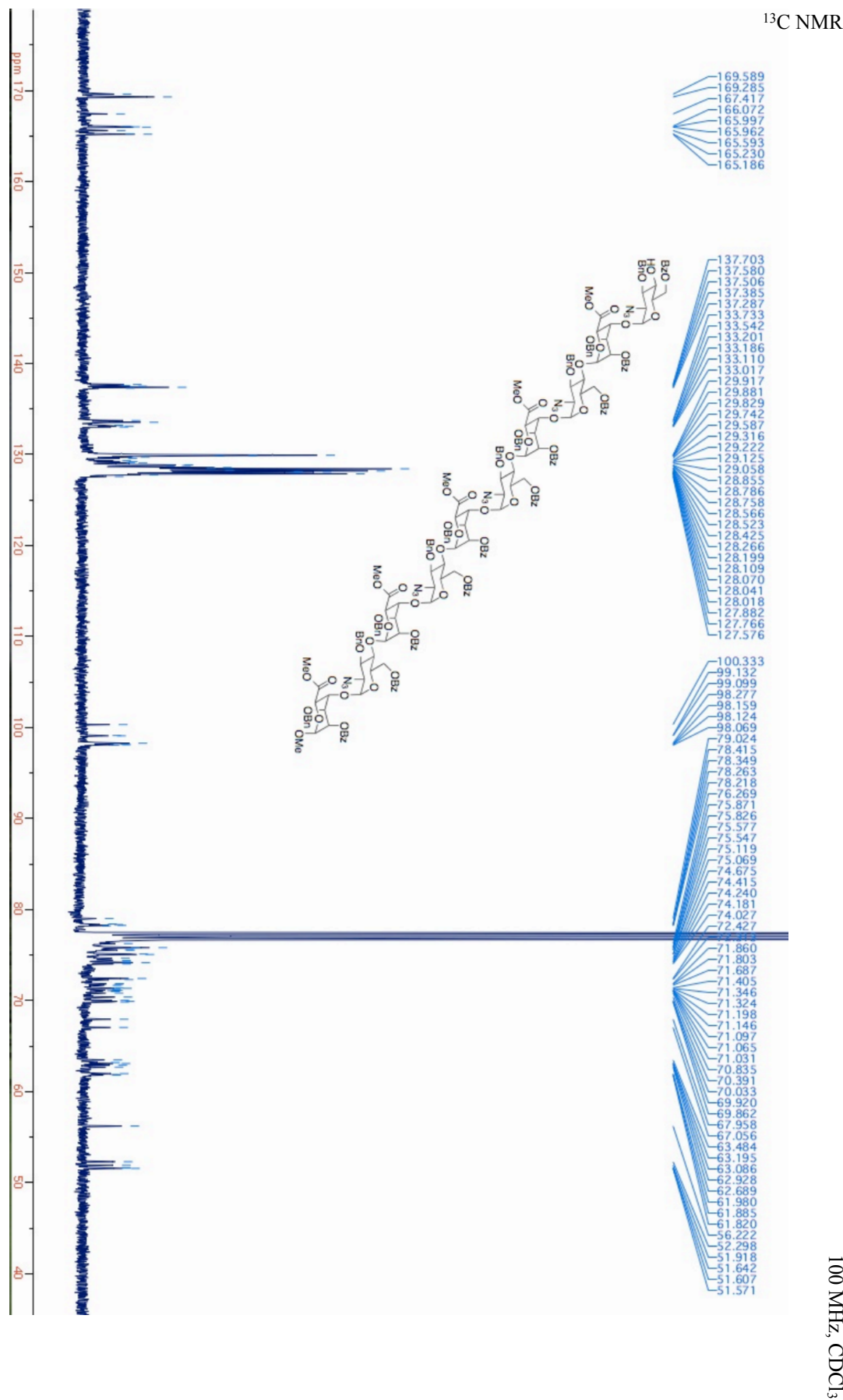
Decasaccharide 17 ESMS and Isotope Pattern



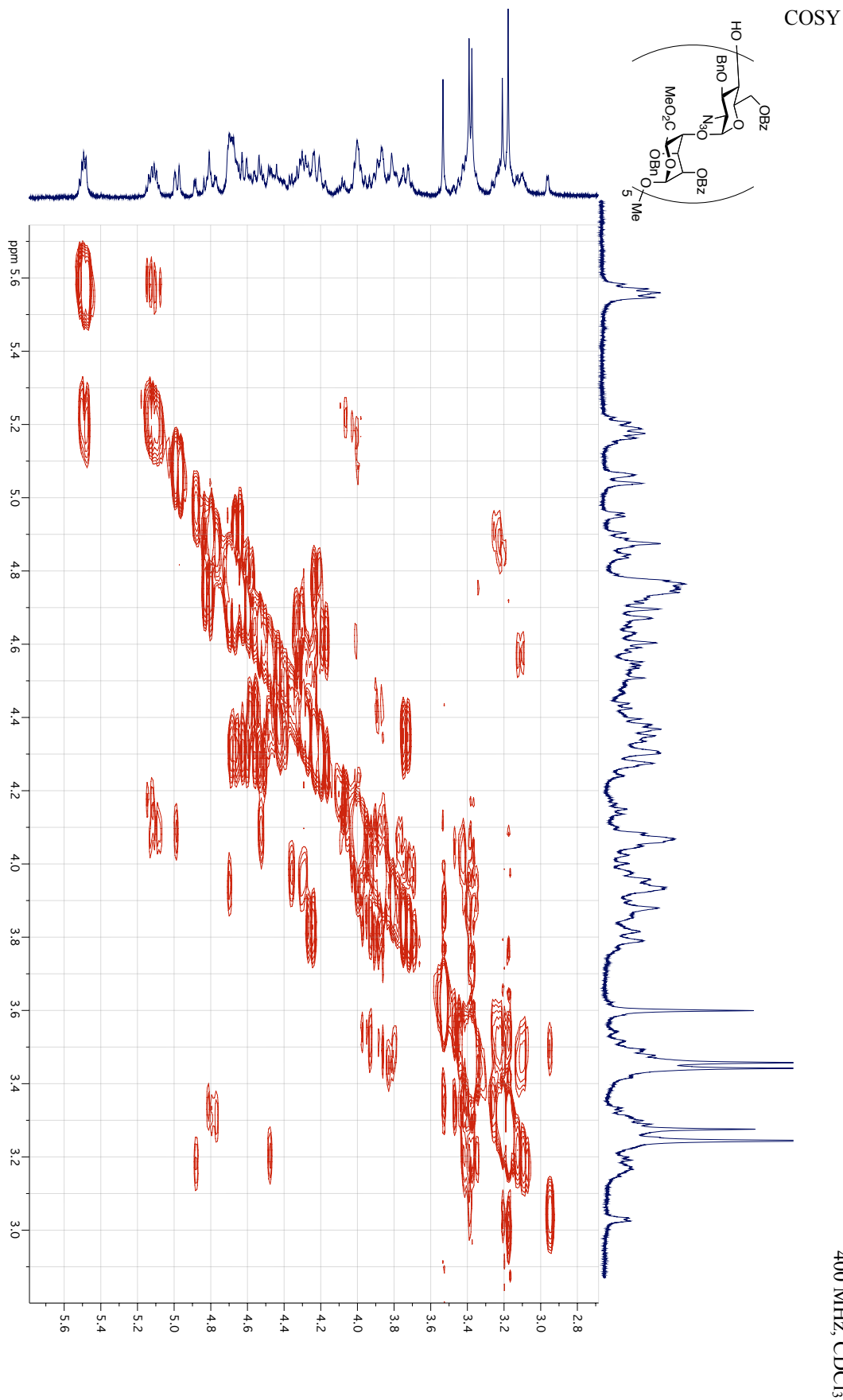
Decasaccharide 18



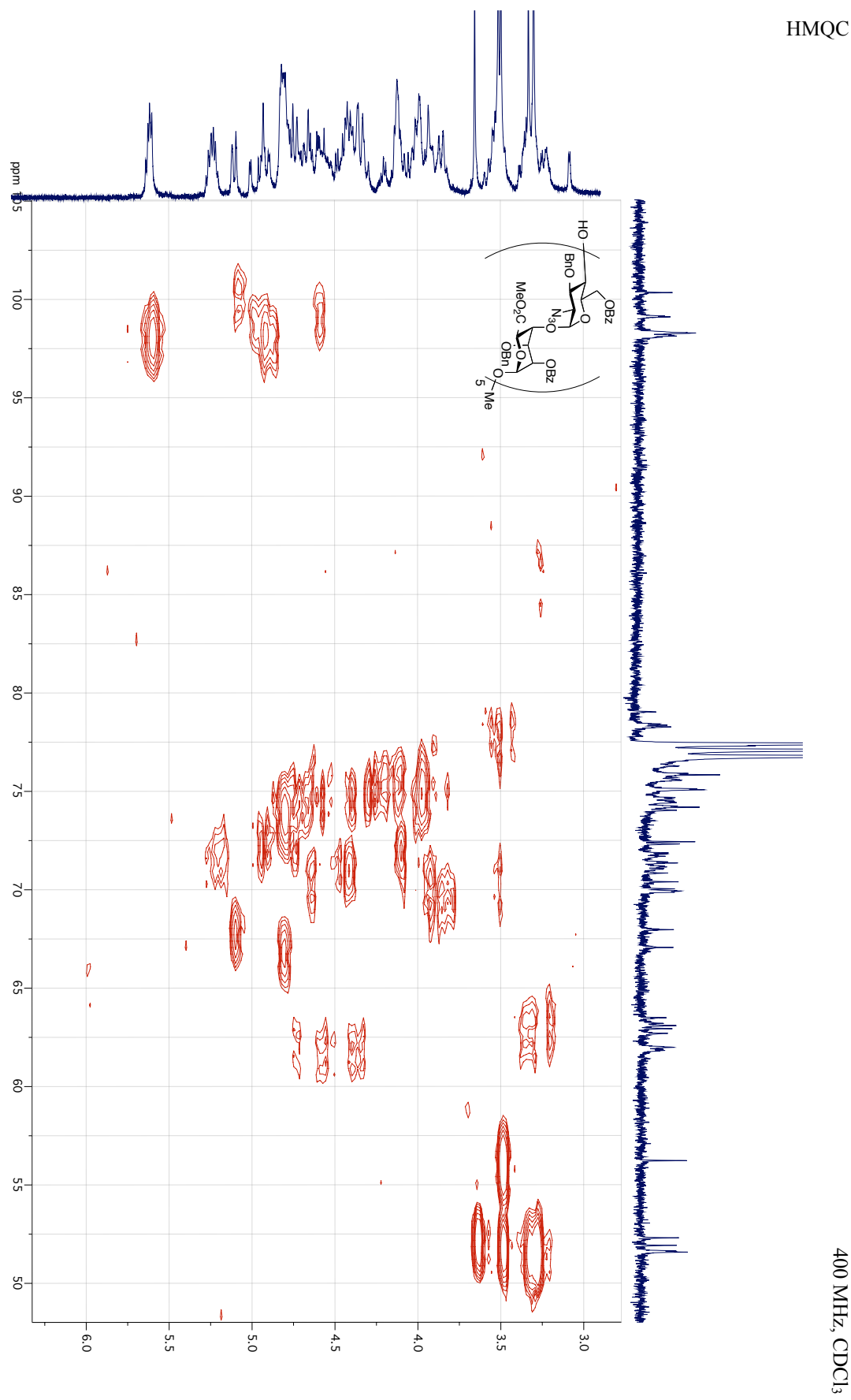
Decasaccharide 18



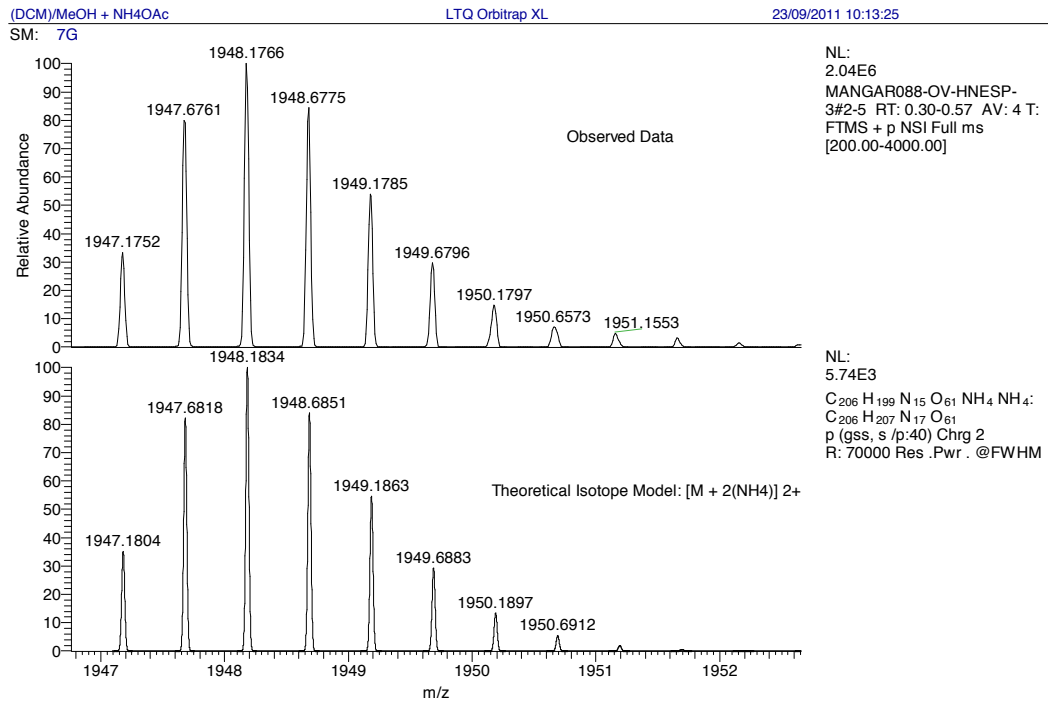
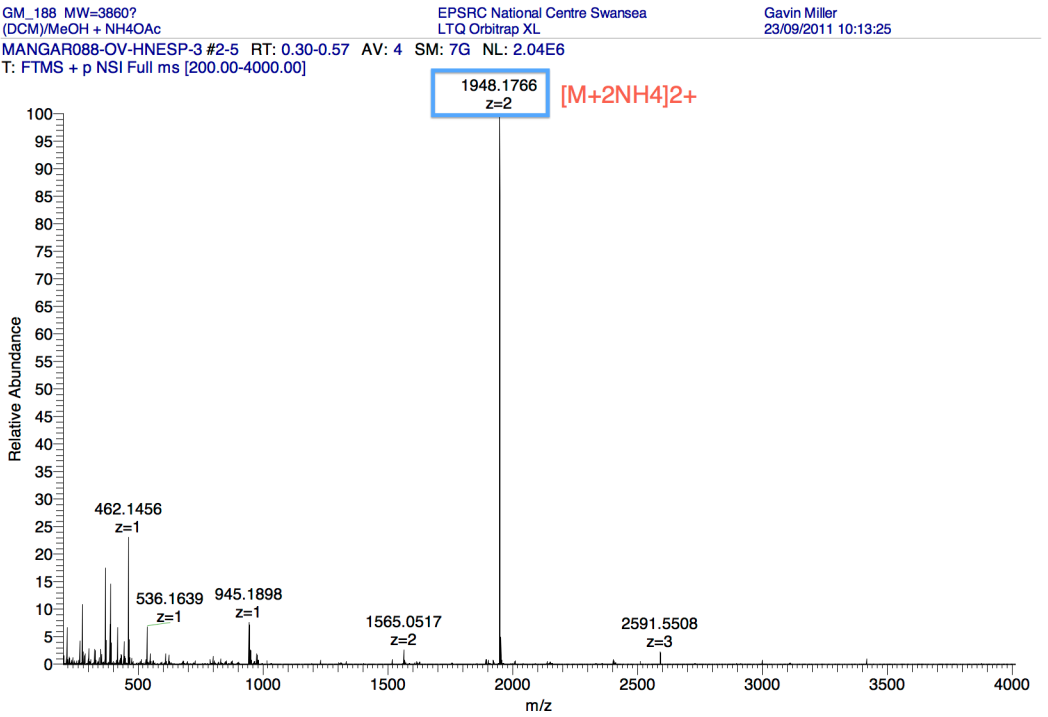
Decasaccharide 18



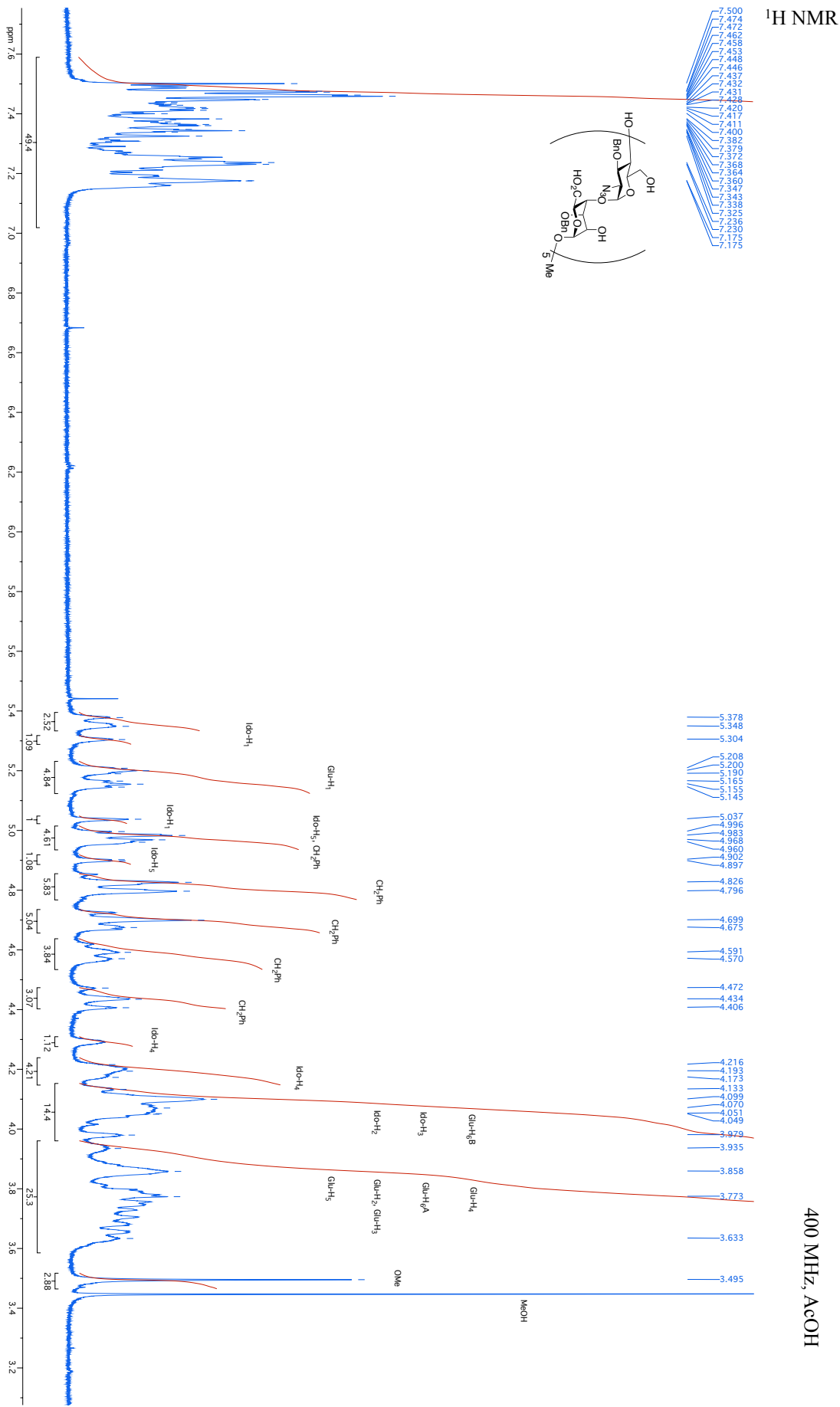
Decasaccharide 18



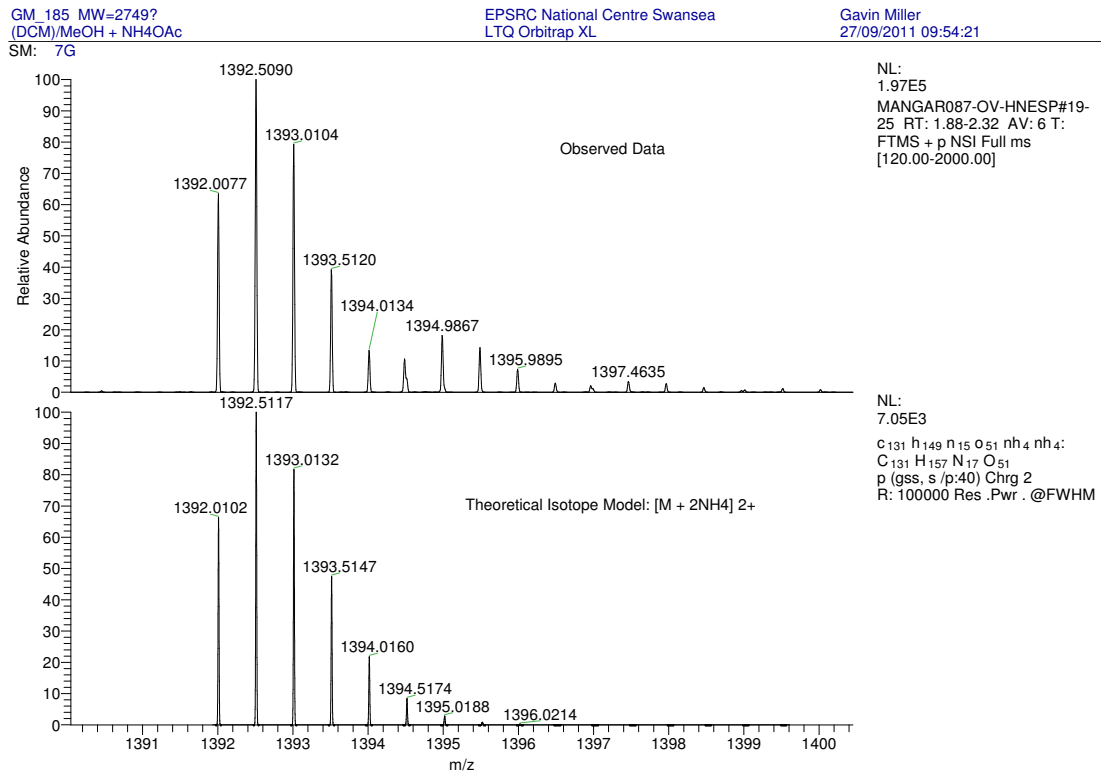
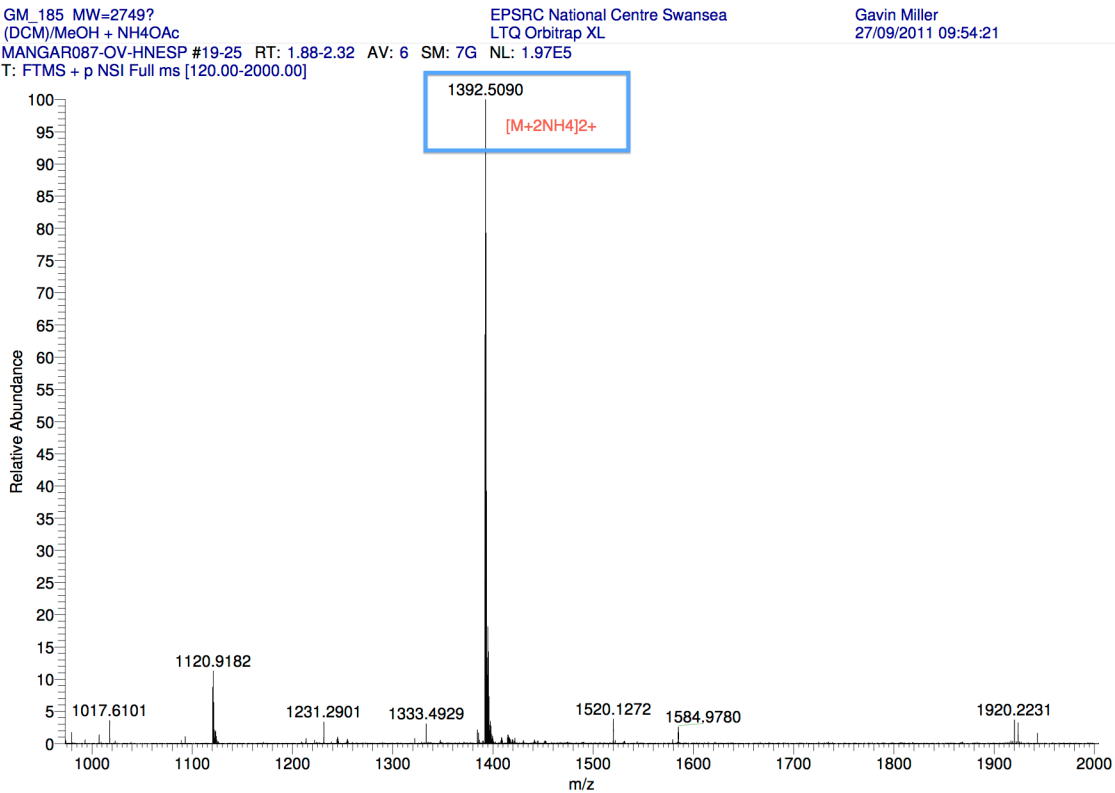
Decasaccharide 18 - FTMS and Isotope Pattern



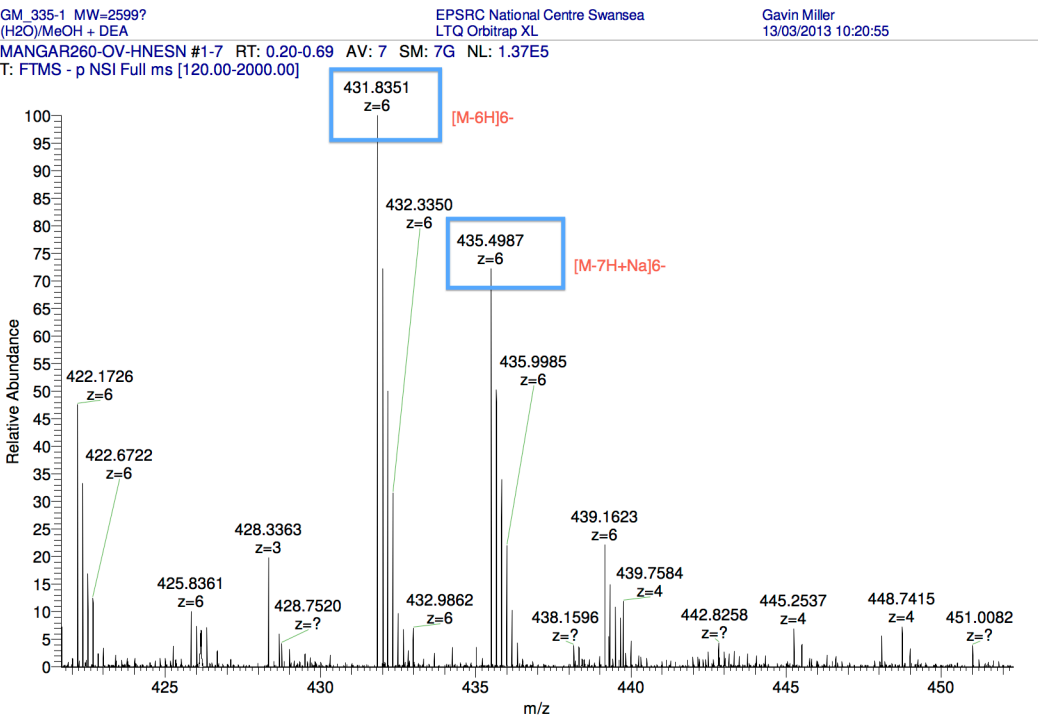
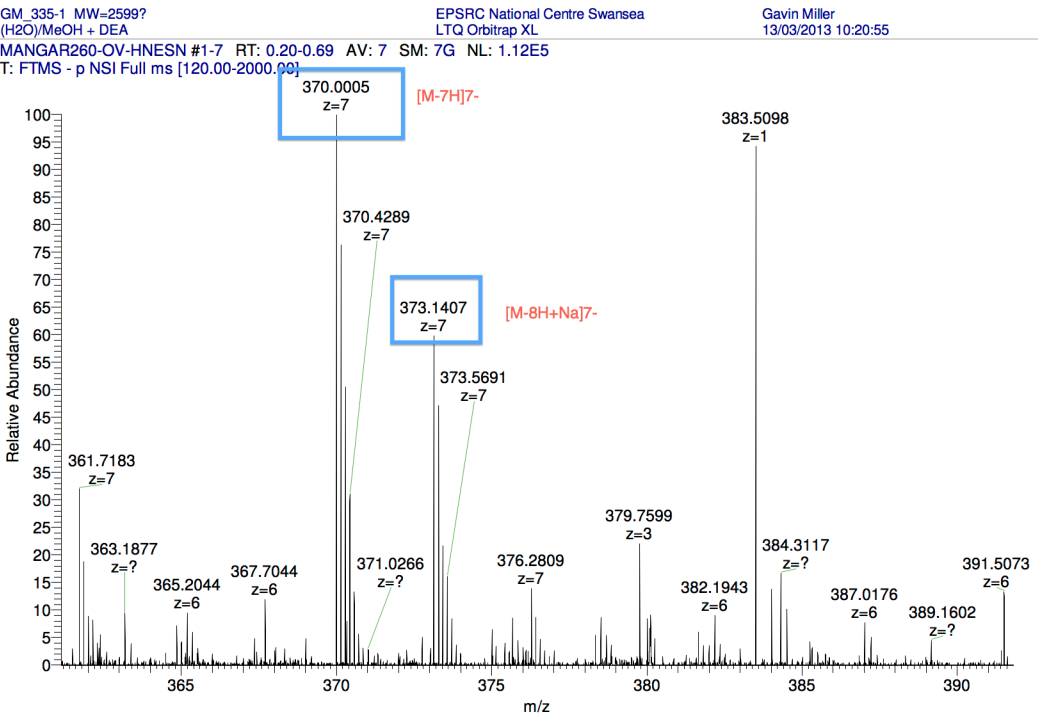
Decasaccharide Saponification 21



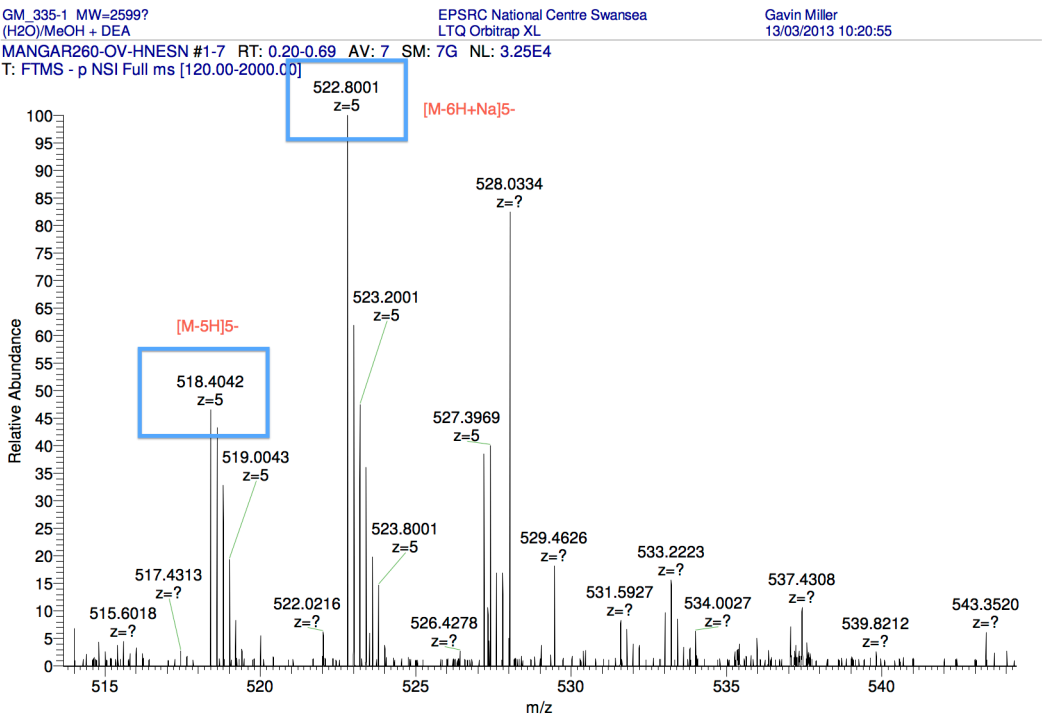
Decasaccharide Saponification 21 FTMS and Isotope Pattern



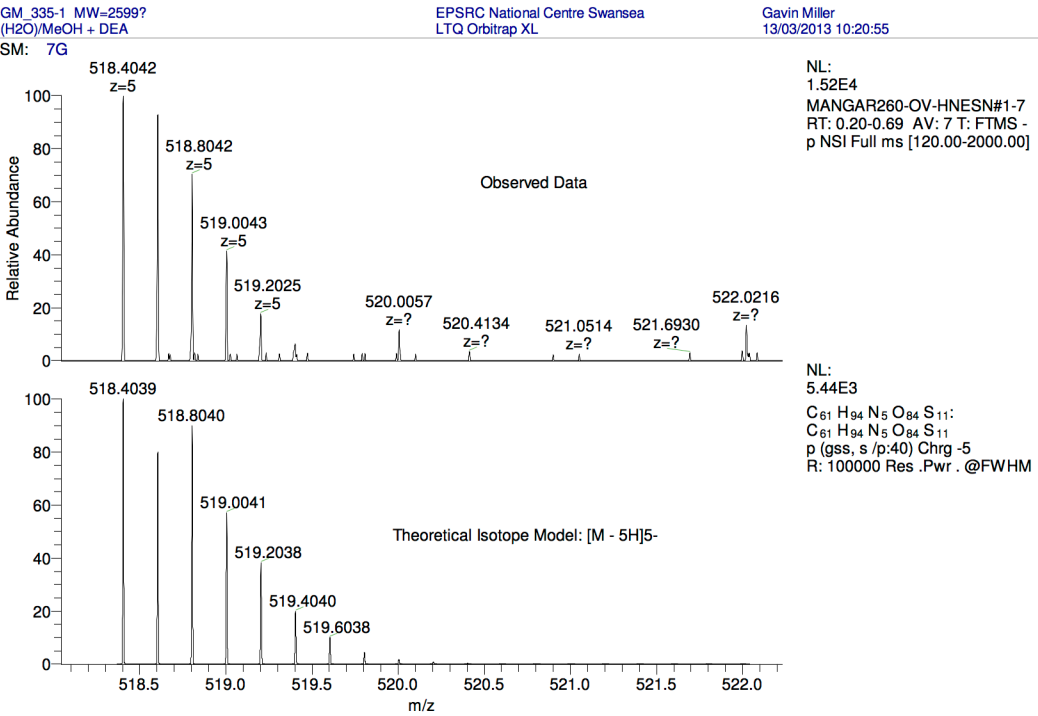
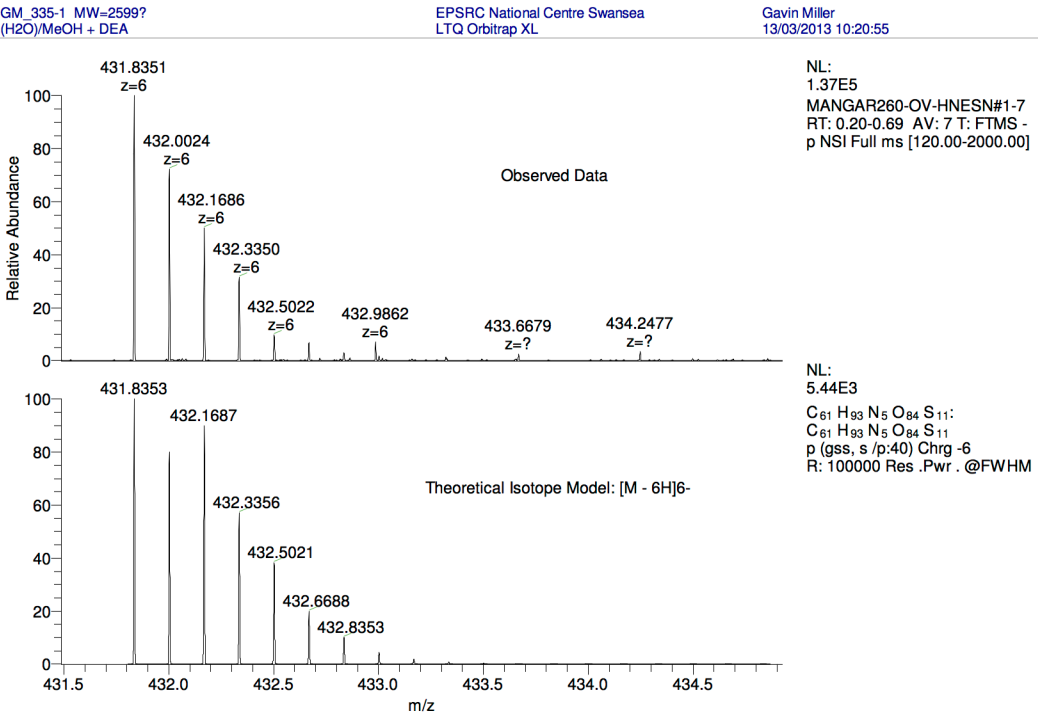
Decasaccharide Hydrogenation 24 - ESI MS



Decasaccharide Hydrogenation 24 - ESI MS

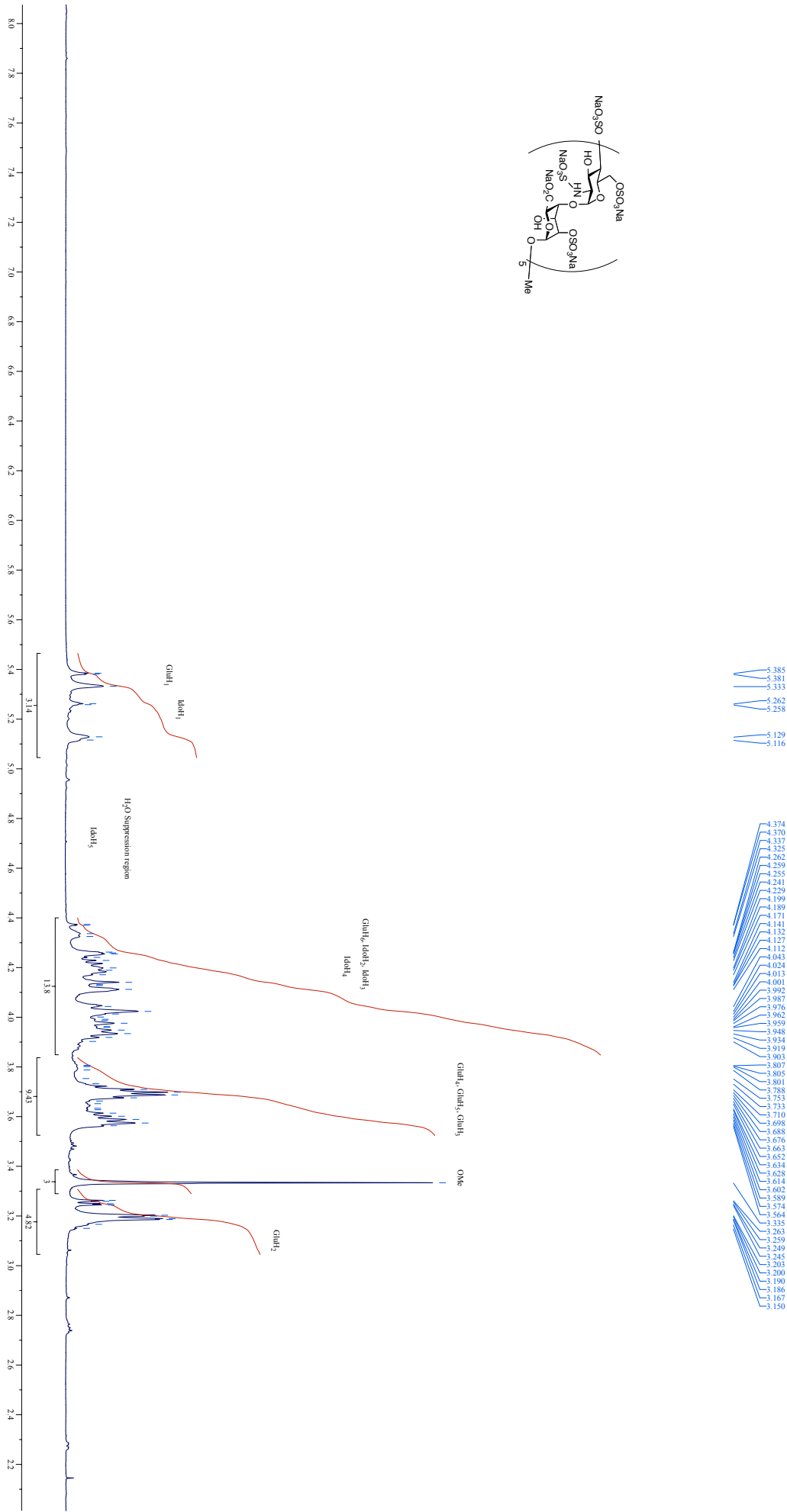


Decasaccharide Hydrogenation 24 - ESI MS Isotope Patterns

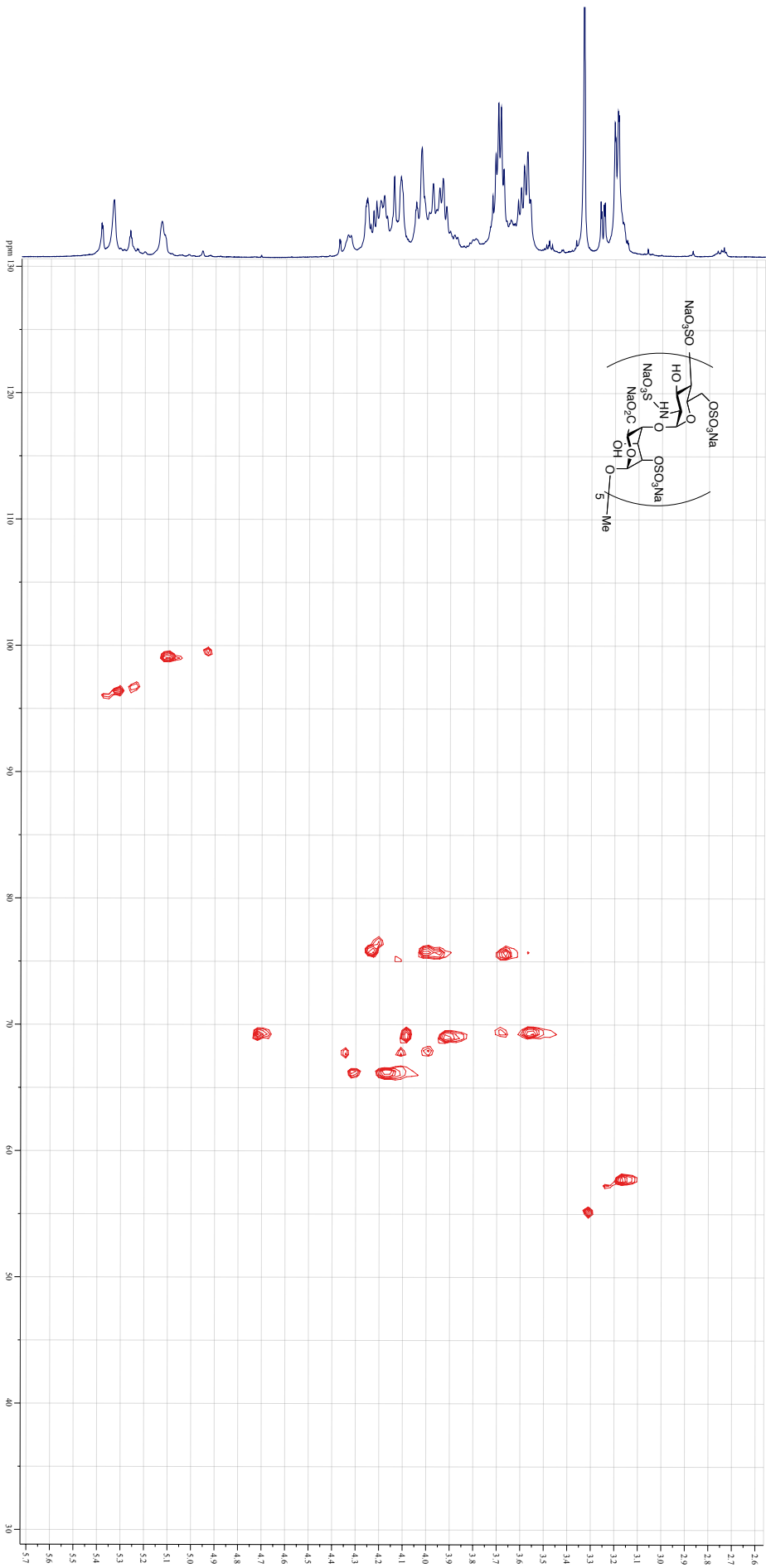


Decasaccharide *N*-sulfation 27

¹H NMR

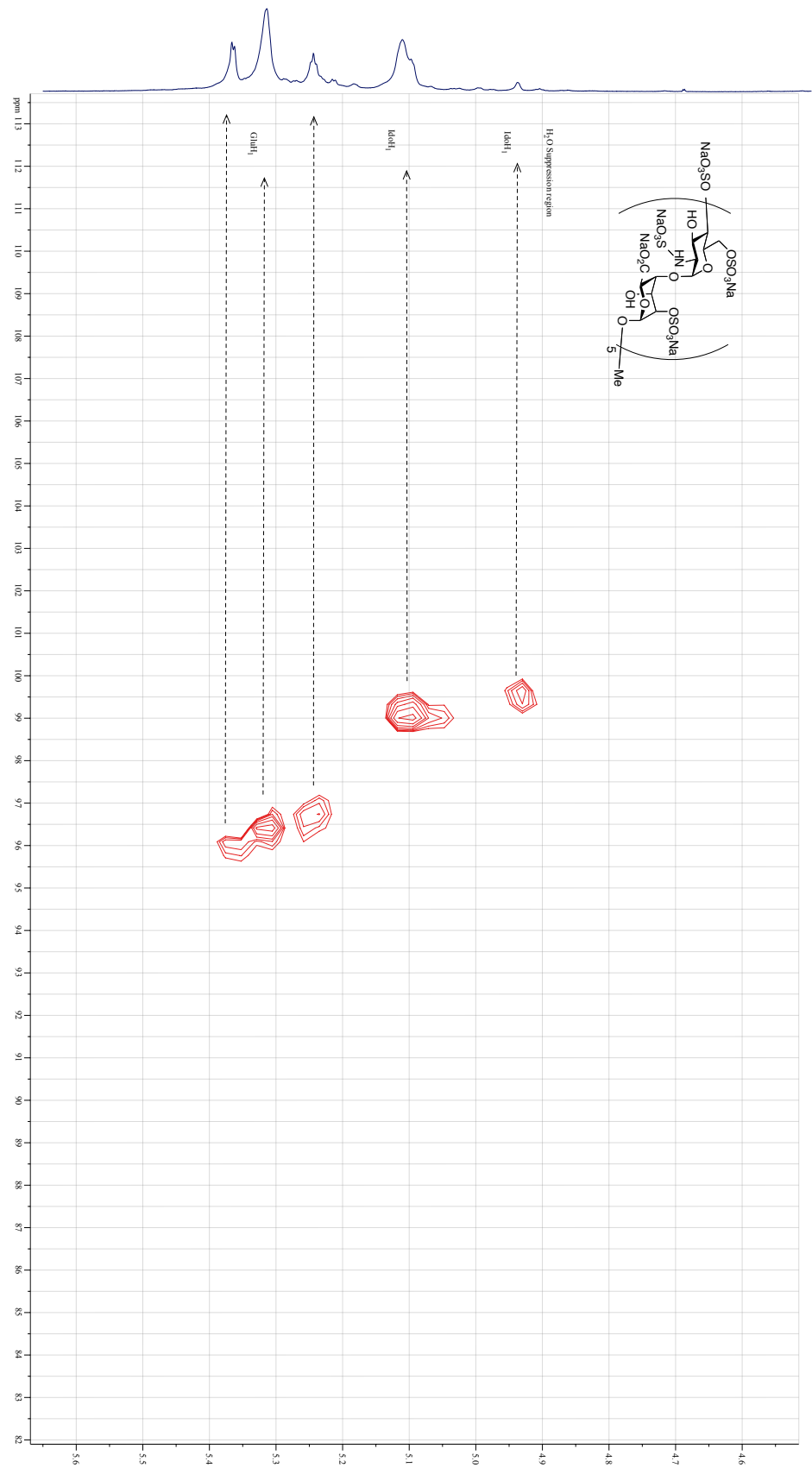


Decasaccharide *N*-sulfation 27



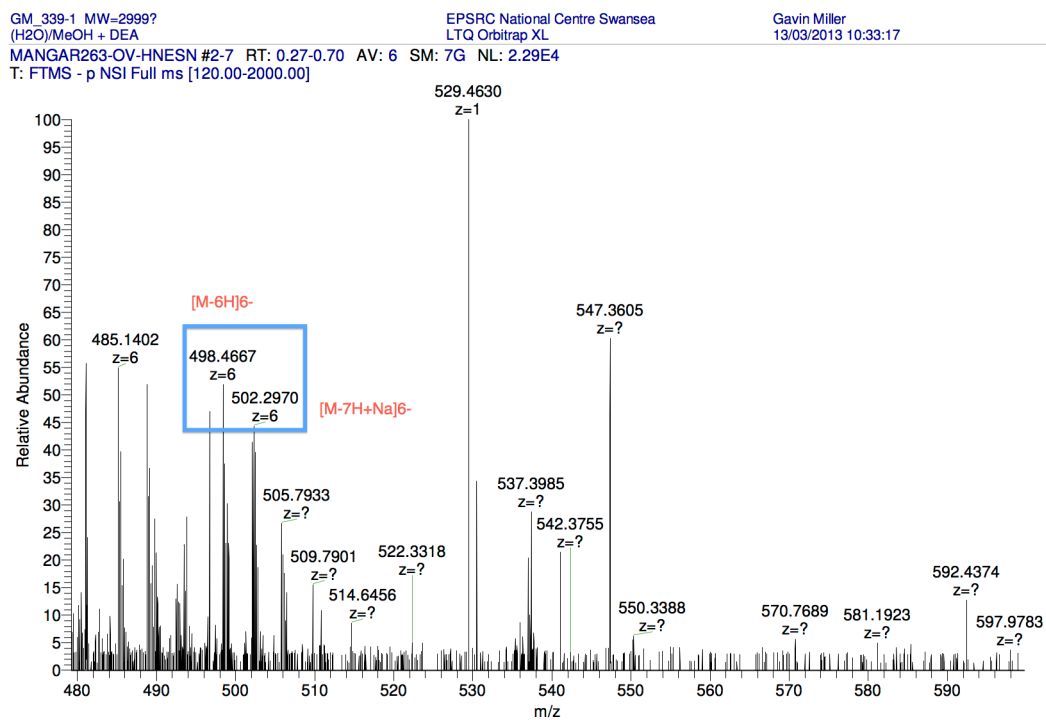
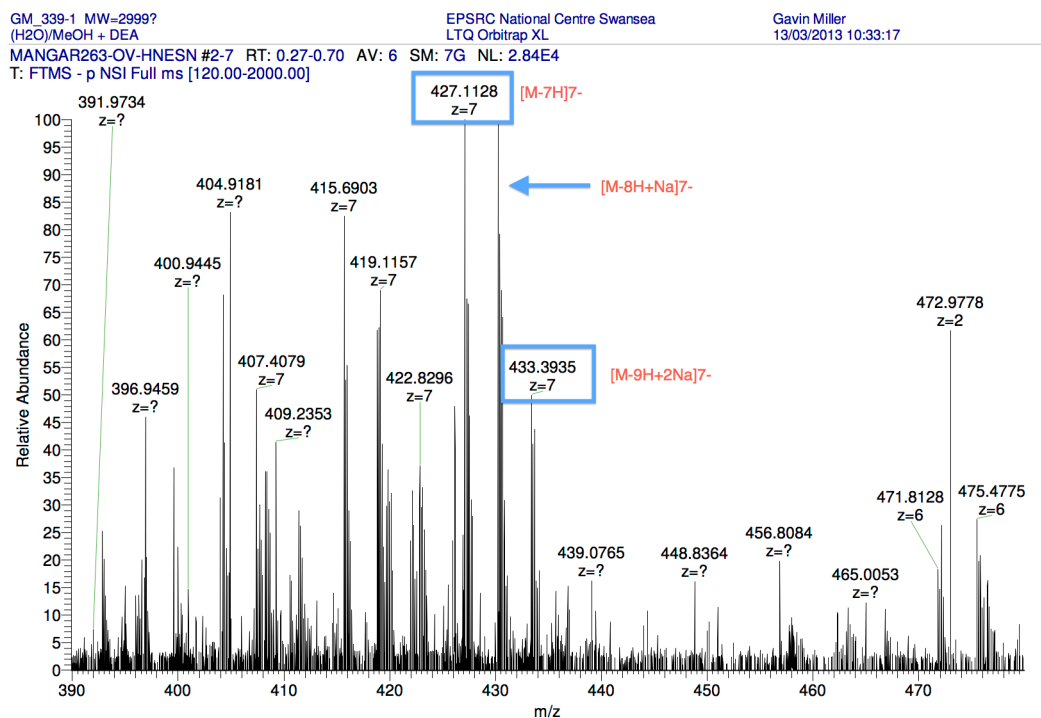
Decasaccharide *N*-sulfation 27 - HSQC Expanded Anomeric Centres Region

HSQC

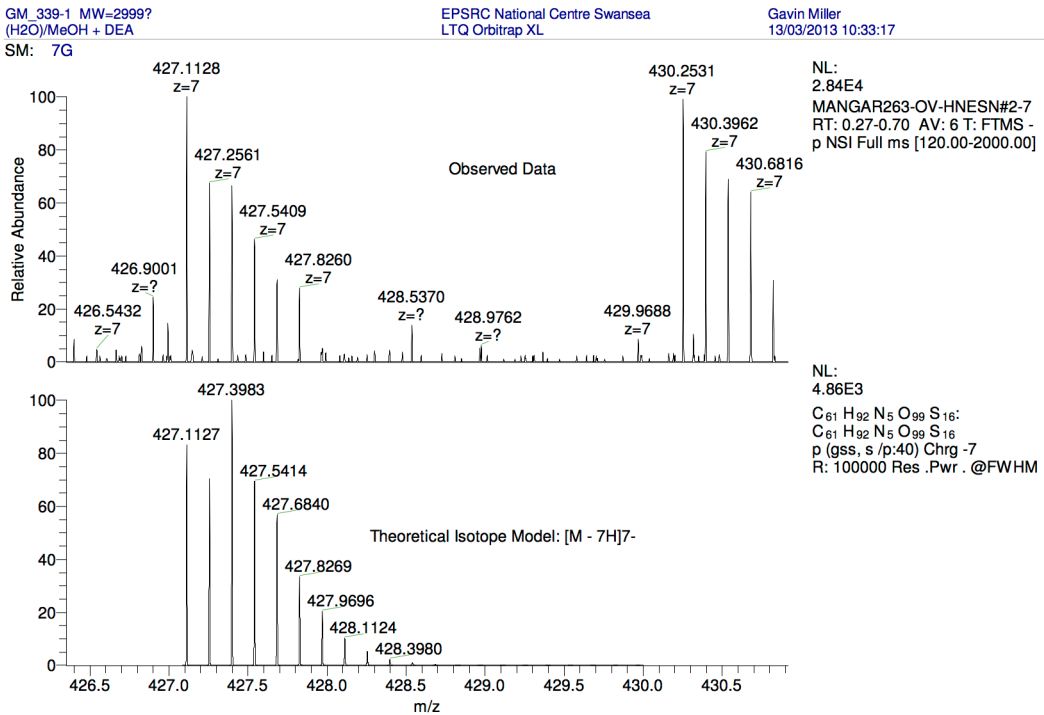


800 MHz, D₂O

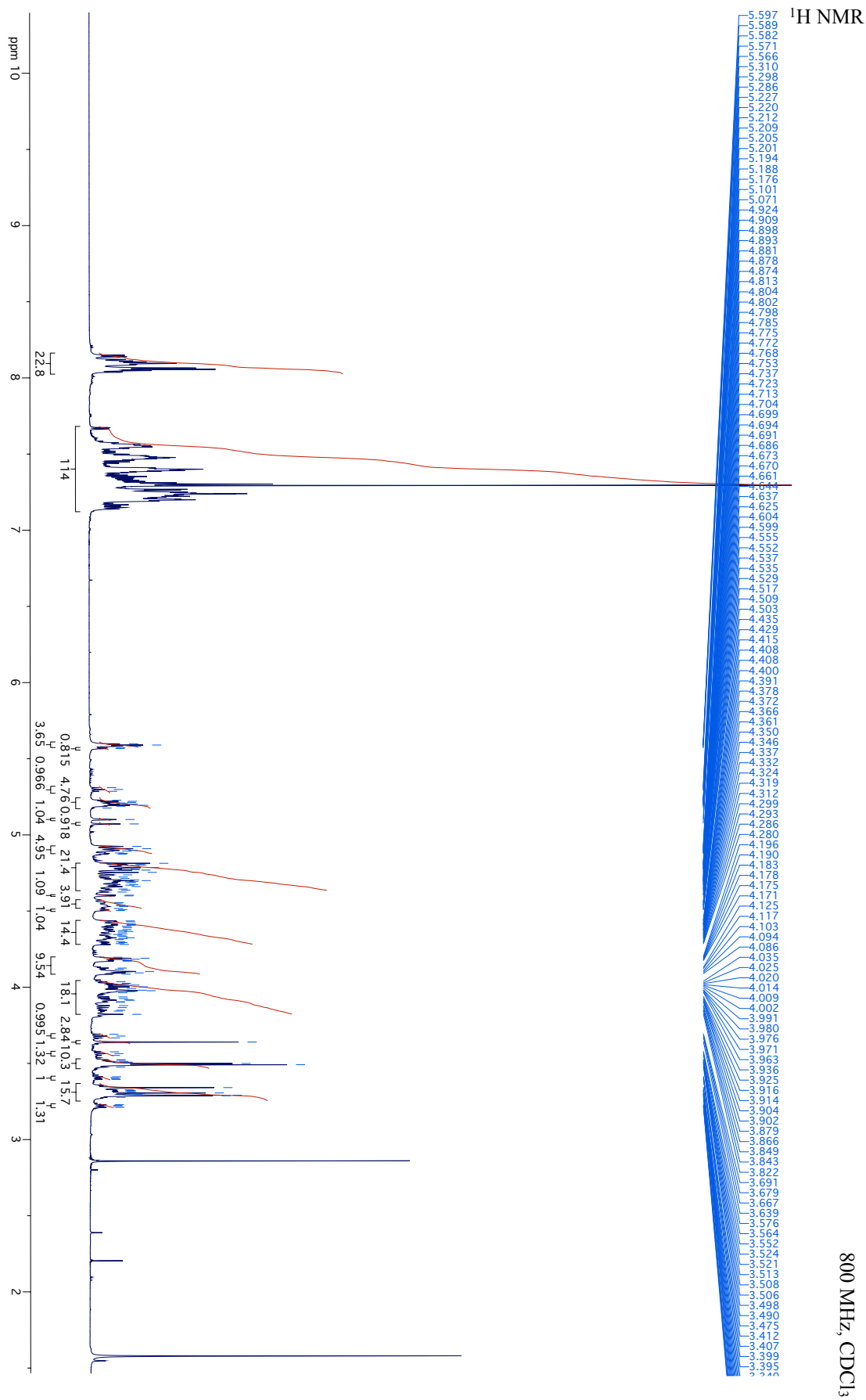
Decasaccharide *N*-sulfation 27 ESI MS



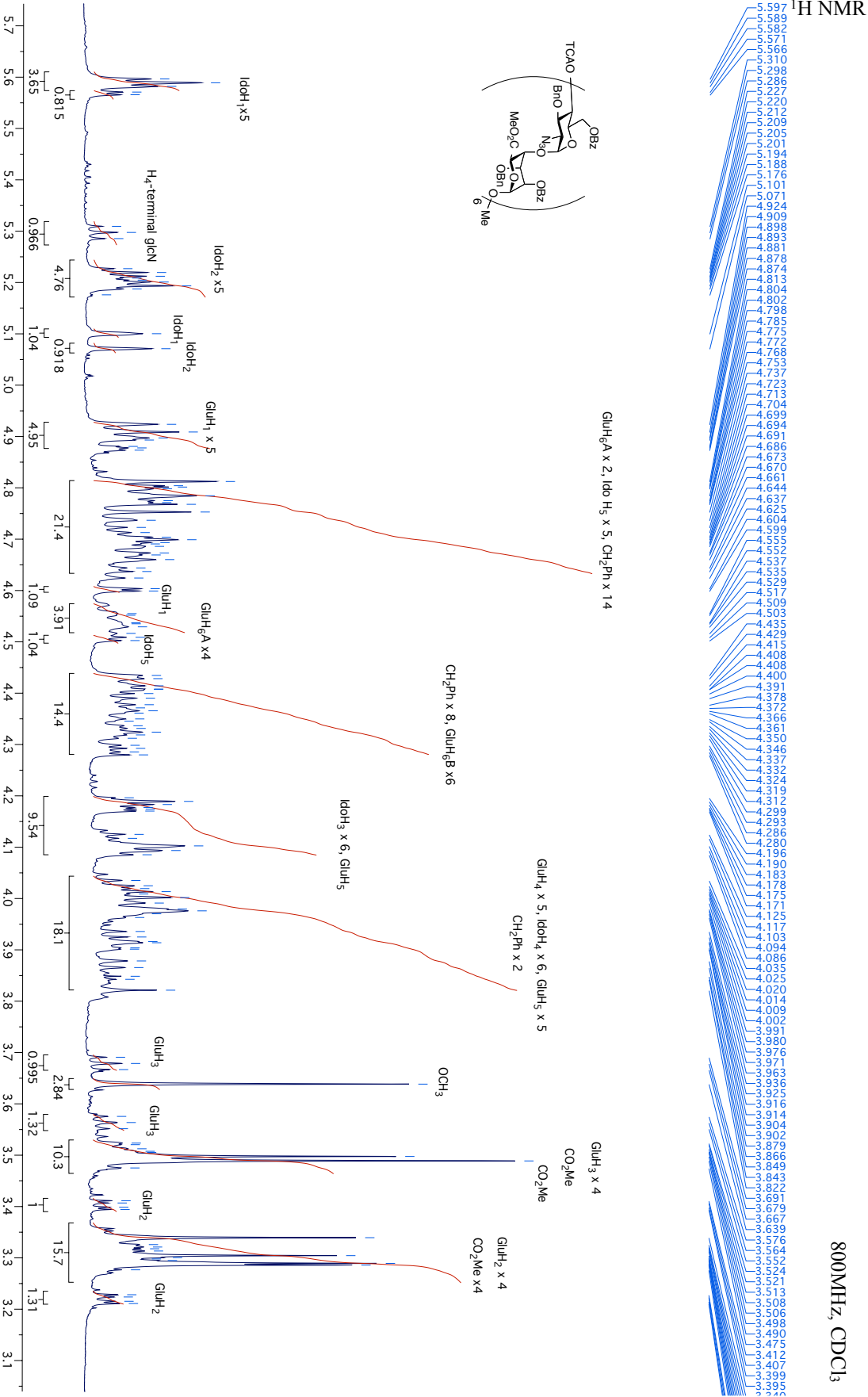
Decasaccharide *N*-sulfation 27 ESI MS Isotope Pattern



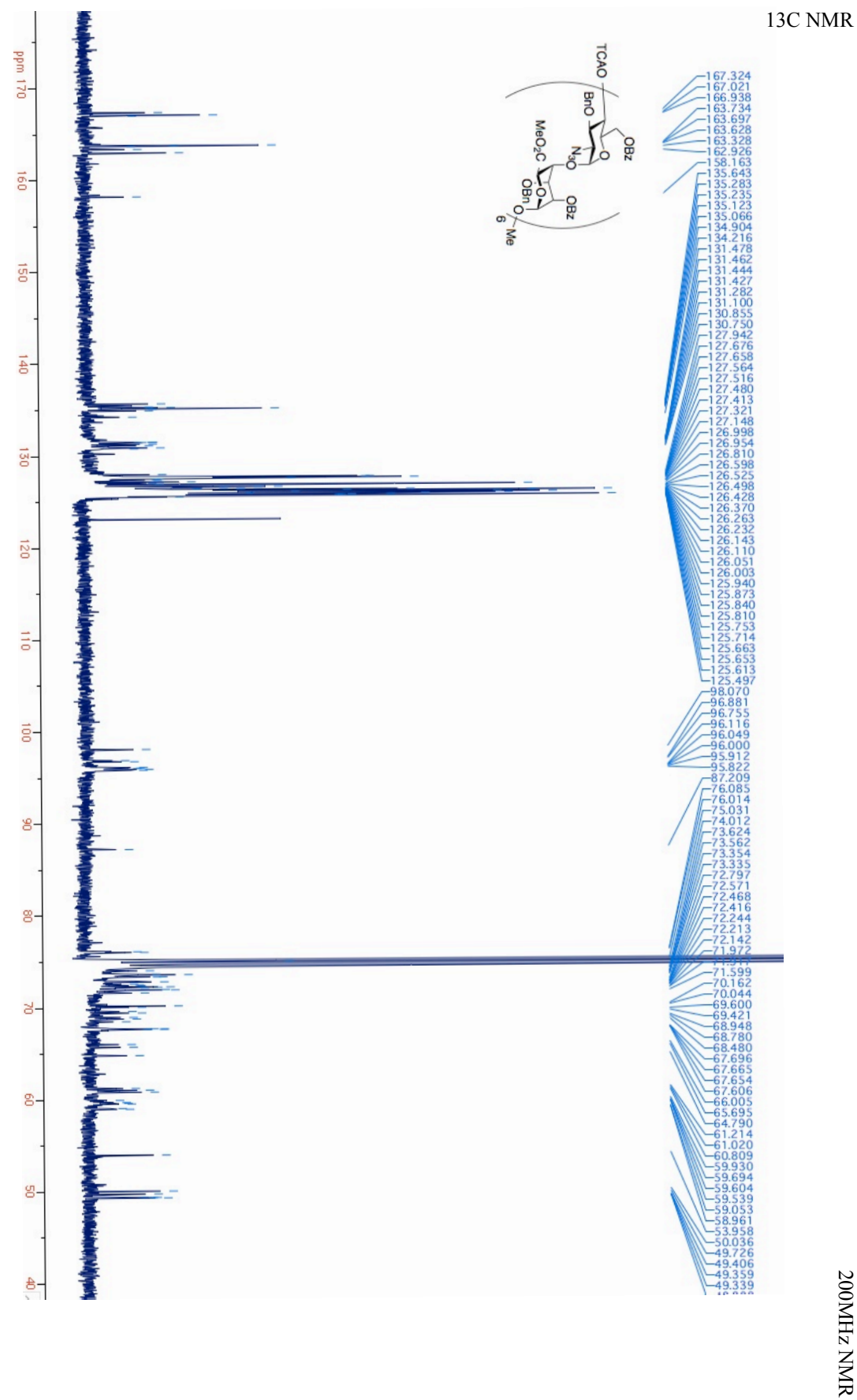
Dodecasaccharide 19



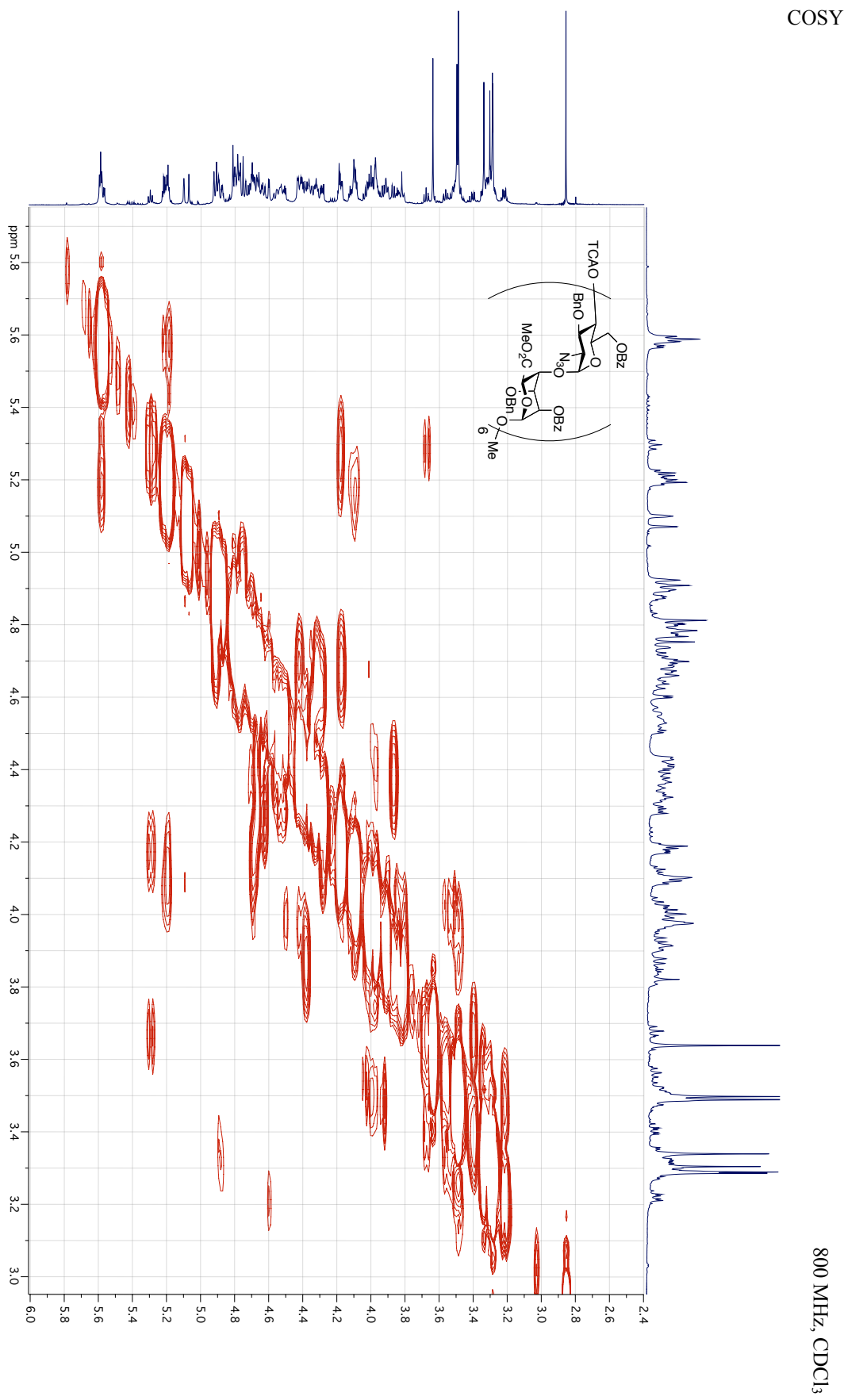
Dodecasaccharide 19 - 1H NMR expansion



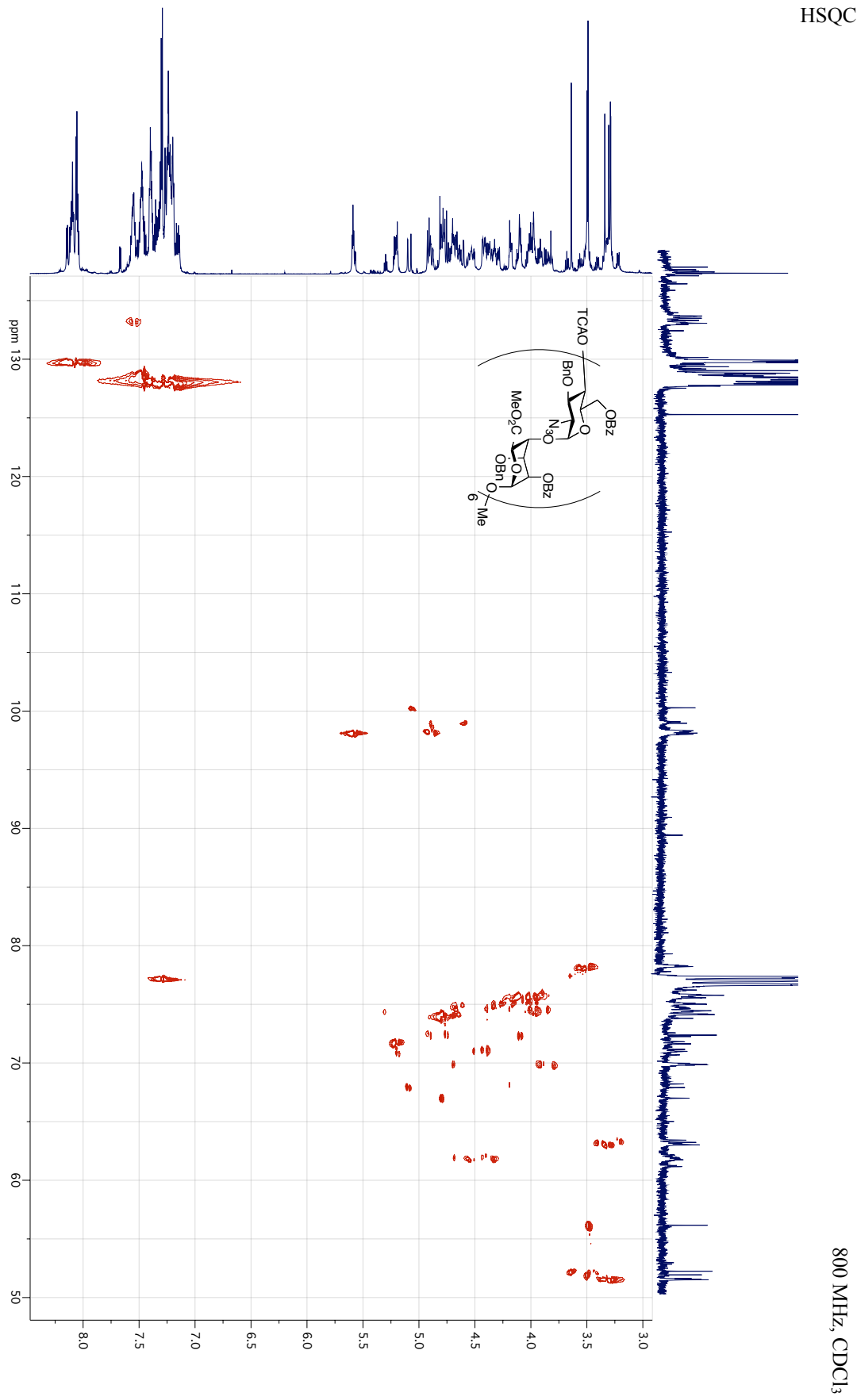
Dodecasaccharide 19



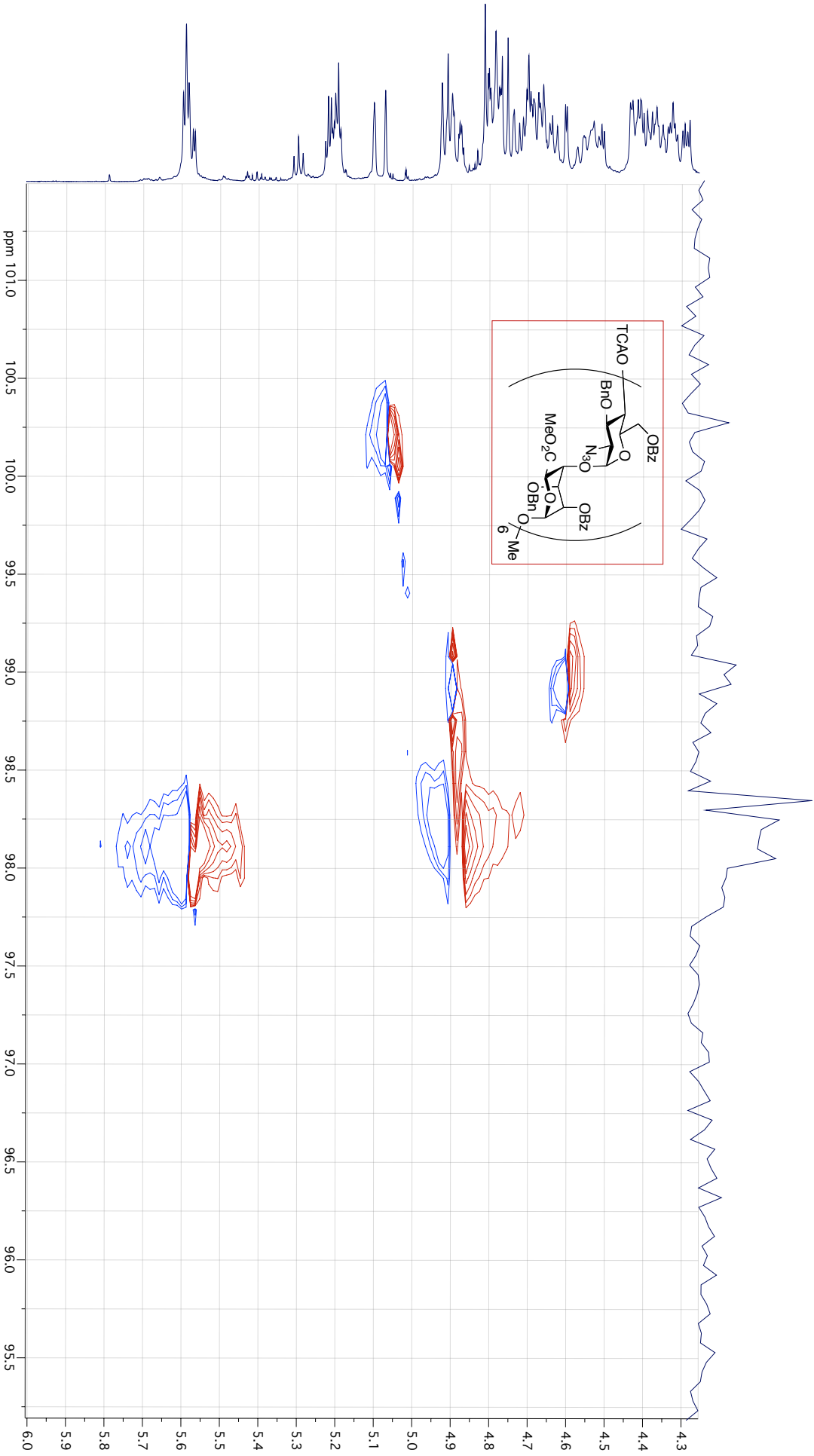
Dodecasaccharide 19



Dodecasaccharide 19



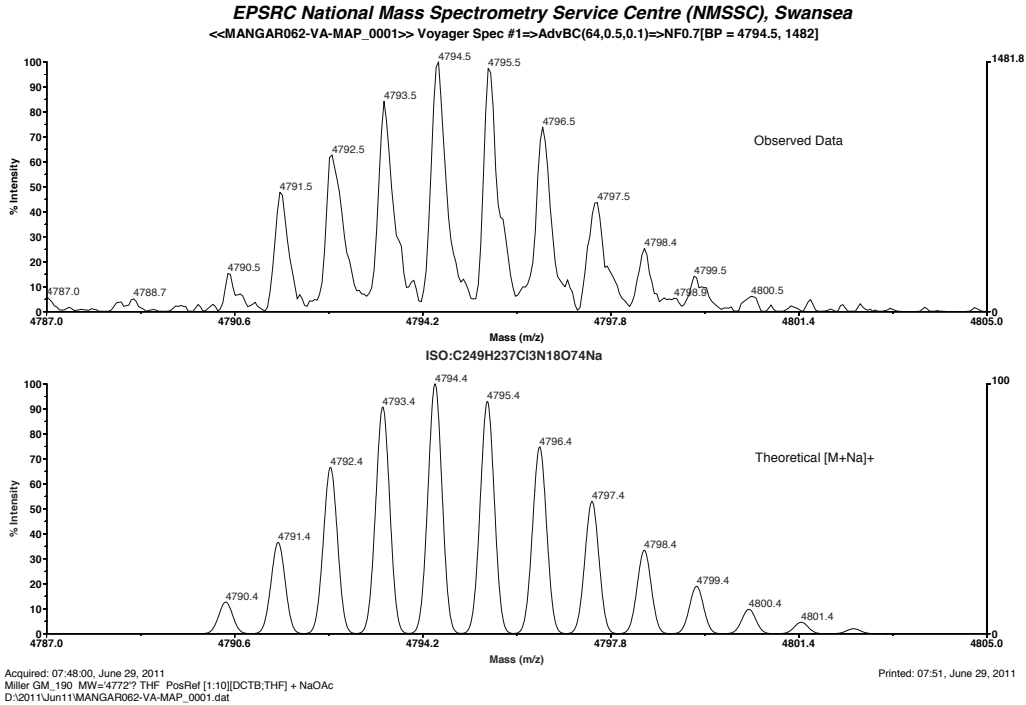
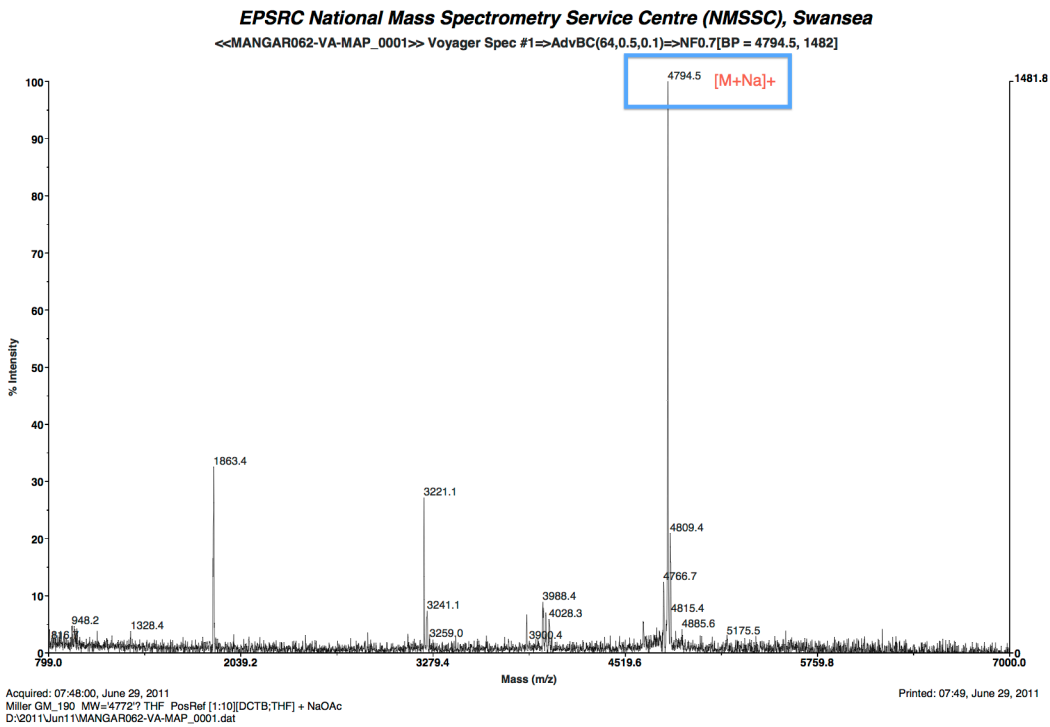
Dodecasaccharide 19 HSQC Expanded Anomeric Centres Region



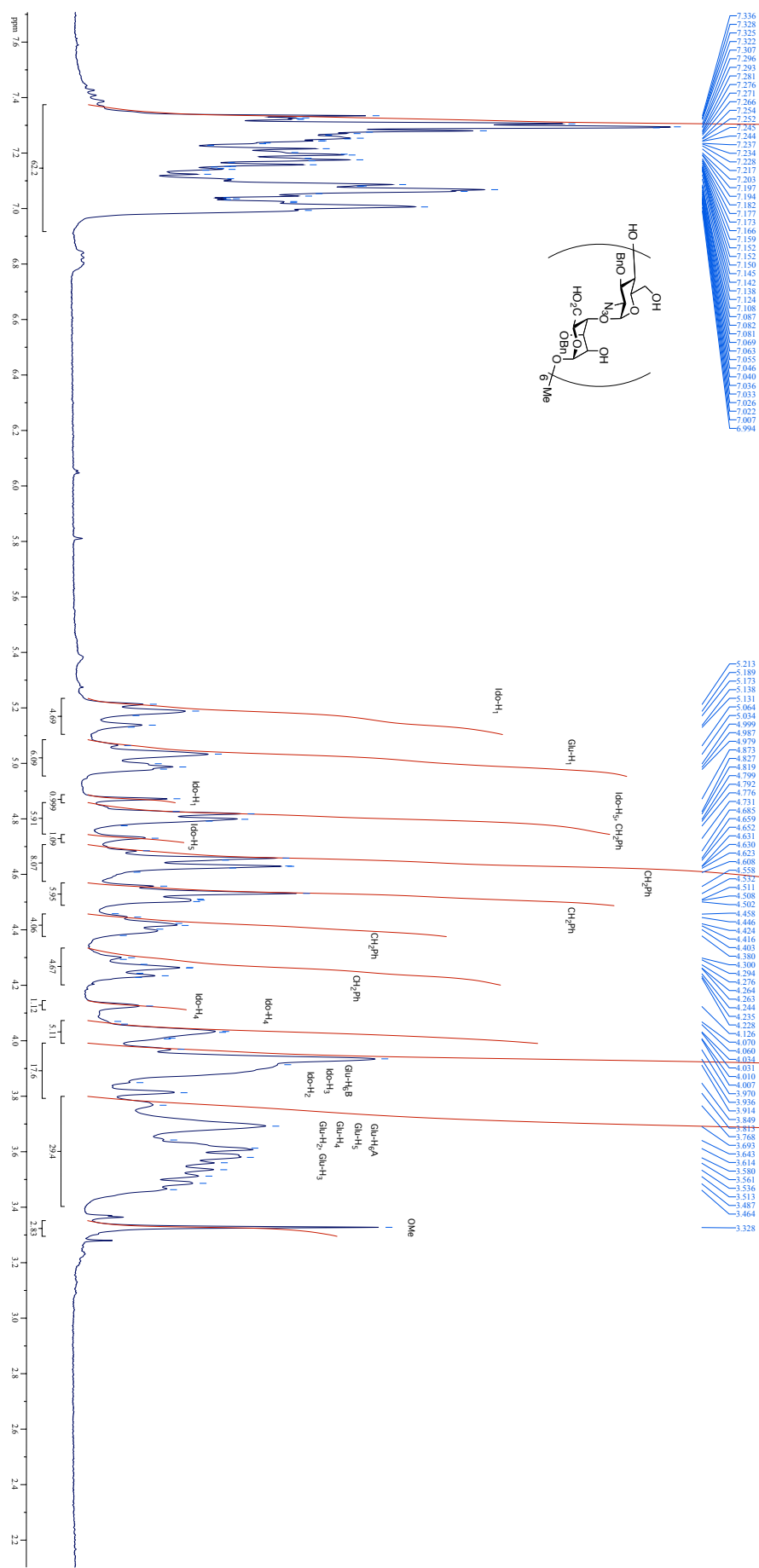
HSQC

800 MHz, CDCl₃

Dodecasaccharide 19 - MALDI MS and Isotope Pattern



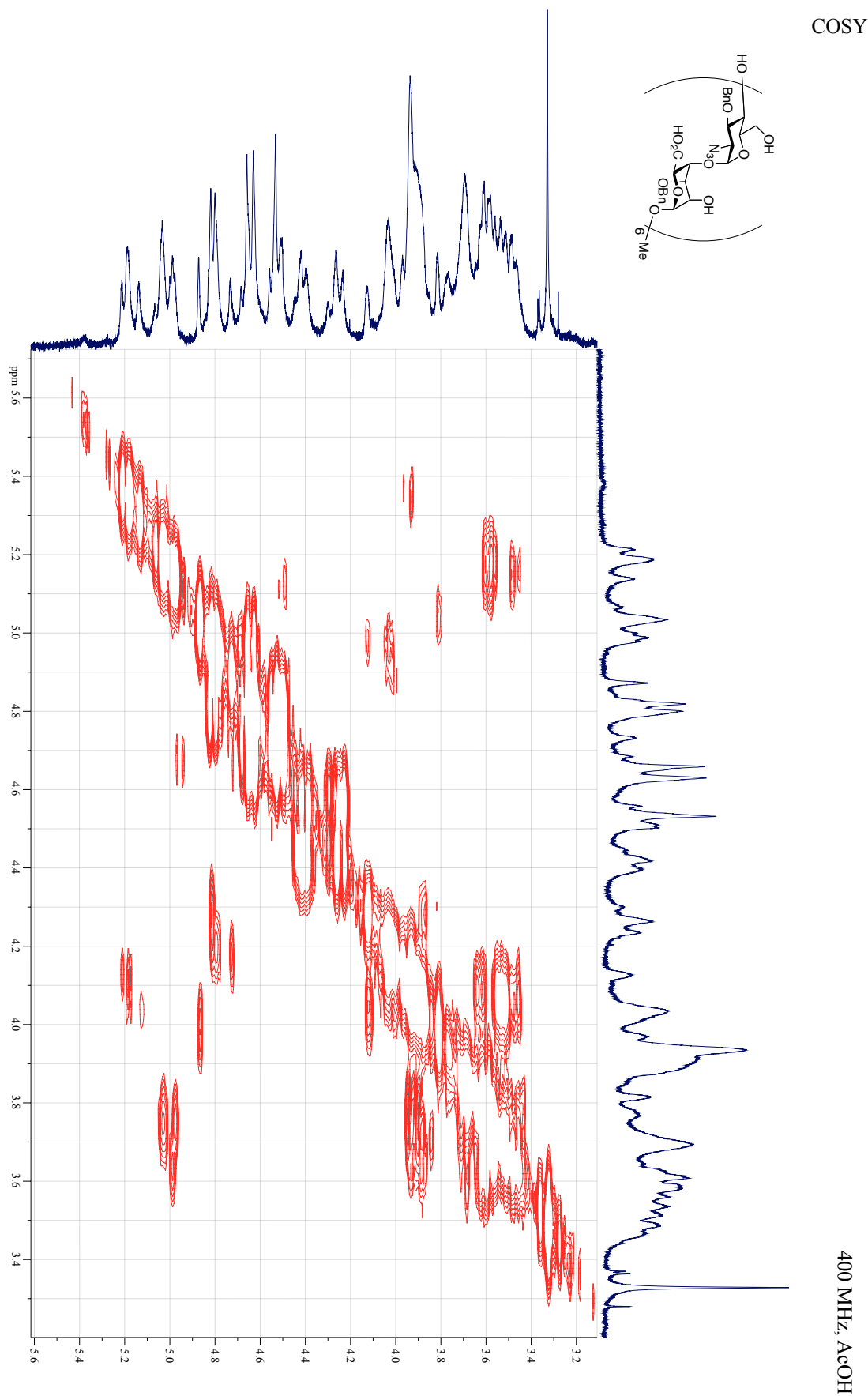
Dodecasaccharide Saponification 22



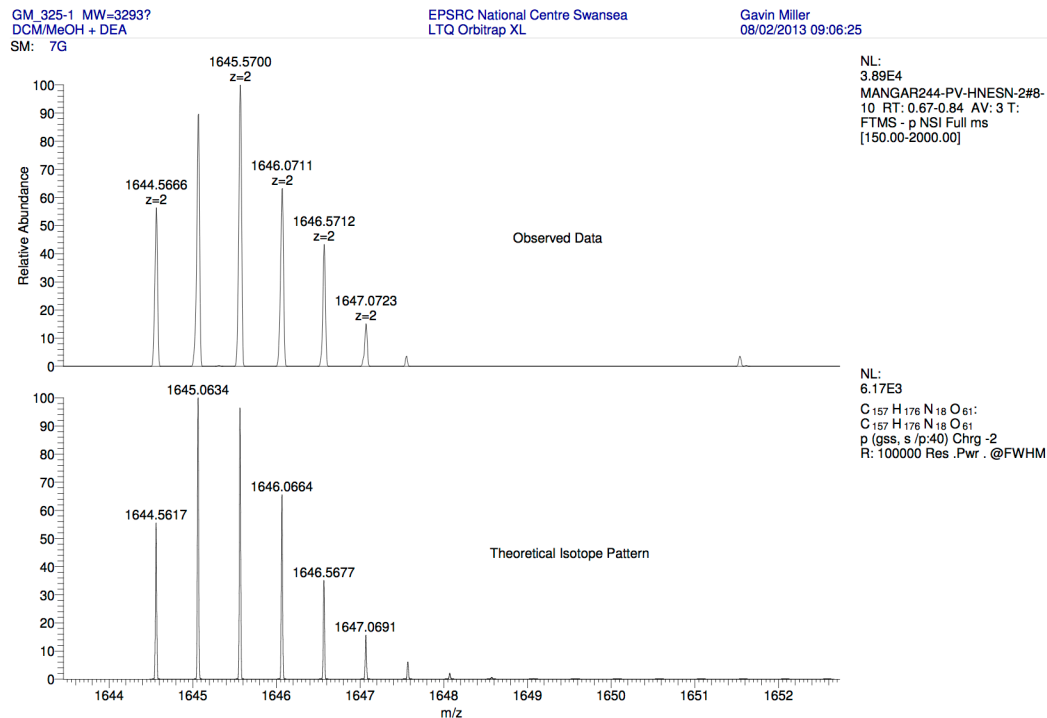
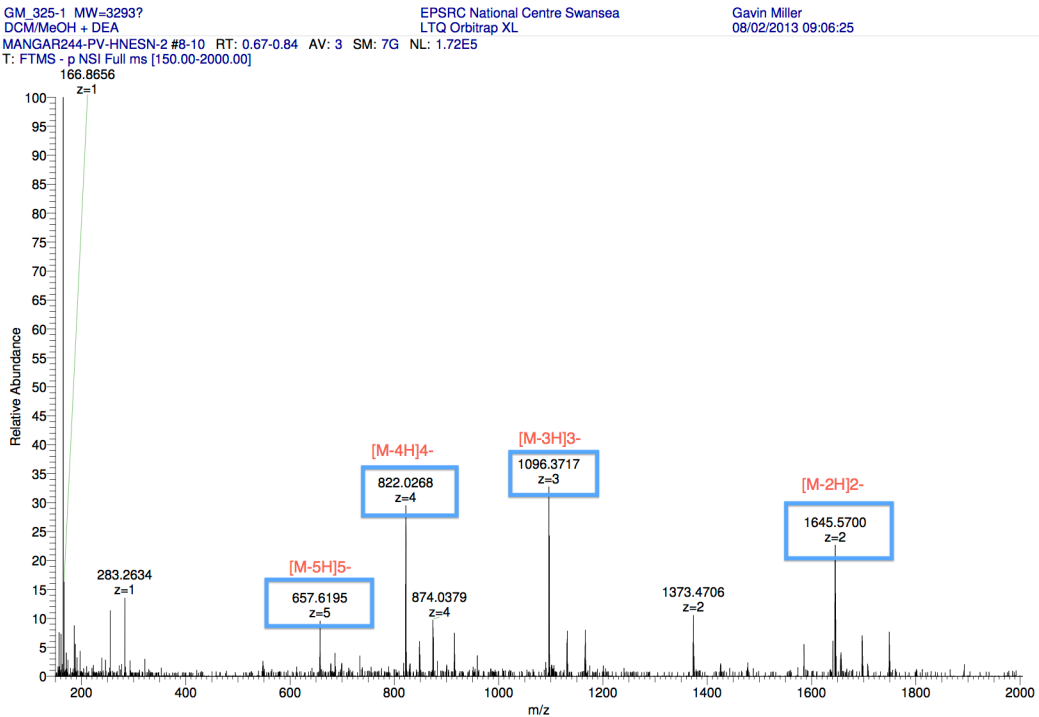
¹H NMR

400 MHz, AcOH

Dodecasaccharide Saponification 22

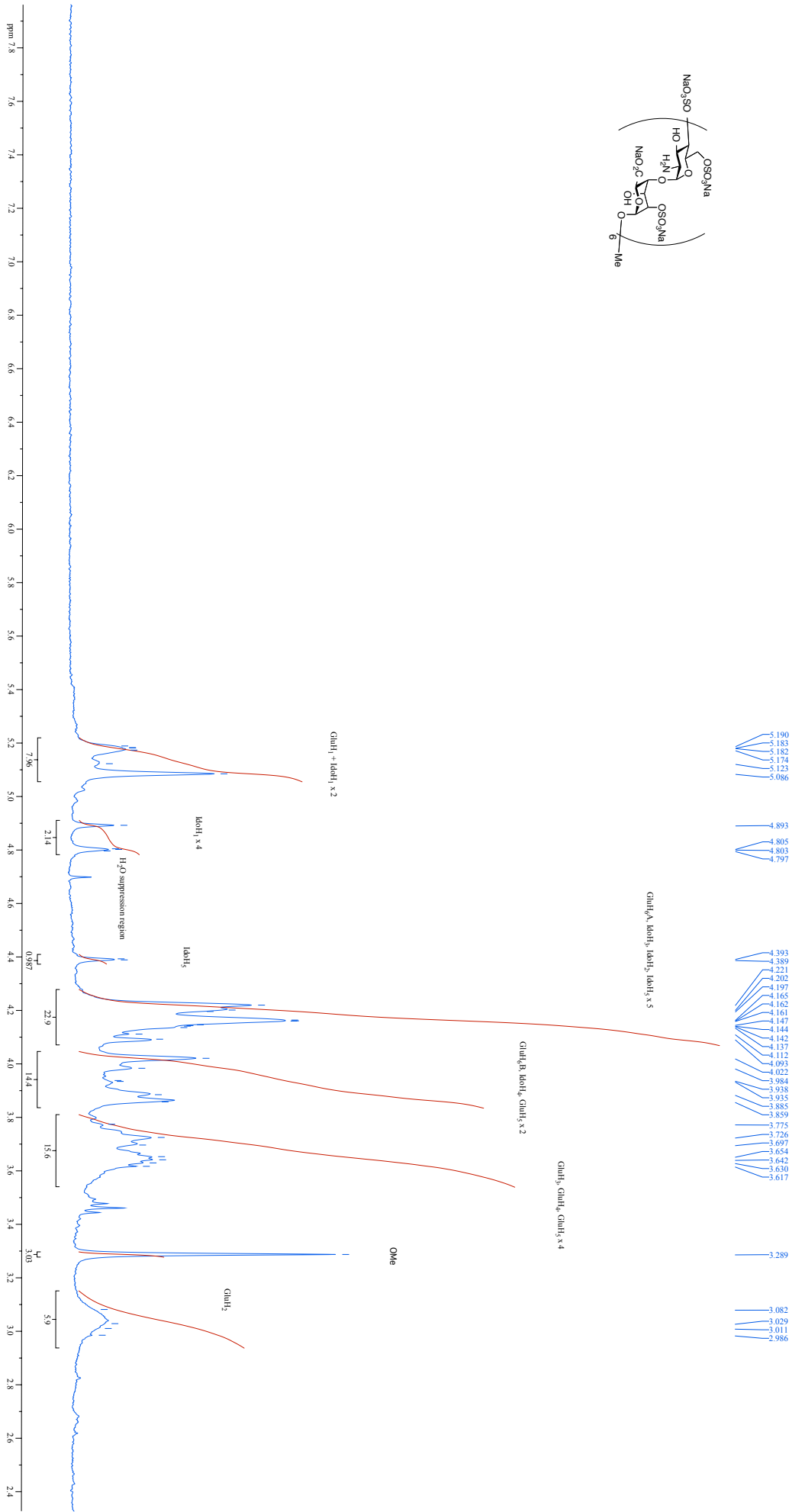


Dodecasaccharide Saponification 22 - ESI MS and isotope pattern

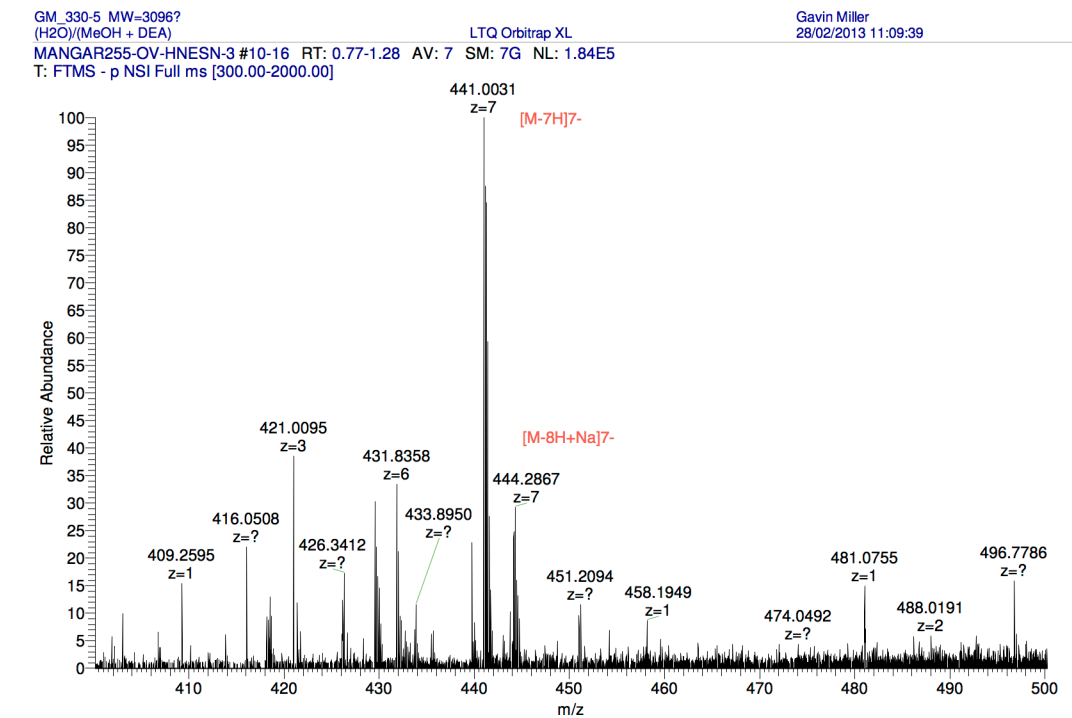
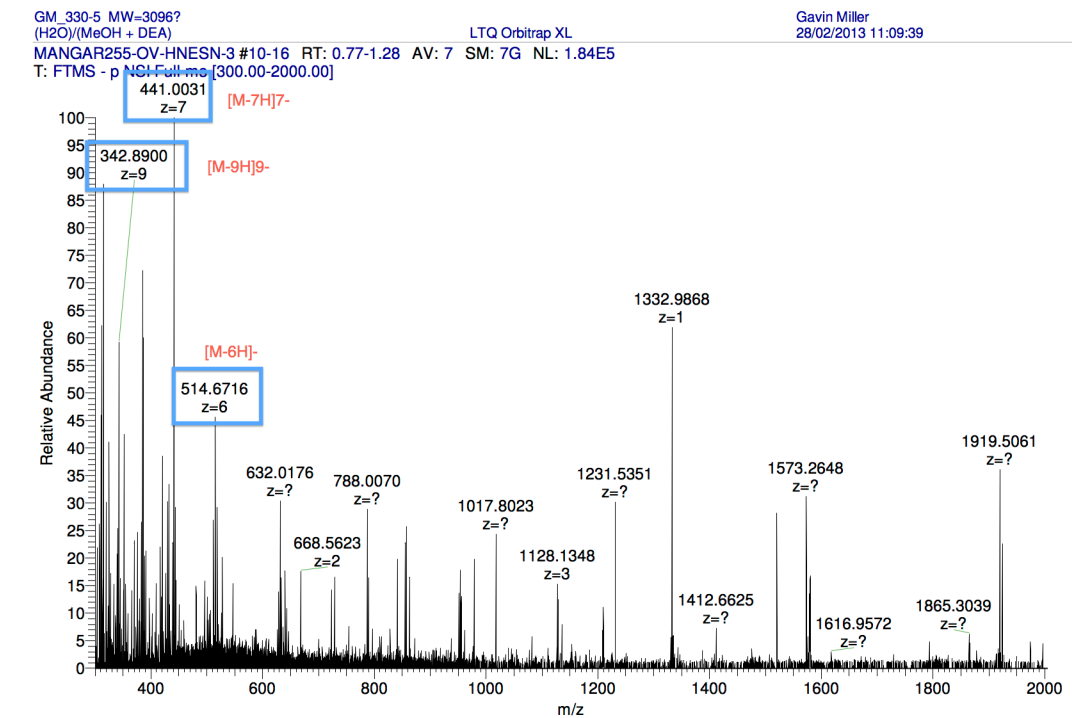


Dodecasaccharide Hydrogenation 25

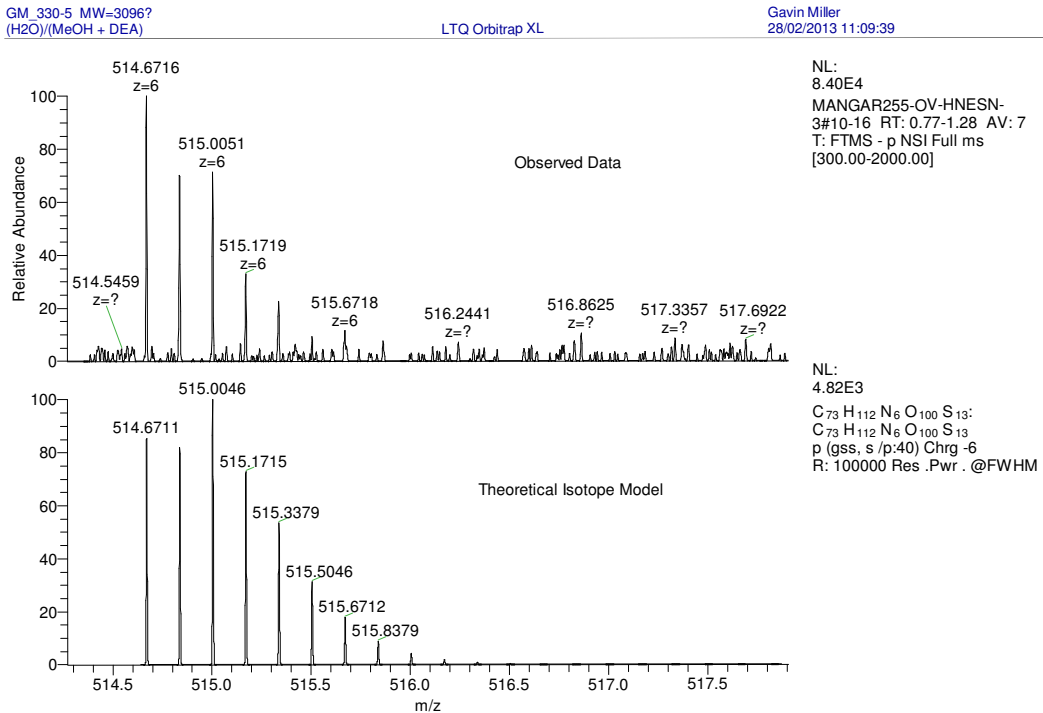
¹H NMR



Dodecasaccharide Hydrogenation 25 - ESI MS



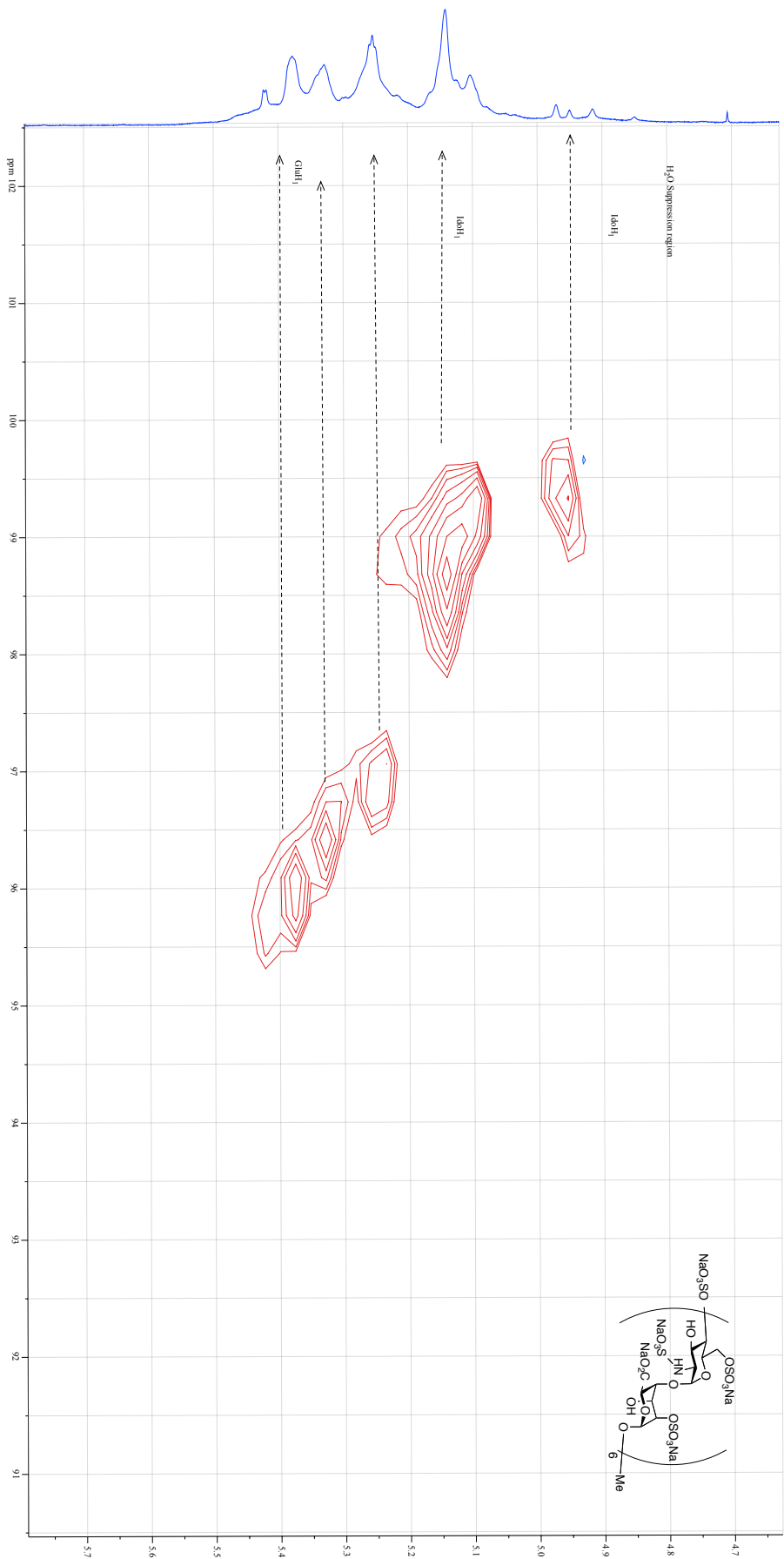
Dodecasaccharide Hydrogenation 25 - MS Isotope Pattern



800 MHz, D₂O

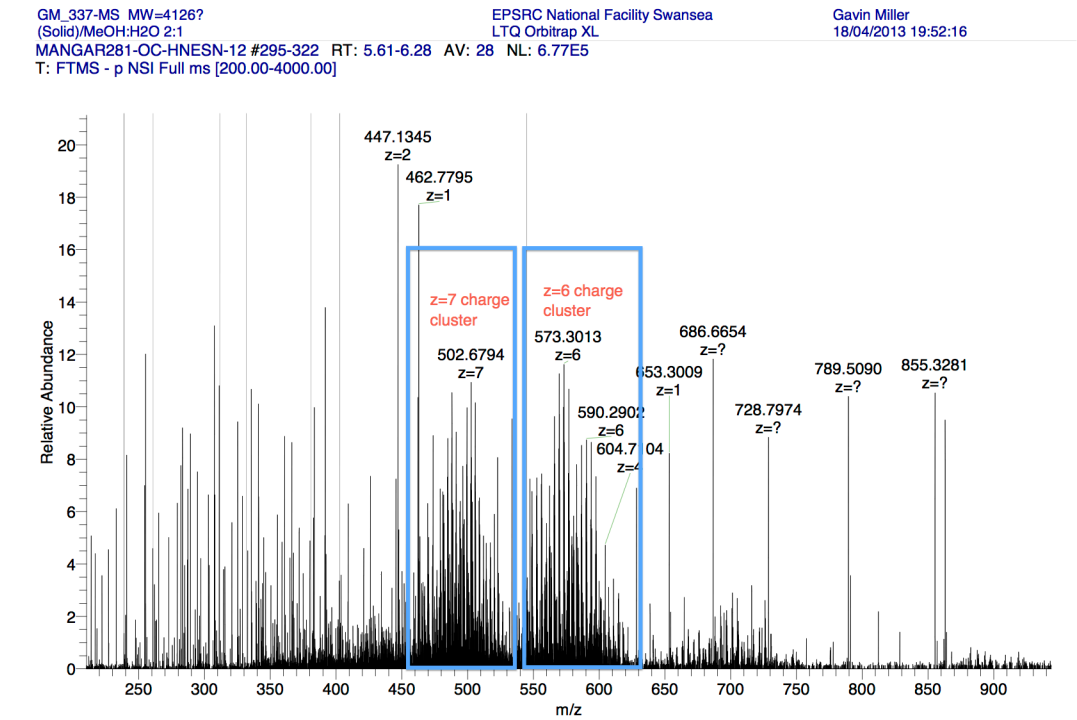
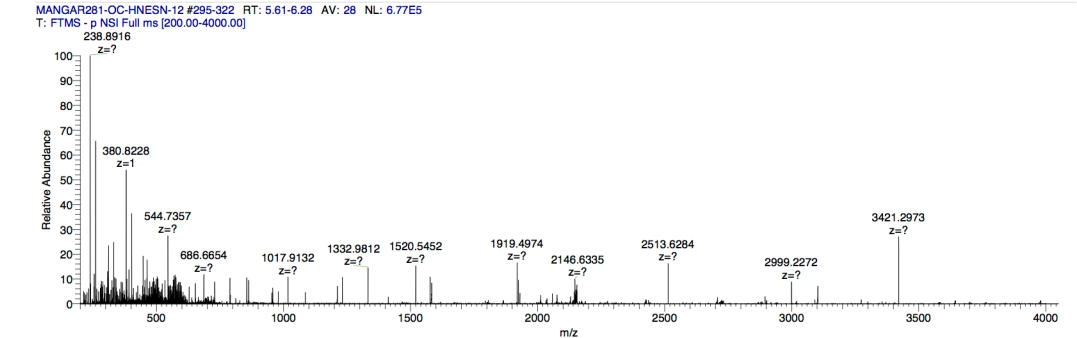
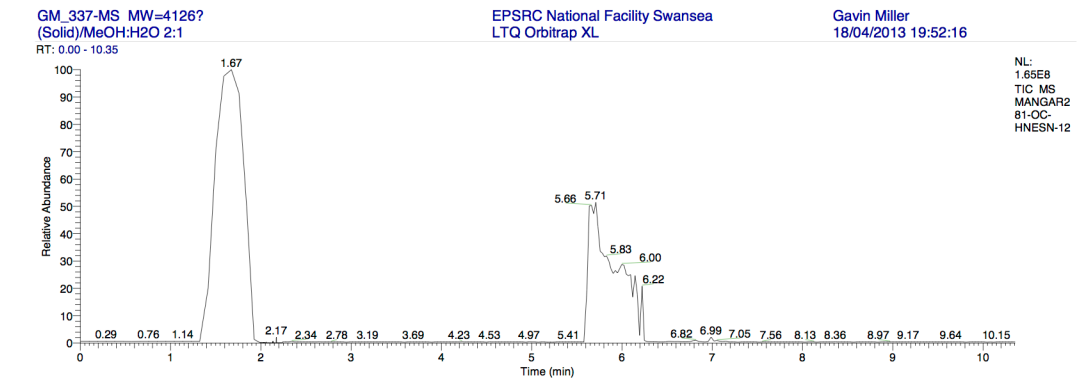
Dodecasaccharide *N*-sulfation 28 - Expanded Anomeric Centres Region

HSQC

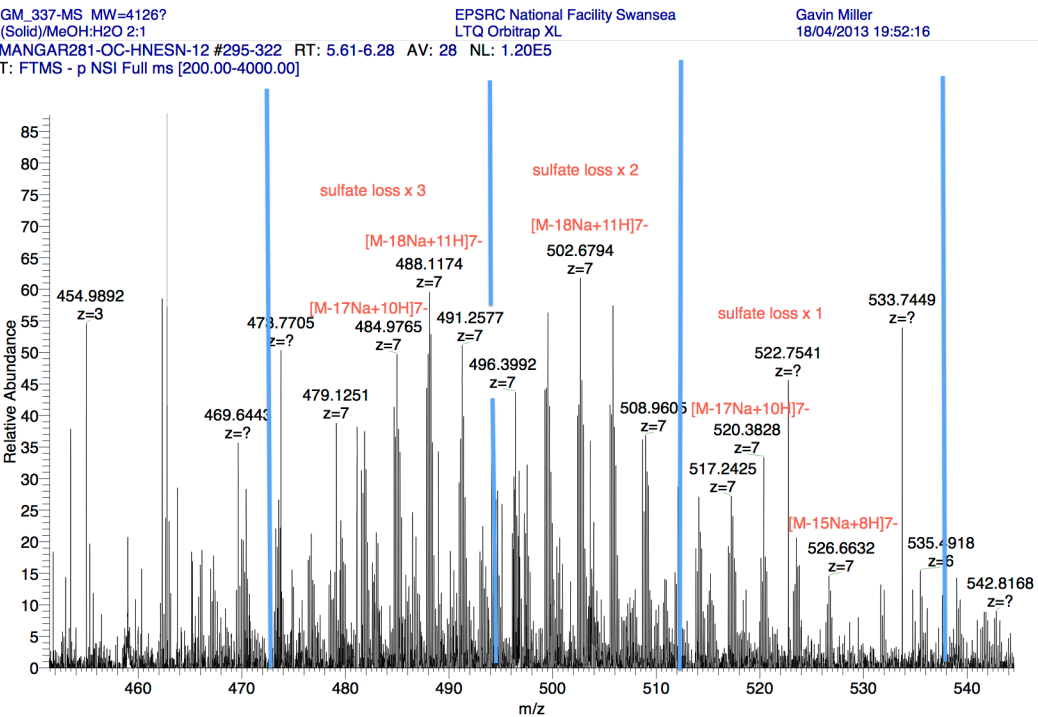
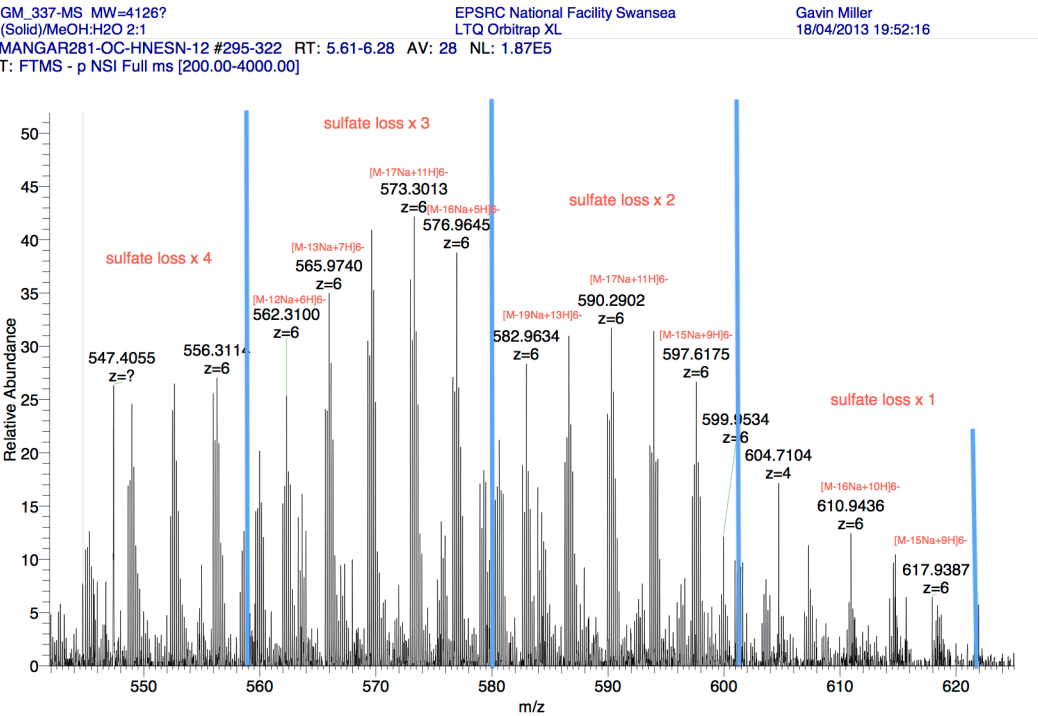


800 MHz, D₂O

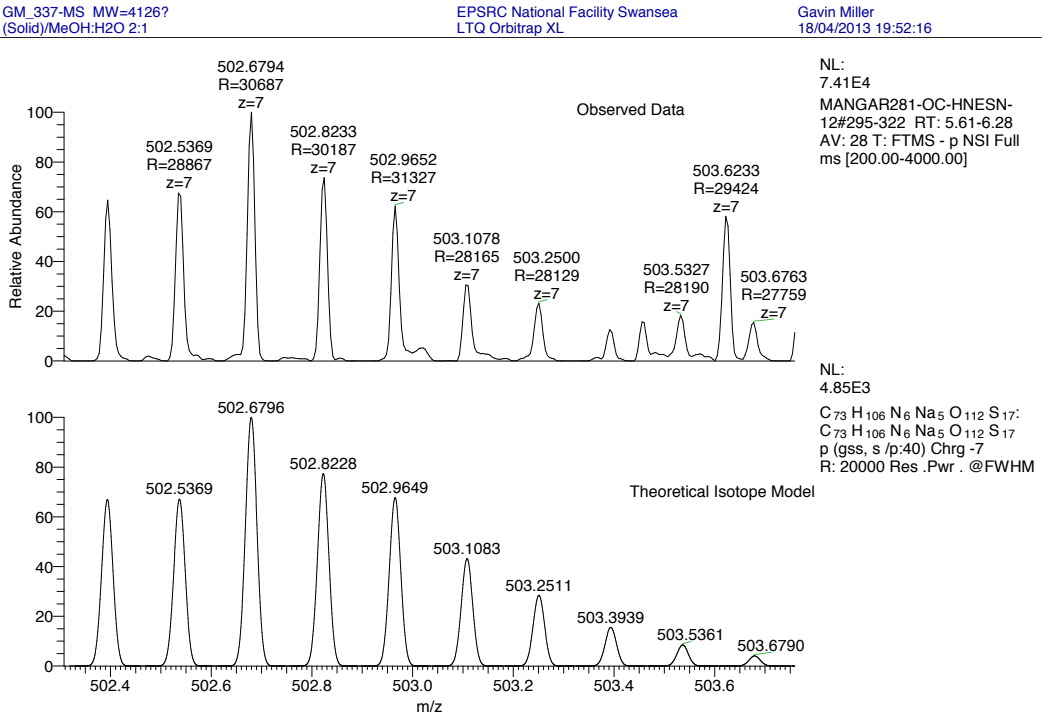
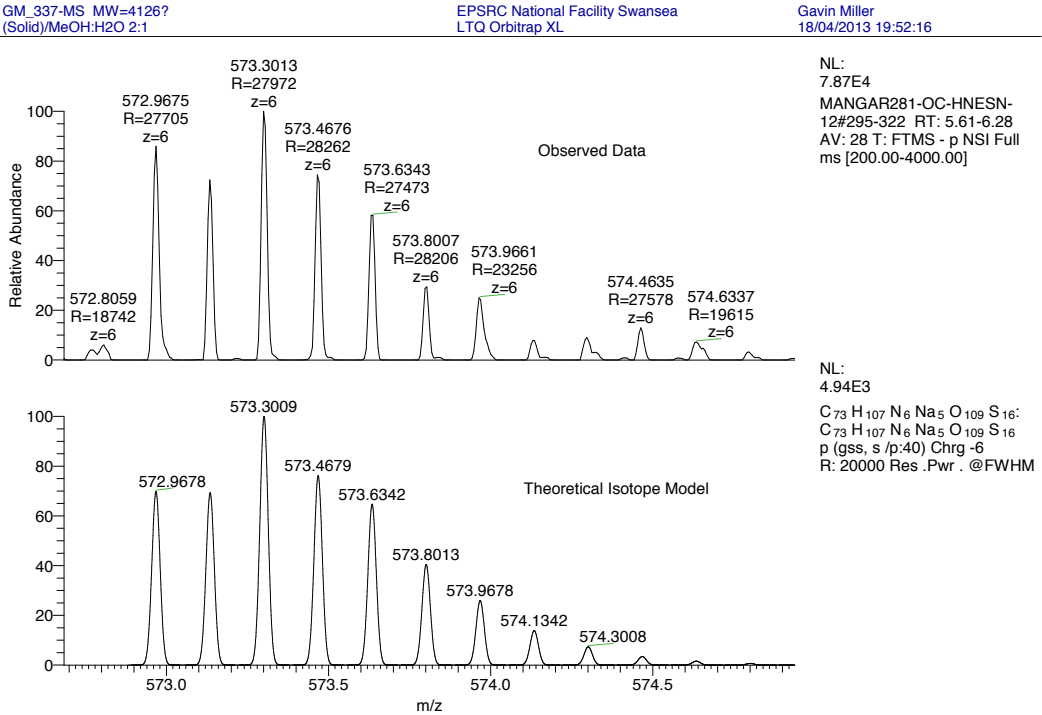
Dodecasaccharide *N*-sulfation 28 - ESI MS



Dodecasaccharide *N*-sulfation 28 - ESI MS



Dodecasaccharide *N*-sulfation 28 -MS Isotope Pattern



¹H NMR overlay showing mixture of furanoside/pyranoside isomers (precursors to 3 and 4)

