

Supplementary Data

Synthesis of chondroitin/dermatan sulfate-like oligosaccharides and evaluation of their protein affinity by fluorescence polarization

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Angulo, José L. de Paz,* Pedro M. Nieto**

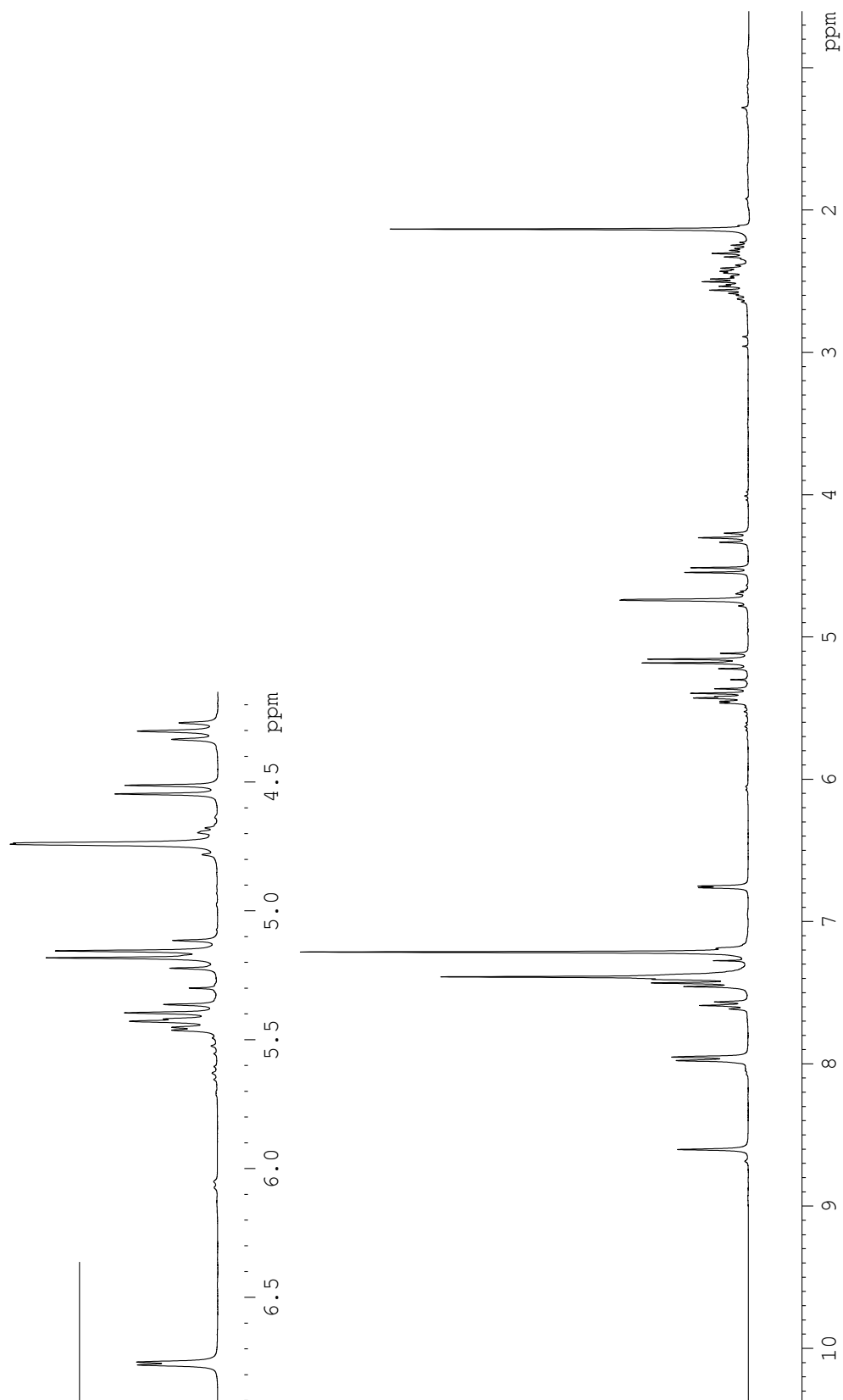
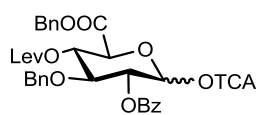
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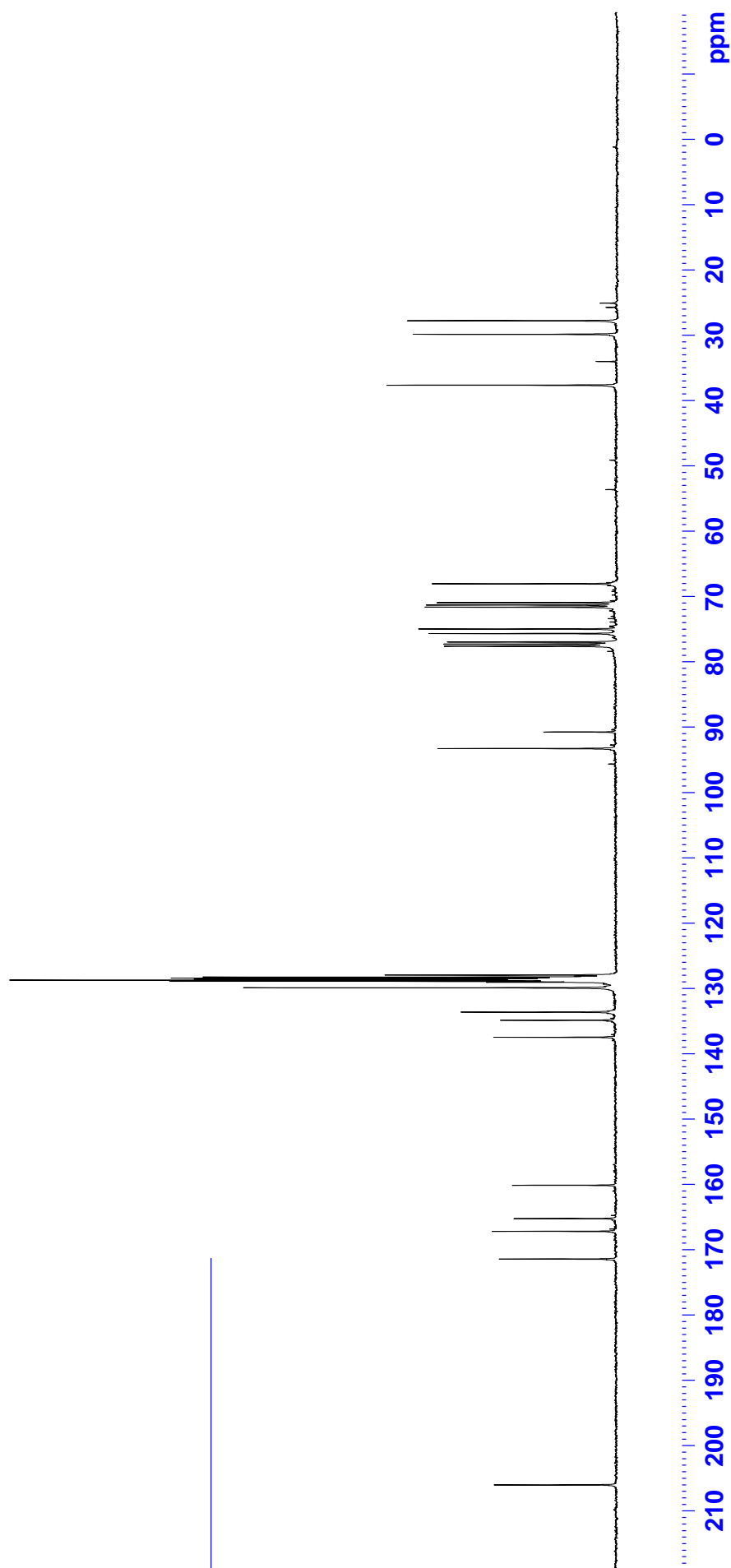
NMR spectra

Compound 2	S-3
Compound 6	S-5
Compound 7	S-7
Compound 8	S-9
Compound 10	S-11
Compound 11	S-13
Compound 3	S-15
Compound 12	S-17
Compound 13	S-18
Compound 14	S-20

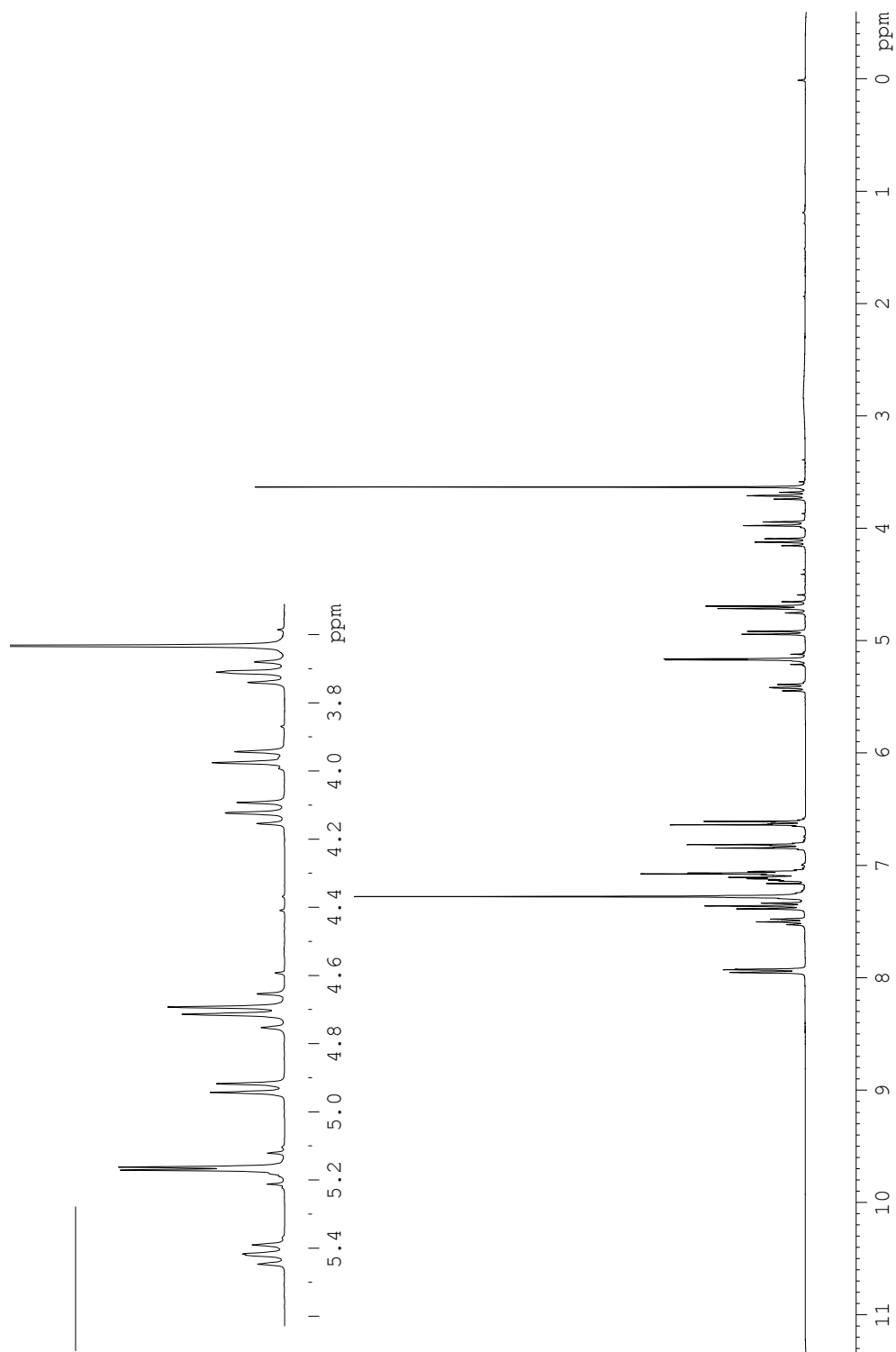
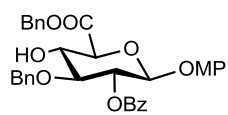
Compound 15	S-21
Compound 16	S-23
Compound 18	S-25
Compound 4	S-27
Compound 19	S-29
Compound 20	S-31
Compound 22	S-33
Compound 23	S-35
Compound 24	S-37
Compound 25	S-39
Compound 26	S-41
Compound 30	S-43
Compound 1	S-45
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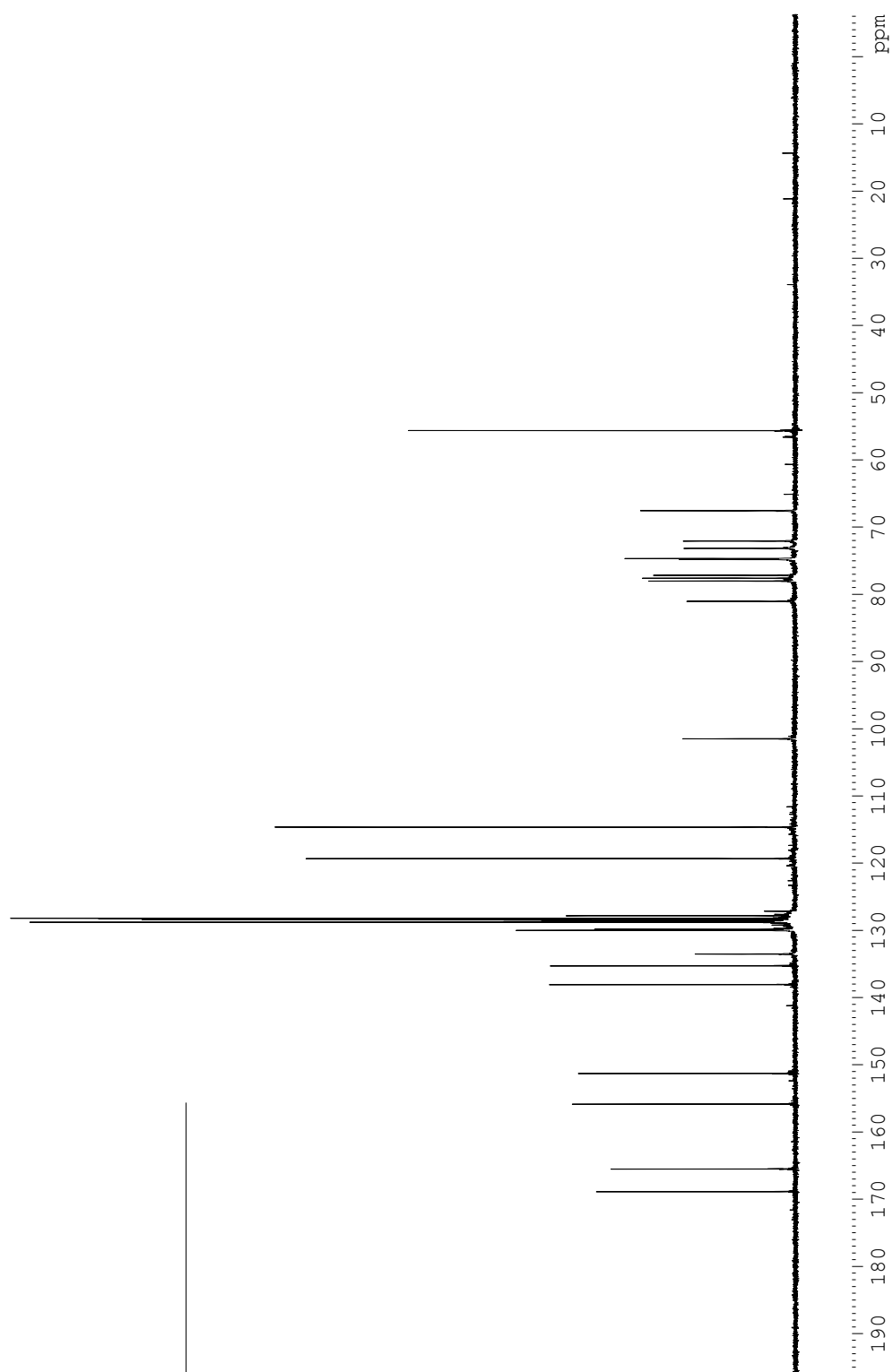
Compound 2



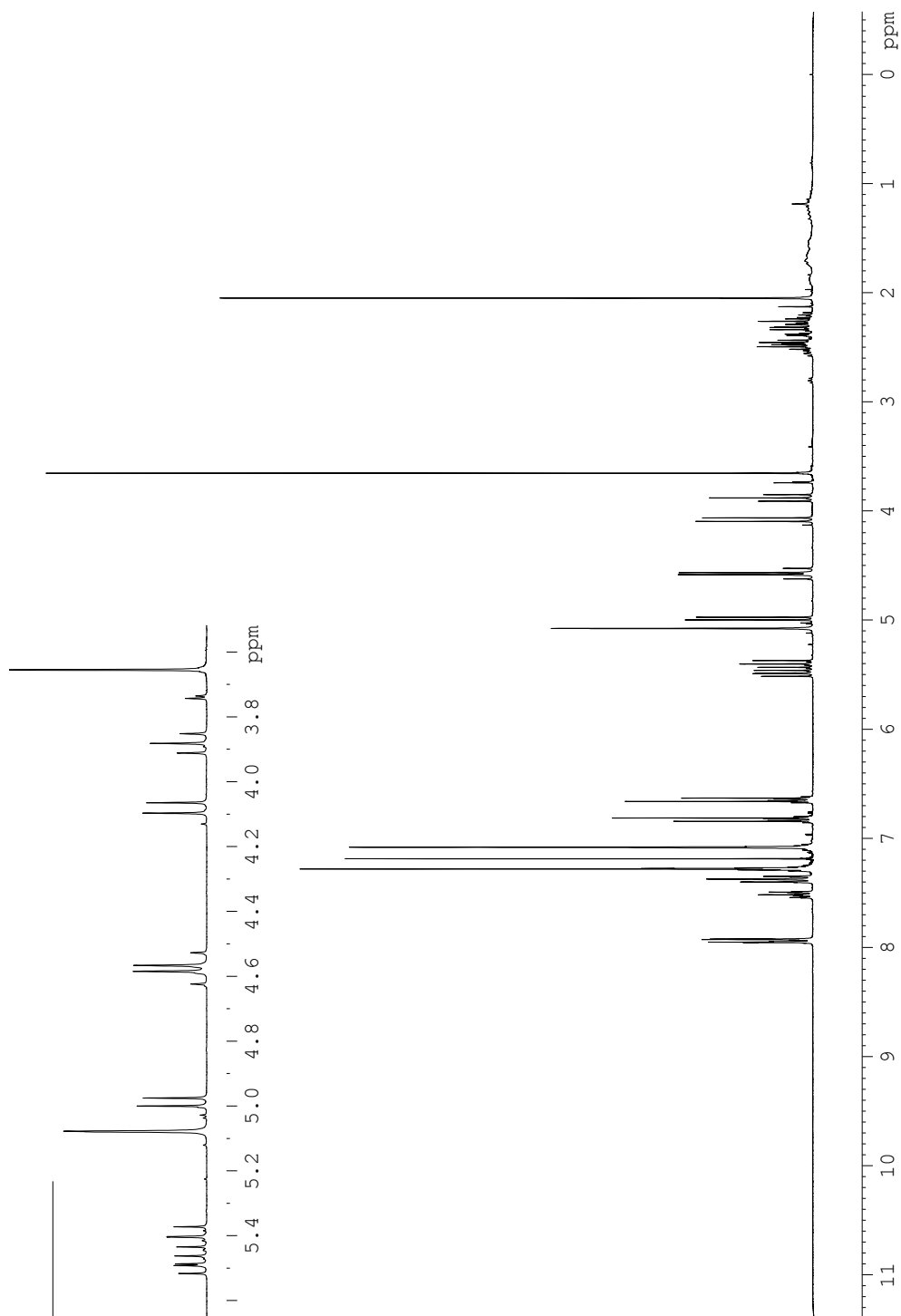
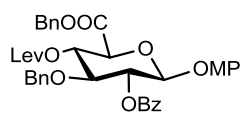


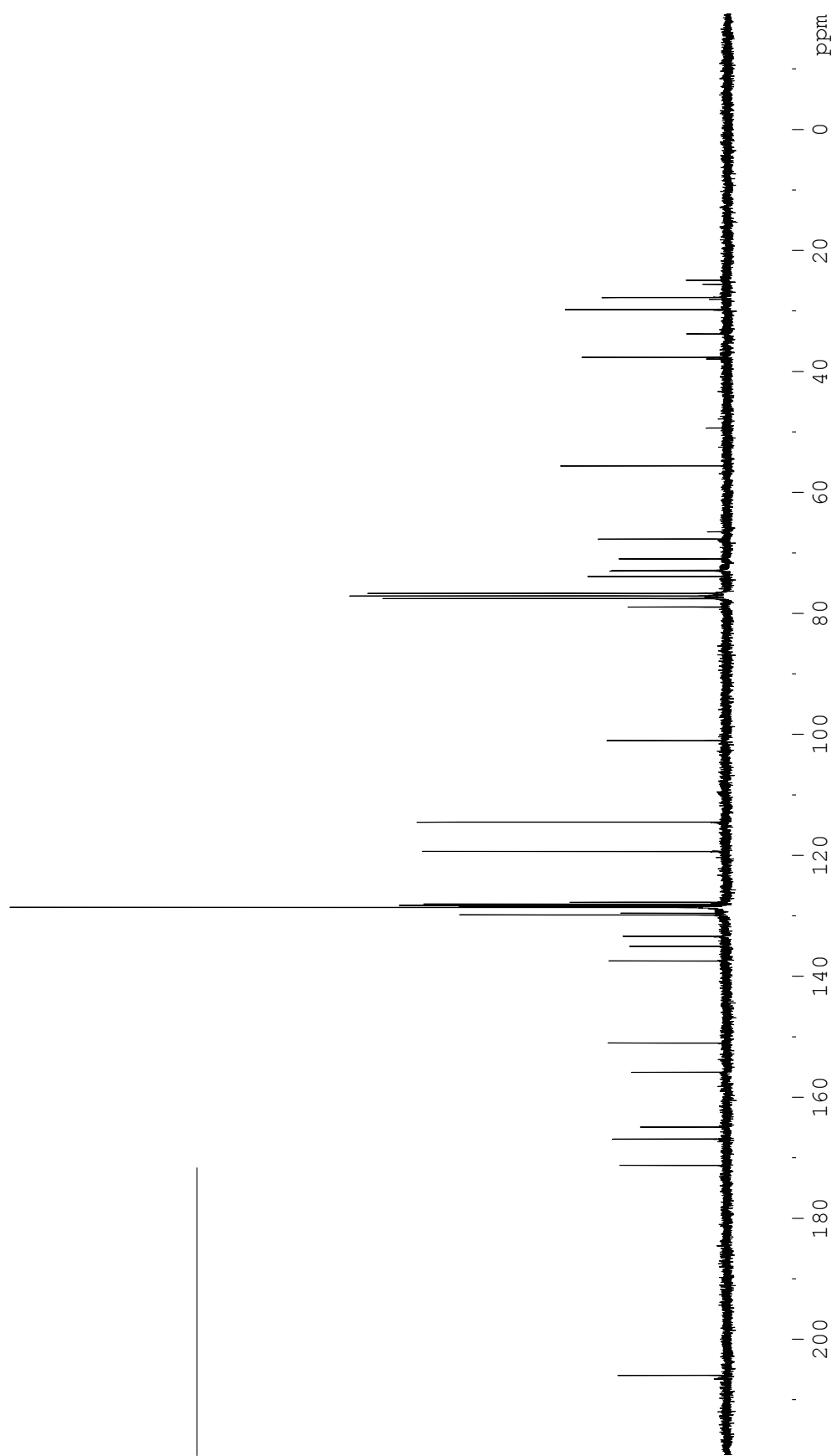
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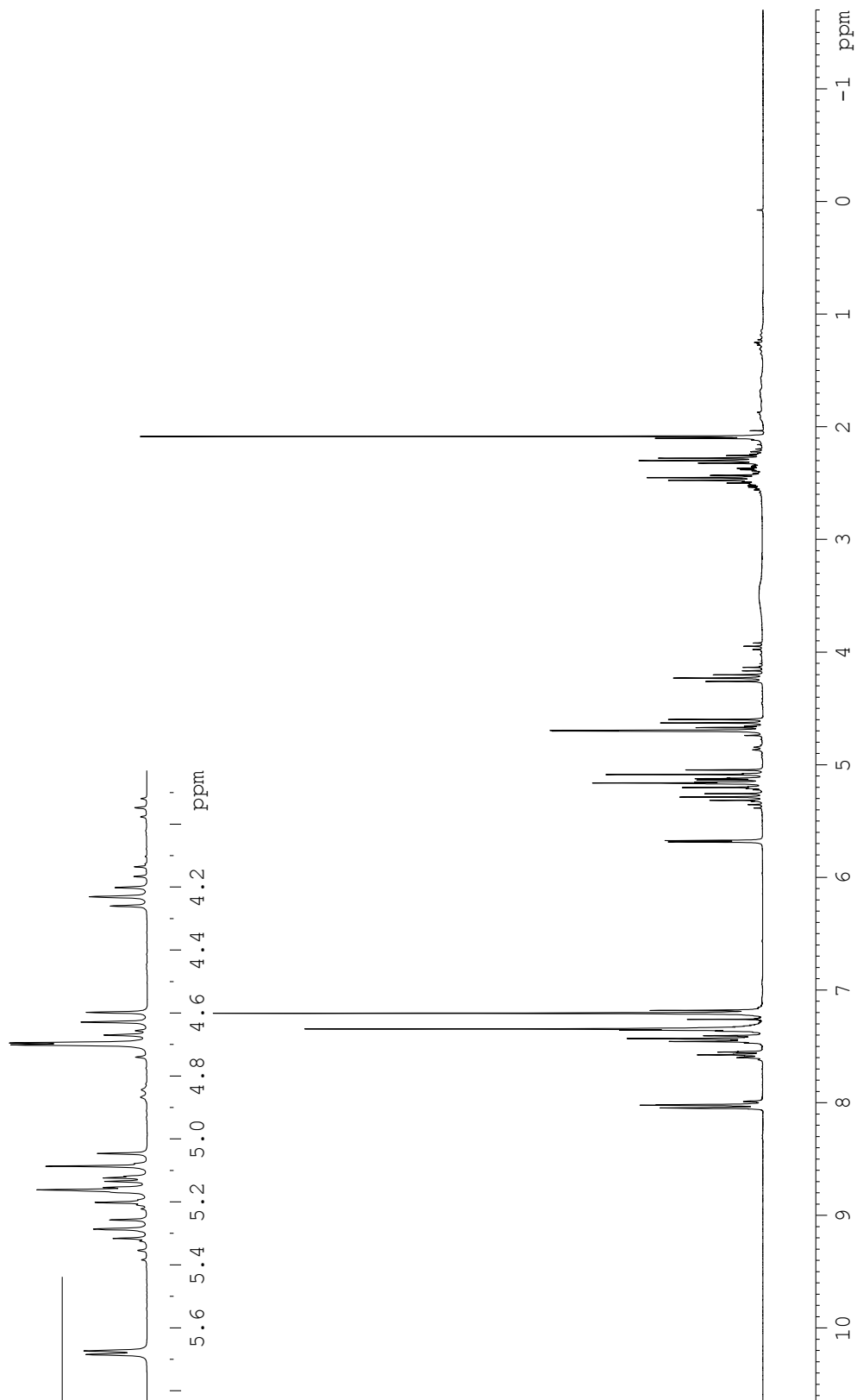
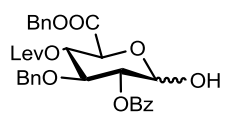


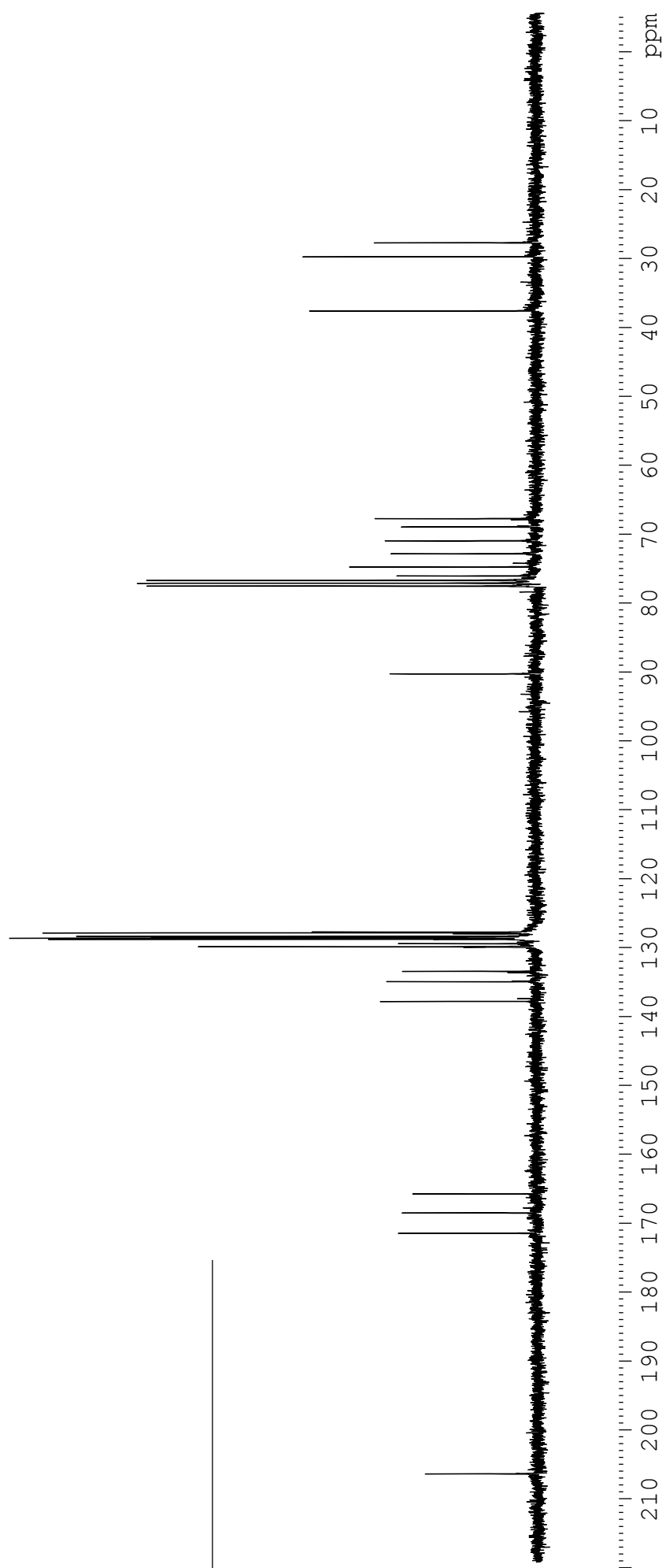
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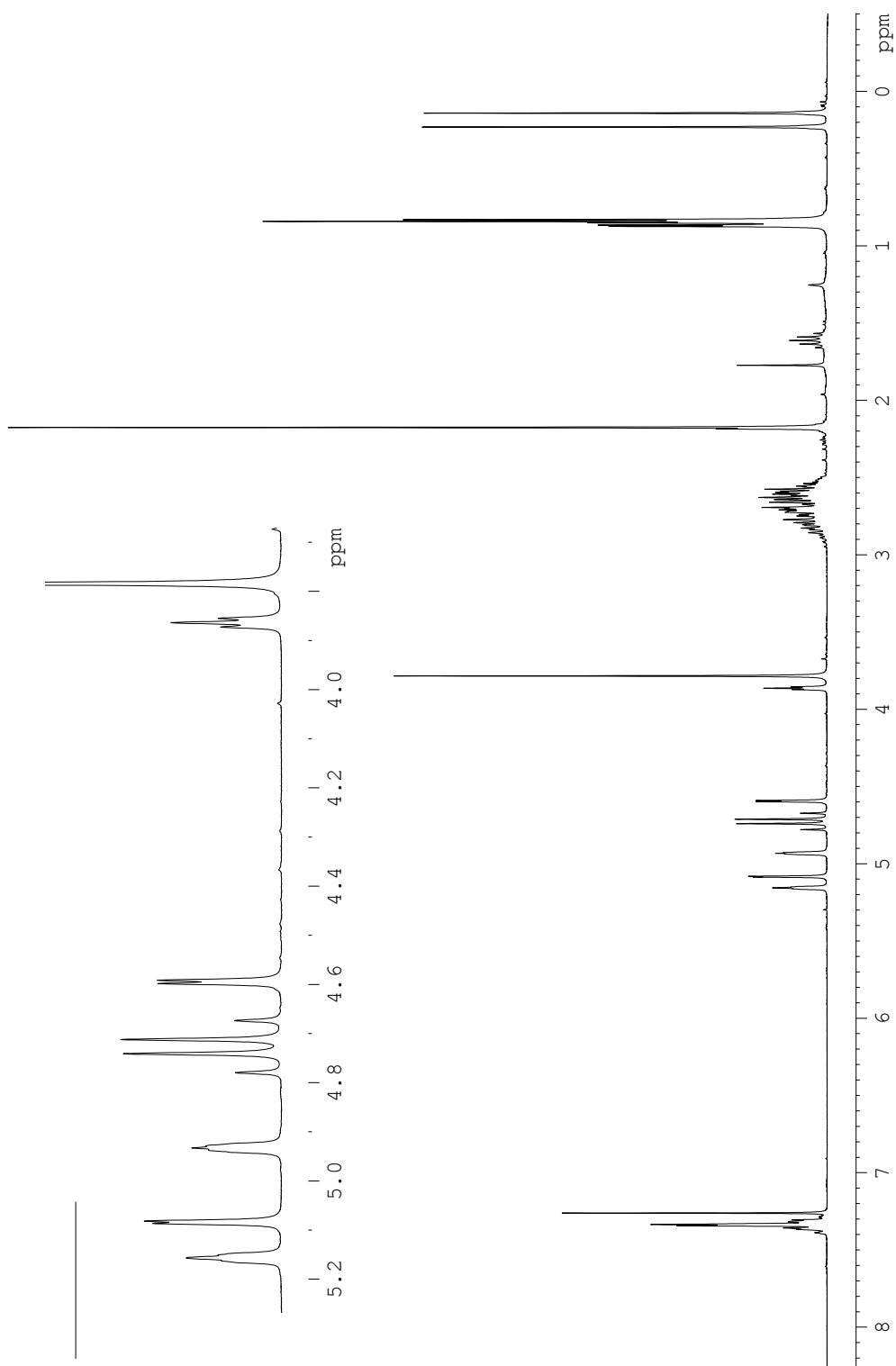
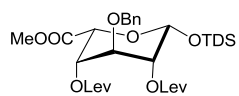


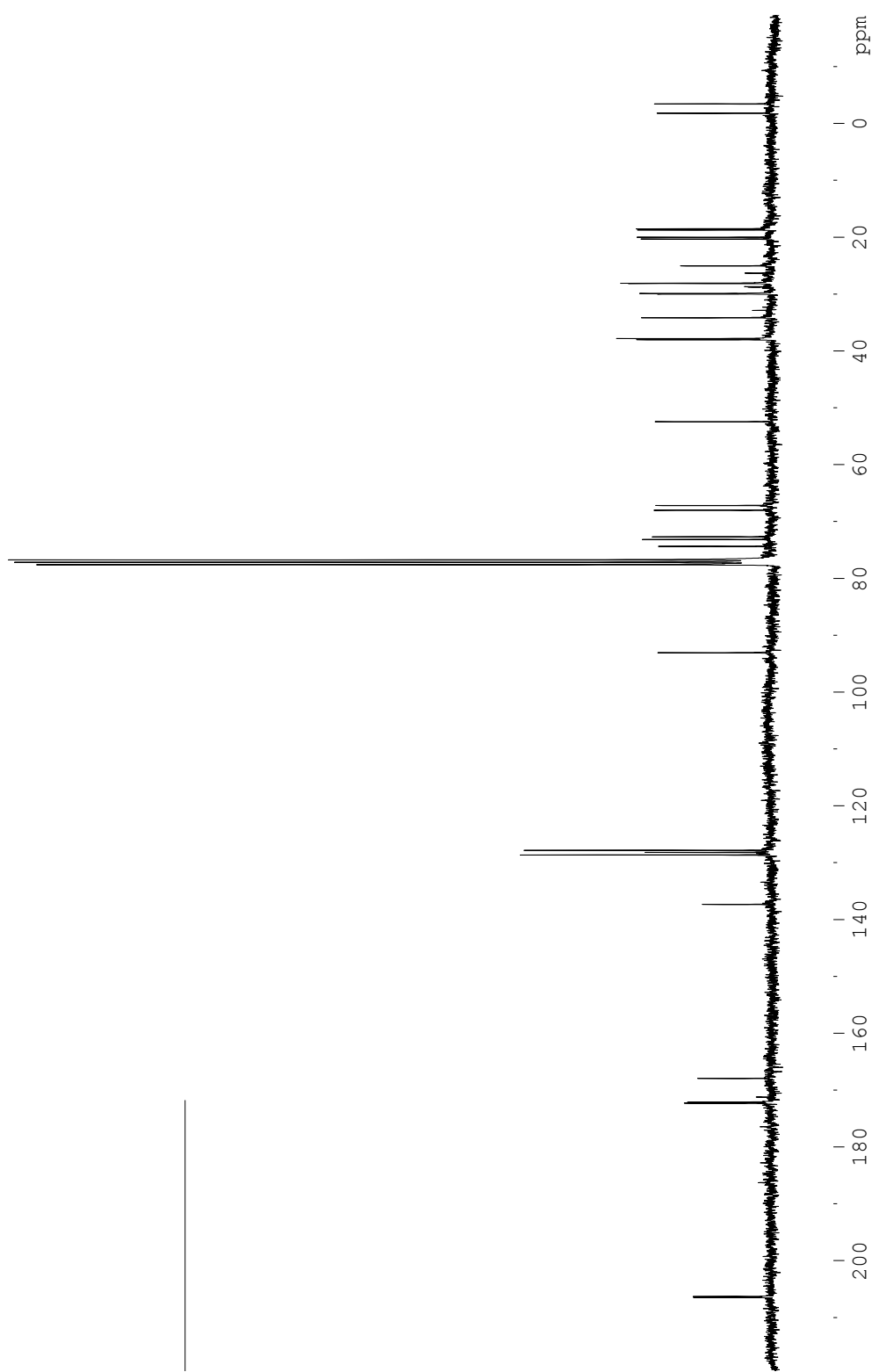
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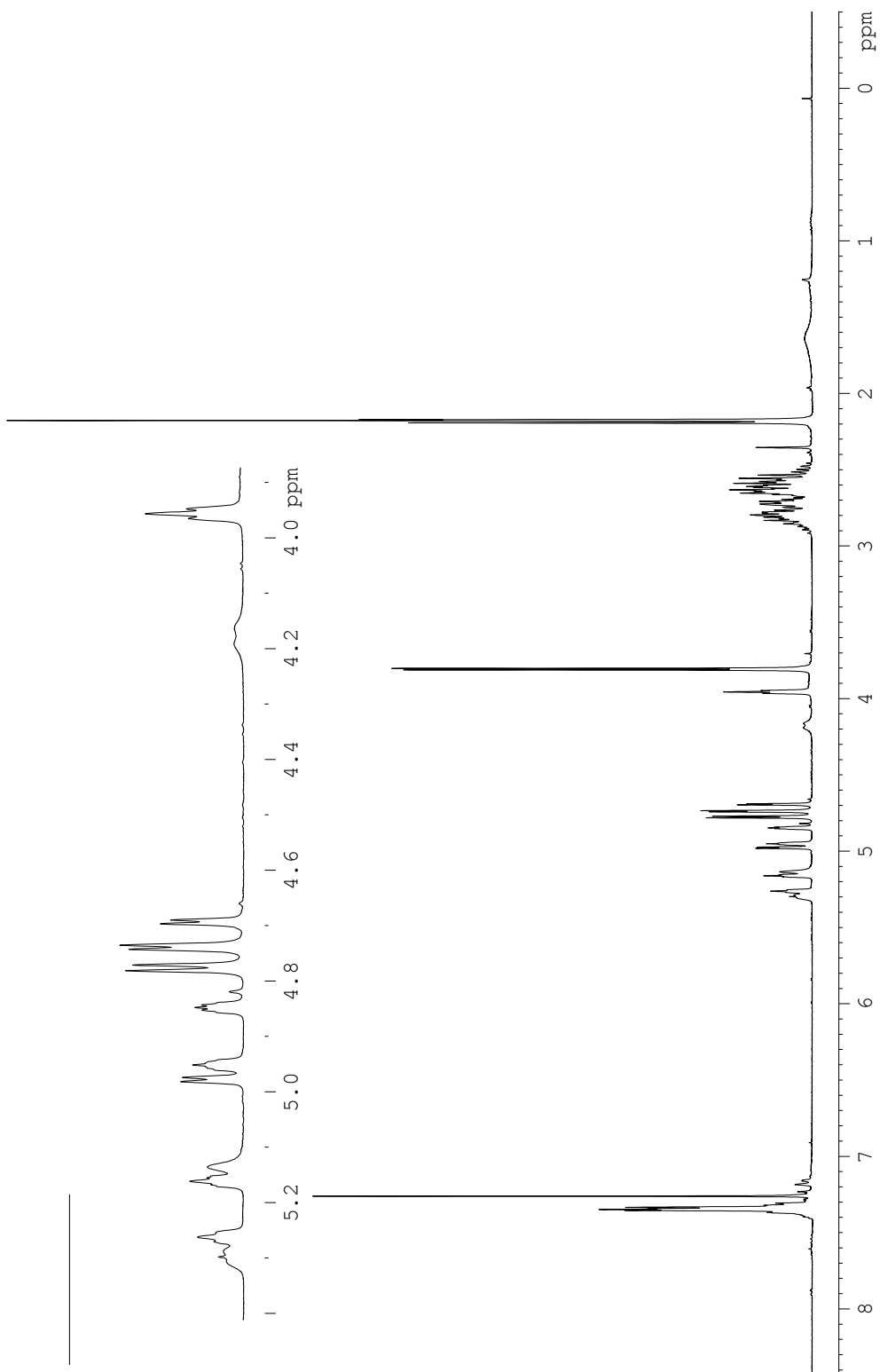
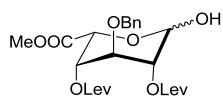


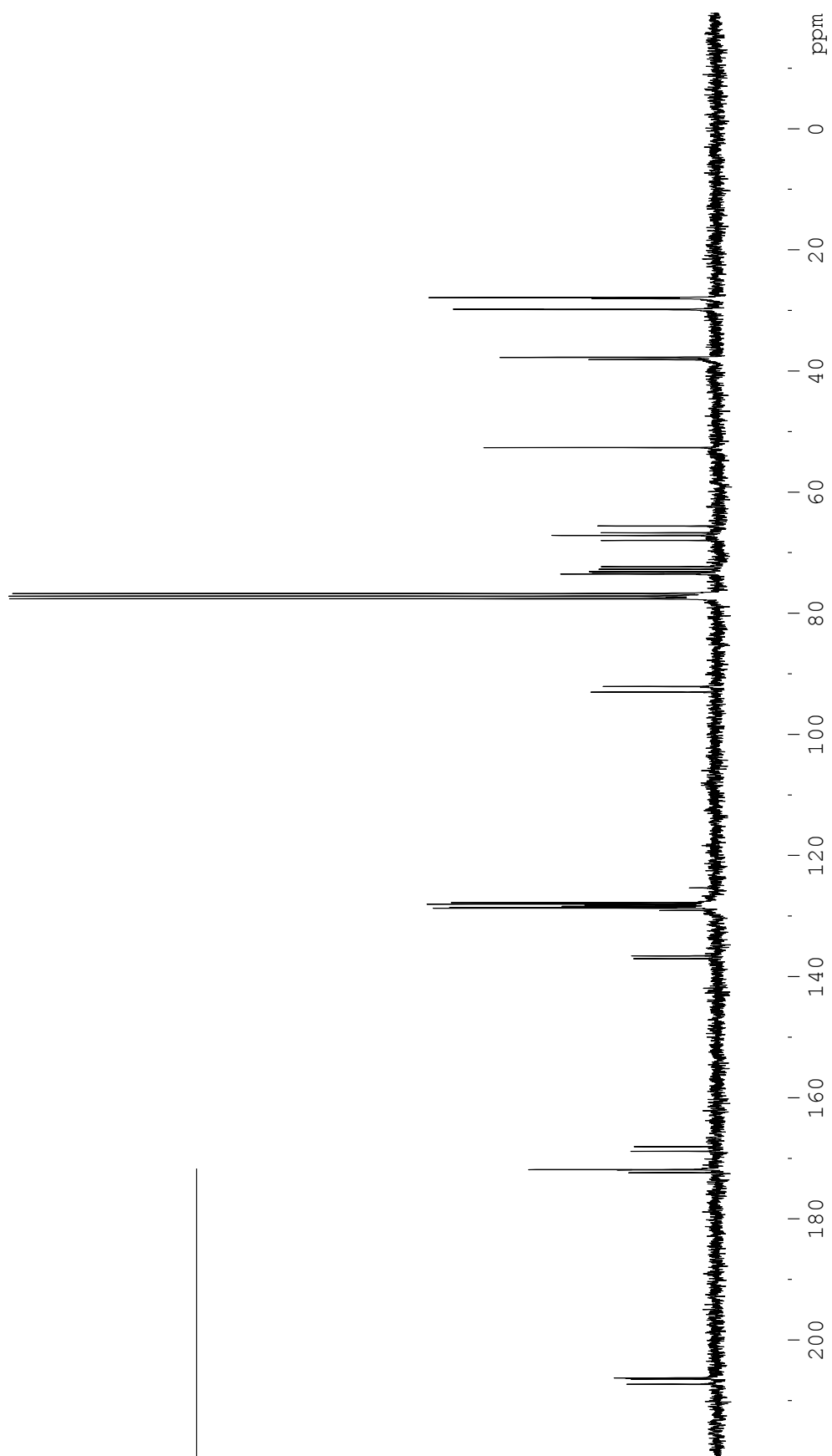
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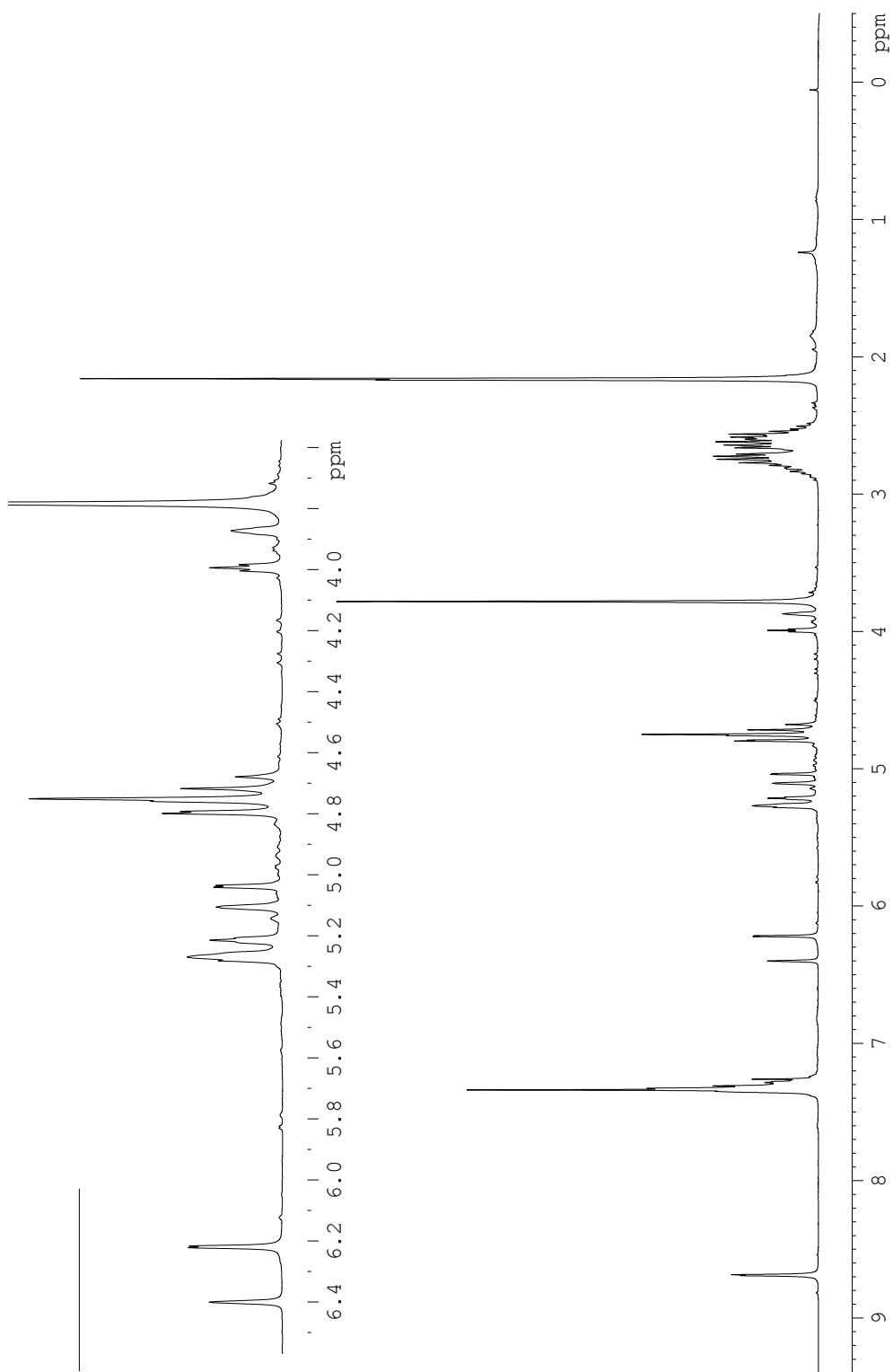
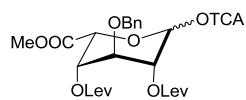


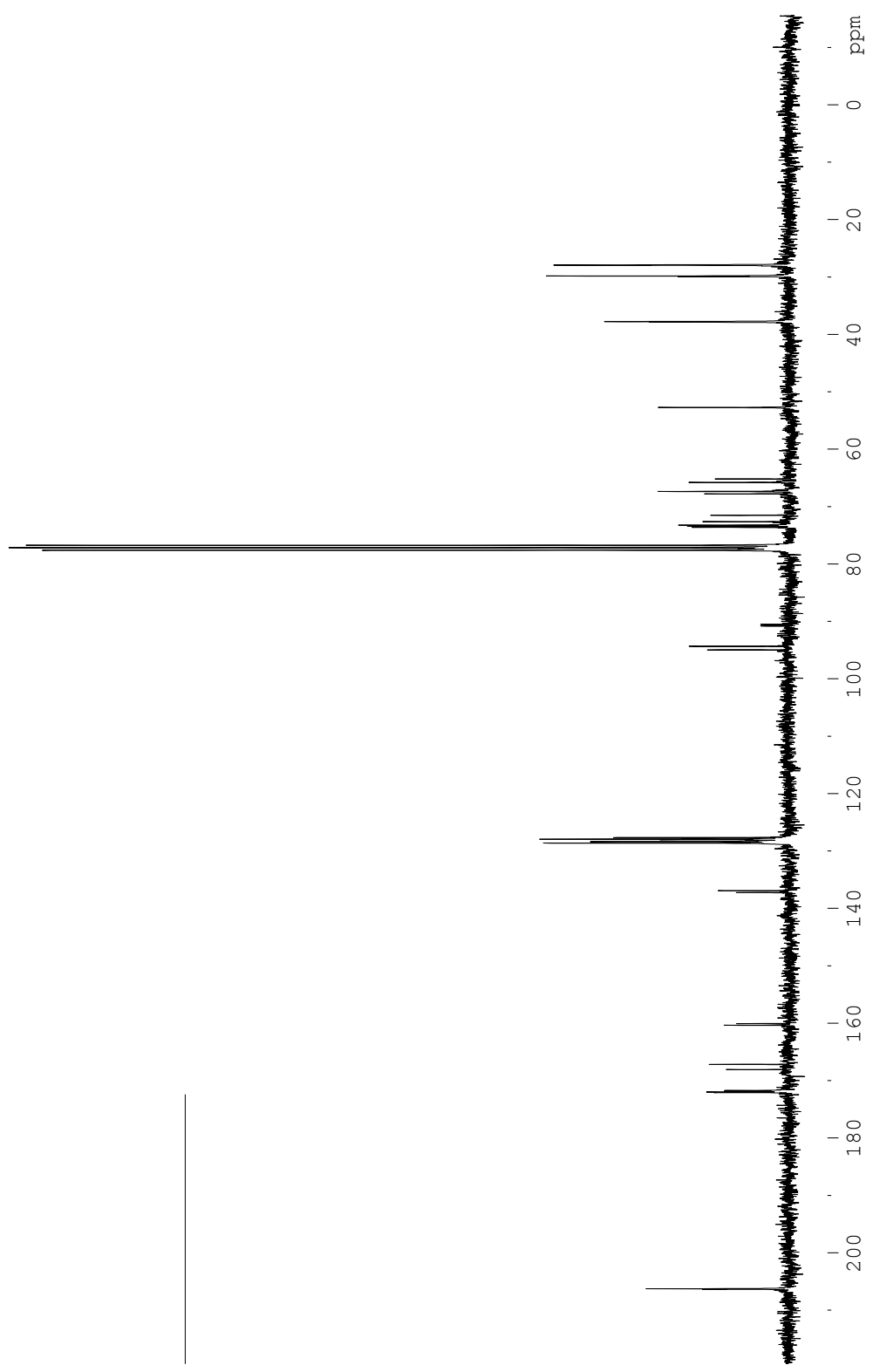
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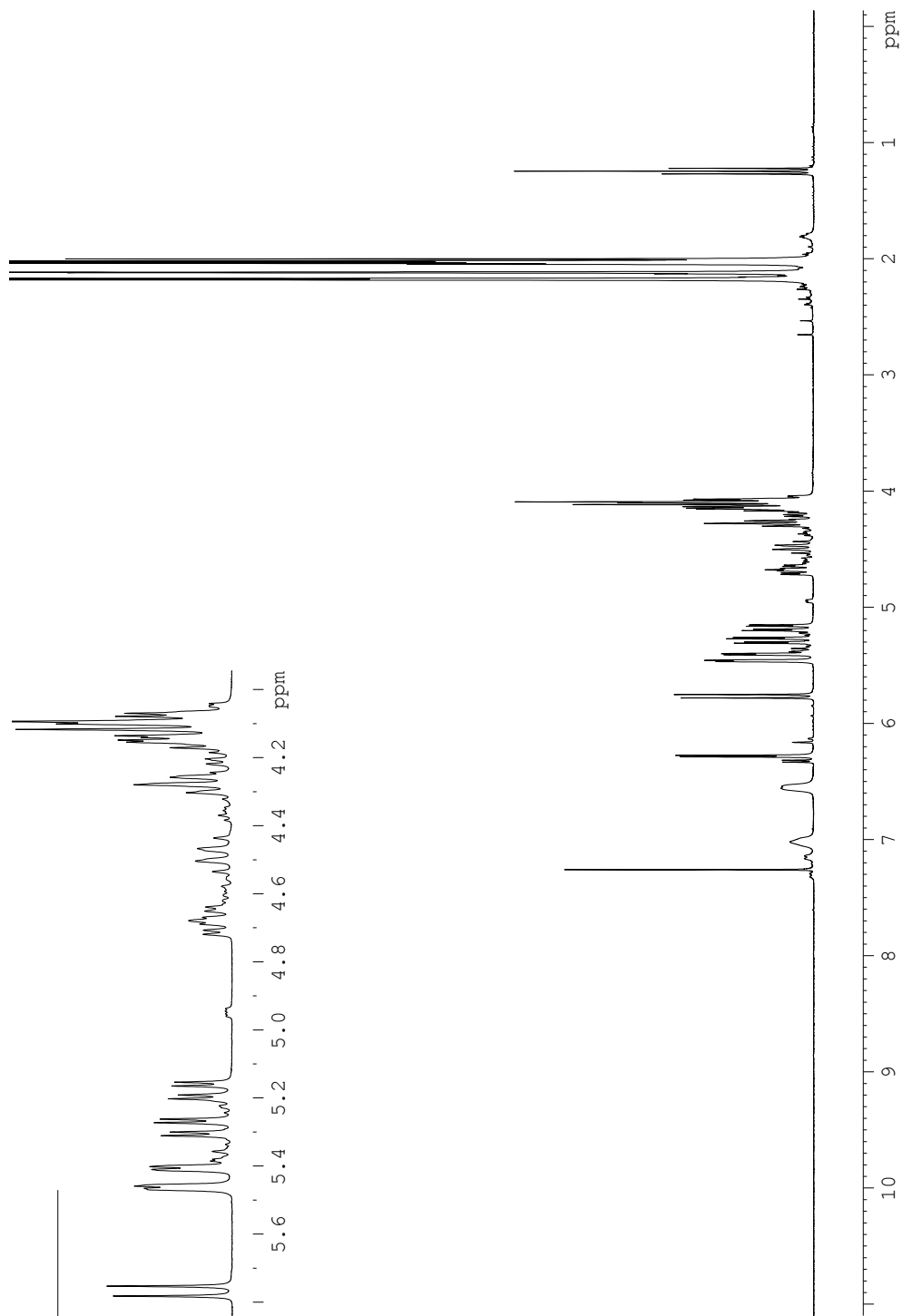
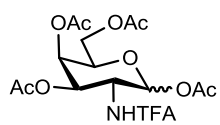


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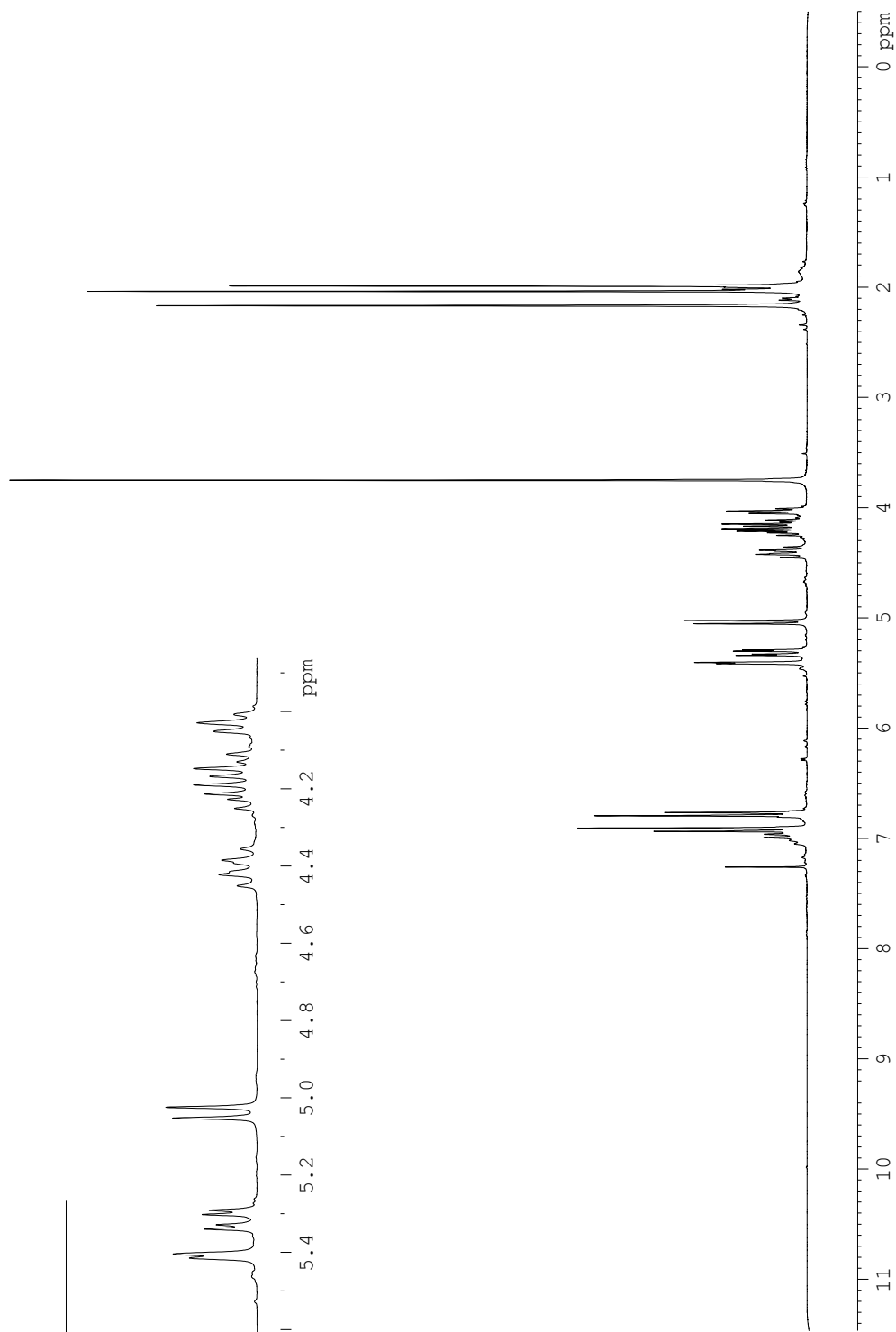
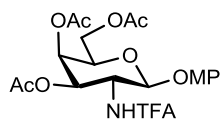


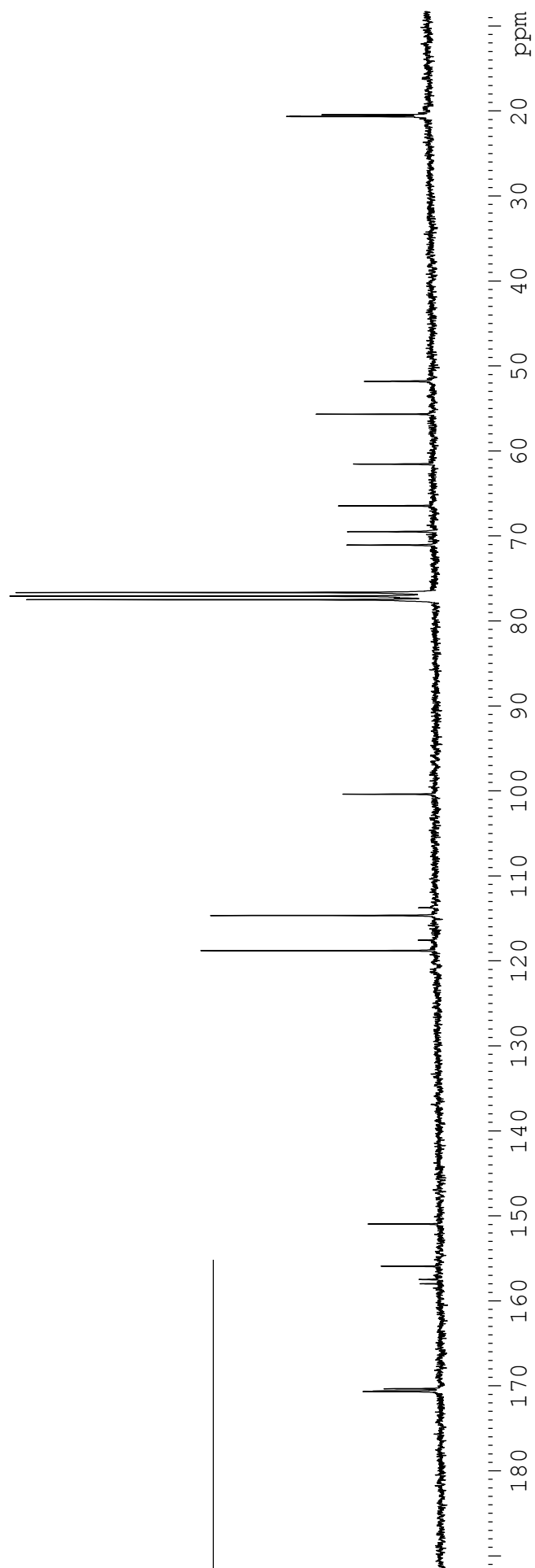


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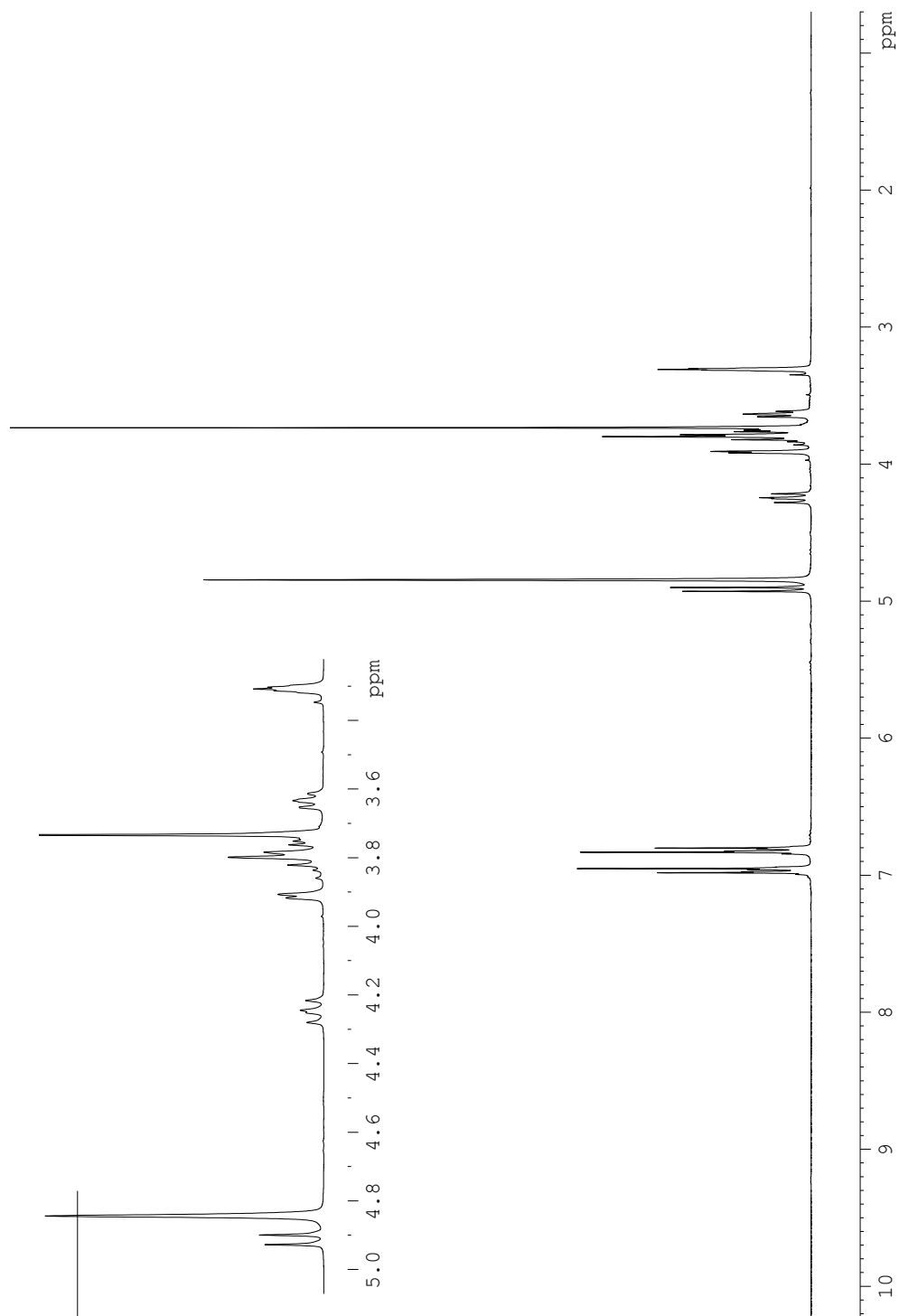
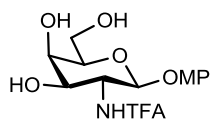


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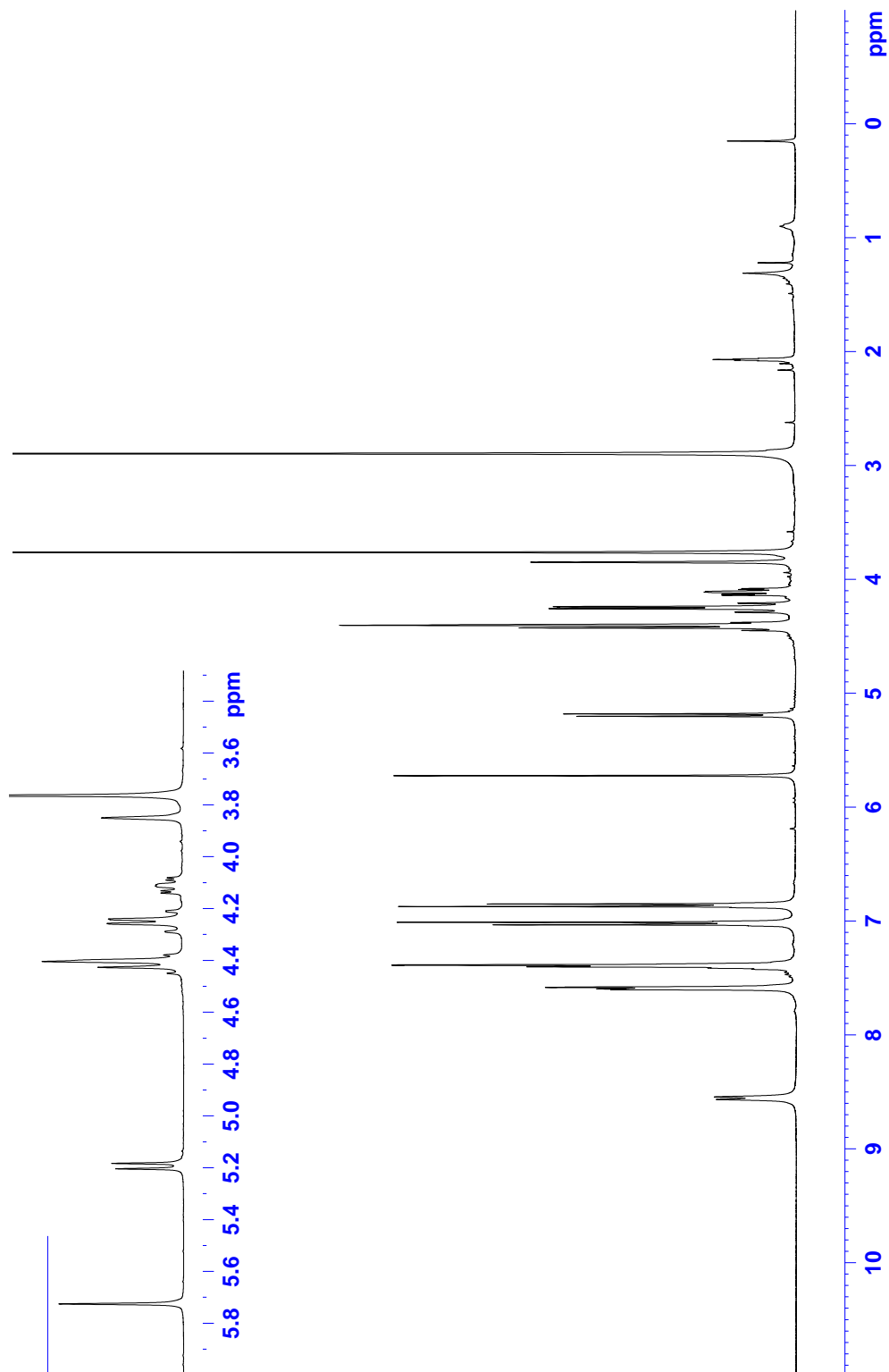
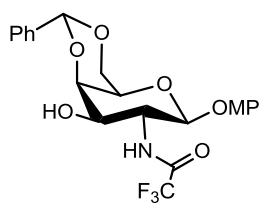


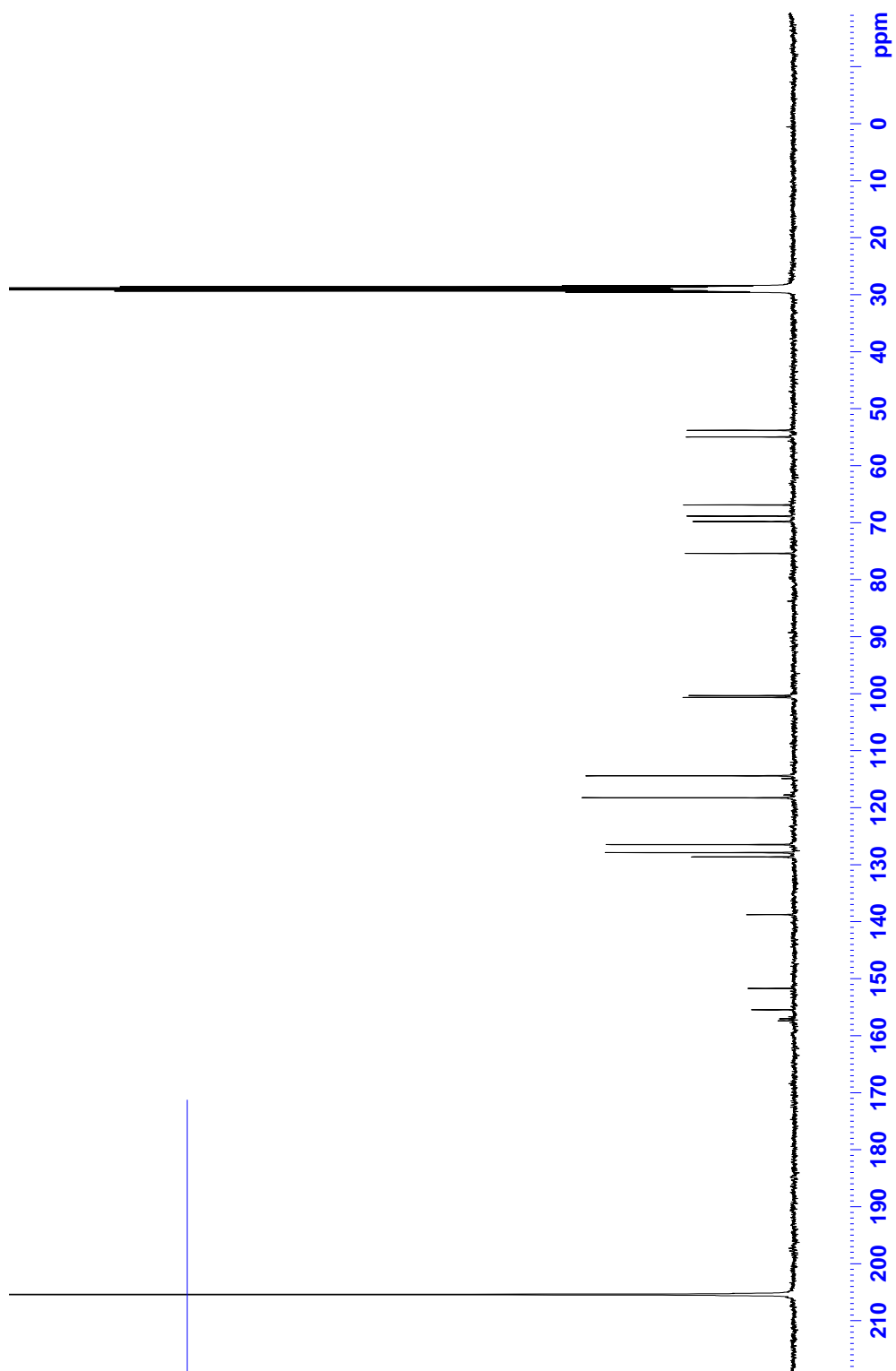


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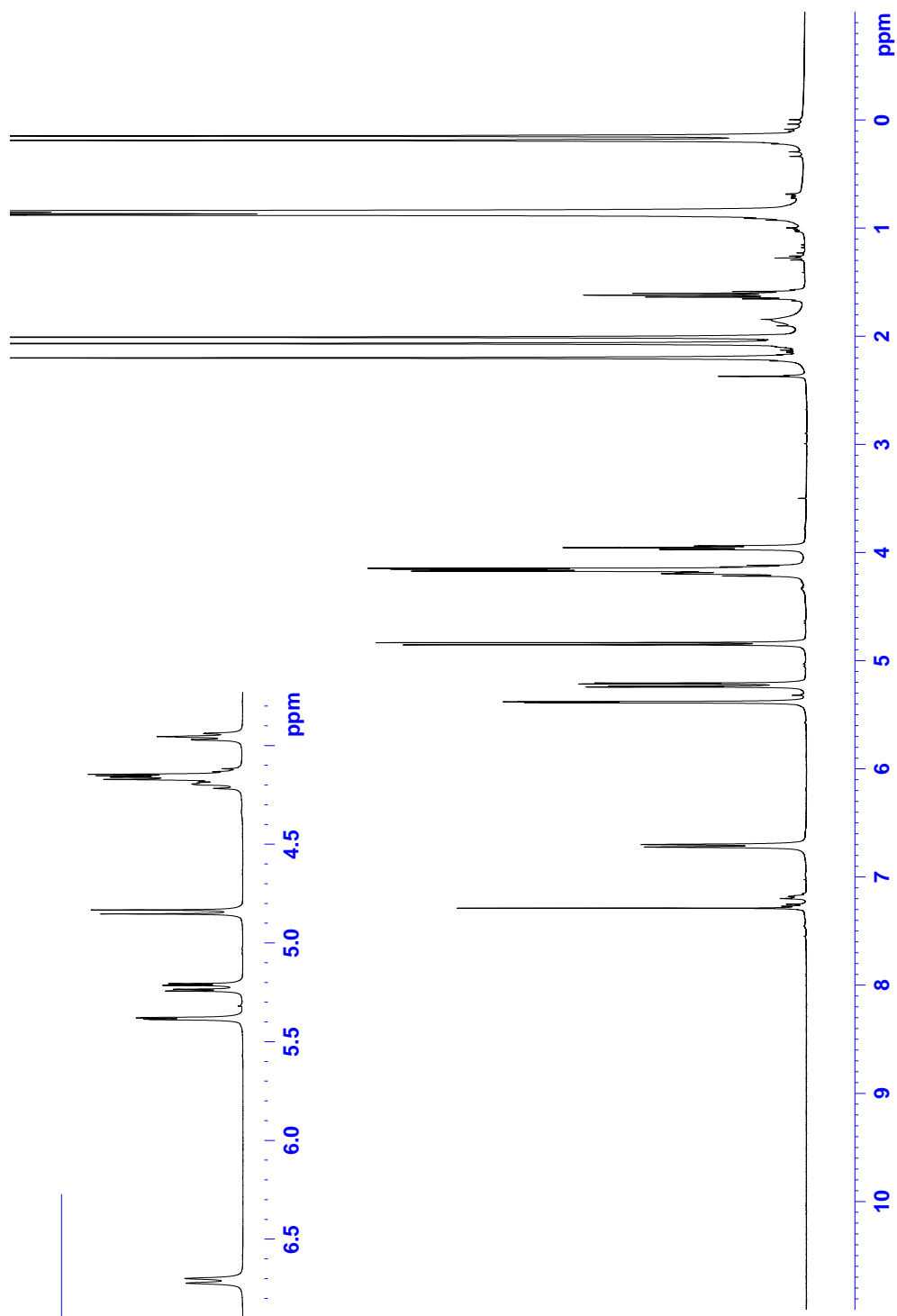
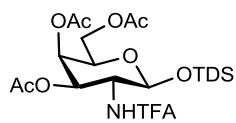


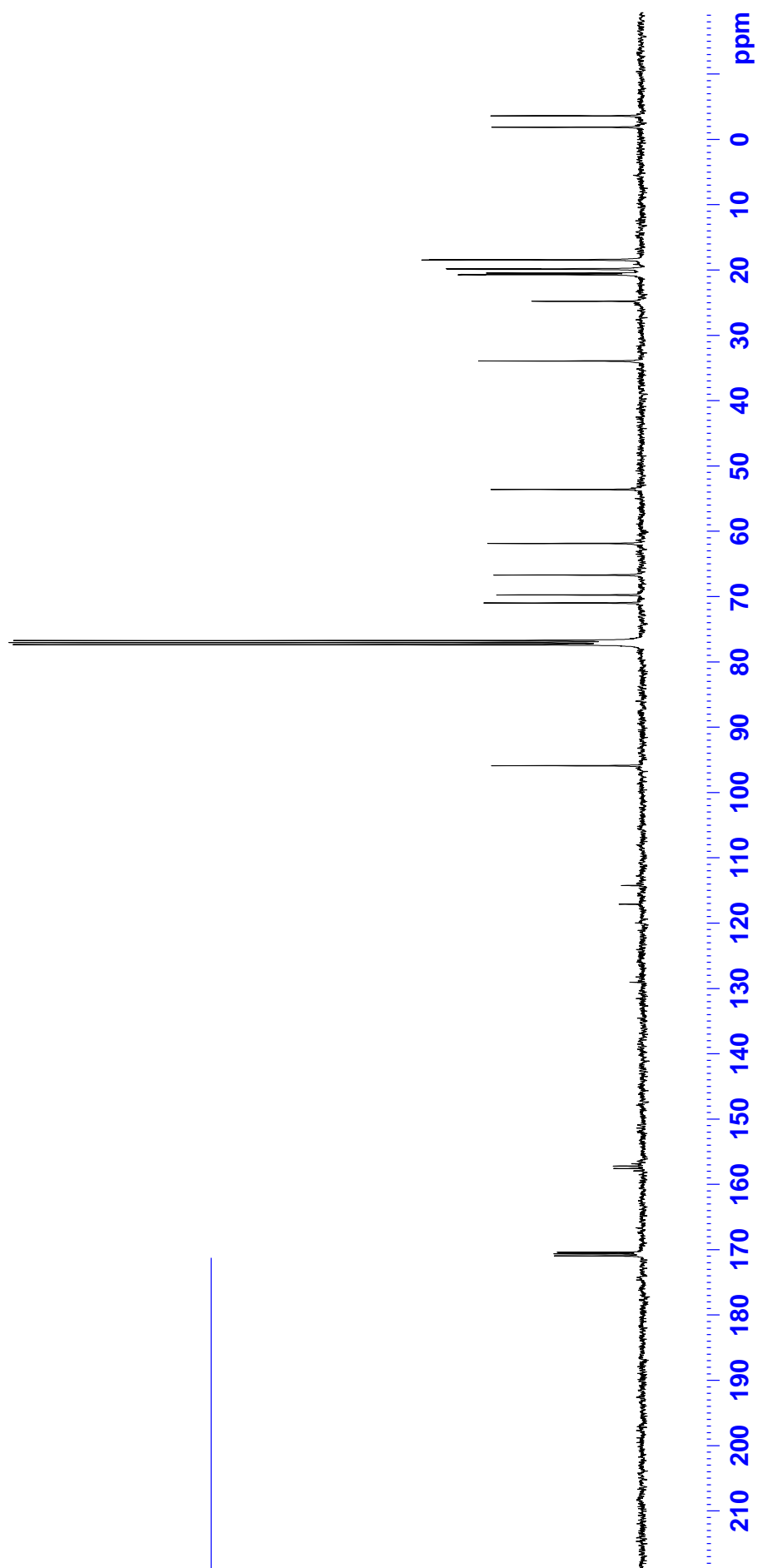
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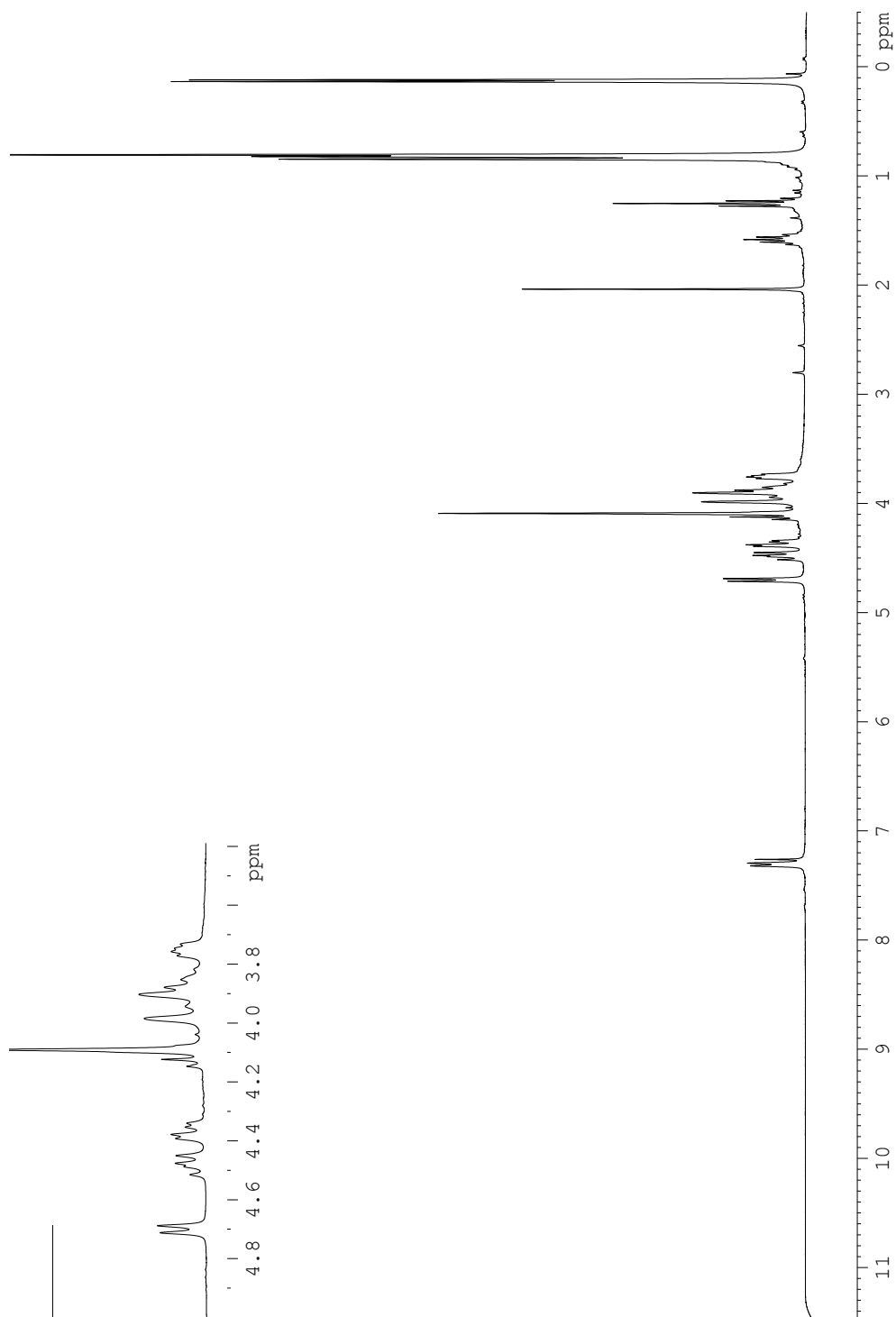
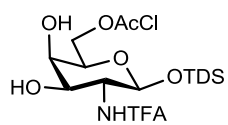


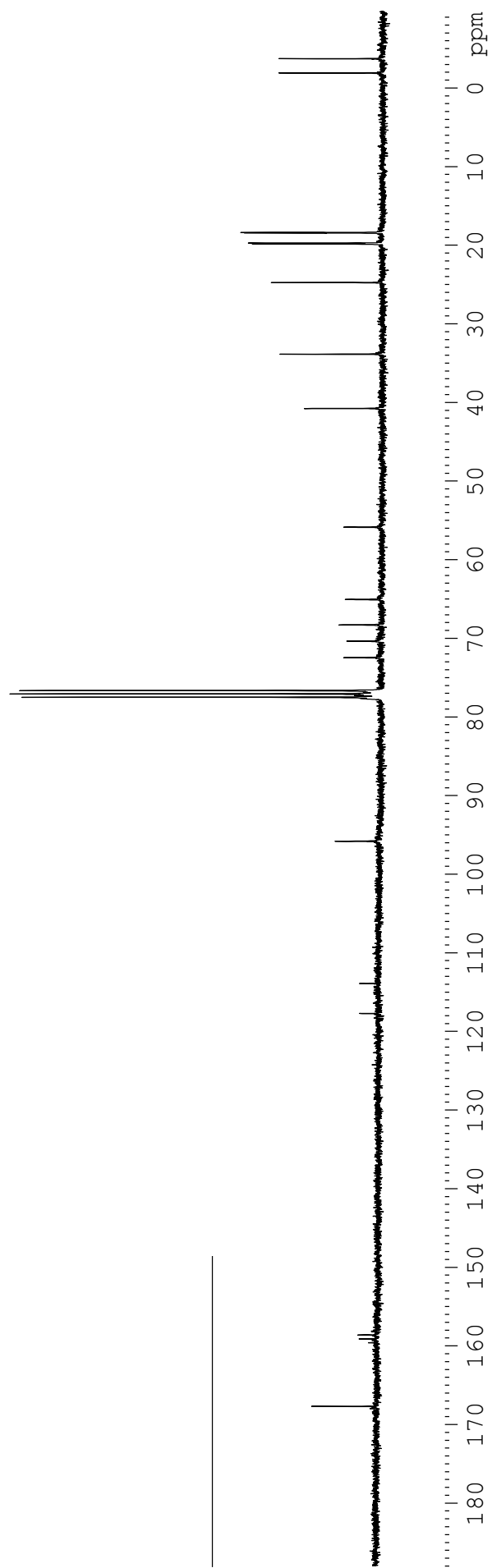
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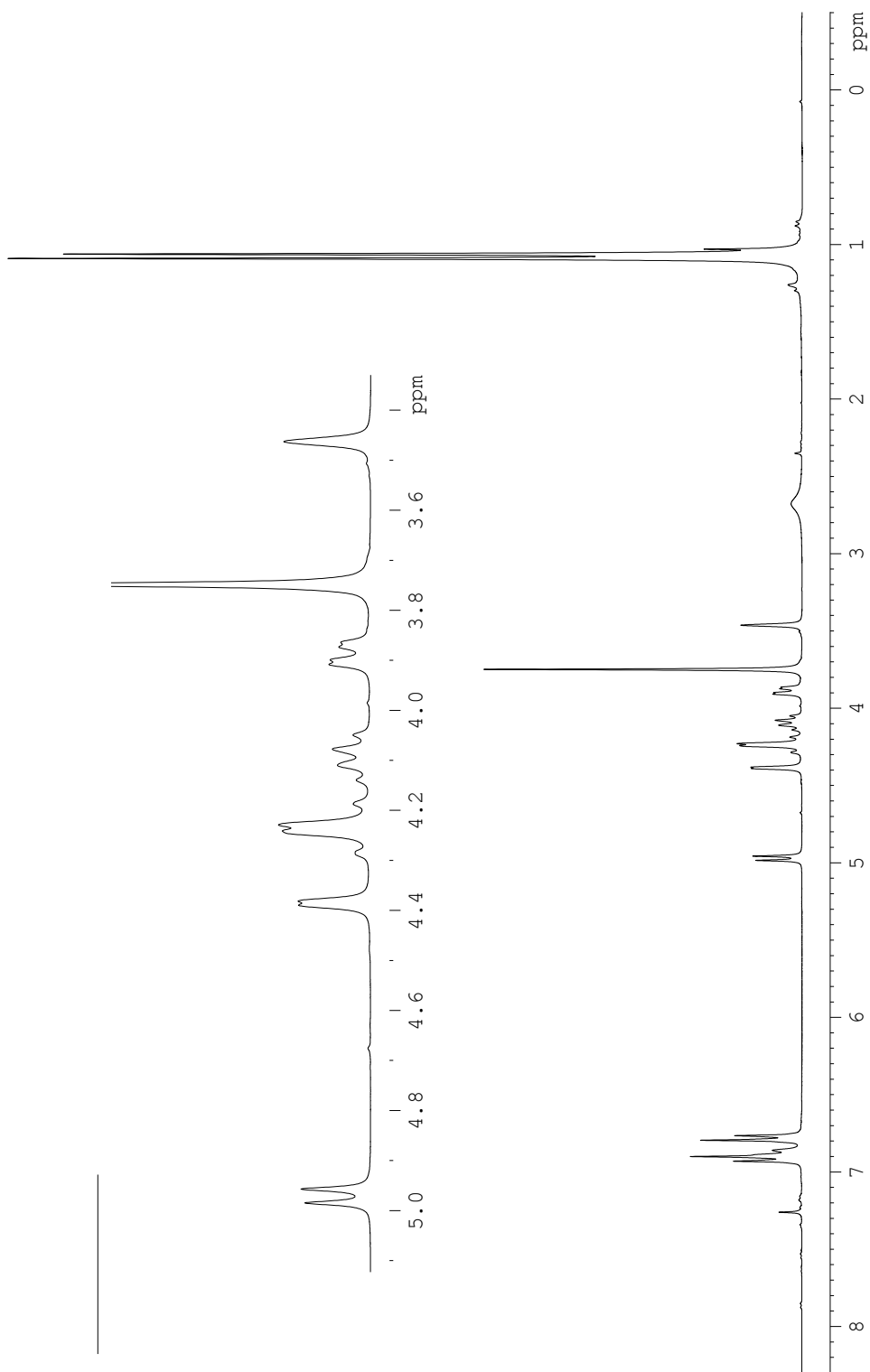
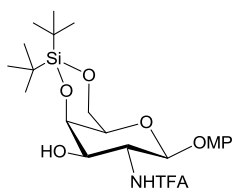


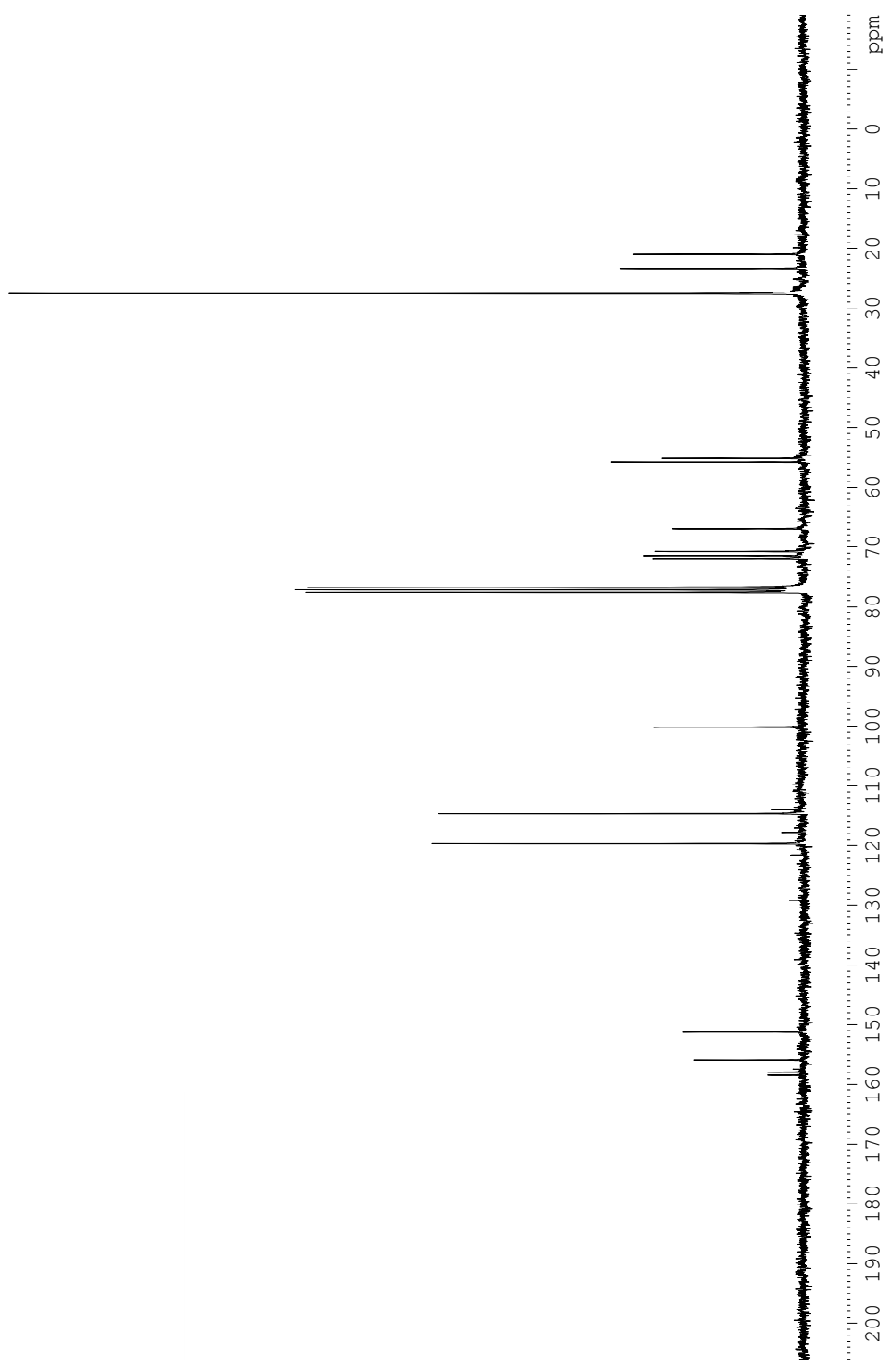
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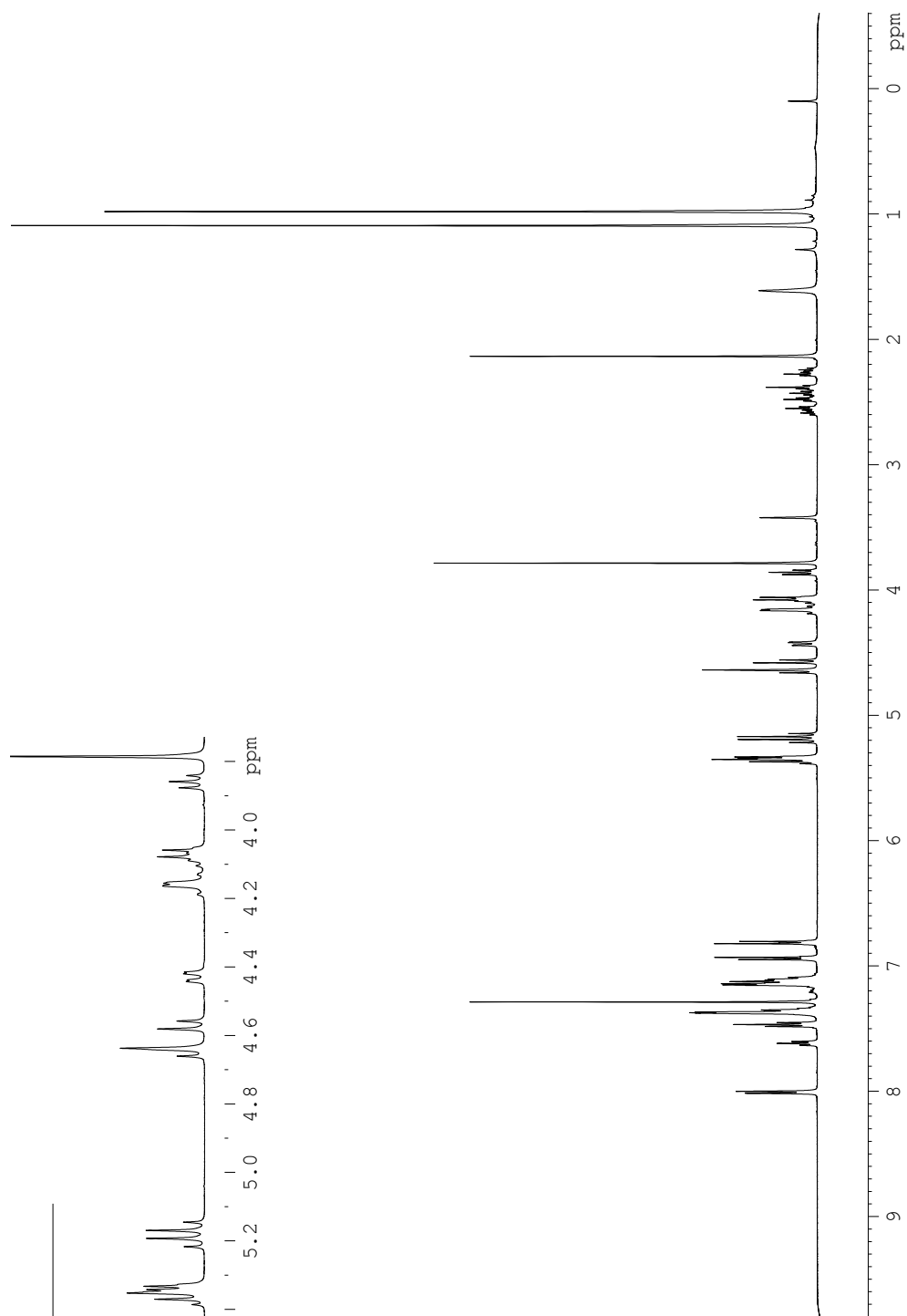
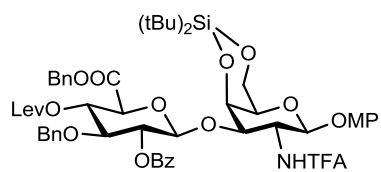


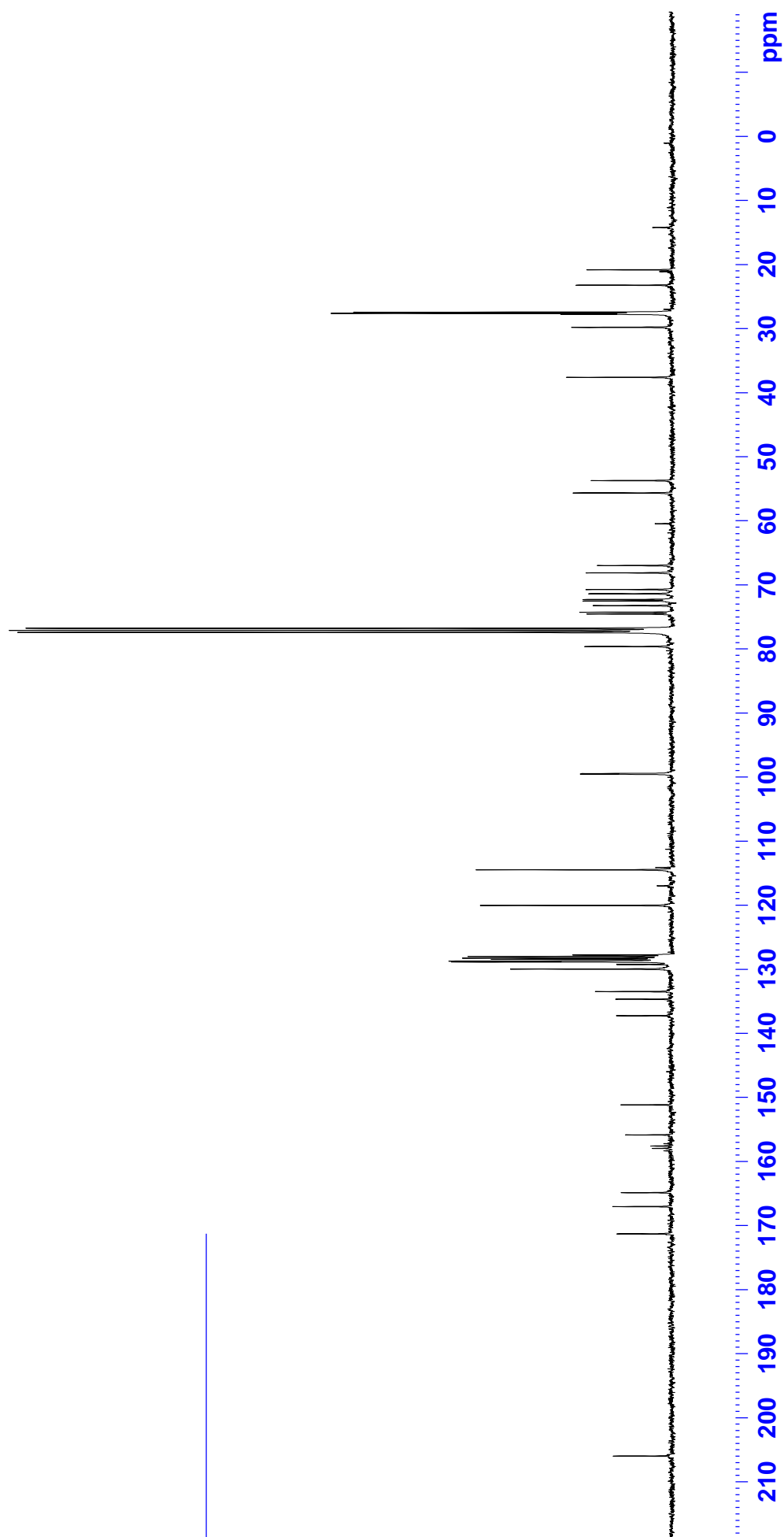
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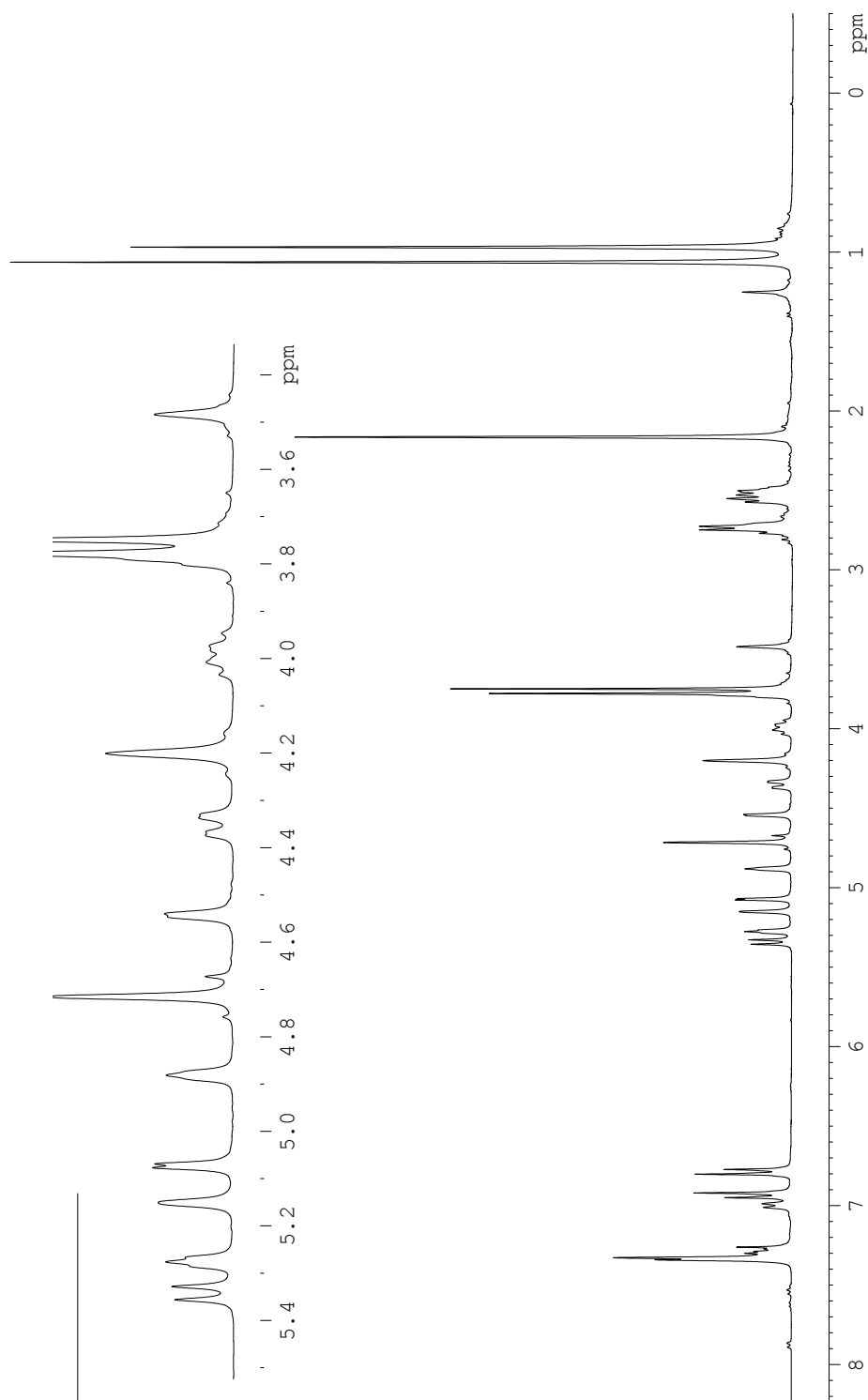
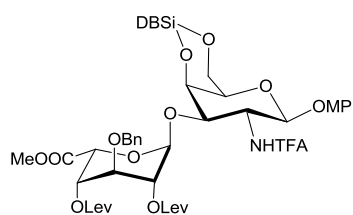


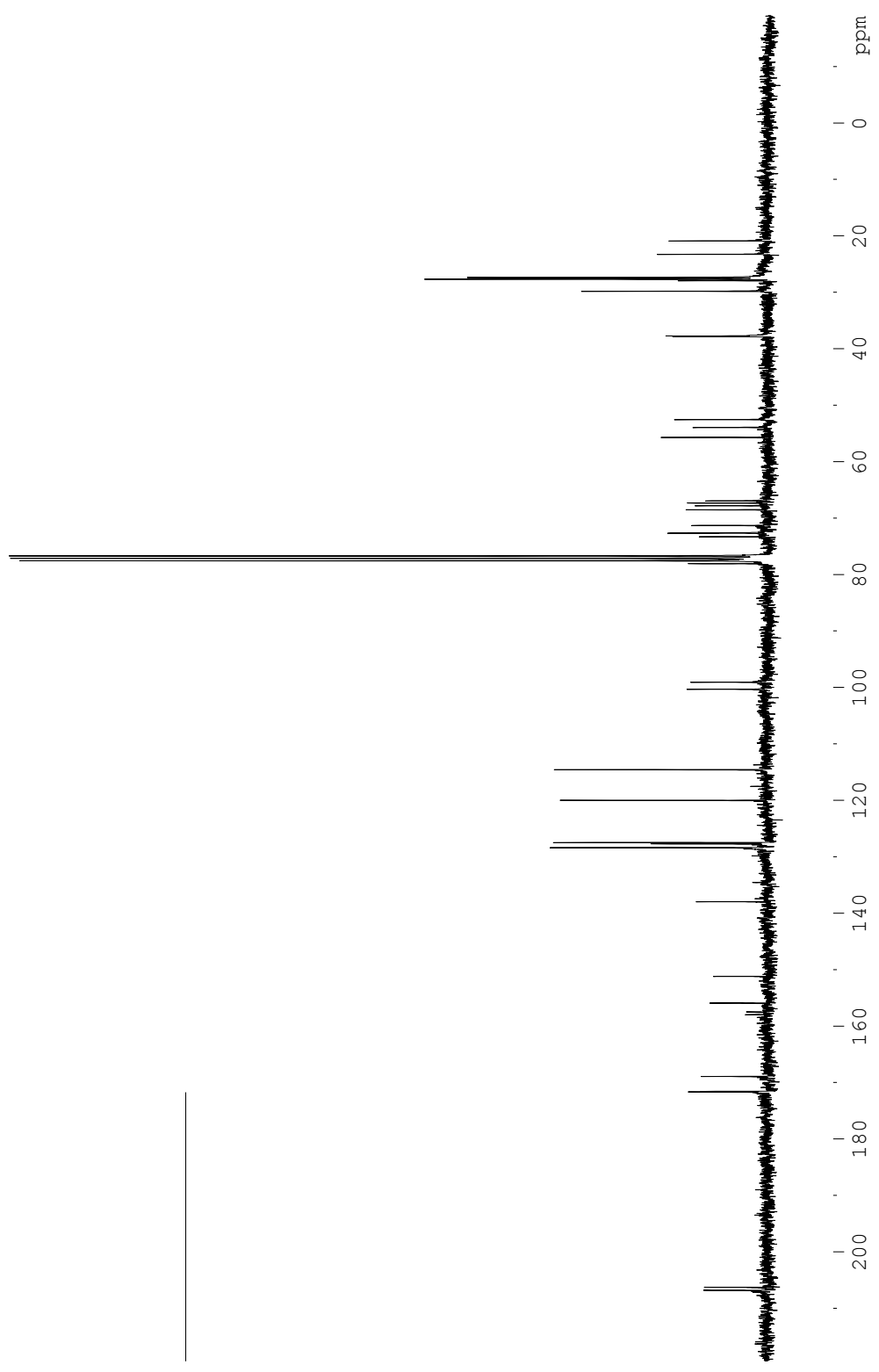
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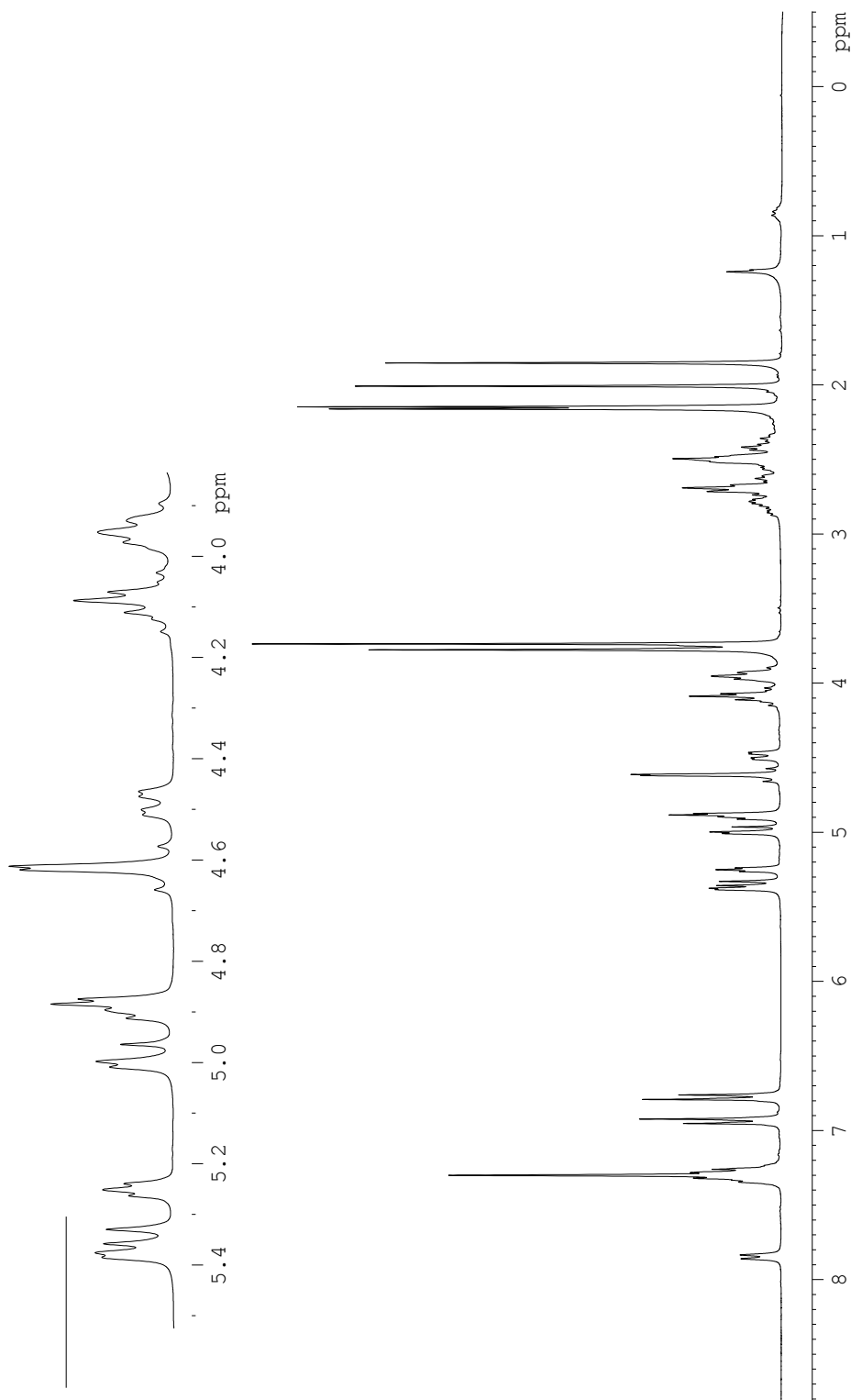
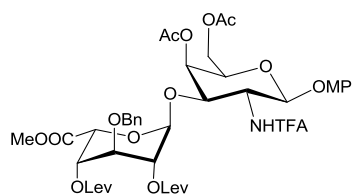


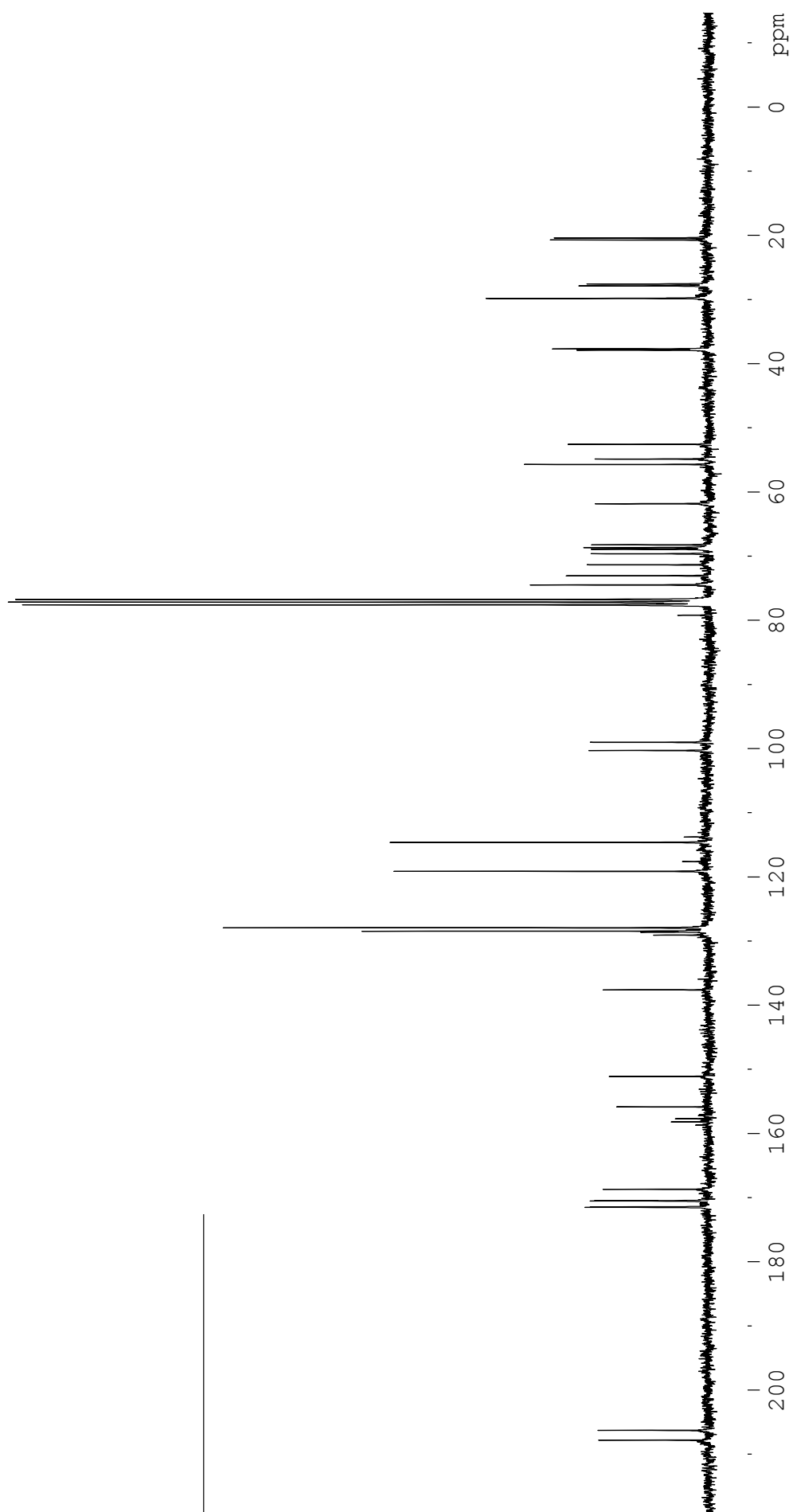
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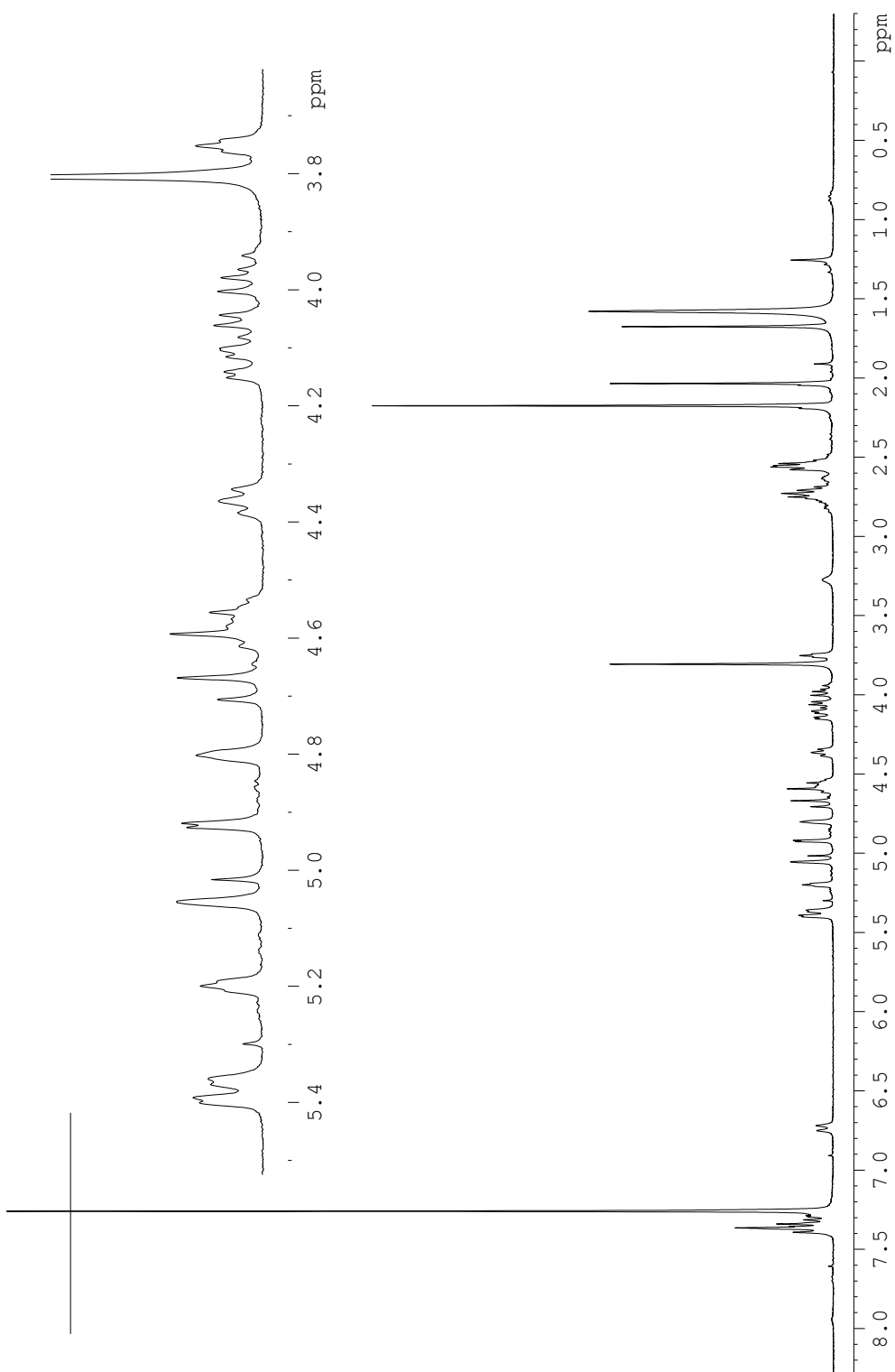
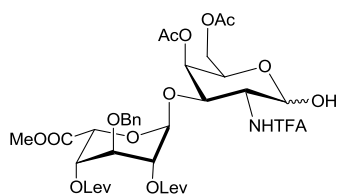


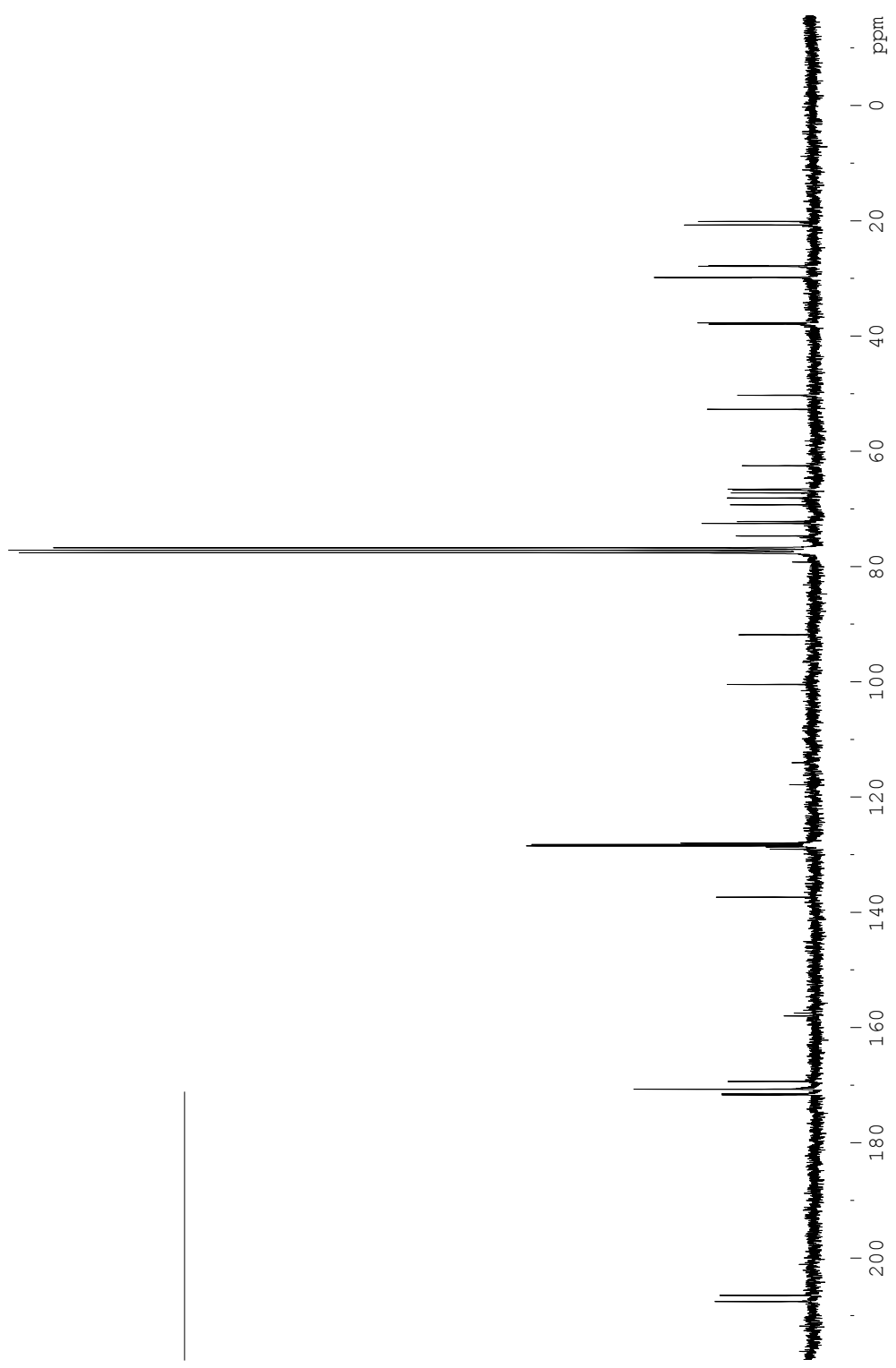
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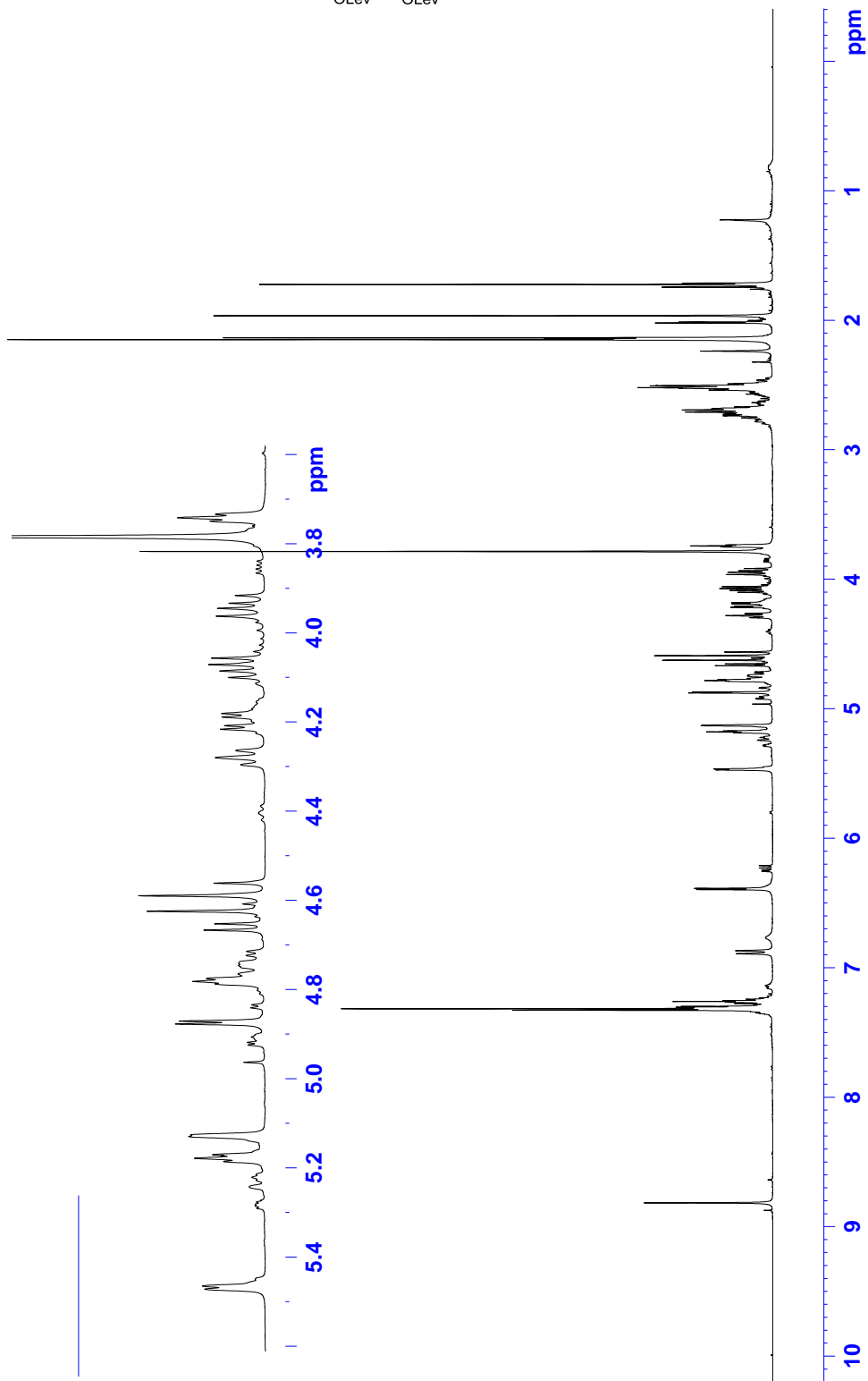
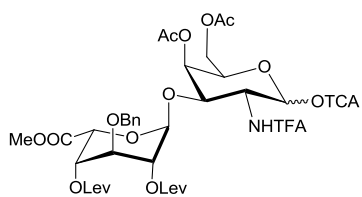


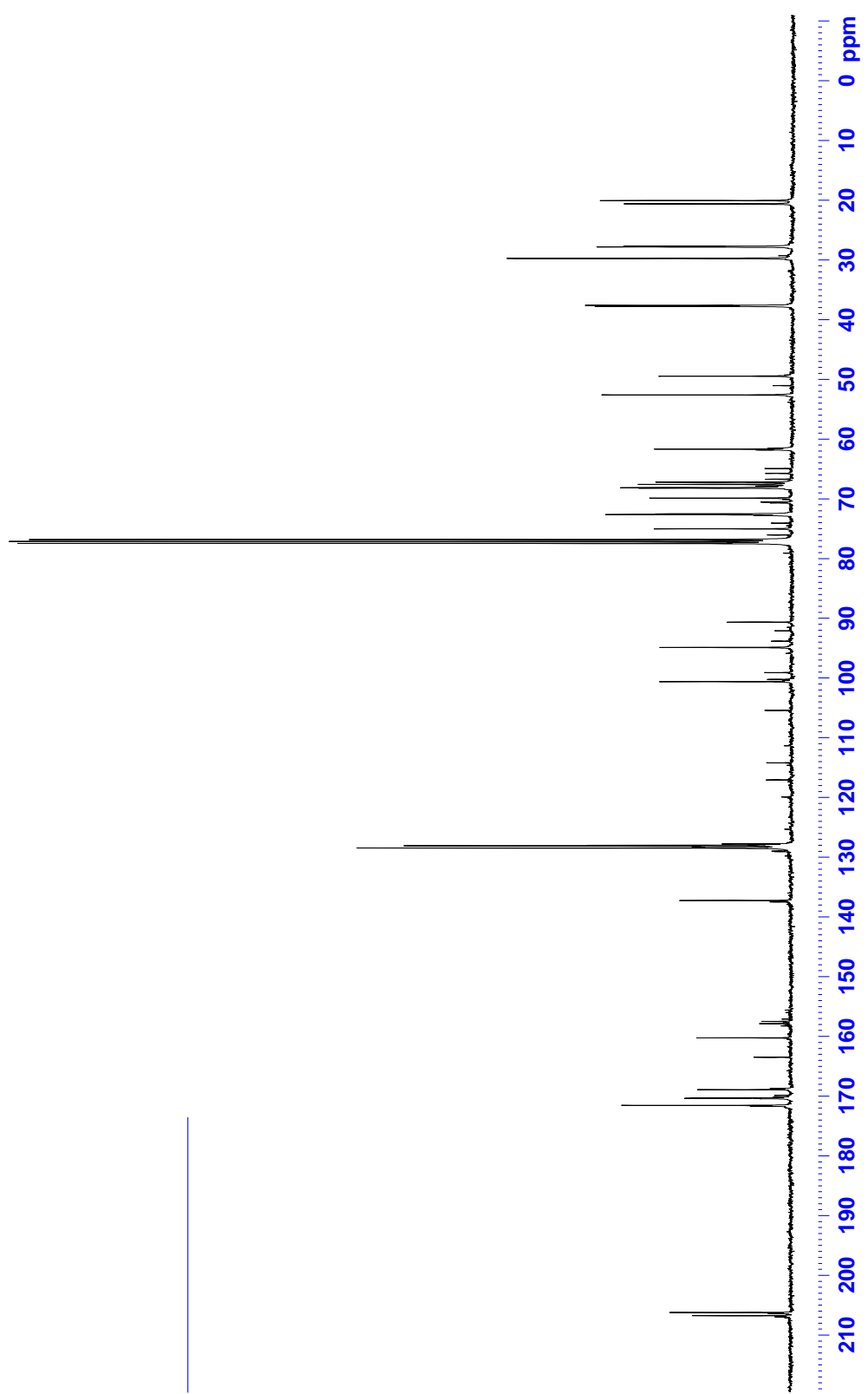
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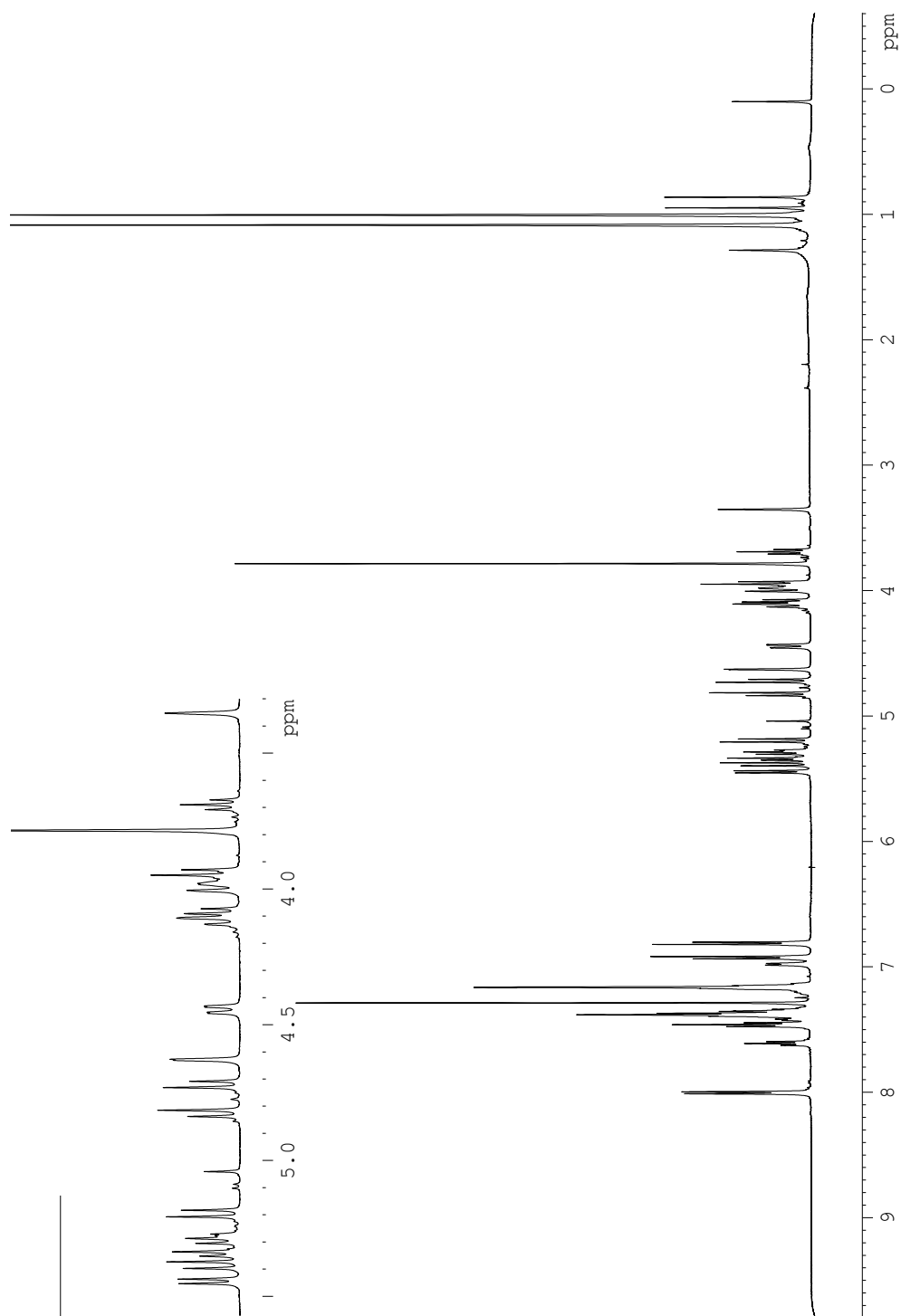
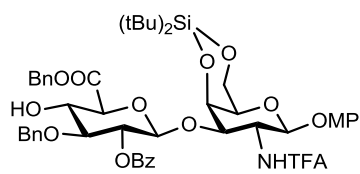


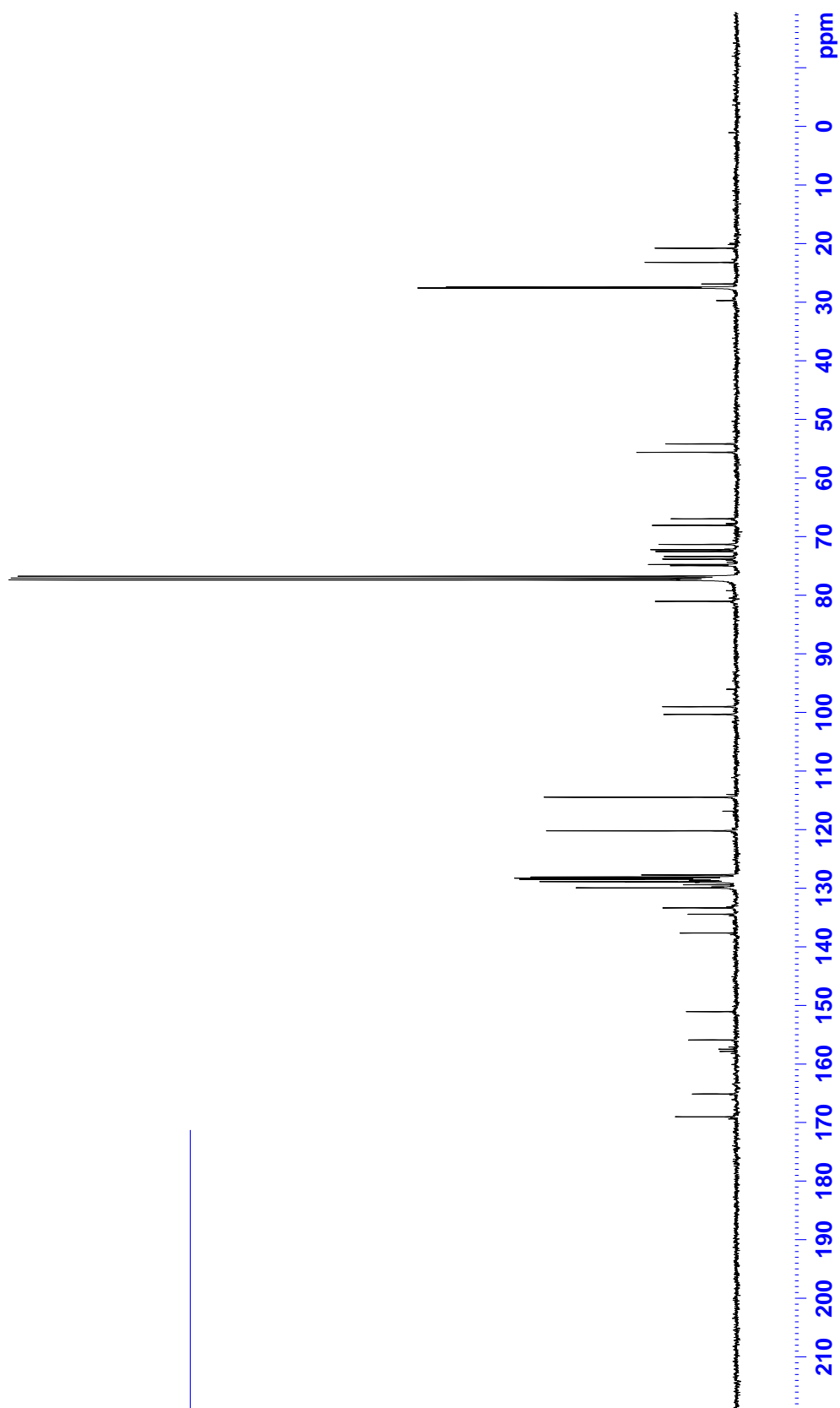
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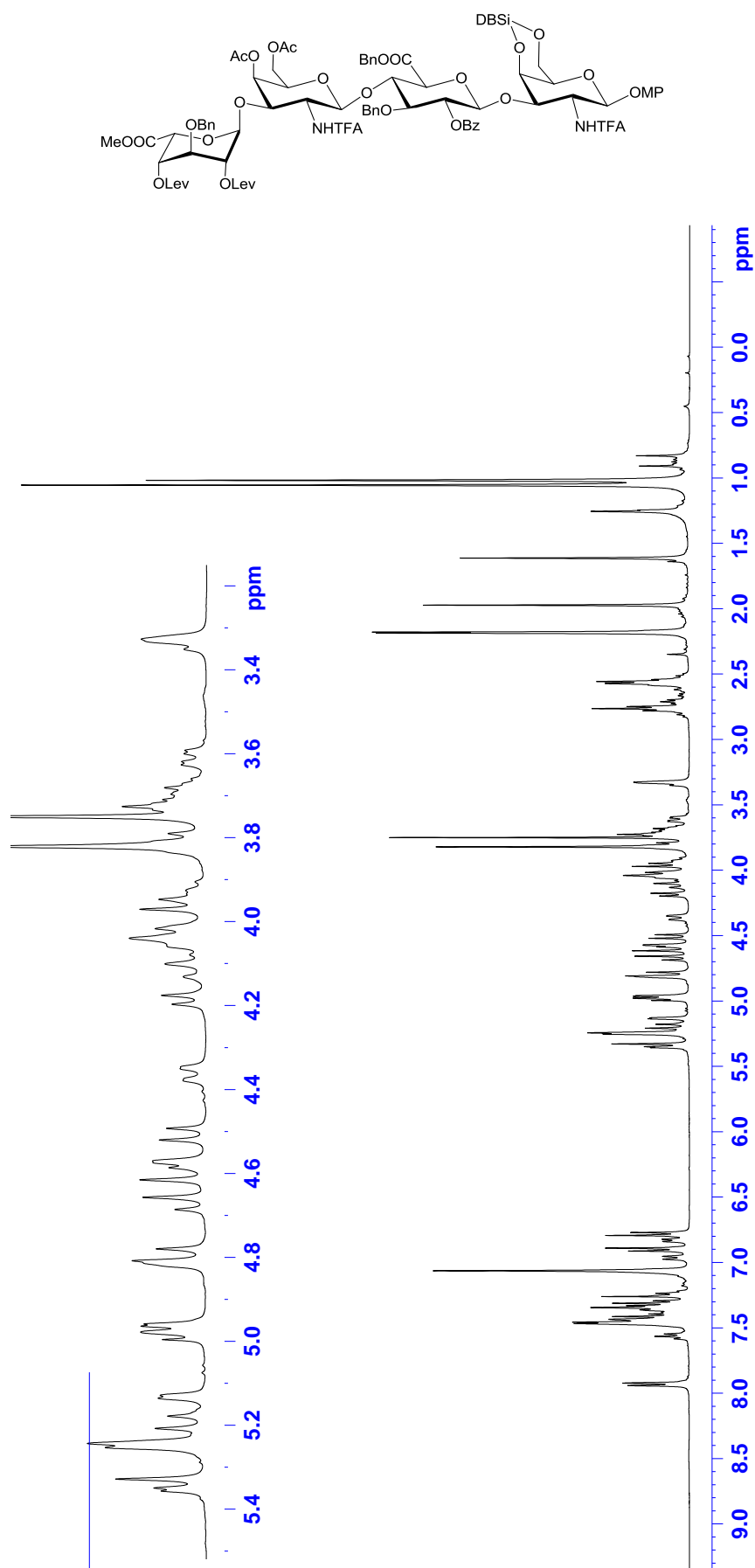


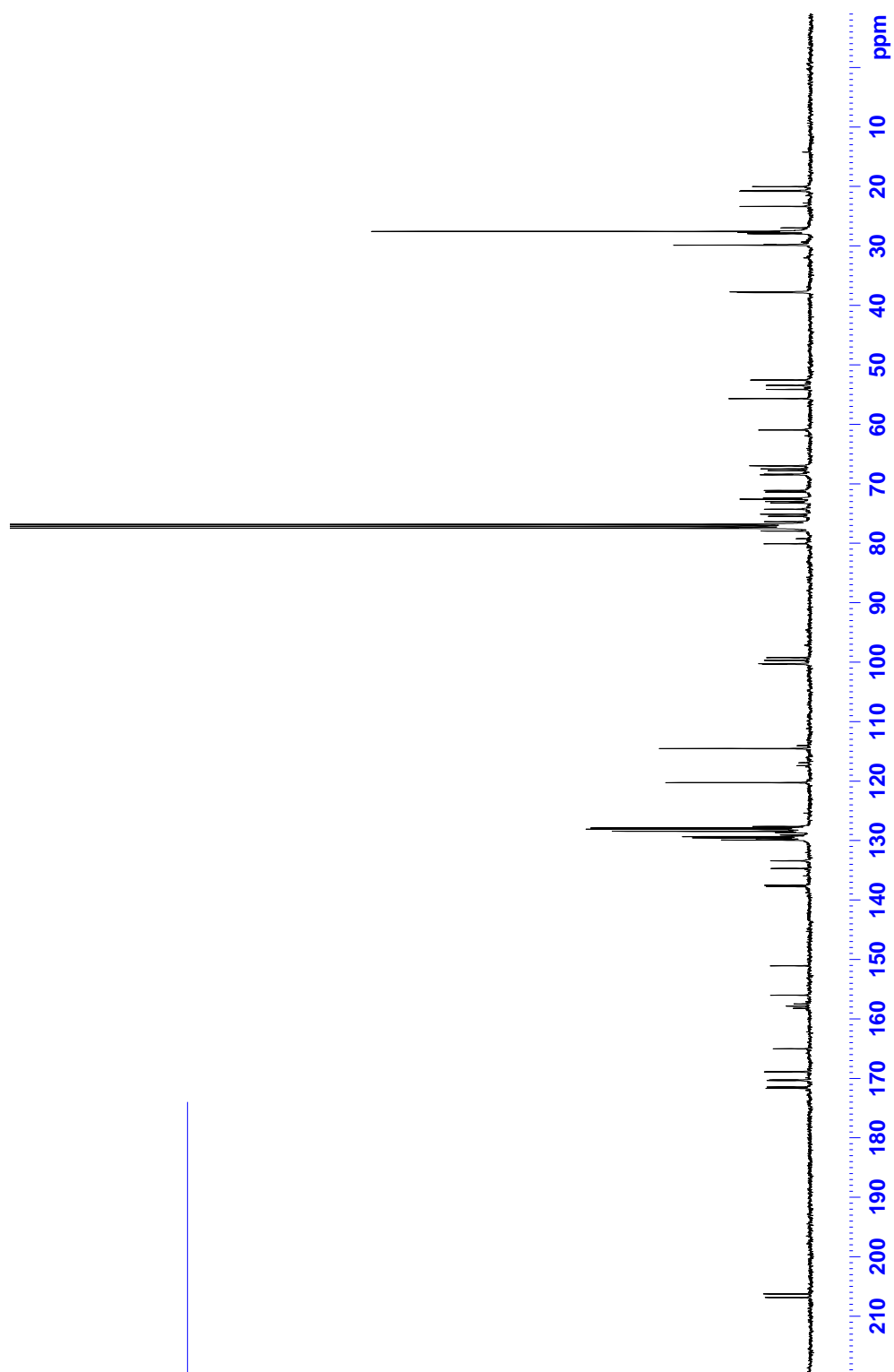
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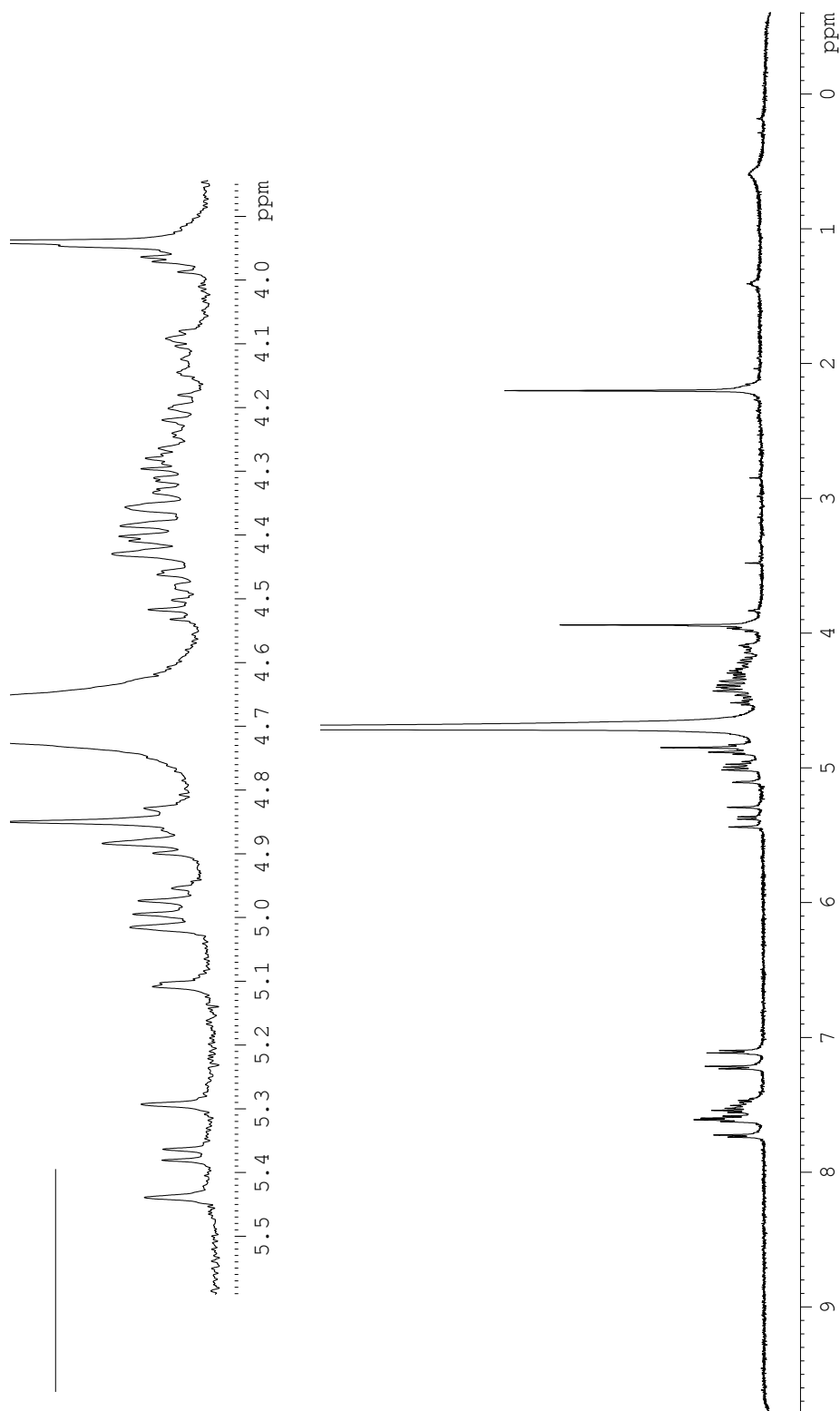
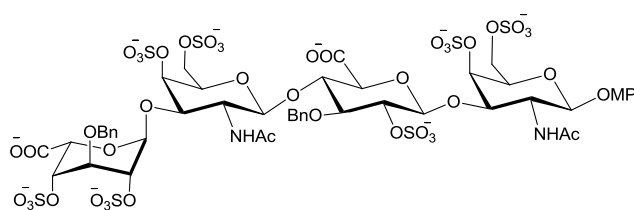


Compound 26

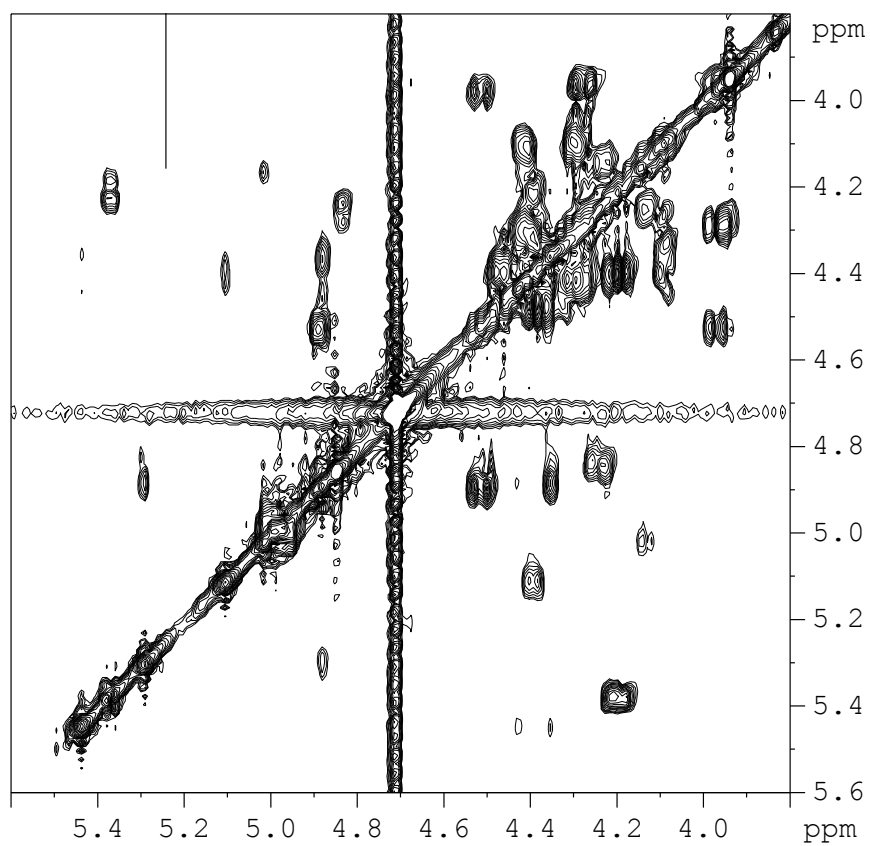




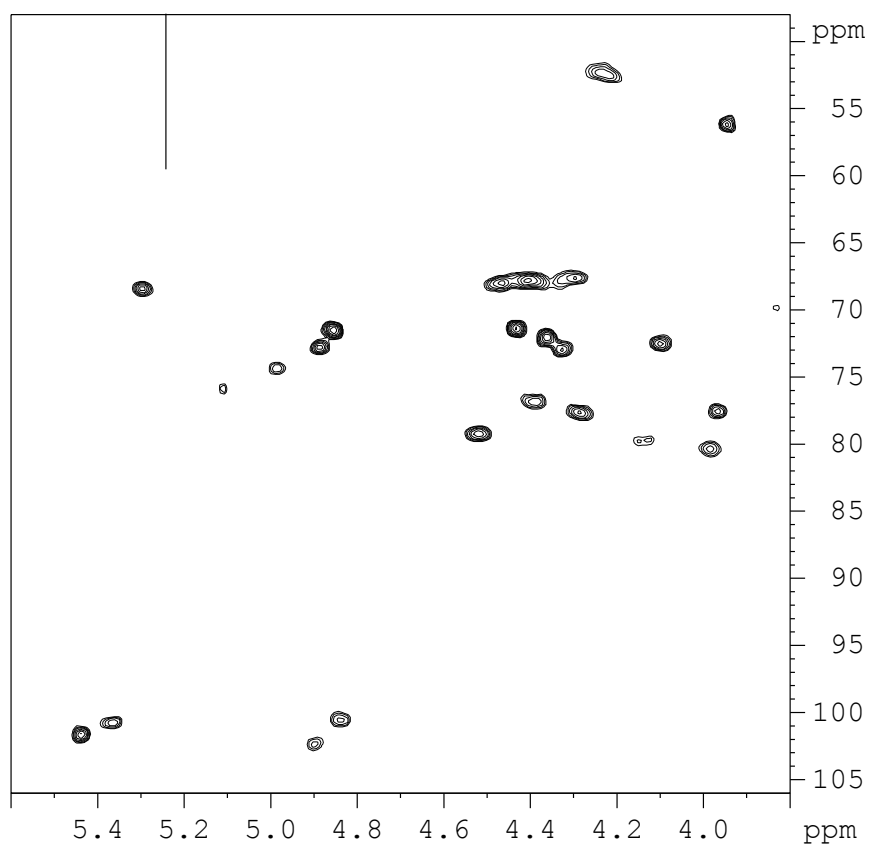
Compound 30 (calcium salt, 40°C)



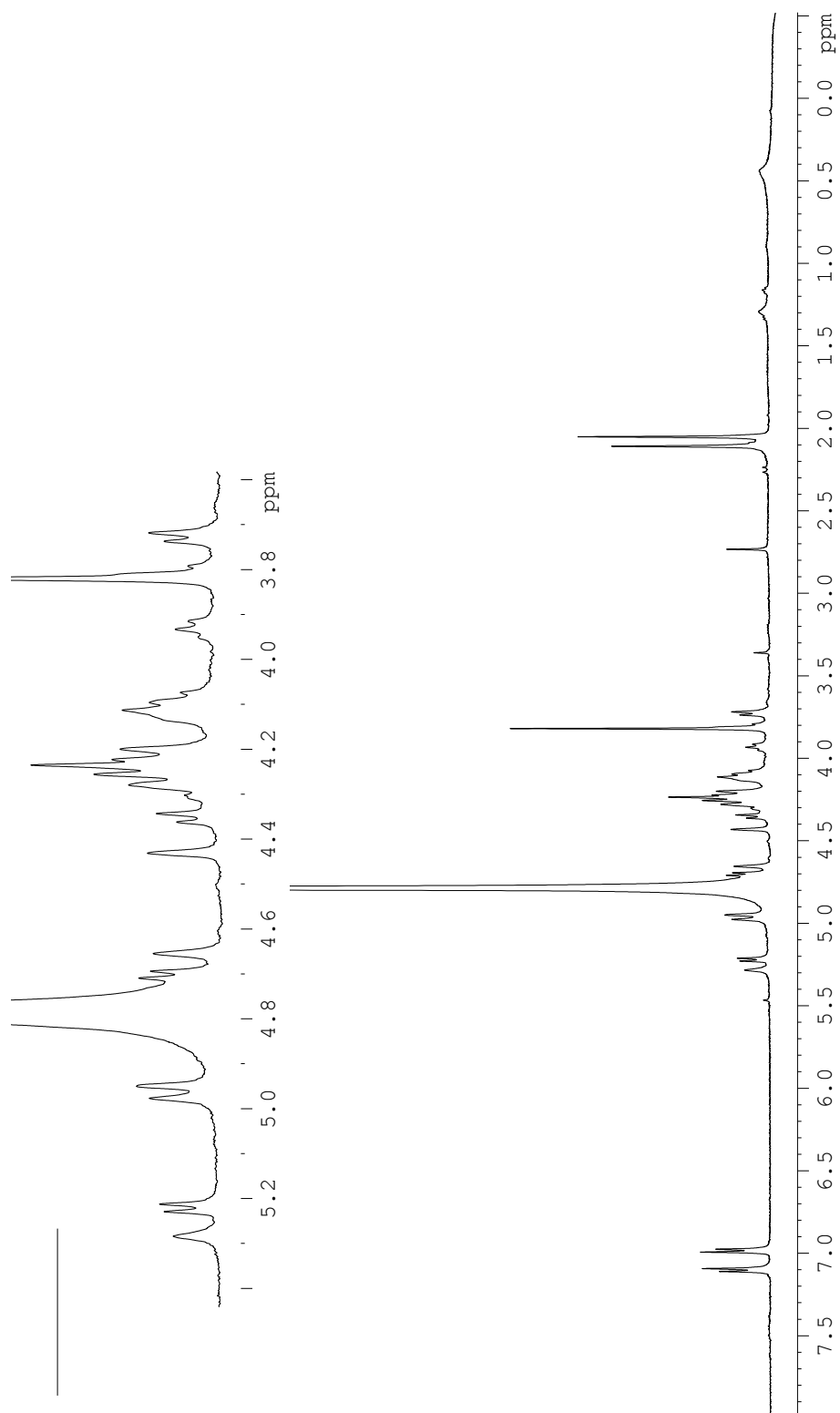
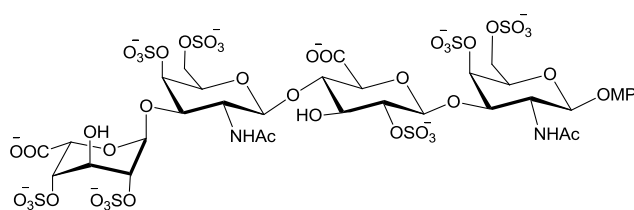
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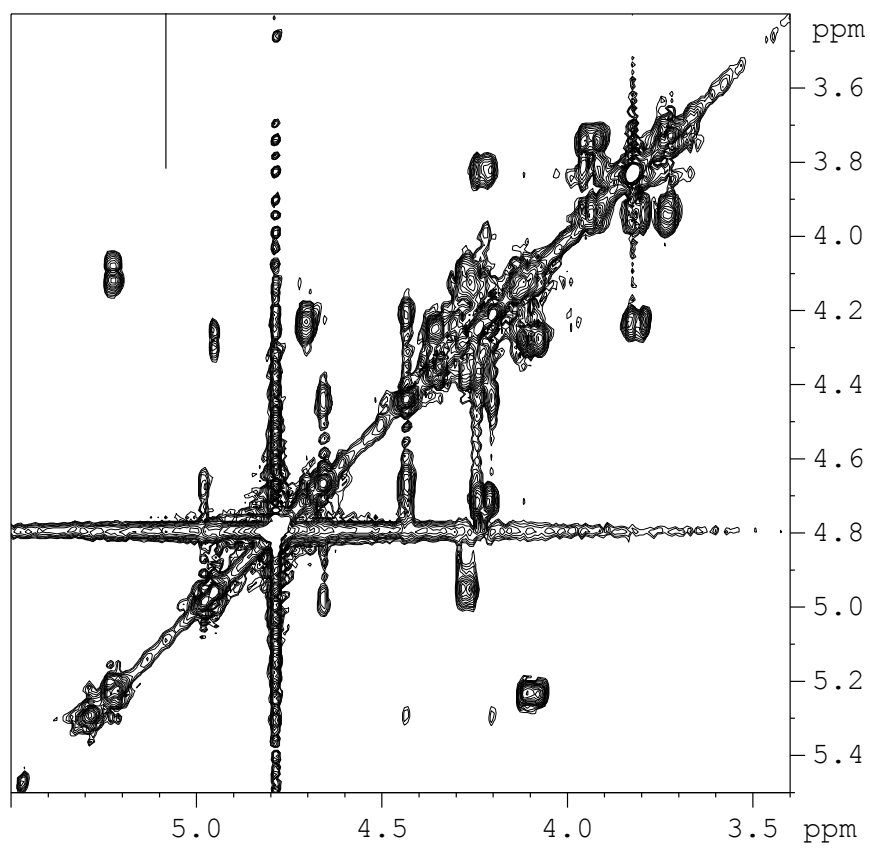
HSQC



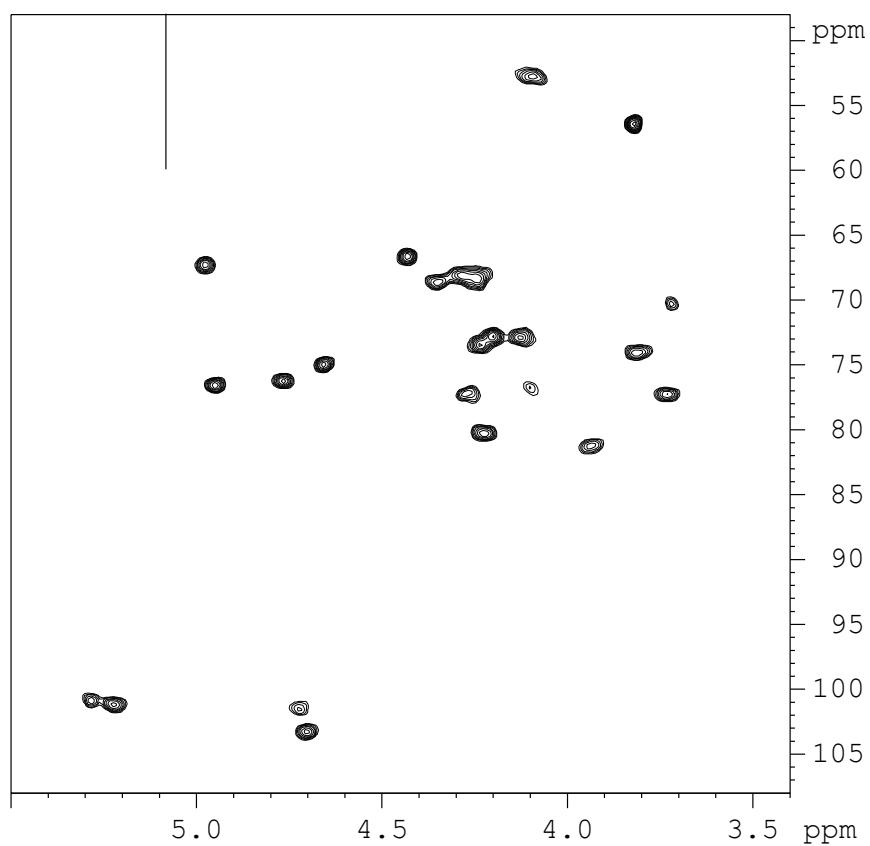
Compound 1



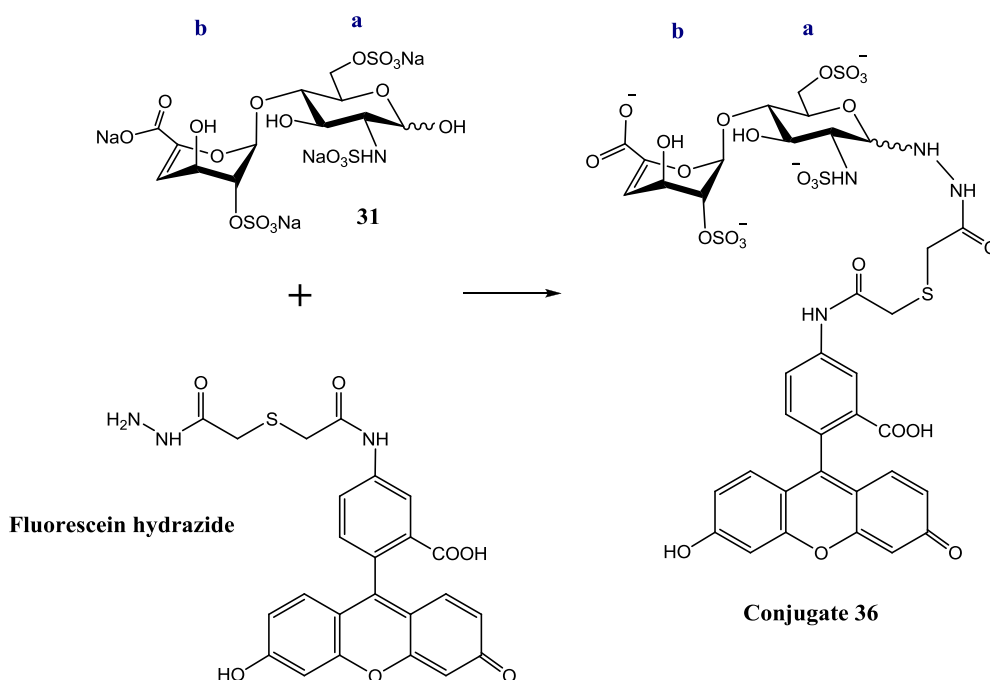
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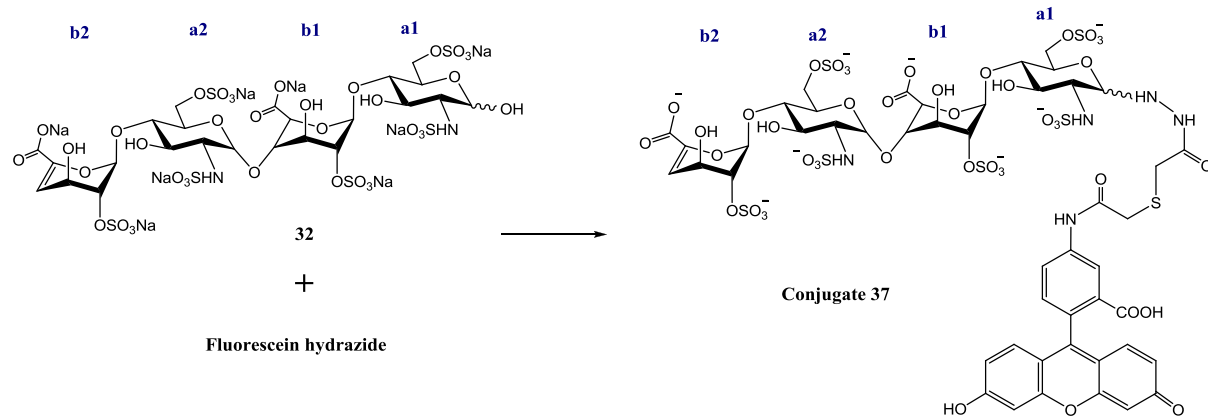
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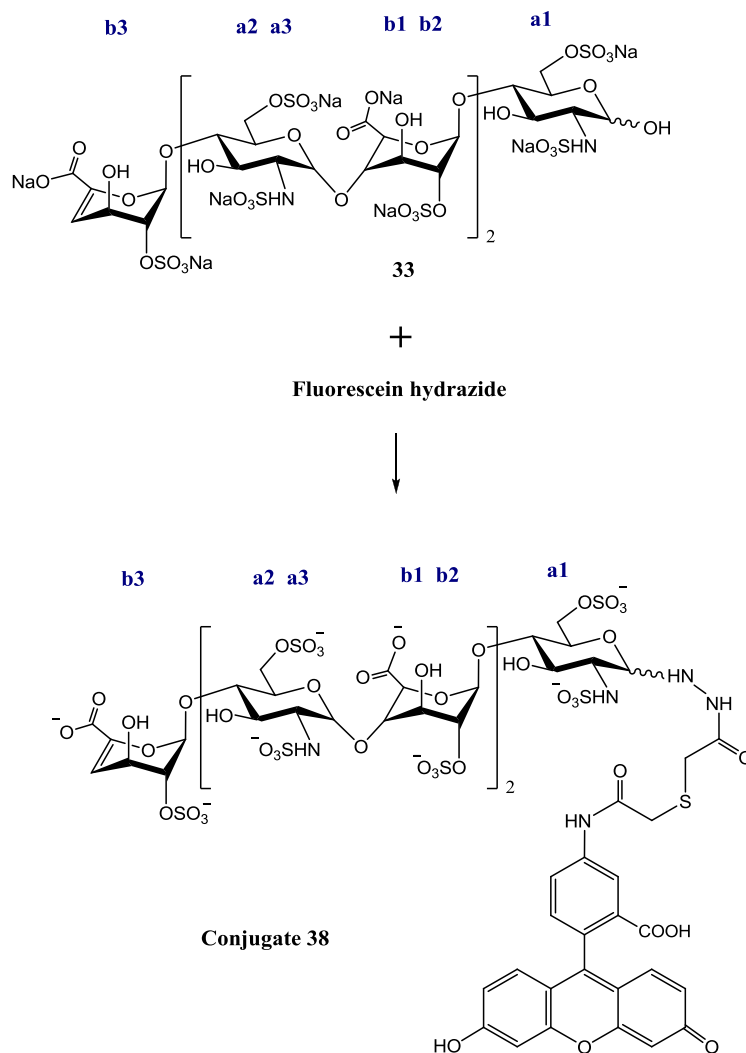
Synthesis of fluorescent conjugates 36-40:



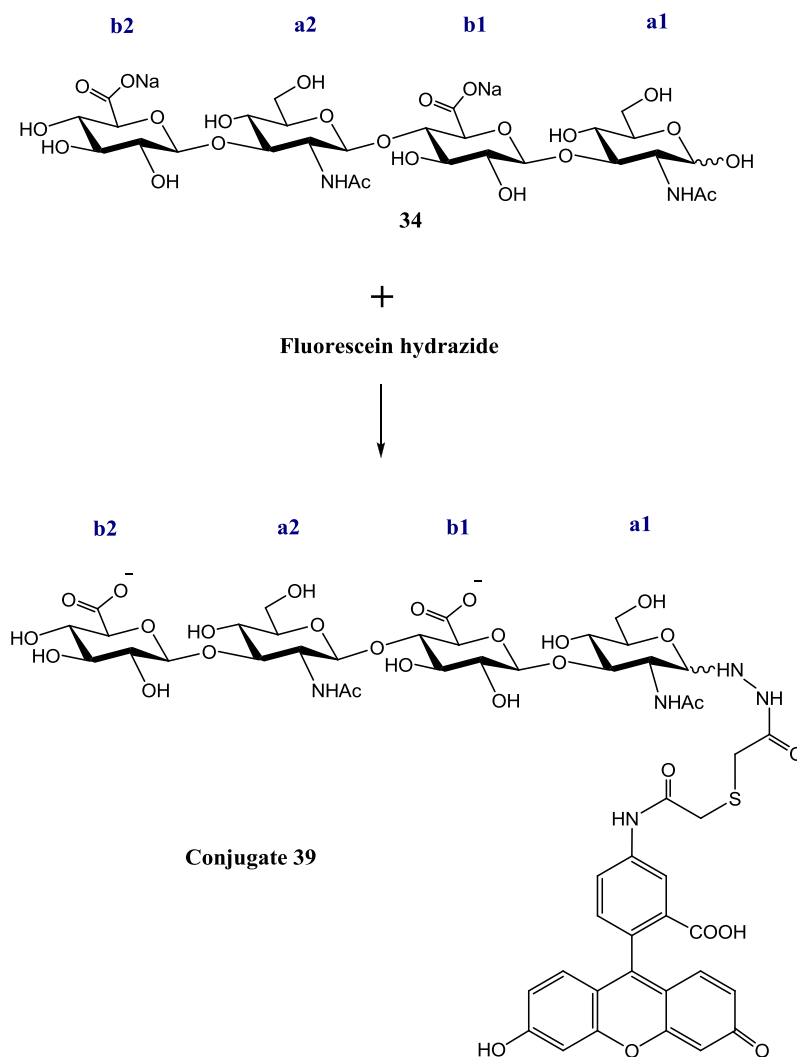
Fluorescent conjugate 36: A solution of heparin disaccharide **31** (from Dextra, 4.0 mg, 6.0 μmol) and fluorescein hydrazide (8.7 mg, 18 μmol) in a mixture of DMSO (450 μL) and phosphate buffer (450 μL , pH 5.5, 500mM) was stirred at 30°C in the dark, boiling off slowly the water under vacuum (15 mbar). After 24 h, DMSO was removed at 35°C and 0.1 mbar. The crude was redissolved in anhydrous DMSO and the mixture was concentrated to dryness (35°C, 0.1 mbar). The residue was purified by C-18 reverse phase chromatography (AcOH-Et₃N buffer (pH 7.5, 10mM)/acetonitrile 100:0 \rightarrow 80:20) to yield, after complete removal of triethylammonium acetate by lyophilisation, conjugate **36** (8mg, 91%, triethylammonium salt) as an α/β mixture (0.6:1). TLC (EtOAc/Py/H₂O/AcOH 6:5:3:1) R_f 0.35; ¹H-NMR (500MHz, D₂O) δ 7.87 (m, 1.6H, fluorescein), 7.72 (m, 1.6H, fluorescein), 7.31 (m, 1.6H, fluorescein), 7.23 (m, 3.2H, fluorescein), 6.63 (m, 6.4H, fluorescein), 5.92 (d, $J_{4,3}=4.5\text{Hz}$, 0.6H, H_{4b α}), 5.90 (d, $J_{4,3}=4.5\text{Hz}$, 1.0H, H_{4b β}), 5.45 (d, $J_{1,2}=3.3\text{Hz}$, 1.6H, H_{1b($\alpha+\beta$)}), 4.86 (d, $J_{1,2}=4.8\text{Hz}$, 0.6H, H_{1a α}), 4.54 (t, $J_{2,3}=J_{2,1}=3.0\text{Hz}$, 0.6H, H_{2b α}), 4.49 (t, $J_{2,3}=J_{2,1}=3.0\text{Hz}$, 1.0H, H_{2b β}), 4.34 (m, 0.6H, H_{6a α}), 4.28 (m, 1.6 H, H_{3b($\alpha+\beta$)}), 4.26 (m, 2H, H_{6a β} +H_{6a' β}), 4.15 (m, 0.6H, H_{6a' α}), 4.13 (d, $J_{1,2}=9.5\text{Hz}$, 1.0H, H_{1a β}), 3.84-3.62 (m, 4.8H, H_{3a($\alpha+\beta$)}+H_{4a($\alpha+\beta$)}+H_{5a($\alpha+\beta$)}), 3.56 (s, 3.2H, NHCOCH₂S), 3.38 (s, 3.2H, NHCOCH₂S), 3.37 (m, 0.6H, H_{2a α}), 3.13 (q, $J=7.5\text{Hz}$, 3.84H, CH₂ Et₃NH), 3.08 (t, $J_{2,3}=J_{2,1}=9.7\text{Hz}$, 1.0H, H_{2a β}), 1.21 (t, $J=7.5\text{Hz}$, 57.6H, CH₃ Et₃NH). ESI MS m/z : calcd for C₃₆H₃₂N₄O₂₅S₄Na: 357.0; found: 356.6 [M+Na]³⁻.



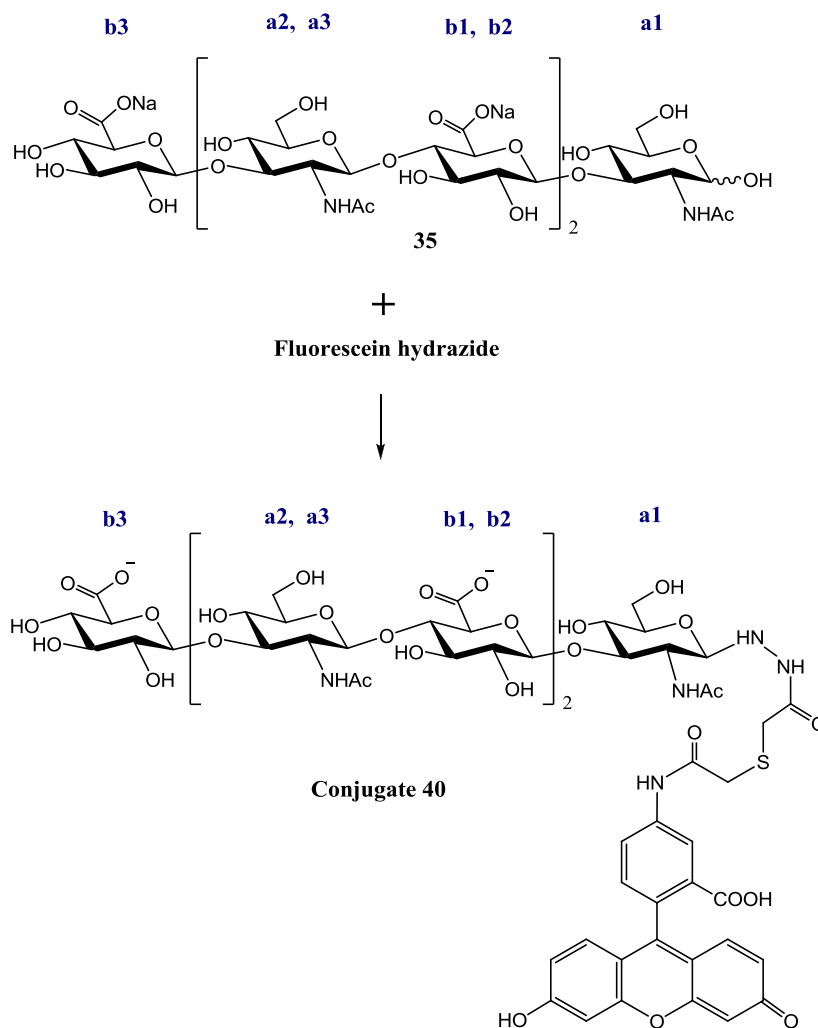
Fluorescent conjugate 37: A solution of heparin tetrasaccharide **32** (from Iduron, 6.0 mg, 4.5 μmol) and fluorescein hydrazide (7.0 mg, 14 μmol) in a mixture of DMSO (325 μL) and phosphate buffer (325 μL , pH 5.5, 500mM) was stirred at 30°C in the dark, boiling off slowly the water under vacuum (15 mbar). After 24 h, DMSO was removed at 35°C and 0.1 mbar. The crude was redissolved in anhydrous DMSO and the mixture was concentrated to dryness (35°C, 0.1 mbar). The residue was purified by C-18 reverse phase chromatography (AcOH-Et₃N buffer (pH 7.5, 10mM)/acetonitrile 100:0 \rightarrow 80:20) to yield, after complete removal of triethylammonium acetate by lyophilisation, conjugate **37** (10mg, 91%) as an α/β mixture (0.3:0.7). ¹H-NMR (500MHz, D₂O) δ 7.94 (m, 1H, fluorescein), 7.74 (m, 1H, fluorescein), 7.22 (m, 3H, fluorescein), 6.67 (m, 4H, fluorescein), 5.94 (bs, 1H, H_{4b2}($\alpha+\beta$)), 5.53-5.22 (m, 3H, H_{1b1}($\alpha+\beta$), H_{1b2}($\alpha+\beta$), H_{1a2}($\alpha+\beta$)), 4.86-4.72 (m, 1H, H_{5b1}($\alpha+\beta$)), 4.54 (bs, 1.3H, H_{2b2}($\alpha+\beta$)+H_{1a1} α), 4.36-3.95 (m, 8.7H, H_{6,6'a1}($\alpha+\beta$), H_{6,6'a2}($\alpha+\beta$), H_{3b1}($\alpha+\beta$), H_{3b2}($\alpha+\beta$), H_{2b1}($\alpha+\beta$), H_{4b1}($\alpha+\beta$), H_{1a1} β), 3.50-3.39 (m, 6H, H_{3a1}($\alpha+\beta$), H_{3a2}($\alpha+\beta$), H_{4a1}($\alpha+\beta$), H_{4a2}($\alpha+\beta$), H_{5a1}($\alpha+\beta$), H_{5a2}($\alpha+\beta$)), 3.55 (bs, 2H, NHC(=O)CH₂S), 3.36 (bs, 2H, NHC(=O)CH₂S), 3.38-3.17 (m, 1.3H, H_{2a2}($\alpha+\beta$)+H_{2a1} α), 3.13 (q, $J = 7.5$ Hz, 48H, CH₂ Et₃NH), 3.00 (m, 0.7H, H_{2a1} β), 1.21 (t, $J = 7.5$ Hz, 72H, CH₃ Et₃NH).



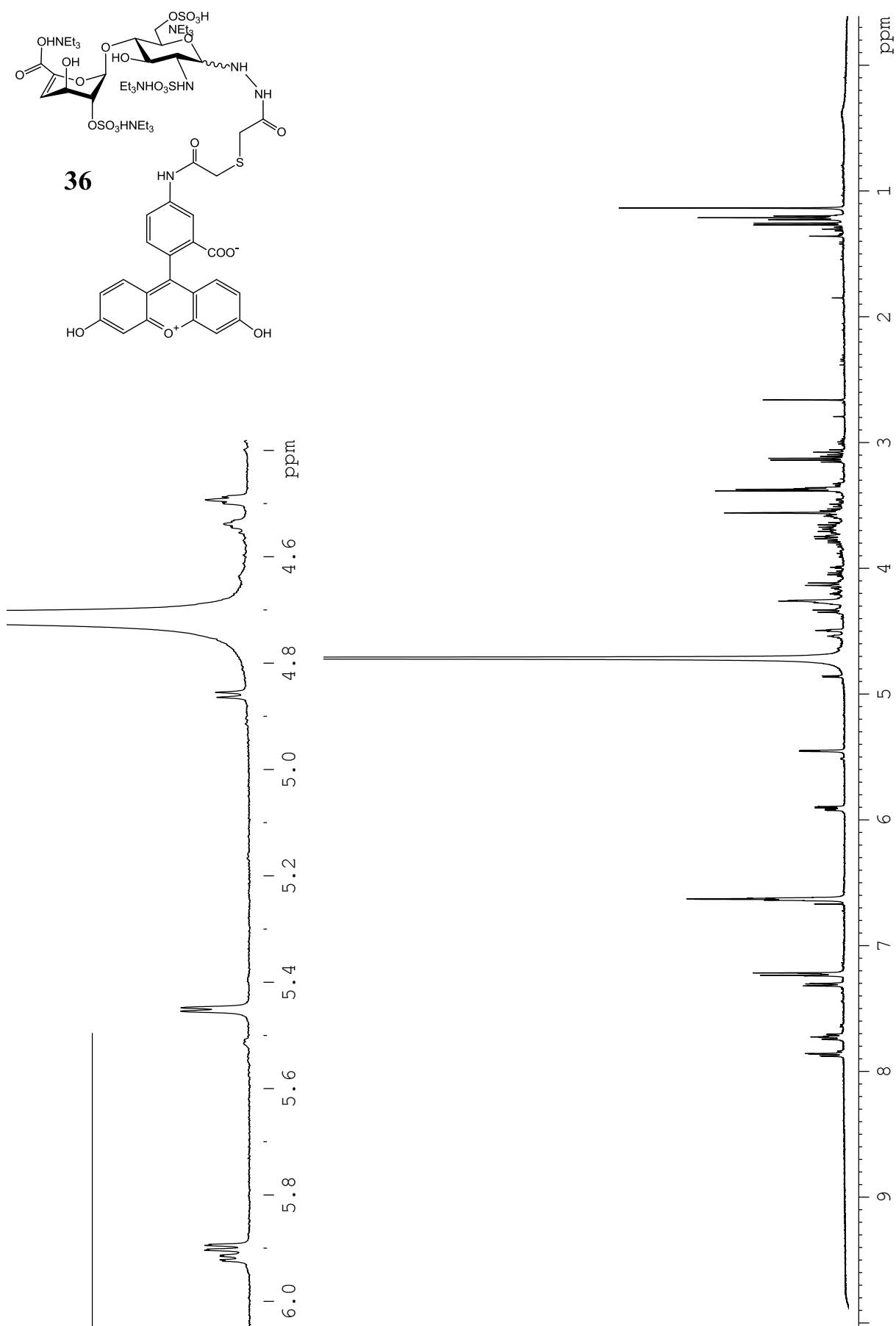
Fluorescent conjugate 38: A solution of heparin hexasaccharide **33** (from Iduron, 6.0 mg, 3.0 μmol) and fluorescein hydrazide (4.5 mg, 9.1 μmol) in a mixture of DMSO (215 μL) and phosphate buffer (215 μL , pH 5.5, 500mM) was stirred at 30°C in the dark, boiling off slowly the water under vacuum (15 mbar). After 24 h, DMSO was removed at 35°C and 0.1 mbar. The crude was redissolved in anhydrous DMSO and the mixture was concentrated to dryness (35°C, 0.1 mbar). The crude was purified by C-18 reverse phase chromatography (AcOH-Et₃N buffer (pH 7.5, 10mM)/acetonitrile 100:0 → 80:20) to yield, after complete removal of triethylammonium acetate by lyophilisation, conjugate **38** (9.7 mg, 94%) as an α/β mixture (0.3:0.7). ¹H-NMR (500MHz, D₂O) δ 8.01 (m, 1H, fluorescein), 7.78 (m, 1H, fluorescein), 7.28 (m, 3H, fluorescein), 6.81 (m, 4H, fluorescein), 5.95 (bd, $J_{4,3} = 4.5\text{Hz}$, 1H, H_{4b3}($\alpha+\beta$)), 5.54-5.15 (m, 5H, H_{1b1}($\alpha+\beta$), H_{1b2}($\alpha+\beta$), H_{1b3}($\alpha+\beta$), H_{1a2}($\alpha+\beta$), H_{1a3}($\alpha+\beta$)), 4.94-4.76 (m, 2H, H_{5b1}($\alpha+\beta$), H_{5b2}($\alpha+\beta$)), 4.54 (bs, 1.3H, H_{2b3}($\alpha+\beta$), H_{1a1} α), 4.35-4.02 (m, 13.7H, H_{6,6'a1}($\alpha+\beta$), H_{6,6'a2}($\alpha+\beta$), H_{6,6'a3}($\alpha+\beta$), H_{3b1}($\alpha+\beta$), H_{3b2}($\alpha+\beta$), H_{3b3}($\alpha+\beta$), H_{2b1}($\alpha+\beta$), H_{2b2}($\alpha+\beta$), H_{4b1}($\alpha+\beta$), H_{4b2}($\alpha+\beta$), H_{1a1} β), 4.01-3.56 (m, 9H, H_{3a1}($\alpha+\beta$), H_{3a2}($\alpha+\beta$), H_{3a3}($\alpha+\beta$), H_{4a1}($\alpha+\beta$), H_{4a2}($\alpha+\beta$), H_{4a3}($\alpha+\beta$), H_{5a1}($\alpha+\beta$), H_{5a2}($\alpha+\beta$), H_{5a3}($\alpha+\beta$)), 3.55 (bs, 2H, NHCOCH₂S), 3.37 (bs, 2H, NHCOCH₂S), 3.34-3.17 (m, 2.3H, H_{2a2}($\alpha+\beta$), H_{2a3}($\alpha+\beta$), H_{2a1} α), 3.14 (q, $J = 7.5\text{Hz}$, 72H, CH₂ Et₃NH), 3.01 (m, 0.7H, H_{2a1} β), 1.22 (t, $J = 7.5\text{Hz}$, 108H, CH₃ Et₃NH).

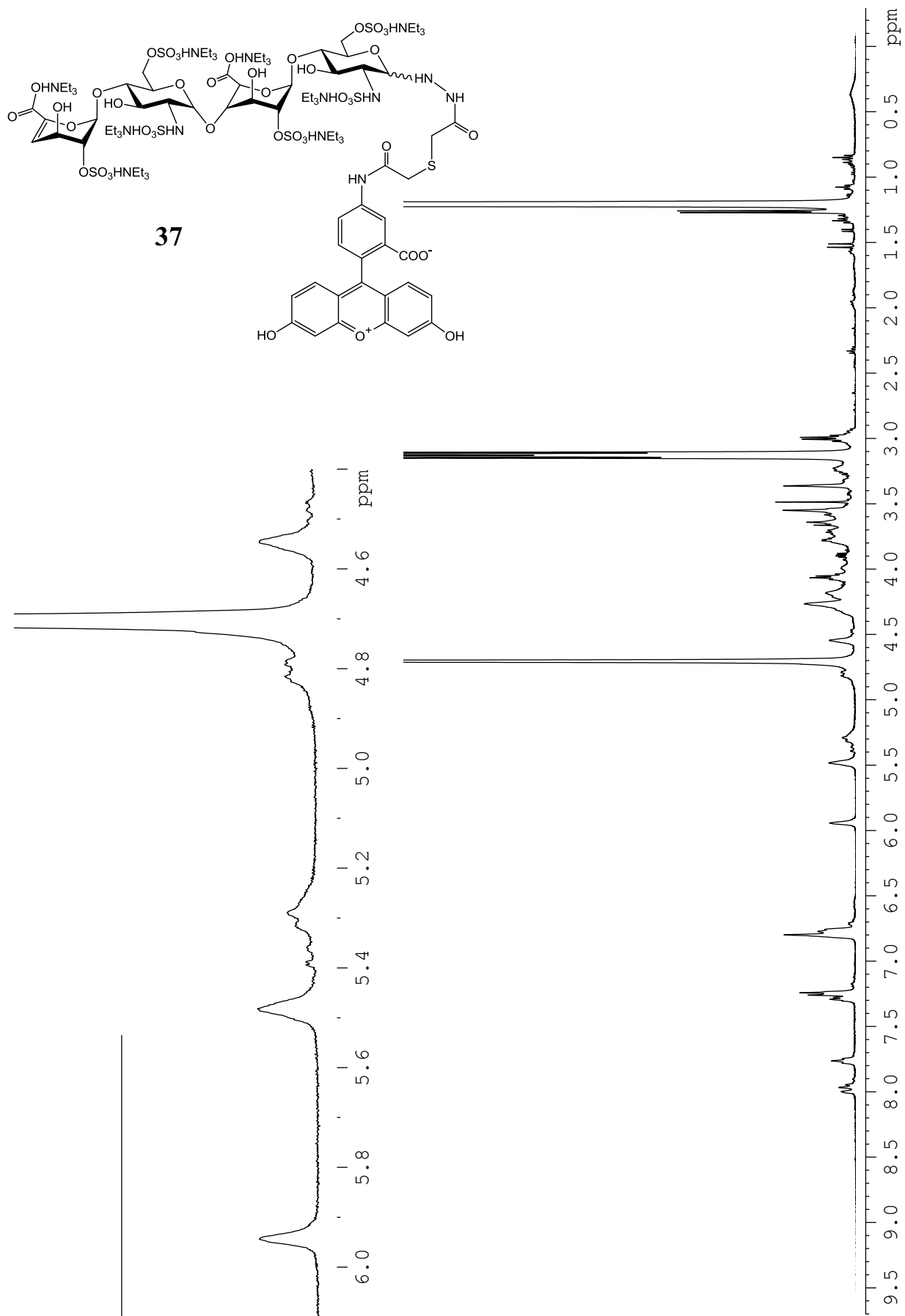


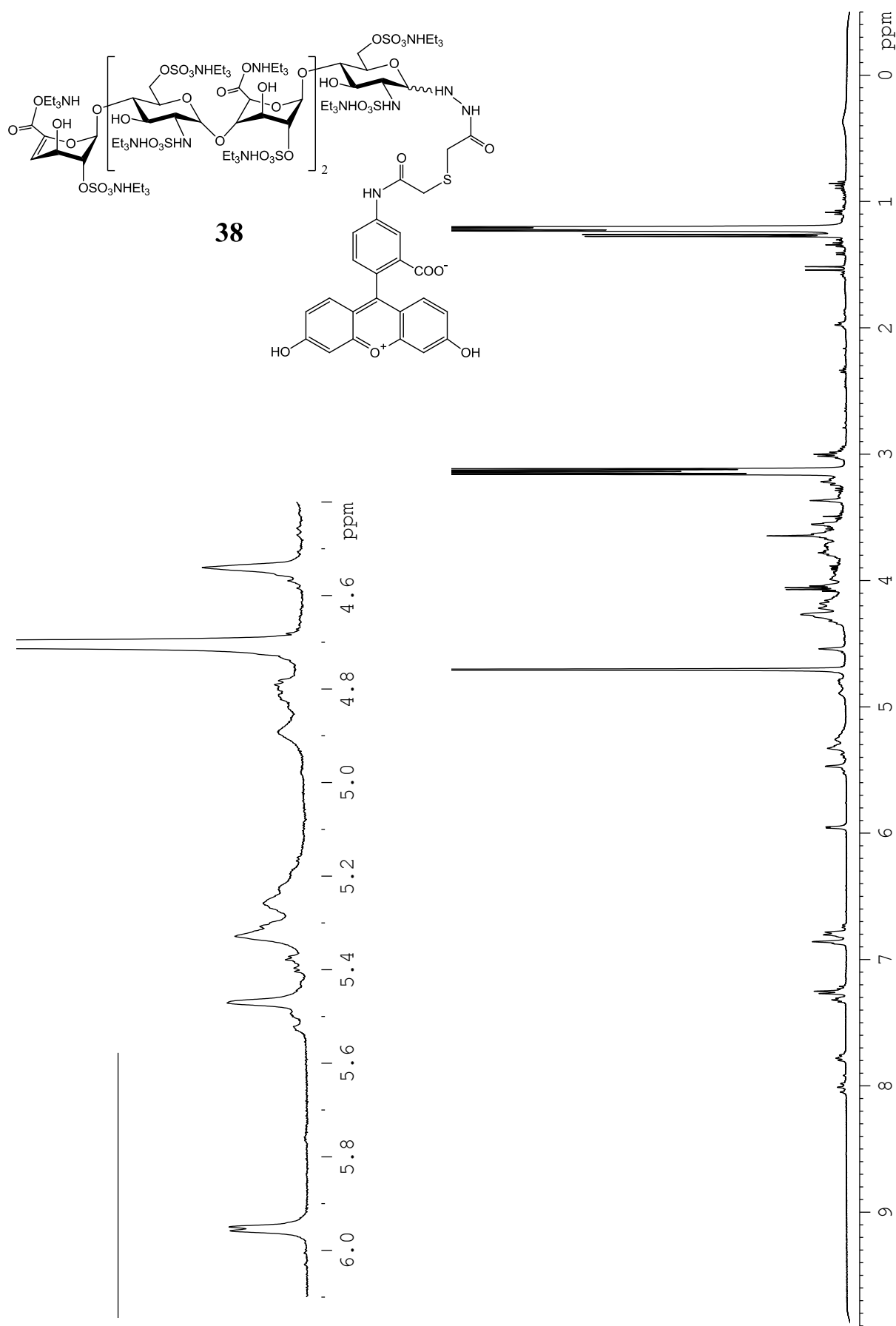
Fluorescent conjugate 39: A solution of hyaluronic acid tetrasaccharide **34** (from Contipro, 5.0 mg, 6.1 μmol) and fluorescein hydrazide (9 mg, 18 μmol) in a mixture of DMSO (420 μL) and phosphate buffer (420 μL , pH 5.5, 500mM) was stirred at 30°C in the dark, boiling off slowly the water under vacuum (15 mbar). After 24 h, DMSO was removed at 35°C and 0.1 mbar. The crude was redissolved in anhydrous DMSO and the mixture was concentrated to dryness (35°C, 0.1 mbar). The crude was purified by C-18 reverse phase chromatography (AcOH-Et₃N buffer (pH 7.5, 10mM)/acetonitrile 100:0 → 80:20) to yield, after complete removal of triethylammonium acetate by lyophilisation, conjugate **39** (8.2 mg, 92%) as an α/β mixture (0.2:0.8). ¹H-NMR (500 MHz, D₂O) δ 7.93 (m, 1H, fluorescein), 7.72 (m, 1H, fluorescein), 7.17 (m, 3H, fluorescein), 6.70 (m, 4H, fluorescein), 4.56 (bd, $J_{1,2} = 4.5\text{Hz}$, 0.2H, H_{1a1 α}), 4.48 (m, 1H, H_{1a2($\alpha+\beta$)}), 4.39 (m, 2H, H_{1b1($\alpha+\beta$)}, H_{1b2($\alpha+\beta$)}), 4.20 (bd, $J_{1,2} = 9.5\text{Hz}$, 0.8H, H_{1a1 β}), 4.05 (m, 0.2H, H_{2a1 α}), 3.88 (t, $J = 10.0\text{Hz}$, 0.8H, H_{2a1 β}), 3.87-3.61 (m, 11H, H_{3a1($\alpha+\beta$)}, H_{3a2($\alpha+\beta$)}, H_{2a2($\alpha+\beta$)}, H_{6,6' α 1($\alpha+\beta$)}, H_{6,6' α 2($\alpha+\beta$)}, H_{4b1($\alpha+\beta$)}, H_{4b2($\alpha+\beta$)}, H_{5b1($\alpha+\beta$)}, H_{5b2($\alpha+\beta$)}), 3.53-3.23 (m, 12H, H_{2b1,2b2($\alpha+\beta$)}, H_{3b1,3b2($\alpha+\beta$)}, H_{4a1,4a2($\alpha+\beta$)}, H_{5a1,5a2($\alpha+\beta$)}, 2xNHC(O)CH₂S), 3.12 (q, $J = 7.5\text{Hz}$, 12H, CH₂ Et₃NH), 1.95 (bs, 3H, CH₃CONH), 1.94 (bs, 3H, CH₃CONH), 1.20 (t, $J = 7.5\text{Hz}$, 18H, CH₃ Et₃NH).

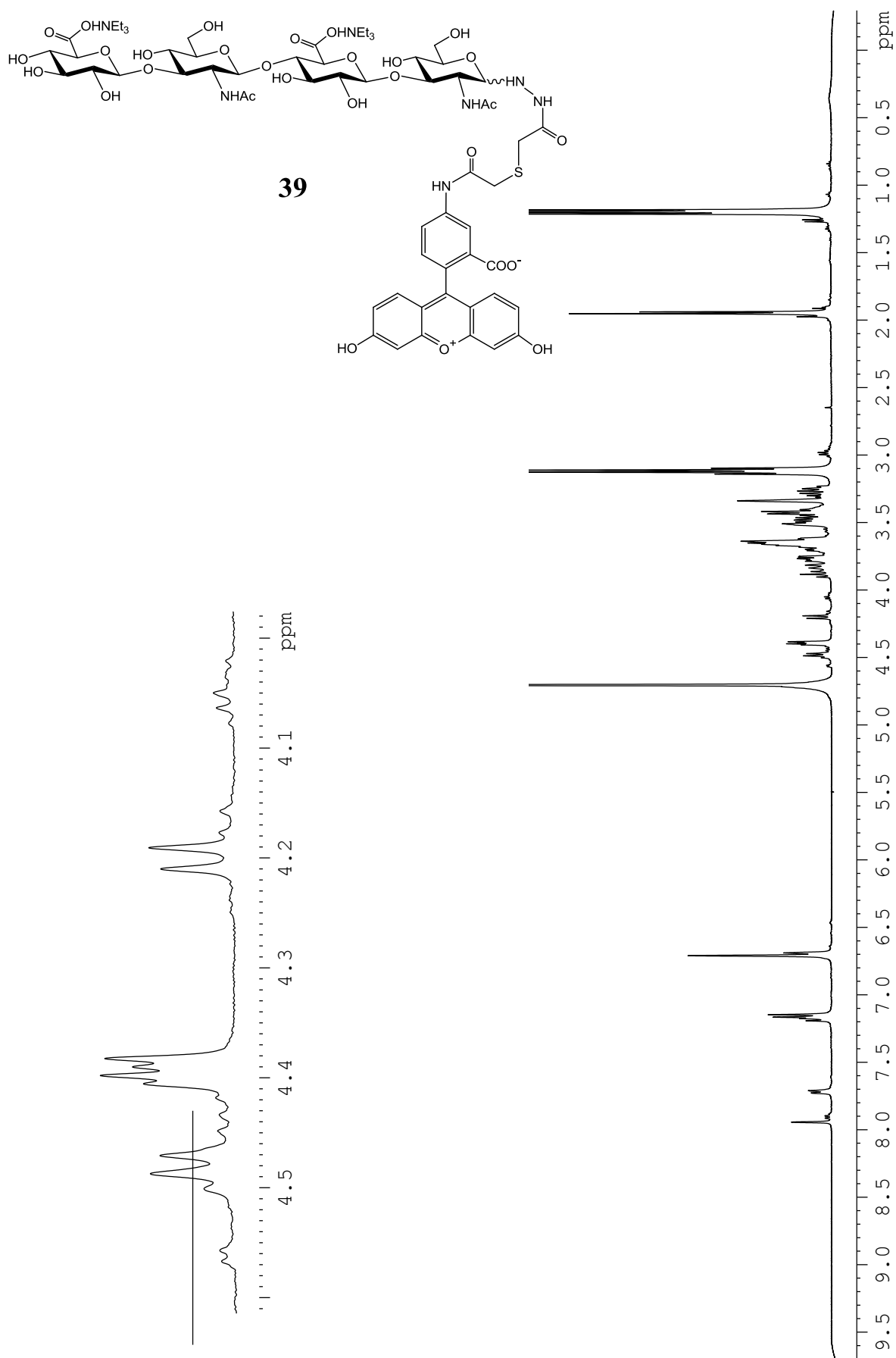


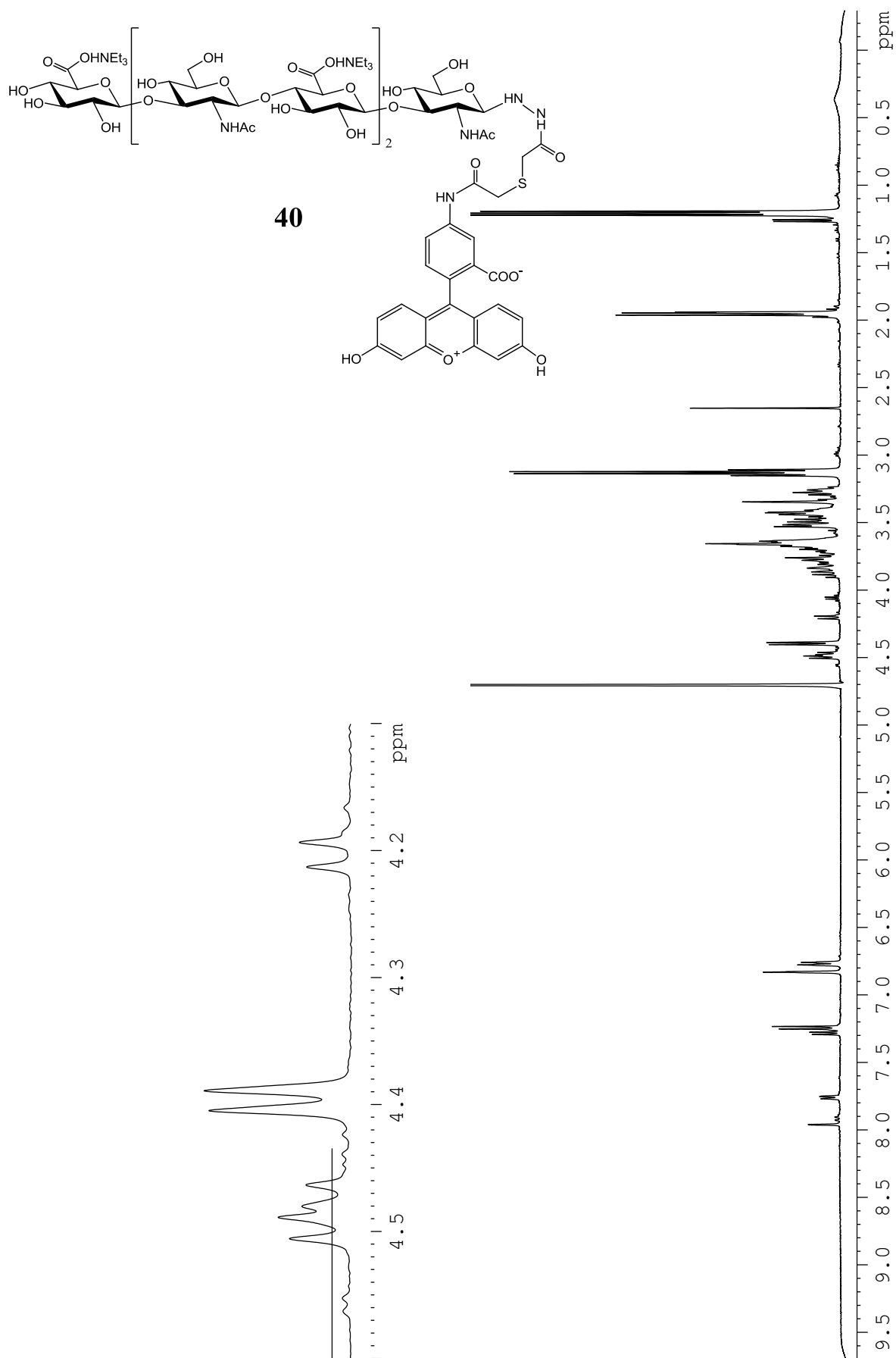
Fluorescent conjugate 40: A solution of hyaluronic acid hexasaccharide **35** (from Contipro, 6.0 mg, 4.9 μmol) and fluorescein hydrazide (7.4 mg, 15 μmol) in a mixture of DMSO (375 μL) and phosphate buffer (375 μL , pH 5.5, 500mM) was stirred at 30°C in the dark, boiling off slowly the water under vacuum (15 mbar). After 24 h, DMSO was removed at 35°C and 0.1 mbar. The crude was redissolved in anhydrous DMSO and the mixture was concentrated to dryness (35°C, 0.1 mbar). The crude was purified by C-18 reverse phase chromatography (AcOH-Et₃N buffer (pH 7.5, 10mM)/acetonitrile 100:0 → 80:20) to yield, after complete removal of triethylammonium acetate by lyophilisation, conjugate **40** (9.0 mg, 95%) as β -isomer. ¹H-NMR (500MHz, D₂O) δ 7.93 (m, 1H, fluorescein), 7.76 (m, 1H, fluorescein), 7.26 (m, 3H, fluorescein), 6.83 (bs, 2H, fluorescein), 6.77 (bd, 2H, fluorescein), 4.48 (m, 2H, H_{1a2,1a3}), 4.40 (bd, $J_{1,2}$ = 8.0Hz, 3H, H_{1b1,1b2,1b3}), 4.20 (d, $J_{1,2}$ = 9.5Hz, 1H, H_{1a1}), 3.88 (t, J = 10.0Hz, 1H, H_{2a1}), 3.86-3.60 (m, 17H, H_{6,6'}a1,a2,a3, H_{2a2,2a3}, H_{3a1,3a2,3a3}, H_{4b1,4b2,4b3}, H_{5b1,5b2,5b3}), 3.54-3.33 (m, 13H, H_{3b1,3b2,3b3}, H_{4a1,4a2,4a3}, H_{5a1,5a2,5a3}, 2xNHCOCH₂S), 3.28 (m, 3H, H_{2b1,2b2,2b3}), 3.13 (q, J = 7.5Hz, 18H, CH₂ Et₃NH), 1.96 (bs, 3H, CH₃CONH), 1.95 (bs, 3H, CH₃CONH), 1.94 (bs, 3H, CH₃CONH), 1.21 (t, J = 7.5Hz, 27H, CH₃ Et₃NH).











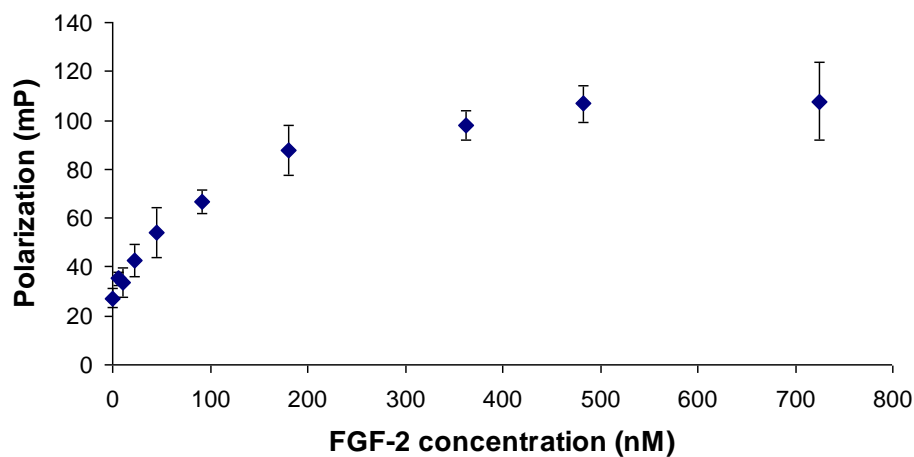


Figure S1. Plot of fluorescence polarization against protein concentration. The polarization values of wells containing probe **38** and increasing concentrations of FGF-2 (from 6 to 725 nM) were recorded. All the measurements are the average of six replicate wells and the error bars show the standard deviations for these measurements. The corresponding binding curve was analyzed as a Langmuir isotherm to determine the K_D of the interaction, assuming a one-site model.