

Supporting Information

New Glucuronic Acid Donors for the Modular Synthesis of Heparan Sulfate Oligosaccharides

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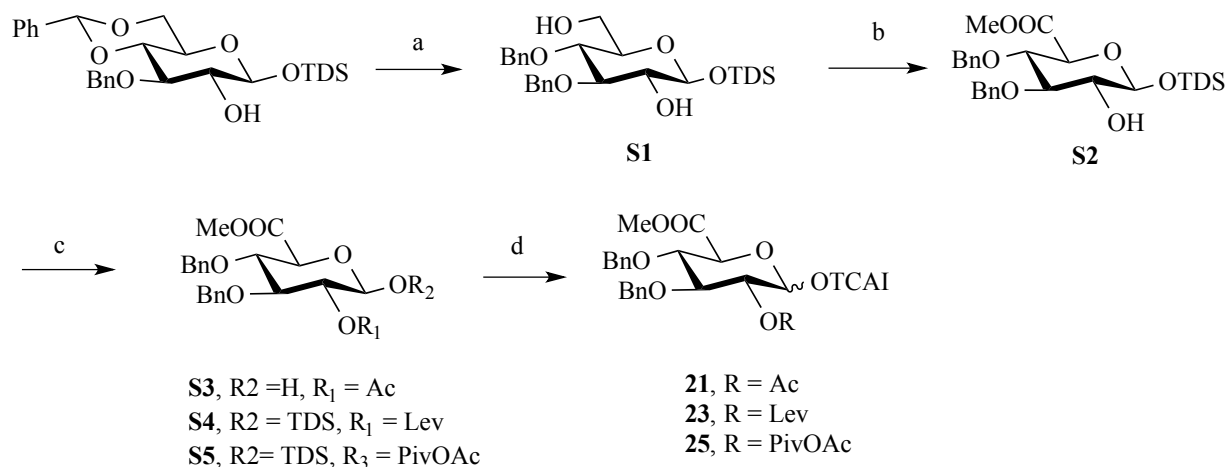
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Preparations and characterizations of compounds **37**, **38**, **S1-S6**

Copies of ¹H and HSQC NMR spectra

Methyl (Phenyl 2-O-levulinoyl-3-O-benzyl-4-O-(9-fluorenylmethoxycarbonyl)-1-thio- α -L-idopyranoside)uronate (37). Compound **37** was prepared according to a literature procedure (S. U. Hansen, G. J. Miller, G. C. Jayson and J. M. Gardiner, *Org. Lett.*, 2013, **15**, 88-91). ^1H NMR (300 MHz, CDCl_3) δ 7.82 – 7.22 (m, 18H, CH Aromatic), 5.64 (s, 1H, H-1), 5.42 (d, $J = 2.1$ Hz, 1H, H-5), 5.22 – 5.09 (m, 2H, H-2, H-5), 4.85 (d, $J = 11.7$ Hz, 1H, CHHBn), 4.79 – 4.68 (d, $J = 11.7$ Hz, 1H, CHHBn), 4.51 (dd, $J = 10.3, 7.3$ Hz, 1H, CHHNap), 4.36 (dd, $J = 10.5, 7.3$ Hz, 1H, CHHNap), 4.22 (t, $J = 7.2$ Hz, 1H, CHNap), 3.95 (t, $J = 2.9$ Hz, 1H, H-3), 3.79 (s, 3H, COOCH_3), 2.77 – 2.37 (m, 4H, CH_2Lev), 2.03 (s, 3H, CH_3Lev). ^{13}C NMR (75 MHz, CDCl_3) δ 130.7, 127.7, 127.5, 124.7, 119.8, 85.8, 73.7, 71.6, 69.3, 69.6, 67.7, 67.5, 49.0, 46.7, 29.9, 28.2. HRMS: m/z : calcd for $\text{C}_{40}\text{H}_{38}\text{O}_{10}\text{SNa}$: 733.2083; found: 733.2090 $[\text{M}+\text{Na}]^+$.

***N*-(Benzyl)-benzyloxycarbonyl-5-aminopentyl-*O*-(methyl-2-*O*-levulinoyl-3-*O*-benzyl- α -L-idopyranosyluronate)-(1 \rightarrow 4)-*O*-2-azido-3-*O*-benzyl-6-*O*-levulinoyl- α -D-glycopyranoside (38).** A suspension of compounds **37** (1.51 g, 2.13 mmol), **30** (1.01 g, 1.42 mmol) and activated molecular sieves (4Å crushed, 150 mg) in dichloromethane (20 mL) was stirred at ambient temperature under an atmosphere of Ar for 1 h. The mixture was cooled to 0 °C followed by addition of NIS (0.35 mg, 2.56 mmol) and AgOTf (0.18 g, 0.71 mmol). TLC analysis (hexane/EtOAc, 1/1, v/v) showed complete consumption of the donor. Et_3N (4 mL) was added and stirred for another 1 h followed by filtration through a pad of Celite and the filtrate was concentrated under reduced pressure. The residue was purified by silica gel column chromatography using a gradient of hexane /EtOAc (2/1 \rightarrow 1/1, v/v) to give compound **38** as oil (1.07 g, 70%). (NMR data reported in: S. Arungundram, K. Al-Mafraji, J. Asong, F. E. Leach, III, I. J. Amster, A. Venot, J. E. Turnbull and G. J. Boons, *J. Am. Chem. Soc.*, 2009, **131**, 17394-17405).



Scheme S1. Synthesis of glucuronic acid donors with C-4 benzyl ether. Reagents and conditions: a) Et₃SiH₃, PhBCl₃, 3Å Molecular sieves, DCM, -78 °C (91%); b) (i) TEMPO, BAIB, rt, DCM, H₂O; (ii) CH₂N₂, Et₂O, (84%); c) (i) Ac₂O, pyridine and then HF:Pyr. THF (79%, **S3**); (ii) levulinic acid, DCC, DMAP, rt, DCM, (89%, **S4**); (iii) PivOAc-Cl, DMAP, Pyr., (88%, **S5**); d) (i) HF:Pyr. THF; (ii) Cl₃CCN, NaH, DCM.

Dimethylthexylsilyl 3,4-O-benzyl-β-D-glucopyranoside (S1). A solution of compound **1** (1.00 g, 2.00 mmol) and activated molecular sieves (3Å, 1.00 g) in dichloromethane (20 mL) was stirred at ambient temperature under an atmosphere of Ar for 1 h. The mixture was cooled to -78 °C followed by addition of Et₃SiH (576 μL, 6.00 mmol) and PhBCl₂ (1.50 mL, 7.00 mmol). After being stirred for 1 h at -78 °C, Et₃N (3 mL) and MeOH (3 mL) were added successively, and the mixture was diluted with CHCl₃ (20 mL) and washed with NaHCO₃ (sat.), dried (MgSO₄), filtered and the filtrate was concentrated under reduced pressure. The residue was purified by silica gel column chromatography using a gradient of CHCl₃/MeOH (95/5 → 80/20, v/v) to give compound **S1** as oil (0.91 g, 91%). ¹H NMR (500 MHz, CDCl₃) δ 7.52 – 7.20 (m, 10H, CH Aromatic), 4.98 (d, *J* = 11.2 Hz, 1H, CHHBn), 4.89 (dd, *J* = 18.4, 11.1 Hz, 2H, CHHBn, CHHBn), 4.67 (d, *J* = 11.0 Hz, 1H, CHHBn), 4.58 (d, *J* = 7.5 Hz, 1H, H-1), 3.86 (dd, *J* = 11.9, 2.9 Hz, 1H, H-6a), 3.76 – 3.53 (m, 3H, H6b, H-4, H-3), 3.52 – 3.37 (m, 2H, H-2, H-5), 1.70 – 1.66 (m, 1H, CH(CH₃)₂), 0.95–0.86 (m, 12H, C(CH₃)₂ and CH(CH₃)₂), 0.21 (s, 6H, Si(CH₃)₂). ¹³C NMR (126 MHz, CDCl₃) δ 128.0, 97.7, 84.2, 76.8, 76.7, 75.5, 75.2, 75.1, 75.0, 62.3, 34.3, 18.5, -1.9. HRMS: *m/z*: calcd for C₂₈H₄₂O₆SiNa: 525.2648; found: 525.2656 [M+Na]⁺.

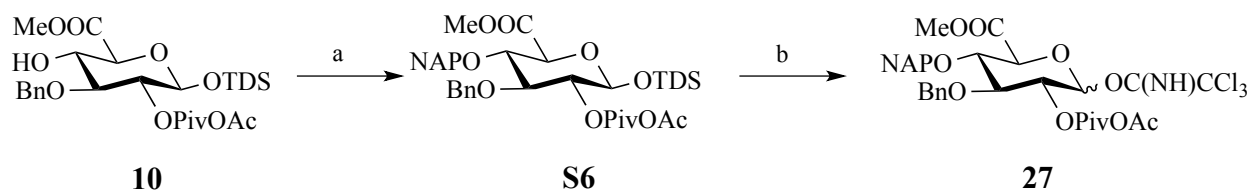
Dimethylhexylsilyl *O*-methyl-3,4-*O*-benzyl- β -D-glucopyranosyluronate (S2). Compound **S2** (355 mg, 84%) was prepared according to the general procedure from compound **S1** (400 mg, 0.80 mmol) using TEMPO (30 mg, 0.20 mmol), BAIB (640 mg, 1.99 mmol) and freshly prepared solution of diazomethane in Et₂O (2 mL). ¹H NMR (600 MHz, CDCl₃) δ 7.39 – 7.33 (m, 6H, *CH* Aromatic), 7.37 – 7.27 (m, 5H, *CH* Aromatic), 4.92 (d, *J* = 11.3 Hz, 1H, *CHHBn*), 4.86 – 4.80 (m, 2H, *CHHBn*, *CHHBn*), 4.62 (d, *J* = 11.3 Hz, 1H, *CHHBn*), 4.53 (d, *J* = 7.4 Hz, 1H, H-1), 3.92 – 3.87 (m, 1H, H-5), 3.84 (d, *J* = 8.9 Hz, 1H, H-4), 3.74 – 3.70 (m, 3H, COOCH₃), 3.59 (t, *J* = 9.0 Hz, 1H, H-3), 3.54 – 3.48 (m, 1H, H-2), 1.55 (d, *J* = 2.8 Hz, 1H, *CH*(CH₃)₂), 0.91 – 0.82 (m, 12H, C(CH₃)₂ and *CH*(CH₃)₂), 0.15 (s, 3H, SiCH₃), 0.17 (s, 3H, SiCH₃), ¹³C NMR (151 MHz, CDCl₃) δ 126.7, 126.1, 125.6, 98.0, 82.7, 78.1, 76.93, 74.3, 74.2, 73.4, 56.4, 51.8, 33.1, 29.1, 20.8, 19.6, -0.3. HRMS: *m/z*: calcd for C₂₉H₄₂O₇SiNa: 553.2597; found: 553.2609 [M+Na]⁺.

Methyl-2-*O*-acetyl-3,4-*O*-benzyl- α/β -D-glucopyranosyluronate (S3). A solution of compound **S2** (90 mg, 0.17 mmol) in a mixture of pyridine and acetic anhydride (4/1, v/v, 0.20 M) was stirred for 2 hr at ambient temperature. The mixture was co-evaporated with toluene *in vacuo* and dried on the membrane pump for 3 hours. To a stirred solution of the resulting crude material in THF (2 mL), 30% HF in pyridine (340 μ L) was added. After stirring at ambient temperature for 18 h, TLC analysis (hexanes/EtOAc, 60/40, v/v) indicated complete consumption of the starting material. The reaction mixture was subsequently diluted with DCM (10 mL), washed with water, NaHCO₃ (sat.), and brine. The organic phase was dried (MgSO₄), filtered, and the filtrate was concentrated under reduced pressure and the residue was purified by silica gel column chromatography using a gradient of hexanes/EtOAc (2/1 \rightarrow 1/1, v/v) to give compound **S3** as oil (57 mg, 79%). ¹H NMR (500 MHz, CDCl₃) δ 7.39 – 7.25 (m, 10H, *CH* Aromatic), 5.50 (d, *J* = 3.6 Hz, 1H, H-1), 4.97 – 4.71 (m, 5H, H-2, 3 \times *CHHBn*), 4.66 (d, *J* = 10.9 Hz, 1H, *CHHBn*), 4.53 (d, *J* = 9.3 Hz, 1H, H-5), 4.09 (t, *J* = 8.5 Hz, 1H, H-3), 3.88 (t, *J* = 8.0 Hz, 1H, H-4), 3.76 (s, 3H, COOCH₃), 2.05 (d, *J* = 9.4 Hz, 3H, COCH₃). ¹³C NMR (126 MHz, CDCl₃) δ 129.8, 128.4, 128.3, 127.7, 90.8, 79.2, 78.7, 75.4, 75.0, 74.5, 72.9, 70.6, 51.9, 21.5. HRMS: *m/z*: calcd for C₂₃H₂₆O₈Na: 453.1525; found: 453.1533 [M+Na]⁺.

Dimethylhexylsilyl O-methyl-2-O-levulinoyl-3,4-O-benzyl-β-D-glucopyranosyluronate (S4). A suspension of DCC (117 mg, 0.57 mmol) and DMAP (1 mg, 0.01 mmol) was added to a solution of compound **S2** (100 mg, 0.19 mmol) and levulinic acid (39 μL, 0.38 mmol) in DCM (1 mL) at 0 °C. After stirring for 6 h at ambient temperature, TLC analysis (hexanes/EtOAc, 70/30, v/v) indicated the consumption of the starting material. The mixture was filtered over pad of Celite and the filtrate was concentrated under reduced pressure. The residue was purified by silica gel column chromatography using a gradient of hexanes/EtOAc (3/1 → 1/1, v/v) to give compound **S4** as oil (105 mg, 89%). ¹H NMR (500 MHz, CDCl₃) δ 7.35 – 7.24 (m, 11H, CH Aromatic), 4.99 (t, *J* = 9.3, 7.2 Hz, 1H, H-2), 4.79 (dd, *J* = 11.5, 5.2 Hz, 2H, CHHBn, CHHBn), 4.75 – 4.61 (m, 3H, H-1, CH₂Bn), 3.98 – 3.90 (m, 2H, H-4, H-5), 3.75 (s, 3H, COOCH₃), 3.68 (t, *J* = 8.4 Hz, 1H, H-3), 2.68 (dt, *J* = 17.5, 6.8 Hz, 2H, CH₂ Lev), 2.49 (t, *J* = 6.8 Hz, 2H, CH₂ Lev), 2.17 (s, 3H, CH₃ Lev), 1.31 (s, 1H, CH(CH₃)₂), 0.88 – 0.81 (m, 12H, C(CH₃)₂ and CH(CH₃)₂), 0.17 (s, 3H, SiCH₃), 0.13 (s, 3H, SiCH₃). ¹³C NMR (151 MHz, CDCl₃) δ 128.2, 127.9, 127.8, 127.7, 96.1, 82.0, 79.0, 74.9, 74.8, 74.5, 37.7, 30.0, 29.4, 27.8, 19.0, 1.3. HRMS: *m/z*: calcd for C₃₄H₄₈O₉SiNa: 651.2965; found: 651.2972 [M+Na]⁺.

Dimethylhexylsilyl O-methyl-2-O-(4-acetoxy-2,2-dimethylbutanoate)-3,4-O-benzyl-β-D-glucopyranosyluronate (S5). To a stirring solution of compound **S2** (100 mg, 0.19 mmol) in pyridine (1 mL), DMAP (30 mg, 0.19 mmol) and 4-acetoxy-2,2-dimethyl butanoyl chloride (45 μL, 0.38 mmol) was added at 0 °C. After stirring for 4hr at ambient temperature, TLC analysis (hexanes/EtOAc, 70/30, v/v) indicated the total consumption of the starting material, after which the reaction mixture was diluted with EtOAc (10 mL) and washed with aqueous NaHCO₃ (10%), H₂O, brine, dried (MgSO₄), filtered and the filtrate was concentrated under reduced pressure. The mixture was concentrated under reduced pressure and was purified by silica gel column chromatography using a gradient of hexanes/EtOAc (4/1 → 1/1, v/v) to compound **S5** as oil (114 mg, 88%). ¹H NMR (600 MHz, CDCl₃) δ 7.36 – 7.14 (m, 10H, CH Aromatic), 5.06 – 4.92 (m, 1H, H-2), 4.85 – 4.59 (m, 5H, 4 × CHHBn, H-1), 4.12 – 4.04 (t, 2H, CH₂ PivOAc), 3.75 – 3.66 (m, 4H, COOCH₃, H-4), 3.46 – 3.40 (t, *J* = 8.5 Hz, 1H, H-3), 1.98 – 1.93 (m, 3H, CH₃ PivOAc), 1.85 (t, *J* = 7.7, 2H, CH₂ PivOAc), 1.61 (m, 1H, CH(CH₃)₂), 1.18 (d, *J* = 4.7 Hz, 6H, 2xCH₃ PivOAc), 0.88 – 0.81 (m, 12H, C(CH₃)₂ and CH(CH₃)₂), 0.18 – 0.11 (m, 6H, Si(CH₃)₂). ¹³C NMR (151 MHz, CDCl₃) δ 128.4, 128.2, 127.7, 127.5, 127.4, 95.9, 82.6, 77.6, 75.3, 75.2, 74.7,

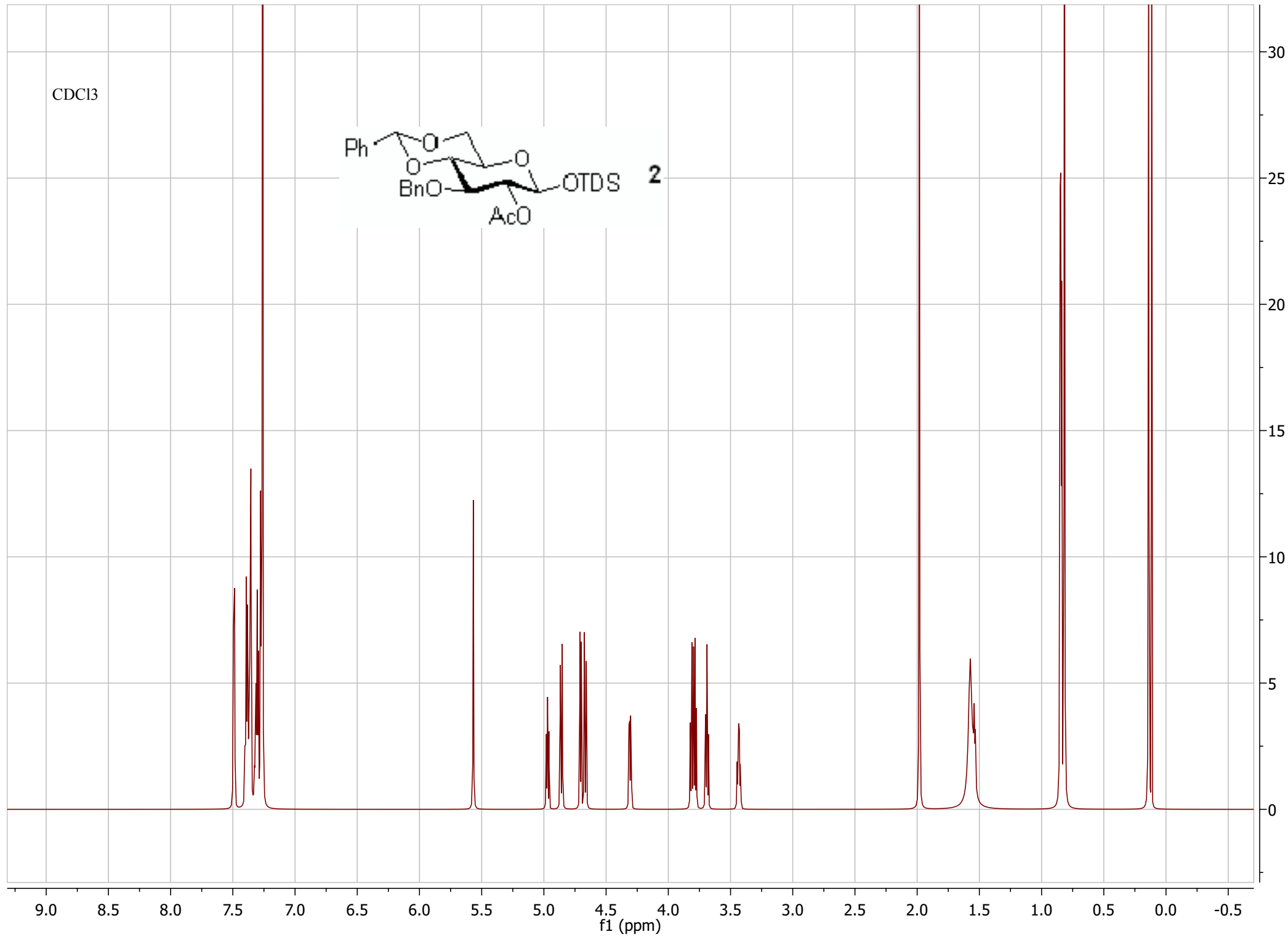
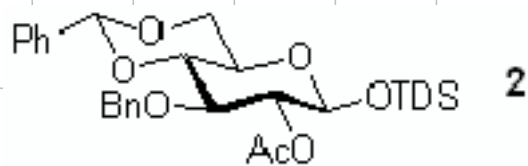
74.4, 61.2, 52.3, 38.0, 33.7, 25.2, 20.9, 19.7. HRMS: m/z: calcd for C₃₇H₅₄O₁₀SiNa: 709.3384; found: 709.3390 [M+Na]⁺.

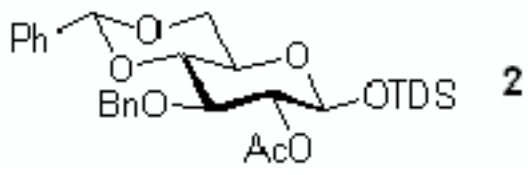


Scheme S2. Synthesis of glucuronic donor with C-4 2-naphthylmethyl ether. Reagents and conditions: a) 2-naphthylmethyl bromide, NaH, TBAI, DMF, -20 °C (70%); b) (i) HF:Pyr. THF; (ii) Cl₃CCN, NaH, DCM.

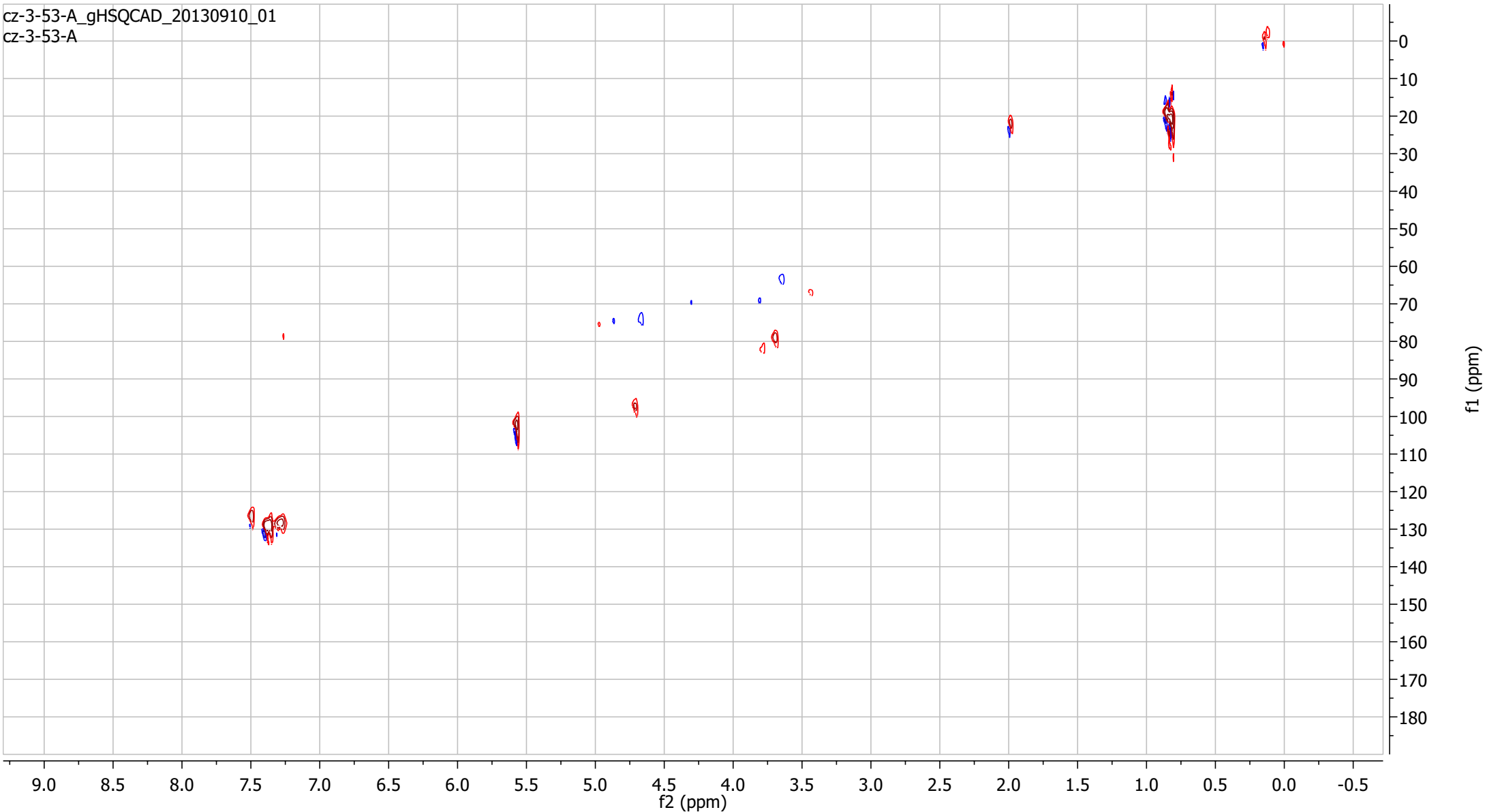
Dimethylhexylsilyl O-methyl-2-O-(4-acetoxy-2,2-dimethylbunoate)-3-O-benzyl-4-O-(2-methyl-naphthyl)-β-L-glucopyranosyluronate (S6). To stirring solution of compound **10** (300 mg, 0.50 mmol) in DMF (5 mL), 2-naphthylmethyl bromide (277 mg, 1.26 mmol) TBAI (2 mg, 0.01 mmol) and NaH (12 mg, 0.50 mmol) were added at -20 °C. After stirring for 30 min at -20 °C, TLC analysis (hexanes/EtOAc, 80/20, v/v) indicated total consumption of starting material. The reaction mixture was quenched with MeOH and was concentrated under reduced pressure. The residue was purified by silica gel column chromatography using a gradient of hexanes/EtOAc (5/1 → 1/1, v/v) to give compound **S6** as oil (259 mg, 70%). ¹H NMR (300 MHz, CDCl₃) δ 7.77 – 7.05 (m, 12H, CH Aromatic), 4.91 (t, *J* = 8.5, 1H, H-2), 4.64 (m, *J* = 11.1 Hz, 6H, CH₂NAP, 3 × CHHBn, H-1), 4.02 – 3.80 (m, 4H, H-4, H-5, CH₂ PivOAc), 3.65 – 3.51 (m, 4H, H-3, COOCH₃), 1.83 (d, *J* = 1.5 Hz, 3H, CH₃ PivOAc), 1.73 (dd, *J* = 8.0, 6.3 Hz, 2H, CH₂ PivOAc), 1.57 – 1.38 (m, 1H, CH(CH₃)₂), 1.06 (s, 4H, 2 × CH₂ PivOAc), 0.79 – 0.61 (m, 12H, C(CH₃)₂ and CH(CH₃)₂), 0.09 – 0.06 (m, 6H, Si(CH₃)₂). ¹³C NMR (126 MHz, CDCl₃) δ 128.3, 127.9, 127.1, 127.0, 126.7, 125.9, 125.8, 96.1, 82.3, 78.9, 74.9, 74.8, 74.4, 74.3, 61.3, 52.4, 38.0, 33.8, 26.0, 25.3, 25.2, 24.7, 24.1, 20.9, 19.9, 18.6. HRMS: m/z: calcd for C₄₁H₅₆O₁₀SiNa: 759.3540; found: 759.3546 [M+Na]⁺.

CDCl₃



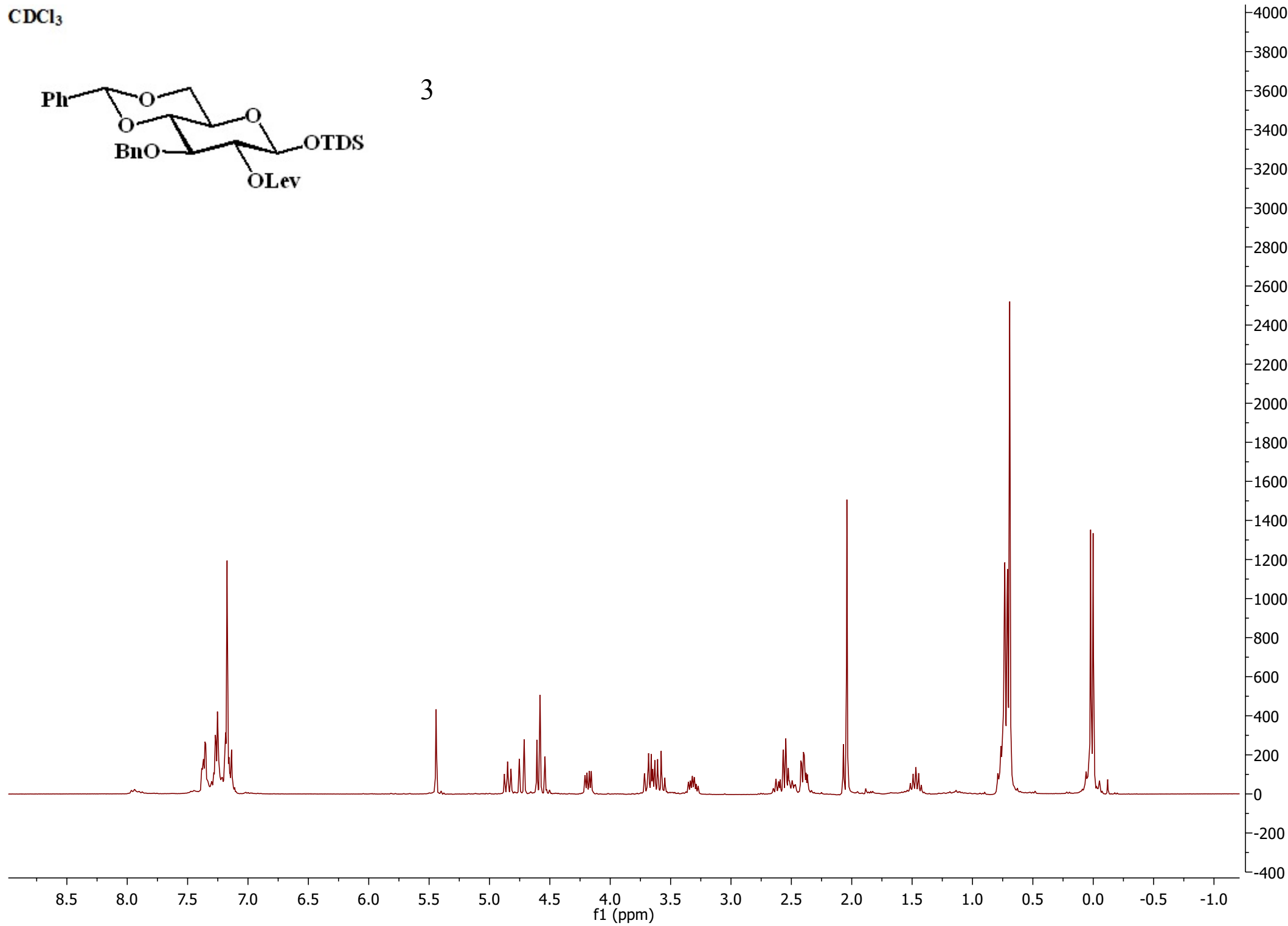
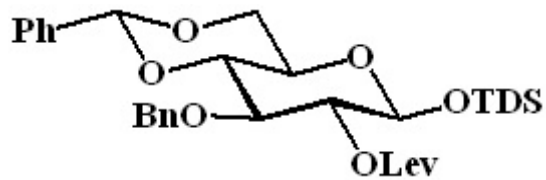


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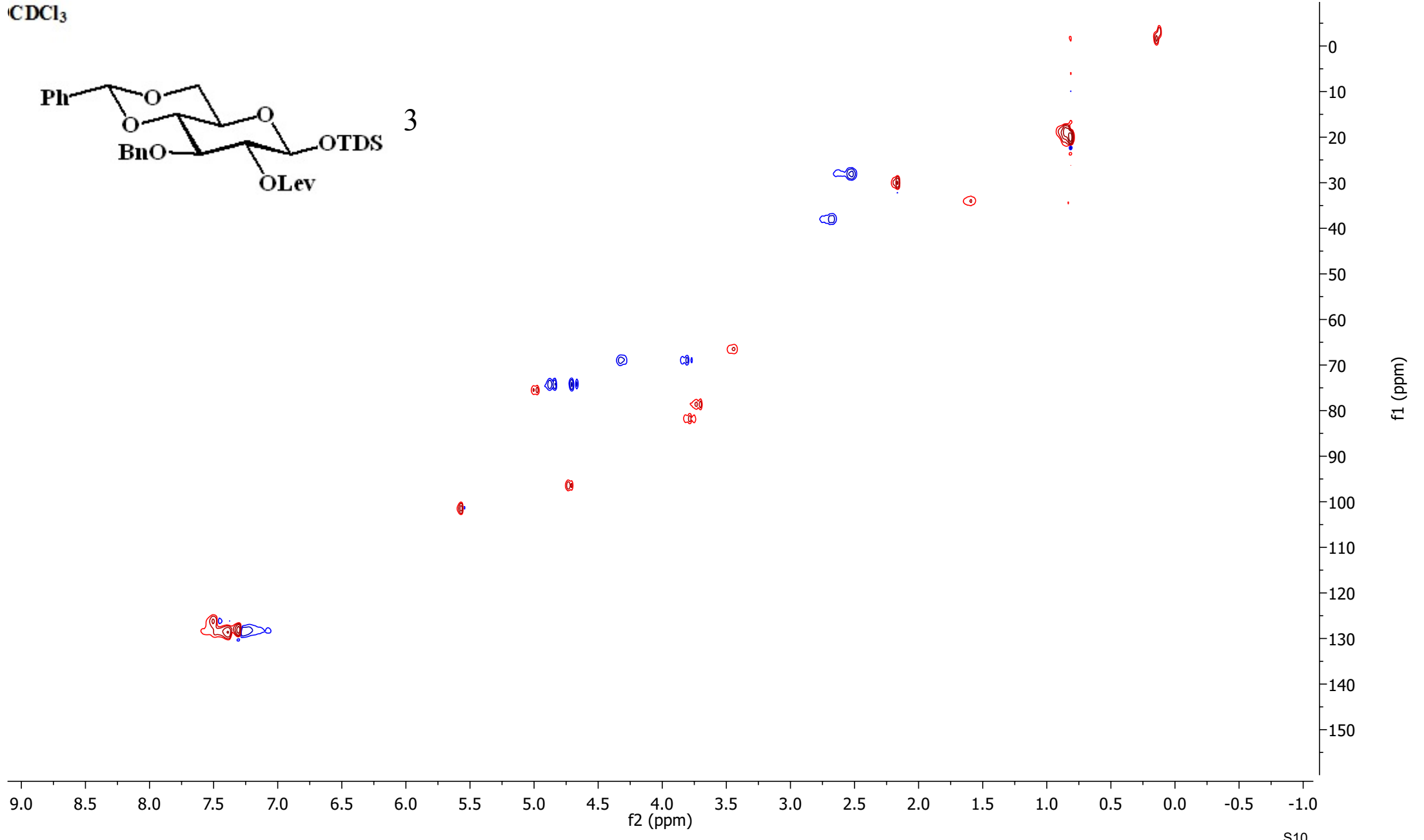
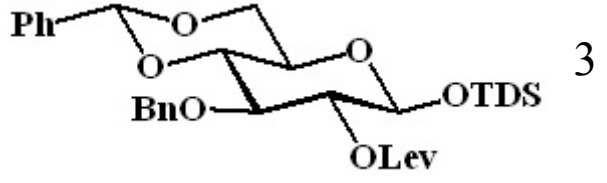


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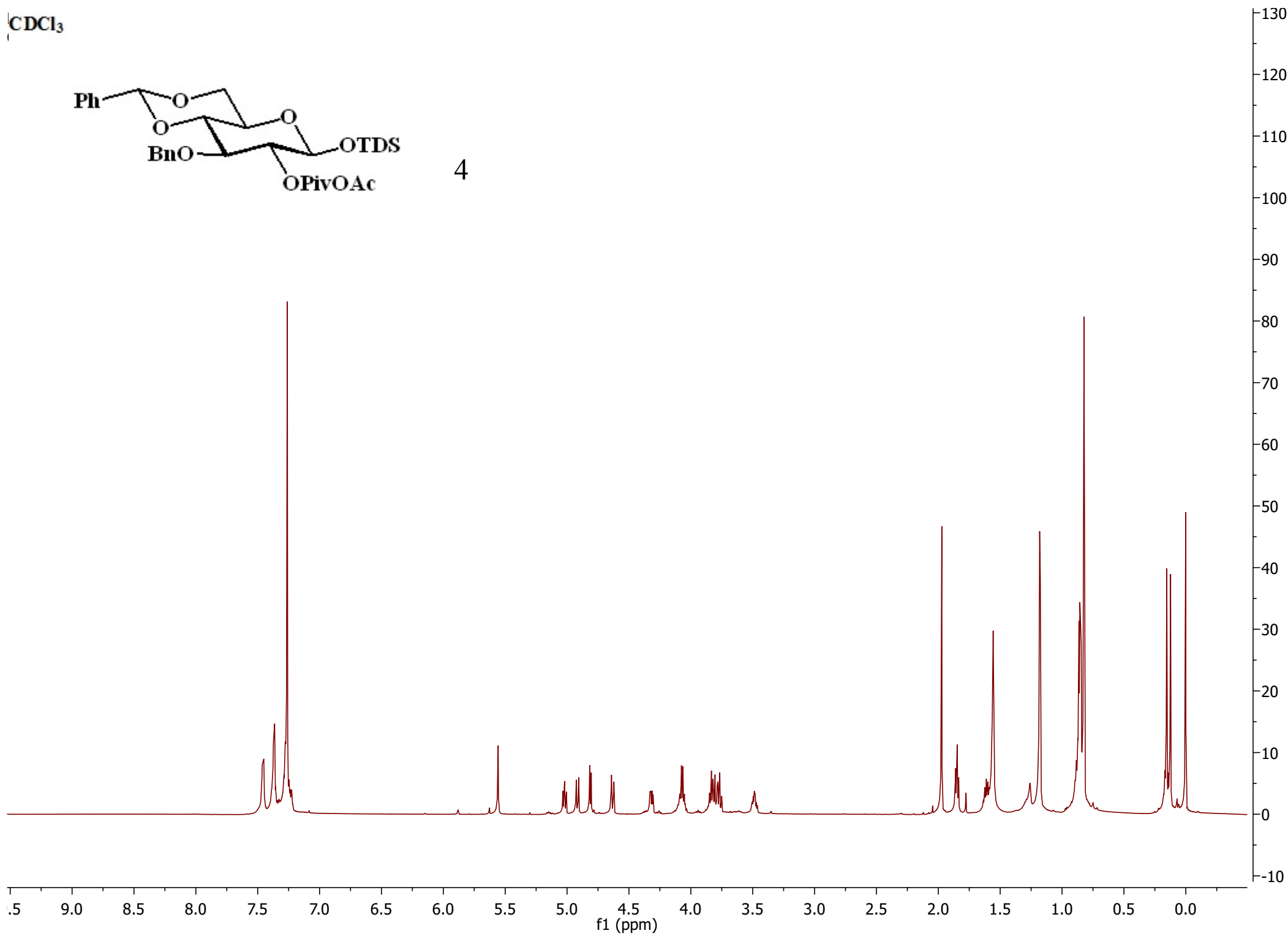
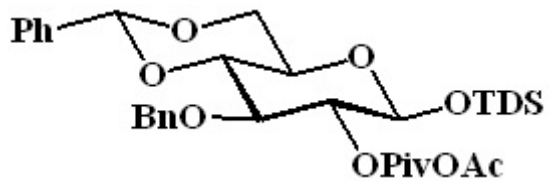
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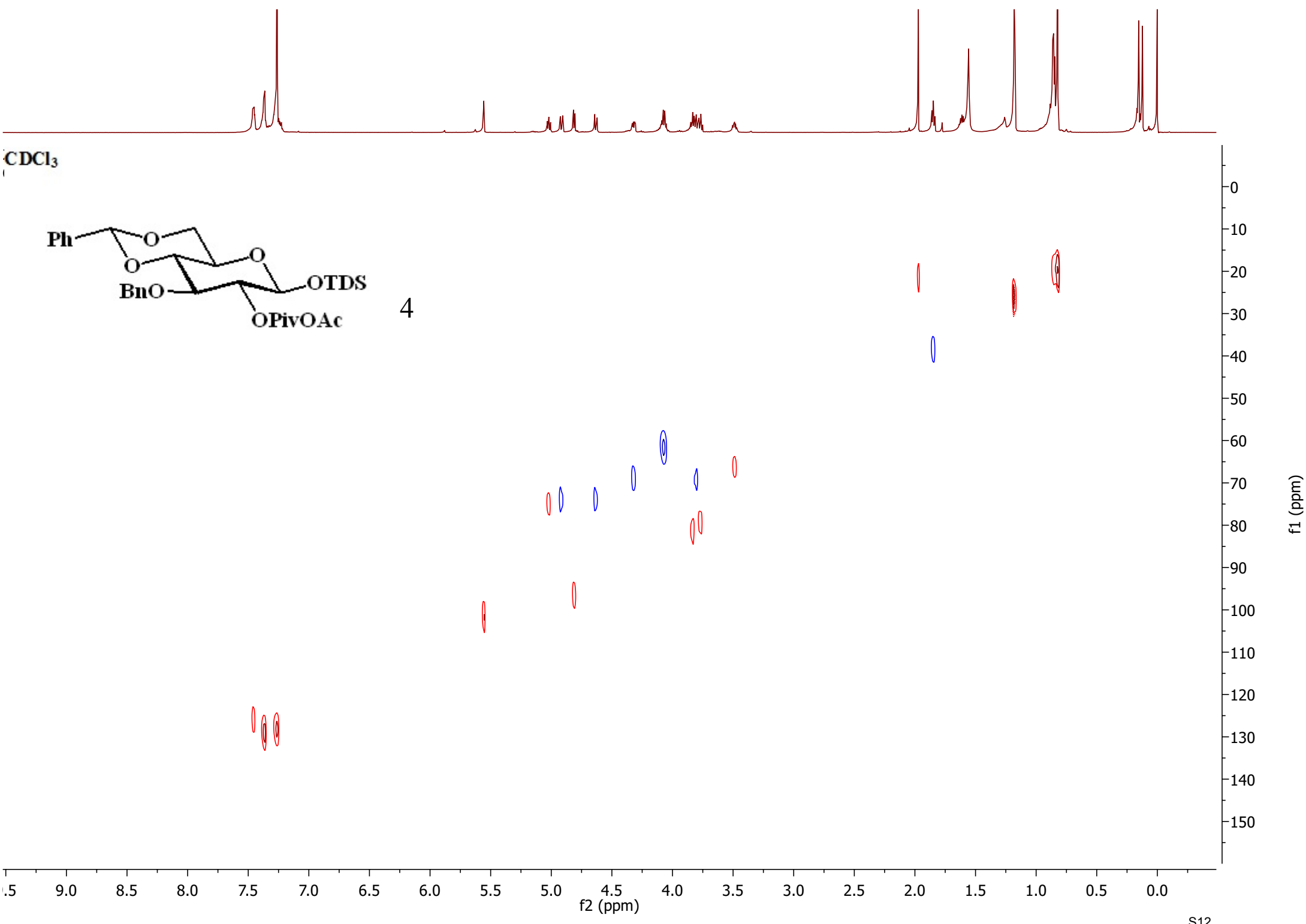
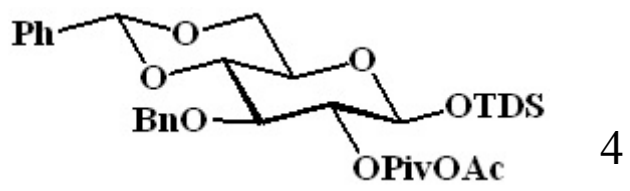
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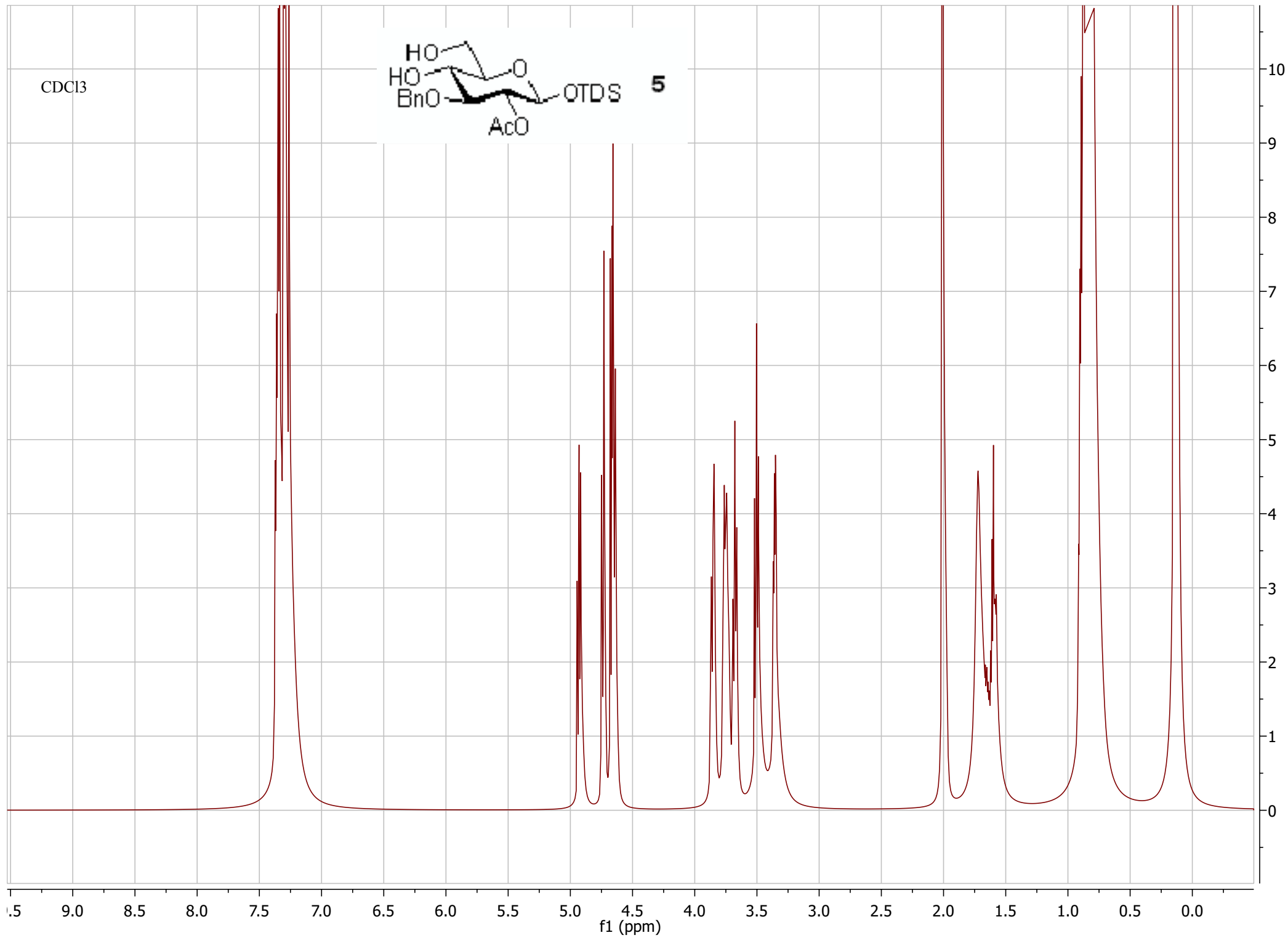
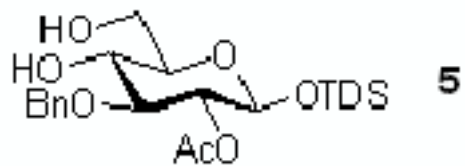
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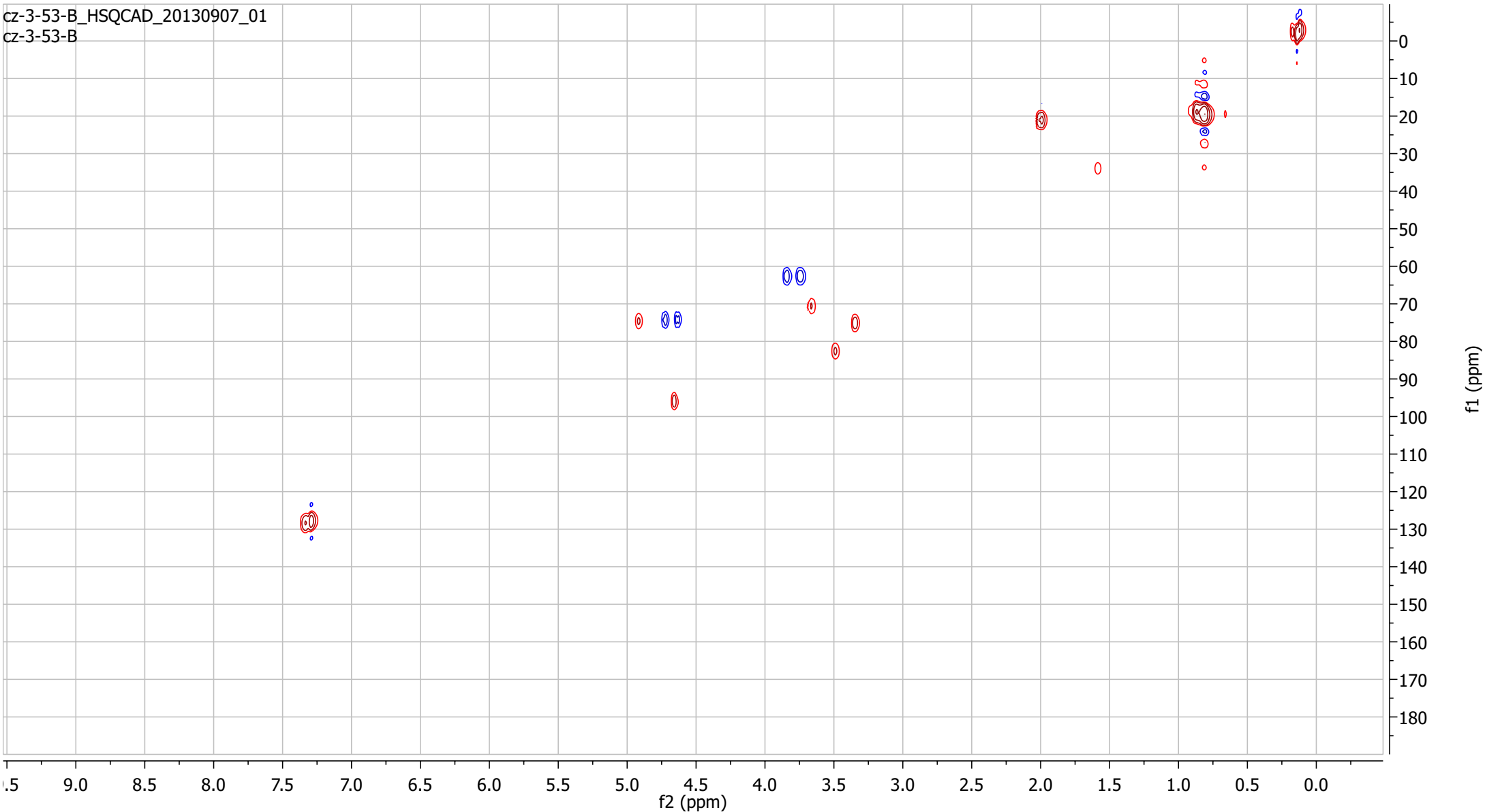
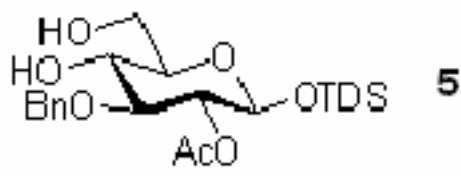
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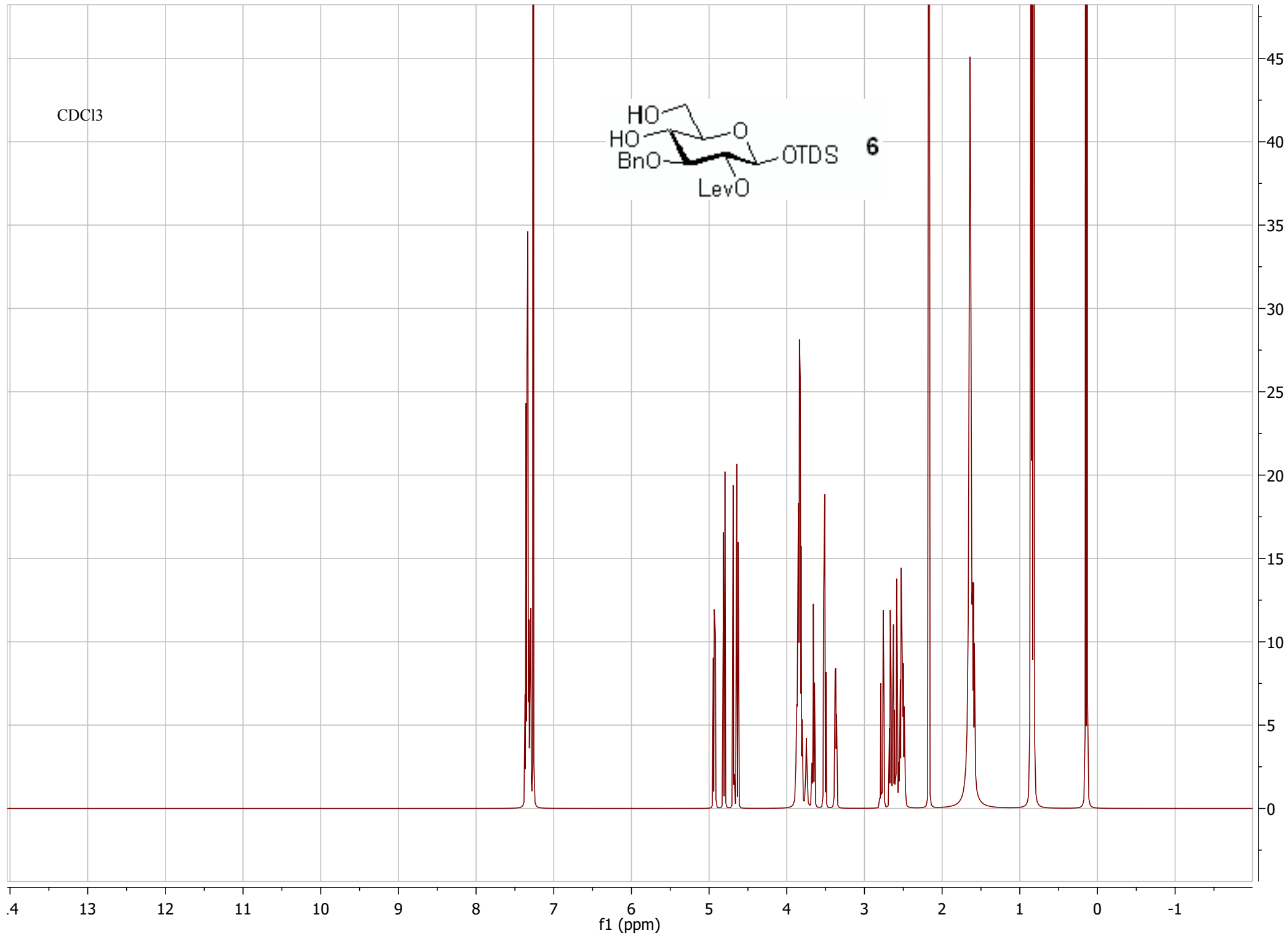
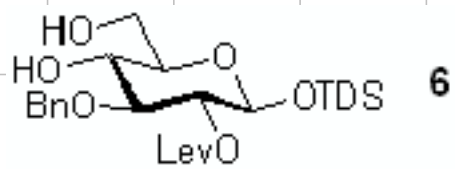
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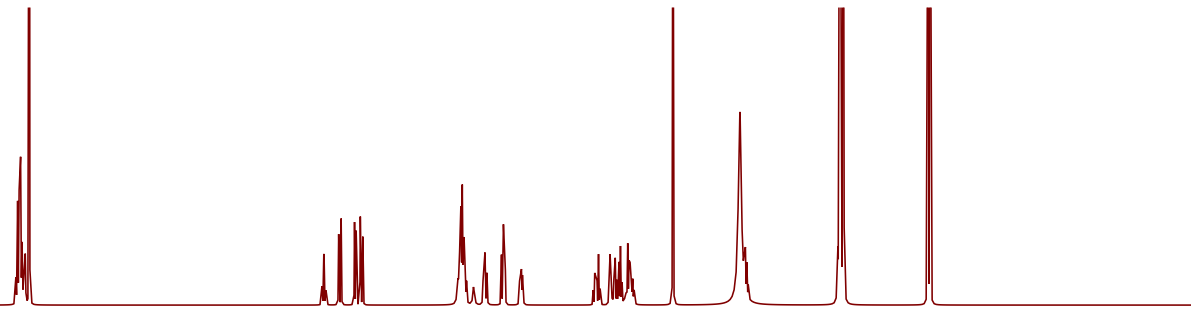
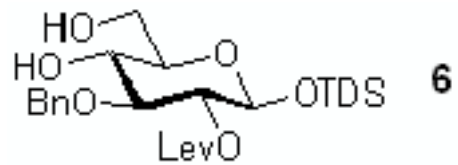
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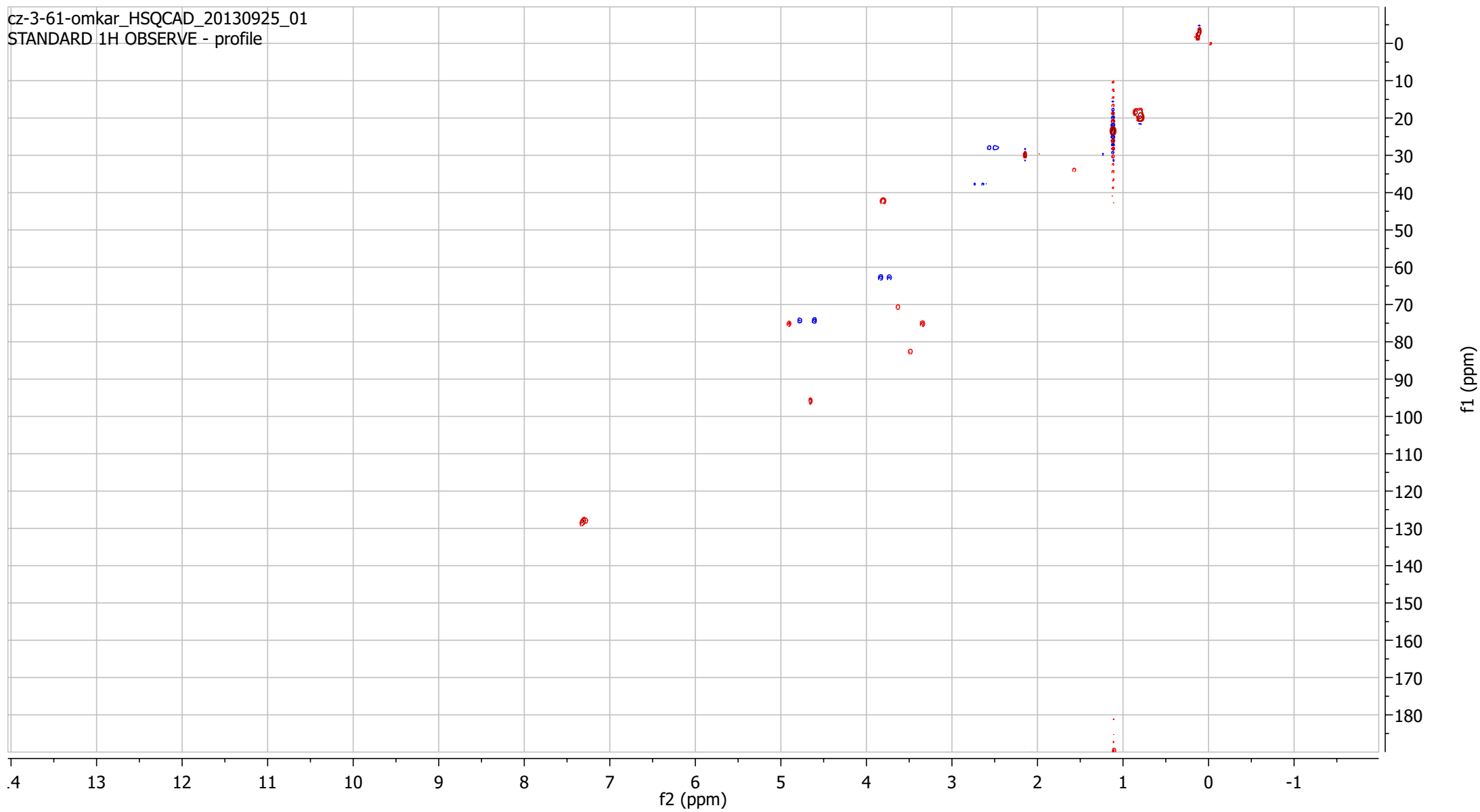
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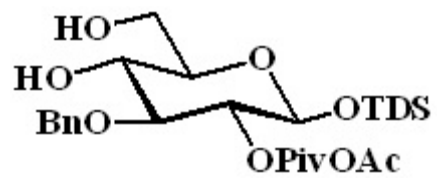
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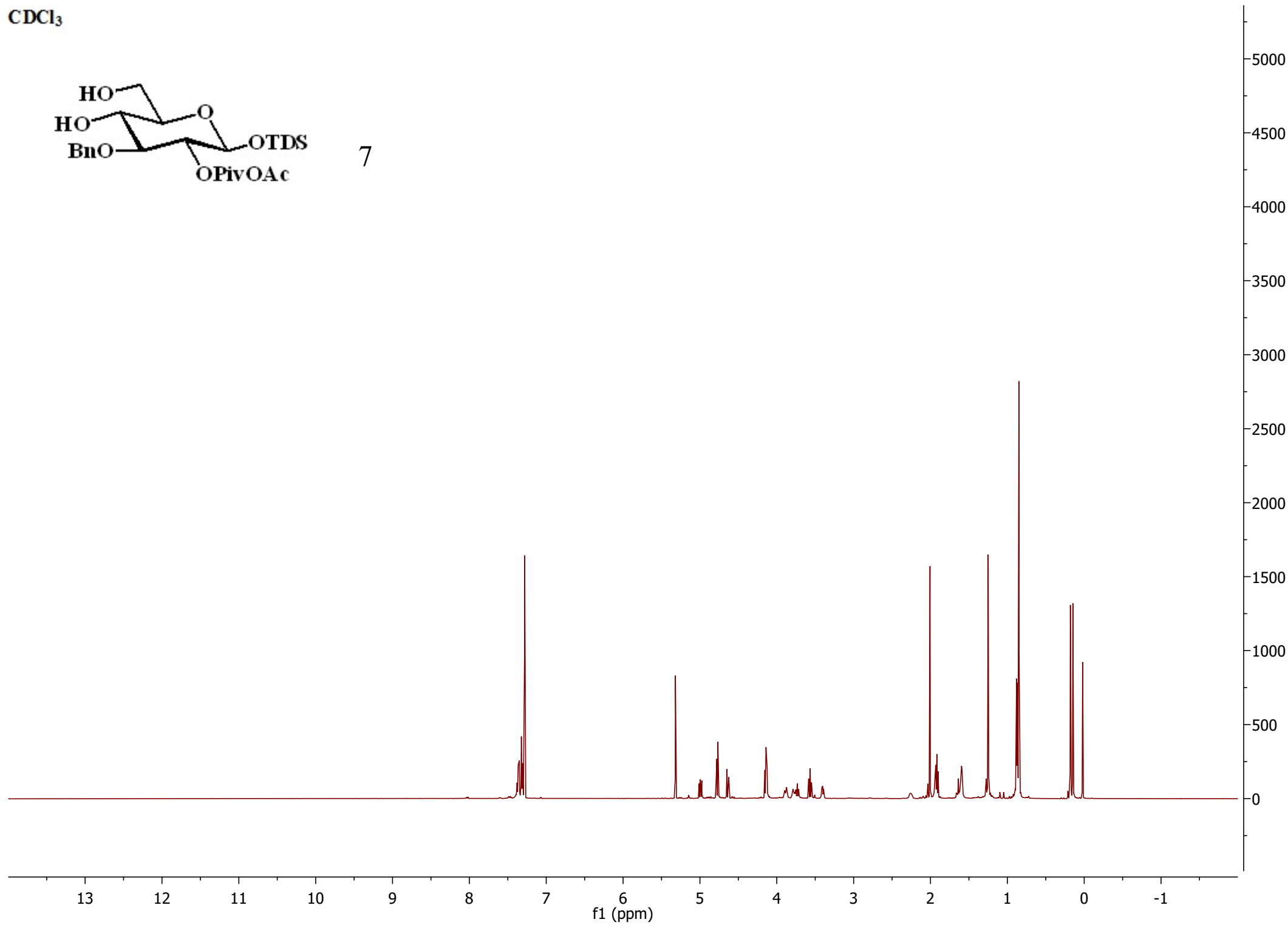
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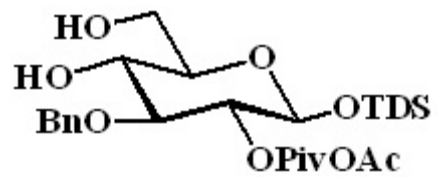
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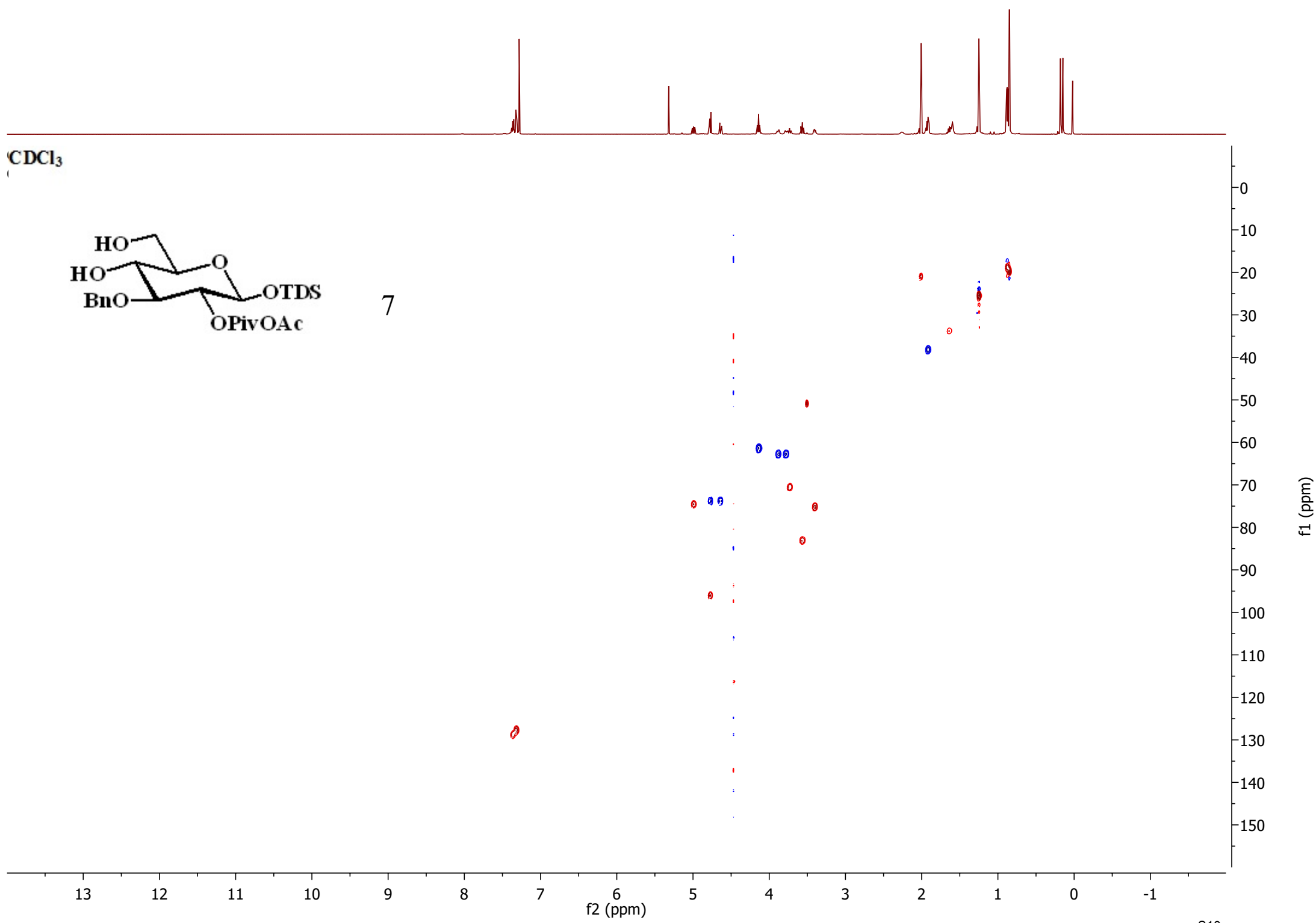
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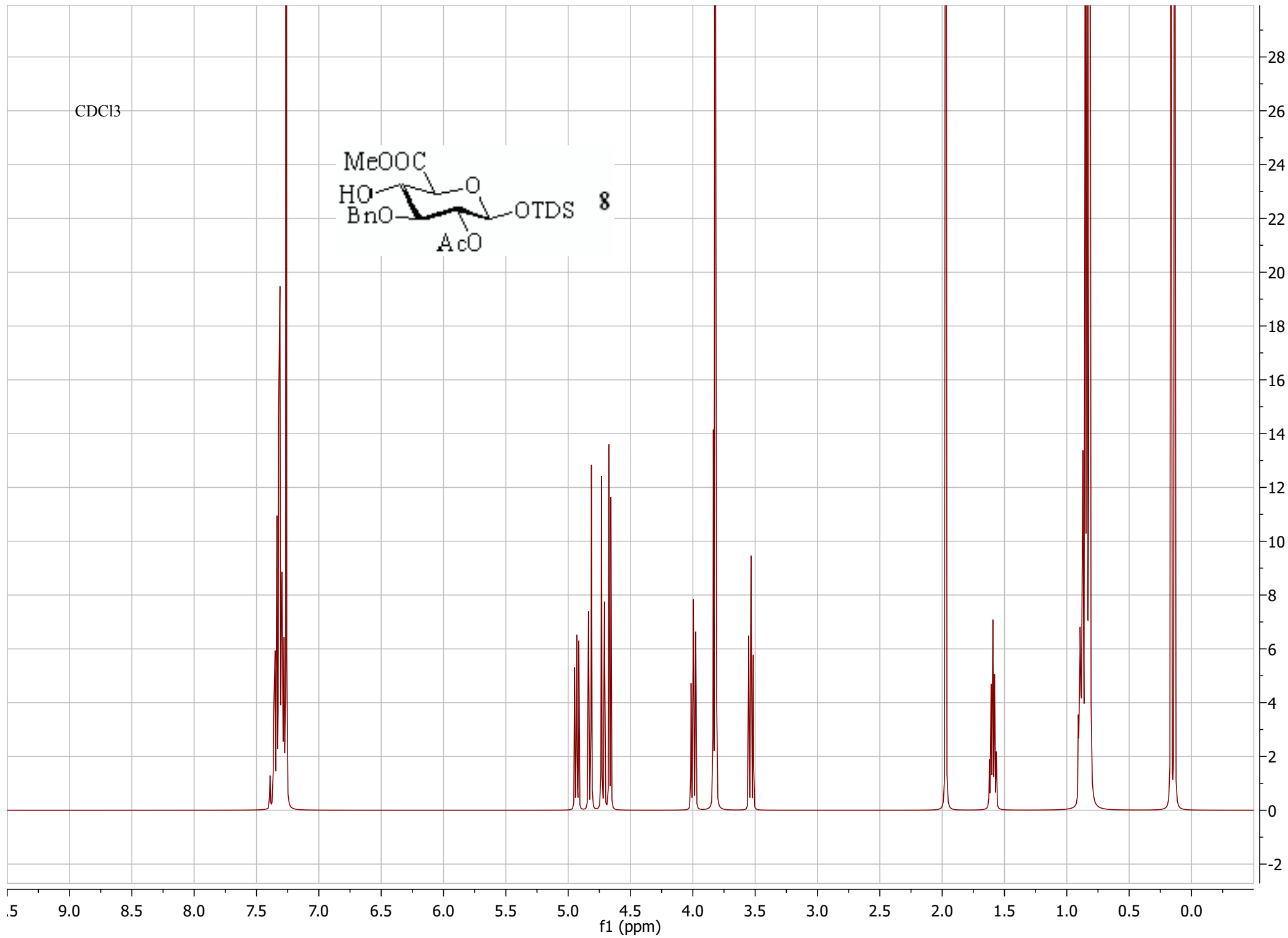


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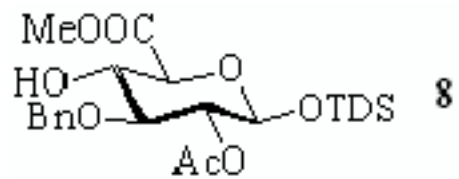


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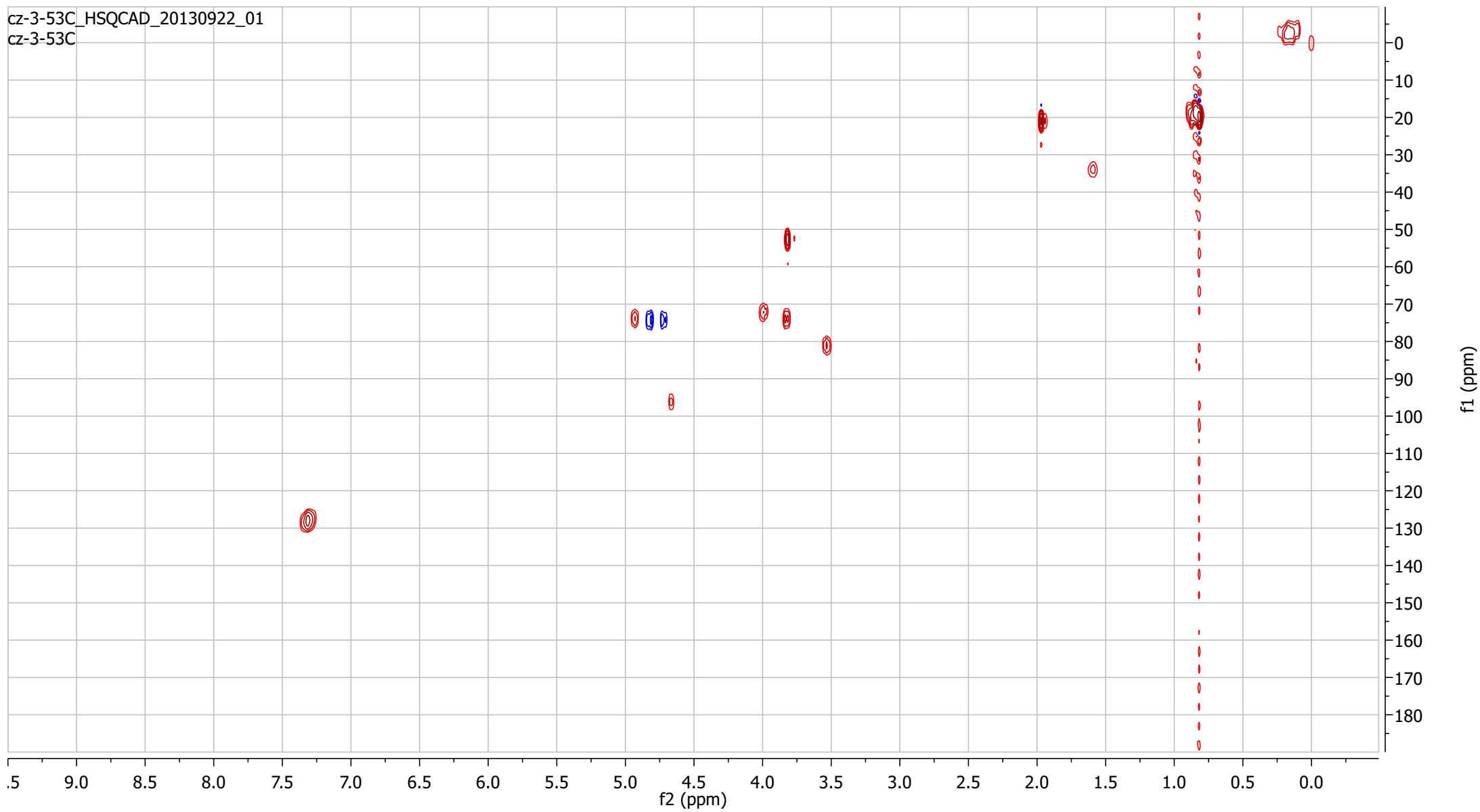




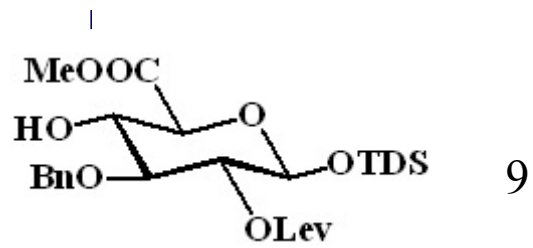
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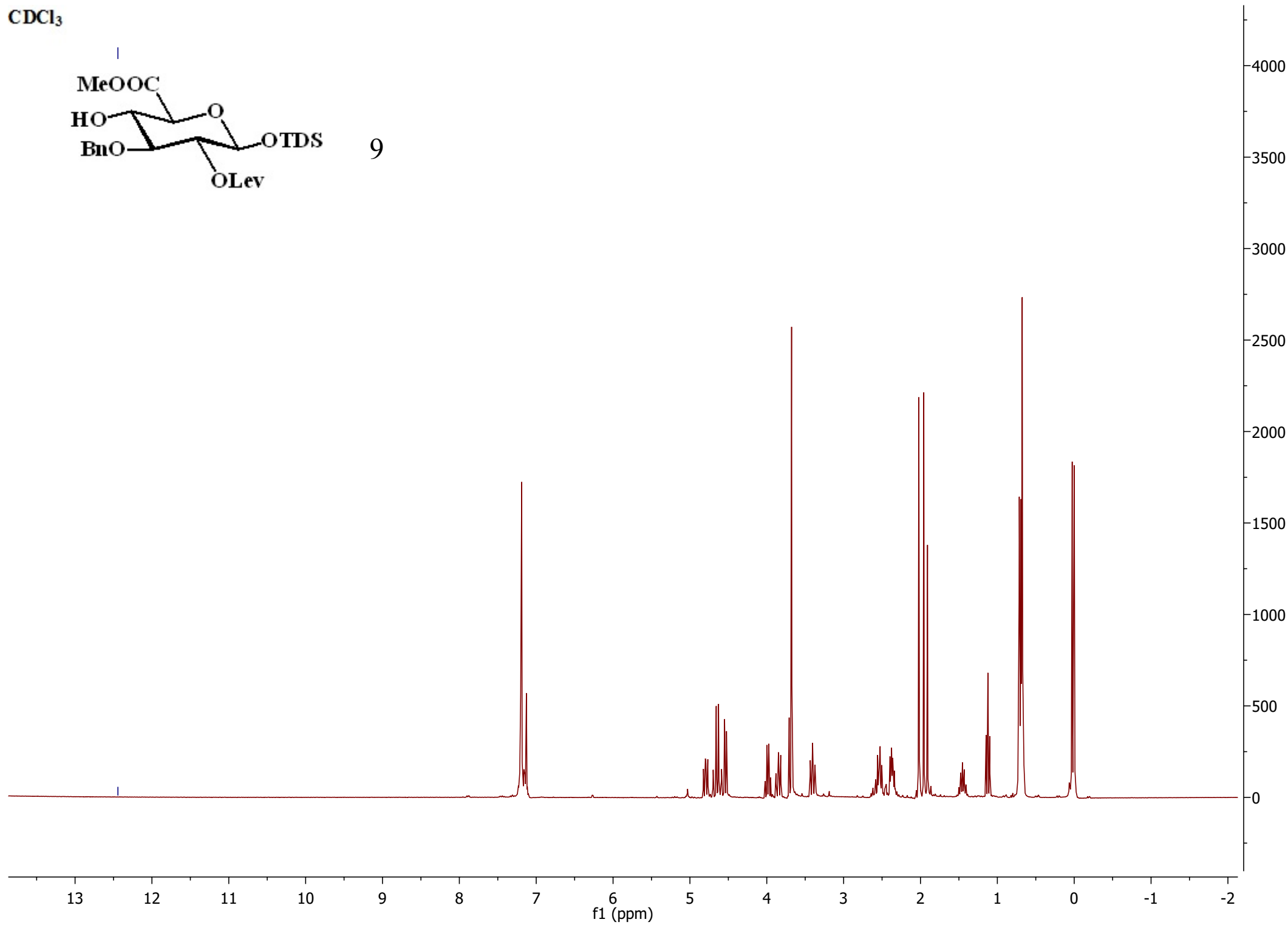
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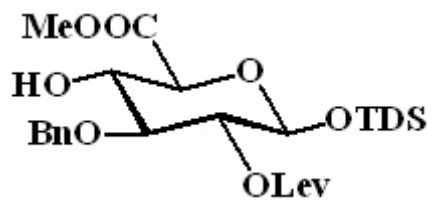


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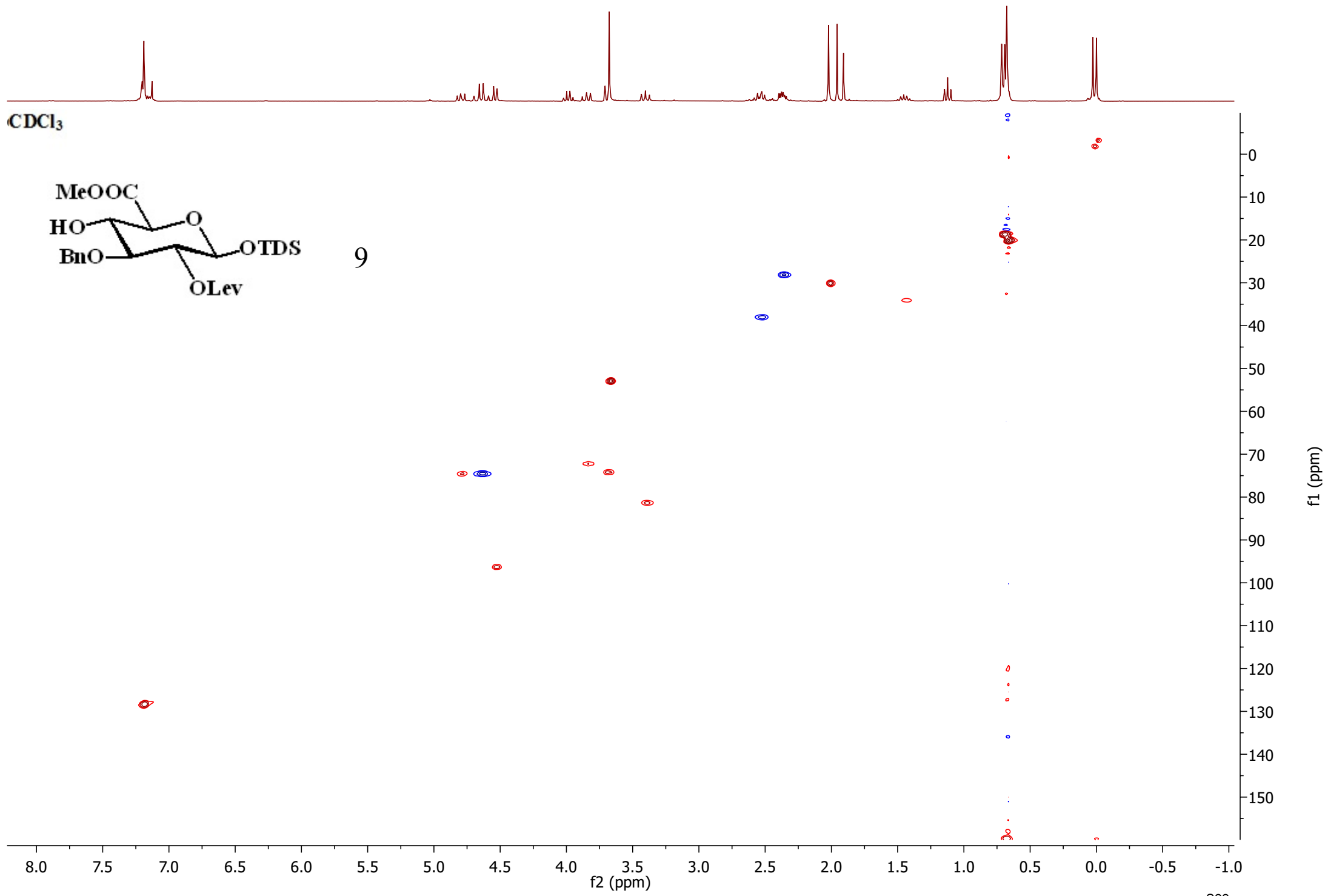


S21

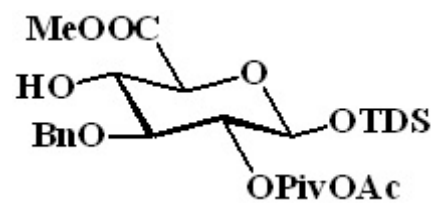
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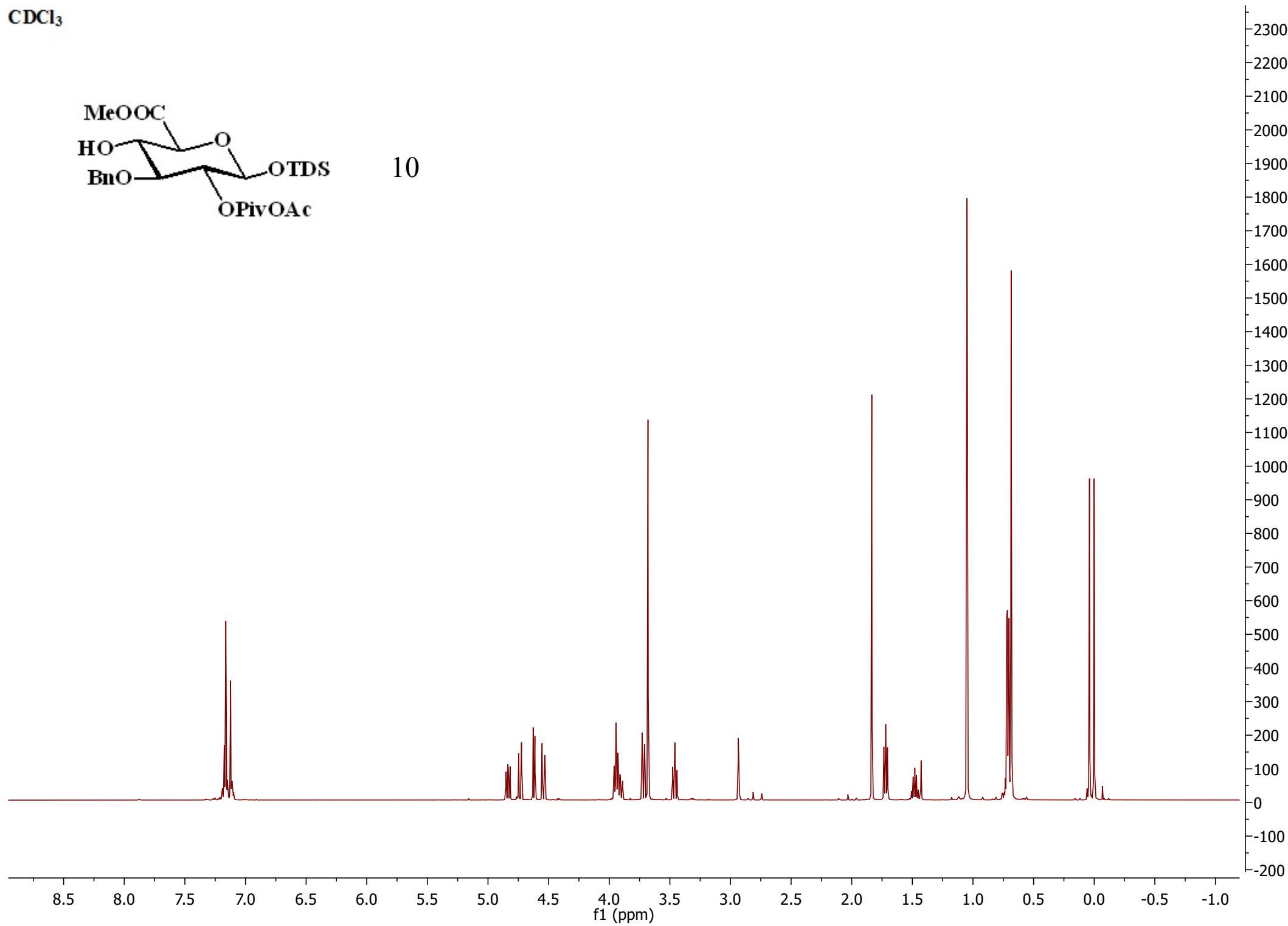
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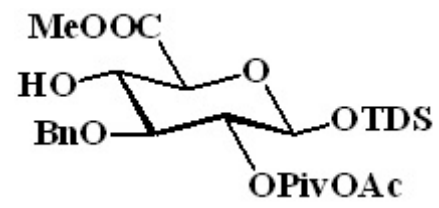
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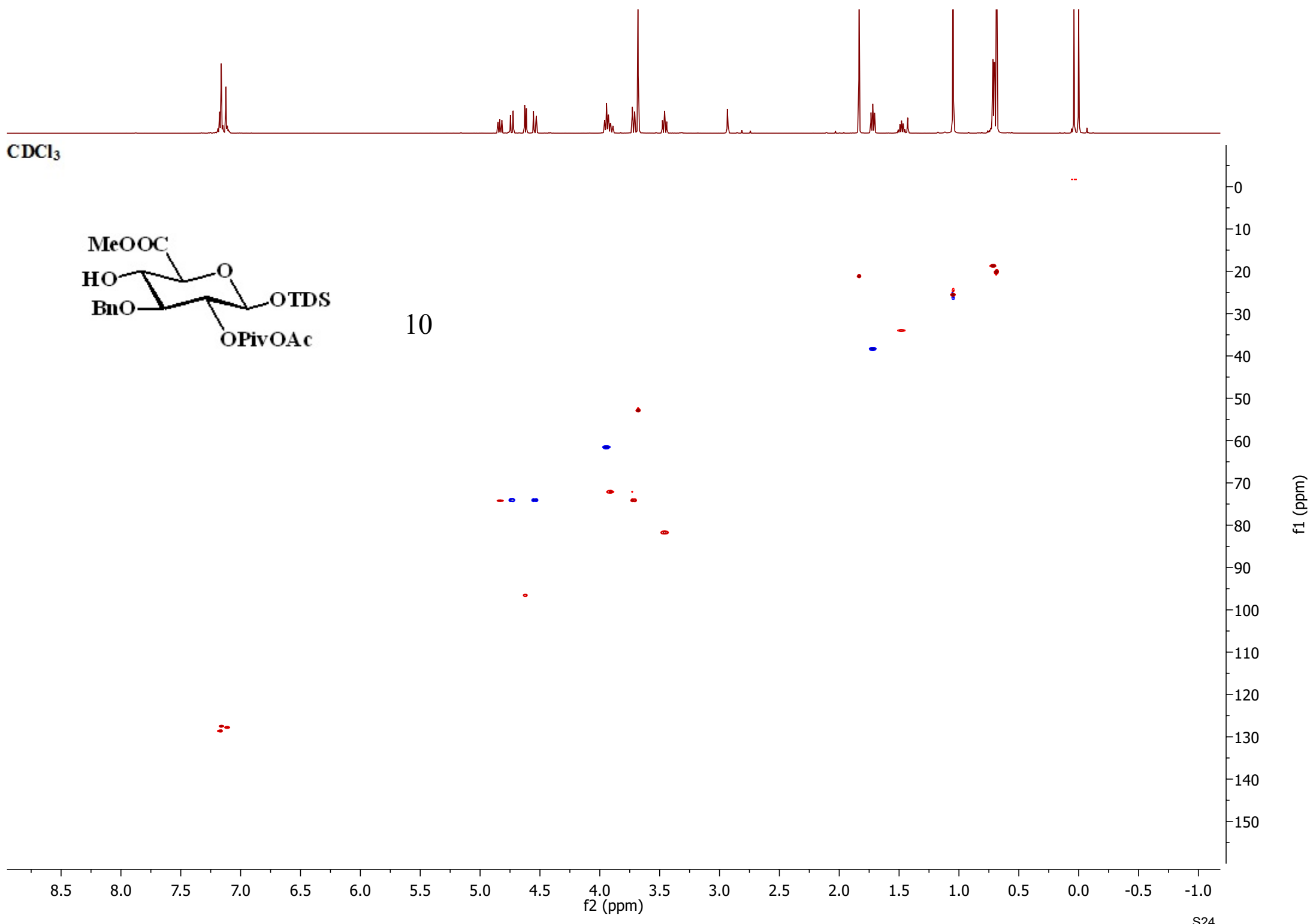
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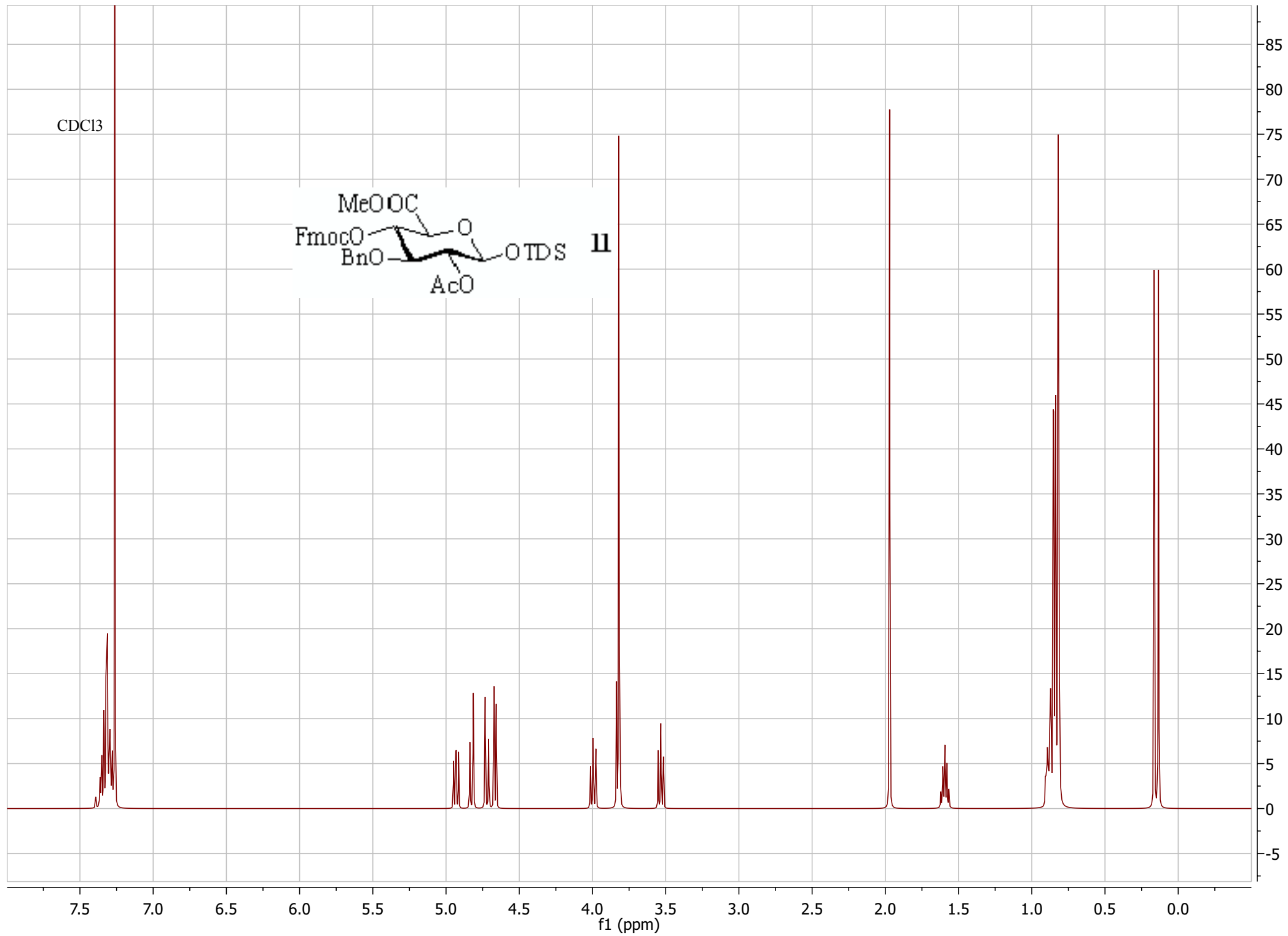


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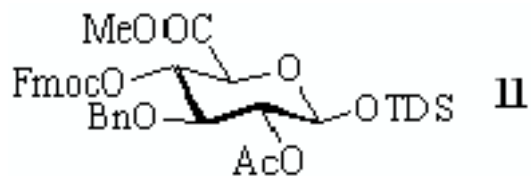


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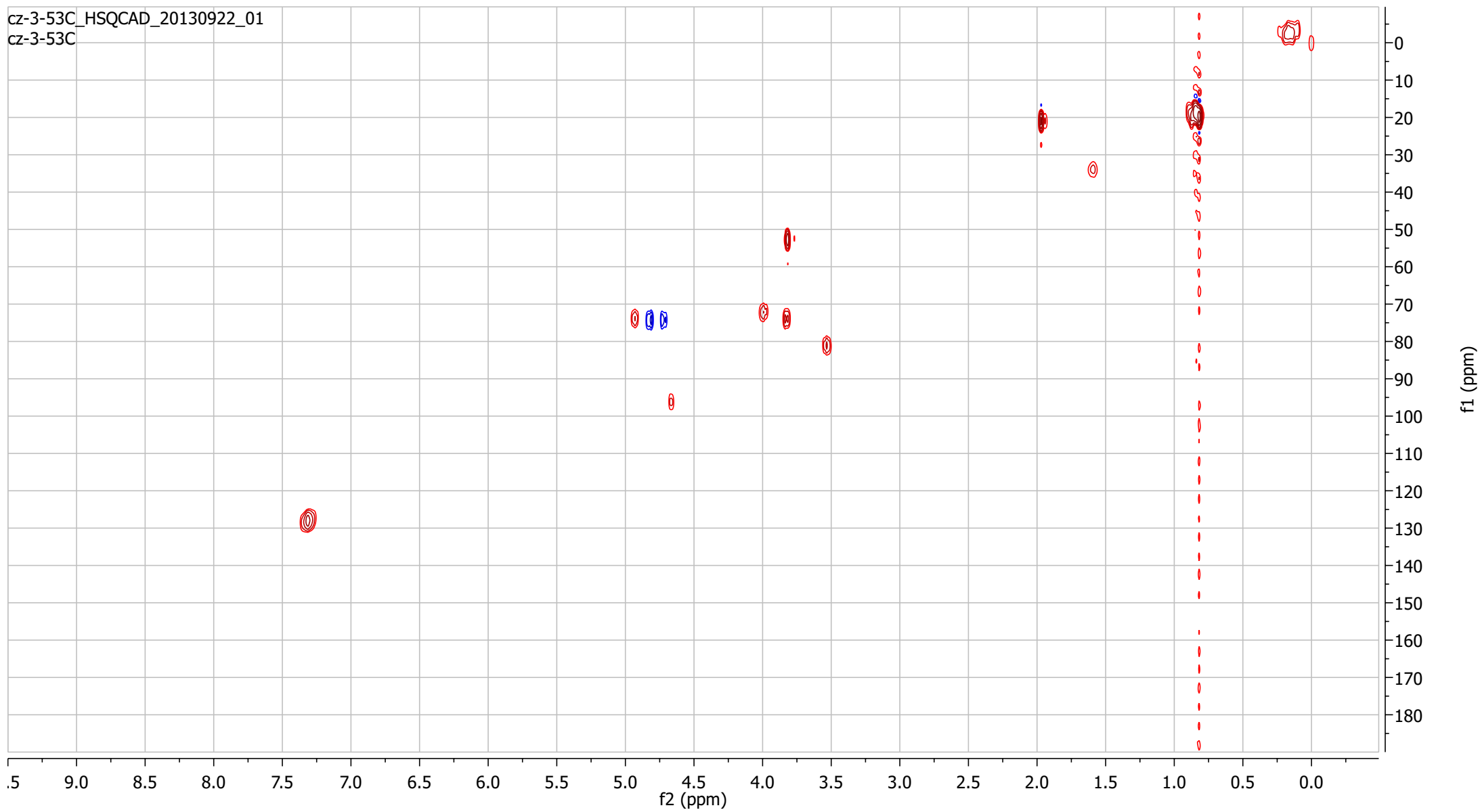




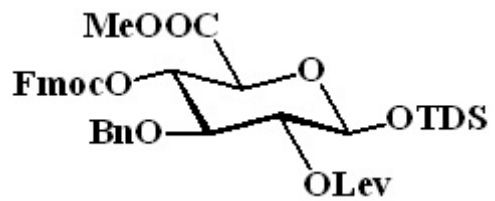
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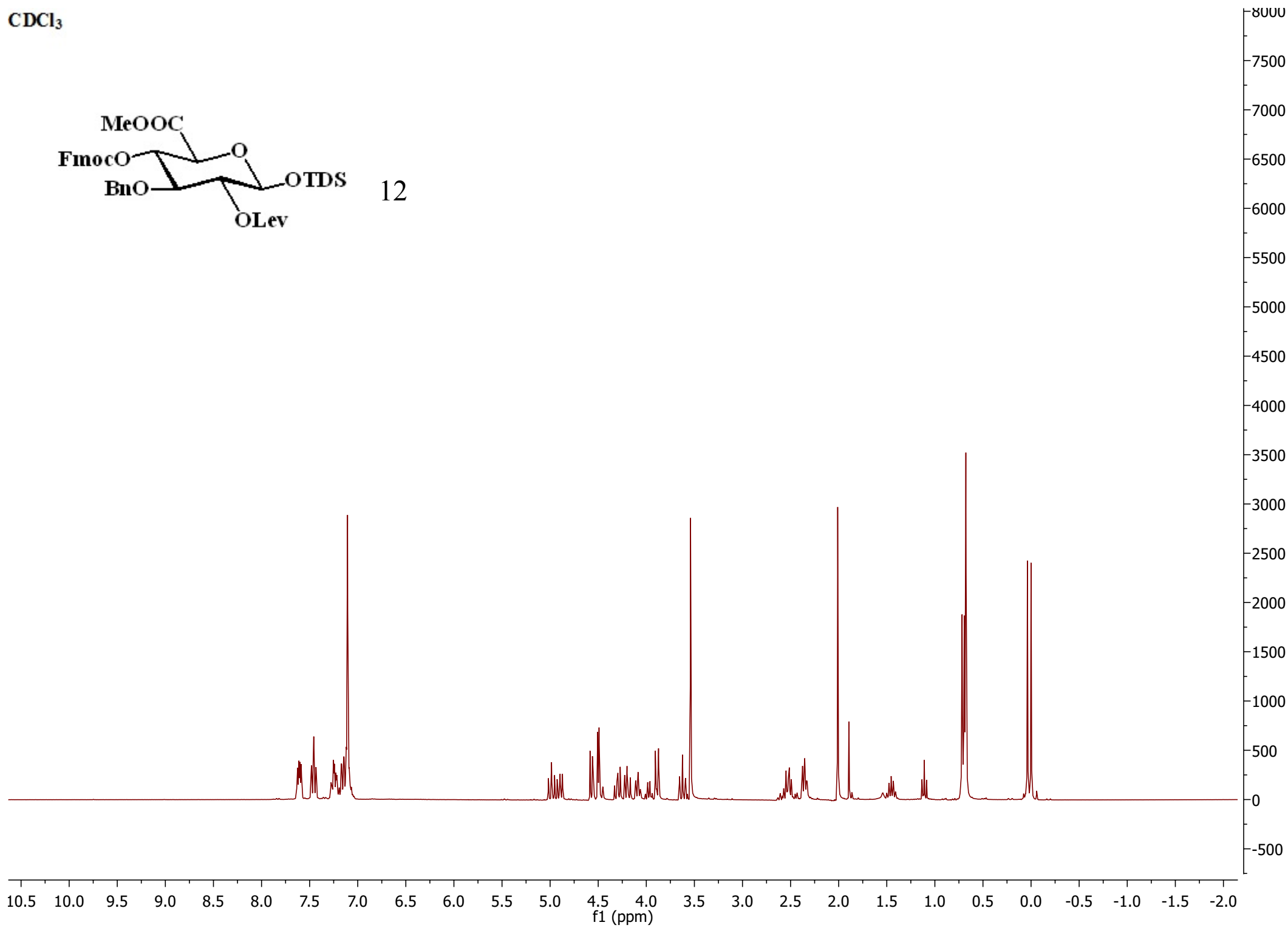
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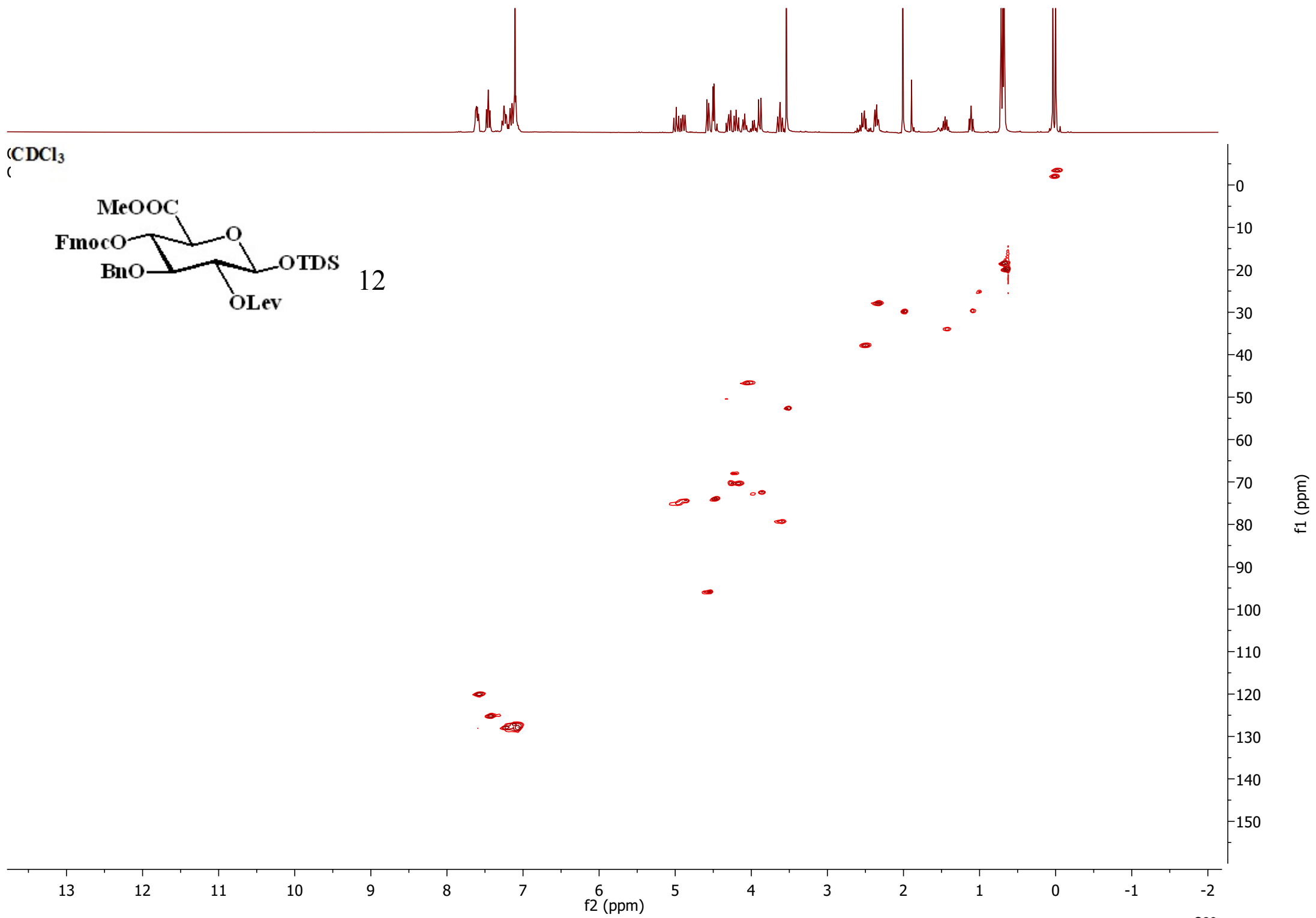


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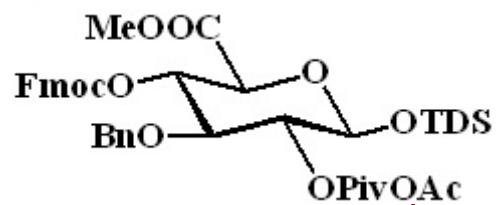


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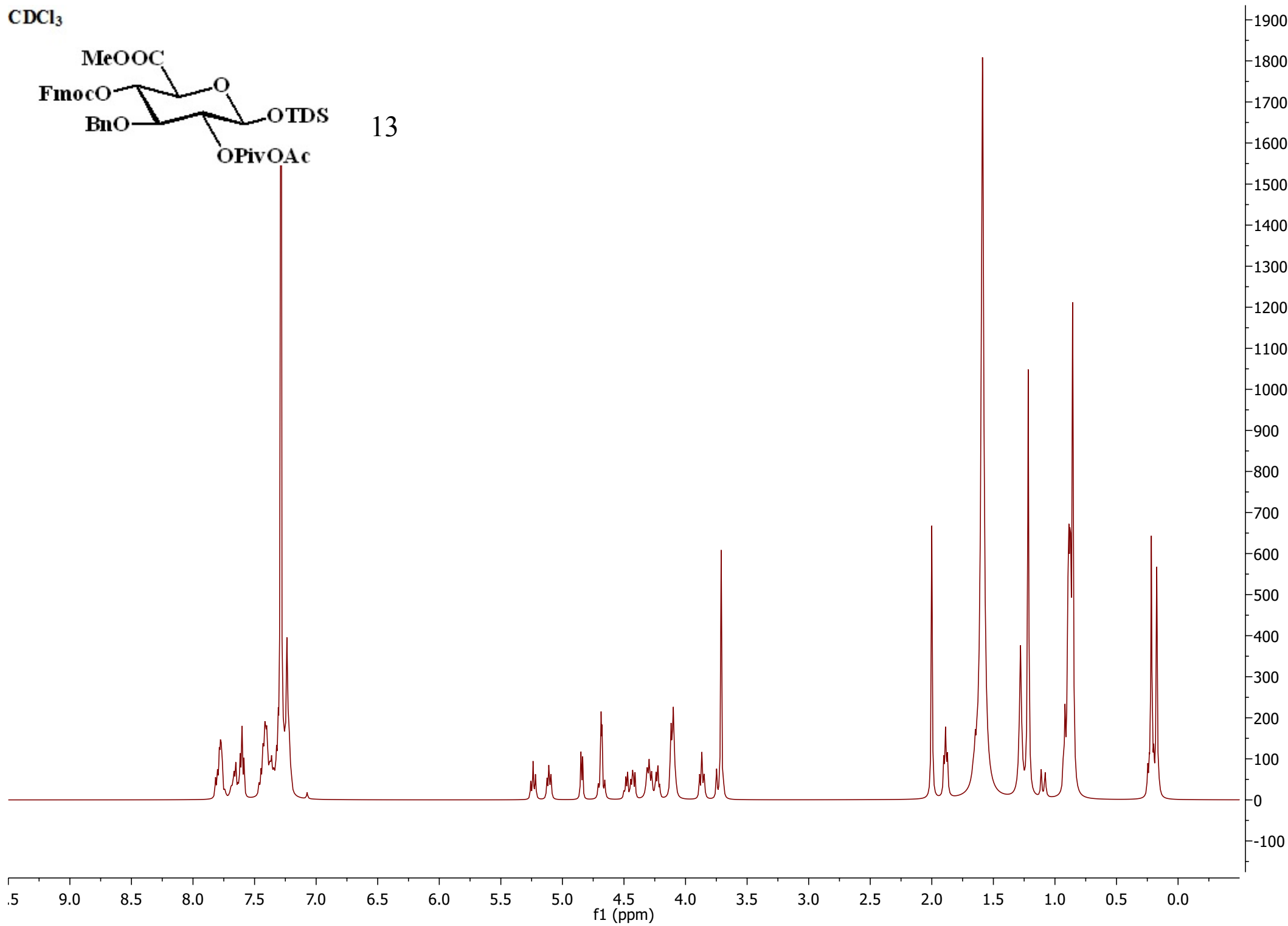




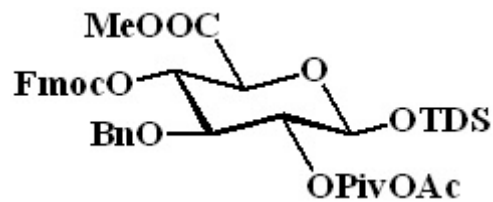
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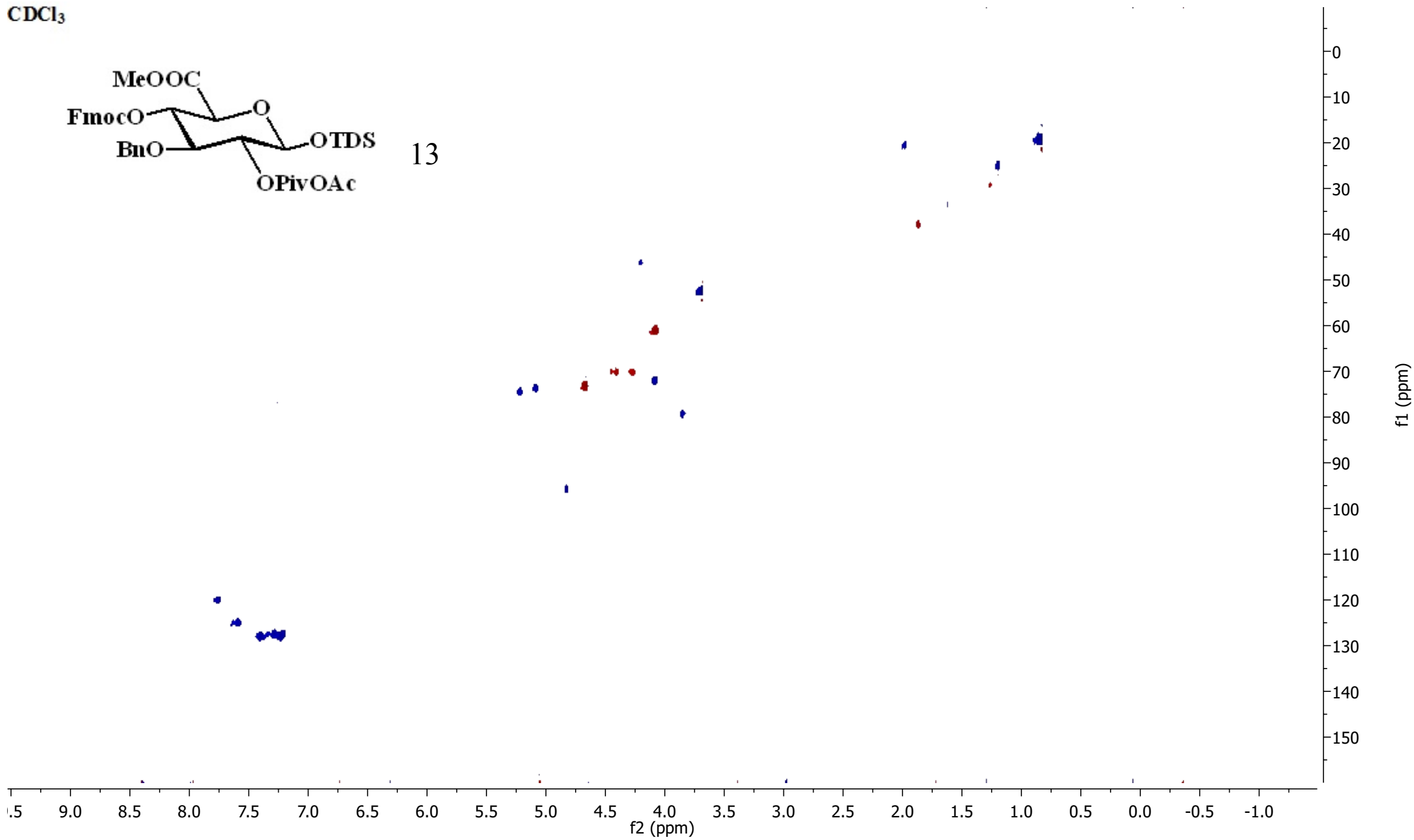
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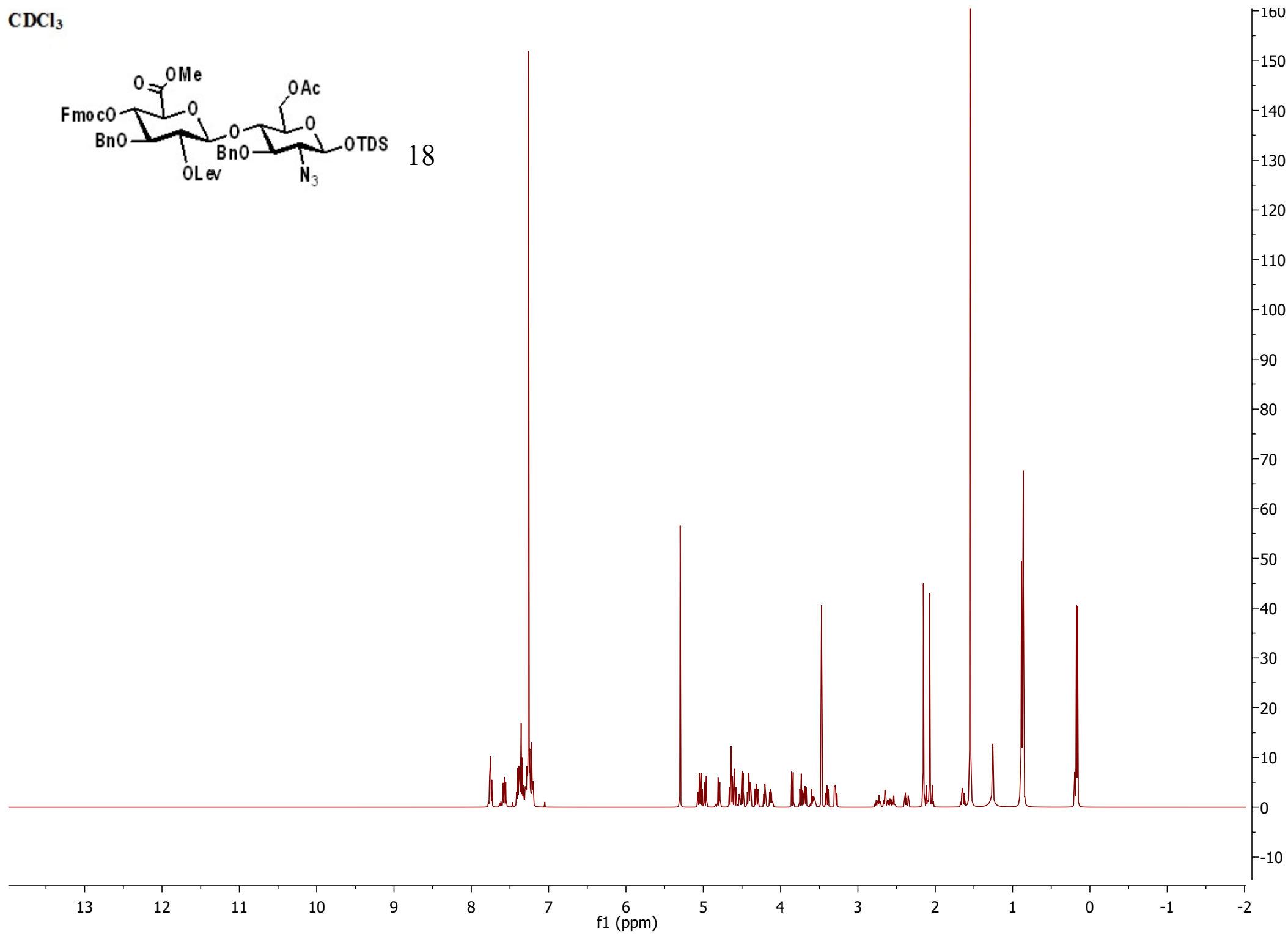
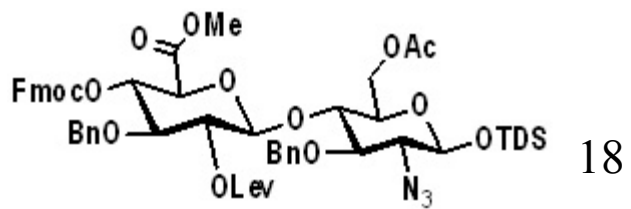
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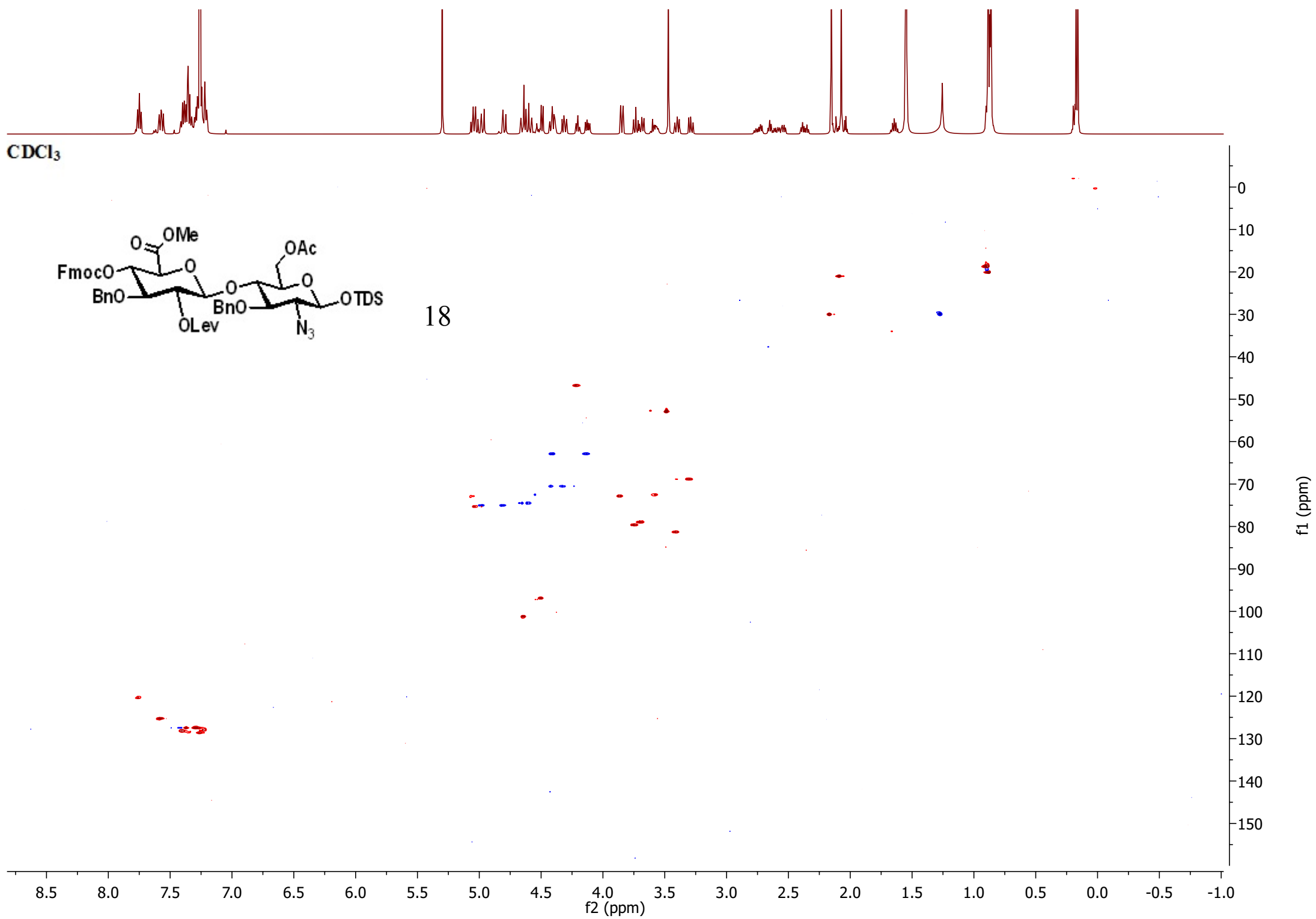
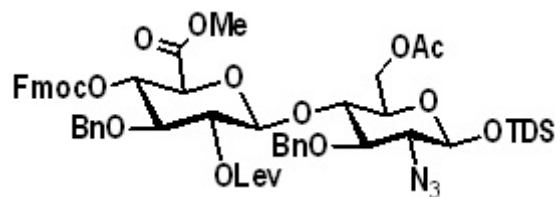
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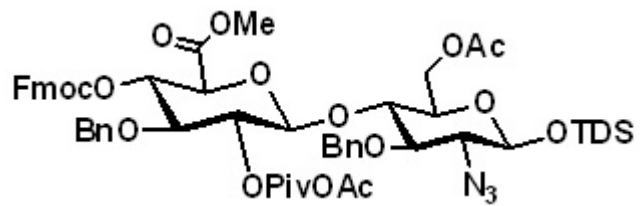
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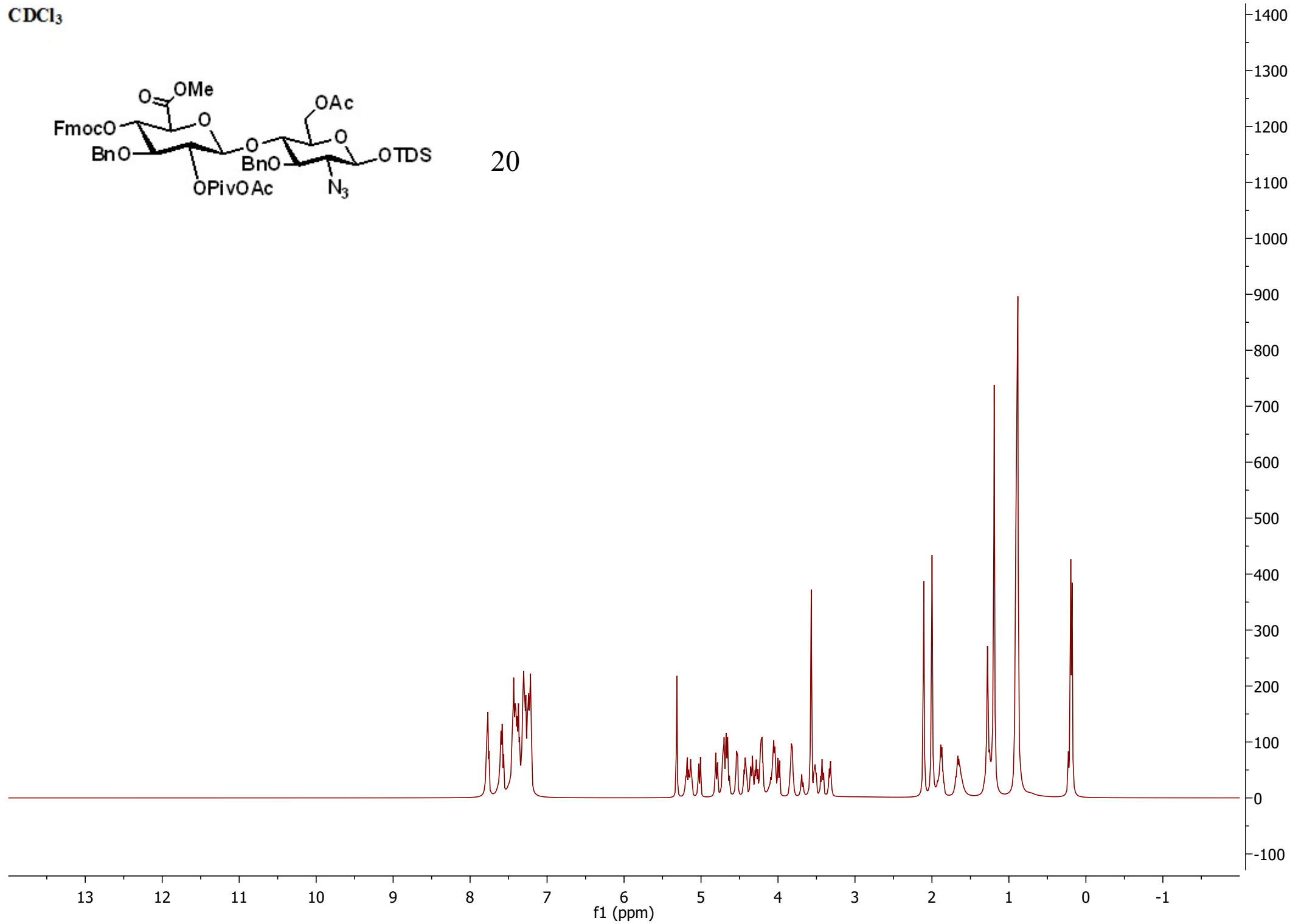
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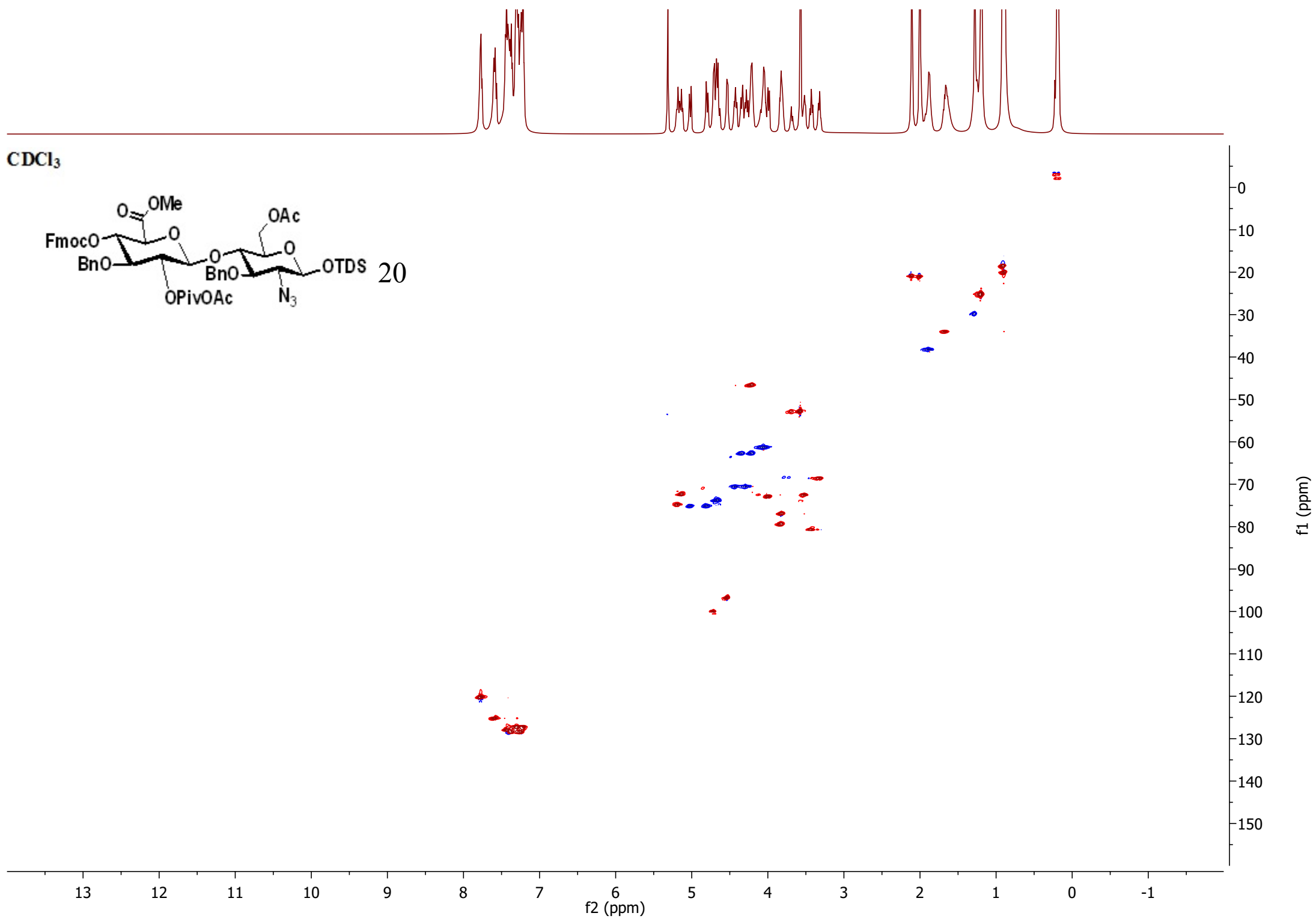
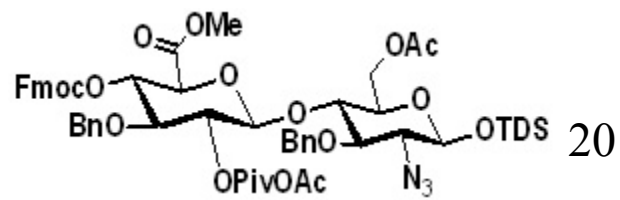
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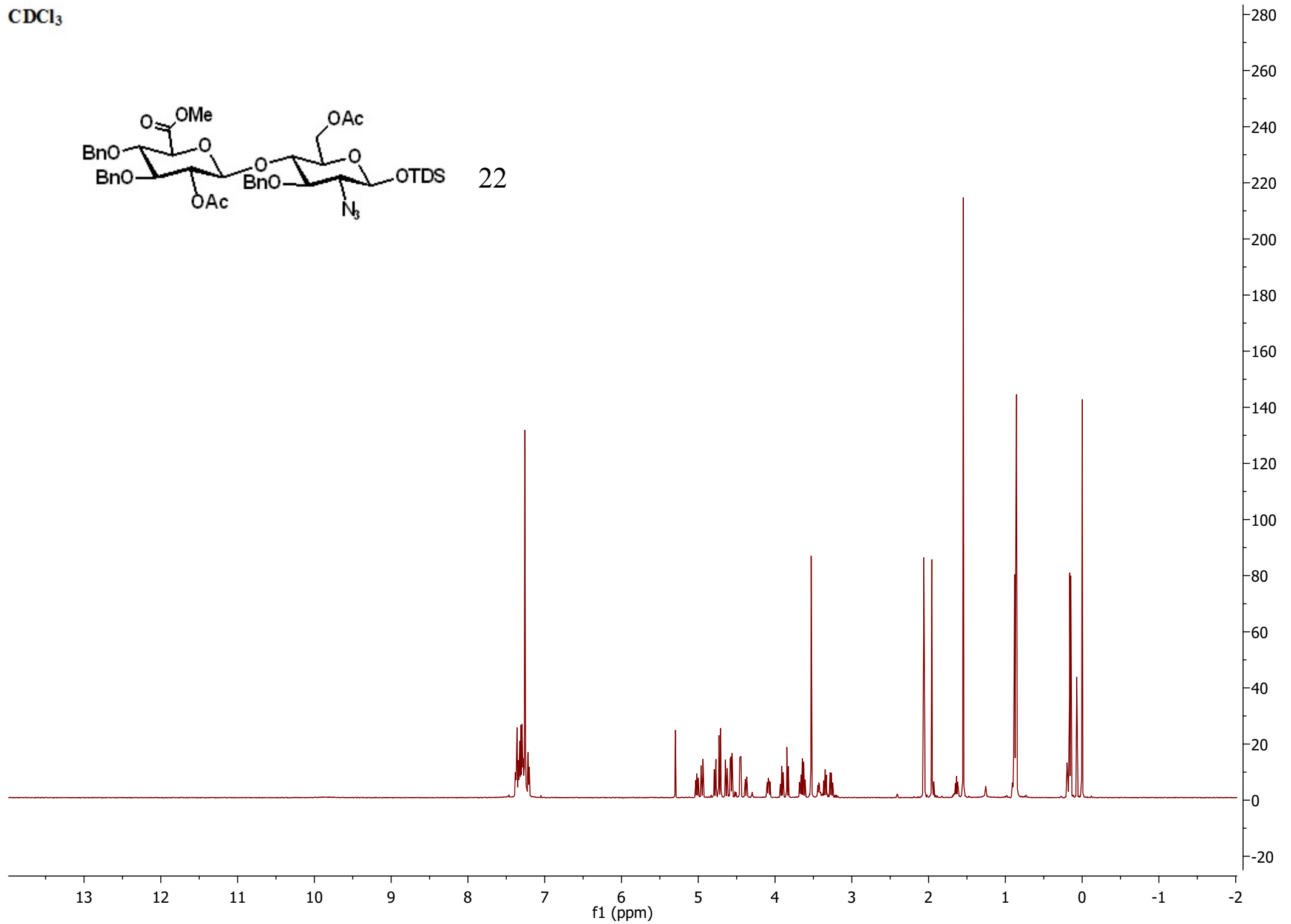
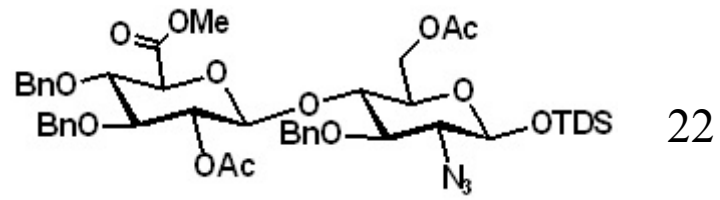
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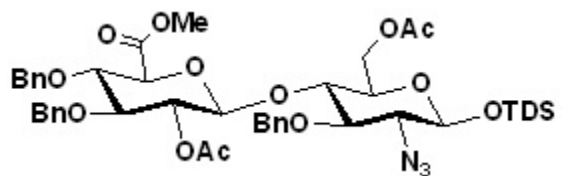
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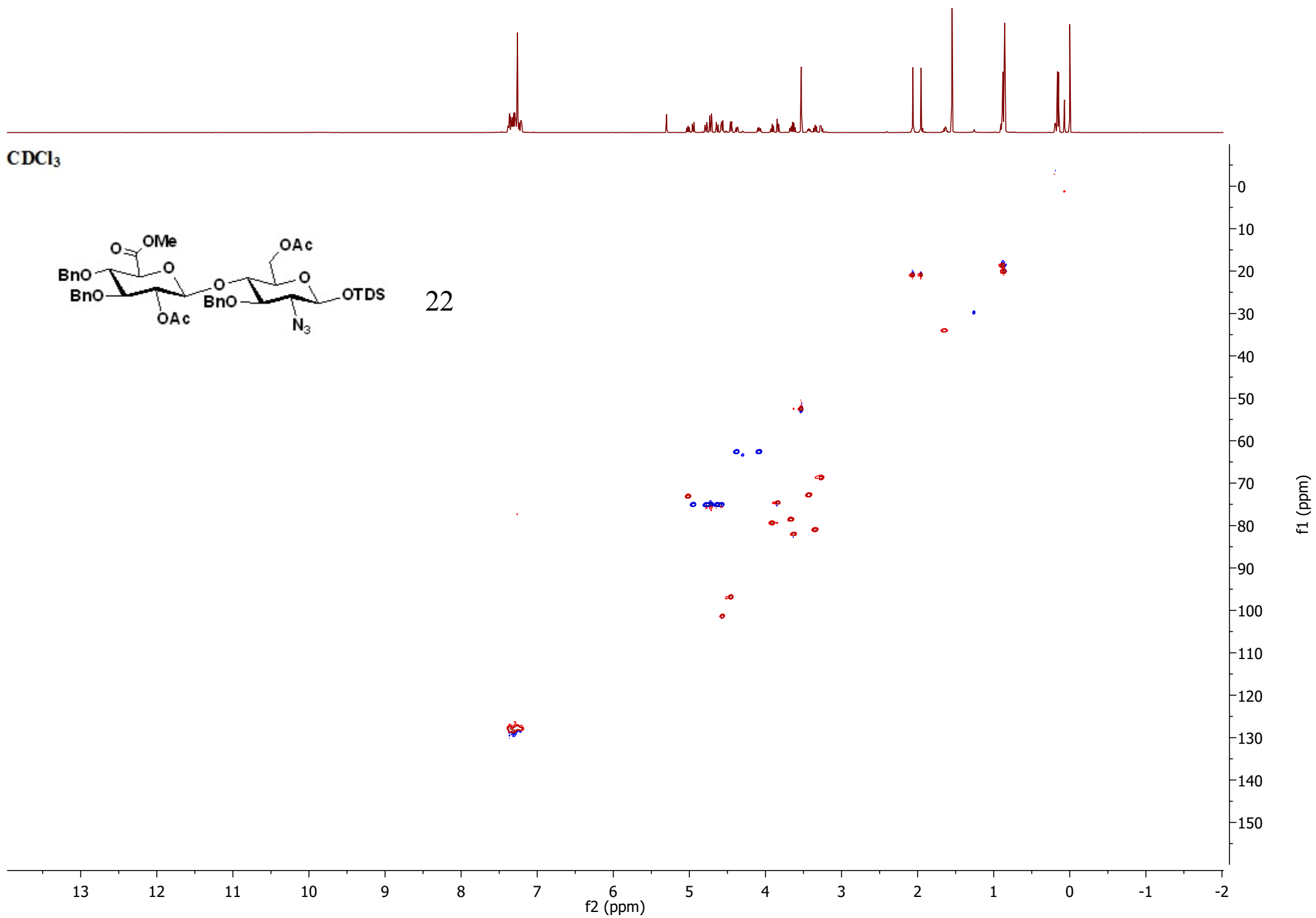
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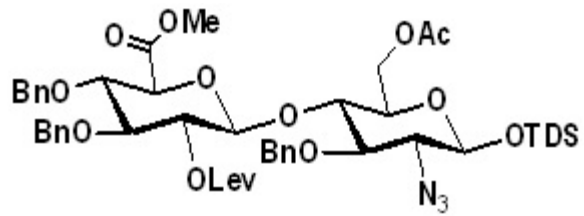
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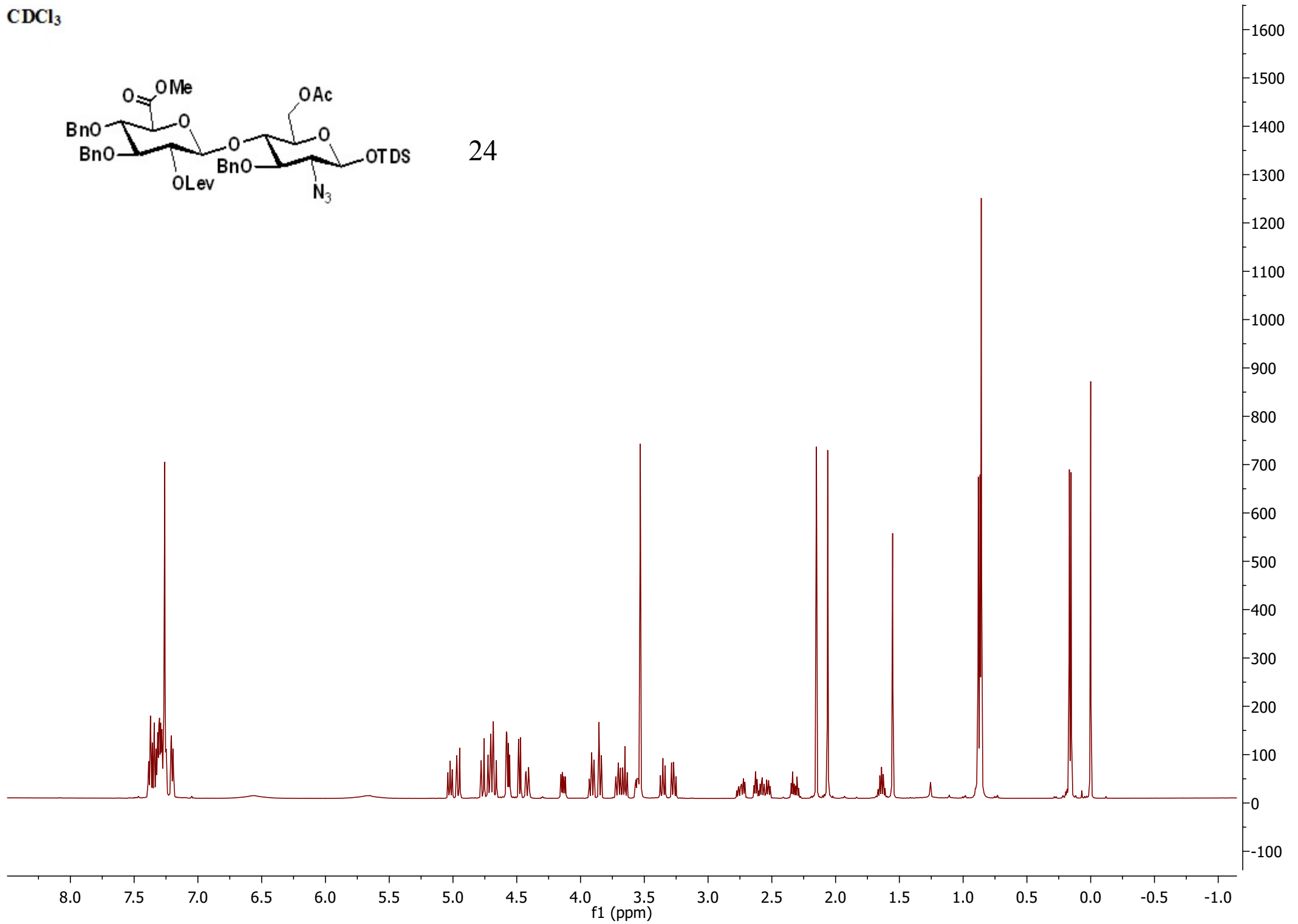
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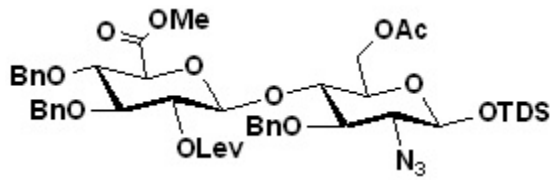
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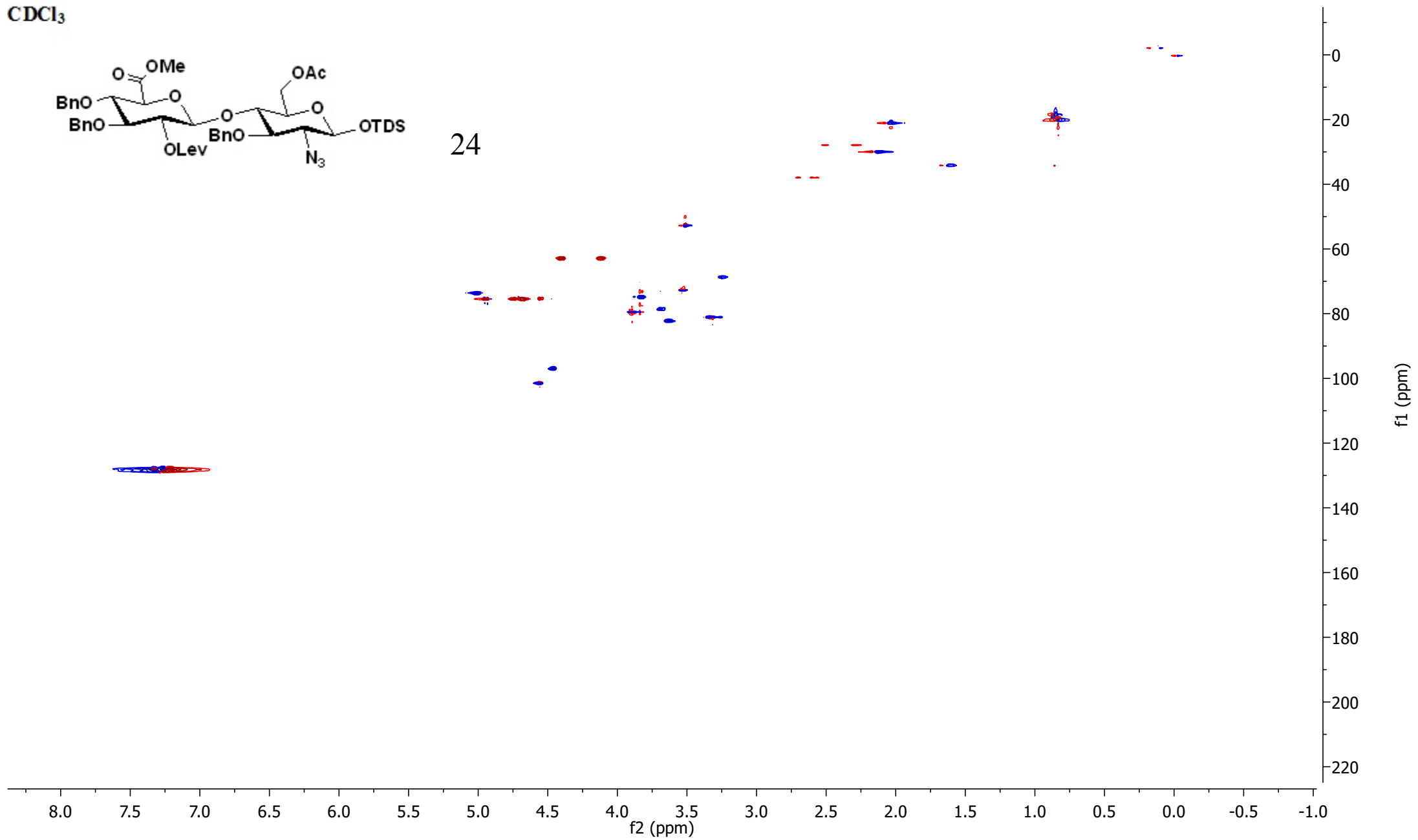
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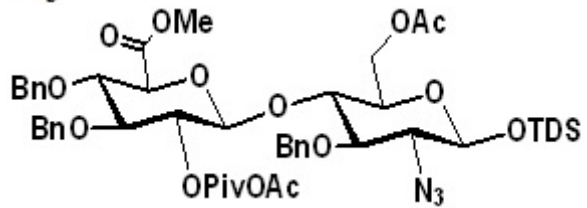
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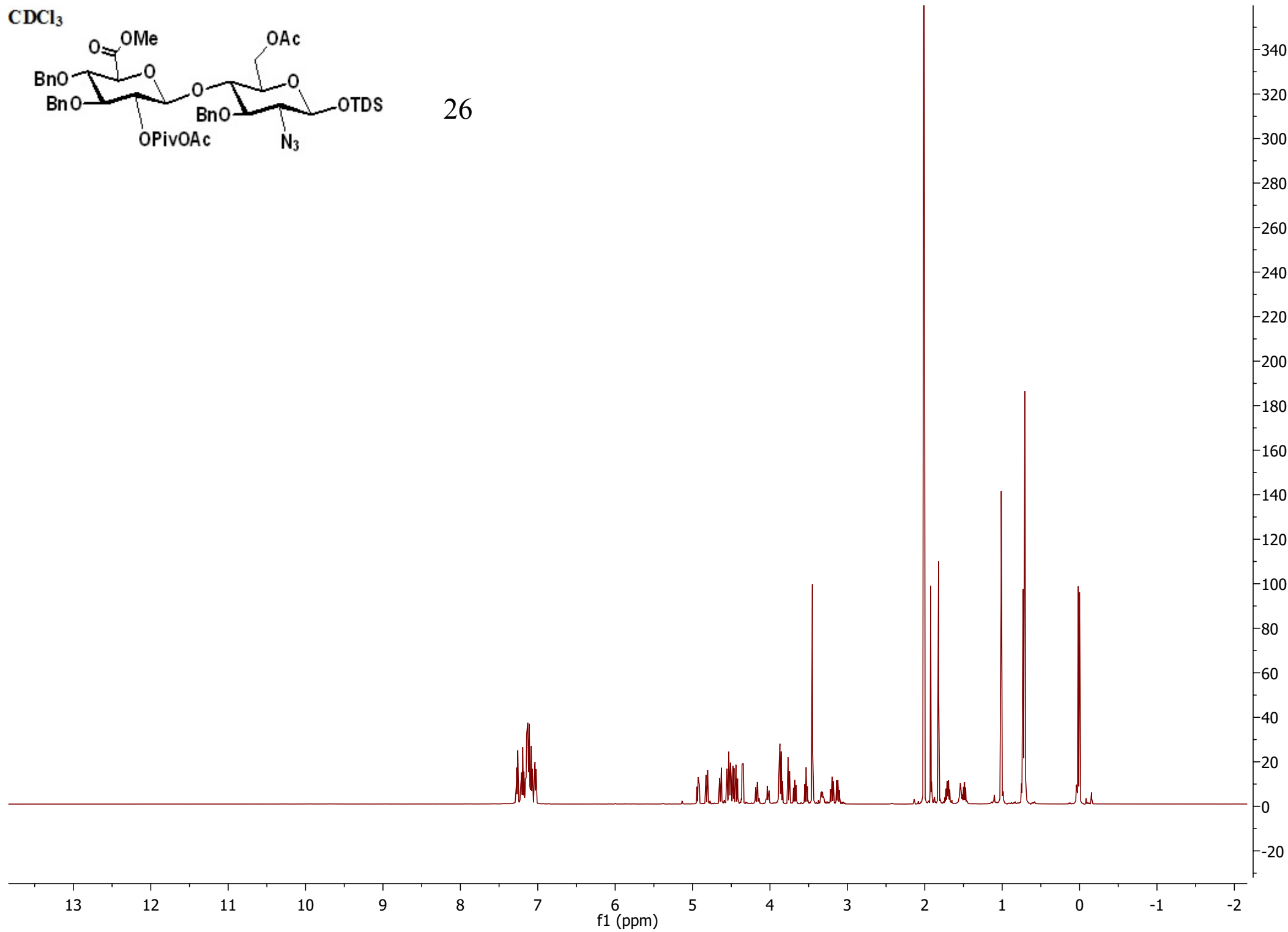
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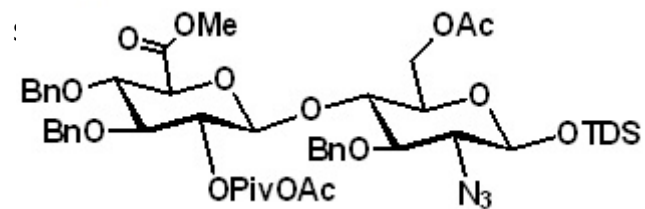
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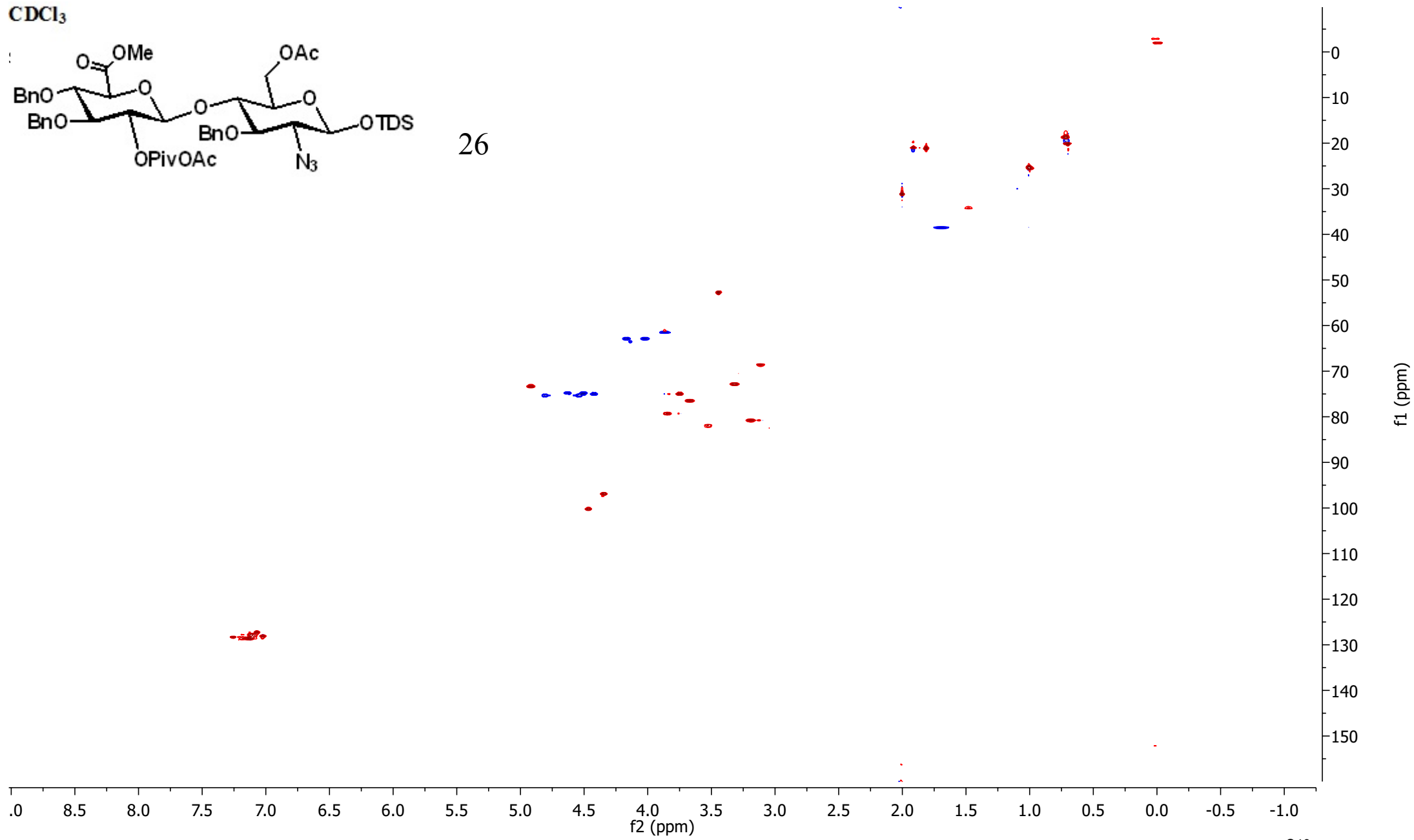
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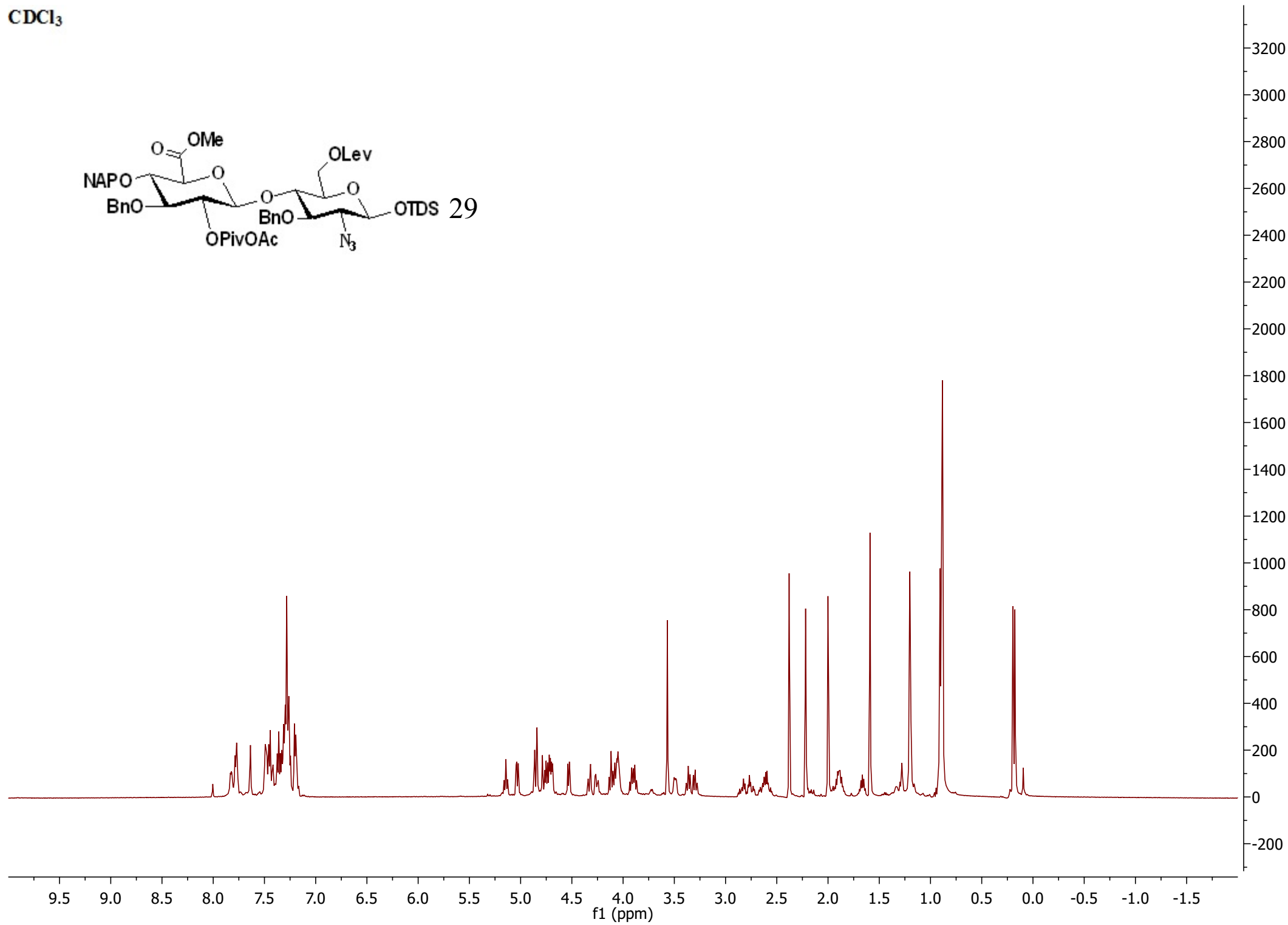
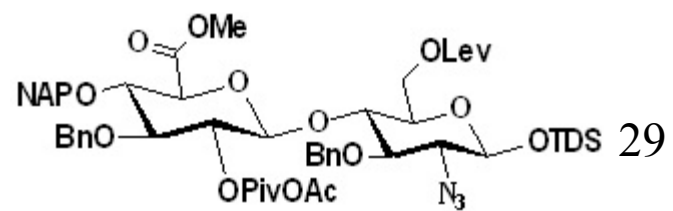
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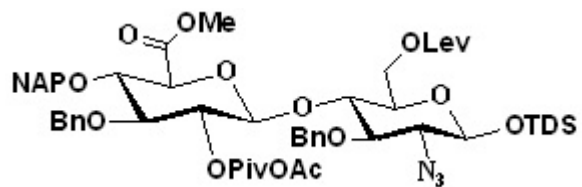
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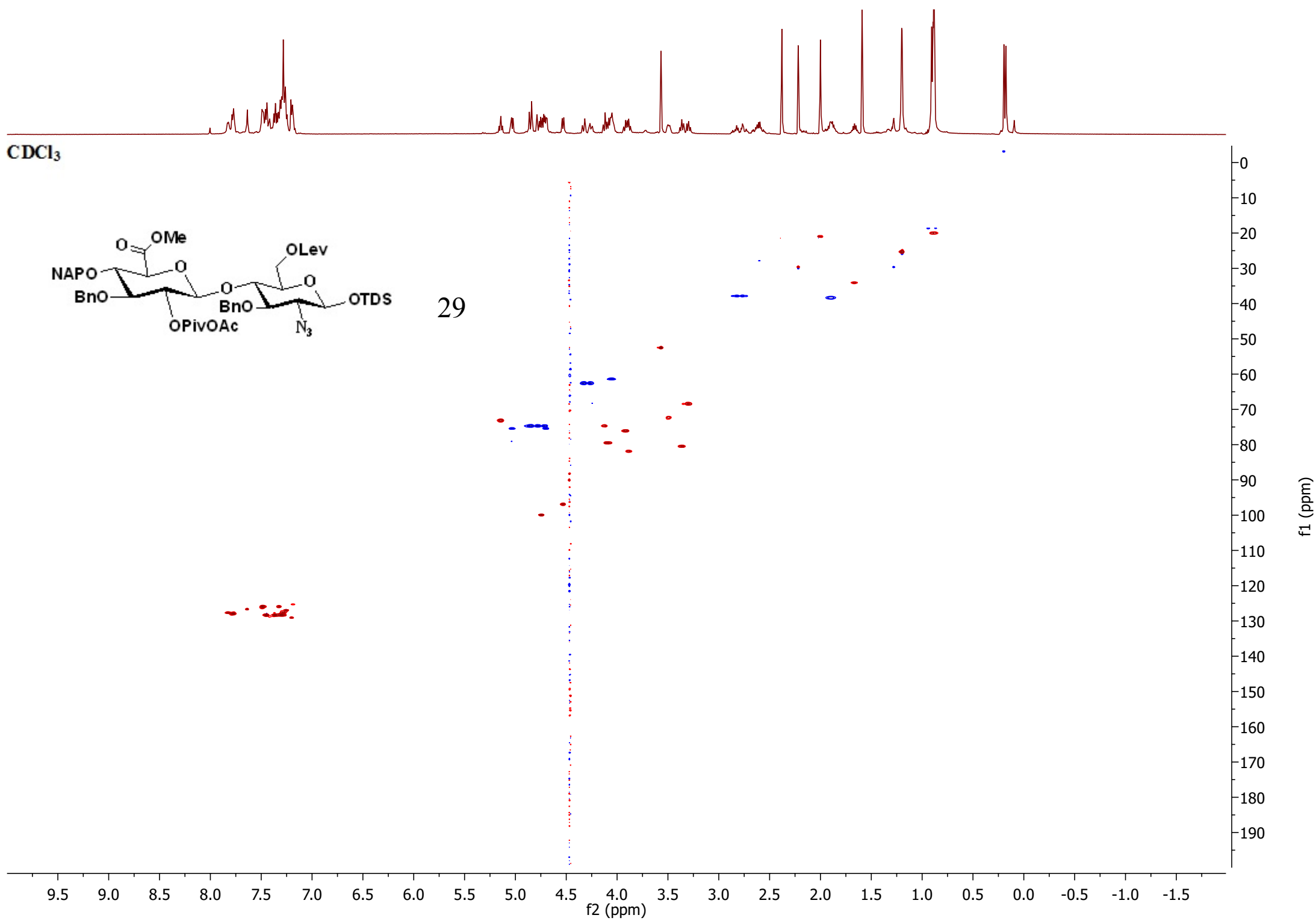
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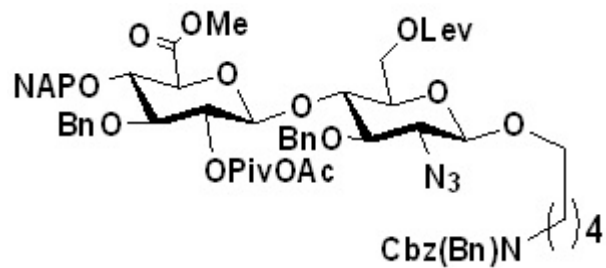
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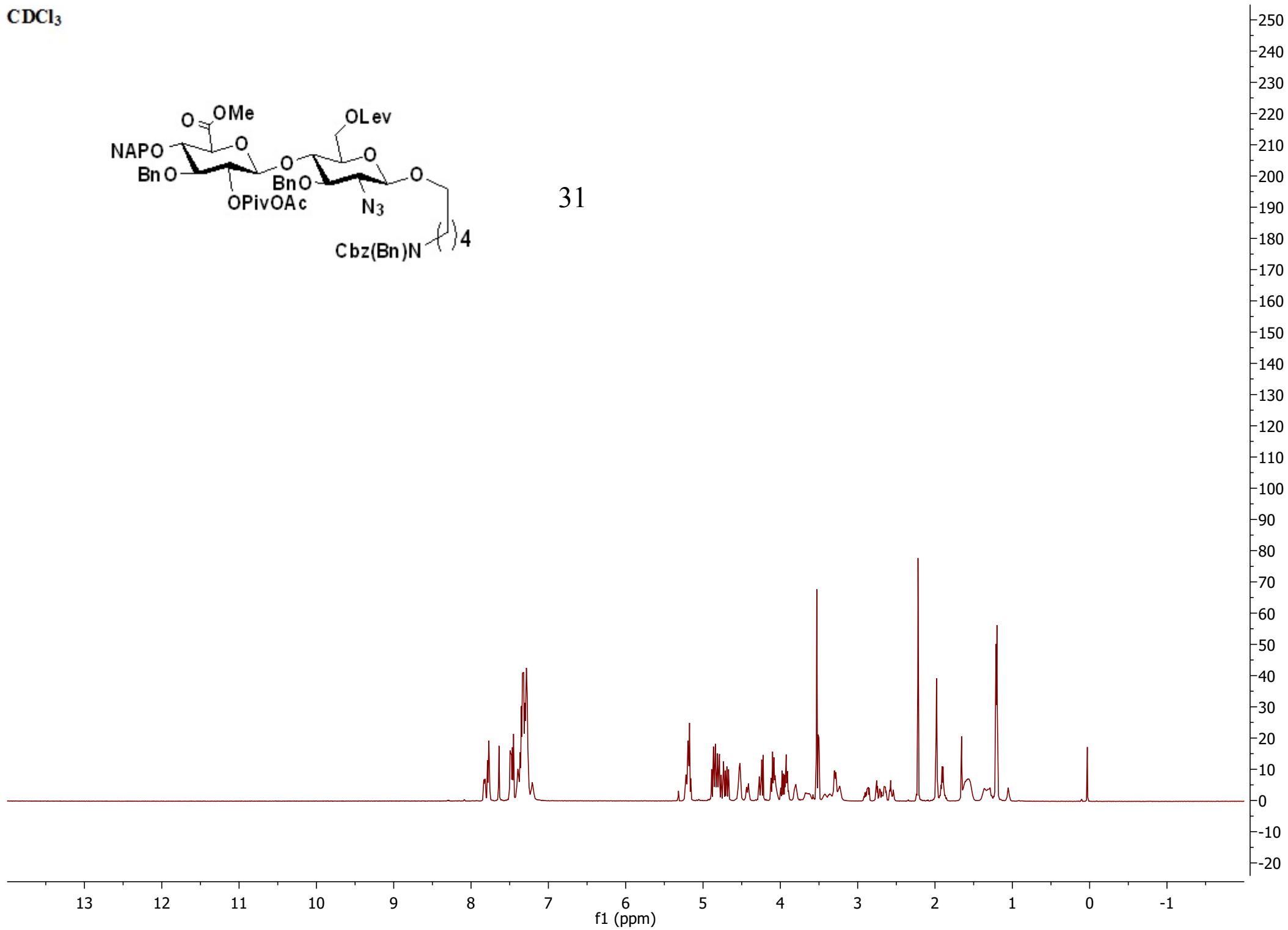
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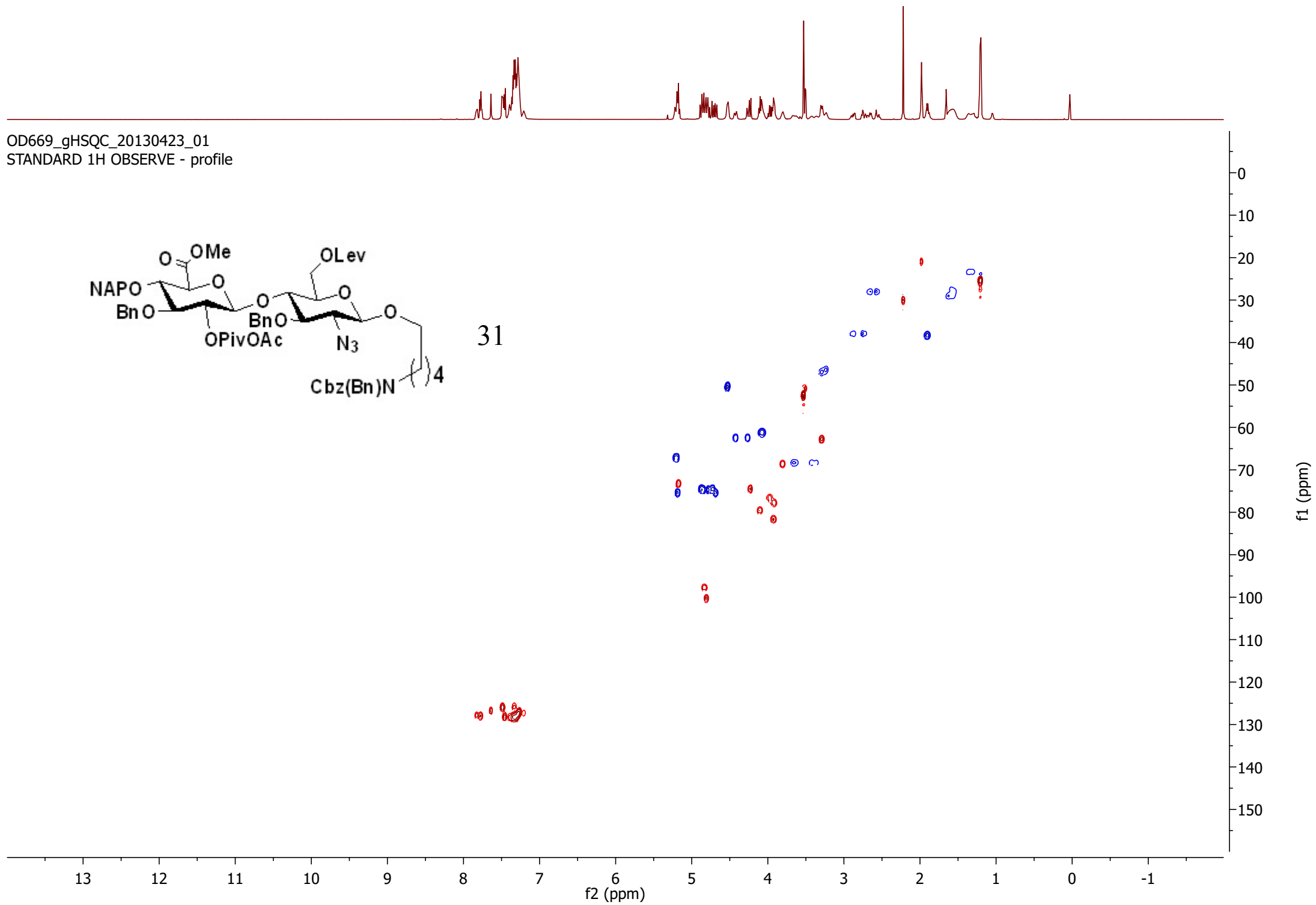
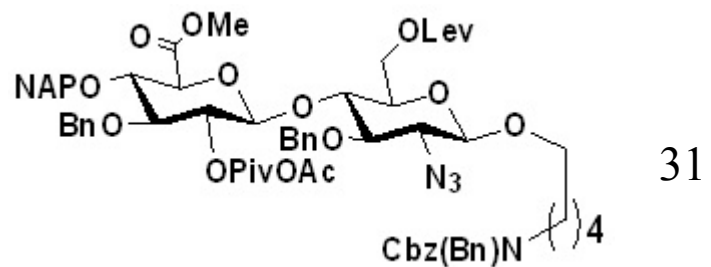
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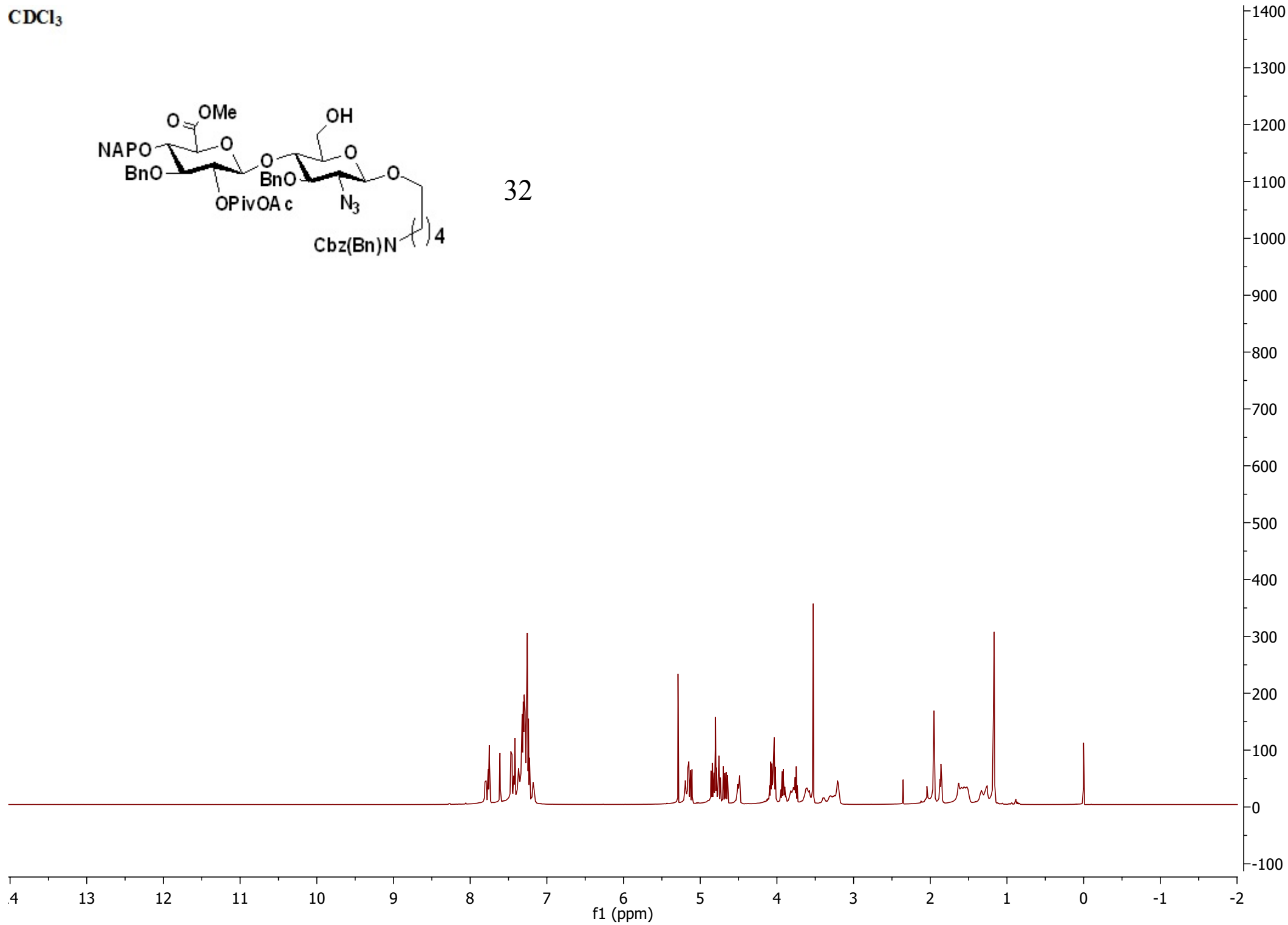
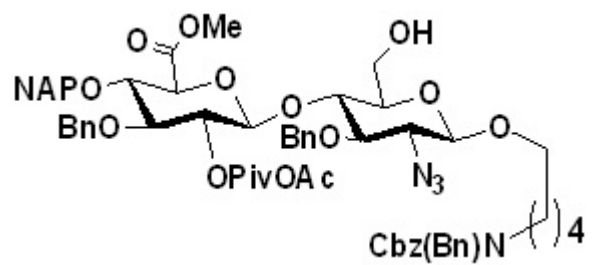
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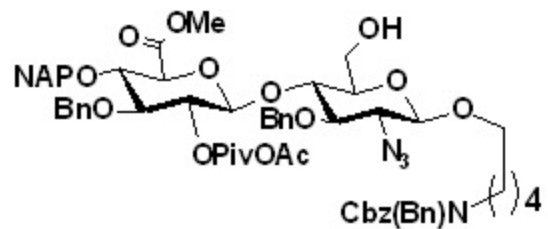
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STANDARD 1H OBSERVE - profile



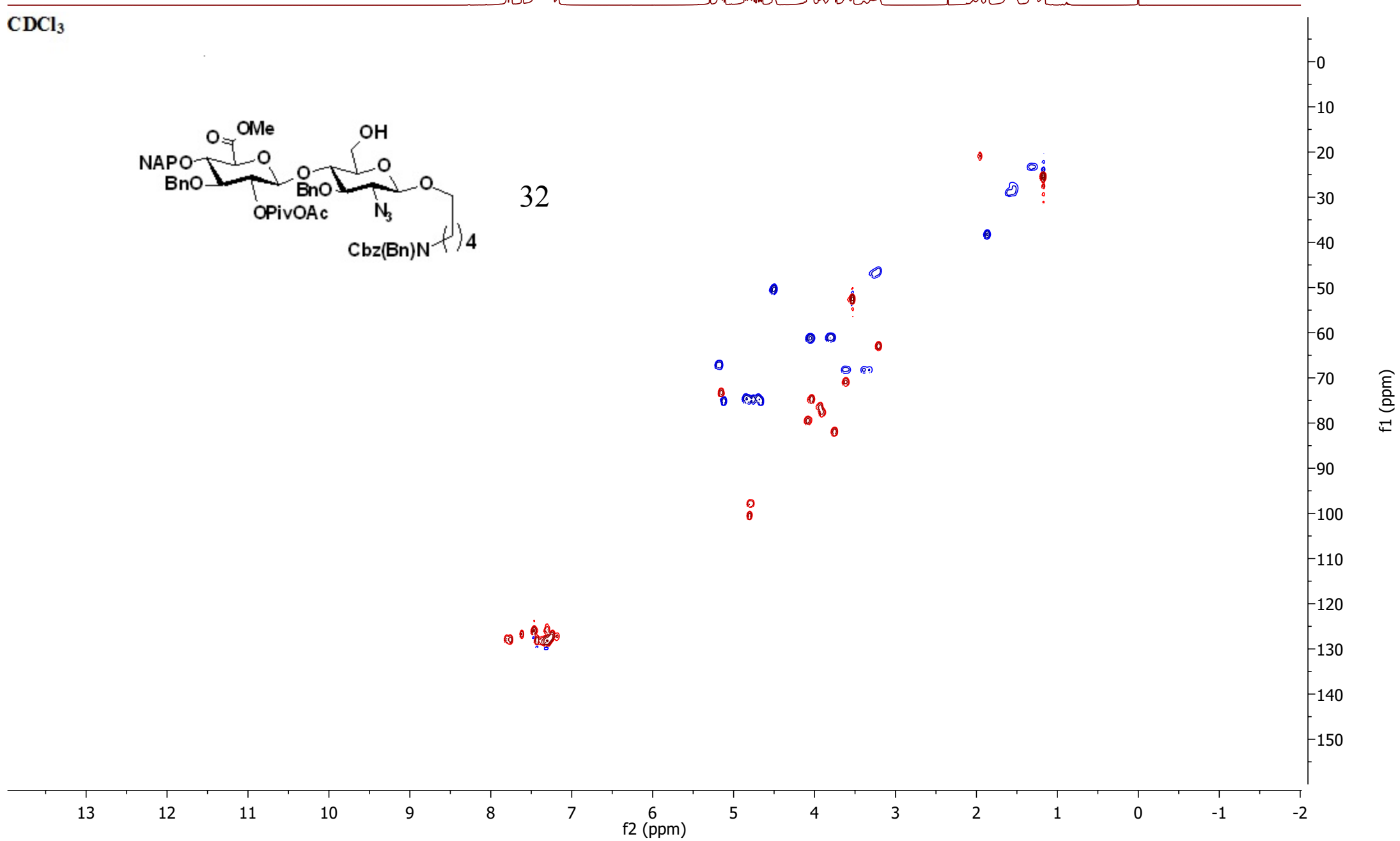
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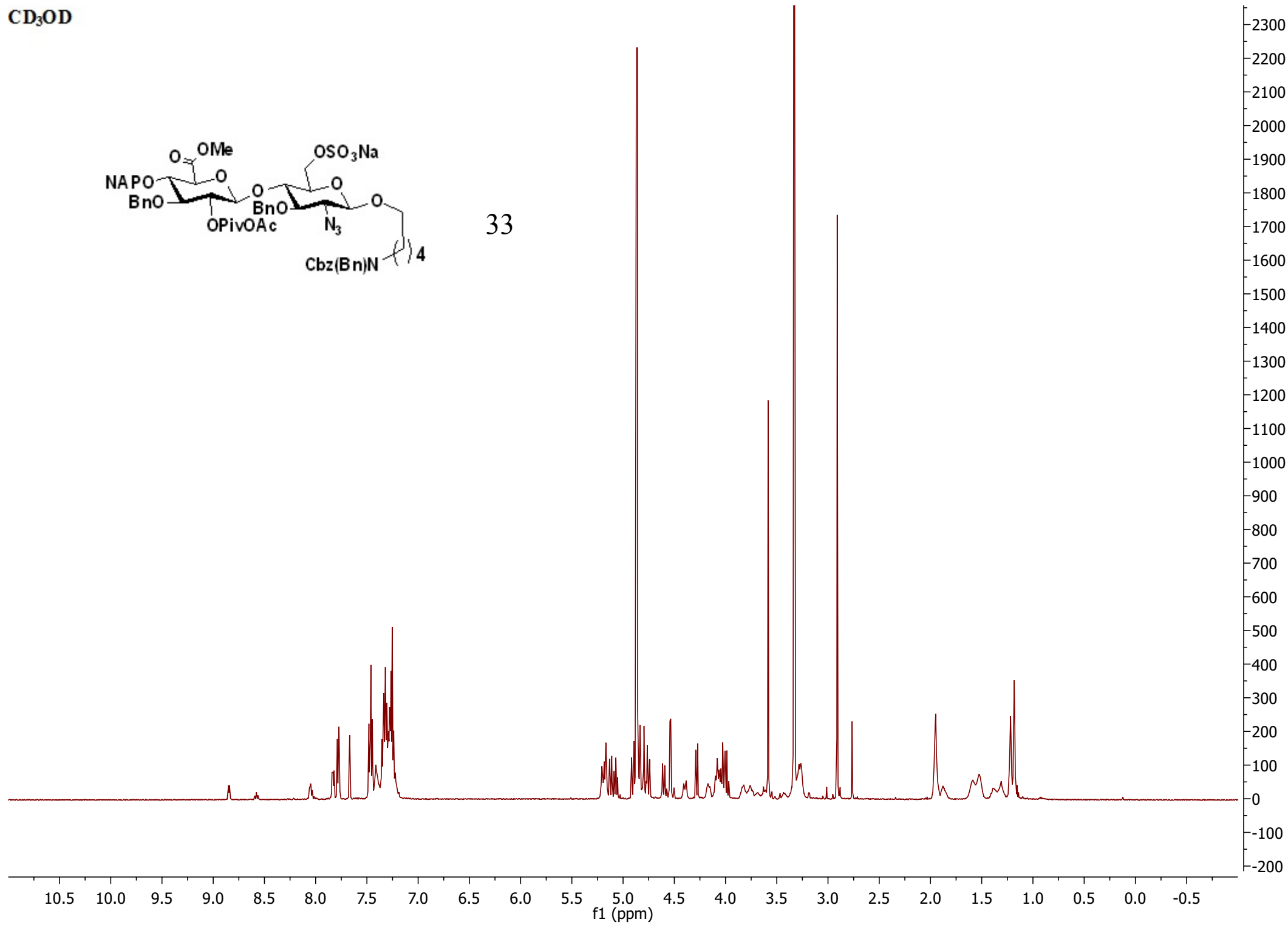
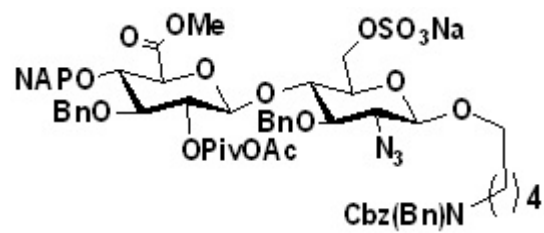
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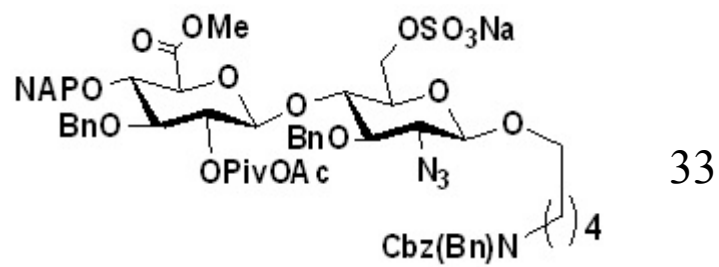
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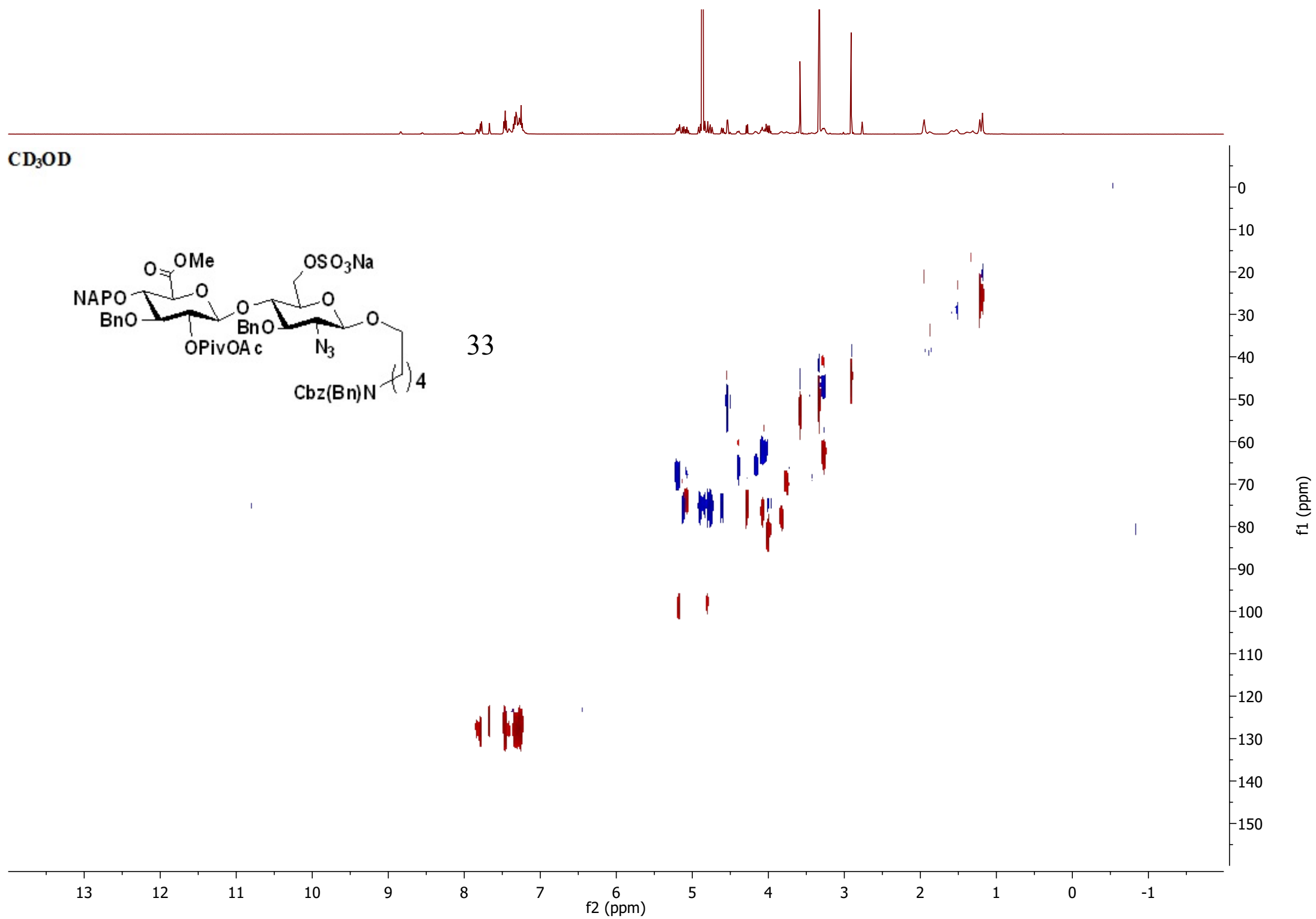
CD₃OD



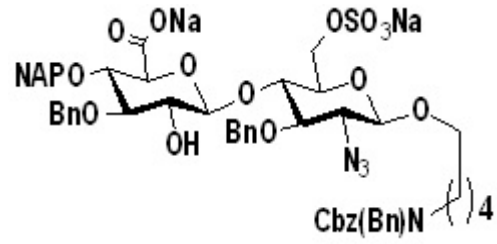
CD₃OD



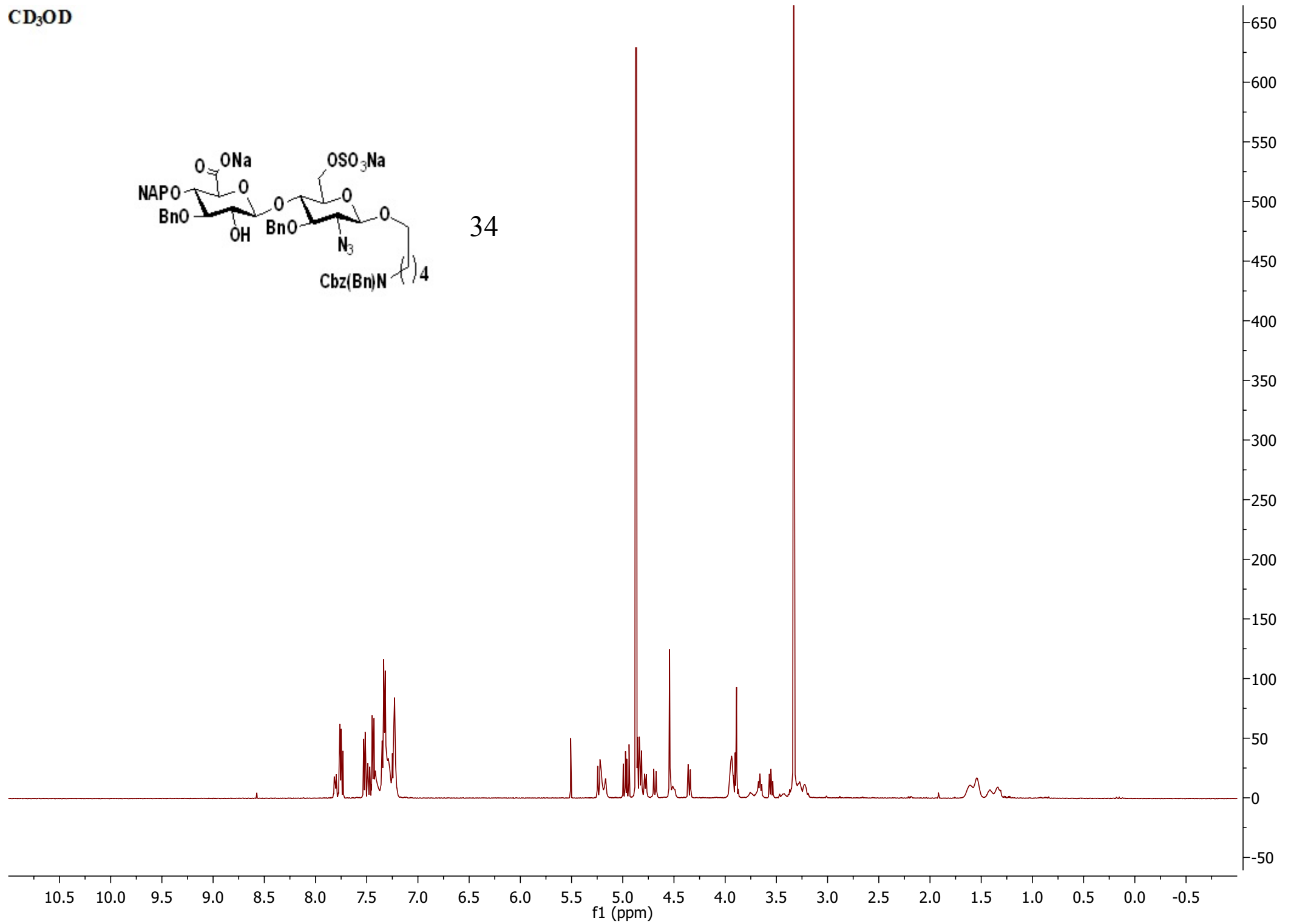
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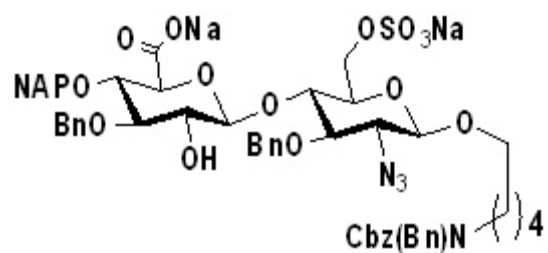
CD₃OD



34



CD₃OD

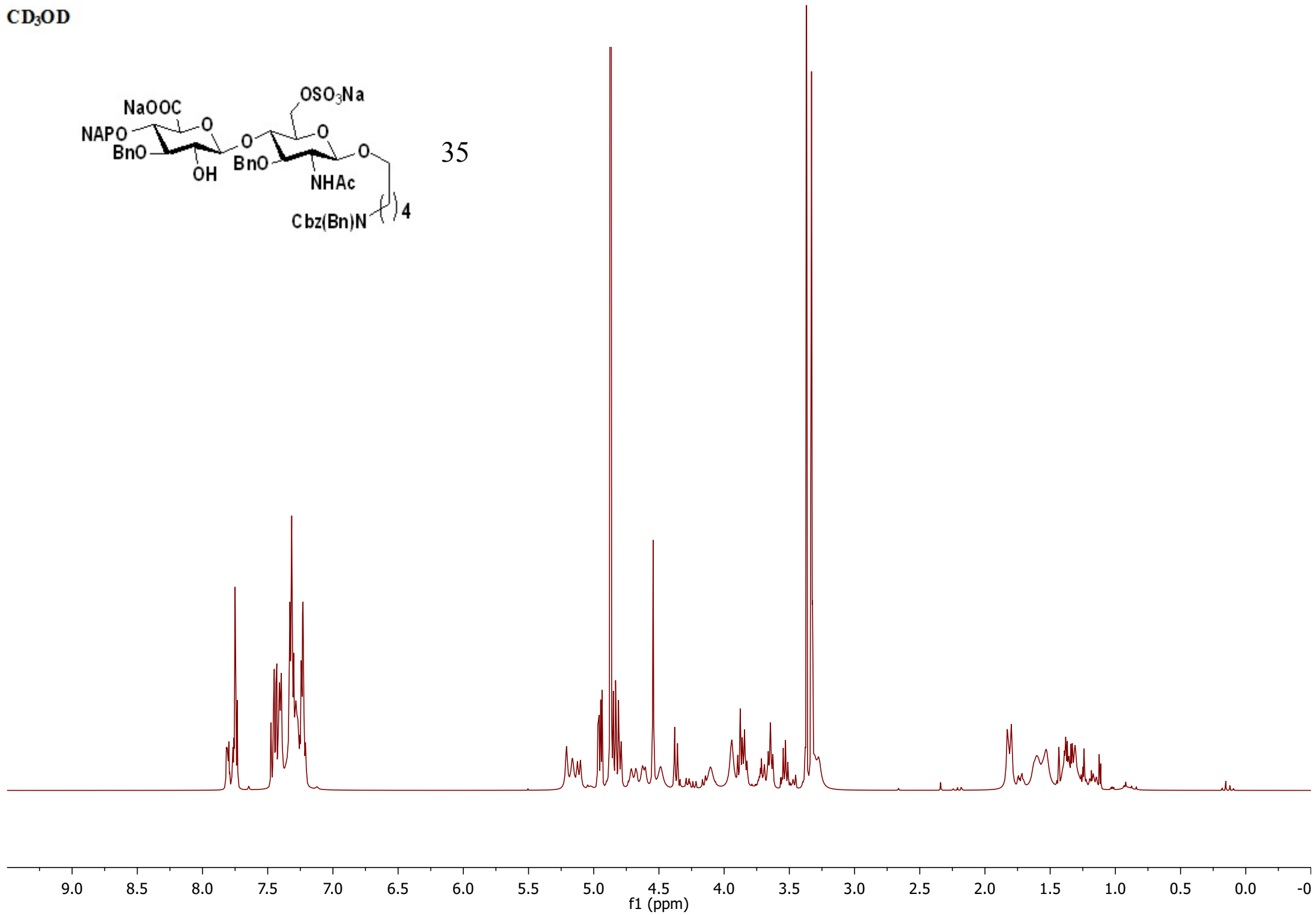
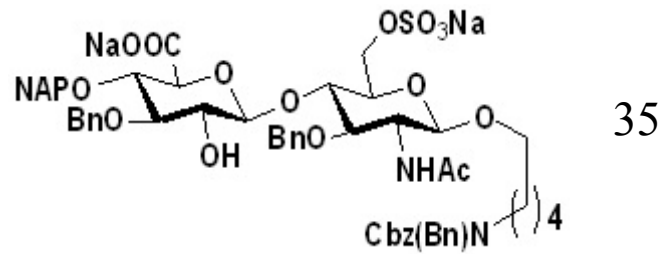


10.5 10.0 9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0 -0.5

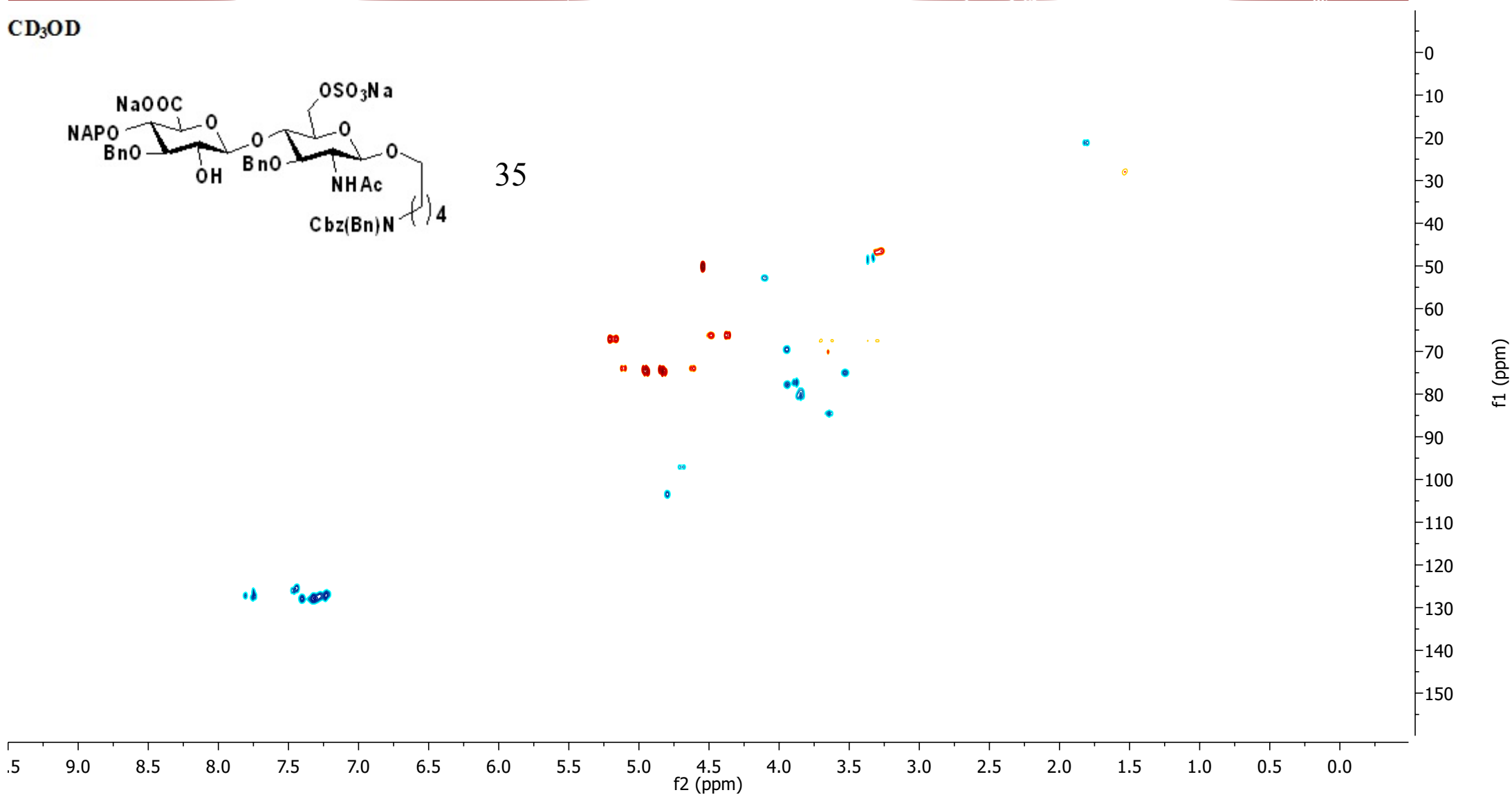
f2 (ppm)

f1 (ppm)

CD₃OD

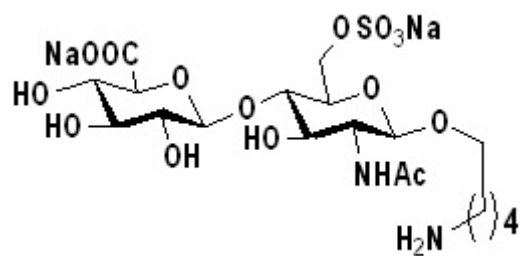


CD₃OD

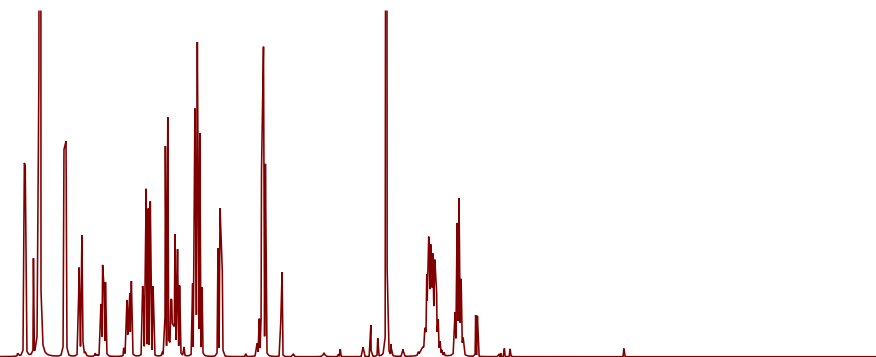


35

D₂O



36

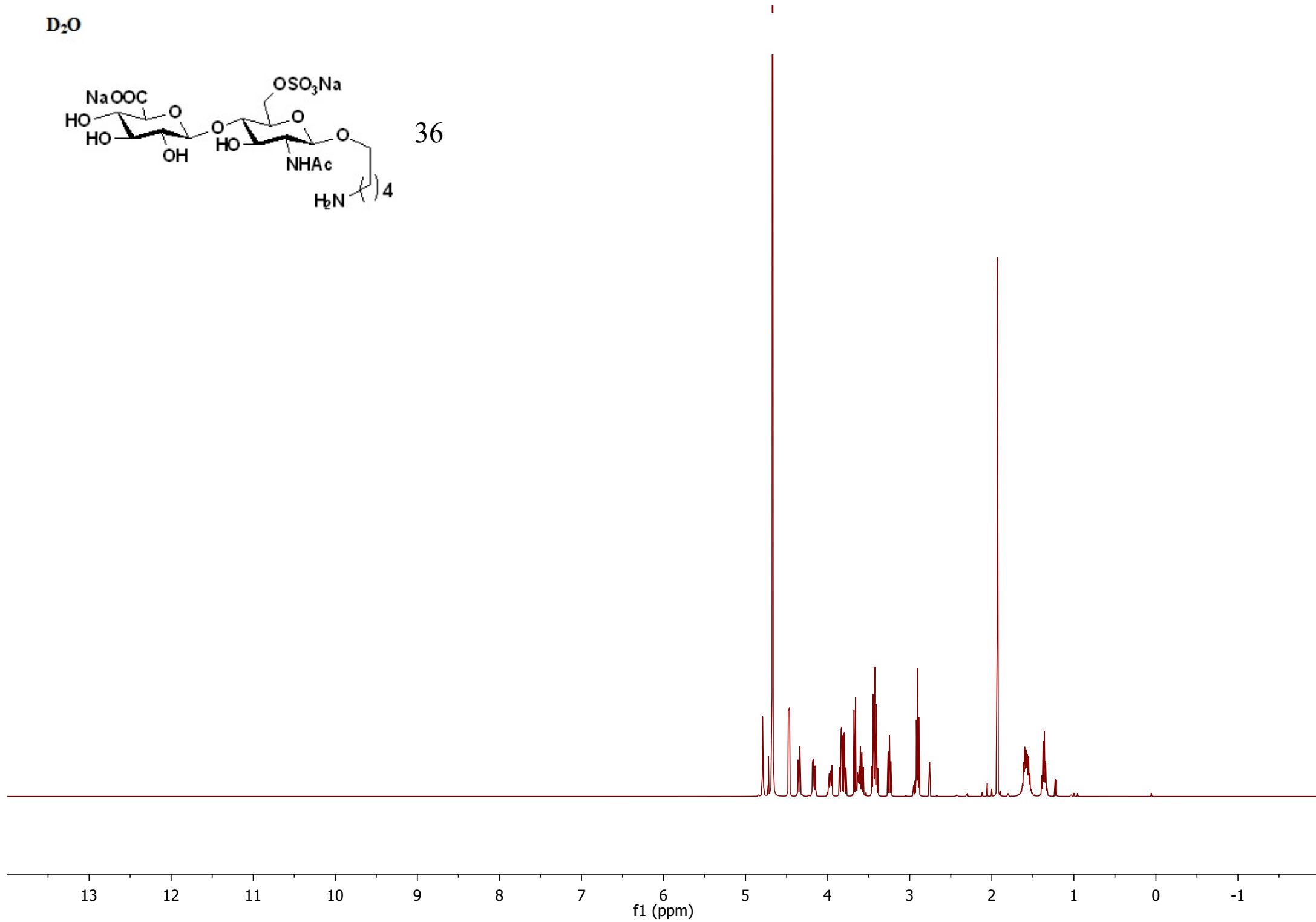
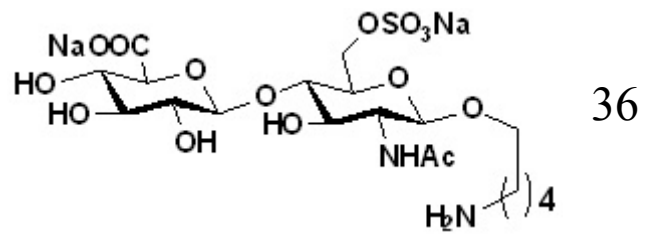


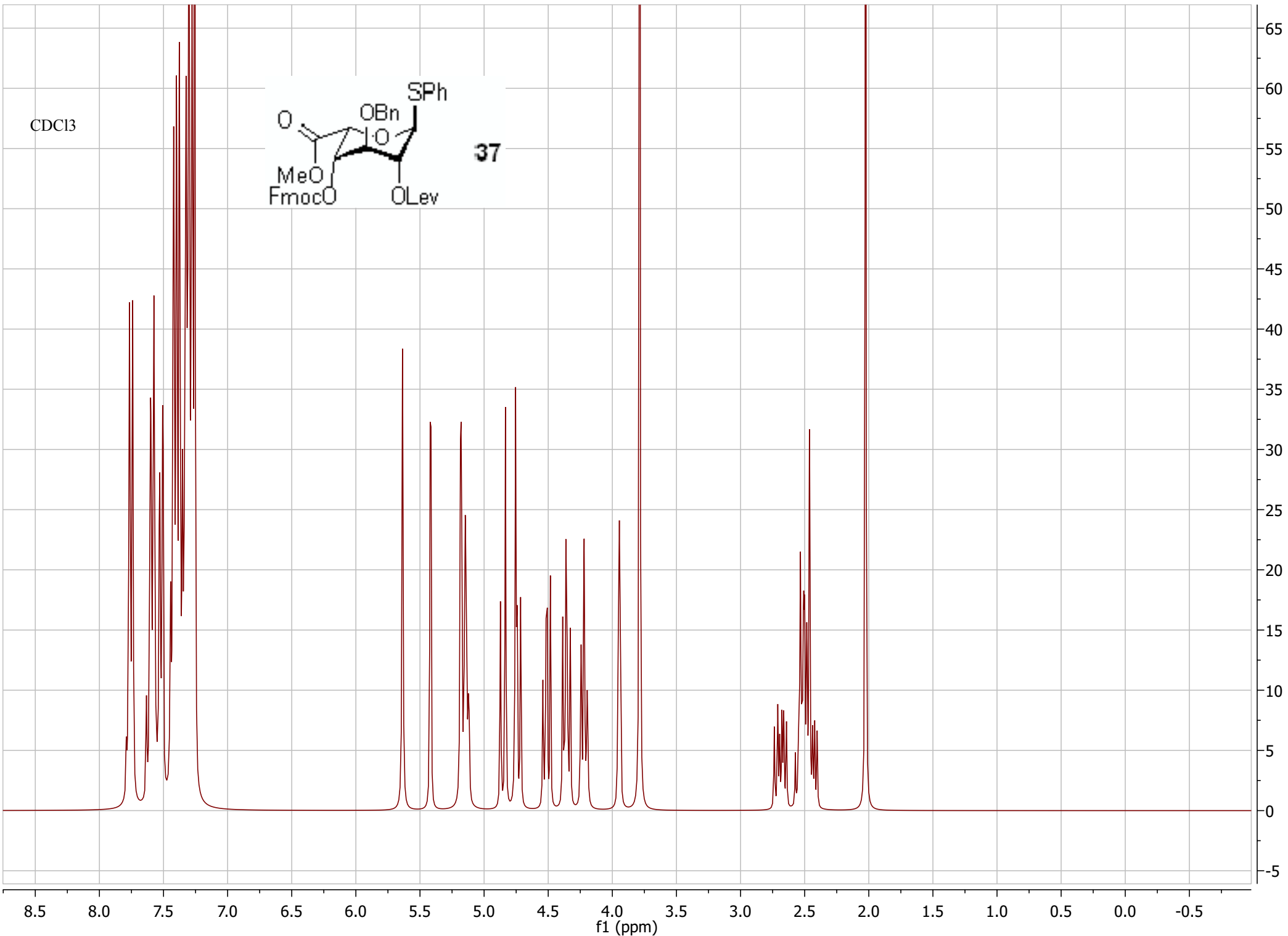
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f2 (ppm)

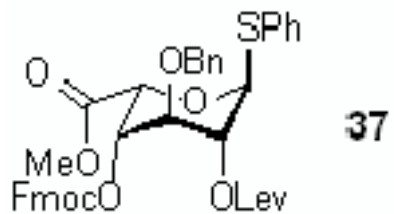
f1 (ppm)

D₂O

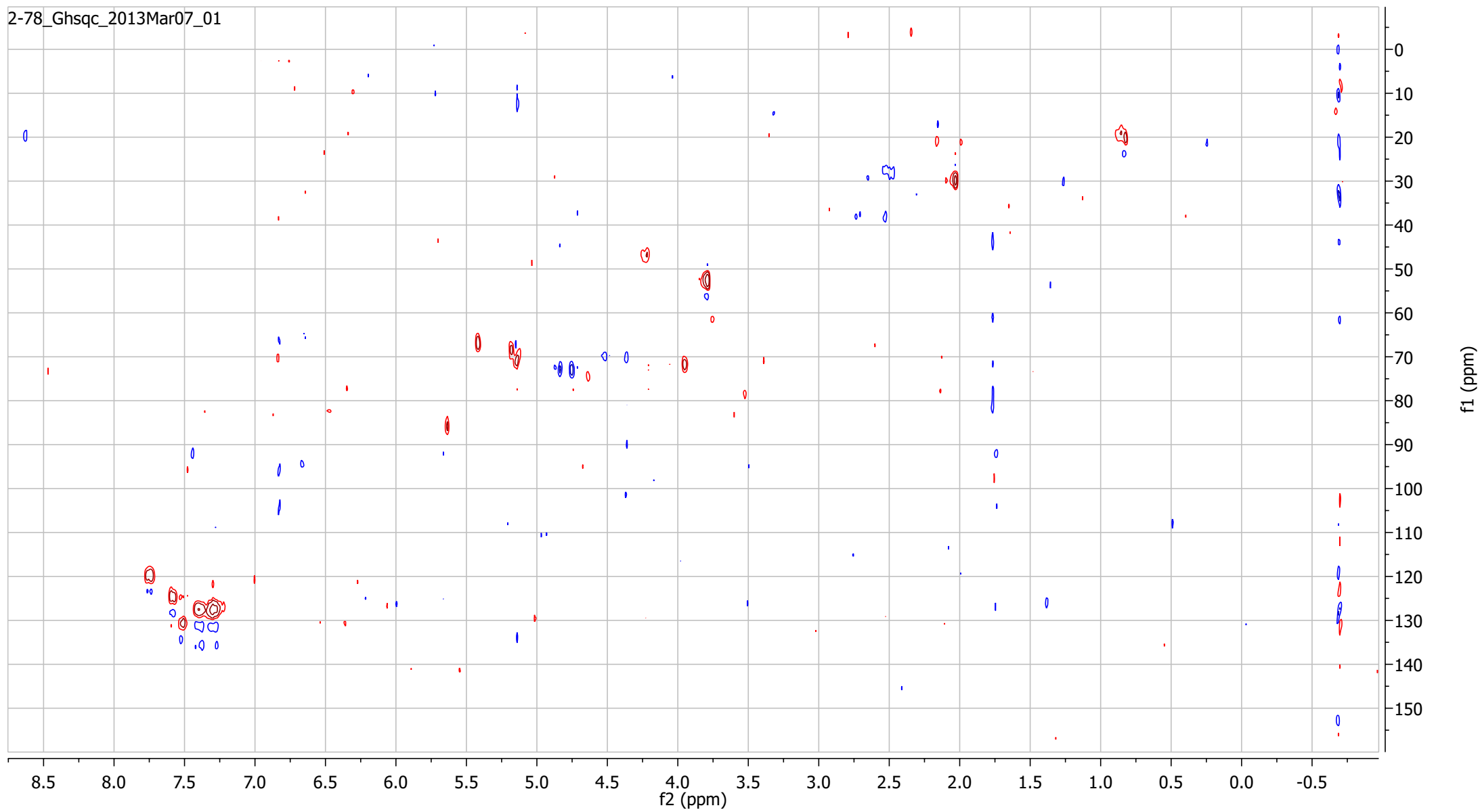


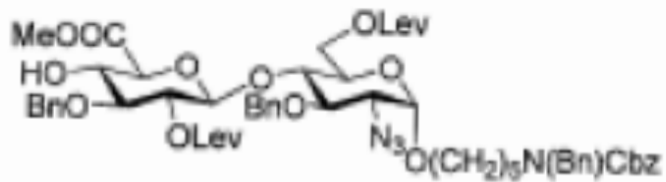


CDCl₃

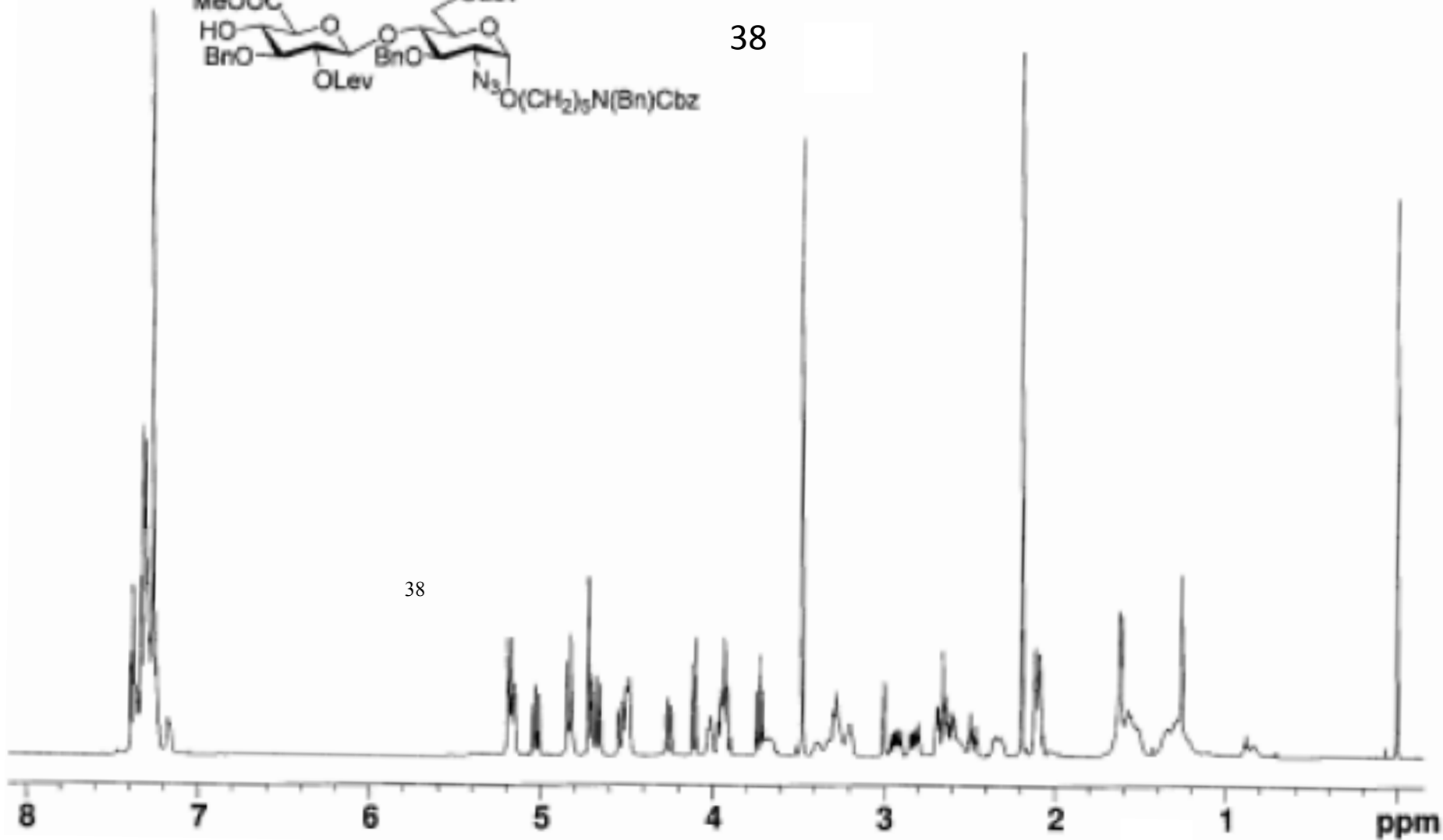


2-78_Ghsqc_2013Mar07_01



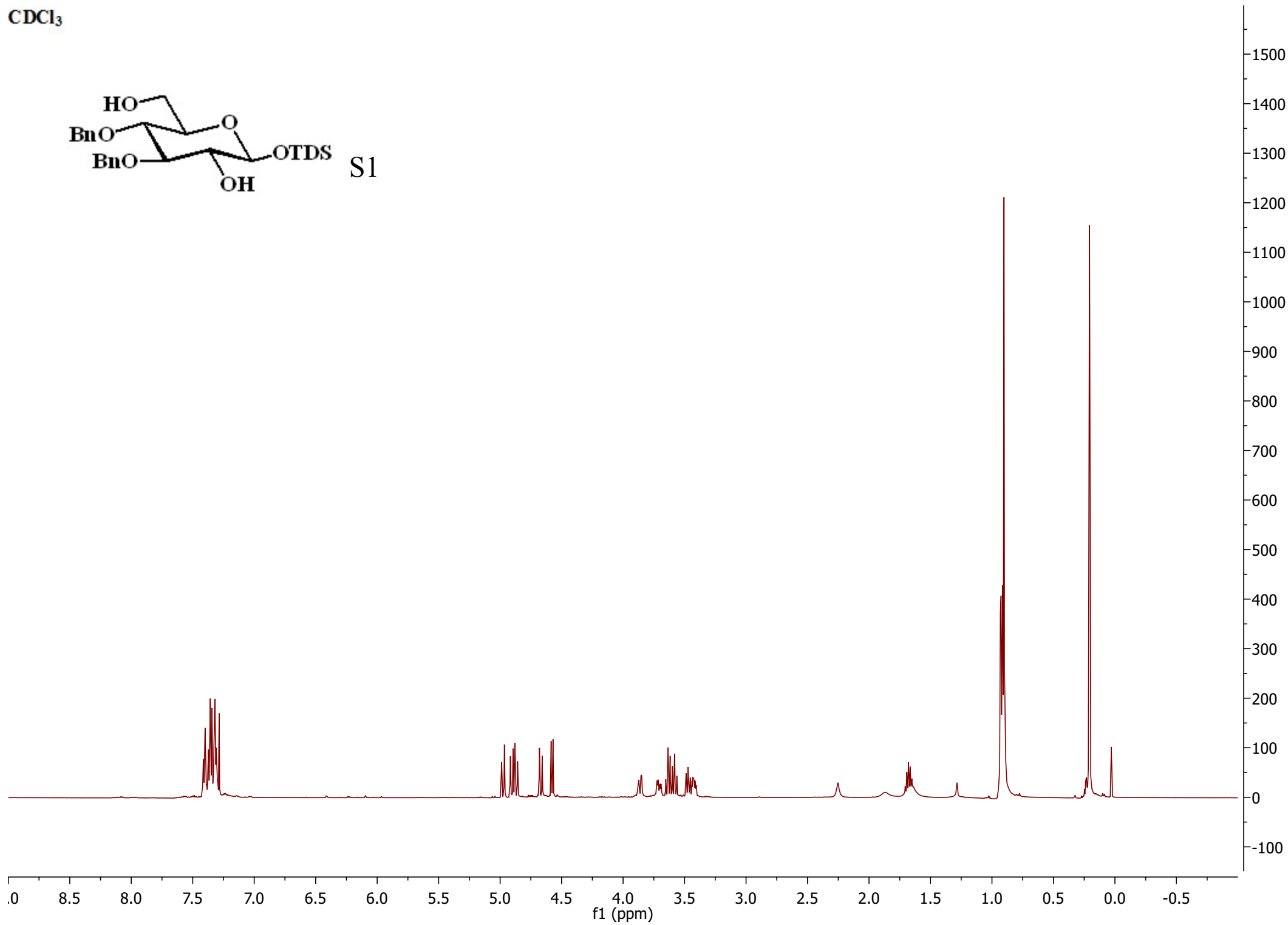
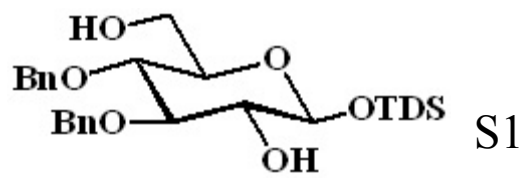


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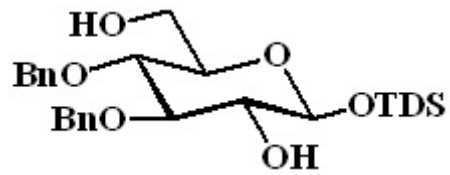


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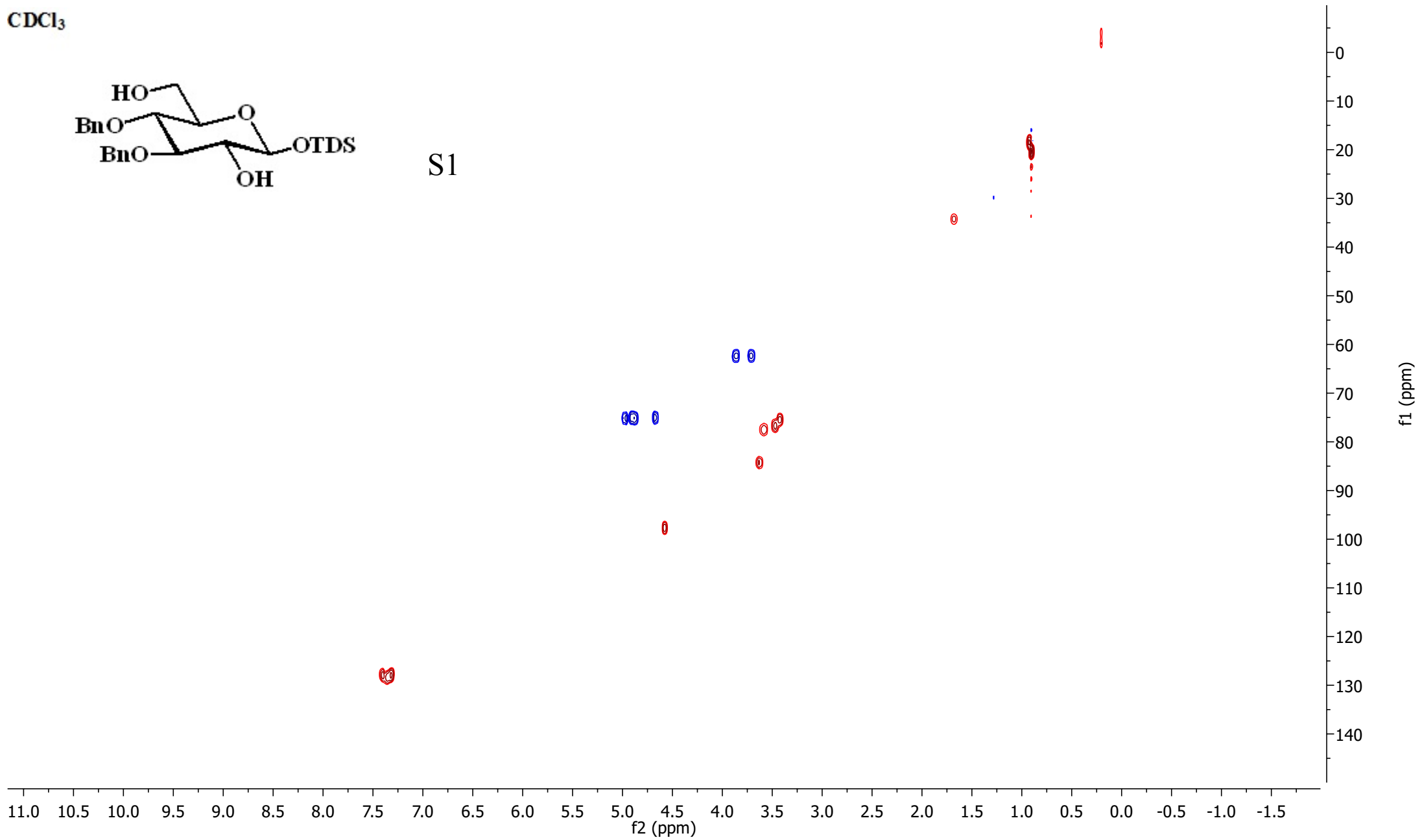
CDCl₃



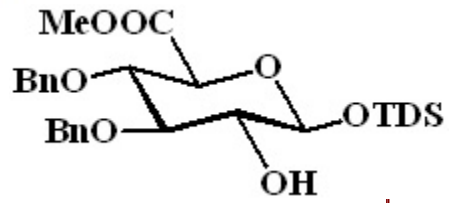
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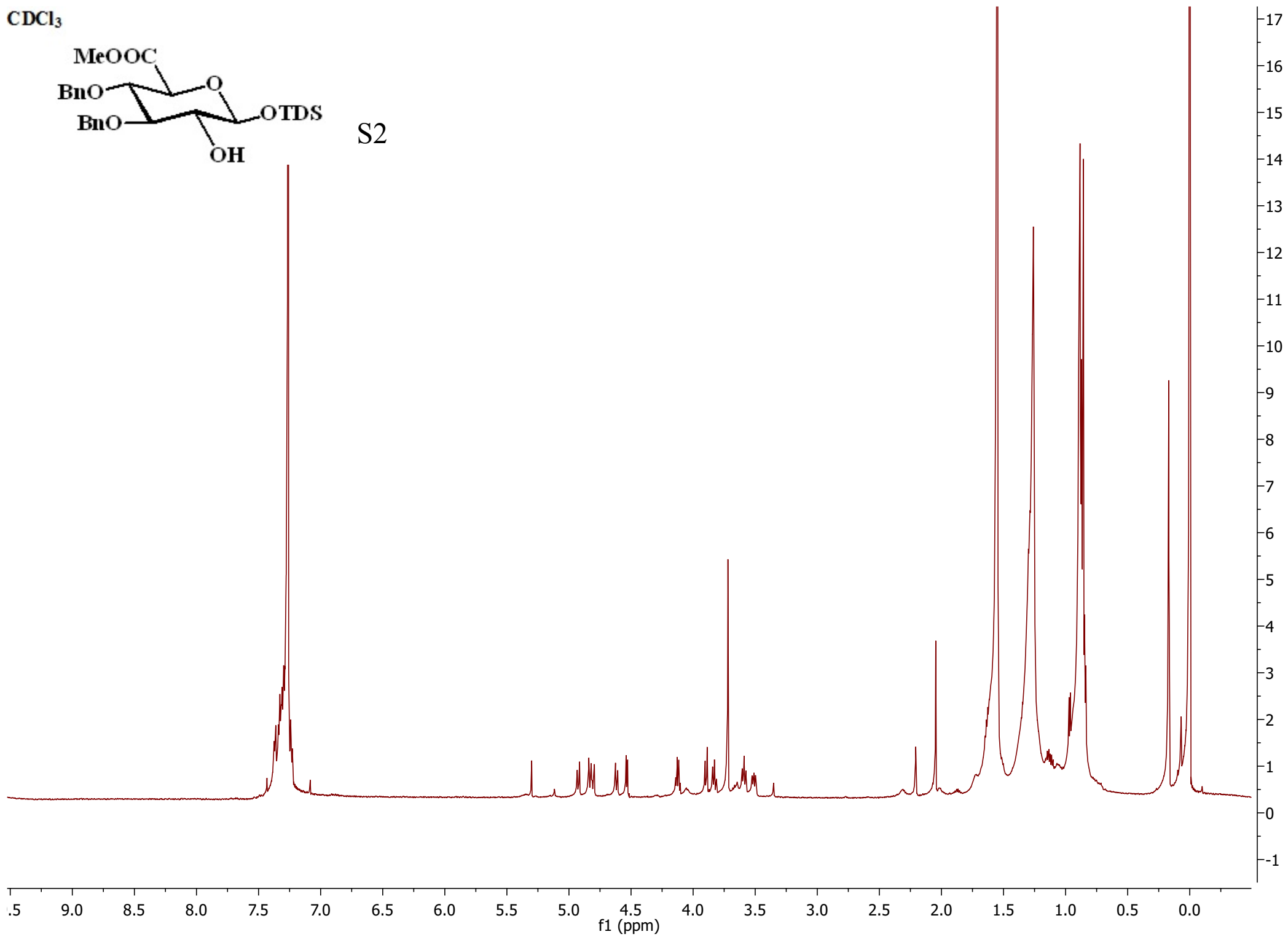
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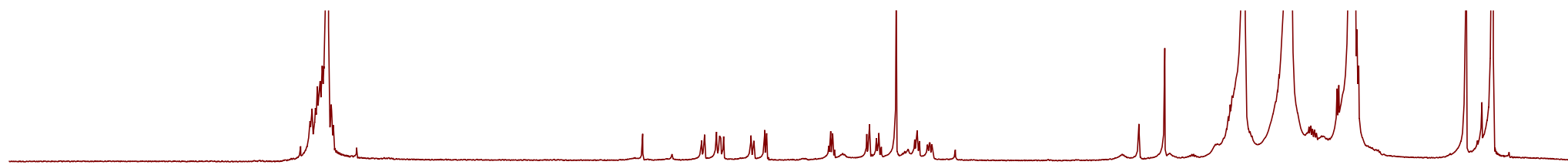


CDCl₃

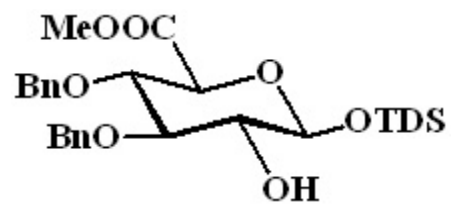


S2

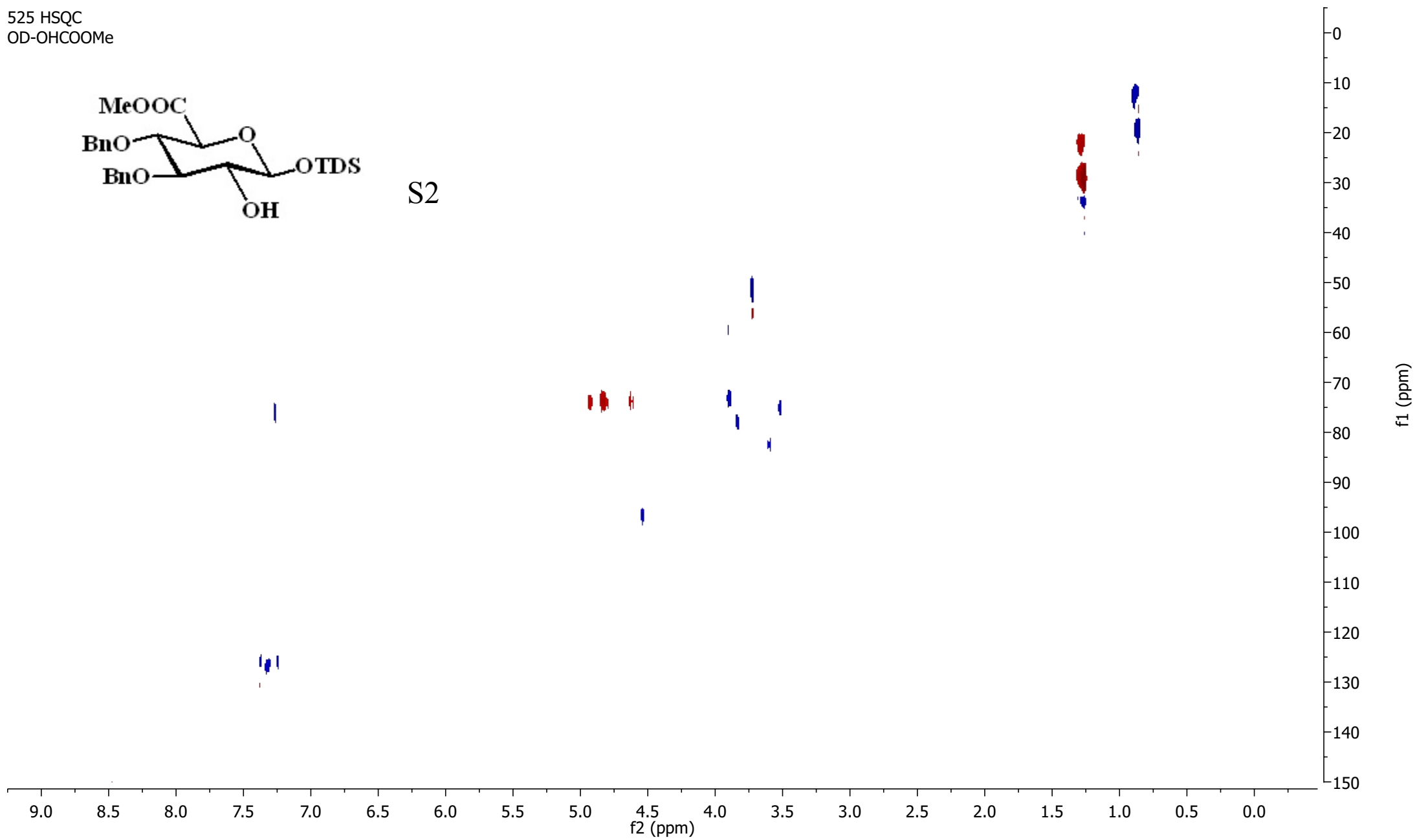




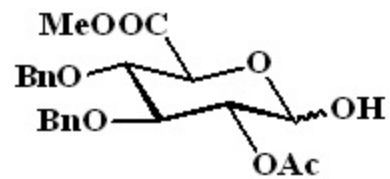
525 HSQC
OD-OHCOOMe



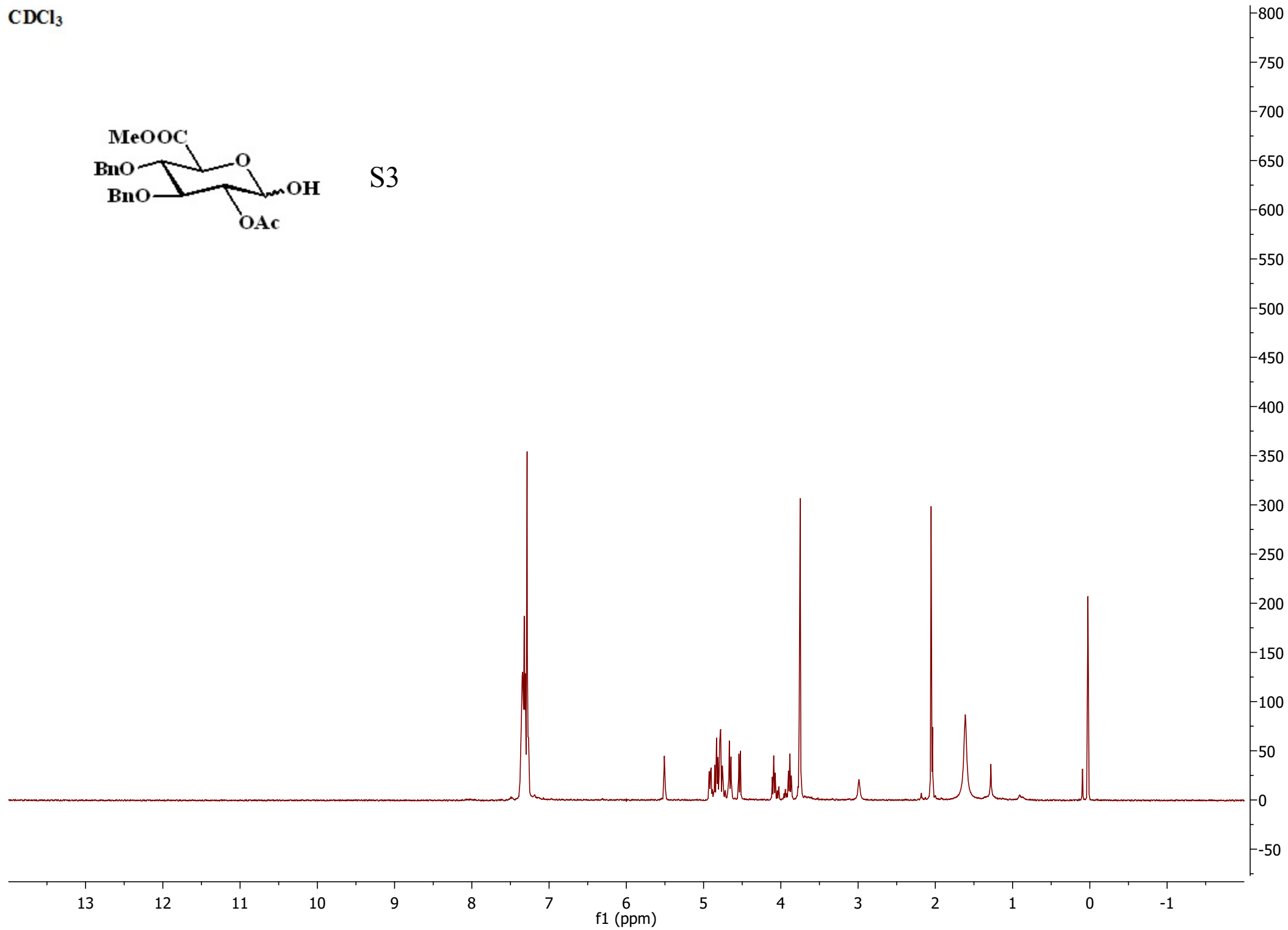
S2



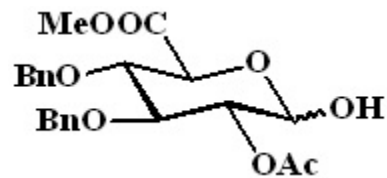
CDCl₃



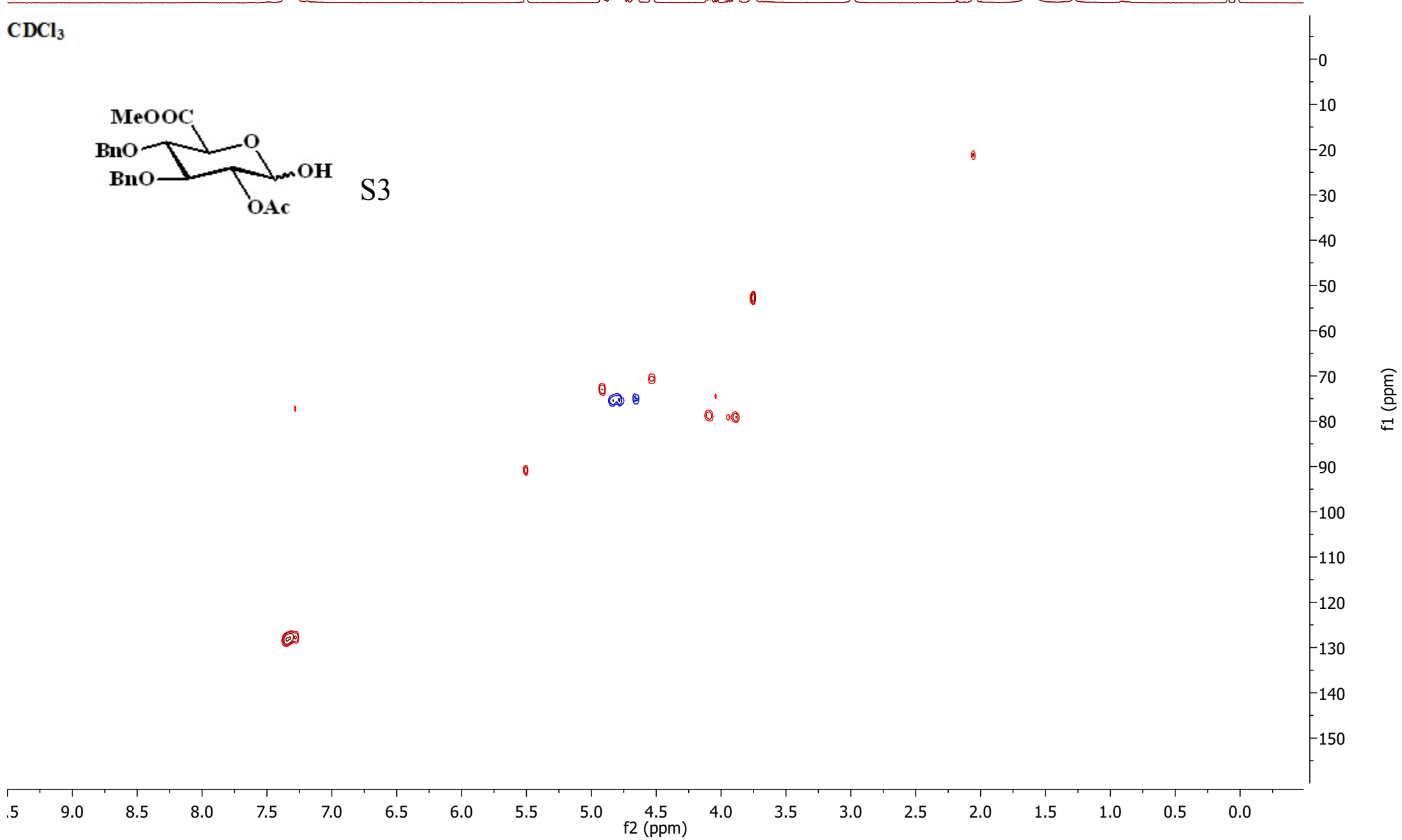
S3



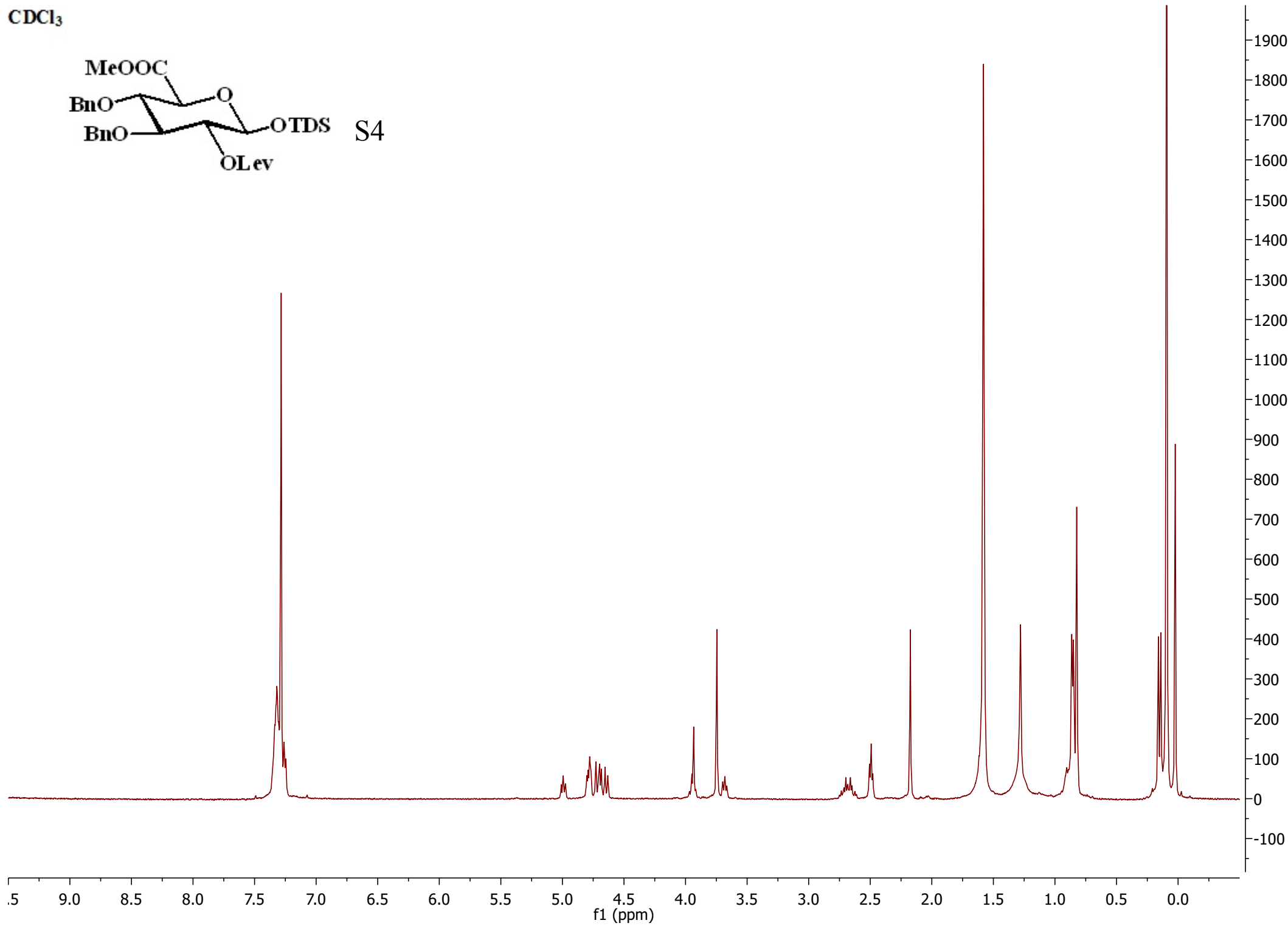
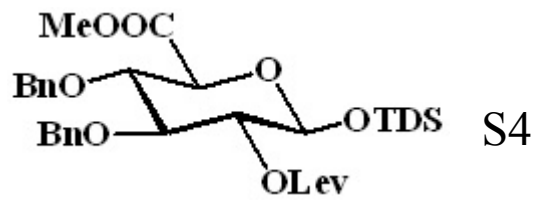
CDCl₃



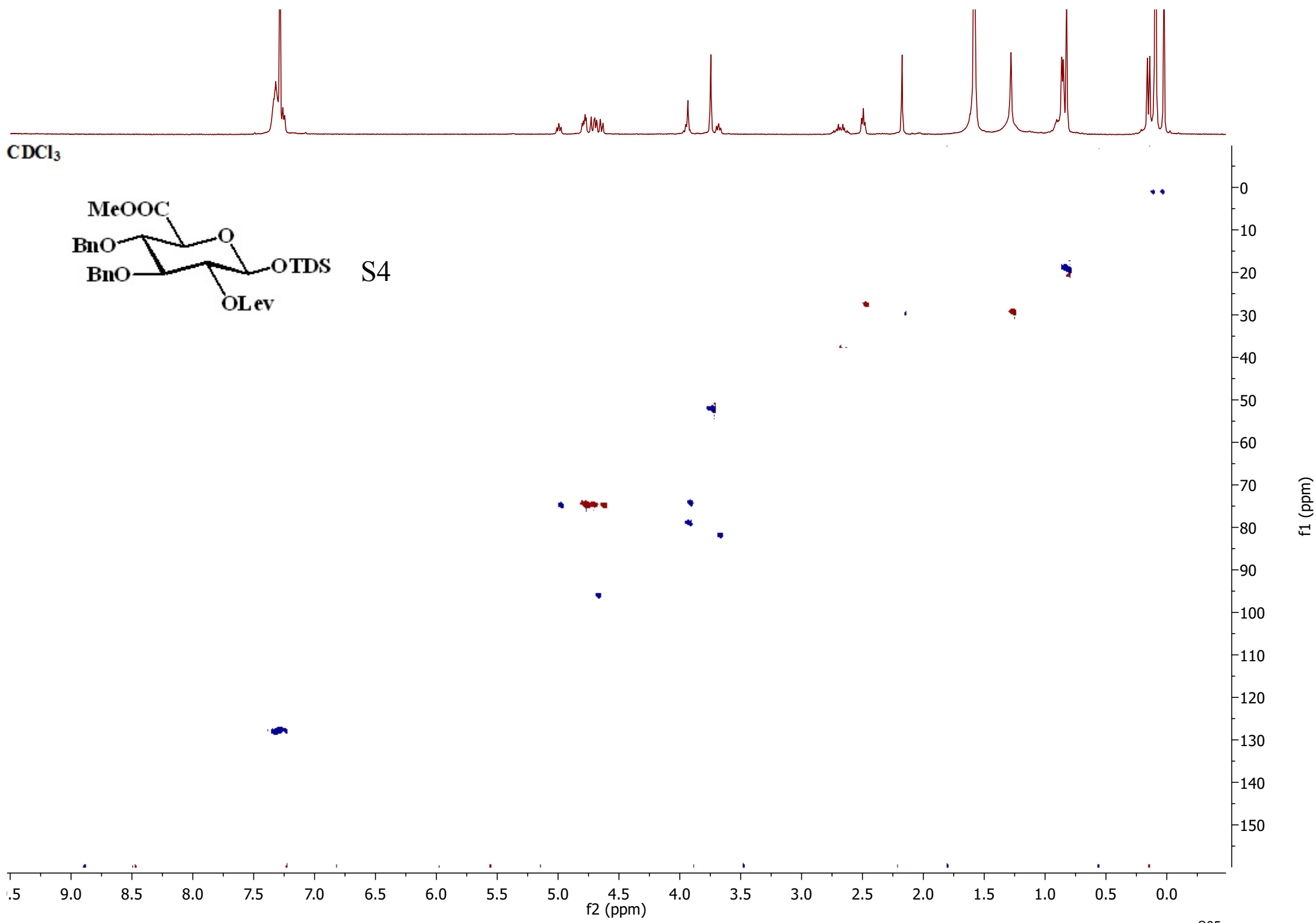
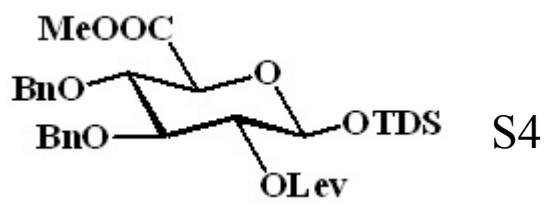
S3



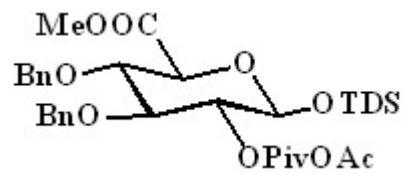
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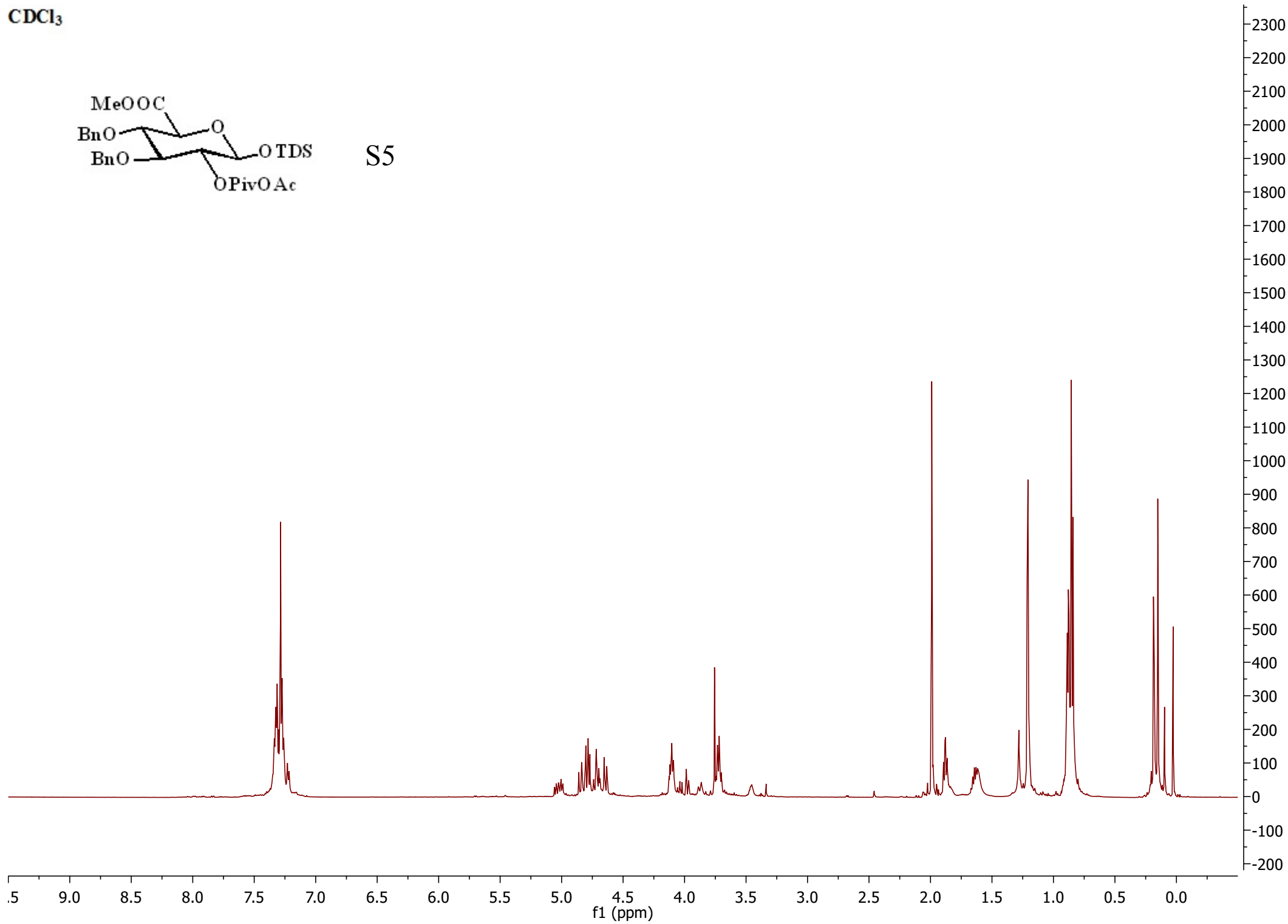
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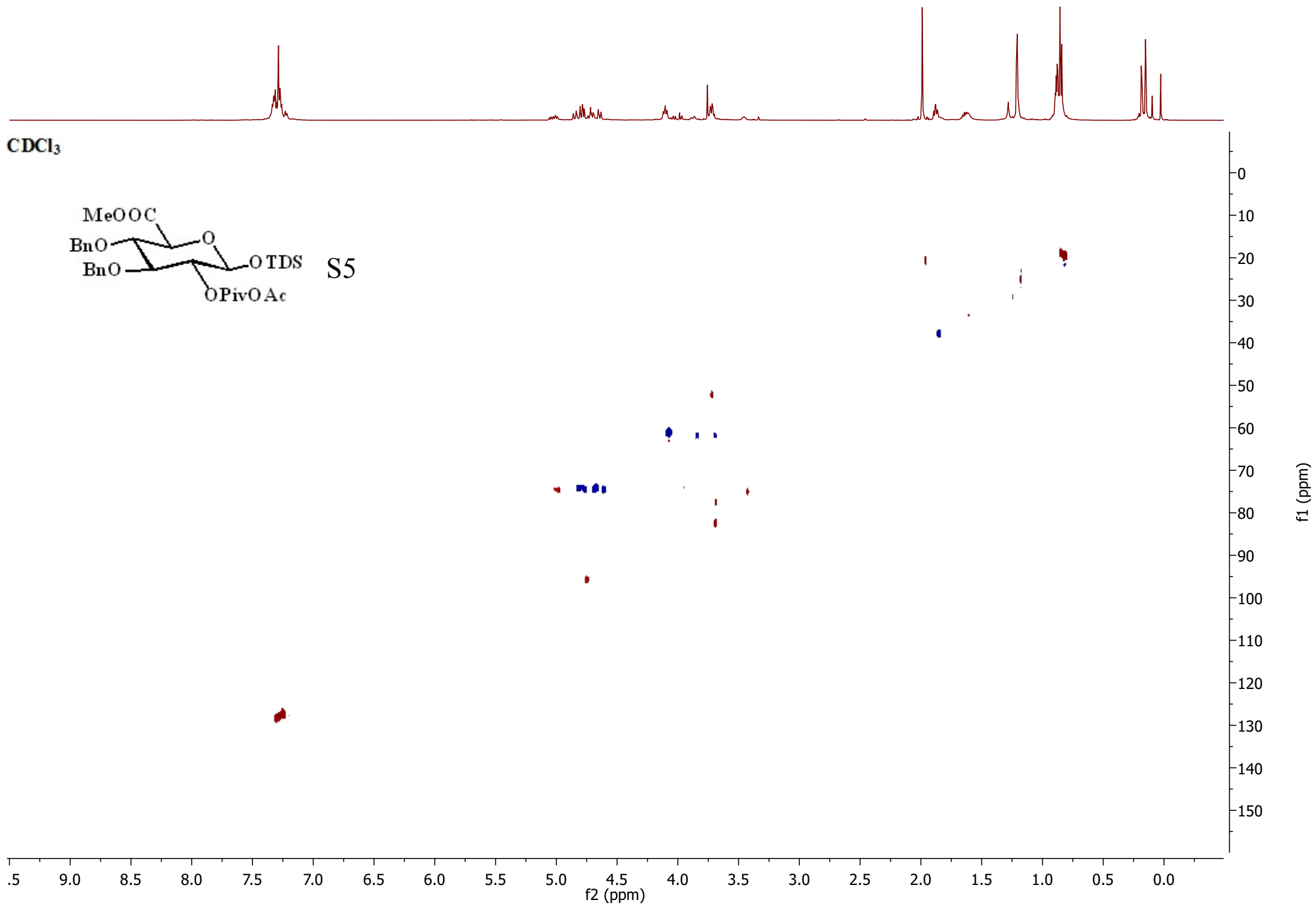
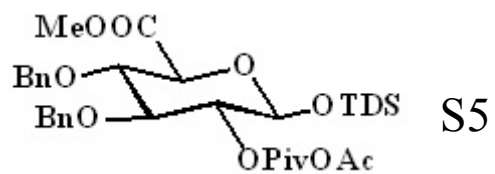
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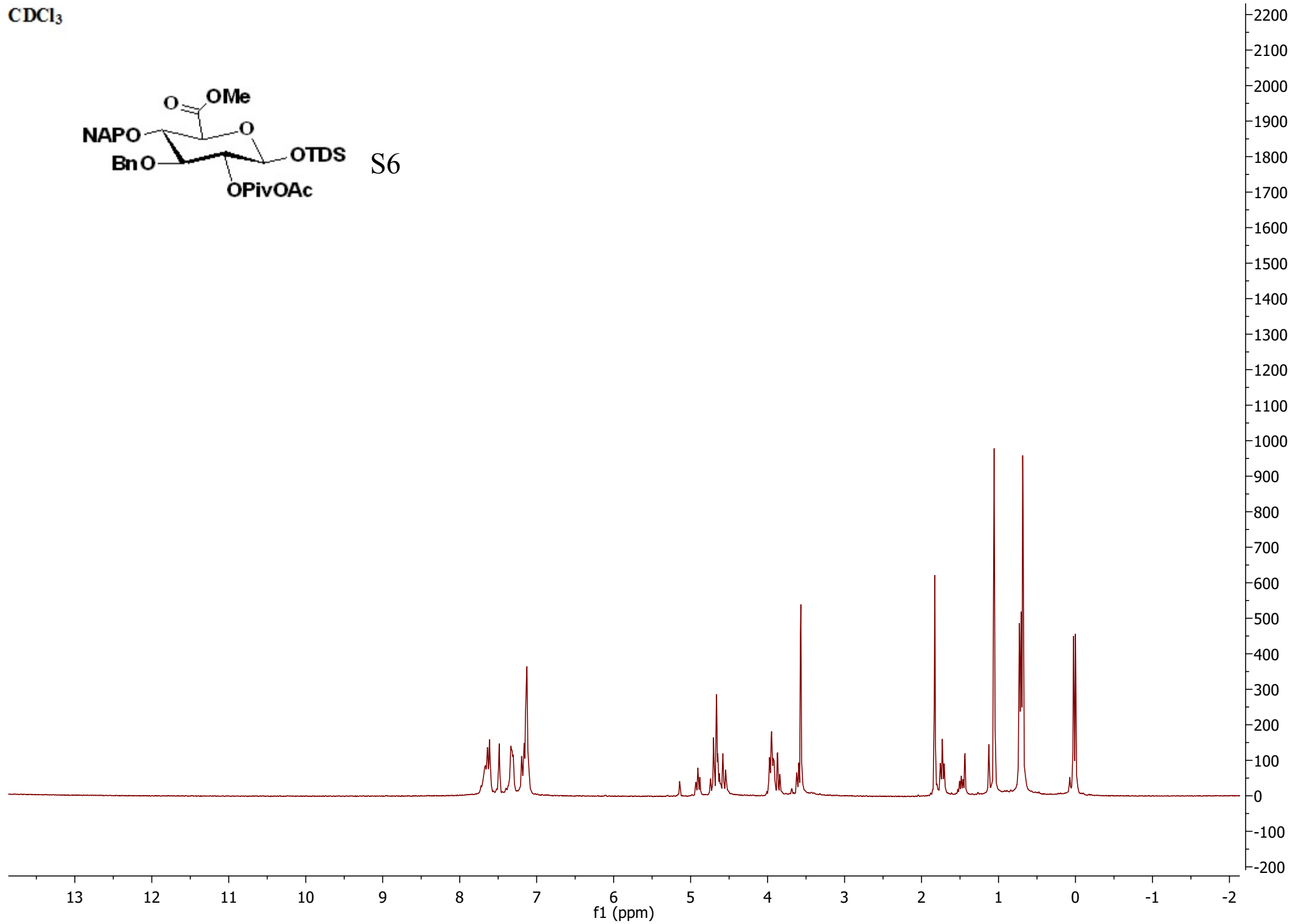
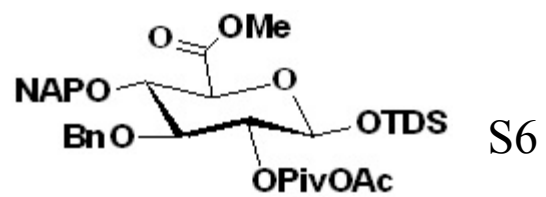
S5



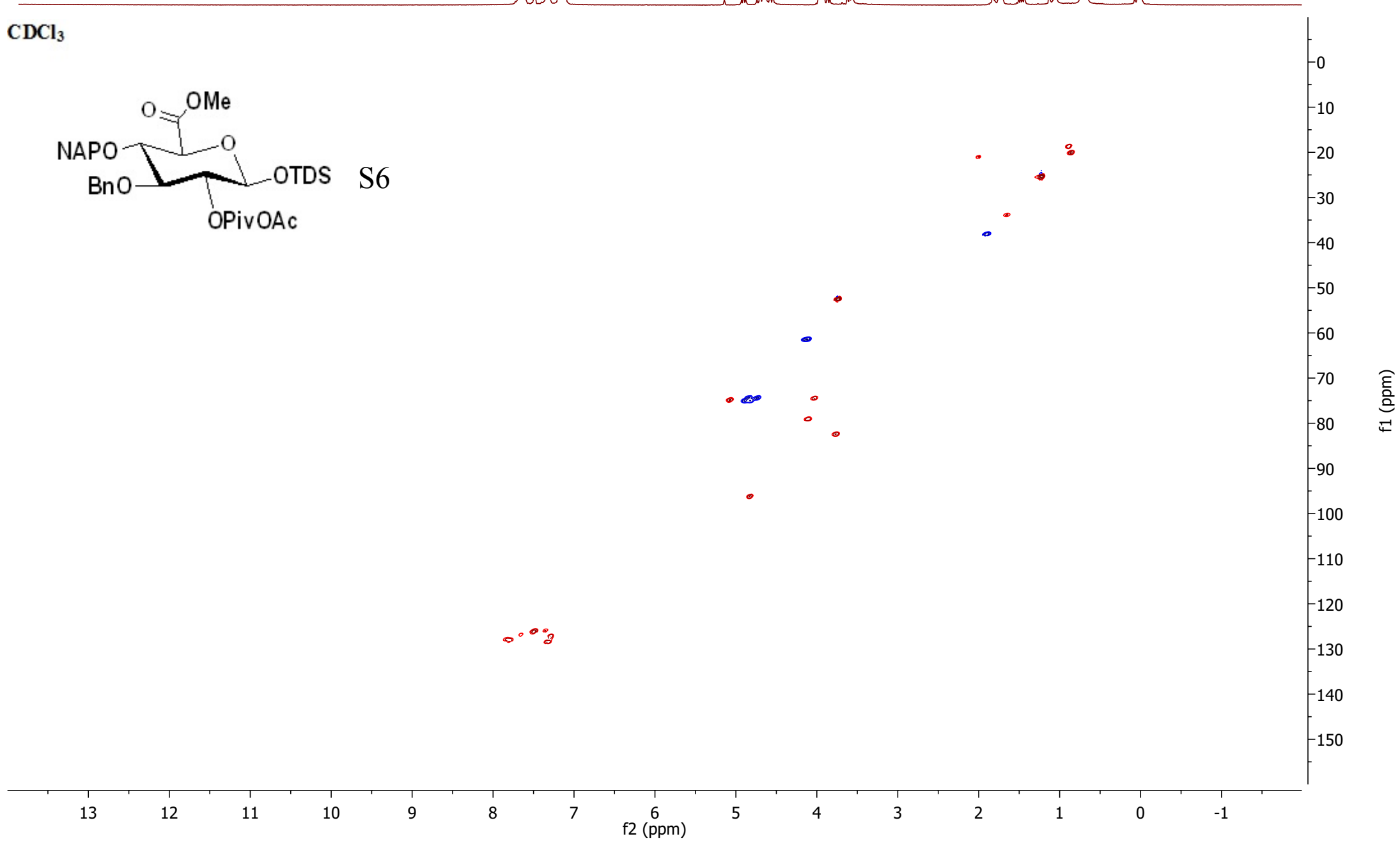
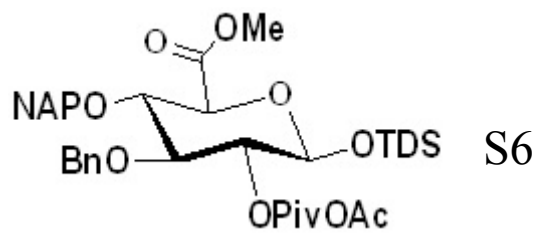
CDCl₃



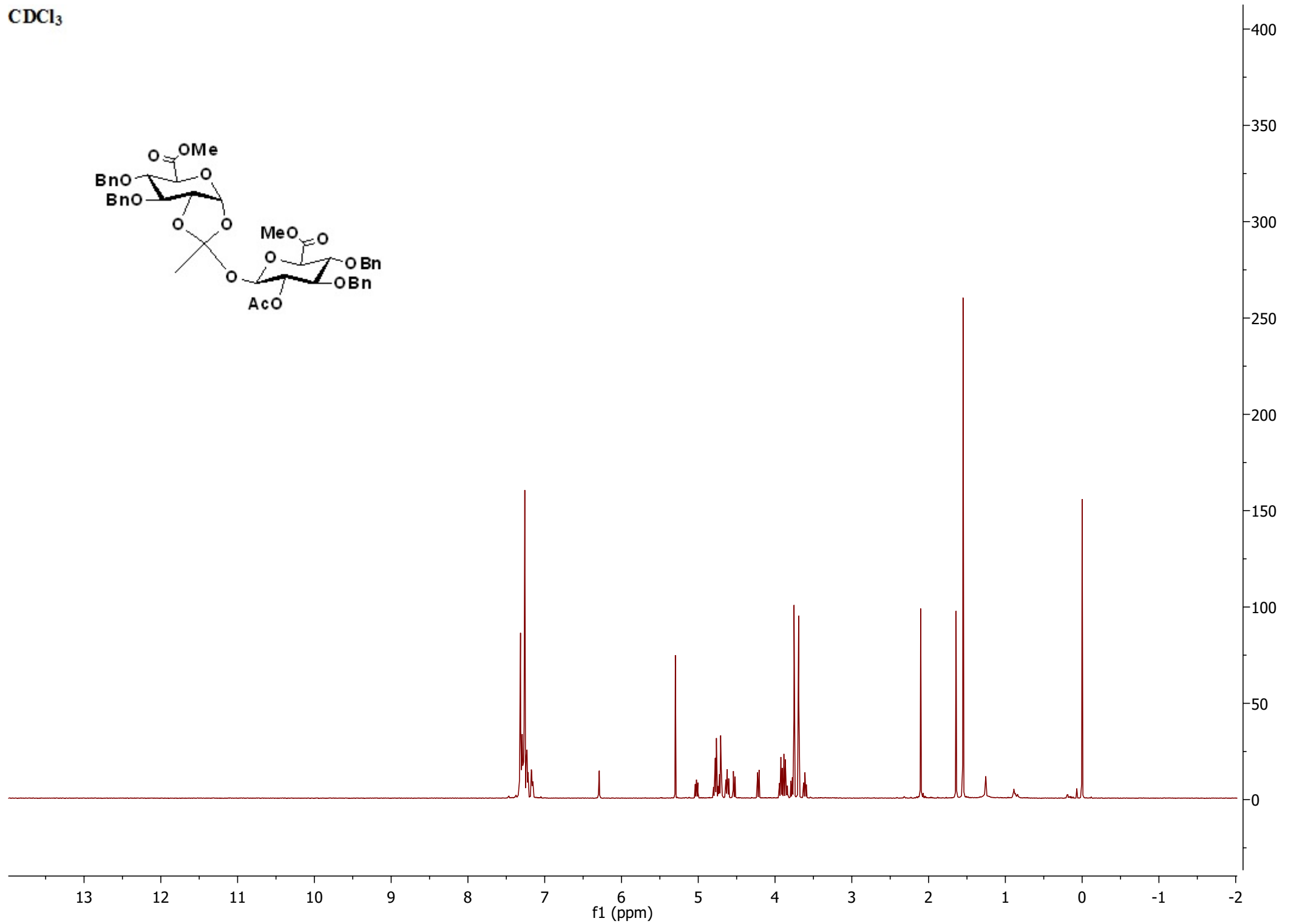
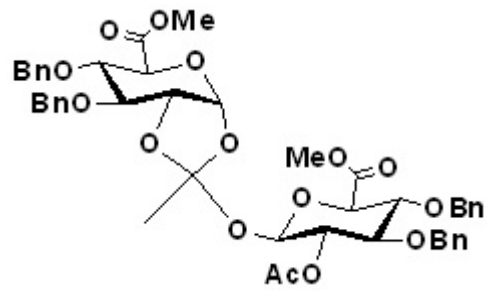
CDCl₃



CDCl₃



CDCl₃



CDCl₃

STANDARD 1H OBSERVE - profile

