Supporting Information

Discovery of Bivalent GalNAc-Conjugated Betulin as Potent ASGPR-Directed Agent Against Hepatocellular Carcinoma

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Table S1. Cytotoxicity of compounds **7** and **8**.

Compound	IC_{50} (μM)			
	HepG2[a]	Huh7	PC3	a549
Betulin	4.2±0.4	5.8±0.5	4.5±0.3	3.7±0.1
7	>50	>50	>50	>50
8	25.9±3.9	47.2±7.5	31.3±4.1	>50
DMSO	n/a	n/a	n/a	n/a
Doxorubicin	0.38±0.07	0.15±0.04	0.11±0.03	0.14±0.06

*n/a – nonactive
[a] - Cell lines in order: hepatocellular carcinomas (HepG2 and Huh7), prostate cancer (PC3), lung adenocarcinoma (a549).

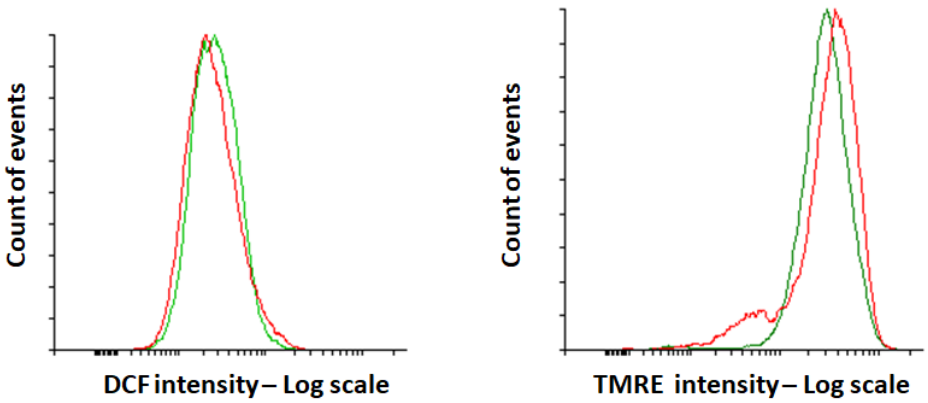


Figure S1. Histograms of distribution of DCF and TMRE in HepG2 cells: green curve – the control cells, red curve – cells treated with compound **8** (72 h at 26 μM, n = 3).

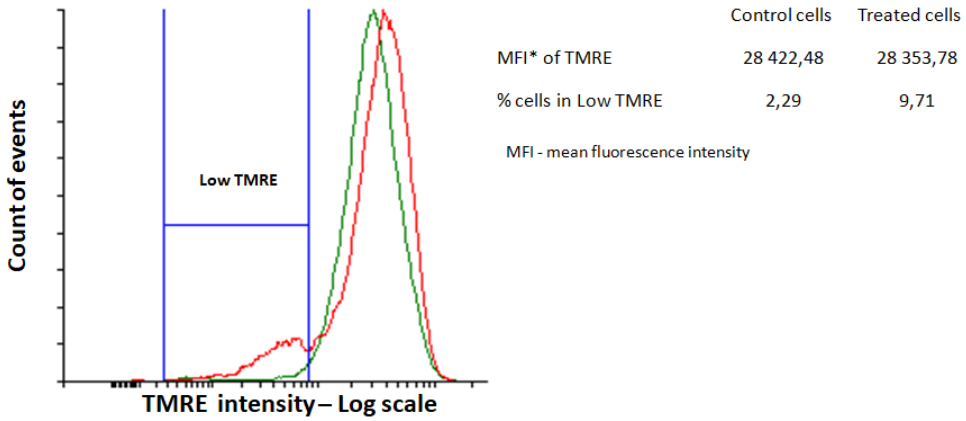


Figure S2. Histograms of distribution of TMRE in HepG2 cells: green curve – the control cells, red curve - treated with compound **8** 72 h (IC_{50}). Low TMRE sub-population in control cells – 2.29%, in treated cells – 9.71%.

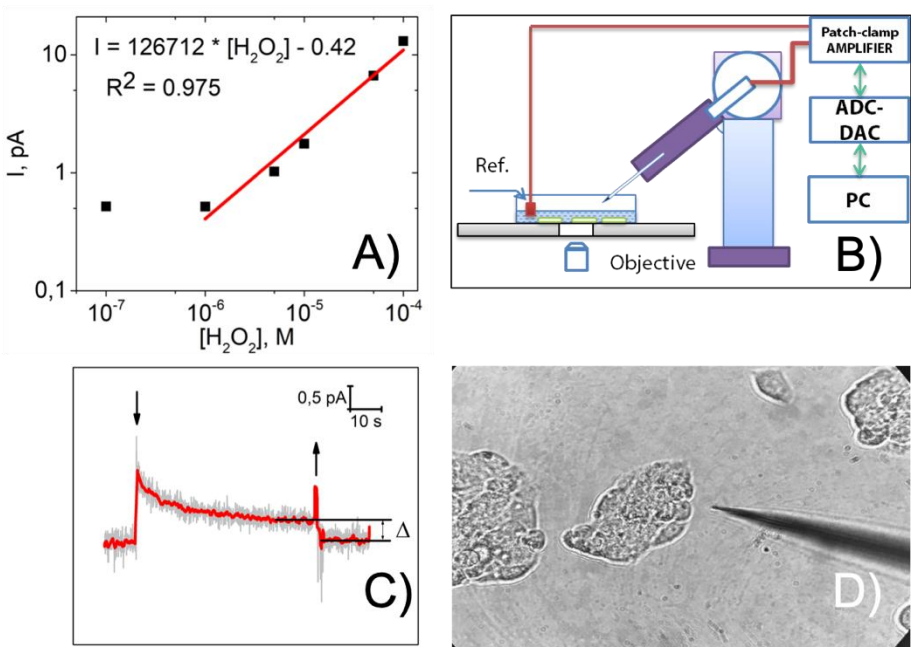


Figure S3. (A) Calibration curve (current vs. H_2O_2 concentration at 800 mV) showing good linear response (R^2 = 0.975). (B) Scheme of the set-up for intracellular measurements. (C) Typical amperometric response of platinized nanoelectrode after penetration into single cell. The moment of penetration into the cell is shown by the down arrow, and the withdrawal of the nanoelectrode from the cell is shown by the up arrow. (D) Optical microscopic view of HepG2 showing the principle of the electrochemical detection of oxidative stress. The nanoelectrode (black shadow on right) used to measure ROS is placed with a micromanipulator.

(A)

(B)

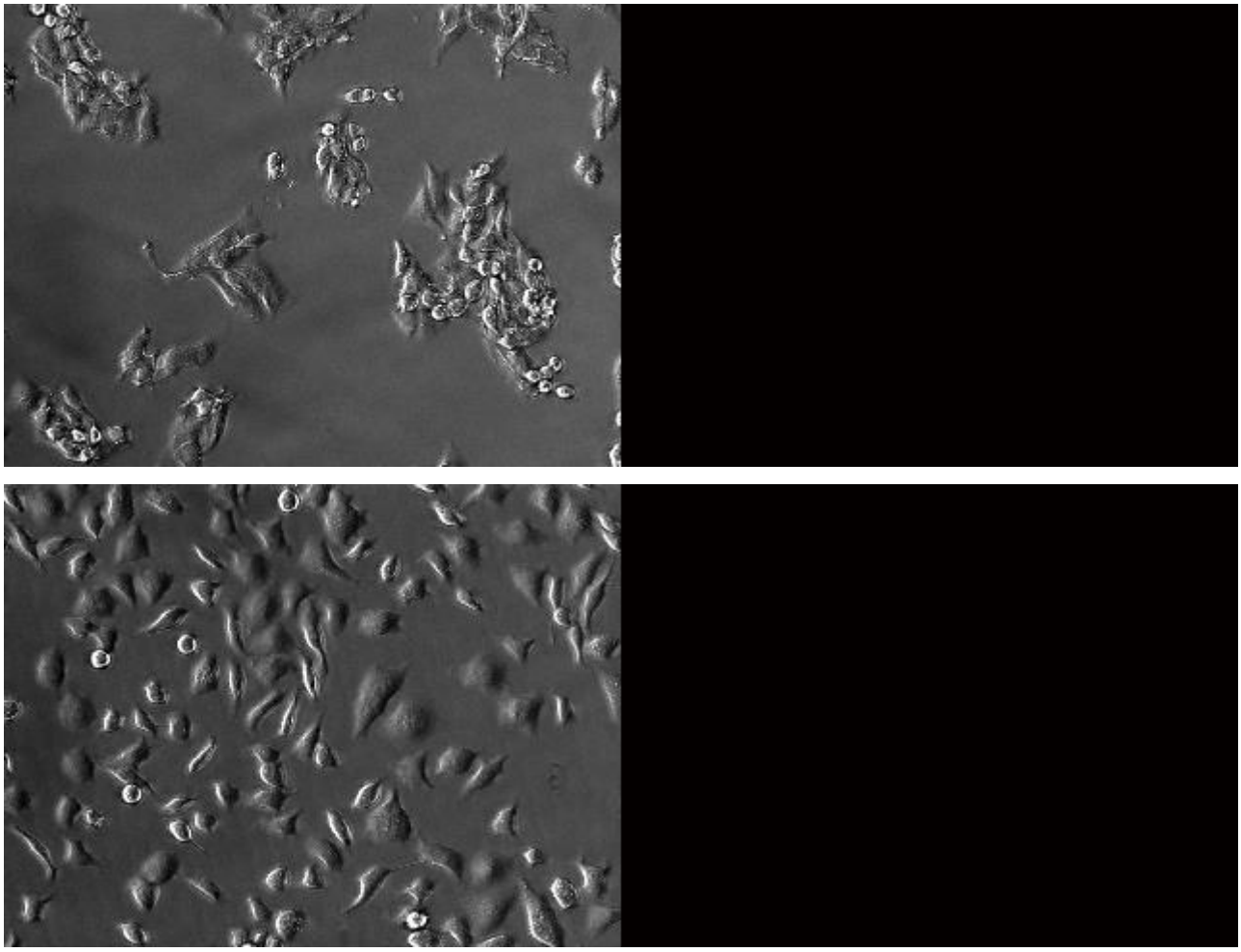


Figure S4. Representative brightfield (left) and fluorescence (right) microscopy images of untreated cells as controls: (A) HepG2 cells; (B) PC3 cells.

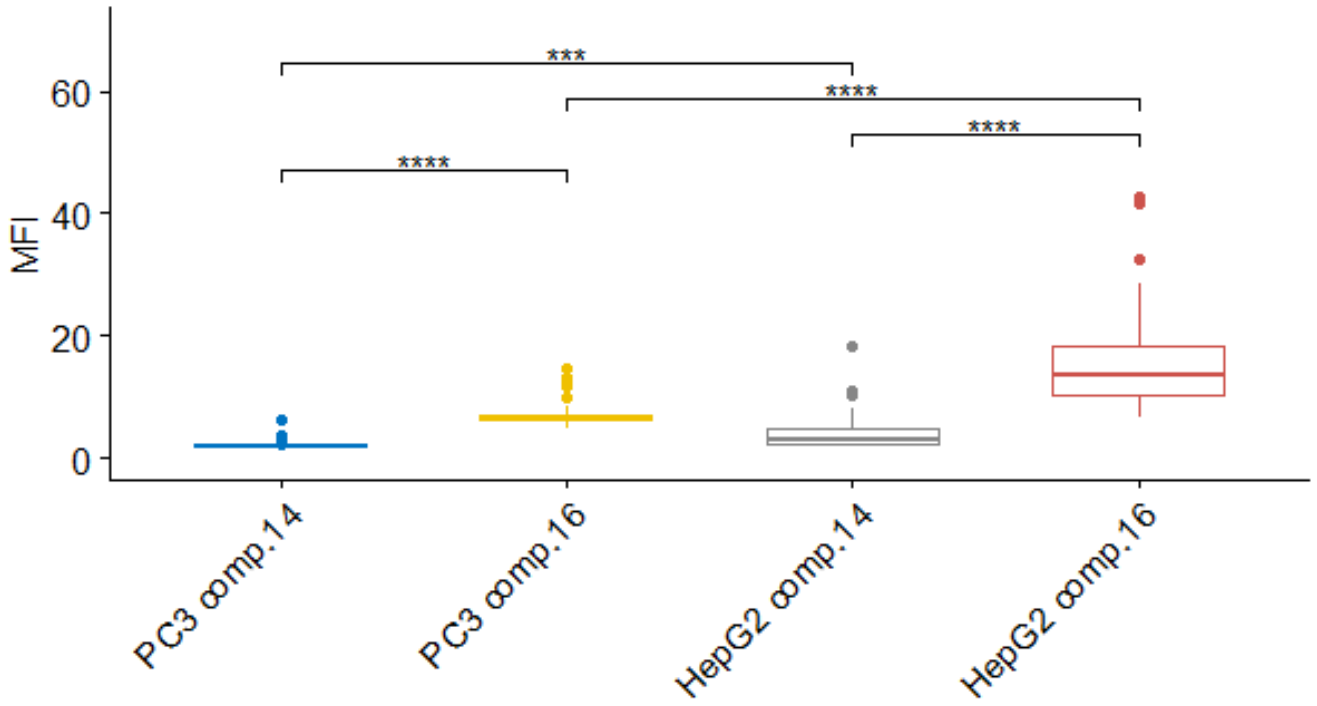


Figure S5. Mean fluorescent intensity (MFI) after HepG2 and PC3 cells incubation with compound **16** and compound **14** at 1 µM for 60 min (n = 3; ANOVA: p <2e-16; *, p < 0.05; **, p < 0.01; ***, p < 0.001, ****, p < 0.0001; t-test, unpaired).

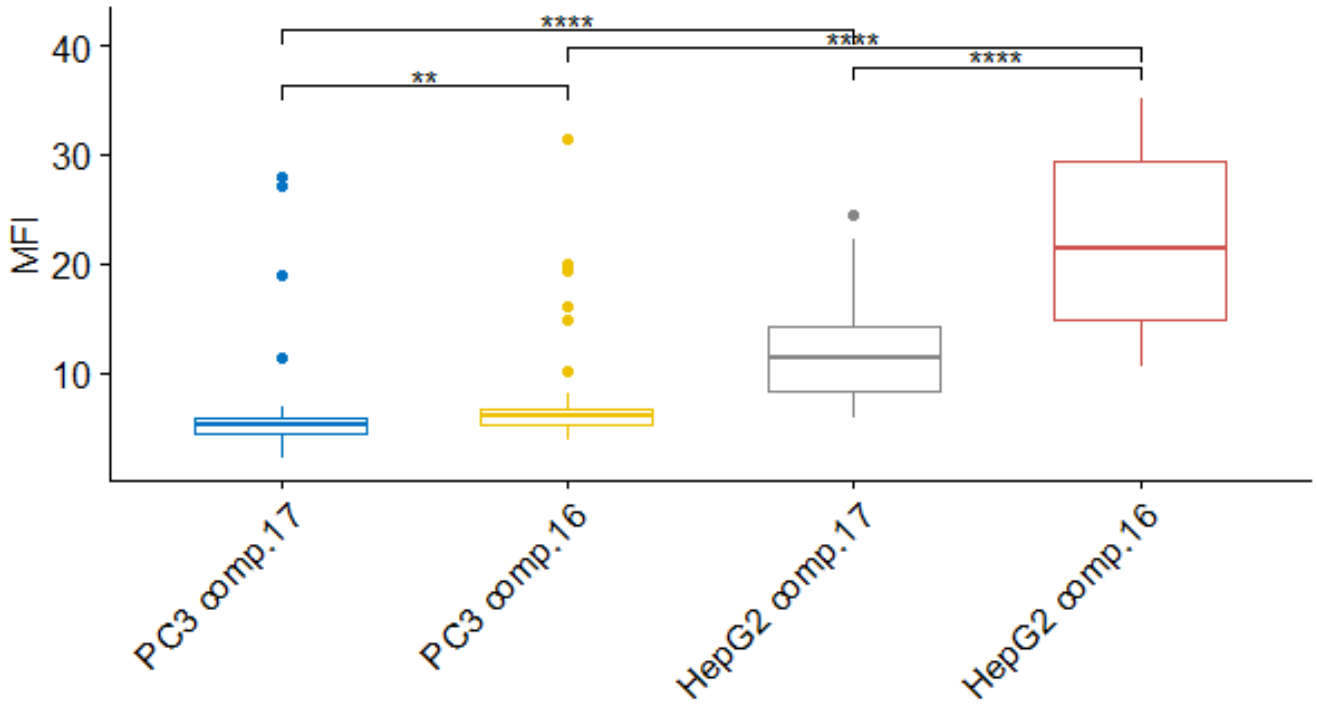
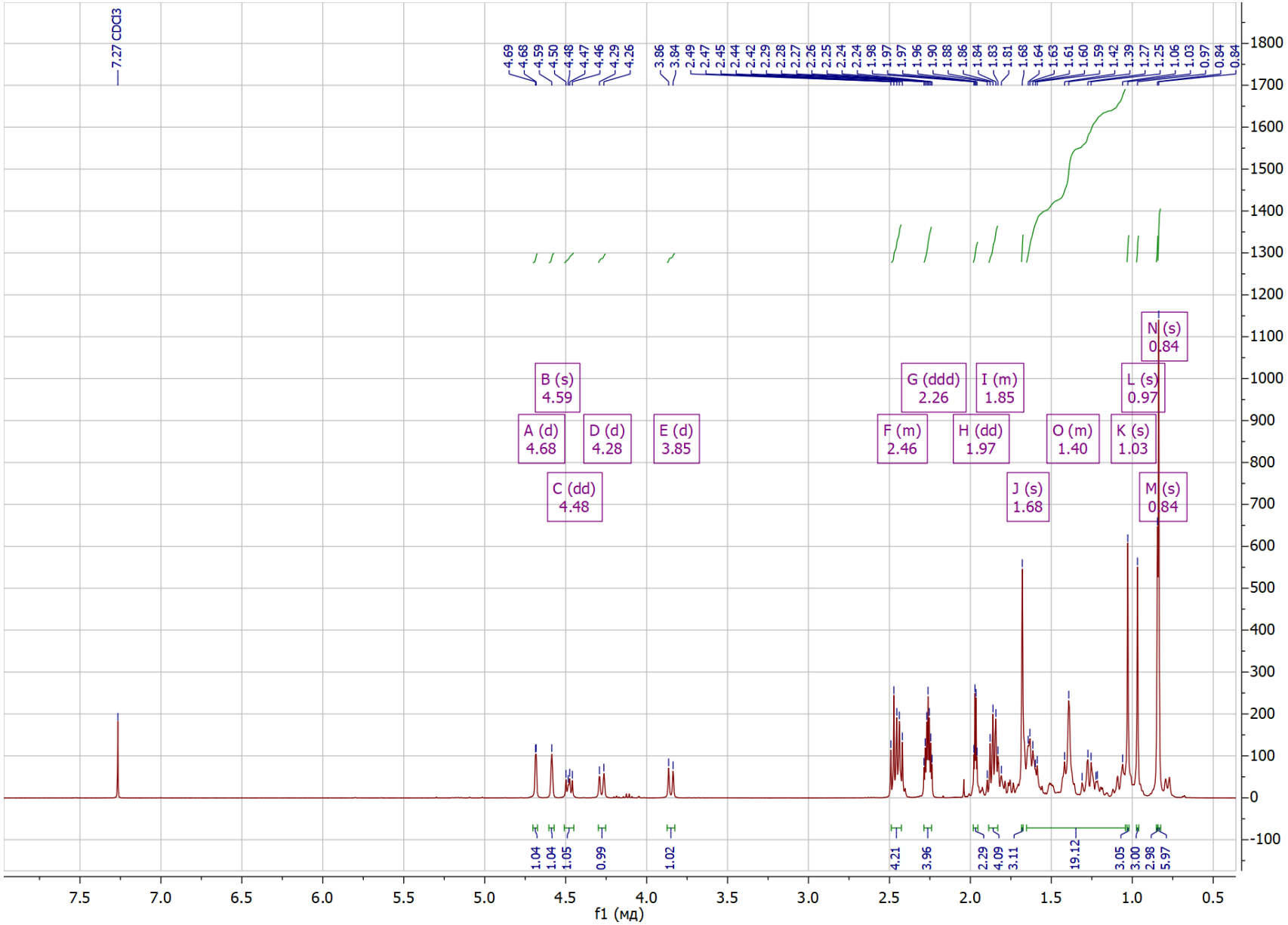
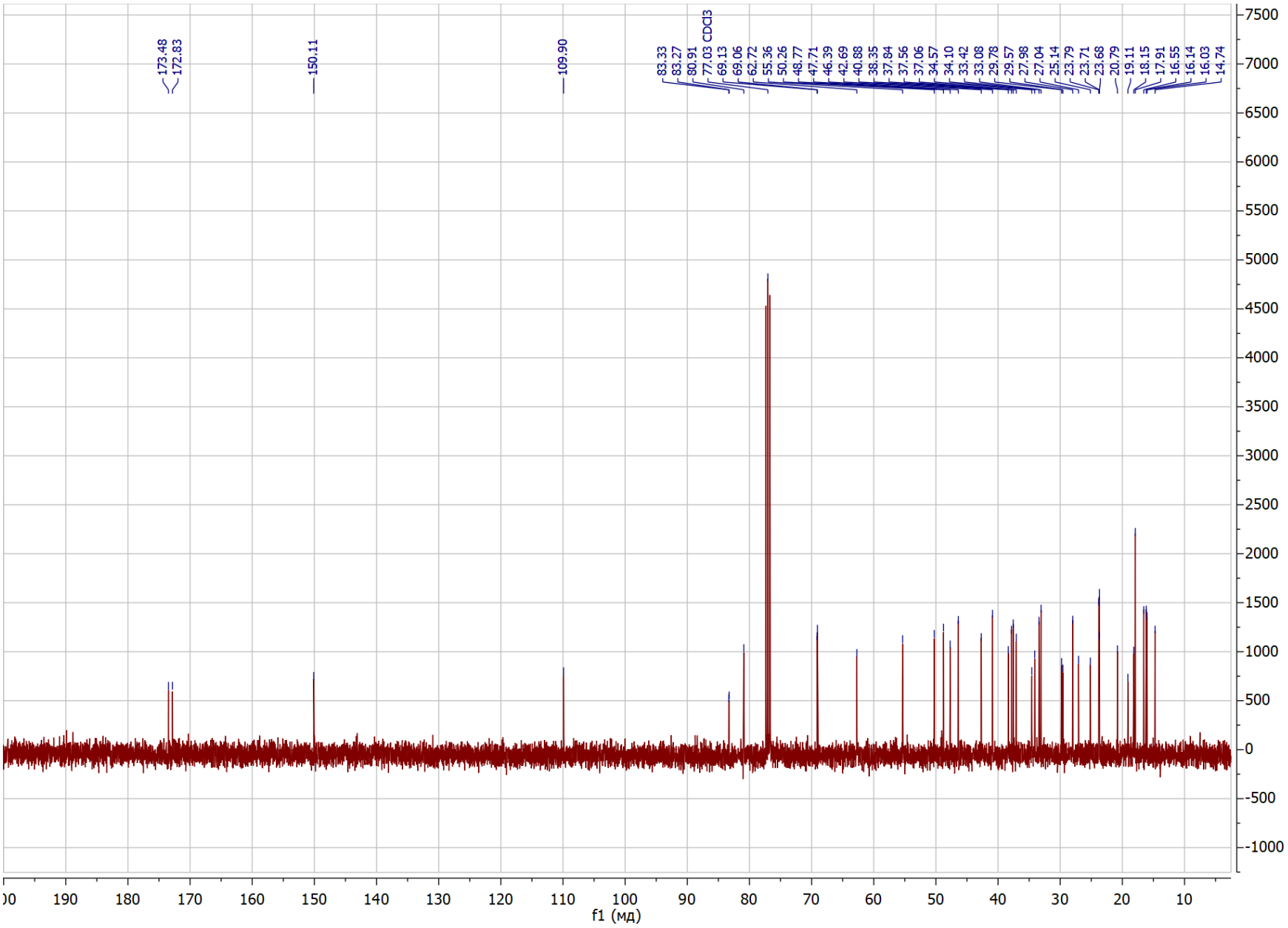


Figure S6. Mean fluorescent intensity (MFI) after HepG2 and PC3 cells incubation with compound **17** and compound **16** at 100 nM for 30 min (n = 3; ANOVA: p <2e-16; *, p < 0.05; **, p < 0.01; ***, p < 0.001, ****, p < 0.0001; t-test, unpaired).



^{1}H NMR of Compound 4



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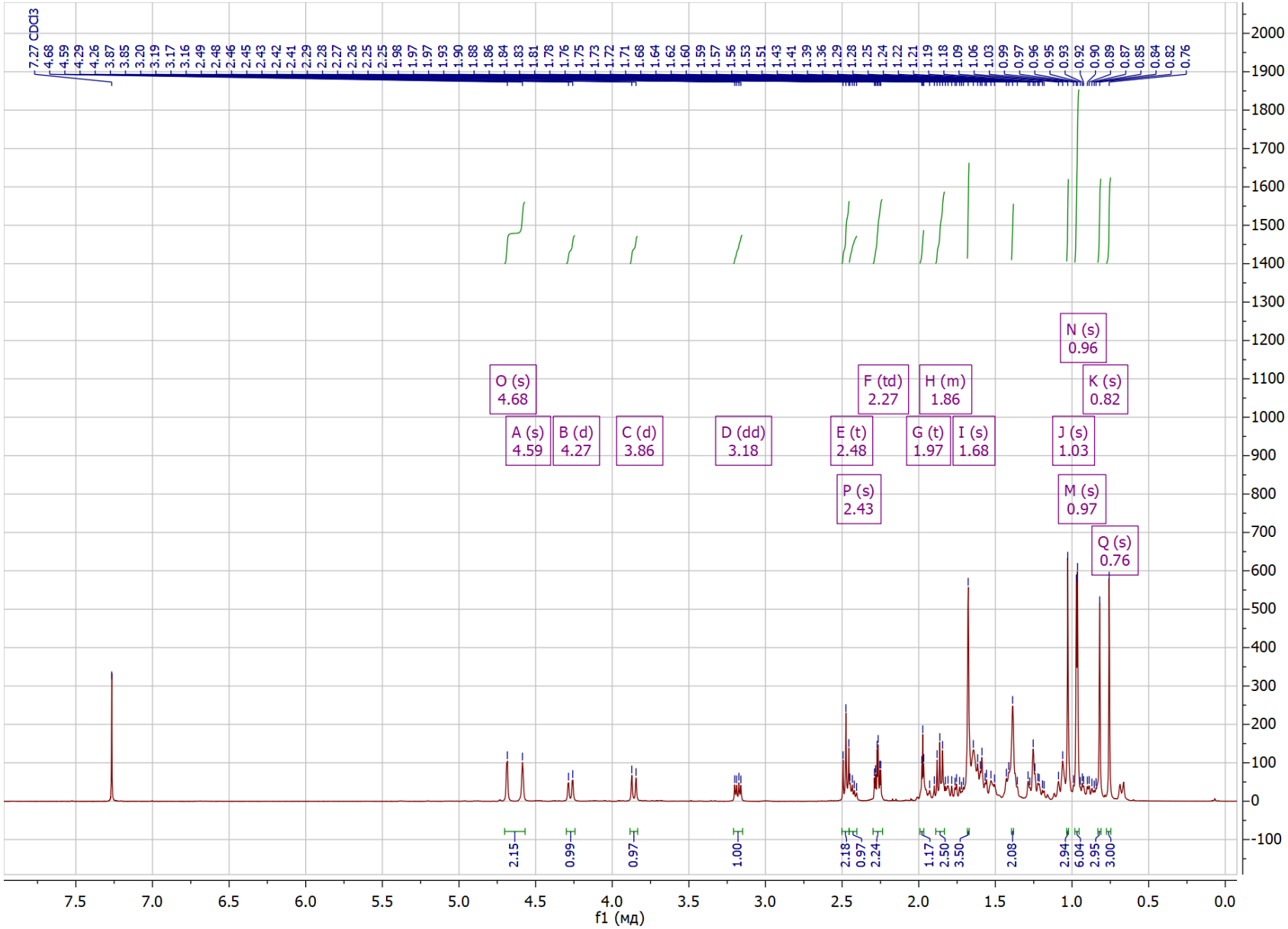

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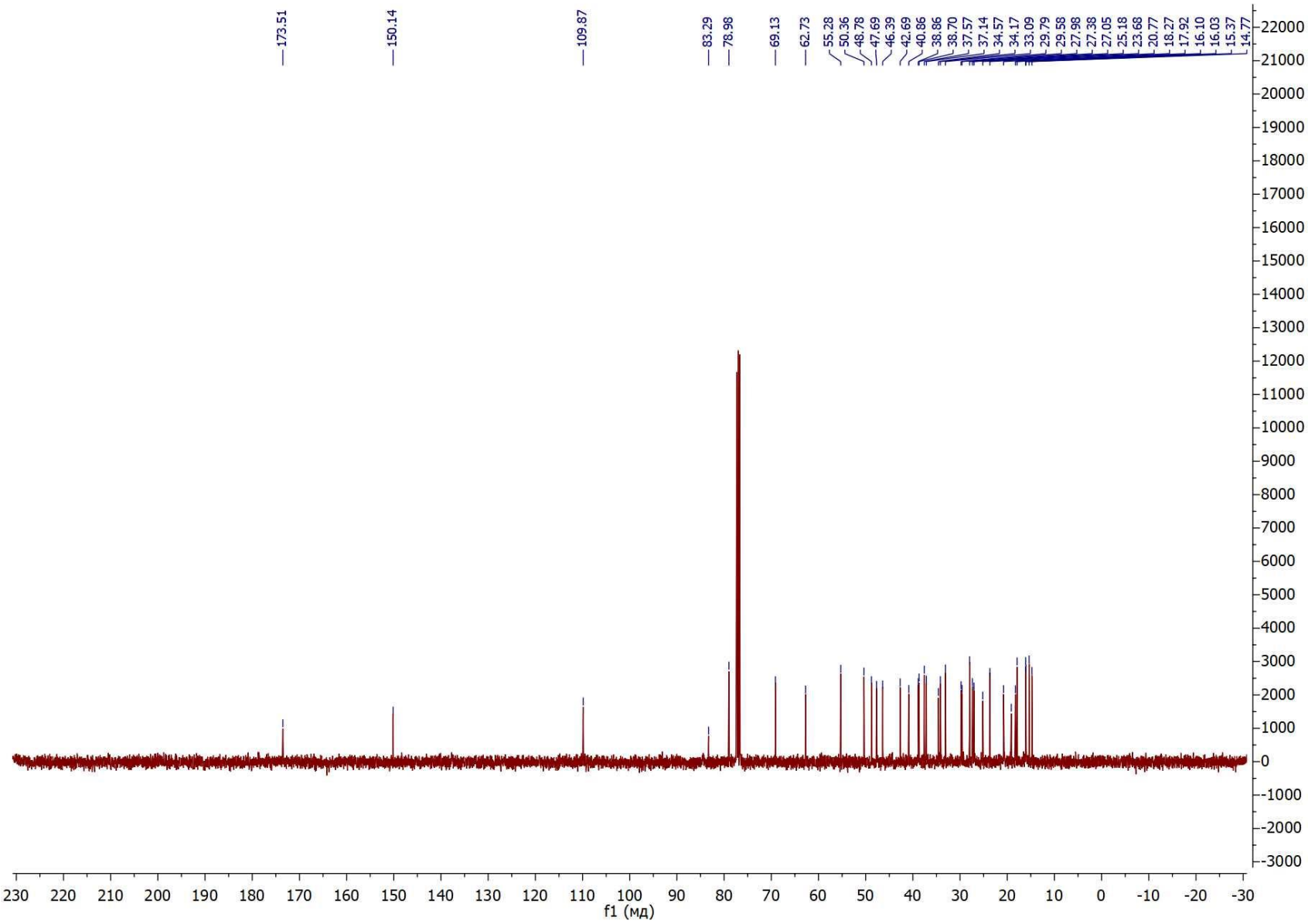
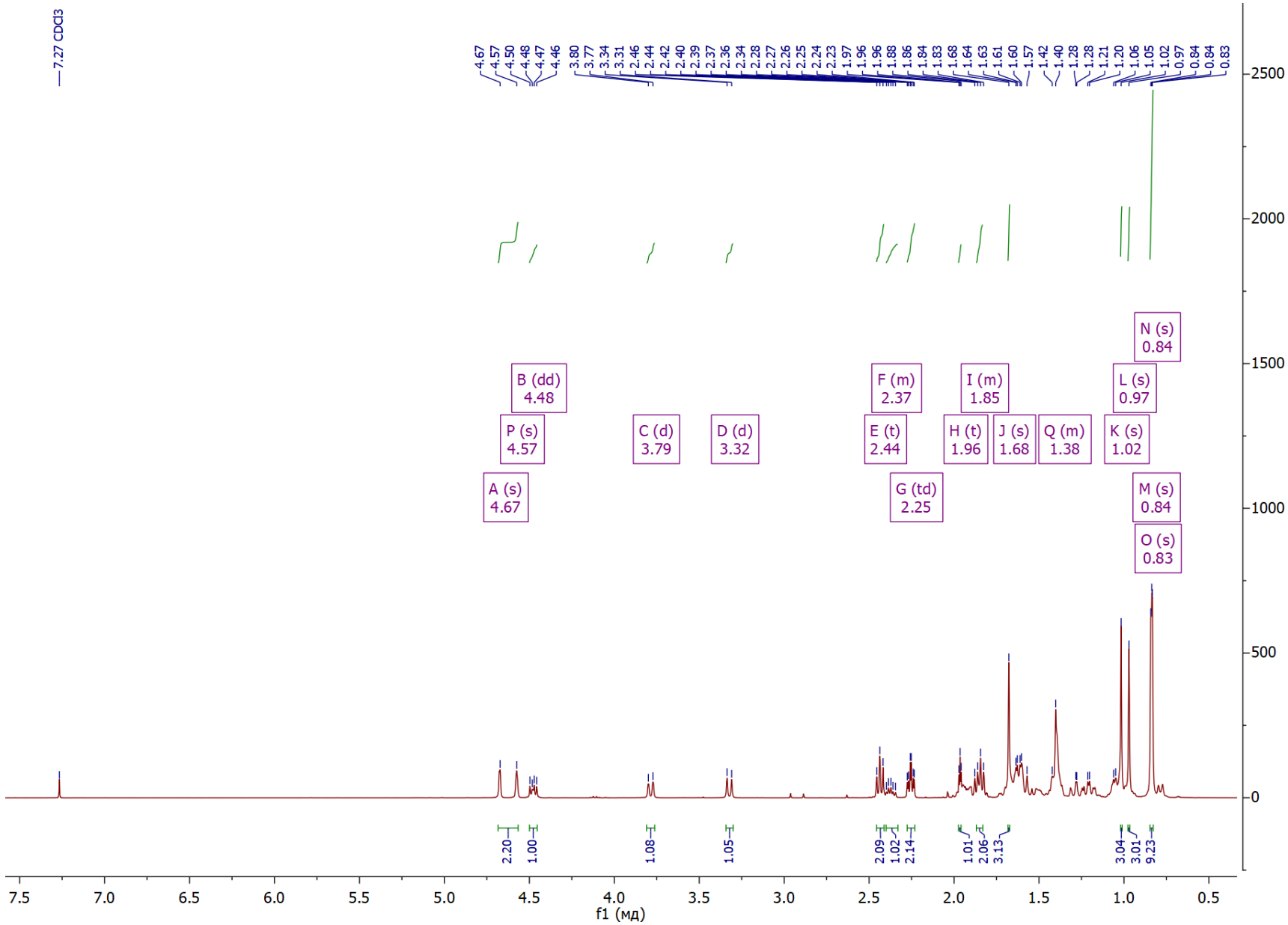


^{13}C NMR of Compound 5



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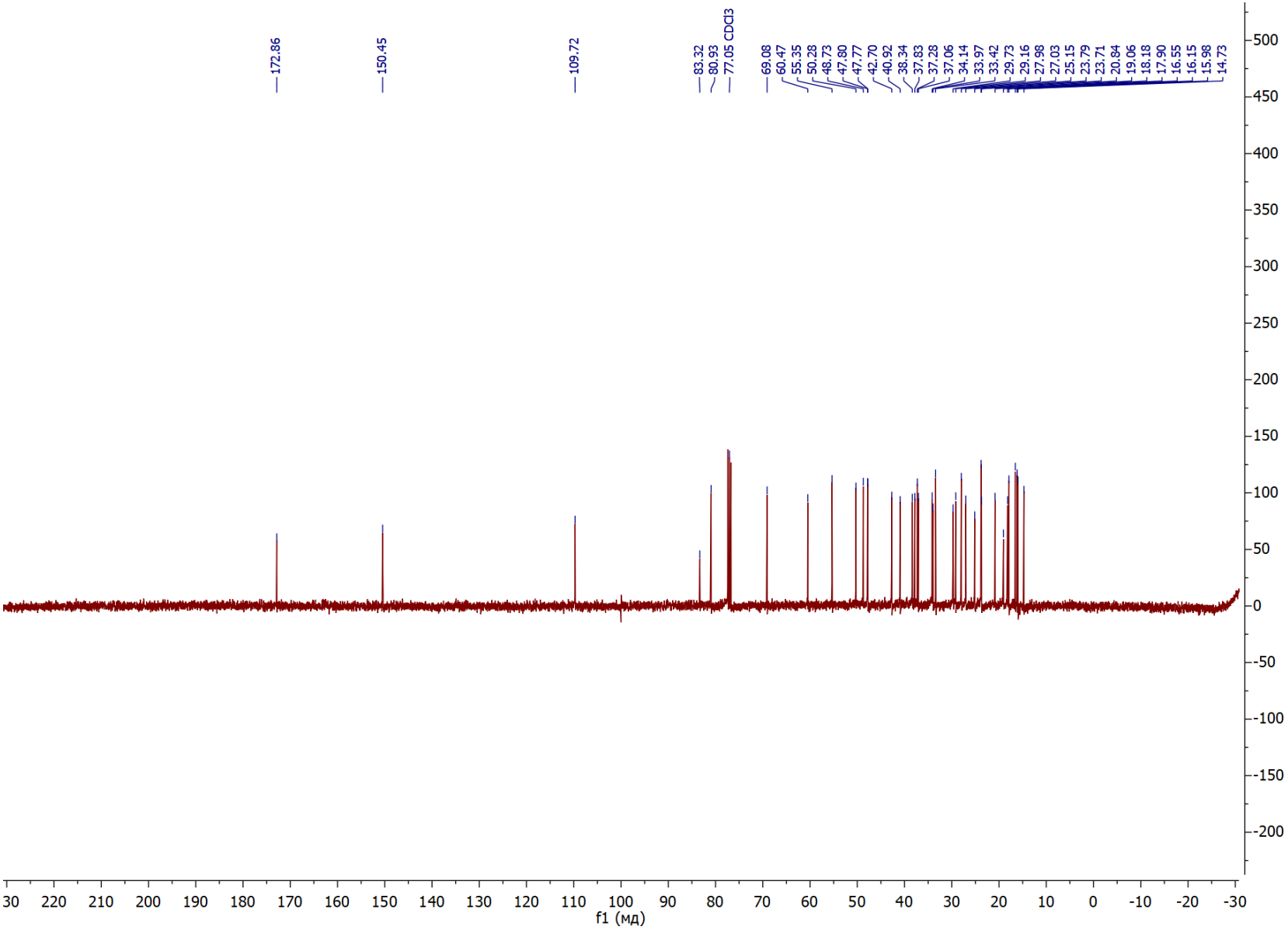

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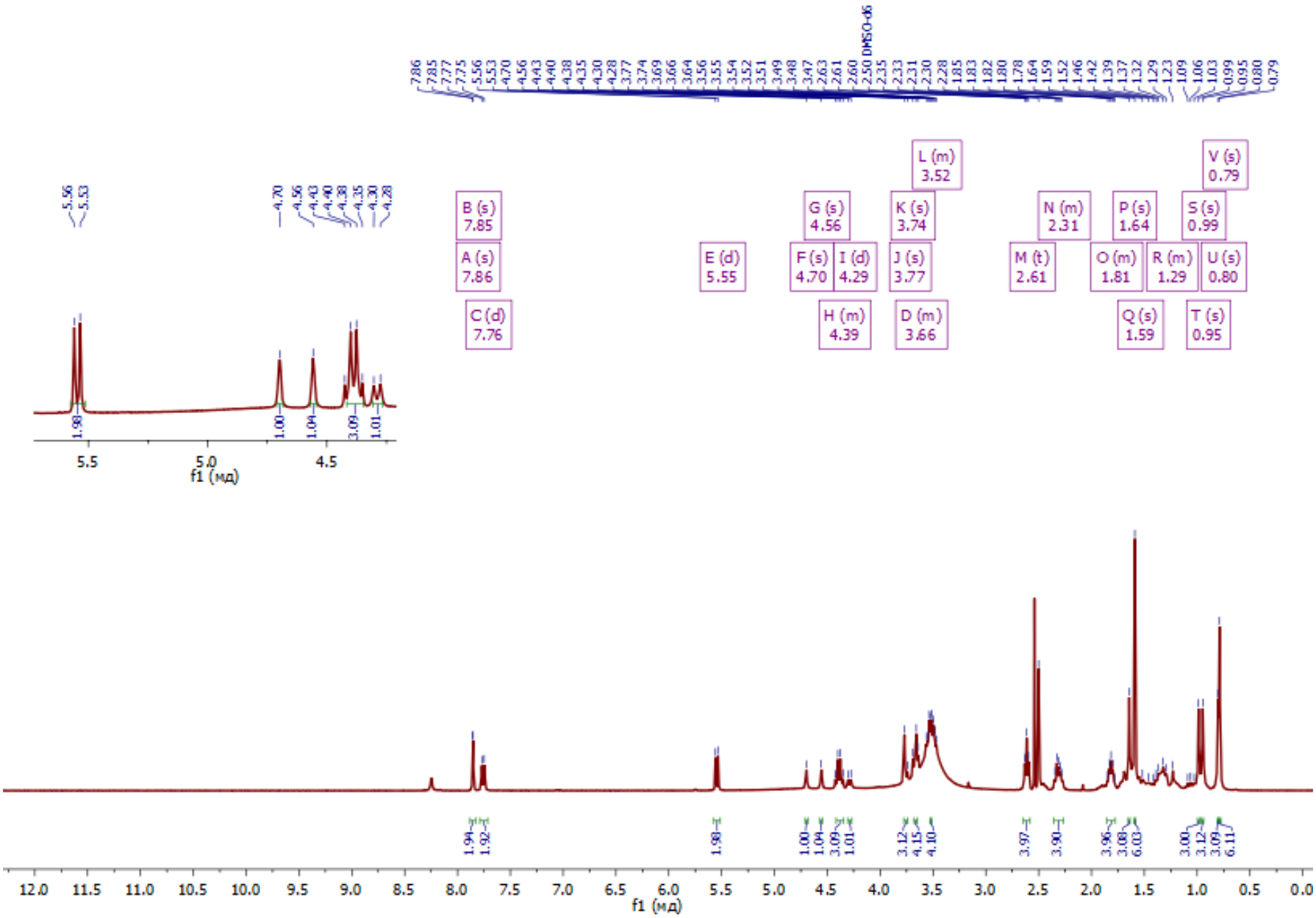
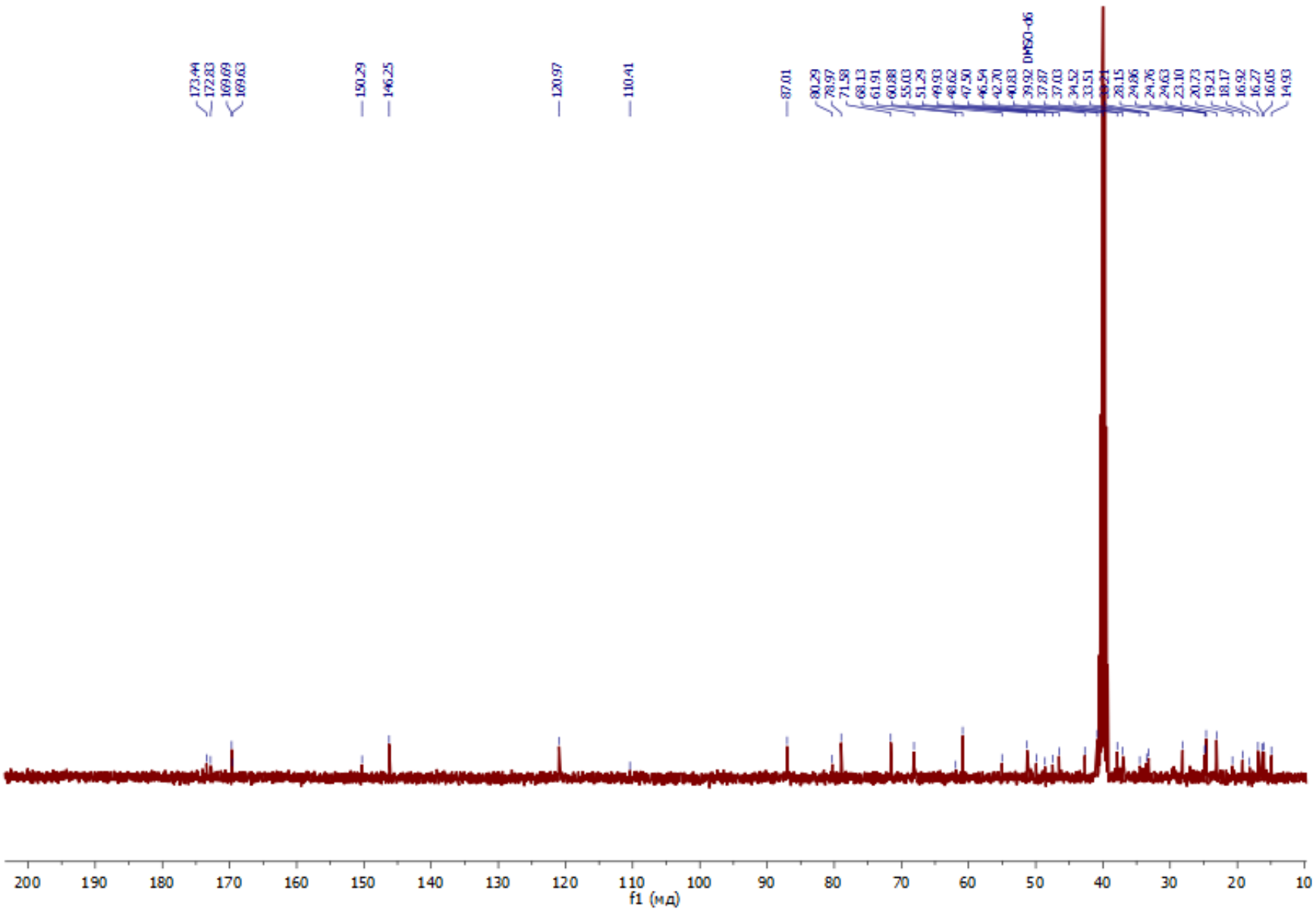


^{1}H NMR of Compound 7



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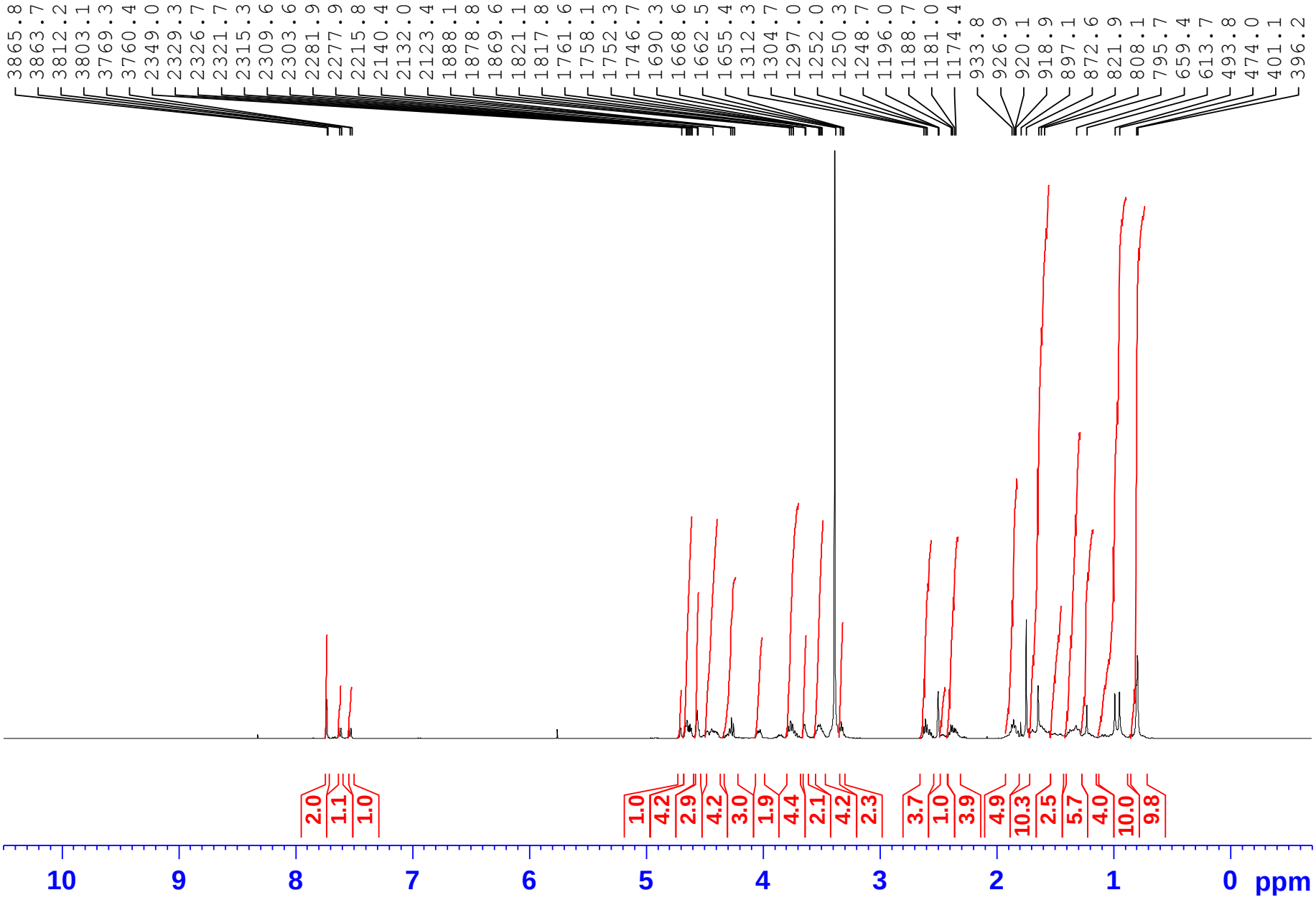
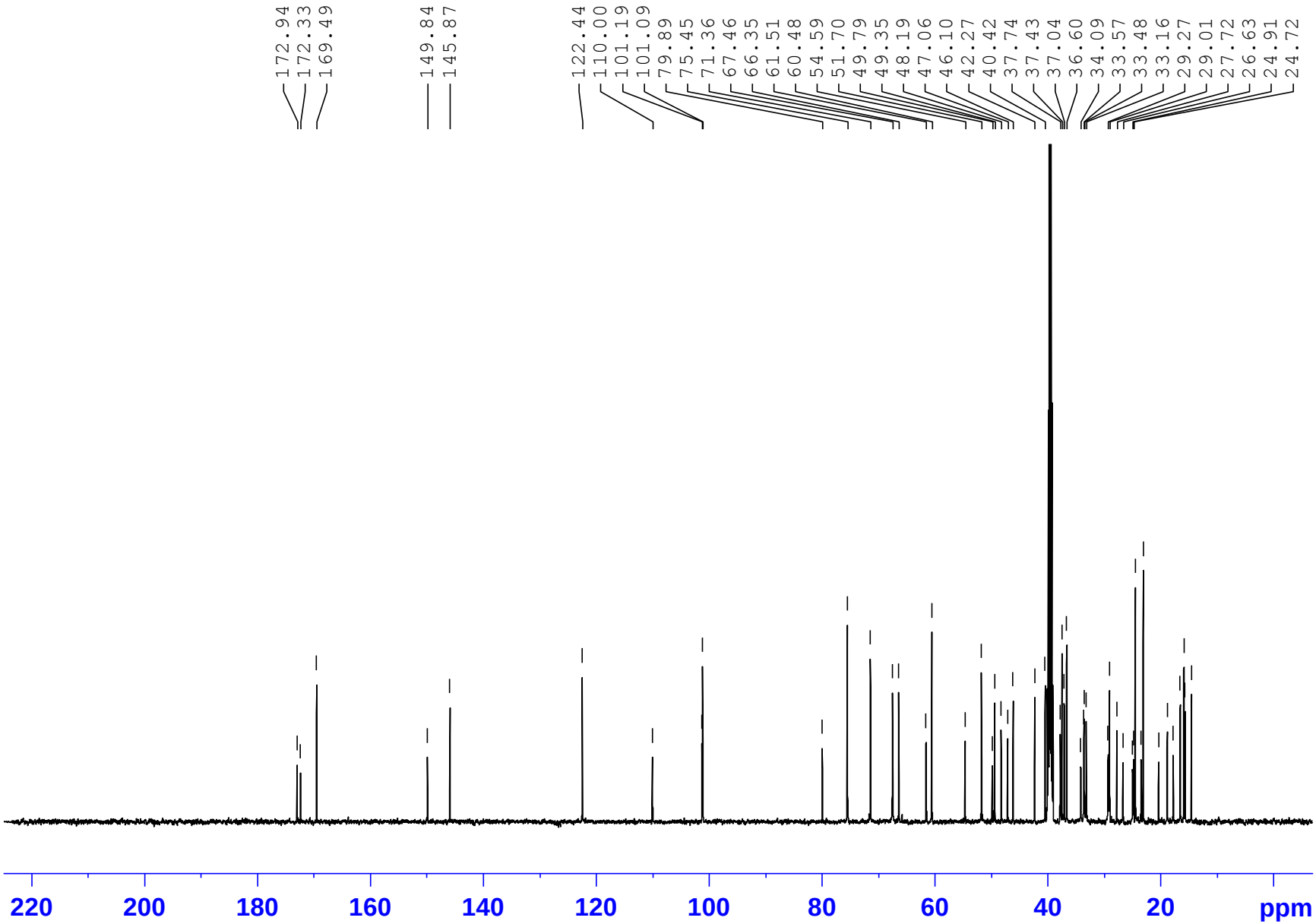
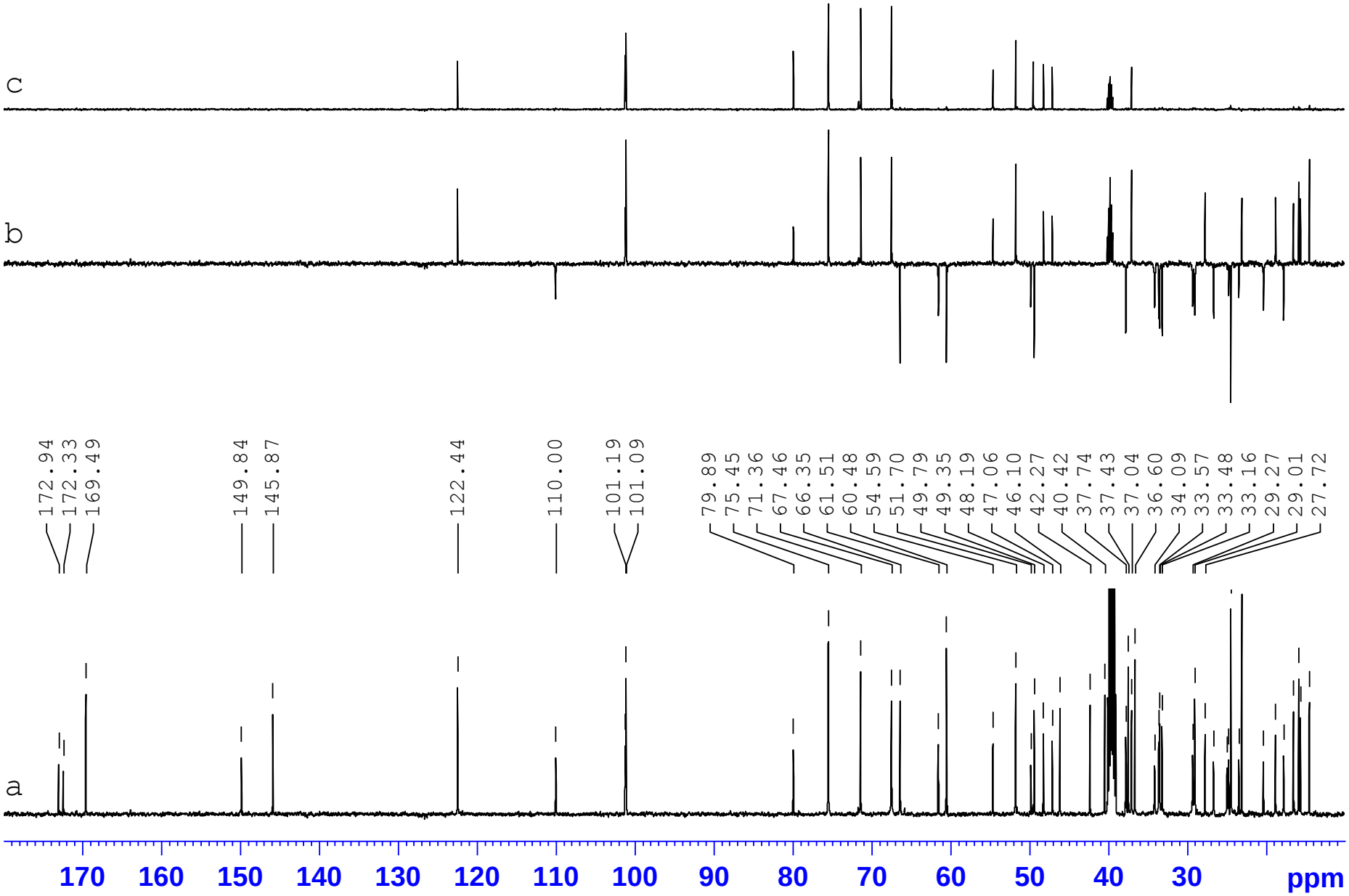


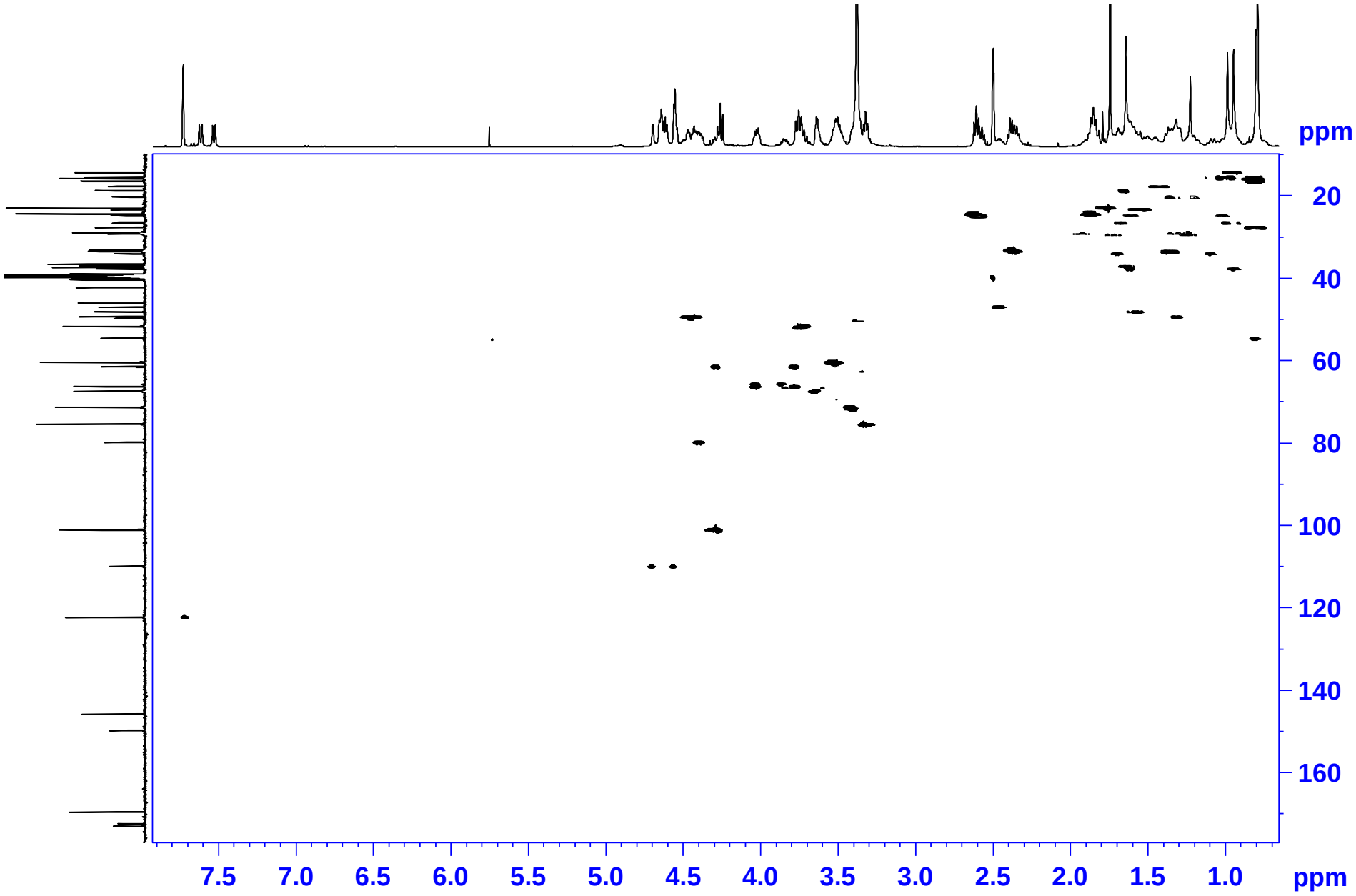
Complete ^{1}H NMR spectrum of Compound 8



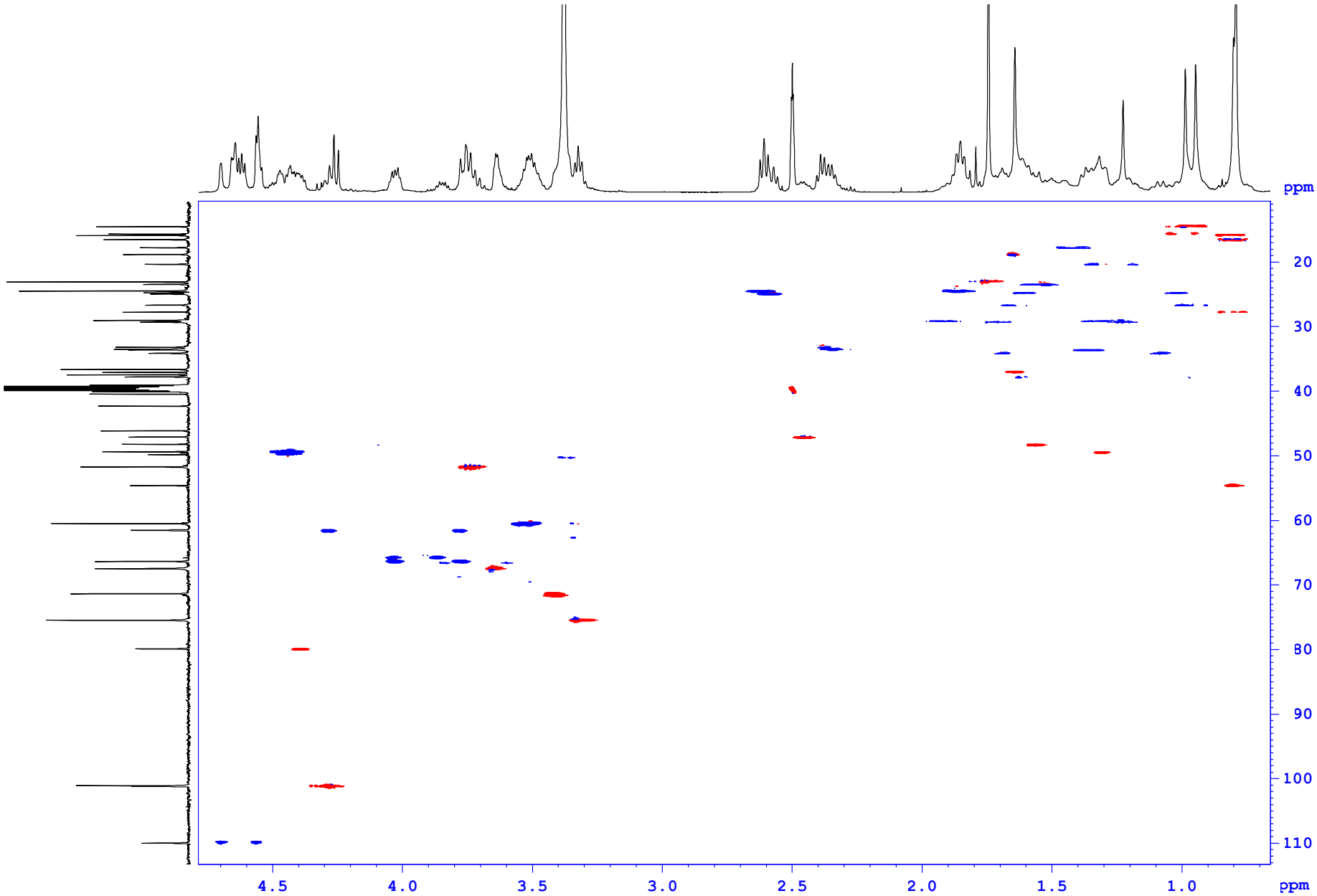
Complete $^{13}C\{^{1}H\}$ spectrum of Compound 8



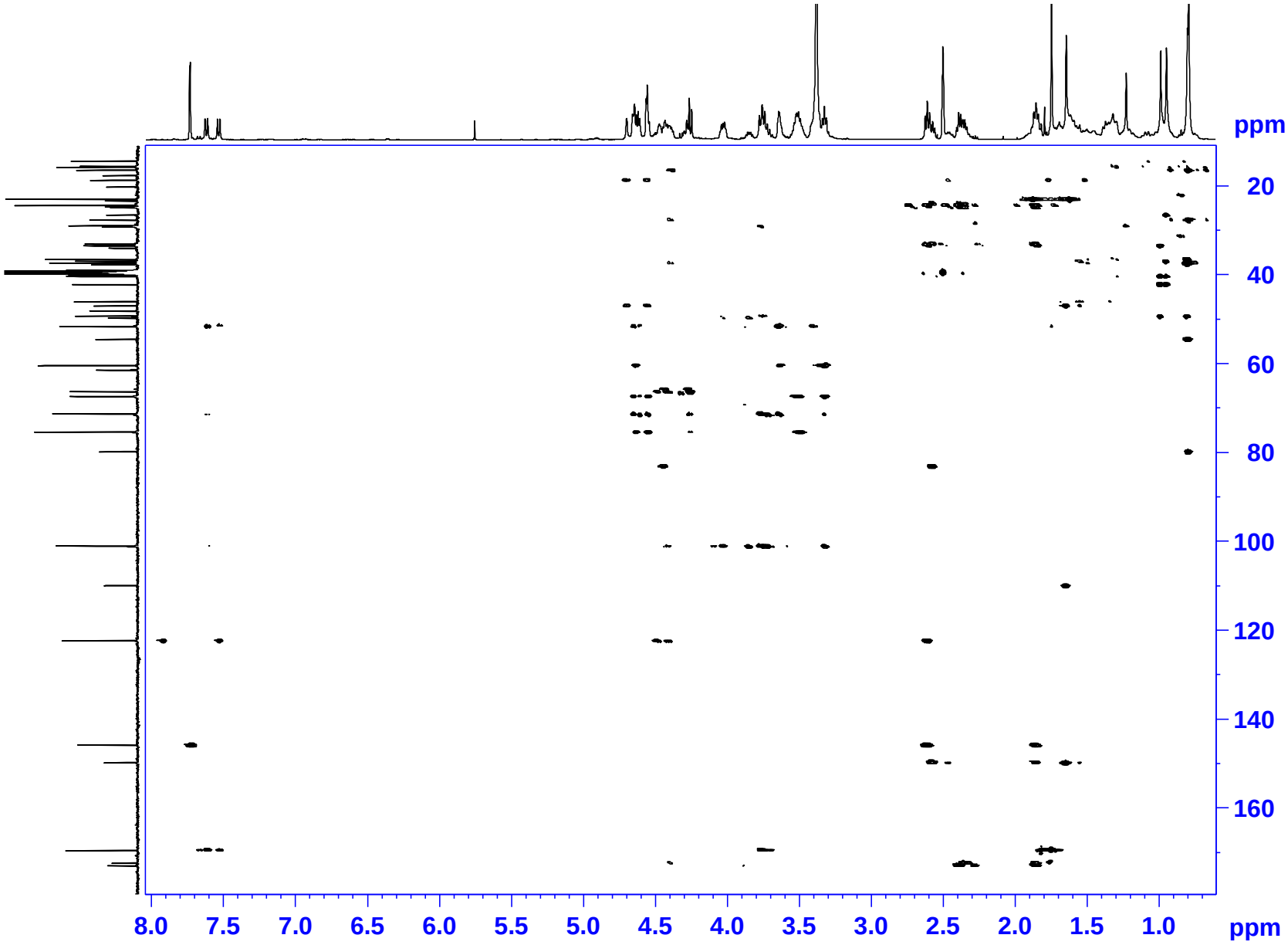
DEPT editing ^{13}C NMR spectrum of compound 8 in DMSO-*d6*: (a) ^{13}C{^{1}H} spectrum; (b) DEPT-135; (c) DEPT-90



{^{1}H, ^{13}C} HSQC spectrum of Compound 8



Expanded {^{1}H, ^{13}C} HSQC spectrum of Compound 8



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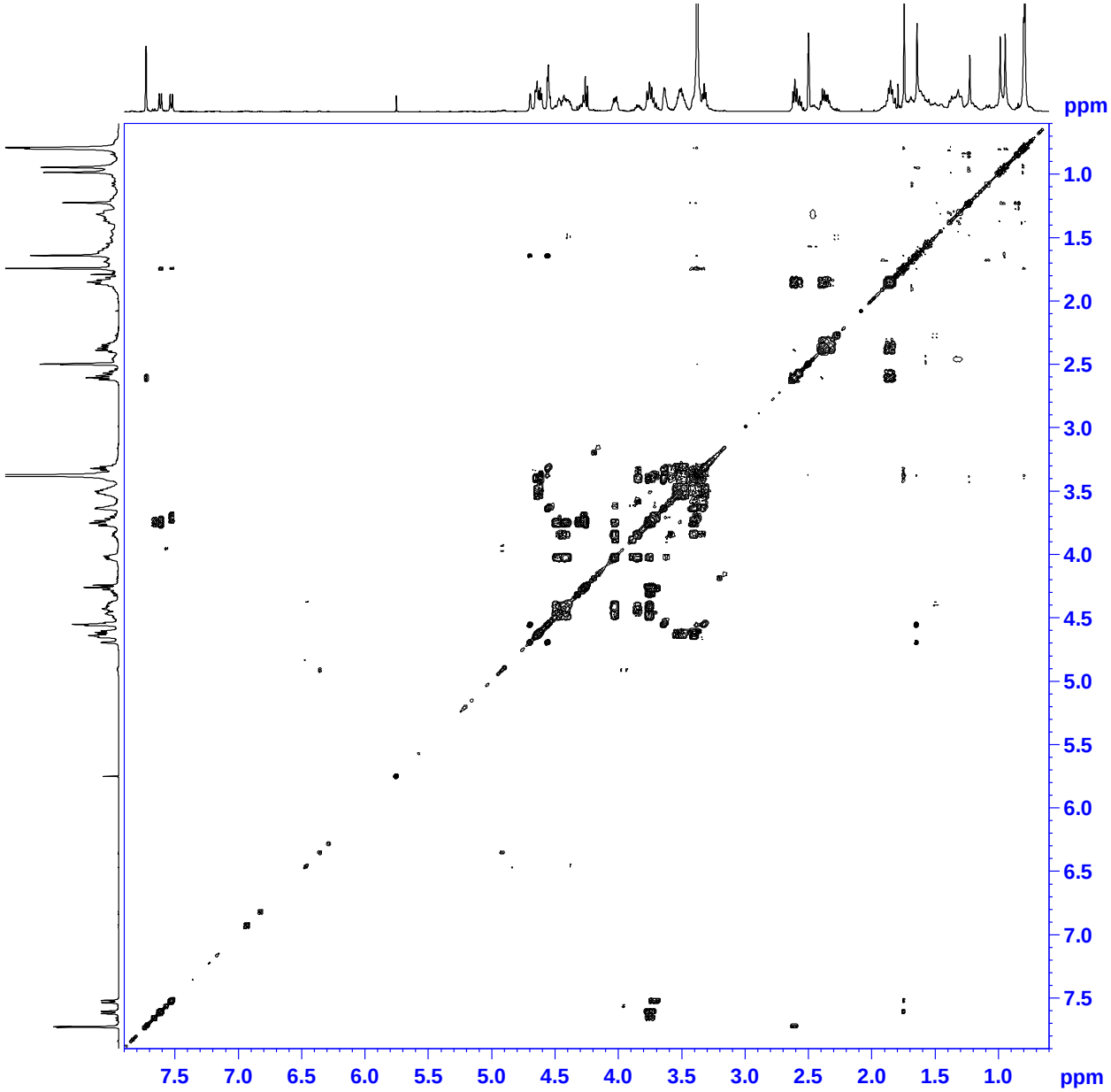
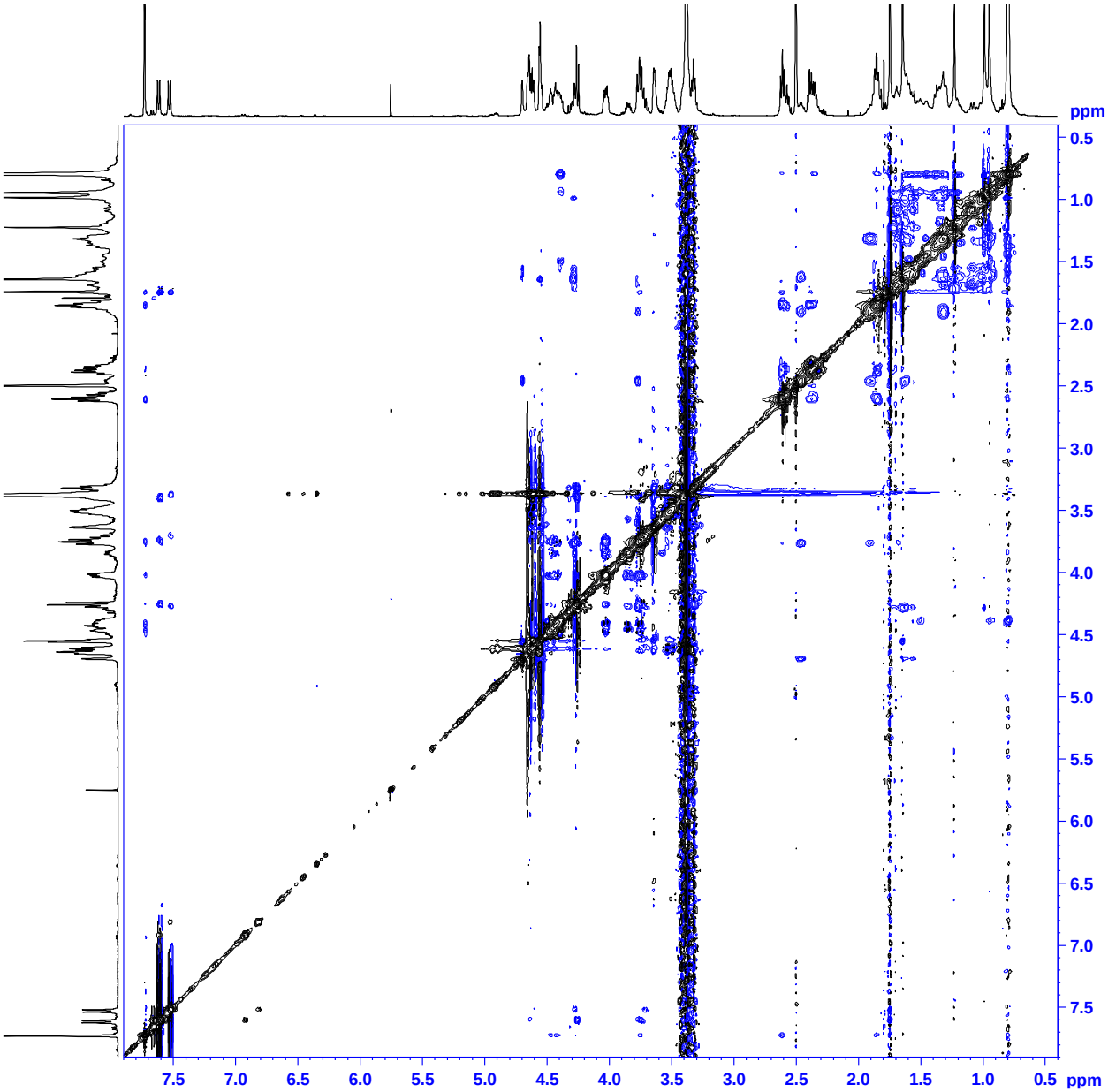
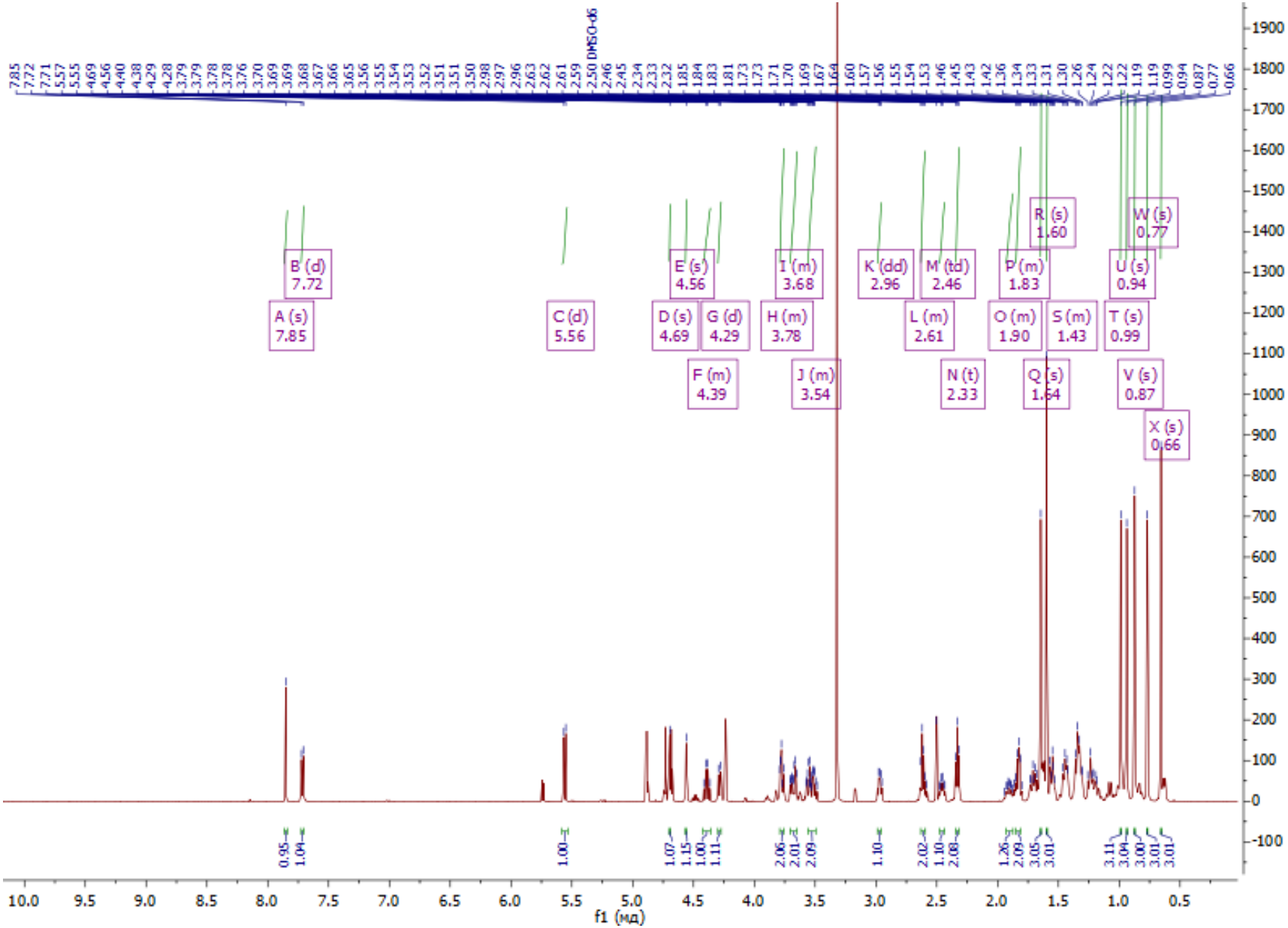


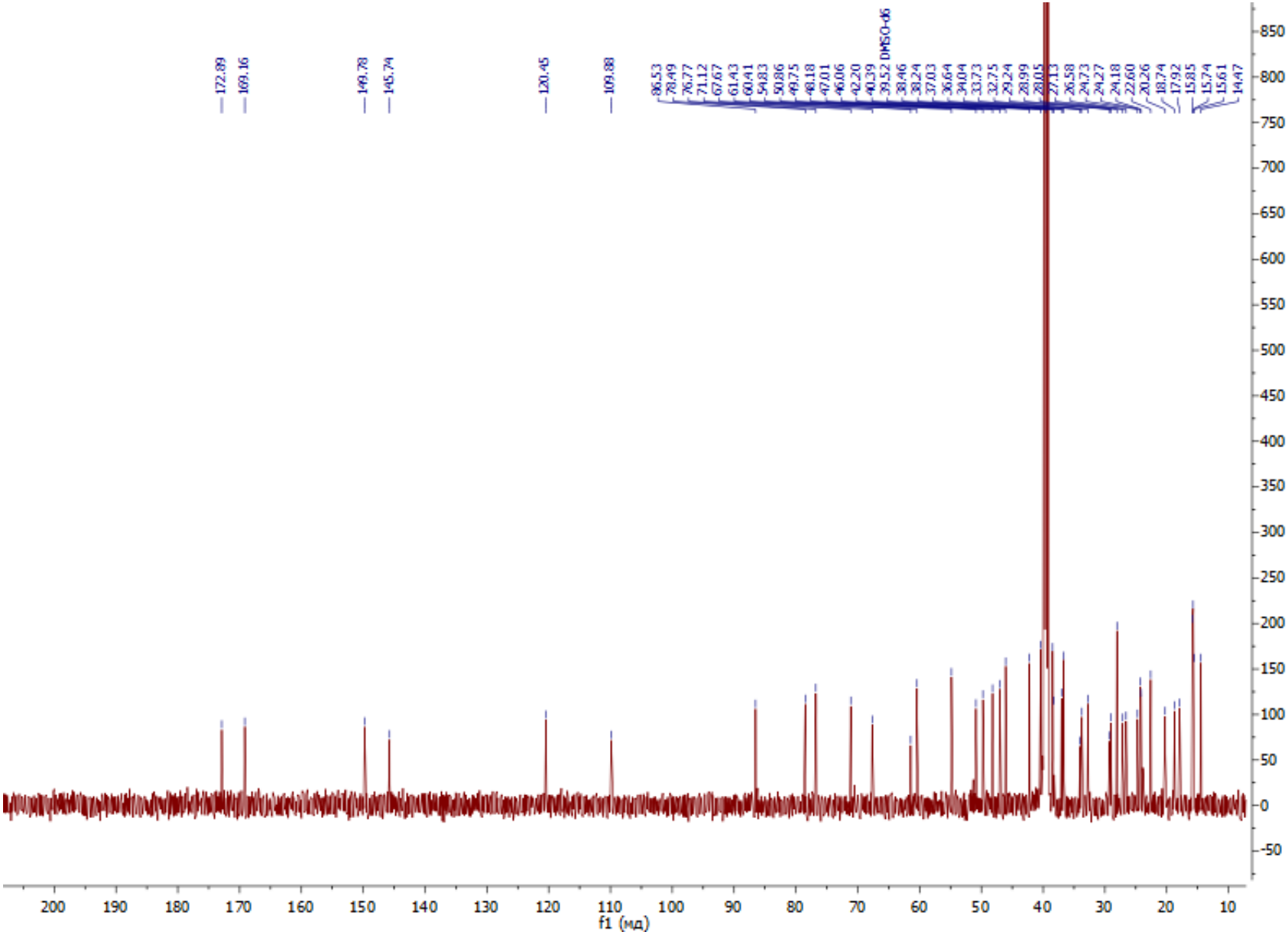
{^{1}H, ^{1}H} COSY-DQF spectrum of Compound 8



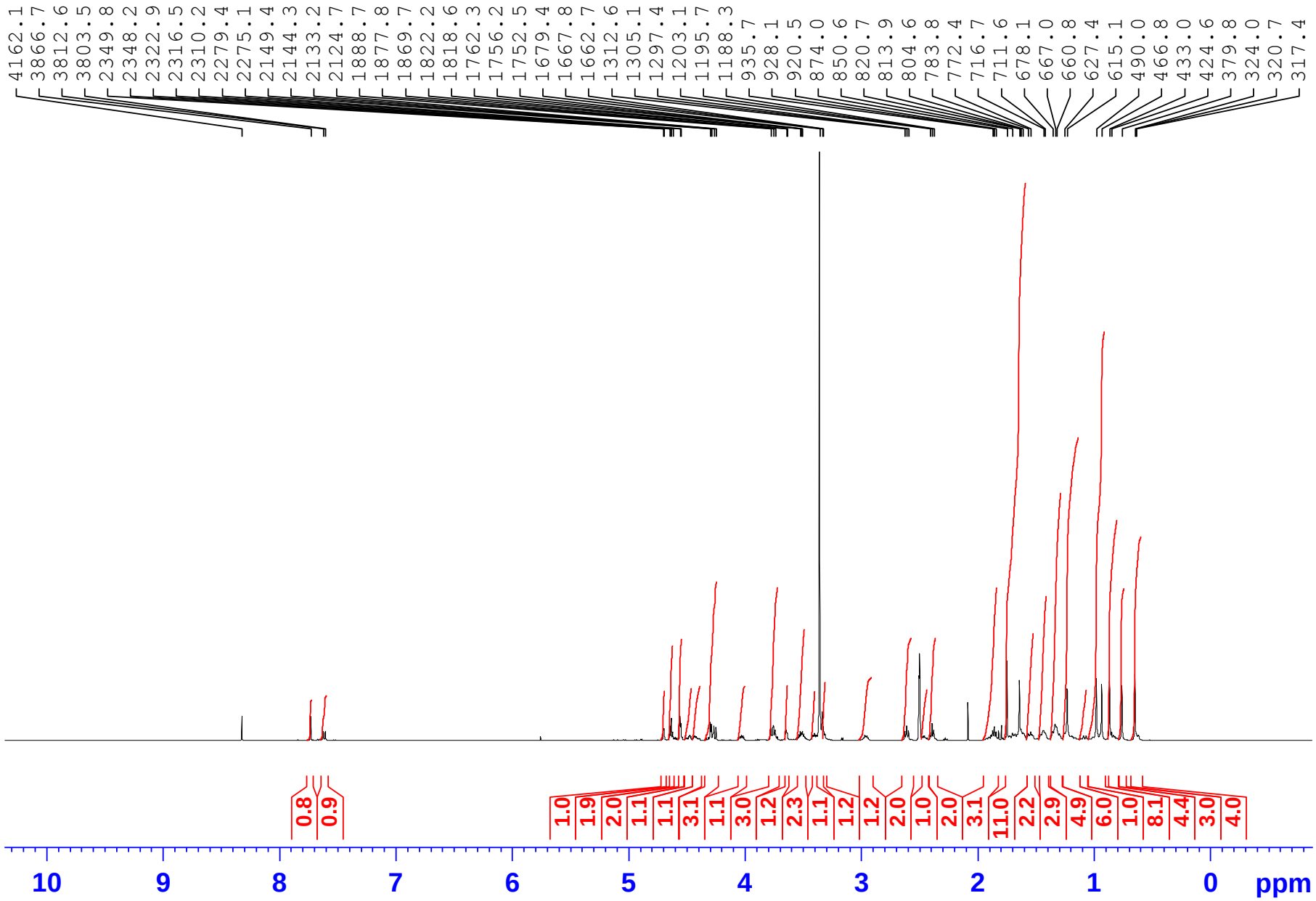
{^{1}H, ^{1}H} ROESY spectrum of Compound 8



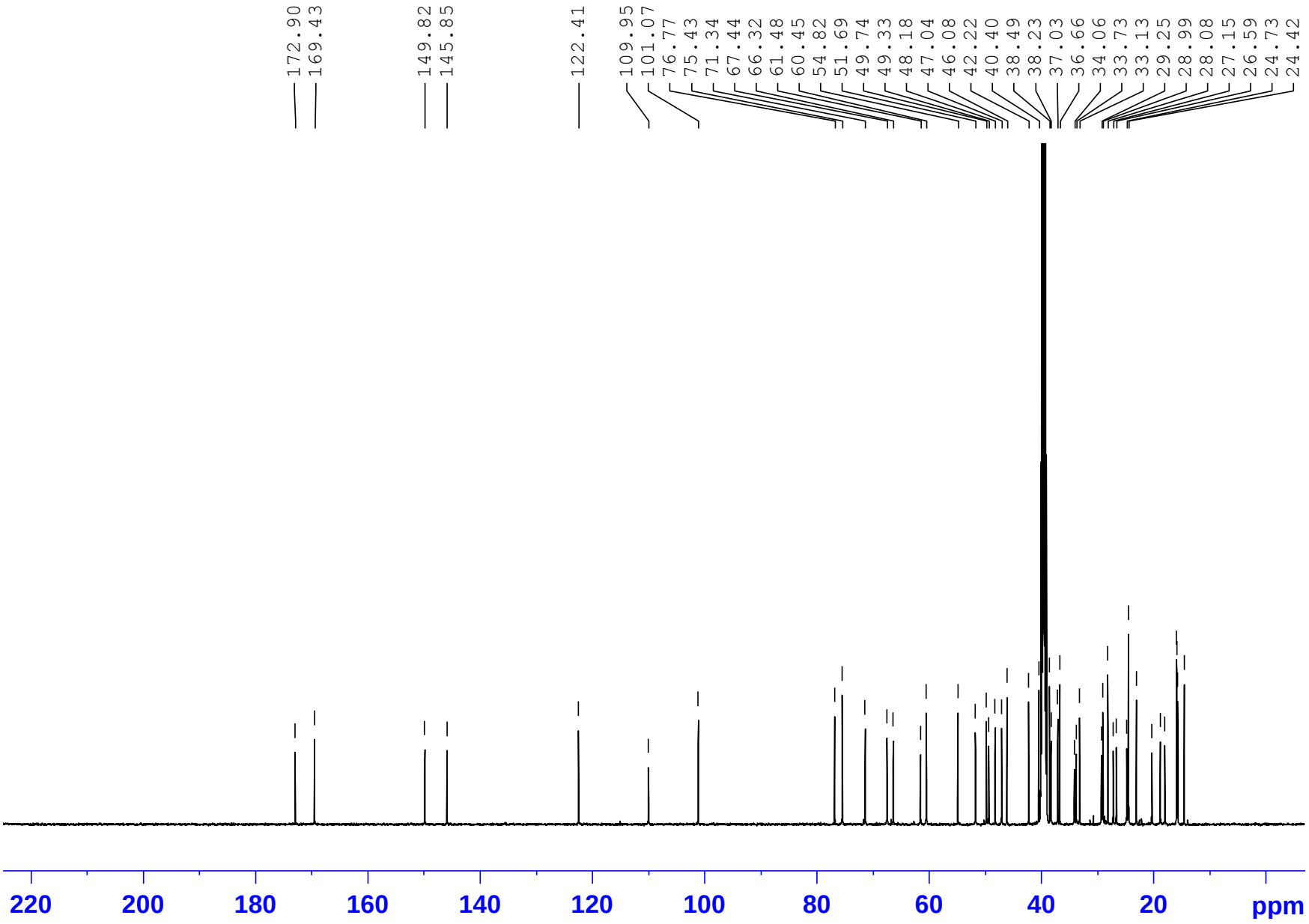
^{1}H NMR of Compound 9



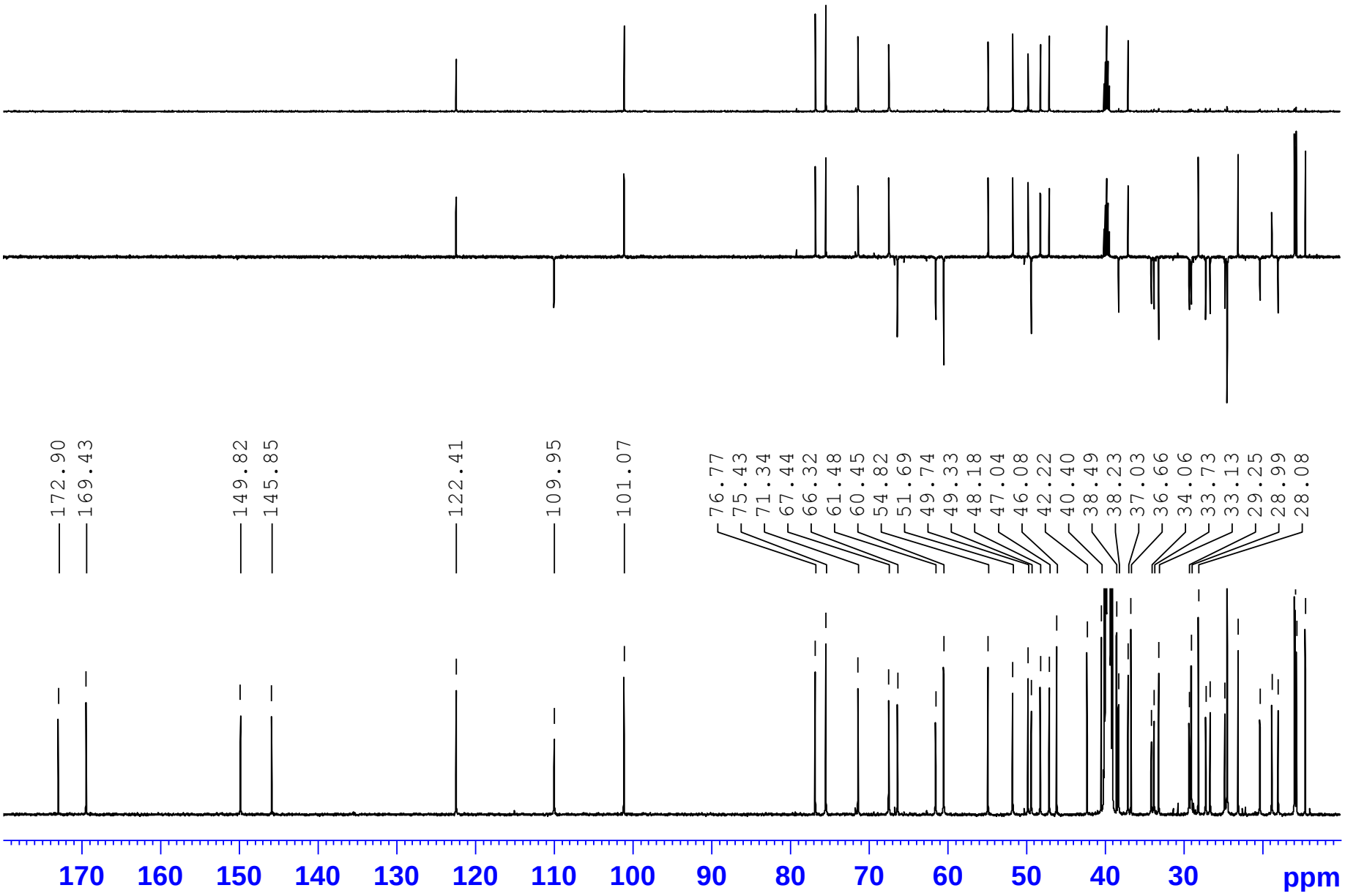
^{13}C NMR of Compound 9



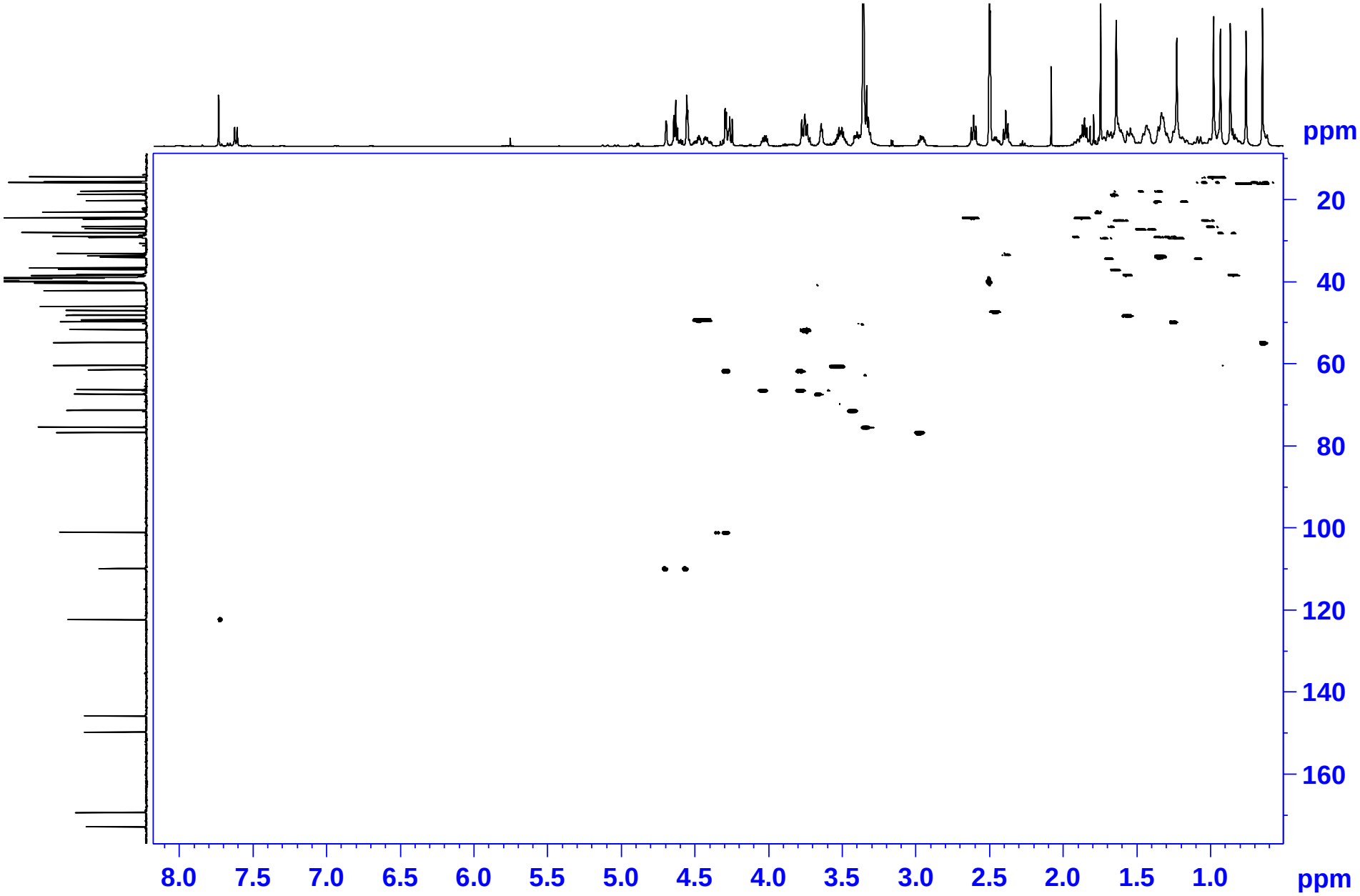
Complete ^{1}H NMR spectrum of Compound 10



Complete $^{13}C\{^{1}H\}$ spectrum of Compound 10



DEPT editing ^{13}C NMR spectrum of compound 10 in DMSO-d6: (a) $^{13}C\{^{1}H\}$ spectrum; (b) DEPT-135; (c) DEPT-90



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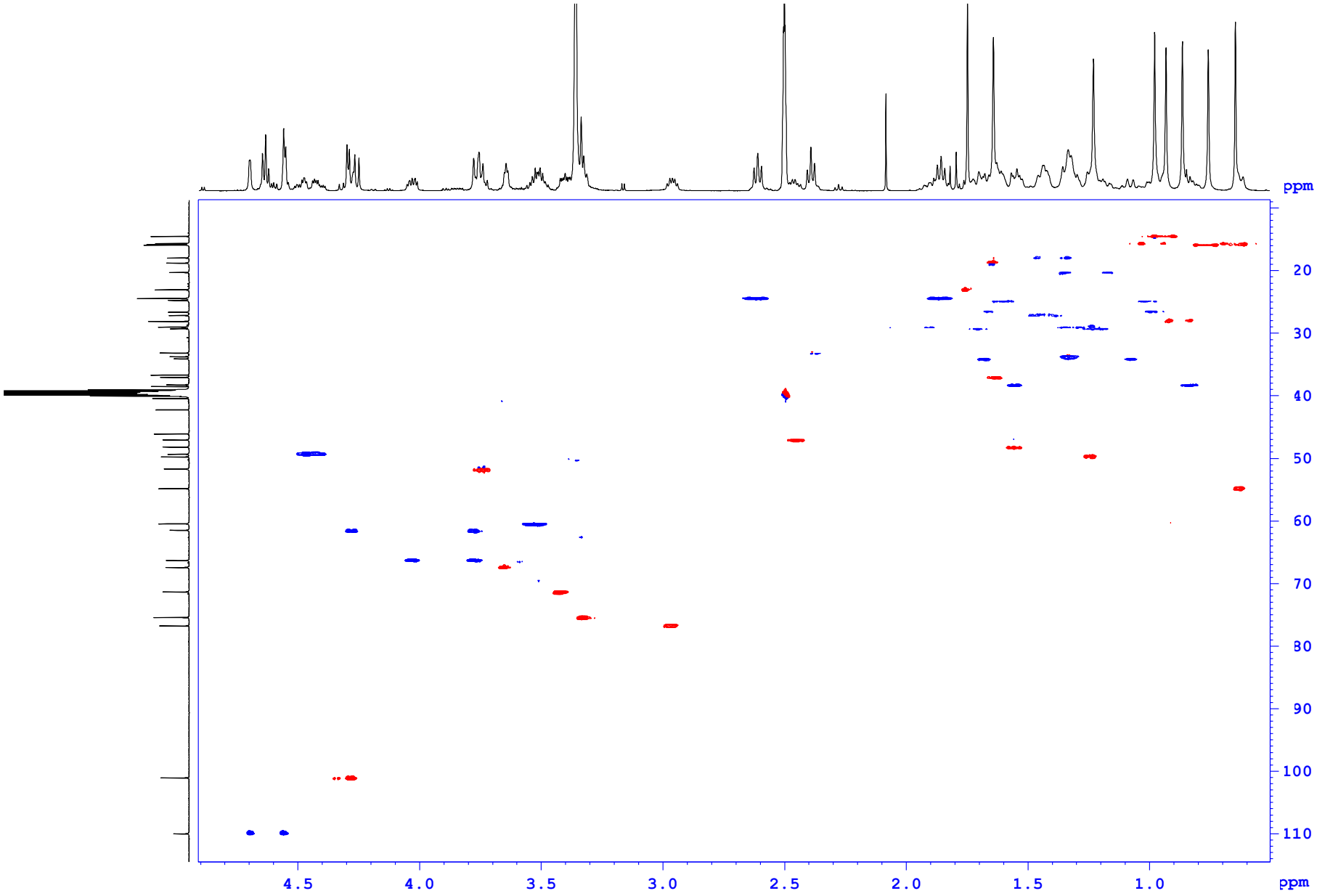

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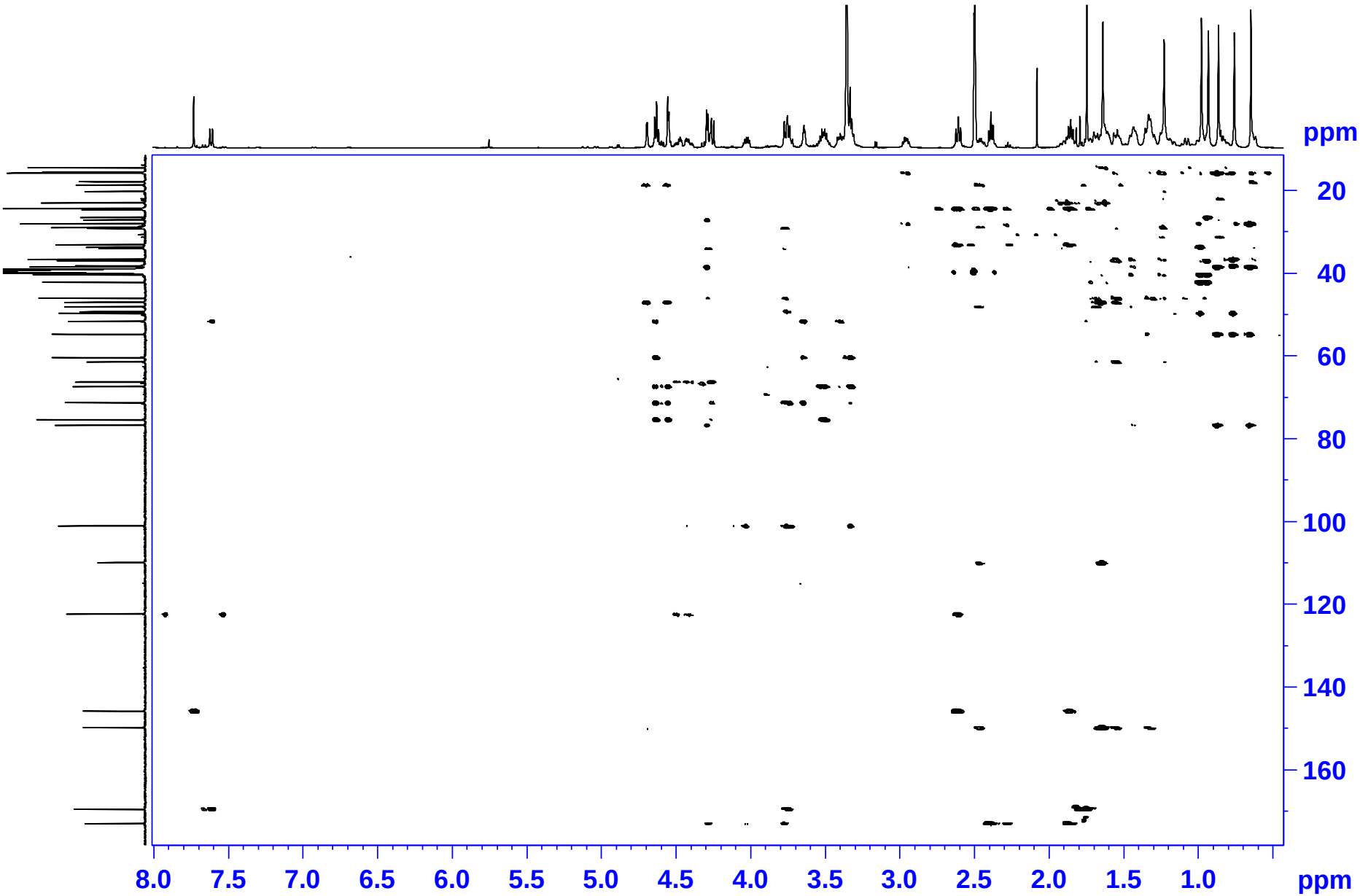

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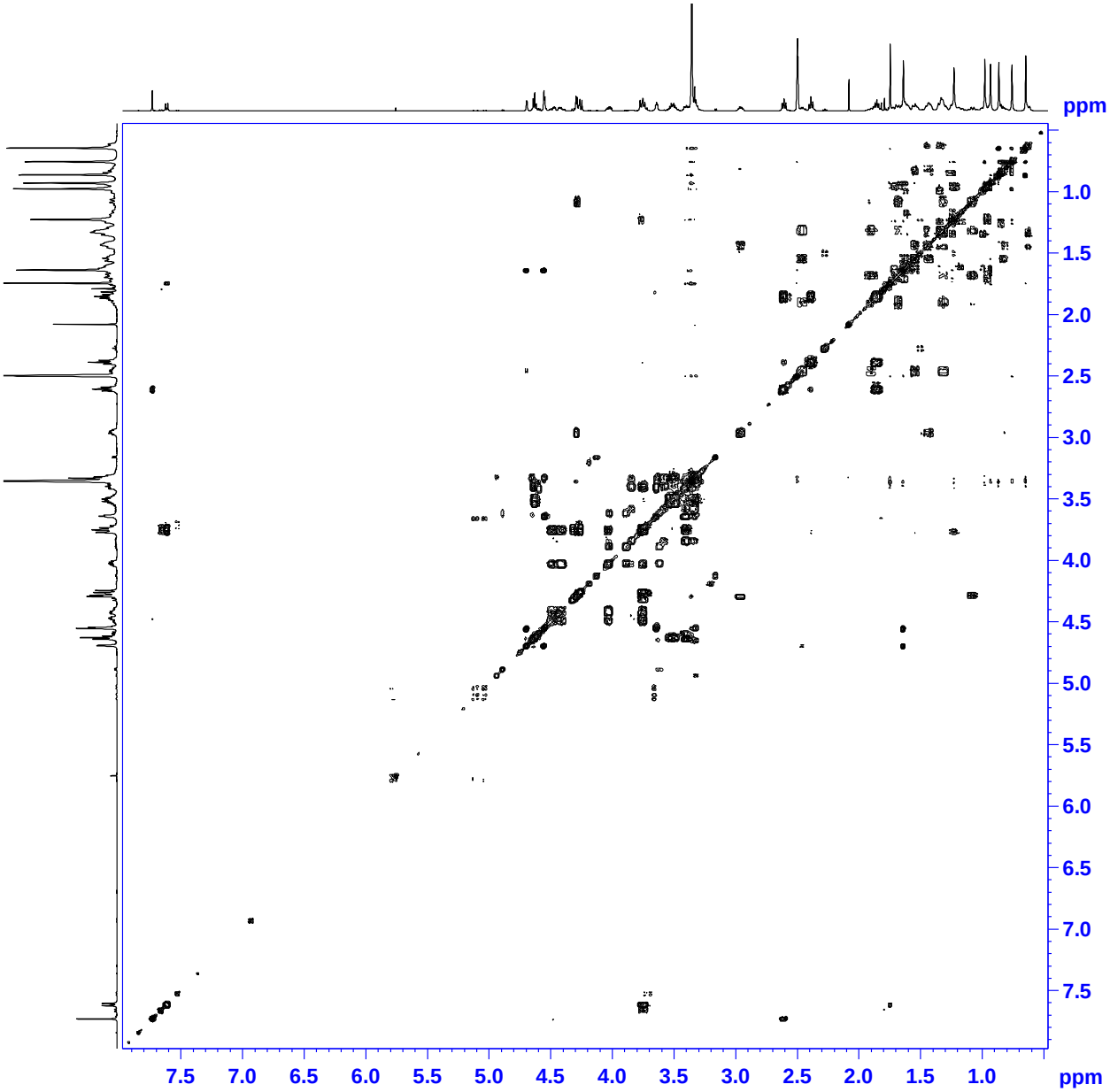

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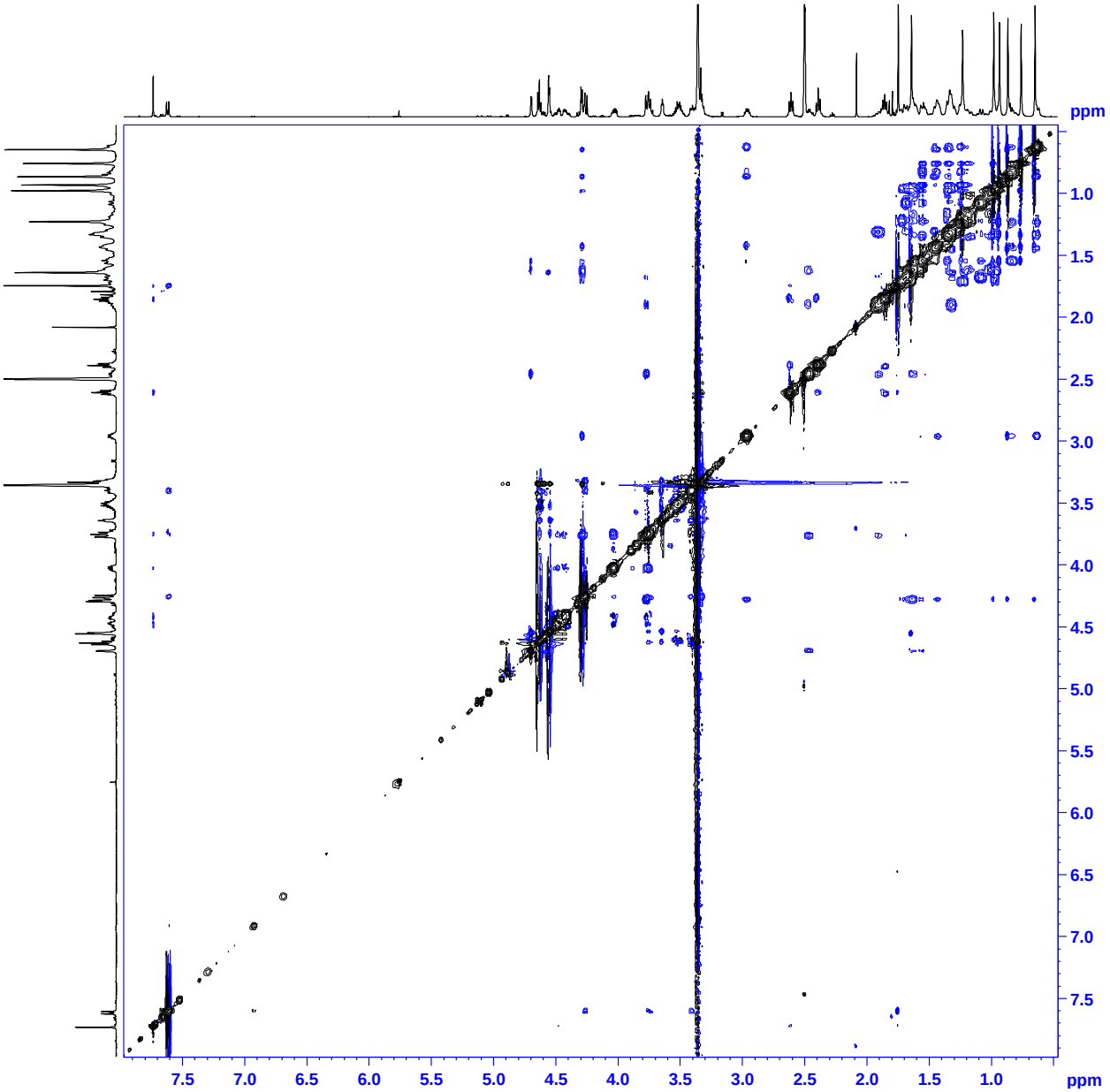

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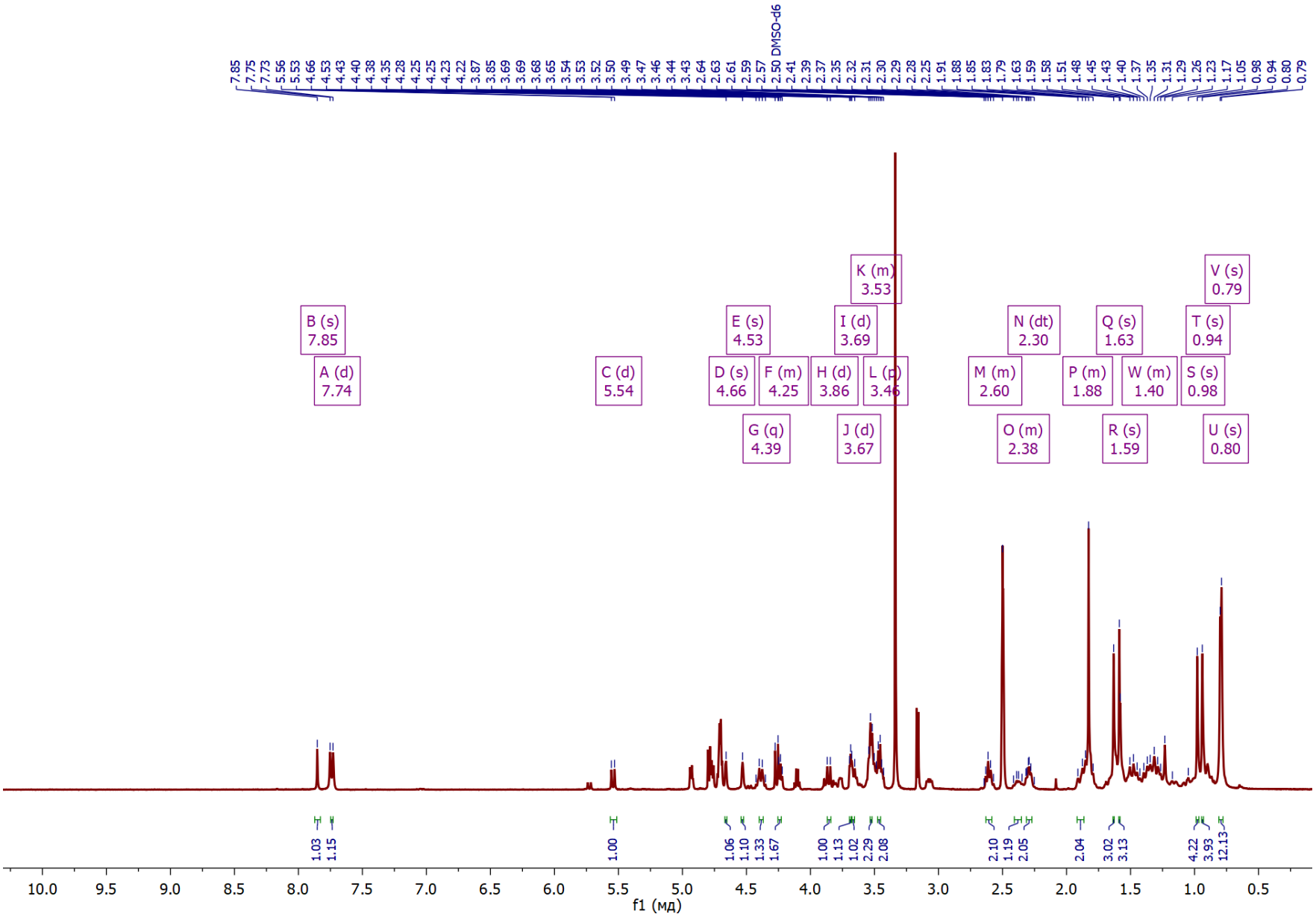

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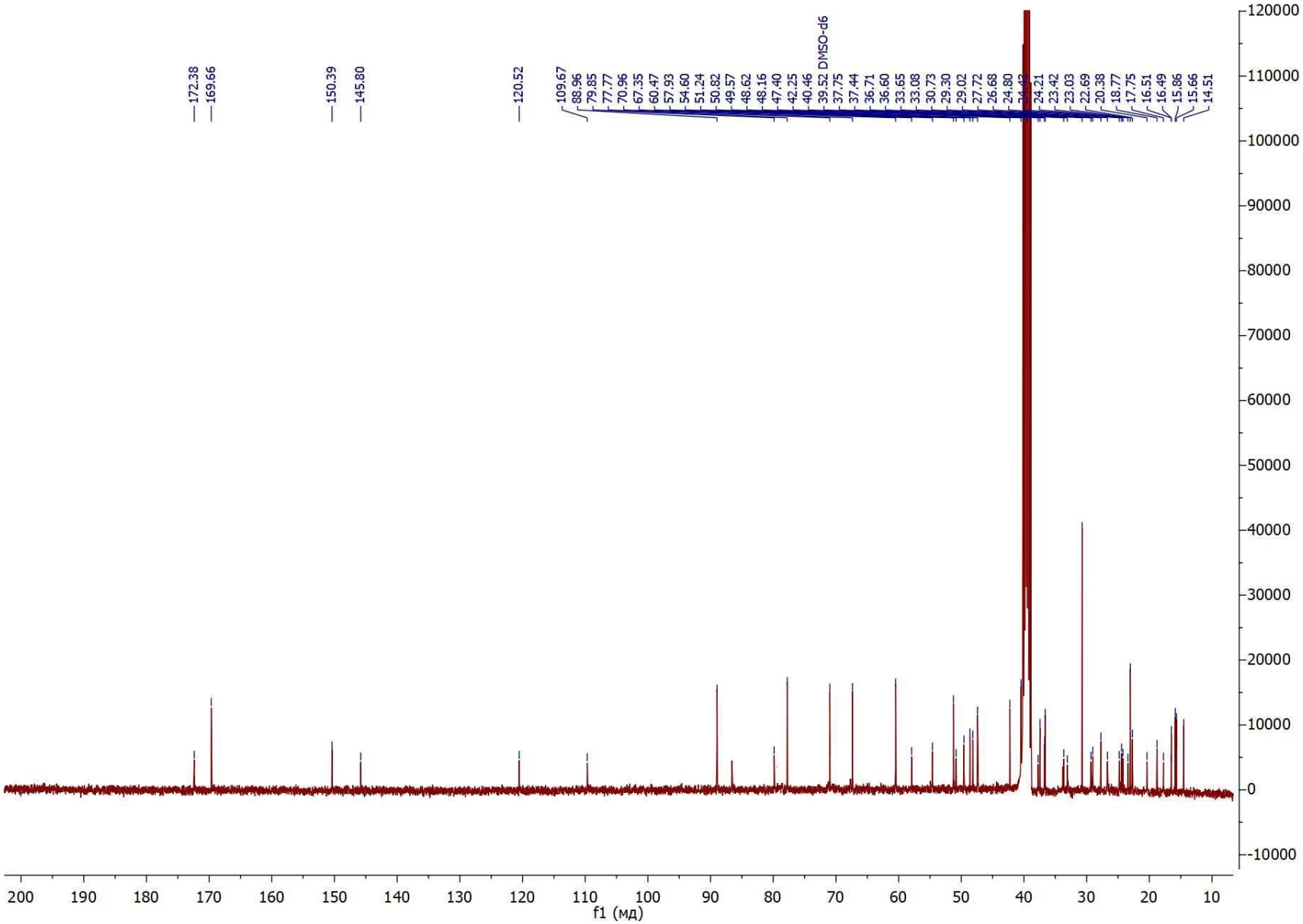

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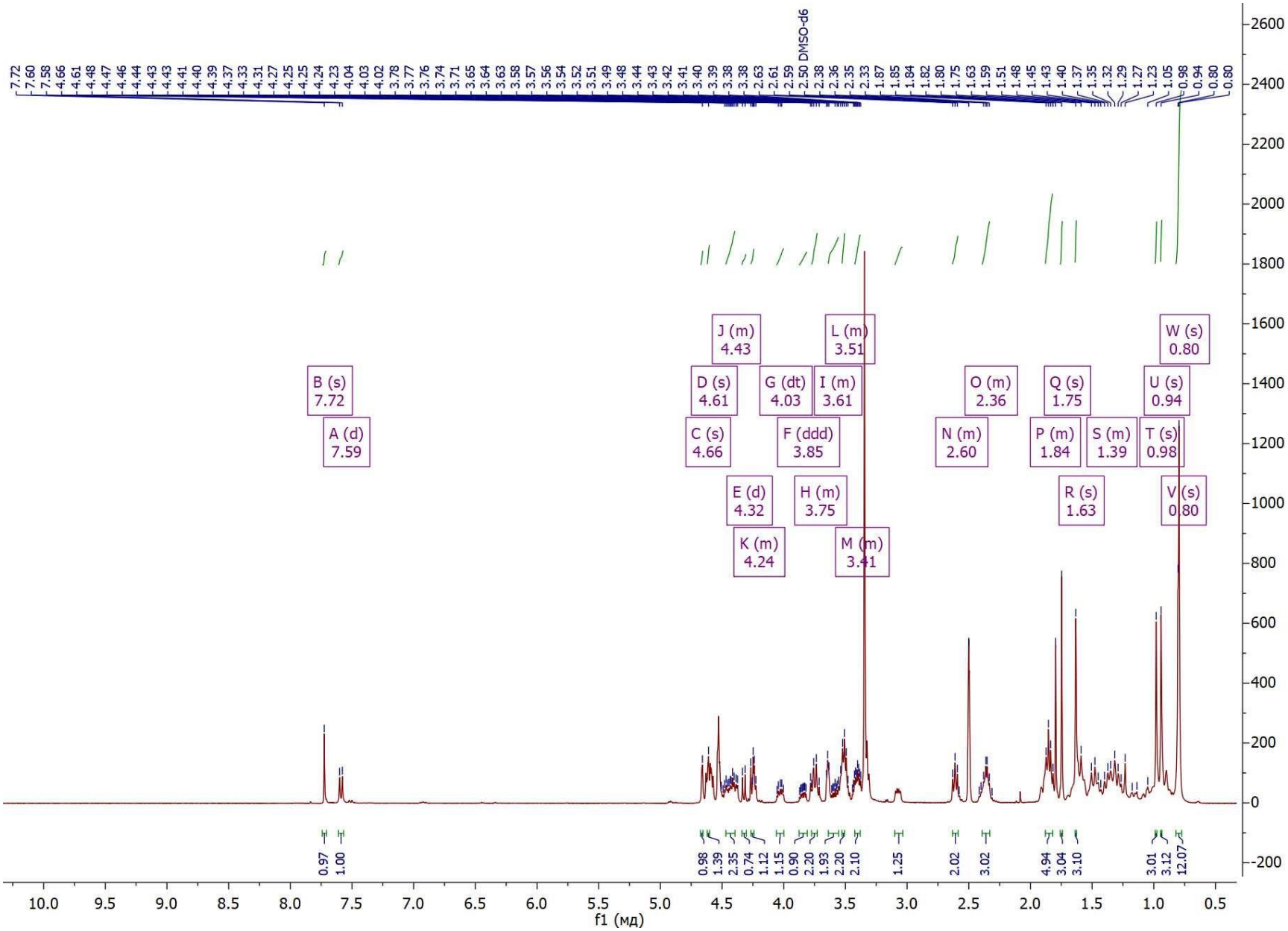

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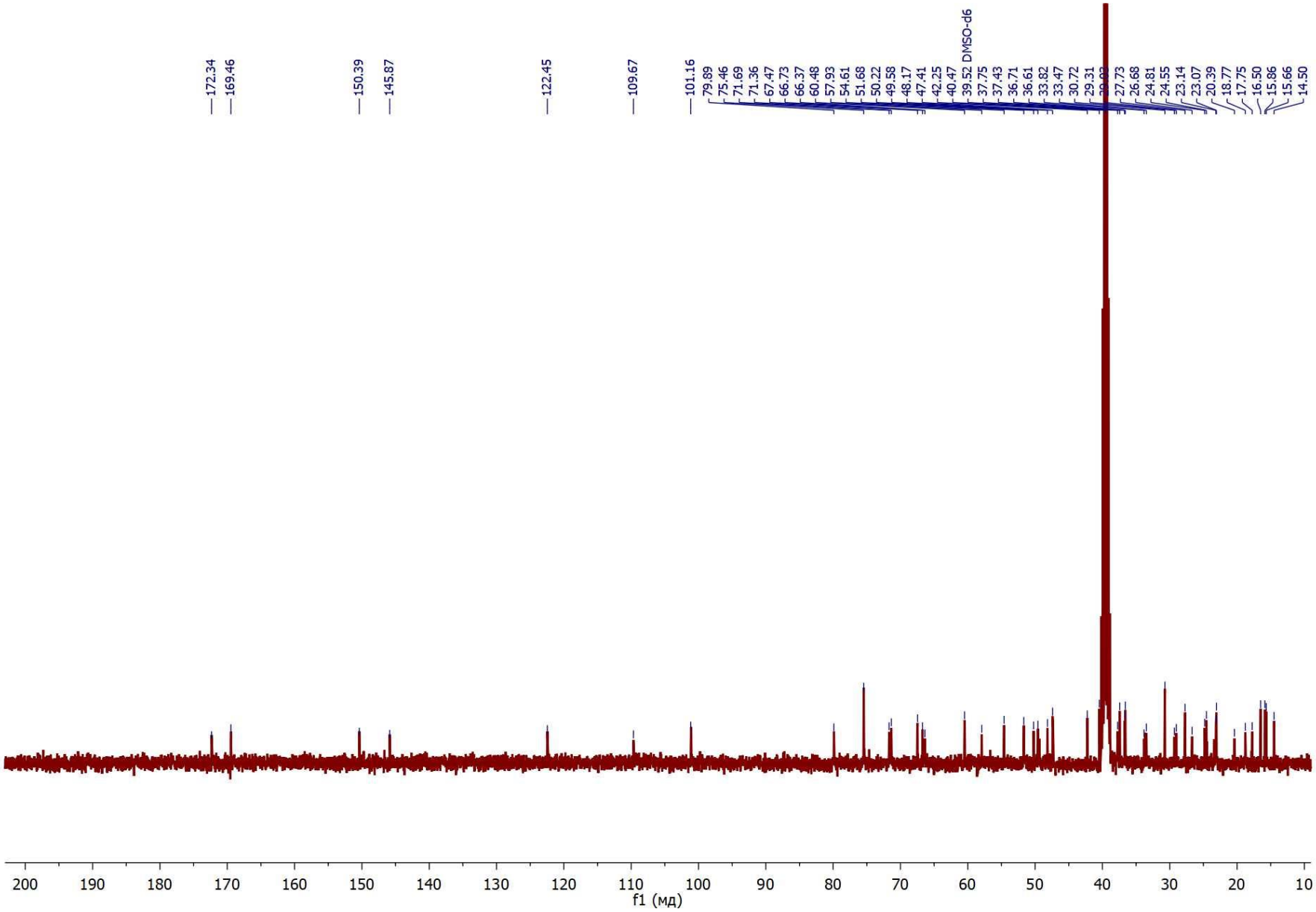

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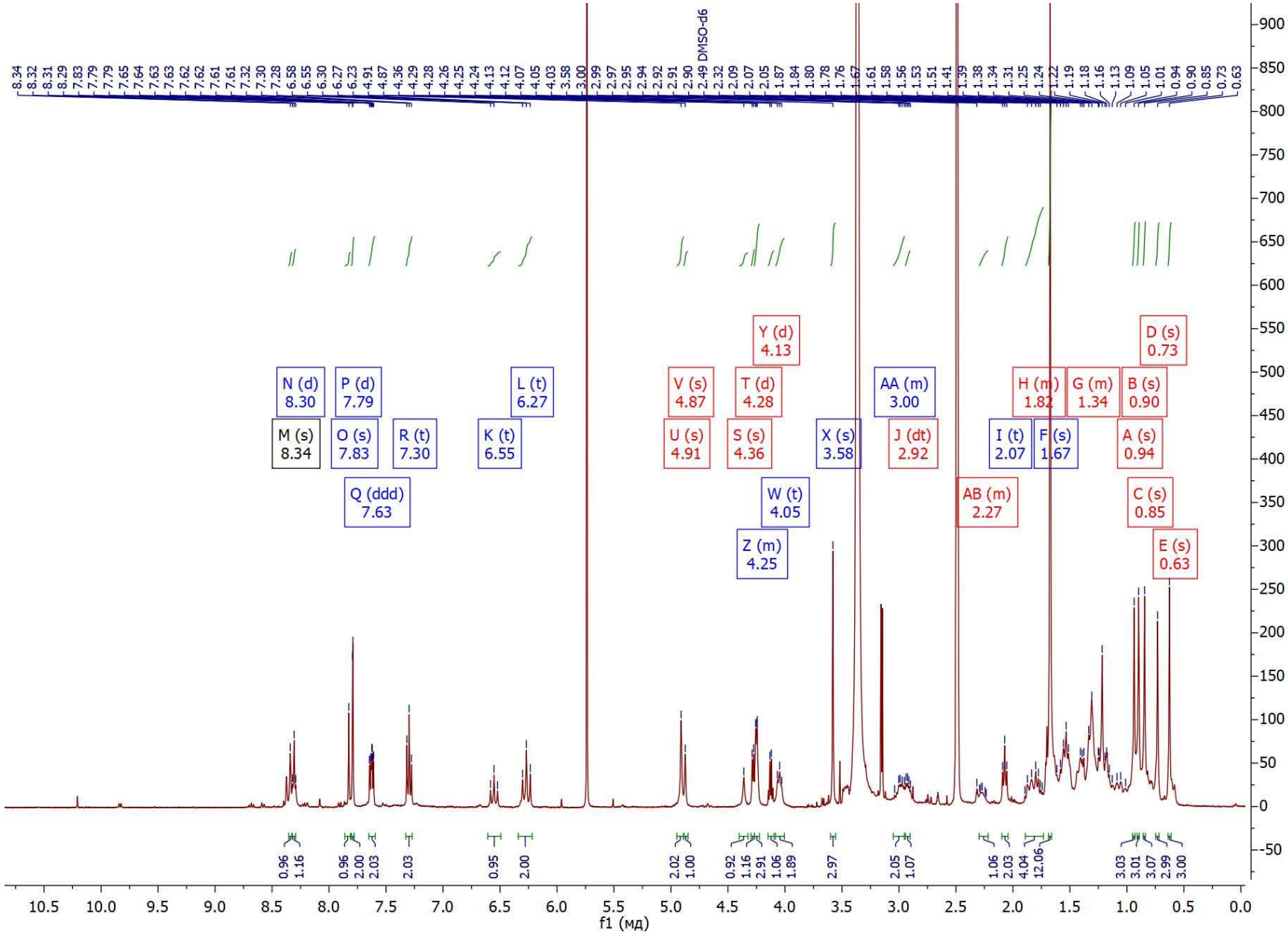
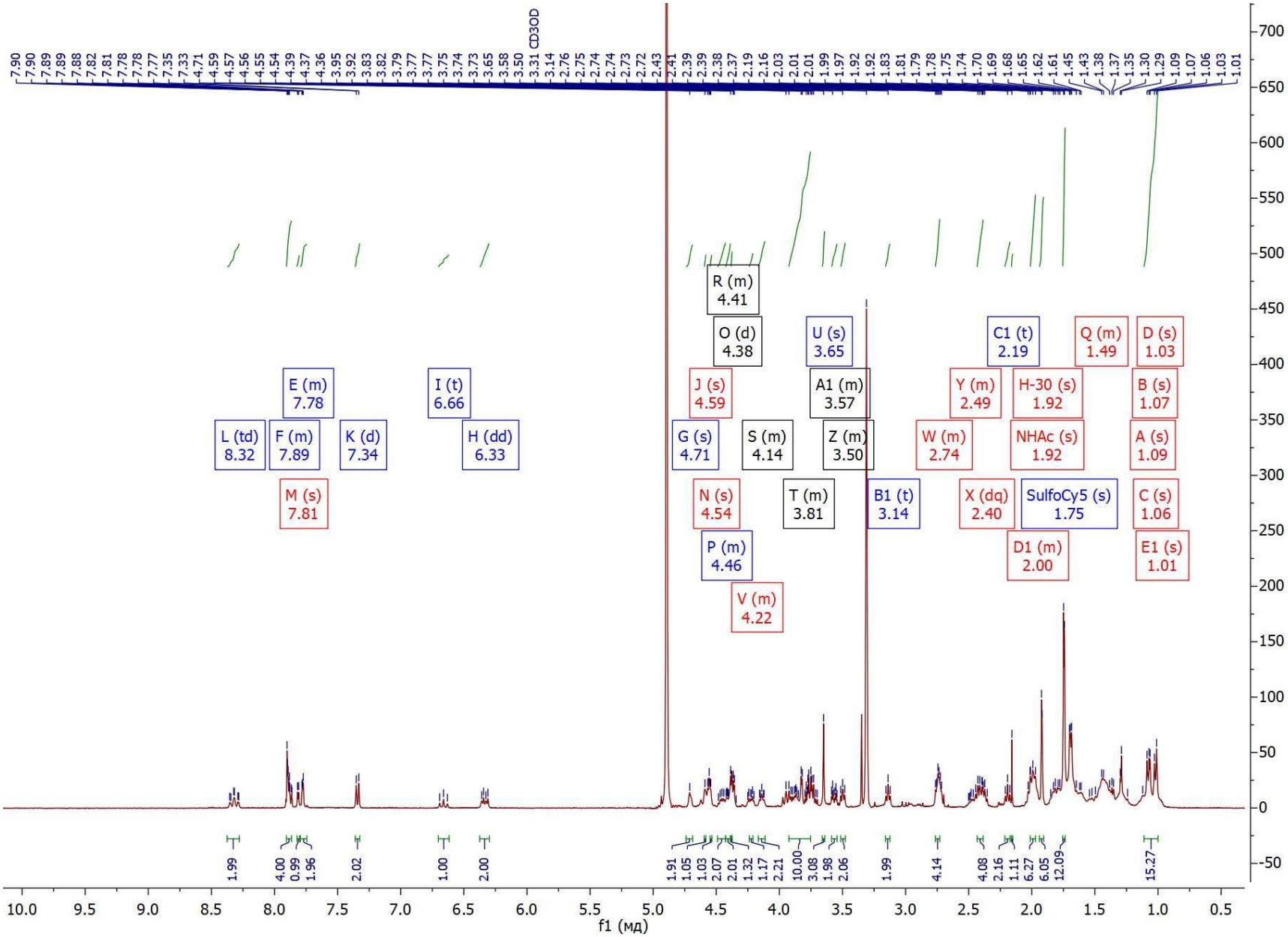


^{1}H NMR of Compound 14



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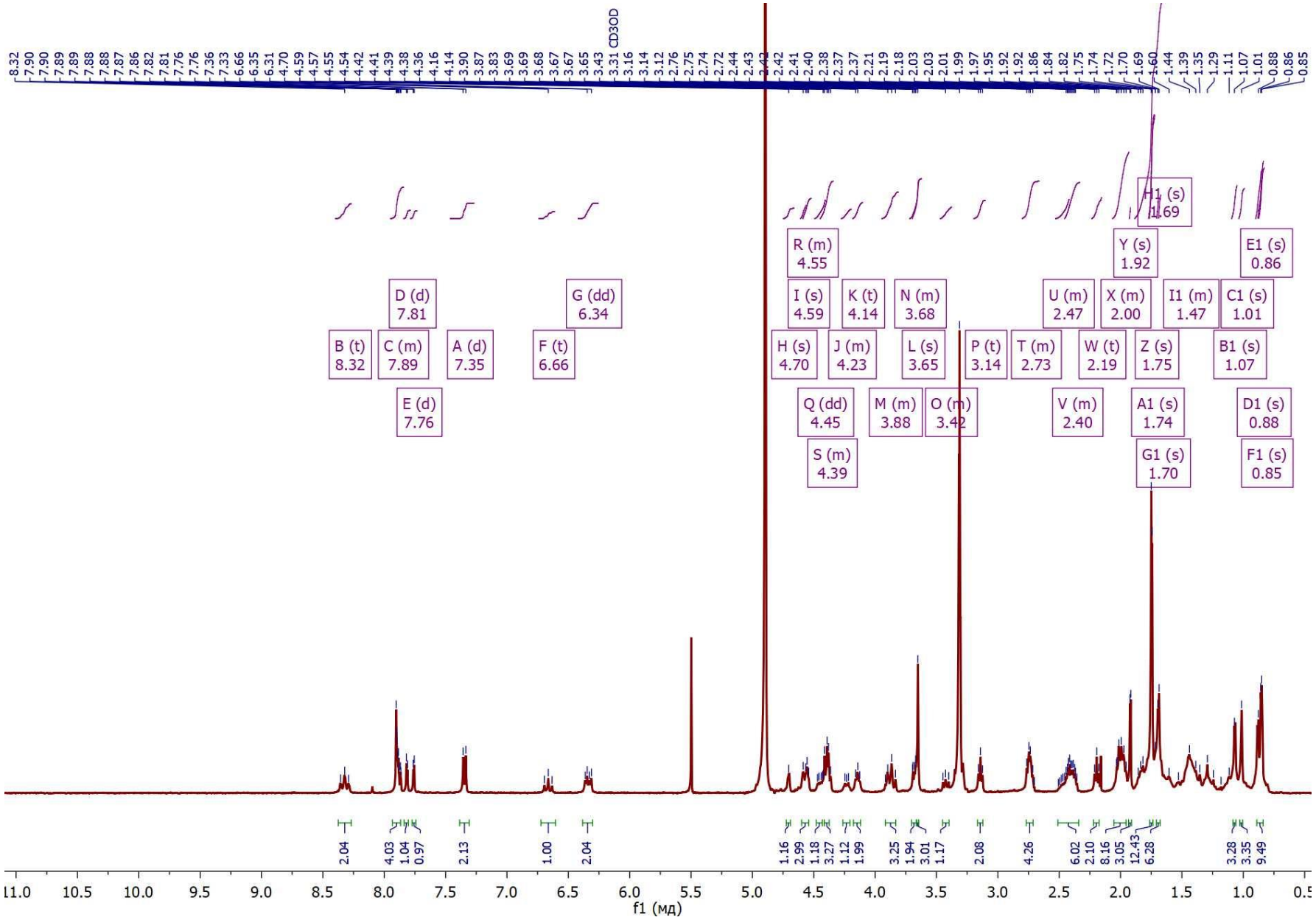
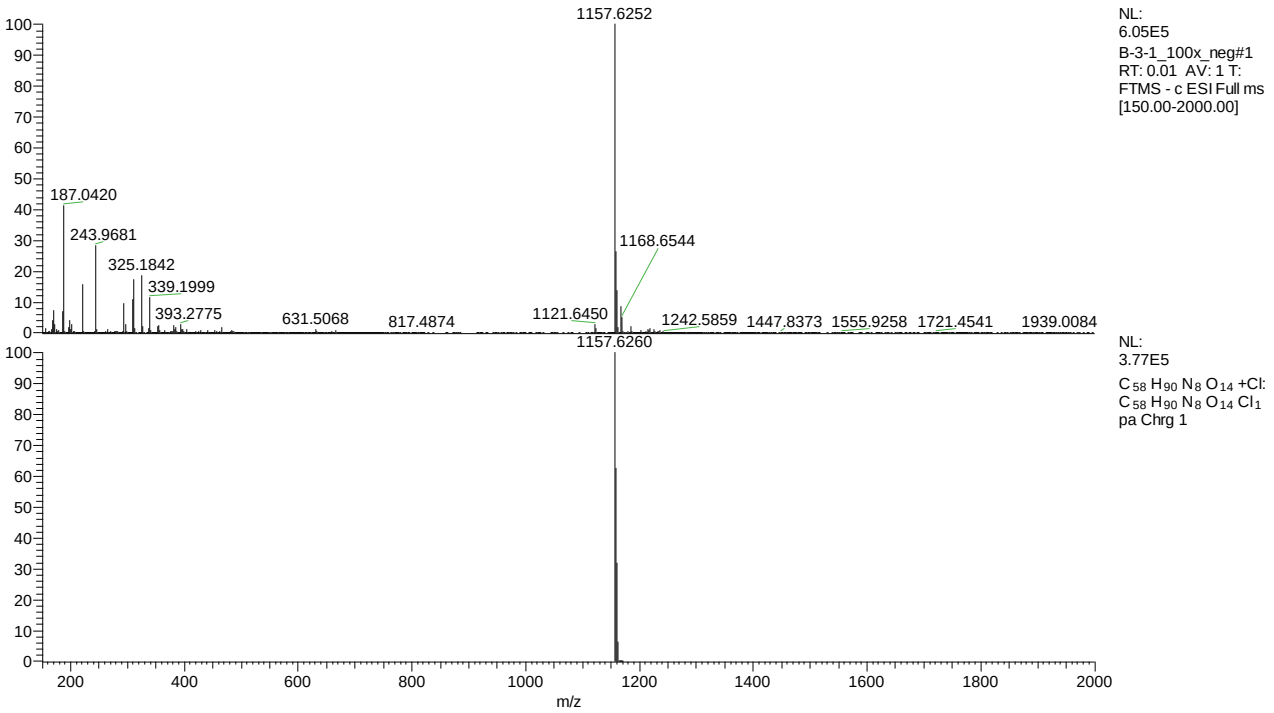
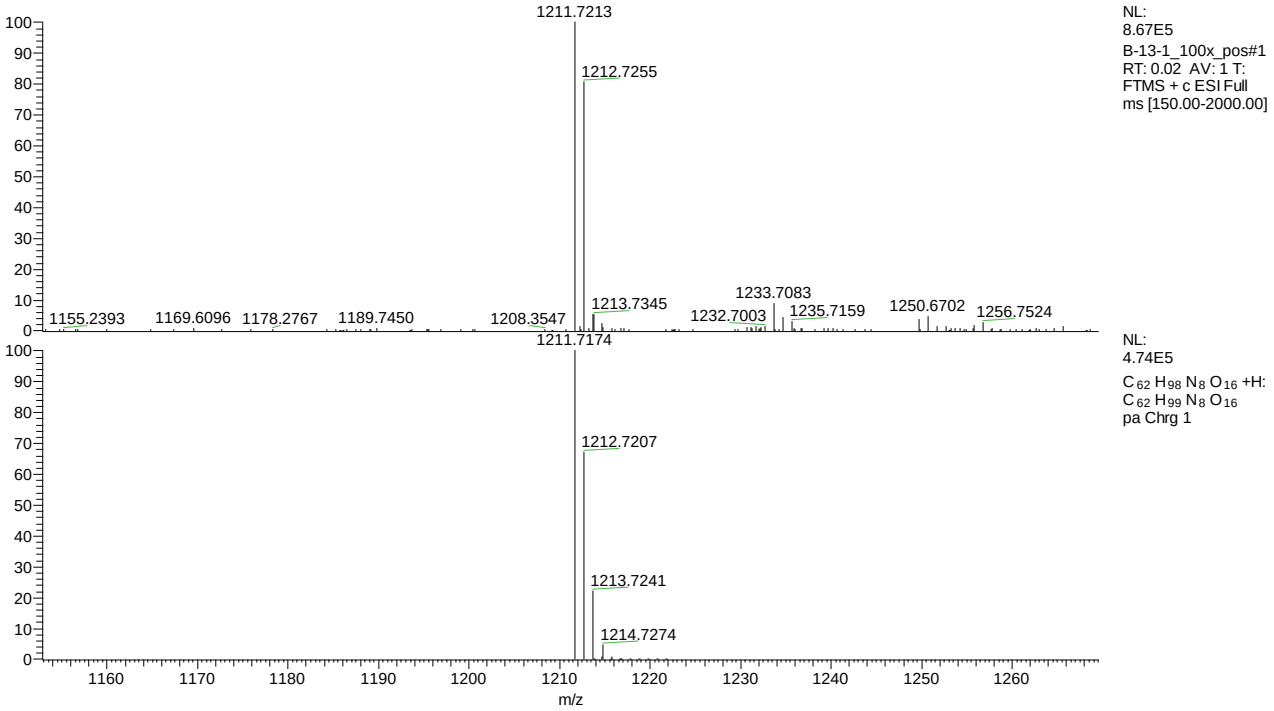


^{1}H NMR of Compound 17

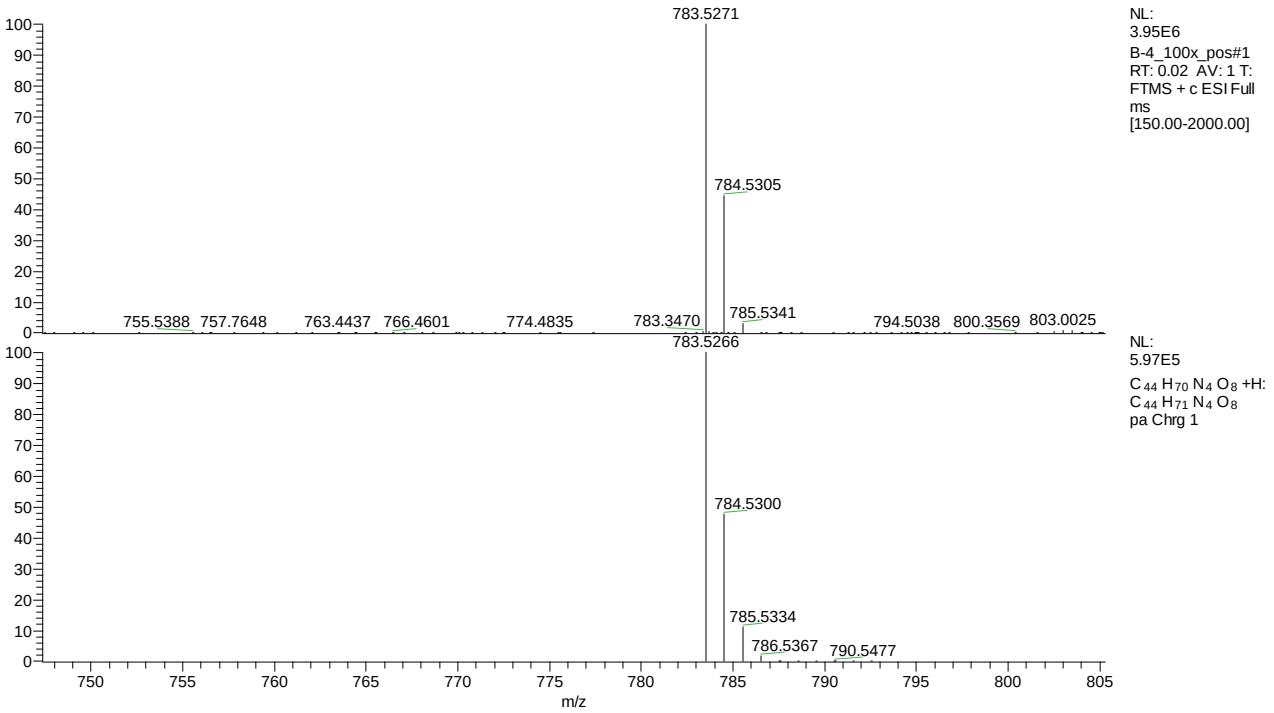
ESI-HRMS spectrum of Compound **7**



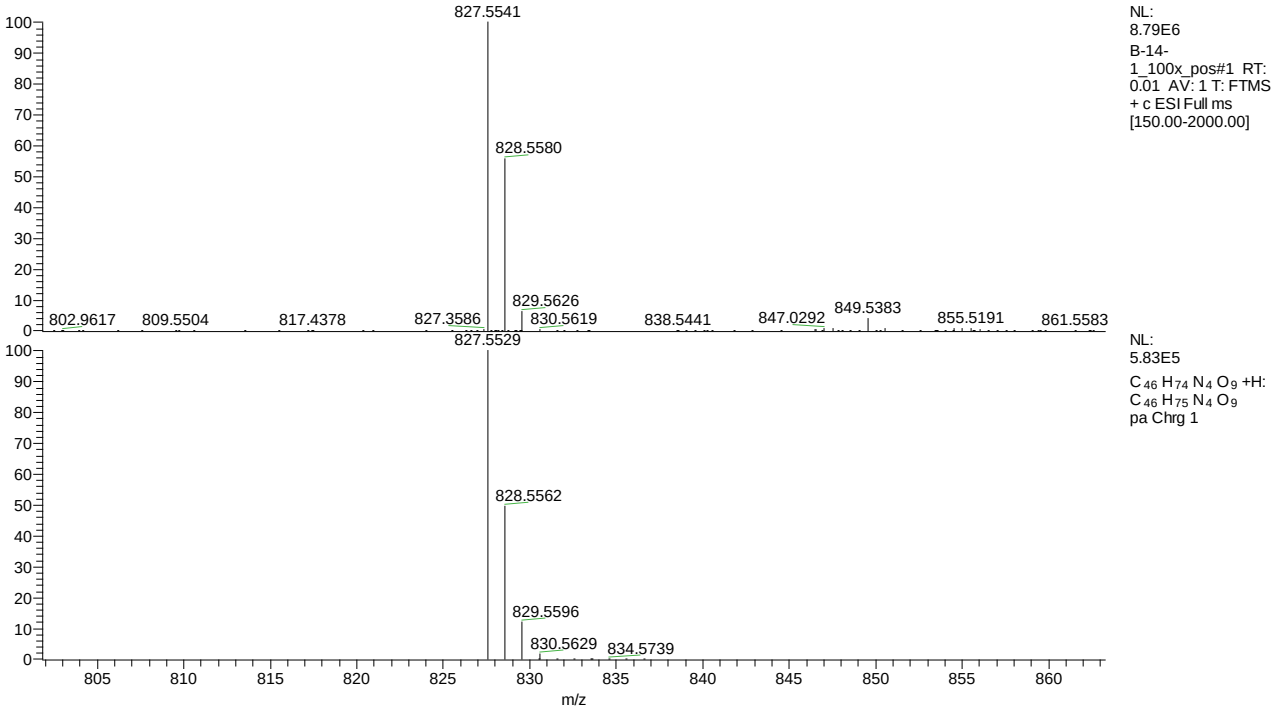
ESI-HRMS spectrum of Compound **8**



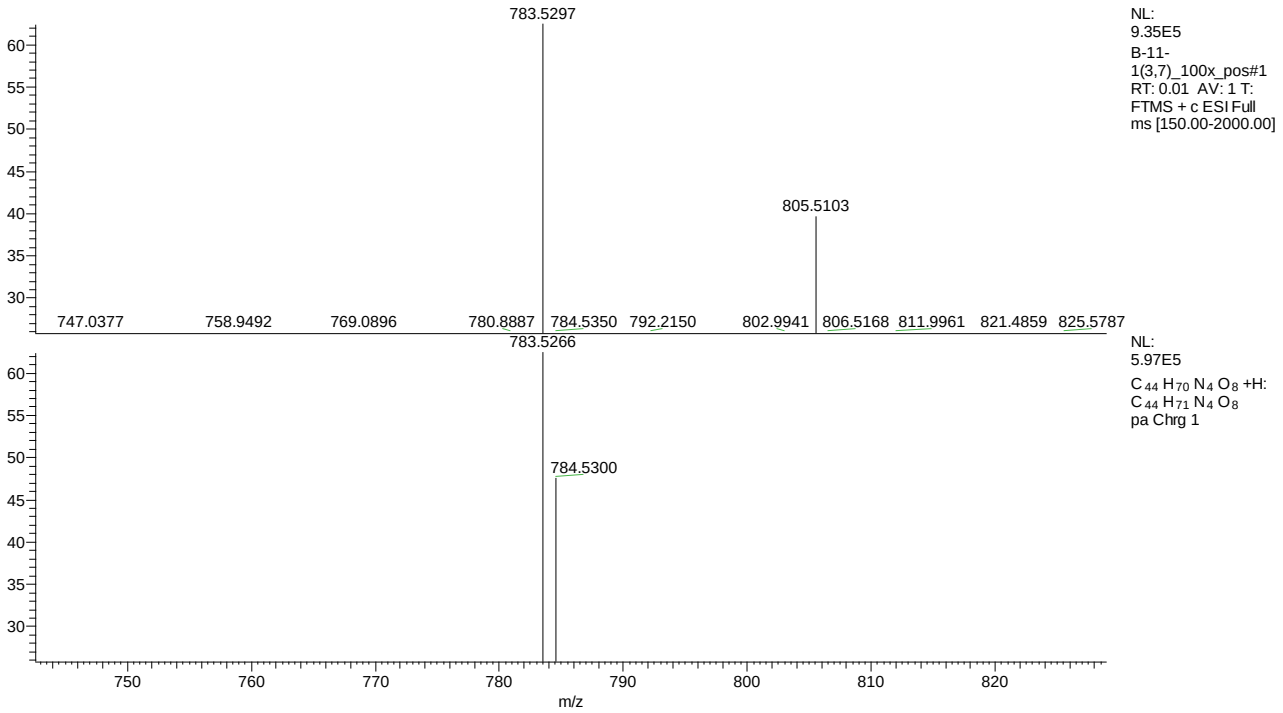
ESI-HRMS spectrum of Compound **9**



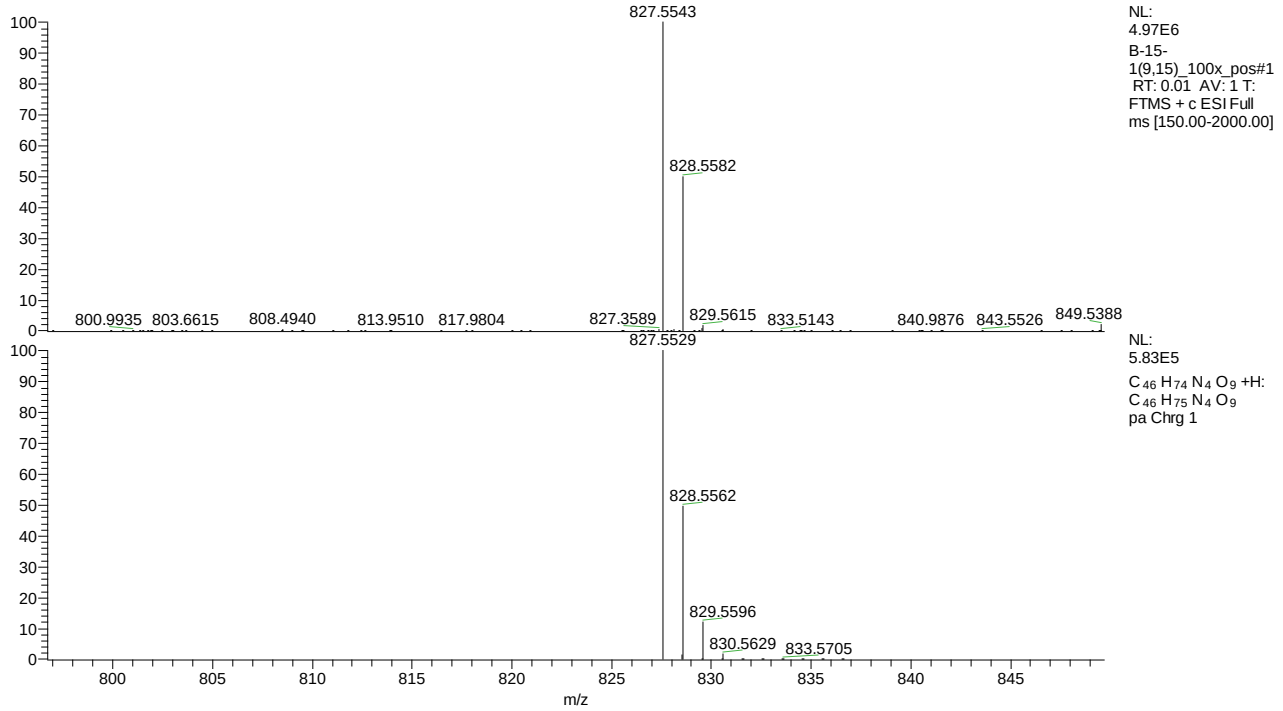
ESI-HRMS spectrum of Compound **10**



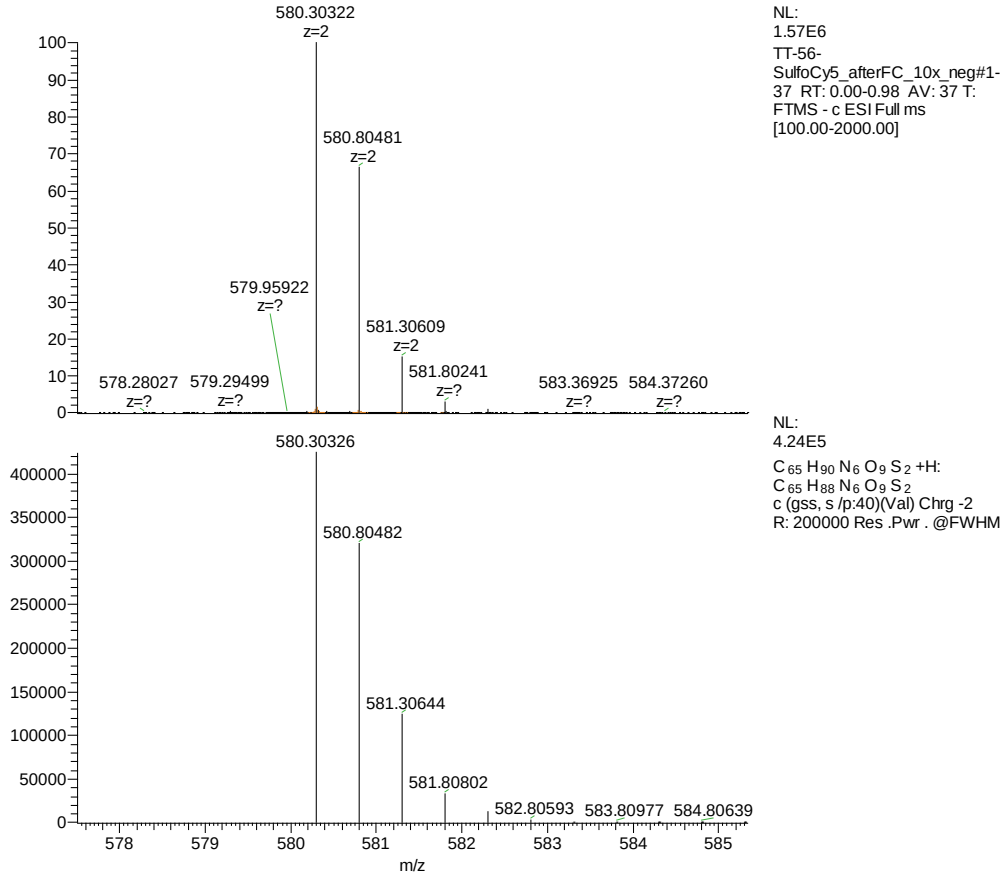
ESI-HRMS spectrum of Compound **11**



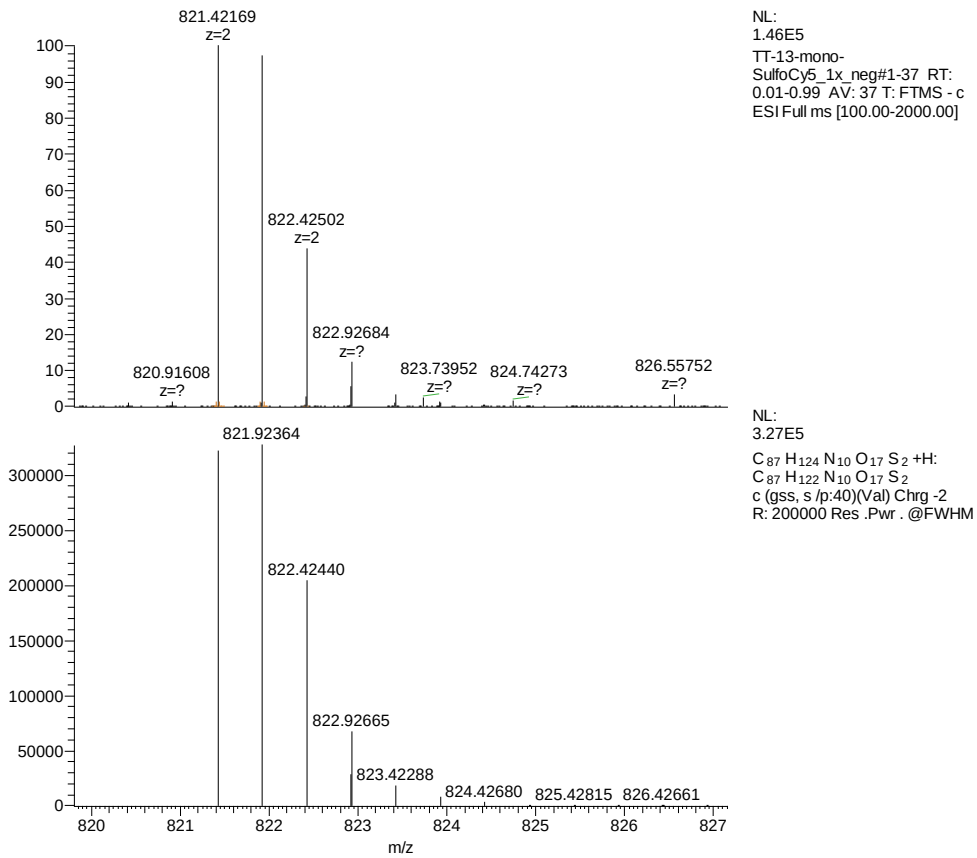
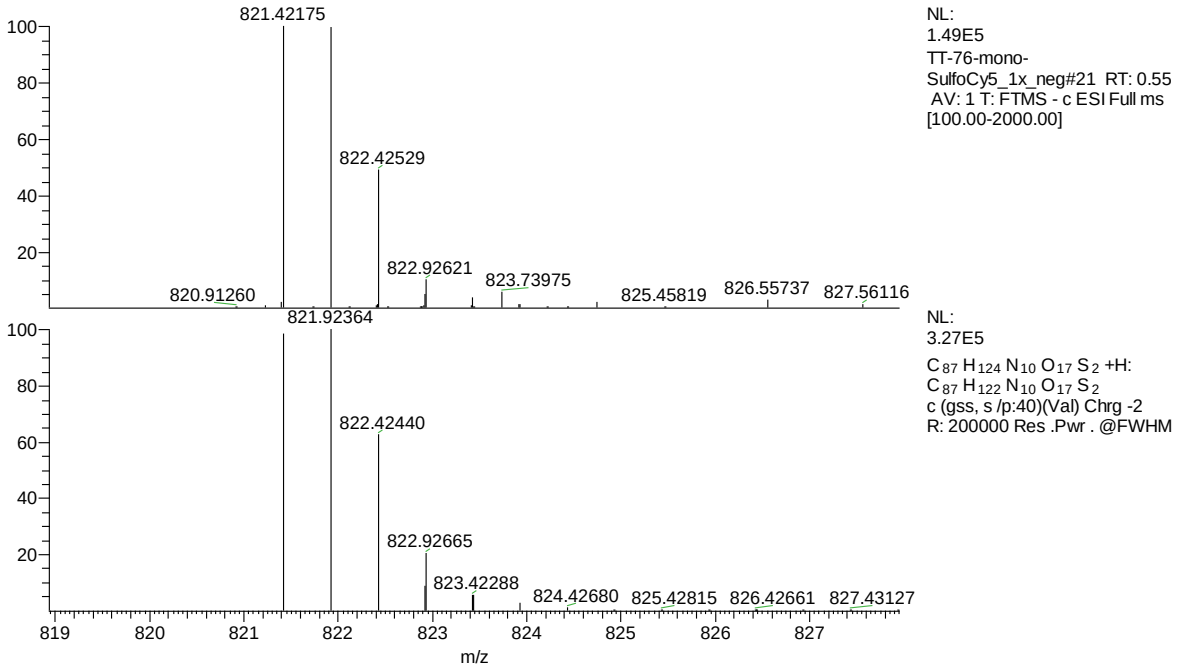
ESI-HRMS spectrum of Compound **12**



ESI-HRMS spectrum of Compound **14**



ESI-HRMS spectrum of Compound **16**



ESI-HRMS spectrum of Compound **17**