Supporting Information

Thienopyrimidinone Derivatives That Inhibit Bacterial tRNA (Guanine37-N^1)-Methyltransferase (TrmD) by Restructuring the Active Site with a Tyrosine-Flipping Mechanism

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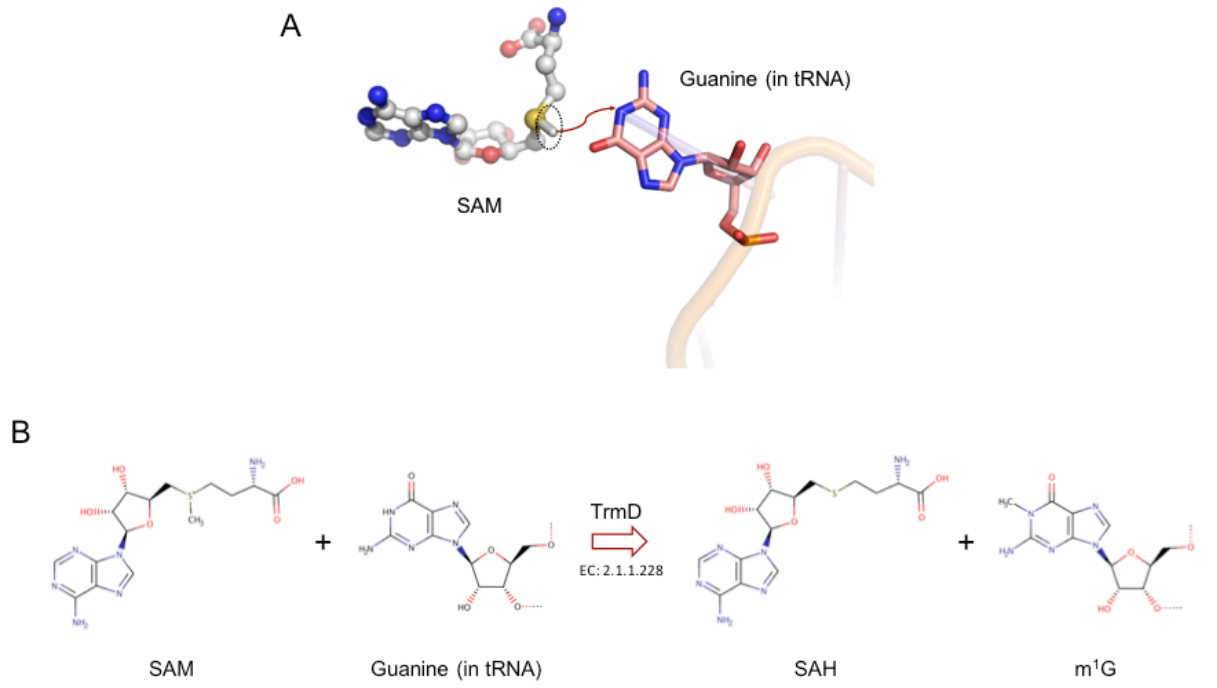


S-adenosyl-L-methionine + guanine(37) in tRNA = S-adenosyl-L-homocysteine + N^1-methylguanine(37) in tRNA

Figure S1. The mechanism of action of TrmD enzyme.

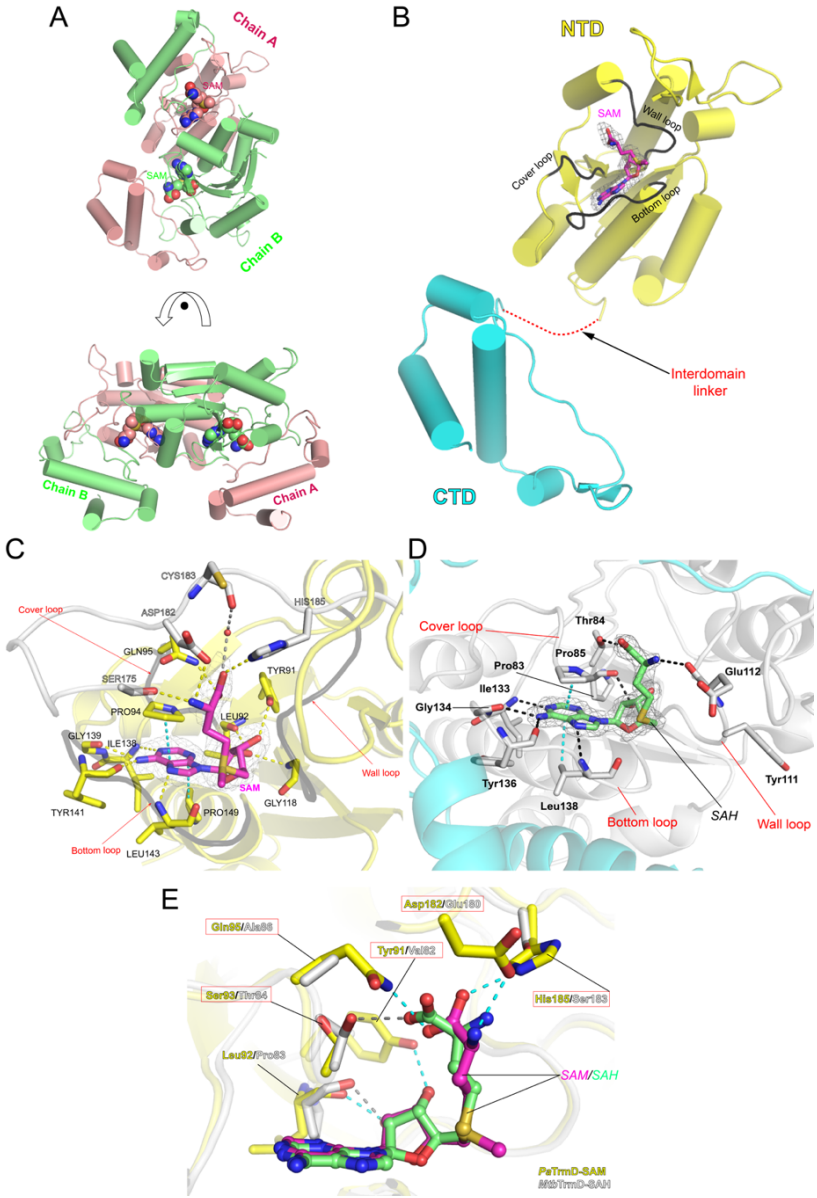


Figure S2. Structures of TrmD from *P. aerginosa* and *M. tuberculosis* (**A**) Two orthogonal views of the *Pa*TrmD-SAM structure (PDB ID 5WYQ) highlighting the dimer architecture and SAM-binding active site (spheres = ligands). (**B**) Close-up of the domain arrangement and active site. Each subunit of the *Pa*TrmD dimer contains two domains: N-terminal domain harbouring the active site (NTD, residues 1-165; yellow), and C-terminal domain (CTD, residues 178-252; cyan). The linker loop or "lid" (residues 166-177) between NTD and CTD is unstructured in *Pa*TrmD-SAM; a likely path is indicated by a red dotted line. Bound SAM is shown as magenta sticks with an unbiased *Fo-Fc* electron density (grey) map contoured at 3.0 σ overlaid on the model. Three loops that are involved in substrate binding are coloured in black: "cover loop" (residues 93-96), "wall loop" (residues 117-124) and "bottom loop" (residues 138-146). (**C, D**) SAM or SAH-binding mode of *Pa*TrmD and *Mtb*TrmD, respectively. Subunit 1 of the *Pa*TrmD dimer is coloured yellow and subunit 2 is coloured grey (PDB ID 5WYQ). Hydrogen bonds formed between *Pa*TrmD and SAM are indicated by yellow dashed lines while the stacking interactions are illustrated as cyan dashed lines. Subunit 1 of the *Mtb*TrmD dimer is coloured grey and subunit 2 is coloured cyan (PDB ID 5ZHJ). Hydrogen bonds formed between *Mtb*TrmD and SAH are indicated by black dashed lines while the stacking interactions are illustrated as cyan dashed lines. (**E**) Active-site superposition of *Pa*TrmD-SAM and *Mtb*TrmD-SAH showing the non-conserved active-site residues.

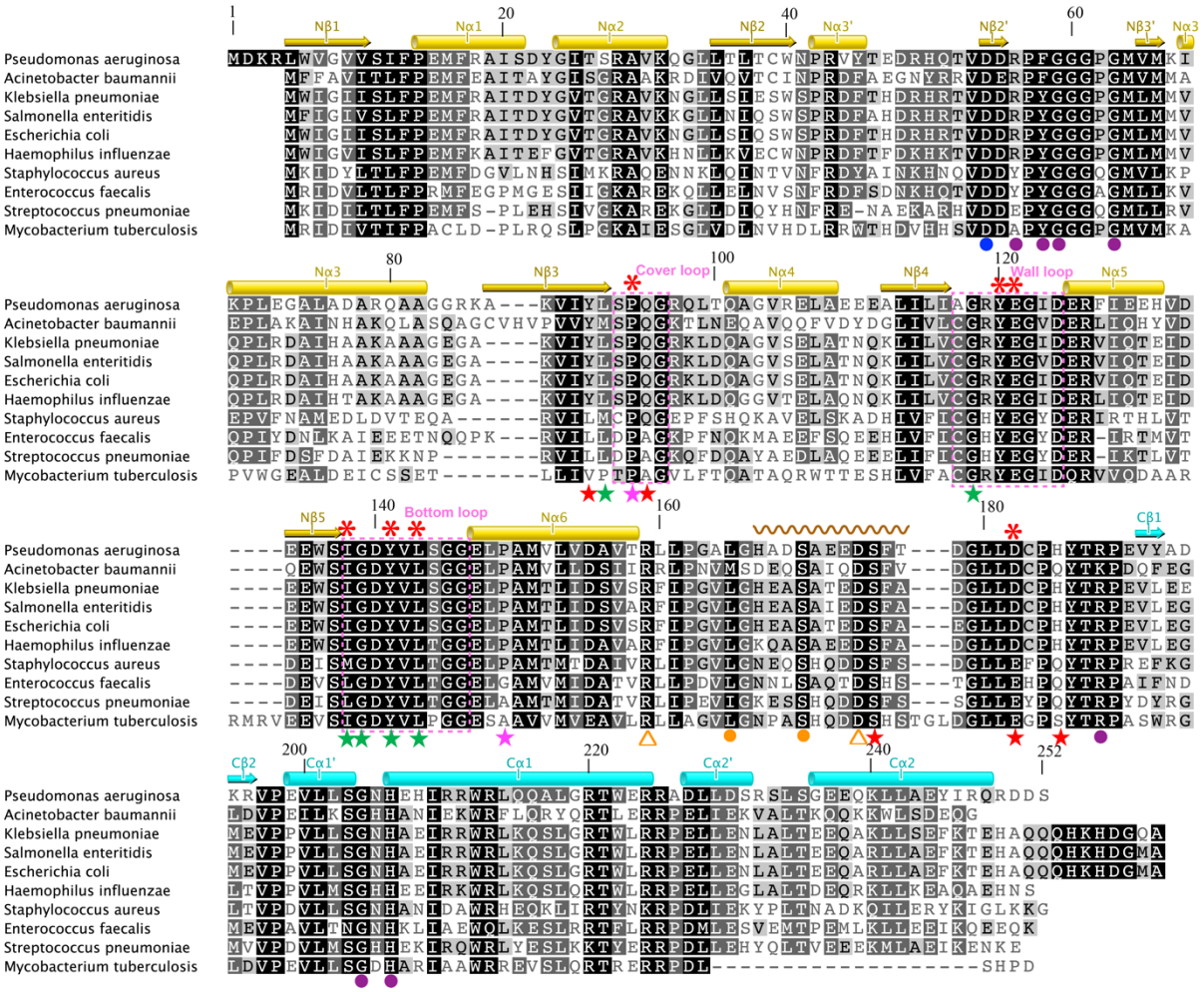


Figure S3. Conserved sequences of TrmD in bacterial pathogens. The conservation of the residues is indicated by different levels of shading. Residue numbers corresponding to *Pa*TrmD are listed above the sequence. Secondary structural elements defined in *Pa*TrmD crystal structure are shown above the sequences (only α-helices and β-strands are shown). Secondary structural elements are labelled in different colours corresponding to their domain regions: N-terminal domain (NTD: yellow) and C-terminal domain (CTD: cyan). The flexible interdomain linker between NTD and CTD is indicated by wave line in brown colour. Three active-site loops (cover loop, wall loop and bottom loop) are highlighted in magenta dashed box. In *Pa*TrmD, the amino acids involved in SAM binding are indicated by star in red (side-chain interacting), green (main-chain interacting), and magenta (stacking interacting), respectively. Compound AZ51-interacting residues are indicated by red asterisks on top of sequence alignment. tRNA-interacting residues determined in *H. influenzae* structure (PDB ID: 4YVI) are highly conserved in the selected sequences and indicated by blue circle (G36-interacting), orange circles (G37-interacting) and purple circles (anti-codon branch-interacting), while G37-interacting catalytic residues are indicated by triangles.

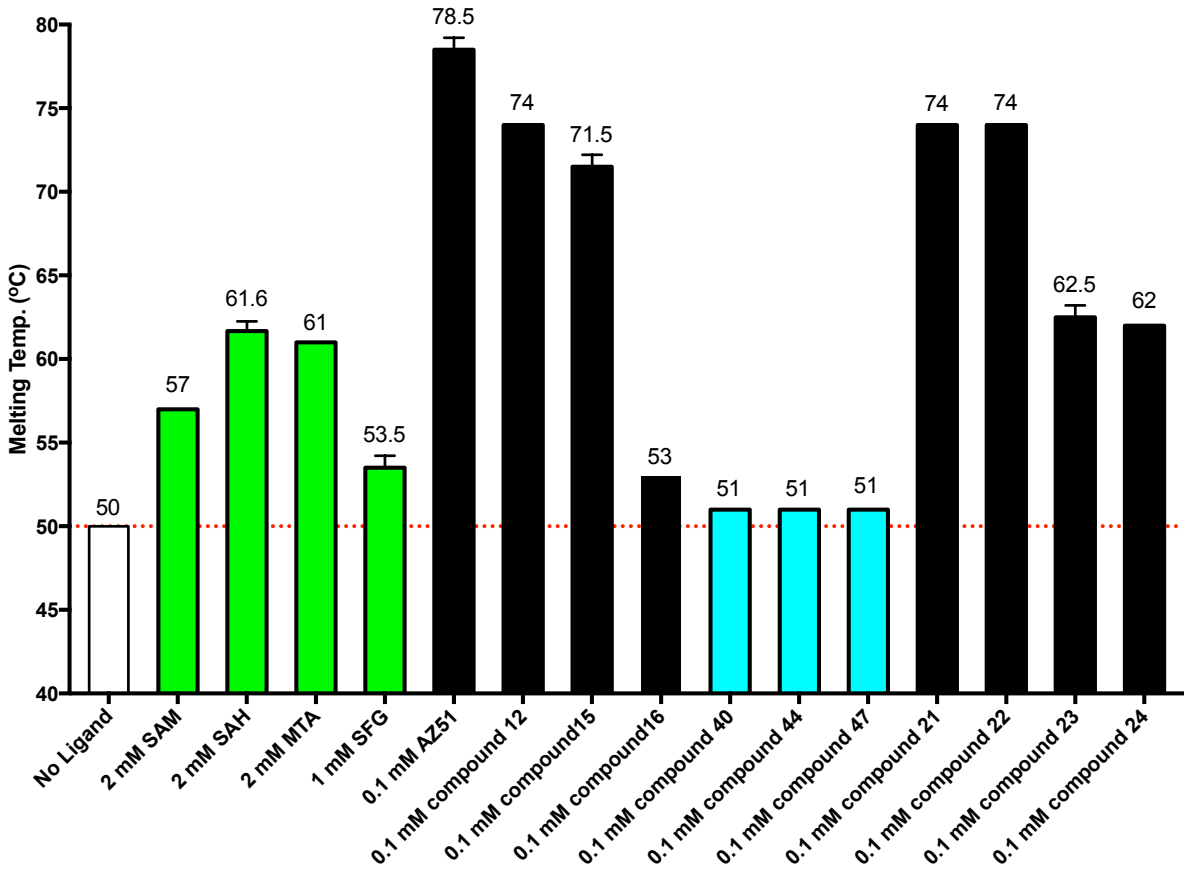


Figure S4. Stabilisation of *Pa*TrmD by binding small molecules. Thermal shift assay results for *Pa*TrmD in the absence (white bar) and the presence of test ligands (colored bars). The melting temperature T_m values (°C) are shown above the bars. All data are mean ± SD for three replicate measurements. Bar colors represent different ligands: black, non-O^6-substituted thienopyrimidone analogues; cyan, O^6-substituted thienopyrimidone analogues; green, non- thienopyrimidone compounds.

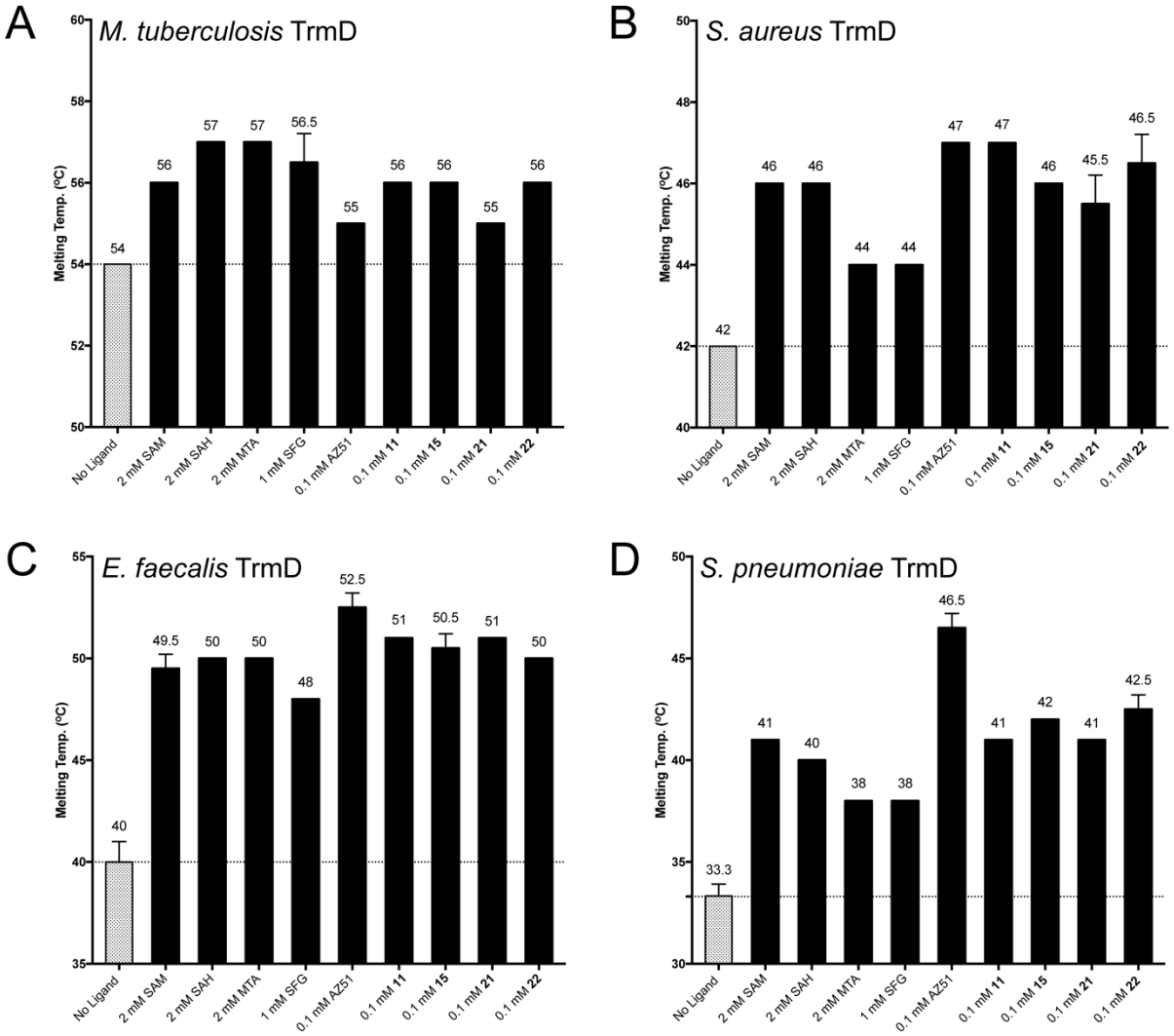


Figure S5. Stabilisation of mycobacterial TrmD and Gram-positive bacterial TrmDs by binding small molecules. The melting temperature T_m values (°C) are shown above the bars. All data are mean ± SD for at least two replicates.

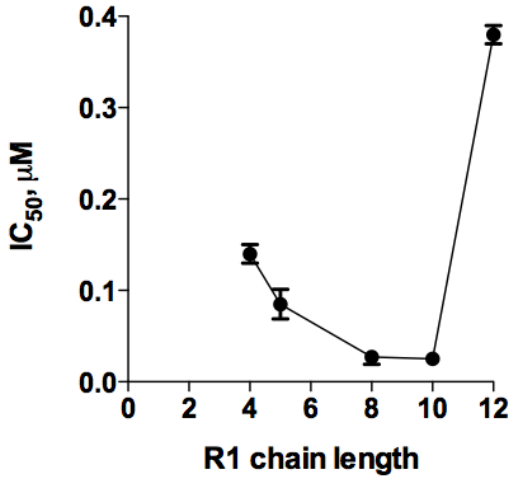


Figure S6. The effect of R1 chain length on TrmD inhibitory activity of compound **15** analogs.

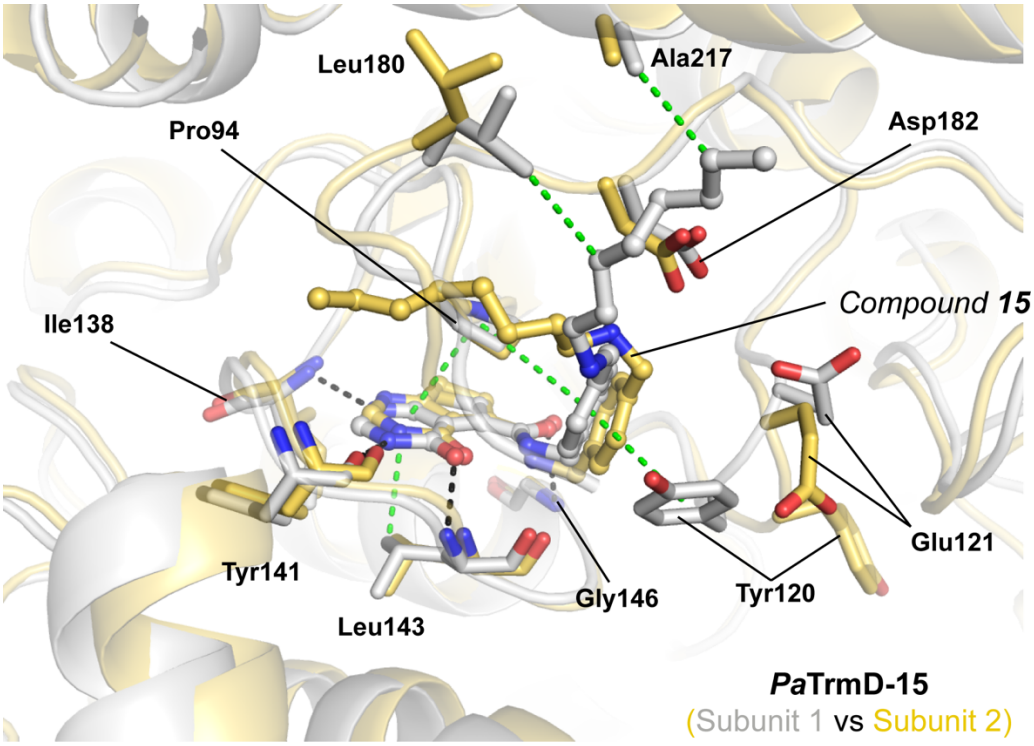


Figure S7. Comparison of the binding-mode differences of **15** at two active sites within a *Pa*TrmD dimer. The polypeptide chains are shown as cartoons, whereas the residues involved in inhibitor binding are shown as stick structures (PDB ID 5ZHN). The compound **15** was shown as sticks with larger atom radius for clarity.

Table S1. Pairwise protein sequence comparisons of TrmDs showing amino-acid identities

	P. aeruginosa	*A. baumannii*	*K. pneumoniae*	*S. enteritidis*	*E. coli*	*H. influenzae*	*S. aureus*	*E. faecalis*	*S. pneumoniae*	*M. tuberculosis*
Pseudomonas aeruginosa		48	64	63	64	62	40	43	43	39
Acinetobacter baumannii	48		52	53	52	54	43	37	37	33
Klebsiella pneumoniae	64	52		95	98	83	43	46	48	39
Salmonella enteritidis	63	53	95		96	82	43	46	49	39
Escherichia coli	64	52	98	96		83	43	45	49	39
Haemophilus influenzae	62	54	83	82	83		43	46	49	37
Staphylococcus aureus	40	43	43	43	43	43		52	55	41
Enterococcus faecalis	43	37	46	46	45	46	52		60	42
Streptococcus pneumoniae	43	37	48	49	49	49	55	60		42
Mycobacterium tuberculosis	39	33	39	39	39	37	41	42	42	

The pairwise sequence analysis was obtained from the program ClustalW2[1,2].
Values are overall percent sequence identities.

Table S2. Haemolytic activity (EC_{50}) of thienopyrimidone compounds

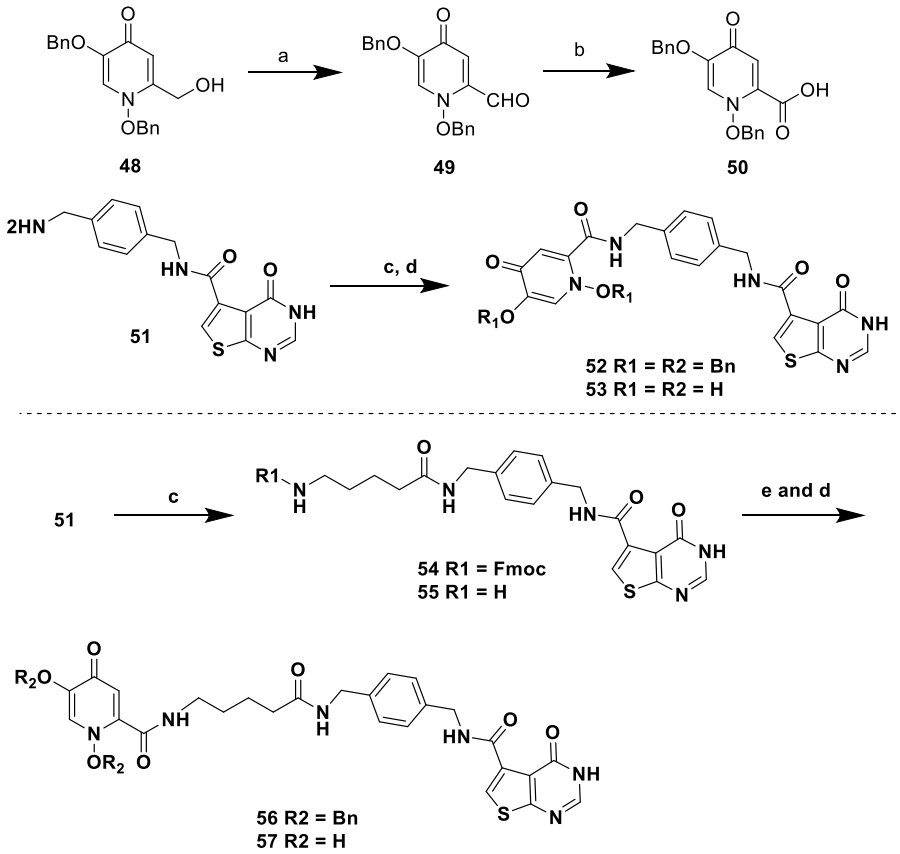
Compounds	EC_{50} (μM)
9	NA[a]
10	NA
11	NA
12	NA
13	NA
14	NA
15	NA
16	NA
17	NA
18	~7%[b]
20	NA
21	NA
22	NA
23	NA
24	~3%[b]
25	NA
30	NA
31	NA
32	NA
33	NA
34	NA
39	NA
40	NA
41	~5%
42	NA
43	~122[c]
44	58 ± 5[d]
45	NA
46	NA
47	~6%[b]
53	NA
57	NA

[a]NA = no activity at 100 μM; [b]Percent hemolysis at 100 μM; [c]Values estimated due to weak activity; [d] Values are mean ± SD for two replicates.

Table S3. Primers and single-stranded synthetic DNA (ssDNA) for *in vitro* transcription of TrmD tRNA substrate

	Sequence (5'-3')
Primers	
T7 primer	GGATCCTAATACGACTCACTATAGGG
$tRNA^{Leu(CAG)}$ reverse primer	TGGTGCCCAGGAGAAGACTC
Single-strand synthetic DNA (template)	
$tRNA^{Leu(CAG)}$	GGATCCTAATACGACTCACTATAGGGGCCCAGGTG GCGGAATTGGTAGACGCACTAGGTTCAGGTCCTAG CGGTGGCAACACCGTGGAAGTTCGAGTCTTCTCCT GGGCACCA

Scheme S1. Synthesis of pyridone siderophore-conjugated analogues



Conditions: (a) DMSO, triethylamine, sulfur trioxide pyridine complex; (b) 1N KOH, THF, ambient temperature; (c) HATU, DMF, ambient temperature, 12 h; (d) 1M BCl_3, dichloromethane; (e) 20% piperidine in DMF.

***General experimental procedure for the synthesis of compounds* 52 *and* 54.** Compound **50** and **51** was synthesized with the known literature conditions. To a solution of acid **50** (1.05 equiv) and amine **51 (**1.0 equiv) in anhydrous DMF (4 mL) was added trimethylamine (5.0 equiv) followed HATU (1.5 equiv). The resulting reaction mixture was stirred under room temperature for overnight. The reaction mixture was diluted with water and extracted with dichloromethane. The solvent was evaporated and purified by column chromatography using 5-15% MeOH in dichloromethane.

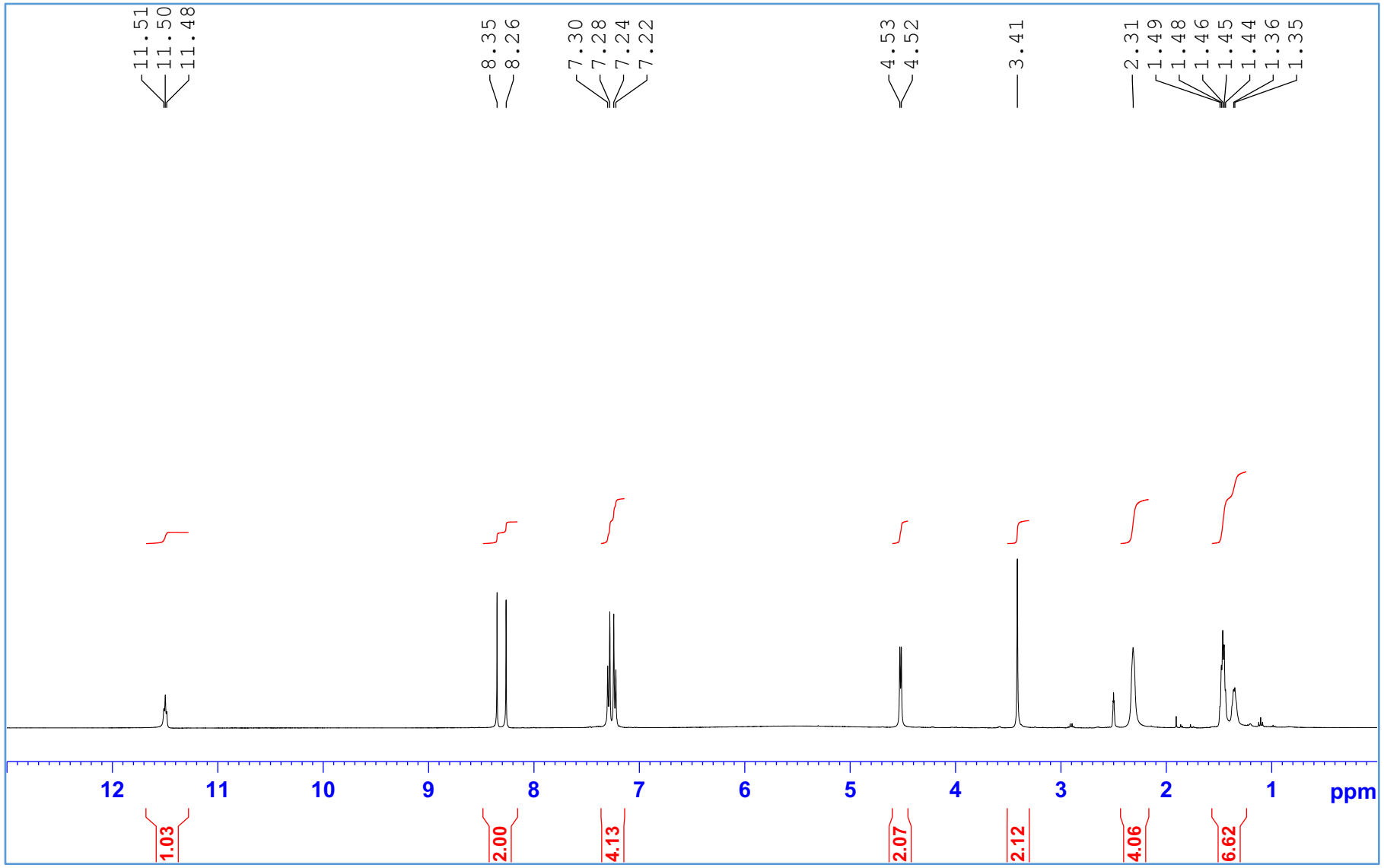
***General experimental procedure for the synthesis of pyridone siderophore-conjugated analog* 53 *and* 57.** To a stirred solution of compound **52** or **56 (**1.0 equiv) in dichloromethane 95 mL) was added BCl_3 **(**7.0 equiv). The resulting reaction mixture was stirred under room temperature for 1h. The reaction mixture cooled to 0 ^oC and quenched with water and solvent was evaporated. The resulting solid triturated with water and the solid was filtered. The solid was washed with diethyl ether to obtain the pure compound in off white solid.

Supplementary References

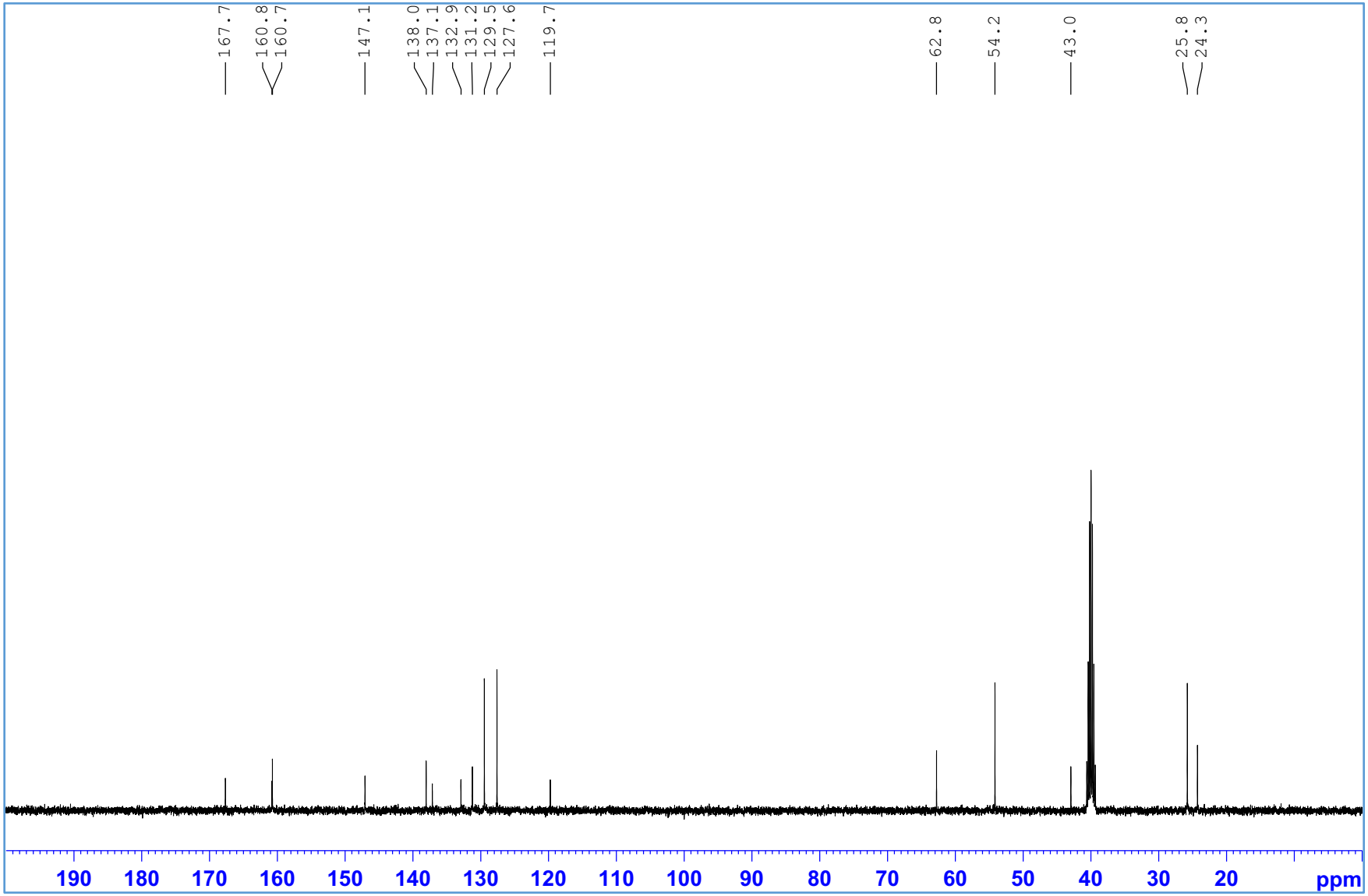
(1) Thompson, J. D.; Higgins, D. G.; Gibson, T. J. CLUSTAL W: Improving the Sensitivity of Progressive Multiple Sequence Alignment Through Sequence Weighting, Position-Specific Gap Penalties and Weight Matrix Choice. *Nucleic Acids Res.* **1994**, *22* (22), 4673–4680.

(2) Larkin, M. A.; Blackshields, G.; Brown, N. P.; Chenna, R.; McGettigan, P. A.; McWilliam, H.; Valentin, F.; Wallace, I. M.; Wilm, A.; Lopez, R.; Thompson, J. D.; Gibson, T. J.; Higgins, D. G. Clustal W and Clustal X Version 2.0. *Bioinformatics* **2007**, *23* (21), 2947–2948.

^{1}H NMR of compound **9** (400 MHz, DMSO-D_6)



^{13}C NMR of compound **9** (100 MHz, DMSO-D_6)



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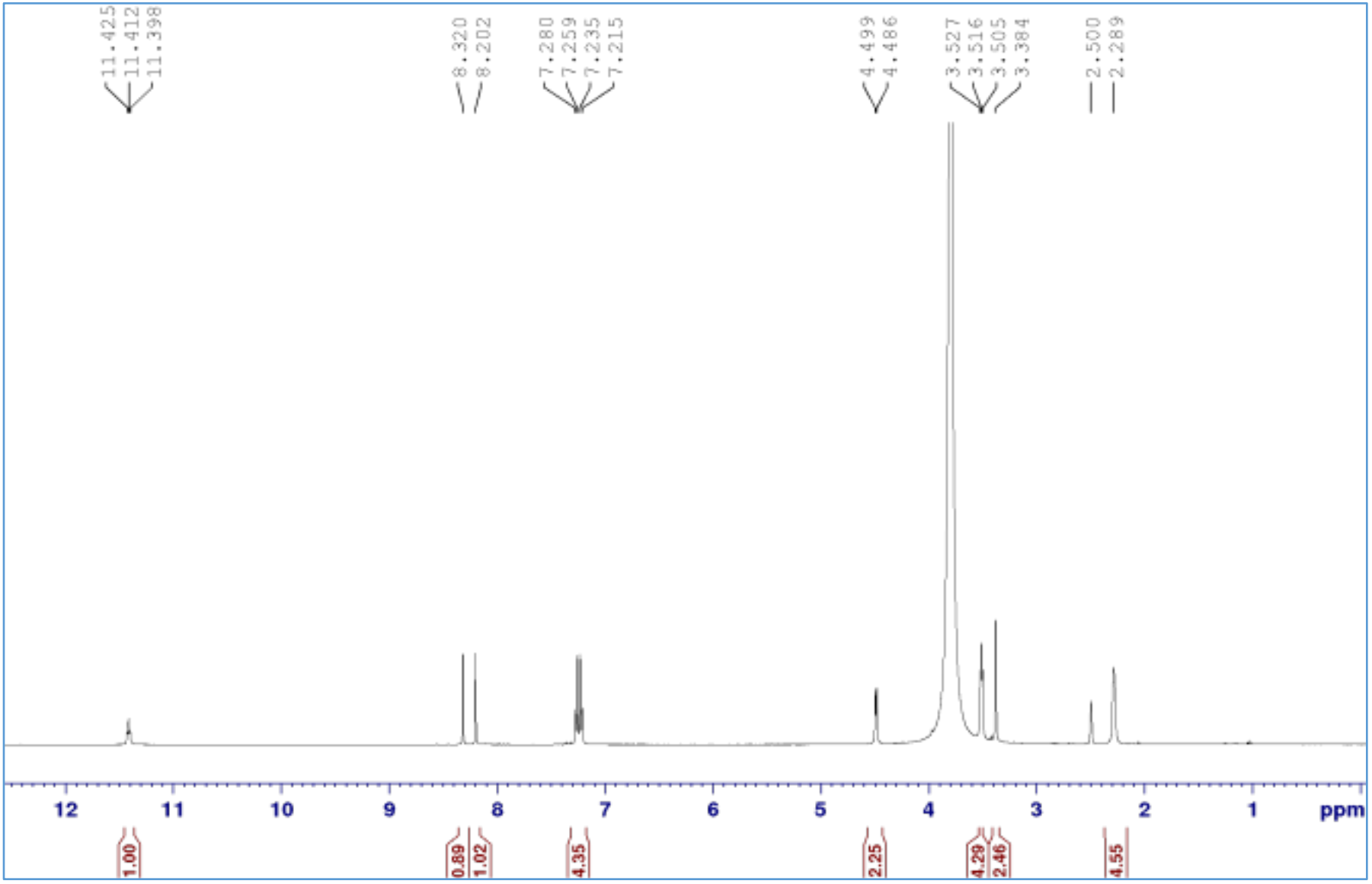

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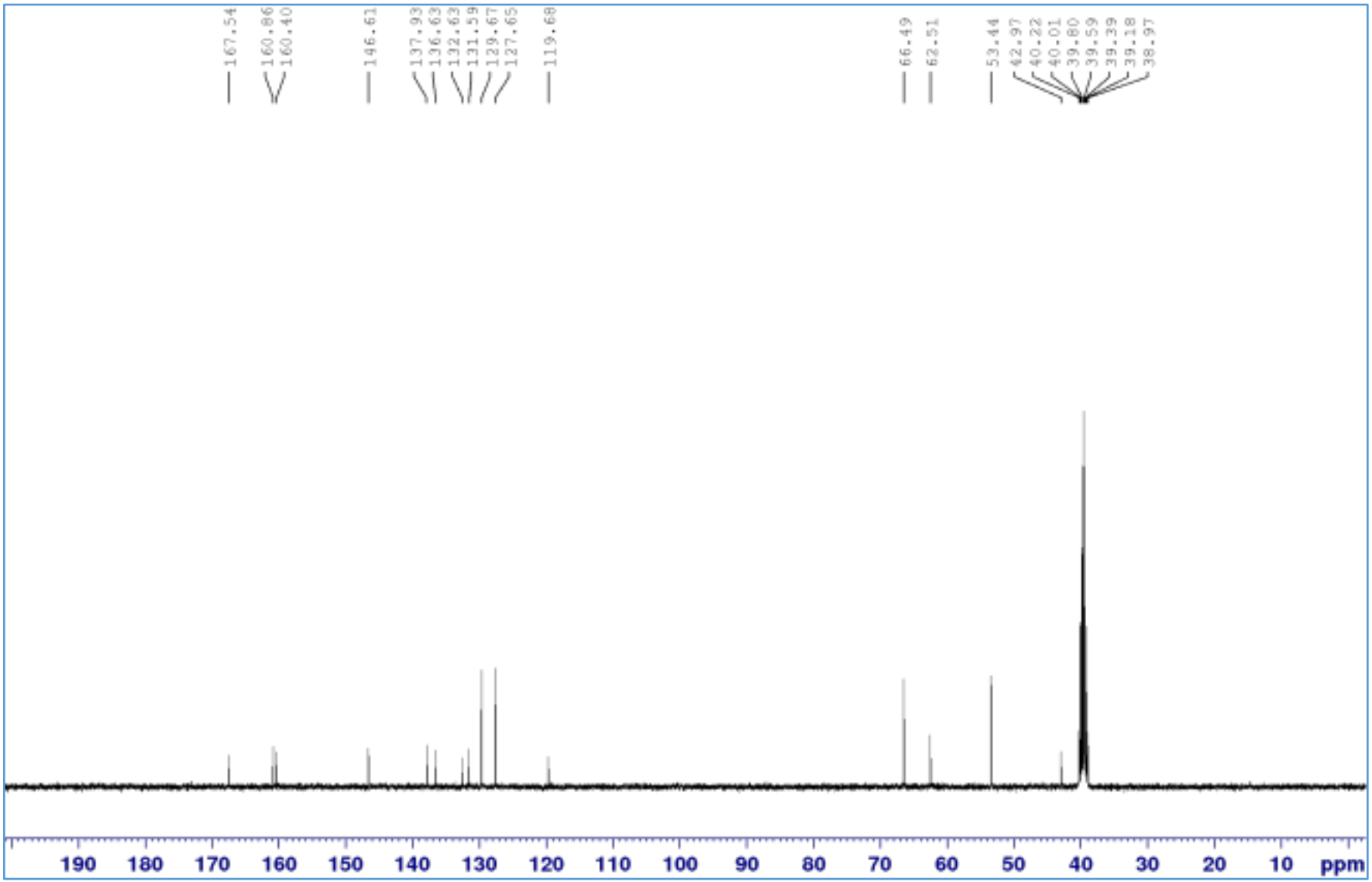

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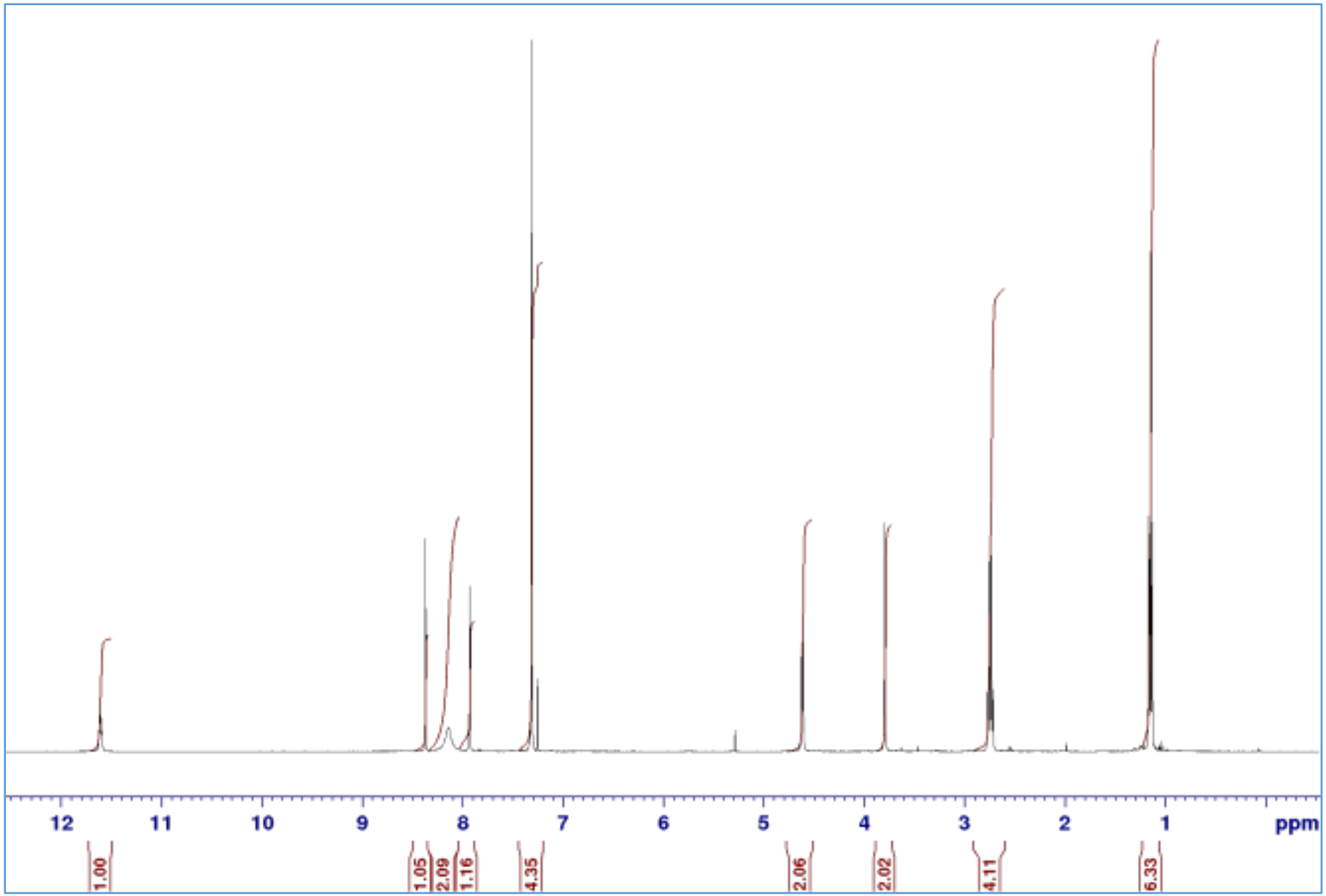

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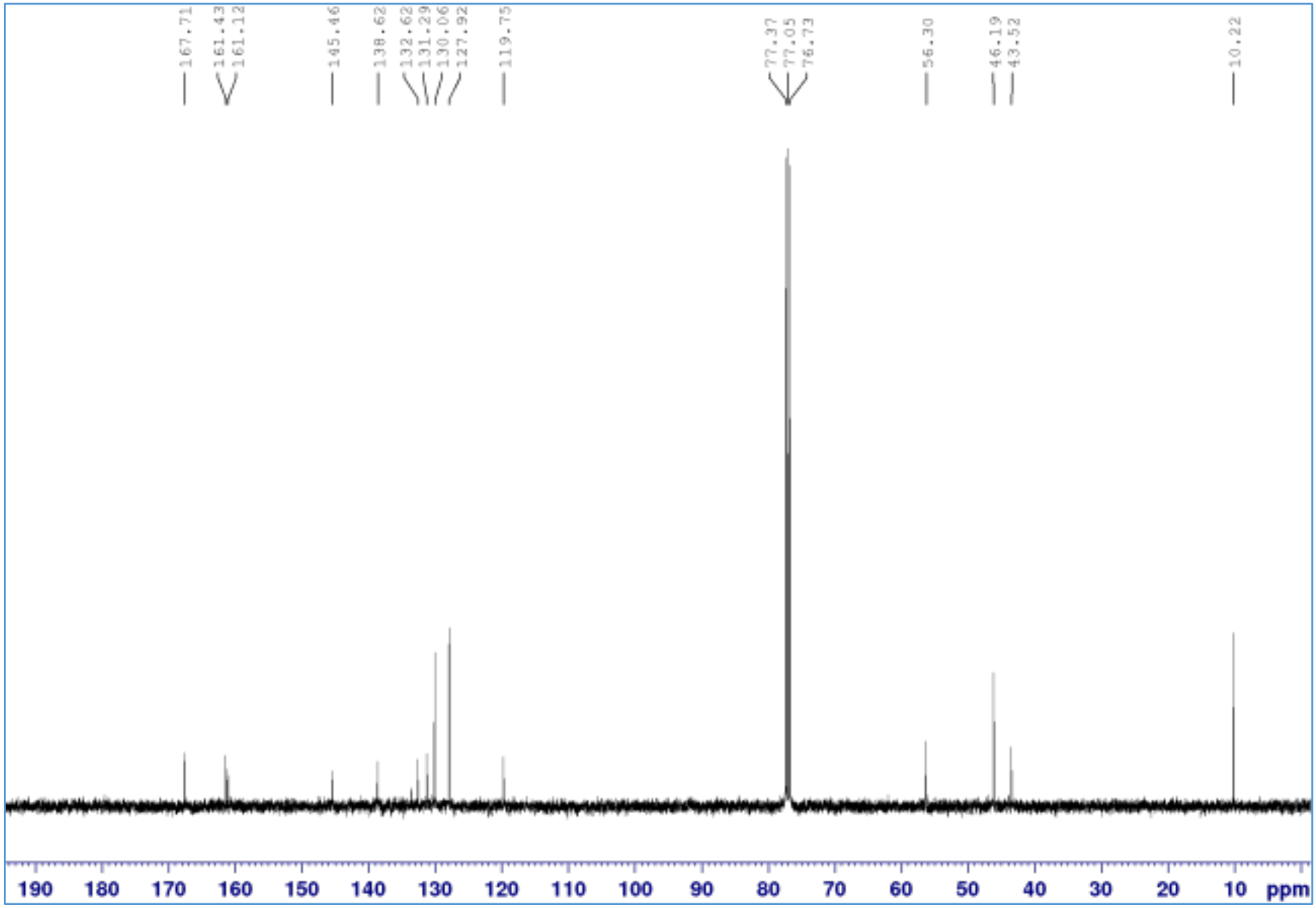

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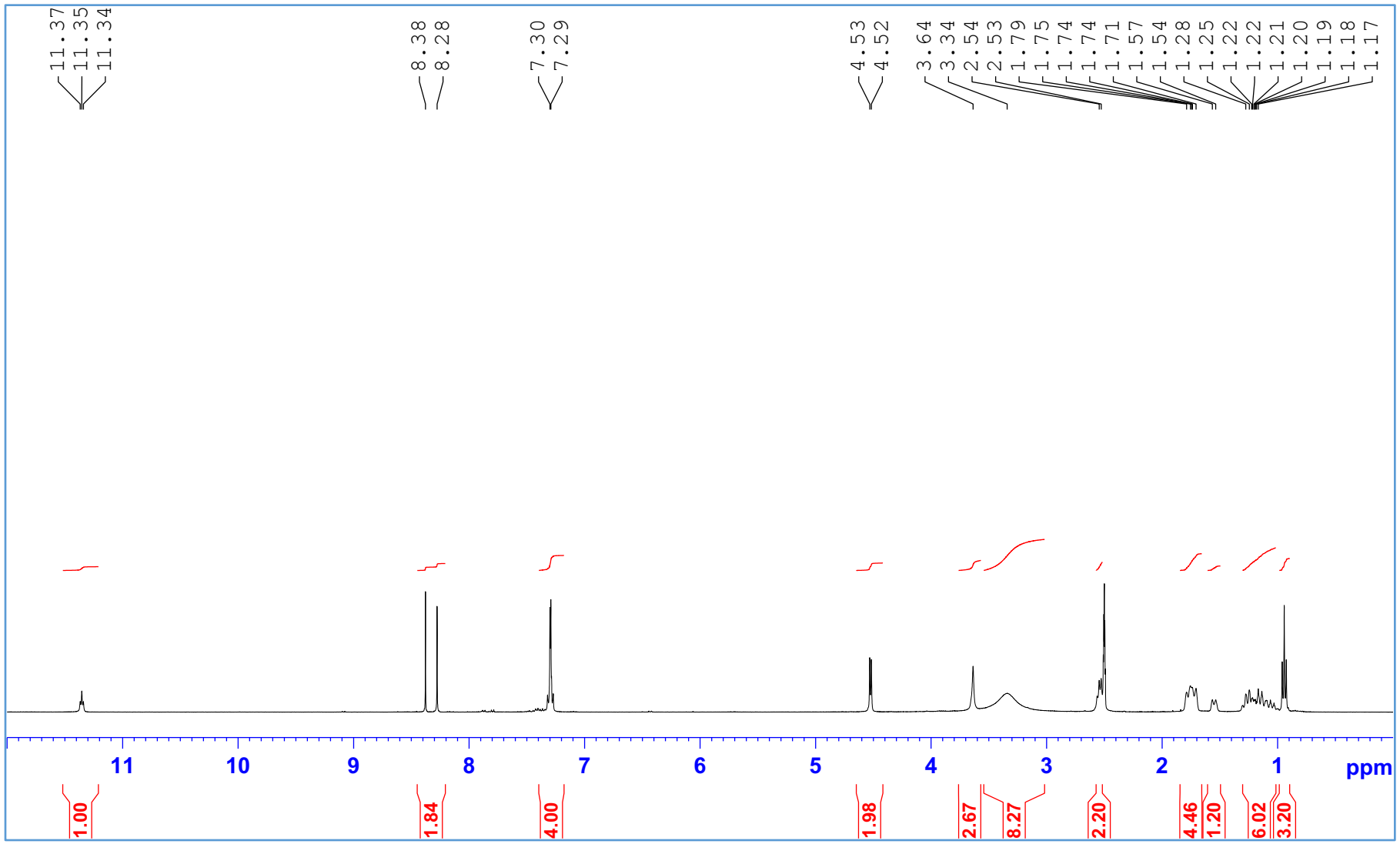

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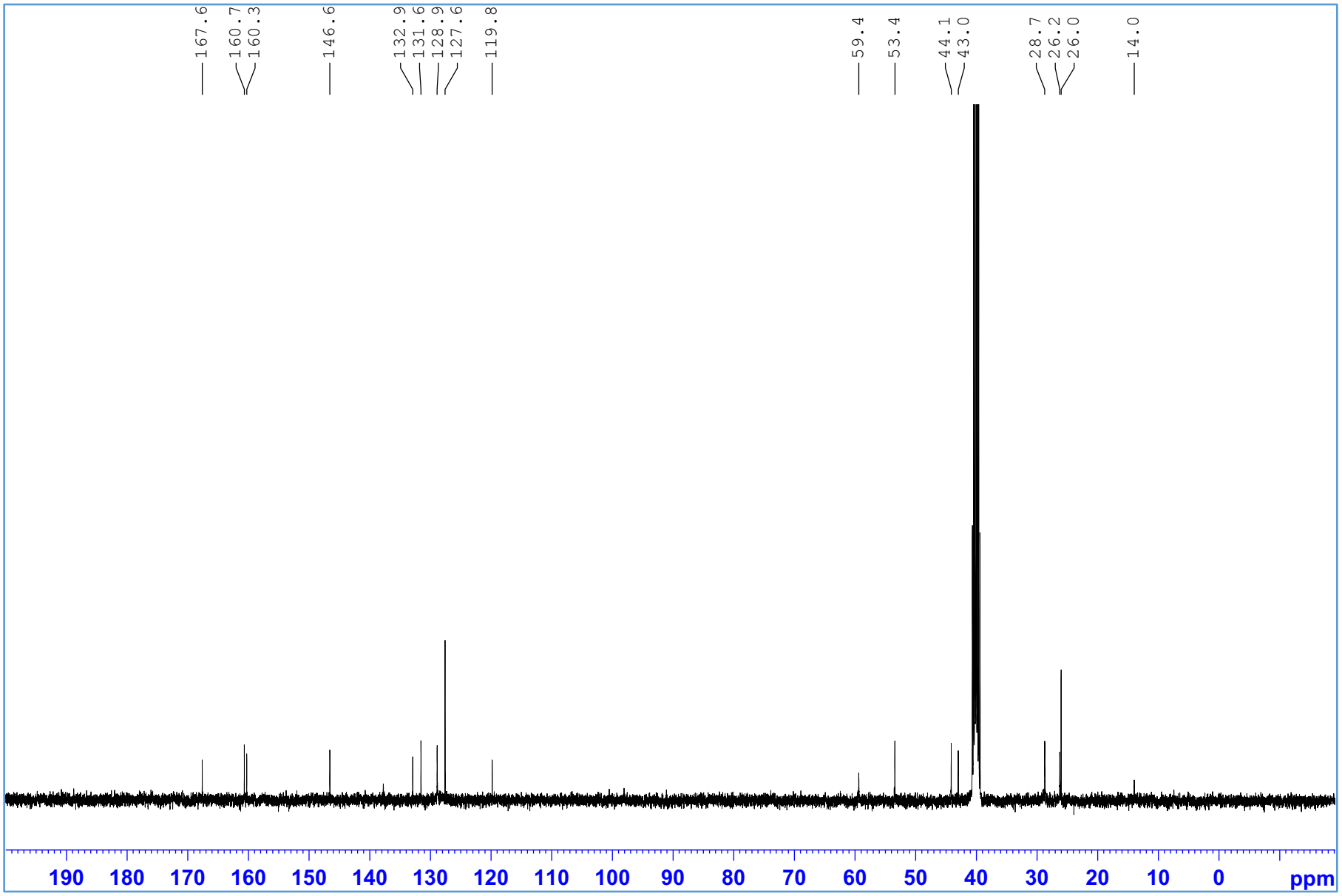

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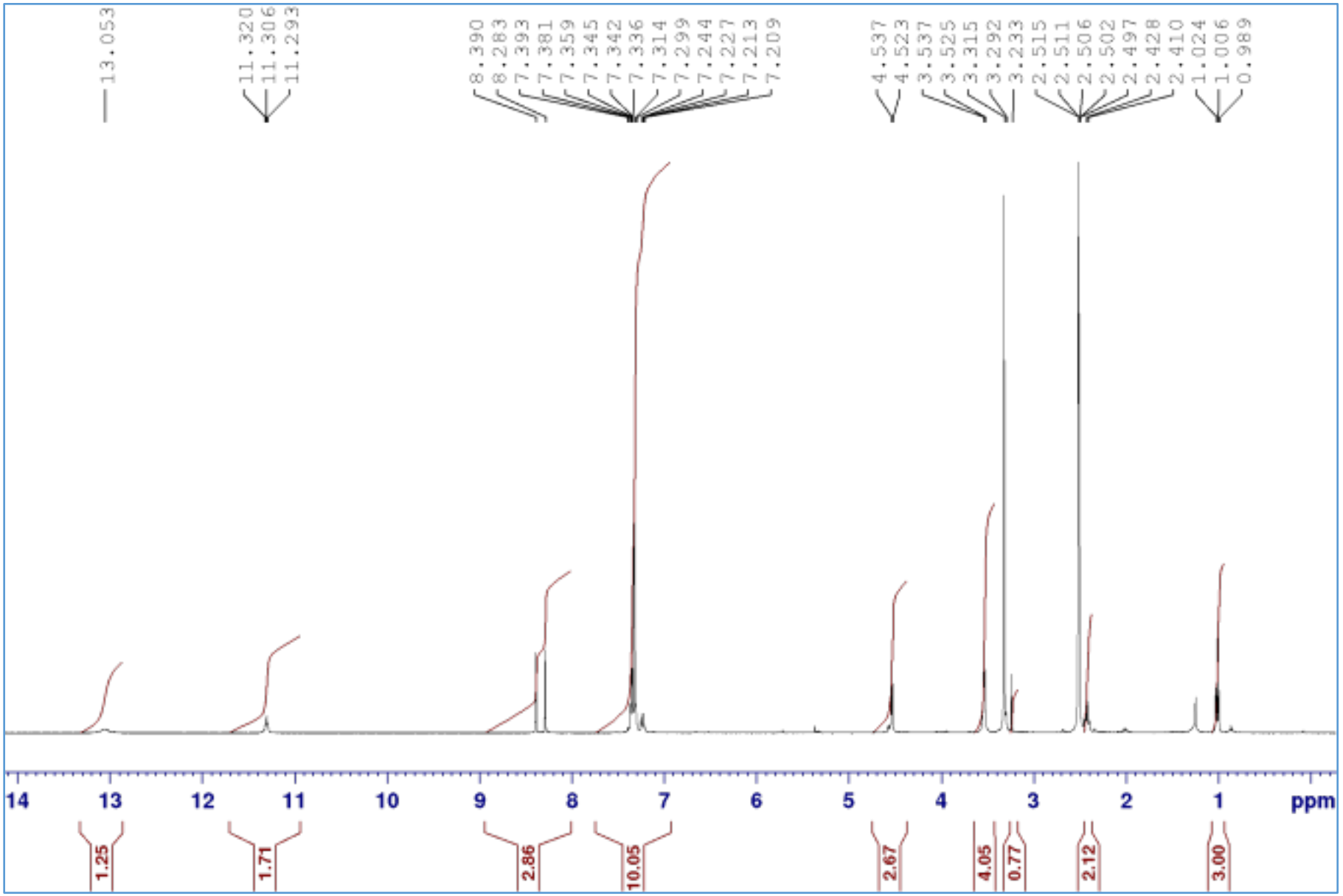

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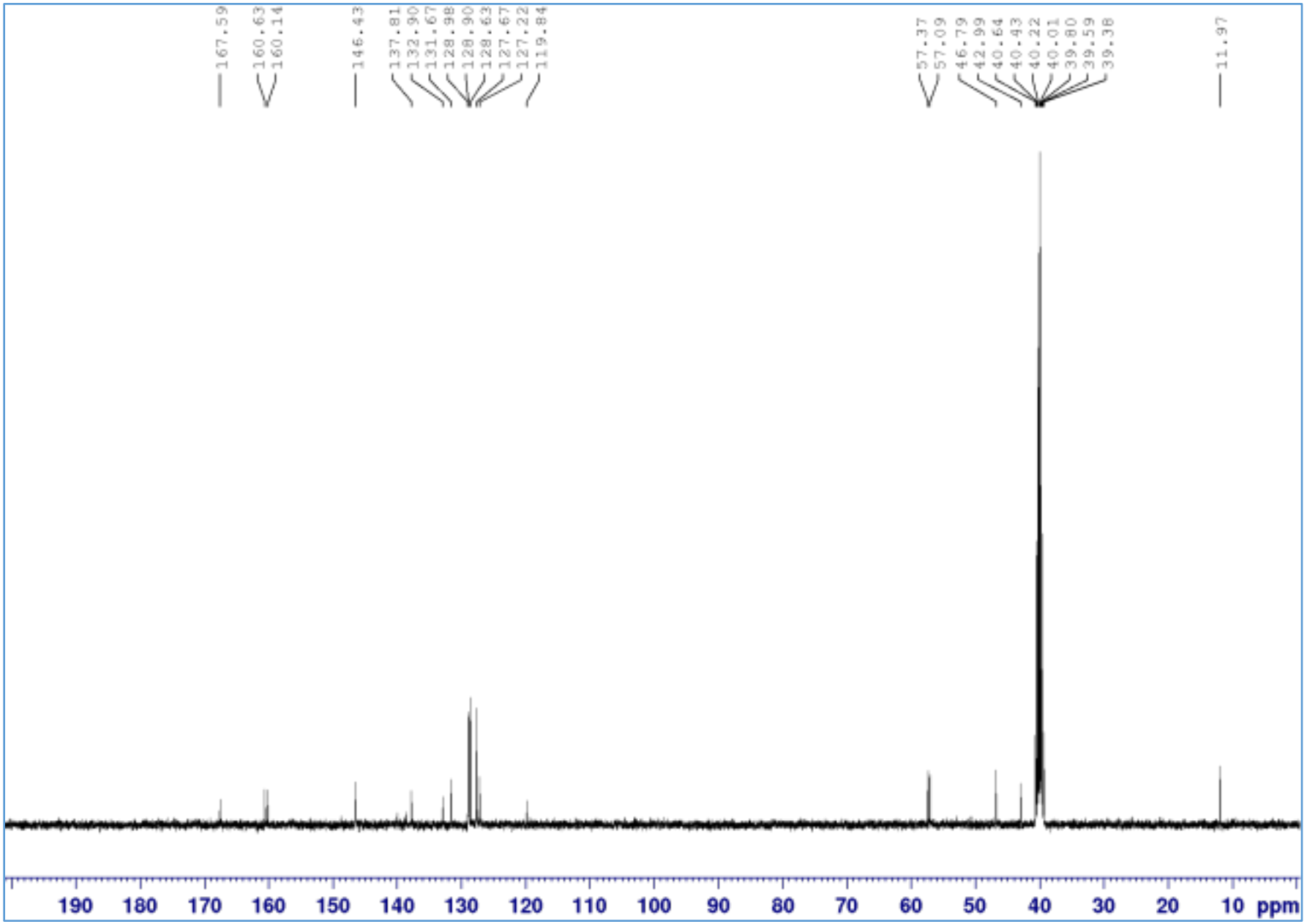

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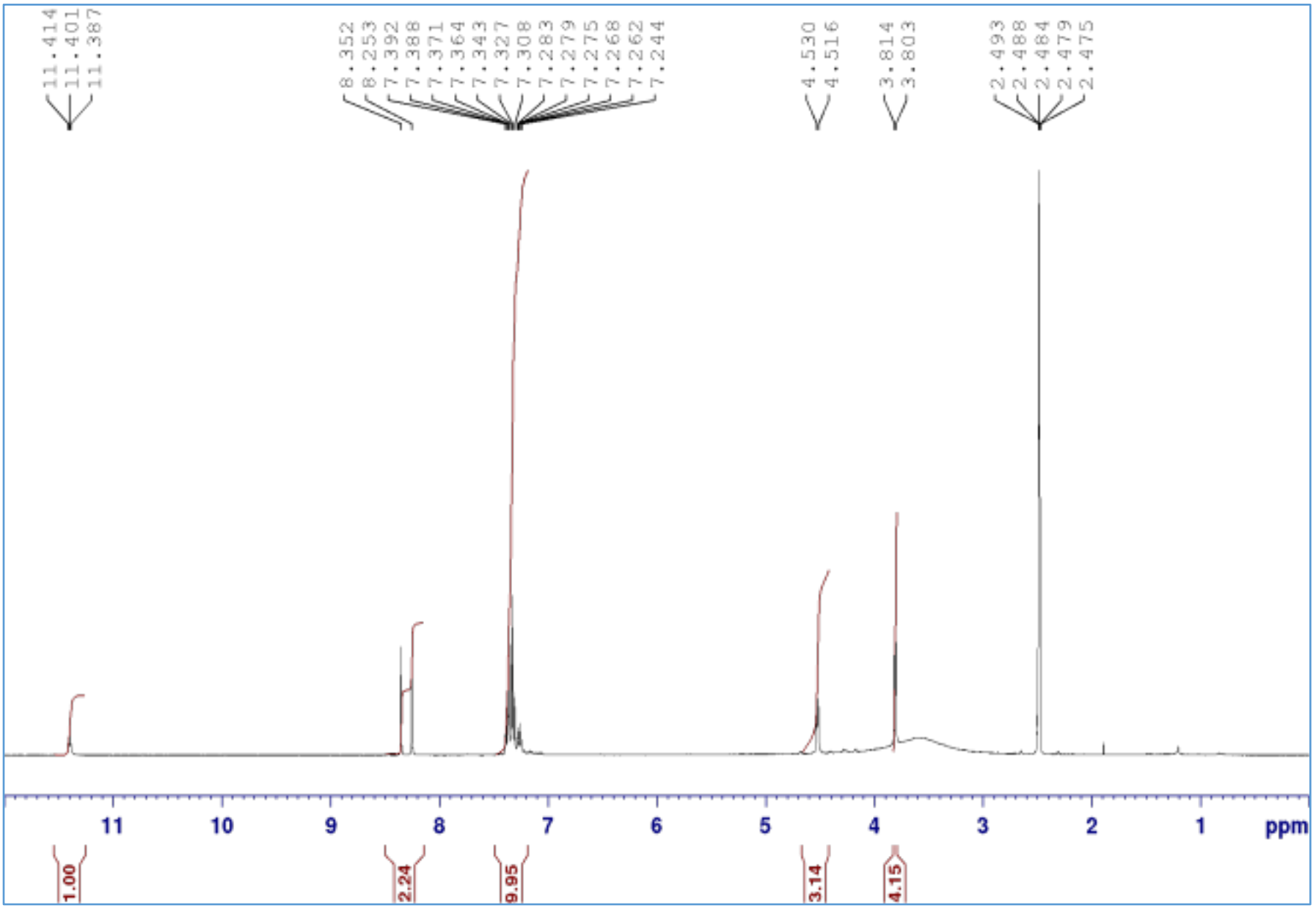

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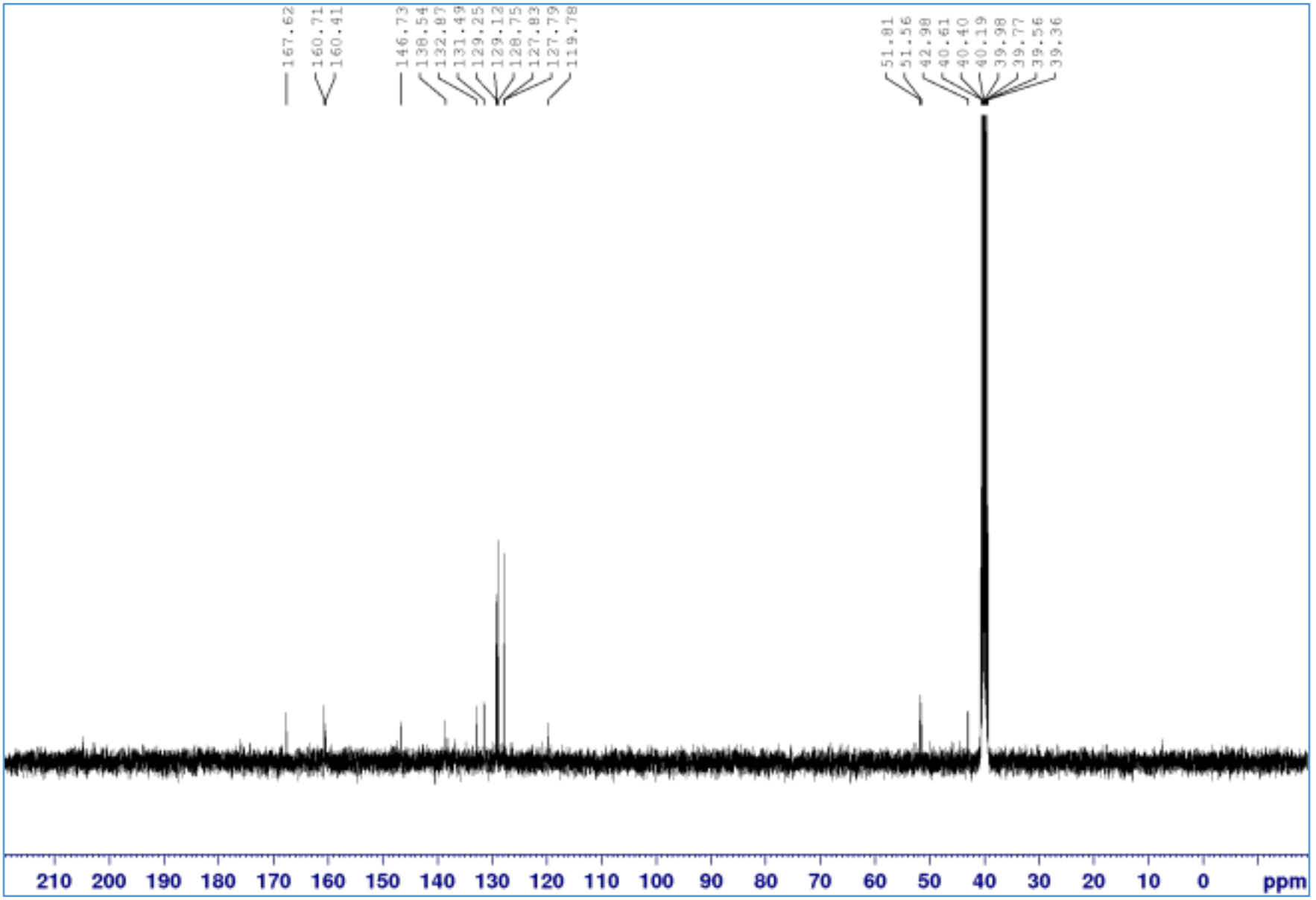

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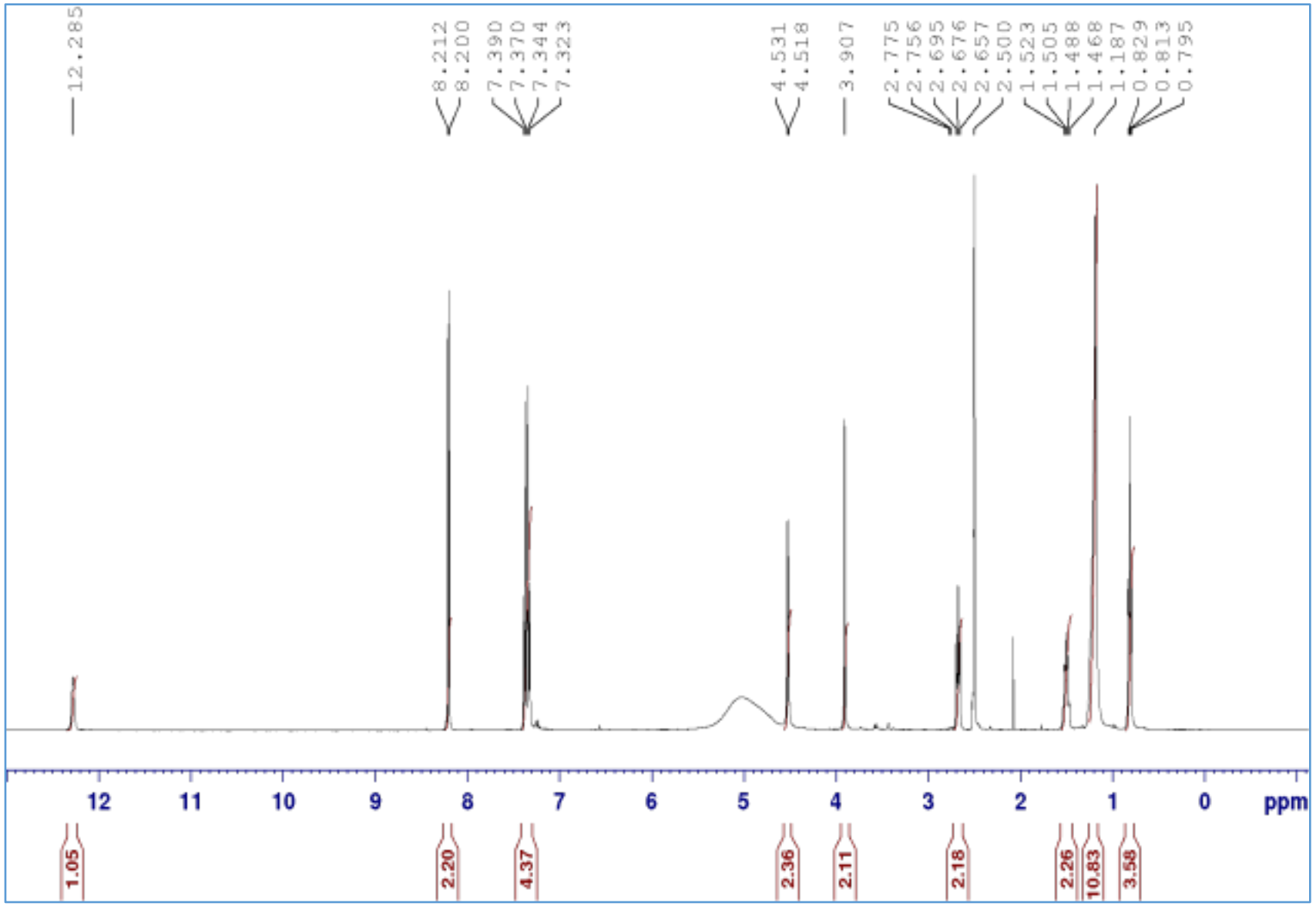

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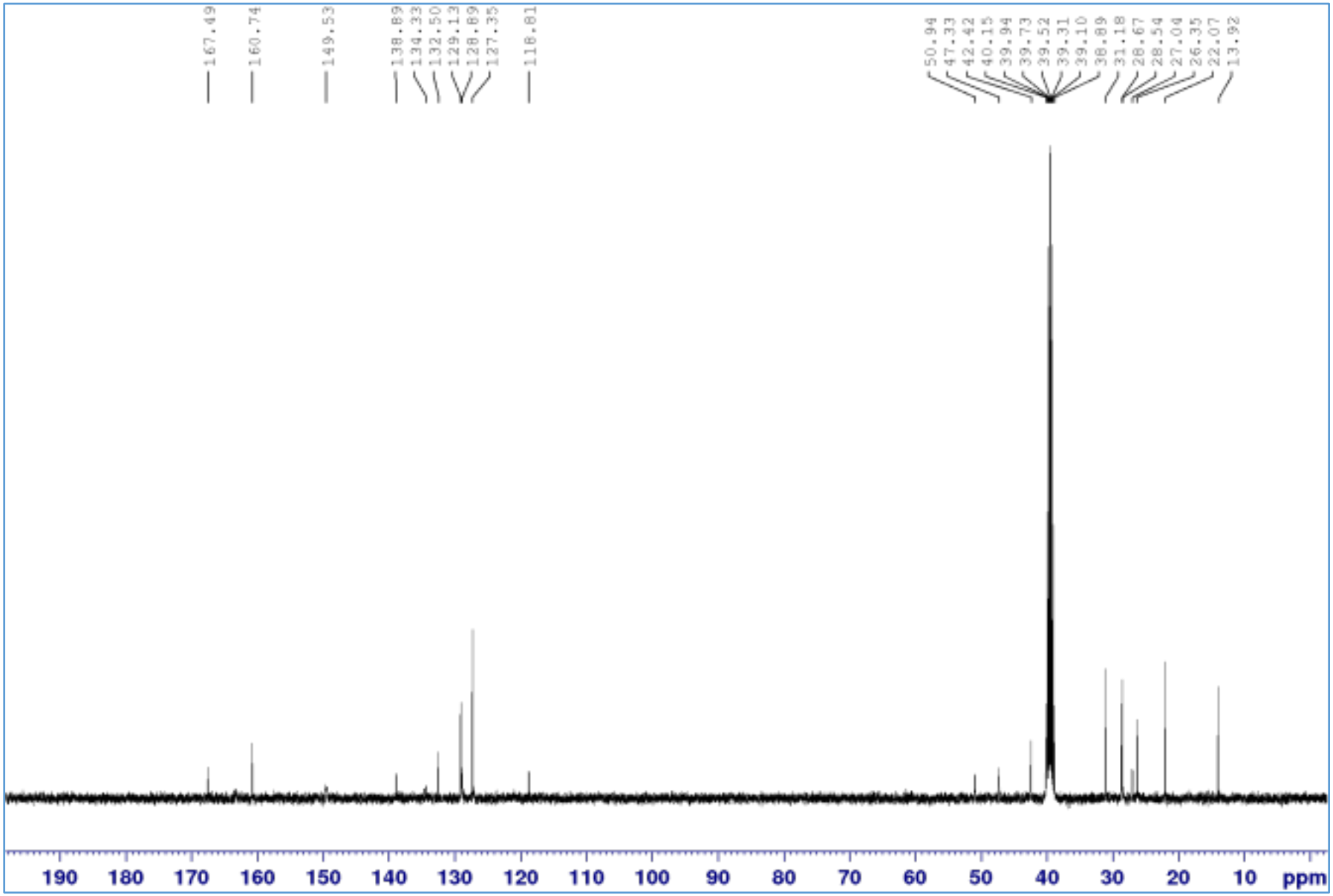

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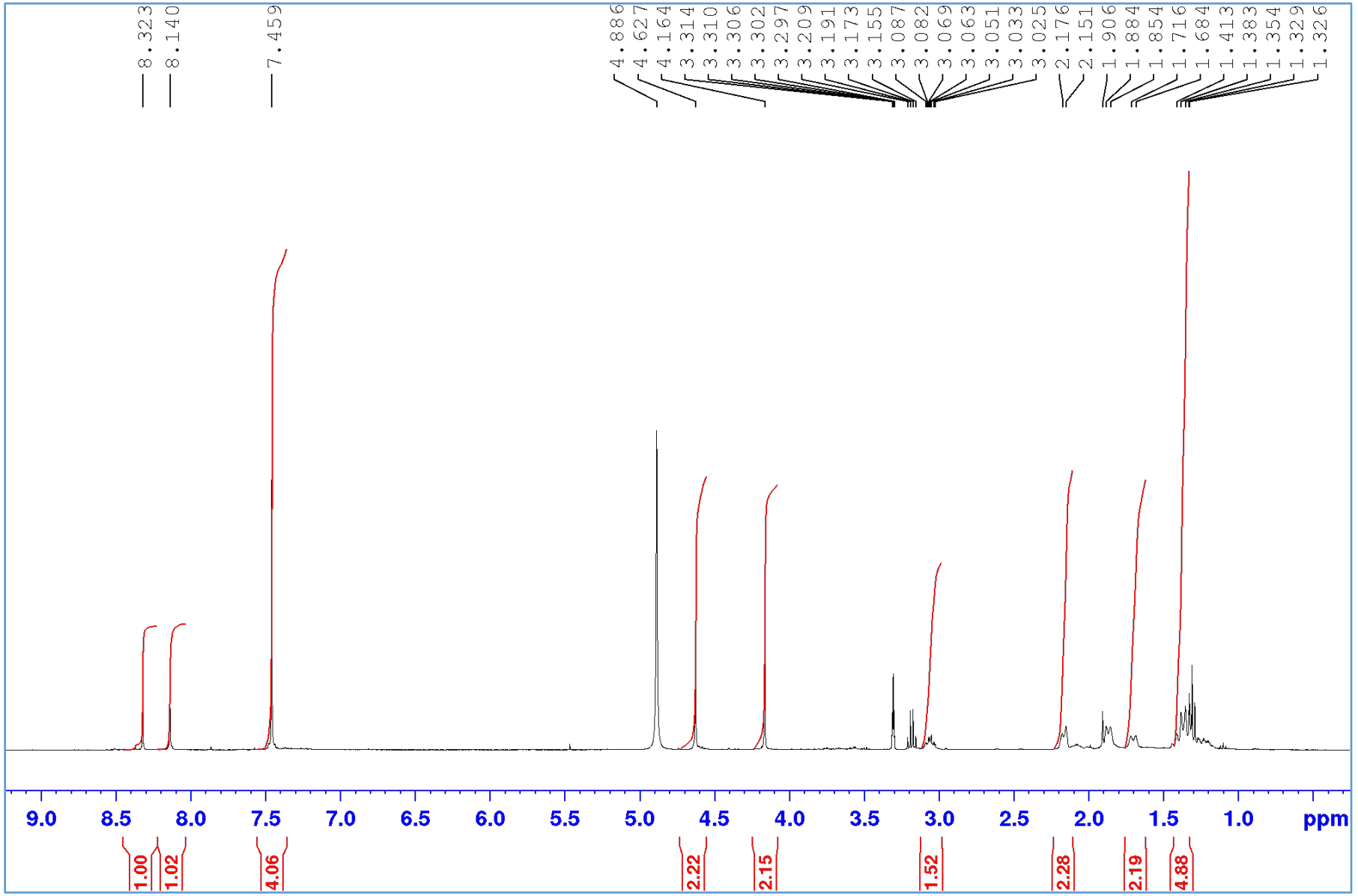

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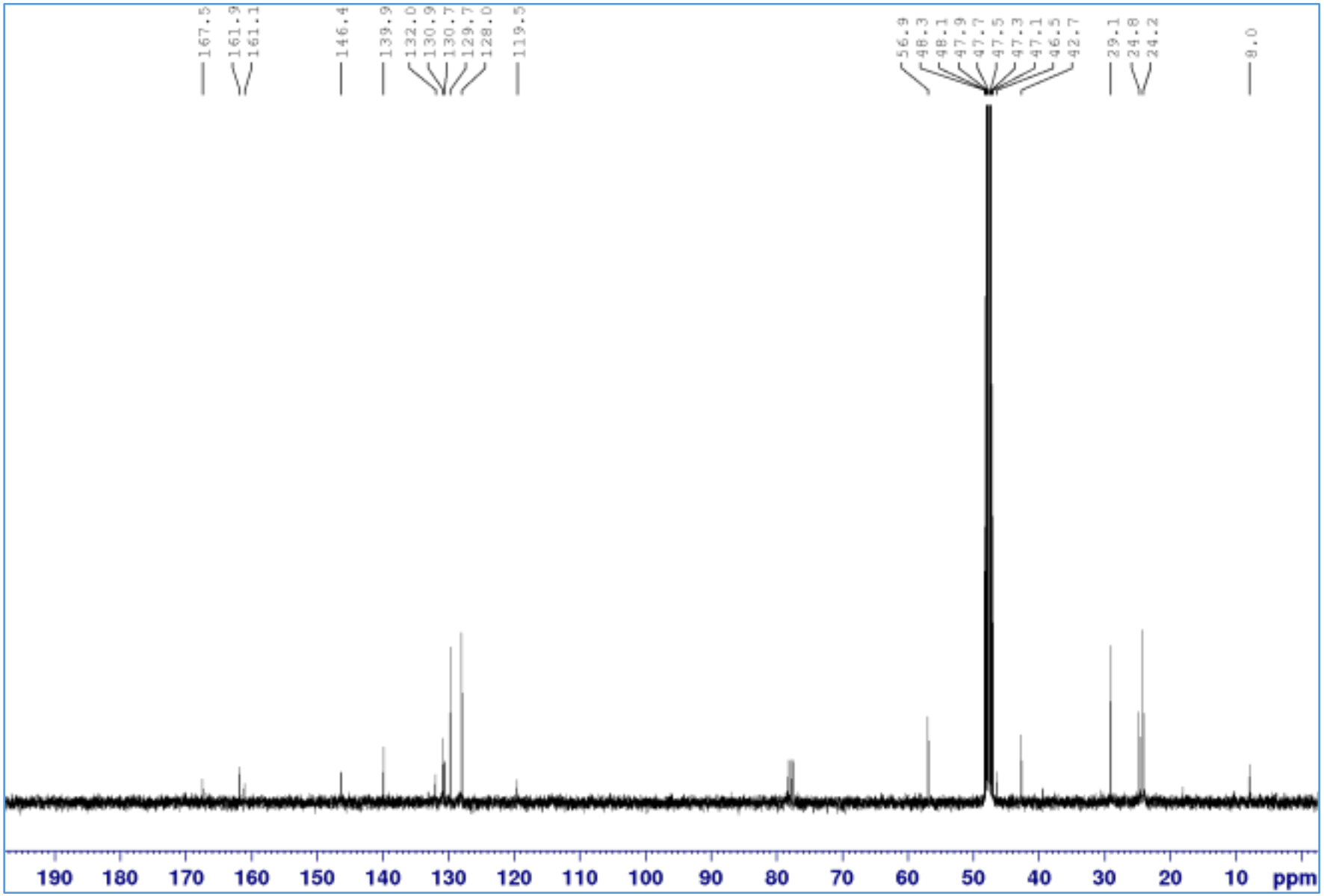

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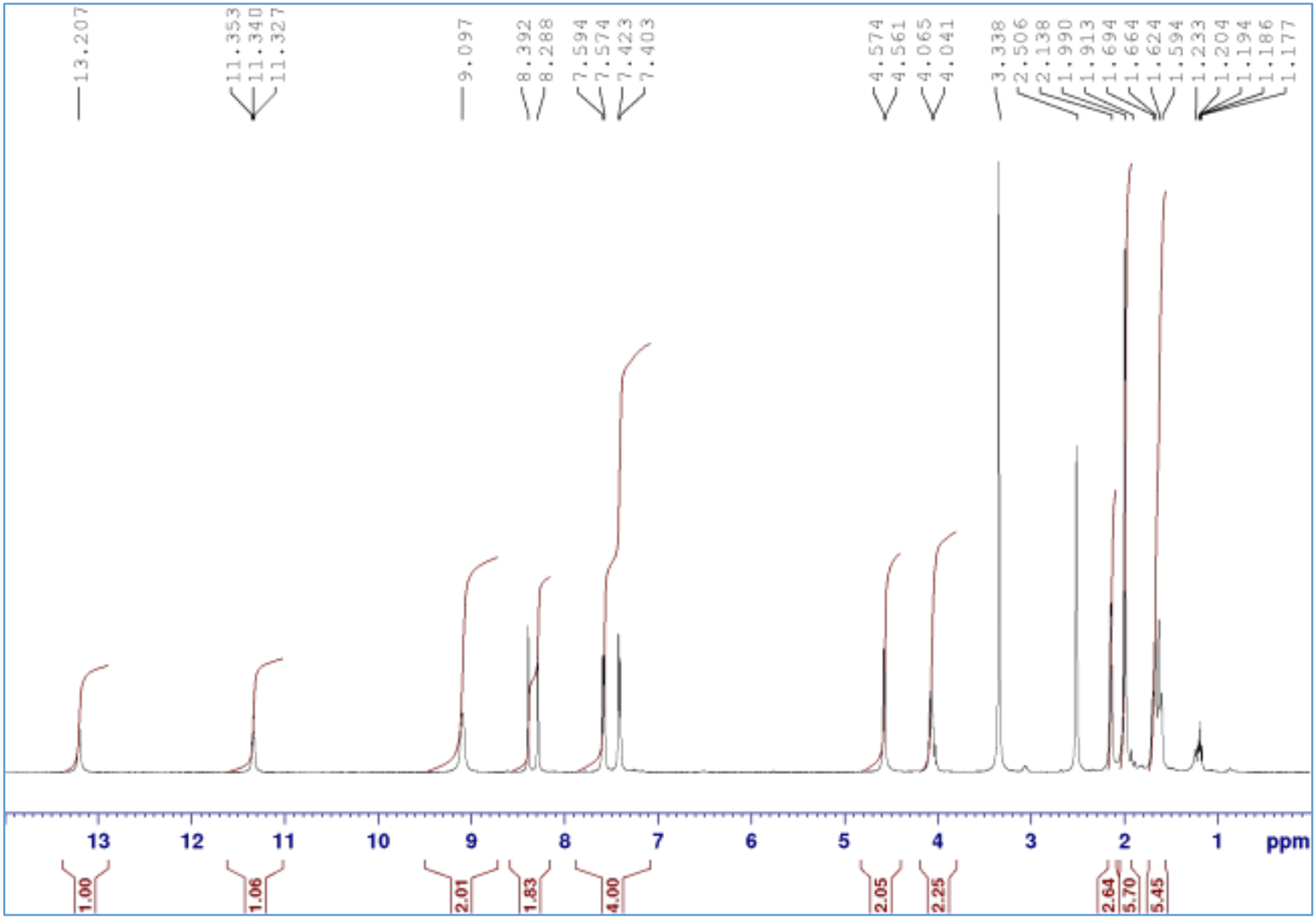

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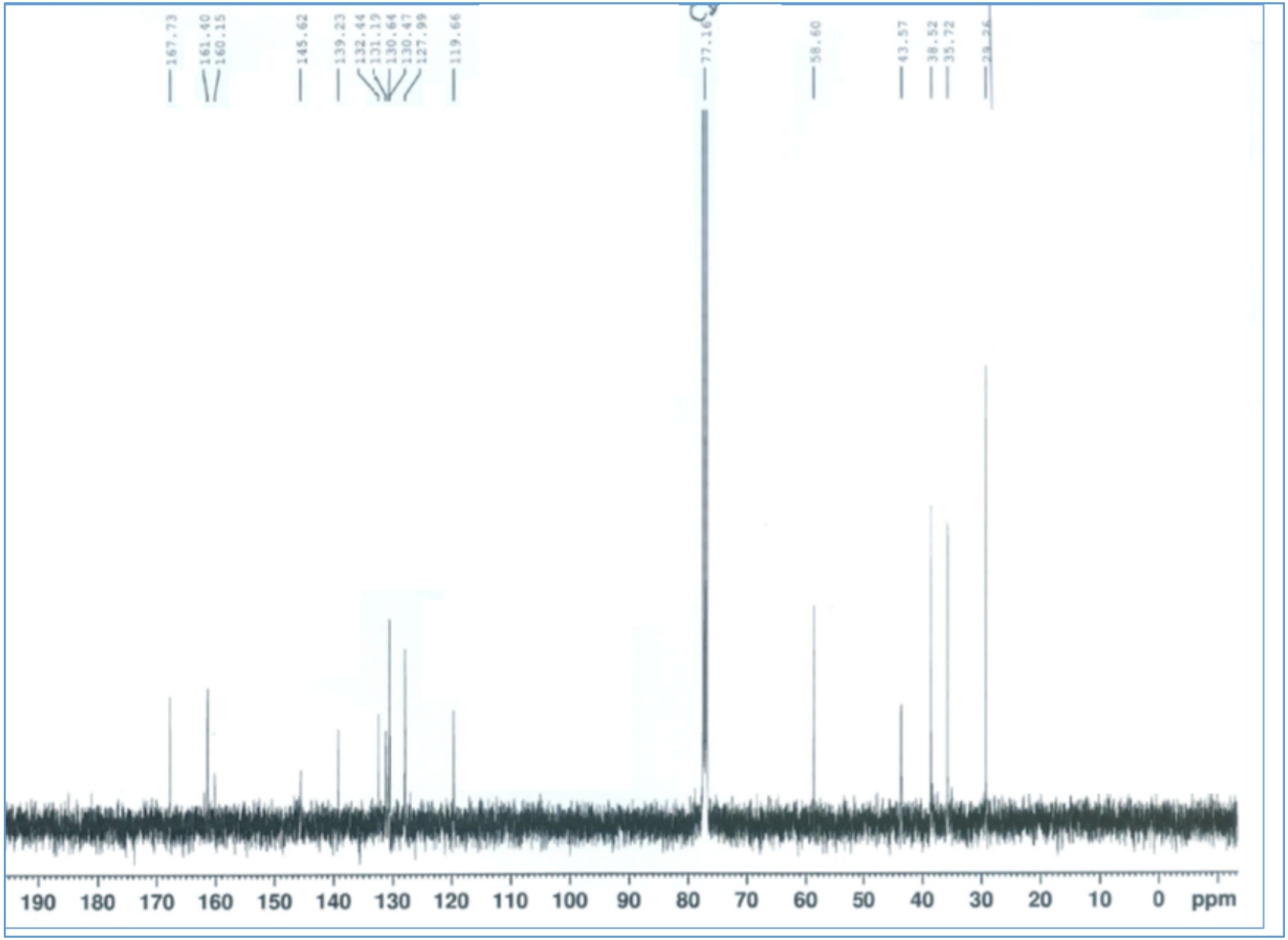

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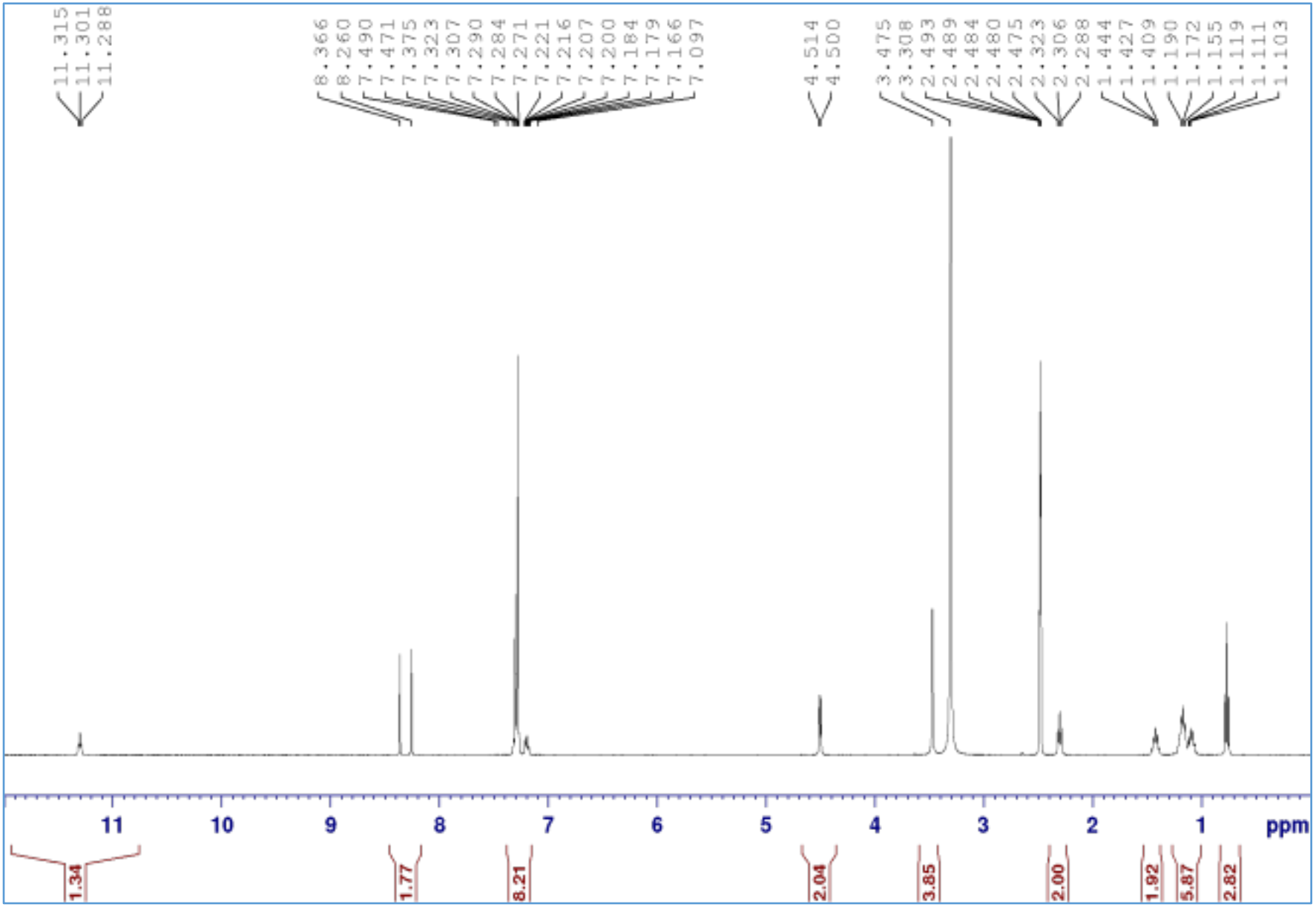

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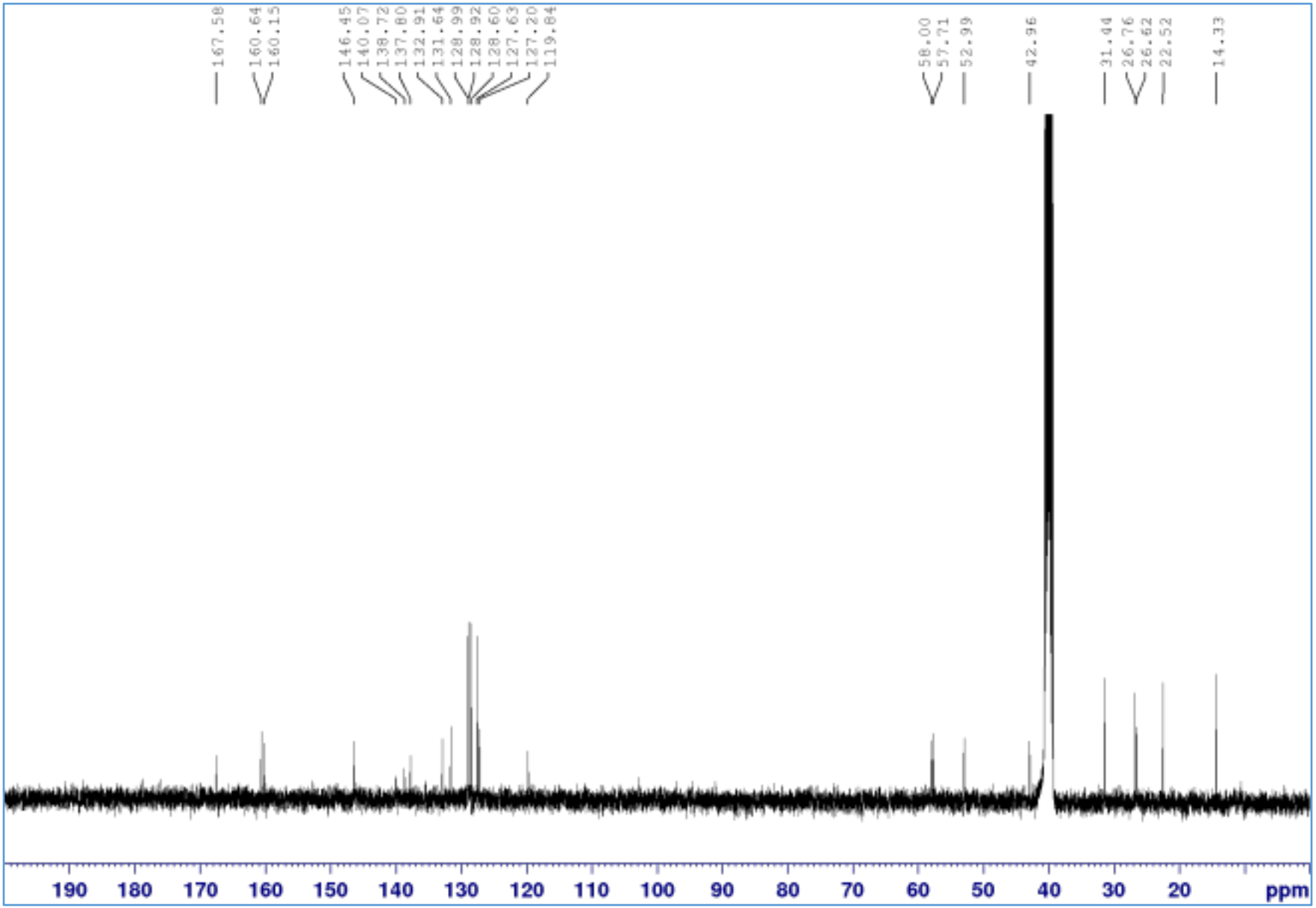

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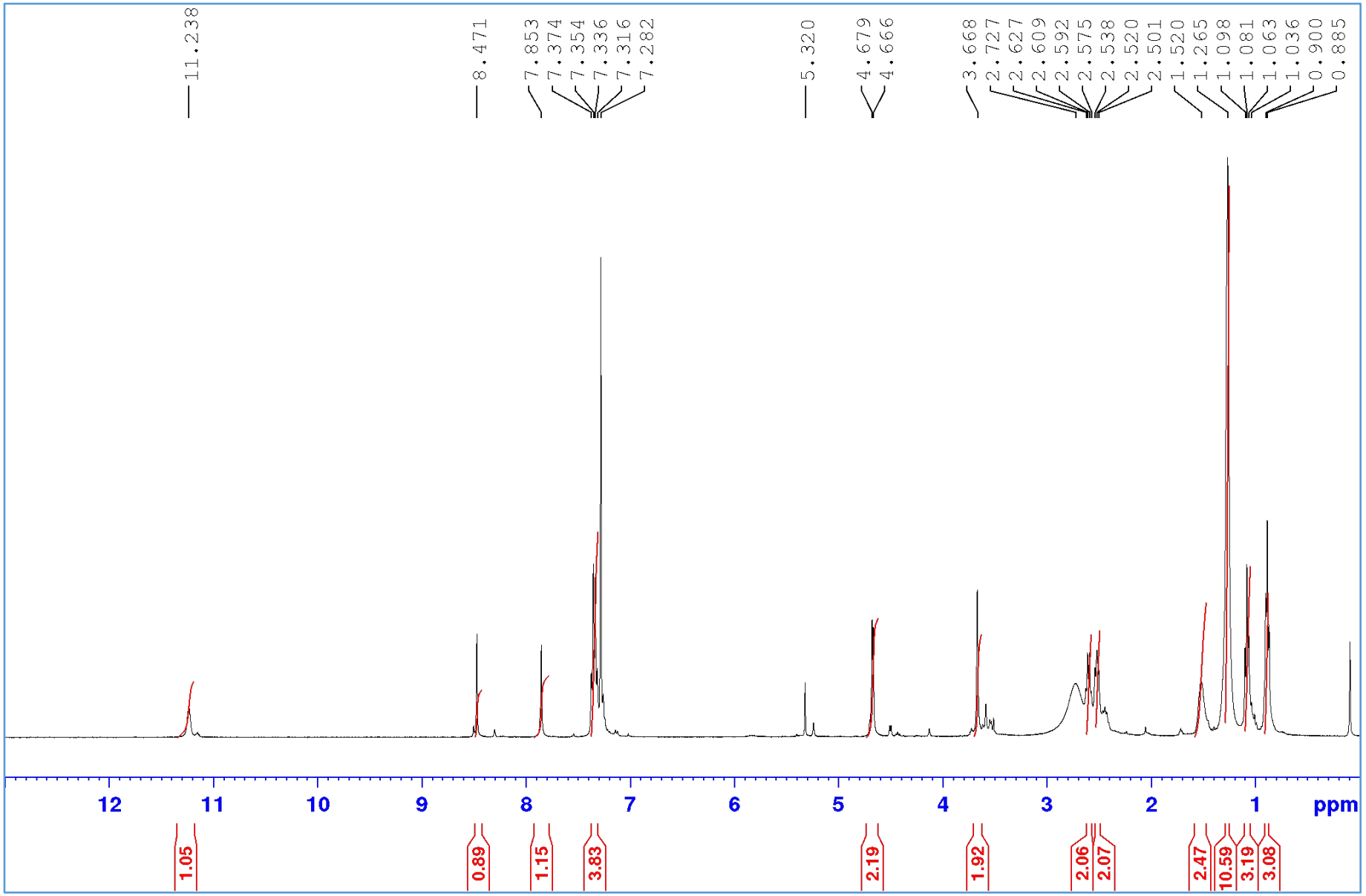

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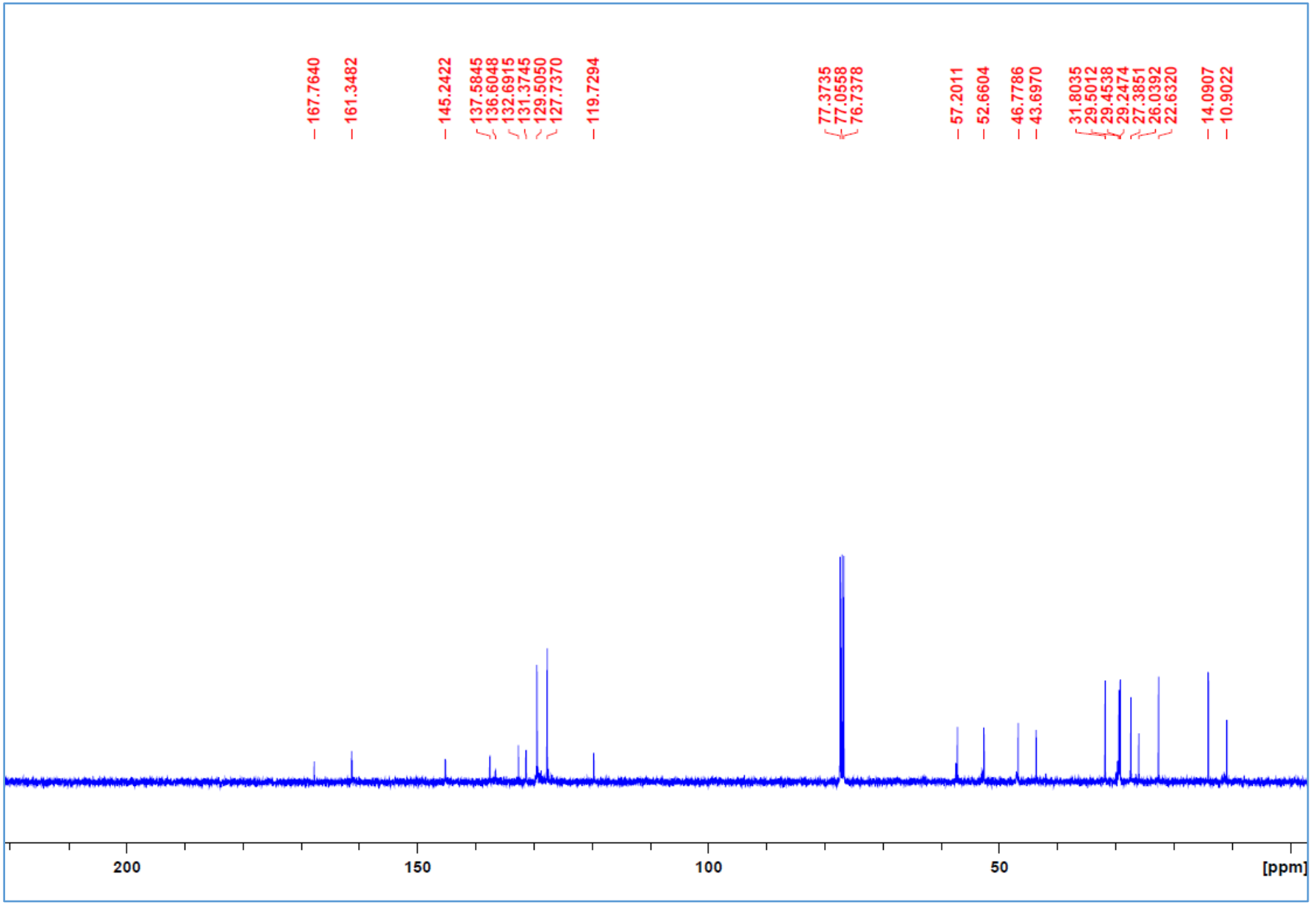

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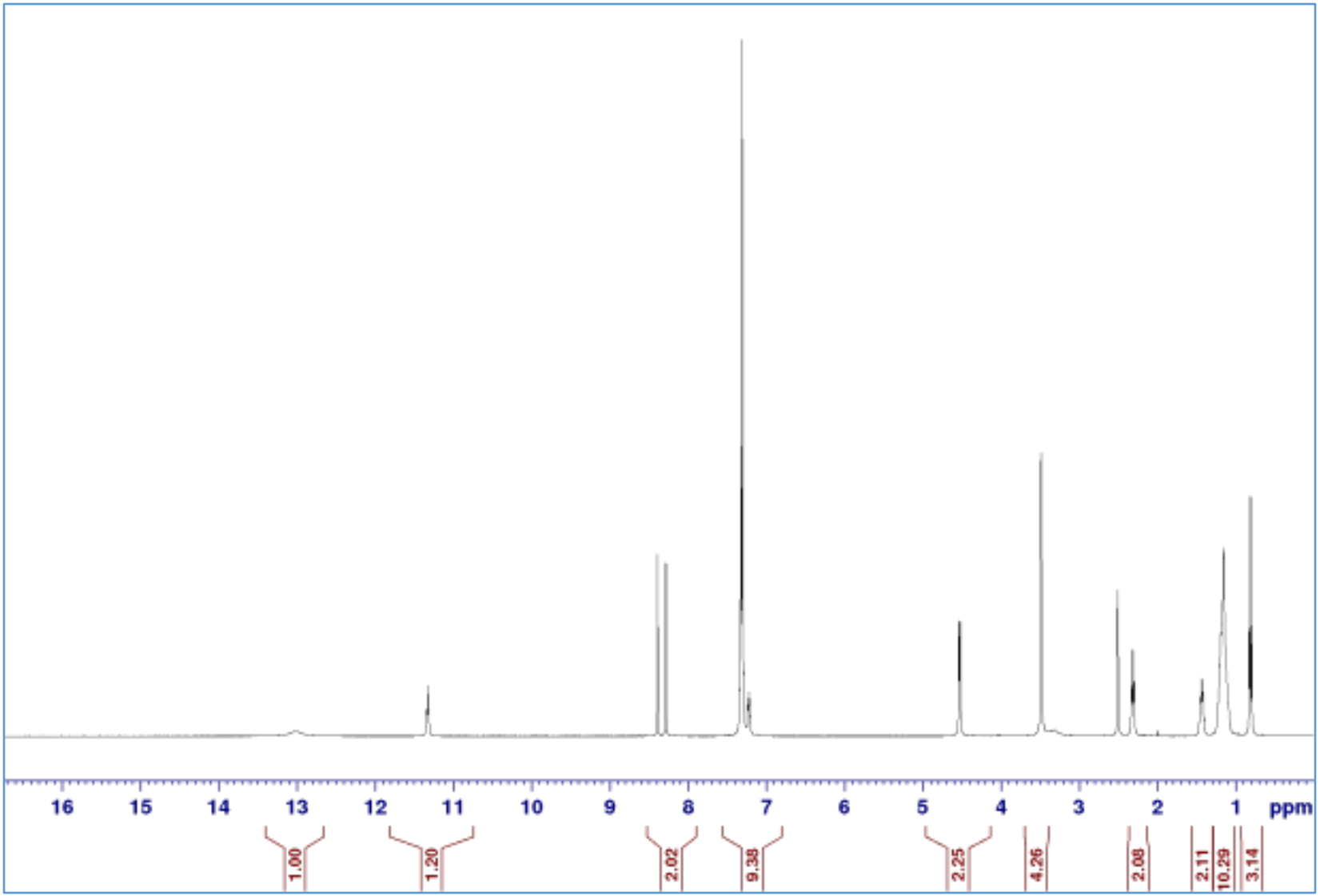

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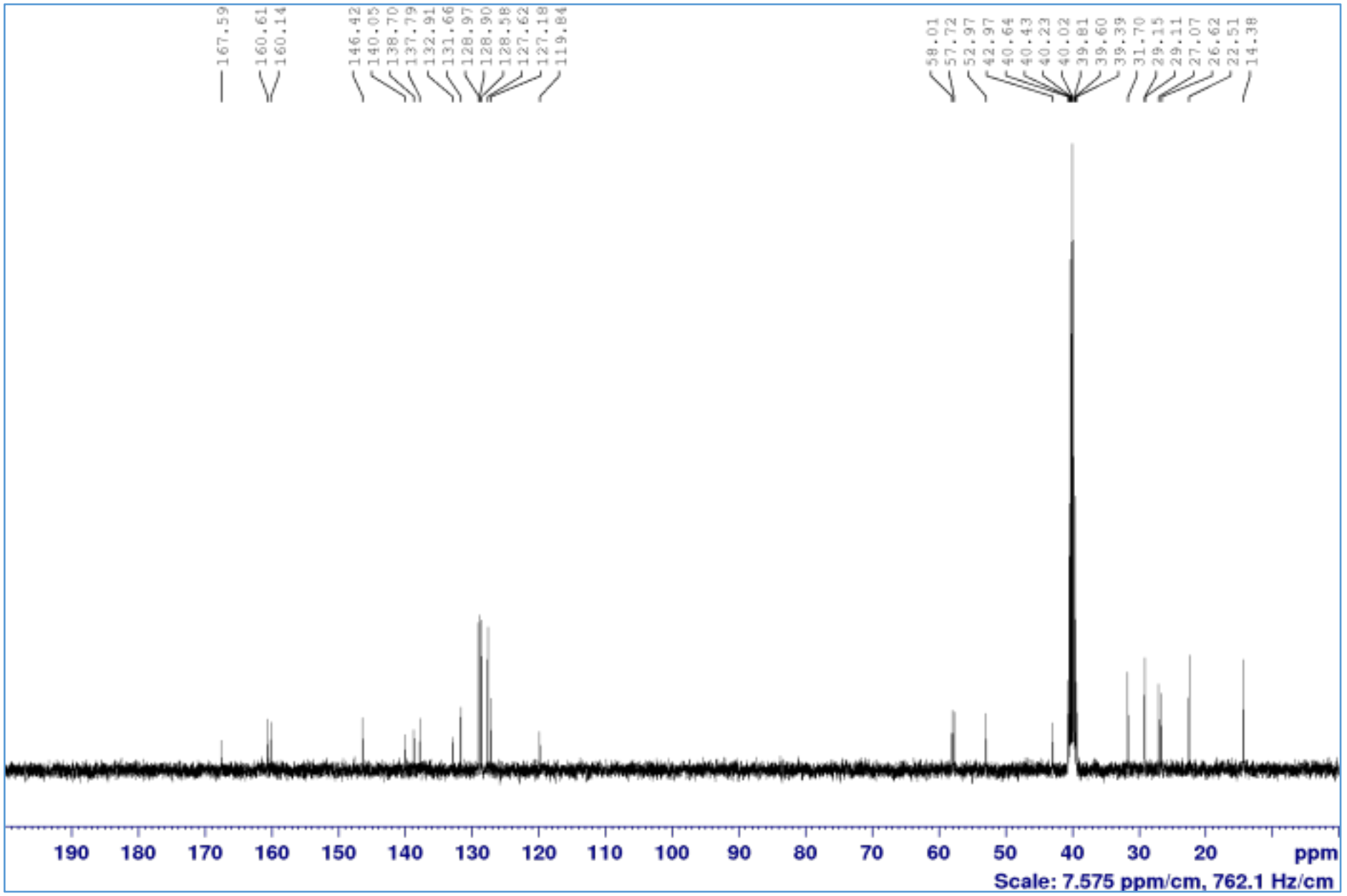

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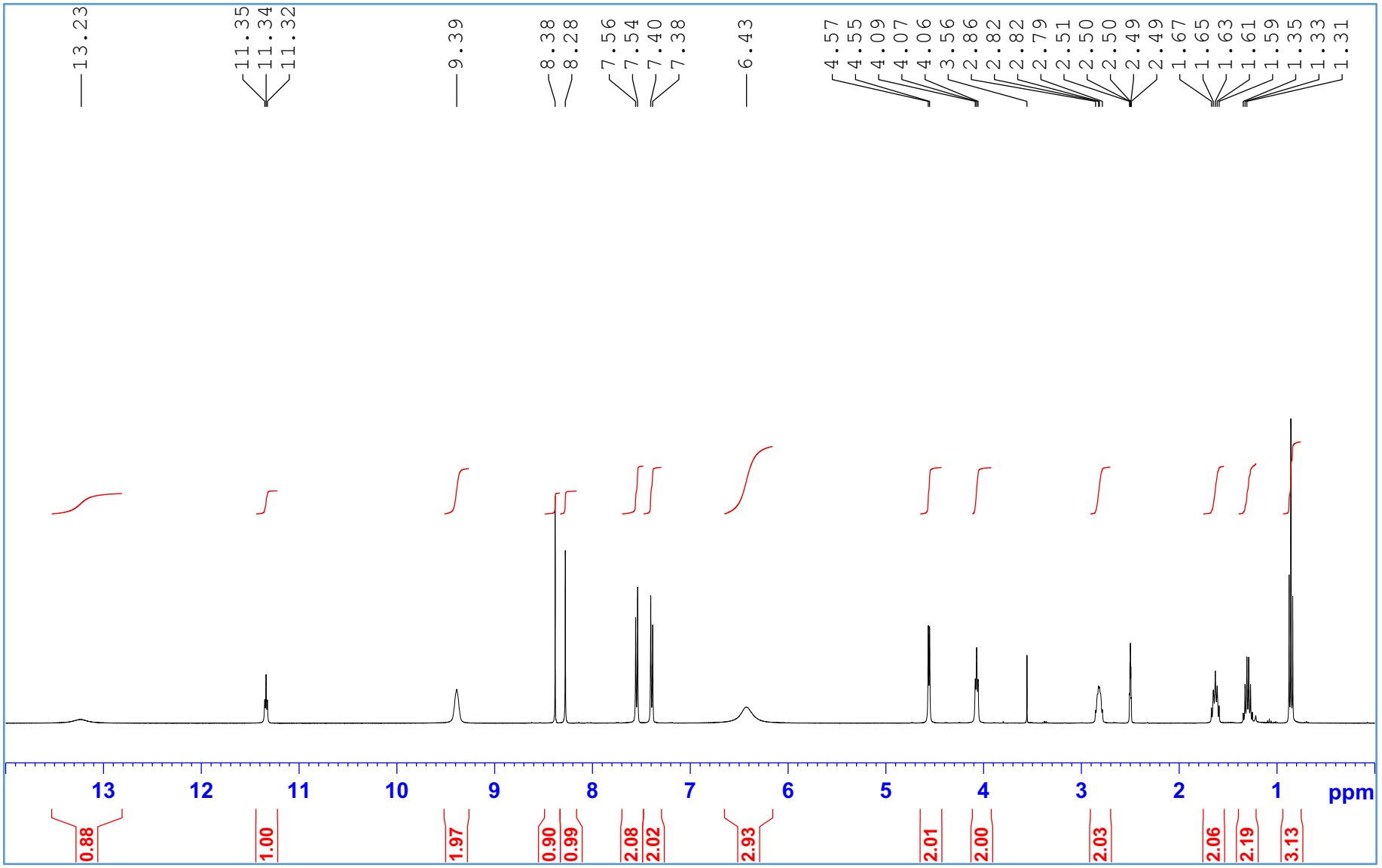

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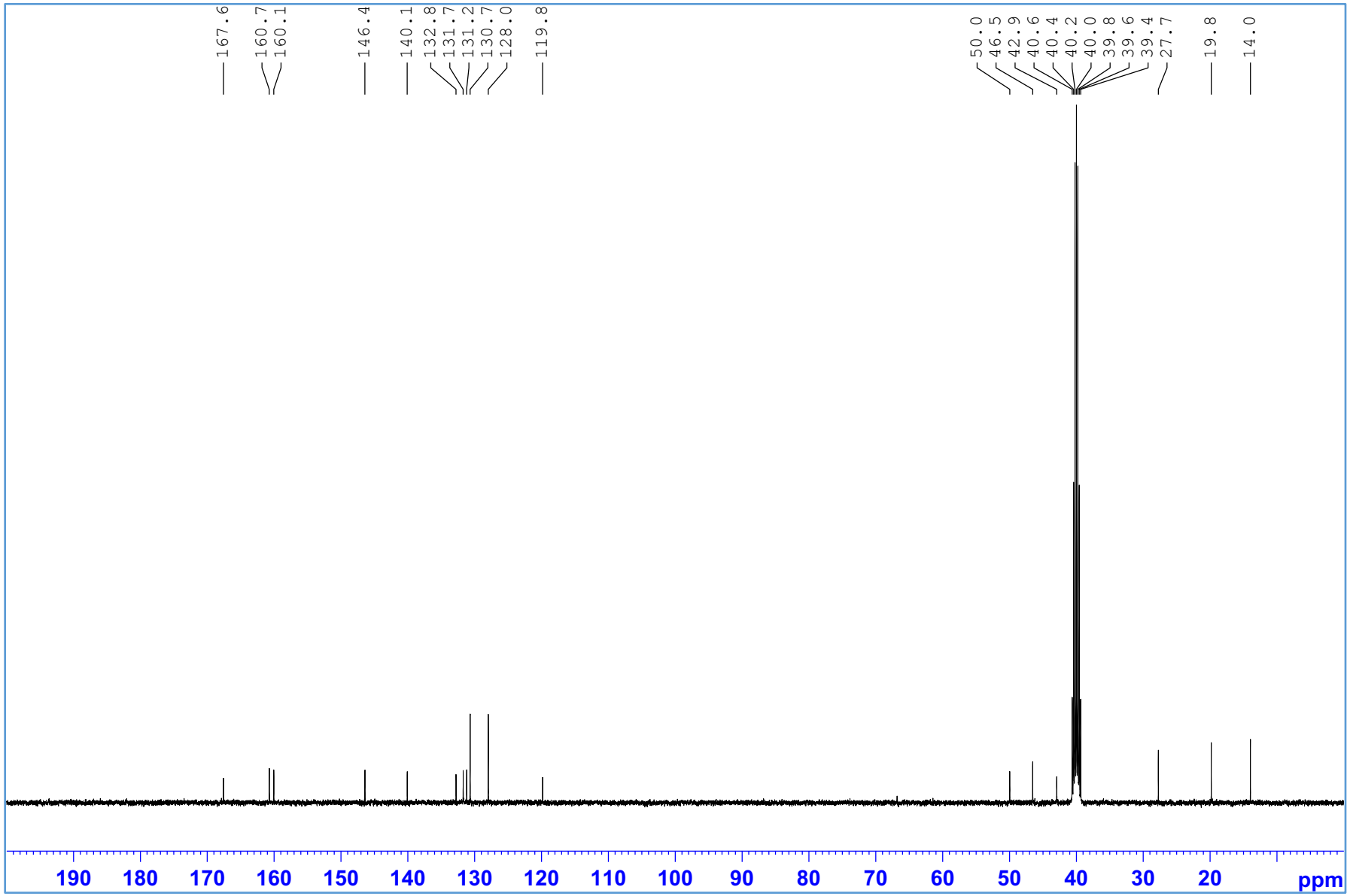
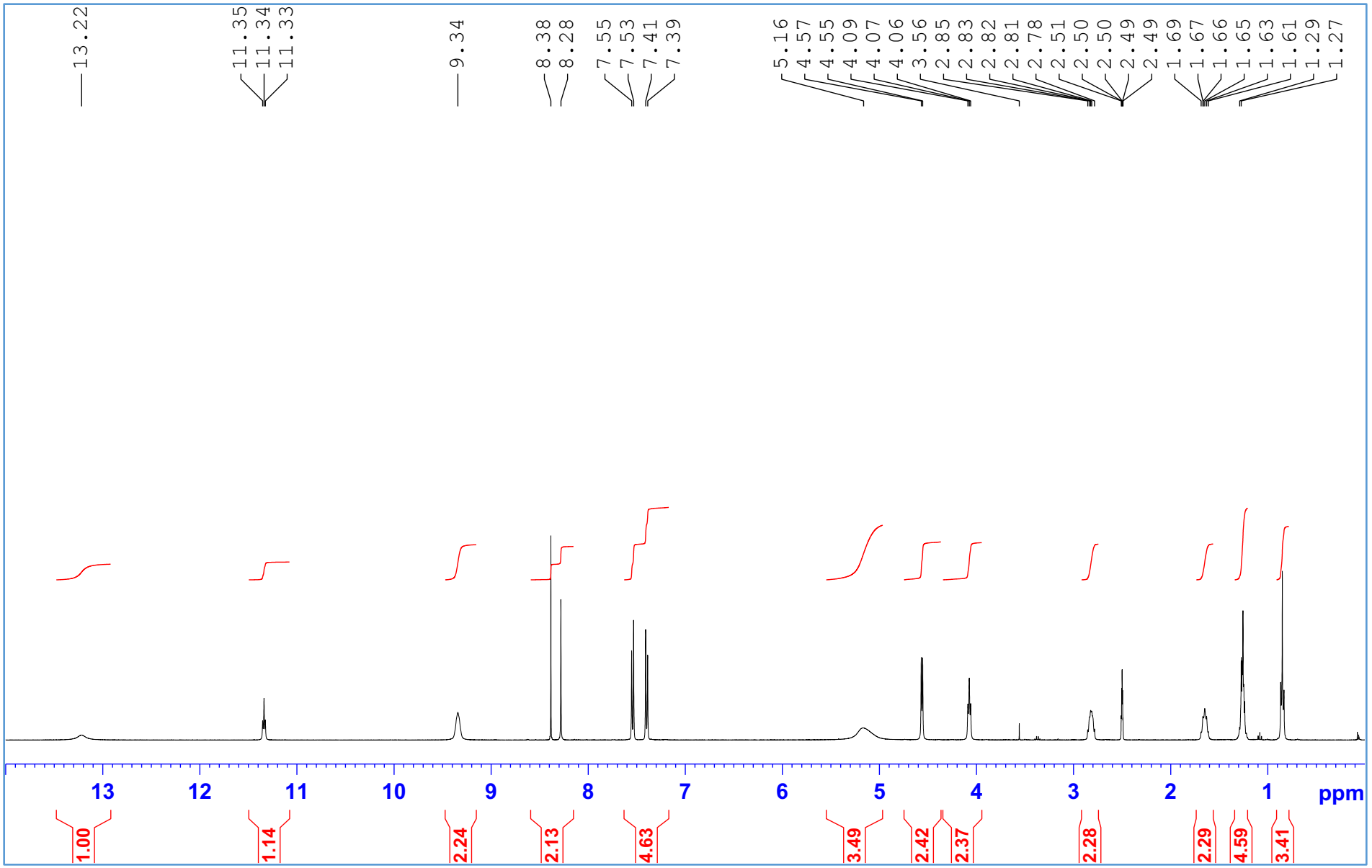
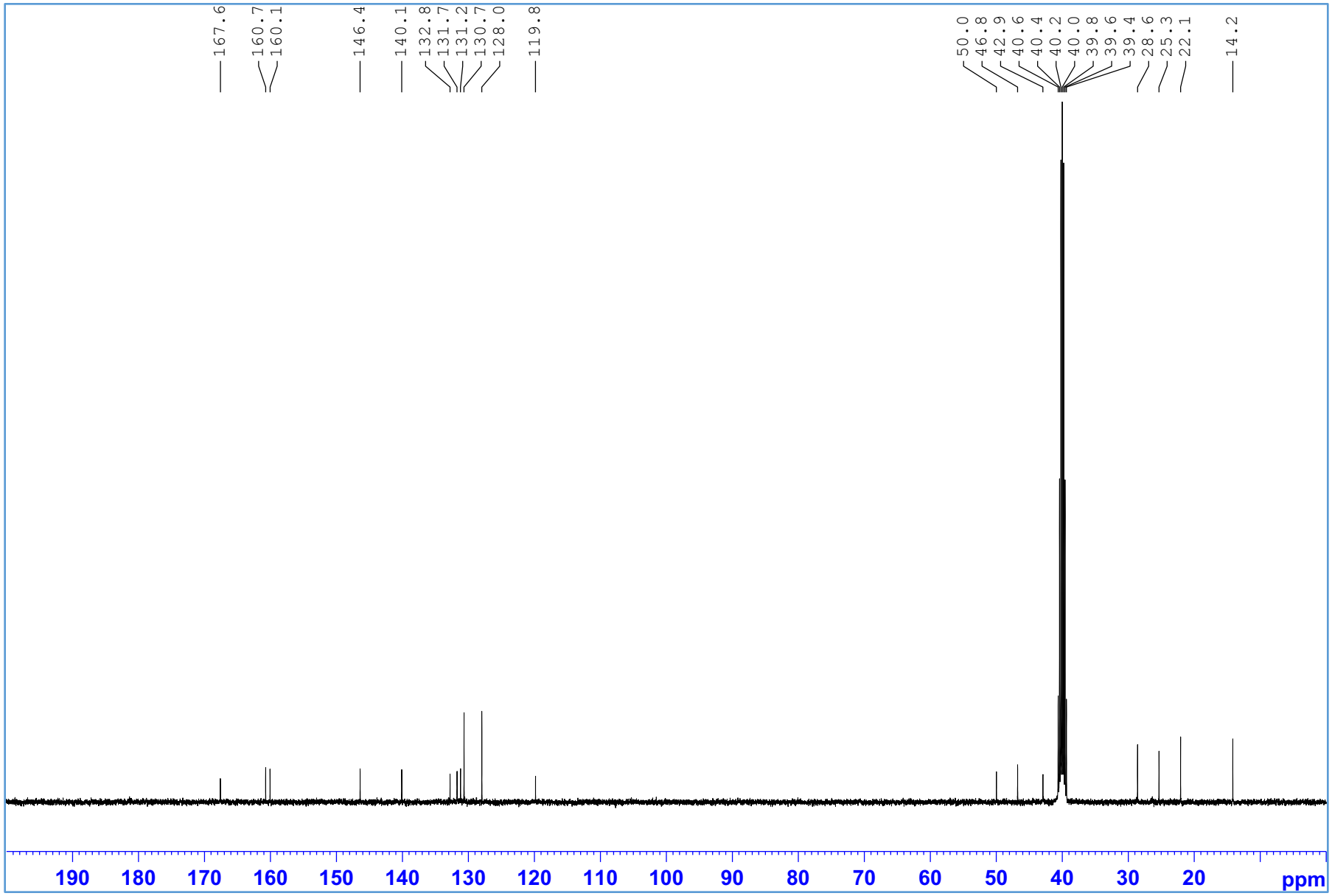


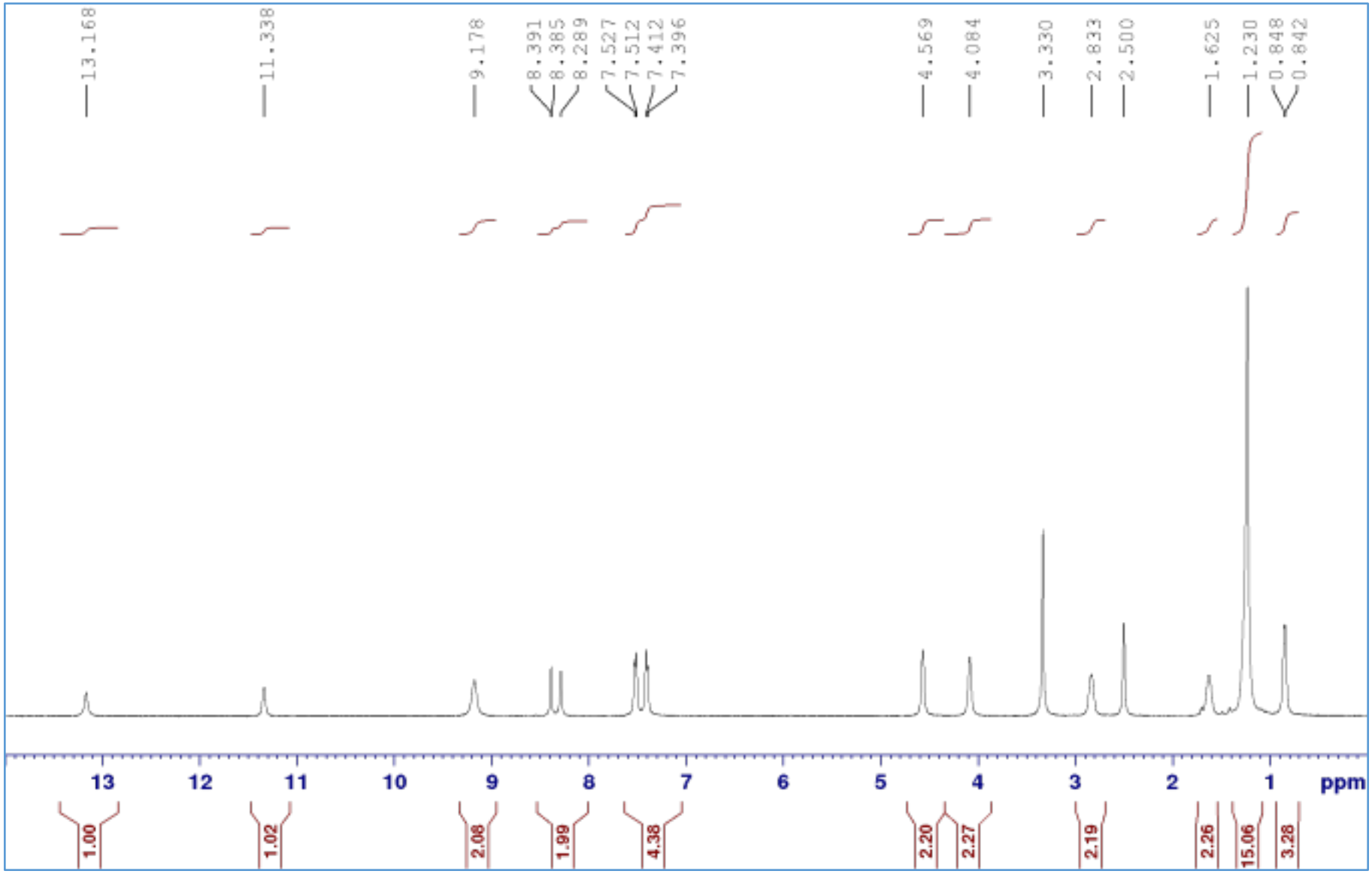
^{1}H NMR of compound **22** (400 MHz, DMSO-D_6)



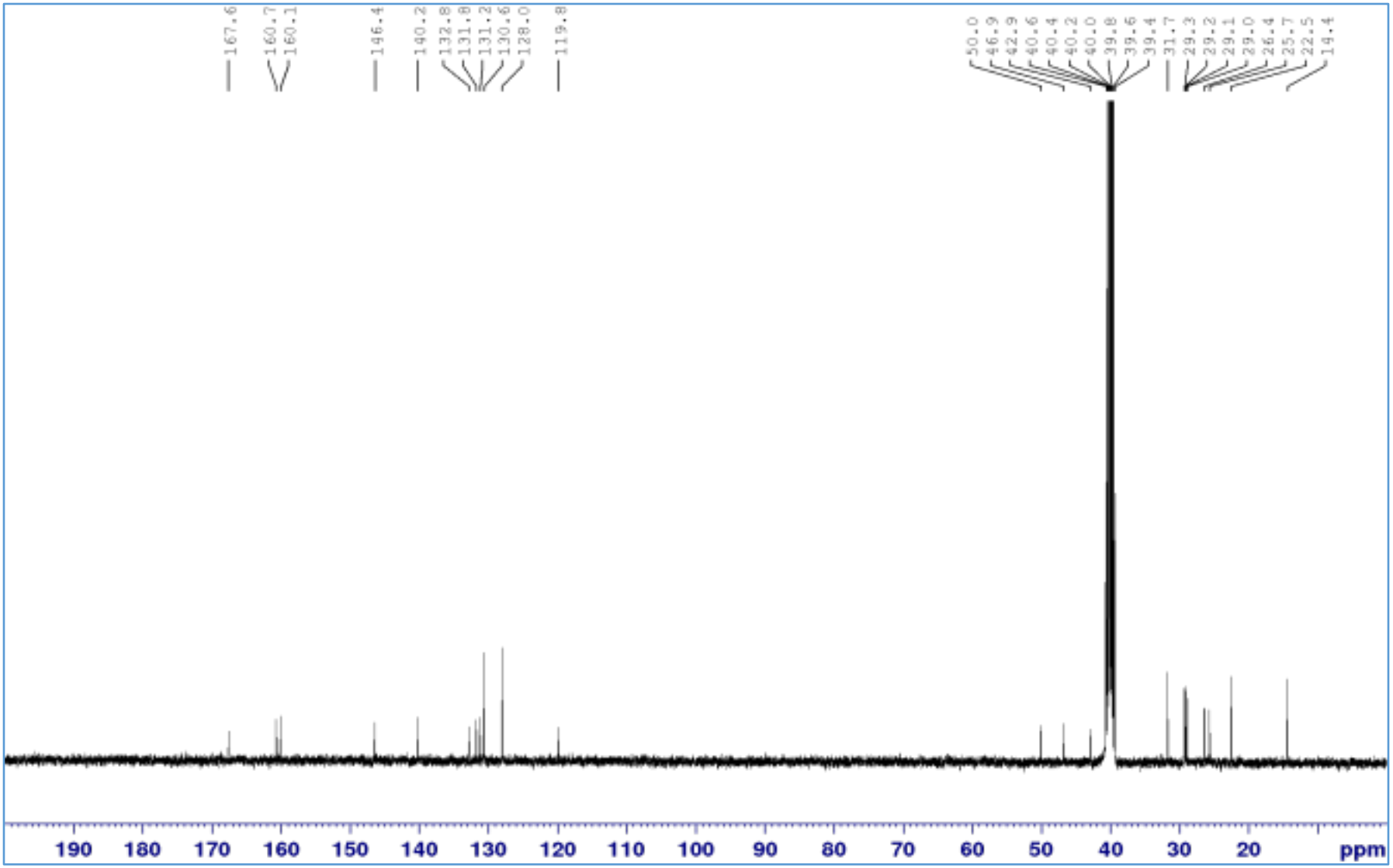
^{13}C NMR of compound **22** (100 MHz, DMSO-D_6)



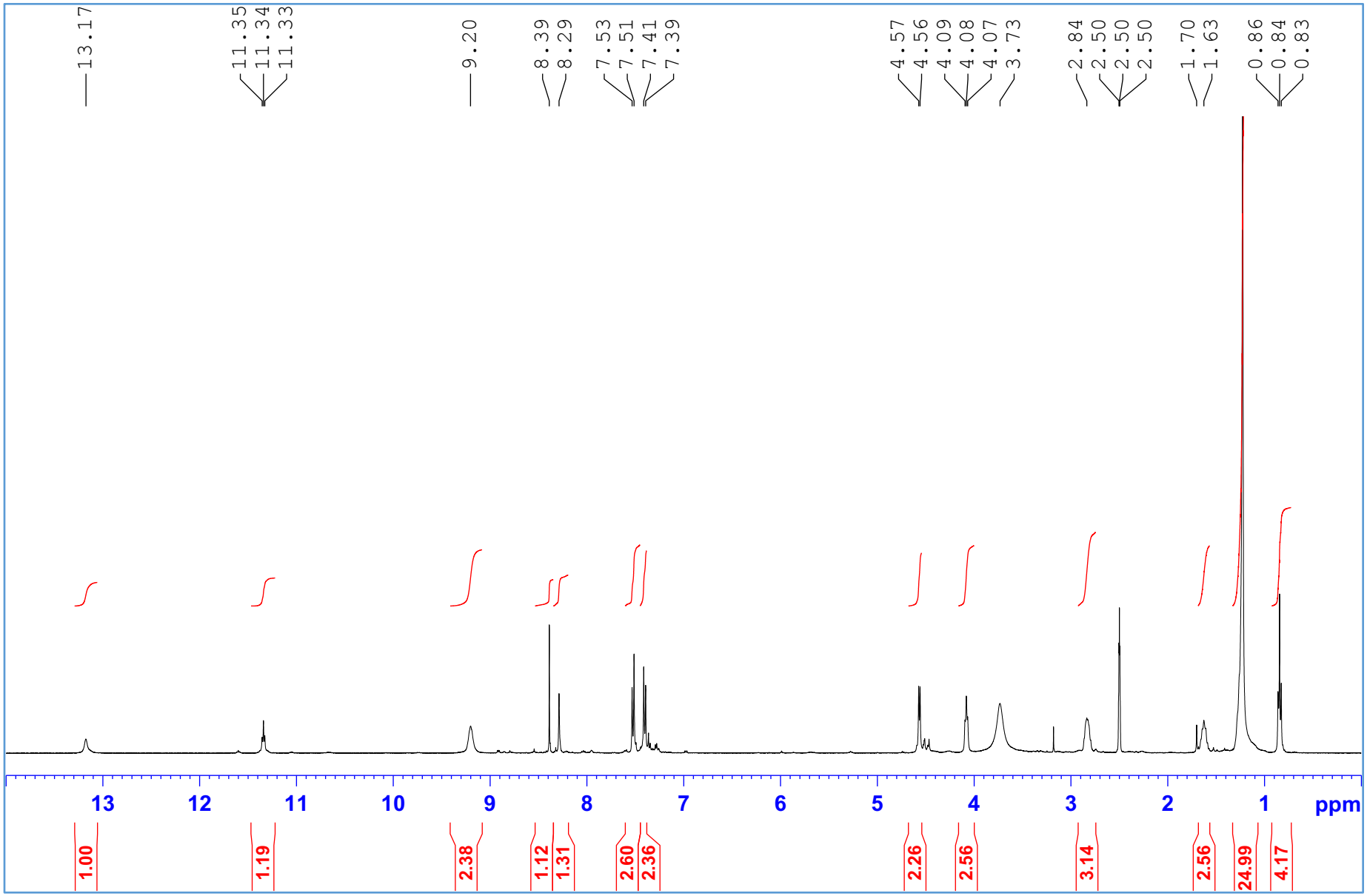
^{1}H NMR of compound **23** (400 MHz, DMSO-D_6)



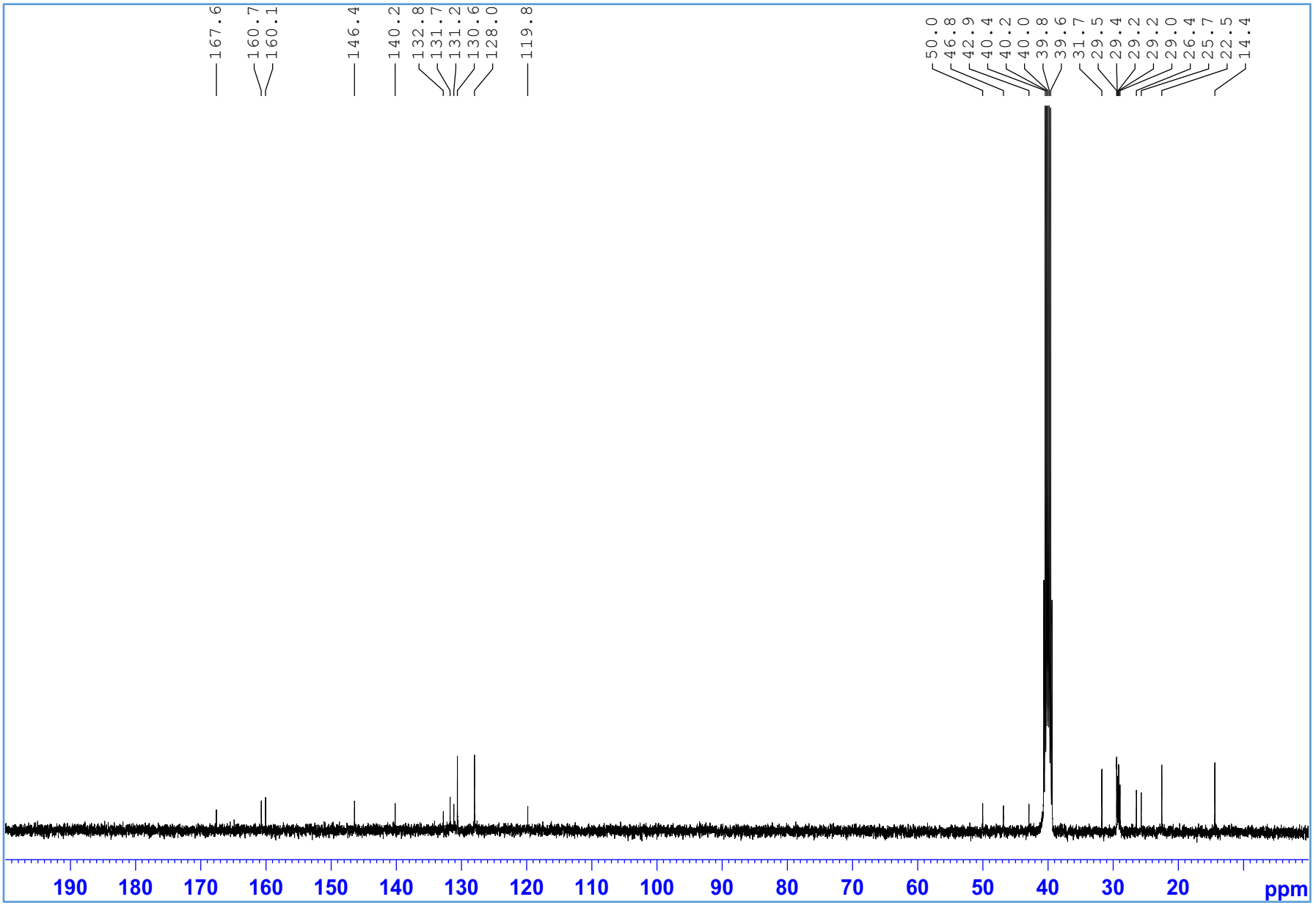
^{13}C NMR of compound **23** (100 MHz, DMSO-D_6)



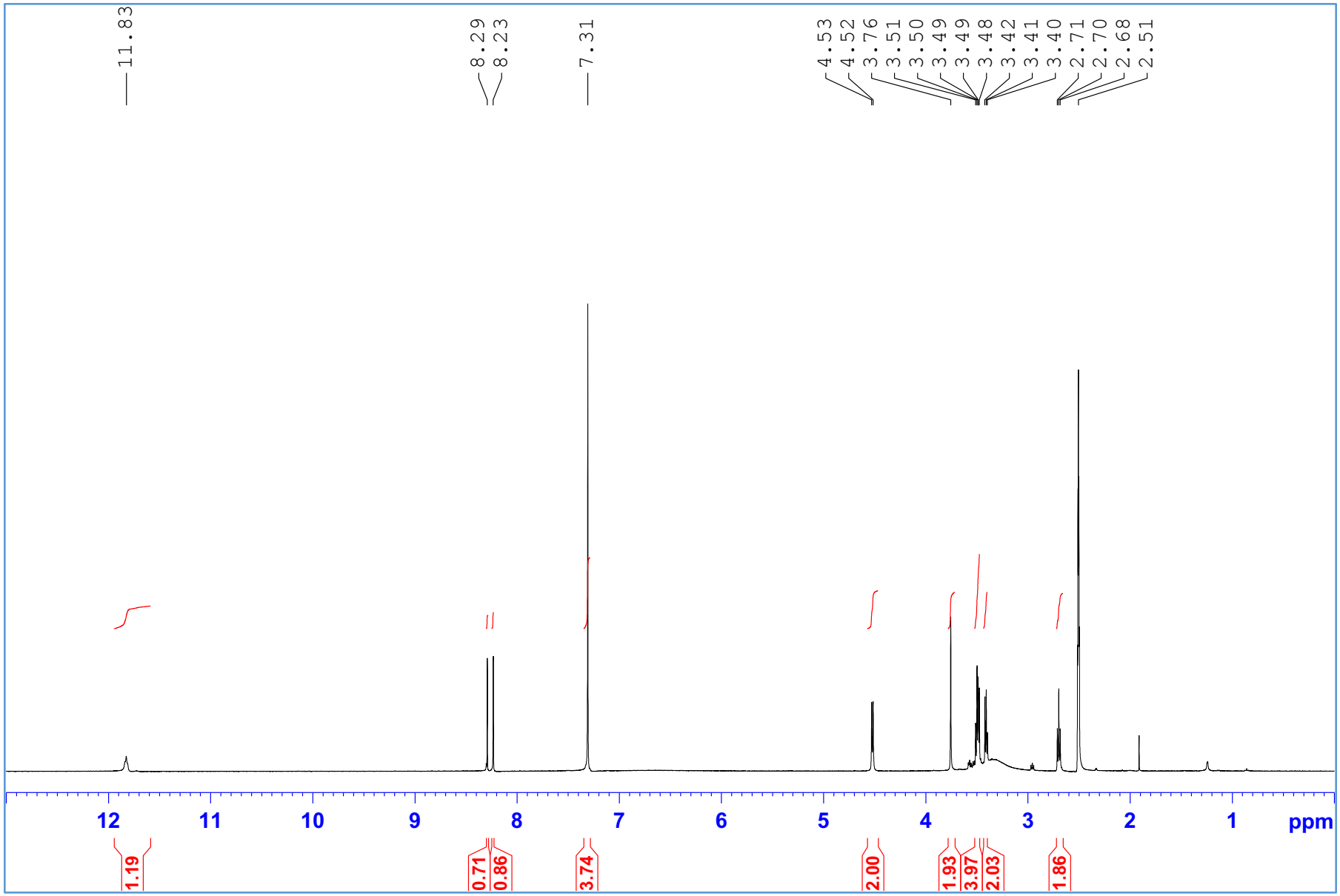
^{1}H NMR of compound **24** (400 MHz, DMSO-D_6)



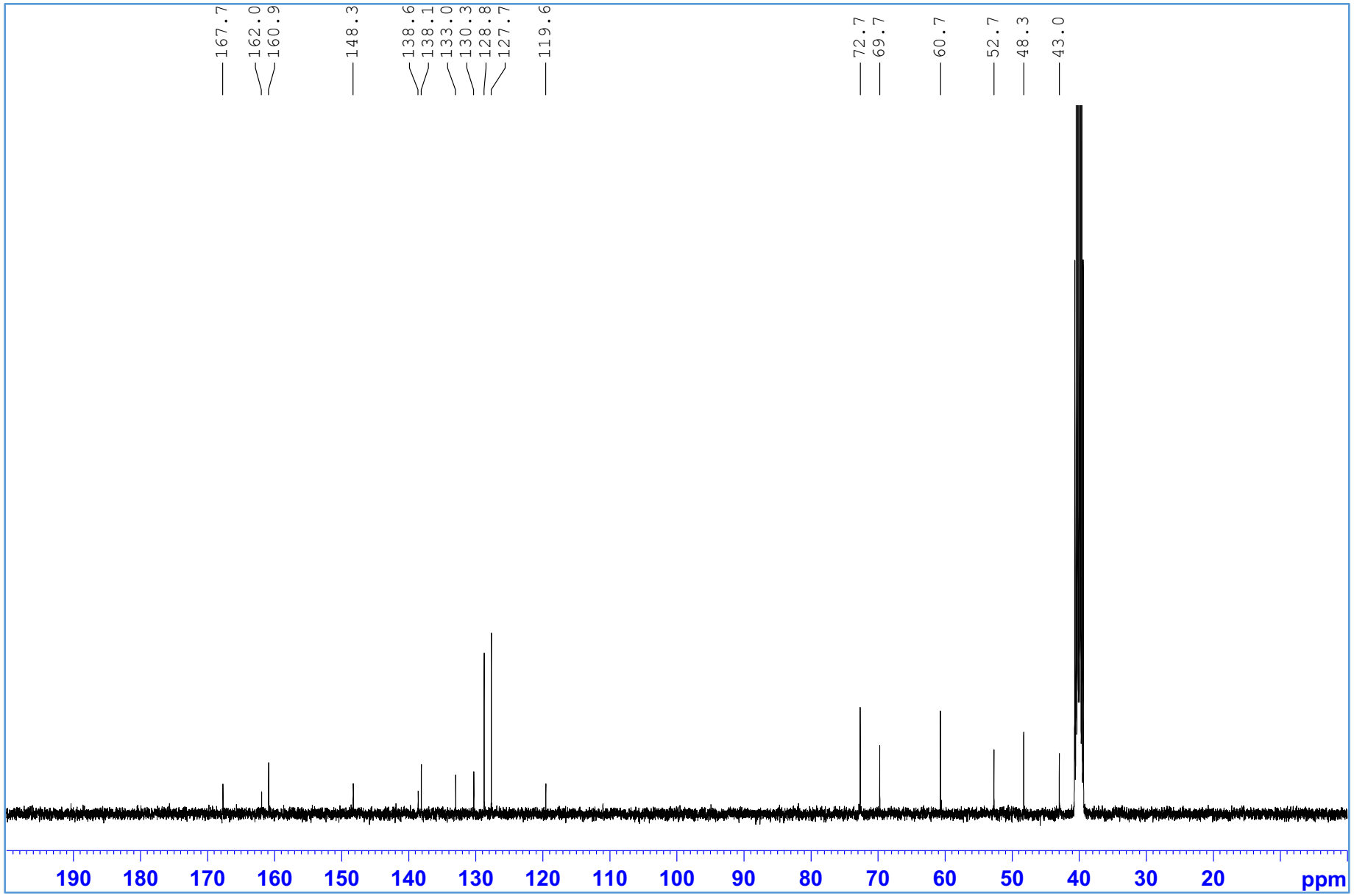
^{13}C NMR of compound **24** (100 MHz, DMSO-D_6)



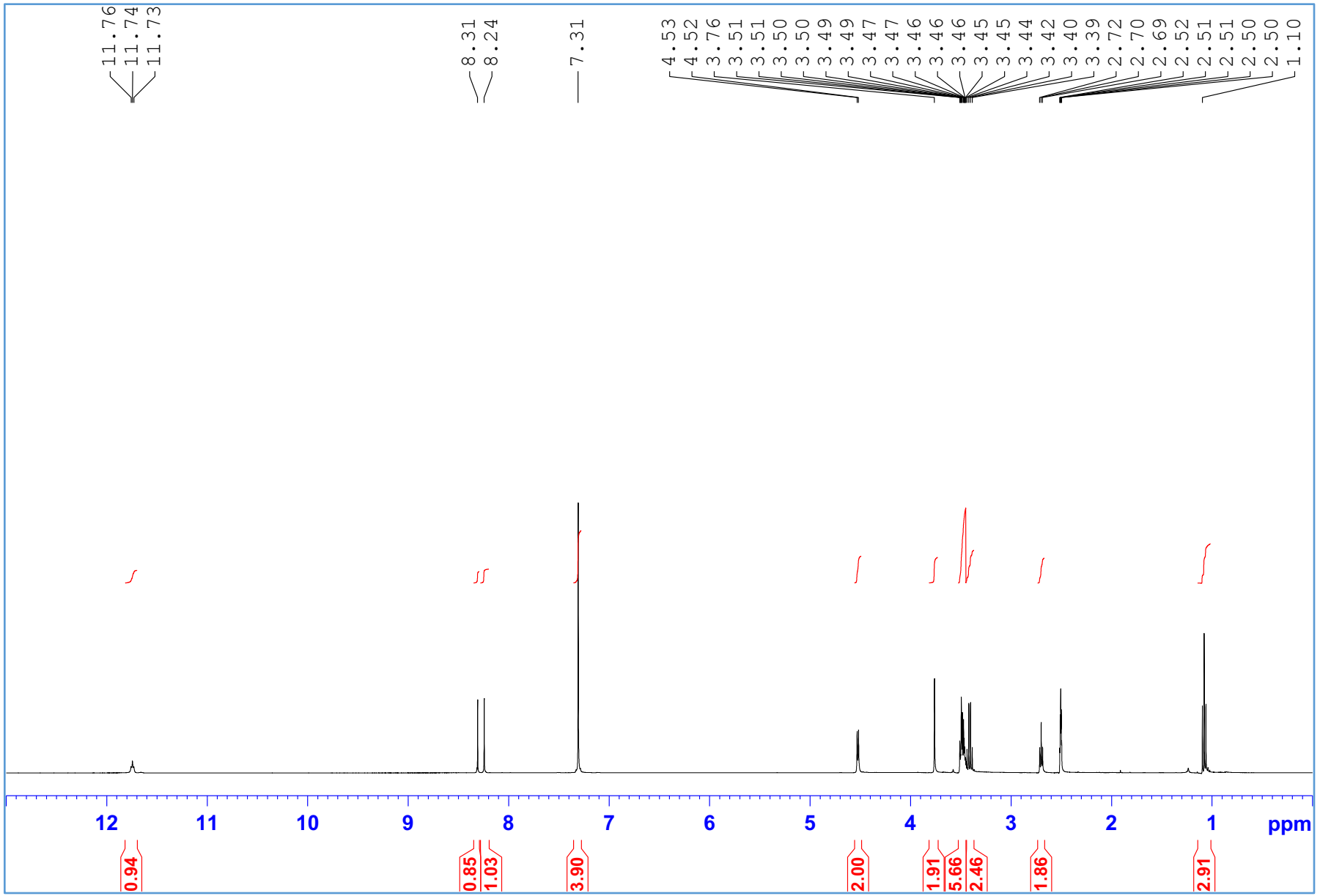
^{1}H NMR of compound **25** (400 MHz, DMSO-D_6)



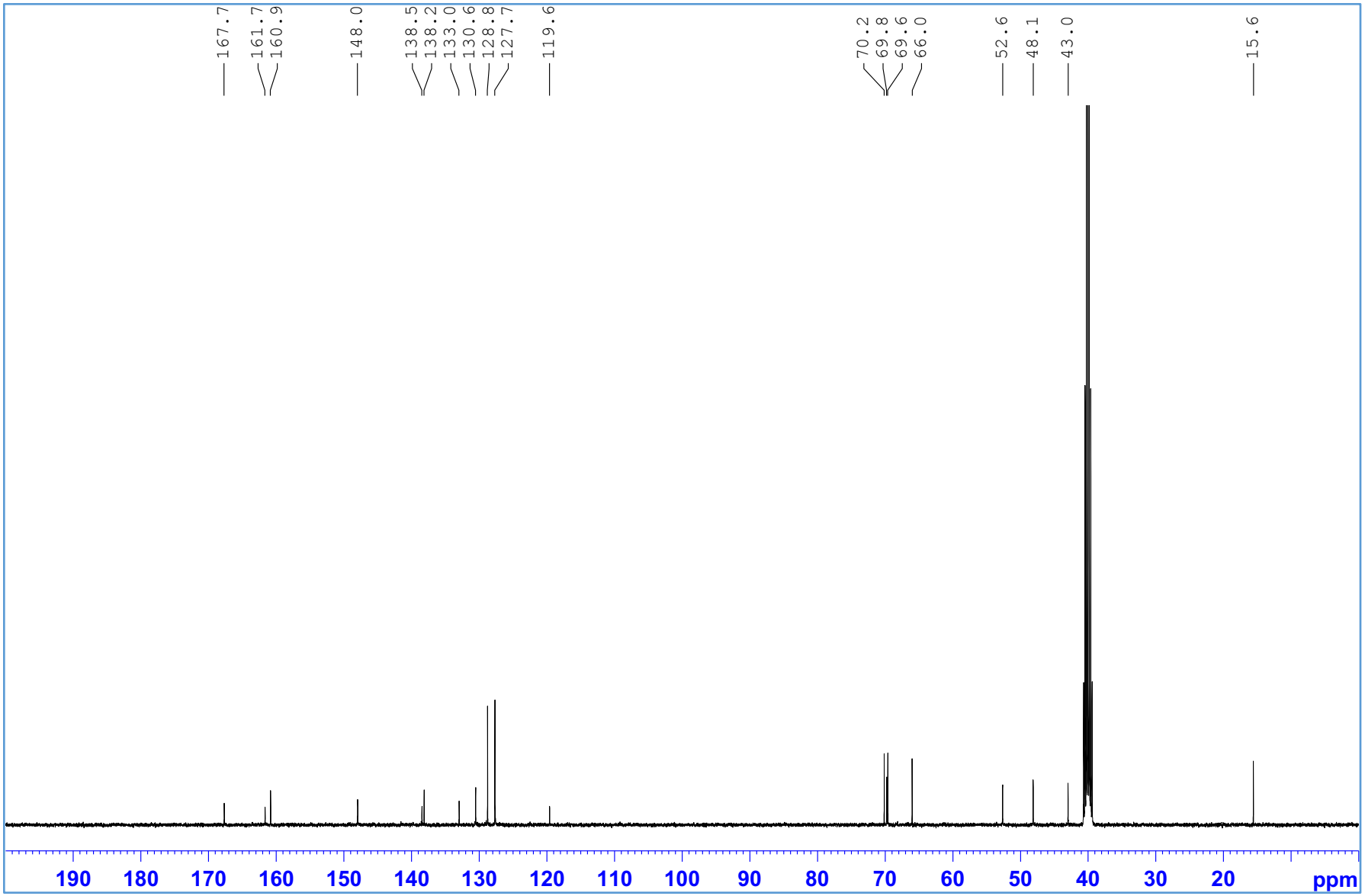
^{13}C NMR of compound **25** (100 MHz, DMSO-D_6)



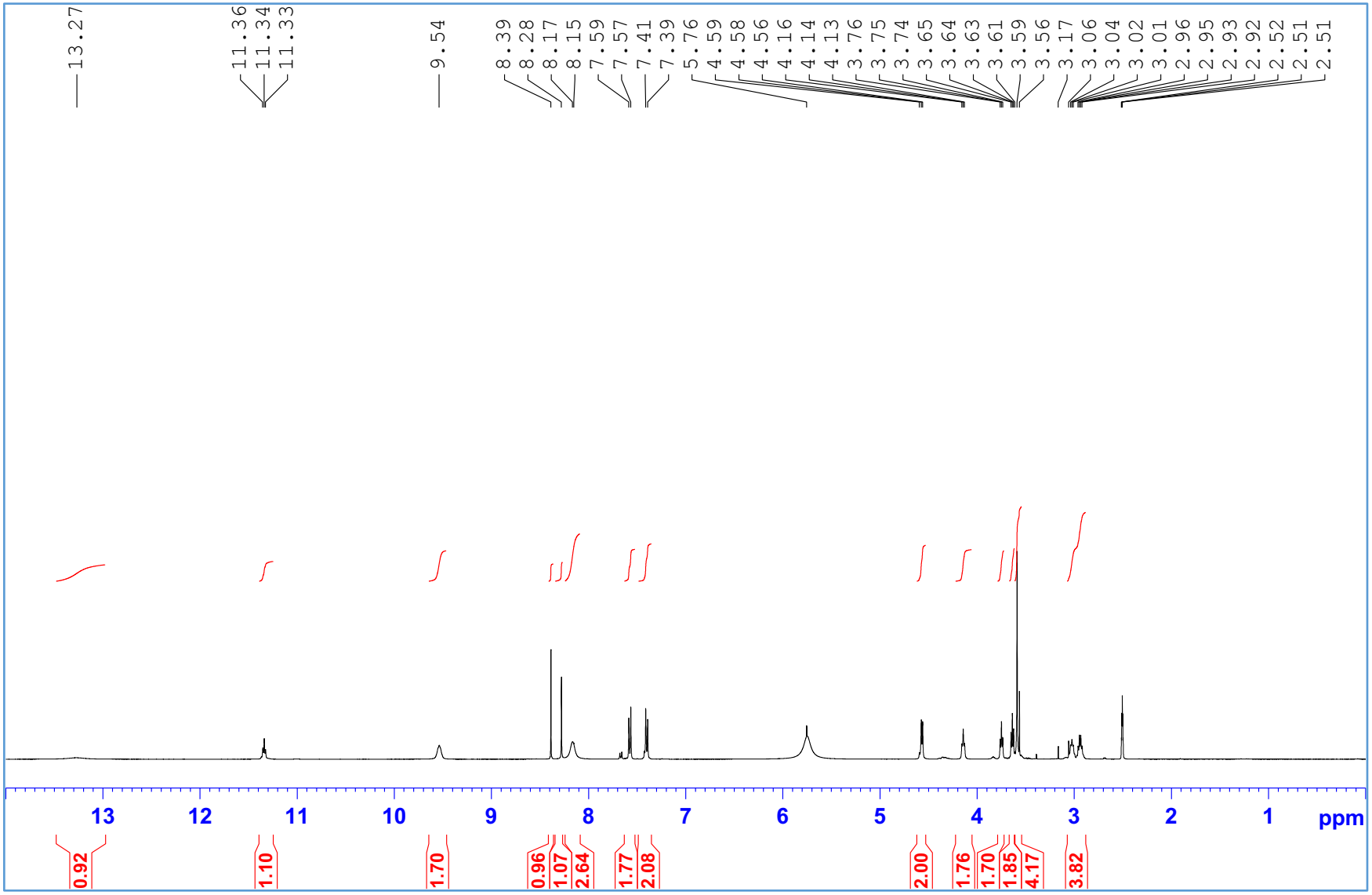
^{1}H NMR of compound **26** (400 MHz, DMSO-D_6)



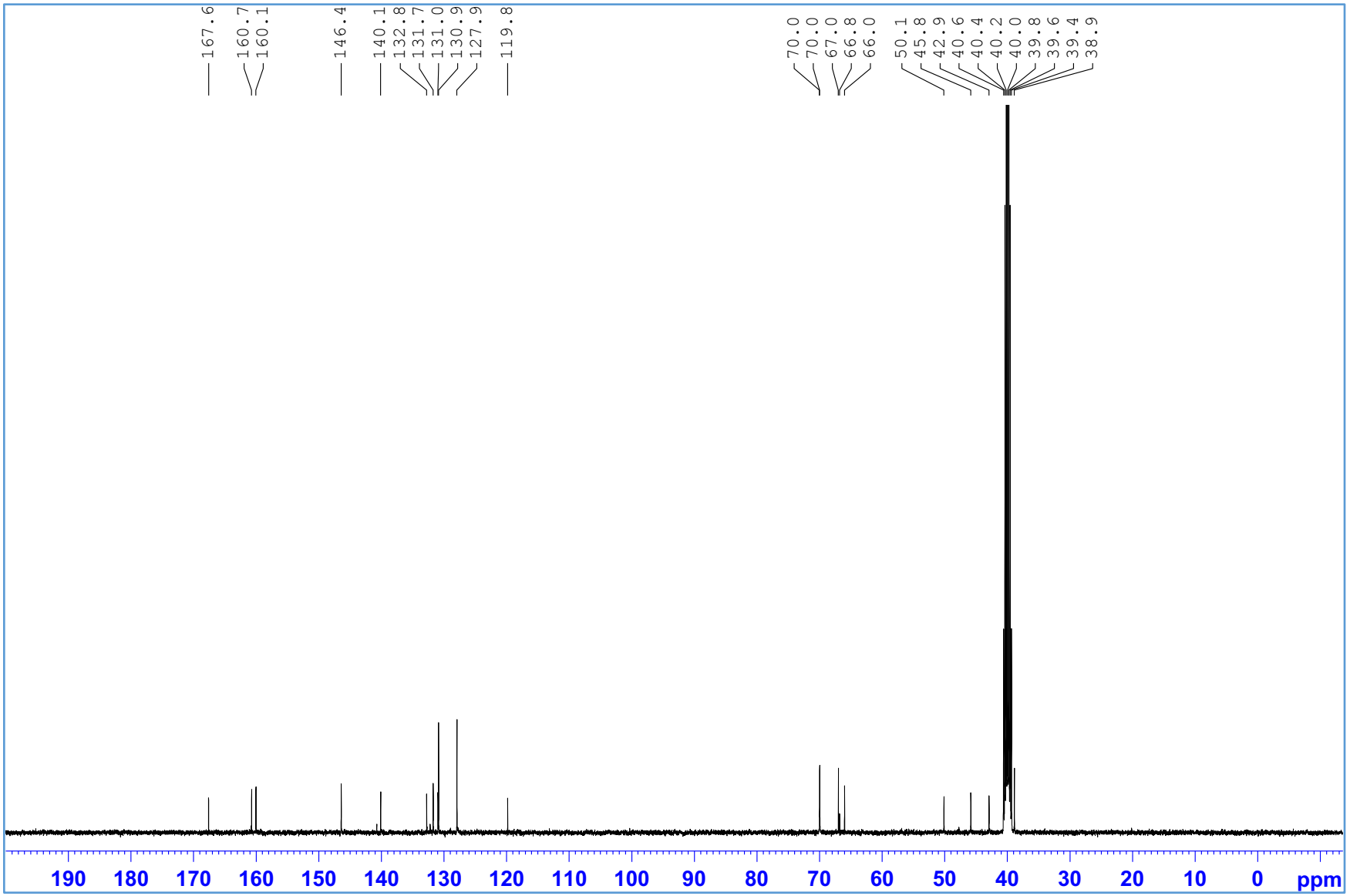
^{13}C NMR of compound **26** (100 MHz, DMSO-D_6)



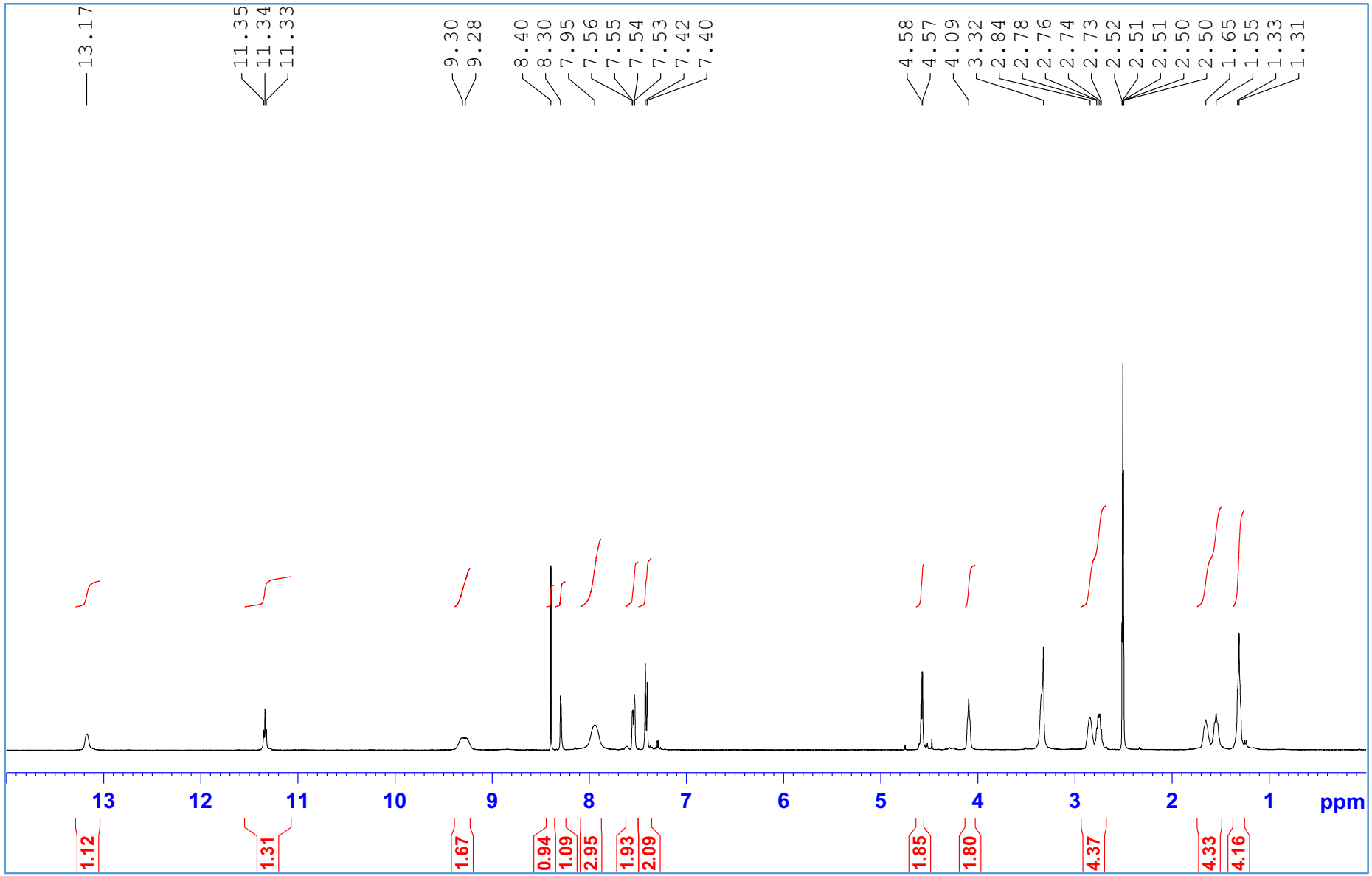
^{1}H NMR of compound **31** (400 MHz, DMSO-D_6)



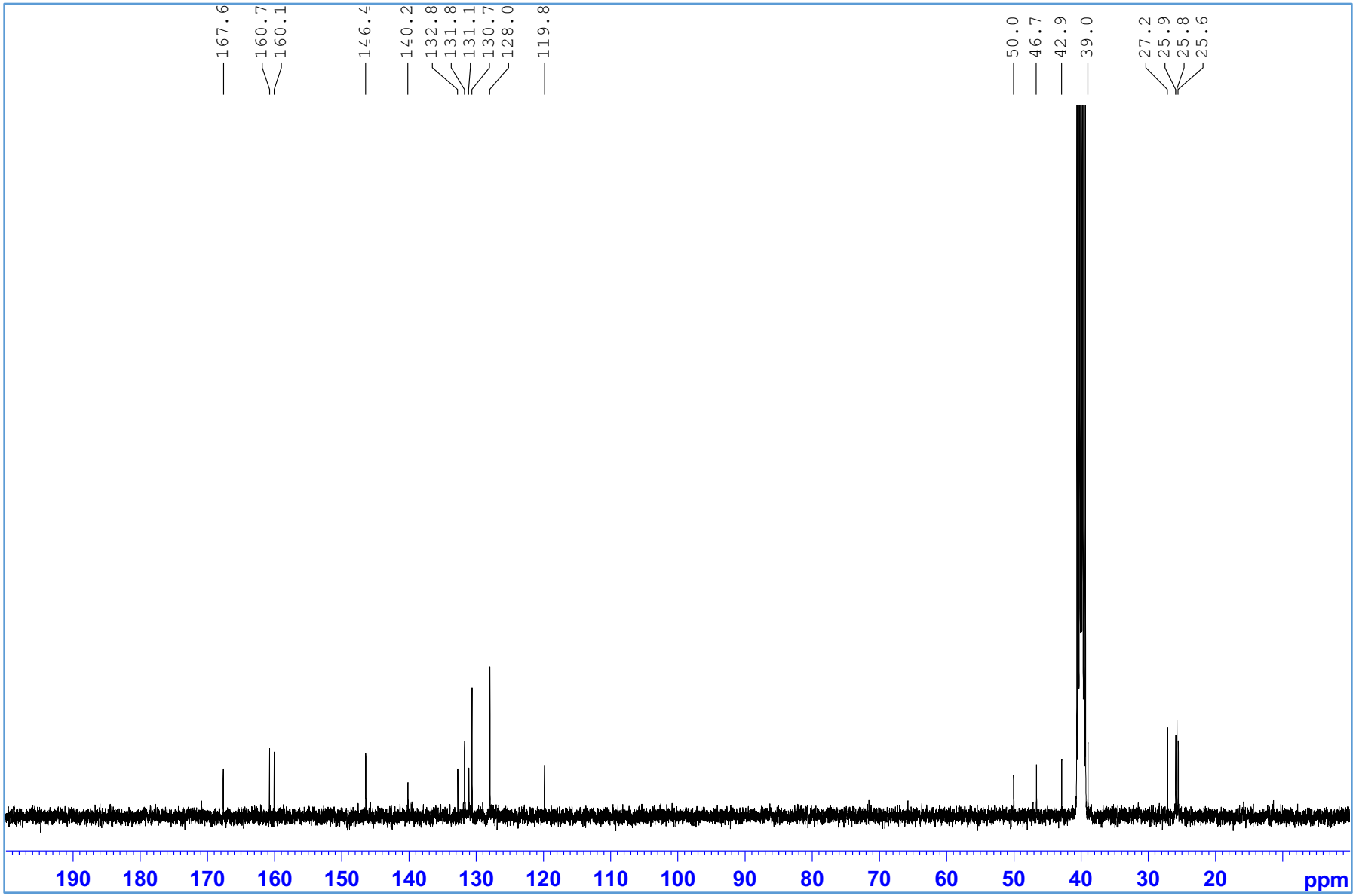
^{13}C NMR of compound **31** (100 MHz, DMSO-D_6)



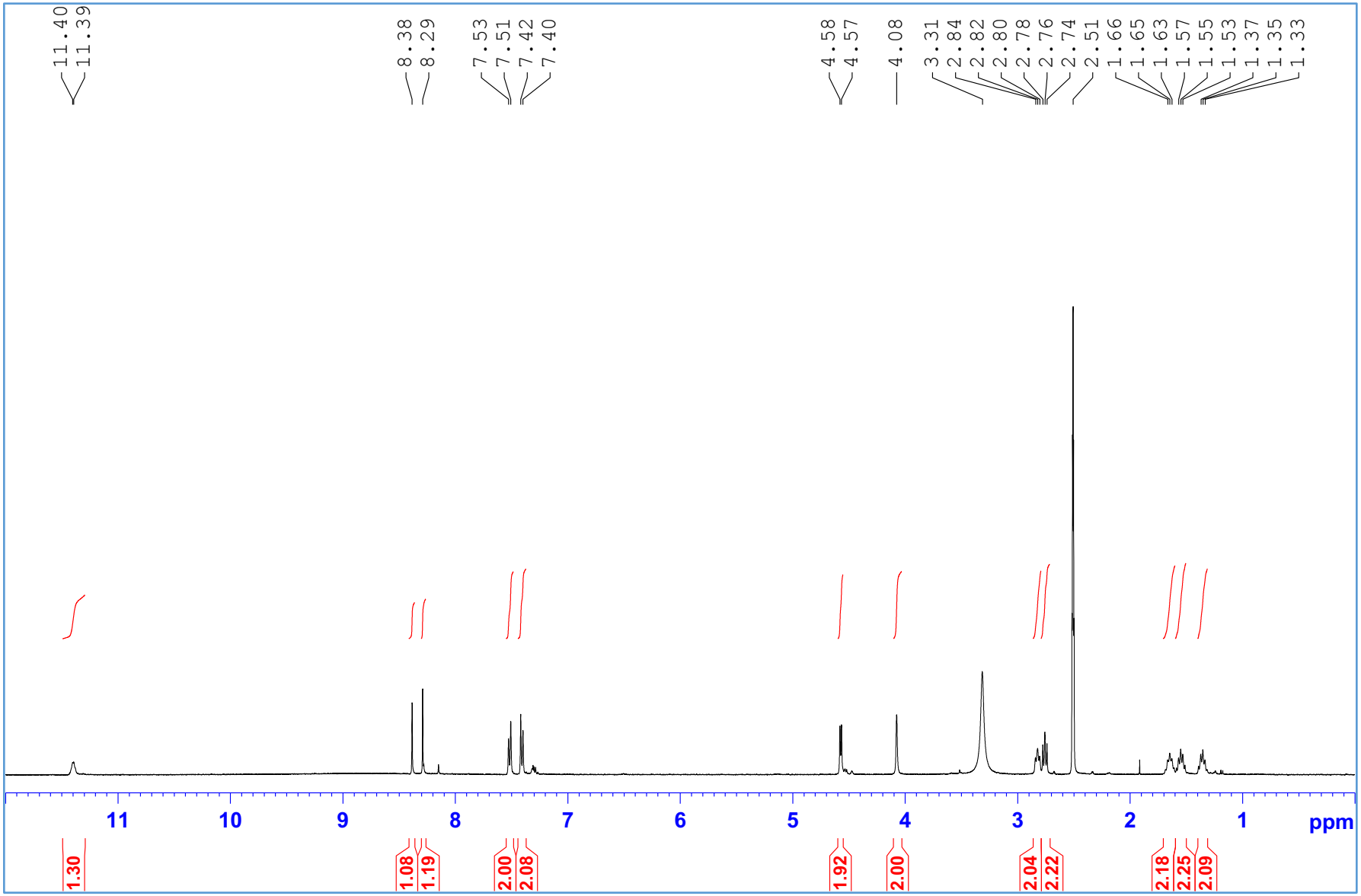
^{1}H NMR of compound **32** (400 MHz, DMSO-D_6)



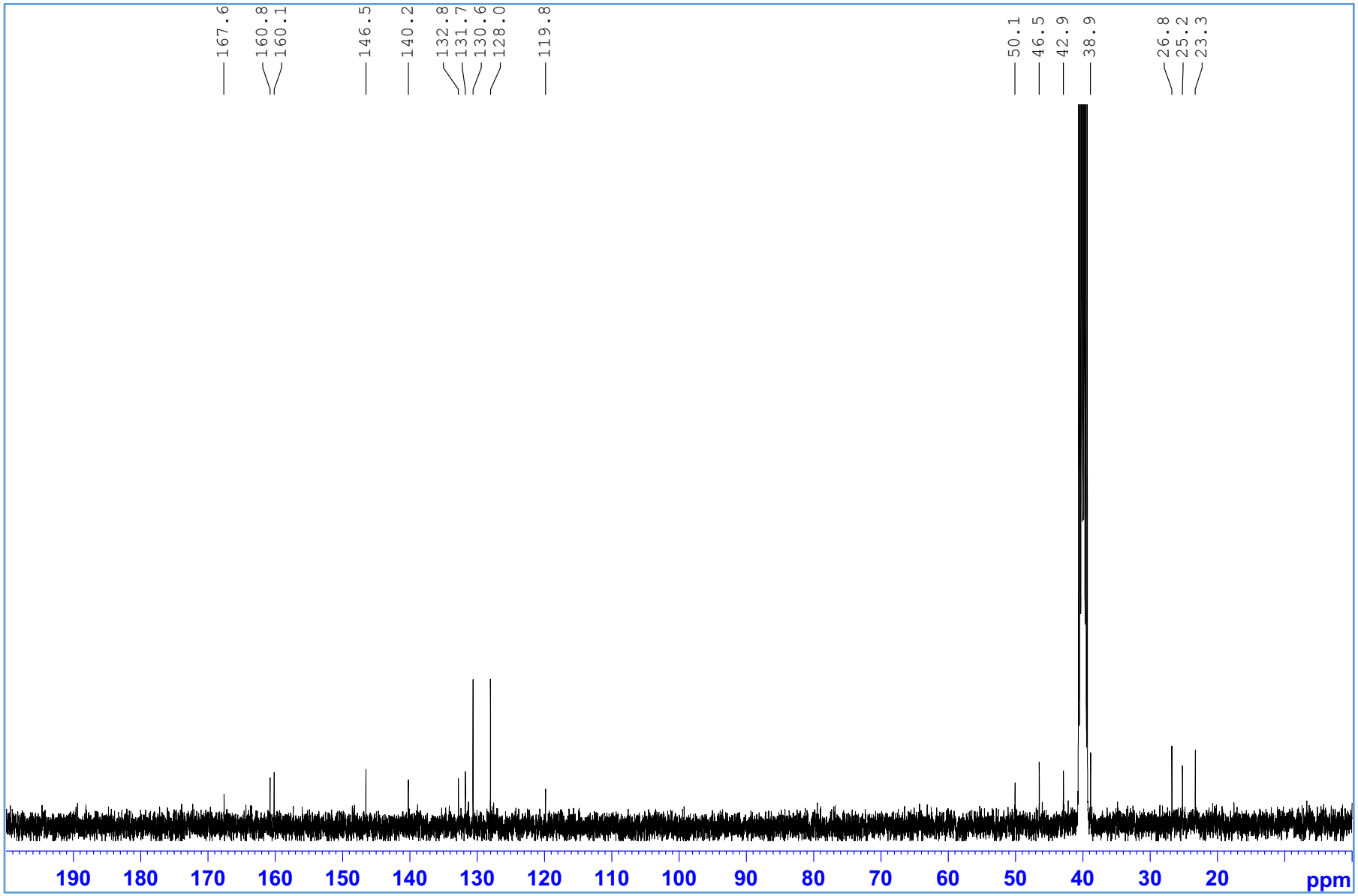
^{13}C NMR of compound **32** (100 MHz, DMSO-D_6)



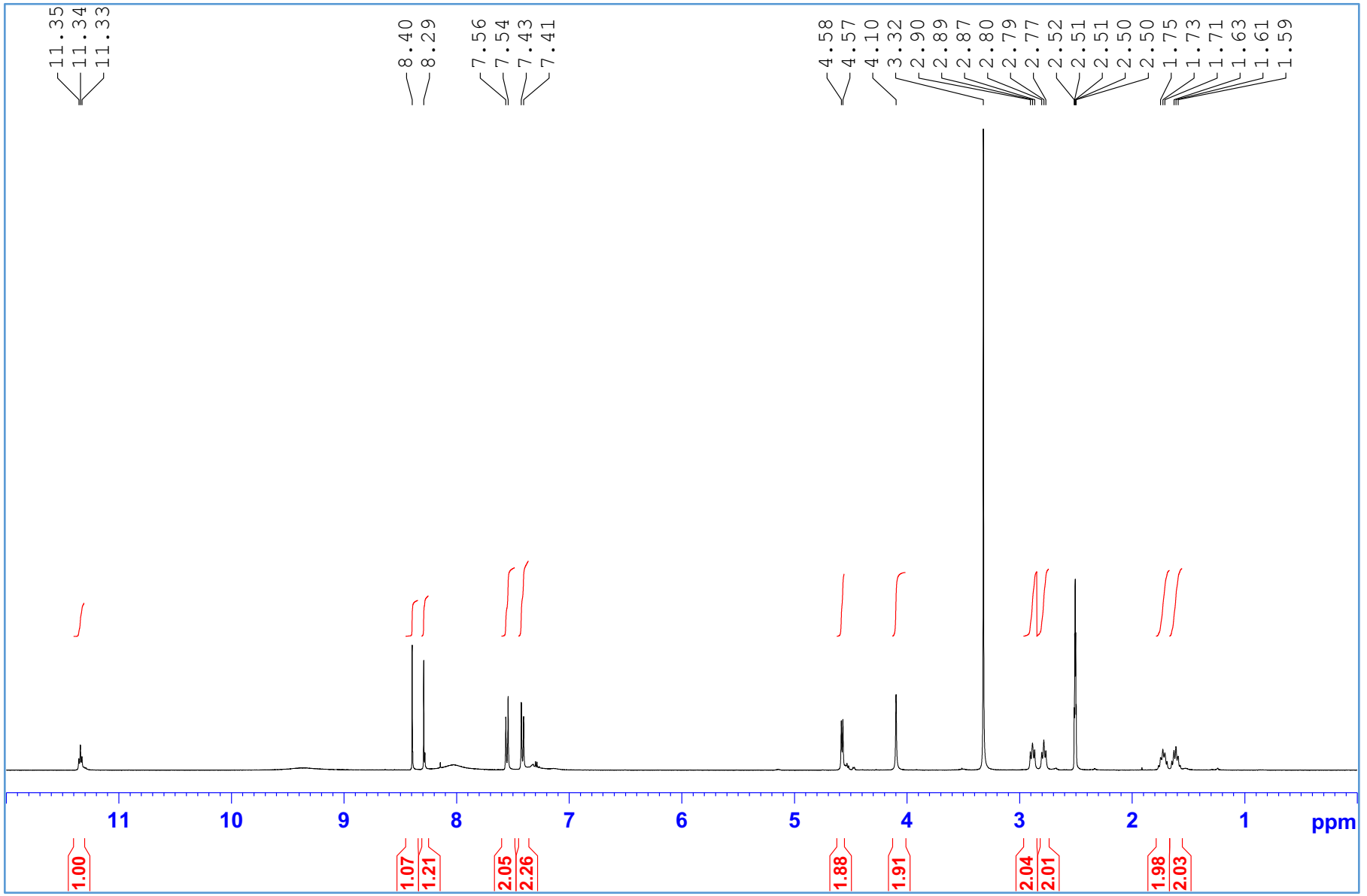
^{1}H NMR of compound **33** (400 MHz, DMSO-D_6)



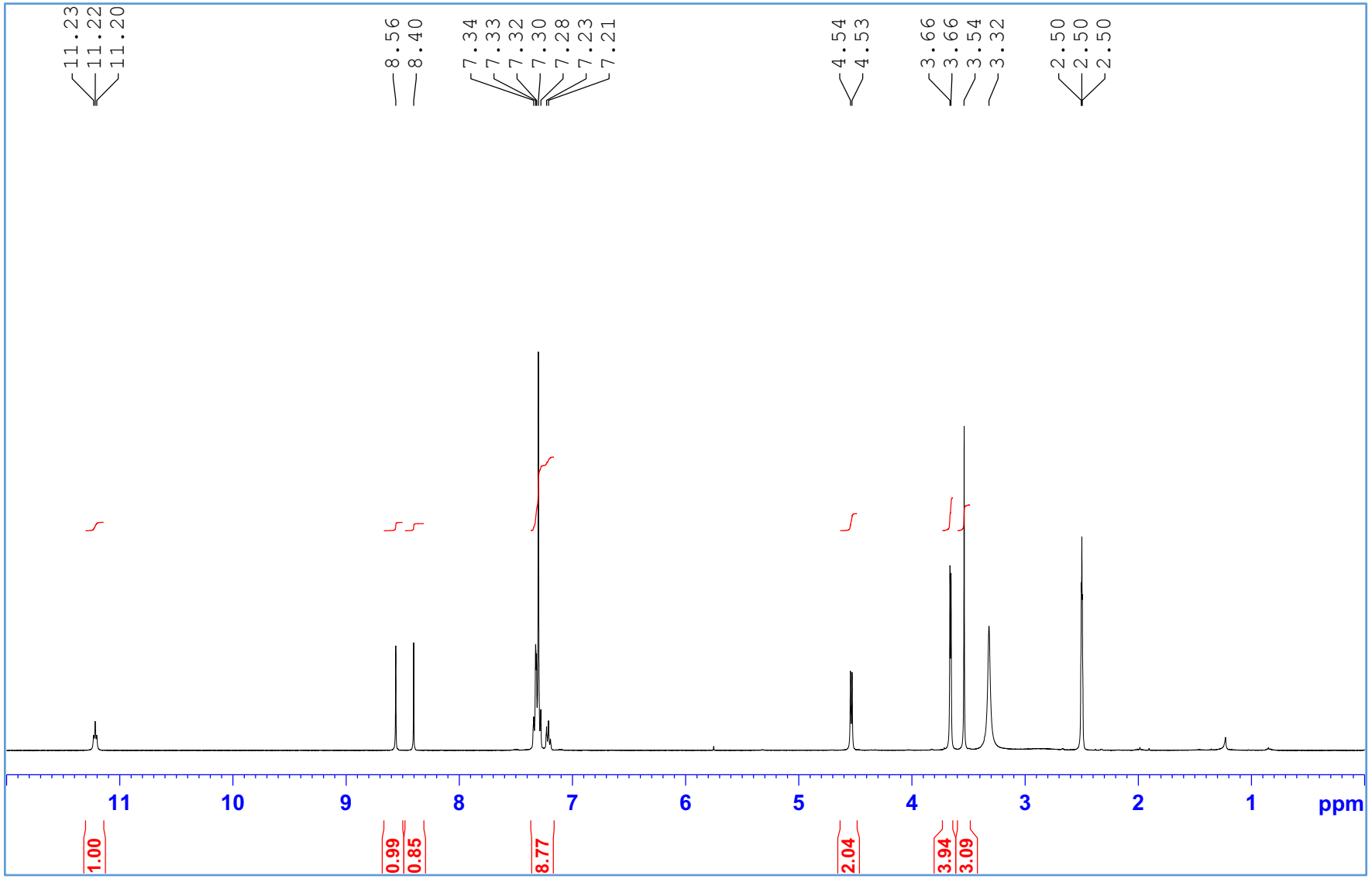
^{13}C NMR of compound **33** (100 MHz, DMSO-D_6)



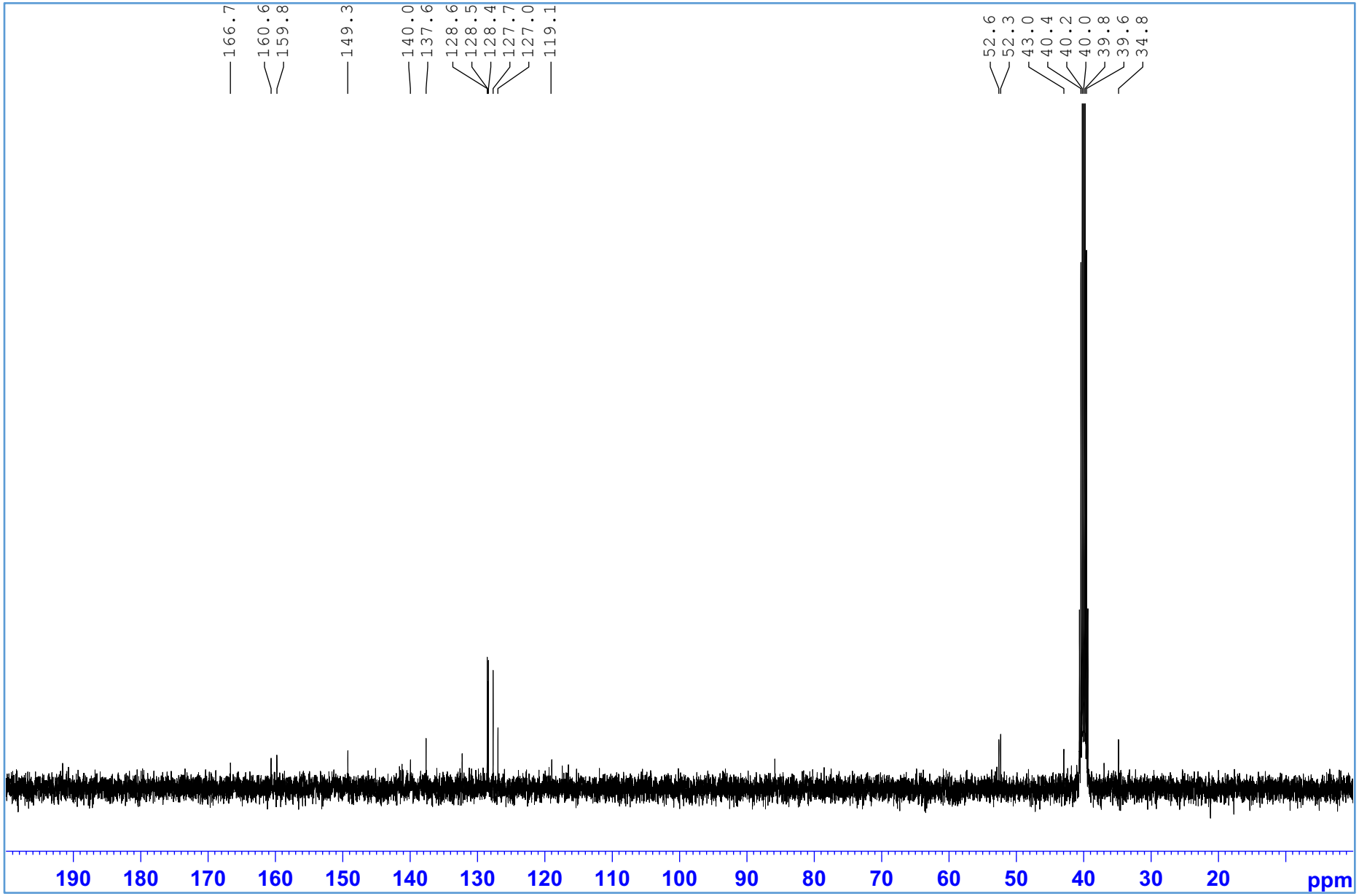
^{1}H NMR of compound **34** (400 MHz, DMSO-D_6)



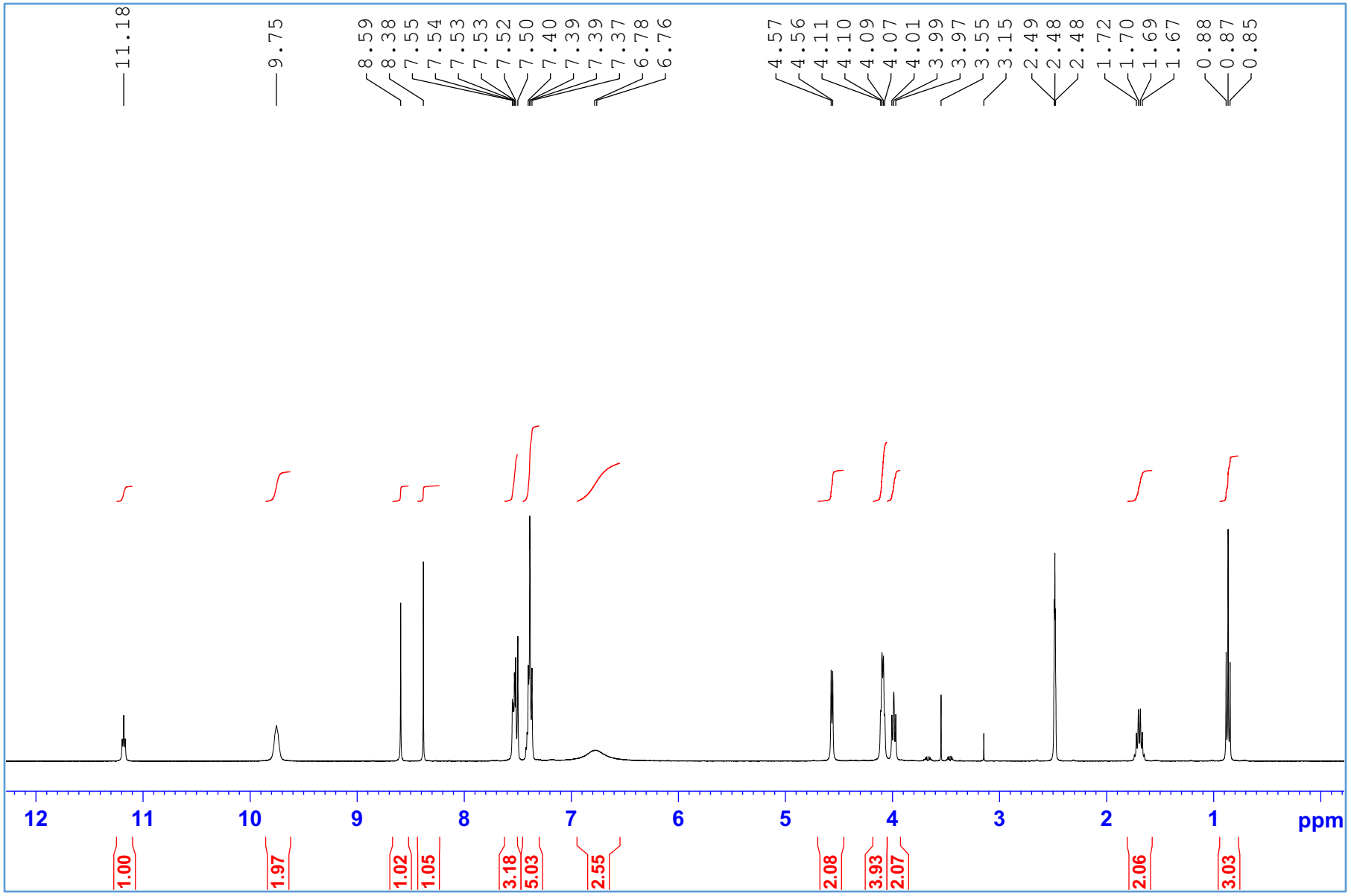
^{1}H NMR of compound **39** (400 MHz, DMSO-D_6)



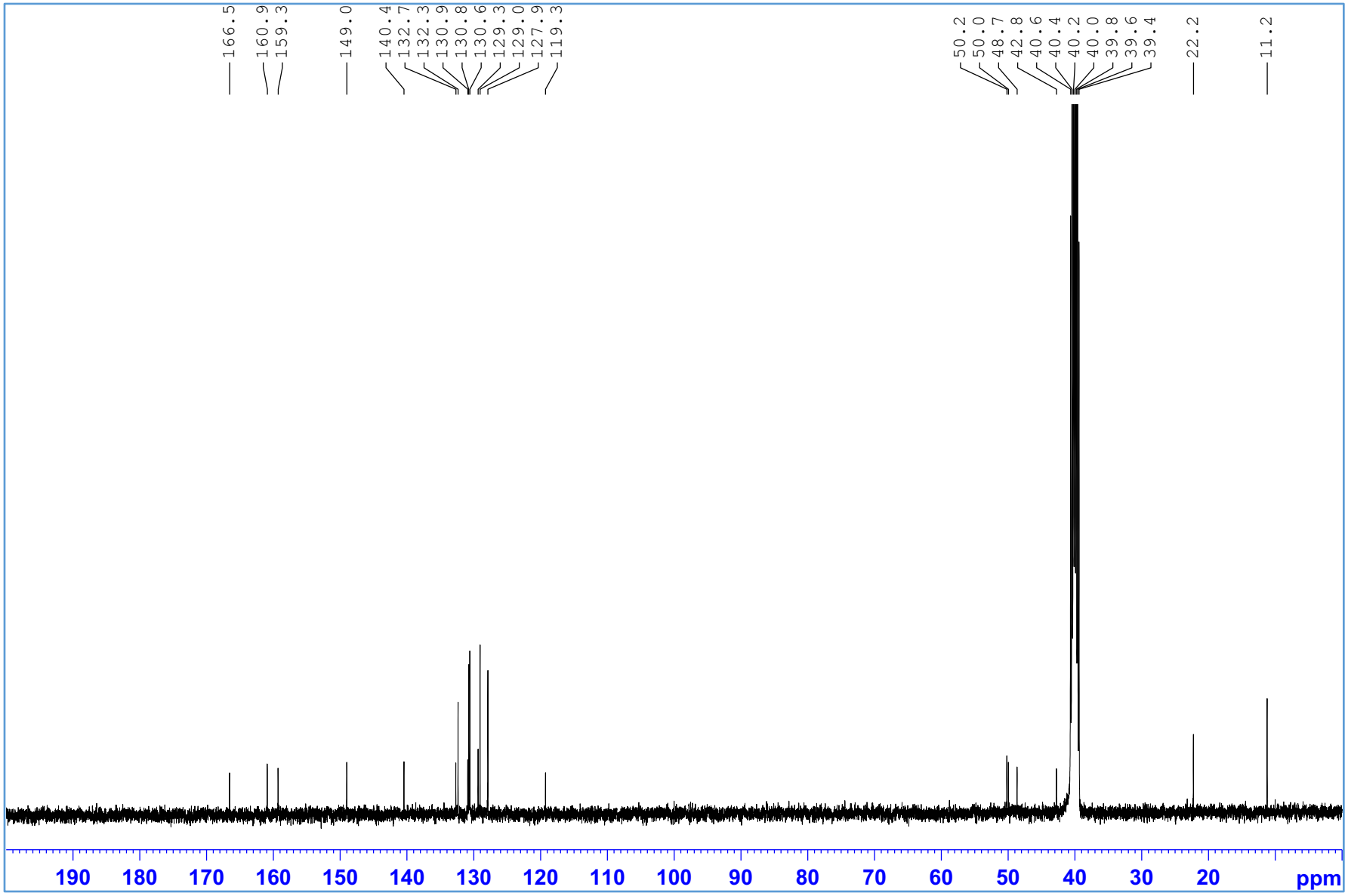
^{13}C NMR of compound **39**(100 MHz, DMSO-D_6)



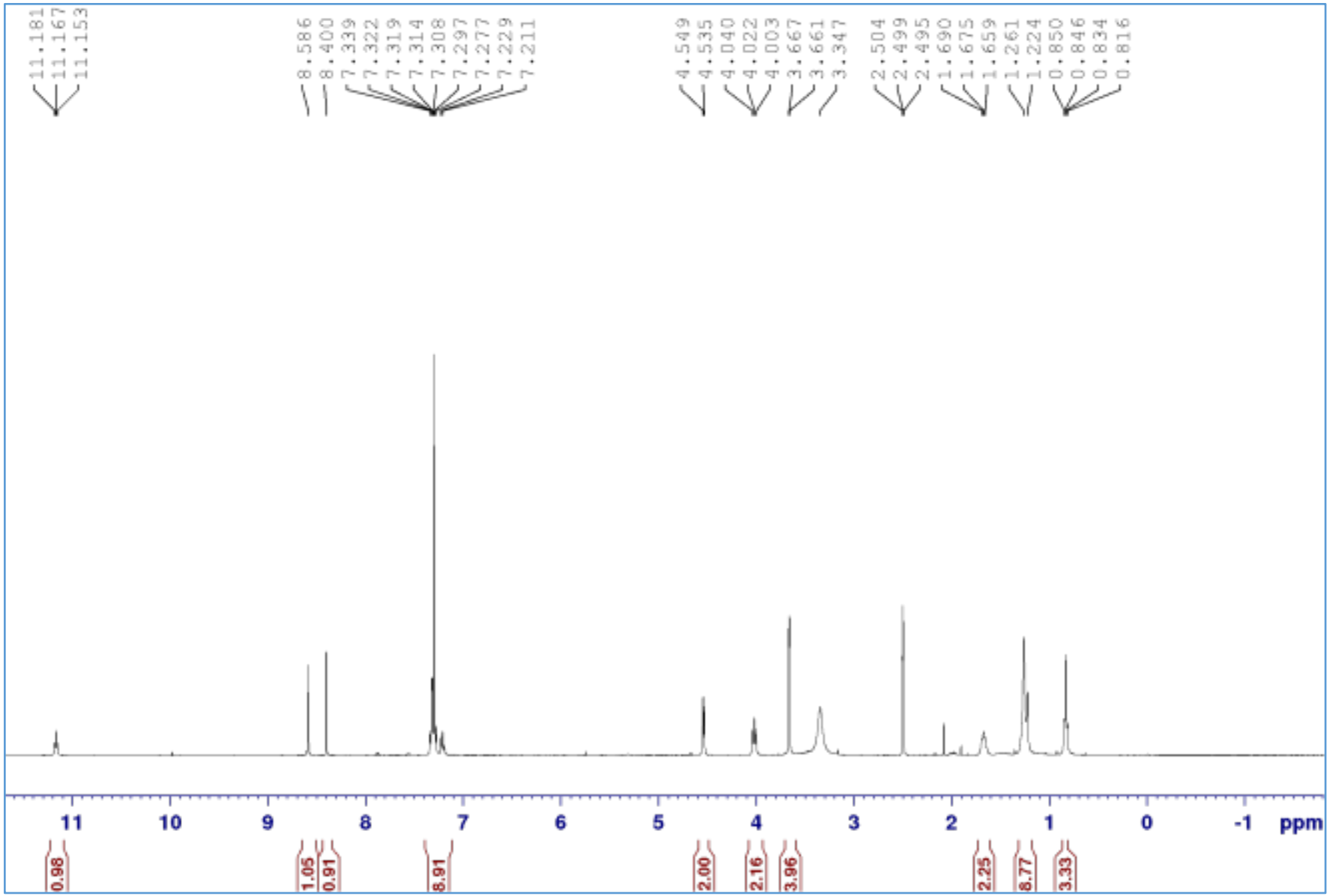
^{1}H NMR of compound **40** (400 MHz, DMSO-D_6)



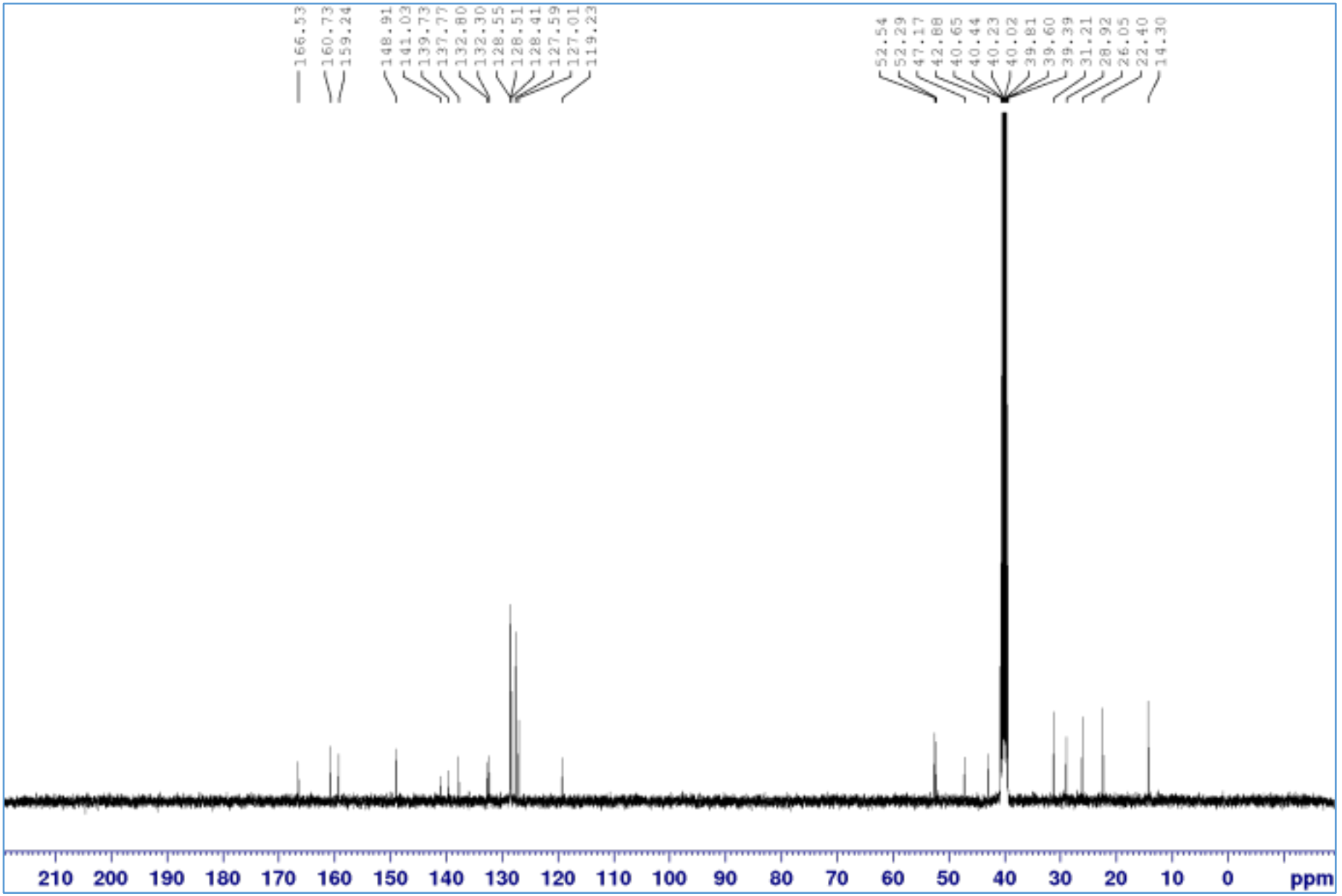
^{13}C NMR of compound **40** (100 MHz, DMSO-D_6)



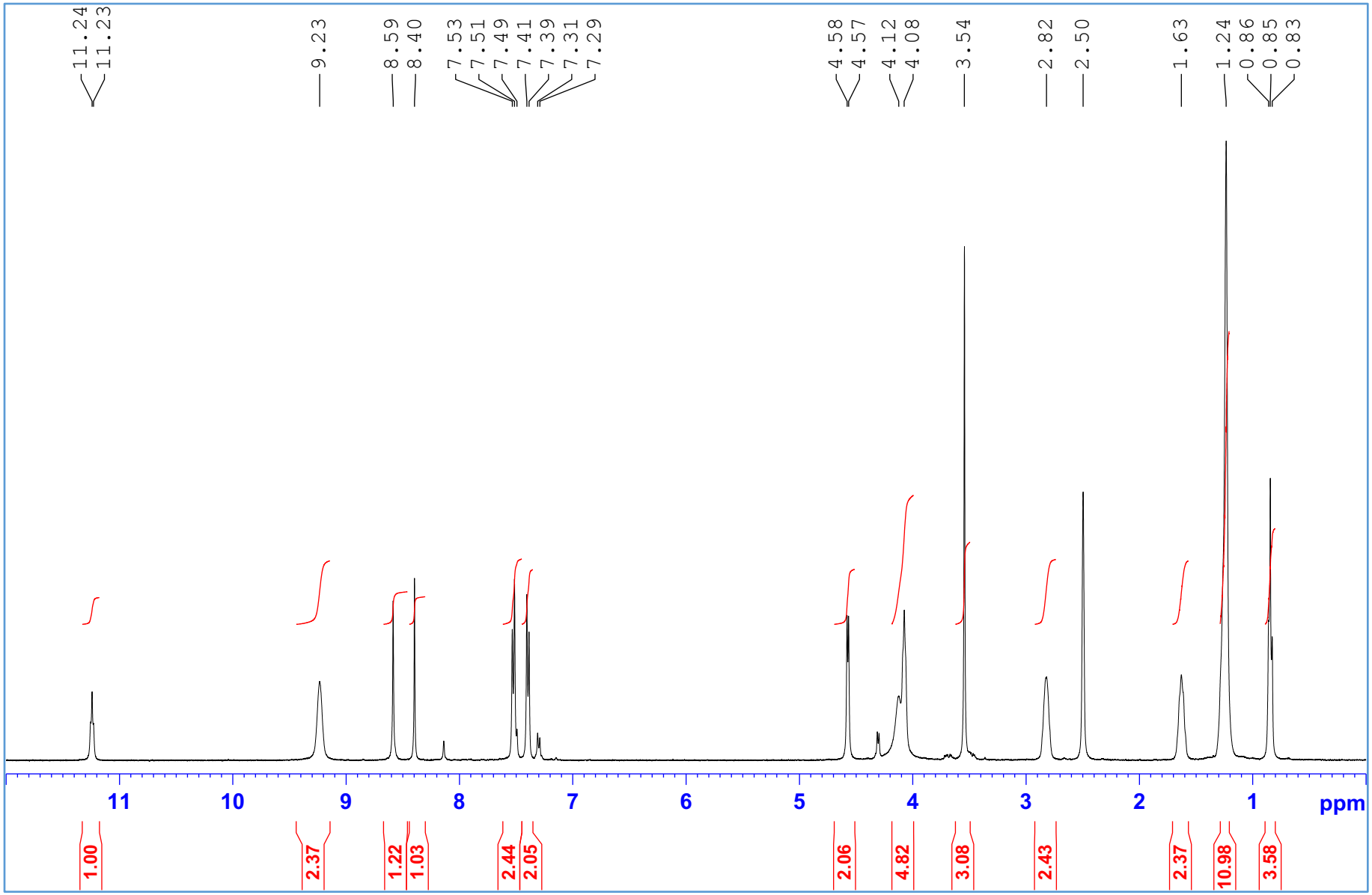
^{1}H NMR of compound **41** (400 MHz, DMSO-D_6)



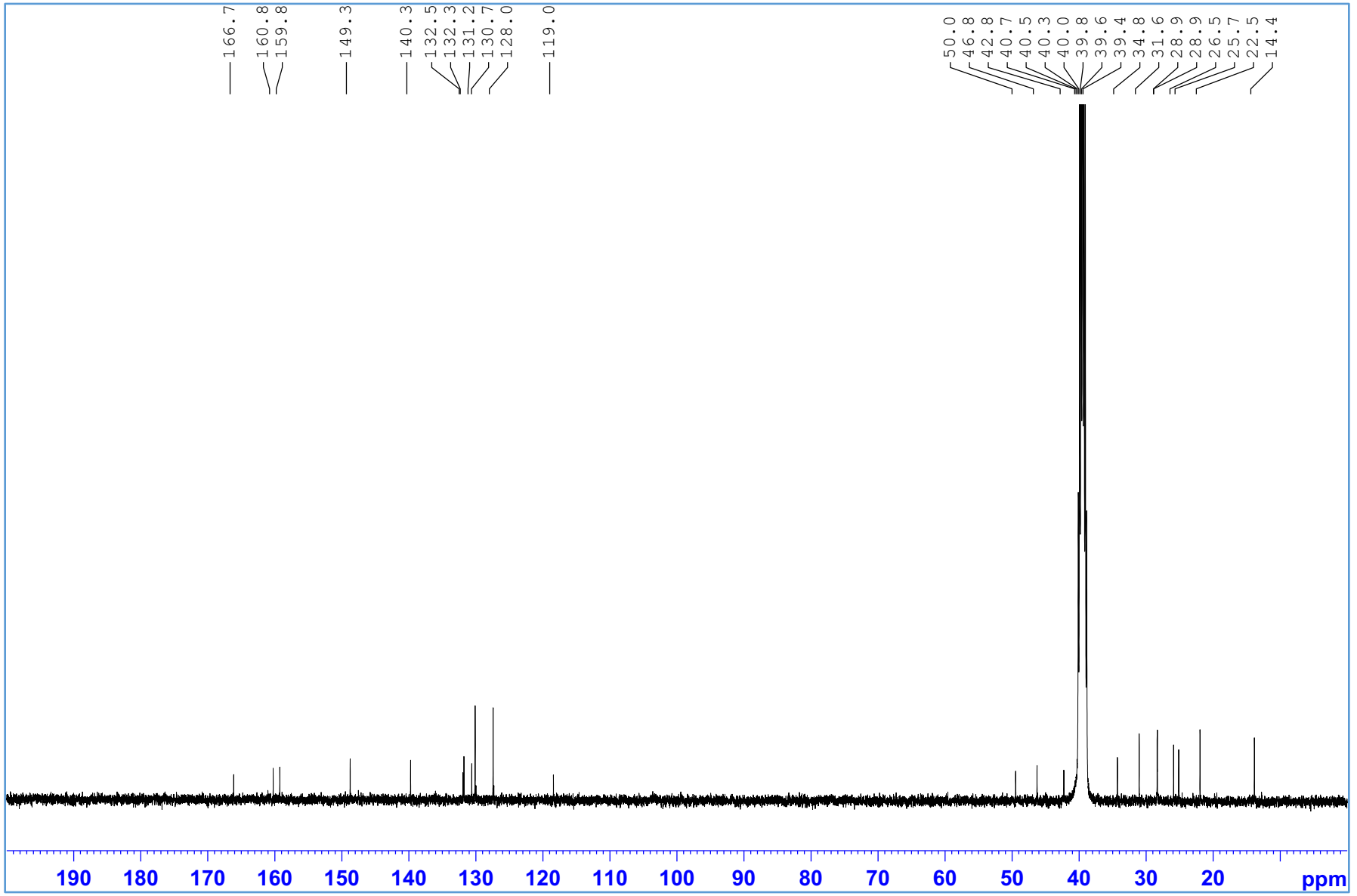
^{13}C NMR of compound **41** (100 MHz, DMSO-D_6)



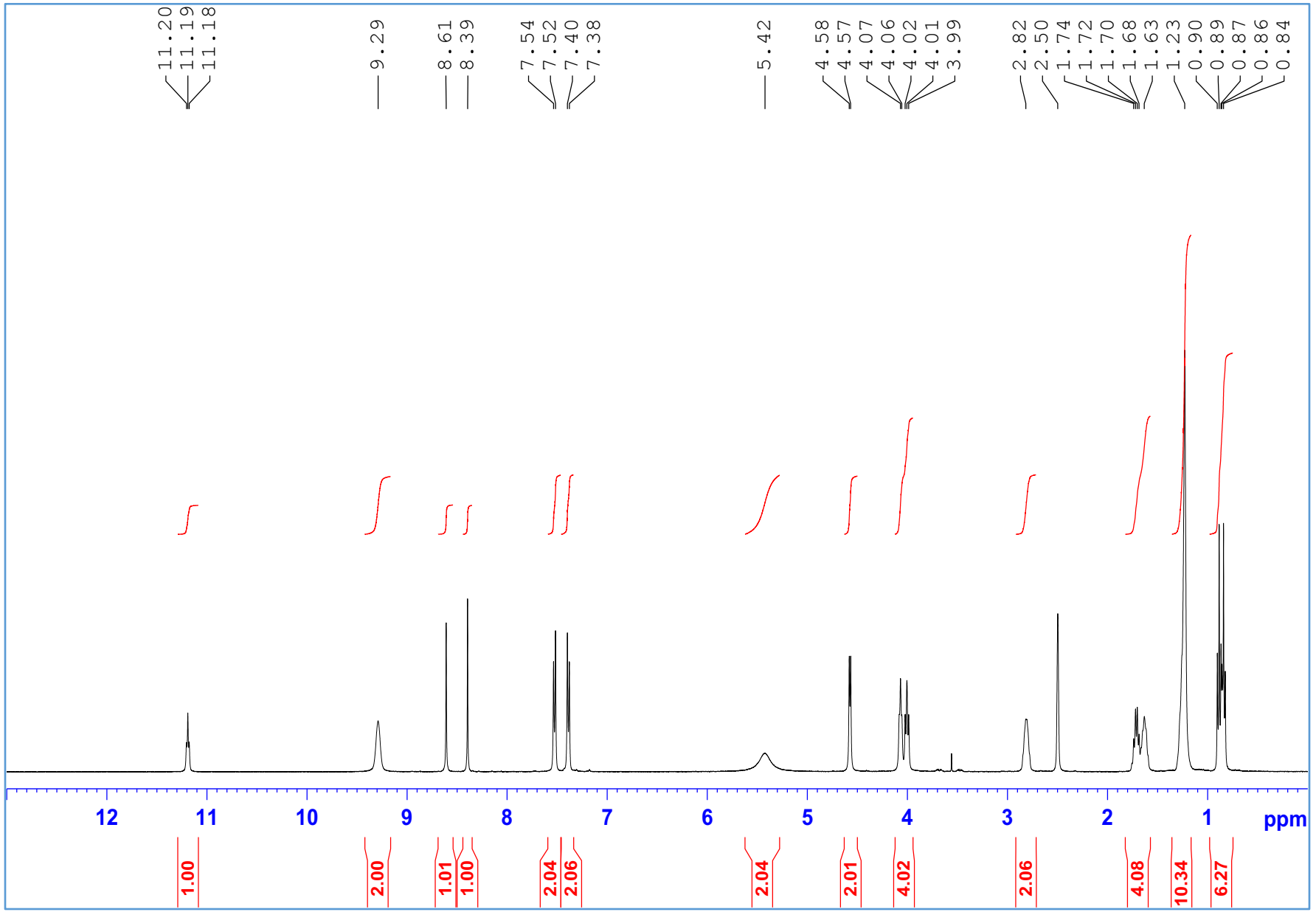
^{1}H NMR of compound **42** (400 MHz, DMSO-D_6)



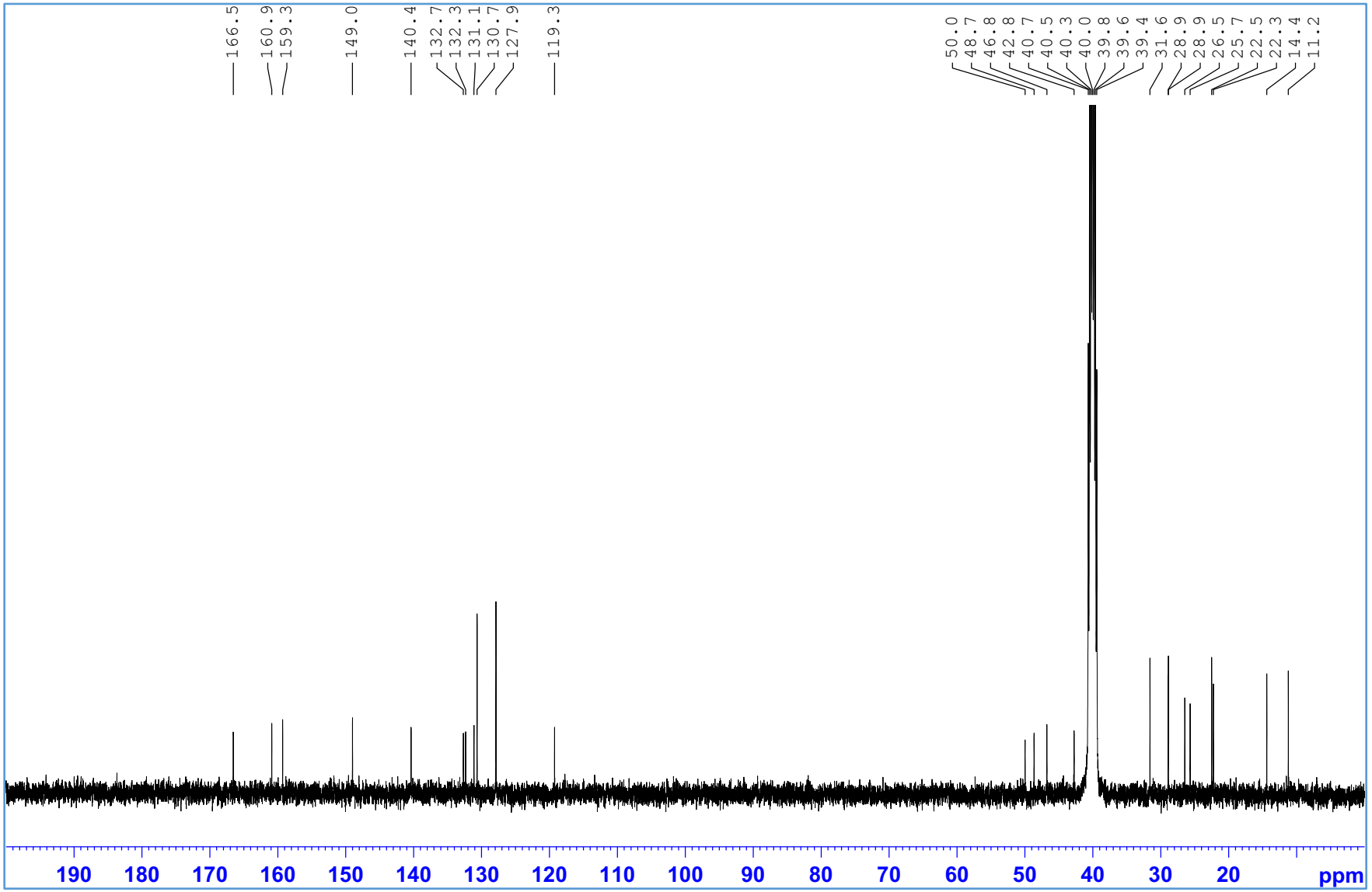
^{13}C NMR of compound **42** (100 MHz, DMSO-D_6)



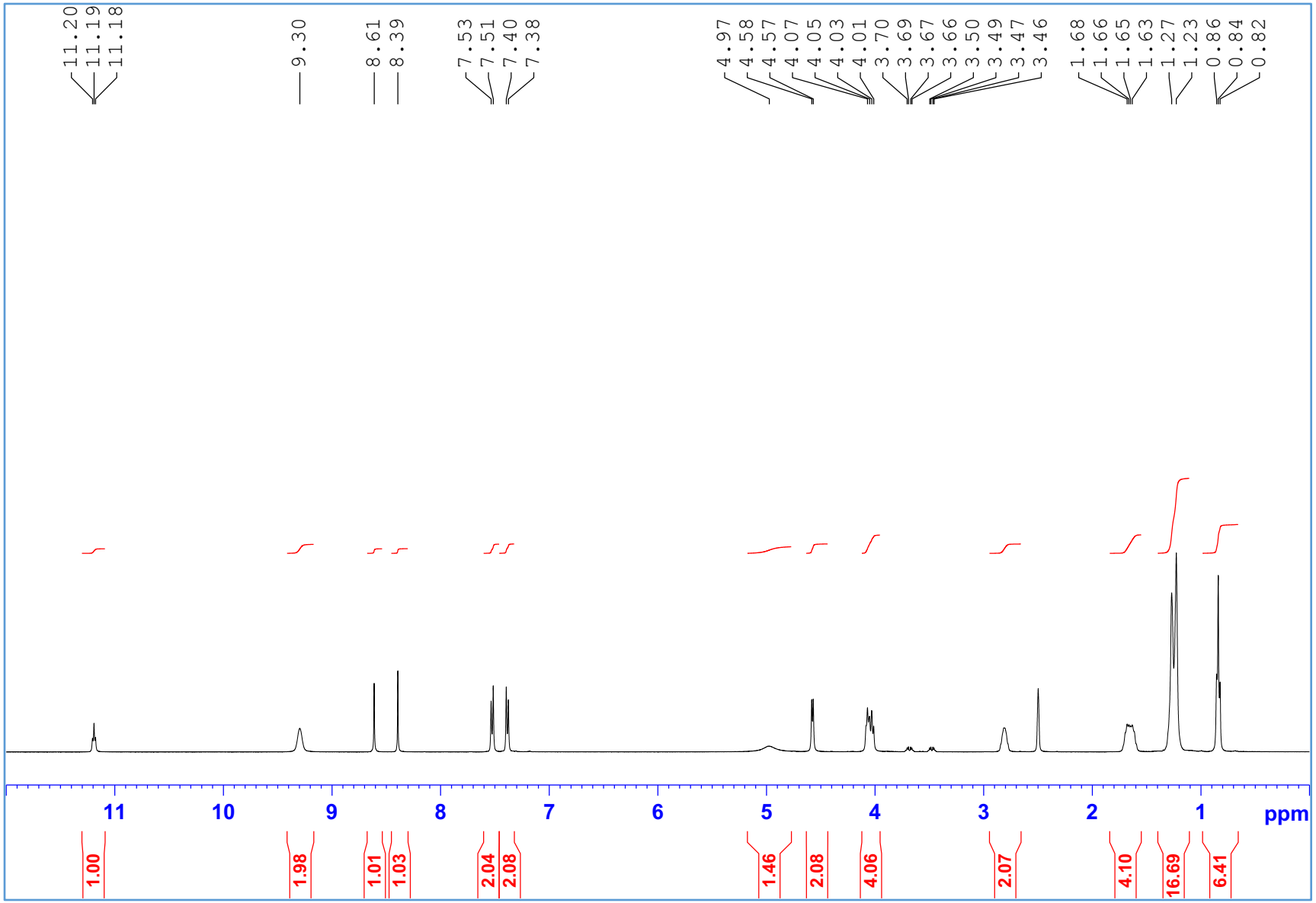
^{1}H NMR of compound **43** (400 MHz, DMSO-D_6)



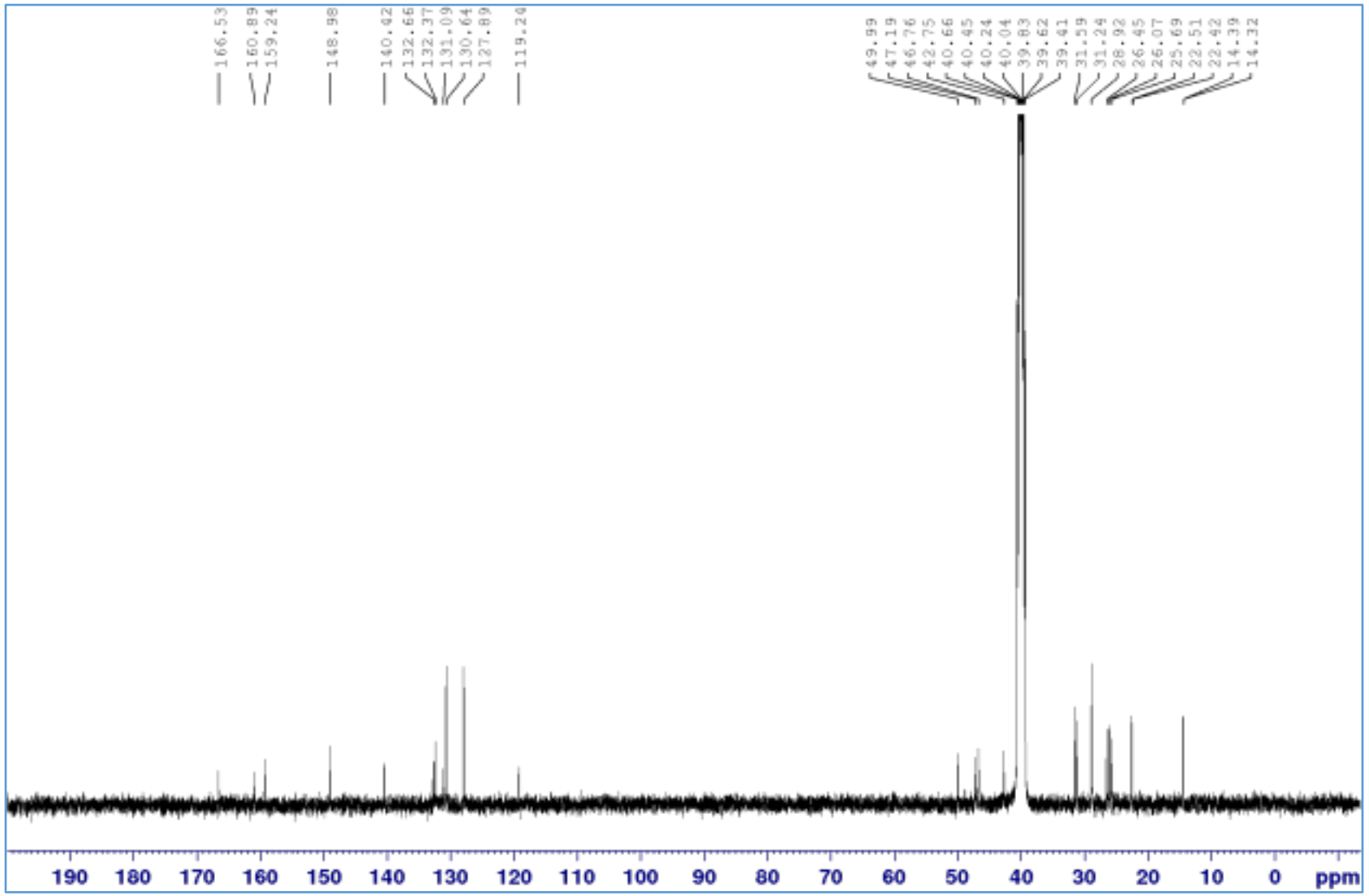
^{13}C NMR of compound **43** (100 MHz, DMSO-D_6)



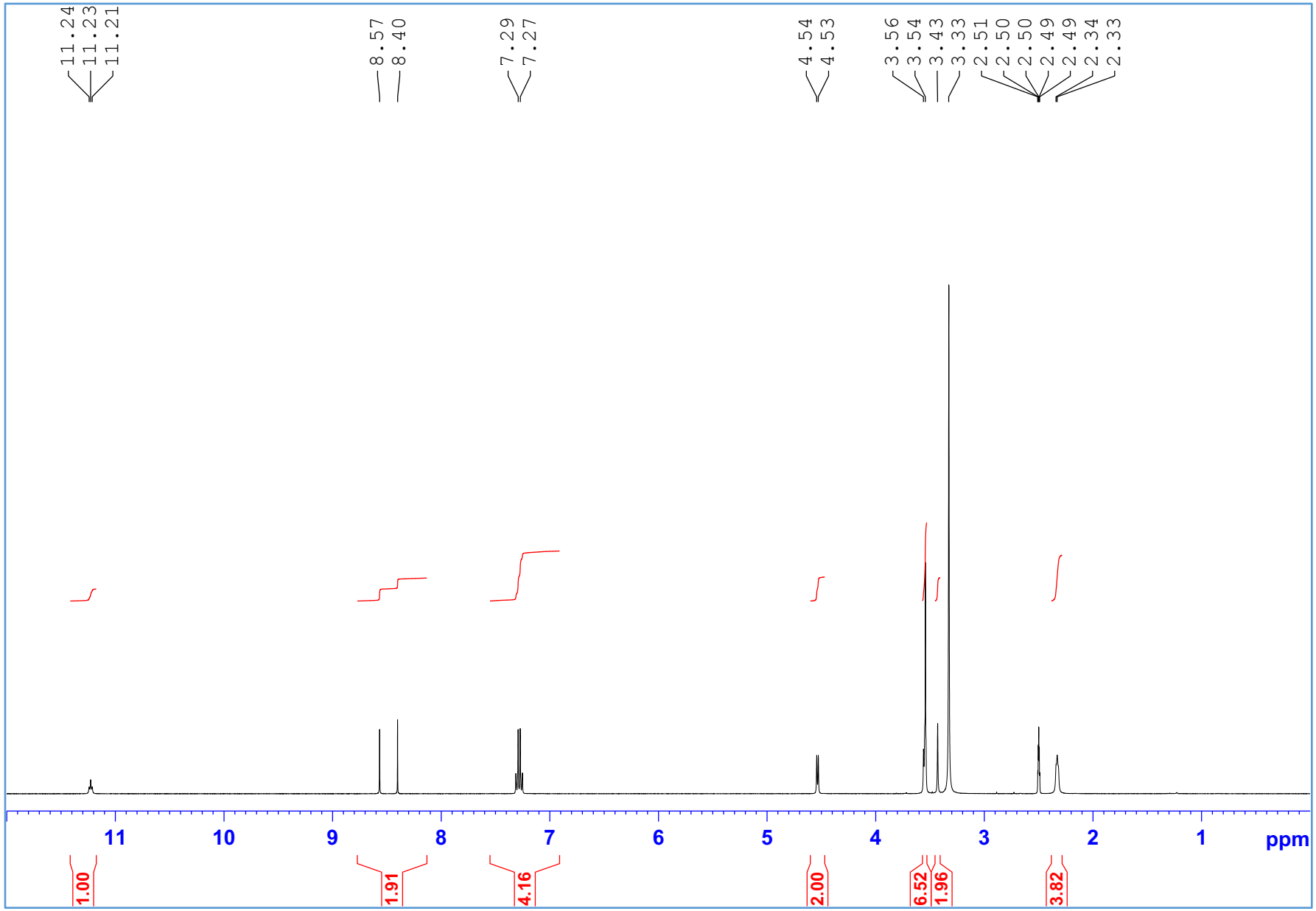
^{1}H NMR of compound **44** (400 MHz, DMSO-D_6)



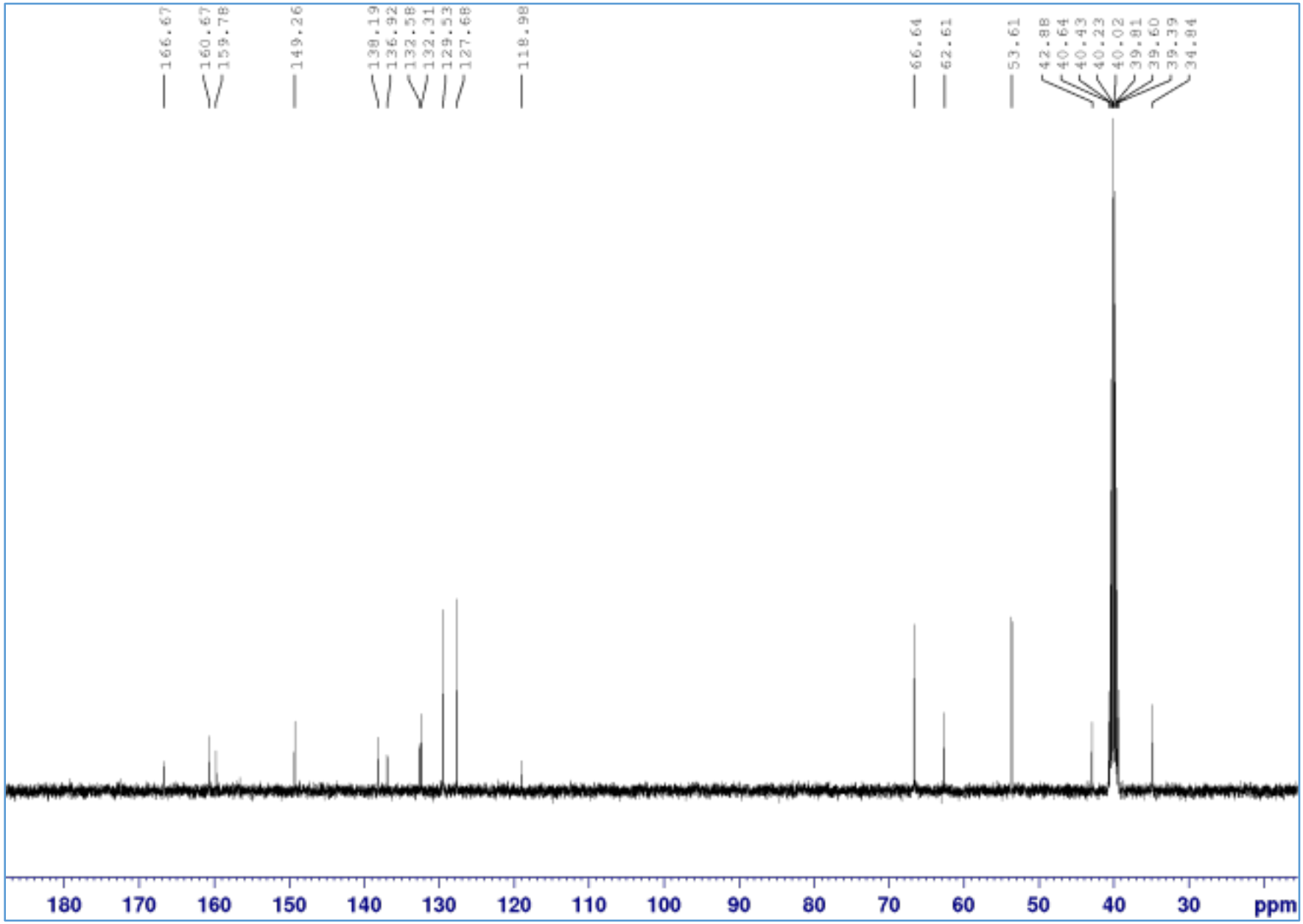
^{13}C NMR of compound **44** (100 MHz, DMSO-D_6)



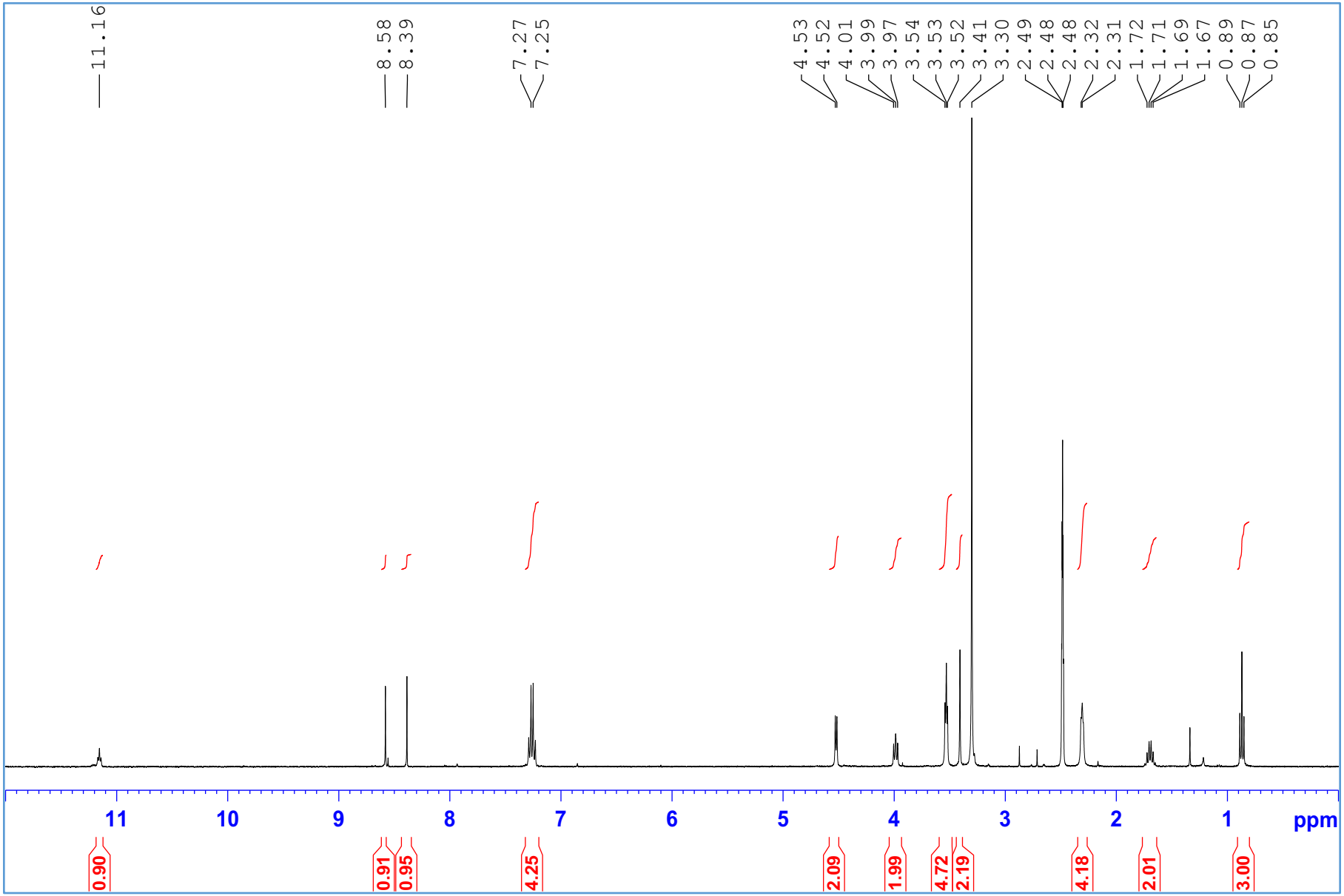
^{1}H NMR of compound **45** (400 MHz, DMSO-D_6)



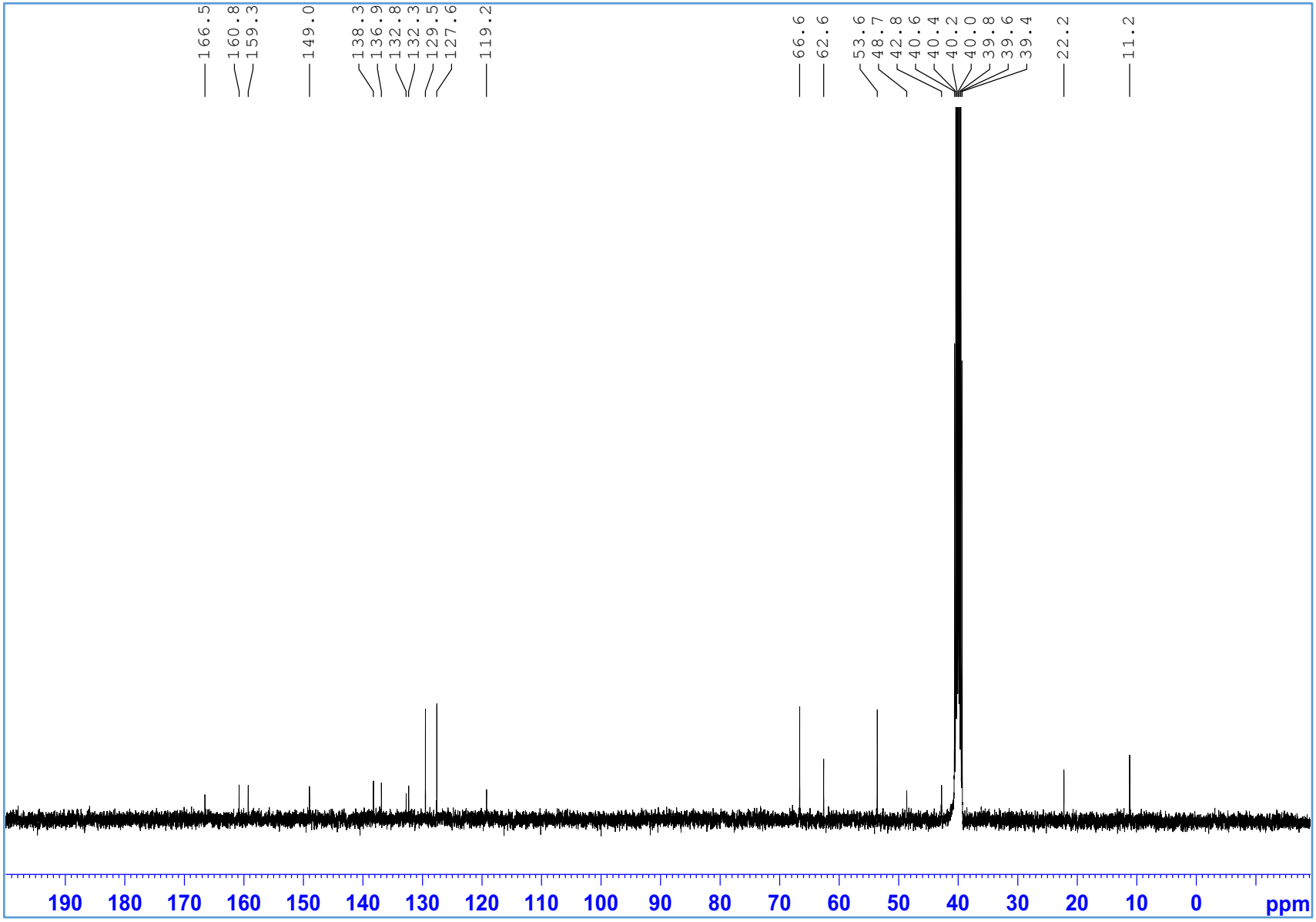
^{13}C NMR of compound **45** (100 MHz, DMSO-D_6)



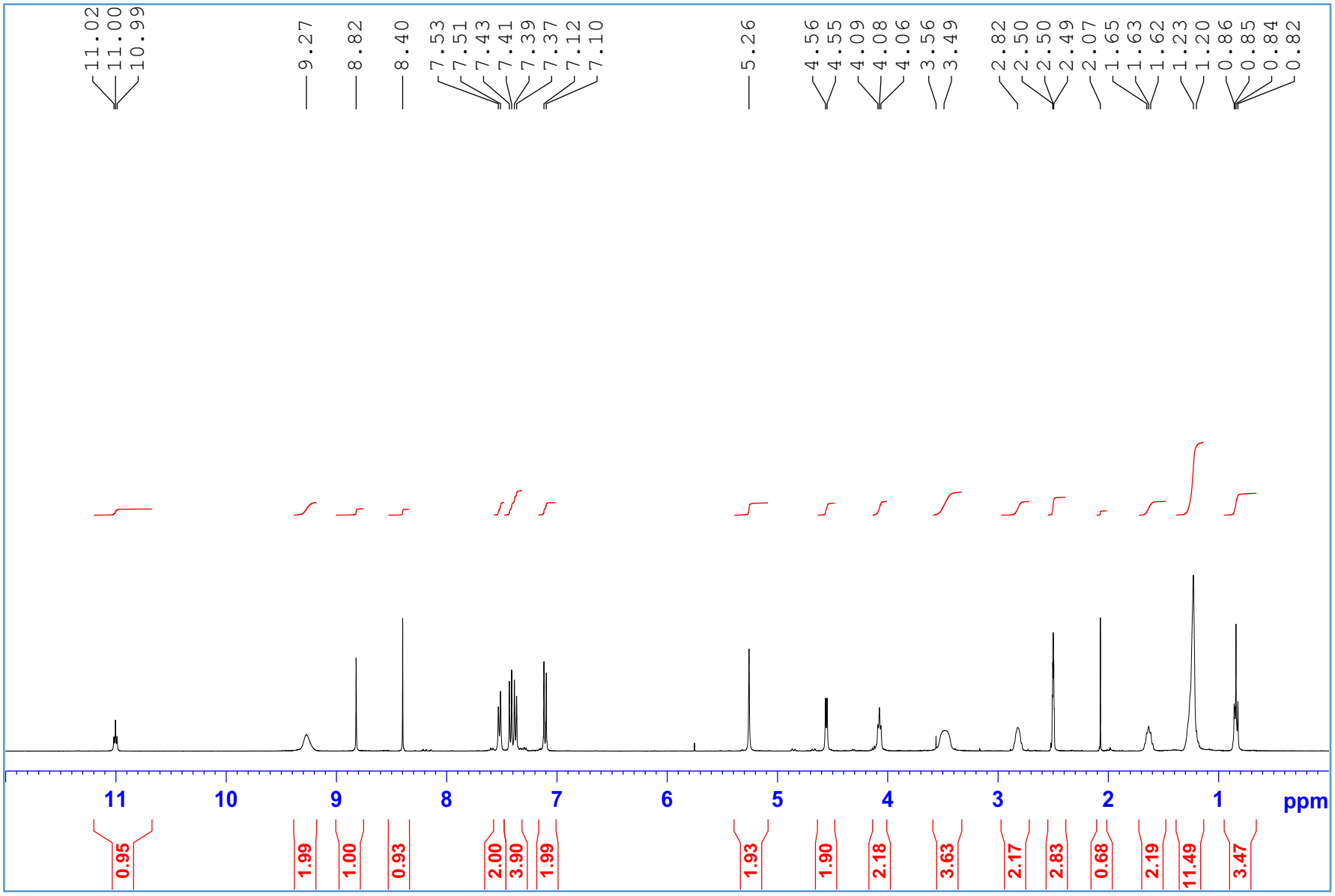
^{1}H NMR of compound **46** (400 MHz, DMSO-D_6)



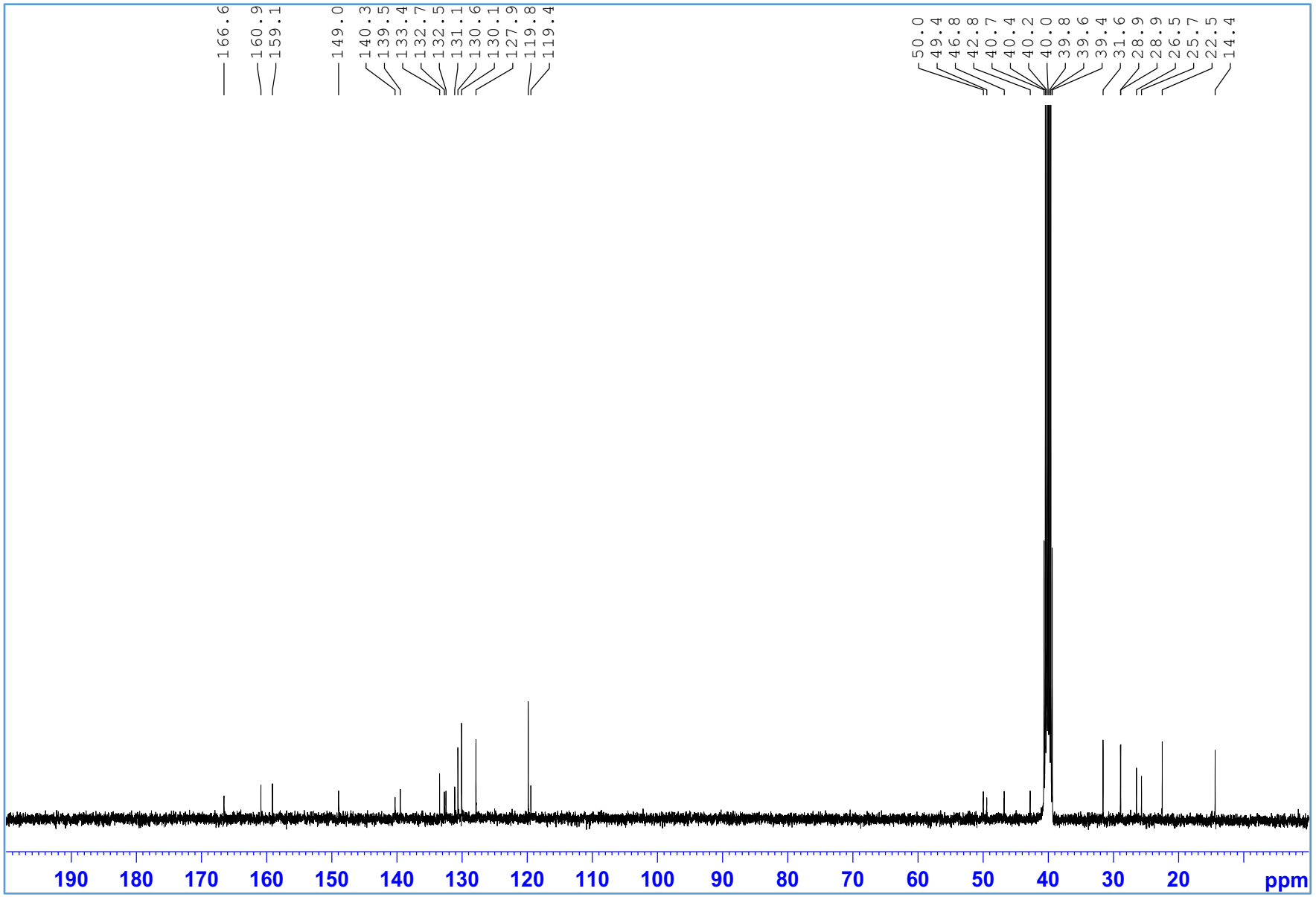
^{13}C NMR of compound **46** (100 MHz, DMSO-D_6)



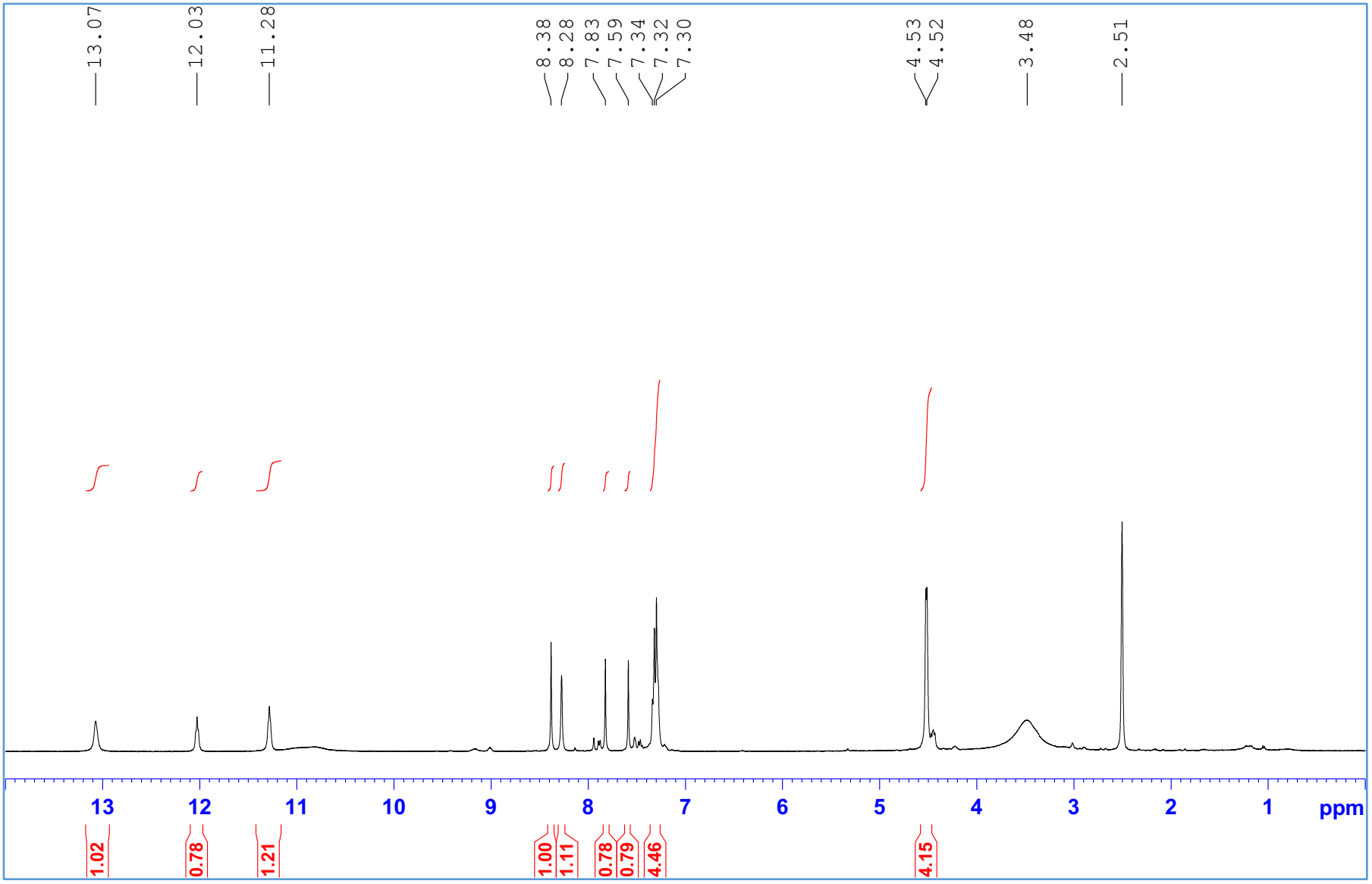
^{1}H NMR of compound **47** (400 MHz, DMSO-D_6)



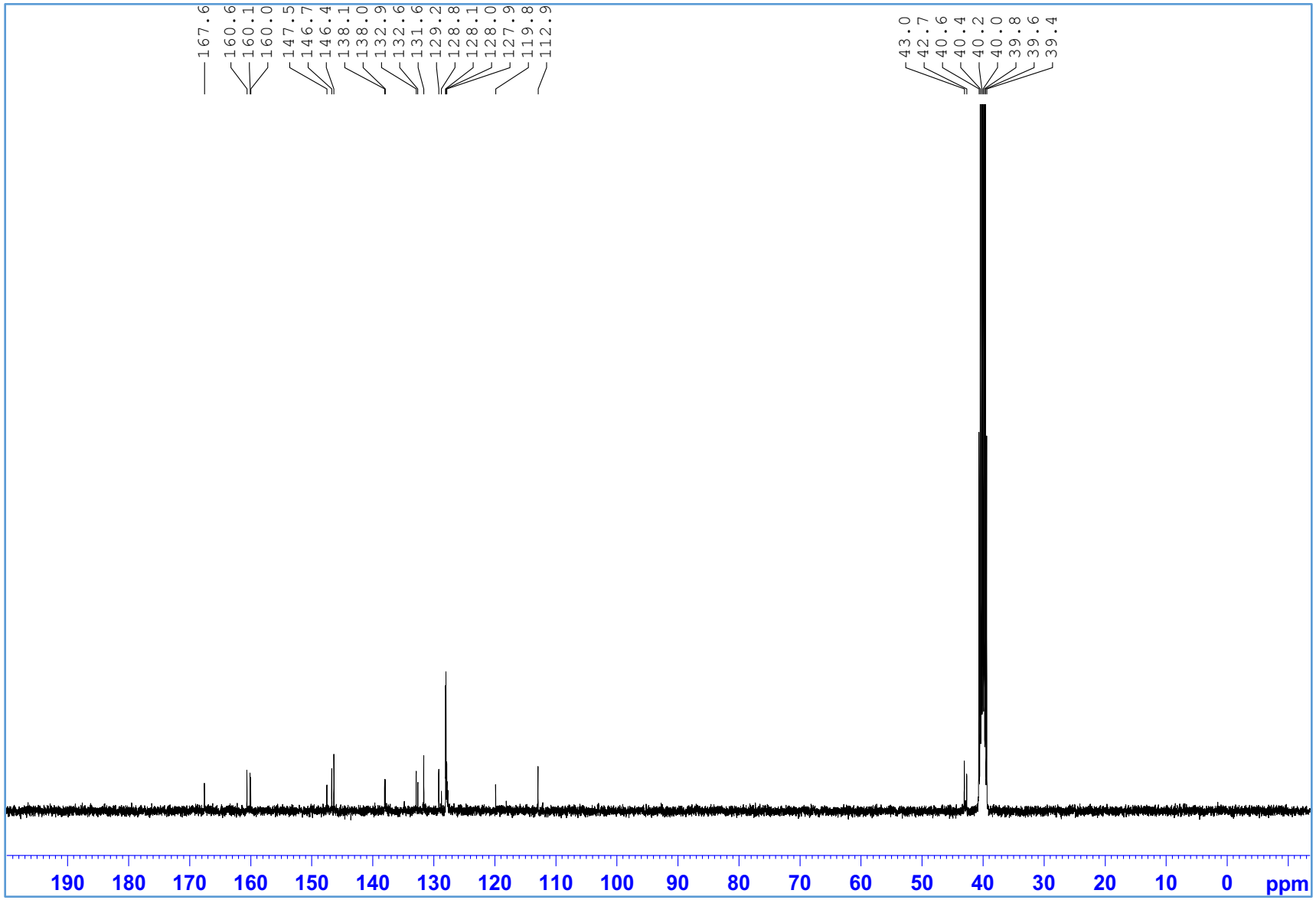
^{13}C NMR of compound **47** (100 MHz, DMSO-D_6)



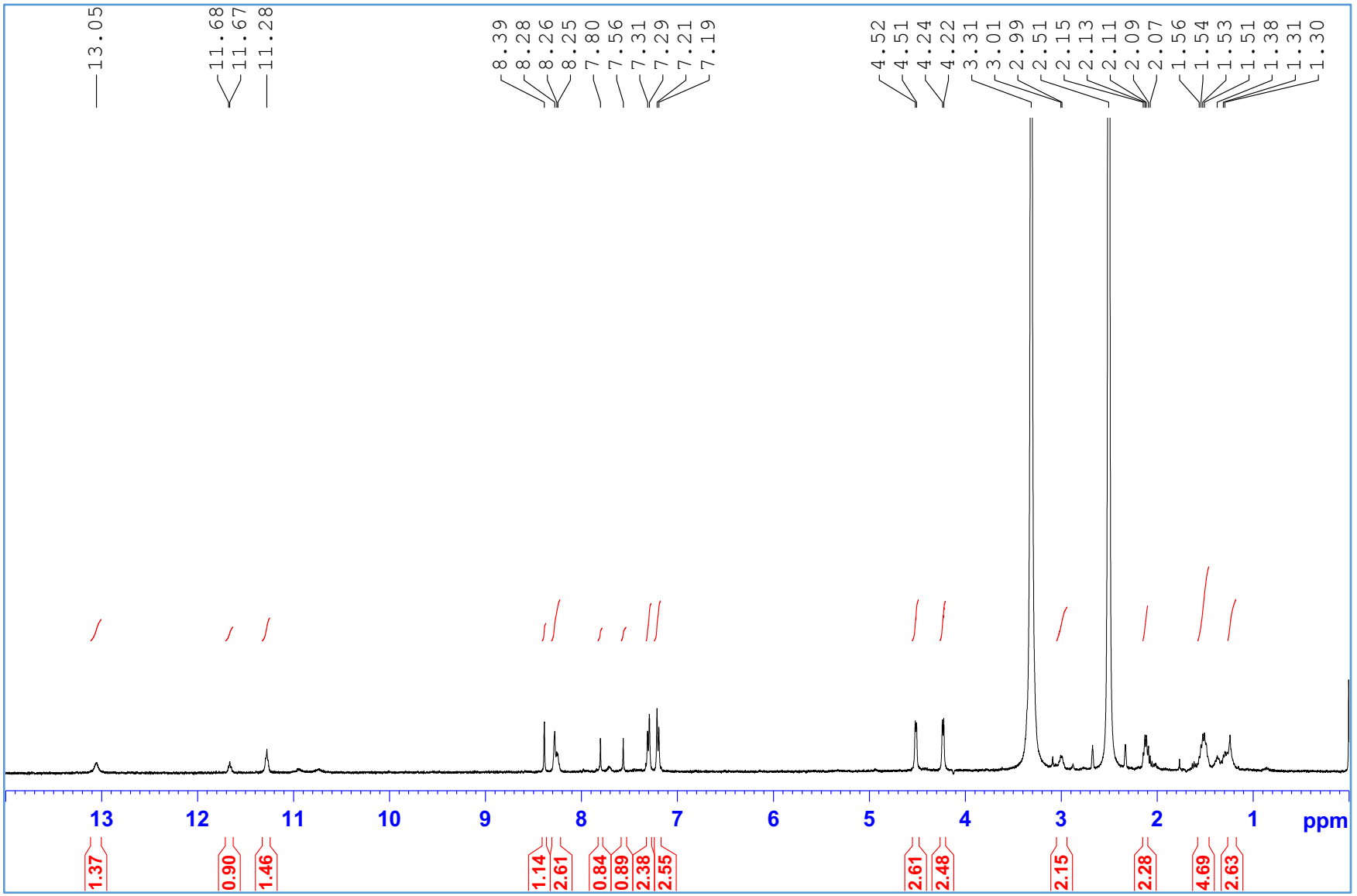
^{1}H NMR of compound **53** (400 MHz, DMSO-D_6)



^{13}C NMR of compound **53** (100 MHz, DMSO-D_6)



^{1}H NMR of compound **57** (400 MHz, DMSO-D_6)



^{13}C NMR of compound **57** (100 MHz, DMSO-D_6)