Supporting Information

Trypanosoma cruzi Malic Enzyme is the target for sulfonamide hits from GSK Chagas Box

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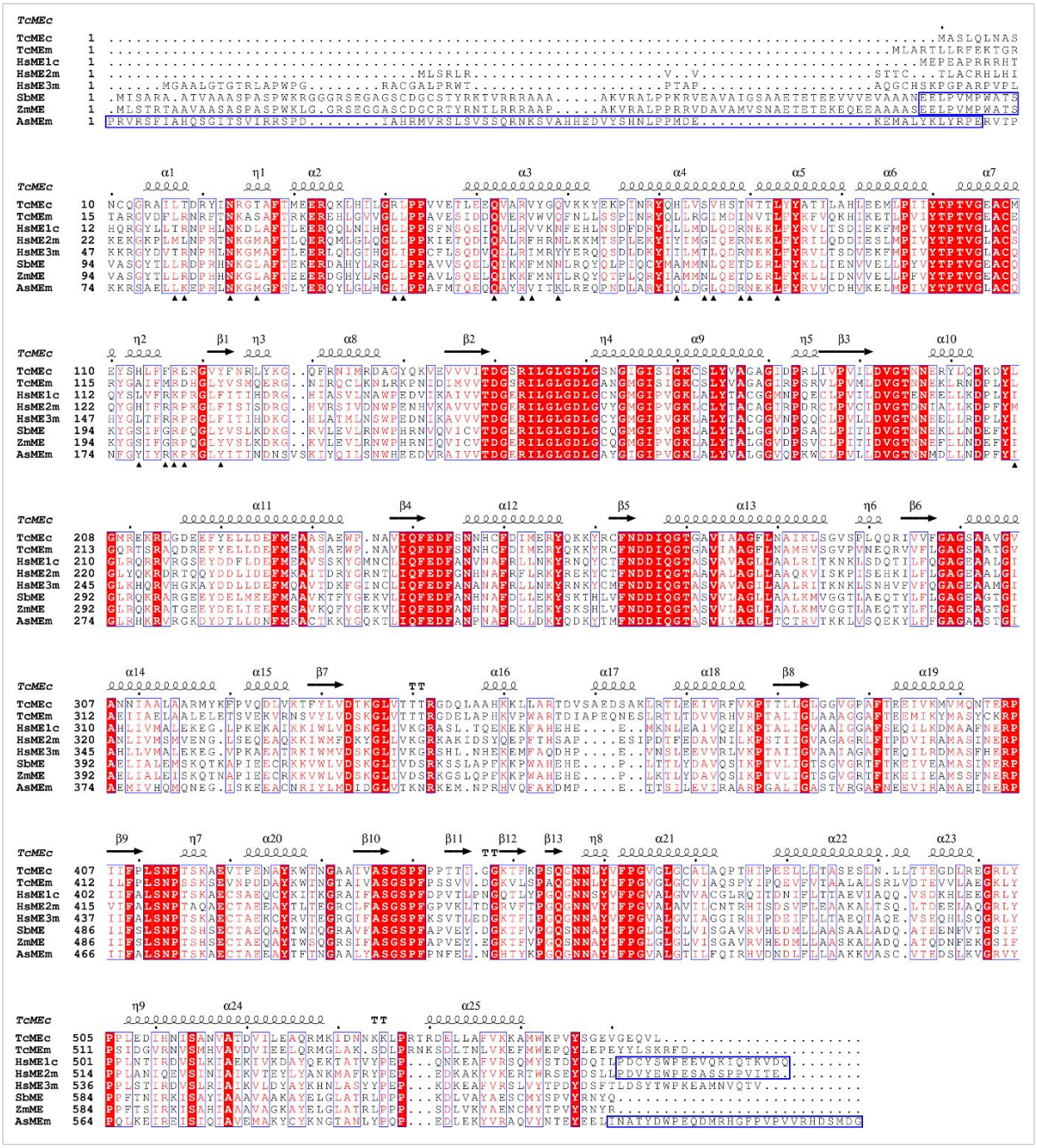


Figure S1. Alignment of aminoacid sequences of eukaryotic malic enzymes (ME). Residues of the cytosolic ME from *Trypanosoma cruzi* that delimit the allosteric inhibition site are indicated (▲). N- or C-terminal extensions known to stabilize the tetrameric structure in eukaryotic enzymes are highlighted (blue box). ***Abbreviations***: **TcMEc**, cytosolic *T. cruzi* ME (UniProt Q4DJ68); **TcMEm**, mitochondrial *T. cruzi* ME (UniProt Q4DJ69); **HsME1c**, cytosolic human ME1 (UniProt P48163); **HsME2m**, mitochondrial human ME2 (UniProt P23368); **HsME3m**, mitochondrial human ME3 (UniProt Q16798); **SbME**, *Sorghum bicolor* ME (UniProt Q84LQ5); **ZmME**, *Zea mays* ME (UniProt P16243); **AsMEm**, mitochondrial *Ascaris suum* ME (UniProt P27443). Figure prepared using Clustal Omega[1] and ESPript 3.0[2].

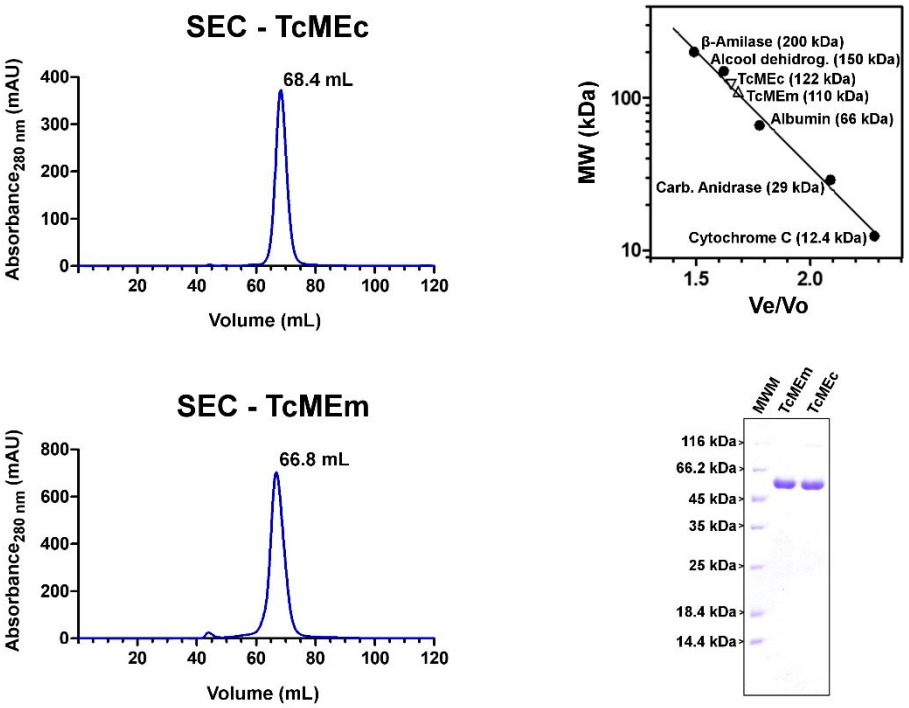


Figure S2. Size exclusion chromatography (SEC) and oligomeric state analysis of *Trypanosoma cruzi* malic enzymes, cytosolic (TcMEc) and mitochondrial (TcMEm) isoforms. Chromatograms obtained for TcMEc and TcMEm (left side) using a Superdex 200 16/600 column (GE Healthcare) show that both proteins eluted with similar elution volumes (Ve). Analysis of the oligomeric state with protein standards of known molecular weights indicate that TcMEc and TcMEm eluted from column in major peaks correspond to dimers (top right). Eletrophoretic analysis in denaturing conditions (SDS-PAGE) shows that the protomers of TcMEc and TcMEm have molecular weight of 64.8 and 65.8 kDa, respectively (bottom-right). Figure adapted from ref.[3].

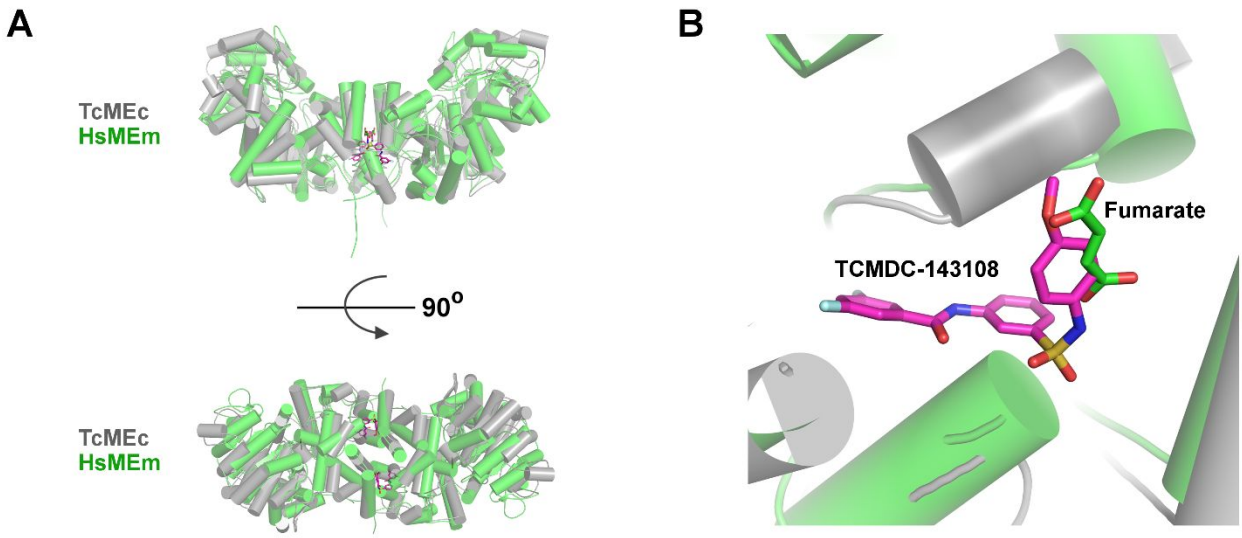


Figure S3. Superposition of Malic Enzymes from *Trypanosoma cruzi* (TcMEc, cytosolic isoform) and *Homo sapiens* (mitochondrial isoform, HsME2m).
Structural alignment of TcMEc dimer (gray cartoon, PDB 6W29) with a HsME2m dimer (green cartoon, PDB 1GZ3) **(A)** showing that the sulfonomide TCMDC-143108 overlaps with fumarate **(B)**, the allosteric activator of the human enzyme. Both the sulfonamide and allosteric activator are located at the dimer interface, apart from the enzyme catalytic center.

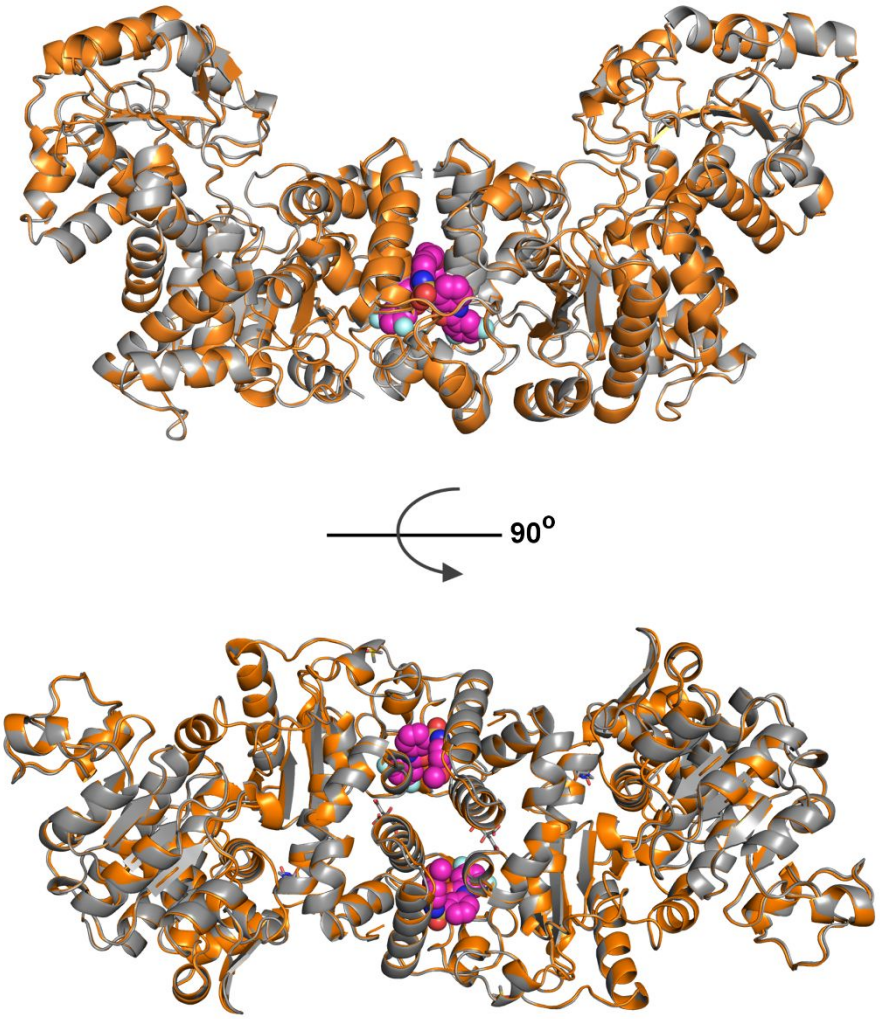


Figure S4. Superposition of structures of TcMEc dimer in ligand-free form (orange cartoon) and bound to sulfonamide inhibitor (gray cartoon with ligand as magenta spheres).
The alignment of TcMEc structures in ligand-bound and ligand-free form shows that the the enzyme enzyme has exactly the same domain and quaternary structure arrangement in these two forms (RMSD of 0.24 Å). This supports the idea that the binding of the inhibitor to the allosteric site locks the enzyme in an inactive conformation.

Table S1. **Effect of TcMEc sulfonamide inhibitors over *T. cruzi* (Tulahuen strain) intracellular amastigotes infecting H9c2**. Molecules described in this table refers to previously reported compounds identified by HTS.[4]

ID	Modifier (#1)	pIC50 *T. cruzi* INF CELL[a]	Modifier (#2)	pIC50 *T. cruzi* AM CELL[b]	Modifier (#3)	pIC50 *T. cruzi* H9C2[c]	SI (AMCELL - H9C2)[d]
ATR3-001	=	4,6	=	4,6	=	5,2	-0,6
ATR3-002	<	4,3	<	4,3	<	4,3	0
ATR3-005	=	4,7	=	4,9	=	4,7	0,2
ATR3-006	=	4,6	=	4,8	<	4,3	0,5
ATR3-007	<	4,3	<	4,3	<	4,3	0
ATR3-008	<	4,3	<	4,3	<	4,3	0
ATR3-014	<	4,3	<	4,3	<	4,3	0
ATR3-016	<	4,3	<	4,3	<	4,3	0
ATR3-021	=	4,5	=	4,6	<	4,3	0,3
ATR3-023	=	4,4	=	4,8	=	4,4	0,4
ATR3-028	=	5,1	=	5,1	=	5,3	-0,2
ATR3-030	=	4,9	=	4,9	=	5,2	-0,3
ATR3-033	=	4,4	=	4,5	=	4,5	0
ATR3-034	=	4,8	=	5,1	=	5,1	0
ATR3-036	=	5,1	=	5,1	=	5	0,1
ATR3-040	<	4,3	<	4,3	<	4,3	0
ATR3-043	=	4,8	=	5	=	5	0
ATR3-045	=	4,8	=	5	=	5	0
ATR3-049	=	4,6	=	4,4	=	4,6	-0,2
ATR3-053	=	5	=	4,9	<	4,3	0,6
ATR3-057	=	4,3	=	4,5	=	4,5	0
ATR3-065	<	4,3	<	4,3	=	4,4	-0,1
ATR3-072	=	4,9	=	5	=	5,2	-0,2
ATR3-073	=	4,7	=	4,8	=	4,6	0,2
ATR3-074	=	5	=	5	=	5,2	-0,2
ATR3-075	=	4,7	=	4,9	=	4,8	0,1
ATR3-076	<	4,3	=	4,4	=	4,4	0
ATR3-080	=	4,8	=	4,9	=	4,9	0
ATR3-084	=	4,6	=	4,5	=	4,6	-0,1
ATR3-086	=	4,8	=	5	=	4,8	0,2
ATR3-087	<	4,3	<	4,3	<	4,3	0
ATR3-088	=	4,4	=	4,5	<	4,3	0,2
ATR3-089	=	4,7	=	4,7	=	4,7	0
ATR3-090	=	4,7	=	4,5	=	4,6	-0,1
ATR3-091	=	5	=	5,2	=	5	0,2
ATR3-092	=	4,8	=	4,9	<	4,3	0,6
ATR3-093	=	4,5	=	4,5	<	4,3	0,2
ATR3-095	<	4,3	<	4,3	<	4,3	0
ATR3-098	=	4,6	=	4,8	=	4,8	0
ATR3-100	=	4,6	=	4,8	=	4,6	0,2
ATR3-102	<	4,3	<	4,3	<	4,3	0

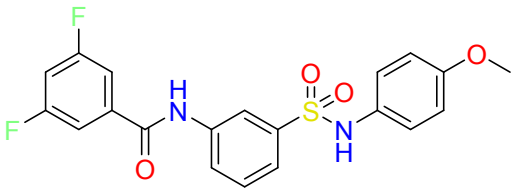
Continues…

Table S1. Continuation…

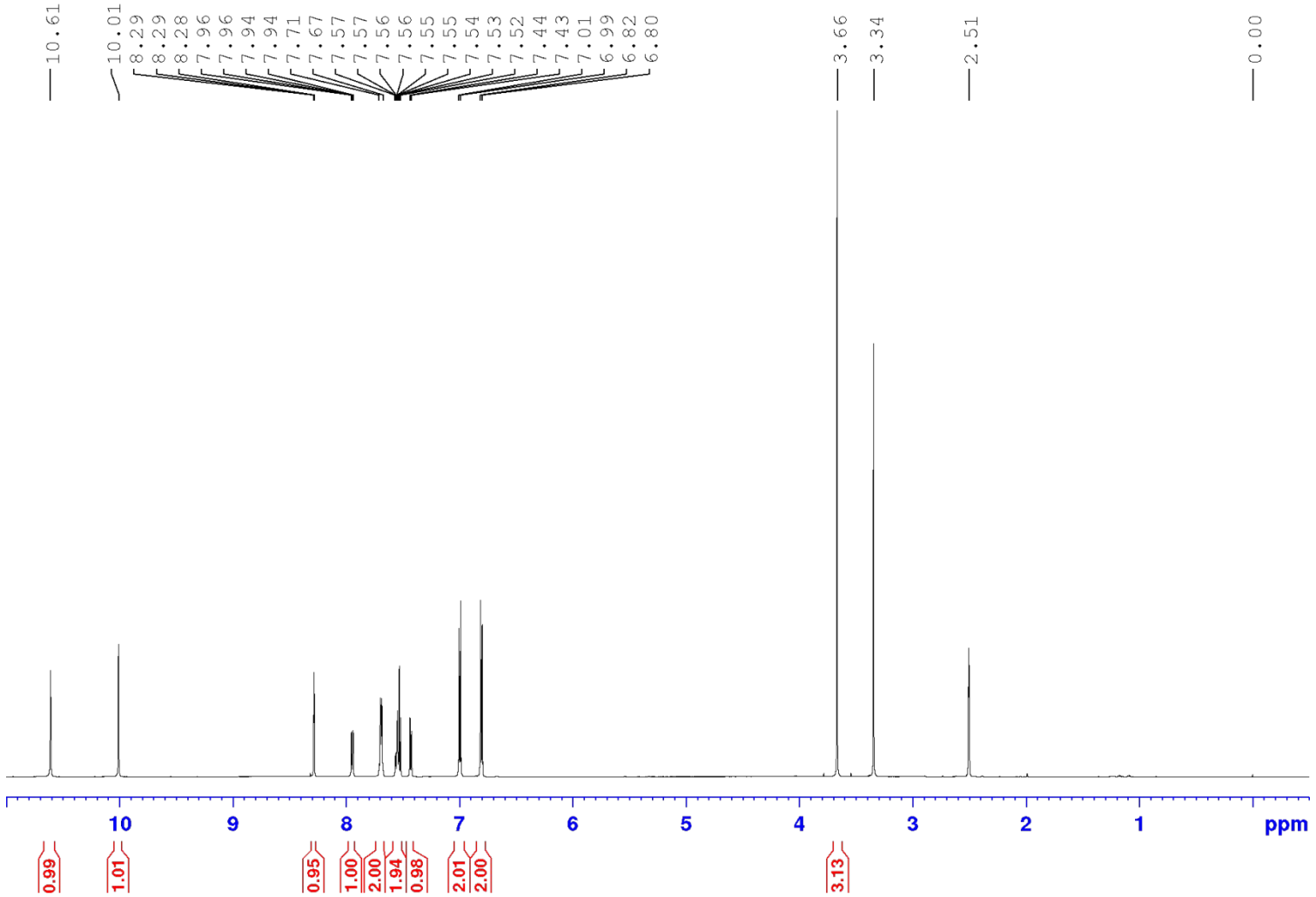
ID	Modifier (#1)	pIC50 *T. cruzi* INF CELL[a]	Modifier (#2)	pIC50 *T. cruzi* AM CELL[b]	Modifier (#3)	pIC50 *T. cruzi* H9C2[c]	SI (AMCELL - H9C2)[d]
ATR3-103	=	4,5	=	4,7	=	4,5	0,2
ATR3-105	=	4,7	=	4,8	=	4,7	0,1
ATR3-107	<	4,3	=	4,3	<	4,3	0
ATR3-112	<	4,3	<	4,3	<	4,3	0
ATR3-114	=	4,7	=	4,8	=	6	-1,2
ATR3-115	=	4,5	=	4,8	=	4,6	0,2
ATR3-124	=	4,3	=	4,4	=	4,3	0,1
ATR3-125	=	4,8	=	5,1	=	5,1	0
ATR3-128	=	4,4	=	4,7	<	4,3	0,4
ATR3-130	=	4,5	=	4,6	=	4,4	0,2
ATR3-132	=	4,6	=	4,6	=	4,5	0,1
ATR3-133	=	5	=	5,4	=	5,1	0,3
ATR3-139	<	4,3	<	4,3	<	4,3	0
ATR3-141	<	4,3	<	4,3	<	4,3	0
ATR3-142	=	4,5	=	4,7	<	4,3	0,4
ATR3-143	<	4,3	<	4,3	<	4,3	0
ATR3-144	=	4,4	=	4,6	<	4,3	0,3
ATR3-146	=	4,3	=	4,5	=	4,5	0
ATR3-152	=	5,2	=	5,5	=	5,3	0,2
ATR3-156	=	4,7	=	5,1	=	5	0,1

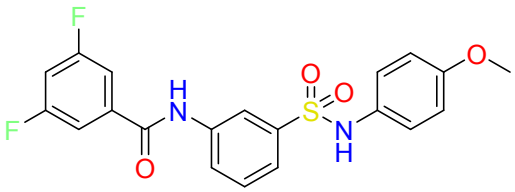
[a] - pIC50 *Trypanosoma cruzi* Imaging: Infected Cells; [b] - pIC50 *Trypanosoma cruzi* Imaging: Amastigotes/Cell; [c] - pIC50 H2c9 Imaging: H9c2 Host Cardiomyocytes; [d] – Selective Index in log scale, calculated by the subtraction pIC50 *T. cruzi* AM CELL less pIC50 *T. cruzi* H9c2.

^{1}H and ^{13}C NMR spectra for compounds **1**, **6**-**43** and **3,5-difluorobenzoyl chloride**

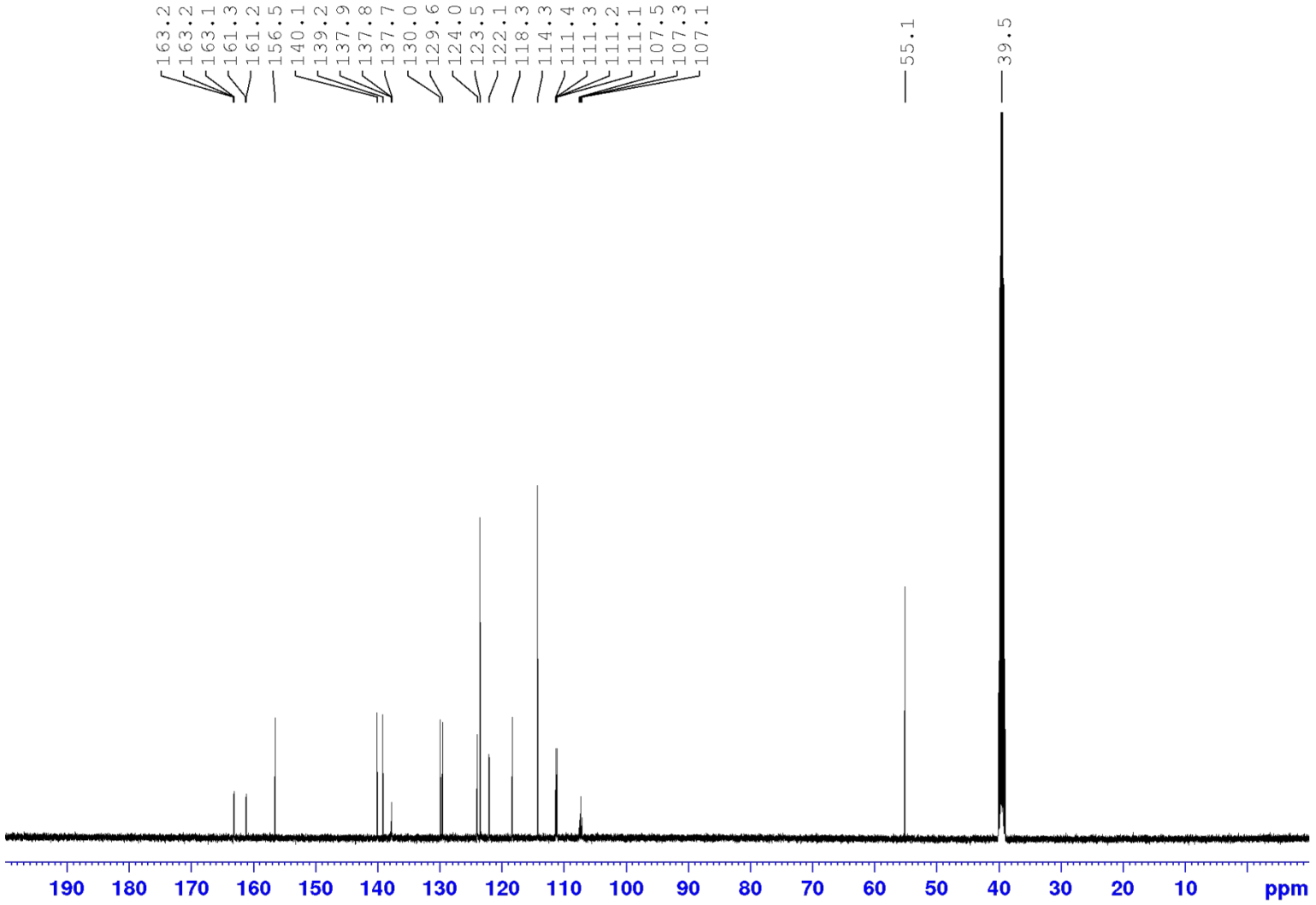


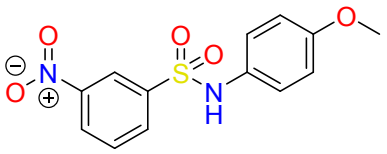
Compound **1** – ^{1}H NMR (600 MHz) – DMSO-d_6



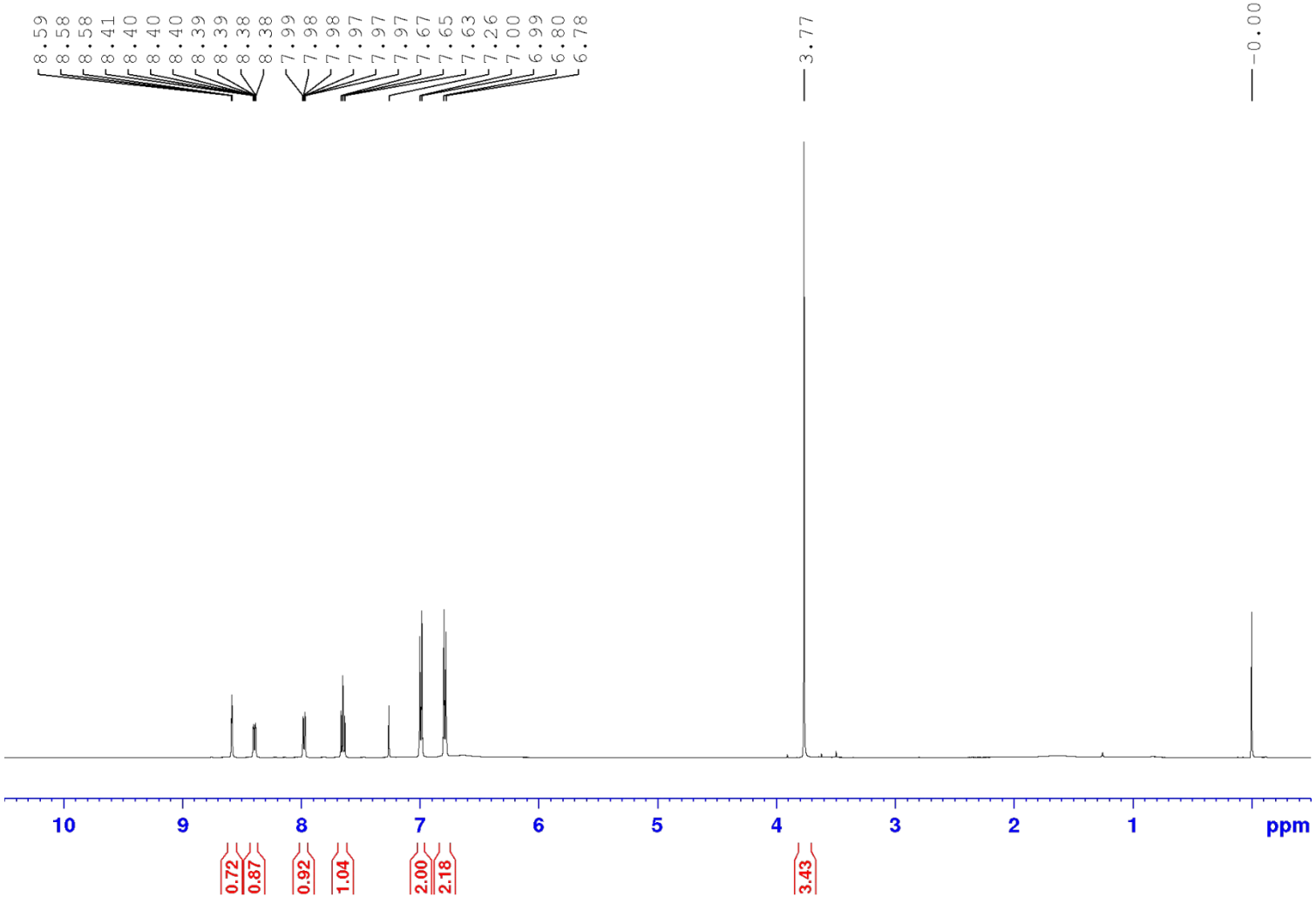


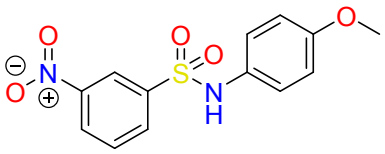
Compound **1** – ^{13}C NMR (150 MHz) – DMSO-d_6



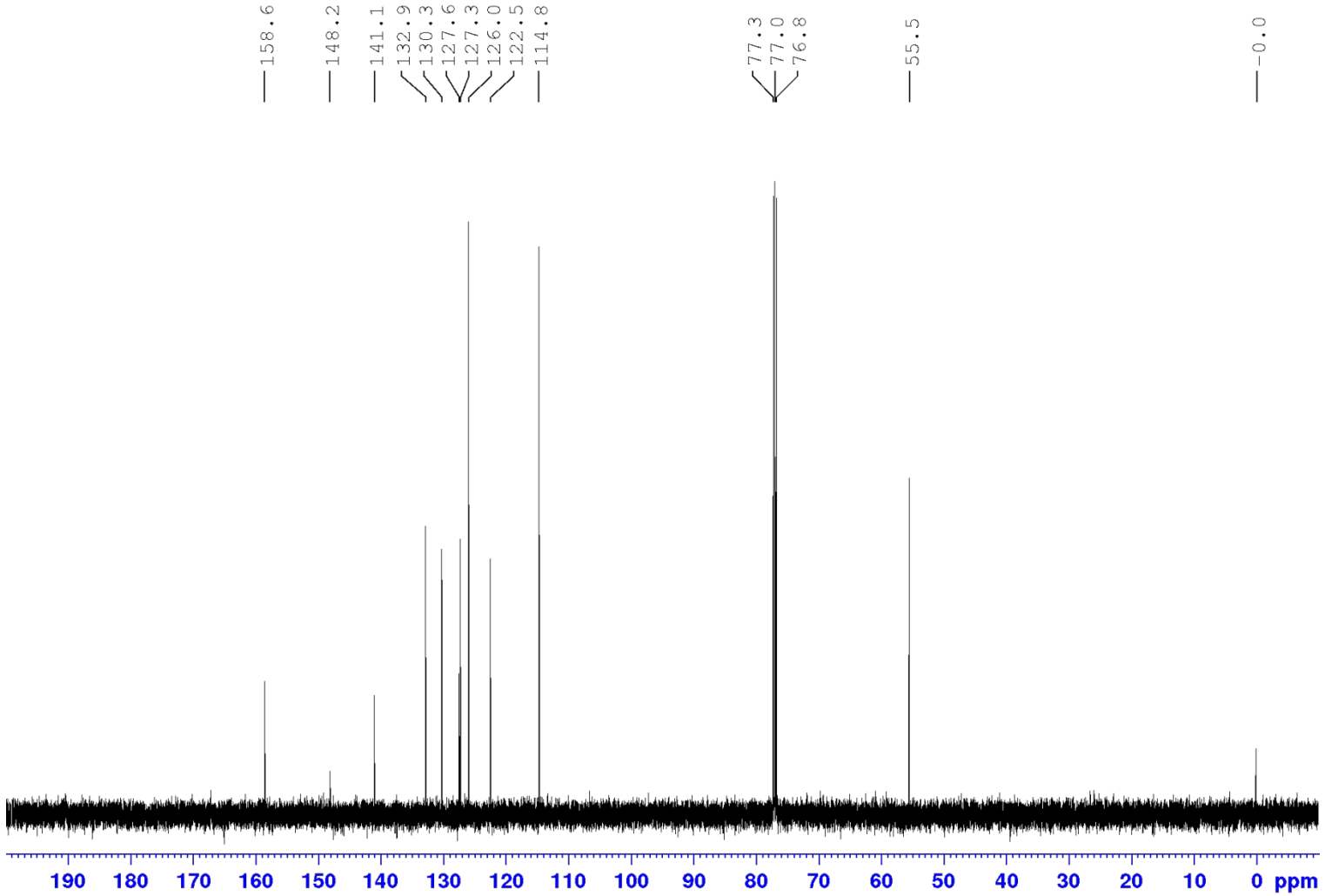


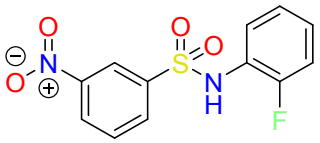
Compound **6** – ^{1}H NMR (500 MHz) – $CDCl_3$



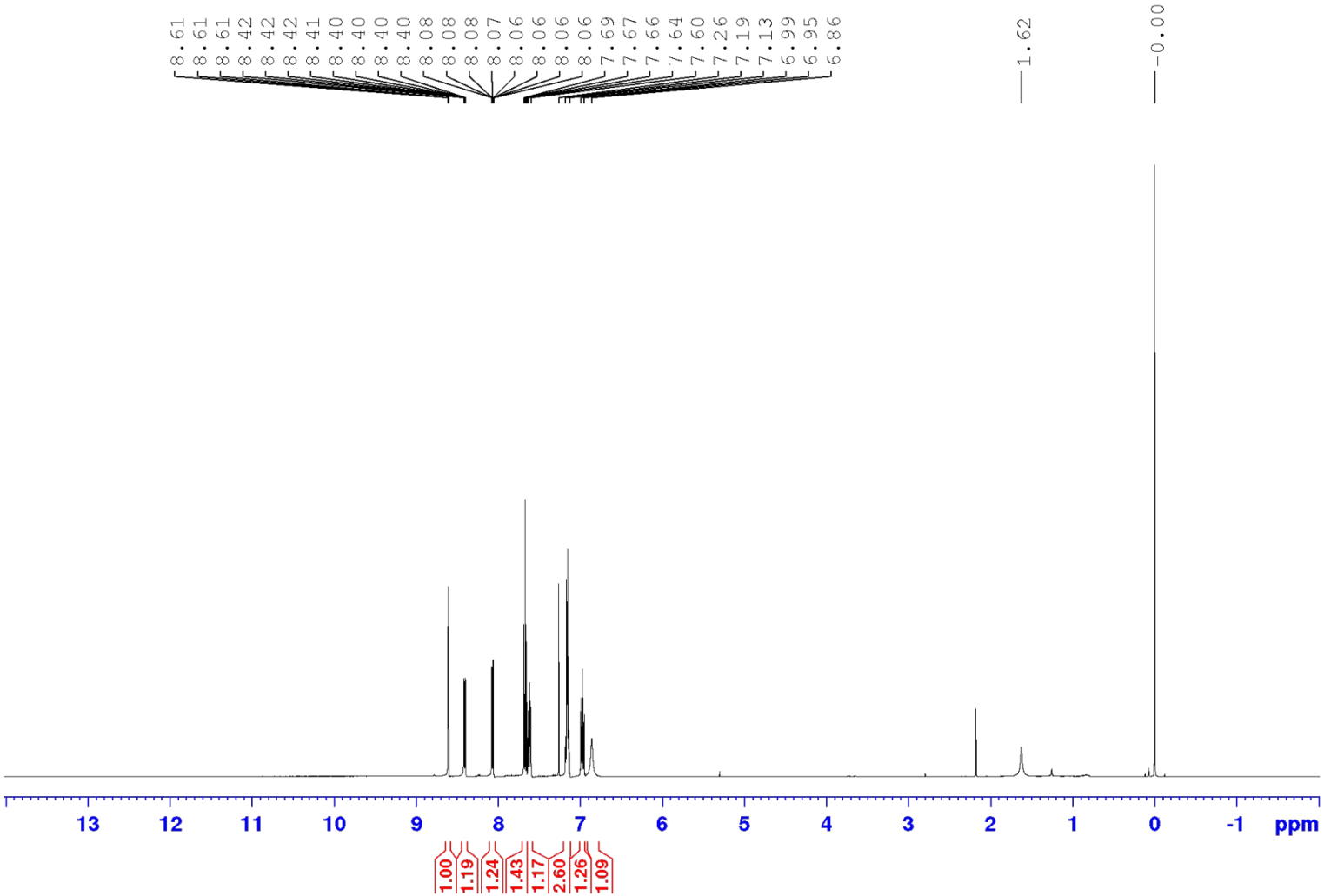


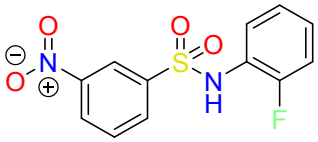
Compound **6** – ^{13}C NMR (125.5 MHz) – $CDCl_3$



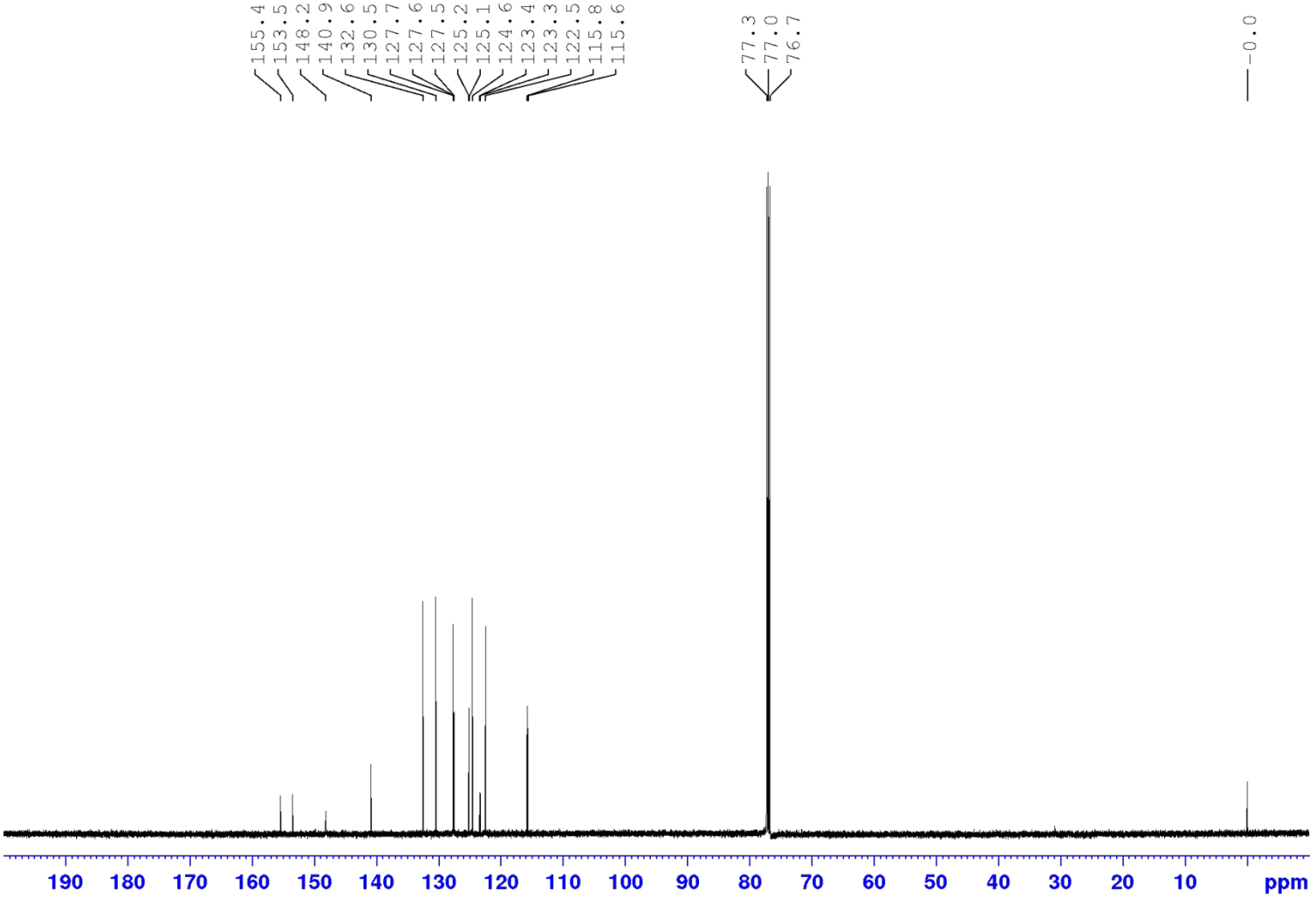


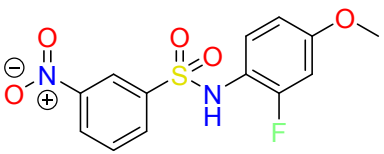
Compound **7** - ^{1}H NMR (500 MHz) – DMSO-d_6



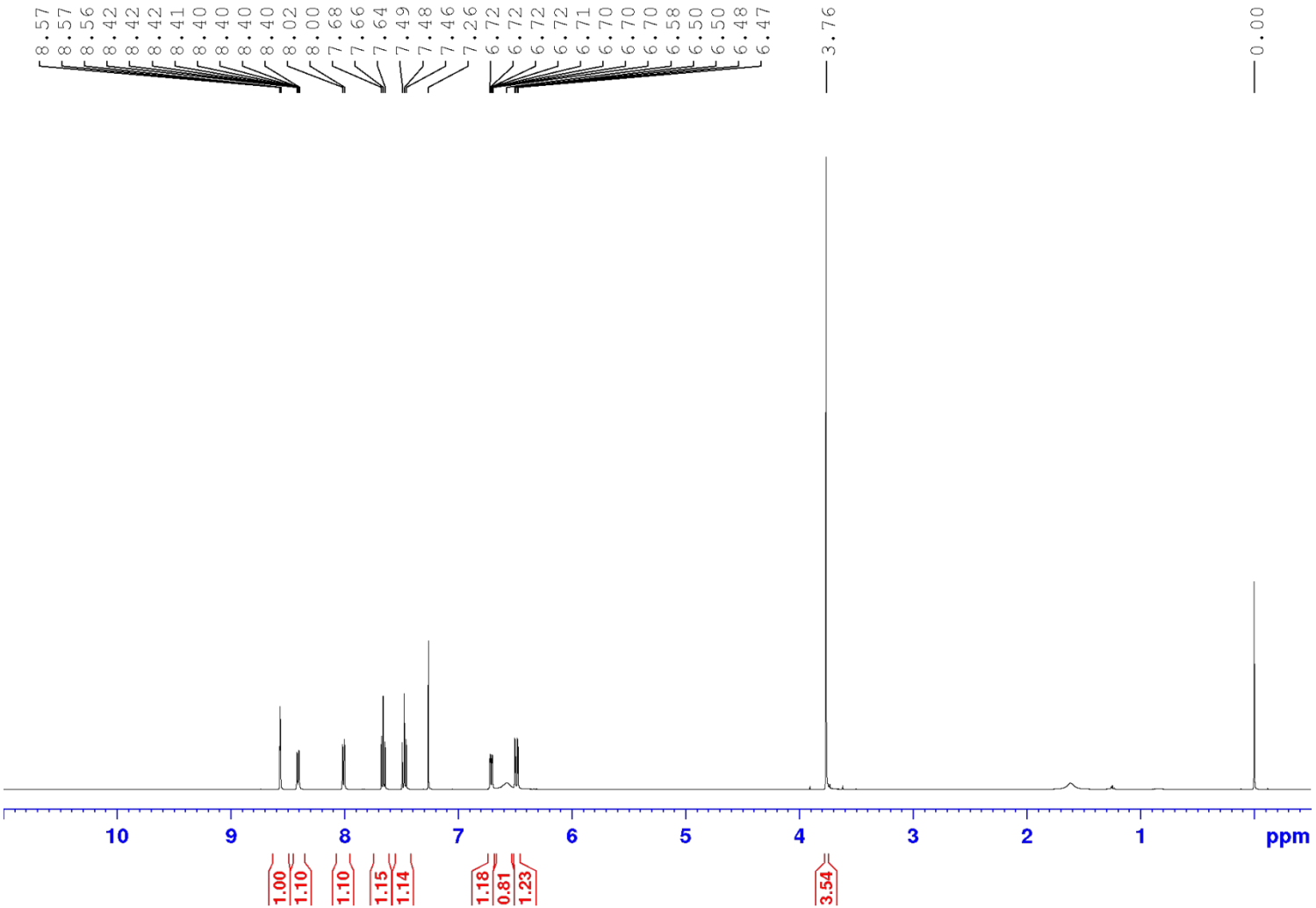


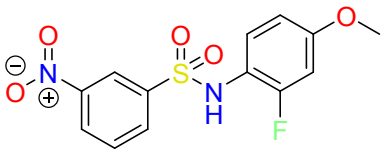
Compound **7** – ^{13}C NMR (125.5 MHz) – DMSO-d_6



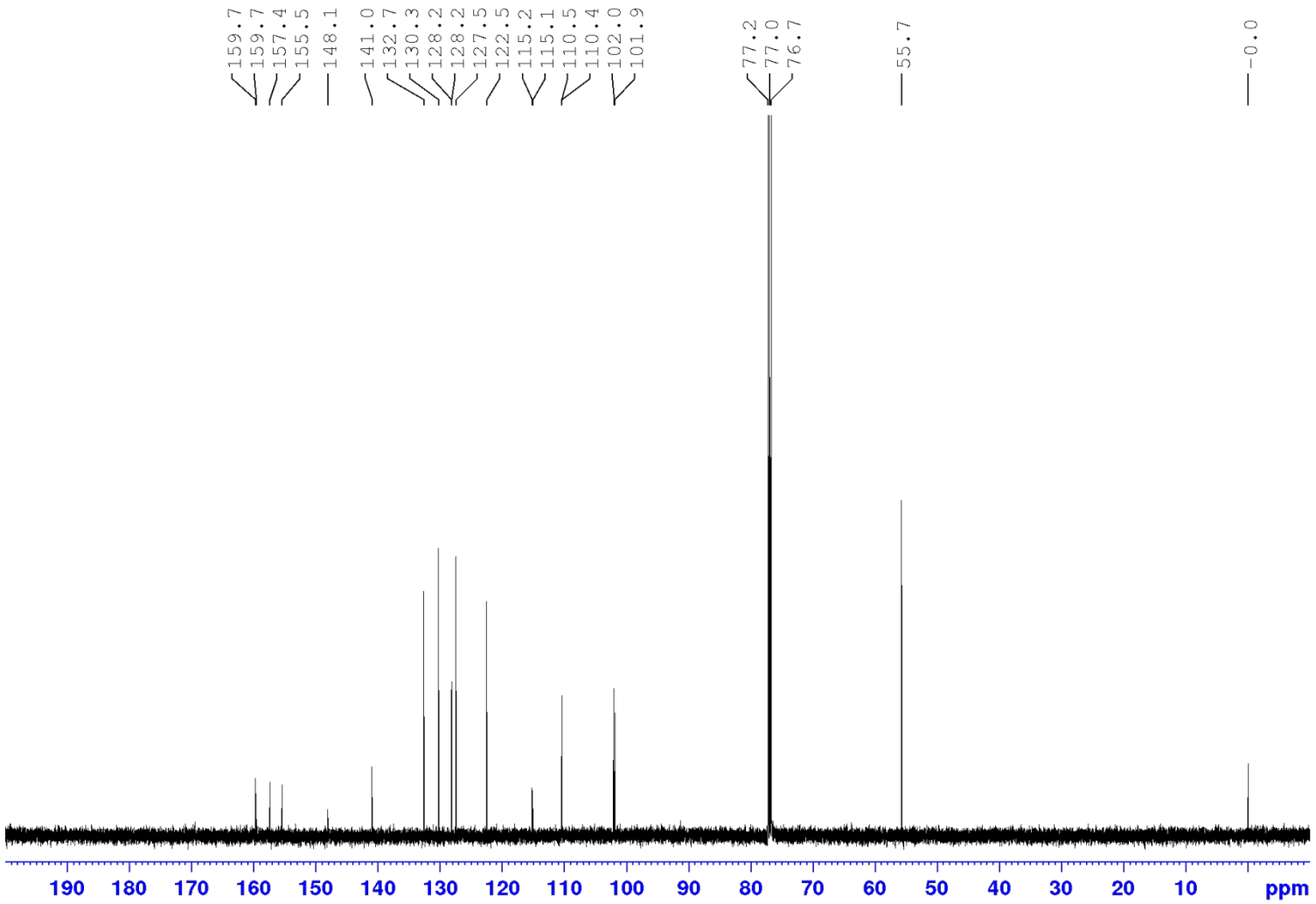


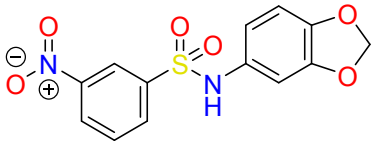
Compound **8** – ^{1}H NMR (500 MHz) – $CDCl_3$



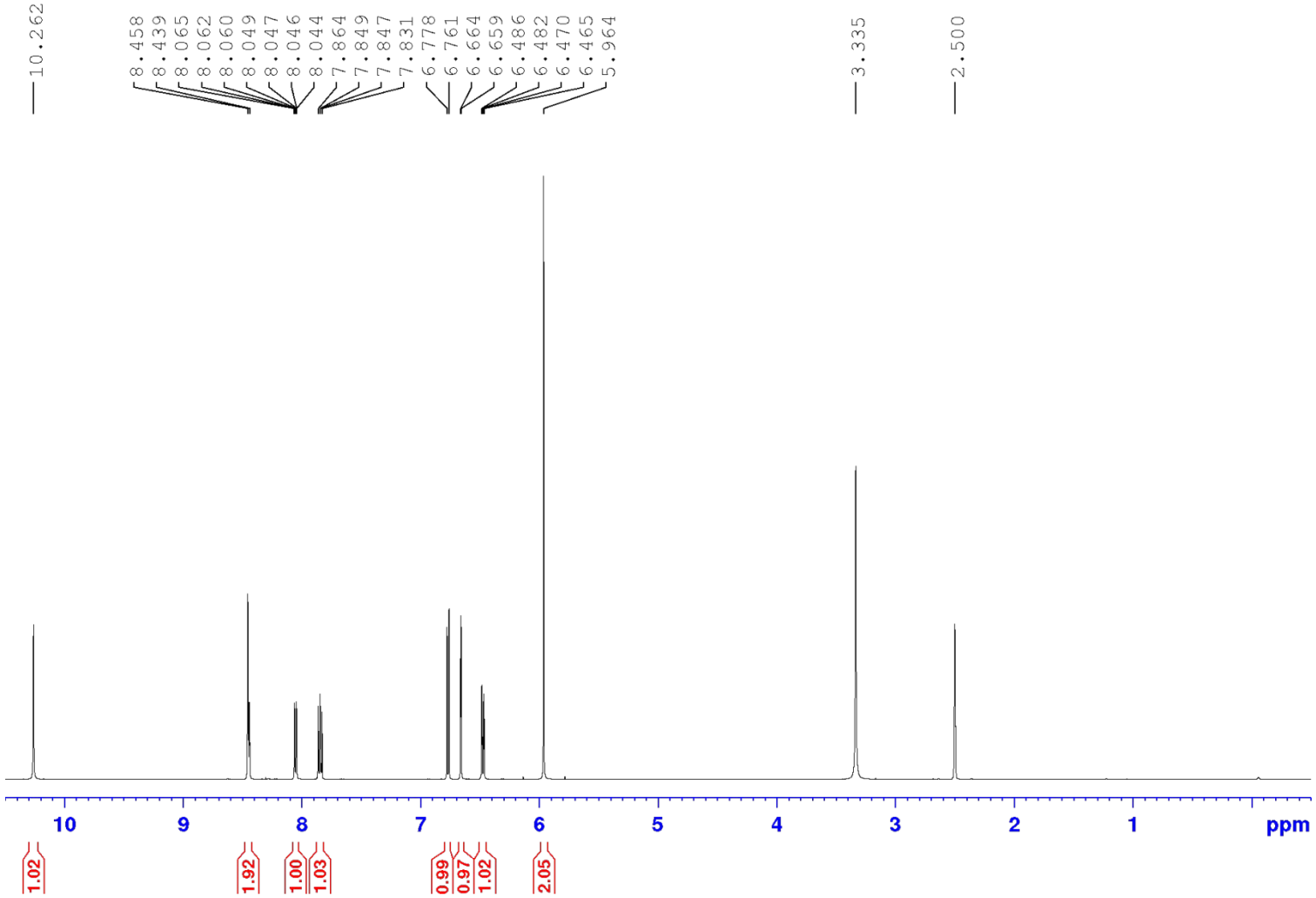


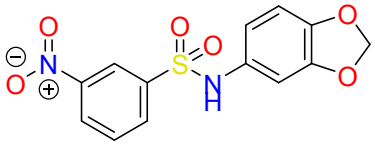
Compound **8** – ^{13}C NMR (125.5 MHz) – $CDCl_3$



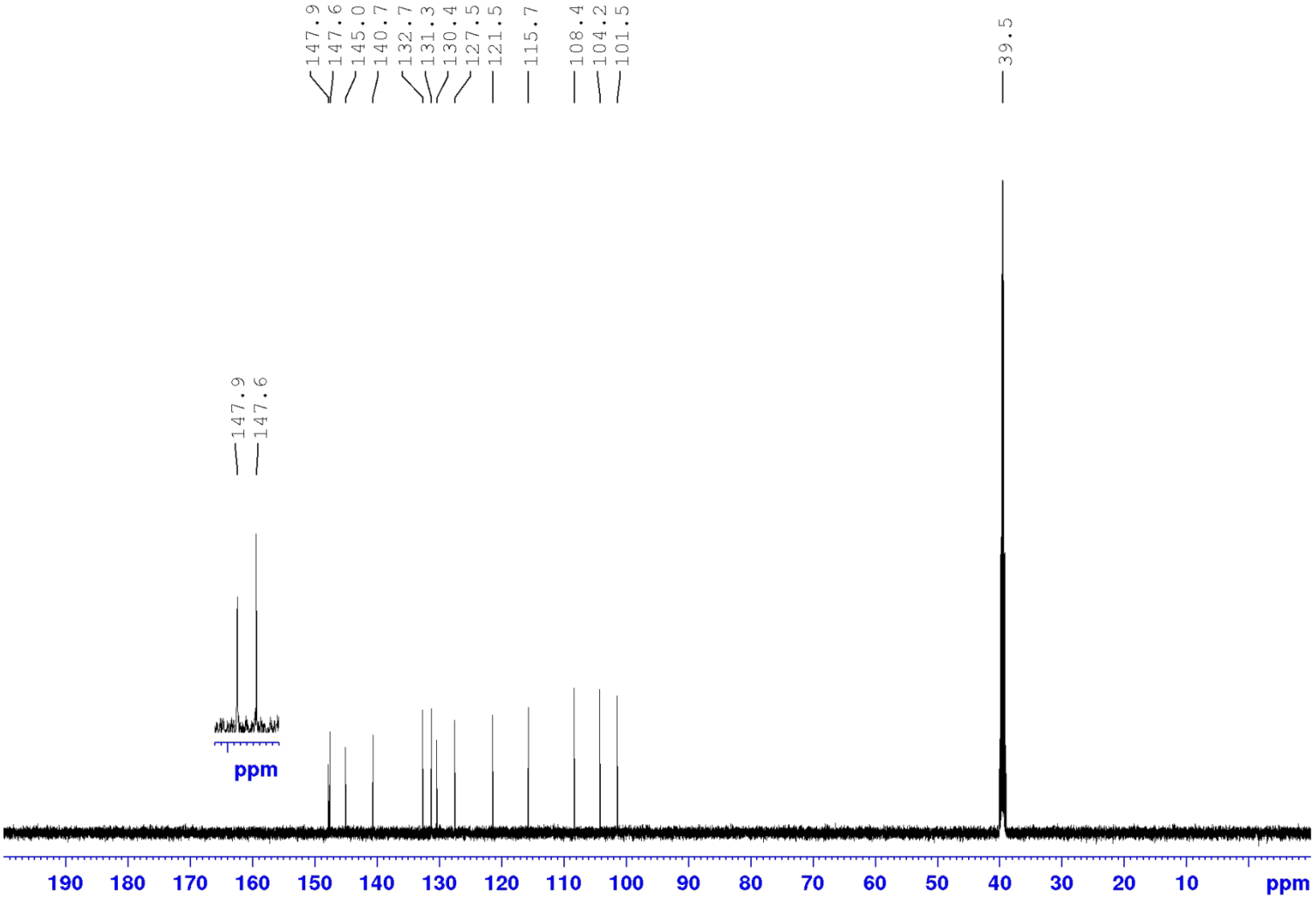


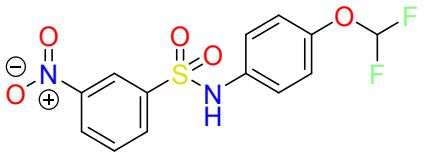
Compound **9** – ^{1}H NMR (500 MHz) – DMSO-d_6





Compound **9** – ^{13}C NMR (125.5 MHz) – DMSO-d_6





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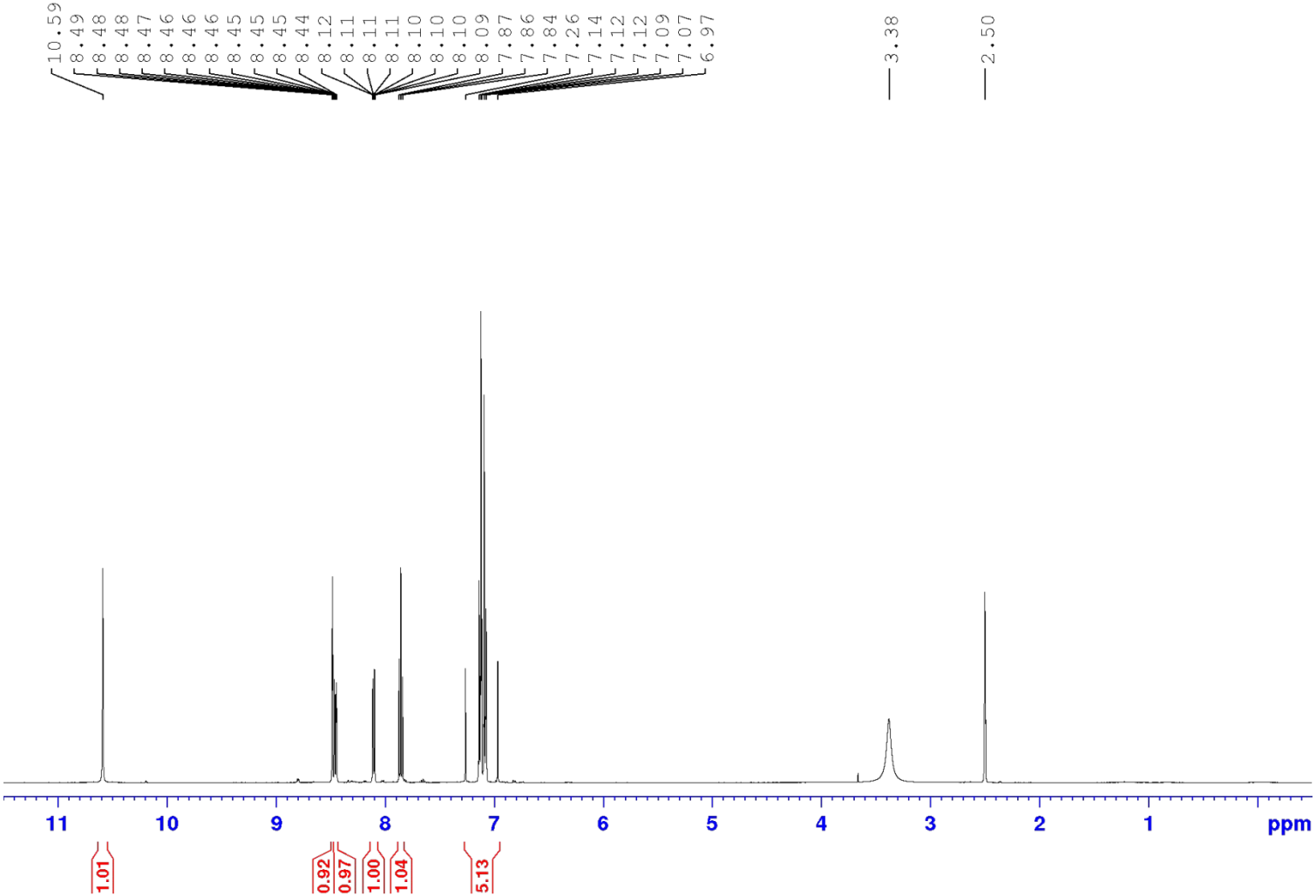
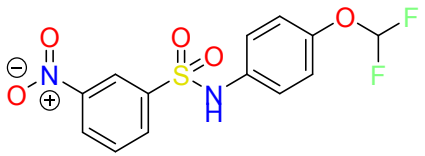
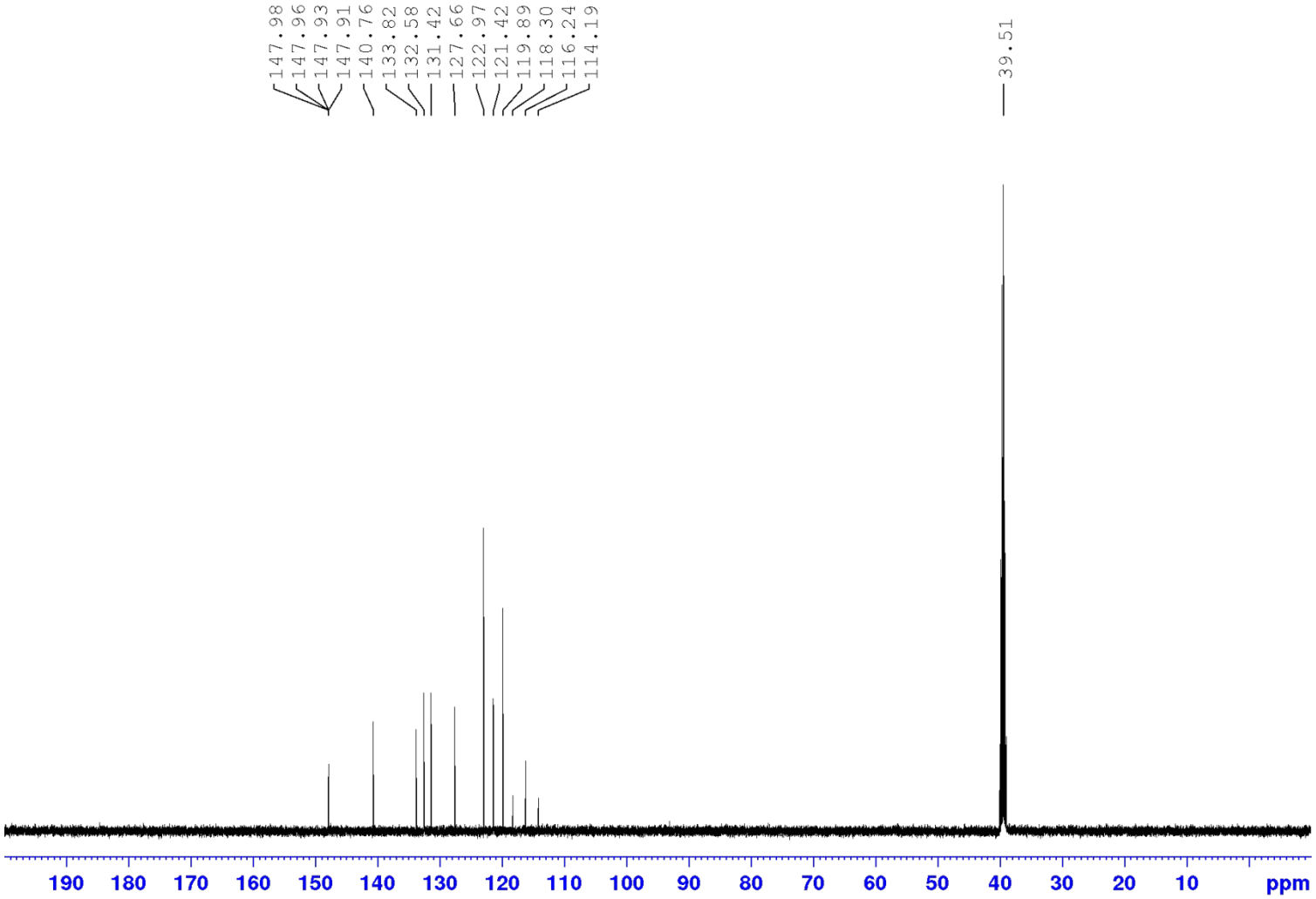
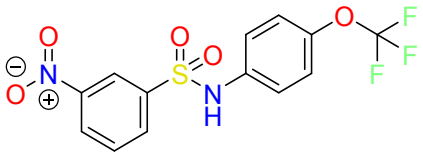


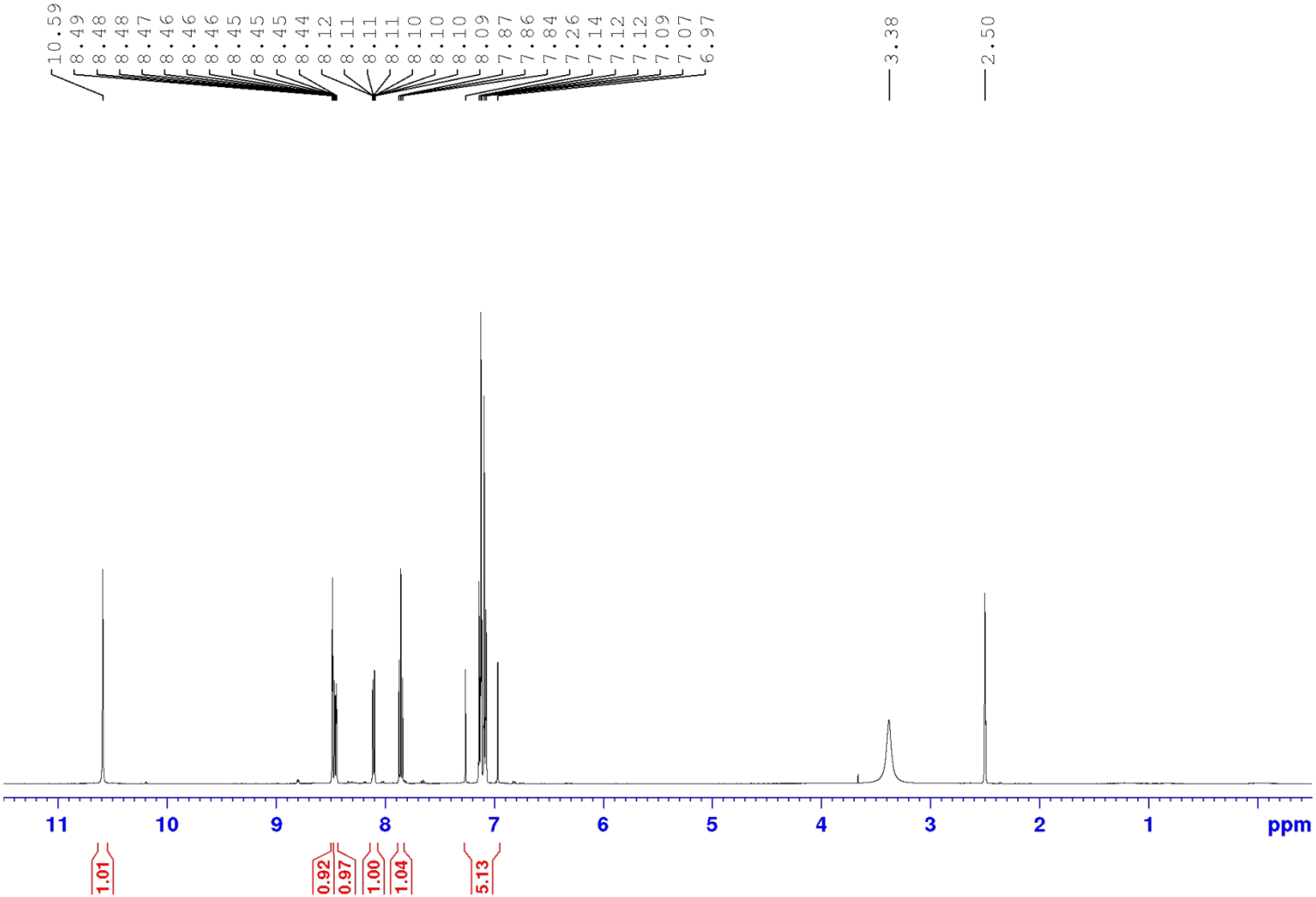


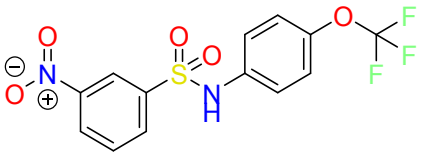
Compound **10** – ^{13}C NMR (125.5 MHz) – DMSO-d_6



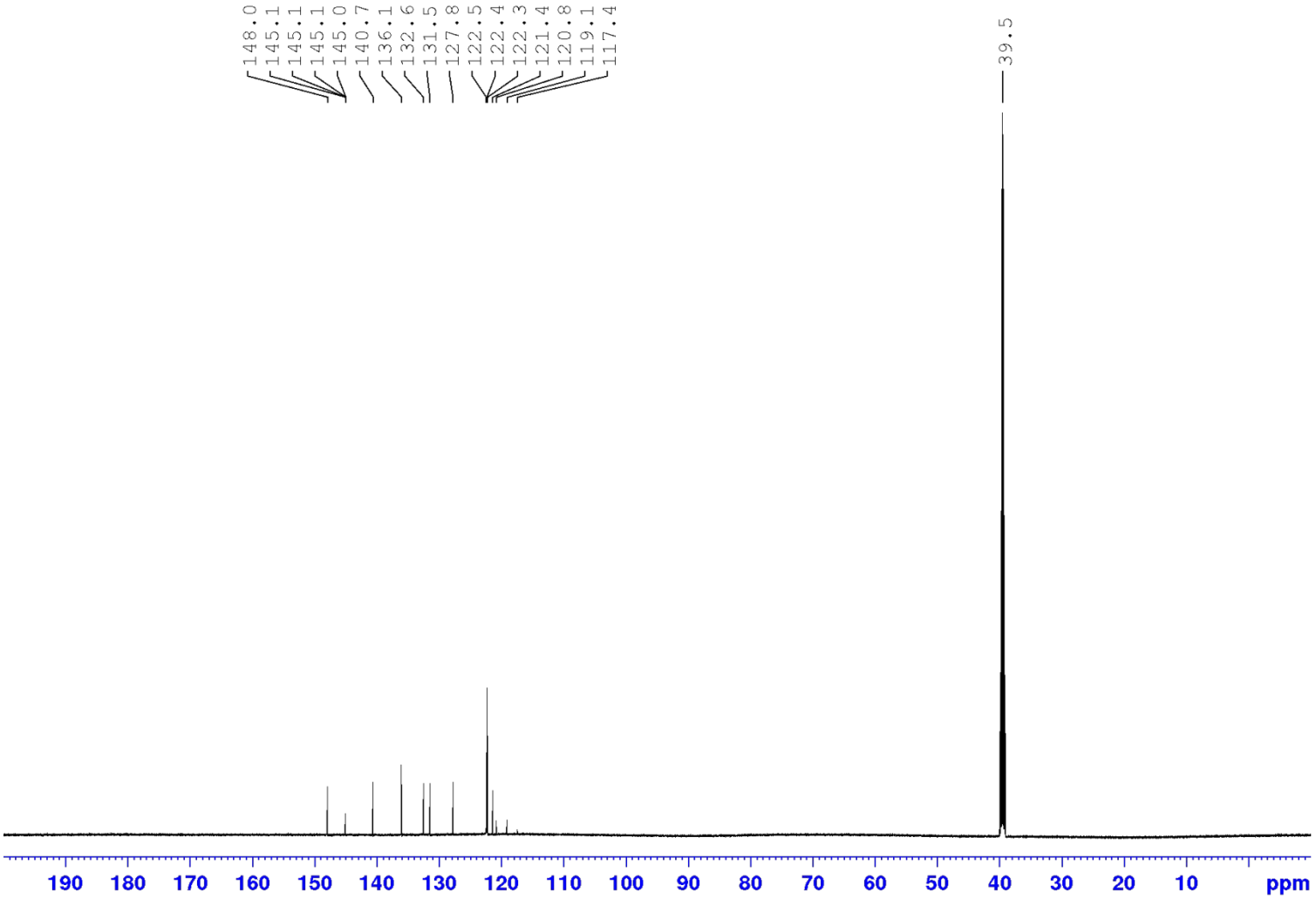


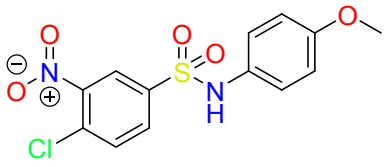
Compound **11** – ^{1}H NMR (500 MHz) – DMSO-d_6



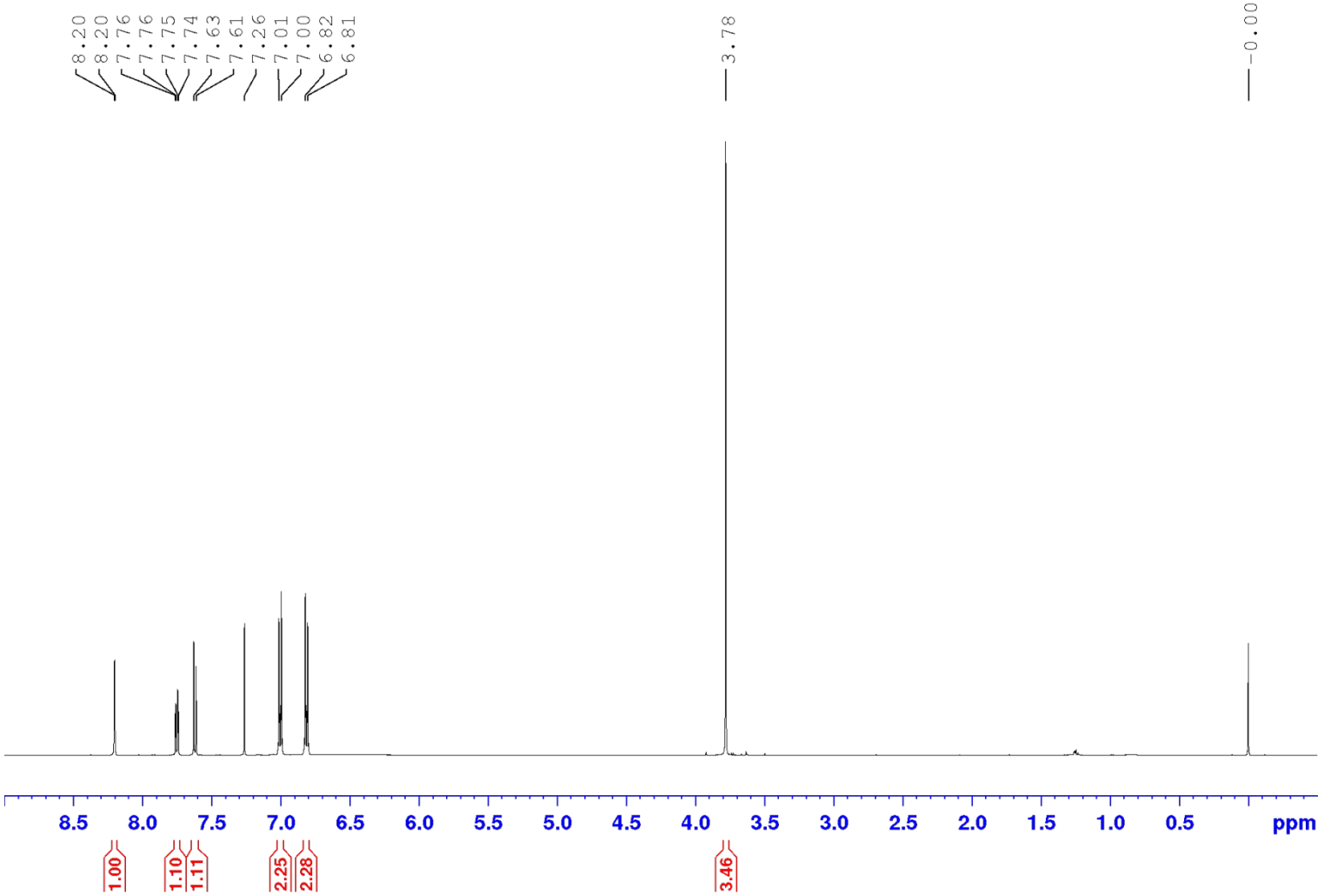


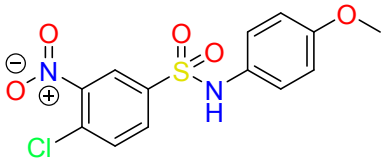
Compound **11** – ^{13}C NMR (150 MHz) – DMSO-d_6



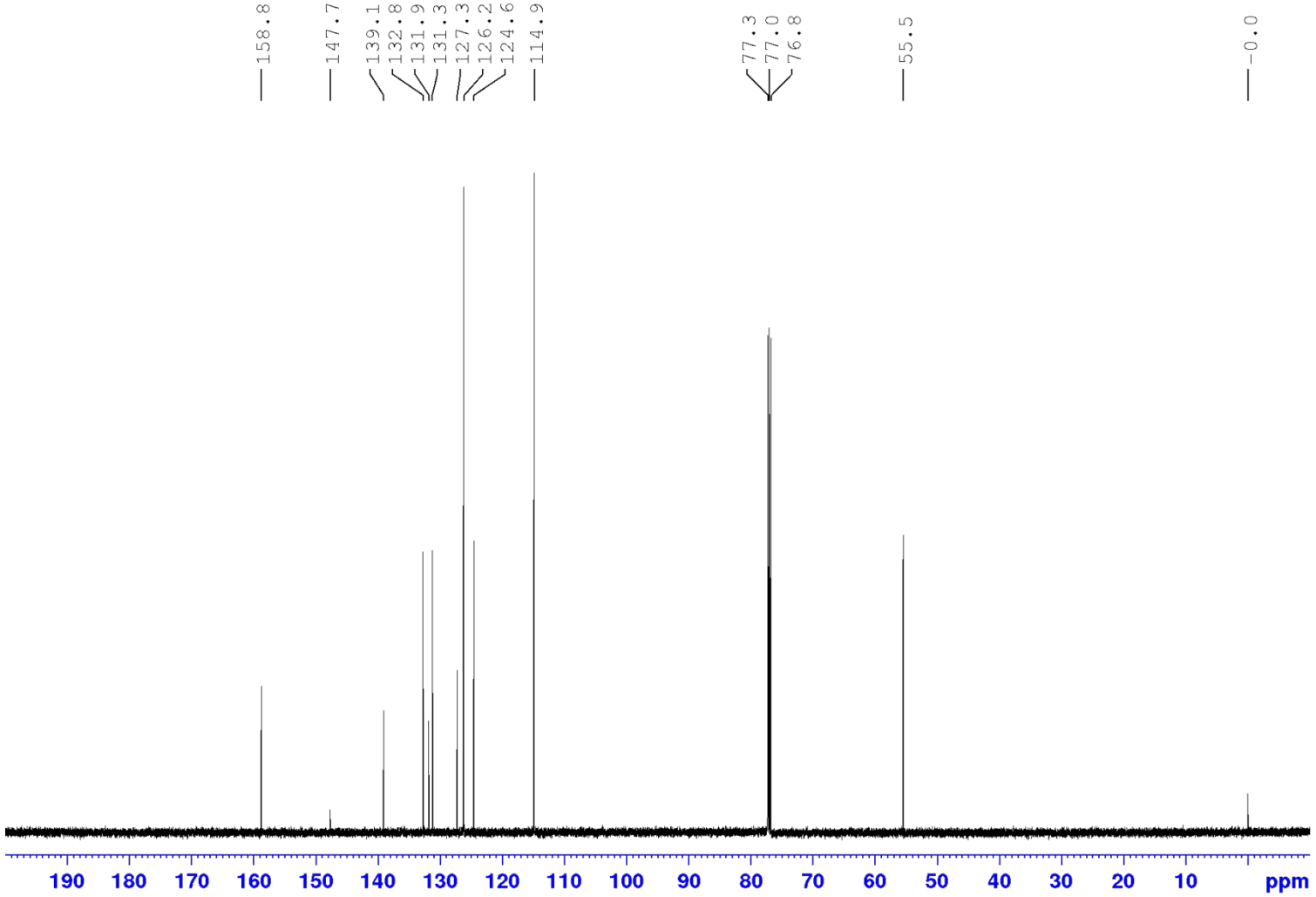


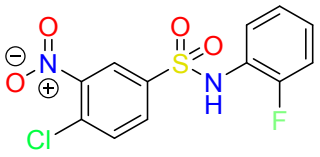
Compound **12** – ^{1}H NMR (500 MHz) – $CDCl_3$



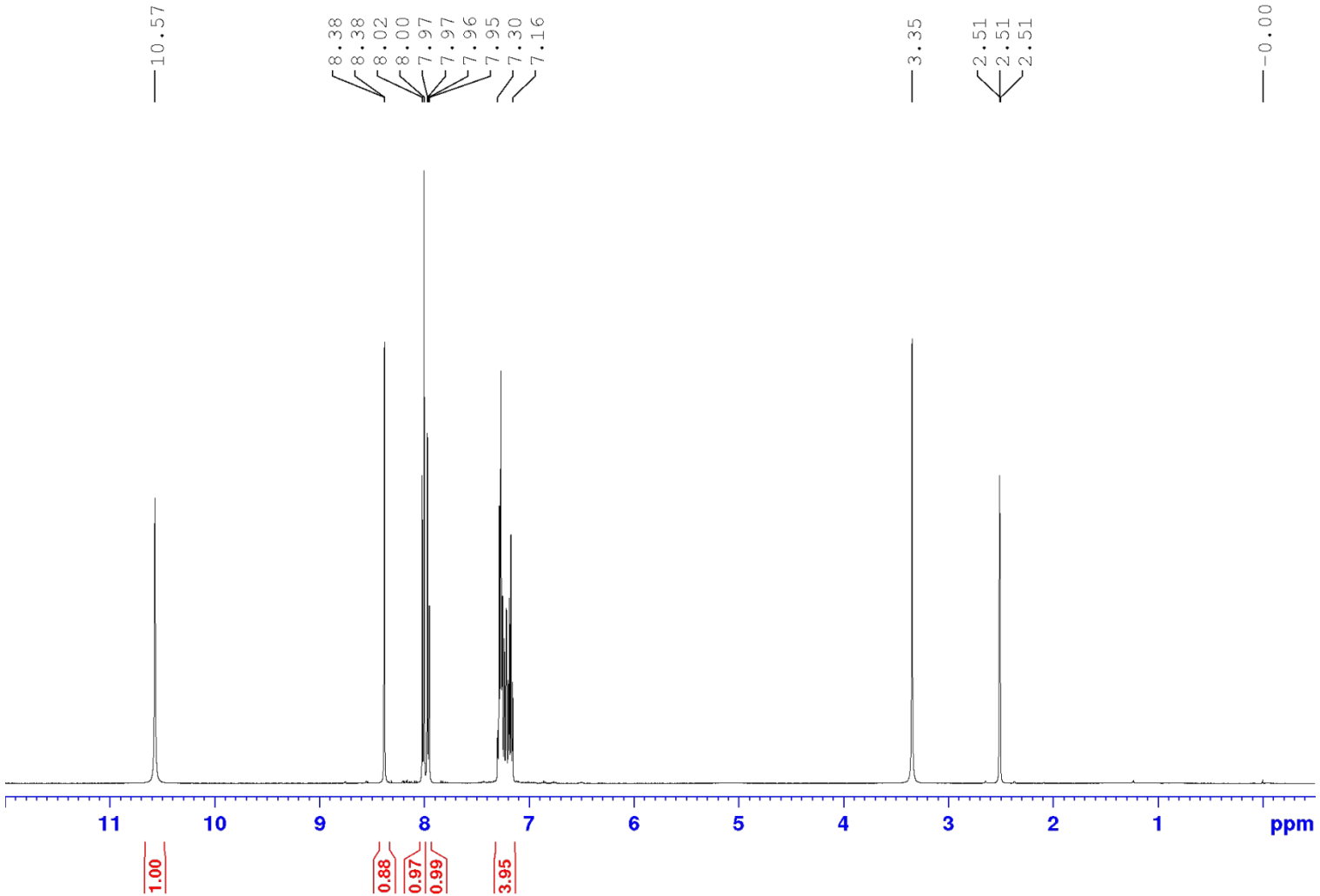


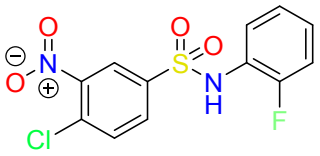
Compound **12** – ^{13}C NMR (125.5 MHz) – $CDCl_3$



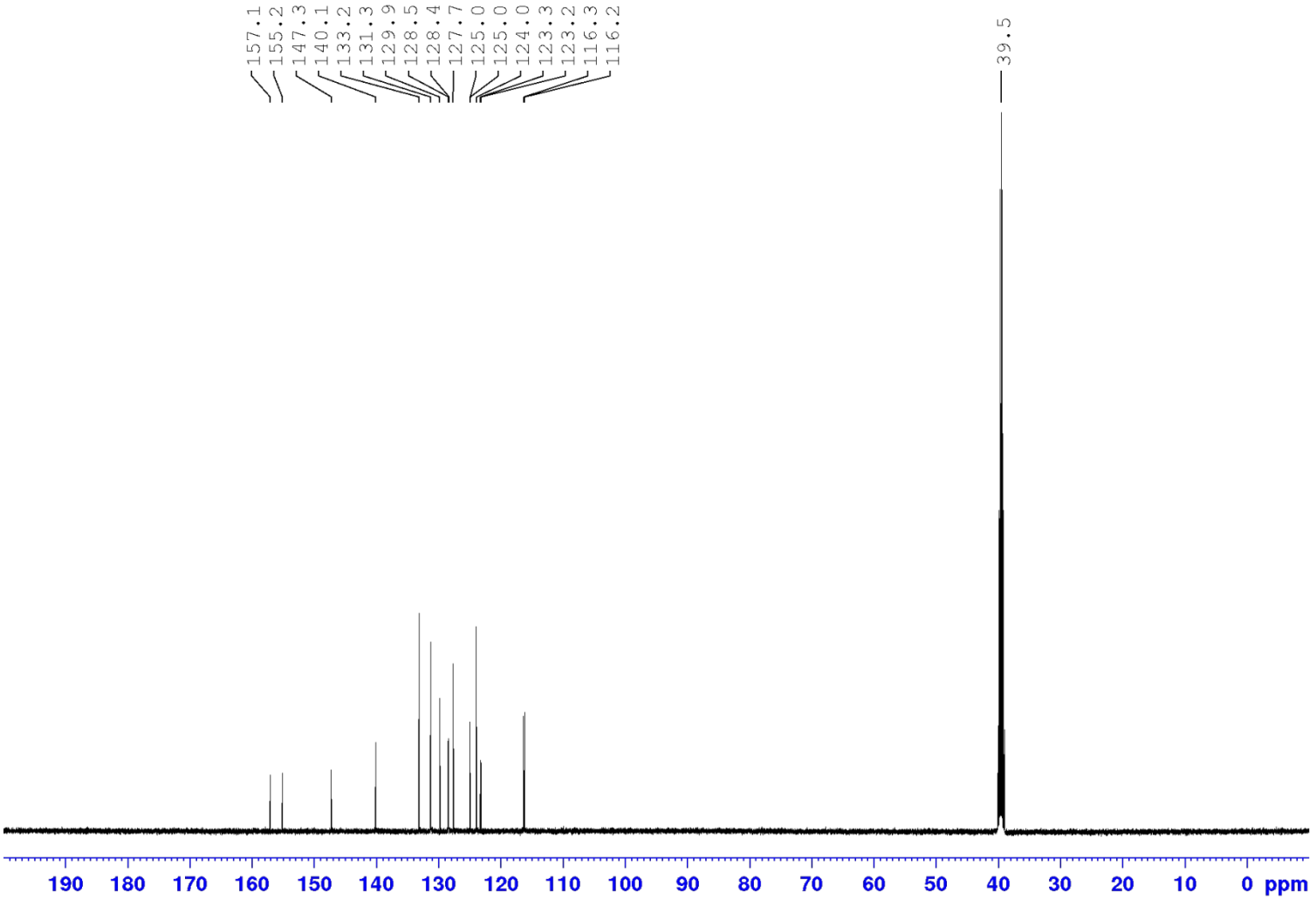


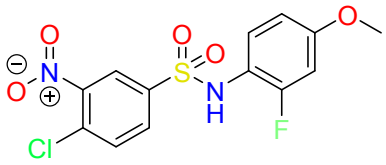
Compound **13** – ^{1}H NMR (500 MHz) – DMSO-d_6



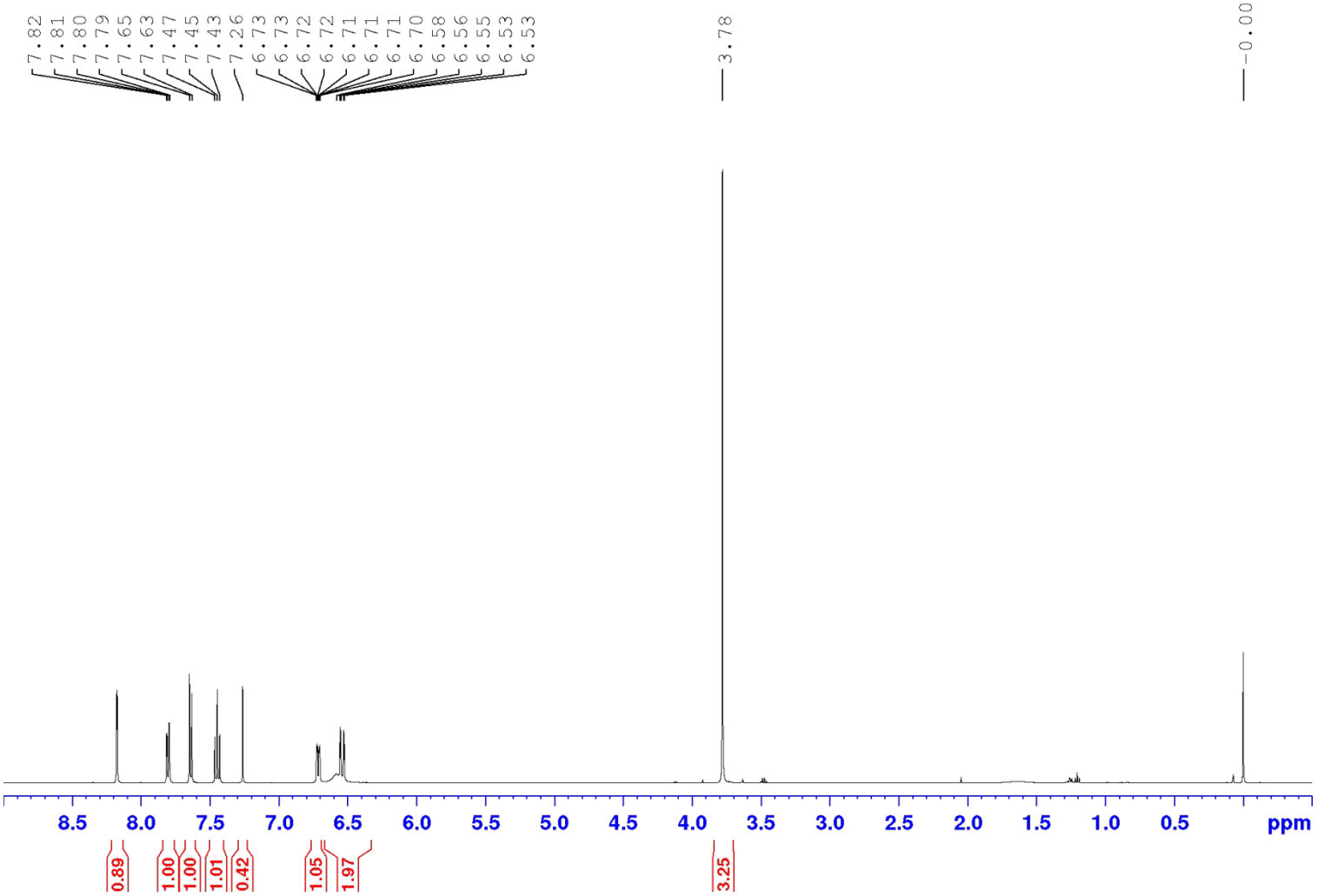


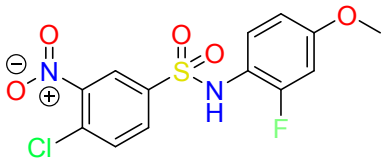
Compound **13** – ^{13}C NMR (125.5 MHz) – DMSO-d_6



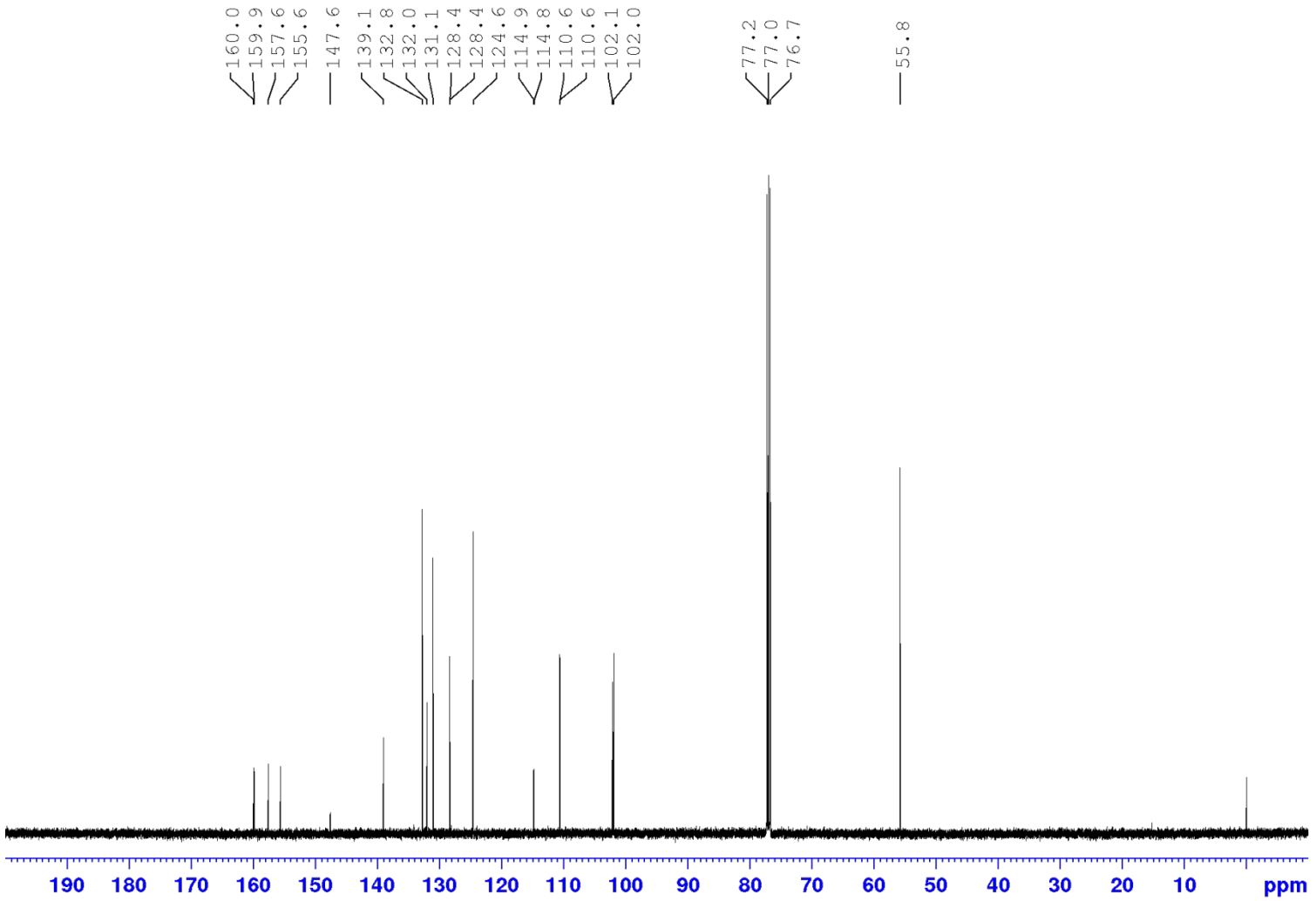


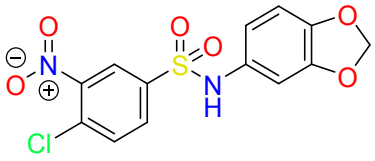
Compound **14** – ^{1}H NMR (500 MHz) – $CDCl_3$



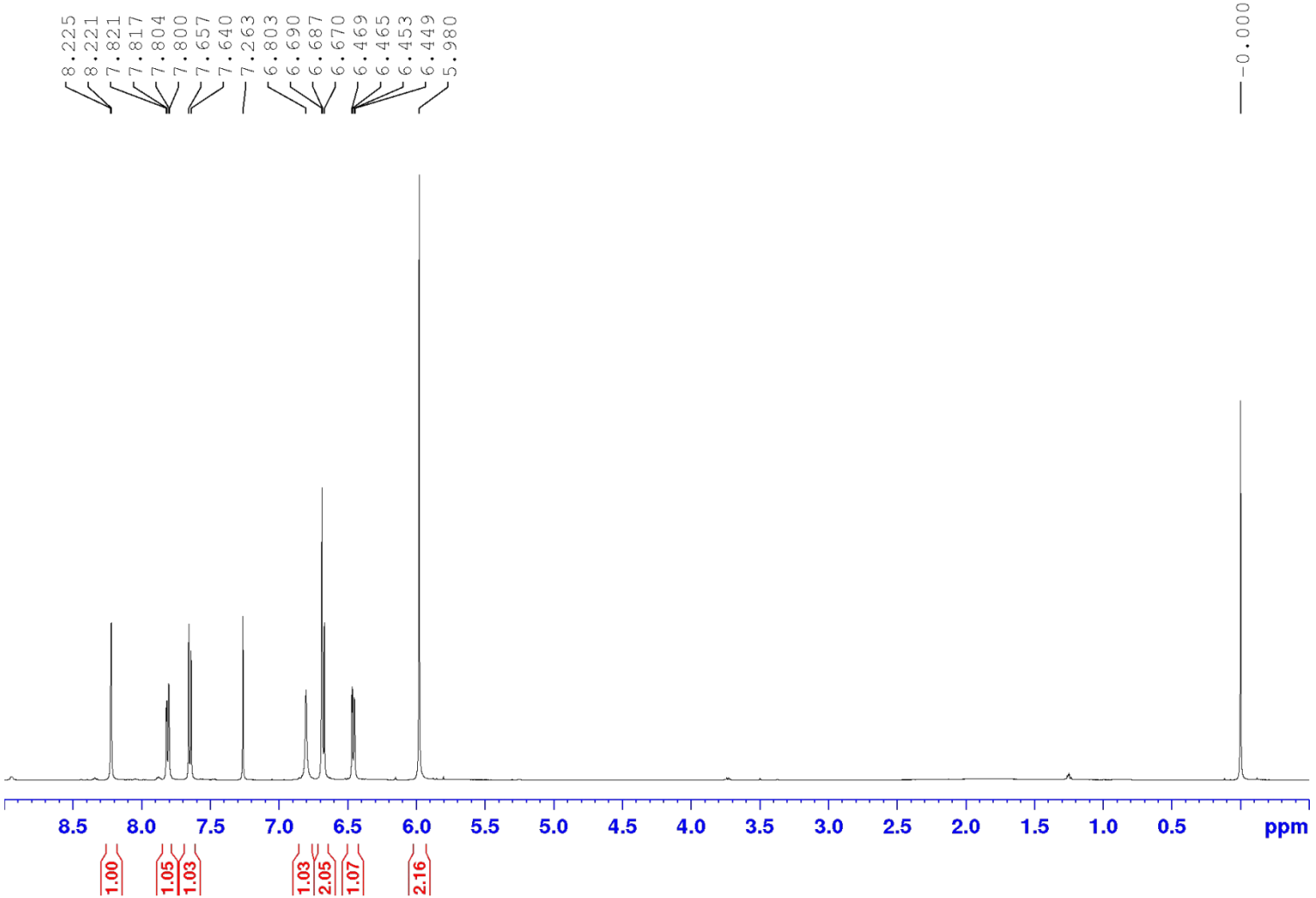


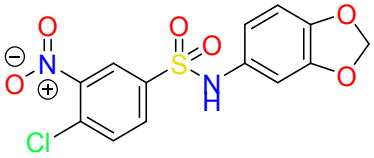
Compound **14** – ^{13}C NMR (125.5 MHz) – $CDCl_3$



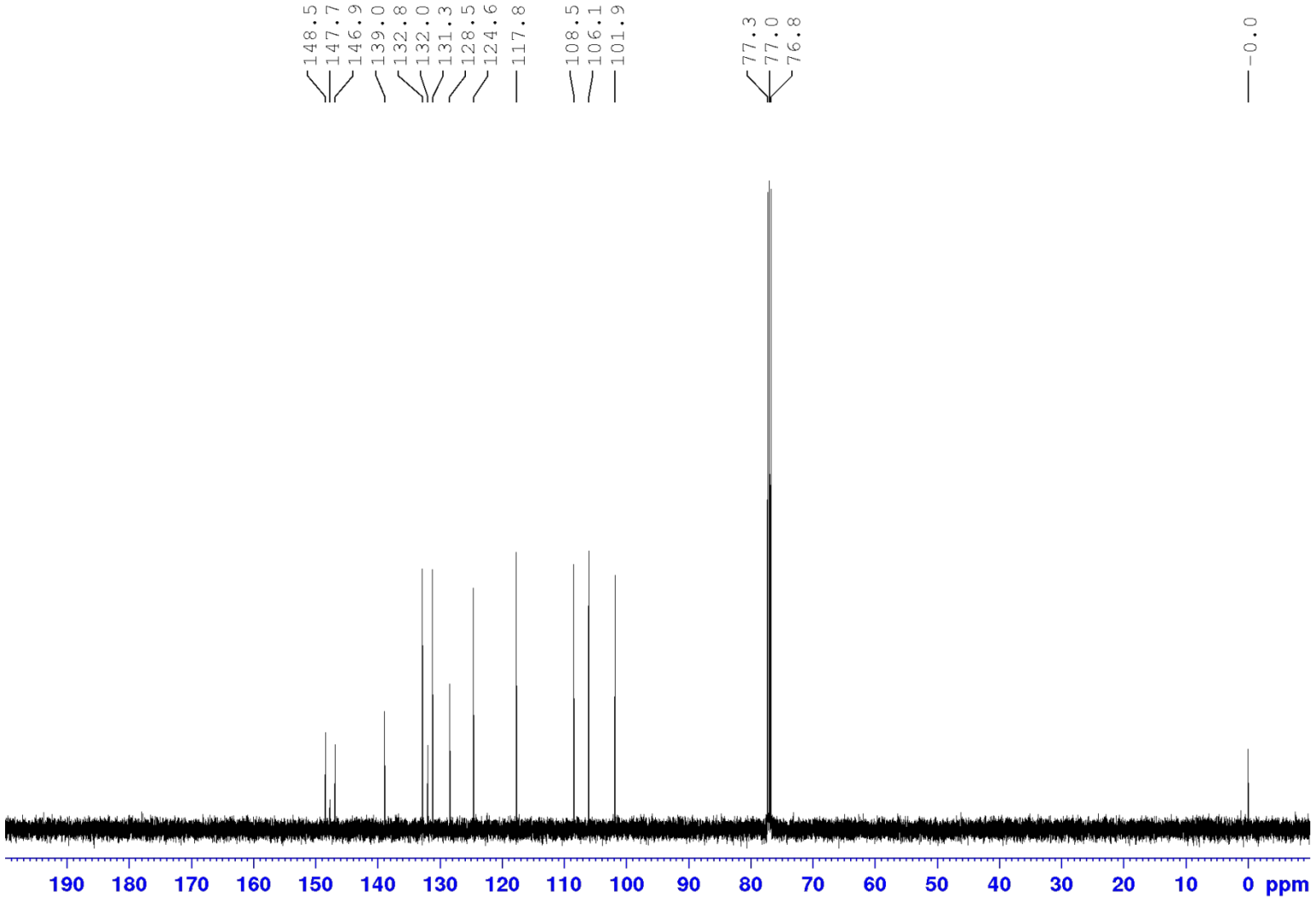


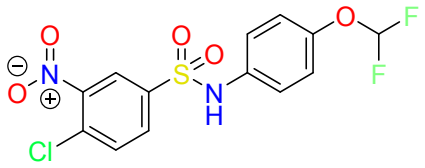
Compound **15** – ^{1}H NMR (500 MHz) – $CDCl_3$



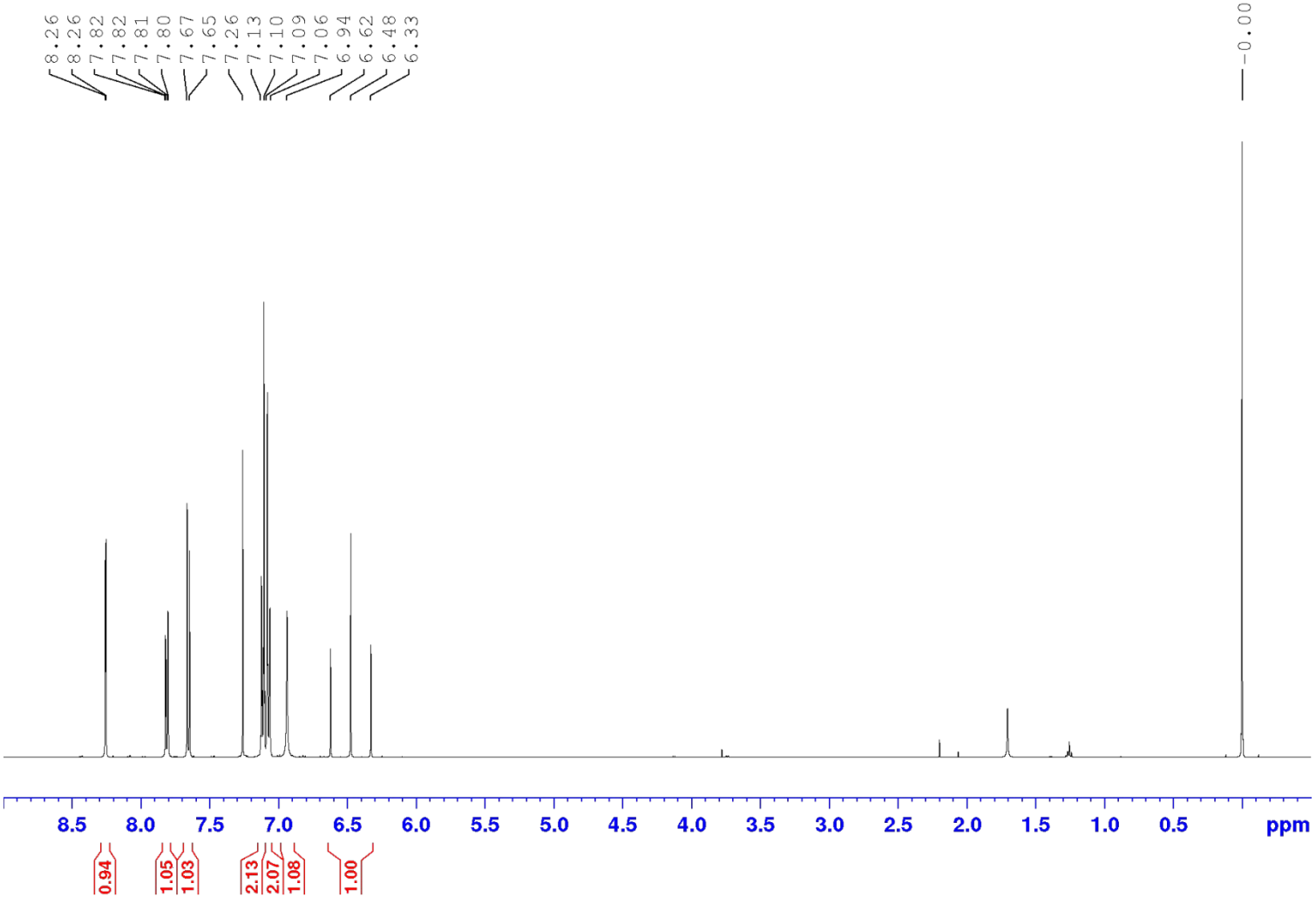


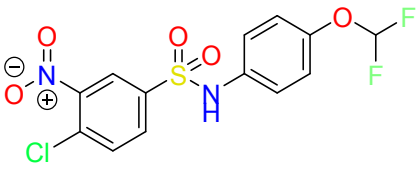
Compound **15** – ^{13}C NMR (125.5 MHz) – $CDCl_3$



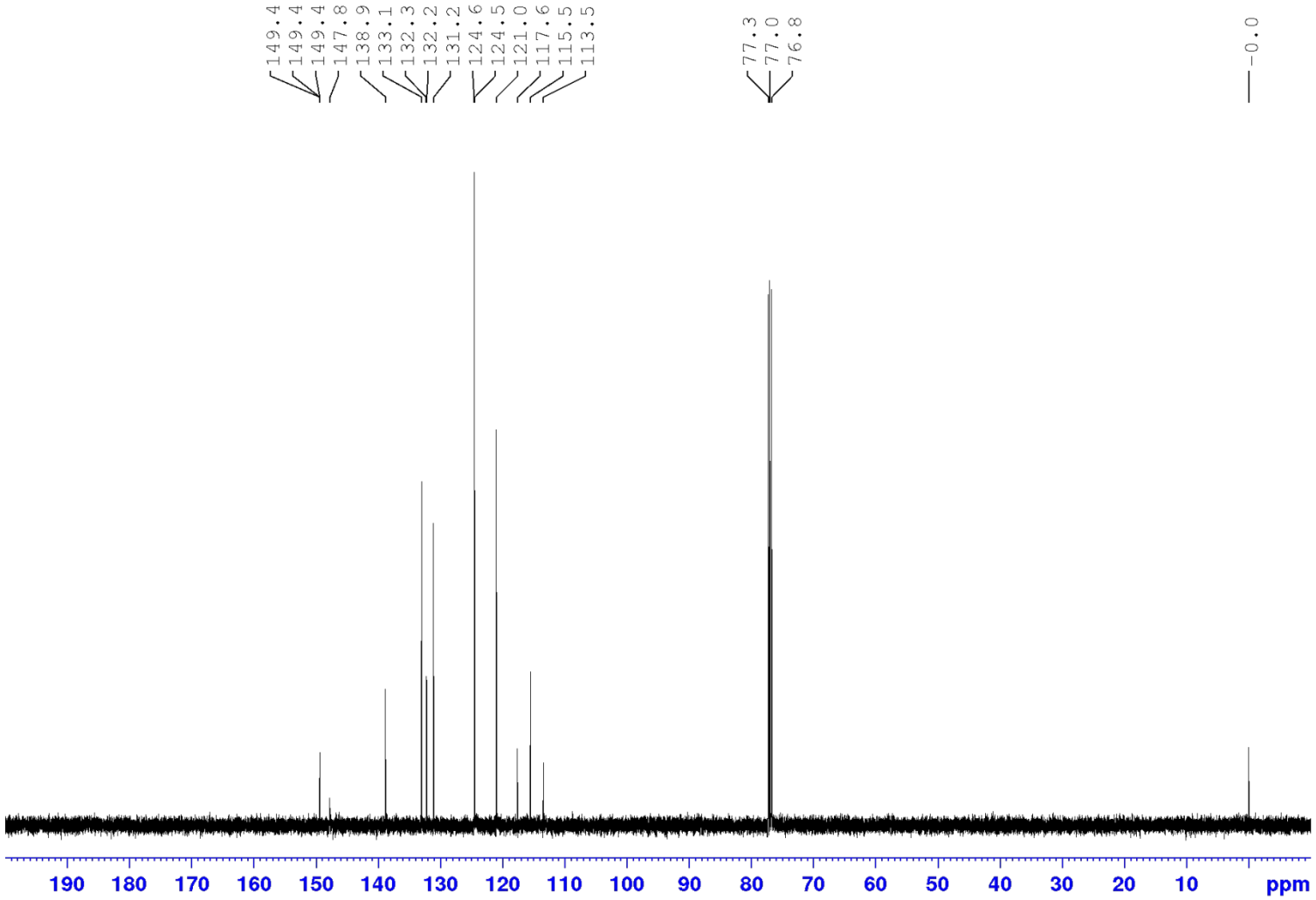


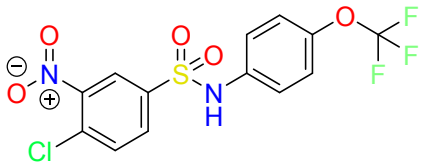
Compound **16** – ^{1}H NMR (500 MHz) – $CDCl_3$



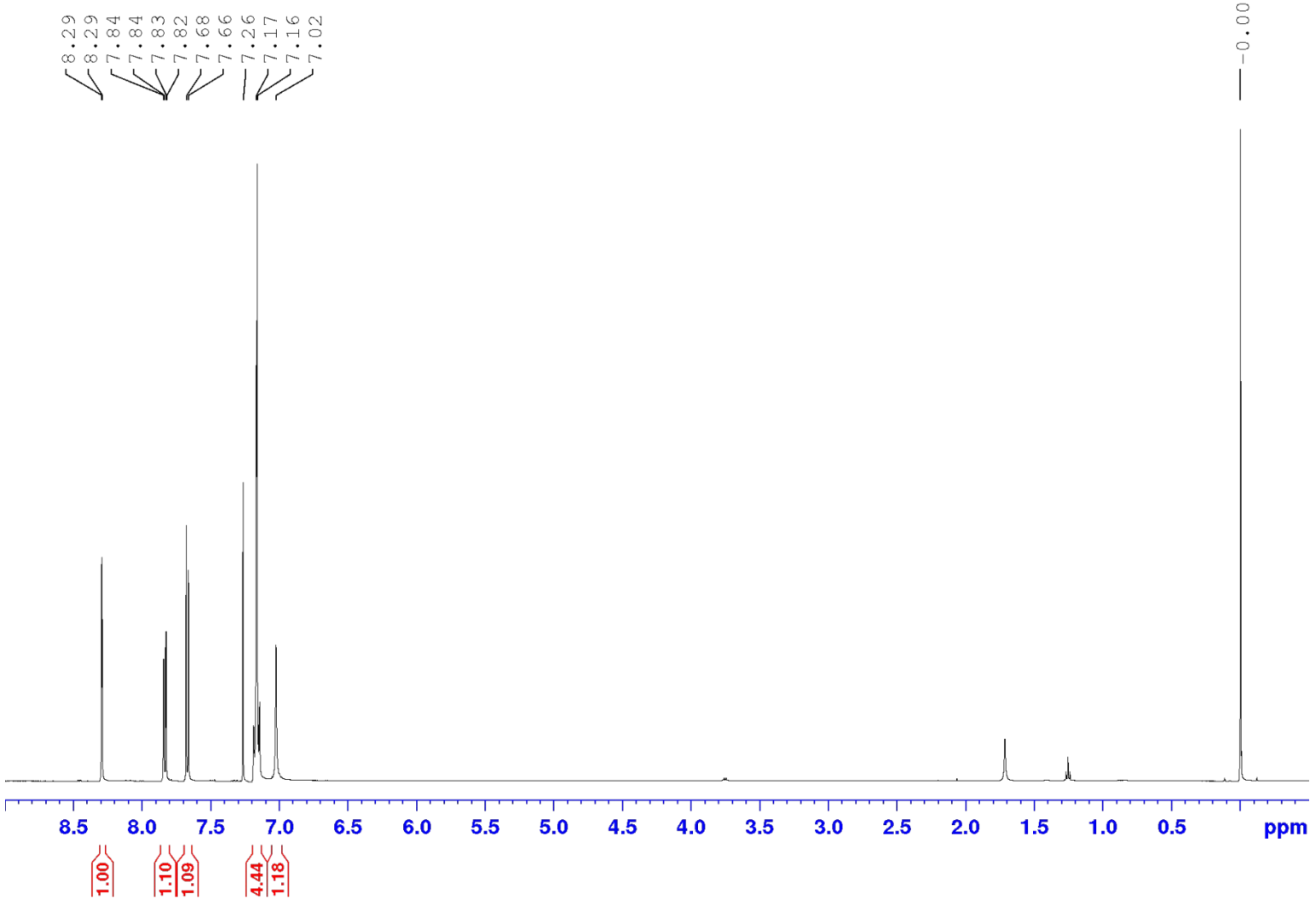


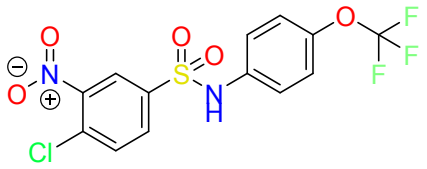
Compound **16** – ^{13}C NMR (125.5 MHz) – $CDCl_3$



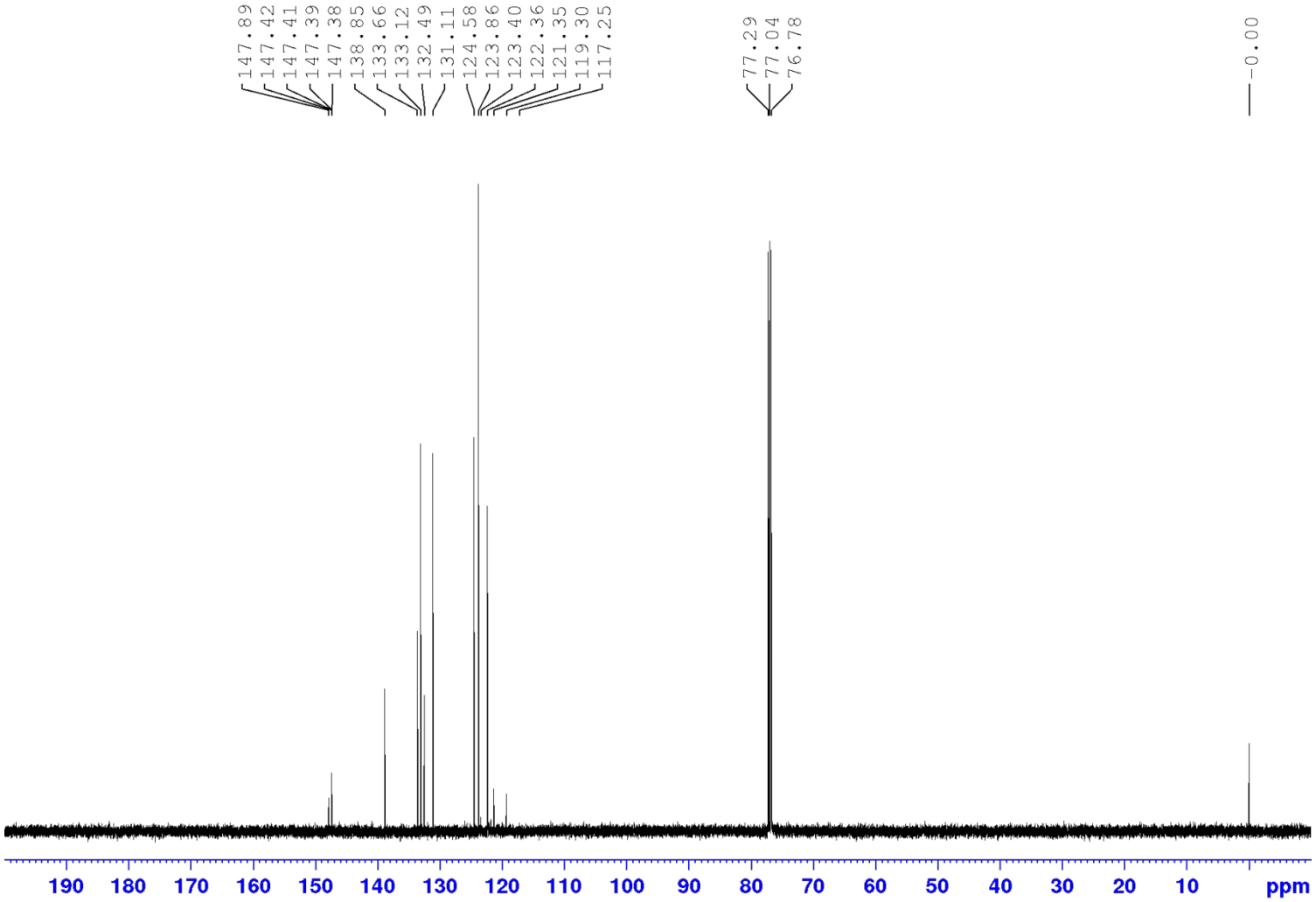


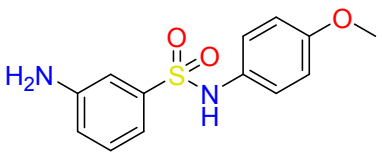
Compound **17** – ^{1}H NMR (500 MHz) – $CDCl_3$



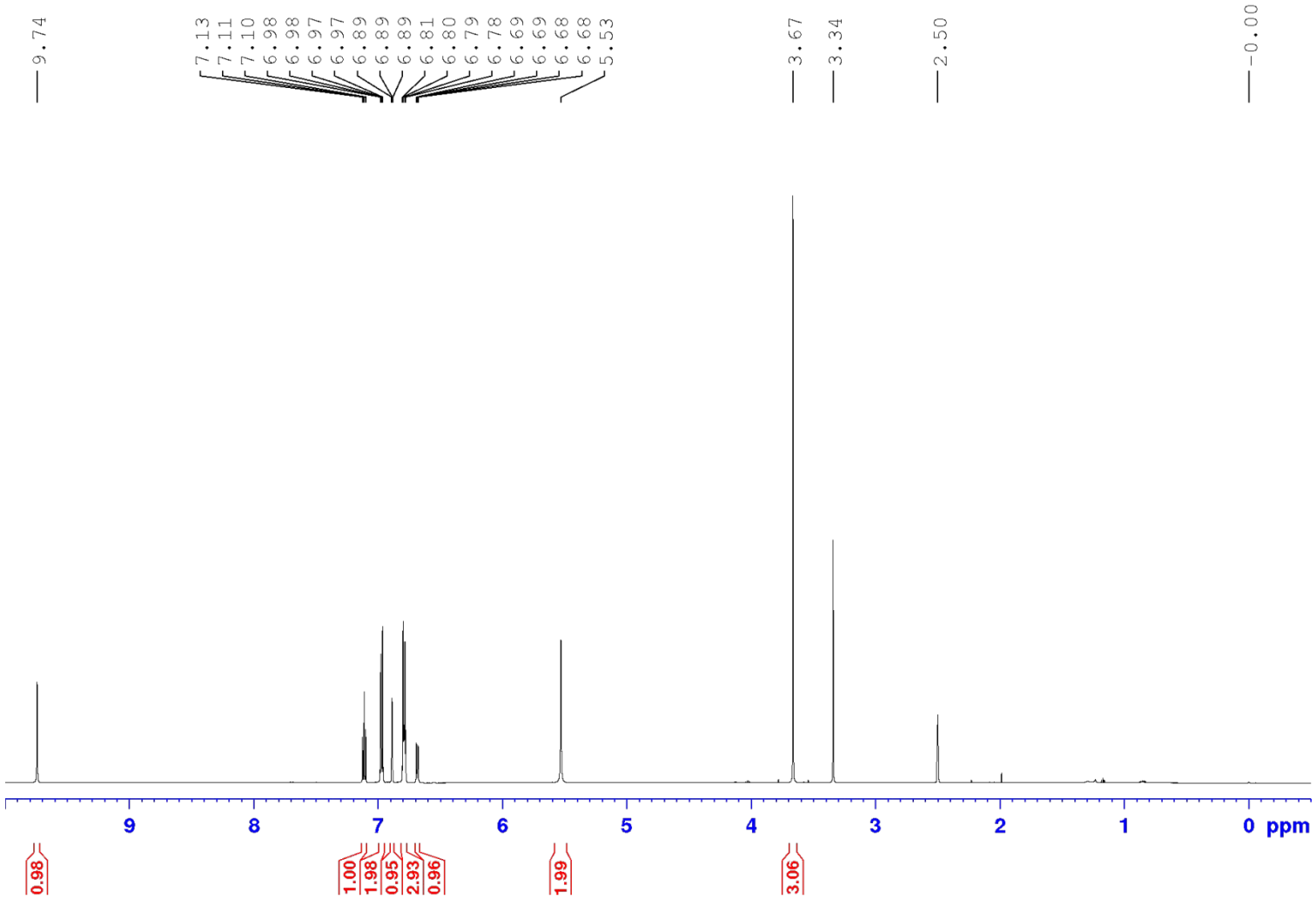


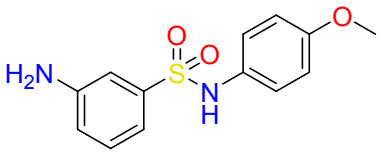
Compound **17** – ^{13}C NMR (125.5 MHz) – $CDCl_3$



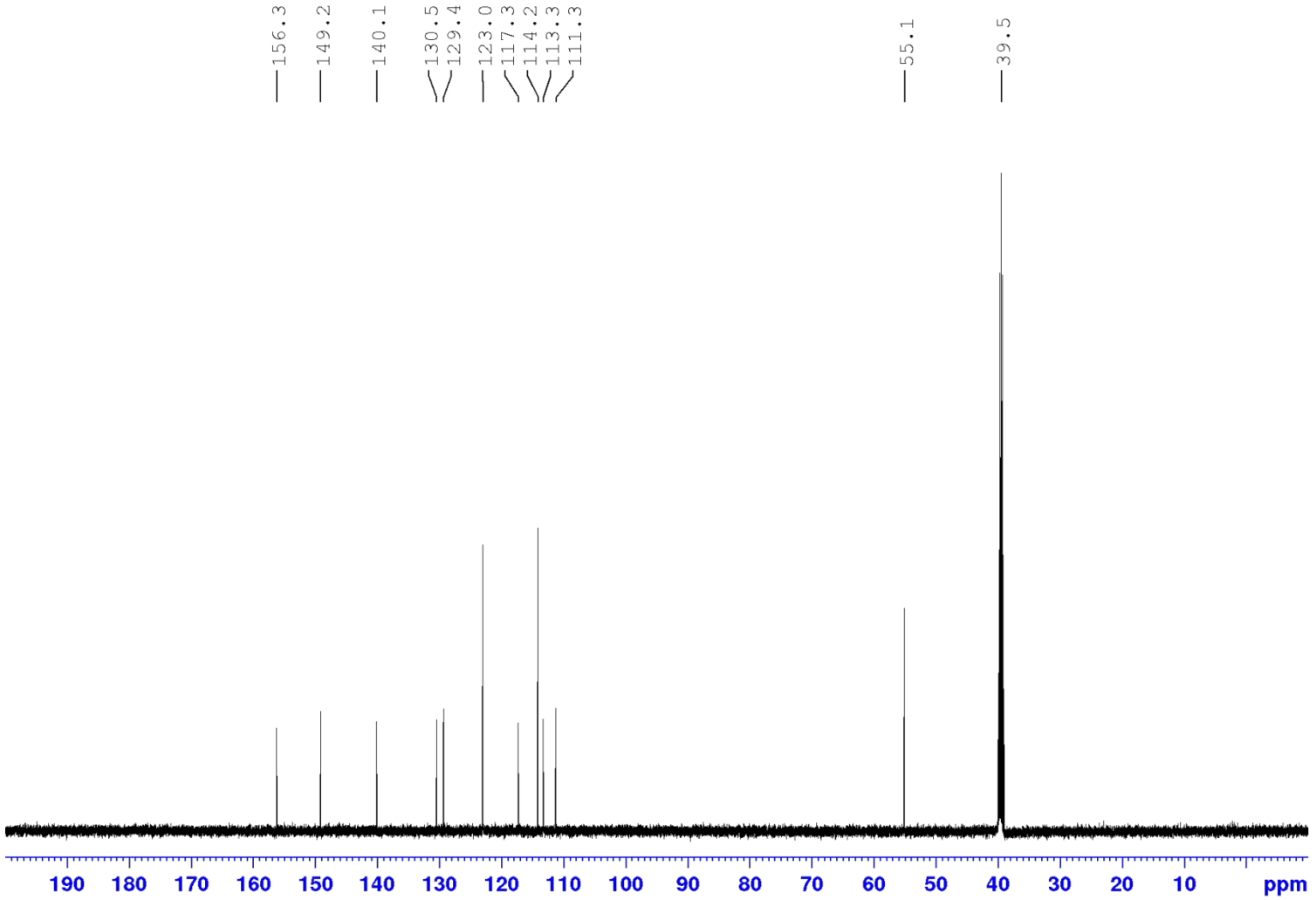


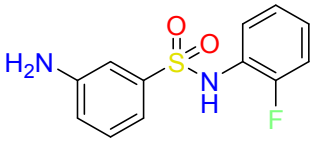
Compound **18** – ^{1}H NMR (600 MHz) – DMSO-d_6



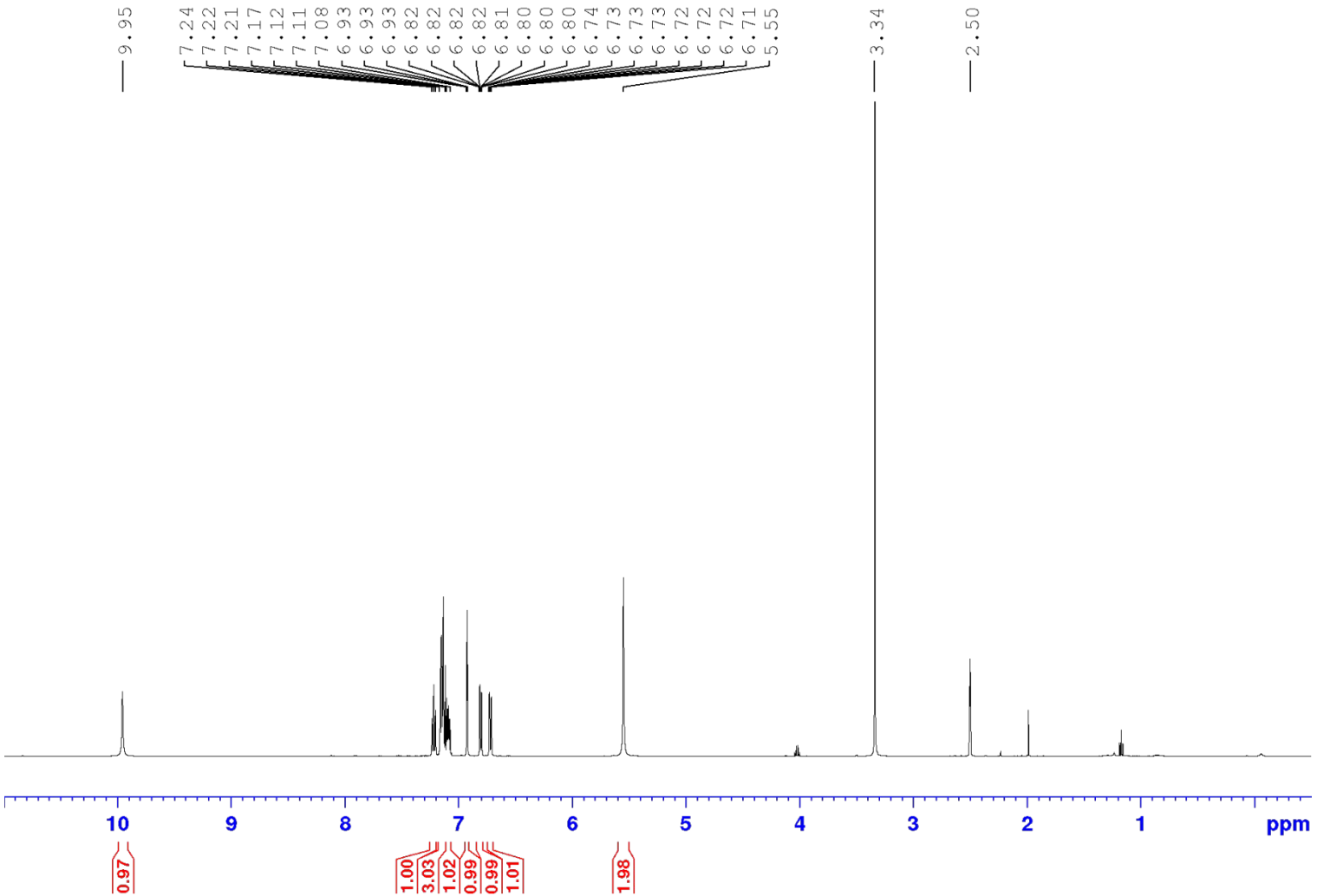


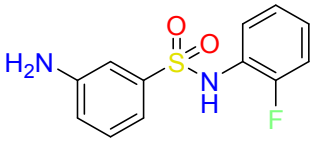
Compound **18** – ^{13}C NMR (150 MHz) – DMSO-d_6



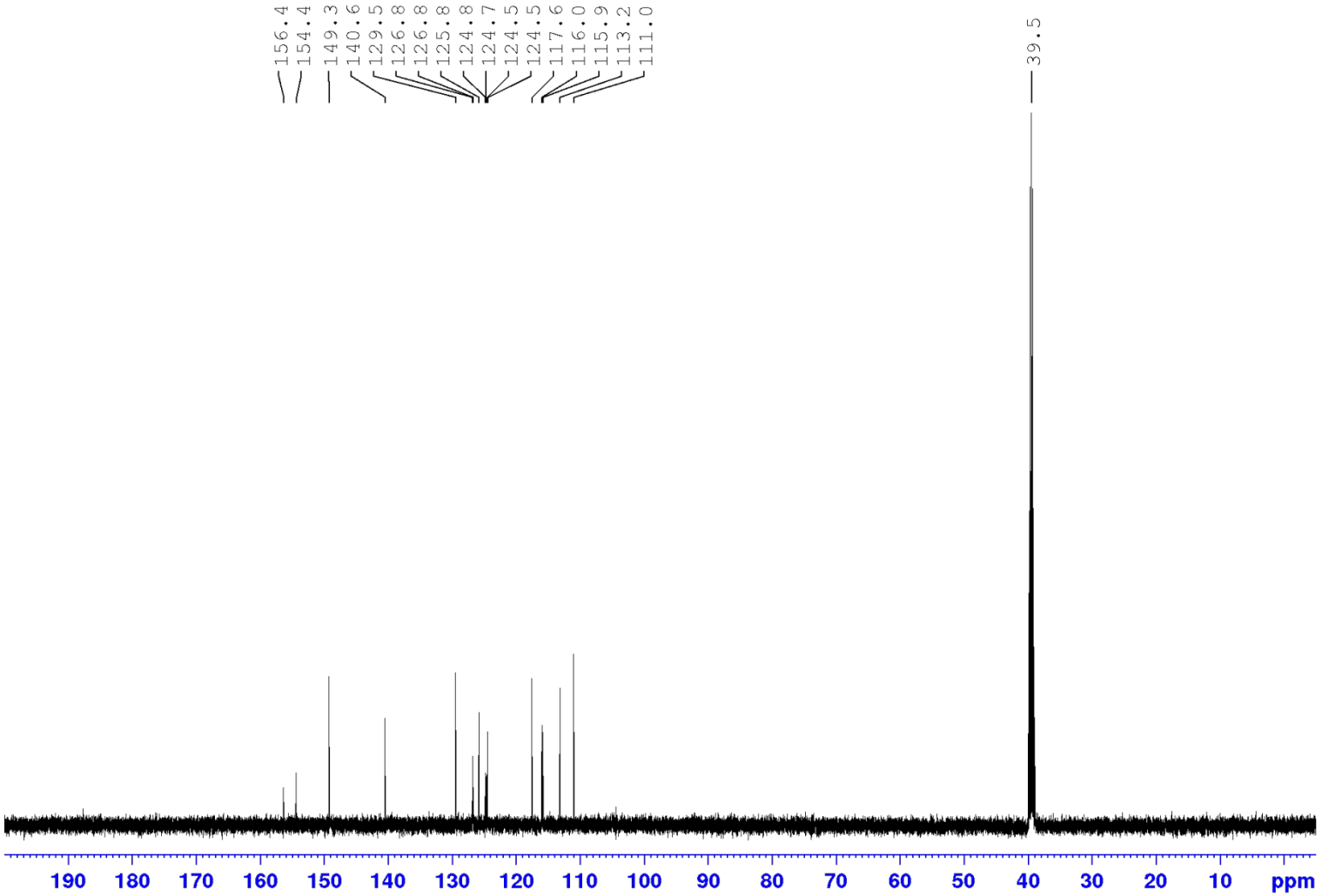


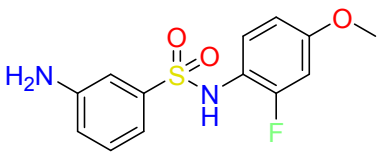
Compound **19** – ^{1}H NMR (500 MHz) – DMSO-d_6



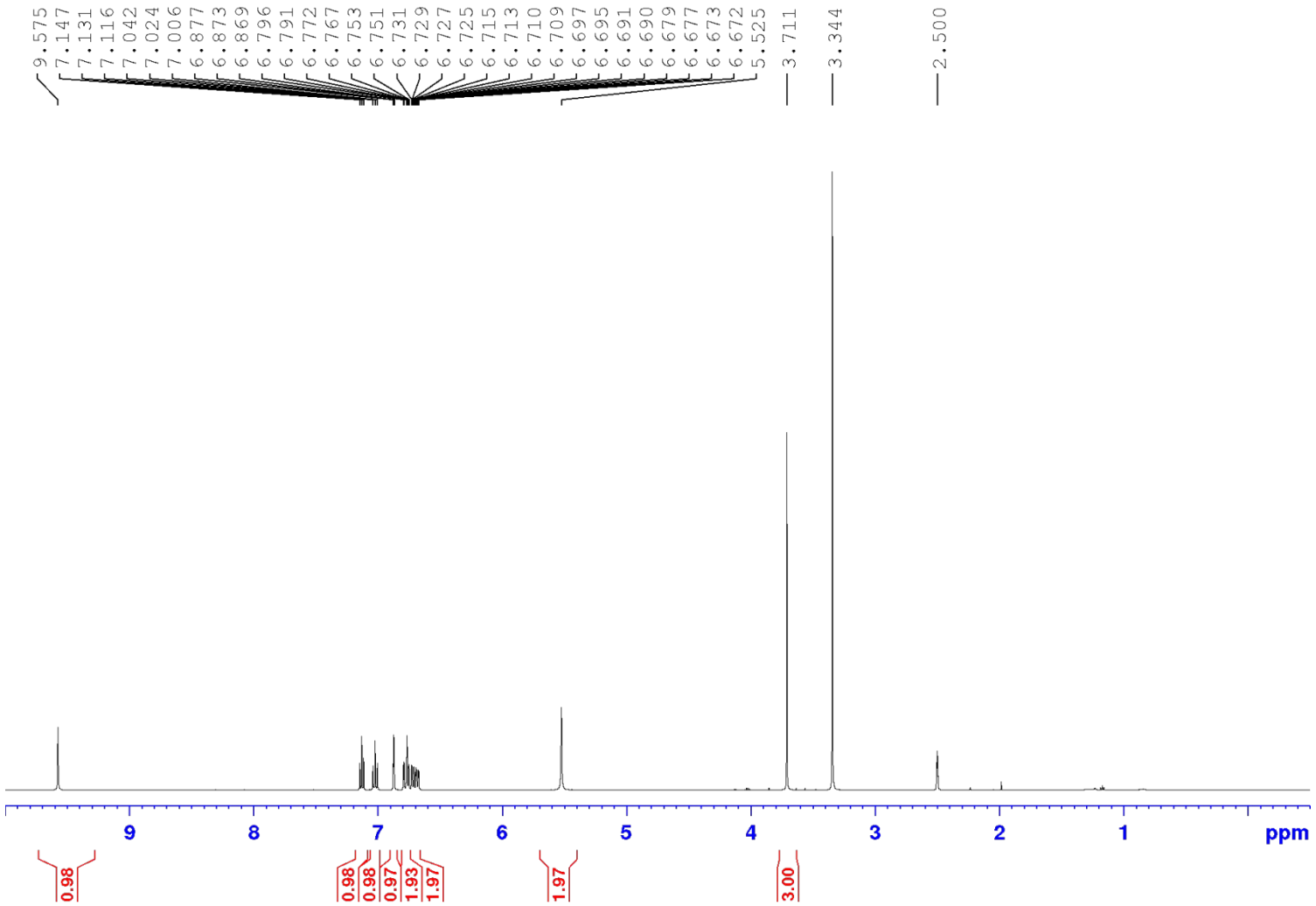


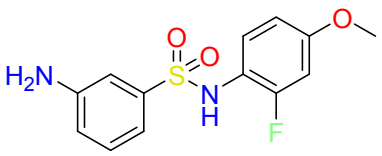
Compound **19** – ^{13}C NMR (125.5 MHz) – DMSO-d_6



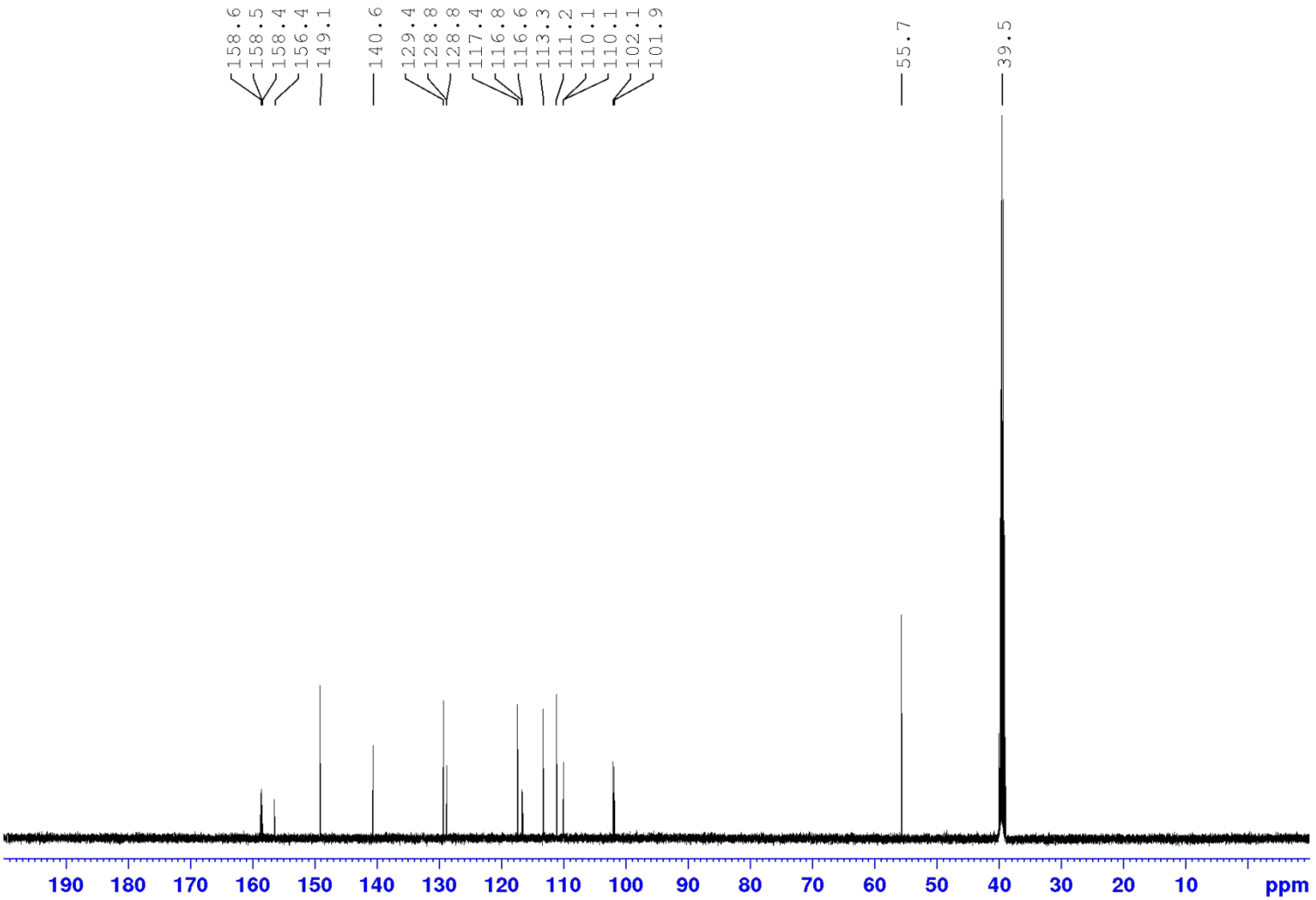


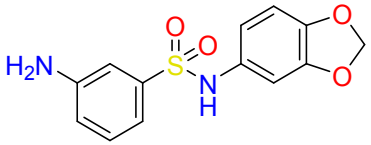
Compound **20** – ^{1}H NMR (500 MHz) – DMSO-d_6



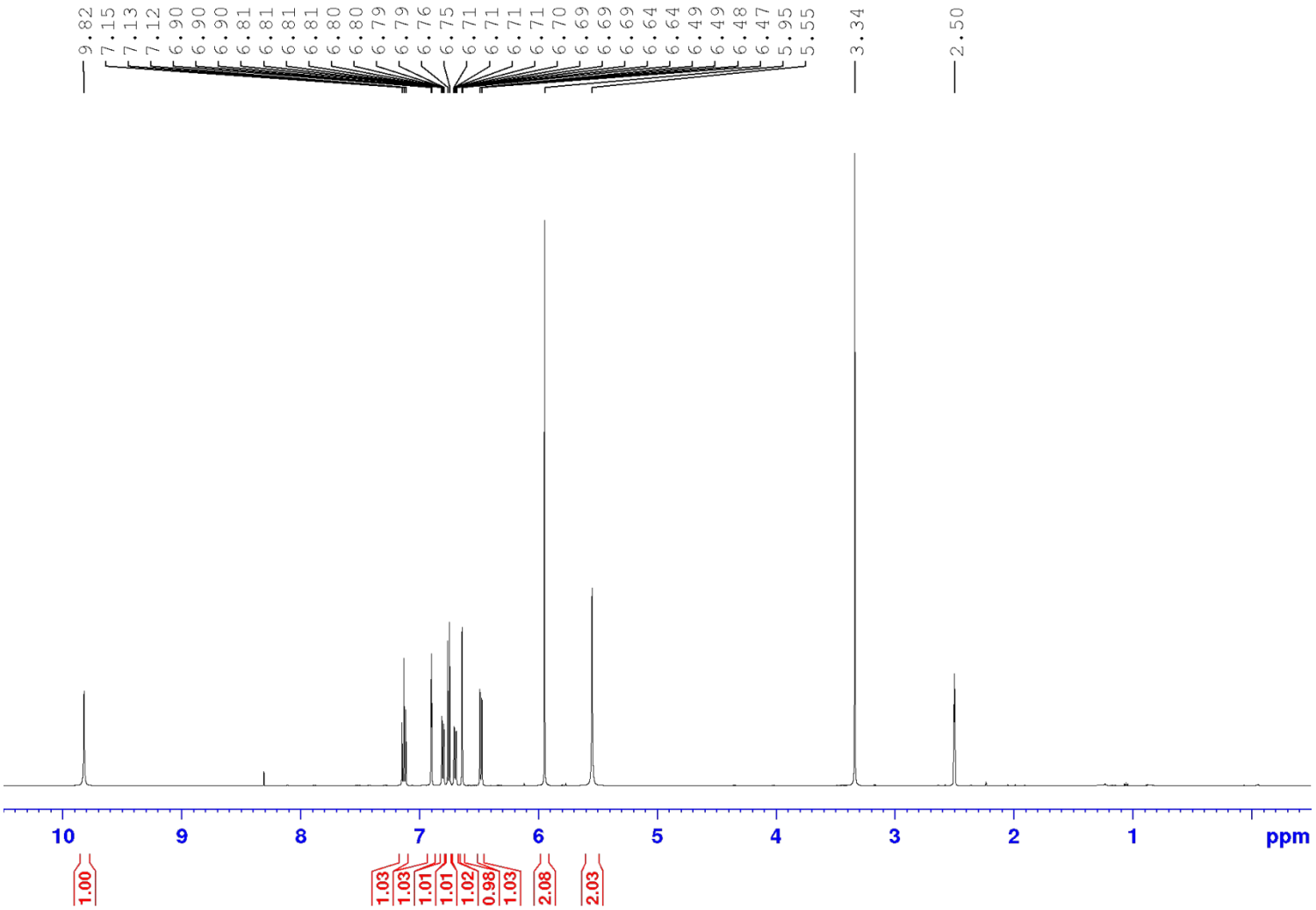


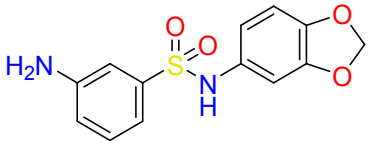
Compound **20** – ^{13}C NMR (125.5 MHz) – DMSO-d_6



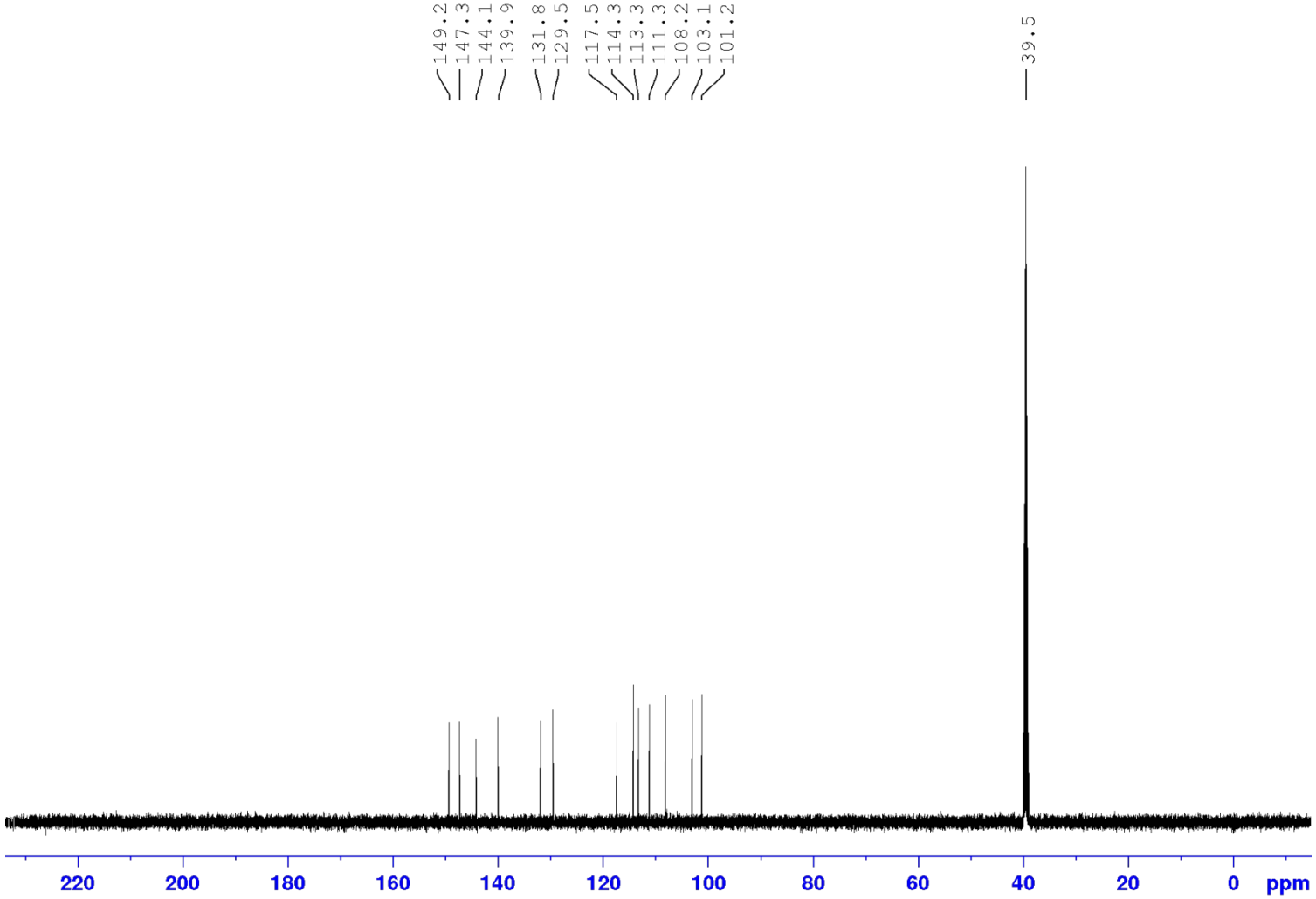


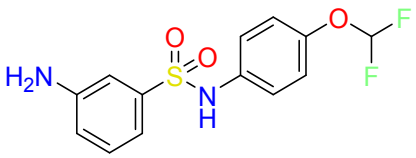
Compound **21** – ^{1}H NMR (500 MHz) – DMSO-d_6



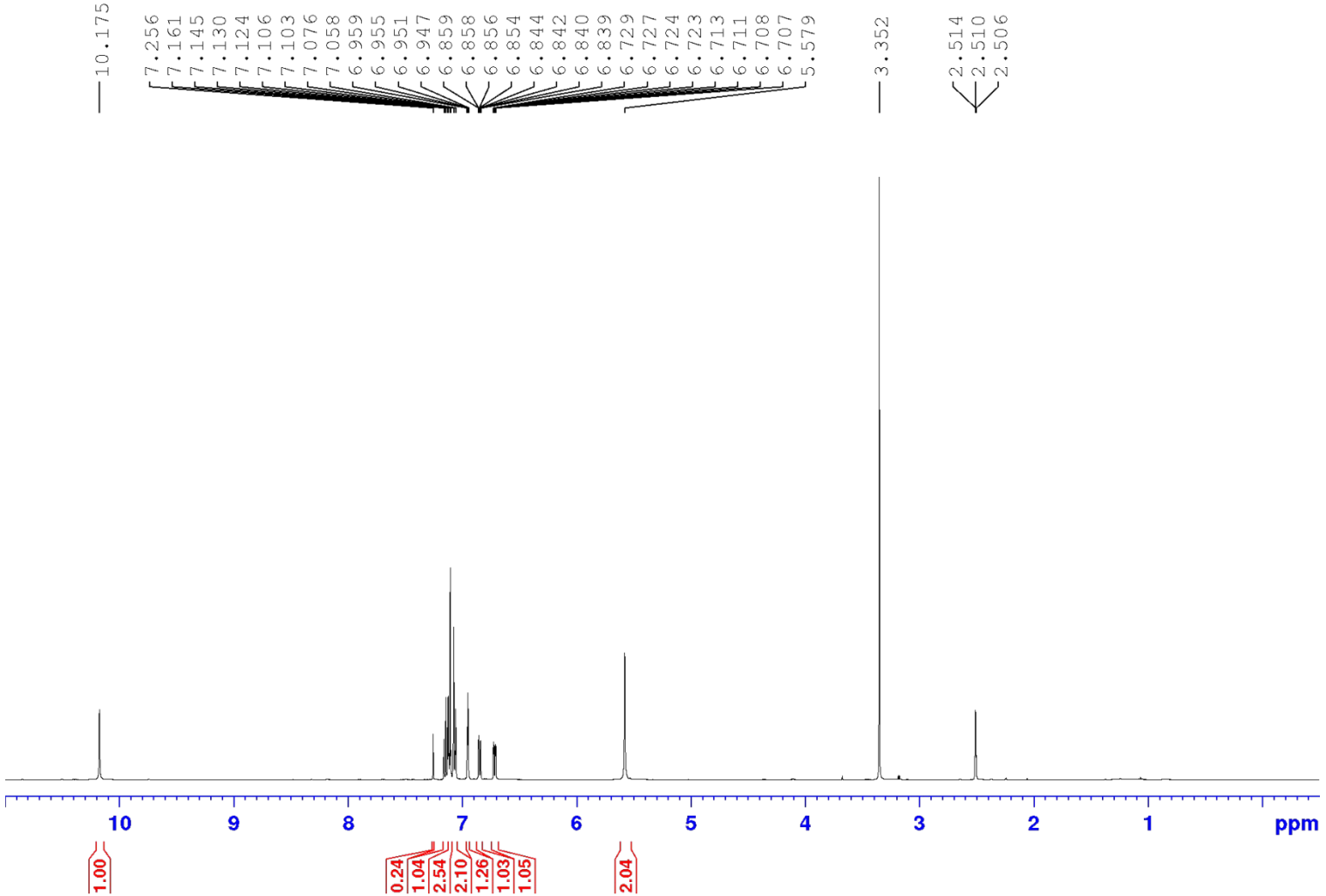


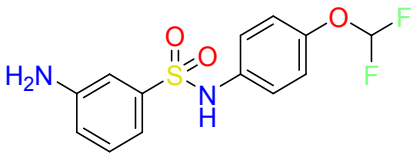
Compound **21** – ^{13}C NMR (125.5 MHz) – DMSO-d_6



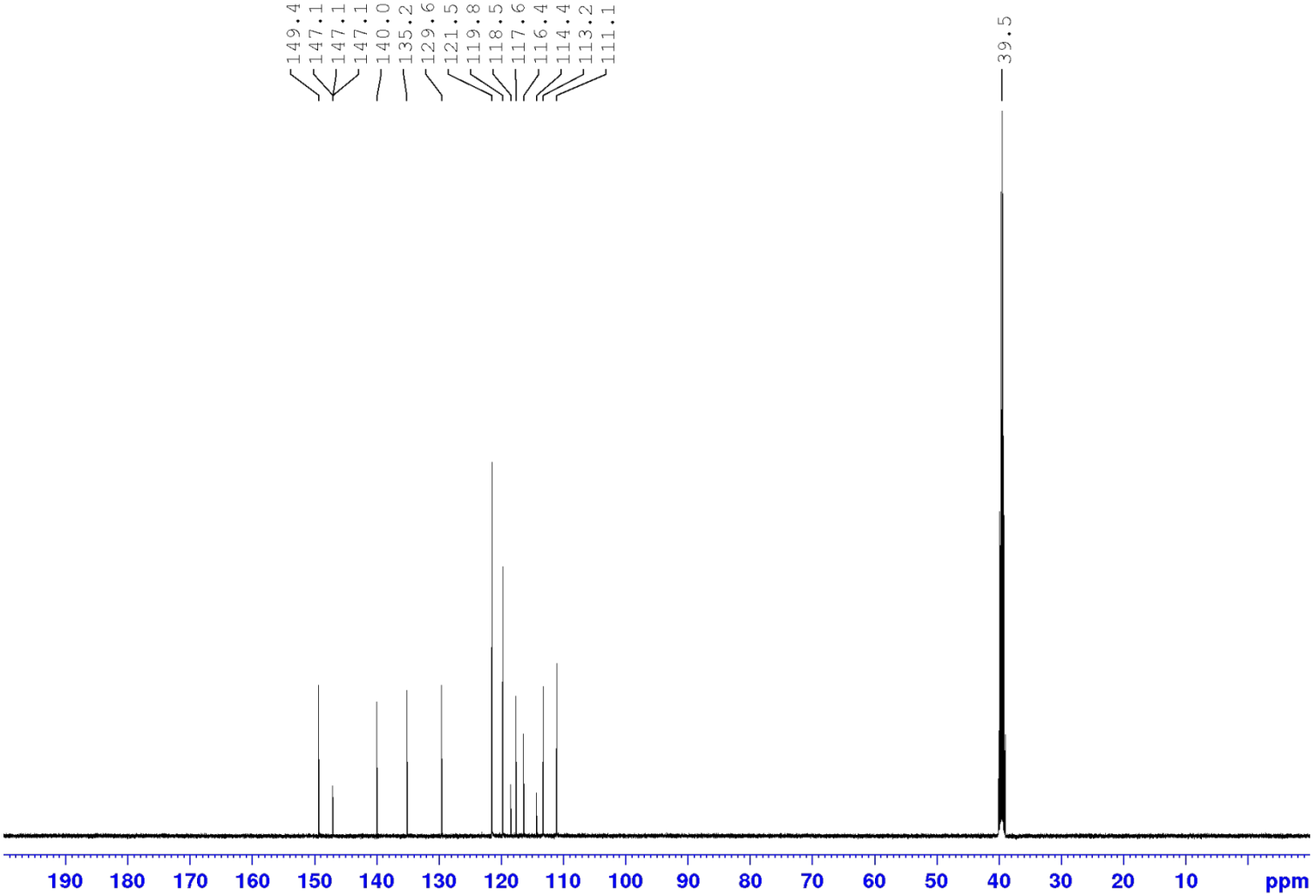


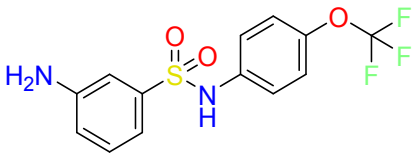
Compound **22** – ^{1}H NMR (500 MHz) – DMSO-d_6



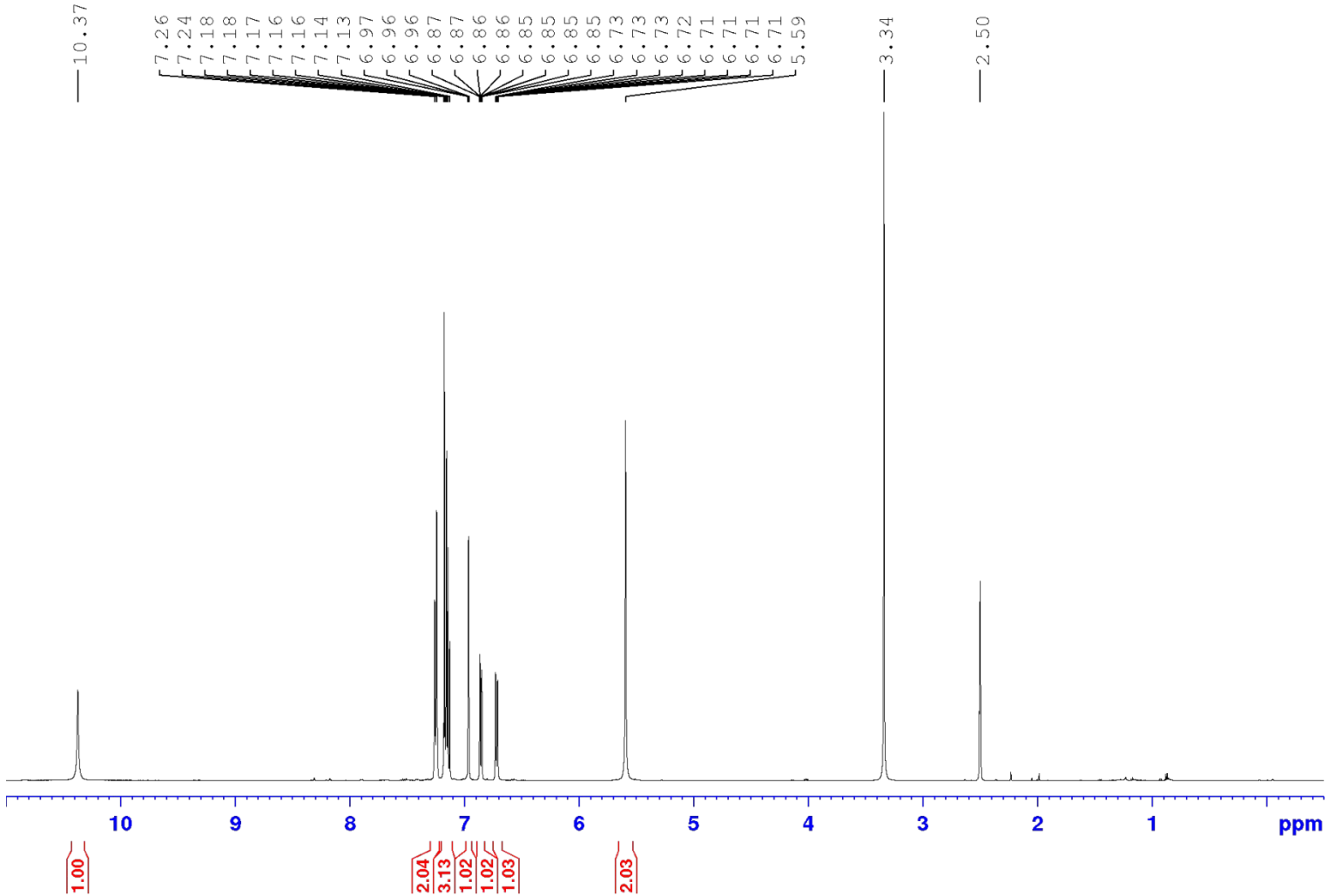


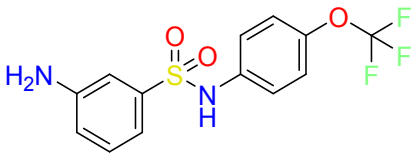
Compound **22** – ^{13}C NMR (125.5 MHz) – DMSO-d_6



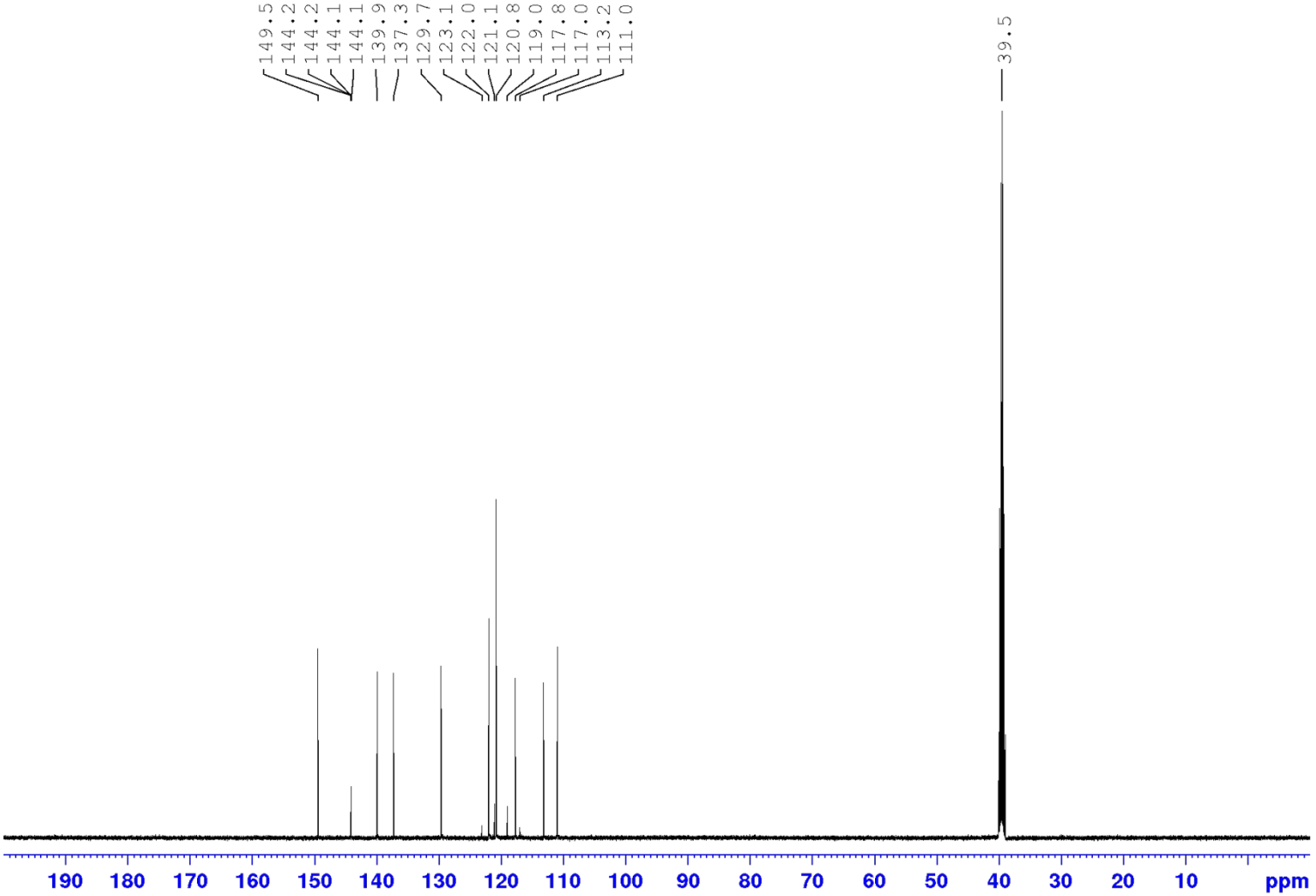


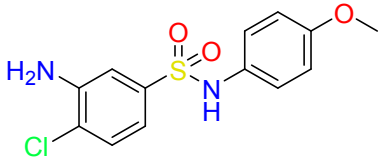
Compound **23** – ^{1}H NMR (500 MHz) – DMSO-d_6



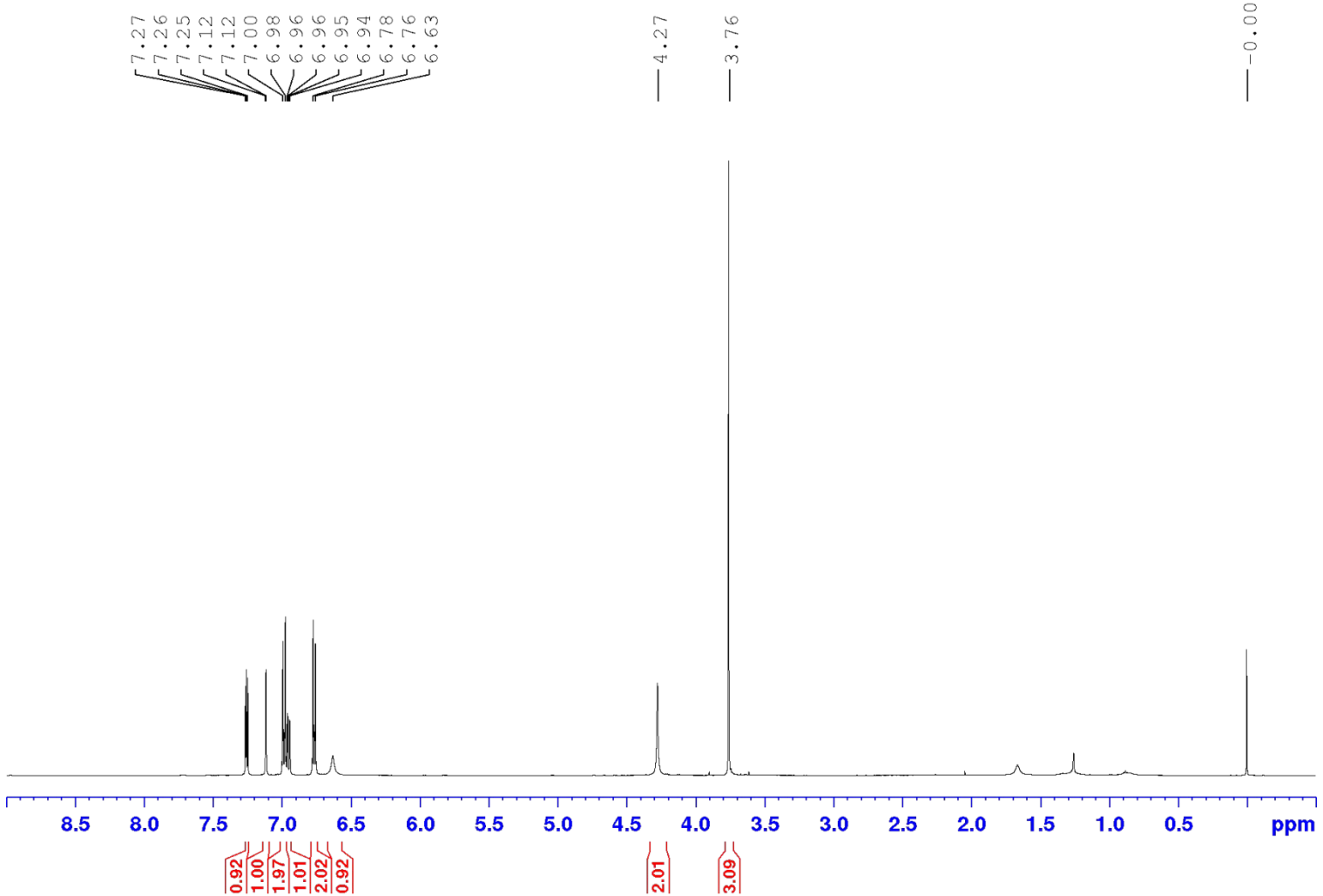


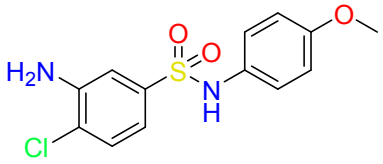
Compound **23** – ^{13}C NMR (125.5 MHz) – DMSO-d_6



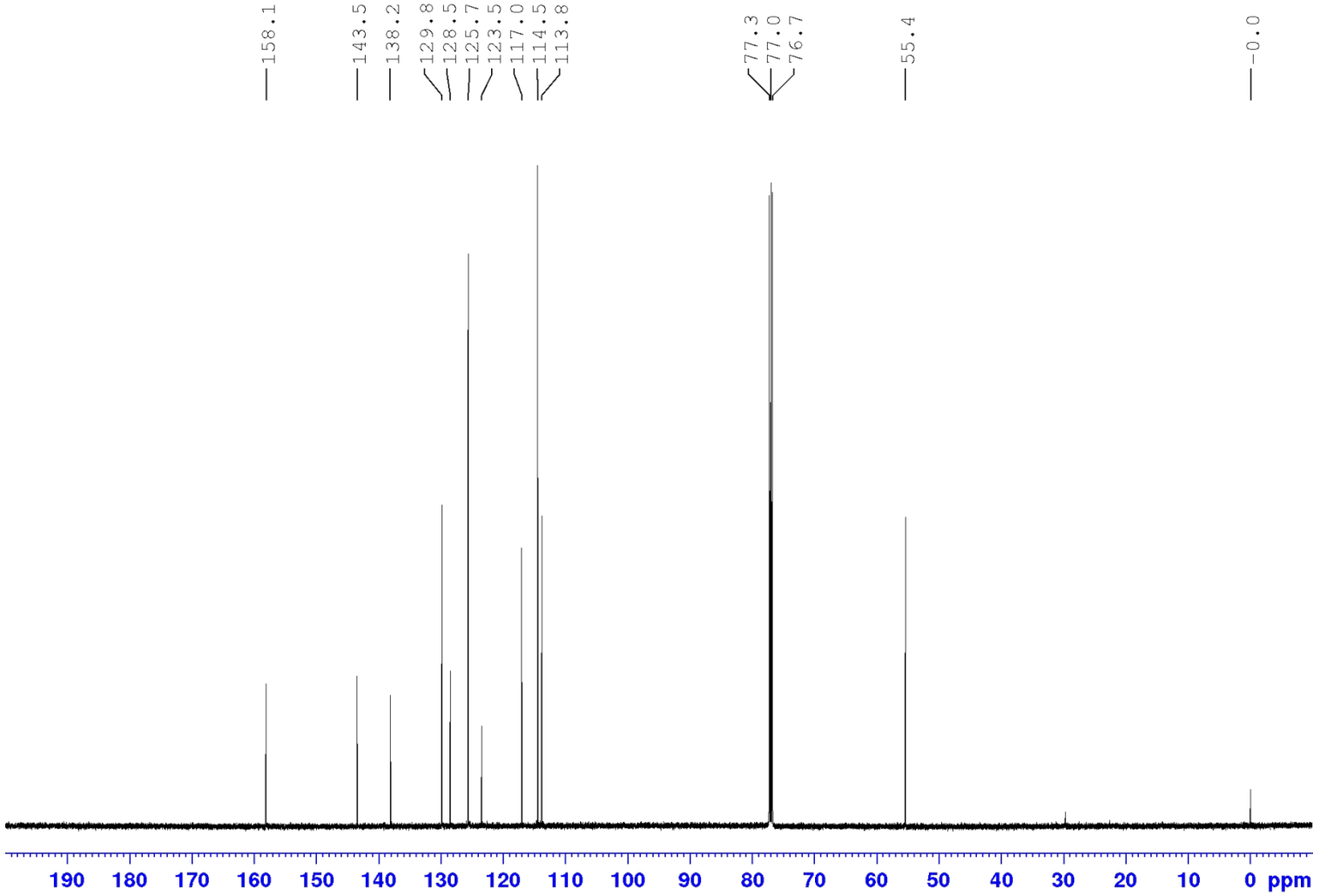


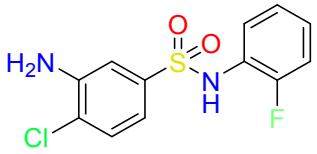
Compound **24** – ^{1}H NMR (500 MHz) – $CDCl_3$



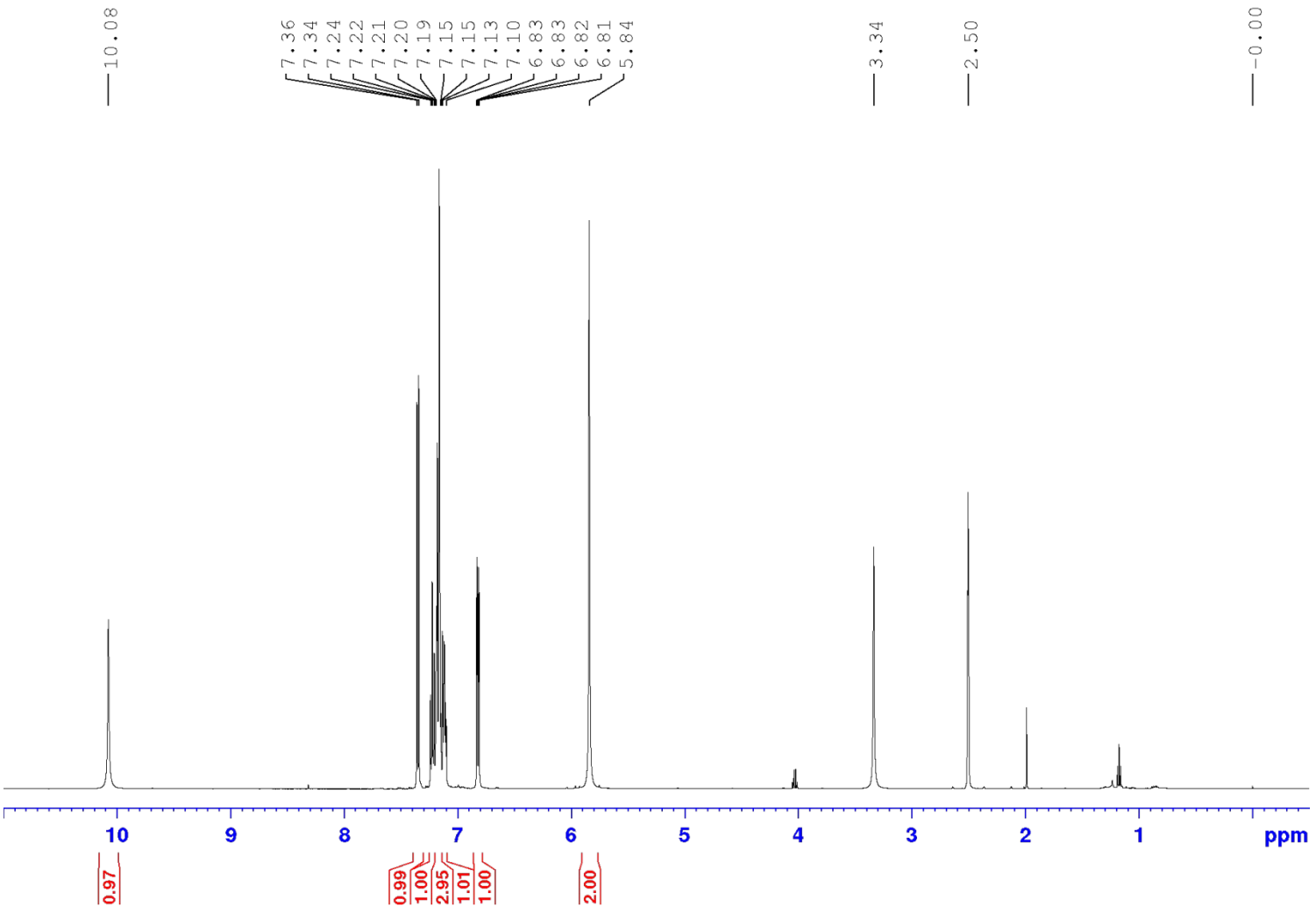


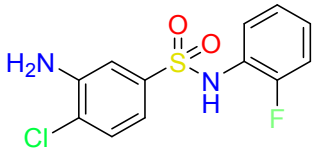
Compound **24** – ^{13}C NMR (125.5 MHz) – $CDCl_3$



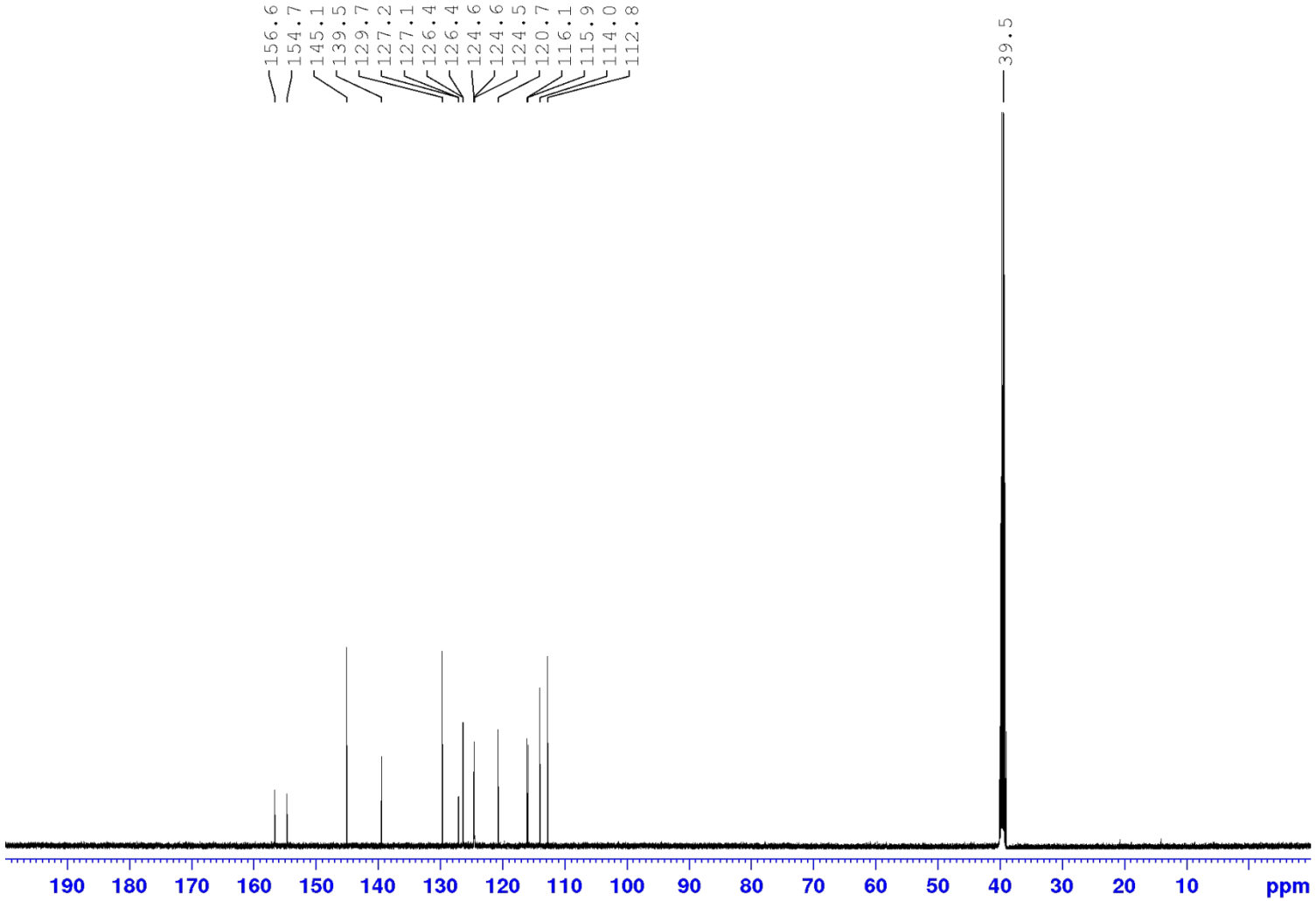


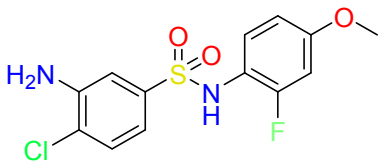
Compound **25** – ^{1}H NMR (500 MHz) – DMSO-d_6



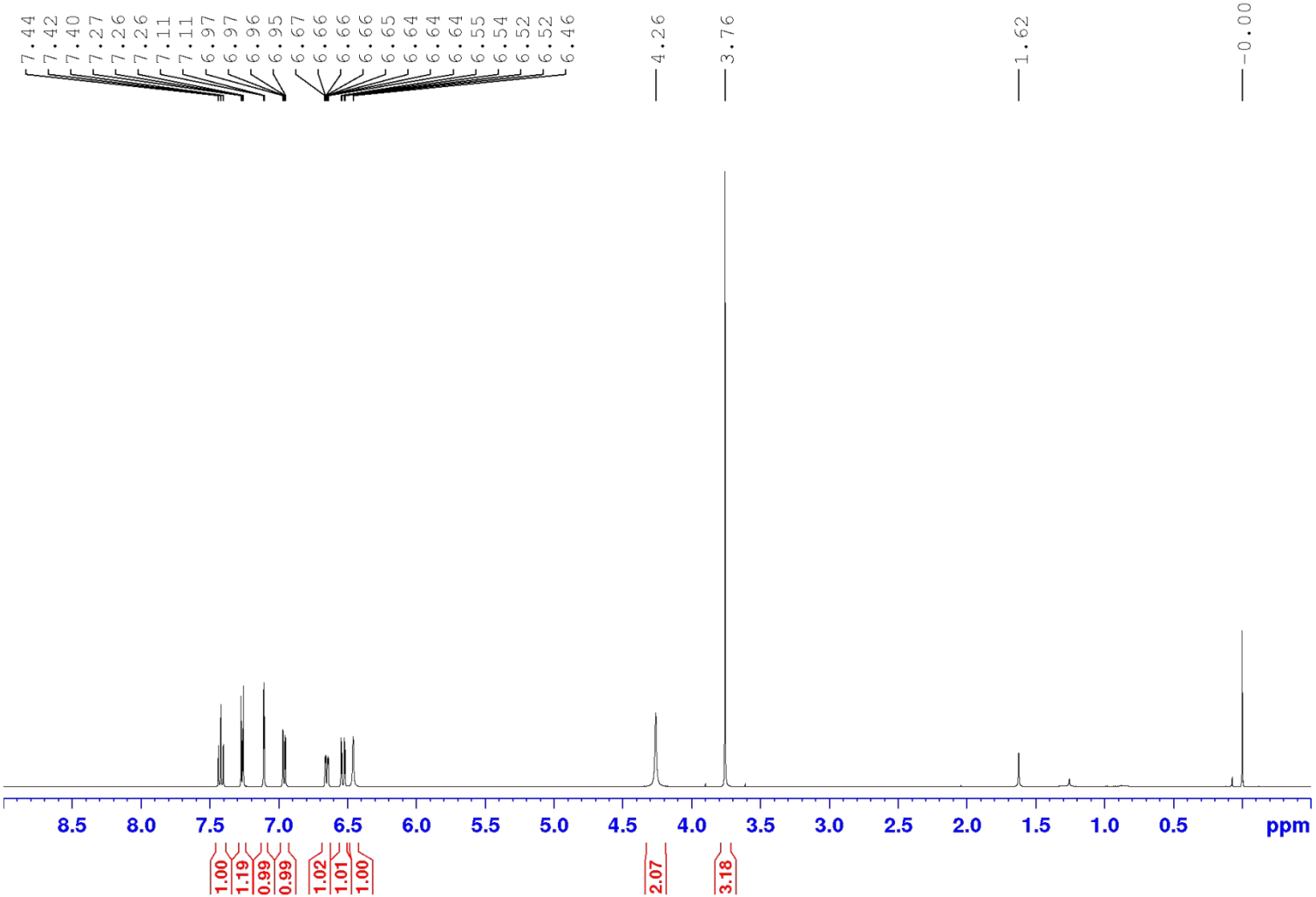


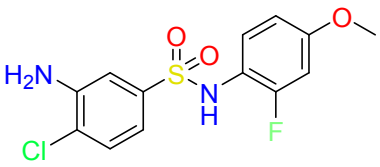
Compound **25** – ^{13}C NMR (125.5 MHz) – DMSO-d_6



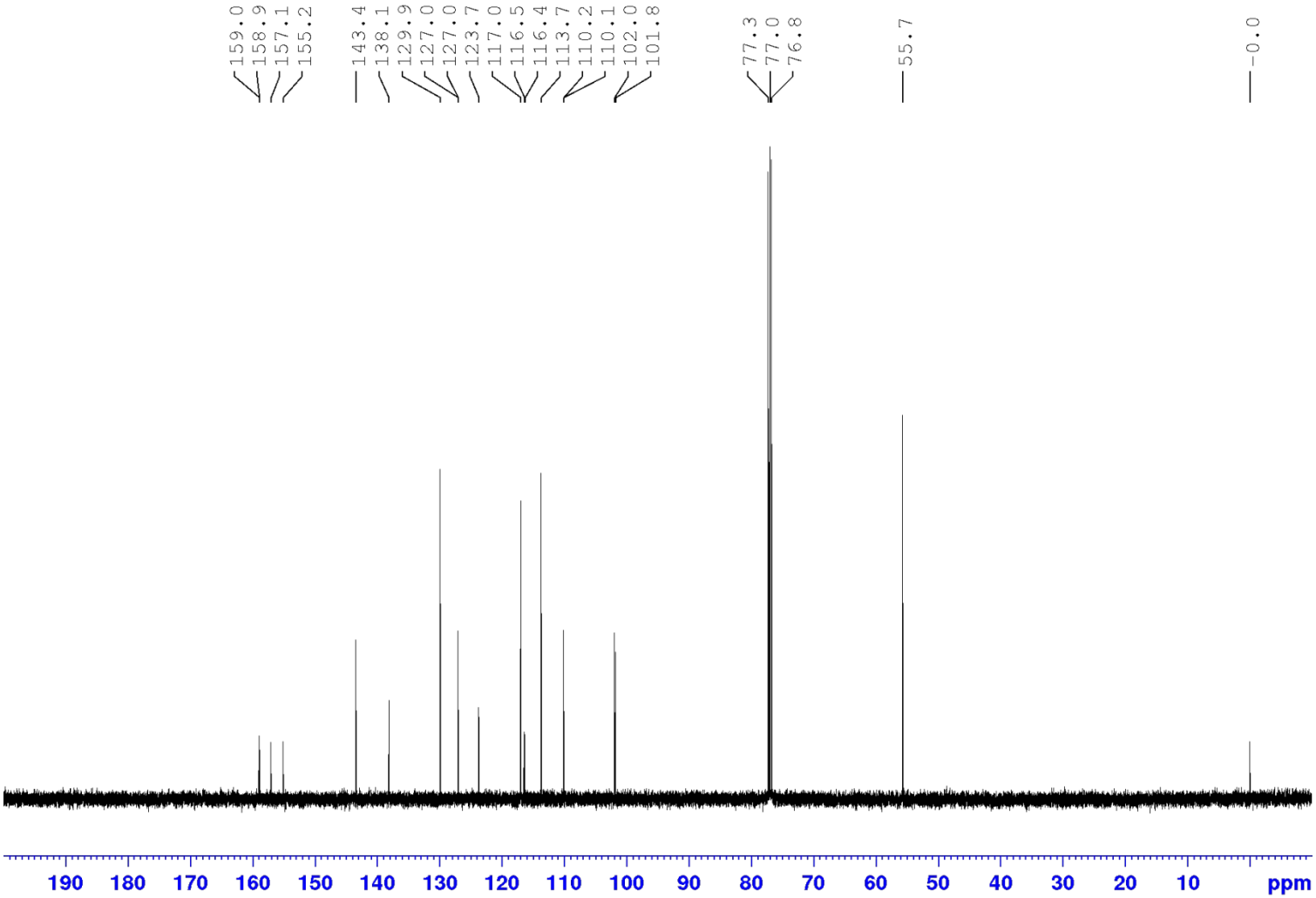


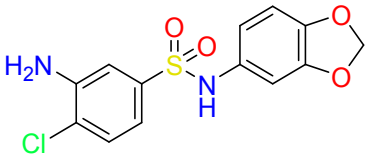
Compound **26** – ^{1}H NMR (500 MHz) – $CDCl_3$



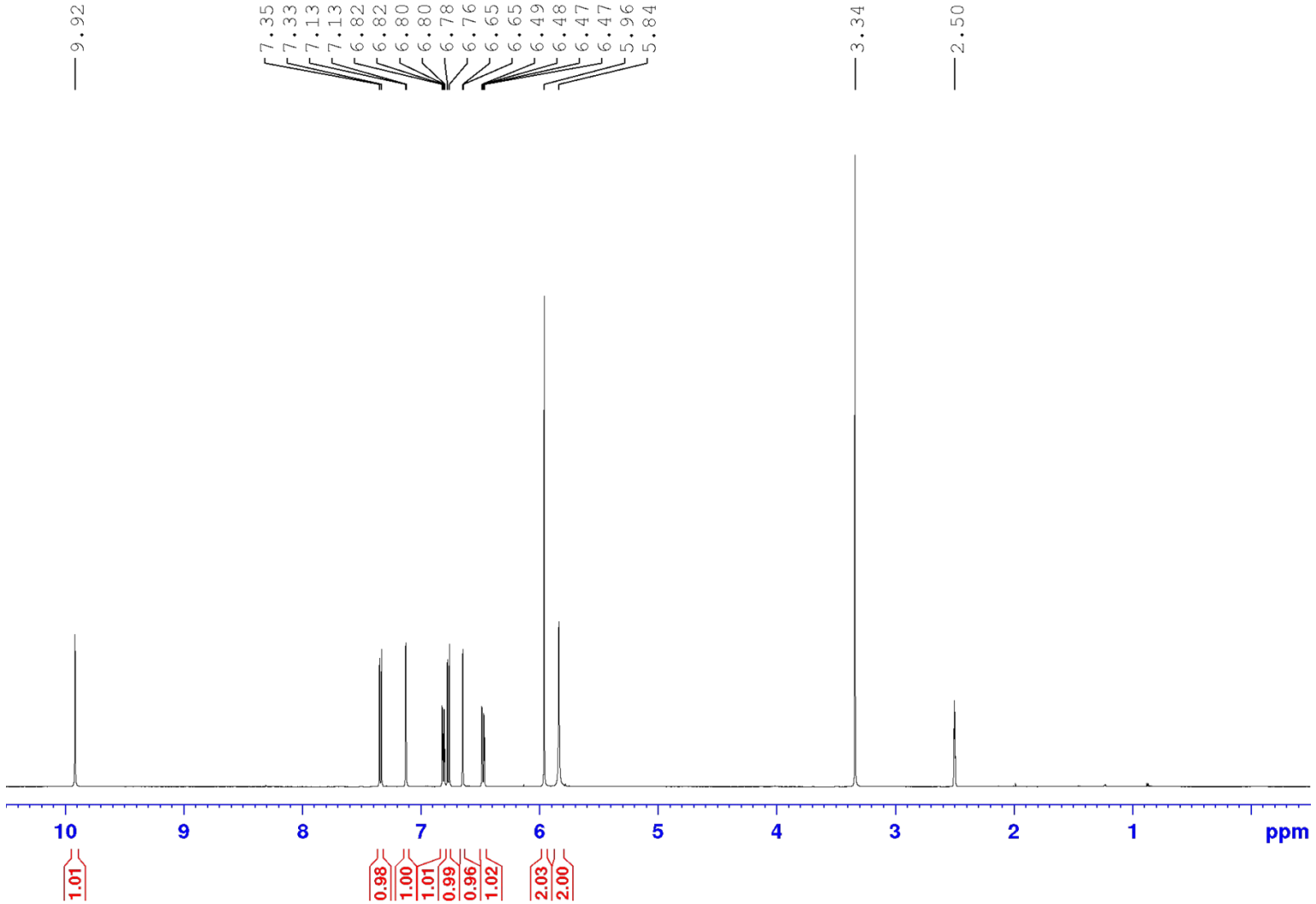


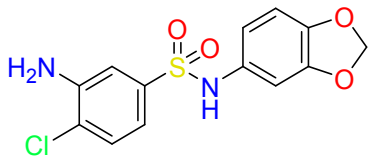
Compound **26** – ^{13}C NMR (125.5 MHz) – $CDCl_3$



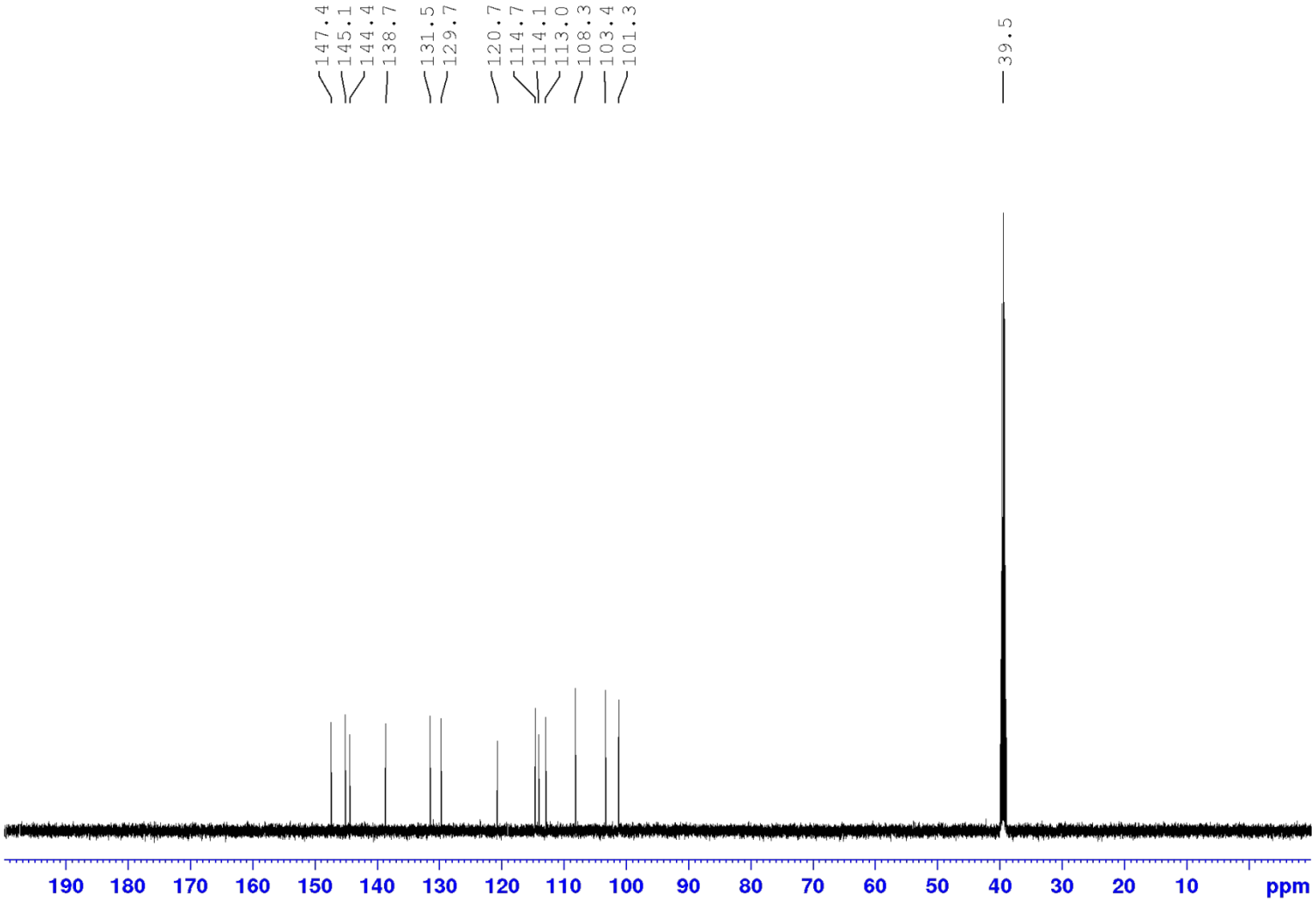


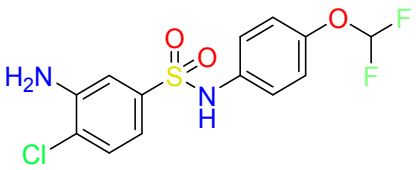
Compound **27** – ^{1}H NMR (500 MHz) – DMSO-d_6



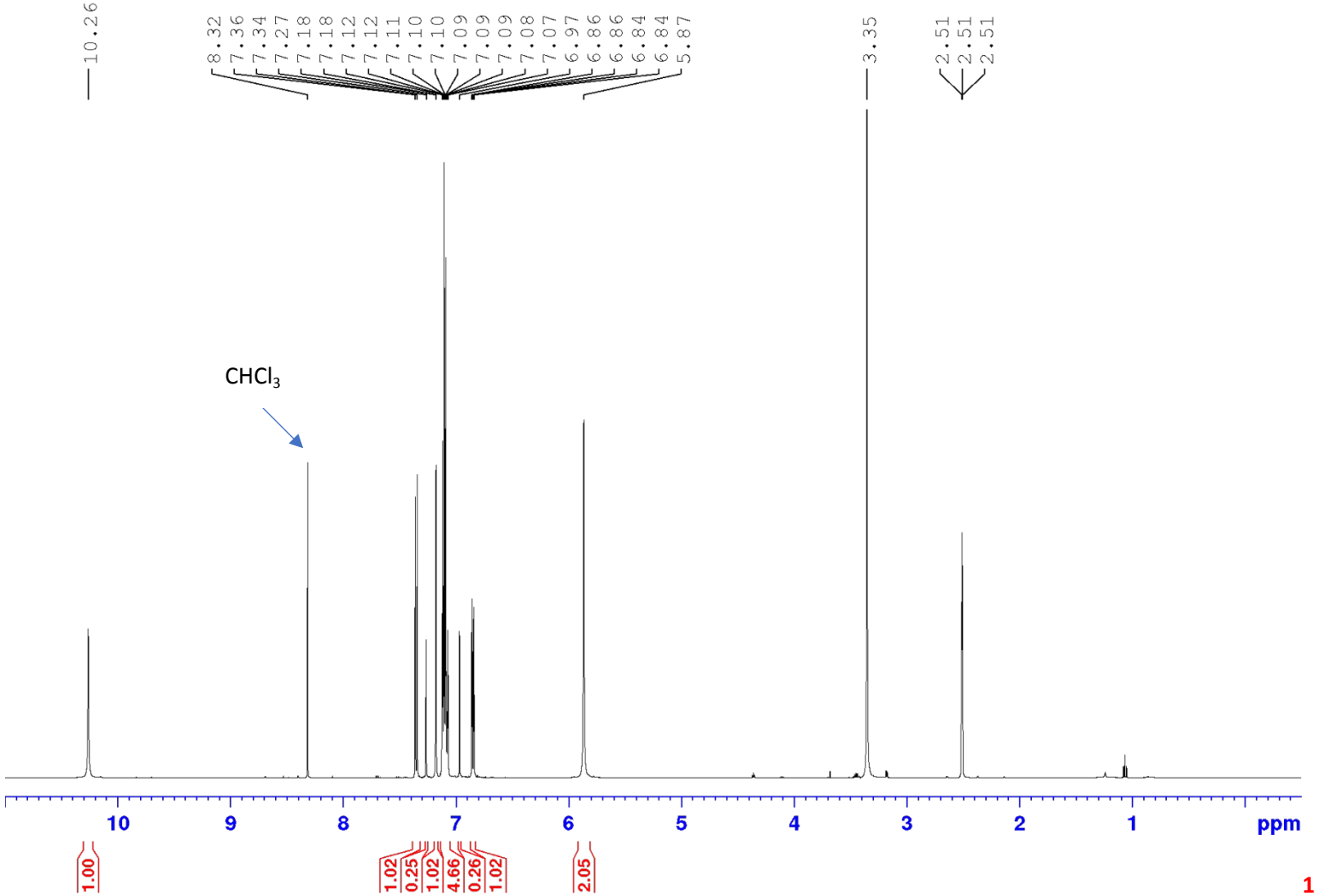


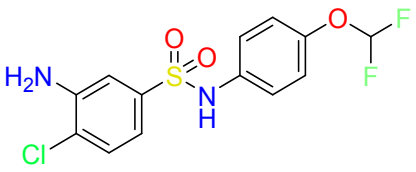
Compound **27** – ^{13}C NMR (125.5 MHz) – DMSO-d_6



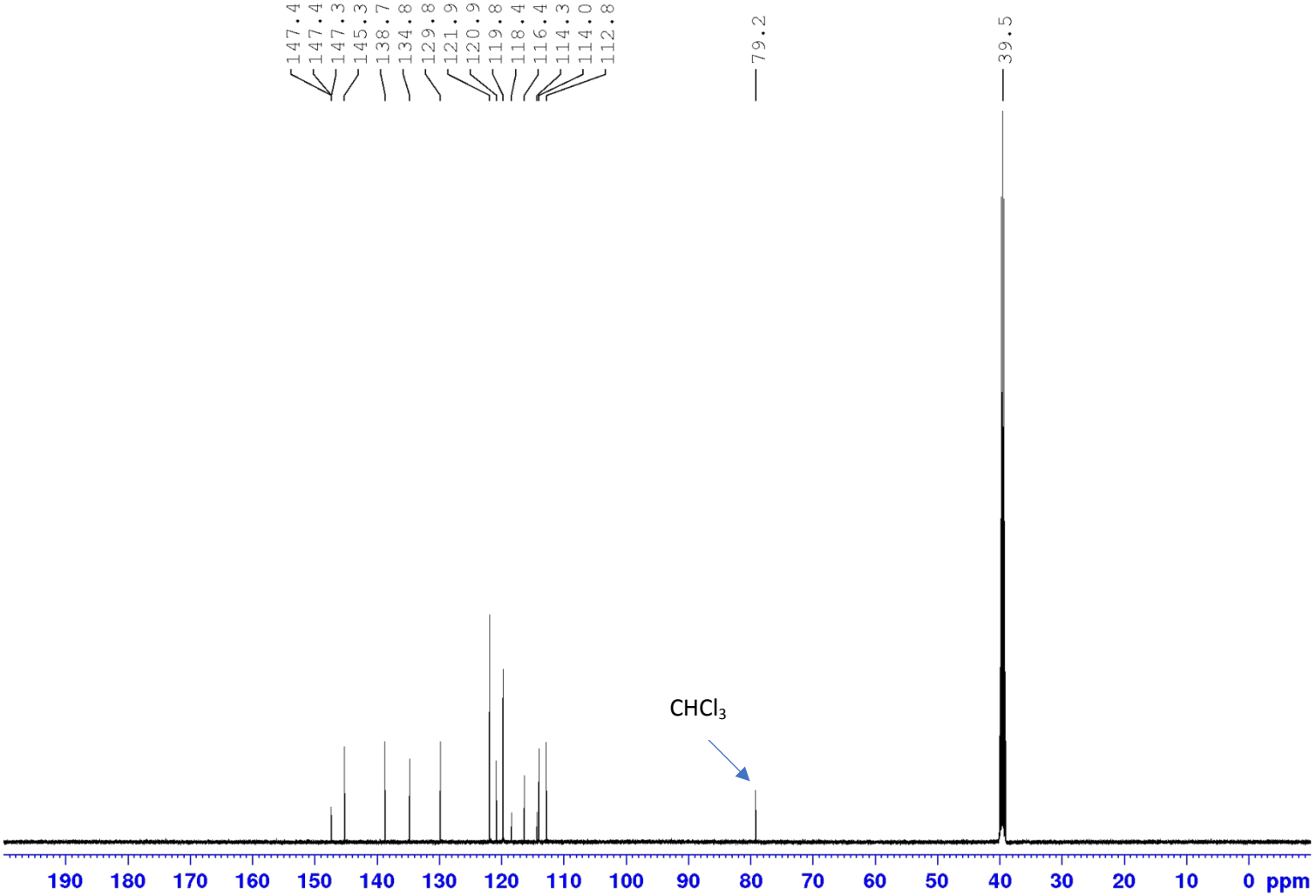


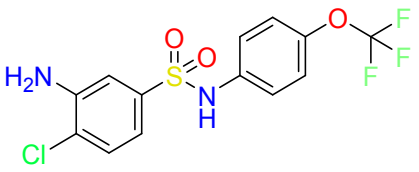
Compound **28** – ^{1}H NMR (500 MHz) – DMSO-d_6



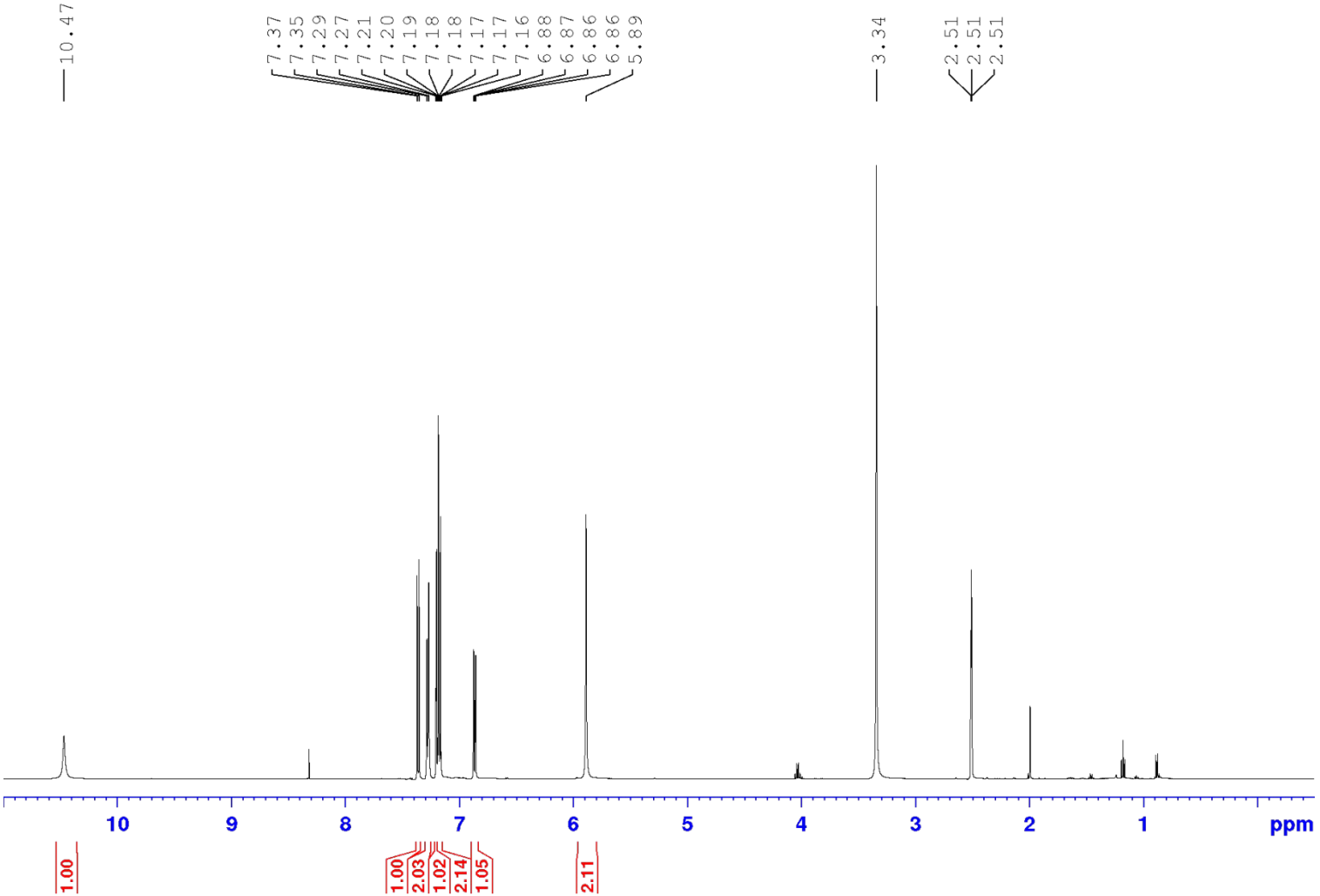


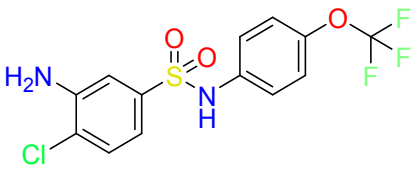
Compound **28** – ^{13}C NMR (125.5 MHz) – DMSO-d_6



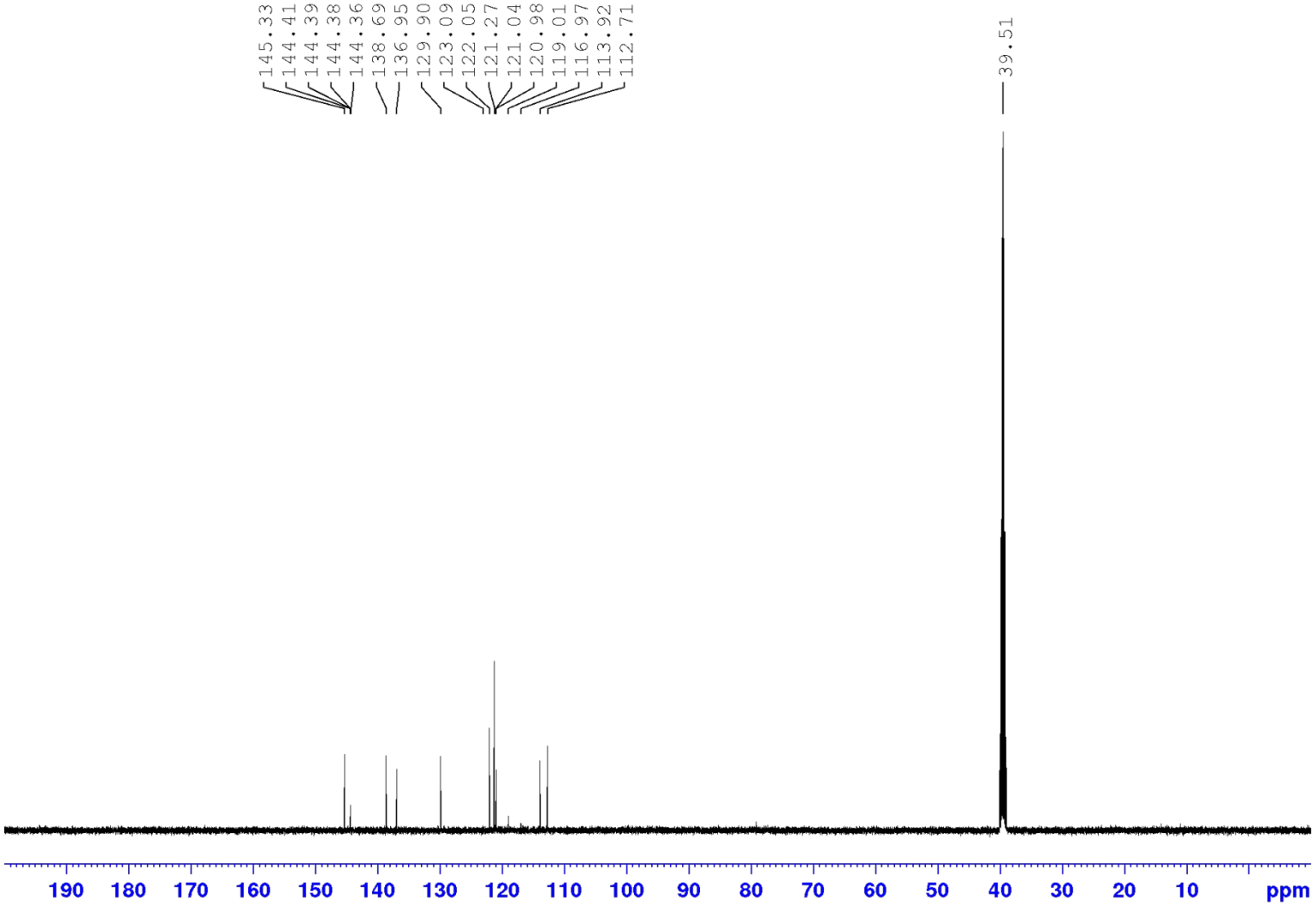


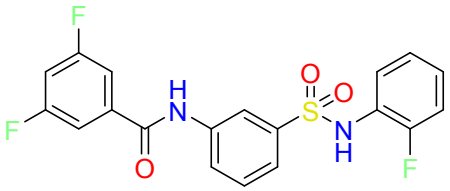
Compound **29** – ^{1}H NMR (500 MHz) – DMSO-d_6



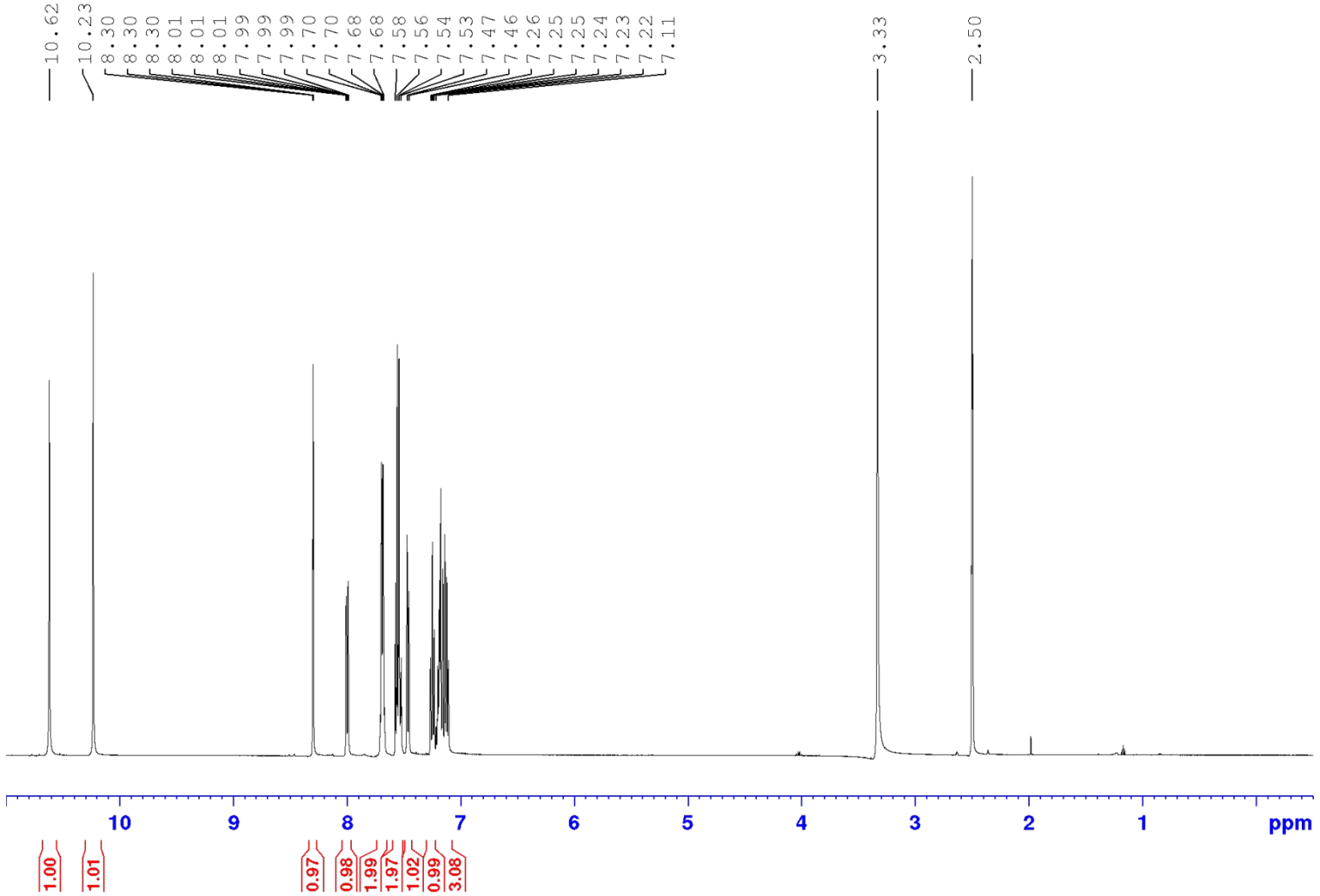


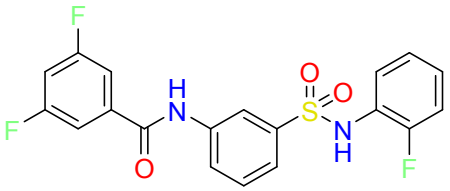
Compound **29** – ^{13}C NMR (125.5 MHz) – DMSO-d_6



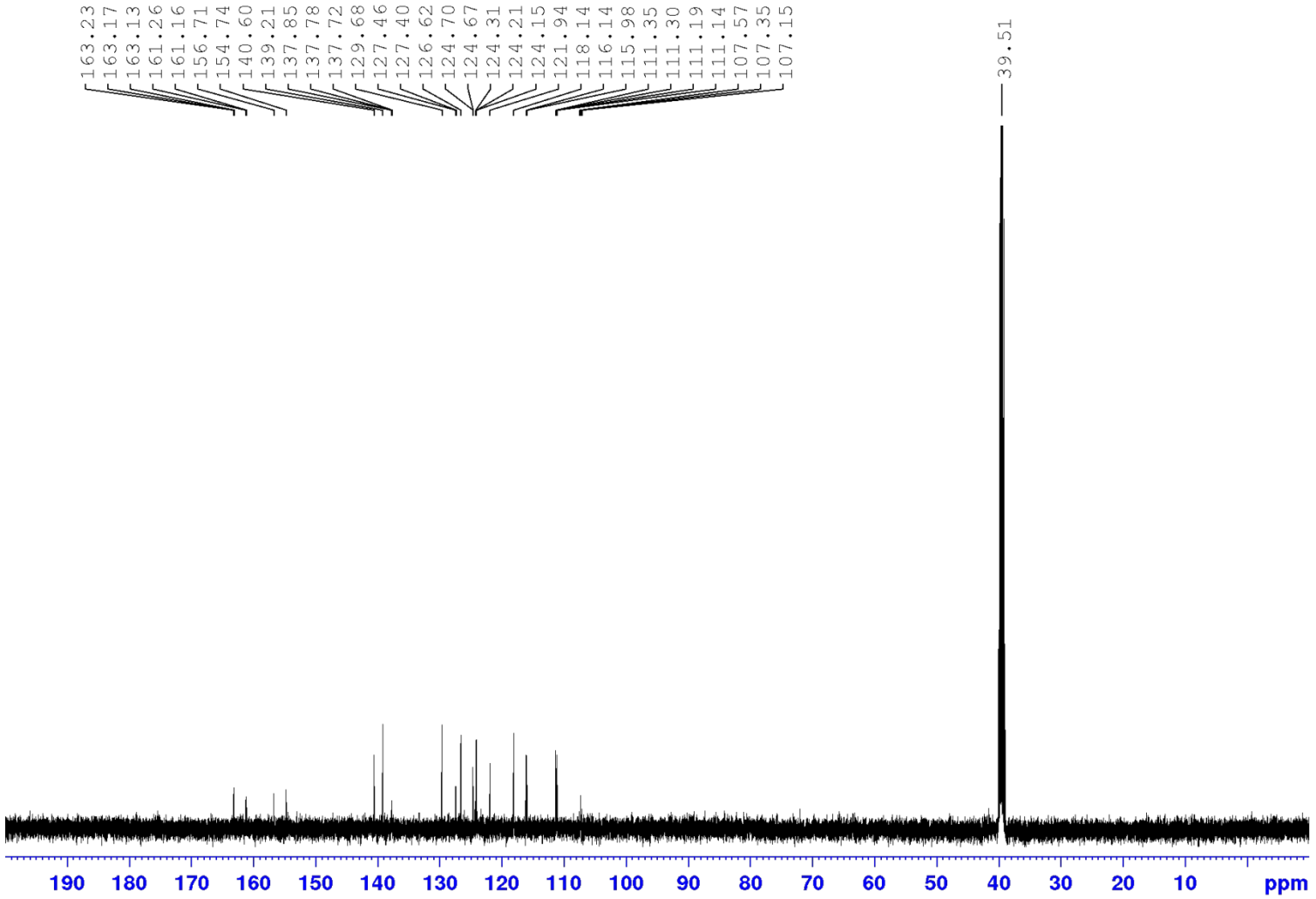


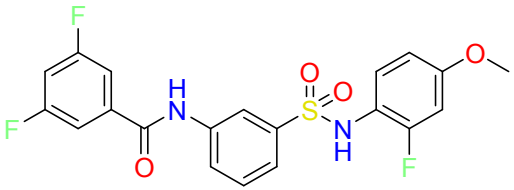
Compound **30** – ^{1}H NMR (500 MHz) – DMSO-d_6



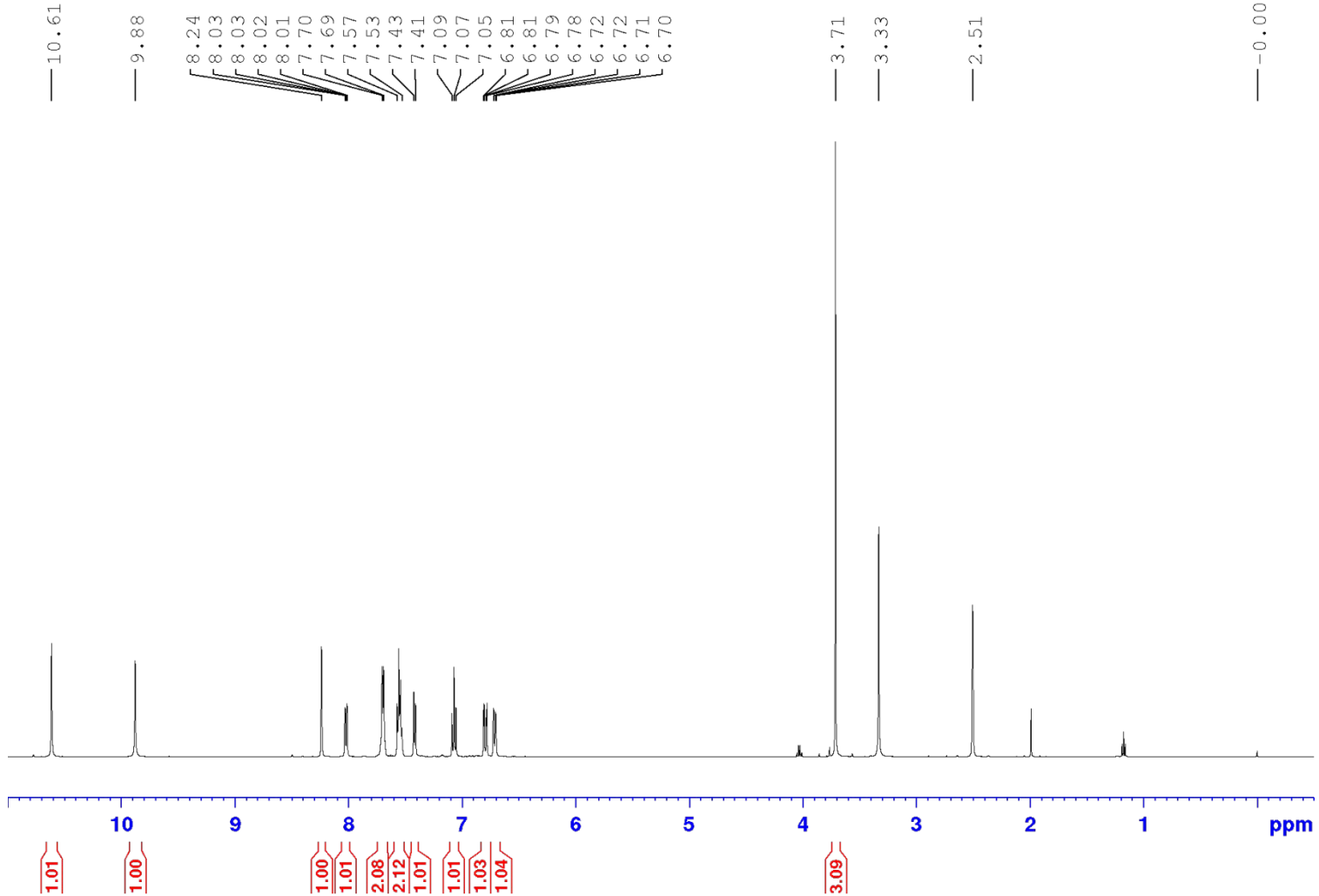


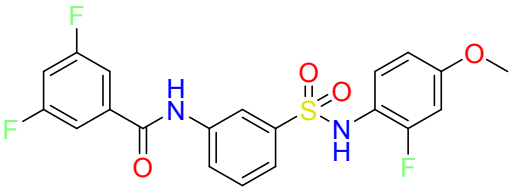
Compound **30** – ^{13}C NMR (125.5 MHz) – DMSO-d_6



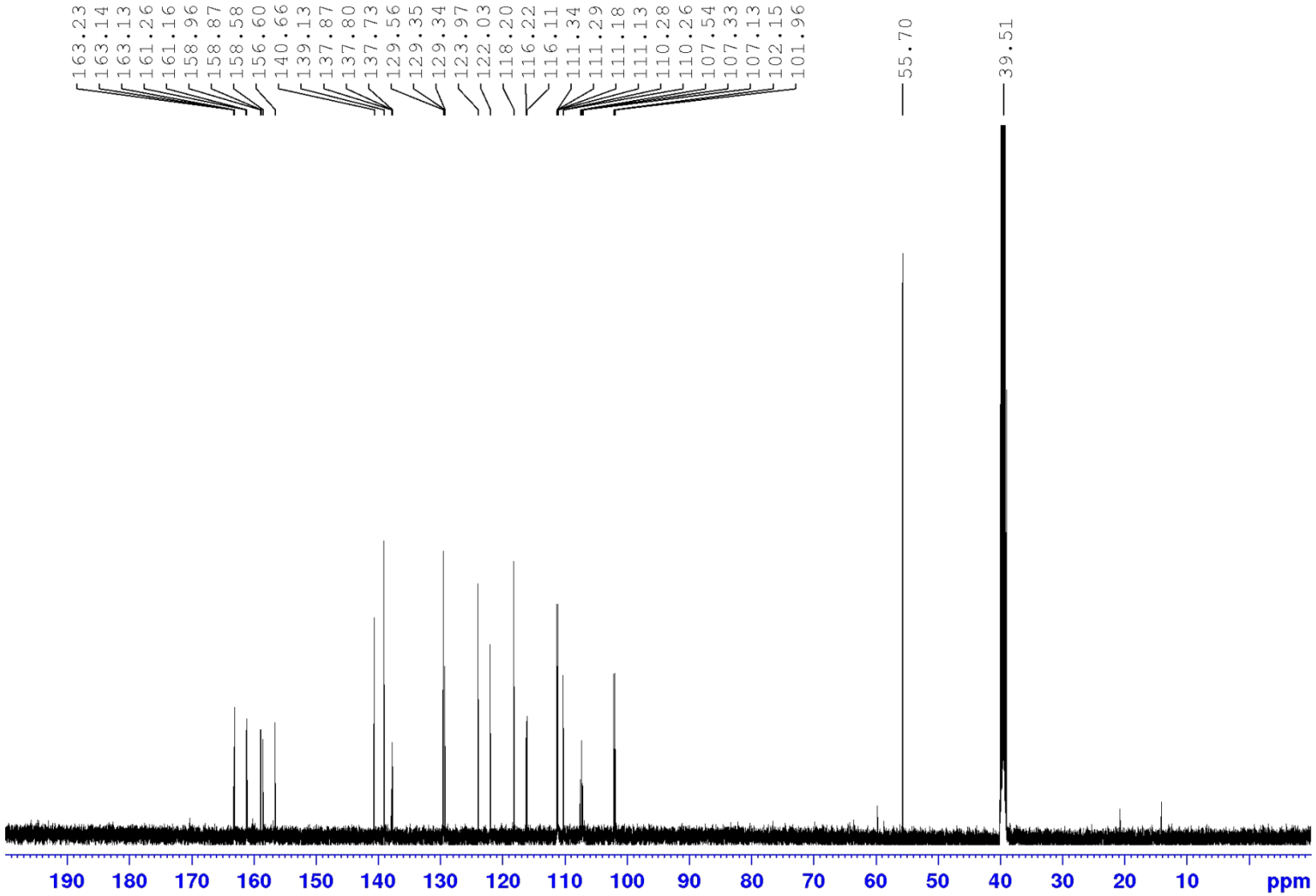


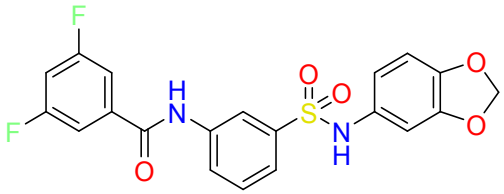
Compound **31** – ^{1}H NMR (500 MHz) – DMSO-d_6



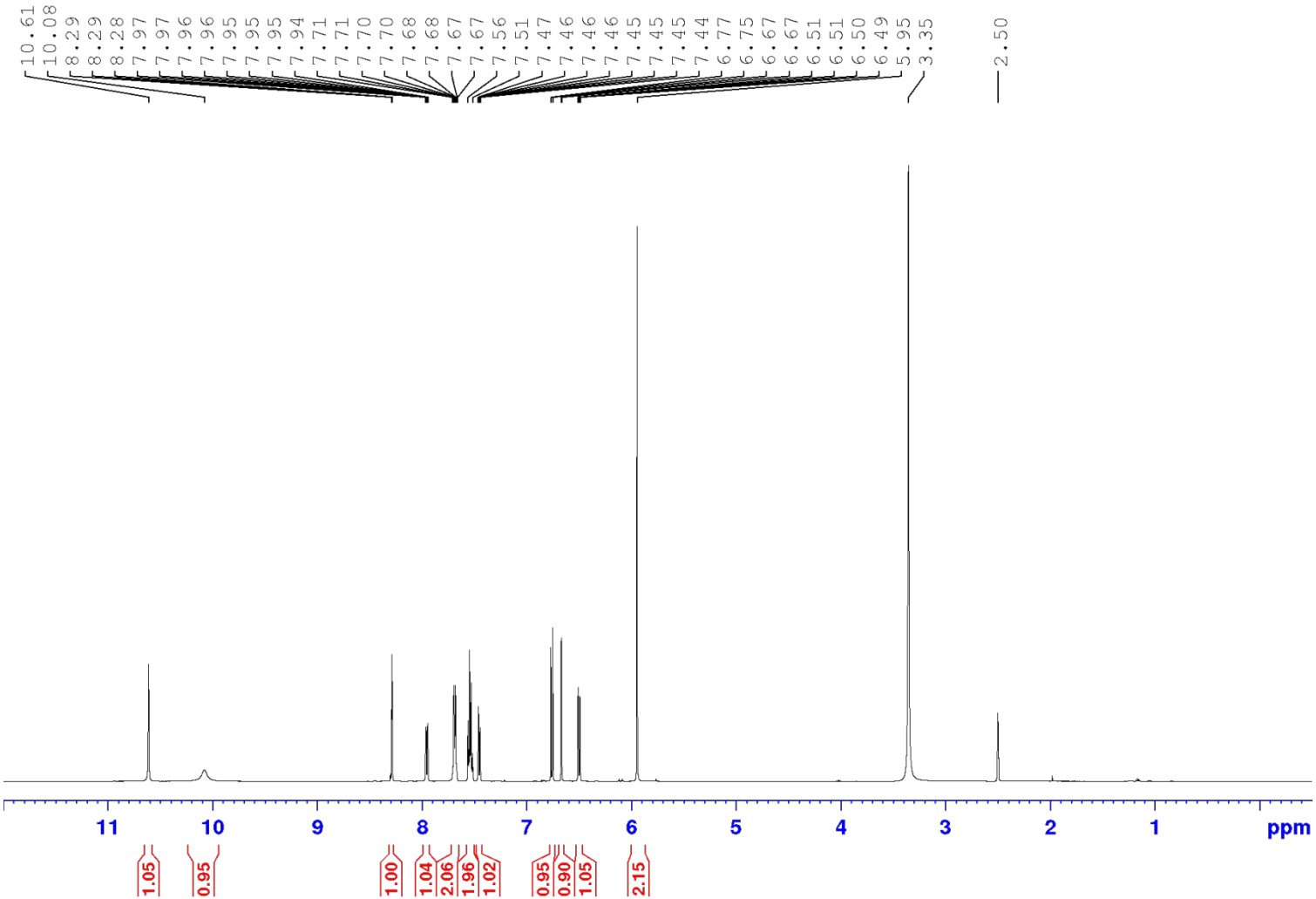


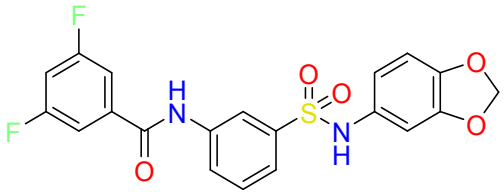
Compound **31** – ^{13}C NMR (125.5 MHz) – DMSO-d_6



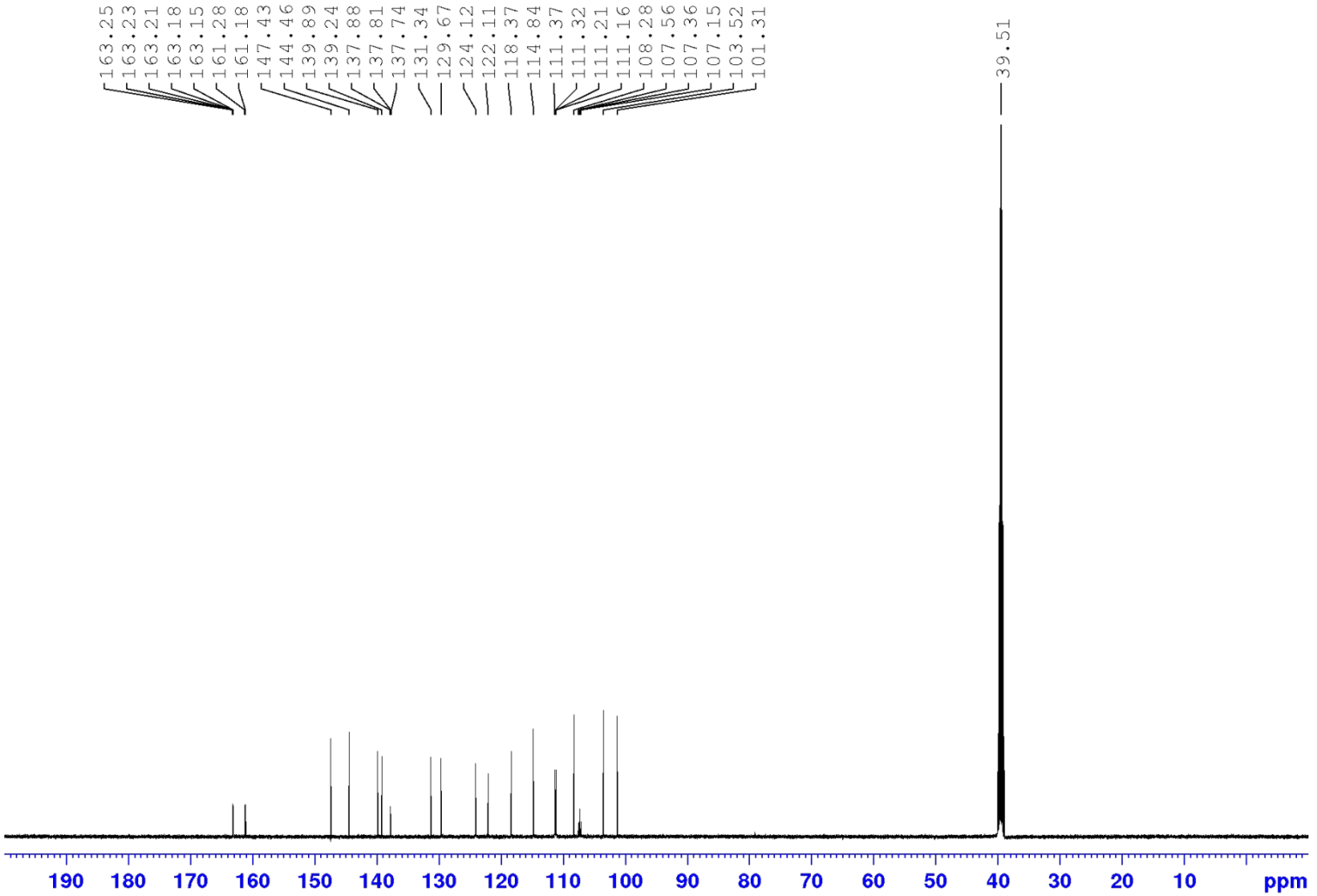


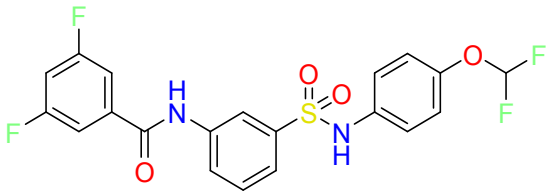
Compound **32** – ^{1}H NMR (500 MHz) – DMSO-d_6



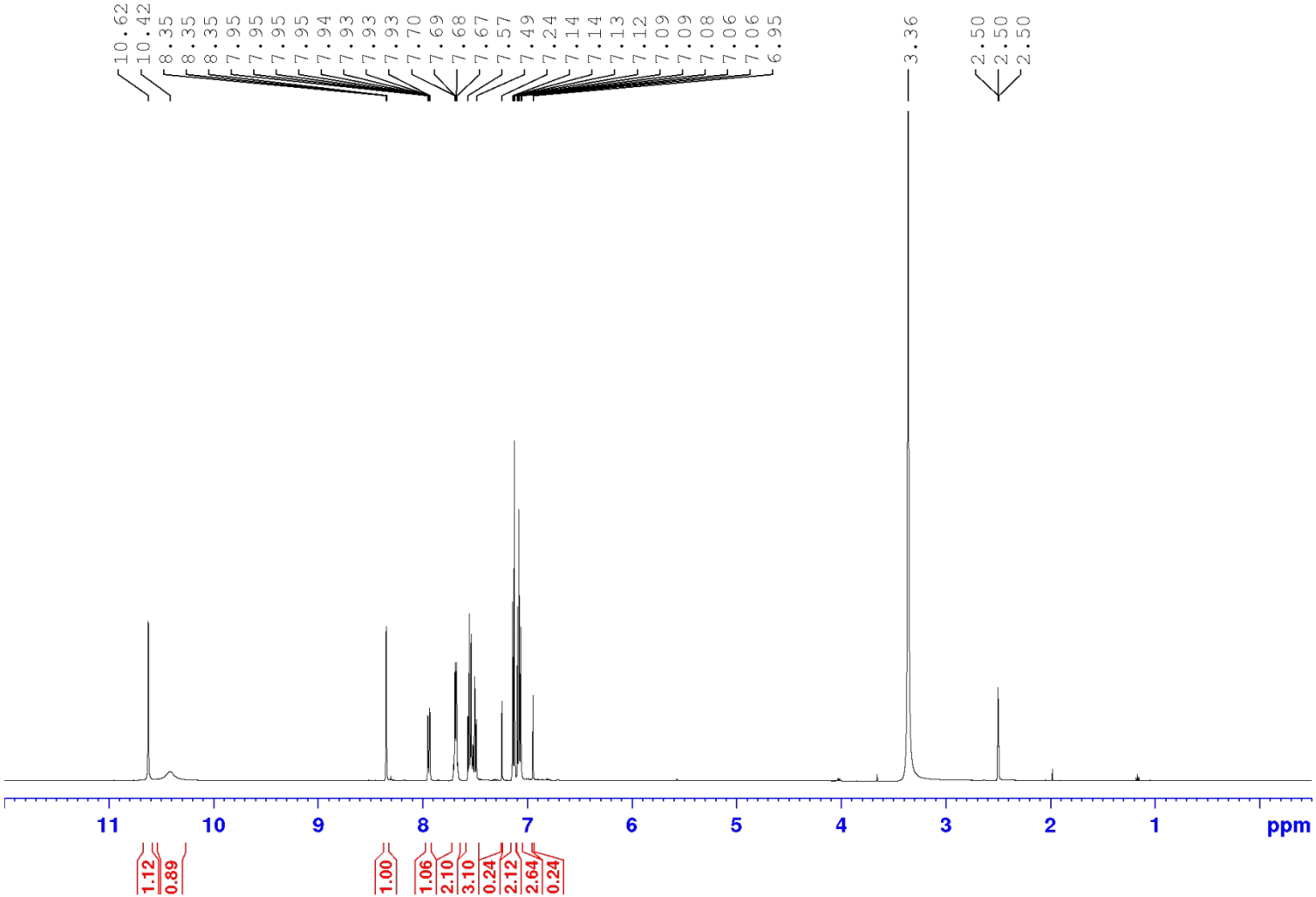


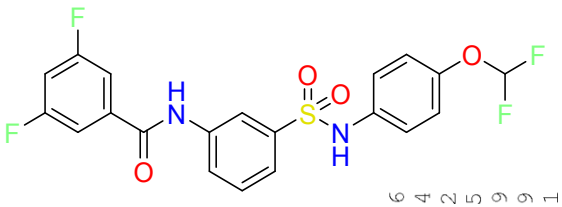
Compound **32** – ^{13}C NMR (125.5 MHz) – DMSO-d_6



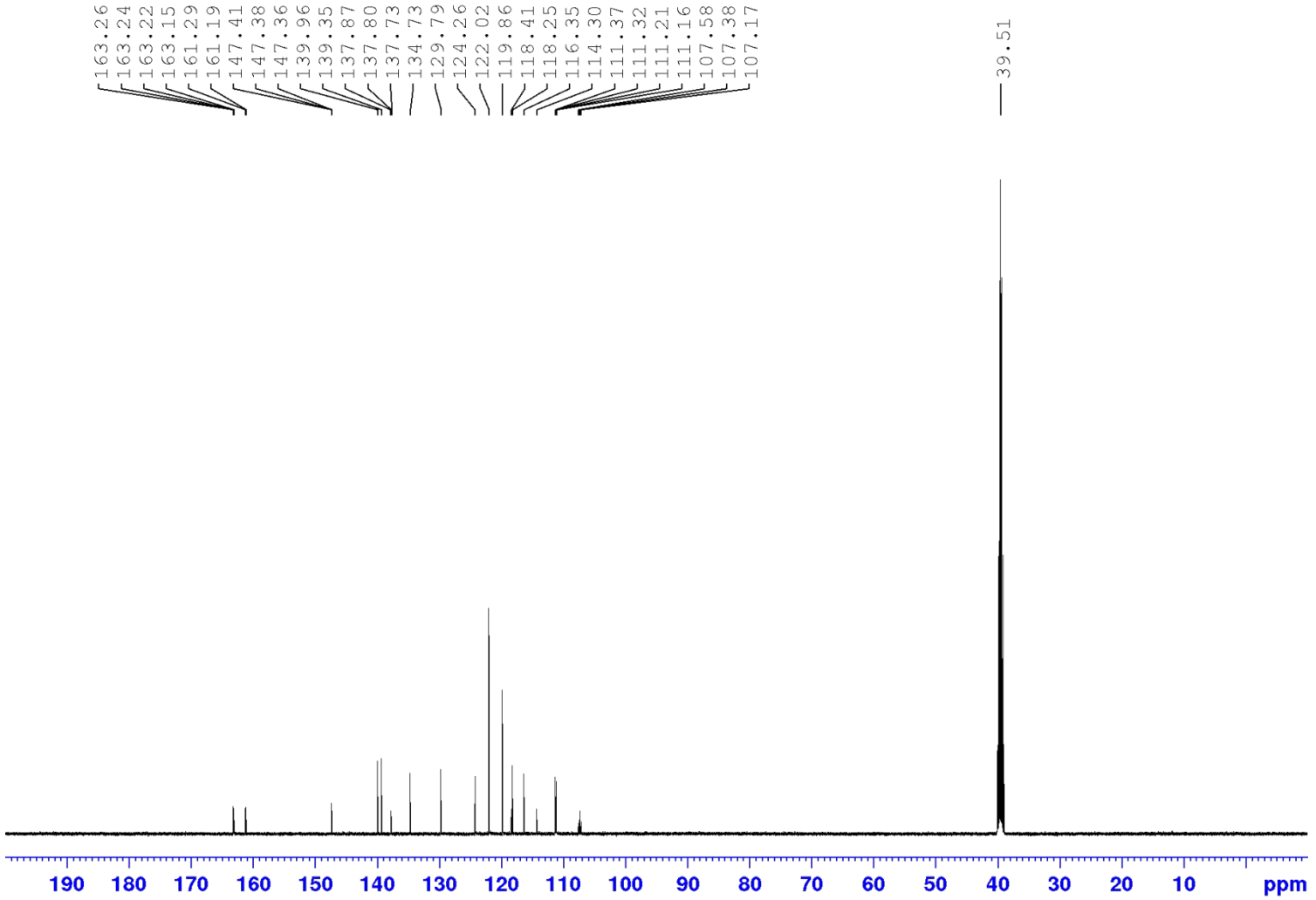


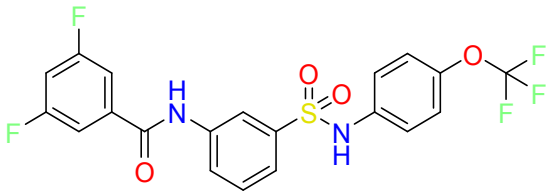
Compound **33** – ^{1}H NMR (500 MHz) – DMSO-d_6



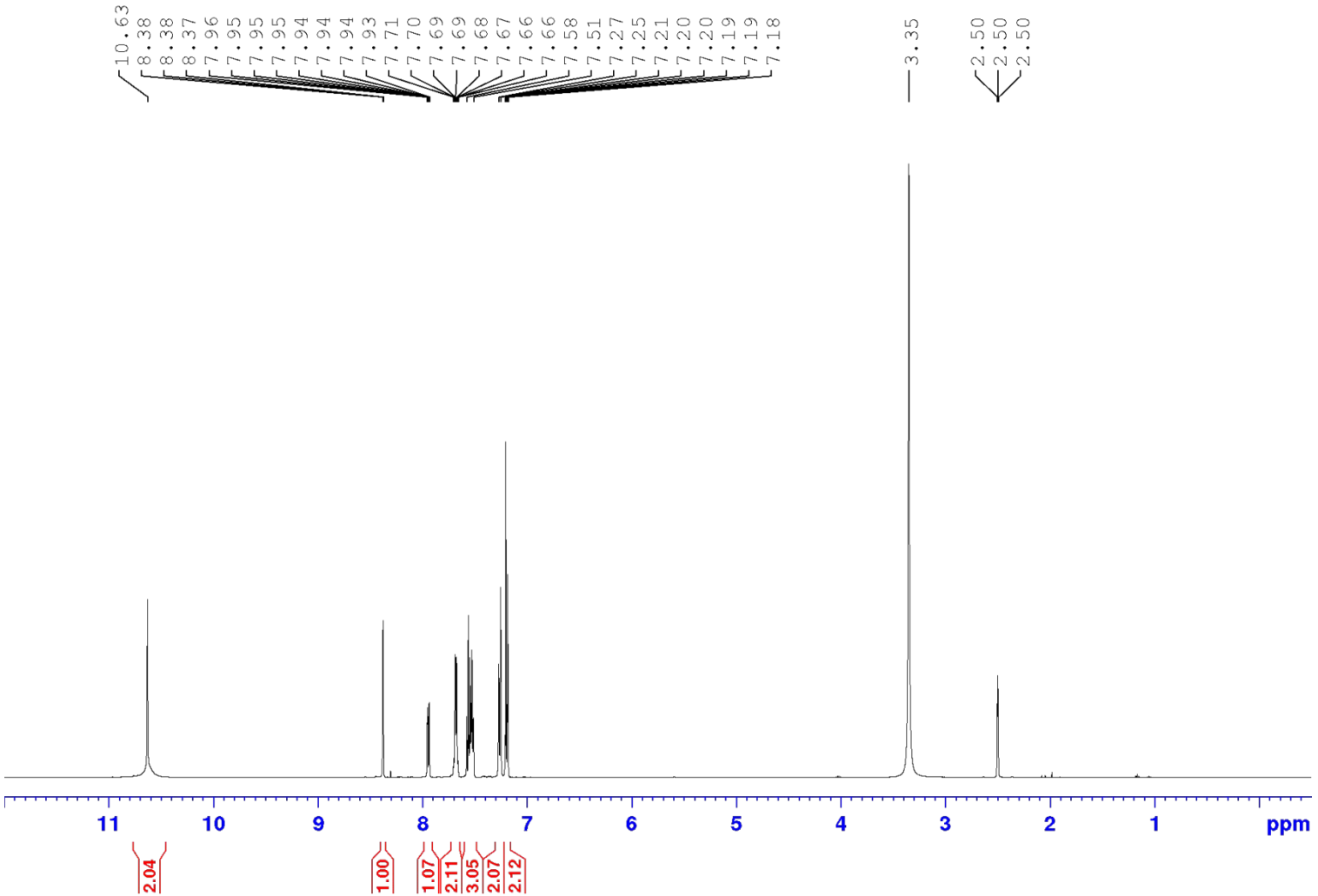


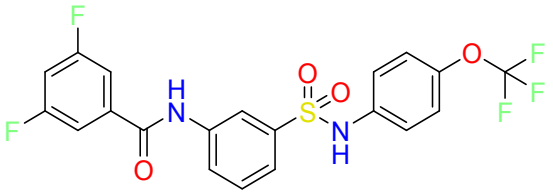
Compound **33** – ^{13}C NMR (125.5 MHz) – DMSO-d_6



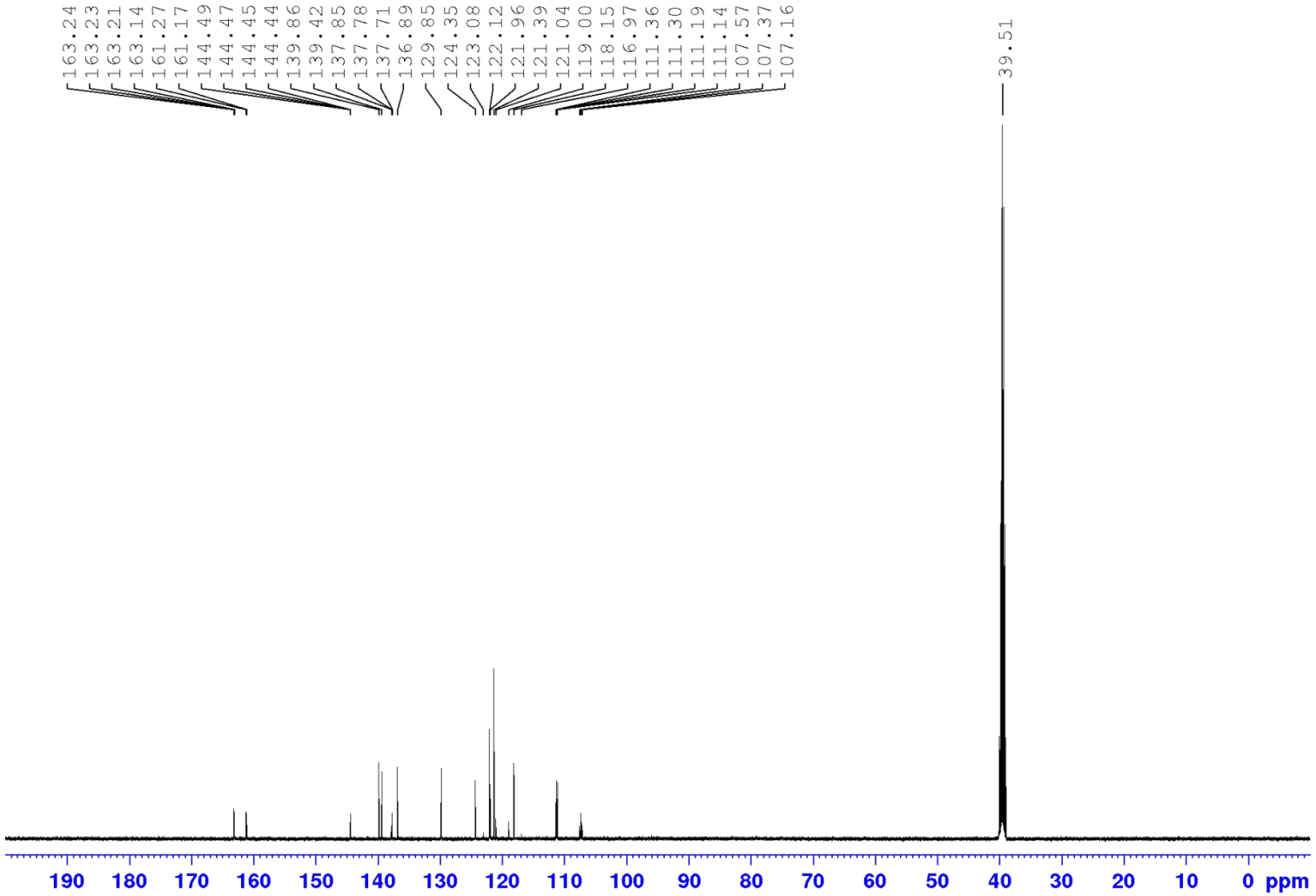


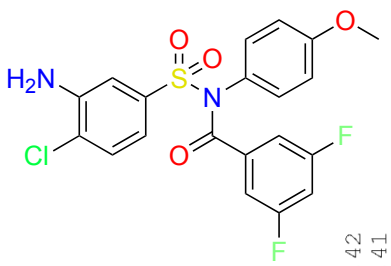
Compound **34** – ^{1}H NMR (500 MHz) – DMSO-d_6



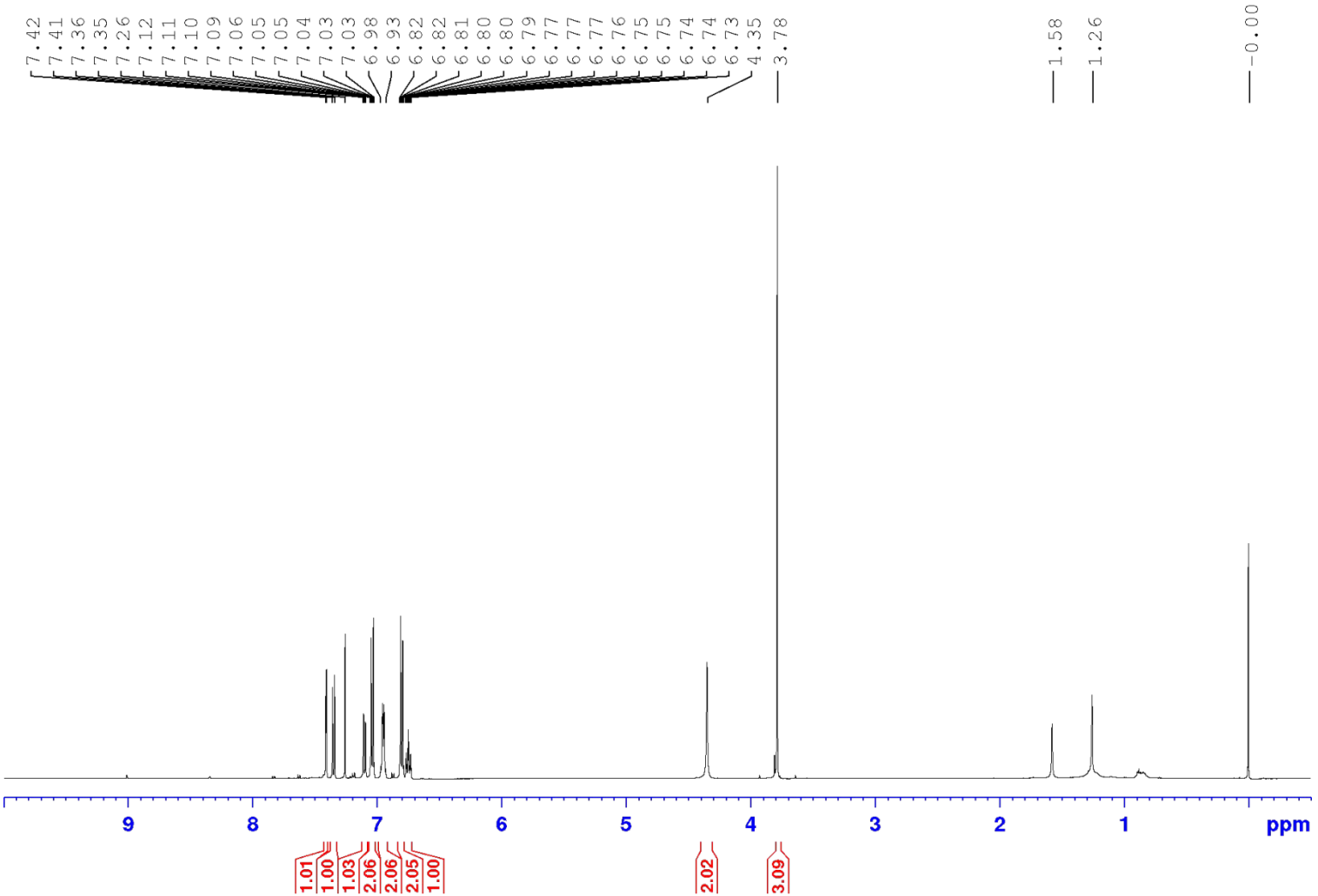


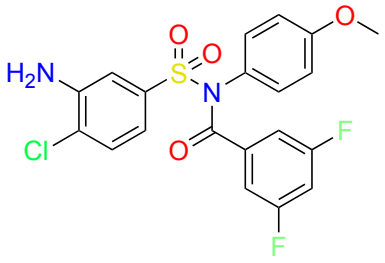
Compound **34** – ^{13}C NMR (125.5 MHz) – DMSO-d_6



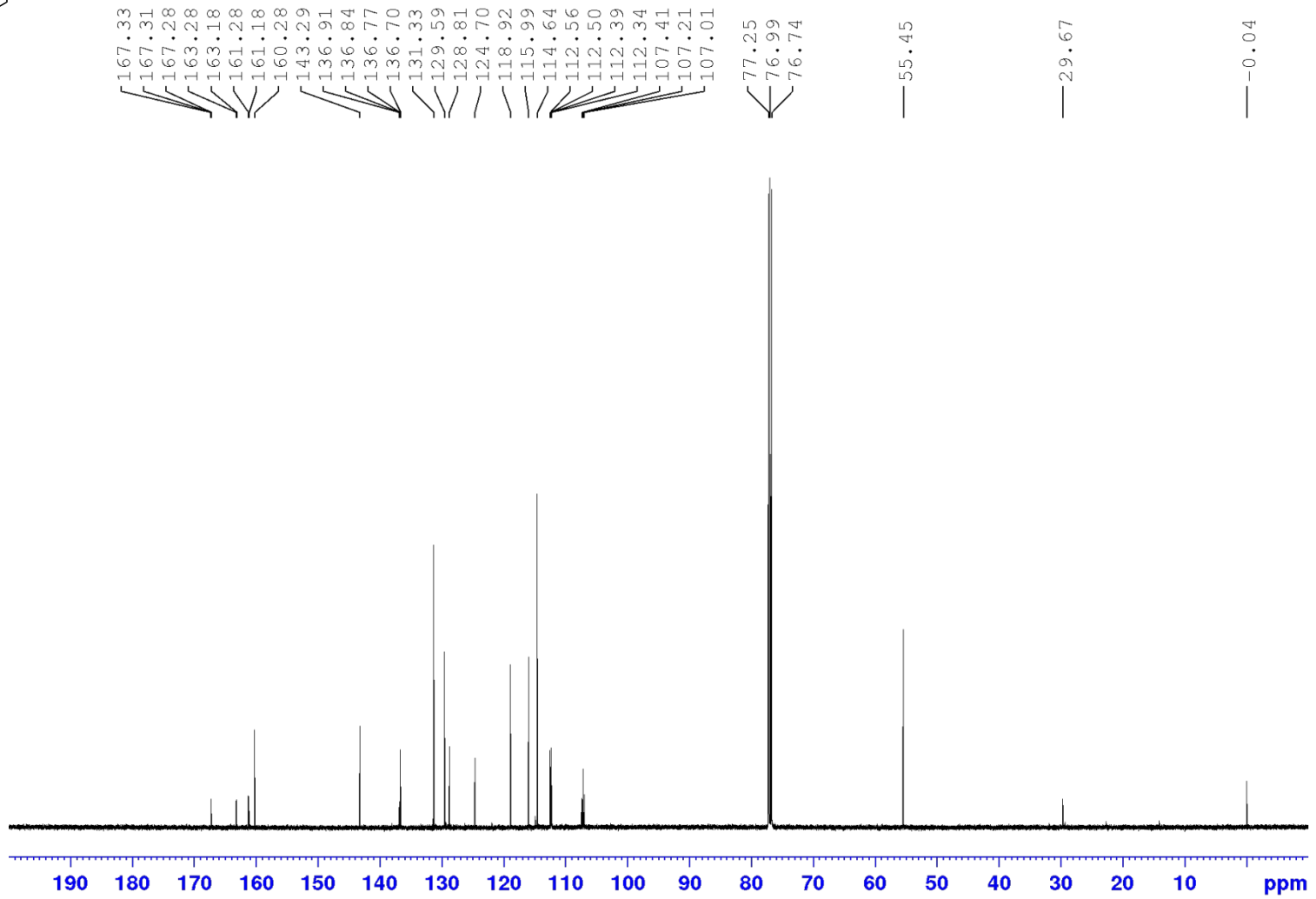


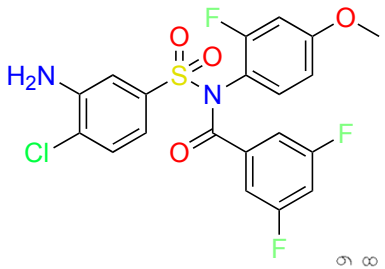
Compound **35** – ^{1}H NMR (500 MHz) – $CDCl_3$



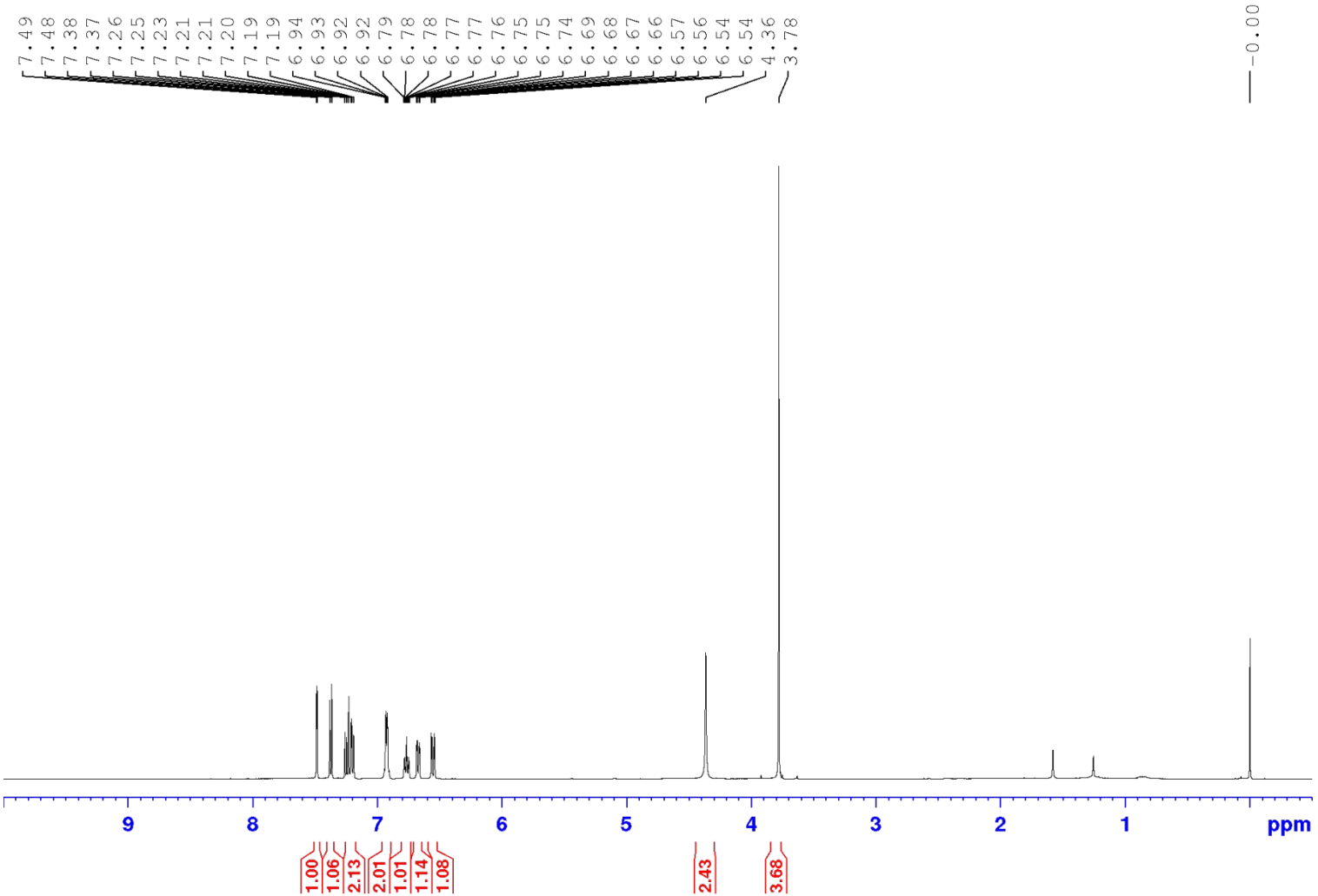


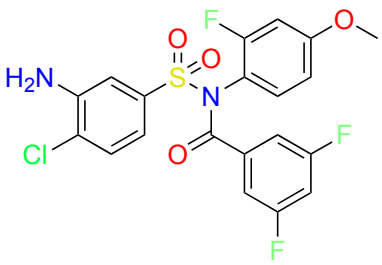
Compound **35** – ^{13}C NMR (125.5 MHz) – $CDCl_3$



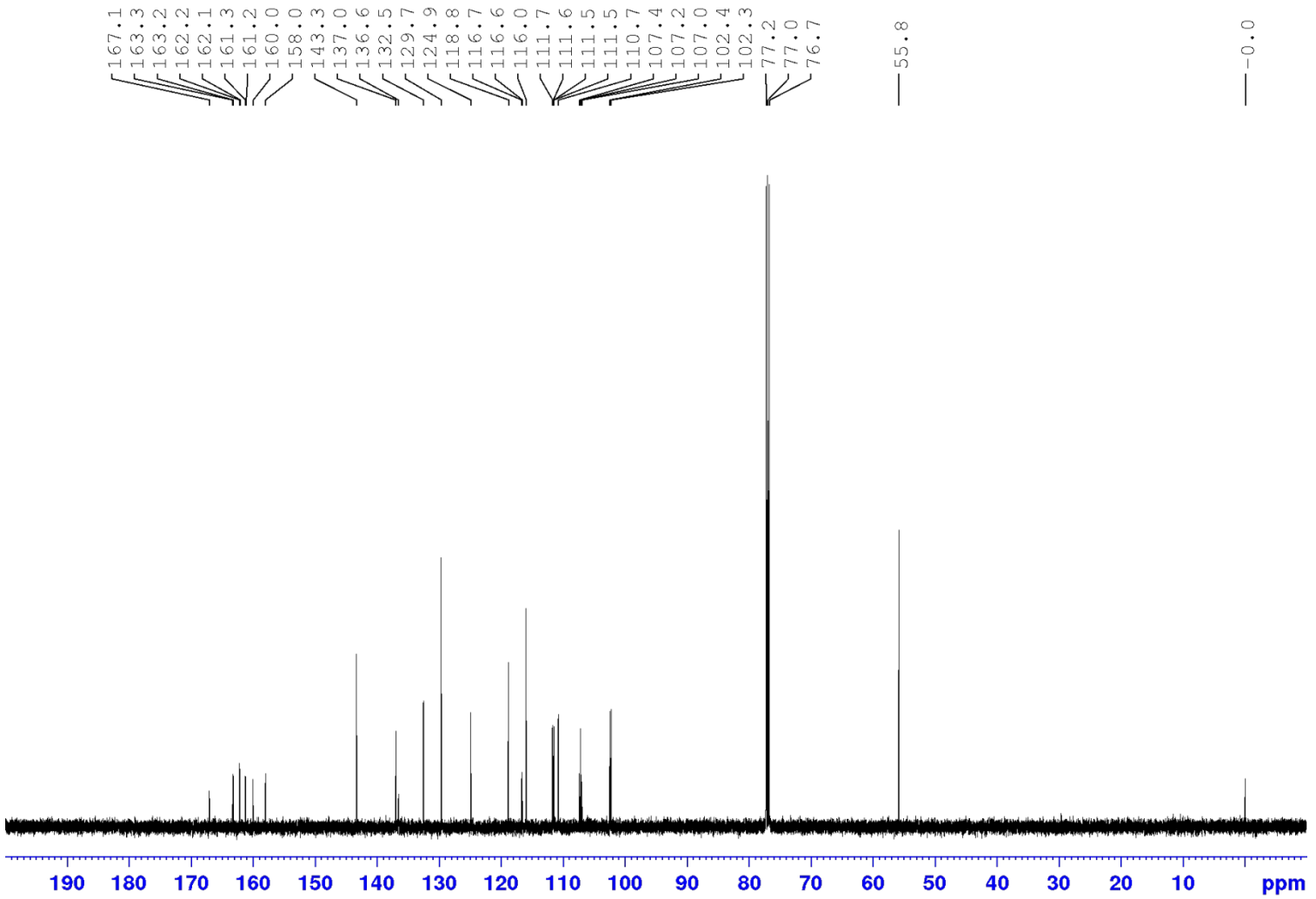


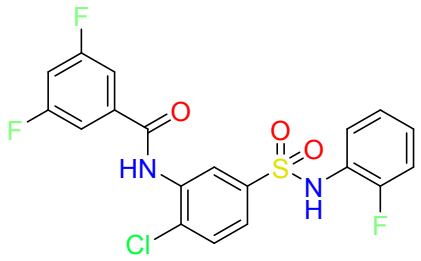
Compound **36** – ^{1}H NMR (500 MHz) – $CDCl_3$



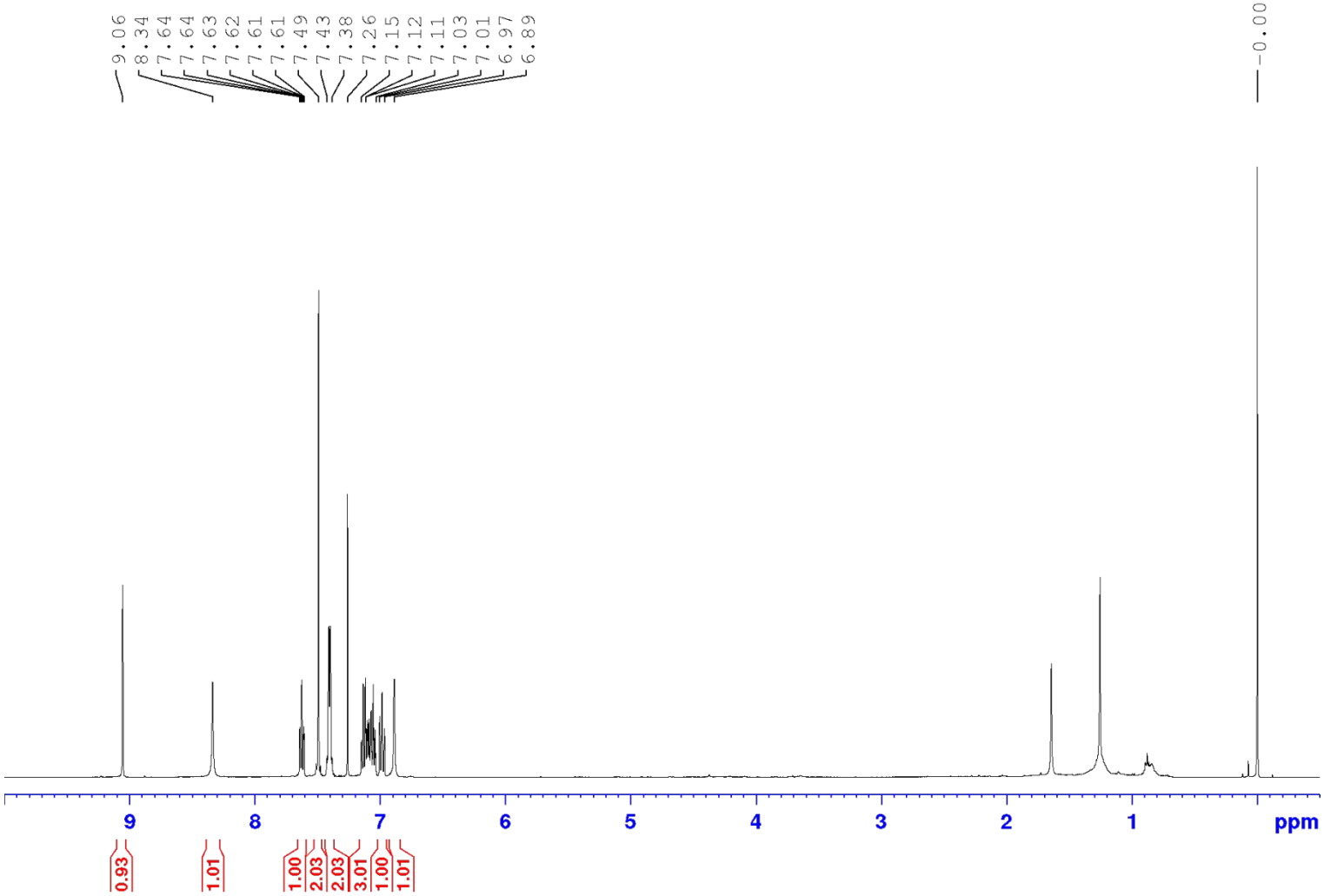


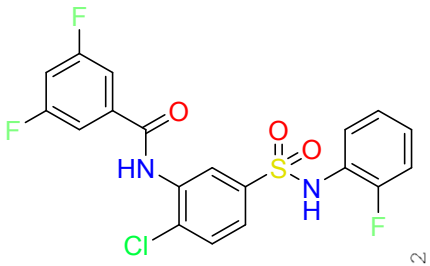
Compound **36** – ^{13}C NMR (125.5 MHz) – $CDCl_3$



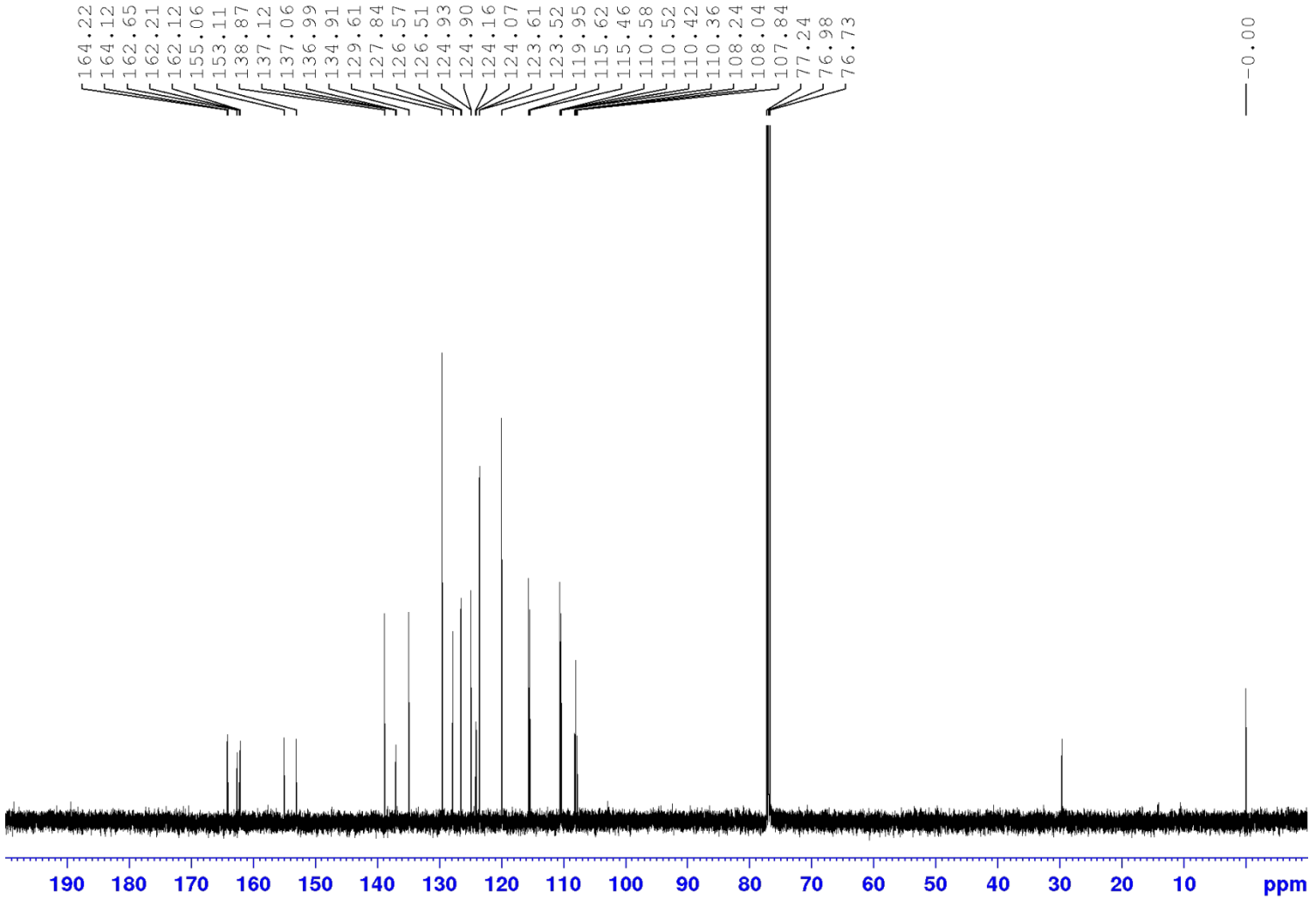


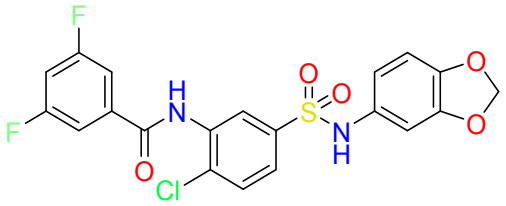
Compound **37** – ^{1}H NMR (500 MHz) – $CDCl_3$



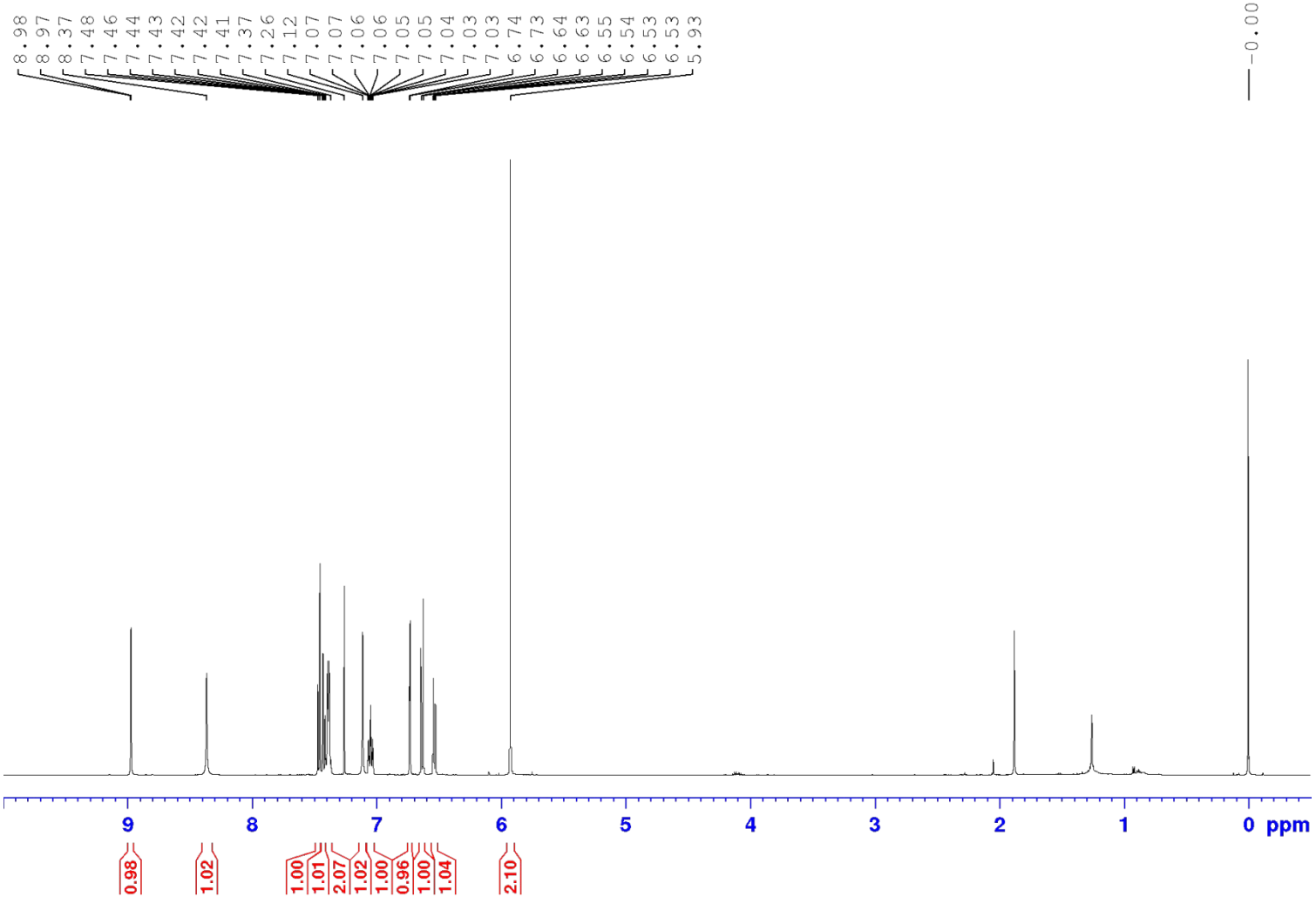


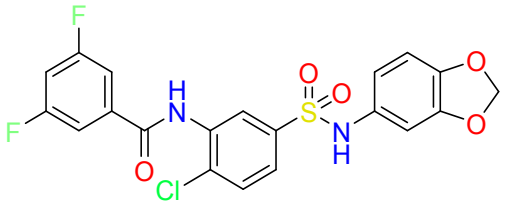
Compound **37** – ^{13}C NMR (125.5 MHz) – $CDCl_3$



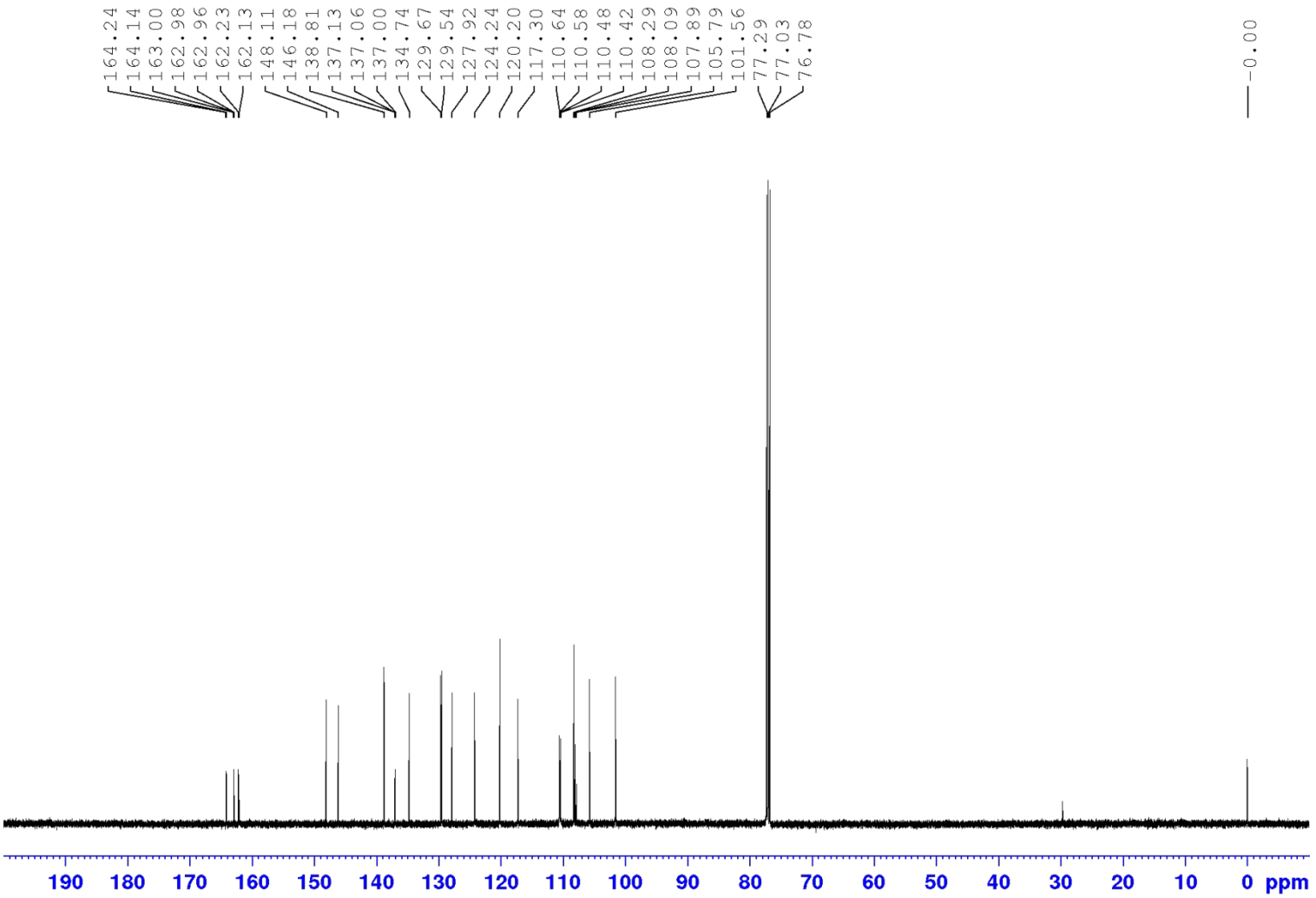


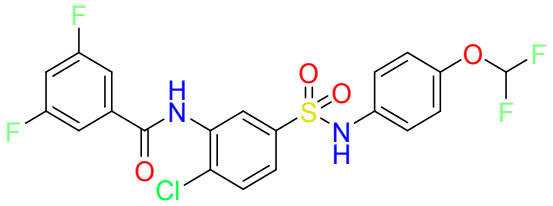
Compound **38** – ^{1}H NMR (500 MHz) – $CDCl_3$



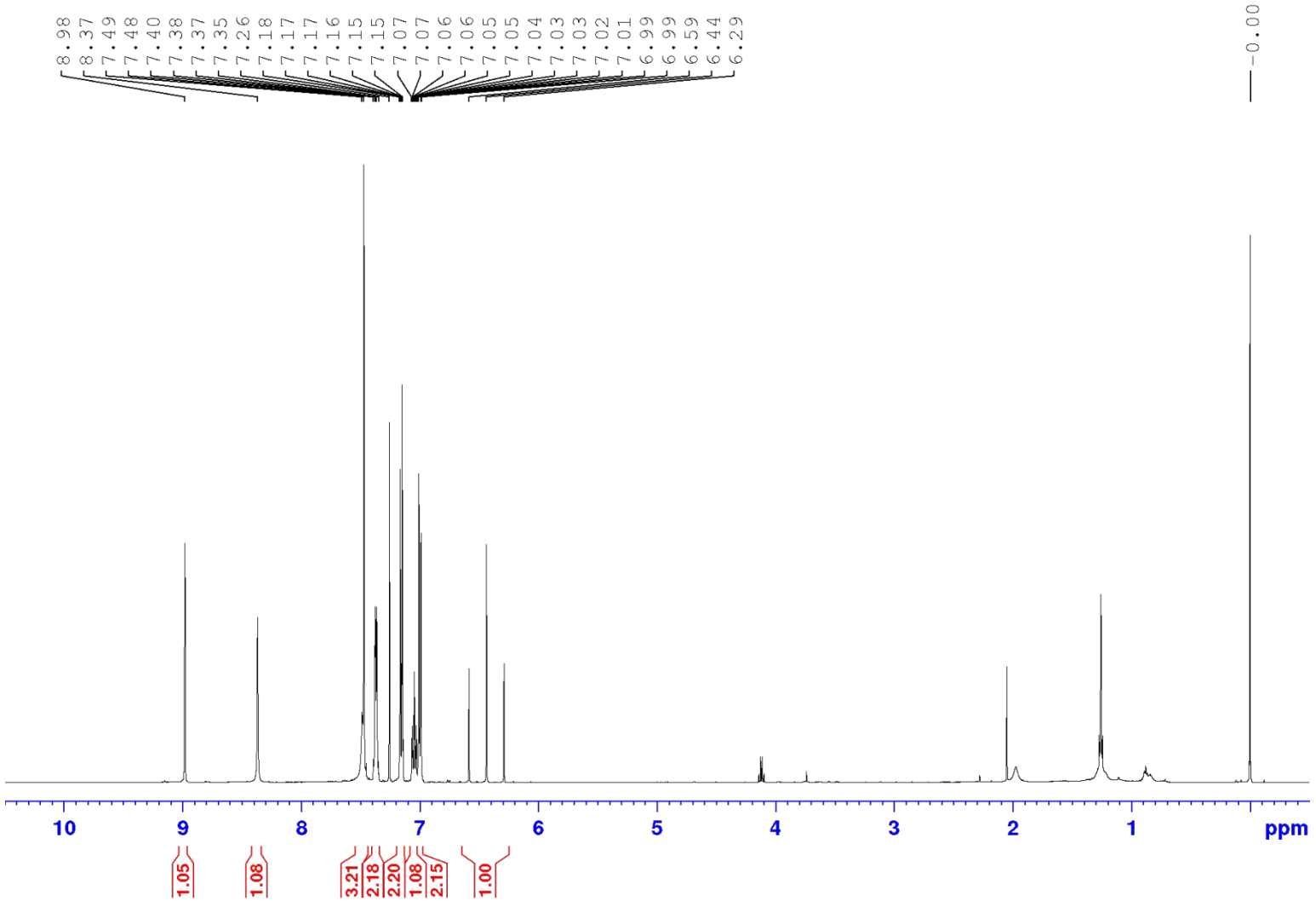


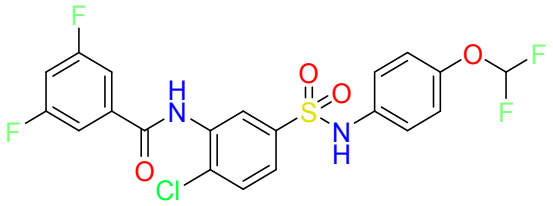
Compound **38** – ^{13}C NMR (125.5 MHz) – $CDCl_3$



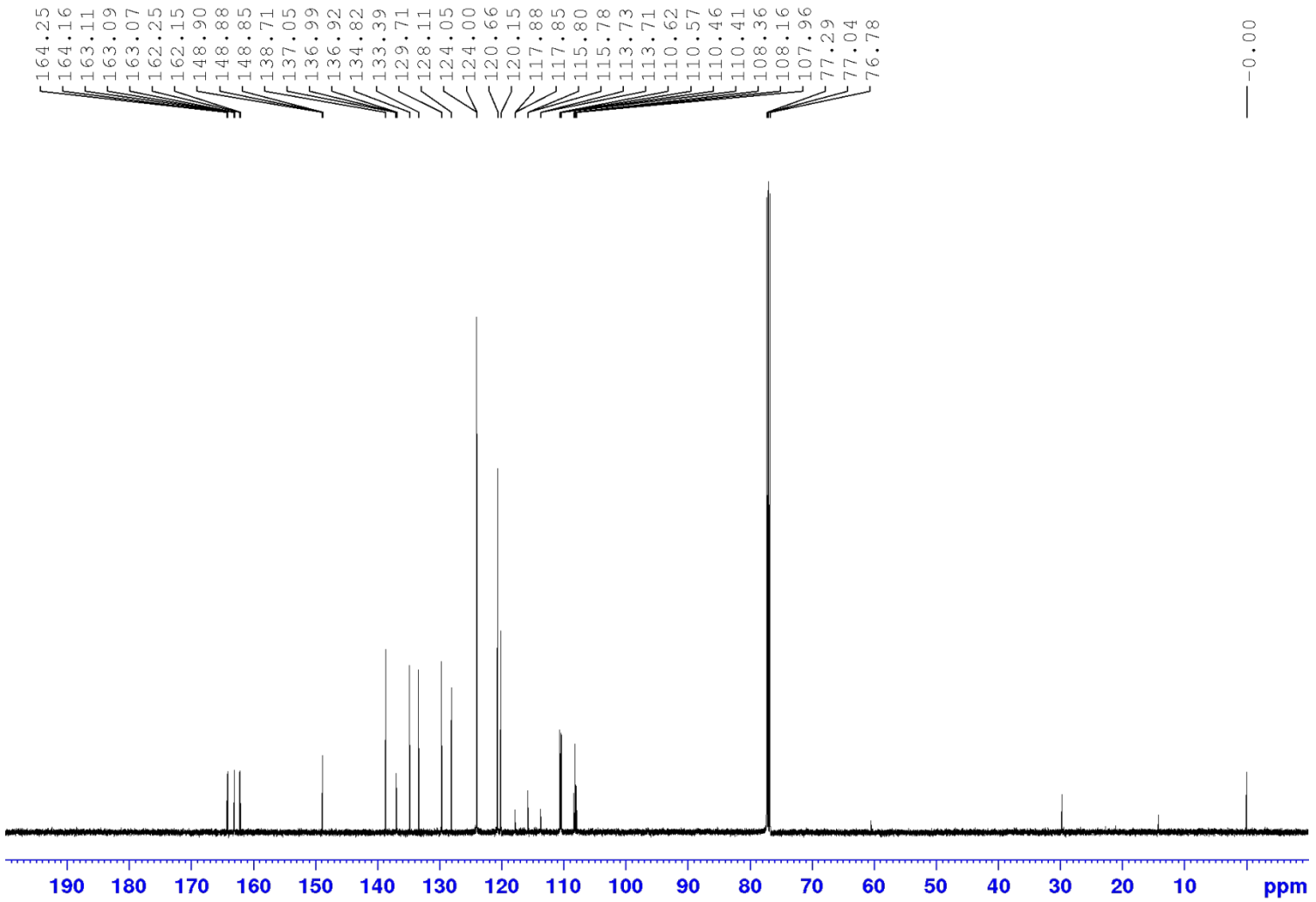


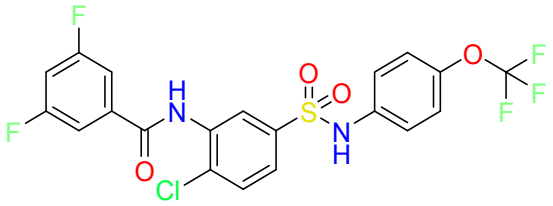
Compound **39** – ^{1}H NMR (500 MHz) – $CDCl_3$



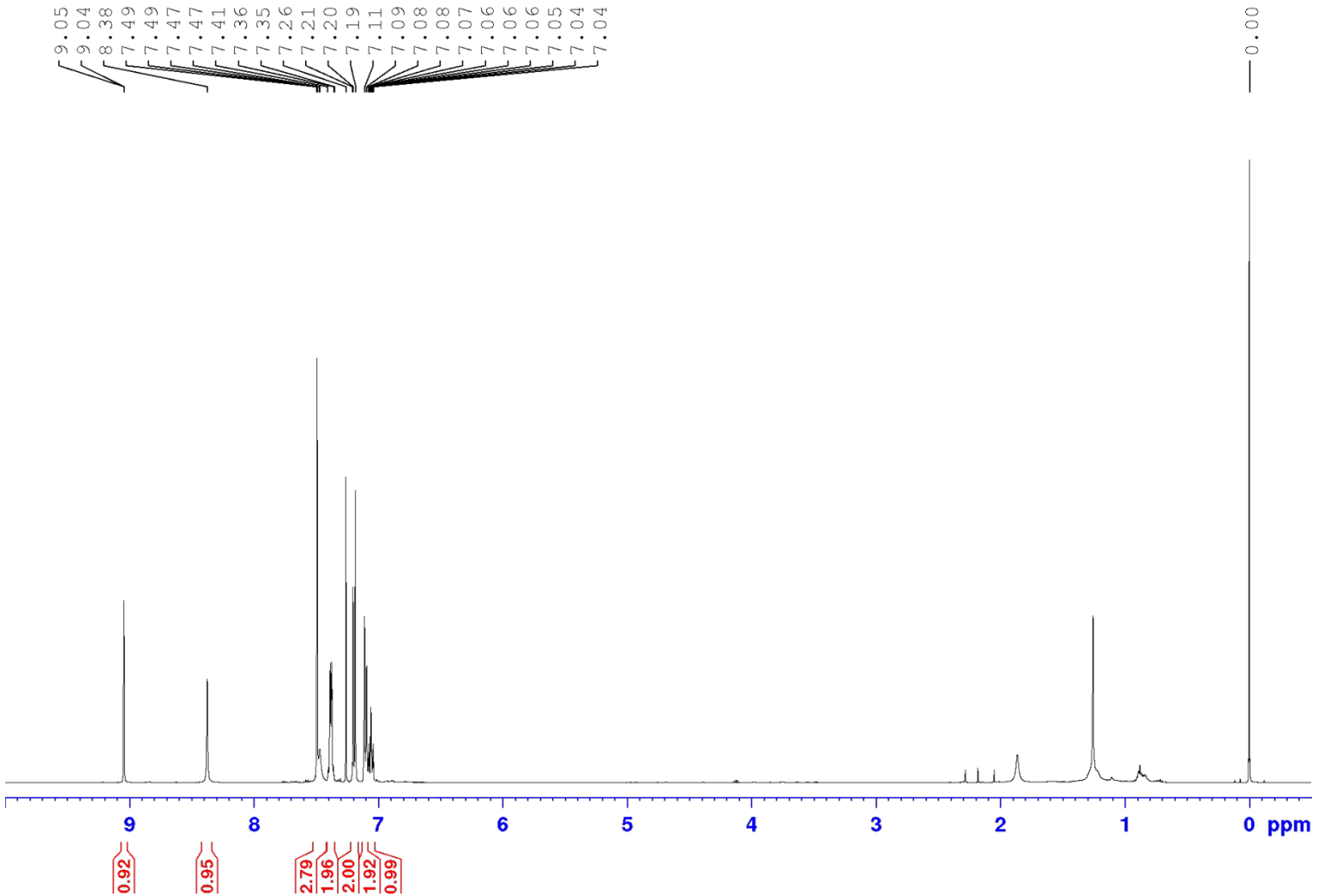


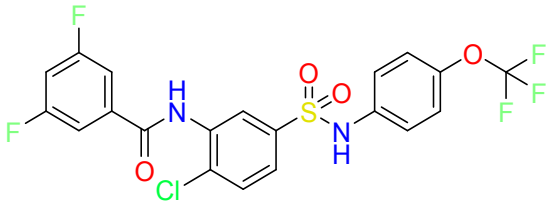
Compound **39** – ^{1}H NMR (500 MHz) – $CDCl_3$



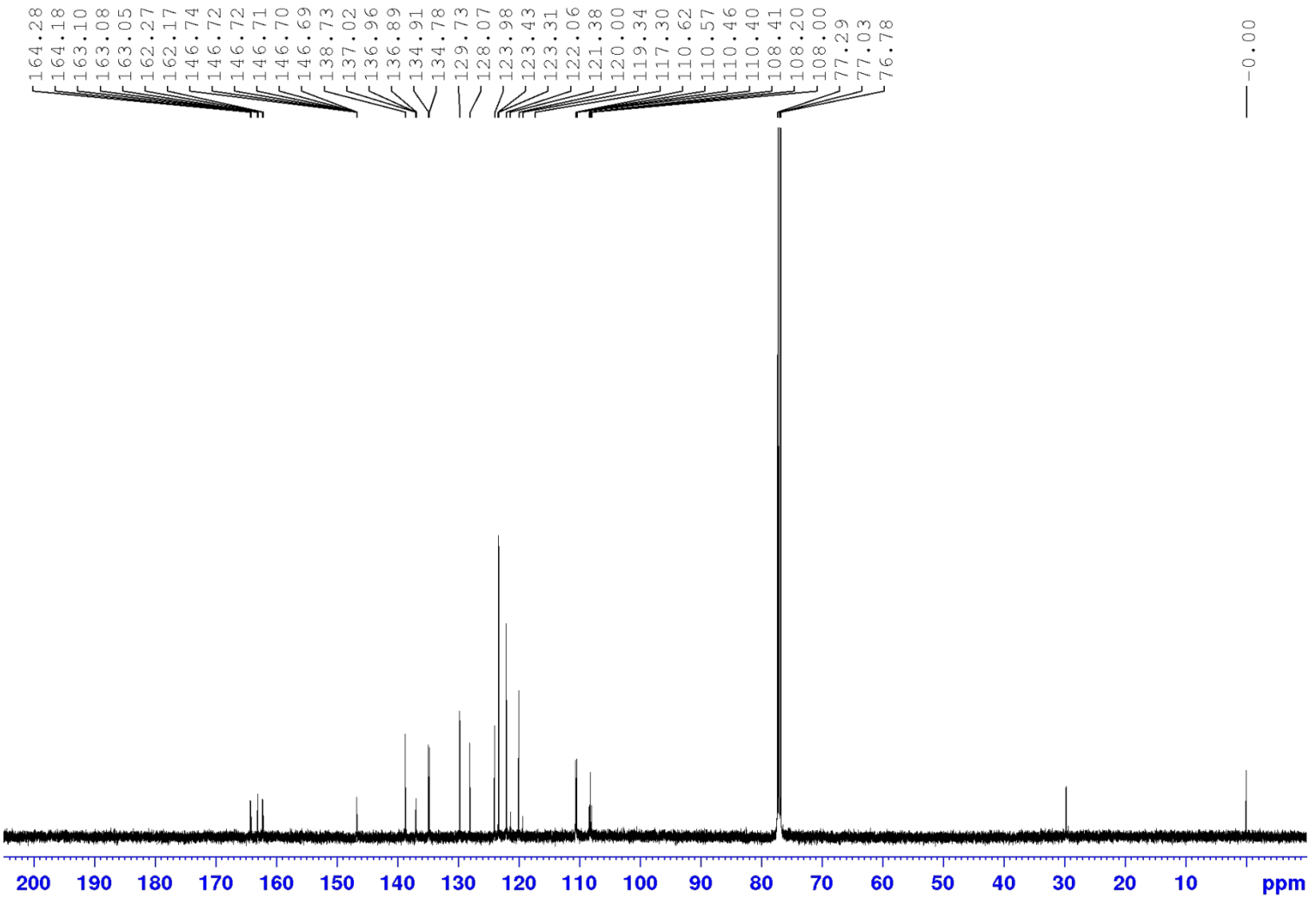


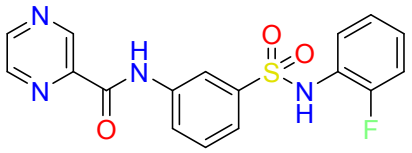
Compound **40** – ^{1}H NMR (500 MHz) – $CDCl_3$



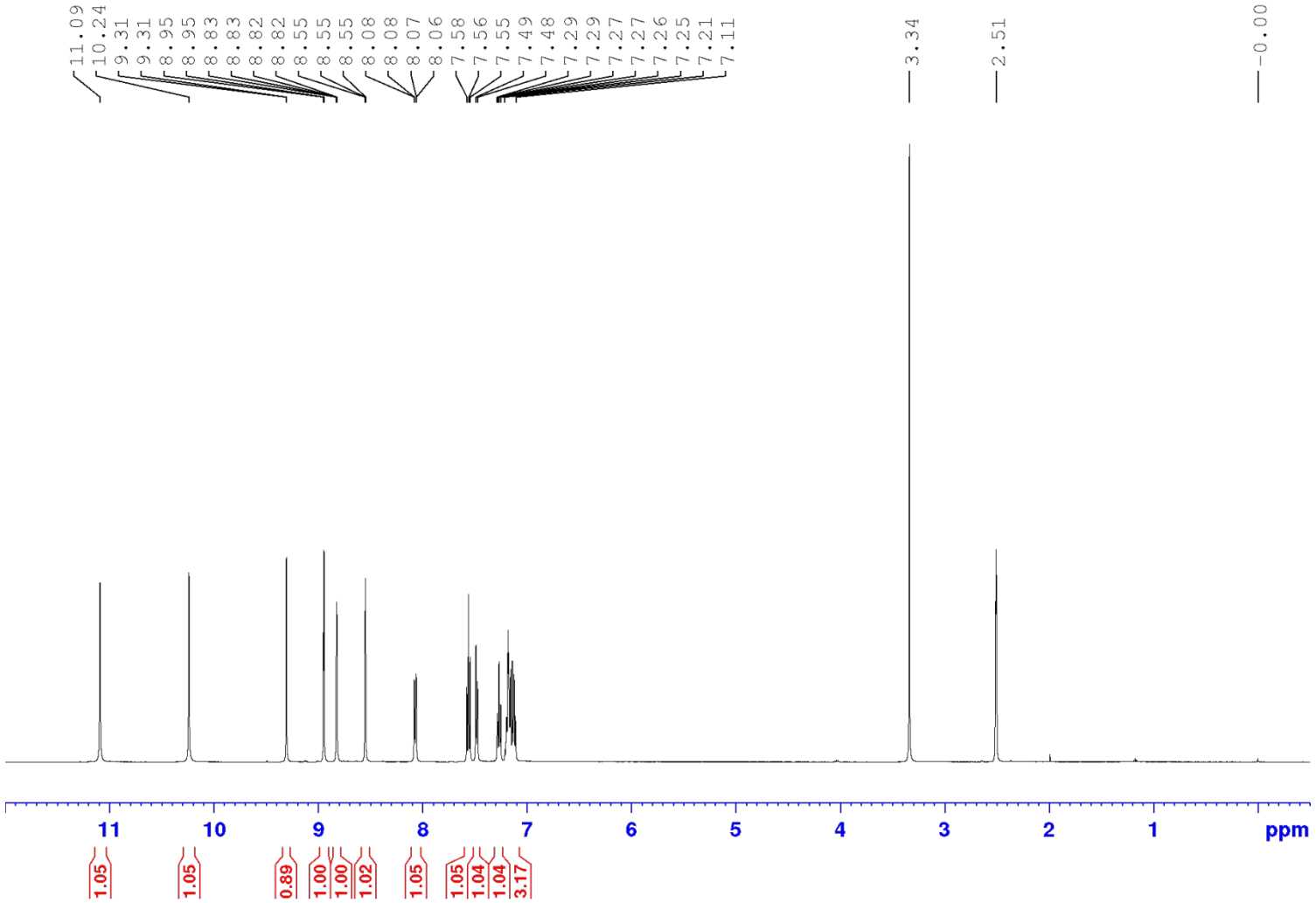


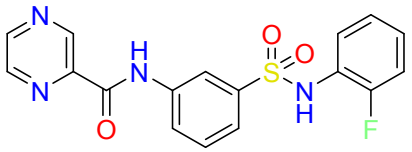
Compound **40** – ^{13}C NMR (125.5 MHz) – $CDCl_3$



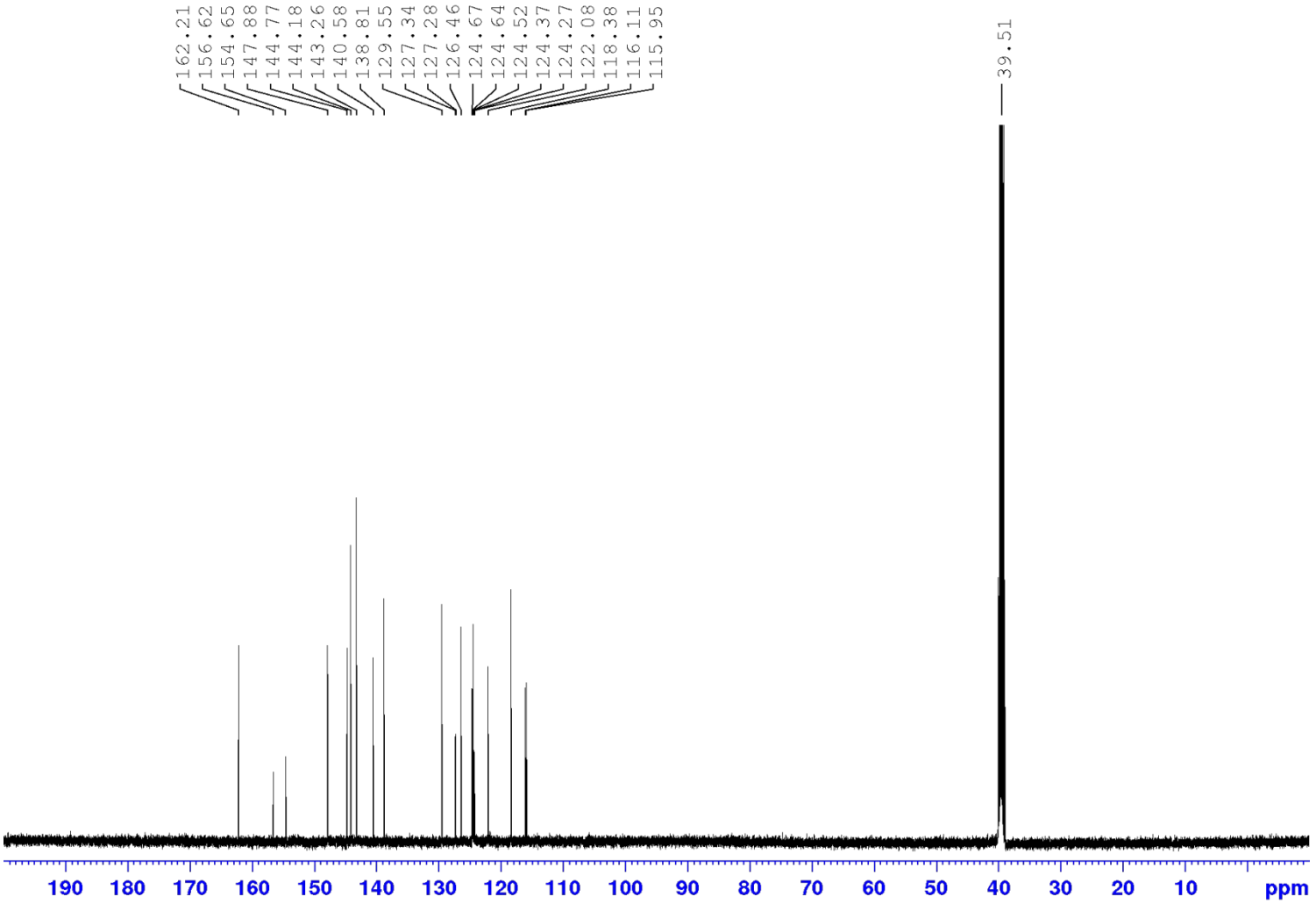


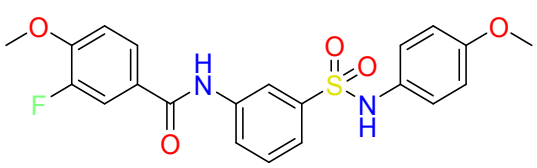
Compound **41** – ^{1}H NMR (500 MHz) – DMSO-d_6



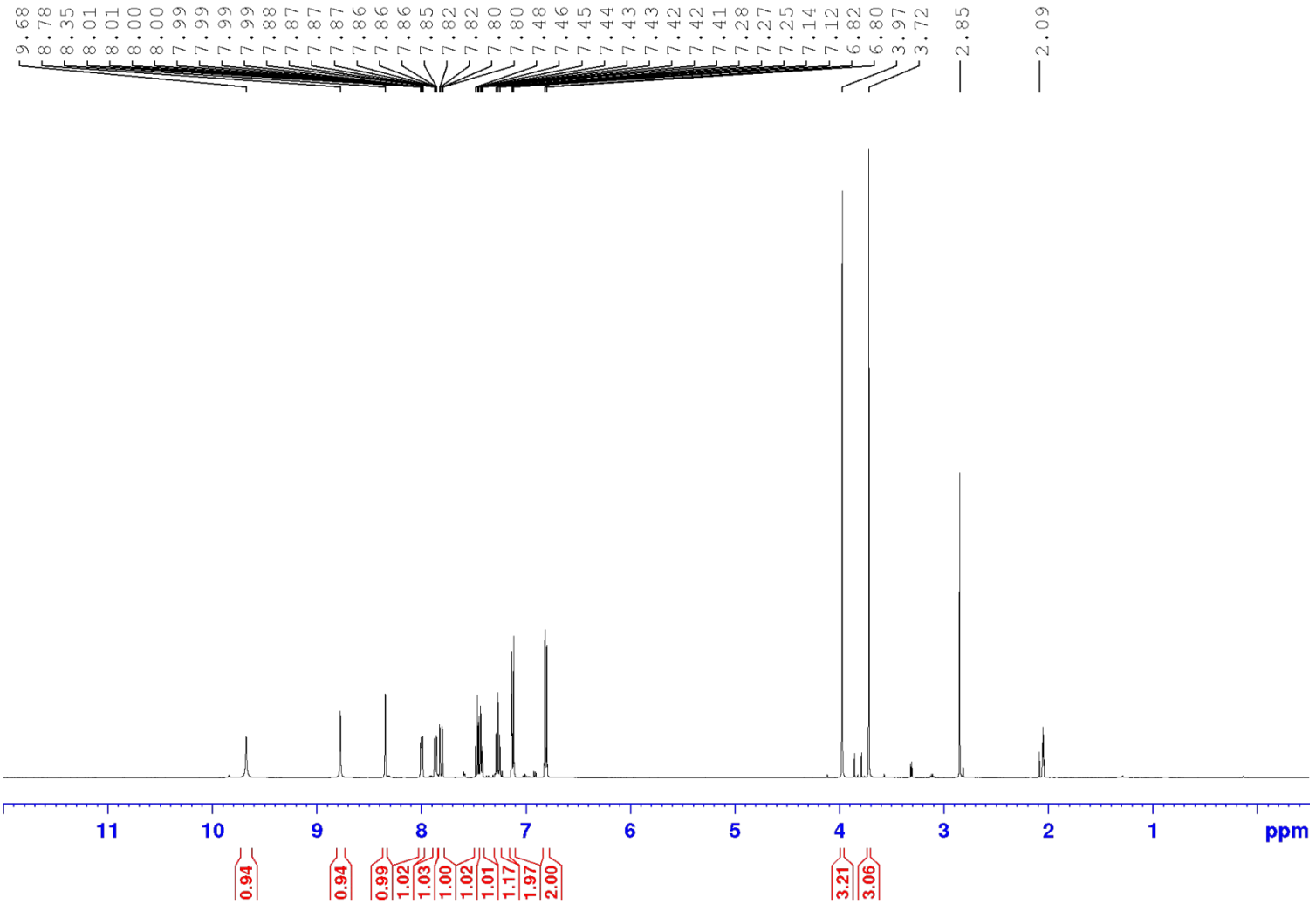


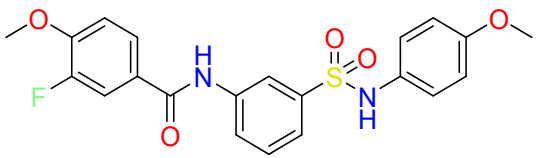
Compound **41** – ^{13}C NMR (125.5 MHz) – DMSO-d_6



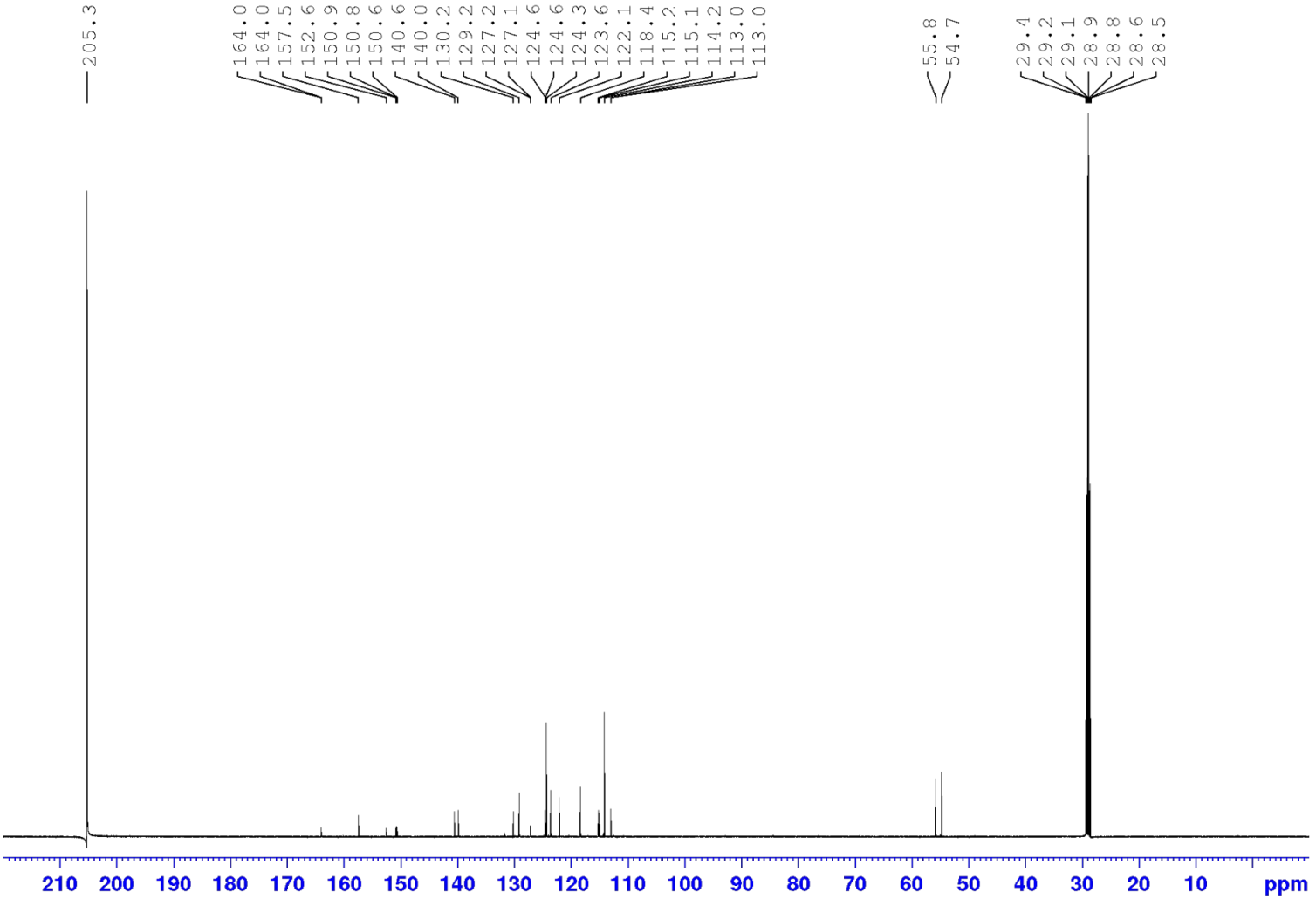


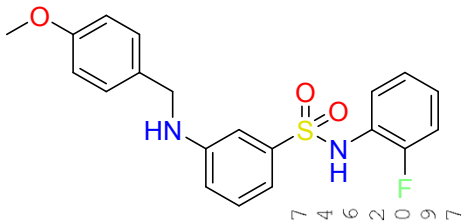
Compound **42** – ^{1}H NMR (500 MHz) – Acetone-d_6



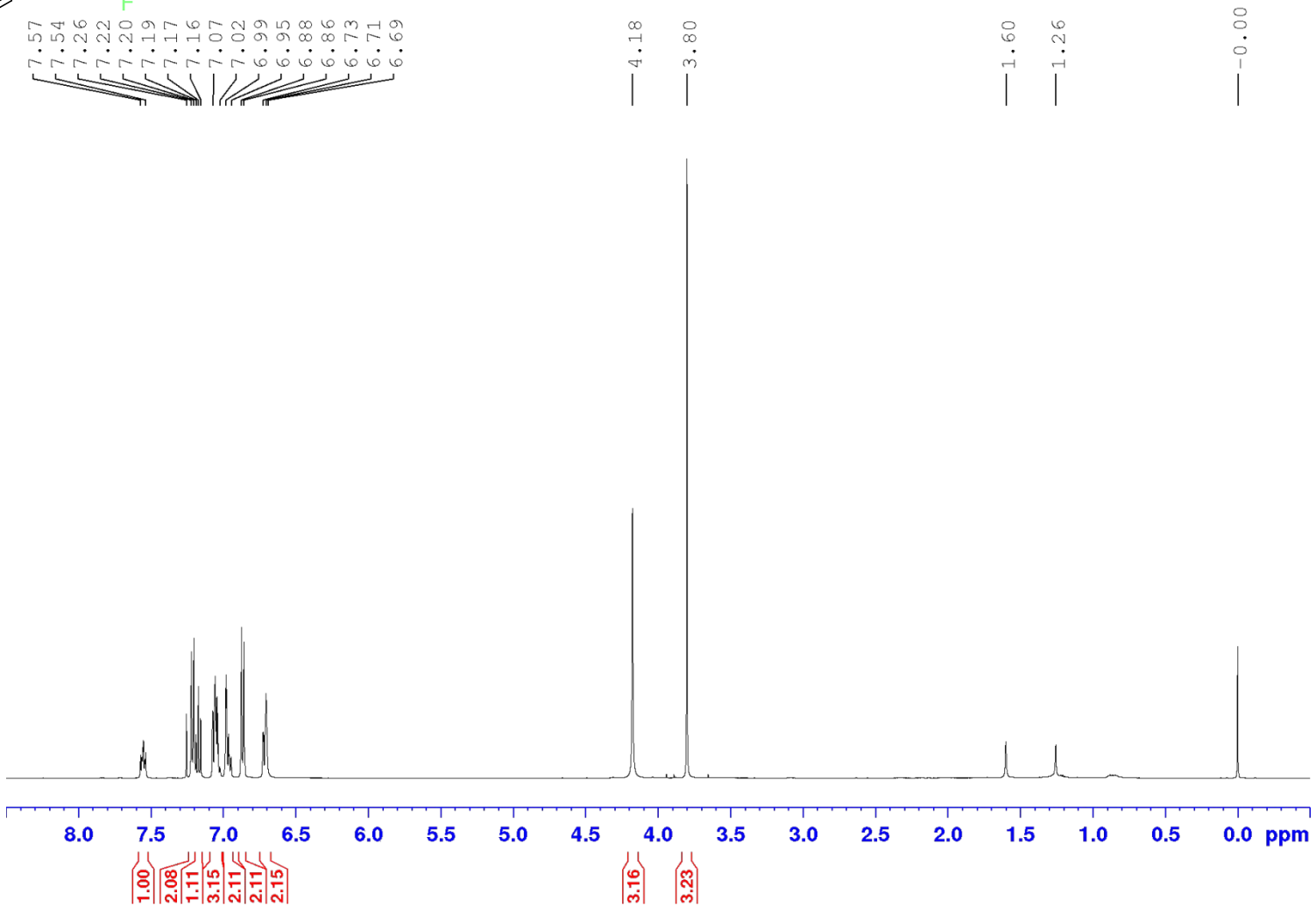


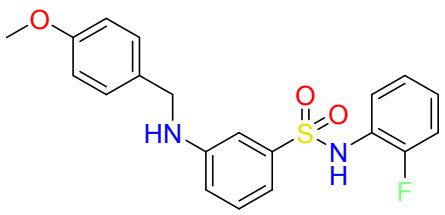
Compound **42** – ^{13}C NMR (125.5 MHz) – Acetone-d_6



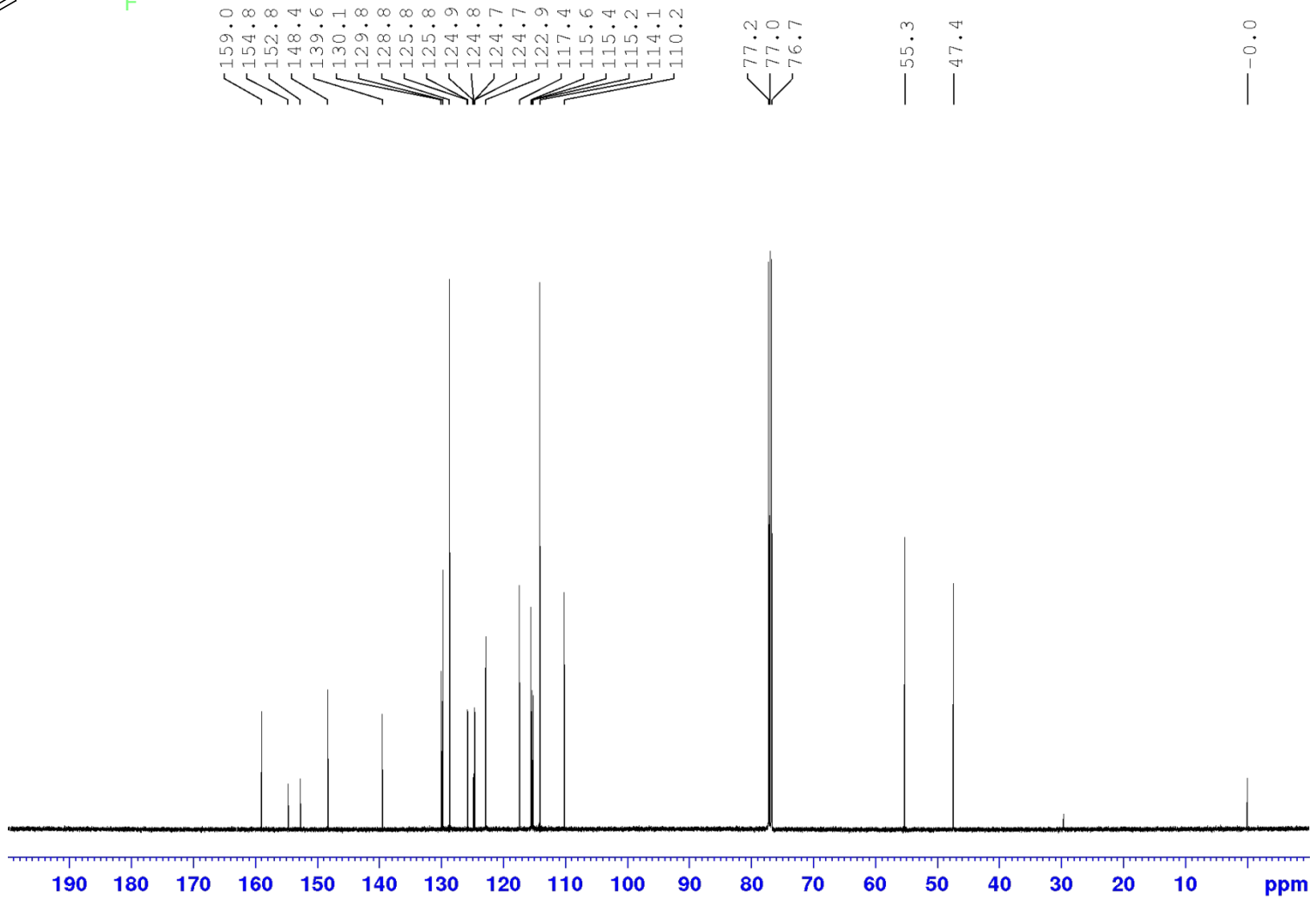


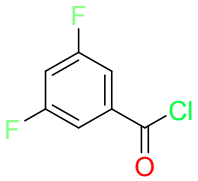
Compound **43** – ^{1}H NMR (500 MHz) – $CDCl_3$



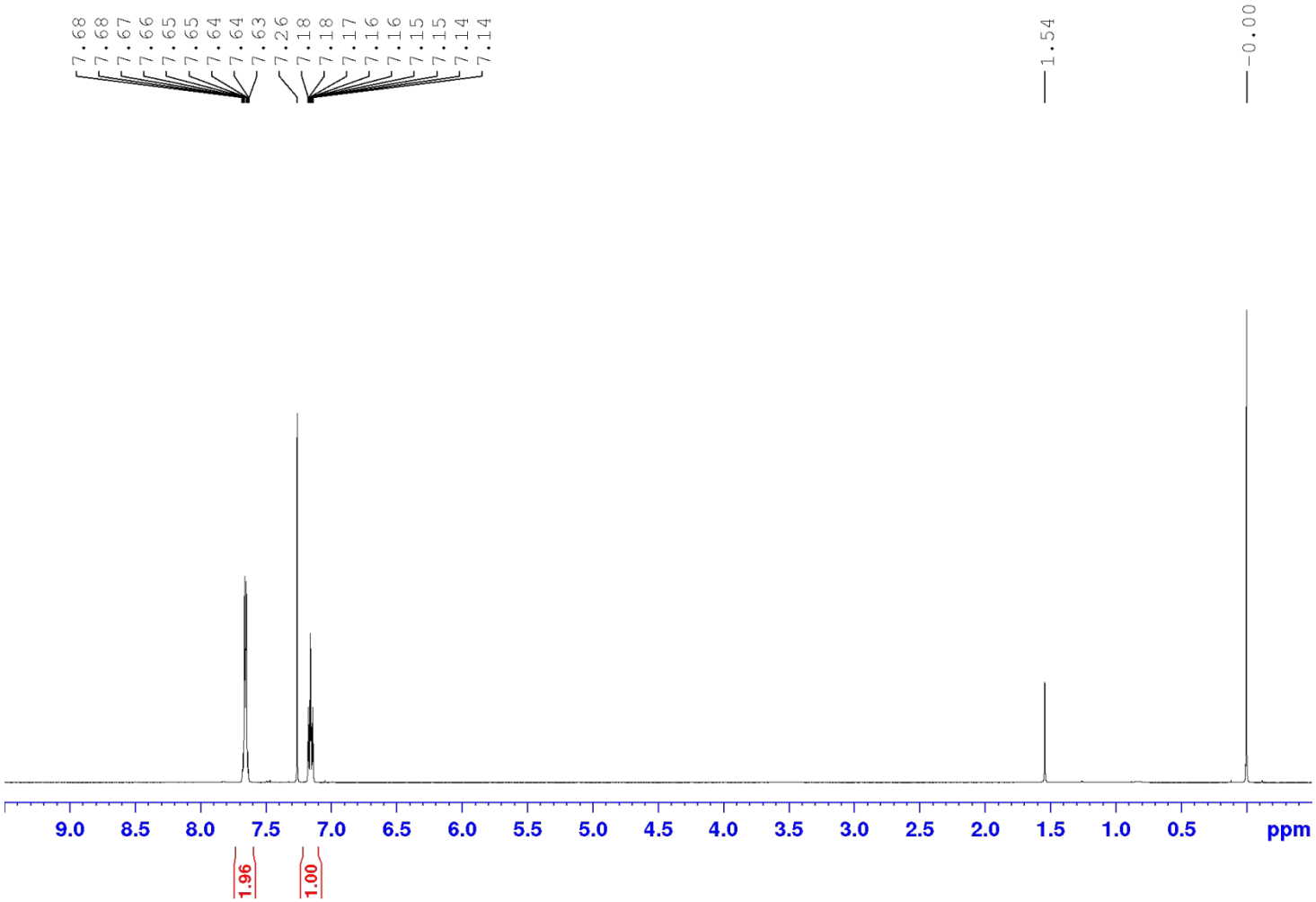


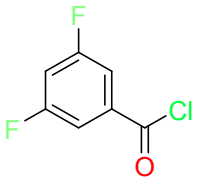
Compound **43** – ^{13}C NMR (125.5 MHz) – $CDCl_3$



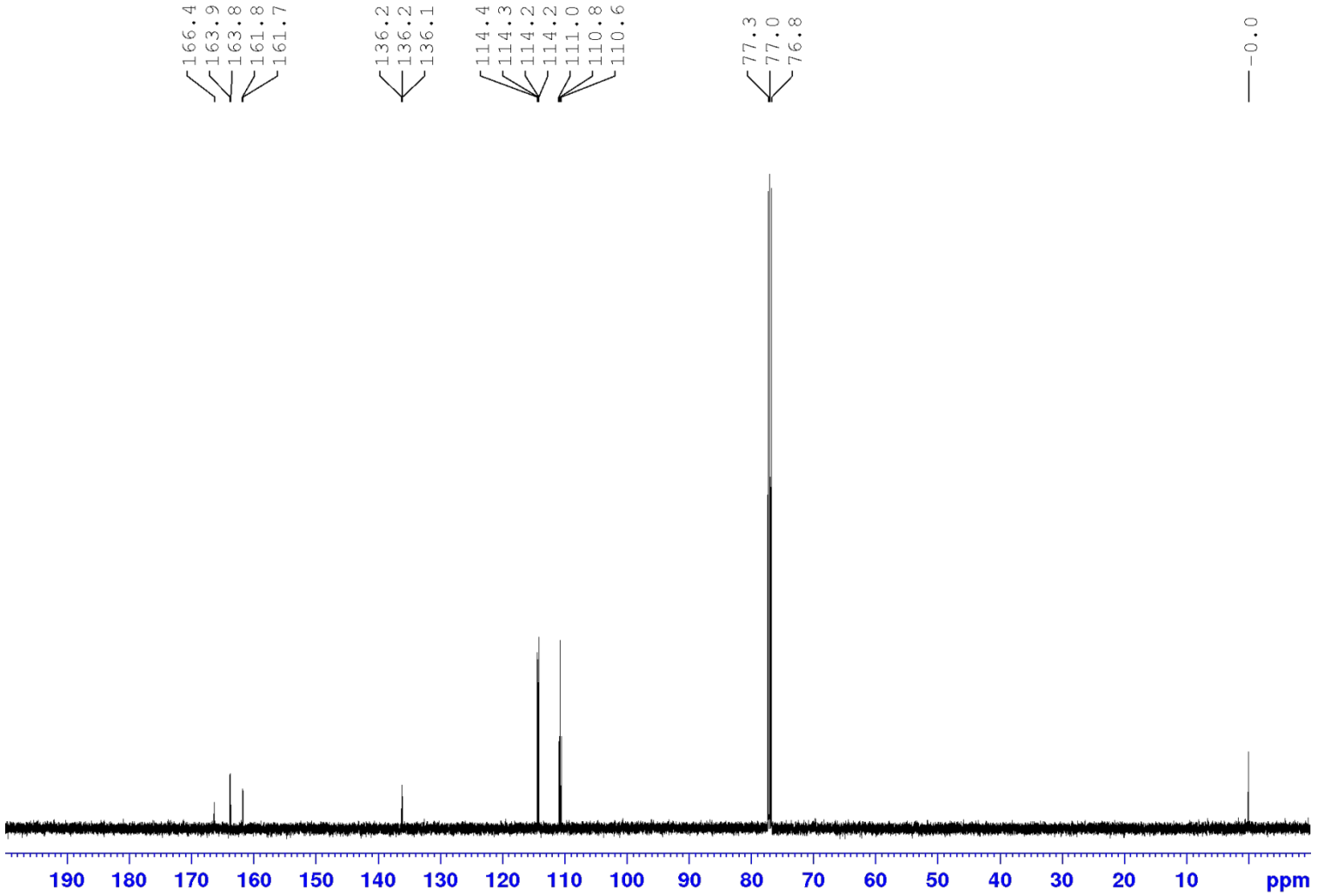


3,5-Difluorobenzoyl chloride – ^{1}H NMR (500 MHz) – $CDCl_3$





3,5-Difluorobenzoyl chloride – ^{13}C NMR (125.5 MHz) – $CDCl_3$



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