Electronic Supplementary Material (ESI) for Organic & Biomolecular Chemistry

Supplementary Information

Synthesis and Evaluation of *M. tuberculosis* Salicylate Synthase (MbtI) Inhibitors Designed to Probe Plasticity in the Active Site

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1. Experimental for Inhibitor Docking.

Calculations were performed on crystal structures of MbtI, using either (*E*)- or (*Z*)-3-phenylacrylate-bound structures, PDB ID: 3RV6. Each structure was prepared with the Protein Preparation Wizard, which is part of the Maestro software package (Maestro v9.2, Schrodinger, LLC, New York, NY, USA). Bond orders and formal charges were added for heteroatomic groups, and hydrogens were added to all atoms in the system. To optimize the hydrogen bond network, histidine tautomers and ionization states were predicted, 180° rotations of the terminal dihedral angle of Asn, Gln, and His residues were assigned, and hydroxyl and thiol hydrogen atoms were sampled. Crystal waters beyond 5 Å of the active site ligand were removed before performing calculations. For both structures, a brief relaxation was performed by using an all-atom constrained minimization carried out with the Impact Refinement module (Impref) (Impact v5.7 Schrodinger, LLC, New York, NY, USA) using the OPLS-2005 force field to alleviate steric clashes that may be present in the original crystal structures. The minimization was terminated when the energy converged or the RMSD reached a maximum cutoff of 0.30 Å for heavy atoms.

3D structures of ligands were generated with LigPrep (LigPrep v2.5, Schrodinger, LLC, New York, NY, USA). Docking calculations were performed with Glide (Glide v5.7, Schrodinger, LLC, New York, NY, USA). Energy grids were generated using the prepared structures described above. Each of the ligands was docked using the Standard Precision (SP) mode of Glide to estimate protein-ligand binding affinities. Z-isomer compounds were docked into the (Z)-3-phenylacrylate-bound structure; and similarly, E-isomer compounds were docked into the (E)-3-phenylacrylate-bound structure. Poses were then rescored with a more physically comprehensive description of binding contributions, using the molecular mechanics (MM) and a continuum solvation model GB/SA to represent water solvent.[1] Here, the Prime-MM-GB/SA protocol (Prime, v3.0, Schrodinger, LLC, New York, NY, USA) was implemented, which minimizes the ligand in the unbound state and minimizes the side chains (within 2 Å of the bound ligand) of complex states using OPLS-2005 and GB/SA.[1] The calculated relative ΔG_{bind} are presented in Table S1.

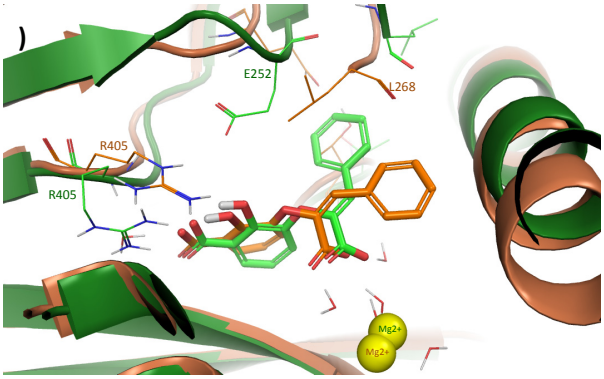
2. Predicted binding affinities of Inhibitors to MbtI

E-isomers:	Compound	ΔG_{bind} *(kcal/mol)*	Compound	ΔG_{bind} *(kcal/mol)*	Compound	ΔG_{bind} *(kcal/mol)*
	m-Br 25	-49.02	**o-Br 26**	**-49.5**	**p-Br 27**	-26.41
	m-Cl 28	-53.03	**o-Cl 29**	**-54.60**	**p-Cl 30**	-31.20
	m-Me 31	-48.87	**o-Me 32**	**-50.39**	**p-Me 33**	-31.73
	m-OH 34	**-56.29**	**o-OH 35**	-38.60	**p-OH 36**	-39.63
	m-CF_3 37	-42.94	**o-CF_3 38**	**-47.90**	**p-CF_3**[1]	-22.96
	Ph 6	-51.90				
Z-isomers:	Compound	ΔG_{bind} *(kcal/mol)*	Compound	ΔG_{bind} *(kcal/mol)*	Compound	ΔG_{bind} *(kcal/mol)*
	m-Br 25	-46.64	**o-Br 26**	**-50.14**	**p-Br 27**	-47.50
	m-Cl 28	**-55.56**	**o-Cl 29**	-48.68	**p-Cl 30**	-50.71
	m-Me 31	-44.5	**o-Me 32**	-34.38	**p-Me 33**	**-46.94**
	m-OH 34	-41.56	**o-OH 35**	**-46.68**	**p-OH 36**	-42.78
	m-CF_3 37	-40.55	**o-CF_3 38**	**-48.45**	**p-CF_3**[1]	-41.86
	Ph 6	-40.70				

[1]**Not tested *in vitro***

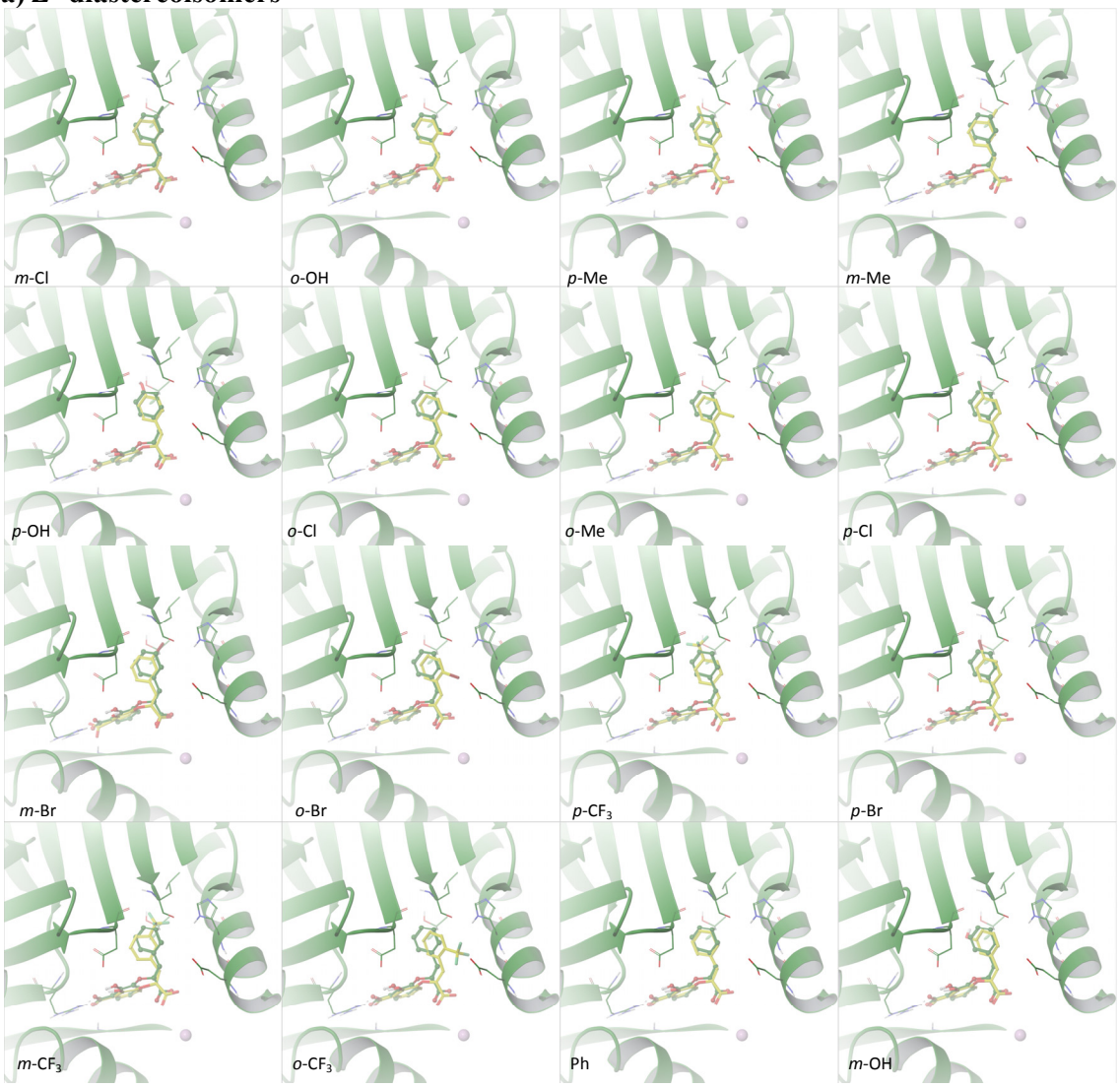
Table S1. Calculated free energies of binding of proposed inhibitors to MbtI.

3. Images of the protein-ligand docking of inhibitors (in the presence of the original diastereoisomer (*E* or *Z*) of the 3-phenylacrylate compound).

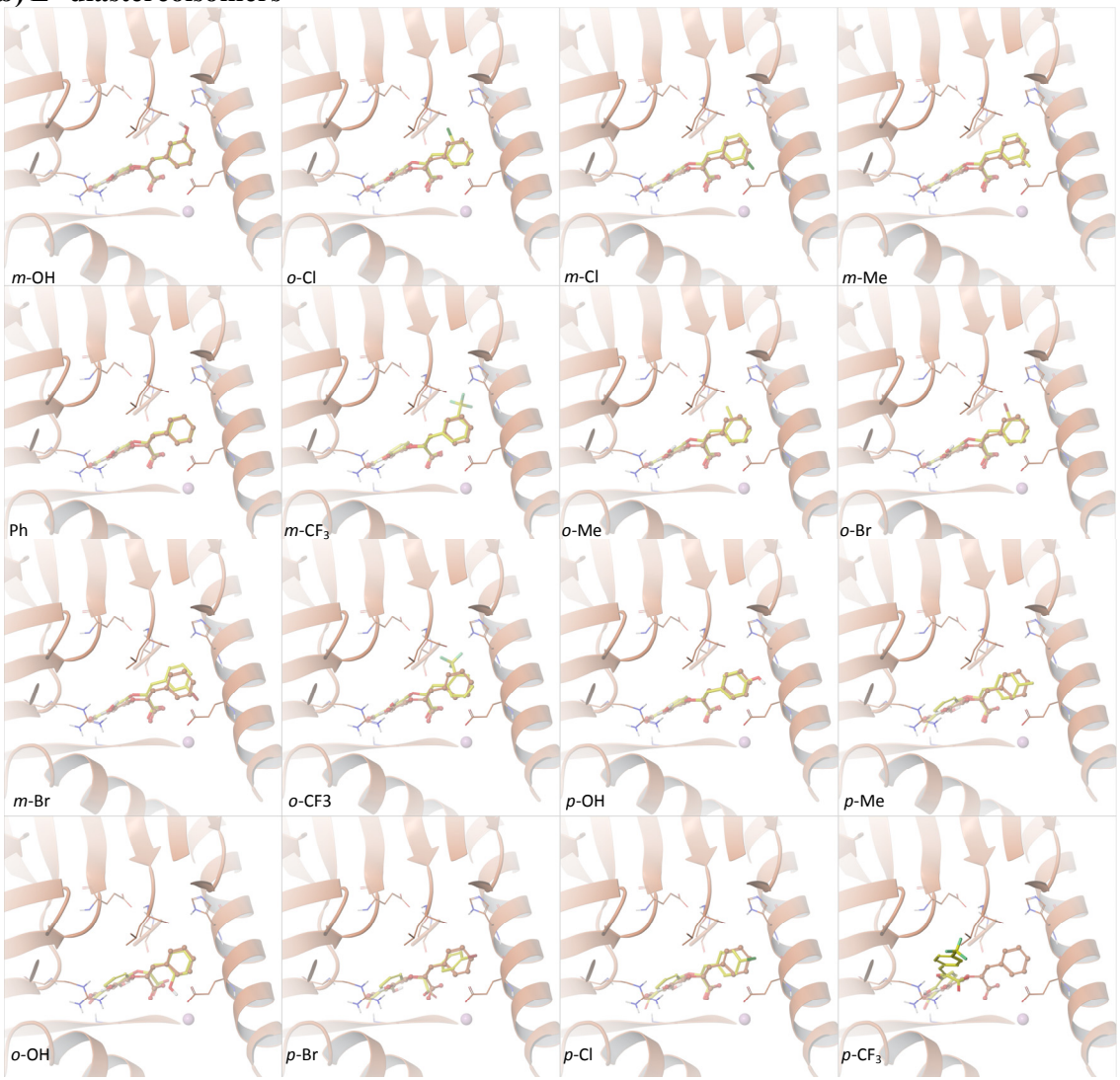


Inhibitor **6** (3-phenylacrylate-based) co-crystal structure: *(Z)*-**6** in green and (*E*)-**6** in orange. The loop region, residues 268-270, is displaced by the side chain.

a) *Z*- diastereoisomers



b) *E*- diastereoisomers



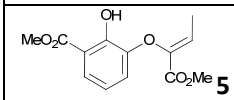
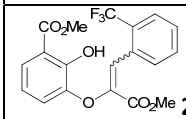
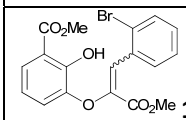
4. Conditions used for iron limiting *M. tuberculosis* whole cell inhibition studies.

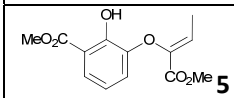
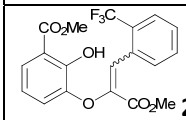
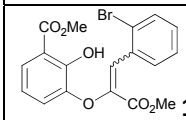
M. tuberculosis H37Ra (ATCC 25177) was grown in low iron media containing 10% OADC (Difco Laboratories, Detroit, MI, USA), 0.15% K_2HPO_4, 0.05% KH_2PO_4, 0.05% $MgSO_4$, 0.40% $(NH_4)_2SO_4$, 0.00005% $CaCl_2$, 0.00001% $ZnSO_4$, 0.00001% $CuSO_4$ and 0.05% Tween-80 in distilled water. For the purpose of iron limitation studies, four different iron concentrations were measured through addition of $FeCl_3$ to the low iron media: 20 mg (0.002% $FeCl_3$), 10 mg (0.001% $FeCl_3$), 1 mg (0.0001% $FeCl_3$) and 0 mg (0% $FeCl_3$). Freshly seeded cultures were grown at 37 °C, for approximately 14 days, to mid-exponential phase (OD600 0.4–0.8) for use in the inhibition assays. The effect of the inhibitors against *M. tuberculosis* growth were measured by a resazurin reduction microplate assay, using the procedure previously described by Taneja and Tyagi.[2] *M. tuberculosis* grown to mid-exponential phase was diluted to OD600 0.01 in the low iron media (10% OADC (Difco Laboratories, Detroit, MI, USA), 0.15% K_2HPO_4, 0.05% KH_2PO_4, 0.05% $MgSO_4$, 0.40% $(NH_4)_2SO_4$, 0.00005% $CaCl_2$, 0.00001% $ZnSO_4$, 0.00001% $CuSO_4$ and 0.05% Tween-80 in distilled water); 96-well microtiter plates were set up with 100 µL inhibitors, serially diluted into low iron media. Diluted *M. tuberculosis* (100 µL, representing ~$2x10^5$ CFU/mL) was added to each well. Plates were incubated for 5 days at 37 °C in a humidified incubator prior to the addition of a 0.02% resazurin solution (30 µL) and 20% Tween-80 (12.5 µL) to each well. Sample fluorescence was measured after 48 h on a BMG Labtech Polarstar Omega instrument with an excitation wavelength of 530 nm and emission at 590 nm. Changes in fluorescence relative to positive control wells (H37Ra with no inhibitor) minus negative control wells (no H37Ra) were plotted for determination of MIC_{50} values.

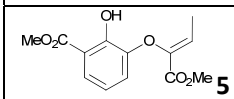
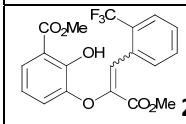
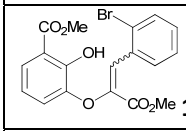
5. Preliminary results from iron limiting *M. tuberculosis* whole cell inhibition studies.

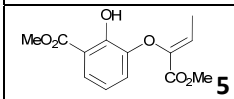
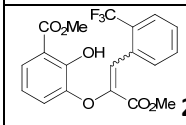
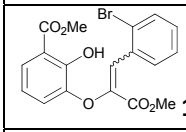
NB: - indicates that the compound did not inhibit more than 50% H37Ra growth at a concentration of 500 μM.

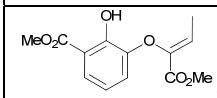
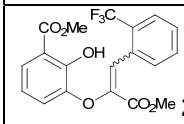
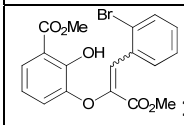
H37Ra OD600=0.01

	Run 1	Run 2	Run 3	Run 4
Compound	**MIC_{50} (μM) 0 mg Fe**	**MIC_{50} (μM) 0 mg Fe**	**MIC_{50} (μM) 0 mg Fe**	**MIC_{50} (μM) 0 mg Fe**
 **5**	-	-	-	-
 **24**	-	-	-	-
 **12**	200	120	-	-
Rifampicin (starting 0.02 μM)		1.7 nM	3 nM	15 nM
Rifampicin (starting 0.1 μM)			2.5 nM	8 nM

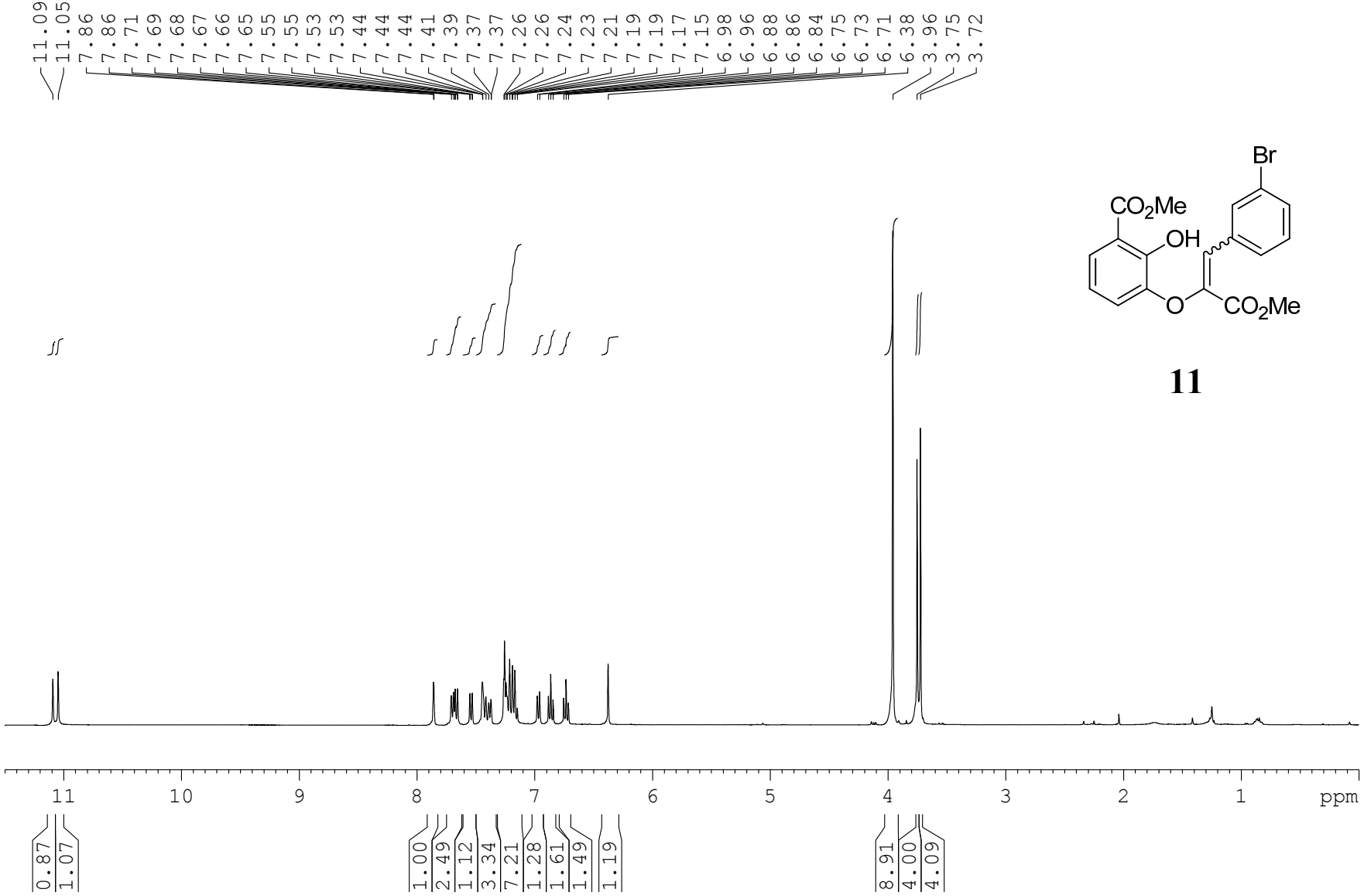
	Run 1	Run 2	Run 3	Run 4
Compound	**MIC_{50} (μM) 1 mg Fe**	**MIC_{50} (μM) 1 mg Fe**	**MIC_{50} (μM) 1 mg Fe**	**MIC_{50} (μM) 1 mg Fe**
 **5**	-	-	-	-
 **24**	-	-	-	-
 **12**	195	180	-	-
Rifampicin (starting 0.02 μM)		1.4 nM	2.5 nM	4 nM
Rifampicin (starting 0.1 μM)			2.5 nM	3 nM

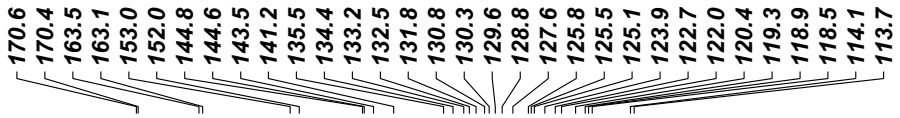
	Run 1	Run 2	Run 3	Run 4
Compound	**MIC_{50} (μM) 10 mg Fe**	**MIC_{50} (μM) 10 mg Fe**	**MIC_{50} (μM) 10 mg Fe**	**MIC_{50} (μM) 10 mg Fe**
 **5**	-	-	-	-
 **24**	-	-	-	-
 **12**	190	90	-	-
Rifampicin (starting 0.02 μM)		1.5 nM	2 nM	5 nM
Rifampicin (starting 0.1 μM)			2 nM	3 nM

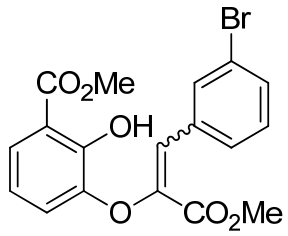
	Run 1	Run 2	Run 3	Run 4
Compound	**MIC_{50} (μM) 20 mg Fe**	**MIC_{50} (μM) 20 mg Fe**	**MIC_{50} (μM) 20 mg Fe**	**MIC_{50} (μM) 20 mg Fe**
 **5**	-	-	-	-
 **24**	-	-	-	-
 **12**	175	125	-	-
Rifampicin (starting 0.02 μM)		1.6 nM	2 nM	4 nM
Rifampicin (starting 0.1 μM)			2 nM	10 nM

	Run 1	Run 2	Run 3	Run 4
Compound	**MIC_{50} (µM) 7H9S media**	**MIC_{50} (µM) 7H9S media**	**MIC_{50} (µM) 7H9S media**	**MIC_{50} (µM) 7H9S media**
 **5**	-	-	-	-
 **24**	195	160	165	190
 **12**	-	240	-	-
Rifampicin (starting 0.02 µM)		7.5 nM	10 nM	16.5 nM
Rifampicin (starting 0.1 µM)			16 nM	20 nM

6. ^{1}H and ^{13}C NMR spectra of novel compounds 11-38







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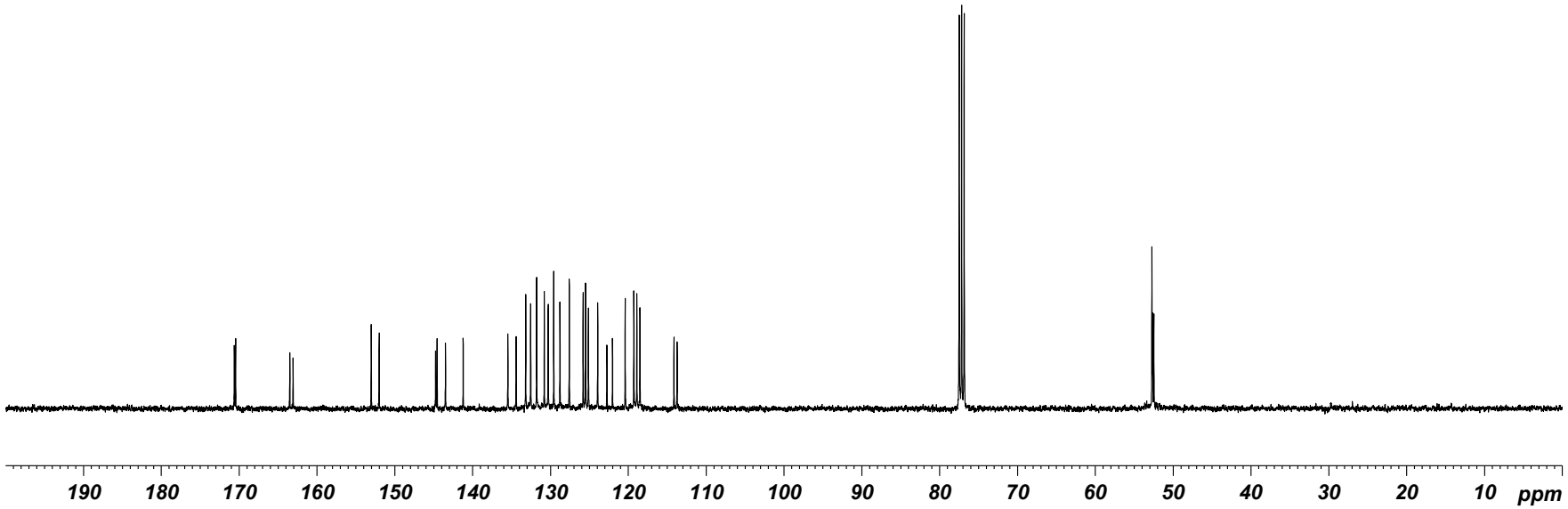
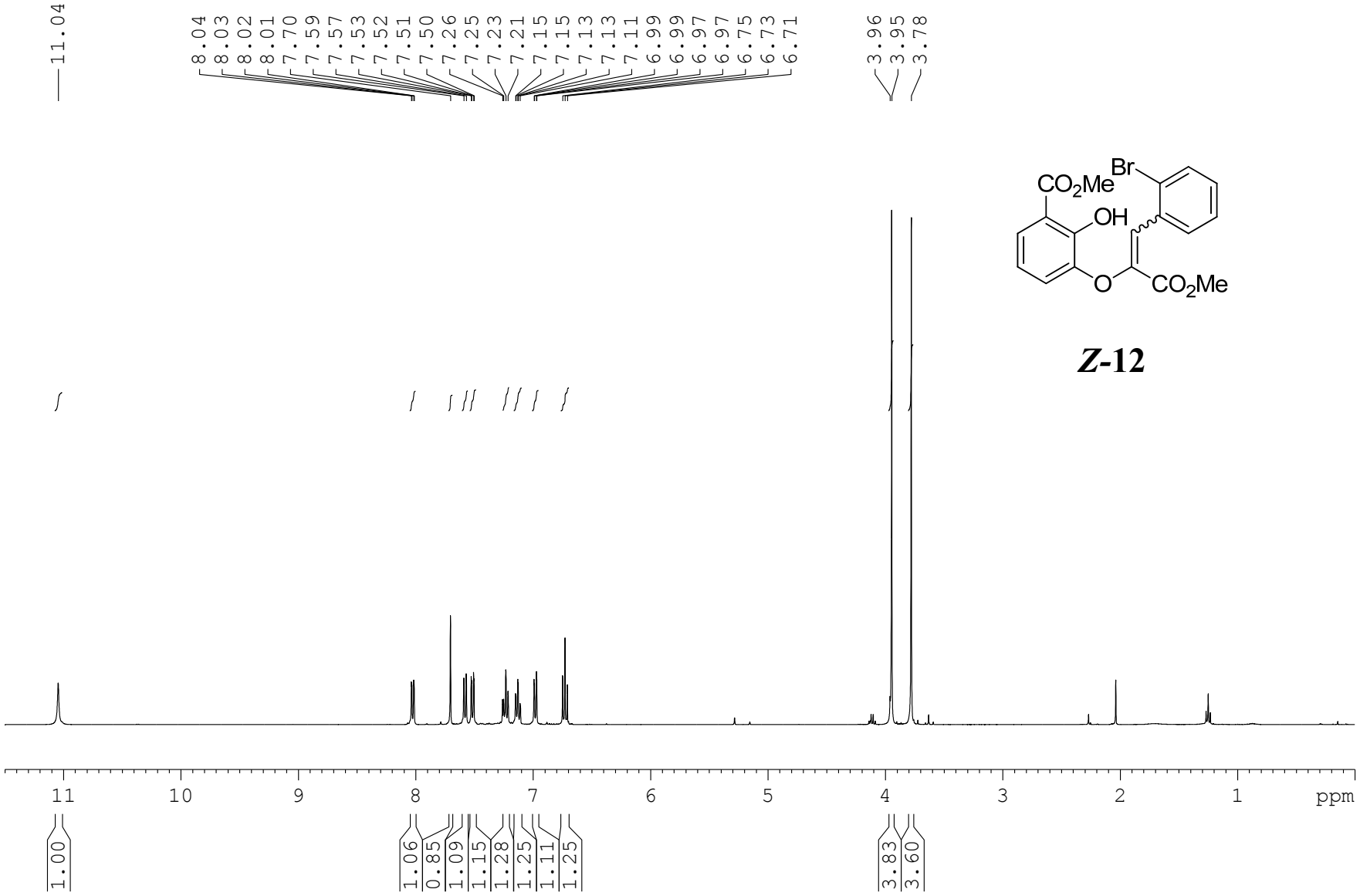
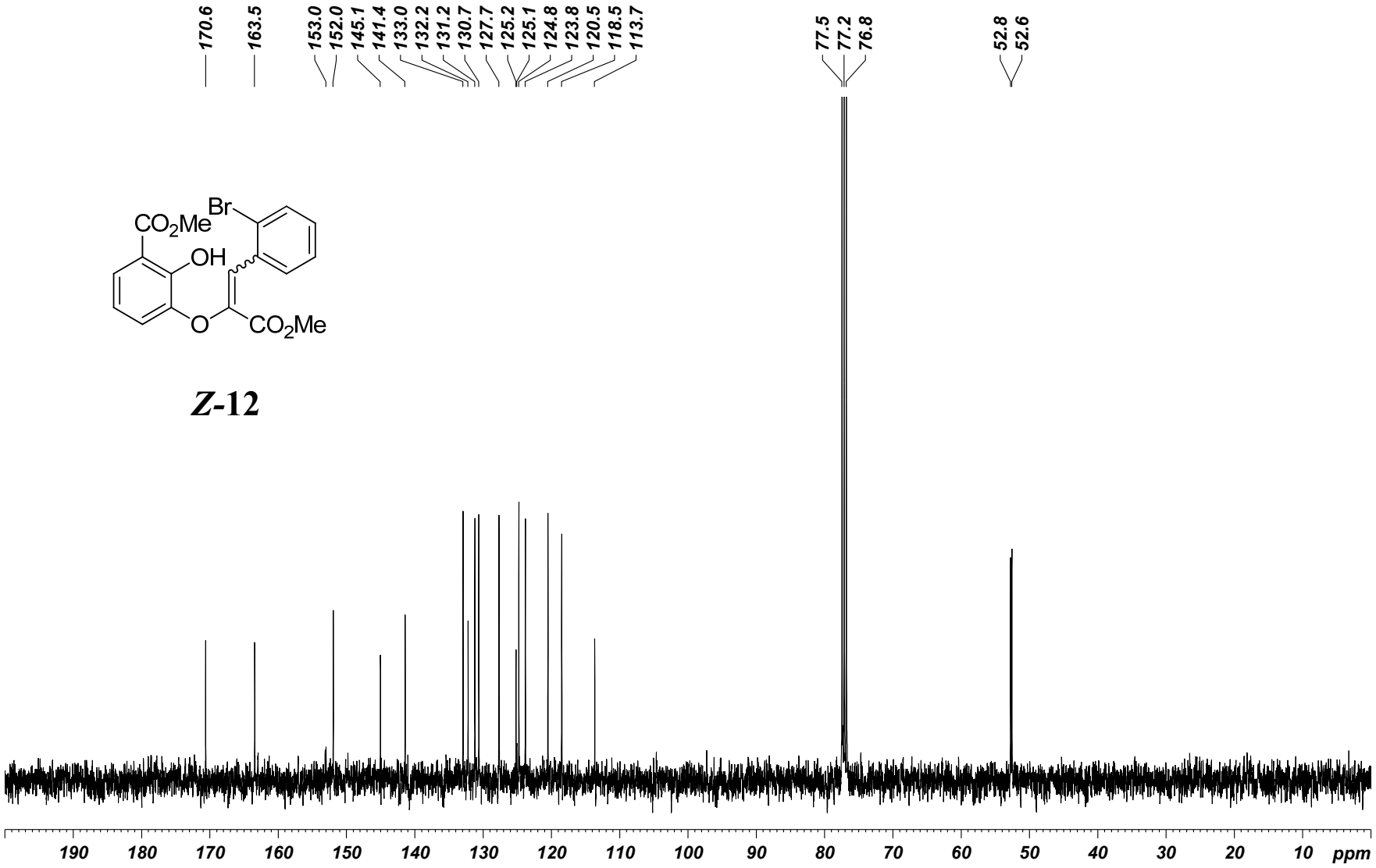
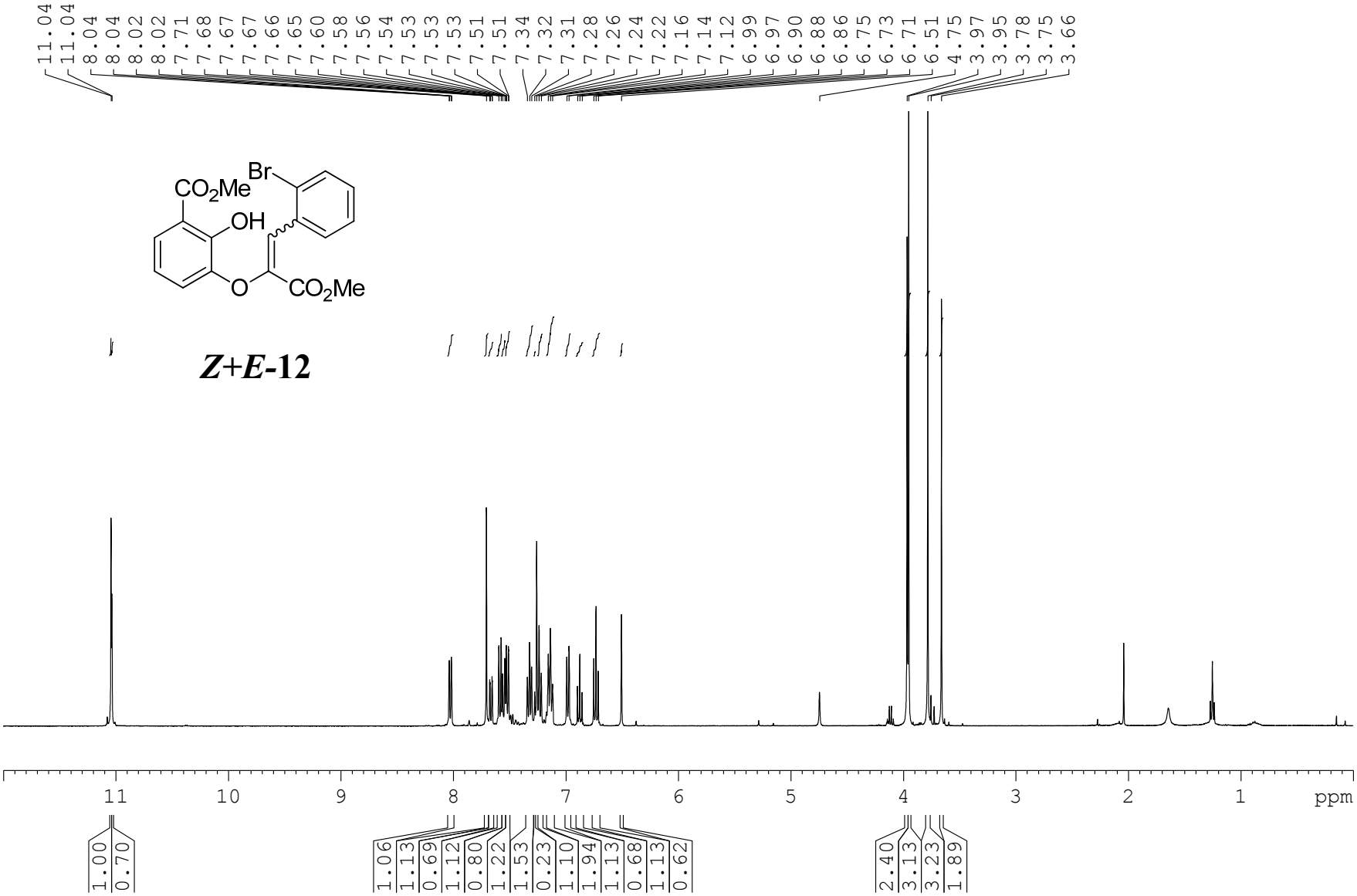


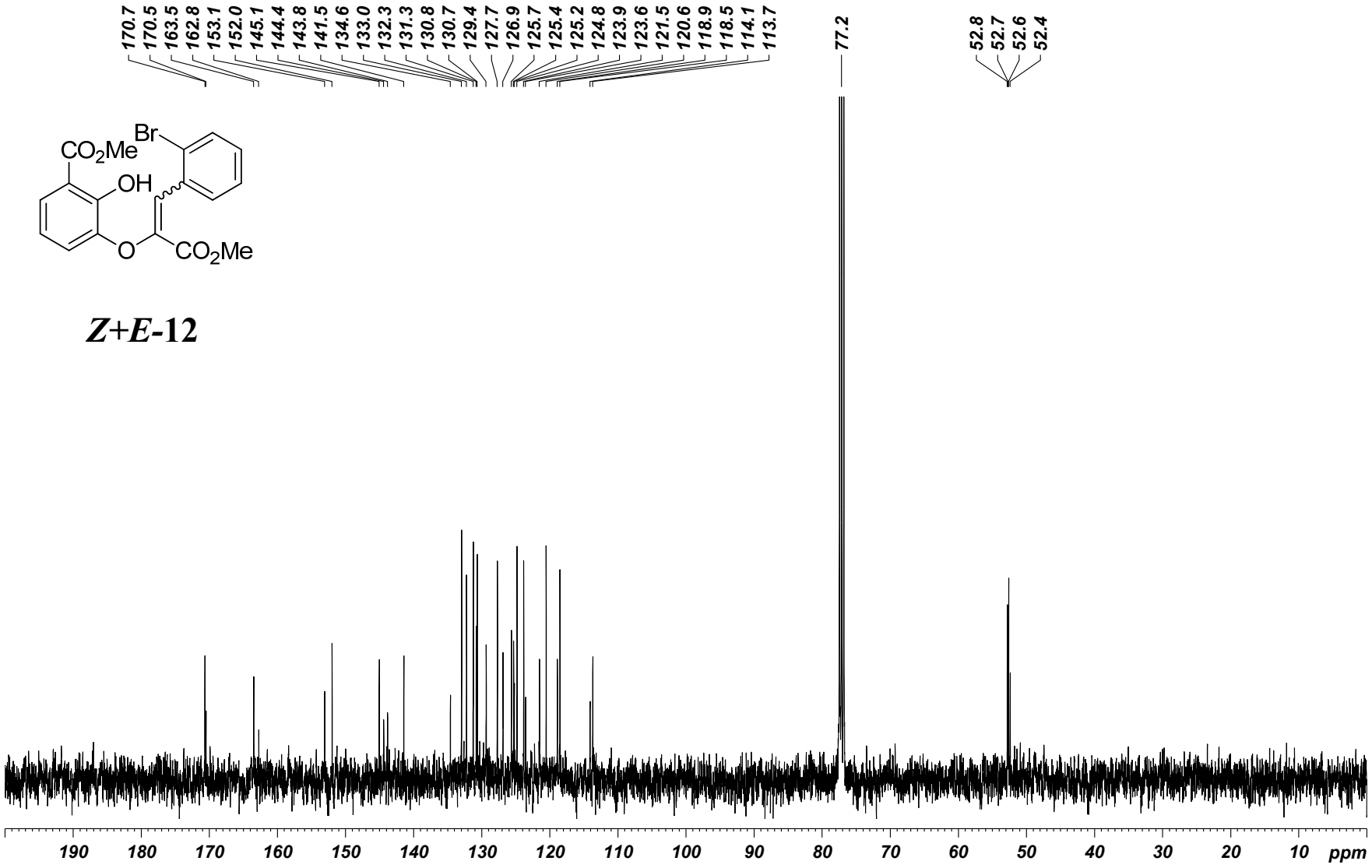




***Z*-12**

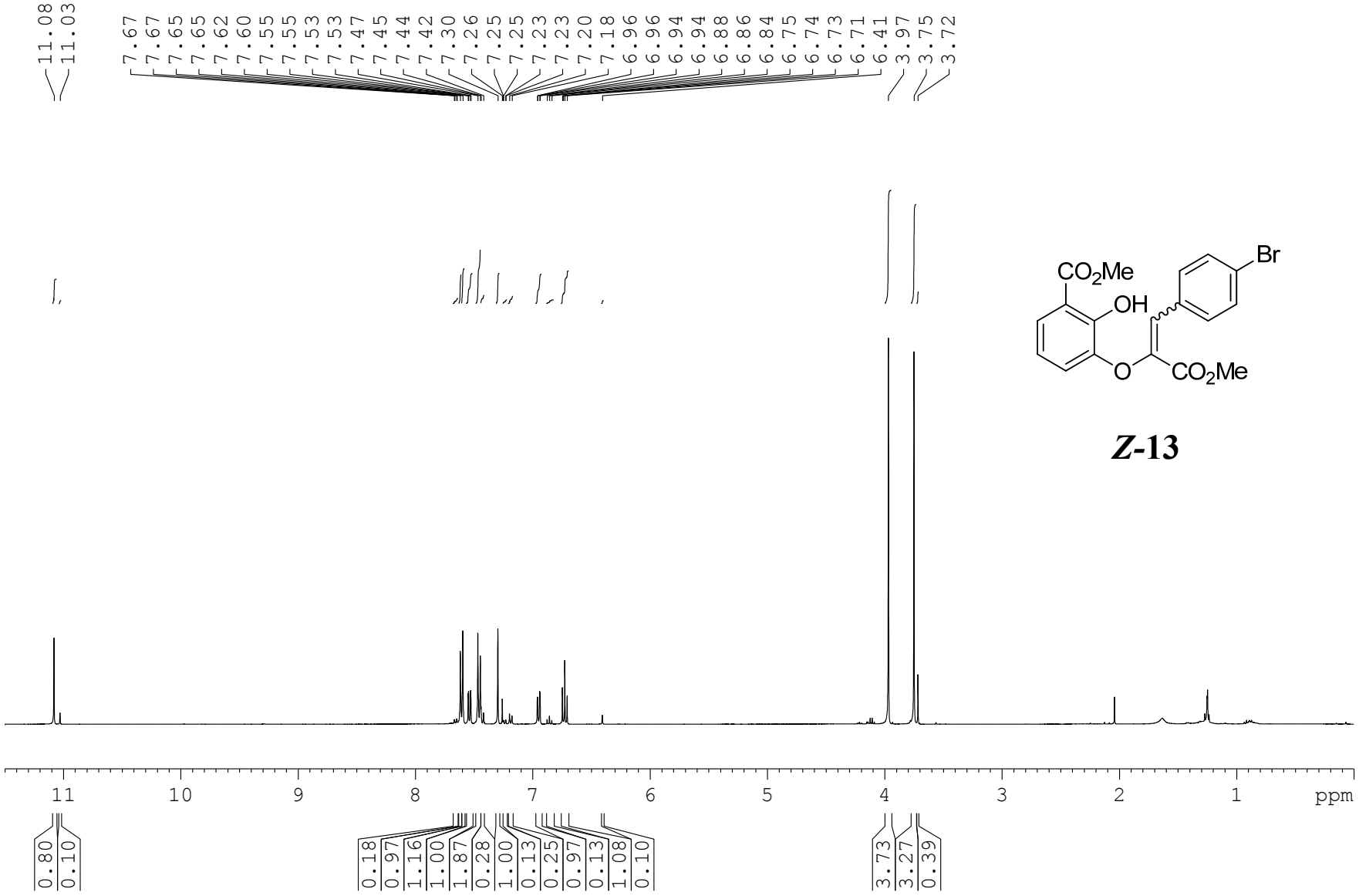


Note: Pure *Z*-isomer was isolated by column chromatography, and the ^{1}H and ^{13}C NMR spectra of the mixed *E* + *Z* fractions were used to assign the *E* diastereomer.



***Z*+*E*-12**

Note: Pure *Z*-isomer was isolated by column chromatography, and the ^{1}H and ^{13}C NMR spectra of the mixed *E* + *Z* fractions were used to assign the *E* diastereomer.



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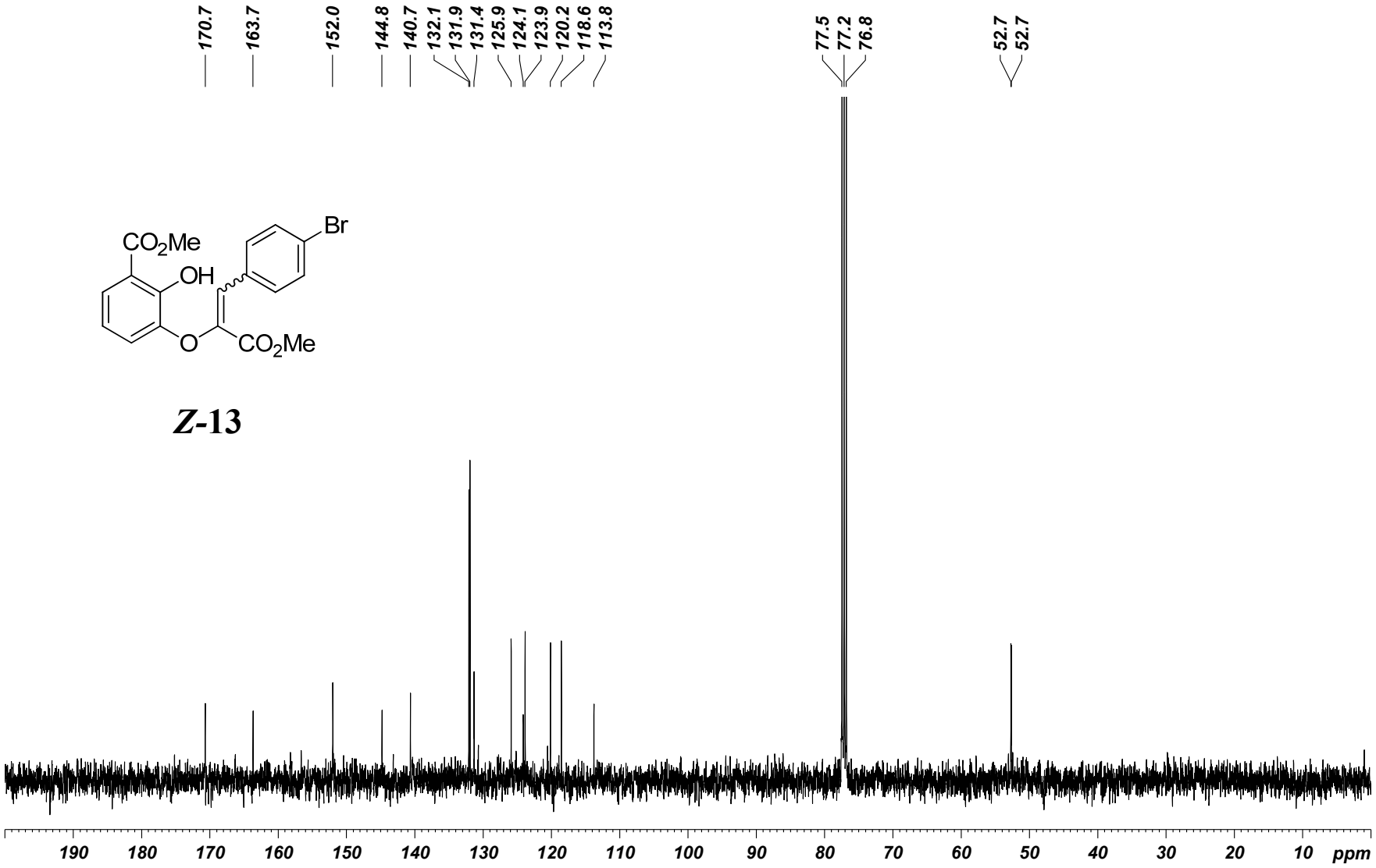

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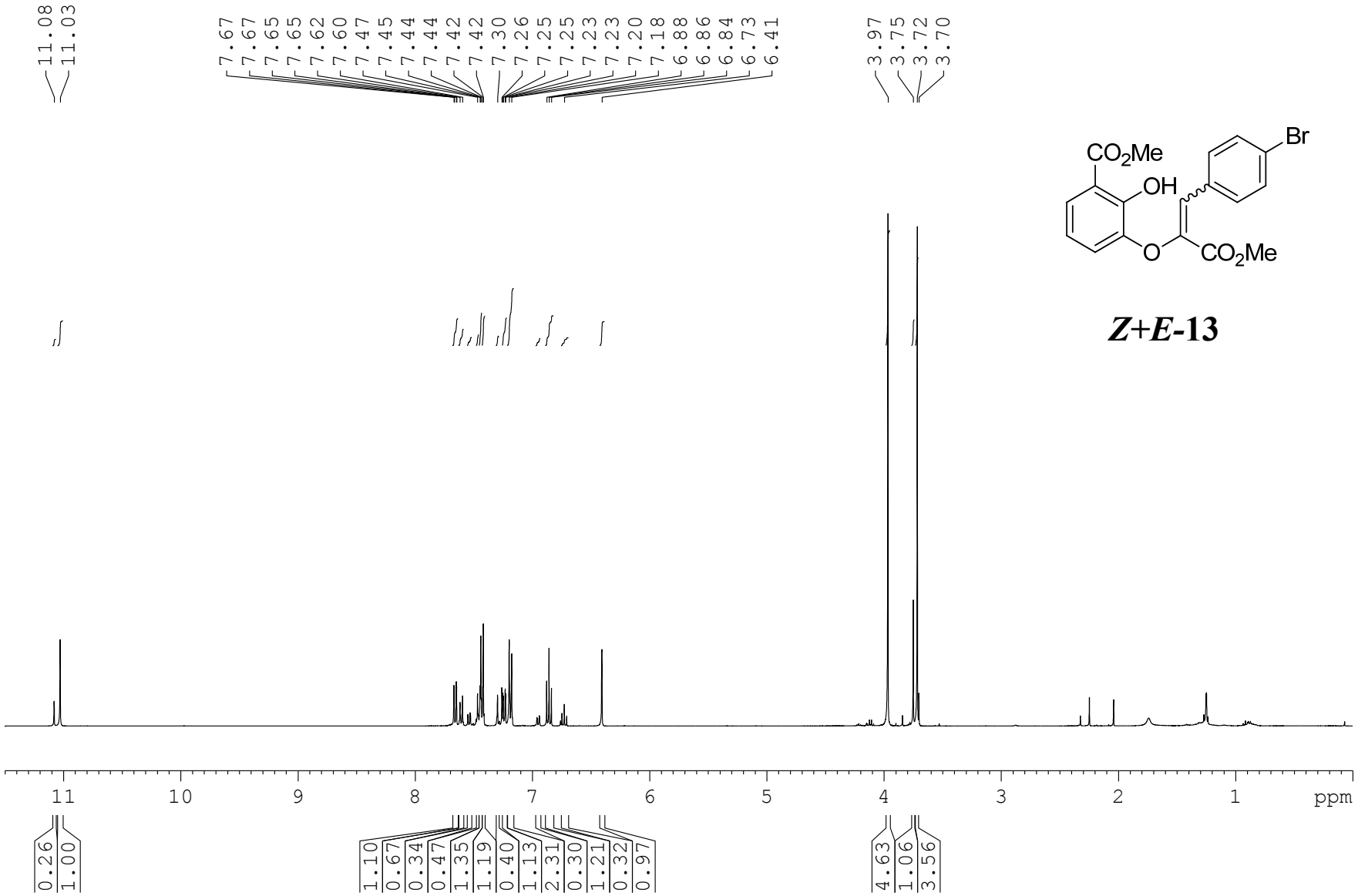
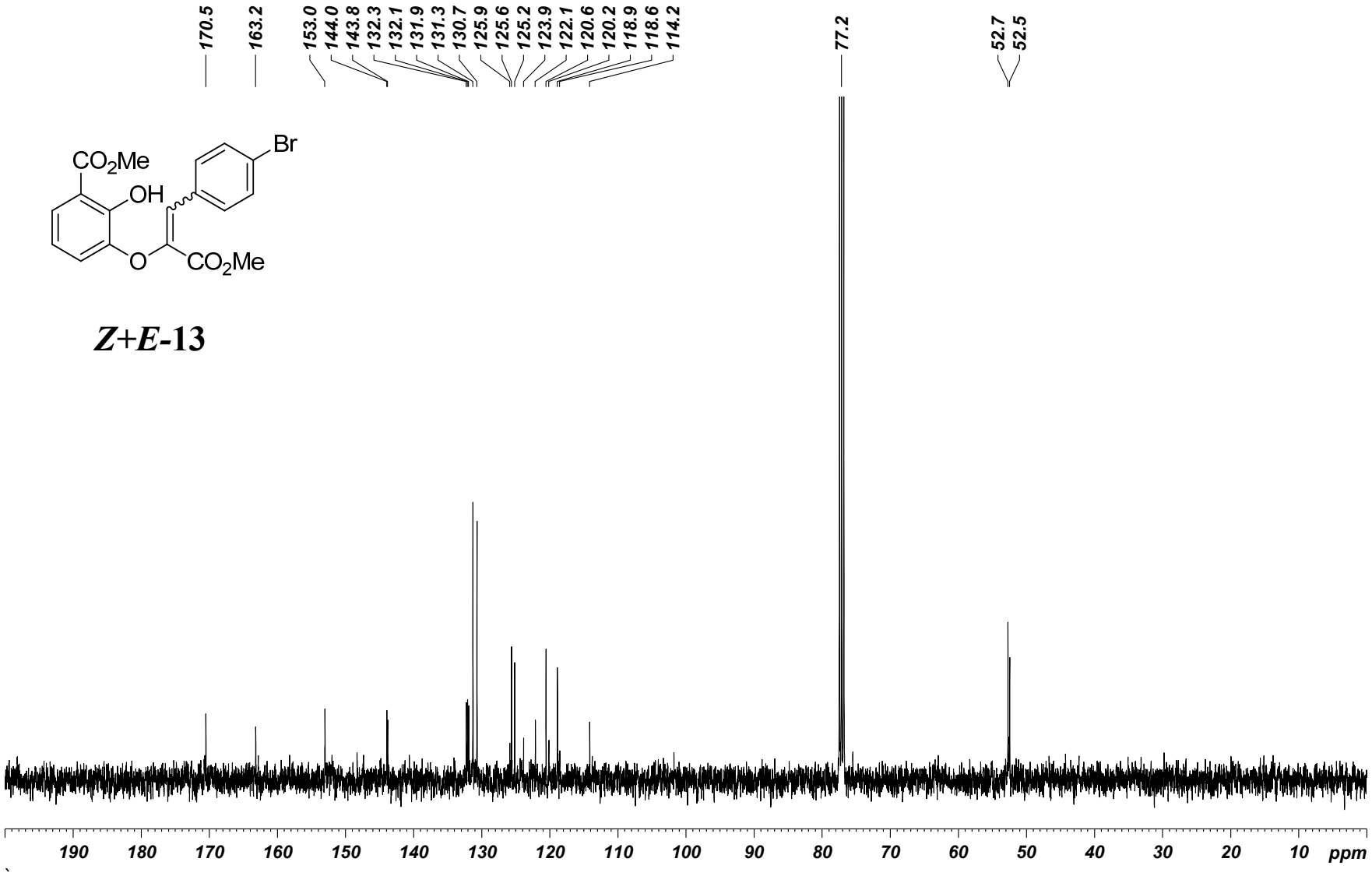
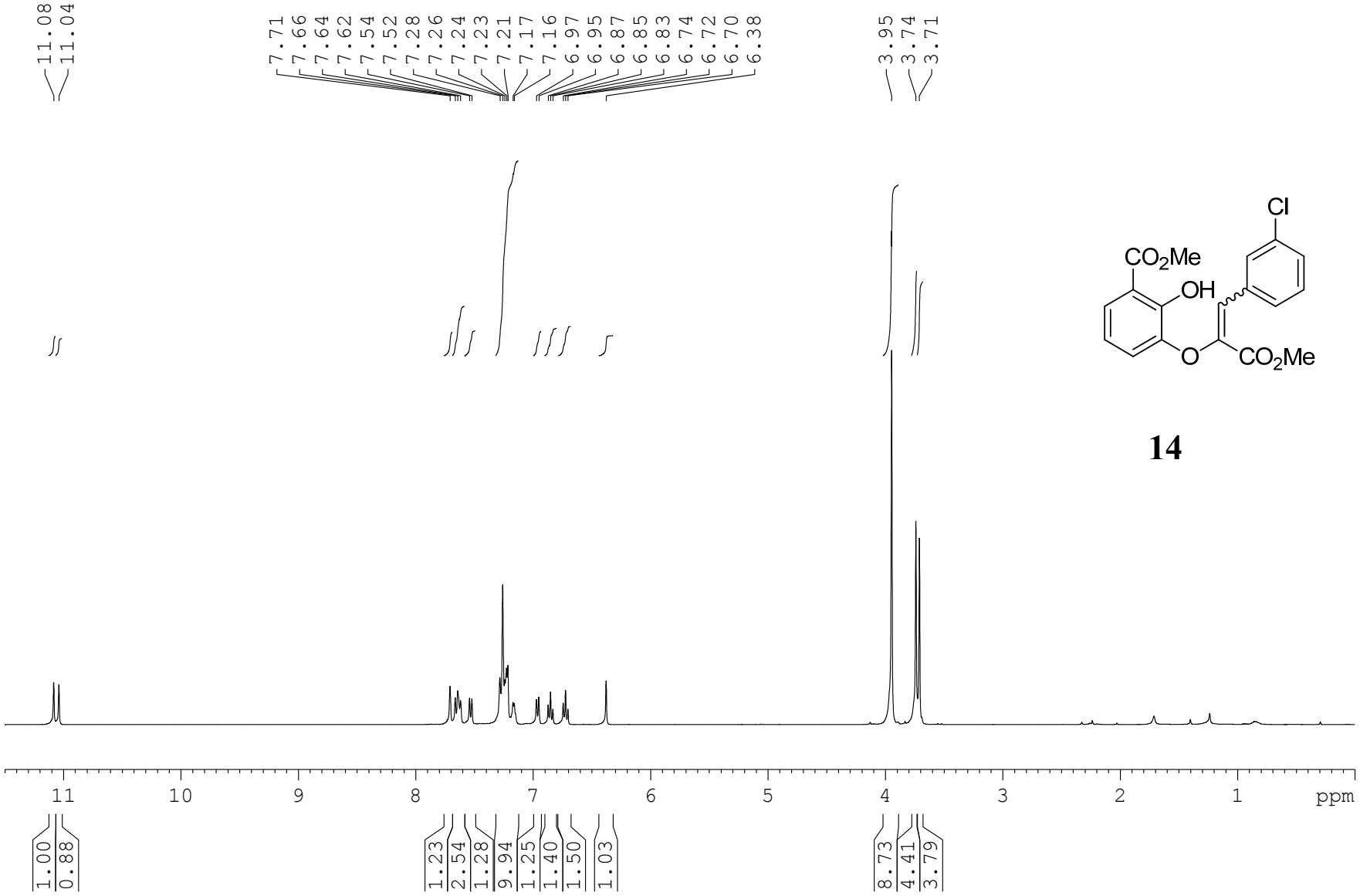


Note: Pure *Z*-isomer was isolated by column chromatography, and the ^{1}H and ^{13}C NMR spectra of the mixed *E* + *Z* fractions were used to assign the *E* diastereomer.



***Z*+*E*-13**

Note: Pure *Z*-isomer was isolated by column chromatography, and the ^{1}H and ^{13}C NMR spectra of the mixed *E* + *Z* fractions were used to assign the *E* diastereomer.



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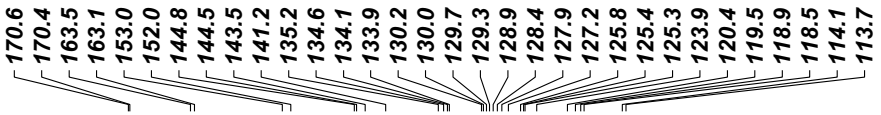

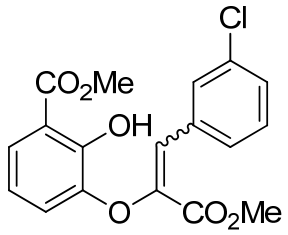

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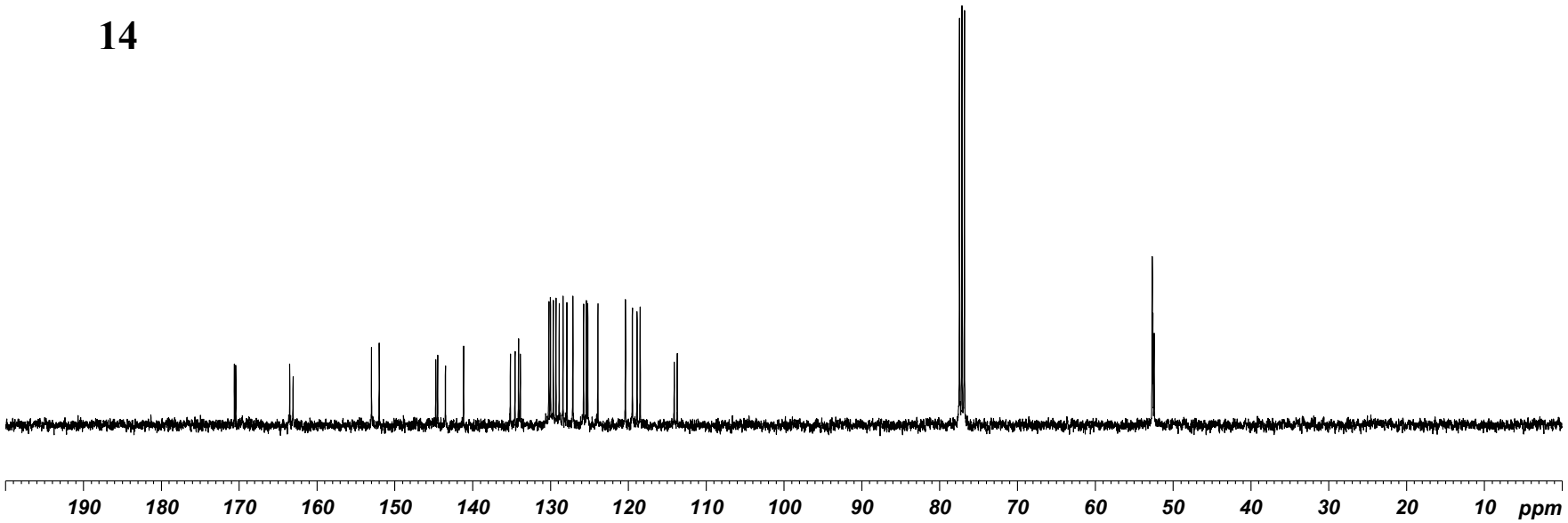

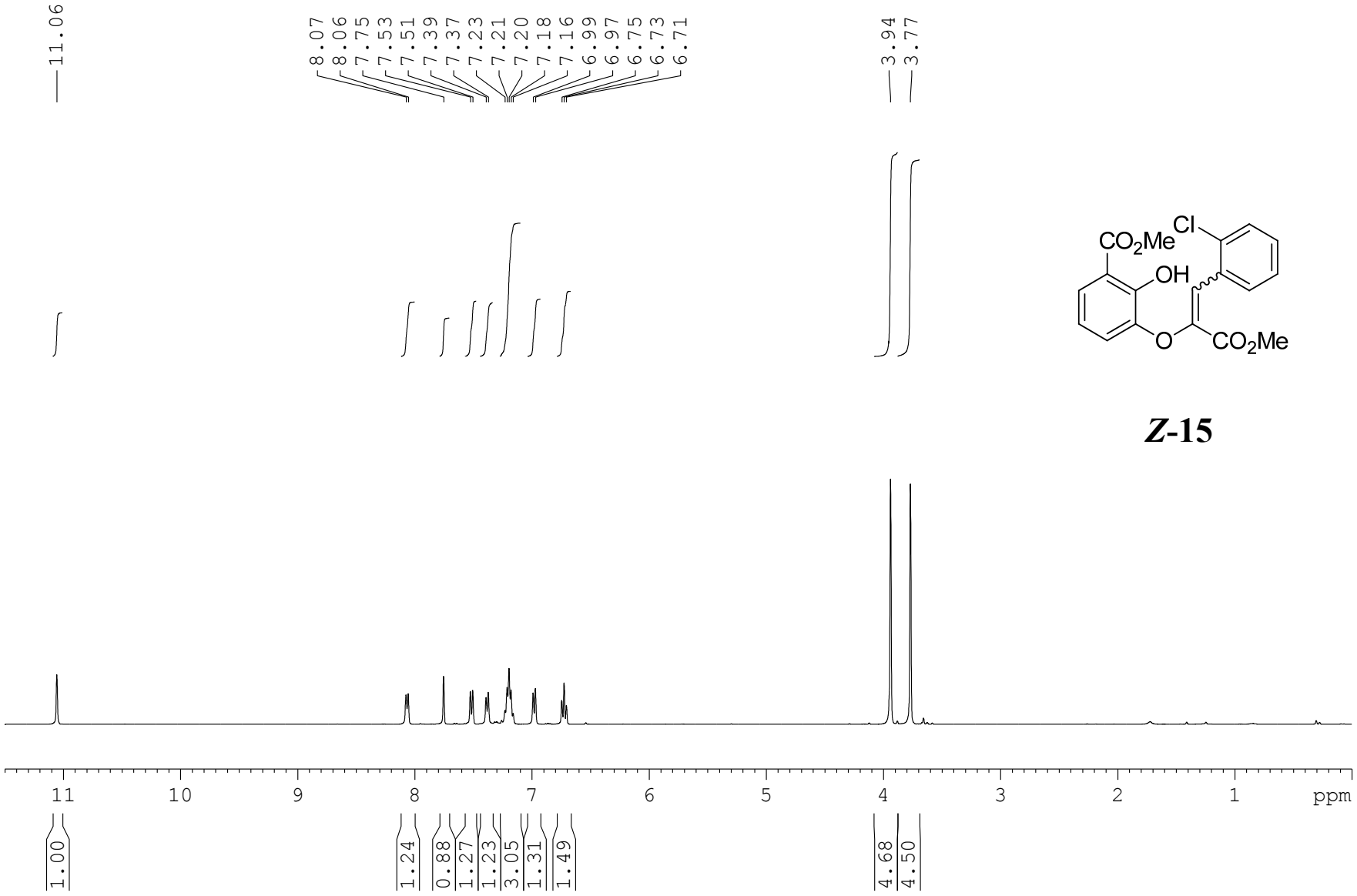

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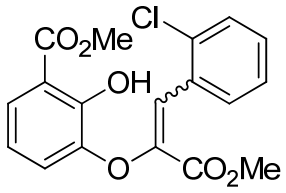
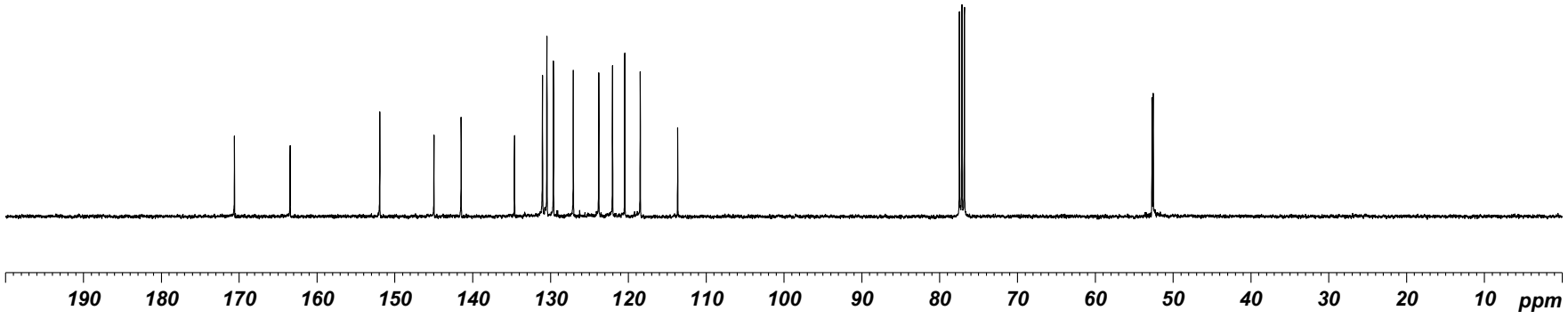
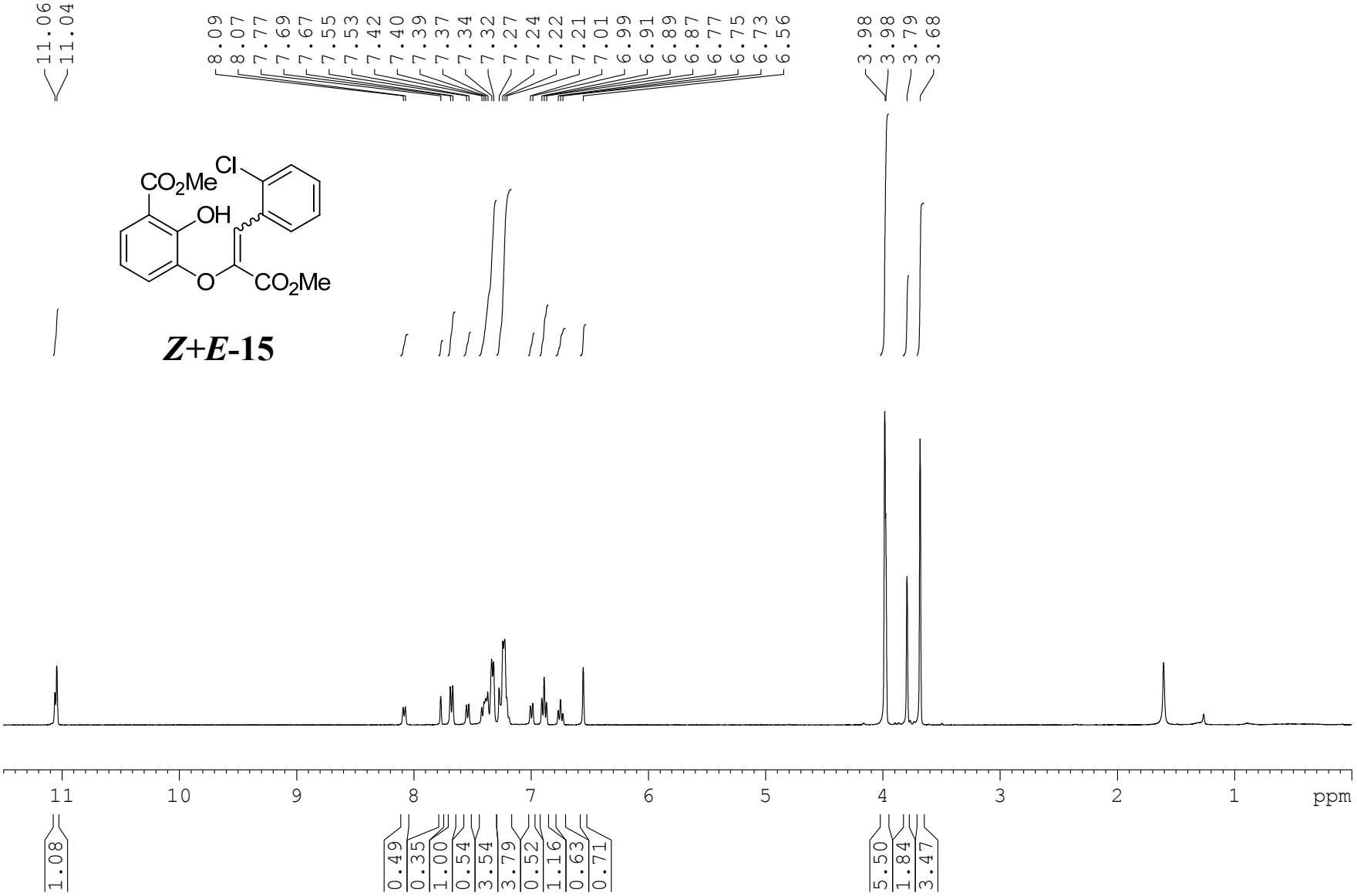
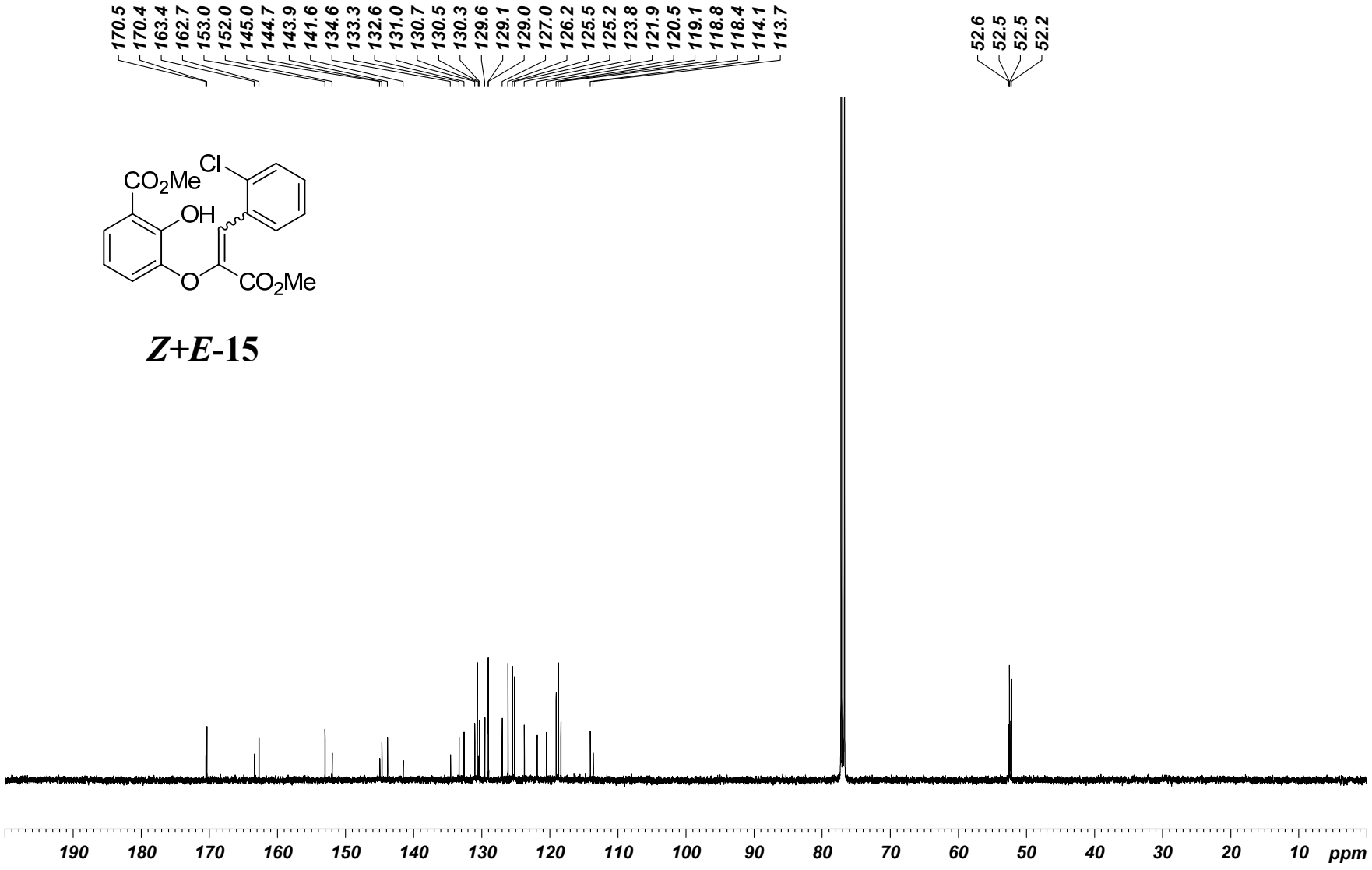


***Z*-15**



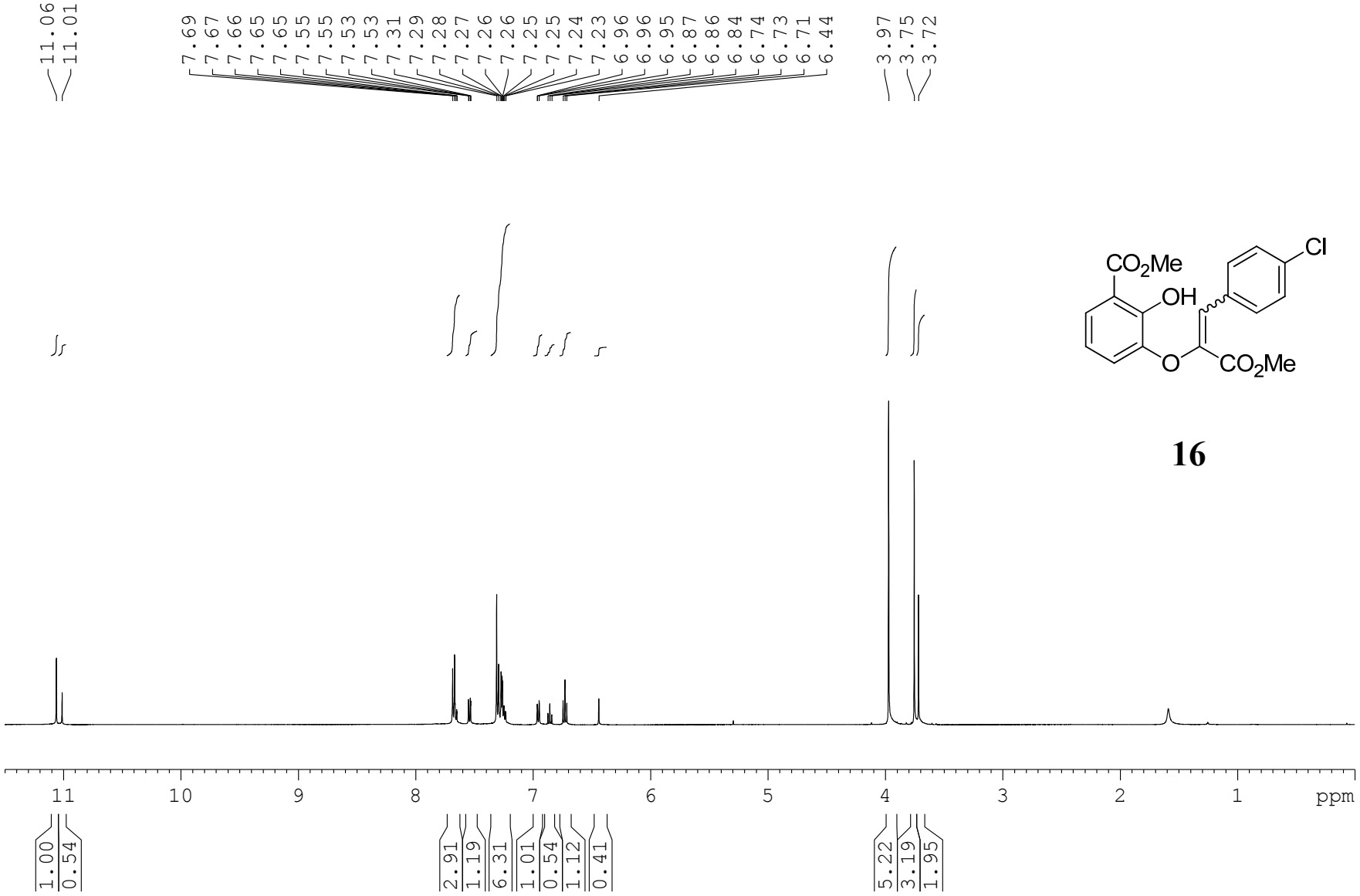


Note: Pure *Z*-isomer was isolated by column chromatography, and the ^{1}H and ^{13}C NMR spectra of the mixed *E* + *Z* fractions were used to assign the *E* diastereomer.



***Z*+*E*-15**

Note: Pure *Z*-isomer was isolated by column chromatography, and the ^{1}H and ^{13}C NMR spectra of the mixed *E* + *Z* fractions were used to assign the *E* diastereomer.



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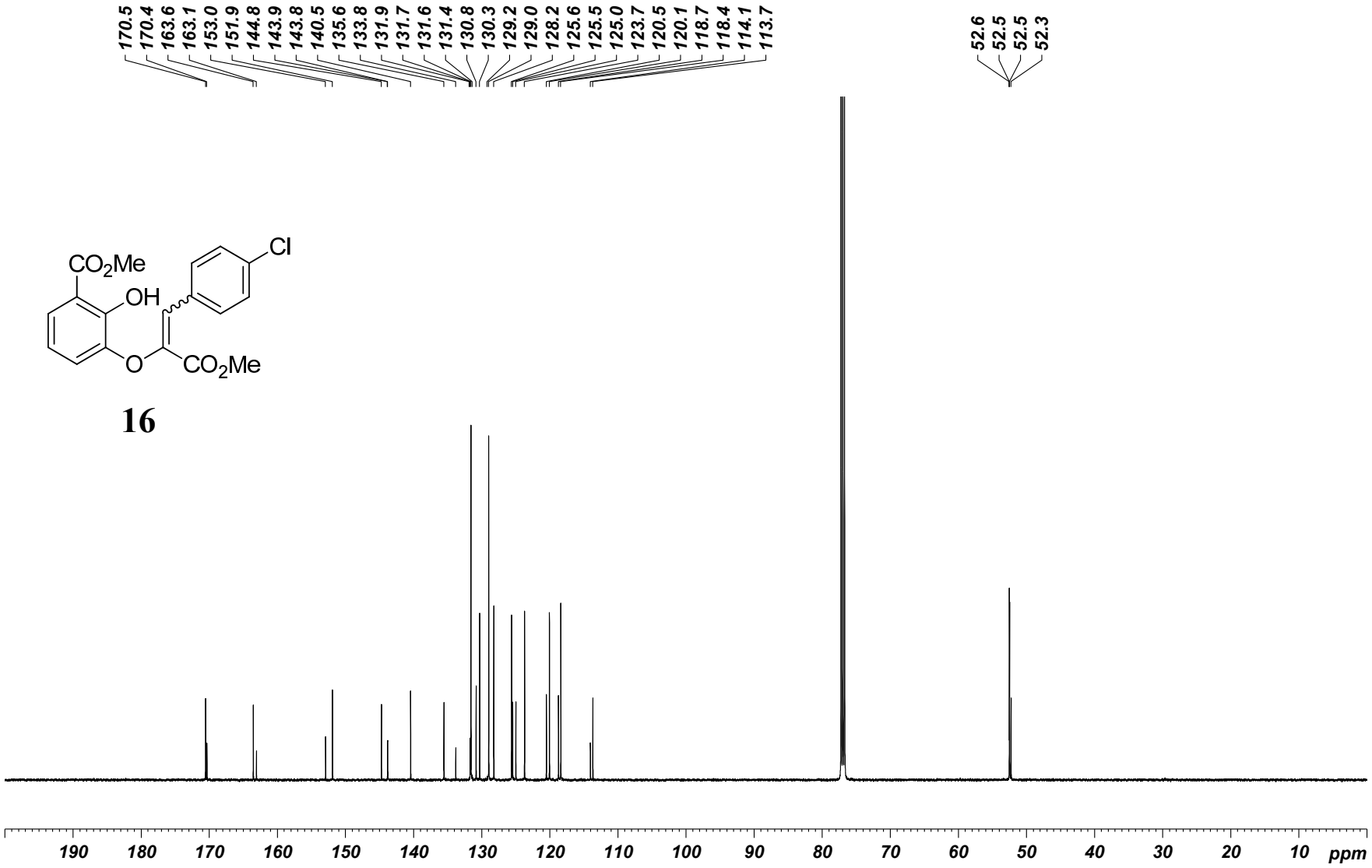

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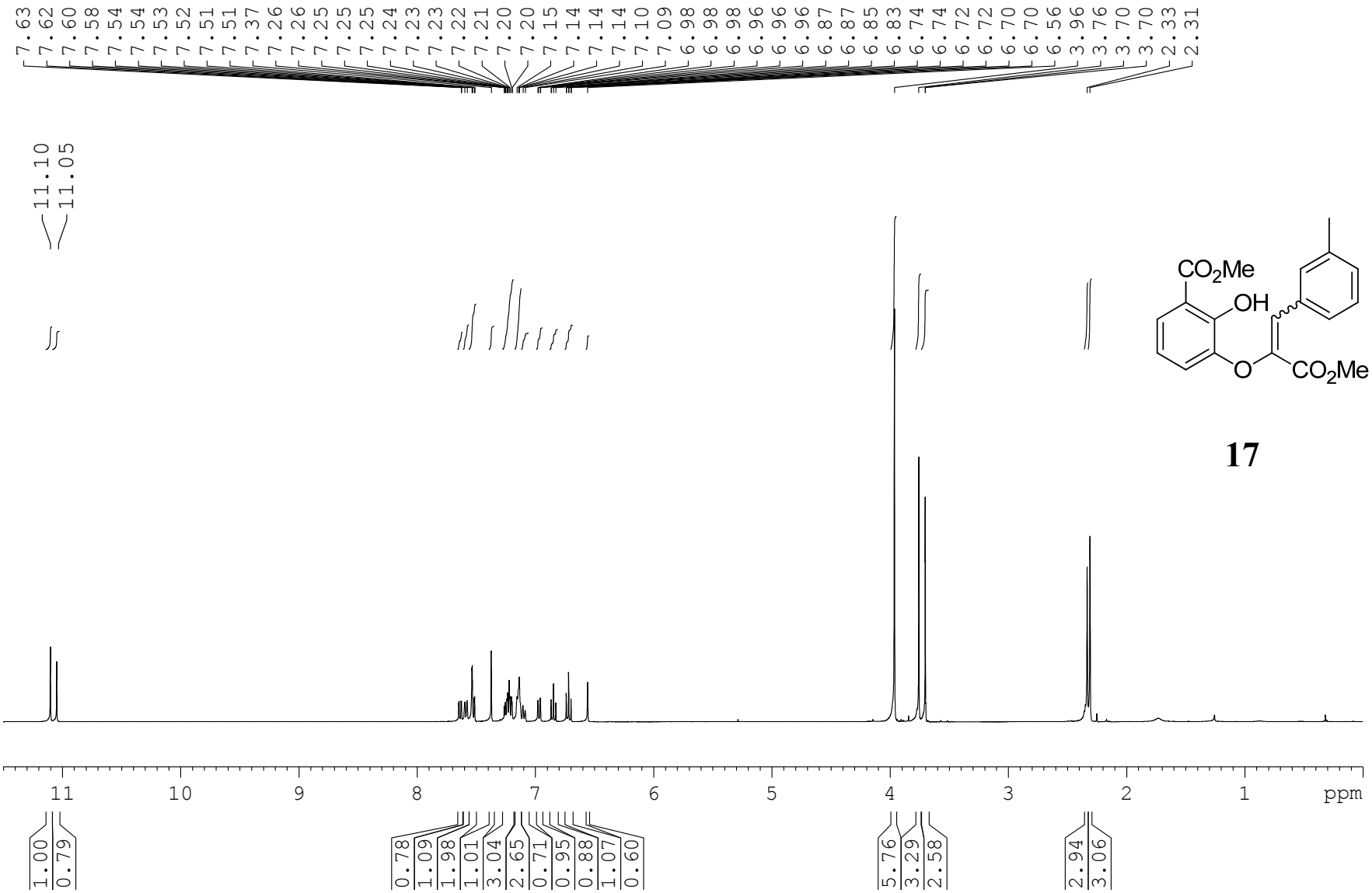

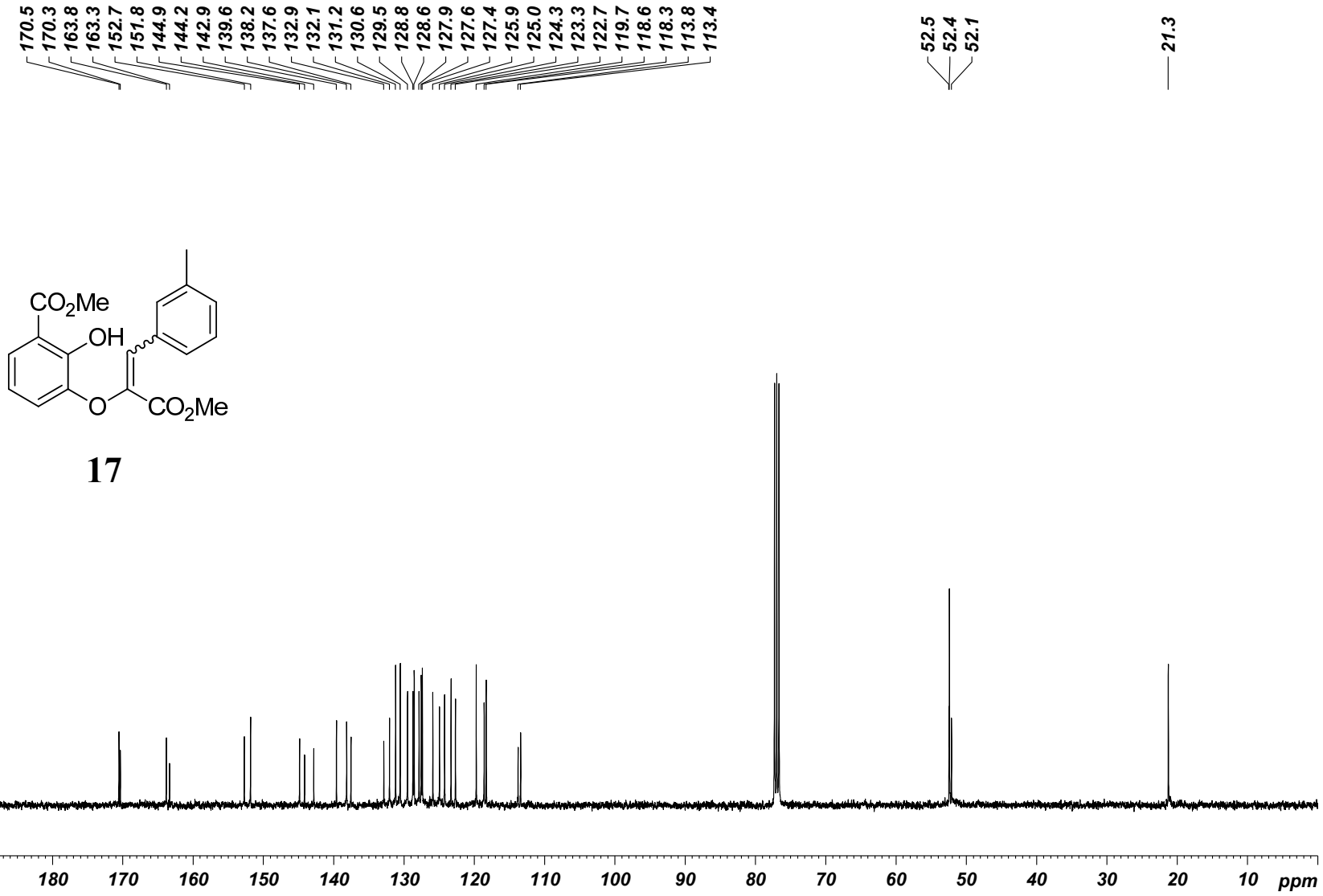

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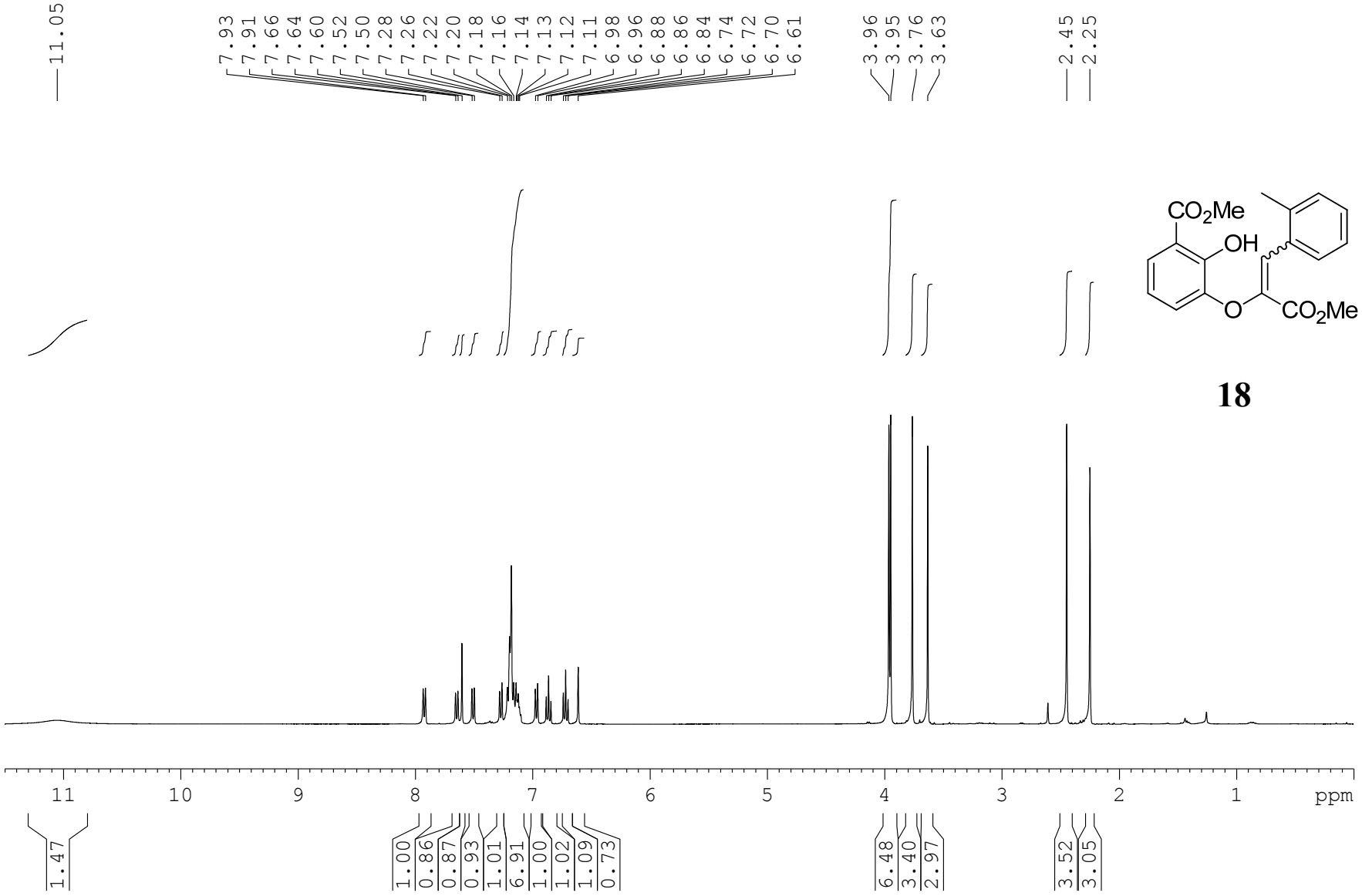

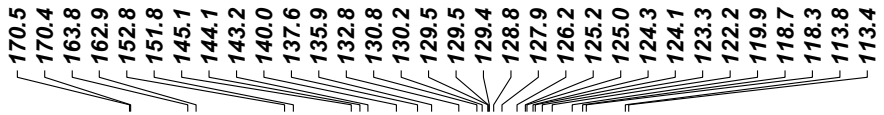

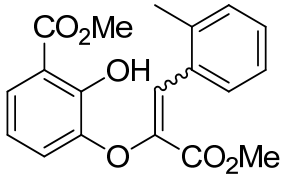

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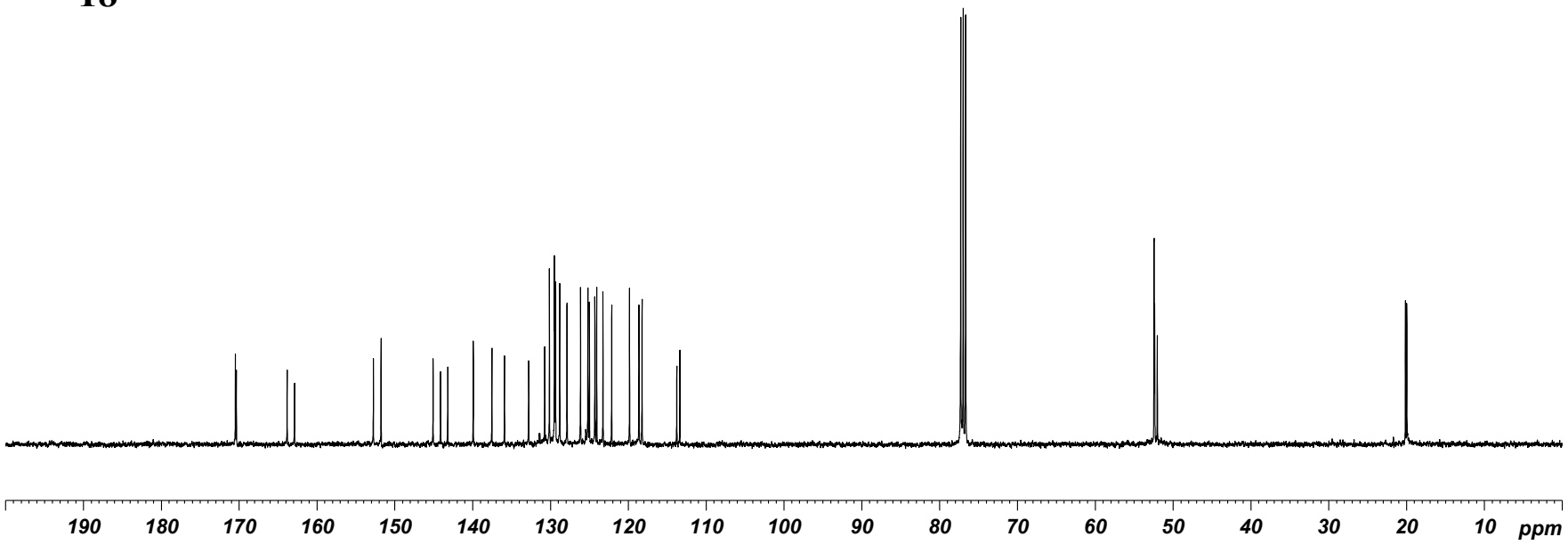

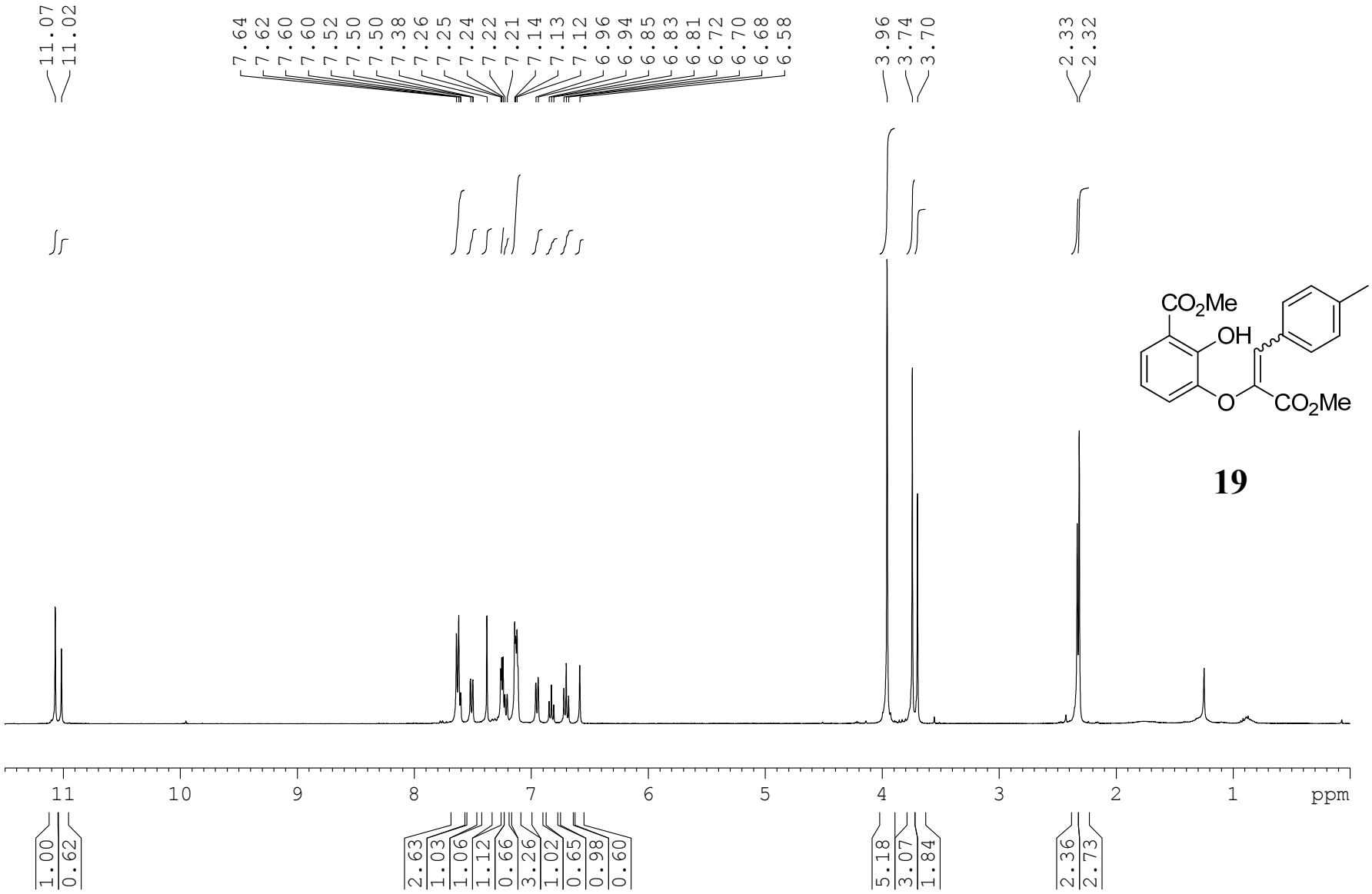

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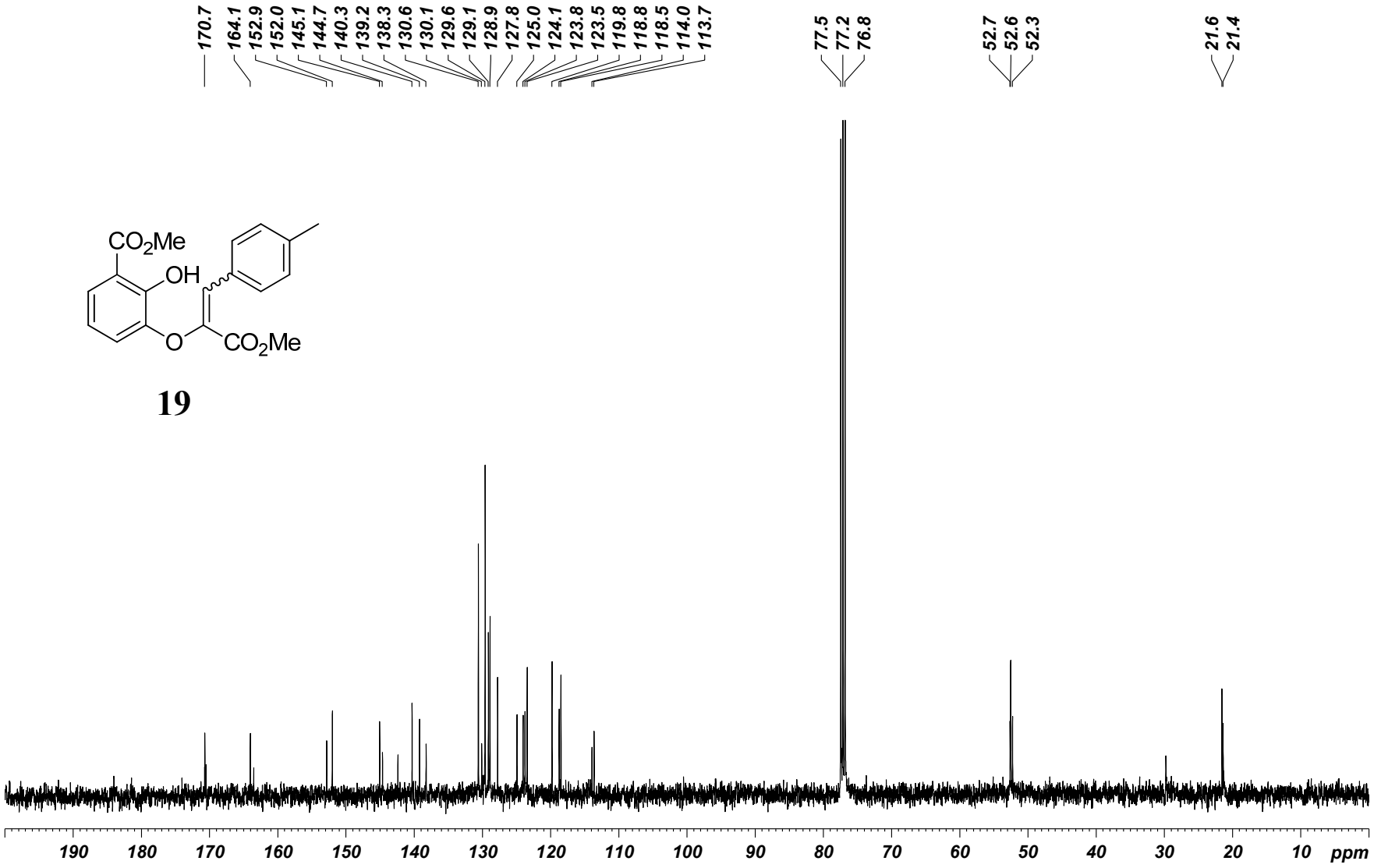

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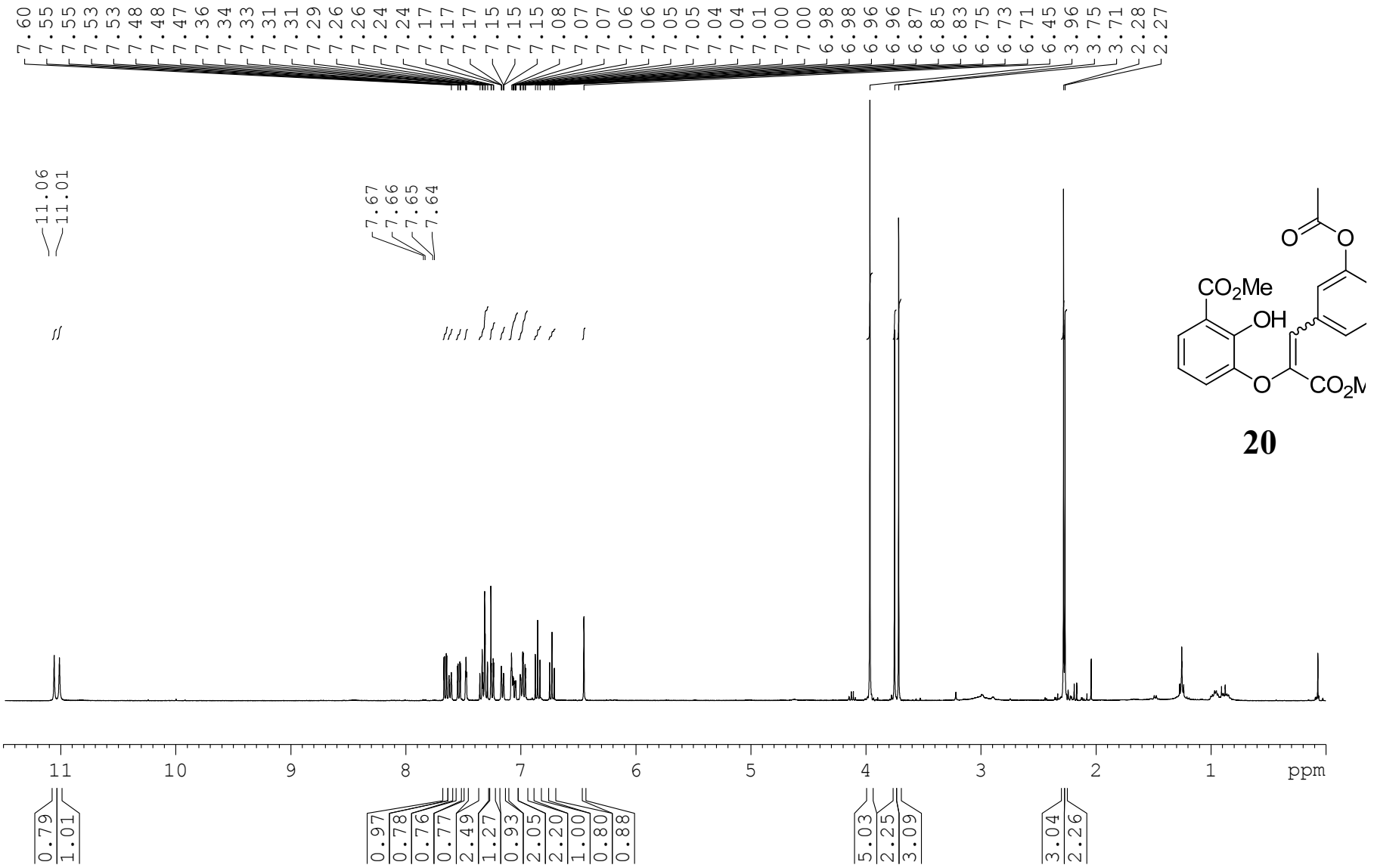

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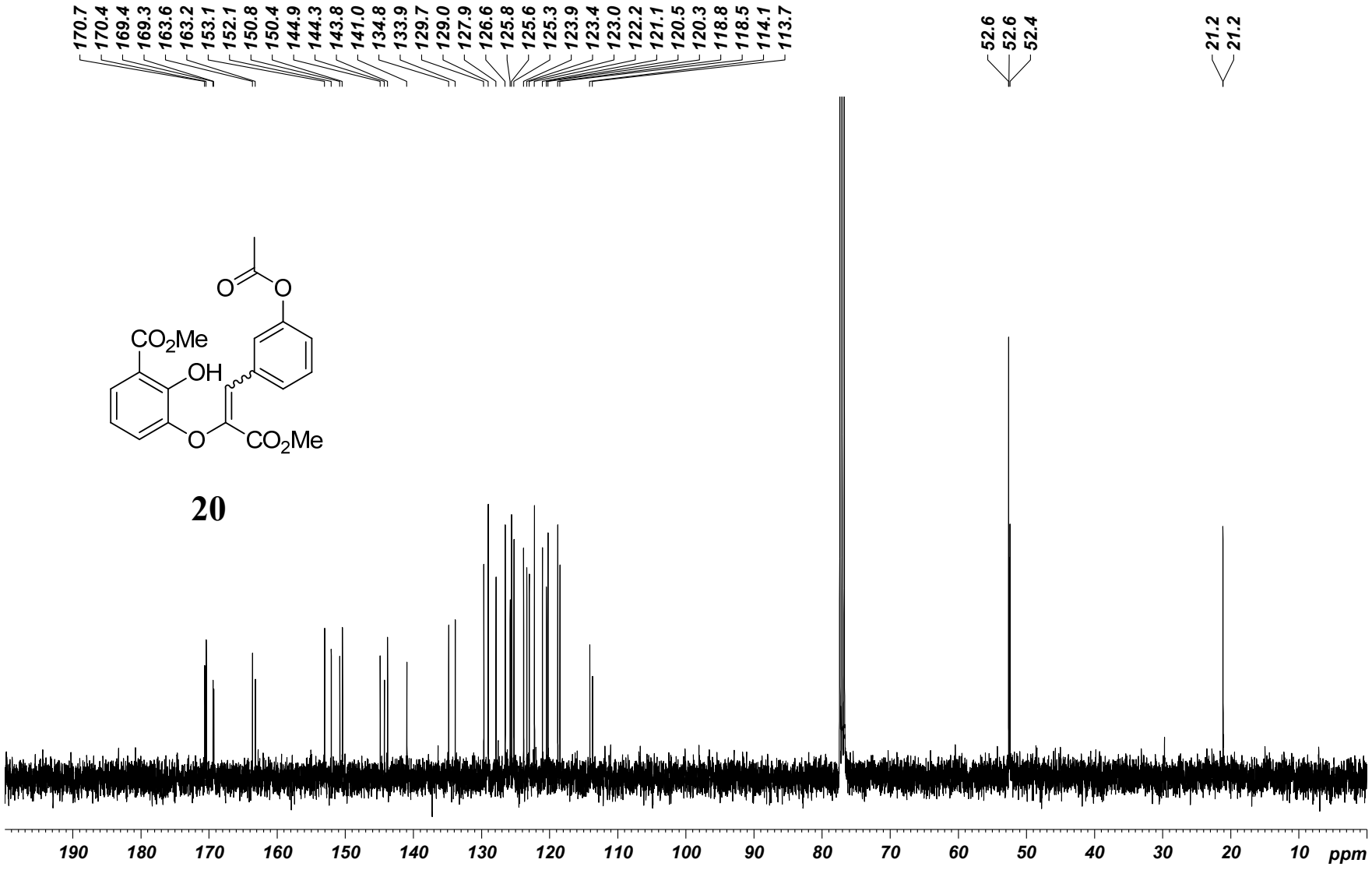

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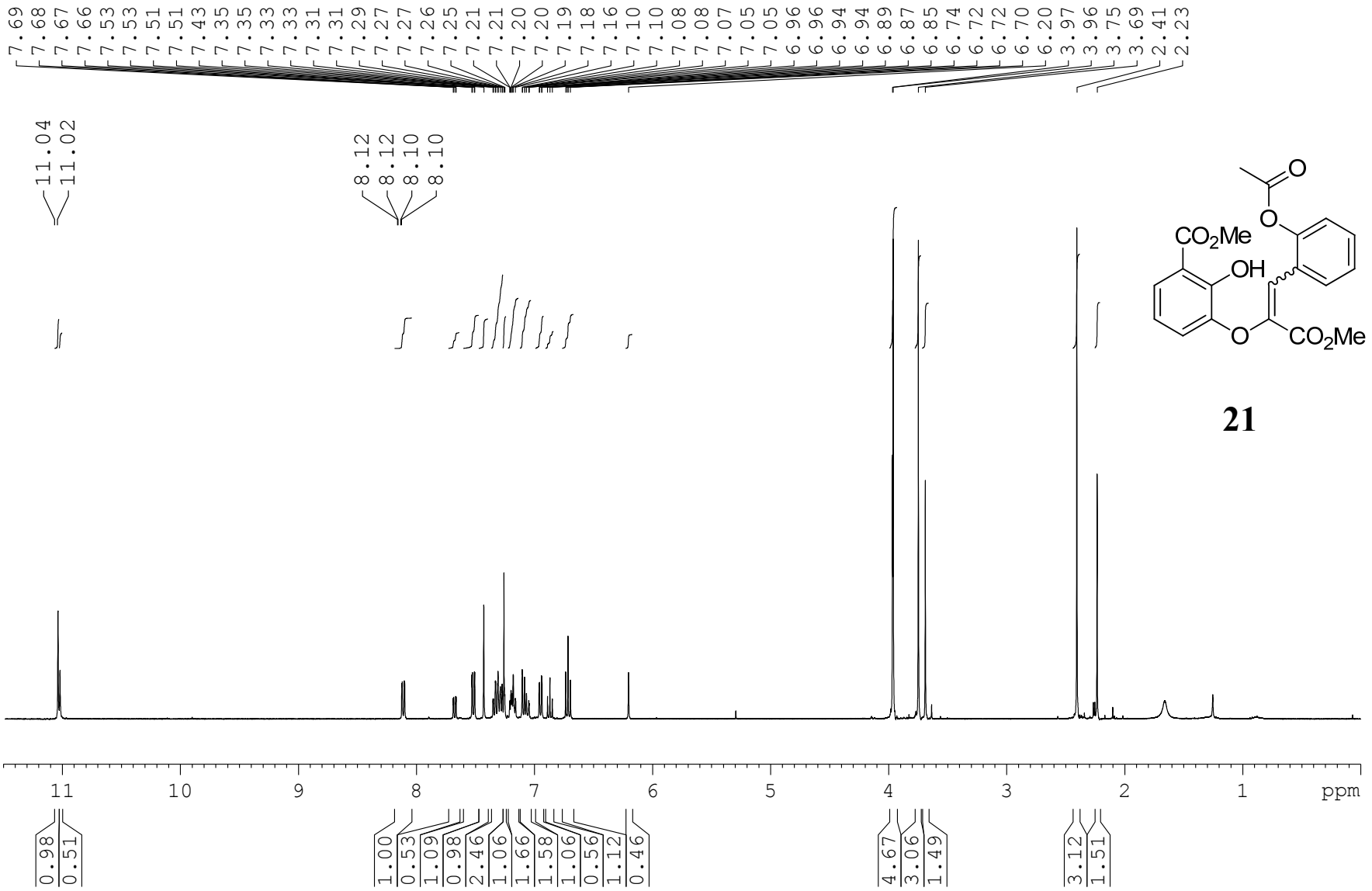

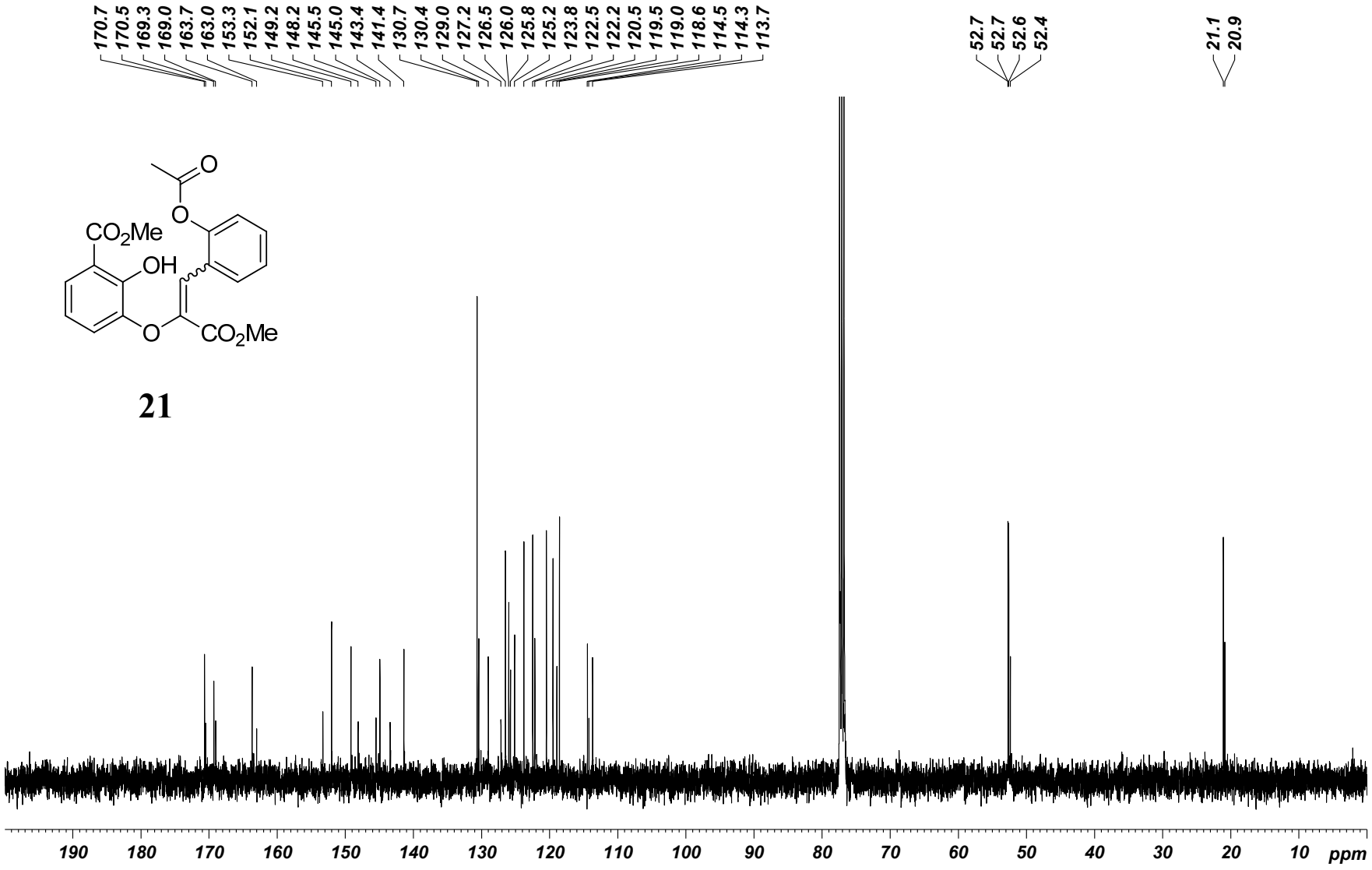

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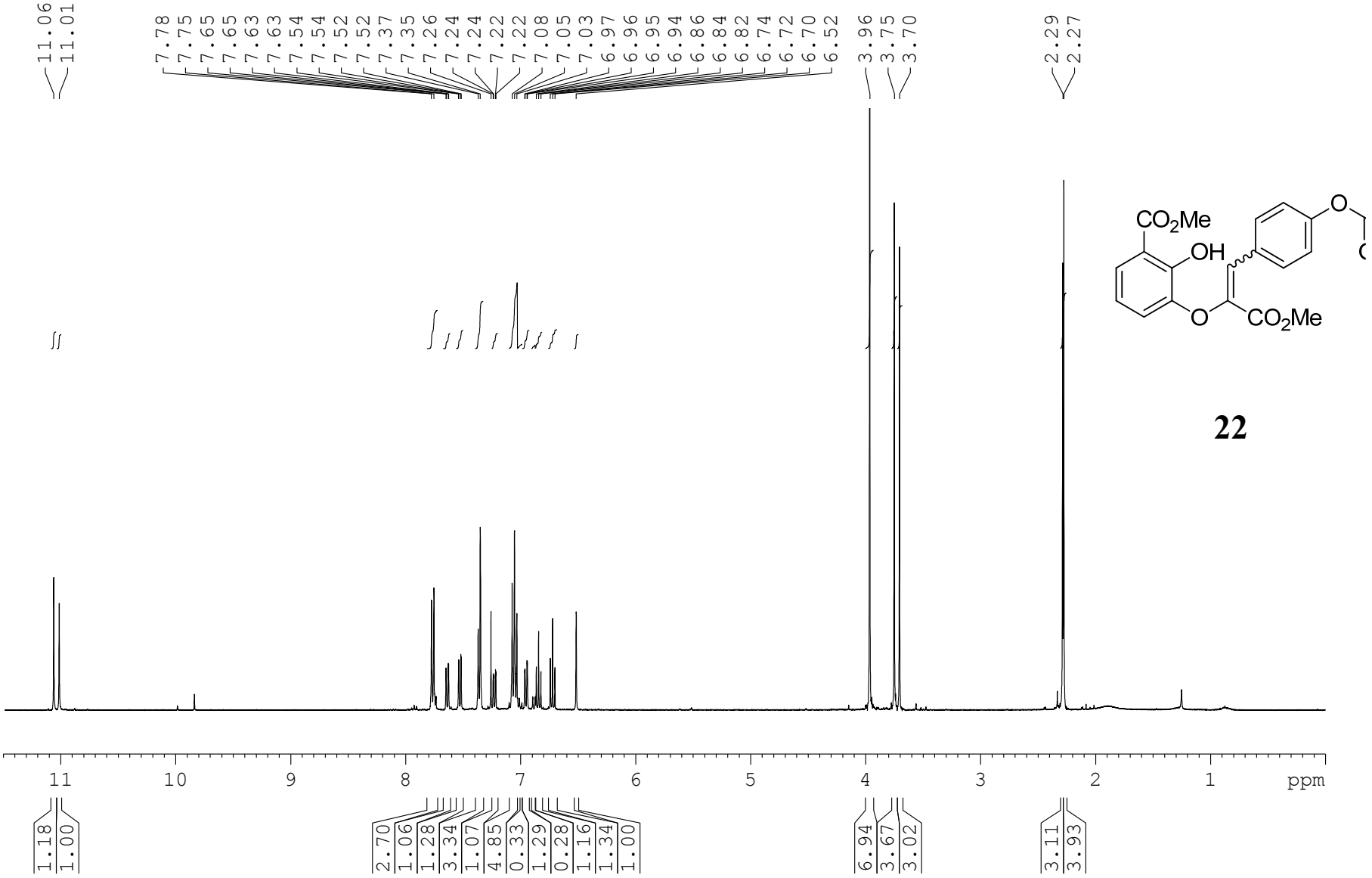

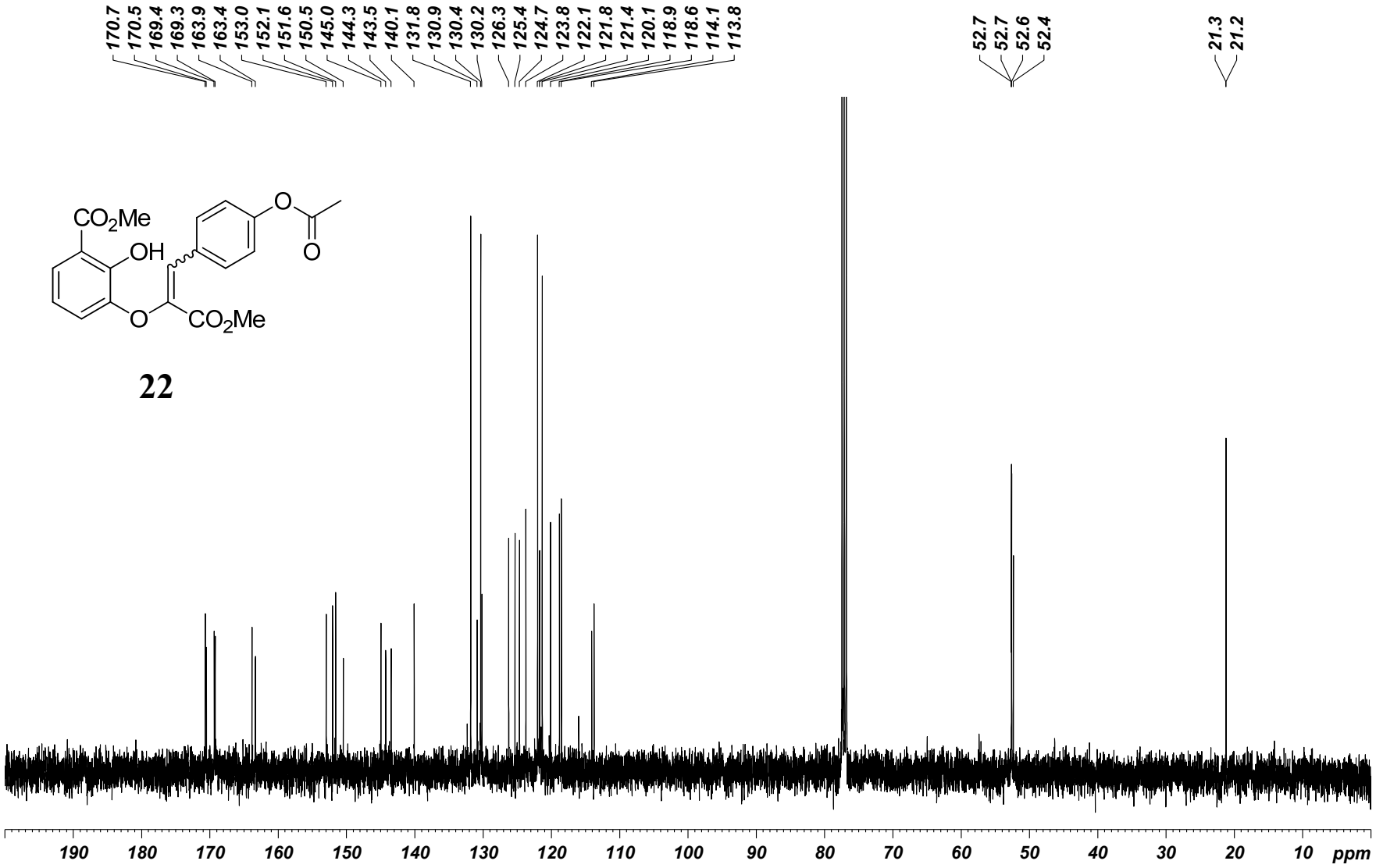

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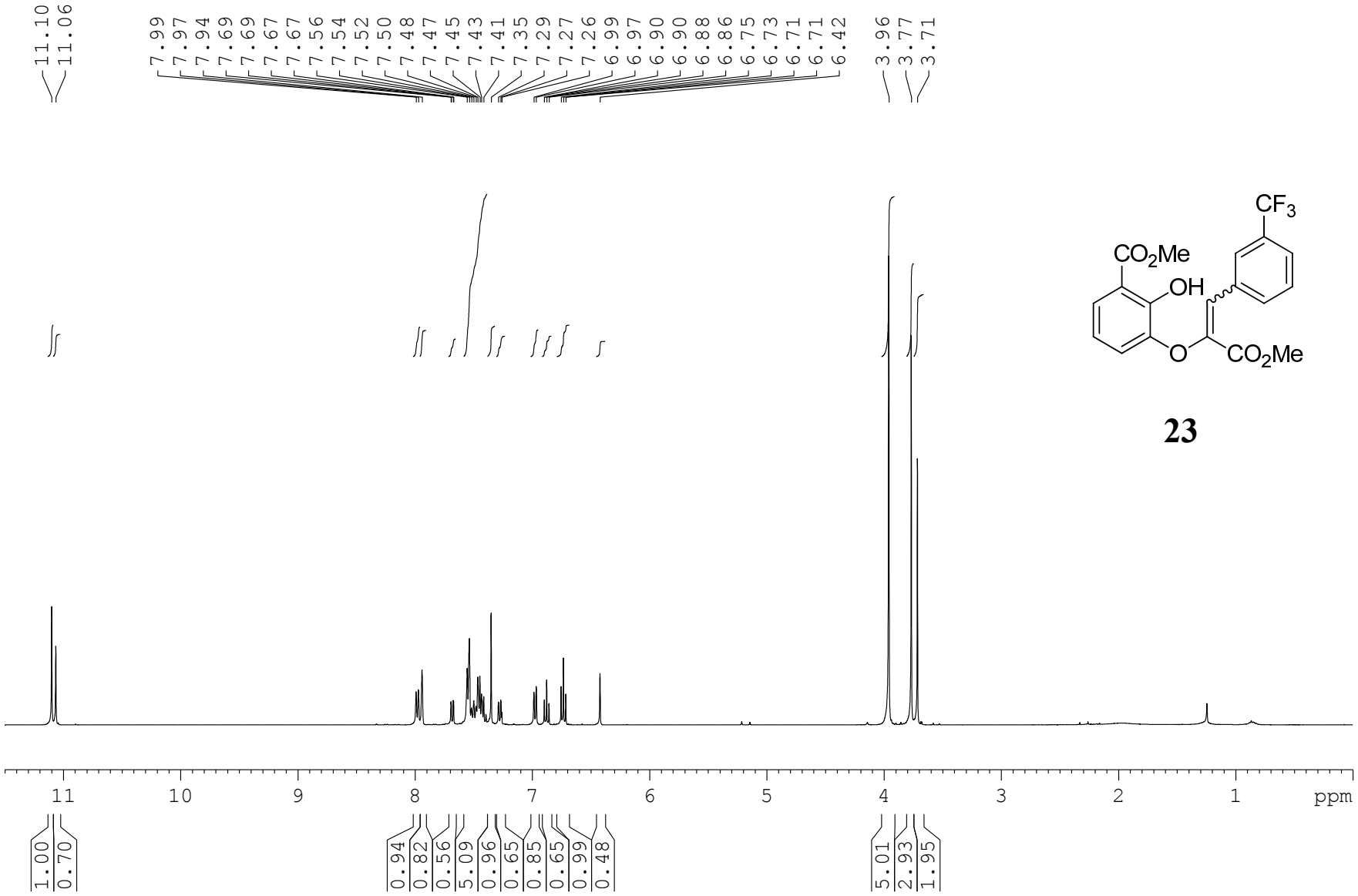

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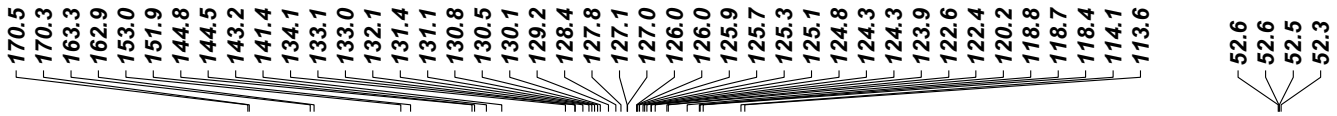

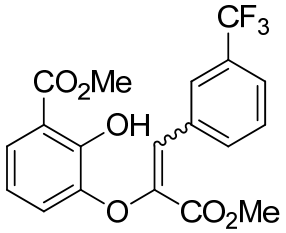

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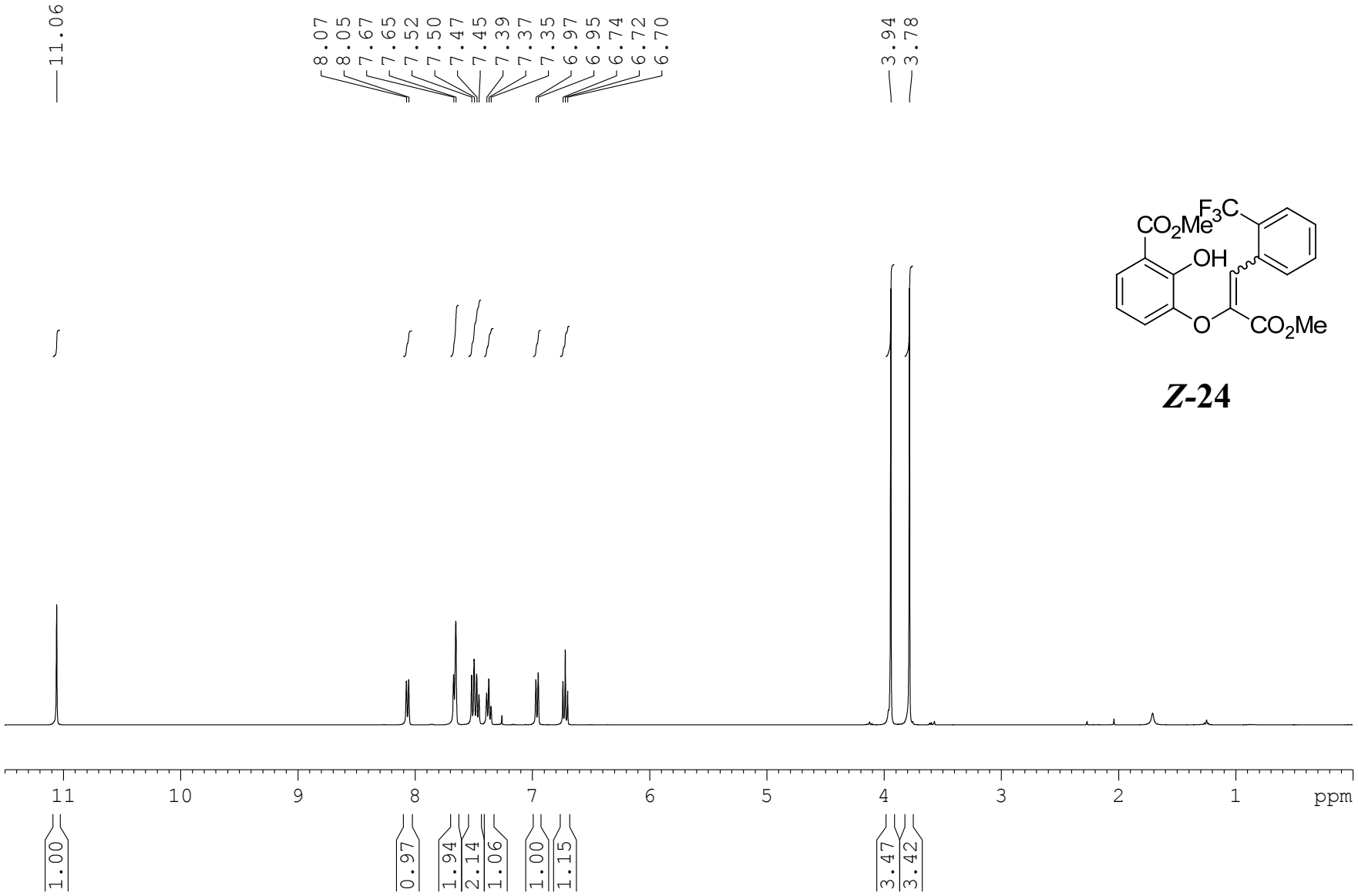
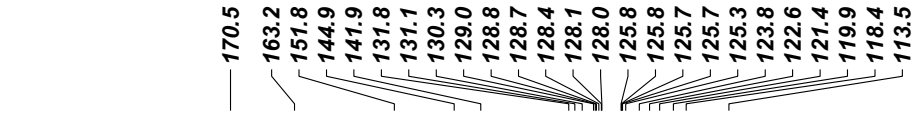
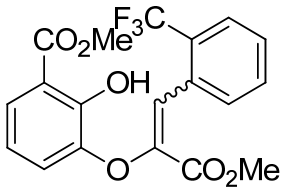
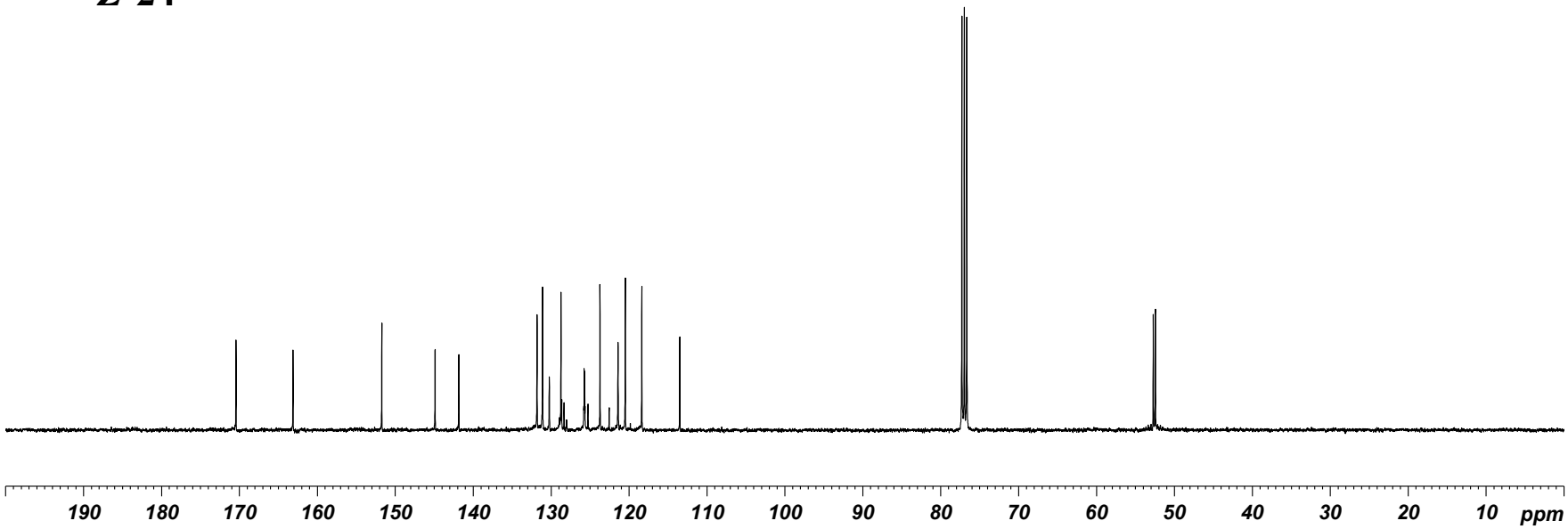


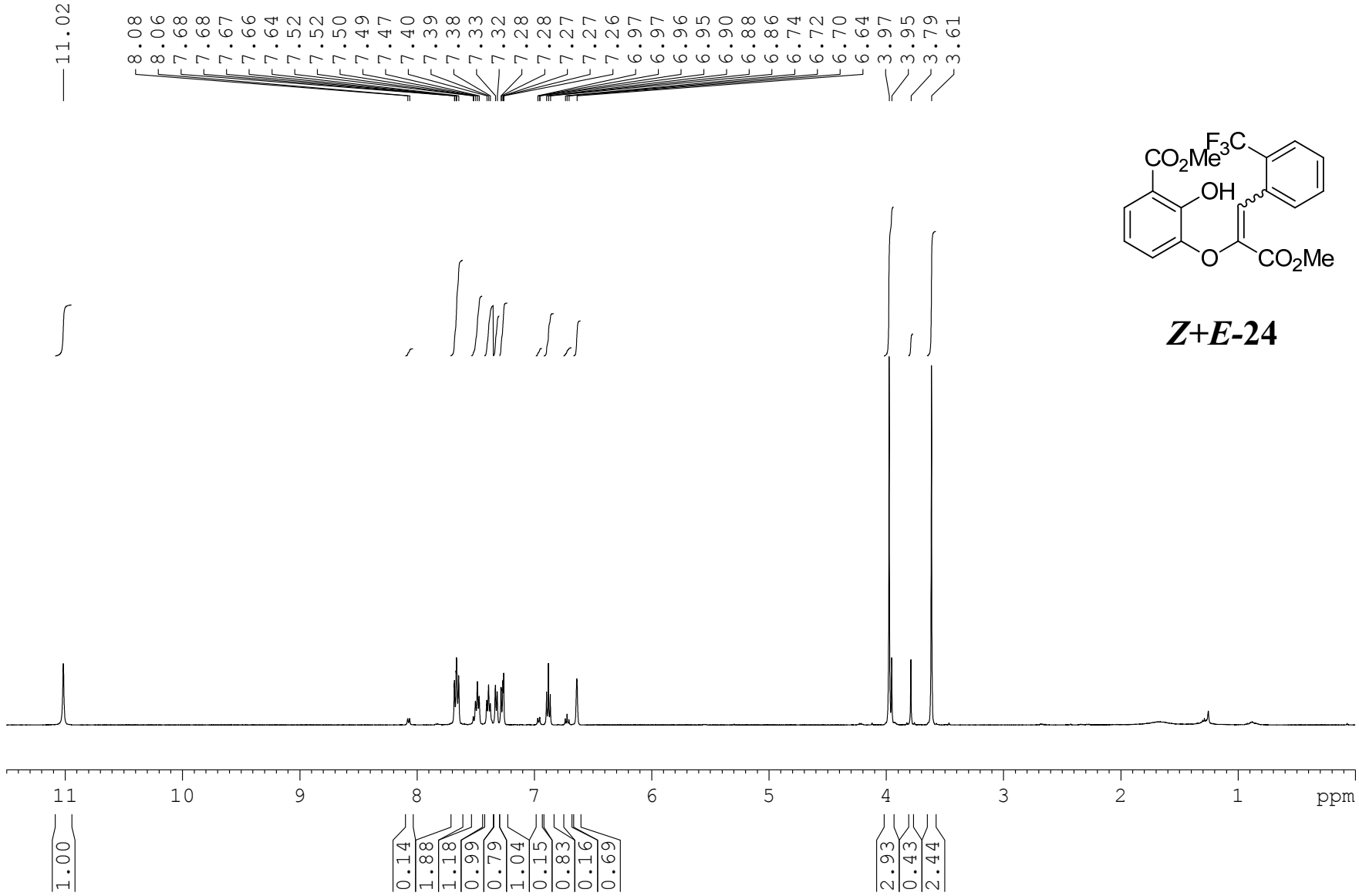
***Z*-24**





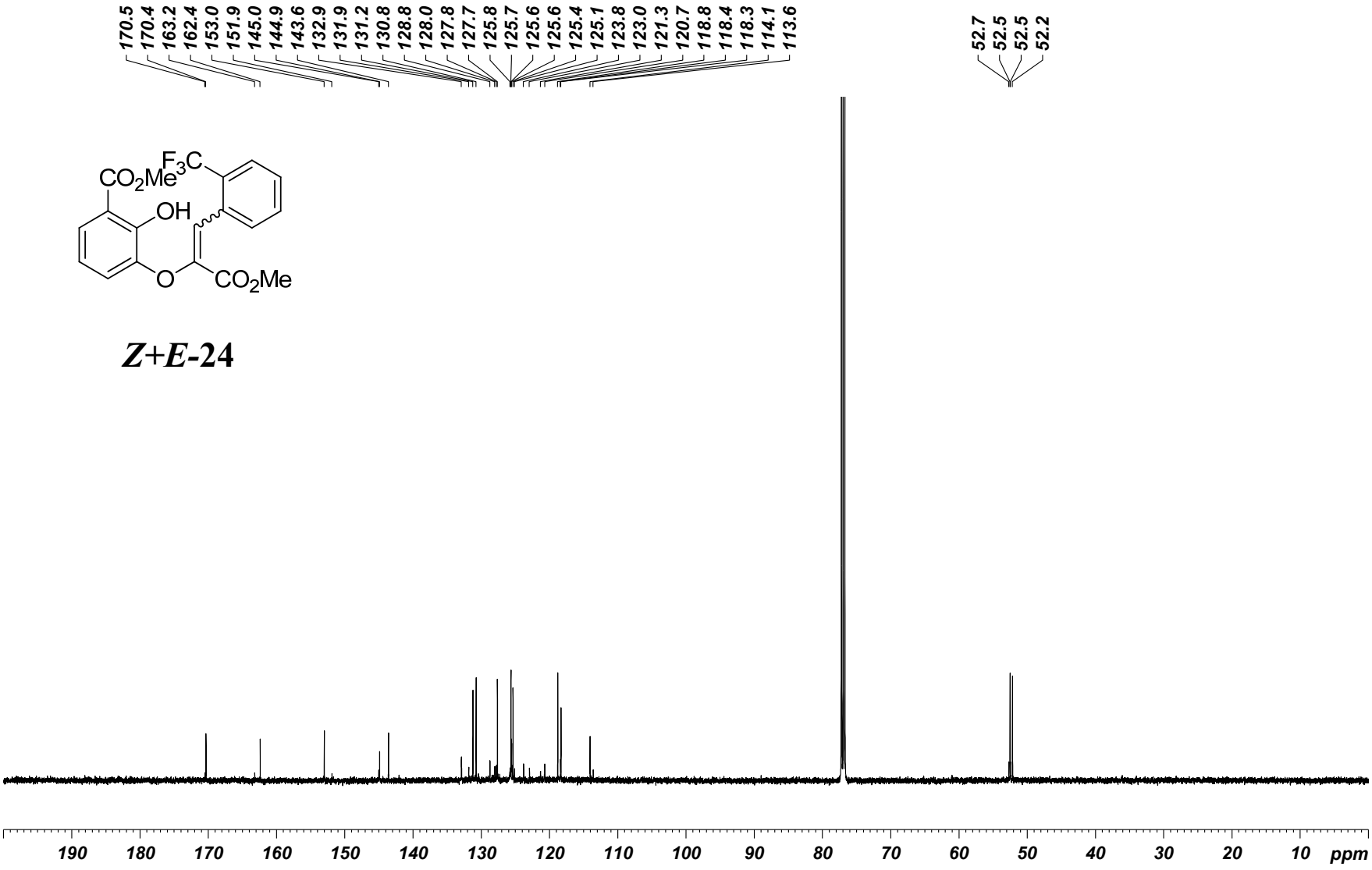
***Z*-24**





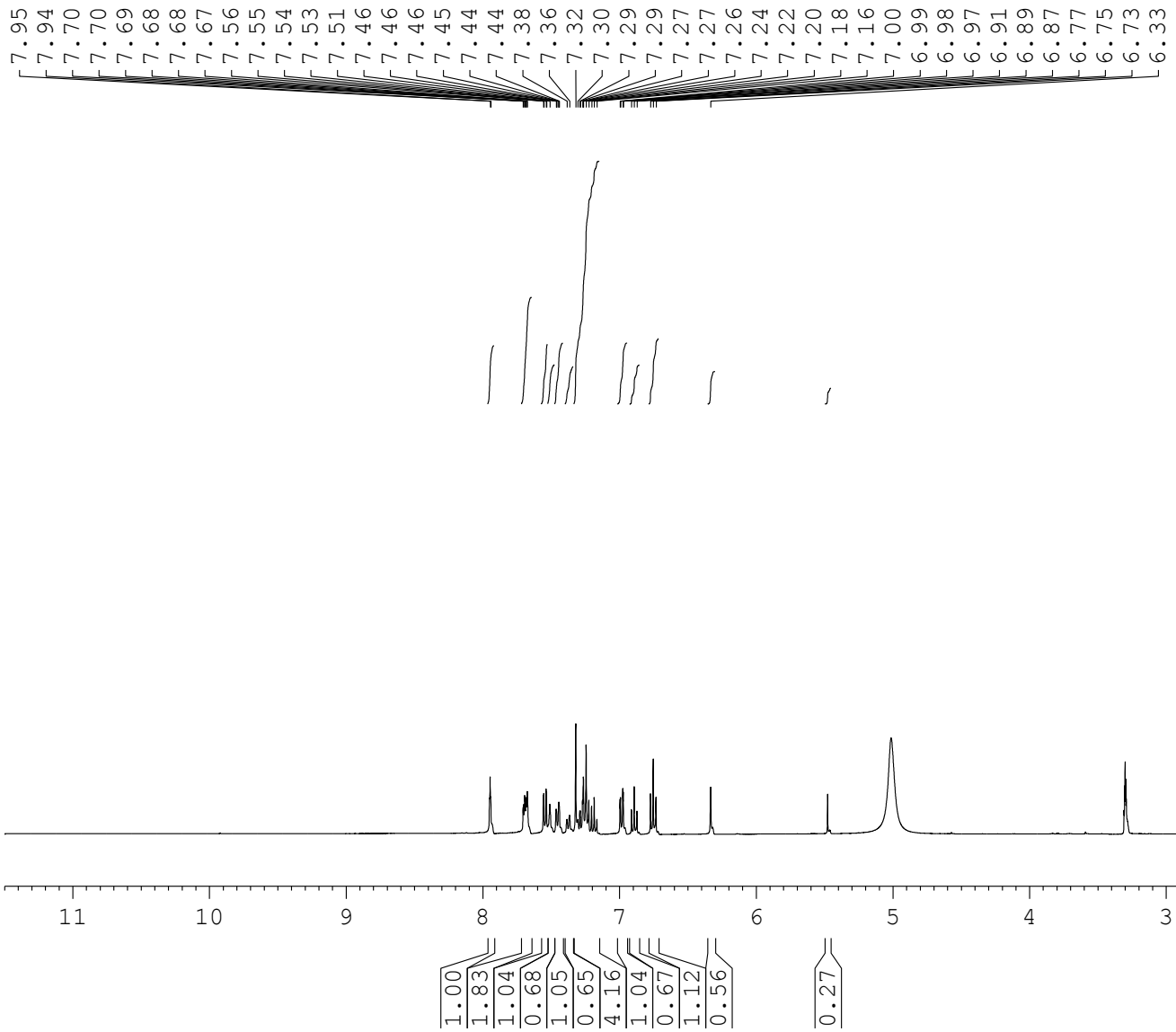
***Z*+*E*-24**

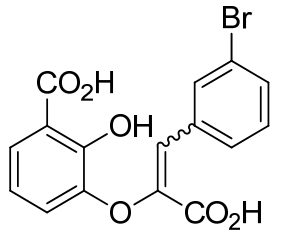
Note: Pure *Z*-isomer was isolated by column chromatography, and the ^{1}H and ^{13}C NMR spectra of the mixed *E* + *Z* fractions were used to assign the *E* diastereomer.



***Z*+*E*-24**

Note: Pure *Z*-isomer was isolated by column chromatography, and the ^{1}H and ^{13}C NMR spectra of the mixed *E* + *Z* fractions were used to assign the *E* diastereomer.





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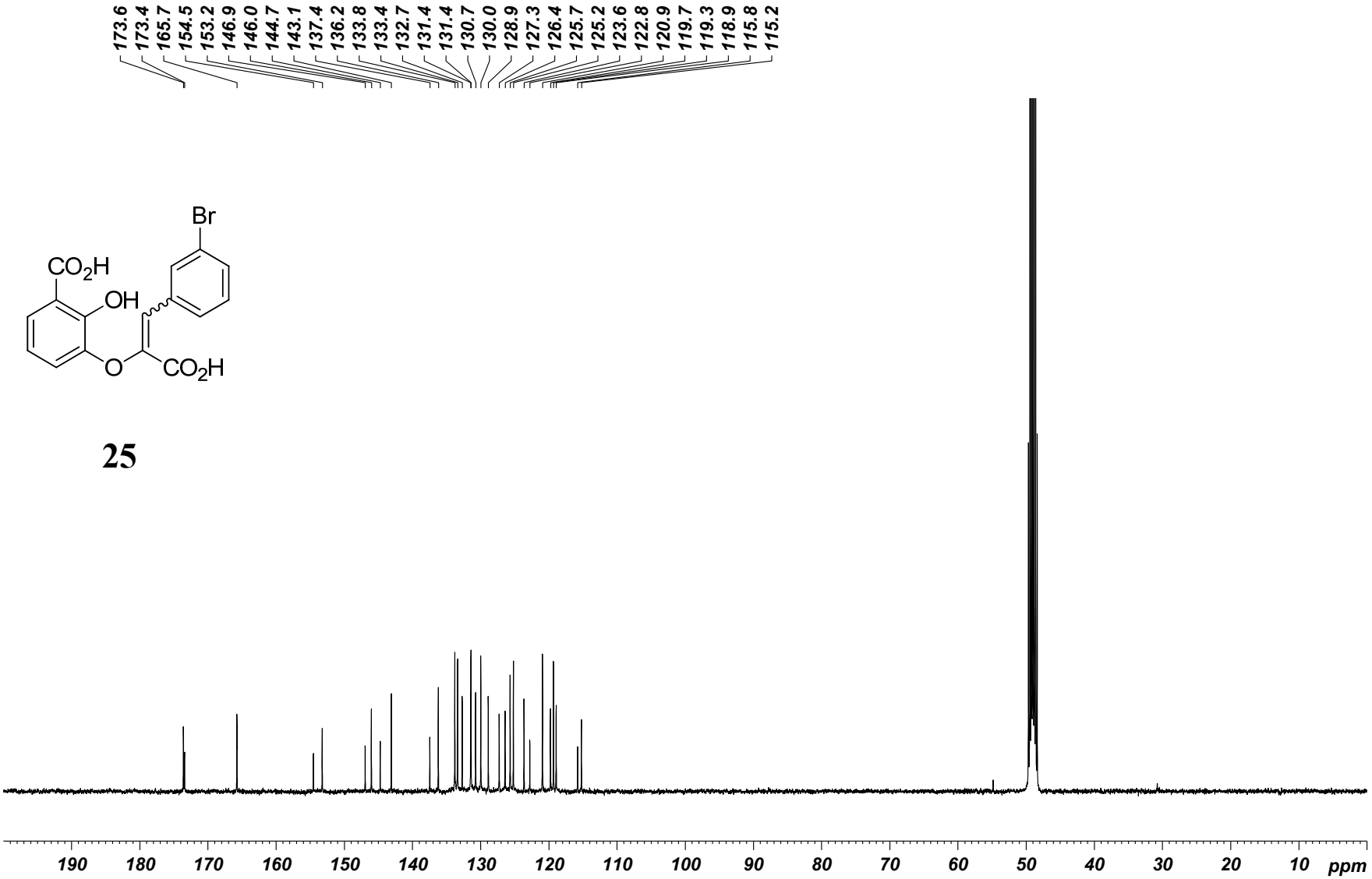

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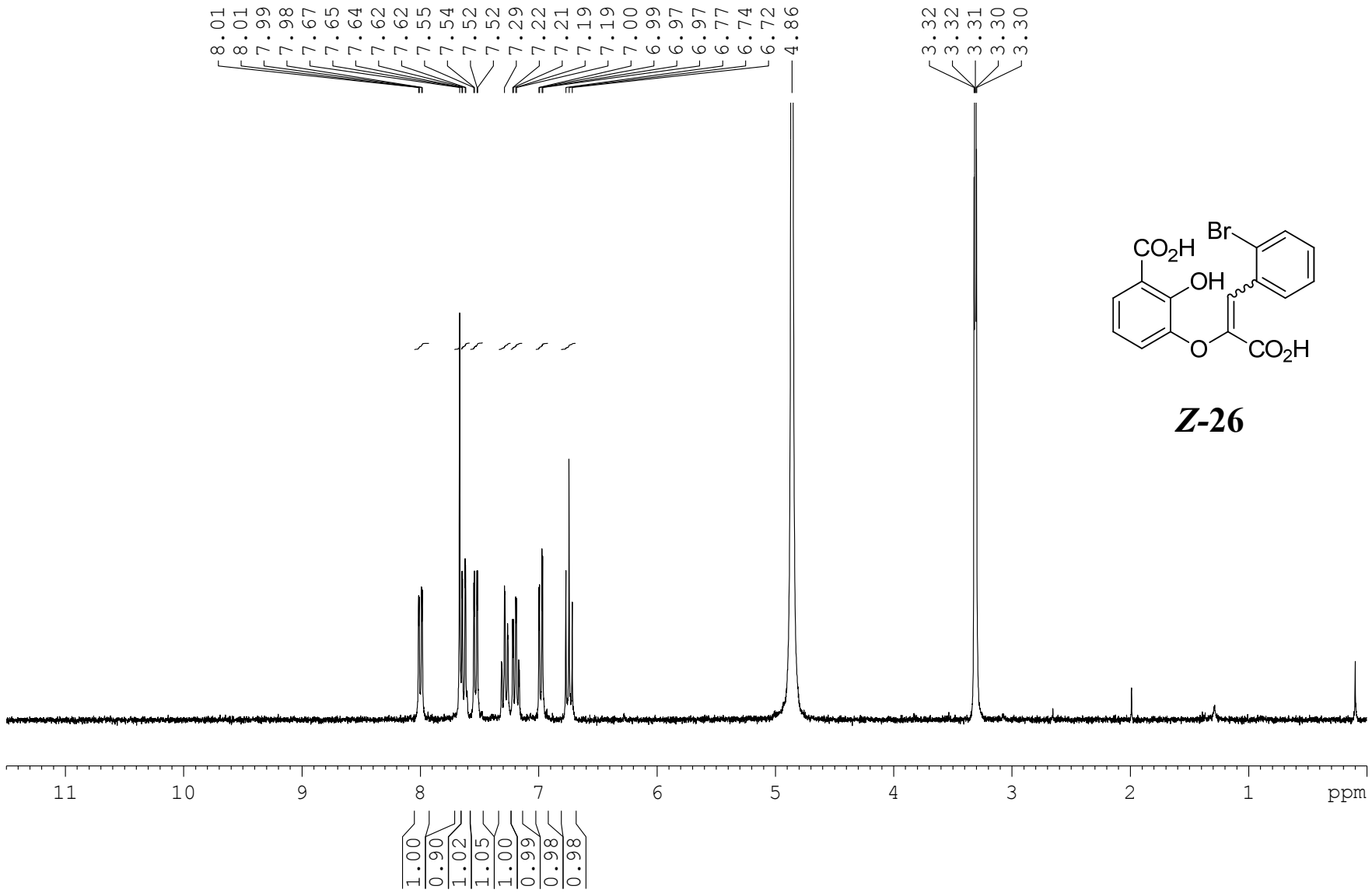
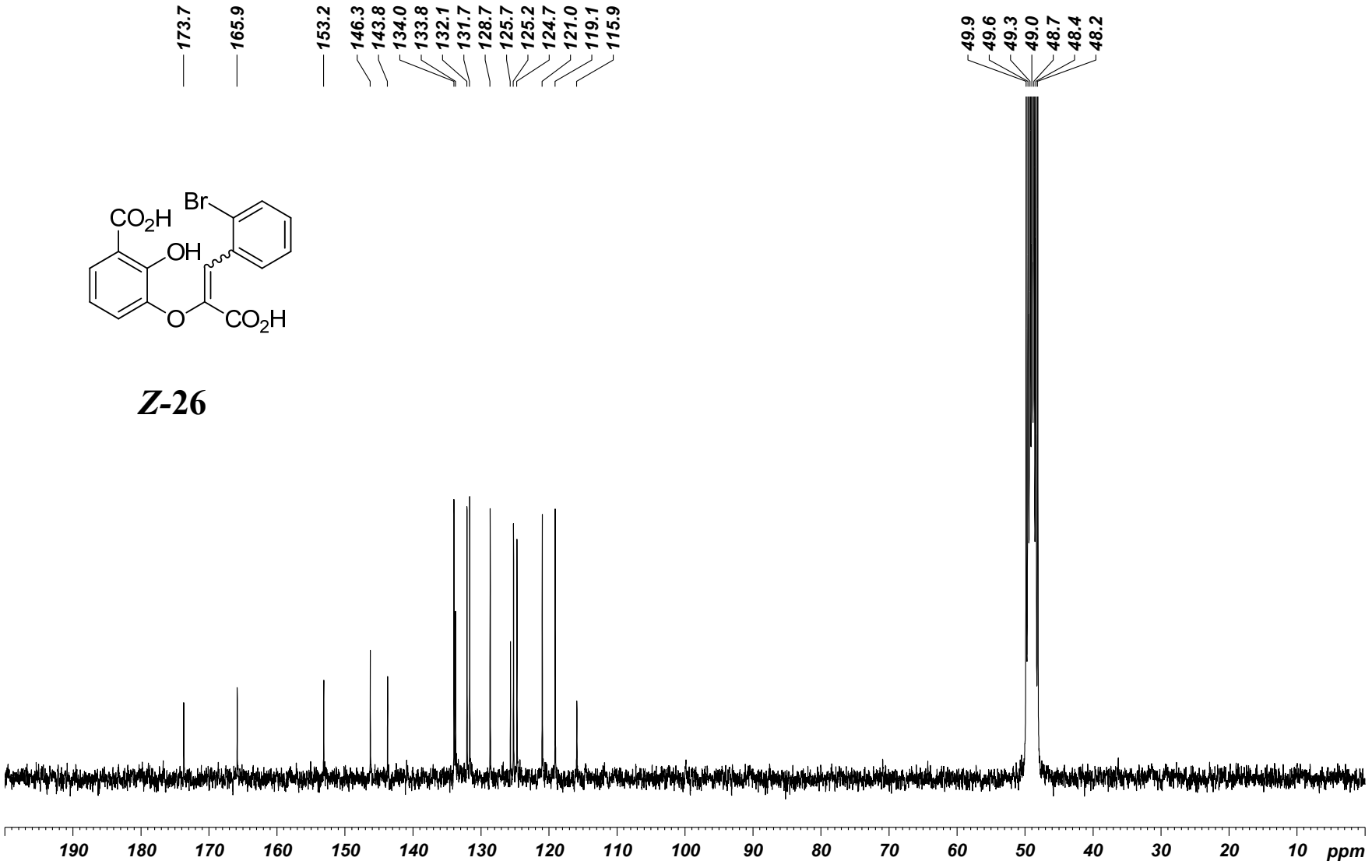
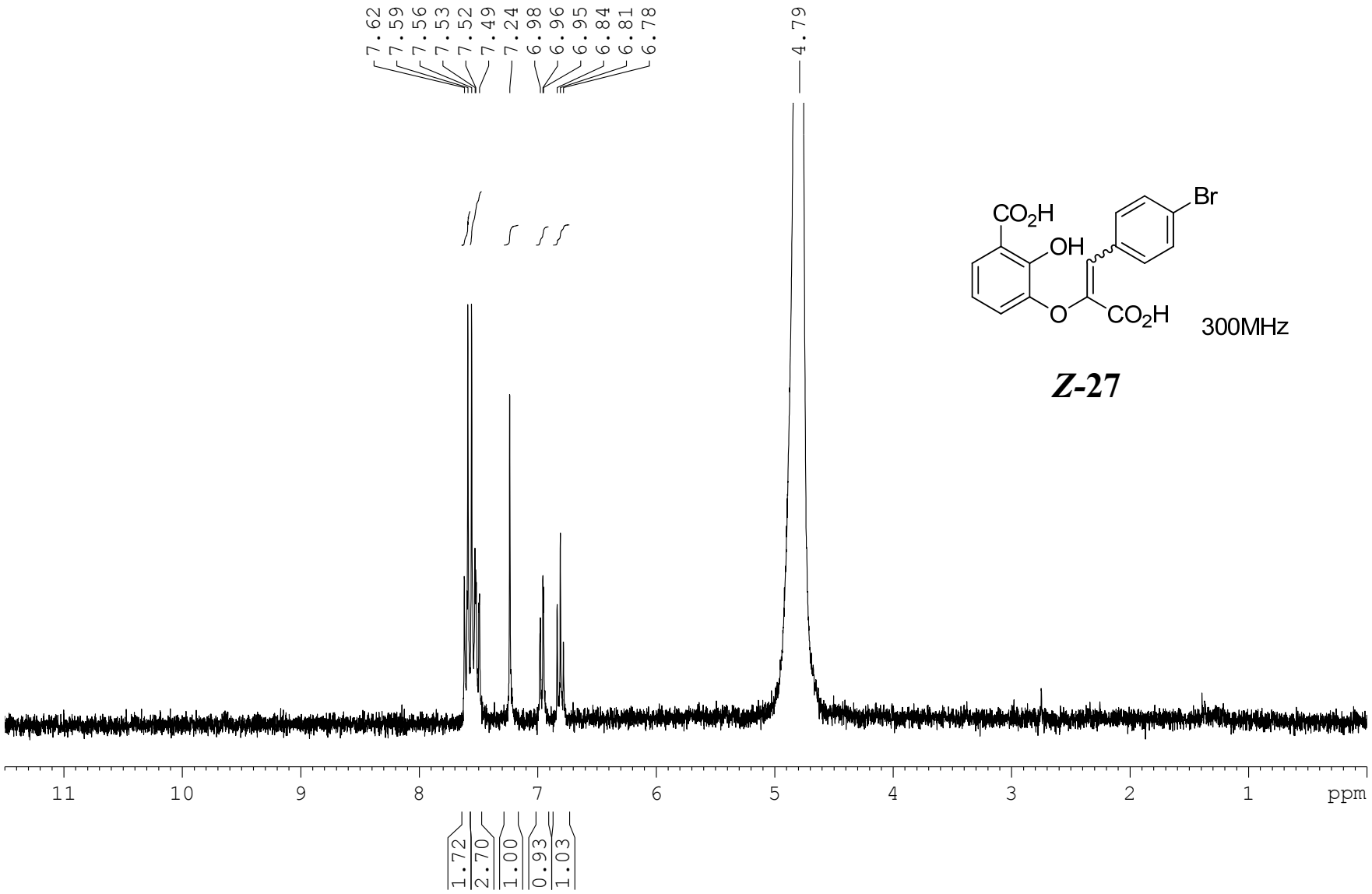


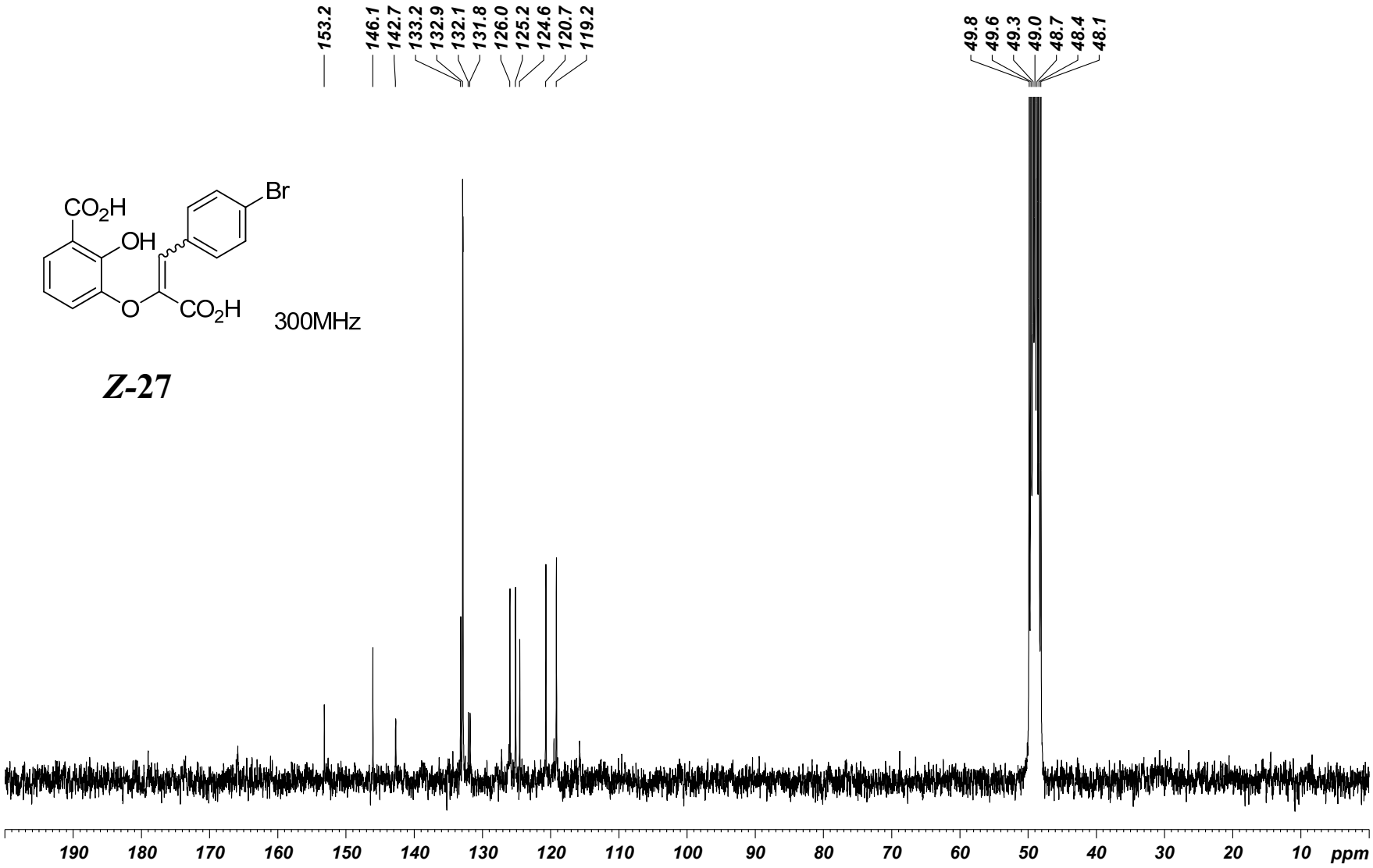
***Z*-26**



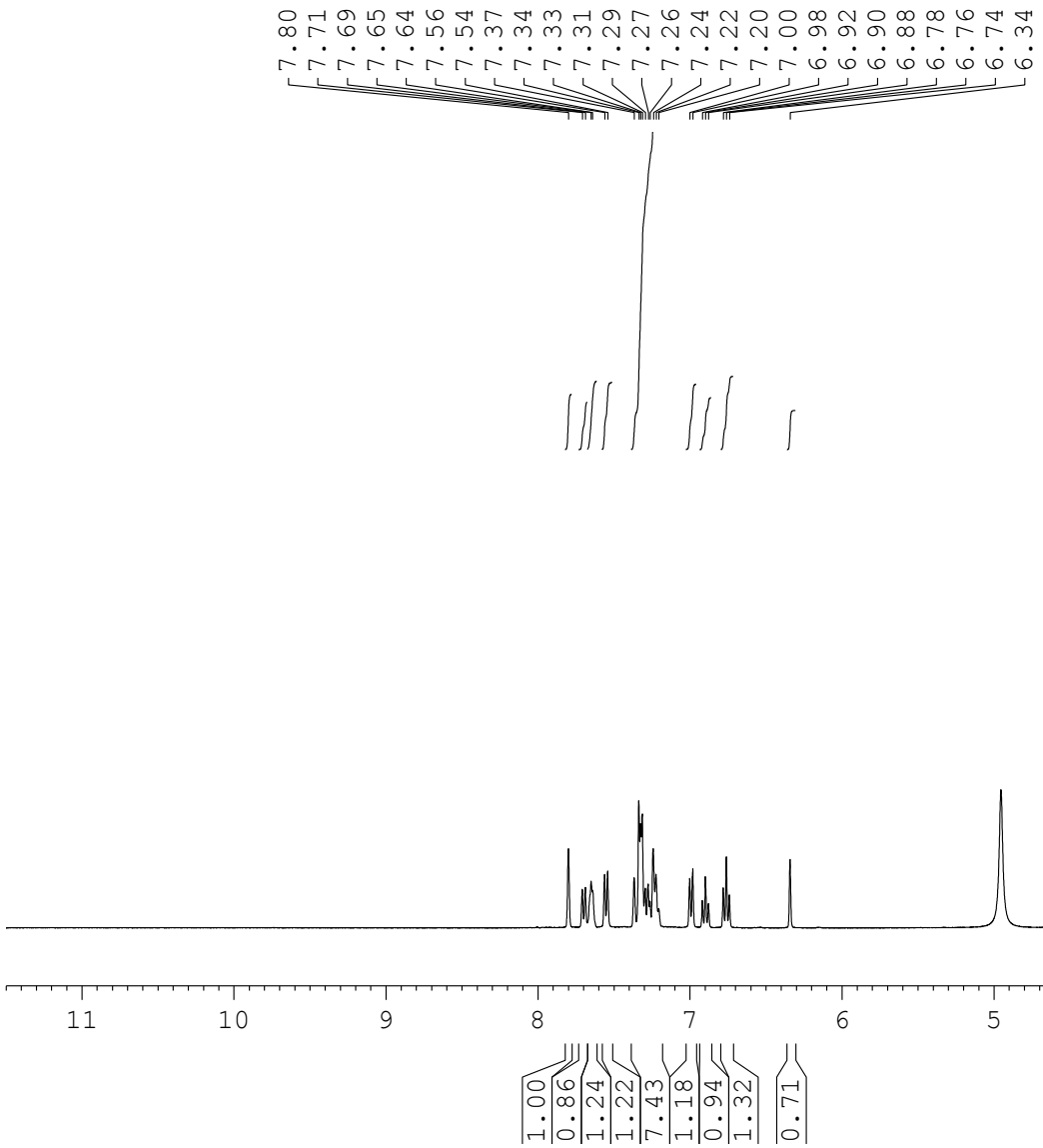
***Z*-26**

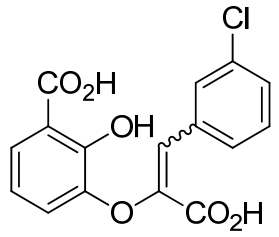


***Z*-27**



***Z*-27**





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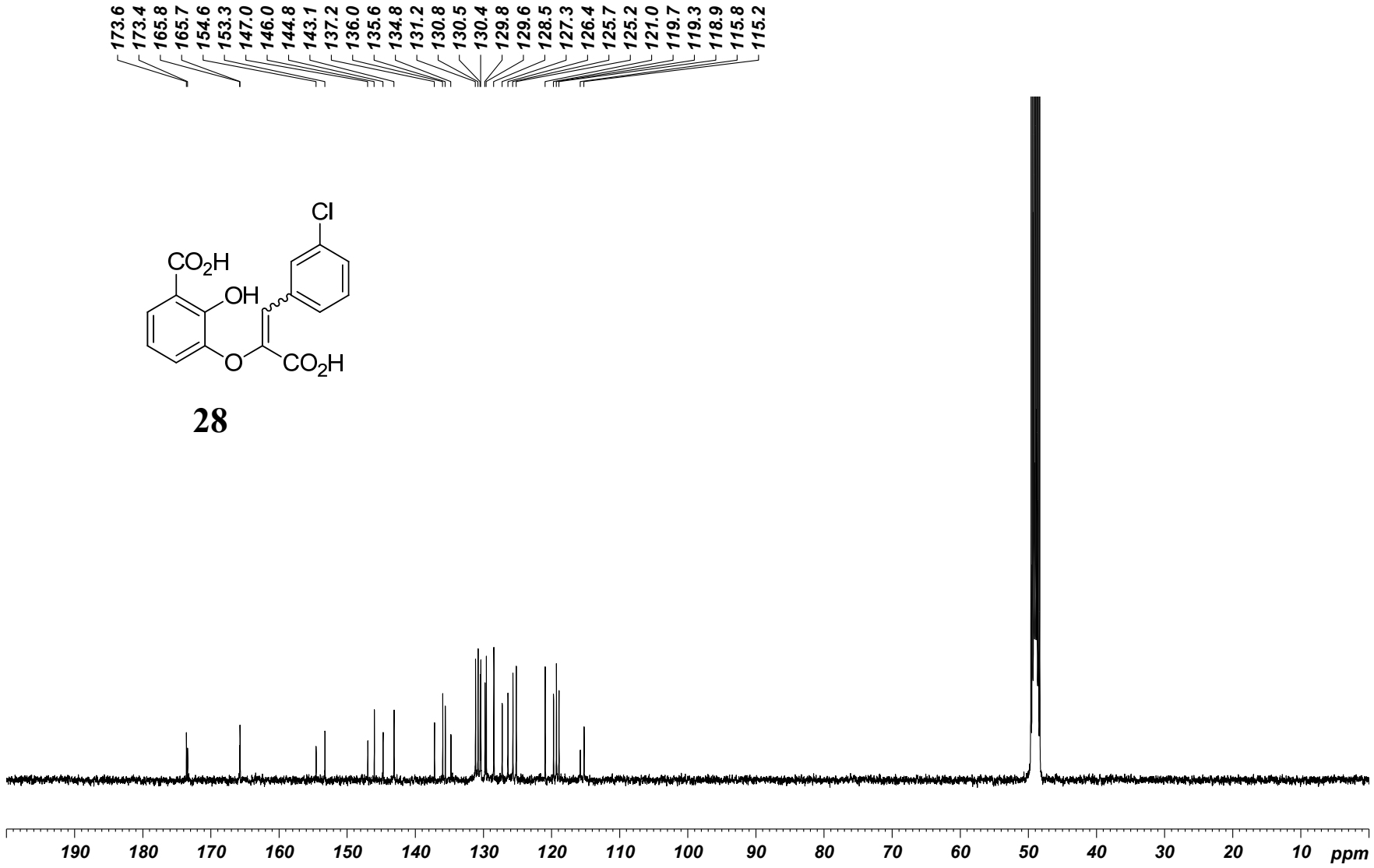

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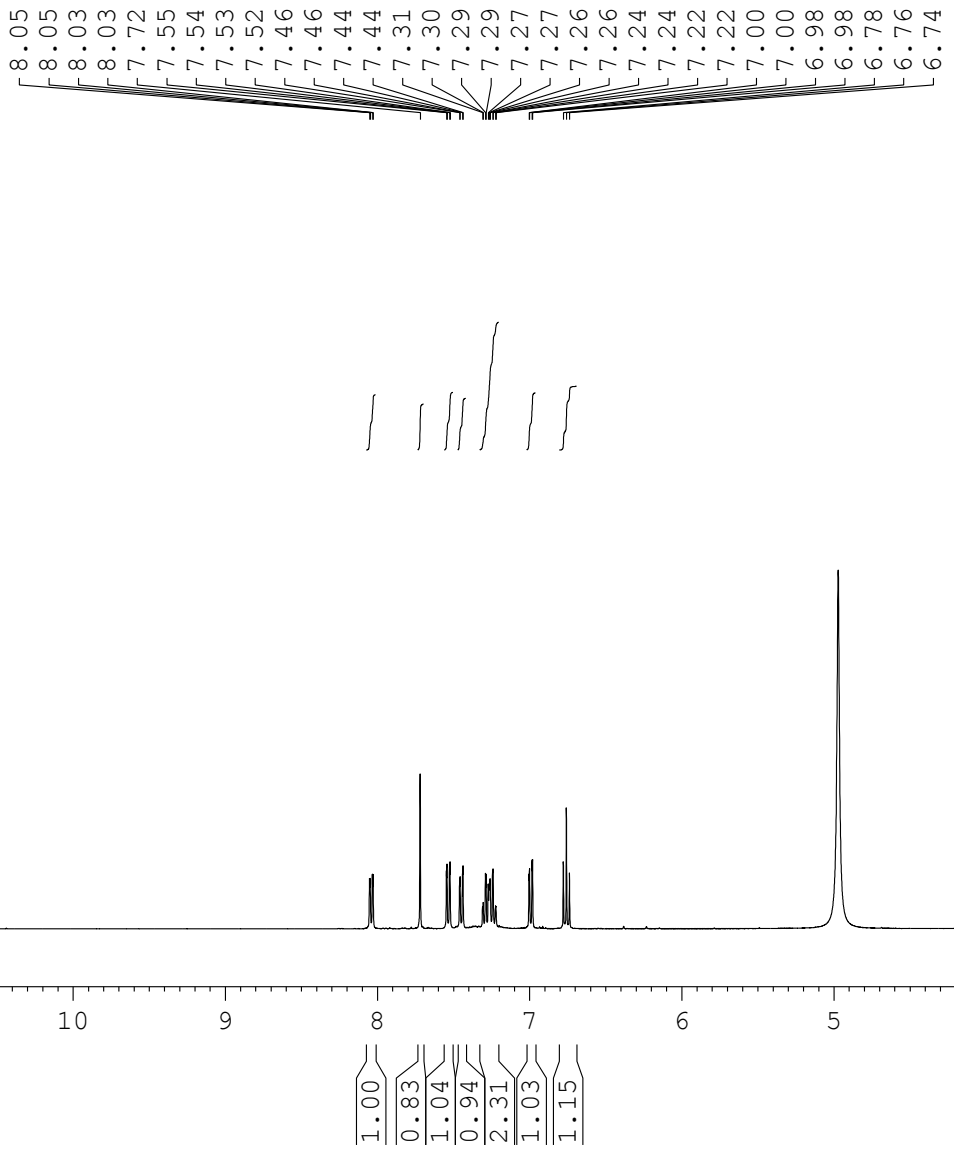

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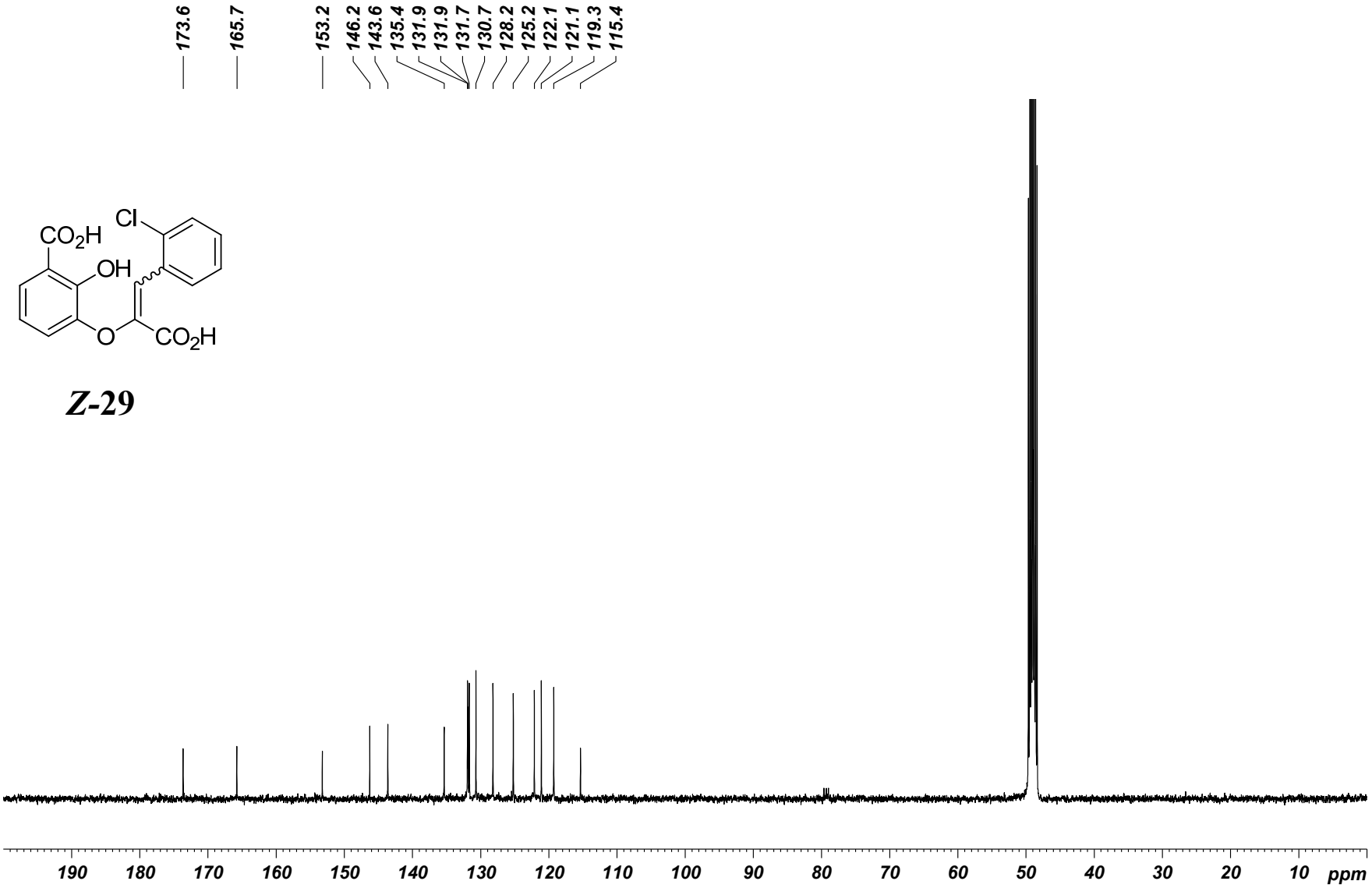
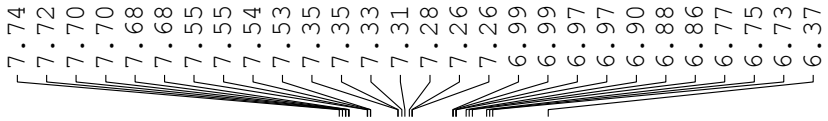
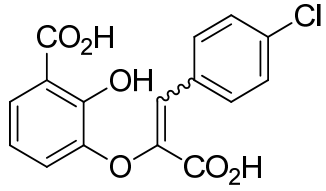


***Z*-29**





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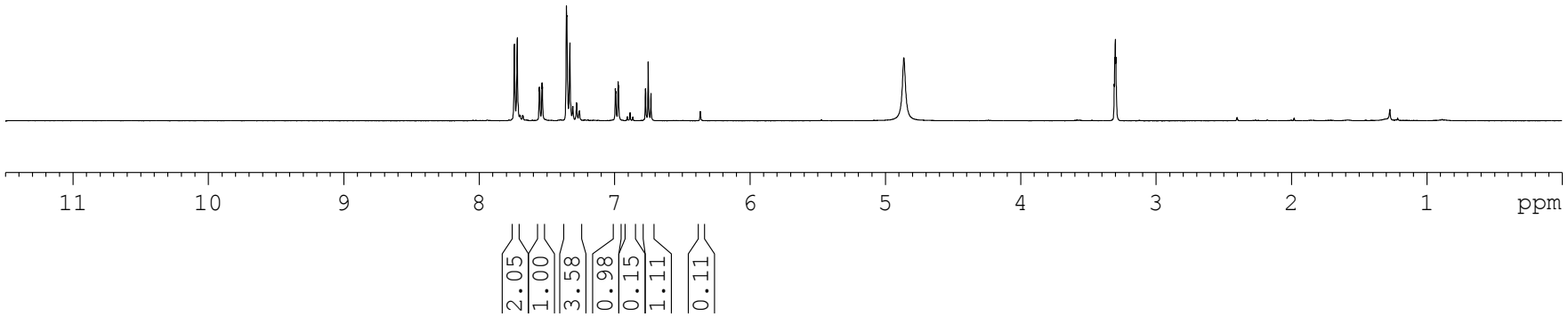

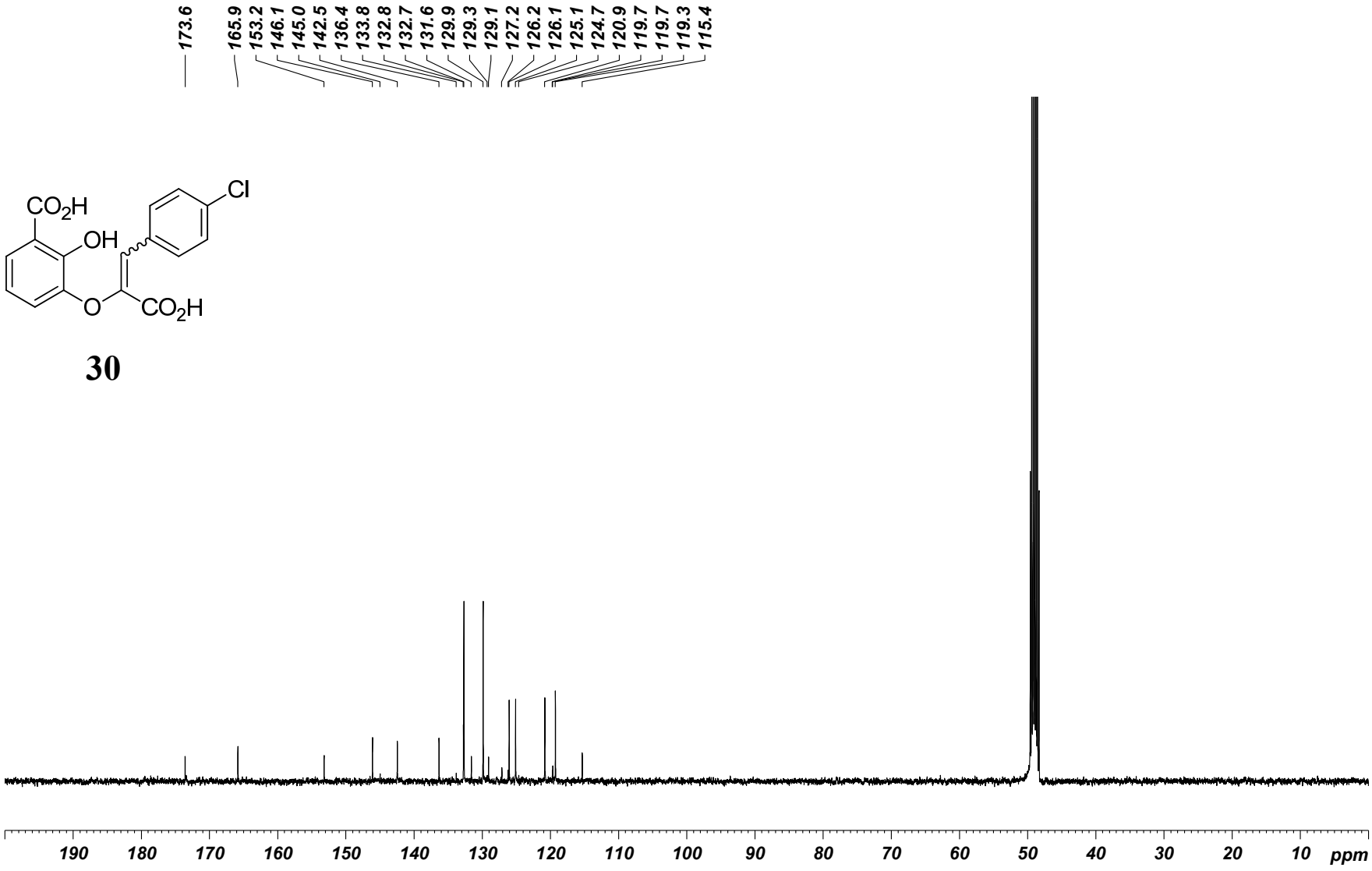

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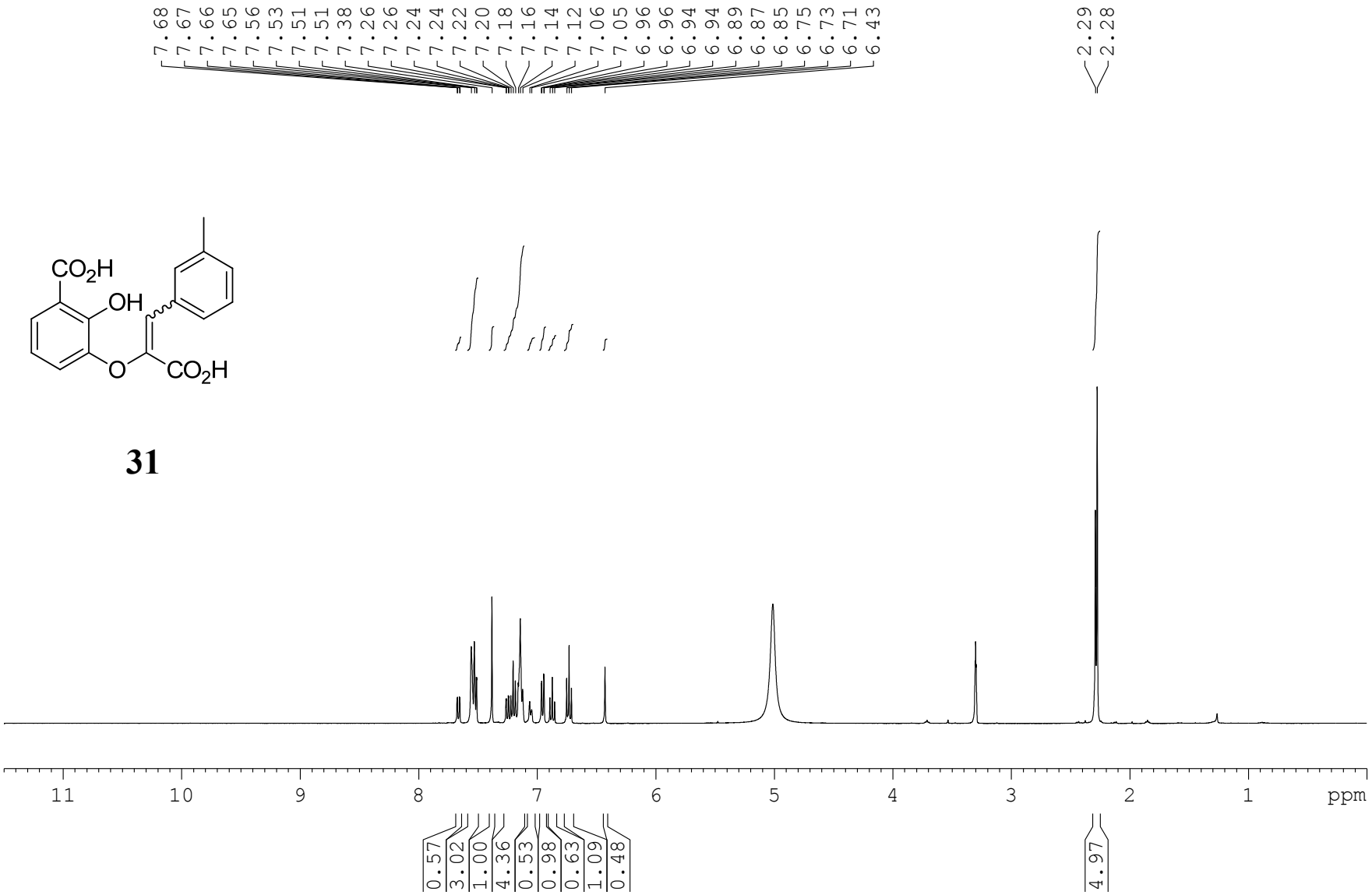

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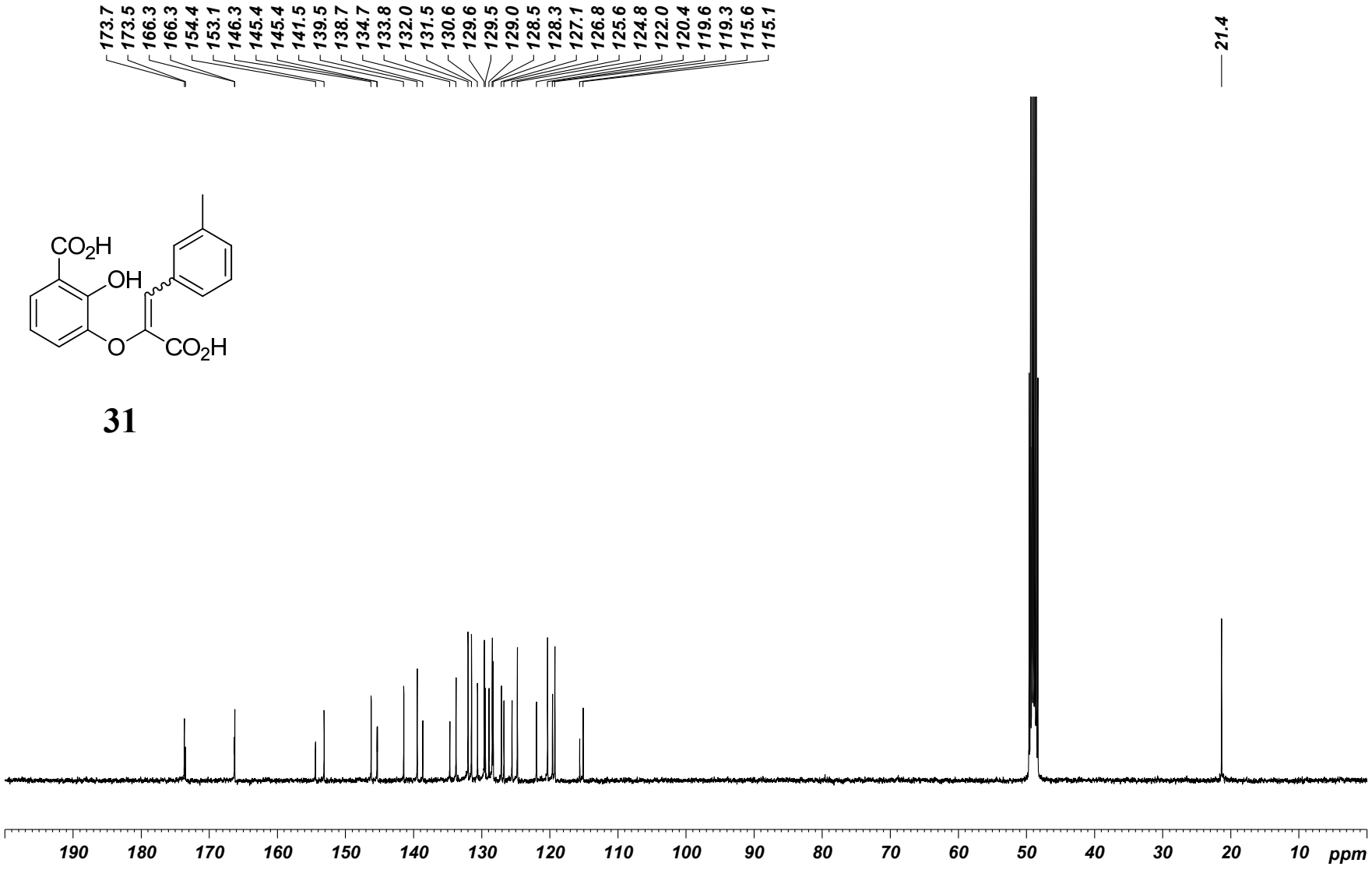

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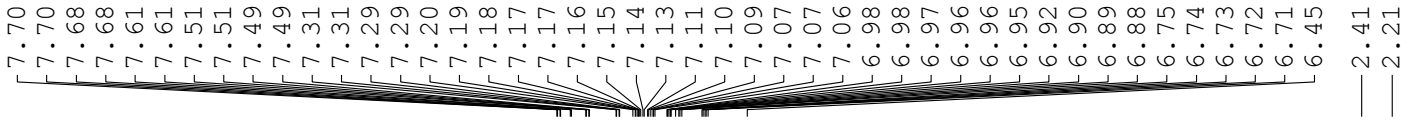

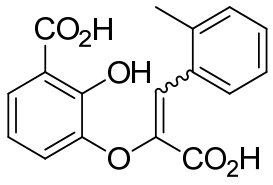

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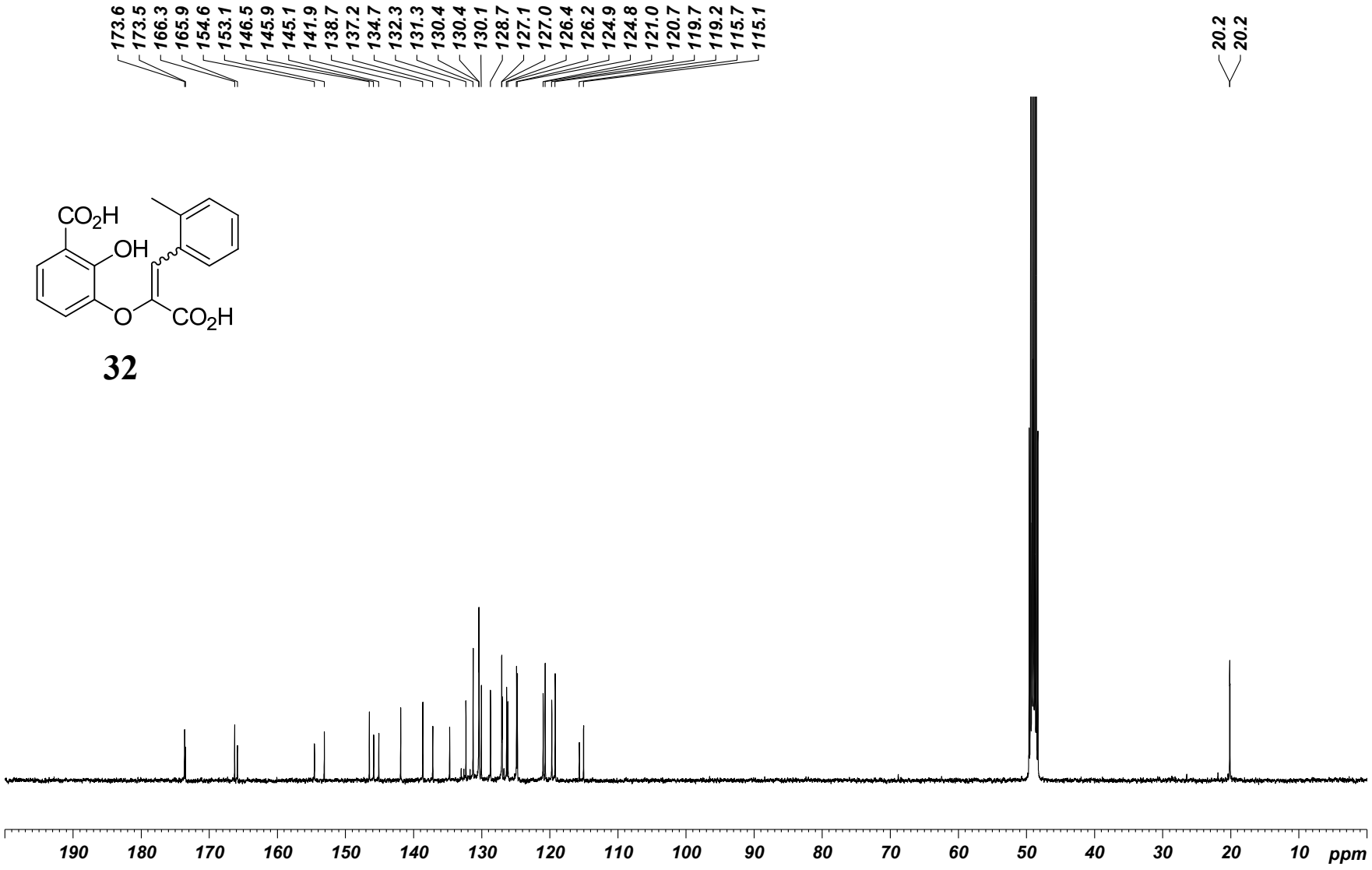

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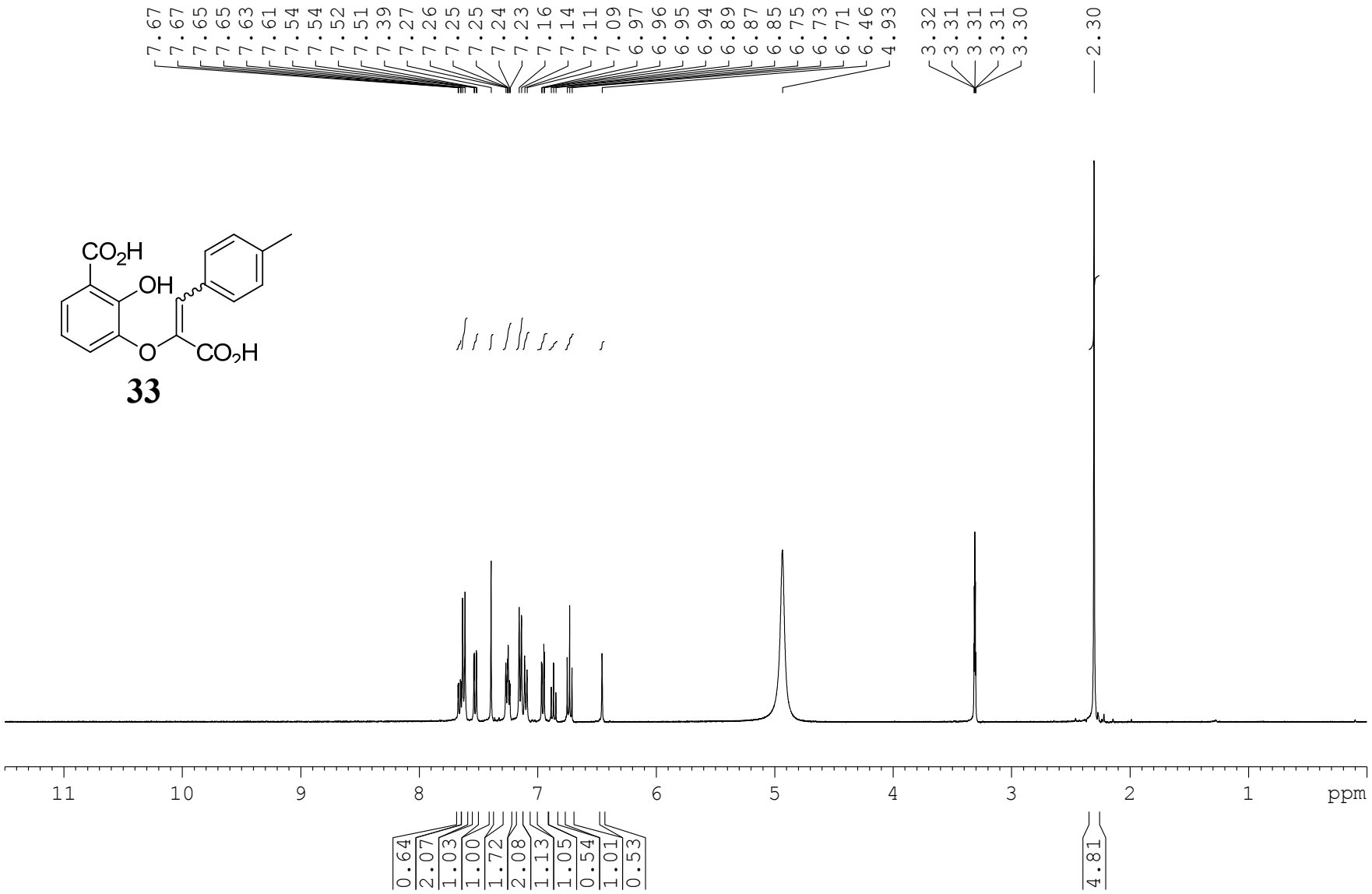

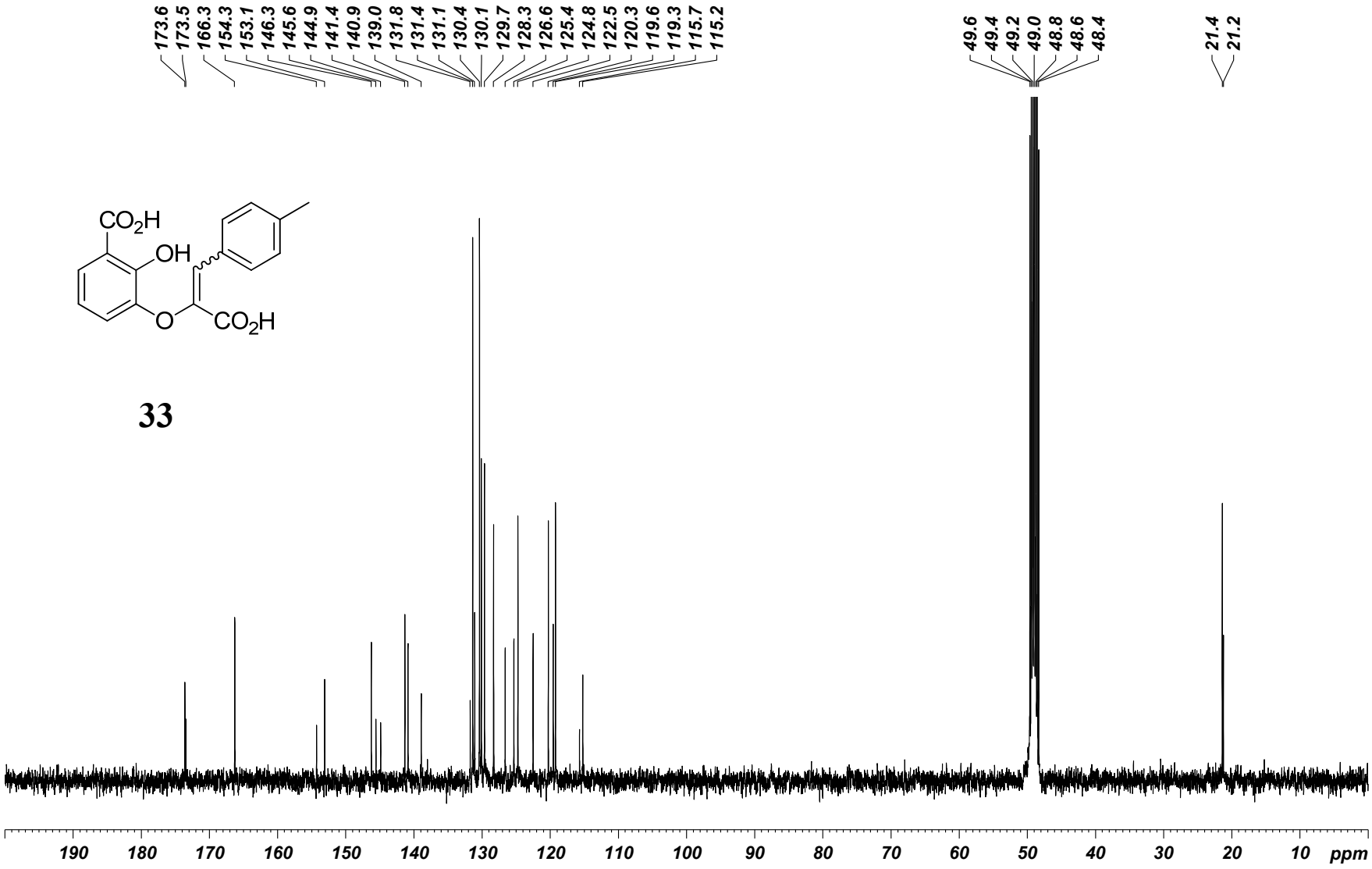

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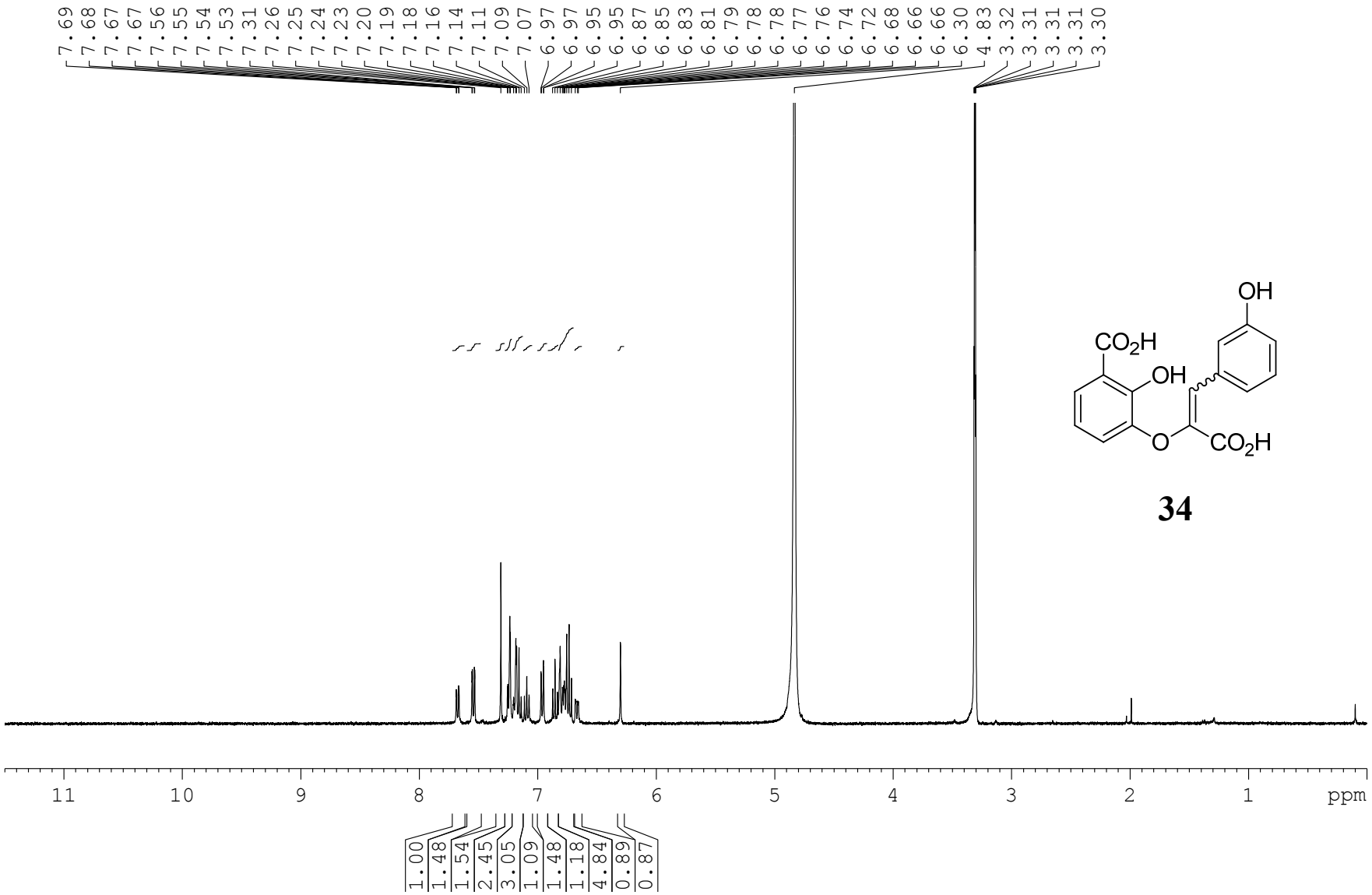

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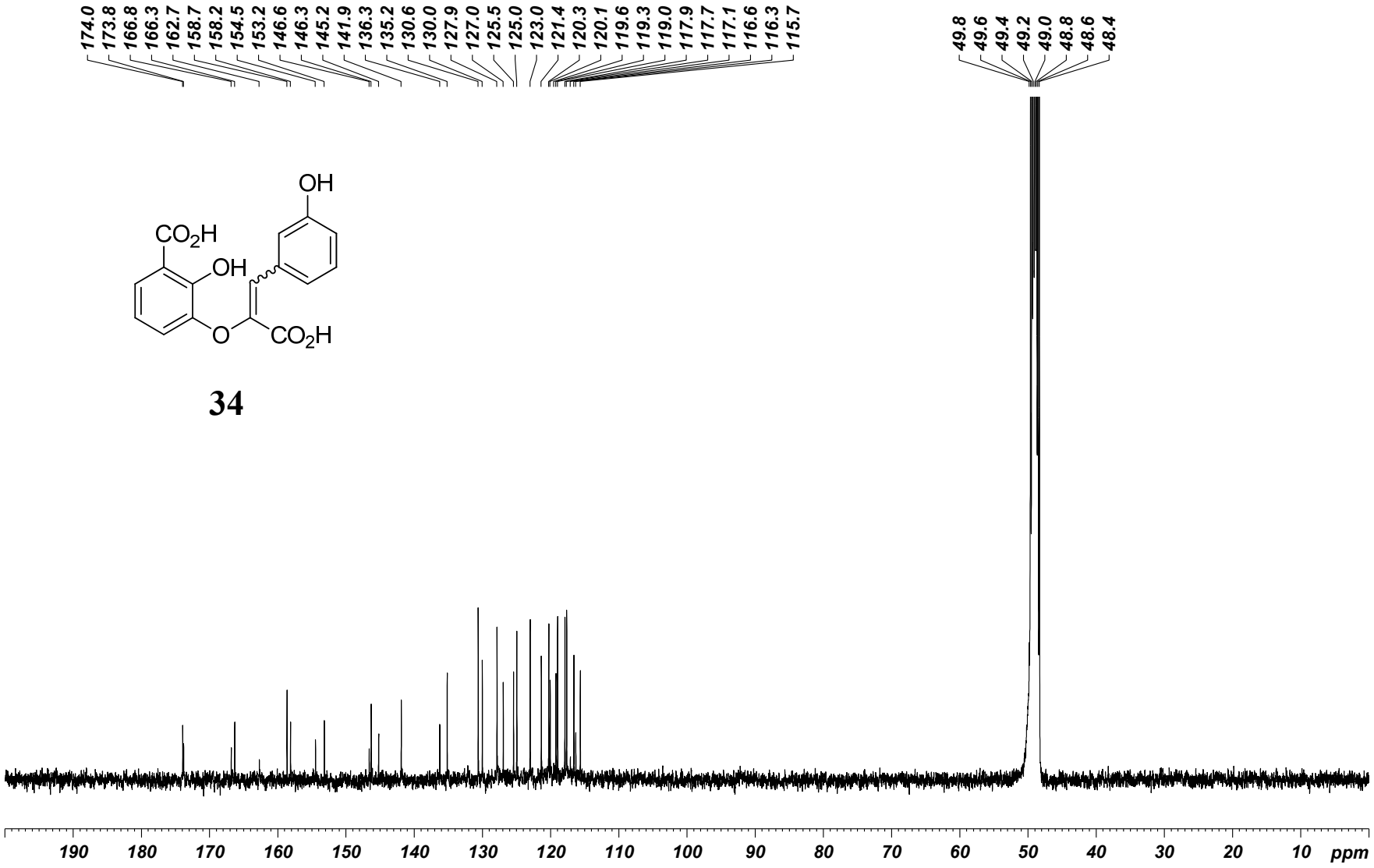

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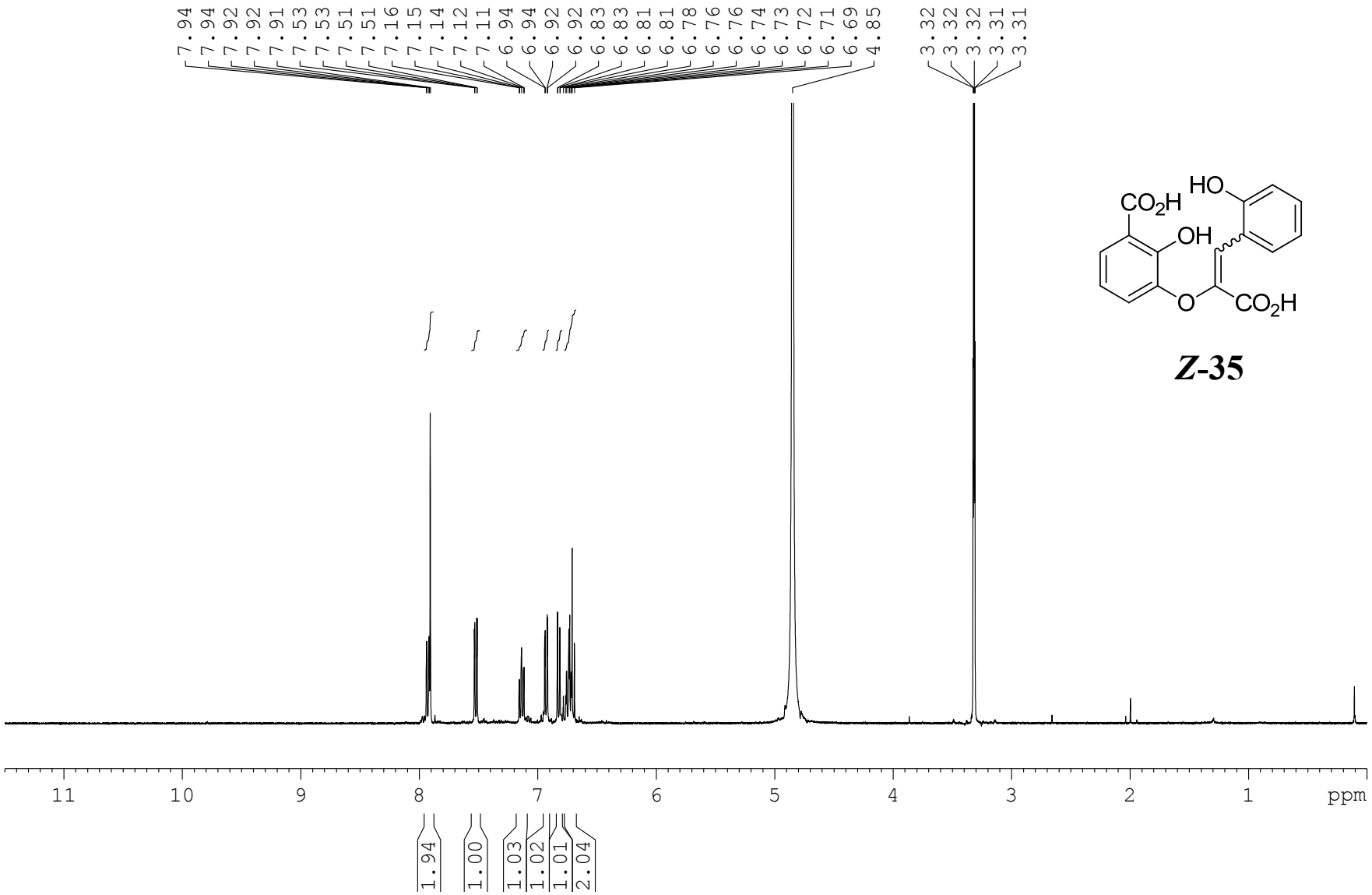
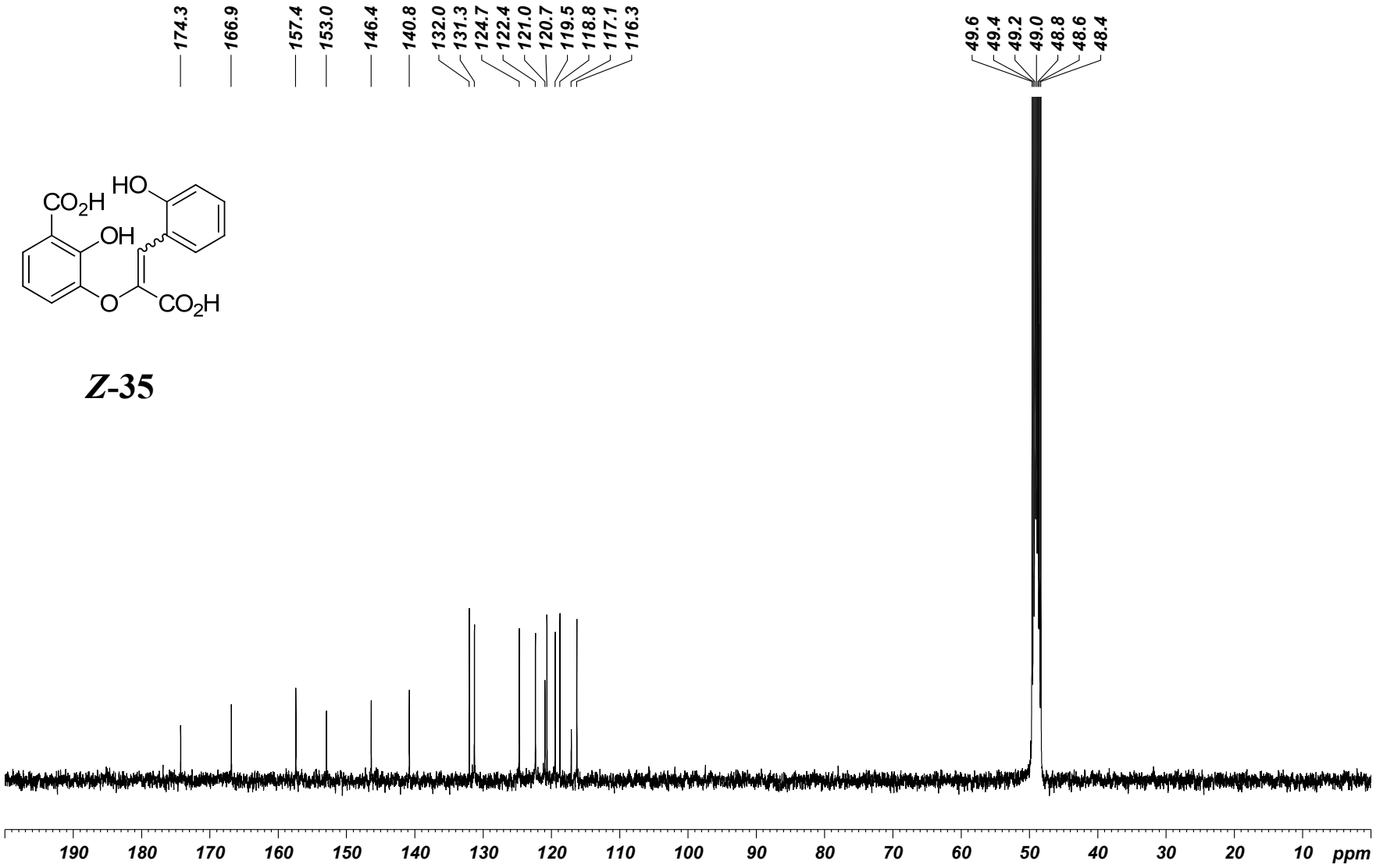


***Z*-35**



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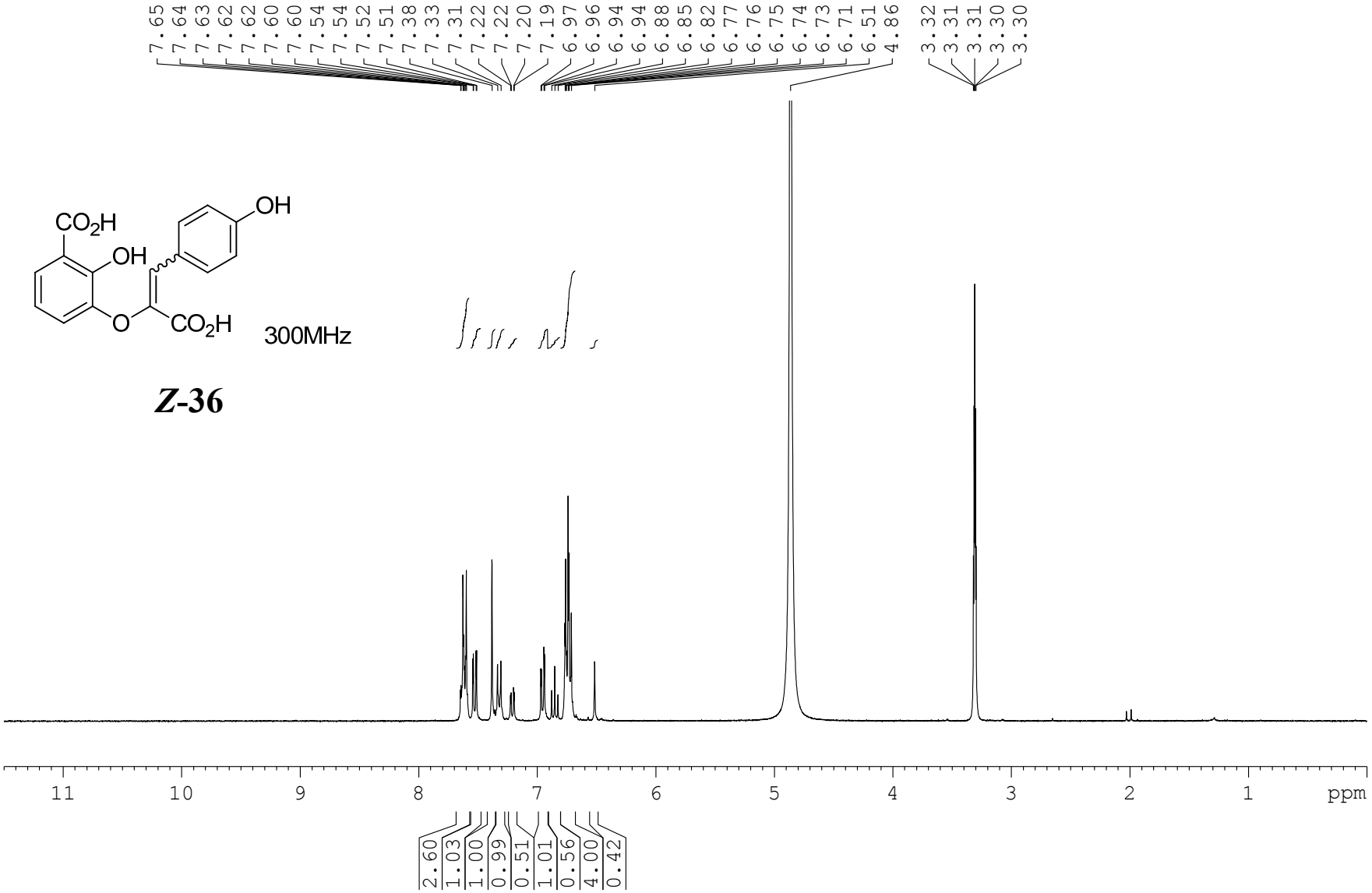

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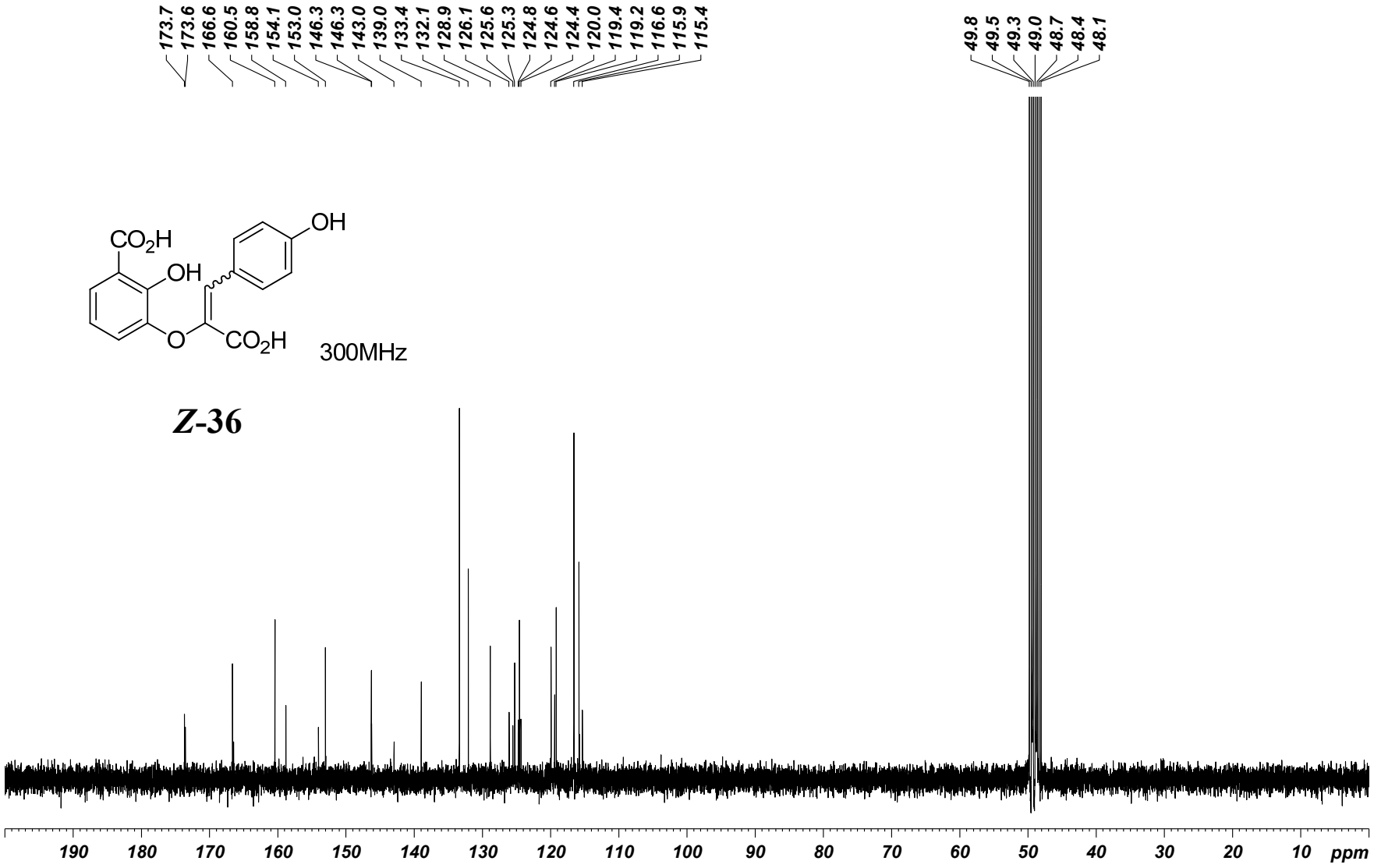

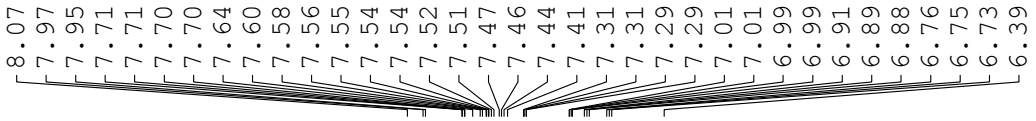

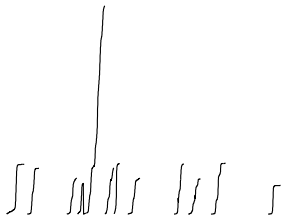

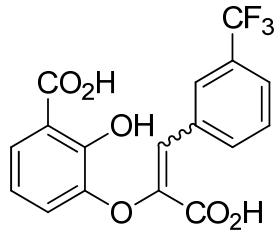

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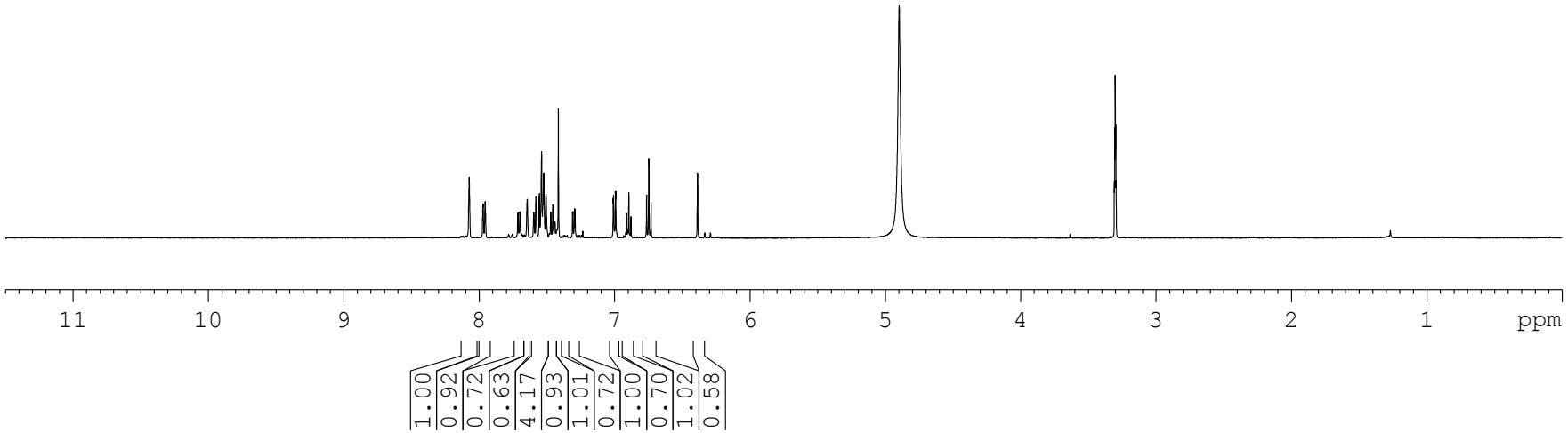

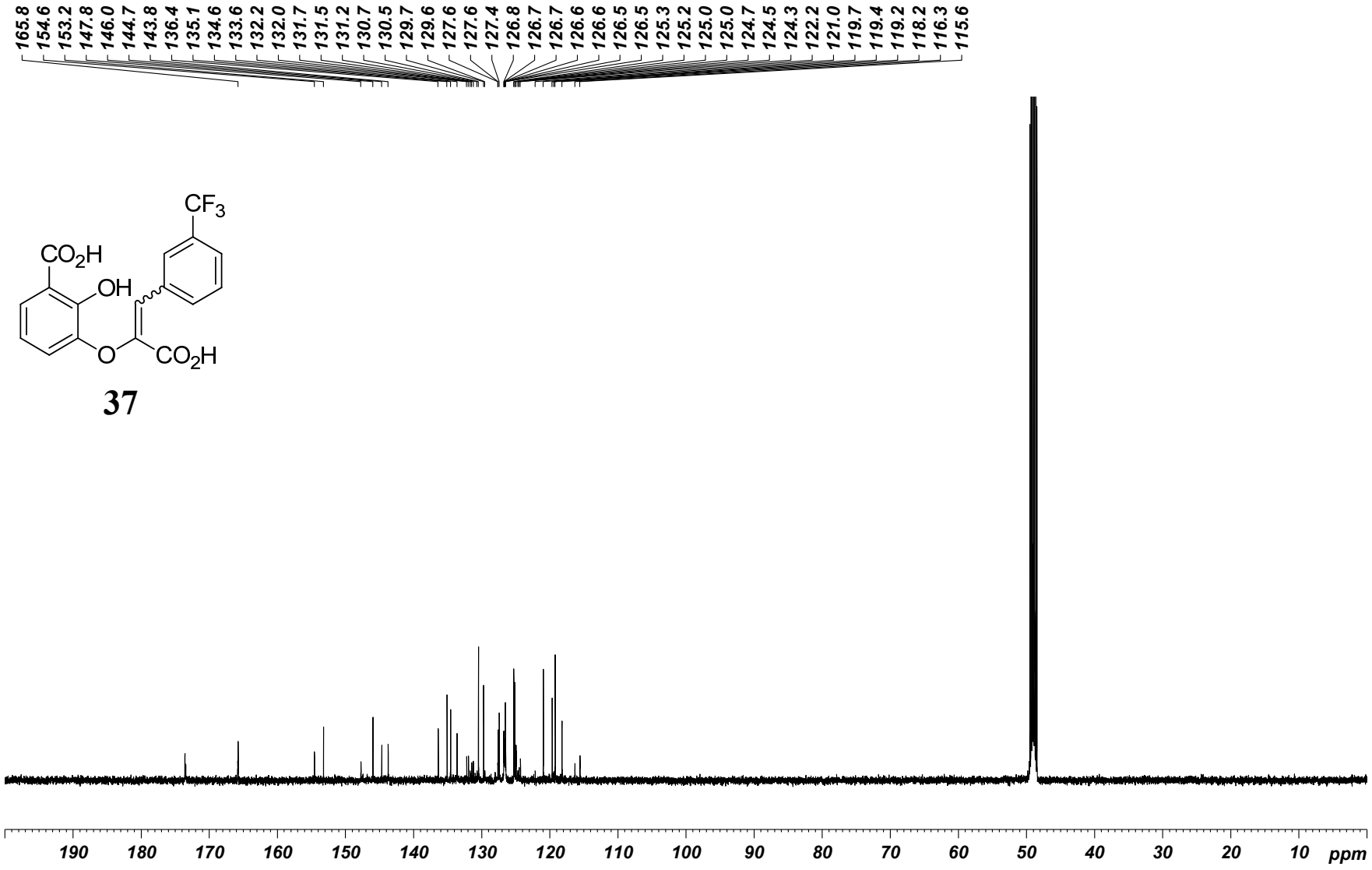

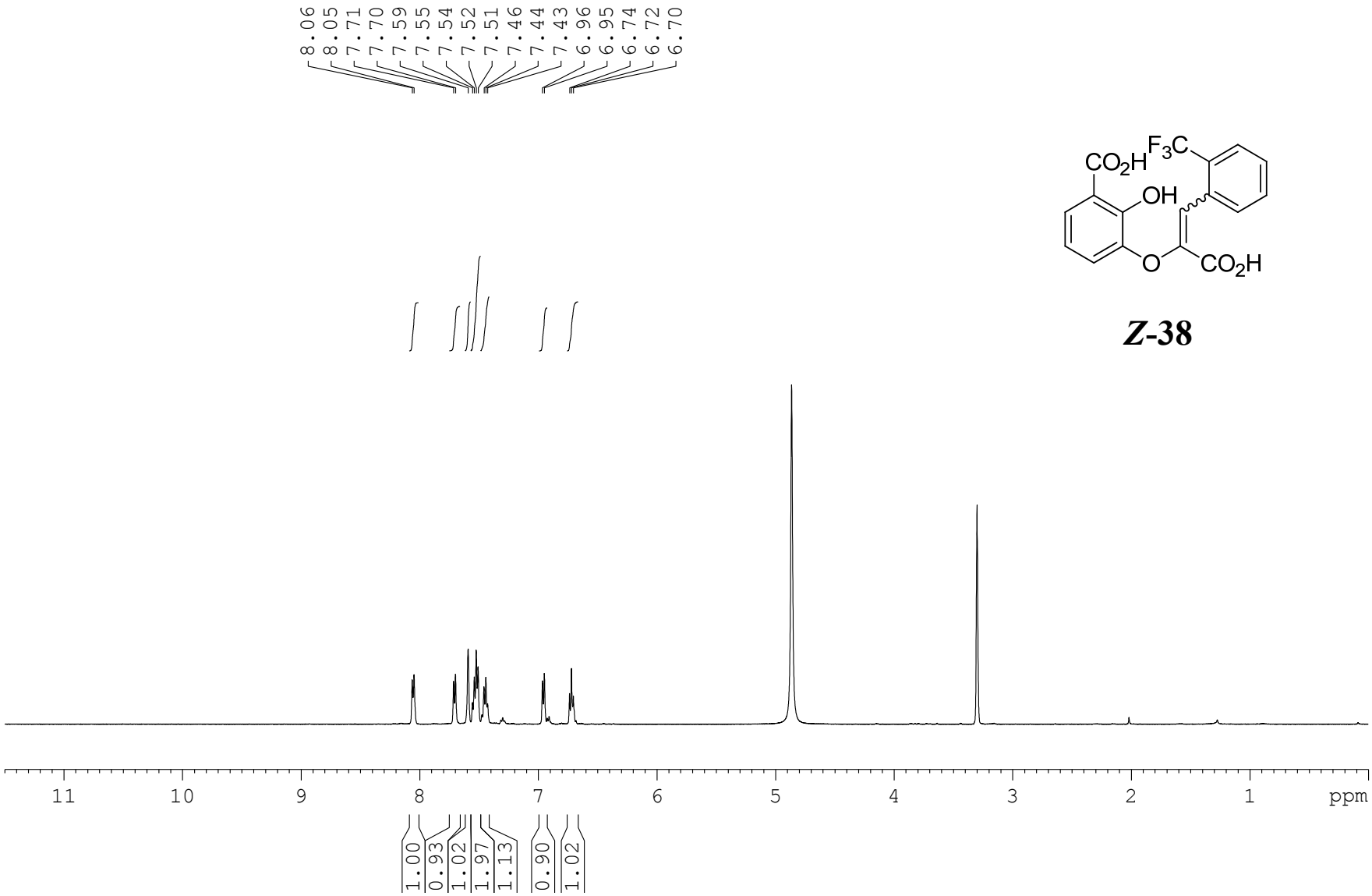

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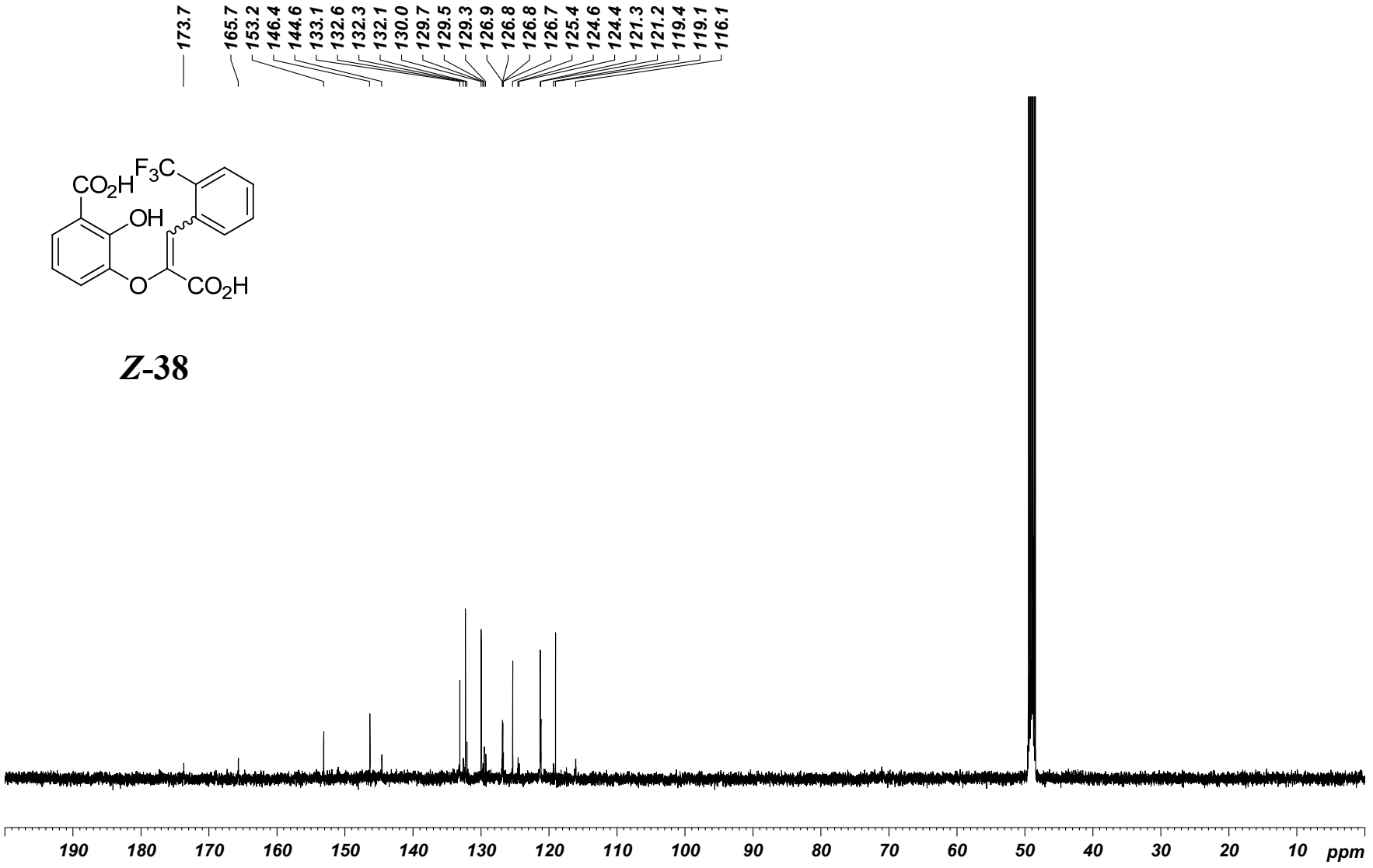

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