

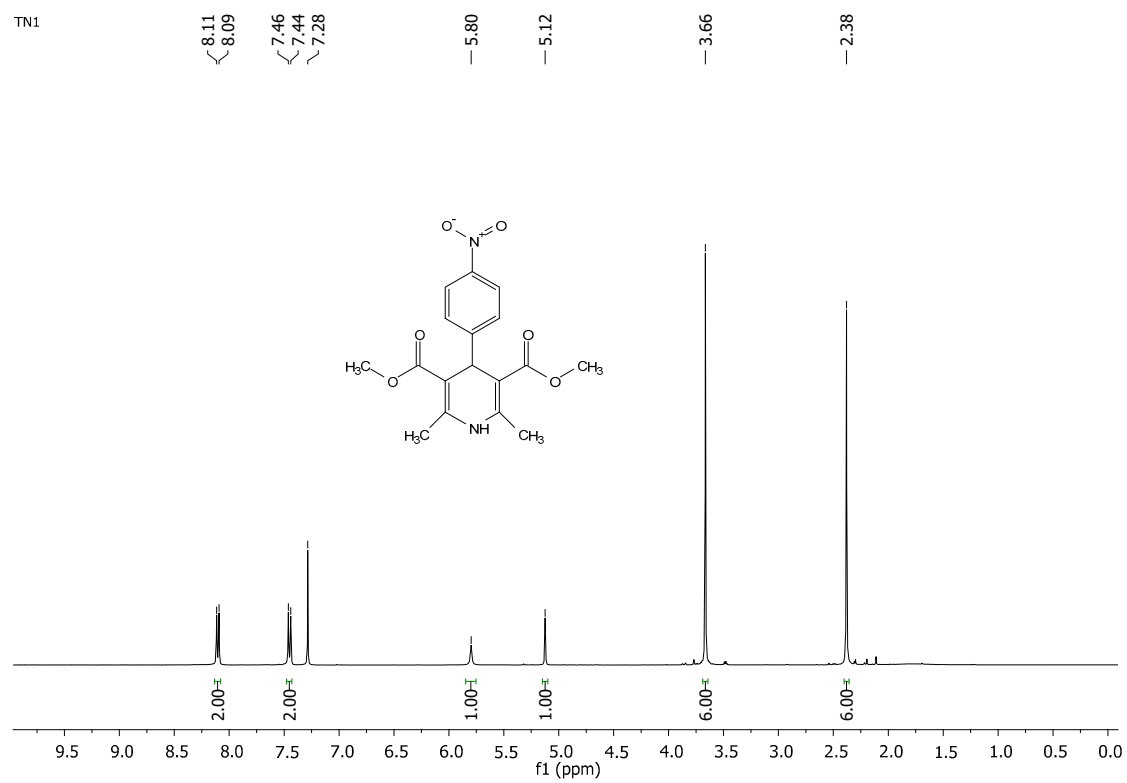
SUPPORTING INFORMATION

Exploring the Potential of Sulfonamide-Dihydropyridine Hybrids as Multitargeted Ligands for Alzheimer's Disease Treatment

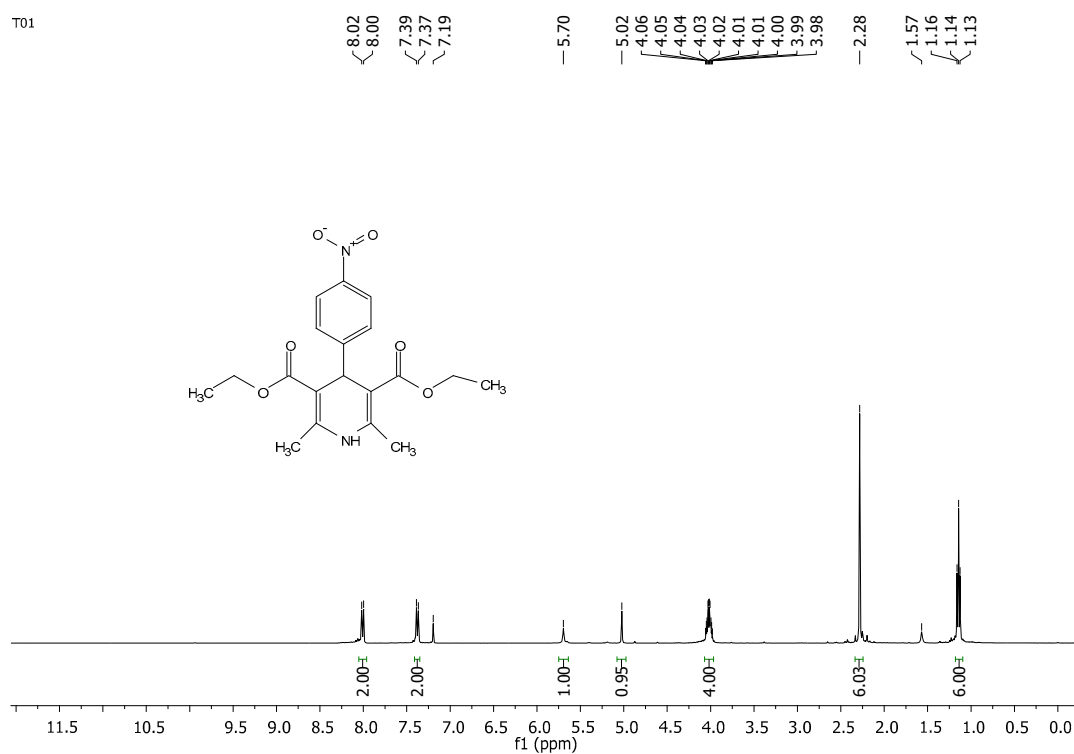
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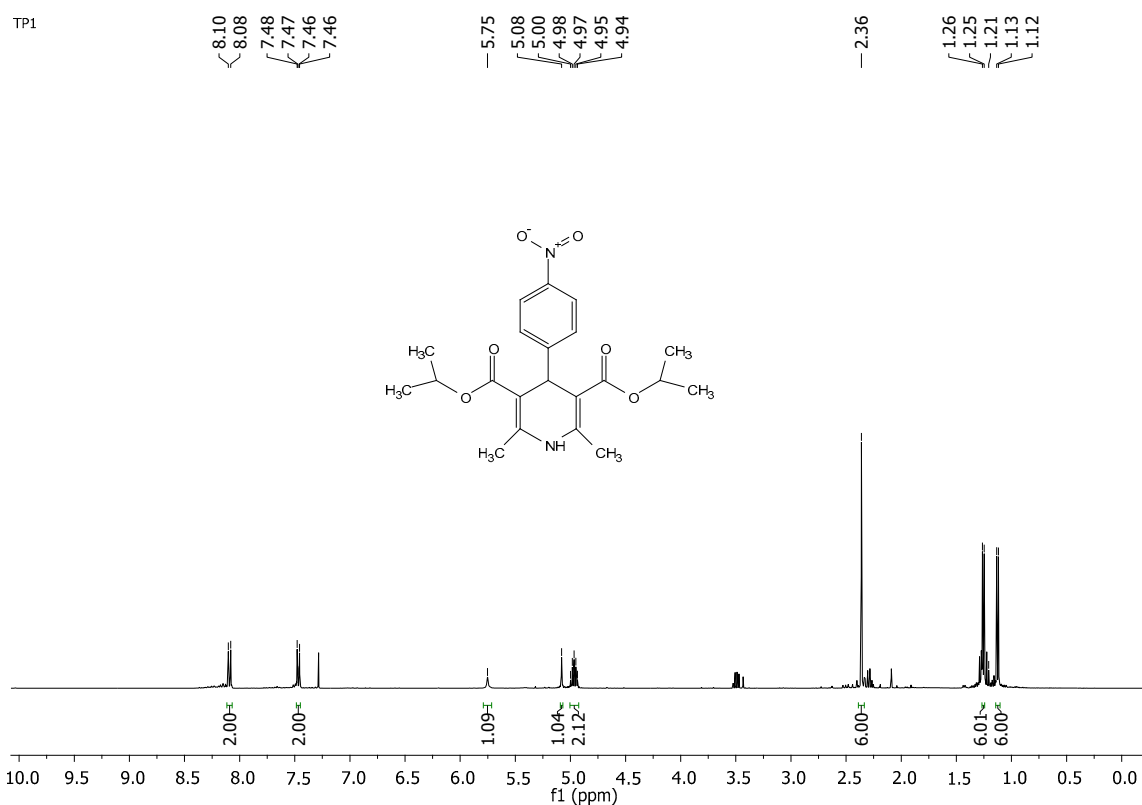
¹H NMR spectrum of compound **2a**



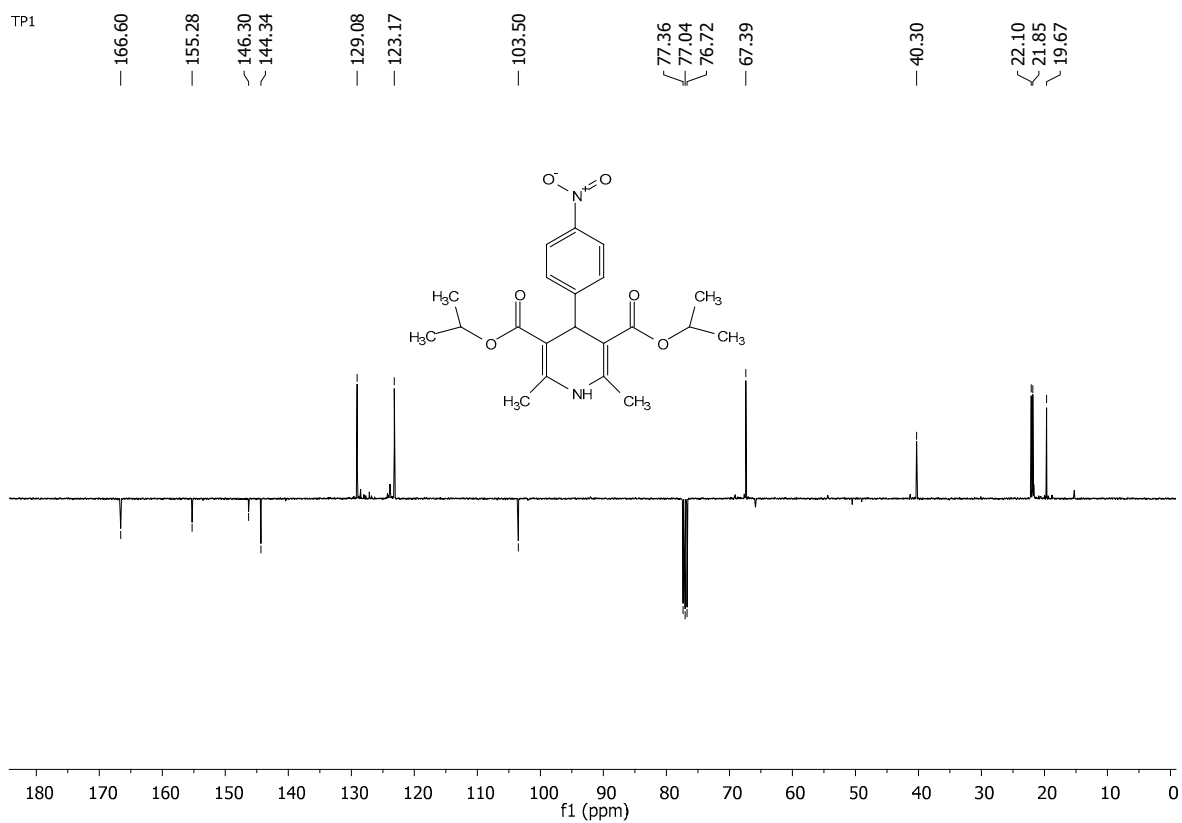
¹H NMR spectrum of compound **2b**



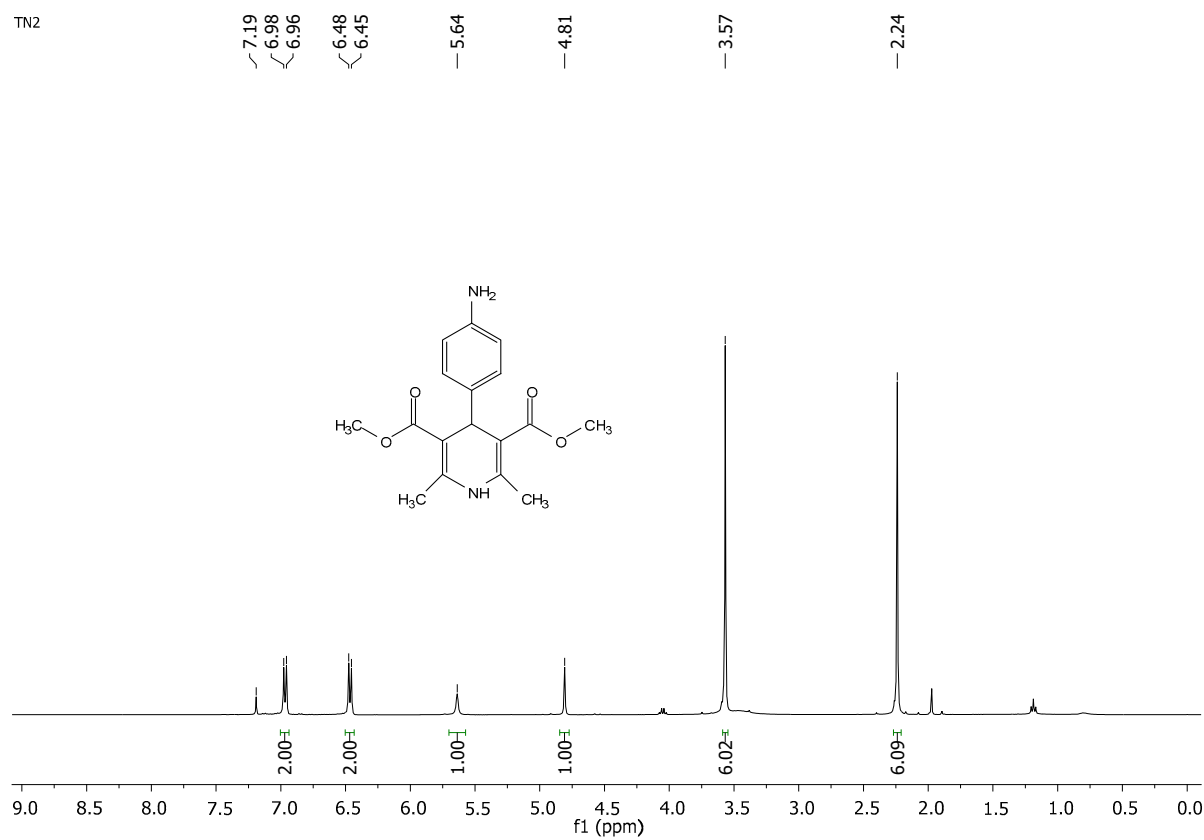
¹H NMR spectrum of compound **2c**



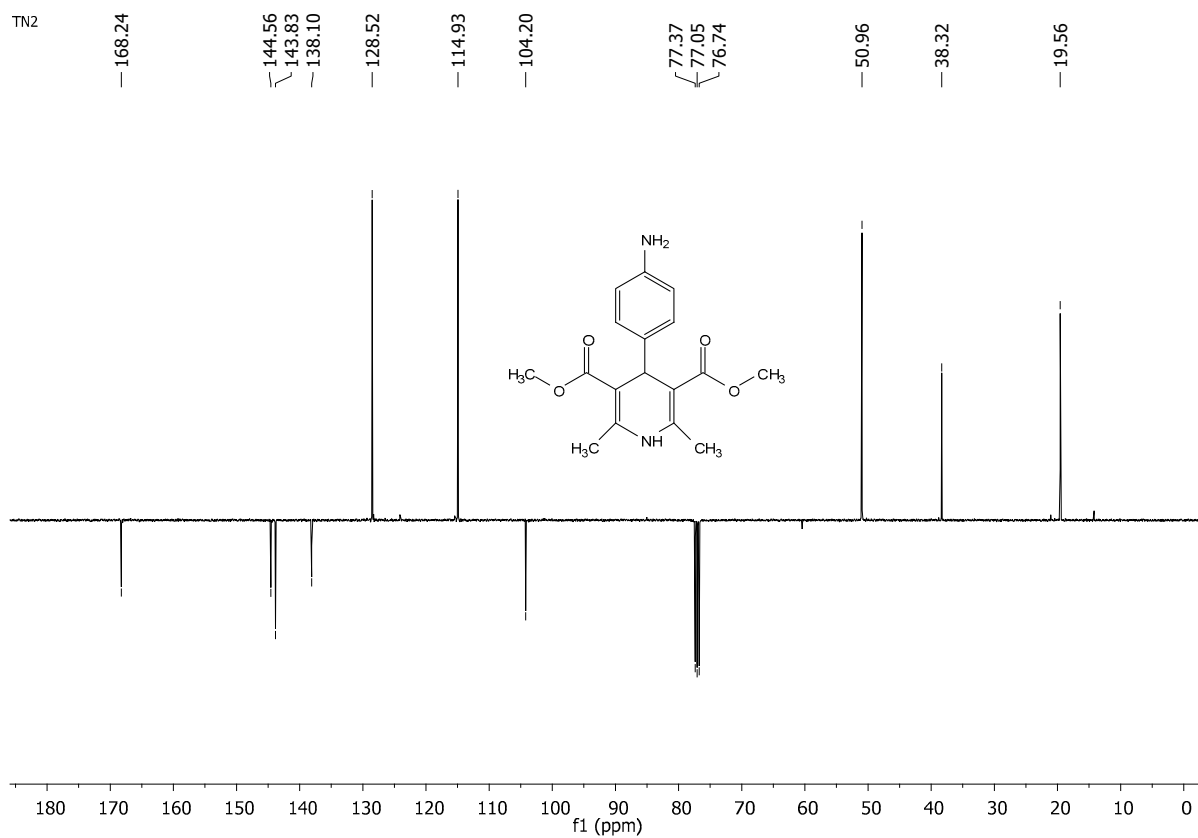
¹³C NMR spectrum of compound **2c**



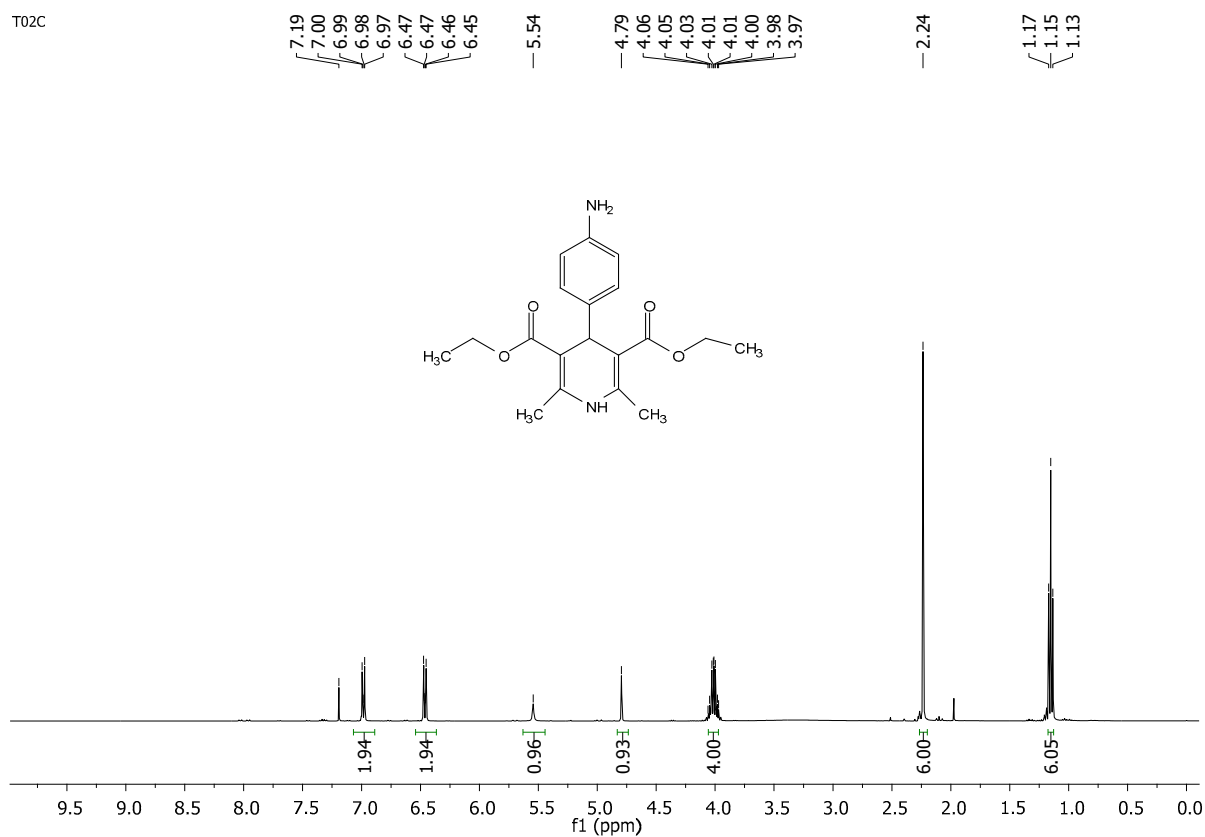
¹H NMR spectrum of compound **3a**



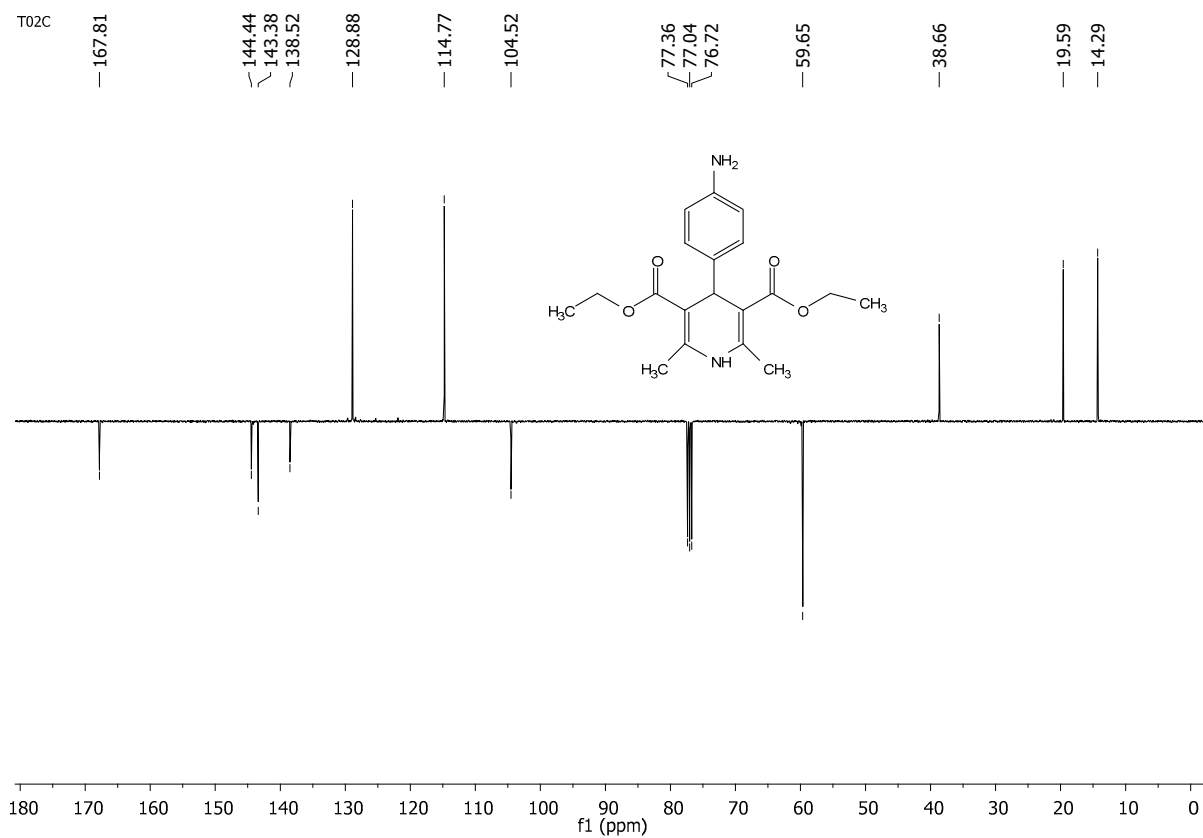
¹³C NMR spectrum of compound **3a**



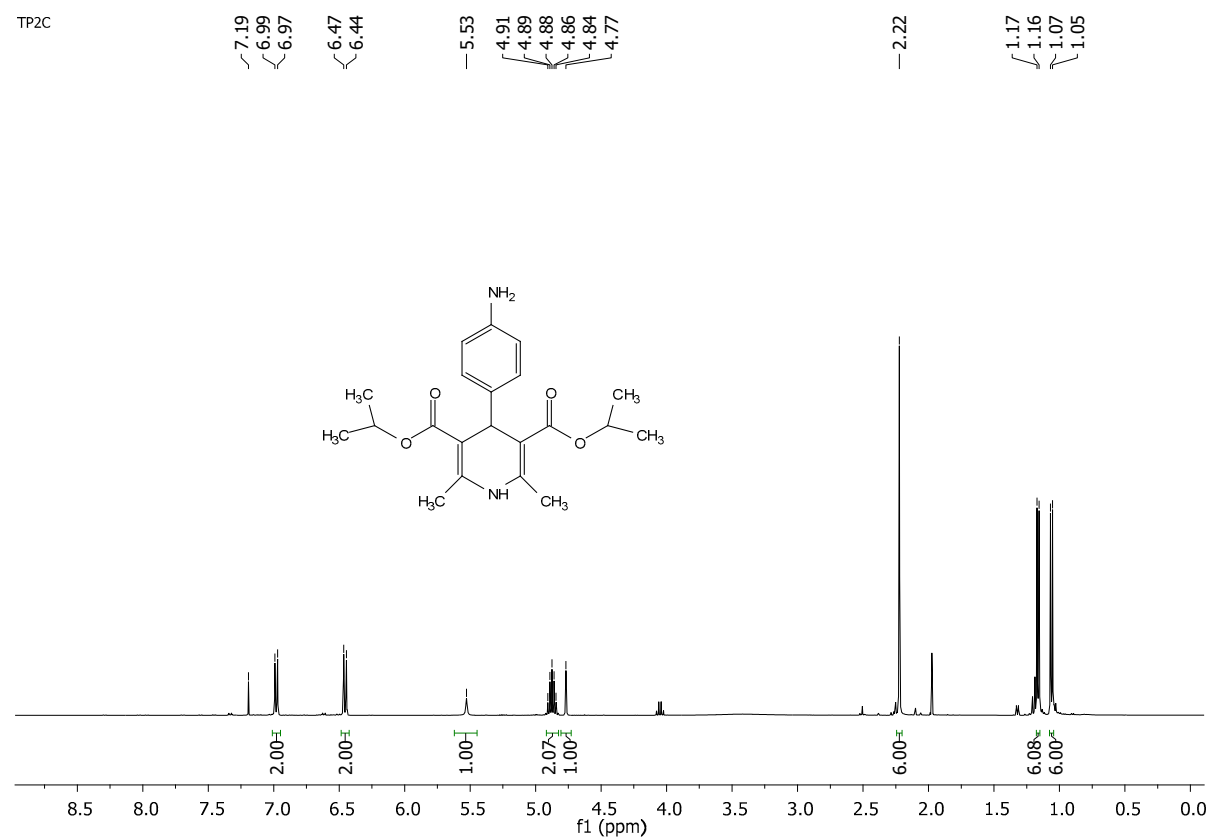
¹H NMR spectrum of compound **3b**



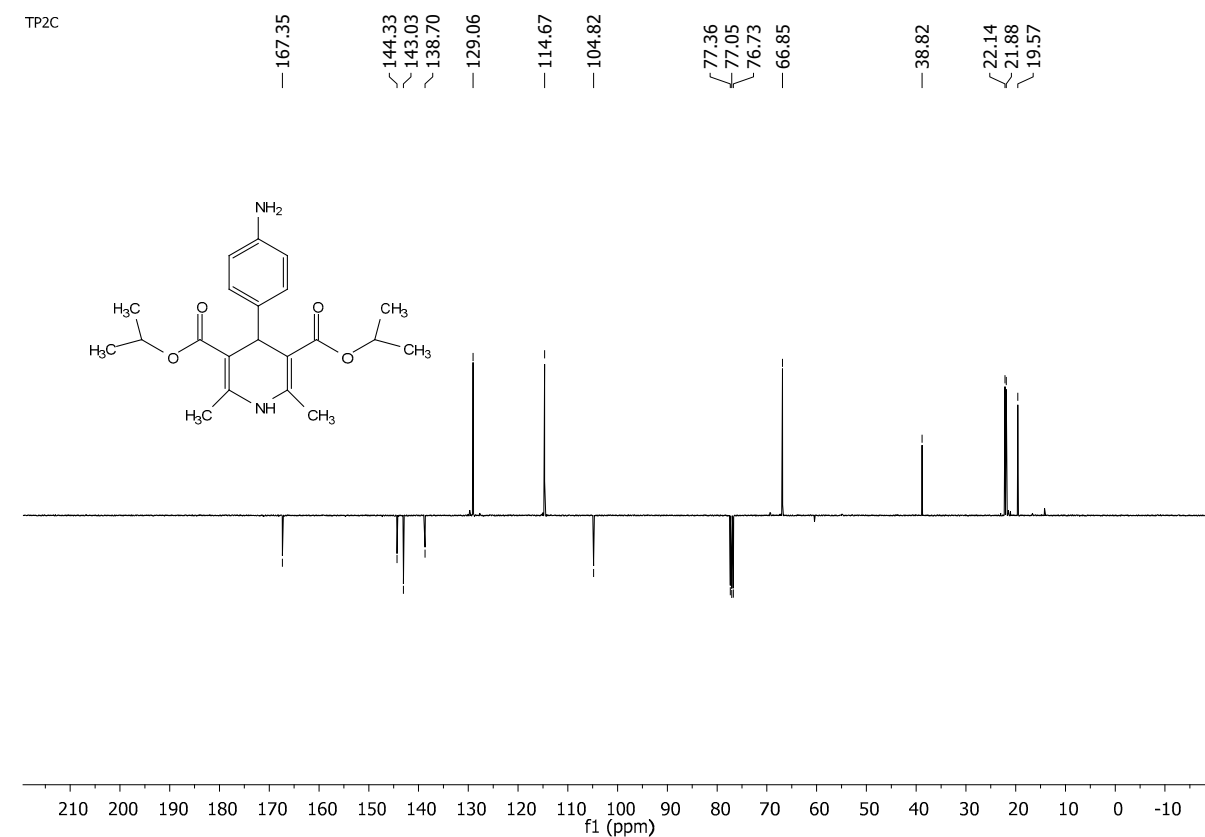
¹³C NMR spectrum of compound **3b**



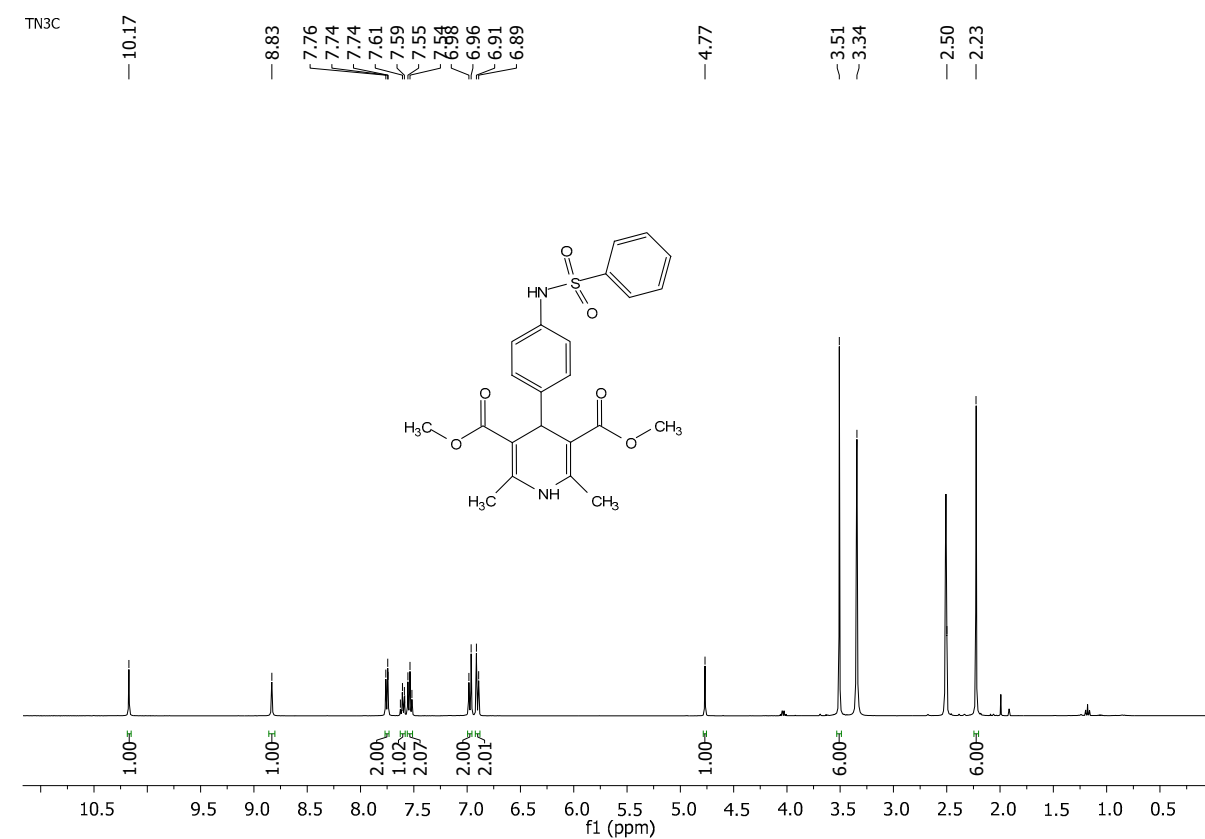
¹H NMR spectrum of compound **3c**



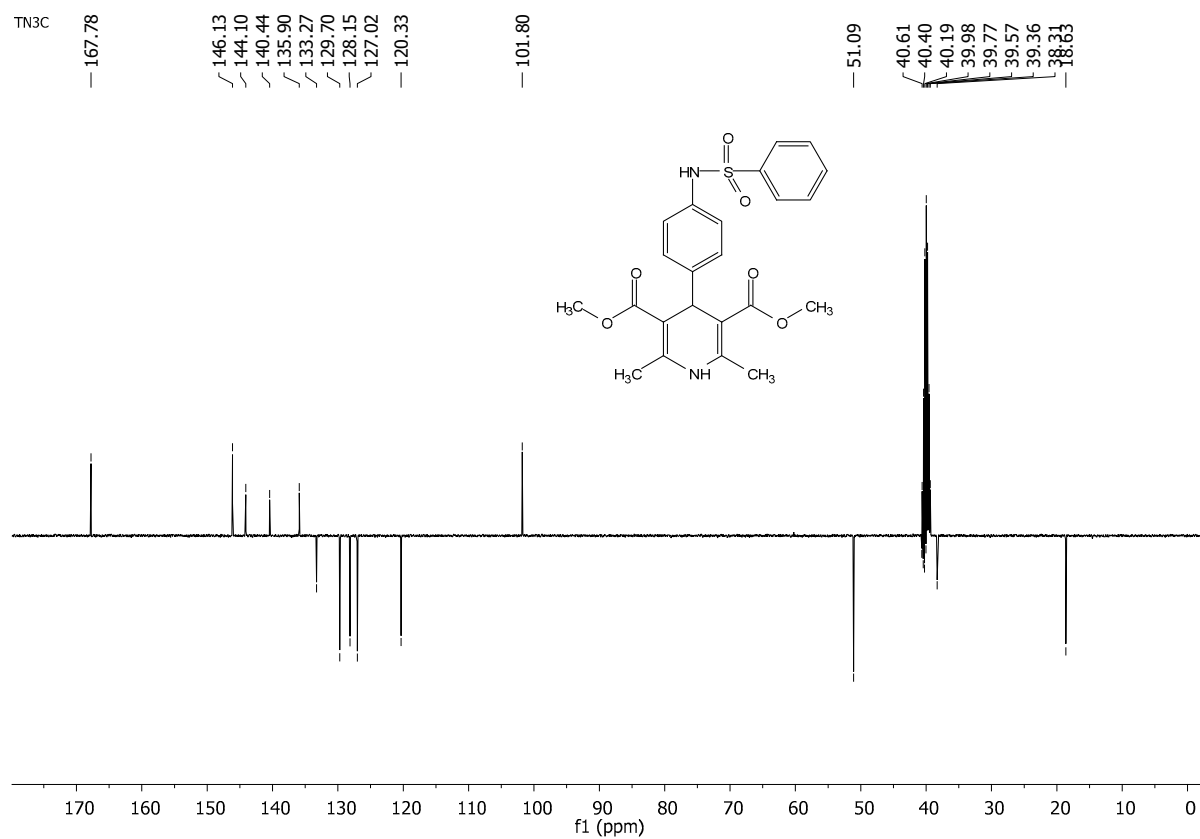
¹³C NMR spectrum of compound **3c**



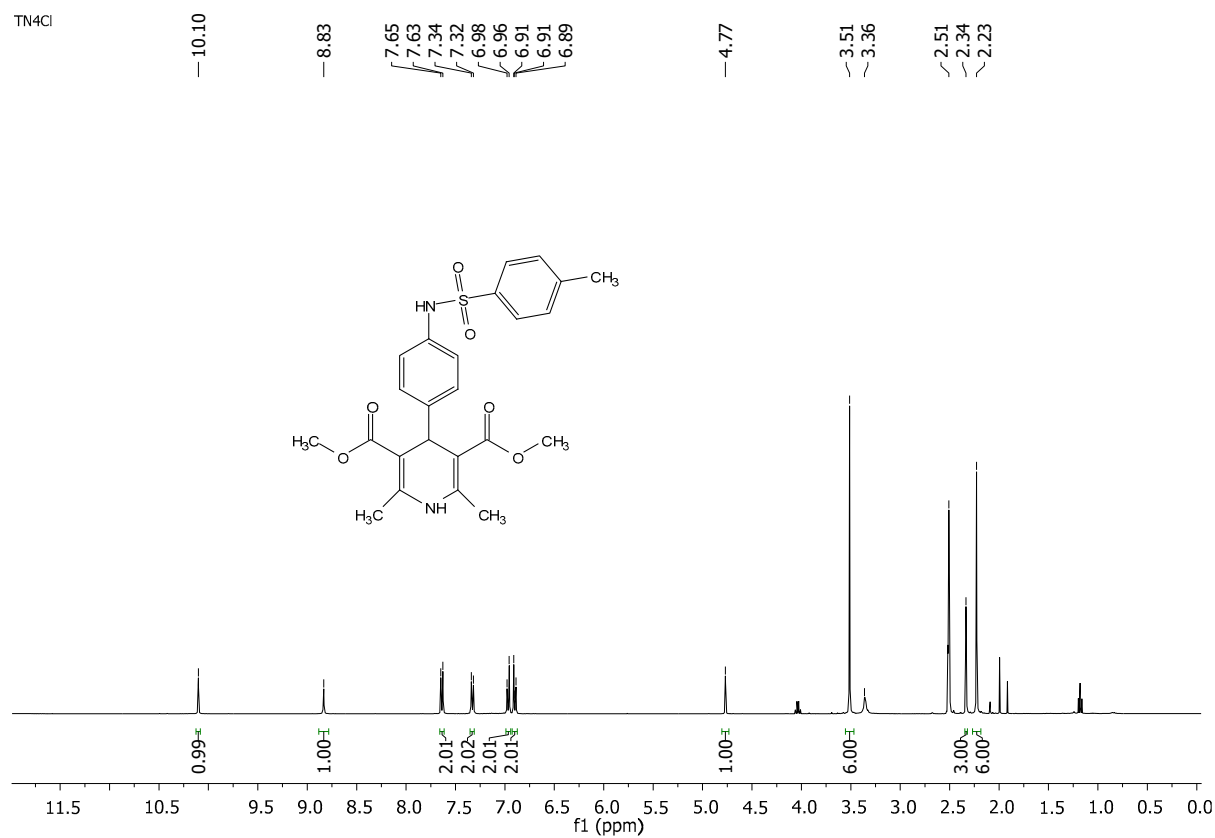
¹H NMR spectrum of compound **4a**



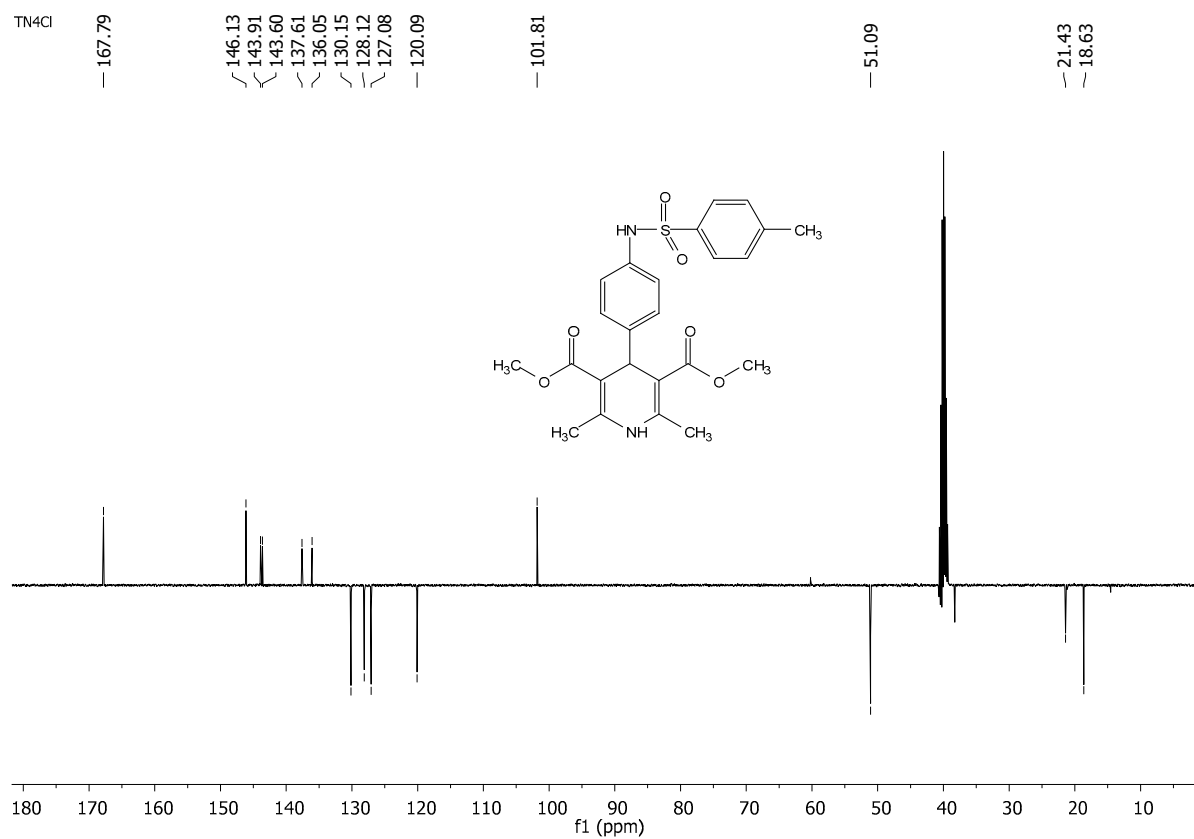
¹³C NMR spectrum of compound **4a**



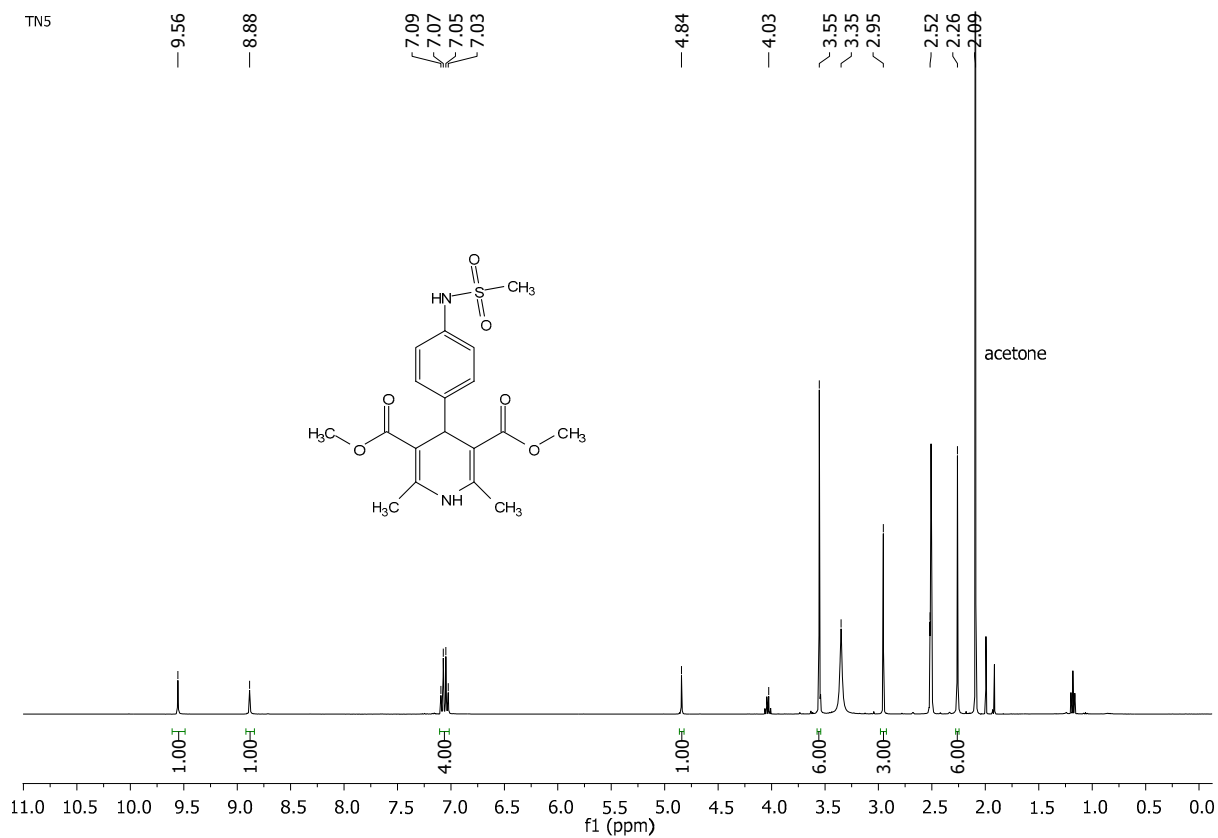
¹H NMR spectrum of compound **4b**



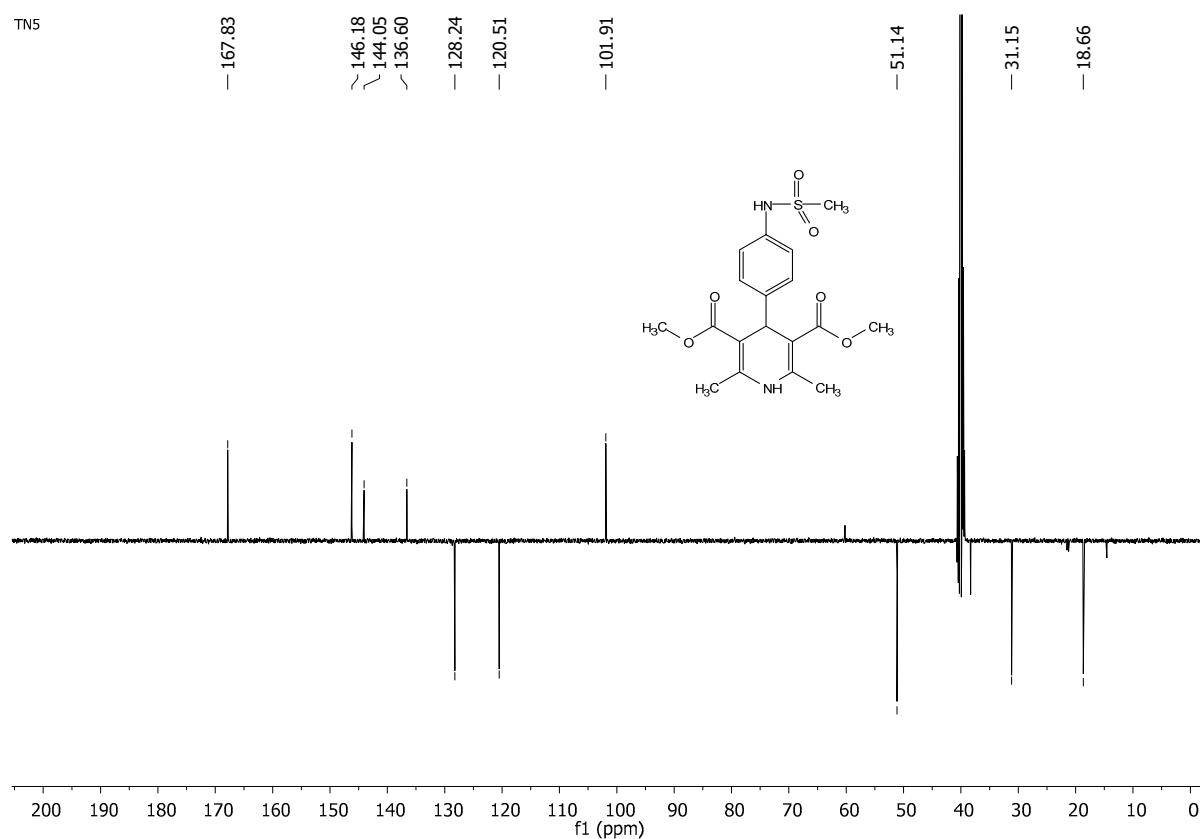
¹³C NMR spectrum of compound **4b**



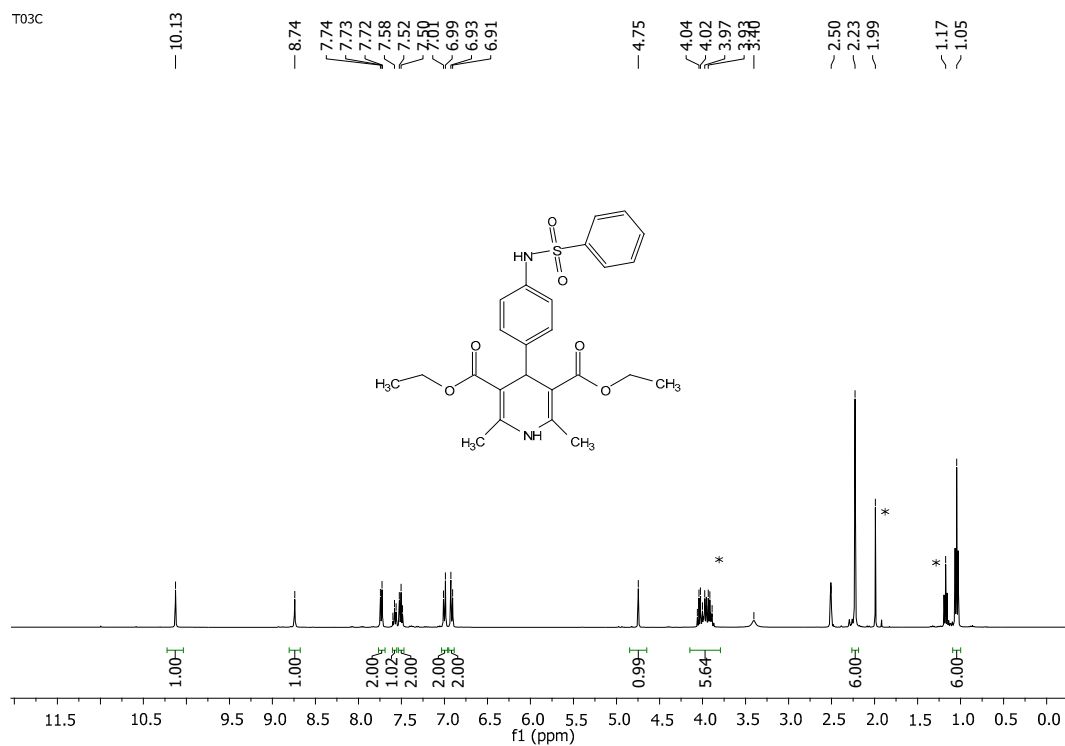
¹H NMR spectrum of compound **4c**



¹³C NMR spectrum of compound **4c**

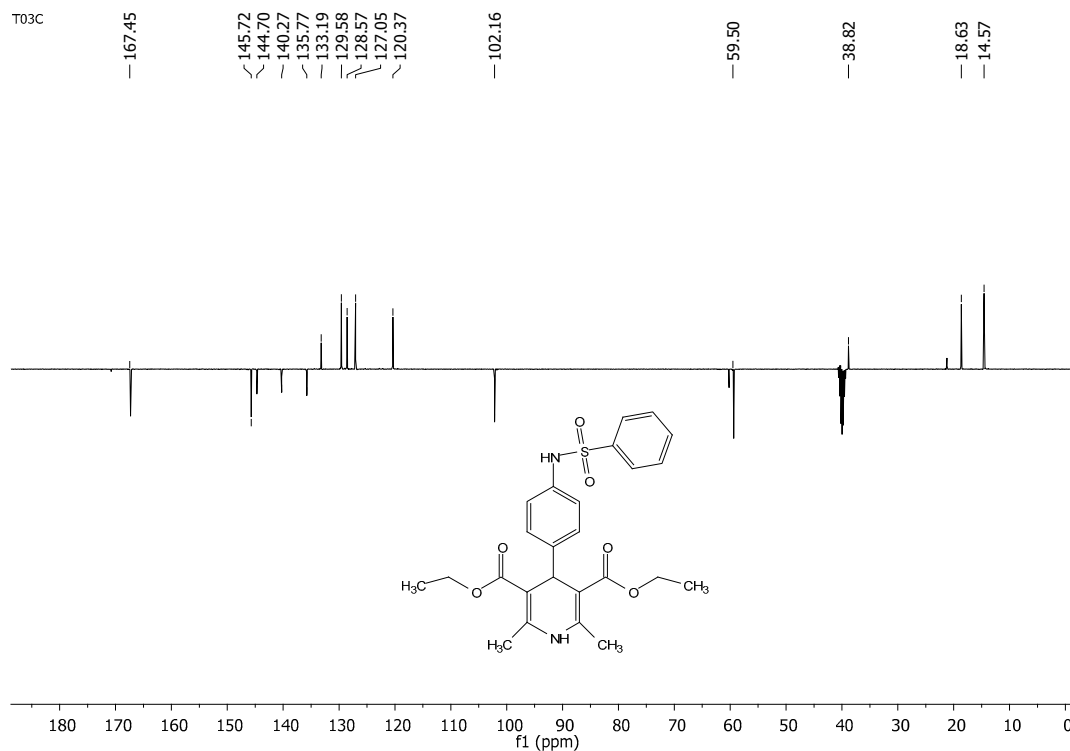


¹H NMR spectrum of compound **4d**

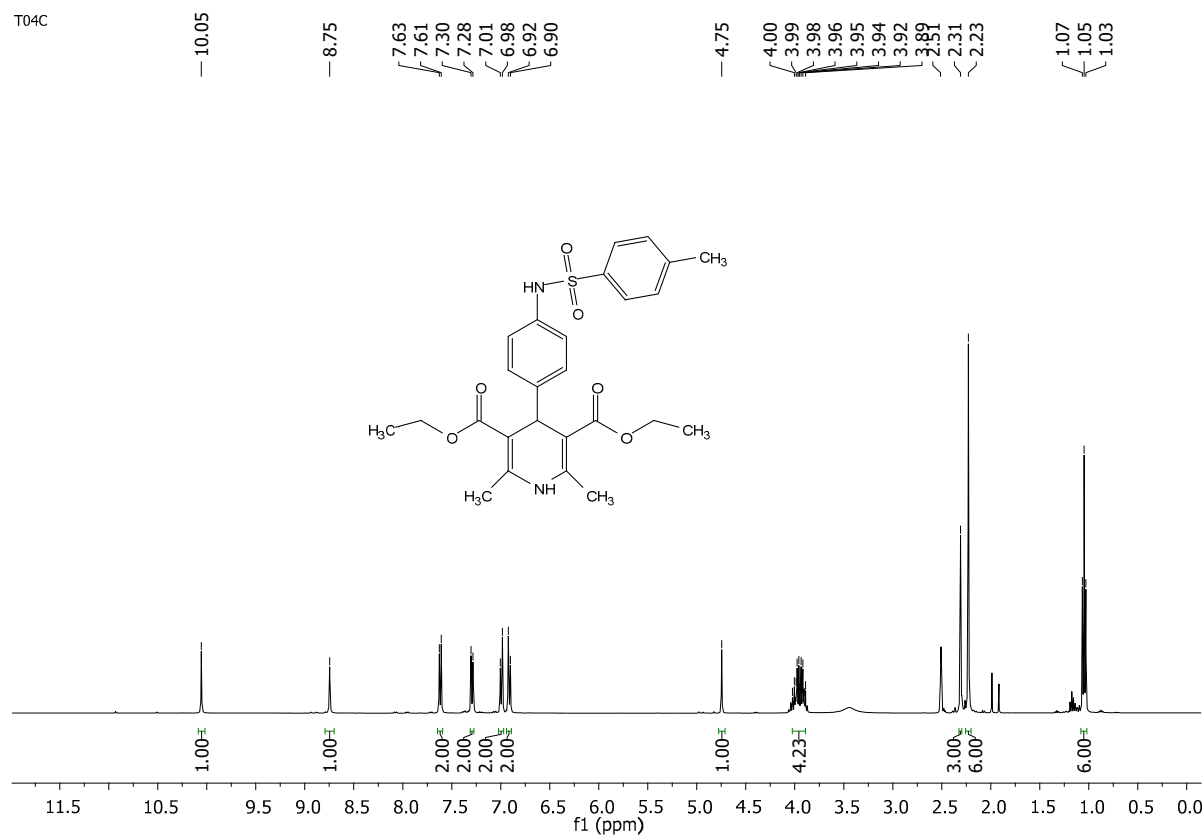


solvent ethyl acetate *

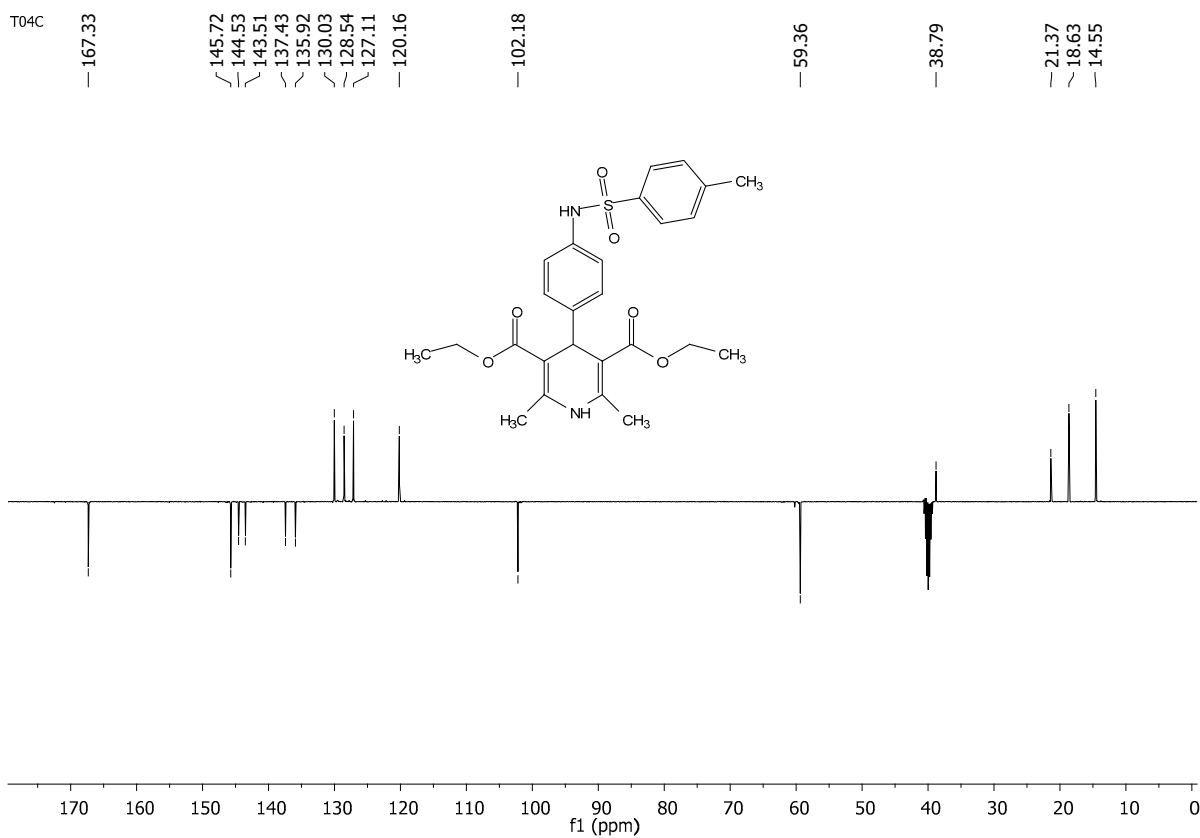
¹³C NMR spectrum of compound **4d**



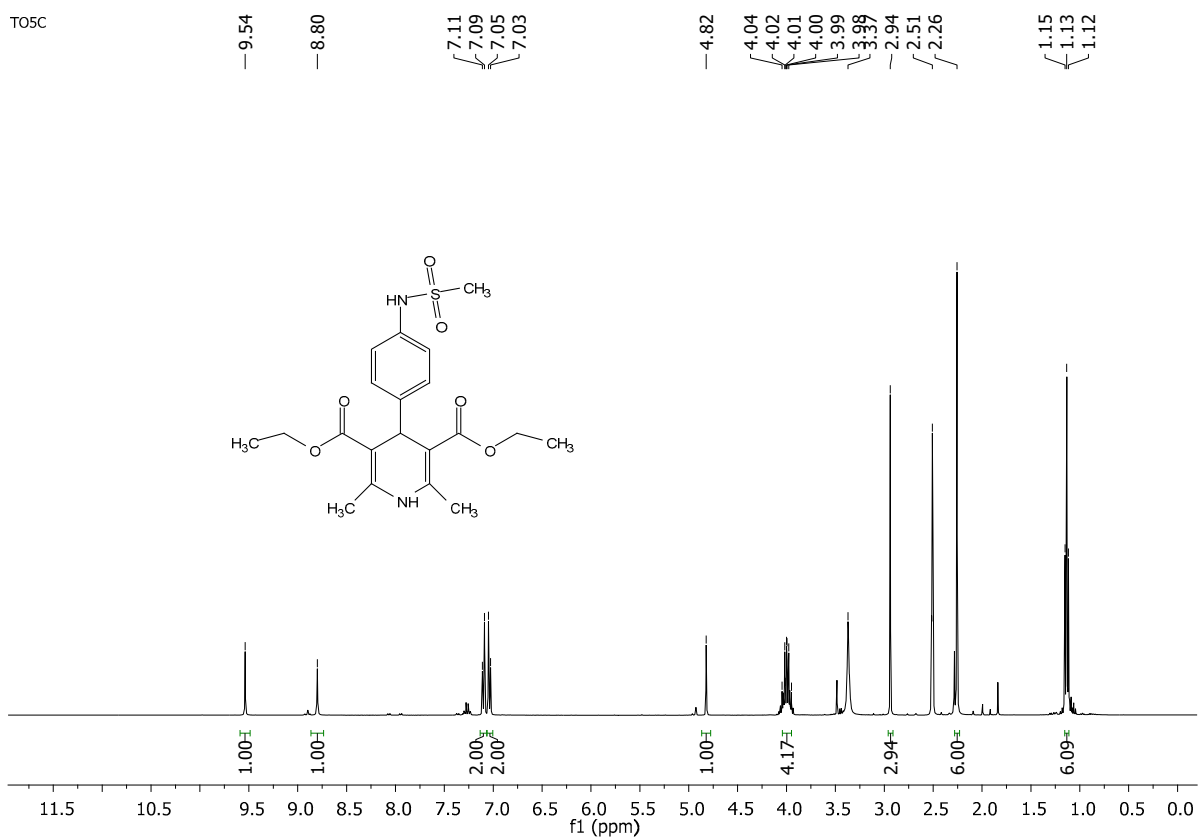
¹H NMR spectrum of compound **4e**



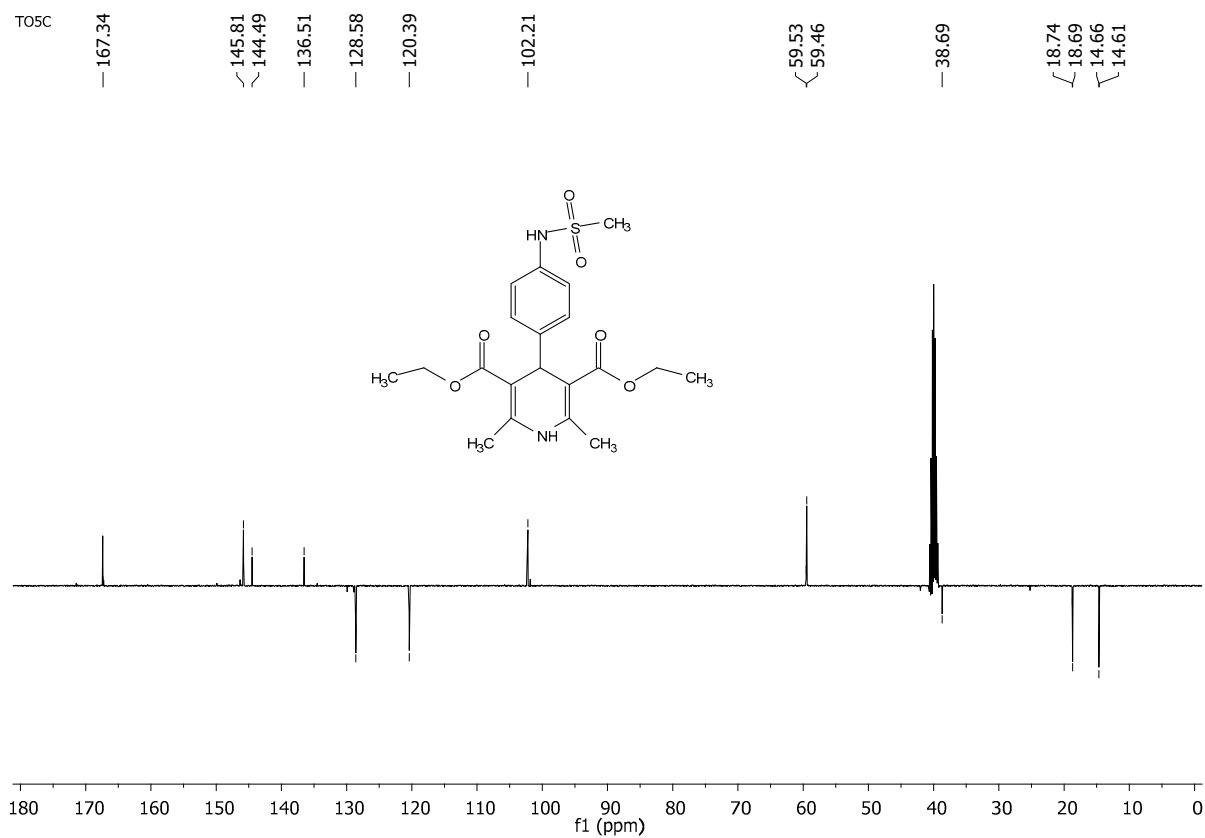
¹³C NMR spectrum of compound **4e**



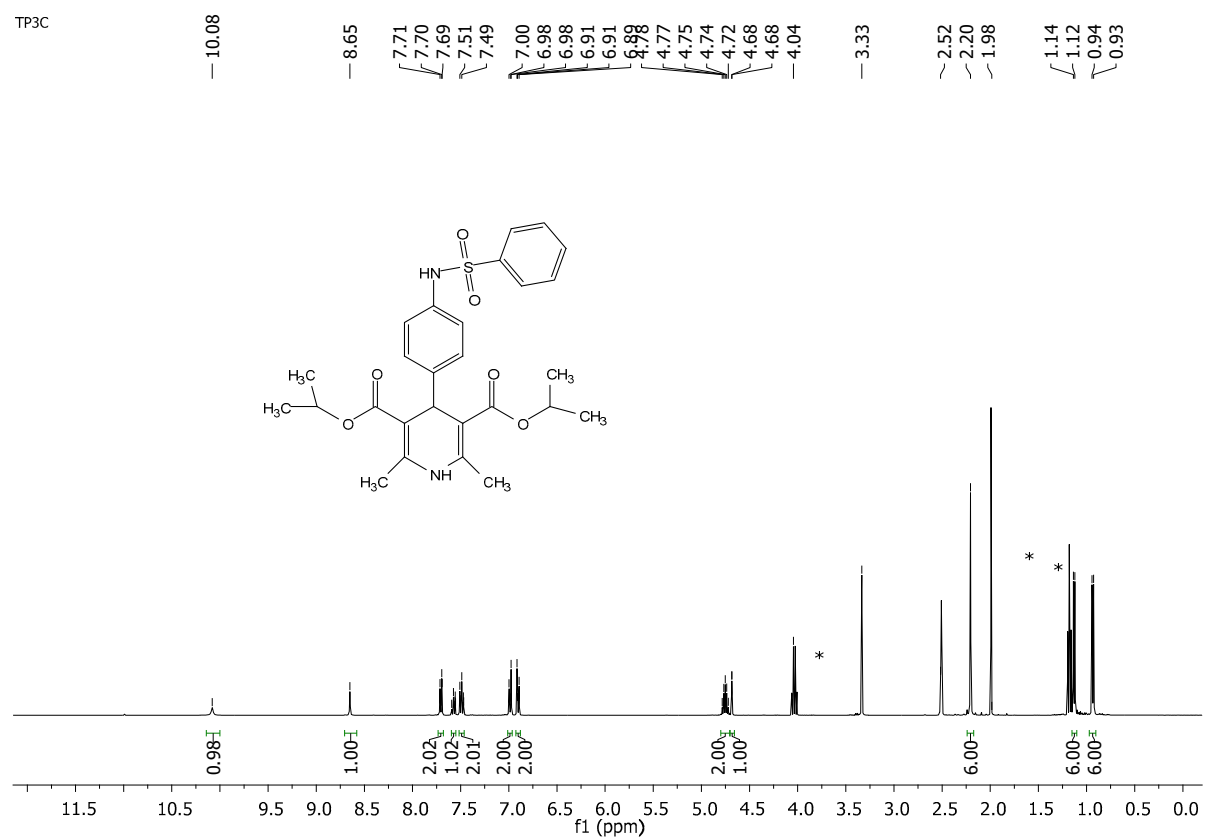
¹H NMR spectrum of compound **4f**



¹³C NMR spectrum of compound **4f**

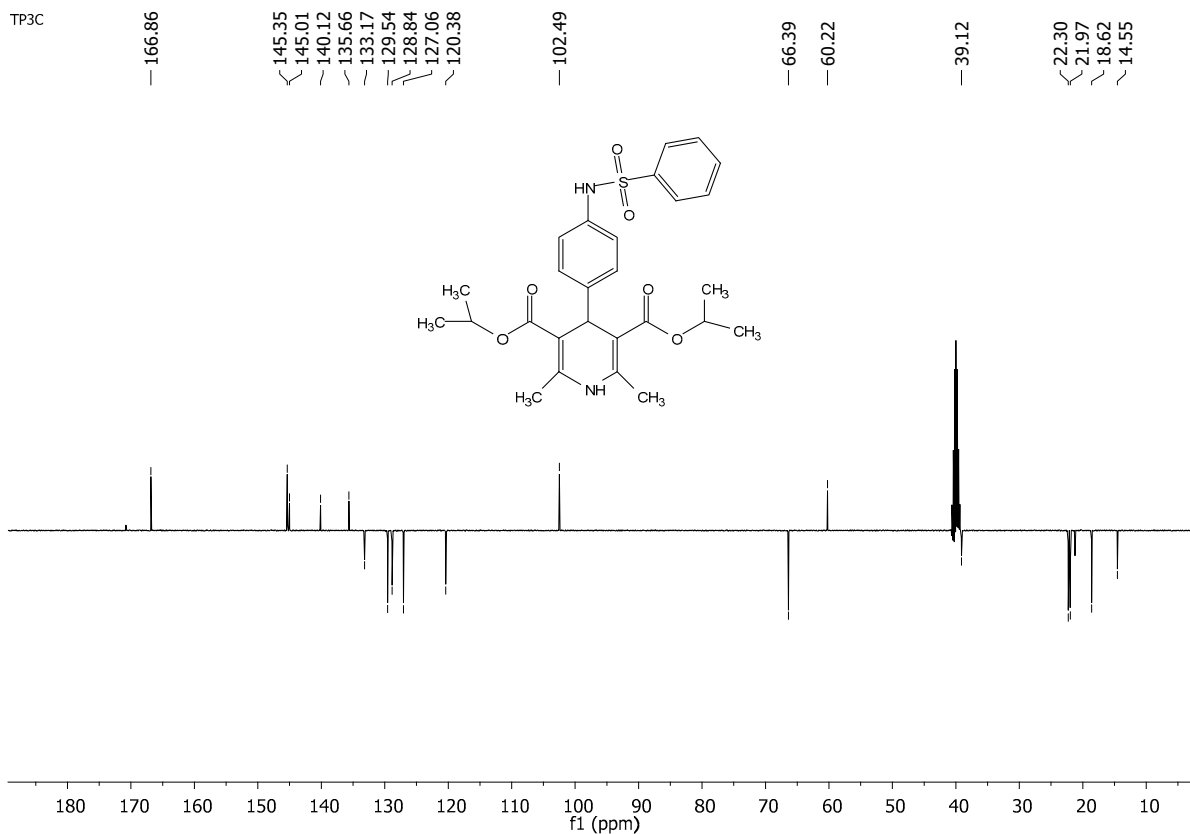


¹H NMR spectrum of compound **4g**

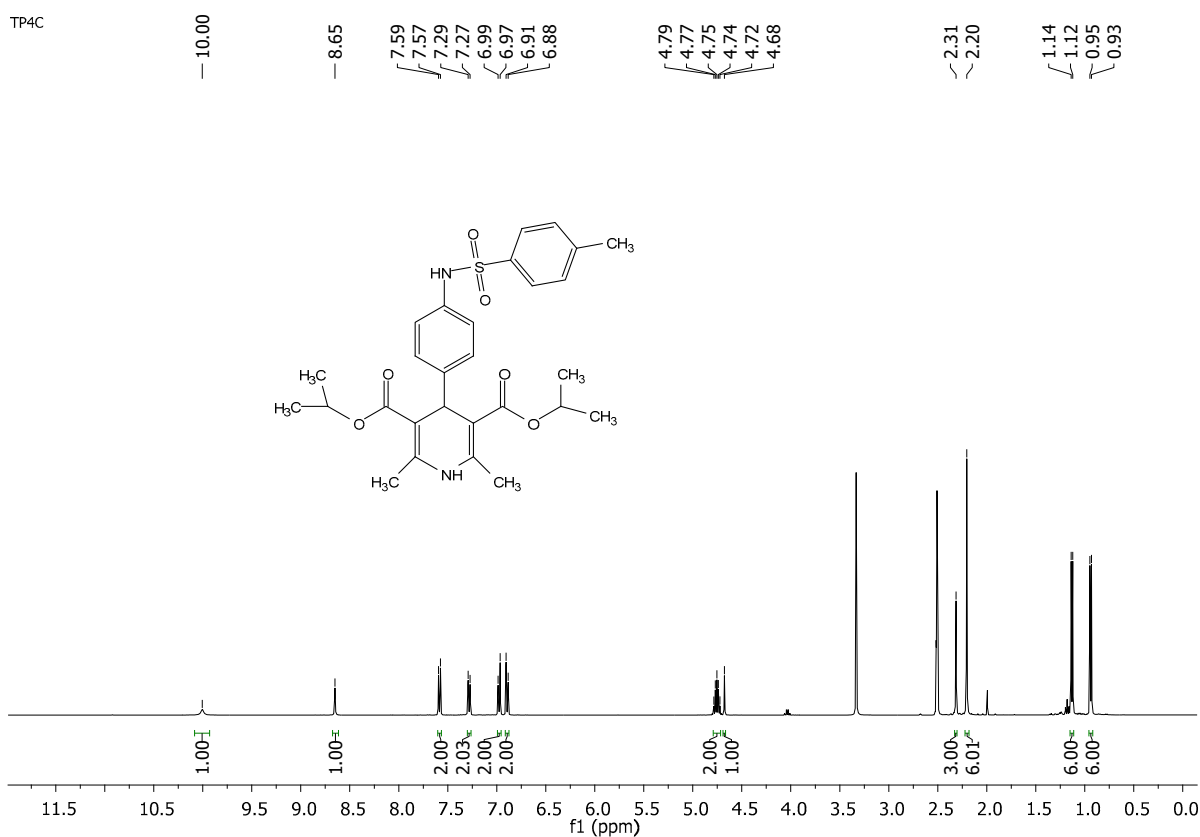


Ethyl acetate *

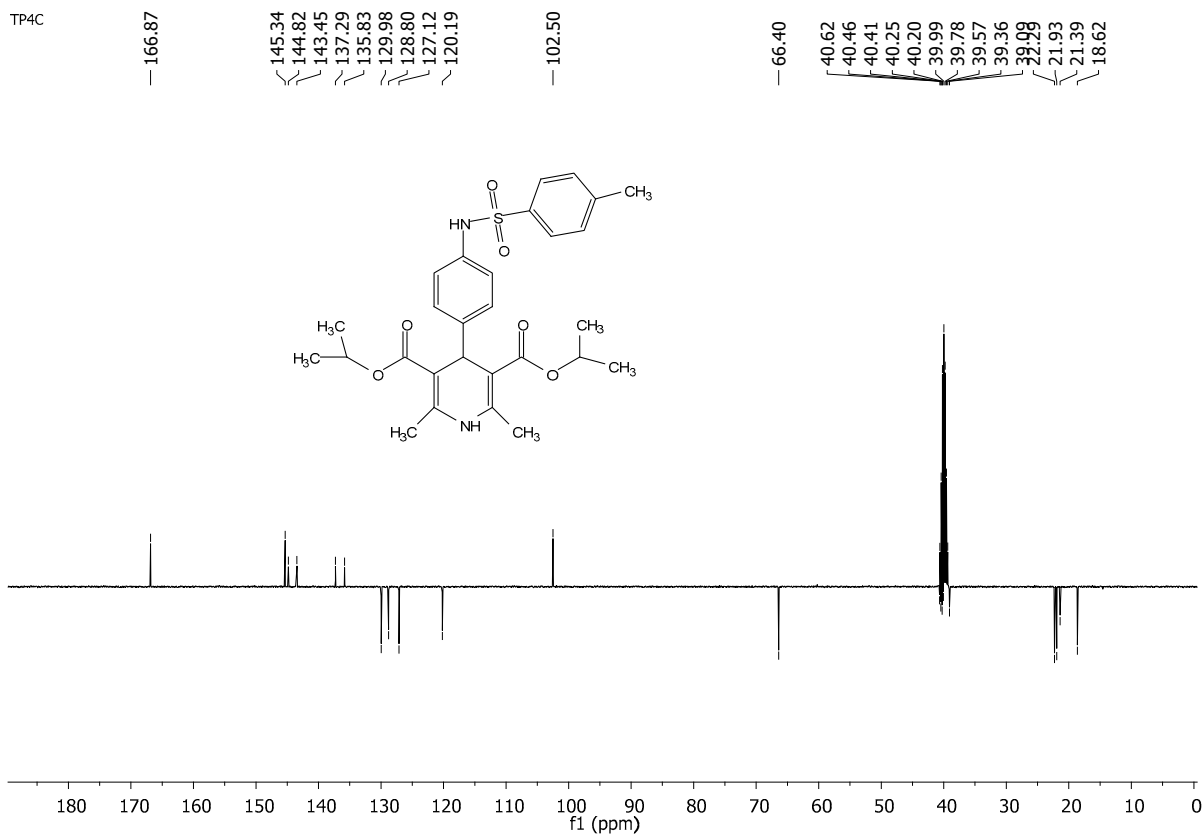
¹³C NMR spectrum of compound **4g**



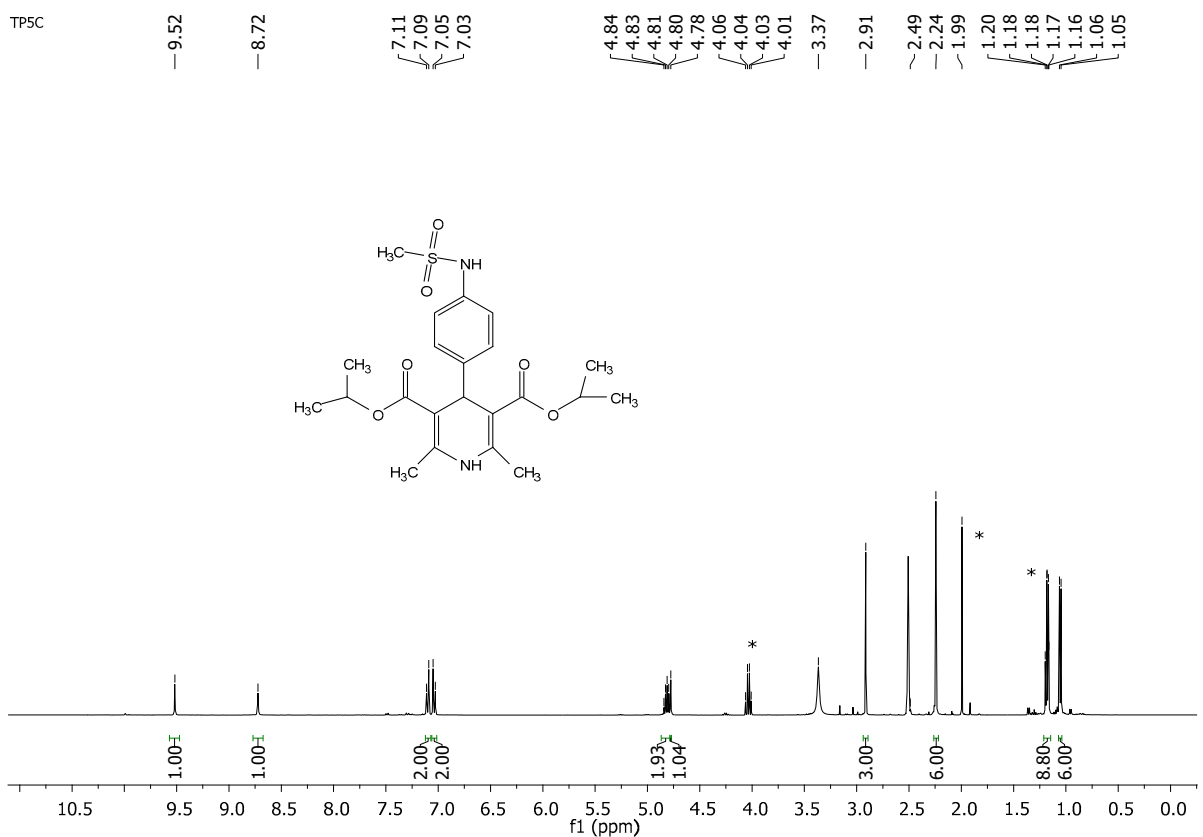
¹H NMR spectrum of compound **4h**



¹³C NMR spectrum of compound **4h**

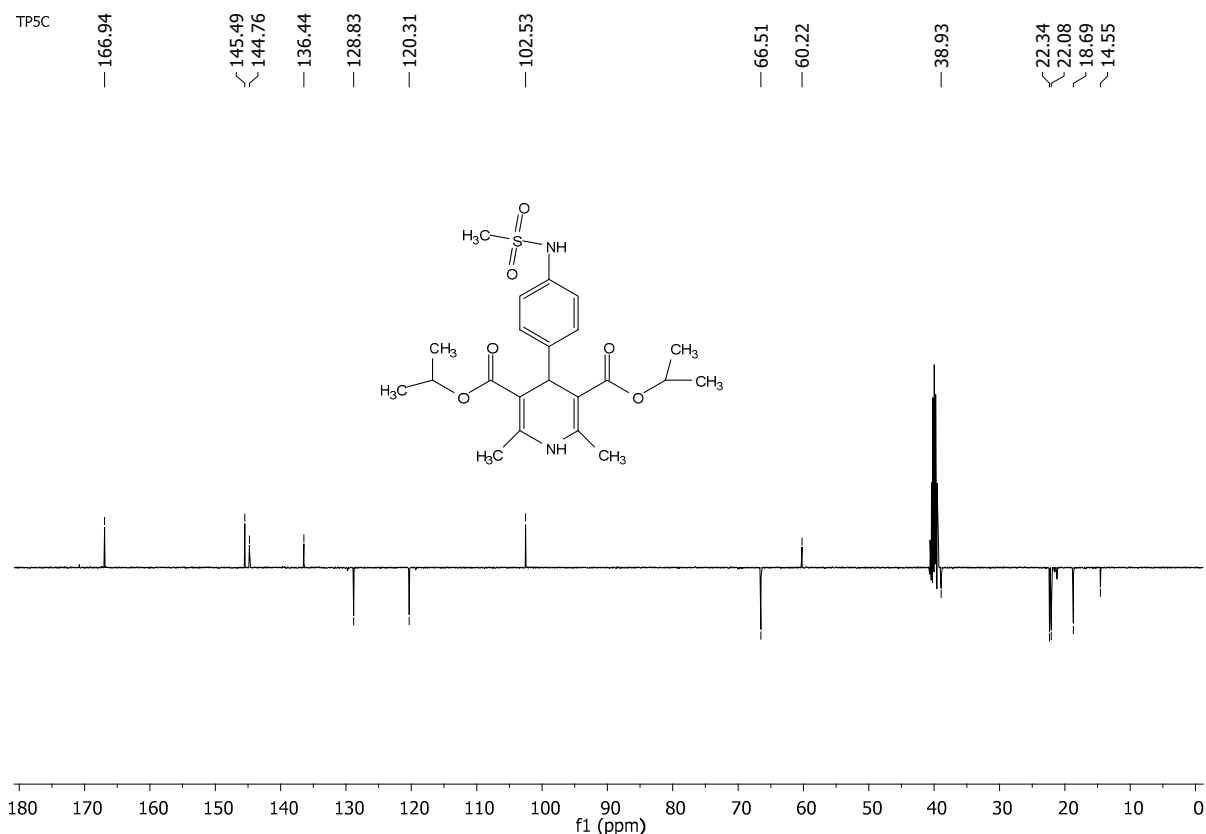


^1H NMR spectrum of compound **4i**



*solvent ethyl acetate

^{13}C NMR spectrum of compound **4i**

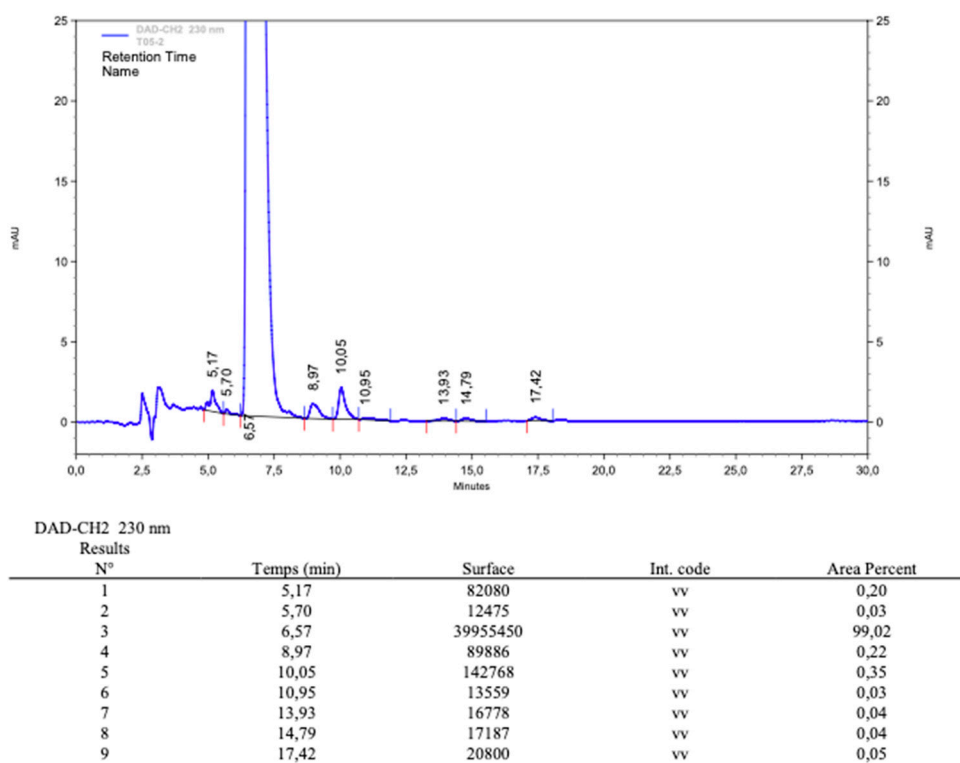


Inhibition of *Ee*AChE and *eq*BuChE

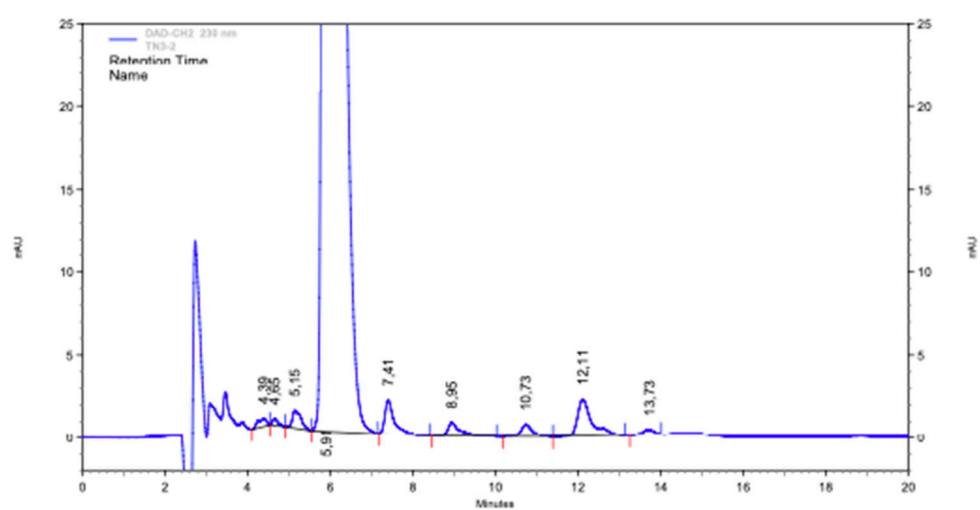
Inhibitory activity assessment of the TFAHs on ChEs was performed following the spectrophotometric method of Ellman using purified AChE from *Electrophorus electricus* (Type V-S, Sigma-Aldrich) or BuChE from horse serum (lyophilized powder, Sigma-Aldrich). Enzymes were first dissolved in 0.1 M phosphate buffer (pH= 8.0) and then aliquoted in small vials. Compounds stock solutions in DMSO (10 mM) were further diluted with DMSO to prepare nine serial dilutions of each compound. The reaction occurs in a final volume of 3 mL of a 0.1 M phosphate-buffered solution at pH= 8.0, containing 5,5'-dithiobis-2-nitrobenzoic acid (DTNB, 2625 μ L, 0.35 mM, final concentration), *Ee*AChE (29 μ L, 0.035 U/mL, final concentration) or *eq*BuChE (60 μ L, 0.05 U/mL, final concentration), tested compound (3 μ L, 0.1-10 μ M, final concentrations). Inhibition curves were built by pre-incubating this blend at room temperature with six concentrations of each compound for 10 min. A control with no compound was always present to determine the percent of enzymatic activity. After this pre-incubation period, acetylthiocholine iodide (105 μ L, 0.35 mM, final concentration) or butyrylthiocholine iodide (150 μ L, 0.5 mM, final concentration) was added, allowing 15 min of additional incubation time, where the DTNB produces the

yellow anion 5-thio-2-nitrobenzoic acid as an indicator of enzymatic activity. After 15 min, absorbances were measured at 412 nm in a spectrophotometer plate reader (iEMS Reader MF, Labsystems). Color generation would be reduced as the compound inhibits the enzymes. IC₅₀ values were determined graphically from log concentration–% inhibition curves (GraphPad Prism 5.3 software, GraphPad Software Inc.). Data are expressed as means ± SEM of at least three different experiments in quadruplicate.

Chromatogram of 4a



Chromatogram of 4f



DAD-CH2 230 nm

Results					
N°	Temps (min)	Surface	Int. code	Area Percent	
1	4,39	30068	vv	0,09	
2	4,65	15196	vv	0,05	
3	5,15	64524	vv	0,20	
4	5,91	31373300	vv	98,06	
5	7,41	134071	vv	0,42	
6	8,95	71946	vv	0,22	
7	10,73	62131	vv	0,19	
8	12,11	218469	vv	0,68	
9	13,73	24179	vv	0,08	