Electronic Supplementary Material (ESI) for New Journal of Chemistry.

Study of structure-bioactivity relationship of three new pyridine Schiff bases: Synthesis, spectral characterization, DFT calculations and biological assays

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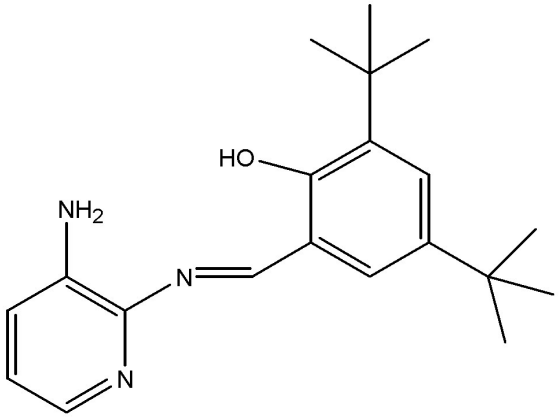
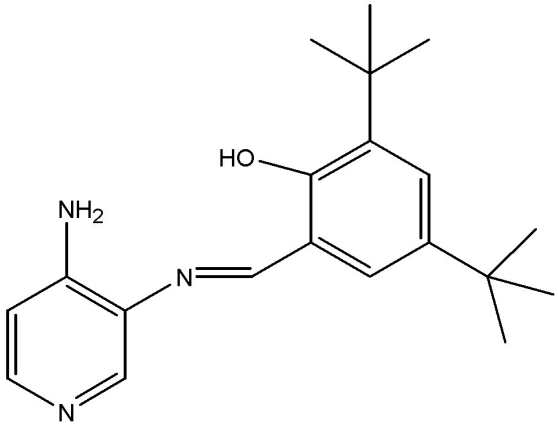
SUPPLEMENTARY SCHEMES



L1



L2

Scheme S1. Chemical structure of (E)-2-{[(2-aminopyridin-3-yl)imino]-methyl}-4,6-di-*tert*-butyl-phenol (**L1**), and (E)-2-{[(3-aminopyridin-4-yl)imino]-methyl}-4,6-di-*tert*-butyl-phenol (**L2**). These pyridine Schiff bases were already reported.[1, 2]

SUPPLEMENTARY TABLES

Table S1. Characteristic constants of Schiff bases **L3** to **L6**.

Compound	M.F.	M.W. (g mol^{-1})	Yield (%)	M.P. (°C)	Solid color	TLC R_f
L3	$C_{12}H_9N_3OCl_2$	282.0	73	223.2—224.9	orange yellow	0.38
L4	$C_{12}H_{10}N_3OCl$	247.5	80	214.4—216.1	yellow	0.40
L5	$C_{13}H_{13}N_3O$	227.0	81	136.8—138.2	yellow	0.45
L6	$C_{21}H_{28}N_2O$	324.0	78	141.8—142.7	orange yellow	0.89

M.F.: Molecular formula

M.W.: Molecular weight

M.P.: Melting point

TLC: Thin layer chromatography

Table S2. FTIR (ATR) properties of de Schiff bases series (**L1** to **L6**)

Compound	% Yield	νOH	νNH_2	νC=N (Azomethine)	νC=C
L1[1, 2]	70	3468	3265 3132	1608	1589
L2[1]	82	3643 3477	3323	1612	1591
L3	80	3626 3300	3109	1612	1593
L4	80	3465 3281	3059	1638	1589
L5	81	3332 3204	3066	1607	1570
L6	78	3486 3388	2966 2952	1614	1570

Table S3. ^{1}HNMR results in DMSO-$_{d6}$ for a Schiff bases series **L2** to **L6**

	H1	H2	H3	H4	H5	H6	H7	-NH_2	-OH
L2[1, 2]	7.92 d	6.68 d	7.98 s	8.81 s	7.47 s	7.37 s	---	5.94	13.18
L3	7.96 d	6.67 d	8.05 s	8.89 s	7.77 d	7.68 d	---	6.24	---
L4	7.94 d	6.64 d	7.99 s	8.83 s	7.41 d	6.99 dd	7.85 d	6.07	---
L5	7,96 d	6.67 d	8.02 s	8.83 s	7.49 d	7.29 d	6.93 dd	6.00	12.87
L6	6.63 dd	6.81 d	7.16 d	8.85 s	7.49 s	7.38 s		4.99	13.71

Table S4. 1D TOCSY experiments and its respective assignment for **L3** and **L4**.

Compound	Irradiated proton	Proton observed
L3	H2 (6.67 ppm)	H1 (7.97 ppm) and → NH_2 (6.23 ppm)
	H3 (8.00 ppm)	H1 (7.96 ppm), H2 (6.68 ppm) and → NH_2 (6.23 ppm)
	H5 (7.78 ppm)	H6 (7.70 ppm)
L4	H2 (6.65 ppm)	H1(7.94 ppm) and → NH_2 (6.11 ppm)
	H3 (8.00 ppm)	H2 (6.64 ppm) and → NH_2 (6.08 ppm)
	H5 (7.00 ppm)	H6 (7.41 ppm) and H7 (7.85 ppm)

Table S5. Most important transition energies calculated for the **L3** to **L6** in gas phase (black), acetonitrile (red), and DMSO (blue).

Compound	λ (nm)	f	Assignment
L3	264.3 275.1 283.4	2.3×10^{-1} 1.3×10^{-1} 1.5×10^{-1}	HOMO-4→LUMO (n → π*) HOMO-5→LUMO (n → π*) HOMO-4→LUMO (n → π*)
	301.5 300.0 296.5	7.3×10^{-2} 8.8×10^{-1} 6.5×10^{-2}	HOMO-2→LUMO (n → π*) HOMO-3→LUMO (n → π*) HOMO-3→LUMO (n → π*)
	343.6 360.8 354.2	6.5×10^{-2} 1.5×10^{-2} 3.3×10^{-2}	HOMO-1→LUMO (π → π*) HOMO-2→LUMO (π → π*) HOMO-2→LUMO (π → π*)
	381.1 397.4 402.3	1.2×10^{-1} 9.5×10^{-2} 9.9×10^{-2}	HOMO→LUMO (π → π*) HOMO→LUMO (π → π*) HOMO-1→LUMO (π → π*)
L4	292.6 284.1 305.7	2.0×10^{-1} 2.6×10^{-1} 1.4×10^{-1}	HOMO-4→LUMO (n → π*) HOMO-5→LUMO (n → π*) HOMO-4→LUMO (n → π*)
	308.1 295.5 325.7	2.2×10^{-2} 1.2×10^{-2} 1.1×10^{-1}	HOMO-3→LUMO (n → π*) HOMO-4→LUMO (n → π*) HOMO-4→LUMO (n → π*)
	336.2 319.4 365.7	4.7×10^{-2} 1.8×10^{-1} 3.7×10^{-2}	HOMO-1→LUMO (π → π*) HOMO→LUMO (π → π*) HOMO-1→LUMO (π → π*)
	371.6 360.0 383.4	2.7×10^{-1} 2.0×10^{-1} 2.0×10^{-1}	HOMO→LUMO (π → π*) HOMO→LUMO (π → π*) HOMO→LUMO (π → π*)
L5	287.8 290.6 307.2	2.3×10^{-1} 1.3×10^{-1} 3.3×10^{-1}	HOMO-4→LUMO (n → π*) HOMO-4→LUMO (n → π*) HOMO-3→LUMO (n → π*)
	298.1 280.5 315.0	1.5×10^{-2} 2.8×10^{-2} 1.3×10^{-2}	HOMO-2→LUMO (n → π*) HOMO-3→LUMO (n → π*) HOMO-2→LUMO (n → π*)
	326.8 300.8 333.9	3.7×10^{-2} 5.8×10^{-2} 1.2×10^{-1}	HOMO-1→LUMO (π → π*) HOMO→LUMO (π → π*) HOMO-1→LUMO (π → π*)
	357.9 376.1	2.8×10^{-1} 3.0×10^{-1}	HOMO→LUMO (π → π*) HOMO→LUMO (π → π*)

Compound	λ (nm)	f	Assignment
	380.5	1.3×10^{-1}	HOMO-1→LUMO (π → π*)
L6	265.5 255.2 284.2	3.9×10^{-1} 2.3×10^{-1} 2.3×10^{-1}	HOMO-3→LUMO (n → π*) HOMO-3→LUMO (n → π*) HOMO-3→LUMO (n → π*)
	298.4 300.1 325.6	4.0×10^{-2} 2.3×10^{-2} 2.3×10^{-2}	HOMO-2→LUMO (n → π*) HOMO-3→LUMO (n → π*) HOMO-2→LUMO (n → π*)
	330.6 315.2 356.7	1.0×10^{-1} 2.3×10^{-2} 2.3×10^{-1}	HOMO-1→LUMO (π → π*) HOMO-1→LUMO (π → π*) HOMO→LUMO (π → π*)
	381.6 400.0 412.3	2.7×10^{-1} 2.3×10^{-1} 2.3×10^{-2}	HOMO→LUMO (π → π*) HOMO-1→LUMO (π → π*) HOMO→LUMO (π → π*)

SUPPLEMENTARY FIGURES

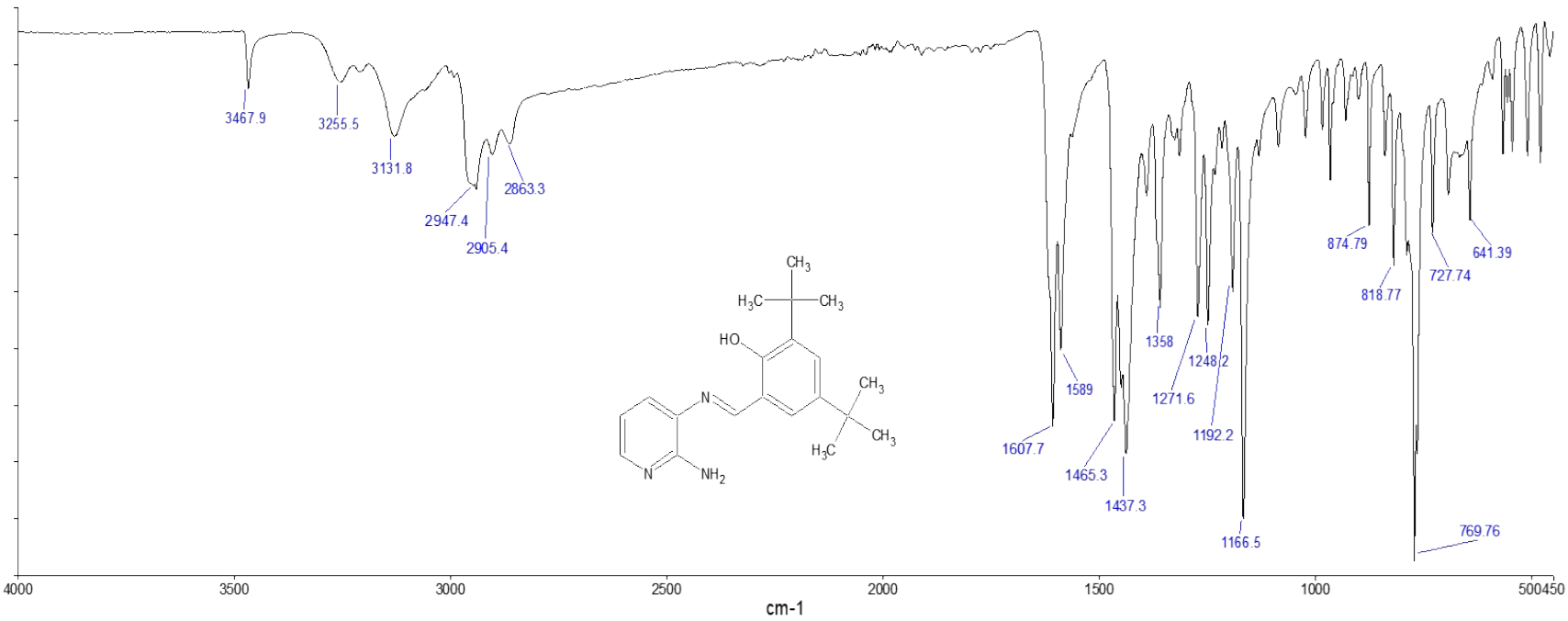


Figure S1. FTIR of **L1**.

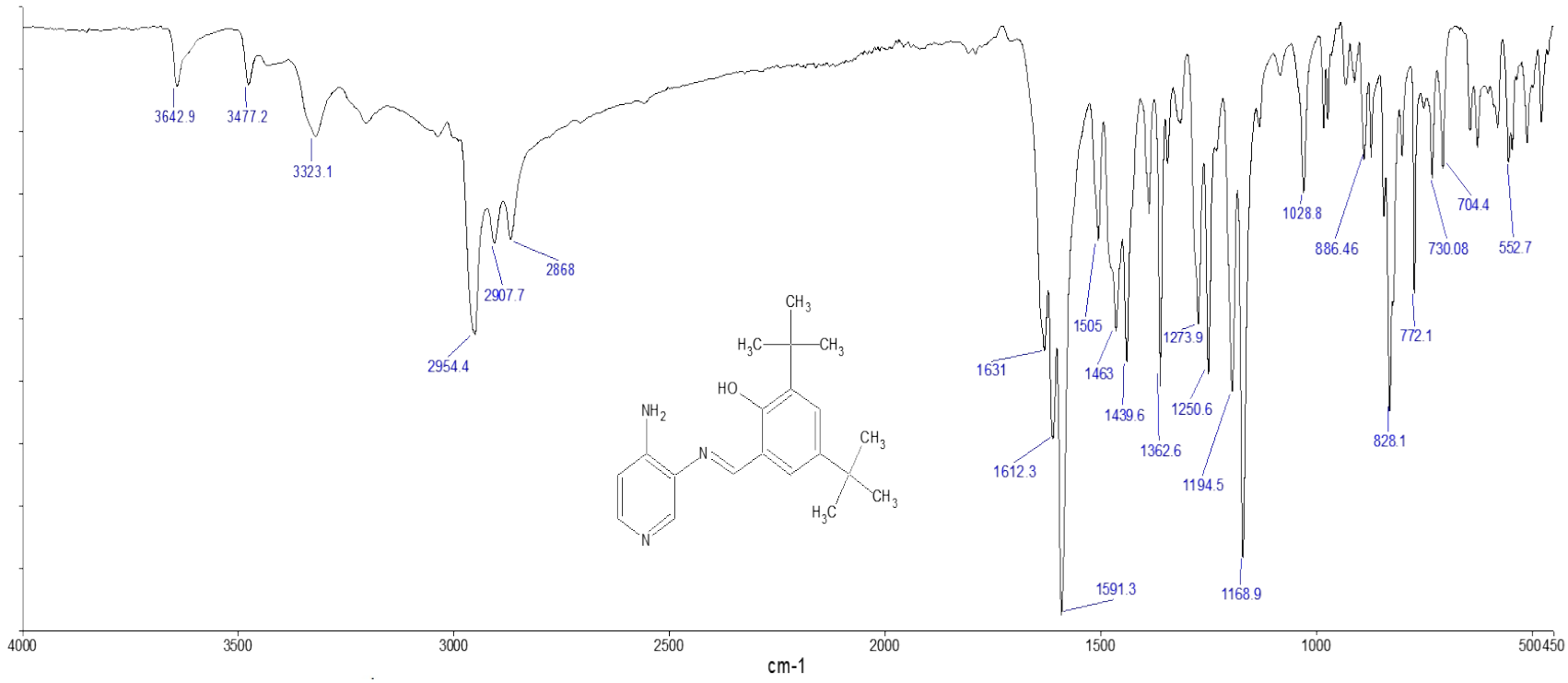


Figure S2. FTIR of **L2**.

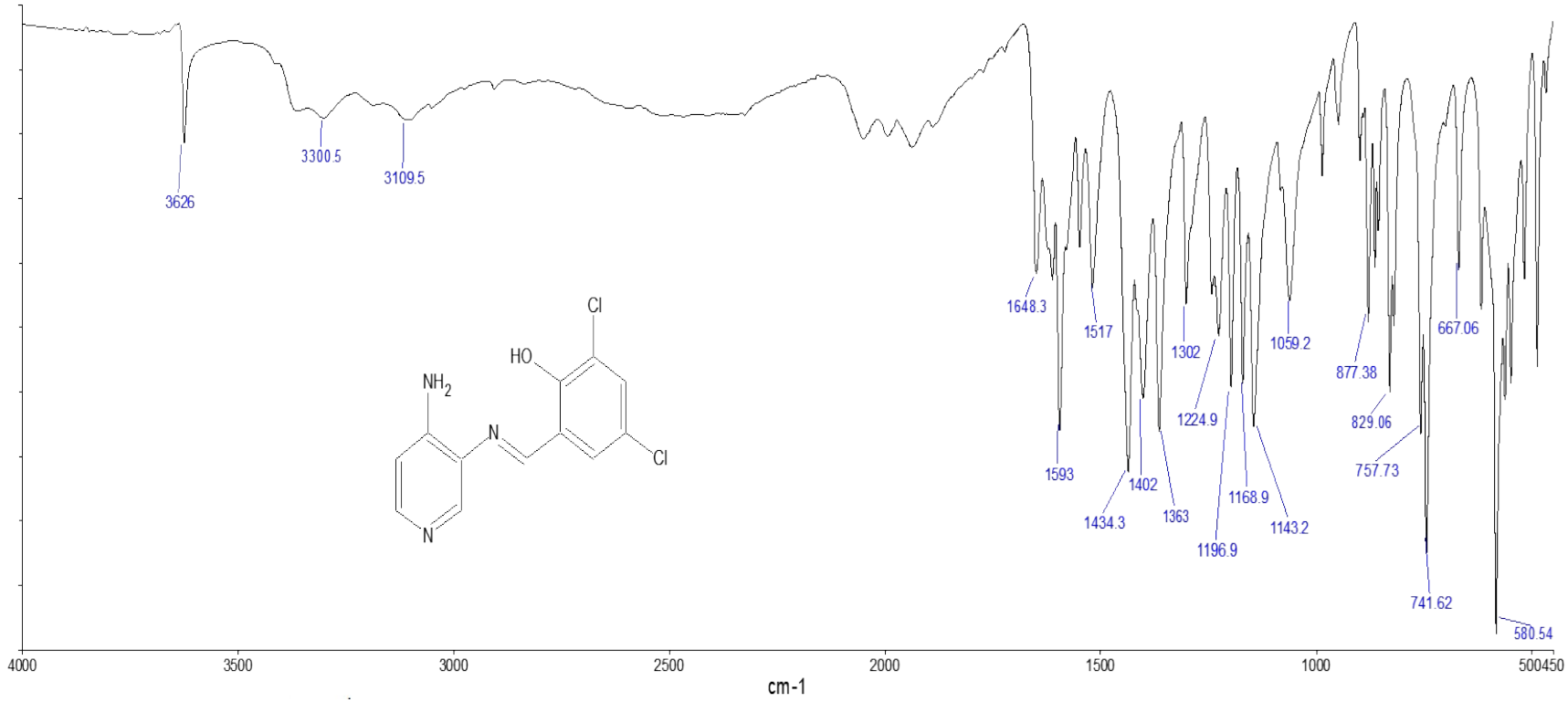


Figure S3. FTIR of **L3**.

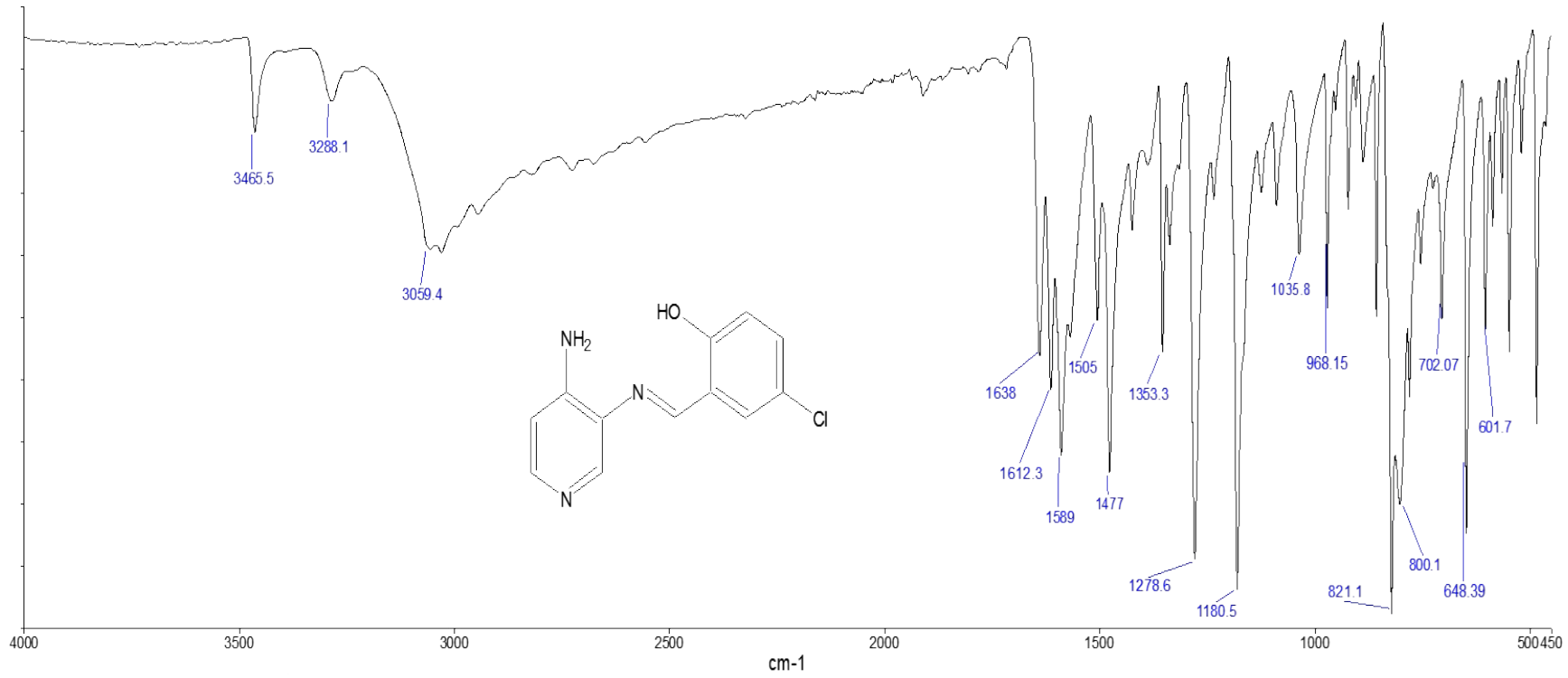


Figure S4. FTIR of **L4**.

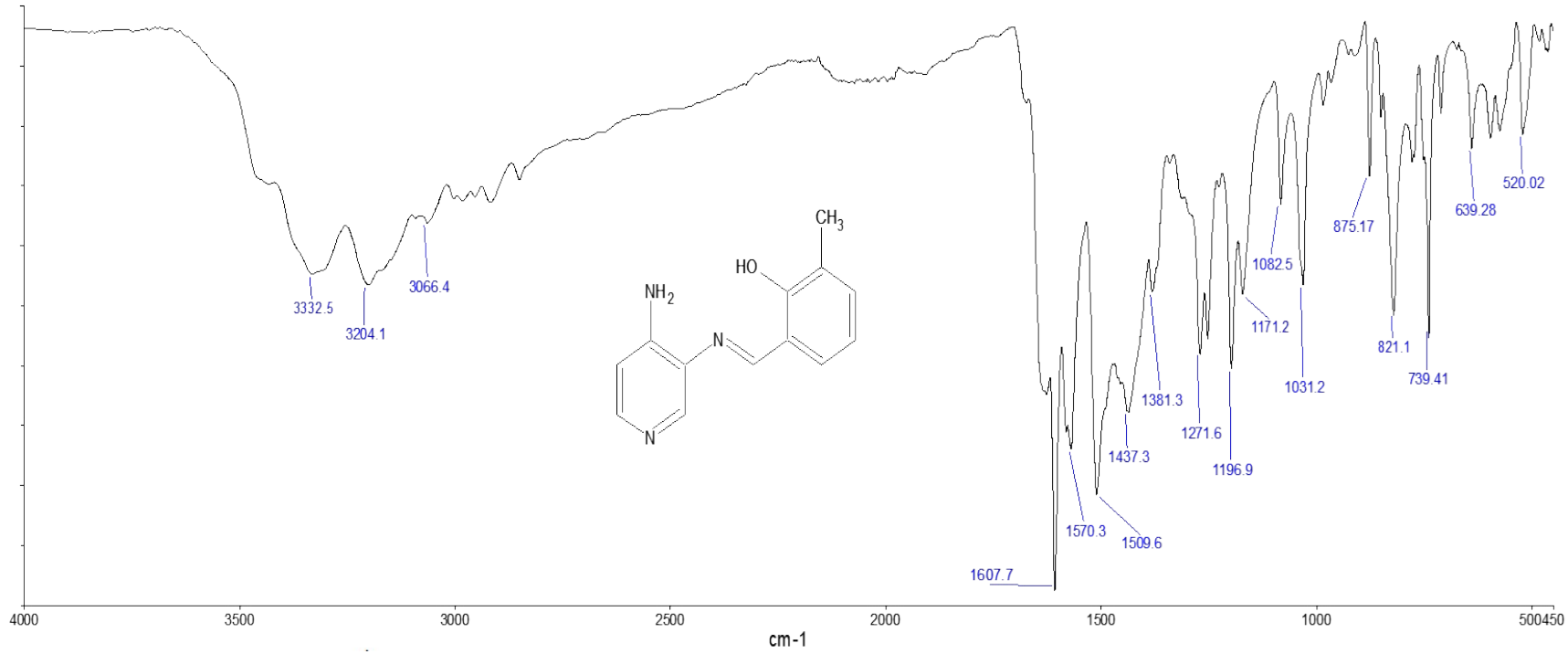


Figure S5. FTIR of **L5**.

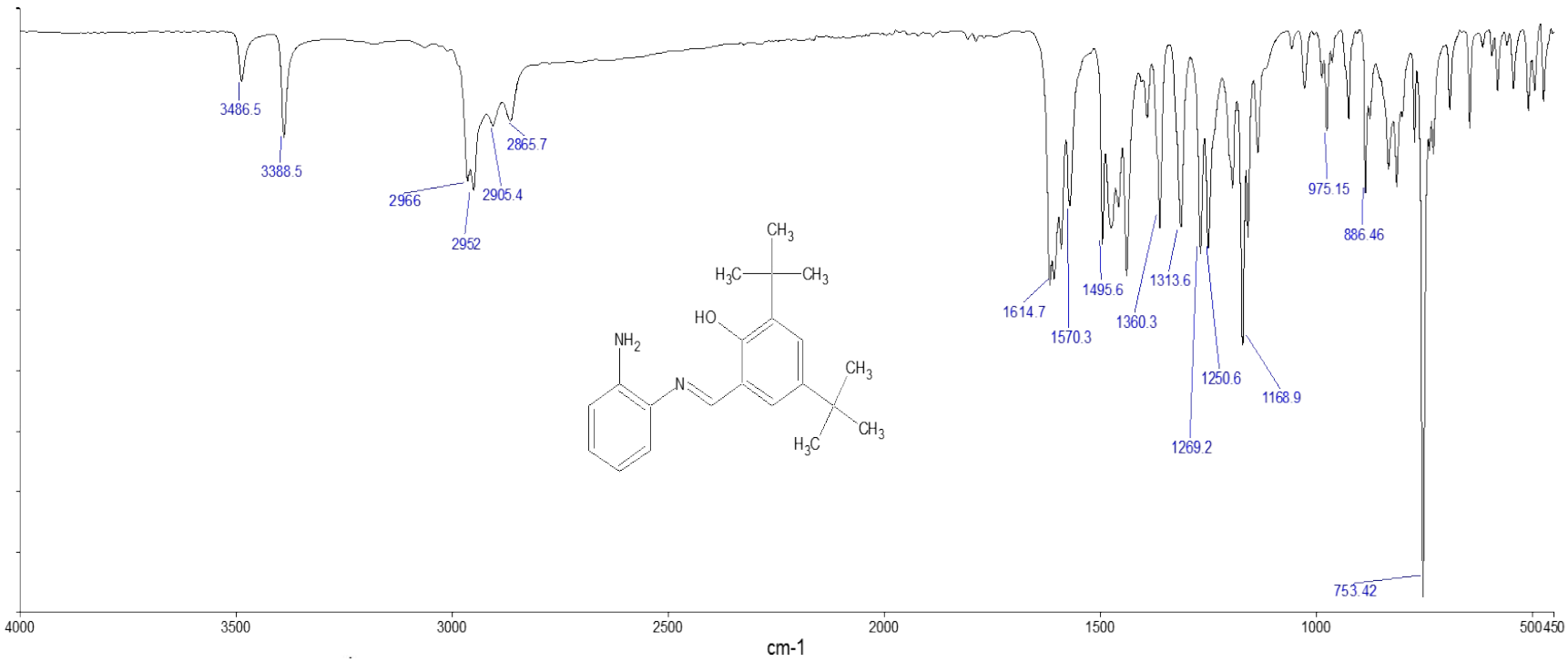


Figure S6. FTIR of **L6**.

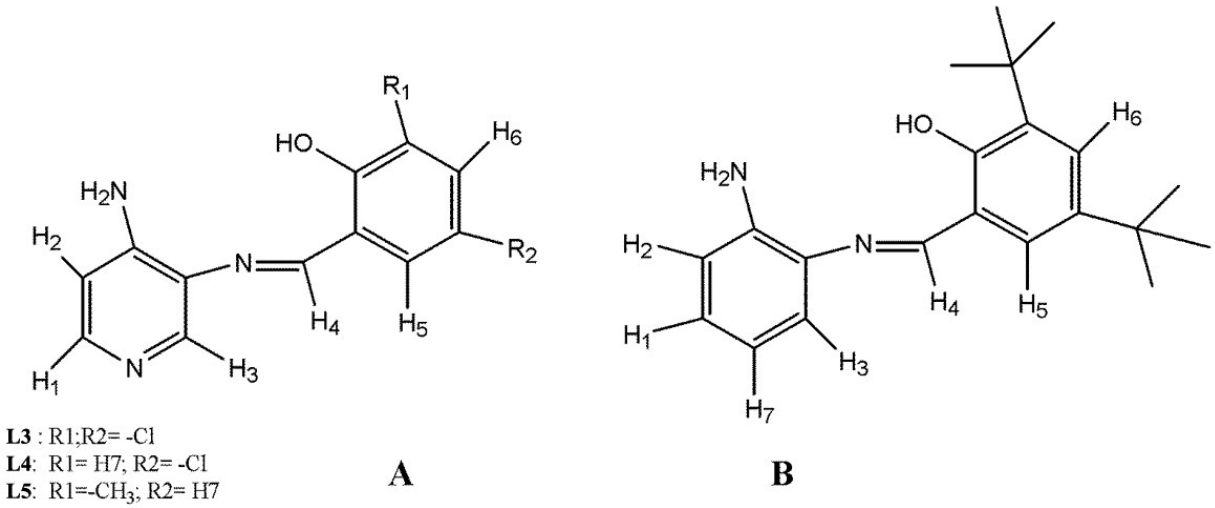


Figure S7. Assignment of ^{1}HNMR signals in the structure proposed; **A**: **L3** to **L5; B**: **L6**.

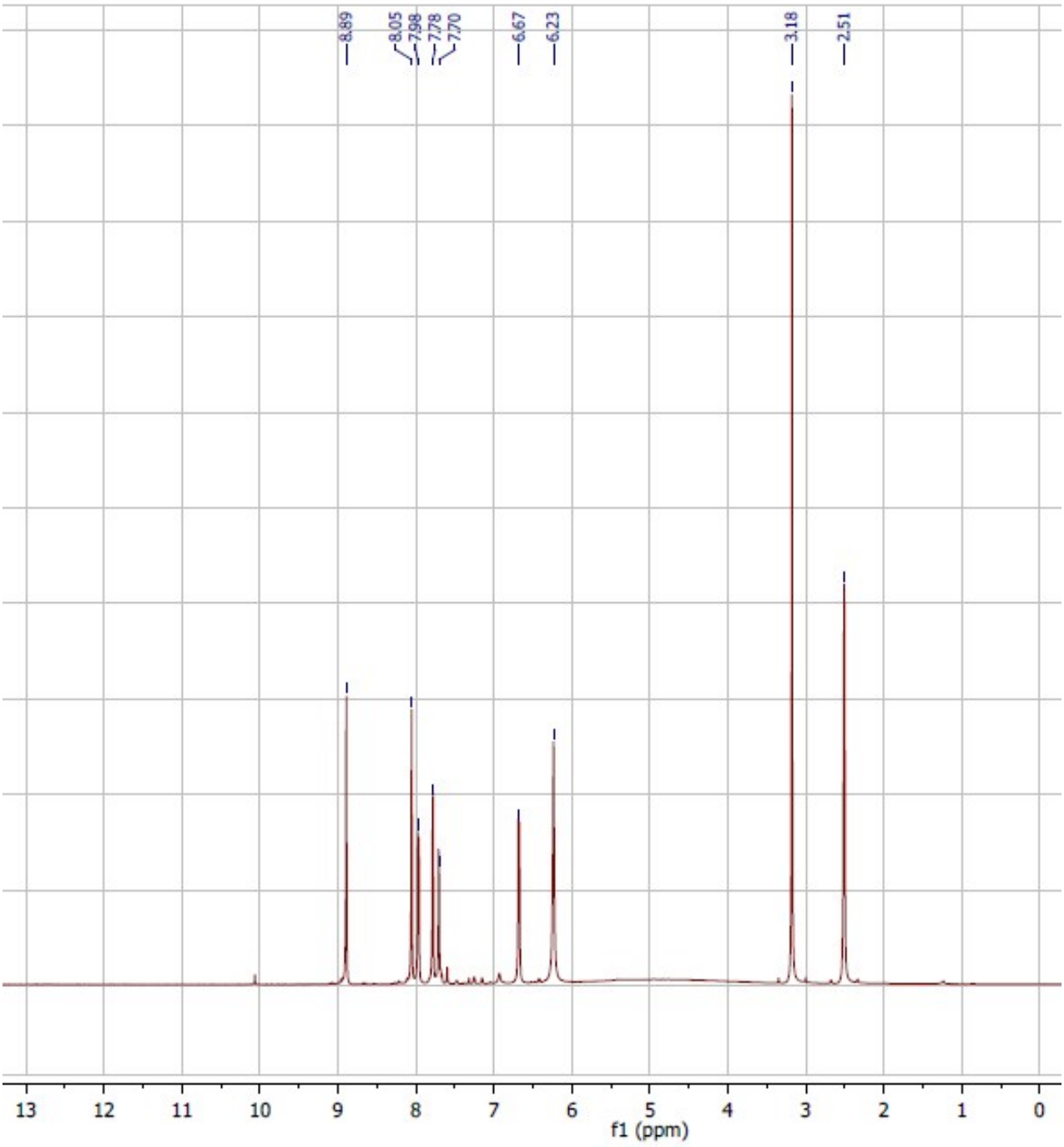


Figure S8. ^{1}HNMR of **L3** in DMSO-$_{d6}$

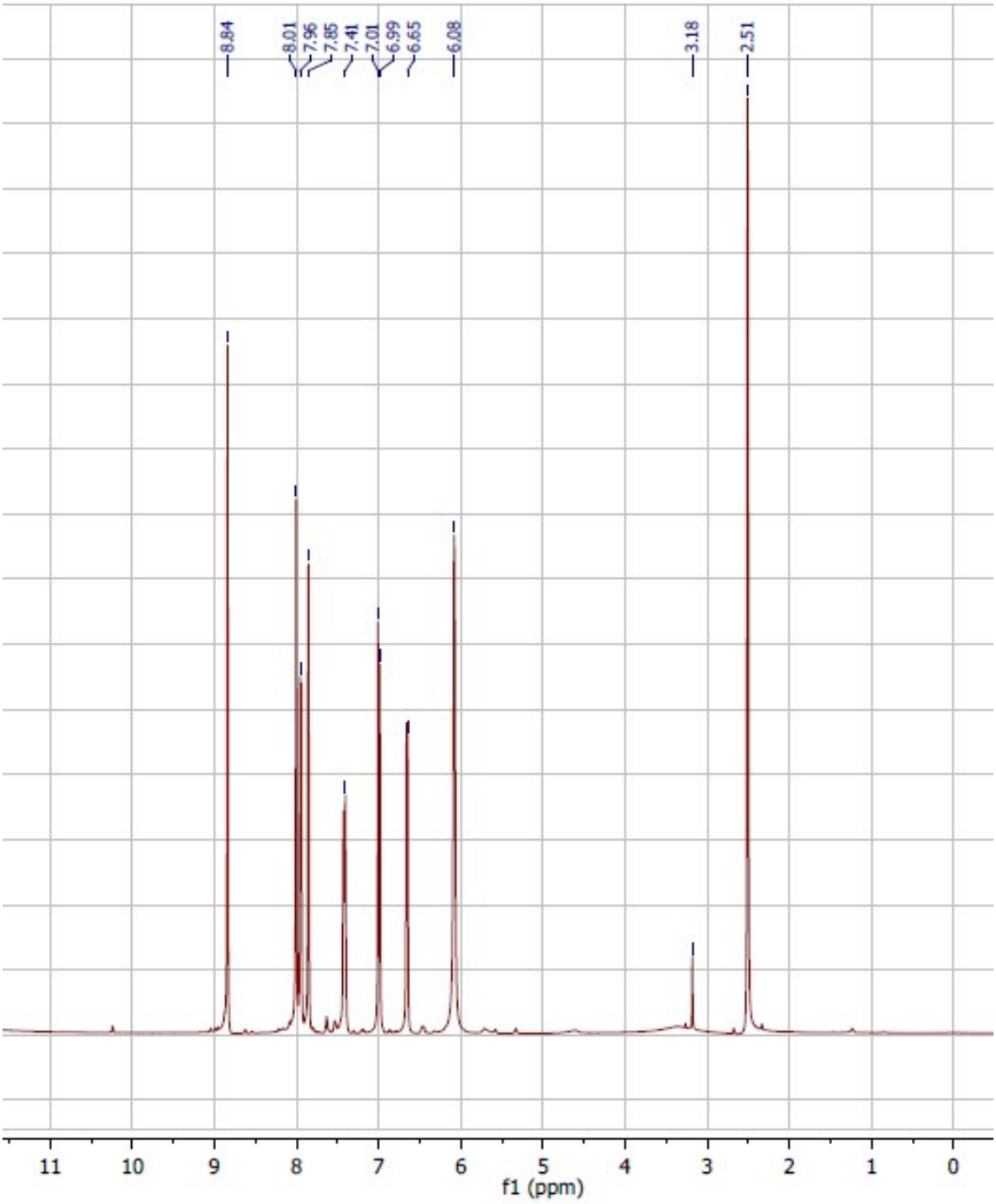


Figure S9. ^{1}HNMR of **L4** in DMSO-$_{d6}$.

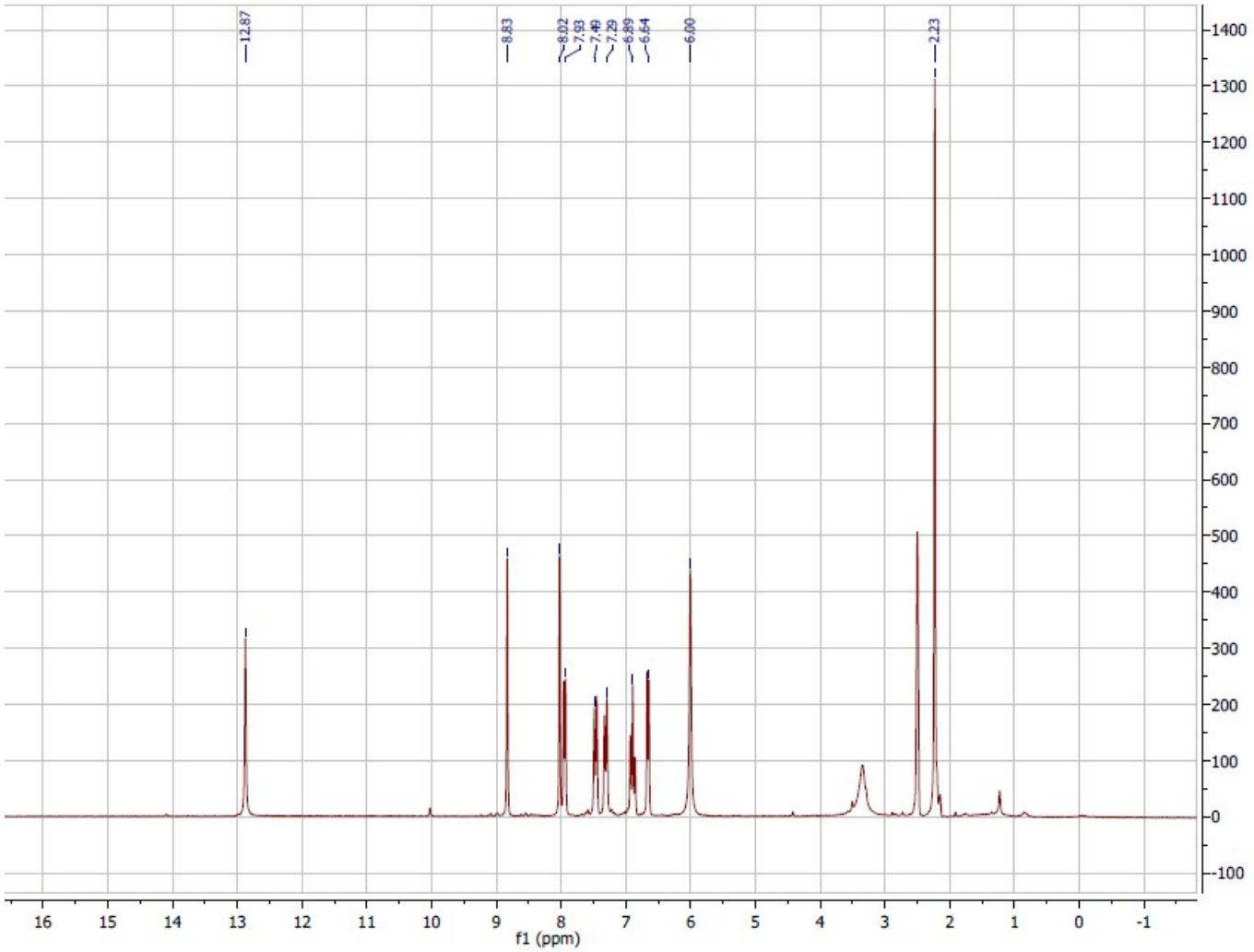


Figure S10. ^{1}HNMR of **L5** in DMSO-$_{d6}$.

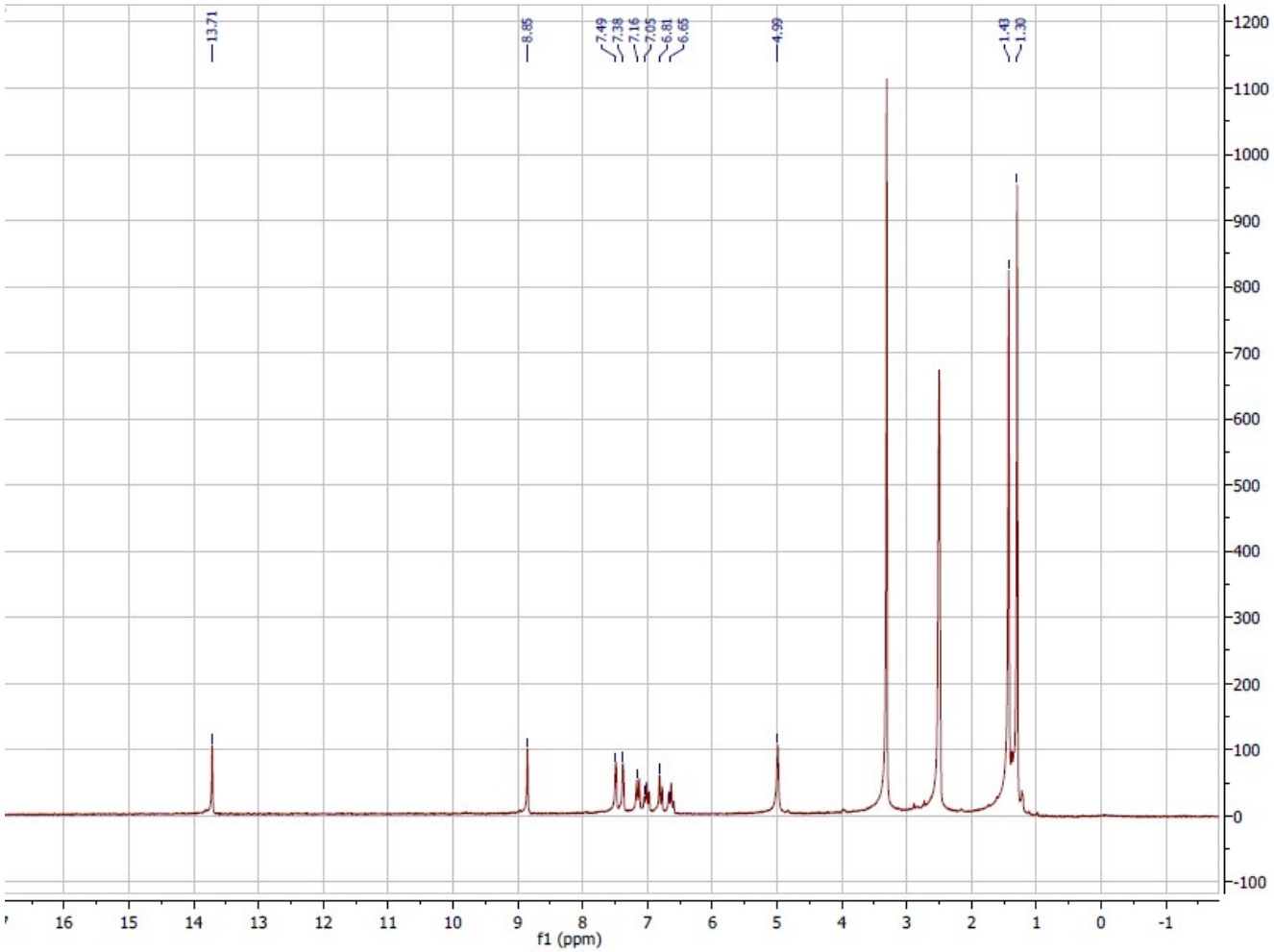


Figure S11. ^{1}HNMR of **L6** in DMSO-$_{d6}$.

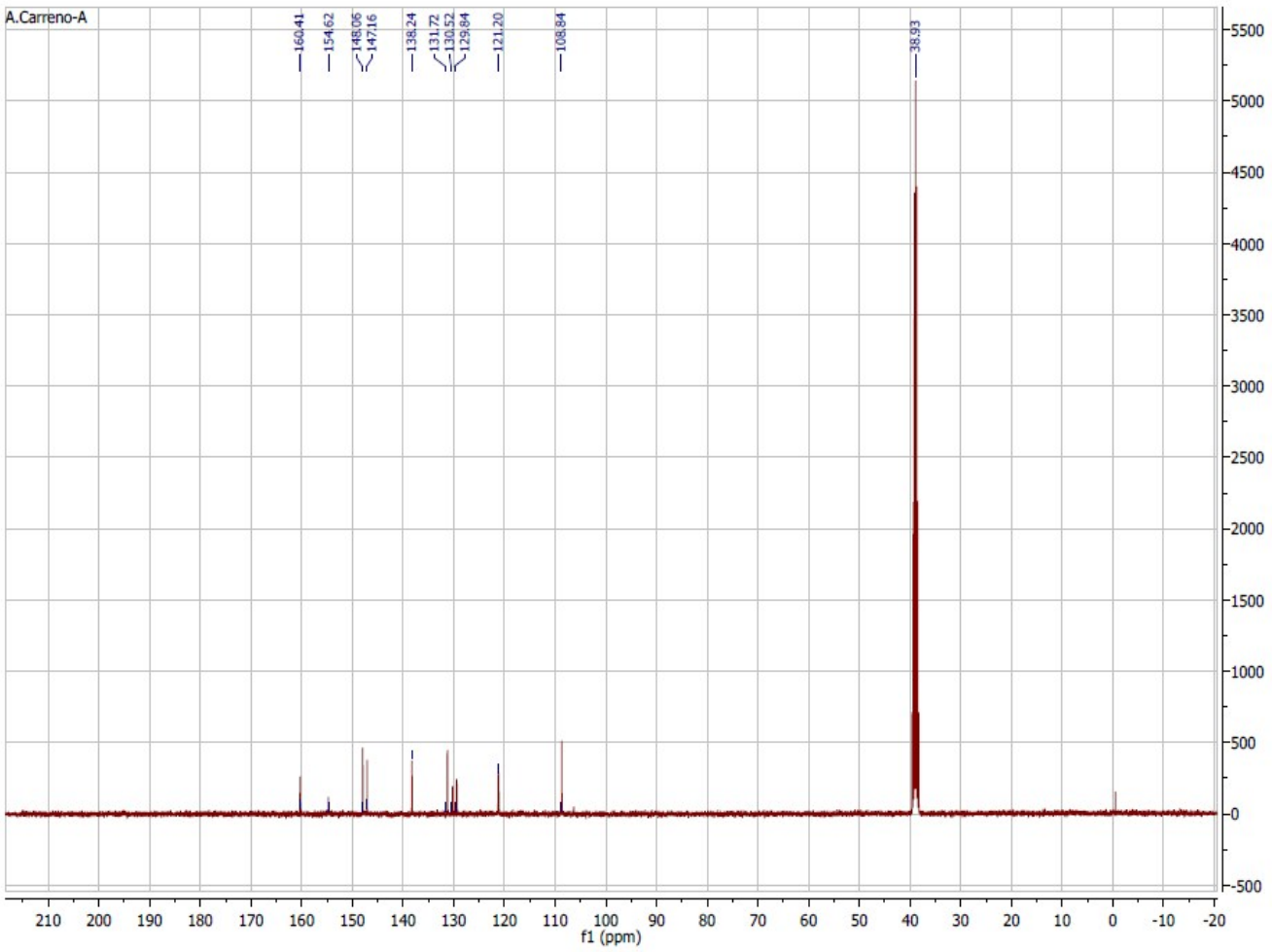


Figure S12. ^{13}CNMR of **L3** in DMSO-$_{d6}$.

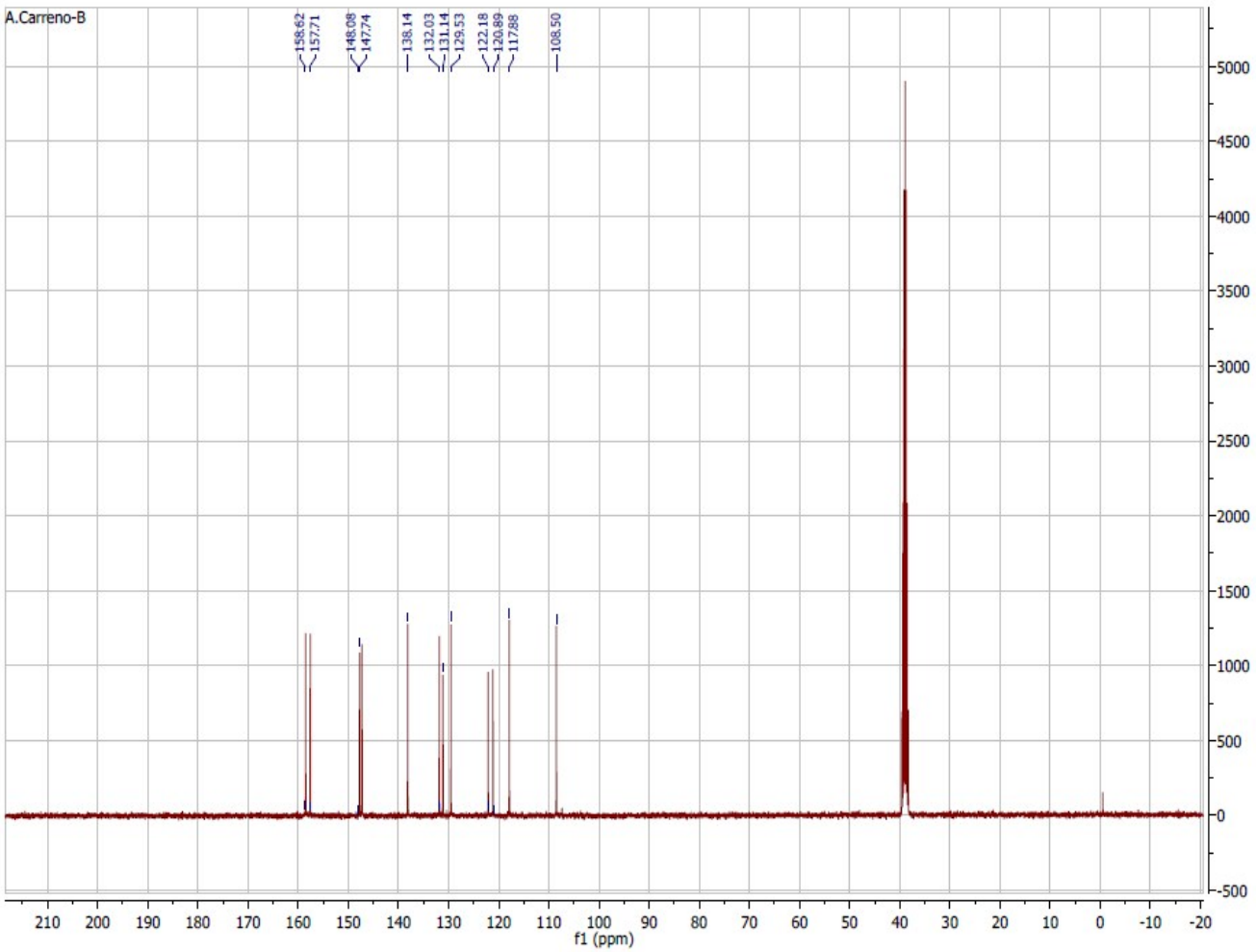


Figure S13. ^{13}CNMR of **L4** in DMSO-$_{d6}$.

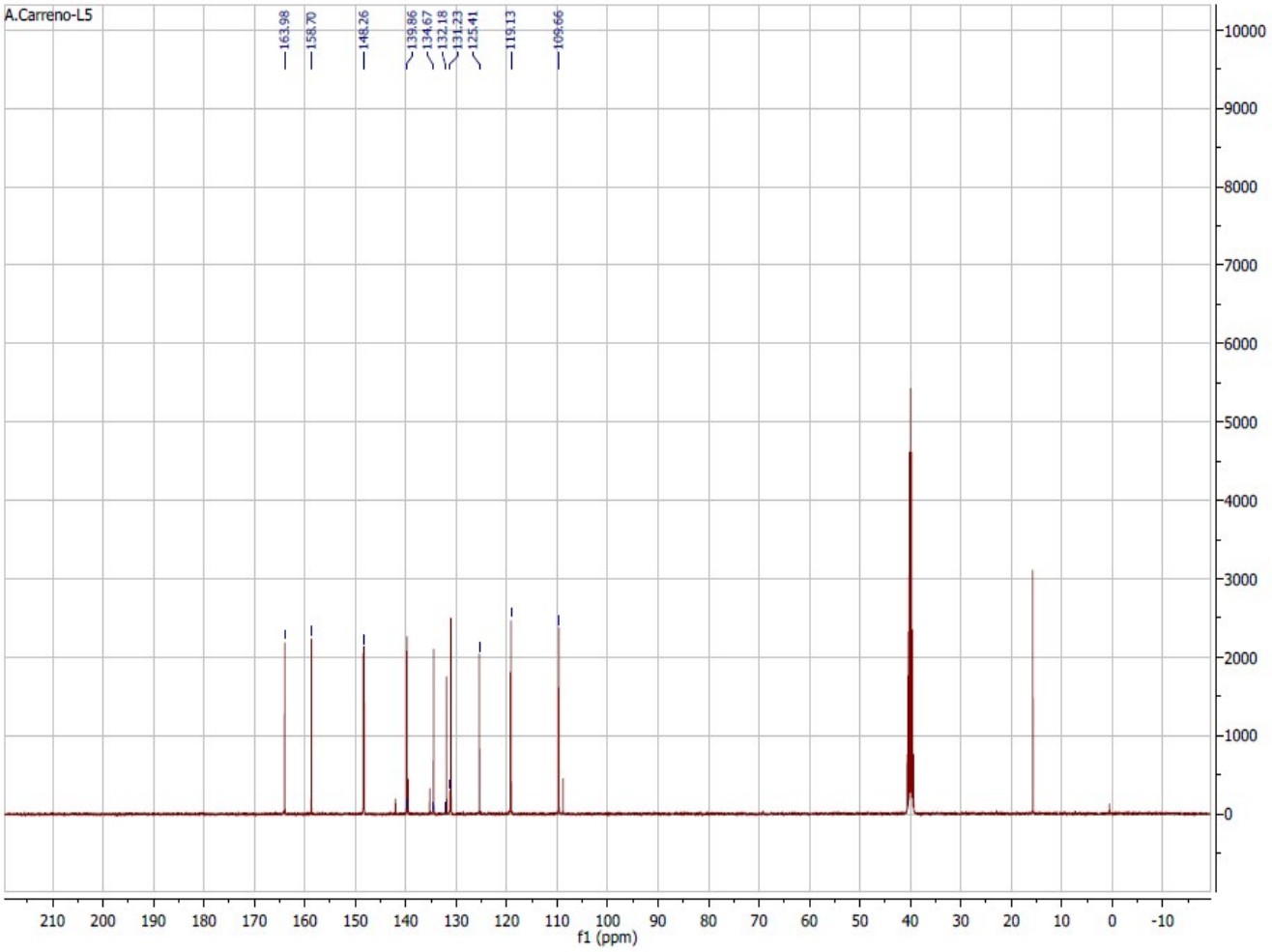


Figure S14. ^{13}CNMR of **L5** in DMSO-$_{d6}$.

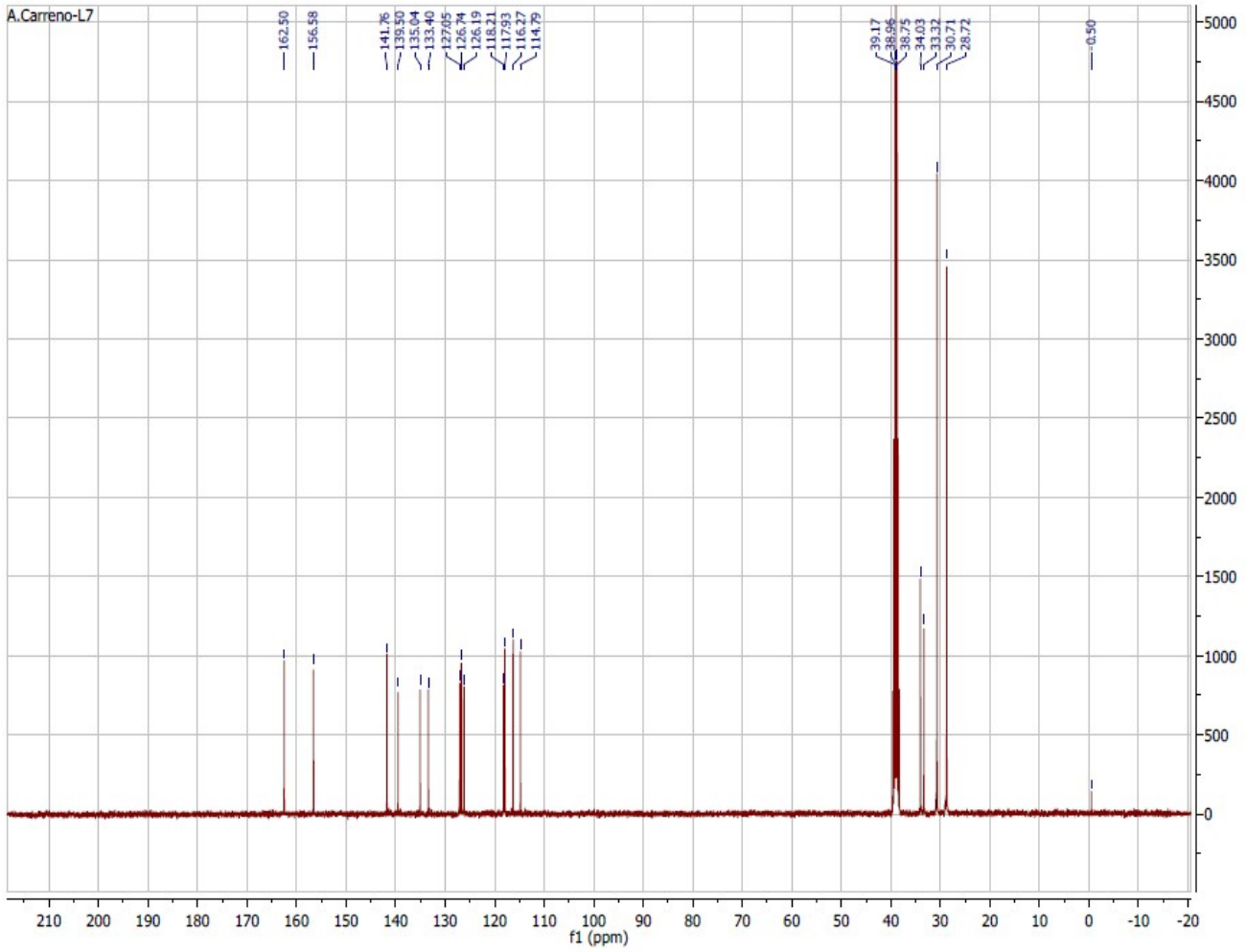


Figure S15. ^{13}CNMR of **L6** in DMSO-$_{d6}$.

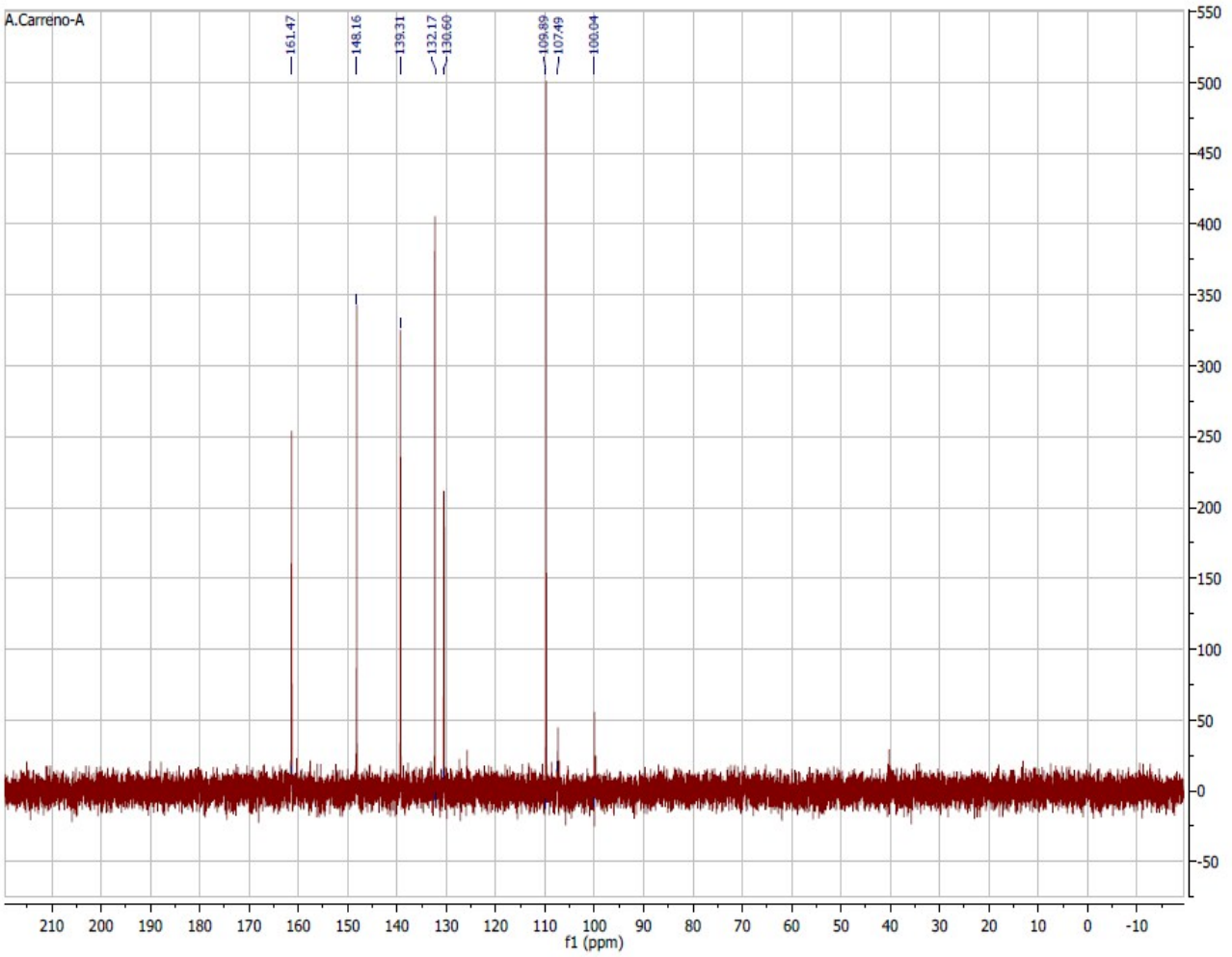


Figure S16. DEPT of **L3** in DMSO-$_{d6}$.

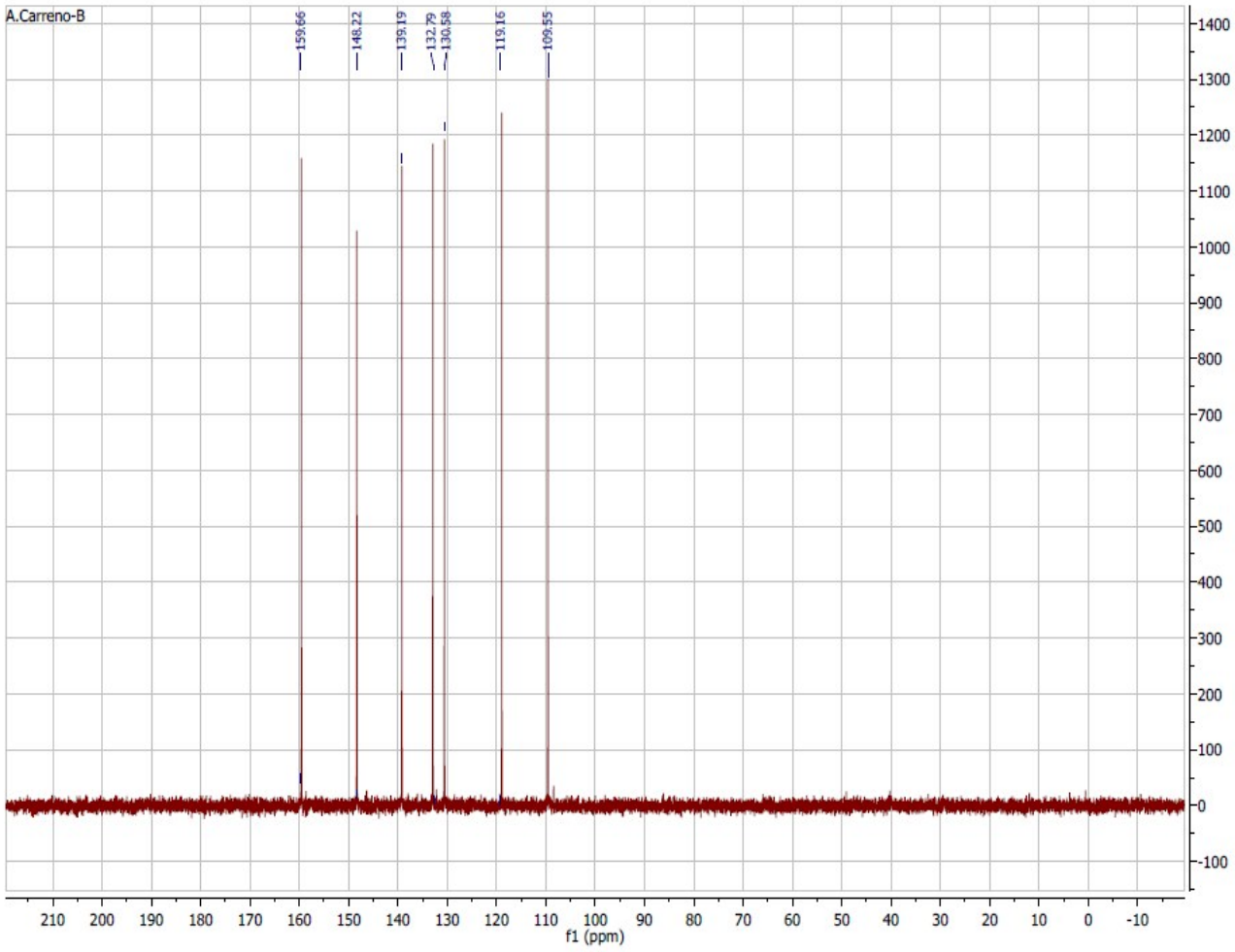


Figure S17. DEPT of **L4** in DMSO-$_{d6}$.

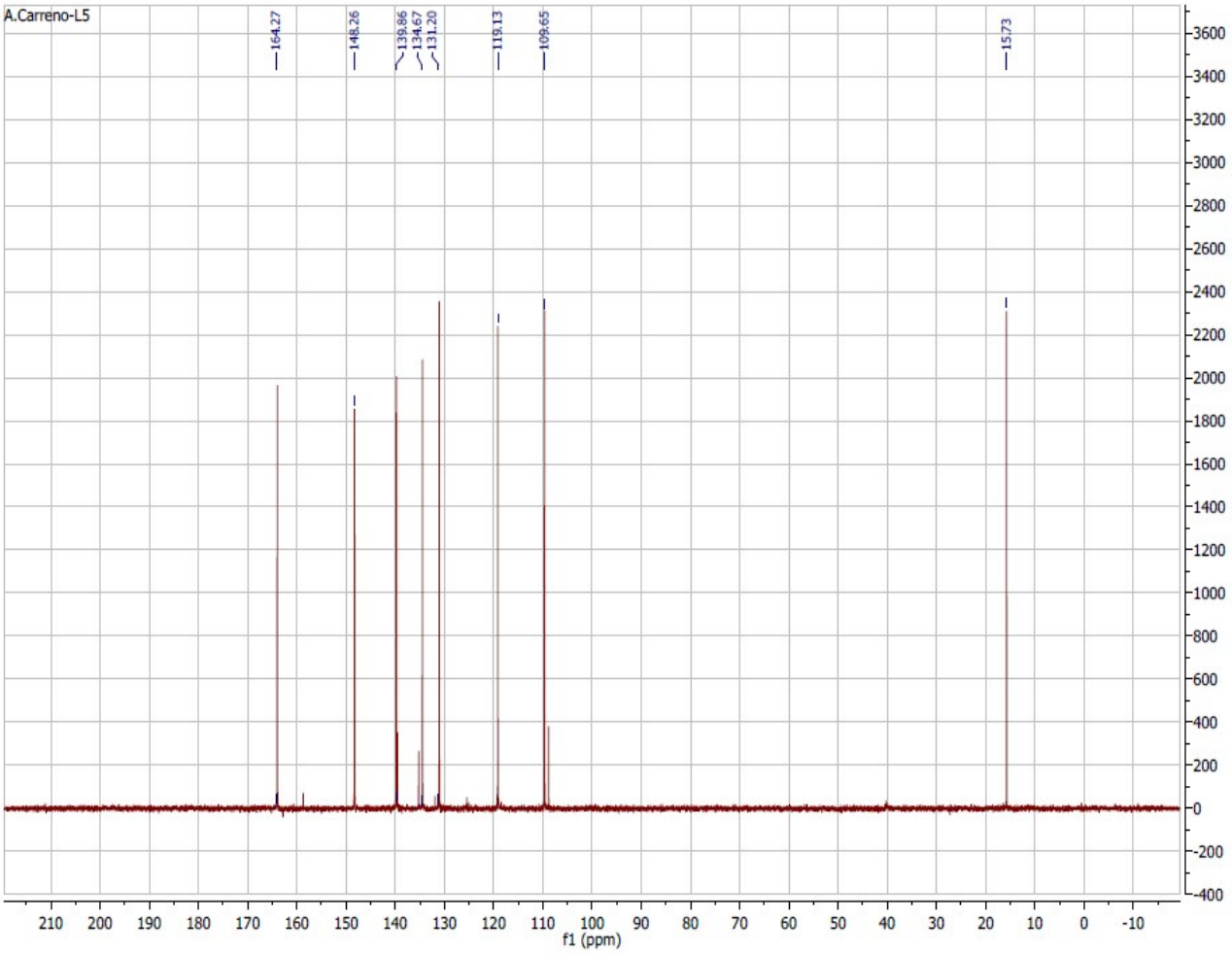


Figure S18. DEPT of **L5** in DMSO-$_{d6}$.

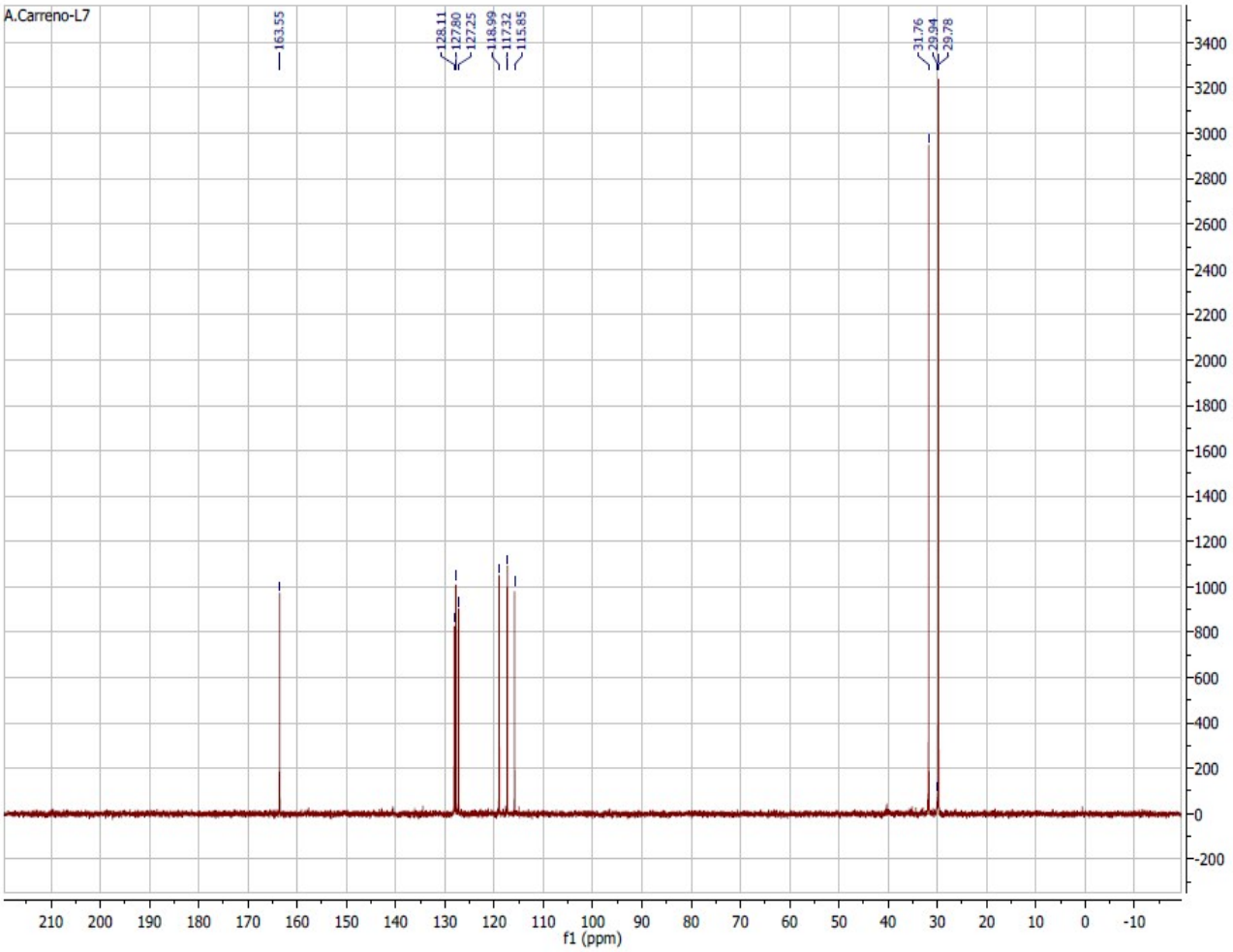


Figure S19.DEPT of **L6** in DMSO-$_{d6}$.

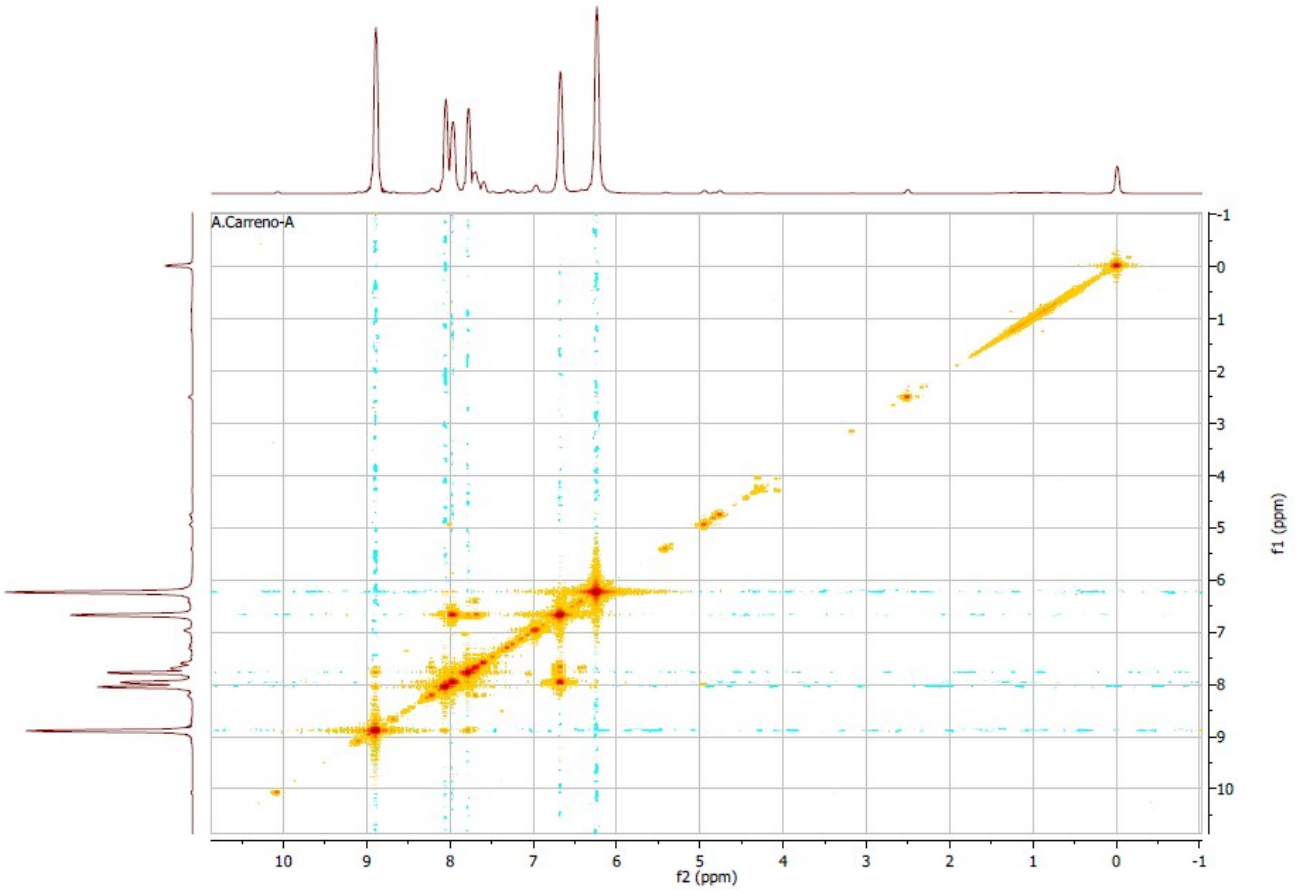


Figure S20.HHCOSSY of **L3** in DMSO-$_{d6}$.

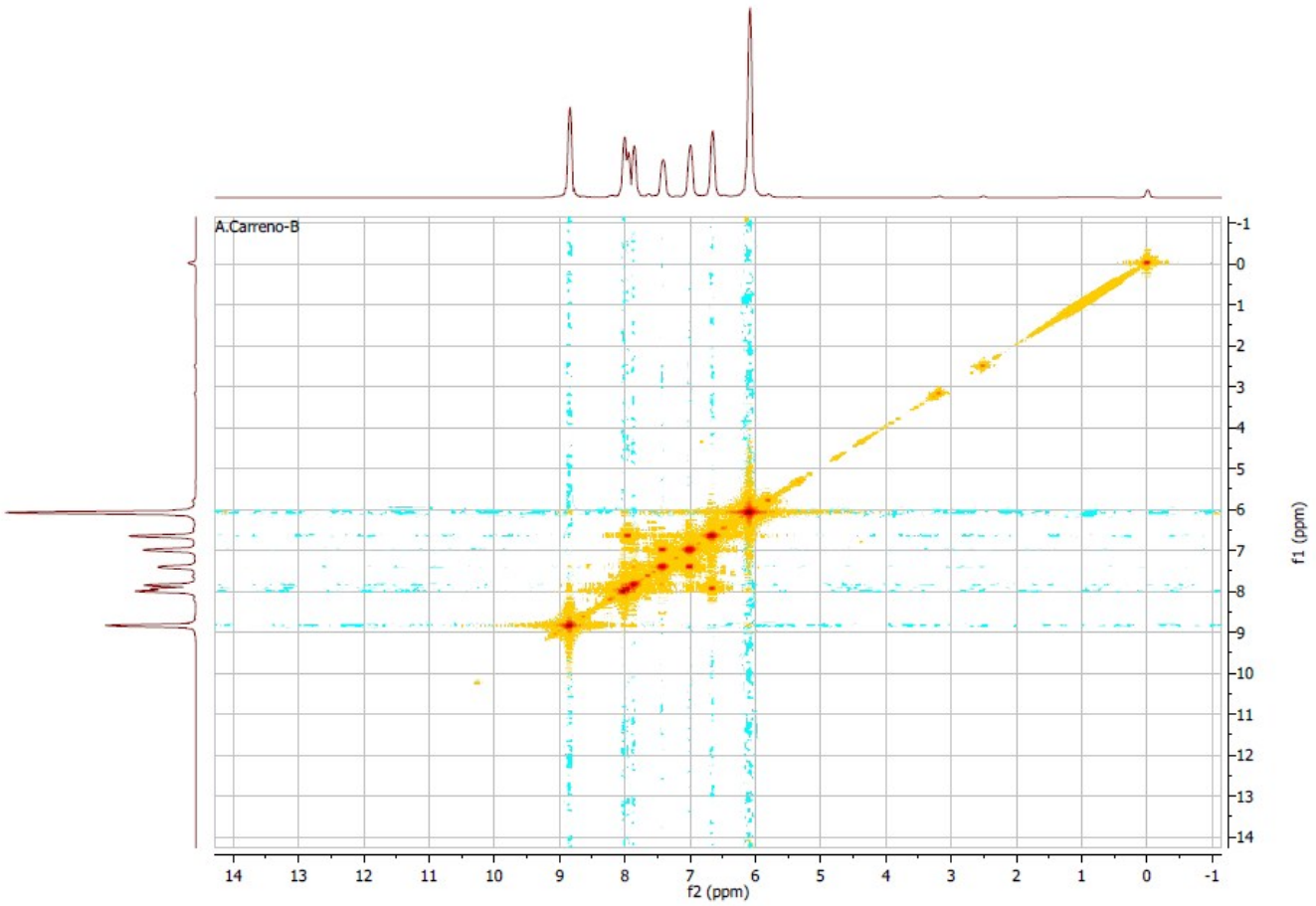


Figure S21.HHCOSSY of **L4** in DMSO-$_{d6}$.

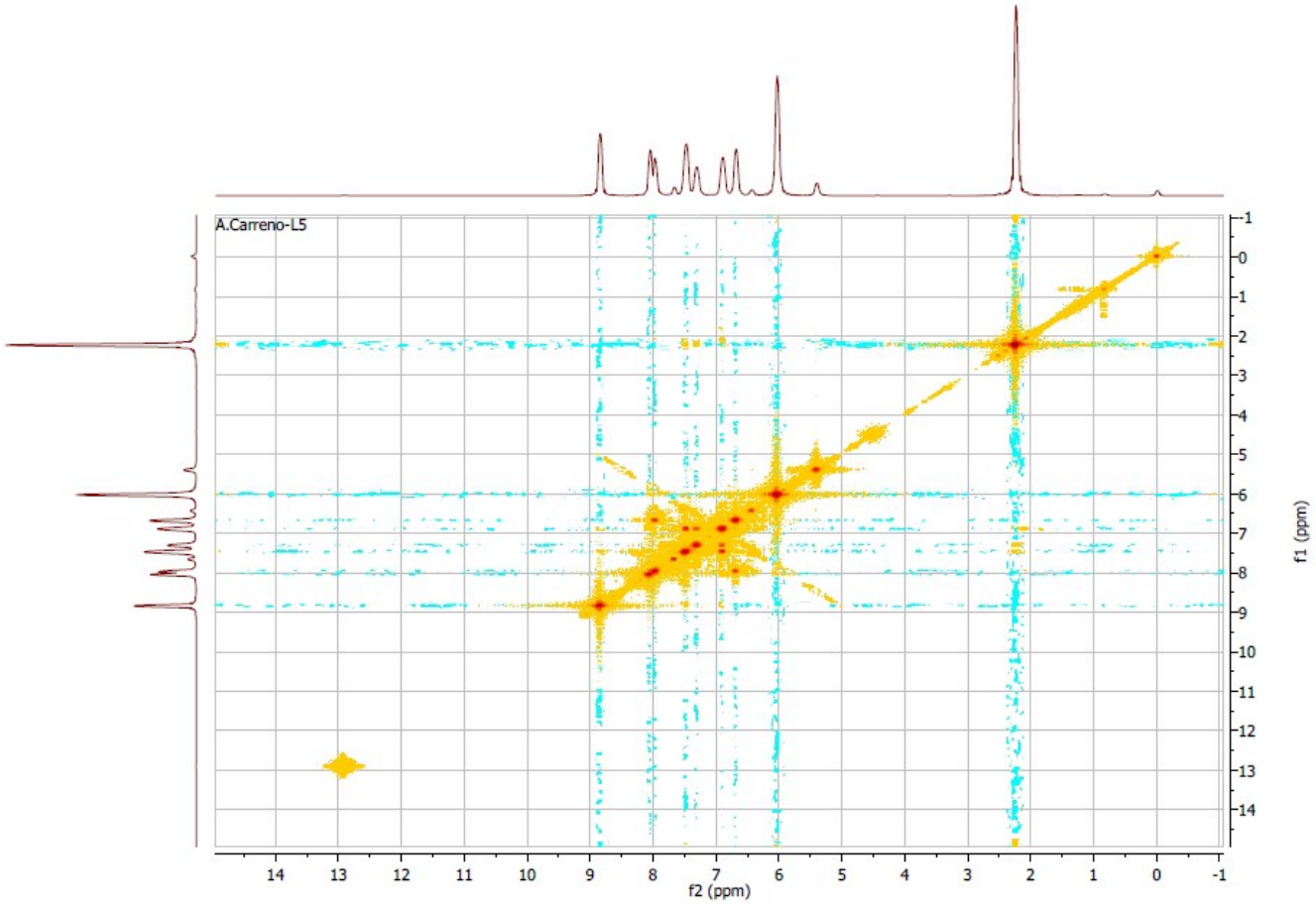


Figure S22.HHCOSSY of **L5** in DMSO-$_{d6}$.

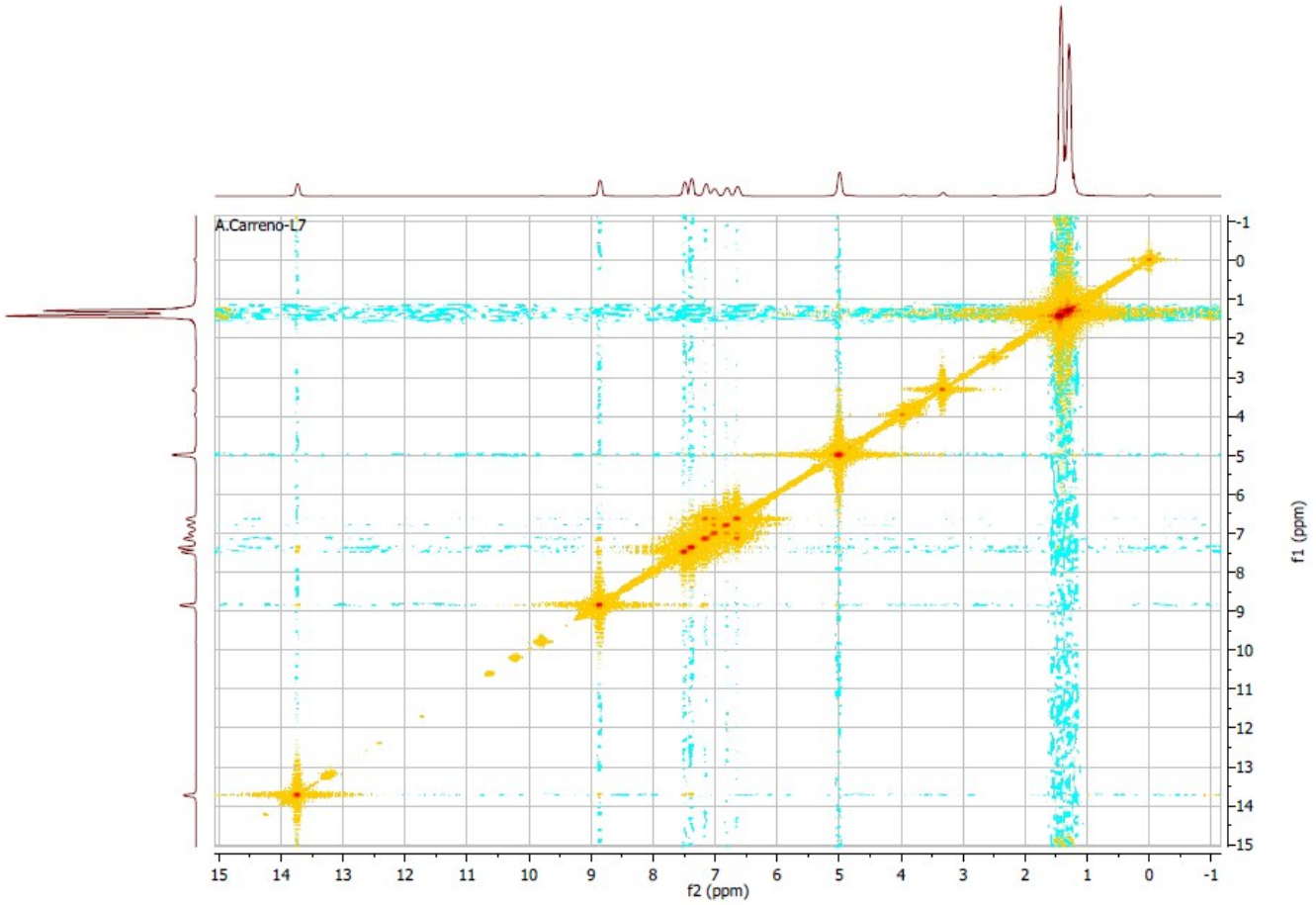


Figure S23.HHCOSSY of **L6** in DMSO-$_{d6}$.

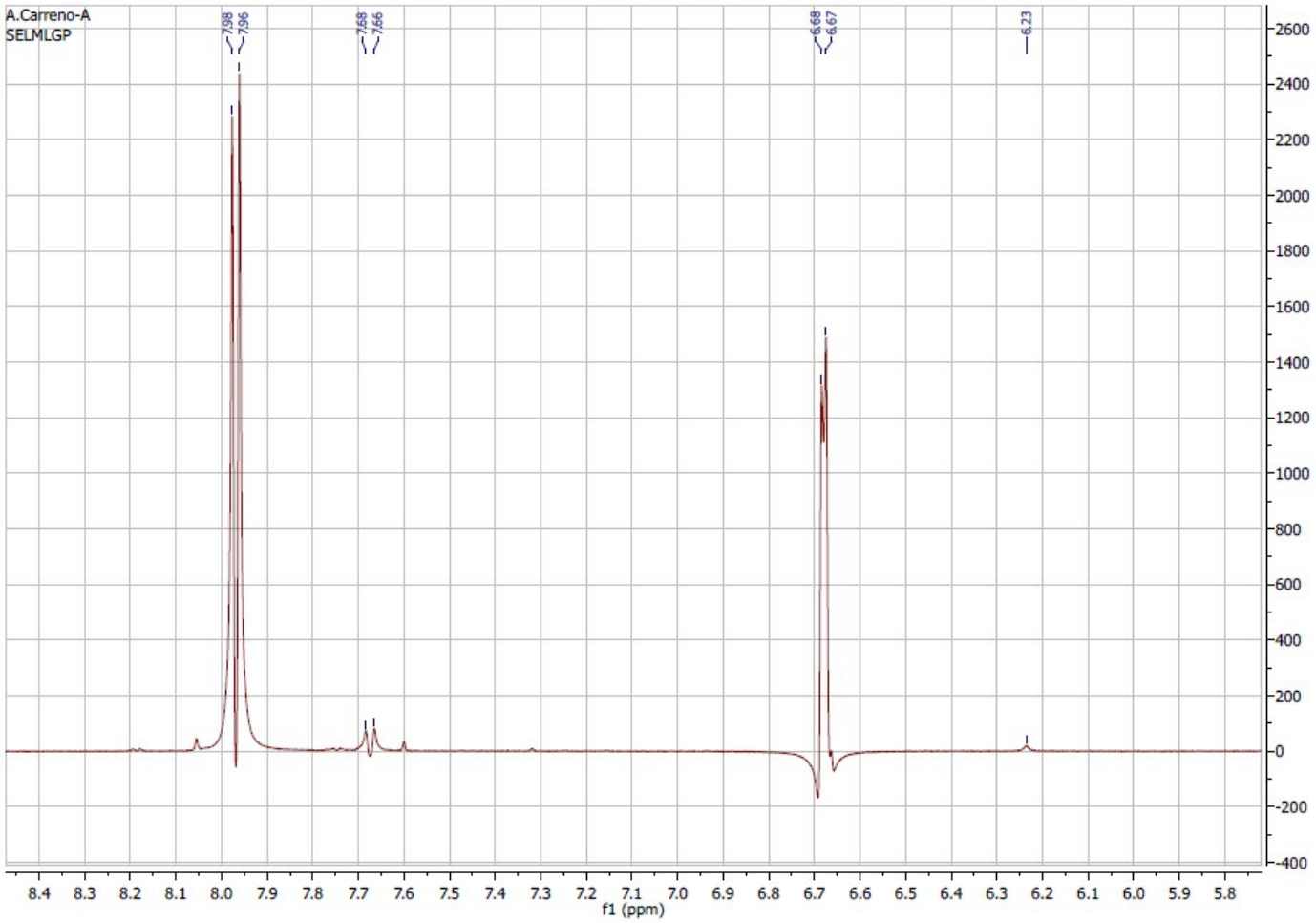


Figure S24. 1D TOCSY experiment of **L3** in DMSO-$_{d6}$, irradiating at 2672.62 Hz (6.67 ppm).

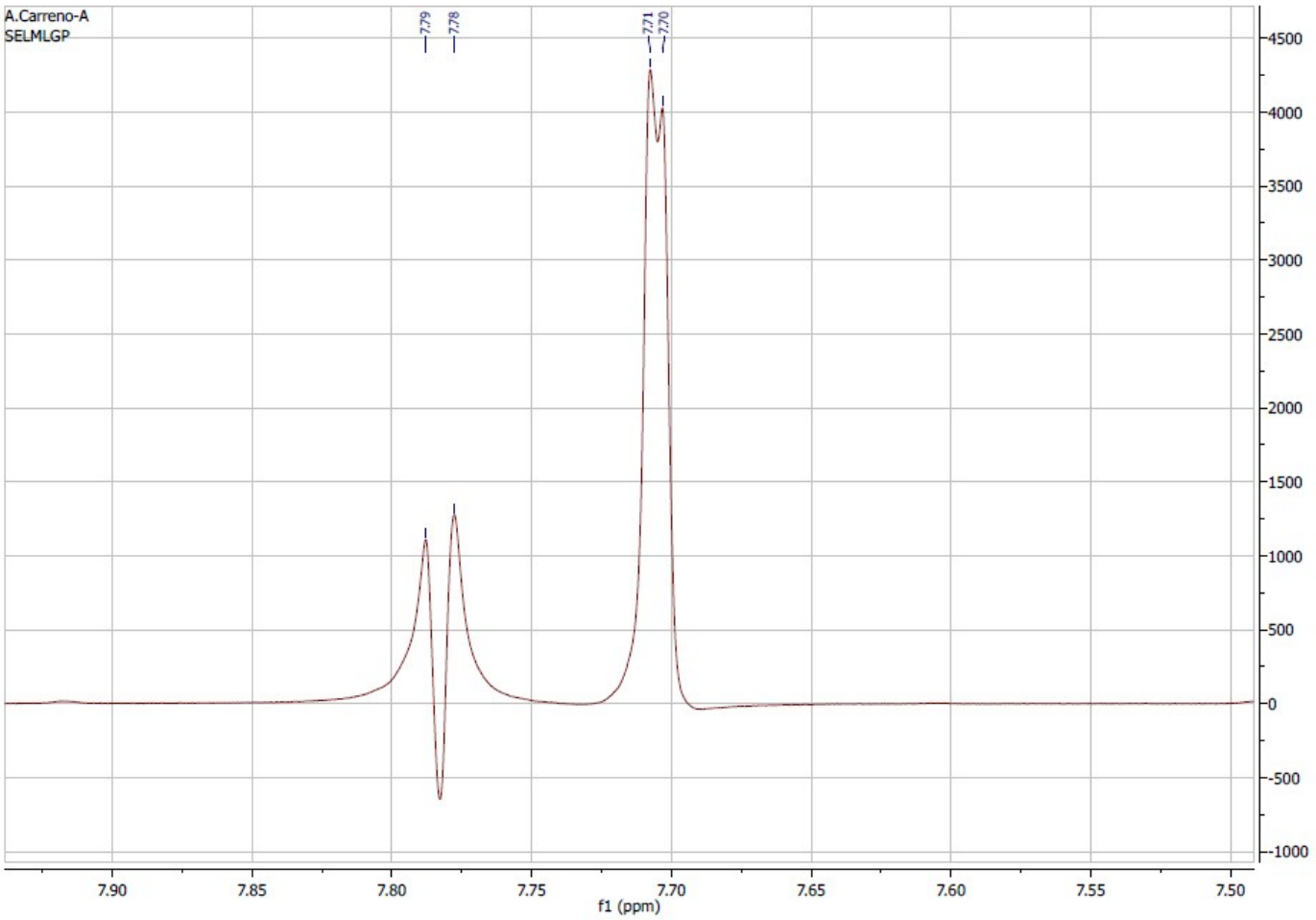


Figure S25. 1D TOCSY experiment of **L3** in DMSO-$_{d6}$, irradiating at 3114.25 Hz (7.78 ppm, H5).

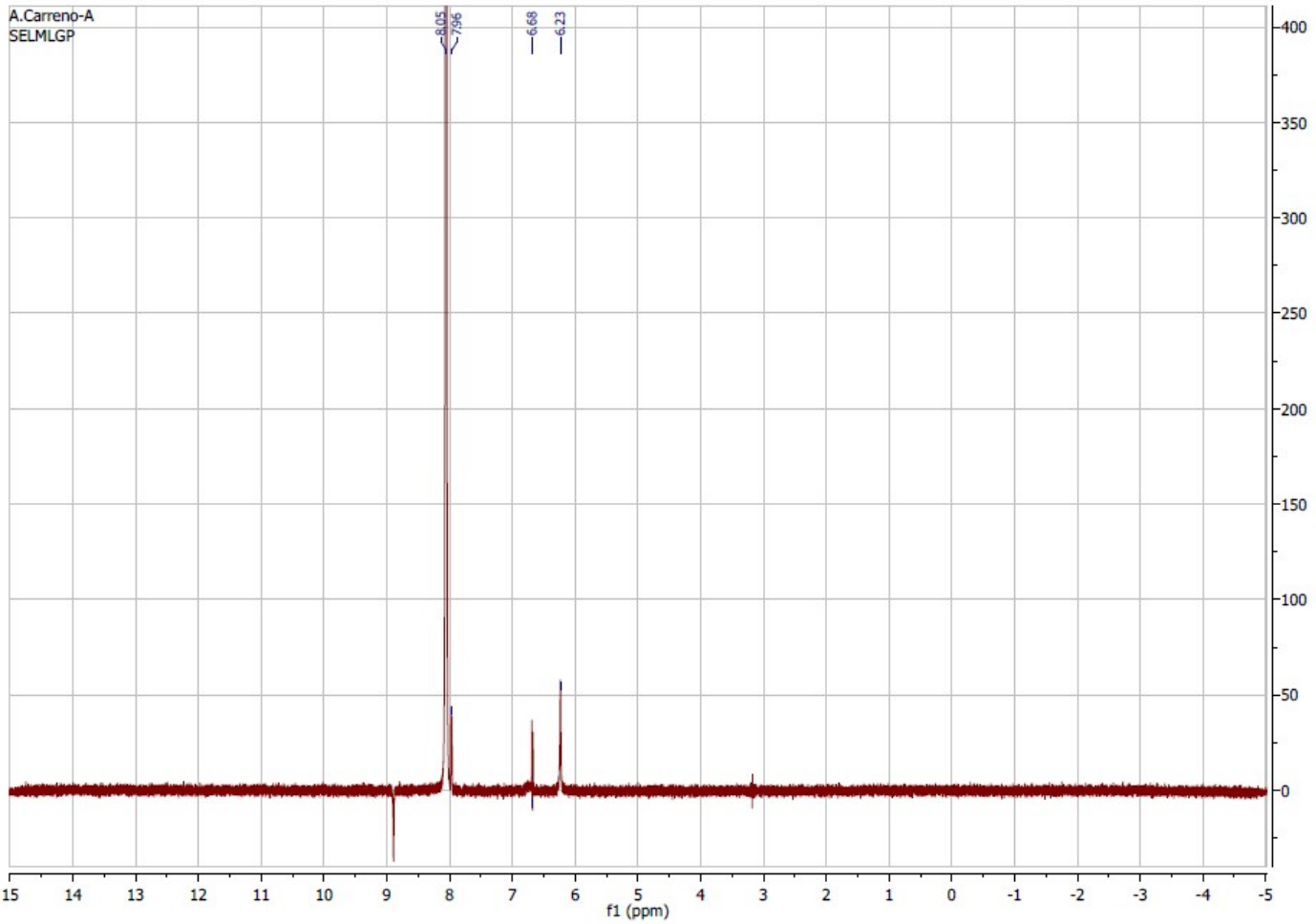


Figure S26. 1D TOCSY experiment of **L3** in DMSO-$_{d6}$, irradiating at 3200.10 Hz (8.00 ppm, H3).

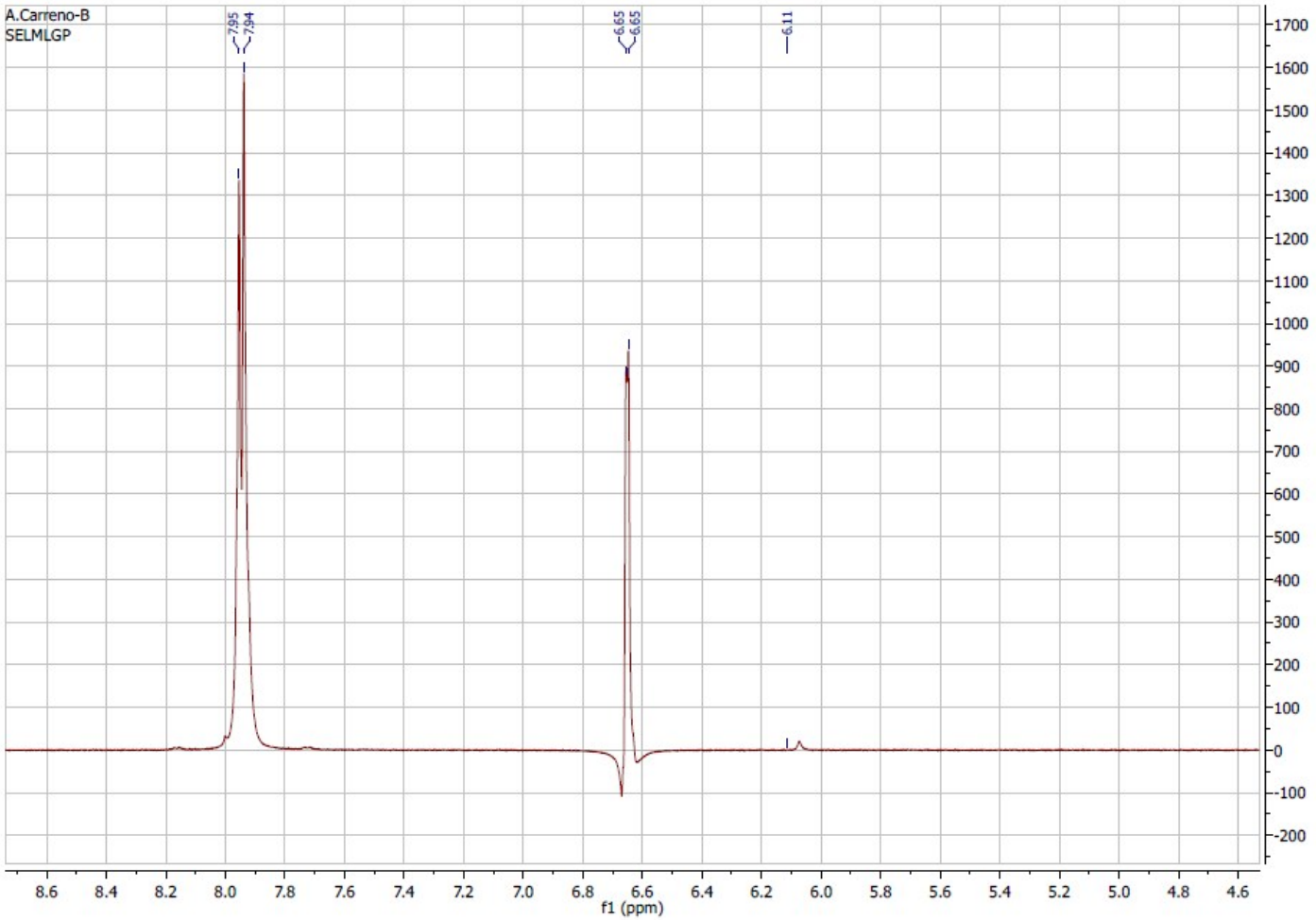


Figure S27. 1D TOCSY experiment of **L4** in DMSO-$_{d6}$, irradiating at 2662.82 Hz (6.65 ppm, H2).

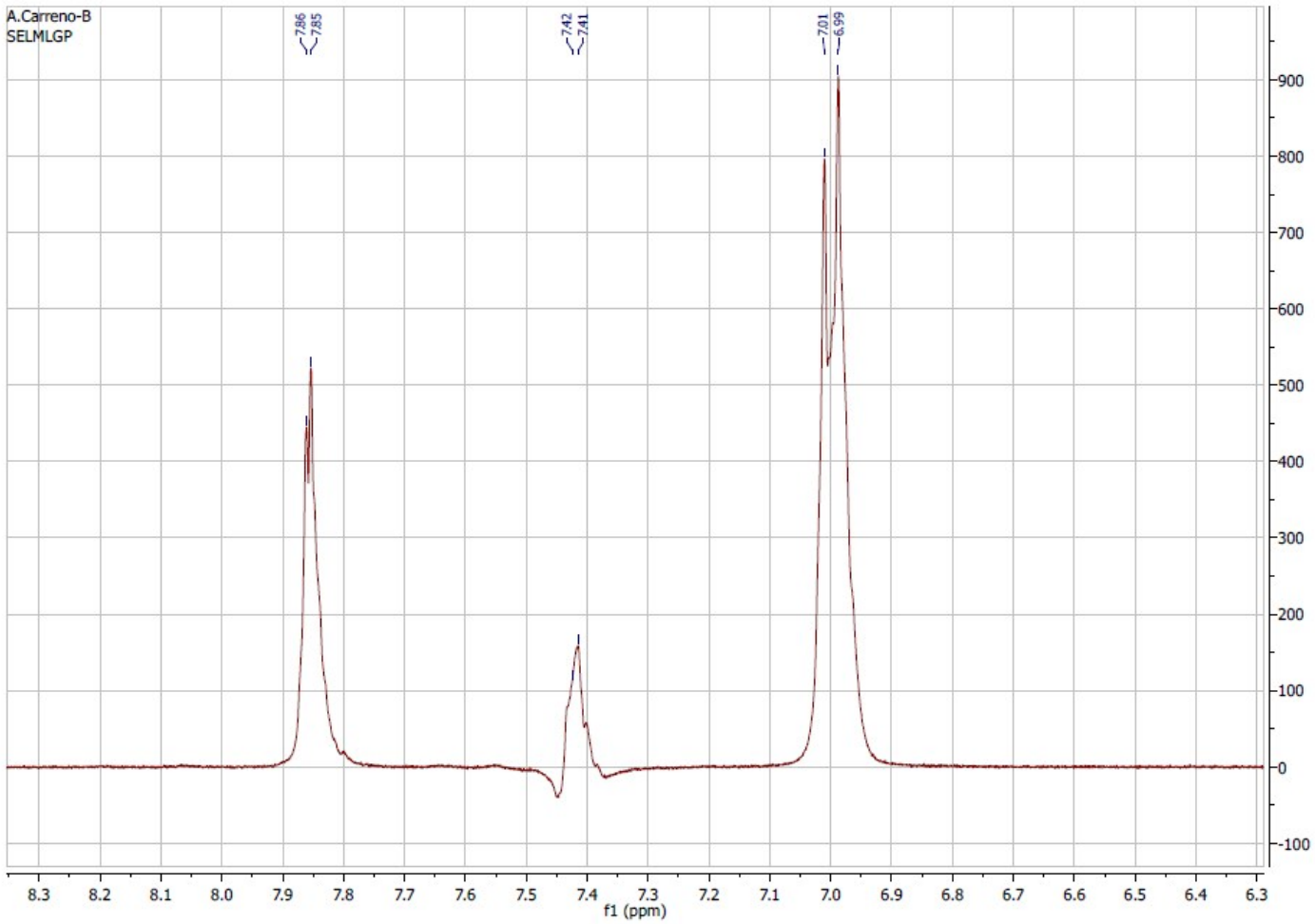


Figure S28. 1D TOCSY experiment of **L4** in DMSO-$_{d6}$, irradiating at 2799.82 Hz (7.00 ppm, H5).

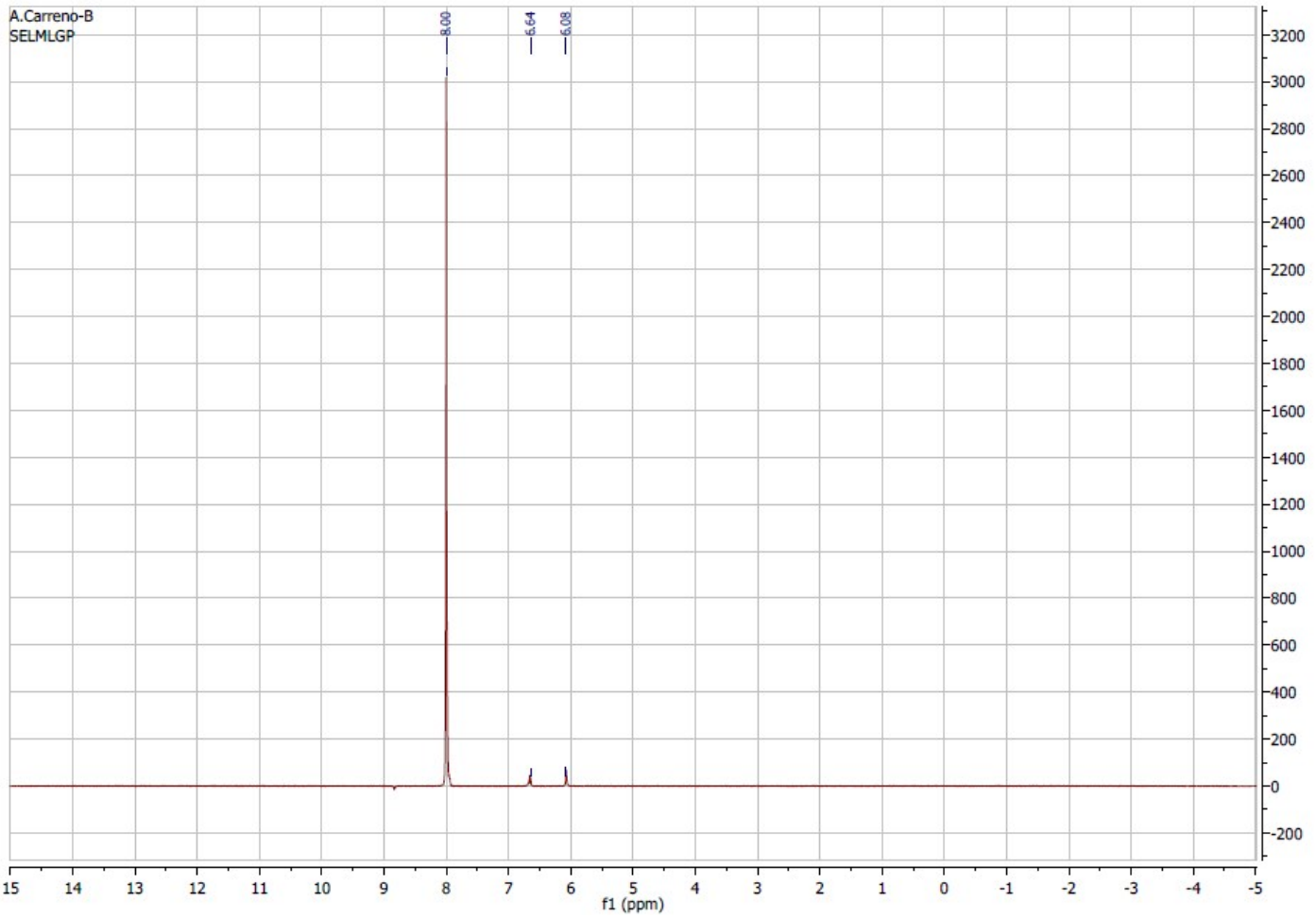


Figure S29. 1D TOCSY experiment of **L4** in DMSO-$_{d6}$, irradiating at 3188.02 Hz (8.00 ppm, H3).

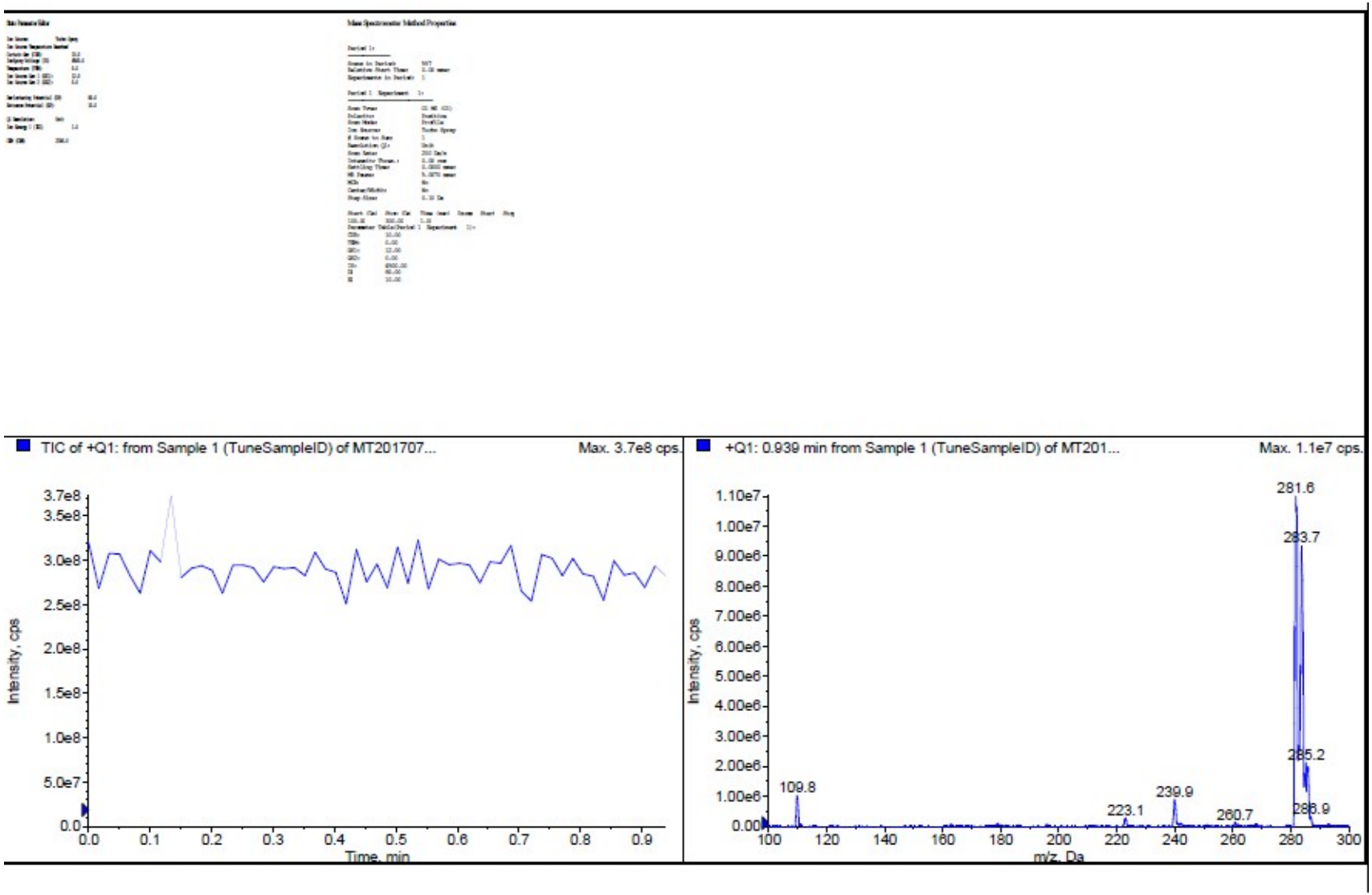


Figure S30. Mass spectra of **L3**.

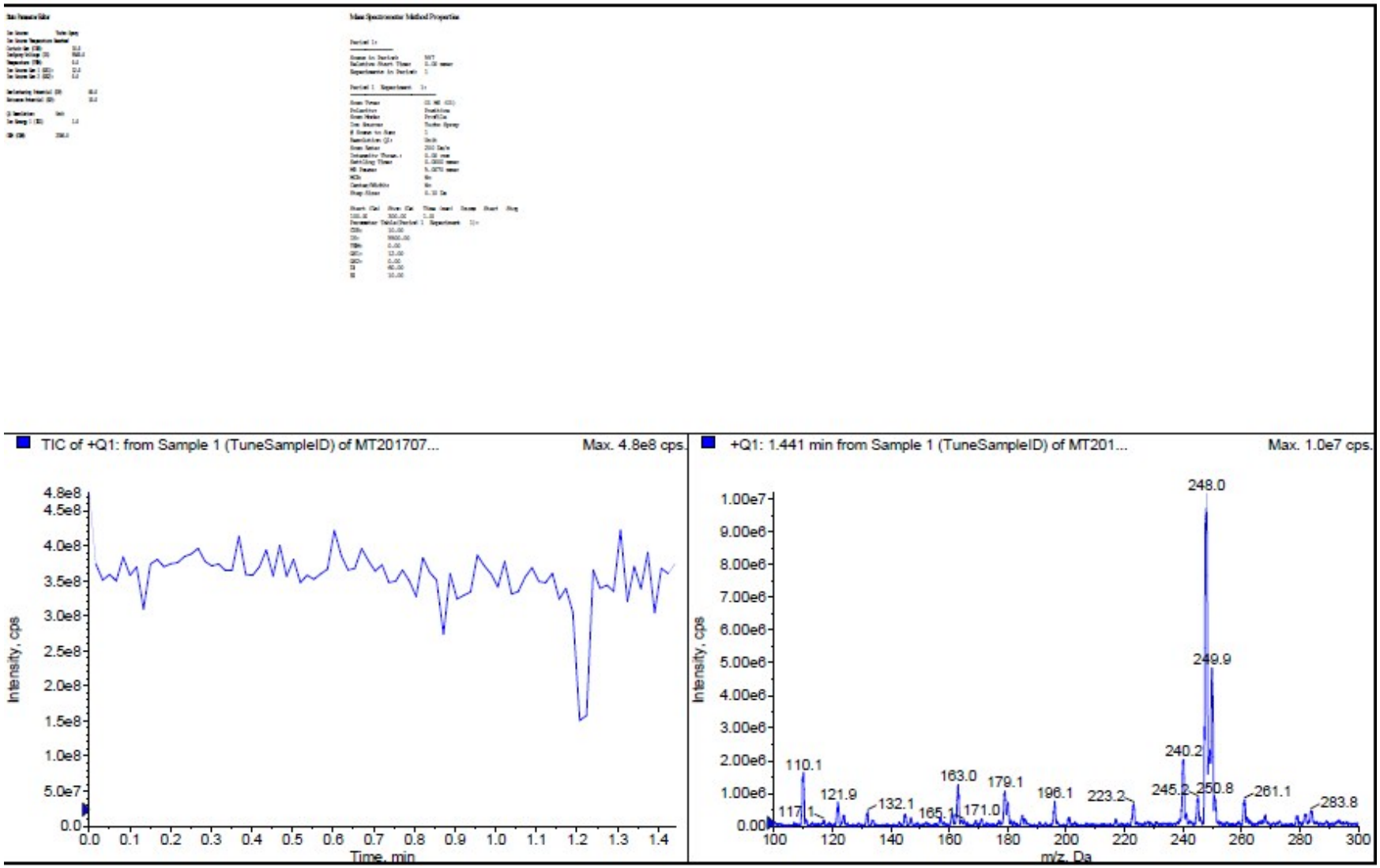


Figure S31. Mass spectra of **L4**.

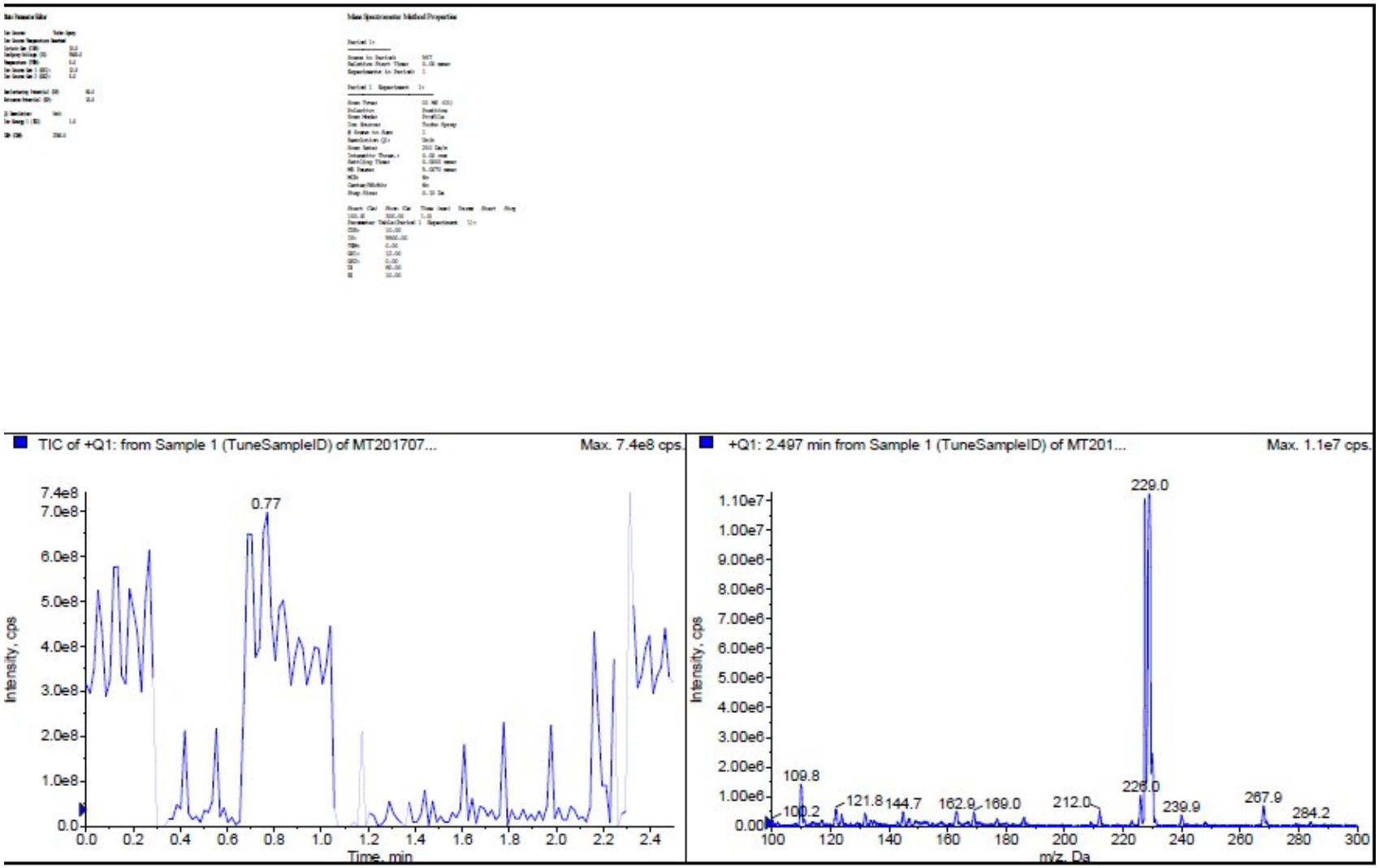


Figure S32. Mass spectra of **L5**.

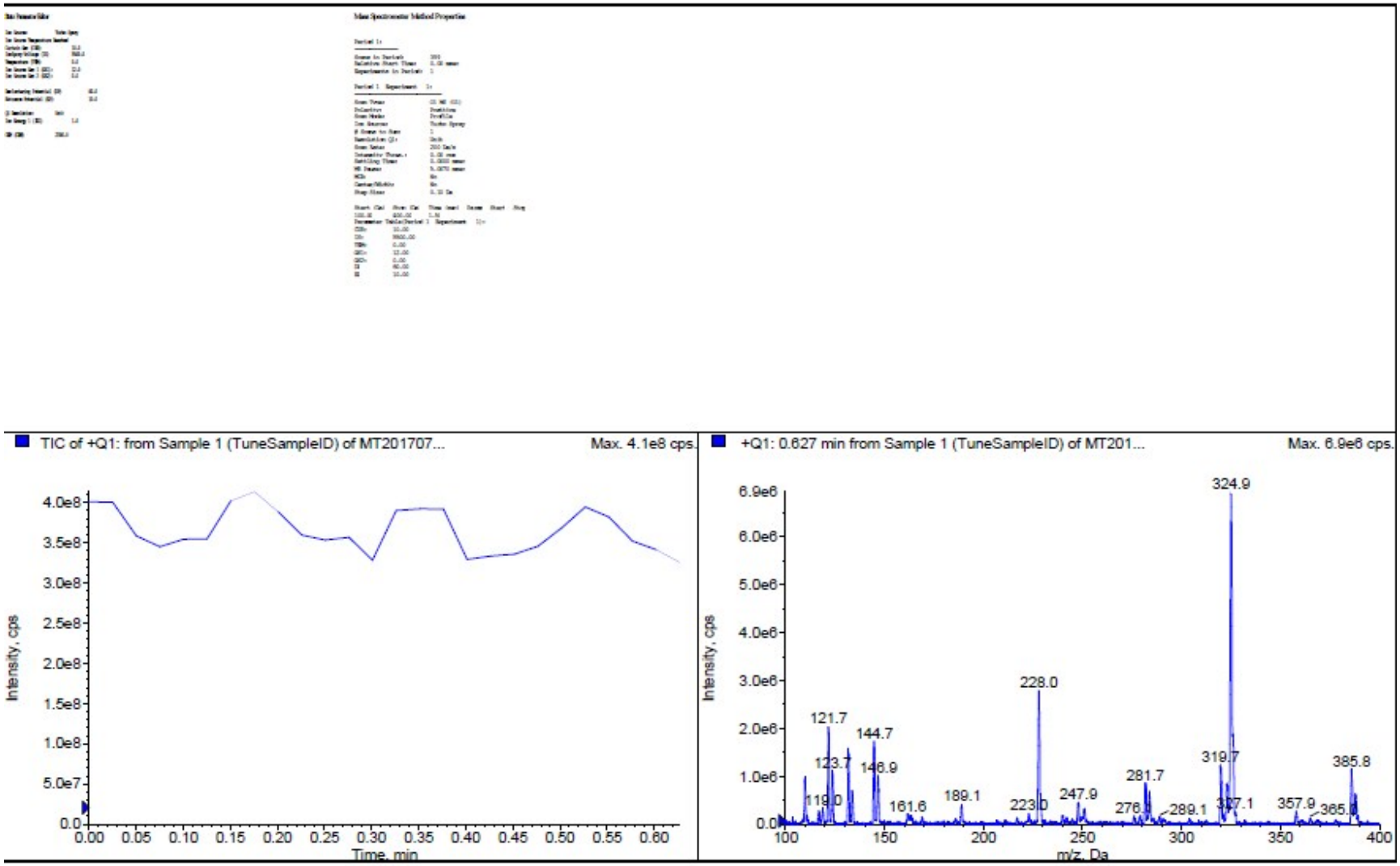


Figure S33. Mass spectra **L6**.

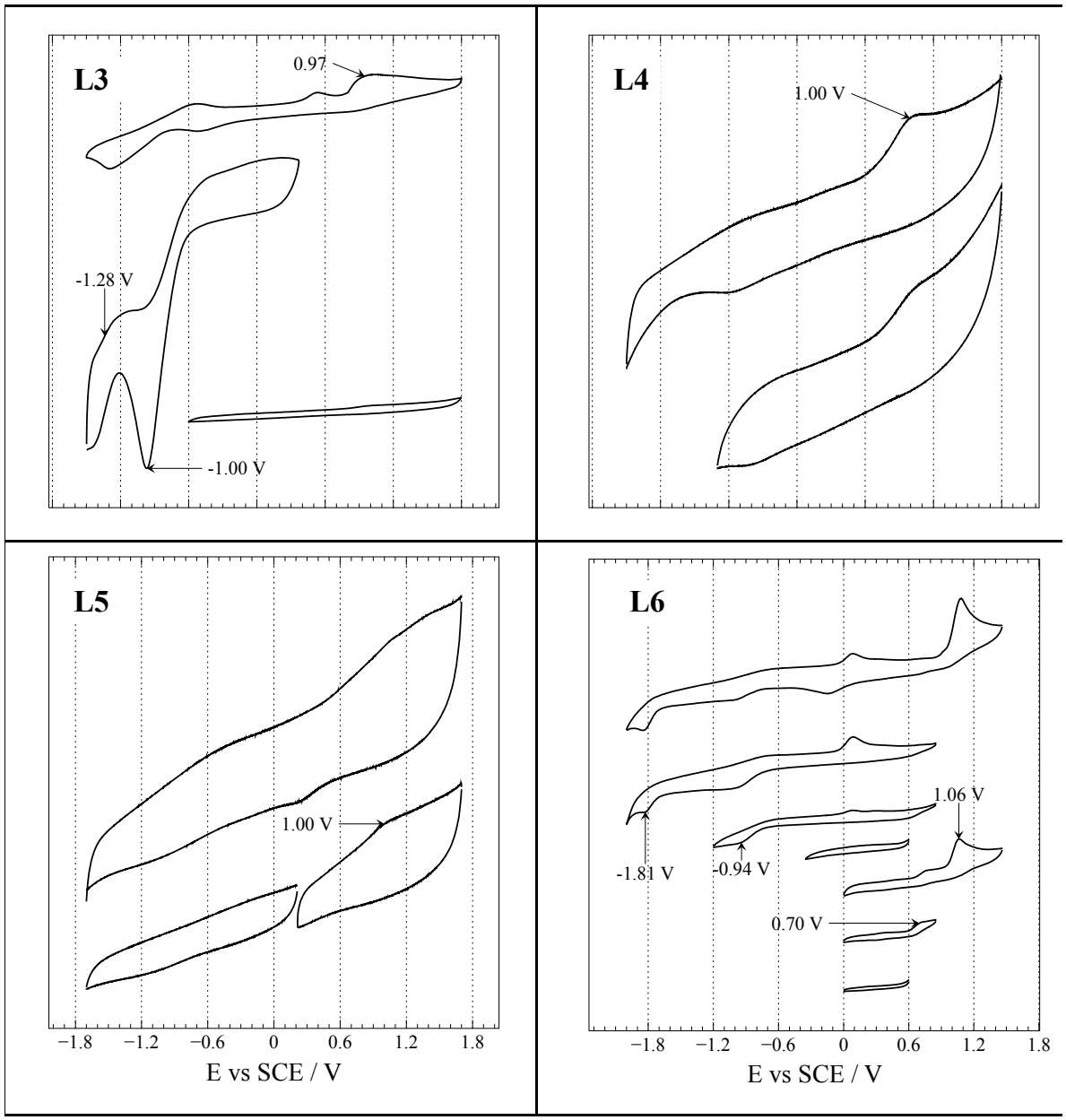


Figure S34. Electrochemical working window study during cyclic voltammetry experiments for compounds. Interface: Same as **Figure 2**.

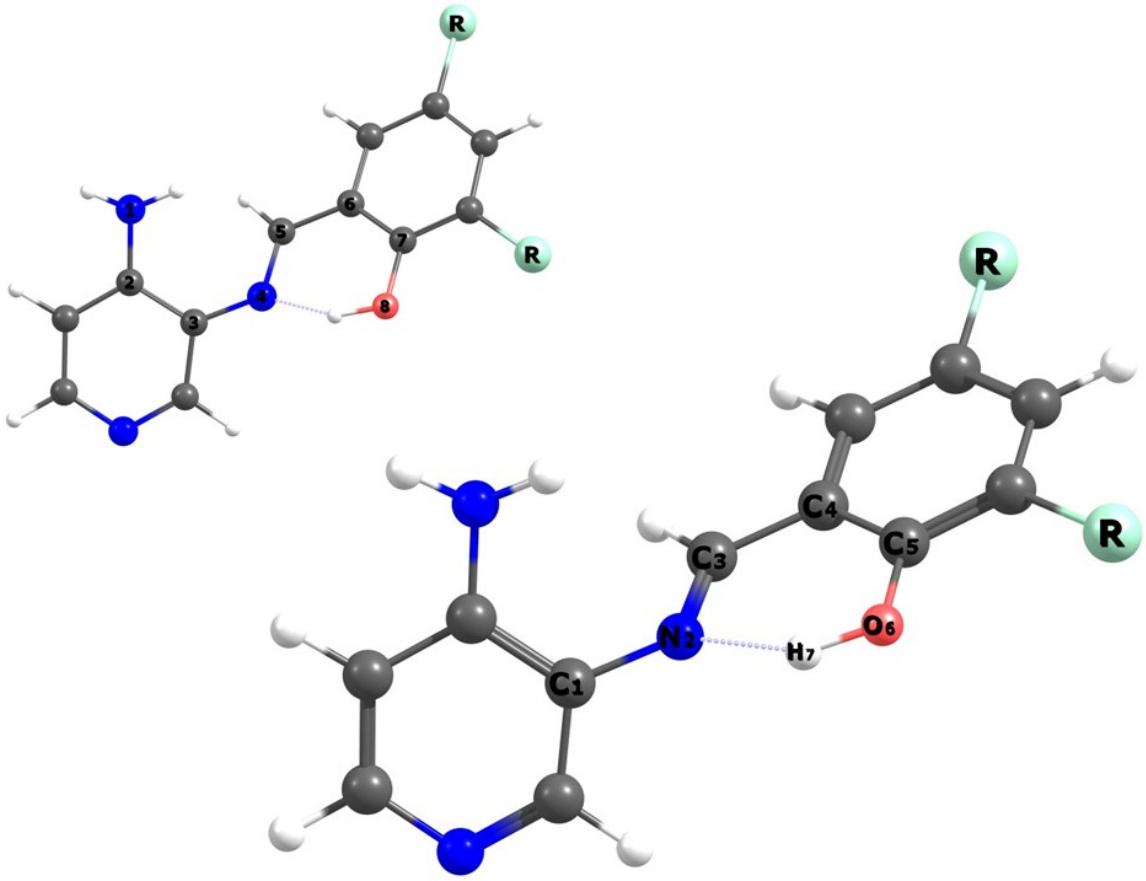


Figure S35. General model for the Schiff bases compounds showing the numbering of the atoms used in this study.

Cell viability assays (L1 to L6)

A cytotoxicity assay of **L3** to **L6** Schiff bases was performed in HeLa (epithelial cell line)[3] through a MTT viability assay.[4-6]

Hela cells were cultured in Dulbecco's Modified Eagle's Medium (DMEM) containing 10% fetal bovine serum (FBS), 2 mM L-glutamine, 100 units/mL penicillin and 100 µg/mL streptomycin. Cells were maintained in 75 cm^2 flasks in a 5% CO_2-humidified atmosphere at 37 °C. Passage took place every 2–3 days. All cell culture ingredients were purchased from Sigma-Aldrich. Toxicity of the respective compounds was determined using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) cell viability assay after 24 h of incubation with **L3** to **L6**. MTT is a yellow compound that, when reduced by functioning mitochondria, produces purple formazan crystals that can be measured spectrophotometrically.[4-6] For this purpose, MTT (Sigma-Aldrich) was dissolved in phosphate buffered saline (PBS) to a concentration of 5 mg/mL and further diluted in culture medium (1:11). Cells were incubated with this MTT-solution for 3 h under normal culture conditions. Afterwards, 155 µL of the solution were rejected and 90 µL of DMSO were added. To completely dissolve the formazan salts, plates were incubated for 10 min on a shaker and prior to being quantified by measuring the absorbance at 535 nm with a ELISA microplate reader. Cell viability was calculated as percentage of surviving cells compared to untreated control cells. Data were analyzed using a two-way ANOVA test.

Our results showed that **L6** exerted no effect against HeLa cells (**Figure S36**). On the other hand, **L3, L4** and **L5** were slightly more cytotoxic than the vehicle alone (DMSO) (1 µg/mL and 0.5 µg/mL for both **L3** and **L5**; 50 µg/mL and 1 µg/mL for **L4**), where **L5** was the less cytotoxic (compare black and grey bars, **Figure S36**). These

differences can be attributed to changes in the phenolic ring, where the presence of chlorine substituents in the 4 and/or 6 positions, apparently slightly increase cytotoxicity. Altogether, our results suggest that this kind of Schiff bases exert their effect through different mechanisms in fungi and in epithelial cells, where the pyridine and the substituted phenolic rings play differential roles in these cell models. In this sense, more experimentation is necessary to completely understand this phenomenon.

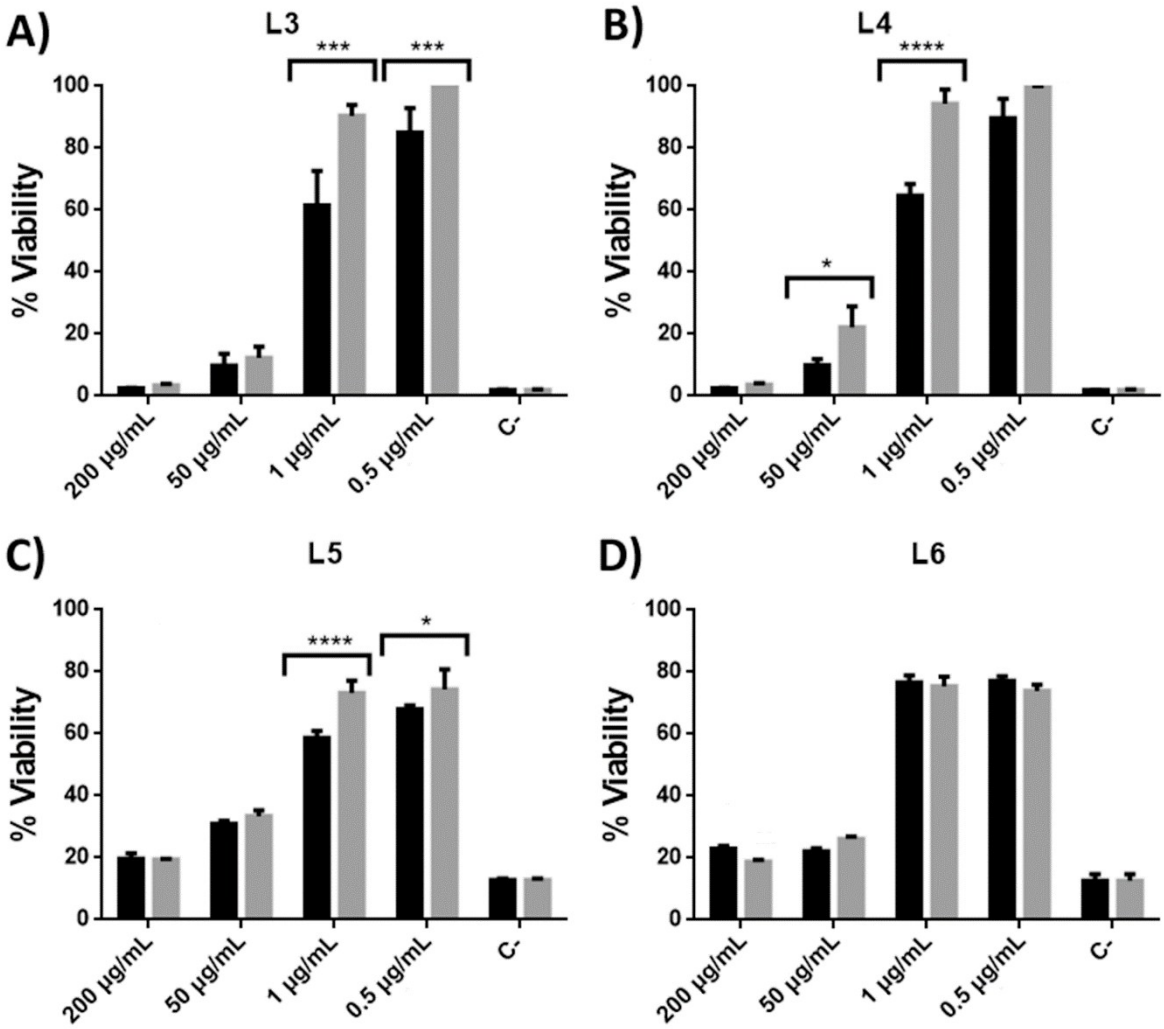


Figure S36. MTT Assay in HeLa cells. Cells were incubated 24 h with the compounds (A, **L3**; B, **L4**; C, **L5**; D, **L6**) prior to measuring cell viability. In all cases, compounds were tested at different concentrations (200 µg/mL + DMSO 50% v/v, 100 µg/mL + DMSO 25% v/v, 50 µg/mL + DMSO 12.5% v/v, or 25 µg/mL + DMSO 6.3% v/v) (black bars). The vehicle alone was also tested (50% v/v, 25% v/v, 12.5% v/v, or 6.3% v/v, respectively). Culture medium alone was used to set 100% viability. C- corresponded to DMSO 100% v/v. * p<0.05; ** p<0.01; *** p<0.001; **** p<0.0001 (ANOVA). All these experiments were performed in biological triplicate.

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