Supporting Information

for

Monofunctional Platinum(II) Anticancer Complexes Based on Multidentate Phenanthridine-Containing Ligand Frameworks

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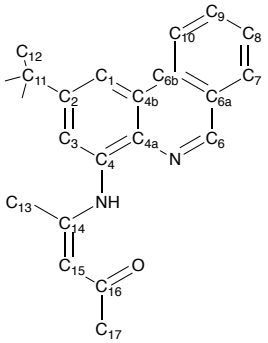
TABLE OF CONTENTS

EXPERIMENTAL DETAILS

Air-sensitive manipulations were carried either in a N_2-filled glove box or using standard Schlenk techniques under Ar. 4-amino-(2-*tert*-butyl)phenanthridine[1] and 4-amino-(2-trifluoromethyl)phenanthridine[2] were synthesized according to literature procedures. All other reagents were purchased from commercial suppliers as noted and used without further purification. Organic solvents were dried and distilled using appropriate drying agents, and distilled water was degassed prior to use. 1- and 2D NMR spectra were recorded on Bruker Avance 300 MHz or Bruker Avance – III 500 MHz spectrometers. ^{1}H and $^{13}C\{^{1}H\}$ NMR spectra were referenced to residual solvent peaks. Infrared spectra of the proligands were collected using a Bruker Invenio R FTIR spectrometer. High resolution mass spectra of Pt(II) complexes were recorded using a Bruker microOTOF-QIII. Elemental analysis was performed at the University of Manitoba using a Perkin Elmer 2400 Series II CHNS/O Elemental Analyzer.

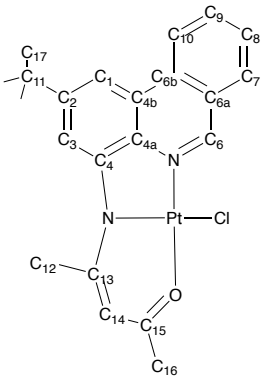
Synthesis of *t*Bu-phenanthridine-nacac proligand (L1): A Teflon-stoppered flask was charged with 4-amino-(2-*tert*-butyl)phenanthridine (1.01 g, 3.03 mmol), acetylacetone (1.30 mL, 12.7 mmol), *p*-tosic acid (0.050 g, 0.27 mmol), toluene (10 mL) and 4 Å molecular sieves. The reaction was heated to reflux for 48 h, then cooled to room temperature and filtered over Celite. The solvent was then evaporated to leave an oily dark brown residue, which was redissolved in a minimal amount of CH_2Cl_2 and passed through a short plug of silica. The solvent was evaporated to isolate a beige solid. Yield = 0.858 g (64%). ^{1}H NMR ($CDCl_3$, 300 MHz, 22 °C): δ 13.44 (s, 1H; N*H*), 9.31 (s, 1H; C_6-*H*), 8.63 (d, J_{HH} = 8.3 Hz, 1H; C_{10}-*H*), 8.32 (d, J_{HH} = 1.9 Hz, 1H; C_1-*H*), 8.06 (d, J_{HH} = 7.3 Hz, 1H; C_7-*H*), 7.86 (ddd, J_{HH} = 1.3 Hz, 7.7



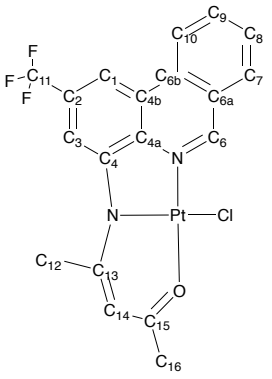
Hz, 8.5 Hz, 1H; C_8-*H*), 7.71 (ddd overlapped, J_{HH} = 0.9 Hz, 7.5 Hz, 8.5 Hz, 1H; C_9-*H*), 7.60 (d, J_{HH} = 1.9 Hz, 1H; C_3-*H*), 5.35 (s, 1H; C_{15}-*H*), 2.23 (s, 3H; C_{13}-*H*), 2.18 (s, 3H; C_{17}-*H*), 1.49 ppm (s, 9H; C_{12}-*H*). $^{13}C\{^1H\}$ NMR ($CDCl_3$, 75 MHz, 22 °C): δ 196.33 (C_{16}), 158.65 (C_{14}), 152.42 (C_6), 149.74 (C_2), 136.91 (C_4), 136.12 (C_{4a}), 132.74 (C_{4b}), 130.97 (C_8), 129.06 (C_7), 127.65 (C_9), 126.83 (C_{6a}), 124.39 (C_{6b}), 122.16 (C_{10}), 119.68 (C_3), 113.88 (C_1), 99.74 (C_{15}), 35.46 (C_{11}), 31.57 (C_{12}), 29.54 (C_{17}), 21.09 ppm (C_{13}). IR (ATR): ν_{max} 3100-2800 (C-H stretch, b, w), 1617 (C=O stretch, n, m), 1570 cm^{-1} (N-H bend, n, s).

Synthesis of CF_3-phenanthridine-nacac proligand (L2): An identical procedure to the synthesis of **L1** was followed using 4-amino-(2-trifluoromethyl)phenanthridine (1.09 g, 3.15 mmol), acetylacetone (1.30 mL, 12.7 mmol), *p*-tosic acid (0.040 g, 0.23 mmol), toluene (10 mL), and 4 Å molecular sieves. **L2** was isolated as an off-white solid. Yield = 0.911 g (64%). ^1H NMR ($CDCl_3$, 300 MHz, 22 °C): δ 13.72 (s, 1H; N*H*), 9.44 (s, 1H; C_6-*H*), 8.60 (d, J_{HH} = 8.3 Hz, 1H; C_{10}-*H*), 8.49 (s, 1H; C_1-*H*), 8.12 (d, J_{HH} = 7.9 H, 1H; C_7-*H*), 7.93 (ddd, J_{HH} = 1.2 Hz, 7.7 Hz, 8.7 Hz, 1H; C_8-*H*), 7.79 (ddd overlapped, J_{HH} = 0.8 Hz, 7.5 Hz, 8.7 Hz, 1H; C_9-*H*), 7.68 (d, J_{HH} = 1.4 Hz, 1H; C_3-*H*), 5.41 (s, 1H; C_{14}-*H*), 2.36 (s, 3H; C_{12}-*H*), 2.21 ppm (s, 3H; C_{16}-*H*). $^{13}C\{^1H\}$ NMR ($CDCl_3$, 75 MHz, 22 °C): δ 197.22 (C_{15}), 156.59 (C_{13}), 154.58 (C_6), 138.73 (C_4), 137.90 (C_2), 132.34 (C_{6a}), 131.90 (C_8), 129.32 (C_7), 128.73 (C_9), 126.91 (C_{6b}), 124.71 (C_{4a}), 122.47 (C_{4b}), 122.34 (C_{10}), 114.08 (q overlapped, C_1), 113.94 (q overlapped, C_3), 101.79 (C_{14}), 29.84 (C_{16}), 21.59 ppm (C_{12}), C_{11} - not observed. ^{19}F NMR ($CDCl_3$, 470 MHz, 22 °C): δ -62.25 ppm. IR (ATR): ν_{max} 3100-2800 (C-H stretch, b, w), 1634 (C=O stretch, n, s), 1579 cm^{-1} (N-H bend, n, s).

Synthesis of [Pt(*t*Bu-phenanthridine-nacac)Cl] (1): A solution of **L1** (0.501 g, 1.51 mmol) in THF (25 mL) was added to a suspension of $PtCl_2$ (0.407 g, 1.53 mmol), Ag_2O (0.179 g, 0.774 mmol) and 4 Å molecular sieves in THF (25 mL). The mixture was protected from light and heated to reflux at 60 °C for 16 h. The mixture was then cooled and filtered over Celite. The solvent was then evaporated under vacuum to leave an orange solid, which was redissolved in $CHCl_3$ and recrystallized via diffusion of diethylether vapours into the filtered solution. Yield = 0.739 g (87%). ^{1}H NMR ($CDCl_3$, 500 MHz, 22°C): δ 10.03 (s, 1H, $^3J_{PtH}$ = 39 Hz; C_6-*H*), 8.57 (d, J_{HH} = 8.3 Hz, 1H; C_{10}- *H*), 8.10 (d, J_{HH} = 1.2 Hz, 1H; C_1-*H*), 8.07 (d, J_{HH} = 7.9 Hz, 1H; C_7-*H*), 7.99 (ddd, J_{HH} = 7.5 H, 9 Hz, 1H; C_8-*H*), 7.78 (d, J_{HH} = 1.3 Hz, 1H; C_3-*H*), 7.74 (ddd, J_{HH} = 7.5 Hz, 8.5 Hz, 1H; C_9-*H*), 5.32 (s, 1H; C_{14}-H), 2.46 (s, 3H; C_{12}-H), 2.07 (s, 3H; C_{16}-H), 1.50 ppm (s, 9H; C_{17}-H). ^{13}C{^{1}H} NMR ($CDCl_3$, 125 MHz, 22 °C): 179.80 (C_{15}), 157.85 (C_{13}), 155.01 (C_6), 151.96 (C_2), 148.78 (C_4), 139.22 (C_{4a}), 133.54 (C_8), 132.36 (C_{6a}), 130.18 (C_7), 128.83 (C_9), 126.38 (C_{6b}), 125.19 (C_{4b}), 122.48 (C_{10}), 119.57 (C_3), 114.02 (C_1), 106.66 (C_{14}), 35.72 (C_{11}), 31.61 (C_{17}), 25.98 (C_{16}), 25.66 ppm (C_{12}). HRMS (APCI-TOF) m/z: $[M + H]^+$ Calcd for $C_{22}H_{23}ClN_2OPt$ 561.1198; Found 561.1085.



Synthesis of [Pt(CF_3-phenanthridinenacac)Cl] (2): An identical procedure to the synthesis of **1** was followed using **L2** (0.501 g, 1.46 mmol) in THF (25 mL) and a suspension of $PtCl_2$ (0.398 g, 1.50 mmol), Ag_2O (0.174 g, 0.752 mmol) and 4 Å molecular sieves in THF (25 mL). Dark red crystals were obtained by diffusion of diethylether vapours into a $CHCl_3$ solution of **1**. Yield = 0.722 g (86%). ^{1}H NMR ($CDCl_3$, 500 MHz,



22 °C): δ 10.20 (s, 1H, $^{3}J_{PtH}$ = 39 Hz; C_6-*H*), 8.57 (d, J_{HH} = 7.9 Hz, 1H; C_{10}-*H*), 8.33 (s, 1H; C_1-*H*), 8.18 (d, J_{HH} = 7.6 Hz, 1H; C_7-*H*), 8.10 (ddd, J_{HH} = 7.0 H, 9.4 Hz, 1H; C_8-*H*), 7.88 (s overlapping, 1H; C_3-*H*), 7.87 (ddd overlapping, 1H; C_9-*H*), 5.39 (s, 1H; C_{14}-*H*), 2.46 (s, 3H; C_{12}-*H*), 2.09 ppm (s, 3H; C_{16}-*H*). $^{13}C\{^{1}H\}$ NMR ($CDCl_3$, 125 MHz, 22 °C): δ 181.84 (C_{15}), 158.39 (C_{13}), 158.01 (C_6), 150.00 (C_4), 142.00 (C_{6b}), 134.70 (C_8), 132.10 (C_{6a}), 130.70 (C_7), 130.06 (C_{4a}), 126.49 (C_2), 125.78 (C_9), 124.74 (C_{6b}), 122.66 (C_{4b}), 122.57 (C_{10}), 116.40 (q, J_{CF} = 3.6 Hz; C_3), 115.13 (q, J_{CF} = 3.6 Hz; C_1), 107.63 (C_{14}), 25.98 (C_{16}), 25.96 ppm (C_{12}). ^{19}F NMR ($CDCl_3$, 470 MHz, 22°C): δ -62.14 ppm. HRMS (APCI-TOF) m/z: $[M + H]^+$ Calcd for $C_{19}H_{14}ClF_3N_2OPt$ 573.0446; Found 573.0327. Anal. Calcd for $Pt_1C_{19}H_{14}F_3N_2O_1Cl_1$: C, 39.77; H, 2.46. Found: C, 39.55; H, 2.79.

X-Ray Crystallography Experimental

X-ray crystal structure data was collected from multi-faceted crystals of suitable size and quality selected from a representative sample of crystals of the same habit using an optical microscope. Crystals were mounted on MiTiGen loops with data collection carried out in a cold stream of nitrogen (150 K; Bruker D8 QUEST ECO). Diffractometer manipulations were carried out using Bruker APEX3 software.[3] Structure solution and refinement was carried out using XS, XT and XL software, embedded within the Bruker SHELXTL suite.[4] For each structure, the absence of additional symmetry was confirmed using ADDSYM incorporated in the PLATON program.[5] CCDC Nos. 1959703 (**L1**) and 1959704 (**1**) contain the supplementary crystallographic data for this paper. The data can be obtained free of charge from The Cambridge Crystallographic Data Centre via www.ccdc.cam.ac.uk/structures.

Crystal structure data for L1 (CCDC Deposit #1959703): X-ray quality crystals were grown following diffusion of diethylether vapor into $CHCl_3$ at room temperature. Crystal structure parameters: $C_{22}H_{24}N_2O_1$ 332.43 g/mol, triclinic, space group $P2_1/c$; a = 13.0840(8) Å, b = 15.8238(9) Å, c = 8.6839(5) Å, $\alpha = \gamma = 90°$, β = 98.385(3)°, V = 1778.68(18) Å^3; Z = 4, ρ_{calcd} = 1.241 g cm^{-3}; crystal dimensions 0.300 x 0.100 x 0.090 mm; diffractometer Bruker D8 QUEST ECO CMOS; Mo K_α radiation, 150(2) K, $2\theta_{max}$ = 2.698 to 27.568°; 23438 reflections, 4082 independent (R_{int} = 0.0479), direct methods; absorption coeff (μ = 0.076 mm^{-1}), absorption correction semi-empirical from equivalents (SADABS); refinement (against F_o^2) with SHELXTL V6.1, 231 parameters, 0 restraints, R_1 = 0.0594 ($I > 2\sigma$) and wR_2 = 0.1499 (all data), Goof = 1.101, residual electron density 0.266/−0.245 e Å^{-3}.

Crystal structure data for 1 (CCDC Deposit #1959704): X-ray quality crystals were grown following diffusion of diethylether vapor into $CHCl_3$ at room temperature. Crystal structure parameters: $C_{23}H_{24}Cl_4N_2O_1Pt_1$ 681.33 g/mol, monoclinic, space group $P2_1/n$; a = 6.9467(3) Å, b = 18.7502(6) Å, c = 18.4435(6) Å, $\alpha = \gamma = 90°$, β = 100.0770(10)°, V = 2365.24(15) Å^3; Z = 4, ρ_{calcd} = 1.913 g cm^{-3}; crystal dimensions 0.150 x 0.059 x 0.047 mm; diffractometer Bruker D8 QUEST ECO CMOS; Mo K_α radiation, 150(2) K, $2\theta_{max}$ = 2.243 to 27.535°; 44379 reflections, 5419 independent (R_{int} = 0.0646), direct methods; absorption coeff (μ = 6.403 mm^{-1}), absorption correction semi-empirical from equivalents (SADABS); refinement (against F_o^2) with SHELXTL V6.1, 285 parameters, 0 restraints, R_1 = 0.0326 ($I > 2\sigma$) and wR_2 = 0.0677 (all data), Goof = 1.075, residual electron density 1.442/−1.154 e Å^{-3}.

Theoretical Calculations

DFT calculations were performed using ORCA version 4.1.2.[6] Tight convergence criteria (TightSCF and TightOpt) were used and the SCF and energy integration grids were set to 4 and 5, respectively (Grid4 Finalgrid5). The ligands were optimized in the gas phase using the PBE0 functional[7, 8] and the def2-TZVP basis set[9] on all atoms using the crystal structure coordinates of **L1** as starting input. The resolution of identity (split-RI-J) with chain-of-spheres approximation (RIJCOSX) was employed to speed up the calculation (Intaccx:4.01,4.01,4.34; Gridx: 1,1,2). Frequency analyses were subsequently performed to confirm all structures were at a minimum on the ground state potential energy surface, and to interpret experimental IR spectra.

Cell Cultures

A2780 and A2780cis cell lines were cultured in RPMI 1640 with L-glutamine supplemented with 10% FBS and 1% PS. SKOV-3, MDA-MB-231, A549, and HEK293 cell line were cultured in DMEM 1g/L glucose, with L-glutamine & sodium pyruvate supplemented with 10% FBS and 1% PS. MET5A cell line was cultured in M199 supplemented with 10% FBS, 1% PS, insulin, 0.1% Trace Elements B, 4 mM Hydrocortisone, and 4 ng/mL EGF. All cell lines were incubated at 37°C under an atmosphere containing 5% CO_2. Cells were passaged by trypsinization and split in a 1:5 ratio.

Cytotoxicity Assays

MTT assays were completed to determine the cytotoxicity of compounds. 100 µL of cell solution containing 2 x 10^4 cells/ mL was seeded in a 96-well plate. The plate was incubated for at 37 °C under an atmosphere containing 5% CO_2 for 24 hr to allow for adherence of cells. 50 µL of cell

medium with varying concentrations of compounds was added, and the cells were incubated for 72 hr. After 72 hr, 30 μL of 5.0 mg/mL MTT in PBS was added to the cell medium. After 2-4 h, the medium was aspirated, and 200 μL of DMSO was added to each well. DMSO content in media was kept below 0.5 % at all times. The plate was shaken for 10 minutes at room temperature. The absorbance of purple formazan was recorded at 562 nm with a BioTek ELx800 plate reader. IC_{50} values were determined using Origin software. All experiments were performed in triplicate.

Uptake

SKOV-3 cells were plated in a 6-well and at 37 °C under an atmosphere containing 5% CO_2 for 24 hr. Cells were then treated with 2 μM Pt compound for 24 hr. After incubation with Pt, cells were collected by trypsinization and washed with 1 mL PBS. Cells were counted, recollected, and resuspended in 200 μL 65% nitric acid. Samples were stored shaking at room temperature overnight. After the cell pellet was dissolved, the solutions were diluted with water and analyzed by GFAAS. All experiments were performed in triplicate.

Apoptosis

SKOV-3 cells were seeded in a 6-well plate at a concentration of 4 x 10^5 cells/well. Cells were then incubated at 37°C under an atmosphere containing 5% CO_2 for 24 hr. Cells were treated with compound for 72 hr (15 μM cisplatin, 1 μM phenanthriplatin, 1 μM **2**). Cells were harvested, the supernatant was then discarded, and cells were resuspended in 1 mL PBS. Cells were resuspended in 1X binding buffer using the FITC Annexin V Apoptosis Detection Kit 1 (BD Biosciences, Franklin Lakes, NJ, USA) reaching a concentration of 10^6 cells/ mL. 100 μL of

this cell solution was transferred to a 5 mL culture tube. Then, 5 μL of Annexin V-FITC and 10 μL propidium iodide (PI) solutions were added to cells. Cells were incubated for 15 min at RT in the dark and then brought to a final volume of 400 μL with 1X binding buffer. Cells were then analyzed with FITC and PerCP-Cy5-5 channels on FACSAria™II (BD Biosciences, Franklin Lakes, NJ, USA) and data was processed using FlowJo software.

FIGURES

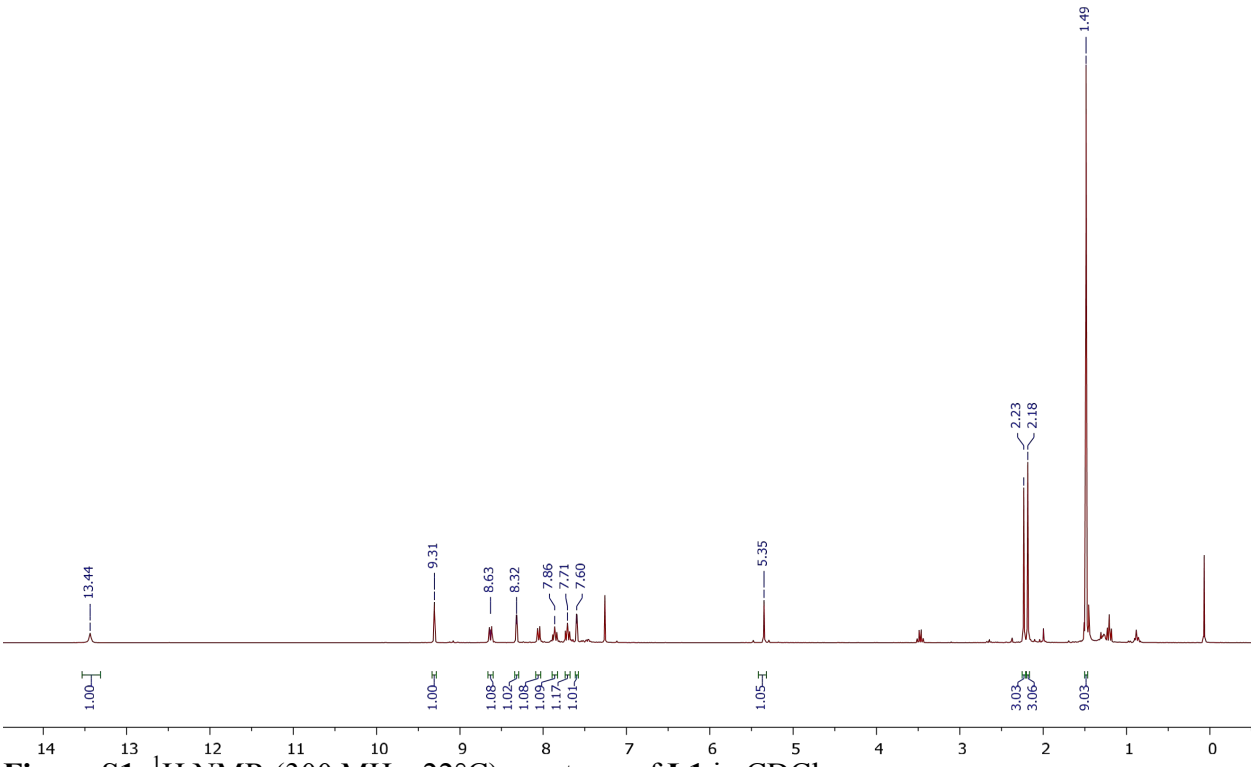


Figure S1. ^{1}H NMR (300 MHz, 22°C) spectrum of **L1** in $CDCl_3$.

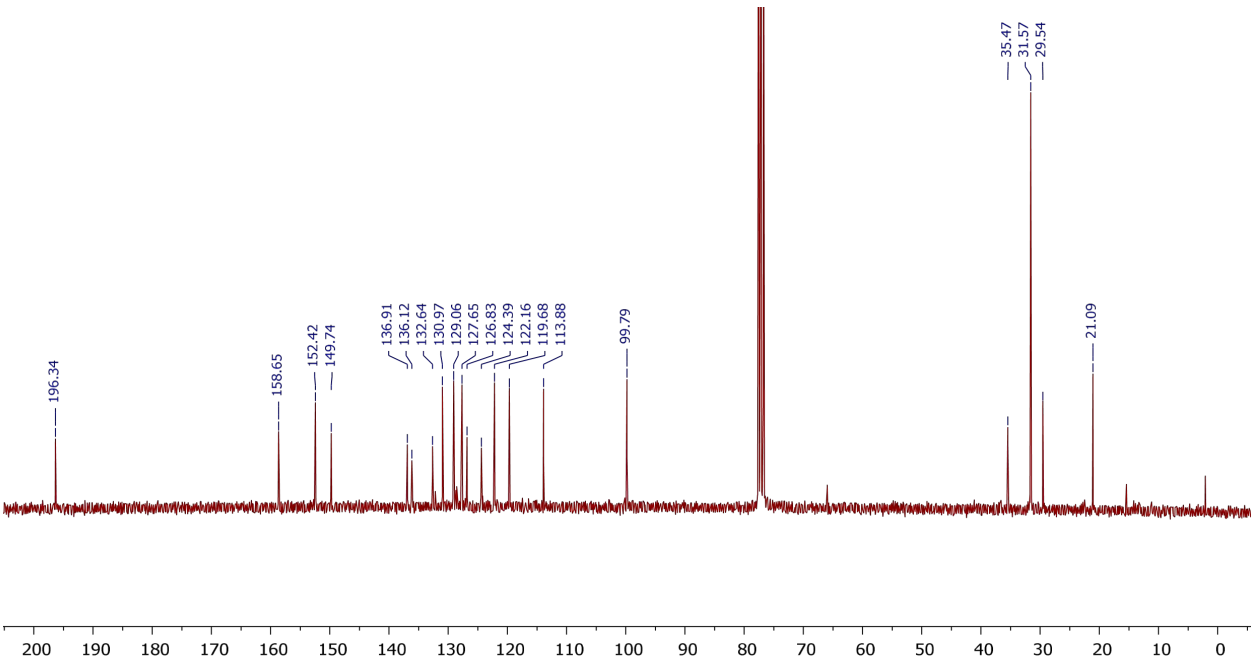


Figure S2. $^{13}C\{^{1}H\}$ NMR (75 MHz, 22°C) spectrum of **L1** in $CDCl_3$.

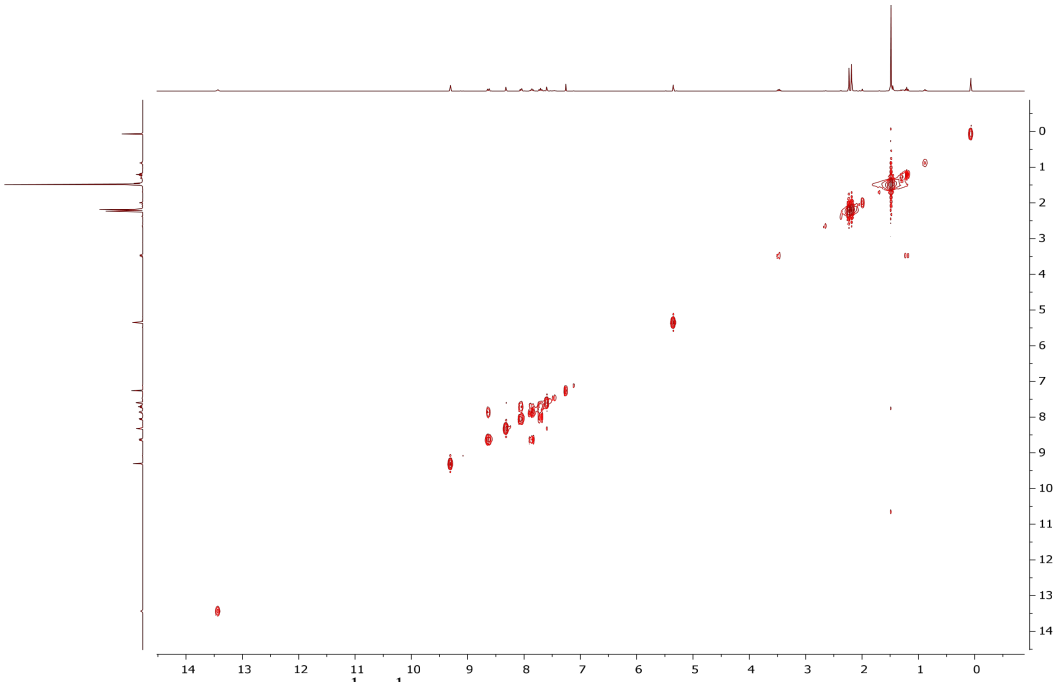


Figure S3. ^{1}H-^{1}H COSY spectrum of **L1** in $CDCl_3$.

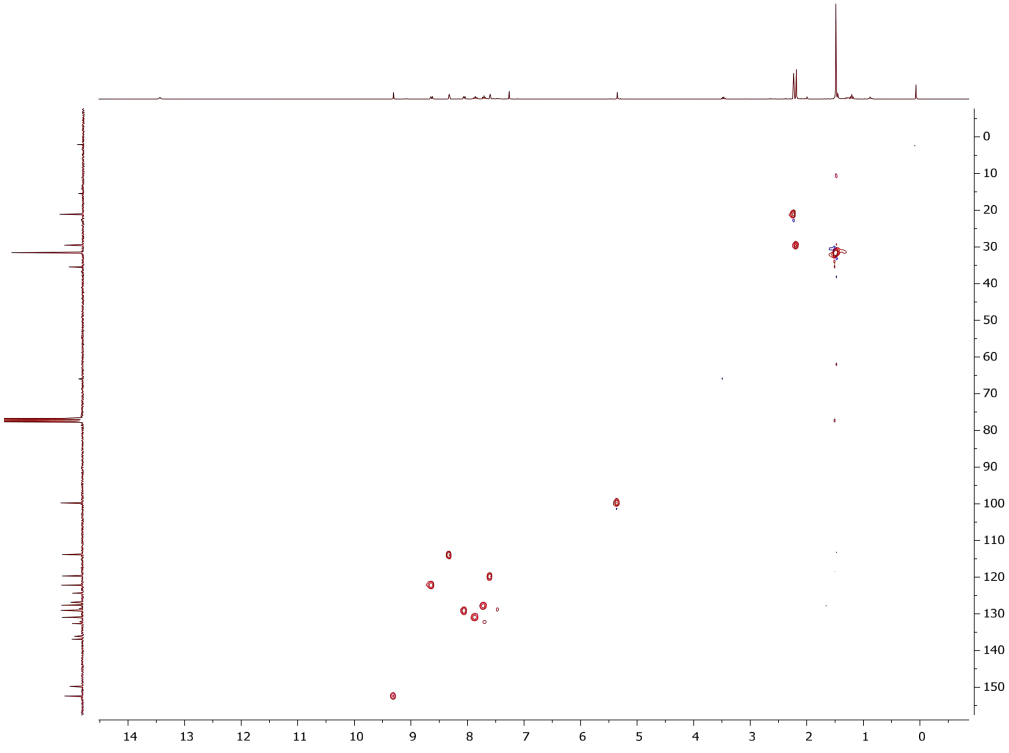


Figure S4. HSQC spectrum **L1** in $CDCl_3$.

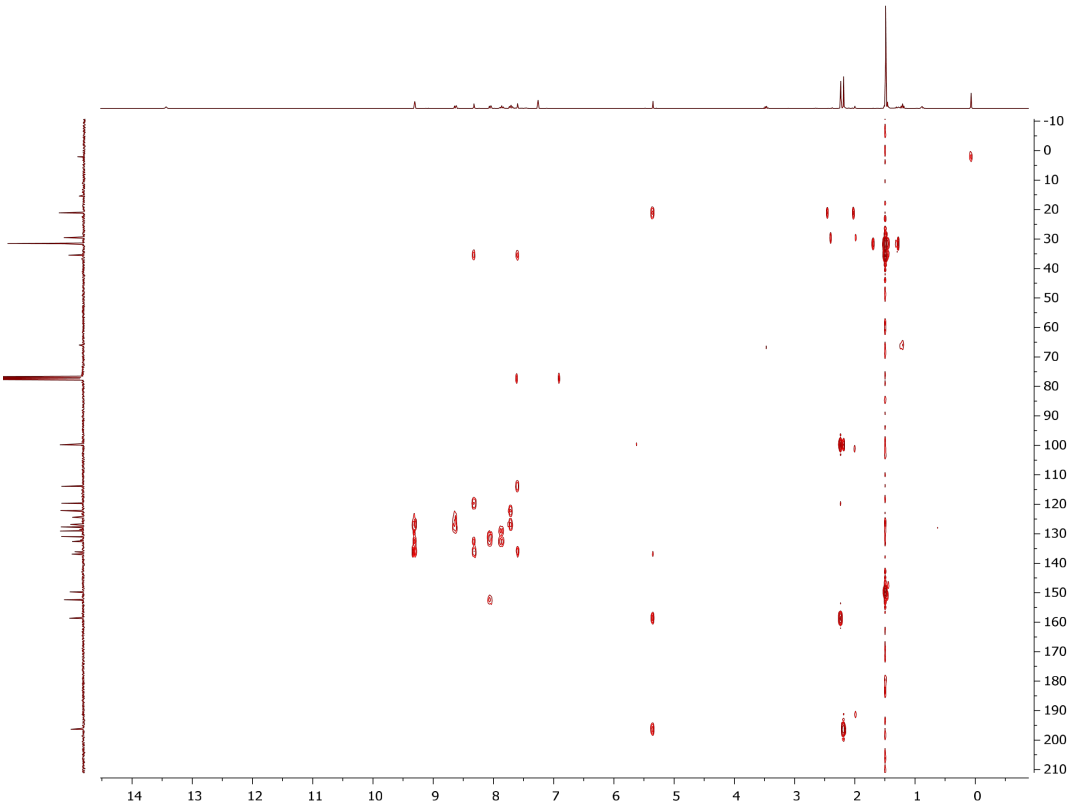


Figure S5. HMBC spectrum of **L1** in $CDCl_3$.

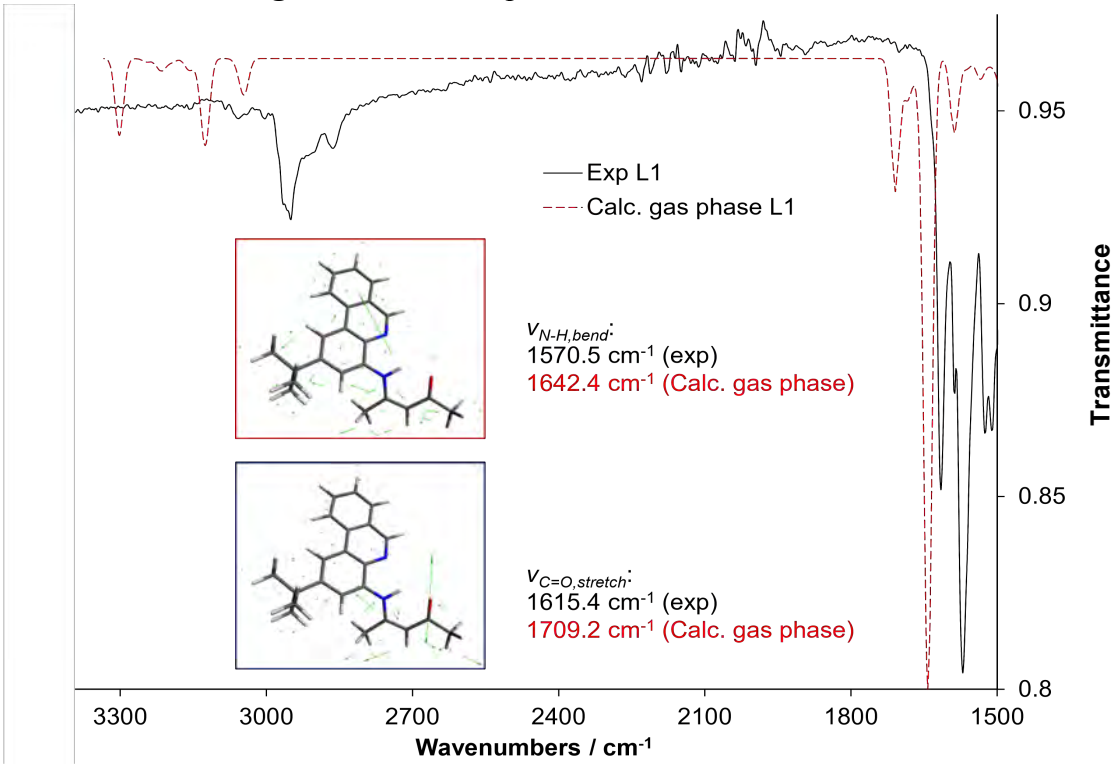


Figure S6. ATR-IR spectrum of **L1**.

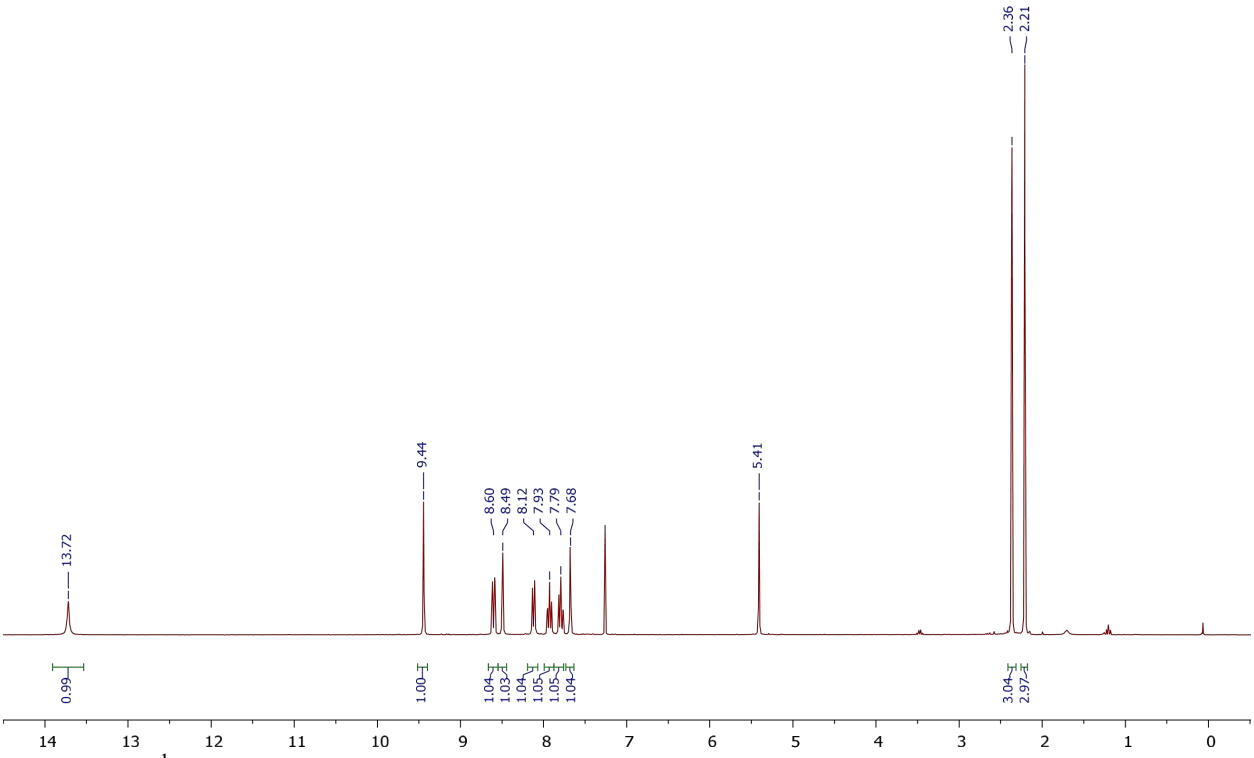


Figure S7. ^{1}H NMR (300 MHz, 22°C) spectrum of **L2** in $CDCl_3$.

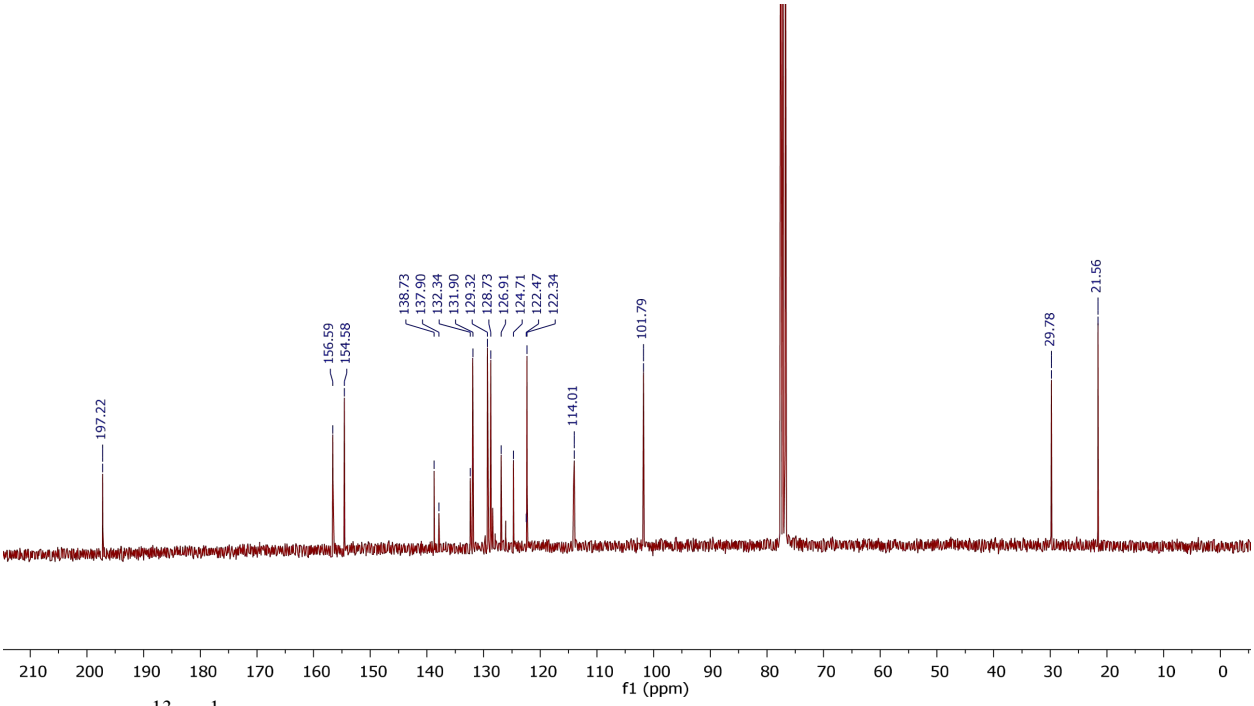


Figure S8. $^{13}C\{^{1}H\}$ NMR (75 MHz, 22°C) spectrum of **L2** in $CDCl_3$.

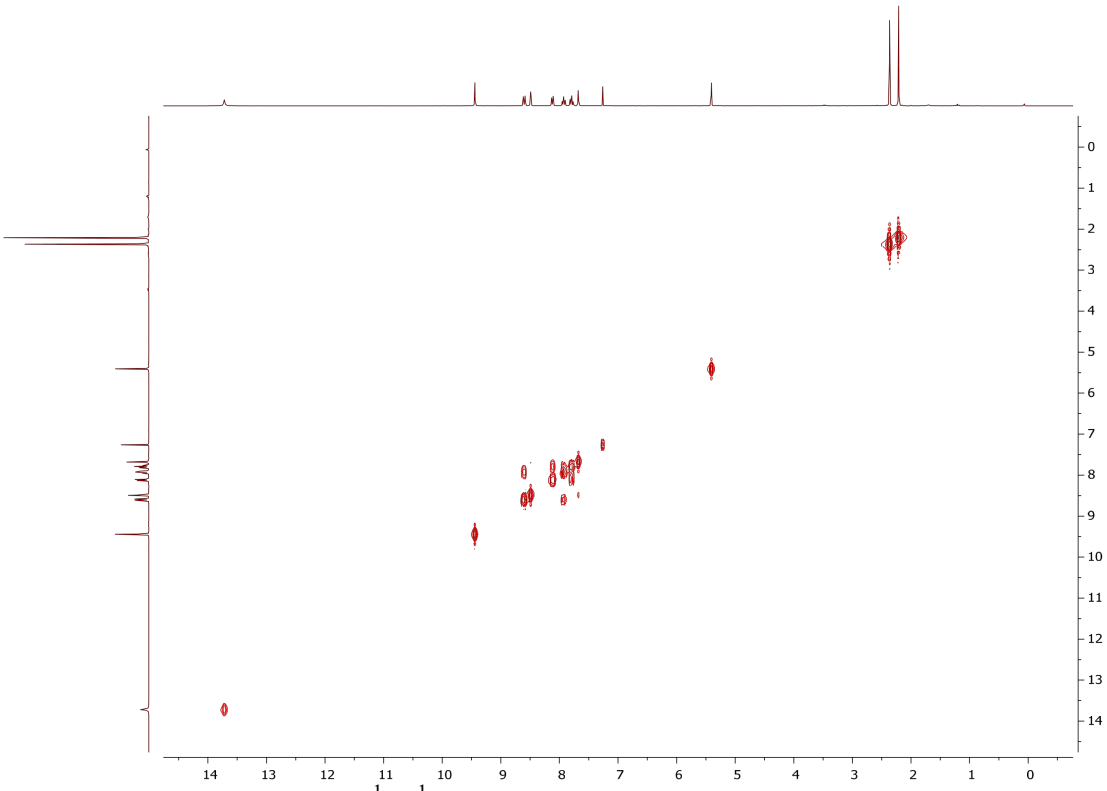


Figure S9. ^{1}H-^{1}H COSY spectrum of **L2** in $CDCl_3$.

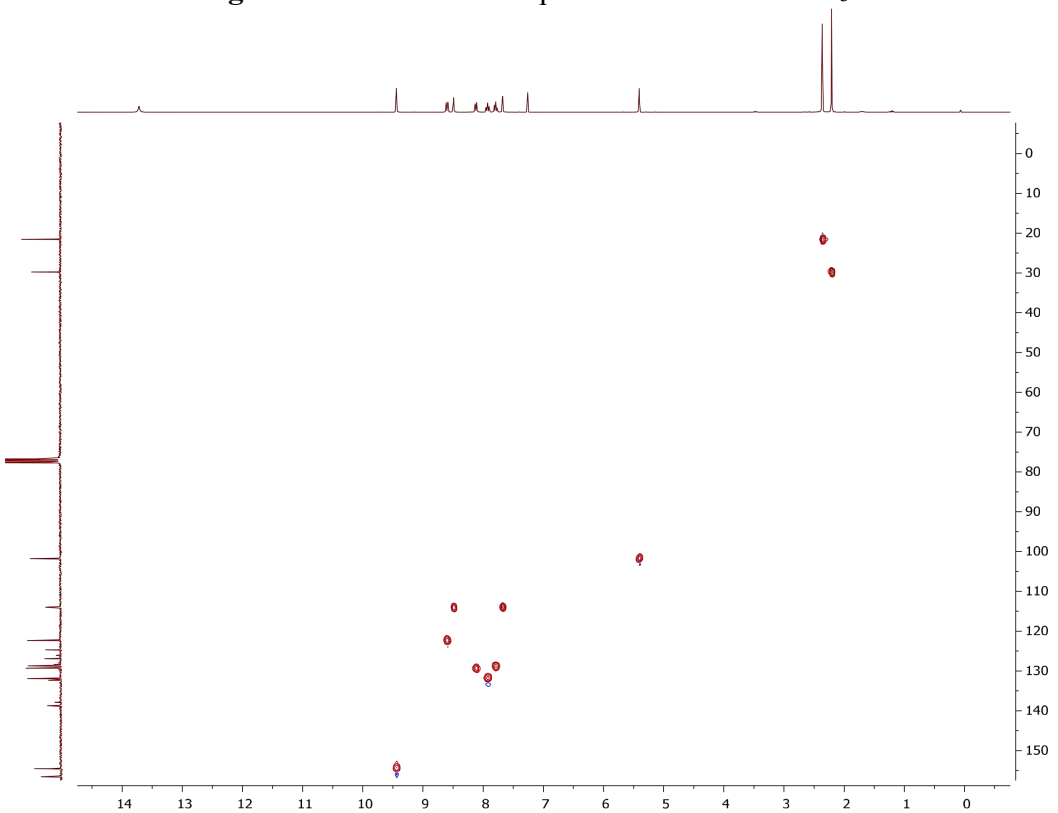


Figure S10. HSQC spectrum of **L2** in $CDCl_3$.

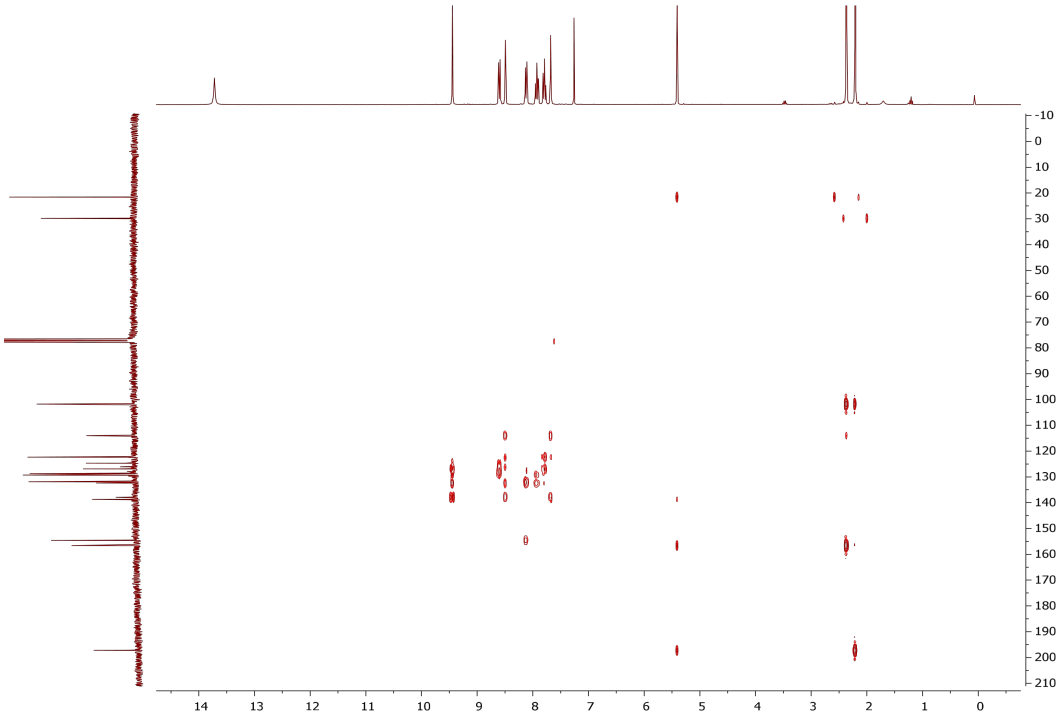


Figure S11. HMBC spectrum of **L2** in $CDCl_3$.

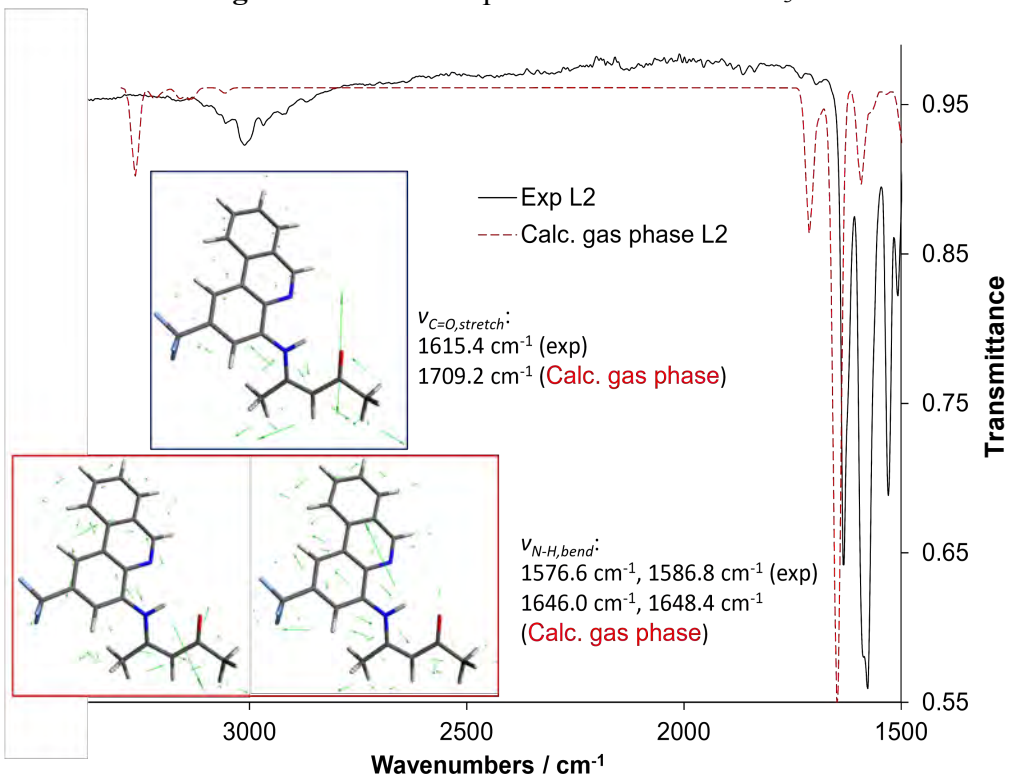


Figure S12. ATR-IR spectrum of **L2**.

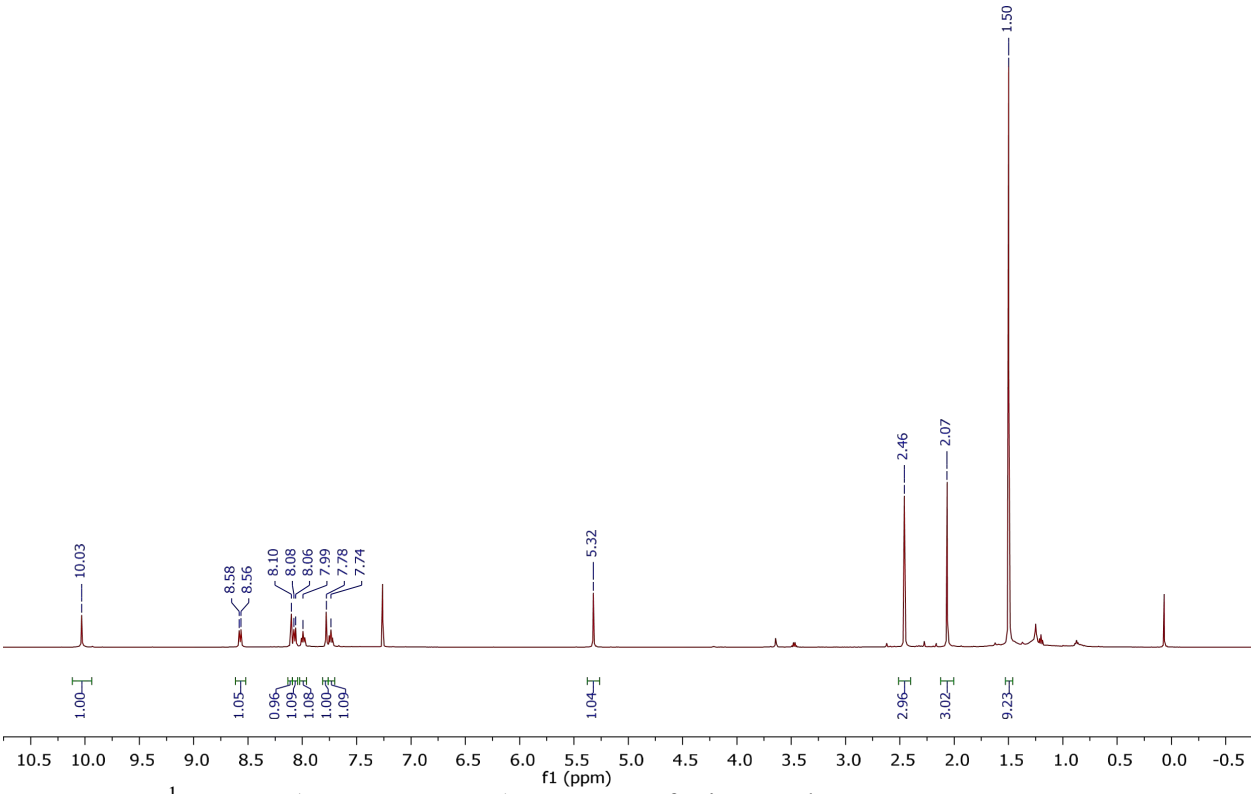


Figure S13. ^{1}H NMR (500 MHz, 22°C) spectrum of **1** in $CDCl_3$.

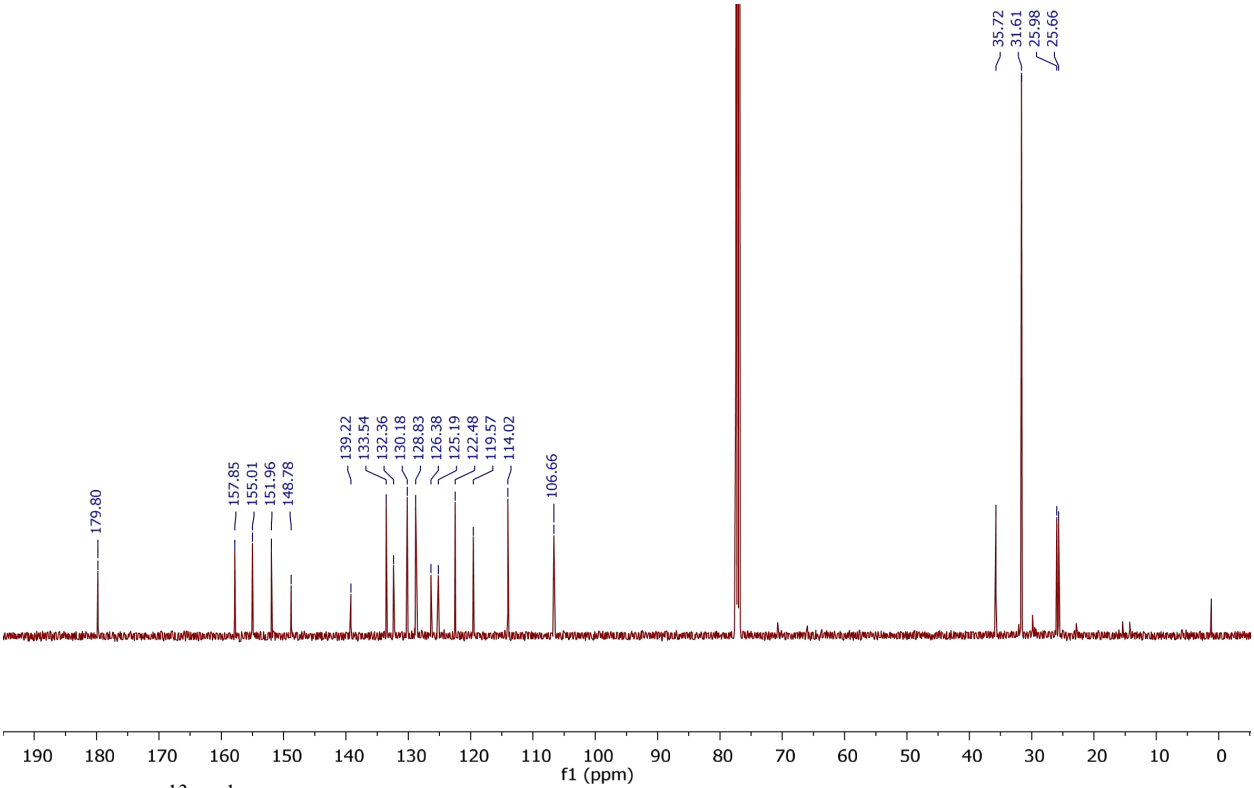


Figure S14. $^{13}C\{^{1}H\}$ NMR (125 MHz, 22°C) spectrum of **1** in $CDCl_3$.

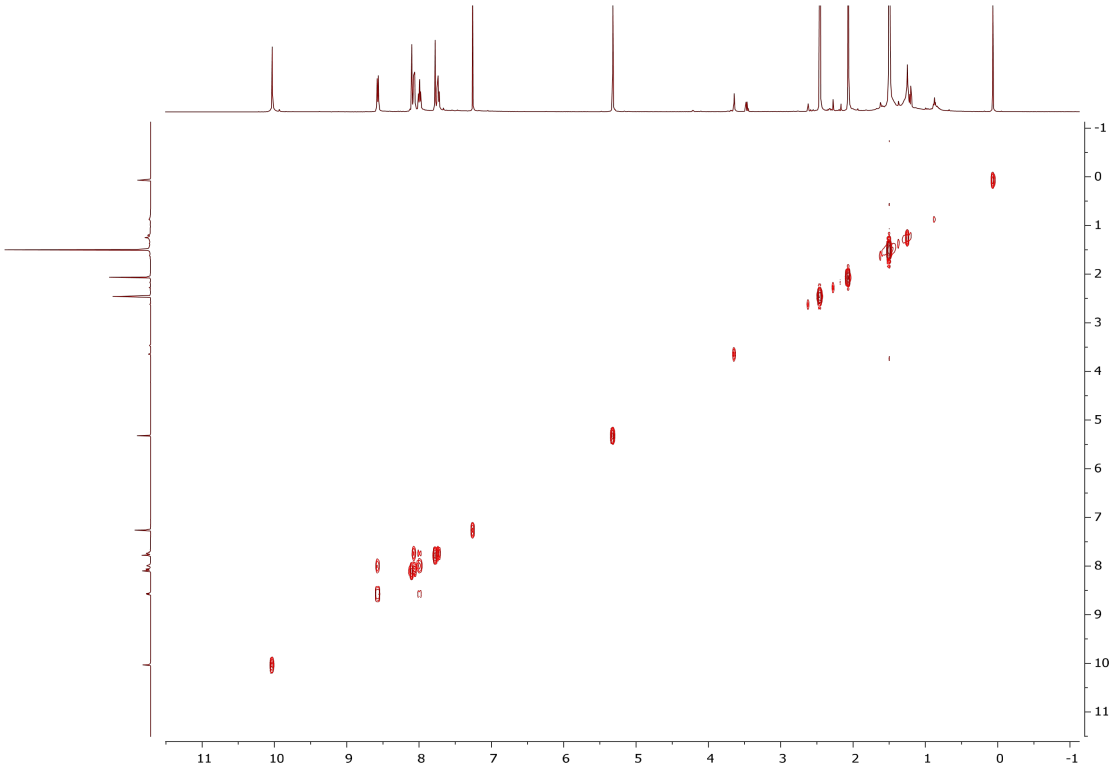


Figure S15. ^{1}H-^{1}H COSY spectrum of **1** in $CDCl_3$.

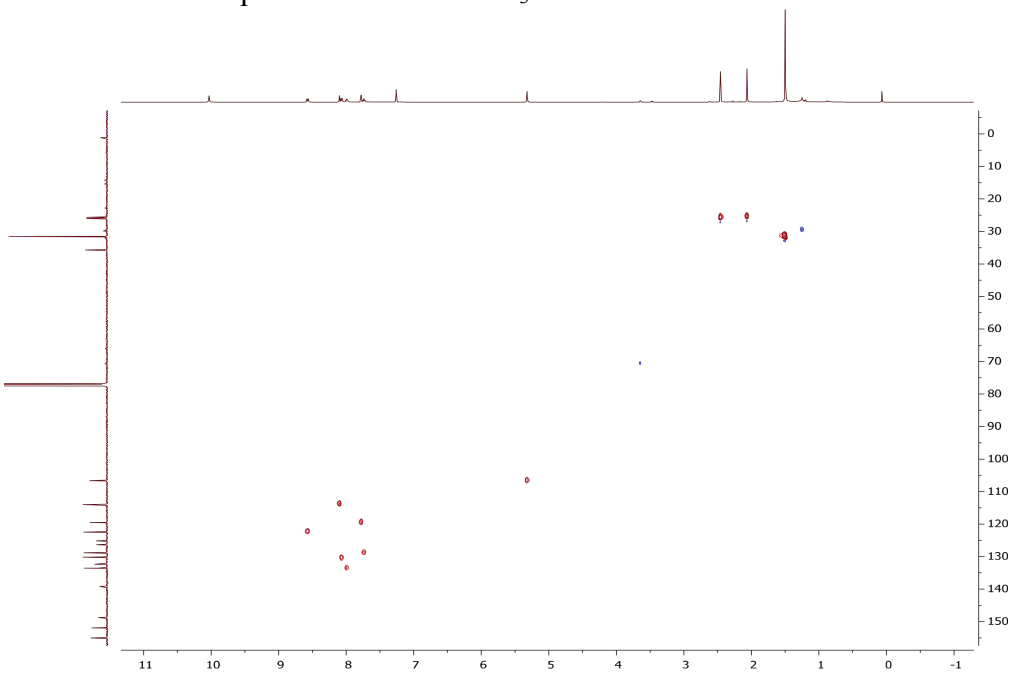


Figure S16. HSQC spectrum of **1** in $CDCl_3$.

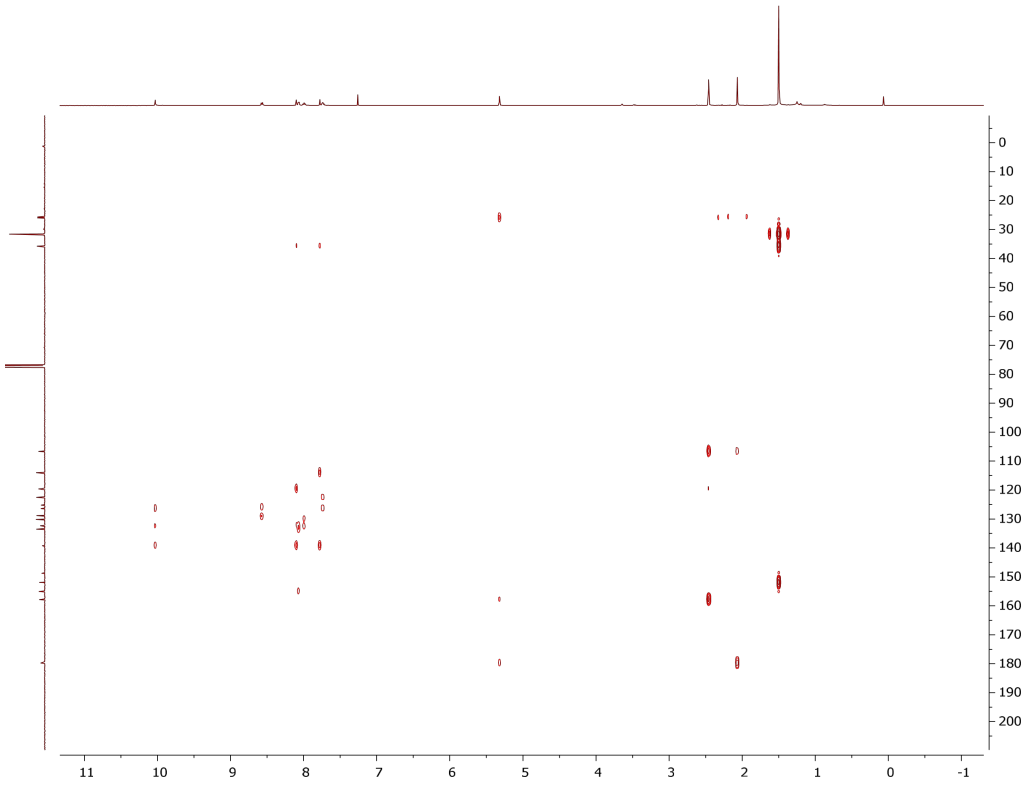


Figure S17. HMBC spectrum of **1** in $CDCl_3$.

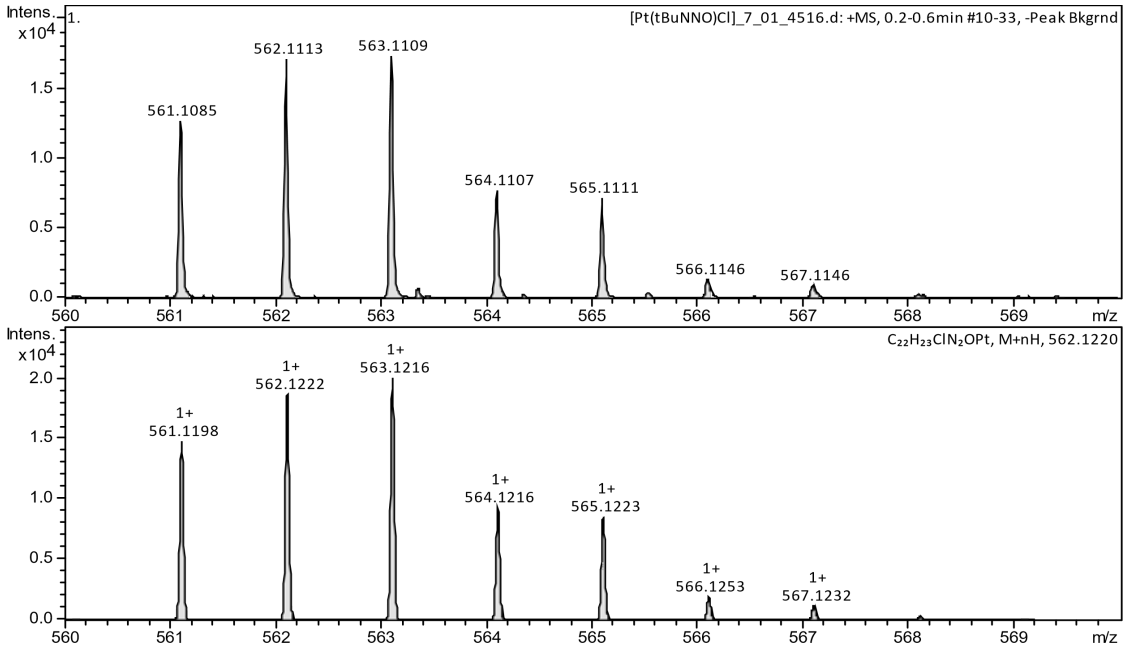


Figure S18. HRMS spectrum of **1**.

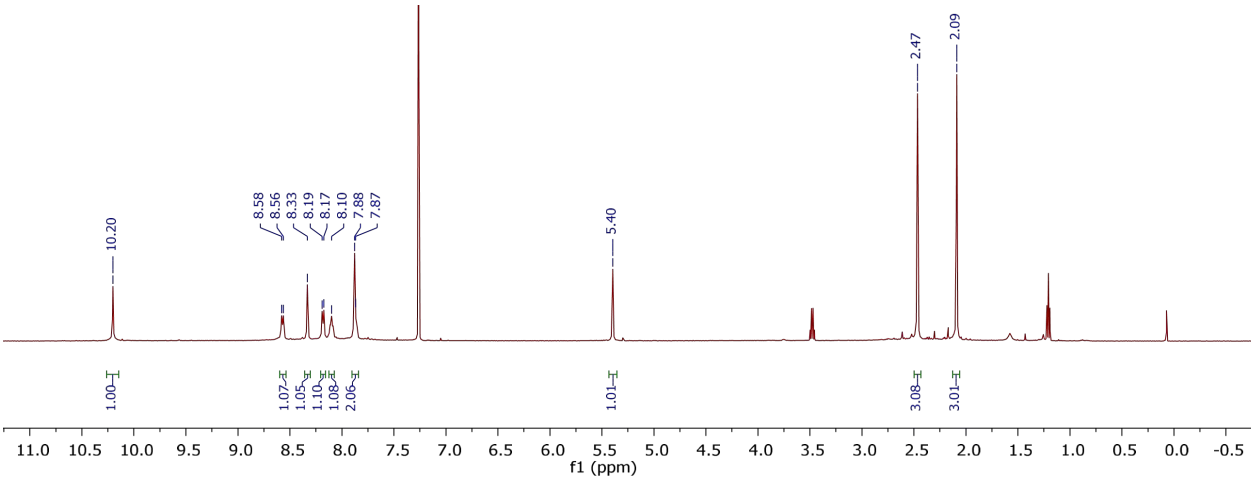


Figure S19. ^{1}H NMR (500 MHz, 22°C) spectrum of **2** in $CDCl_3$.

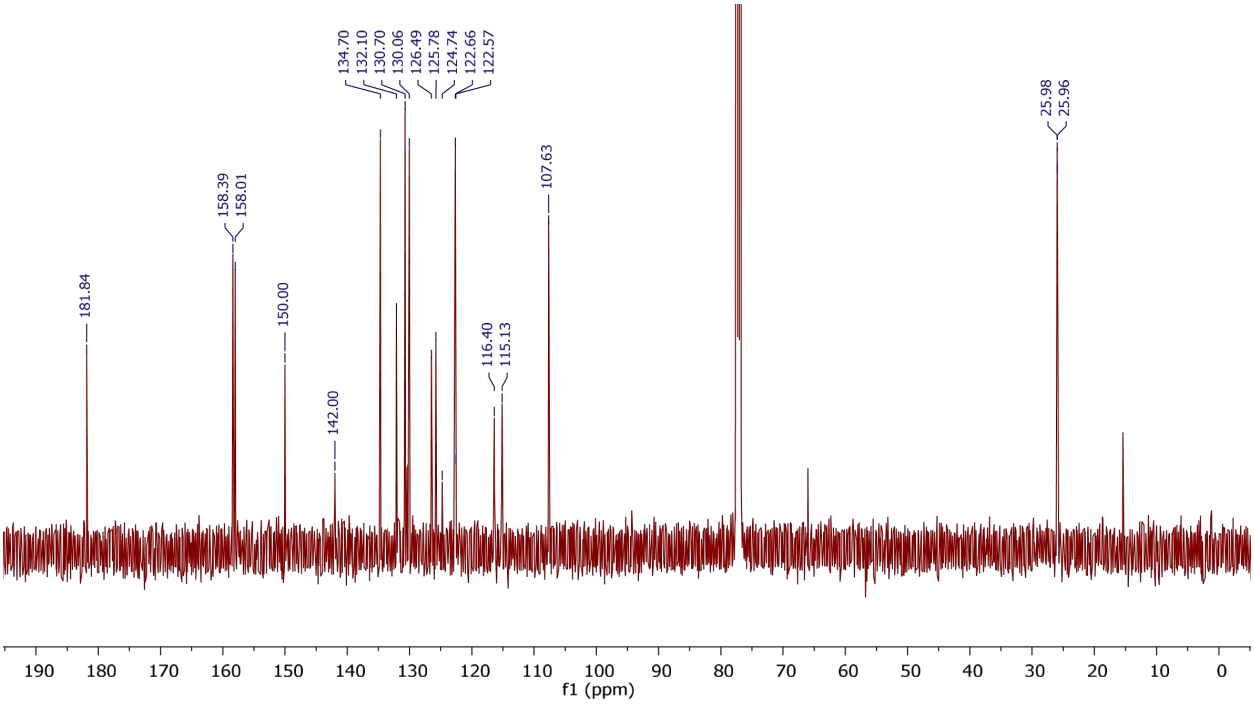


Figure S20. $^{13}C\{^{1}H\}$ NMR (125 MHz, 22°C) spectrum of **2** in $CDCl_3$.

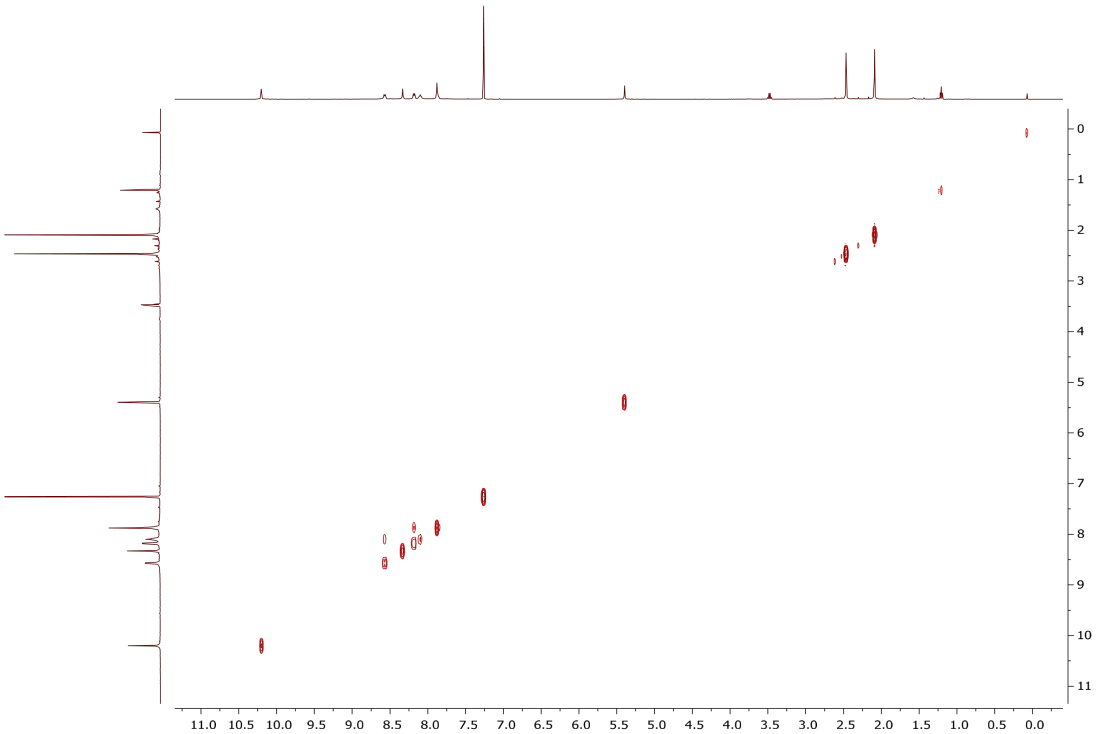


Figure S21. ^{1}H-^{1}H COSY spectrum of **2** in $CDCl_3$.

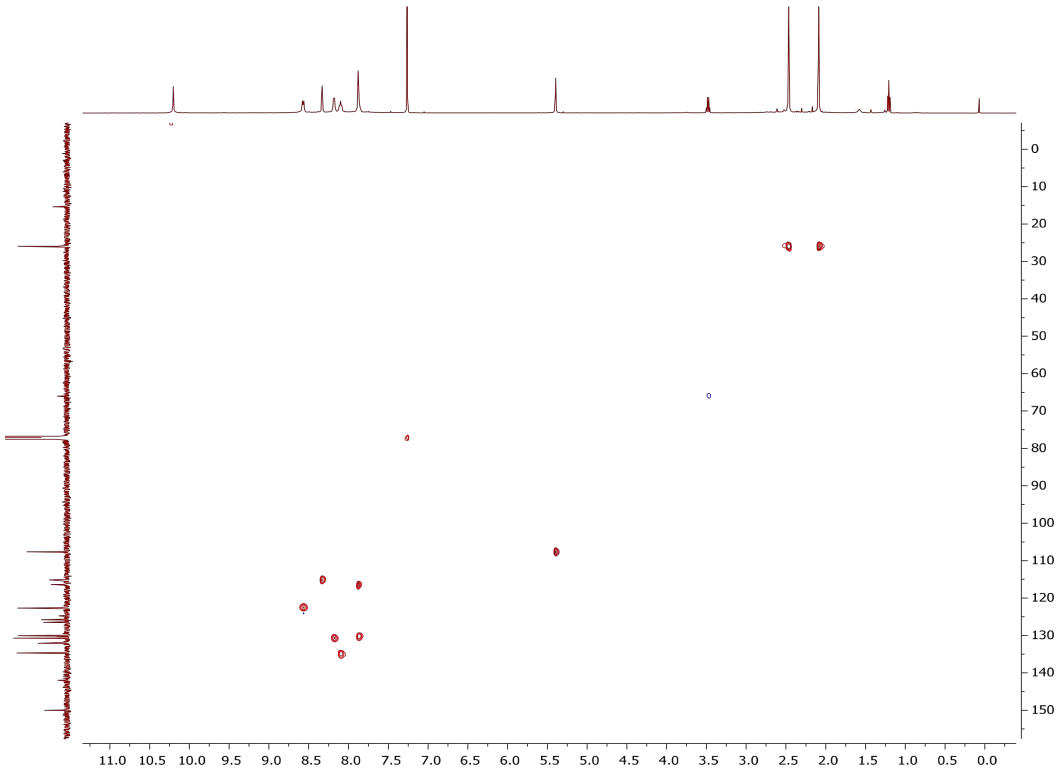


Figure S22. HSQC spectrum of **2** in $CDCl_3$.

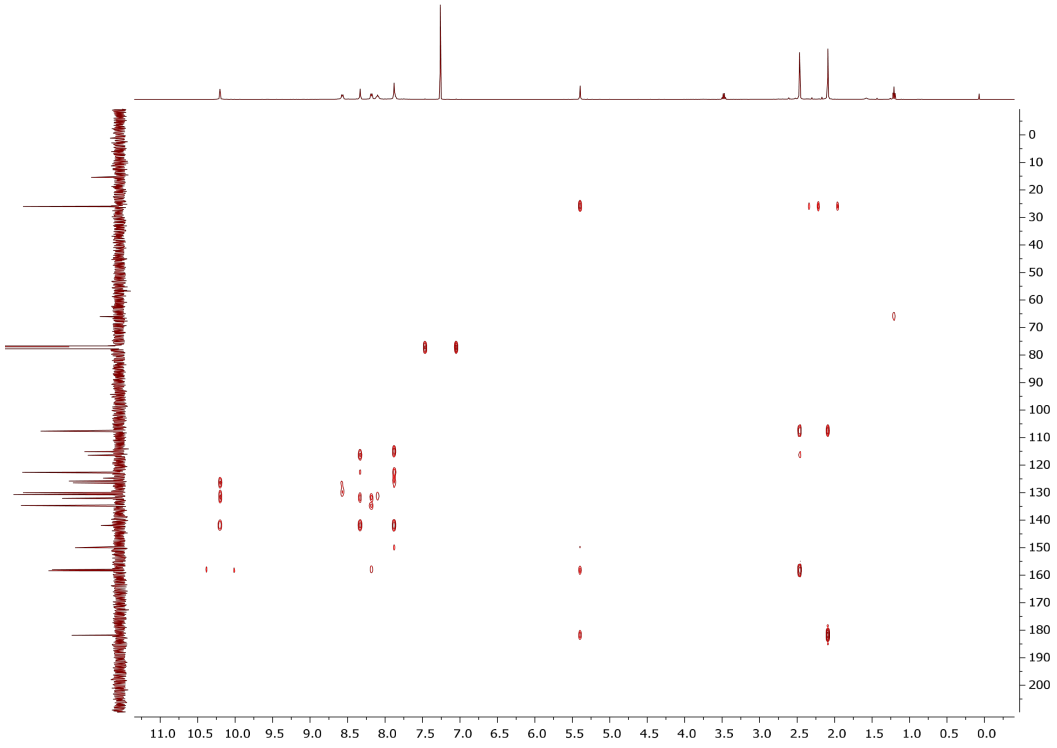


Figure S23. HMBC spectrum of **2** in $CDCl_3$.

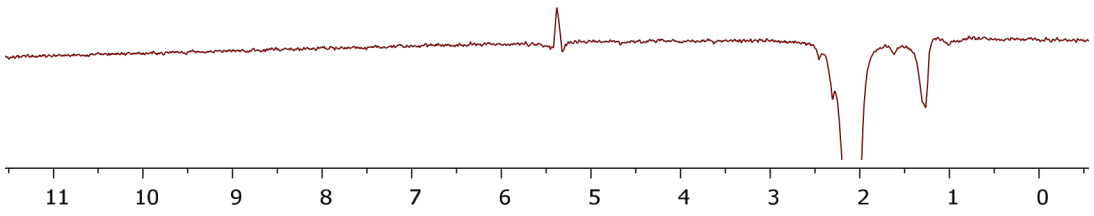


Figure S24. 1D selective gradient TOCSY (120 ms, freq: 2.07 ppm, 128 transients, 4 Hz line broadening) spectrum of **1** in $CDCl_3$.

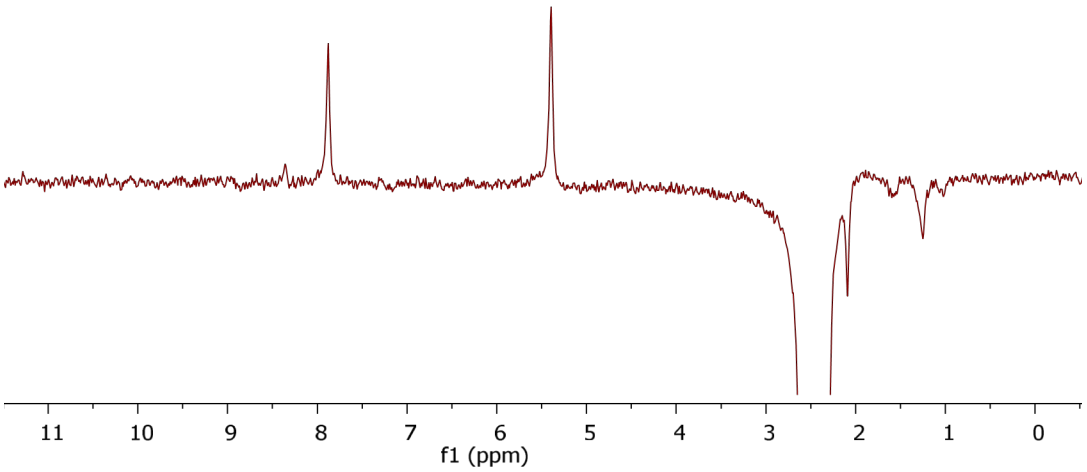


Figure S25. 1D selective gradient TOCSY (120 ms, freq: 2.46 ppm, 128 transients, 4 Hz line broadening) spectrum of **2** in $CDCl_3$.

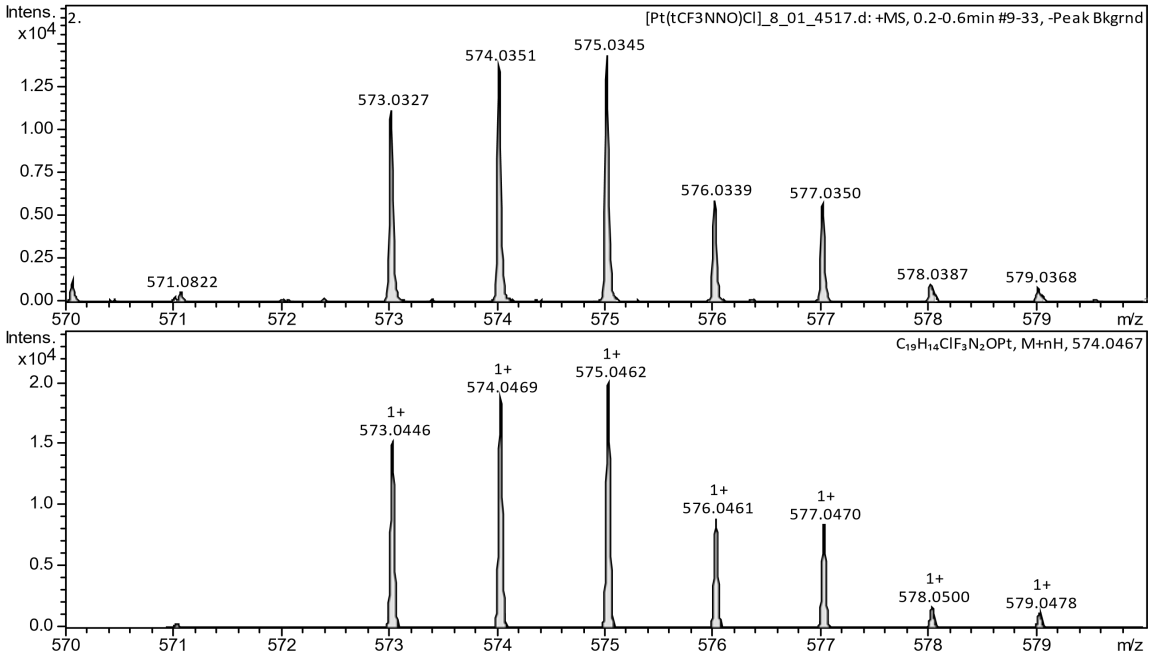


Figure S26. HRMS spectrum of **2**.

Table S1. MTT cytotoxicity assay data A2780 and A2780cis cell lines.

Compound	A2780 IC_{50} (μM)	A2780cis IC_{50} (μM)
cisplatin	1.41 ± 0.54	32.94 ± 8.82
1	2.81±0.168	4.57±1.41
2	0.48 ± 0.17	0.55 ± 0.22
L2	7.22±2.07	20.1±7.17
4-amino-(2-*tert*-butyl)phenanthridine	8.38±2.83	20.5±6.07
4-amino-(2-trifluoromethyl)phenanthridine	19.3±12.1	>100 μM

Table S2. Cellular uptake of cisplatin, phenthriplatin, and **2** in SKOV3 ovarian cancer cells ([Pt] = 2 μM, 24 h at 37 °C, 5% CO_2).

Compound	log([Pt]) Conc (pmol/million cells)
cisplatin	2.12 ± 0.129
phenanthriplatin	2.88 ± 0.023
2	4.09 ± 0.138

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