Electronic Supplementary Material (ESI) for Chemical Communications.

Excitation modulation of Eu:BPEPC based complexes as low-energy excitation reference standards for circularly polarised luminescence (CPL).

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Reagents

All reagents were purchased and used as received. Analytical solvents were purchased from Fisher Scientific and Sigma Aldrich and were HPLC grade. Anhydrous solvents were freshly distilled over the appropriate drying agent and stored under argon in a septum-capped bottle or purchased and used as received. Water was purified by the 'Purite$_{STILL}$plus' system, with conductivity of ≤ 0.04 µS cm^{-1}. Air sensitive reactions were carried out under an atmosphere of argon using Schlenk-line techniques.

Analytical Methods

Thin layer Chromatography were carried out on neutral aluminium silica plates (Merck 5554) or neutral aluminium oxide plates (Merck 5550) and visualised under UV irradiation (254/365 nm). Preparative column chromatography was performed using silica gel (Merck Silica Gel 60, 230-400 mesh) or neutral aluminium oxide (Merck 90, 70-320 mesh).

NMR. Proton (^{1}H) and carbon (^{13}C) NMR spectra were recorded on Bruker Avance III-HD-400 spectrometers with operating frequencies of 399.95 MHz or 400.07 MHz for ^{1}H and 100.57 MHz or 100.60 MHz for ^{13}C or Bruker Neo-400 spectrometer with operating frequencies of 400.20 MHz for ^{1}H and 100.63 MHz for ^{13}C. The operating temperature of the spectrometers (usually 295 K) was measured with the aid of an internal calibration solution of ethylene glycol.

Melting point were recorded using a Gallenkamp (Sanyo) apparatus and are uncorrected.

Reverse phase preparative HPLC purification was performed at 295 K using a Shimadzu system consisting of a Degassing Unit (DGU-20A$_{5R}$), a Prominence Preparative Liquid Chromatograph (LC-20AP), a Prominence UV/Vis Detector (SPD-20A) and a Communications Bus Module (CBM-20A). An XBridge C18 OBD 19 x 100 mm, i.d. 5 µM column was used with a flow rate of 17 mL/min (prep). The solvent was Ammonium bicarbonate buffer (25 mM, pH = 7.55) / Acetonitrile (isocratic 10% acetonitrile in buffer (3 min), linear gradient to 100% acetonitrile (10 min), isocratic 100% acetonitrile (5 min).

Mass spectrometry analysis were performed on a QToF Premier equipped with an Acquity UPLC (Waters Corp.). Reverse phase gradient separation was achieved using an Acquity UPLC BEH C18 column 1.7 µm (2.1 mm x 100 mm) (Waters Corp.). The 0.4 mL/min solvent gradient ran from 100% water containing 0.1% formic acid to 100% acetonitrile over 5 minutes. Positive ions from the electrospray ion source were recorded as a full MS spectrum. The desired precursor ion was mass selected by the quadrupole with an isolation window that transmitted the full isotopic envelope. A reference spray provided a 'lock mass' that enable a calibration correction for accurate mass determination of the MS data.

Optical measurements

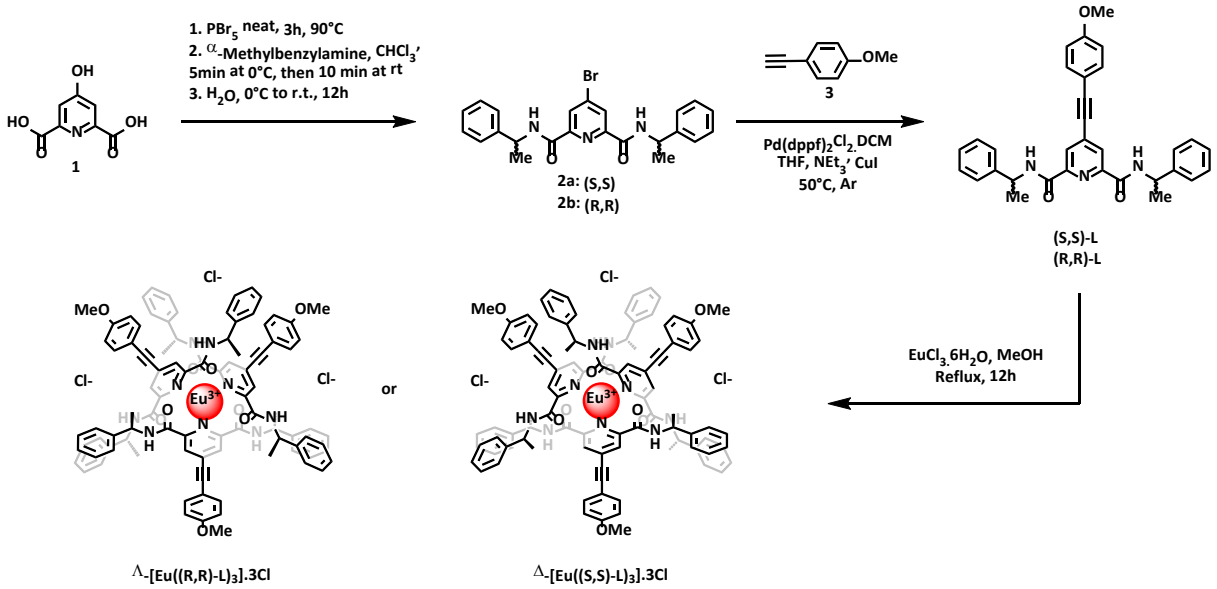
Absorption spectroscopy UV/Vis absorption measurements were recorded using a Perkin-Elmer Lambda 900 absorption spectrophotometer, using matched quartz cells.

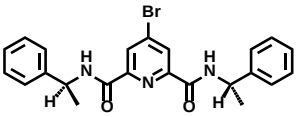
Luminescence. Emission spectra and luminescence quantum yields f were measured using a Horiba-Jobin Yvon Fluorolog-3® spectrofluorimeter coupled with a Quanta-ϕ integrating sphere. The steady-state luminescence was excited by unpolarised light from a 450W xenon CW lamp and detected at an angle of 90° for diluted solution measurements (10 mm quartz cell) by a red-sensitive Hamamatsu R928 photomultiplier tube. Spectra were reference corrected for both the excitation source light intensity variation (lamp and grating) and the emission spectral response (detector and grating). Phosphorescence lifetimes (> 30 μs) were obtained by pulsed excitation using a FL-1040 UP Xenon Lamp. Luminescence decay curves were fitted by least-squares analysis using Origin®.

Ligand **(S,S)-BPEPC**, **(R,R)-BPEPC** and associated Europium complexes were prepared according to the described procedure.[1] Chelidamic acid **1**, (S)-(-)-(α)-methylbenzylamine and (R)-(+)-(α)-methylbenzylamine (both with 99+% e.e.) and 1-ethynyl-4-methoxybenzene **3** were purchased to Sigma Aldrich.

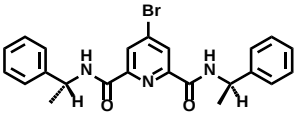
Circularly polarised luminescence: CPL measurements were conducted on a custom built spectrometer[1] (in Durham University) consisting of a laser-driven light source (Energetiq EQ–99 LDLS, spectral range 170 to 2100 nm) coupled to an Acton SP2150 monochromator (600 g/nm, 300 nm Blaze) that allows excitation wavelengths to be selected with a 6 nm FWHM band–pass. The collection of the emitted light was facilitated (90° angle set up, 1 cm path length quartz curette) by a Lock-In Amplifier (Hinds Instruments Signaloc 2100) and Photoelastic Modulator (Hinds Instruments PEM–90). The differentiated light was focused onto an Acton SP2150 monochromator (1200 g/nm, 500 nm Blaze) equipped with a high sensitivity cooled Photo Multiplier Tube (H10723–20 Extended red–multialkali PMT based photosensor (5V)). Spectra were recorded using a 5 spectral average sequence in the range of 570–720 nm with 0.5 nm spectral intervals and 500 μs integration time. The recorded CPL spectrum underwent a 25% Fourier transformation smoothening protocol using Origin 8.0 Software (Origin Labs) to enhance appearance (all calculations were carried out using raw spectral data).

Synthesis of the optical isomers of the Europium complexes of tris-(4-((4-methoxyphenyl)ethynyl)-N,N'-bis(1-phenylethyl)-pyridine-2,6-dicarboxamide)



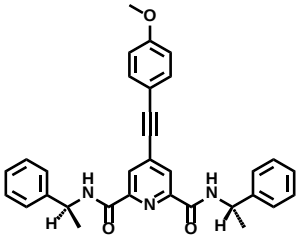


Synthesis of 4-bromo-(S,S)-N^2,N^6-bis(1-phenylethyl)pyridine-2,6-dicarboxamide 2a. To a round bottom flask under argon were added chelidamic acid **1** (0.20 g, 1.1 mmol) and neat Phosphorus pentabromide (1.41 g, 3.3 mmol). The reaction mixture was stirred at 90°C for 3 hours. The melted mixture was then cooled down to 0°C using ice bath and was diluted in anhydrous $CHCl_3$ (10 mL). (S)-(-)-(α)-methylbenzylamine (99+% e.e., 0.57 mL, 4.4 mmol) was added slowly at 0°C and the reaction mixture was stirred at 0°C during 5 minutes and then 10 min at room temperature. $CHCl_3$ was distilled off and the residual oil was poor into cold water (100 mL) and left under stirring for 12 hours. CH_2Cl_2 (100 mL) was added and the aqueous layer was extracted and washed with CH_2Cl_2 (2 x 50 mL). Combined organic layer were dried other Na_2SO_4, filtered and concentrated to dryness. Column chromatography (SiO_2, CH_2Cl_2 100% to 1% MeOH in CH_2Cl_2) yielded pure compound **2a** (0.38 mg, 77%) was as a white solid. R_f = 0.25 (2% MeOH in CH_2Cl_2); m.p. 165-167°C; UPLC (water, 5 to 95 % CH_3CN over 5 min, 0.1% formic acid) t_r = 3.05 min; ^{1}H NMR (298 K, 400 MHz, $CDCl_3$) δ_H 8.36 (s, 2H), 7.94 (d, 2H, J = 8.0 Hz), 7.30 – 7.10 (m, 10H), 5.17 (dq, 2H, J = 8.0 Hz, J = 7.0 Hz), 1.41 (d, 6H, J = 7.0 Hz); ^{13}C NMR (298 K, 100 MHz, $CDCl_3$) δ_C 161.6, 149.8, 142.6, 136.3, 128.8, 128.6, 127.6, 126.1, 49.1, 21.6; (HRMS+) *m/z* 452.0977 $[M+H]^+$ ($C_{23}H_{23}N_3O_2Br$ requires 452.0974); elemental analysis calculated for (%): C=61.07, H=4.90, N=9.29, measured: C=60.45, H=4.90, N=9.10

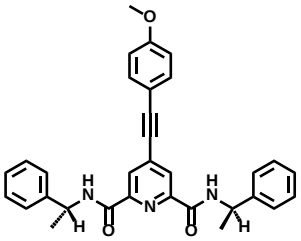


Synthesis of 4-bromo-(R,R)-N^2,N^6-bis(1-phenylethyl)pyridine-2,6-dicarboxamide 2b. This compound was synthesised following the same procedure as **2a** using (R)-(+)-(α)-methylbenzylamine (99+% e.e.) to obtain compound **2b** (0.42 mg, 84%). R_f = 0.25 (2% MeOH in CH_2Cl_2); m.p. 165-167°C; UPLC (water, 5 to 95 %

CH_3CN over 5 min, 0.1% formic acid) t_r = 3.04 min; ^1H NMR (298 K, 400 MHz, $CDCl_3$) δ_H 8.37 (s, 2H), 7.98 (d, 2H, J = 8.0 Hz), 7.30 – 7.10 (m, 10H), 5.19 (dq, 2H, J = 8.0 Hz, J = 7.0 Hz), 1.42 (d, 6H, J = 7.0 Hz); ^{13}C NMR (298 K, 100 MHz, $CDCl_3$) δ_C 161.6, 149.8, 142.6, 136.3, 128.8, 128.6, 127.6, 126.1, 49.1, 21.6; (HRMS+) *m/z* 452.0968 $[M+H]^+$ ($C_{23}H_{23}N_3O_2Br$ requires 452.0974); elemental analysis calculated for (%): C=61.07, H=4.90, N=9.29, measured: C=61.04, H=4.89, N=9.28.



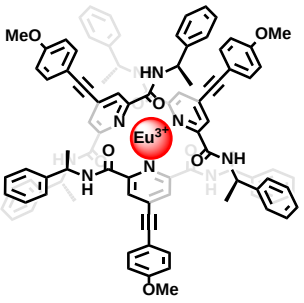
Synthesis of 4-((4-methoxyphenyl)ethynyl)-(S,S)-N^2,N^6-bis(1-phenylethyl)pyridine-2,6-dicarboxamide (S,S)-L. Compound **2a** (100 mg, 0.22 mmol) and 1-Ethynyl-4-methoxybenzene **3** (36 µL, 0.28 mmol) were dissolved in dry THF (2 mL) then NEt_3 (160 µL, 1.14 mmol) was added. The solution was degased 3 times using freeze thaw cycles and $Pd(dppf)Cl_2.CH_2Cl_2$ (21 mg, 0.03 mmol) and CuI (12 mg, 0.06 mmol) were added and the solution was degased once more. The reaction mixture was stirred at 60°C under argon for 12 h. After complete conversion, the reaction mixture was concentrated to dryness and the dark brown residue was dissolved in CH_2Cl_2 (20 mL) and water (20 mL). Aqueous layer was extracted and washed with CH_2Cl_2 (3 × 10 mL). Combined organic layers were dried over Na2SO4, filtered and concentrated to dryness. The residual oil was purified by column chromatography (SiO_2, 25% EtOAc in Hexane to 50% EtOAc in Hexane) to give pure ***(S,S)-L*** (68 mg, 62 %) as a pale yellowish solid. R_f (Hexane/EtOAc, 75/25) = 0.11; m.p. 233-234°C; UPLC (water, 5 to 95 % CH_3CN over 5 min, 0.1% formic acid) t_r = 3.45 min; ^1H NMR (298 K, 400 MHz, $CDCl_3$) δ_H 8.36 (s, 2H), 7.82 (d, 2H, J = 8.0 Hz), 7.51 (dm, 2H, J = 9.0 Hz), 7.41 – 7.28 (m, 10H), 6.91 (dm, 2H, J = 9.0 Hz), 5.32 (dq, 2H, J = 8.0 Hz, J = 7.0 Hz), 3.84 (s, 3H), 1.61 (d, 6H, J = 7.0 Hz); ^{13}C NMR (298 K, 100 MHz, $CDCl_3$) δ_C 162.2, 160.7, 148.9, 142.9, 135.5, 133.9, 128.9, 127.7, 126.6, 126.1, 114.3, 113.6, 97.2, 85.2, 55.4, 49.2, 21.9; (HRMS+) *m/z* 504.2301 $[M+H]^+$ ($C_{32}H_{30}N_3O_3$ requires 504.2287); elemental analysis calculated for (%): C=76.32, H=5.80, N=8.34, measured: C=74.03, H=5.68, N=8.09.



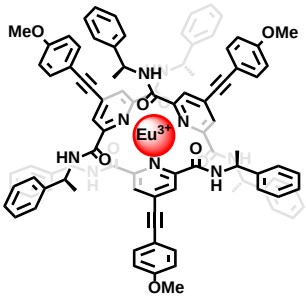
Synthesis of 4-((4-methoxyphenyl)ethynyl)-(R,R)-N^2,N^6-bis(1-phenylethyl)pyridine-2,6-dicarboxamide (R,R)-L. Ligand ***(R,R)-L*** (60 mg, 55%) was synthesised following the procedure described for ***(S,S)-L***. R_f (Hexane/EtOAc, 75/25) = 0.12; m.p. 233-234°C; UPLC (water, 5 to 95 % CH_3CN over 5 min, 0.1% formic acid) t_r = 3.66 min; ^1H NMR (298 K, 400 MHz, $CDCl_3$) δ_H 8.36 (s, 2H), 7.85 (d, 2H, J = 8.0 Hz), 7.51 (apparent d, 2H, J = 9.0 Hz), 7.40 – 7.28 (m, 10H), 6.91 (apparent d, 2H, J = 9.0 Hz), 5.31 (apparent p, 2H, J = 7.0 Hz), 3.84 (s, 3H), 1.60 (d, 6H, J = 7.0 Hz); ^{13}C NMR (298 K, 100 MHz, $CDCl_3$) δ_C 162.2, 160.7, 148.9, 142.9, 135.5, 133.8, 128.9, 127.7, 126.6, 126.1, 114.3, 113.6, 97.2, 85.2, 55.4, 49.1, 21.9; (HRMS+) *m/z* 504.2291 $[M+H]^+$ ($C_{32}H_{30}N_3O_3$ requires

504.2287); elemental analysis calculated for (%): C=76.32, H=5.80, N=8.34, measured: C=75.06, H=5.76, N=8.28.

Europium complex of tris-4-((4-methoxyphenyl)ethynyl)-N^2,N^6-bis(1-phenylethyl)pyridine-2,6-dicarboxamide $[EuL_3]^{3+}$. The ligand ***L*** ((S,S): 58 mg, 110 µmol; (R,R): 50 mg, 95 µmol), was dissolved in MeOH (2 mL), and $EuCl_3.6H_2O$ ((S,S): 13.6 mg, 36.7 µmol and (R,R): 11.75 mg, 31.7 µmol) was added and the solution was stirred at 60°C during 12 h. Solvent was removed *in vacuo* and the complexes ***$[Eu((S,S)\text{-}L)_3]^{3+}$*** (45 mg, 70%) or ***$[Eu((R,R)\text{-}L)_3]^{3+}$*** (38 mg, 68 %) were isolated in their trichloride salts using crystallisation by slow diffusion of ^{t}BuOMe in hot MeOH. $\tau_{Acetonitrile}$= 1.21 ms; q = 0; λ_{max} = 360 nm; $\varepsilon_{Acetonitrile}$ = 55000 $M^{-1}.cm^{-1}$; $\phi_{Acetonitrile}$ = 12 %

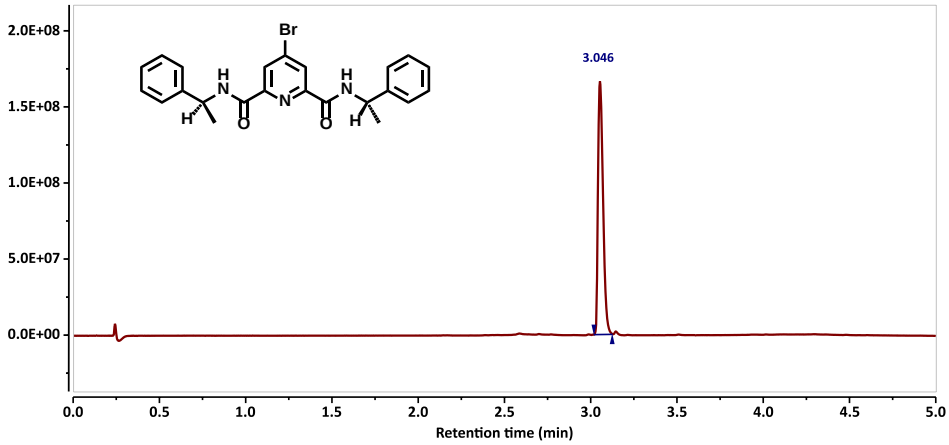


Λ-[Eu(R,R)L^2]

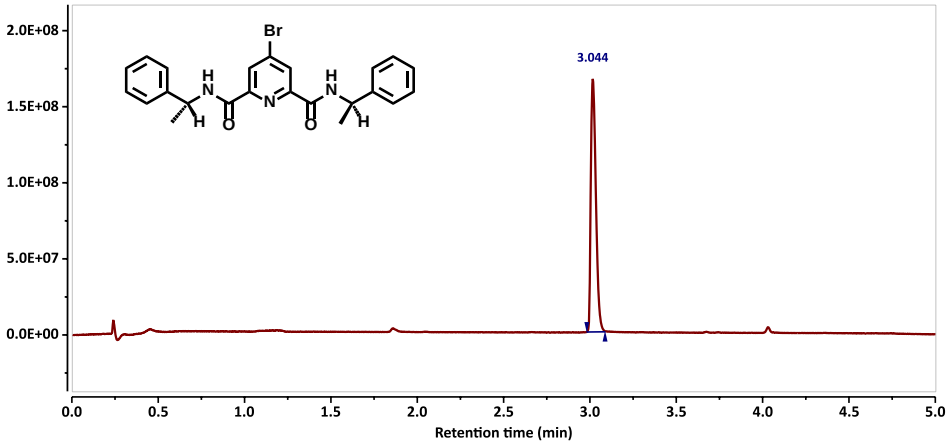


Δ-[Eu(S,S)L^2]

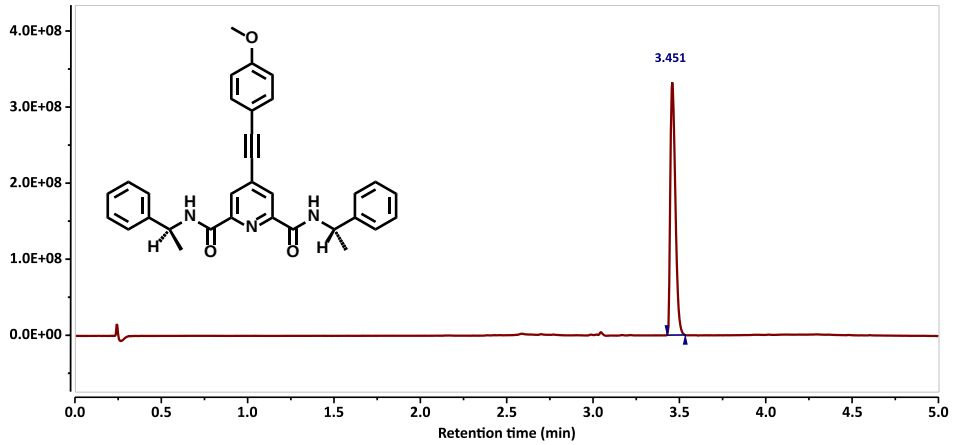
UPLC Chromatograms



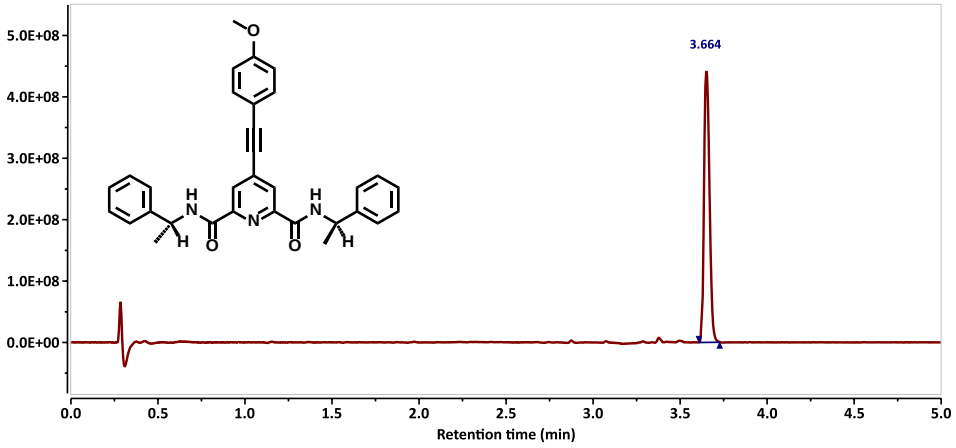
Compound 2a



Compound 2b



Ligand (S,S)-L



Ligand (R,R)-L

X-ray diffraction

X-ray diffraction experiments (**Table S1**) were carried out on a Bruker 3-circle D8 Venture diffractometer with a PHOTON 100 CMOS area detector, using Mo-K_α radiation (λ=0.71073 Å) from an Incoatec IμS microsource with focussing mirrors. Crystals were cooled using a Cryostream (Oxford Cryosystems) open-flow N_2 gas cryostat. The data were processed using APEX3 v.2016.1-0 and reflection intensities integrated using SAINT v8.38A software (Bruker AXS, 2016). The data were corrected for absorption by numerical integration based on crystal face-indexing, using SADABS program.[2] The structures were solved by dual-space intrinsic phasing method using SHELXT 2018/2 program,[3] and refined by full-matrix least squares using SHELXL 2018/3 software [4] on OLEX2 platform.[5] Absolute configurations were determined from anomalous scattering, using Flack-Parsons method.[6]

Curiously, while the crystal packing patterns of the cations in the two materials are mirror images of each other, disposition and amount of the solvent and disorder of the anions are different. The asymmetric unit of each structure contains two complex cations (with some of the outlying groups disordered), six chloride anions and two MeOBu^t molecules, that of *Λ*-enantiomer also contains 9

methanol and one water molecule, whereas that of *Δ*-enantiomer contains 6 methanol and 2 water molecules. Generally, *Δ*-enantiomer shows much more disorder in the structure and much higher crystal mosaicity.

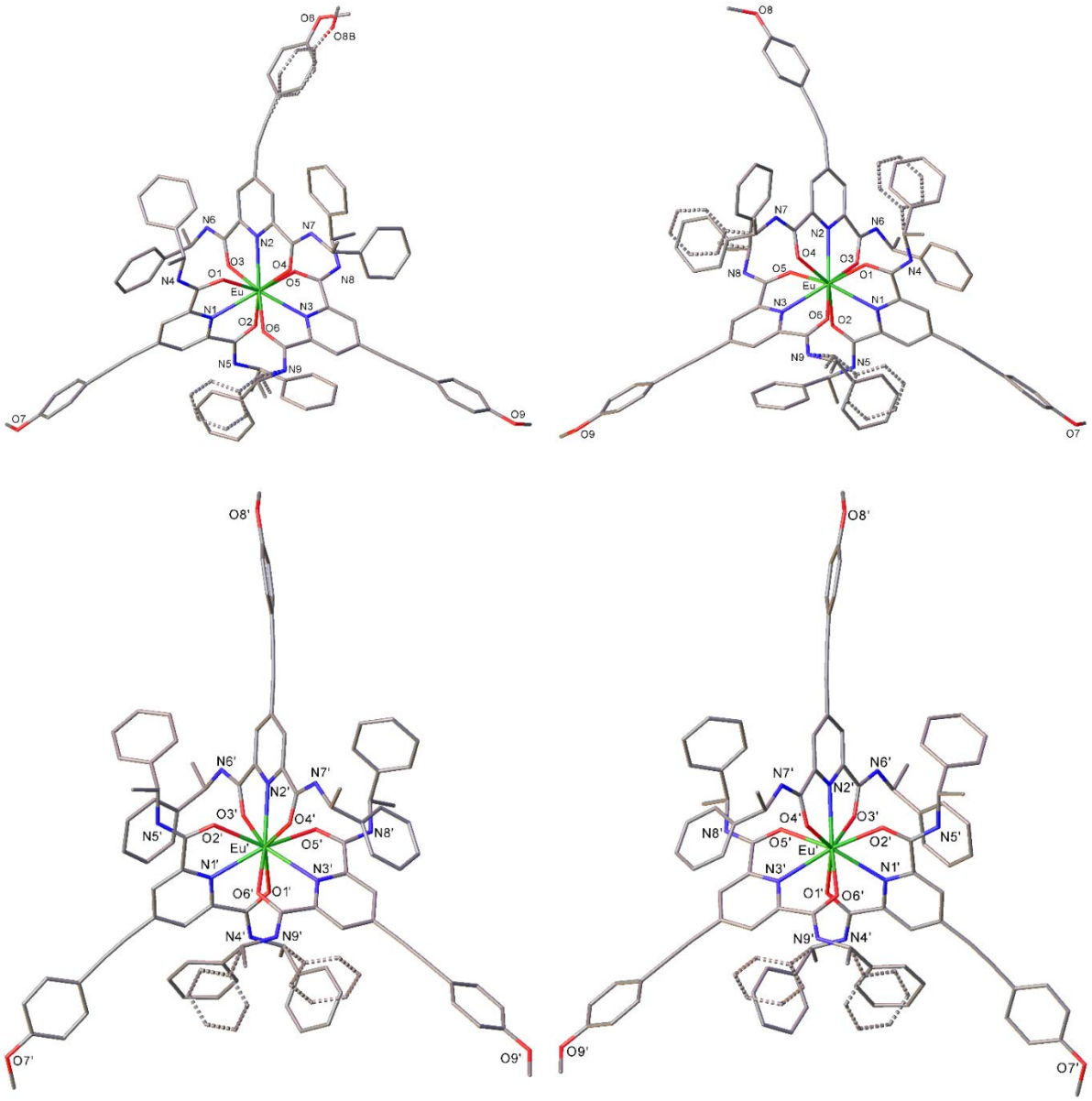
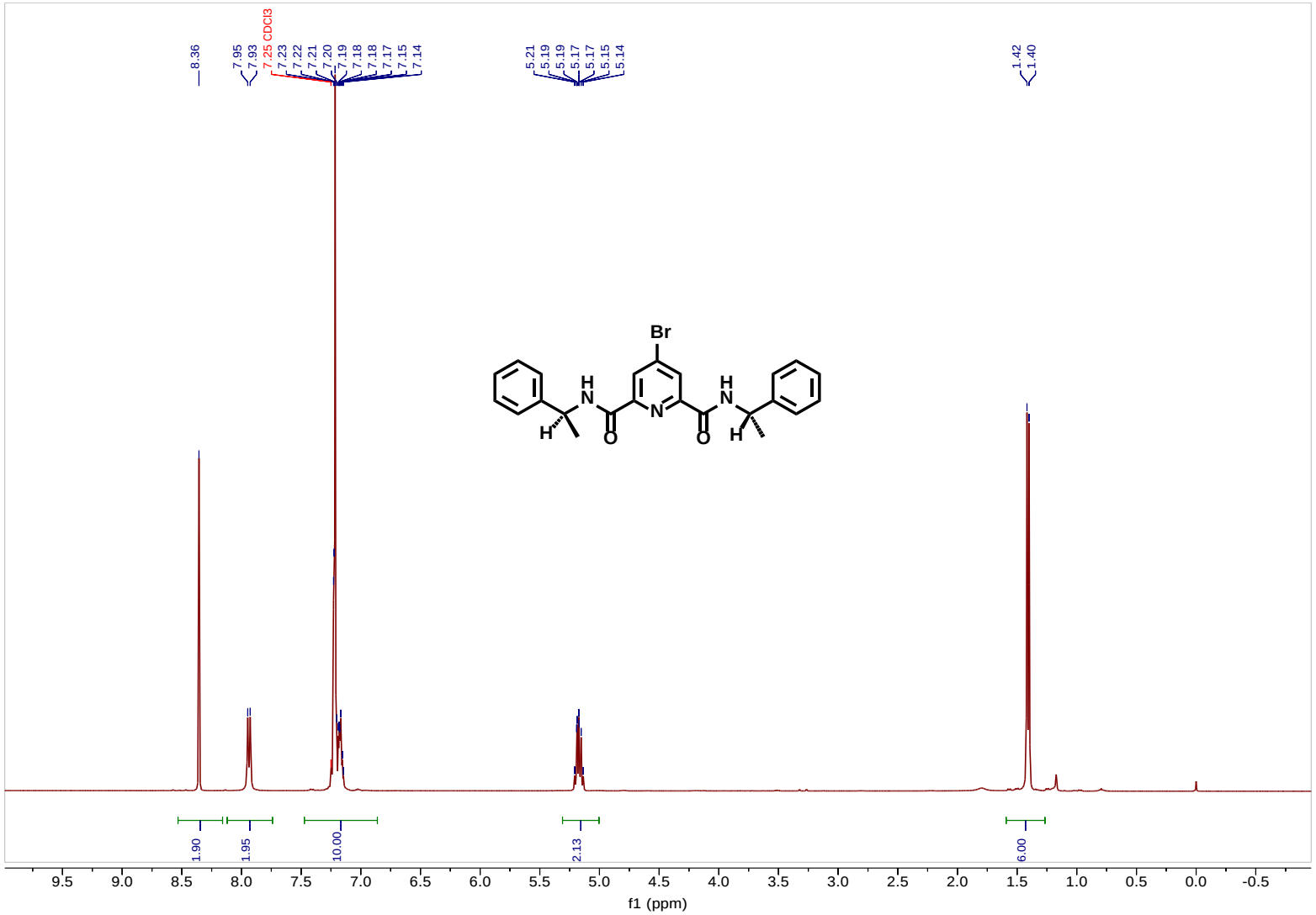


*Figure S1. Two independent cations in the crystals of Λ– (left) and Δ– (right) enantiomers of [Eu**L**$_3$]Cl$_3$, showing the disorder. H atoms are omitted for clarity.*

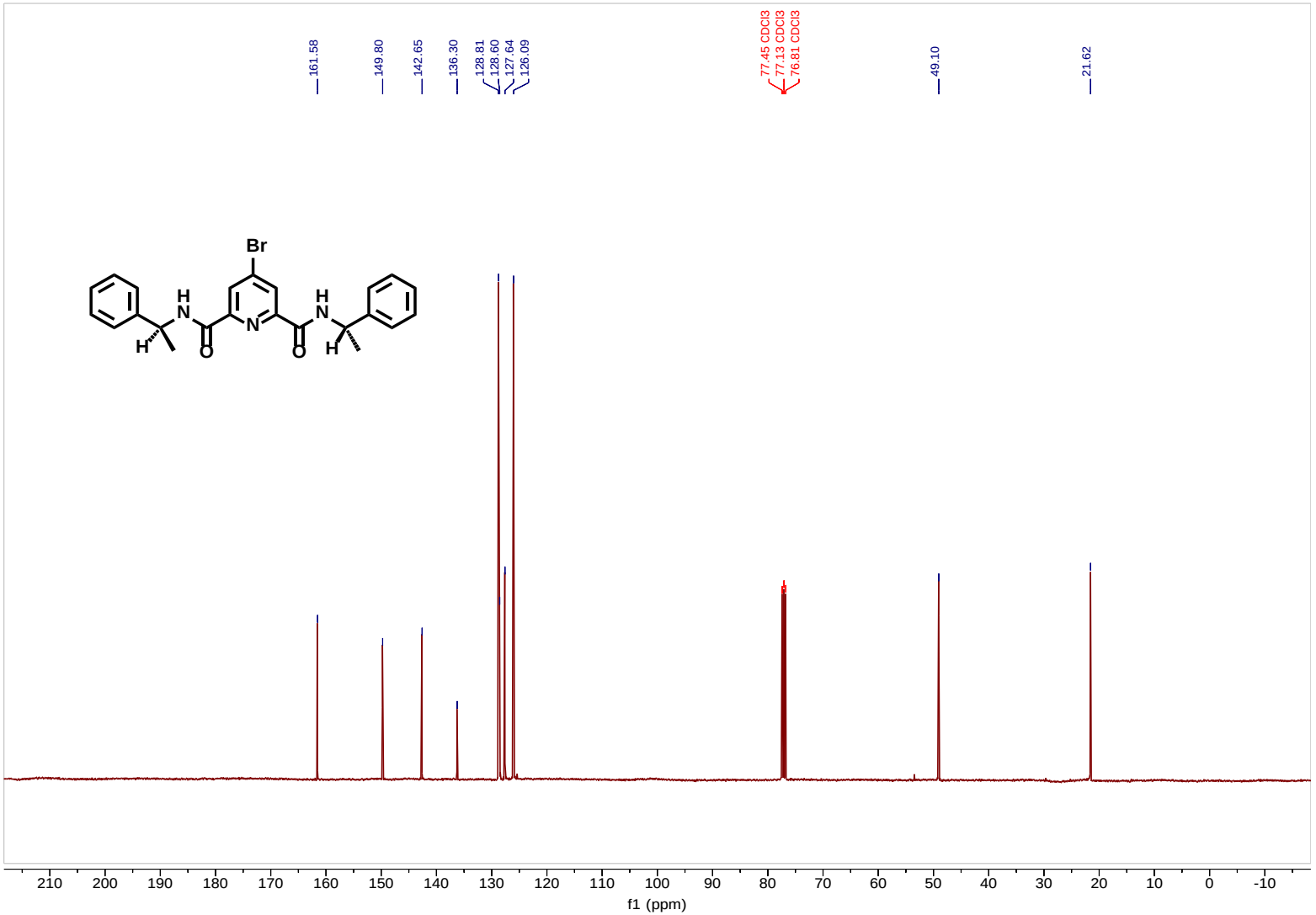
Table S1. Crystal data and experimental details

Compound	*Λ*-[Eu((*R*,*R*)-L)$_3$].Cl$_3$	*Δ*-[Eu((*S*,*S*)-L)$_3$].Cl$_3$
	19srv028	19srv002
CCDC	1946560	1952741
Formula	$[C_{96}H_{87}EuN_9O_9]^{3+}(Cl^-)_3$ $\cdot C_5H_{12}O\cdot 4.5CH_4O\cdot ½H_2O$	$[C_{96}H_{87}EuN_9O_9]^{3+}(Cl^-)_3$ $\cdot C_5H_{12}O\cdot 3CH_4O\cdot H_2O$
$D_{calc.}$/ g cm^{-3}	1.291	1.265
μ/mm^{-1}	0.75	0.75
Formula Weight	2010.39	1971.34
T/K	120	120
Crystal System	monoclinic	monoclinic
Flack Parameter	-0.0231(15)	0.006(3)
Space Group	$P2_1$ (no. 4)	$P2_1$ (no. 4)
a/Å	19.9608(11)	19.884(8)
b/Å	23.1000(12)	23.010(10)
c/Å	22.9095(13)	23.107(11)
β/°	101.7747(18)	101.685(16)
V/Å^3	10341.2(10)	10353(8)
Z	4	4
$2\theta_{max}$/°	60.3	55
Reflections measured	233259	241190
unique	60952	44258
with $I > 2\sigma(I)$	49312	35028
R_{int}	0.0582	0.0986
Parameters	2559	2493
Restraints	78	2191
$\Delta\rho_{max,min}$/ eÅ^{-3}	0.61, -0.50	1.87, -0.77
Goodness of fit	1.012	1.035
R_1, wR_2 [$I > 2\sigma(I)$]	0.039, 0.074	0.050, 0.105
R_1, wR_2 (all data)	0.062, 0.080	0.076, 0.114

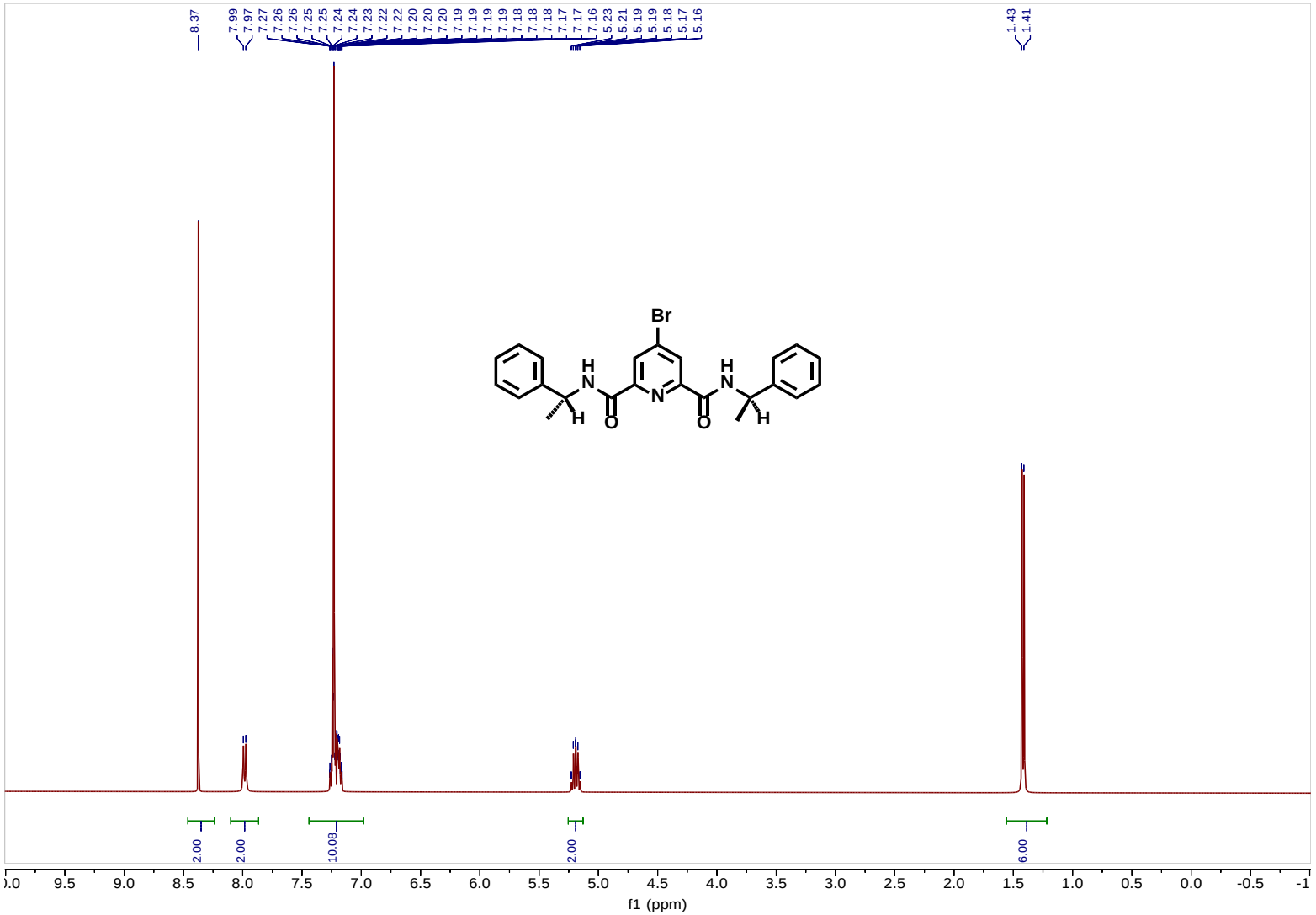
NMR Spectra



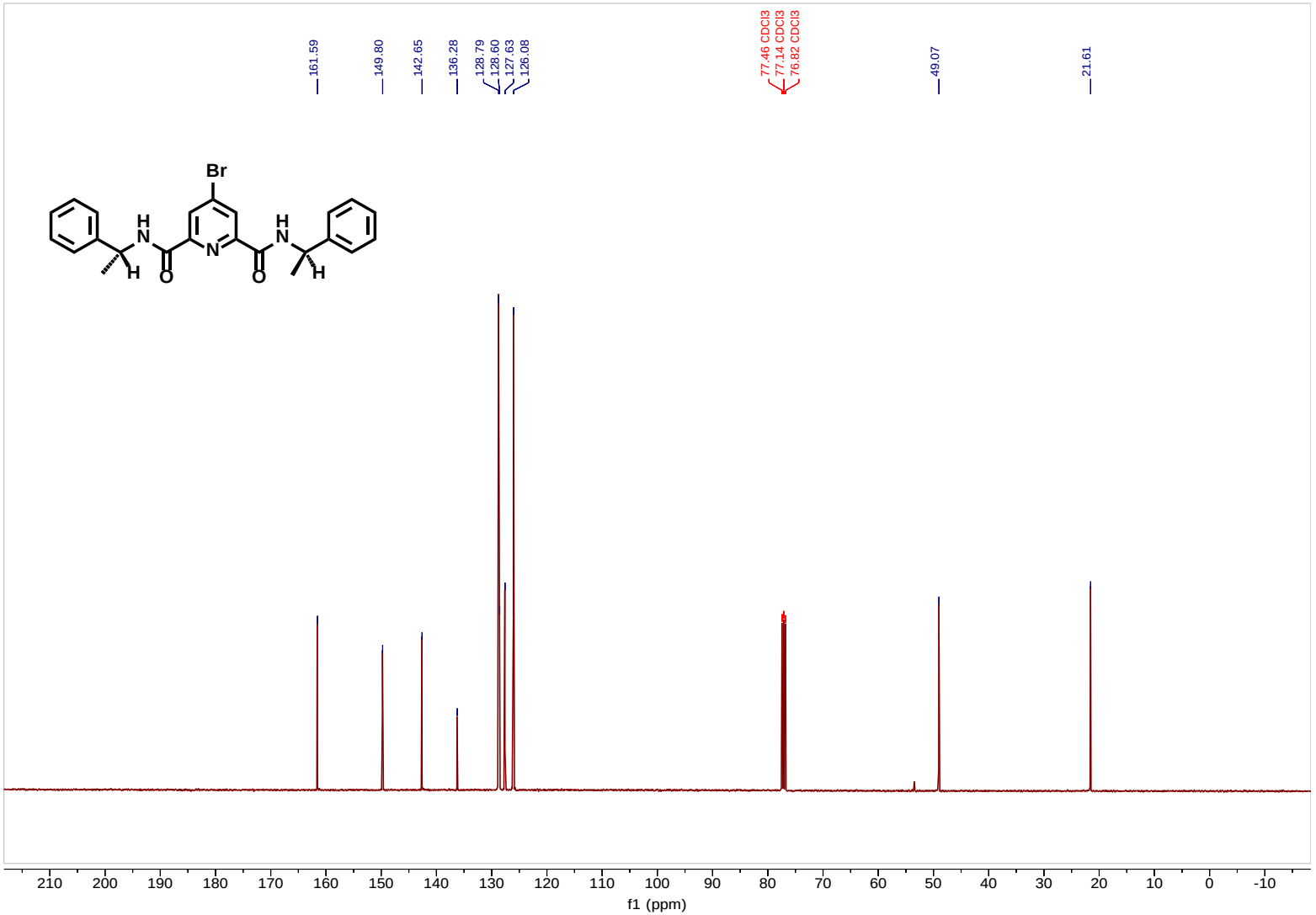
^{1}H NMR of compound 2a (298 K, $CDCl_3$, 400 MHz)



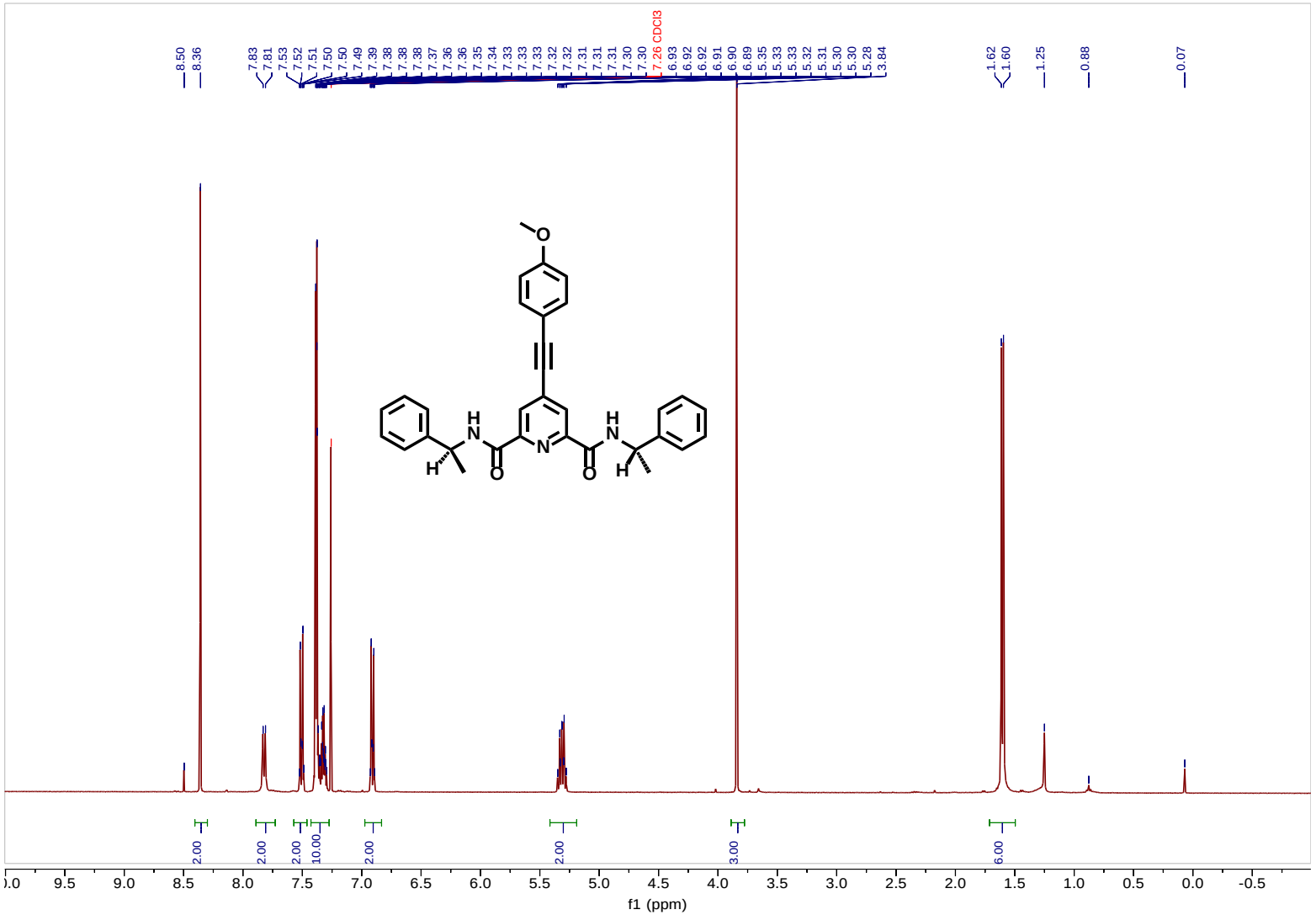
^{13}C NMR of compound 2a (298 K, 100 MHz, $CDCl_3$)



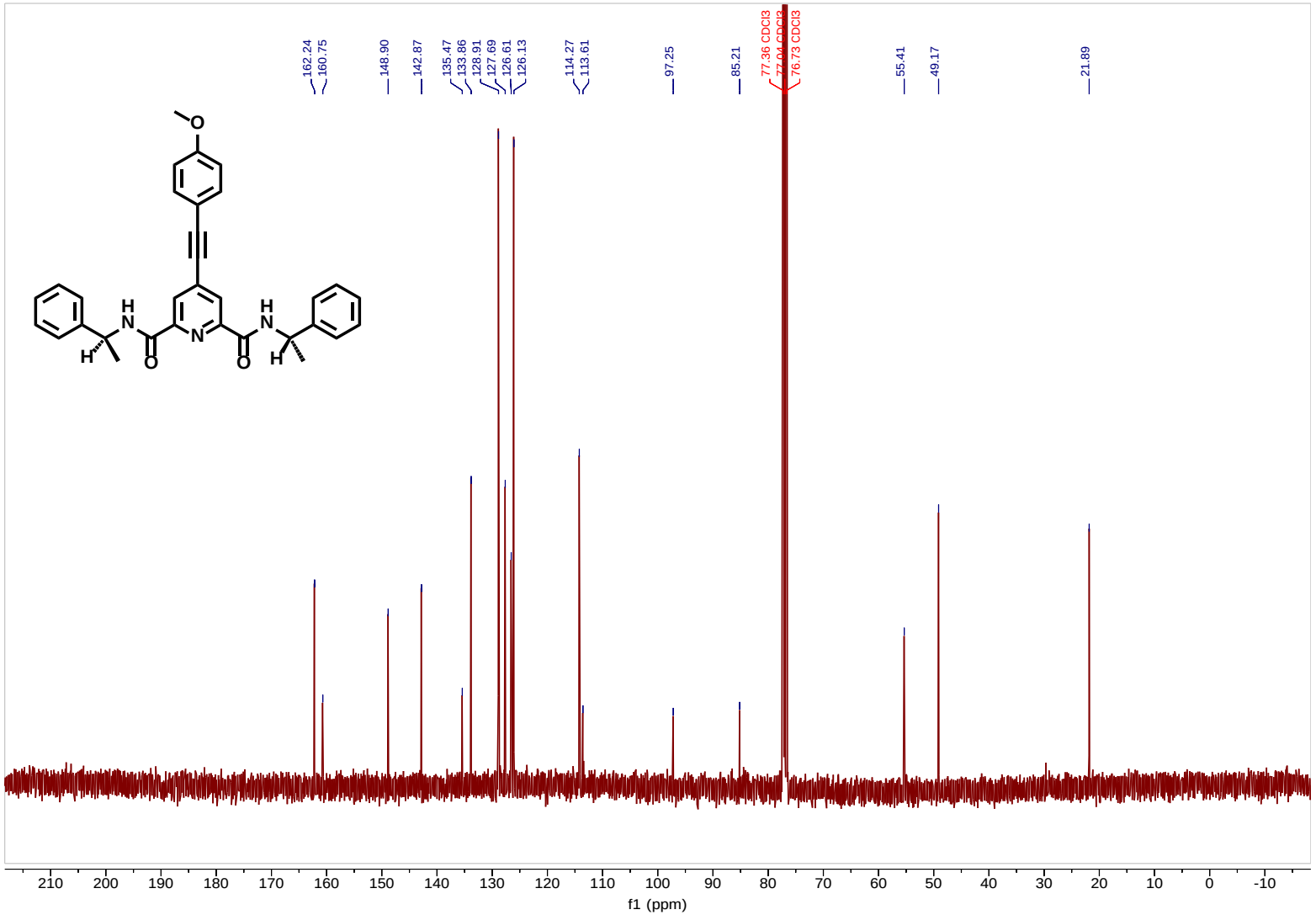
^{1}H NMR of compound 2b (298 K, $CDCl_3$, 400 MHz)



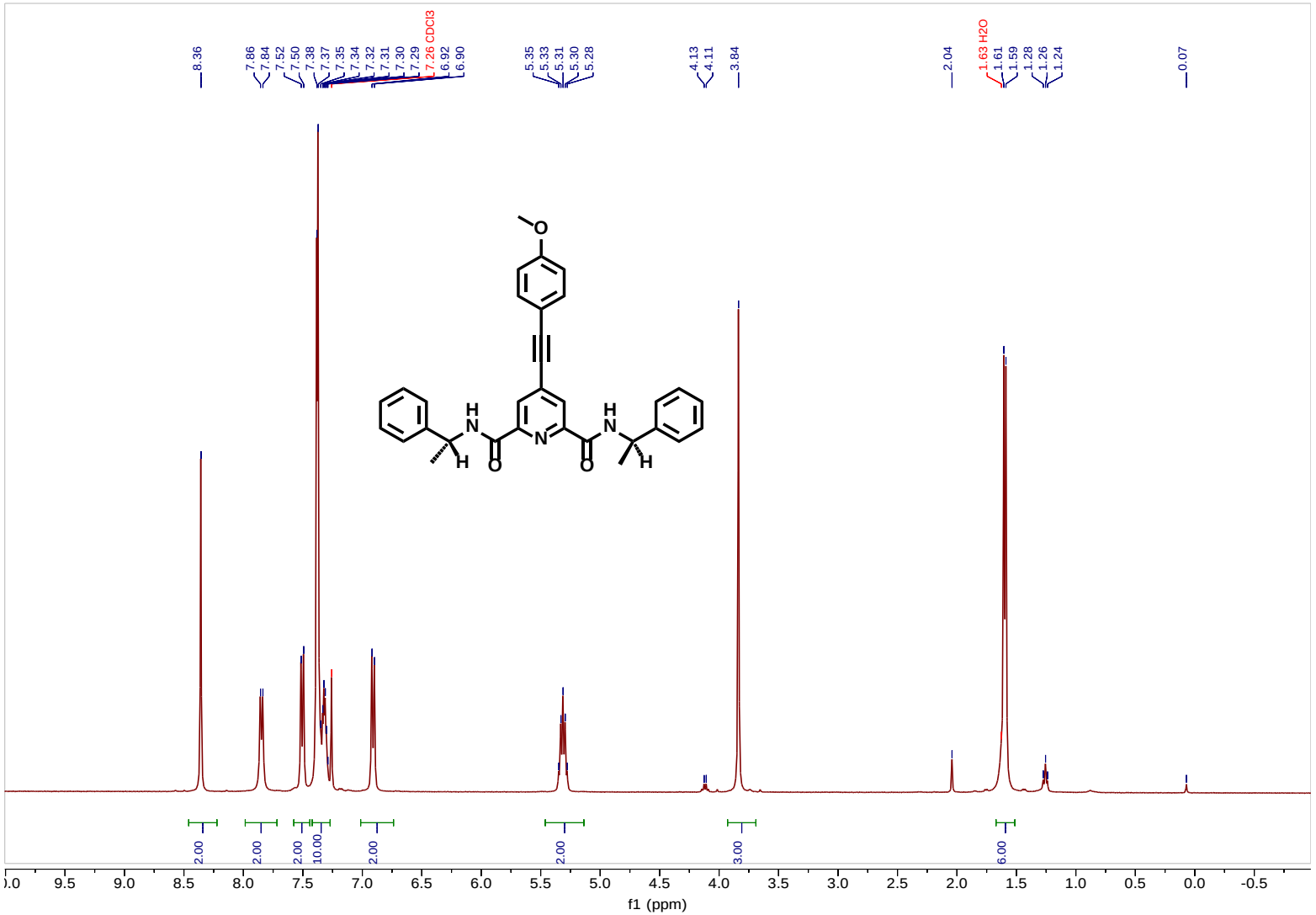
^{13}C NMR of compound 2b (298 K, 100 MHz, $CDCl_3$)



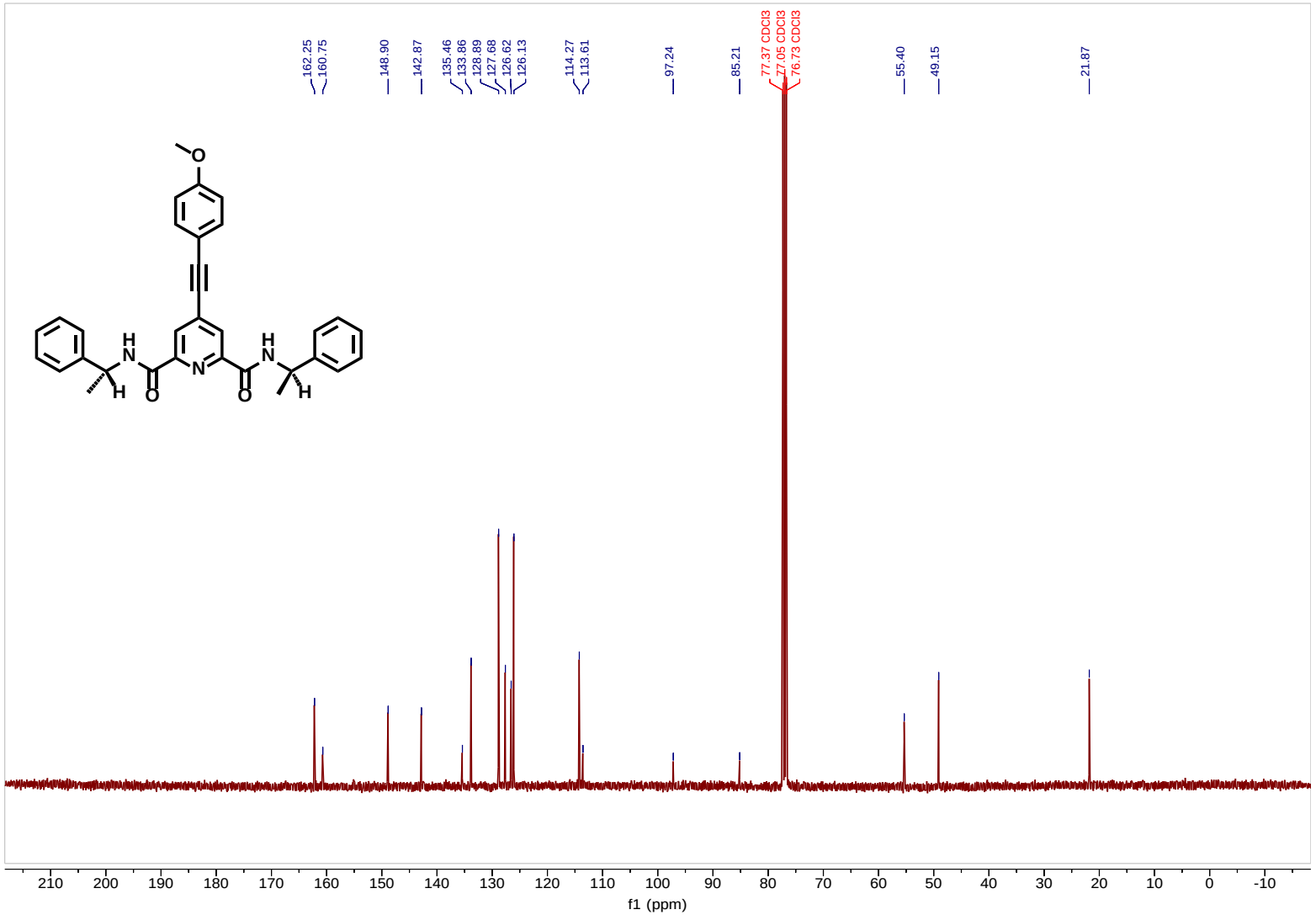
^{1}H NMR of ligand (S,S)-L^{2} (298 K, $CDCl_3$, 400 MHz)



^{13}C NMR of Ligand (S,S)-L^{2} (298 K, 100 MHz, CDCl$_3$)



^{1}H NMR of ligand (R,R)-L^2 (298 K, $CDCl_3$, 400 MHz)



^{13}C NMR of Ligand (R,R)-L^2 (298 K, 100 MHz, $CDCl_3$)

Photophysical studies of Europium complexes

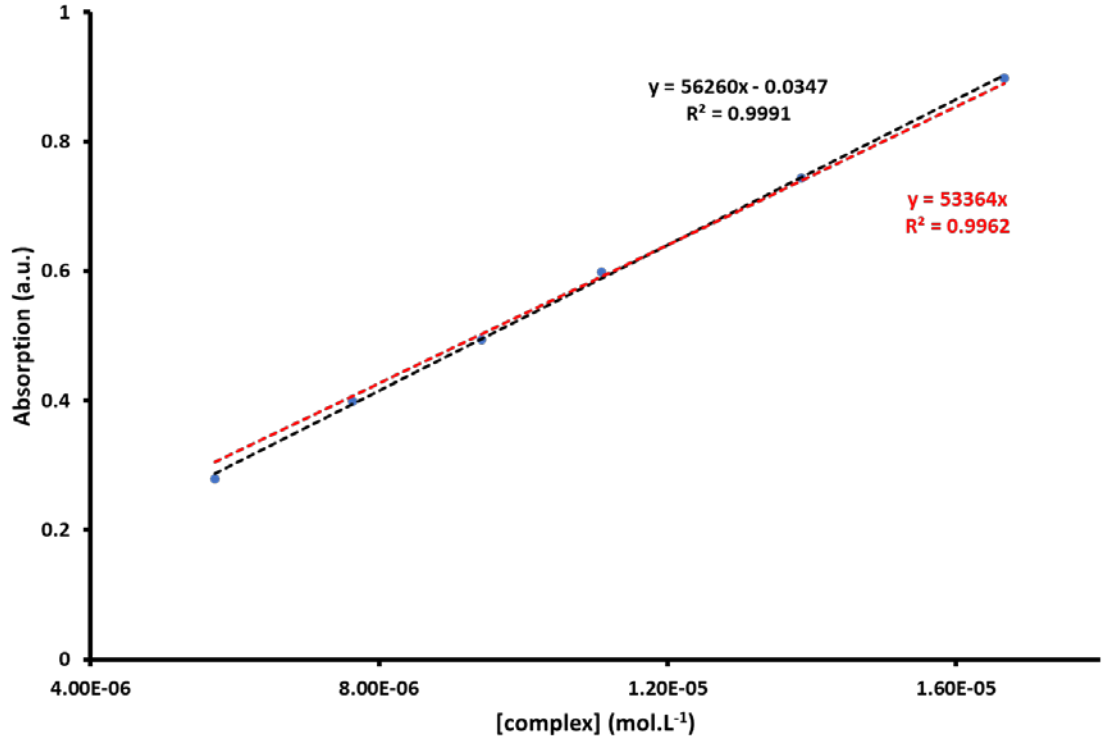


Figure S2. Extinction coefficient determination of $[Eu(\mathbf{L})_3]^{3+}$ (Initial concentration C = 16.67 µM in acetonitrile)

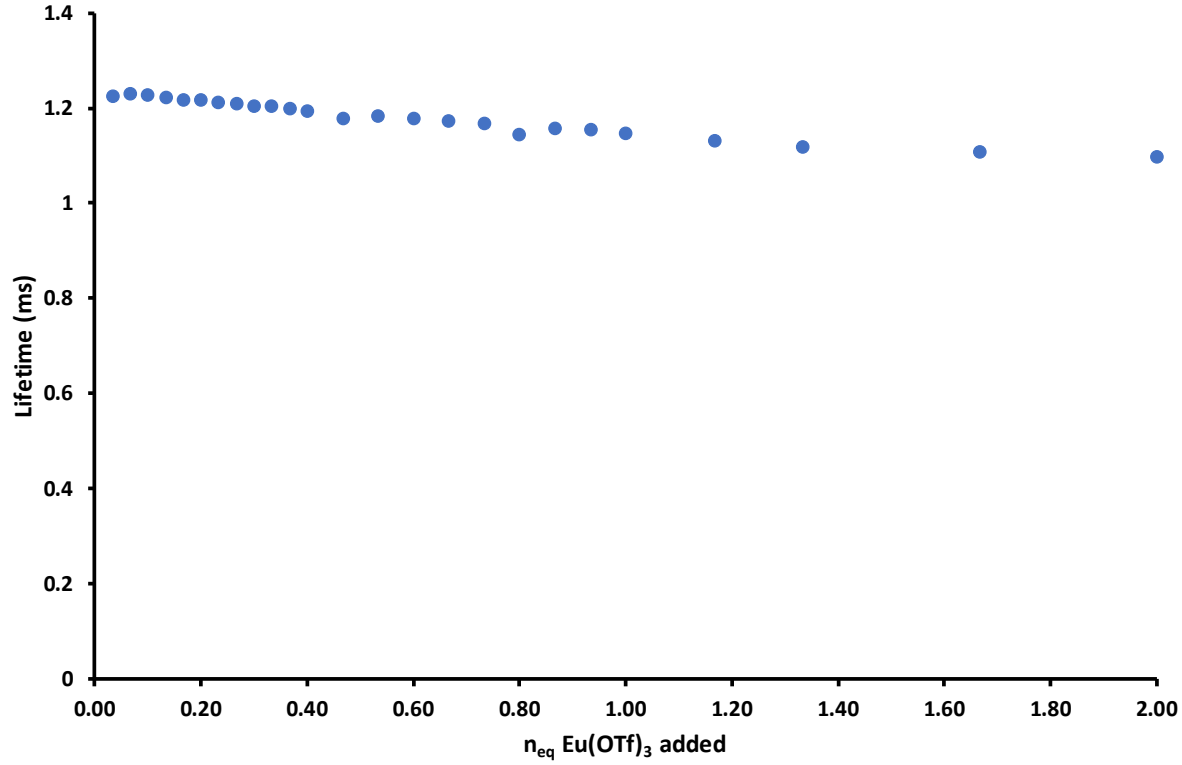


Figure S3. Mono exponential lifetime decay evolution of Eu-containing species formed upon addition of a solution of $Eu(OTf)_3$ ($c_{Eu} = 5.61\times10^{-4}$ M) to a solution of ligand L ($c_L = 5.55\times10^{-5}$ M) in acetonitrile. Stirring time of 10 minutes between addition and measurement.

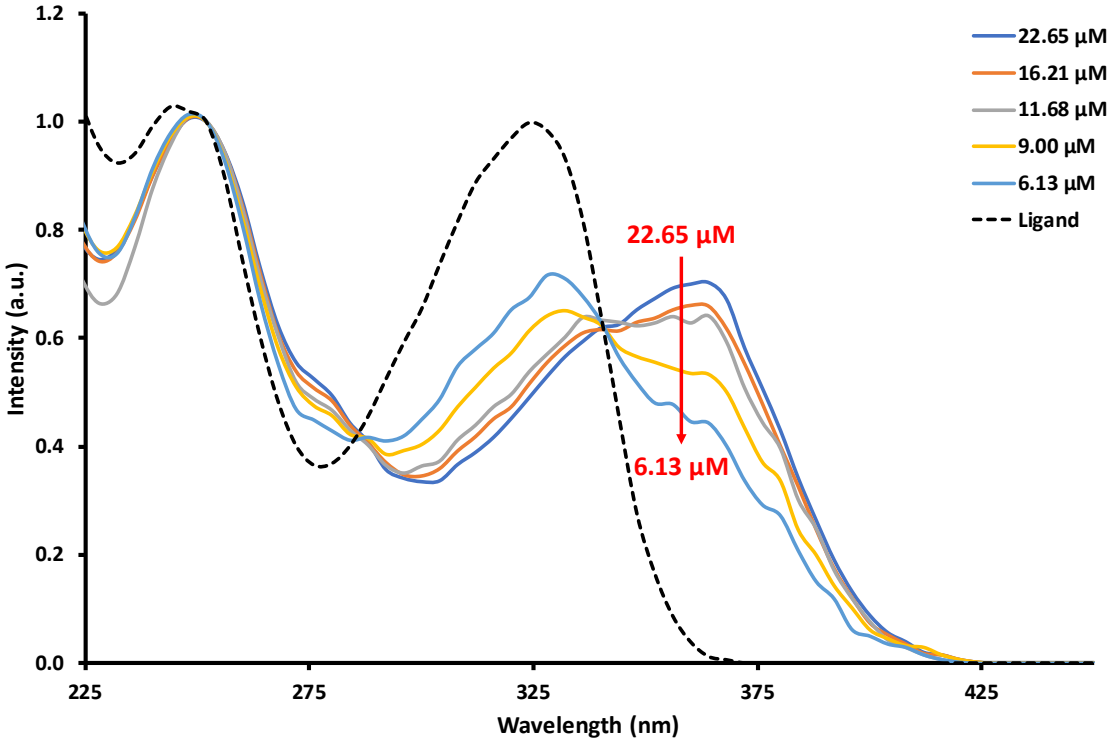


Figure S4. Variation of absorption at 360 nm vs $[Eu(L)_3]^{3+}$ along the dilution. Black dash line corresponds to the absorption of the free ligand (55 µMol.L-1) in acetonitrile. All absorption spectra are normalised with the absorption band at 250 nm corresponding to the n→π electronic transition.*

The dilution of the complex solution favour the ligand dissociation monitored by the decrease of the absorption band at 360 nm corresponding to the maximum absorption wavelength of the complex and the increase of the absorption band at 330 nm corresponding to the free ligand in solution

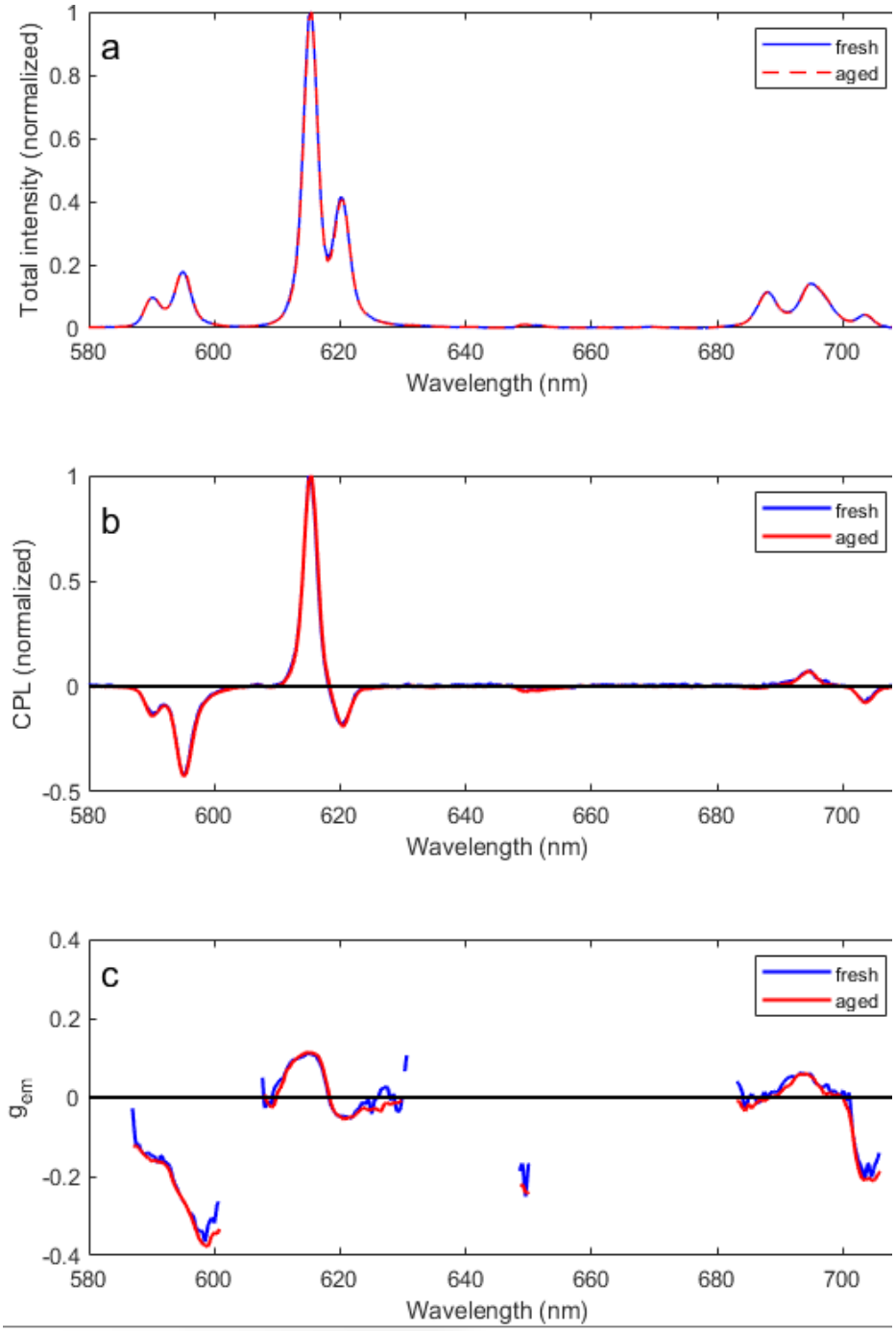


*Figure S5. Comparison of CPL spectra from a solution of Λ-[Eu((R,R)-**L**)$_3$]$^{3+}$ (c = 15×10^{-6} M) at t=0 and t = 4 months. a. total intensity emission, b. CPL spectra, c. g_{lum} values*

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