Choline-Geranate (CAGE) Ionic Liquids Potentiate The Anticancer Activity Of Platinum-Based Drugs

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Electronic Supplementary Information (ESI)

Materials and methods

Potassium tetrachloroplatinate(II) (K_2PtCl_4), geranic acid, dry n-hexane, hydrogen peroxide 30%, succinic anhydride, anhydrous N,N-dimethylformamide(DMF) and potassium oxalate monohydrate were supplied by Fisher Scientific. Choline bicarbonate, sodium acetate, dry toluene, 2-(2,4-difluorophenyl) pyridine (dfppy), bis(diphenylphosphino)ethane (dppe) and cisplatin were acquired from Merck. Glacial acetic acid was purchased from Scharlab while cisplatin was obtained from TCI chemicals.
All reagents employed in this study were of commercial grade and sourced from newly unsealed containers. Milli-Q water underwent purification using a Milipore Direct-Q® 3 UV apparatus. Solvents for NMR spectroscopy were obtained from Euroisotop.

Nuclear Magnetic Resonance (NMR). ^{1}H and ^{13}C NMR spectra were recorded in 5 mm NMR tubes using standard pulse programs and a Bruker Fourier 300 NMR spectrometer at room temperature. ^{19}F, ^{31}P NMR spectra were recorded using standard pulse programs either in a Bruker AVANCE NEO 400 NMR or 500 NMR spectrometer. The ^{1}H–^{19}F HOESY spectra were recorded with the hoesy1d, with different mixing time 40, 60, 120 ms, 512 scans, recycling delay 1 s. The chemical shifts (δ, quoted in parts per million, ppm) in ^{1}H and ^{13}C NMR were internally referenced to the residual peak of the corresponding solvents: D_2O (^{1}H: 4.79 ppm), $CDCl_3$ (^{1}H: 7.26) and DMSO-*d*^{6} (^{1}H: 2.50 ppm and ^{13}C: 39.52 ppm). Data processing was carried out using MestreNova, version 11.0 (Mestrelab Research, S.L.)

Mass Spectrometry (MS). The analysis consisted in a chromatographic separation in an ultrahigh performance liquid chromatography (UPLC, Acquity system from Waters Cromatografia S.A.) coupled to a high-resolution mass spectrometer (Synapt G2 from Waters Cromatografia S.A., time of flight analyser (TOF)) by an electrospray ionization source in negative mode (ESI-).
The chromatographic separation was achieved using an Acquity UPLC BEH C18 column (1.7 µm, 2.1×50 mm i.d.) with an Acquity UPLC BEH C18 1.7 µm VanGuard pre-column (2.1×5 mm) (Waters Cromatografia S.A.) and a binary A/B gradient (solvent A: water with 0.1 % formic acid and solvent B: methanol). The gradient program was established as follows: initial conditions were 5 % B, raised to 100 % B over 2.5 min, held at 100 % B until 4 min, decreased to 5 % B over the next 0.1 min, and held at 5 % B until 5 min for re-equilibration of the system prior to the next injection. A flow rate of 0.25 mL/min was used, the column temperature was 30 °C, the autosampler temperature 4 °C and the injection volume 7,5 µL.
High resolution mass data were acquired in SCAN mode, using a mass range 100–1200 u in resolution mode (FWHM ≈ 20,000) and a scan time of 0.1 s. The source temperature was set to 120 °C and the desolvation temperature to 350 °C. The capillary voltage was 0.7 kV and the cone voltage 15 V. Nitrogen was used as the desolvation and cone gas at flow rates of 600 L/h and 10 L/h, respectively. Before analysis, the mass spectrometer was calibrated with a sodium formate solution. A leucine-enkephalin solution was used for the lock mass correction, monitoring the ions at mass-to-charge ratio (m/z) 556.2771. All of the acquired spectra were automatically corrected during acquisition based on the lock mass. Samples were dissolved in DMSO at a concentration of 1 mg/ml and diluted at 50 µg/mL for the analysis.

Infrared spectroscopy

FTIR spectra were recorded at room temperature over the range of 600–4000 cm^{-1} using a JASCO 6300 spectrometer equipped with an attenuated total reflectance (ATR) accessory. Each spectrum was acquired at a resolution of 4 cm^{-1}, averaging 200 scans per measurement. No baseline correction or smoothing was applied to the spectra.

Thermogravimetric analysis (TGA)

The moisture content of the sample was determined by thermogravimetric analysis (TGA) using a TA Instruments Q500 thermobalance. The analysis was carried out with the Hi-Res 4 program (resolution: 4; sensitivity: 1.0; heating rate: 50 °C/min from 40 to 800 °C under a nitrogen atmosphere). The resulting thermogram was analyzed with TA Universal Analysis software. Moisture content was calculated based on the weight loss observed in the first step of the thermogram (40–100 °C), corresponding to the release of water. The content of CAGE ILs in the sample was determined from the weight loss occurring between 100 and 225 °C. For each sample, the onset temperature (Ton) was also determined.

UPLC-MS

Chromatography for **2**–**6** (0.1 mM) was performed in an Acquity UPLC system using an Acquity BEH C18 column (100x 2.1 mm, 1.7 µm) from Waters (Milford, MA, USA) and equipped with photodiode array detector (PDA). The gradient elution buffers were A (1% TFA in water) and B (1% TFA in acetonitrile). The gradient method was: 0–1 minutes, isocratic at 100% A; 1–10 minutes, gradient to 20% A; 10–11 minutes, gradient to 100% A; 11–14 minutes of stabilization at 100% A. The UV detector wavelength was set at 254 nm and the injection volume was 10 µL. Total run time was 14 minutes while the flow rate was set at 300 $\mu L \cdot min^{-1}$. The mass spectrometry detection was carried out using a time-of-flight mass spectrometer (ESI-TOF) LCT Premier XE from Waters (Milford, MA, USA) with an electrospray ionization source, working in positive/V mode. The MS range acquired was between m/z 50–1.000. The capillary and cone voltages were set at 3.000 and 50 V, respectively. Desolvation gas temperature was 300 °C and source temperature was 120 °C. The desolvation gas flow was set at 600 $L \cdot h^{-1}$ and cone gas flow was set at 50 $L \cdot h^{-1}$. For data analysis, Masslynx v4.1 software was used (Waters, Milford, MA, USA). All the analytes were identified by mass spectrometry. The raw data of chromatograms were exported to .csv file and plotted in Origin Pro 2020 software to obtain clearer and comparable graphs.

Computational details

Atomistic MD simulations were performed using periodic boundary conditions with the GROMACS package (version 2023).[1] Initial systems with a 50% m/m composition and a concentration of 2.5 mM were created using Packmol (version 20.14.3),[2] specifying the number of molecules of each type, as well as the initial box size and geometry (8.7x8.7x8.7 nm^3). The dfppy molecule was always placed at the center, while the remaining molecules were randomly distributed. The RESP procedure was followed for obtaining the parameters of the dfppy, geranic acid, geranate and choline. First, each molecule was optimized, and the electrostatic potential (ESP) charges were calculated using Gaussian16 package[3] (B3LYP/6-31G(d,p)). Subsequently, the restrained electrostatic potential (RESP) charges[4] and GAFF parameters[5] for

these compounds were obtained by a two-stage fitting procedure using Antechamber, distributed with Ambertools.[6] Water was described by the TIP3P water model.
Once the initial system (comprising dfppy, CAGE 1:2, and water molecules) was set up, an SD minimization was carried out for 100,000 steps or until the energy difference fell below 100 kJ/mol, which was typically achieved before reaching the step limit.
Electrostatic interactions were treated using the Particle Mesh Ewald (PME) method, while van der Waals interactions were handled using a cut-off approach. Constraints were imposed on all H-bonds using the linear constraints solver algorithm (LINCS). The system was then equilibrated in the NVT ensemble by gradually heating from 298 K to 600 K at a rate of 0.151 K/ps. The system was maintained at the high temperature for 6 ns, followed by a cooling phase back to 298 K at the same rate, resulting in a total NVT equilibration time of 10 ns. The high-temperature equilibration phase was followed by an NPT ensemble equilibration for 2 ns. The V-rescale thermostat[7] and a C-rescale barostat[8] were used in the equilibrations.
The production phase was conducted in the NVT ensemble using a V-rescale thermostat at 298 K and leap-frog algorithm with a 2-fs time-step. Five independent 20 ns trajectories were generated, each containing one dfppy, 455 ion pairs and 11,113 water molecules, starting from different initial geometries. We save frames every 5 ps. The resulting trajectories were collectively analyzed using TRAVIS to obtain the overall structure of the system via Radial Distribution Functions (RDFs).[9,10]

In vitro cell studies

Cell culture

U87-MG cells were purchased from American Type Culture Collection (ATCC, VA, USA) and used as in vitro model of glioblastoma cancer cells. U87-MG cells were maintained in Dulbecco's Modified Eagle Medium (DMEM) High Glucose w/o sodium pyruvate (ECM0101L, Euroclone, Milan, Italy) supplemented with 10% fetal bovine serum (FBS, ECS0180L, Euroclone, Milan, Italy), 4 mM L-glutamine (ECB3000D, Euroclone, Milan, Italy), and 100 U(mL penicillin/streptomycin (ECB3001B, Euroclone, Milan, Italy).

Cell Viability assay

The effect of CAGE formulations and platinum complexes on cell viability were assessed by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay.[11] U87-MG cells were seeded in 96-well plates at a density of 1×10^4 cells/well for 48 hours. Different doses of CAGE ILs (0.5-2.5 mg/mL) or different doses of platinum derivatives (1.5-2.5 µM), free or dissolved in CAGE 1:2, were added to the culture medium and incubated for up to 48 hours at 37°C, 5% CO_2.
Tthe assay was performed as per the manufacturer's protocol and absorbance was measured at 570 nm using a microplate reader (SPECTROstar Nano, BMG LABTECH, Ortenberg, Germany). Cell viability was calculated compared with untreated cells used as control (100 % viability).
Results are presented as the mean± standard deviation of three independent experiments.

Synthesis and Characterization

Synthesis of CAGE (1:1)

CAGE 1:1 was prepared by following the procedure reported by Zakrewsky *et al.*[12] One equivalent of pure geranic acid (0.059 moles), previously recrystallized five times at –70°C from a solution containing 70 wt% geranic acid and 30 wt% acetone, was placed into a 500-mL round-bottom flask. Then, one equivalent of choline bicarbonate (80 wt% solution, 0.059 mol) was added. The mixture was stirred at room temperature until no more CO_2 evolved. The solvent was then removed by rotary evaporation at 60°C for 20 minutes, and the resulting product was dried in a vacuum oven for 48 hours at 60°C.

^{1}H NMR (300 MHz, Chloroform-d), δ (ppm): 5.68 (m, 1H), 5.09 (m ,1H), 4.05 (m, 2H), 3.64 (m, 2H), 3.29 (s, 9H), 2.05 (m, 7H), 1.66 (s, 3H), 1.59 (s, 3H); IR (cm^{-1}): 3182 cm^{-1} (O–H stretching vibrations); 1551 cm^{-1} (C=O stretching vibrations); 956 cm^{-1} (C–O stretching vibrations).

TGA: Moisture content (40-100 °C): 5% by weight; CAGE 1:1 content (100-225 °C): 95% by weight; Onset degradation temperature (T_{on}): 168 °C.

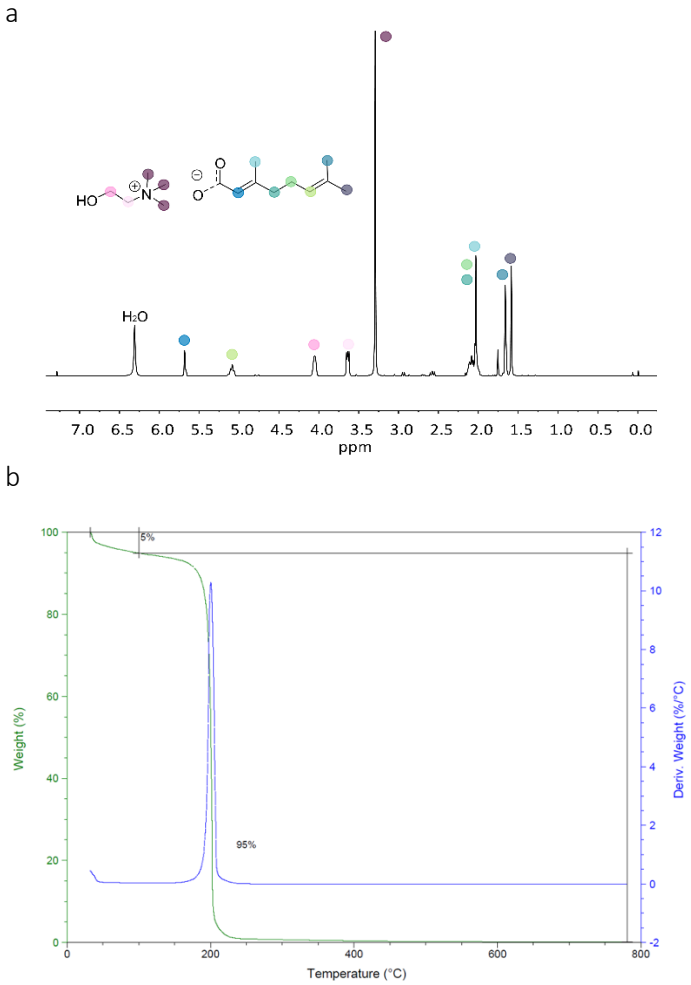


Fig. S1 (a) ^{1}H NMR (300 MHz, Chloroform-d) and (b) TGA analysis for CAGE 1:1.

Synthesis of CAGE 1:2

CAGE 1:2 was prepared following the procedure described above, but with two equivalents of geranic acid (0.059 moles) and one equivalent of choline bicarbonate (0.029 moles).

^{1}H NMR (300 MHz, Chloroform-d), δ (ppm): 5.72 (m, 2H), 5.09 (m, 2H), 4.08 (m, 2H), 3.67 (m, 2H), 3.30 (s, 9H), 2.10 (m, 14H), 1.67 (s, 6H), 1.60 (s, 6H); IR (cm^{-1}): 3367,3182 cm^{-1} (O–H stretching vibrations); 1551 cm^{-1} (C=O stretching vibrations); 956 cm^{-1} (C–O stretching vibrations).

TGA: Moisture content (40-100 °C): 3% by weight; CAGE 1:1 content (100-225 °C): 97% by weight; Onset degradation temperature (T_{on}): 150 °C.

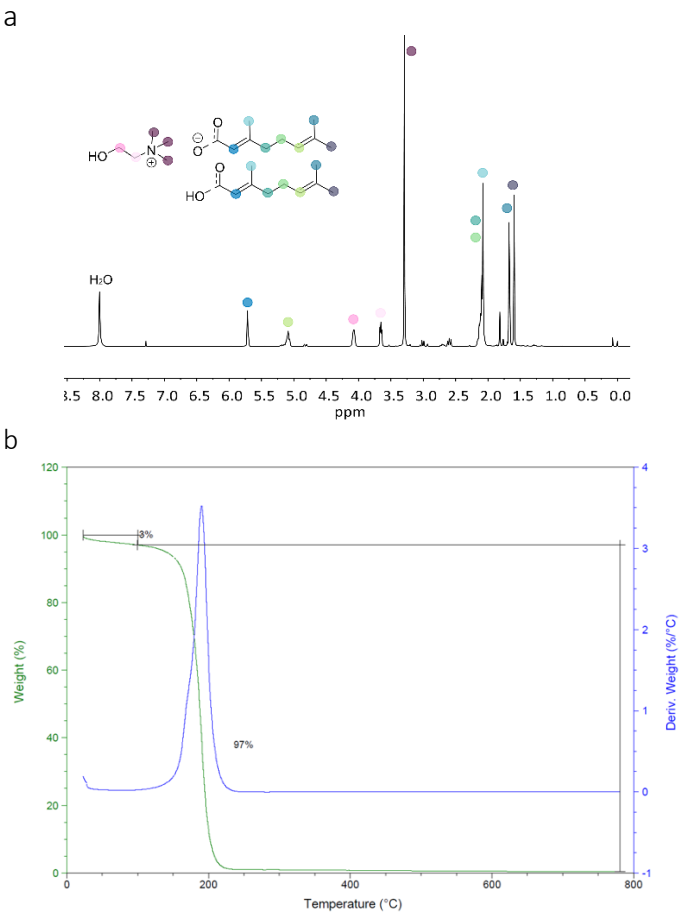


Fig. S2 (a) ^{1}H NMR (300 MHz, Chloroform-d) and (b) TGA analysis for CAGE 1:2.

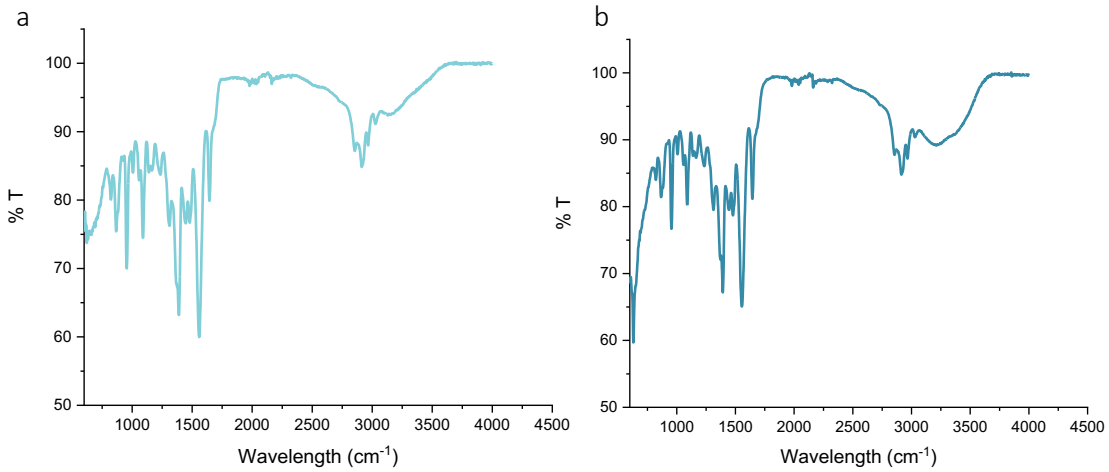


Fig. S3 FT-IR spectra of (a) CAGE 1:1 and (b) CAGE 1:2.

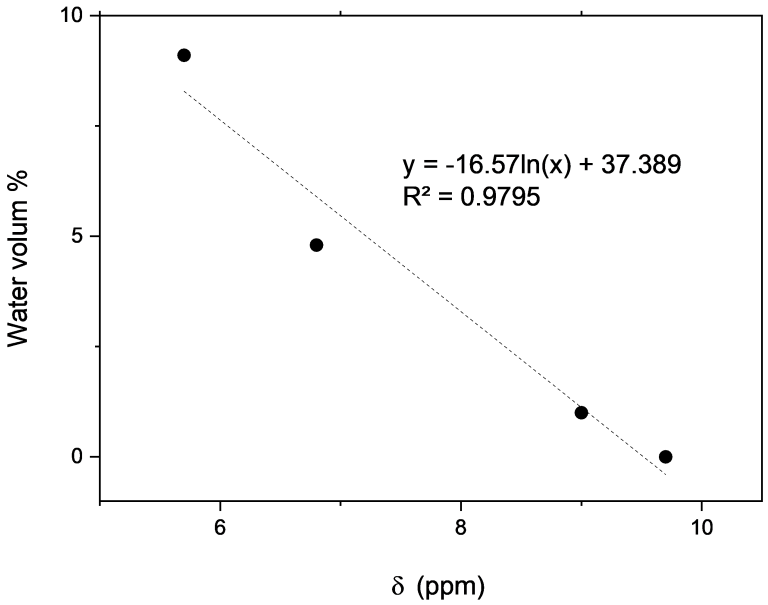


Fig. S4 Calibration curve for the estimation of water content (%) based on ^{1}H NMR chemical shift.[13]

Synthesis Of Complexes

Cisplatin (**1**) was obtained from commercial sources (Merck).

Synthesis of [Pt(dfppy)(Cl)(DMSO)] (2)

Complex **2** was prepared according to the method described by Shahsavari et al.[14] An aqueous solution of K_2PtCl_4 (2 g, 4.81 mmol in 16 ml) was filtered through paper into a 50 ml beaker to remove impurities such as metallic Pt and/or K_2PtCl_6. Subsequently, DMSO (1.024 ml, 14.43 mmol) was added, and the solution was gently hand-mixed before being left to stand at room temperature until yellow needles fully precipitated. The precipitate was then filtered and washed with multiple 10 ml portions of water, ethanol, and diethyl ether. The resulting solid (**A**) was dried under vacuum for 4 hours. Next, the dfppy ligand (45.12 mg, 0.236 mmol) and NaOAc (19.36 mg, 0.236 mmol) were added to a solution of **A** (100 mg, 0.236 mmol) in dry toluene. This mixture was refluxed at 110°C for 24 hours under an argon atmosphere. The solvent was removed under reduced pressure, yielding a yellow-green solid that was washed with dry n-hexane (3 × 2 ml) and dried under vacuum. Yield: 79 %.

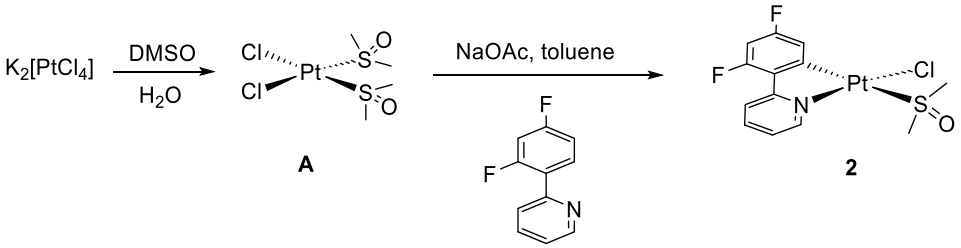
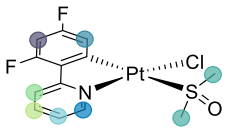


Figure S5: Synthesis of **2** following the procedure described by Shahsavari et al.[14]



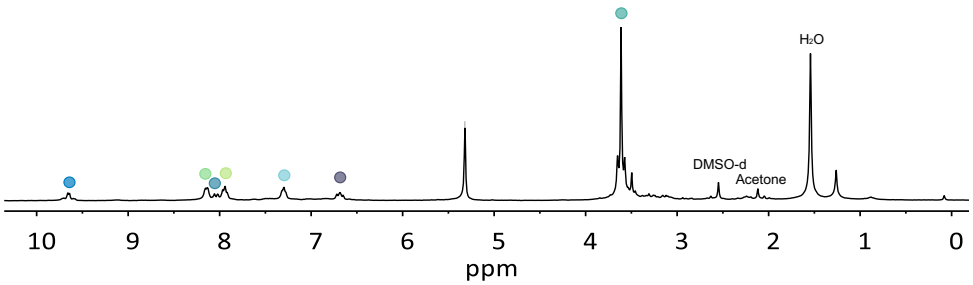


Figure S6: ^{1}H NMR (300 MHz, CD_2Cl_2) of **2**: δ 9.66 (dt, *J* = 23.3, 11.0 Hz, 1H), 8.14 (d, *J* = 8.1 Hz, 1H), 8.04 (dd, *J* = 10.8, 2.4 Hz, 1H), 7.94 (t, *J* = 7.8 Hz, 1H), 7.30 (t, *J* = 6.6 Hz, 1H), 6.68 (ddd, *J* = 11.8, 8.5, 2.5 Hz, 1H), 3.61(s, 6H).

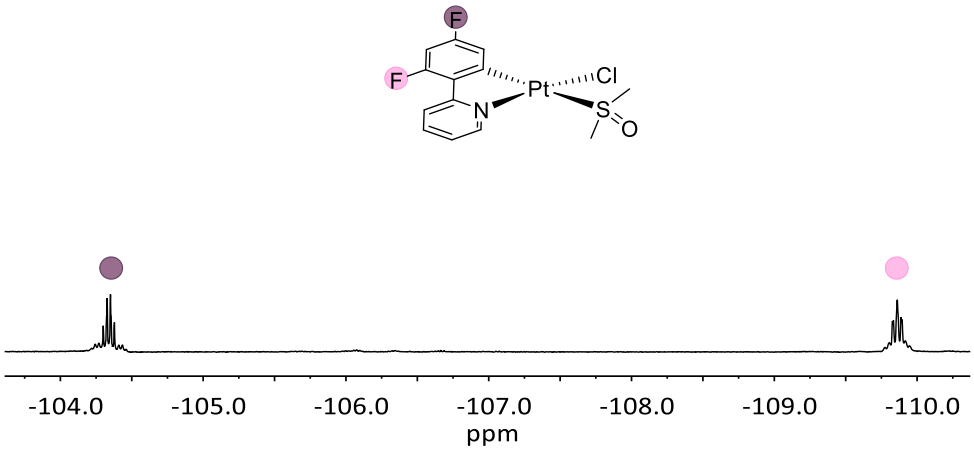


Figure S7. ^{19}F NMR (376 MHz, Chloroform-*d*) of **2**: δ -104.19 – -104.48 (m), -109.87 (tq, *J* = 20.5, 11.2, 10.3 Hz).

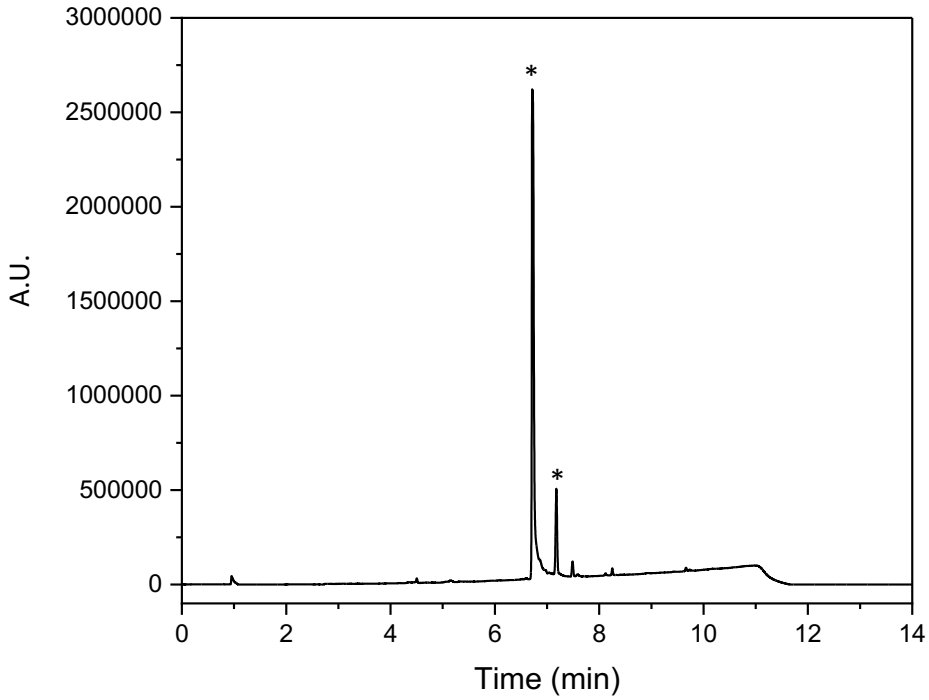


Figure S8. UPLC-MS chromatogram at 254 nm of compound **2.** Peaks labeled with an asterisk (*), eluting at 6.72 and 7.18 minutes, correspond to an ion at m/z = 466.9679, consistent with the fragment $[Pt(dfppy)(CH_3CN)_2]^+$. These arise from the in-source fragmentation of two distinct speciation products of **2** (i.e., Cl and DMSO loss), generated under the UPLC conditions.

Synthesis of [Pt(dfppy)(dppe)(Cl)] (3)

Complex **3** was obtained following the procedure reported by Shahsavari et al.[15] In brief, the dppe ligand (77 mg, 0.2 mmol) was added to a solution of **2** (100 mg, 0.2 mmol) in acetone (15 mL) and the reaction mixture was stirred for 3 h at room temperature. Next, the solution volume was concentrated to ~ 2 mL by solvent evaporation and n-hexane (5 mL) was added to precipitate **3** as a greenish solid. Yield: 81%.

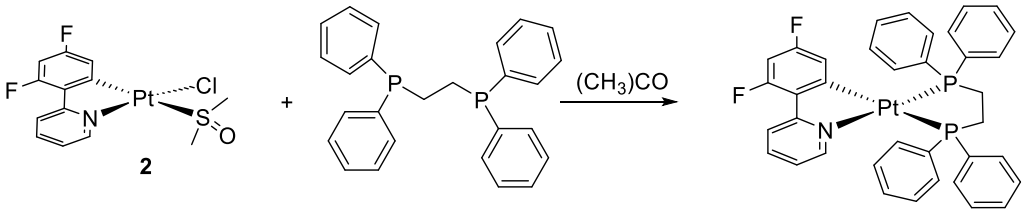


Figure S9. Synthesis of **3** following the procedure of Shahsavari et al.[15]

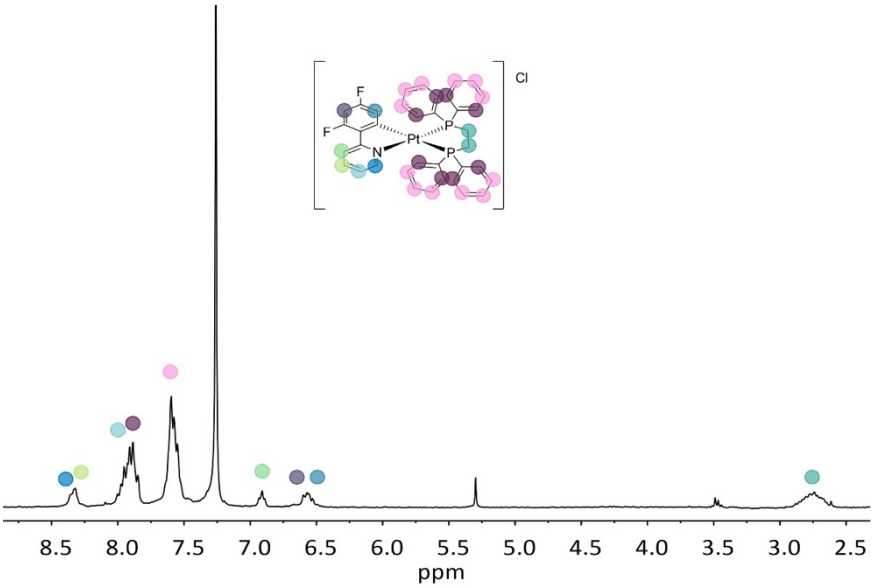


Figure S10. ^{1}H NMR (300 MHz, $CDCl_3$) of **3**: δ 8.34 (m, 2H), 7.92 (m, 9H), 7.59 (m, 12H), 6.91 (t, *J* = 6.7 Hz, 1H), 6.56 (m, 2H), 2.75 (m, 4H).

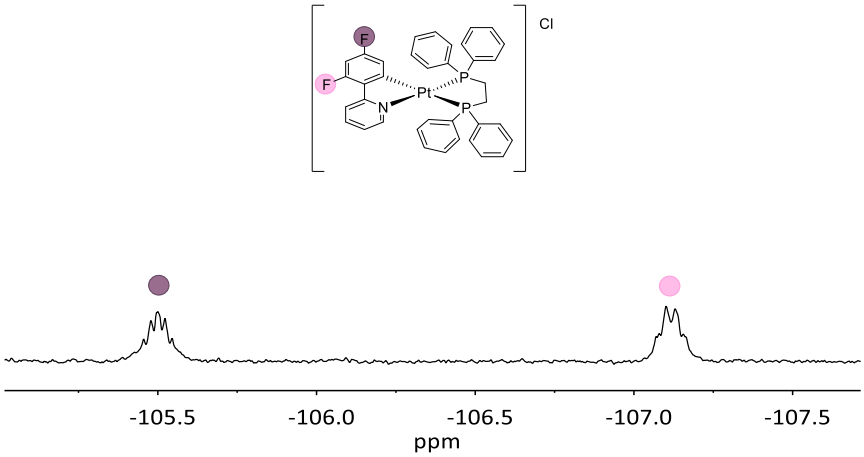


Figure S11. ^{19}F NMR (376 MHz, $CDCl_3$) of **3**: δ -105.50 (m, 1F), -107.12 (m, 1F).

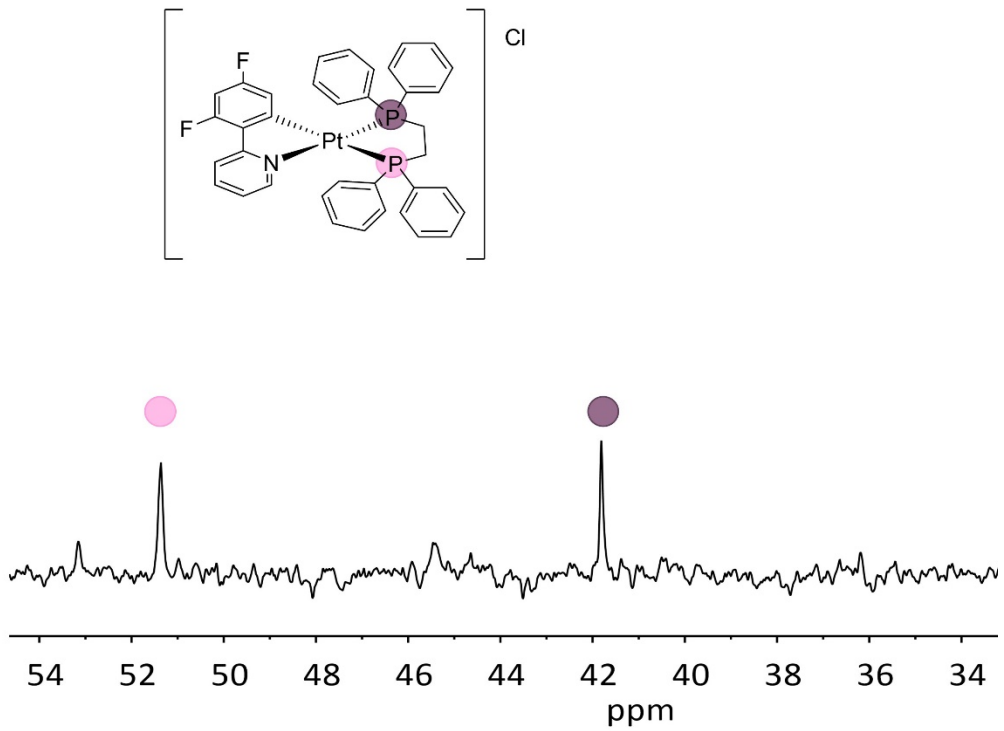


Figure S12. ^{31}P NMR (162 MHz, $CDCl_3$) of **3**: δ 51.36 (s,1P), 41.81 (s, 1P).

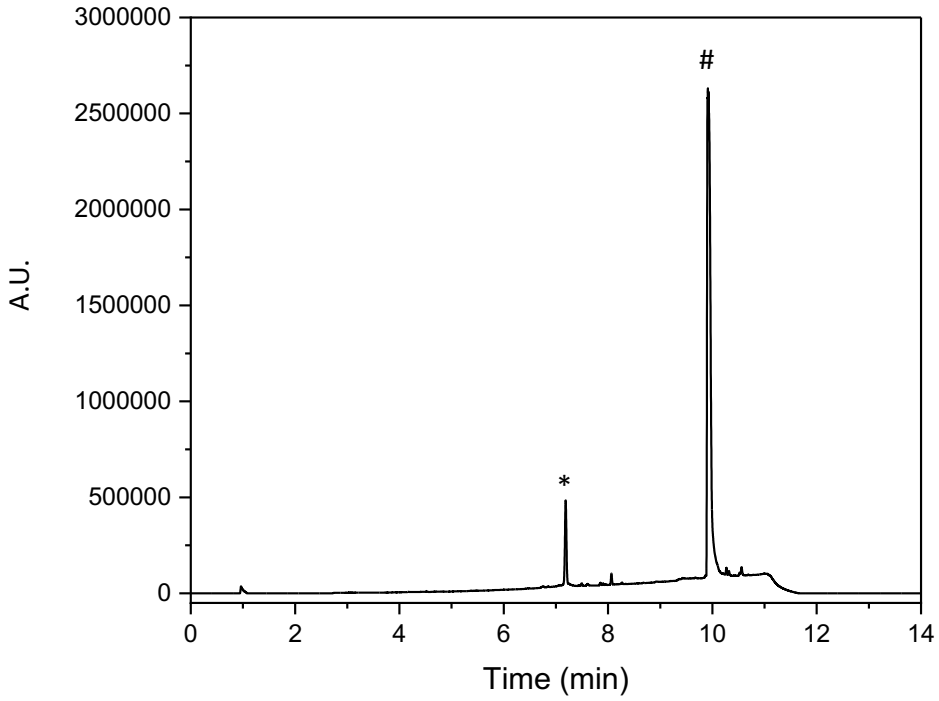


Figure S13. UPLC-MS chromatogram at 254 nm of **3**. The peak labeled with a hash symbol (#), eluting at 9.91 minutes, corresponds to an ion at m/z = 783.9408, consistent with the molecular ion $[M]^+$.Peak labeled with an asterisk (*), eluting at 7.18 minutes, corresponds to an ion at m/z = 466.9679, consistent with the fragment $[Pt(dfppy)(CH_3CN)_2]^+$. This arises from the in-source fragmentation of a speciation product of **3** (i.e., dppe loss), generated under the UPLC conditions.

Synthesis of [Pt(dfppy)(oxalate)] (4)

Complex **2** (100 mg, 0.20 mmol) was dissolved in 1.5 mL of DMF, while potassium oxalate (110.58 mg, 0.60 mmol) was dissolved separately in 1.5 mL of water. Both solutions were then combined in a round-bottom flask and heated to 50°C. Promptly, the solution turned green, and the reaction was allowed to proceed for 3 hours. The solution volume was subsequently reduced by rotary evaporation at 40°C to remove water, resulting in a color change from green to yellow. The addition of diethyl ether led to the precipitation of a greenish solid, which was washed repeatedly with acetone and diethyl ether. Yield: 60%.

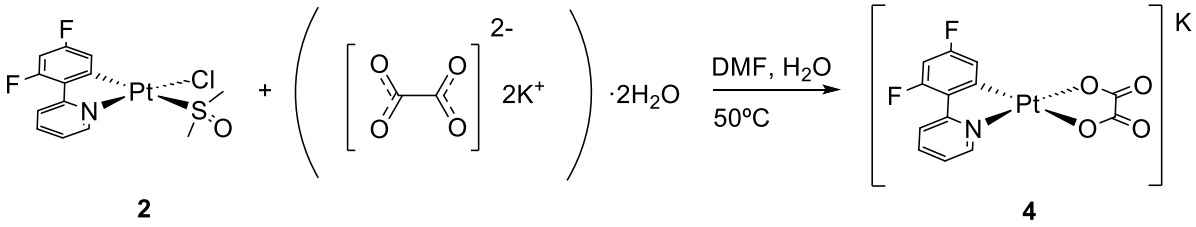


Figure S14: Synthesis of **4**.

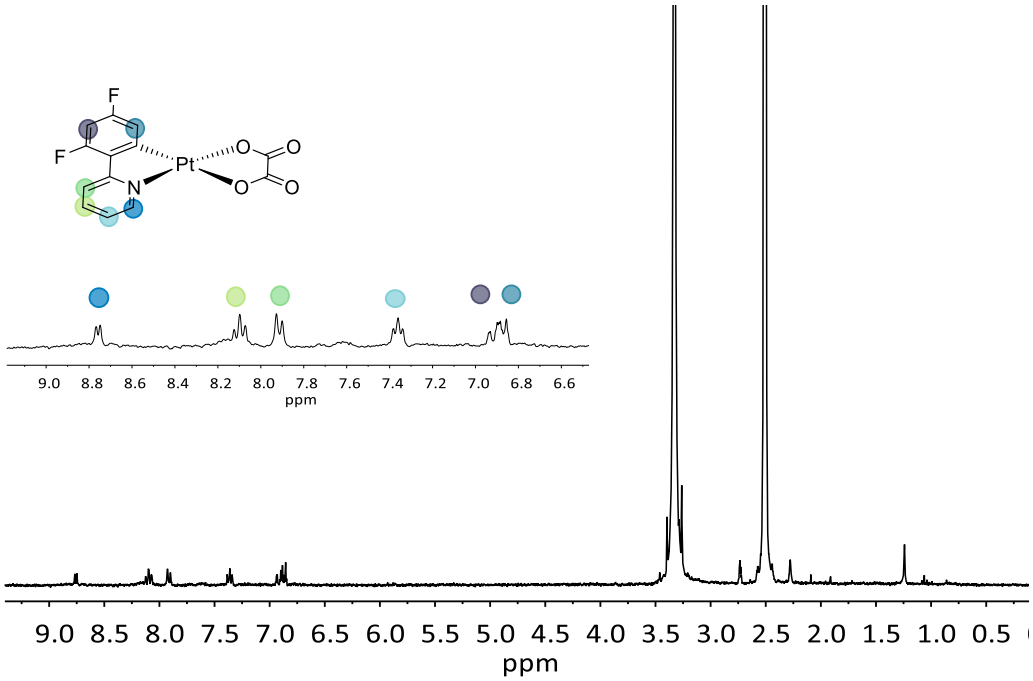


Figure S15: ^{1}H NMR (300 MHz, DMSO-d_6) of **4**: δ 8.76 (d, *J* = 5.8 Hz, 1H), 8.10 (t, *J* = 7.8 Hz, 1H), 7.91 (d, *J* = 8.2 Hz, 1H), 7.36 (t, *J* = 6.7 Hz, 1H), 6.89 (dd, *J* = 13.9, 9.9 Hz, 2H).

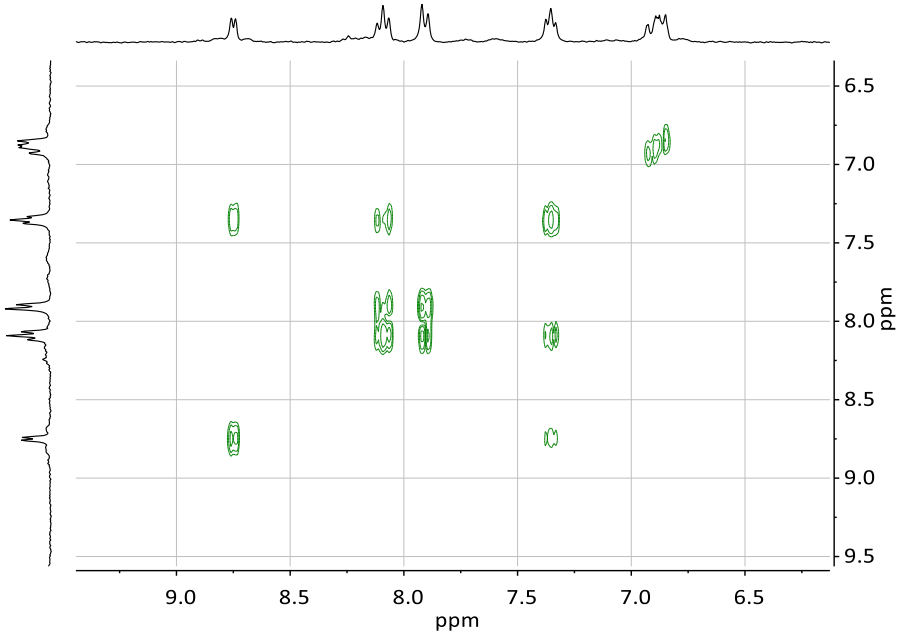


Figure S16. ^{1}H-^{1}H COSY of **4** in DMSO-d_6.

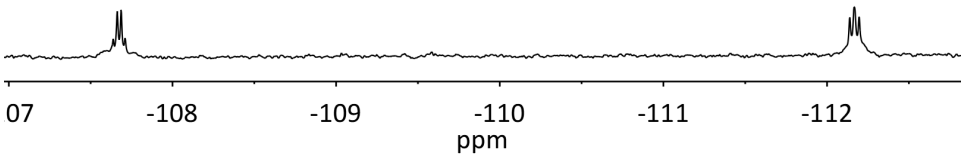


Figure S17. ^{19}F NMR (376 MHz, DMSO-d_6) of **4**: δ –107.67 (q, *J* = 9.6 Hz,1F), –112.17 (t, *J* = 11.1 Hz, 1F).

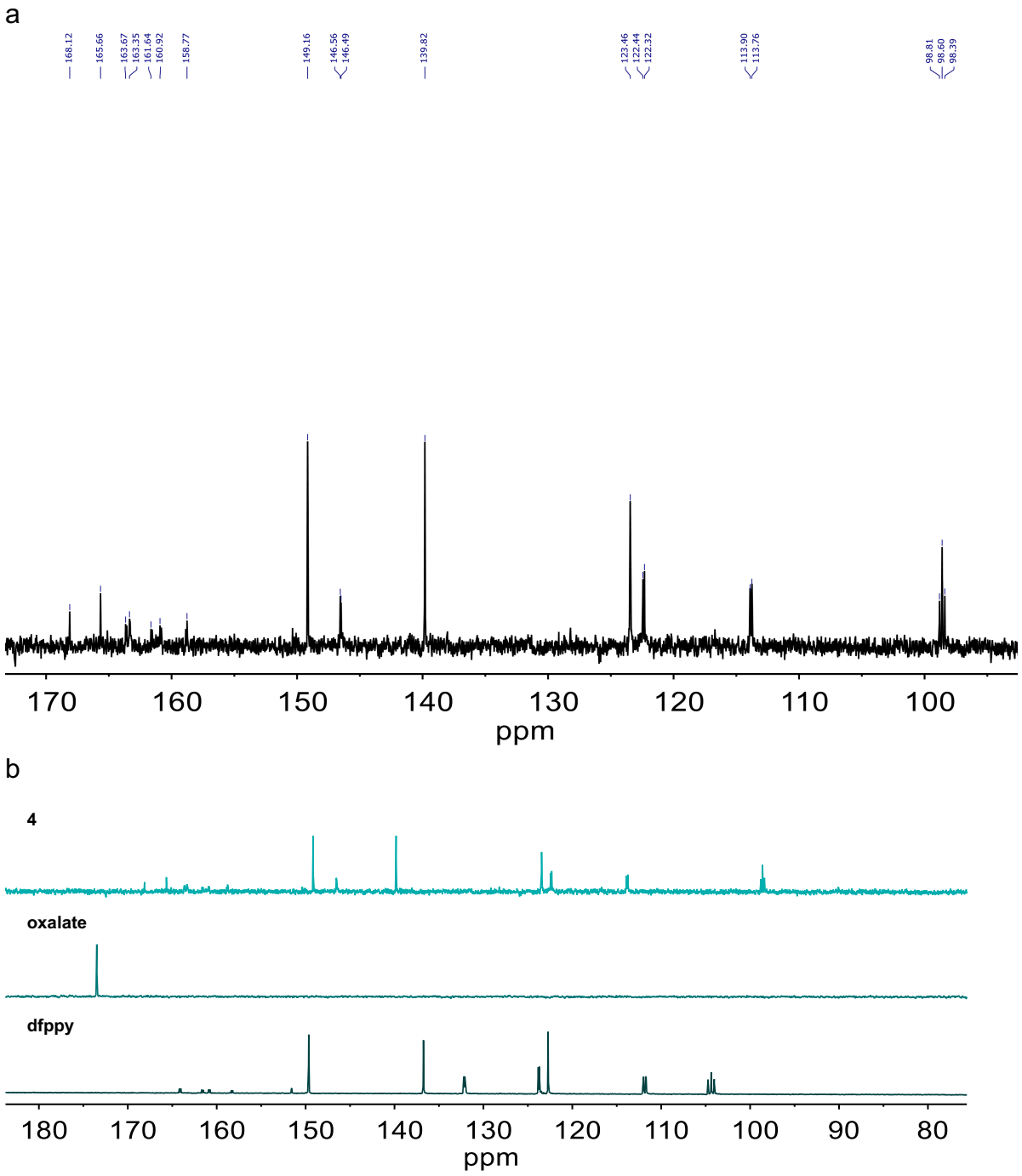


Figure S18. (a) ^{13}C NMR (126 MHz, DMSO-d_6) of **4**: δ 161.75, 150.36, 149.17, 144.00, 142.81, 139.85, 123.55, 122.90 (d), 122.42, 116.61 (d), 113.76, 101.59 (t), 98.62; (b) Comparison between ^{13}C NMR spectra of dfppy (DMSO-d_6), sodium oxalate (D_2O) and 4 (DMSO-d_6).

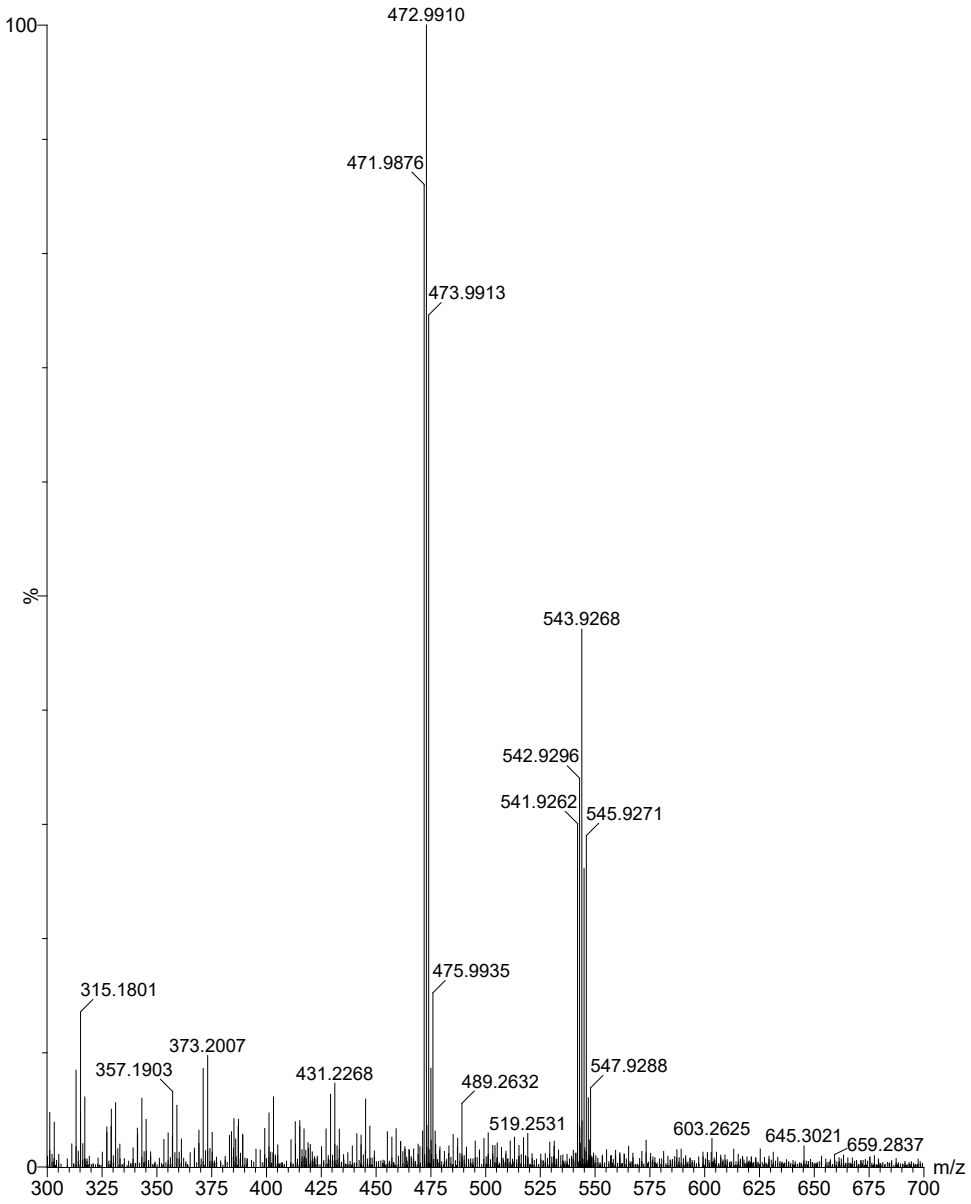


Figure S19. HR-MS (ESI-) spectrum of **4**. m/z $[M]^-$ calculated for $C_{13}H_6F_2NO_4Pt^-$: 472.9913; found 472.9910.

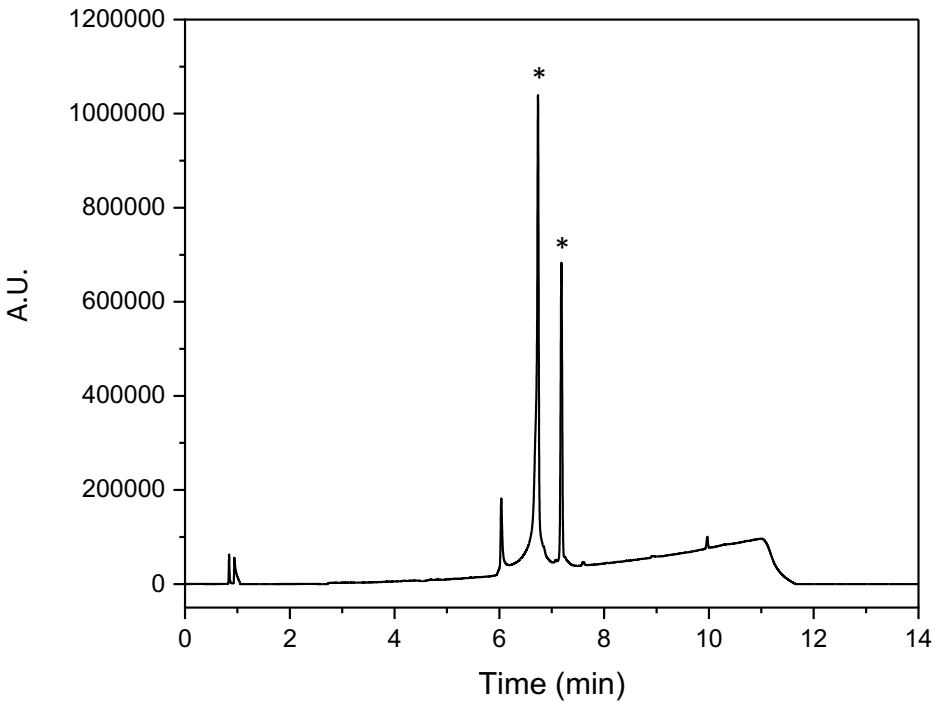


Figure S20. UPLC-MS chromatogram at 254 nm of compound **4**. Peaks labeled with an asterisk (*), eluting at 6.74 and 7.18 minutes, correspond to an ion at m/z = 466.9679, consistent with the fragment $[Pt(dfppy)(CH_3CN)_2]^+$. These arise from the in-source fragmentation of two distinct speciation products of **4** (i.e., oxalate loss), generated under the UPLC conditions.

Synthesis of *cis,cis,trans*-$[Pt(NH_3)_2Cl_2(O_2CC_2H_4COOH)_2]$ (5)

Complex **5** was obtained by adapting the synthesis method described by Tolan et al.[16]

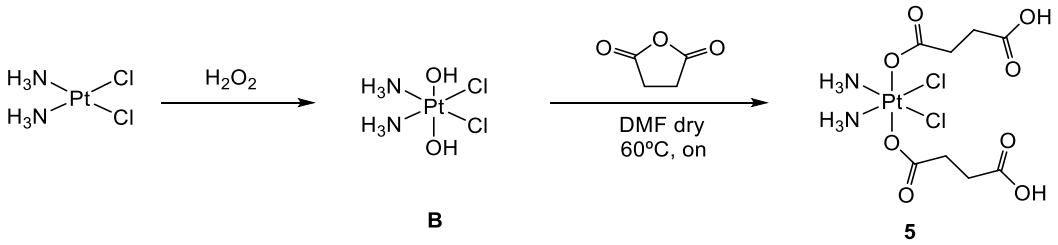


Figure S21. Reaction scheme for the synthesis of *cis,cis,trans*-$[Pt(NH_3)_2Cl_2(succinate)_2]$ (**5**).

cis,cis,trans-$[Pt(NH_3)_2Cl_2(OH)_2]$ **(B)**: Hydrogen peroxide (30% solution, 15 ml) was added dropwise to a round bottom flask containing cisplatin (250 mg; 0.833 mmol). The reaction mixture was heated to 75 ºC for 5 hours. The resulting bright yellow solution was left at room temperature in the dark overnight to allow the product to crystallize. The crystals were separated by centrifugation, washed with cold water, ethanol, and ether, then dried under vacuum.

cis,cis,trans-$[Pt(NH_3)_2Cl_2(succinate)_2]$ (**5**): In a 5 mL round bottom flask 55 mg (0.146 mmol) of **B** and 88 mg (0.878 mmol) of anhydride succinic were dissolved in 2 mL of dry DMF under N_2 atmosphere and the mixture was left stirring at 60 ºC overnight to give a clear yellow solution. The next day, DMF was removed under reduced pressure and the remaining oil was, first, dissolved in the minimum quantity of acetone and then precipitated with diethyl ether (3 x 5 mL). We recovered the final product as a clear yellow solid by centrifugation (70 mg). Yield: 84%.

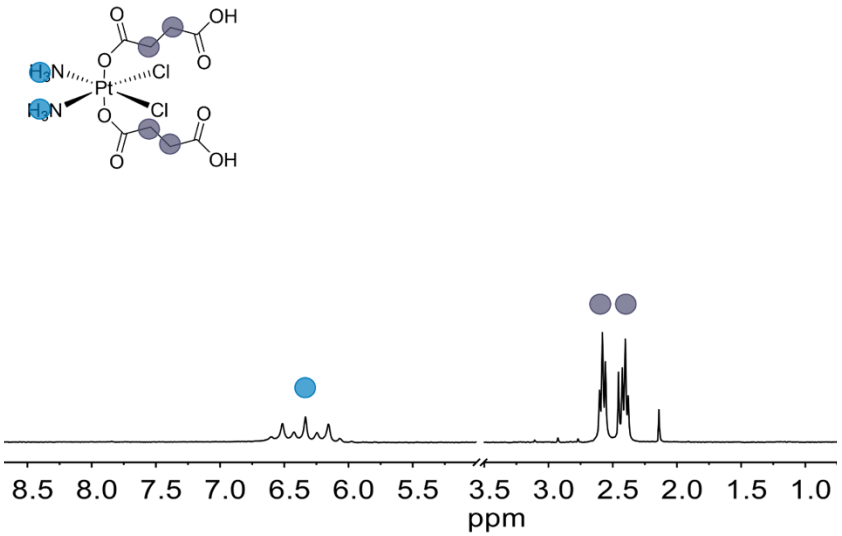


Figure S22. ^{1}H NMR (300 MHz, PBS/D_2O) of **5**: δ 6.38 (broad, 6H), δ 2.58 (t, *J* = 6.7 Hz, 4H), 2.42 (t, *J* = 6.8 Hz, 4H).

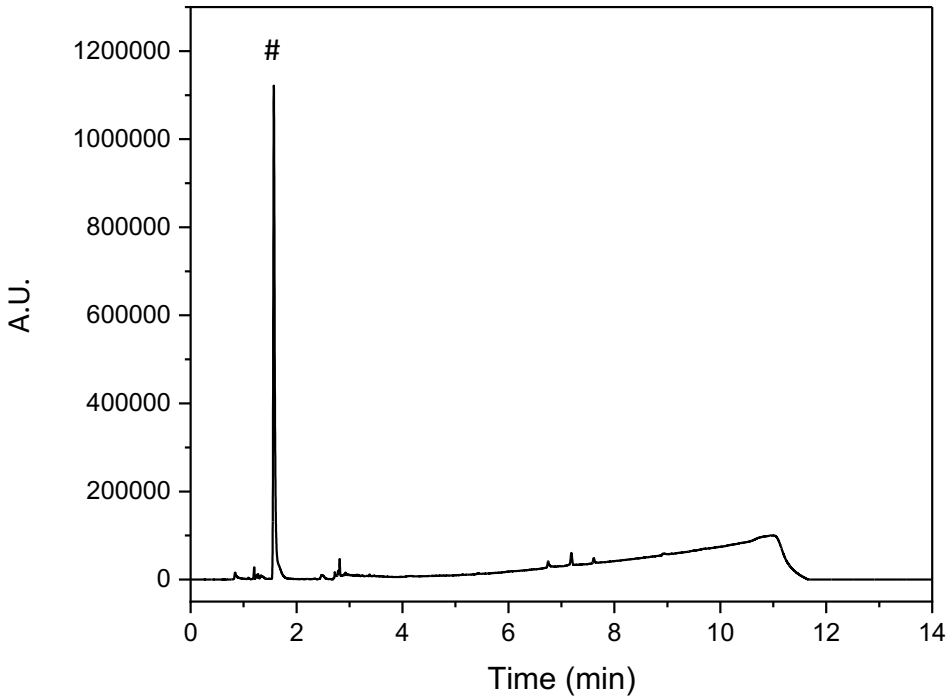


Figure S23. UPLC-MS chromatogram at 254 nm of **5**. The peak labeled with a hash symbol (#), eluting at 1.57 minutes, corresponds to an ion at m/z = 534.8907, consistent with the molecular ion $[M + H]^+$.

Synthesis of *cis,cis,trans*-$[Pt(NH_3)_2Cl_2(CH_3CO_2)(O_2CC_2H_4COOH)]$ (6)

Complex **6** was obtained adapting the synthesis method described by Tolan et al.[16]

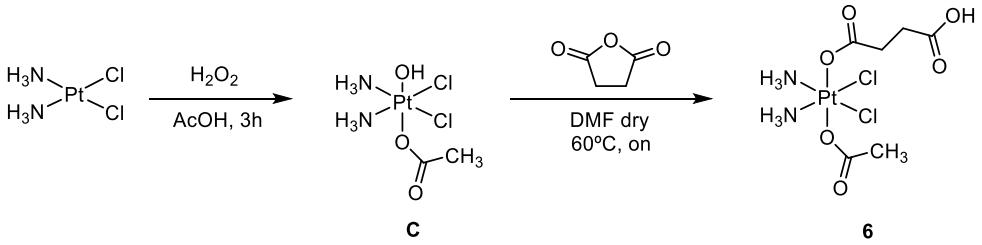


Figure S24. Reaction scheme for the synthesis of *cis,cis,trans*-$[Pt(NH_3)_2Cl_2(acetate)(succinate)]$ (**6**).

cis,cis,trans-$[Pt(NH_3)_2Cl_2(acetate)OH]$ **(C)**: 200 mg of cisplatin were dissolved in 100 mL of acetic acid and 2 mL of H_2O_2 30% (25 eq) were added dropwise. The resulting mixture was kept stirring in dark conditions for 3 hours. Afterwards, the unreacted cisplatin was removed by centrifugation and the solution was concentrated in the rotavapor, forming a clear yellow oil, which was precipitated in diethyl ether and washed with acetone and dichloromethane to obtain 170 mg of a white solid. Yield: 68%.

cis,cis,trans-$[Pt(NH_3)_2(Cl_2)(acetate)(succinate)]$: In a 5 mL round bottom flask 55 mg (0.146 mmol) of **C** and 88 mg (0.878 mmol) of anhydride succinic were dissolved in 2 mL of dry DMF under N_2 atmosphere and the mixture was left stirring at 60ºC overnight to give a clear yellow solution. Next day the DMF was removed under reduced pressure and the remaining oil was precipitated with diethyl ether (3 x 5 mL) by previously dissolving in the minimum quantity of acetone. Afterwards, the product was recovered as a clear yellow solid by centrifugation (70 mg). Yield: 84%.

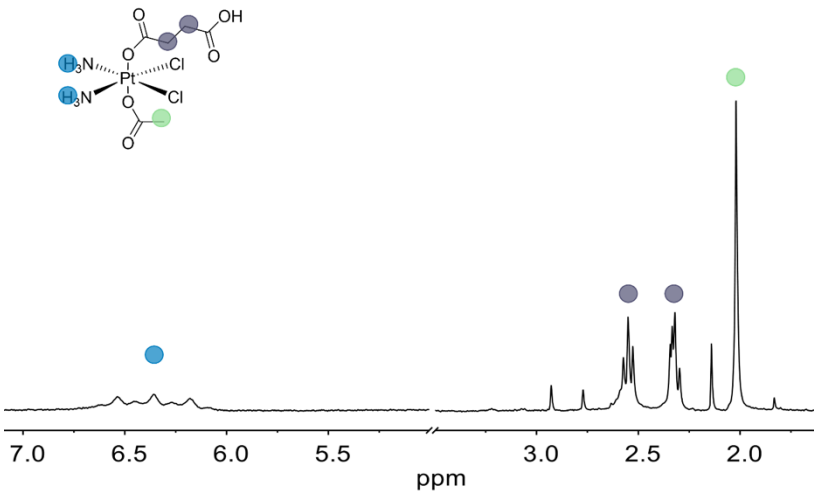


Figure S25: ^{1}H NMR (300 MHz, PBS/D_2O) of **6**: δ 6.36 (broad, 6H), 2.55 (t, *J* = 6.9 Hz, 2H), 2.32 (m, 2H), 2.02 (s, 3H).

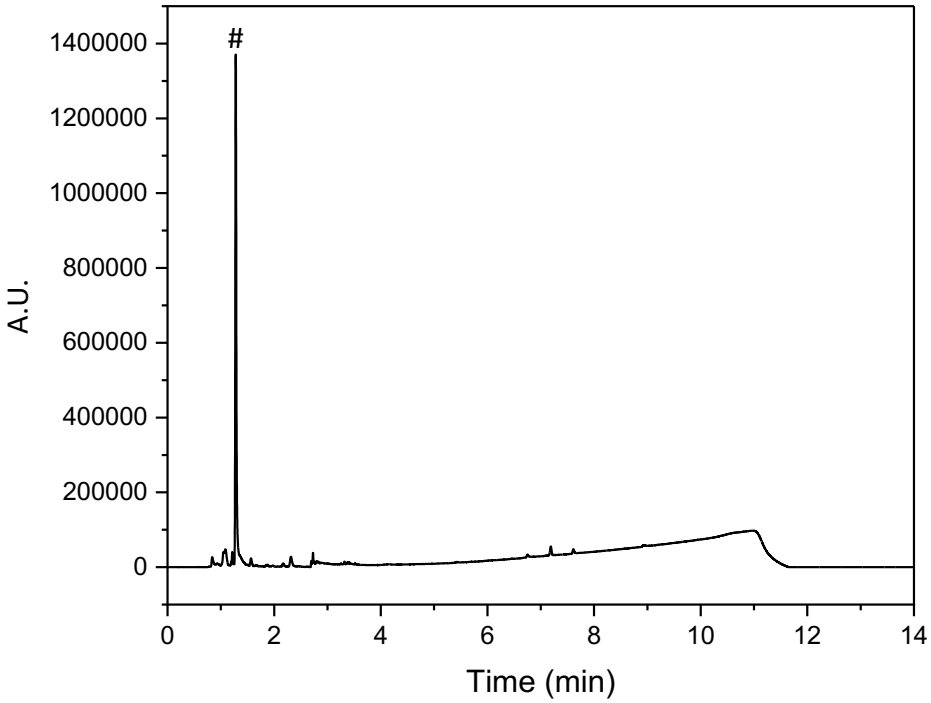


Figure S26. UPLC-MS chromatogram at 254 nm of **6**. The peak labeled with a hash symbol (#), eluting at 1.28 minutes, corresponds to an ion at m/z = 476.8864, consistent with the molecular ion $[M + H]^+$.

Table S1. Solubilities of **1**–**6** in CAGE 1:1 and CAGE 1:2.

Compound	CAGE 1:1	CAGE 1:2
1	48mM	48mM
2	100 mM	48 mM
3	21 mM	26 mM
4	26 mM	< 21 mM
5	48 mM	< 21 mM
6	100 mM	26 mM

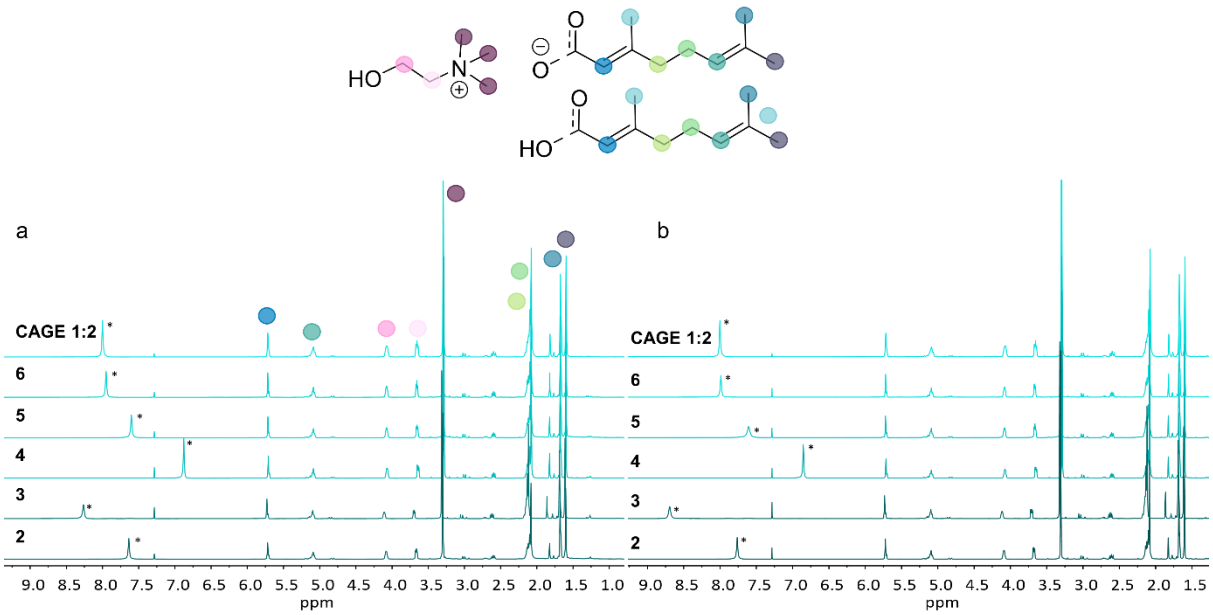


Figure S27. ^{1}H NMR (400 MHz, $CDCl_3$) spectra of **2**–**6** in $CDCl_3$-CAGE mixture at (a) t = 0 and (b) t = 48 h. (* Water peak).

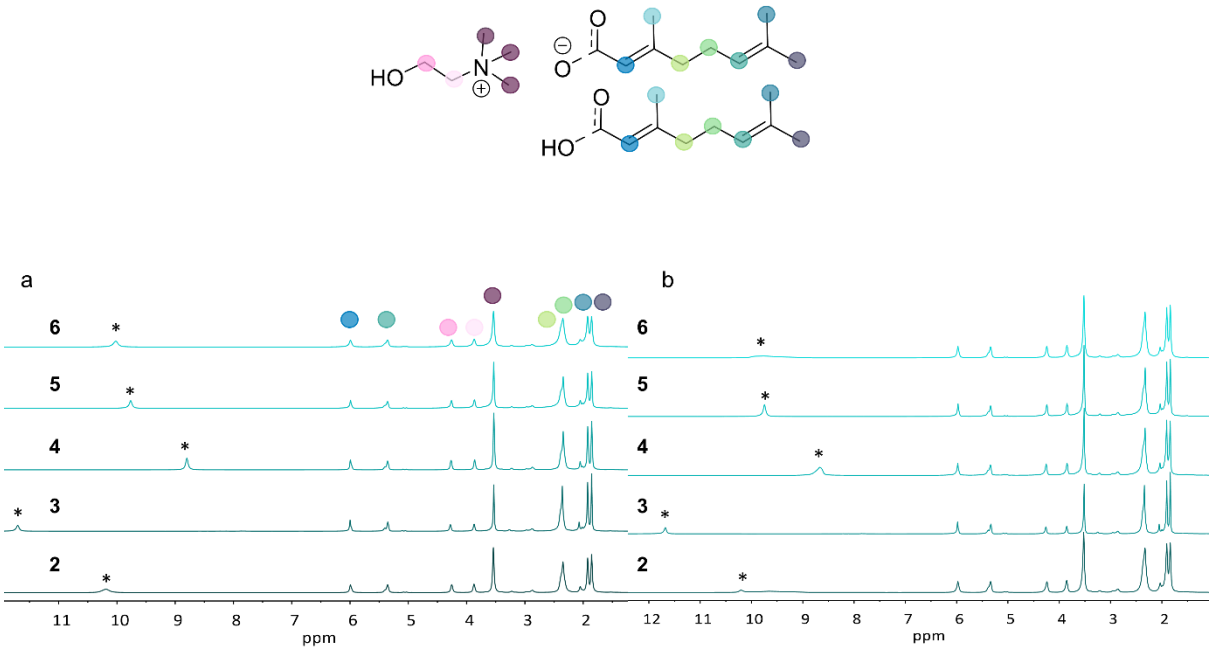


Figure S28. ^{1}H NMR (400 MHz, $CDCl_3$) spectra of **2**–**6** in neat CAGE at (a) t = 0 and (b) t = 48 h. (* Water peak).

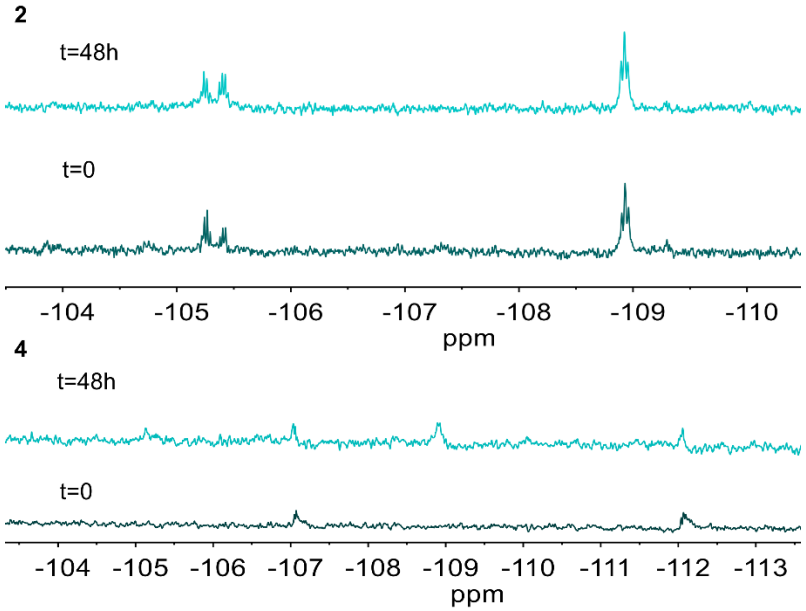


Figure S29. ^{19}F NMR (400 MHz, $CDCl_3$) spectra of **2** and **4** in $CDCl_3$-CAGE 1:2 mixture over time.

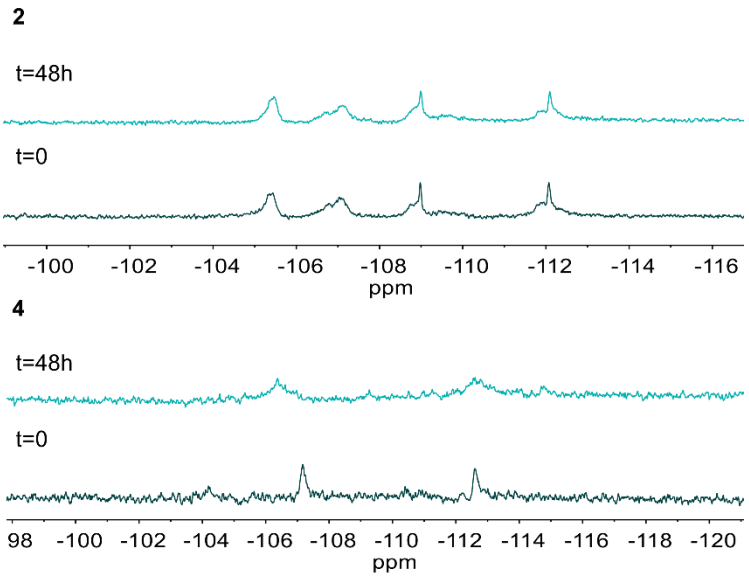


Figure S30. ^{19}F NMR (500 MHz, neat) spectra of **2** and **4** in neat CAGE 1:2.

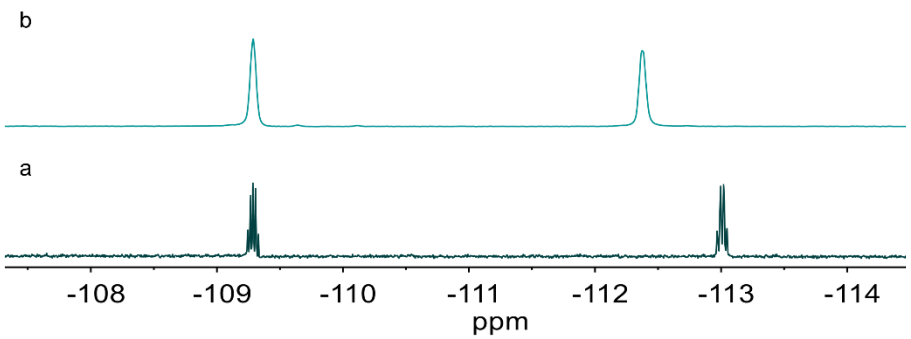


Figure S31. ^{19}F NMR (400 MHz) spectra of dfppy in (a) $CDCl_3$-CAGE 1:2 mixture and (b) neat CAGE 1:2.

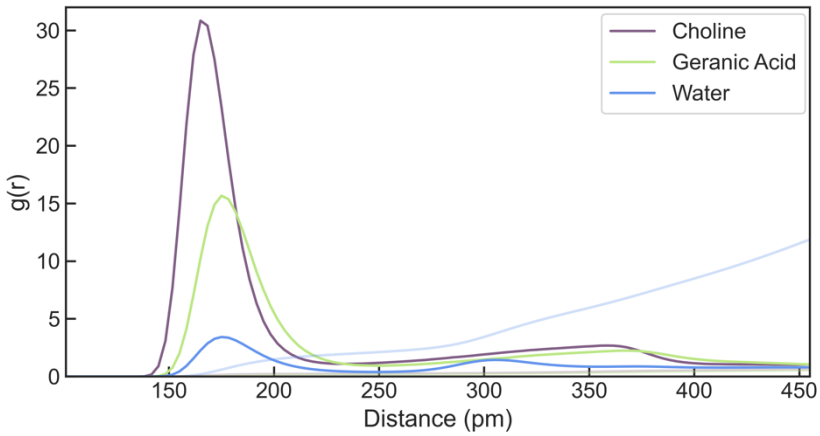


Figure S32. Radial distribution function (rdf) of the hydroxyl H of choline, geranic acid and water, around the oxygen atoms of the geranate, together with their integral values.

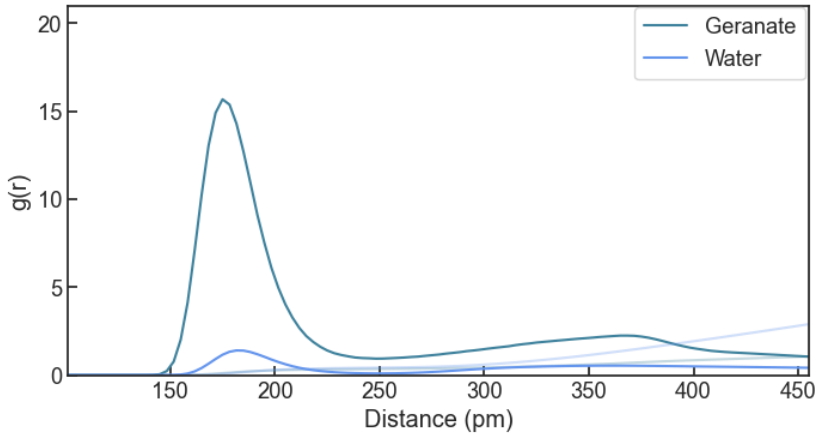


Figure S33. Radial distribution function (rdf) of the oxygen atoms of geranate and water around the hydroxyl H atom of the geranic acid, together with their integral values.

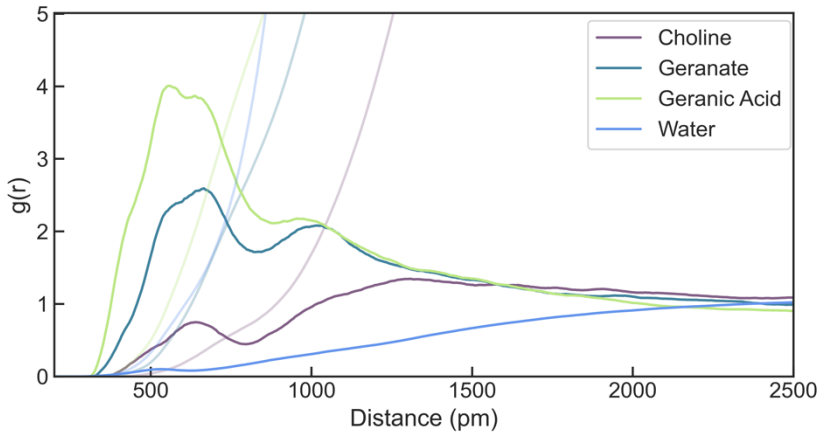


Figure S34. Radial distribution function (rdf) of the hydrophobic moieties of choline, geranate, geranic acid, and water, around the center of mass of the fluorinated ring of dfppy ligand, together with their integral values.

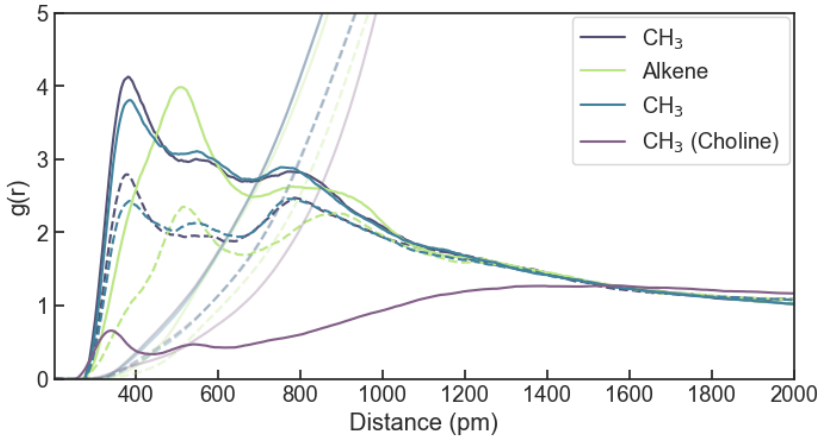


Figure S35. Radial distribution function (rdf) of the hydrophobic moieties of geranate (dashed lines), geranic acid (solid lines) and choline around the 2'-F atom of dfppy, together with their integral values.

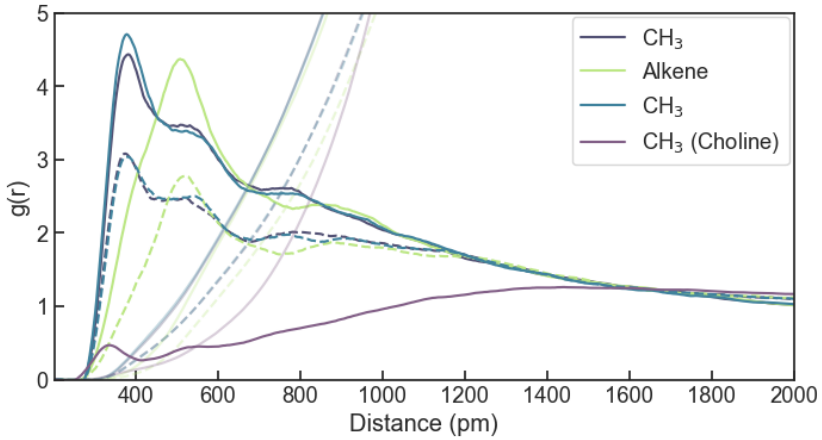


Figure S36. Radial distribution function (rdf) of the hydrophobic moieties of geranate (dashed lines), geranic acid (solid lines) and choline around the 4'-F atom of dfppy, together with their integral values.

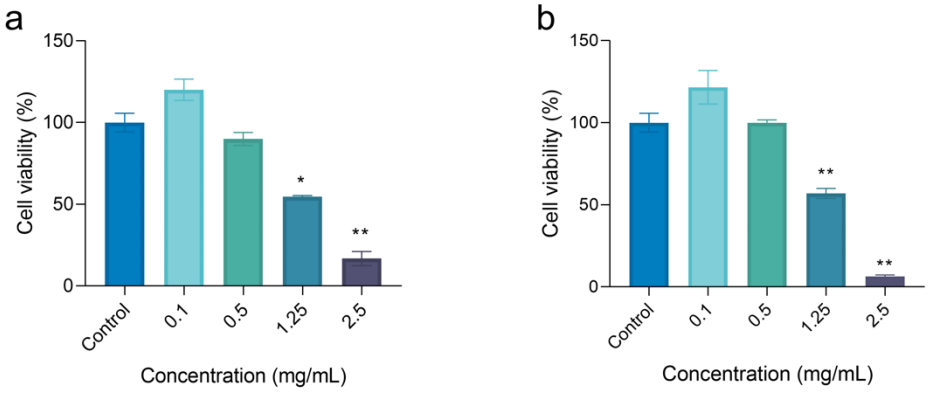


Figure S37. Cell viability of U87-MG cells after 48 hours of incubation with CAGE (1:1) (a) and CAGE (1:2) (b) at concentrations ranging from 0.1 to 2.5 mg/mL (**P <0.007; *P < 0.23; one-way ANOVA followed by Welch's t test). Control: untreated cells.

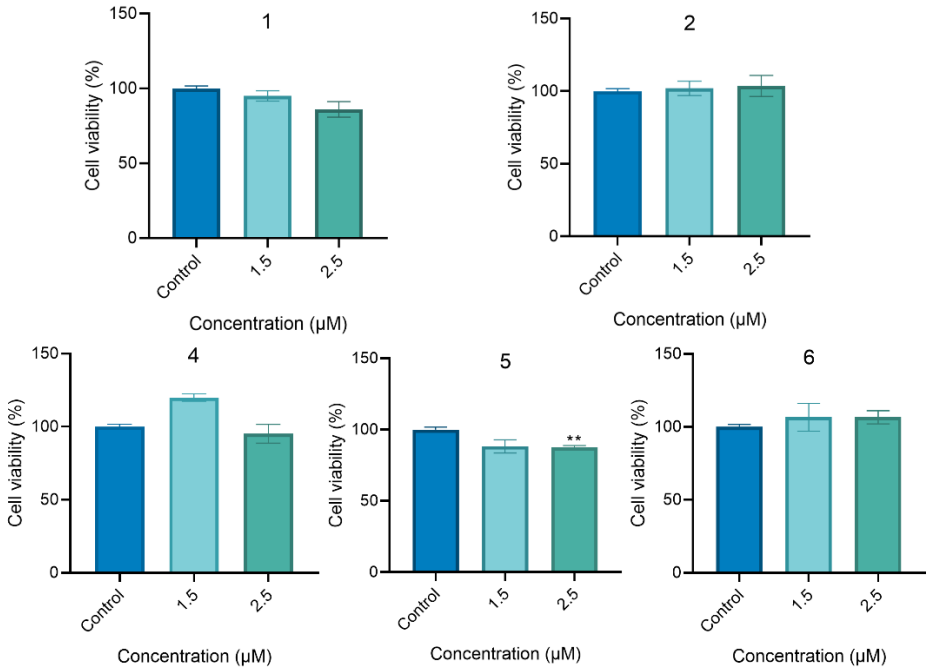


Figure S38. Cell viability of U87-MG cells after 48 hours of incubation with **1**, **2** **4**–**6** at concentrations of 1.5 and 2.5 μM. (**P < 0.0057; one-way ANOVA followed by Welch's t test). Control: untreated cells.

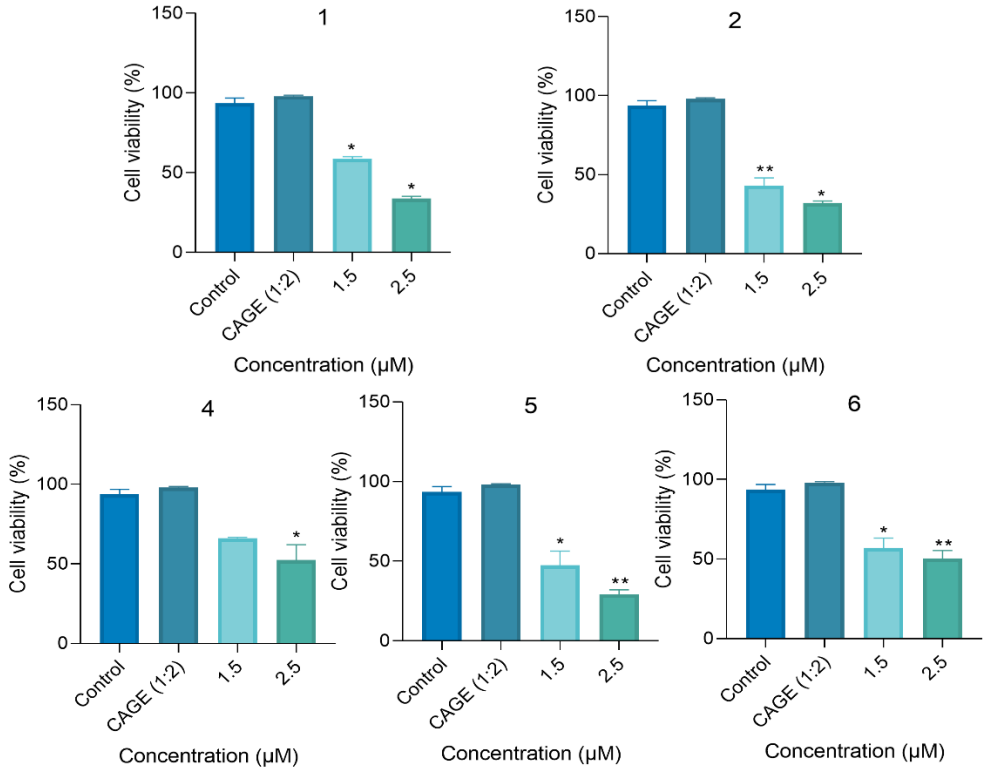


Figure S39. Cell viability of U87-MG cells after 48 hours of incubation with **1**, **2** **4**–**6** at concentrations of 1.5 and 2.5 μM, dissolved in CAGE 1:2 (**P< 0.0052; *P < 0.23; one-way ANOVA followed by Welch's t test). Control: untreated cells.

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