Supporting Information

Biotinylated Pt(IV) prodrugs with elevated lipophilicity and cytotoxicity

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Vita Nikitina: CVA measurements: Lead;
Nikolay Ul'yanovskiy: HPLC data: Lead
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Alevtina Semkina: ICP MS Methodology: Equal; Data Curation: Equal;
Ksenia Vlasova: ICP MS Methodology: Equal; Data Curation: Equal;
Nikolay Zyk: Supervision: Equal
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Experimental Procedures

Materials: Cisplatin, N,N'-Dicyclohexylcarbodiimide (DCC), N-Hydroxysuccinimide (NHS), hexafluorophosphate (HBTU), dimethylaminopyridine (DMAP), H_2O_2 37% w/w were purchased from commercial sources (Aldrich, Alfa, TCI, etc.), and used without further purification. Naproxen was isolated from the pounded tablets by acidification of the suspension and extraction with methylene chloride. Hydrochloric acid, N,N-dimethylformadide (DMF), toluene, MeOH, EtOH, CH_2Cl_2, DMEM, PBS, TEA and PI were purchased in Sigma Aldrich (USA), dimethylsylfoxide (DMSO) was purchased from VMR (Life Science). Flash column chromatography (300–400 mesh silica gel) was performed for compound purification. Analytical thin-layer chromatography (TCL) was performed on Merck silica gel aluminum plates with F-254 indicator. Compounds were visualized by irradiation with UV light or iodine staining.

Instrumentation: ^{1}H, ^{13}C and ^{195}Pt NMR spectra were recorded on a Brucker-Avance instrument at 400 MHz (^{1}H), 101 MHz (^{13}C) and 86 MHz (^{195}Pt). Deuterochloroform ($CDCl_3$), deuteromethanol (CD_3OD) and dimethylsulfoxide-d6 (DMSO-d6) were used solvents. Chemical shifts are given in ppm on the δ scale relative to hexamethyldisiloxane as an internal standard for ^{1}H, ^{13}C NMR, $K_2[PtCl_6]$ solution for ^{195}Pt NMR. High-resolution mass spectra were recorded on an Orbitrap Elite mass spectrometer (Thermo Scientific). IR spectra were obtained on ThermoNicolet ISFT-IR (USA). Liquid chromatography–mass spectrometry was performed by a Shimadzu Prominence LC-20 system with a column oven and fraction collector coupled to a single quadrupole mass spectrometer Shimadzu LCMS-2020 with a dual DUIS-ESIAPCI ionization source. XANES spectra were measured at the L3 edge of platinum at the STM station of the Kurchatov Center of Synchrotron Radiation. The storage ring of the synchrotron is powered by an energy of 2.5 GeV, the average current in the ring is about 80 mA. P content was determined by inductively coupled plasma mass spectrometry using an ICP-MS 7500a instrument (Agilent Technologies). The device was set up to work with solutions that have a low total concentration of solutes (RF Power 1500Vt, Carrier Gas Flow Rate 1.10 l/min, Sample Flow Rate 0.4 ml/min, Sampling Depth 4mm, t°C, 2°C). Flow cytometry data were obtained using a NovoCyte 2000R flow cytometer (ACEA Biosciences, USA), and the obtained data were analyzed using NovoExpress v.1.2.4 software.

Synthetic procedures

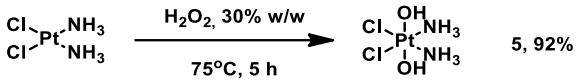
Synthesis of Biotin-N-hydroxysuccinimide ester (1). Biotin NHS ester was synthesized according to the previous report.[1] Briefly, 500 mg (2.05 mmol, 1 equiv.) of biotin, 588 mg of EDC (3.07 mmol, 1.5 equiv.) and 271 mg of NHS (2.35 mmol, 1.15 equiv.) were dissolved in 15 ml of anhydrous DMF. The solution was stirred at r.t. for 24 hours and then added to 100 ml of ice-water mixture. The precipitate was collected, washed twice with water and methanol, and then air-dried. 425 mg of biotin-NHS-ester were obtained as a white powder. Yield: 61%. ^{1}H NMR (400 MHz, DMSO-d_6, δ ppm): 6.42 (s, 1H), 6.36 (s, 1H), 4.29 (br. s., 1H), 4.19-4.07 (m, 1H), 3.12-3.05 (m, 1H), 2.91-2.75 (m, 5H), 2.66 (t, 2H, J = 7.4 Hz), 2.57 (d, 1H, J = 12.5 Hz), 1.68-1.52 (m, 3H), 1.42 (d, 3H, J = 6.2 Hz).

Synthesis of ctc-$[Pt(NH_3)_2$(biotin)$(OH)(Cl)_2$ (2). Oxoplatin (1 equiv) and the corresponding NHS ester (1.2 equiv) were dissolved in DMSO (690 µl/10 mg of **1**) and the mixture was stirred at 60°C overnight. The unreacted oxoplatin **1** was separated by centrifugation, the solution was added dropwise to the excess of diethyl ether (10-15 ml of ether per 1 ml of DMSO). After shaking the mixture, the two phases system was obtained, and the diethyl ether phase was removed. The procedure was repeated until sticky beige precipitate was obtained which was suspended in methanol and precipitated by diethyl ether. The monocarboxylate complex was isolated by centrifugation, washed with diethyl ether and air-dried. From 80 mg (0.24 mmol) of oxoplatin 82 mg of **2** were obtained as beige powder following the general procedure for monocarboxylated platinum(IV) prodrugs. Yield: 61%. ^{1}H NMR (400 MHz, DMSO-d_6, δ ppm): 6.40 (s, 1H), 6.35 (s, 1H), 6.15-5.77 (m, 6H), 4.36-4.27 (m, 1H), 4.15-4.07 (m, 1H), 3.12-3.05 (m, 1H), 2.81 (dd, 1H, J_1 = 5.0, J_2 = 12.5 Hz), 2.58 (s, 1H), 2.14 (t, 2H, J = 7.3 Hz), 1.65-1.53 (m, 1H), 1.51-1.26 (m, 6H). HRMS (m/z): calcd for $C_{10}H_{23}Cl_2N_4O_4PtS$ $(M + H)^+$, 560.0459; found 560.0475. Purity (HPLC): 100%.

Synthesis of ctc-$[Pt(NH_3)_2$(biotin)(NPX)$(Cl)_2$ (3). 40 mg (0.071 mmol, 1 equiv.) of **2** and 54 mg (0.122 mmol, 1.7 equiv.) of naproxen anhydride were dissolved in 1.3 ml of anhydrous DMF and the solution was stirred overnight at r.t. The solvent was evaporated under reduced pressure, the residue was suspended in several drops of methanol and precipitated with diethyl ether. The precipitate was isolated by centrifugation, washed with diethyl ether, and air-dried. 18 mg of **3** were obtained as a beige powder. Yield: 33%. ^{1}H NMR (400 MHz, DMSO-d_6, δ ppm): 7.81-7.66 (m, 3H), 7.46 (d, 1H, J = 8.9 Hz), 7.25 (s., 1H), 7.11 (d, 1H, J = 6.8 Hz), 6.86-6.09 (m, 8H), 4.29 (m, 1H), 4.12 (m., 1H), 3.87-3.77 (m, 4H), 3.07 (m, 2H), 2.79 (d, 1H, J = 5.1 Hz), 2.56 (d, 1H, J = 12.5 Hz), 2.34-2.13 (m, 3H), 1.79-1.02 (m, 9H). ^{195}Pt NMR (86MHz, DMSO-d_6, δ ppm): 1220.66. HRMS (m/z): calcd for $C_{24}H_{35}Cl_2N_4O_6PtS$ $(M + H)^+$, 772.1297; found 772.1303. Purity (HPLC): 95.11%.

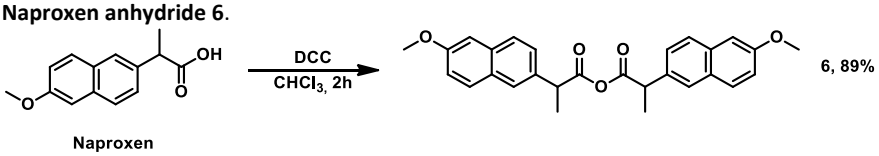
Synthesis of ctc-$[Pt(NH3)_2$(biotin)$(OC(O)C_{17}H_{35})(Cl)_2$ (4). Monocarboxylated Pt(IV) prodrug (1 equiv.) and stearic anhydride (1.5 equiv.) were dissolved in DMF (122 µl/10 mg of stearic anhydride) and mixture was stirred at 60°C overnight. The reaction was cooled to r.t., the precipitated stearic anhydride was separated by centrifugation. The DMF solution was evaporated under reduced pressure, then several drops of methanol were added to the residue followed by addition of 6-8 ml of diethyl ether. The precipitate was removed by centrifugation, the solution was evaporated. The residue was suspended in petroleum ether, the precipitate was isolated by centrifugation and air-dried. From 40 mg (0.071 mmol) 40 mg of **4** were obtained as a beige powder following the general procedure. Yield: 68%. ^{1}H NMR (400 MHz , DMSO-d_6, δ ppm): 6.40 (br. s., 8H), 4.35-4.26 (m, 1H), 4.14 (d, J = 4.5 Hz, 1H), 3.08 (d, 1H, J = 4.9 Hz), 2.89-2.86 (m, 1H), 2.81 (dd, 1H, J_1 = 5.1, J_2 = 12.3 Hz), 2.63-2.52 (m, 1H), 2.28-2.15 (m, 4H), 1.45 – 1.31 (m, 8H), 1.22 (s, 33H), 0.87-0.81 (m, 3H). ^{195}Pt NMR (86MHz, DMSO-d_6, δ ppm): 1227.28. HRMS (m/z): calcd for $C_{28}H_{57}Cl_2N_4O_5PtS$ $(M + H)^+$, 826.3069; found 826.3081. Purity (HPLC): 97.05%.

Oxoplatin 5



To 500 mg (1.67 mmol) of cisplatin, 30 ml of 30% hydrogen peroxide solution were added dropwise. The mixture was stirred at 75°C for 5 hours. The solution was left overnight at room temperature to form a yellowish precipitate. The precipitate formed was separated, the solvent was removed under reduced pressure, the residue was collected with a small amount of water and washed with water, methanol, and diethyl ether. 515 mg (92%) of oxoplatin **5** was obtained as a light-yellow powder.
IR: 3459 (OH), 3149, 1583 (c), 1440 (c), 1378 (s), 1074 (s, Pt-OH), 860 (s), 574 (br. s).

Naproxen anhydride 6.



Naproxen

The mixture of naproxen (500 mg, 2.17 mmol) and DCC (0.6 equiv) was dissolved in 20 ml of anhydrous CH2Cl2 and stirred for 2 hours at r.t. The dicyclohexylurea precipitate was filtered, the filtrate was evaporated under reduced pressure and the residue was suspended in EtOAc. The mixture was filtered, the filtrate was evaporated under reduced pressure. The crude product was purified by flash chromatography with DCM as eluent. 427 mg of naproxen anhydride **6** was obtained as a white powder. Yield: 89%. ^{1}H NMR (400 MHz ,CDCl3, δ ppm): 7.53 (d, 1H, J = 7.07 Hz), 7.50 (d, 1H, J = 7.40 Hz), 7.43 (d, 1H, J = 1.3 Hz), 7.16 (dd, 1H, J1 = 8.5 Hz, J2 = 1.8 Hz), 7.12 (dd 1H, J1 = 8.9 Hz, J2 = 2.5 Hz), 7.05 (d, 1H, J = 2.5 Hz), 3.94 (s, 3H), 3.82 (quad, 1H, J = 7.07 Hz), 1.52 (d, 3H, J = 7.1 Hz). ^{13}C NMR (101 MHz, CDCl3, δ ppm): 169.64, 157.35, 133.36, 133.24, 128.84, 128.40, 126.92, 125.91, 125.42, 118.65, 105.13, 54.88, 45.88, 17.39.

Stability studies. Platinum prodrug **3** in the presence of 0.8% of Tween 20, and 0.2% of F-127 was incubated in 90% PBS and 10% DMSO ($4.8*10^{-5}$ M, $4.8*10^{-5}$ M, $3.57*10^{-5}$ M and $7.14*10^{-5}$ M, respectively) at 37°C. Absorbance of the resulting solutions was measured at different time marks.

Log K_w determination. Lipophilicity studies of each compound were carried out under the same HPLC conditions, by varying the composition of the mobile phase in such a way that the retention times were acceptable (analyte did not elute with the front and did not stay on the column for a long time). (Table 4) After determining the retention factor of the analyte at different compositions of the mobile phase (alcohol fraction), the dependence of the logarithm of this factor on the content of the organic modifier (alcohol) in the mobile phase was plotted. Extrapolation to the Y-axis was used to determine the logarithm of the retention factor at 100% water content, which in turn characterizes the lipophilicity of the analyte.

Electrochemical study. Cyclic voltammetry was performed on a potentiostat/galvanostat PalmSens 3 (PalmSens, Netherlands) at r.t. with a scan rate at 100 mV/s. A three-electrode cell contained glassy carbon working (2.0 mm diameter) and auxiliary electrodes, and an Ag/Ag+ reference electrode. The surface of glassy carbon electrodes (r =1 mm) in Teflon bodies were polished before each measurement using Al_2O_3 (10 and 0.05 µm) and wet microcloth pad in distill water. Between the individual polishing steps, the electrodes were rinsed with distilled water. The electrochemical cell was filled with a 3:1 mixture of DMSO and H2O with dissolved triethylbenzylammonium chloride TEBAC (0.1 mol/L) as a supporting electrolyte. The working electrode compartment was filled with ligand or complex (1-2 mM) solution prepared in the same supporting electrolyte. Prior to the measurement the cell was purged with argon for 3-5 min. A trace amount of ferrocene was added to the supporting electrolyte solution as an internal reference in a blank experiment.

Cell culture

MCF7, VA13, and A549 cell lines were maintained in DMEM/F-12 media containing 10% fetal bovine serum (FBS), 50u/ml penicillin and 0.05 mg/ml streptomycin (all products from Thermo Fisher, USA) at 37°C in 5% CO2. MCF10A cell line was maintained in DMEM/F12 (Thermo Fisher) with 5% Horse Serum (Invitrogen), 20ng/m EGF (Invitrogen), 0.5 mg/ml Hydrocortisone (Sigma-Aldrich), 100 ng/ml Cholera Toxin (Sigma-Aldrich), 10µg/ml Insulin (Paneco),1x Pen/Strep (Thermo Fisher). A549 is non-small cell lung carcinoma line; VA13 is WI38 subline 2RA, immortalized lung fibroblasts, HEK293T is highly transfectable derivative of human embryonic kidney 293 cells, and contains the SV40 T-antigen. Cell cultures were genotyped by STR and tested for the absence of mycoplasma.
The CDDP-resistant and parental human ovarian cancer cell lines (SKOV3/CDDP and SKOV3) were purchased from G. A. Posypanova, PhD, National Research Centre "Kurchatov Institute", Russian Federation) and American Type Culture Collection (Manassas, VA) respectively. Cells were propagated in Dulbecco's modified Eagle's medium supplemented with 10% fetal bovine serum (HyClone, Logan, UT), 2 mM L-glutamine, 100 U/mL penicillin and 100 µg/mL streptomycin (PanEco, Russia) at 37°C, 5% CO2 in a humidified atmosphere. Cells in logarithmic phase of growth were used in the experiments.

MTT assay. The cytotoxicity of substances was evaluated using the MTT (3-(4,5-dimethylthiazol-2-yl)2,5-diphenyl tetrazolium bromide) assay. For this, 5,000 MCF-7 cells were seeded in 96-well plate in 100 µL of full DMEM (Gibco, USA) per well and incubated overnight. Drugs were dissolved in DMSO to 10 mM and then diluted with full DMEM to reach 50 nM -100 µM concentrations (final DMSO concentration in the media was less than 1%) and transferred to cells for 72 hours. The MTT reagent (Paneco LLC, Russia) then was added to cells up to final concentration of 0.5 g/l and incubated for 2 hours at 37ºC at 5% CO_2. The MTT solution was then discarded and 100 µl of DMSO (PharmaMed LLC, Russia) was added. The plates were swayed on a shaker (60 rpm) to solubilize the formazan. The absorbance was measured using a microplate reader (VICTOR X5 Light Plate Reader, PerkinElmer, USA) at a wavelength of 565 nm (in order to measure

formazan concentration). The results were used to construct a dose-response graph and to estimate IC_{50} value (GraphPad Software, Inc., San Diego, CA).

Flow cytometry staining of MCF-7 and MCF-10A cells with Ab140974 antibodies. MCF-7 and MCF-10A cells were detached with trypsin-EDTA, then inactivated by media with FBS. After inactivation cells were washed with PBS and fixed 10 minutes with 3.7% formaldehyde in PBS. Then cells were washed with PBS and thoroughly resuspended in PBST (PBS+ 0.1% Tween 20) with 1% bovine serum albumin (BSA) for 30 minutes to block unspecific binding of the antibodies. The procedure was followed by incubation of cells with antibodies 10 µg/ml ab140974 (Abcam) in 1% BSA in PBST for 1 hour at r.t. After 1 hours the cells were washed two times in PBS, 5 minutes each wash and incubated with the secondary antibodies 20 µg/ml QL230437 (ThermoFisher Scientific) in 1% BSA for 1 hour at r.t. in the dark. At the end cells were washed three times with PBS for 5 minutes each in the dark, resuspended in PBS, and analyzed with MACSQuant Flow cytometer.

Cellular platinum accumulation and distribution.

Cell preparation and incubation. Human breast adenocarcinoma cells MCF-7 was maintained in DMEM/F12 (Gibco, #31331028) supplemented with 10% FBS (Gibco, #10270106) at 37°C with the air atmosphere of 5% CO_2. MCF-7 cells were seeded in T25 plates (2×10^5 cells/1 mL, 5 mL/plate, 3 technical replicates). After 24 hours incubation, 5 µL stock solutions of Cisplatin, Pt(IV) prodrugs were added (10 µM in DMSO) with DMSO as a control group and incubated for 3.5 hours. After treatment, cells were washed with PBS, trypsinized and collected. After that, the cell concentration was calculated using EVE Automated Cell Counter (NanoEntek, Korea).

Subcellular fractionation procedure. Cell samples were subjected to three freezing/thawing cycles at -80°C, treated with 200 µl of PBS supplemented with 0.1 M NaOH and 0.05% Tween-20, and vigorously vortexed to lyse the cells. The homogenate was centrifuged at 600 g for 15 minutes at 4°C to isolate the nuclear fraction. Then, the supernatant was carefully transferred to a new tube and centrifuged at 15000 g for 15 minutes at 4°C to isolate the mitochondria fraction. The supernatant was a cytosol fraction, which was carefully transferred to another tube. All three fractions were treated with highly pure nitric acid (100 µl for nuclear and mitochondria fractions, and 300 µl for cytosol fraction) for at least 48 hours until complete dissolution.

ICP-MS. Platinum detection was performed using mass-spectrometer with inductively coupled plasma Aurora Elite (Bruker, Bremen, Germany). The following parameters of mass-spectrometer were used: RF power 1.45 kW, Sampling depth 5.5 mm, Plasma flow 17.0 L/min, Auxiliary flow 1.75 L/min, Sheath gas 0.20 L/min, Nebulizer flow 0.98 L/min, Dwell time 10 ms. Detection was carried out using ^{195}Pt ions for platinum and ^{209}Bi for an internal standard. Mass spectrometer control, data collection and processing were performed using Quantum software (Bruker, Bremen, Germany). Mass-spectrometer calibration (burner position, peak shape and resolution, detector voltage) was carried out in automatic mode by feeding into the spectrometer ICP-MS tune solution (AnalytikJena, Jena, Germany), containing Be, Mg, Co, In, Ba, Pb, Th, Ce, Tl. Concentration of each element was 1 µg/l. Quantitation of platinum was carried out using external standard method. For calibration dependence plotting the standard platinum solutions Platinum Standard for ICP-MS (Sigma-Aldrich, Buchs, Switzerland) with concentrations 0.01, 0.1, 1.0 and 10 µg/l were used. As an internal standard the bismuth standard solution Custom standard (AnalytikJena, Jenna, Germany) with bismuth concentration 1 µg/l which was injected into the spectrometer on the second line during the whole period of analysis. Sample preparation was performed as follows. Sample aliquot of 50 µl was diluted to 10 ml with deionized water. The sample was then injected into the spectrometer and the concentration of platinum was determined. The standard deviation was calculated based on 5 measurements.

XANES study of drug release in A549 tumor cells (Cell preparation). Human lung carcinoma A549 cells were seeded in T25 culture flasks (700000 cells per flask). After 40 hours incubation and reaching 80% confluence, 10 µM of Pt(IV) prodrug **3** was added for 3 hours or 20 hours. Following the incubation, the drug-containing medium was removed, and the cells were washed thoroughly with ice cold PBS solution (pH 7.5, SIGMA) to remove residual medium. The cells were trypsinised and centrifuged at 2500 rpm for 3 min, the supernatant was removed, and the cells were resuspended in a solution of ammonium acetate (100 mM, 5 mL) and centrifuged again. Finally, the cell pellets were washed with a 70% ethanol solution, yielding a dry cell pellet. The samples were immediately freeze-dried for 24 hours and stored in a desiccator until they were analyzed. At the time of analysis, the freeze-dried cells were packed into a polycarbonate sample holder, and the open face secured with a Kapton tape (Kapton) window for XANES analysis. XANES spectra of L3 edge of platinum were recorded in the fluorescence signal detection mode.

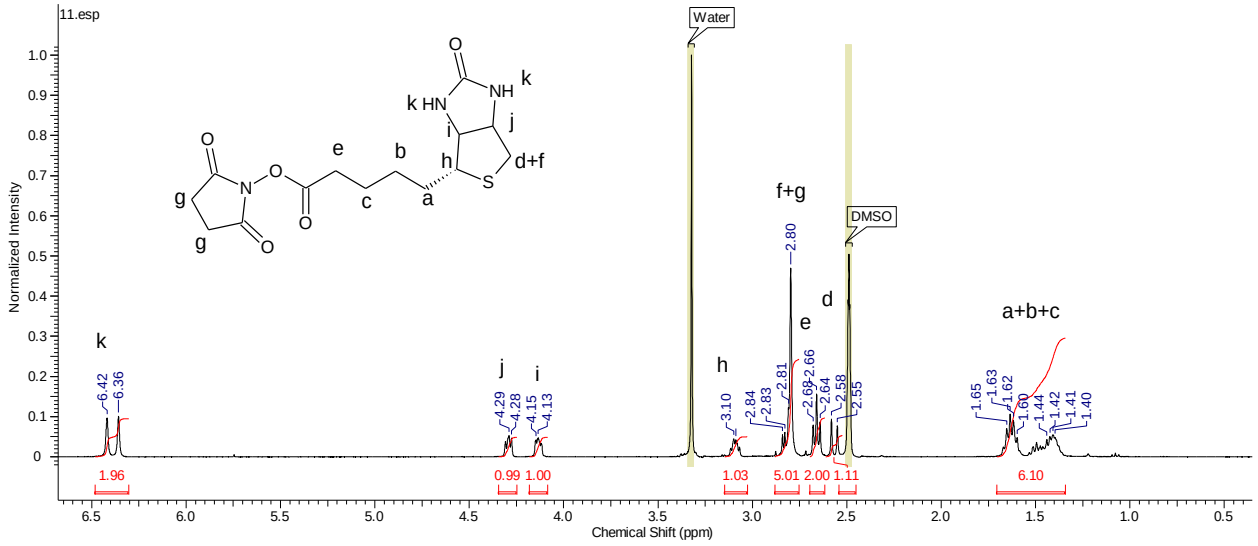


Figure S1. ^{1}H NMR spectrum of compound 1.

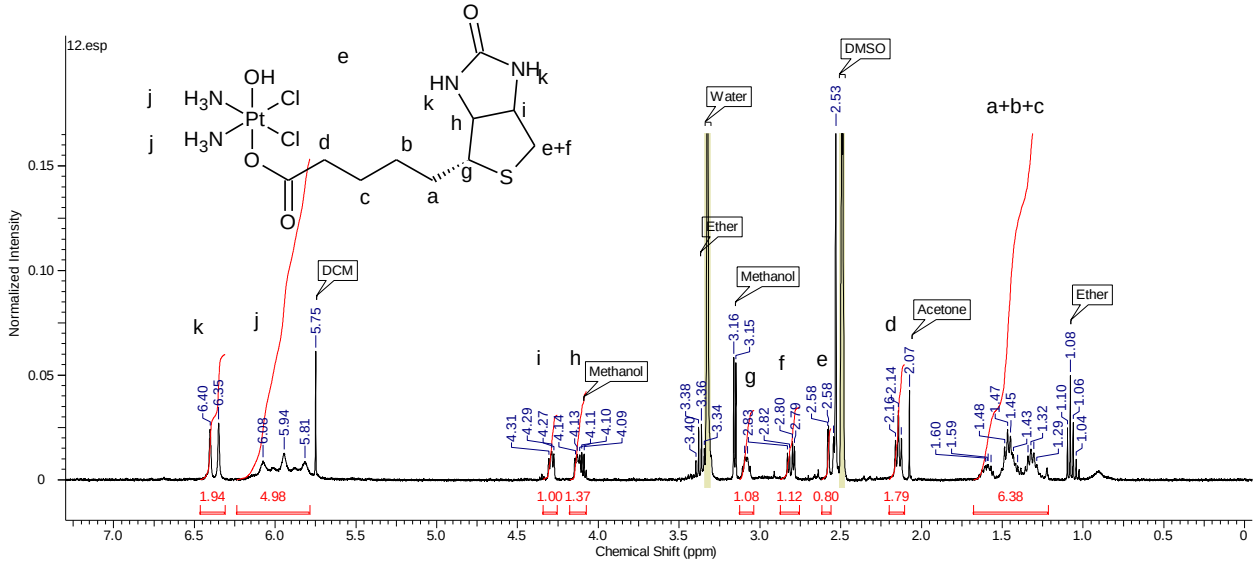


Figure S2. ^{1}H NMR spectrum of compound 2.

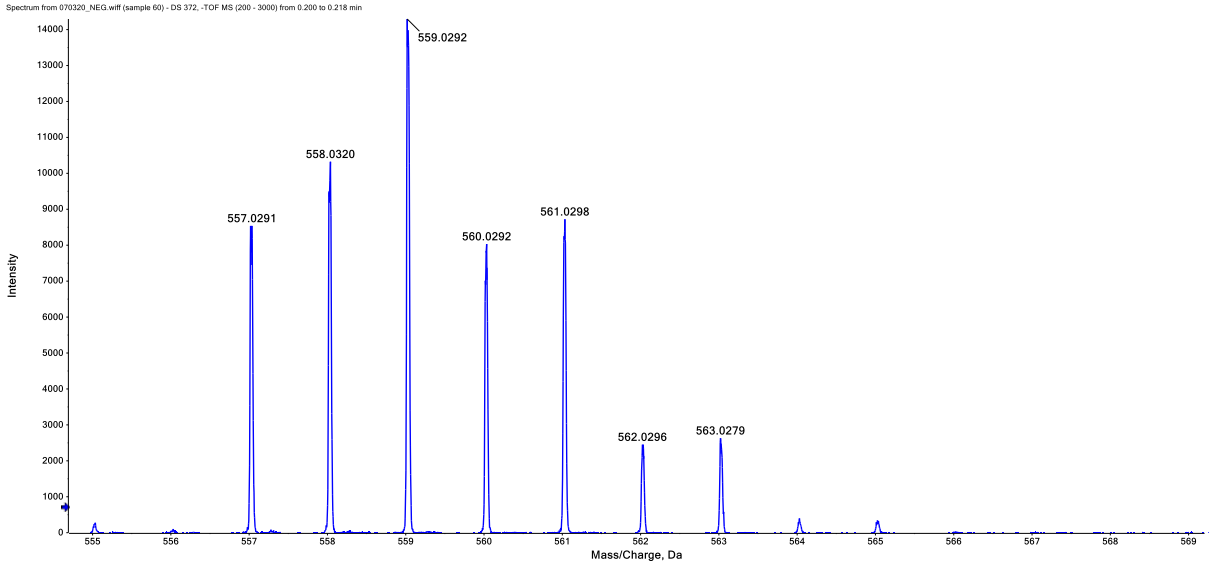


Figure S3. HRMS spectrum of compound 2.

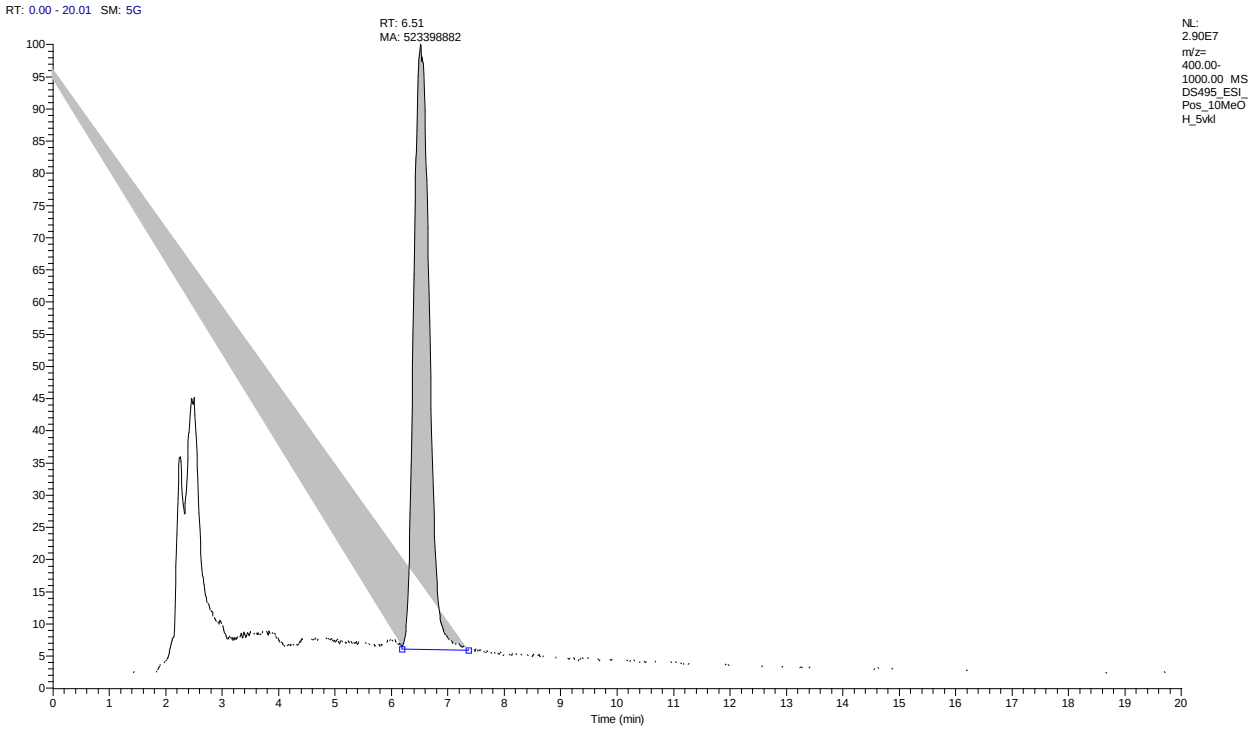


Figure S4. RP-HPLC chromatogram of compound 2.

Table S1. HPLC track of compound 2.

RT: 0.00 - 20.01

Number of detected peaks: 1

Apex RT	Start RT	End RT	Area	%Area	Height	%Height
6.51	6.19	7.37	523398881.9	100	27196558.2	100

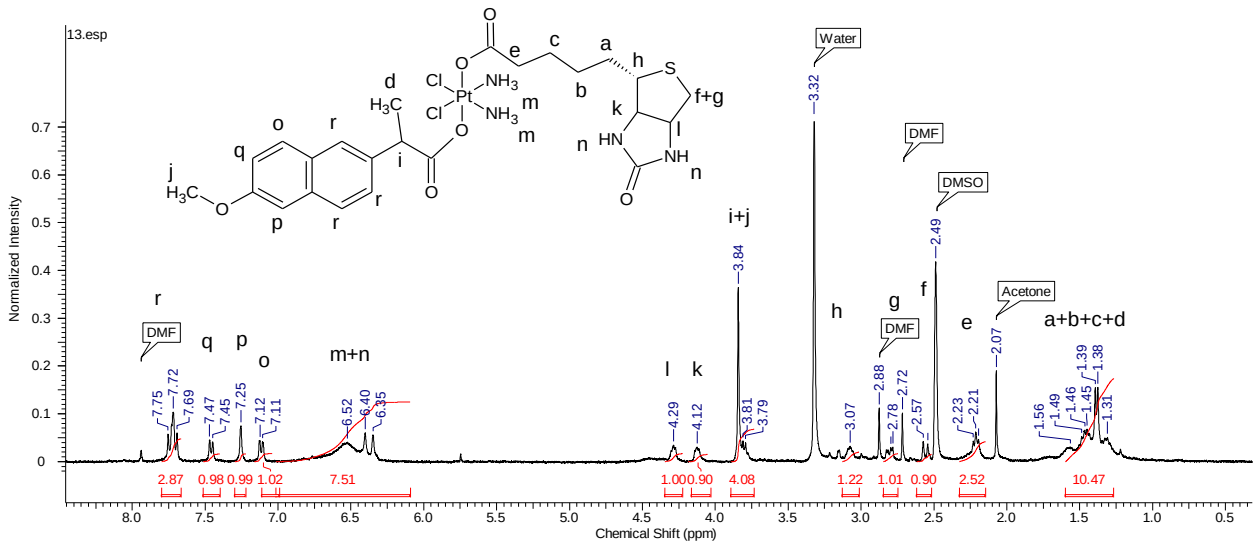


Figure S5. 1H NMR spectrum of compound 3.

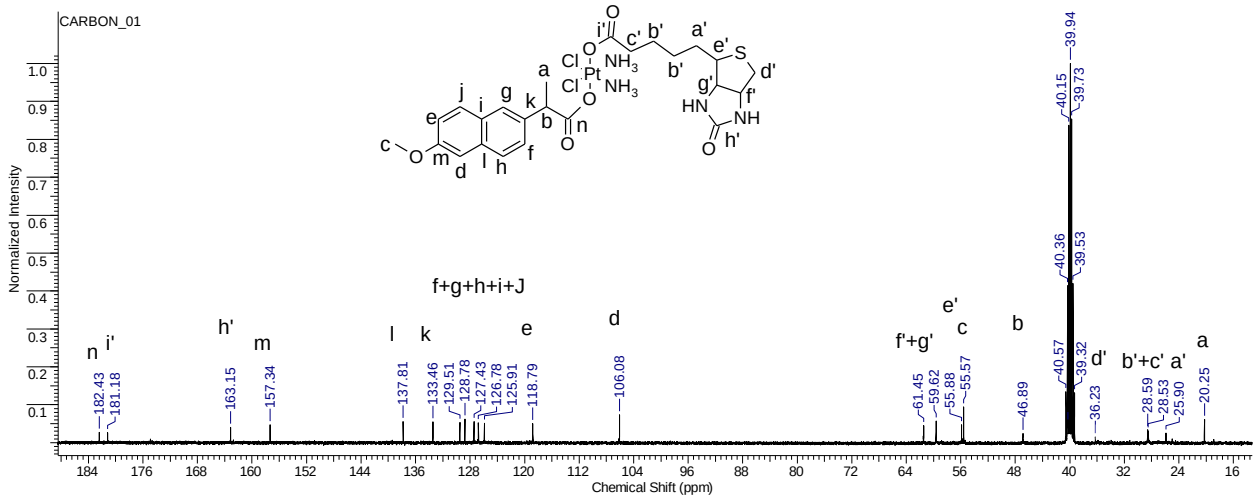


Figure S6. ^{13}C NMR spectrum of compound 3.

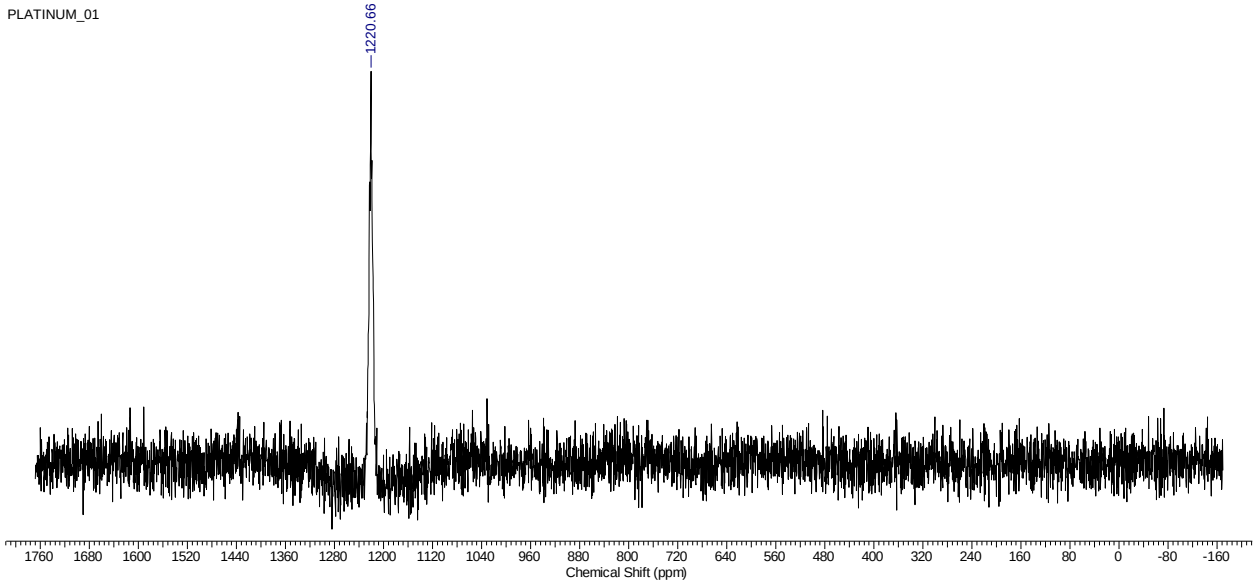


Figure S7. ^{195}Pt NMR spectrum of compound 3.

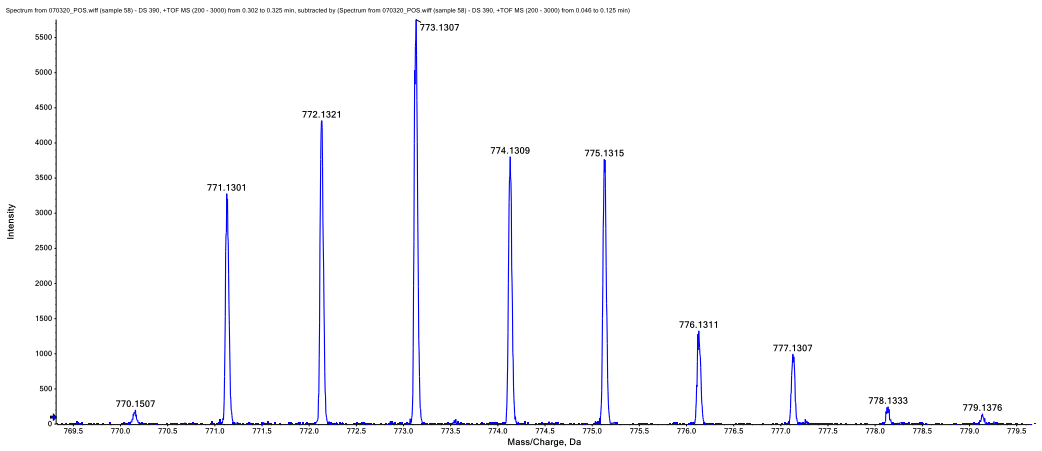


Figure S8. HRMS spectrum of compound 3.

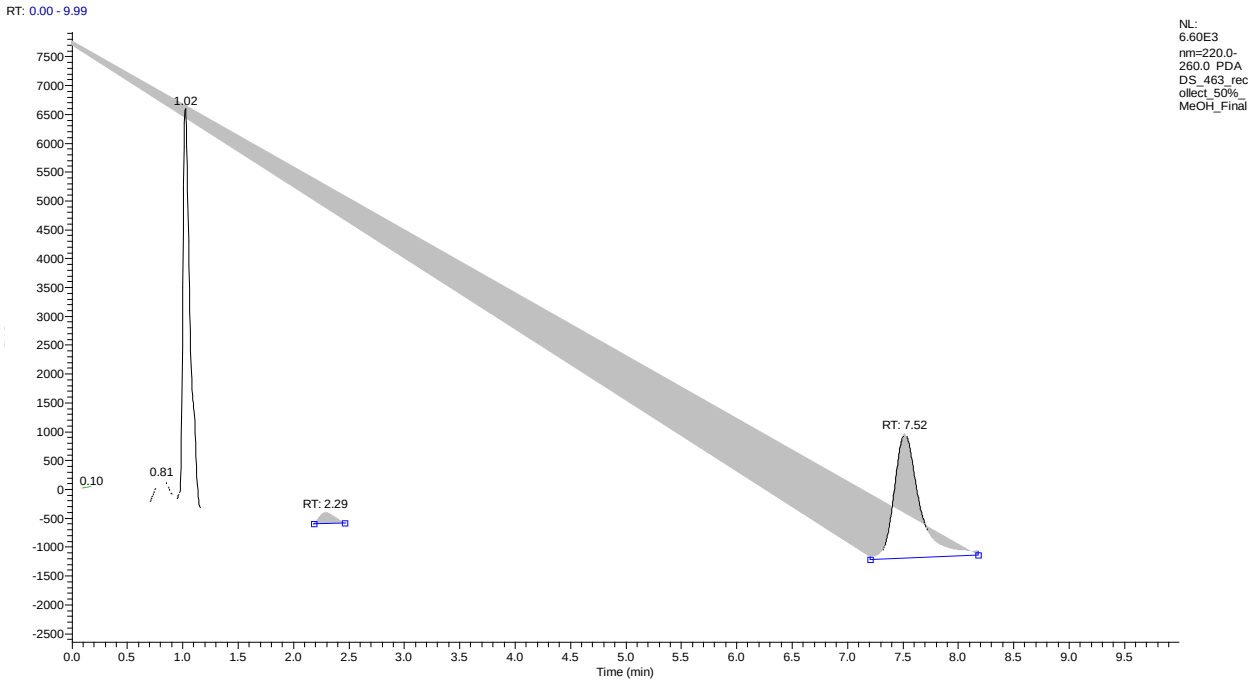


Figure S9. RP-HPLC chromatogram of compound 3.

Table S2. HPLC track of compound 3.

RT: 0.00 - 10.02

Number of detected peaks: 2

Apex RT	Start RT	End RT	Area	%Area	Height	%Height
2.29	2.2	2.46	1852.557	4.89	194.59	8.15
7.52	7.2	8.18	36050.4	95.11	2194.456	91.85

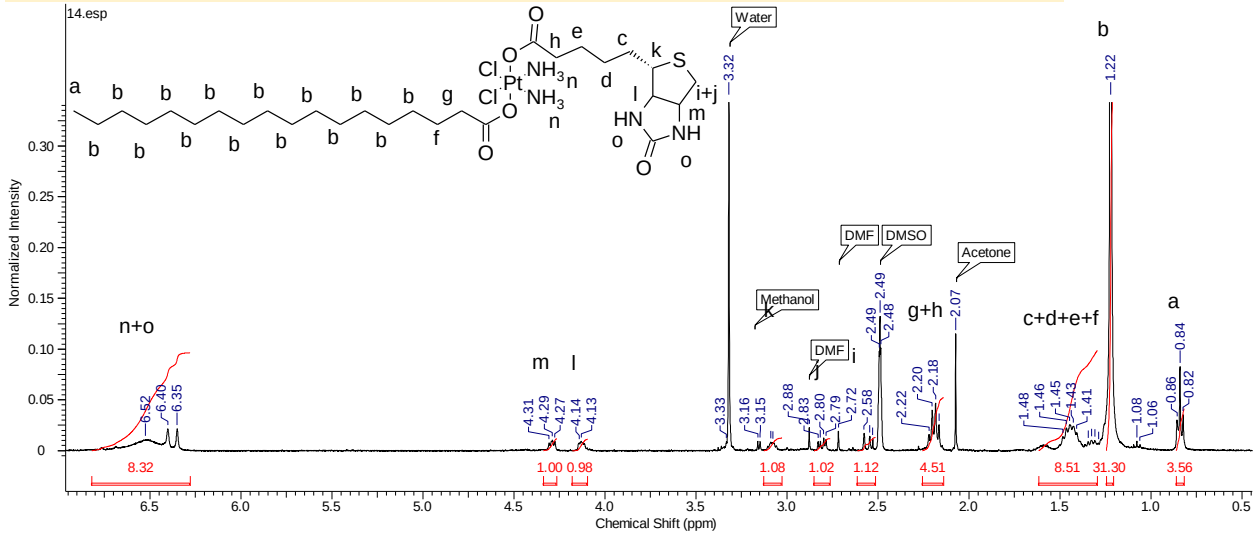


Figure S10. ^{1}H NMR spectrum of compound 4.

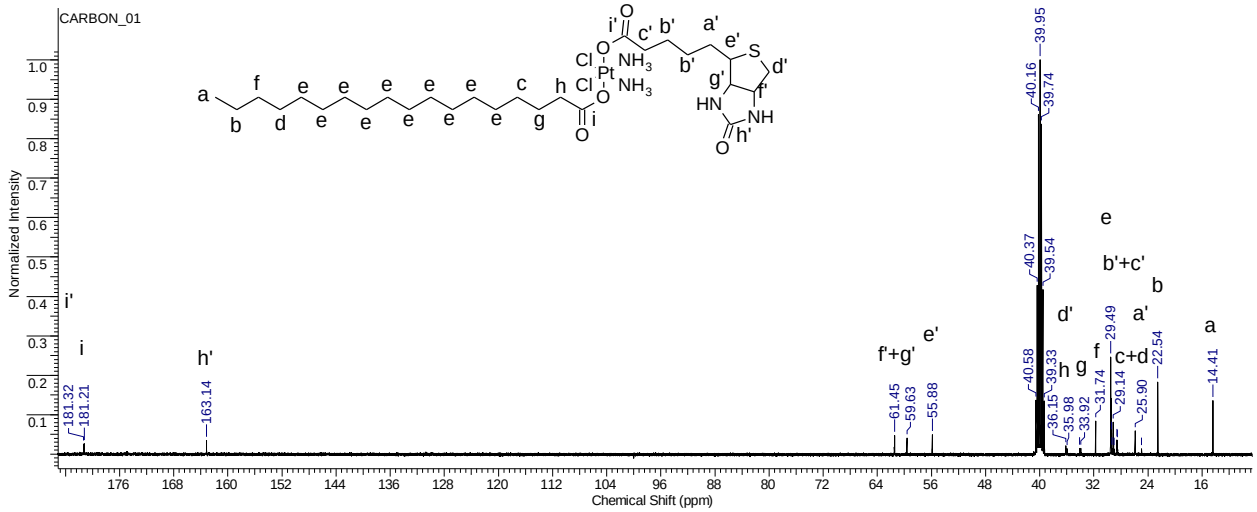


Figure S11. ^{13}C NMR spectrum of compound 4.

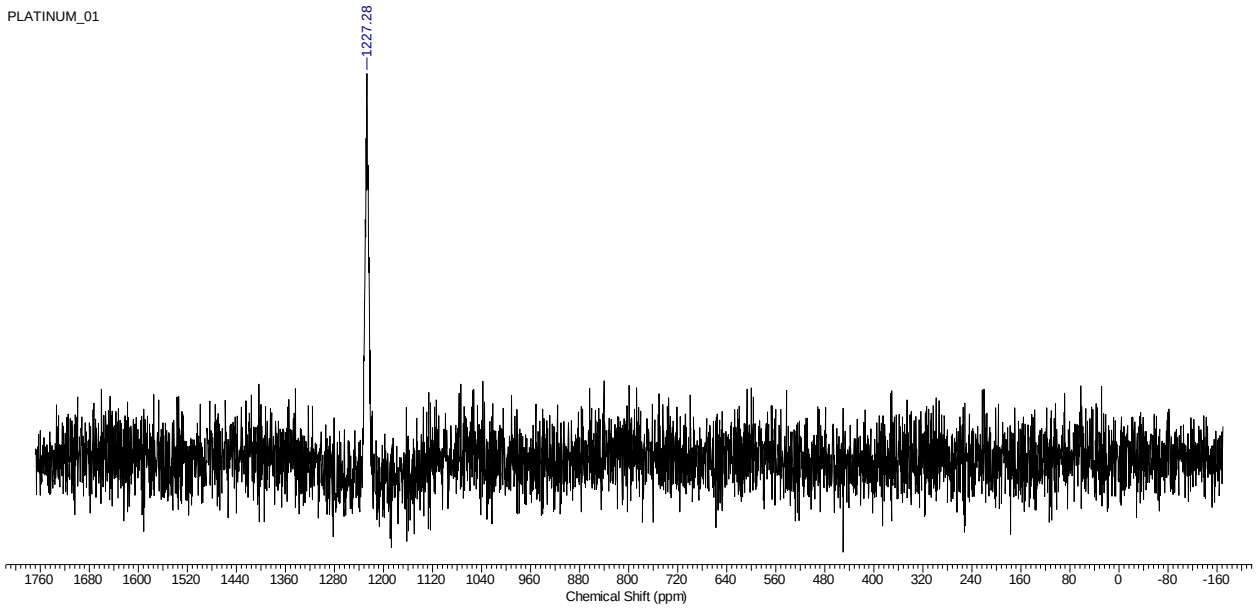


Figure S12. ^{195}Pt spectrum of compound 4.

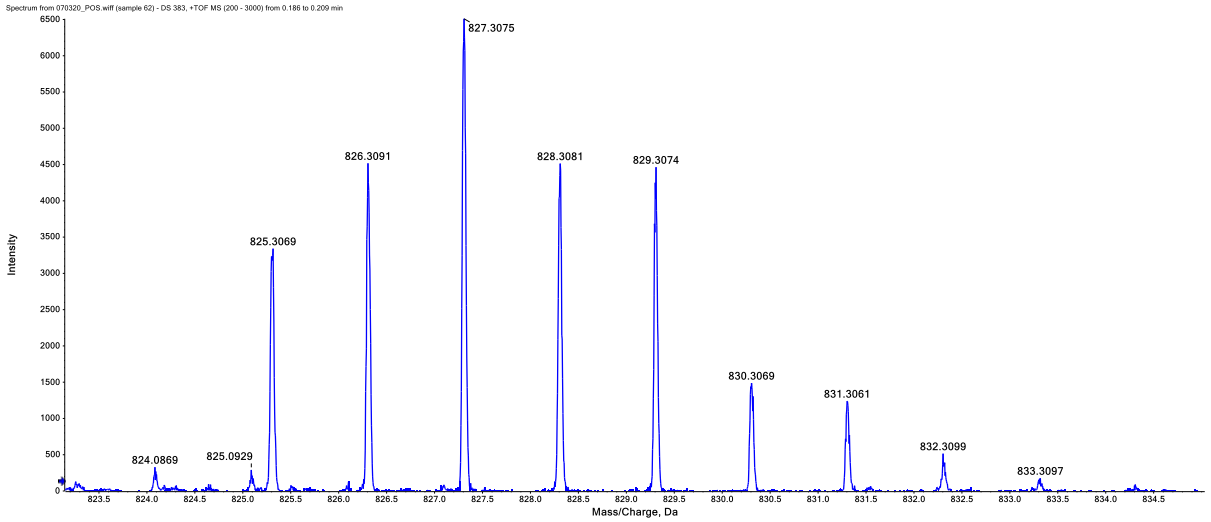


Figure S13. HRMS spectrum of compound 4.

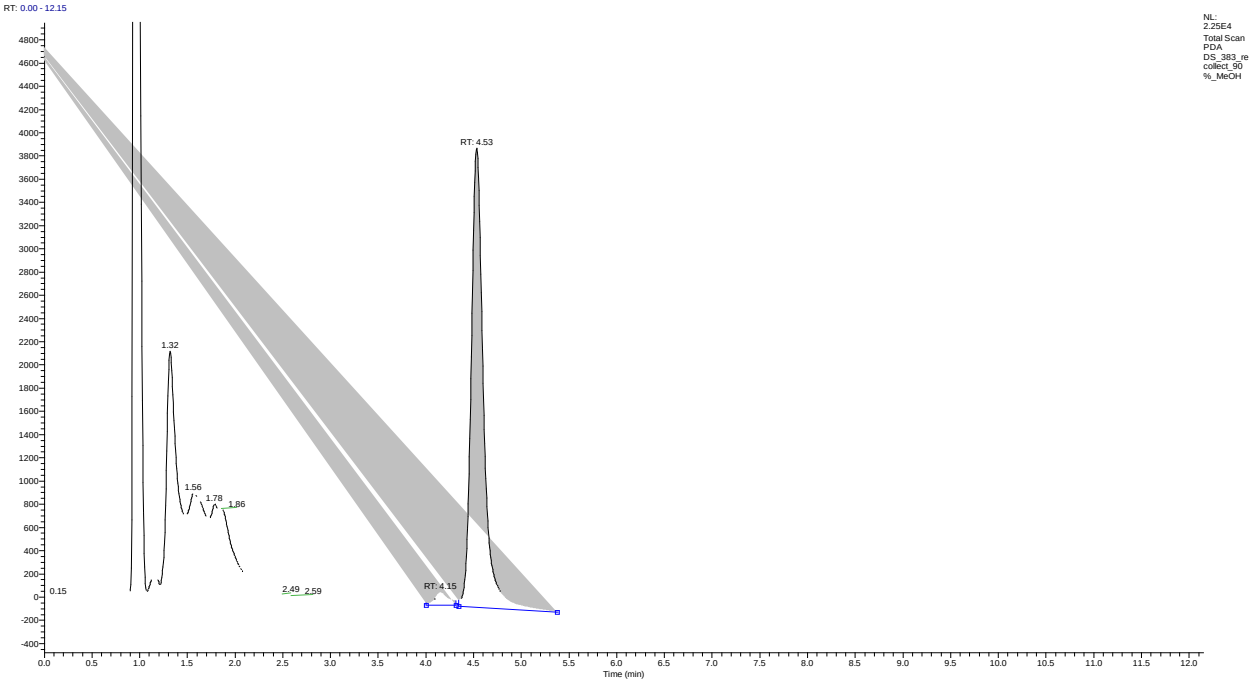


Figure S14. **RP-HPLC chromatogram of compound** 4**.**

Table S3. HPLC track of compound 4

RT: 0.00 - 12.15
Number of detected peaks: 2

Apex RT	Start RT	End RT	Area	%Area	Height	%Height
4.15	4	4.32	1085.619	2.95	108.397	2.67
4.53	4.34	5.38	35664.6	97.05	3953.212	97.33

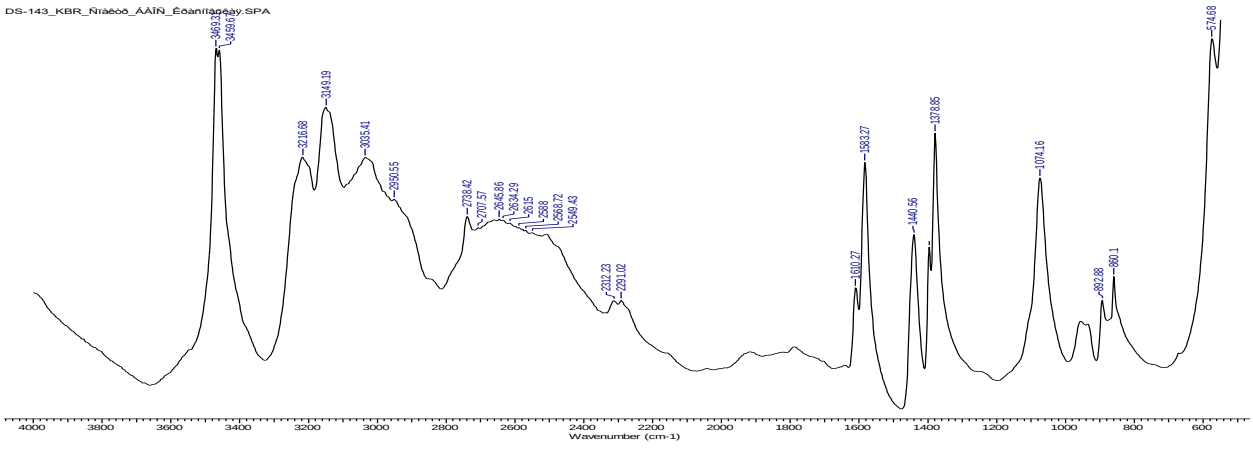


Figure S15. IR spectrum of oxoplatin 5.

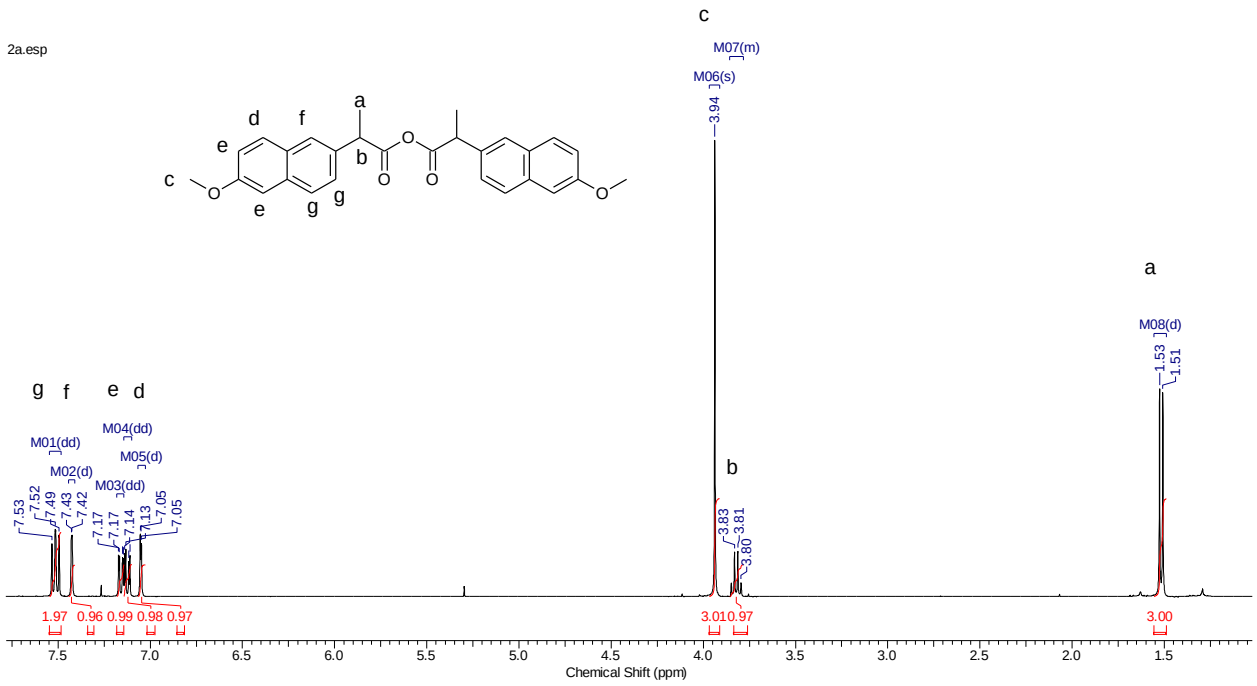


Figure S16. ^{1}H NMR spectrum of compound 6.

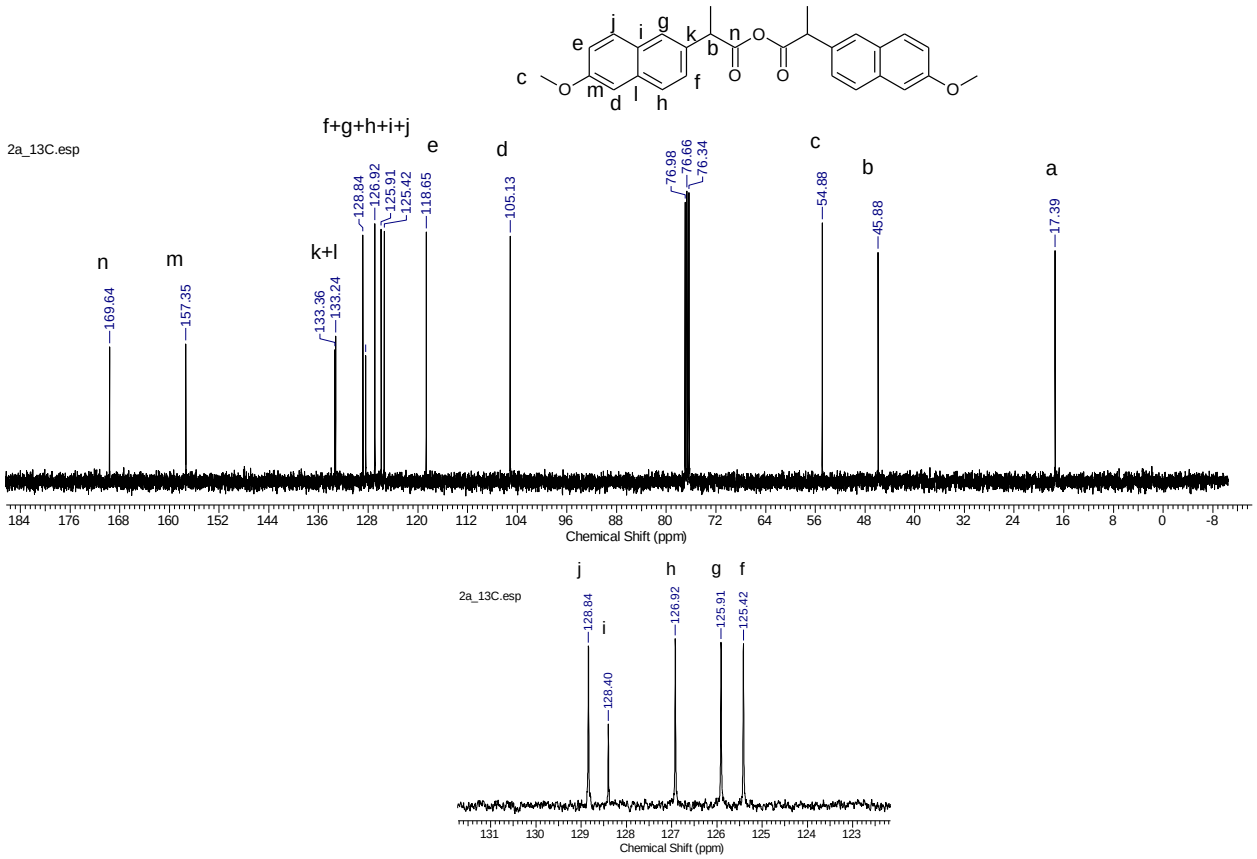


Figure S17. ^{13}C NMR spectrum of compound 6.

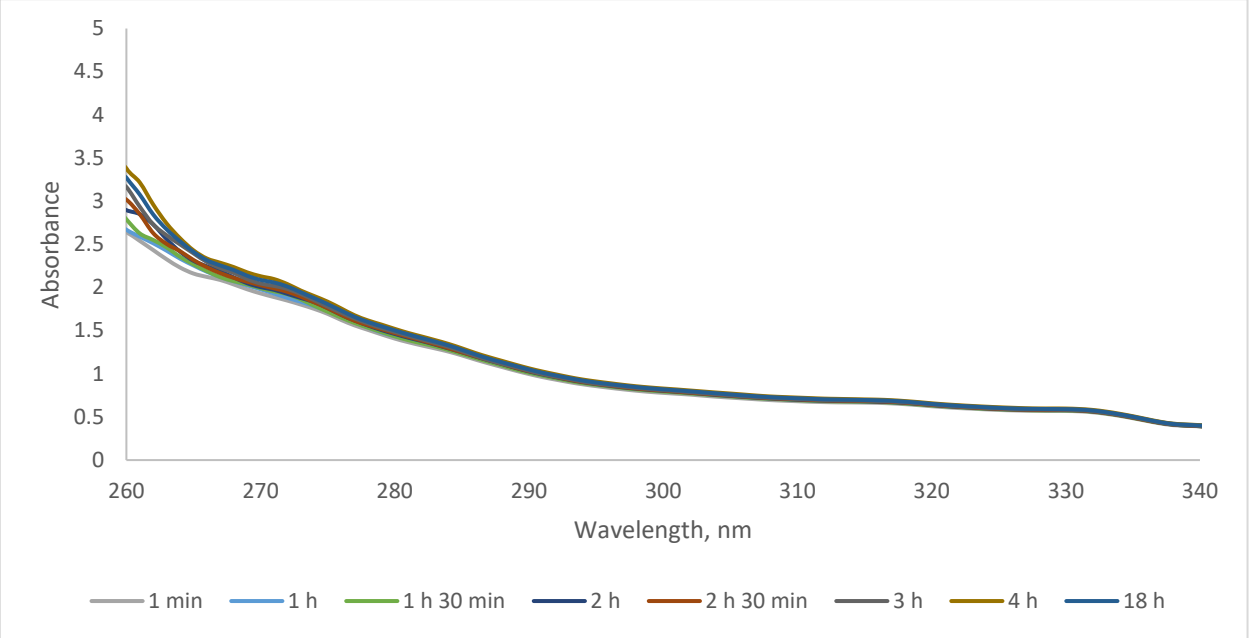


Figure S18. Stability of Pt(IV) prodrugs 3 in 10% DMSO, 0,8% Tween 20, 0,2% F-127, 89% PBS solution. Concentration of 3: $7.14 \cdot 10^{-5}$ M.

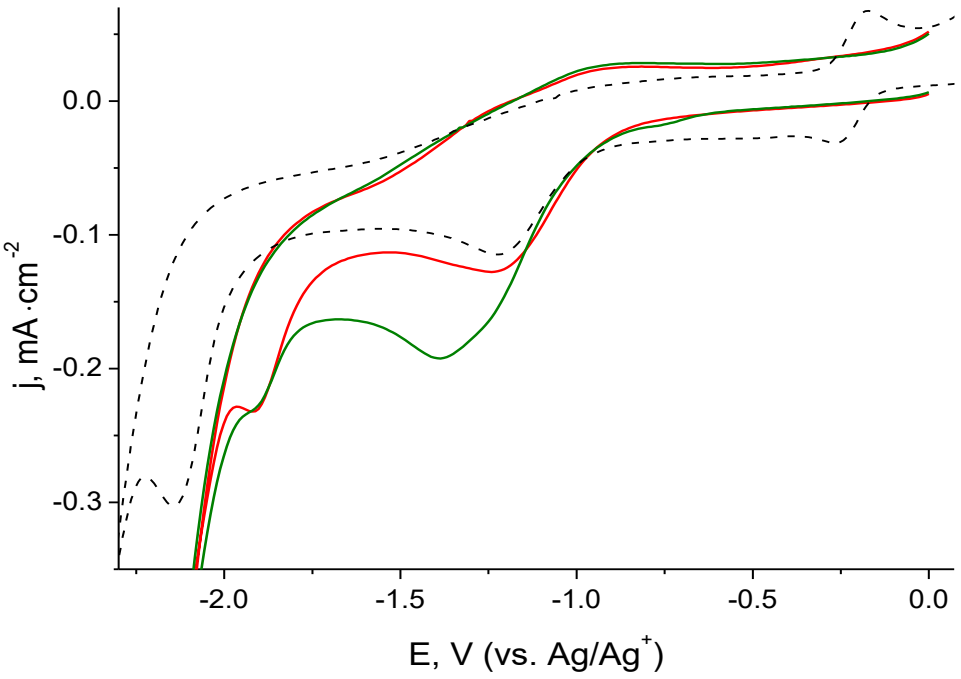


Figure S19. Cyclic voltammograms for model complex (— red) and 2 (— green), DMSO: H_2O (3:1), 0.1 M TEBAC, 100mV/s, blank ferrocene solution in the supporting electrolyte (---).

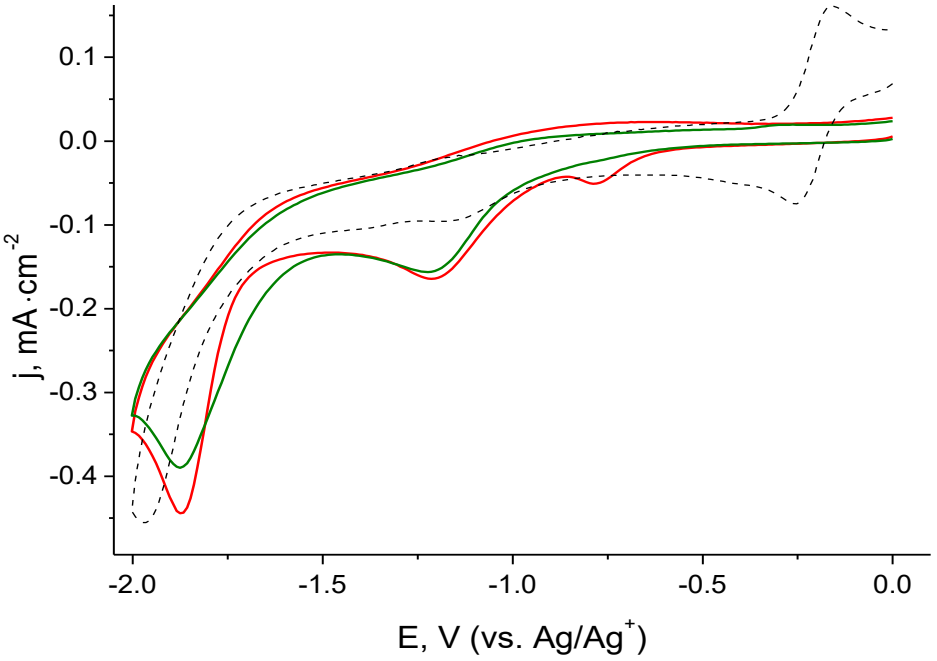


Figure S20. Cyclic voltammograms for complex 4 (— red) and biotin (— green), DMSO: H_2O (3:1), 0.1 M TEBAC, 100mV/s, blank ferrocene solution in the supporting electrolyte (---).

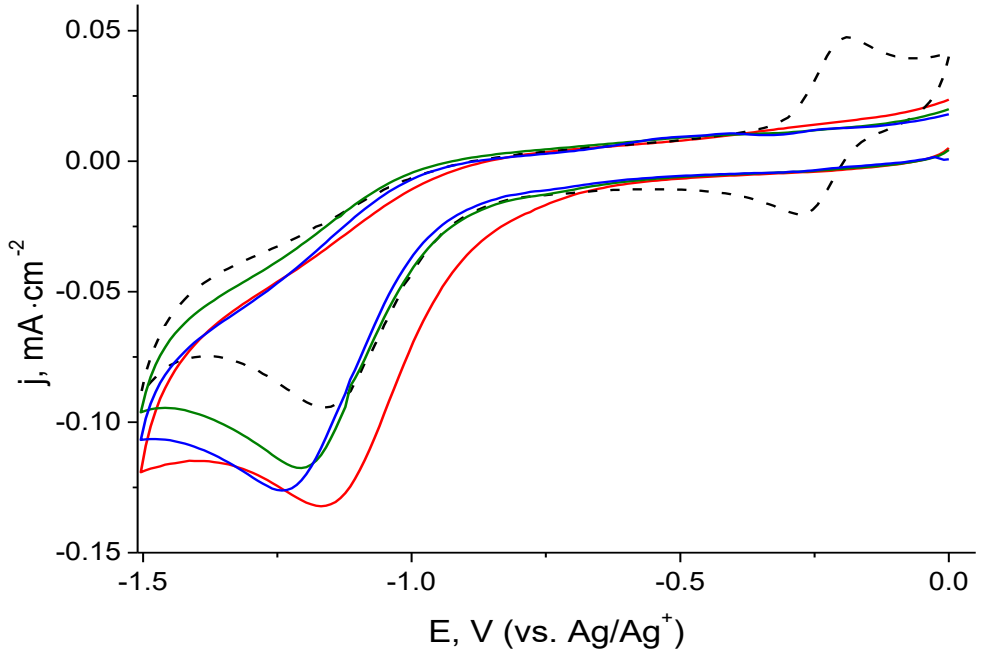


Figure S21. Cyclic voltammograms for complex 3 (— red) and naproxen (— green), biotin (— blue), DMSO: H_2O (3:1), 0.1 M TEBAC, 100 mV/s, blank ferrocene solution in the supporting electrolyte (---).

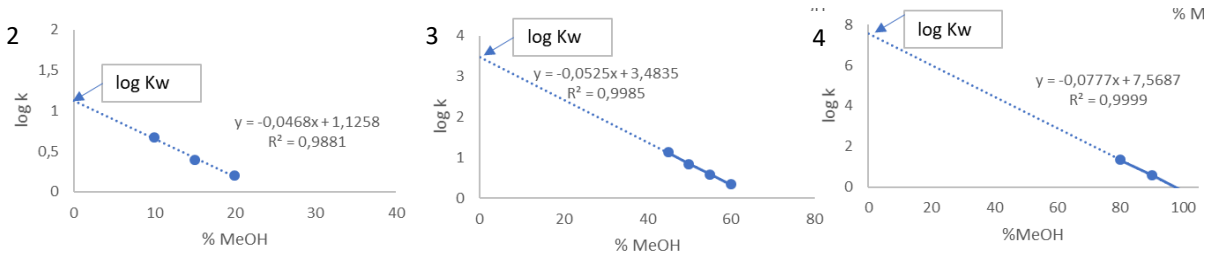


Figure S22. The log K-%MeOH dependence for compounds 2 – 4. Intersection of a trend line with the y axis indicate the log kw value.

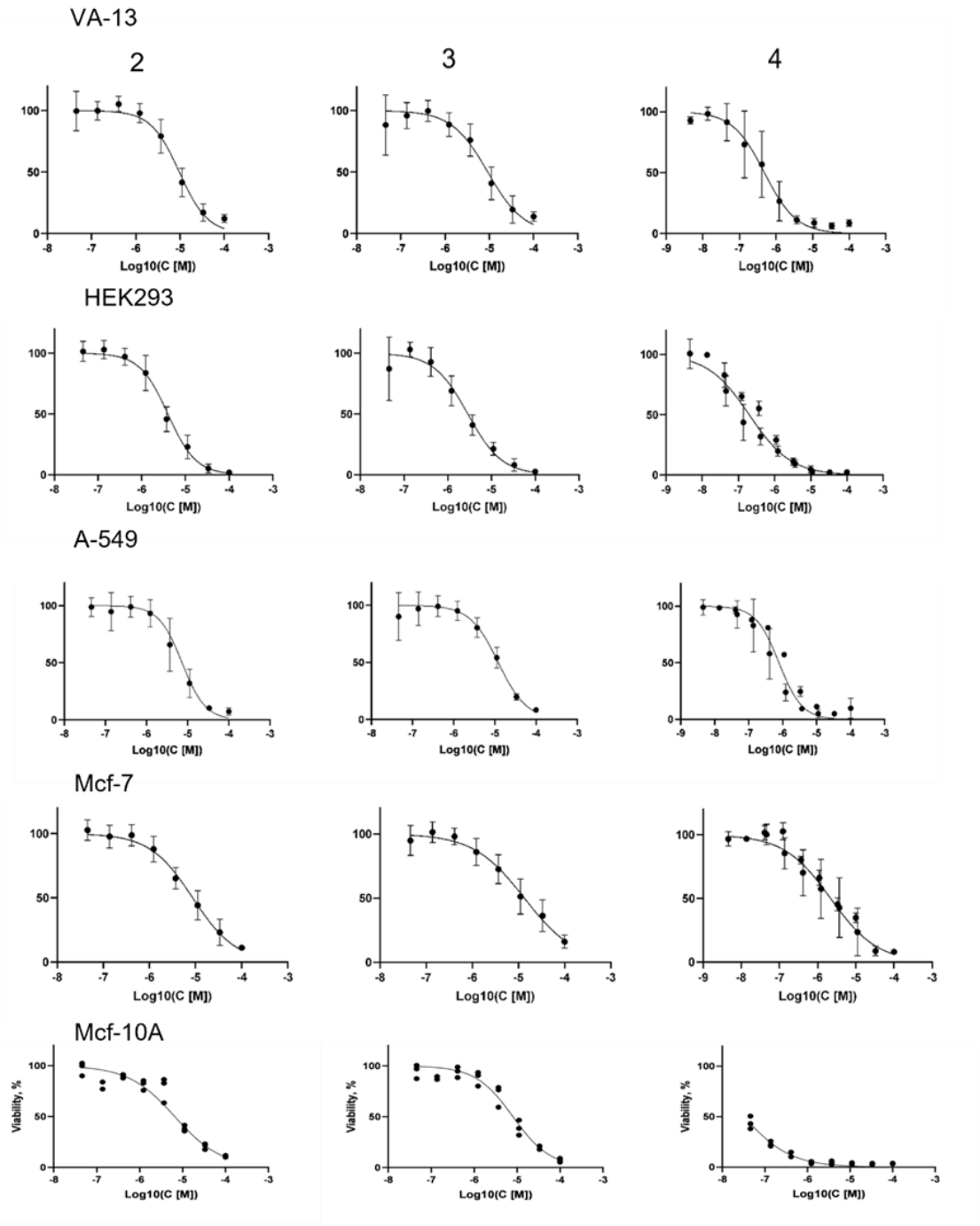


Figure S23. Cytotoxicity curves of Pt(IV) prodrugs 2-4 on the VA-13, MCF-7, MCF-10A, A549 cell lines.

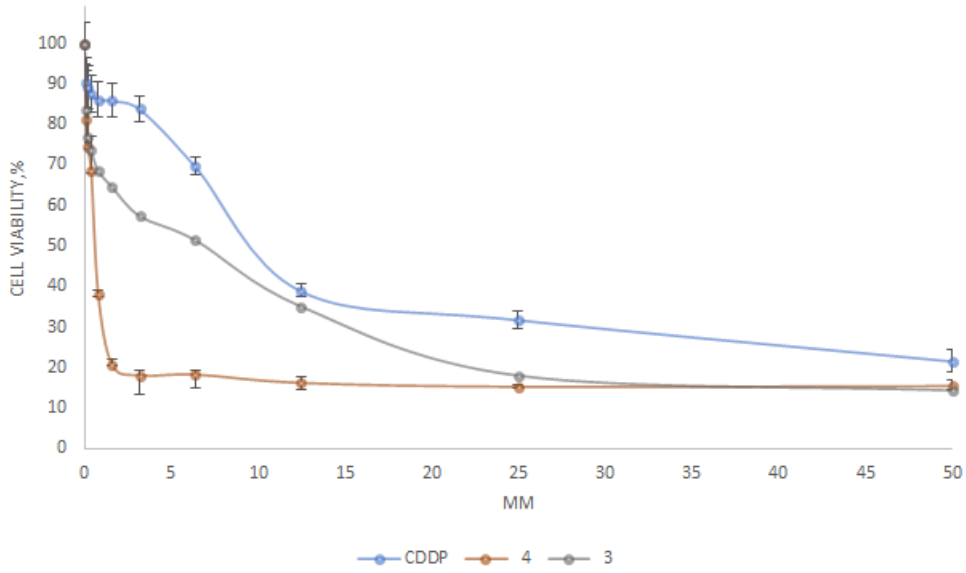


Figure S24. Cytotoxicity curve of Pt(IV) prodrugs 3, 4 on the wild type SKOV-3 cell line.

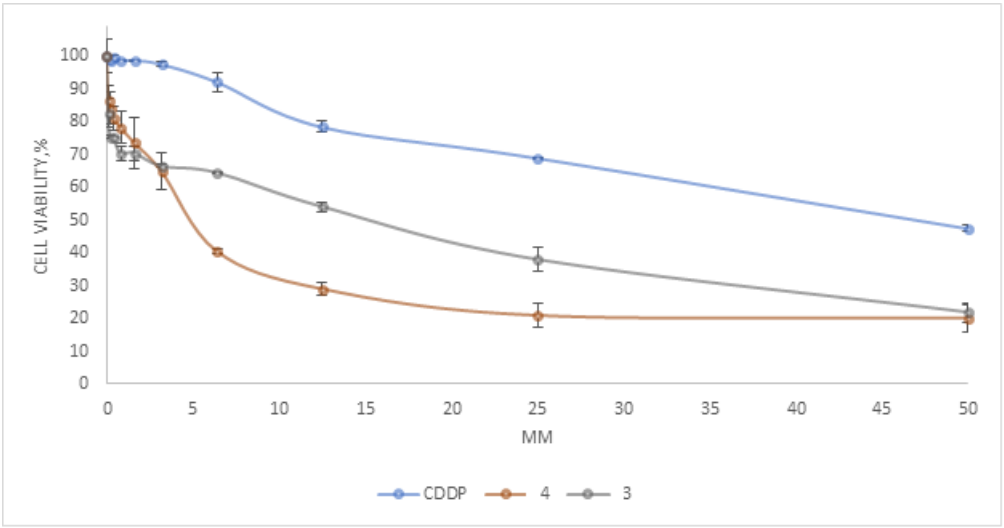


Figure S25. Cytotoxicity curves of Pt(IV) prodrugs 3, 4 on CDDP-resistant SKOV-3 cell line.

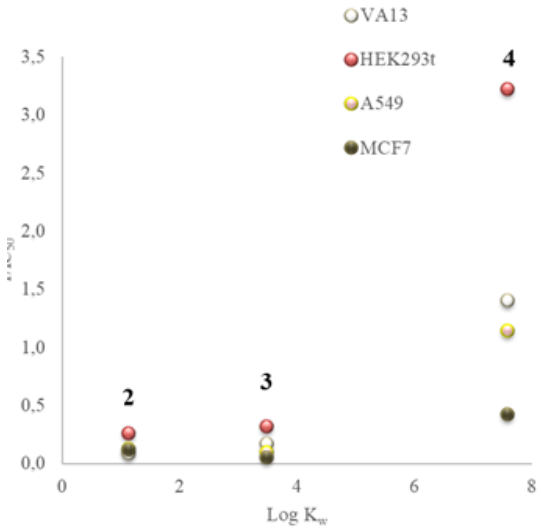


Figure S26. Correlation between cytotoxic activity ($1/IC_{50}$ values) and capacity factor (log Kw values) for Pt(IV) prodrugs **2-4** with biotin in axial position.

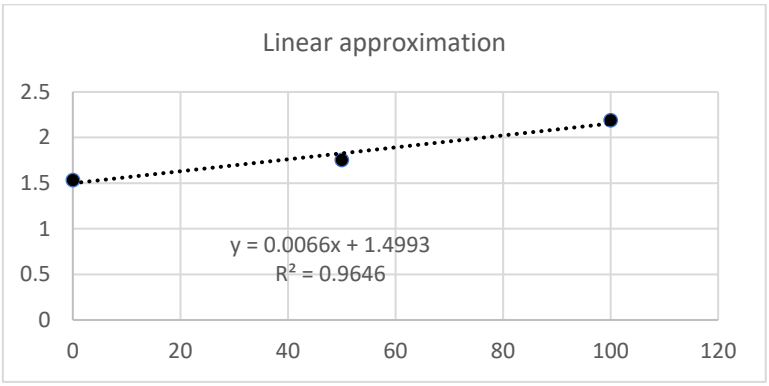


Figure S27. Linear dependence of *a/b* ratio for solid Pt^{IV} prodrug **3**, Cisplatin Pt^{II}, and equimolar mixture Pt^{II}+Pt^{IV} 50:50.

Table S4. Variations of % methanol in the mobile phase and the corresponding Log K values for prodrugs **2-4.**

Pt(IV) prodrug	% Methanol in mobile phase	Log k
2	10%	0.673069434
	15%	0.394881584
	20%	0.205546238
3	60%	0.342842492
	55%	0.584379348
	50%	0.84509804
	45%	1.131359258
4	100%	-0.20969986
	90%	0.583174647
	80%	1.344935481

Table S5. HPLC conditions for purification of prodrugs 2-4.

Pt(IV) prodrug	**% Methanol in mobile phase**	**Retention time, apex point, min**
2	10%	6.51

3	60%	7.52
4	90%	4.53

Table S6. Reduction potentials $E_c(Pt^{IV}/Pt^{II})$ of Pt(IV) prodrugs 2 and 4*.

Compound	Ec (Pt(IV) / Pt (II) (vs. Fc^+/Fc)	Axial Ligands
2	-1.169 V	Biotin
4	-0.572 V	Biotin, stearic acid

1-2 mM in DMSO/water 3:1

Table S7. Intracellular accumulation and distribution of cisplatin (CDDP) and Pt(IV) prodrug 4 in MCF-7 cells after 3.5 hours of incubation, ng Pt/10^6 cells.

	CDDP	4
Cytoplasm	0.833±0.08	0.82±0.04
Mitochondria	0.01±0.01	0.42±0.07
Nucleus	0.03±0.01	1.09±0.03
Total	0.87±0.11	2.33±0.42

Table S8. Summary of extrapolated percentage of unreduced platinum(IV) for A549 cells incubated with CDDP and 3. (Calculated using the corresponding derived linear function (Figure S27)

	3 (Solid)	CDDP (Solid)	1:1 Mixture
a	1,98	1,47	1,64
b	0,86	0,92	0,90
a/b	2,30	1,60	1,82

References

1. Muhammad, N.; Sadia, N.; Zhu, C.; Luo, C.; Guo, Z.; Wang, X. Biotin-Tagged Platinum(IV) Complexes as Targeted Cytostatic Agents against Breast Cancer Cells. *Chem. Commun.* **2017**, *53* (72), 9971–9974. https://doi.org/10.1039/c7cc05311h.