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Platinum(IV) prodrugs with heavy-atom-free BODIPY in axial position: instant photoactivation, enhanced biosafety and improved phototoxicity

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Materials and methods

Synthesis. Complex acetatodiamminedichloridohydroxidoplatinum(IV) was synthesized following the procedures found elsewhere[S1,S2]. DCC (dicyclohexylcarbodiimide), DCM, EtOAc, DMF, azidoacetic acid, 6-azidohexanoic acid, 3-bromothiophene, acetyl chloride, aluminum chloride, ethyl isocyanoacetate, acetic acid, copper(I) iodide, cesium carbonate, potassium hydroxide, ethylene glycol, trifluoroacetic acid, DDQ, triethylamine, boron trifluoride etherate, tetrakis(acetonitrile)copper(I) tetrafluoroborate, tris[(1-benzyltriazol-4-yl)methyl]amine (TBTA), acetone, sodium sulfate, petroleum ether and silica gel. were from commercial sources (AKSci, Sigma Aldritch, Thermo Fisher, etc.). The solvents were purified following the literature procedures[S3,S4], other reagents were used without further purification.

Analytical thin-layer chromatography (TLC) was performed on Merck silica gel aluminum plates with F-254 indicator. Compounds were visualized by irradiation with UV light (254, 365 nm).

Preparative column chromatography was performed using Acros brand silica gel (60–200 mesh).

The NMR spectra were recorded on a Bruker Avance 400 MHz spectrometer (USA) in DMSO-d_6 and $CDCl_3$ with TMS as an internal standard for ^1H and ^{13}C and K2PtCl6 for ^{195}Pt NMR. All ^{13}C spectra were ^1H decoupled.

HPLC-DAD-HRMS analysis was performed with Vanquish liquid chromatograph (Thermo Fisher Scientific, USA) and a high-resolution mass spectrometer based on the Orbitrap Fusion Lumos Tribrid (Thermo Scientific, USA) equipped with an electrospray ionization source (ESI). To separate the components of the analyzed solution, a Shim-pack GIST C18-Aq chromatographic column (3 x 150 mm, 3 μm, Shimadzu, Japan) filled with a reverse-phase sorbent with polar-endcapping was used. The column temperature was maintained at 35 °C throughout the analysis using a thermostat. A 0.1% aqueous solution of formic acid (A) and acetonitrile (B) were used as eluents. An isocratic elution mode was used with a ratio of eluents A and B of 15:85%. The flow rate of the mobile phase was 0.4 ml/min. The injected sample volume was 1.0 μl. The analysis time was 10 minutes. The ESI-MS conditions on the Orbitrap Fusion Lumos Tribrid were as follows: mode for recording positively and negatively charged molecules; the capillary voltage of the ionization source was 3500 V for the positive mode and -2500 V for the negative mode; ion source chamber temperature - 350 °C; ion transfer interface temperature - 325 °C; gas pressure for solvent atomization in the ion source (nitrogen) - 50, auxiliary gas pressure - 10, curtain gas pressure - 1. Scanning range m/z: 200-1700 Da. The resolution of the mass spectrometer for analysis is not less than 15000. The mass spectrometer was calibrated immediately prior to sample analysis using CalMix PierceTM calibration mixtures (Thermo Scientific, USA). Data processing was performed using Xcalibur 4.6 software (Thermo Scientific, USA). To determine the relative amount of Pt(IV) prodrugs **Pt-1** and **Pt-2** and cisplatin in the studied solutions peak area was measured for positive ions in the ranges m/z 898-908 for **Pt-1,** m/z 954-964 for **Pt-2**, and m/z 340-348 for cisplatin.

[Pt(OAc)(2-azidoacetate)(Cl_2(NH_3)$_2$)] 2. 110 mg (0.84 mmol, 5.05 equiv.) of DCC was dissolved in 1.5 ml of DMF and 54 mg (0.8 mmol 5 equiv.) of 2-azidoacetic acid was added. The reaction mixture was suspended in an ultrasonic bath for 15 min. Then, the formed precipitate was separated by centrifugation. The resulting solution of 2-azidoacetic anhydride in DMF was mixed with suspension of 40 mg (0.1 mmol, 1 equiv.) of compound **1** in 2 ml of DMF. The reaction mixture was stirred at 40°C for 4 hours. After that, the solution was removed under reduced pressure and the crude product was suspended in 0.5 ml of MeOH and precipitated by addition of 6-8 ml of diethyl ether. The precipitate was separated by centrifugation and was purified by flash chromatography (CH_2Cl_2:MeOH, 10:1). [Pt(OAc)(2-azidoacetate)(Cl_2(NH_3)$_2$)] **2** was obtained as a beige powder. Yield: 36 mg (66%). ^1H NMR (400 MHz, DMSO-d_6, δ, ppm.): 6.65-6.35 (m, 6H, NH_3), 3.87 (s, 2H, C(O)CH_2N_3), 1.90 (s, 3H, C(O)-CH_3). HRMS: calc. for $C_4H_{11}Cl_2N_5NaO_4Pt^+$ 480.9734 (**2**+Na)$^+$; found $C_4H_{11}Cl_2N_5NaO_4Pt^+$ 480.9735 (**2**+Na)$^+$.

Pt(OAc)(6-azidohexanoate)($Cl_2(NH_3)_2$)] 3. 89 mg (0.57 mmol, 3.5 equiv.) of 6-azidohexanoic acid were dissolved in 4 ml of DCM, 59 mg (0.28 mmol, 1.8 equiv.) of DCC were added, the reaction was stirred at room temperature for 12 hours. The precipitate was removed by filtration and washed by DCM, the solvent was removed under reduced pressure, the residue was suspended in EtOAc and filtered again. The solvent was again removed under reduced pressure, the residue was dissolved in 0.9 ml of dry DMF and 60 mg (0.16 mmol, 1 equiv.) of complex **1** was added. The reaction mixture was stirred at rt for 24 hours, the solvent was removed under reduced pressure, the residue was suspended in 0.5 ml of MeOH, precipitated with ether, the crude product was purified by flash chromatography (CH_2Cl_2:MeOH, 10:1). Pt(OAc)(6-azidohexanoate)($Cl_2(NH_3)_2$)] **3** was obtained as a beige powder. Yield: 57 mg (69%). ^{1}H NMR (400 MHz, DMSO-d_6, δ, ppm.): 6.65-6.35 (m, 6H, NH_3), 3.30 (m, 2H, COOPt-C$\underline{H}_2$), 2.21 (t, 2H, J=7.4 Hz, CH_2-N_3), 1.89 (s, 3H, CH_3), 1.53-1.42 (m, 4H, β-CH_2, δ-CH_2), 1.32 (m, 2H, γ-CH_2).

3-Bromo-2-acetylthiophene 4.[S5] 100 ml of dry DCM were cooled using ice bath, then 4.9 g (36.8 mmol, 6 equiv.) of $AlCl_3$ were added under Ar. Then the solution of 2.68 ml of acetyl chloride (39.2 mmol, 6.4 equiv.) in 30 ml of DCM was added over 10 minutes, then solution was stirred for 30 minutes at 0°C. 0.574 ml of 3-bromothiophene (6.13 mmol, 1 equiv.) in 30 ml of DCM were added over 10 minutes, the reaction was stirred for 30 minutes at 0°C and allowed to cool to rt within 1 hour. Then the reaction was again cooled to 0°C , diluted with DCM, water was added carefully. The organic layer was separated, the water layer was extracted with DCM, then the combined organic layer was washed with $NaHCO_3$ solution, brine, dried with sodium sulfate. The solvent was removed under reduced pressure, 3-bromo-2-acetylthiophene **4** was obtained as a dark brown liquid. Yield: 1.1 g (89%). ^{1}H NMR (400 MHz, $CDCl_3$, δ, ppm.): 7.51 (d, 1H, J=5.3 Hz, α-H), 7.10 (d, 1H, J=5.3 Hz, β-H), 2.69 (s, 3H, CH_3). ^{13}C NMR (101 MHz, $CDCl_3$, δ, ppm): 190.09, 139.12, 133.58, 133.21, 114.30, 29.64.

Ethyl 6-methyl-4*H*-thieno[3,2-*b*]pyrrole-5-carboxylate 5.[S6] 1.02 g (5 mmol, 1 equiv.) of thiophene **4**, 3.25 g (10 mmol, 2 equiv.) of Cs_2CO_3 and 95 mg (0.5 mmol, 0.1 equiv.) of CuI were mixed in 3 ml of DMSO and stirred under Ar for 30 minutes at room temperature. Then 622 mg (5.5 mmol, 1.1 equiv.) of ethyl isocyanoacetate were added dropwise and the mixture was stirred for 4 hours at 50°C. The mixture was diluted with DCM, washed twice with brine, dried, the solvent was removed under reduced pressure, the crude product was purified with column chromatography (eluent hexane:EtOAc 10:1). Ethyl 6-methyl-4H-thieno[3,2-b]pyrrole-5-carboxylate **5** was obtained as a white solid. Yield: 370 mg (35%). ^{1}H NMR (400 MHz, $CDCl_3$-d6, δ, ppm.): 9.01 (br. s., 1H, NH), 7.31 (d, 1H, J=5.3 Hz, α-H(Th)), 6.92 (d, 1H, J=5.3 Hz, β-H(Th)), 4.38 (q, 2H, J=7.2 Hz, CH_2), 2.53 (s, 3H, CH_3), 1.41 (t, 3H, J=7.2 Hz, CH_2-C$\underline{H}_3$). ^{13}C NMR (101 MHz, $CDCl_3$, δ, ppm): 161.92, 139.08, 128.68, 126.13, 122.86, 119.82, 110.94, 59.92, 14.11, 11.86.

6-Methyl-4*H*-thieno[3,2-*b*]pyrrole 6. 370 mg (1.77 mmol, 1 equiv.) of thienopyrrole **5** and 692 mg (10.6 mmol, 6 equiv.) of KOH were dissolved in 8.9 ml of ethylene glycol and the reaction mixture was refluxed under Ar for 2 hours with TLC control. When all starting compound **5** was consumed, the mixture was cooled to room temperature, put into water, extracted with DCM, the organic layer was dried and the solvent was removed under reduced pressure. 6-methyl-4H-thieno[3,2-b]pyrrole **6** was obtained as a light-yellow oil. Yield: 210 mg (86%). ^{1}H NMR (400 MHz, $CDCl_3$, δ, ppm.): 7.99 (br. s., 1H, NH), 7.09 (d, 1H, J=5.1 Hz, α-H(Th)), 6.92 (d, 1H, J=5.1 Hz, β-H(Th)), 6.77 (s, 1H, α-H(Pyr)), 2.26 (s, 3H, CH_3). ^{13}C NMR (101 MHz, $CDCl_3$, δ, ppm): 181.99, 138.32, 123.34, 120.42, 112.05, 111.39, 11.27.

5,5-Difluoro-10,12-dimethyl-11-(4-(prop-2-yn-1-yloxy)phenyl)-5*H*-4λ^4,5λ^4-thieno[2',3':4,5]pyrrolo-[1,2-*c*]thieno[2',3':4,5]pyrrolo[2,1-*f*][1,3,2]diazaborinine 7. 190 mg (1.39 mmol, 2 equiv.) of thienopyrrole 6 and 107 mg (0.68 mmol, 1 equiv.) of 4-(prop-2-yn-1-yloxy)benzaldehyde were dissolved in 140 ml of DCM and few drops of TFA were added. The reaction mixture was stirred for 18 hours under Ar at rt, then 190 mg of DDQ in 30 ml of DCM were added in one portion. The reaction was stirred at rt for 30 minutes, cooled down to 0°C on an ice bath, then 2.16 ml of Et_3N were added, reaction mixture was stirred for 30 minutes at 0°C, then 1.48 ml of BF_3*Et_2O were slowly added and the reaction was stirred overnight at rt. The reaction was filtered over layer of silica gel, the filtrate was washed trice with water, organic layer was dried, then the solvent was removed under reduced pressure. The crude product was purified using flash chromatography, eluent DCM:MeOH 10:1. 5,5-difluoro-10,12-dimethyl-11-(4-(prop-2-yn-1-yloxy)phenyl)-5*H*-4λ^4,5λ^4-thieno[2',3':4,5]pyrrolo[1,2-*c*]thieno-[2',3':4,5]pyrrolo[2,1-*f*][1,3,2]diazaborinine 7 was obtained as a green powder. Yield: 79 mg (25%). ^{1}H NMR (400 MHz, $CDCl_3$-d_6, δ, ppm.): 7.61-7.60 (m, 2H, $H_{3,4}$(Ar)), 7.30-7.28 (m, 2H, α-H(Th)), 7.17-7.14 (m, 4H, β-H(Th), $H_{2,6}$(Ar), 4.79 (d, 2H, J=2.4 Hz, CH_2), 2.58 (t, 1H, J=2.4 Hz, C≡CH), 1.63 (s, 6H, CH_3). ^{13}C NMR (101 MHz, $CDCl_3$, δ, ppm): 158.55, 156.98, 145.53, 140.21, 133.92, 129.55, 126.92, 115.92, 114.16, 76.05, 56.08, 14.21.

General procedure for the synthesis of BODIPY-conjugated Pt^{IV} prodrugs
24.2 mg (0.052 mmol, 1.2 equiv.) of BODIPY **1**, 7 mg of TBTA (0.012 mmol, 0.3 equiv.) and 4.2 mg of $Cu(CH_3CN)_4BF_4$ (0.012 mmol, 0.3 equiv.) were dissolved in 2 ml of DMF under Ar, the reaction was stirred for 10 minutes at room temperature, then 0.04 mmol (1 equiv.) of the corresponding Pt^{IV} prodrug was added and the reaction was stirred for 2 hours at room temperature with TLC control. The solvent was then removed under reduced pressure, the crude product was purified using column chromatography, eluent DCM:MeOH 10:1.

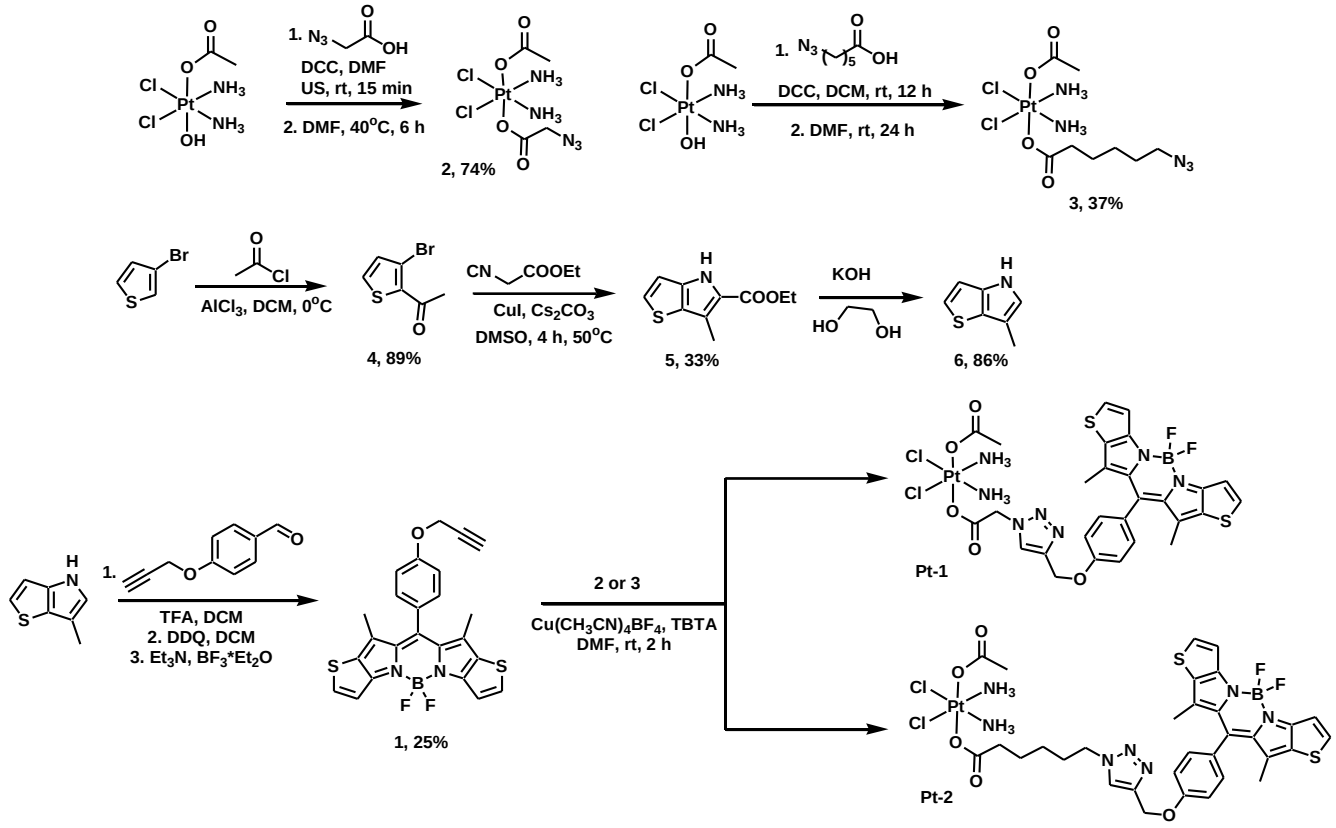
Pt-1. From 20 mg of [Pt(OAc)(2-azidoacetate)($Cl_2(NH_3)_2$)] **2** 20 mg (50%) of **Pt-1** was obtained as a dark purple powder. **^{1}H NMR** (400 MHz, DMSO-d_6, δ, ppm): 8.18 (s, 1H, N-CH=C), 8.08 (d, 2H, J=5.3 Hz, α-H(Th)), 7.42 (d, 2H, J=8.6 Hz, $H_{3,4}$(Ar)), 7.30 (d, 2H, J=8.6 Hz, $H_{2,6}$(Ar)), 7.06 (d, 2H, J=5.3 Hz, β-H(Th)), 6.53 (br.s., 6H, NH_3), 5.24 (s, 4H, O-CH_2, N-CH_2-COO), 1.91 (s, 3H, CH_3-COO), 1.62 (s, 6H, 1,7-CH_3). **^{195}Pt NMR** (86 MHz, DMSO-d_6 δ, ppm): 1232.55. **HRMS:** calc. $C_{28}H_{28}BCl_2FN_7O_5PtS_2$, 901.0695 (**Pt-1**+H-HF)$^+$; found $C_{28}H_{28}BCl_2FN_7O_5PtS_2$ 901.0684 (**Pt-1**+H-HF)$^+$.

Pt-2. From 28 mg of [Pt(OAc)(6-azidohexanoate)($Cl_2(NH_3)_2$)] **3** 20 mg (38%) of **Pt-2** was obtained as a dark purple powder. ^{1}H NMR (400 MHz, DMSO-d_6, δ, ppm): 8.29 (s, 1H, N-CH=C), 8.08 (d, 2H, J=5.3 Hz, α-H(Th)), 7.42 (d, 2H, J=8.6 Hz, $H_{3,4}$(Ar)), 7.30 (d, 2H, J=8.6 Hz, $H_{2,6}$(Ar)), 7.06 (d, 2H, J=5.3 Hz, β-H(Th)), 6.53 (br.s., 6H, NH_3), 5.24 (s, 2H, O-CH_2), 4.37 (t, 2H, J=6.9 Hz, N-CH_2), 2.21 (t, 2H, J=8.1 Hz, CH_2-COO), 1.90 (s, 3H, CH_3-COO), 1.84-1.79 (m, 2H, C$\underline{H_2}$-CH_2-N), 1.62 (s, 6H, 1,7-CH_3), 1.52-1.46 (m, 2H, CH_2-C$\underline{H_2}$-CH_2), 1.31-1.24 (m, 2H, CH_2-C$\underline{H_2}$-CH_2). **^{195}Pt NMR** (86 MHz, DMSO-d_6 δ, ppm): 1232.07. **HRMS:** calc. $C_{32}H_{36}BCl_2FN_7O_5PtS_2$, 957.1321 (**Pt-2**+H-HF)$^+$; found $C_{32}H_{36}BCl_2FN_7O_5PtS_2$ 957.1314 (**Pt-2**+H-HF)$^+$.

Stability and reduction rate. Platinum(IV) prodrugs **Pt-1** and **Pt-2** were dissolved in the DMSO:MeOH:H_2O 60:30:10 mixture to achieve 1 mM concentration. For reduction studies, the stock solution of 100 mM NaAsc was used so that the final NaAsc concentration in the experimental mixture was 10 mM. The resulting solutions were kept in the dark at room temperature. At 0, 5 and 24 hours' time points aliquots were taken and diluted 16.7 times with methanol for analysis. **Photoreduction**. For light-induced photoreduction of **Pt-1** and **Pt-2** the 1 mM solutions of Pt^{IV} prodrugs containing 10 mM NaAsc were irradiated with 530 nm light (3.8 mW/cm^2), the aliquots were taken at 0 min (before irradiation), 1 min, 2 mins, 5 mins and 10 mins.

Cytotoxicity. Immortalized human fibroblasts WI-26 were seeded in a 96-well plate (5×10^4 cells/well) in complete RPMI-1640 medium and incubated overnight. Then working solutions of the studied compounds (0.05-100 μM) were added to the cells (100 μl) for 2 h in a CO_2 incubator. Then, to determine dark toxicity, the cells were left in the dark for another 72 h. Light toxicity was assessed using two approaches. In the first case, after 2 h of incubation, the medium was replaced with fresh one and then irradiated with a dose of 1 J/cm^2 (450 nm, 80 s). In the second case, irradiation (450 nm, 1 J/cm^2, 80 s) was carried out in a medium containing the tested compounds. Cytotoxicity was measured after 72 h using the MTT assay, the viability of control cells was taken as 100%.

Scheme S1.



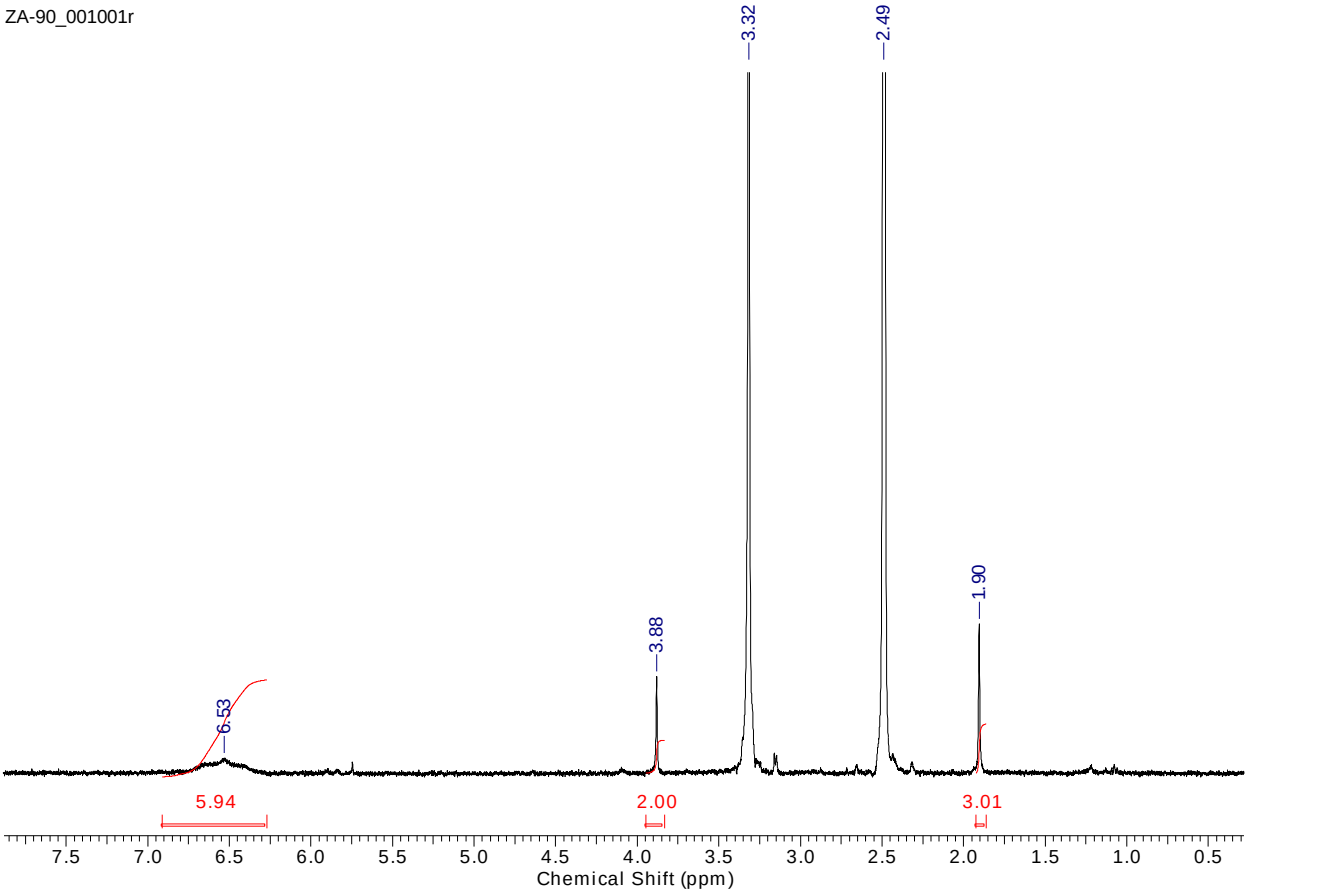


Figure S1. ^{1}H NMR spectrum of compound $(NH_3)_2Cl_2(AcO)Pt(OH)$**.**

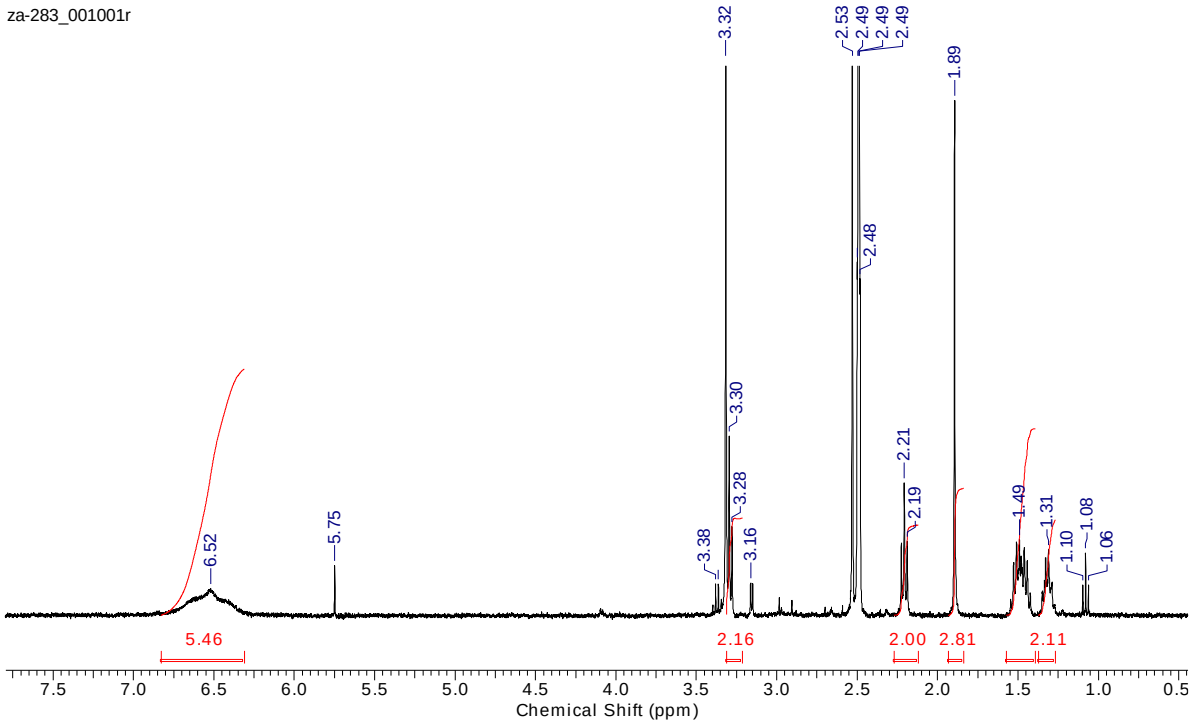


Figure S2. ^{1}H NMR spectrum of compound **3.**

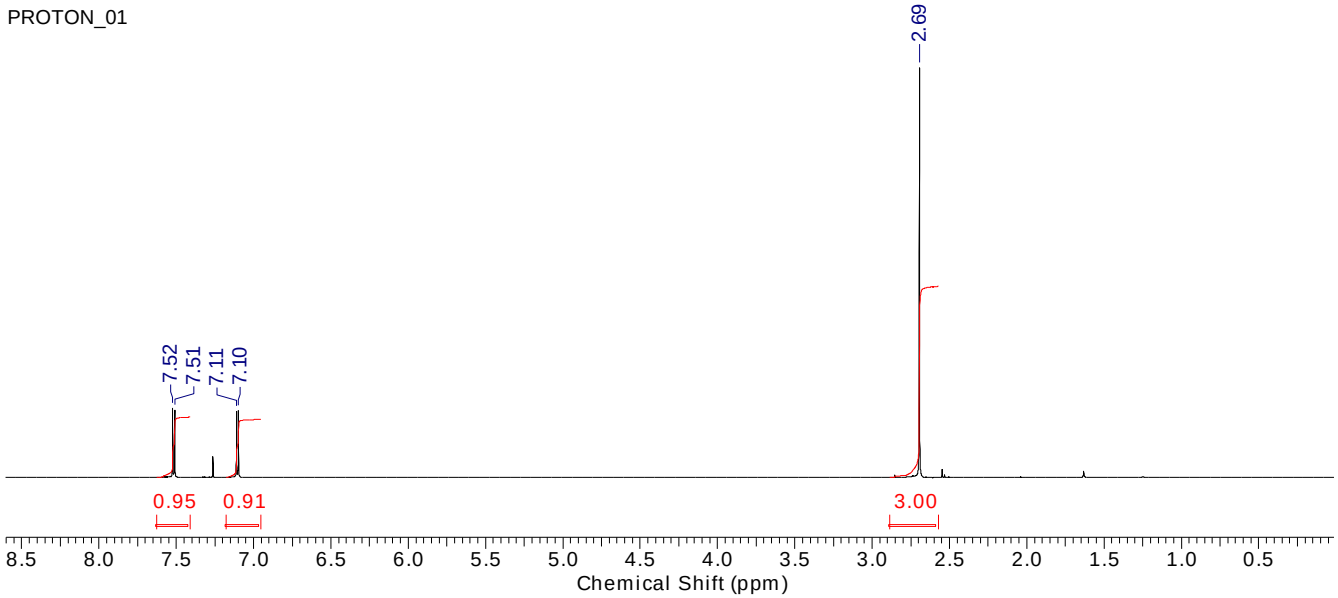


Figure S3. ^{1}H NMR spectrum of compound **4.**

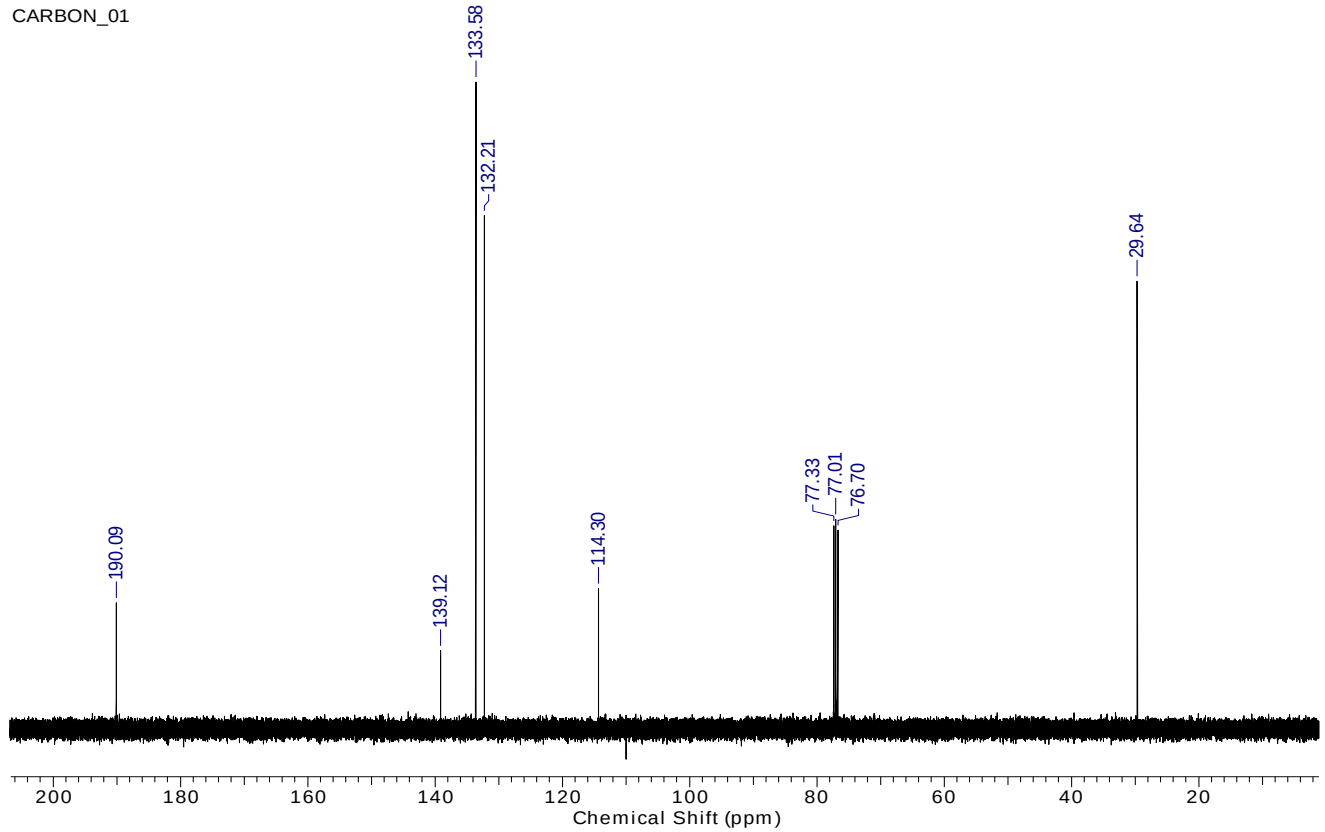


Figure S4. ^{13}C NMR spectrum of compound **4.**

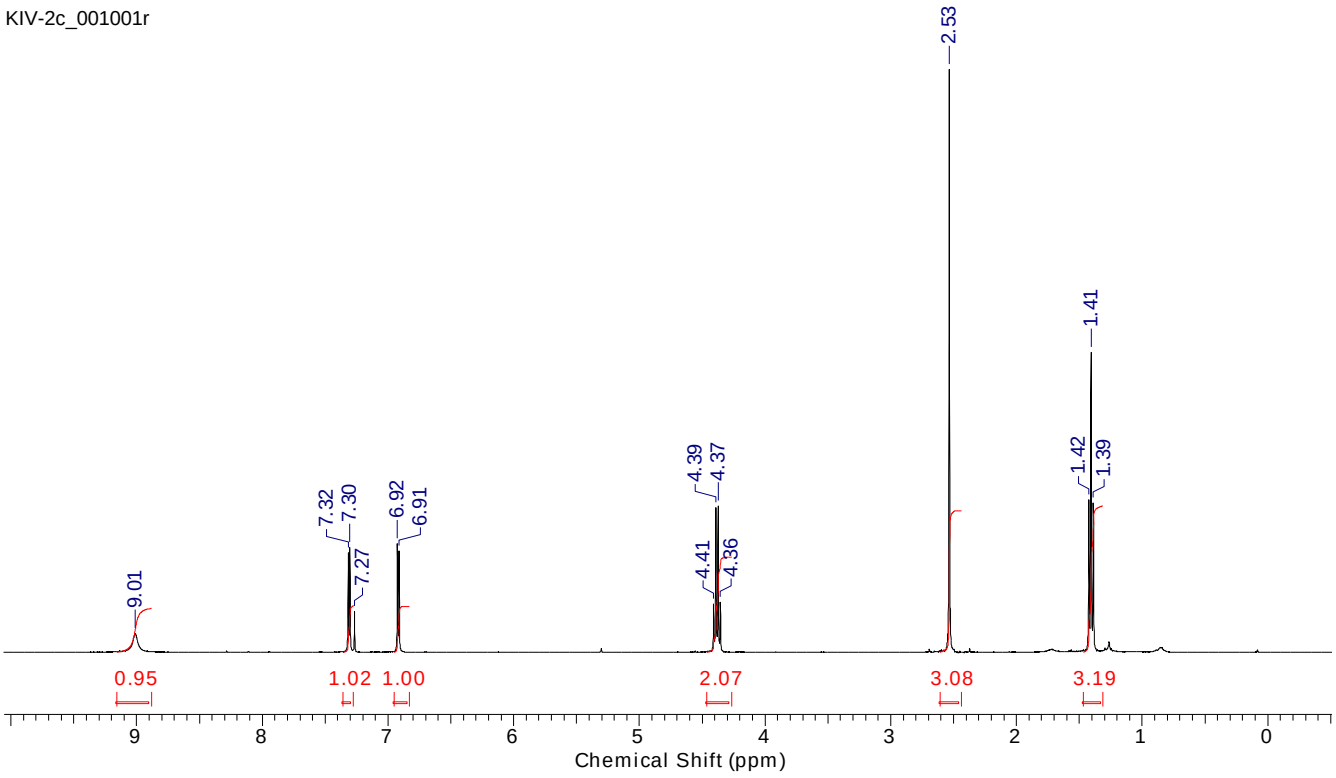


Figure S5. ^{13}C NMR spectrum of compound **5.**

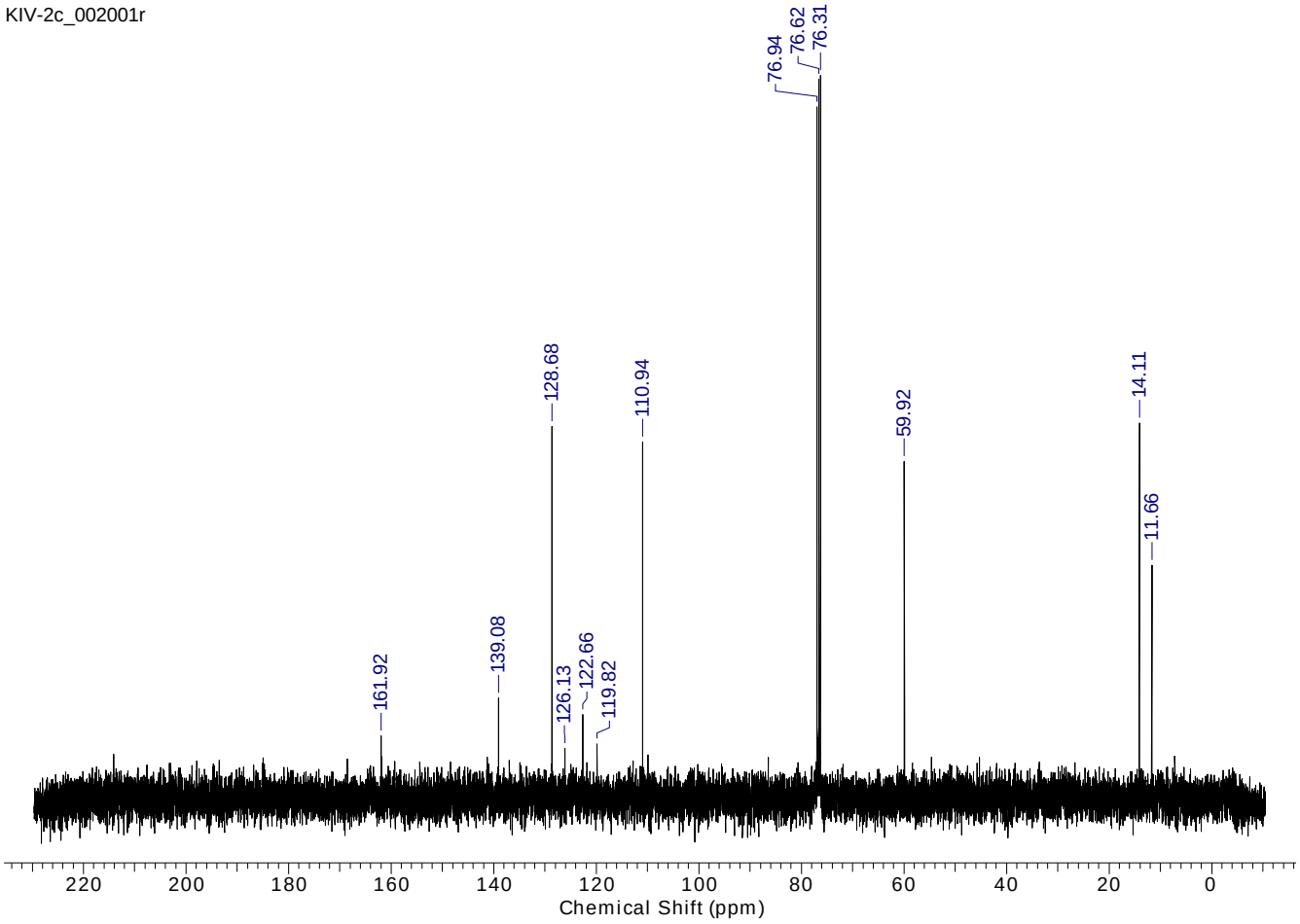


Figure S6. ^{13}C NMR spectrum of compound **5.**

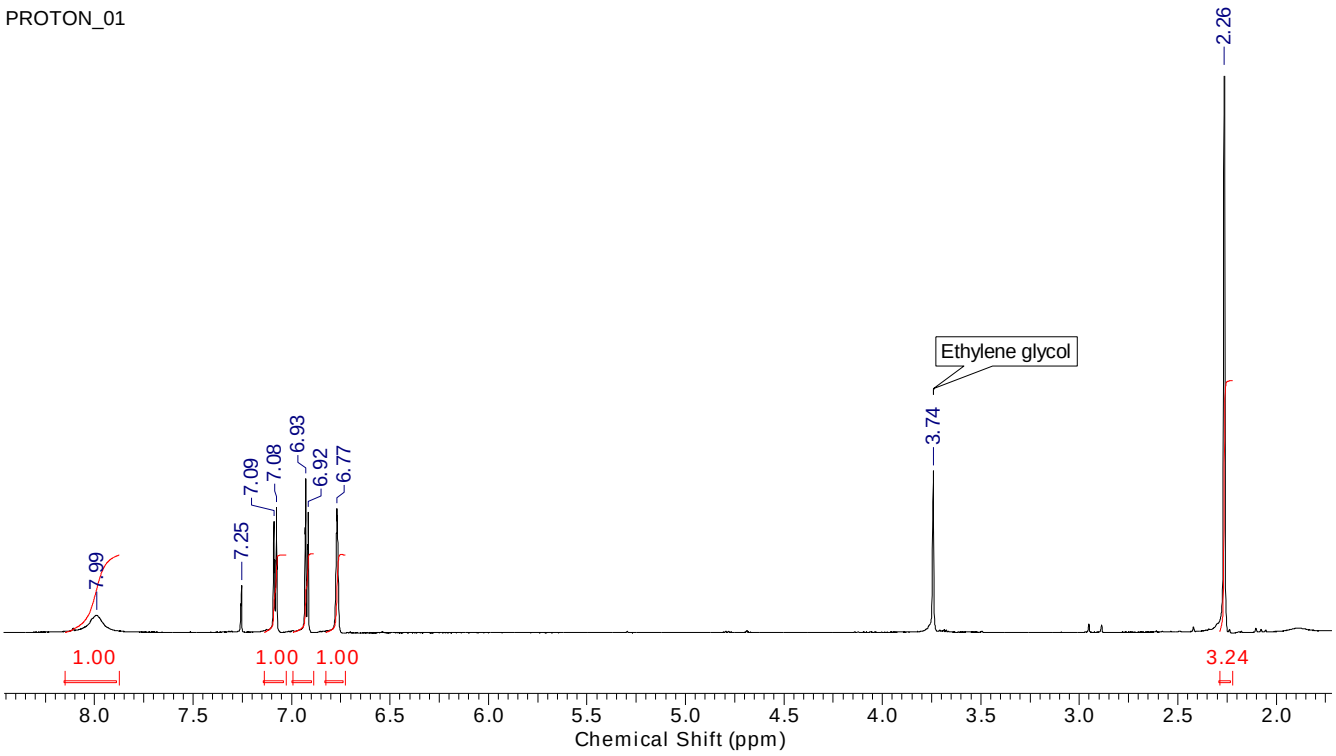


Figure S7. ^{13}C NMR spectrum of compound **6.**

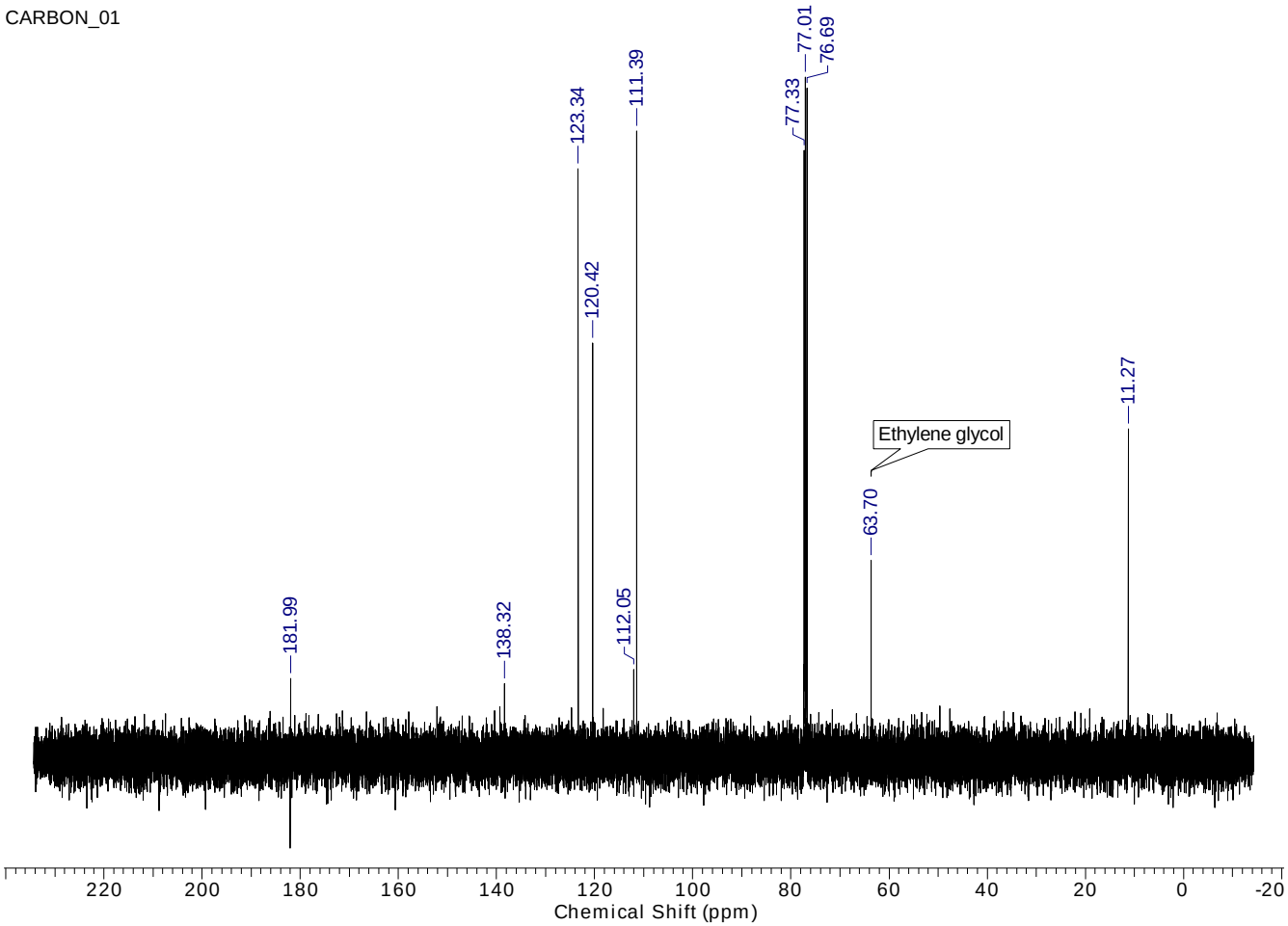


Figure S8. ^{13}C NMR spectrum of compound **6.**

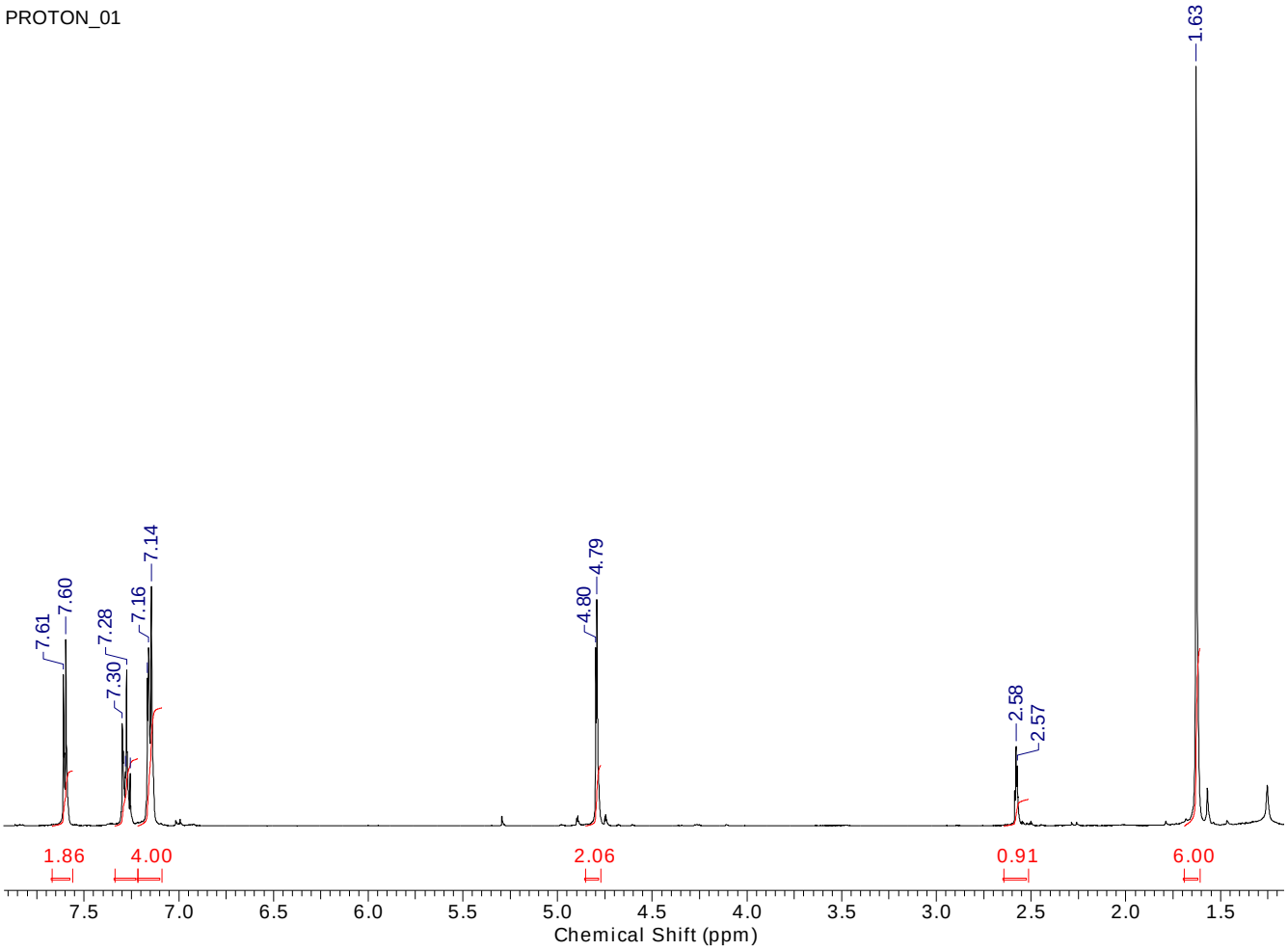


Figure S9. ^{1}H NMR spectrum of compound **1.**

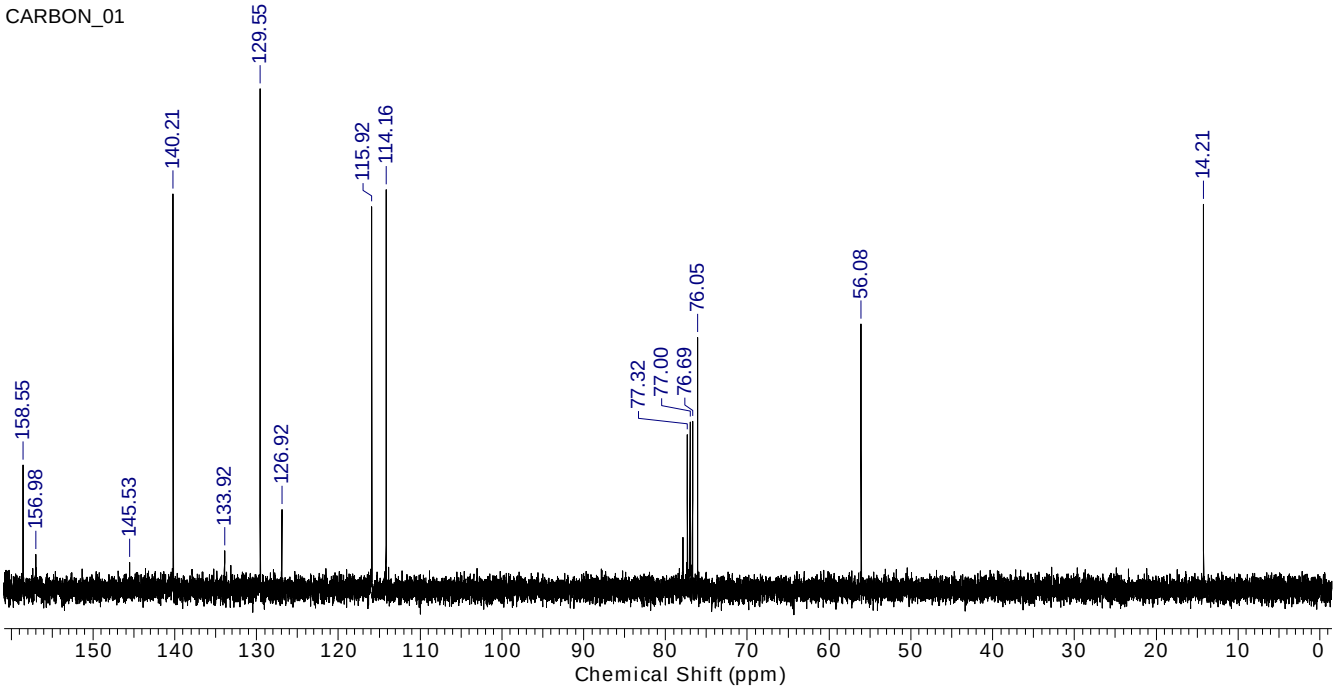


Figure S10. ^{13}C NMR spectrum of compound **1.**

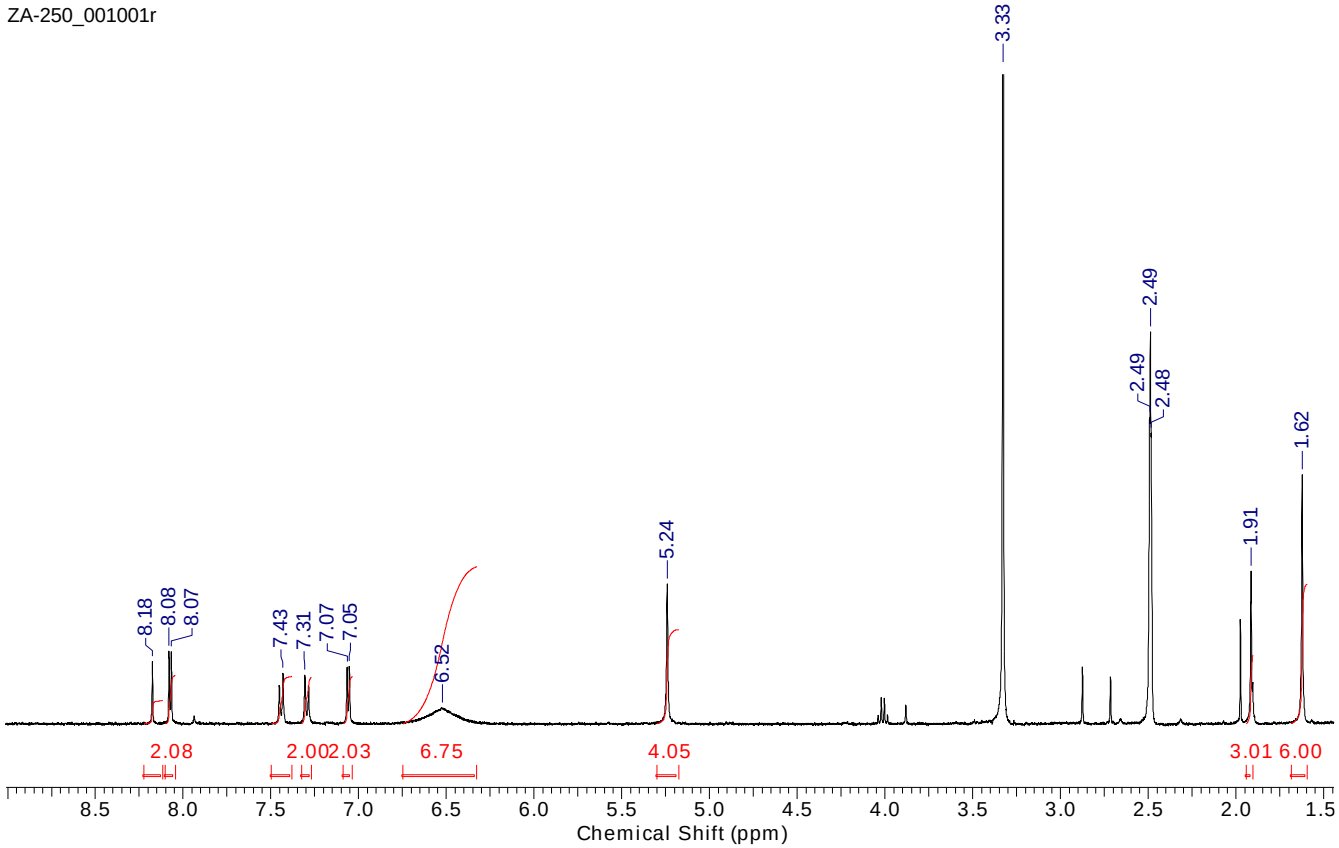


Figure S11. ^{1}H NMR spectrum of the Pt^{IV} prodrug **Pt-1.**

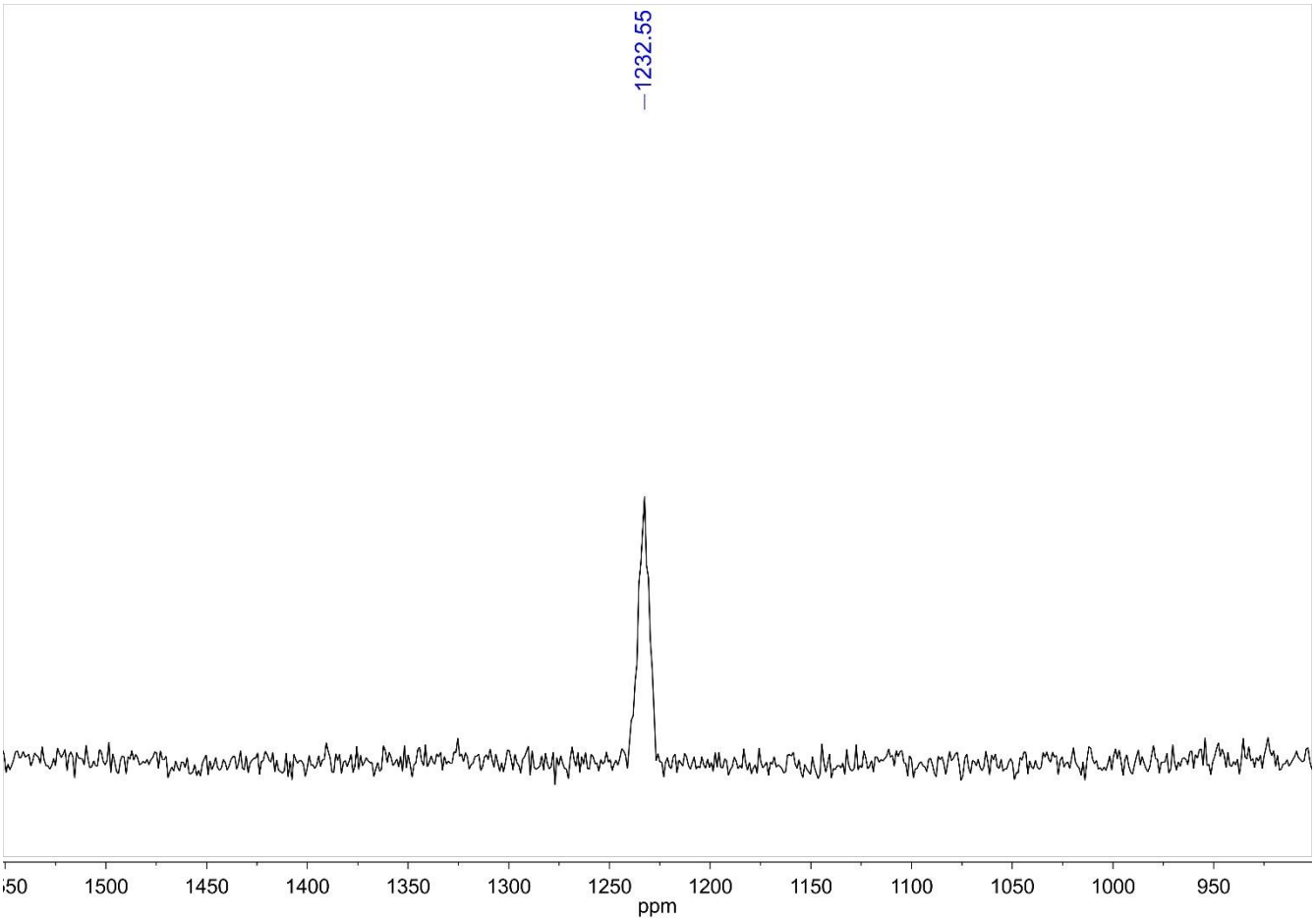


Figure S12. ^{195}Pt NMR spectrum of the Pt^{IV} prodrug **Pt-1.**

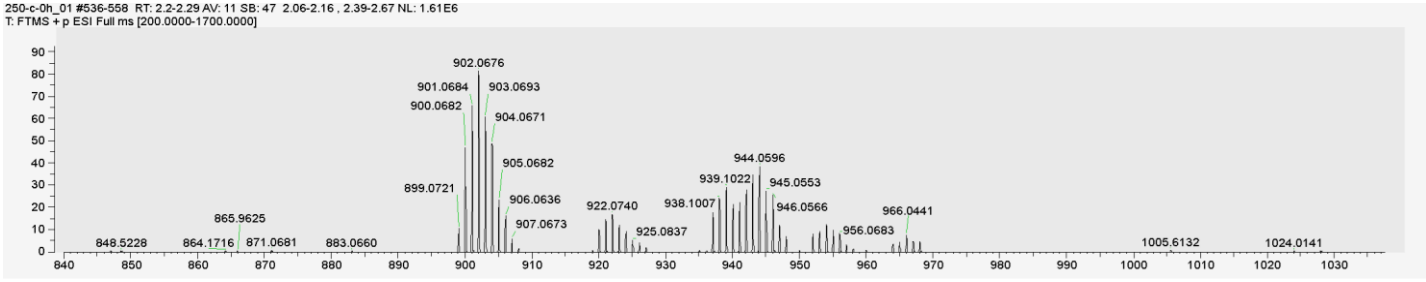


Figure S13. HRMS spectrum of the Pt^{IV} prodrug **Pt-1.**

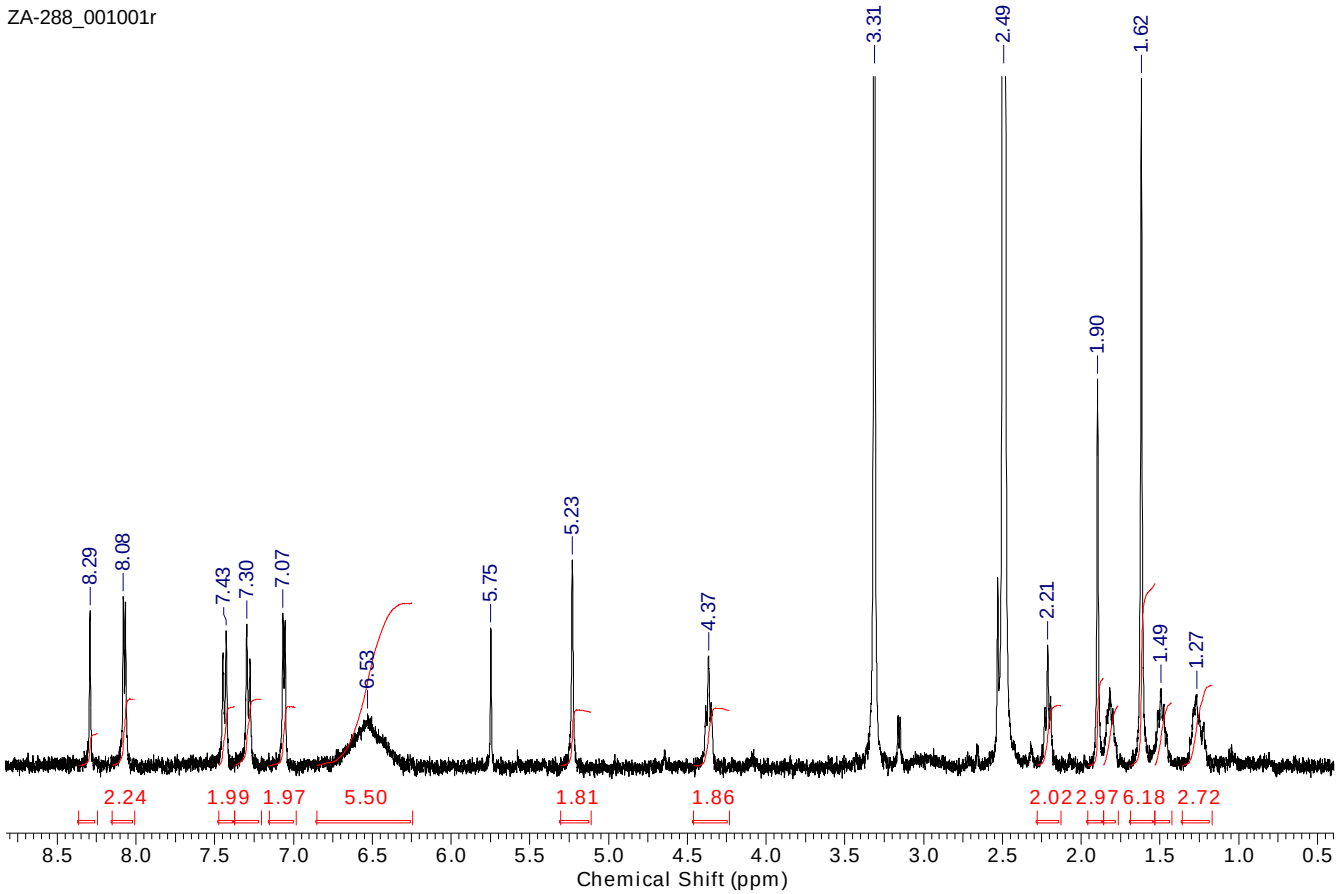


Figure S14. ^{1}H NMR spectrum of the Pt^{IV} prodrug **Pt-2.**

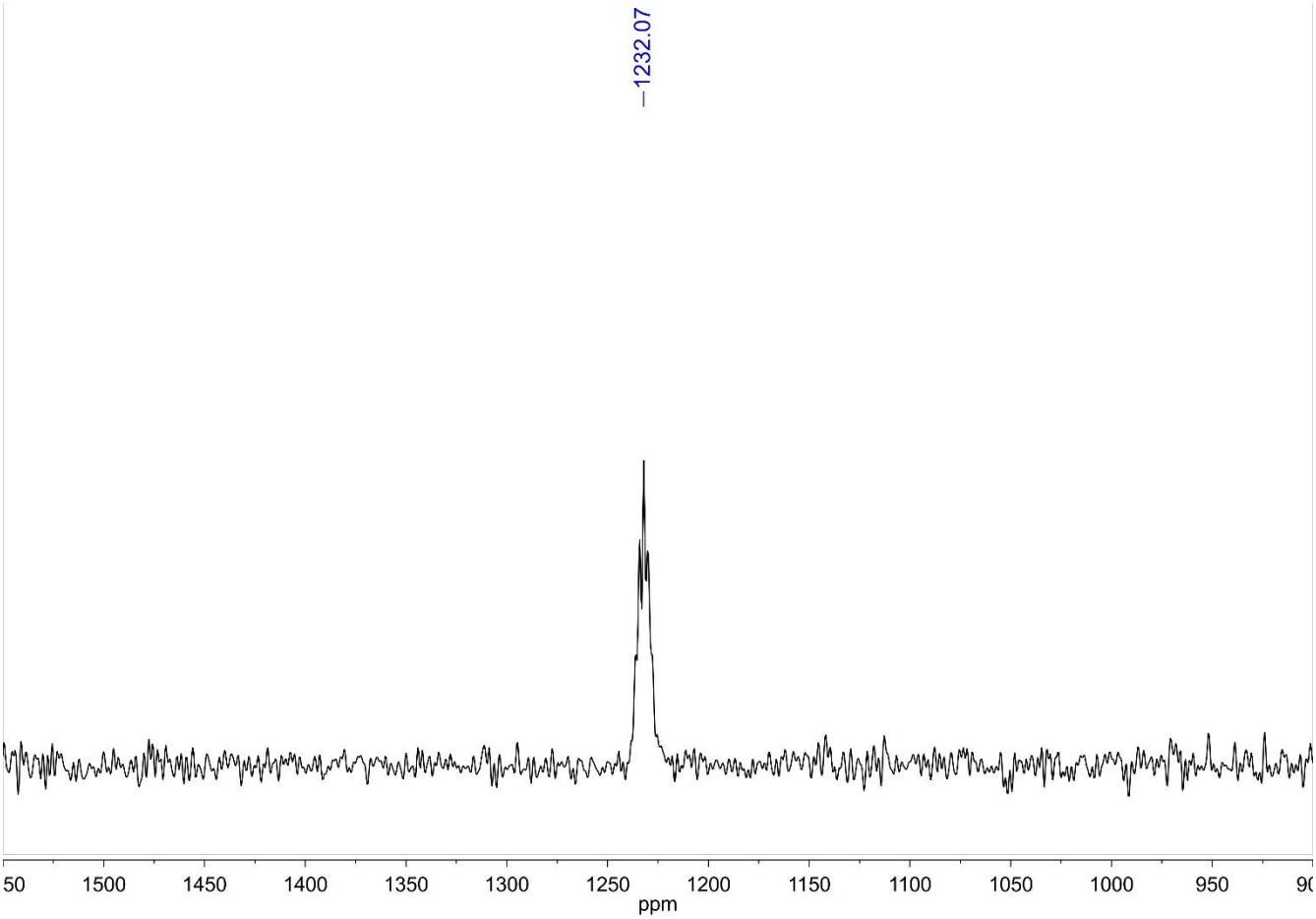


Figure S15. ^{195}Pt NMR spectrum of the Pt^{IV} prodrug **Pt-2.**

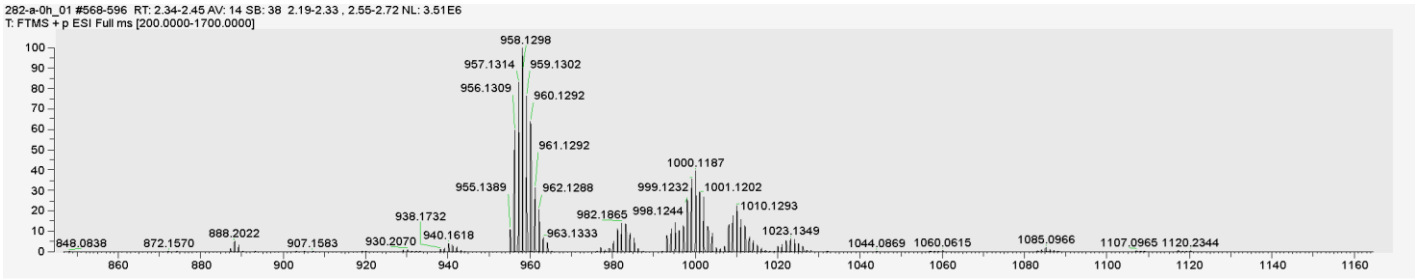


Figure S16. HRMS spectrum of the Pt^{IV} prodrug **Pt-2.**

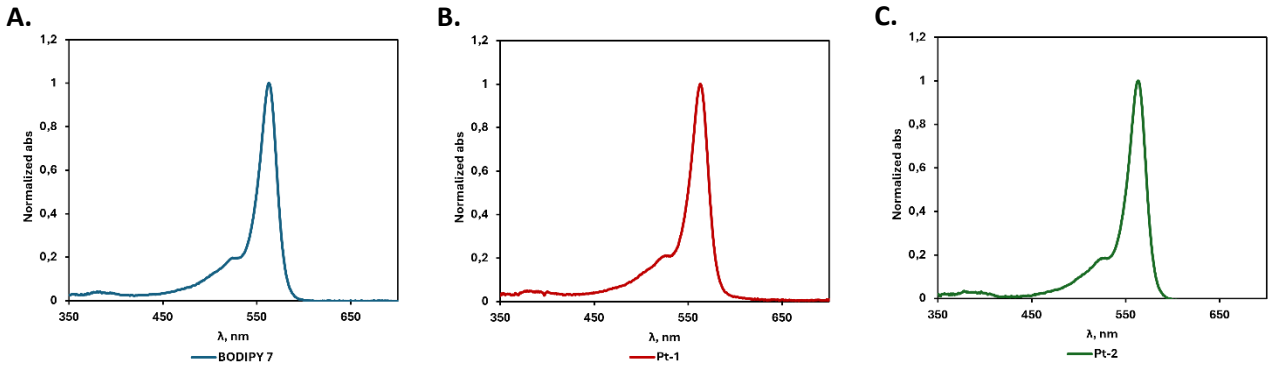


Figure S17. Normalized absorption spectra of BODIPY **1 (A)**, **Pt-1 (B)** and **Pt-2 (C)** Pt(IV) prodrugs

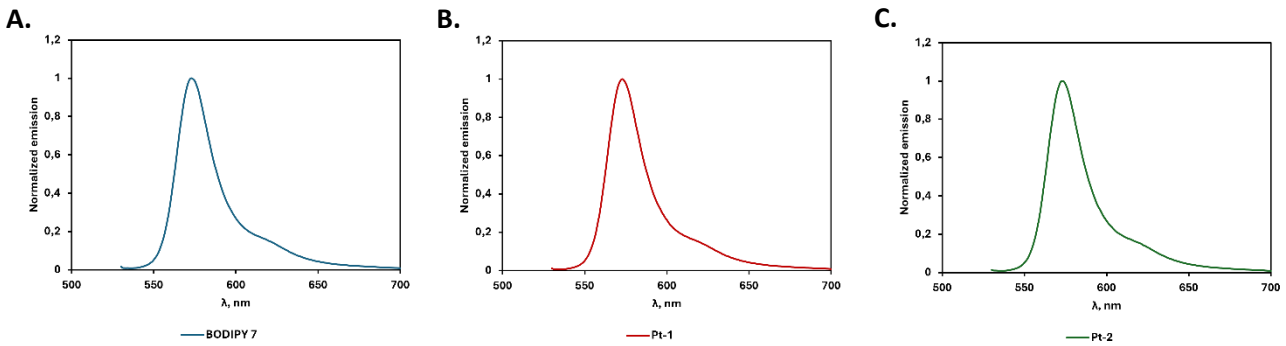


Figure S18. Normalized emission spectra of BODIPY **1 (A)**, **Pt-1 (B)** and **Pt-2 (C)** Pt(IV) prodrugs. λ_{ex} = 525 nm, slits 5/5.

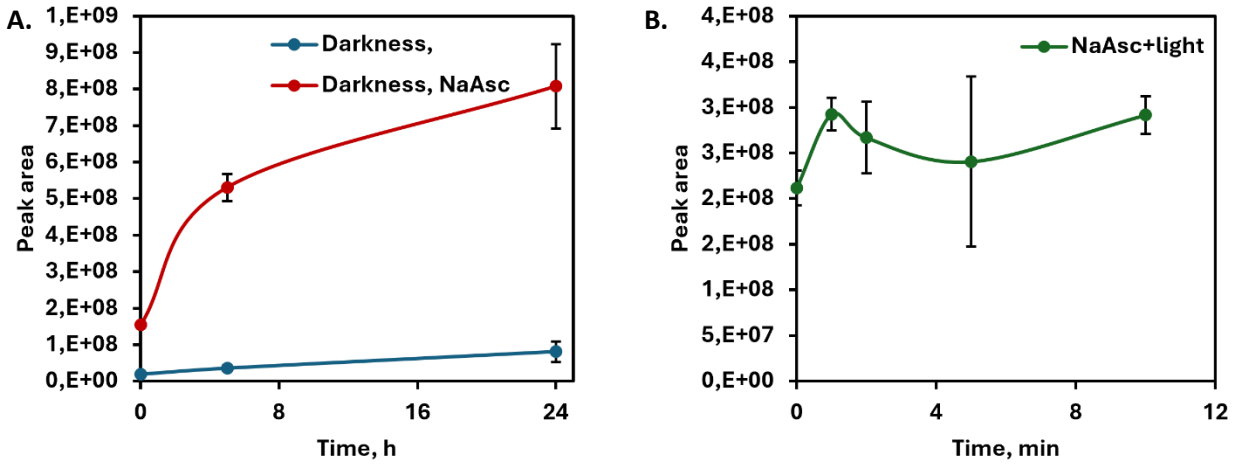


Figure S19. CDDP accumulation in **Pt-1** solutions based on the 342.0100 ion peak area in LCMS **A.** In the absence of light **B.** Under green light irradiation (530 nm, 3.8 mW/cm^2).

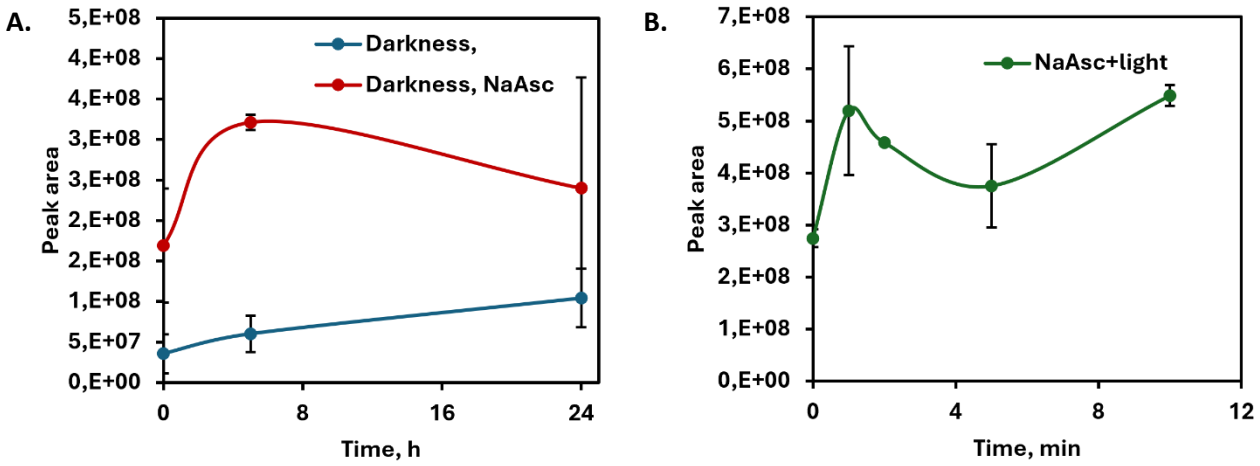


Figure S20. CDDP accumulation in **Pt-2** solutions based on the 342.0100 ion peak area in LCMS **A.** In the absence of light **B.** Under green light irradiation (530 nm, 3.8 mW/cm^2).

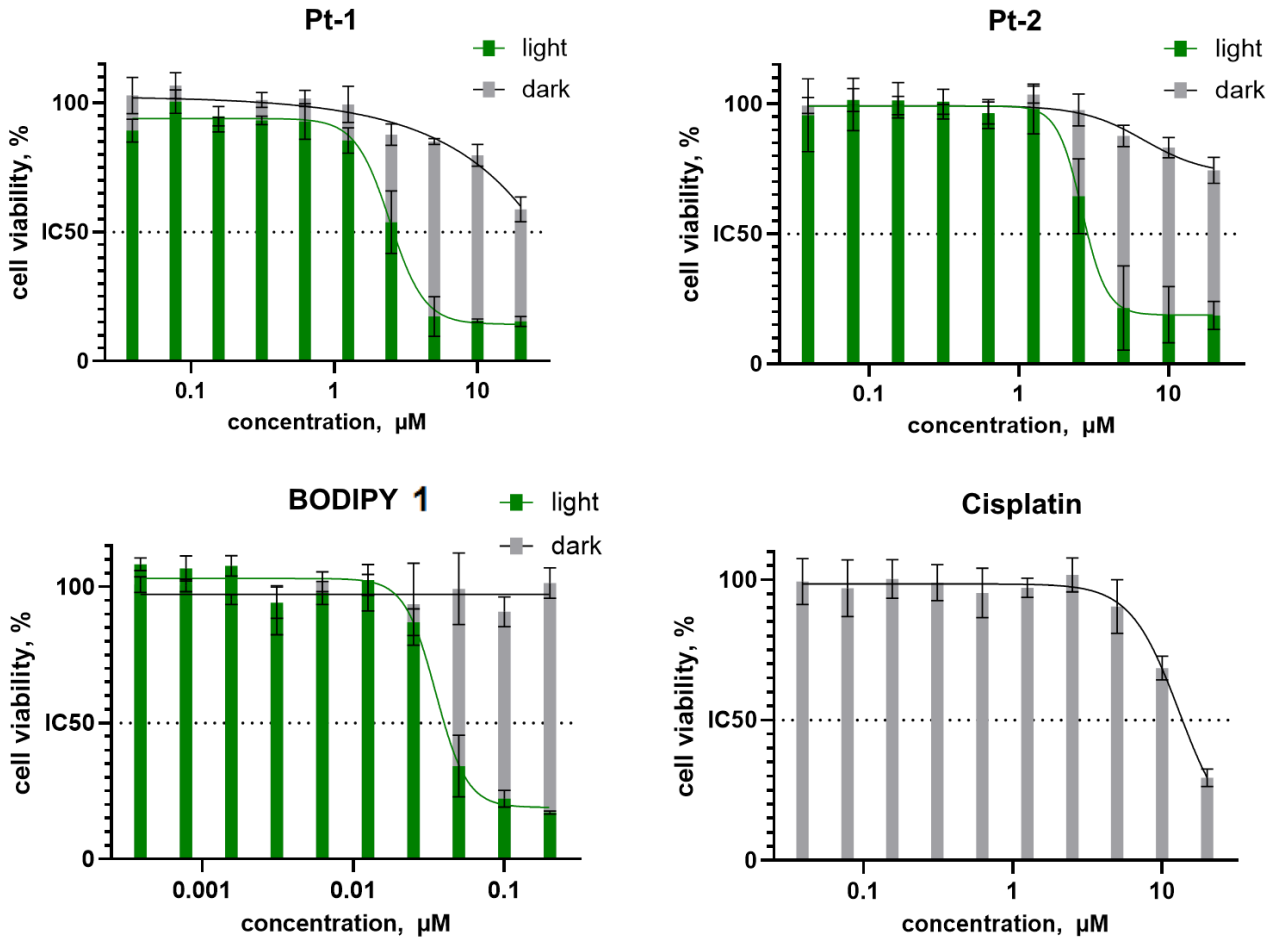


Figure S21. Sk-Br-3 cell viability curves incubated with **Pt-1**, **Pt-2**, BODIPY **1** and cisplatin in the darkness and under green light (530 nm, 3.8 mW/cm^2, 4 min 20 s).

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