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Photoactive platinum(IV) complex conjugated to a cancer-cell-targeting cyclic peptide

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Experimental Section

Materials and instruments. Pyridine (py) was purchased from Fischer Scientific UK, $KPtCl_4$, NaN_3, H_2O_2 (30%), succinic anhydride, *N*-hydroxysuccinimide (NHS), 4-dimethylaminopyridine (DMAP), 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDC), *N*,*N*-diisopropylethylamineand (DIPEA) and other chemicals were from Sigma Aldrich and used without further purification. Roswell Park Memorial Institute media (RPMI-1640) supplemented with 10% v/v foetal calf serum (FCS) and 1% v/v penicillin/streptomycin was used in *in vitro* experiments.

The cyclic disulphide peptide c(CRWYDENAC) was purchased from Cambridge Research Biochemicals (Billingham, UK) with a purity of >95% (HPLC/MS), all of amino acids in the peptide have L-configuration.

NMR spectra were recorded on a Bruker Avance III 400 MHz spectrometer using the residual signal of the solvent as a chemical shift reference. ESI-HR-MS data were collected on a Bruker microTOF instrument at 298 K with a scan range of m/z 50-2000 in positive mode. Samples were prepared in methanol solution.

Electronic absorption spectra were recorded on a Varian Cary 300 UV-vis spectrophotometer in a 1 cm quartz cuvette and referenced to neat solvent. The spectral width was 200–600 nm and the bandwidth was 1.0 nm; the scan rate was set to 600 nm/min.

Analytical reversed-phase HPLC analyses were carried out on an Agilent ZORBAX Eclipse XDB-C18 column (250×4.6 mm, 5 μm, flow rate: 1 mL/min), by using linear gradients of 0.1% v/v TFA in H_2O (solvent A) and 0.1% v/v TFA in CH_3CN (solvent B). LC-MS was carried out on a Bruker Amazon X connected online to an Agilent 1260 HPLC.

A LZC-ICH2 photoreactor (Luzchem Research Inc.) equipped with 8 Luzchem LZC-420 lamps without light filtration was used as the light sources for photoactivation. A blue LED light source with λ_{max} = 465 nm was used for *in vitro* growth inhibition and cellular accumulation.

Analysis of platinum content was carried out on ICP-OES 5300DV (Perkin Elmer) or ICP-MS 7500cx (Agilent) instruments. The emission wavelength used for Pt ICP-OES detection was 265.945 nm, and ^{195}Pt was detected in ICP-MS using ^{166}Er (50 ppb) as an internal standard.

Synthesis and characterisation. *Caution!* No problem was encountered during the work reported here, but due care and attention with appropriate precautions should be taken in the synthesis and handling of heavy metal azides. All syntheses and purifications were carried out in the dark with minimal light exposure.

Freshly prepared *trans*, *trans*, *trans*-[Pt(py)$_2$(N$_3$)$_2$(OH)(succ-NHS)] (**FM-NHS**) (14.1 mg, 21.1 μmol) was stirred with c(CRWYDENAC) (9.0 mg, 7.7 μmol) and DIPEA (40 μL) in DMF (2 mL) under nitrogen at 298 K for 36 h. After evaporation to dryness, the excess Pt(IV) precursor and DIPEA were removed by washing with DCM and H_2O. The yellow solid was dried and characterised by LC-MS. Yield: 36%. ESI-HRMS: $[M + 2\,H]^{2+}$ (*m/z*): 855.7493 and $[M + H]^+$: 1710.4919. HPLC R_t = 12.7 min (analytical gradient: 10 to 80% CH_3CN in 30 min). $\varepsilon_{290\ nm}$ = 30435 $M^{-1}\,cm^{-1}$, $\varepsilon_{260\ nm}$ = 27442 $M^{-1}\,cm^{-1}$ (5% DMSO + 95% RPMI-1640).

Dark stability and photodecomposition in aqueous solution. The dark stability and photodecomposition of conjugate **Pt-cP** in phenol red-free RPMI-1640 with 5% DMSO was monitored by UV-vis spectroscopy in the dark or in the presence of blue light (420 nm) at different time intervals at 298 K.

Photoreactions with 5′-GMP. 2 mol equiv of guanosine 5′-monophosphate disodium salt hydrate (5′-GMP-Na$_2$) were mixed with 30 μM **Pt-cP** in aqueous solution with 1% DMSO. The solution was irradiated for 1 h by blue light (420 nm) without prior incubation and analysed immediately. HPLC analysis was carried out on an Agilent ZORBAX Eclipse XDB-C18 column (250 × 4.6 mm, 5 μm, flow rate: 1 mL/min). The mobile phases A (0.1 % v/v formic acid in HPLC grade H_2O) and B (0.1 % v/v formic acid in HPLC grade acetonitrile) were used in HPLC. A Bruker Amazon X MS instrument connected online with HPLC analysed the Pt adducts.

Cell culture. A2780 ovarian carcinoma, A549 lung adenocarcinomic, PC3 prostate human cancer cell lines and normal MRC lung cell line were obtained from the European Collection of Animal Cell Culture (ECACC), Salisbury, UK. All cell lines used in this work were grown in fully prepared RPMI-1640. The adherent monolayers of cells were grown at 310 K in a humidified atmosphere containing 5% CO_2 and passaged regularly at *ca*. 80% confluence.

Photo-dark cytotoxicity. Approximately 10000 cells were seeded per well in 96-well plates. Independent duplicate plates were used, one for dark samples, the other for irradiation experiments. The cells were pre-incubated in drug-free medium with phenol red at 310 K for 24 h. Complexes were dissolved first in DMSO and then diluted in phenol red-free RPMI-1640 cell culture medium to make the stock solution of the drug. These stock solutions were further diluted using phenol-red free cell culture medium to achieve working concentrations. The maximum DMSO concentration was < 0.5% v/v in all solutions. Cells were exposed to various concentrations of the drugs for 1 h. Then one plate was irradiated for 1 h using blue light (4.8 mW cm^{-2} per LED at 465 nm), while the dark plate was kept in the incubator. After irradiation,

supernatants of both plates were removed by suction and washed with PBS buffer. Photocytotoxicity was determined after another 24 h recovery at 310 K in drug-free phenol red-containing medium by comparison to untreated controls which were exposed only to the vehicle. Untreated controls between the irradiated and the non-irradiated plates were also compared to ensure that the differences in cell survival were statistically relevant, hence guaranteeing that the differences in cell viability observed were not caused by the light source. The SRB assay was used to determine cell viability.[1] Absorbance measurements of the solubilised dye (on a Promega microplate reader) allowed the determination of viable treated cells compared to untreated controls. IC_{50} values (concentrations which caused 50% of cell death), were determined as the average of triplicates and their standard deviations were calculated. Stock concentrations for all metal complexes used in these biological assays were adjusted by ICP-OES metal quantification.

Platinum accumulation in cancer cells. For Pt cellular accumulation studies, *ca*. 5×10^6 cancer cells were plated in 100 mm Petri dishes and allowed to attach for 24 h. (i) For photo-dark cellular accumulation, independent duplicate plates were used. The plates were exposed to complexes at equipotent concentrations equal to the photoactive IC_{50} values in the corresponding cell line. After 1 h of incubation in the dark at 310 K, one of the plates was irradiated by blue light (465 nm) for 1 h, while the other one was left in the dark. (ii) For cellular accumulation at the same concentration, cells were treated with complexes at 10 μM for 1 h at 310 K in the dark. Additional plates were incubated with medium alone as a negative control. The cells were rinsed three times with cold phosphate-buffered saline (PBS) and harvested by trypsinisation. The number of cells in each sample was counted manually using a haemocytometer. Then the cells were centrifuged to obtain the whole cell pellet for ICP-MS analysis. All experiments were conducted in triplicate.

ICP-MS sample preparation. The whole cell pellets were dissolved in concentrated 72% v/v nitric acid (200 μL), and the samples were then transferred to Wheaton v-vials (Sigma-Aldrich) and incubated in an oven at 343 K overnight. The vials were then allowed to cool, and each cellular sample solution was transferred into a vial and diluted with Milli-Q water (3.8 mL), to give a final HNO_3 concentration of *ca*. 3.6% v/v.

Table S1. Photoproducts from reaction between conjugate **Pt-cP** and 5′-GMP (guanosine monophosphate) upon blue light irradiation (420 nm, 1 h) detected by LC-MS (positive-ion mode).

Peak	**Formula**	**Structure**	**Calculated (m/z)**	**Found (m/z)**
G1	$C_{10}H_{15}N_5O_8P$	([5'-GMP+H]$^+$)	364.07	364.09
G2	$C_{21}H_{25}N_7O_{10}PPt$	$\{Pt^{II}(py)_2(OC(O)H)(GMP)\}^+$	761.10	762.16
G3	$C_{20}H_{24}N_{10}O_8PPt$	$\{Pt^{II}(py)_2(N_3)(GMP)\}^+$	758.12	758.22
G4	$C_{13}H_{13}N_2O_6Pt$	$\{Pt^{IV}(py)_2(OC(O)H)_3\}^+$	488.04	488.23
G5	$C_{52}H_{69}N_{14}O_{19}S_2$		1257.43	1257.28

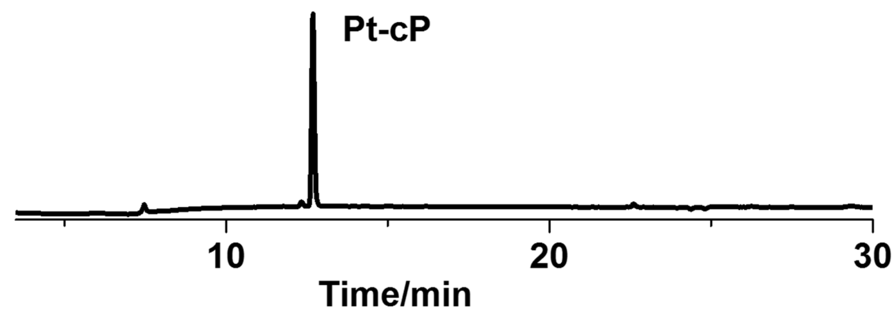

Figure S1. HPLC purity of conjugate **Pt-cP** detected at 254 nm using CH_3CN/H_2O as mobile phase.

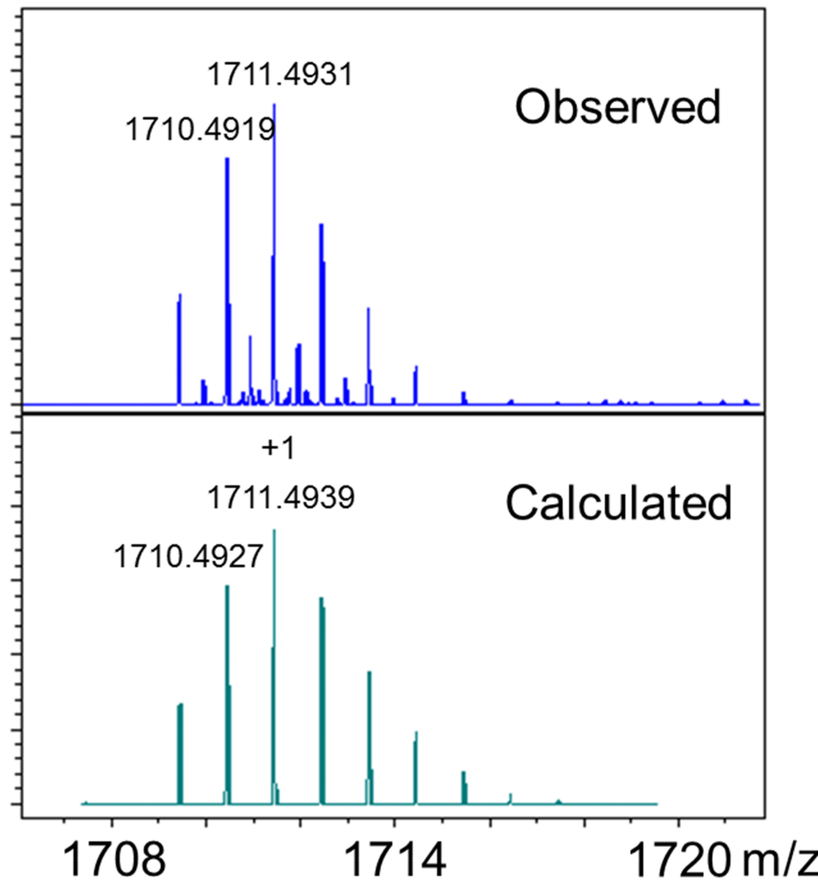

Figure S2. Observed and calculated HR-ESI mass spectra of the molecular ion ($[M + H]^+$) of **Pt-cP**.

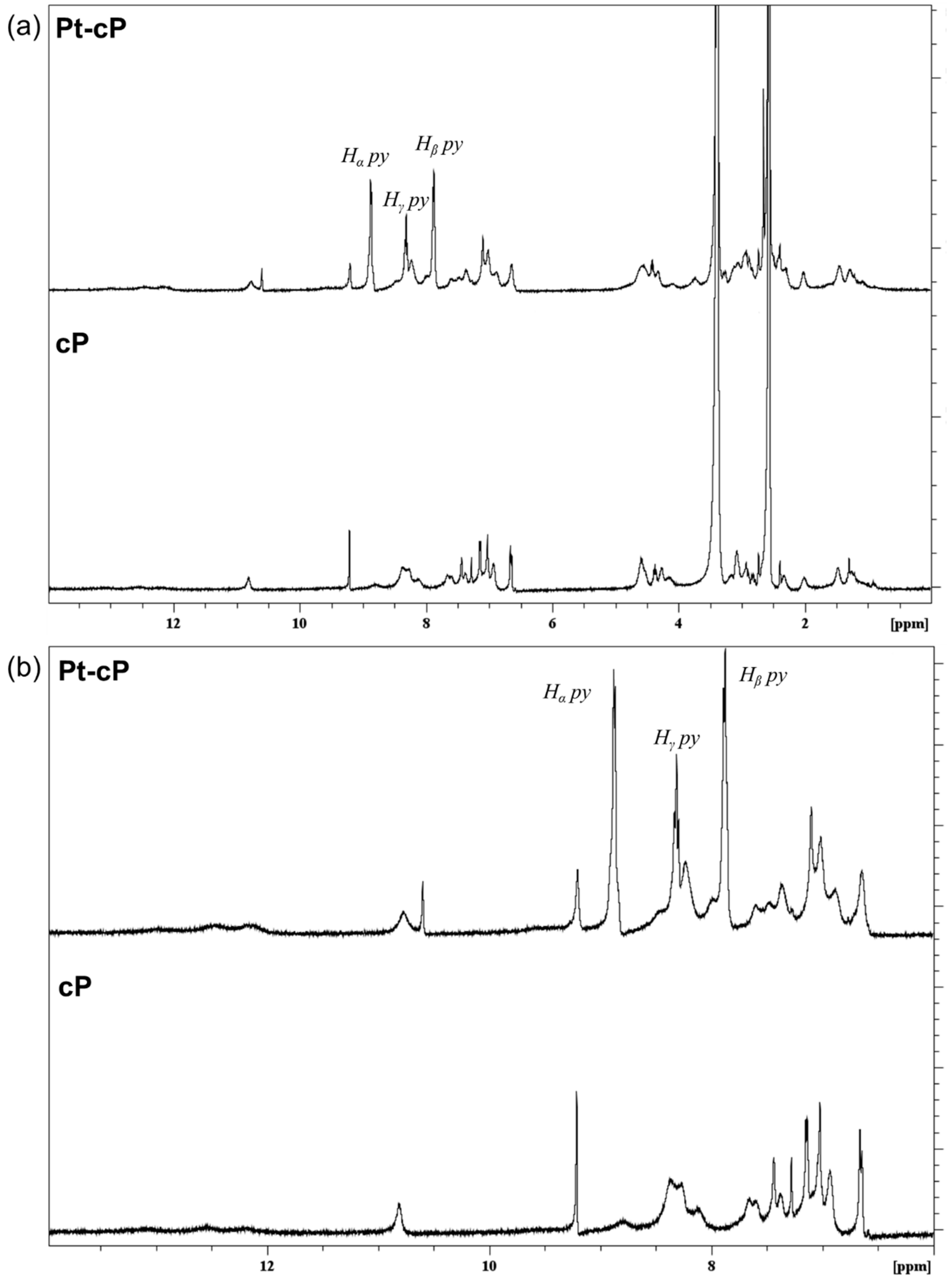

Figure S3. (a) Full ^{1}H NMR spectrum, and (b) low-field region of the spectrum of conjugate **Pt-cP** and cyclic peptide c(CRWYDENAC) (**cP**) in DMSO–d_6. No attempt was made to assign all of the peaks. The peaks at very low field are likely to arise from NH and OH protons.

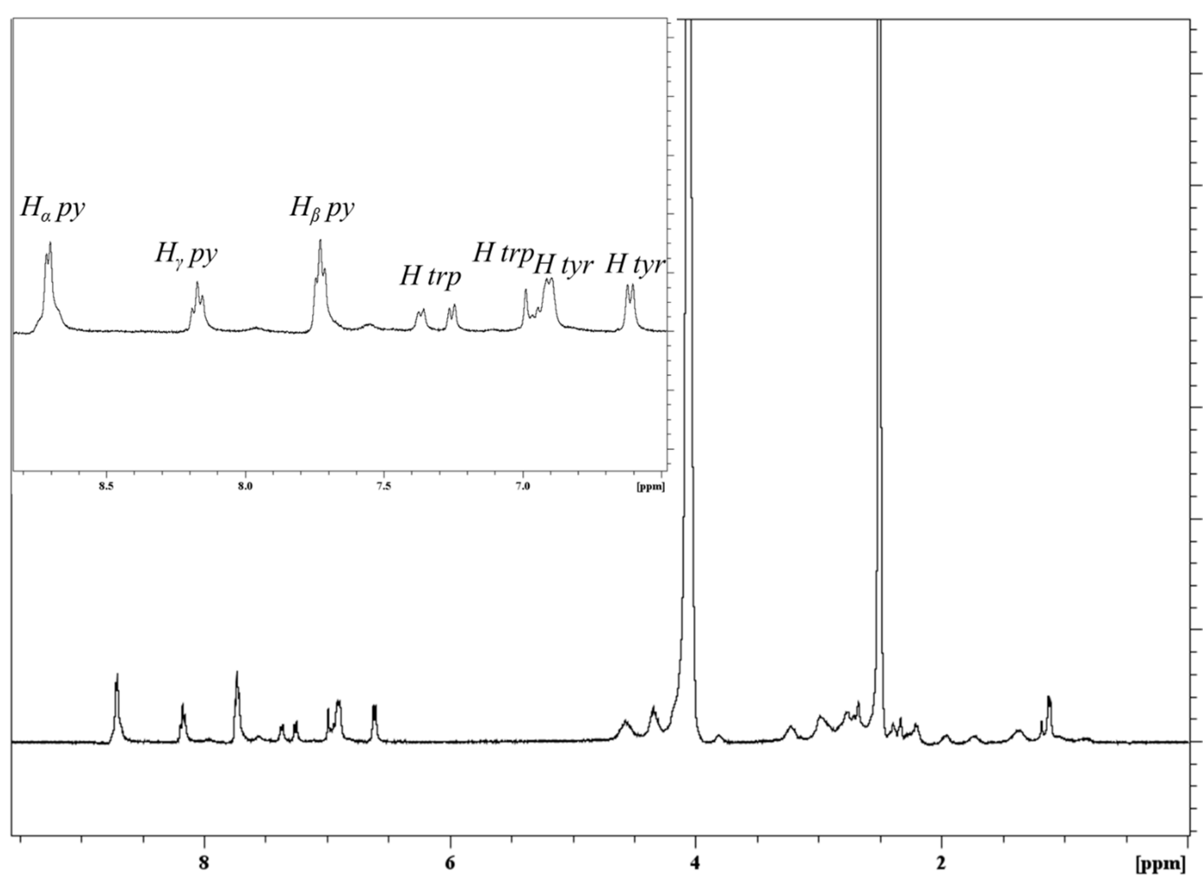

Figure S4. ^{1}H NMR spectrum of conjugate **Pt-cP** in 80% DMSO–d_6/20% D_2O.

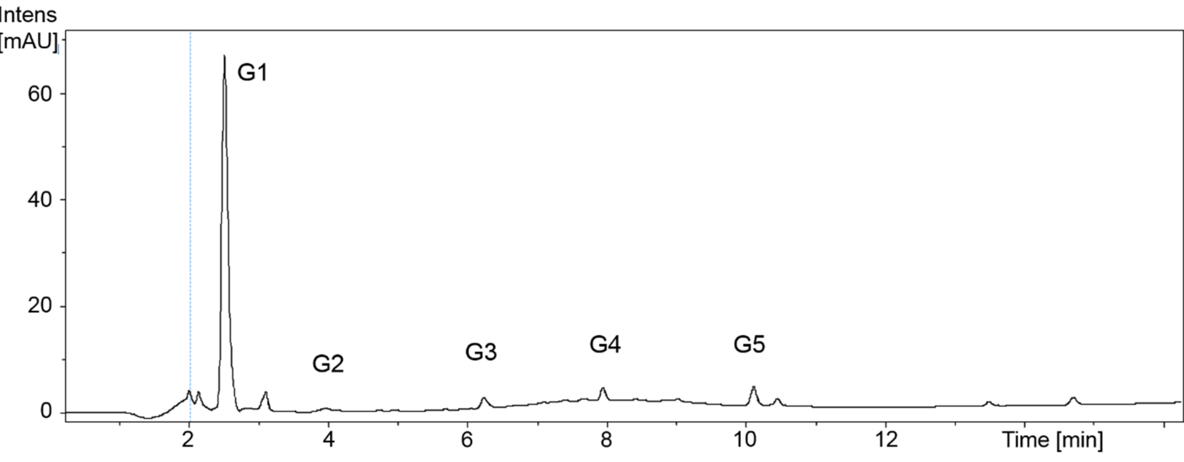

Figure S5. Photoproducts from reaction of **Pt-cP** with 2 mol equiv 5′-GMP upon blue light irradiation (420 nm, 1 h) monitored by HPLC (detecting wavelength 254 nm), **G1**–**G5** refer to the species listed in Table **S1**.

Reference

1. V. Vichai, K. Kirtikara, *Nat. Protoc.*, 2006, **1**, 1112–1116.