Electronic Supplementary Material (ESI) for Polymer Chemistry.

SUPPORTING INFORMATION

Dual-drug delivery based charge-conversional polymeric micelles for enhanced cellular uptake and combination therapy

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Methods and Characterization

Synthesis of 2-(4-imidazolyl) methyl methacrylate (IMMA)

A mixture of methacryloyl chloride (7.77 g, 74.32 mmol), 4-imidazolemethanol hydrochloride (5.0 g, 37.16 mmol) were added to a 10 mL of round-flask and heated to 75 °C. After 5 h of incubation, the mixture was diluted with methanol (5 mL), and precipitated into excess cold diethyl ether (100 mL), the white solid was separated by filtration and washed with cold acetone. The final product was dried under vacuum and IMMA was obtained as a brown powder (6.25 g, 83% yield). ^{1}H NMR (500 MHz, D_2O): δ 8.68 (s, 1H, =CH-N=), 7.53 (s, 1H, -N-CH=N), 6.07 (s, 1H, CH_2=C2), 5.67 (s, 1H, CH_2=C2), 5.24 (s, 2H, -O-CH_2-C-), 1.83 (s, 3H, -CH_3). ^{13}C NMR (126 MHz, D_2O) δ 168.77 (C=O), 135.25 (N-**C**2), 134.30 (=C-), 127.85 (N-C=N), 127.69 (CH_2=C), 118.92 (N-CH=C), 55.84 (CH_2-O), 17.28 (CH_3).

Synthesis of 2-aminoethyl methacrylate hydrochloride (AMA)

AMA monomer was prepared according to a previously reported protocol.[1] A mixture of methacryloyl chloride (10.78 g, 103.1 mmol), 2-aminoethanol hydrochloride (5.0 g, 51.55 mmol) and hydroquinone (52.1 mg, 0.52 mmol) were added to a 50 mL of three-necked round-flask. The esterification reaction was incubated in the melted at 80 °C for 2 h. Subsequently, the mixture was cooled to room temperature, diluted with tetrahydrofuran (THF, 10 mL), and precipitated into excess cold diethyl ether (100 mL), the white solid was separated by filtration and washed with cold diethyl ether. The final product was dried under reduced pressure to afford the white solid powder (8.42 g, 99% yield). ^{1}H NMR (500 MHz, D_2O): δ 6.16 (s, 1H, CH_2=C2), 5.73 (s, 1H, CH_2=C2), 4.45-4.32 (m, 2H, -O-CH_2-), 3.36-3.34 (m, 2H, -CH_2-N), 1.90 (s, 3H,-CH_3). ^{13}C NMR (126 MHz, D_2O) δ 169.12 (C=O), 135.33 (C=CH_2), 127.65 (CH_2=C), 61.30 (-O-CH_2-), 38.57 (-CH_2-N), 17.35 (CH_3).

Synthesis of small molecular prodrug (*cis*-Pt(IV)-COOH) based on *cis*-platinum (Pt(II))

Small molecular prodrug *cis*-Pt(IV)-COOH was synthesized according to the previously reported protocols.[2, 3] In brief, to a suspension of Pt(II) (0.5 g, 1.67 mmol) in DI water (12.5 mL), a 10-fold excess of H_2O_2 (30% w/v, 17.5 mL, 15.0 mmol) was added. The oxidizing reaction was carried out at 50 °C for 4 h with continuous stirring. After recrystallization *in situ*, oxoplatin (Pt(IV)) was collected by filtration, and washed with cold DI water, ethanol and ether. The yellow-green solid residues were dried under vacuum with an isolated yield of 56.4% (334 mg, 1.0 mmol). ^{1}H NMR (500 MHz, DMSO) δ 5.45 (s, 6H).

Subsequently, Pt(IV) (315 mg, 0.946 mmol) was suspended in N,N-dimethylformamide (DMF, 15 mL), to which succinic anhydride (SA, 104 mg, 1.04 mmol) was added in the dark. The mixture was stirred at room temperature for 24 h and then diluted with DI water. The solution was lyophilized and acetone was added to precipitate the pale yellow solid, and then washed with acetone, ethanol and ether to obtain the grey white powder with an isolated yield of 52.64% (216 mg, 0.498 mmol). ^{1}H NMR (500 MHz, DMSO) δ 11.82 (s, 1H), 7.31-4.89 (m, 6H), 2.37 (t, J = 7.0 Hz, 4H). ^{13}C NMR (126 MHz, DMSO) δ 180.09 (s), 31.19-30.65 (m), 30.41 (s).

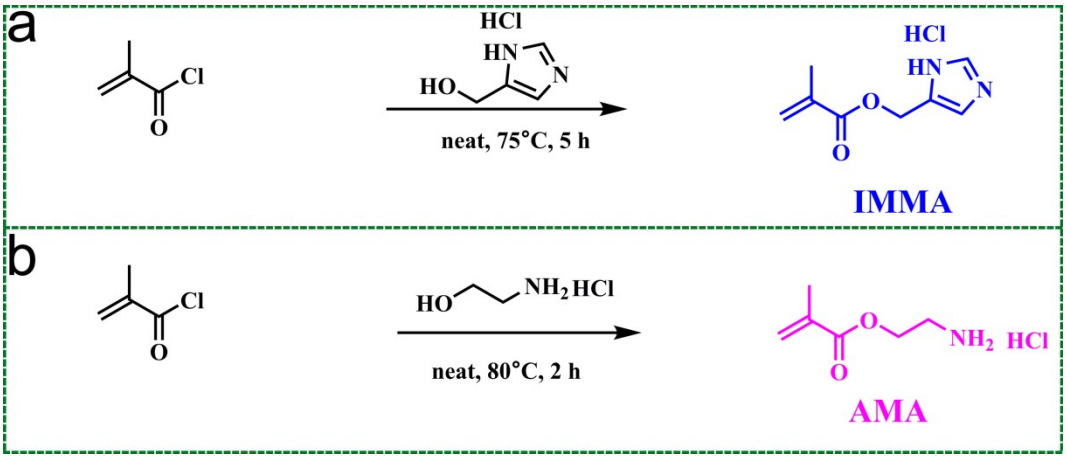
Synthesis of small molecular prodrug (PTX-SS-COOH) based on PTX

Another small molecular prodrug PTX-SS-COOH was synthesized through esterification using a previously reported protocol.[4, 5] 3,3'-Dithiodipropionic acid (DPA) was used as the donor to induce the disulfide to the PTX in preparing the PTX-SS-COOH. Briefly, equal amount (0.5 mmol) of DPA (105 mg), EDCI (96 mg) and DMAP (61 mg) were dissolved in CH_2Cl_2 (4 mL), and were added to a flask under nitrogen. The mixture was stirred for 0.5 h to activate the carboxyl of DPA at 0 °C. PTX (214 mg, 0.25 mmol) in CH_2Cl_2 (1 mL) was added drop-wise to the reaction, the mixture was stirred at room temperature for 24 h. Progress of the esterification was monitored by thin-layer chromatography (TLC), and then the crude product was washed with 0.01 M HCl twice and DI

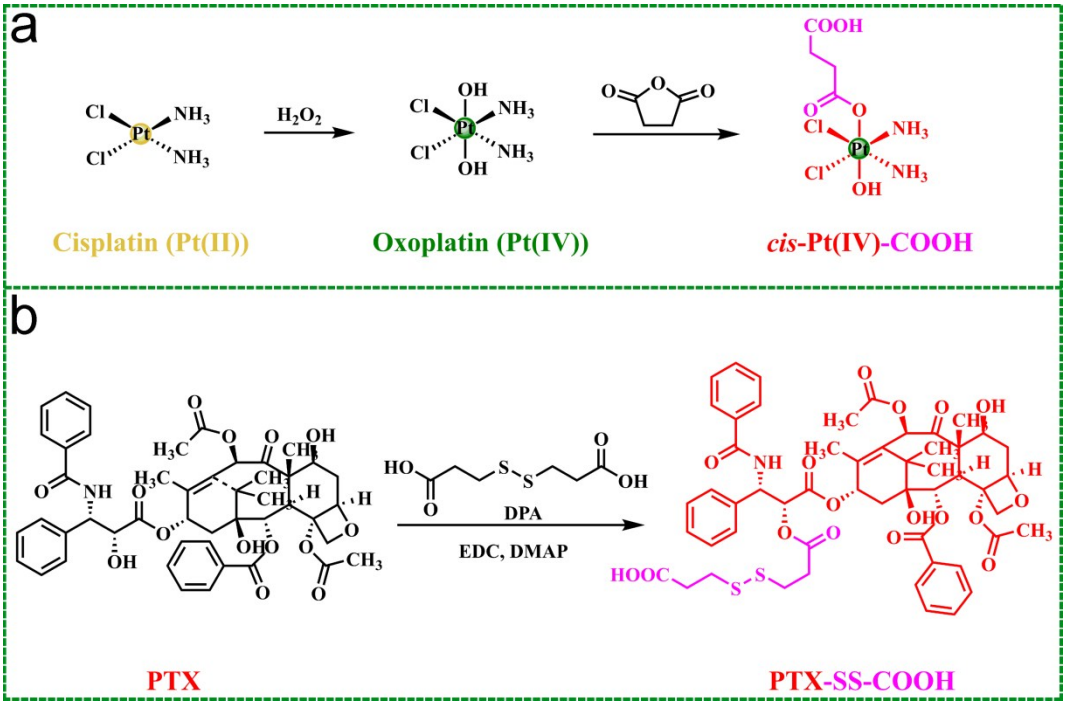
water for three times and dried under vacuum. The final product was purified by silica gel column chromatography (CH_2Cl_2 : CH_3OH = 40 : 1, R_f = 0.18) to obtain the pure PTX-SS-COOH. Yield: 88.7% (232 mg, 0.22 mmol).

^{1}H NMR characterization of *cis*-Pt(IV)-COOH and PTX-SS-COOH

As shown in Fig. S5 and S6, all the labelled proton assignments agree well with the Pt(IV) and *cis*-Pt(IV)-COOH molecule structures, respectively. Compared with spectrum of Pt(IV), there exist new characteristic peaks at δ 2.36 - 2.39 ppm in *cis*-Pt(IV)-COOH, which suggested the successful acylation reaction between the hydroxy of Pt(IV) and SA. In addition, Fig. S8 collects the ^{1}H NMR spectra of PTX and PTX-SS-COOH, by comparison with the spectrum of PTX, resonances at δ 6.17 ppm (assigned to 2'-OH) in PTX-SS-COOH disappeared, while there exist two new peaks at δ 2.74 and 2.89 ppm (assigned to DPA), respectively, which indicated the successful esterification reaction between PTX and DPA.



Scheme S1 The synthetic procedure of IMMA (a) and AMA (b) monomers.



Scheme S2 The synthetic procedure of small molecular prodrugs: *cis*-Pt(IV)-COOH (a) and PTX-SS-COOH (b).

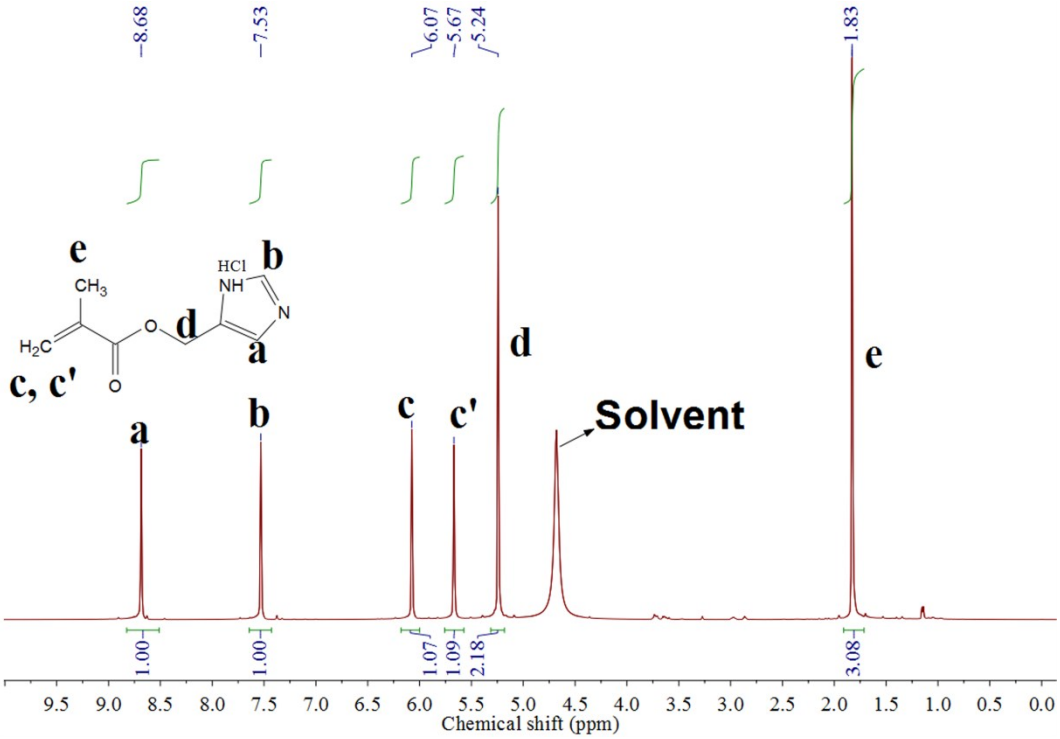


Fig. S1 ^{1}H NMR spectrum of IMMA in D_2O.

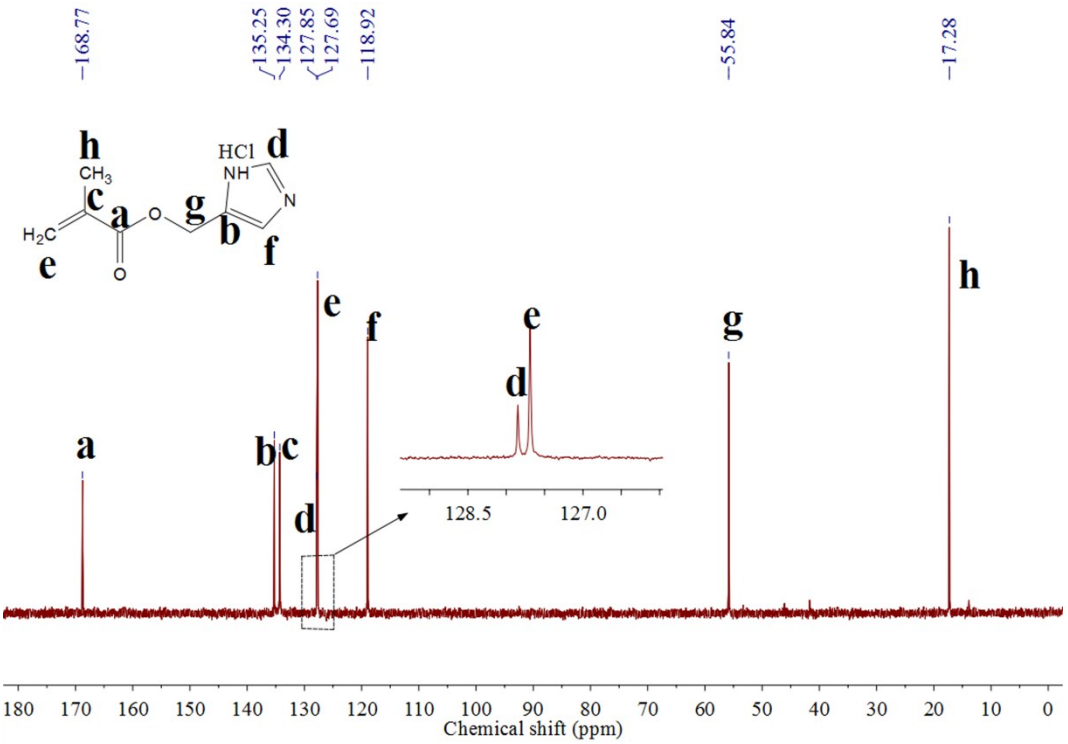


Fig. S2 ^{13}C NMR spectrum of IMMA in D_2O.

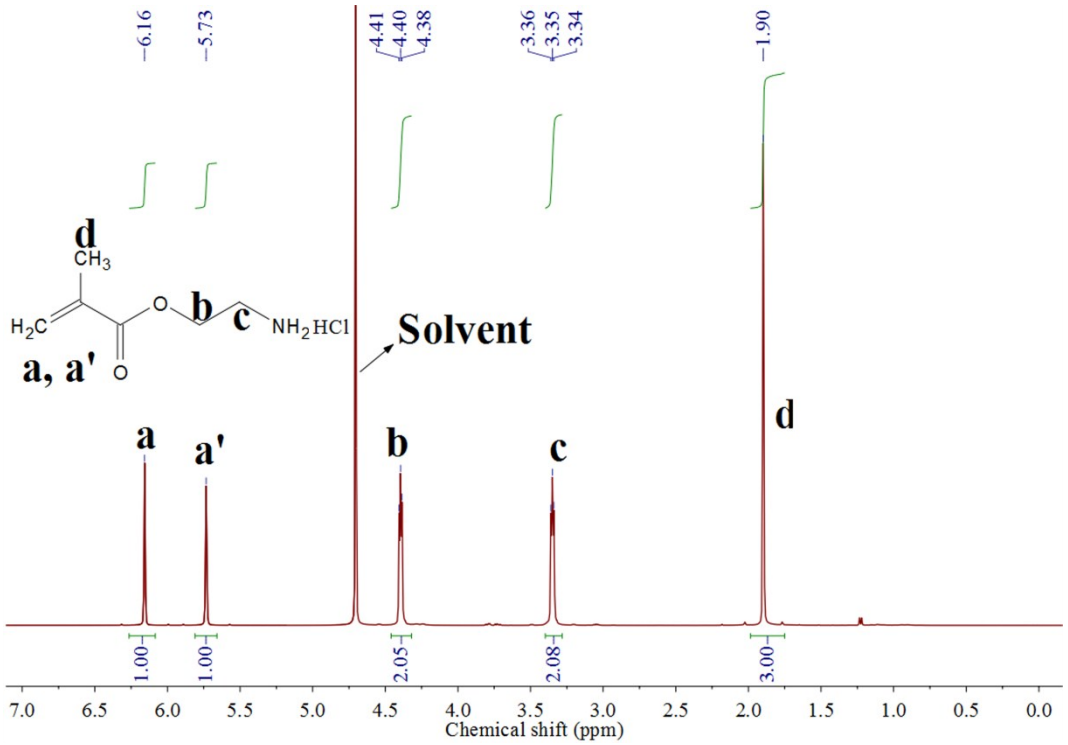


Fig. S3 ^{1}H NMR spectrum of AMA in D_2O.

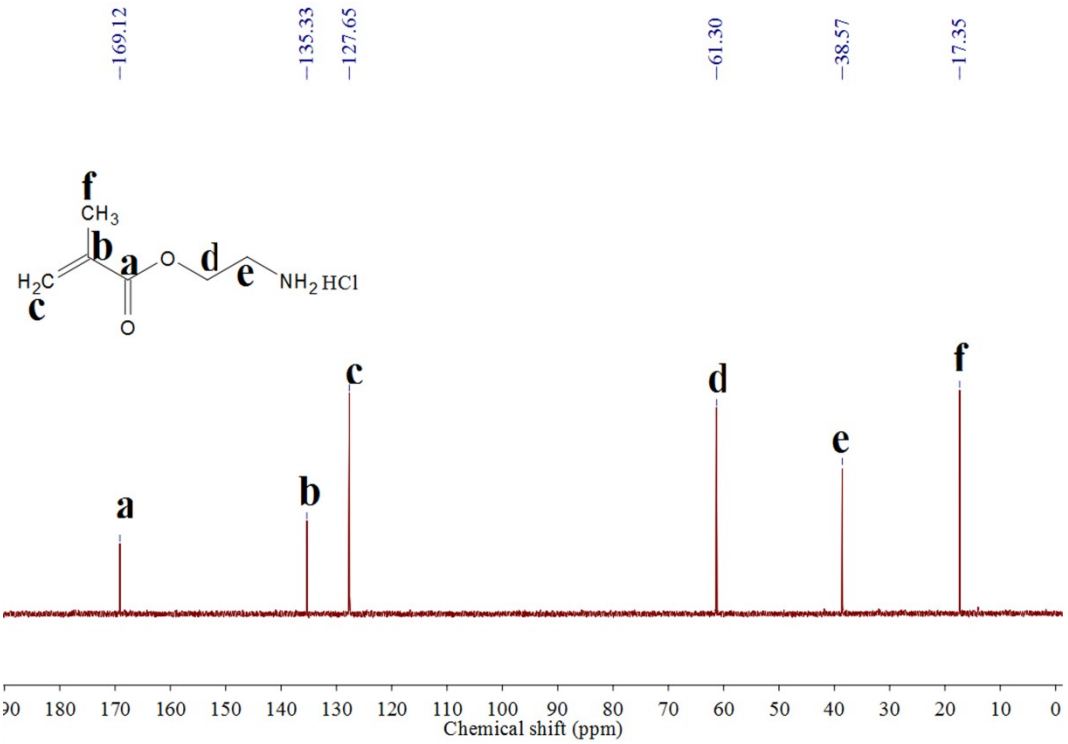


Fig. S4 ^{13}C NMR spectrum of AMA in D_2O.

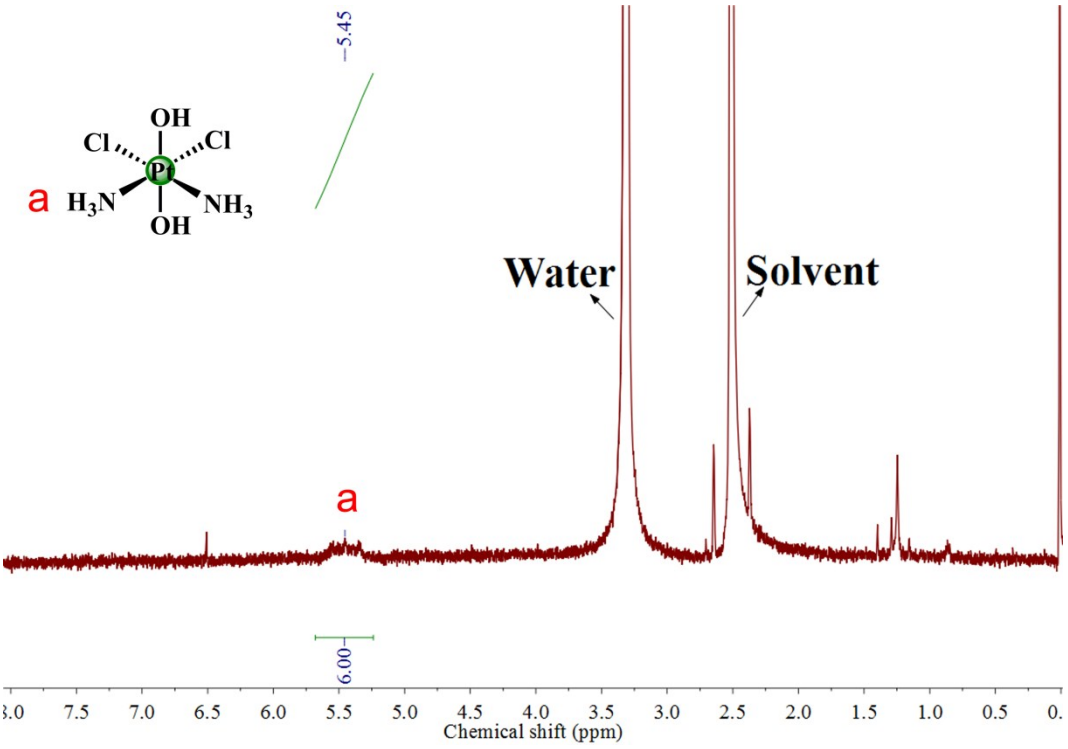


Fig. S5 ^{1}H NMR spectrum of Pt(IV) in DMSO-d_6.

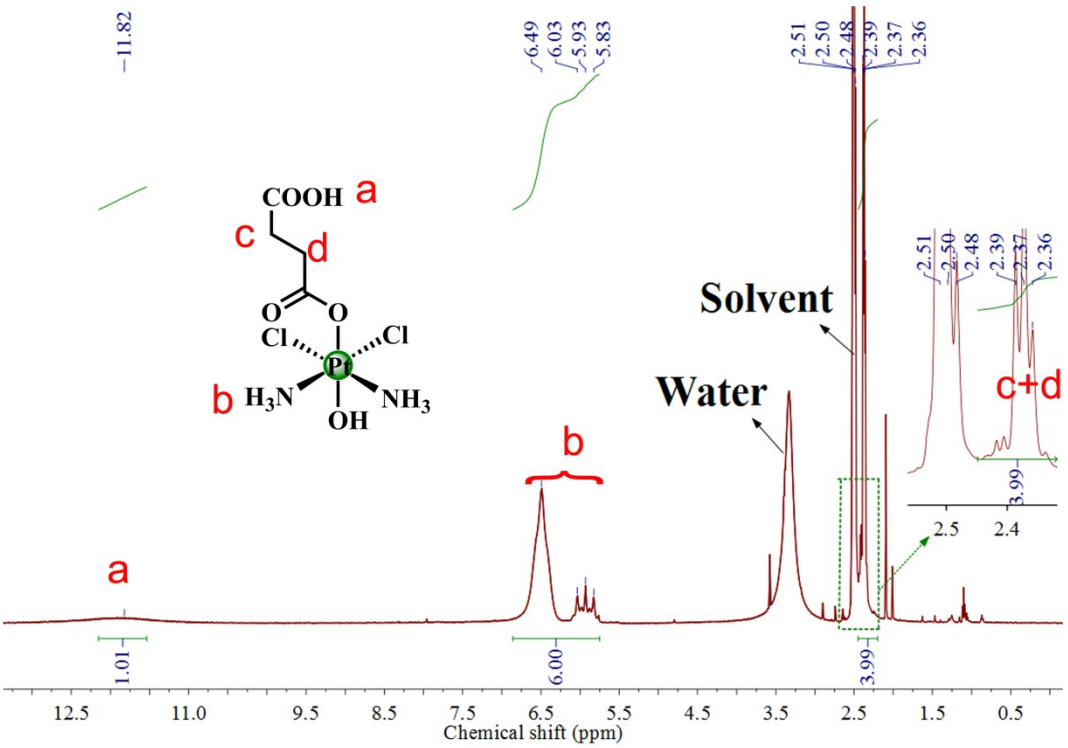


Fig. S6 ^{1}H NMR spectrum of *cis*-Pt(IV)-COOH in DMSO-d_6.

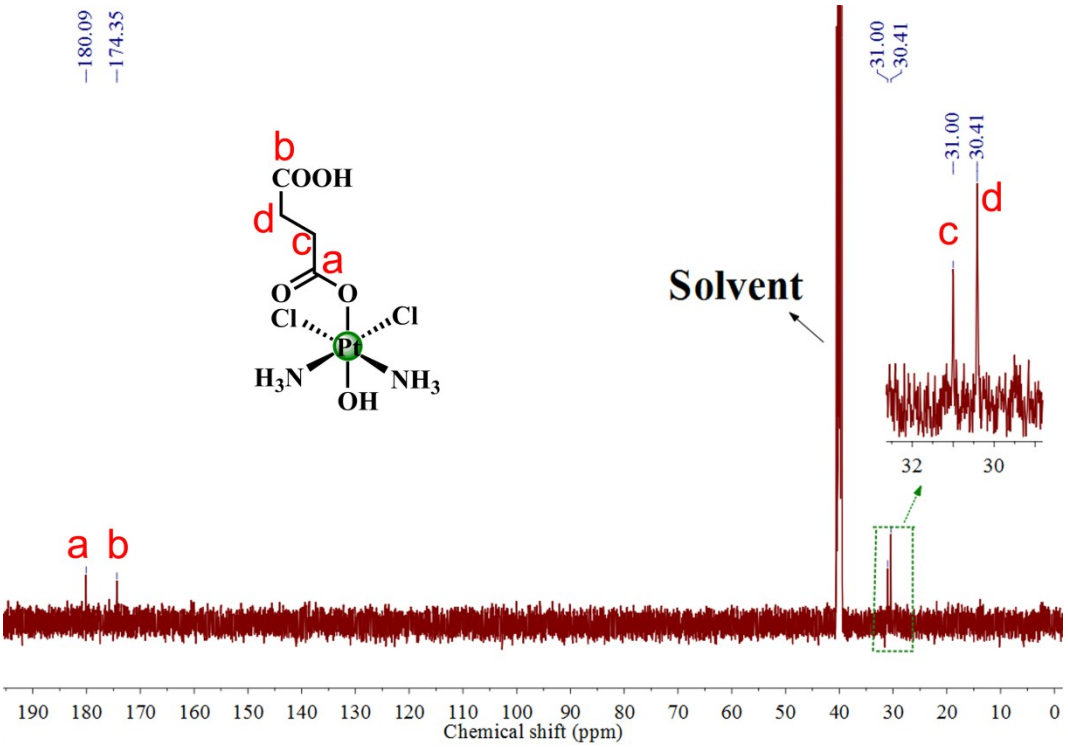


Fig. S7 ^{13}C NMR spectrum of *cis*-Pt(IV)-COOH in DMSO-d_6.

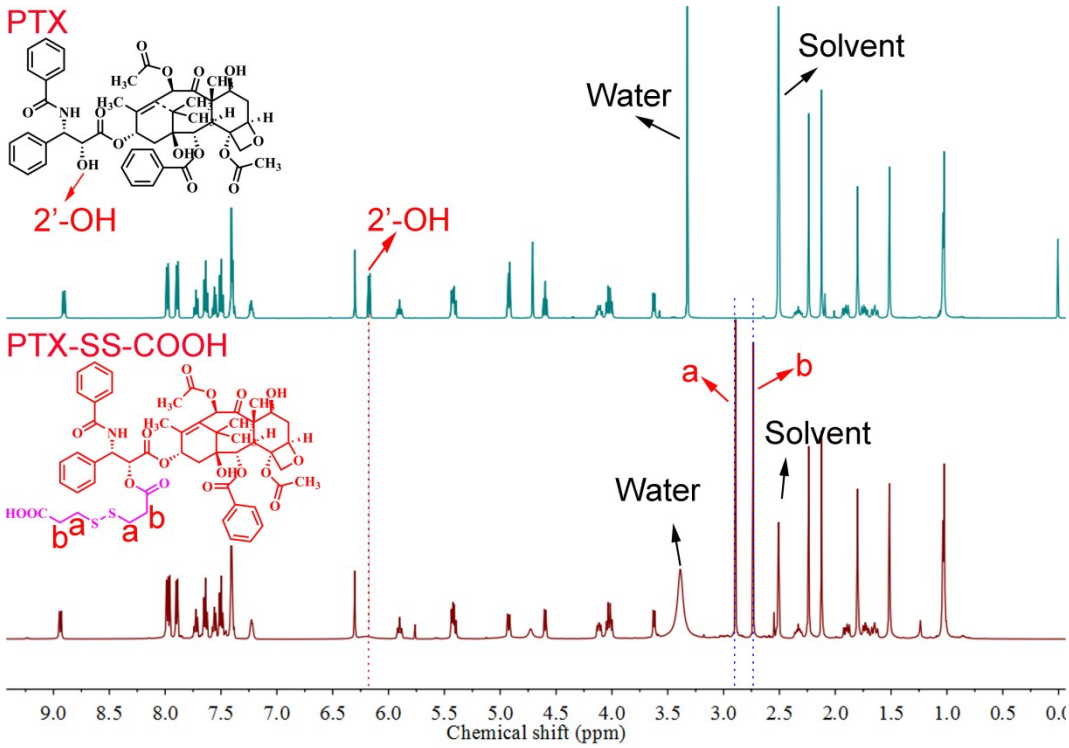


Fig. S8 ^{1}H NMR spectra of PTX and PTX-SS-COOH in DMSO-d_6.

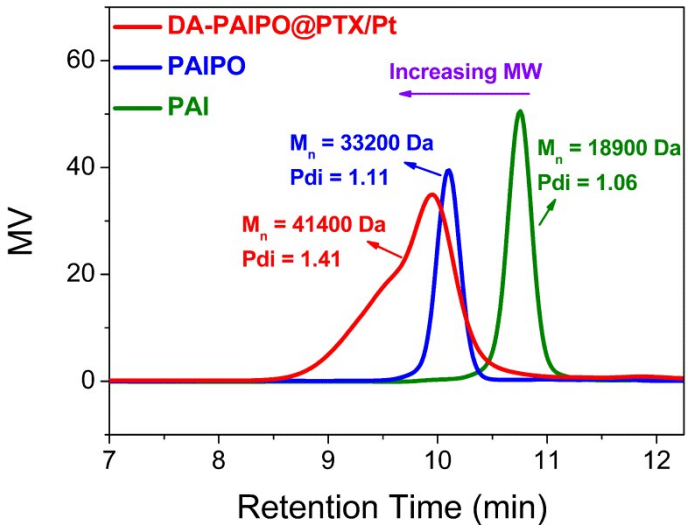


Fig. S9 GPC cures of PAI, PAIPO and DA-PAIPO@PTX/Pt conjugates. (GPC analysis was performed on a Waters 1525 using a Breeze 2 HPLC system, and equipped with a Styrage® HR 4 DMF 7.8 × 300 mm column.) Polystyrene standards were used for calibration, and the column was eluted with DMF (HPLC) that contained 10 mM LiBr and at 35 °C at a flow rate of 1.0 mL/min.

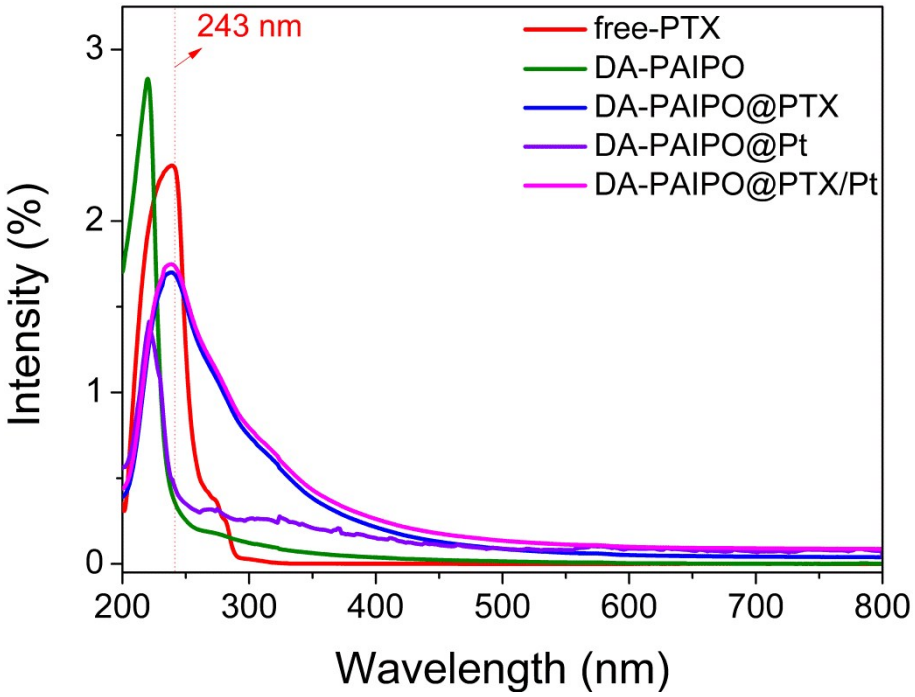


Fig. S10 UV-visible scanning spectrophotometry of free-PTX and PTX-conjugated prodrugs.

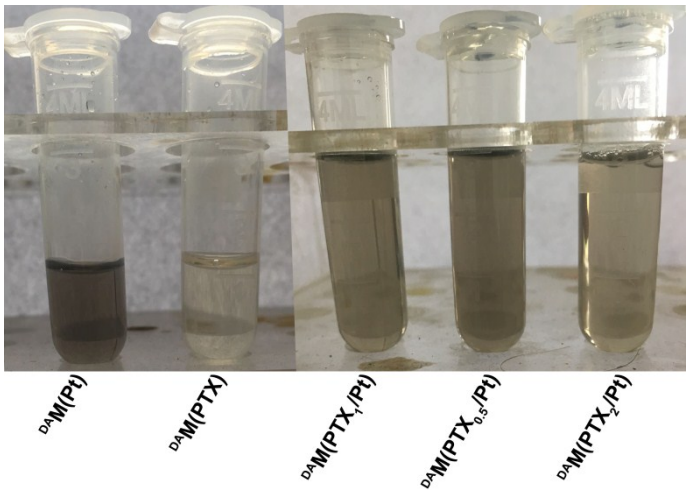


Fig. S11 Representative digital photograph of micellar solution at an equal prodrug concentration (1 mg/mL).

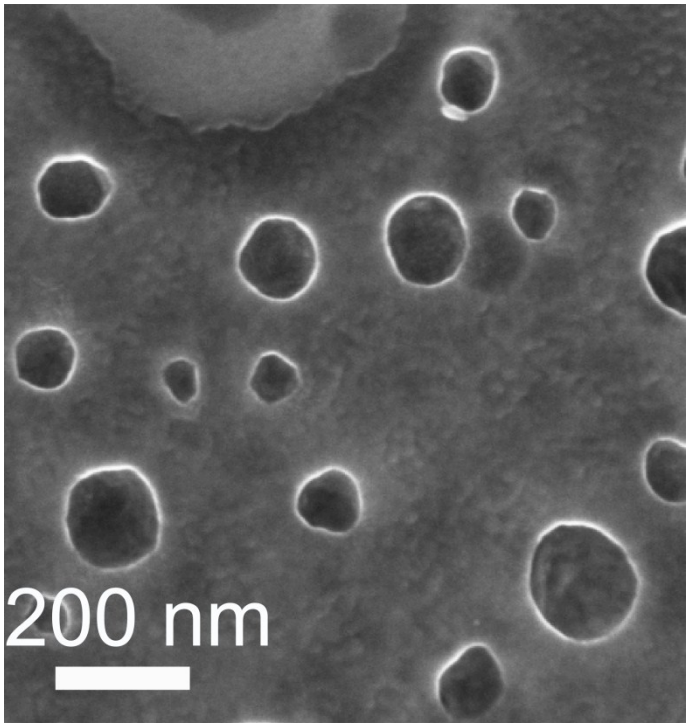


Fig. S12 Representative SEM images of ^{DA}M(PTX$_1$/Pt).

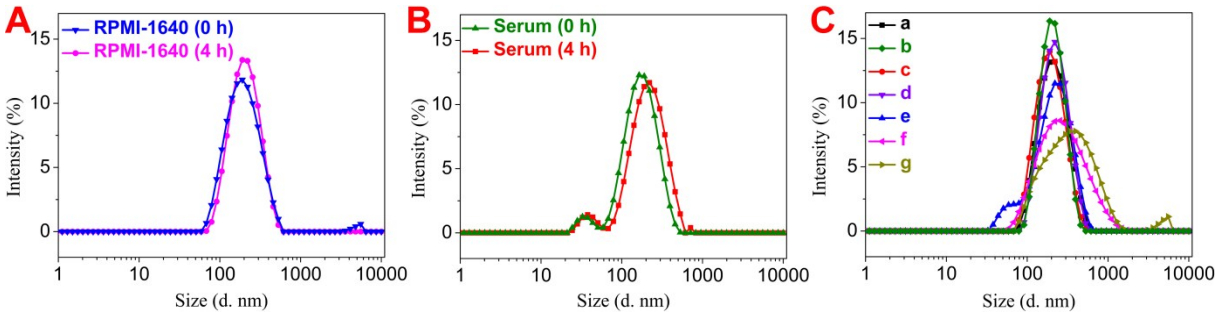


Fig. S13 Size change of ^{DA}M(PTX$_1$/Pt) determined by DLS after incubating in RPMI-1640 (A), serum (B), and in PBS (C) at pH 7.4 (a), NaASc 50 μM + GSH 20 μM at pH 7.4 (mimicking physiological microenvironment) for 4 h (b) or in NaASc 5 mM + GSH 10 mM at pH 5.0 (mimicking intracellular microenvironment) for 0 h (c), 0.5 h (d), 1 h (e), 2 h (f), 4 h (g), respectively.

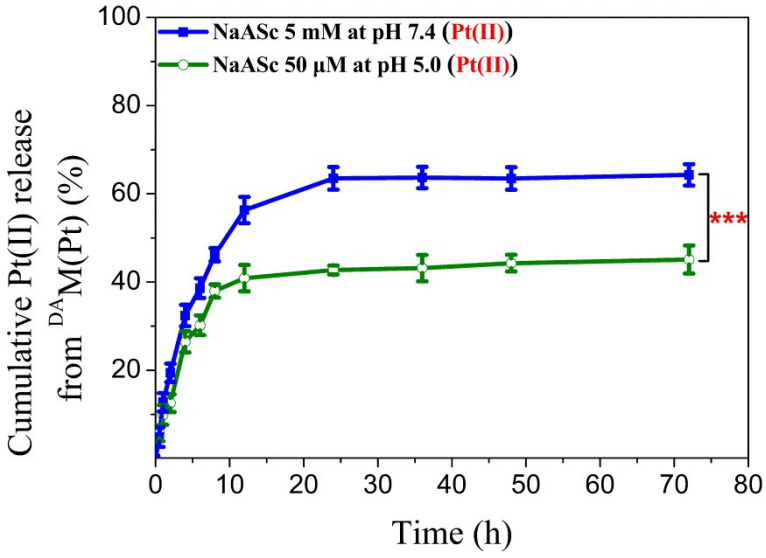


Fig. S14 Pt(II) release profiles from ^{DA}M(Pt) in PBS (NaASc 50 μM at pH 5.0) or PBS (NaASc 5 mM at pH 7.4) (***P < 0.001).

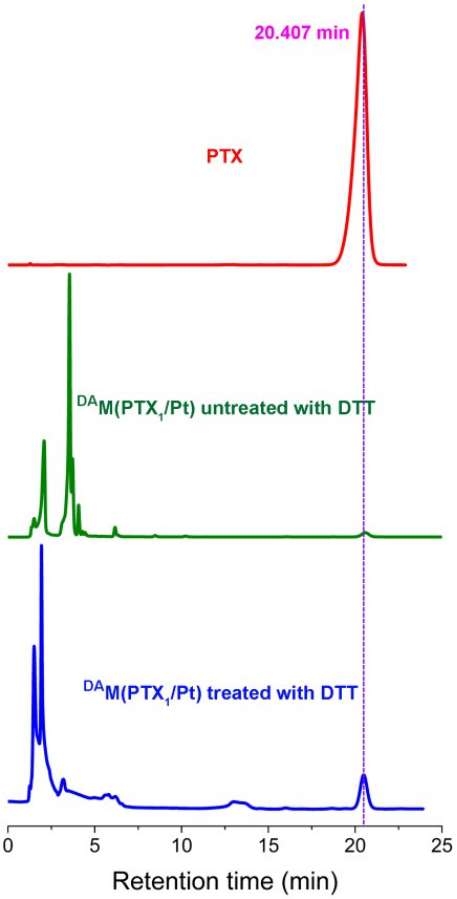


Fig. S15 HPLC analysis for the PTX release profiles from ^{DA}M(PTX$_1$/Pt).

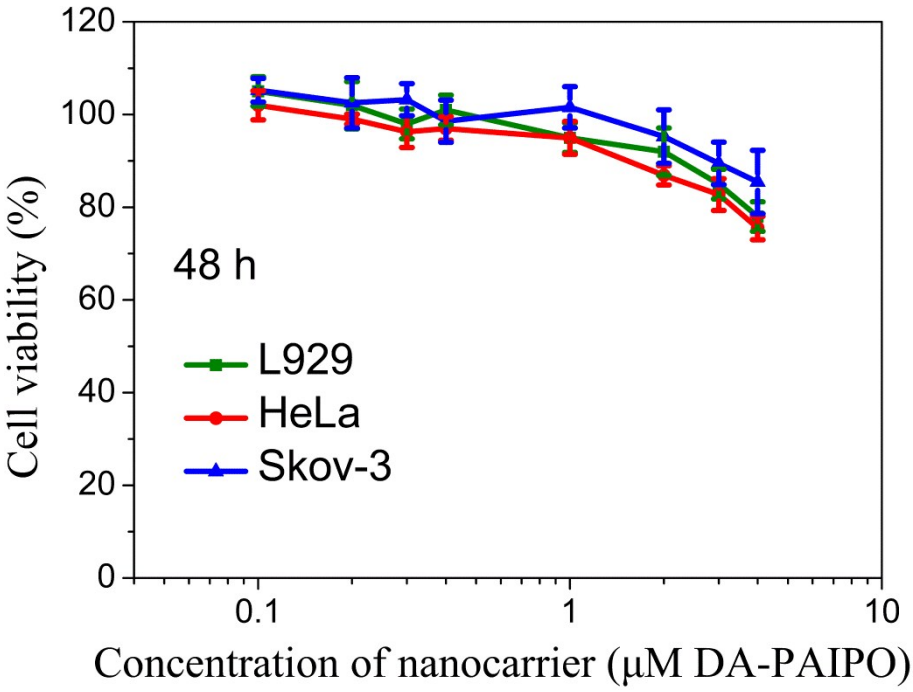


Fig. S16 Cytotoxicity of DA-PAIPO conjugate against L929, HeLa and Skov-3 cells after 48 h of incubation.

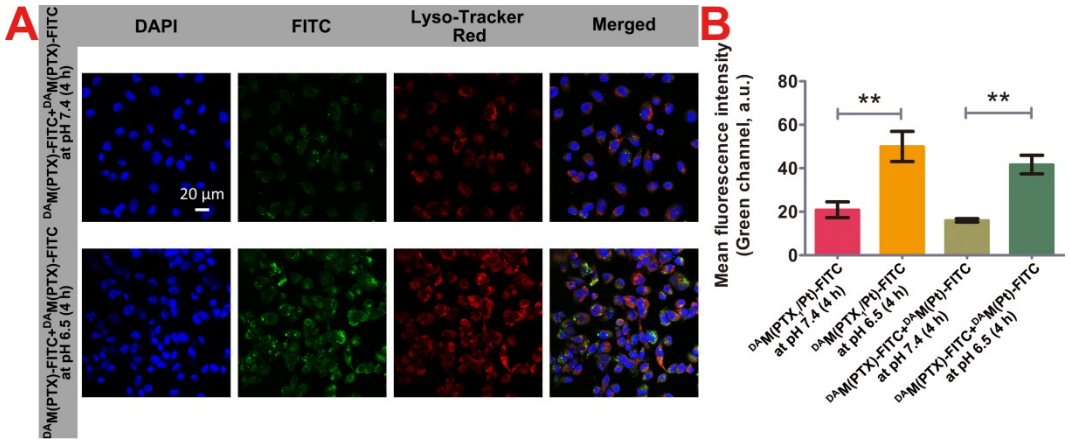


Fig. S17 CLSM images (A) of Skov-3 cells after incubation with physical mixture of ^{DA}M(PTX)-FITC and ^{DA}M(Pt)-FITC at an equal dosage of FITC (20 μg/mL), and at pH 7.4 or 6.5 for 4 h. Scale bars = 20 μm. The mean fluorescence intensity (Green channel) were shown on the right (B) (0.001 < **P < 0.01).

Reference

1. R. Seto, K. Matsumoto and T. Endo, *Journal of Polymer Science Part A Polymer Chemistry*, 2013, **51**, 4522-4529.
2. Y. Shi, S. A. Liu, D. J. Kerwood, J. Goodisman and J. C. Dabrowiak, *Journal of Inorganic Biochemistry*, 2012, **107**, 6-14.
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