

## Electronic Supplementary Information

# A pH responsive complexation-based drug delivery system for oxaliplatin

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### General experimental details.

All reagents were purchased commercially and used without further purification unless otherwise noted. **CP5A** and **CP6A** were synthesized and purified according to our previously reported procedure.<sup>S1</sup> Oxaliplatin (98%) was purchased from TCI (Shanghai) Development Co., Ltd. Roswell Park Memorial Institute (RPMI) 1640 medium and Dulbecco's Modified Eagle Medium (DMEM) were purchased from Hyclone (Beijing). Fetal bovine serum (FBS), penicillin-streptomycin and phosphate buffered saline (PBS, for cell incubation) were purchased from Invitrogen (CA, USA). <sup>1</sup>H NMR (400 MHz) and <sup>13</sup>C NMR (100 MHz) spectra were recorded using a JNM-ECA-400 spectrometer. Variable-temperature <sup>1</sup>H NMR spectra were recorded with a Bruker Avance 500 MHz spectrometer. Plasma stability was determined by analytical reversed-phase high-performance liquid chromatography (RP-HPLC) using an Shimadzu LC-10AT system and a RP-C8 column (Waters XBridge<sup>TM</sup>, 5  $\mu$ m, 4.6  $\times$  250 mm). For the *in vivo* stability studies, the platinum content in plasma and red cell blood cells was measured by inductively coupled plasma mass spectrometry (ICP-MS) using an Agilent 7500ce instrument. The ITC experiments were performed on a MicroCal Auto-iTC200 isothermal titration micro-calorimeter. Phosphate buffer solutions (20 mM) with different pH values for the ITC experiments were prepared by mixing disodium hydrogen phosphate water solution (20 mM) and sodium dihydrogen phosphate water solution (20 mM) according to the calculated volume ratios. Water was obtained from a Millipore water purification system and had a minimum resistivity of 18.2 M $\Omega$ ·cm. The pH values of the buffer solutions were verified on a pH-meter calibrated with two standard buffer solutions.

### ITC experiments.

A thermostatted ITC was used for all microcalorimetric experiments. All titration experiments involving studies of **CP6A** and **OX** were performed in aqueous phosphate buffer solutions at 298.15 K and at atmospheric pressure. Before the titration experiment, each sample was degassed and thermostatted using the manufacturer's supplied ThermoVac accessory. Twenty-nine sequential injections were made for each ITC experiment. The first injection was 2  $\mu$ L; then, a constant volume (10  $\mu$ L/injection) of guest solution (7.50–10.1 mM) was injected into the cell filled with **CP6A** solution (0.10 mM). Figures S4–S7 show

typical titration curves. Each titration of **OX** produced a reaction heat profile, as would be expected were a **CP6A – OX** complex being formed. Control experiments were carried out to measure the dilution heat by injecting guest solution into pure buffer solution. The dilution heat was subtracted from the heat determined in the titration experiments to get the net reaction heat. The results were analyzed using the “one set of binding sites” model; this provided the association constant ( $K_a$ ), standard molar reaction enthalpy ( $\Delta H^\circ$ ), and entropy changes ( $\Delta S^\circ$ ).

#### **Plasma stability as studied by HPLC.**

In the plasma stability experiments, HPLC analyses were carried out to evaluate the stability of **OX** in the absence and presence of **CP6A**. The plasma samples were obtained by cryogenic speed centrifuge from rat blood. **OX** and the putative **OX-CP6A** complex with concentrations of 940  $\mu\text{M}$  were dissolved in diluted plasma (rat plasma diluted with water (1:3, v/v)) and incubated at 37 °C. At different time intervals, samples (50  $\mu\text{L}$ ) were withdrawn and treated with  $\text{CH}_3\text{OH}$  (50  $\mu\text{L}$ ) as a precipitant to remove plasma protein. Then, 20  $\mu\text{L}$  of each sample was analyzed by RP-HPLC with an RP-C8 column (Waters XBridge<sup>TM</sup>, 5  $\mu\text{m}$ , 4.6  $\times$  250 mm) and a gradient of acetonitrile (70%  $\text{CH}_3\text{CN}/\text{H}_2\text{O}$ ) and deionized water with a flow rate of 1 mL/min at a wavelength of 205 nm. Every sample was tested three times and average value of the **OX**'s peak area was calculated. The degradation process was monitored for 36 h.

#### ***In vivo* stability as studied by ICP-MS.**

As part of the *in vivo* stability analysis, ICP-MS studies were carried out to evaluate the stability of **OX** in the absence and presence of **CP6A**. Rats were obtained from Beijing Vital River Laboratory Animal Technology Co., Ltd. All experimental procedures were approved by the Animal Care and Use Committee of National Beijing Center for Drug Safety Evaluation and Research. Ten rats were randomly divided into two groups with 5 rats in each group. Depending on the group, the rats were injected with **OX** alone or a 1:1 mixture of **OX** and **CP6A** through the tail vein at an **OX** equivalent dose of 15 mg/kg. Blood samples were

collected into heparin tubes from the orbit at time intervals of 15 min and 1 h after injection. The samples were centrifuged to separate plasma and red blood cells. The resulting plasma and red blood cell samples were dissolved in 65% (v:v) nitric acid in polytetrafluoroethylene digestion tanks, respectively, and heated at 140 °C. The platinum content in the samples was then measured by ICP-MS.

### ***In vitro* cell assay.**

The relative cytotoxicity *in vitro* of **CP6A** against 293T (normal cell), HepG-2, MCF-7, and A549 cell lines, as well as **OX** and the 1:1 mixture of **OX** and **CP6A** against the MCF-7, A549, and HepG-2 cell were assessed using the Cell Counting Kit-8 (CCK-8, Dojindo Laboratories) according to the manufacturer's instructions. To test **CP6A**, 293T cells were seeded in 96-well plates at a density of 5000 cells/well in 100  $\mu$ L of complete DMEM containing 10% fetal bovine serum, supplemented with 50 U mL<sup>-1</sup> penicillin and 50 U mL<sup>-1</sup> streptomycin. Likewise, MCF-7, A549, and HepG-2 cells were seeded 96-well plates at a density of 5000 cells/well in 100  $\mu$ L of RPMI 1640 medium supplemented with 10% fetal bovine serum (FBS), 100 mg/mL of streptomycin, and 100 mg/mL of penicillin, and cultured for 24 h in 5% CO<sub>2</sub> at 37 °C. **CP6A** was dissolved in the culture medium and then diluted to the required concentration. It was then added to the cell-containing wells, and further incubated at 37 °C under 5% CO<sub>2</sub> for 48 h. Subsequently, 10  $\mu$ L of CCK-8 was added into each cell and incubated for another 1 h. The plates were then measured at 450 nm using a SpectraMax<sup>®</sup> M5 (Molecular Devices, WI, USA) plate reader. Cells without **CP6A** were used as a negative control. All experiments were carried out three times. For **OX** and the 1:1 mixture of **OX** and **CP6A**, the same procedures were performed with cytotoxicity determined by varying the concentration of the species in question.

### ***In vivo* antitumor efficacy.**

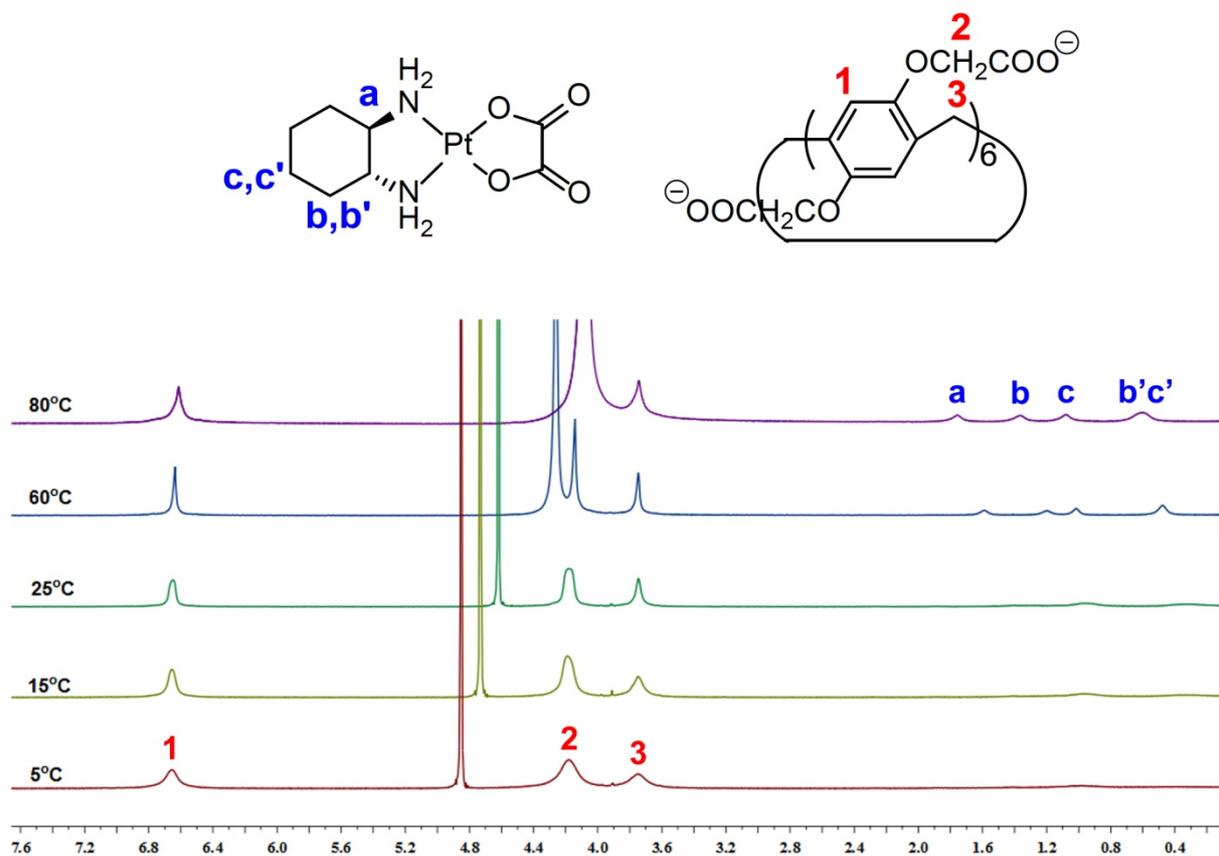
Male Kunming mice, initially weighing 20-22 g, were obtained from Beijing Vital River Laboratory Animal Technology Co., Ltd. All experimental procedures were conducted in accordance with the Guide for the Care and Use of Laboratory Animals of the AAALAC, and

were approved by the Animal Care and Use Committee of the National Beijing Center for Drug Safety Evaluation and Research. S180 cells with a density of  $1 \times 10^7$  cells/ mice (0.2 mL) were implanted into mice by subcutaneous injection. After 24 h, the mice were randomly divided into four groups with 12 mice in each group. The mice were then injected through the tail vein at different time points (on days 1, 3, 5, and 7) with saline being used as the negative control and **OX** as the positive control with a total dose of 35 mg/kg. The **OX + CP6A** mixture was administered at a total dosage of 15 mg/kg and 35 mg/kg (in **OX**). The weights of the animals were recorded after administration at different time point. The mice were sacrificed on day 8 and the tumors were separated from the animals and weighed.

#### **Acute toxicity assay.**

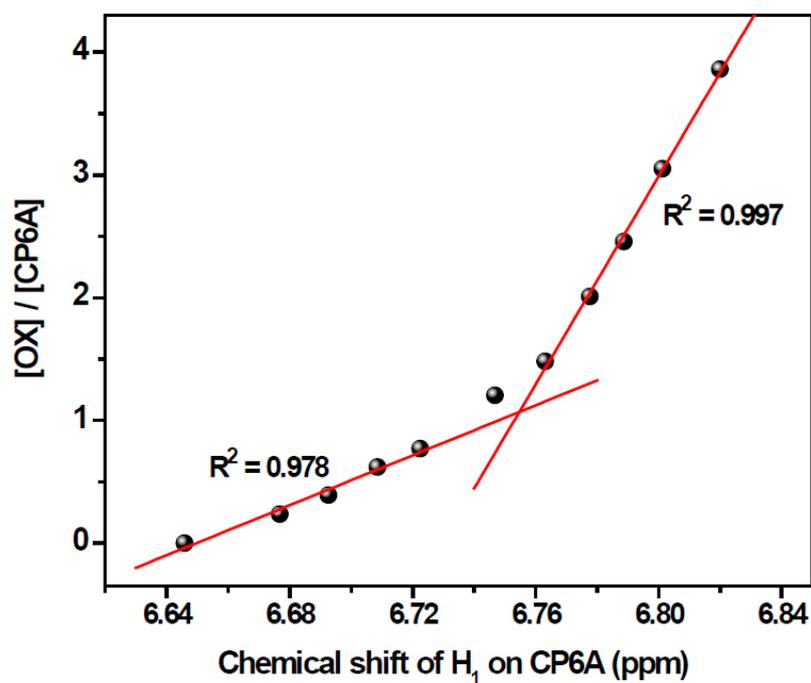
Kunming (KM) mice were obtained from Beijing Vital River Laboratory Animal Technology Co., Ltd. They were provided with free water and food at room temperature on a standard 12 h light/dark cycle. All experimental procedures were conducted in accordance with the Guide for the Care and Use of Laboratory Animals of the AAALAC, and were approved by the Animal Care and Use Committee of National Beijing Center for Drug Safety Evaluation and Research. The mice were randomly divided into seven groups to allow for testing of every agent and mixture considered in this study with 10 mice (5 male and 5 female) in each group. All experiments were performed during daylight hours. **CP6A** in saline at doses of 80, 160, 320, 360, 420, 640, 1280 mg/kg, as well as **OX** and the 1:1 **OX + CP6A** complex in saline at doses of 1.75, 5.50, 17.5, 30.9, 41.3, 55.0, 158 mg/kg (in **OX**), were injected into the mice via the tail vein. The mice were observed and deaths recorded over the ensuing 14 days. The LD<sub>50</sub> values were calculated using a program obtained from SPSS (Statistical Product and Service Solutions).

Variable-temperature  $^1\text{H}$  NMR spectra of OX + CP6A (1:1 mixture).



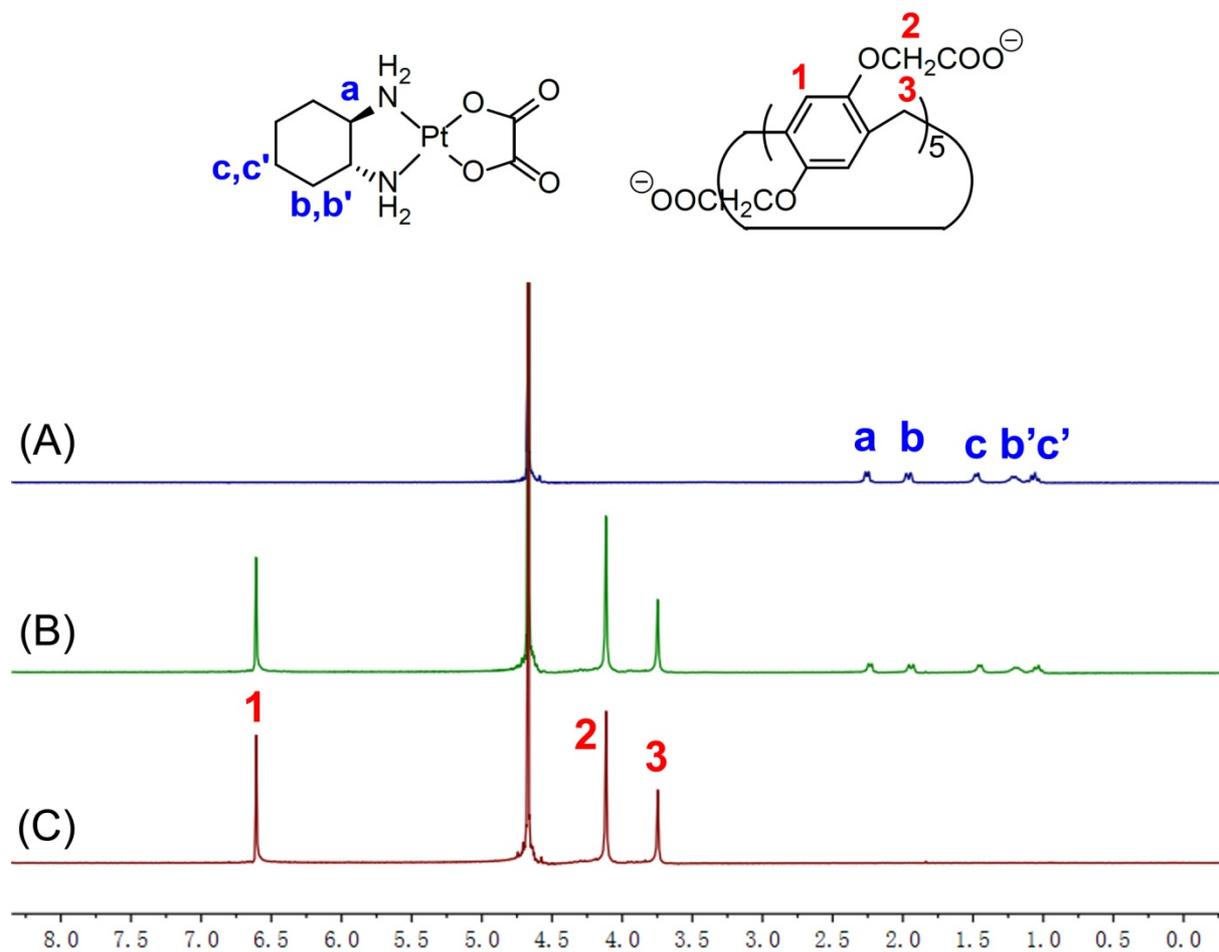
**Figure S1.** Variable-temperature  $^1\text{H}$  NMR spectra (500 MHz) of a 1:1 mixture of **OX** and **CP6A** (5.0 mM) in  $\text{D}_2\text{O}$ . The temperatures were 5, 15, 25, 60 and 80 °C.

**Mole ratio plot for CP6A and OX.**



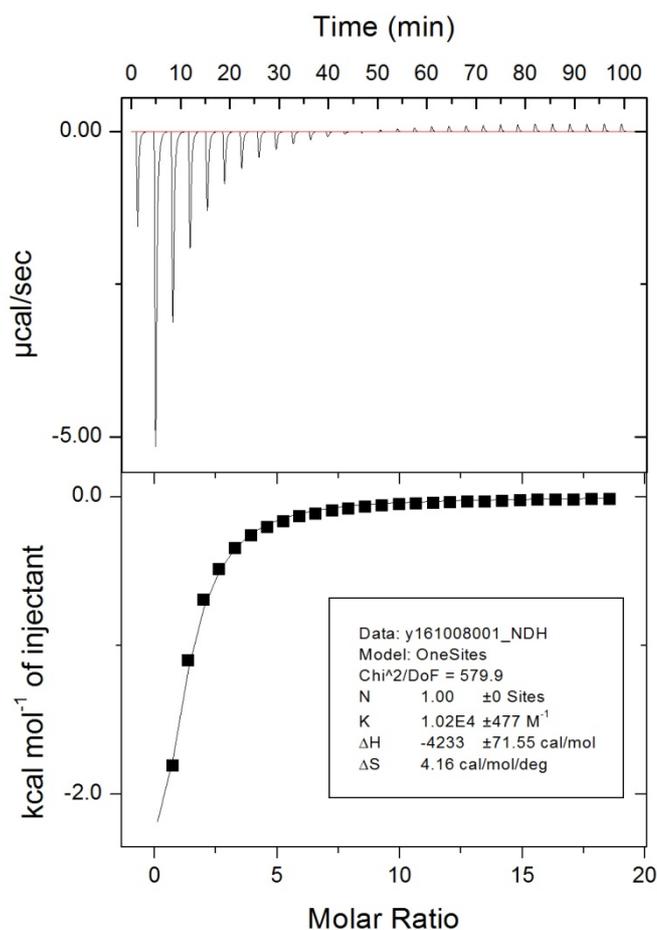
**Figure S2.** Mole ratio plot for CP6A and OX from <sup>1</sup>H NMR (500 MHz, 296 K) experiments, wherein CP6A (at a fixed concentration) in D<sub>2</sub>O was treated different molar equivalents of OX. The results are consistent with a 1 : 1 binding stoichiometry.

**$^1\text{H}$  NMR spectra of OX in the absence and presence of CP5A.**

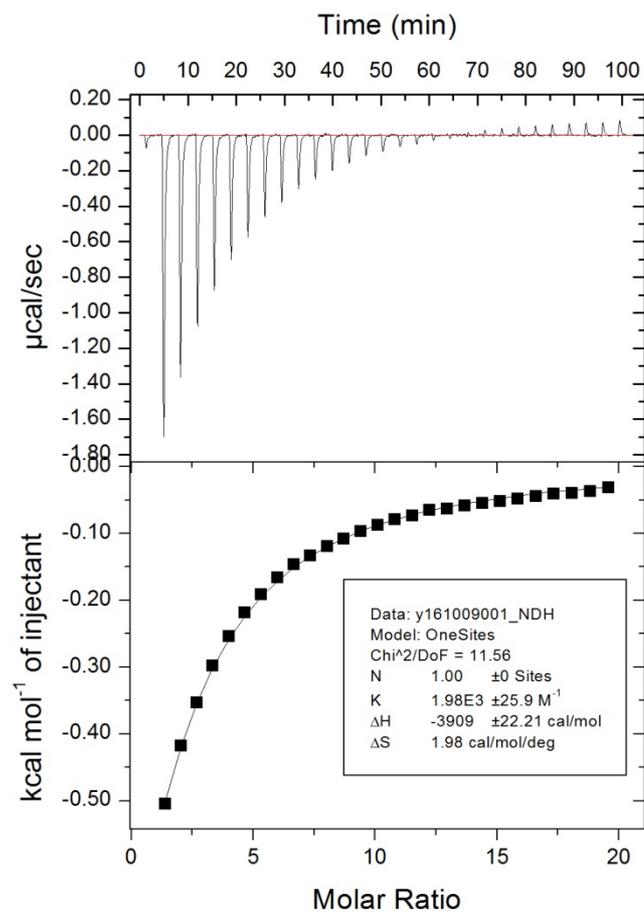


**Figure S3.**  $^1\text{H}$  NMR (400 MHz, 293 K) spectra of (A) OX, (B) OX + CP5A, and (C) CP5A in  $\text{D}_2\text{O}$  at 4.7–5.0 mM.

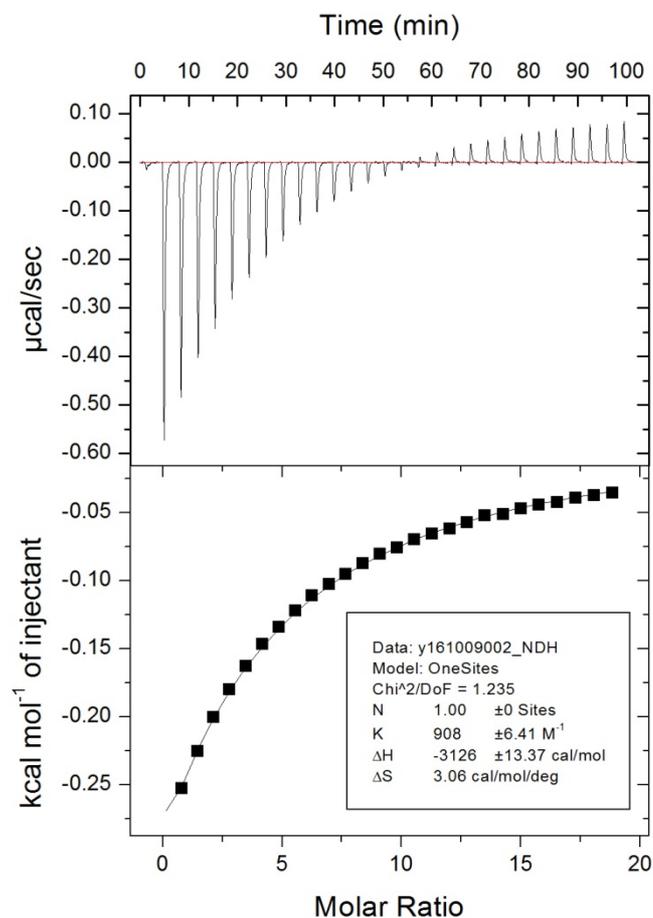
### ITC titration curves at different pH values.



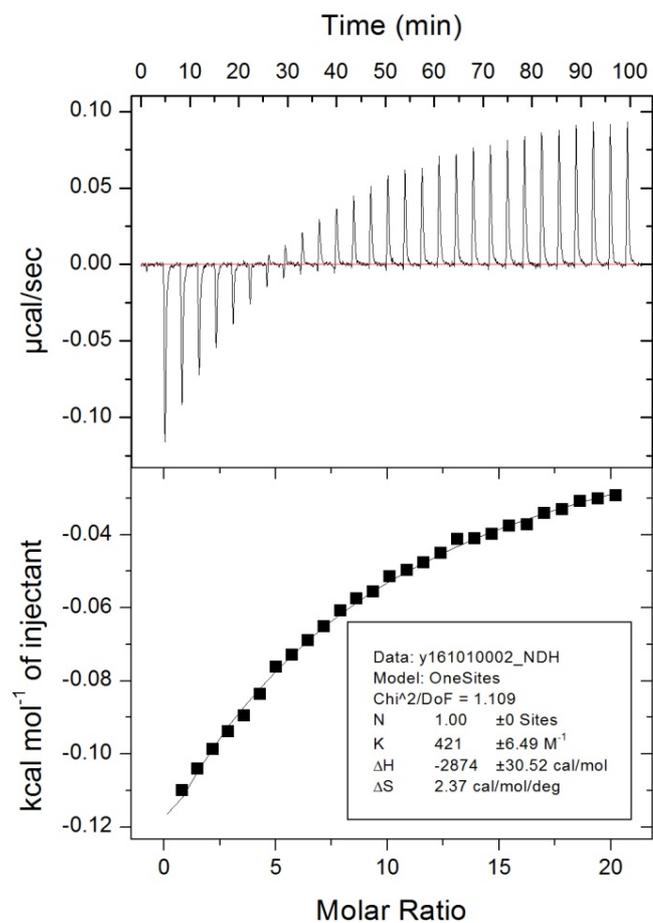
**Figure S4.** ITC experiments of CP6A with OX at pH 7.4 in aqueous phosphate buffer solution at 298.15 K. Top: Raw ITC data for 29 sequential injections (10  $\mu\text{L}$  per injection except the first injection) of an OX solution into a CP6A solution. Bottom: Net reaction heat obtained from the integration of the calorimetric traces after correction for the heat of dilution.



**Figure S5.** ITC experiments corresponding to the treatment of **CP6A** with **OX** at pH 6.5 in aqueous phosphate buffer solution at 298.15 K. Top: Raw ITC data for 29 sequential injections (10 µL per injection except the first injection) of **OX** solution into a **CP6A** solution. Bottom: Net reaction heat obtained from the integration of the calorimetric traces after correction for the heat of dilution.

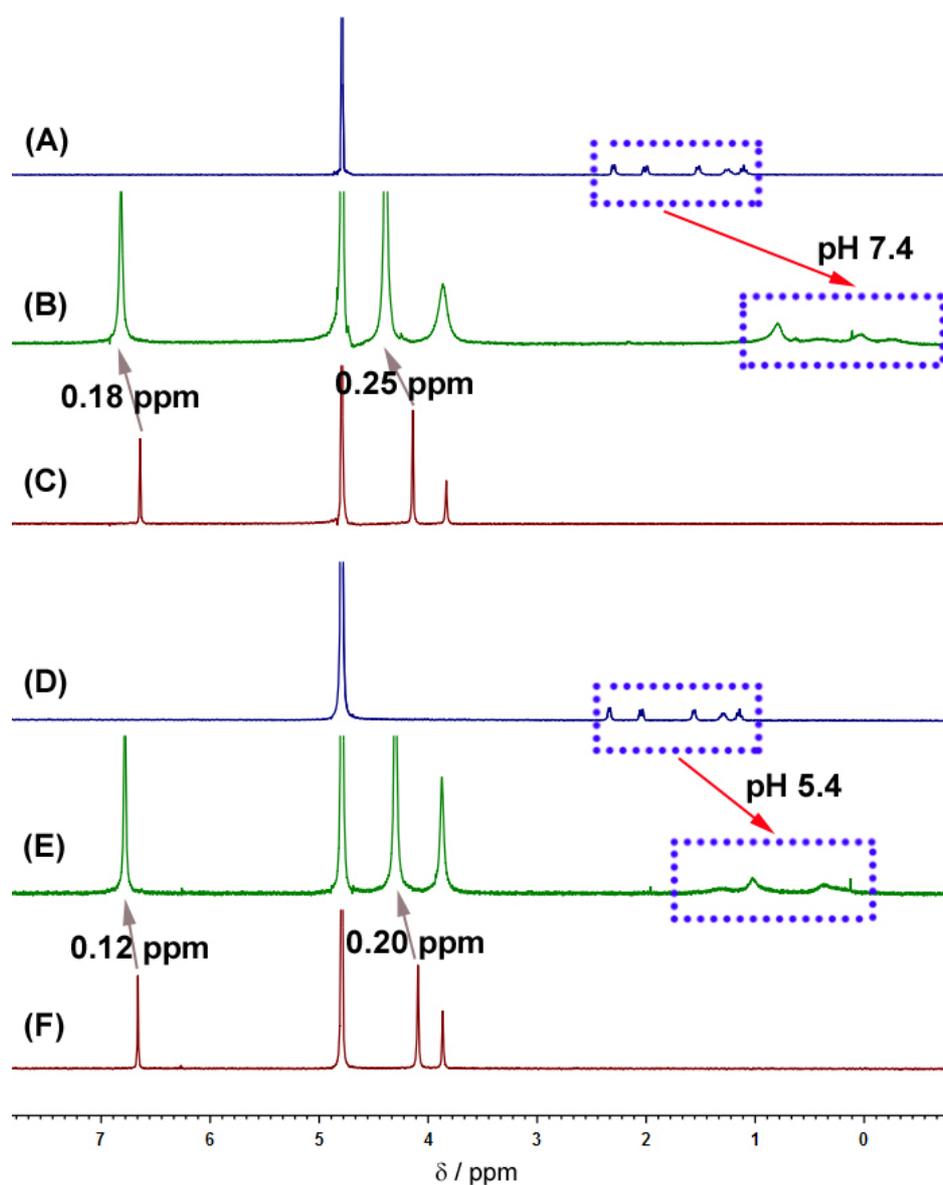


**Figure S6.** ITC experiments corresponding to the treatment of **CP6A** with **OX** at pH 6.0 in aqueous phosphate buffer solution at 298.15 K. Top: Raw ITC data for 29 sequential injections (10  $\mu\text{L}$  per injection except the first injection) of **OX** solution into a **CP6A** solution. Bottom: Net reaction heat obtained from the integration of the calorimetric traces after correction for the heat of dilution.



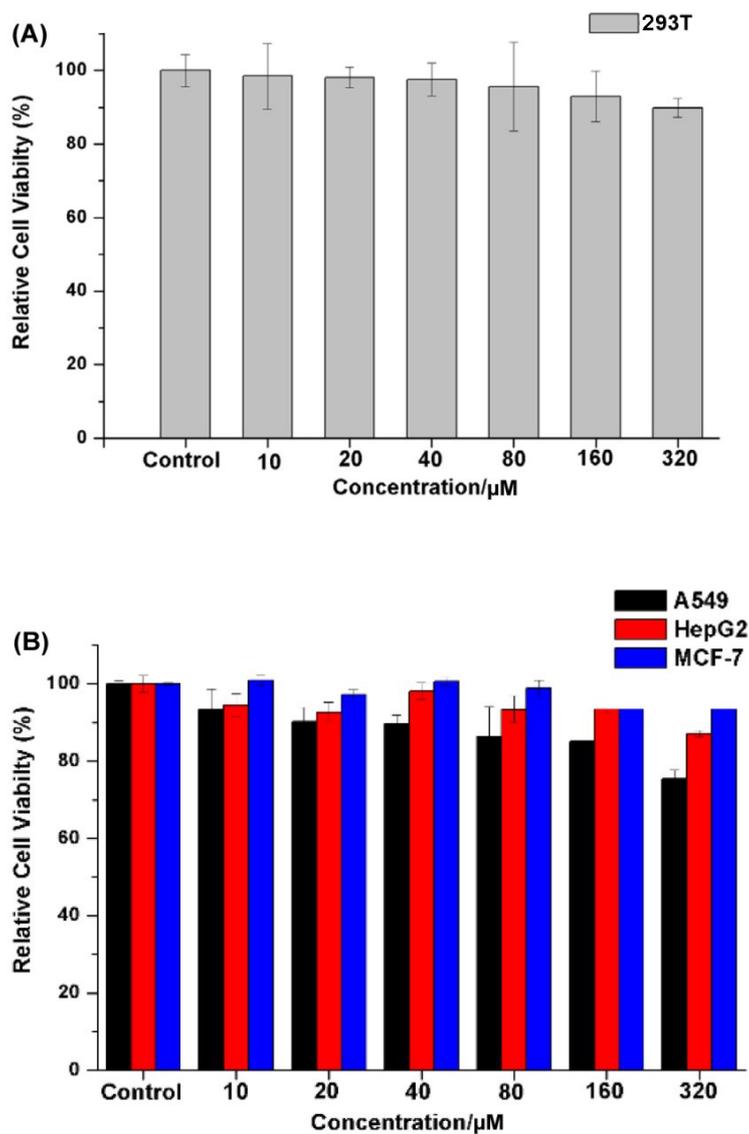
**Figure S7.** ITC experiments corresponding to the treatment of **CP6A** with **OX** at pH 5.4 in aqueous phosphate buffer solution at 298.15 K. Top: Raw ITC data for 29 sequential injections (10 µL per injection except the first injection) of **OX** solution into a **CP6A** solution. Bottom: Net reaction heat obtained from the integration of the calorimetric traces after correction for the heat of dilution.

**<sup>1</sup>H NMR spectra of 1:1 OX and CP6A at pD values of 7.4 and 5.4.**



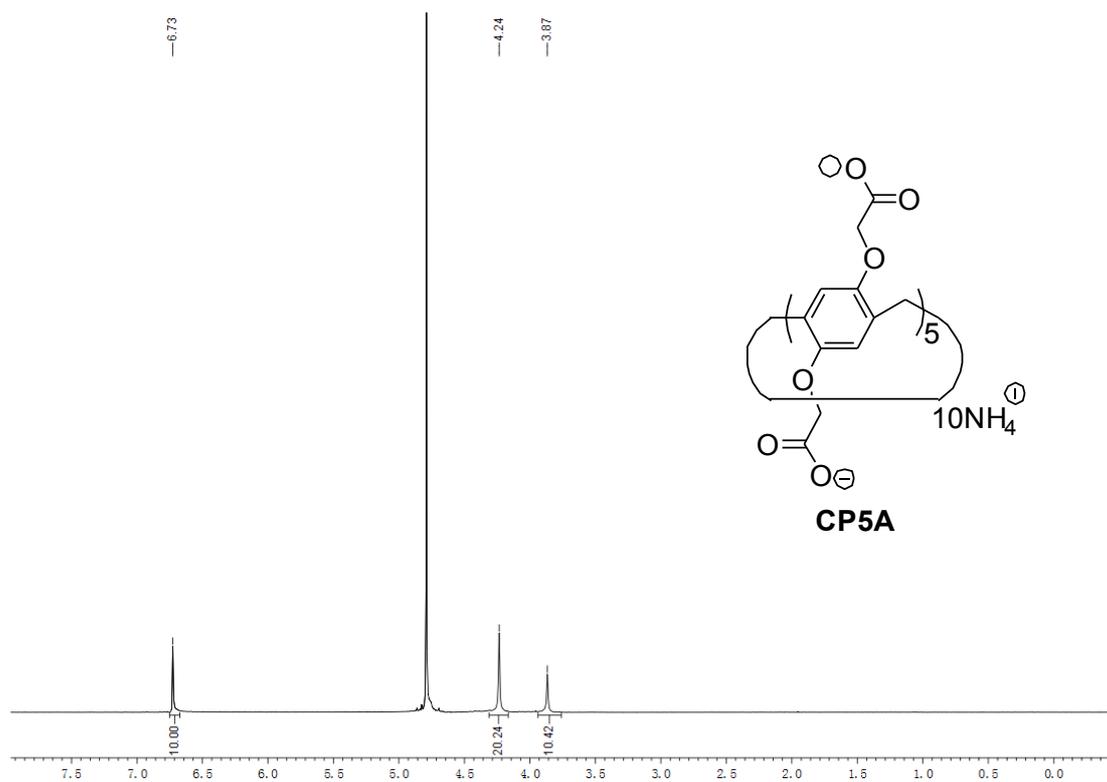
**Figure S8.** <sup>1</sup>H NMR (400 MHz, 298 K) spectra of (A) **OX**, (B) a 1:1 mixture of **OX** + **CP6A**, and (C) **CP6A** recorded at a pD = 7.4 and (D) **OX**, (E) 1:1 **OX** + **CP6A**, and (F) **CP6A** recorded at pD = 5.4 in deuterated phosphate buffer solution. The concentrations of **OX** and **CP6A** were 5.0 mM.

***In vitro* cytotoxicity assay of CP6A.**

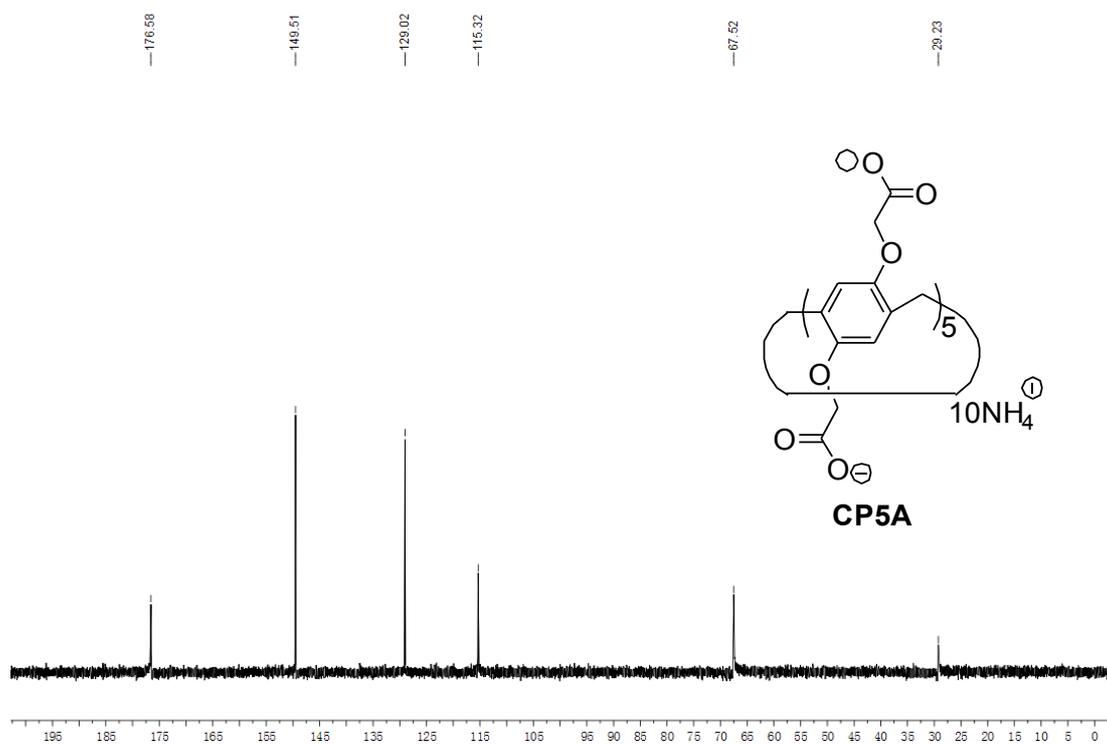


**Figure S9.** (A) Relative cell viabilities of 293T cells after incubation for 48 h with CP6A at various concentrations (mean  $\pm$  SD, n = 3). (B) Relative cell viabilities of A549, HepG-2, and MCF-7 after incubation for 48 h with CP6A at various concentrations (mean  $\pm$  SD, n = 3).

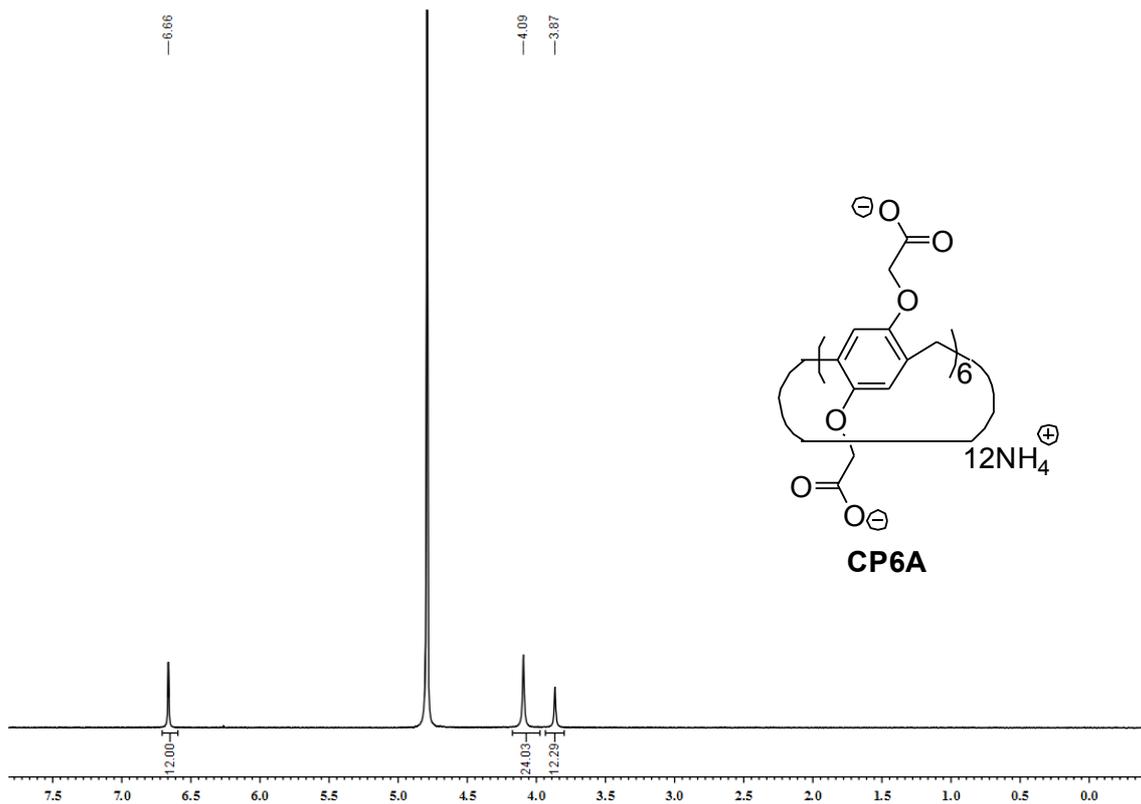
**$^1\text{H}$  NMR and  $^{13}\text{C}$  NMR spectra of CP5A and CP6A.**



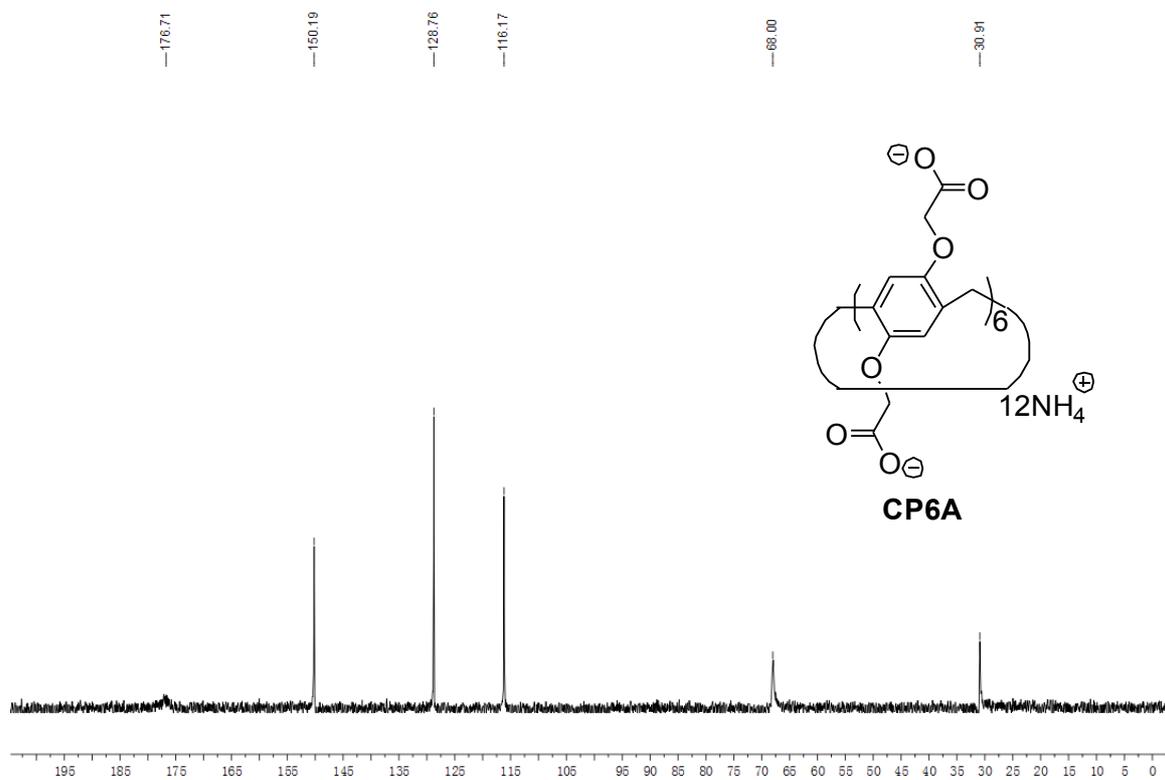
**Figure S10.**  $^1\text{H}$  NMR spectrum (400 MHz) of CP5A in  $\text{D}_2\text{O}$ .



**Figure S11.**  $^{13}\text{C}$  NMR spectrum (100 MHz) of CP5A in  $\text{D}_2\text{O}$ .



**Figure S12.** <sup>1</sup>H NMR spectrum (400 MHz) of CP6A in D<sub>2</sub>O.



**Figure S13.** <sup>13</sup>C NMR spectrum (100 MHz) of CP6A in D<sub>2</sub>O.

## Supporting Reference

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S1 Chunju Li, Xiaoyan Shu, Jian Li, Songhui Chen, Kang Han, Min Xu, Bingjie Hu, Yihua Yu and Xueshun Jia, *J. Org. Chem.*, 2011, **76**, 8458–8465.