Supramolecular chemotherapeutic drug constructed from pillararene-based supramolecular amphiphile†

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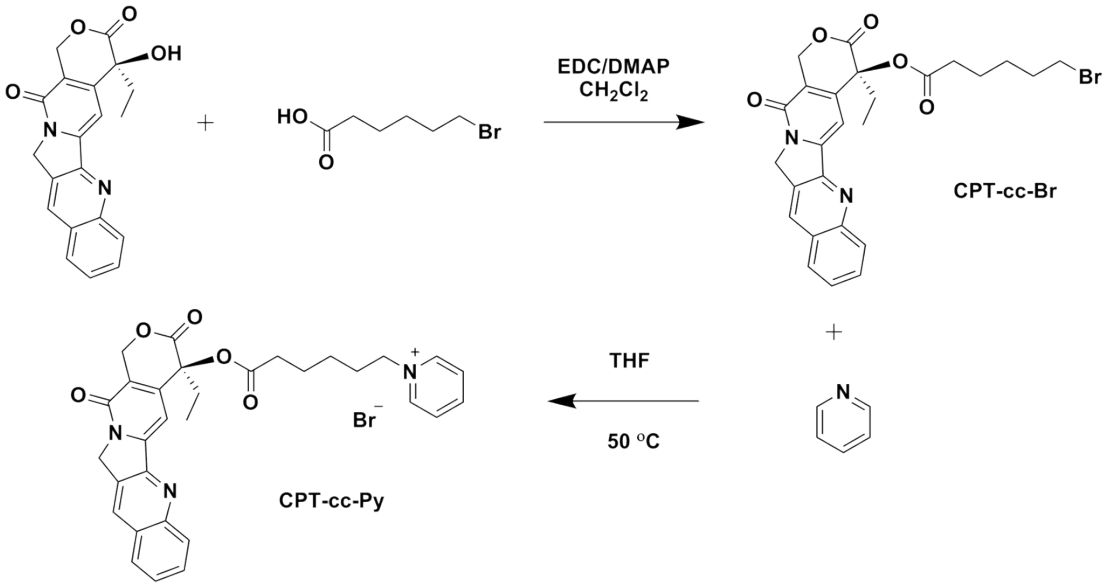
Electronic Supplementary Information (15 pages)

1. Materials and methods

All reagents were commercially available and used as supplied without further purification. Solvents were either employed as purchased or dried according to procedures described in the literature. The model compound (1-(ethoxycarbonylmethyl)pyridinium chloride, **M**) was purchased from Sigma. Water-soluble pillar[5]arene (**P5**) was synthesized according to literature procedures.[S1] ^{1}H NMR and ^{13}C NMR spectra were recorded on a Bruker Avance DMX 300 spectrophotometeror. The ^{1}H NMR spectra about the host–guest complexation were recorded on a Bruker Avance Ⅲ-400 spectrometry. The 2D NOESY NMR spectra were recorded on a Bruker Avance DMX 600 spectrophotometer with TMS as the internal reference. Transmission electron microscopy (TEM) investigations were carried out on a HT-7700 instrument. Dynamic light scattering (DLS) measurements were performed on a Nano-ZS ZEN3600 instrument. UV-vis-NIR spectra were taken on a Shimadzu UV-3150 spectrophotometer. The isothermal titration calorimetry (ITC) experiments were performed on a VP-ITC micro-calorimeter (Microcal, USA). The cell images were taken by a confocal laser scanning microscopy (CLSM, Radiance2100, Bio-Rad) with a 100 × oil immersion lens.

*2. The synthesis of **CPT-cc-Py***



Scheme S1. Synthetic route to **CPT-cc-Py**.

Synthesis of **CPT-cc-Br**: EDC (167 mg, 0.87 mmol) and DMAP (4.88 mg, 0.04 mmol) were added to a suspension of CPT (100 mg, 0.29 mmol) in 20 mL of DCM, followed by 6-bromohexanoic acid (169 mg, 0.87 mmol) pre-dissolved in 5 mL of DCM. After being stirred for 24 h at room temperature, the reaction mixture was poured into 40 mL of water. The organic phase was collected. The aqueous phase was extracted with DCM three times. The DCM fractions were combined and dried over anhydrous Na_2SO_4. Upon removal of DCM by rotary evaporation, the obtained **CPT-cc-Br** was further purified by column chromatography (silica gel; DCM/ethyl acetate = 8:1 to obtain **CPT-cc-Br** as a blue solid (150 mg, 85%). The ^{1}H NMR spectrum of **CPT-cc-Py** is shown in Fig. S1. ^{1}H NMR (300 MHz, $CDCl_3$, room temperature) δ (ppm): 8.43 (s, 1H), 8.27−8.24 (d, J = 9.0 Hz, 1H), 7.99−7.96 (d, J = 9.0 Hz, 1H), 7.90−7.84 (m, 1H), 7.73−7.68 (m, 1H), 7.24 (s, 1H), 5.74−5.69 (d, J = 15.0 Hz, 1H), 5.47−5.41 (d, J = 18.0 Hz, 1H), 5.32 (m, 2H), 3.45−3.36 (m, 2H), 2.58−2.52 (m, 2H), 2.41−2.29 (m, 1H), 2.22−2.15 (m, 1H), 1.95−1.85 (m, 2H), 1.75−1.67 (m, 2H), 1.58−1.48 (m, 2H), 1.04−0.99 (t, J = 9.0 Hz, 3H). The ^{13}C NMR spectrum of **CPT-cc-Br** is shown in Fig. S2. ^{13}C NMR (75 MHz, $CDCl_3$, room temperature) δ (ppm): 166.65, 156.78, 152.86,

151.57, 148.21, 145.85, 145.25, 130.76, 130.25, 128.95, 127.65, 119.63, 95.72, 77.47, 66.42, 65.90, 63.12, 49.54, 40.25, 40.11, 36.20, 35.98, 31.27, 7.04.

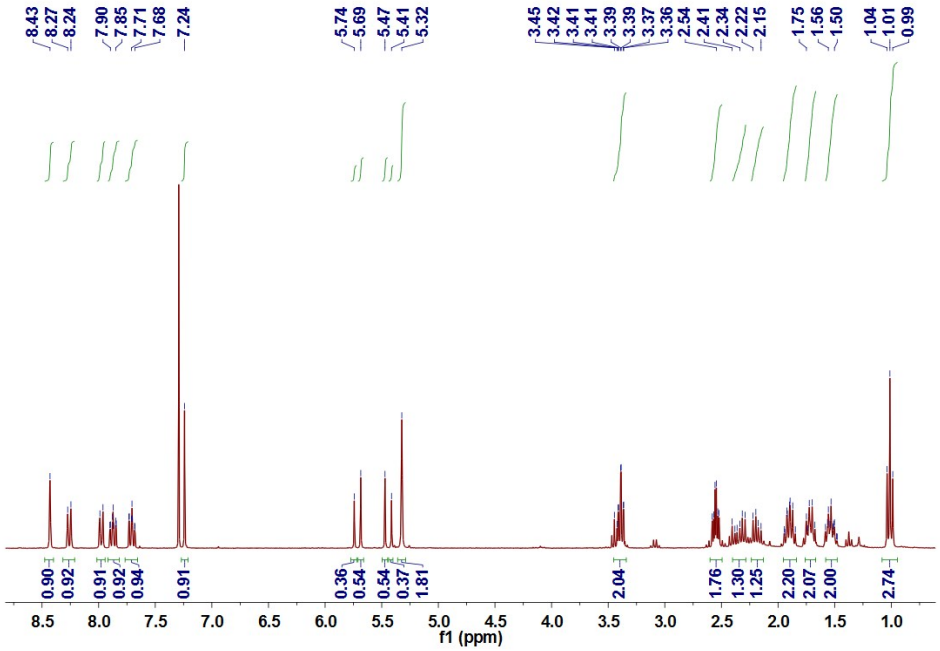


Fig. S1 ^{1}H NMR spectrum (300 MHz, $CDCl_3$, room temperature) of **CPT-cc-Br**.

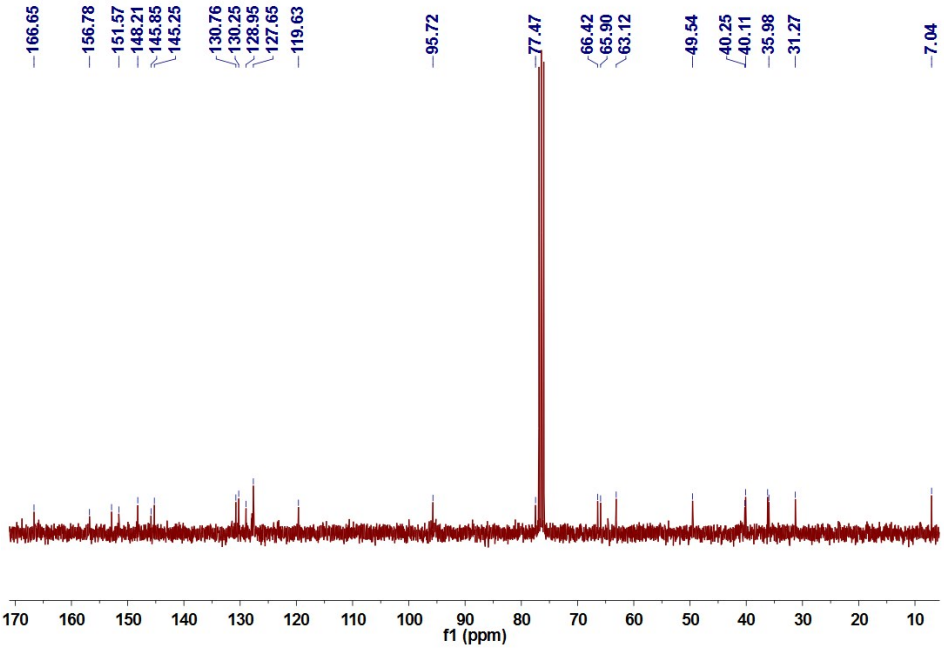


Fig. S2 ^{13}C NMR spectrum (75 MHz, $CDCl_3$, room temperature) of **CPT-cc-Br**.

Synthesis of **CPT-cc-Py**: Pyridine (79 mg, 1.0 mmol) was added to a solution of **CPT-cc-Br** (50 mg, 0.1 mmol) in THF (10 mL). The mixture was heated under nitrogen at 50 °C for 24 h. Upon removal of THF by rotary evaporation, the obtained mixture was further purified by column chromatography (silica gel; methanol:DCM = 1:1) to yield **CPT-cc-Py** as a bule solid (24 mg, 40%). The ^{1}H NMR spectrum of **CPT-cc-Py** is shown in Fig. S3. ^{1}H NMR (300 MHz, DMSO-d_6, room temperature) δ (ppm): 8.69（s, 1H), 8.59−8.58 (d, J = 3.0 Hz, 2H), 8.19−8.12 (m, 2H), 7.90−7.70 (m, 3H), 7.41−7.37 (m, 2H), 7.06 (s, 1H), 5.50 (s, 2H), 5.29 (s, 2H), 3.49−3.43 (m, 2H), 2.61−2.46 (m, 2H), 2.21−2.13 (m, 2H), 1.90−1.74 (m, 2H), 1.63−1.56 (m, 2H), 1.48−1.40 (m, 2H), 0.96−0.91 (t, J = 9.0 Hz, 3H). The ^{13}C NMR spectrum of **CPT-cc-Py** is shown in Fig. S4. ^{13}C NMR (75 MHz, DMSO-d_6, room temperature) δ (ppm): 172.04, 167.34, 156.64, 152.40, 149.69, 147.96, 146.09, 145.45, 136.21, 131.67, 129.89, 129.01, 128.63, 128.09, 127.80, 123.99, 119.00, 94.78, 75.73, 66.38, 50.32, 34.85, 33.03, 31.96, 30.35, 27.02, 23.60, 7.64.

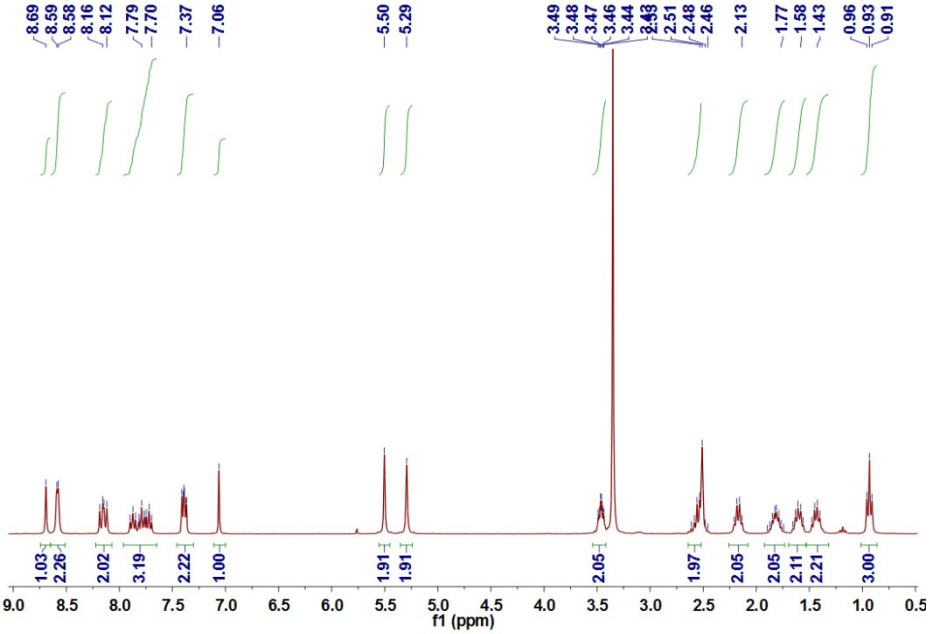


Fig. S3 ^{1}H NMR spectrum (300 MHz, DMSO-d_6, room temperature) of **CPT-cc-Py**.

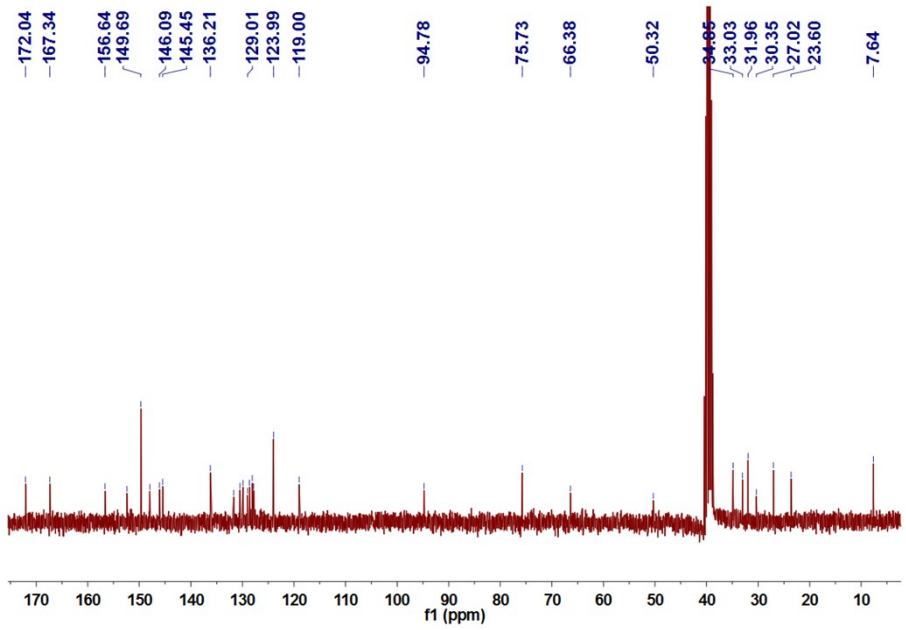
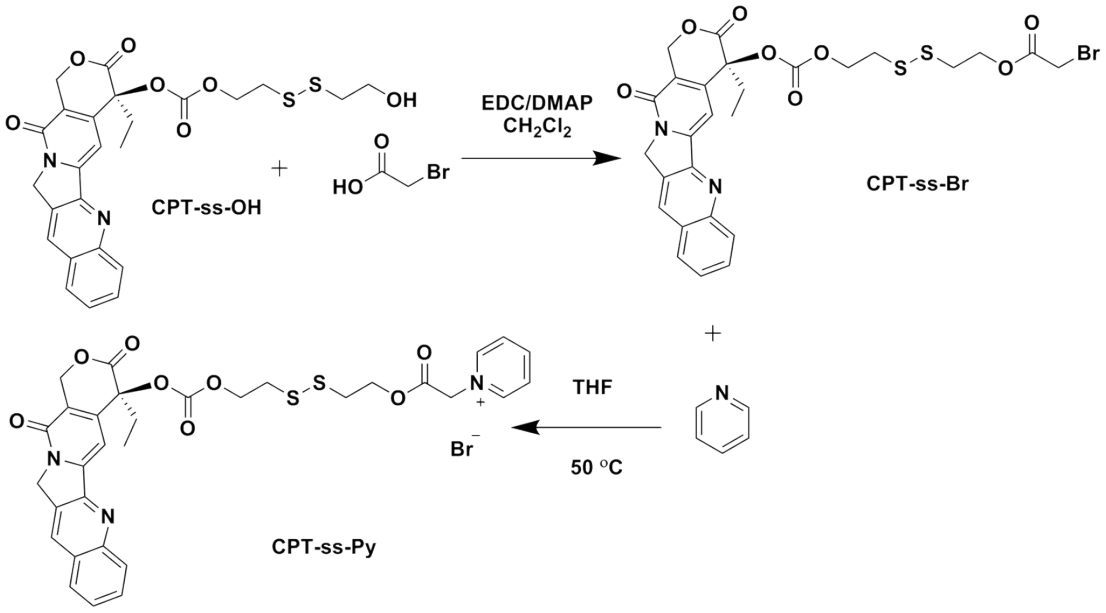


Fig. S4 ^{13}C NMR spectrum (75 MHz, DMSO-d_6, room temperature) of **CPT-cc-Py**.

*3. The synthesis of **CPT-ss-Py***



Scheme S2. Synthetic route to **CPT-ss-Py**.

Synthesis of **CPT-ss-Br**: To a suspension of CPT-ss-OH (100 mg, 0.19 mmol) in 20 mL of DCM were added EDC (110 mg, 0.57 mmol) and DMAP (4.0 mg, 0.03 mmol), followed by 2-bromoacetic acid (79 mg, 0.57mmol) pre-dissolved in 5 mL of DCM. After being stirred for 24 h at room temperature, the reaction mixture was added 40 mL of water. The organic phase was collected. The aqueous phase was extracted with

DCM three times. The DCM fractions were combined and dried over anhydrous Na_2SO_4. Upon removal of DCM by rotary evaporation, the obtained mixture was further purified by column chromatography (silica gel; DCM:ethyl acetate = 8:1) to obtain **CPT-ss-Br** as a bule solid (92 mg, 75%). The ^{1}H NMR spectrum of **CPT-ss-Br** is shown in Fig. S5. ^{1}H NMR (300 MHz, $CDCl_3$, room temperature) δ (ppm): 8.46 (s, 1H), 8.29−8.26 (d, J = 9.0 Hz, 1H), 8.01−7.98 (d, J = 9.0 Hz, 1H), 7.92−7.86 (m, 1H), 7.75−7.70 (m, 1H), 7.40 (s, 1H), 5.78−5.72 (d, J = 18.0 Hz, 1H), 5.46−5.41 (d, J = 15.0 Hz, 1H), 5.36 (s, 2H), 4.45−4.39 (m, 4H), 4.18 (s, 1H), 4.08 (s, 1H), 3.01−2.94 (m, 4H), 2.37−2.16 (m, 2H), 1.08−1.03 (t, J = 9.0 Hz, 3H). The ^{13}C NMR spectrum of **CPT-ss-Br** is shown in Fig. S6. ^{13}C NMR(75 MHz, $CDCl_3$, room temperature) δ (ppm): 169.26, 167.26, 167.12, 157.38, 153.49, 152.25, 148.87, 146.50, 145.79, 131.32, 130.84, 129.62, 128.52, 128.25, 128.20, 120.28, 96.22, 78.09, 77.24, 67.07, 66.51, 63.74, 50.13, 40.85, 40.72, 36.83, 36.61, 31.91, 7.66.

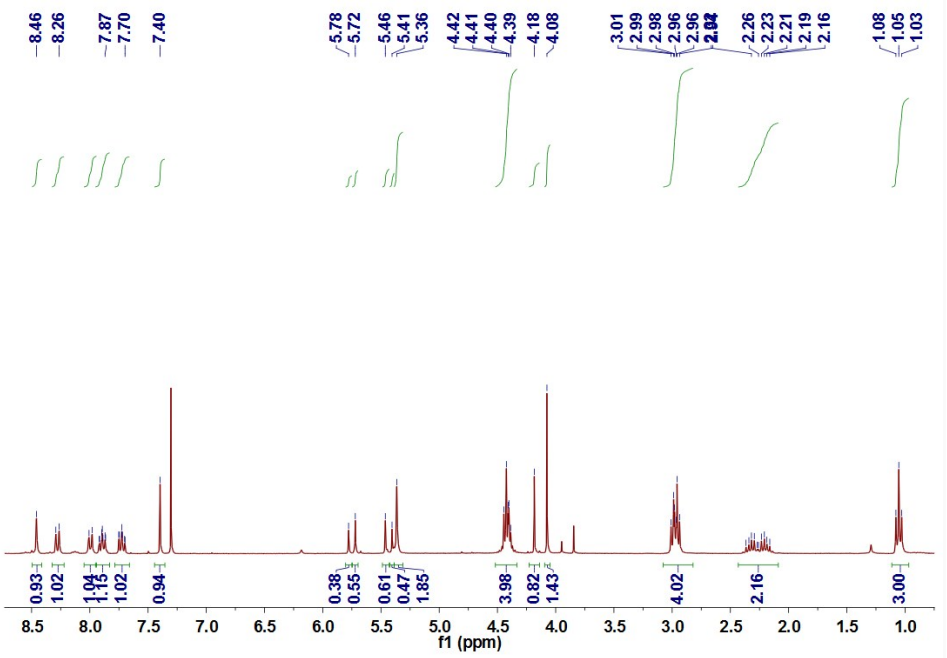


Fig. S5 ^{1}H NMR spectrum (300 MHz, $CDCl_3$, room temperature) of **CPT-ss-Br**.

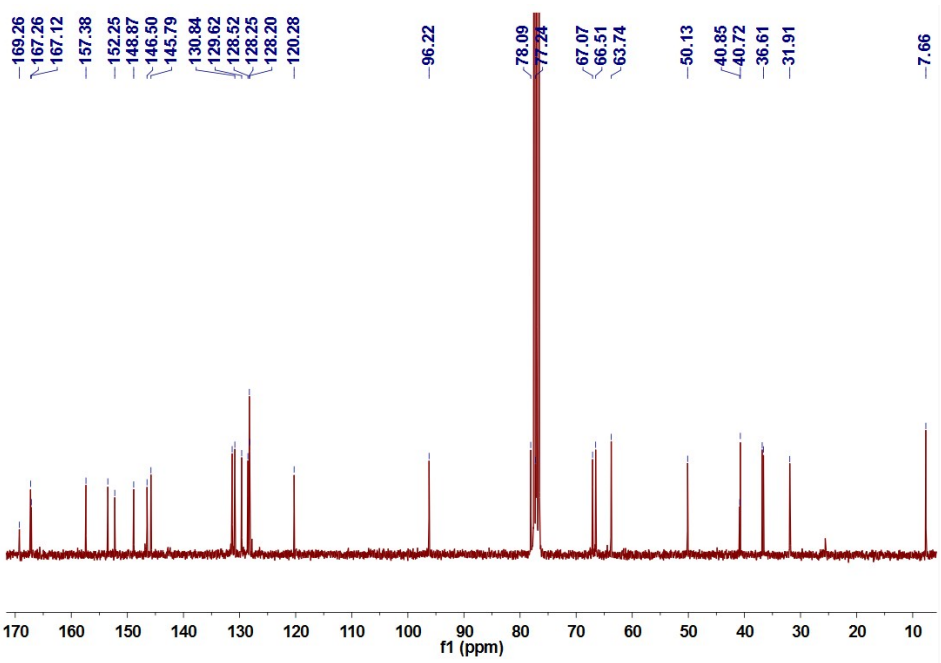


Fig. S6 ^{13}C NMR spectrum (75 MHz, $CDCl_3$, room temperature) of **CPT-ss-Br**.

Synthesis of **CPT-ss-Py**: Pyridine (63 mg, 0.80 mmol) was added to a solution of **CPT-ss-Br** (50 mg, 0.08 mmol) in THF (10 mL). The mixture was heated under nitrogen at 50 °C for 24 h. Upon removal of THF by rotary evaporation, the obtained mixture was further purified by column chromatography (silica gel; methanol:DCM = 1:1) to yield **CPT-ss-Py** as a bule solid (23 mg, 40%). The ^{1}H NMR spectrum of **CPT-ss-Py** is shown in Fig. S7. ^{1}H NMR (300 MHz, DMSO-d_6, room temperature) δ (ppm): 8.69 (m, 1H), 8.59 (m, 2H), 8.18−8.12 (m, 2H), 7.88−7.72 (m, 3H), 7.43−7.39 (m, 2H), 7.11 (m, 1H), 5.54 (m, 2H), 5.30 (m, 2H), 4.35−4.27 (m, 4H), 3.04−2.97 (m, 4H), 2.52−2.51 (m, 2H), 2.20−2.19 (m, 2H), 0.95−0.94 (m, 3H). The ^{13}C NMR spectrum of **CPT-ss-Py** is shown in Fig. S8. ^{13}C NMR (75 MHz, DMSO-d_6, room temperature) δ (ppm): 169.08, 167.60, 167.52, 156.95, 153.29, 152.66, 149.97, 148.34, 146.71, 145.21, 136.73, 132.07, 130.91, 130.22, 129.43, 128.99, 128.47, 128.22, 124.41, 119.65, 94.86, 78.38, 66.92, 66.73, 63.64, 50.79, 41.96, 41.42, 36.64, 36.48, 30.78, 28.51, 8.02.

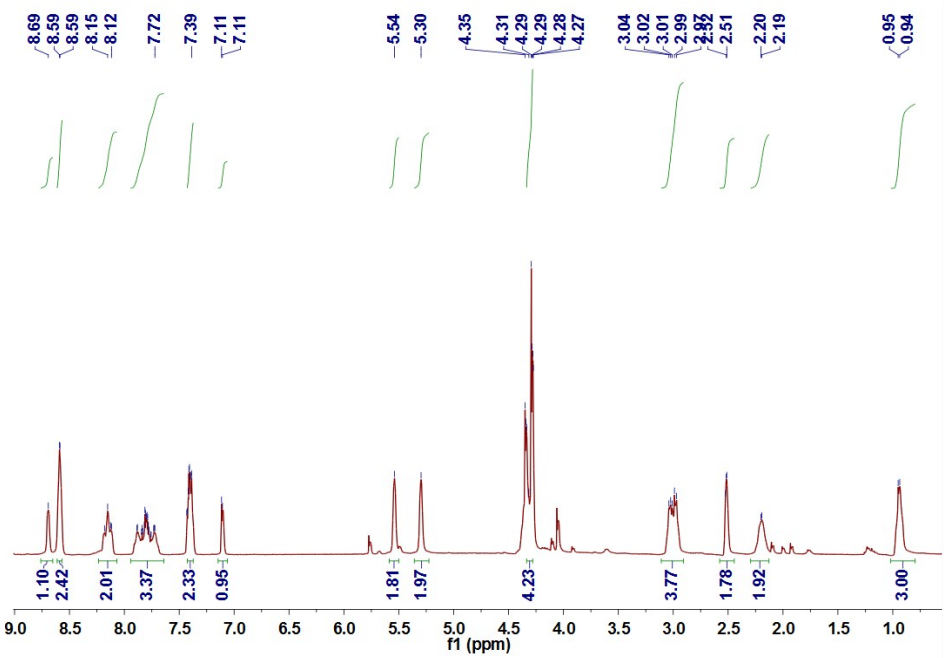


Fig.S7 ^{1}H NMR spectrum (300 MHz, DMSO-d_6, room temperature) of **CPT-ss-Py**.

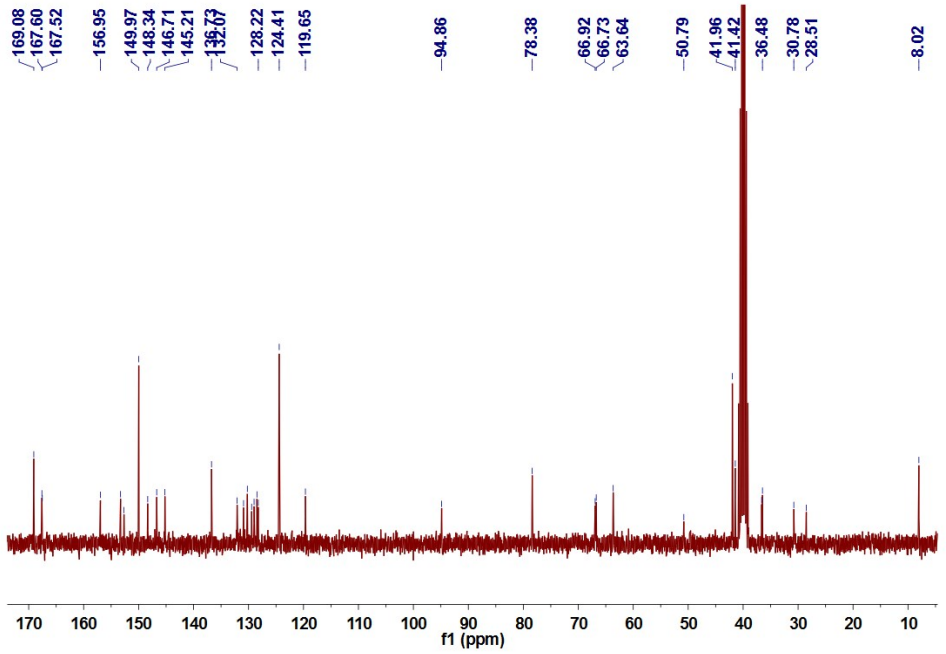


Fig. S8 ^{13}C NMR spectrum (75 MHz, DMSO-d_6, room temperature) of **CPT-ss-Py**.

*4. 2D NOESY NMR spectra of **P5** and **G** in H_2O*

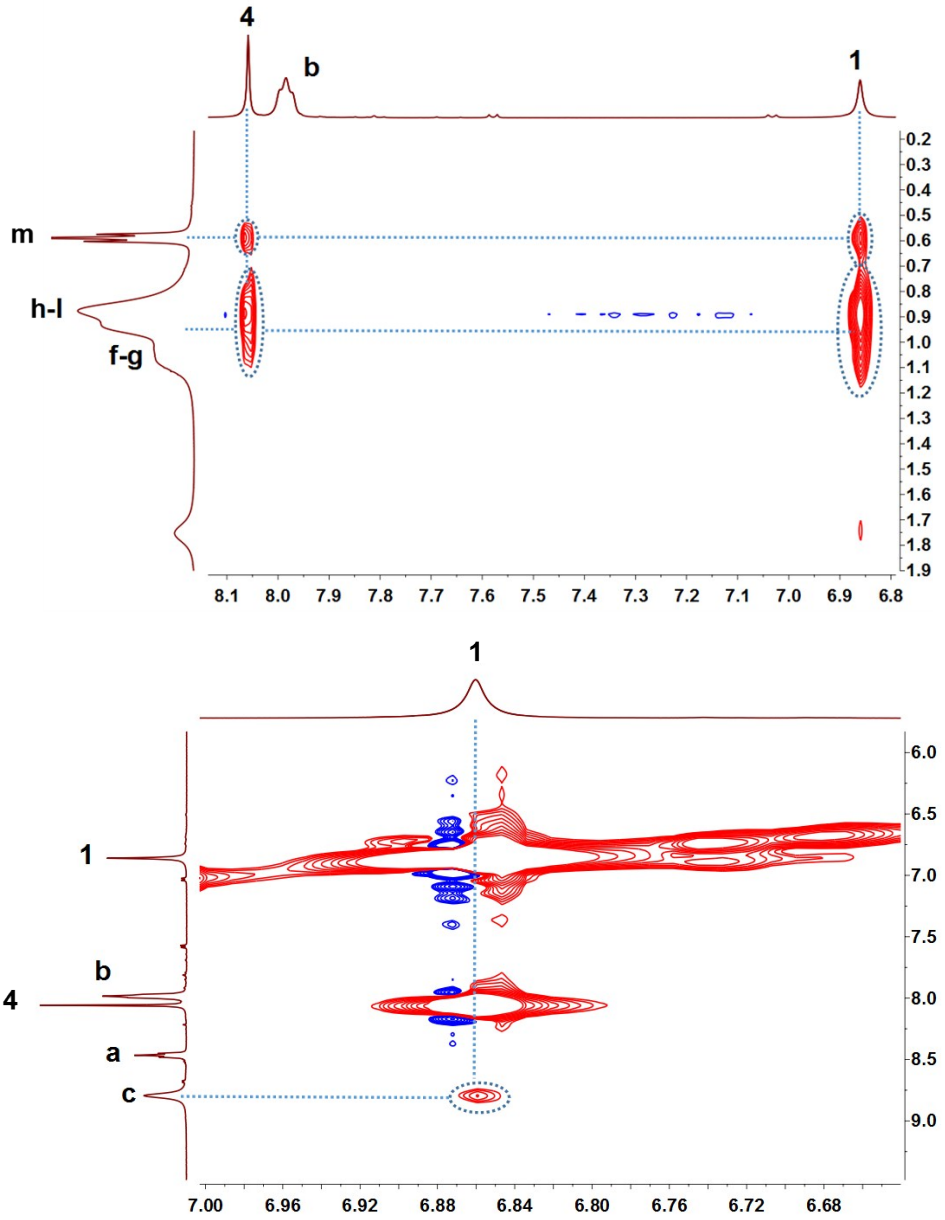


Figure S9. Partial NOESY NMR spectra (600 MHz, D_2O, room temperature) of **P5** (2.0 mM) and **G** (2.0 mM).

*5. ITC investigations of host−guest complexation between **P5** and **G**.*

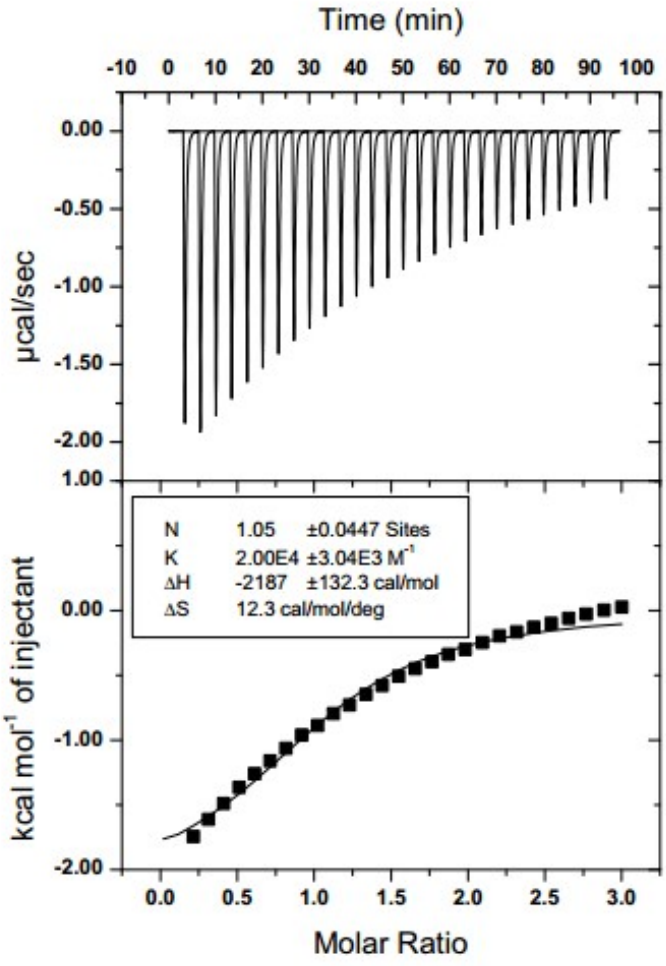


Figure S10. Microcalorimetric titration of **P5** (2.00 mM, 10 µL per injection) with **G** (0.100 mM) in water at 298.15 K.

6. Cytotoxicity evaluation.

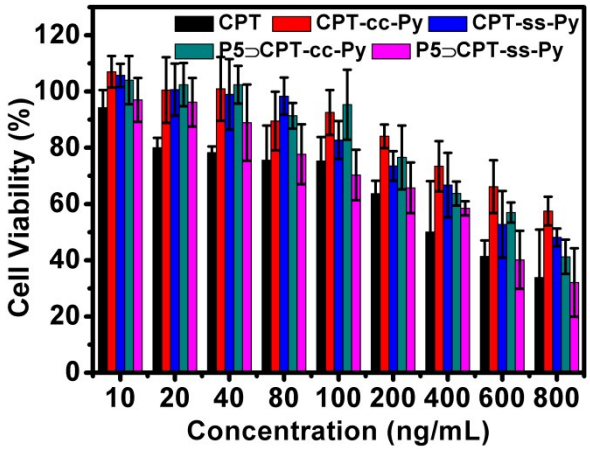
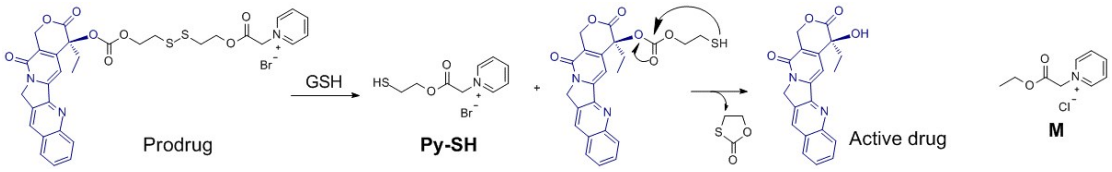


Figure S11. Cytotoxicity of B16F10 cells by culturing with the CPT, **CPT-cc-Py**, **CPT-ss-Py**, **P5⊃CPT-cc-Py** and **P5⊃CPT-ss-Py** with different concentrations for 24 h.



Scheme S3. GSH-triggered drug release and the model compound (**M**) used.

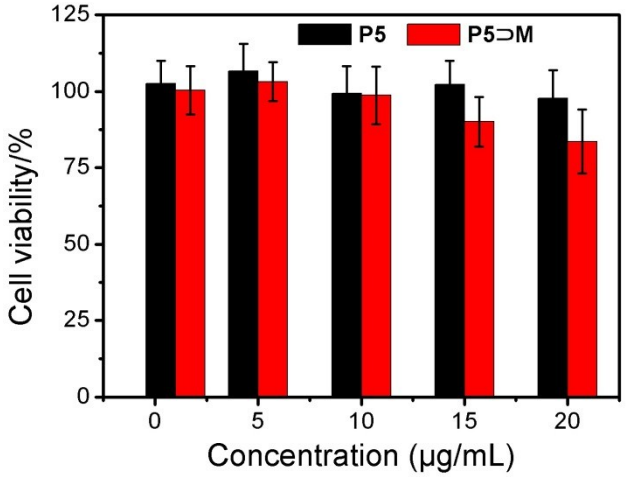


Figure S12. Cytotoxicity of **P5** and **P5⊃M** against HeLa cells with different concentrations for 24 h.

In order to mimic the cytotoxicity of **Py-SH** generated after the GSH-triggered drug release, a model compound (**M**) was used. We evaluated the cytotoxicity of **P5⊃M** using MTT assay. As shown in Fig. S12, the cell viability was higher than 80% when the concentration of **P5⊃M** was 20 μg/mL, which confirmed that the cytotoxicity of the by-product was low. Additionally, the cytotoxicity of **P5** was also evaluated by MTT assay. As shown in Fig. S12, negligible changes in cell viability was observed even when the concentration of **P5** reached 20 μg/mL, suggesting its cytotoxicity was low.

Critical aggregation concentration (CAC) determination of CPT-ss-Py and P5⊃CPT-ss-Py. The dependence of the solution conductivity on the solution concentration is used to determine the critical aggregation concentration. Typically,

the slope of conductivity versus the concentration below CAC is steeper than the slope above the CAC. Therefore, the junction of the conductivity-concentration plot represents the CAC value. To measure the CAC values of **CPT-ss-Py** and **P5⊃CPT-ss-Py**, the conductivities of the solutions at different concentrations (from 0 to 0.01 mM) were determined. By plotting the conductivity versus the concentration, we estimated the CAC values of **CPT-ss-Py** and **P5⊃CPT-ss-Py**.

TEM and DLS Studies. The morphology of the SNPs was revealed using TEM. TEM samples were prepared by drop-coating the solution of SNPs onto a carbon-coated copper grid. The corresponding solution was left to stand overnight and the insoluble precipitate was eliminated by using a microporous membrane before DLS tests. Dynamic light scattering (DLS) measurements were carried out using a 200 mW polarized laser source Nd:YAG (λ = 532 nm). The polarized scattered light was collected at 90° in a self-beating mode with a Hamamatsu R942/02 photomultiplier. The signals were sent to a Malvern 4700 submicrometer particle analyzer system.

Controlled Release Studies. The release of CPT from **SNPs** was determined using a dialysis strategy. Briefly, **SNPs** was dissolved in phosphate buffered saline (PBS) to prepare a solution with the concentration of 100 μg mL^{-1} . The solution (2 mL) was transferred into a dialysis cassettes the molecular weight cut-off of 1 kDa and dialyzed against PBS (25 mL) with/without GSH for 24 h. 100 μL of the

solution was taken at pre-determined times from dialysate for HPLC measurement. At the same time, fresh PBS (100 μL) was added back into the dialysate. The released CPT was detected by HPLC using 30% acetonitrile containing 0.1% TFA as fluent with the flow rate of 1 mL min^{-1} (UV detector at 366 nm). The controlled release experiments were evaluated in triplicate.

Cell Cultures. The cell lines including HeLa, A549 and B16F10 cells were purchased from ATCCand cultured in Dulbecco's modified Eagle's medium (DMEM) containing 10% fetal bovine serum (FBS) and 1% penicillin/streptomycin. The cells were harvested from the cell culture medium by incubating in a trypsin solution (0.5% *w*/*v* in PBS) for 2 min. The cells were centrifuged, and the supernatant was discarded. The cells were resuspended in serum-supplemented DMEM at a concentration of 1×10^4 cells/mL. Cells were cultured at 37 °C and 5% CO_2.

Evaluation of Cytotoxicity. The cytotoxicity of CPT, **CPT-cc-Py**, **CPT-ss-Py**, **P5⊃CPT-cc-Py** and **P5⊃CPT-ss-Py** were evaluated by using MTT assay. HeLa cells were seeded in 96-well plates at 8×10^3 cells/well, allowed to adhere overnight and incubated with serum-free culture media containing CPT, **CPT-cc-Py**, **CPT-ss-Py**, **P5⊃CPT-cc-Py** and **P5⊃CPT-ss-Py** at a serious of concentration. After 24 h incubation, the media was removed and washed with PBS for three times. The cells were incubated in 100 μL DMEM medium containing 0.5 mg/mL MTT reagent for an

additional 4 h, 100 μL DMSO was added to each well to dissolve formazan crytal. Eventually, each well was measured using a scanning spectrophotometer (Model 550, Bio-Rad) at a wavelength 570 nm.

Cellular uptake assay. HeLa cells were cultured in the chambers at a density of 1 × 10^5 per well for 24 h. The cells were incubated with **P5⊃CPT-ss-Py** SNPs (CPT concentration: 0.1 μg/mL) at 37 °C for 1 h and 24 h respectively, followed by staining with 1.0 mM Lyso-Tracker Red for 5 min, The cells were washed three times with PBS and fixed with fresh 4.0% formaldehyde at room temperature for 15 min. The images were taken using a LSM-510 confocal laser scanning microscope (Radiance2100, Bio-Rad) (100 × oil immersion lens).

***Reference**:*

S1. Z. Li, J. Yang, G. Yu, J. He, Z. Abliz and F. Huang, *Org. Lett.*, 2014, **16**, 2066−2069.