Supporting Information

Supramolecular Drug-Drug Complex Vesicles Enable Sequential Drug Release for Enhanced Combination Therapy

Chengfei Liu,[#a] Chunpu Li,[#b] Cui Pang,[c] Muqiong Li,[c] Huixin Li,[a] Pengxiang Li,[a] Li Fan,*[c] Hong Liu*[b] and Wei Tian*[a]

[a] Shanxi Key Laboratory of Macromolecular Science and Technology, MOE Key Laboratory of Material Physics and Chemistry under Extraordinary Conditions, School of Chemistry and Chemical Engineering, Northwestern Polytechnical University, Xi'an, 710072, China.

[b] State Key Laboratory of Drug Research, Shanghai Institute of Materia Medica, Chinese Academy of Sciences, 555 Zu Chong Zhi Road, Shanghai, 201203, China

[c] Department of Pharmaceutical chemistry and analysis, School of Pharmacy, Airforce medical university, 169th Changle West Road, 710032 Xi'an, Shaanxi, China

Corresponding Author

xxfanny@fmmu.edu.cn (L. Fan), hliu@simm.ac.cn (H. Liu), happytw_3000@nwpu.edu.cn (W. Tian)

[#] These authors contributed equally to this work.

Table of Contents

1. Materials and methods

1.1 Materials

All the reagents and solvents were commercially available and used as received unless specified otherwise. All aqueous solutions were prepared with distilled water. Compounds pillar[5]arene-NH_2, c,c,t-$Pt(NH_3)_2Cl_2(OOCCH_2CH_2COOH)_2$ were prepared according to the literature procedures. camptothecin (CPT), cisplatin ($Pt(NH_3)_2Cl_2$), N,N-Diisopropylethyl-amine (DIPEA), 4-dimethyl-aminopyridine (DMAP) and 2-bromoisobutyryl bromide were purchased from J&K Scientific Ltd, China. Pillar[5]arene, BBr_3 and Succinic anhydride were purchased from Energy Chemical, China. N,N,N′,N″,N″-pentamethyldiethylenetriamine (PMDETA) and 1-hydroxybenzotriazole (HOBt) were purchased form Adamas, China. CuBr was purchased from Energy Chemical and purified by washing with acetic acid and methanol before use.

1.2 Structure characterization methods

IR spectra were recorded on a TENSOR27 spectrometer and are reported in cm^{-1}. UV-Vis spectra were measured using an Agilent Cary-100 spectrophotometer. Fluorescence spectra were recorded on a Horiba Fluorolog-3 spectrometer. ^1H NMR, ^{13}C NMR, ^1H-^1H COSY, NOESY spectra were recorded on a Bruker AVANCE III 400 MHz spectrometer or a Bruker Avance DMX 500 spectrophotometer with use of the deuterated solvent as the lock and the residual solvent or TMS as the internal reference. The two-dimensional diffusion-ordered NMR spectra were recorded on a Bruker DRX600 spectrometer. Electrospray Ionization (ESI) mass spectra were acquired with Bruker micr OTOF-Q II electrospray instrument. DLS was performed on a Zeta sizer Nano ZS90 before sample was filtered. SEM was carried out using Verios G4 microscope. AFM images were obtained on a Dimension Fast Scan and Dimension Icon instrument. The sample for transmission electron microscopy (TEM) measurement was prepared by dropping the solution onto a copper grid with uranyl acetate and the sample was examined by a FEI Talos F200X instrument.

1.3 Critical aggregation concentration (CAC) measurement

4 μL of pyrene ethanol solution (6×10^{-4} mol L^{-1}) was added to 4 mL of **SDDC** aggregates aqueous solution with different concentrations, while the theoretically concentration of pyrene in each flask

was kept at 6×10^{-7} mol L^{-1}. The fluorescence emission spectra of all samples were recorded on a Horiba Fluorolog-3 spectrometer at 332 nm excitation wavelength and 5 nm slit width. The I_3/I_1 values of all solution were recorded.

1.4 Preparation specimen of SVs for TEM, SEM and AFM

In order to display the most closed original morphology and size of **SV**s, transmission electron microscopy (TEM) specimens were prepared by freeze-dried method according to the literature procedure.[1] Firstly, positing a drop of diluted solution onto a carbon-coated copper grid. The excess solution on the copper grid was quickly absorbed by filter paper after deposition. To prepare a freeze-dried specimen, the copper grid was immediately freeze-dried by the liquid nitrogen bath. Then, the obtained frozen specimen was immediately transferred into a freeze dryer and dried. Other conventional specimens were allowed to dry under ambient conditions.

1.5 *In vitro* drug release from SVs

The in vitro release of CPT and CDDP from the **SV**s was studied as a function of time. 1mg **SM**s were dispersed in 10 mL of ten different PBS buffer solution: (1) pH 7.4; (2) pH 7.4 + esterase (30 U/mL); (3) pH 7.4 + GSH (1 mg/ml); (4) pH 7.4 + spermin (0.1M) ; (5) pH 7.4 + esterase (30 U/mL) + GSH (1 mg/ml); (6) pH 5.0; (7) pH 5.0 + esterase (30 U/mL); (8) pH 5.0 + GSH (1 mg/ml); (9) pH 7.4 + spermin (0.1M) ; (10) pH 5.0 + esterase (30 U/mL) + GSH (1 mg/ml) at 37 °C and stirred at 100 rpm with a membrance tubing (MWCO = 300) . At determined time intervals, 3ml of solution was collected with 0.5ml DMSO to completely dissolve the drugs and replaced by fresh PBS (3 mL). The amount of CPT in the solution was determined by UV/Vis spectrometry at λ = 370 nm. The amount of CDDP in the solution was determined by ICP-AES. The experiments were all repeated in triplicate.

1.6 *In vitro* cell experiment

1.6.1 Cellular uptake of SVs by HepG2 cells

Live cell confocal microscopy was first introduced to assess the cellular uptake and intracellular fate of the **SV**s with cell densities of 5×10^4 cells/mL in dishes (35 mm confocal imaging dish, ibidi, Cat. No. 81156) and incubated for 24 hours before introducing **SV**s, after that the original **SV**s -free medium were discarded and the fresh prepared **SV**s-containing (50 μg/mL) medium were added. All of the **SV**s and free CPT were dispersed in the medium by slightly ultrasonication right before their introduction to the cells. After incubation for another 24 hrs, stained the cells with Lyso Tracker Green DND-26 for 40 min at 37 °C. The live cell samples were observed and imaged by Nikon A1R fluorescence microscopy.

Flow cytometry was also introduced to evaluate the intracellular uptake efficiency of SVs. HepG2 cells were seeded in 6-well plates with cell densities of 2×10^5 cells/mL and cultured for 24 h. Then discard the medium and replaced with **SV**s mediums at a final concentration of 30μM. Incubated the cells with the SVs solutions for specific time durations, 4, 12 and 24h, following by replacing the medium with PBS and washed for 3 times. Collected the cells with typsin and performed the flow cytometry assay. Data for 1.0×10^4 gated events were collected and analysis by Beckman FC500 flow cytometer.

1.6.2 *In vitro* cytotoxicity studies of SVs

CellTiter-Glo Luminescence Assay (Promega-G7573) was used to determine *in vitro* cytotoxicity for each cell line after treatment with compounds. The HepG2 cells, HeLa cells and A549 cells were used to evaluate the anticancer activity of **SV**s. The free drug CPT and CDDP and the mixture of CPT and CDDP were used as control. The cells were seeded into 96-well plates at 2×10^3 cells per well in 100 μL of culture medium. After 24 h incubation, the medium was removed and replaced with 100 μL of a medium containing serial dilutions of **SV**s, free CPT and CDDP, or the CPT/CDDP mixture from 0.12 to 24 μM. The cells without the treatment were used as control. The cells were grown for another 72h at 37 °C. After equilibration at room temperature for approximately 10 minutes, 20 μL of CellTiter-Glo reagent was added to each well and luminescence was measured using a MD SpectraMax Paradigm reader.

1.6.3 Cell apoptosis and cell cycle assay

HepG-2 cells were seeded in 6-well plates at 6.0 × 10^5 cells per well in 2 mL of complete RPMI-1640 and cultured for 24h. The cells were treated with free CPT, CDDP, CPT/CDDP mixture and **SV**s at the same concentration (24 μM) for 24 h. HepG2 cells without the treatment were used as a control. For quantitative measurement of apoptosis, treated cells were harvested and washed twice with 4°C PBS, stained with FITC annexin V and PI according to the manufacturer's instructions. For cell cycle determination, treated cells were collected, washed for twice with 4°C PBS, fixed with 70% ethanol at 4 °C overnight, followed by PI/RNase staining for 15 min in the dark. Both cell apoptosis and cycle were analyzed by flow cytometry beckman FC500, and 1 × 10^6 cells per sample were counted.

1.7 *In vivo* anticancer experiment

1.7.1 Pharmacokinetics and biodistribution

For pharmacokinetic studies, SD rats (~200 g) were randomly divided into free CPT, CDDP and **SV**s groups (n = 3). The aqueous solutions of free CPT, CDDP and **SV**s were intravenously injected via tail vein at a dose of 3μmol/ Kg. The blood samples (0.5 mL) were taken from the eye socket at the 0.5, 1 h, 4 h, 8 h, and 12 h time points after injection. The plasma was obtained by centrifugation at 3,000 rpm for 10 min and stored at -20 °C. We treated 500 μL of plasma two times with 1mL of ethyl acetate. The solvent solutions separated by centrifugation were pooled. The samples of free CPT and **SV**s were directly examined by using fluorescence spectroscopy, and the samples of free CDDP were measured by use of ICP-AES. The amounts of free CPT, CDDP and **SV**s were obtained from standard curves previously obtained by analysis of blood samples containing known amounts of free CPT, CDDP and **SV**s.

To assess the tissue distribution of free CPT, CDDP and **SV**s, the HepG2 tumor-bearing mice were intravenously injected via tail vein with free CPT, CDDP and **SV**s at a dose of 3μmol/ Kg. Mice were sacrificed by cervical vertebra dislocation at1 h and 8 h after drug administration (n = 3 at each time point), and the heart, liver, spleen, lung, kidney and tumor were collected. Tissue samples were rinsed in saline, blotted using paper towel, weighed and immediately being homogenized. Free CPT and **SV**s were extracted from the homogenate using 2 mL of ethyl acetate. Free CDDP were extracted from the homogenate using 2 mL of ethyl acetate and methanol (v/v:

2:1). The solvent solutions separated by centrifugation were pooled. The samples of free CPT and SVs were directly examined by using fluorescence spectroscopy, and the samples of free CDDP were measured by use of ICP-AES. The amounts of free CPT, CDDP and **SV**s were obtained from standard curves previously obtained by analysis of blood samples containing known amounts of free CPT, CDDP and **SV**s.

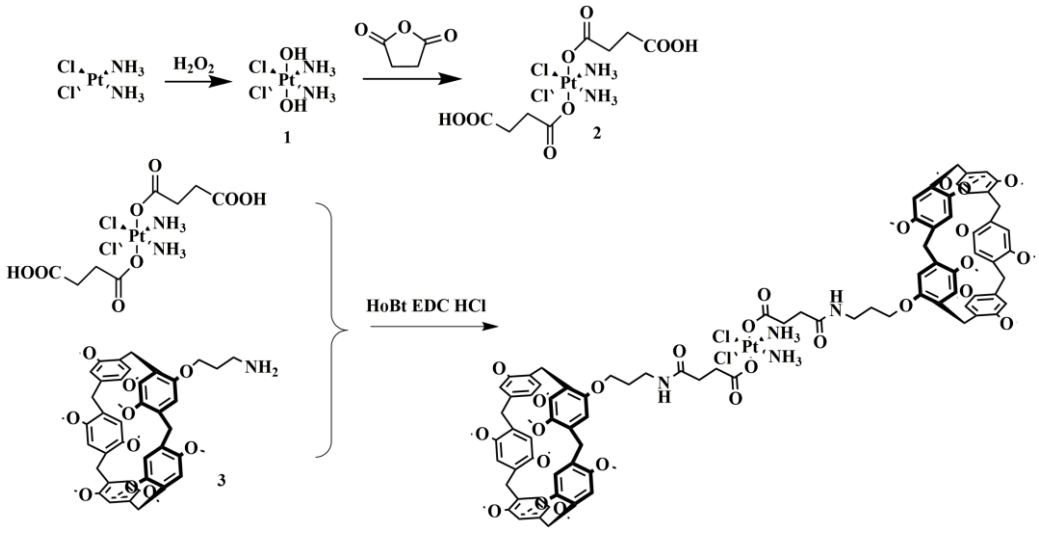
1.7.2 *In vivo* optical imaging

HepG2 tumor-bearing mice were randomly separated into two groups and injected intravenously via tail vein with 200 μL of Cy5-loaded SVs and free Cy5 possessed similar absorption intensity of Cy5. The fluorescence distribution was monitored at 12, 24, 48 and 72 h using an *in vivo* imaging system with appropriate wavelength (λ_{ex} = 649 nm, λ_{em} = 670 nm) which detected by IVIS Lumina 2.

1.7.3 *In vivo* anticancer activity

The HepG2 tumor-bearing nude were randomly divided into five groups, and mice in different treatment groups were intravenously injected via the tail vein with PBS, free CPT (3μmol/ Kg), CDDP (3μmol/ Kg), CPT/CDDP mixture (3μmol/ Kg, The molar ratio of CPT and CDDP was 2:1.) and **SV**s (3μmol/ Kg) once every 3 days for 21 days. Each mouse of different group was earmarked and followed individually throughout the whole experiments. The length and width of the tumor and the body weight of mice were measured before every injection by the end of experiment. Tumor volume(V) was calculated using the formula: V (mm^3) = 1/2 × length (mm) × width $(mm)^2$.After 21 days post-injection, mice were sacrificed, and tumors were separated, weighted and photographed. In addition, the tumors were cut into small pieces, fixed in 10% formalin and embedded in paraffin. Then the tissues embedded in paraffin were sectioned for histopathological analysis with H&E staining.

2 Synthesis of bis(pillar[5]arene)-amine-cisplatin (CDDP(P5)$_2$)



Scheme S1. Synthetic routes of CDDP(P5)$_2$.

2.1 Synthesis of c,c,t-Pt(NH$_3$)$_2$Cl$_2$(OH)$_2$ (1)

Compound1 was prepared as stated previously, with slight modifications.[2] In this method, cisplatin (2.00 g, 6.67 mmol) was dissolved in hydrogen peroxide (H_2O_2; 30 wt%, 11.40 mL, 100.0 mmol) and the reaction mixture was heated to 70℃ with vigorous magnetic stirring for 5 h in a dark chamber. The mixture was then cooled and stirring was continued overnight. The reaction mixture was concentrated, and the product was washed 3 times with ice cold H_2O, ethanol and diethyl ether, and vacuum dried. The product was obtained as a bright yellow powder and the yield was about 1.91 g (85.8 %).

2.2 Synthesis of c,c,t-Pt(NH$_3$)$_2$Cl$_2$(OOCCH$_2$CH$_2$COOH)$_2$ (2).

A mixture of c,c,t-Pt(NH$_3$)$_2$Cl$_2$(OH)$_2$ (1.00 g, 3.00 mmol) and succinic anhydride (1.50 g, 15.0 mmol) in DMF (30 mL) was heated at 70 ℃ in the dark for 24 h with magnetic stirring. During this time the solid material dissolved to form a yellow-brown solution. DMF was then removed under reduced pressure, and the product was purified and filtered to a pale-yellow solid. The product was dried in vacuo.

^{1}H NMR (400 MHz, DMSO-d_6) δ = 11.94, 6.50, 2.36.^{13}C NMR (100 MHz, DMSO-d_6) δ =180.11, 174.23, 30.90, 30.29. HRMS (ESITOF) ($C_8H_{16}Cl_2N_2O_8Pt$) m/z: calcd. for $[M+Na]^+$ 556.9806; found 556.9836, error 5.3 ppm.

2.3 Synthesis of Pillar[5]arene-NH_2 (3)

Compound 3 was synthesized according to the literature procedure. [3]

^{1}H NMR (400 MHz, DMSO-d_6) δ = 6.82-6.75 (m, 10H), 3.89 (t, J=6.0, 2H), 3.66 (s, 43H), 2.7-2.71 (m, 2H), 1.80-1.77 (m, 2H).^{13}C NMR (100 MHz, $CDCl_3$) δ = 151.02, 150.98, 150.94, 150.90, 150.84, 150.17, 128.67, 128.61, 128.48, 128.37, 115.12, 114.37, 114.22, 114.17, 113.99, 66.33, 56.09, 56.03, 55.94, 55.90, 38.59, 33.55, 29.96, 29.90, 29.82, 29.63. HRMS (ESI-TOF) ($C_{47}H_{55}NO_{10}$) m/z: calcd. for $[M+H]^+$ 794.3898; found 794.3899, error 0.1ppm.

2.4 Synthesis of bis(pillar[5]arene)-amine-cisplatin (CDDP(P5)$_2$)

Compound 2 (1.34 g, 2.50 mmol), EDC·HCl (0.60 g, 3.00 mmol) and HOBt (0.40 g, 3.00 mmol) were dissolved in anhydrous DMF (30 mL) and stirred for 1 h at 0 °C. Then, compound 3 (2.40 g, 3.00 mmol) was added and the reaction mixture was stirred for 12 h at room temperature. The solvent was reduced via distillation and the product was dissolved by CH_2Cl_2. The reaction mixture was poured into saturated brine (100 mL) and the resulting solution was extracted with dichloromethane (30 mL×3). The combined organic phase was concentrated and purified by flash column chromatography (CH_2Cl_2 /CH_3OH, 10:1 v/v) to afford compound CDDP(P5)$_2$ as a yellow solid (2.15 g, 43%).

^{1}H NMR (400 MHz, $CDCl_3$) δ = 6.71 (m, 20H), 6.14 (s,5H), 3.86-3.82 (m, 4H), 3.78-3.53 (m, 74H), 3.39 (d, J=5.1, 4H), 2.53 (s, 4H), 2.45 (s, 4H), 1.90-1.87 (m, 4H).^{13}C NMR (100 MHz, $CDCl_3$) δ = 182.58, 173.20, 151.13-150.85, 149.79, 128.67-128.18, 114.97, 114.57-114.06, 66.68, 56.21-55.84, 37.73, 32.29, 30.07, 29.92-29.20. HRMS (ESITOF) ($C_{102}H_{122}Cl_2N_4O_{26}Pt$) m/z: calcd. for $[M+Na]^+$ 2107.7276; found 2107.7325, error 2.3 ppm.

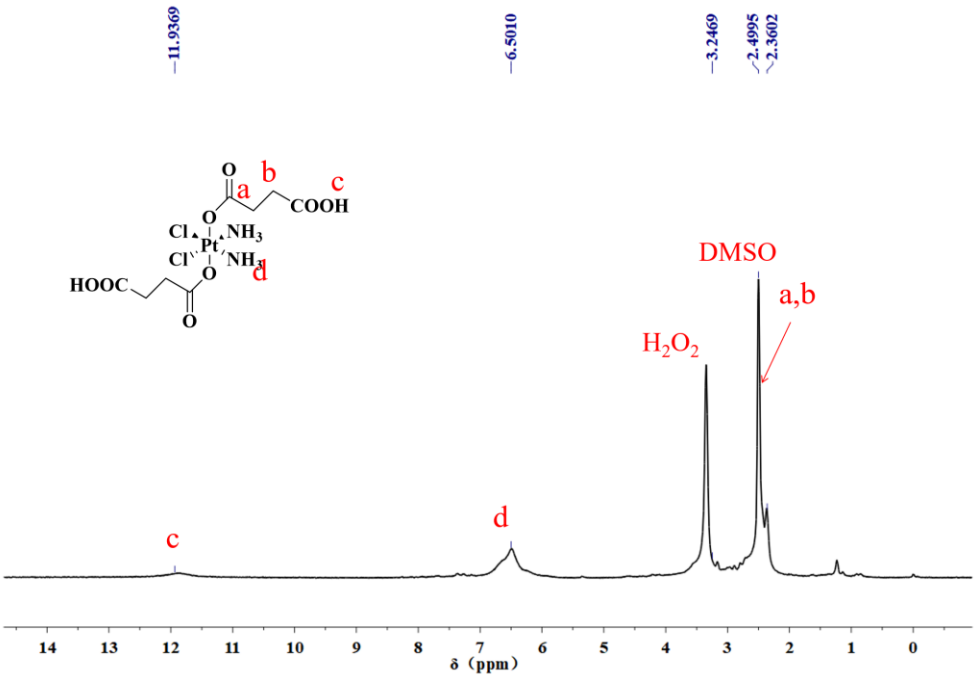


Figure S1. ^{1}H NMR spectrum recorded (400 MHz, $CDCl_3$, RT) for c,c,t-$Pt(NH_3)_2Cl_2(OOCCH_2CH_2COOH)_2$.

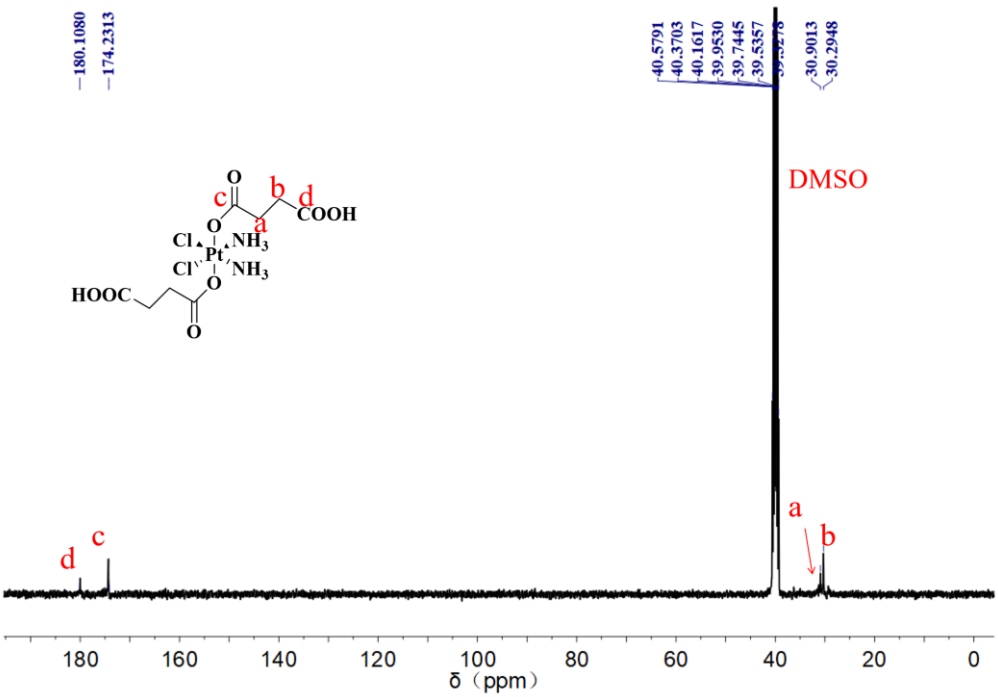


Figure S2. ^{13}C NMR spectrum recorded (400 MHz, $CDCl_3$, RT) for c,c,t-$Pt(NH_3)_2Cl_2(OOCCH_2CH_2COOH)_2$.

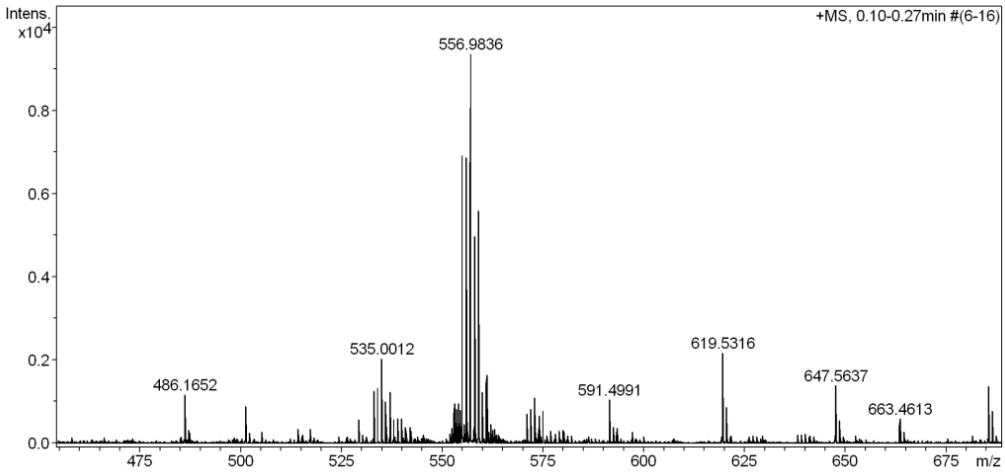


Figure S3. High-resolution electrospray ionization mass spectrum of c,c,t-$Pt(NH_3)_2Cl_2(OOCCH_2CH_2COOH)_2$.

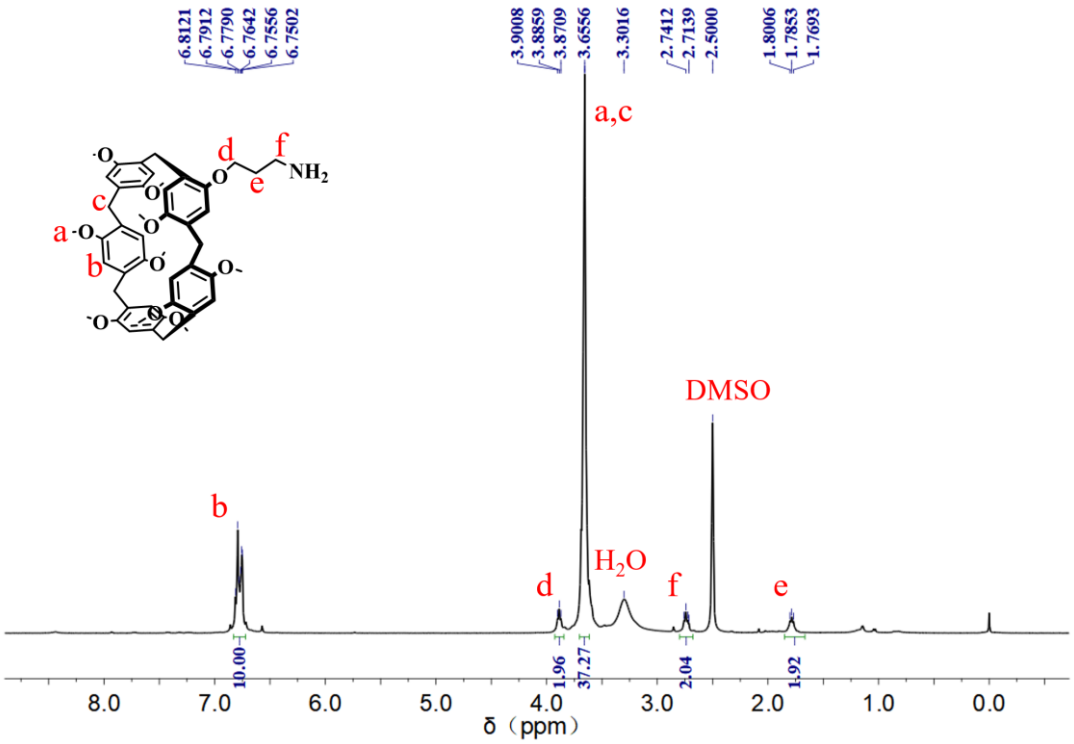


Figure S4. ^{1}H NMR spectrum recorded (400 MHz, $CDCl_3$, RT) for pillar[5]arene-NH_2.

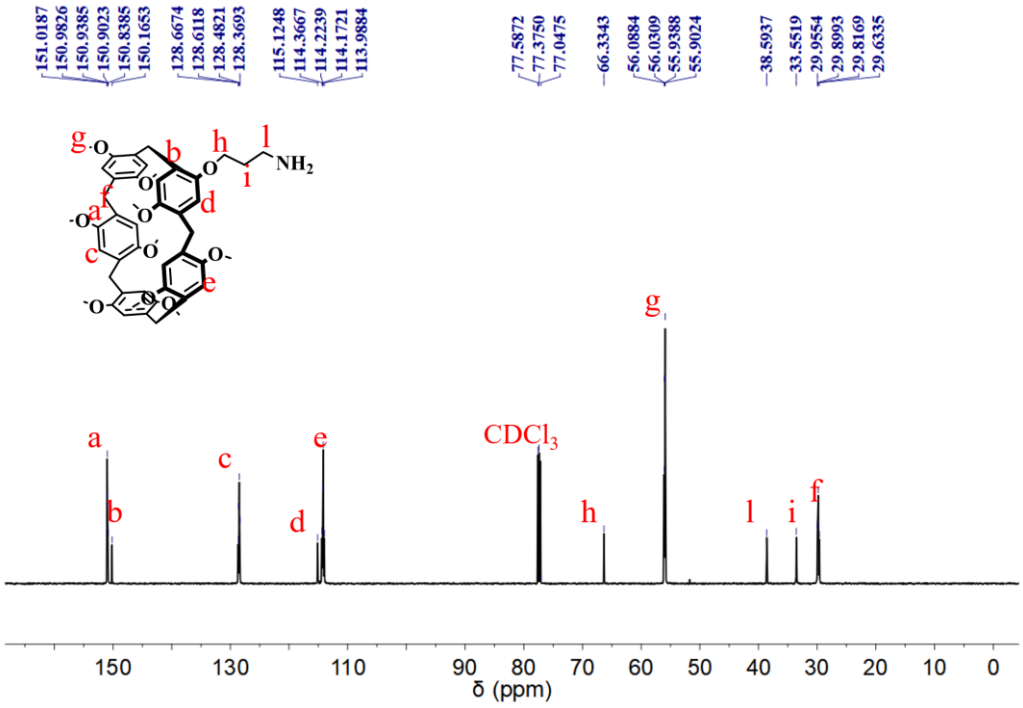


Figure S5. ^{13}C NMR spectrum recorded (400 MHz, $CDCl_3$, RT) for pillar[5]arene-NH_2.

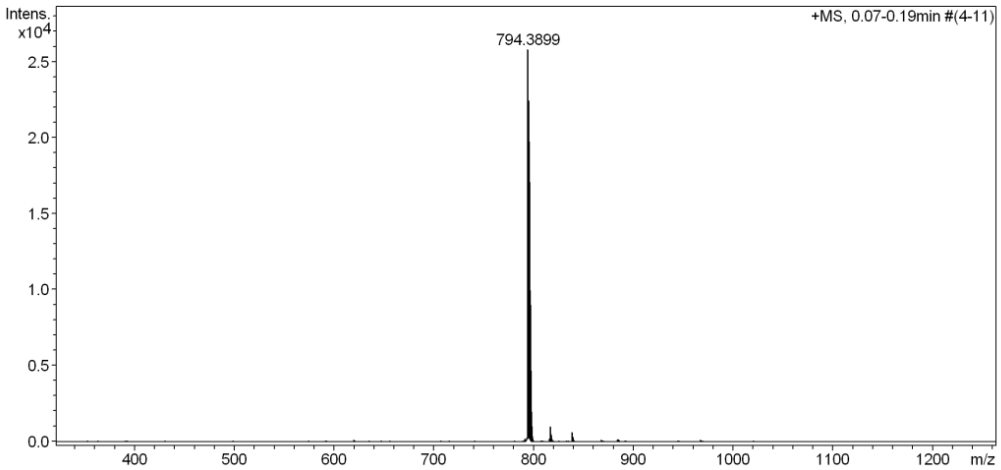


Figure S6. High-resolution electrospray ionization mass spectrum of pillar[5]arene-NH_2.

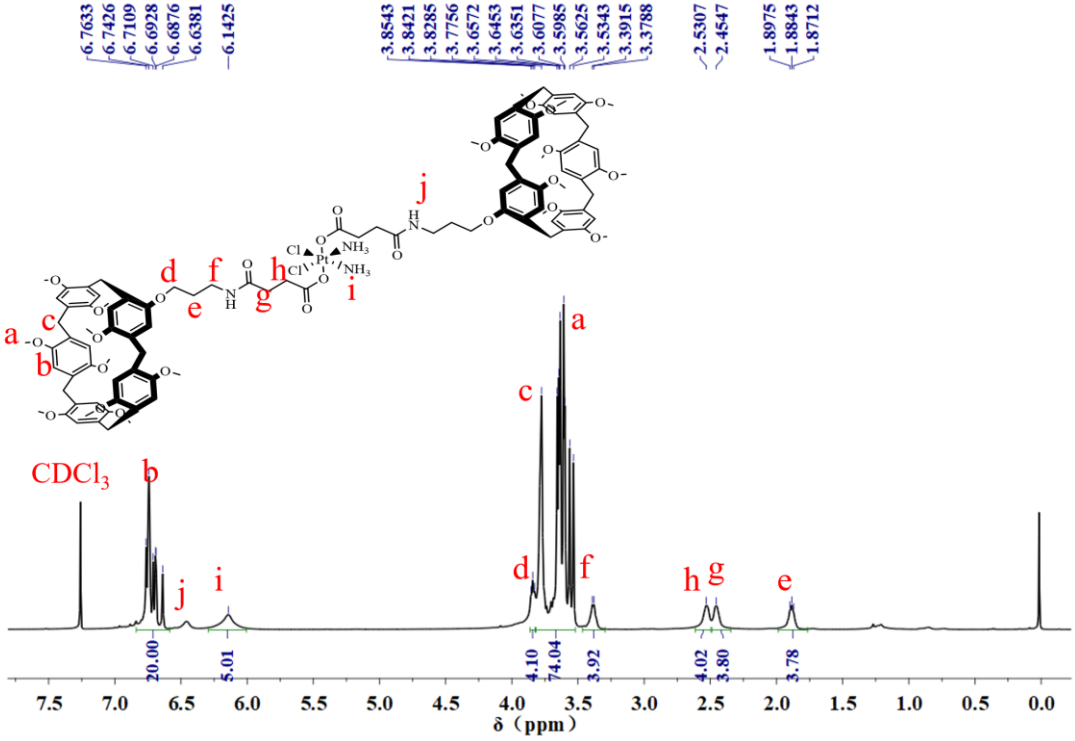


Figure S7. ^{1}H NMR spectrum recorded (400 MHz, $CDCl_3$, RT) for CDDP(P5)$_2$.

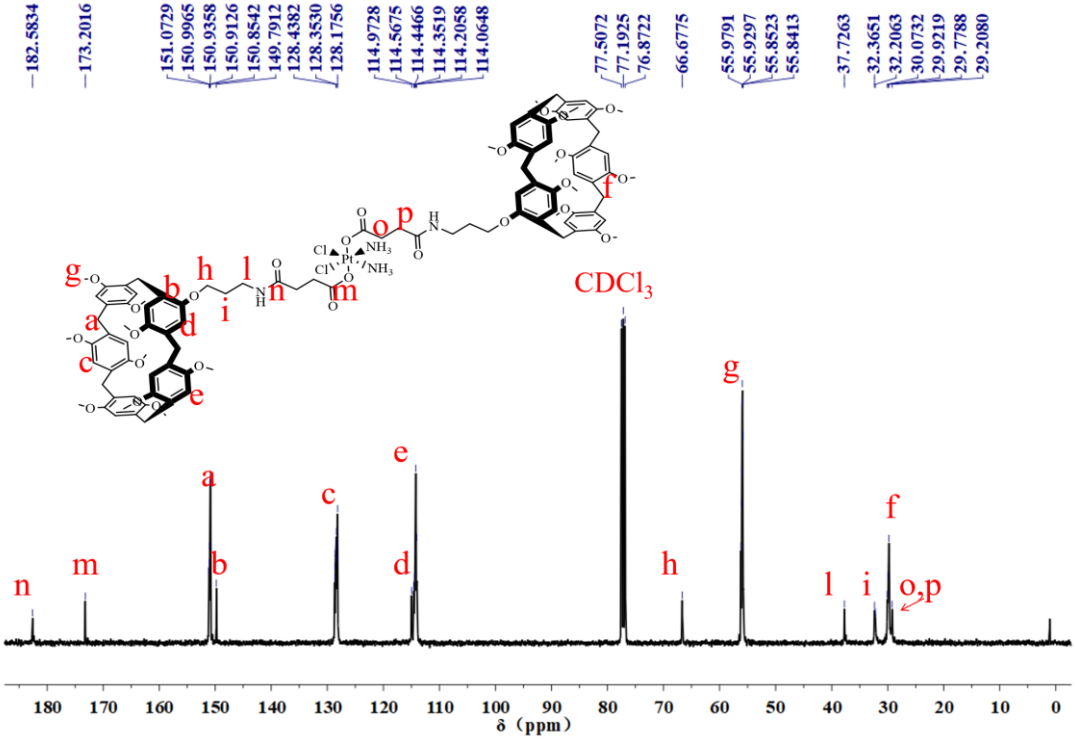


Figure S8. ^{13}C NMR spectrum recorded (400 MHz, $CDCl_3$, RT) for CDDP(P5)$_2$.

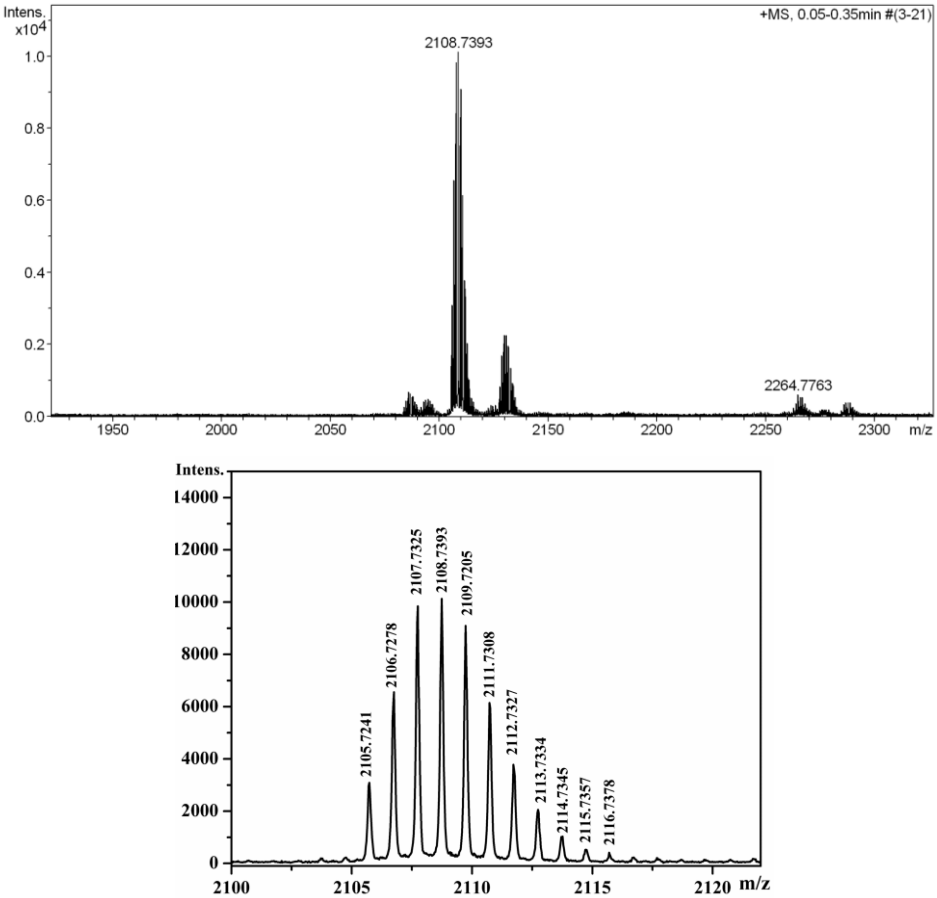


Figure S9. High-resolution electrospray ionization mass spectrum of CDDP(P5)$_2$.

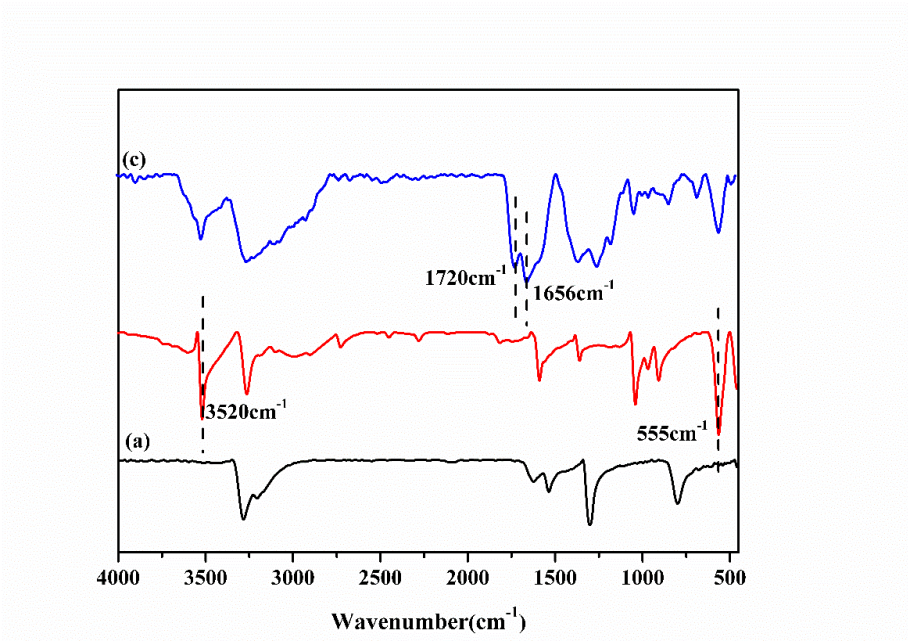
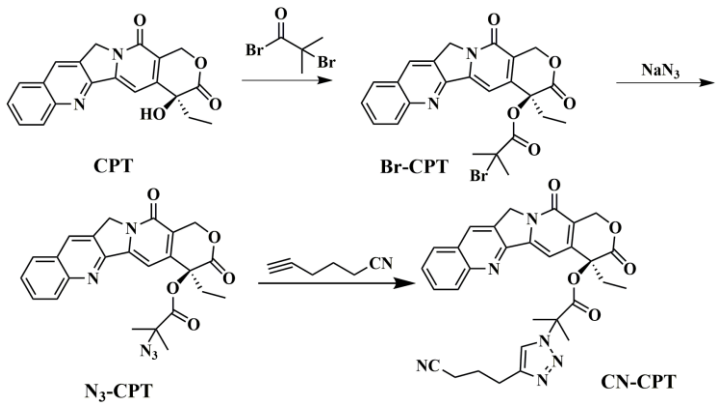


Figure S10. FT-IR spectra of (a) cisplatin (b) c,c,t-[$Pt(NH_3)_2Cl_2(OH)_2$]) and (c) c,c,t-[$Pt(NH_3)_2Cl_2(OOCCH_2CH_2COOH)_2$].

c,c,t-[$Pt(NH_3)_2Cl_2(OH)_2$] displays a sharp and intense peak at 3520 cm^{-1}(OH stretching) and a new $Pt(OH)_2$ stretch at 555 cm^{-1}, respectively, compared with the cisplatin peak. After reacting with succinic anhydride, the 3461cm^{-1} band is weakened, and two peaks appear (1720 cm^{-1} and 1656 cm^{-1}) that are characteristic of the coordinated carboxyl group (1656 cm^{-1}) and free carboxyl group. Comparison of the three FTIR spectra indicates that c,c,t-[$Pt(NH_3)_2Cl_2(OH)_2$ has reacted with succinic anhydride to afford c,c,t-[$Pt(NH_3)_2Cl_2(OOCCH_2CH_2COOH)_2$.[4]

3 Synthesis of cyano-methylpropionyl-camptothecin (CN-CPT)



Scheme S2. Synthetic routes of CN−CPT.

3.1 Synthesis of bromo-methylpropionyl-Camptothecin (Br-CPT)

The CPT was acylated with 2-bromopropionyl bromide in the presence of DIPEA and DMAP in CH_2Cl_2. CPT (1.00 g, 2.87 mmol) was added to dry CH_2Cl_2 (40 mL) before excess DIPEA (1.48 g, 11.48 mmol) and DMAP (1.40 g, 11.48 mmol) was added. After cooling the solution in an ice bath, 2-bromoisobutyryl bromide (2.63 g, 11.48 mmol) was added dropwise to the mixture using a dropping funnel over a period of 1 h at 0 ℃, then at room temperature for 5 h. After that, the resulting viscous yellowish liquid was washed with 0.1 M $NaHCO_3$ to hydrolysis of unreacted 2-bromoisobutyryl bromide. Then, the solution was extracted three times with dichloromethane, and the supernatant liquid was dried with anhydrous sodium sulfate. The combined organic phase was concentrated and purified by flash column chromatography ($CH_3COOC_2H_5$ /PE, 1:1 v/v) to afford compound Br-CPT as a white solid (0.72 g, 50%).

^{1}H NMR (400 MHz, $CDCl_3$) δ = 8.39 (s, 1H), 8.24 (d, J=8.5, 1H), 7.93 (d, J=8.2, 1H), 7.82 (t, J=7.4, 1H), 7.66 (t, J=7.4, 1H), 7.47 (s, 1H), 5.71 (d, J=17.2, 1H), 5.41 (d, J=17.2, 1H), 5.28 (d, J=3.4, 2H), 2.38-2.15 (m, 2H), 2.10 (s, 3H), 1.94 (s, 3H), 1.04 (t, J=7.5, 3H).^{13}C NMR (100 MHz, $CDCl_3$) δ = 170.24 , 166.95, 157.27, 152.25, 148.87, 146.49, 145.53, 130.98, 130.48, 129.86, 128.35, 128.07, 119.81, 95.85, 67.19, 54.74, 49.85, 31.55, 30.46, 7.67. HRMS (ESI-TOF) ($C_{24}H_{21}BrN_2O_5$) m/z: calcd. for $[M+Na]^+$ 521.0509; found 521.0510, error 0.2 ppm.

3.2 Synthesis of azide-methylpropionyl-Camptothecin (N_3-CPT)

A mixture of compound Br-CPT (1.00 g, 2.00 mmol) and sodium azide (0.52 g, 8.00 mmol) in a solution of N,N-Dimethylformamide (50 mL) was stirred at 50℃ for 12h. After that, the solvent was reduced via distillation and the product was dissolved by CH_2Cl_2. The reaction mixture was poured into saturated brine (100 mL) and the resulting solution was extracted with dichloromethane (30 mL×3). The combined organic phase was concentrated and purified by flash column chromatography ($CH_3COOC_2H_5$ /PE, 1:1 v/v) to afford compound N_3-CPT as a white solid (0.41 g, 45%).

^{1}H NMR (400 MHz, $CDCl_3$) δ = 8.37 (s, 1H), 8.21 (d, J=8.5, 1H), 7.91 (d, J=8.1, 1H), 7.81 (t, J=7.3, 1H), 7.64 (t, J=7.5, 1H), 7.14 (s, 1H), 5.69 (d, J=17.2, 1H), 5.39 (d, J=17.2, 1H), 5.24 (d, J=3.5, 2H), 2.35-2.17 (m, 2H), 1.62 (s, 3H), 1.54 (s, 3H), 1.03 (t, J=7.5, 3H).^{13}C NMR (100 MHz, $CDCl_3$) δ = 171.68, 166.92, 157.29, 152.02, 148.79, 146.58, 145.46, 131.23, 130.68, 129.69, 128.40, 128.17, 128.10, 120.00, 95.36, 66.95, 62.87, 50.00, 31.58, 24.19, 7.69. HRMS (ESI-TOF) ($C_{24}H_{21}N_5O_5$)

m/z: calcd. for $[M+H]^+$ 460.1609; found 460.1615. calcd. for $[M+Na]^+$ 482.1433; found 482.1434, error 0.2 ppm.

3.3 Synthesis of cyano-methylpropionyl-Camptothecin (CN-CPT)

A mixture of N_3-CPT (2.30 g, 5.00 mmol) and 5-cyano-1-pentyne (0.93 g, 10.00 mmol) with PMDETA (5.20 g, 1.40 mmol) was dissolved in 100 mL anhydrous DMF. The mixture was degassed via three freeze–evacuate–thaw cycles, and CuBr (0.15 g, 1.00 mmol) was added. After 16 h of stirring under an N_2 atmosphere at 45 °C, the reaction was quickly quenched in an ice bath, opened and exposed to air. The solvent was reduced via distillation and the product was dissolved by CH_2Cl_2. The mixture was then passed through a neutral alumina to remove copper catalysts. The reaction mixture was poured into saturated brine (100 mL) and the resulting solution was extracted with dichloromethane (30 mL×3). The combined organic phase was concentrated and purified by flash column chromatography (($CH_3COOC_2H_5$ /PE, 2:1 v/v) to afford compound CN-CPT as a white solid (1.10 g, 40%).

^{1}H NMR (400 MHz, $CDCl_3$) δ = 8.39 (s, 1H), 8.27 (d, J=8.5, 1H), 7.92 (d, J=8.1, 1H), 7.84 (t, J=7.5, 1H), 7.67-7.62 (m, 2H), 7.12 (s, 1H), 5.68 (d, J=17.2, 1H), 5.37 (d, J=17.2, 1H), 5.24 (s, 2H), 2.90 (t, J=7.1, 2H), 2.42 (t, J=7.1, 2H), 2.08 (m, 10H), 0.90 (t, J=7.4, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ = 170.35, 166.75, 157.17, 152.00, 148.80, 146.74, 145.67, 145.29, 131.17, 130.61, 129.74, 128.35, 128.08, 120.50, 119.44, 95.20, 67.06, 64.27, 49.99, 31.41, 25.66, 25.37, 24.94, 24.32, 16.49, 7.55. HRMS (ESI-TOF) ($C_{30}H_{28}N_6O_5$) m/z:calcd. for $[M+Na]^+$ 575.2033; found 575.2013, error 3.6 ppm.

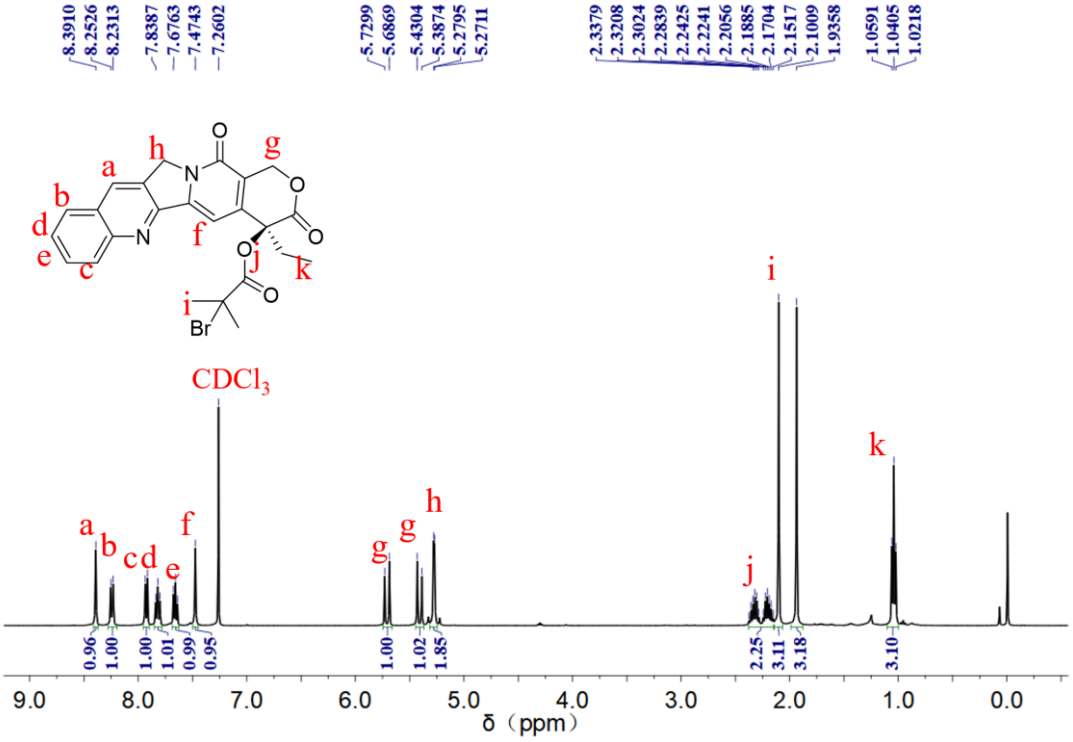


Figure S11. ^{1}H NMR spectrum recorded (400 MHz, $CDCl_3$, RT) for Br-CPT.

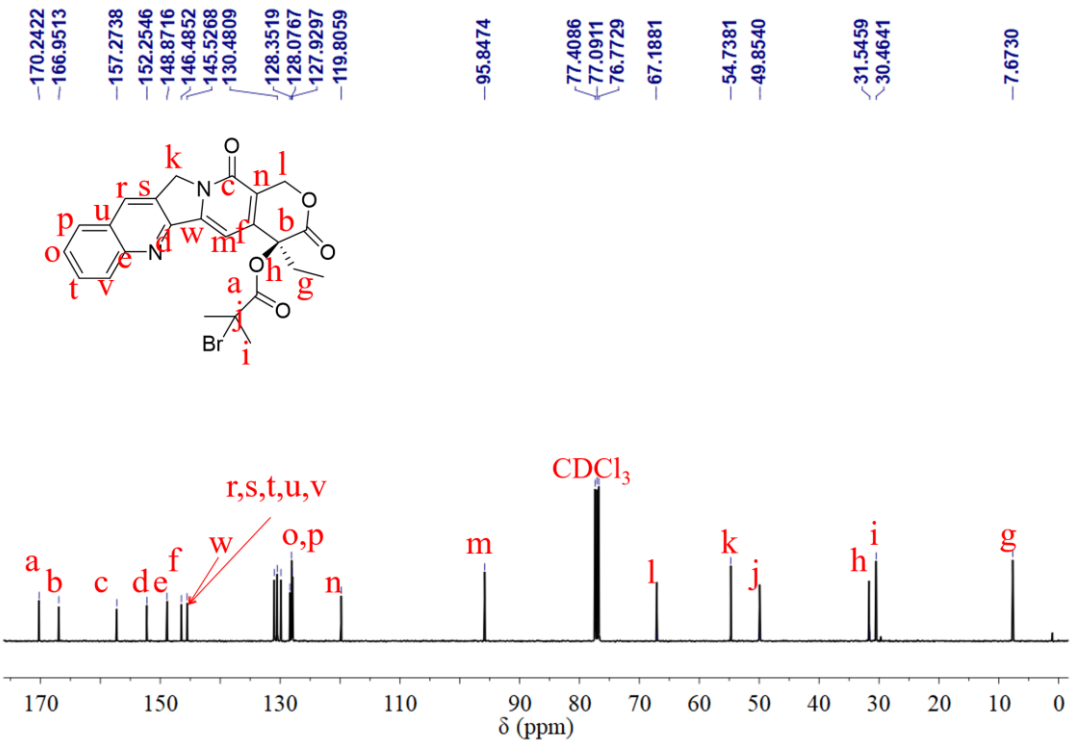


Figure S12. ^{13}C NMR spectrum recorded (100 MHz, $CDCl_3$, RT) for Br-CPT.

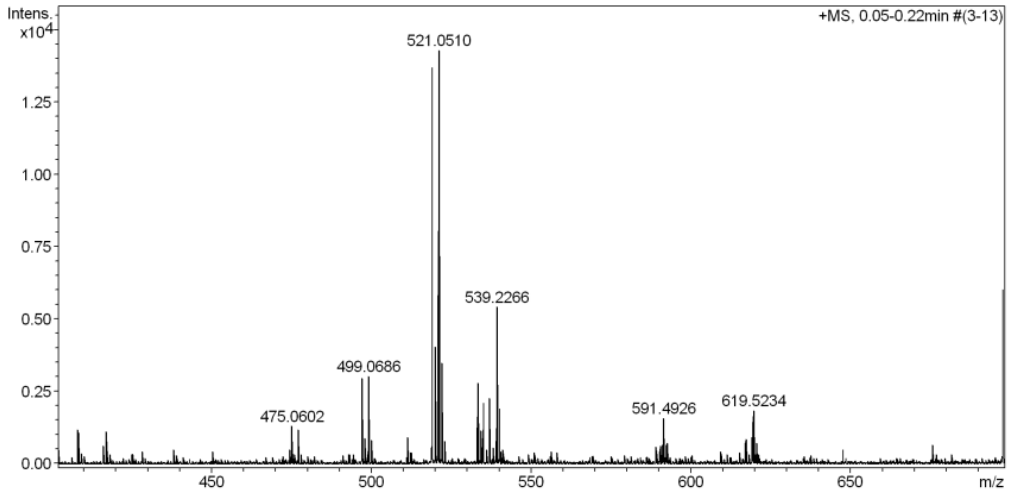


Figure S13. High-resolution electrospray ionization mass spectrum of Br-CPT.

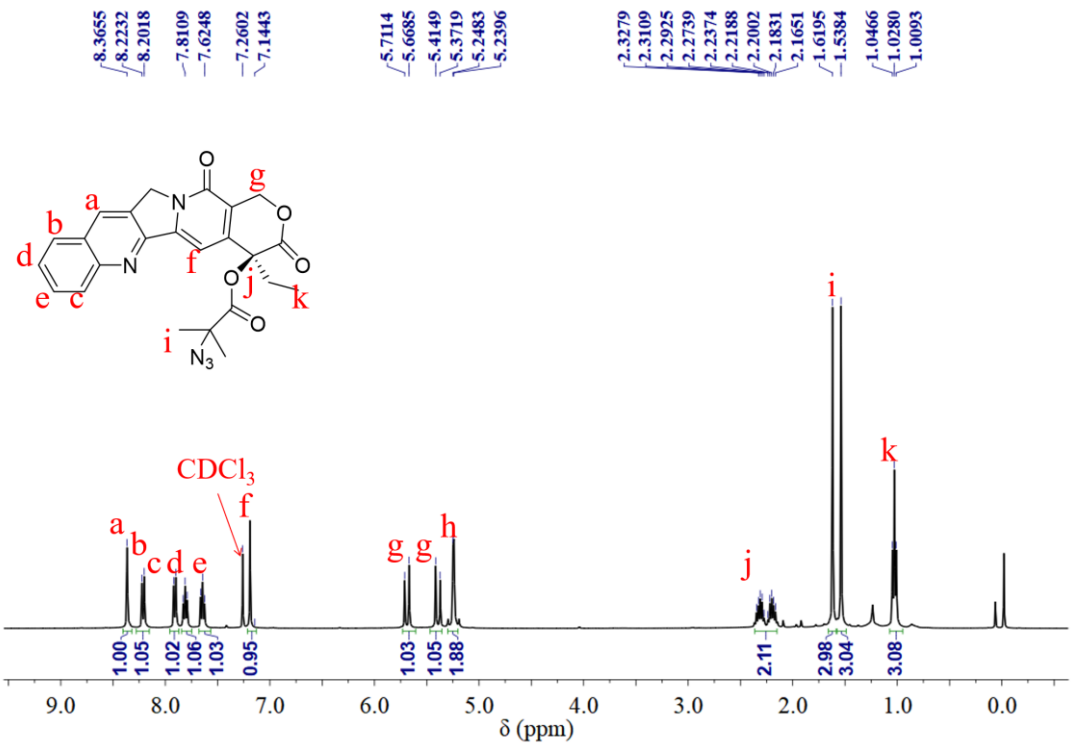


Figure S14. ^{1}H NMR spectrum recorded (400 MHz, $CDCl_3$, RT) for N_3-CPT.

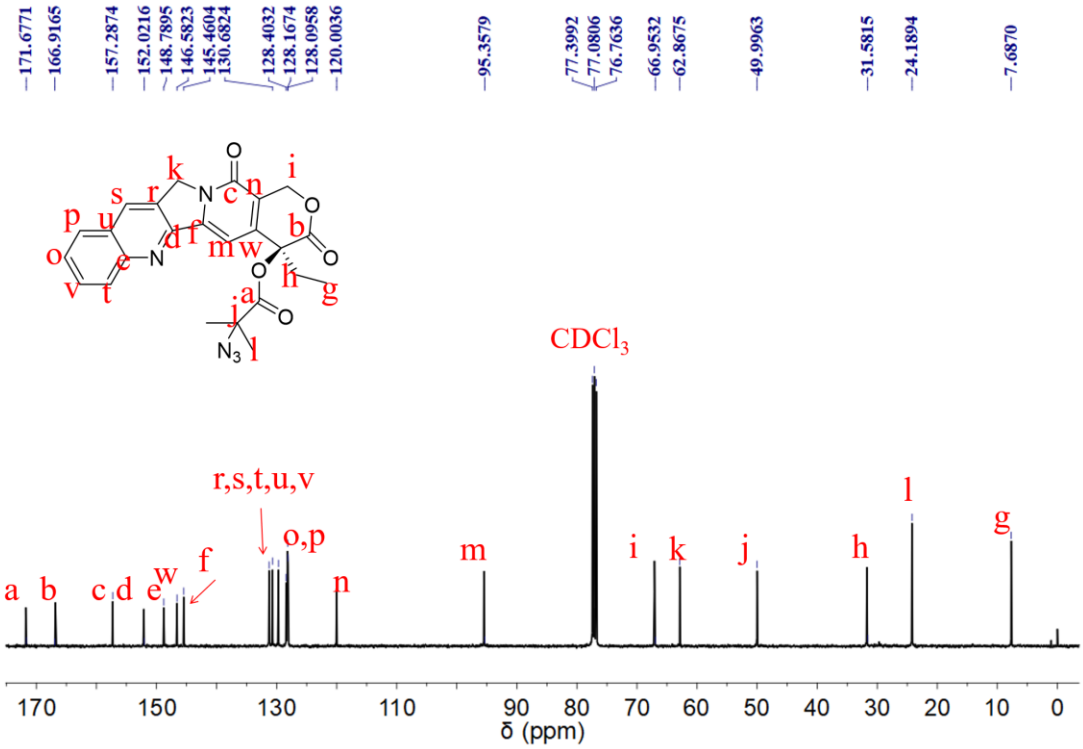


Figure S15. ^{13}C NMR spectrum recorded (100 MHz, $CDCl_3$, RT) for N_3-CPT.

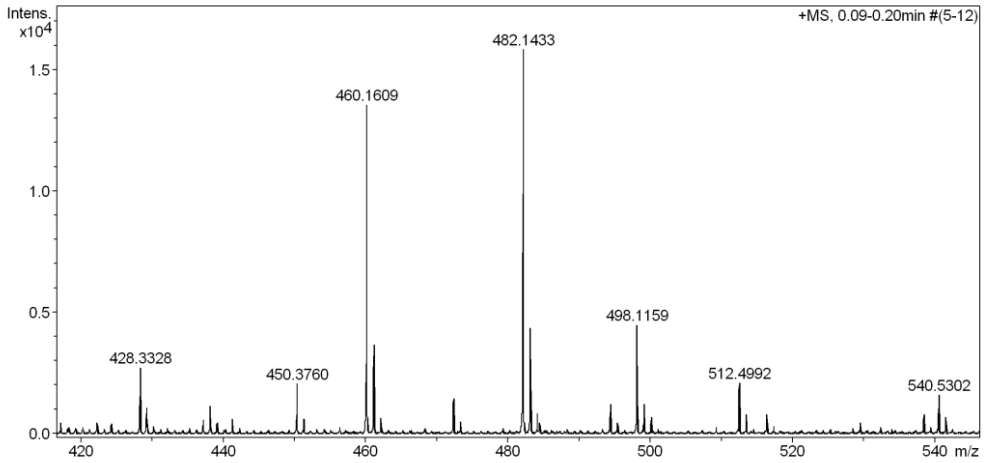


Figure S16. High-resolution electrospray ionization mass spectrum of N_3-CPT.

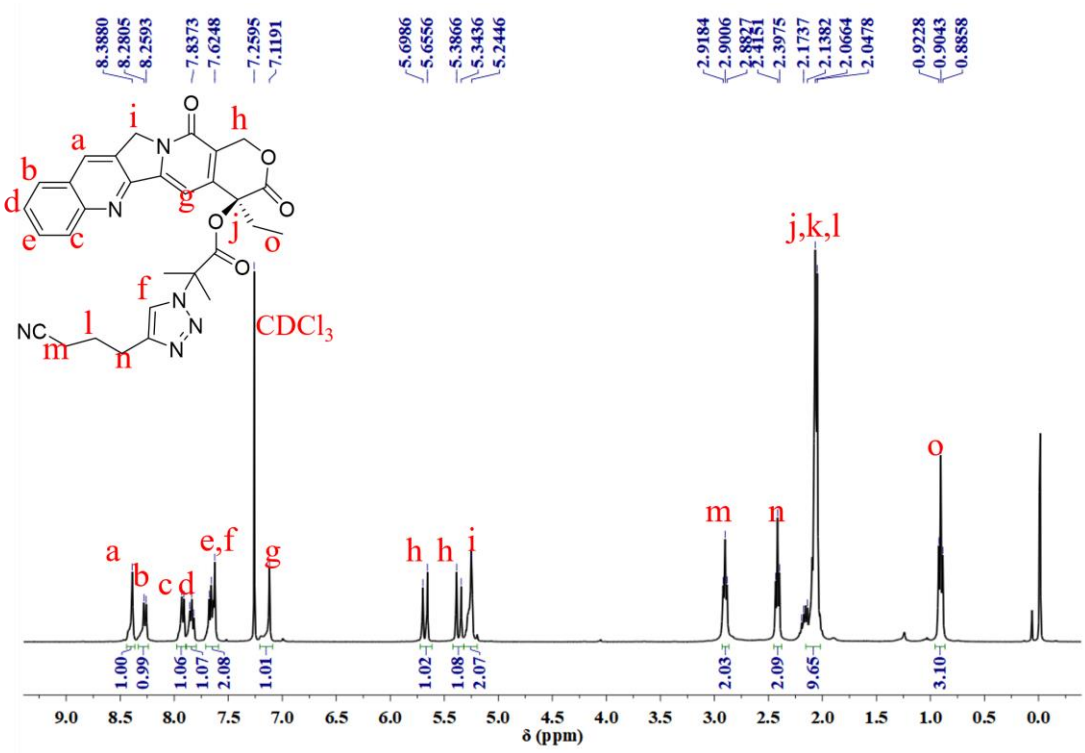


Figure S17. ^{1}H NMR spectrum recorded (400 MHz, $CDCl_3$, RT) for CN-CPT.

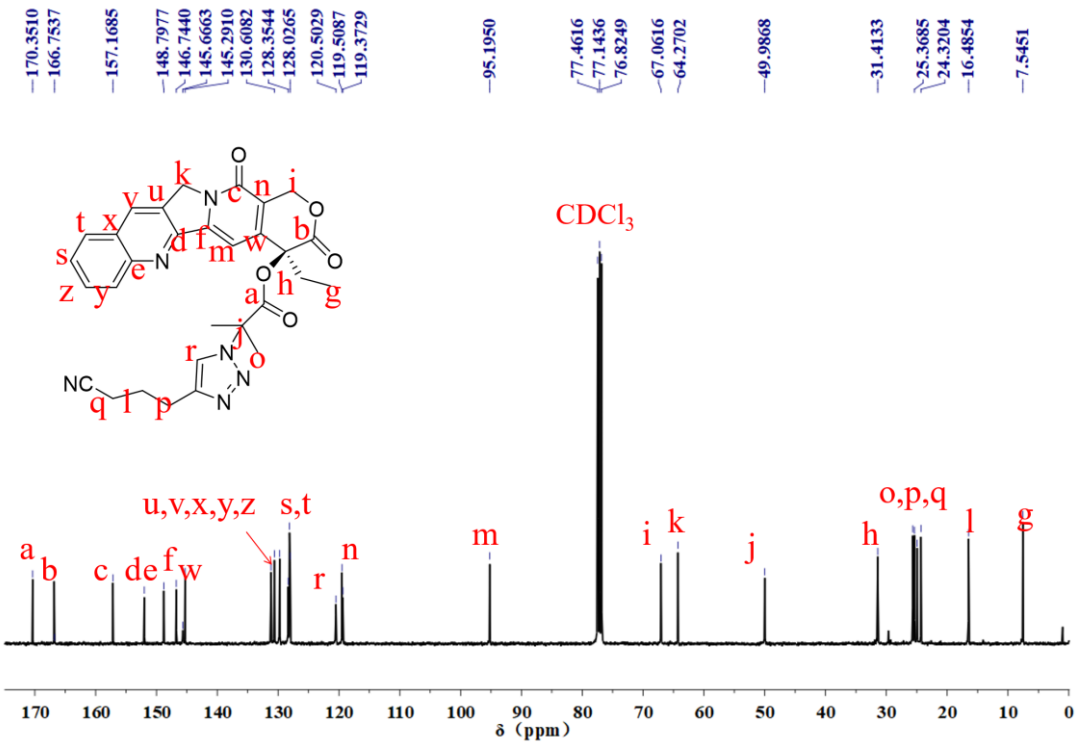


Figure S18. ^{13}C NMR spectrum recorded (400 MHz, $CDCl_3$, RT) for CN-CPT.

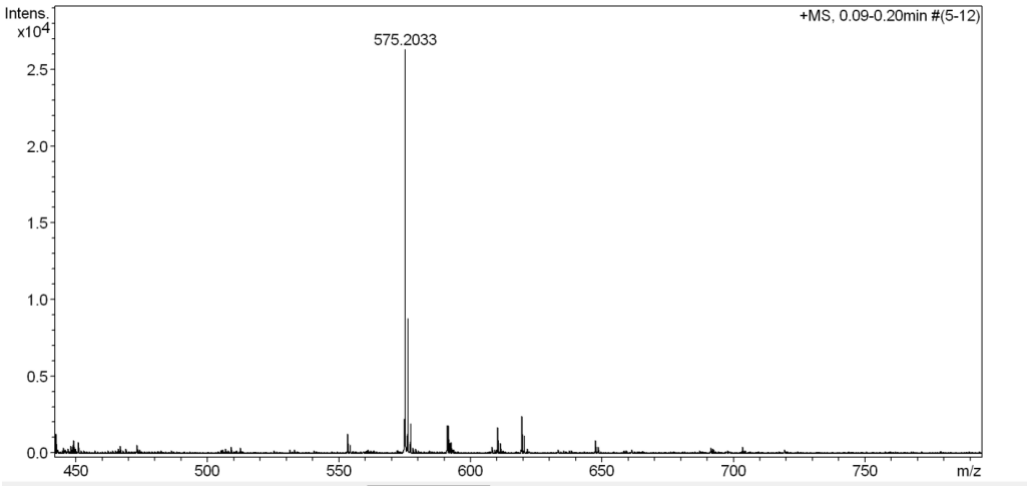


Figure S19. High-resolution electrospray ionization mass spectrum of CN-CPT.

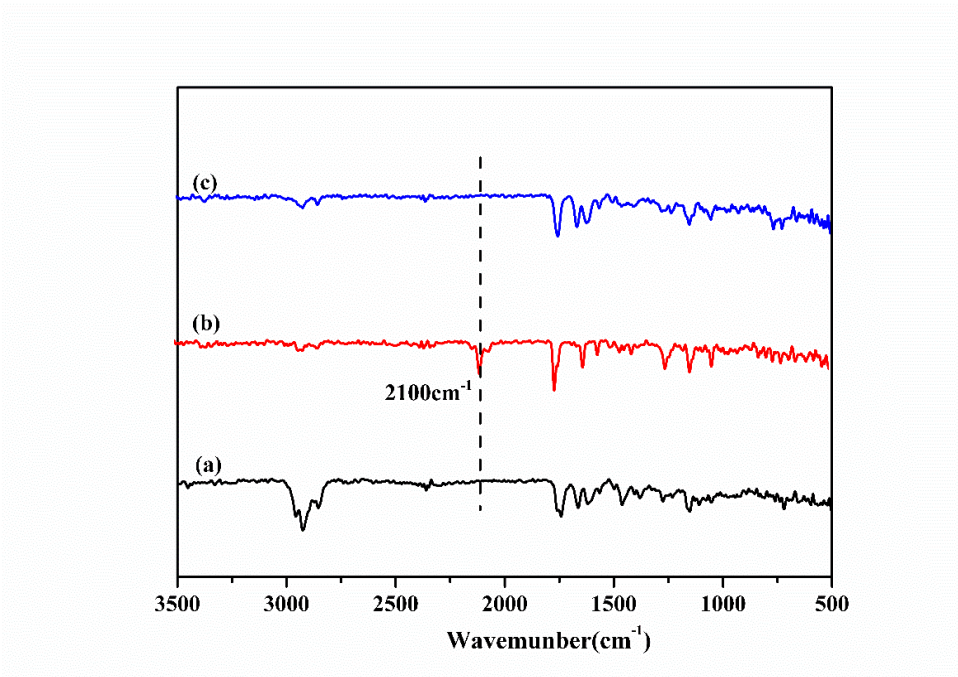


Figure S20. FT-IR spectra of (a) Br-CPT, (b) N_3-CPT, (c) CN-CPT

N_3-CPT, which is obtained by sodium azide with Br-CPT, displays a sharp and intense peak at 2100 cm^{-1}, compared with the Br-CPT peak. After reacting with 5-cyano-1-pentyne by click reaction, the 2100 cm^{-1} band is disappeared, which indicates that N_3-CPT has reacted with 5-cyano-1-pentyne to afford CN-CPT.

4. Host-guest interaction behavior between CDDP(P5)$_2$ and CN-CPT.

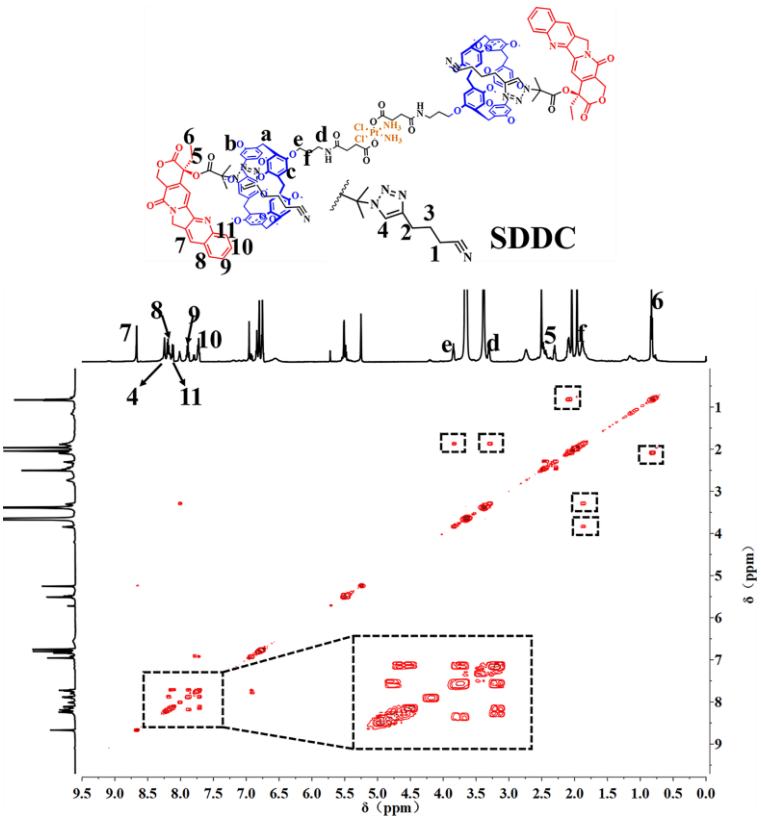


Figure S21. COSY ^{1}H NMR spectrum recorded (600 MHz, DMSO-d_6, 298 K) for CDDP(P5)$_2$ (12 mM) with 2 equiv of CN-CPT.

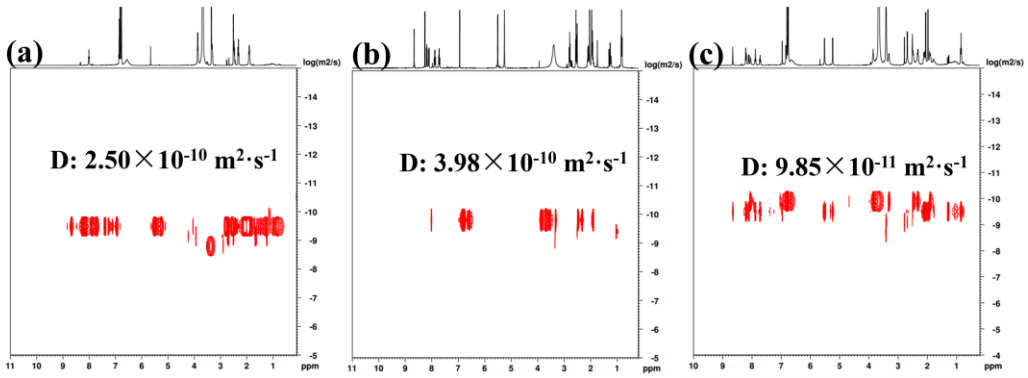


Figure S22. Representative DOSY spectra (600 MHz, DMSO-d_6, 298 K) of a solution of (a) CDDP(P5)$_2$ (25 mM), (b) CN-CPT (50 mM), (C) 1:2 molar ratio of CDDP(P5)$_2$ (25 mM) and CN-CPT.

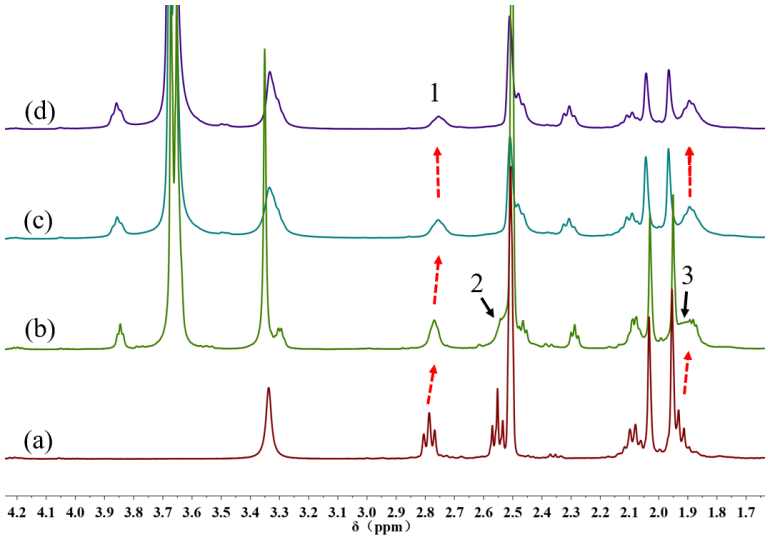


Figure S23. ^{1}H NMR spectra recorded (400 MHz, 298 K, DMSO-d_6) of individual CN-CPT (a); CN-CPT at a concentration of 15.00 mM in the presence of (b) 0.25, (c) 0.5, (d) 1.0 equiv of CDDP(P5)$_2$.

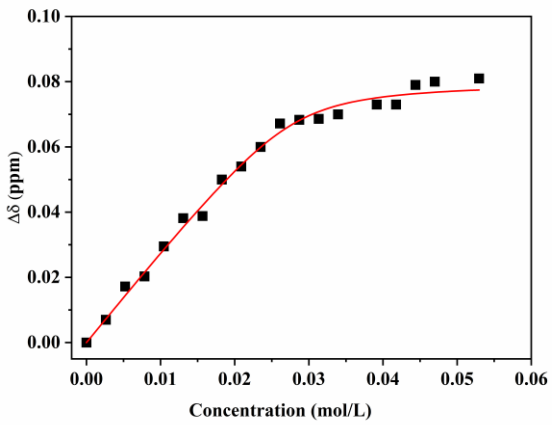


Figure S24. Non-linear fitting curve of the chemical shift changes of CN-CPT (H_1) versus the concentration of **P5** of CDDP(P5)$_2$. The association constant *K*a was calculated according to the following equation:[5]

$$\Delta\delta = (\Delta\delta_{max}/[G]_0)\ (0.5[H] + 0.5([G]_0+1/Ka) - (0.5([H]^2+(2[H](1/Ka-[G]_0)) + (1/Ka +[G]_0)^2)^{0.5}))$$

Where $\Delta\delta$ is the chemical shift change of H_1 on CDDP(P5)$_2$ upon titration, $\Delta\delta_{max}$ is the chemical shift change of H_1, $[G]_0$ is the fixed concentration of CN-CPT (0.028 mol/L), [H] is the concentration of added **P5** of CDDP(P5)$_2$. Based on the above equation, the association constant (*K*a) of SDDP was calculated to be 1.02(±1.0) × 10^3 M^{-1}.

5. Preparation and characterization of SDDC-based supramolecular vesicles.

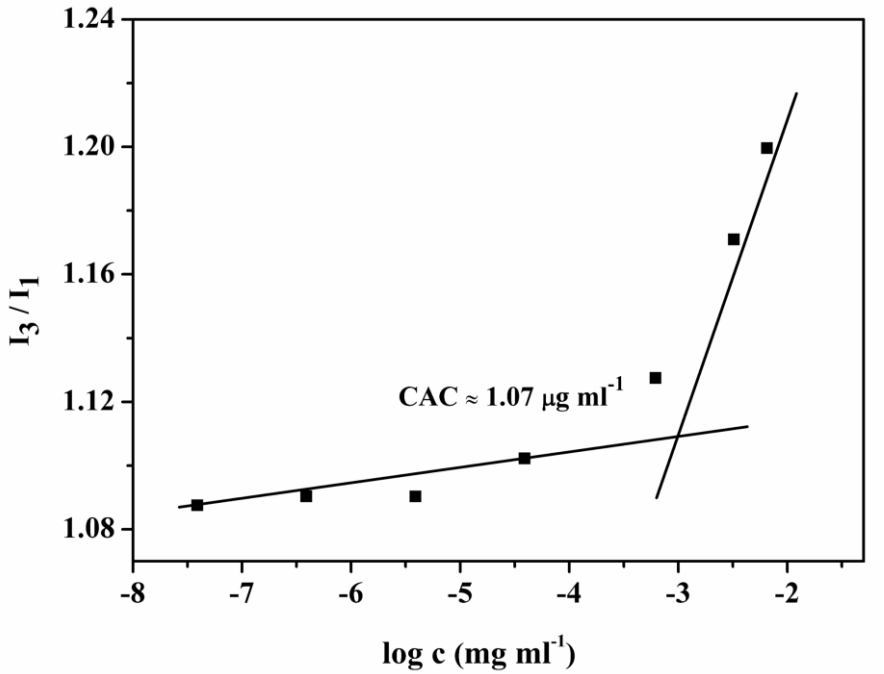


Figure S25. Relationship between the fluorescent intensity ratio (I_3/I_1) and **SDDC** aggregates concentration in water. The CAC value is about 1.07 μg mL^{-1}.

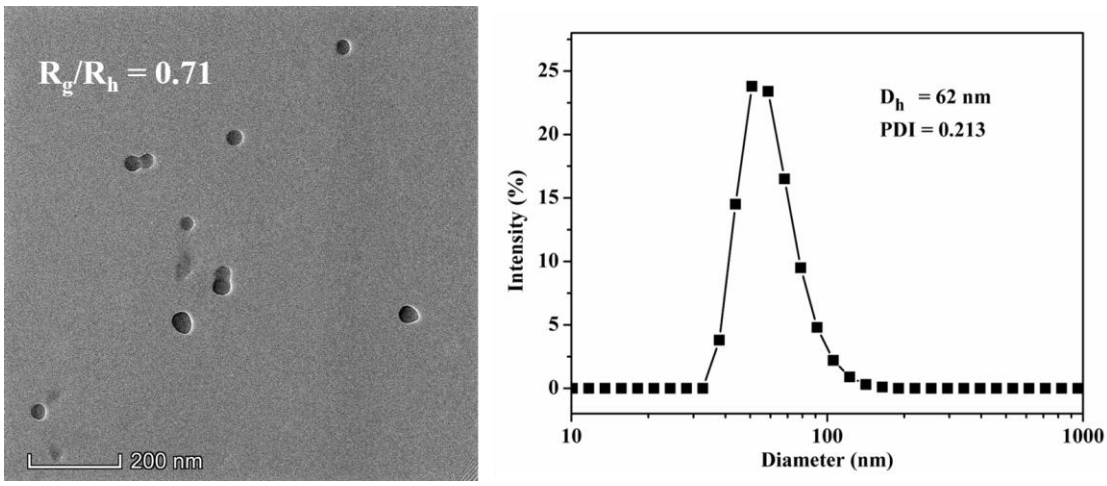


Figure S26. TEM image and DLS of **SDDC** aggregates with $CDDP(P5)_2$ in the presence of 1.0 equiv of CN-CPT.

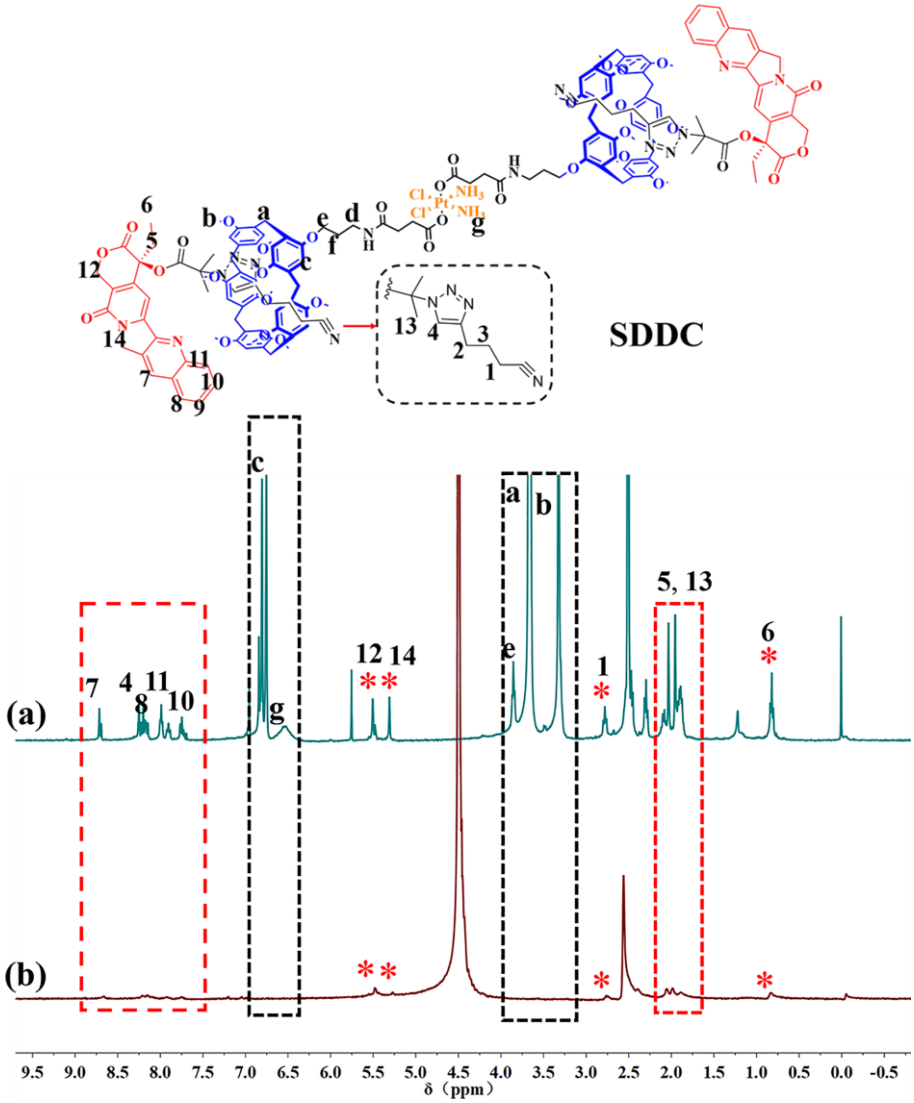


Figure S27. ^{1}H NMR spectra recorded (400 MHz, 298 K) of a mixture of CDDP(P5)$_2$ and CN-CPT (molar ratio 1: 2) in (a) DMSO-d_6, (b) D_2O: DMSO-d_6 = 9:1, v/v.

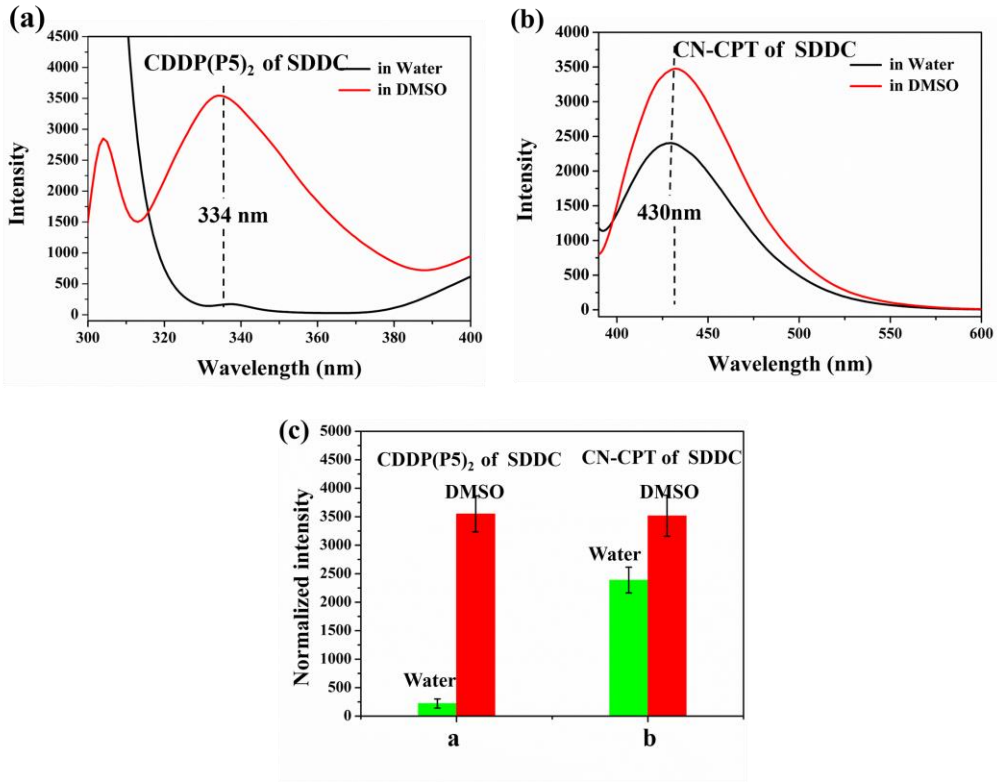


Figure S28. (a) Fluorescence spectra of **SDDC** in water and DMSO for $CDDP(P5)_2$ (λ_{ex} = 305 nm, λ_{em} = 334 nm). (b) Fluorescence spectra of **SDDC** in water and DMSO for CN-CPT (λ_{ex} = 370 nm, λ_{em} = 430 nm). (c) Normalized fluorescence intensity in different media.

6. *In vitro* drug release and stability of supramolecular vesicles (SVs)

6.1 *In vitro* stability investigation.

The solution stability of **SV**s was evaluated by detecting the change of morphology and size at different conditions. As shown in Figures S29, S30, and Table S1, the morphology and size of **SV**s were almost no obvious change at different concentration, and even were still retained after storing for 10 days in aqueous solution (with 5% DMSO) (Figure S31). Then, the DLS measurements were performed at a series of different time intervals to further study the stability of **SV**s. No significant change in their D_h and PDI was observed in water (with 5% DMSO) for 16 days (Figure S32a). Additionally, the D_h of these vesicles also remained unchanged in PBS buffer within 48 h. While a little increasing in D_h after cell culture medium for 48 h might be attributed to protein adsorption in the presence of fetal bovine serum (Figure S32b, c). Thus, all of these results demonstrated that although no surfactants or excipients were used, **SV**s still showed very stability in aqueous solution, which might have a great potential arrive to tumour sites in intact form by EPR effect during circulation.

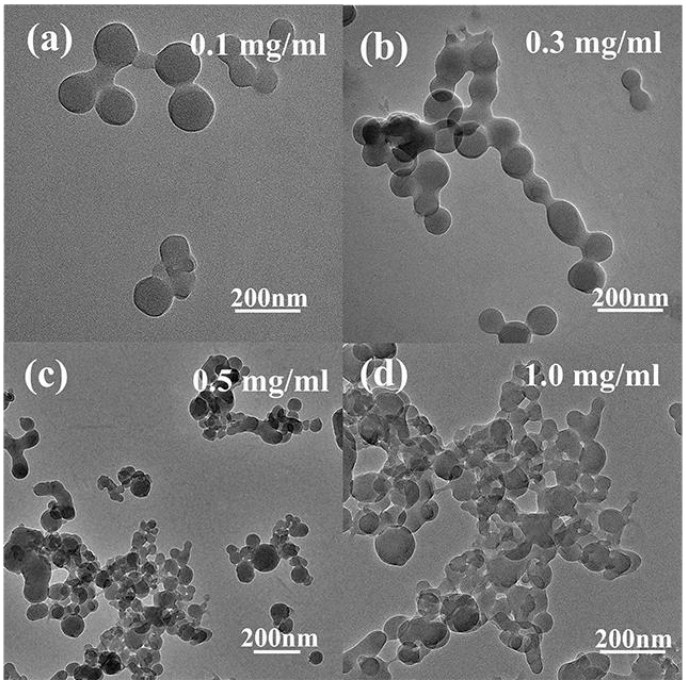


Figure S29. TEM images of **SV**s at different concentration (a) 0.1 mg/mL, (B) 0.3 mg/mL, (C) 0.5 mg/mL, and (D) 1.0 mg/mL.

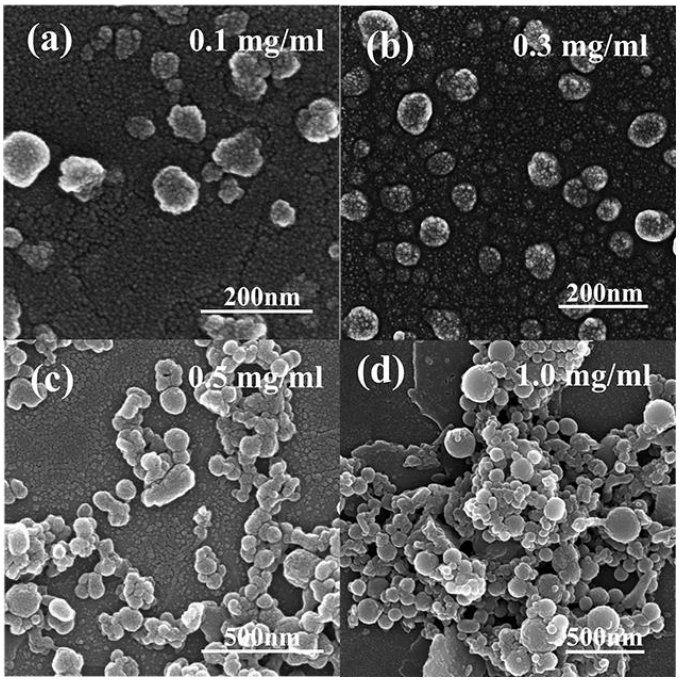


Figure S30. SEM images of **SV**s at different concentration (a) 0.1 mg/mL, (B) 0.3 mg/mL, (C) 0.5 mg/mL, and (D) 1.0 mg/mL.

Table S1. Size of **SV**s at different concentration in aqueous solution (with 5% DMSO).

Code	Concentration (mg/mL)	$D_{av,TEM}$[a] (nm)	$D_{av,SEM}$[b] (nm)	D_z[c] (nm)	PDI[d]	Morphology
1	0.1	90.1 ± 3.4	100.3 ± 4.7	117	0.201	Spherical
2	0.3	89.3 ± 5.4	102.1 ± 2.8	115	0.192	Spherical
3	0.5	91.3 ± 4.8	101.3 ± 4.2	118	0.352	Spherical
4	1.0	91.7 ± 5.2	102.6 ± 2.9	120	0.582	Spherical

[a] Average diameter determined by TEM. [b] Average diameter determined by SEM. [c] Z-Average diameter determined by DLS. [d] Polydispersity of particles' diameter determined by DLS.

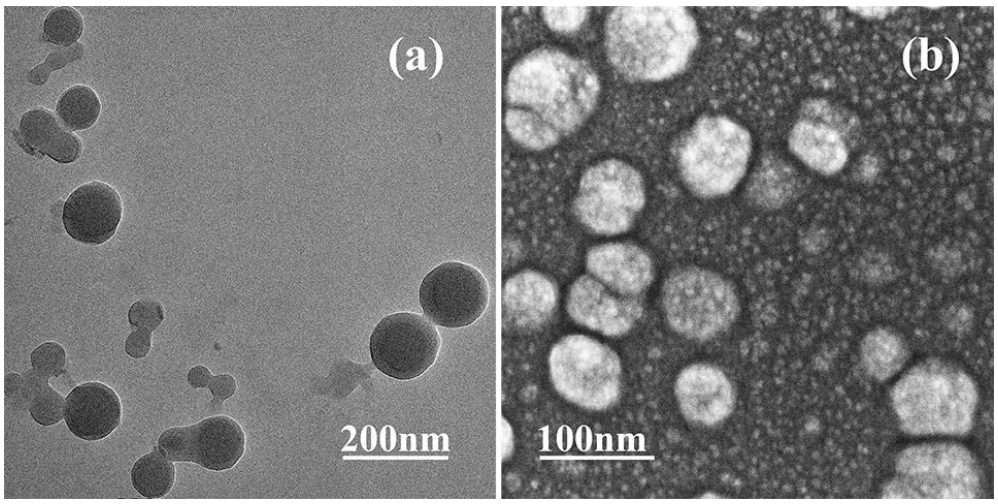


Figure S31. TEM (a) and SEM (b) images of **SV**s after storing for 10 days.

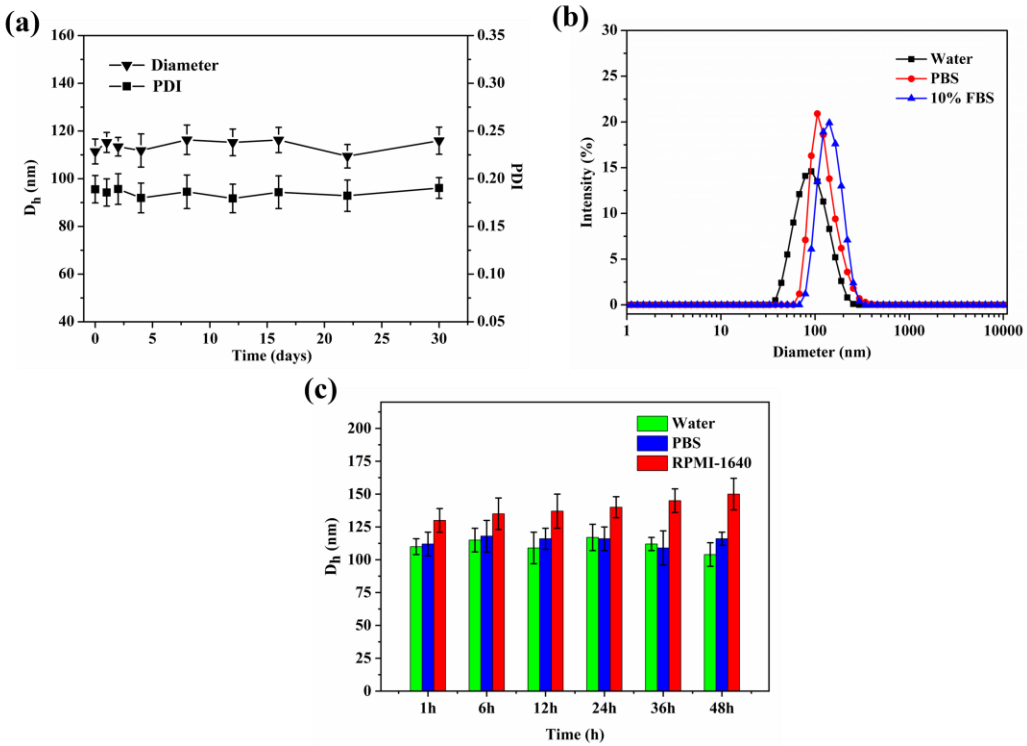


Figure S32: Stability of **SV**s in vitro through D_h and PDI. (a) Influence of storage on D_h and PDI of **SV**s after storing for 16 days. (b, c) Change of D_h of **SV**s in water, PBS and cell culture medium after incubation for 48 h.

6.2 *In vitro* drug release

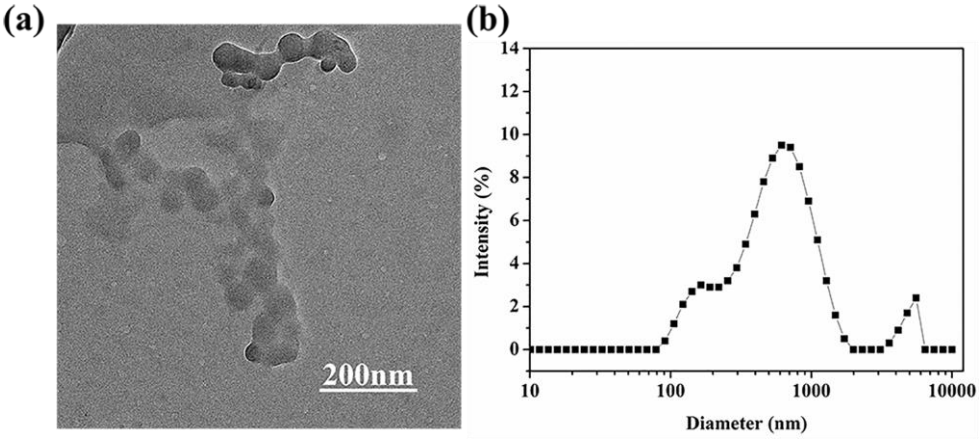


Figure S33. TEM image (a) and Hydrodynamic diameter (D_h) distribution (b) of **SV**s with spermine as a function of time over a period of 48 h.

The spermine had a high association constant with P5 as competitive replacement to destroy the supramolecular interactions. **SV**s solution containing spermine was gradually ruptured and aggregated, indicating that the host-gust interaction between CDDP$(P5)_2$ and CN-CPT could be destroyed by the competitive replacement of spermine.

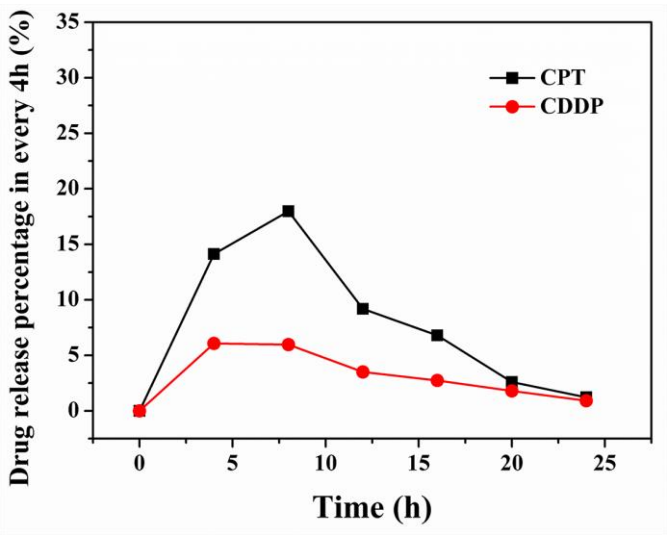


Figure S34. CPT and CDDP release percentage in PBS (pH 5.0 and spermine) in every 4 hrs.

The release speed of CPT increased rapidly in 8h incubation. After that the release speed decreased, but the relatively rapid release behavior also lasted until 16 h incubation. However, the general trend of CDDP's release speed was relatively flat except for the slightly fast release speed at first.

6.3 Release kinetics of CPT and CDDP from SVs.

The release mechanism of CPT and CDDP from **SV**s was thus proposed to elucidate the release behavior by studying the release kinetics with a simple semiempirical equation [Eq. (1)] and a modified equation [Eq. (2)] to describe the release behavior of polymeric micelles.[6]

$$M_t/M_\infty = kt^n \quad (1)$$

$$\ln r = \ln k + n \ln t,\ r = M_t/M_\infty \quad (2)$$

in which M_t and M_∞ indicate the cumulative amounts of guest molecule released at time t and infinity, respectively; k describes the release constant; k' indicates the constant proportional to k; and n is the kinetic and release mechanism.

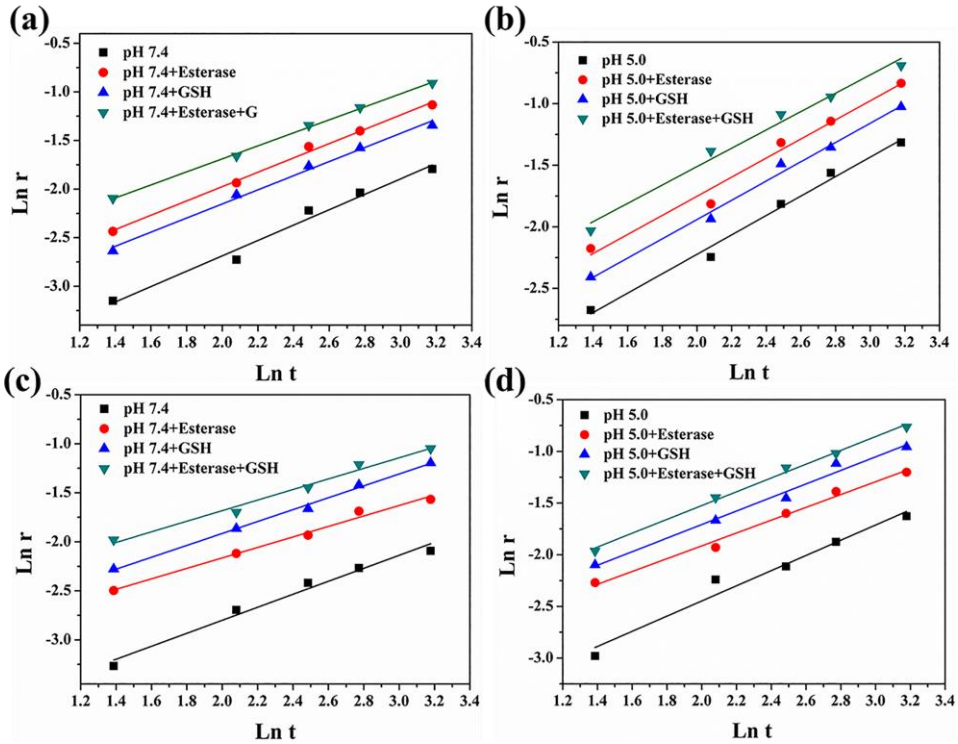


Figure S35. Cumulative release curves of CPT (a, b) and CDDP (c, d) from **SV**s as a function of time from 4 h to 24 h.

Table S2. Release kinetics parameters of CPT from **SV**s with or without esterase or glutathione at 37℃ fitted with Peppas' formula.[a]

Release experiment	Fitting equation	n^b	k^b	R^{2b}
pH7.4	$\ln r = 0.791 \ln t - 4.268$	0.791	0.014	0.977
pH7.4+Esterase	$\ln r = 0.736 \ln t - 3.447$	0.736	0.031	0.994
pH7.4+GSH	$\ln r = 0.725 \ln t - 3.603$	0.725	0.027	0.992
pH7.4+Esterase+GSH	$\ln r = 0.669 \ln t - 3.027$	0.669	0.048	0.998
pH5.0	$\ln r = 0.788 \ln t - 3.798$	0.788	0.022	0.985
pH5.0+Esterase	$\ln r = 0.774 \ln t - 3.300$	0.774	0.036	0.975
pH5.0+GSH	$\ln r = 0.781 \ln t - 3.502$	0.781	0.030	0.989
pH5.0+ Esterase+GSH	$\ln r = 0.745 \ln t - 3.000$	0.745	0.049	0.981

[a] All experiments were conducted at 37℃. [b] Calculated by using Equation (2).

Table S3. Release kinetics parameters of CDDP from **SV**s with or without esterase or glutathione at 37℃ fitted with Peppas' formula.[a]

Release experiment	Fitting equation	n^b	k^b	R^{2b}
pH7.4	$\ln r = 0.458 \ln t - 3.547$	0.458	0.029	0.993
pH7.4+Esterase	$\ln r = 0.533 \ln t - 3.231$	0.533	0.039	0.985
pH7.4+GSH	$\ln r = 0.606 \ln t - 3.127$	0.606	0.043	0.995
pH7.4+Esterase +GSH	$\ln r = 0.539 \ln t - 2.761$	0.539	0.063	0.979
pH5.0	$\ln r = 0.734 \ln t – 3.915$	0.734	0.020	0.969
pH5.0+Esterase	$\ln r = 0.619 \ln t - 3.150$	0.619	0.043	0.986
pH5.0+GSH	$\ln r = 0.655 \ln t - 3.017$	0.655	0.049	0.981
pH5.0+ Esterase +GSH	$\ln r = 0.667 \ln t - 2.859$	0.667	0.057	0.994

[a] All experiments were conducted at 37℃. [b] Calculated by using Equation (2).

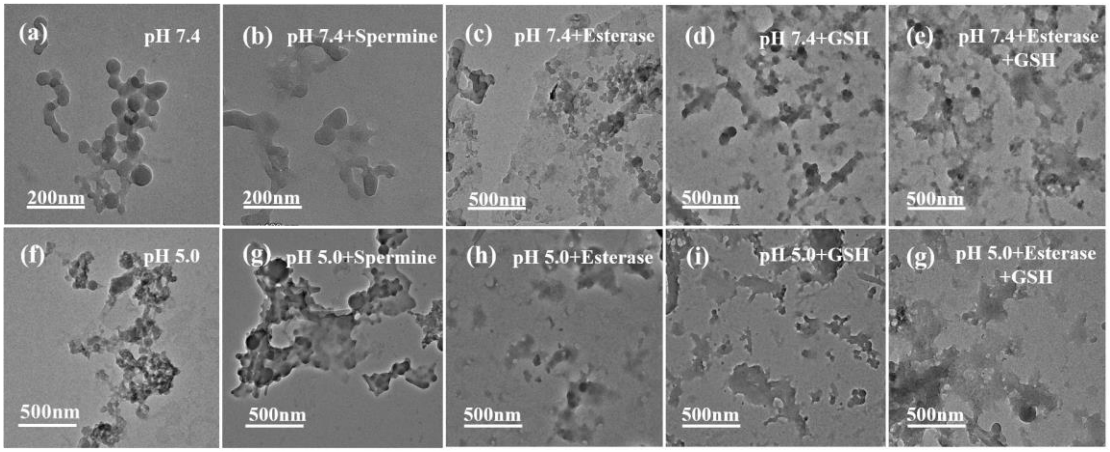


Figure S36. TEM images of **SV**s as a function of time over a period of 72 h: (a) pH 7.4; (b) pH 7.4 + Spermine; (c) pH 7.4 + Esterase; (d) pH 7.4 + GSH; (e) pH 7.4 + Esterase + GSH; (f) pH 5.0; (g) pH 5.0 + Spermine; (h) pH 5.0 + Esterase; (i) pH 5.0 + GSH; (g) pH 5.0 + Esterase + GSH, respectively at 37 °C.

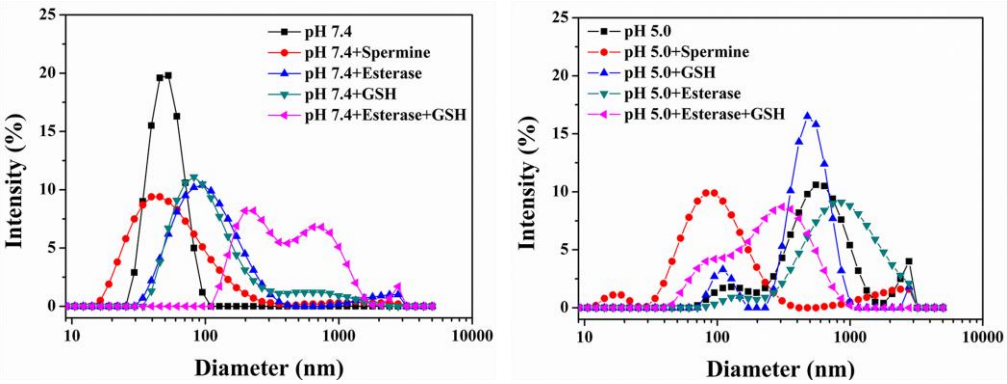


Figure S37. DLS images of **SV**s as a function of time over a period of 72 h: pH 7.4; pH 7.4 + Spermine; pH 7.4 + Esterase; pH 7.4 + GSH; pH 7.4 + Esterase + GSH; pH 5.0; pH 5.0 + Spermine; pH 5.0 + Esterase; pH 5.0 + GSH; pH 5.0 + Esterase + GSH, respectively at 37 °C.

7. Biological properties of SVs

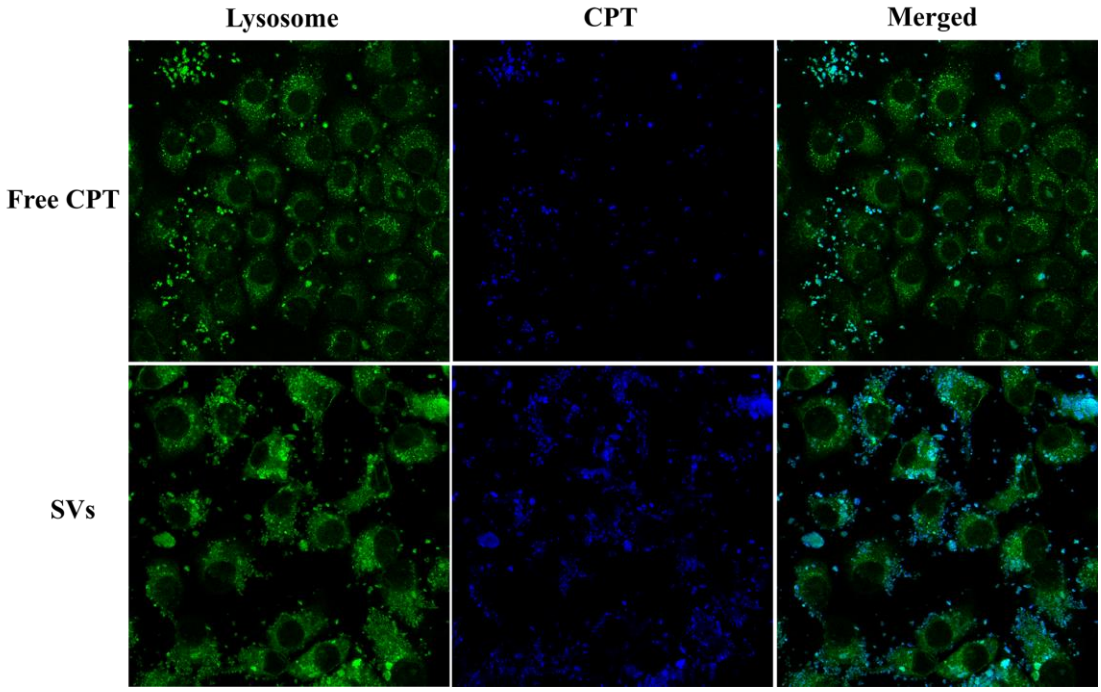


Figure S38. Intracellular uptake study of **SV**s was carried out by Confocal microscopy. **SV**s (100 μM) were incubated with HepG-2 cells for 24 h. The lysosomes inside the cells were stained by lysotracker in green color, while the CPT signals represented the **SV**s in blue color. Free CPT was used as a control.

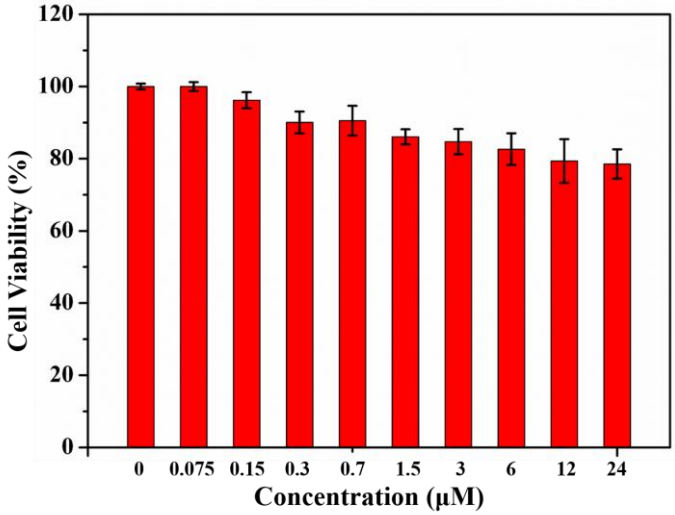


Figure S39. Cytotoxicity of **SV**s at different concentrations to L929 cells for 72 h of incubation.

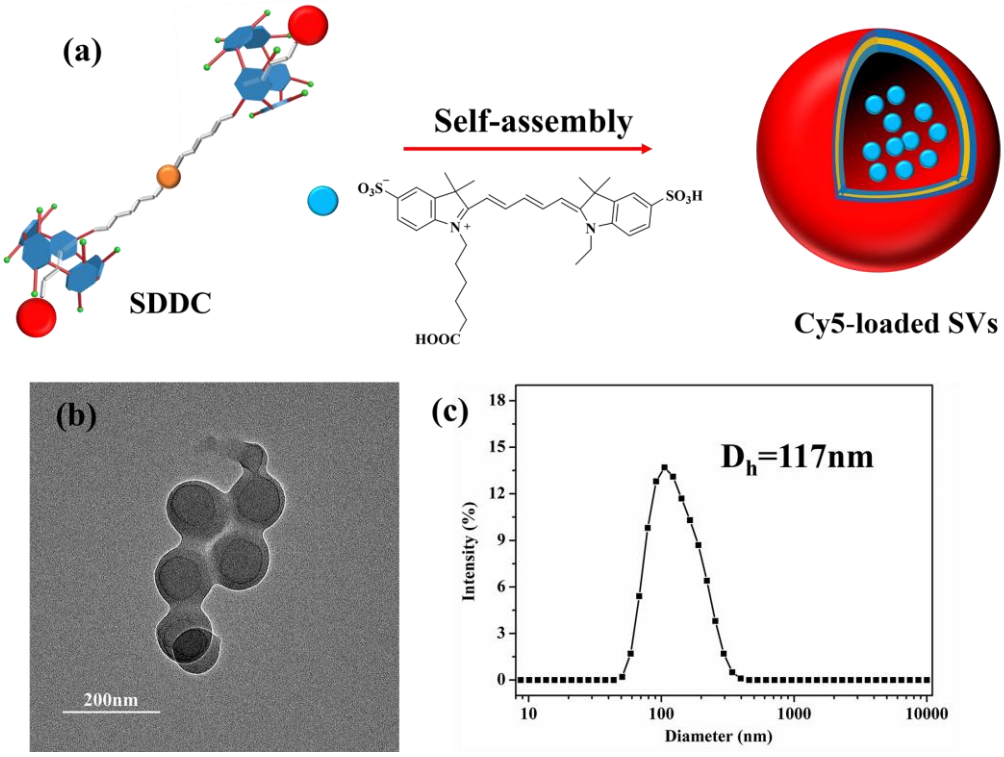


Figure S40. (a) Schematic illustration of preparation of Cy5-doped **SV**s for NIRF imaging. (b) The TEM image and (c) DLS of Cy5-loaded **SV**s.

8. References

[1] Wang, W.; Zhang, K.; Chen, D. From Tunable DNA/Polymer Self-Assembly to Tailorable and Morphologically Pure Core−Shell Nanofibers. *Langmuir*. **2018**, *34*, 15350-15359.

[2] Anil, K.; Hou, S.; Zhang., X. Neuropilin-1-Targeted Gold Nanoparticles Enhance Therapeutic Efficacy of Platinum (IV) Drug for Prostate Cancer Treatment. *ACS NANO*. **2014**, *8*, 4205-4220.

[3] Nazarova, A. A.; Yakimova, L. S.; Klochkov, V. V.; Stoikov, I. I. Monoaminophosphorylated pillar[5]arenes as hosts for alkaneamines. *New Journal of Chemistry*. **2017**, *41*, 1820-1826.

[4] Rieter, W. J. Nanoscale Coordination Polymers for Platinum-Based Anticancer Drug Delivery. *J Am Chem Soc*. **2008**, *130*, 11584-11585.

[5] Zhang, M.; Yin, S.; Zhang, J.; Zhou, Z.; Saha, M. L.; Lu, C.; Stang, P. J. Metallacycle-cored Supramolecular Assemblies with Tunable Fluorescence Including White-light Emission. *Proc Natl Acad Sci U S A*. **2017**, *114*, 3044-3049.

[6] Yao, H.; Qi, M.; Liu, Y.; Tian, W. Host–Guest Binding-Site-Tunable Self-Assembly of Stimuli Responsive Supramolecular Polymers. *Chem.Eur.J*. **2016**, *22*, 8508-8519.