Electronic Supplementary Information

Bifunctional supramolecular prodrug vesicles constructed from camptothecin derivative with water-soluble pillar[5]arene for cancer diagnosis and therapy

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1. General information and experimental procedure

1) General information:

All reactions were performed in air atmosphere unless otherwise stated. The commercially available reagents and solvents were either employed as purchased or dried according to procedures described in the literatures. Column chromatography was performed with silica gel (200 – 300 and 300 – 400 mesh) produced by Qingdao Marine Chemical Factory, Qingdao (China). All yields were given as isolated yields. NMR spectra were recorded on a Bruker DPX 400 MHz spectrometer with internal standard tetramethylsilane (TMS) and solvent signals as internal references at room temperature, and the chemical shifts (δ) were expressed in ppm and *J* values were given in Hz. High-resolution electrospray ionization mass spectra (HR-ESI-MS) were recorded on an Agilent 6540Q-TOF LCMS equipped with an electrospray ionization (ESI) probe operating in the positive-ion mode with direct infusion. Low-resolution electrospray ionization mass spectra (LR-ESI-MS) were obtained on Finnigan Mat TSQ 7000 instruments. Transmission electron microscope (TEM) investigations were carried out on a JEM-2100 instrument. Dynamic light scattering (DLS) measurements were carried out on a Brookhaven BI-9000AT system (Brookhaven Instruments Corporation, USA), using a 200-mW polarized laser source (λ = 514 nm). Zeta-potential measurements were performed at 25 ℃ on a Zetasizer Nano Z apparatus (Malvern Instruments Ltd., UK) using the Smoluchowski model for the calculation of the Zeta-potential from the measured electrophoretic mobility. The UV-Vis absorption spectra were measured on a Perkin Elmer Lambda 35 UV-Vis Spectrometer. The confocal laser scanning microscopy (CLSM) investigations were carried out on a Zeiss LSM 710 instrument. The excitation and emission spectra were recorded on a Hitachi F-7000 Fluorescence Spectrometer.

2) Experimental procedure:

Fabrication of the WP5⊃DNS-CPT supramolecular prodrug vesicles. WP5⊃DNS-CPT prodrug vesicles were prepared as follows: The stock solution of **DNS-CPT** (0.02 M, dissolved in DMSO) and **WP5** (0.42 mM, dissolved in water) were prepared. Then, 480 μL of **WP5** solution was added into a volumetric flask (5 mL) and 4.52 mL of water was added to dilute **WP5** solution. Finally, 50 μL of **DNS-CPT** was quickly injected into the above solution. The ultimate concentrations of **WP5** and **DNS-CPT** were 0.04 mM and 0.2 mM, respectively. After standing

overnight, the prepared **WP5⊃DNS-CPT** prodrug vesicles were purified by dialysis (molecular weight cutoff 10000) in distilled water for several times until the water outside the dialysis tube exhibited negligible fluorescence.

The drug encapsulation efficiency of SN-38 was calculated based on equation (1):

$$\text{Encapsulation efficiency (\%)} = (m_{\text{SN-38-loaded}}/m_{\text{SN-38}}) \times 100 \quad (1)$$

where $m_{\text{SN-38-loaded}}$ and $m_{\text{SN-38}}$ are mass of SN-38 encapsulated into the vesicles and mass of prodrug **DNS-CPT** added, respectively. The mass of SN-38 was measured by a fluorescence spectrophotometer at 556 nm.

GSH-responsive behavior of WP5⊃DNS-CPT prodrug vesicles. 0, 1, 5 and 10 mM GSH were used as drug release media to simulate the normal physiological condition and the intracellular condition of tumor cells. In a typical release experiment, 8.4 mL of **WP5⊃DNS-CPT** vesicles was added into 1.6 mL of appropriate release medium at 37 ℃. At selected time intervals, 2 mL of the release media was taken out for measuring the concentrations of released SN-38 by the fluorescence technique, and then was returned to the original release media. A nearly 100% release of SN-38 from **WP5⊃DNS-CPT** prodrug vesicles was obtained by adding Triton X-100 to the vesicle solution.

***In vitro* cytocompatibility and cytotoxicity assay.** The relative *in vitro* cytocompatibility of **WP5**, **DNS-CPT**, and **WP5⊃DNS-CPT** vesicles against NIH 3T3 normal cells, and the relative *in vitro* cytotoxicity of SN-38 and **WP5⊃DNS-CPT** vesicles against MCF-7 and HepG2 cancer cells were assessed by using the MTT assay, respectively (NIH 3T3, MCF-7, and HepG2 cell lines were supplied by KeyGen Biotech Co. Ltd, Nanjing, China). Firstly, the cells were seeded in 96-well plates at a density of 1×10^4 cells per well in 100 μL of DMEM containing 10% fetal bovine serum (Gibco), 50 U·mL^{-1} penicillin, and 50 U·mL^{-1} streptomycin (Hyclone), and cultured in 5% CO_2 at 37 ℃ for 24 h. Then, the original medium was removed and 100 μL of fresh culture medium containing different concentrations of **WP5**, **DNS-CPT**, and **WP5⊃DNS-CPT** vesicles (for NIH 3T3 normal cells) or SN-38 and **WP5⊃DNS-CPT** vesicles (for MCF-7 and HepG2 cancer cells) was added into each well, and the cells were further incubated for 24, 48, and 72 h, respectively.

Subsequently, 100 μL of MTT solution (0.5 mg/mL) was added into each well and incubated for another 4 h. After that, the medium containing MTT was removed, and dimethyl sulfoxide (DMSO, 100 μL) was added to each well to dissolve the MTT formazan crystals. Finally, the

plates were shaken for 30 min, and the absorbance of formazan product was measured at 490 nm by a microplate reader (Thermo Scientific). Untreated cells in medium were used as the blank control. All experiments were carried out with four replicates. The cytocompatibility and cytotoxicity were expressed as the percentage of the cell viability relative to the blank control.

Cellular uptake and intracellular localization observed by CLSM. The cellular uptake and intracellular localization of SN-38 were examined in HepG2 cancer cells. Briefly, HepG2 cancer cells were plated onto glass-bottomed Petri dishes in 400 μL of complete DMEM culture medium at a density of 1×10^5 cells mL^{-1} for 24 h before treatment. Then, cells were incubated with **WP5⊃DNS-CPT** vesicles solution (equivalent to 2 μM SN-38) for 1 and 4h, respectively. Subsequently, the culture medium was removed and cells were washed with PBS thrice. Then, DAPI (Cell Signaling Technology, USA) was added to the medium for 15 min to stain nuclei. Finally, the cells were washed three times with PBS and investigated by confocal laser scanning microscopy (LSM710, CarlZeiss). The fluorescence characteristic of SN-38 was used to directly monitor the drug release without utilizing additional dye.

Flow cytometric analysis. HepG2 cancer cells were seeded in 6-well plates at a density of 2×10^5 cells per well in DMEM culture medium (1.2 mL) and cultured for 24 h, and then the culture medium was removed. SN-38 and **WP5⊃DNS-CPT** vesicles dissolved in DMEM culture medium at a final concentration of 8 μM were added into the wells and the cells were incubated at 37 ℃ for 24 h, respectively. HepG2 cancer cells without incubation with **WP5⊃DNS-CPT** vesicles were used as a control. After incubation for 24 h, the cell solutions were centrifuged at 2000 rpm for 5 min. Culture medium was removed and the cells were washed with PBS twice. After removal of the supernatants, the cells were resuspended in binding buffer (400 μL). The apoptosis cells were determined by staining using an Annexin V-FITC apoptosis detection kit according to the manufacturer's protocol: Annexin V-FITC (5 μL) was added to the cell suspensions, after incubation in the dark for 15 min, cell apoptosis was detected by flow cytometry at specific wavelengths (Ex = 488 nm; Em = 530 nm).

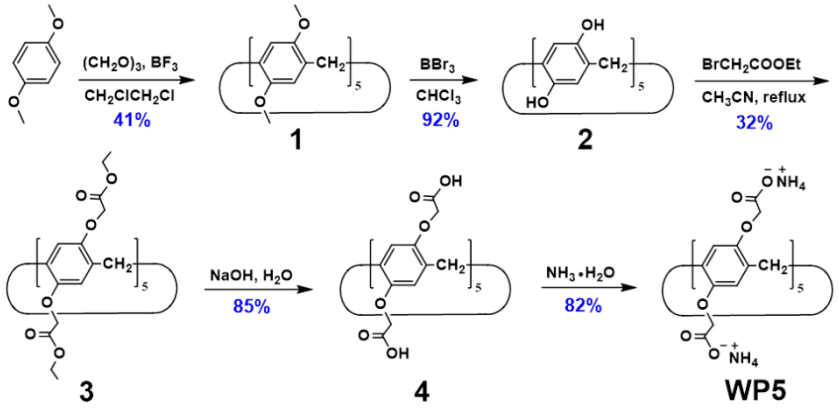
Animals and tumor models. Female BALB/c nude mice (4-5 weeks old) were purchased from Jiangsu KeyGen Biotech Co. Ltd and maintained in a pathogenfree environment under controlled temperature (25 ℃). Animal care and handling procedures were in agreement with the guidelines evaluated and approved by the ethics committee of Jiangsu Province. The mice were injected

subcutaneously in the right flank region with 200 μL of cell suspension containing 1×10^7 HepG2 cancer cells. The fluorescent imaging was carried out at different time intervals after intravenous administration when tumors reached 100~200 mm^3.

2. Synthesis of host molecule WP5 and guest molecule DNS-CPT

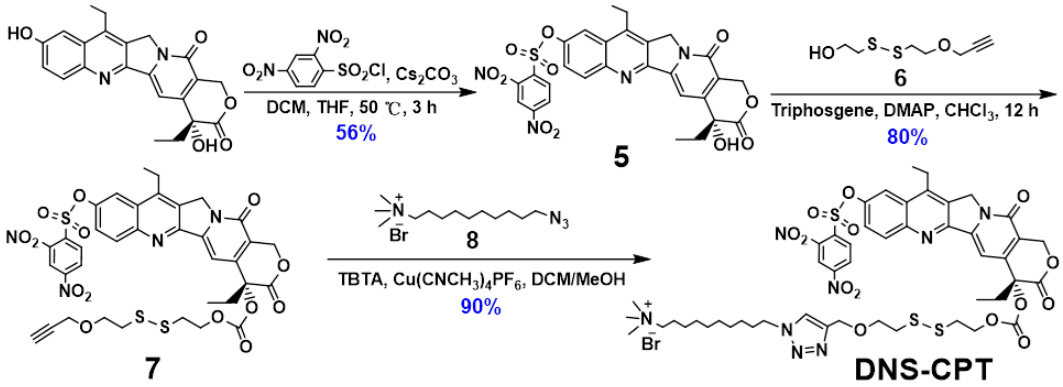
1) Synthesis of host molecule WP5

WP5 was synthesized and purified according to previously reported procedures (Scheme S1).[S1-S6]



Scheme S1. Synthesis route of host molecule **WP5.**

2) Synthesis of guest molecule DNS-CPT



Scheme S2. Synthesis route of guest molecule **DNS-CPT.**

Synthesis of compound 5

7-Ethyl-10-hydroxyl-camptothecin (0.52 g, 1.43 mmol) and trimethylamine (0.6 mL, 4.32 mmol) was dissolved in dichloromethane (DCM, 100 mL) and stirred for 0.5 h under ice bath. Then, the solution of 2,4-dinitrobenzene sulfonyl chloride (0.45 g, 1.67 mmol, 20 mL) was added dropwise into the above mixture during 0.5 h. The mixture was further stirred for 2 h and

quenched with saturated $NaHCO_3$. The separated organic phase were dried over Na_2SO_4, filtered, and concentrated. The crude product was purified by silica gel chromatography using DCM/MeOH (40:1, *v/v*) as the eluent to afford compound **5** as a slight yellow solid (0.50 g, 0.80 mmol, 56%). ^{1}H NMR (400 MHz, DMSO-d_6, 298 K) δ (ppm): 9.16 (d, *J* = 2.0 Hz, 1H), 8.61 (dd, *J* = 8.8, 2.4 Hz, 1H), 8.34 (d, *J* = 8.8 Hz, 1H), 8.26 (d, *J* = 9.6 Hz, 1H), 8.06 (d, *J* = 2.4 Hz, 1H), 7.67 (dd, *J* = 9.6, 2.8 Hz, 1H), 7.34 (s, 1H), 5.45 (s, 2H), 5.35 (s, 2H), 3.15 (q, 2H), 1.92-1.81 (m, 2H), 1.19 (t, *J* = 7.6 Hz, 3H), 0.89 (t, *J* = 7.2 Hz, 3H).

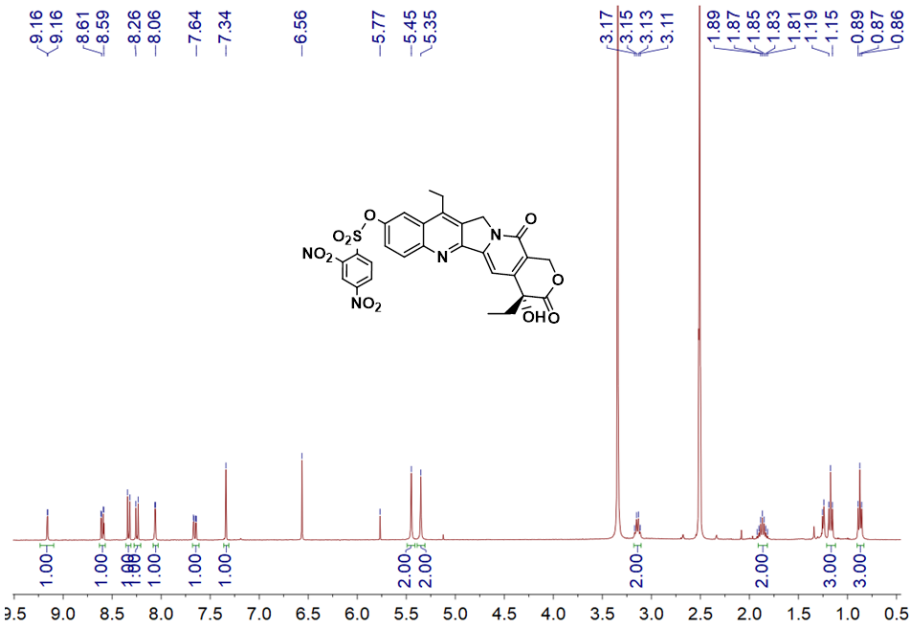


Fig. S1 ^{1}H NMR spectrum (400 MHz, DMSO-d_6, 298 K) of compound **5**.

Synthesis of compound 6

Bis(2-hydroxyethyl) disulfide (3.85 g, 25 mmol) and propargyl bromide (1 mL, 12.5 mmol) were dissolved in THF (100 mL). Then, NaH powder (60 wt %, 1.0 g, 25 mmol) was added into the reaction mixture in three successive batches under argon atmosphere within 1 h at 0 ℃. The mixture was further stirred for another 8 h at room temperature, and then a few drops of water were added to quench the reaction. The crude product was purified by column chromatography, using petroleum ether/ethyl acetate (PE/EA = 5:1, *v/v*) as eluent to afford a pale-yellow clear oil (2.81 g, 15 mmol, 60 %). ^{1}H NMR (400 MHz, $CDCl_3$, 298 K) δ (ppm): 4.21 (d, *J* = 2.4 Hz, 2H), 3.91 (t, *J* = 5.8 Hz, 2H), 3.83 (t, *J* = 6.4 Hz, 2H), 2.93 (t, *J* = 6.4 Hz, 2H), 2.90 (t, *J* = 5.6 Hz, 2H), 2.47 (t, *J* = 2.4 Hz, 1H), 1.74 (s, 1H).

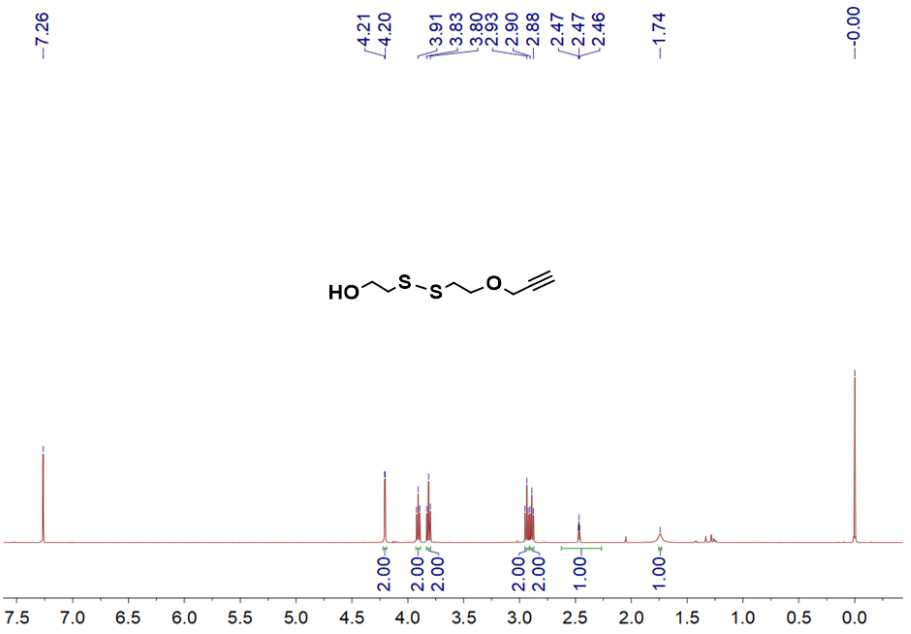


Fig. S2 ^{1}H NMR spectrum (400 MHz, $CDCl_3$, 298 K) of compound **6**.

Synthesis of compound 7

A mixture of compound **5** (0.24 g, 0.38 mmol) and dimethylaminopyridine (DMAP, 0.16 g, 1.34 mmol) in 10 mL of anhydrous DCM was stirred under argon atmosphere for 0.5 h at room temperature. Then a solution of triphosgene (0.11 g, 0.38 mmol) in anhydrous DCM (10 mL) was added slowly. The resulting mixture was stirred at room temperature for additional 0.5 h, and then the solution of compound **6** (0.22 g, 1.15 mmol) and diisopropylethylamine (DIEA, 0.4 g, 3.06 mmol) in DCM was added. The resulting mixture was stirred at room temperature for 0.5 h. After removal of the solvent under reduced pressure, the crude product was purified by silica gel chromatography using PE/EA (1:3, *v*/*v*) as the eluent to afford compound **7** as a pale yellow solid (0.26 g, 0.3 mmol, 80 %). ^{1}H NMR (400 MHz, DMSO-d_6, 298 K) δ (ppm): 9.16 (d, *J* = 2.4 Hz, 1H), 8.62 (dd, *J* = 8.4, 2.0 Hz, 1H), 8.35 (d, *J* = 8.8 Hz, 1H), 8.25 (d, *J* = 9.2 Hz, 1H), 8.07 (d, *J* = 2.8 Hz, 1H), 7.68 (dd, *J* = 9.2, 2.4 Hz, 1H), 7.07 (s, 1H), 5.54 (q, 2H), 5.38 (q, 2H), 4.35-4.29 (m, 2H), 4.08 (d, *J* = 2.4 Hz, 2H), 3.59 (t, *J* = 6.4 Hz, 2H), 3.40 (t, *J* = 2.4 Hz, 1H), 3.16 (q, 2H), 3.01 (t, *J* = 6.0 Hz, 2H), 2.88 (t, *J* = 6.4 Hz, 2H), 2.22-2.13 (m, 2H), 2.0 (s, 1H), 1.16 (t, *J* = 6.8 Hz, 3H), 0.92 (t, *J* = 7.2 Hz, 3H). ^{13}C NMR (100 MHz, DMSO-d_6, 298 K) δ (ppm): 167.5, 156.9, 153.4, 153.3, 152.1, 148.7, 147.7, 147.3, 146.7, 146.6, 145.2, 134.2, 133.1, 131.1, 129.8, 128.0, 127.5, 124.9, 121.6, 120.1, 117.4, 95.2, 80.5, 78.3, 77.8, 67.6, 66.9, 66.7, 57.7, 50.2, 38.0, 36.6, 31.6, 22.7, 14.2, 8.0. LR-ESI-MS: m/z $[M + H]^+$ calcd for $[C_{36}H_{33}N_4O_{14}S_3]^+$ 841.11, found 841.10, m/z

$[M + Na]^+$ calcd for $[C_{36}H_{32}N_4NaO_{14}S_3]^+$ 863.10, found 863.05. HR-ESI-MS: m/z $[M + H]^+$ calcd for $[C_{36}H_{33}N_4O_{14}S_3]^+$ 841.1150, found 841.1147, m/z $[M + Na]^+$ calcd for $[C_{36}H_{32}N_4NaO_{14}S_3]^+$ 863.0969, found 863.0972.

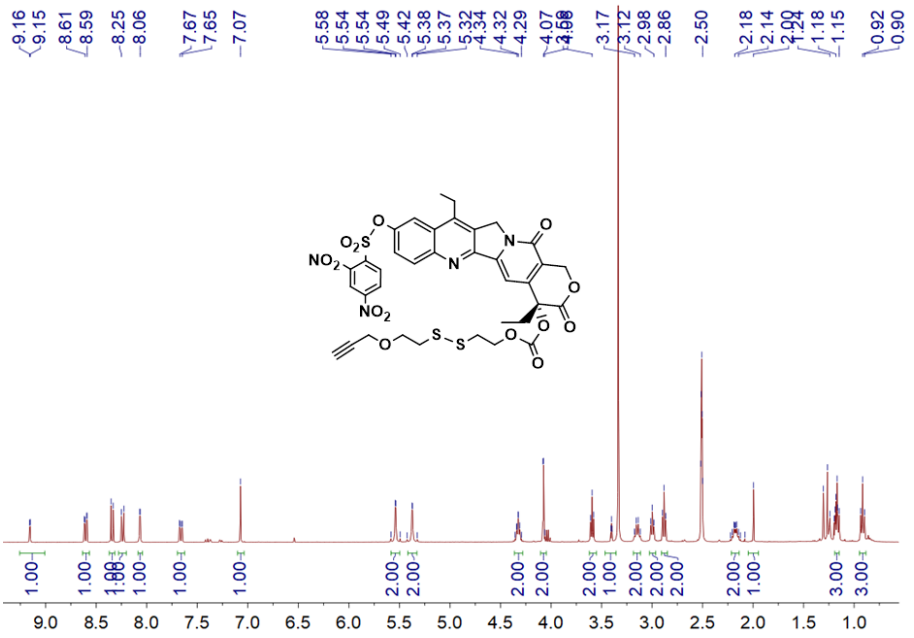


Fig. S3 ^{1}H NMR spectrum (400 MHz, DMSO-d_6, 298 K) of compound **7**.

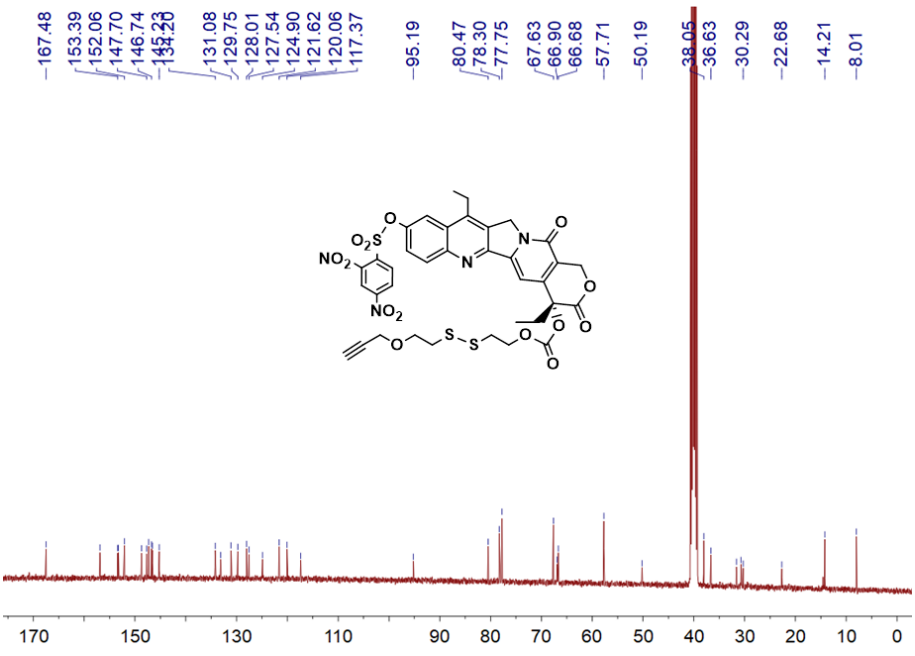


Fig. S4 ^{13}C NMR spectrum (100 MHz, DMSO-d_6, 298 K) of compound **7**.

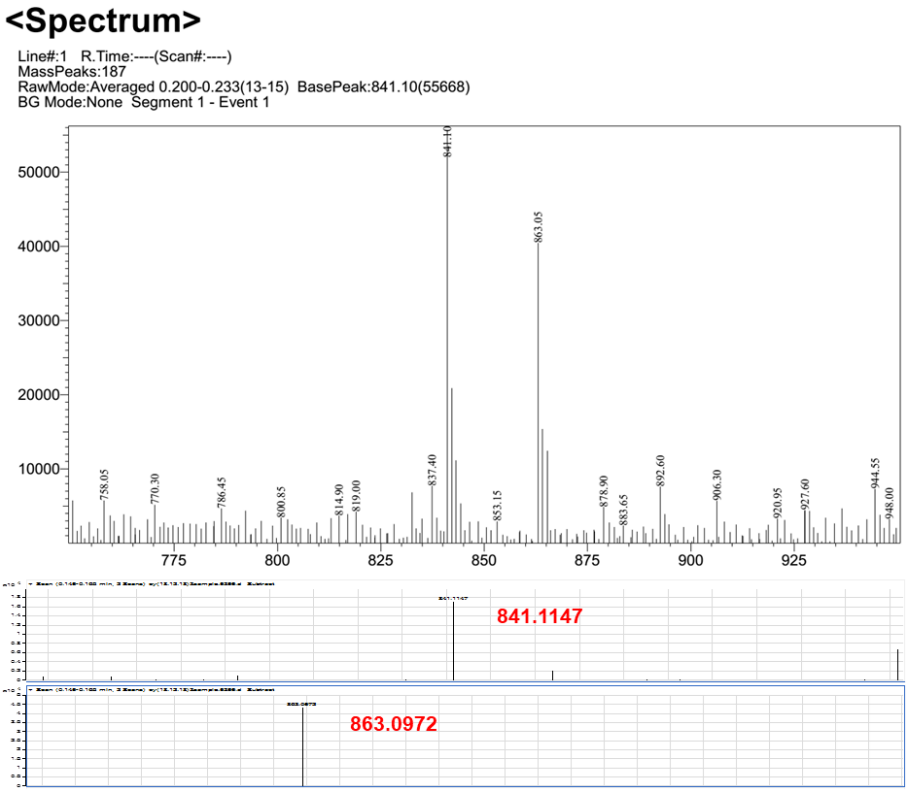


Fig. S5 LR-ESI-MS and HR-ESI-MS spectra of compound **7**.

Synthesis of compound 8

1,10-Dibromodecane (5.7 g, 19 mmol) was dissolved in DMF (100 mL). Then, NaN_3 (1.23 g, 19 mmol) was added into the mixture over 30 min and stirred at room temperature for 72 h. Water (1000 mL) was added to quench the reaction. The aqueous layer was extracted with dichloromethane (3 × 200 mL), and the organic layer was washed by water (1000 mL) and saturated brine (500 mL), respectively. Finally, the organic layer was dried over Na_2SO_4, filtered, and concentrated to afford 1-azido-10-bromodecane as colorless oil. Further, excess trimethylamine in ethanol (1 mL, 3.6 mmol) was added into the solution of 1-azido-10-bromodecane (0.15 g, 0.57 mmol, 15 mL, $CHCl_3$) and refluxed overnight. The mixture were concentrated under reduced pressure and washed by anhydrous ether to give compound **8** as a white solid (0.18 g, 0.56 mmol, 99%). ^{1}H NMR (400 MHz, D_2O, 298 K) δ (ppm): 3.25-3.20 (m, 4H), 3.01 (s, 9H), 1.72-1.65 (m, 2H), 1.55-1.48 (m, 2H), 1.28-1.23 (m, 12H). ^{13}C NMR (100 MHz, D_2O, 298 K) δ (ppm): 66.7, 52.8, 52.74, 52.70, 51.2, 28.4, 28.3, 28.2, 28.1, 28.0, 25.9, 25.4, 22.2. LR-ESI-MS: m/z $[M - Br]^+$ calcd for $[C_{13}H_{29}N_4]^+$ 241.24, found 241.15. HR-ESI-MS: m/z $[M - Br]^+$ calcd for $[C_{13}H_{29}N_4]^+$ 241.2387, found 241.2389.

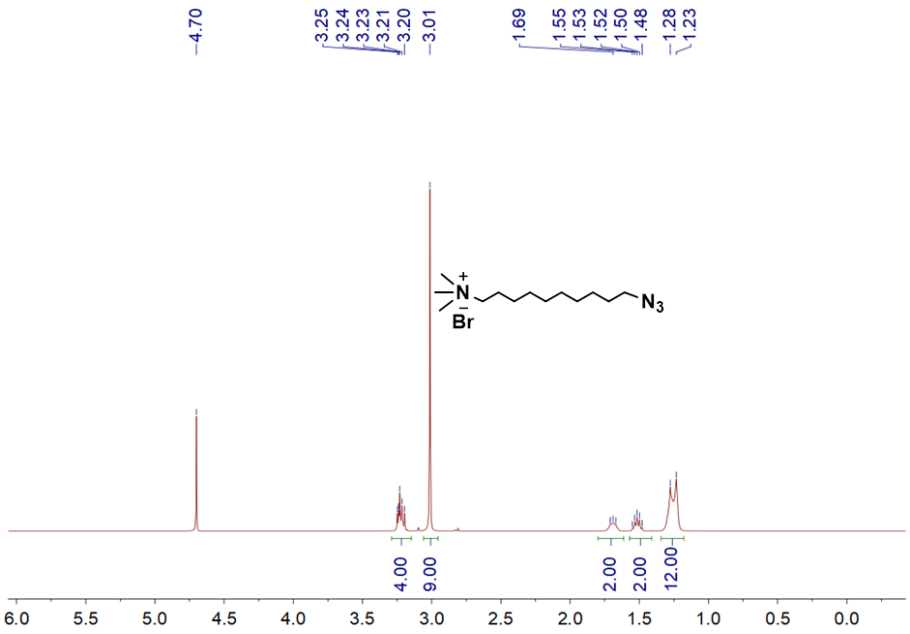


Fig. S6 ^{1}H NMR spectrum (400 MHz, D_2O, 298 K) of compound **8**.

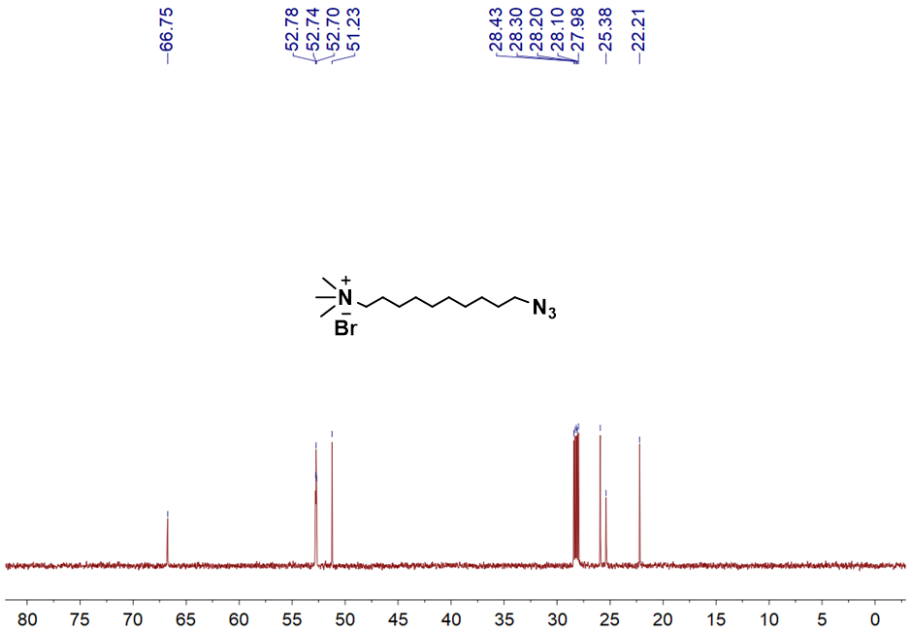


Fig. S7 ^{13}C NMR spectrum (100 MHz, D_2O, 298 K) of compound **8**.

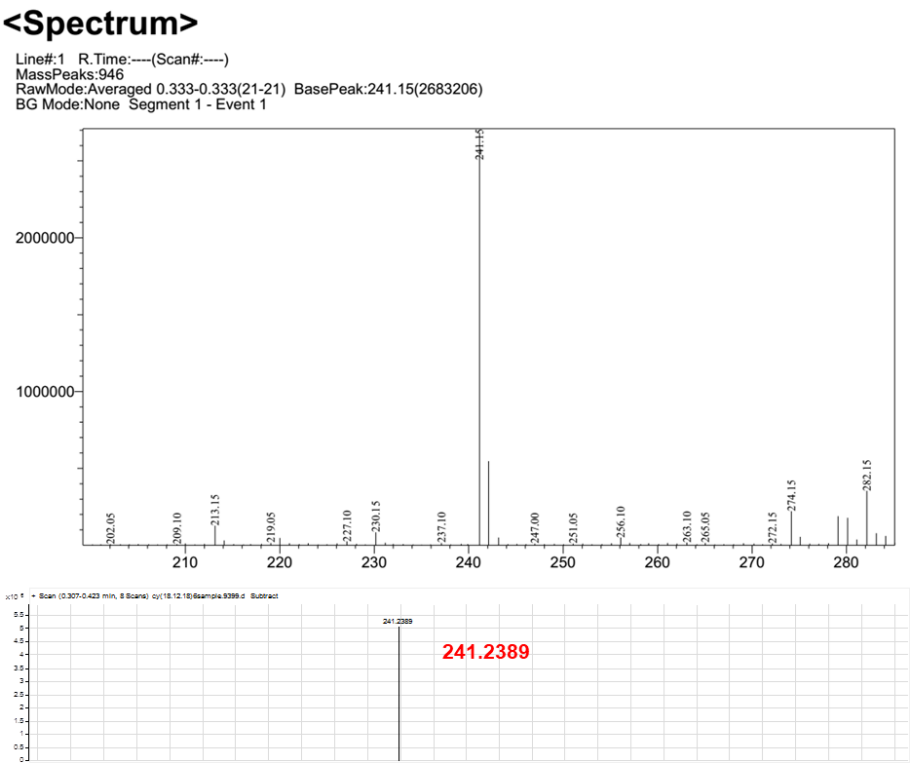


Fig. S8 LR-ESI-MS and HR-ESI-MS spectra of compound **8**.

Synthesis of compound DNS-CPT

A mixture of compound **7** (0.32 g, 0.38 mmol), tris-(benzyltriazolylmethyl)amine (TBTA, 0.02 g, 0.038 mmol), $Cu(CNCH_3)_4PF_6$ (0.014 g, 0.038 mmol) and **8** (0.16 g, 0.51 mmol) in DCM/MeOH (1:1, *v*/*v*, 20 mL) was stirred under argon atmosphere for 24 h at room temperature. After removal of the solvent under reduced pressure, the crude product was purified by silica gel chromatography using DCM/MeOH (8:1, *v*/*v*) as the eluent to afford compound **DNS-CPT** as a pale yellow solid (0.39 g, 0.34 mmol, 90%). ^{1}H NMR (400 MHz, DMSO-d_6, 298 K) δ (ppm): 9.15 (d, *J* = 2.0 Hz, 1H), 8.61 (dd, *J* = 8.4, 2.0 Hz, 1H), 8.35 (d, *J* = 8.8 Hz, 1H), 8.24 (d, *J* = 9.2 Hz, 1H), 8.06 (d, *J* = 2.4 Hz, 1H), 8.02 (s, 1H), 7.66 (dd, *J* = 9.2, 2.4 Hz, 1H), 7.06 (s, 1H), 5.54 (q, 2H), 5.36 (s, 2H), 4.42 (s, 2H), 4.31-4.27 (m, 2H), 3.60 (t, *J* = 6.0 Hz, 2H), 3.26-3.21 (m, 4H), 3.17 (q, 2H), 3.02 (s, 9H), 2.98 (t, *J* = 6.0 Hz, 2H), 2.88 (t, *J* = 6.0 Hz, 2H), 2.20-2.13 (m, 2H), 1.79-1.72 (m, 2H), 1.56-1.49 (m, 2H), 1.33-1.22 (m, 12H), 1.17 (t, *J* = 7.2 Hz, 3H), 0.92 (t, *J* = 7.6 Hz, 3H). ^{13}C NMR (100 MHz, DMSO-d_6, 298 K): δ (ppm) = 167.5, 156.9, 153.4, 153.3, 152.0, 148.7, 147.7, 147.3, 146.7, 146.6, 145.3, 144.0, 134.2, 133.1, 131.1, 129.7, 129.2, 128.0, 127.5, 124.9, 121.6, 120.0, 117.4, 95.2, 78.3, 68.0, 66.9, 66.7, 65.8, 63.6, 52.64, 52.60, 52.57, 51.1, 49.7, 38.2, 36.6, 30.7, 29.3, 29.1, 28.94, 28.91, 28.74, 28.68, 26.6, 26.2, 22.5, 14.2, 8.0. LR-ESI-MS:

m/z [M – Br]$^+$ calcd for [$C_{49}H_{61}N_8O_{14}S_3$]$^+$ 1081.35, found 1081.30. HR-ESI-MS: m/z [M – Br]$^+$ calcd for [$C_{49}H_{61}N_8O_{14}S_3$]$^+$ 1081.3464, found 1081.3484.

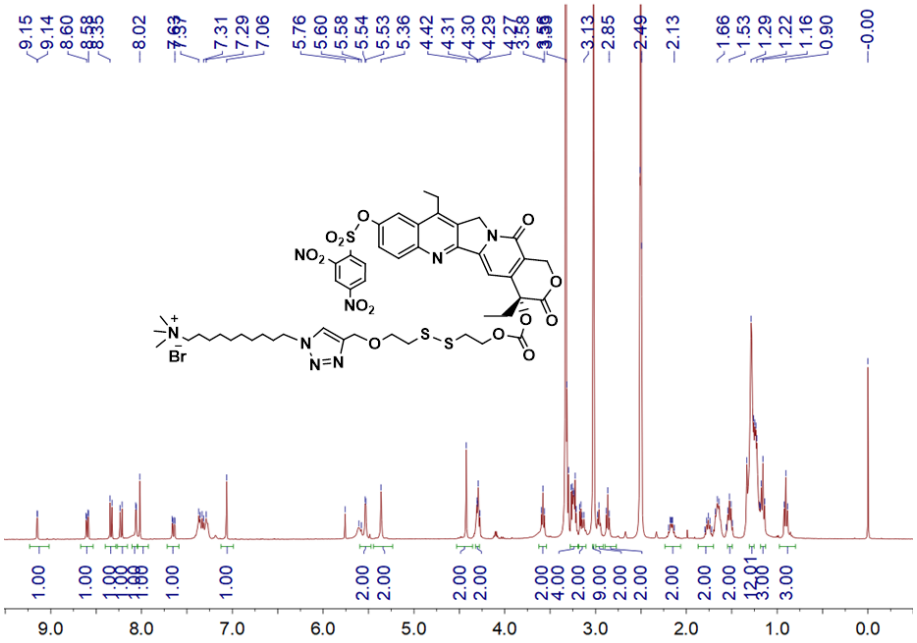


Fig. S9 ^{1}H NMR spectrum (400 MHz, DMSO-d_6, 298 K) of compound **DNS-CPT**.

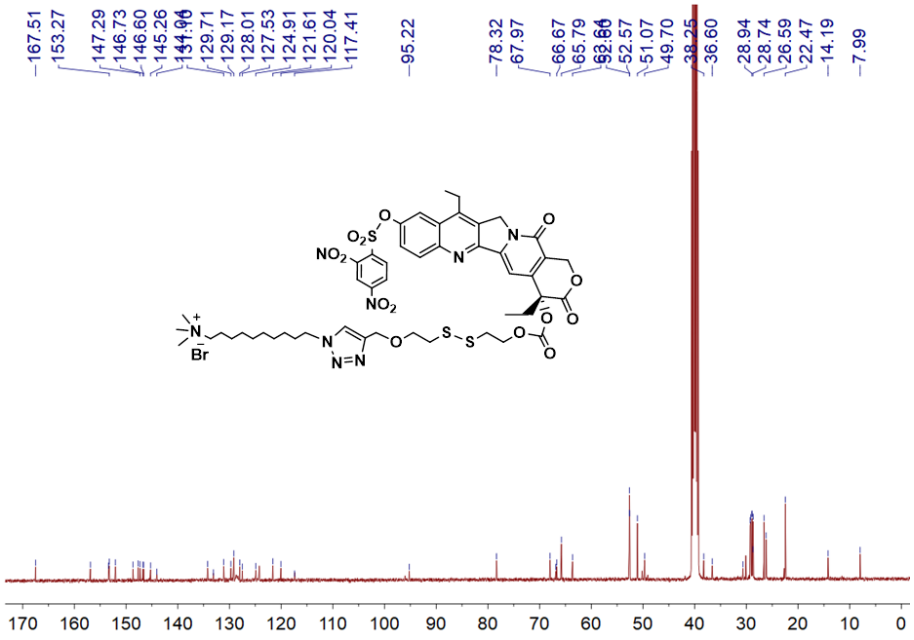


Fig. S10 ^{13}C NMR spectrum (100 MHz, DMSO-d_6, 298 K) of compound **DNS-CPT**.

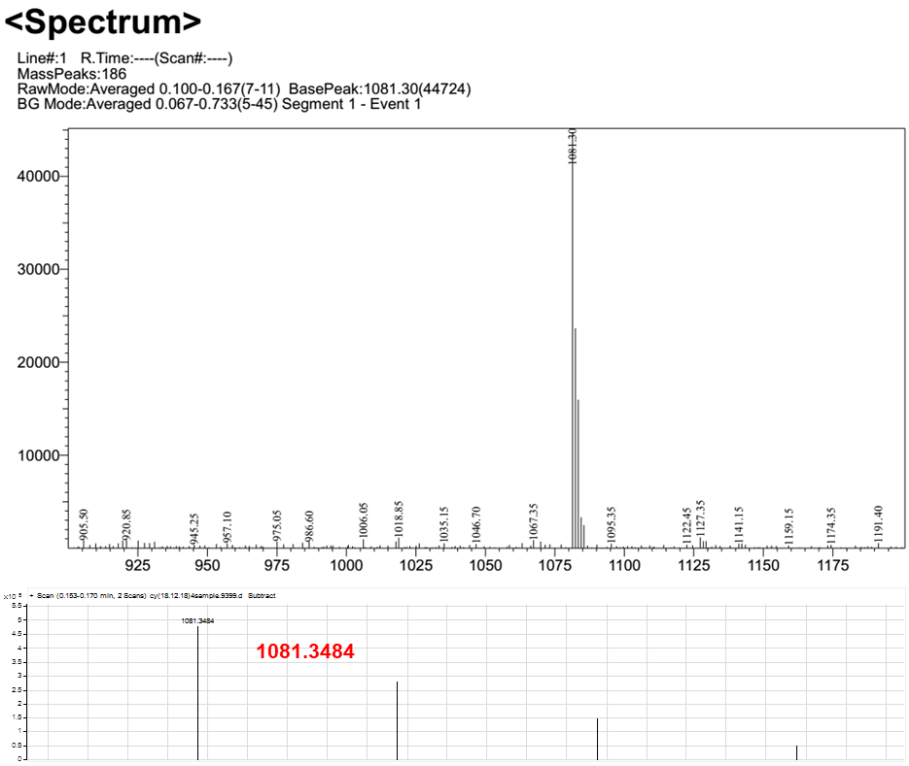
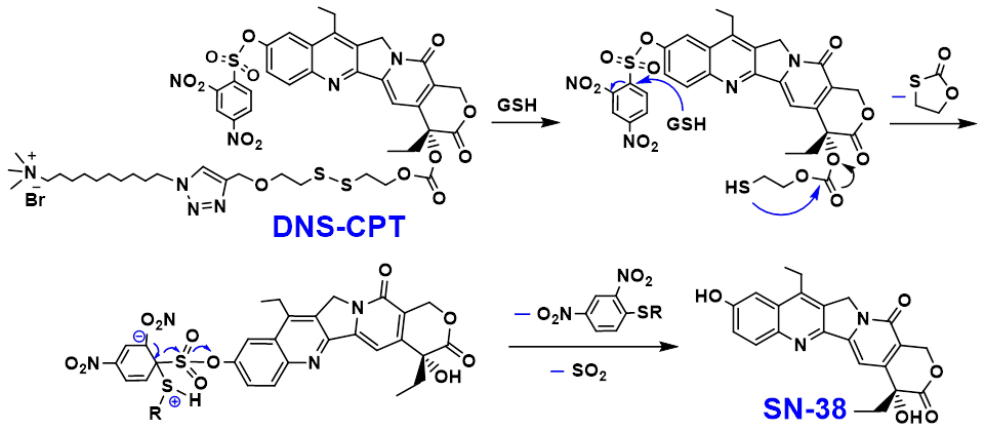


Fig. S11 LR-ESI-MS and HR-ESI-MS spectra of compound **DNS-CPT**.

3. The mechanism for GSH-triggered SN-38 release from guest DNS-CPT



Scheme S3. The mechanism for GSH-triggered SN-38 release from guest **DNS-CPT**.

4. Host-guest interaction between WP5 and DNS-CPT

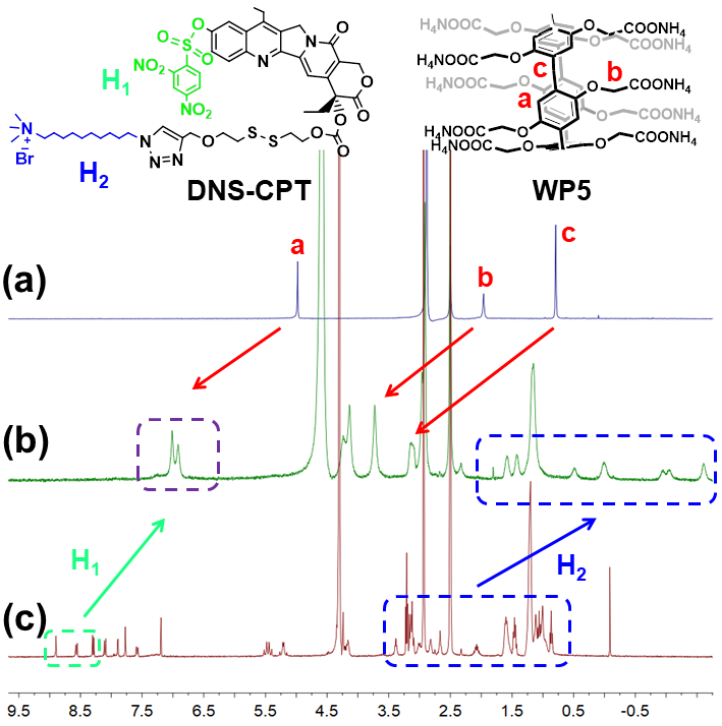


Fig. S12 ^{1}H NMR (400 MHz, D_2O and DMSO-d_6, v/v = 4:6, 298 K) spectra: (a) **WP5** (8.0 mM), (b) **DNS-CPT** (4.0 mM) and **WP5** (8.0 mM), and (c) **DNS-CPT** (8.0 mM).

5. Tyndall effect of WP5⊃DNS-CPT vesicles

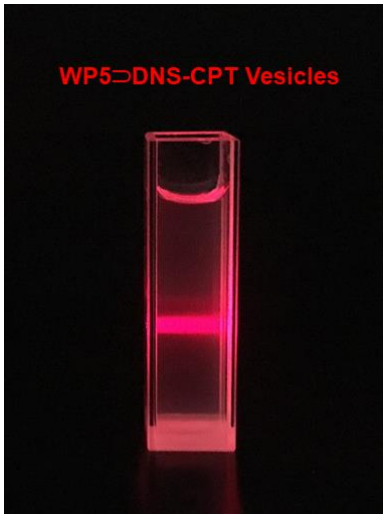


Fig. S13 Tyndall effect of **WP5⊃DNS-CPT** vesicles.

6. Determination of the best molar ratio of WP5 and DNS-CPT leading to aggregation

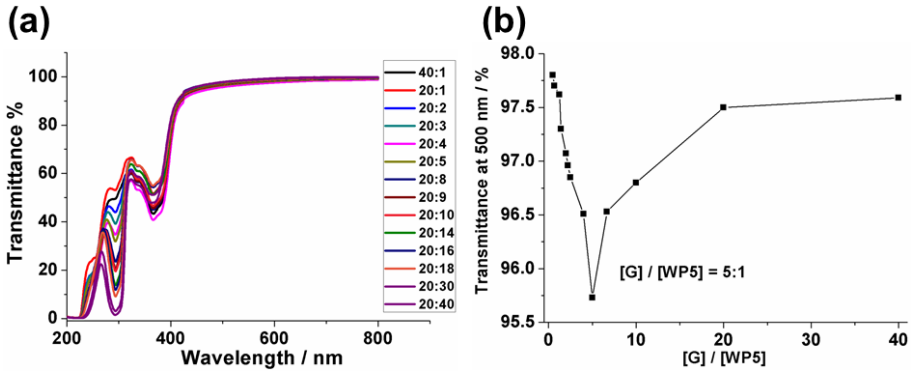


Fig. S14 (a) Optical transmittance of a mixture of **WP5** and **DNS-CPT** in water with a constant **DNS-CPT** concentration (0.1 mM) on increasing the concentration of **WP5** (0.025 – 2 equiv.) at 25°C. (b) Dependence of the relative optical transmittance at 500 nm on the **WP5** concentration with a fixed concentration of **DNS-CPT** (0.1 mM) at 25 ℃.

7. Critical aggregation concentration (CAC) determination of WP5⊃DNS-CPT

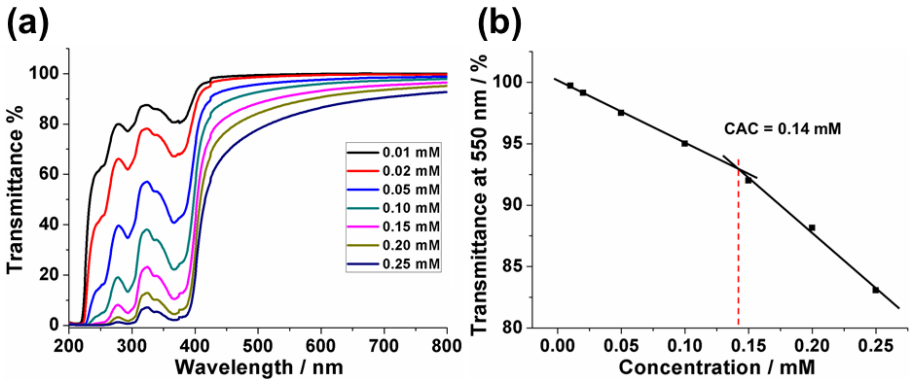


Fig. S15 Optical transmittance of a mixture of **WP5** and **DNS-CPT** in water with the best molar ratio of **WP5** and **DNS-CPT** (**[DNS-CPT]/[WP5]** = 5:1) on increasing the concentration of **DNS-CPT** (0.01 mM – 0.25 mM.) at 25 ℃.

8. Stability of WP5⊃DNS-CPT vesicles in water

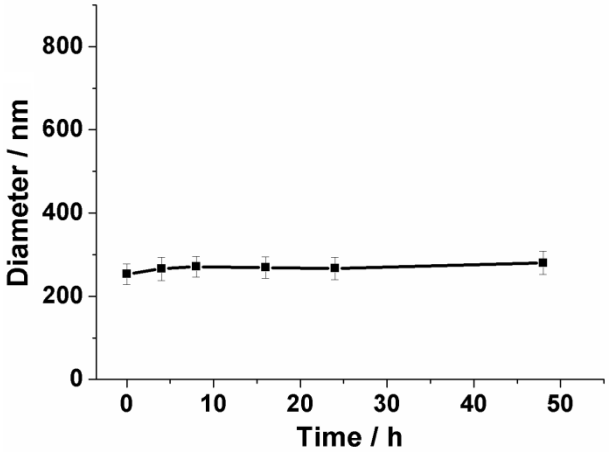


Fig. S16 Time-dependent size changes of **WP5⊃DNS-CPT** vesicles in water.

9. Zeta-potentials of the aggregates formed by WP5⊃DNS-CPT

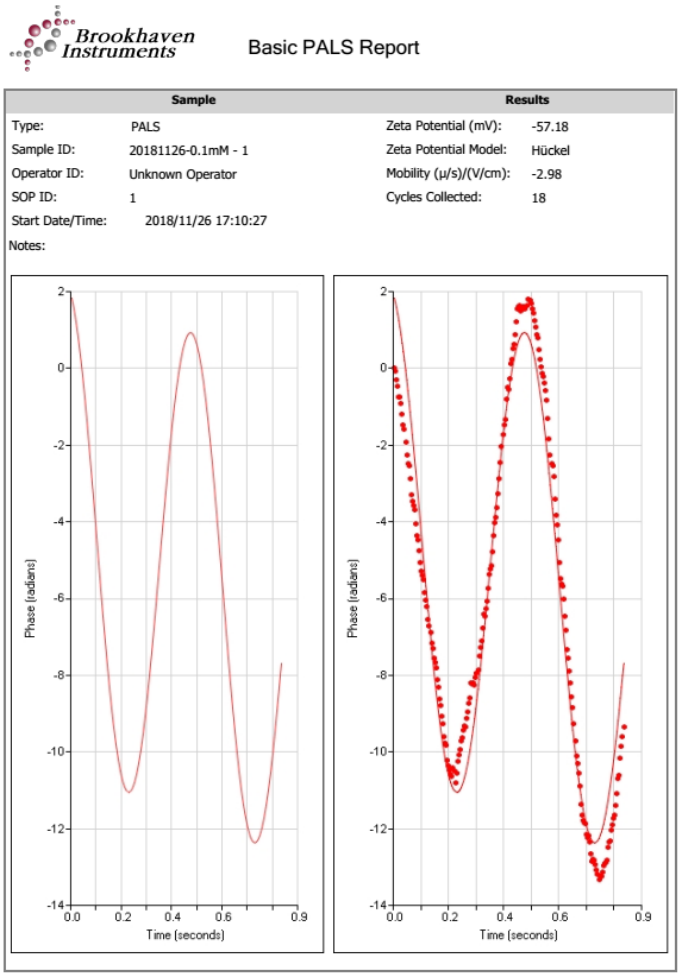


Fig. S17 Zeta-potentials of **WP5⊃DNS-CPT** vesicles: [**DNS-CPT**]/[**WP5**] = 5:1.

10. Investigation of the pH-responsiveness of WP5⊃DNS-CPT vesicles

As shown in Fig. S18, after incubation at pH = 5.0 for 24 h, DLS result showed that the size of the obtained **WP5⊃DNS-CPT** vesicles became very small (0.13 nm) and TEM image indicated no vesicle structure could be observed. More importantly, no obvious fluorescent signal could be observed under acidic condition (pH = 7.4 or 5.0), but when 10 mM GSH was added into the above solution, strong fluorescent signal of SN-38 could be observed, indicating that GSH responsiveness was the key factor to trigger the release of SN-38. Although pH responsiveness could not directly result in the releasing of SN-38, it was still useful for the disassembly of **WP5⊃DNS-CPT** prodrug vesicles to expose guest **DNS-CPT** and facilitate the release SN-38. Therefore, the results showed that SN-38 could be released much faster at pH=5.0 than that of pH=7.0 (Fig. S18c).

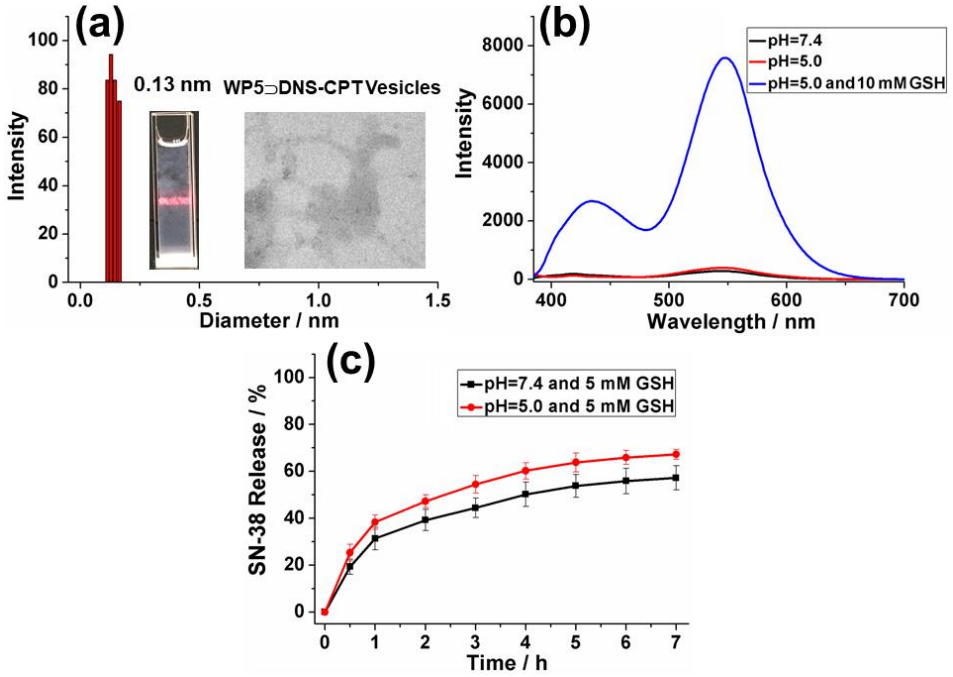


Fig. S18 (a) DLS data of **WP5⊃DNS-CPT** vesicles after incubation at pH = 5.0 for 24 h. Inset: Photo showing the Tyndall effect and TEM image of **WP5⊃DNS-CPT** vesicles after incubation at pH = 5.0 for 24 h. (b) Fluorescence spectra of **WP5⊃DNS-CPT** vesicles after being incubated under different conditions. (c) Time-dependent SN-38 release from **WP5⊃DNS-CPT** vesicles after being incubated at pH = 7.4 and 5.0 with the presence of 5 mM GSH for different time periods.

11. Investigation of the GSH-responsiveness of WP5⊃DNS-CPT vesicles

The disulfide bond and the DNS group of **DNS-CPT** are very sensitive to glutathione (GSH), which could directly lead to the efficient release of free SN-38 from **WP5⊃DNS-CPT** prodrug vesicles. Therefore, with increasing concentration of GSH, SN-38 will be released much faster. Moreover, considering the GSH concentration of cancer cells based on previous reports,[S7-S9] 2 μM, 2 mM, and 10 mM GSH were chosen to investigate the GSH-responsive drug release of the **WP5⊃DNS-CPT** vesicles, respectively. As shown in Fig. S19, no obvious fluorescent signal of SN-38 could be observed after incubating with 2 μM GSH, and obvious SN-38 release could be detected with the presence of 2 mM GSH, but it always lower than that of 10 mM GSH. The above results indicated that the release of SN-38 with the presence of 10 mM GSH is much more efficient.

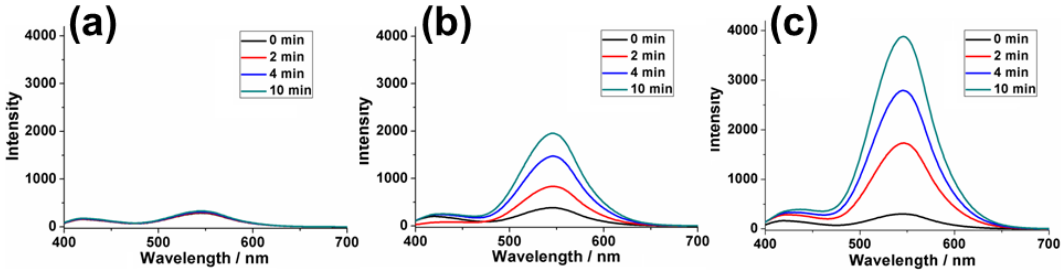


Fig. S19 Fluorescence spectra of **WP5⊃DNS-CPT** vesicles after being incubated with GSH for different time periods: (a) 2 μM GSH, (b) 2 mM GSH, and (c) 10 mM GSH.

12. DLS and TEM images of WP5⊃DNS-CPT vesicles under GSH-stimulus

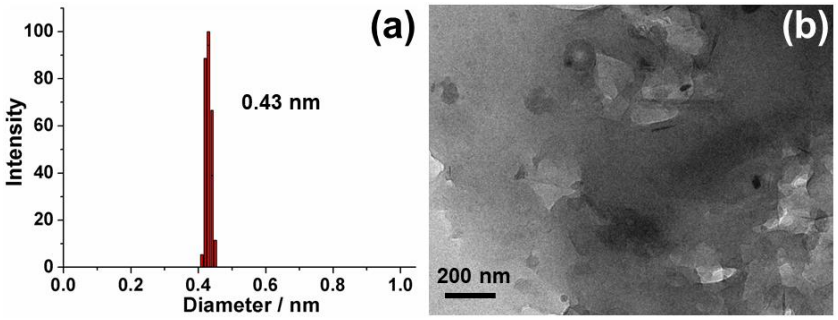


Fig. S20 DLS and TEM images of **WP5⊃DNS-CPT** vesicles with the presence of GSH (10.0 mM).

13. *In vitro* cytocompatibility of WP5, DNS-CPT, and WP5⊃DNS-CPT vesicles

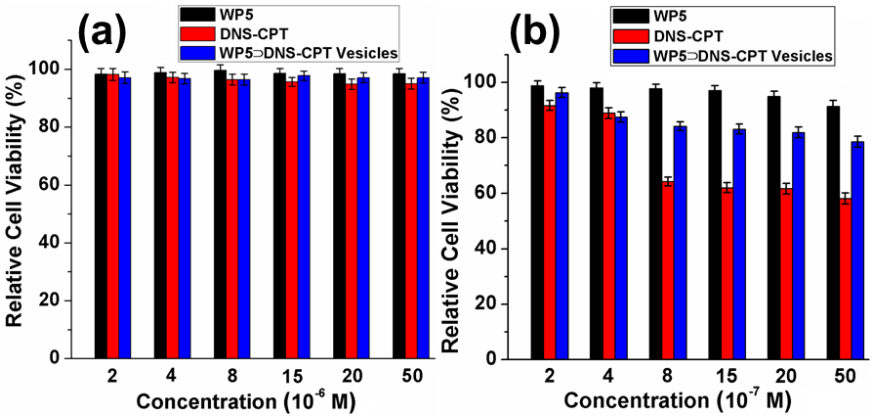


Fig. S21 *In vitro* cytocompatibility of **WP5**, **DNS-CPT**, and **WP5⊃DNS-CPT** vesicles against NIH 3T3 normal cells after incubation for (a) 24 h and (b) 48 h, respectively.

14. References

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