Supplementary Information

Dimeric Drug Polymeric Nanoparticles with Exceptionally High Drug Loading and Quantitative Loading Efficiency

Kaimin Cai,† Xi, He,[†,‡] Ziyuan Song,[†] Qian Yin,[†] Yanfeng Zhang,[†] Fatih M. Uckun,[§,⊥] Chen, Jiang,[*,‡] Jianjun Cheng[*,†]

†Department of Materials Science and Engineering, University of Illinois at Urbana-Champaign, Urbana, IL 61801, United States
‡Key Laboratory of Smart Drug Delivery, Ministry of Education, Department of Pharmaceutics, School of Pharmacy, Fudan University, Shanghai 201203, China
§Division of Hematology-Oncology, Systems Immunobiology Laboratory, Children's Center for Cancer and Blood Diseases, Children's Hospital Los Angeles, Los Angeles, CA 90027, United States
⊥Department of Pediatrics and Norris Comprehensive Cancer Center, University of Southern California Keck School of Medicine, Los Angeles, CA 90027, United States

Materials. Chemicals were purchased and used as received unless otherwise specified. Anhydrous dimethylformamide (DMF) was dried with a column packed with 4Å molecular sieves. Tetrahydrofuran (THF) were dried with a column packed with alumina. 2,6-bis(hydroxymethyl)aniline (BHA),[1] was synthesized according to literature report. mPEG-PLA (Mw 5000-3500) was purchased from Laysan Bio Inc. Camptothecin, 3-chloro-1-propanol, sodium azide, *N,N*-dimethylamino pyridine, dithiothreitol(DTT), triphosgene, and human serum (from platelet poor human plasma) were purchased from Sigma-Aldrich. Human serum was filtered through 0.22 μm PVDF filter to remove large aggregates. Phosphate buffered saline (PBS) was purchased from Mediatech, Inc. A Corning Subsidiary. The HeLa cells (ATCC, Manassas, VA, USA) used in the MTT (3-(4,5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide) assay were cultured in Dulbecco's modified Eagle medium (DMEM) containing 10% fetal bovine serum, 1000 units/mL aqueous penicillin G, and 100 μg/mL streptomycin at 37 ℃ under 5% CO_2 humidified atmosphere.

Instrumentation. NMR spectra were recorded on a Varian U500 (500 MHz) or VXR-500 (500 MHz) spectrometer. All chemical shifts were reported in part per million (ppm). Particle sizes and polydispersities were measured with a ZetaPlus dynamic light scattering (DLS) detector (15 mW laser, incident beam = 676 nm, detecting angle = 90 °, CONTIN algorithm, Brookhaven Instruments, Holtsville, NY, USA). HPLC was performed on a System Gold system (Beckman Coulter, Fullerton, CA, USA) equipped with a 126P solvent module, a System Gold 128 UV detector, and an analytical C18 column (Luna C18, 250 mm × 4.6 mm, 5 μm, Phenomenex). The UV wavelength for detecting camptothecin-based compounds was set at 369 nm. Analysis of Dox-SS-Dox and CPT-SS-CPT solubility was performed on a Shimadzu HPLC system (LC-20AT) connected with PDA detector (SPD-M20A) and fluorescence detector (RF-20A). Shimadzu C18 column (3 μm, 50 mm × 4.6 mm) was used for analysis. Gradient method was adopted using 0.1 % TFA-H_2O and acetonitrile as mobile phase (Figure S8d). TEM samples were prepared on 200 mesh carbon film supported copper grids. One drop of the nanoparticle solution (~ 10 μL) was placed on the grid and allowed to stand for 10 min. Filter paper was then used to remove the residual solution. The resulting sample was imaged using JEOL 2100 Cryo TEM at 200 kV. Fluorescence was measured on Perkin-Elmer LS55 fluorescence spectrometer. MTT absorption was measured on Perkin Elmer Victor3 multi-label readers at λ_{abs} = 570 nm.

CPT-SS-CPT water solubility test

1 mg CPT-SS-CPT was suspended in 3 mL water and stirred vigorously at room temperature for 24 hour. The suspension was filtered through 0.45 μm PVDF memberane to remove insoluble solid. The solution was diluted 1:1 with 0.1 % TFA-water then subject to HPLC analysis. No CPT-SS-CPT peak was detected in the solution by fluorescence detector. The fluorescence detector could detect CPT-SS-CPT down to 5 ng/mL as shown in Figure S8c. Therefore, the

saturated CPT-SS-CPT concentration is lower than 10 ng/mL.

Nanoparticle Preparation.
Drug or dimeric prodrug was first dissolved in DMF with mPEG-PLA at designated weight ratio and the drug concentration was 10 mg/mL. For CPT/mPEG-PLA encapsulation, mPEG-PLA concentration was 10 mg/mL and CPT concentration was adjusted accordingly except that for 1:1 CPT/mPEG-PLA experiment, concentration was both 3 mg/mL due to the poor solubility of CPT in DMF. 100 μL above solution was added dropwise into 2.0 mL DI water with mild stirring (550 rpm) using a magnetic bar. Nanoparticle size and distribution was analyzed by DLS directly without further purification.

Nanoparticle Formulation Study
Drug aggregation test by post mPEG-PLA coating (described in Figure 2a):
$mPEG_{5k}$-PLA_{3k} solution was first prepared by mixing 25 μL $mPEG_{5k}$-PLA_{3k} solution (20 mg/mL in DMF) with 2.0 mL DI H_2O. 25 μL DMF solution of CPT-SS-CPT (10 mg/mL) was added into 2.0 mL PBS at 550 rpm mixing at once (denoted as solution A). 500 μL solution A was mixed with 1.0 mL as-prepared mPEG-PLA solution 10 seconds or 30 seconds after the drug dimer addition. The leftover solution A (without mPEG-PLA) was diluted with 1.0 mL H_2O. The particle sizes of all the solutions were monitored by DLS. The experiment was done in triplicate and the figure is presented as mean value ± standard deviation.

The post addition experiment described in Figure 2b-c
50 μL DMF solution of $mPEG_{5k}$-PLA_{3k} (20 mg/mL) was added dropwise into 2.0 mL DI water with mild stirring (550 rpm) using a magnetic bar. The micelle size was then measured by DLS. To the micelle solution was then added 12.5 μL DMF solution of CPT-SS-CPT (5 mg/mL) under stirring. Extra 37.5 μLCPT-SS-CPT solution was added in 3 three portions and the size was monitored by DLS right after the mixing. 4 independent entries were done and the results were presented as mean value ± standard deviation.

Nanoparticle stability test.
The freshly prepared nanoparticle solution was diluted with PBS 10, 100 and 1000 fold to test its stability in the presence of salt and dilution effect. The size and distribution of the nanoparticle was measured by DLS. For serum stability test, 1.0 mL nanoparticle solution (~400 μg/mL) was diluted with 1.0 mL 1:1 human serum/PBS and characterized by DLS. No significant size change was observed for all groups over 24 hours.

Drug loading and loading efficiency measurement.

The freshly prepared nanoparticle solution was centrifuged at 3000 rpm for 10 minutes to remove large aggregate. An aliquot of the supernatant was dissolved in DMF and the CPT conjugate content was quantified by UV-vis absorption at λ_{max} through standard curve. The detection wavelength for CPT, CPT-SS-CPT, were 369, 364 nm respectively. Drug loading was calculated as DL = *w*(drug)/[*w*(drug conjugates)+ *w*(polymer)] and drug loading efficiency was calculated as LE = *w*(drug in NP)/[*w*(initial drug added)].

For Dox-SS-Dox nanoparticles, the freshly prepared nanoparticle solution was centrifuged at 3000 rpm for 10 minutes to remove large aggregate. An aliquot of the supernatant was dissolved in 5 fold acetonitrile and the Dox-SS-Dox content was quantified by HPLC standard curve at UV absorption 234 nm. Drug loading and loading efficiency was calculated accordingly.

Critical micelle concentration (CMC) determination of $mPEG_{5k}$-PLA_{3k}

mPEG-PLA was first dissolved in PBS to designated concentration. Then 1.0 mg/mL DMSO solution of Nile Red was added to a final concentration of 2×10^{-4} mg/mL (6×10^{-7} M). The fluorescence of the solution was then measured by fluorescence spectrometer. (λ_{ex}=557 nm, λ_{em} = 601 nm) The CMC was determined by extending the linear FL intensity of both the high and low concentration region.

CPT release from CPT-SS-CPT/mPEG-PLA nanoparticle (SS NP).

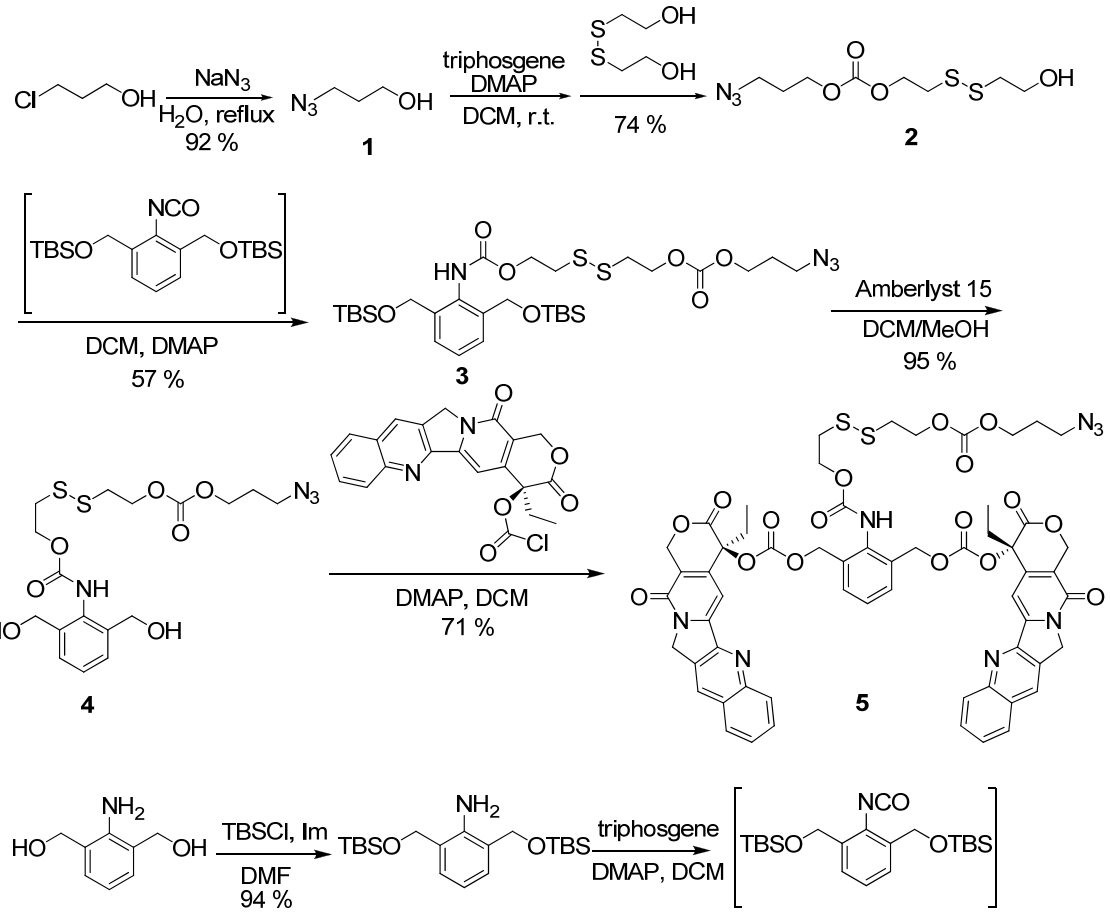
The SS NPs were diluted with PBS to 2 μg/mL. Then 10 mM DTT was added and the clear solution was incubated at 37 ℃. At specified time point, 1.0 mL solution was collected, centrifuged at 15 krpm to remove particles and the supernatant was subject to HPLC analysis after 1:1 dilution with 0.1% TFA-H_2O to quantify the drug release content (λ_{abs} = 369 nm). Gradient method (0.1% TFA-H_2O/Acetonitrile, see Figure S8d) was adopted to elute both CPT and CPT-SS-CPT in single run. Blank control was carried out without the addition of DTT.

***In vitro* cytotoxicity of SS NP**.

Standard MTT protocol was followed to evaluate the cytotoxicity of the high loading nanoparticles. Briefly, HeLa cells were seeded in 96-well plate at 1000 cells/well in 100 μL DMEM medium and were allowed to attach overnight. Free CPT and irinotecan was first dissolved in DMSO and diluted with PBS to the concentration desired. For the highest concentration, the DMSO content was 5% for CPT and 1% for irinotecan respectively. SS NPs were formulated in PBS from 10/2.5 mg/mL CPT-SS-CPT/mPEG-PLA DMF solution through nanoprecipitation. The particles were then washed with PBS via ultrafiltration 5 times (3 krpm, 5 min each) and diluted with PBS, filtered through 0.45 μm PVDF filter to sterilize. No significant NP size change was observed throughout the process as characterized by DLS. 10 μL drug or nanoparticle solution was added into the well to the designated final concentration and incubated at 37 ^{o}C for 72 hours. PBS and 5% DMSO-PBS was taken as 100 % control. 20 μL 5 mg/mL

MTT solution was added to the medium and incubated at 37 ℃ for 3 hours. Then the medium was carefully removed and the violet crystal was dissolved in 100 μL DMSO and quantified by absorption at λ_{abs} = 570 nm. Cytotoxicity of N_3-SS-BHA was conducted following the same procedure except that N_3-SS-BHA was first dissolved in DMSO as 1 mg/mL solution and then diluted with PBS. The cell viability data was fitted by dose-response curve as follows, in which parameter c_0 is the IC_{50} value.

$$y = A_1 + \frac{A_2 - A_1}{1+10^{(\log c_0/c)p}}$$



Scheme S1. Synthesis of CPT-SS-CPT

Synthesis of 3-azido-1-propanol (**1**)

An aqueous solution (150 mL) of 3-chloro-1-propanol (9.45 g, 100 mmol) was refluxed with sodium azide (9.75 g, 150 mmol, 1.5 equiv) for 22 h. The product was extracted with 100 mL methylene chloride three times, dried over sodium sulfate and concentrated as colorless oil (9.31 g, 92 % yield). ^{1}H NMR (500 MHz, $CDCl_3$), δ 3.76 (t, 2H, J = 5.7 Hz), 3.46 (t, 2H, J = 6.5 Hz), 1.84 (m, 2H). ^{13}C NMR (126 MHz, $CDCl_3$), δ 59.63, 48.31, 31.30. HRMS-EI (*m/z*): $[M]^+$ calcd for $C_3H_7N_3O$, 101.0589; observed, 101.0589.

Synthesis of 3-azidopropyl 2-((2-hydroxyethyl)disulfanyl)ethyl carbonate (**2**)

5 mL DCM solution of **1** (1.13 g, 11.2 mmol) was added slowly to a phosgene-toluene solution (15 wt%, 30 mL, 45 mmol, 4.0 equiv) at 0 ^{o}C. After stirring at room temperature for 17 h, solvent and excessive phosgene was removed under vacuum. The as prepared chloroformate was redissolved in 10 mL DCM, which was added into a DCM/THF solution (10/40 mL) of 2,2'-hydroxylethyl disulfide (8.64 g, 56 mmol, 5.0 equiv) and DMAP (2.8 g, 23 mmol, 2.0 equiv). After stirring for 2 h, the mixture was concentrated under vacuum, extracted with 100 mL EtOAc, washed excessively with water (100 mL each, 6 times). The organic layer was dried over sodium sulfate and solvent was removed under vacuum affording a light yellow oil as the product (2.34 g, 74 % yield). ^{1}H NMR (500 MHz, $CDCl_3$) δ 4.41 (t, 2 H, *J* = 6.7 Hz), 4.25 (t, 2 H, *J* = 6.1 Hz), 3.90 (m, 2 H), 3.45 (t, 2 H, *J* = 6.7 Hz), 2.96 (t, 2 H, *J* = 6.7 Hz), 2.89 (t, 2 H, *J* = 5.7 Hz), 1.93 (m, 2H). ^{13}C NMR (126 MHz, $CDCl_3$), δ 154.87, 65.79, 65.05, 60.23, 47.85, 41.57, 36.75, 28.15. HRMS-EI (*m/z*): $[M]^+$ calcd for $C_8H_{15}N_3O_4S_2$, 281.0504; observed, 281.0513.

Synthesis of N_3-SS-BHA-OTBS (**3**)

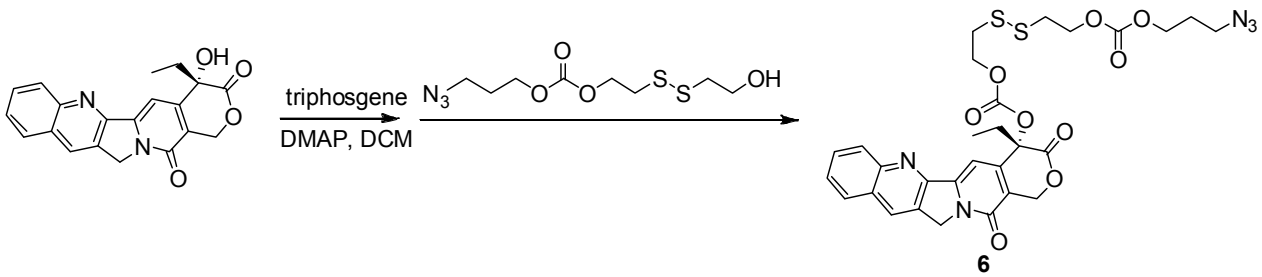
To 2 mL DCM solution of 2,6-bis((tert-butyldimethylsilyloxy)methyl)aniline (110 mg, 0.28 mmol) and DMAP (88 mg, 0.70 mmol, 2.5 equiv) was added triphosgene (29 mg, 0.090 mmol, 0.35 equiv). White precipitates formed and dissolved gradually over 20 minutes. After stirred for 2 h, 2 mL DCM solution of **2** (77 mg, 0.28 mmol, 1.0 equiv) was added. The mixture was subject to column after the reaction finished as monitored by TLC. The mixture was subject to chromatography directly. (Hexane: EtOAc 10:1) The product was obtained as a colorless oil (110 mg, 57 % yield). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.58 (br 1H), 7.34 (d, 2H, *J* = 7.5 Hz), 7.21 (t, 1H, *J* = 7.5 Hz), 4.70 (s, 4H), 4.40 (m, 2H+2H), 4.21 (t, 2H, *J* = 6.2 Hz), 3.41 (d, 2H, *J* = 6.7 Hz), 2.97 (m, 2H+2H), 1.92 (m, 2H), 0.92 (s, 18H), 0.09 (s, 12H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 154.71, 153.86, 136.07, 132.56, 126.94, 125.97, 65.62, 64.87, 62.89, 62.83, 47.75, 37.60, 36.87, 28.08, 25.84, 18.23, -5.36. HRMS-ESI (*m/z*): $[M+H]^+$ calcd for $C_{29}H_{53}N_4O_7S_2Si_2$, 689.2894; observed, 689.2894.

Synthesis of N_3-SS-BHA (**4**)

110 mg **3** was stirred with 100 mg amberlyst-15 in a mixture of DCM/MeOH (8/6 mL) overnight. After the reaction completed, the suspension was subject to flash column (hex:EtOAc 4:1 to EtOAc) giving a white solid (70 mg, 95 % yield). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.60 (br 1H), 7.38 (d, 2H, *J* = 7.5 Hz), 7.27 (t, 1H, *J* = 7.5 Hz), 4.63 (d, 4H, *J* = 5.0 Hz), 4.44 (m, 2H+2H), 4.21 (t, 2H, *J* = 6.2 Hz), 3.41 (d, 2H, *J* = 6.7 Hz), 3.01 (m, 6H), 1.92 (m, 2H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 155.58, 154.53, 136.41, 133.04, 128.82, 126.79, 65.35, 64.77, 63.07, 61.77, 47.47, 37.18, 36.54, 27.73. HRMS-ESI (*m/z*): $[M+H]^+$ calcd for $C_{17}H_{25}N_4O_7S_2$, 461.1165; observed, 461.1165.

Synthesis of CPT-SS-CPT (**5**)

A DCM suspension (3 mL) of camptothecin (63.5 mg, 0.182 mmol, 2.4 equiv), DMAP (49 mg, 0.40 mmol, 5.3 equiv) and triphosgene(18.9 mg, 0.064 mmol, 0.84 equiv) was stirred at room temperature for 15 minutes, during which the camptothecin dissolved gradually. Then 1 mL DCM solution of **4** was added once. After completion of the reaction as monitored by TLC, the solution was subject to chromatography. DCM:MeOH 50:1, EtOAc:MeOH 20:1 giving an off-white powder (65 mg, 71 % yield). ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.39 (s, 2H), 8.23 (d, *J* = 8.5 Hz, 2H), 7.93 (d, *J* = 8.1 Hz, 2H), 7.84 (s, 2H), 7.66 (s, 2H), 7.35 (d, *J* = 7.5 Hz, 3H), 7.30 (s, 2H), 7.23 (t, *J* = 7.5 Hz, 1H), 5.50 (dd, *J* = 151 Hz, 17Hz, 4H), 5.25 (s, 4H), 5.11 (s, 4H), 4.26 (s, 2H), 4.14 (s, 4H), 3.35 (m, 4H), 2.81 (m, 2H), 2.73 (t, *J* = 6.5 Hz, 2H), 2.18 (dq, *J* = 53.5 Hz, 6.5 Hz, 4H), 1.85 (m, 2H), 0.98 (t, *J* = 6.5 Hz, 6H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 167.40, 157.31, 154.81, 154.37, 153.60, 152.19, 148.73, 146.47, 145.68, 133.15, 132.60, 131.50, 130.95, 129.78, 129.58, 128.59, 128.34, 128.27, 128.22, 127.96, 120.27, 96.12, 78.14, 77.41, 77.15, 76.90, 67.12, 67.01, 65.69, 65.00, 63.46, 50.14, 47.88, 37.27, 36.79, 31.90, 28.15, 7.74. HRMS-ESI (*m/z*): $[M+H]^+$ calcd for $C_{59}H_{53}N_8O_{17}S_2$, 1209.2970; observed, 1209.2961.

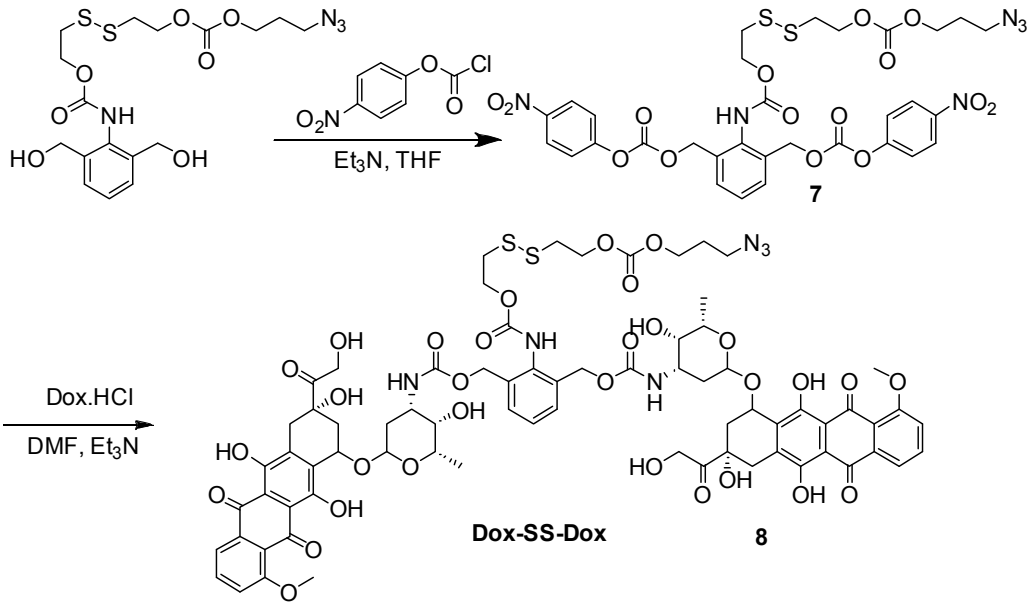


Scheme S2. Synthesis of CPT-SS

Synthesis of CPT-SS (**6**)

A DCM suspension (1 mL) of camptothecin (34.8 mg, 0.10 mmol, 1.0 equiv), DMAP (26.8 mg, 0.22 mmol, 2.2 equiv) and triphosgene(11.0 mg, 0.037 mmol, 0.37 equiv) was stirred at room temperature for 10 minutes, during which the camptothecin dissolved gradually. Then 1 mL DCM solution of **2** (33.8 mg, 0.12mmol, 1.2 equiv) was added once. After completion of the reaction as monitored by TLC, the reaction was quenched by 1 drop of water and subject to chromatography (pure EtOAc) giving an off-white powder (43 mg, 66 % yield).

^{1}H NMR (500 MHz, $CDCl_3$) δ 8.41 (s, 1H), 8.24 (d, *J* = 8.5 Hz, 1H), 7.95 (d, *J* = 8.1 Hz, 1H), 7.85 (dd, *J* = 8.5 Hz, 8.1 Hz, 1H), 7.68 (dd, *J* = 8.5 Hz, 8.1 Hz, 1H), 7.35 (s, 1H), 5.55 (dd, *J* = 155.6, 17.2 Hz, 2H), 5.31 (s, 2H), 4.35 (m, 4H), 4.20 (t, *J* = 6.2 Hz, 2H), 3.40 (t, *J* = 6.6 Hz, 2H), 2.96 (d, *J* = 6.2 Hz, 2H), 2.92 (d, *J* = 6.2 Hz, 2H), 2.22 (dq, *J* = 58.0 Hz, 6.6 Hz, 2H), 1.92 (tt, *J* = 6.6 Hz, 6.2 Hz, 2H), 1.01 (t, *J* = 6.6 Hz, 3H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 167.35, 157.34, 154.79, 153.54, 152.28, 148.79, 146.45, 145.65, 131.44, 130.92, 129.63, 128.59, 128.31, 128.28, 128.25, 120.41, 96.22, 78.13, 67.17, 66.61, 65.64, 65.00, 50.11, 47.90, 37.09, 36.62, 31.96, 28.22, 7.75. HRMS-ESI (*m/z*): $[M+H]^+$ calcd for $C_{29}H_{30}N_5O_9S_2$, 656.1485; observed, 656.1488.



Scheme S3. Synthesis of Dox-SS-Dox

Synthesis of nP-SS-BHA (**7**)

To a stirred DCM solution of *p*-nitrophenyl chloroformate (101 mg, 0.50 mmol, 5.0 equiv in 1mL DCM) was added a mixture of **4**(N_3-SS-BHA) (46 mg, 0.10 mmol, 1.0 equiv), triethylamine (84 uL, 0.60 mmol, 6.0 equiv) in 1 mL DCM. The solution was stirred at room temperature overnight and then subject to silica chromatography. Hexane: EtOAc 3:1 to 2:1. The product was obtained as a white powder after being crystallized in DCM/hexane (32 mg, 41 % yield). ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.26 (d, 4H, *J* = 14 Hz), 7.58 (d, 2H, *J* = 7.5 Hz), 7.44 (t, 1H, *J* = 7.5 Hz), 7.37 (d, 4H, *J* = 14 Hz), 7.2 (br 1H), 5.36 (s, 4H), 4.44-4.39 (m, 2H+2H), 4.20 (t, 2H, *J* = 6.0 Hz), 3.38 (d, 2H, *J* = 6.5 Hz), 2.99 (m, 6H), 1.90 (m, 2H).^{13}C NMR (126 MHz, $CDCl_3$) δ 155.43, 154.93, 154.58, 152.63, 145.62, 134.06, 132.44, 131.13, 128.15, 125.44, 121.86, 67.46, 65.80, 65.14, 63.56, 47.91, 37.73, 36.94, 28.21. HRMS-ESI (*m/z*): $[M+H]^+$ calcd for $C_{31}H_{31}N_6O_{15}S_2$, 791.1289; observed, 791.1290.

Synthesis of Dox-SS-Dox (**8**)

Dox-HCl salt (24.9mg, 0.043 mmol, 2.2 equiv) was dissolved in 1.2 mL DMF with triethylamine (24 μL, 0.18 mmol, 9.0 equiv). 600 μL DMF solution of **7** was dropwise added into the above solution. After the clear solution was stirred at room temperature overnight, 10 mL DCM was added and washed with 0.1 M HCl (aq) 20 mL, dried over Na_2SO_4, concentrated, subject to chromatography. (DCM:MeOH 25:1 to 15:1) The product was obtained as a red powder (17 mg, 54 % yield). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.99-7.77 (m, 4H), 7.37 (d, *J* = 7.5 Hz, 2H), 7.28 (m, 2H), 7.17 (t, *J* = 7.5 Hz, 1H), 5.47 (s, 2H), 5.29-5.23 (m, 4H), 5.06 (m, 2H), 4.95 (m, 2H), 4.75 (s,

4H), 4.52 (s, 1H), 4.30 (m, 2H), 4.17 (m, 2H+4H), 4.06 (s, 6H), 3.76 (m, 2H), 3.65 (m, 2H), 3.38 (t, J = 6.0 Hz, 2H), 3.12 (m, 2H), 2.88 (m, 4H+2H+2H), 2.34 (d, J = 12.5 Hz, 2H), 2.16 (d, J = 12.5 Hz, 2H), 1.89 (m, 4H+2H), 1.32 (d, 6H). HRMS-ESI (*m/z*): $[M-H]^-$ calcd for $C_{73}H_{77}N_6O_{31}S_2$, 1597.4075; observed, 1597.4070.

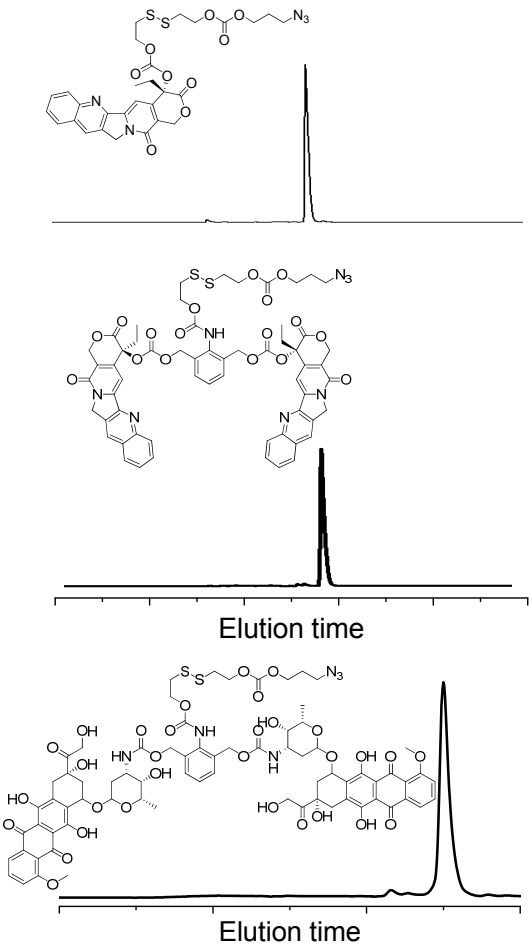


Figure S1. HPLC trace of synthesized CPT-SS, CPT-SS-CPT, and Dox-SS-Dox. λ_{abs} = 369 nm for CPT-SS, CPT-SS-CPT, and λ_{abs} = 234 nm for Dox-SS-Dox, respectively.

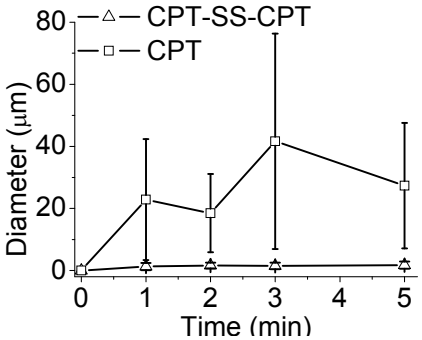
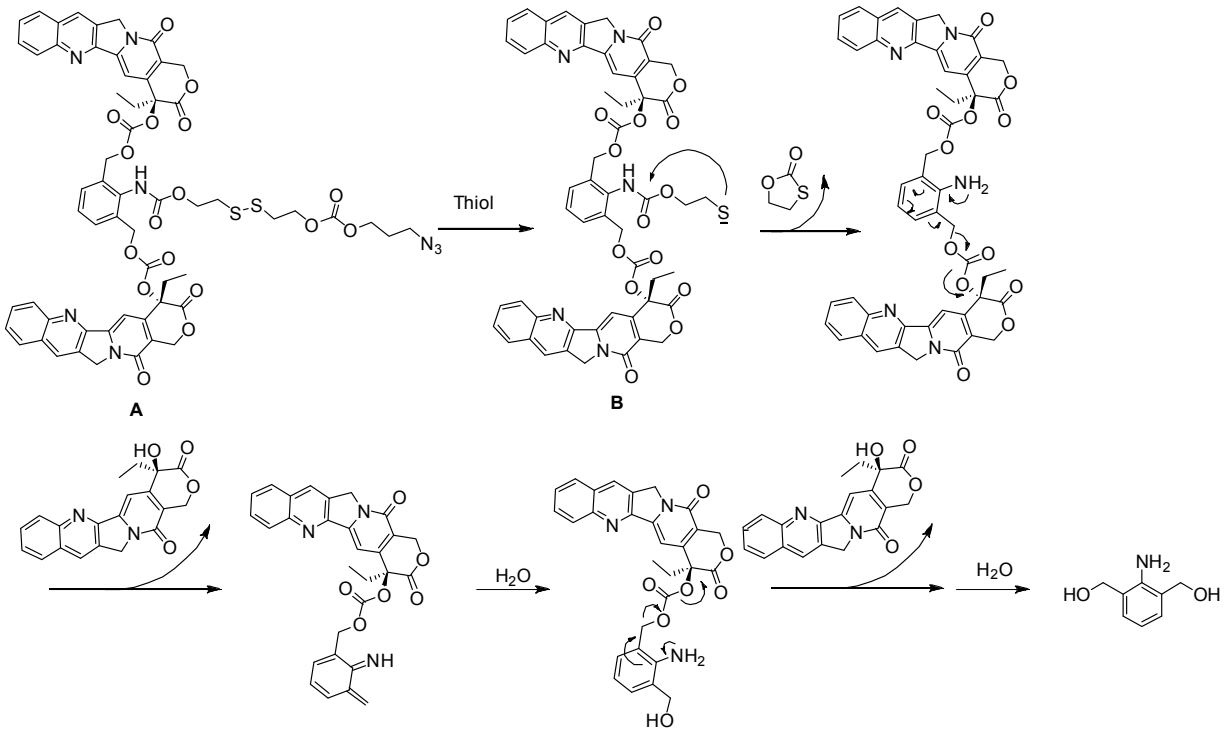


Figure S2. Size evolution of CPT and CPT-SS-CPT aggregates over time without amphiphilic polymers. Data are presented as mean diameter ± half peak width.

Table S1. Formulation results of drugs with mPEG-PLA via nanoprecipitation (DMF-H_2O).[a]

Entry	Drug	Drug/ mPEG-PLA	*d* (nm)	PDI	LE(%)[b]	DL(%)[c]
1	CPT	1.0	2669[d]	0.28	-	-
2	CPT	0.1	4588[d]	0.36	-	-
3	CPT-SS-CPT	1.0	171	0.09	> 99	28.8
4	CPT-SS-CPT	2.0	161	0.16	> 99	38.4
5	CPT-SS-CPT	3.0	173	0.15	> 99	43.2
6	CPT-SS-CPT	4.0	174	0.13	> 99	46.1
7	CPT-SS-CPT	6.0	165	0.13	> 99	49.3
8	CPT-SS-CPT	8.0	169	0.07	> 99	51.2
9	CPT-SS-CPT	10.0	181	0.12	> 99	52.3
10	CPT-SS	1.0	726[d]	0.30	-	-
11	None[e]	0	45	0.22	-	-

a, 100 μL DMF solution of drug/mPEG-PLA was added dropwise into 2.0 mL DI water. *b*, Drug loading efficiency. *c*, Drug loading of CPT. *d*, large aggregates were observed. *e*, mPEG-PLA without drugs was formulated.



Scheme S4. Proposed release mechanism of thiol triggered drug release.

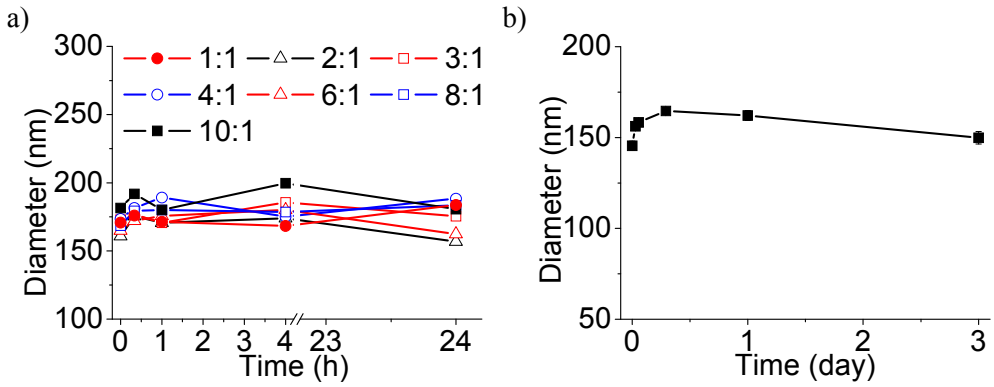


Figure S3. (a) Hydrodynamic diameter of SS NPs with different CPT-SS-CPT/mPEG-PLA feed ratio. 100 μL DMF solution of CPT-SS-CPT (10 mg/mL) with corresponding mPEG-PLA (weight ratio shown in figure) was added dropwise into 2.0 mL DI water. Particle size was measured by DLS without purification. (b) SS NP size change in the presence of 1:1 human serum.

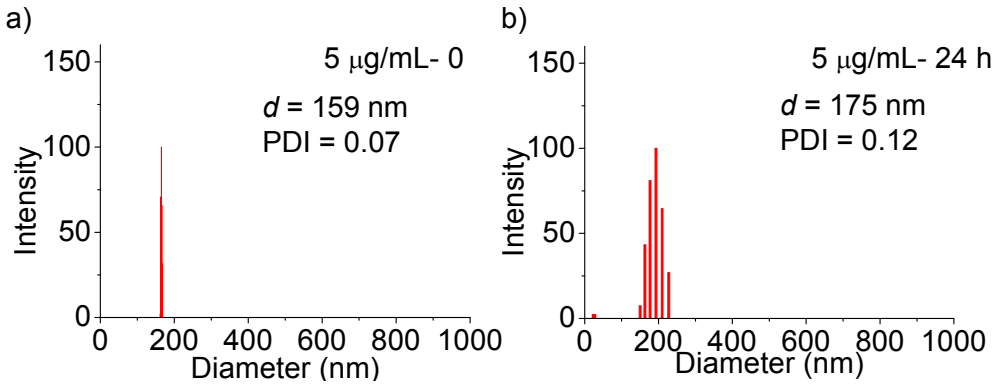


Figure S4. 5 μg/mL CPT-SS-CPT nanoparticle size distribution before (a) and after (b) 24 hour incubation in PBS. Note the equivalent mPEG-PLA concentration is 0.8 μg/mL and the CMC of the mPEG-PLA is 69 μg/mL. Formulation procedure: 100 μL DMF solution of CPT-SS-CPT/mPEG-PLA (10/1.7 mg/mL) was added dropwise into 2.0 mL DI water, then diluted with PBS to 5 μg/mL CPT-SS-CPT for DLS test.

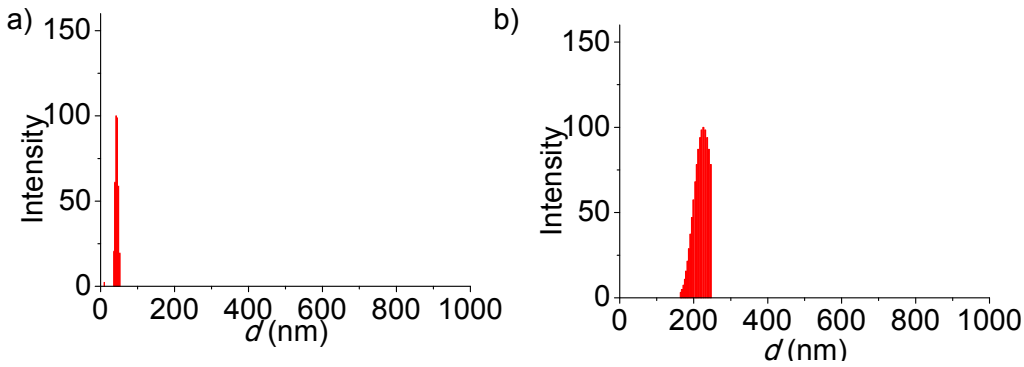


Figure S5. Nanoparticle size distribution of mPEG-PLA solution before (a) and after (b) the addition of DMF solution of CPT-SS-CPT.

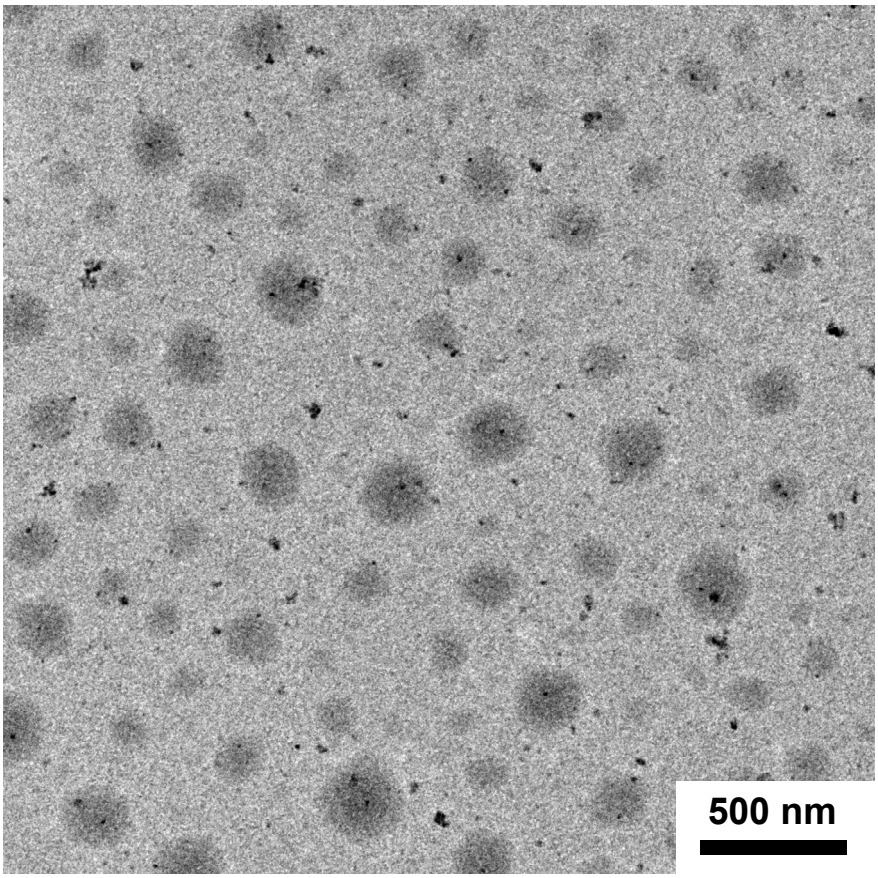


Figure S6. Broad view of TEM image of CPT-SS-CPT/mPEG-PLA nanoparticles prepared through post drug addition described in Figure 2c. Drug/mPEG-PLA ratio = 1:4.

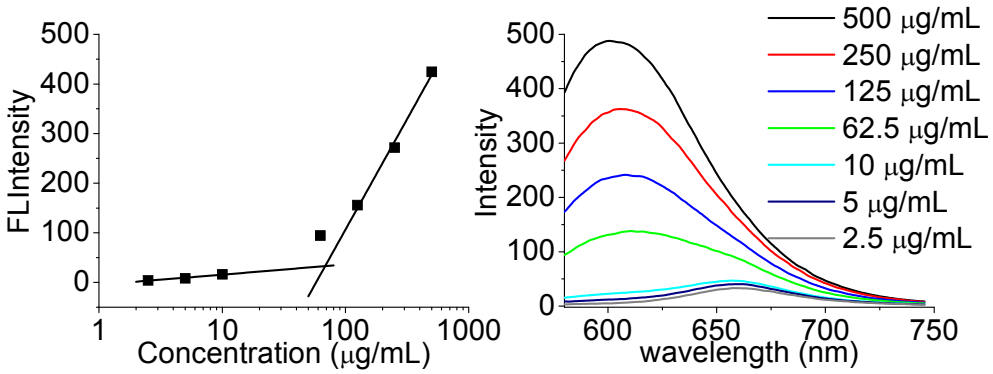


Figure S7. Nile Red Fluorescence intensity change versus concentrations of $mPEG_{5k}$–PLA_{3k}. The Nile Red concentration was fixed at 6×10^{-7} M. The CMC in PBS was determined to be 69 μg/mL.

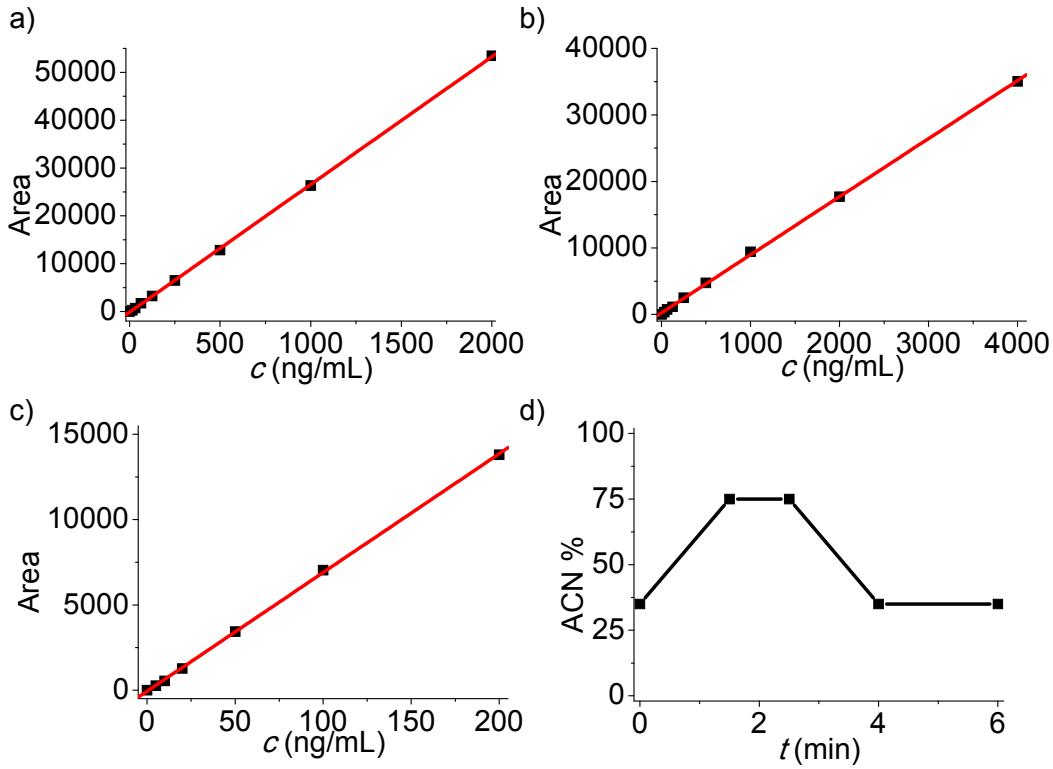


Figure S8. HPLC Standard curve of CPT (a) and CPT-SS-CPT (b) based on UV absorption at 369 nm. (c) HPLC Standard curve of CPT-SS-CPT based on fluorescence (λ_{ex} = 369 nm, λ_{em} = 442 nm) (R^2 = 0.9997). (d) Time line of HPLC method as shown by acetonitrile gradient change.

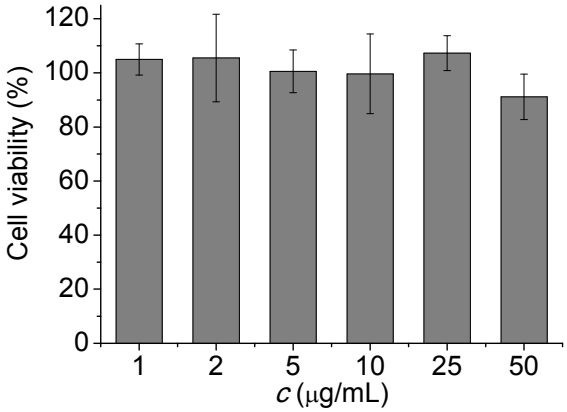


Figure S9. Cytotoxicity of N_3-SS-BHA in HeLa cells. No significant toxicity was observed up to 50 μg/mL.

^{1}H NMR and ^{13}C NMR of new compound

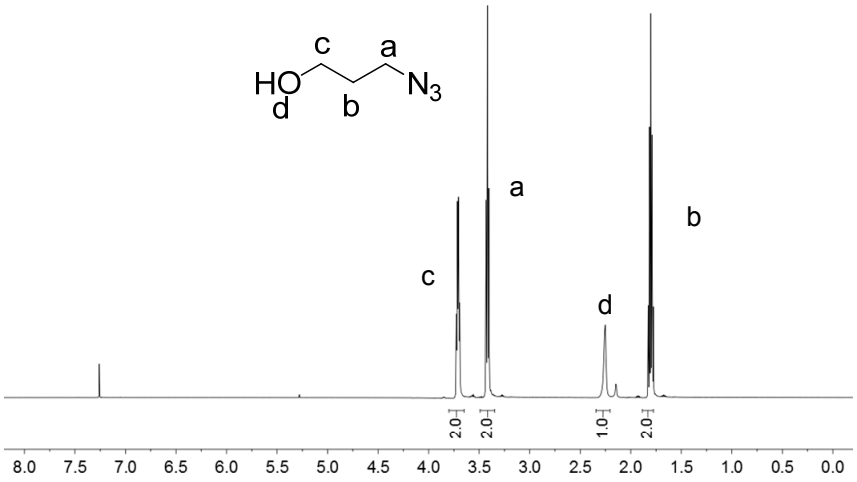


Figure S10. ^{1}H NMR of 3-azidopropanol.

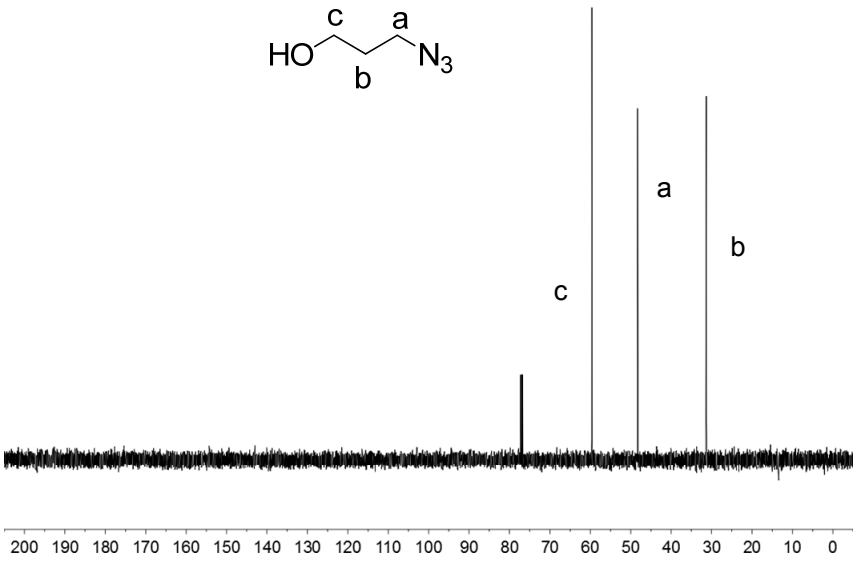


Figure S11. ^{13}C NMR of 3-azidopropanol.

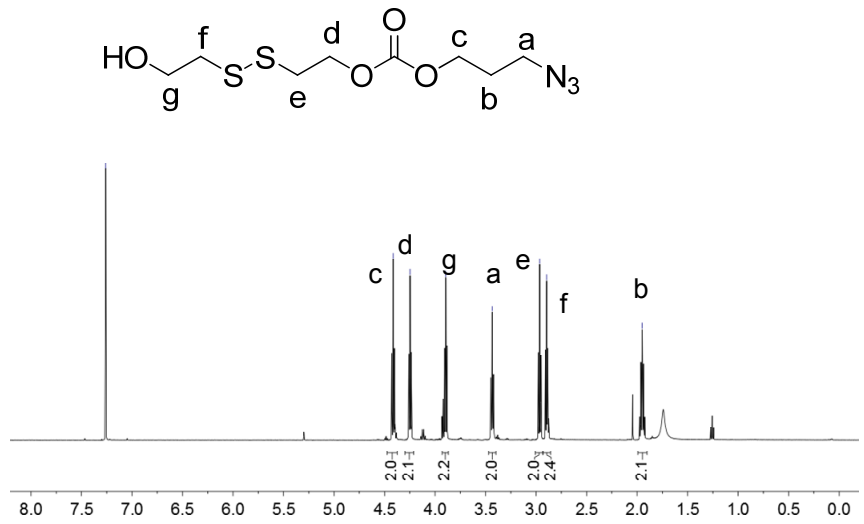


Figure S12. [1]H NMR of 3-azidopropyl 2-((2-hydroxyethyl)disulfanyl)ethyl carbonate.

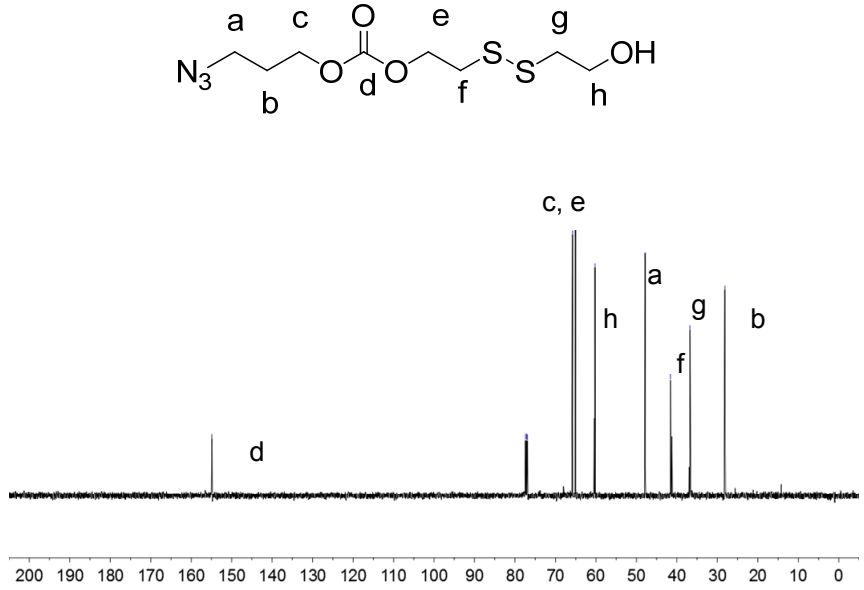


Figure S13. [13]C NMR of 3-azidopropyl 2-((2-hydroxyethyl)disulfanyl)ethyl carbonate.

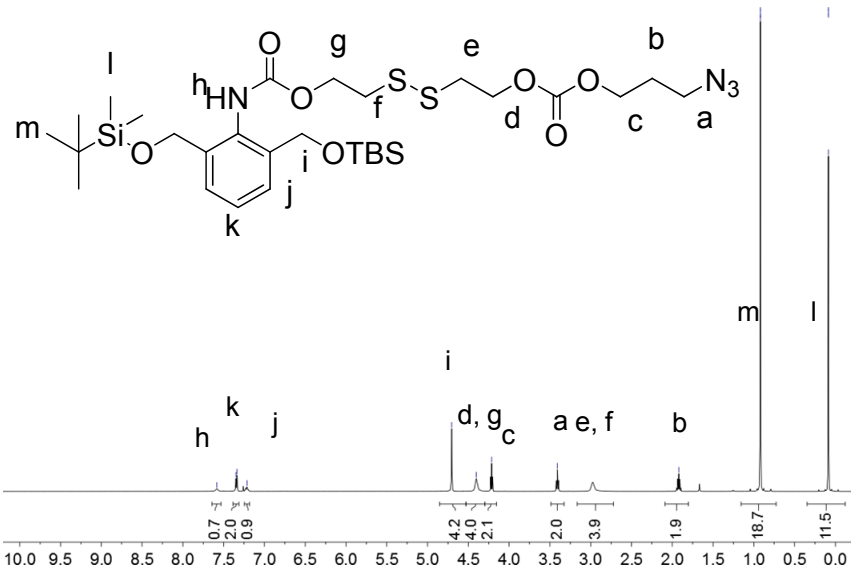


Figure S14. ^{1}H NMR of N_3-SS-BHA-OTBS.

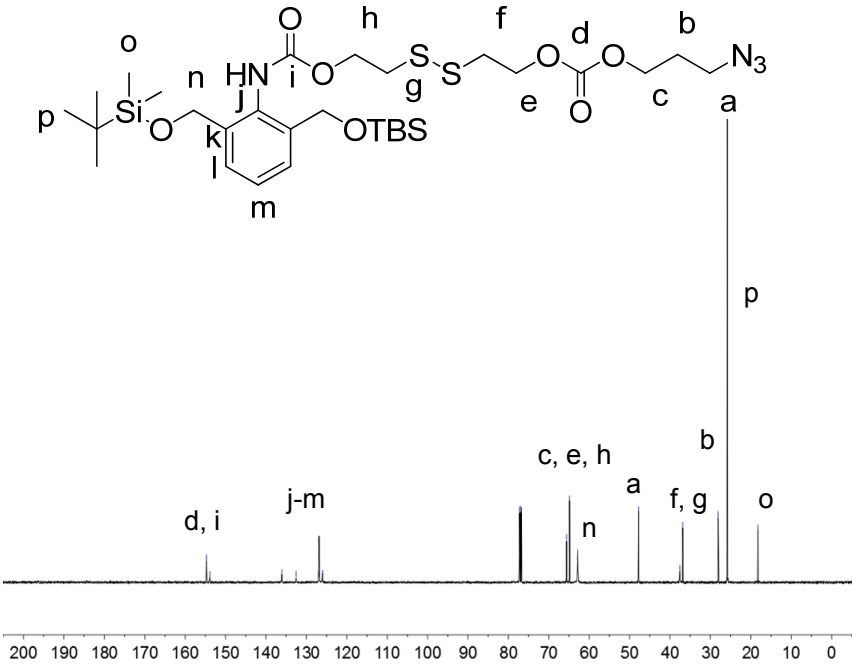


Figure S15. ^{13}C NMR of N_3-SS-BHA-OTBS.

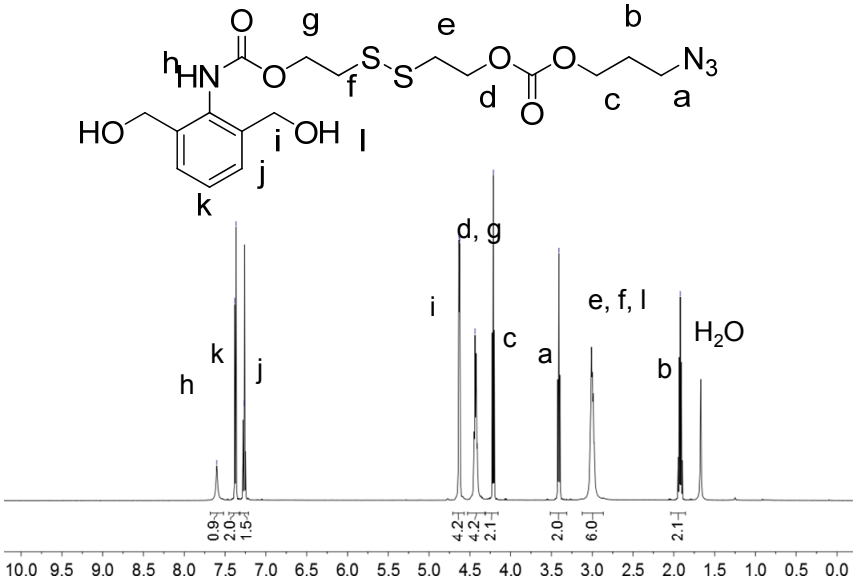


Figure S16. ^{1}H NMR of N_3-SS-BHA.

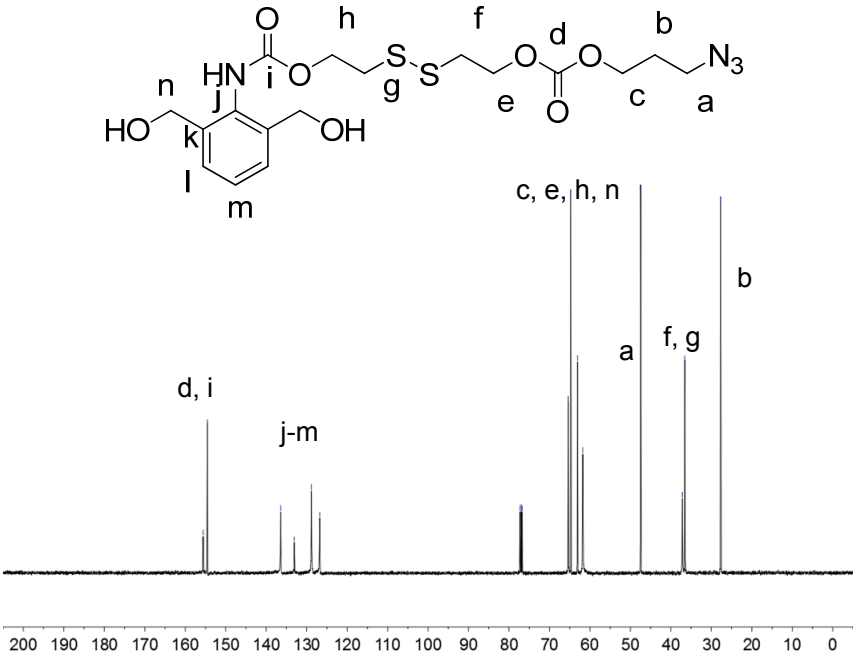


Figure S17. ^{13}C NMR of N_3-SS-BHA.

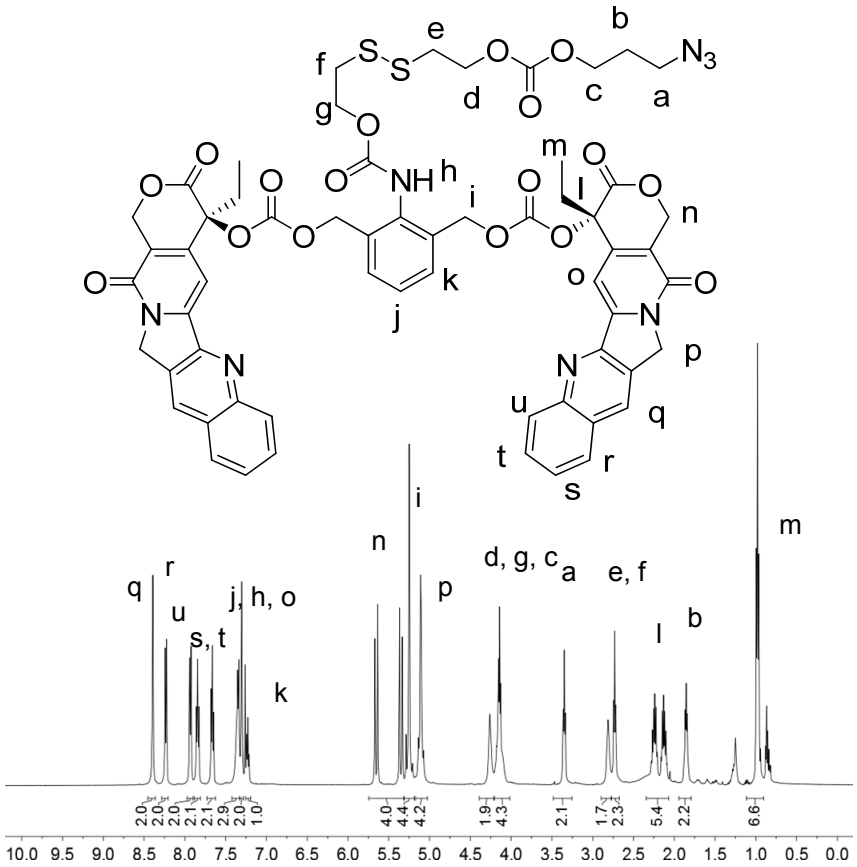


Figure S18. ^{1}H NMR of CPT-SS-CPT.

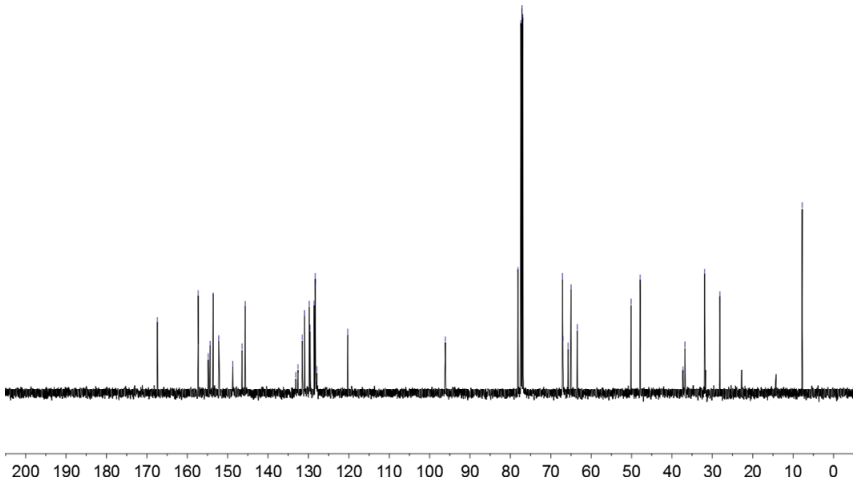


Figure S19. ^{13}C NMR of CPT-SS-CPT.

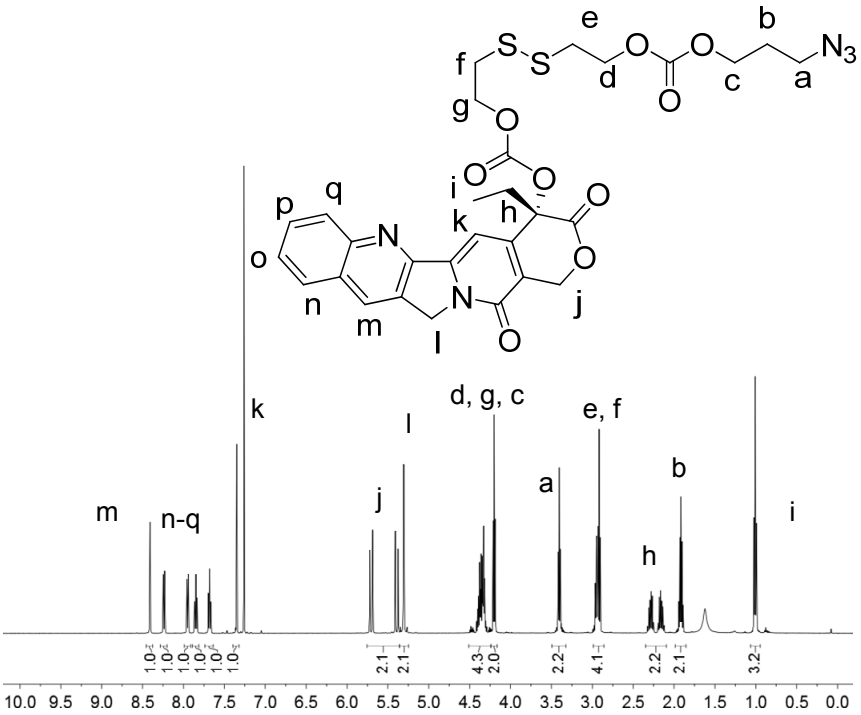


Figure S20. ^{1}H NMR of CPT-SS.

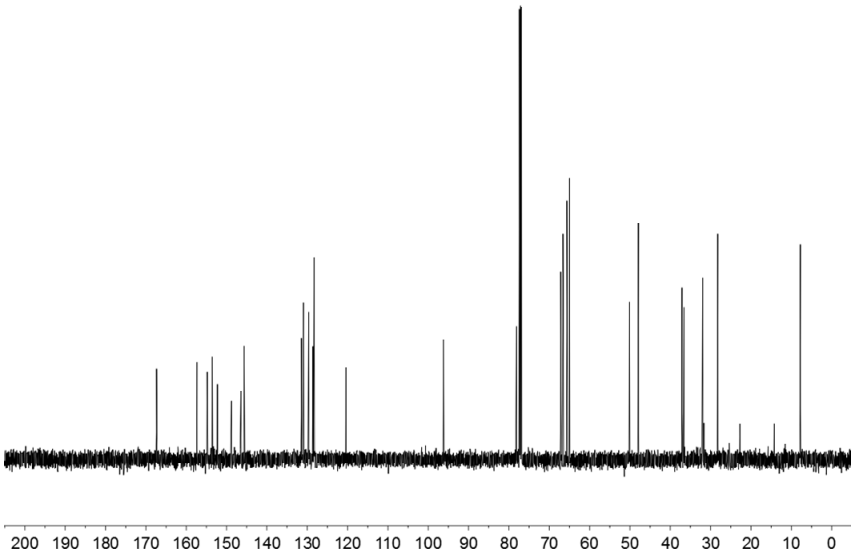


Figure S21. ^{13}C NMR of CPT-SS.

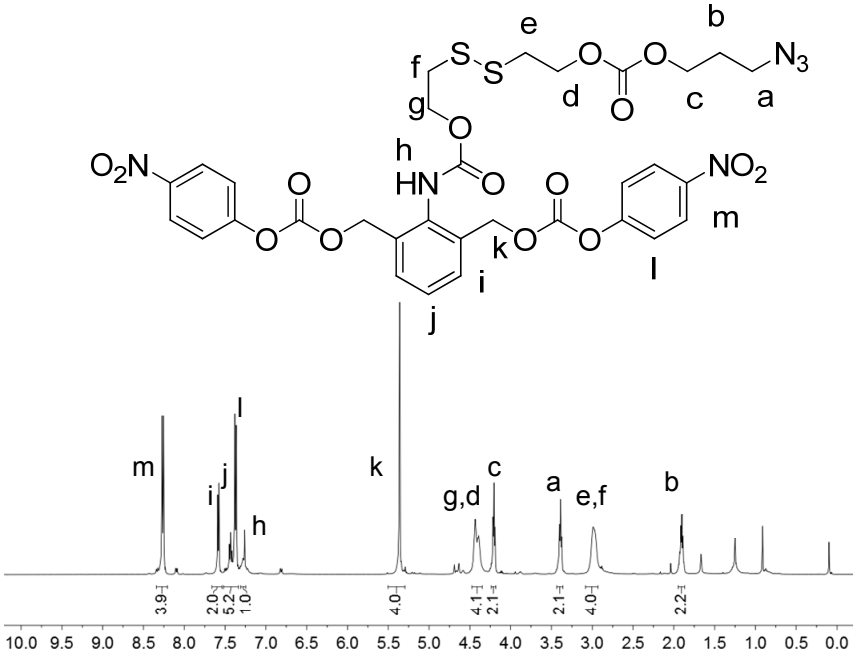


Figure S22. ^{1}H NMR of nP-SS-BHA.

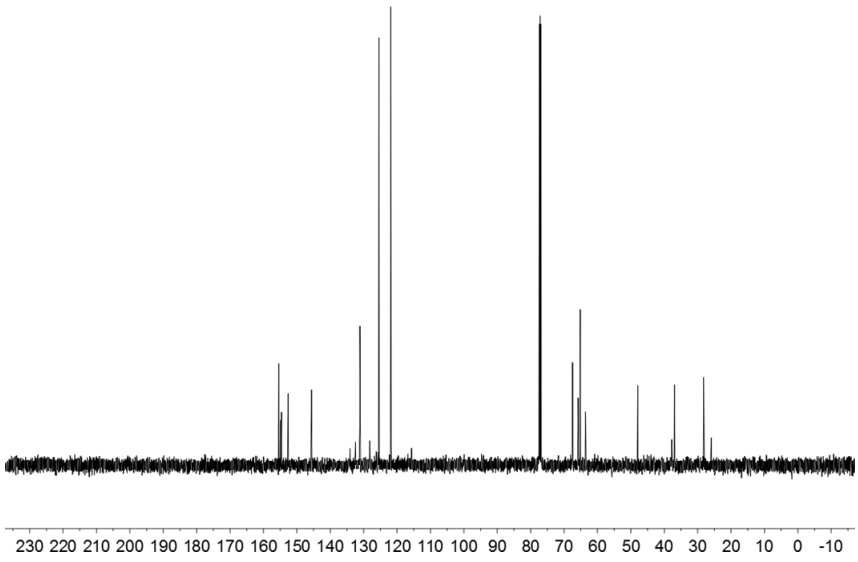


Figure S23. ^{13}C NMR of nP-SS-BHA.

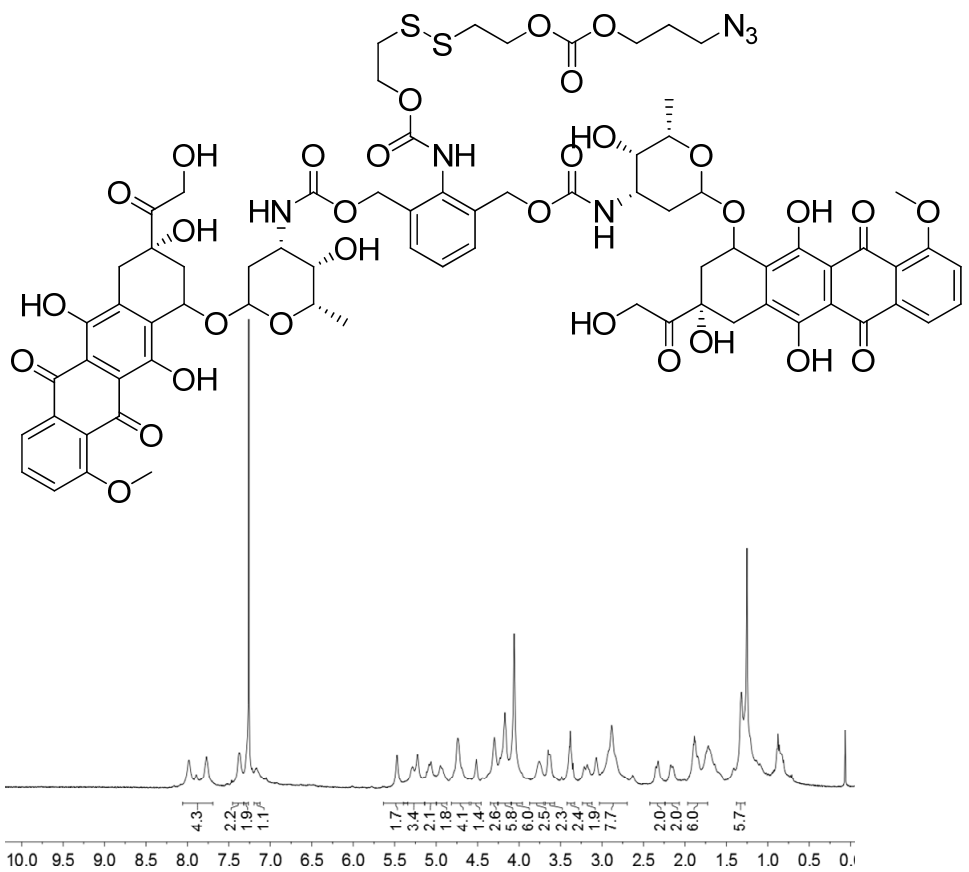


Figure S24. ^{1}H NMR of Dox-SS-Dox.

Reference

(1) Zhang, Y.; Ma, L.; Deng, X.; Cheng, J. *Polym. Chem.* **2013**, *4*, 224-228.