Electronic Supplementary Material (ESI) for Biomaterials Science.

Supporting Information

Self-Boosting Stimulus Activation of Polyprodrug with Cascade Amplification for Enhanced Antitumor Efficacy

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Experimental Section

1.Materials.

3-Methyl-4-nitrobenzoate, *N*-bromosuccinimide (NBS), benzoyl peroxide (BPO), potassium carbonate (K_2CO_3), 4-hydroxybenzyl alcohol, tert-butyldimethylchlorosilane, tetrabutylammonium fluoride (TBAF), imidazole, *N, N*′-carbonyldiimidazole (CDI), 4-dimethylaminopyridine (DMAP), zinc powder, acetic acid (AcOH), sodium bicarbonate ($NaHCO_3$), diisobutyl aluminium hydride (DIBAL), Rochelle salt, phenylchloroformate, dibutyltin dilaurate (DBTL) were purchased from Aladdin Industrial, Inc. (Shanghai, China). Doxorubicin hydrochloride (DOX·HCl) was purchased from Dalian Meilun Biotechnology Co. LTD. Amine terminated methoxy poly(ethylene glycol) (mPEG-NH_2) was purchased from JenKen Co. LTD. (Beijing, China).

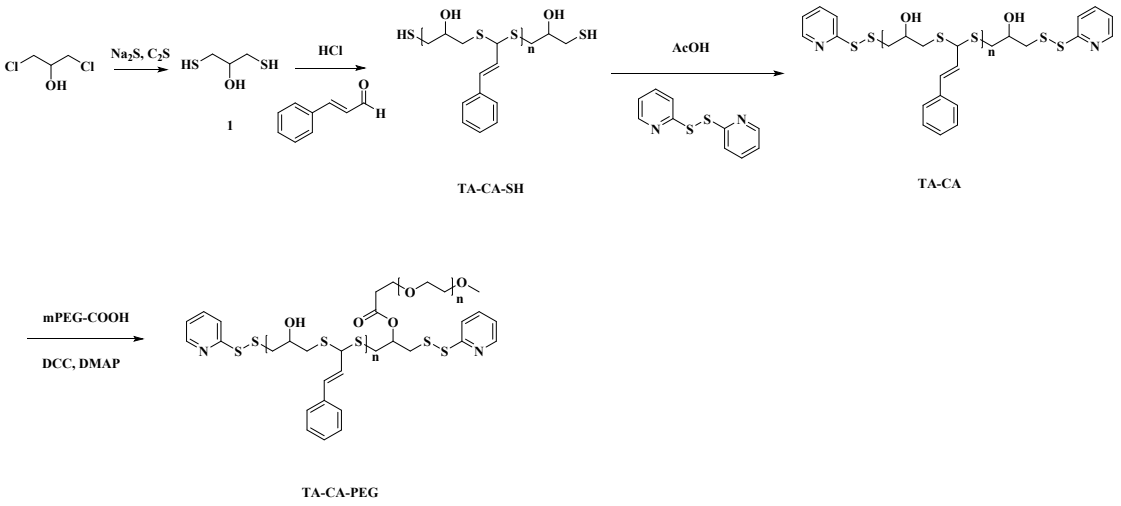
Dulbecco's modifed eagle's medium (DMEM), trypsin-EDTA and penicillin−streptomycin were purchased from Gibco BRL (Eggenstein, Germany). Fetal bovine serum (FBS) was obtained from ExCell Biology, Inc (Shanghai, China). Hoechst 33342 were obtained from Life Technologies. Intracellular reactive oxygen species (ROS) fluorescent probe 2,7-dichlorodi-hydrofluorescein diacetate (DCFH-DA) and methylthiazolyldiphenyl-tetrazolium bromide (MTT) was obtained from Sigma-Aldrich.

2.Characterization.

The polymer dispersity index (PDI) of polymer was determined by gel permeation chromatography (GPC) measurements on a Waters GPC system (Waters, Milford, MA). The system was equipped with a Waters 2414 refractive index detector and a Waters 1515 HPLC solvent pump. Four Waters styragel high-resolution columns (HR_4, HR_2, HR_1, and $HR_{0.5}$) were also equipped with molecular weights of 5000−600,000, 500−20,000, 100−5000, and 0−1000, respectively. HPLC grade *N*, *N*-dimethylformamide (DMF) was used as eluent at 35 °C with a flow rate of 1.0 mL min^{-1}. Monodispersed polystyrene standards with a molecular weight range of 1.31×10^3 to 5.51×10^4 were used to generate the calibration curve. Bruker ARX 400 NMR spectrometer (Bruker, Billerica, MA) was used

to record ^{1}H NMR spectra. Deuterated chloroform or deuterated dimethyl sulfoxide was used as the solvent for NMR measurements. Deuterated chloroform or deuterated dimethyl sulfoxide was used as the solvent for NMR measurements. Nanoparticle hydrodynamic diameters (Rh) measurements were carried out in aqueous solution using a Malvern ZS90 dynamic light scattering instrument (DLS) with a He-Ne laser (633 nm) and 90° collecting optics. The data were analyzed using Malvern Dispersion Technology Software 7.0.2. Photoluminescence (PL) spectra were measured on a Shimadzu RF-6000 spectrofluorometer (Shimadzu UV-2600, Japan). Absorbance and fluorescence intensity were measured by multi-functional microporous plate analysis system (Biotek Cytation5, BioTek, United States). Obtaining confocal image under confocal microscope (CLSM, Nikon Ti-E A1, Japan). Ultrapure water was obtained from Millipore ultrapure water meter machine (18.2 MU, Bedford). The mice were imaging by *In-Vivo* Xtreme (Bruker, German).

3. Synthesis of TA-CA-PEG



Scheme S1. Synthetic procedures of TA-CA-PEG.

Synthesis of 1,3-dimercapto-2-propanol (1). CS_2 (13.2 mL, 220 mmol) was added to a solution of $Na_2S{\cdot}9H_2O$ (48 g, 200 mmol) in 100 mL water and stirred at 40 °C for 5 h. Removed the excess CS_2 and the remaining solution was attenuated with 300 mL water to get a ~ 33% solution of sodium trithiocarbonate. Subsequently, 1,3-dichloro-2-

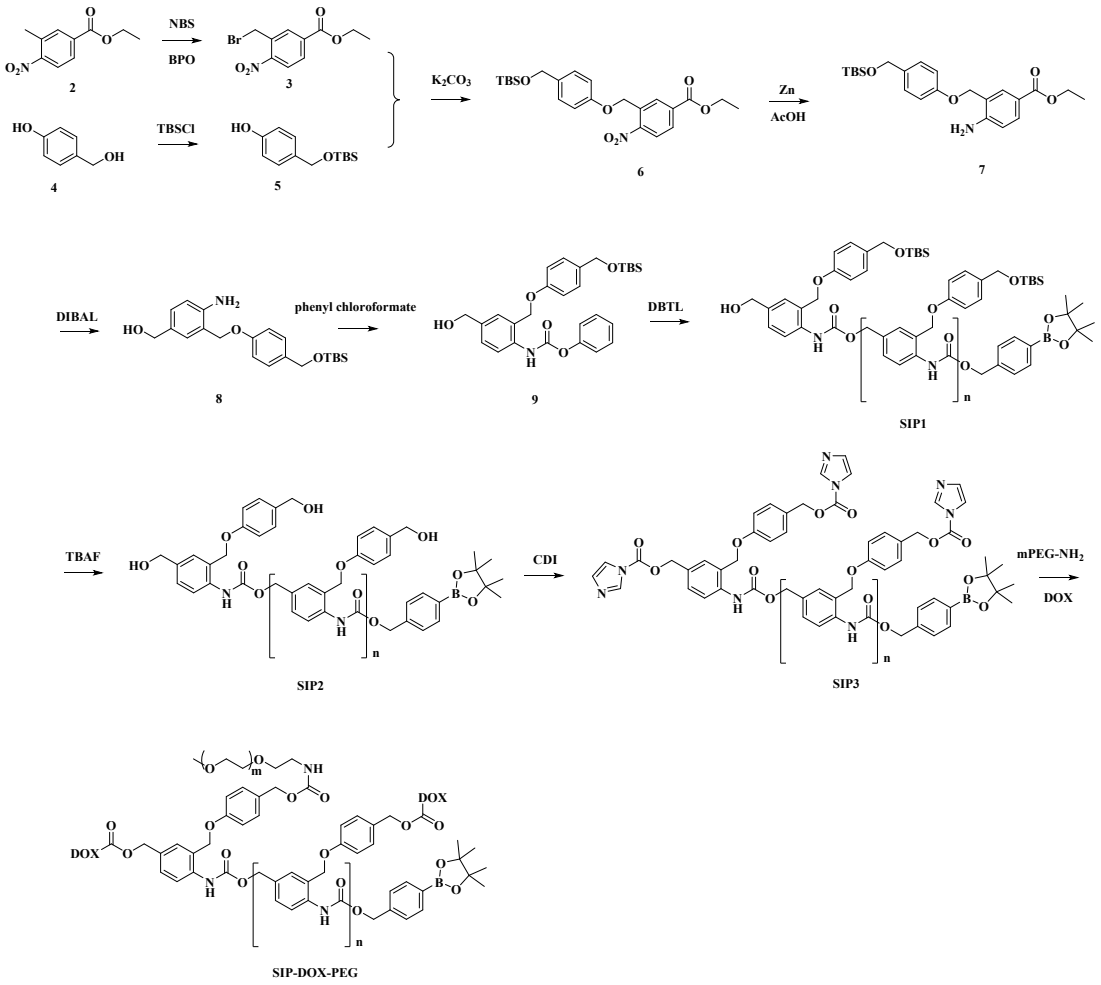
propanol (6.62 mL, 70 mmol) was added drop-wise to the solution at 0 °C then stirred at 60 °C for 5 h. Cool to room temperature and then washed with ethyl acetate (5 ×200 mL). Collected aqueous part and acidified by adding concentrated sulphuric acid slowly then extracted with diethyl ether (3 ×200 mL). After purified by vacuum distillation at 90 °C the product **1** was obtained as a light-yellow liquid (3.51 g, 40.5%).

Synthesis of TA-CA-SH. Compound **1** (1.04 g, 8.36 mmol), CA (1.08 g, 8 mmol) and hydrochloric acid (32 μL, 38.4 mmol) was added into a glass flask and stirred at 0 °C for 30 min under argon atmosphere. Washed viscous solution by water to remove most of the hydrochloric acid and then dissolved in 4 mL of THF and precipitated from excess of cold hexane. The polymer purified by gel column (1% crosslinked: separation of M_W 600-14,000 lipophilic polymers) using THF as eluents and dried under vacuum to obtain TA-CA-SH as a light-yellow waxy solid (0.7 g, 33.6%).

Synthesis of TA-CA. TA-CA-SH (0.3 g, 0.08 mmol) and 2,2'-dithiodipyridine (0.04 g, 0.16 mmol) were dissolved in 10 mL dry degassed THF and 2 drop of acetic acid was added as the catalyst, then stirred at 25 °C under argon atmosphere for 24 h. Afterwards, concentrated the solution and then purified *via* a gel column using THF as eluents and dried under vacuum to obtain TA-CA as a light-yellow solid (0.26 g, 85.5%).

Synthesis of TA-CA-PEG. The TA-CA-SH (140 mg, 0.025 mmol), mPEG-COOH (60 mg, 0.03 mmol), DCC (19 mg, 0.09 mmol) and DMAP (11 mg, 0.09 mmol) was dissolved in DMF (2 mL), stirred at room temperature under argon atmosphere for 24 h. Afterwards, concentrated the solution and then purified by a gel column using DMF as eluents and dried under vacuum to obtain TA-CA-PEG as a solid (0.13 g, 65%).

4. Synthesis of SIP-DOX-PEG



Scheme S2. Synthetic procedures of SIP-DOX-PEG.

Synthesis of ethyl 3-(bromomethyl)-4-nitrobenzoate (3). A suspension of 3-methyl-4-nitrobenzoate (**2**) (50.0 g, 239 mmol) and *N*-bromosuccinimide (NBS, 49.3 g, 277 mmol) in CCl_4 (600 mL) was heated for 30 min in a three necked flask equipped with a Dean-Stark apparatus to remove any traces of water. After cooling and removal of the Dean-Stark apparatus, a 20 mg portion of benzoyl peroxide (BPO) was added and the reaction mixture was heated under reflux for 24 h. Addition of BPO followed by 24 h of heating was repeated until TLC (EtOAc: hexane = 1:4, R_f = 0.5) indicated nearly complete conversion of the starting material. The solids were removed by filtration over celite then washed with CH_2Cl_2, and the filtrate was concentrated in vacuo. The dark brown residue was crystallized from EtOAc/hexane affording pure compound **3** as a light-yellow solid (44.3 g, 64%). ^{1}H NMR (400 MHz,

Chloroform-*d*) δ 8.25 (d, *J* = 1.8 Hz, 1H), 8.15 (dd, *J* = 8.5, 1.8 Hz, 1H), 8.08 (d, *J* = 8.5 Hz, 1H), 4.85 (s, 2H), 4.46 (q, *J* = 7.1 Hz, 2H), 1.45 (t, *J* = 7.1 Hz, 3H).

Synthesis of ethyl 4-(((tert-butyldimethylsilyl)oxy)methyl)phenol (5). 4-Hydroxybenzyl alcohol (**4**) (5.0 g, 0.04 mol) and tert-butyldimethylchlorosilane (TBSCl) (7.9 g, 0.05 mol) were dissolved in 15 mL DMF, and the mixture was stirred at room temperature under argon atmosphere for 10 min. Then, imidazole (5.5 g, 0.09 mol) was introduced to the mixture and stirred at 25 °C for 16 h. The solvent was then evaporated under reduced pressure and the residue was subjected to silica gel chromatography with hexane: ethyl acetate = 15:1 as eluent (R_f = 0.7), affording product **5** as a white solid (7.32 g, 76%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.42 (s, 1H), 7.19 – 6.94 (m, 2H), 6.87 – 6.61 (m, 2H), 4.56 (s, 2H), 0.87 (s, 9H).

Synthesis of ethyl 3-((4-(((tert-butyldimethylsilyl)oxy)methyl)phenoxy)methyl)-4-nitrobenzoate (6). Compound **3** (4.0 g, 13.72 mmol) and compound **5** (2.5 g, 10.49 mmol) were added to 50 mL acetonitrile followed by K_2CO_3 (2.5 g, 18.09 mmol), then the solution was stirred at 70 °C for 12 h. The solvent was cooled to room temperature and the solids were removed by filtration. Then the solvent was evaporated to dryness, the residue was subjected to silica gel chromatography with hexane: ethyl acetate = 10:1 as eluent (R_f = 0.4), affording product **6** as a white solid (4.2 g, 68%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.32 (d, *J* = 1.9 Hz, 1H), 8.24 (d, *J* = 8.5 Hz, 1H), 8.13 (dd, *J* = 8.5, 1.9 Hz, 1H), 7.29 – 7.17 (m, 2H), 7.04 – 6.96 (m, 2H), 5.49 (s, 2H), 4.64 (s, 2H), 4.36 (q, *J* = 7.1 Hz, 2H), 1.33 (t, *J* = 7.1 Hz, 3H), 0.88 (d, *J* = 3.1 Hz, 9H), 0.06 (s, 6H).

Synthesis of ethyl 4-amino-3-((4-(((tert-butyldimethylsilyl)oxy)methyl)ph-enoxy)methyl)benzoate (7). Compound **6** (4.2 g, 9.42 mmol) and zinc powder (2.9 g, 44.35 mmol) were dissolved in 150 mL CH_2Cl_2 and stirred at room temperature for 10 min. Then 5.35 mL acetic acid was added into the reaction mixture stirred at 25 °C for 1 h. The solids were removed by filtration and solvent was then evaporated under reduced pressure, the residue was subjected to silica gel chromatography with hexane: ethyl acetate=7:1 as eluent (R_f = 0.35), affording product

7 as a white solid (3.15 g, 80%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.80 (d, *J* = 2.2 Hz, 1H), 7.63 (dd, *J* = 8.5, 2.1 Hz, 1H), 7.27 – 7.15 (m, 2H), 7.08 – 6.94 (m, 2H), 6.70 (d, *J* = 8.5 Hz, 1H), 5.99 (s, 2H), 4.97 (s, 2H), 4.62 (s, 2H), 4.20 (q, *J* = 7.1 Hz, 2H), 1.26 (t, *J* = 7.1 Hz, 3H), 0.88 (s, 9H), 0.05 (s, 6H).

Synthesis of (4-amino-3-((4-(((tert-butyldimethylsilyl)oxy)methyl)phenoxy)me-thyl)phenyl)methanol (8). A solution of compound **7** (3.15 g, 7.58 mmol) in anhydrous tetrahydrofuran (THF) (80 mL) was cooled to -78 °C and a 1 M solution of diisobutylaluminium hydride (DIBAL) in THF (22.7 mL) was added dropwise within 75 min. The reaction mixture was stirred for 24 h at -78 °C and then poured into 50 mL of a saturated solution of Rochelle salt. Subsequently, the mixture was extracted with diethyl ether (3 × 50 mL), the organic layers were dried over Na_2SO_4, and the solvent was evaporated. The residue was purified by silica gel chromatography with hexane: ethyl acetate= 5:1 as eluent (R_f = 0.2), affording product **8** as a white solid (1.5 g, 56%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.25 – 7.18 (m, 2H), 7.13 (d, *J* = 2.1 Hz, 1H), 7.03 – 6.94 (m, 3H), 6.65 (d, *J* = 8.1 Hz, 1H), 4.94 (d, *J* = 4.4 Hz, 4H), 4.91 (d, *J* = 5.6 Hz, 1H), 4.62 (s, 2H), 4.31 (d, *J* = 5.6 Hz, 2H), 0.89 (s, 9H), 0.06 (s, 6H).

Synthesis of phenyl (2-((4-(((tert-butyldimethylsilyl)oxy)methyl)phenoxy)me-thyl)-4-(hydroxymethyl)phenyl)carbamate (9). Compound **8** (1.5 g, 4.02 mmol) was suspended in a 15 mL mixture of THF: sat. $NaHCO_3$: water (ratio 2:2:1) and phenylchloroformate (0.52 mL, 4.16 mmol) was added dropwise over 5 minutes. The reaction was monitored to completion by TLC (EtOAc: Hex =1:1). EtOAc was then added and the organic phase washed twice with saturated NH_4Cl solution. The solvents were removed under reduced pressure and the crude product purified by silica gel chromatography with hexane/ethyl acetate=25:22 as eluent (R_f = 0.4), affording product **9** (1.0 g, 50%) as a white solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.38 (s, 1H), 7.18 – 7.13 (m, 2H), 7.12 – 7.05 (m, 3H), 6.97 – 6.88 (m, 3H), 6.74 – 6.66 (m, 3H), 6.59 (d, *J* = 8.1 Hz, 1H), 4.88 (s, 2H), 4.83 (t, *J* = 5.6 Hz, 1H), 4.56 (s, 2H), 4.25 (d, *J* = 5.5 Hz, 2H), 0.83 (s, 9H).

Synthesis of the self-immolative polymer 1 (SIP1). Compound **9** (306 mg, 0.62 mmol) and dibutyltin dilaurate

(DBTL) (5% mol/mol) were dissolved in dry DMF (1 mL), pre-heated to 110 °C, under argon atmosphere and the solution was stirred for 15 minutes. (4-(4,4,5,5-Tetramethyl-1,3,2-dioxaborolan-2-yl)phenyl)methanol (**10**) (145 mg, 0.62 mmol), dissolved in dry DMF (310 µL), was then added and the reaction stirred for additional 30 minutes. After cooling to room temperature, the polymer was precipitated from MeOH, filtered and dried under reduced pressure for a few hours. **SIP1** was obtained as a white powder (150 mg, 49%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.13 (s, 20H), 7.52 (d, *J* = 6.1 Hz, 36H), 7.37 (dd, *J* = 23.6, 7.8 Hz, 35H), 7.16 (t, *J* = 11.1 Hz, 86H), 6.91 (d, *J* = 8.4 Hz, 65H), 5.25 – 4.98 (m, 92H), 4.57 (d, *J* = 13.9 Hz, 60H), 1.30 (s, 12H), 0.93 – 0.78 (m, 294H), 0.08 (s, 197H).

Synthesis of SIP2. SIP1 (60 mg, 0.012 mmol) was dissolved in dry DMF (3 mL), followed by 1 M solution of tetrabutylammonium fluoride (TBAF) in THF (180 µL, 0.69 mmol) and the solution was stirred for 2 h. After completion of the reaction, the polymer was precipitated from MeOH, filtered and dried under reduced pressure for a few hours. **SIP2** was obtained as a white powder (20 mg, 47%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.16 (s, 21H), 7.61 – 7.32 (m, 67H), 7.20 (q, *J* = 11.9, 10.3 Hz, 88H), 6.92 (dd, *J* = 12.3, 7.8 Hz, 71H), 5.16 – 4.93 (m, 131H), 4.39 (t, *J* = 5.6 Hz, 60H), 1.20 (d, *J* = 35.3 Hz, 11H), 0.87 (s, 40H).

Synthesis of SIP3. SIP2 (100 mg, 0.013 mmol) and *N, N*′-carbonyldiimidazole (CDI, 500 mg, 3 mmol) were dissolved in DMF and the solution was stirred at room temperature for 12 h under argon atmosphere. The viscous solution precipitated to excess of cold diethyl ether twice. Then dried under vacuum to obtain **SIP3** as a white solid which was used without further purification (0.095 g, 71%).

Synthesis of SIP-DOX-PEG. Triethylamine (30 mg, 0.30 mmol) and DOX (58 mg, 0.10 mmol) dissolved in DMF and the solution was stirred at room temperature for 4 h under argon atmosphere. Then, mixture of SIP3 (50 mg, 0.005 mmol), 4-dimethylaminopyridine (DMAP) (10 mg, 0.08 mmol) and mPEG-NH_2 (100 mg, 0.05 mmol) in DMF (2 mL) was added and the mixture was stirred at room temperature for 24 h under argon atmosphere. The solution was precipitated from excess of cold diethyl ether twice and dried under vacuum to obtain SIP-DOX-PEG as a red

solid (0.07 g, 34%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.16 (s, 20H), 7.90 (q, *J* = 4.4, 3.7 Hz, 33H), 7.65 (dt, *J* = 7.7, 3.8 Hz, 20H), 7.56 – 7.40 (m, 35H), 7.27 – 7.11 (m, 54H), 6.99 – 6.87 (m, 39H), 5.15 – 5.02 (m, 58H), 4.98 – 4.91 (m, 37H), 4.59 (s, 40H), 3.99 (s, 50H), 3.24 (s, 15H), 2.15 (qd, *J* = 14.7, 13.8, 4.1 Hz, 38H), 1.88 (td, *J* = 12.7, 3.6 Hz, 19H), 1.69 (dd, *J* = 12.5, 4.4 Hz, 19H), 1.16 (d, *J* = 6.5 Hz, 47H).

5. Preparation nanoparticles of TA_{CA}, SIP_{DOX} and $TS_{CA/DOX}$.

Dissolve TA-CA-PEG, SIP-DOX-PEG or TA-CA-PEG + SIP-DOX-PEG mixture (10 mg) in 1 mL of DMSO and then dropwise added to 9 mL of ultra-pure water. The mixture was stirred at room temperature for 3 h. And then transferred into the dialysis membrane tubing (MWCO=3500 Da) and dialyzed against water for 24 h. The DOX concentration was determined by a microplate system (Molecular Devices) at 480 nm by UV absorption.

6. Characterization of NPs.

The changes of nanoparticle hydrodynamic diameters (Rh) in ROS condition and PBS were investigated by a Malvern ZS90 dynamic light scattering instrument (DLS) to verify the ROS-responsiveness of nanoparticles; H_2O_2, Fenton reagent, and NaClO in PBS were used as ROS sources. Specifically, nanoparticles (1 mg/mL) were mixed with 0.5 mL of ROS reagent and incubated at 37 °C in a constant temperature water bath oscillator. Hydroxyl radical (•OH) was formed by mixing Fenton's Reagent with H_2O_2. Sodium hypochlorite (NaClO) at pH 6.02 to form hypochlorous acid (ClO^-), C_{ClO^-} = Abs_{292nm} /0.39 (mM). Nanoparticles in PBS were utilized as control. The nanoparticles size was recorded by DLS at the predetermined time interval.

7. Study on the release of DOX and CA from NPs.

The release profile of DOX and CA from nanoparticles were determined by dialysis method. Nanoparticles were dissolved in phosphate-buffered saline (PBS) (2 mg mL^{-1}, 1 mL) and transferred into a dialysis bag (MWCO: 3500 Da), followed by incubation with 25 mL buffer solution under various conditions (with 10 mM H_2O_2 or without H_2O_2) in a shaking water bath at 37 °C. At regular intervals, collected 1 mL of buffer solution from the outside of

the dialysis bag, and replaced with 1 mL of fresh buffer solution. The DOX and CA concentration was determined by a microplate system (Molecular Devices) at 480 nm and 292 nm by UV absorption.

5 mg of $TS_{CA/DOX}$ was dispersed in 1 mL of PBS containing 10 mM H_2O_2, placing at room temperature for 24 h before freeze-drying. 0.5 mL of DMF was added to dissolve, followed by centrifugal precipitation. The drugs release and activation were examined by High Performance Liquid Chromatography (HPLC). The HPLC isocratic mobile phase consisted of acetonitrile and Acetic acid solution (5%, v/v) in volume ratio of 75:25, and the UV/vis detector was set at 292 nm and 480 nm for CA and DOX analysis, respectively.

8. Cell culture.

Mouse breast cancer cell line 4T1 cells were cultured in DMEM medium (37 °C, 5% CO_2), supplemented with 10% fetal bovine serum and 1% penicillin (100 IU mL^{-1}), streptomycin (100 μg mL^{-1}).

9. Detection of intracellular reactive oxygen species (ROS) generation.

The intracellular ROS was detected by using 2',7'-dichlorofluorescin diacetate (DCFH-DA) as indicator and studied by confocal images. 4T1 cells (2×10^5 cells per well) were cultured on glass coverslips for 12 h, then exposure to PBS, CA, DOX, CA+DOX, TA_{CA}, SIP_{DOX} and $TS_{CA/DOX}$ (DOX: 2 μg mL^{-1}, CA: 30 μg mL^{-1}). After incubation for 6 h, the medium was removed and washed with PBS for three times, stained by DCFH-DA (20 μM) for 30 mins at 37 °C. After staining, the probe was rinsed three times with PBS and fixed with 4% formaldehyde. Finally, cells were visualized through CLSM.

4T1 cells (1×10^5 cells per well) were cultured on 24-well culture plates. After treated with PBS, CA, DOX, CA+DOX, TA_{CA}, SIP_{DOX} and $TS_{CA/DOX}$ (DOX: 2 μg mL^{-1}, CA: 30 μg mL^{-1}) for 4 h, then stained by DCFH-DA (20 μM). Cells were rinsed three times with PBS, collected and analyzed by Flow Cytometry.

4T1 cells (1×10^4 cells/well) were cultured in 96-well black culture plates. After treated with TA_{CA}, SIP_{DOX} and $TS_{CA/DOX}$ (DOX: 2 μg mL^{-1}, CA: 30 μg mL^{-1}) at the desired concentrations, cells were washed by PBS for three times,

then stained by DCFH-DA (20 μM). After staining, the probe was rinsed three times with PBS then placed in PBS. The ROS level was analyzed using a microplate system (DCF, E_x/E_m = 488/525 nm).

10. Intracellular DOX release.

Cultured 4T1 cells (1×10^5 cells per well) on 24 well plates, then treated with DOX, SIP_{DOX} and $TS_{CA/DOX}$ in equivalent doses (DOX 2 μg mL^{-1}) for 2 and 4 h. Cells were rinsed with PBS for three times, fixed with 4% formaldehyde, and visualized by CLSM.

Cultured 4T1 cells (2×10^5 cells per well) on 12 well plates, treated with SIP_{DOX} and $TS_{CA/DOX}$ at an equivalent dose (4 μg mL^{-1} DOX). After incubated for different periods of time, lysed cells in deionized water containing 1% Triton X-100 at ultrasound for 1 h. Then freeze-dried cell lysates and redissolved it in acetonitrile. The concentration of DOX in cell lysates was determined by HPLC analyses and normalized to the total protein content of the cells determined *via* the BCA Protein Assay Kit (KeyGEN BioTECH).

11. Cytotoxicity assay.

4T1 cells were seeded in 96-well culture plates (1×10^4 cells/well) and incubated for 12 h. The cells were then treated with TA_{CA}, SIP_{DOX} and $TS_{CA/DOX}$ at the desired concentrations. The cell viability was measured by the standard commercial methyl thiazolyl tetrazolium (MTT) assay after 48 h of cell culture.

12. Cell cycle and apoptosis analysis detection.

4T1 cells were seeded in six well plates, then treated with PBS, CA, DOX, CA+DOX, TA_{CA}, SIP_{DOX} and $TS_{CA/DOX}$ (DOX: 2 μg mL^{-1}, CA: 30 μg mL^{-1}) for 12 h. Then 4T1 cells were stained with Annexin V-APC (5 μL for one sample)/7-AAD (5 μL for one sample) (Annexin V-APC Apoptosis Detection Kit with 7-AAD, KeyGEN BioTECH) for 30 min at room temperature and then added 500 μL binding buffer to analyze by a flow cytometry.

To investigate the influence of toxicity and apoptosis on cell cycle, 4T1 cells were seeded in 24-well plates at a density of 1×10^5 cells and incubated for 12 h. Afterward, $TS_{CA/DOX}$ (DOX: 2 μg mL^{-1}, CA: 30 μg mL^{-1}) was added and

incubated for 12 h, and then washed by PBS, followed by detachment via trypsin. Then the cells were washed with PBS and fixed using the mixture of 75% ethanol at 4°C for 12 h. Afterwards, the cells were washed with PBS again and incubated with 100 μg mL^{-1} 7-AAD/RNase solution at 37°C for 30 min ready for analysis using flow cytometry.

13. Animals and tumor model.

Female BALB/c mice (20 ± 2 g, 6 weeks old) were purchased from Hunan SJA Laboratory Animal Co., Ltd (Hunan, China). 4T1 cells (1×10^6) were injected into the right mammary fat pads to establish an orthotopic 4T1 tumor model. At the end of experiments, all mice were killed by CO_2 inhalation. All animal experiments were approved by the Ethics Committee of the South China University of Technology.

14. *In vivo* biodistribution.

When the tumor volume reached 200 mm^3, the tumor-bearing mice were randomly divided into two groups (n=3), intravenously injected with 100 μL of PBS, IR-780 (an NIR dye) encapsulated in SIP_{DOX} NP and $TS_{CA/DOX}$ NP (1 mg kg^{-1}). All the mice were imaged at 6, 12 and 24 h after injection, using a *in vivo* Xtreme (Bruker, German) instrument to collect the body fluorescence images (E_x/E_m = 750/830 nm). The mice were killed at 24 h after administration, the major organs (liver, kidney, lung, spleen, and heart) and tumor were taken to detect the distribution of IR-780 *ex vivo*.

15. Tumor growth inhibition.

When the tumor volume reached 100 mm^3, 4T1 tumor-bearing mice were randomly divided into five groups (n=5). The mice were treated with PBS, DOX, TA_{CA}, SIP_{DOX} and $TS_{CA/DOX}$ (5 mg kg^{-1} DOX equivalent) by intravenous injection once every two days, four times in all. The tumor volumes and body weight were measured every other day. Using this formula to calculate the volume of tumor: $V=L\times W^2/2$ (L, the longest dimension; W, the shortest dimension).

16. Study on ROS level *in vivo*.

The tumor bearing mice were injected with PBS, DOX, TA_{CA}, SIP_{DOX} and $TS_{CA/DOX}$ (5 mg kg^{-1} DOX equivalent) for 24 hours, DCFH-DA probe (2.5 mg kg^{-1}) was intratumorally injected for ROS detecting, and the tumors were collected after 4 hours, frozen sectioned at 7 μm thickness, observed by using CLSM examination.

17. Immunohistochemical analysis.

The tumors were harvested, 4% paraformaldehyde fixed, paraffin embedded, sliced and stained with hematoxylin and eosin (H&E). Tumor sections were also stained with terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL) apoptosis detection kit assay then observed by CLSM.

18. Statistical analysis.

All the obtained data were expressed as mean ± standard deviation. Statistical significance of the treatment groups was evaluated using unpaired two-tailed Student's t-test. ns, non-significant. $*P < 0.05$, $**P < 0.01$, and $***P < 0.001$ were considered statistically significant in analyses.

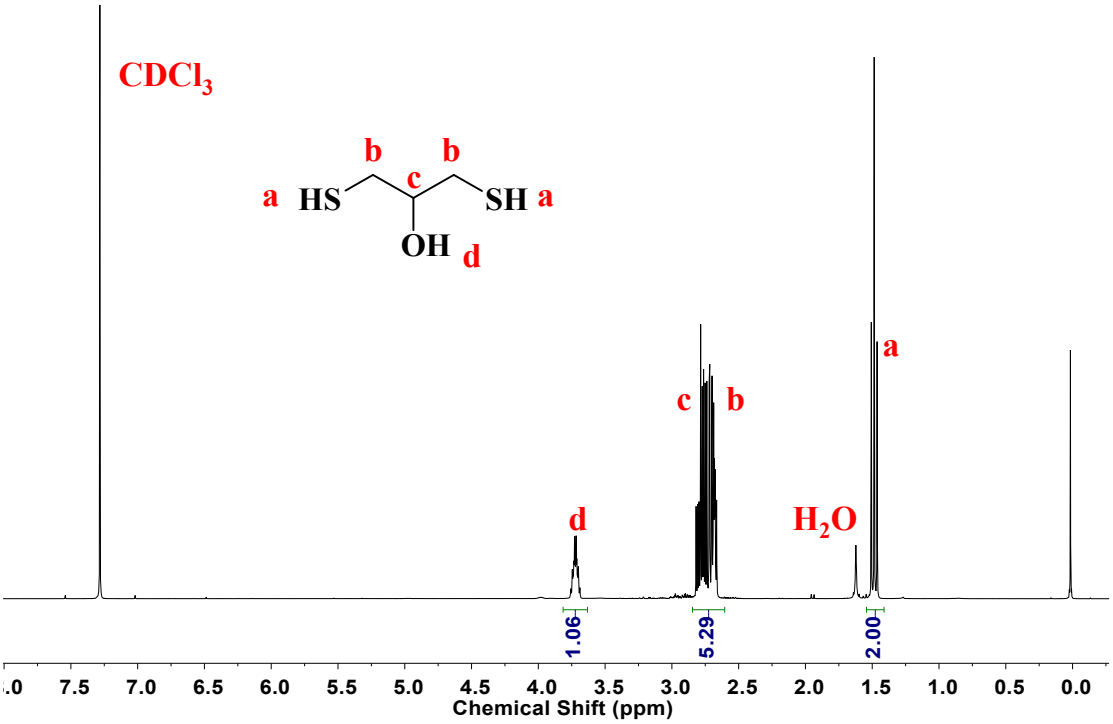


Figure S1. ^{1}H NMR spectrum recorded for compound **1** in $CDCl_3$.

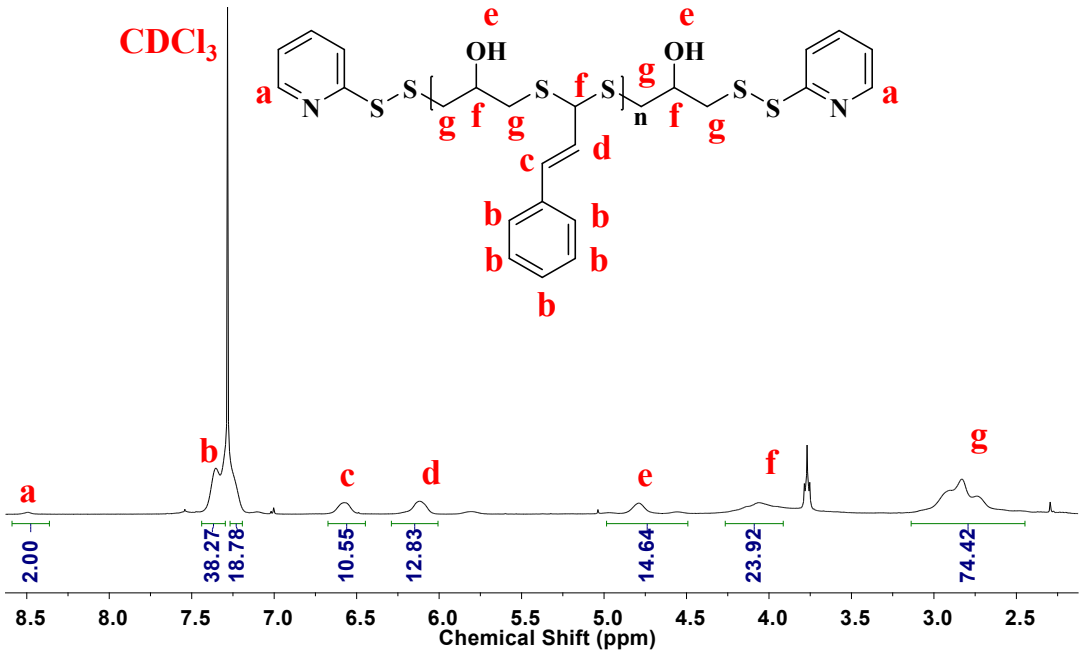


Figure S2. ^{1}H NMR spectrum recorded for TA-CA in $CDCl_3$.

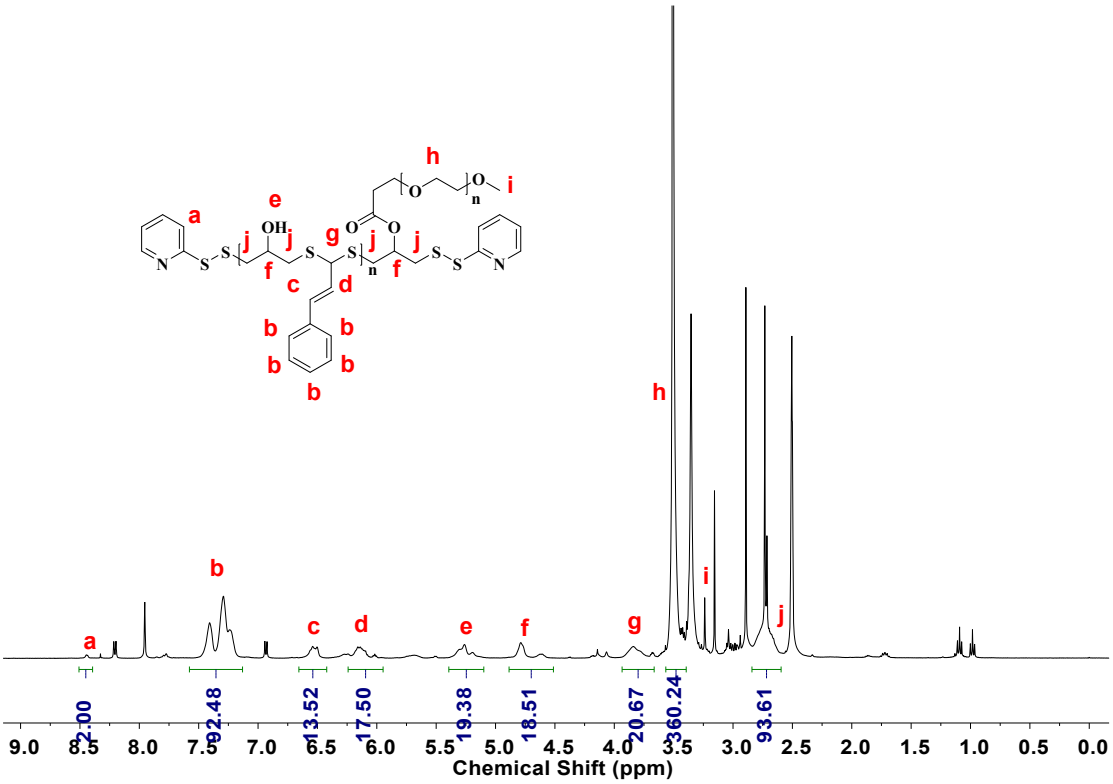


Figure S3. ^{1}H NMR spectrum recorded for TA-CA-PEG in DMSO-d_6.

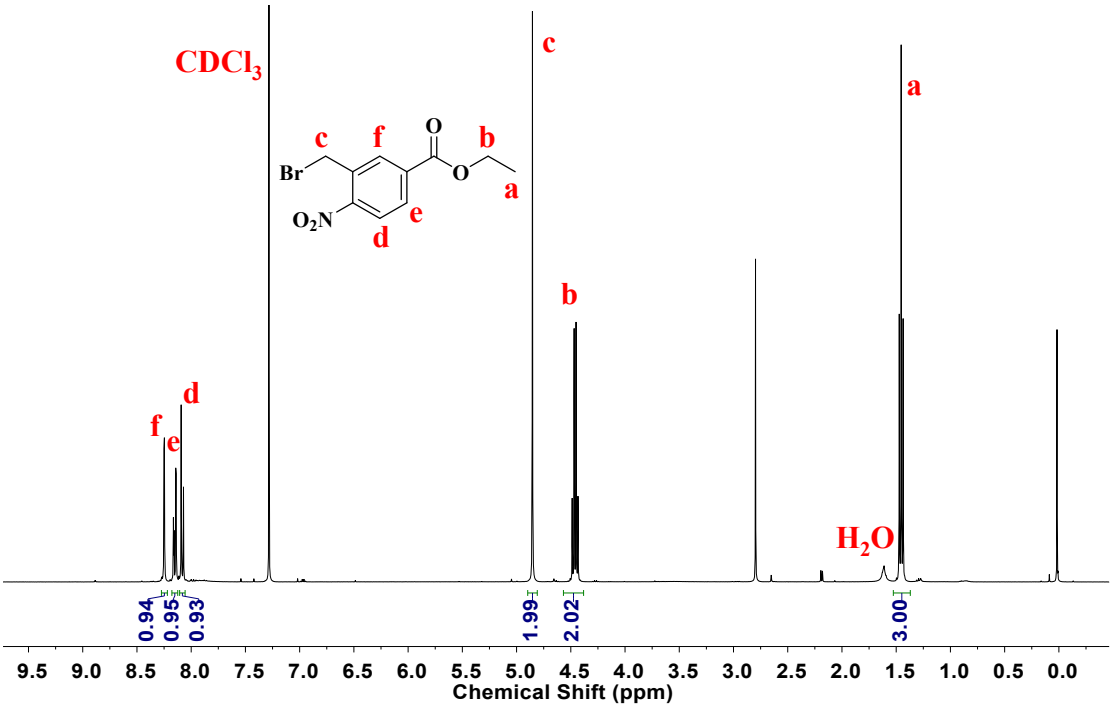


Figure S4. ^{1}H NMR spectrum recorded for compound **2** in $CDCl_3$.

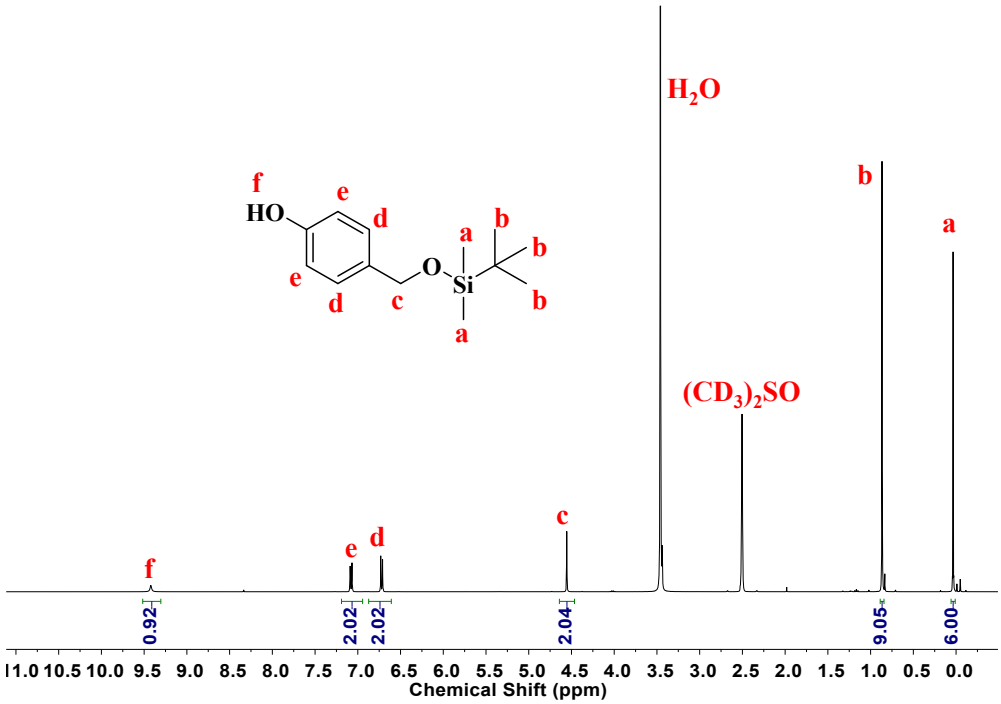


Figure S5. ^{1}H NMR spectrum recorded for compound **4** in DMSO-d_6.

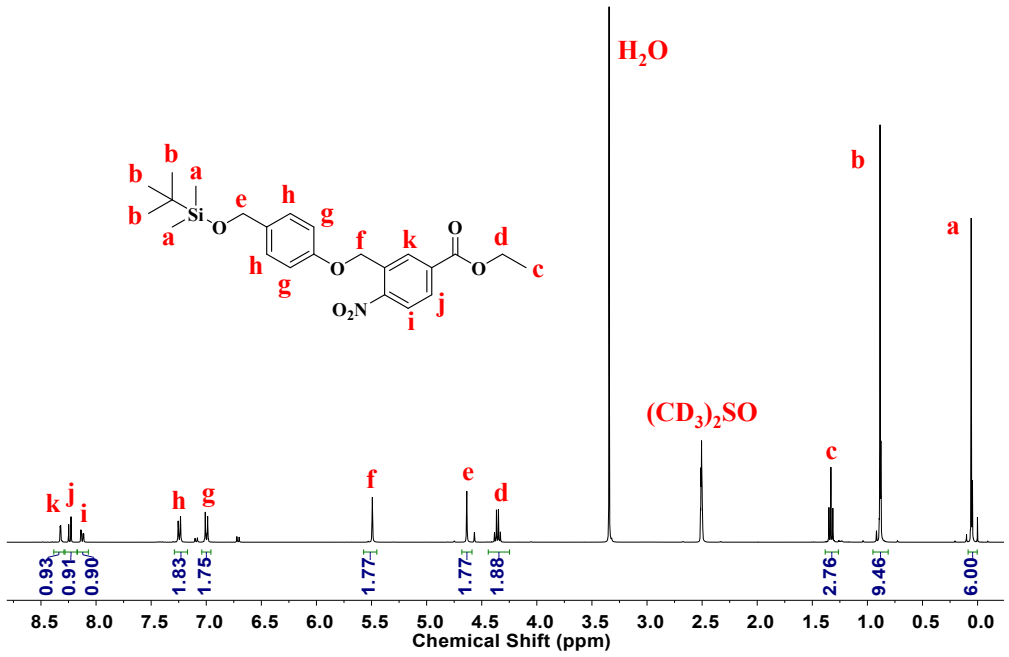


Figure S6. ^{1}H NMR spectrum recorded for compound **5** in DMSO-d_6.

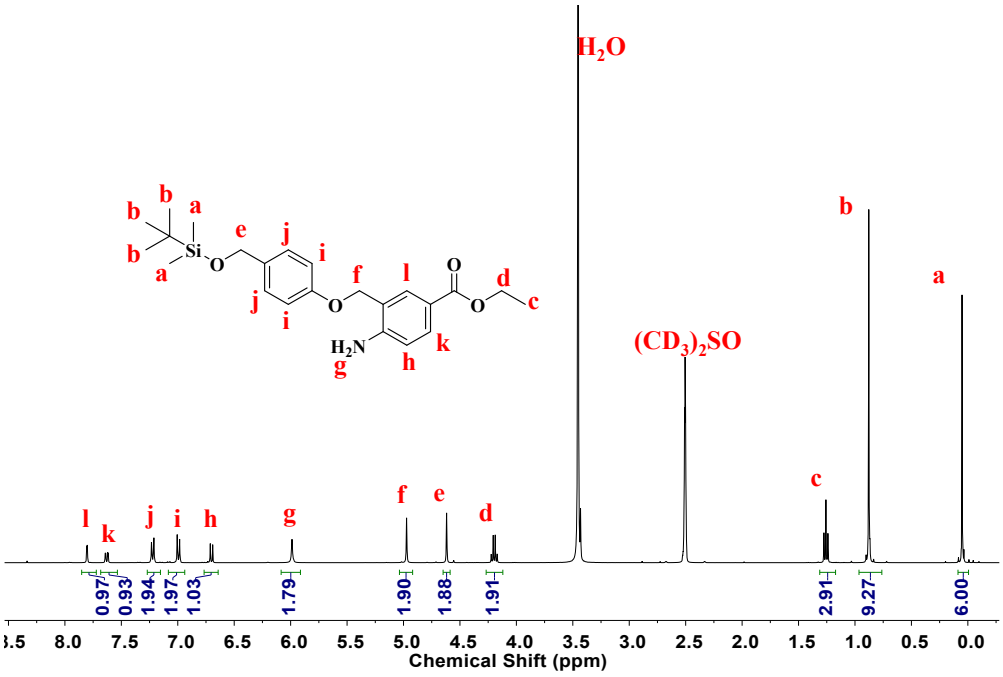


Figure S7. ^{1}H NMR spectrum recorded for compound **6** in DMSO-d_6.

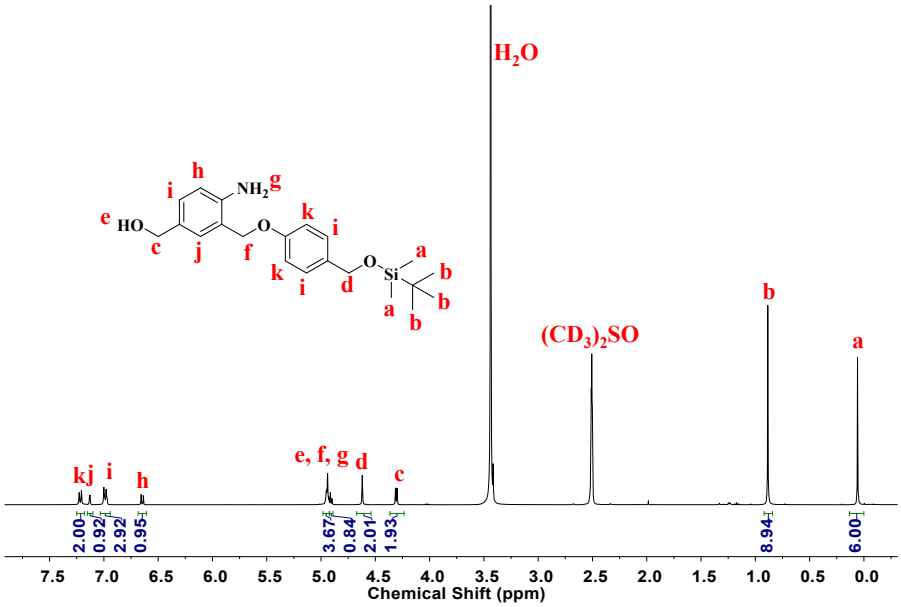


Figure S8. ^{1}H NMR spectrum recorded for compound **7** in DMSO-d_6.

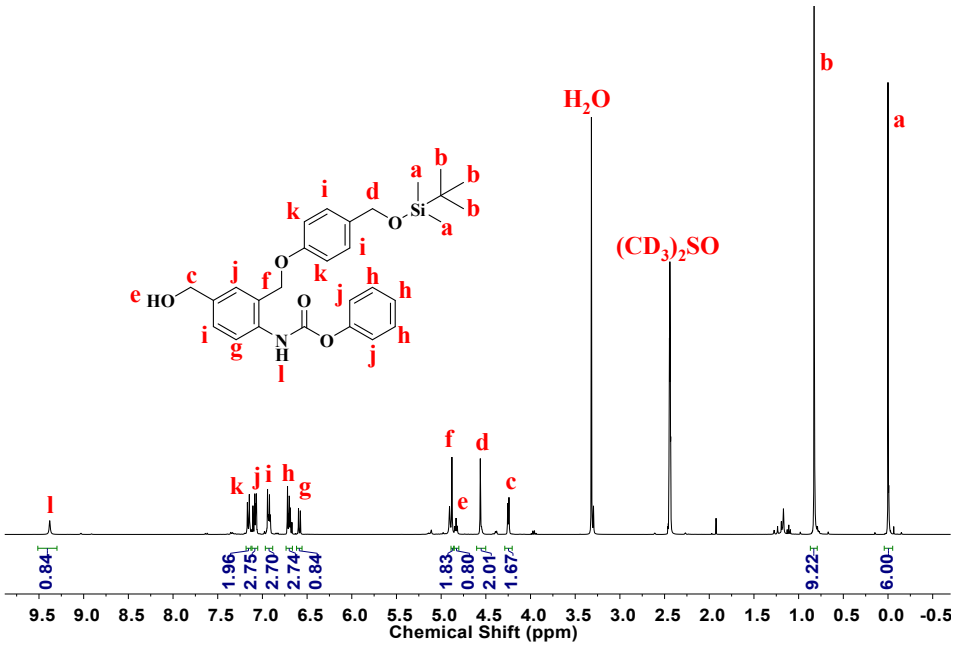


Figure S9. ^{1}H NMR spectrum recorded for compound **8** in DMSO-d_6.

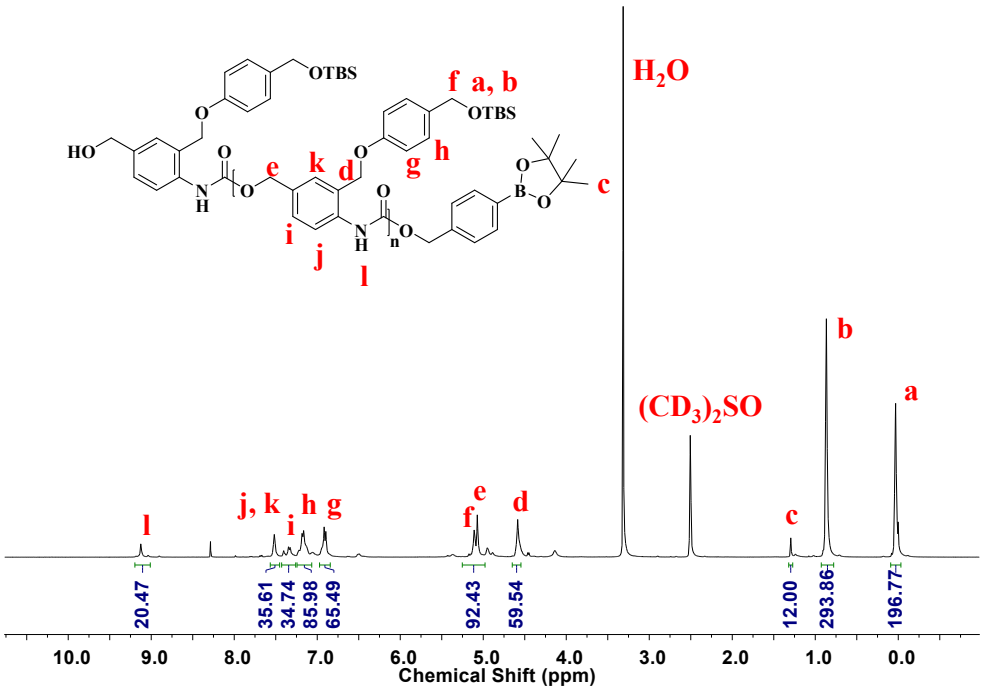


Figure S10. ^{1}H NMR spectrum recorded for **SIP1** in DMSO-d_6.

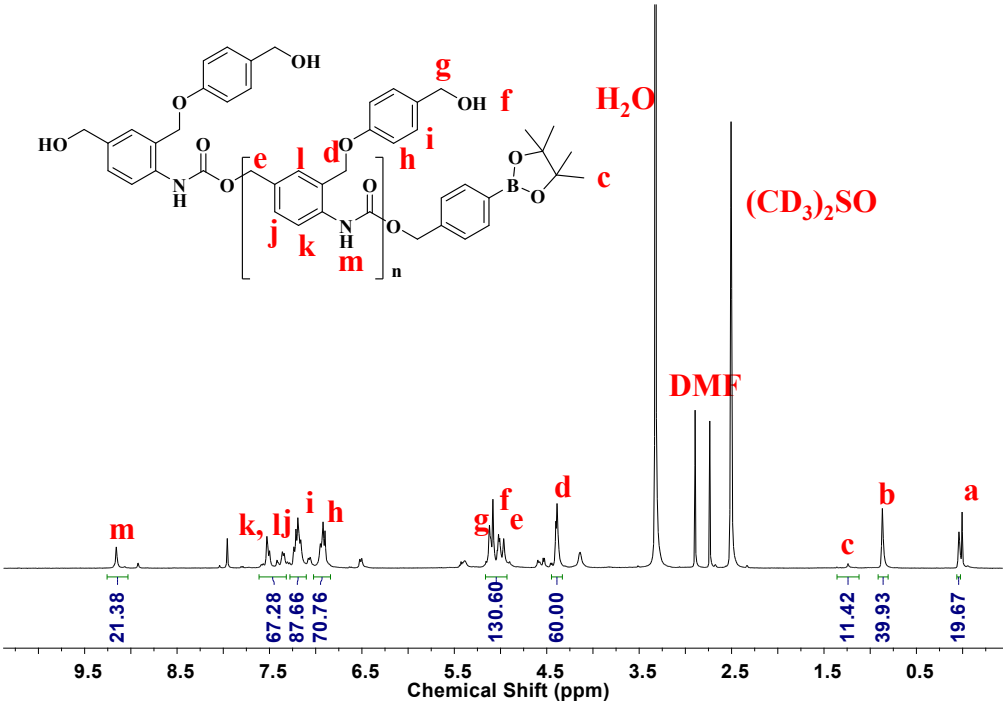


Figure S11. ^{1}H NMR spectrum recorded for **SIP2** in DMSO-d_6.

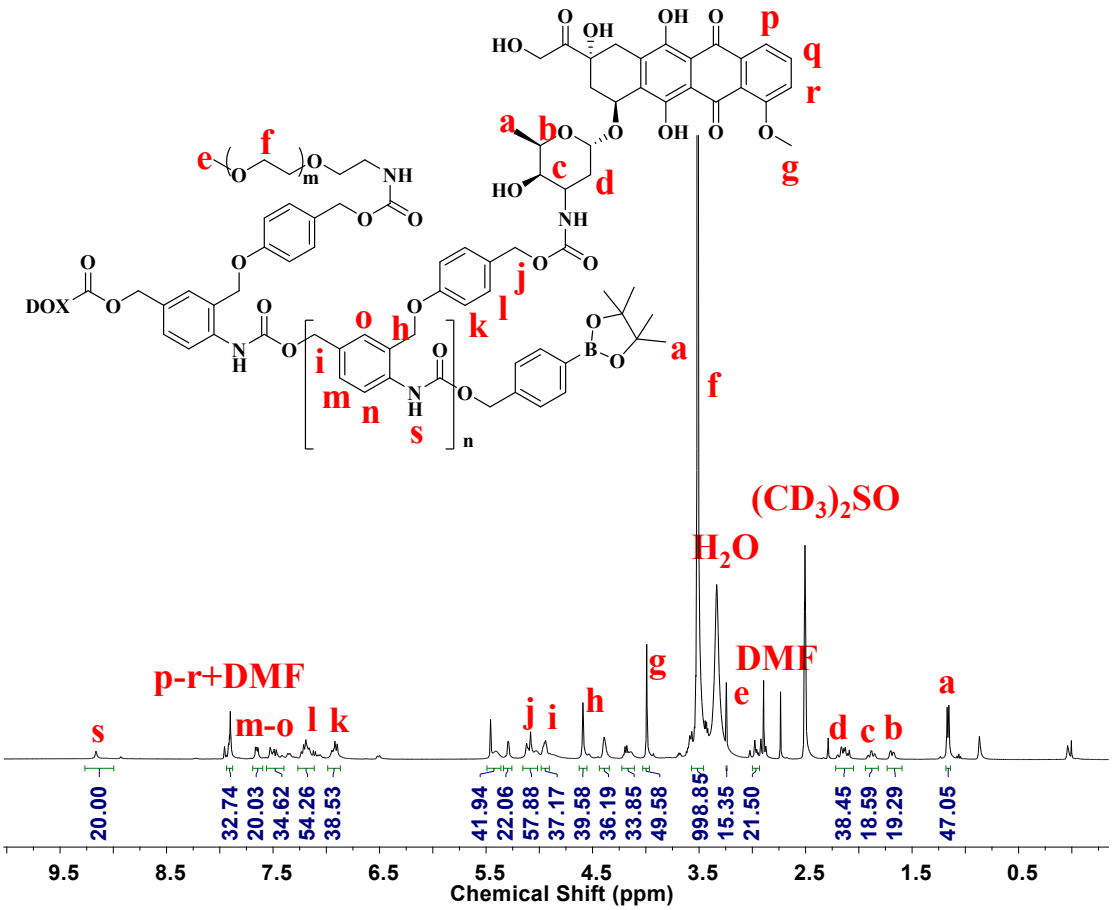


Figure S12. ^{1}H NMR spectrum recorded for SIP-DOX-PEG in DMSO-d_6.

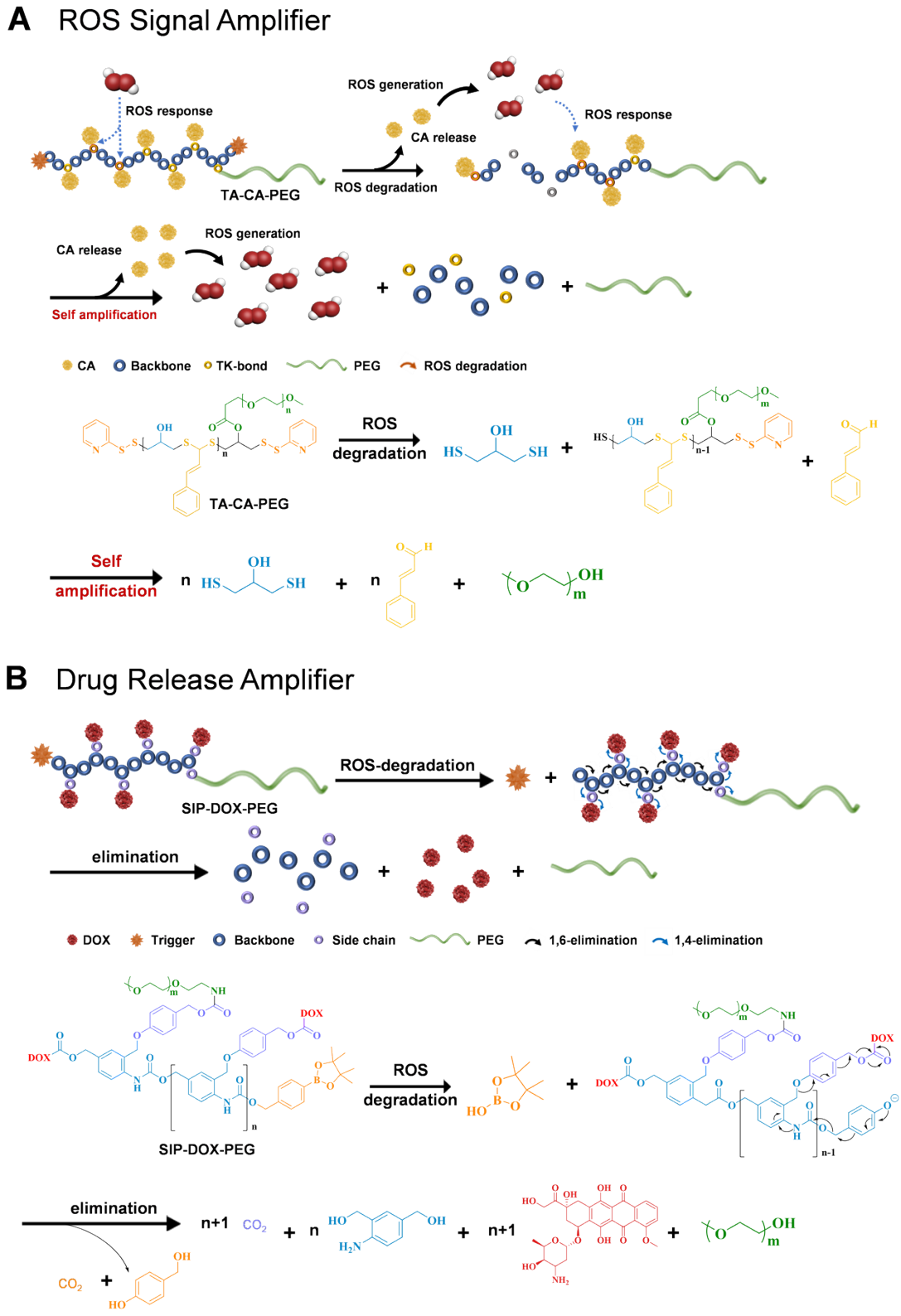


Figure S13. The degradation mechanism of TA-CA-PEG (A) and SIP-DOX-PEG (B) reacted with ROS.

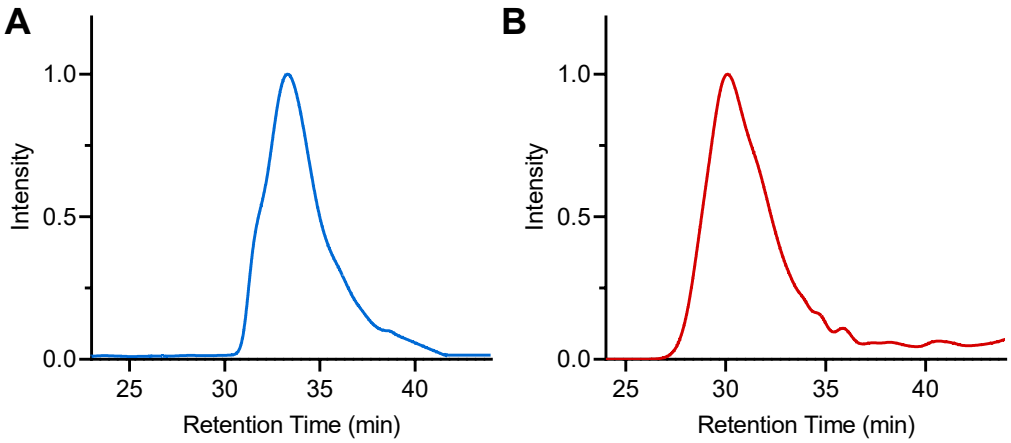


Figure S14. GPC analysis of TA-CA-PEG (A), SIP-DOX-PEG (B).

Table S1. Characterization of PCS.

Samples	M_n (g/mol) (GPC)	M_w (g/mol) (GPC)	PDI
SIP	10380	8240	1.26
TA-CA-PEG	5930	5540	1.07
SIP-DOX-PEG	22130	15650	1.35

Evaluated by GPC with polystyrene standards.

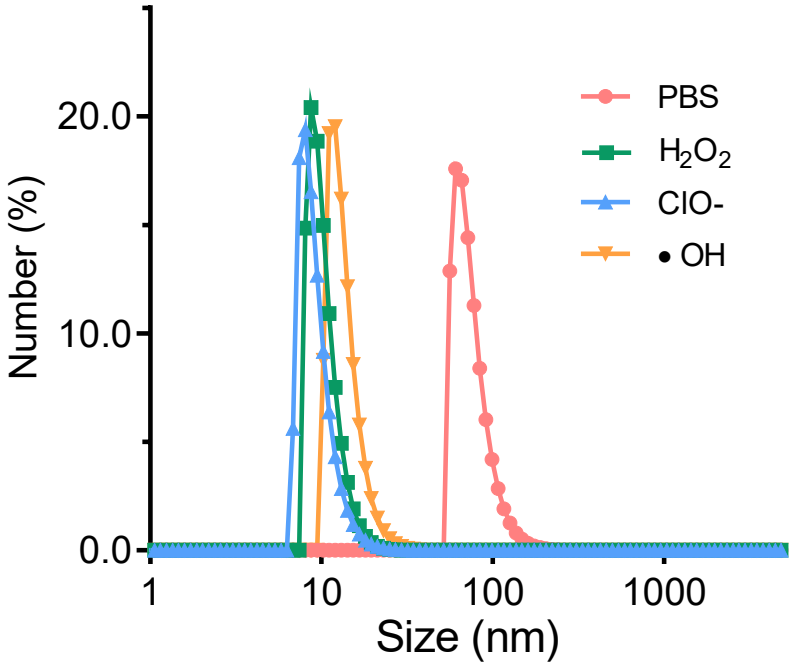


Figure S15. Size changes of $TS_{CA/DOX}$ in the presence of various ROS (H_2O_2: 10 mM, ClO^-: 5 nM, •OH: 3.3 mM).

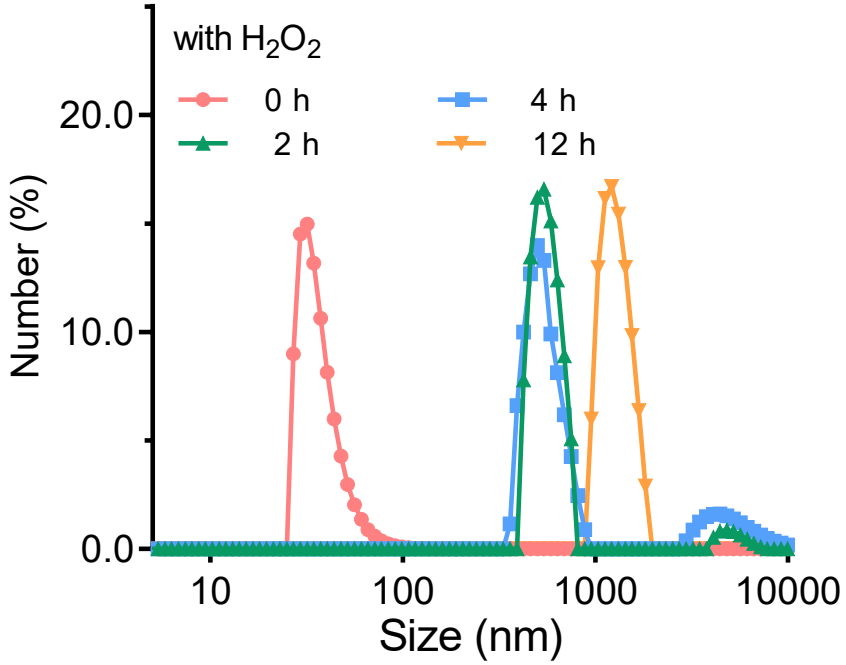


Figure S16. Size changes of SIP_{DOX} after the addition of H_2O_2 (10 mM) in PBS for different time.

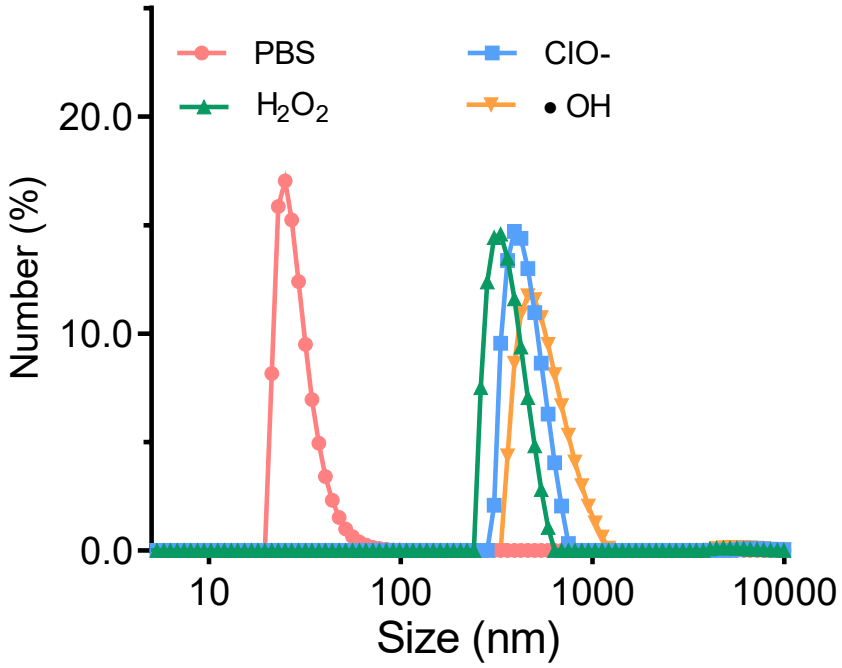


Figure S17. Size changes of SIP_{DOX} in the presence of various ROS (H_2O_2: 10 mM, ClO^-: 5 nM, •OH: 3.3 mM).

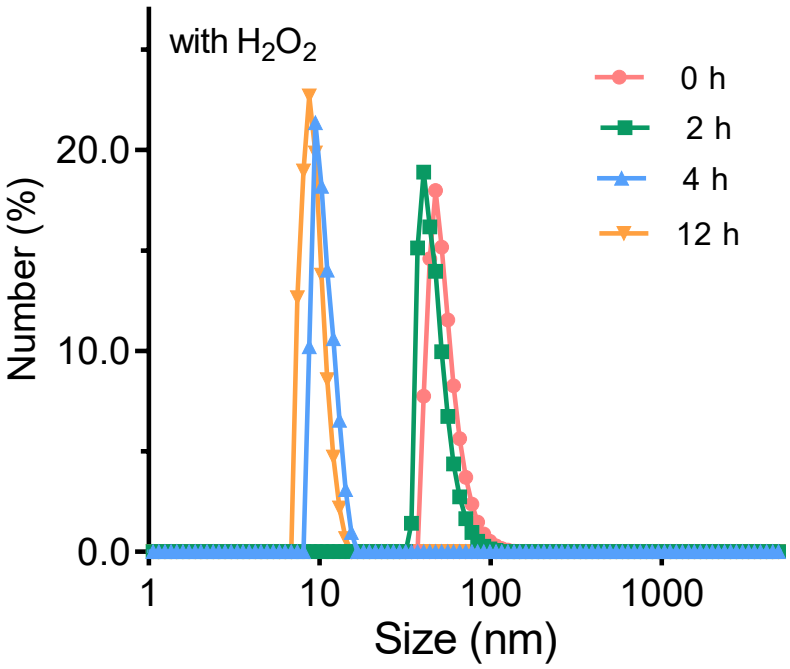


Figure S18. Size changes of TA_{CA} after the addition of H_2O_2 (10 mM) in PBS for different time.

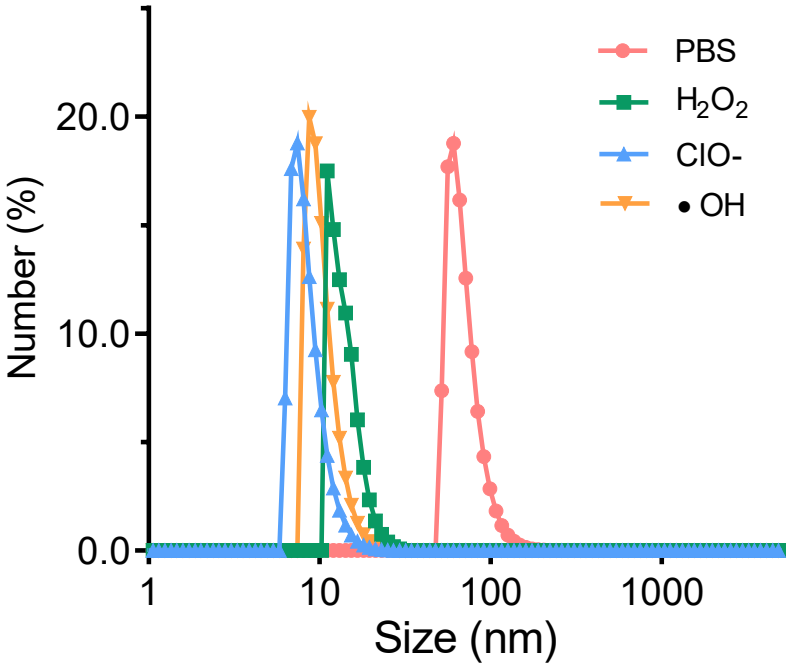


Figure S19. Size changes of TA_{CA} in the presence of various ROS (H_2O_2: 10 mM, ClO^-: 5 nM, •OH: 3.3 mM).

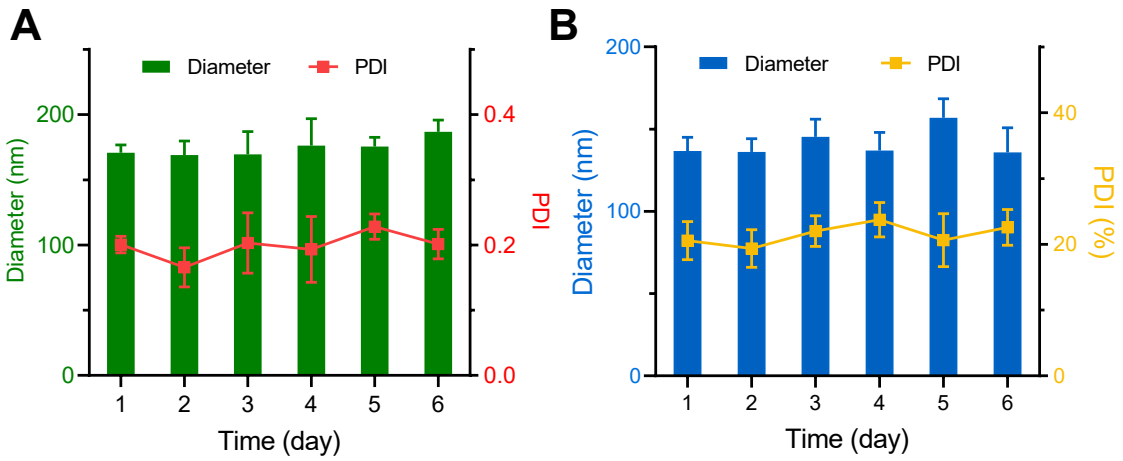


Figure S20. Size and polydispersity index (PDI) changes of $TS_{CA/DOX}$ (A) and SIP_{DOX} (B) after incubation in PBS for 7 days.

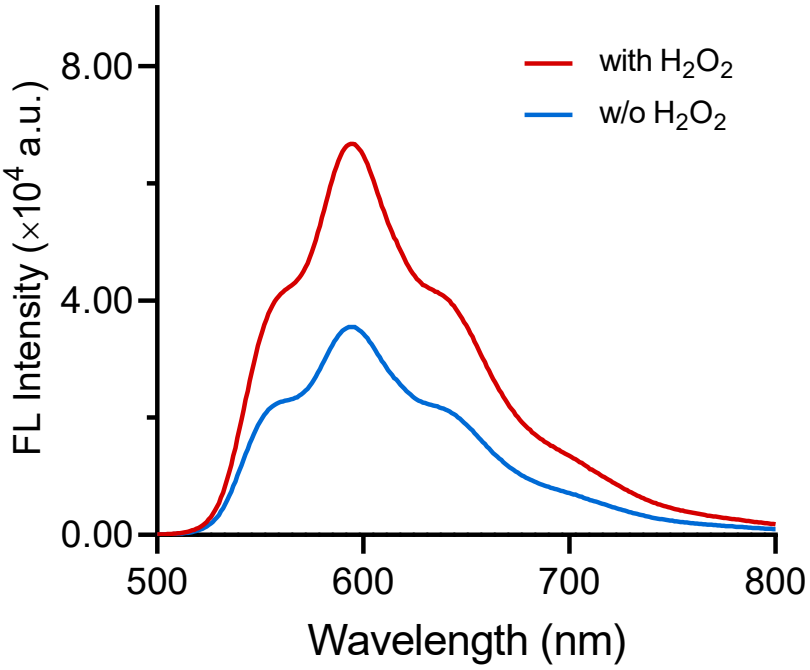


Figure S21. FL data of $TS_{CA/DOX}$ in PBS with or without H_2O_2 (10 mM) treatment.

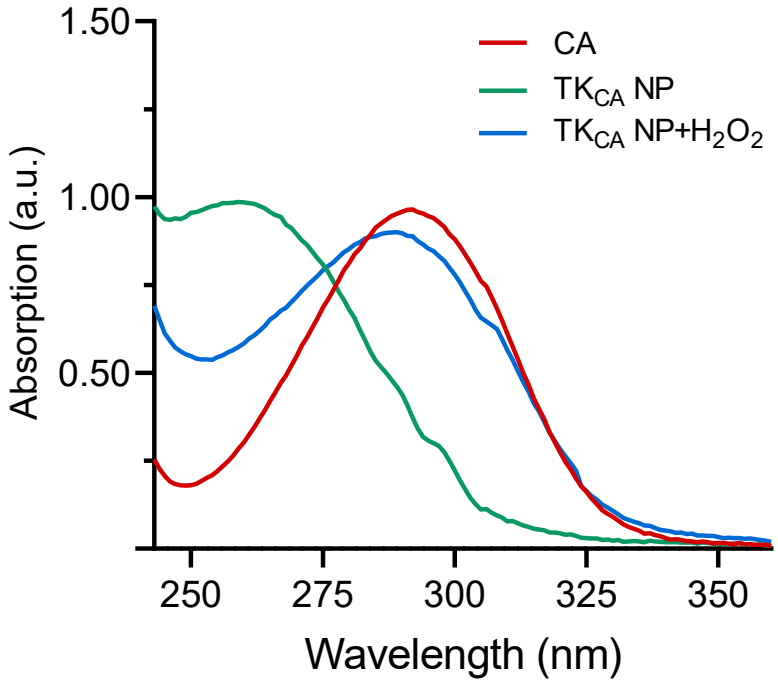


Figure S22. UV data of $TS_{CA/DOX}$ in PBS with or without H_2O_2 (10 mM) treatment.

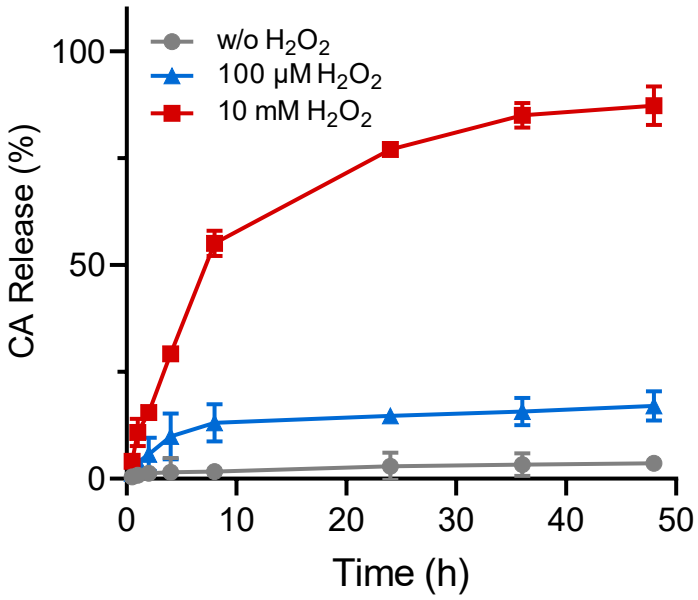


Figure S23. CA release profile from $TS_{CA/DOX}$ in the absence or presence of H_2O_2 (100 μM, 10 mM).

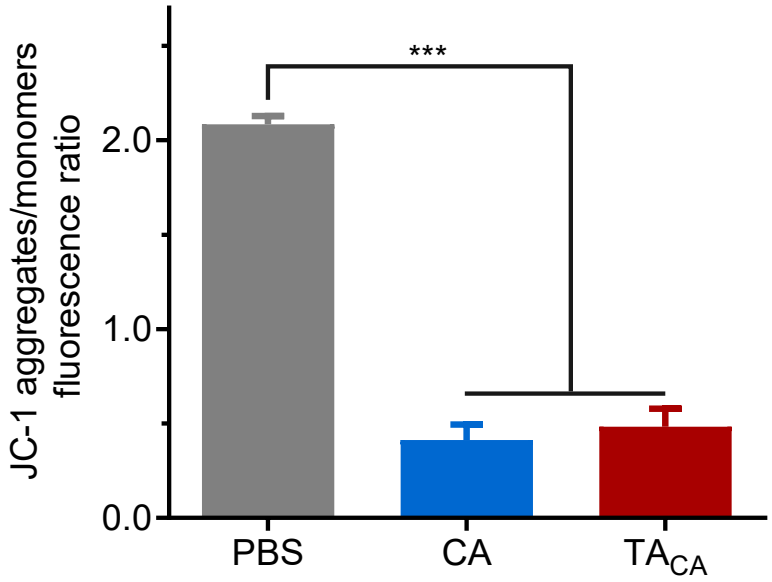


Figure S24. Quantitatively investigate of mitochondrial membrane potential by flow cytometry.

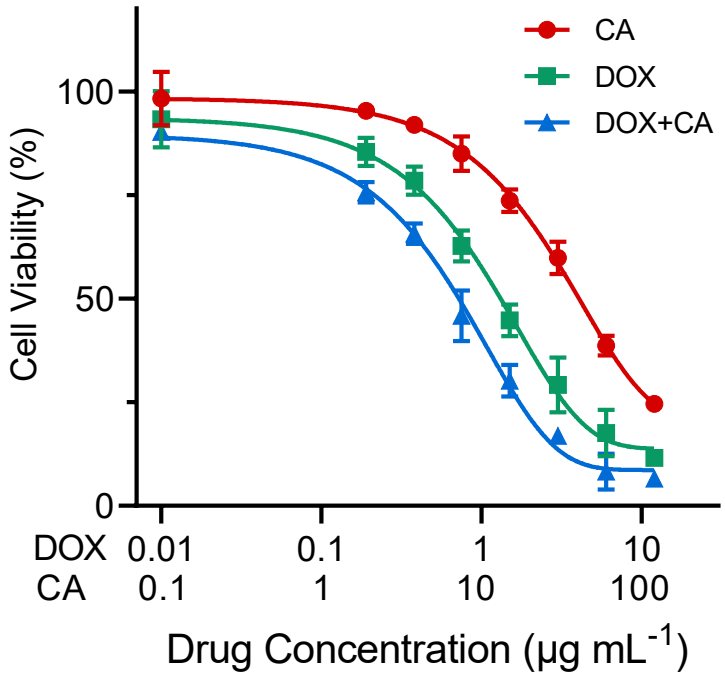


Figure S25. *In vitro* dose–effect cytotoxicity profiles of various ratios of free drugs (CA, DOX and DOX+CA).

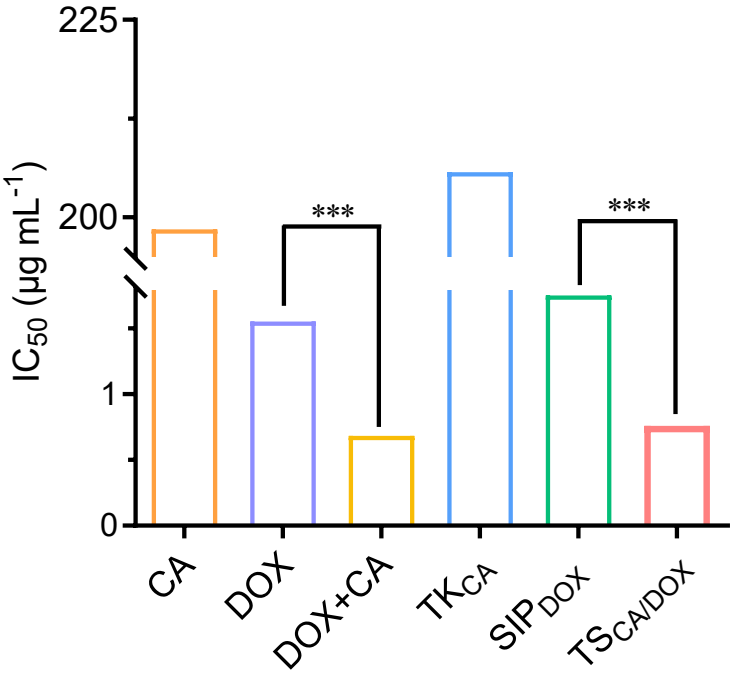


Figure S26. IC_{50} values of 4T1 cells after diverse treatments for 24 h.

Figure S27. (A) Flow cytometry analysis and (B) relative quantified results of the cell cycle of 4T1 cells treated with $TS_{CA/DOX}$ for 12 h.

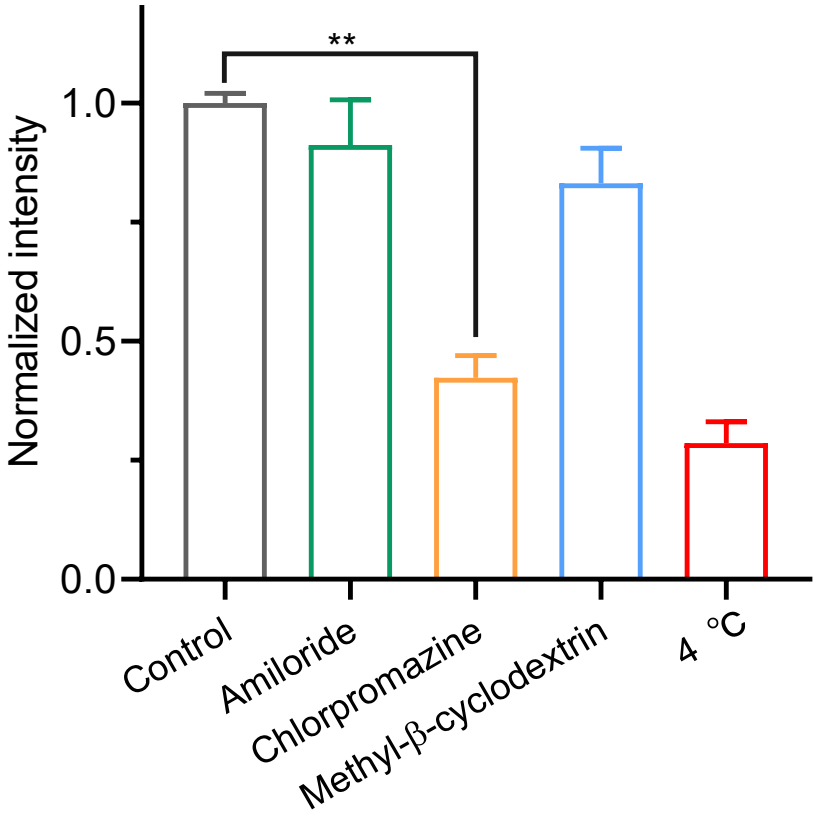


Figure S28. Normalized internalized amounts of $TS_{CA/DOX}$ by 4T1 cells treated with phosphate buffer solution (PBS) (control), chlorpromazine (10.0 μg mL^{-1}), methyl-β-cyclodextrin (5.0 mg mL^{-1}), amiloride (100.0 μg mL^{-1}) at 37 °C, and PBS at 4 °C, respectively.

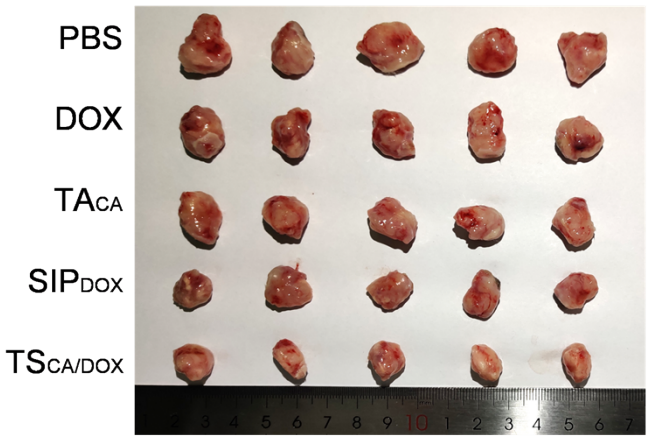


Figure S29. Picture of tumors after intravenous injection with PBS, DOX, TA_{CA}, SIP_{DOX}, $TS_{CA/DOX}$.