Supporting Information for:

Endogenous reactive oxygen species burst induced and spatiotemporally controlled multiple drugs release by traceable nanoparticles for enhancing antitumor efficacy

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1. Synthesis

1.1 Synthesis of PEG_{2K}-NH-N-DOX prodrug:

CH_3-PEG-OH (2.0 g, 1 mmol) and 40 mL absolute tetrahydrofuran (THF) were added to a 100 mL completely dry round-bottomed flask under dry N_2 protection. NaH (72 mg, 3 mmol) was quickly added into the flask with vigorous stirring at RT for 6 h until no bubbles emerged. Then, ethyl 2-bromoacetate (250 mg, 1.5 mmol) was slowly injected into the mixture. The reaction mixture was stirred under N_2 overnight at 35 °C. Then, the mixture was filtered. To the filtrate, 4 mL of water was added. The mixture was concentrated to remove THF by rotary evaporation. Then, the residue was extracted with dichloromethane (30 mL×3). The organic layers were combined, washed with water and brine, dried over anhydrous Na_2SO_4, filtered, and concentrated under reduced pressure. The crude product was further purified with n-hexane precipitation three times and then **PEG–O–CH_2COOEt** (1.3 g, yield 63.4%) was obtained as a white solid and confirmed by ^{1}H NMR.

^{1}H NMR (500 MHz, $CDCl_3$): δ/ppm = 4.15 (m, 2H, -CH_2-); 4.08 (s, 2H, -CH_2-); 3.58 (s, 185H, -CH_2-CH_2O-); 3.31 (s, 3H, CH_3-); 1.22 (t, 3H, CH_3-).

PEG–O–CH_2COOEt (1.05 g, 0.5 mmol) and hydrazine hydrate (2.5 mL, 50 mmol) were mixed with 50 mL THF in a 100 mL flask, and the mixture was refluxed with vigorous stirring for 7 h. Then the organic phase was collected and the solvent was removed under vacuum. The crude product was precipitated with ether. **PEG-O-CH_2CONHNH_2** was obtained (0.95 g, yield 90.4%) and confirmed by ^{1}H NMR.
^{1}H NMR (500 MHz, $CDCl_3$): δ/ppm = 4.11 (s, 2H, -CH_2-); 3.64 (s, 185H, -CH_2-CH_2O-); 3.24 (3H, CH_3-).

PEG-O-CH_2CONHNH_2 (420 mg, 0.2 mmol) and DOX.HCl (120 mg, 0.2 mmol) were dissolved in 10 mL of anhydrous methanol, and 20 μl trifluoroacetic acid was added as a catalyst. The mixture was stirred in the dark at room temperature for 48 h. Then 0.1 g triethylamine (TEA) was added and the mixture was dialyzed against PBS buffer (pH 8.0) using a dialysis membrane with a molecular weight cut-off of 1500 Da at room temperature for 48 h. After freeze-drying, the crude product was dissolved in 10 mL of DCM, filtered, and concentrated under reduced pressure. **PEG_{2K}-NH-N-DOX** was obtained (426 mg, yield 79 %) as a red powder and confirmed by ^{1}H NMR and MALDI-TOF-MS.

^{1}H NMR (500 MHz, DMSO-d6): δ/ppm = 7.5-8.2 (CH of aromatic ring); 3.51 (PEG, -CH_2-CH_2O-); 3.38 (3H, CH_3-); 1.19 (3H, CH_3-).

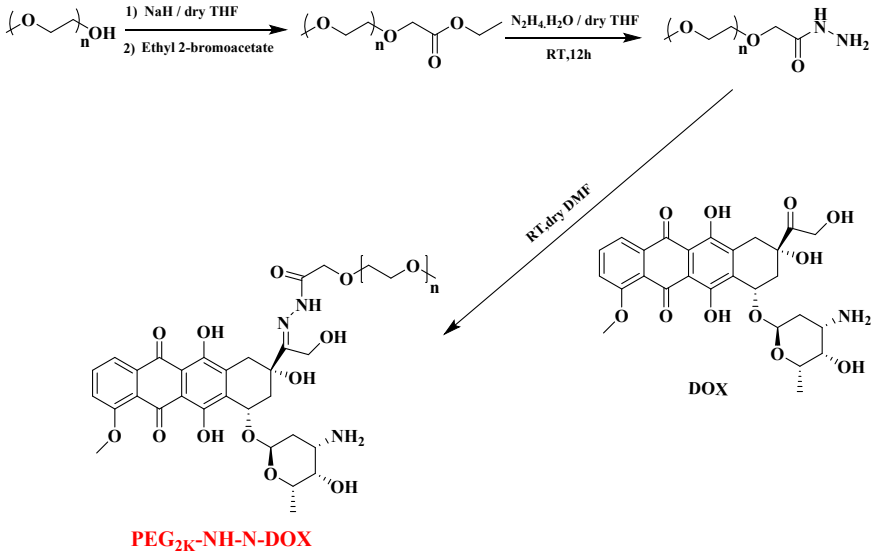
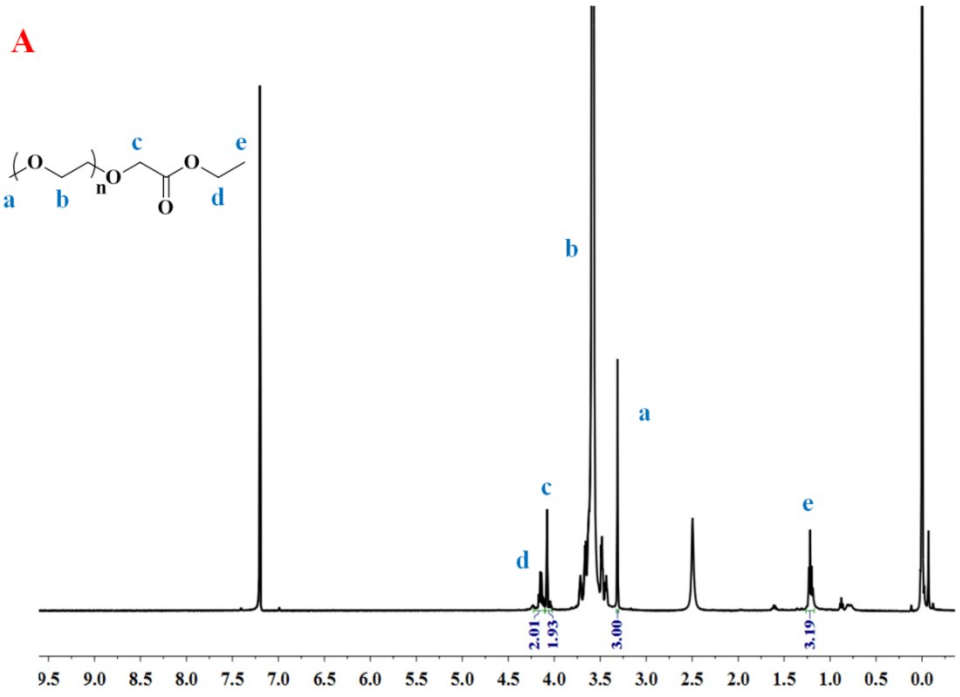
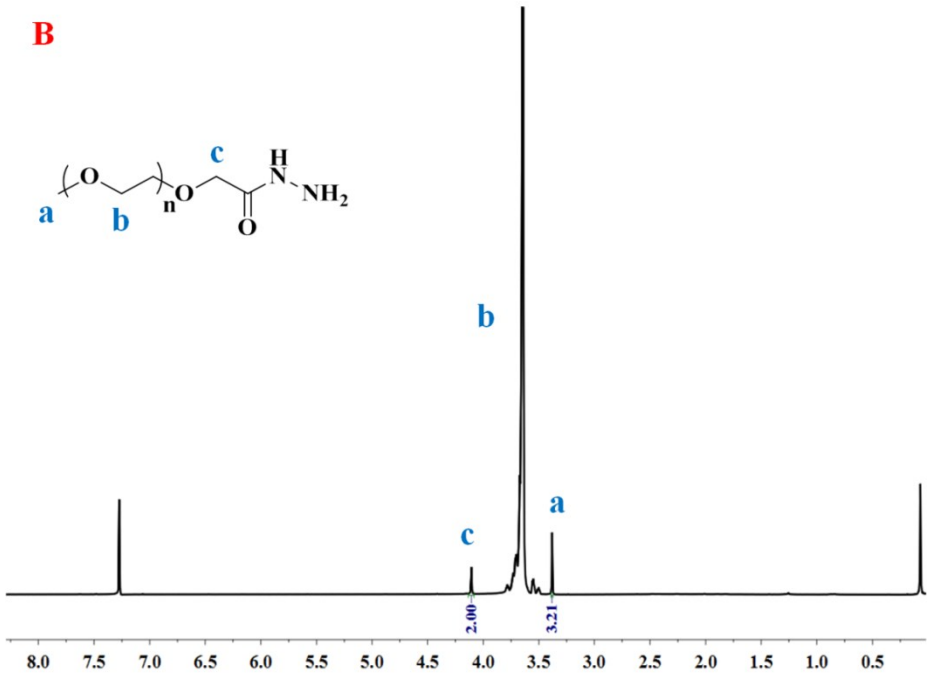


Fig. S1. Synthetic route of **PEG_{2K}-NH-N-DOX** prodrug.





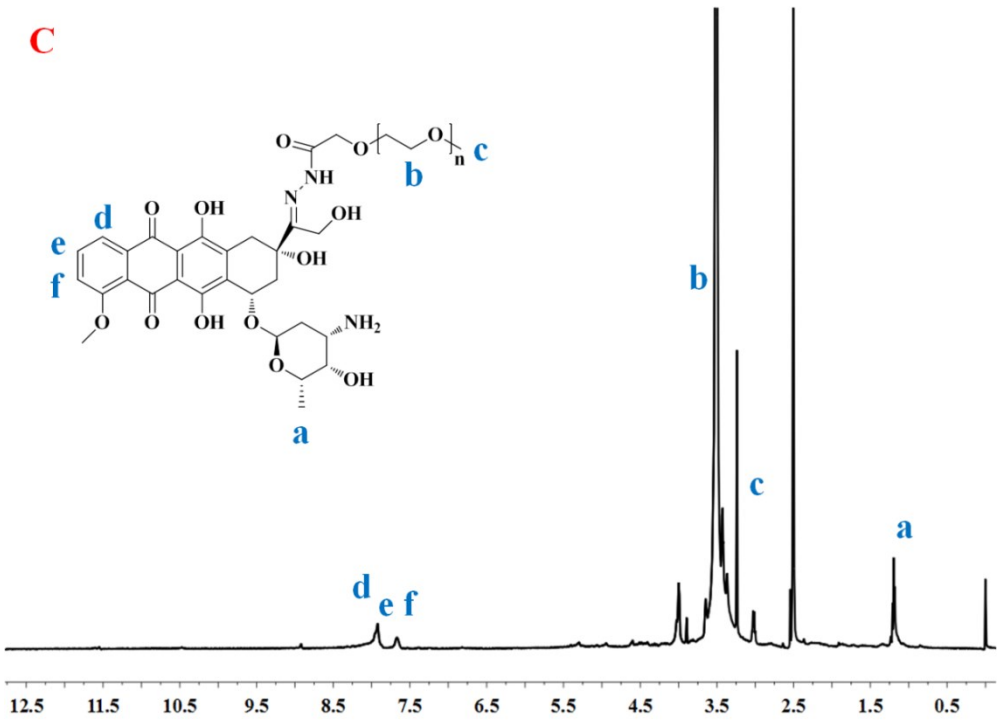


Fig. S2. ^{1}H-NMR spectra of (A) **PEG–O–CH_2COOEt**, (B) **PEG-O-CH_2CONHNH_2** and (C) **PEG_{2K}-NH-N-DOX.**

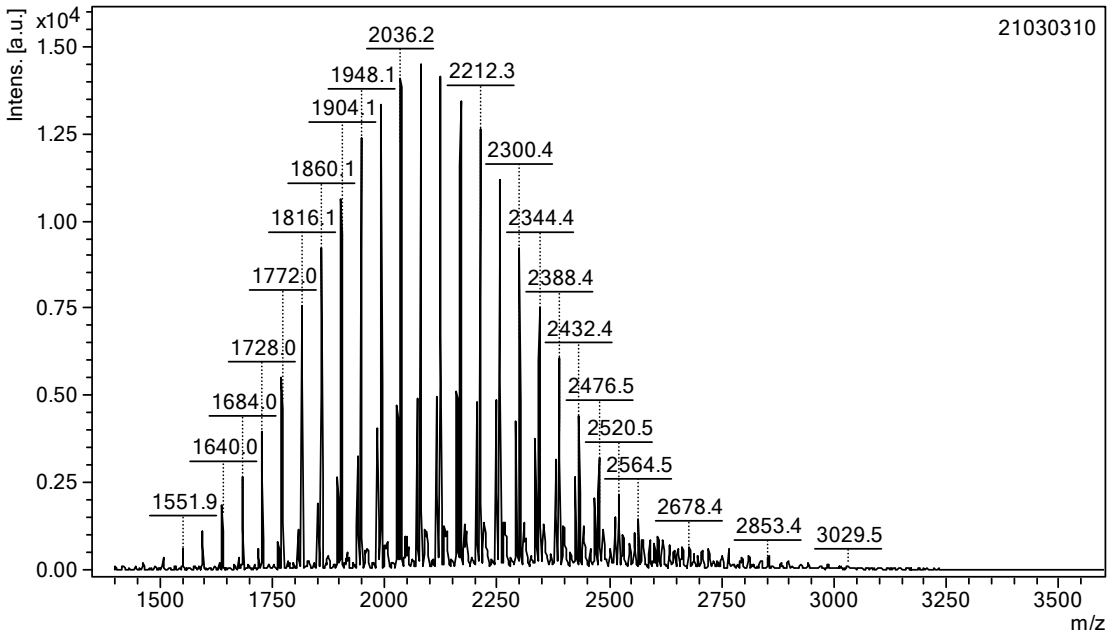


Fig. S3. MALDI-TOF-MS spectra of **PEG_{2K}-NH-N-DOX.**

1.2. Synthesis of PEG_{2K}-S-S-CPT-ROS Prodrug:

Anhydrous 3-mercaptopropionic acid (5.2 g, 49.1 mmol) and anhydrous acetone (5.8 g, 98.2 mmol) were purged with dry HCl gas under stirring at room temperature for 6 h. Afterward, the mixture was crystallized in an ice bath. The collected crystals were washed with hexane and cold water, and vacuum dried to obtain 5, 5-dimethyl-4, 6-dithia-nonanedioic acid (**COOH-S-S-COOH-ROS**) as a white powder (9.16 g, yield 74.1%). The successful synthesis of the thioketal linker was confirmed by ^{1}H-NMR and MS examination, respectively.

^{1}H NMR (500 MHz, $CDCl_3$): δ/ppm = 11.99 (2H, -COOH); 2.83 (4H, -CH_2-); 2.62 (4H, -CH_2-); 1.53 (6H, CH_3-). MS: M=252; m/z 250.59 is $[M-H]^-$.

COOH-S-S-COOH-ROS (504 mg, 2 mmol) was dissolved in anhydrous THF (50 mL) and cooled in an ice bath. $LiAlH_4$ (1 g, 27 mmol) was slowly added over 30 min and the mixture was refluxed for 1 h. Then the reaction mixture was slowly added 15% NaOH aqueous solution until no gas was generated. Afterward, the mixture was filtered. The filtrate was evaporated to dryness. DCM was added followed by Na_2SO_4 to remove water. The mixture was filtered and evaporated to obtain hydroxyl residue ROS-cleavable thioketal linker (**OH-S-S-OH-ROS**, 313 mg, yield 80%). The successful synthesis of **OH-S-S-OH-ROS** was confirmed by ^{1}H-NMR.

^{1}H NMR (500 MHz, $CDCl_3$): δ/ppm = 3.74 (4H, -CH_2-); 2.75 (4H, -CH_2-); 1.61 (6H, CH_3-).

CPT (0.5 g, 1.43 mmol) and triphosgene (0.157 g, 0.53 mmol) were mixed with 40 mL anhydrous DCM in a 100 mL flask under dry N_2 protection, a DCM(10 mL) solution containing DMAP (0.53 g, 4.35 mmol) was slowly injected into the mixture. After stirring at room temperature for 30 min, **OH-S-S-OH-ROS** (310 mg, 1.58 mmol) in anhydrous THF (5 mL) was added. The solution was stirred at room temperature 24 h. The mixture was washed with 0.1 M HCl (3 × 40 mL), brine (1 × 80 mL) and then with water (1 × 80 mL). The organic layer was separated and dried over anhydrous Na_2SO_4. After filtration, the solvent was removed in vacuum and the residue was purified by silica gel chromatography (CH_2Cl_2/CH_3OH: 20:1) to give **CPT-S-S-OH-ROS** as a yellow powder (600 mg, yield 73.4%) and confirmed by ^1H NMR and MS.

^1H NMR (500 MHz, $CDCl_3$): δ/ppm = 8.36 (d, 1H,); 8.22 (m, 1H); 7.94 (m, 1H); 7.82 (m, 1H); 7.67 (m, 1H); 7.34 (m, 1H); 5.71 (m, 1H); 5.35 (m, 1H); 5.29 (t, 2H); 4.2 (m, 2H); 3.74 (m, 2H); 2.67 (m, 4H); 2.21 (m, 2H); 1.59 (s, 3H); 1.51 (t, 3H) ;0.98 (m, 3H). MS: M=570; m/z 551.86 is $[M-H_2O-H]^+$.

The solution of mPEG (2 g, 0.1 mmol) and DMAP (15 mg, 0.12 mmol) was dissolved in 40 mL of anhydrous 1,4-dioxane under a nitrogen atmosphere at RT. Succinic anhydride (50 mg, 0.5 mmol) was added for the further reaction of 24 h. Afterward, the solution was concentrated under vacuum, the residue was washed with brine, extracted with dichloromethane (30 mL×3), dried over anhydrous Na_2SO_4, filtered, concentrated by rotary evaporation and precipitated in cold ethyl ether three times, then **mPEG-COOH** (1.6 g, yield 80%) was obtained as a white solid and confirmed by ^1H NMR and IR.

^1H NMR (500 MHz, $CDCl_3$): δ/ppm = 3.61 (PEG, -CH_2-CH_2O-); 3.27 (3H, CH_3-).

IR: the characteristic peak at 1723 cm^{-1}corresponds to the absorption of the C=O.

mPEG-COOH (400 mg, 0.2 mmol) was dissolved in anhydrous DCM (10 mL). The solution of EDCI (60 mg, 0.3 mmol), DMAP (36 mg, 0.3 mmol) in anhydrous DCM (5 mL) was added to the above-mentioned solution and the mixture was stirred at room temperature for 2 h. Next, the solution of **CPT-S-S-OH-ROS** (120 mg, 0.21 mmol) in 15 mL of anhydrous DCM was added and the reaction was stirred for 24 h at room temperature under dry N_2 protection. Afterward, the mixture was washed with 0.1 M HCl, brine and water, dried over anhydrous Na_2SO_4 and concentrated to the crude product, which was purified by silica gel chromatography (CH_2Cl_2/CH_3OH: from 50:1 to 25:1 and then 10:1 ratio in sequence) to

give **PEG_{2K}-S-S-CPT-ROS** as a pale yellow powder (200 mg, yield 44.4%) and confirmed by ^1H NMR and MALDI-TOF-MS.

^1H NMR (500 MHz, $CDCl_3$): δ/ppm = 8.34 (s, 1H,); 8.16 (d, 1H); 7.88 (d, 1H); 7.78 (t, 1H); 7.61 (t, 1H); 7.26 (s, 1H); 5.61(m, 1H); 5.33 (d, 1H); 5.24 (s, 2H); 4.17 (m, 4H); 3.58 (PEG); 3.48 (m, 4H); 2.23 (m, 1H); 2.1 (m, 1H); 1.52 (d, 3H); 1.48 (d, 3H); 0.92 (t, 3H).

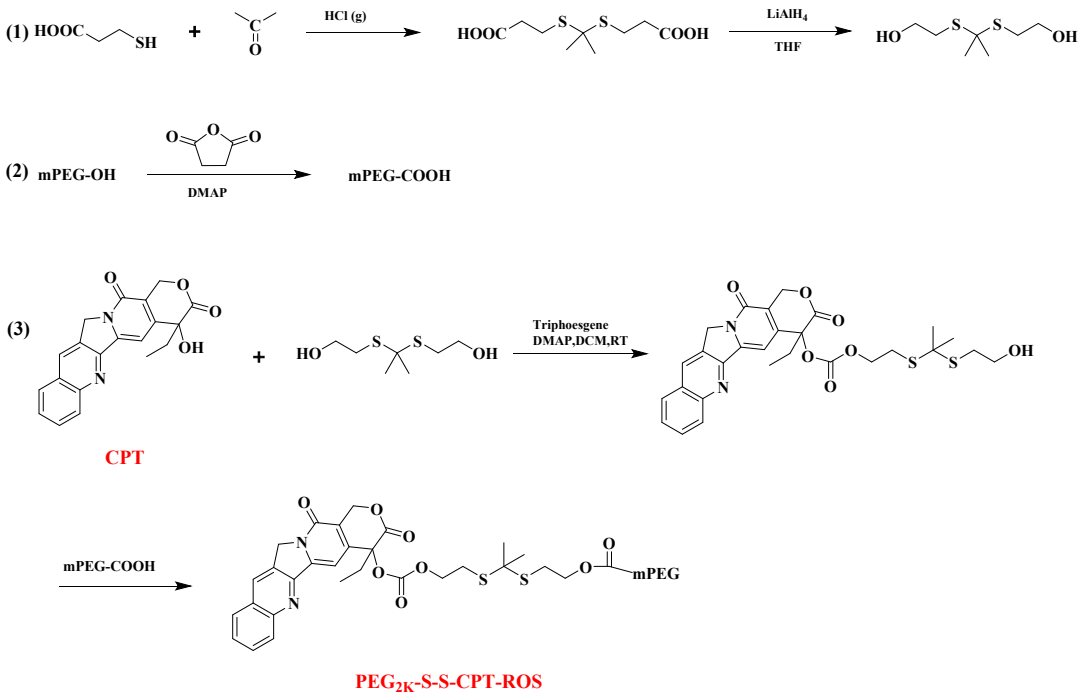
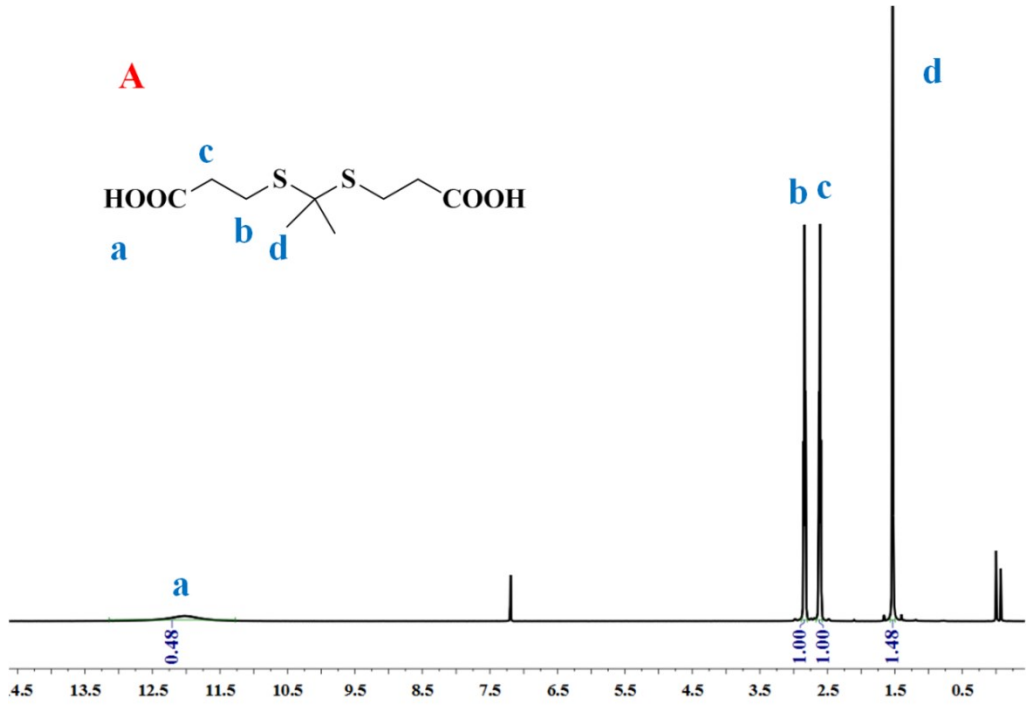


Fig. S4. Synthetic route of **PEG_{2K}-S-S-CPT-ROS** prodrug.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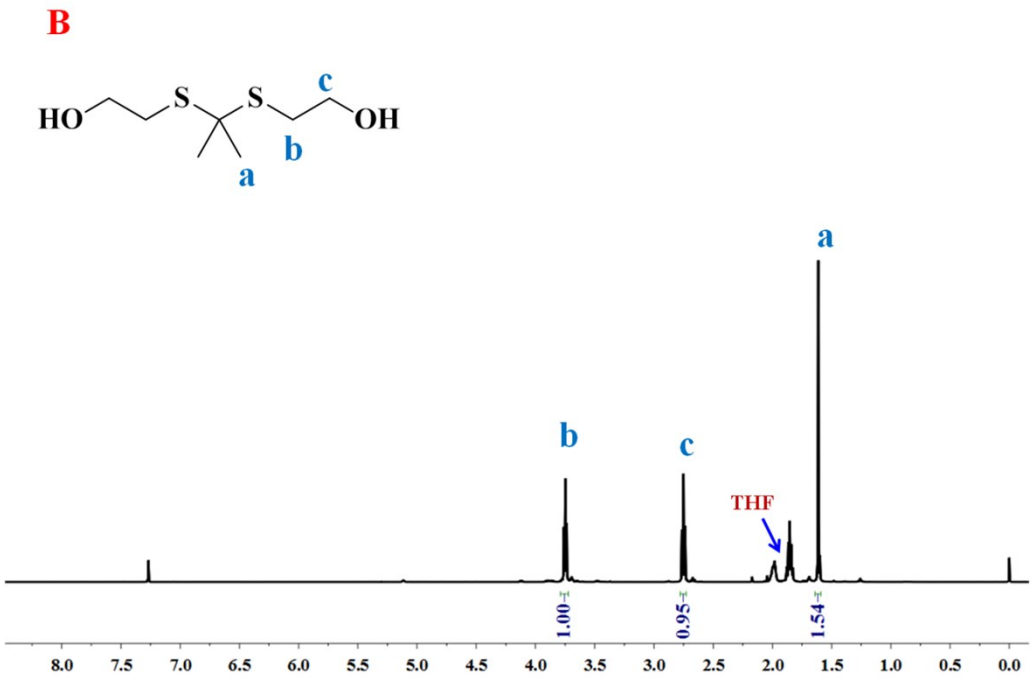
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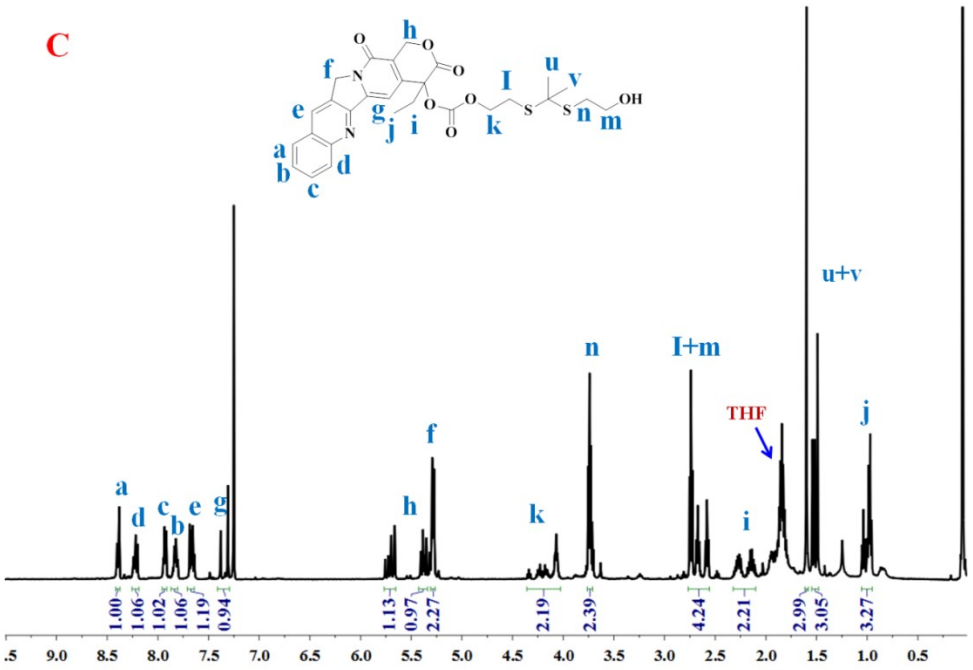
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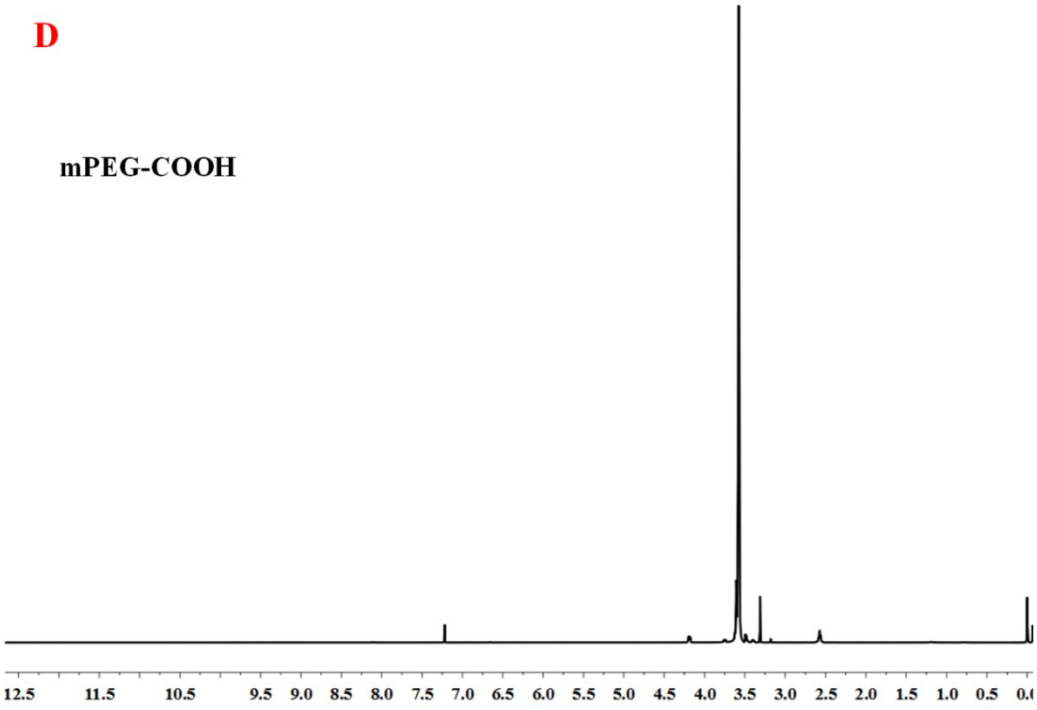
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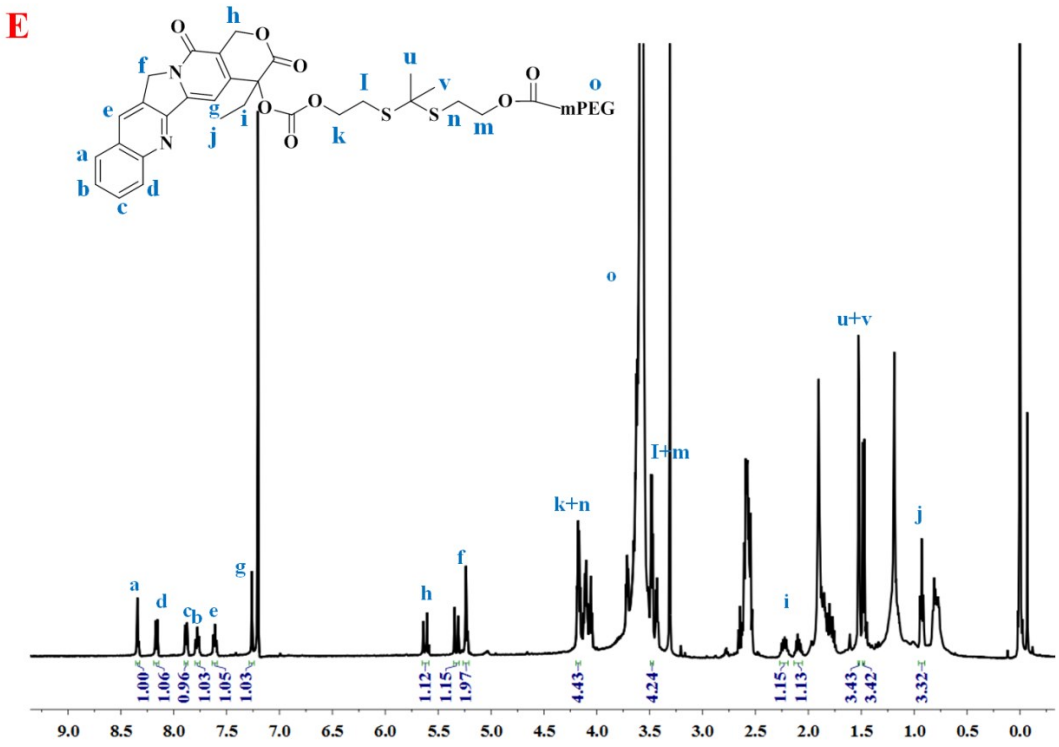
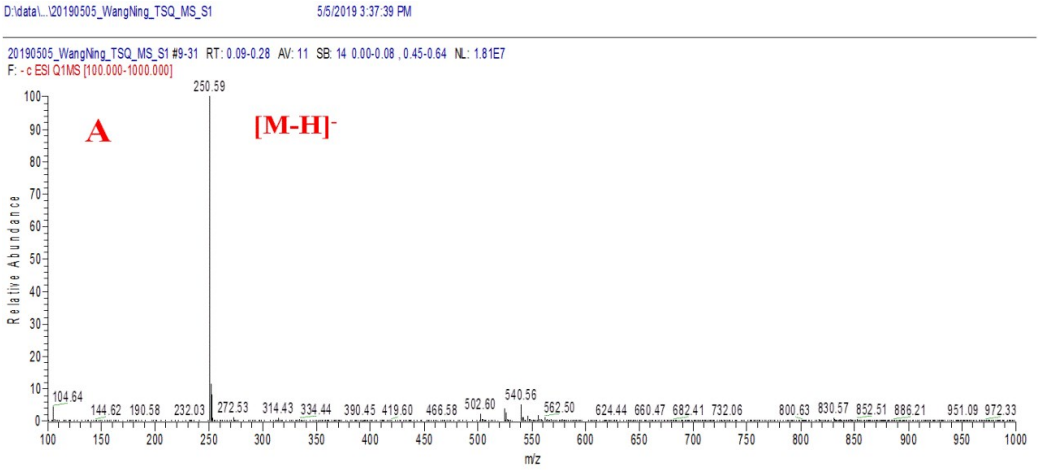


Fig. S5. [1]H-NMR spectra of (A) **COOH-S-S-COOH-ROS**, (B) **OH-S-S-OH-ROS**, (C) **CPT-S-S-OH-ROS**, (D) **mPEG-COOH** and (E) **PEG_{2K}-S-S-CPT-ROS.**



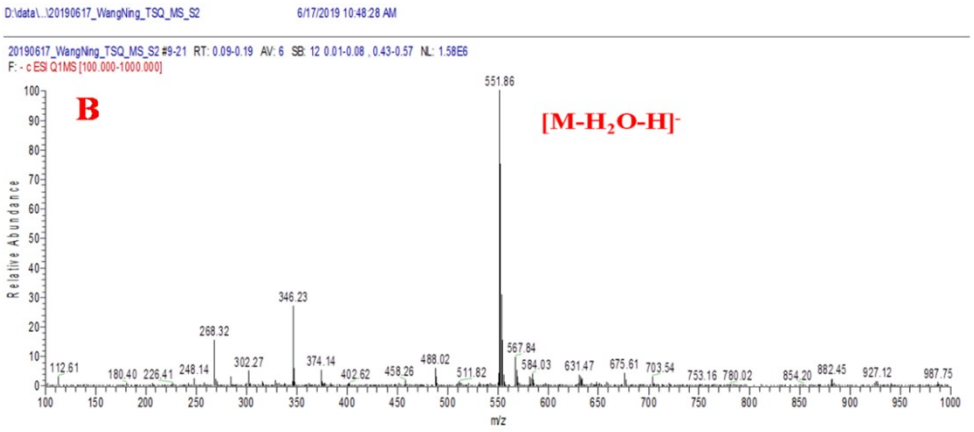


Fig. S6. MS spectra of (A) **COOH-S-S-COOH-ROS**, (B) **CPT-S-S-OH-ROS**.

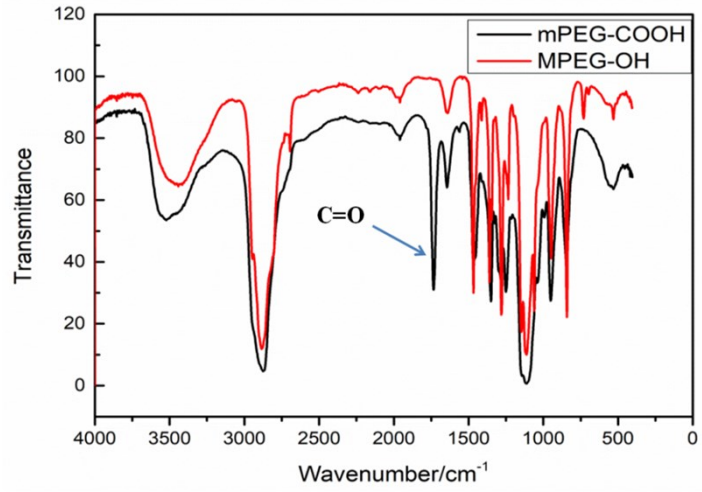


Fig. S7. IR spectra of **mPEG-COOH.**

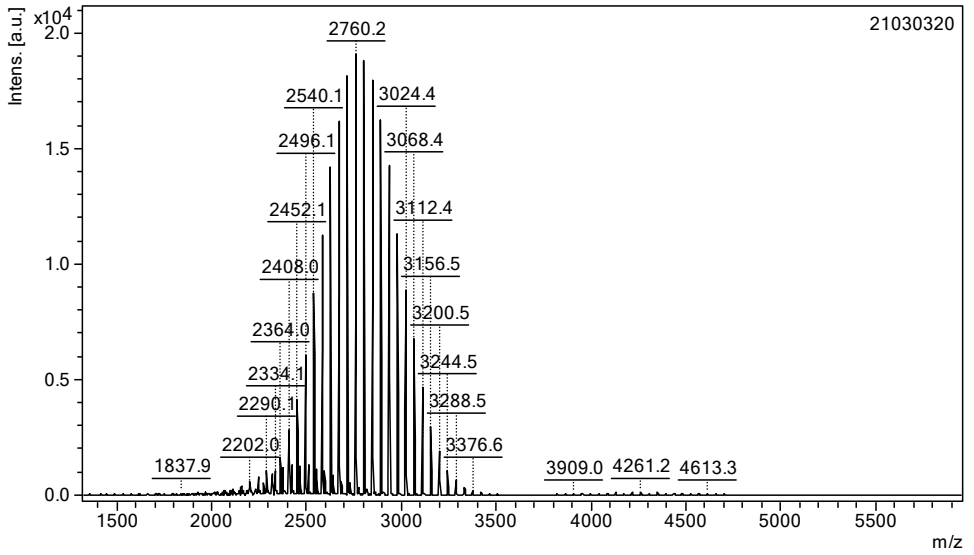


Fig. S8. MALDI-TOF-MS spectra of **PEG_{2K}-S-S-CPT-ROS.**

1.3 Synthesis of TPP-PEG_{2K}-TOS :

Dichloromethane (20 mL) containing 1.01 mL (3 g, 246 mmol) of methanesulfonyl chloride was added dropwise with stirring to an ice-cold solution of 8.0 g (4.0 mmol) of poly(ethylene glycol) 2000 and 3.6 mL (2.6 g, 26 mmol) of triethylamine in 50 mL of dichloromethane. After 4 h of stirring, the ice bowl was removed and a total of 2 mL of water was added. Then 30 mL of dichloromethane was added to dilute the reaction. The organic phase was washed twice with 2 M HCl and twice with brine, dried (Na_2SO_4), and evaporated. The crude product was dissolved in ethyl acetate (50 mL) with warming and diethyl ether (50 mL) was then added. After incubation at 4 °C for 24 h, the precipitation was filtered, washed with cold (ethyl acetate/diethyl ether: 1:1) and ether. The target product was obtained as a white solid (7.8 g, yield 90%)

A total of 4 g of poly(ethylene glycol) 2000 1, ω-bis methanesulfonate was dissolved in 150 mL of aqueous ammonia solution. After incubation at room temperature for 120 h, the solution was extracted three times with dichloromethane (50 mL each), the organic layers were combined, dried with Na_2SO_4 and evaporated, then dried under vacuum. The crude product was purified by silica gel chromatography ($CHCl_3$/CH_3OH: from 30:1 to 15:1 and then 5:1 ratio in sequence) to give **NH_2-PEG_{2K}-NH_2** as a white solid (1.61 g, yield 45%) and confirmed by ^{13}C NMR.

D-α-Tocopherol Succinate (265 mg, 0.5 mmol) was dissolved in anhydrous DMF (5 mL). The solution of EDCI (120 mg, 0.6 mmol), DMAP (75 mg, 0.6 mmol) in anhydrous DMF (5 mL) was added to the above-mentioned solution and the mixture was stirred at room temperature for 2 h. Next, the solution of **NH_2-PEG_{2K}-NH_2** (1 g, 0.5 mmol) in 10 mL of anhydrous DMF was added and the reaction was stirred for 24 h at room temperature under dry N_2 protection. Afterward, the crude product was purified by dialyzing in a membrane (MWCO 1000) against DMF for 48 h to remove the excess EDCI, DMAP. The product was finally dialyzed against the distilled water for 24 h to remove DMF. The **TOS-PEG_{2K}-NH_2** was obtained as a white solid (787 mg, yield 63%) by freeze-drying and confirmed by ^1H NMR. ^1H NMR (500 MHz, $CDCl_3$): δ/ppm = 3.61 (PEG); 0.7-2.9 (TOS).

(4-Carboxybutyl)triphenylphosphonium bromide (97 mg, 0.22 mmol) was dissolved in anhydrous DMF (5 mL). The solution of EDCI (52 mg, 0.26 mmol), DMAP (32 mg, 0.26 mmol) in anhydrous DMF (5 mL) was added to the above mentioned solution and the mixture

was stirred at room temperature for 2 h. Next, the solution of **TOS-PEG$_{2K}$-NH$_2$** (253 mg, 0.1 mmol) in 10 mL of anhydrous DMF was added and the reaction was stirred for 24 h at room temperature under dry N_2 protection. Afterward, the crude product was purified by dialyzing in a membrane (MWCO 1000) against DMF for 2 days and deionized water for 24 h, respectively, and lyophilized to obtain the final product **TPP-PEG$_{2K}$-TOS** (177 mg, yield 59.2%). The successful synthesis of **TPP-PEG$_{2K}$-TOS** was confirmed by ^{1}H-NMR and MALDI-TOF-MS.

^{1}H NMR (500 MHz, $CDCl_3$): δ/ppm = 7.6-7.8 (TPP); 3.6 (PEG); 0.7-2.9 (TOS).

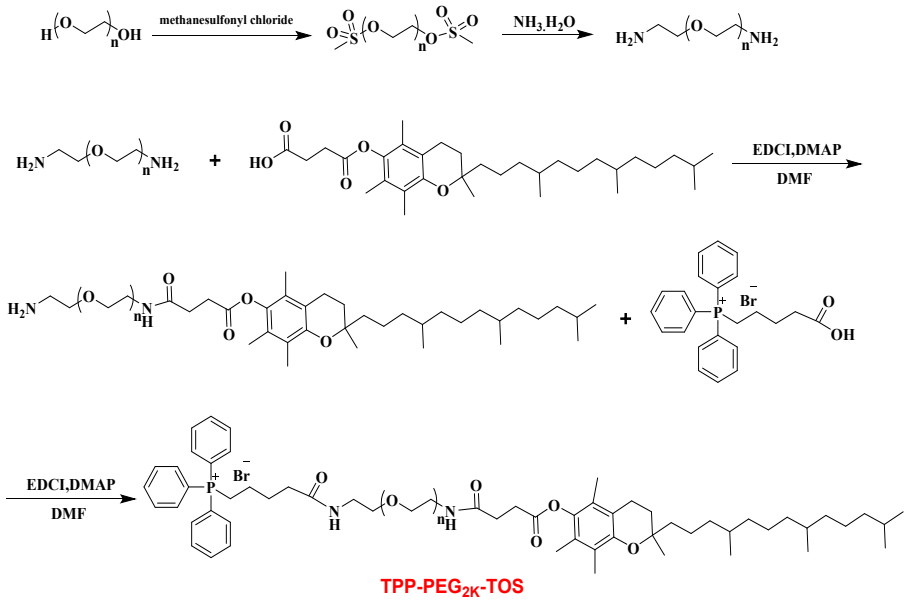


Fig. S9. Synthetic route of **TPP-PEG$_{2K}$-TOS.**

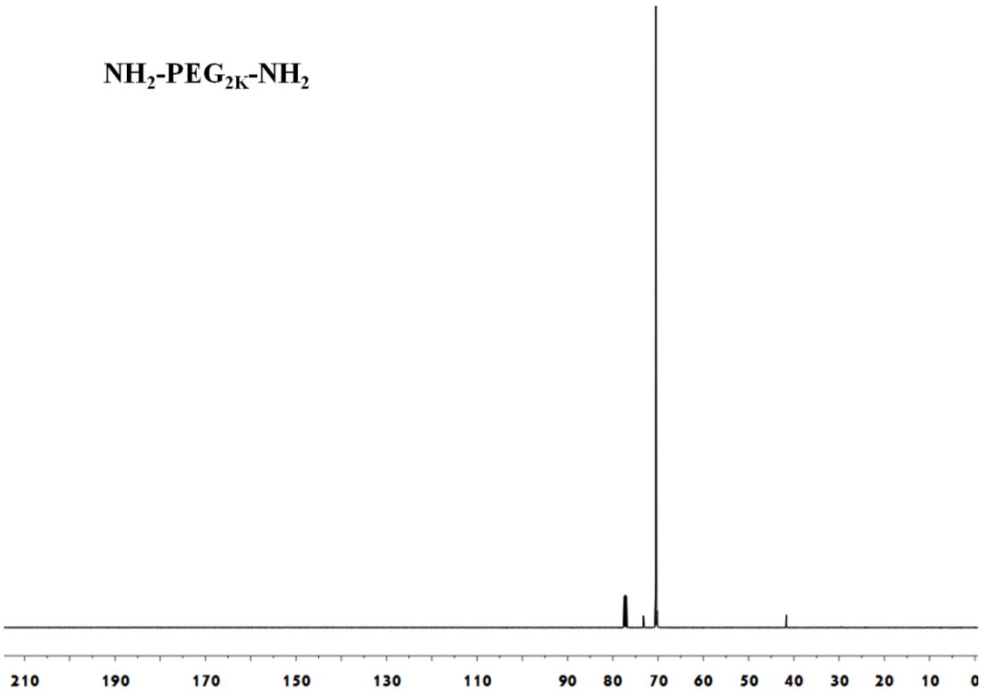
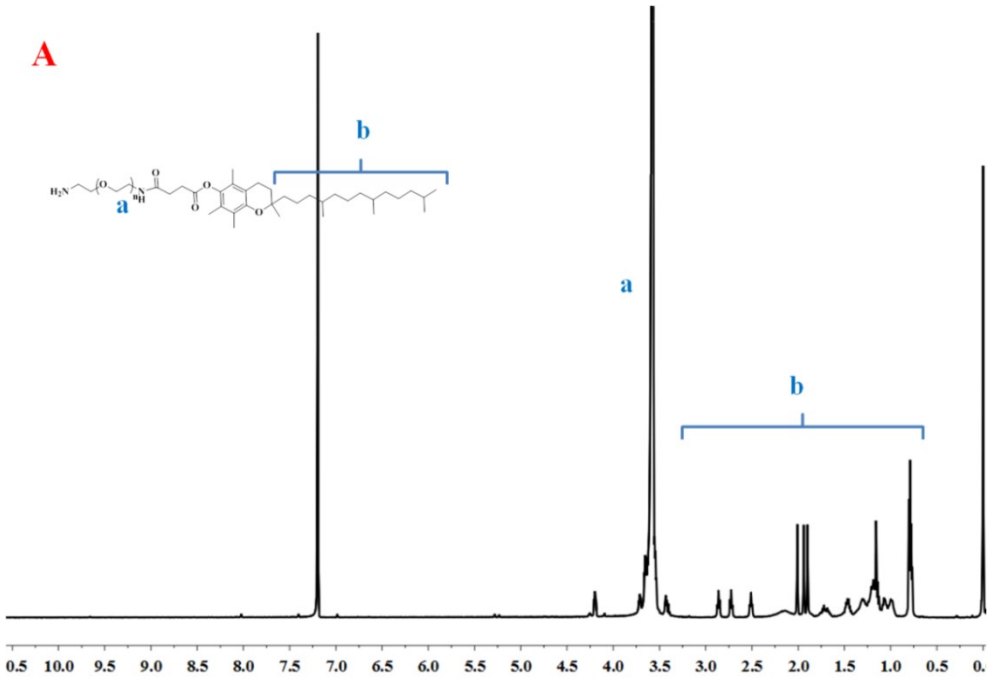


Fig. S10. ^{13}C-NMR spectra of **NH_2-PEG_{2K}-NH_2**.



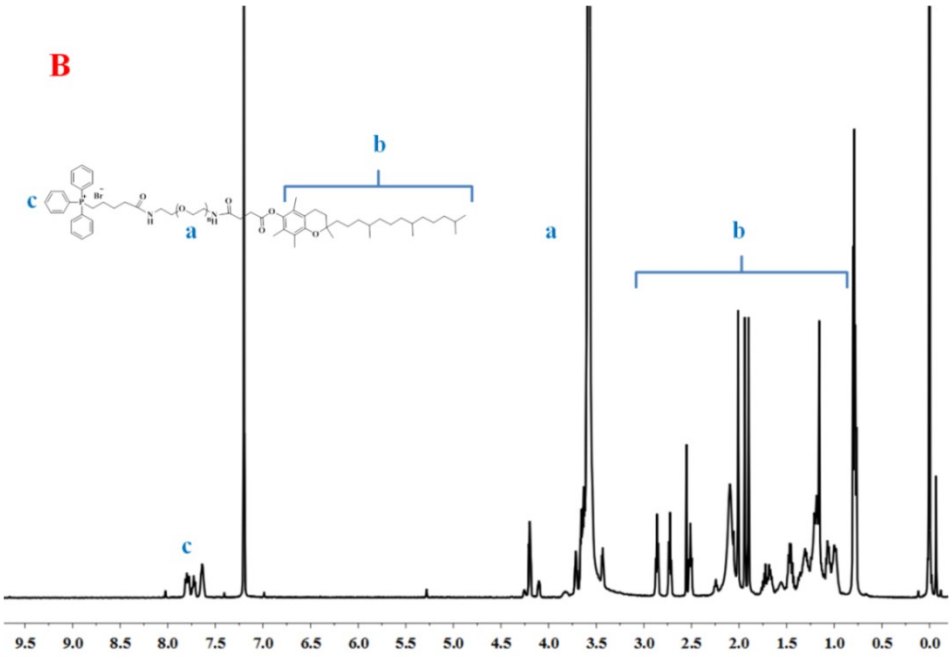


Fig. S11. ^{1}H-NMR spectra of (A) **TOS-PEG$_{2K}$-NH$_2$** and (B) **TPP-PEG$_{2K}$-TOS.**

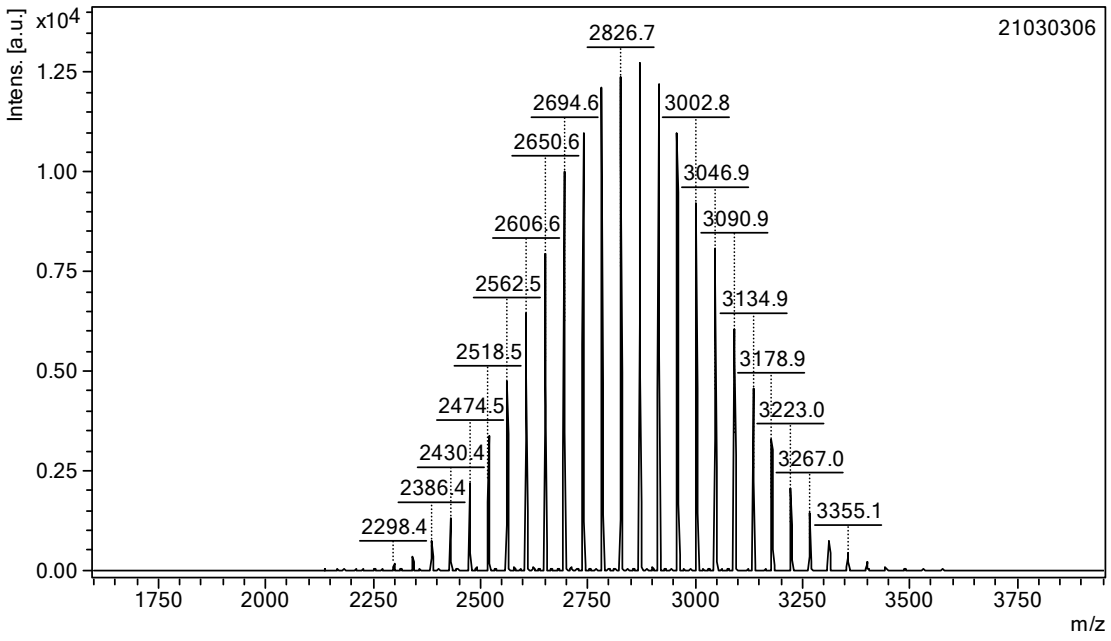


Fig. S12. MALDI-TOF-MS spectra of **TPP-PEG$_{2K}$-TOS**.

1.4 Synthesis of TPP-PEG$_{2K}$-LND:

(4-Carboxybutyl) triphenylphosphonium bromide (97 mg, 0.22 mmol) was dissolved in anhydrous DMF (5 mL). The solution of EDCI (52 mg, 0.26 mmol), DMAP (32 mg, 0.26 mmol) in anhydrous DMF (5 mL) was added to the above mentioned solution and the mixture

was stirred at room temperature for 2 h. Next, the solution of **NH_2-PEG$_{2K}$-NH_2** (440 mg, 0.22 mmol) in 10 mL of anhydrous DMF was added and the reaction was stirred for 24 h at room temperature under dry N_2 protection. Afterward, the crude product was purified by dialyzing in a membrane (MWCO 1000) against DMF for 48 h to remove the excess EDCI, DMAP and uncoupled (4-Carboxybutyl)triphenylphosphonium bromide. The product was finally dialyzed against the distilled water for 24 h to remove DMF. The **TPP-PEG$_{2K}$-NH_2** was obtained as a white solid (332 mg, yield 63%) by freeze-drying and confirmed by ^{1}H NMR and MALDI-TOF-MS.

^{1}H NMR (500 MHz, $CDCl_3$): δ/ppm = 7.6-7.8 (TPP); 3.6 (PEG).

Lonidamine (39 mg, 0.12 mmol) was dissolved in anhydrous DMF (5 mL). The solution of EDCI (28 mg, 0.14 mmol), DMAP (17 mg, 0.14 mmol) in anhydrous DMF (5 mL) was added to the above-mentioned solution and the mixture was stirred at room temperature for 2 h. Next, the solution of **TPP-PEG$_{2K}$-NH_2** (250 mg, 0.1 mmol) in 10 mL of anhydrous DMF was added and the reaction was stirred for 48 h at room temperature under dry N_2 protection. Afterward, the crude product was purified by dialyzing in a membrane (MWCO 1000) against DMF for 2 days and deionized water for 48 h, respectively, and lyophilized to obtain the final product **TPP-PEG$_{2K}$-LND** (137 mg, yield 49.23%). The successful synthesis of **TPP-PEG$_{2K}$-LND** was confirmed by ^{1}H-NMR and MALDI-TOF-MS.

^{1}H NMR (500 MHz, $CDCl_3$): δ/ppm = 7.6-7.8 (TPP); 5.7 (-CH_2-); 3.6 (PEG).

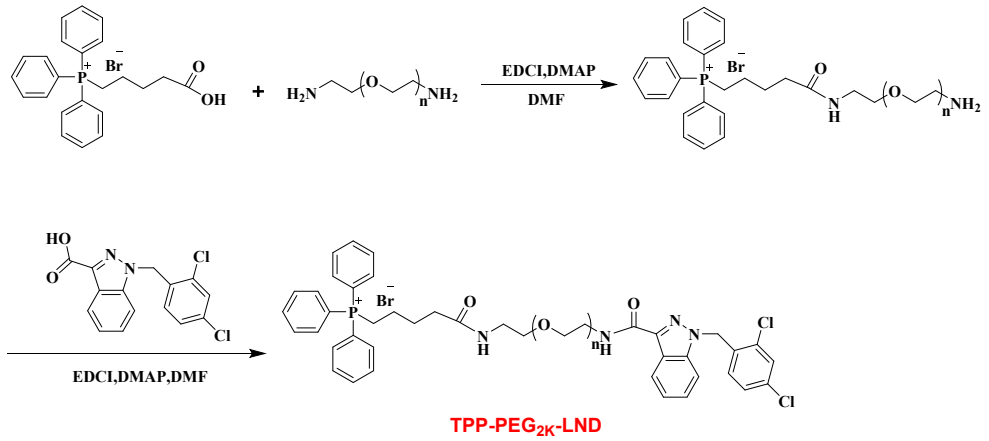


Fig. S13. Synthetic route of **TPP-PEG$_{2K}$-LND.**

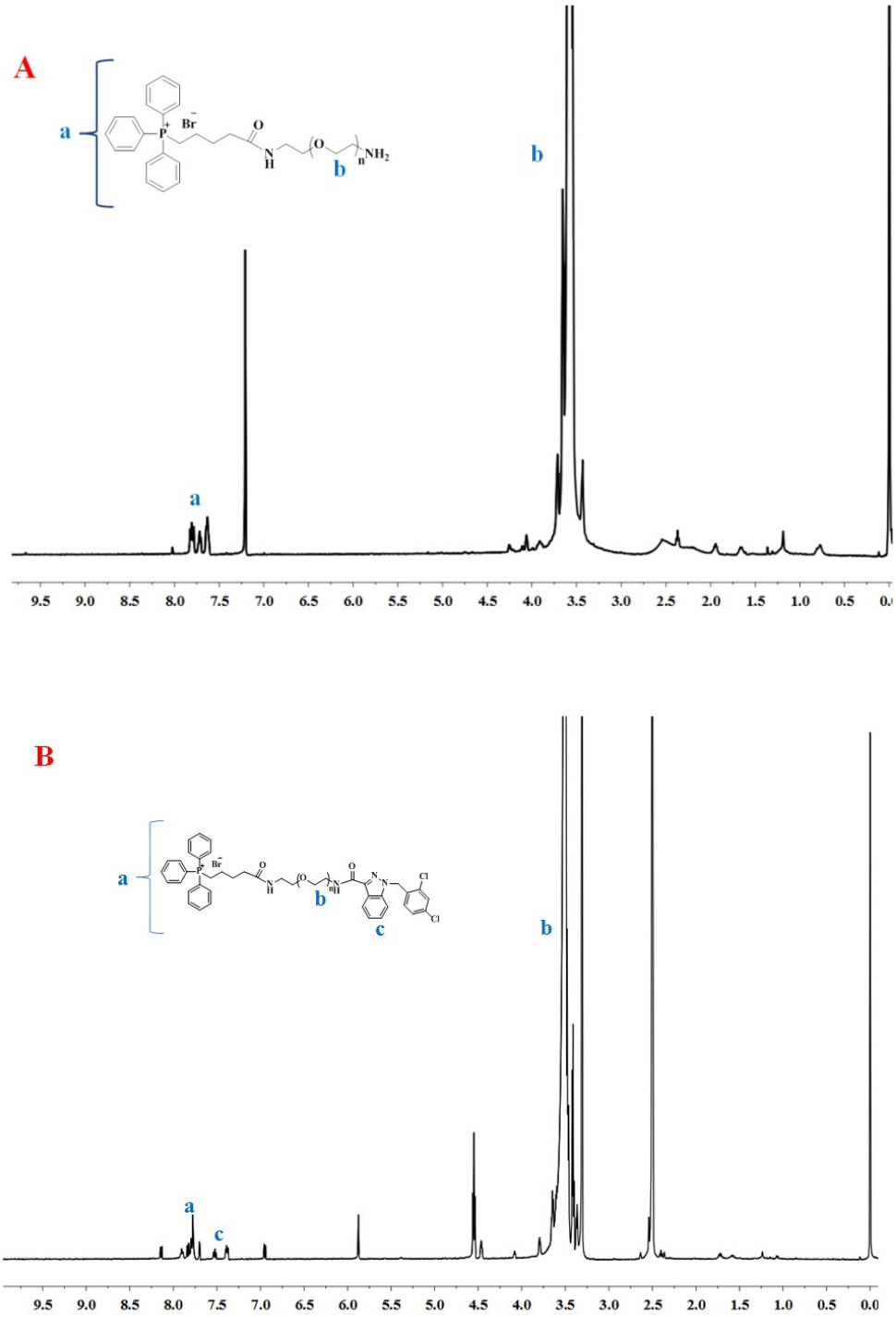


Fig. S14. ^{1}H-NMR spectra of (A) **TPP-PEG$_{2K}$-NH$_2$** and (B) **TPP-PEG$_{2K}$-LND.**

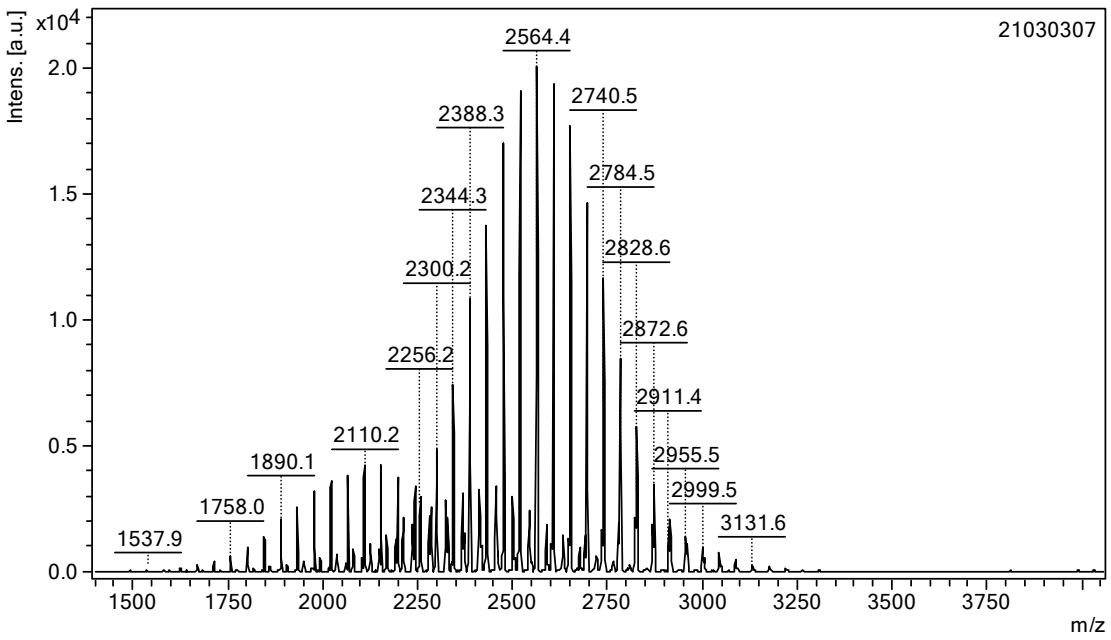


Fig. S15. MALDI-TOF-MS spectra of **TPP-PEG$_{2K}$-LND**.

2. Experimental details

2.1. Materials

Methanol (purity 99.8%), ethanol (purity 99.8%), dichloromethane (purity 99.8%), petroleumether and diethylether (purity 99.8%) were purchased from Tianjin fuyu fine chemical co. LTD, China. Polyethylene glycol monomethyl ether (CH_3-PEG_{2K}-OH, 2000 Da, AR), polyethylene glycol (OH-PEG_{2K}-OH, 2000 Da, AR), N-(3-Dimethylaminopropyl)-N-ethylcarbodiimide (EDCI) were purchased from TCI. Hydrazine hydrate, sodium hydride (NaH), (4-Carboxybutyl)triphenylphosphonium bromide, ethyl 2-bromoacetate, 4-dimethylaminopyridine (DMAP), 3-mercaptopropionic acid, anhydrous acetone, doxorubicin hydrochloride (DOX.HCl), (S)-(+)-Camptothecin (CPT), triphosgene, lithium aluminium hydride ($LiAlH_4$), succinic anhydride, D-α-tocopherol succinate (TOS) were purchased from Shanghai Aladdin biochemical technology co. LTD, China. CombrestatinA4 (CA4) was purchased from Shanghai Haoyun Chemical Technology Co., LTD, China. LysoTracker Green, Reactive Oxygen Species Assay Kit, Rhodamine 123, Mito-Tracker Green, Caspase-3/Caspase-9 antibody was purchased from Beyotime. MTT was purchased from KGI Biotech. MitoSOX Red was purchased from Thermo Fisher Scientific.

2.2. Preparation of NPs

NPs were prepared by a thin-film hydration method. Compositions of the prepared NPs were as follows: (1) Traceable NPs (**TLDCAG**), **TPP-PEG_{2K}-TOS /TPP-PEG_{2K}-LND/ PEG_{2K}-NH-N-DOX/PEG_{2K}-S-S-CPT-ROS/CA4/Gd-DTPA-N16-16** (mass ratio = 2: 2: 1.2: 2.5: 0.8: 2); (2) Traceable NPs without **CA4** (**TLDCG**), **TPP-PEG_{2K}-TOS/TPP-PEG_{2K}-LND/PEG_{2K}-NH-N-DOX/PEG_{2K}-S-S-CPT-ROS/Gd-DTPA-N16-16** (mass ratio = 2: 2: 1.2: 2.5: 2); (3) Traceable NPs without **PEG_{2K}-NH-N-DOX** (**TLCAG**), **TPP-PEG_{2K}-TOS/TPP-PEG_{2K}-LND/PEG_{2K}-S-S-CPT-ROS/CA4/Gd-DTPA-N16-16** (mass ratio = 2: 2: 2.5: 0.8: 2);

(4) Traceable NPs without **PEG_{2K}-S-S-CPT-ROS** (**TLDAG**), **TPP-PEG_{2K}-TOS/TPP-PEG_{2K}-LND/PEG_{2K}-NH-N-DOX/CA4/Gd-DTPA-N16-16** (mass ratio = 2: 2: 1.2: 0.8: 2); (5) Traceable NPs without, **TPP-PEG_{2K}-TOS** and **TPP-PEG_{2K}-LND** (**DCAG**), **PEG_{2K}-NH-N-DOX/PEG_{2K}-S-S-CPT-ROS/CA4/Gd-DTPA-N16-16** (mass ratio = 1.2: 2.5: 0.8: 2); (6) Traceable NPs without **CA4**, **TPP-PEG_{2K}-TOS** and **TPP-PEG_{2K}-LND** (**DCG**), **PEG_{2K}-NH-N-DOX/PEG_{2K}-S-S-CPT-ROS/Gd-DTPA-N16-16** (mass ratio = 1.2: 2.5: 2); (7) Traceable NPs without **CA4**, **PEG_{2K}-NH-N-DOX**, **PEG_{2K}-S-S-CPT-ROS** (**TLG**), **TPP-PEG_{2K}-TOS/TPP-PEG_{2K}-LND/Gd-DTPA-N16-16** (mass ratio = 1: 1: 1); (8) Traceable NPs without **CA4**, **PEG_{2K}-NH-N-DOX**, **Gd-DTPA-N16-16** (**TLC**), **TPP-PEG_{2K}-TOS/TPP-PEG_{2K}-LND/PEG_{2K}-S-S-CPT-ROS** (mass ratio = 2: 2: 2.5); (9) Empty NPs, **Gd-DTPA-N16-16**. All materials and drugs were dissolved in the mixture of chloroform and methanol (v/v = 1:2), and then the organic solvent was removed by rotary evaporation to form a NPs film. The film was further dried and stored in a vacuum overnight. The obtained film was hydrated in PBS (pH =7.4) under 37 °C for 30 min. Subsequently, the hydrated solution was treated by an ultrasonic signal generator to form NPs (parameters: work 2 s, suspend 5 s, 100 W, total time 20 min).

2.3. Characterization of TLDCAG NPs

The hydrodynamic size, polydispersity (PDI) and morphology of **TLDCAG** NPs were measured using a dynamic light scattering (DLS) instrument (Malvern Zetasizer Nano ZS) and transmission electron microscope (TEM, HT7700 Exaens, Hitachi), respectively. Serum stability was measured by incubating **TLDCAG** NPs in PBS (pH =7.4) containing 10% fetal bovine serum (FBS) at 37 °C. At each time point, the mean diameter and PDI of **TLDCAG** NPs were detected by DLS. To measure the pH-sensibility of **TLDCAG** NPs, **TLDCAG** NPs was incubated in PBS of different pH values at 37 °C for 12 h, and the mean diameter and PDI of the **TLDCAG** were measured by DLS. ROS-sensibility of **TLDCAG** NPs was

measure by incubating **TLDCAG** NPs in PBS (pH =7.4) containing (50 mM H_2O_2, 1 μM $FeSO_4$). At each time point, the mean diameter and PDI of **TLDCAG** NPs were detected by DLS.

2.4. Release of three drugs from TLDCAG NPs

The vitro release of DOX and CA4 were performed in PBS (pH 7.4, 6.5 and 5.0). Briefly, 1 mL **TLDCAG** NPs solution removed free CA4 was suspended in a dialysis bag (MWCO 3500), then the dialysis bags were placed in 100 mL PBS (pH 7.4, 6.5 and 5.0). At predetermined time intervals, PBS (2 mL) was removed, and the same volume of fresh PBS was added. The concentration of CA4 in the PBS was detected using HPLC assay. The concentration of DOX in the PBS was detected by fluorescence spectrophotometer. Similarly, 1 mL **TLDCAG** NPs solution removed free CA4 was added in dialysis bag (MWCO 3500), then the dialysis bags were placed in 100 mL pH 7.4, 6.5, 5.0, (7.4 + 1 mM H_2O_2 + 1 μM $FeSO_4$), (7.4 + 50 mM H_2O_2 + 1 μM $FeSO_4$), (6.5 + 1 mM H_2O_2 + 1 μM $FeSO_4$), (6.5 + 50 mM H_2O_2 + 1 μM $FeSO_4$), (5.0 + 1 mM H_2O_2 + 1 μM $FeSO_4$), (5.0 + 50 mM H_2O_2 + 1 μM $FeSO_4$) PBS buffer. At predetermined time intervals, PBS (2 mL) was removed, and the same volume of fresh PBS was added. The concentration of CPT in the PBS was measured via fluorescence spectrophotometer.

2.5. Hemolysis test of materials

Fresh blood was diluted 50 times with 0.9% NaCl solution and added to a 24-well plate with a volume of 1.0 mL per well. Various concentrations of materials solution were added for 4 h incubation at 37 °C. After 4 h, 0.8 mL of blood was transferred to a test tube and centrifuged at 2000 rpm for 5 min to remove intact red blood cells. Transfer 200 μL of the supernatant to a 96-well plate, and measure the absorbance at 540 nm using a microplate

reader (Multiskan FC, Thermo, USA). Treat red blood cells with 1% triton X-100 and set it as a positive control.

2.6. Cell culture

The human umbilical vein endothelial cells (HUVECs) and human breast cancer cells (MCF-7) were obtained from the school of pharmaceutical science and technology, Dalian university of technology (Dalian, China). The culture conditions were listed as follows: MCF-7 cells: DMEM containing 10% (v/v) FBS; HUVECs cells: DMEM/F12 medium with 10% (v/v) FBS. The cells were cultured in an incubator at 37 °C under an atmosphere of 5% CO_2.

2.7. In vitro cellular uptake

In vitro cell uptake was studied using confocal laser scanning microscopy (CLSM) and flow cytometry (FCM).

For quantitative experiments, MCF-7 cells were plated in six-well plates (50,000 cells per well) and cultured 24 h at 5% CO_2, 37 °C. The medium was replaced and **TLDCG** NPs, DOX.HCl was incubated with MCF-7 cells for 1, 2, 3, 4 h under 5% CO_2, 37 °C. After medium removal and subsequent washing three times with cold PBS, the cells were trypsinized and resuspended in 0.2 mL PBS for FCM quantitative analysis (FACSCanto, BD, USA). The concentrations of both CPT and DOX were 5 μg/mL and 5 μg/mL, respectively.

For qualitative experiments, MCF-7 cells were plated in 35-mm glass-bottom culture dish at a density of 1×10^5 cells per well and cultured overnight at 5% CO_2, 37 °C. The medium was replaced and **TLDCG** NPs was added. After incubation for different time intervals, cells were washed three times with PBS and followed by staining with Lysotracker green at 37 °C. Afterwards, the cells were washed three times with cold PBS, and 1 mL

DMEM was added. Finally, the cells were imaged by CLSM (FV1000, Olympus, USA). The concentrations of CPT and DOX were 5 μg/mL and 5 μg/mL, respectively.

2.8. Intracellular ROS quantification

MCF-7 cells were seeded at 1 × 10^5 cells/well into a 35-mm glass-bottom culture dish and cultured overnight at 5% CO_2, 37 °C. The medium was replaced with fresh DMEM containing 10% FBS, followed by the addition of **TLDCG** NPs. The control experiment was a blank culture medium. After 4, 6, 12 h incubation, the culture medium was removed, the cells were washed three times with cold PBS, and then the cells were stained with fresh DMEM medium (1 mL) containing DCFH-DA (10 μM). After incubating 30min, the cells were washed with ice-cold PBS for 3 times. The fluorescence of dichlorofluorescein (DCF) decomposed from DCFH-DA stimulated by ROS was observed by CLSM and FCM. The concentrations of DOX, CPT, **TPP-PEG$_{2K}$-LND**, **TPP-PEG$_{2K}$-TOS**, **Gd-DTPA-N16-16** were 5 μg/mL, 5 μg/mL, 50 μg/mL, 50 μg/mL, 50 μg/mL, respectively.

2.9. Mitochondrial superoxide detection

MitoSOX Red is used as the mitochondrial superoxide indicator. MitoSOX Red could penetrate the living cell membrane and selectively enter mitochondria. Once it enters mitochondria, it can be rapidly oxidized by superoxide instead of other ROS and reactive nitrogen species (RNS), and produce a large amount of fluorescence.

MCF-7 cells were plated in 35-mm glass-bottom culture dish (50,000 cells per well) and cultured 24 h at 5% CO_2, 37 °C. The medium was replaced and **TLC** NP_S, was added. After incubation for 4 h, cells were washed three times with PBS and followed by staining with MitoSOX Red at 37 °C for 15 min. Afterwards, the cells were washed three times with PBS, and 1 mL DMEM was added. Finally, the cells were imaged by CLSM. The concentrations of

CPT, **TPP-PEG$_{2K}$-LND**, **TPP-PEG$_{2K}$-TOS** were 5 μg/mL, 50 μg/mL, 50 μg/mL, respectively.

2.10. CPT release inside cells

MCF-7 cells were seeded into 6-well plates at 1 × 10^5 cells/well incubated for 24 h. Next, the medium was replaced with fresh DMEM containing 10% FBS, followed by addition of **TLDCG** NPs with **DCG** NPs (CPT and DOX equivalent concentration of 5 μg/mL; **TPP-PEG$_{2K}$-LND**, **TPP-PEG$_{2K}$-TOS**, **Gd-DTPA-N16-16** equivalent concentration of 50 μg/mL). After 24 h incubation, the culture medium was removed, and the cells were washed three times by ice-cold PBS. Subsequently, the cells were trypsinized and transferred to a 5 mL EP tube. The EP tube was placed in an ice bath, and the cell suspension was treated under ultrasound (ultrasonic operating parameters: work 2 s, pause 5 s, 100 W, total time 20 min). Released CPT drugs were then extracted with $CHCl_3$/CH_3OH (1:1, v/v) and centrifuged at 2000 g for 10 min. The solvents were collected and removed by volatilization. The residue was redissolved in methanol and measured by HPLC.

2.11. Mitochondria membrane potential (Δψm) depolarization

The Δψm depolarization was measured following a previous report. Briefly, MCF-7 cells were seeded at 1 × 10^5 cells/well into a 35-mm glass-bottom culture dish and cultured overnight at 5% CO_2 at 37 °C. The medium was replaced with fresh DMEM containing 10% FBS, followed by addition of **TLDCG** NPs (DOX and CPT equivalent concentration of 0.5 μg/mL; **TPP-PEG$_{2K}$-LND**, **TPP-PEG$_{2K}$-TOS**, **Gd-DTPA-N16-16** equivalent concentration of 5 μg/mL). The control experiment was a blank culture medium. After 12 and 24 h incubation, the culture medium was removed, the cells were washed with PBS two times, and then the cells were stained with Rhodamine 123 (Beyotime, Shanghai agent) for 30 min at 5% CO_2, 37 °C. Cell images were collected on a CLSM.

2.12. Western blot analysis

MCF-7 cells were seeded in six-well plates for 24 h incubation. The medium was replaced with fresh DMEM containing 10% FBS and was treated with different formulations (**TLDCG** NPs, **DCG** NPs). The control experiment was a blank culture medium. After incubation 24 h, the cells were lysed in RIPA buffer containing protease inhibitor. 20 μg of protein were separated by SDS-PAGE and transferred onto PVDF membrane. Primary antibodies against, caspase 9, caspase 3 were used. β-actin was used as a control. The concentrations of DOX, CPT, **TPP-PEG$_{2K}$-LND**, **TPP-PEG$_{2K}$-TOS**, **Gd-DTPA-N16-16** were 5 μg/mL, 5 μg/mL, 50 μg/mL, 50 μg/mL, 50 μg/mL, respectively.

2.13. Cytotoxicity study in vitro

The cytotoxicity of different NPs formulations was measured with MTT assay. Briefly, HUVEC$_S$ and MCF-7 cells were seeded in 96-well plates (100 μL medium) and cultured overnight at 5% CO_2, 37 °C. When the cell density reached around 60-70%, the medium was replaced with a fresh one (90 μL), and then NP$_S$ solution (10 μL each) of various concentrations was added for 24 h incubation. The medium was pipetted out, then 100 μL medium and 50 μL MTT were added into each well incubated for 4 h. Finally, the medium was replaced with 150 μL DMSO. Absorbance was measured by a microplate reader at 492 nm. The control experiment was a blank culture medium.

2.14. Blood pharmacokinetics

All animal experiments in this paper were performed in accordance with the guidelines of the National Institutes of Health and approved by the Animal Ethics Committee of Dalian University of technology.

To determine pharmacokinetics, the SD rats were randomly divided into 2 groups, and injected intravenously through the tail vein with the **TLDCAG** NPs and free drugs

respectively, at the equivalent dose of 2.0 mg DOX/kg, 1 mg CA4/kg and 2.0 mg CPT/kg. Blood samples (500 μL) were collected from eye puncture and taken into centrifuge tube containing EDTA-2Na at 10 min, 0.5, 1, 1.5, 2, 4, 8, and 24 h post injection. To extract DOX, CPT and CA4, acetone and methanol was added respectively to the blood. The samples were vortexed and the solution was immediately centrifuged at 1,800 g for 10 min. The concentrations of DOX, CPT and CA4 in supernatant were measured by fluorescence spectrophotometry and HPLC method, respectively.

2.15. In vivo therapy on MCF-7 solid tumors

MCF-7 cells (5×10^7) mixed with a certain amount of matrigel were injected into the right forelimb of female nude mice (5-7 weeks old). When the tumor volume reached 100 mm^3, the mice were randomly divided into 6 groups (n=4). The first group was injected with PBS (control); the second group was injected with **TLG** NPs; the third group was injected with DOX/CPT mixed solution; the fourth group was injected with **DCG** NPs; the fifth group was injected with **TLDCG** NPs; the sixth groups were injected with **TLDCAG** NPs. The mice were injected via the tail vein every 3 days, and the body weight and tumor volume were recorded. The tumor volume was calculated by the following formula: $V=L\times W^2/2$, where L is the longest dimension of the tumor, and W is the shortest dimension of the tumor.

2.16. In vivo MRI-tracking studies

Nude mice with tumor volume reached about 1500 mm^3, Gd-DTPA and **TLDCAG** NPs were injected through the tail vein (the concentration of Gd: 2 mM). Subsequently, the Gd-DTPA and **TLDCAG** NPs in the tumor of nude mice were traced by MesoQMR23-060H-I magnetic resonance imager at different time points (0, 30, 60, 90, 120, 150, and 180 min).

2.17. Fluorescence imaging studies in vivo

To further test the tissue distribution of **TLDCAG** NPs, **TLDCAG** NPs and DOX.HCl were injected into tumor-bearing nude mice through the tail vein, respectively. Subsequently, real-time imaging was performed with Berthold LB983NC100 in vivo imaging system. The mice were sacrificed at 24 h, the tumors and major organs (heart, lung, kidney, spleen, and liver) were collected, washed with cold saline and fluorescently imaged to determine the biological distribution of DOX.HCl and **TLDCAG** NPs. DOX equivalent was 10 mg/kg body weight, respectively.

2.18. Blood biochemistry tests of nude mice after treatments

After 30 days of treatment, 0.5 mL whole blood was collected from the tumor bearing mice. The levels of hematology and serum biochemistry were measured by dry biochemical analyzer (FDC nx500ivc +) and animal blood analyzer (Mairi 2600).

2.19. H&E Assays

The mice were sacrificed after treatment with PBS, DOX/CPT, **TLG** NPs, **DCG** NPs, **TLDCG** NPs, **TLDCAG** NPs for 30 days. The tumors and major organs were collected, and the tissue sections were stained with hematoxylin and eosin (H&E) for histological analysis.

2.20. Statistical analysis

All values were expressed as mean ± S.D. Each value is the average of at least three repeated experiments. One-way ANOVA was used to determine significant differences. Statistical significance was set as $p<0.05$ (*$p<0.05$, **$p<0.01$, ***$p<0.001$).

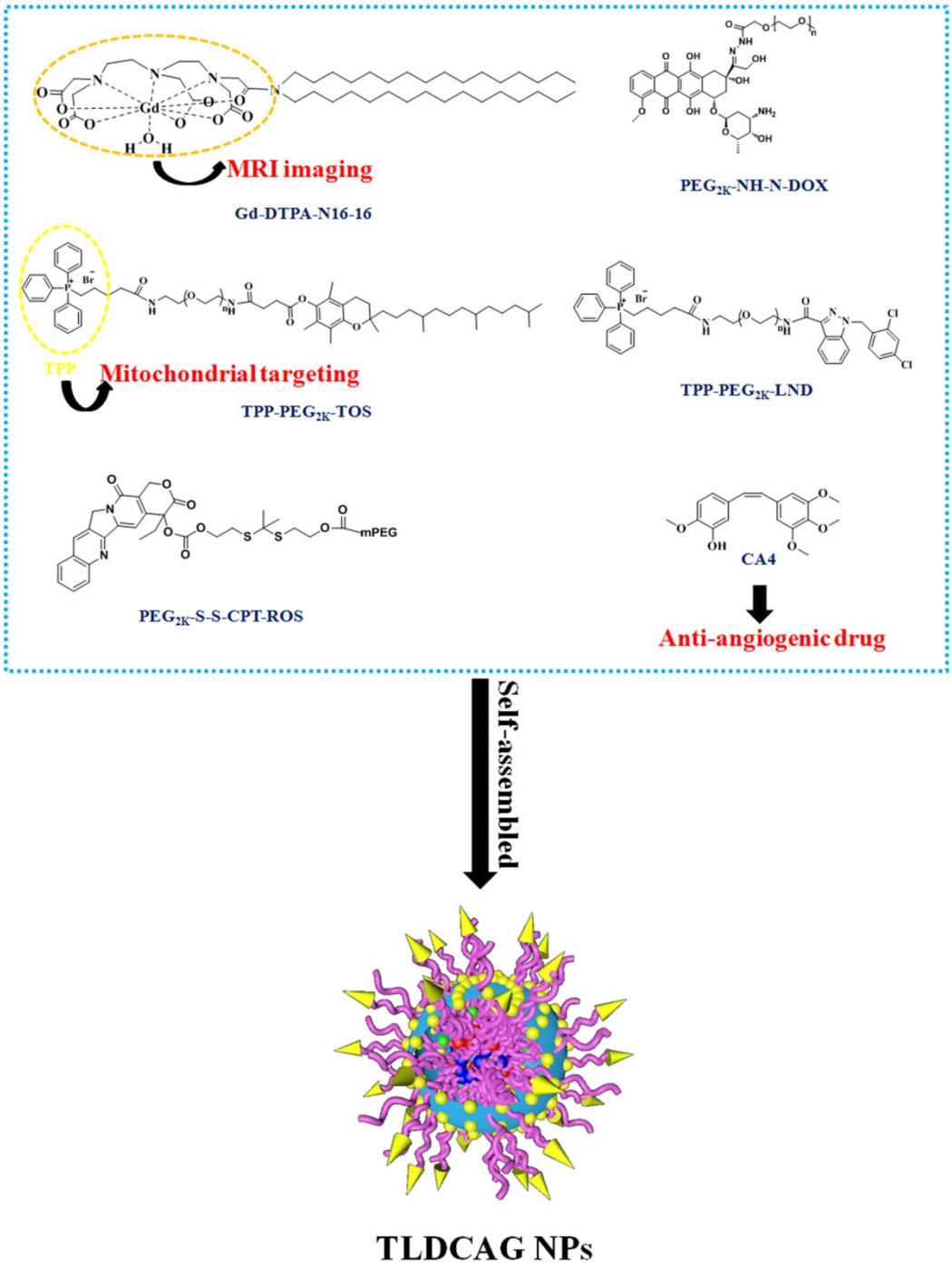


Fig. S16. The structural composition of **TLDCAG** NPs

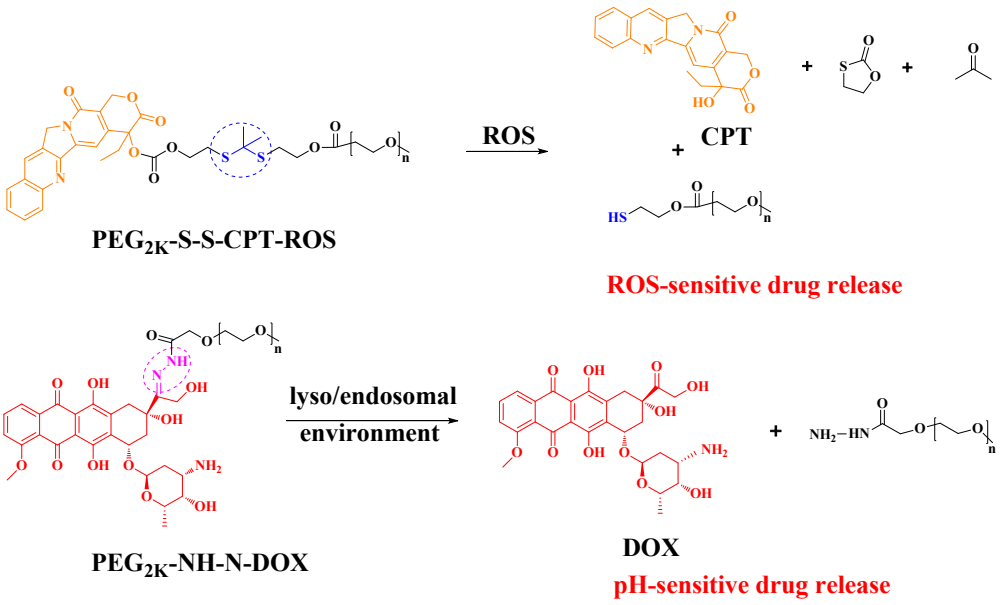


Fig. S17. The mechanism of prodrug response

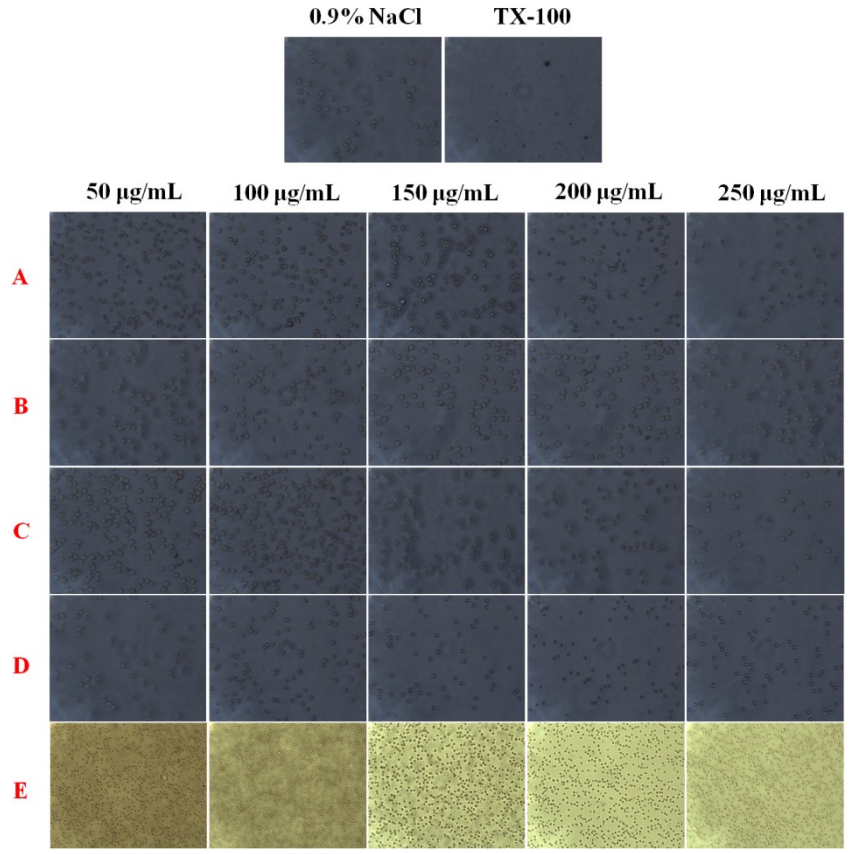


Fig. S18. Microscope images of erythrocyte of (A) **TPP-PEG$_{2K}$-TOS**, (B) **TPP-PEG$_{2K}$-LND**, (C) **PEG$_{2K}$-S-S-CPT-ROS,** (D) **Gd-DTPA-N16-16** and (E) **PEG$_{2K}$-NH-N-DOX.**

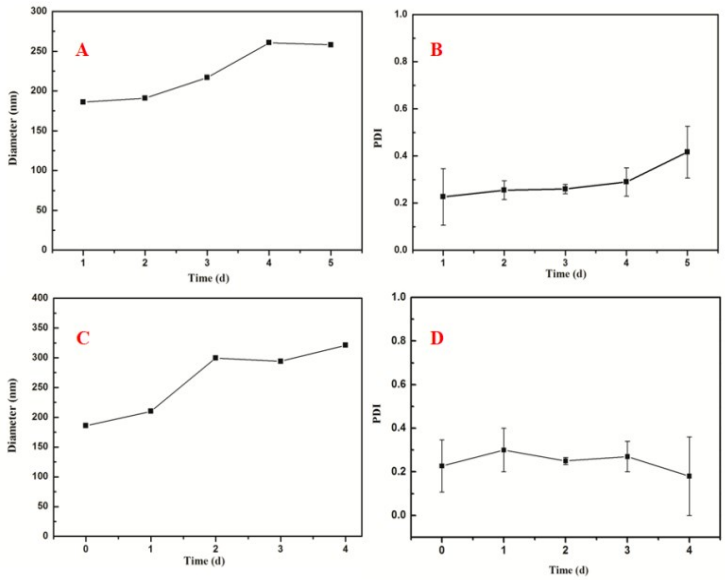


Fig. S19. (A) (B) The average diameter and size PDI of **TLDCAG** NPs in pH=7.4 at different time. (C) (D) The average diameter and size PDI of **TLDCAG** NPs in PBS (pH=7.4) contain10% FBS at different time.

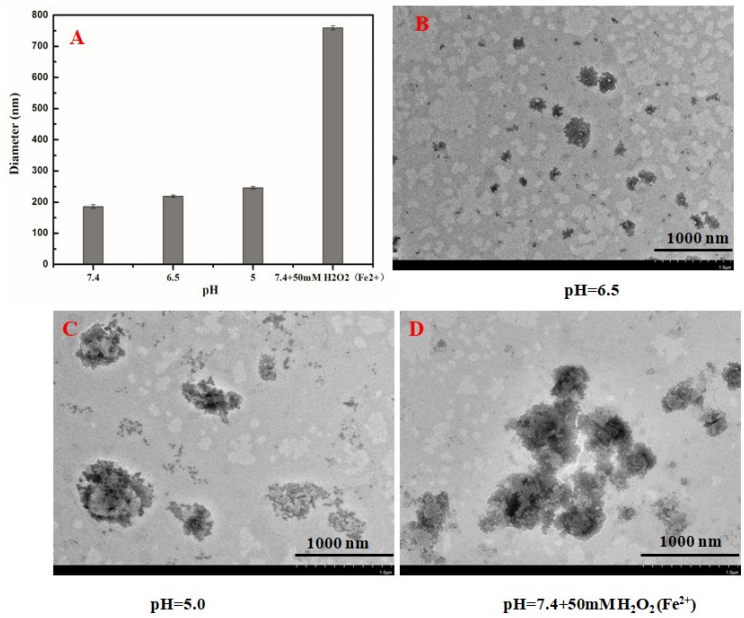


Fig. S20. (A) The average diameter of **TLDCAG** NPs at different conditions. TEM image of the **TLDCAG** NPs at (B) pH=6.5, (C) pH=5.0, (D) pH=7.4+50 mM H_2O_2 (1 μM Fe_2O_4). Scale bar = 1000 nm.

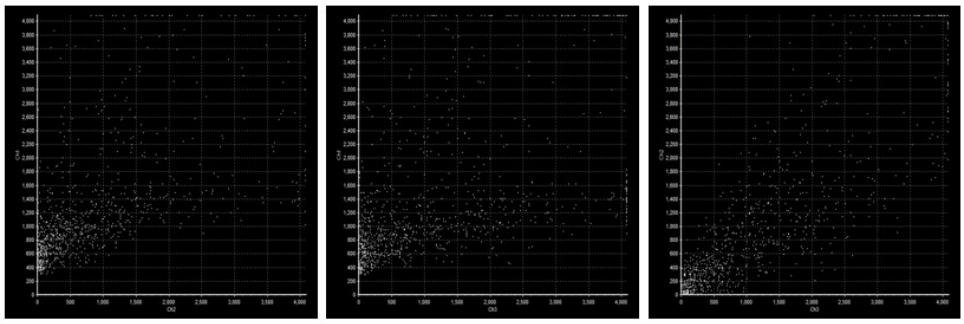


Fig. S21. Pearson's coefficient graph of overlay.

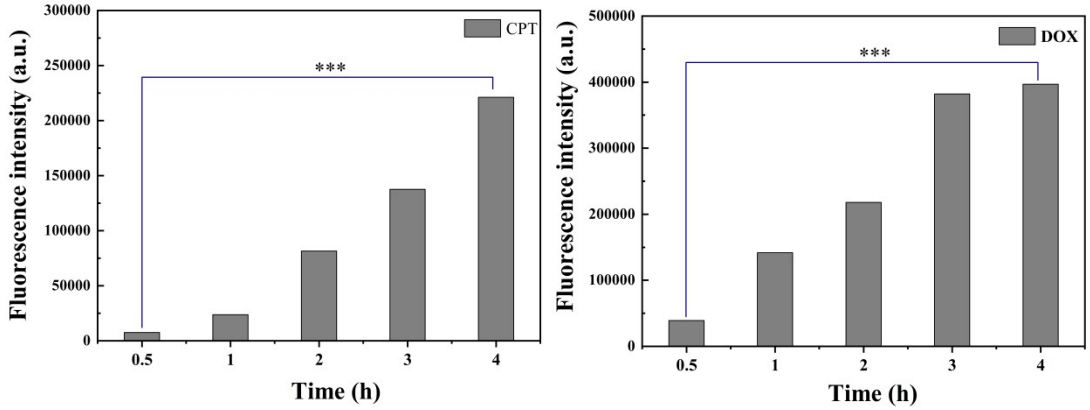


Fig. S22. Quantified intracellular CPT and DOX by measuring the blue and red fluorescence intensity of Fig. 3B and summarized in the bar graph.

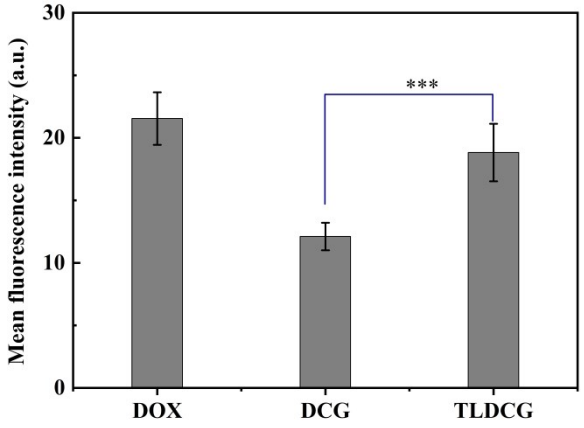


Fig. S23. Quantified intracellular DOX by measuring the red fluorescence intensity of Fig. 4A and summarized in the bar graph.

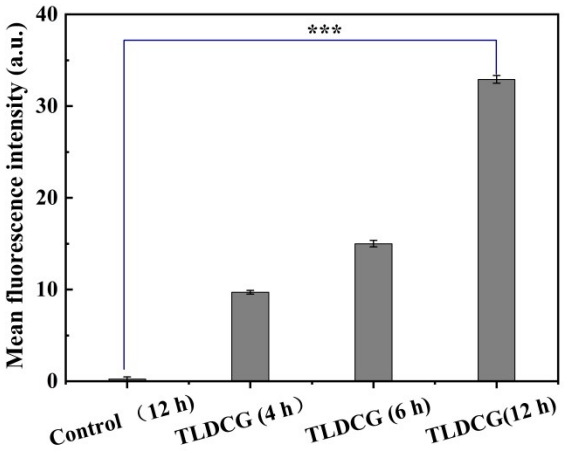


Fig. S24. Quantified ROS by measuring the green fluorescence intensity of Fig. 4B and summarized in the bar graph.

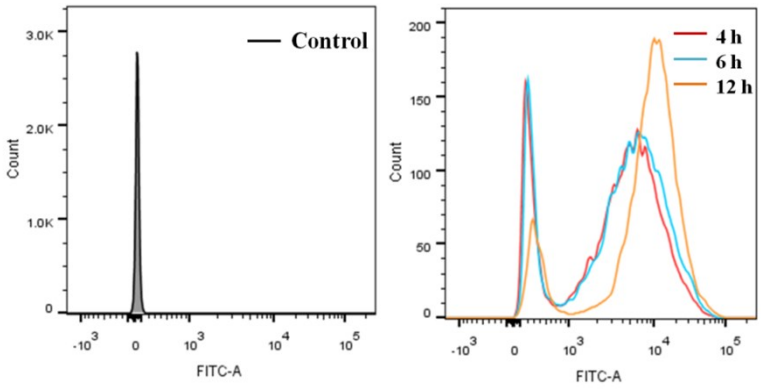


Fig. S25. The flow cytometry images of endogenous ROS generation in MCF-7 cells treated with **TLDCG** NPs**.**

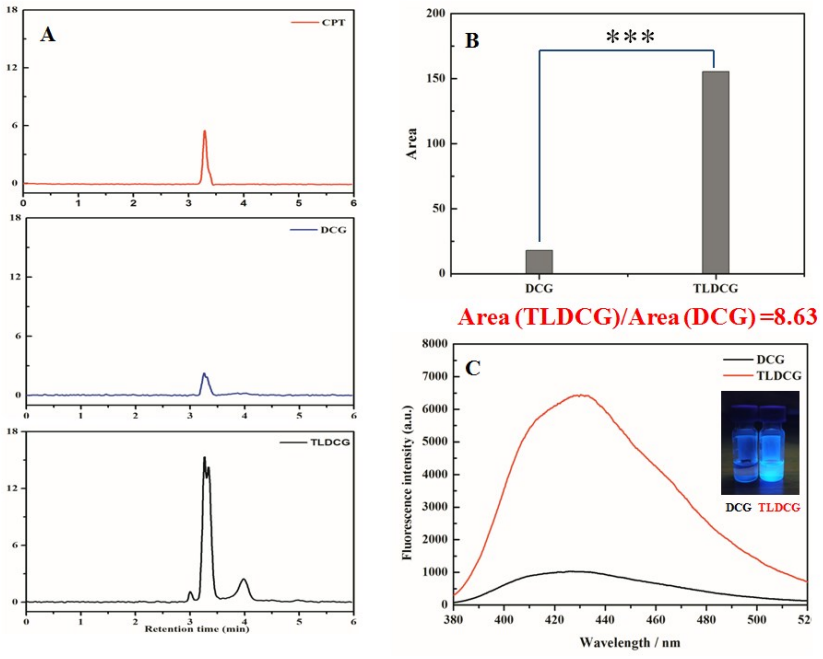


Fig. S26. (A) (B) HPLC and (C) fluorescence spectroscopy monitor the content of CPT in the cells **DCG** NPs and **TLDCG** NPs were incubated with MCF-7 cells for 24 h. Inset: images from left to right show the fluorescence intensity of intracellular CPT in MCF-7 cells treated with **DCG** NPs and **TLDCG** NPs.

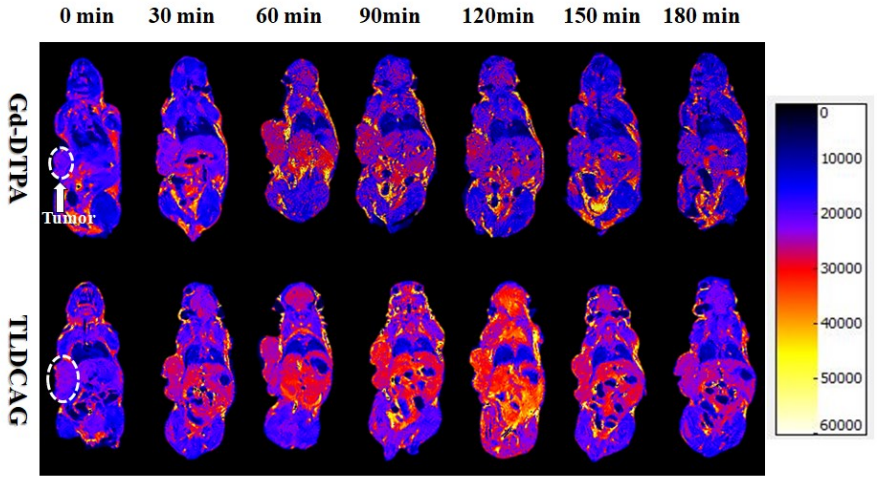


Fig. S27. MR pseudocolor images of tumor-bearing mice recorded at the different time following the injection of **TLDCAG** NPs and Gd-DTPA of the same concentration of Gd ions.

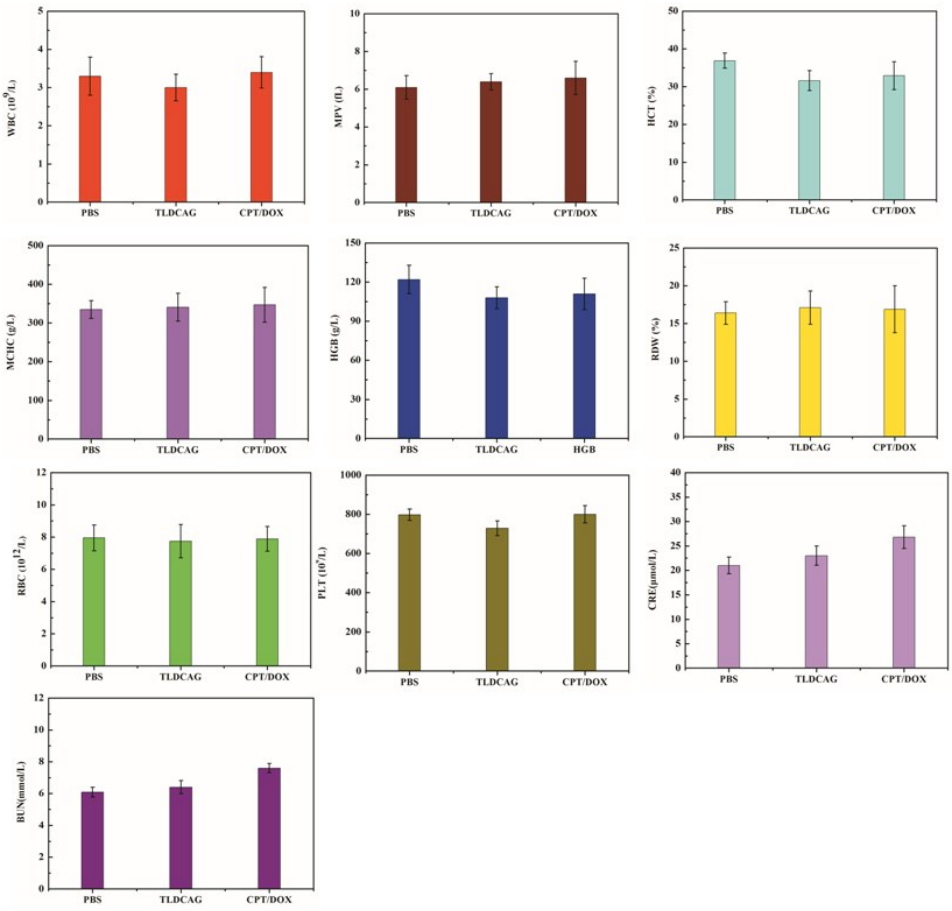


Fig. S28. Hematology and serum biochemistry test of the tumor-bearing mice after treatment with PBS, DOX/CPT, and **TLDCAG** NPs for 30 days.