Supporting Information

Amphiphilic triblock copolymer prodrug for tumor-specific pH/reduction dual-triggered drug delivery: Effect of self-assembling behaviors

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Table of Contents

1. Experimental details.

Materials. polyethylene glycol monomethyl ether (PEG, MW ~ 2000) were obtained from Aladdin Chemical Co. Ltd. 2, 2′-Azobis (2-methylpropionitrile) (AIBN, 98%) was purchased from Sinopharm Chemical Reagent Co. Ltd., and re-crystallized in ethanol. 2-Mercaptoethanol, 2,2′-dithiodipyridine (99%, HEOWNS), methacryloyl chloride (98%, HEOWNS), glacial acetic acid (99%, Guangfu regeant), triethylamine (TEA), 2-(diisopropylamino) ethyl methacrylate (DPA), 2-iminothiolane hydrochloride (98%) were purchased from Aladdin Chemical Co. Ltd. Doxorubicin hydrochloride (DOX·HCl, Beijing Huafeng United Technology Co., Ltd., China) were used as received. Other reagents and solvent were analytical grade and used as received.

Characterizations

^{1}H NMR spectra were recorded on a JEOL ECS (400 M) spectrometer.

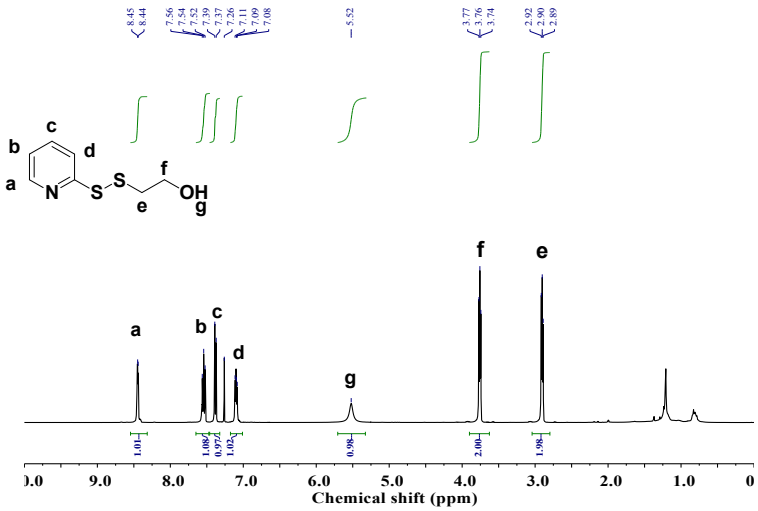
The drug content and drug release data of the prodrugs were obtained by TU-1109 UV/vis-spectrophotometer (Beijing Purkinje General Instrument Co. Ltd, Beijing, China).

The morphology observation was conducted on transmission electron microscope (TEM, JEM1200), sampling with aqueous dispersion (1.0 mg/mL).

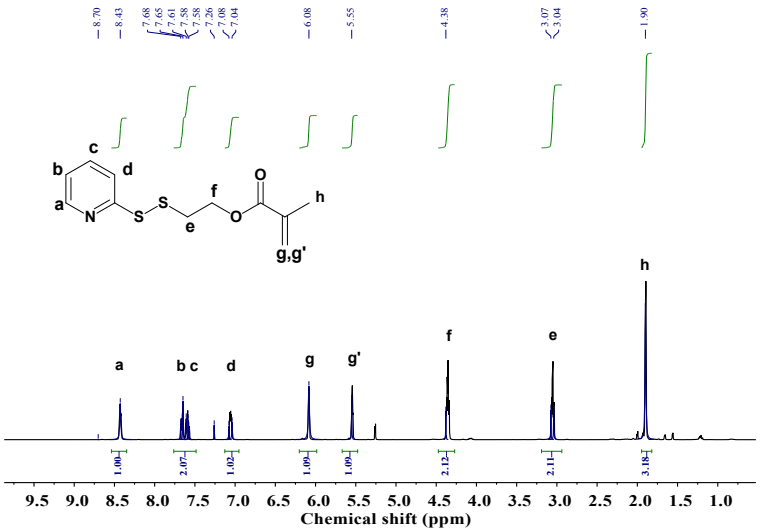
The diameter and distribution of the prodrug nanoparticles were measured with a dynamic light scattering (DLS) using a BI-200SM equipment (Brookhaven Instruments Corporation, USA).

Confocal fluorescence photos of cell were taken on research-grade inverted fluorescence microscope OLYMPUS, IX71.

Synthesis of compound of pyridyldisulfide ethanol. Aldrithiol-2 (4.4g, 0.02 mol) was dissolved in 40 mL of methanol, and 1 mL of glacial acetic acid was added. Then 0.78 g of 2-mercaptoethanol (0.01 mmol) in 7 mL of methanol was added dropwise. After stirring for additional 3 h, the solvent was evaporated to get a yellow oily crude product, which was purified by flash column chromatography using silica gel as a stationary phase and a mixture of ethyl acetate/hexane as eluent to obtain a pure colorless oil. Yield, 60%. ^{1}H NMR (400 MHz, Chloroform-*d*) δ 8.44 (d, J = 2.9 Hz, 1H), 7.65 – 7.47 (m, 1H), 7.38 (d, J = 7.9 Hz, 1H), 7.18 – 7.01 (m, 1H), 5.52 (s, 1H), 3.90 – 3.63 (m, 2H), 3.04 – 2.80 (m, 2H).



Synthesis of PDSM. Compound of pyridyldisulfide ethanol (1.31 g, 7 mmol) was dissolved in 30 mL of dry dichloromethane with 1.6 g (16 mmol) of trimethylamine. The mixture was pre-cooled in an ice-bath. Then 947 mg of methacryloyl chloride (9 mmol) in 10 mL of dichloromethane was added dropwise. After continuous stirring for another 6 h, the reaction mixture was washed with 3 × 30 mL of distilled water and then with 30 mL of brine. The organic layer was collected, dried with anhydrous $MgSO_4$ and concentrated to get a yellow oily crude product. It was purified by column chromatography using silica gel as the stationary phase and a mixture of ethyl acetate/hexane as eluent. The pure product was obtained as a colorless oil (yield: 71%).^{1}H NMR (400 MHz, Chloroform-*d*) δ 8.43 (s, 1H), 7.76 – 7.49 (m, 2H), 7.06 (d, *J* = 15.5 Hz, 1H), 6.08 (s, 1H), 5.55 (s, 1H), 4.38 (s, 2H), 3.06 (d, *J* = 14.3 Hz, 2H), 1.90 (s, 3H).



Drug release profiles. *In vitro* drug release from the PEG_{43}-$PDPA_{35}$-P(MAL-DOX)$_3$ prodrug nanoparticles were performed in pH 7.4 PBS, pH 7.4 PBS +10 μM GSH, pH 5.0 ABS, pH 5.0 ABS + 10 mM GSH and pH 5.0 ABS + 10 mM GSH + 0.1% Tween at 37 °C using dialysis bag (MWCO=1000 Da). 10.0 mL of the dispersion (0.2 mg/mL) of the PEG_{43}-$PDPA_{35}$-P(MAL-DOX)$_3$ prodrug nanoparticles was transferred into the dialysis bag and immersed in corresponding buffer solution (100 mL). 5 mL of the incubated solution was taken out at different time intervals, and the same amount of fresh buffer was added to keep the volume constant. DOX-SH release profiles were characterized by measuring the UV absorbance of the solutions at 485 nm with the help of a calibration curve of DOX-SH in the same buffer solution.

***In vitro* cytotoxicity assay.** The cytotoxicity of the PEG_{43}-$PDPA_{35}$-P(MAL-DOX)$_3$ nanoparticles and free DOX toward the HepG 2 cells or L20 cells were evaluated using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay. Cells were seeded into a 96-well plate at a density of 1×10^5 cells per well in the medium containing 10% FBS and cultured for 24 h at 37 ℃ in a 5% CO_2 atmosphere. Then, the PEG_{43}-$PDPA_{35}$-P(MAL-DOX)$_3$ nanoparticles or free DOX with different concentrations were loaded into wells separately. After 48 h of incubation, 20 μL of MTT (5 mg/mL) solution was added and incubated for another 4 h. The medium was carefully removed, and 150 μL of DMSO was added into each well to dissolve crystals.

The absorbance of the solution in well at 490 nm was measured using a microplate reader (Bio-Rad Laboratories, iMark). The results are expressed as viability (%) relative to a control without any treatment. Data were presented as average±SD (n = 5).

Cellular uptake and intracellular biodistribution. HepG 2 cells were seeded into a 96-well plate at a density of 1×10^5 cells per well, the cells were then incubated with PEG_{43}-$PDPA_{35}$-P(MAL-DOX)$_3$ nanoparticles (15 μg/mL) for 24 h at 37 ℃. Then, the culture media were removed, and the cells were washed 3 times with PBS and fixed with 4% paraformaldehyde. The cells were then rinsed 3 times with PBS and dyed with 4', 6-diamidino-2-phenylindole (DAPI), after washed with PBS 3 times, the prepared cells were examined by inverted fluorescence microscope (OLYMPUS, IX71).

2. Supporting Figures

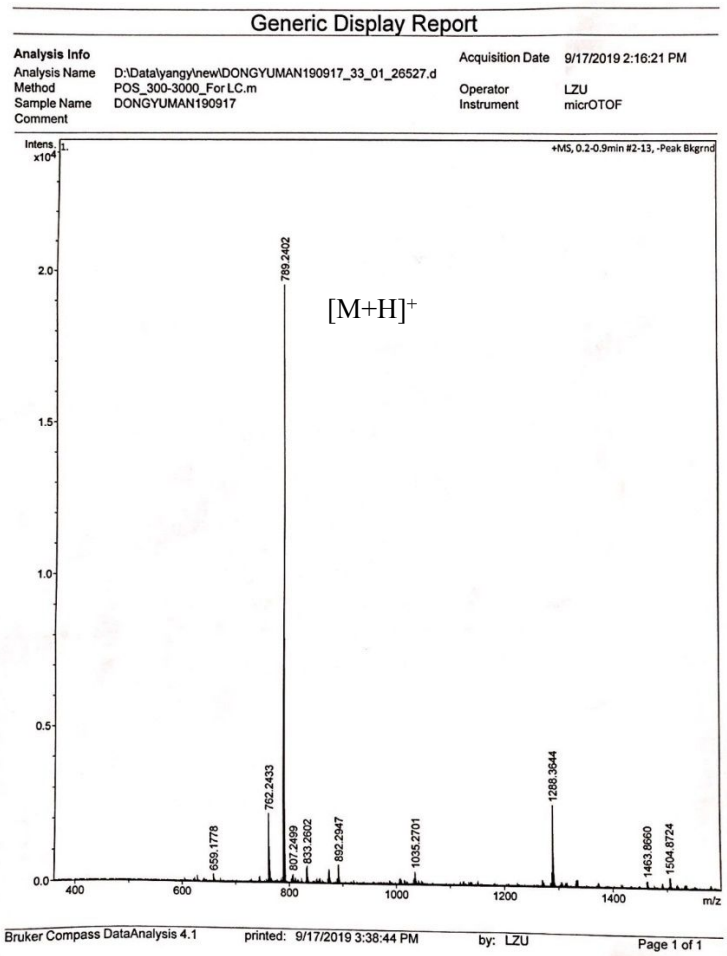


Figure S1. TOF-MS spectrum of MAL-DOX.

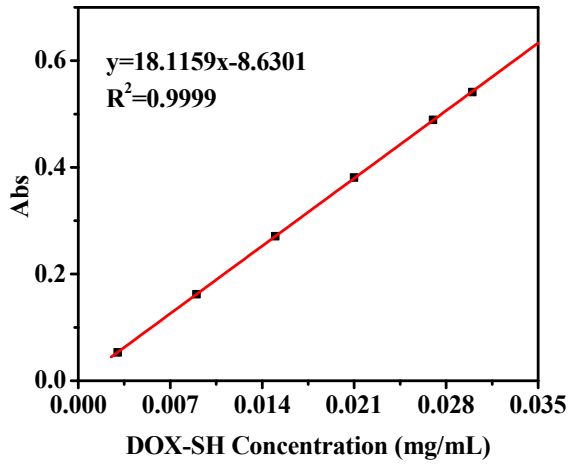


Figure S2. Calibration curve of DOX-SH in DMSO.

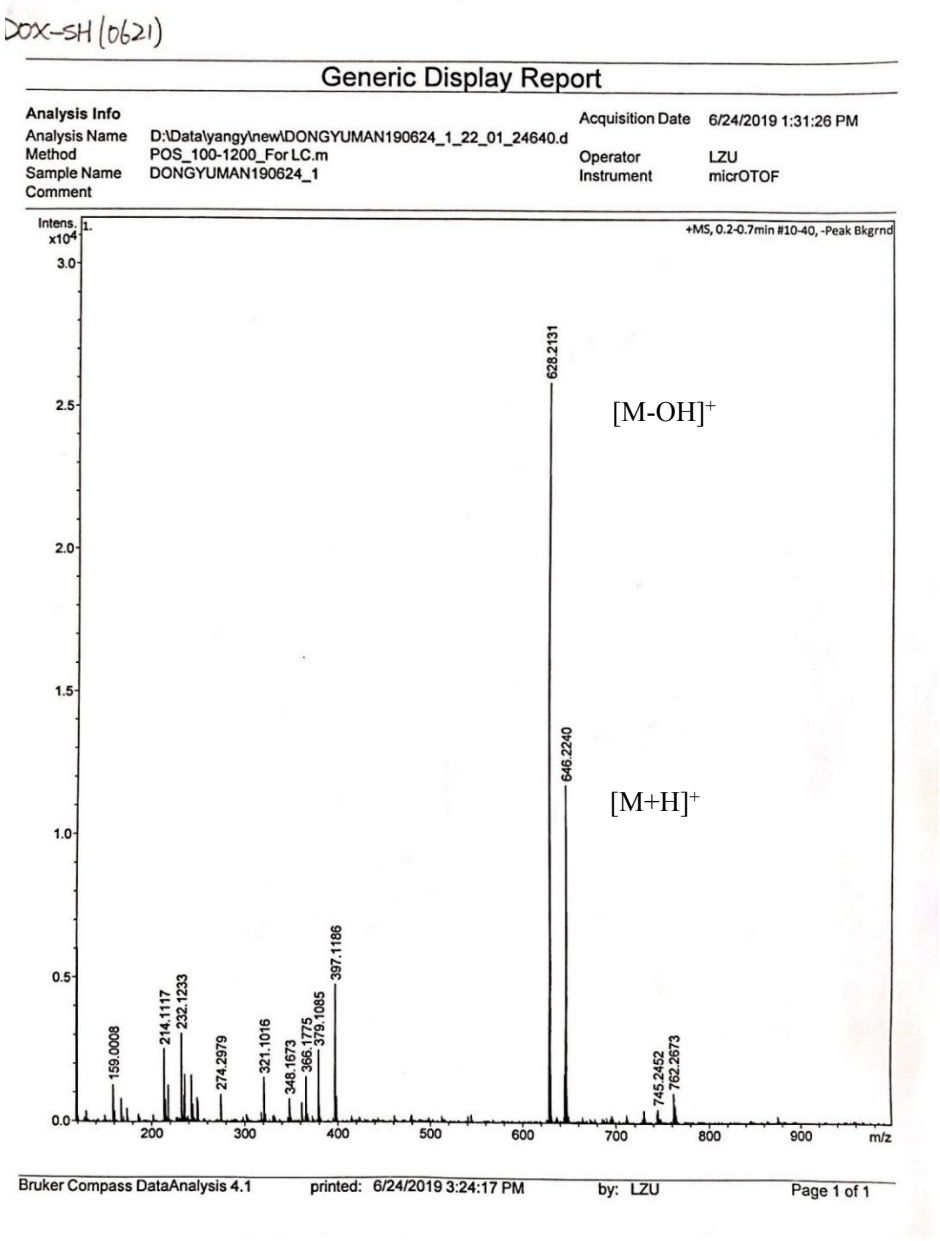
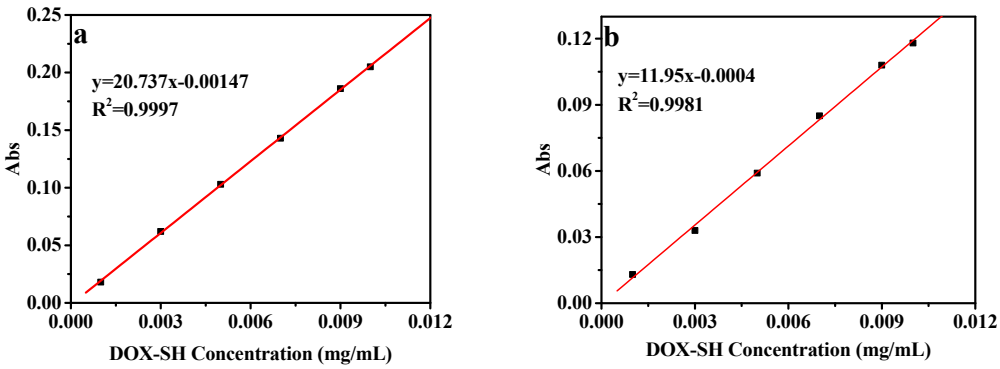


Figure S3. TOF-MS spectrum of DOX-SH.



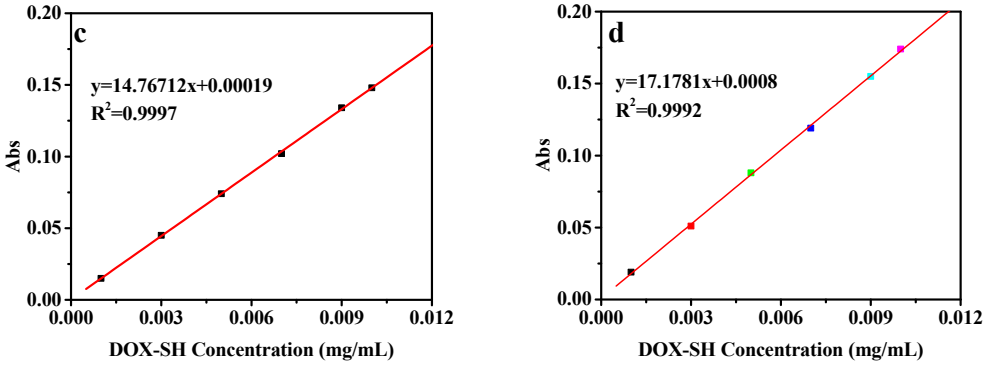


Figure S4. Calibration curves of DOX-SH in pH 5.0 ABS (a), pH 7.4 PBS (b), pH 5.0 ABS+10 mM GSH (c) and pH 7.4 PBS+10 μM GSH (d).