Electronic supplementary information

Reduction/pH dual-responsive biodegradable camptothecin polymeric prodrugs combined with doxorubicin for synergistic anticancer efficiency

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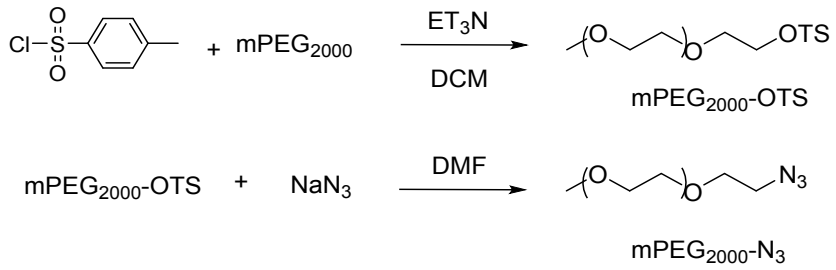
Synthesis of branched chains.

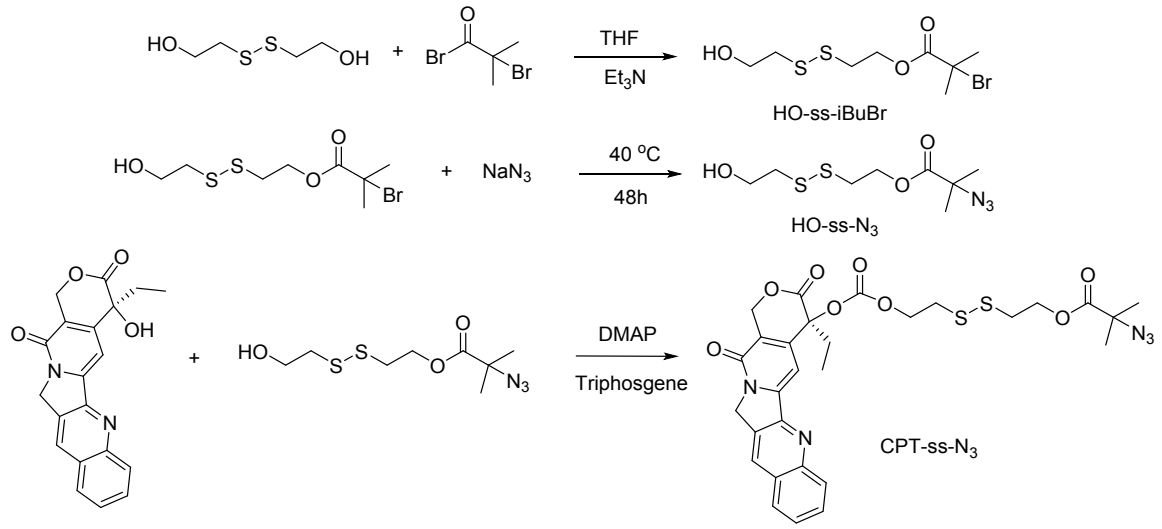


Scheme S1. Synthesis of $mPEG_{2000}$-N_3.

Synthesis of Azido end-functional poly(ethylene glycol) methyl ether ($mPEG_{2000}$–N_3).

mPEG (Mn = 2000 Da) (10 mmol) and triethylamine (30 mmol) were added in 50 mL dichloromethane. Under magnetic stirring at room temperature for 24 h, The dichloromethane solution was washed successively by water (50 mL × 2), Brine (50 mL × 2), 1 M HCL (50 mL × 1), Saturated sodium bicarbonate solution (50 mL × 1), water (50 mL × 2) and dried over anhydrous magnesium sulfate, and filtered. Then crude product purified *via* excess adding of cold ether absolute, which finally dried at room temperature in a vacuum oven overnight after filtration to yield a white powder mPEG-OTS. mPEG-OTS (5 mM) was dissolved in 40 mL DMF and sodium azide (15 mM) were then added. The reaction was stirred at room temperature for 24 h. Then DMF was removed *via* vacuum evaporation, and 50 mL toluene was added with subsequently filtration to remove undissolved solid. The toluene solution was removed *via* vacuum evaporation.[1] Then product mPEG-N_3 precipitated by excess adding of cold ether absolute, which finally dried at room temperature in a vacuum oven overnight to yield a white powder. Yield of $mPEG_{2000}$-N_3 are 78.3%.

^{1}H NMR (400 MHz, $CDCl_3$, TMS) δ: 3.68–3.65 (m, 184H, methylene in mPEG), 3.38 (s, 3H, –OC*H*3 end group). ^{13}C NMR (100MHz, $CDCl_3$) δ: 72.0, 70.6, 70.0, 59.0, 50.7.



Scheme S2. Synthesis of CPT-ss-N_3.

Synthesis of 2-Hydroxyethyl-2'-(bromoisobutyryl)ethyl Disulfide (HO-ss-iBuBr).

Triethylamine (25 mmol) and 2,2'-dithiodiethanol (30 mol) were dissolved in 100 mL of dry THF in ice bath. α-Bromoisobutyryl bromide (20 mol) dissolved in 40 mL of THF, was added dropwise into the solution. The solution was stirred in ice bath for 1 h and at room temperature for 16 h. The resulting mixture was filtered and removed THF by rotary evaporation, dichloromethane was added and the solution was washed by 3% HCl solution for three times, 2% NaOH solution for three times and water for three times. The organic phase was collected and dried over magnesium sulfate anhydrous for 12 h. The crude solution was concentrated by rotary evaporation and purified by column chromatography(4:1 petroleum ether–ethyl acetate) resulting in a yellow powder in 51.8% yield.

^{1}H NMR, δ (400 MHz, $CDCl_3$, TMS, ppm): 4.48-4.44 (t, 2H, CH_2-OH), 3.92-3.88 (t, 2H, CH_2-O-C=O), 2.99-2.96 (t, 2H, HO-CH_2-CH_2-S), 2.91-2.88 (t, 2H, O=C-O-CH_2-CH_2-S), 1.95 (s, 6H, CH_3). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 171.6, 63.6, 60.2, 55.5, 41.6, 36.5, 30.7. HR-MS (ESI): Calcd for: $C_8H_{15}BrO_3S_2$: 324.9544, 326.9524 ($[M + Na]^+$), Found 324.9531, 326.9529 ($[M + Na]^+$).

Synthesis of 2,2'-dithiobis[1-(2-azido-2-methylpropionyloxy) ethane] (HO-ss-N_3).

HO-ss-iBuBr (10 mmol) and NaN_3 (30 mmol) were dissolved in 100 mL of DMF in one 100 mL of flask. The reaction was performed at 40 °C for 48 h. The mixture was passed through a short

column of basic Al_2O_3 and DMF was evaporated. The crude product was dissolved in 100 mL of water and extracted with 200 mL CH_2Cl_2 for three times. The organic layer was dried over anhydrous Na_2SO_4 for 12 h and the filtrate was dried by rotary evaporation. Finally, the product was dried under vacuum at 25 °C for 24 h to give a yellow viscous liquid in 93.6% yield

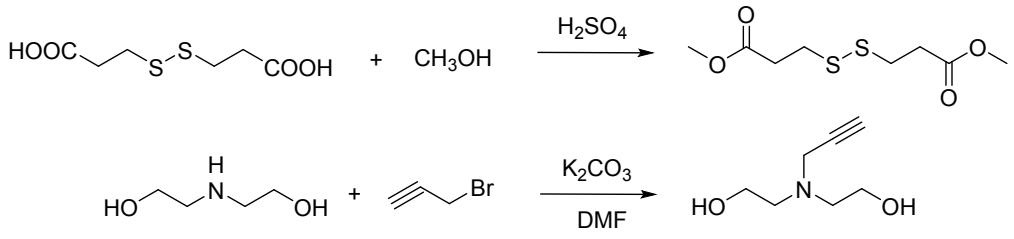
^{1}H NMR, δ (400 MHz, $CDCl_3$, TMS, ppm): 4.48-4.43 (t, 2H, C*H*$_2$-OH), 3.92-3.88 (t, 2H, C*H*$_2$-O-C=O), 2.99-2.96 (t, 2H, HO-CH$_2$-C*H*$_2$-S), 2.91-2.88 (t, 2H, O=C-O-CH$_2$-C*H*$_2$-S), 1.49 (s, 6H, C*H*$_3$). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 172.7, 63.3, 60.3, 55.5, 41.6, 37.0, 36.8, 24.5. HR-MS (ESI): Calcd for: $C_8H_{15}N_3O_3S_2$: 288.0453 ([M + Na]$^+$), Found 288.0446 ([M + Na]$^+$).

Synthesis of reduction-responsive and clickable CPT derivative (CPT-ss-N_3).

CPT (2.87 mmol) and DMAP (8.65 mmol) were suspended in 150 mL of dry DCM in ice bath under a argon atmosphere. Triphosgene (0.96 mmol) was added and the mixture was stirred for 30 min. After that, HO-ss-N_3 (4.26 mmol) in 15 mL of dry DCM was added dropwise into the reaction. The reaction was further stirred at 25 °C for 16 h. After filtration and the removal of the solvents by rotary evaporation, the residue was diluted with 100 mL of DCM and washed with water for once, 1.0 M HCl solution for twice, and brine for three times, respectively. The organic layer was collected and dried over anhydrous Na_2SO_4 for 12 h.[2] The filtrate was concentrated and the crude product was purified by silica column chromatography (4:1 DCM–ethyl acetate) resulting in a yellow powder in 38.5% yield.

HR-MS (ESI): Calcd for: $C_{29}H_{29}N_5O_8S_2$: 662.1356 ([M + Na]$^+$), Found 662.1349 ([M + Na]$^+$).

Synthesis of various monomers



Scheme S3. Synthesis of *N*-propargyldiethanolamine and dimethyl 3,3'-Dithiopropionate.

Synthesis of *N*-propargyldiethanolamine.

Anhydrous K_2CO_3 (60 mmol) and Diethanolamine (30 mmol) in 50 mL DMF and the mixture was cooled in an ice bath. Once cooled propargyl bromide (36 mmol) was added. The reaction flask was let slowly let warm to 80 °C for 4 h. After removing the K_2CO_3 by filtering, and evaporated under reduced pressure.[3] The final product was obtained by silica gel column chromatography (CH_2Cl_2 to 95:5 CH_2Cl_2–MeOH) resulting in an orange liquid in 46.5% yield.

^{1}H NMR (400 MHz, $CDCl_3$, TMS) δ: 3.67–3.64 (m, 4H, –NCH_2CH_2–), 3.49 (d, J = 2.3 Hz, 2H, –$NCH_2C{\equiv}CH$), 2.76–2.74 (m, 4H, –NCH_2CH_2–), 2.22–2.21 (t, J = 2.3 Hz, 1H, –$NCH_2C{\equiv}CH$). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 78.4, 73.1, 59.2, 55.1, 42.3. HR-MS (ESI): Calcd for: $C_7H_{13}NO_2$: 144.1024 ($[M + H]^+$), Found 144.1004 ($[M + H]^+$).

Synthesis of dimethyl 3,3'-Dithiopropionate.

3,3'-Dithiodipropionic acid (20 mmol) were added in 100 mL methanol. Under magnetic stirring at 80 °C for 4 h , and the methanol evaporated under reduced pressure. The final product was obtained by silica gel column chromatography (4:1 petroleum ether–ethyl acetate), resulting in dimethyl 3,3'-Dithiopropionate as an colorless liquid in 88.1% yield.

^{1}H NMR (400 MHz, $CDCl_3$, ppm) δ: 3.71 (s, 6H, CH_3O–), 2.95-2.93 (t, 4H, –SCH_2CH_2–), 2.77-2.73 (t, 4H, –SCH_2CH_2–). ^{13}C NMR (100 MHz, $CDCl_3$, ppm) δ: 172.1, 51.9, 33.9, 33.0. HR-MS (ESI): Calcd for: $C_8H_{14}O_4S_2$: 239.0412 ($[M + H]^+$), Found: 239.0404 ($[M + H]^+$).

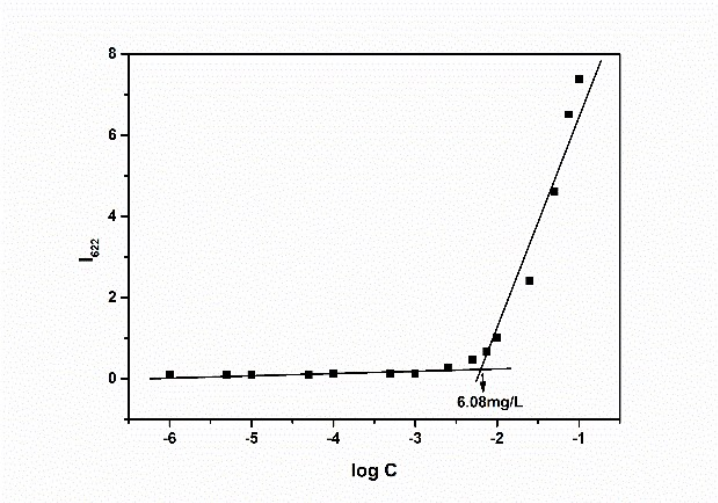


Figure S1. Plots of the intensity ratio I_{622} from the Nile red emission spectra versus the logarithm of the concentration of CPT Prodrugs.

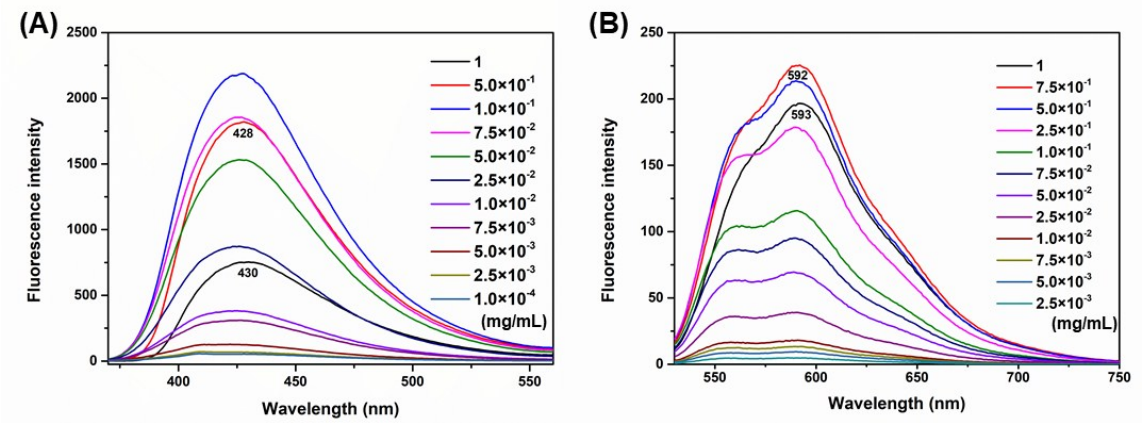


Figure S2. Fluorescence emission spectra of (A) CPT and (B) DOX at different concentrations of (A) CPT Prodrugs and (B) DOX@CPT Prodrugs.

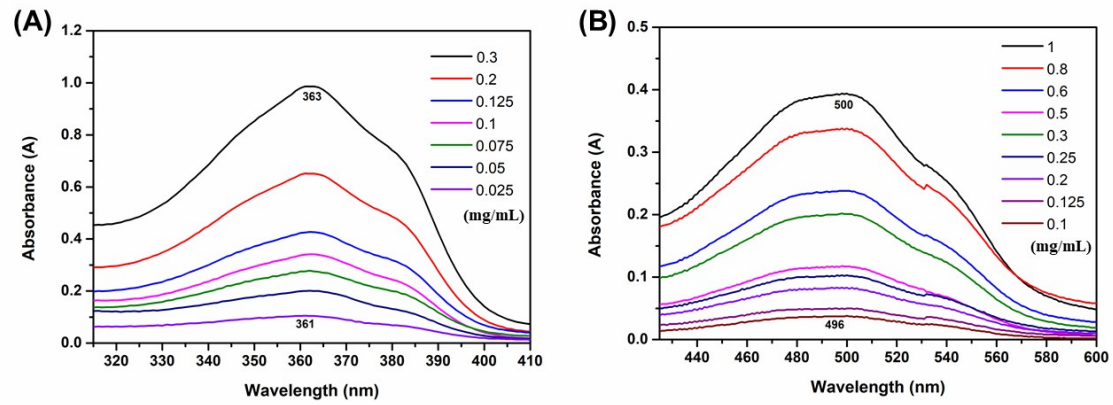


Figure S3. UV-vis absorption spectra of (A) CPT and (B) DOX at different concentrations of (A) CPT Prodrugs and (B) DOX@CPT Prodrugs.

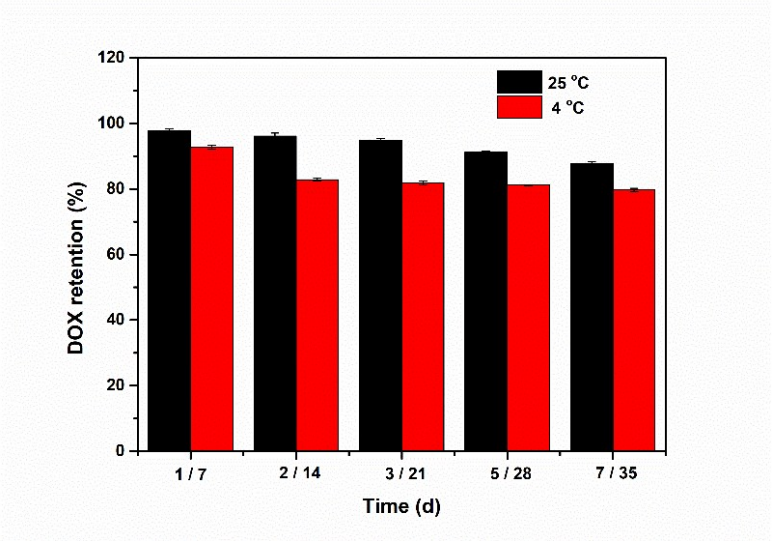


Figure S4. Storage stability of DOX@CPT Prodrugs were stored at 25 °C for 7 days and 4 °C for 35 days. Data represent mean ± SD (n = 3).

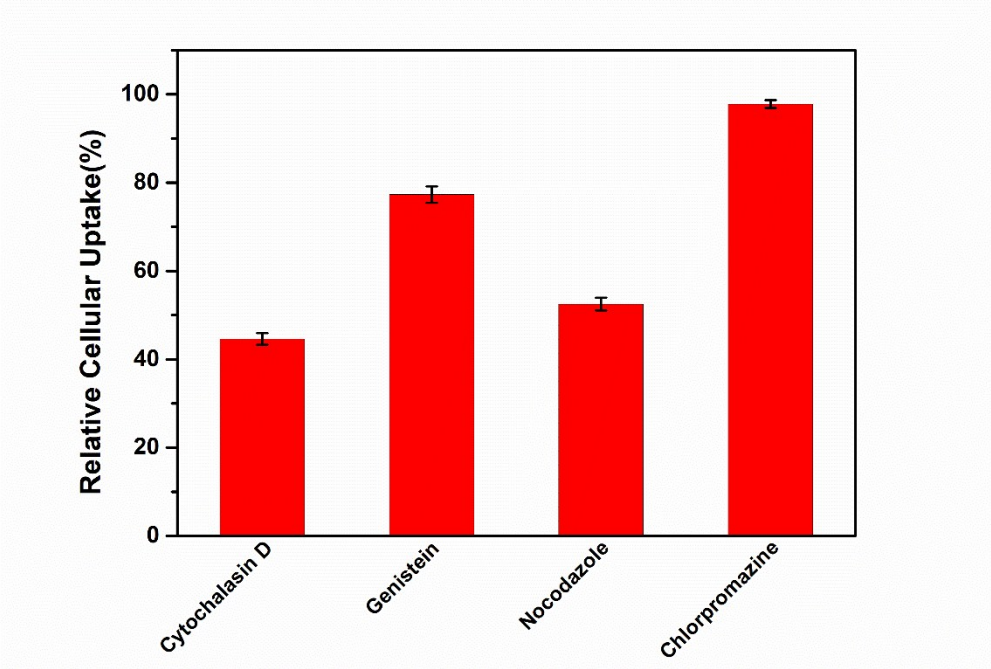


Figure S5. Relative cellular uptake of DOX@CPT Prodrugs in HepG2 cells in the presence of various endocytic inhibitors.

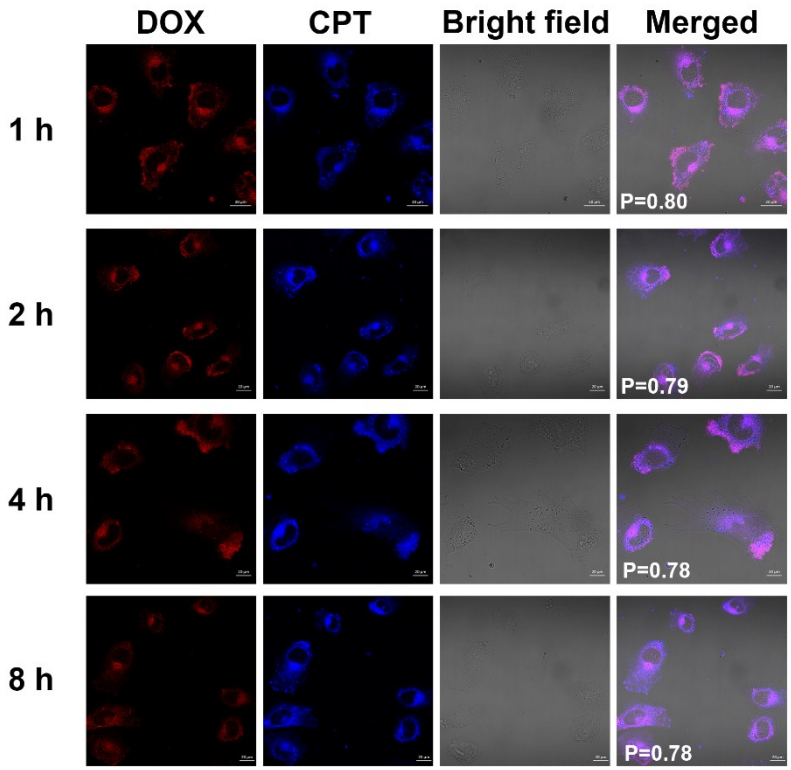


Figure S6. CLSM images of HL-7702 cells incubated with DOX@CPT Prodrugs for different time. The scale bar is 20 μm.

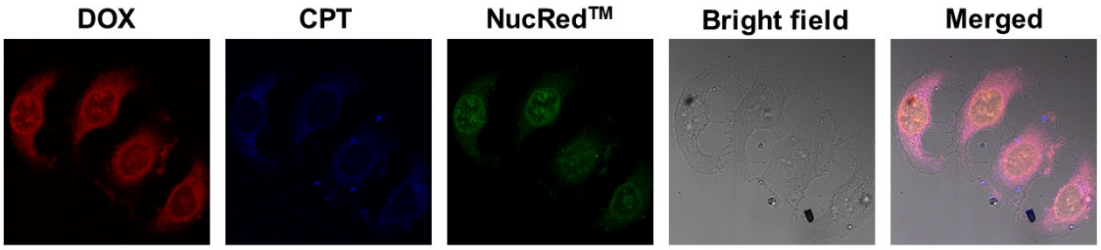


Figure S7. CLSM images of HepG2 cells incubated with DOX@CPT Prodrugs and NucRed™ Live 647 for 12 h.

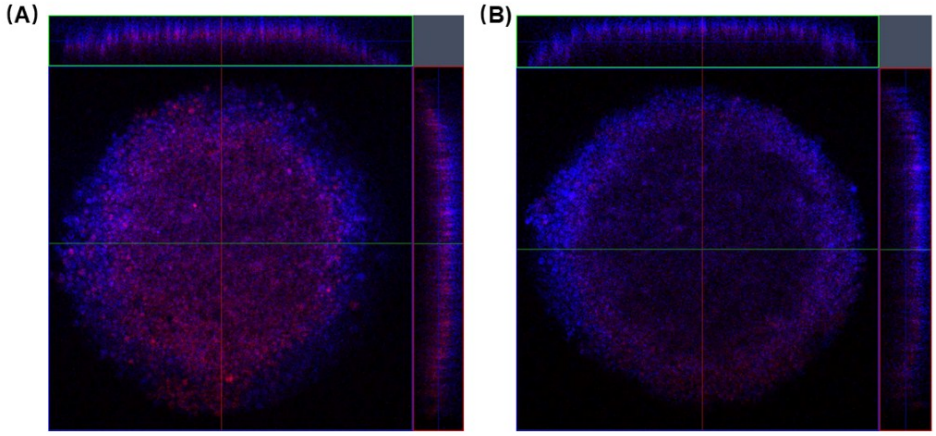


Figure S8. Cross-section images of X and Y-axis of CLSM in 3D spheroids incubating (A) DOX@CPT Prodrugs and (B) Free DOX/CPT.

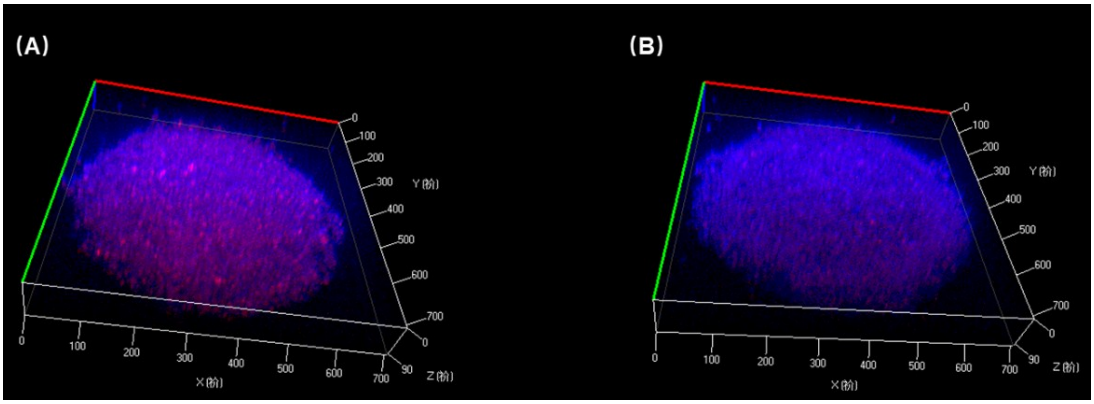


Figure S9. Three-dimensional reconstruction of CLSM in 3D spheroids incubating (A) DOX@CPT Prodrugs and (B) Free DOX/CPT.

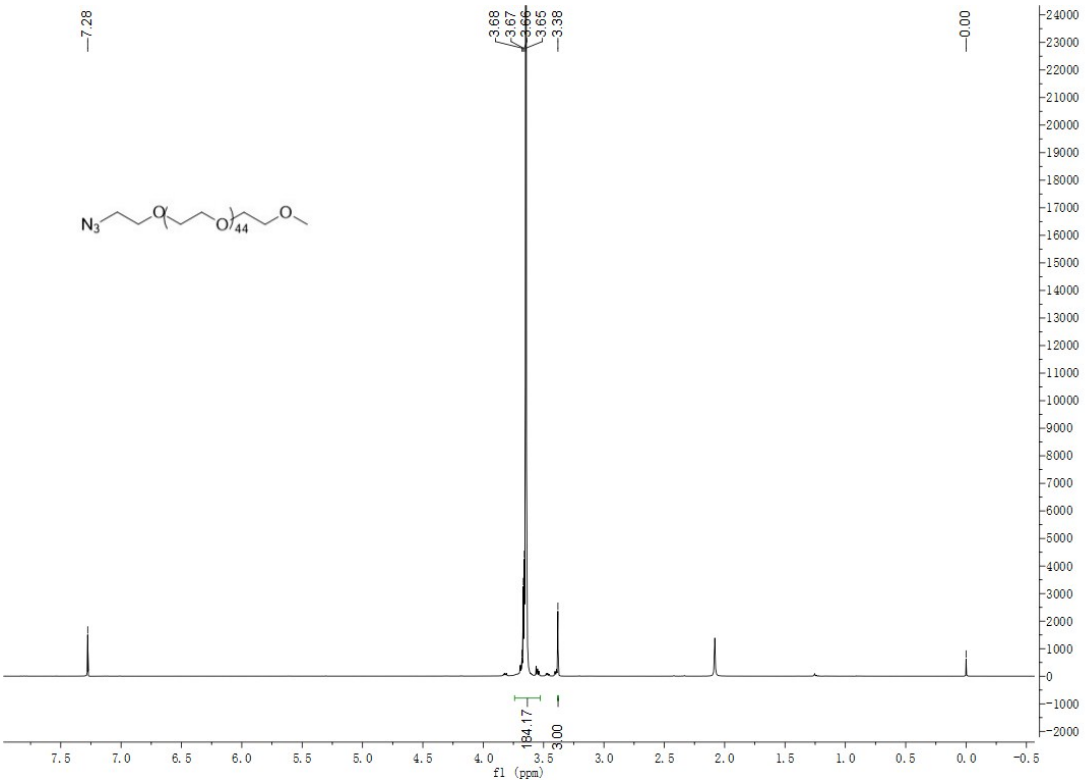


Figure S10. ^{1}H NMR spectrum of $mPEG_{2000}$-N_3 in $CDCl_3$.

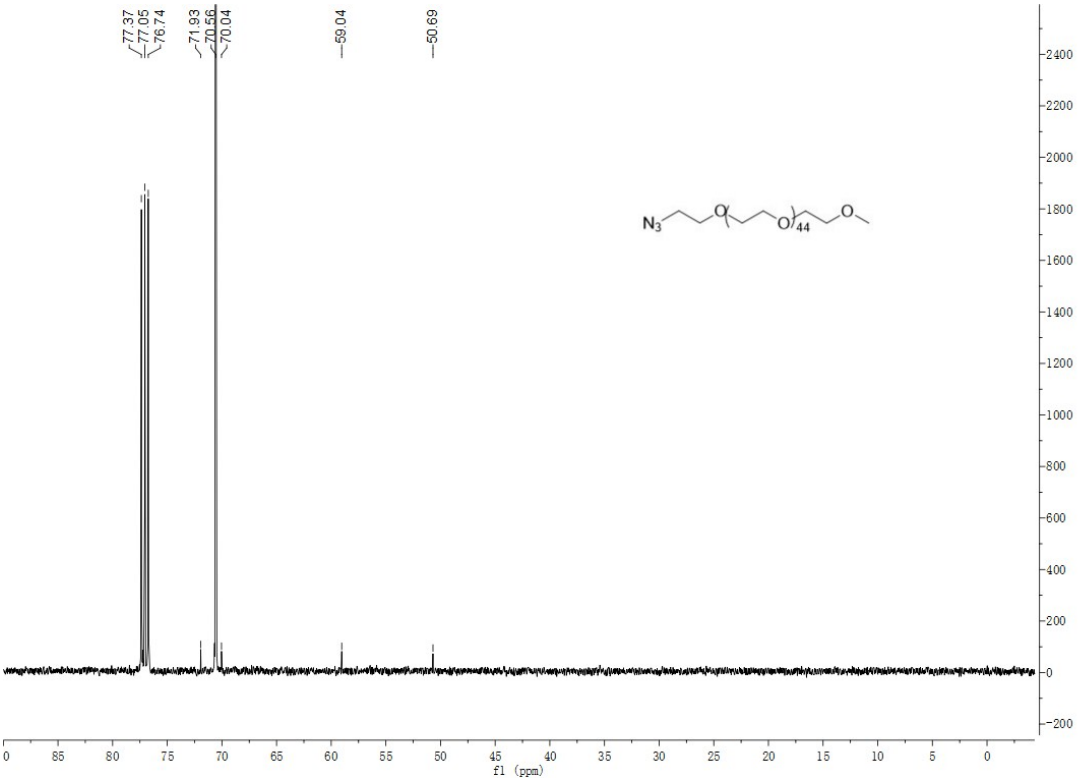


Figure S11. ^{13}C NMR spectrum of $mPEG_{2000}$-N_3 in $CDCl_3$.

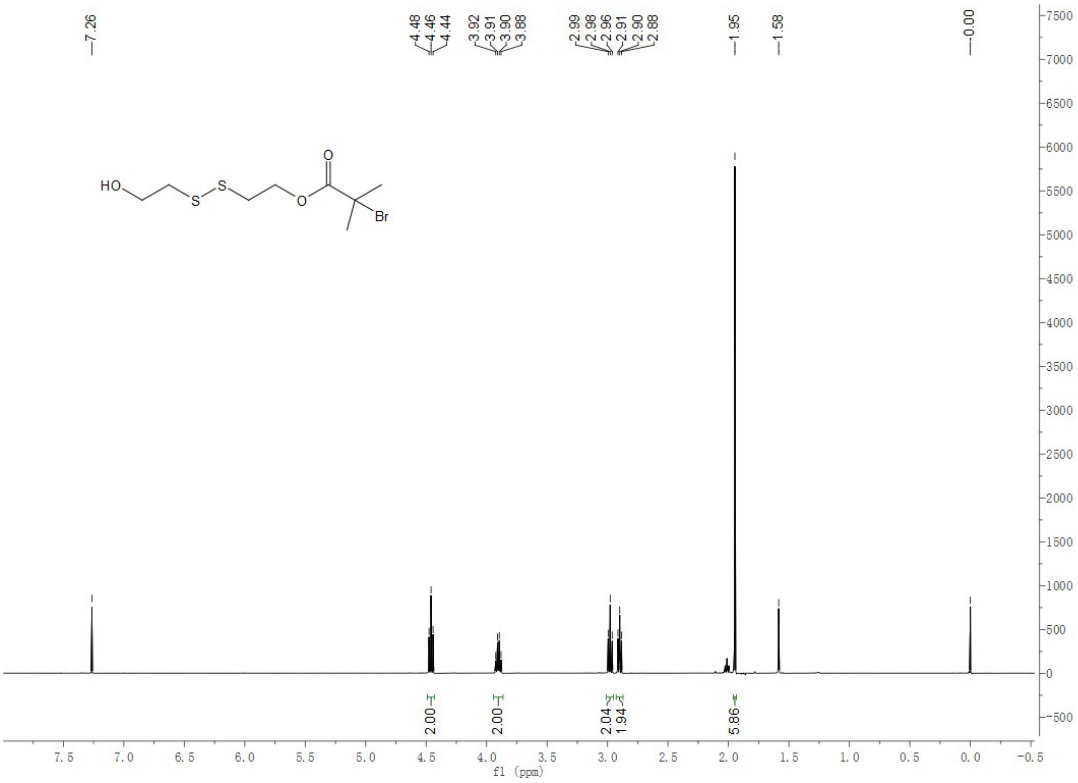


Figure S12. ^{1}H NMR spectrum of HO-ss-iBuBr in $CDCl_3$.

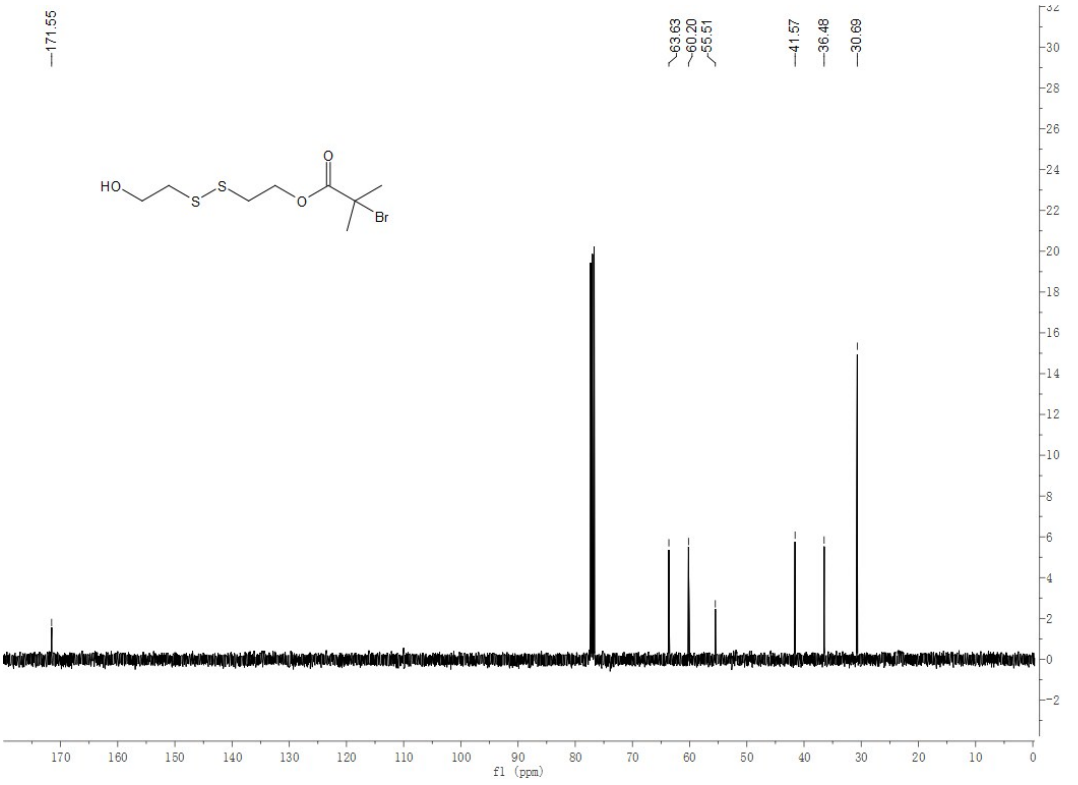


Figure S13. ^{13}C NMR spectrum of HO-ss-iBuBr in $CDCl_3$.

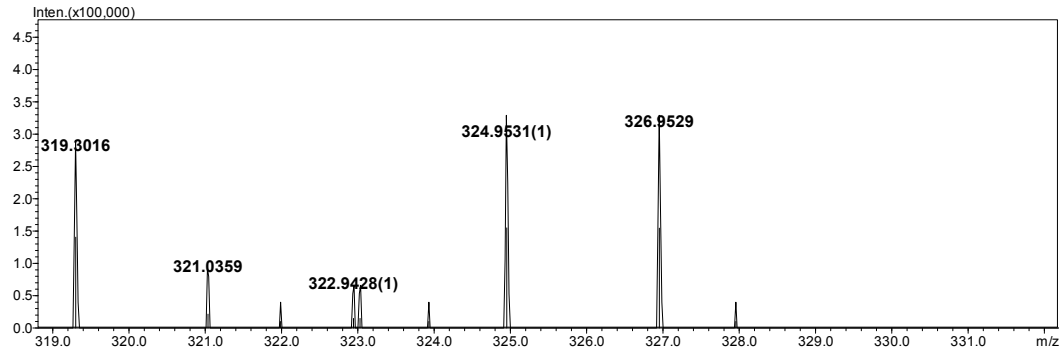


Figure S14. HR-MS of HO-ss-iBuBr.

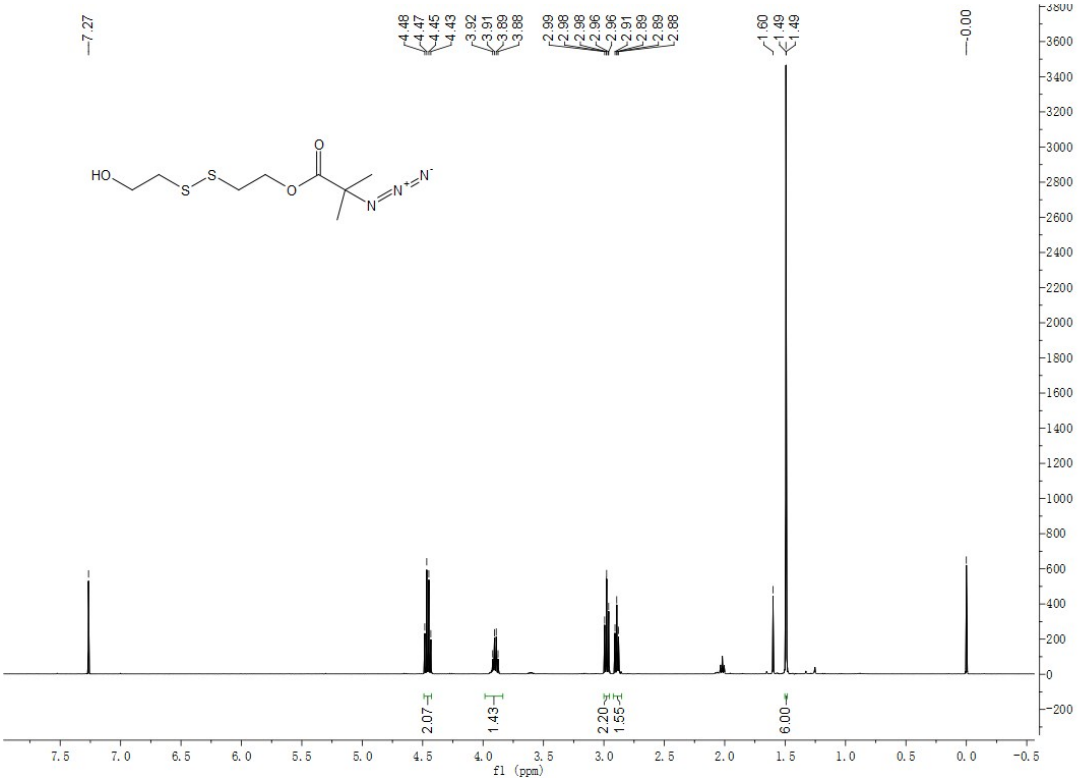


Figure S15. ^{1}H NMR spectrum of HO-ss-N_3 in $CDCl_3$.

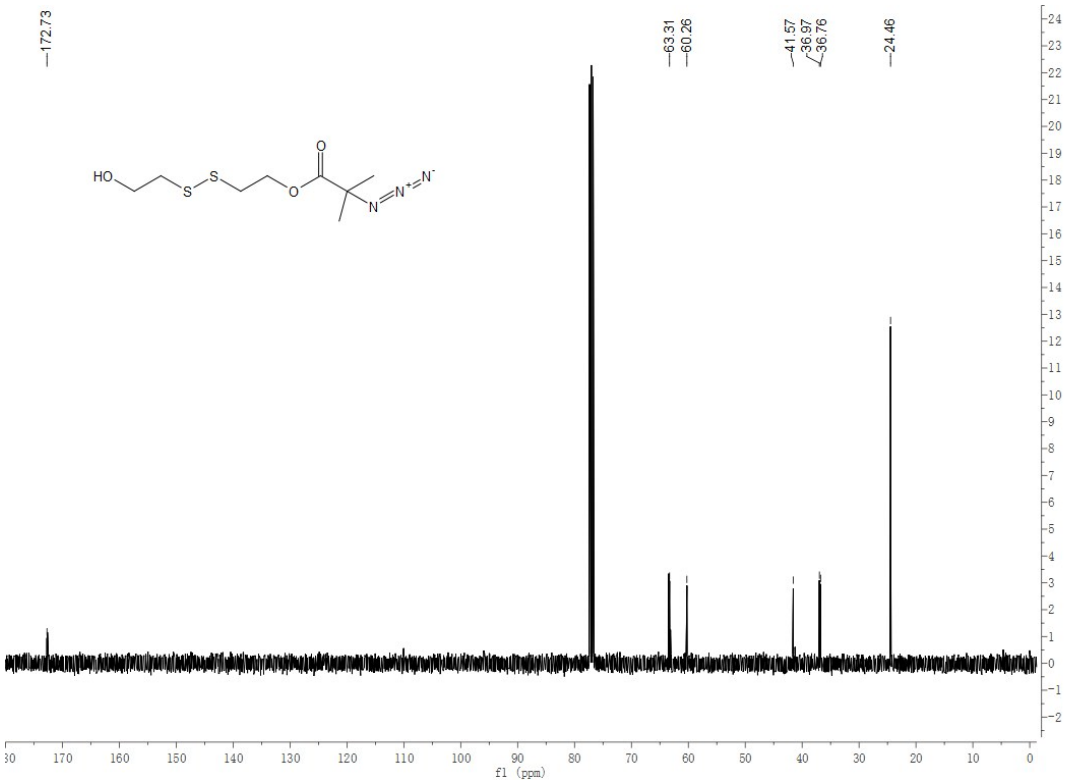


Figure S16. ^{13}C NMR spectrum of HO-ss-N_3 in $CDCl_3$.

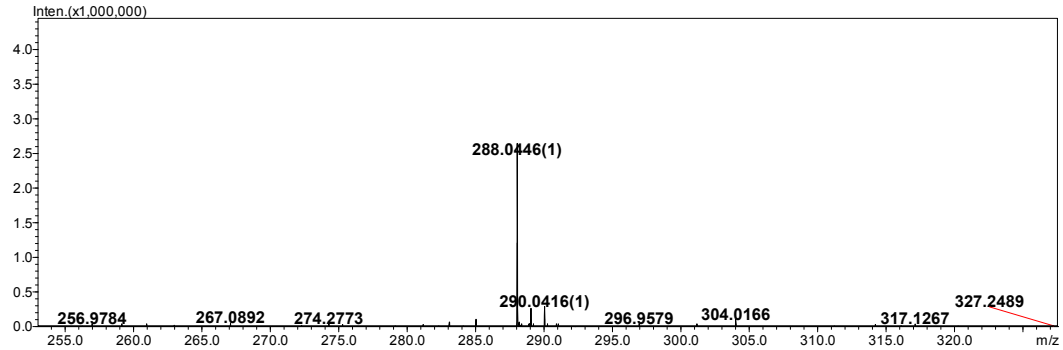


Figure S17. HR-MS of HO-ss-N_3.

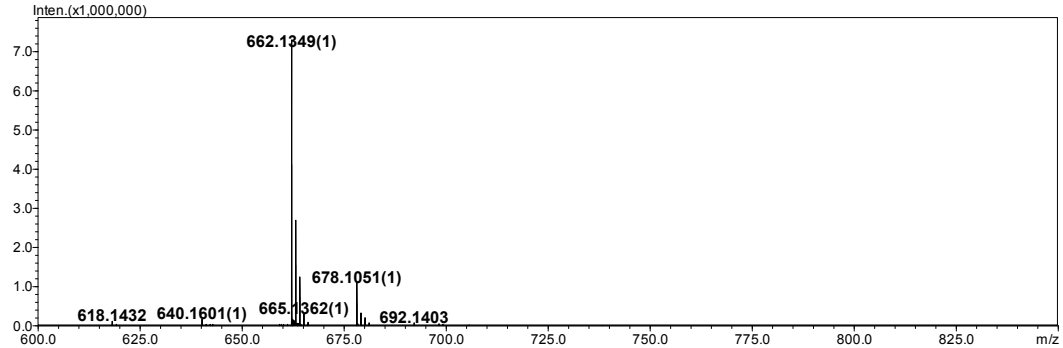


Figure S18. HR-MS of CPT-ss-N_3.

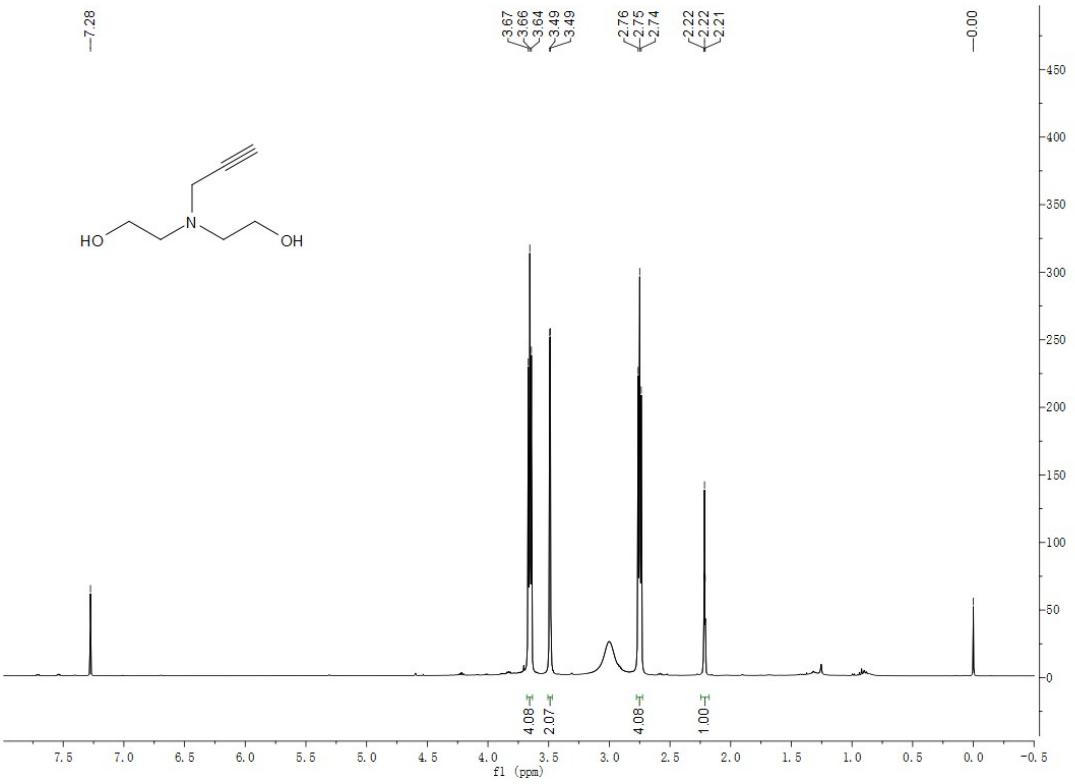


Figure S19. ^{1}H NMR spectrum of *N*-propargyldiethanolamine in $CDCl_3$.

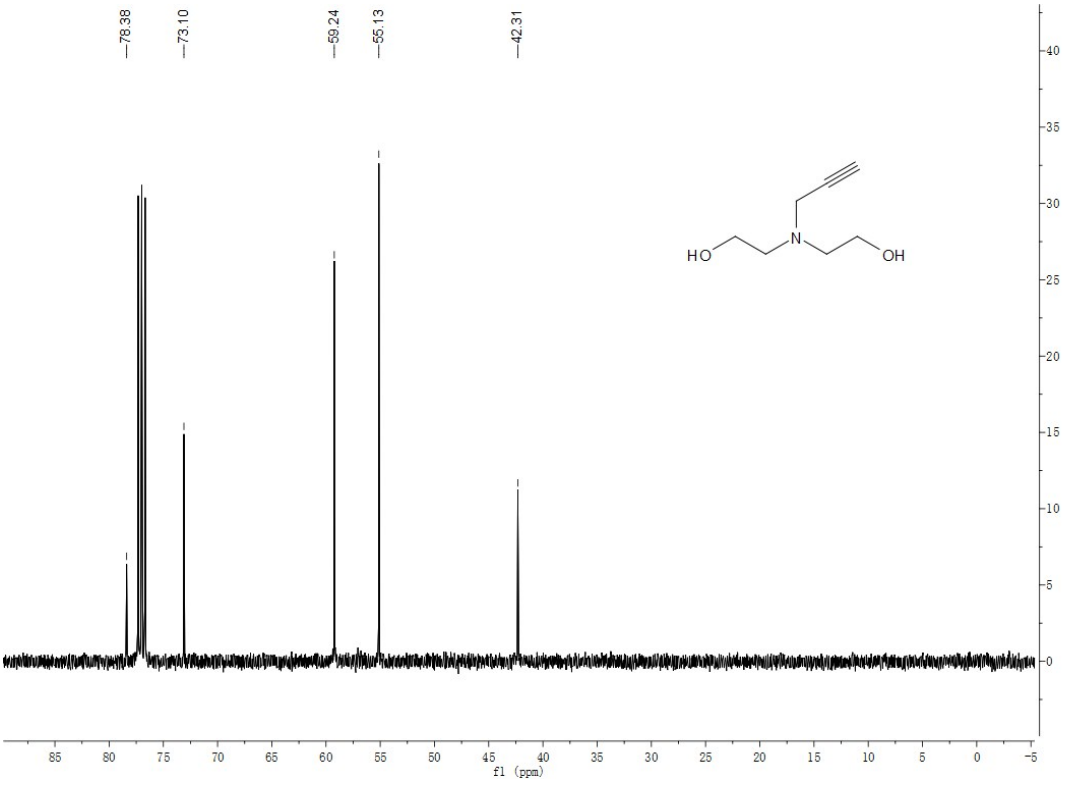


Figure S20. ^{13}C NMR spectrum of *N*-propargyldiethanolamine in $CDCl_3$.

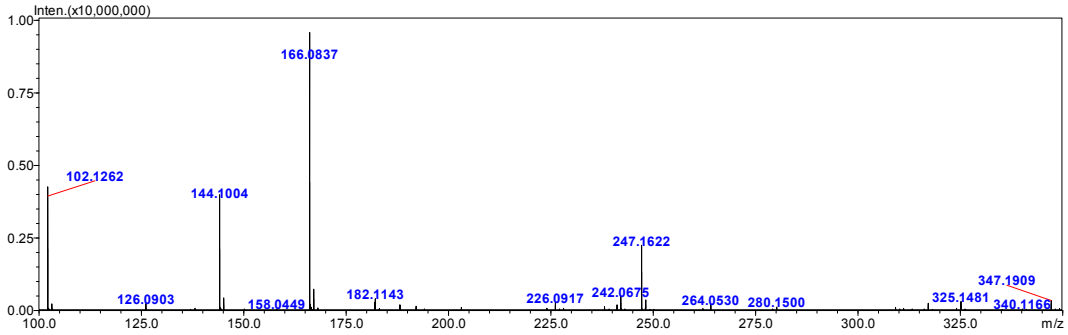


Figure S21. HR-MS of *N*- propargyldiethanolamine.

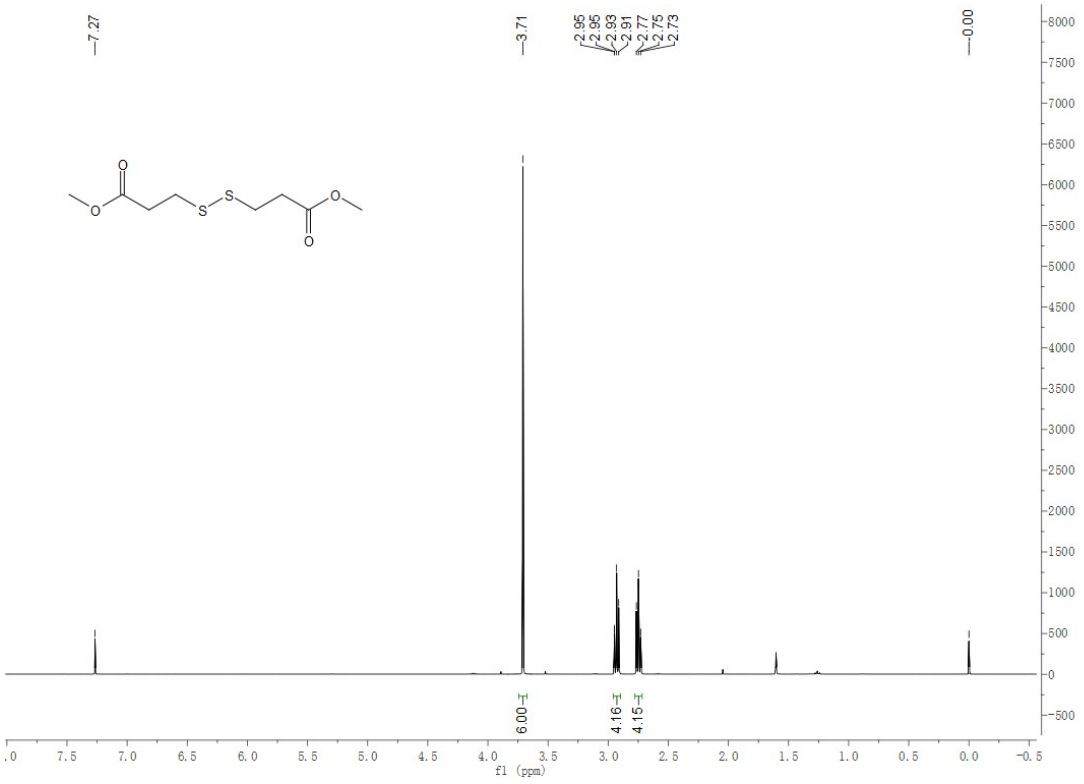


Figure S22. ^{13}C NMR spectrum of dimethyl 3,3'-Dithiopropionate in $CDCl_3$.

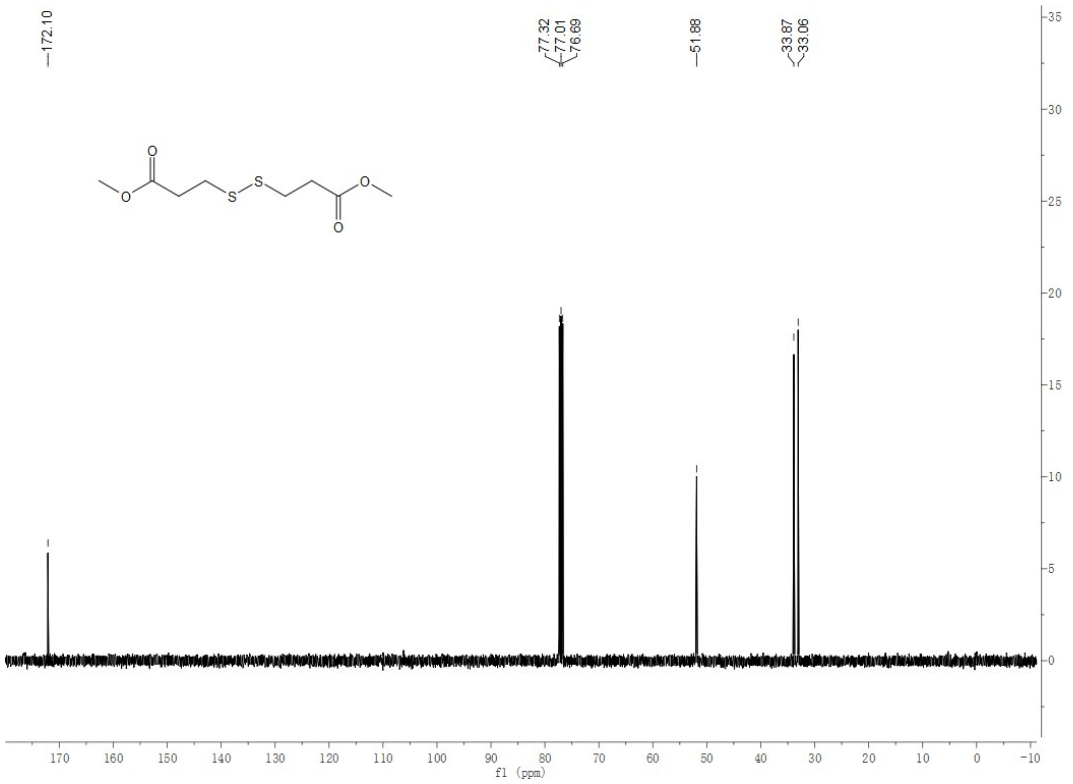


Figure S23. ^{13}C NMR spectrum of dimethyl 3,3'-Dithiopropionate in $CDCl_3$.

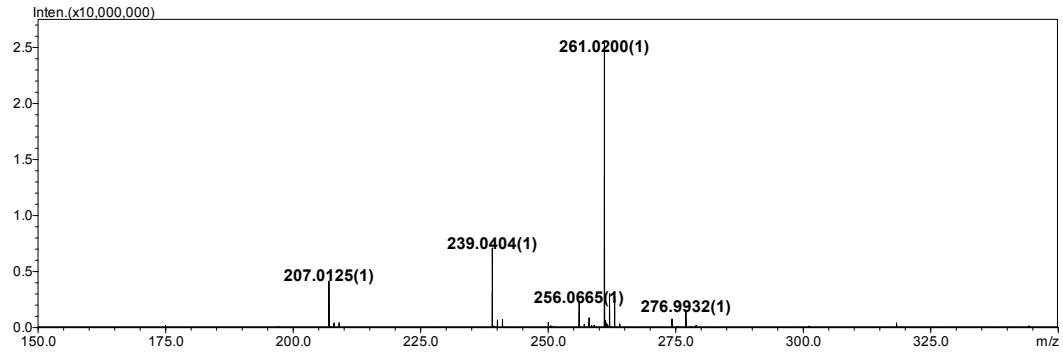


Figure S24. HR-MS of dimethyl 3,3'-Dithiopropionate.

References

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2. X. Q. Du, Y. Sun, M. Z. Zhang, J. L. He and P. H. Ni, ACS Appl. Mater. Interfaces, 2017, 9, 13939-13949.

3. B. Boens, T. S. Ouk, Y. Champavier and R. Zerrouki, *Nucleos. Nucleo. Nucl.,* 2015, 34, 500-514.