Supporting Information

for

Reduction-responsive amphiphilic polymeric prodrugs of camptothecin-polyphospoester for cancer chemotherapy

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Synthesis of N-cyclohexyl-N'-(3,5-bis(trifluoromethyl)phenyl)thiourea (TU)

TU was synthesized were synthesized and purified according to the previous report. Firstly, in a flame-dried 50 mL flask, 3,5-bis(trifluoromethyl)phenylisothiocyanate (2.00 g, 7.4 mmol) was dissolved in 10 mL dry THF under argon atmosphere. Secondly, Cyclohexylamine (0.73 g, 7.2 mmol) was added dropwise at room temperature to the stirring solution. After the reaction mixture was stirred for 5 h, the solvent was removed in vacuo. Thirdly, the colorless residue was recrystallized from boiling chloroform. It was filtered hot and cooled down. After filtration, colorless needles filtrate was concentrated. Finally, the product was washed with cold chloroform and dried in vacuo. (1.77 g, 4.8 mmol, yield: 67%)

^{1}H NMR (300 MHz, DMSO-d_6): δ 9.84 (s, 1H), 8.23 (s, 1H), 8.17 (s, 2H), 7.72 (s, 1H), 4.11 (s, 1H), 1.94-1.15 (m, 10H).

Synthesis of CPTO

Firstly, in a 200 mL three-neck flask, CPT (1.00 g, 2.87 mmol), DMAP (1.12 g, 9.19 mmol) and triphosgene (0.315 g, 1.06 mmol) were dissolved in 75 mL anhydrous DCM under argon atmosphere and the mixture was stirred at room temperature for 30 mins. Secondly, a solution of diethylene glycol (3.05 g, 28.7 mmol) in 10 mL anhydrous THF was added dropwise into the above flask and further stirred overnight at room temperature. Thirdly, the mixture was washed with 0.1 M HCl aqueous solution, NaCl aqueous solution and deionic water in turn. The organic layer was dried with anhydrous $MgSO_4$ for 12 h. After filtration, the filtrate was concentrated and further purified by silica gel column chromatography using DCM containing 10% MeOH as the eluent. Finally, some light yellow powder was obtained (CPTS: 1.05 g, yield: 72 %).

ESI-MS m/z (M^+) calcd 480.4, found 503.1 ($M + Na^+$).

^{1}H NMR (400 MHz, $CDCl_3$): δ 8.42 (s, 1H), 8.24 (dd, 1H), 7.95 (dd, 1H), 7.85 (m, 1H), 7.69 (m, 1H), 7.40 (s, 1H), 5.72 (d, 1H), 5.38 (d, 1H), 5.30 (t, 2H), 4.33 (m, 2H), 3.75 (m, 2H), 3.71 (m, 2H), 3.60 (m, 2H), 2.26 (m, 1H), 2.14 (m, 1H), 1.02 (t, 3H) (Figure 1a). ^{13}C NMR (400 MHz, $CDCl_3$): δ 167.3, 157.2, 153.6, 152.2, 148.6, 146.2, 146.0, 131.4, 130.8, 129.3, 128.5, 128.2, 128.1, 128.0, 120.0, 96.2, 77.9, 72.5, 68.4, 67.6, 66.9, 61.4, 49.9, 31.7, 7.6. IR (cm^{-1}): 2950, 1746, 1665, 1616, 1564, 1457, 1272, 1234, 1161, 1134, 1045 cm^{-1}.

Synthesis of CPTOP

In a 50 mL round bottom flask, CPTO (0.481 g, 1 mmol) and TEA (0.101 g, 1 mmol) were dissolved in 20 mL anhydrous THF and stirred at -20 °C. A solution of COP (0.141 g, 1 mmol) in 10 mL THF was added dropwise into the above flask at -20 °C and kept further stirring for 6 h.

When the mixture came back to room temperature, the white precipitate was filtered by a Schlenk funnel with a layer of dried silica gel (thickness: 5 mm). The filtrate was concentrated by rotary evaporation under the high vacuum to produce some yellow solid. The crude product was purified by the re-precipitation in anhydrous diethyl ether, some yellow powder was achieved (CPTOP: 0.511 g, yield: 87%).

^{1}H NMR (400 MHz, $CDCl_3$): δ 8.42 (s, 1H), 8.24 (dd, 1H), 7.95 (dd, 1H), 7.85 (m, 1H), 7.69 (m, 1H), 7.40 (s, 1H), 5.72 (d, 1H), 5.38 (d, 1H), 5.30 (t, 2H), 4.33 (m, 2H), 3.75 (m, 2H), 3.71 (m, 2H), 3.60 (m, 2H), 3.1(m, 4H), 2.26 (m, 1H), 2.14 (m, 1H), 1.02 (t, 3H);

^{31}P NMR (400 MHz, $CDCl_3$): 18.70.

Polymerization of EP

PEP was prepared by the ROP of EP in bulk at 0 °C with benzyl alcohol as the initiator and TBD as the catalyst. The typical polymerization proceeded as follows: In a glove box under N_2 atmosphere (<0.1 ppm H_2O), benzyl alcohol (61.4 mg, 1.10 mmol) and EP (1.434 g, 10 mmol) were introduced into a 25 ml pre-dry tube. TBD was freeze-dried with benzene once before use and a stock solution in an anhydrous DCM (10mg TBD in 1.1 mL DCM) prepared. Both tubes were removed out the glove box and cooled down to 0 °C. the polymerization was initiated by the addition of the TBD solution (0.5 mL, TBD 26 mg, 0.19 mmol) to the stirred monomer/initiator solution via syringe. The polymerization was quenched after 5 min by addition of acetic acid. The polymers were purified by precipitation from DCM into diethyl ether three times, them centrifuged (10 min, 4500 rpm, 0 °C), the supernatant decanted and dried in vacuo.

^{1}H NMR (400 MHz, $CDCl_3$): δ 7.39-7.26 (m, 5H, Ar), 6.12 (s, 2H, ArCH_2O), 4.18-4.13 (m, 196H, OCH_2CH_2O), 4.12-4.03 (m, 98H, OCH_2CH_3), 3.56 (t, 2H, $POCH_2CH_2OH$), 1.26 (t, 148H, OCH_2CH_3)

^{31}P NMR (400 MHz, $CDCl_3$): δ -0.20

Synthesis of polymeric prodrug PCPTOP-co-PEEP

The polymeric prodrugs were synthesized by ring opening copolymerization of CPTOP and EEP. Typically, in a glove box under N_2 atmosphere (< 0.1 ppm H_2O), benzyl alcohol (0.011 g, 1 mmol), TU (0.037 g, 0.1 mmol), CPTOP (0.293 g, 5 mmol) and EEP (? g, 45 mmol) were added into a flamed and argon-purged vial. A solution of DBU (0.037, 0.1 mmol) in 1ml dichloromethane was added to the above flask to initiate the copolymerization. The reaction was stirred at room temperature for 12 hours to allow for complete conversion. When the reaction was completed, the catalysts were quenched by addition of approximately 0.1ml of acetic acid and the crude copolymer was purified by dialysis (3kDa MWCO) against DMSO and water for approximately 64 hours. The solvent was lyophilized to afford copolymer as dark brown waxy oil.

^{1}H NMR (400 MHz, $CDCl_3$): δ 8.43 (s, 1H), 8.23 (dd, 1H), 7.96 (dd, 1H), 7.86 (m, 1H), 7.69 (m, 1H), 7.44 (s, 1H), 7.40-7.30 (br, 5H), 5.73 (d, 1H), 5.37 (d, 1H), 5.31 (t, 2H), 4.5-4.2 (br, OPOOCH_2CH_2OOPO), 4.2-4.0(br, OPOOCH_2CH_3),3.7(m, 4H), 2.17 (m, 1H), 1.47-1.23(br, $OPOOCH_2CH_3$), 1.03 (t, 3H)

^{31}P NMR (400 MHz, $CDCl_3$): δ 0.18, -0.20.

Table S1 The preparation and characterization data of PCPTOP-*co*-PEEP

Sample	Feed molar ratio $[M_1]$: $[M_2]$:$[I]^a$	DP $[M_1]$: $[M_2]$:I^b	$M_{n,th}{}^b$	$M_{n,GPC}{}^c$	$M_w/M_n{}^c$	*DC (%)*^{d}	Yield (%)
PCPTOP-co-PEEP	45::5:1	50:4:1	9900	9000	1.25	25.1	89.1

[a] M_1, M_2 and I represent EP, CPTOP and benzyl alcohol, respectively. [b] Calculated from ^{1}H NMR spectra. [c] Evaluated by GPC. [d] Determined by UV-Vis spectrum.

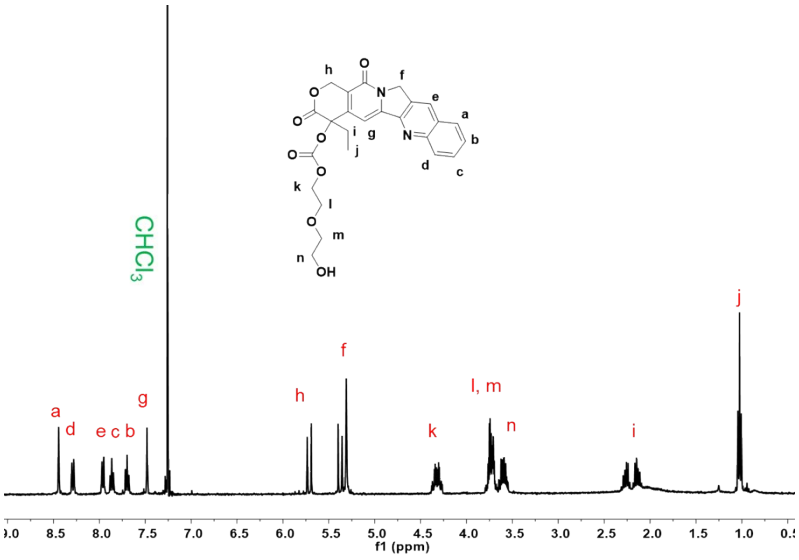

Figure S1. ^{1}H NMR spectrum of CPTO

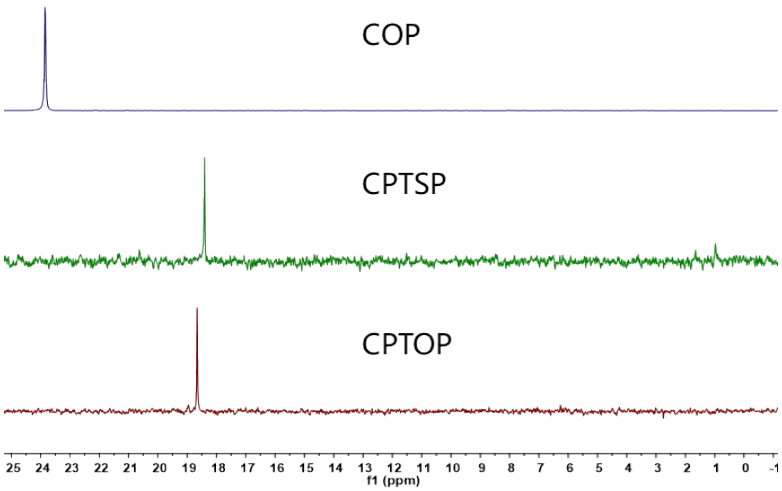

Figure S2. ^{31}P NMR spectra of COP, CPTSP and CPTOP

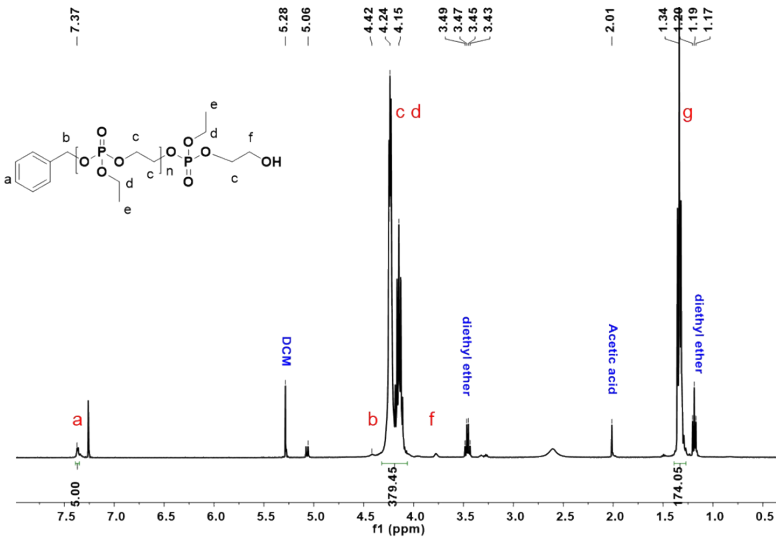

Figure S3. ^{1}H NMR spectrum of PEEP

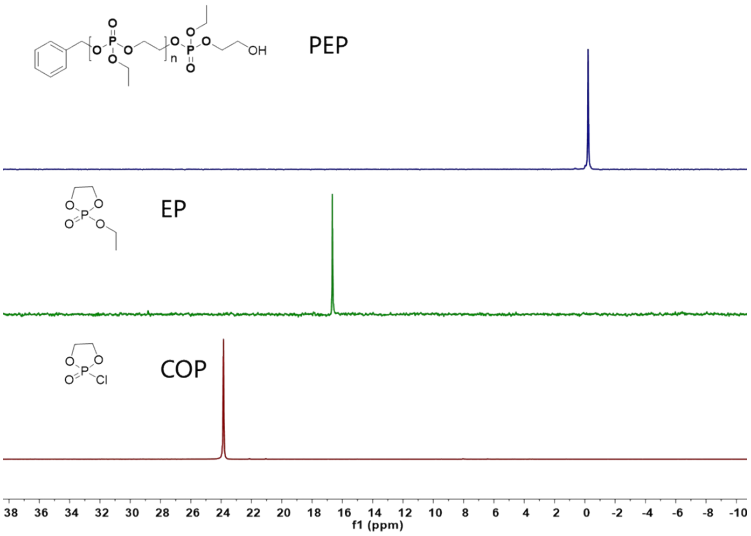

Figure S4. ^{31}P NMR spectra of COP, EP and PEEP

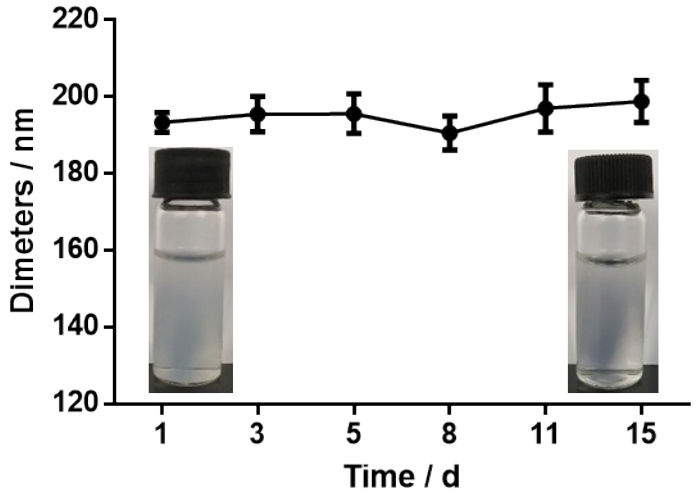

Figure S5. Influence of storage on diameter of PCPTSP-*co*-PEEP3 micelles. The solution of PCPTSP-*co*-PEEP3 micelles was stored at 4 °C in refrigerator for 28 days. At different time intervals (1, 3, 5, 8, 11 and 15 days), the average size were determined. Samples were measured in triplicates. The values are the mean ± SD. Inset: a digital photograph of PCPTSP-*co*-PEEP3 micelle solution, exhibiting a stable, transparent bluish solution.

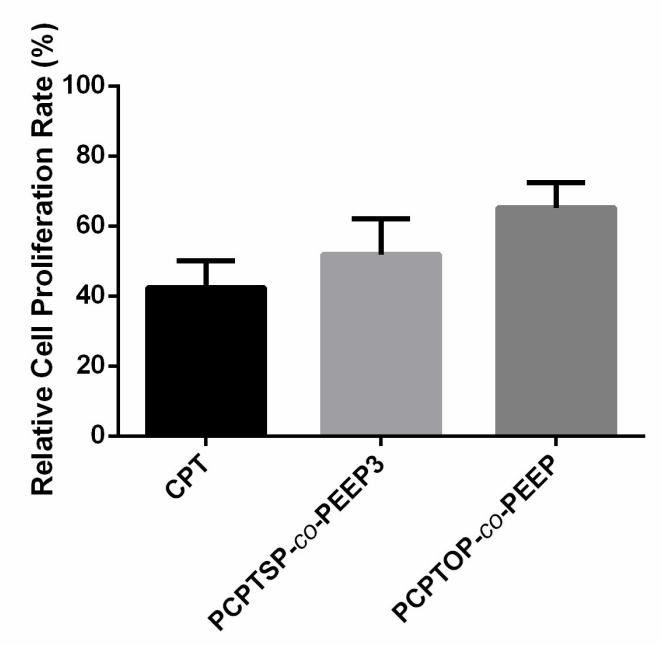

Figure S6. The relative cell proliferation rate (P%) of HT29 cells treated with CPT PCPTSP-*co*-PEEP3 and PCPTOP-*co*-PEEP (0.8 μM). Calculated from cell viability assay by the following formula: P% = optical density (OD) of drug treated group / OD of PBS treated group × 100%

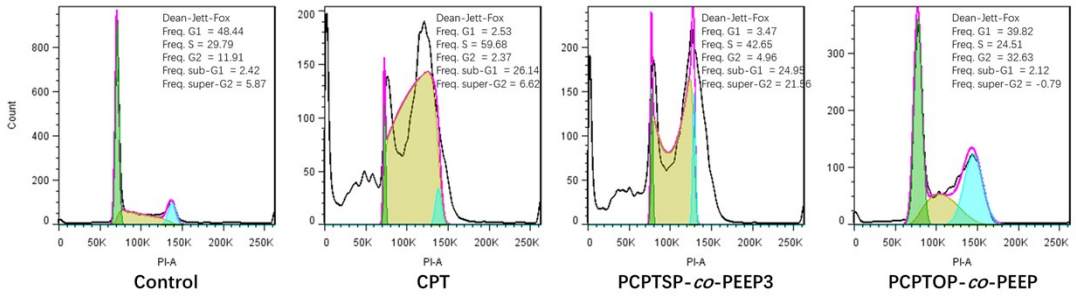

Figure S7. The cell cycle distribution histograms of HT29 cells treated with PBS, CPT, PCPTSP-*co*-PEEP3 and PCPTOP-*co*-PEEP after incubation for 48 h.

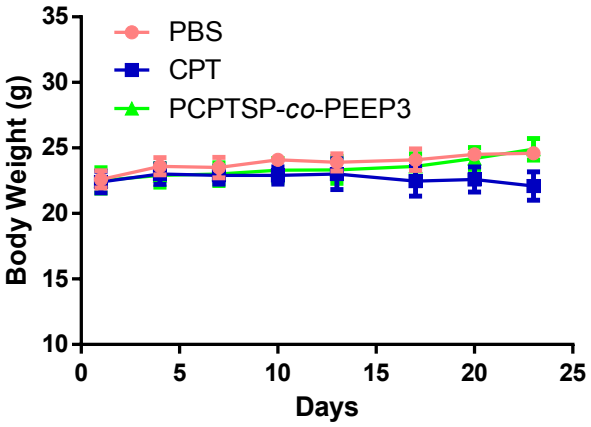

Figure S8. The body weights of HT29-bearing nude mice after treatment with PBS, CPT and PCPTSP-*co*-PEEP3 micelles for the different time.