Supporting Information

Fine-Tuning the Sequential Drug Release of Nano-Formulated Mutual Prodrugs Dictates the Combination Effects

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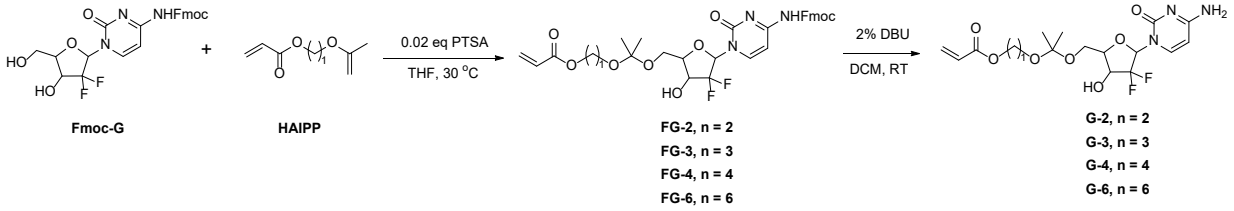
Materials

All chemicals were purchased from commercial sources and used as received unless otherwise mentioned. Paclitaxel (PTX) was obtained from Dalian Meilun Biotechnology Co., Ltd. (Dalian, CHN). Gemcitabine (Gem) was purchased from Nanjing Chemlin Chemical Industry Co., Ltd. Tetrahydrofuran (THF) was distilled from sodium/benzophenone under nitrogen until the solvent turned deep blue in color. Cell Counting Kit-8 (CCK-8) was ordered from Dojindo laboratories (Shanghai, CHN). BeyoClick™ EdU-488 kit, Tubulin-tracker Red, Lyso-tracker green, Ki67 Rabbit Monoclonal Antibody, Colorimetric TUNEL Apoptosis Assay Kit, Hoechst 33342, and DAPI were purchased from Beyotime Biotechnology (Shanghai, CHN). A2780 human ovarian cancer cells were obtained from BNCC (Beijing, CHN), and PANC02 murine pancreatic cancer cell lines and A549 non-small cell lung carcinoma cell lines were kindly gifted by Prof. Chenggang Li's laboratory. All cell lines were cultured in Dulbecco's Modified Eagle's Medium (DMEM, Hyclone) supplemented with 10% fetal bovine serum and 1% penicillin/streptomycin, and incubated in a CO_2 incubator at 37 °C in a humidified atmosphere containing 5% CO_2. Female BALB/c athymic nude mice (6–8 weeks old, weight ~20 g) were supplied by Beijing Vital River Laboratory Animal Technology Co., Ltd (Beijing, CHN). Animal experiments were carried out in accordance with the guidelines approved by the Institutional Animal Care and Use Committee of Nankai University.

Instrumentation

^{1}H NMR and ^{13}C NMR data were acquired from a Bruker AVANCE III 400 Hz spectrometer and analyzed by MestReNova 9.0 software. All chemicals containing isopropenyl ether or ketal moiety that dissolved in deuterated chloroform ($CDCl_3$) were added 1% Et_3N (v/v) to prevent acidolysis. High resolution mass spectrum (HR-MS) were recorded on a Varian 7.0T FTMS spectrometer by ESI source. Dynamic light scattering (DLS) and ζ potential measurements were carried out on Zetasizer nano ZS90 (Malvern Panalytical Ltd. Malvern, UK) with a He-Ne laser (633 nm). Scattered light was detected at 90° with 1 mL of sample in a DTS1070 cell. Data was analyzed by Zetasizer software. All measurements were repeated three times. Transmission electron microscopy (TEM) images were acquired from a FEI Talos F200C transmission electron microscope (Thermo Fisher Scientific. CA, USA) with accelerator voltage of 20-200 kV. Samples were prepared on carbon film supported copper grids and negative stained by 2% solution of sodium phosphotungstate. The liquid chromatographic analysis was carried out on an Agilent high performance liquid chromatography (HPLC) system (Agilent Technologies, CA, USA) with a Venusil MP C18 column (4.6 × 250 mm, 5 μm) maintained at 30 °C. The mobile phase was composed of water and acetonitrile and the flow rate was 1 mL/min. Gemcitabine was detected at a detection wavelength of 268 nm with a retention time of 1.6 min. Paclitaxel was detected at a detection wavelength of 227 nm with a retention time of 5.5 min. Confocal microscopy images were acquired from a Leica TCS SP8 laser scanning confocal microscope (Leica Microsystems, Berlin, GER).

Synthesis of prodrug precursors of Gem and PTX



Scheme S1. Synthesis of Gem prodrug precursors.

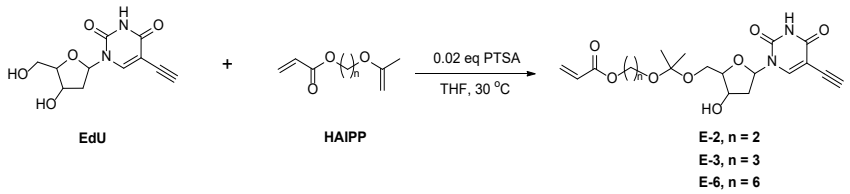
Fmoc-G and HAIPP were synthesized by previously reported methods.[1] To a 100 mL Schlenk flask, 4.12 mmol Fmoc-G and 24.71 mmol HAIPP were added and flushed with N_2. Then 0.04 mmol *p*-toluenesulfonic acid (PTSA) and 40 mL redistilled THF were added to the flask and the reaction was stirred at 30 °C for 20 min. Afterwards, 0.5 mL Et_3N was added to quench the reaction. The crude products were not isolated and were poured to 100 mL DCM, then treated with 2.02 g 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU). 5 min later, the organic phase of four precursors were washed by saturated $NaHCO_3$ and dried by sodium sulfate, removed by rotary evaporation, and the four precursors (G-2, G-3, G-4, G-6) were purified by silica gel column (eluents: DCM/MeOH = 20/1, 1% Et_3N (v/v) was added).

G-2 (n = 2, yield 72%): ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.71 (d, J = 7.5 Hz, 1H), 6.39 (dd, J = 17.3, 1.4 Hz, 1H), 6.29 (t, 1H), 6.12 (dd, J = 17.3, 10.4 Hz, 1H), 5.89 – 5.79 (m, 2H), 4.35 – 4.20 (m, 3H), 4.10 – 3.99 (m, 1H), 3.85 (d, J = 10.7 Hz, 1H), 3.77 – 3.59 (m, 3H), 1.38 (s, 6H); ^{13}C NMR δ 166.46, 166.14, 156.15, 140.46, 131.22, 128.24, 122.54, 119.97, 100.53, 95.82, 69.27, 63.93, 59.09, 58.54, 24.63.

G-3 (n = 3, yield 70%): ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.77 (d, J = 7.5 Hz, 1H), 6.43 – 6.30 (m, 2H), 6.09 (dd, J = 17.3, 10.4 Hz, 1H), 5.82 (dd, J = 10.4, 1.5 Hz, 1H), 4.26 (q, J = 6.4, 5.2 Hz, 3H), 4.06 – 3.98 (m, 1H), 3.85 – 3.79 (m, 1H), 3.68 (dd, J = 11.2, 3.4 Hz, 1H), 3.50 (tq, J = 7.0, 3.0 Hz, 2H), 1.92 (p, J = 6.3 Hz, 2H), 1.38 (s, 6H); ^{13}C NMR δ 166.62, 166.14, 156.19, 140.71, 130.98, 128.48, 100.47, 95.71, 79.62, 62.13, 58.60, 57.51, 29.16, 24.82, 24.77.

G-4 (n = 4, yield 80%): ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.77 (d, J = 7.5 Hz, 1H), 6.37 (dd, J = 17.3, 1.5 Hz, 1H), 6.27 (t, J = 7.1 Hz, 1H), 6.09 (dd, J = 17.3, 10.4 Hz, 1H), 5.91 (d, J = 7.5 Hz, 1H), 5.80 (dd, J = 10.4, 1.5 Hz, 1H), 4.32 (q, J = 11.5 Hz, 1H), 4.15 (t, J = 6.6 Hz, 2H), 4.09 – 4.00 (m, 1H), 3.82 (d, J = 11.1 Hz, 1H), 3.72 – 3.61 (m, 1H), 3.42 (t, J = 6.4 Hz, 2H), 1.79 – 1.67 (m, 2H), 1.65 – 1.53 (m, 2H), 1.36 (s, 6H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 166.59 , 166.07 , 156.15 , 140.76 , 130.84 , 128.60 , 100.36 , 95.57 , 79.67 , 64.60 , 60.46 , 58.57 , 29.81 , 26.41 , 25.79 , 24.90.

G-6 (n = 6, yield 78%): ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.76 (d, J = 7.5 Hz, 1H), 6.38 (dd, J = 17.4, 1.5 Hz, 1H), 6.31 (t, 1H), 6.10 (dd, J = 17.3, 10.4 Hz, 1H), 5.86 – 5.77 (m, 2H), 4.34 – 4.22 (m, 1H), 4.13 (t, J = 6.8 Hz, 2H), 4.04 (dd, J = 7.2, 4.0 Hz, 1H), 3.82 (d, J = 10.4 Hz, 1H), 3.66 (dd, J = 11.2, 3.5 Hz, 1H), 3.38 (t, J = 6.6 Hz, 2H), 1.71 – 1.60 (m, 2H), 1.53 (m, 2H), 1.37 (m, 10H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 166.58, 166.14, 156.22, 140.65, 130.70, 128.64, 125.26, 122.69, 120.11, 100.29, 95.76, 79.68, 64.74, 60.94, 58.55, 29.88, 28.60, 26.06, 25.88, 24.89, 24.83.



Scheme S2. Synthesis of EdU prodrug precursors.

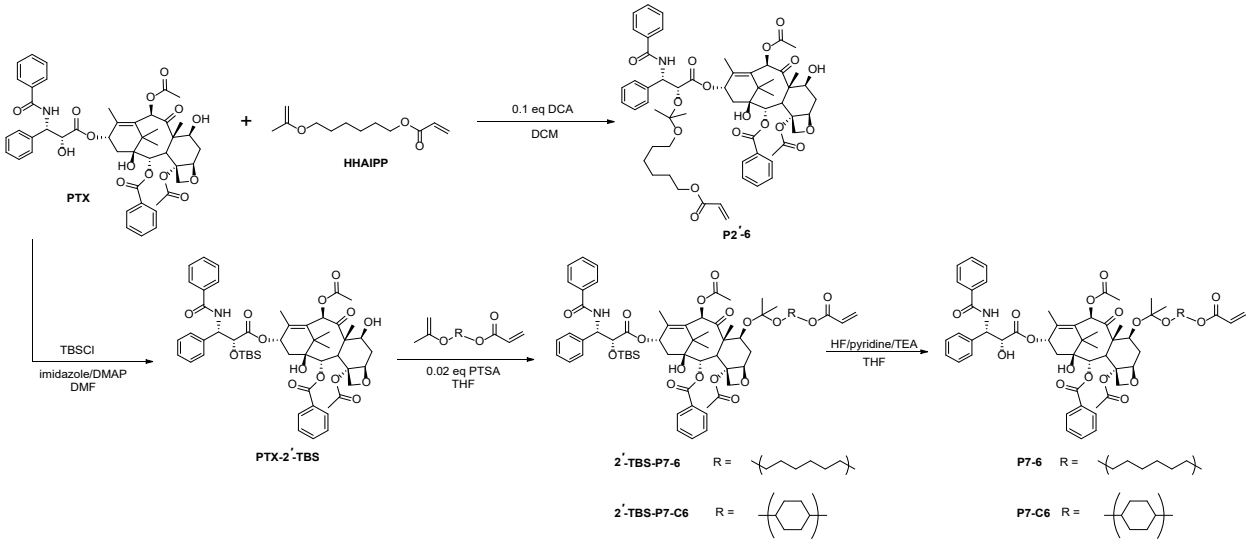
EdU precursors were synthesized using the same method as Gem precursors by replacing Fmoc-G with 5-ethynyl-2′-deoxyuridine (EdU), and E-2, E-3, E-6 were purified by silica gel column (eluents: DCM/MeOH = 20/1, 1% Et_3N (v/v) was added).

E-2 (*n* = 2, yield 74%): ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.25 (s, 1H), 6.89 – 6.80 (m, 1H), 6.44 (dd, *J* = 17.3, 1.4 Hz, 1H), 6.30 (t, *J* = 6.1 Hz, 1H), 6.15 (dd, *J* = 17.3, 10.4 Hz, 1H), 5.88 (dd, *J* = 10.4, 1.4 Hz, 1H), 4.51 (q, *J* = 4.8 Hz, 1H), 4.40 – 4.22 (m, 2H), 4.15 – 4.03 (m, 1H), 3.84 – 3.60 (m, 5H), 3.34 – 3.24 (m, 1H), 3.16 (s, 1H), 2.47 – 2.38 (m, 1H), 2.21 (dt, *J* = 13.9, 6.1 Hz, 1H), 1.46 (d, *J* = 3.9 Hz, 5H).

E-3 (*n* = 3, yield 70): ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.25 (s, 1H), 6.41 (dd, *J* = 17.4, 1.5 Hz, 1H), 6.33 (t, *J* = 6.3 Hz, 1H), 6.12 (dd, *J* = 17.3, 10.4 Hz, 1H), 5.84 (dd, *J* = 10.4, 1.5 Hz, 1H), 4.46 (m, 3.9 Hz, 1H), 4.27 (t, *J* = 6.3 Hz, 2H), 4.11 (q, *J* = 2.9 Hz, 1H), 3.64 (dd, *J* = 10.9, 2.5 Hz, 1H), 3.53 (dt, *J* = 6.7, 3.4 Hz, 2H), 3.16 (s, 1H), 2.42 (m, 6.2, 4.1 Hz, 1H), 2.18 (m, 6.2 Hz, 1H), 1.95 (m, 7.5 Hz, 3H), 1.43 (d, *J* = 8.5 Hz, 6H).

E-6 (*n* = 6, yield 65%): ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.28 (s, 1H), 6.43 – 6.31 (m, 2H), 6.16 – 6.07 (m, 1H), 5.86 – 5.79 (m, 1H), 4.47 (dt, *J* = 6.2, 3.2 Hz, 1H), 4.22 – 4.11 (m, 3H), 3.73 (dd, *J* = 10.9, 2.6 Hz,

1H), 3.64 (dd, J = 10.9, 2.4 Hz, 1H), 3.41 (td, J = 6.7, 2.0 Hz, 2H), 3.31 – 3.21 (m, 1H), 2.46 – 2.38 (m, 1H), 2.22 – 2.14 (m, 1H), 1.72 – 1.63 (m, 2H), 1.60 – 1.51 (m, 2H), 1.46 – 1.34 (m, 10H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 166.56, 163.69, 150.77, 143.98, 130.72, 128.64, 100.43, 98.81, 86.57, 85.86, 81.36, 71.82, 64.62, 61.16, 60.62, 41.92, 29.89, 29.77, 28.63, 26.06, 25.85, 25.04, 24.94.



Scheme S3. Synthesis of PTX prodrug precursors.

To synthesize P2′-6, PTX (1.17 mmol) and HHAIPP (3.51 mmol) were added into a 50 mL round bottom flask and dissolved with 8 mL dry DCM. After that, 0.117 mmol DCA in 1 mL DCM was added dropwise to the flask. The mixture was stirred at 30 °C for 1 h and was added 100 μL Et_3N. The solution was then concentrated and the crude product was refined using high performance preparative liquid chromatography (acetonitrile/water = 65/35, with 0.1 % Et_3N (v/v) added) to afford P2′-6 as a white solid.

To obtain P7-6 and P7-C6, PTX-2′-TBS was first synthesized as previously reported.[2] To a 100 mL Schlenk flask, 2.0 mmol PTX-2′-TBS, 12.0 mmol HHAIPP or HCHAIPP were added and flushed with N_2. Then 0.04 mmol *p*-toluenesulfonic acid (PTSA) and 20 mL redistilled THF were added to the flask and the reaction was stirred at 30 °C for 6 h. Afterwards, 0.2 mL Et_3N was added to quench the reaction. The solvents were evaporated and the product 2′-TBS-P7-6/C6 were obtained as a white powder by silica gel column chromatography (eluents: PE/EA = 2/1, 1% Et_3N (v/v) was added). Then, 2′-TBS-P7-6/C6 were dissolved in 5 mL dry THF and 20 eq HF/pyridine (added with 50% Et_3N (v/v)) were added to the solution, and stirred for 24 h. Afterwards, the solution was washed by saturated $NaHCO_3$ solution. The organic phase was dried by sodium sulfate, removed by rotary evaporation, and P7-6/C6 was purified by silica gel column chromatography (eluents: PE/EA = 1/1, 1% Et_3N (v/v) was added).

P2′-6: (yield 46%): ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.17 – 8.08 (d, 2H), 7.76 (dt, *J* = 7.0, 1.4 Hz, 2H), 7.65 – 7.57 (m, 1H), 7.56 – 7.45 (m, 3H), 7.44 – 7.33 (m, 6H), 7.30 – 7.26 (m, 1H), 7.19 (d, *J* = 8.1 Hz, 1H), 6.40 (dd, *J* = 17.3, 1.5 Hz, 1H), 6.29 (s, 1H), 6.20 (t, *J* = 8.9 Hz, 1H), 6.12 (dd, *J* = 17.3, 10.4 Hz, 1H), 5.82 (dd, *J* = 10.4, 1.5 Hz, 1H), 5.68 (d, *J* = 7.1 Hz, 1H), 5.63 – 5.56 (m, 1H), 4.97 (dd, *J* = 9.8, 2.3 Hz, 1H), 4.67 (d, *J* = 3.6 Hz, 1H), 4.42 (q, *J* = 7.2, 5.5 Hz, 1H), 4.32 (d, *J* = 8.4 Hz, 1H), 4.21 (d, *J* = 8.4 Hz, 1H), 4.13 (t, *J* = 6.7 Hz, 2H), 3.81 (d, *J* = 7.0 Hz, 1H), 3.18 – 3.10 (m, 1H), 2.31 – 2.25 (m, 1H), 2.22 (s, 3H), 2.09 (dd, *J* = 15.3, 8.8 Hz, 1H), 1.94 – 1.87 (m, 4H), 1.68 (s, 3H), 1.65 – 1.58 (m, 2H), 1.32 (s, 3H), 1.23 (s, 6H), 1.18 – 1.10 (s, 6H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 203.78, 171.58, 171.15, 169.96, 167.11,

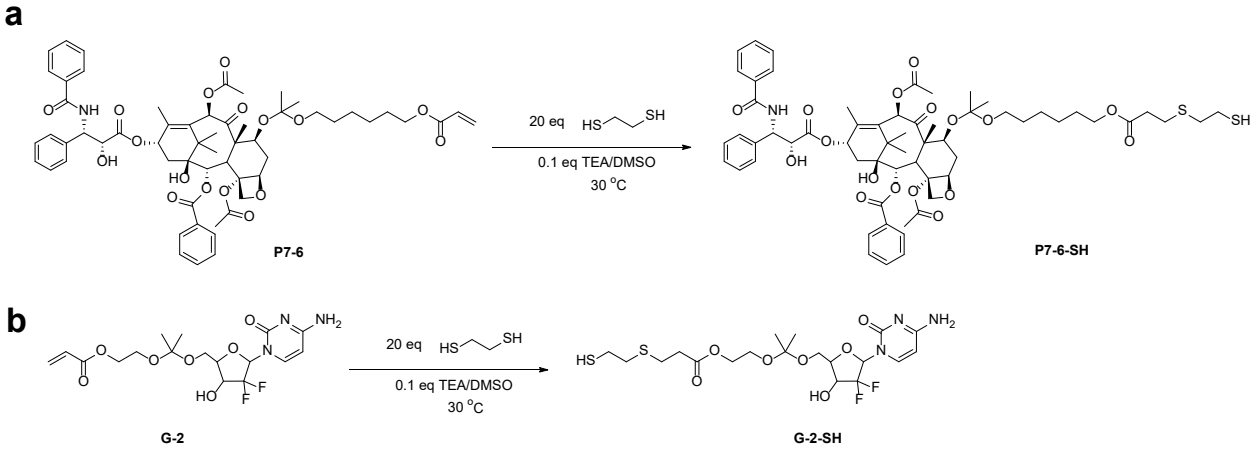
166.95, 166.30, 142.65, 138.56, 134.29, 133.70, 132.83, 131.71, 130.60, 130.18, 129.27, 128.75, 128.69, 128.62, 128.57, 128.08, 127.13, 126.98, 102.10, 84.48, 81.13, 79.10, 75.61, 75.09, 72.90, 72.09, 71.34, 64.45, 62.03, 58.51, 55.60, 53.06, 46.05, 45.61, 43.23, 35.77, 35.63, 29.84, 29.69, 28.54, 26.77, 25.78, 25.56, 24.72, 22.93, 22.12, 20.85, 15.11, 10.07, 10.01, 9.64, 8.15.

P7-6 (yield 44%): ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.15 – 8.09 (m, 2H), 7.79 – 7.74 (m, 2H), 7.65 – 7.58 (m, 1H), 7.54 – 7.46 (m, 5H), 7.41 (t, *J* = 7.5 Hz, 4H), 7.37 – 7.33 (m, 1H), 7.05 (d, *J* = 8.9 Hz, 1H), 6.44 – 6.33 (m, 2H), 6.22 – 6.14 (m, 1H), 6.10 (dd, *J* = 17.4, 10.4 Hz, 1H), 5.83 – 5.75 (m, 2H), 5.67 (d, *J* = 6.9 Hz, 1H), 4.91 (d, *J* = 8.9 Hz, 1H), 4.78 (d, *J* = 2.6 Hz, 1H), 4.39 (dd, *J* = 10.6, 6.5 Hz, 1H), 4.30 (d, *J* = 8.4 Hz, 1H), 4.18 (d, *J* = 8.4 Hz, 1H), 4.15 – 4.09 (m, 2H), 3.84 (d, *J* = 6.9 Hz, 1H), 3.47 – 3.39 (m, 1H), 3.34 (p, *J* = 7.5 Hz, 1H), 2.99 – 2.85 (m, 1H), 2.37 (s, 3H), 2.31 (q, *J* = 7.0, 6.0 Hz, 2H), 2.19 (s, 3H), 1.85 (d, *J* = 1.4 Hz, 4H), 1.75 (s, 3H), 1.64 (t, *J* = 7.2 Hz, 2H), 1.50 (s, 3H), 1.47 – 1.42 (m, 2H), 1.30 (s, 5H), 1.26 (d, *J* = 4.1 Hz, 3H), 1.22 (d, *J* = 3.0 Hz, 6H), 1.19 (s, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 201.81, 172.81, 170.46, 169.41, 167.02, 166.77 (d, *J* = 71.5 Hz), 139.51, 138.46, 134.16, 133.88, 133.80, 132.00, 130.51, 130.31, 129.36 , 129.02, 128.78 , 128.32, 127.19, 101.30, 84.53, 81.47, 78.83, 75.05, 74.81, 73.36, 72.45, 72.25, 64.71, 60.98, 57.74, 55.28, 47.34, 43.39, 35.63, 34.95, 29.91, 28.71, 26.78, 26.00, 25.16, 23.97, 22.85, 21.13, 21.05, 14.67, 11.07.

P7-C6 (yield 42%): ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.18 – 8.07 (m, 2H), 7.76 (dt, *J* = 8.6, 1.5 Hz, 2H), 7.65 – 7.57 (m, 1H), 7.56 – 7.45 (m, 5H), 7.45 – 7.37 (m, 4H), 7.37 – 7.30 (m, 1H), 7.05 (t, *J* = 8.5 Hz, 1H),

6.48 – 5.63 (m, 6H), 5.00 – 4.67 (m, 3H), 4.45 – 4.08 (m, 3H), 4.02 – 3.65 (m, 3H), 2.95 – 2.73 (m, 1H), 2.39 (d, J = 7.5 Hz, 3H), 2.35 – 2.26 (m, 2H), 2.18 (d, J = 4.8 Hz, 3H), 2.03 – 1.54 (m, 15H), 1.53 – 1.45 (m, 2H), 1.45 – 1.34 (m, 1H), 1.32 – 1.16 (m, 9H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 201.46, 172.65, 170.37, 169.22, 167.00, 165.61, 139.48, 138.38, 134.29, 133.96, 133.78, 133.57, 131.80, 130.19, 129.93, 129.49, 129.11, 128.90, 128.68, 128.64, 128.19, 127.13, 127.10, 101.88, 84.34, 84.19, 81.68, 81.53, 81.33, 78.80, 76.47, 73.33, 72.60, 72.26, 72.03, 67.90, 57.74, 56.90, 55.19, 47.84, 47.33, 46.39, 43.43, 43.35, 36.15, 35.71, 31.75, 31.30, 29.76, 29.18, 29.09, 26.74, 26.67, 26.58, 26.35, 24.97, 22.66, 21.11, 21.02, 20.80, 20.67, 14.41, 11.65, 11.01, 10.78.

Synthesis of mutual ketal prodrugs



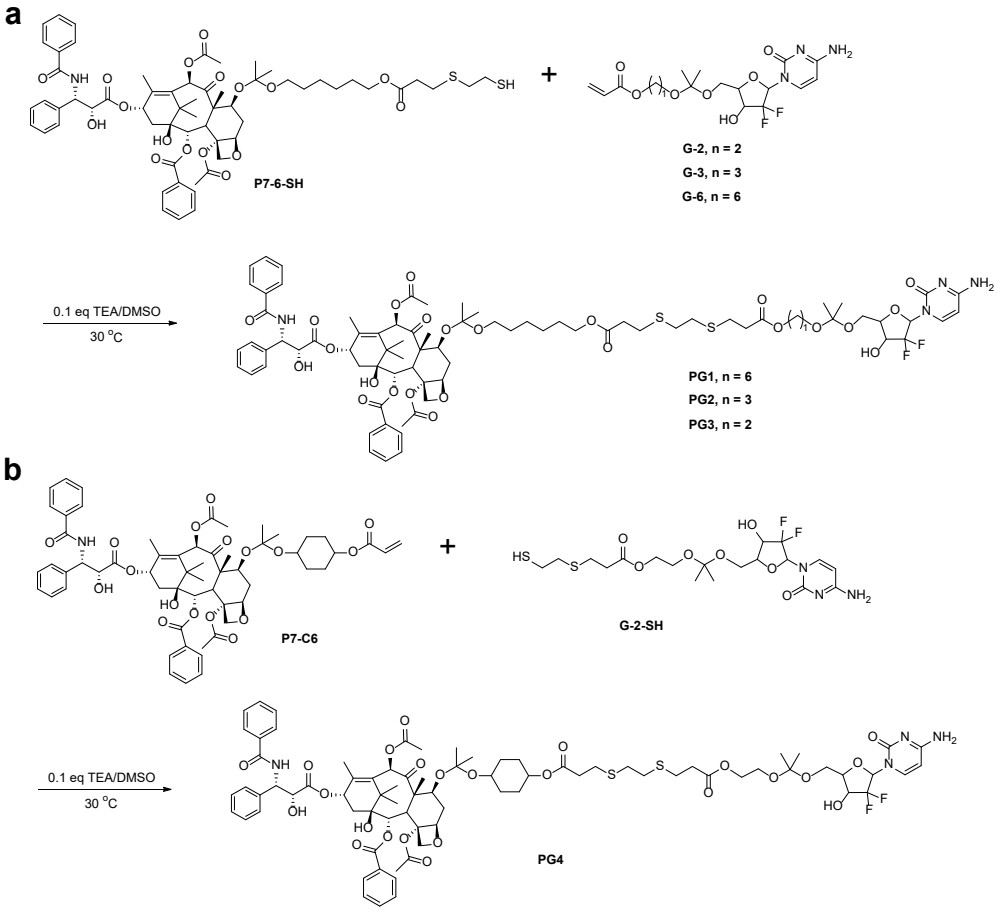
Scheme S4. Synthesis of P7-6-SH and G-2-SH.

To obtain PG prodrugs, P7-6-SH and G-2-SH were first synthesized by Michael addition of acrylate and large excess amounts of by 1,2-ethanedithiol. To a 10 mL Schlenk flask, 0.05 mmol P7-6 or G-2, 1.0

mmol 1,2-ethanedithiol dissolved in 1 mL dry DMSO were added and then flushed with N_2. Then 10 mmol% of anhydrous Et_3N were added to the flask and the reaction was stirred at 30 °C for 1 h. After the completion of reaction monitored by TLC, the solution was poured to 50 mL EtOAc and washed with saturated $NaHCO_3$ and NaCl solution. The organic phase was dried with Na_2SO_4. P7-6-SH or G-2-SH was isolated by silica gel column chromatography (1% Et_3N (v/v) was added).

P7-6-SH (yield 91%): ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.11 (d, 2H), 7.75 (d, 2H), 7.60 (t, 1H), 7.53 – 7.45 (m, 5H), 7.44 – 7.36 (m, 4H), 7.35 – 7.30 (m, 1H), 7.08 (d, *J* = 8.9 Hz, 1H), 6.25 – 6.12 (m, 1H), 5.77 (dd, *J* = 8.8, 2.6 Hz, 1H), 5.66 (d, *J* = 6.9 Hz, 1H), 4.90 (d, *J* = 9.8, 2.1 Hz, 1H), 4.77 (d, *J* = 2.7 Hz, 1H), 4.39 (dd, *J* = 10.6, 6.4 Hz, 1H), 4.29 (d, *J* = 8.4 Hz, 1H), 4.18 (d, *J* = 8.4 Hz, 1H), 4.06 (t, *J* = 6.7 Hz, 2H), 3.83 (d, *J* = 6.8 Hz, 1H), 3.42 (q, *J* = 9.2, 6.7 Hz, 1H), 3.32 (q, *J* = 9.3, 7.1 Hz, 1H), 2.90 (ddd, *J* = 15.7, 9.9, 6.4 Hz, 1H), 2.81 – 2.67 (m, 6H), 2.58 (t, *J* = 7.3 Hz, 2H), 2.41 – 2.26 (m, 5H), 2.18 (s, 3H), 1.88 – 1.80 (m, 4H), 1.74 (s, 3H), 1.60 (t, *J* = 7.2 Hz, 2H), 1.50 (s, 3H), 1.46 – 1.39 (m, 2H), 1.36 – 1.27 (m, 4H), 1.24 – 1.19 (m, 6H), 1.18 (s, 3H).

G-2-SH (yield 88%): ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.73 (d, *J* = 7.5 Hz, 1H), 6.36 – 6.27 (m, 1H), 5.83 (d, *J* = 7.6 Hz, 1H), 4.37 – 4.16 (m, 3H), 4.11 – 4.02 (m, 1H), 3.86 (d, *J* = 11.0 Hz, 1H), 3.71 (dd, *J* = 11.2, 3.5 Hz, 1H), 3.63 (q, *J* = 5.1 Hz, 2H), 2.83 – 2.68 (m, 6H), 2.67 – 2.60 (m, 5H), 1.40 (s, 6H).



Scheme S5. Synthesis of mutual prodrugs PG1, PG2, PG3, and PG4.

To a 25 mL Schlenk flask was added 0.05 mmol P7-6-SH and 0.075 mmol G-2, G3 or G6 and purged with N_2 (for PG4, the reactants were 0.075 mmol P7-C6 and 0.05 mmol G-2-SH). Then 1 mL dry DMSO containing 0.005 mmol Et_3N were added to the flask and the solution were stirred at 30 °C for 6 h. The solution was poured to 50 mL EtOAc and washed with saturated $NaHCO_3$ and NaCl solution. The organic phase was dried with Na_2SO_4. The product was purified by flash column chromatography.

PG1 (yield 82%): ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.07 (d, 2H), 7.83 (d, 3H), 7.73 (d, J = 7.5 Hz, 1H), 7.58 (t, J = 7.3 Hz, 1H), 7.49 (t, J = 7.0 Hz, 4H), 7.43 (m, 1H), 7.35 (t, J = 7.5 Hz, 4H), 6.37 (s, 1H), 6.34 –

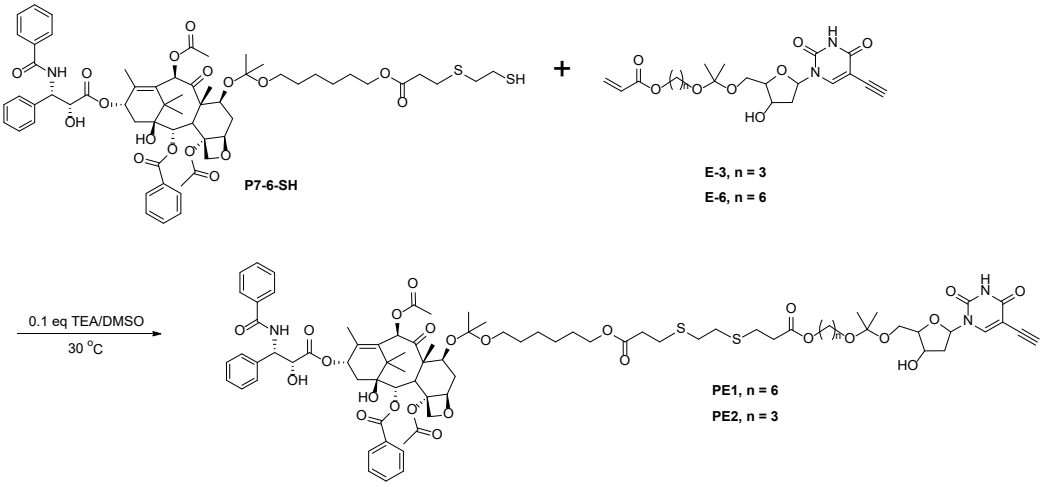
6.26 (m, 1H), 6.13 (t, *J* = 9.0 Hz, 1H), 5.84 – 5.71 (m, 2H), 5.64 (d, *J* = 6.9 Hz, 1H), 4.93 – 4.84 (m, 1H), 4.79 (d, *J* = 3.7 Hz, 1H), 4.38 (dd, *J* = 10.5, 6.4 Hz, 1H), 4.26 (d, *J* = 8.7 Hz, 2H), 4.16 (d, *J* = 8.5 Hz, 1H), 4.05 (q, *J* = 7.0 Hz, 5H), 3.80 (d, *J* = 7.8 Hz, 2H), 3.65 (dd, *J* = 11.3, 3.3 Hz, 1H), 3.44 – 3.23 (m, 4H), 2.86 (dd, *J* = 14.3, 6.2 Hz, 1H), 2.78 (td, *J* = 7.3, 1.6 Hz, 4H), 2.71 (s, 5H), 2.57 (dt, *J* = 7.3, 3.6 Hz, 5H), 2.36 (s, 3H), 2.30 – 2.22 (m, 1H), 2.16 (s, 4H), 2.06 (d, *J* = 2.9 Hz, 1H), 1.94 (d, *J* = 2.5 Hz, 0H), 1.84 (s, 3H), 1.72 (s, 3H), 1.65 – 1.55 (m, 4H), 1.52 (s, 1H), 1.45 – 1.39 (m, 2H), 1.36 (s, 8H), 1.30 – 1.22 (m, 6H), 1.22 – 1.15 (m, 9H); ^{13}C NMR (101 MHz, $CDCl_3$) δ: 201.84, 173.01, 171.88, 169.32, 167.24, 139.82, 138.69, 133.62, 131.61, 130.18, 128.71, 128.46, 127.94, 127.34, 101.18, 100.17, 94.90, 84.40, 81.16, 79.79, 78.63, 75.05, 74.76, 73.67, 72.31, 71.67, 64.84, 60.85, 58.49, 57.57, 55.91, 47.21, 43.29, 35.46, 34.98, 34.84, 32.17, 29.79, 28.54, 27.15, 26.64, 25.98, 25.87, 25.78, 25.07, 24.87, 23.89, 22.72, 21.12, 20.93, 14.50, 10.96.

PG2 (yield 86%): ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.08 (d, *J* = 7.3 Hz, 2H), 7.80 (d, 2H), 7.74 (d, *J* = 7.5 Hz, 1H), 7.64 (d, *J* = 8.7 Hz, 1H), 7.61 – 7.56 (m, 1H), 7.55 – 7.43 (m, 5H), 7.38 (t, *J* = 7.6 Hz, 4H), 6.40 (s, 1H), 6.37 – 6.29 (m, 1H), 6.15 (t, 1H), 5.77 (dd, *J* = 8.8, 3.4 Hz, 1H), 5.70 (d, *J* = 7.5 Hz, 1H), 5.66 (d, *J* = 6.9 Hz, 1H), 4.91 (d, 1H), 4.82 (d, *J* = 3.5 Hz, 1H), 4.39 (dd, *J* = 10.5, 6.5 Hz, 1H), 4.34 – 4.13 (m, 5H), 4.12 – 3.98 (m, 3H), 3.85 – 3.76 (m, 2H), 3.70 – 3.62 (m, 1H), 3.55 – 3.38 (m, 3H), 3.37 – 3.26 (m, 1H), 2.96 – 2.83 (m, 1H), 2.79 (t, *J* = 7.2 Hz, 4H), 2.72 (s, 4H), 2.62 – 2.56 (m, 4H), 2.36 (s, 3H), 2.29 – 2.23 (m, 2H), 2.19 (s, 3H), 2.14 (s, 1H), 1.88 (d, *J* = 6.3 Hz, 6H), 1.74 (s, 3H), 1.65 – 1.53 (m, 2H), 1.48 (s, 3H), 1.46 – 1.41 (m, 2H), 1.39 (s, 6H), 1.36 – 1.26 (m, 4H), 1.24 – 1.16 (m, 9H); ^{13}C NMR (101 MHz,

$CDCl_3$) δ: 173.15, 171.99, 170.46, 167.42, 165.58, 140.82, 139.74, 133.88, 133.83, 133.60, 131.69, 130.17, 128.76, 128.64, 128.51, 128.01, 127.29, 101.18, 100.33, 95.22, 84.40, 81.17, 78.48, 75.07, 74.72, 73.60, 72.34, 71.77, 64.89, 62.17, 60.87, 58.48, 57.58, 57.41, 55.91, 47.18, 43.32, 35.40, 34.96, 34.87, 32.15, 29.77, 29.10, 28.53, 27.10, 26.65, 25.86, 25.09, 24.82, 24.78, 23.89, 22.73, 21.15, 20.97, 14.52, 10.97.

PG3 (yield 80%): ^{1}H NMR (400 MHz, $CDCl_3$) 8.12 – 8.06 (m, 2H), 7.84 – 7.78 (m, 2H), 7.72 (d, *J* = 7.5 Hz, 1H), 7.60 (dd, *J* = 8.2, 6.2 Hz, 2H), 7.54 – 7.44 (m, 5H), 7.43 – 7.34 (m, 4H), 7.30 (d, *J* = 7.4 Hz, 1H), 6.38 (d, *J* = 15.2 Hz, 2H), 6.14 (t, *J* = 8.8 Hz, 1H), 5.78 (dd, *J* = 8.7, 3.5 Hz, 1H), 5.68 (dd, *J* = 18.4, 7.2 Hz, 2H), 4.90 (d, *J* = 9.3 Hz, 1H), 4.83 (d, *J* = 3.6 Hz, 1H), 4.39 (dd, *J* = 10.6, 6.4 Hz, 1H), 4.23 (ddd, *J* = 24.5, 19.2, 8.5 Hz, 5H), 4.06 (q, *J* = 6.2, 5.8 Hz, 3H), 3.83 (dd, *J* = 15.9, 9.2 Hz, 2H), 3.73 (dd, *J* = 11.1, 3.5 Hz, 1H), 3.68 – 3.58 (m, 2H), 3.43 (q, *J* = 6.9 Hz, 1H), 3.37 – 3.26 (m, 1H), 2.94 – 2.84 (m, 1H), 2.80 (td, *J* = 7.2, 3.5 Hz, 4H), 2.73 (s, 4H), 2.62 (dd, *J* = 17.1, 7.1 Hz, 3H), 2.36 (s, 3H), 2.24 (t, *J* = 8.1 Hz, 1H), 2.19 (s, 3H), 2.00 – 1.91 (m, 2H), 1.85 (s, 3H), 1.74 (s, 3H), 1.59 (q, *J* = 6.8 Hz, 2H), 1.41 (s, 8H), 1.38 – 1.16 (m, 18H); ^{13}C NMR (101 MHz, $CDCl_3$) δ: 201.96, 173.15, 171.96, 166.94, 165.58, 155.77, 140.79, 138.66, 133.62, 131.70, 130.18, 128.77, 128.65, 128.52, 128.02, 127.30, 101.18, 100.52, 95.26, 84.41, 81.17, 78.50, 75.07, 74.72, 73.58, 72.34, 71.75, 64.90, 64.00, 60.87, 59.10, 58.61, 57.58, 55.93, 47.19, 43.32, 35.39, 34.96, 34.84, 32.18, 32.14, 29.78, 28.53, 27.12, 26.98, 26.65, 25.87, 25.09, 24.77, 23.89, 22.73, 21.14, 20.97, 14.52, 10.97.

PG4 (yield 55%): ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.08 (d, *J* = 7.7 Hz, 2H), 7.97 – 7.76 (m, 3H), 7.70 (dd, *J* = 7.5, 4.8 Hz, 1H), 7.59 (q, *J* = 7.6 Hz, 1H), 7.49 (t, *J* = 8.8 Hz, 5H), 7.42 – 7.34 (m, 4H), 7.33 – 7.28 (m, 1H), 6.42 (d, *J* = 5.5 Hz, 1H), 6.35 (t, *J* = 7.7 Hz, 1H), 6.23 – 6.05 (m, 1H), 5.84 – 5.61 (m, 3H), 4.97 – 4.65 (m, 3H), 4.41 (dd, *J* = 10.6, 6.6 Hz, 1H), 4.33 – 4.13 (m, 5H), 4.09 – 3.98 (m, 1H), 3.91 – 3.55 (m, 6H), 2.82 – 2.75 (m, 5H), 2.72 (d, *J* = 4.4 Hz, 4H), 2.66 – 2.57 (m, 5H), 2.40 – 2.28 (m, 4H), 2.24 – 2.12 (m, 5H), 2.00 – 1.33 (m, 25H), 1.29 – 1.16 (m, 10H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 173.19, 171.94, 170.44, 169.43, 166.90, 165.59, 155.77, 140.80, 138.61, 133.88, 133.60, 131.70, 130.17, 129.36, 128.76, 128.64, 128.51, 128.01, 127.32, 101.78, 100.52, 95.26, 84.31, 81.18, 79.64, 78.48, 77.29, 73.58, 72.48, 71.86, 70.53, 67.83, 64.00, 59.10, 58.60, 57.56, 56.16, 55.75, 47.18, 43.32, 36.11, 35.31, 34.83, 32.18, 32.07, 31.47, 29.62, 29.26, 29.16, 27.89, 27.77, 27.23, 26.98, 26.64, 26.43, 25.03, 24.94, 24.77, 22.74, 21.20, 20.99, 14.49, 11.04.



Scheme S6. Synthesis of mutual prodrugs PE1 and PE2.

PE prodrugs were synthesized using the same method as PG1 by replacing G-2 and G-6 with E-3 and E-6, and the PE prodrugs were purified by silica gel column (eluents: DCM/MeOH = 20/1, 1% Et_3N (v/v) was added).

PE1(yield 62%): ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.16 (s, 1H), 8.04 (d, *J* = 7.7 Hz, 2H), 7.71 (d, *J* = 7.7 Hz, 2H), 7.53 (t, *J* = 7.4 Hz, 1H), 7.42 (q, *J* = 7.1, 6.5 Hz, 5H), 7.32 (t, *J* = 7.5 Hz, 4H), 7.27 – 7.21 (m, 2H), 6.33 (s, 1H), 6.24 (t, *J* = 6.5 Hz, 1H), 6.10 (t, *J* = 9.1 Hz, 1H), 5.74 – 5.66 (m, 1H), 5.59 (d, *J* = 6.9 Hz, 1H), 4.65 (s, 6H), 4.34 (m, 2H), 4.22 (d, *J* = 8.4 Hz, 1H), 4.12 (d, *J* = 8.6 Hz, 1H), 4.06 – 3.95 (m, 5H), 3.76 (d, *J* = 6.8 Hz, 1H), 3.67 – 3.60 (m, 1H), 3.54 (d, *J* = 10.7 Hz, 1H), 3.34 (t, *J* = 6.6 Hz, 3H), 3.26 (q, *J* = 7.6 Hz, 1H), 3.08 (s, 1H), 2.83 (m, 1H), 2.77 – 2.69 (m, 4H), 2.66 (d, *J* = 1.2 Hz, 4H), 2.30 (s, 5H), 2.12 (s, 3H), 1.90 – 1.86 (m, 1H), 1.67 (s, 3H), 1.53 (m, 5H), 1.42 (s, 2H), 1.34 (d, *J* = 9.9 Hz, 9H), 1.17 – 1.08 (m, 10H).

PE2 (yield 68%): ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.26 (s, 1H), 8.16 – 8.07 (m, 2H), 7.85 – 7.76 (m, 2H), 7.62 (t, *J* = 7.4 Hz, 1H), 7.51 (m, 4H), 7.41 (m, 3H), 7.35 – 7.30 (m, 2H), 6.40 (s, 1H), 6.31 (t, *J* = 6.3 Hz, 1H), 6.16 (t, *J* = 8.9 Hz, 1H), 5.78 (d, *J* = 7.8 Hz, 1H), 5.66 (d, *J* = 6.9 Hz, 1H), 4.92 (d, *J* = 9.6 Hz, 1H), 4.80 (d, *J* = 3.0 Hz, 1H), 4.52 – 4.44 (m, 1H), 4.42 – 4.36 (m, 1H), 4.31 (d, *J* = 8.4 Hz, 1H), 4.25 – 4.18 (m, 2H), 4.13 (d, *J* = 2.8 Hz, 1H), 4.07 (t, *J* = 6.6 Hz, 2H), 3.86 – 3.79 (m, 1H), 3.73 (dd, *J* = 10.8, 2.6 Hz, 1pH), 3.67 – 3.60 (m, 1H), 3.51 (m, 2H), 3.42 (m, 1H), 3.33 (q, *J* = 8.1, 7.7 Hz, 2H), 2.86 – 2.77 (m, 4H),

2.73 (s, 3H), 2.38 (s, 3H), 2.28 (m, 1H), 2.20 (s, 3H), 1.90 (t, J = 6.2 Hz, 1H), 1.85 (s, 3H), 1.74 (s, 3H), 1.60 (m, 1H), 1.50 (s, 3H), 1.43 (d, J = 6.9 Hz, 6H), 1.35 - 1.16 (m, 15H).

Preparation of NPs

NPs were prepared by nanoprecipitation process. DSPE-PEG_{2k} was used to co-assemble with PGs to form stable NPs. Generally, 10 mg of PGs and 1mg of DSPE-PEG_{2k} were dissolved in 1 mL acetone with 0.1% Et_3N (v/v), then the solution was added dropwise to 10 mL PB buffer (pH 8.0, 10 mM) under 500 rpm stirring. After 2 h evaporation of acetone, the nanoformulations were filtered through a 0.45 μm PES membrane. The obtained PG NPs were characterized by DLS and TEM. Stability of PG NPs were monitored by measuring their size by DLS every day for 5 days.

Hydrolysis kinetics of Gem and PTX precursors

In 5 mL tubes, 50 μM Gem precursors (G-2, G-3, G-4, and G-6) or PTX precursors (P2′-6, P7-6, and P7-C6) in 3 mL PB buffer (pH 5.0 and 7.4, 30 mM, 40% was added to increase the solubility) and incubated in a shaker at 37 °C with a shaking rate of 100 rpm (n = 4). At predetermined time points, aliquots (200 μL) of buffer were taken, terminated by 200 μL PB at pH 8.0 (200 mM), and then analyzed by HPLC. The concentrations of Gem and PTX and precursors were determined by standard curves. The half-lives ($t_{1/2}$) of Gem or PTX were calculated by $t_{1/2} = 0.693/k$, and k is determined by pseudo-first-order kinetics as the following formula:

$$\ln(C_0 - C_t) = -kt + \ln(C_0)$$

where k represents the hydrolysis rate constant, C_0 is the initial concentration of prodrug precursors, C_t is the concentration of Gem or PTX at predetermined time points, t means reaction time.

Drug release experiments of PG NPs

20 μL of PG NPs (1 mg/mL) were added to 1.5 mL tubes, then 180 μL PB buffer (pH 5.0 and 7.4, 30 mM) were added to each tube all the tubes were incubated in a shaker at 37 °C with a shaking rate of 100 rpm (n = 4). At predetermined time points, 200 μL PB at pH 8.0 (200 mM) were added to the selected tubes, and 400 μL ACN were added to increase the solubility of PGs. The concentration of prodrugs and released Gem and PTX were analyzed by HPLC. The half-lives of PG and Gem or PTX were determined by pseudo first order kinetics with the same methods as described in the previous section.

Cell cytotoxicity assay

A2780, A549, and PANC02 cells were seeded in 96-well plates at a density of 5×10^3 cells per well and incubated for 24 h. Then culture media were replaced by serial dilutions of PG NPs, Gem, PTX or equal molar ratio mixture of Gem and PTX in 100 μL DMEM media (equivalent concentration ranged from 0.1 nM to 1 mM). Cells were incubated for 72 h, then culture media were replaced by 100 μL fresh DMEM media, followed by adding 10 μL CCK-8 to each well. After incubation for 1.5 h, the absorbance at 450

nm was measured by a TECAN spark Multimode Reader Platform. The half-maximal inhibitory of drug concentration (IC_{50}) was determined by GraphPad Prism 8.0 with the fitting mode of inhibitor vs. response -- Variable slope (four parameters). The combination indexes were determined according to the Chou–Talalay method[3], the CI value were calculated by the data of cell viabilities from cytotoxicity experiments by the following formula and simulated in the software Calcusyn 2.0:

$$CI = \frac{(D)_1}{(D_x)_1} + \frac{(D)_2}{(D_x)_2}$$

where $(D_x)_1$ is the dose of Drug1 alone that inhibits x% of cell viability, and likewise the $(D_x)_2$ is for Drug2. $(D)_1$ is the portion of Drug1 in combination $(D)_1 + (D)_2$ also inhibits x%, and $(D)_2$ for Drug2 likewise.

Intracellular drug release of NPs

A2780 cells (5×10^4 cells/well) were seeded in 24-well slices and placed in 24-well plates in 500 μL DMEM media and incubated for 24 h. Then culture media was replaced by 500 μL DMEM media containing EdU (10 μM) or PE NPs (10 μM EdU contained). After 1 h incubation with EdU, 6 h incubation with PE NPs culture media were discarded, and cells were washed by PBS for 3 times, then fixed with 4% paraformaldehyde for 15 min. Then cells were stained by Azide-488 following the protocol of BeyoClick™ EdU-488 kit. Afterwards, cells were washed by PBS for 3 times. After the tubulin staining, nucleus was stained by Hoechst 33342 for 40 seconds. Fluorescence was observed by confocal laser scanning microscope.

In vivo anticancer efficacy

Small pieces of A549 tumor tissues (2 mm^3) were subcutaneously implanted into the right flank of female BALB/c nude mice. When tumors reached 50-100 mm^3, mice were randomized to seven groups (n = 5) and intravenously administered with PBS, Gem (6.4 mg/kg) and PTX Mic, PTX Mic + Gem, PG NPs (20 mg/kg paclitaxel equiv. dose) respectively. The day on which mice received treatment was set as day 0. Tumor volume and body weight were monitored every other day. Tumor volume was calculated by the following formula: $V = (a \times b^2)/2$, where V is tumor volume, a is the length of tumor, and b is the width. When the tumor size reached 2,000 mm^3, mice were sacrificed. All mice were sacrificed at day 21, blood was collected for biochemical analysis, while tumors and major organs (heart, liver, spleen, lung, kidney) were harvested, fixed with 4% paraformaldehyde for histological analyses.

The fixed tumors and major organs were dehydrated and embedded in paraffin blocks to prepare tissue sections. The sections were stained with hematoxylin and eosin (H&E), Ki67, and terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL) using standard protocols. Histology images were collected using microscope (Olympus) and analyzed using ImageJ 1.4. Briefly, Ki67 or TUNEL positive cells were determined by the positively stained color analyzed by the plugin *IHC_Profiler* in ImageJ 1.4 from at least five scopes of tumor slices.

Supplementary data

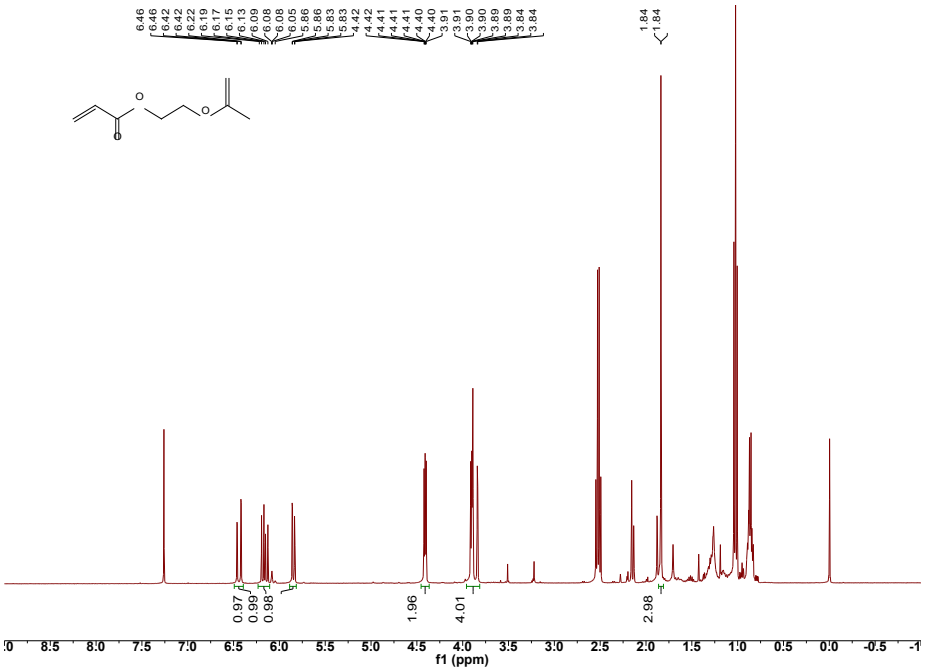


Figure S1. ^{1}H NMR spectrum of **HEAIPP** ($CDCl_3$).

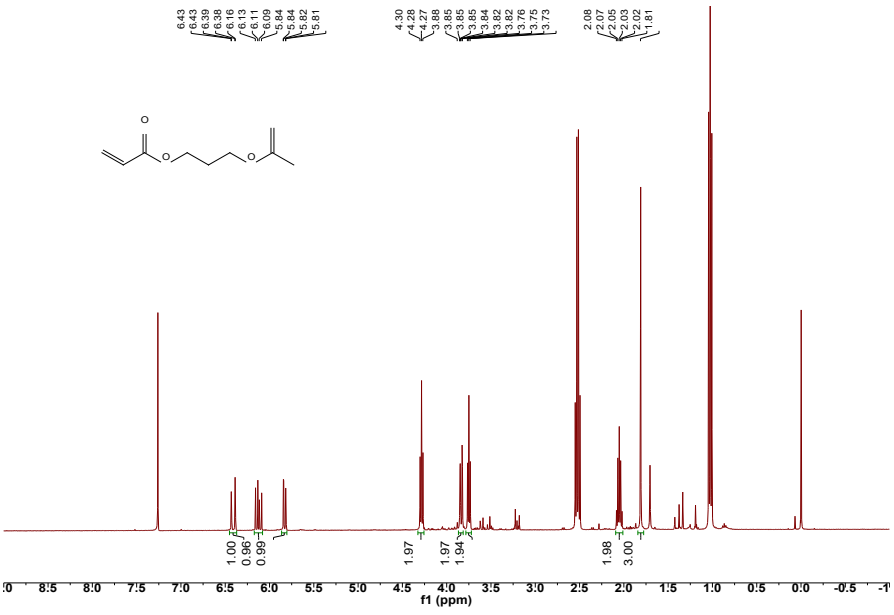


Figure S2. ^{1}H NMR spectrum of **HPAIPP** ($CDCl_3$).

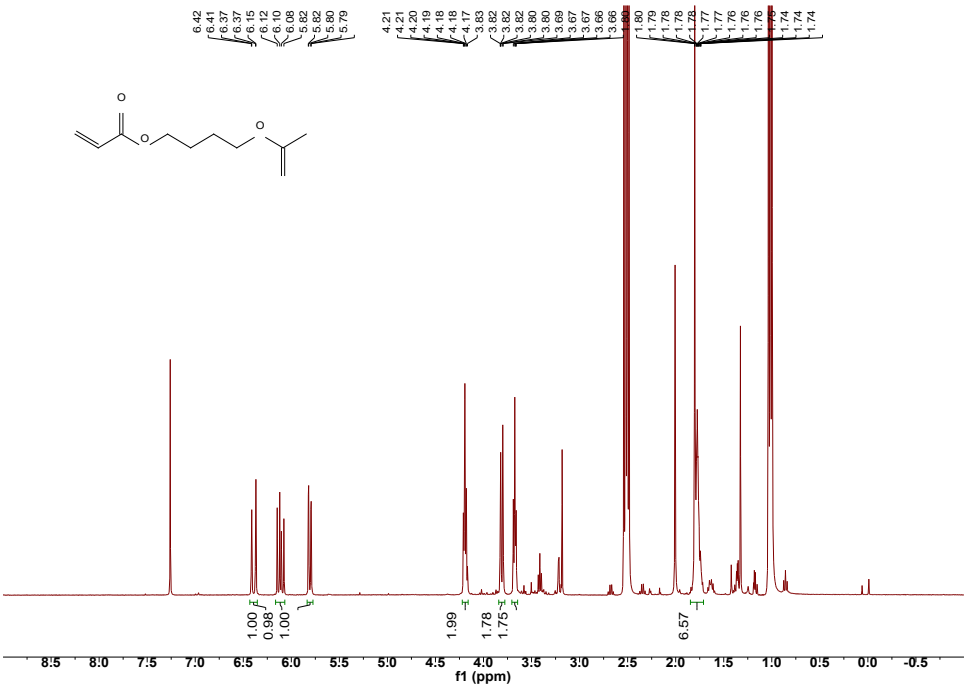


Figure S3. ^{1}H NMR spectrum of **HBAIPP** ($CDCl_3$).

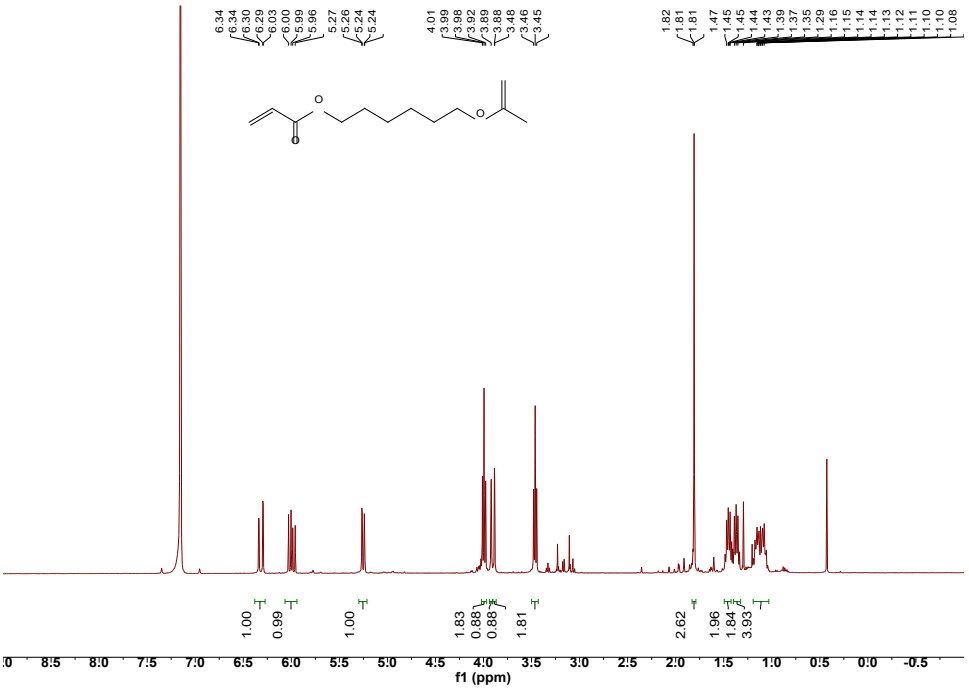


Figure S4. ^{1}H NMR spectrum of **HHAIPP** (C_6D_6).

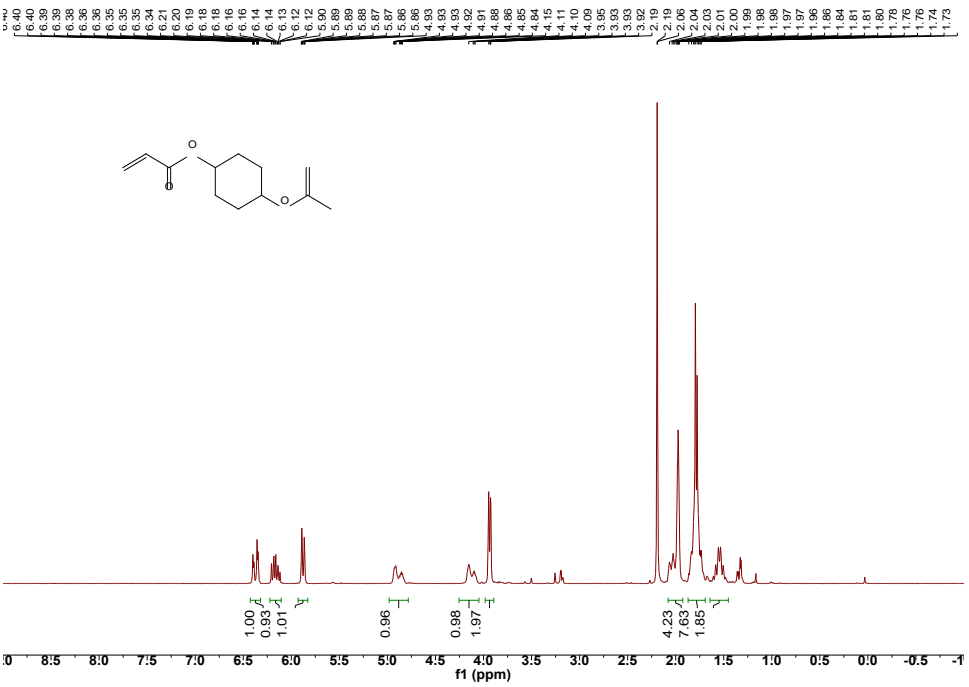


Figure S5. ^{1}H NMR spectrum of **HCHAIPP** (Acetone-d_6).

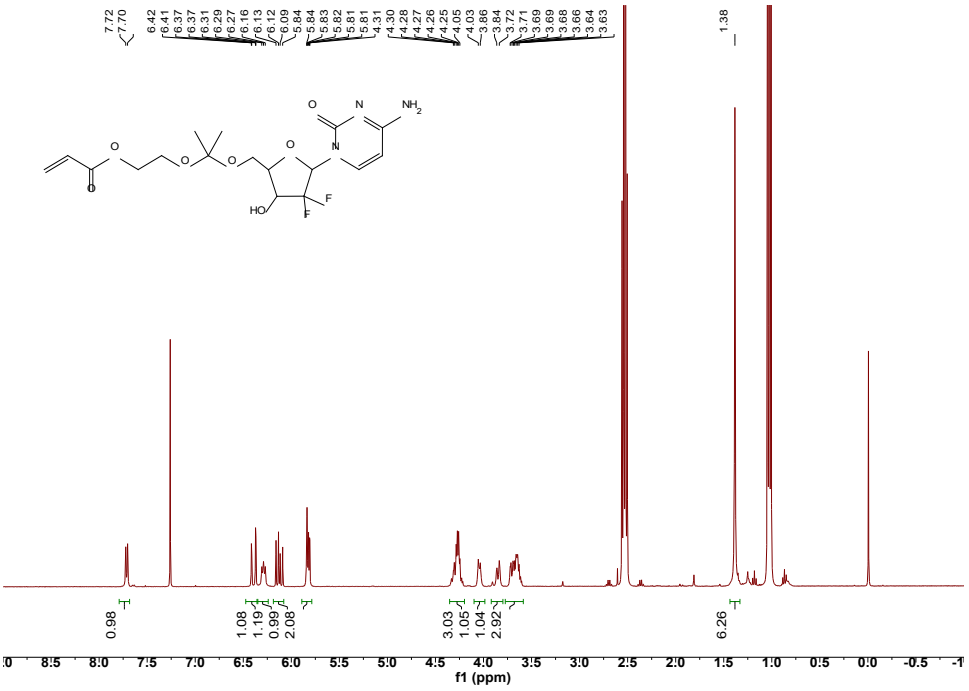


Figure S6. ^{1}H NMR spectrum of **G-2** ($CDCl_3$).

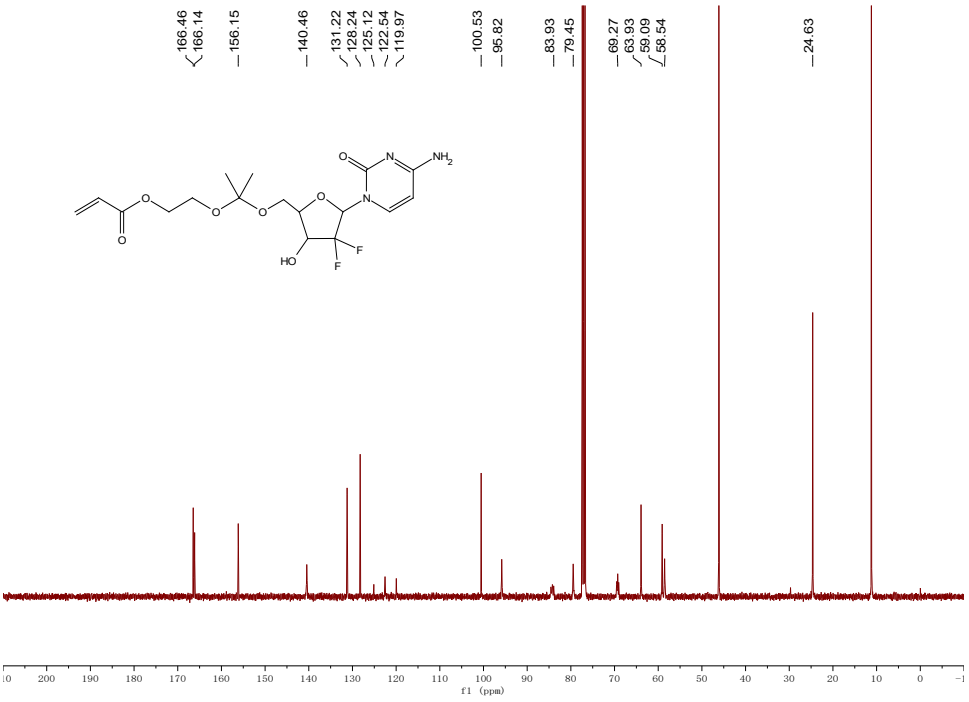


Figure S7. ^{13}C NMR spectrum of **G-2** ($CDCl_3$).

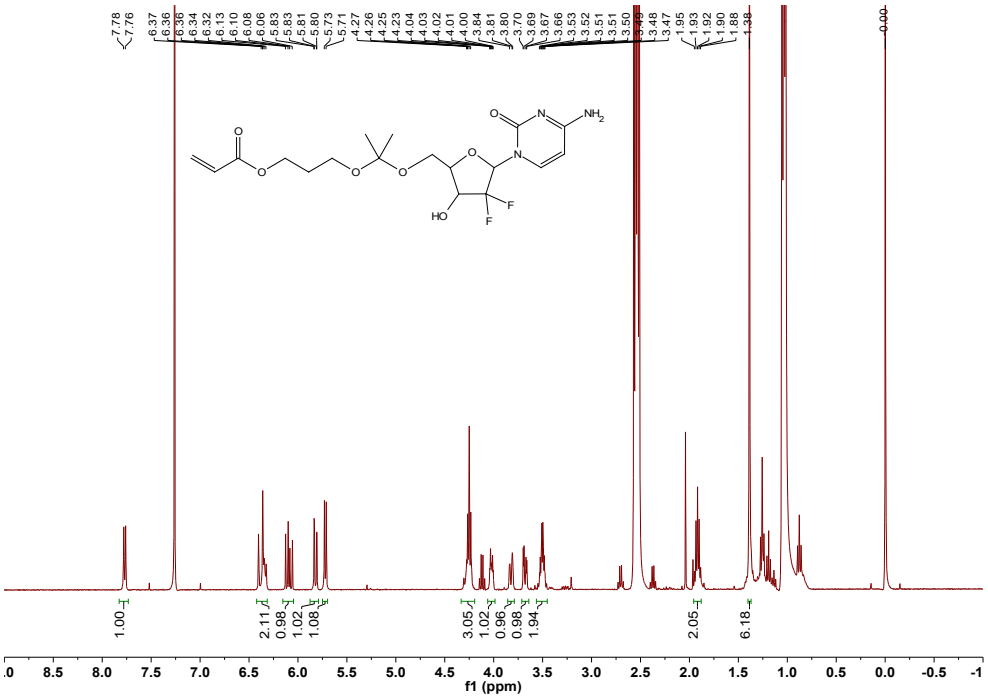


Figure S8. ^{1}H NMR spectrum of **G-3** ($CDCl_3$).

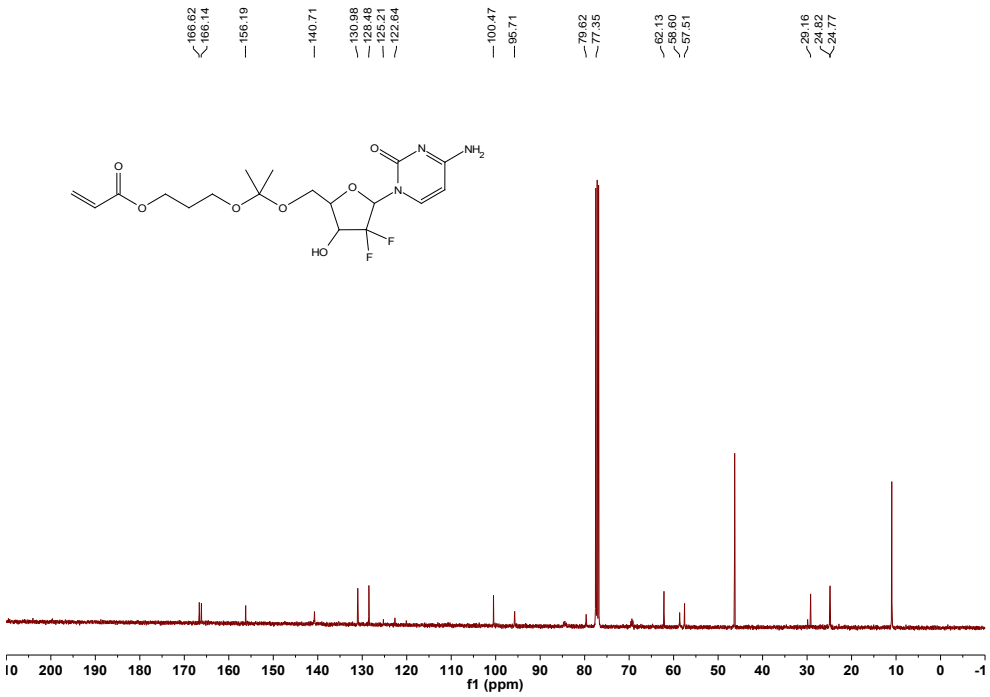


Figure S9. ^{13}C NMR spectrum of **G-3** ($CDCl_3$).

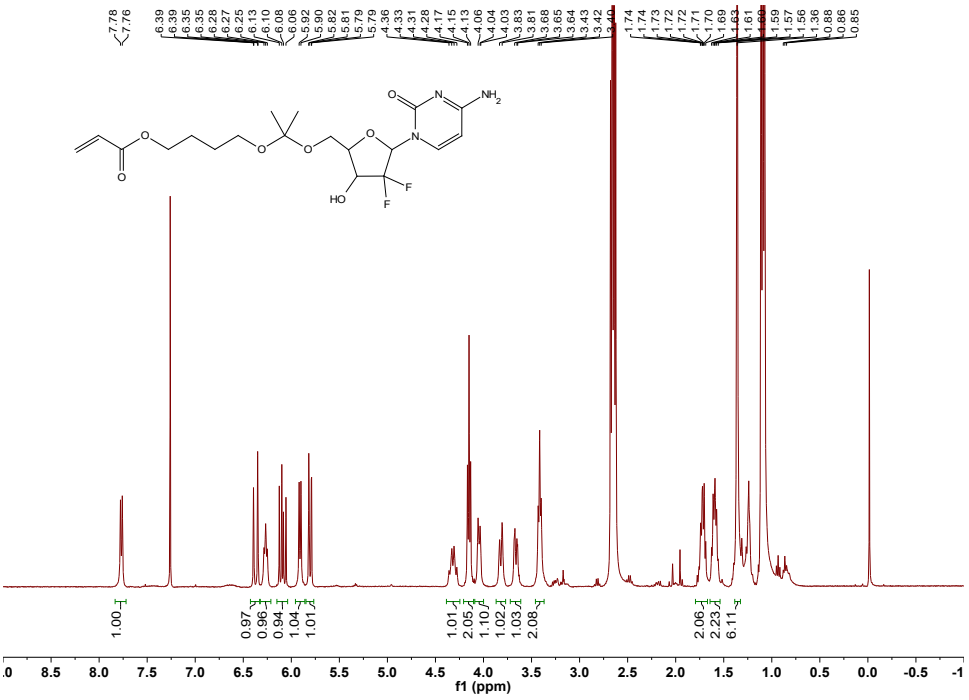


Figure S10. ^{1}H NMR spectrum of **G-4** ($CDCl_3$).

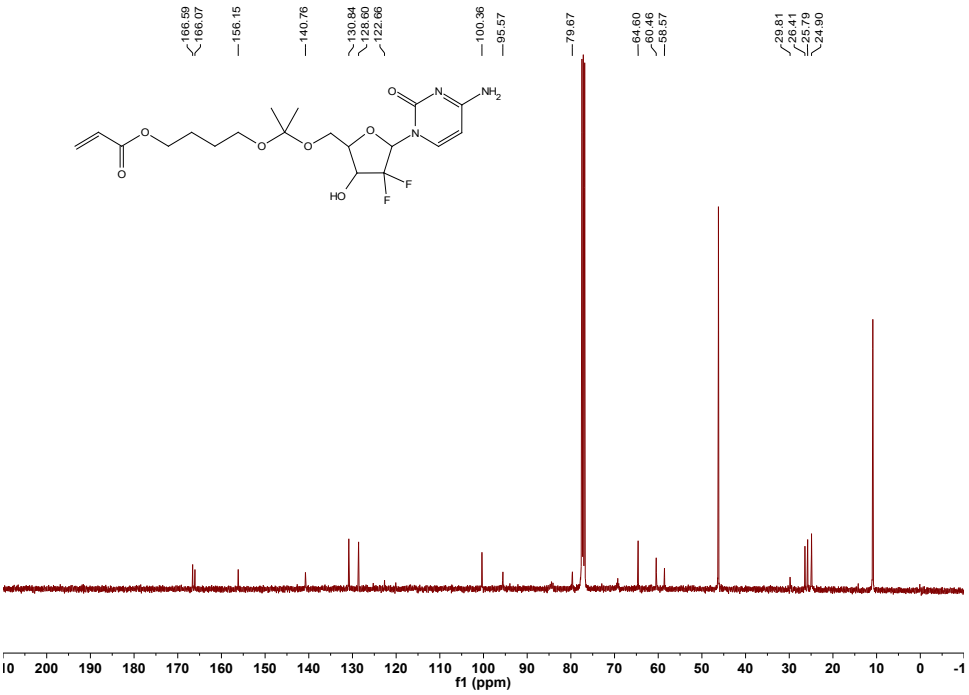


Figure S11. ^{13}C NMR spectrum of **G-4** ($CDCl_3$).

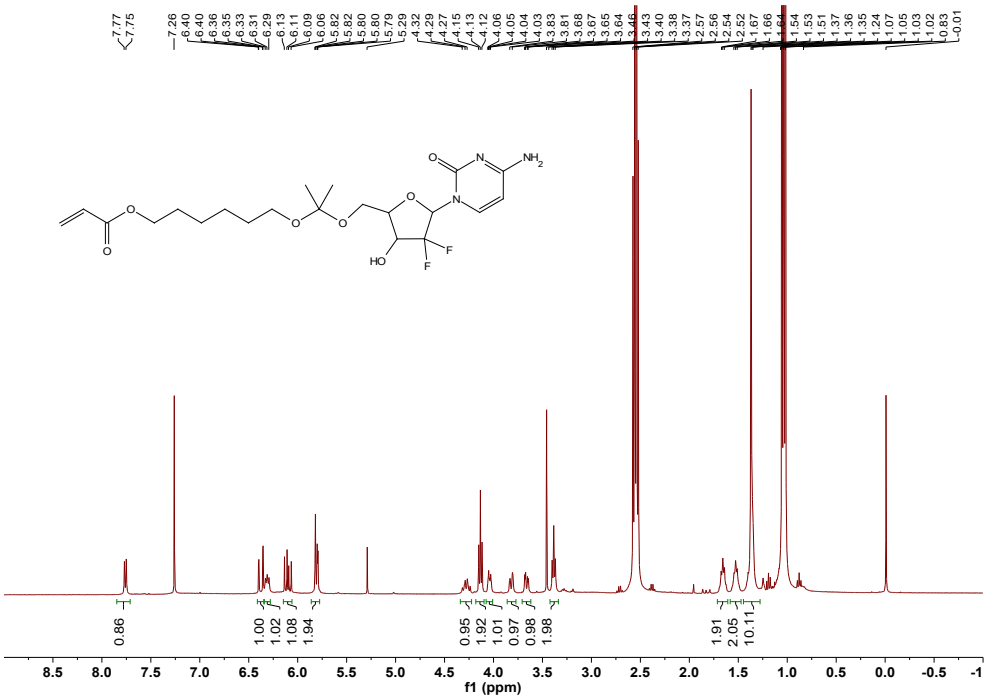


Figure S12. ^{1}H NMR spectrum of **G-6** ($CDCl_3$).

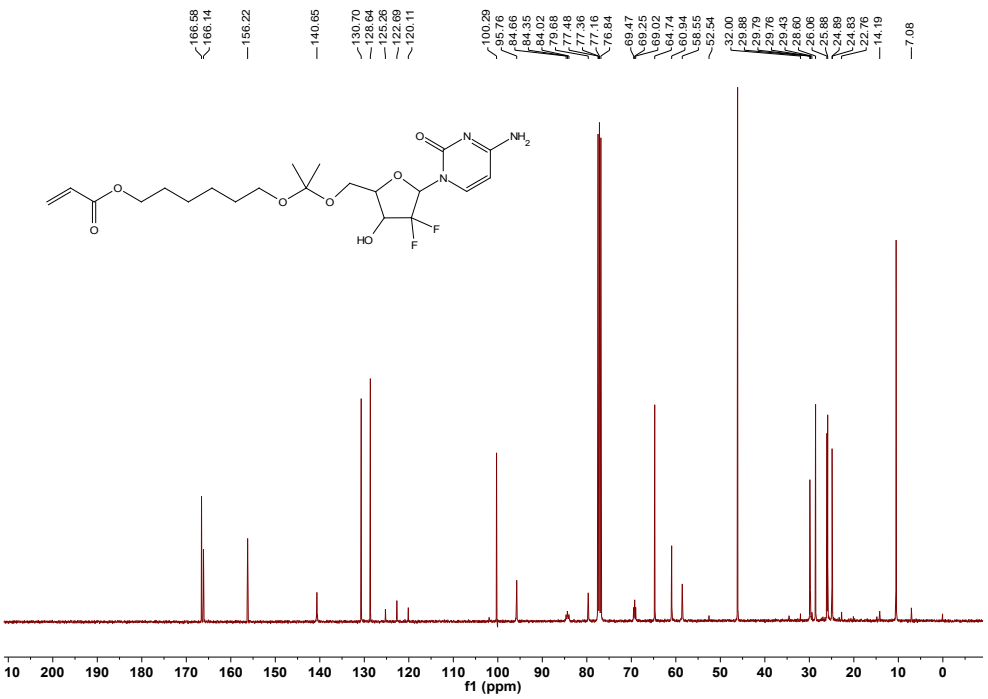


Figure S13. ^{13}C NMR spectrum of **G-6** ($CDCl_3$).

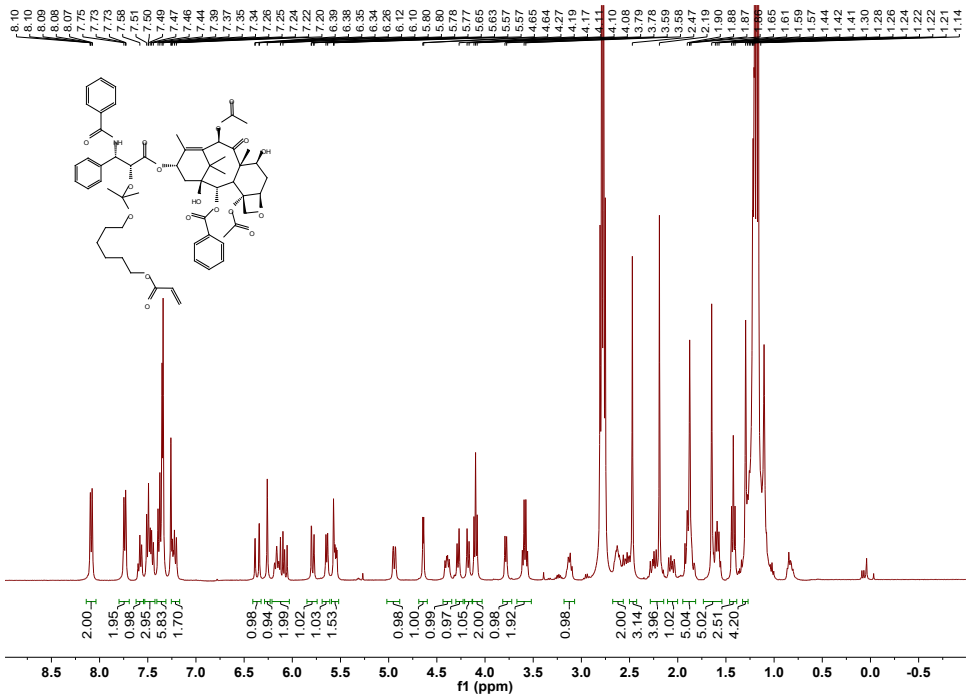


Figure S14. ^{1}H NMR spectrum of **P2′-6** ($CDCl_3$).

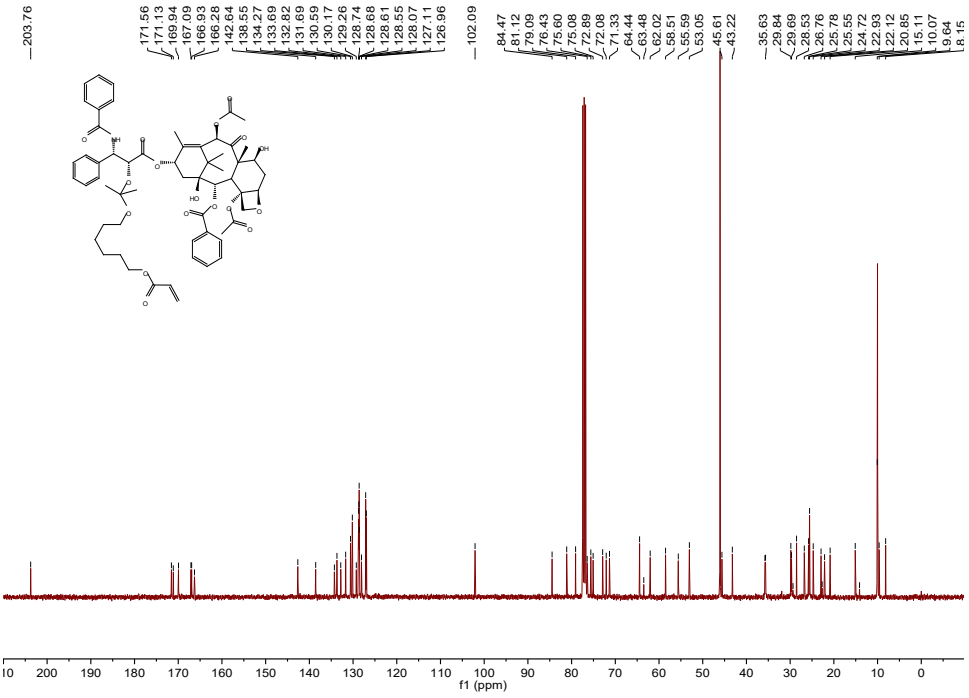


Figure S15. ^{13}C NMR spectrum of **P2′-6** ($CDCl_3$).

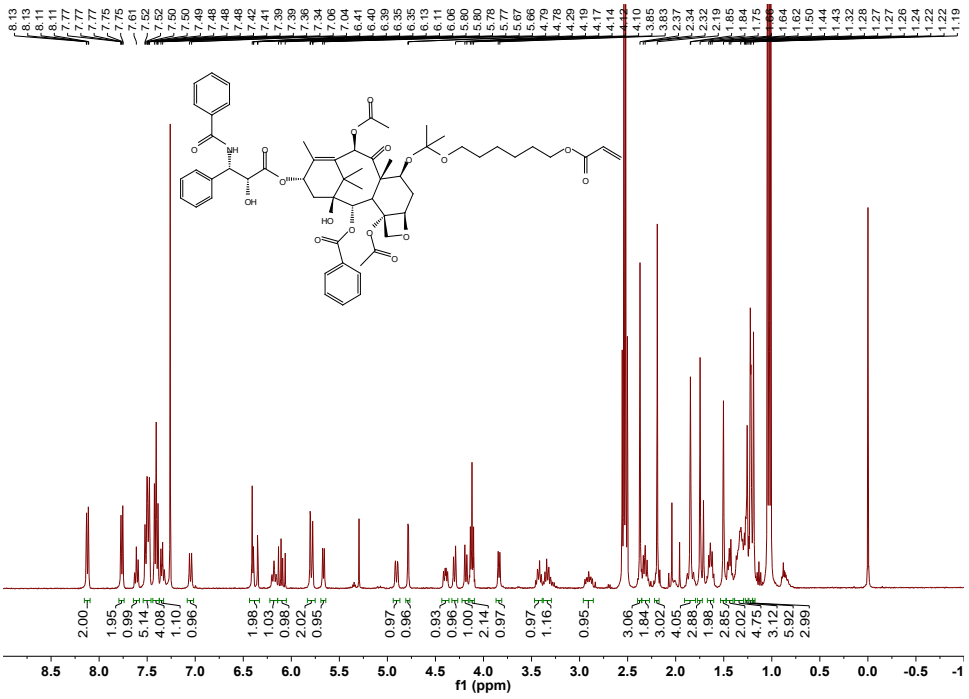


Figure S16. ^{1}H NMR spectrum of **P7-6** ($CDCl_3$).

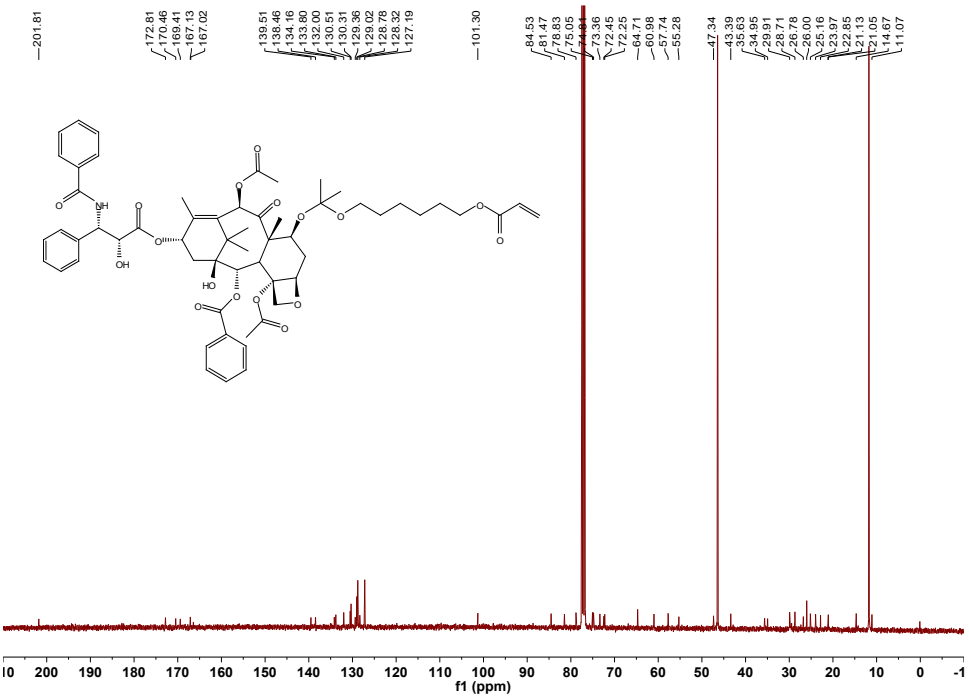


Figure S17. ^{13}C NMR spectrum of **P7-6** ($CDCl_3$).

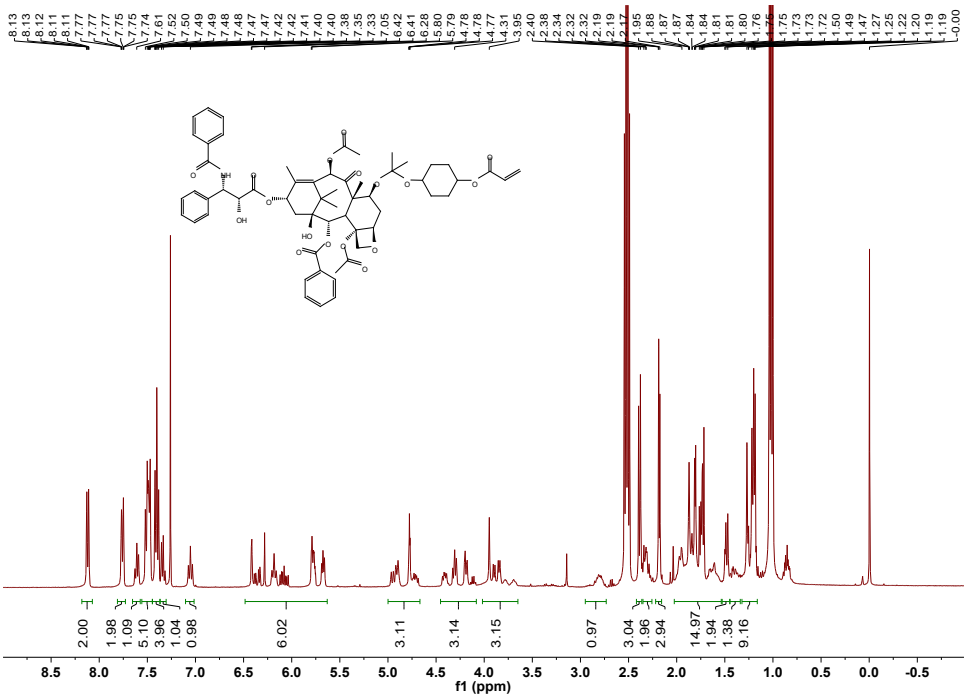


Figure S18. ^{1}H NMR spectrum of **P7-C6** ($CDCl_3$).

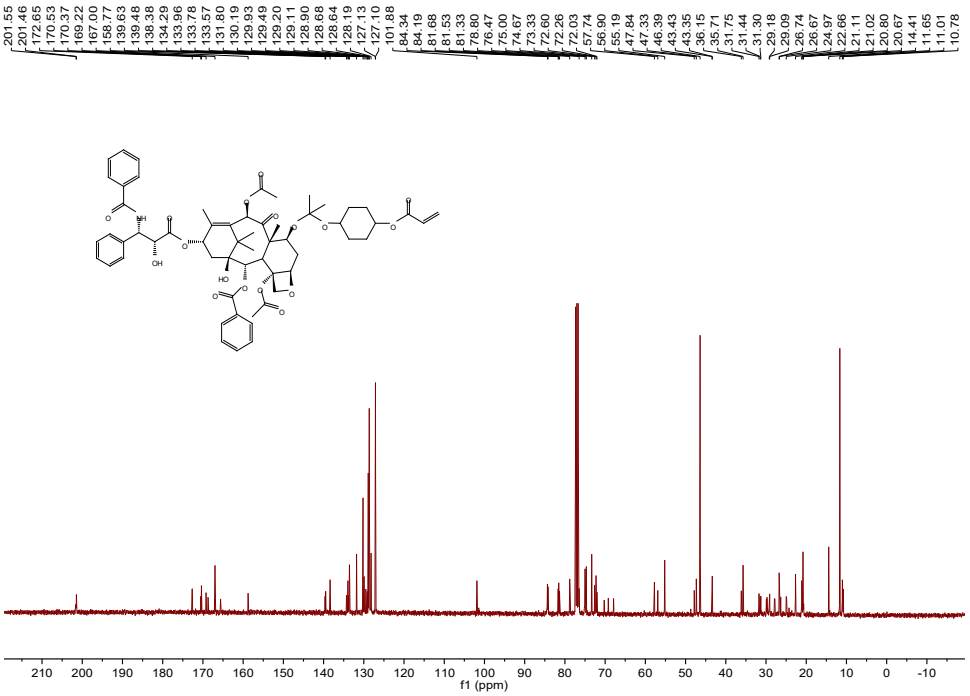


Figure S19. ^{13}C NMR spectrum of **P7-C6** ($CDCl_3$).

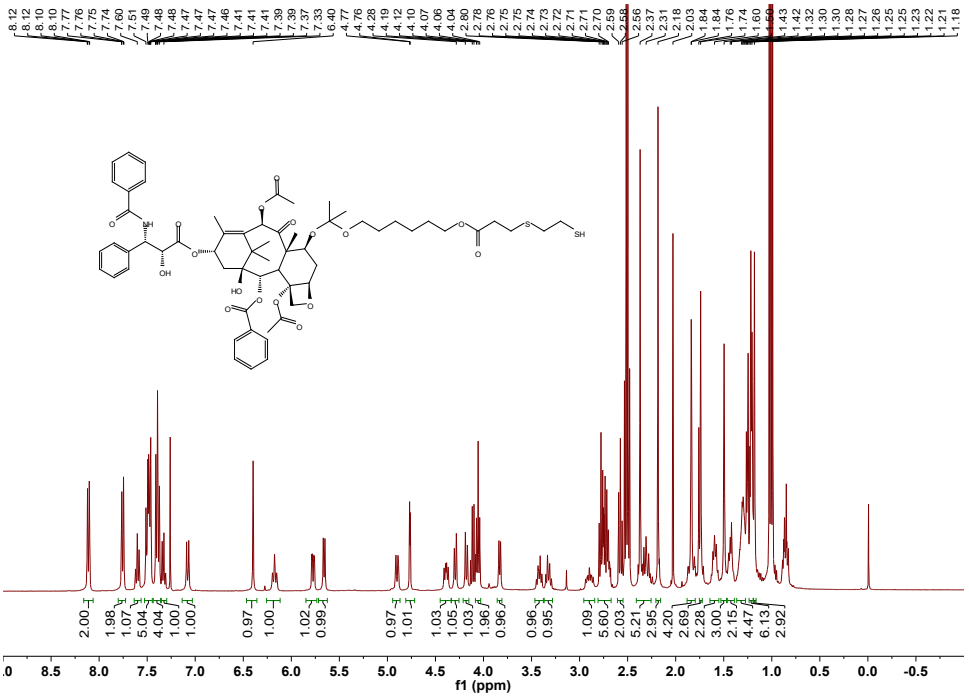


Figure S20. ^{1}H NMR spectrum of **P7-6-SH** ($CDCl_3$).

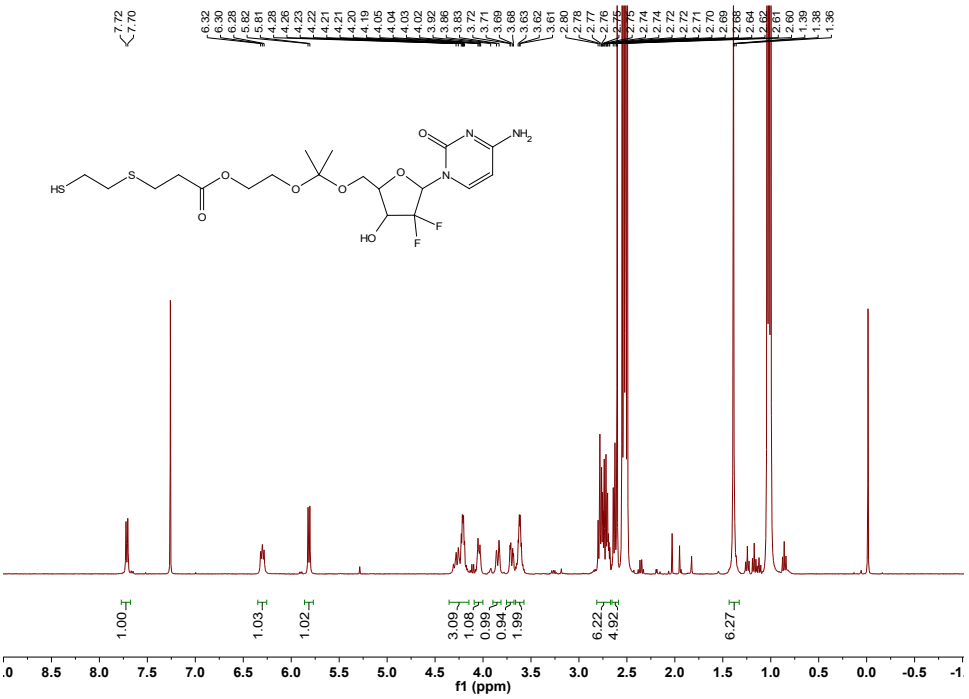


Figure S21. ^{1}H NMR spectrum of **P7-6-SH** ($CDCl_3$).

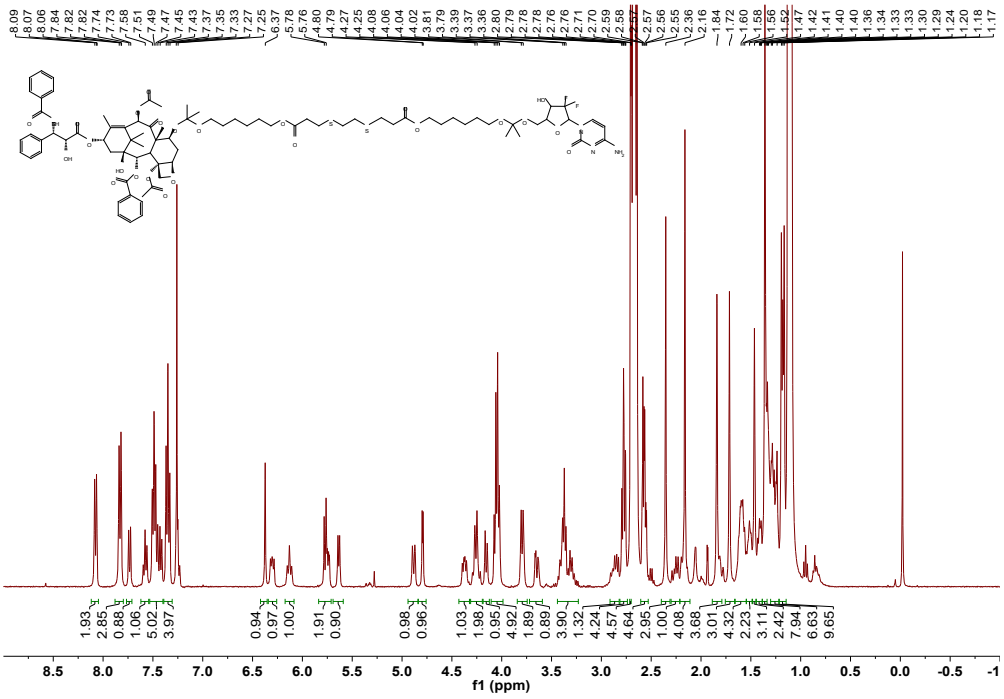


Figure S22. ^{1}H NMR spectrum of **PG1** ($CDCl_3$).

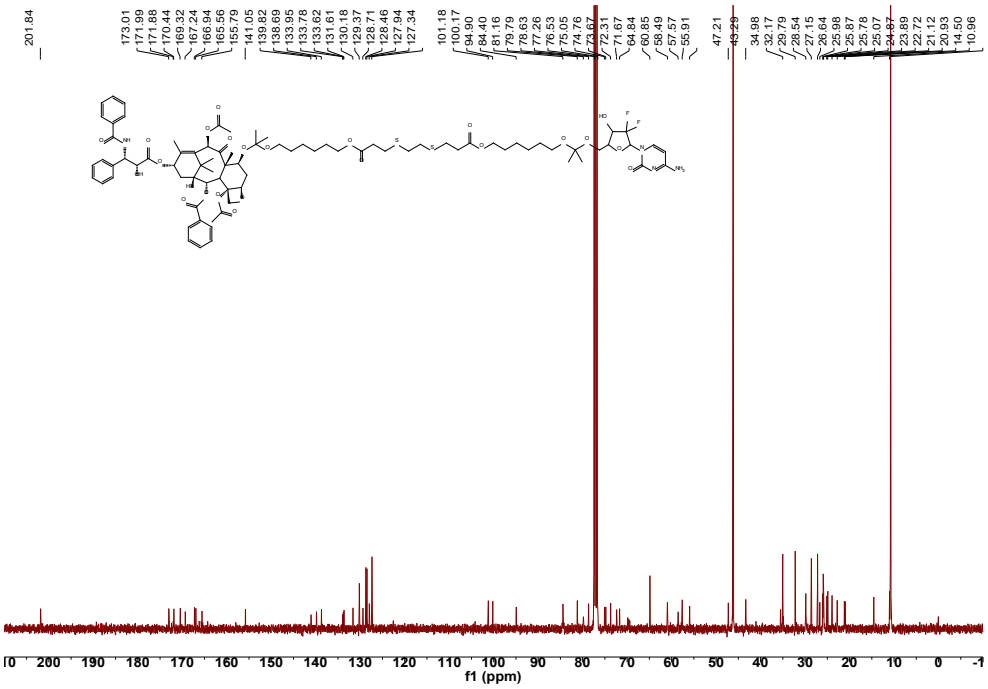


Figure S23. ^{13}C NMR spectrum of **PG1** ($CDCl_3$).

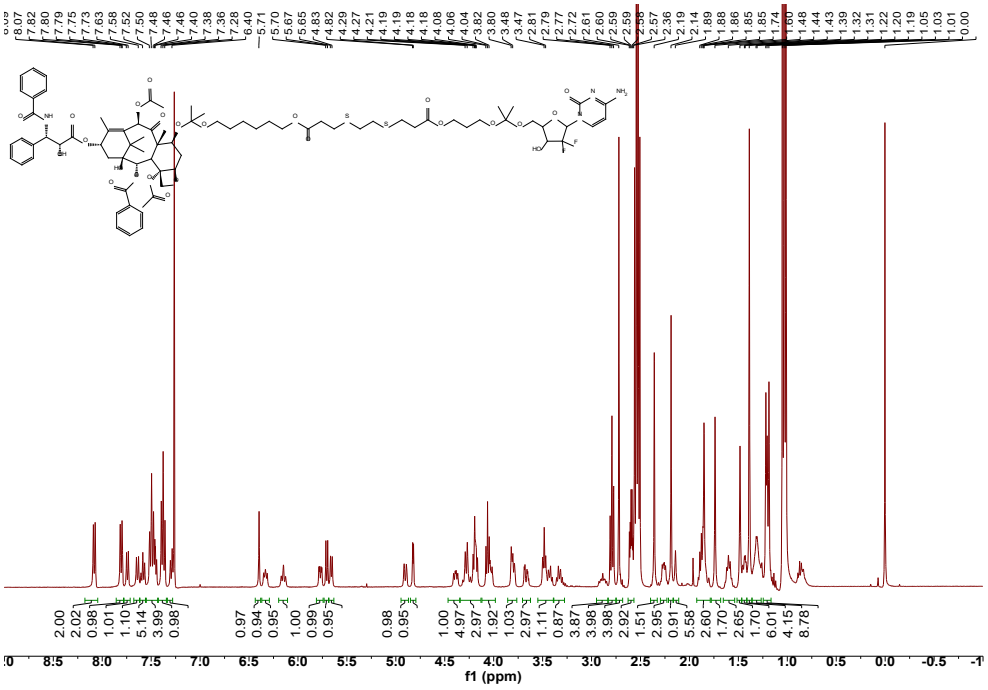


Figure S24. ^{1}H NMR spectrum of **PG2** ($CDCl_3$).

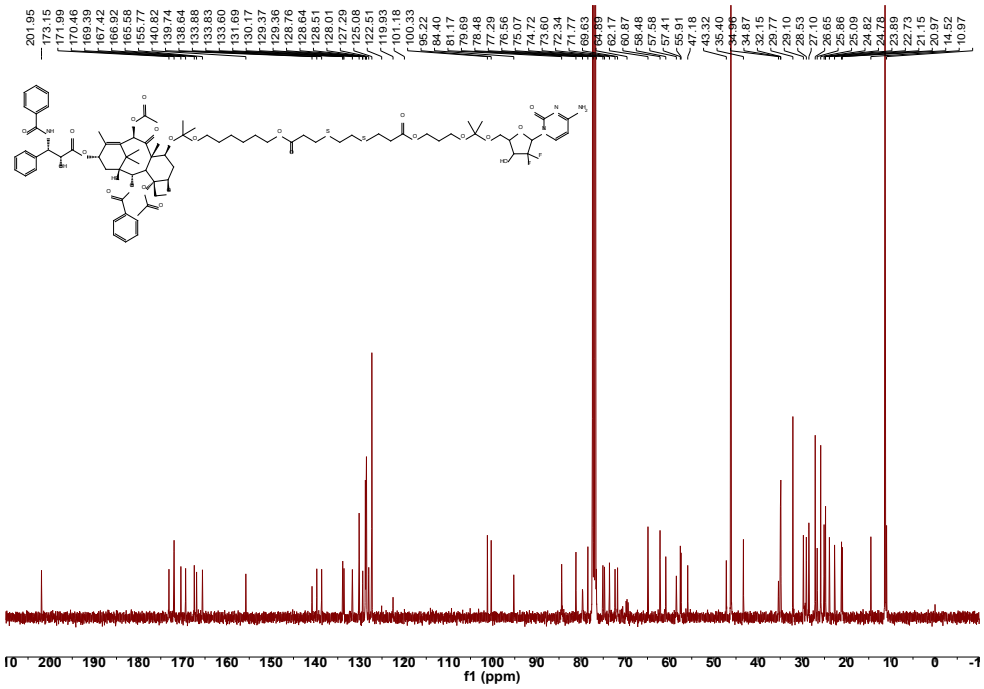


Figure S25. ^{13}C NMR spectrum of **PG2** ($CDCl_3$).

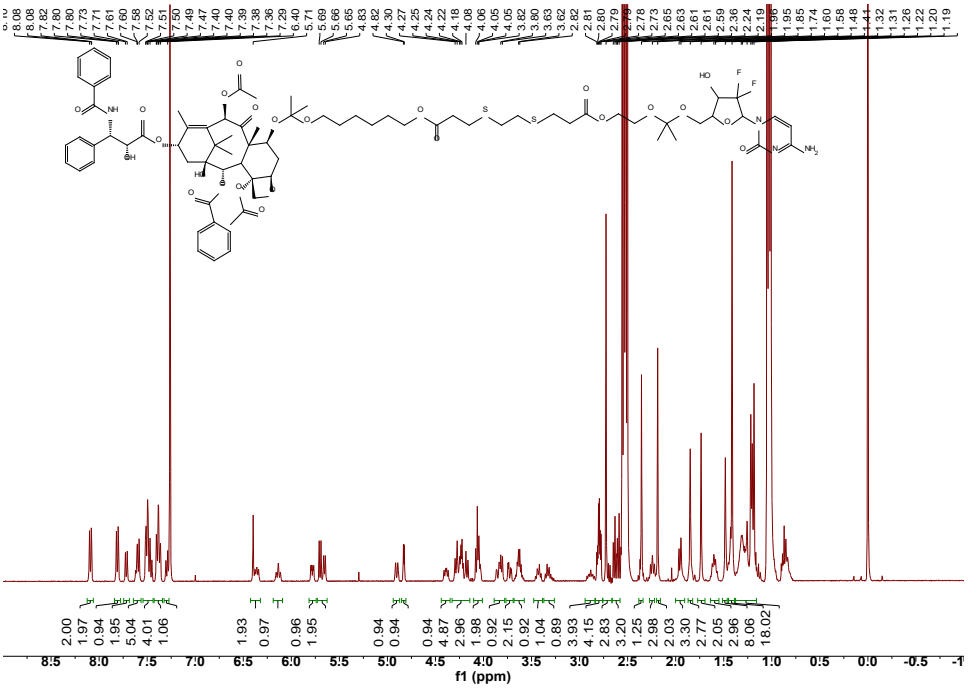


Figure S26. ^{1}H NMR spectrum of **PG3** ($CDCl_3$).

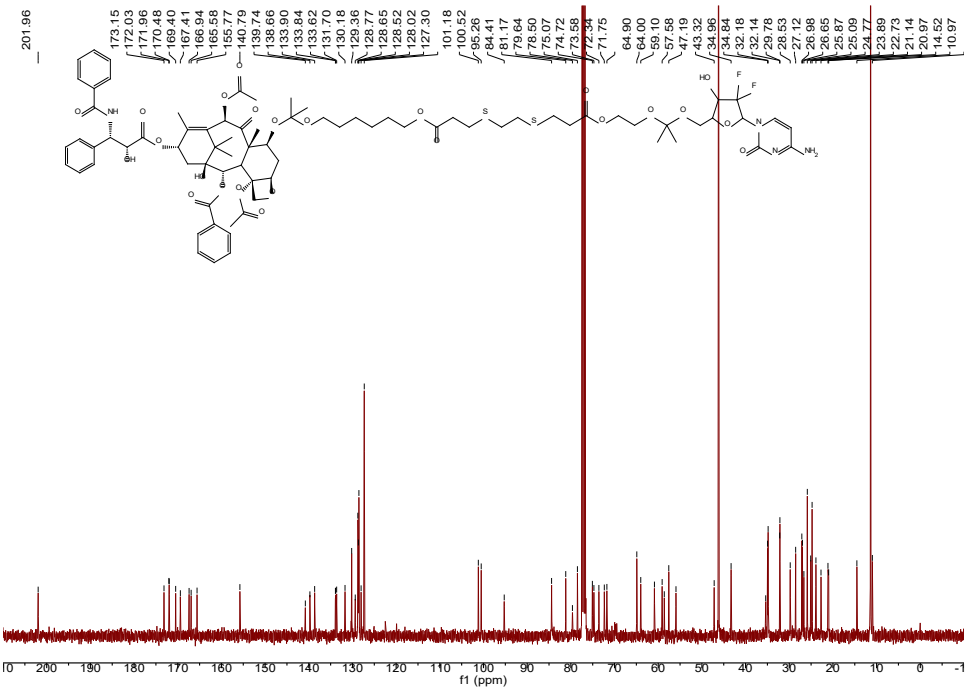


Figure S27. ^{13}C NMR spectrum of **PG3** ($CDCl_3$).

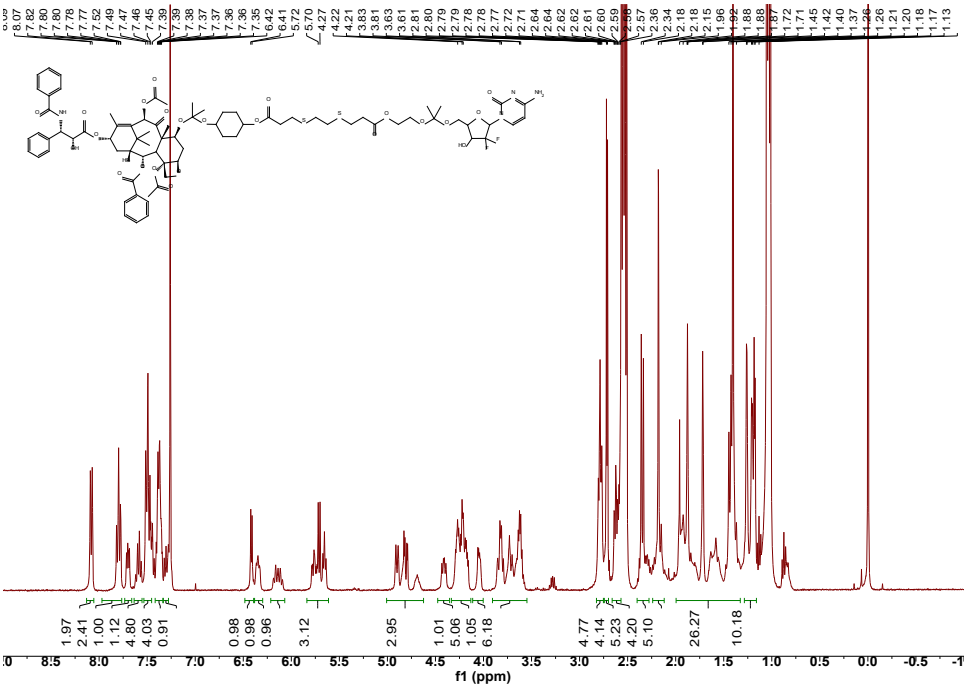


Figure S28. ^{1}H NMR spectrum of **PG4** ($CDCl_3$).

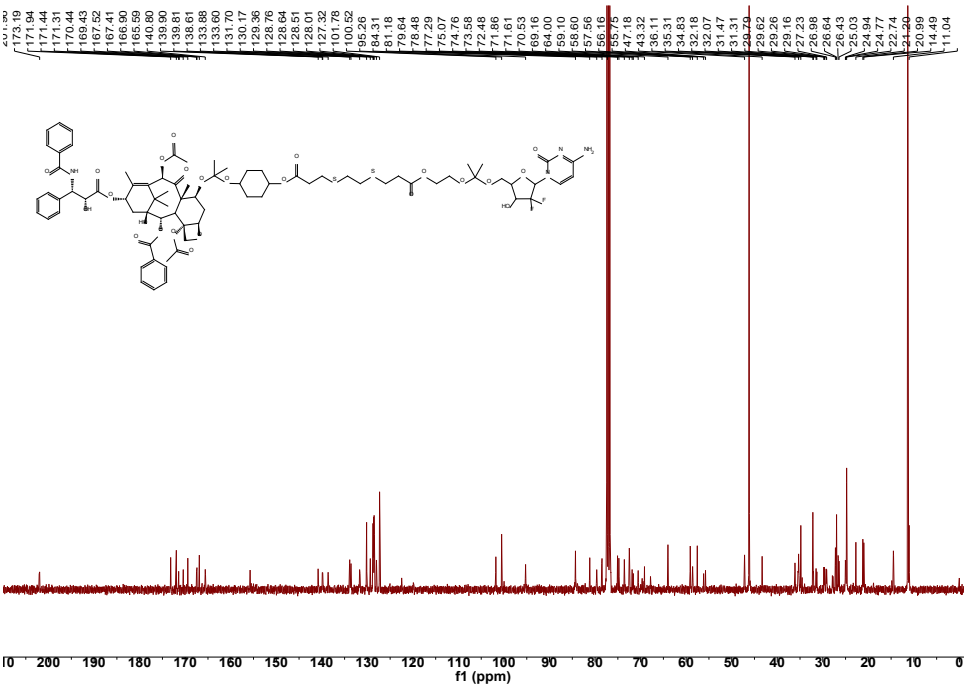


Figure S29. ^{13}C NMR spectrum of **PG4** ($CDCl_3$).

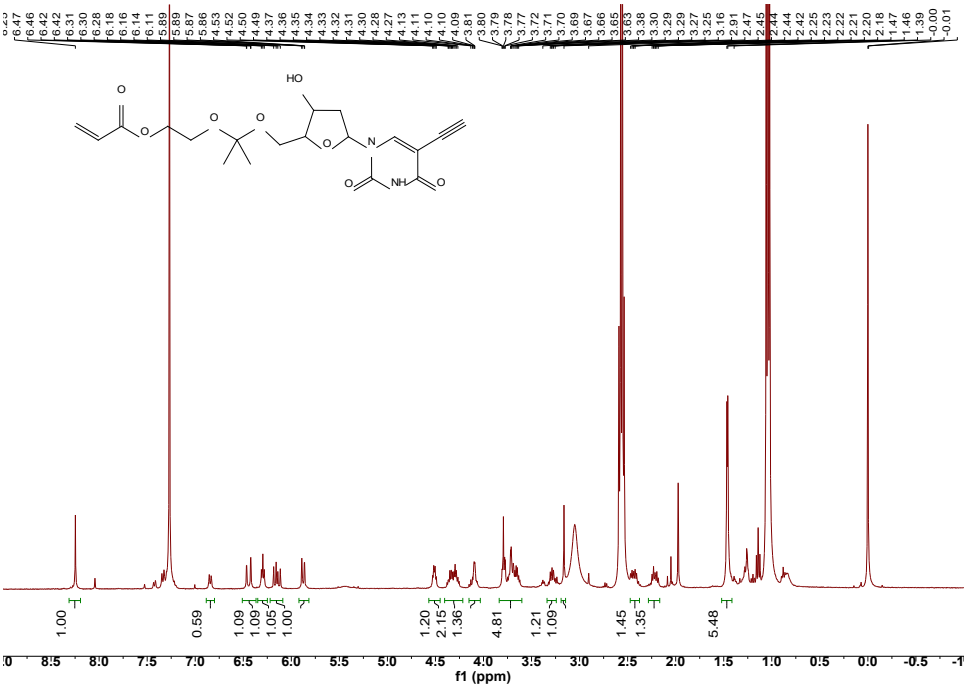


Figure S30. ^{1}H NMR spectrum of **E-2** ($CDCl_3$).

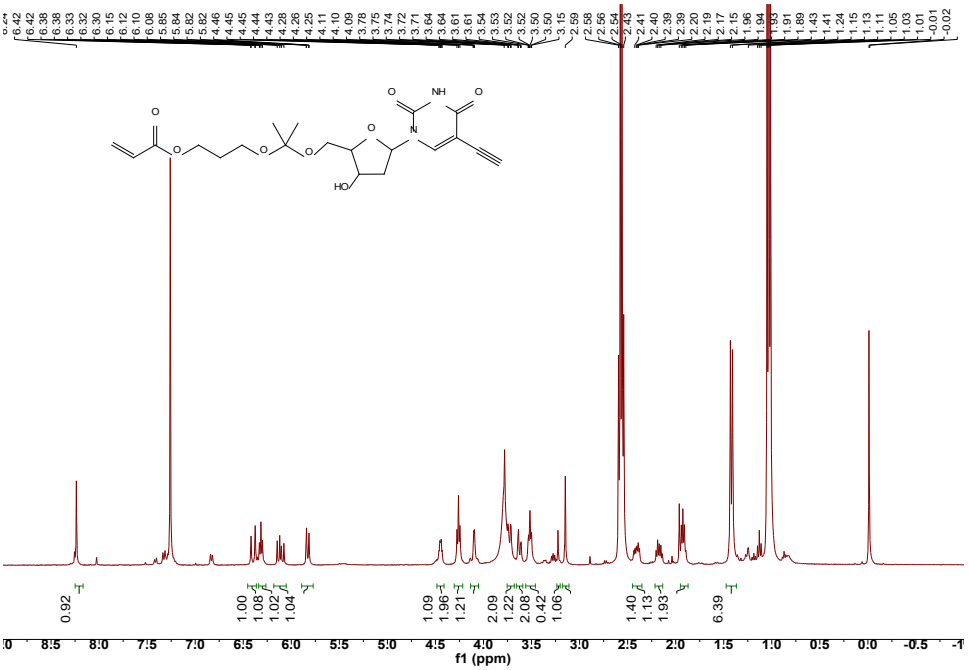


Figure S31. ^{1}H NMR spectrum of **E-3** ($CDCl_3$).

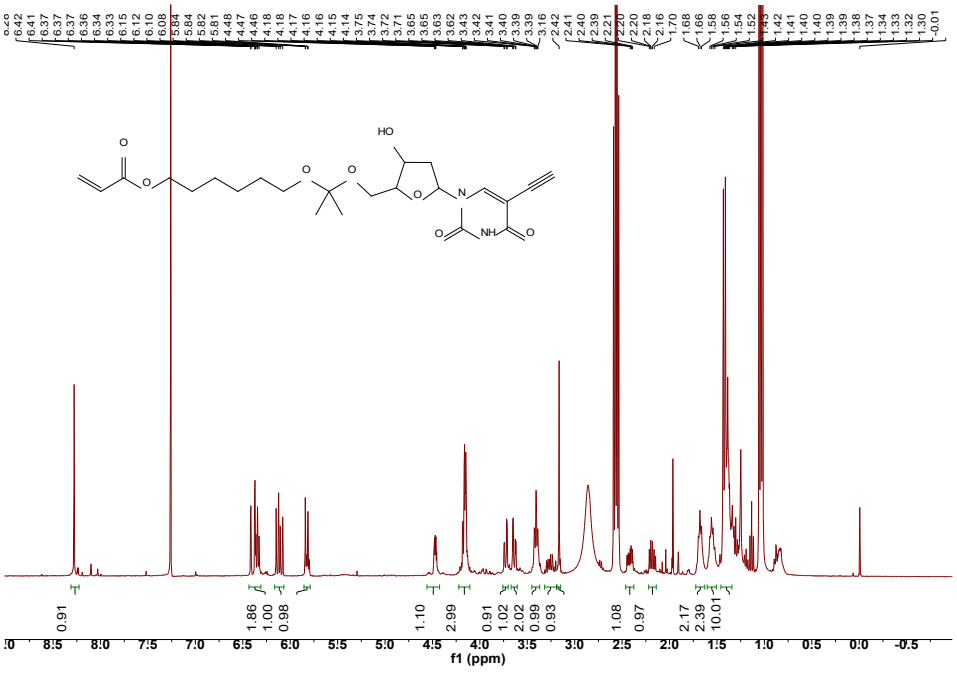


Figure S32. ^{1}H NMR spectrum of **E-6** ($CDCl_3$).

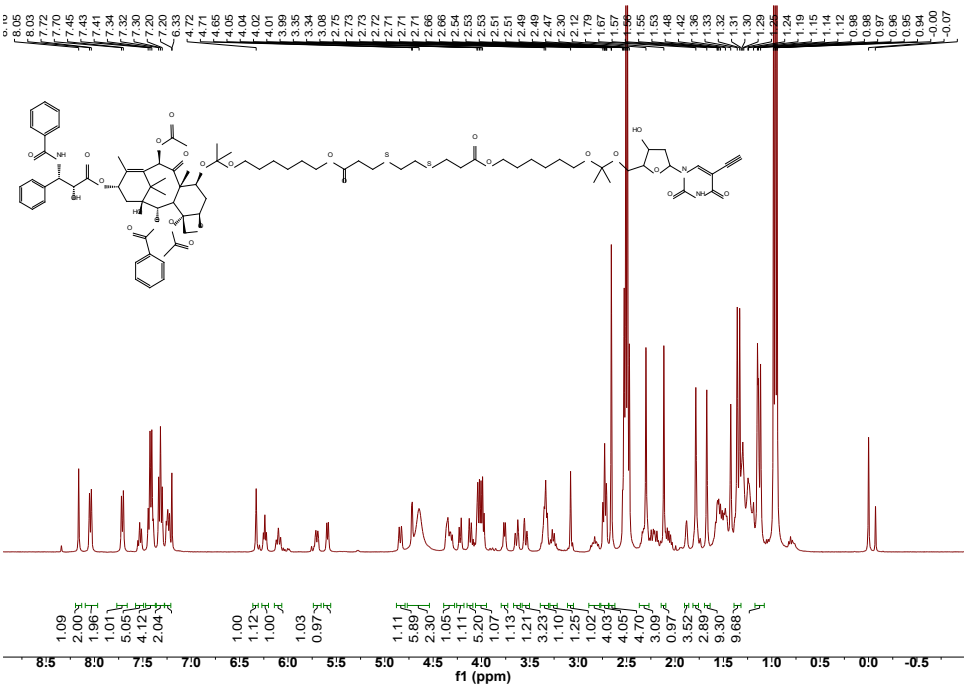


Figure S33. ^{1}H NMR spectrum of **PE1** ($CDCl_3$).

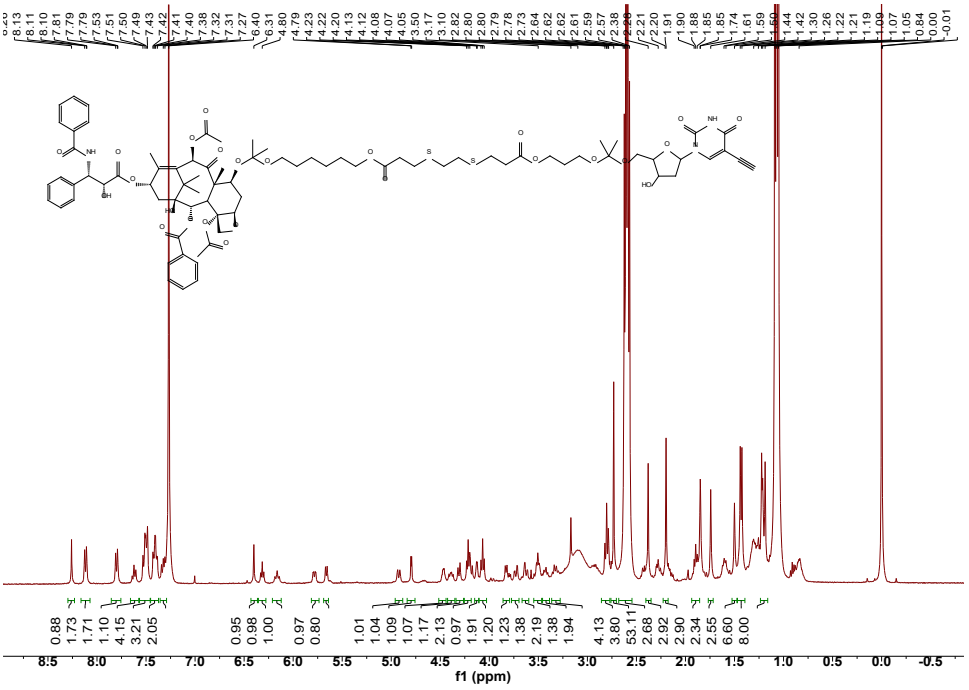


Figure S34. ^{1}H NMR spectrum of **PE2** ($CDCl_3$).

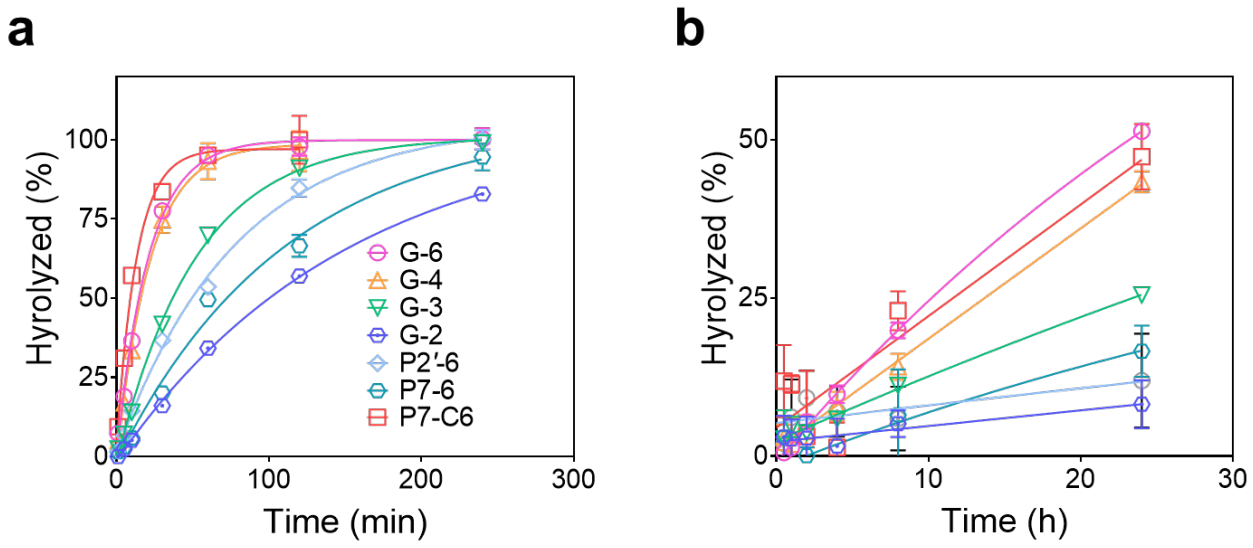


Figure S35. Hydrolysis kinetics of Gem and PTX precursors at (a) pH 5.0 and (b) 7.4 (n = 4).

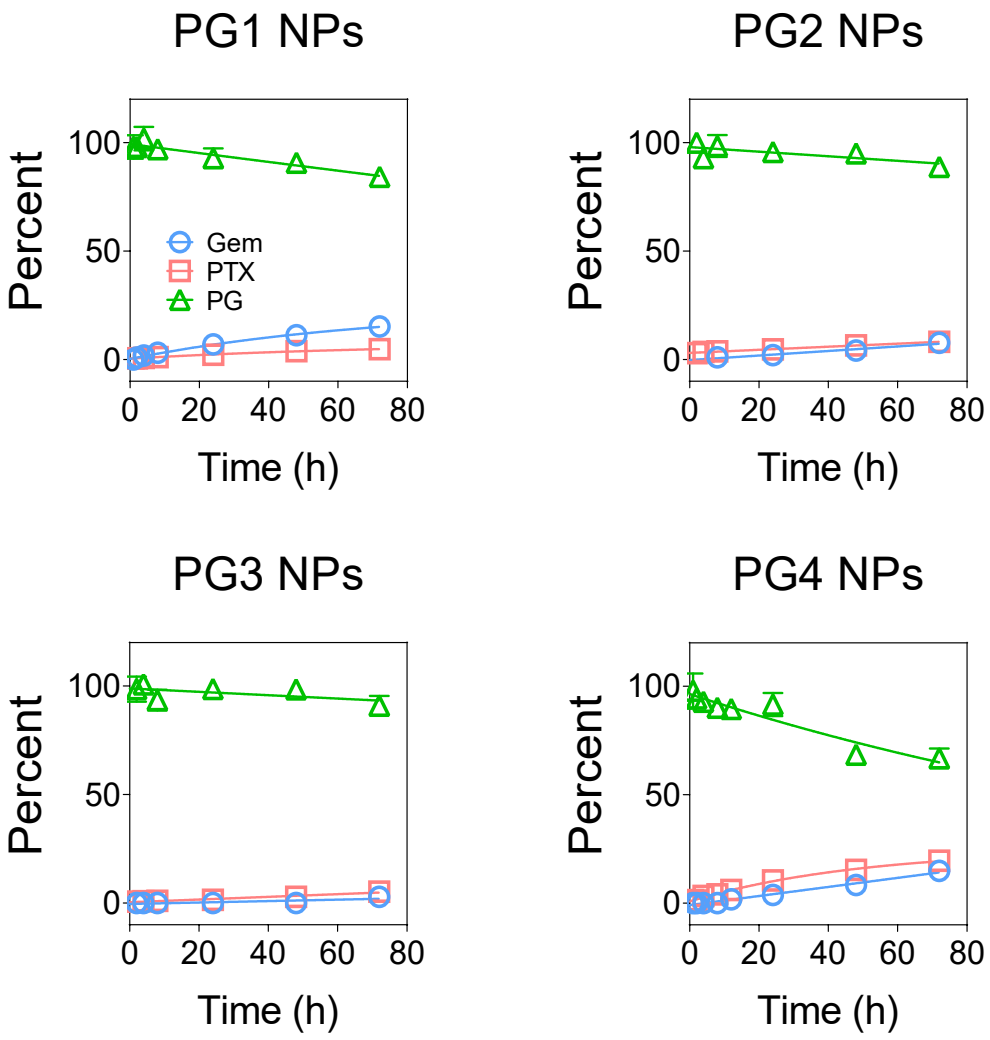


Figure S36. Hydrolysis kinetics of PG NPs at pH 7.4 determined by HPLC (n = 4).

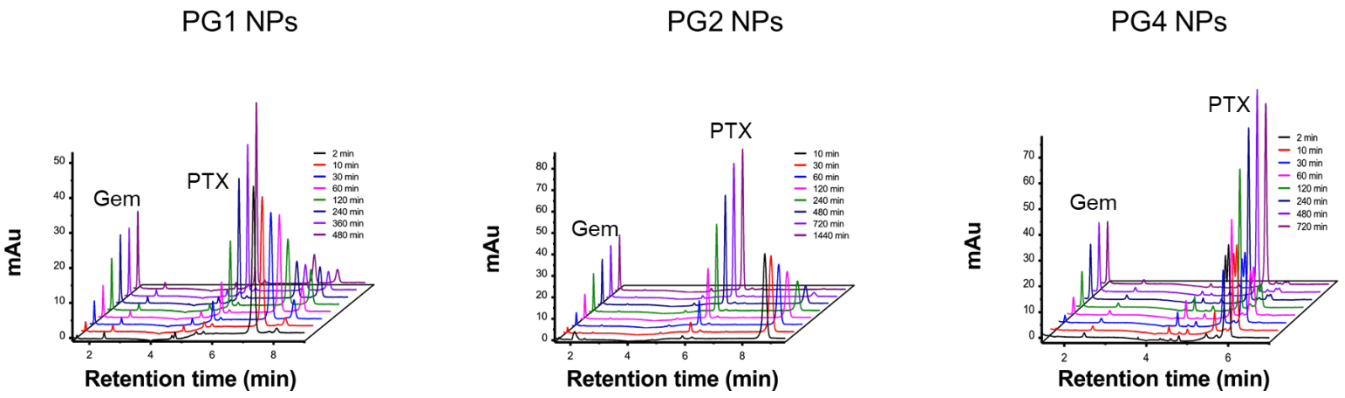


Figure S37. The HPLC chromatograms of PG NPs at pH 5.0 over 480 min. Mobile phase was ACN/H_2O (70/30, v/v); HPLC was run at a flow rate of 1 mL/min. Retention time: R_t of Gem = 1.6 min; R_t of PTX = 5.5 min. R_t of PG1, PG2 and PG4 was 7.2 min, 8.3 min, and 6.9 min.

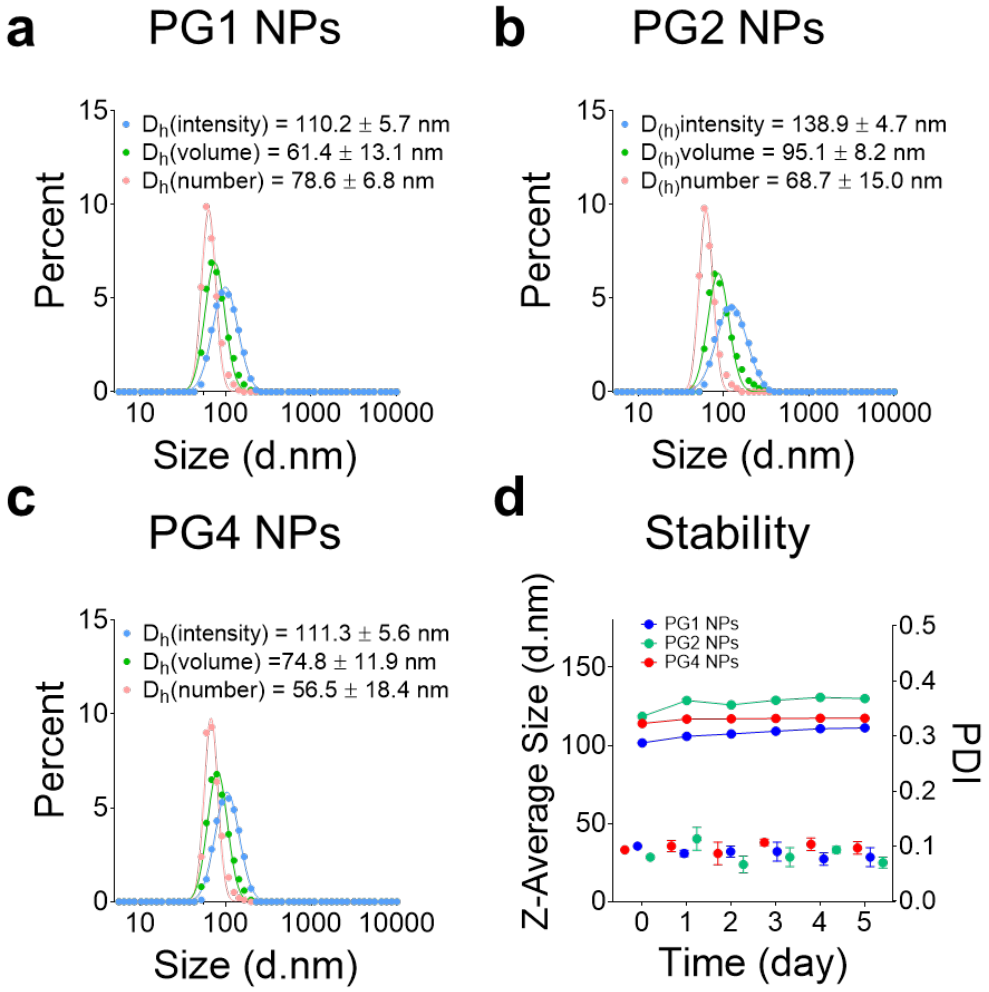
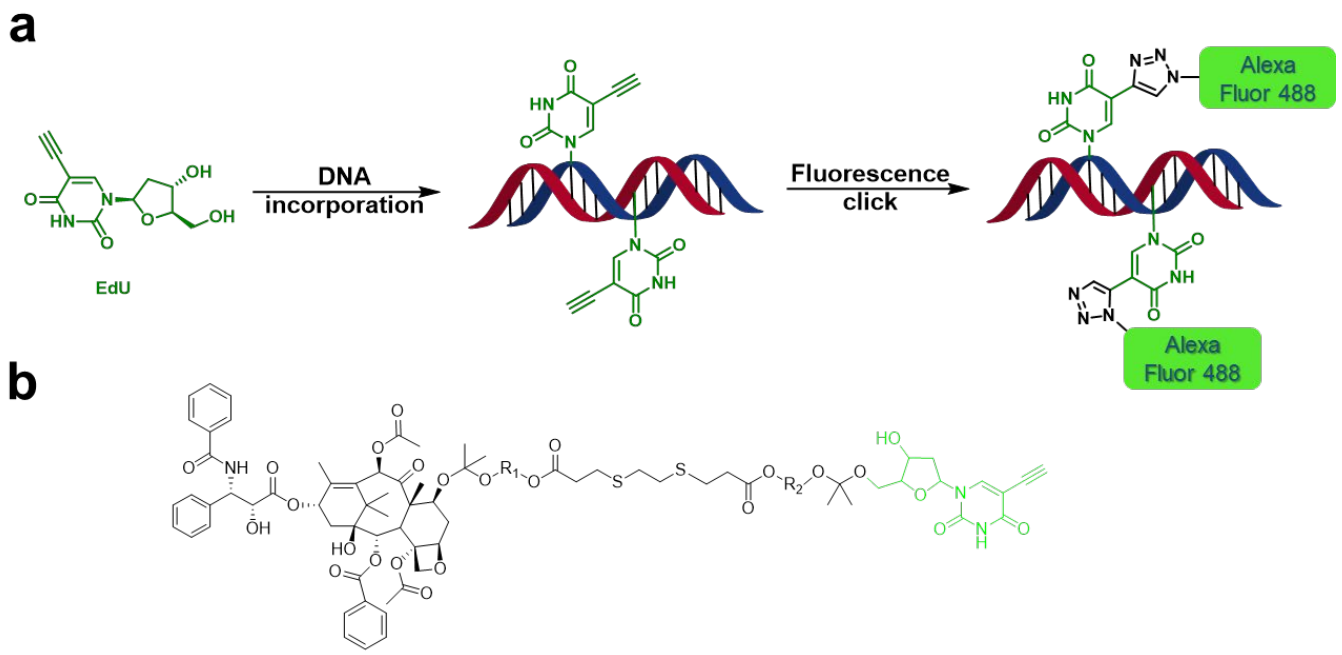


Figure S38. The size distribution of PG NPs (a-c) and stability during 5 days storage under 4 °C determined by DLS (d).



Dimer	R_1	R_2
PE_f		
PE_s		

Figure S39. (a) Schematic illustration of DNA incorporation of EdU and detection by azide-488 fluorescent dye. (b) The structure of PE prodrugs.

Table S1. IC_{50} values of each formulation in different cell lines.

Drugs	IC_{50} in PANC02 cell line (nM)	IC_{50} in A549 cell line (nM)	IC_{50} in A2780 cell line (nM)
P_sG_f NPs	28.3 ± 3.6	3.5 ± 1.5	13.2 ± 2.9
P_iG_i NPs	30.6 ± 5.1	5.6 ± 1.1	10.6 ± 4.2
P_fG_s NPs	47.5 ± 6.3	3.2 ± 1.5	21.5 ± 2.7
PTX + Gem	20.8 ± 5.9	7.4 ± 1.0	6.0 ± 3.4
PTX	83.3 ± 11.4	20.6 ± 1.3	8.2 ± 3.8
Gem	34.1 ± 6.8	15.1 ± 1.6	4.7 ± 2.5

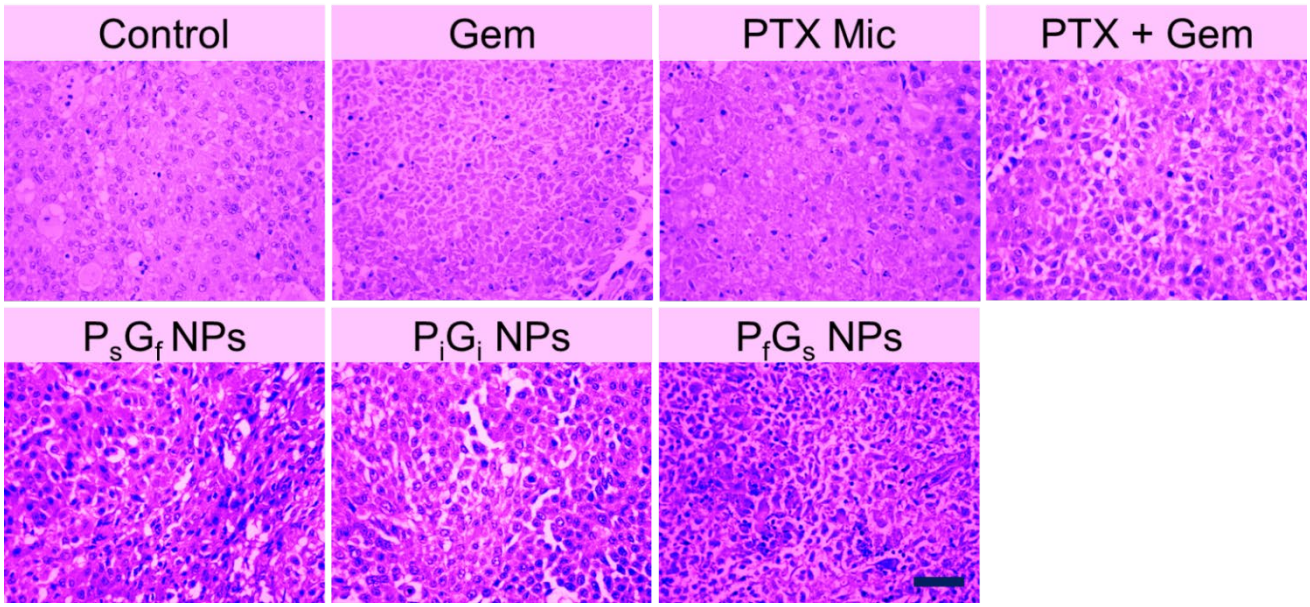


Figure S40. H&E staining of tumor tissues from A549 xenografted mice treated with PBS, Gem, PTX Mic, PTX Mic + Gem, and PG NPs at the end of treatment. Scale bar: 50 μm.

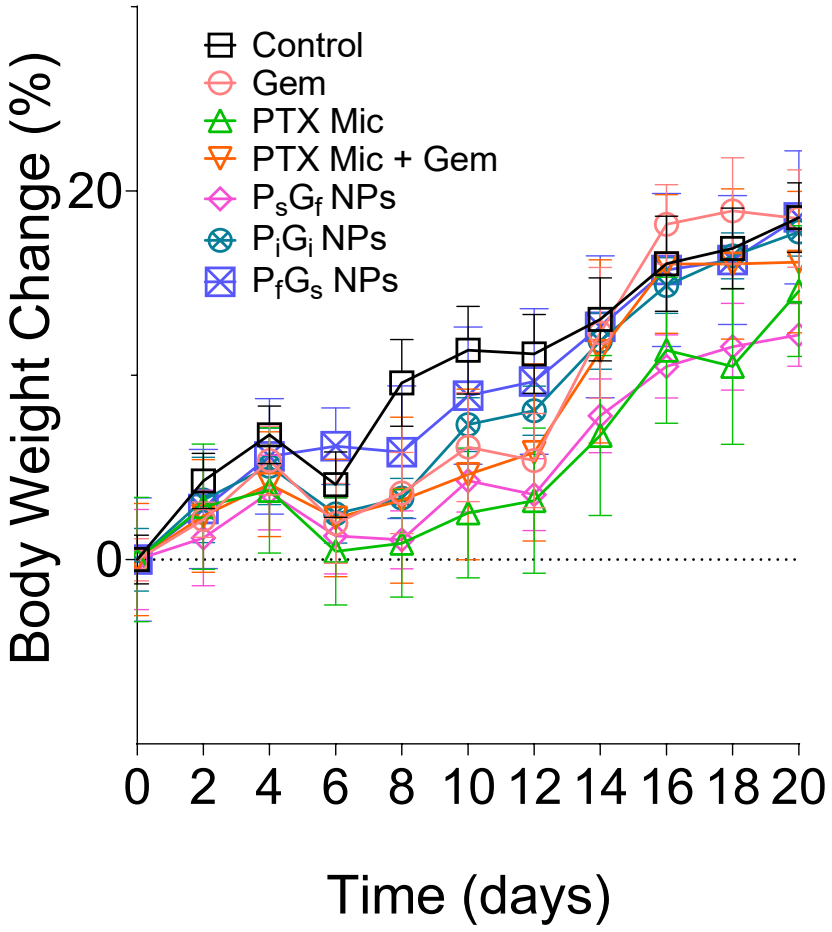


Figure S41. Body weight change of A549 xenografted mice during the treatment with PBS, Gem, PTX Mic, PTX Mic + Gem, and PG NPs (n = 5).

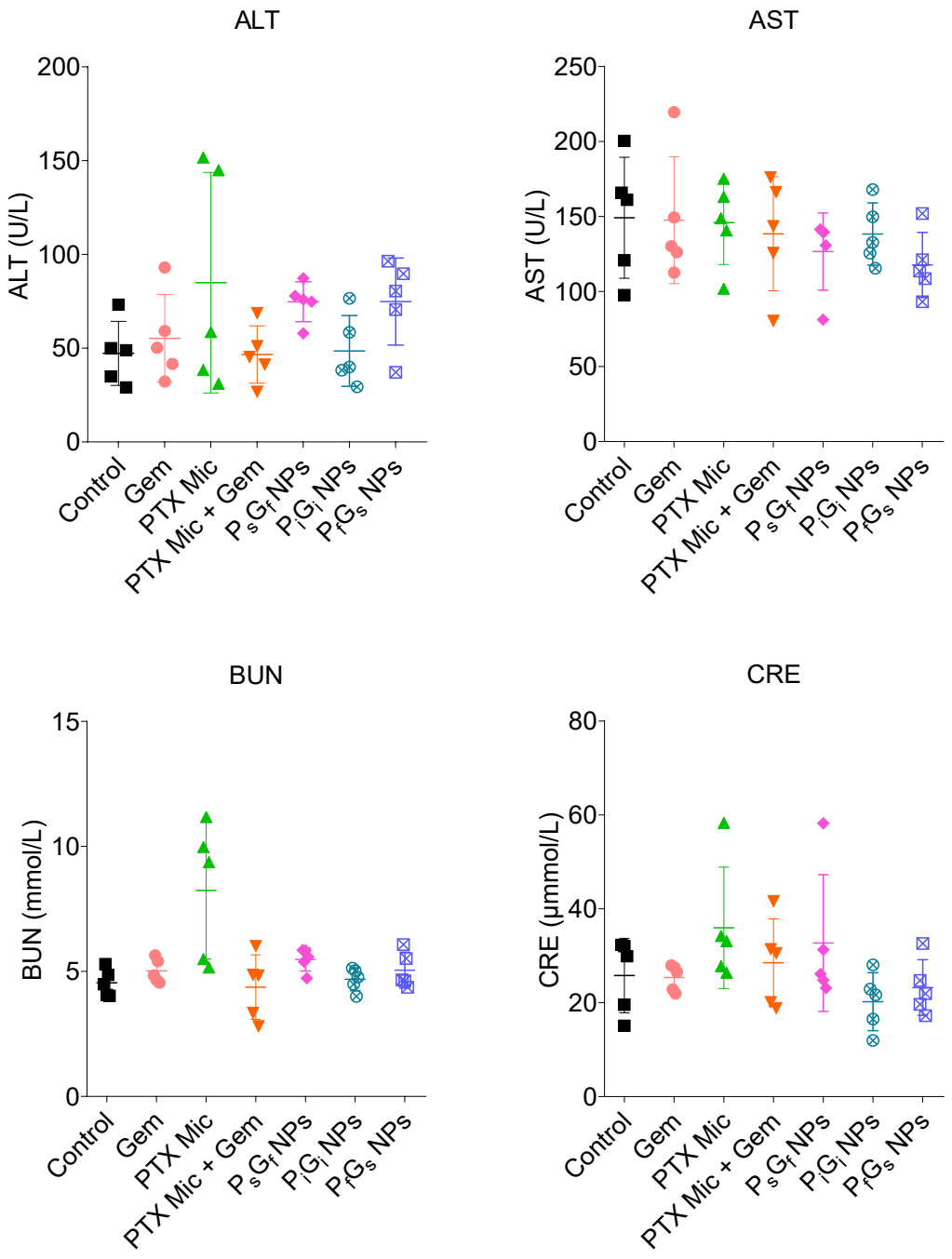


Figure S42. Biochemical analysis of blood samples from the mice treated with PBS, Gem, PTX Mic, PTX Mic + Gem, and PG NPs at the end of treatment (n = 5).

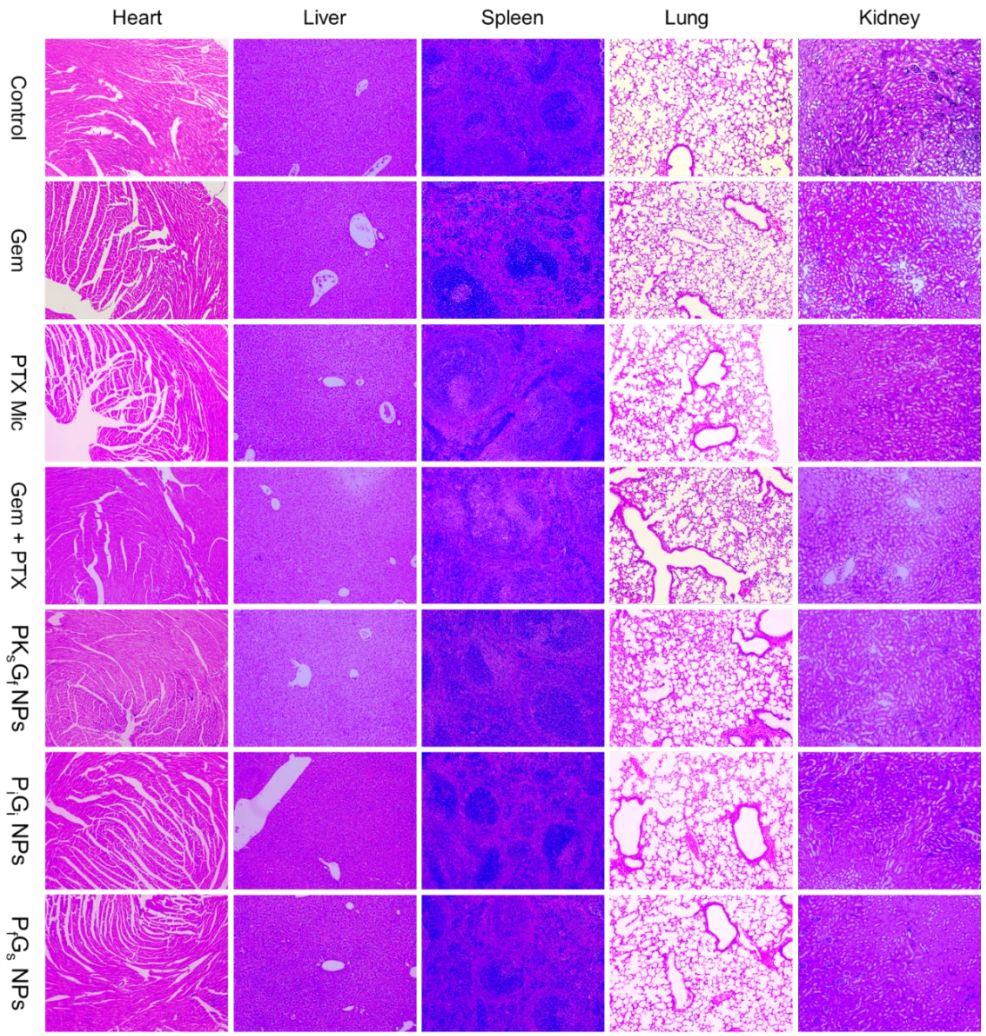


Figure S43. H&E staining of major organs from A549 xenografted mice treated with PBS, Gem, PTX Mic, PTX Mic + Gem, and PG NPs at the end of treatment. The magnification fold is 100×.

References

1. H. Zhong, J. Mu, Y. Du, Z. Xu, Y. Xu, N. Yu, S. Zhang and S. Guo, *Biomacromolecules*, 2020, **21**, 803-814.
2. J. D. Gibson, B. P. Khanal and E. R. Zubarev, *J. Am. Chem. Soc.*, 2007, **129**, 11653-11661.
3. T. C. Chou, *Cancer Res*, 2010, **70**, 440-446.