## Materials

Anhydrous chloroform, dichloromethane (DCM), tetrahydrofuran (THF), dimethylformamide (DMF), toluene and dimethyl sulfoxide (DMSO) were available commercially from Aldrich and used as received. Ethylene glycol and diethylene glycol were dried by molecular sieves. Poly(ethylene glycol) methyl ether (5 kDa, mPEG) was dried by azeotropical distillation from anhydrous toluene just before use. 2,2'-Dithiodiethanol was purchased from TCI and dried by molecular sieves. Camptothecin (CPT) and bis(pentafluorophenyl)carbonate were obtained from Matrix Scientific and used as received. $CDCl_3$ and $d_6$-DMSO were purchased from Cambridge Isotope Laboratories. SYTOX® green stain was obtained from Invitrogen (Grand Island, NY). Clear polystyrene tissue culture treated 6-well and 96-well plates were obtained from Corning Costar. All other solvents and reagents were available commercially from Aldrich and used as received unless otherwise noted.

## Methods

Nuclear magnetic resonance (NMR) spectra were collected in $CDCl_3$ and $d_6$-DMSO on a 400 MHz Varian NMR and the residual solvent peak ($CDCl_3$ $\delta$ = 7.26 or 77.0 ppm; $d_6$-DMSO $\delta$ = 2.50 or 39.4 ppm) was used as an internal chemical shift reference. Gel permeation chromatography (GPC) was performed on an OmniSEC system equipped with a GMHxl column and a tetra detector (refractive index, ultraviolet, right-angle light scattering, and low-angle light scattering). DMF+ 0.1% LiBr was used as the mobile phase at a flow rate of 1 mL $min^{-1}$ and column

temperature of 40 °C. Samples were filtered through a nylon syringe filter with 0.45 μm size pores before injection. High performance liquid chromatography (HPLC) was performed on a LC10 (Shimadzu Scientific Instruments; Columbia, MD) system equipped with a 03A-0355-E0 column. Acetonitrile/PBS mixture was used as the mobile phase at a flow rate of 1 mL $min^{-1}$. Matrix-assisted laser desorption ionization-time of flight (MALDI-TOF) mass spectrometry was performed on an Ultraflex II TOF/TOF mass spectrometer (Bruker Daltonics, Billerica, MA) equipped with a Nd:YAG laser. A total of 100 laser shots were used to acquire each mass spectrum. The following instrument parameters were used: an ion source 1 voltage of 25.00 kV, an ion source 2 voltage of 23.40 kV, a lens voltage of 6.50 kV for the 5k polymer; an ion source 1 voltage of 25.00 kV, an ion source 2 voltage of 22.00 kV, a lens voltage of 6.75 kV for the 12k polymer, pulsed ion extraction of 100 ns for the 5k polymer and 150 ns for the 12k polymer. Saturated MALDI matrix solutions were prepared in water/acetonitrile solutions (30/70 v/v) containing 0.1% trifluoroacetic acid. Sinapinic acid was used as matrix. Dynamic light scattering (DLS) was recorded on a DynaProTM Plate Reader (Wyatt Technology; Santa Barbara, CA) to measure the hydrodynamic radius. Samples were filtered through an AnotopTM syringe filter with 0.2 μm size pores (Whatman; Florham Park, NJ) prior to analysis. Liquid chromatography–mass spectrometry (LC–MS) was performed on a reverse-phase high pressure liquid chromatography using a Supelco Ascentis (5 cm × 1 mm, 3 μm) C18 column over 11 min with an elution gradient of 25-75% buffer B (5% $H_2O$, 95% acetonitrile). MS data was collected using an Agilent 1100 series quadrupole ion trap

mass spectrometer (Agilent Technologies, Santa Clara, CA). Cryogenic transmission electron microscopy (cryo-TEM) experiments were performed at Duke University's Shared Materials Instrumentation Facility (Durham, NC). Lacey holey carbon grids (Ted Pella, Redding, CA) were glow discharged in a PELCO EasiGlow Cleaning System (Ted Pella, Redding, CA). A 200 μM sample in a 3 μL drop was deposited onto the grid, blotted for 3 s with an offset of -3 mm, and vitrified in liquid ethane using the Vitrobot Mark III (FEI, Eindhoven, Netherlands). Prior to vitrification, the sample chamber was maintained at 22 °C and 100% relative humidity to prevent sample evaporation. Grids were transferred to a Gatan 626 cryoholder (Gatan, Pleasenton, CA) and imaged with an FEI Tecnai G2 Twin TEM (FEI, Eindhoven, Netherlands). Fluorescence spectroscopy was performed on a Cary Eclipse spectrophotometer equipped with a Xenon flash lamp (Varian Instruments; Palo Alto, CA). UV-Vis spectrophotometry was performed on a Victor$^{3\ TM}$ microplate reader (Perkin Elmer; Waltham, MA). Fourier transform infrared spectroscopy (FT-IR) was performed on a Thermo Electron Nicolet 8700 FT-IR spectrometer using reflectance mode.

**Cell culture**

C26 cells were cultured in RPMI-1640 (R8758; Sigma, St. Louis, MO), supplemented to 10% fetal bovine serum (F0392; Sigma, St. Louis, MO), 4.5 g L-1 D-glucose (G8769; Sigma), 10 mM HEPES (15630-080; Invitrogen; Carlsbad, CA), and 1 mM sodium pyruvate (11360-070, Invitrogen). 4T1 cells were cultured in D6429 (Sigma,

St. Louis, MO), supplemented with 10% fetal bovine serum (F0392; Sigma, St. Louis, MO). Cells were incubated at 37 °C with 5% carbon dioxide and maintained in T-75 flasks (Corning; Corning, NY) and passaged twice a week after washing with 7 mL Dulbecco's PBS without calcium or magnesium (14190-144; Invitrogen) and 2 mL of 0.05% trypsin + 0.5 mM EDTA (25300-054; Invitrogen).

**Animal experiments**

All animals were treated in accordance with National Institute of Health Guide for the Care and Use of Laboratory Animals under protocols approved by the Duke University Institutional Animal Care and Use Committee. Female Balb/C mice (7 weeks, ~18 g weight) were used for all studies.

**Synthesis of Carb-$C_6F_5$**

Carb-$C_6F_5$ was synthesized according to previous report.[1] A 150 mL round bottom flask was charged with 2,2-bis(hydroxymethyl)propionic acid (3.65 g, 27.2 mmol), bis-(pentafluorophenyl)carbonate (26.8 g, 68.0 mmol), CsF (0.826 g, 5.44 mmol), and 90 mL of anhydrous THF. Initially the reaction was heterogeneous, but after one hour a clear homogeneous solution was formed that was allowed to stir for 20 h. The solvent was then removed *in vacuo*, and the residue was re-dissolved in DCM and the precipitated pentafluorophenol byproduct was removed by filtration. The filtrate was extracted with sodium bicarbonate and water and was dried with anhydrous $MgSO_4$. The solvent was evaporated *in vacuo* and the product was re-crystallized twice from

ethyl acetate/hexane mixture (1:1/v:v) to give Carb-$C_6F_5$ as a white crystalline powder. Yield: 6.34 g (72 % yield). $^1$H NMR (400 MHz, $CDCl_3$): δ 4.85 (d, 2H, $J$ = 11.2 Hz), 4.36 (d, 2H, $J$ =11.2 Hz), 1.56 (s, 3H) (Figure S1). $^{13}$C NMR (400 MHz, $CDCl_3$): δ 167.9, 146.7, 142.2~136.7, 128.0, 72.4, 41.0, 17.5. IR ($cm^{-1}$): 1783, 1737, 1519, 1185, 1103, 1085, 994 $cm^{-1}$.

**Synthesis of intermediate 1**

A 50 mL round bottom flask was charged with chlorambucil (3.04 g, 10.0 mmol), ethylene glycol (3.10 g, 50.0 mmol), dicyclohexylcarbodiimide (2.27 g, 11.0 mmol), 4-dimethylaminopyridine (DMAP) (0.305 g, 2.50 mmol) and 15 mL of anhydrous DMF. After stirring for 48 hours, the mixture was filtered and the filtrate was precipitated into water. The crude product was re-dissolved in DCM and dried with anhydrous $MgSO_4$. The solvent was removed *in vacuo* and the residue was purified by column chromatography (silica, 1:1 ethyl acetate/chloroform). The desired product was obtained as viscous oil. Yield: 2.44 g (70 % yield). ESI-MS $m/z$ ($M^+$) calcd 348.3, found 371.0 ($M + Na^+$). $^1$H NMR (400 MHz, $CDCl_3$): δ 7.07 (d, 2H, $J$ = 8.8 Hz), 6.63 (d, 2H, $J$ = 8.8 Hz), 4.21 (m, 2H), 3.82 (m, 2H), 3.70 (m, 4H), 3.63 (m, 4H), 2.57 (t, 2H, $J$ = 3.6 Hz), 2.37 (t, 2H, $J$ = 7.6 Hz), 1.93 (m, 2H) (Figure S2). $^{13}$C NMR (400 MHz, $CDCl_3$): δ 173.5, 143.8, 129.8, 129.2, 111.6, 65.3, 60.1, 52.9, 40.2, 33.4, 32.9, 26.2. IR ($cm^{-1}$): 2950, 1730, 1615, 1519, 1388, 1354, 1276, 1250, 1180, 1149 $cm^{-1}$.

**Synthesis of CarbCL**

Intermediate 1 (890 mg, 2.56 mmol) was combined with Carb-$C_6F_5$ (834 mg, 2.56 mmol) and CsF (582 mg, 3.83 mmol) in 20 mL of anhydrous THF in a 50 mL round bottom flask and stirred for 36 h at room temperature. The reaction mixture was concentrated and re-dissolved in DCM. After ~10 min, the pentafluorophenol byproduct precipitated from solution and was removed by filtration. The mother liquid was washed with aqueous sodium bicarbonate (3 × 50 mL), brine (1 × 50 mL) and water (1 × 50 mL). The organic layer was separated and dried over anhydrous $MgSO_4$. The solvent was removed *in vacuo* and the residue was purified by column chromatography (silica, 1:1 ethyl acetate/hexane). The final material was obtained as a viscous liquid. Yield: 652 mg (52 % yield). ESI-MS *m*/*z* ($M^+$) calcd 490.3, found 490.1 ($M^+$). $^1$H NMR (400 MHz, $CDCl_3$): δ 7.07 (d, 2H, *J* = 8.4 Hz), 6.63 (d, 2H, *J* = 8 Hz), 4.68 (d, 2H, *J* = 10.4 Hz), 4.41 (t, 2H, *J* = 4 Hz), 4.31 (t, 2H, *J* = 5.6 Hz), 4.19 (d, 2H, *J* = 10.8 Hz), 3.70 (t, 4H, *J* = 7.2 Hz), 3.62 (t, 4H, *J* = 6.4 Hz), 2.56 (t, 2H, *J* = 7.6 Hz), 2.35 (t, 2H, *J* = 7.6 Hz), 1.90 (m, 2H), 1.32 (s, 3H) (Figure S3). $^{13}$C NMR (400 MHz, $CDCl_3$): δ 173.0, 170.8, 147.2, 144.0, 129.4, 111.9, 72.6, 63.5, 61.1, 53.2, 40.3, 39.9, 33.6, 33.0, 26.3, 17.0. IR ($cm^{-1}$): 1760, 1737, 1615, 1519, 1463, 1403, 1355, 1237, 1178, 1140, 1102, 737 $cm^{-1}$.

**Synthesis of intermediate 3**

CPT (1.00 g, 2.87 mmol) and triphosgene (0.315 g, 1.06 mmol) were suspended in anhydrous DCM (75 mL) under argon. DMAP (1.12 g, 9.19 mmol) was added drop wise to DCM (5 mL). After stirring for 30 min, diethylene glycol (3.05 g, 28.7 mmol)

in anhydrous THF (10 mL) was added and the reaction mixture was stirred overnight at room temperature. The mixture was washed with 0.1 M HCl aqueous solution (3 × 60 mL), brine (1 × 60 mL) and water (1 × 60 mL). The organic layer was separated and dried over anhydrous $MgSO_4$. The solvent was removed *in vacuo* and the residue was purified by re-crystallization from chloroform/methanol (1:10/v:v). Yield: 1.05 g (76 % yield). ESI-MS *m*/*z* ($M^+$) calcd 480.4, found 503.1 ($M + Na^+$). $^1H$ NMR (400 MHz, $CDCl_3$): δ 8.42 (s, 1H), 8.24 (dd, 1H, *J* = 0.4 Hz, *J* = 8.4 Hz), 7.95 (dd, 1H, *J* = 0.8 Hz, *J* = 8.4 Hz), 7.85 (m, 1H), 7.69 (m, 1H), 7.40 (s, 1H), 5.72 (d, 1H, *J* = 17.2 Hz), 5.38 (d, 1H, *J* = 17.2 Hz), 5.30 (t, 2H, *J* = 1.2 Hz), 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, *J* = 7.6 Hz) (Figure S4). $^{13}C$ 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 Carb-O-CPT**

Intermediate 3 (200 mg, 0.416 mmol) was combined with Carb-$C_6F_5$ (163 mg, 0.500 mmol) and 1,8-bis(dimethylamino)naphthalene (proton sponge) (89.2 mg, 0.416 mmol) in 3 mL of anhydrous DMSO in a 25 mL round bottom flask, and stirred for 24 h at room temperature. The reaction mixture was precipitated into water and then re-dissolved in DCM. The liquid was washed with aqueous sodium bicarbonate (3 × 50 mL), brine (1 × 50 mL) and water (1 × 50 mL). The organic layer was separated

and dried over anhydrous $MgSO_4$. The solvent was removed *in vacuo* and the residue was purified by column chromatography (silica, 4:1 DCM/acetone). Yield: 85.5 mg (33 % yield). ESI-MS *m/z* ($M^+$) calcd 622.5, found 645.0 ($M + Na^+$). $^1H$ NMR (400 MHz, $CDCl_3$): δ 8.41 (s, 1H), 8.22 (dd, 1H, *J* = 0.4 Hz, *J* = 8.4 Hz), 7.95 (dd, 1H, *J* = 0.8 Hz, *J* = 8.4 Hz), 7.85 (m, 1H), 7.68 (m, 1H), 7.33 (s, 1H), 5.70 (d, 1H, *J* = 17.2 Hz), 5.38 (d, 1H, *J* = 17.6 Hz), 5.31 (s, 2H), 4.69 (qd, 2H, *J* = 2 Hz, *J* = 5.6 Hz, *J* = 11.2 Hz, *J* = 16.8 Hz), 4.34 (m, 2H), 4.26 (m, 2H), 4.19 (m, 2H), 3.69 (m, 4H), 2.28 (m, 1H), 2.17 (m, 1H), 1.35 (s, 3H), 1.00 (t, 3H, *J* = 7.6 Hz) (Figure S5). $^{13}C$ NMR (400 MHz, $CDCl_3$): δ 170.9, 167.2, 157.0, 153.4, 152.0, 148.5, 147.3, 146.2, 145.3, 131.1, 130.5, 129.1, 128.3, 128.1, 127.9, 127.8, 120.0, 95.6, 77.7, 72.7, 68.5, 68.3, 67.6, 66.8, 64.4, 49.8, 39.9, 31.5, 17.2, 7.4. IR ($cm^{-1}$): 1748, 1664, 1616, 1463, 1403, 1270, 1235, 1180, 1134, 1101, 1047, 774 $cm^{-1}$.

**Synthesis of intermediate 5**

CPT (1.50 g, 4.31 mmol) and triphosgene (0.473 g, 1.59 mmol) were suspended in anhydrous DCM (110 mL) under argon. DMAP (1.68 g, 13.8 mmol) was added drop wise to DCM (10 mL). After stirring for 30 min, 2,2'-dithiodiethanol (6.64 g, 43.1 mmol) in anhydrous THF (15 mL) was added and the reaction mixture was stirred overnight at room temperature. The mixture was washed with 0.1 M HCl aqueous solution (3 × 80 mL), brine (1 × 80 mL) and water (1 × 80 mL). The organic layer was separated and dried over anhydrous $MgSO_4$. The solvent was removed *in vacuo* and the residue was purified by re-crystallization from chloroform/methanol

(3:10/v:v). Yield: 1.48 g (65 % yield). ESI-MS *m*/*z* ($M^+$) calcd 528.5, found 551.2 (M + $Na^+$). $^1$H NMR (400 MHz, $CDCl_3$): δ 8.43 (s, 1H), 8.23 (dd, 1H, *J* = 0.4 Hz, *J* = 8.4 Hz), 7.96 (dd, 1H, *J* = 0.8 Hz, *J* = 8.4 Hz), 7.86 (m, 1H), 7.69 (m, 1H), 7.44 (s, 1H), 5.73 (d, 1H, *J* = 17.2 Hz), 5.37 (d, 1H, *J* = 17.2 Hz), 5.31 (t, 2H, *J* = 1.2 Hz), 4.38 (m, 2H), 3.91 (m, 2H), 2.93 (m, 4H), 2.29 (m, 1H), 2.17 (m, 1H), 1.03 (t, 3H, *J* = 7.6 Hz) (Figure S6). $^{13}$C NMR (400 MHz, $d_6$-DMSO): δ 167.5, 156.9, 153.8, 153.2, 148.3, 146.6, 145.2, 132.0, 130.9, 130.1, 129.6, 129.4, 129.0, 128.9, 119.6, 94.8, 78.3, 66.8, 59.7, 50.7, 41.6, 36.6, 30.8, 8.0. IR ($cm^{-1}$): 2950, 1748, 1663, 1603, 1456, 1403, 1266, 1234, 1160, 1053, 438, 422 $cm^{-1}$.

**Synthesis of Carb-SS-CPT**

In a 25 mL round bottom flask, intermediate 5 (200 mg, 0.378 mmol) was combined with Carb-$C_6F_5$ (148 mg, 0.454 mmol) and proton sponge (81.1 mg, 0.378 mmol) in 3 mL of anhydrous DMSO and stirred for 24 h at room temperature. The reaction mixture was precipitated into water and then re-dissolved in DCM. The liquid was washed with aqueous sodium bicarbonate (3 × 50 mL), brine (1 × 50 mL) and water (1 × 50 mL). The organic layer was separated and dried over anhydrous $MgSO_4$. The solvent was removed *in vacuo* and the residue was purified by column chromatography (silica, 4:1 DCM/acetone). Yield: 76.1 mg (30 % yield). ESI-MS *m*/*z* ($M^+$) calcd 670.7, found 693.4 (M + $Na^+$). $^1$H NMR (400 MHz, $CDCl_3$): δ 8.42 (s, 1H), 8.22 (dd, 1H, *J* = 0.4 Hz, *J* = 8.4 Hz), 7.95 (dd, 1H, *J* = 0.8 Hz, *J* = 8.4 Hz), 7.85 (m, 1H), 7.68 (m, 1H), 7.33 (s, 1H), 5.70 (d, 1H, *J* = 17.2 Hz), 5.39 (d, 1H, *J* = 17.2 Hz),

5.32 (s, 2H), 4.65 (d, 2H, $J$ = 11.2 Hz), 4.41 (t, 2H, $J$ = 6.4 Hz), 4.35 (m, 2H), 4.16 (d, 2H, $J$ = 12 Hz), 2.93 (m, 4H), 2.28 (m, 1H), 2.17 (m, 1H), 1.32 (s, 3H), 1.01 (t, 3H, $J$ = 7.2 Hz) (Figure S7). $^{13}$C NMR (400 MHz, $CDCl_3$): δ 170.8, 167.2, 157.2, 153.4, 152.2, 148.9, 146.5, 145.5, 131.2, 130.7, 129.5, 128.5, 128.2, 128.1, 128.0, 120.1, 95.8, 78.1, 72.8, 67.0, 66.4, 63.4, 50.0, 40.2, 36.8, 36.5, 31.8, 17.5, 7.6. IR ($cm^{-1}$): 1749, 1665, 1616, 1463, 1403, 1267, 1235, 1171, 1135, 1102 $cm^{-1}$.

**Copolymerization of CarbCL and trimethylene carbonate (TMC)**

Typically, CarbCL (98.0 mg, 0.2 mmol), TMC (163.2 mg, 1.6 mmol), and mPEG-5k (100 mg, 0.02 mmol) were dissolved in anhydrous dichloromethane (1 mL) under argon, and TBD (1.39 mg, 0.01 mmol) was added to this solution to initiate polymerization. A series of samples were taken and quenched with an excess of acetic acid for $^1$H NMR and GPC analyses to determine the conversion of CarbCL and TMC as well as the molecular weight of the resulting polymer. $^1$H NMR (400 MHz, $CDCl_3$): δ 7.06 (d, 26H, $J$ = 8.8 Hz), 6.62 (d, 26H, $J$ = 8 Hz), 4.24 (m, 290H), 3.69 (s, 52H), 3.64 (s, 454H), 3.62 (s, 52H), 3.38 (s, 3H), 2.55 (t, 26H, $J$ = 6.8 Hz), 2.33 (t, 26H, $J$ = 6.8 Hz), 2.05 (m, 92H), 1.90 (m, 26H), 1.27 (br, 39H) (Figure S8b). $^{13}$C NMR (400 MHz, $CDCl_3$): δ 172.8, 154.5, 154.2, 143.9, 130.0, 129.3, 111.7, 104.7, 70.2, 63.9, 62.6, 61.3, 53.1, 46.1, 40.2, 33.5, 32.9, 27.6, 27.5, 26.2. IR ($cm^{-1}$): 2871, 1745, 1519, 1460, 1234, 1105, 1033, 949, 791, 438 $cm^{-1}$. mPEG-poly(TMC-CL) with different drug loading were synthesized by adjusting CarbCL/mPEG molar feed ratio. The ratio of peak area at 4.68 ppm (assigned to the protons of one of the methylene in the cyclic

carbonate) vs. 7.07 ppm (assigned to the aromatic protons) in the $^1$H NMR spectrum of the crude product after predetermined reaction time was used to calculate CarbCL conversion. The ratio of peak area at 2.15 ppm (assigned to –$CH_2CH_2CH_2$- in TMC) vs. 2.05 ppm (assigned to –$CH_2CH_2CH_2$- in the polymer backbone) in the $^1$H NMR spectrum of the crude product was used to calculate TMC conversion. The ratio of peak area at 4.27 ppm (assigned to the ester and carbonate bonds) vs. 3.38 ppm (assigned to the terminal methyl protons in mPEG) was used to determine the DP of CarbCL in mPEG-poly(TMC-CL). The ratio of peak area at 2.05 ppm (assigned to –$CH_2CH_2CH_2$- in the polymer backbone) vs. 3.38 ppm (assigned to the terminal methyl protons in mPEG) was used to determine the DP of TMC in mPEG-poly(TMC-CL).

**Copolymerization of Carb-O-CPT and TMC**

Typically, Carb-O-CPT (62.3 mg, 0.1 mmol), TMC (40.8 mg, 0.4 mmol), and mPEG-5k (50 mg, 0.01 mmol) were dissolved in anhydrous chloroform (0.5 mL) under argon, and TBD (0.696 mg, 0.005 mmol) was added to this solution to initiate polymerization. The reaction was quenched with excess acetic acid and the crude product was purified by twice precipitation from chloroform into diethyl ether. Yield: 126 mg (82 % yield). $^1$H NMR (400 MHz, $CDCl_3$): δ 8.41 (br, 9H), 8.21 (br, 9H), 7.94 (br, 9H), 7.84 (br, 9H), 7.66 (br, 9H), 7.34 (br, 9H), 5.69 (br, 9H), 5.38 (br, 9H), 5.30 (br, 18H), 4.30 (br, 18H), 4.24 (t, 236H, $J$ = 6 Hz), 3.64 (s, 454H), 3.38 (s, 3H), 2.28 (br, 9H), 2.17 (br, 9H), 2.05 (br, 90H), 1.25 (br, 27H), 0.99 (br, 27H) (Figure

S10). IR ($cm^{-1}$): 2871, 1745, 1519, 1460, 1243, 1105, 1033, 949, 791, 438 $cm^{-1}$. mPEG-poly(TMC-$CPT_O$) with different drug loading contents was synthesized by adjusting Carb-O-CPT/mPEG molar feed ratio.

**Copolymerization of Carb-SS-CPT and TMC**

Carb-SS-CPT (33.5 mg, 0.05 mmol), TMC (40.8 mg, 0.4 mmol), and mPEG-5k (50 mg, 0.01 mmol) were dissolved in anhydrous chloroform (0.5 mL) under argon, and TBD (0.696 mg, 0.005 mmol) was added to this solution to initiate polymerization. After achieving quantitative conversion of Carb-SS-CPT determined by $^1$H NMR spectrum, the reaction catalysts were quenched with excess acetic acid and the crude product was purified by twice precipitation from chloroform into diethyl ether. Yield: 104 mg (84 % yield). $^1$H NMR (400 MHz, $CDCl_3$): δ 8.39 (br, 4H), 8.18 (br, 4H), 7.93 (br, 4H), 7.82 (br, 4H), 7.66 (br, 4H), 7.33 (br, 4H), 5.76 (br, 4H), 5.35 (br, 4H), 5.33 (br, 8H), 4.30 (br, 8H), 4.24 (t, 202H, $J$ = 6 Hz), 3.64 (s, 454H), 3.38 (s, 3H), 2.92 (br, 16H), 2.28 (br, 4H), 2.17 (br, 4H), 2.05 (br, 88H), 1.25 (br, 12H), 1.00 (br, 12H) (Figure S11). IR ($cm^{-1}$): 2871, 1745, 1664, 1519, 1460, 1243, 1105, 1033, 949, 791, 438 $cm^{-1}$.

**Copolymerization of CarbCL, Carb-SS-CPT, and TMC**

CarbCL (24.5 mg, 0.05 mmol), Carb-SS-CPT (16.8 mg, 0.025 mmol), TMC (43.4 mg, 0.425 mmol), and mPEG-5k (50 mg, 0.01 mmol) were dissolved in anhydrous chloroform (0.5 mL) under argon, and TBD (0.696 mg, 0.005 mmol) was added to

this solution to initiate polymerization. After achieving quantitative conversion of CarbCL and Carb-SS-CPT determined by $^{1}$H NMR spectrum, the reaction catalysts were quenched with excess acetic acid and the crude product was purified by twice precipitation from chloroform into diethyl ether. Yield: 108 mg (80 % yield). $^{1}$H NMR (400 MHz, $CDCl_3$): δ 8.42 (br, 2H), 8.22 (br, 2H), 7.95 (br, 2H), 7.85 (br, 2H), 7.68 (br, 2H), 7.33 (br, 2H), 7.06 (d, 10H, $J$ = 8 Hz), 6.62 (d, 10H, $J$ = 8 Hz), 5.70 (br, 2H), 5.39 (br, 2H), 5.32 (br, 4H), 4.31 (br, 24H), 4.23 (t, 222H, $J$ = 6 Hz), 3.69 (br, 20H), 3.64 (s, 454H), 3.62 (br, 20H), 3.38 (s, 3H), 2.92 (br, 8H), 2.55 (t, 10H, $J$ = 7.2 Hz), 2.33 (t, 10H, $J$ = 7.2 Hz), 2.28 (br, 2H), 2.17 (br, 2H), 2.05 (br, 94H), 1.91 (br, 10H), 1.26 (br, 21H), 1.00 (br, 6H) (Figure S12). IR ($cm^{-1}$): 2871, 1745, 1460, 1244, 1104, 1033, 948, 791, 430, 405 $cm^{-1}$.

**Synthesis of mPEG-poly(TMC)**

TMC (204 mg, 2 mmol) and mPEG-5k (200 mg, 0.04 mmol) were dissolved in anhydrous chloroform (2 mL) under argon, and TBD (2.78 mg, 0.02 mmol) was added to this solution to initiate polymerization. After stirring for 1 hour at room temperature, the solution was precipitated into diethyl ether. Yield: 347 mg (86 % yield). $^{1}$H NMR (400 MHz, $CDCl_3$): δ 4.22 (t, 186H, $J$ = 6.4 Hz), 3.63 (s, 454H), 3.36 (s, 3H), 2.03 (br, 92H) (Figure S13). $^{13}$C NMR (400 MHz, $CDCl_3$): δ 154.7, 70.4, 64.1, 27.8. IR ($cm^{-1}$): 2872, 1744, 1462, 1405, 1246, 1099, 1034, 946, 793, 417 $cm^{-1}$.

**End-group derivatization of mPEG-poly(TMC-CL)**

*N, N'*-carbonyldiimidazole (16.2 mg, 0.1 mmol) and mPEG-poly($TMC_{17}$-$CL_{10}$) (58.2 mg, 5 μmol) were dissolved in anhydrous dichloromethane (0.5 mL) under argon, and this solution was stirred at room temperature for 24 h. Then the reaction solution was extracted thrice by water to remove the water-soluble byproduct imidazole and the un-reacted *N, N'*-carbonyldiimidazole. The final product was obtained by precipitation from dichloromethane into diethyl ether. $^1$H NMR (400 MHz, $CDCl_3$): δ 8.15 (s, 1H), 7.43 (s, 1H), 7.06 (d, 20H, $J$ = 8.8 Hz), 6.62 (d, 20H, $J$ = 8 Hz), 4.24 (m, 110H), 3.69 (s, 40H), 3.64 (s, 454H), 3.62 (s, 40H), 3.38 (s, 3H), 2.55 (t, 20H, $J$ = 6.8 Hz), 2.33 (t, 20H, $J$ = 6.8 Hz), 2.05 (m, 34H), 1.90 (m, 20H), 1.27 (br, 30H) (Figure S8c).

**Preparation of micelles**

5 mg polymer prodrug in 0.5 mL DMSO was slowly added into 1.25 mL deionized water and followed by stirring for another 0.5 hour. Subsequently, the solution was dialyzed against deionized water (2 × 4000 mL) for 24 hours (MWCO = 7.5 kDa). To prepare drug loaded mPEG-poly(TMC) micelles, the same procedure was used except the addition of CL or CPT into DMSO.

To measure the drug content, the micelle solution was lyophilized and then re-dissolved in DMF. The UV absorbance of the solution at 303 nm (CL) and 360 nm (CPT) was measured and the total loading of drugs was determined by using a linear standard curve. Drug loading content was calculated according to the following formula:

Drug loading content (wt%) = (weight of drug/weight of polymer) × 100%

**Critical micelle concentration (CMC) measurement**

Pyrene was used as the fluorescence probe to investigate the CMC of polymer prodrugs.[2] Firstly polymer was dispersed in the aqueous solution of pyrene. Then the solution was diluted to various desired concentrations from 0.5 mg $mL^{-1}$ to $1.0 \times 10^{-5}$ mg $mL^{-1}$ with a constant pyrene concentration of $6.0 \times 10^{-7}$ mol $L^{-1}$. The excitation spectra of all solutions were recorded. The emission wavelength was set at 375 nm. The $I_{339.2}/I_{334.9}$ ratio values of all solutions were recorded. As shown in Figure 1c, the intensity ratio was ~0.36 at concentrations' of mPEG-poly(TMC-CL) below ~1 μg $mL^{-1}$, that is characteristic of pyrene in a hydrophilic environment. With an increase in polymer concentration, the intensity ratio increased dramatically and finally reached ~1.0, which is characteristic of pyrene in a hydrophobic environment, suggesting partitioning of pyrene into the hydrophobic core of a micelle.

**Drug release**

0.3 mL of mPEG-poly(TMC-$CPT_{SS}$) micelle solution (1 mg $mL^{-1}$) was transferred into a dialysis membrane tubing (MWCO = 7.5 kDa). The tubing was immersed into a glass tube containing 30 mL of PBS (pH 7.4) or PBS with 10 mM of glutathione (GSH) and incubated with shaking in the dark at 37 °C. Both nonresponsive mPEG-poly(TMC-$CPT_O$) micelle incubated with 10 mM GSH and mPEG-poly(TMC-CL) in PBS (pH 7.4) were used as negative controls. At

predetermined time intervals, the total released drugs were measured by HPLC. The amount of total released drugs was determined by using a linear standard curve obtained from HPLC. For analysis of the drug release in serum, the micelles were incubated directly in serum. At predetermined time intervals, the solution was added by acetronitrile to precipitate the serum proteins. The concentration of drugs in acetronitrile was then analyzed by HPLC.

It has been reported that the scission of disulfide bond is followed by intramolecular cyclization and cleavage of the neighboring carbonate/carbamate bonds.[3] To confirm the release of free CPT from mPEG-poly(TMC-$CPT_{SS}$) under reducing conditions, the external buffer solution after 10 hours incubation was checked by LC–MS. As shown in Figure S15, the peak at 349.1 m/z demonstrated that the disulfide bond was cleaved by the nucleophilic thiolate of GSH, which induced the breakdown of the neighboring carbonate bond to generate free CPT (Scheme 2c). Because GSH has a micromolar concentration in human plasma, whereas its intracellular concentration is ~10 mM in the cytosol of most cells[4] and the cytosolic GSH concentration in some tumor cell lines is even greater than in in normal cells,[5] we expect that these reduction-sensitive mPEG-poly(TMC-$CPT_{SS}$) micelles will be stable in systemic circulation, but will efficiently release the drug upon internalization by tumor cells. Moreover, the mPEG-poly(TMC-CL) micelles, with an ester bond between the drug and polymer, showed ~55% drug release after incubation for 144 h, showing that there is slow but appreciable degradation of the ester bond at neutral pH (Figure S16a).

The *in vitro* stability of these self-assembled micelles was monitored by DLS. As shown in Figure S16c, the micelles were stable in size and polydispersity over a 48 h period in PBS containing 10% serum, indicating that they should exist in systemic circulation for a significant period of time by virtue of PEGs' stealth-like properties.

**Intracellular drug release**

4T1 cells were seeded in 6-well plates at $7 \times 10^5$ cells per well in 1 mL media and cultured for 24 hours. Cells were rinsed by PBS and incubated at 37 °C for additional 6 hours with mPEG-poly(TMC-$CPT_{SS}$) micelle at a final CPT concentration of 20 μM in culture media. Free CPT at the same concentration was used as a control. The media was removed and cells were rinsed with Hank's balanced salt solution (HBSS). Thereafter, the cells were fixed with 4% formaldehyde for 0.5 hour at room temperature, and the slides were rinsed with HBSS for three times. Finally, the cells were stained with SYTOX® green for 15 minutes at room temperature and the slides were rinsed with HBSS. The slides were mounted and imaged on a Leica SP5 confocal microscope. Images were acquired with a 63× water immersion objective.

As shown in Figure S17, the cells incubated with mPEG-poly(TMC-$CPT_{SS}$) micelles showed more uniform intracellular distribution of blue fluorescence than that of free CPT, which might be attributed to the endosome escape of mPEG-poly(TMC-$CPT_{SS}$) micelles and subsequent CPT release via reduction-triggered cleavage of disulfide bond in cells.

**Biodegradation measurement**

*In vitro* enzymatic degradation was conducted in lipase solution (lipase from *Thermomyces lanuginosus*, EC 3.1.1.3, minimum 100 000 units/g, Sigma-Aldrich, St. Louis, USA) at 37 °C with constant gentle shaking. 180 mg of mPEG-poly(TMC) was dispersed in 18 mL of a 2:1 deionized water/lipase solution, the solution was then divided into six portions and immersed into a temperature controlled shaker at 37 °C. At days 0, 0.5, 1, 2, 4, and 6, each solution was taken out and lyophilized for NMR and GPC analysis. In the $^{1}$H NMR spectrum, the peak area at 4.16 ppm was measured to calculate the degradation rate of polycarbonate backbone.

It is well-known that aliphatic polycarbonates degrade via an enzymatic process and the degradation of polycarbonate produces an alcohol and carbon dioxide.[6] As displayed in Figure S18a, the resonances at 3.66 ppm and 1.81 ppm in the $^{1}$H NMR spectrum of the degradation product from mPEG-poly(TMC) suggested that the poly(TMC) was degraded into corresponding propane-1,3-diol in the presence of lipase. As shown in Figure S18b, the GPC curve of the degraded product after 6 days incubation was closer to that of mPEG5k, indicating the majority of poly(TMC) was degraded. These data agree well with the $^{1}$H NMR results.

***In vitro* cytotoxicity**

The *in vitro* anticancer effect of polymer prodrugs was evaluated against C26 cells and 4T1 cells respectively by thiazolyl blue tetrazolium bromide (MTT) assay.[7] Cells were seeded into 96-well plates at 3 000 cells per well in 100 μL media. After 24

hours incubation, the culture media was replaced by 200 μL fresh prepared culture media containing a series of drug concentrations. The cells were further incubated for 72 hours and followed by adding 20 μL of 5 mg $mL^{-1}$ MTT assays stock solution in PBS. After 4 hours incubation, the media containing unreacted MTT was removed carefully. The obtained blue formazan crystals were dissolved in 200 μL/well DMSO and the absorbance was measured at a wavelength of 490 nm.

Cytotoxicity of mPEG-poly(TMC) without drug cargo was measured to confirm that the carrier materials alone did not contribute to cell death. As shown in Figure S19, no significant cytotoxicity was observed after 72 hours incubation even with concentration up to 1 mg $mL^{-1}$, demonstrating the good biocompatibility of the base polymer.

### *In vivo* pharmacokinetics

To measure pharmacokinetics, mPEG-poly(TMC-$CPT_{SS}$) micelle solution or free CPT dispersed in Tween 80/water (9/1 v/v) was intravenously injected into female Balb/C mice (20 mg CPT Equiv $kg^{-1}$ BW) via the tail vein according to previous protocol.[8,9] A blood sample of 10 μL was collected from the tail vein at 40 s, 0.5, 1, 2, 4, 8, 24 and 48 hours after injection and diluted into PBS with heparin (1,000 U $mL^{-1}$). The blood was centrifuged (14,000 rpm, 10 min, 4 °C) and excess dithiothreitol and 9-fold volume of acetonitrile was added to the plasma. The mixtures were vortexed and kept at room temperature for 24 hours to completely release CPT, followed by centrifuging at 14,000 rpm for 10 min. The drug concentration of the supernatant was

checked by HPLC. Assuming a 42% hematocrit for blood,[10] the amount of CPT was determined by using a linear standard curve obtained from the same media. The dataset was fit to a two-compartment pharmacokinetic model using SAAMII™ (University of Washington, Seattle, WA).

**Table S1.** Summary of all polymer prodrugs [a]

| Entry | Molar feed ratio | M1 | M2 | M3 | M4 | $M_n$ |
|---|---|---|---|---|---|---|
| | M1 : M2 : M3 : M4 : I | conv % | conv % | conv % | conv % | g mol$^{-1}$ |
| 1 | 45 : 5 : 0 : 0 :1 | >99 | >99 | | | 12600 |
| 2 | 43 : 7.5 : 0 : 0 : 1 | >99 | >99 | | | 13900 |
| 3 | 40 : 10 : 0 : 0 : 1 | >99 | >99 | | | 16900 |
| 4 | 35 : 15 : 0 : 0 : 1 | >99 | >99 | | | 18000 |
| 5 | 40 : 0 : 10 : 0 : 1 | >99 | | >99 | | 15800 |
| 6 | 37 : 0 : 3 : 0 : 1 | >99 | | >99 | | 11000 |
| 7 | 40 : 0 : 0 : 5 : 1 | >99 | | | >99 | 12800 |
| 8 | 43 : 5 : 0 : 2.5 : 1 | >99 | >99 | | >99 | 14300 |

[a] Polymerization conditions: 0.02 M mPEG-5k, 0.01 M TBD, $CH_3Cl$, 20 °C. M1, M2, M3, M4, and I are TMC, CarbCL, Carb-O-CPT, Carb-SS-CPT, and mPEG-5k, respectively. The conversion and $M_n$ were calculated by $^1$H NMR.

**Table S2.** Summary of micelle size determined by TEM [a]

| Entry | Mean diameter (+/- SD) nm |
|---|---|
| mPEG-poly($TMC_{46}$-$CL_{13}$) | 40 ± 6 |
| mPEG-poly($TMC_{49}$-$CL_4$) | 32 ± 5 |
| mPEG-poly($TMC_{41}$-$CL_{16}$) | 50 ± 6 |
| mPEG-poly(TMC-$CPT_{SS}$) | 31 ± 9 |

[a] Mean micelle size based on a statistical evaluation.

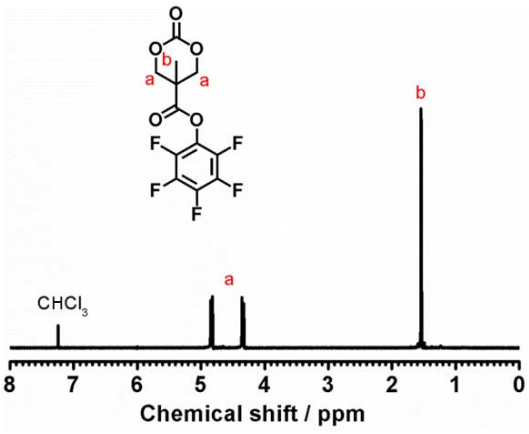


**Figure S1.** $^{1}$H NMR spectrum of Carb-$C_6F_5$.

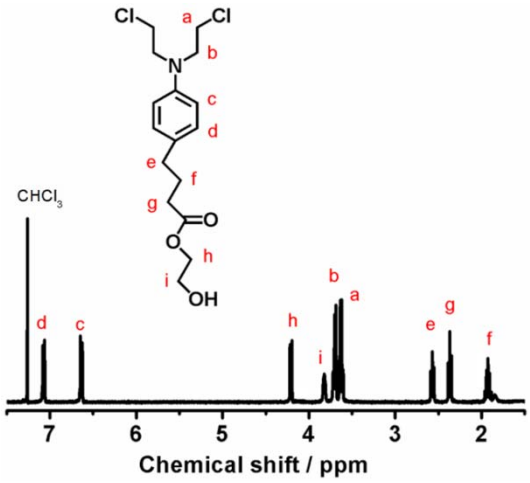


**Figure S2.** $^{1}$H NMR spectrum of intermediate 1.

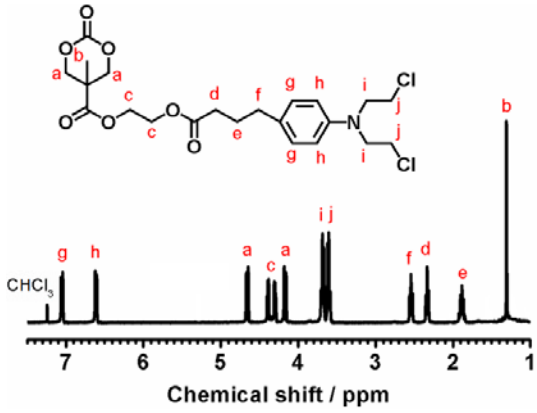


**Figure S3.** $^{1}$H NMR spectrum of CarbCL.

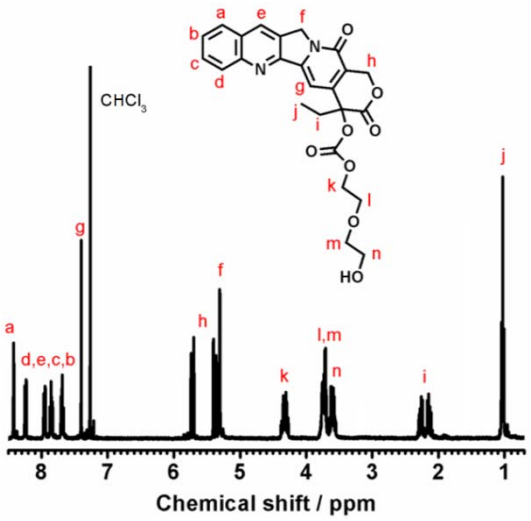


**Figure S4.** $^{1}$H NMR spectrum of intermediate 3.

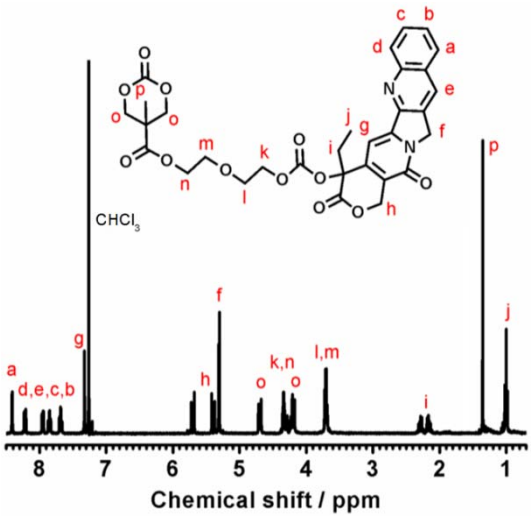


**Figure S5.** $^{1}$H NMR spectrum of Carb-O-CPT.

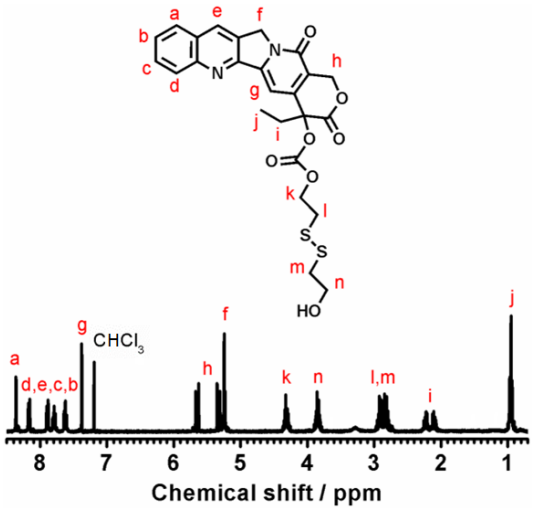


**Figure S6.** $^{1}$H NMR spectrum of intermediate 5.

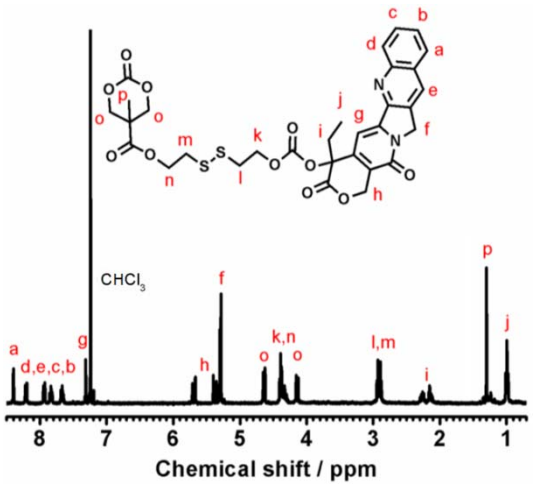


**Figure S7.** $^{1}$H NMR spectrum of Carb-SS-CPT.

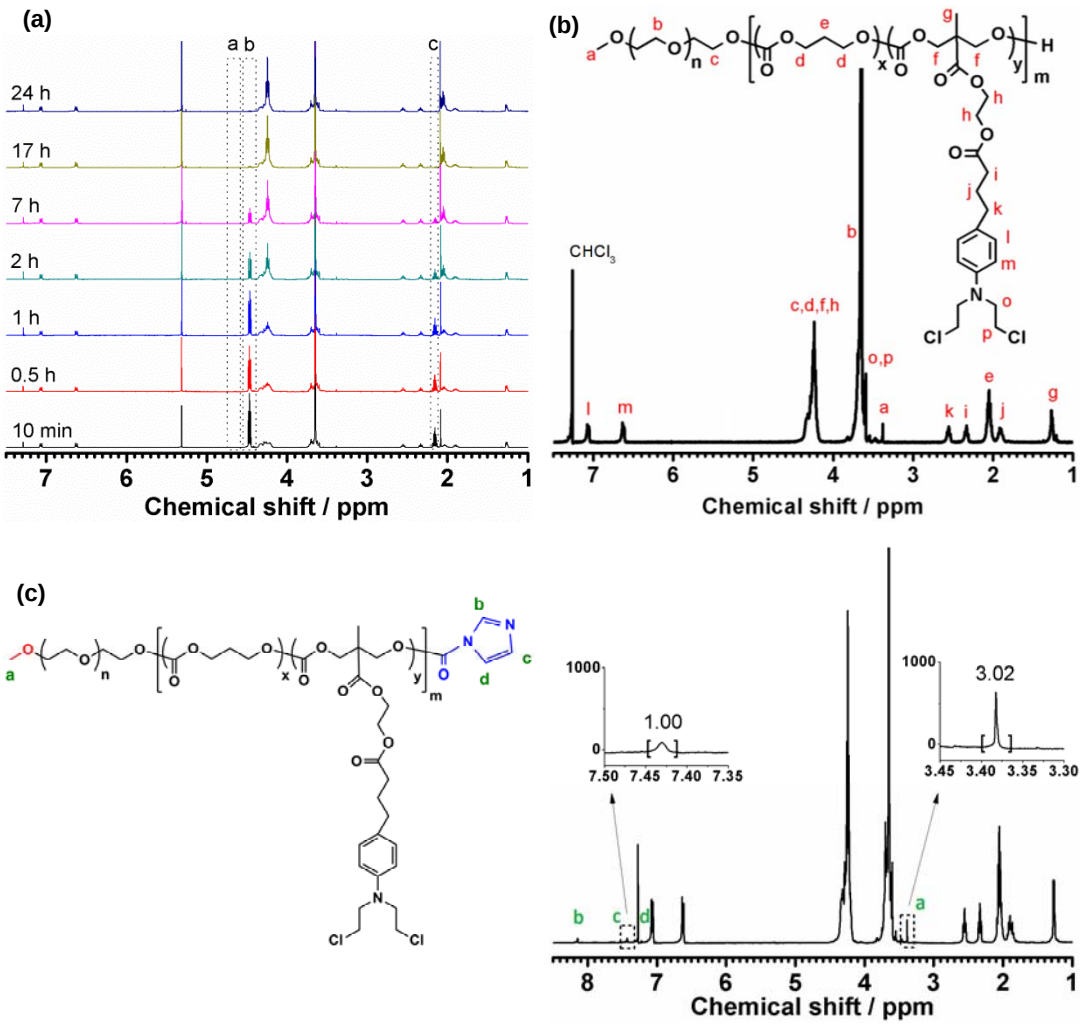


**Figure S8. a**, $^{1}$H NMR spectra of the polymerization solutions at predetermined time intervals (a is assigned to the protons of one of the methylene in the cyclic carbonate of CarbCL, b and c are separately attributed to the –C*H*$_2$CH$_2$C*H*$_2$- and –CH$_2$C*H*$_2$CH$_2$- in TMC, respectively. **b**, $^{1}$H NMR spectrum of the final mPEG-poly(TMC-CL) that was purified by precipitation from chloroform to diethyl ether. **c**, $^{1}$H NMR spectrum of mPEG-poly(TMC$_{17}$-CL$_{10}$) after reaction with *N, N'*-carbonyldiimidazole.

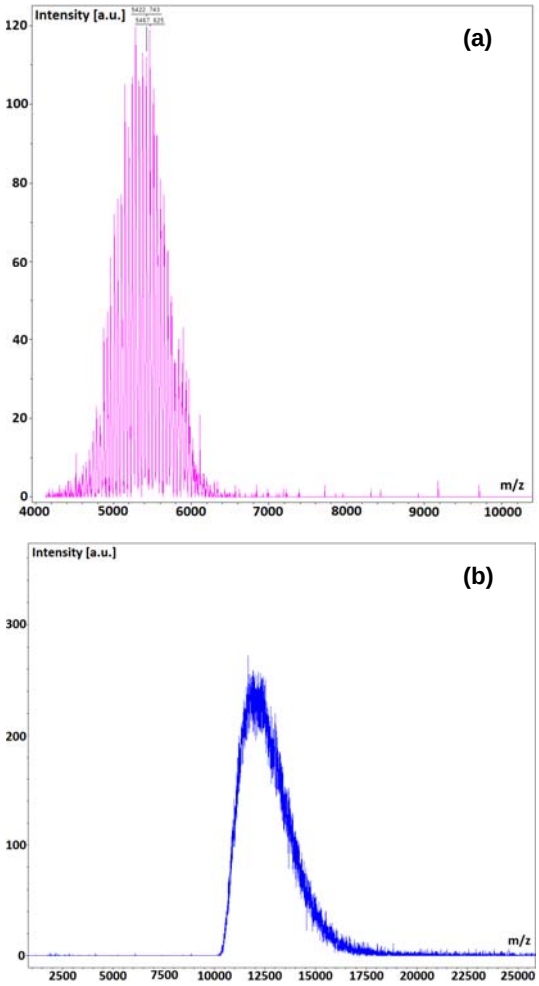


**Figure S9.** MALDI-TOF spectra of (**a**) mPEG-5k and (**b**) mPEG-poly($TMC_{17}$-$CL_{10}$), respectively.

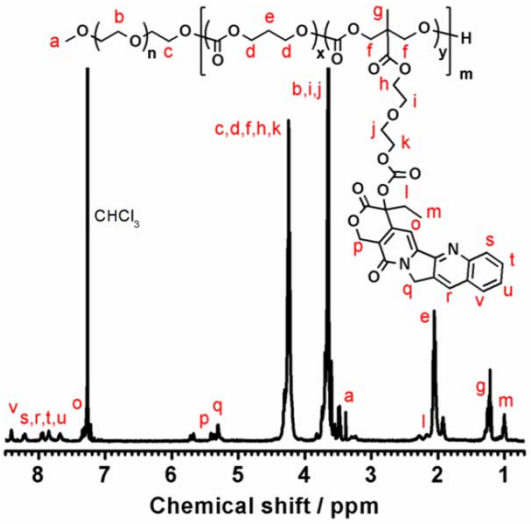


**Figure S10.** $^{1}$H NMR spectrum of mPEG-poly(TMC-CPT$_{O}$).

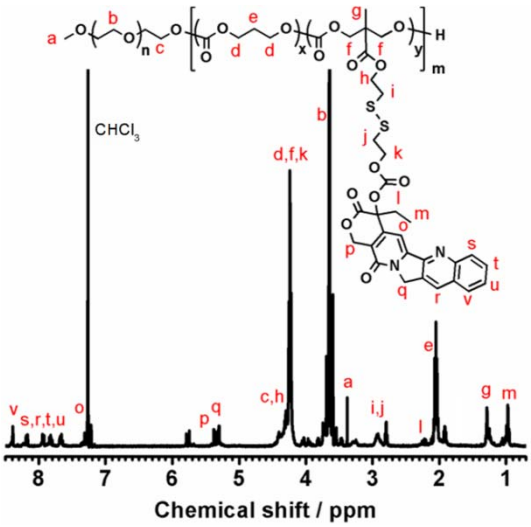


**Figure S11.** $^{1}$H NMR spectrum of mPEG-poly(TMC-CPT$_{SS}$).

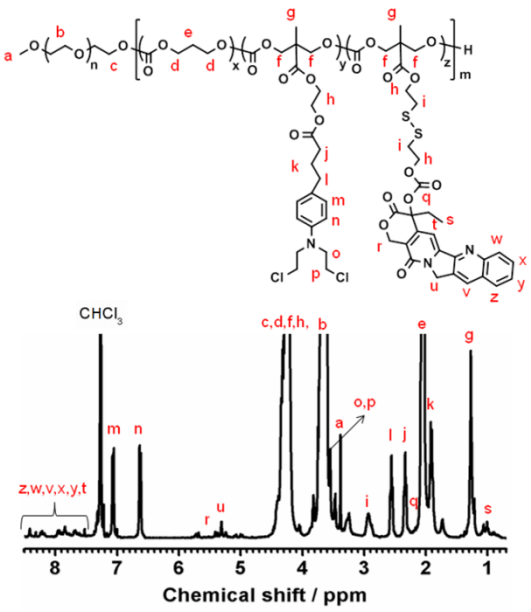


**Figure S12.** $^{1}$H NMR spectrum of mPEG-poly(TMC-CL-CPT$_{SS}$).

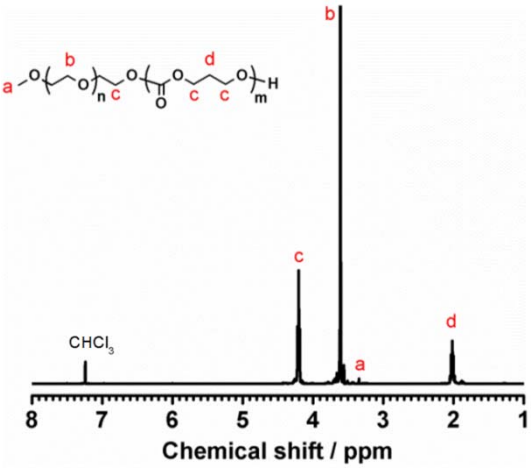


**Figure S13.** $^{1}$H NMR spectrum of mPEG-poly(TMC).

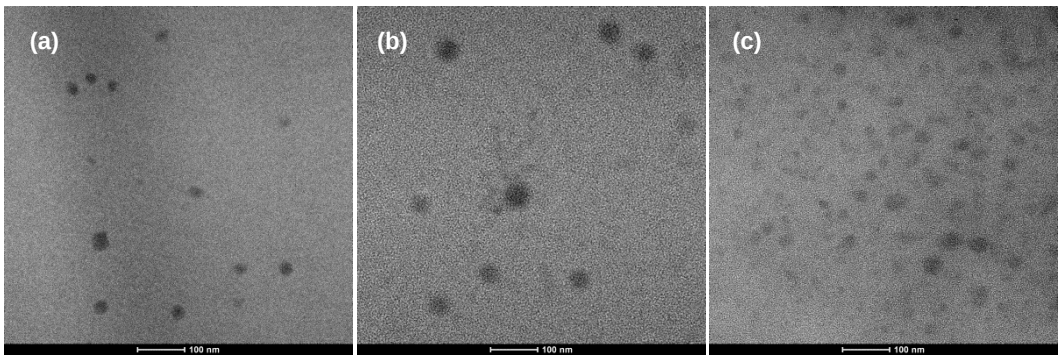


**Figure S14.** TEM images of self-assembled mPEG-poly($TMC_{49}$-$CL_4$) (**a**), mPEG-poly($TMC_{41}$-$CL_{16}$) (**b**) and mPEG-poly(TMC-$CPT_{SS}$) (**c**) micelles, respectively.

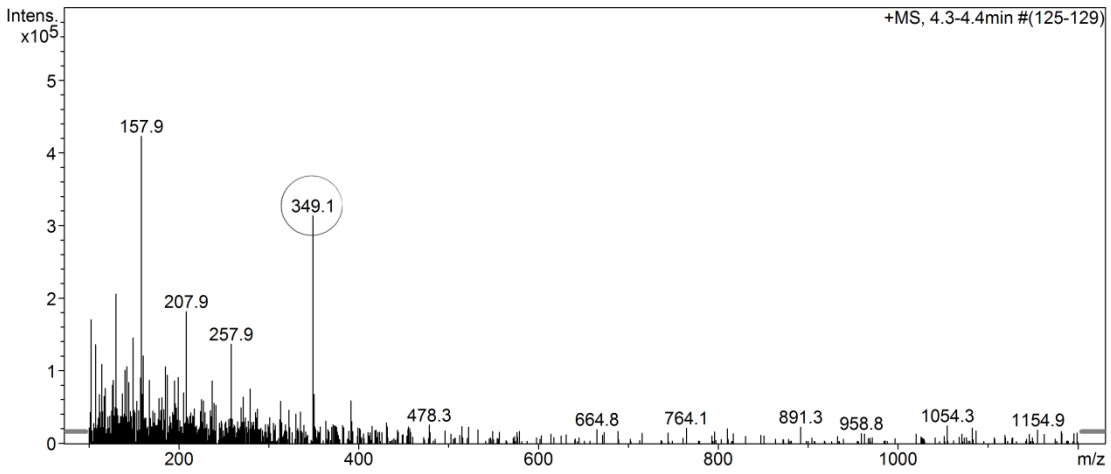


**Figure S15.** Mass spectrum of the released products from mPEG-poly(TMC-$CPT_{SS}$) with the treatment of 10 mM GSH.

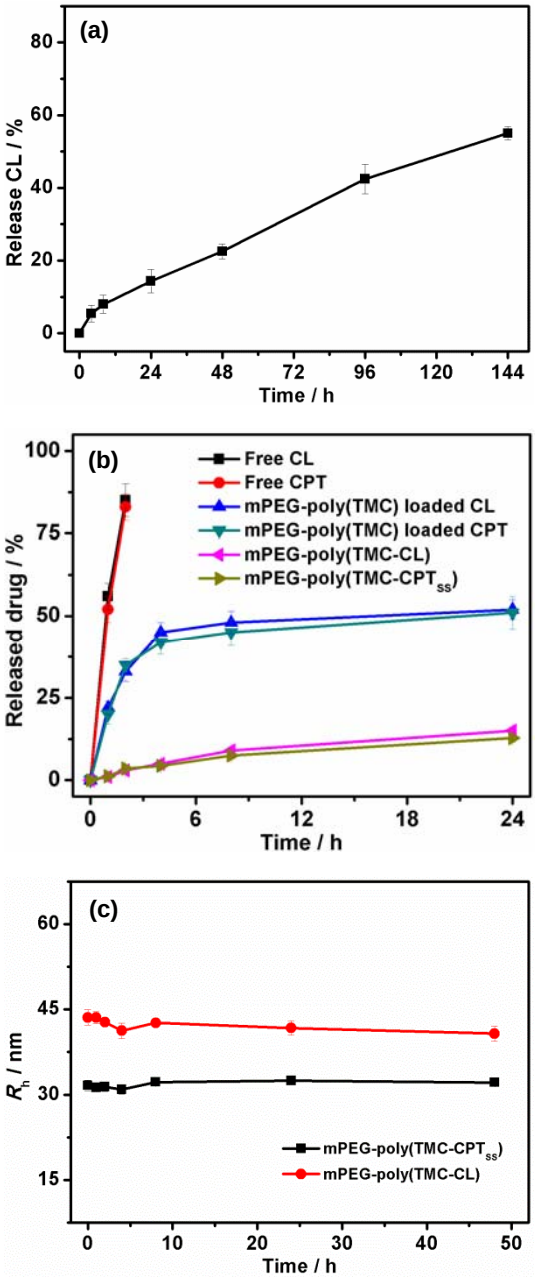


**Figure S16. a**, Cumulative CL release from mPEG-poly(TMC-CL) micelles in PBS (pH 7.4) at 37 °C. **b**, Cumulative drug release in serum at 37 °C. **c**, Stability of mPEG-poly(TMC-CL) and mPEG-poly(TMC-CPT$_{SS}$) micelles in serum at 37 °C.

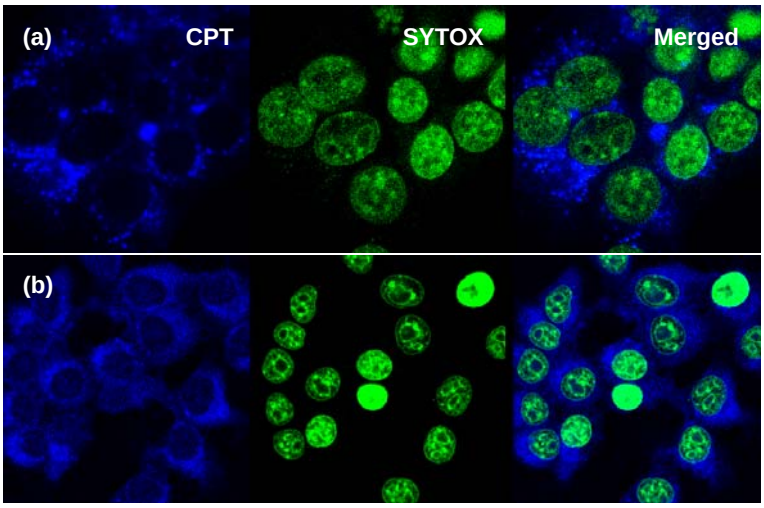


**Figure S17.** Confocal images of 4T1 cells incubated with free CPT (**a**) and mPEG-poly(TMC-$CPT_{SS}$) (**b**) for 6 hours, respectively. The cell nuclei were stained by SYTOX.

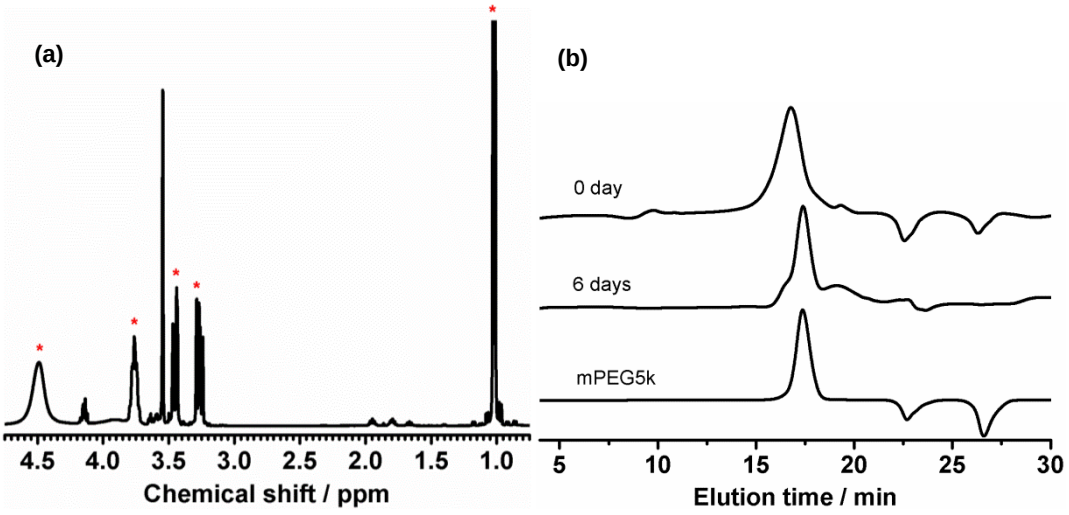


**Figure S18. a**, $^{1}H$ NMR spectrum of the degradation products from mPEG-poly(TMC) after 6 days incubation in lipase solution. The peaks labeled by asterisk refer to the signals of propane-1,2-diol originally from the lipase solution. **b**, GPC curves of mPEG5k, the mPEG-poly(TMC) before and after 6 days incubation in lipase solution.

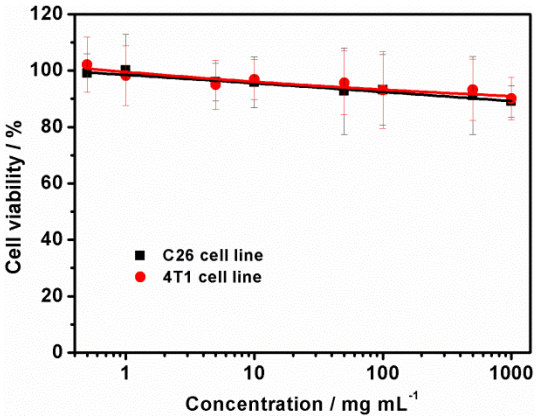


**Figure S19.** Cell viability treated with mPEG-poly(TMC)$_{46}$ for 72 hours. The % cell viability is normalized against the blank cells in the same experiment.

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