Supplementary Information for

Investigation on the controlled synthesis and post-modification of poly-[(*N*-2-hydroxyethyl)-aspartamide]-based polymers

Shixian Lv[a,1], Yuchen Wu[a,1], Jingqi Dang[a], Zhaohui Tang[b], Ziyuan Song[c], Sheng Ma[b], Xiao Wang[a], Xuesi Chen[b], Jianjun Cheng*[c] and Lichen Yin*[a]

[a] Jiangsu Key Laboratory for Carbon-Based Functional Materials and Devices, Institute of Functional Nano and Soft Materials (FUNSOM), Soochow University, Suzhou, 215123, China.

[b] Key Laboratory of Polymer Ecomaterials, Changchun Institute of Applied Chemistry, Chinese Academy of Sciences, Changchun, 130022, China

[c] Department of Materials Science and Engineering, University of Illinois at Urbana-Champaign, Urbana, Illinois, 61801, USA

[1] These authors contributed equally.

Corresponding authors: lcyin@suda.edu.cn (L. Yin); jianjunc@illinois.edu (J. Cheng)

Materials and Methods

Materials

Poly(ethylene glycol) monomethyl ether (mPEG, M_n = 5000) and methyl thiazolyl tetrazolium (MTT) were purchased from Sigma Aldrich (St. Louis, MO, USA) and used as received. mPEG-NH_2 (M_n = 5000) was prepared according to our previous work.[1] γ-Benzyl-$_L$-aspartate-*N*-carboxyanhydride (BLA-NCA) and γ-benzyl-$_L$-glutamate-*N*-carboxyanhydride (BLG-NCA) were synthesized according to the literatures.[2, 3] Paclitaxel (PTX) was purchased from Beijing Huafeng United Technology Corporation (Beijing, China). Podophyllotoxin (PPT) was purchased from Aladdin (Shanghai, China). Succinic anhydride, 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (EDC·HCl), carbonylimidazole (CDI), and dimethylaminopyridine (DMAP) were purchased from Energy Chemical (Shanghai, China). *N*, *N*-Dimethylformamide (DMF) was stored over calcium hydride (CaH_2) and purified by vacuum distillation with CaH_2 before use. Other reagents and solvents were purchased from Sinopharm Chemical Reagent Co. Ltd. (Beijing, China) and used as received.

Characterization

The nuclear magnetic resonance (NMR) spectra were recorded on Agilent 400 MHz. Molecular weight distributions (polydispersity index, PDI = M_w/M_n) of the copolymers were determined by gel permeation chromatography (GPC). For PBLA and mPEG-*b*-PBLA, the GPC analyses were performed on a Waters 1515 GPC

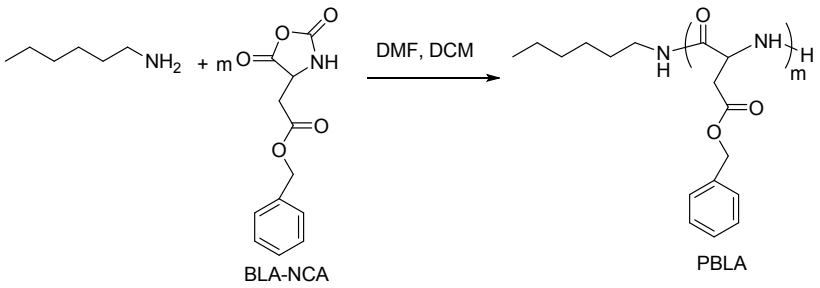
instrument equipped with MZ-gel SDplus columns (500 Å, 10^3 Å, 10^4 Å) following a differential refractive-index detector (RI 2414). DMF with 0.05 mol L^{-1} LiBr was used as the eluant at a flow rate of 0.8 mL min^{-1} at 25 °C and a series of narrow polystyrene standards were used for the calibration of the columns. For water soluble polymers, the measurement was performed on a Waters 1515 HPLC and a 2410 refractive index detector, equipped with PL aquagel-OH MIXED-M columns. 0.1 M acetate buffer (pH 2.8) was used as eluent at a flow rate of 1.0 mL min^{-1}. The sample concentration was 2.0 mg mL^{-1}, and polyethylene glycol with various molecular weights (2 mg mL^{-1}) was used as the standard for determination of the calibration curve. The size and zeta potential of particles in aqueous solution were measured by dynamic light scattering (DLS) on a Malvern Zetasizer Nano ZS90 with a He-Ne laser (633 nm) and 90° collecting optics. Critical micelle concentration (CMC) was measured by fluorescence spectroscopy using pyrene as a probe on a France JY Fluromax 4 fluorescence spectrophotometer with the emission wavelength of 390 nm. The excitation fluorescence at 340 and 335 nm was monitored. CMC was estimated as the cross-point of the tangent to the horizontal line of I_{340}/I_{335} with the relative constant values and the diagonal line with rapidly increased I_{340}/I_{335} ratio.

Cell culture

NIH/3T3 (mouse embryo fibroblast) cells and MCF-7 (human breast carcinoma) cells were purchased from the American Type Culture Collection (Rockville, MD, U.S.A.) and cultured in Dulbecco's modified Eagle's medium (DMEM) with high glucose

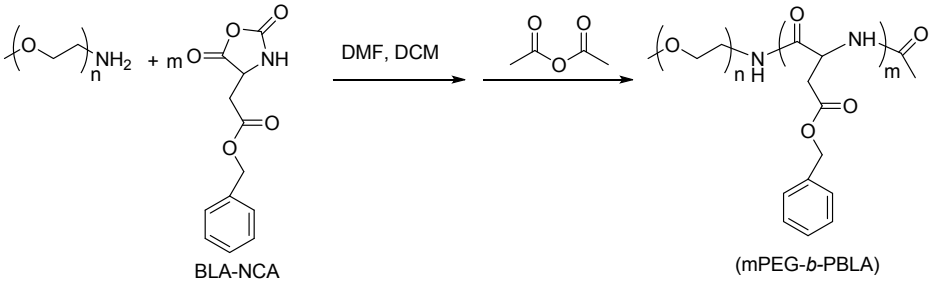
containing 10% FBS, supplemented with 50 U mL^{-1} penicillin and 50 U mL^{-1} streptomycin, and incubated at 37 ^{o}C in 5% CO_2 atmosphere.

Synthesis of poly(β-benzyl-$_L$-aspartate) (PBLA) homopolymers



PBLA with designed DP (50, 100, and 150) were synthesized through the ring opening polymerization (ROP) of BLA-NCA monomer initiated by the terminal primary amino group of hexanamine. Briefly, BLA-NCA (11.16 g, 44.8 mmol) was dissolved in a mixture of dry DMF (11.16 mL) and dichloromethane (DCM, 100.4 mL). Afterwards, different amount of hexanamine was added into the above solution *via* a syringe under argon. The reaction was maintained at 35 °C under gentle stirring for 3 days. PBLA was obtained after precipitation in excess amount of iced diethyl ether (yield: 89%). DP (which represents the DP of amino acid unit unless otherwise specified throughout the manuscript) of the obtained polymers was determined by ^{1}H NMR in trifluoroacetic acid-*d* (TFA-*d*).

Synthesis of methoxy poly(ethylene glycol)-*b*-poly(β-benzyl-$_L$-aspartate) (mPEG-*b*-PBLA) copolymers



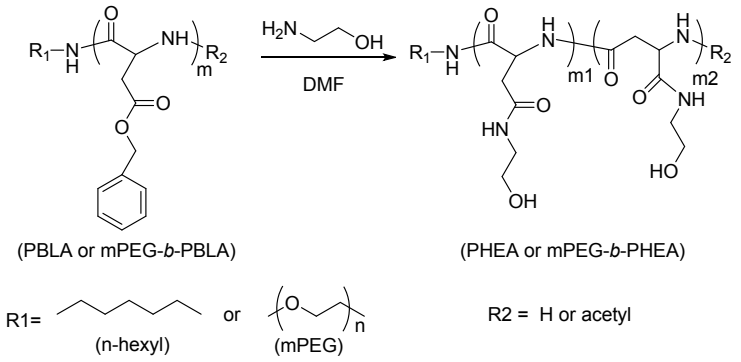
The mPEG-*b*-PBLA block copolymers with designed DP of BLA unit (12, 22, and 32) were synthesized through the ROP of BLA-NCA monomer with mPEG-NH_2 as the macro-initiator. Briefly, BLA-NCA (5.58 g, 22.4 mmol) was dissolved in the mixture of dry DMF (5.58 mL) and DCM (50.2 mL). Different amount of mPEG-NH_2 was dehydrated through an azeotropic process with toluene, and the remaining toluene was removed under vacuum. Afterwards, mPEG-NH_2 dissolved in dry DCM was added into the BLA-NCA solution *via* a syringe under argon. The reaction was maintained at 35 °C under gentle stirring for 3 days. Then excessive acetic anhydride was added to the solution to block the terminal amino group, and the mixture was maintained at 35 °C for another 12 hours. The mixture was concentrated under vacuum at 60 °C. mPEG-*b*-PBLA copolymer was obtained after precipitation in excess amount of iced diethyl ether (yield: 86%). The DP of obtained mPEG-*b*-PBLA was determined by ^{1}H NMR in TFA-*d*.

Investigation of the aminolysis process of PBLA with ethanolamine

PBLA (0.50 g, DP = 50) was suspended in dry DMF (5 mL), and different amount of ethanolamine (1, 3, or 9 equivalents to the BLA unit) was added. The mixture was maintained at 35 °C. At determined time intervals, the reaction mixture was withdrawn and precipitated into diethyl ether. The precipitate was washed thoroughly

by diethyl ether and evaporated under vacuum. Afterwards, ^{1}H NMR measurement was performed to analyze the structure of the intermediate product using TFA-*d* as the solvent.

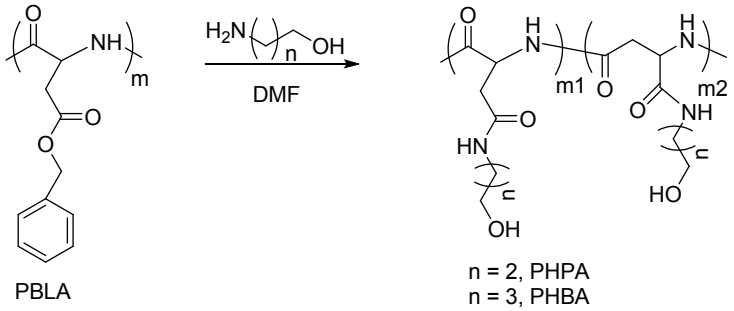
Synthesis of PHEA and mPEG-*b*-PHEA



PHEA and mPEG-*b*-PHEA were synthesized by aminolysis of PBLA (designed DP = 50, 100 or 150) and mPEG-*b*-PBLA (designed DP = 12, 22 or 32) by ethanolamine in DMF based on the above result. Typically, PBLA or mPEG-*b*-PBLA (4.0 g) was suspended or dissolved in DMF (40 mL), and ethanolamine (3 equivalents to BLA unit) was added. The mixture was maintained at 35 °C for 12 h. The solution was precipitated with excess amount of cold diethyl ether to remove unreacted small molecules. The precipitation was washed three times by diethyl ether, and the precipitate was dissolved in DMF and dialyzed against distilled (DI) water. The purified product was obtained as white solid after freeze-drying.

To verify the structures of PHEA and mPEG-*b*-PHEA, the fresh-made or lyophilized materials were dissolved in TFA-*d* for ^{1}H NMR measurement at designed temperatures (25 and 50 °C).

Synthesis of poly-[(*N*-3-hydroxypropyl)-aspartamide] (PHPA) and poly-[(*N*-4-hydroxybutyl)-aspartamide] (PHBA)



PHPA and PHBA were synthesized according to the same procedure for PHEA. In brief, PBLA (2.0 g, DP = 50) was suspended in DMF (20 mL), and 3-amino-1-propanol or 4-amino-1-butanol (3 equivalents to the BLA unit) was added. The mixture was maintained at 35 °C for 12 h. The solution was precipitated with excess amount of cold diethyl ether to remove unreacted small molecules. The precipitate was washed three times by diethyl ether, and white solid of PHPA or PHBA was obtained after freeze-drying. The structures of PHPA and PHBA were confirmed by ^{1}H NMR of PAED using the mixture of TFA-*d* and D_2O (1:9) as the solvent.

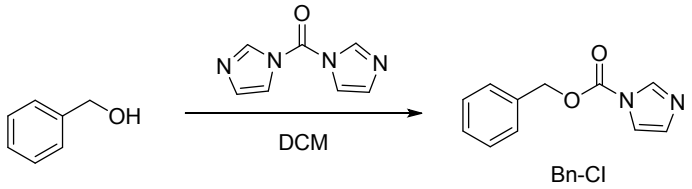
Study of water solubility of the resulting polymers

To test the aqueous solubility of the polymers, 200 mg of lyophilized PHEA, mPEG-*b*-PHEA, PHPA, PHBA, or mPEG-NH_2 were placed in tiny bottles, and different amount of DI water, phosphate buffered saline (PBS, 0.01M, pH = 7.4), 0.1 M HCl or 0.1 M NaOH aqueous solution was added. After complete dissolution, the solution was photographed.

***In vitro* cytotoxicity study**

The *in vitro* cytotoxicity of mPEG-*b*-PHEA was evaluated by the MTT assay. Briefly, NIH/3T3 and MCF-7 cells were seeded in 96-well plates at 7,000 cells/well in 100 μL of DMEM and incubated at 37 °C for 24 h. The medium was replaced with 200 μL fresh DMEM containing mPEG-*b*-PHEA at different concentrations. After 48-h incubation, cells were subjected to viability assessment using the MTT assay. Data were presented as means ± SD (n = 3).

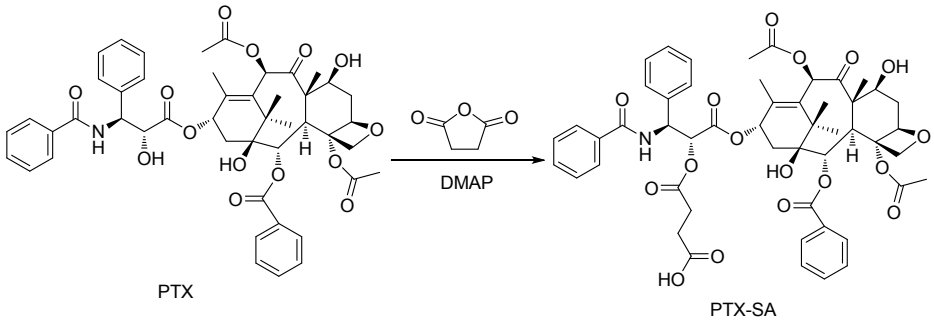
Synthesis of benzyl carbonylimidazole (Bn-CI)



Bn-CI

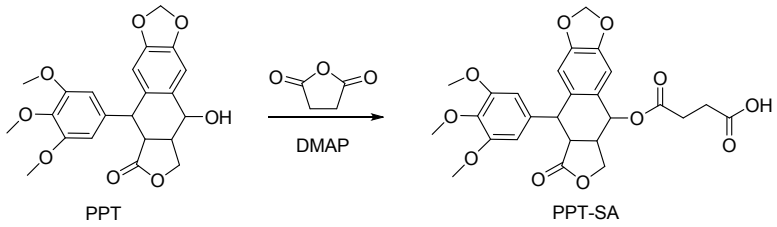
Bn-CI was synthesized according to the literature with minor modification.[4] Briefly, benzyl alcohol (Bn-OH, 5.0 g, 46.2 mmol) was dissolved in anhydrous DCM (50 mL) in a flame-dried 100-mL flask. Carbonyldiimidazole (15 g, 92.5 mmol) was added and the reaction was stirred overnight at room temperature. The mixture was diluted into ethyl acetate (200 mL) and washed with DI water (3 × 100 mL). The organic layer was washed with brine (3 x 50 mL), dried over $MgSO_4$, and concentrated in vacuum to give a light yellow liquid. The structure was determined by ^1H NMR using $CDCl_3$ as the solvent.

Synthesis of 2'-O-succinyl-paclitaxel derivative (PTX-SA)



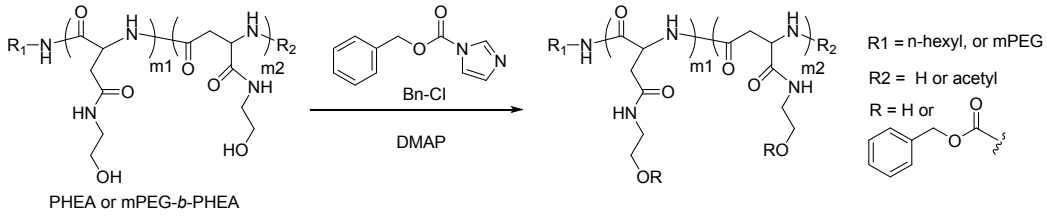
PTX-SA was synthesized according to the literature with minor revision.[5] Briefly, PTX (300 mg), succinic anhydride (38.64 mg), and DMAP (42.6 mg) were dissolved in dry DCM (5 mL) in a flame-dried flask and stirred at room temperature for 48 h. The absence of unreacted drug was verified by TLC. The mixture was then diluted in DCM (100 mL) and washed with DI water (3 × 100 mL). The organic layer was washed with brine (3 × 50 mL) and dried over $MgSO_4$. After the solvent was evaporated, the crude product was purified by preparative TLC (methyl alcohol/DCM = 1/100 to 1: 20). ^{1}H NMR ($CDCl_3$): δ 1.11 [s, CH3], 1.20 [s, CH3], 1.69 [s, CH3], 1.9 [s, CH3], 2.2 [m, OAc], 2.4 [m, OAc], 2.5-2.8 [m, HOOC-CH_2CH_2-COO-PTX], 3.78 [d, CH], 4.17 [d, 20CH2], 4.3 [d, CH2], 4.46 [dd, CH], 4.96 [d,CH], 5.50 [d, CH], 5.67 [d, CH], 5.98 [dd, CH], 6.22 [t, CH], 6.27 [s, CH], 7.25 [s, Ph], 7.4 [m, NBz], 7.5 [m, OBz], 7.73 [d, 30-NBz], 8.1 [d, 2-OBz]. MS (MALDI-TOF): calcd. for $C_{51}H_{56}NO_{17}$ $[M+H]^+$ *m*/*z*, 954.4, found 954.3; calcd. for $C_{51}H_{55}NO_{17}Na$ $[M+Na]^+$ *m*/*z*, 976.3, found 976.3

Synthesis of succinyl-podophyllotoxin derivative (PPT-SA)



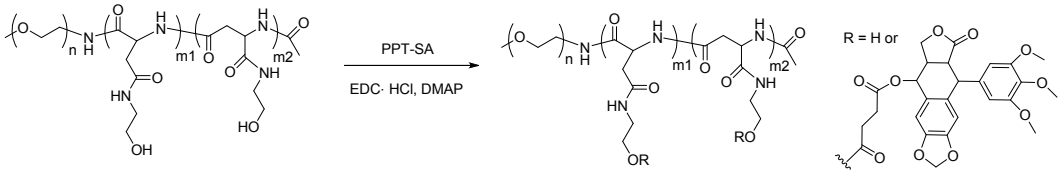
PPT-SA was synthesized according to the similar process for PTX-SA. Briefly, PPT (1.20 g), succinic anhydride (579 mg) and DMAP (707 mg) were dissolved in dry DCM (20 mL) in a flame-dried flask and stirred at room temperature for 48 h. The absence of unreacted drug was verified by TLC. The mixture was then diluted in DCM (300 mL) and washed with DI water (3 × 200 mL). The organics were washed with brine (3 × 200 mL), dried over $MgSO_4$. After the solvent was evaporated, the crude product was purified by preparative TLC (methyl alcohol/DCM = 1/100 to 1: 20). ^{1}H NMR measurement was performed using TFA-*d* as the solvent. MS (ES-): calcd. for $C_{26}H_{25}O_{11}$ $[M-H]^-$ *m*/*z*, 513.1, found 513.3

The reactivity of hydroxyl groups in PHEA and mPEG-*b*-PHEA



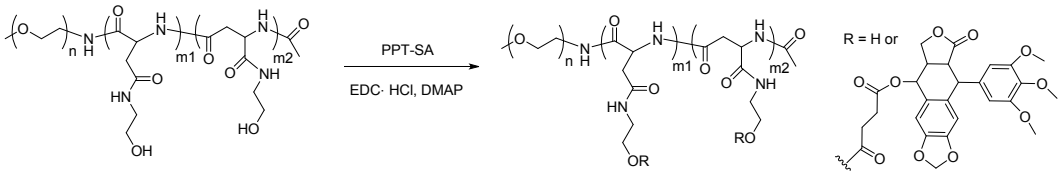
In a round flame-dried flask, 500 mg of PHEA (DP = 50, P1) or mPEG-*b*-PHEA (DP = 21, P5) was added, which was dried under high vacuum for 6 h. Then different amount of Bn-CI and equivalent DMAP were added. Afterwards, dry DMF (6 mL) was added and the reaction was maintained at different temperatures (25, 50, 70, 90 °C). At the designed time intervals, the reaction mixture was withdrawn and

precipitated into diethyl ether. The precipitate was washed by diethyl ether for three times and the residual solvent was evaporated under vacuum. Afterwards, the structure of the resulting PHEA-*g*-Bn or mPEG-*b*-PHEA-*g*-Bn (PG-1) was determined by ^{1}H NMR measurement using TFA-*d* as the solvent.



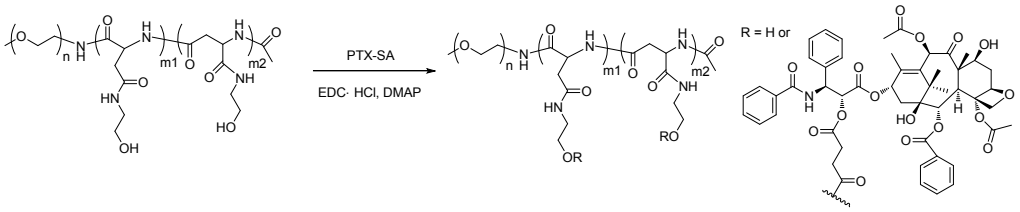
To further test the reaction activity of the pendant hydroxyl groups, mPEG-*b*-PHEA (P5, 500 mg), PPT-SA (93 mg), EDC·HCl (69 mg), and DMAP (22 mg) were added into a round flame-dried flask, and dissolved in dry DMF (10 mL) under vacuum for 6 h. The reaction was maintained at different temperatures (25, 50 °C). At the desired time intervals (12, 24, 48, and 72 h), the reaction mixture was withdrawn and precipitated with excess amount of diethyl ether to remove unreacted small molecules. The residual solvent was evaporated under vacuum. The purified product was obtained as a white solid after freeze-drying. The structures of the resulting mPEG-*b*-PHEA-*g*-PPT conjugates were determined by ^{1}H NMR using TFA-*d* as the solvent. The amount of PPT in the conjugate was measured by UV-Vis spectrometry at 292 nm.

Synthesis of mPEG-*b*-PHEA-*g*-PPT (PG-2)



mPEG-*b*-PHEA (P5, 500 mg), PPT-SA (186 mg), EDC·HCl (138 mg), and DMAP (44 mg) were added into a round flame-dried flask, and dissolved in dry DMF (10 mL) under vacuum for 6 h. The reaction was maintained at 50 °C for 24 h. The mixture was precipitated with excess amount of diethyl ether to remove unreacted PPT-SA and other small molecules. The precipitation was repeated twice before pumping vacuum and the crude product of mPEG-*b*-PHEA-*g*-PPT was obtained. The crude product was re-dissolved in DMF and dialyzed against DI water. The purified product was obtained as a white solid after freeze-drying. The structure was determined by ^{1}H NMR using TFA-*d* as the solvent. The amount of PPT in mPEG-*b*-PHEA-*g*-PPT was measured by UV-Vis spectrometry at 292 nm.

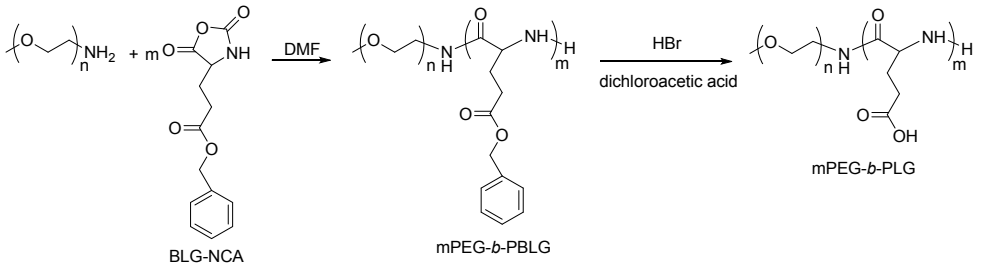
Synthesis of mPEG-*b*-PHEA-*g*-PTX (PG-3)



mPEG-*b*-PHEA (P5, 400 mg), PTX-SA (184 mg), EDC·HCl (74 mg), and DMAP (23.5 mg) were added into a round flame-dried flask, and dissolved in dry DMF (6 mL) under vacuum for 6 h. The reaction was maintained at 50 °C for 24 h. The mixture was precipitated with excess amount of diethyl ether to remove unreacted PTX-SA and other small molecules. The precipitation was repeated twice before pumping vacuum and the crude product of mPEG-*b*-PHEA-*g*-PTX was obtained. The crude product was re-dissolved in DMF and dialyzed against DI water. The purified

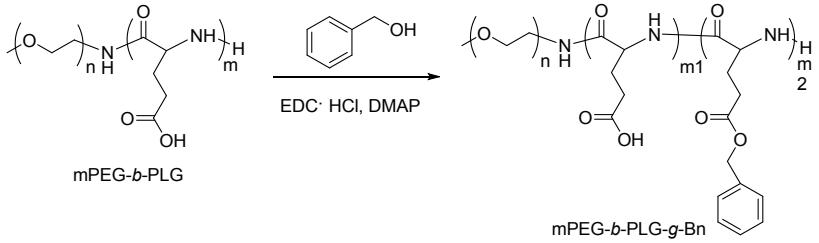
product was obtained as a white solid after freeze-drying. The structure was determined by ^{1}H NMR using a mixture of dimethylsulfoxide-*d*^{6} (DMSO-*d*^{6}) and TFA-*d* (1:1) as the solvent.

Synthesis of methoxy poly(ethylene glycol)-*b*-poly(L-glutamic acid) (mPEG-*b*-PLG)



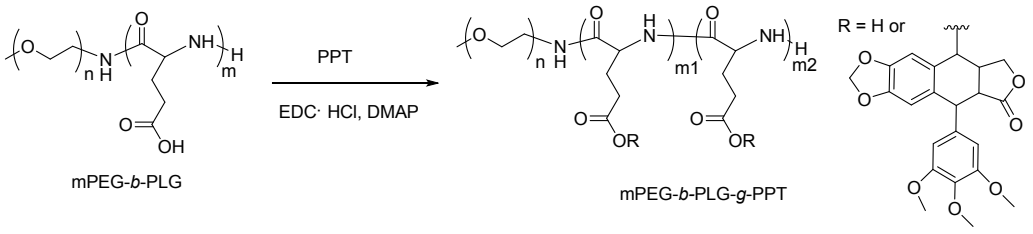
mPEG-*b*-PLG was synthesized through the ROP of BLG-NCA monomer with mPEG-NH_2 as the macro-initiator followed by deprotection of benzyl groups according to the literature.[6] Typically, BLG-NCA (3.472 g, 13.2 mmol) and mPEG-NH_2 (3.0 g, 0.6 mmol) were dissolved in dry DMF. The polymerization was performed at 25 °C for 3 days. Then, the solution was precipitated into excess amount of cold diethyl ether to give the methoxy poly(ethylene glycol)-*b*-poly(γ-benzyl-L-glutamate) (mPEG-*b*-PBLG). Subsequently, mPEG-*b*-PBLG (4.0 g) was dissolved in 40 mL of dichloroacetic acid. After addition of 12 mL of HBr/acetic acid (33 wt %), the solution was slowly stirred at 25 °C for 1 h and the final product was precipitated into excessive diethyl ether. The precipitate was dialyzed against DI water and freeze-dried, yielding a white solid. The DP of the glutamic acid unit was determined to be 21 by ^{1}H NMR in TFA-*d*.

Synthesis of mPEG-*b*-PLG-*g*-Bn (PG-4)



Benzyl alcohol was grafted to mPEG-*b*-PLG to give mPEG-*b*-PLG-*g*-Bn. In brief, mPEG-*b*-PLG (500 mg, 0.0649 mmol), benzyl alcohol (91 mg, 0.845 mmol), EDC·HCl (322 mg, 1.69 mmol), and DMAP (103 mg, 0.845 mmol) were dissolved in dry DMF. The reaction was maintained at 25 °C for 3 days. The mixture was precipitated with excess amount of diethyl ether to remove unreacted small molecules. The precipitation was repeated twice before pumping vacuum and the crude product of mPEG-*b*-PLG-*g*-Bn was obtained. The crude product was re-dissolved in DMF and dialyzed against DI water. The purified product was obtained as a white solid after freeze-drying. The structure was determined by ^{1}H NMR measurement using TFA-*d* as the solvent.

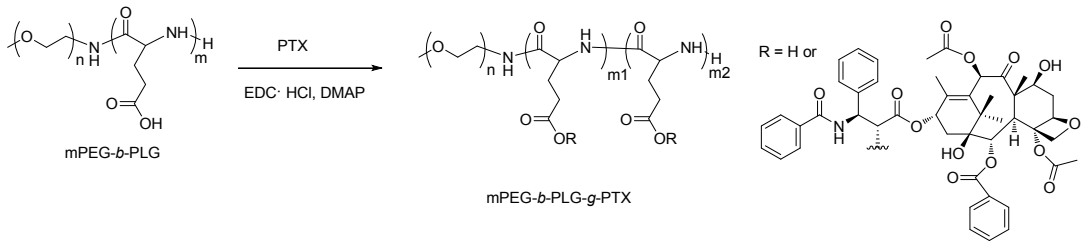
Synthesis of mPEG-*b*-PLG-*g*-PPT (PG-5)



PPT was grafted to mPEG-*b*-PLG to give mPEG-*b*-PLG-*g*-PPT. In brief, mPEG-*b*-PLG (500 mg, 0.0649 mmol), PPT (161 mg, 0.388 mmol), EDC·HCl (148 mg, 0.775 mmol), and DMAP (47.5 mg, 0.388 mmol) were dissolved in dry DMF. The reaction

was maintained at 25 °C for 3 days. The mixture was precipitated with excess amount of diethyl ether to remove unreacted PPT-SA and other small molecules. The precipitation was repeated twice before pumping vacuum and the crude product of mPEG-*b*-PLG-*g*-PPT was obtained. The crude product was re-dissolved in DMF and dialyzed against DI water. The purified product was obtained as a white solid after freeze-drying. The structure was determined by ^{1}H NMR measurement using TFA-*d* as the solvent. The amount of PPT in the conjugate was measured by UV-Vis spectrometry at 292 nm.

Synthesis of mPEG-*b*-PLG-*g*-PTX (PG-6)



PTX was grafted to mPEG-*b*-PLG to give mPEG-*b*-PLG-*g*-PTX. In brief, mPEG-*b*-PLG (400 mg, 0.0519 mmol), PTX (133 mg, 0.156 mmol), EDC·HCl (60 mg, 0.314 mmol), and DMAP (19.2 mg, 0.157 mmol) were dissolved in dry DMF. The reaction was maintained at 25 °C for 3 days. The mixture was precipitated with excess amount of diethyl ether to remove unreacted PTX and other small molecules. The precipitation was repeated twice before pumping vacuum and the crude product of mPEG-*b*-PLG-*g*-PTX was obtained. The crude product was re-dissolved in DMF and dialyzed against DI water. The purified product was obtained as a white solid after freeze-drying. The structure was determined by ^{1}H NMR measurement using a

mixture of dimethylsulfoxide-d^6 (DMSO-d^6) and TFA-*d* (1:1) as the solvent.

Water solubility of the conjugates

To test the aqueous solubility and re-dissolution ability of the conjugates obtained from mPEG-*b*-PHEA, lyophilized mPEG-*b*-PHEA-*g*-Bn (PG-1, 200 mg), mPEG-*b*-PHEA-g-PPT (PG-2, 200 mg), and mPEG-*b*-PHEA-*g*-PTX micelles (PG-3, 10 mg) were placed in tiny bottles. Then different amount of PBS was added. Afterwards, the state was photographed. The conjugates prepared from mPEG-*b*-PLG were used as control groups.

Table S1. Characterizations of PBLA and mPEG-*b*-PBLA.

Entry	Polymer	Designed DP	Obtained DP[a]	PDI[b]
1	PBLA	50	50	--
2	PBLA	100	98	--
3	PBLA	150	140	--
4	mPEG-*b*-PBLA	12	12	1.06
5	mPEG-*b*-PBLA	22	21	1.09
6	mPEG-*b*-PBLA	32	30	1.10

[a] Measured by ^{1}H NMR in TFA-*d*; [b] Determined by GPC using DMF as the solvent.

Table S2. Reaction of mPEG-*b*-PHEA (P5) with Bn-CI and PPT-SA at 25 °C.

Entry	Reaction agent	Molar ratio to polymer	Reaction time (h)	Conversion ratio (%)
1	Bn-CI	10	24	28[a]
2	Bn-CI	10	48	30[a]
3	Bn-CI	10	168	30[a]
4	PPT-SA	3	24	31[b]
5	PPT-SA	3	48	33[b]
6	PPT-SA	3	72	32[b]
7	PPT-SA	6	48	25[b]

[a] Measured by ^{1}H NMR in TFA-*d*. [b] Measured by UV-Vis spectrometer at 292 nm.

Table S3. Reaction of PHEA and mPEG-*b*-PHEA at various temperatures for 24 h.

Entry	Polymer	Reaction agent	Molar ratio to polymer	T (°C)	Conversion ratio (%)
1	P1	Bn-CI	10	70	85 [a]
2	P1	Bn-CI	10	80	84 [a]
3	P5	Bn-CI	10	50	88 [a]
4	P5	Bn-CI	10	70	86 [a]
5	P5	Bn-CI	10	85	87 [a]
6	P5	PPT-SA	3	50	86 [b]

[a] Measured by ^{1}H NMR in TFA-*d*. [b] Measured by UV-Vis spectrometer at 292 nm.

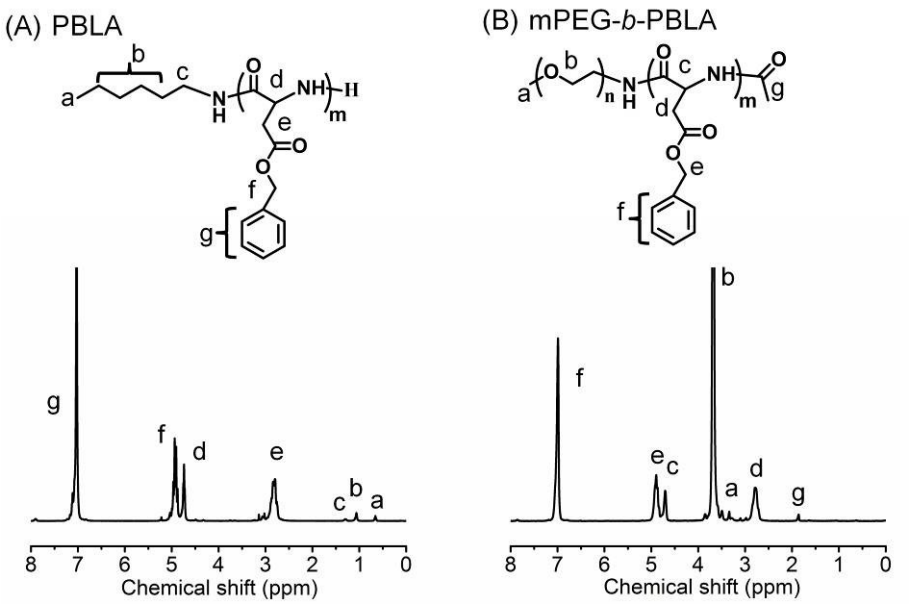


Fig. S1. ^{1}H NMR spectra of PBLA (A) and mPEG-*b*-PBLA (B) in TFA-*d*.

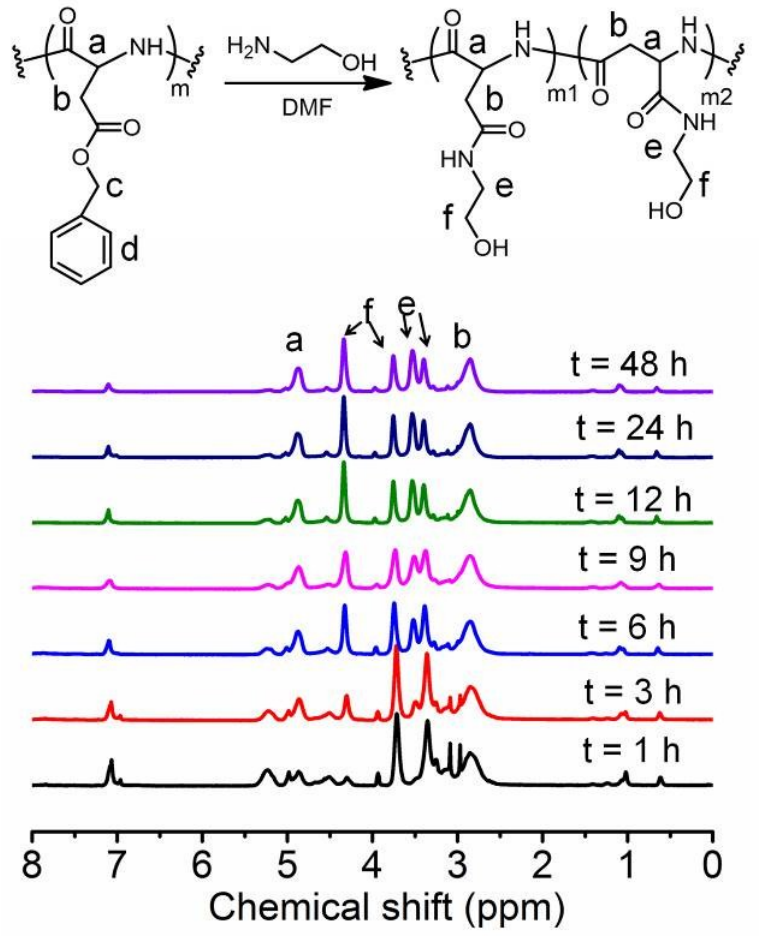


Fig. S2. ^{1}H NMR spectra of PBLA (DP = 50) after aminolysis by ethanolamine (1 equivalent to the BLA unit) for different incubation time (^{1}H NMR solvent: TFA-*d*).

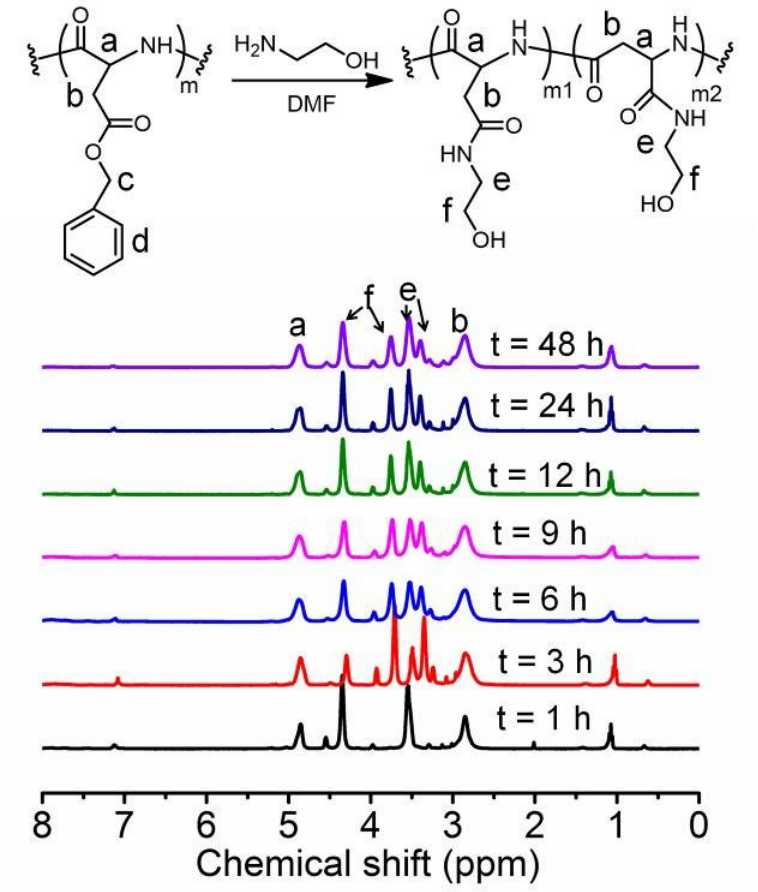


Fig. S3. ^{1}H NMR spectra of PBLA (DP = 50) after aminolysis by ethanolamine (9 equivalents to BLA unit) for different time (^{1}H NMR solvent: TFA-*d*).

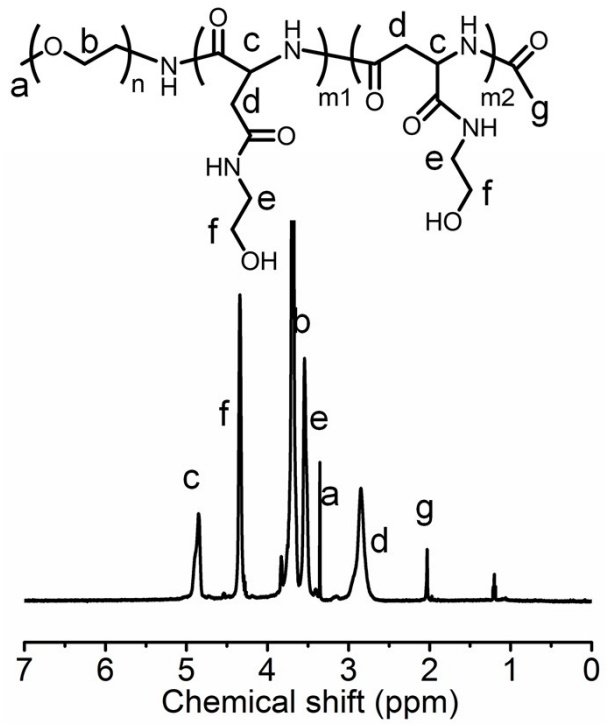


Fig. S4. ^{1}H NMR spectrum of mPEG-*b*-PHEA (DP = 21, P5) in TFA-*d*.

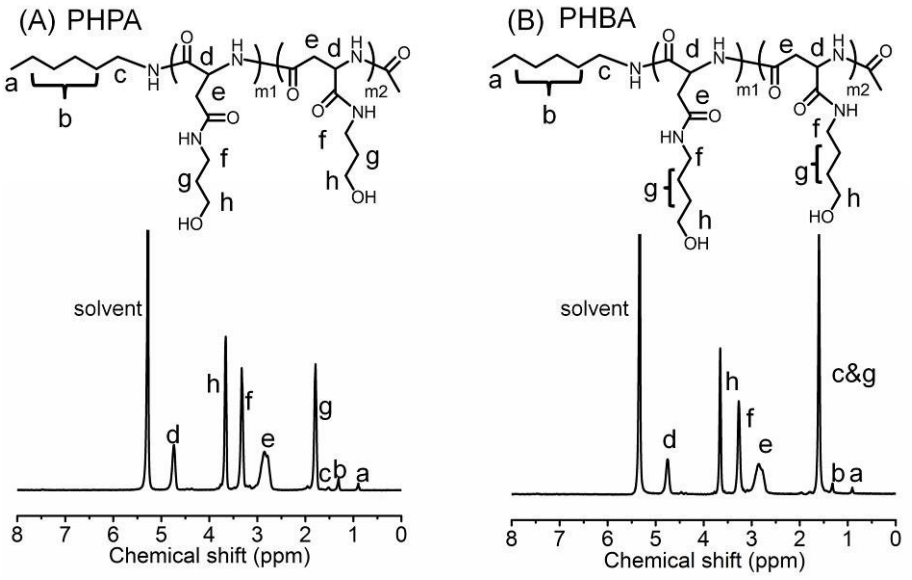


Fig. S5. ^{1}H NMR spectra of PHPA and PHBA in D_2O/TFA-*d* (v:v = 9:1).

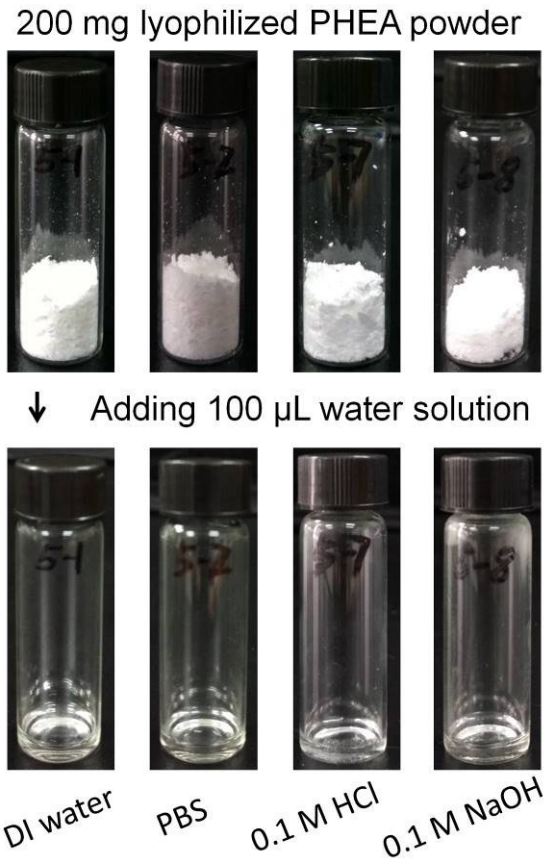


Fig. S6. Solubility of PHEA (P1) in various aqueous solutions.

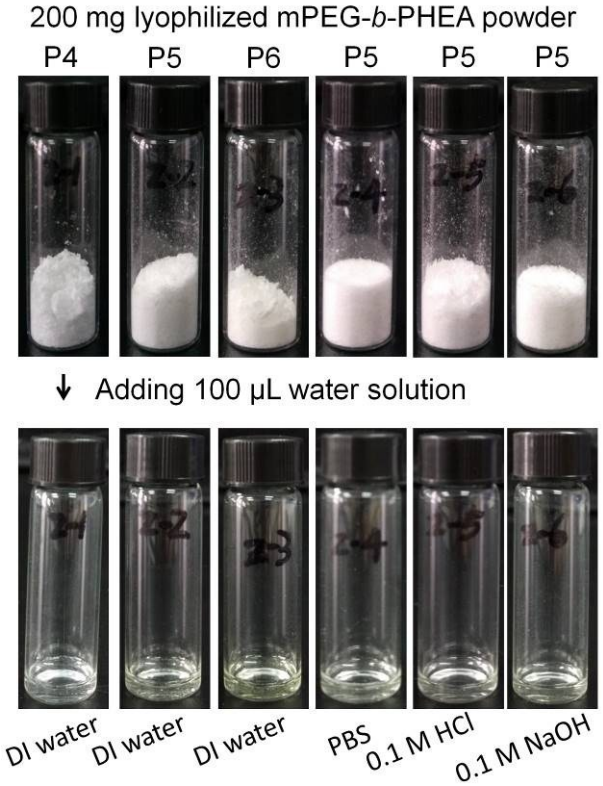


Fig. S7. Solubility of mPEG-*b*-PHEA (P4, P5, and P6) in various aqeuous solutions.

Fig. S8. Solubility of PHPA and PHBA in various aqeuous solutions.

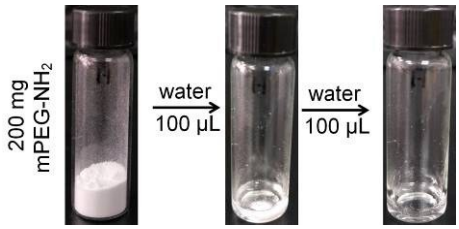


Fig. S9. Solubility of mPEG-NH_2 (M_n = 5000) in DI water.

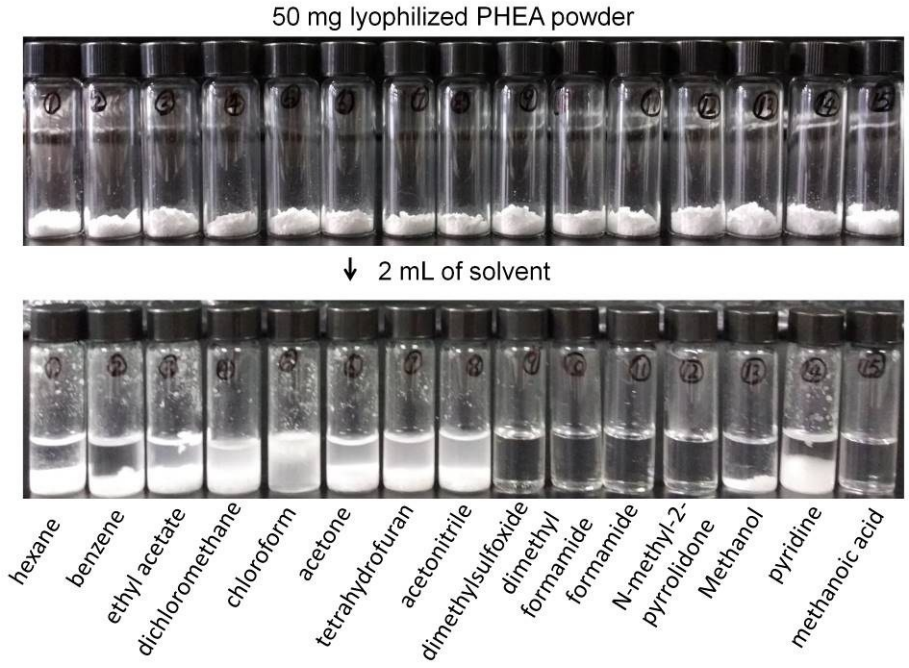


Fig. S10. Solubility of PHEA (P1) in various organic solvents.

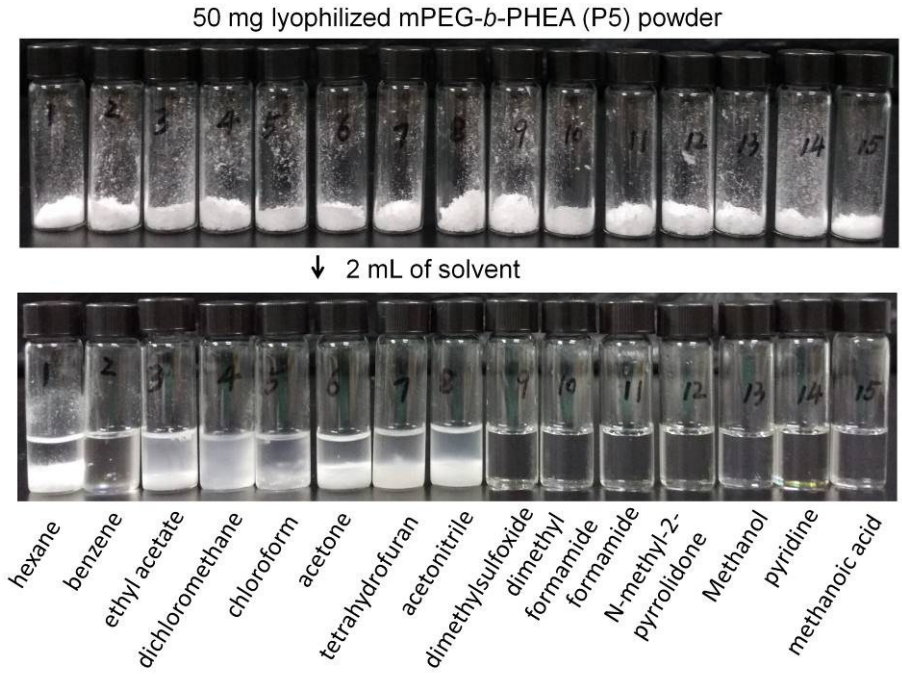


Fig. S11. Solubility of mPEG-*b*-PHEA (P5) in various organic solvents.

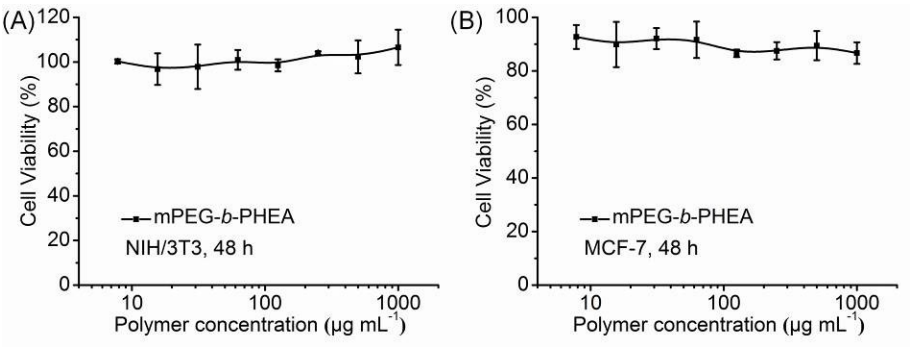


Fig. S12. *In vitro* cytotoxicity of mPEG-*b*-PHEA (P5) toward NIH/3T3 and MCF-7 cells.

The safety and biocompatibility of PHEA have already been reported,[5] and therefore, we herein mainly evaluated the *in vitro* cytotoxicity of mPEG-*b*-PHEA. mPEG-*b*-PHEA showed minimal cytotoxicity to normal cells (NIH/3T3) or cancer cells (MCF-7) at high concentrations up to 1 mg mL^{-1}.

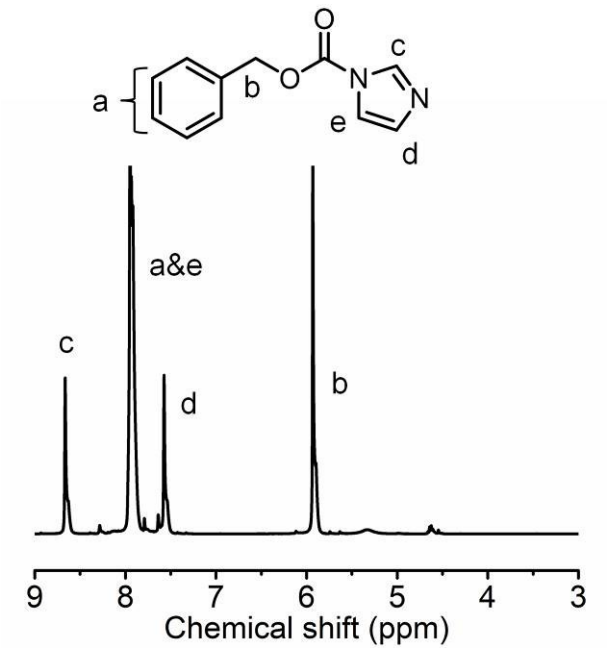


Fig. S13. ^{1}H NMR spectrum of Bn-CI in $CDCl_3$.

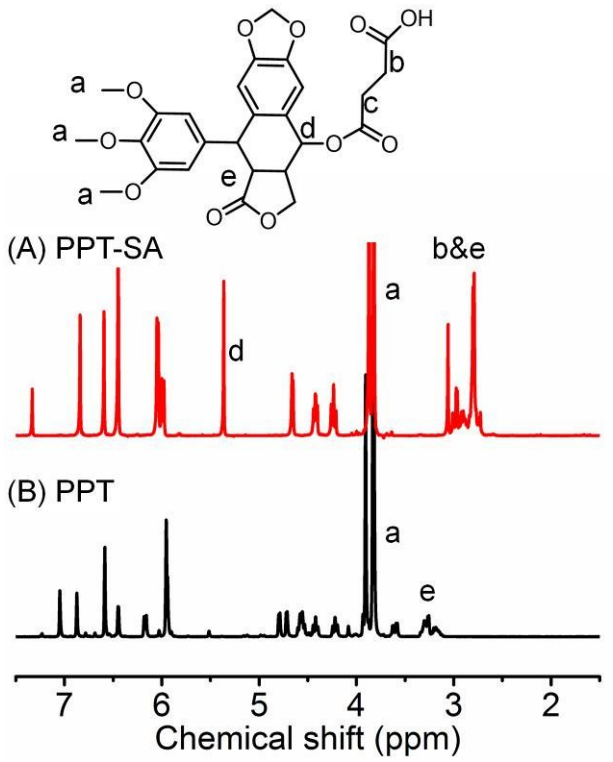


Fig. S14. ^{1}H NMR spectra of PPT and PPT-SA in TFA-*d*.

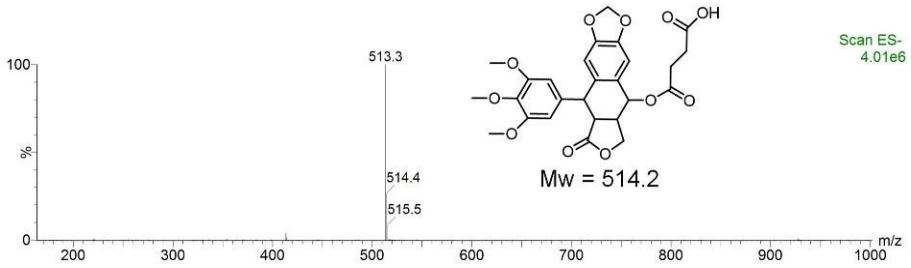


Fig. S15. Mass spectrum of PPT-SA.

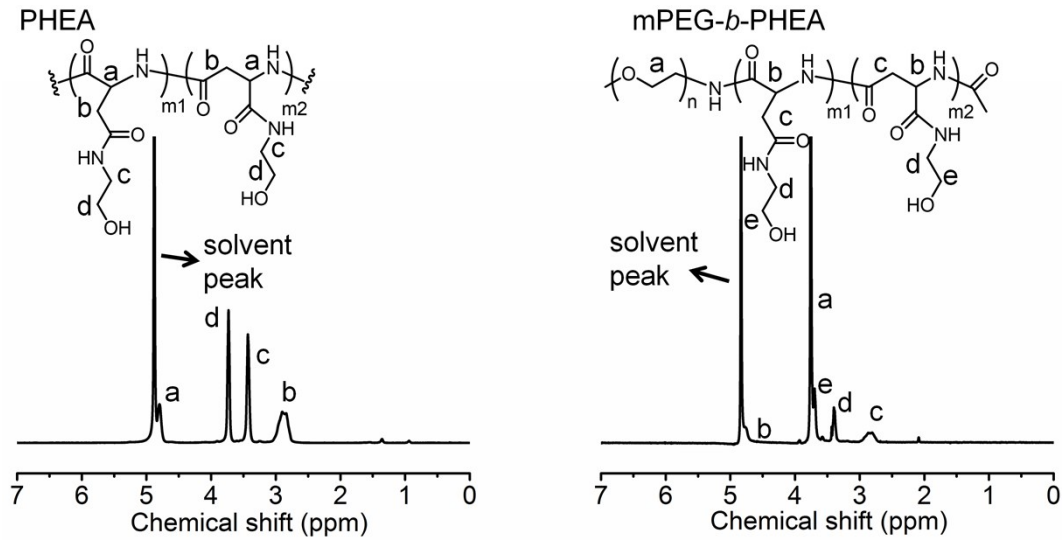


Fig. S16. ^{1}H NMR spectra of PHEA (P1) and mPEG-*b*-PHEA (P5) in D_2O.

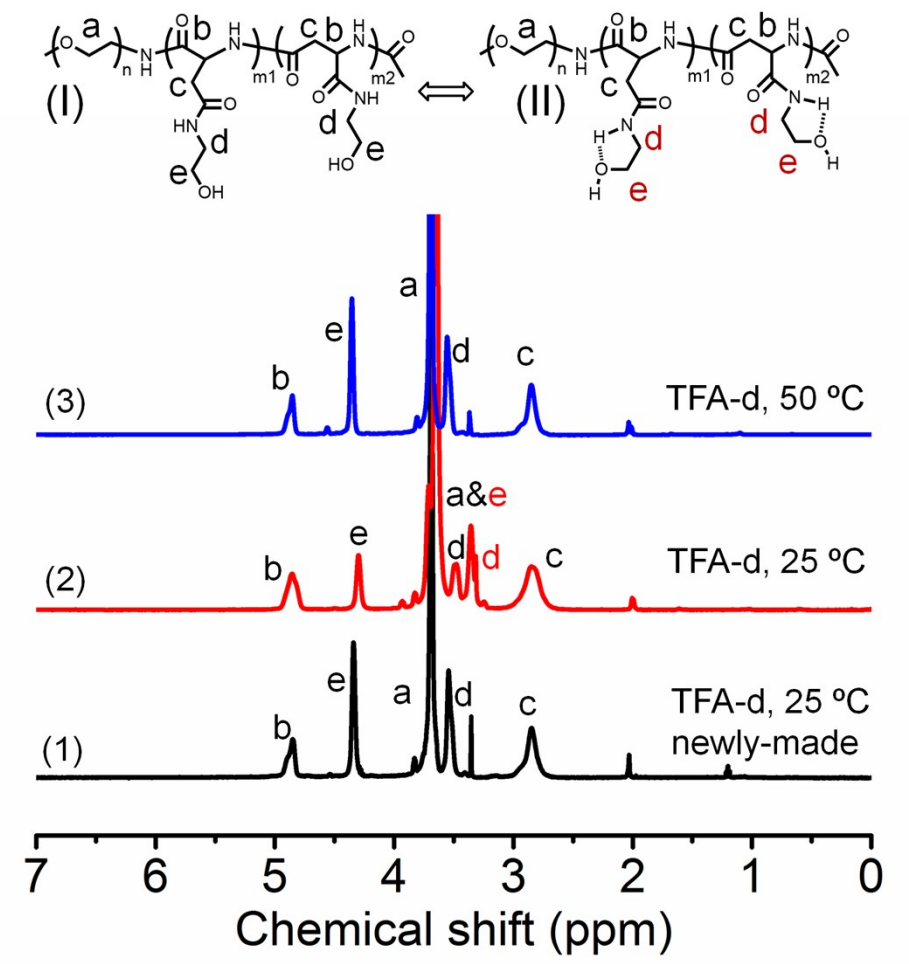


Fig. S17. Proposed chemical structures of mPEG-*b*-PHEA (I and II), and ^{1}H NMR spectra of mPEG-*b*-PHEA in TFA-*d* under different conditions (1, newly made mPEG-*b*-PHEA determined at 25 ºC; 2, mPEG-*b*-PHEA determined at 25 ºC after 24-h placement; 3, mPEG-*b*-PHEA determined at 50 ºC after 24-h placement).

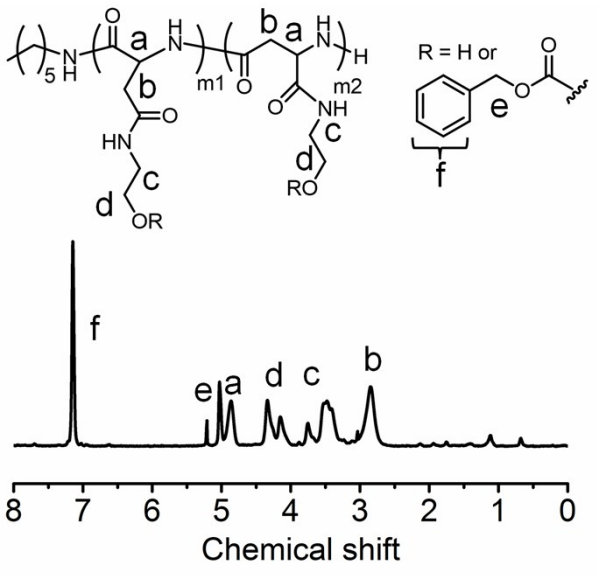


Fig. S18. ^{1}H NMR spectrum of PHEA-*g*-Bn after reaction of P1 with Bn-CI at 50 ºC for 12 h (NMR solvent: TFA-*d*).

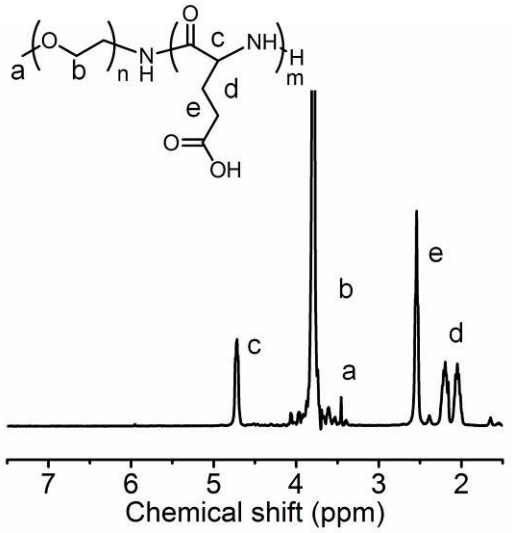


Fig. S19. ^{1}H NMR spectrum of mPEG-*b*-PLG in TFA-*d*.

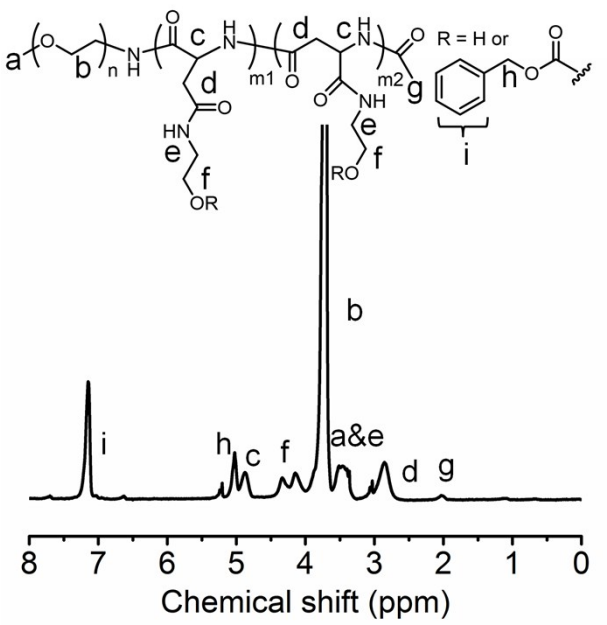


Fig. S20. ^{1}H NMR spectrum of mPEG-*b*-PHEA-*g*-Bn (PG-1) in TFA-*d*.

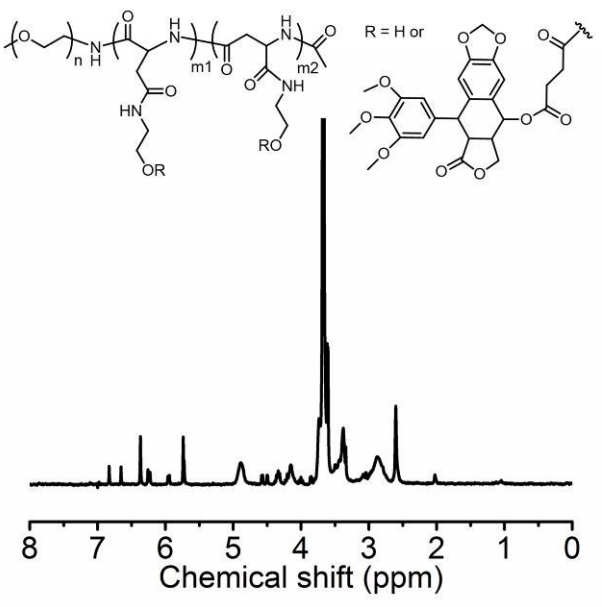


Fig. S21. ^{1}H NMR spectrum of mPEG-*b*-PHEA-*g*-PPT (PG-2) in TFA-*d*.

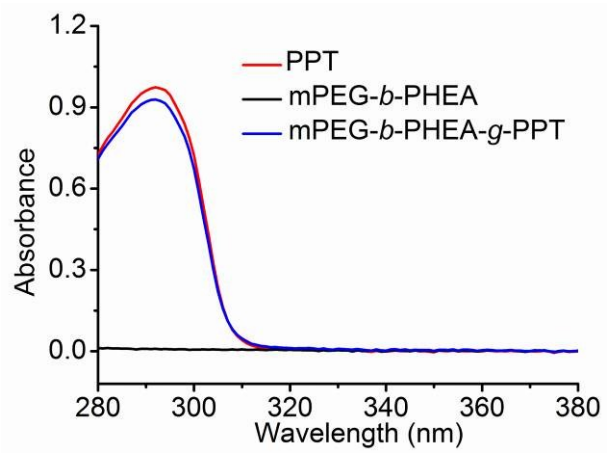


Fig. S22. UV-Vis measurements of mPEG-*b*-PHEA (0.5 mg mL^{-1}), free PPT (0.1 mg mL^{-1}), and mPEG-*b*-PHEA-*g*-PPT (PG-2, 0.5 mg mL^{-1}) in DMF.

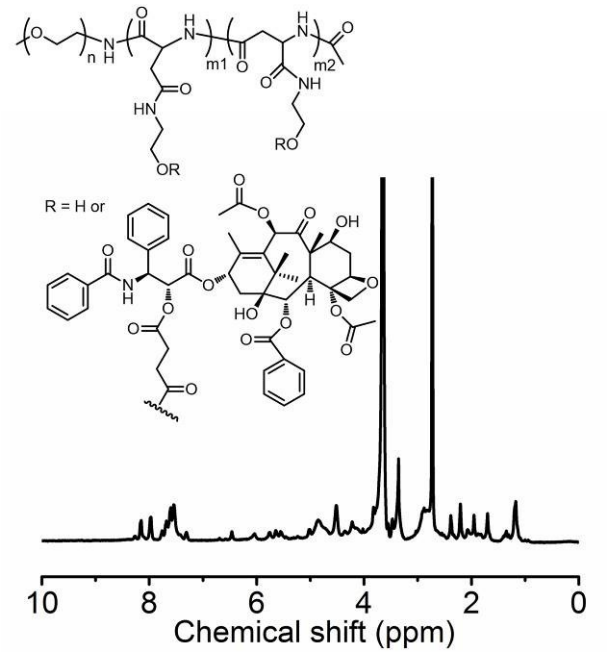


Fig. S23. ^{1}H NMR spectrum of mPEG-*b*-PHEA-*g*-PTX (PG-3) in the mixture of DMSO-d^{6} and TFA-*d* (1:1, v/v).

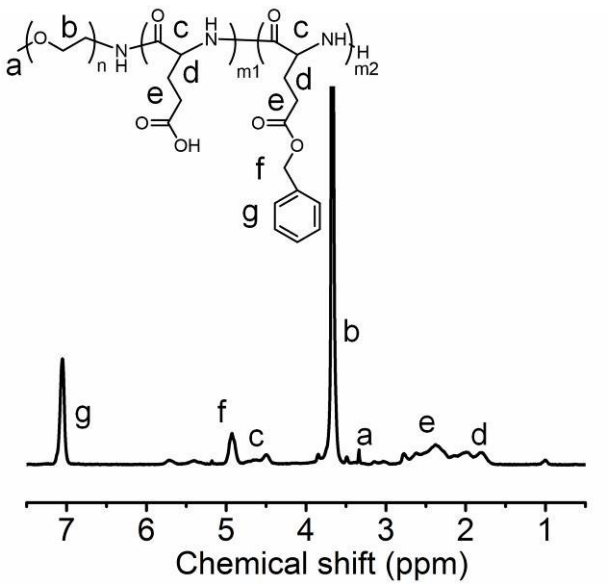


Fig. S24. ^{1}H NMR spectrum of mPEG-*b*-PLG-*g*-Bn (PG-4) in TFA-*d*.

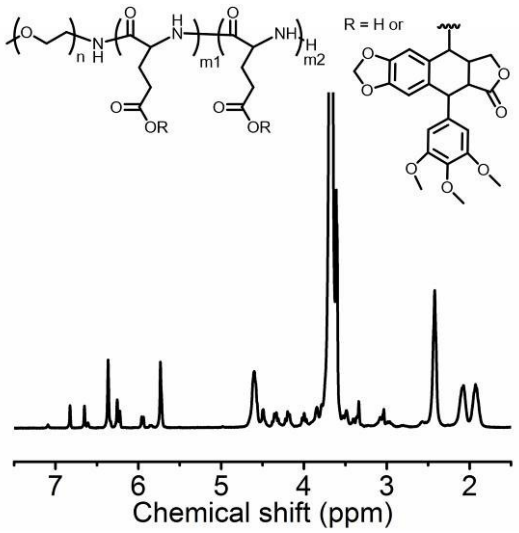


Fig. S25. ^{1}H NMR spectrum of mPEG-*b*-PLG-*g*-PPT (PG-5) in TFA-*d*.

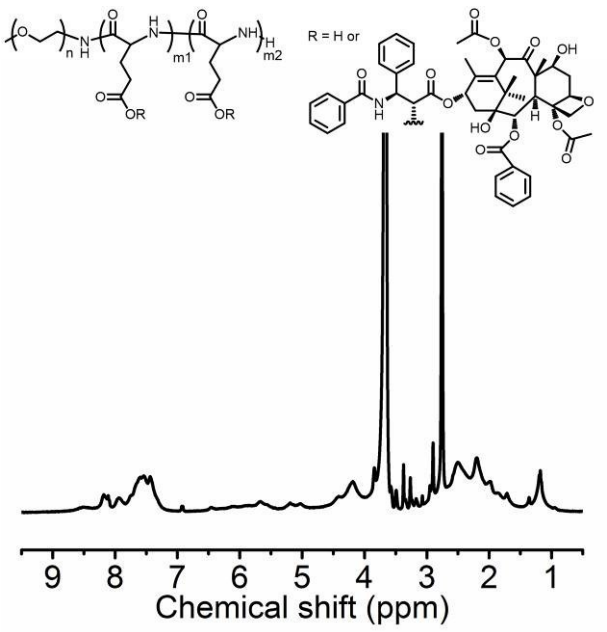


Fig. S26. ^{1}H NMR spectrum of mPEG-*b*-PLG-*g*-PTX (PG-6) in the mixture of DMSO-d^{6} and TFA-*d* (1:1, v/v).

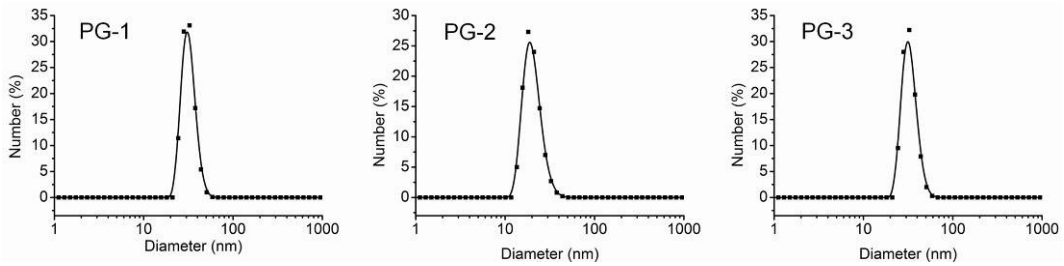


Fig. S27. Size distributions of mPEG-*b*-PHEA-*g*-Bn (PG-1), mPEG-*b*-PHEA-*g*-PPT (PG-2), and mPEG-*b*-PHEA-*g*-PTX (PG-3) micelles in PBS.

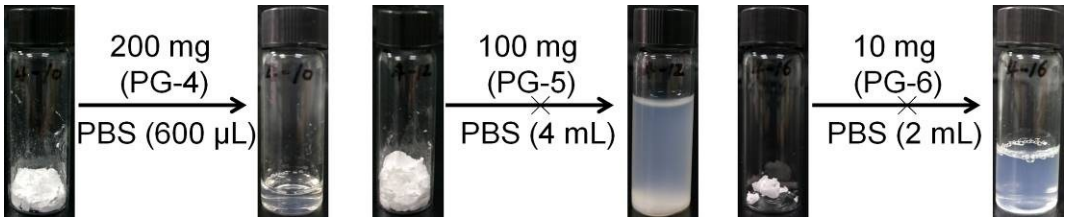


Fig. S28. Solubility of mPEG-*b*-PLG-*g*-Bn (PG-4), mPEG-*b*-PLG-*g*-PPT (PG-5), and mPEG-*b*-PLG-*g*-PTX (PG-6) micelles in PBS after lyopholization.

References

1. H. Y. Tian, C. Deng, H. Lin, J. R. Sun, M. X. Deng, X. S. Chen and X. B. Jing, *Biomaterials*, 2005, **26**, 4209-4217.
2. A. Harada and K. Kataoka, *Macromolecules*, 1995, **28**, 5294-5299.
3. A. Demarre, H. Soyez, E. Schacht and J. Pytela, *Polymer*, 1994, **35**, 2443-2446.
4. G. Bertolini, G. Pavich and B. Vergani, *J. Org. Chemi.*, 1998, **63**, 6031-6034.
5. G. Cavallaro, M. Licciardi, P. Caliceti, S. Salmaso and G. Giammona, *Eur. J. Pharm. Biopharm.*, 2004, **58**, 151-159.
6. M. Li, W. Song, Z. Tang, S. Lv, L. Lin, H. Sun, Q. Li, Y. Yang, H. Hong and X. Chen, *Acs Appl. Mater. Inter.*, 2013, **5**, 1781-1792.