PEG-Polypeptide Dual Brush Block Copolymers: Synthesis and Application in Nanoparticle Surface PEGylation

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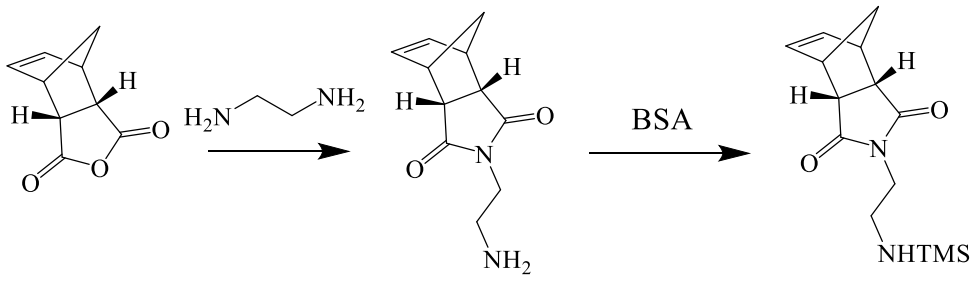
Experimental section

Materials. All chemicals were purchased from Sigma-Aldrich (St. Louis, Mo) and used as received unless otherwise specified. Anhydrous *N*,*N*'-dimethylformamide (DMF) was dried by a column packed with 4Å molecular sieves and stored in a glovebox. Tetrahydrofuran (THF) and hexane were dried by a column packed with alumina and stored in a glove box. (S)-γ-benzyl-$_{L}$-glutamate-*N*-carboxyanhydride (Glu-NCA),[1] (S)-ε-carbobenzoxy-$_{L}$-lysine-*N*-carboxyanhydride (Lys-NCA),[1] (3,5-dioxo-4-azatricyclo[5.2.1.0]-dec-8-en-4-yl)ethanoic acid,[2] Grubbs catalyst C1,[3] and (*N*-benzyl)-5-norbornene-endo-2,3-dicarboximide (M2)[4] were prepared by following the previously reported procedures. Paclitaxel-polylactide (Ptxl-LA, DP = 100, M_n = 12.7 × 10^3 g/mol, M_w/M_n = 1.03) was synthesized via ring-opening polymerization (ROP) of $_{L}$-lactide using [(BDI)ZnN-$(TMS)_2$](BDI = 2-((2,6-diisopropylphenyl)amino)-4-((2,6-diisopropylphenyl)imino)-2-pentene, TMS = trimethylsilyl) as catalyst and Paclitaxel (Ptxl) as initiator.[5, 6] Poly(ethylene glycol)-*block*-poly($_{L}$-lactide) ($mPEG_{113}$-PLA_{194}, PEG-PLA, M_n = 19.2 × 10^3 g/mol, M_w/M_n = 1.08) was synthesized via ROP of $_{L}$-lactide using stannous(II) octanoate ($Sn(Oct)_2$) as catalyst and poly(ethylene glycol) methyl ether with M_n of 5.0 × 10^3 g/mol as initiator.[7] Poly(ethylene glycol)-*block*-poly(γ-benzyl-$_{L}$-glutamate) ($mPEG_{226}$-$PBLG_{215}$ or PEG-PBLG, M_n = 57.1 × 10^3 g/mol, M_w/M_n = 1.18) was synthesized via ROP of Glu-NCA using methoxy-poly(ethylene glycol)-amine ($mPEG_{226}$-NH_2) with MW of 10 kDa as initiator.

Instrumentation. ^{1}H and ^{13}C NMR spectra were recorded on a Varian UI400 MHz, a UI500NB MHz or a VXR-500 MHz spectrometer. Tandem gel permeation chromatography (GPC) experiments were performed on a system equipped with an isocratic pump (Model 1100, Agilent Technology, Santa Clara, CA), a DAWN HELEOS 18-angle laser light scattering detector (also known as multi-angle laser light scattering (MALLS) detector, Wyatt Technology, Santa Barbara,

CA) and an Optilab rEX refractive index detector (Wyatt Technology, Santa Barbara, CA). The detection wavelength of HELEOS was set at 658 nm. Separations were performed using serially connected size exclusion columns (100Å, 500Å, 10^3Å and 10^4Å Phenogel columns, 5 μm, 300 × 7.8 mm, Phenomenex, Torrance, CA) at 60°C using DMF containing 0.1 M LiBr as the mobile phase. The MALLS detector is calibrated using pure toluene with no need for external polymer standards and can be used for the determination of the absolute molecular weights. The molecular weights (MWs) of all polymers were determined based on the *d*n/*d*c value of each sample calculated offline by using the internal calibration system processed by the ASTRA V software (version 5.1.7.3, Wyatt Technology, Santa Barbara, CA). Infrared spectra were recorded on a Perkin Elmer 100 serial FTIR spectrophotometer calibrated with polystyrene film. Particle sizes and dispersities were measured with a ZetaPlus dynamic light scattering (DLS) detector (15 mW laser, incident beam = 676 nm, Brookhaven Instruments, Holtsville, NY, USA).



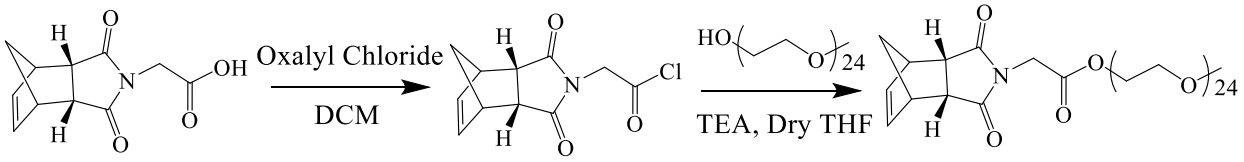
Norbornyl-NH_2 **M1**

Scheme S1. Synthesis of M1.

Synthesis of *N*-aminoethylene-5-norbornene-endo-2,3-dicarboximide (Norbornyl-NH_2). 5-Norbornene-2,3-dicarboxylic anhydride (3.1 g, 20 mmol) was added slowly to a flask containing vigorously stirred ethylenediamine (30 mL, 0.45 mol) at room temperature. The reaction temperature was then raised to 120 ^oC and stirred for 12 h. The excess of ethylenediamine was removed under vacuum after cooling to room temperature and a viscous oil-like residue was

obtained. Saturated NaCl aqueous solution (30 mL) and NaOH aqueous solution (2 M, 5 mL) were added to the viscous oil-like residue. The mixture was extracted by ethyl acetate (3 × 30 mL). The organic phase (ethyl acetate) was combined and washed with DI-water (2 × 10 mL) to remove residual ethylenediamine and then dried over anhydrous Na_2SO_4. The solvent was removed under vacuum to give Norbornyl-NH_2 (2.8 g, yield 70%) of a white solid. ^{1}H NMR ($CDCl_3$, 500 MHz): δ 6.12 (s, 2H, -C<u>H</u>=C<u>H</u>-), 3.42-3.40 (m, 4H, -CHC<u>H</u>CON- and NC<u>H</u>$_2$CH$_2$-), 3.28 (s, 2H, -CHC<u>H</u>CON-), 2.76-2.74 (m, 2H, N $CH_2C\underline{H}_2NH_2$), 1.75-1.54 (dd, 2H, -CHC<u>H</u>$_2$CH-, J = 9.0 Hz), 1.02 (broad, 2H, NH_2). ^{13}C NMR ($CDCl_3$, 500 MHz): δ 178.2, 134.8, 52.5, 46.0, 45.2, 42.0, 40.4. ESI-MS (low resolution, positive mode): calculated for $C_{11}H_{15}N_2O_2$, *m*/*z*, 207.1 $[M + H]^+$; found 207.1 $[M + H]^+$.

Synthesis of *N*-(*N*-trimethylsilylaminoethylene)-5-norbornene-endo-2,3-dicarboximide (M1). In a glovebox, Norbornyl-NH_2 (2.1 g, 10 mmol) and *N,O*-bis(trimethylsilyl) acetamide (BSA), (3.0 mL, 12 mmol) was dissolved in anhydrous THF (50 mL). The mixture was then stirred at room temperature for 48 h under argon. The solvent was removed under reduced pressure to give the crude material, which was recrystallized with diisopropyl ether at −30 ^{o}C twice to give M1 as a light yellow crystalline (2.0 g, yield 72%). ^{1}H NMR ($CDCl_3$, 500 MHz): δ 6.09 (s, 2H, -C<u>H</u>=C<u>H</u>-), 3.38 (s, 2H, -CHC<u>H</u>CON-), 3.32 (t, 2H, CONC<u>H</u>$_2$CH$_2$-), 3.25 (s, 2H, -CHC<u>H</u>CON-), 2.76-2.72 (m, 2H, $CH_2C\underline{H}_2NHTMS$), 1.73-1.54 (dd, 2H, -CHC<u>H</u>$_2$CH-, J = 9.0 Hz), 0.43 (broad, 1H, NH), 0.01 (9H, Si(C<u>H</u>$_3$)$_3$). ^{13}C NMR ($CDCl_3$, 500 MHz): δ 178.1, 134.7, 52.4, 46.0, 45.1, 42.1, 39.8, 0.17. ESI-MS (low resolution, positive mode): calculated for $C_{14}H_{23}N_2O_2Si$, *m*/*z*, 279.1 $[M + H]^+$; found 279.1 $[M + H]^+$.



Scheme S2. Synthesis of NPEG.

Synthesis of Norbornyl-PEG$_{24}$ (NPEG). A stirred mixture of (3,5-dioxo-4-azatricyclo[5.2.1.0]-dec-8-en-4-yl)ethanoic acid (1.0 g, 4.5 mmol) in dichloromethane (30 mL) was cooled to 0 ^{o}C. Oxalyl chloride (0.78 mL, 9.0 mmol) was added followed by DMF (1 drop) which prompted effervescence. The reaction mixture was stirred at room temperature overnight, after which the solvent was evaporated under vacuum to obtain the crude, (3,5-dioxo-4-azatricyclo[5.2.1.0]-dec-8-en-4-yl)ethanoic chloride, This crude was used without further purification. This solution of (3,5-dioxo-4-azatricyclo[5.2.1.0]-dec-8-en-4-yl)ethanoic chloride and triethylamine (5.8 mL, 41.7 mmol) in dry THF (30 mL) was added to a 100 mL round-bottomed flask containing poly(ethylene glycol) monomethyl ether (M_n = 1100, DP = 24) (4.4 g, 4.0 mmol), which was dried under high vacuum for 1 h prior to the reaction. The containing the dry poly(ethylene glycol) monomethyl ether. The mixture was stirred at room temperature for 17 h. The solvent was evaporated under vacuum, and the residues were dissolved in DCM (60 mL). The organic phase was washed with HCl aqueous solution (2 M, 2 × 20 mL) and saturated aqueous $NaHCO_3$ solution (2 × 20 mL), dried over anhydrous Na_2SO_4, filtered, and concentrated under vacuum to afford the norbornyl-PEG$_{24}$ (NPEG) macromonomer as a white solid (4.1 g, yield 79 %). ^{1}H NMR ($CDCl_3$, 500 MHz): δ 6.6 (m 2H, -C<u>H</u>=C<u>H</u>-), 4.18 (s, 2H, NC<u>H</u>$_2$COO-), 4.02 (d, 2H, -COOC<u>H</u>$_2$CH$_2$-), 3.56 (m, 92H, -OC<u>H</u>$_2$C<u>H</u>$_2$O-), 3.45 (d, 2H, -CHC<u>H</u>CON-), 3.28 (m, 5H, -C<u>H</u>CHCON- and C<u>H</u>$_3$O-), 1.62-1.35 (dd, 2H, -CHC<u>H</u>$_2$CH-).

General procedure for the preparation of block brushes

Typical procedures: In a glovebox, Grubbs catalyst C1 in THF (0.01 M, 0.125 mL) was added to the NPEG (32.5 mg, 0.025 mmol) solution in DCM (1 mL) in one portion and stirred at room temperature for 3 h. An aliquot of the solution (0.05 mL) was taken out, diluted to 0.5 mL by DMF, and used for GPC analysis directly. The mixture of M1 (7 mg, 0.025 mmol) and M2 (19 mg, 0.075 mmol) in DCM (0.5 mL) was then added and stirred for 1 h before another aliquot of the solution (0.05 mL) was taken out and diluted for GPC analysis. The solvent was then removed under vacuum for 1 h in the glove-box. The resulting ROMP polymer was dissolved in DMF (1 mL). Glu-NCA or Lys-NCA in DMF was added to the solution, and stirred overnight. The third aliquot of the solution (0.05 mL) was then taken out, diluted to 0.5 mL by DMF, and used for GPC analysis directly. The rest of the reaction solutions were precipitated with diethyl ether (10 mL), collected by centrifuge and dried under vacuum. The brush polymers (Px-*g*-Glu_z or Px-*g*-Lys_z) were obtained as a white solid with high yields (>80%).

General procedure to study the stability of nanoparticles in the PBS buffer

A mixture of Ptxl-LA_{100} (2 mg/mL) and PEG or a PEG-containing polymer (PEG-PLA, PEG-PBLG, Px-*g*-Glu_Z or P1-*g*-Lys_{20} (2 mg/mL)) in DMF (50 μL) were quickly added into a rapidly stirred (1000 rpm) 1× PBS solution via a pipette (2 mL, DMF/PBS =1/40, v/v). The PEG coated NPs were analyzed by DLS and the particle sizes were monitored for over 24 h in PBS.

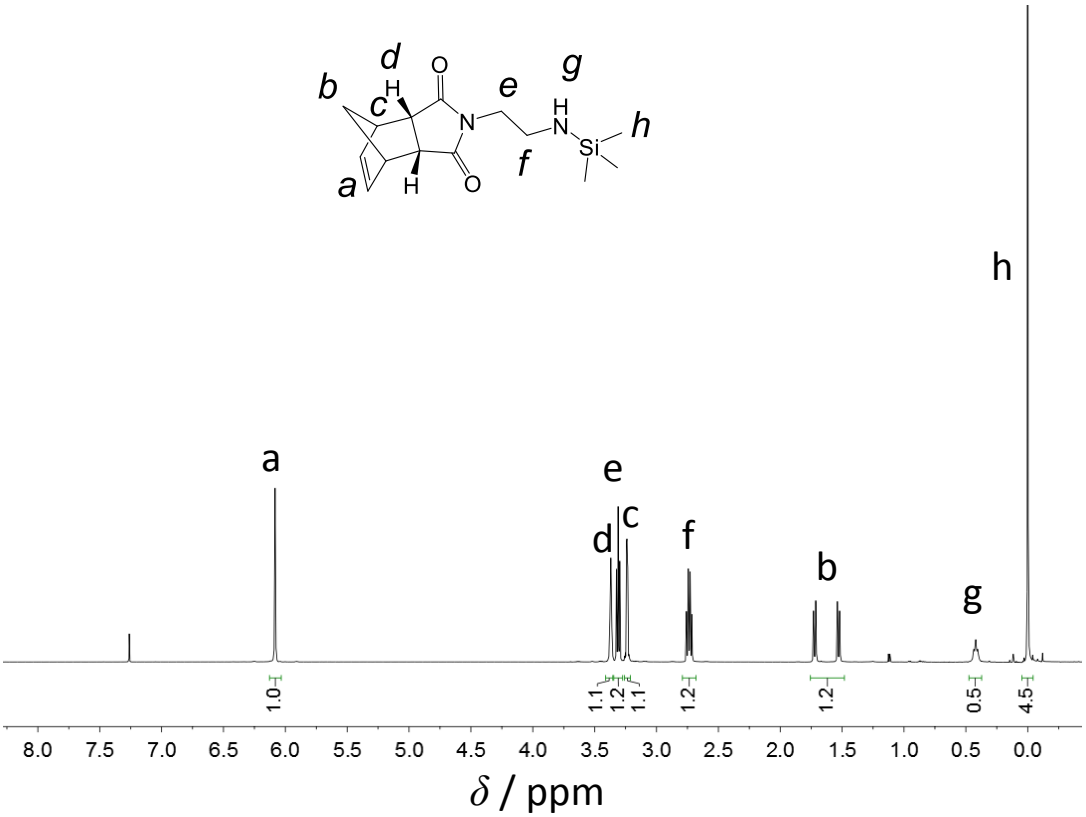


Figure S1. ^{1}H NMR spectrum of M1 in $CDCl_3$.

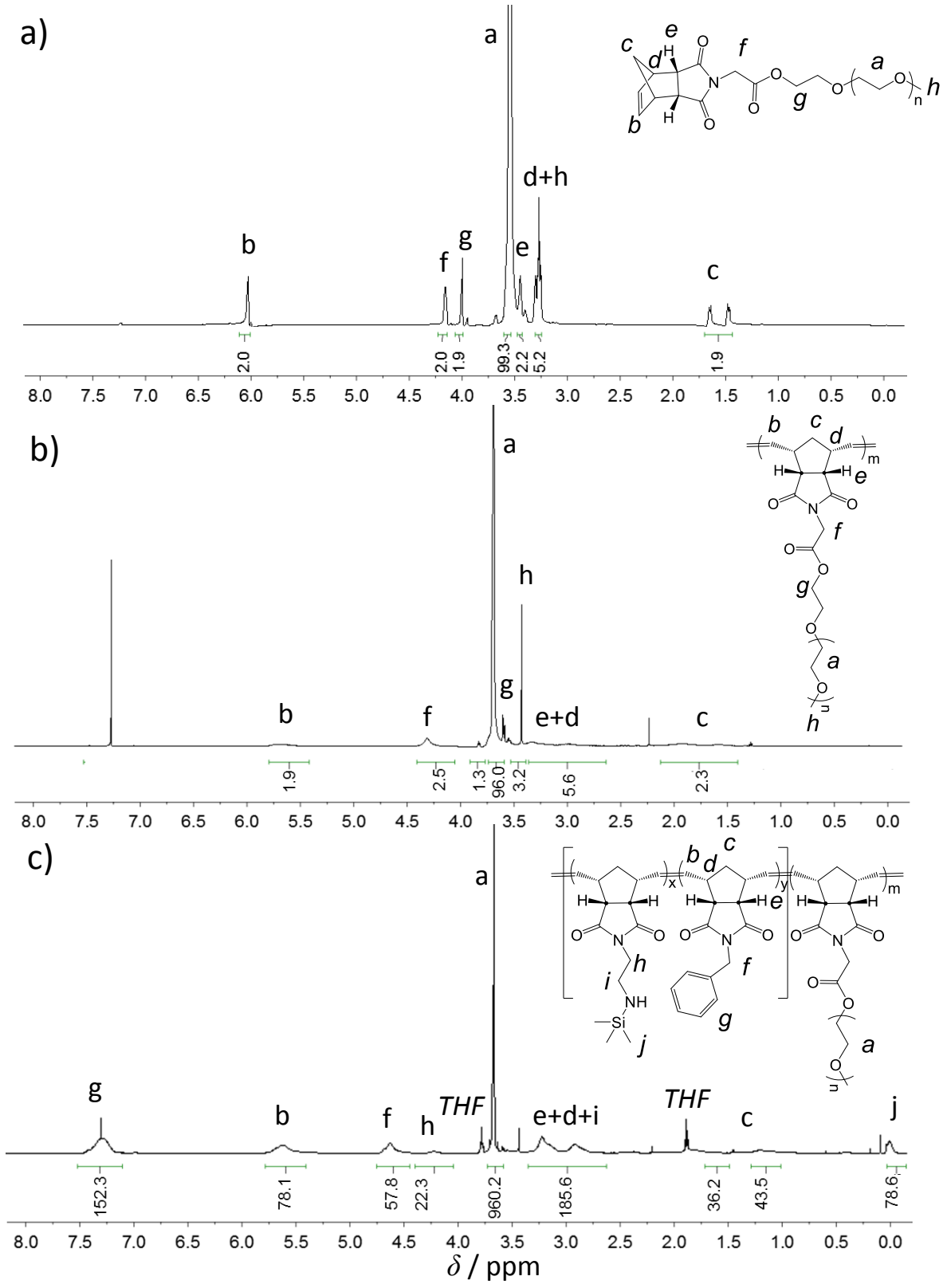


Figure S2: ^{1}H NMR spectra of NPEG (a), PNE_{20} (b) and P1 (c) in $CDCl_3$.

^{1}H NMR spectrum indicated that the esterification reaction was complete (Figure S2a). The signal at 3.56 ppm (peak *a*) can be ascribed to the methylene protons of PEG main chain, whereas signals at 6.6 ppm (peak *b*) was ascribed to -CH=CH- protons of terminal norborny group, respectively. By comparing integral ratios of peaks *b* to that of *a*, the degree of end group functionalization was calculated to be nearly 100%, i.e., a quantitative end group transformation was achieved.

Two PNE with different degree of polymerizations (DPs) were obtained using ROMP of NPEG at monomer/initiator (M/I) ratio of 20 and 40. ^{1}H NMR spectrum indicated that all NPEG were consumed after 3 h polymerization (Figure S2b). Well-defined block copolymers, P1-P3, were obtained using these PNE_{20} and PNE_{40} as macroinitiators and M1 and M2 as monomers. ^{1}H NMR spectrum indicated that the *N*-TMS groups on the monomer M1 can be well preserved during ROMP of a mixture of M1 and M2 in the presence of a Grubbs catalyst (C1), the *N*-TMS groups on the polymer backbone were used as initiators to polymerize NCAs in the next step.

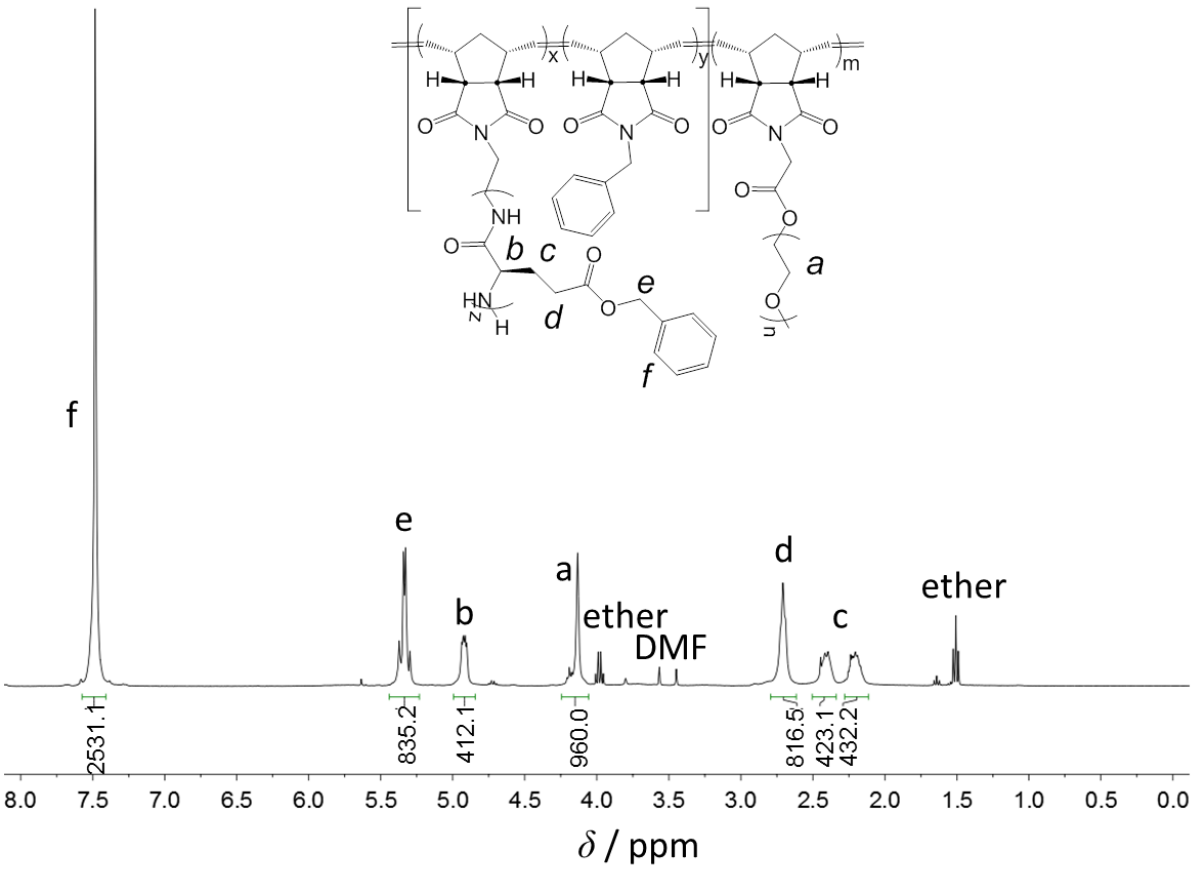


Figure S3: ^{1}H NMR spectrum of P1-*g*-Glu$_{40}$ in TFA-*d*.

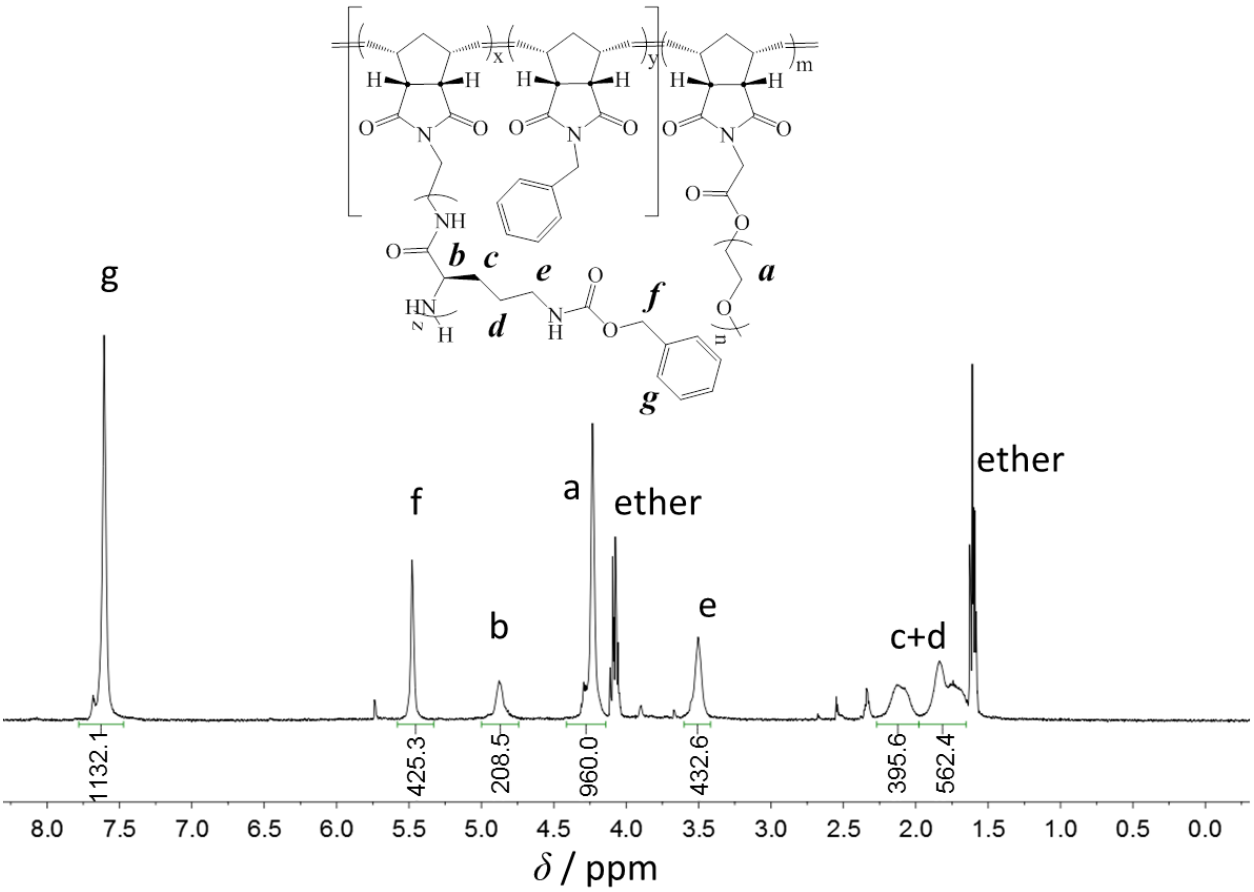


Figure S4: ^{1}H NMR spectrum of P1-*g*-Lys$_{20}$ in TFA-*d*.

Table S1. Stability of Ptxl-LA NPs in PBS (1×) after coated with PEG, PEG-PLA, PEG-PBLG, Px-*g*-Glu_z, or P1-*g*-Lys_{20}.

entry	coating polymer	incubation time (h)	diameter (nm) [a]	PDI[a]
1	PEG	0	106.6	0.098
2	PEG	0.067	217.4	0.362
3	PEG-PLA	0	101.6	0.059
4	PEG-PLA	0.067	195.3	0.125
5	PEG-PLA	1	1562.5	0.446
6	PEG-PBLG	0	124.6	0.135
7	PEG-PBLG	1	1168.5	0.504
8	PEG-PBLG	24	7479.4	0.665
9	P1-*g*-Glu_{20}	0	95.9	0.089
10	P1-*g*-Glu_{20}	1	98.2	0.092
11	P1-*g*-Glu_{20}	24	103.7	0.102
12	P2-*g*-Glu_{20}	0	86.2	0.069
13	P2-*g*-Glu_{20}	1	95.1	0.087
14	P2-*g*-Glu_{20}	24	97.2	0.091
15	P2-*g*-Glu_{40}	0	124.6	0.104
16	P2-*g*-Glu_{40}	1	132.6	0.124
17	P2-*g*-Glu_{40}	24	134.8	0.135
18	P3-*g*-Glu_{20}	0	116.2	0.103
19	P3-*g*-Glu_{20}	1	120.5	0.112
19	P3-*g*-Glu_{20}	24	124.2	0.108
20	P1-*g*-Lys_{20}	0	109.4	0.089
21	P1-*g*-Lys_{20}	1	120.4	0.103
22	P1-*g*-Lys_{20}	24	124.5	0.112

[a]Determined by dynamic light scattering.

Reference:

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