Electronic Supplementary Material (ESI) for Chemical Communications

Supporting Information for:

Dendron-mediated Self-assembly of Highly PEGylated Block Copolymers: A Modular Nanocarrier Platform

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EXPERIMENTAL

Materials

Hydroxyl-terminated poly(ε-caprolactone) (PCL) polymers with two different molecular weights (M_n 3500 and PDI 1.18, M_n 14000 and PDI 1.20) were purchased from Polymer Source Inc. (Montreal, Canada). Generation 3 polyester-8-hydroxyl-1-acetylene bis-MPA dendron (G3 dendron; G3), p-toluenesulfonyl chloride (TsCl), 2-bromoethyl isocyanate (BEI), triethylamine (TEA), trimethylamine hydrochloride (TMA), sodium azide (NaN_3), anhydrous sodium sulfate, *N,N,N′,N″,N″*-pentamethyldiethylenetriamine (PMDETA), copper bromide (CuBr), dibutyltin dilaurate (DBTDL), and *p*-nitrophenyl chloroformate (*p*-NPC) and indomethacin (IMC) were all provided by Sigma Aldrich Co. (St. Louis, USA). Methoxy polyethylene glycol amine (mPEG-NH_2, M_n 2000 and PDI 1.02, M_n 5000 and PDI 1.04) was purchased from JenKem Technology USA Inc. (TX, USA). Regenerated cellulose dialysis membranes (3.5K and 12-14K MWCO) were purchased from Spectrum Labs (CA, USA). All solvents and reagents were used without further purification unless otherwise specified.

Synthesis of PCL3.5K-G3 dendron via click reaction

Tosylation of PCL3.5K. A terminal hydroxyl group of PCL was firstly tosylated prior to introduction of an azide group as previously reported (Figure S1A).[1] PCL3.5K (1 g, 0.286 mmol) along with TEA (200 μl, 1.43 mmol) and trimethylamine hydrochloride (14 mg, 0.143 mmol) were dissolved in 8 mL of dichloromethane. To this solution, TsCl (272 mg, 1.430 mmol, 5 eq.) dissolved in 2 mL of dichloromethane was added dropwise. The reaction was carried out at room temperature for 24 h. Following the reaction, the solvent was evaporated until a viscous liquid remained. The viscous liquid was then precipitated into cold diethyl ether, filtered, and dried *in vacuo* (Yield: 90 %).

Azido-functionalization of PCL3.5K-Ts. The tosyl group of PCL3.5K-Ts was converted into azide for subsequent click chemistry. PCL3.5K-Ts (840 mg, 0.24 mmol) was dissolved in 8 mL of dimethylformamide. To

this solution, sodium azide (312 mg, 4.8 mmol, 20 eq.) was added and reacted at room temperature for 24 h under N_2. The reaction mixture was diluted with 200 mL of dichloromethane and washed three times with 150 mL of deionized water and once with 200 mL of brine. The organic layer was dried over anhydrous sodium sulfate, filtered, concentrated, and precipitated into cold diethyl ether (Yield: 70 %).

Synthesis of PCL3.5K-G3 dendron. PCL3.5K-G3 dendron was synthesized via click chemistry between PCL3.5K-N_3 and G3 dendron bearing an acetylene group in the presence of copper (I) and a base by modification of a previously reported method.[2] PCL3.5K-N_3 (73 mg, 0.021 mmol) and G3 dendron (20 mg, 0.023 mmol, 1.1 eq.) were dissolved in 2 mL of dimethylformamide containing PMDETA (7.3 mg, 0.042 mmol, 2 eq.). After dissolving, copper bromide (6 mg, 0.042 mmol, 2 eq.) was added and the reaction was carried out at 80 °C for 24 h. Products were recovered by precipitation into cold diethyl ether and filtration (Yield: 96 %).

Synthesis of PCL14K-G3 dendron via *click reaction*

Bromination of PCL14K. The terminal hydroxyl group of high molecular weight PCL was easily converted into bromide by reaction with 2-bromoethyl isocyanate (Figure S1B). PCL14K (1 g, 0.071 mmol) was dissolved in 15 mL of toluene containing TEA (11 μl, 0.071 mmol) with a catalytic amount of DBTDL. To this solution, 1 mL of toluene containing 2-bromoethyl isocyanate (108 mg, 0.714 mmol, 10 eq.) was added dropwise and reacted at room temperature for 24 h under N_2. Following the reaction, the solvent was evaporated until a viscous liquid remained. The viscous liquid was then precipitated into cold diethyl ether, filtered and dried *in vacuo* (Yield: 92%).

Azido-functionalization of PCL14K-Br. The bromine group of PCL14K-Br was converted into azide for click chemistry. PCL14K-Br (910 mg, 0.065 mmol) was dissolved in 12 mL of dimethylformamide. To this solution, sodium azide (84 mg, 1.3 mmol, 20 eq.) was added and reacted at room temperature for 24 h under N_2. The reaction mixture was diluted with 200 mL of dichloromethane and washed three times with 150 mL of deionized water and once with 200 mL of brine. The organic layer was dried over anhydrous sodium sul-

fate, filtered, concentrated and precipitated into cold diethyl ether (Yield: 81%).

Synthesis of PCL14K-G3 dendron. PCL14K-G3 dendron was synthesized via click chemistry between PCL14K-N_3 and G3 dendron bearing an acetylene group in the presence of copper (I) and a base by modification of a previously reported method.[2] PCL14K-N_3 (200 mg, 0.0143 mmol) and G3 dendron (16 mg, 0.0157 mmol, 1.1 eq.) were dissolved in 3 mL of dimethylformamide containing PMDETA (4.94 mg, 0.0286 mmol, 2 eq.). After dissolving, copper bromide (4 mg, 0.0286 mmol, 2 eq.) was added and the reaction was carried out at 80 °C for 24 h. Products were recovered by precipitation into cold diethyl ether and filtration (Yield: 95%).

mPEG conjugation to PCL-G3 dendron

mPEG conjugation was accomplished following activation of the peripheral hydroxyl groups on PCL-G3 dendron (Figure S2).[3] PCL-G3 dendron was dissolved in 8 mL of dichloromethane containing pyridine (5 eq.). After adding *p*-NPC (5 eq.) dropwise, the reaction was carried out at room temperature for 24 h. The solvent was evaporated until a viscous liquid remained. The viscous liquid was then precipitated into cold diethyl ether, filtered and dried *in vacuo*. For mPEG conjugation, a solution of the activated PCL-G3 dissolved in 1 mL of dimethylformamide was added dropwise to 3 mL of dimethylformamide containing mPEG-NH_2 (1.2 eq.) and TEA (4 eq.). The reaction was carried out at room temperature for 24 h. The solution was then transferred into a dialysis bag (MWCO 3.5K for mPEG2K-NH_2 and MWCO 12-14K for mPEG5K-NH_2), dialyzed for 2 days, and then freeze dried for 2 days (Yields: > 70%).

Synthesis of linear PCL-mPEG copolymers

300 mg of PCL was dissolved in 8 mL of dichloromethane containing pyridine (5 eq.). After adding *p*-NPC (5 eq.) dropwise, the reaction was carried out at room temperature for 24 h. The solvent was evaporated until a viscous liquid remained. The viscous liquid was then precipitated into cold diethyl ether, filtered and dried

in vacuo (Yields: > 90%). A solution of the activated PCL dissolved in 1 mL of dimethylformamide was added dropwise to 3 mL of dimethylformamide containing mPEG-NH_2 (1.2 eq.) and TEA (4 eq.) and reacted at room temperature for 24 h. The crude product was transferred into a dialysis bag, dialyzed for 1 day and then freeze dried for 2 days (Yields: > 80%). For PCL14K-mPEG copolymers, the extraction method was used due to their low HLB values (Yields: > 60%).

Polymer characterization

^{1}H-NMR spectra were recorded at 400 MHz (DPX-400 NMR spectrometer, Bruker Biospin Co., MA, USA). NMR chemical shifts are reported in ppm with calibration against a solvent signal. FT-IR spectra were recorded using FT-IR spectrophotometer (NEXUS 870, Thermo Nicolet Co., WI, USA). GPC measurements were carried out using a 600 HPLC pump, 717plus Autosampler, 2414 Refractive Index detector (Waters, Milford, MA, USA) and a MiniDAWN™ TREOS triple-angle light scattering detector (Wyatt, Santa Barbara, CA, USA) using THF as the mobile phase at 1 mL/min with Waters Styragel® HR2 and HR4E columns at 30°C.

Additional synthetic description

The terminal hydroxyl groups of both PCL3.5K and PCL14K were completely substituted into a tosyl group and bromide, respectively (Figure S3). The identical method for both molecular weights of PCL was first attempted, however effective substitution was not observed for high molecular weight PCL with *p*-toluenesulfonyl chloride and the alternative method was chosen due to the high reactivity of the isocyanate group with hydroxyl groups as well as the ease of nucleophilic substitution of bromine for azide.

G3 polyester dendron bearing an acetylene functional group at the focal point was reacted with PCL-N_3 *via* click chemistry. Although click chemistry has been shown to be efficient under mild conditions, a variety of reaction conditions have been developed that vary in terms copper species, bases, solvents and temperatures

further increase conjugation yields.[4] The click reaction between PCL-N_3 and G3 dendron was firstly catalyzed by 0.2 equivalent amounts of CuBr and PMDETA based on the feed molar amount of PCL-N_3 in DMF at room temperature as previously reported.[5] However, the conjugation yield was calculated to be less than 10 % by ^{1}H-NMR with no appearance of characteristic protons associated with triazole formation. According to few reports on the failure of click reactions,[6] we investigated the efficiency of the click reaction with a variety of conditions; copper species ($CuSO_4$, CuBr, CuI), sodium ascorbate, bases (PMDETA, diazobicyclo[5.4.0undec-7-ene]), solvents (DMF and THF), temperatures (30, 50, and 80 °C). It was found that the click reaction using CuBr and PMDETA in DMF at 80 °C afforded the desired PCL-G3 products with high conjugation yields (PCL3.5K-G3: 70 %; PCL14K-G3: 80 %).

Micelle preparation and characterization

For blank micelles, 20 mg of polymer was dissolved in 2 mL of dimethylformamide. The solution was dialyzed (MWCO 3.5K) against distilled water for 1 day and freeze dried for 2 days. IMC was chosen as a model hydrophobic drug. IMC-loaded micelles were prepared by a dialysis method. 40 mg of polymer was dissolved in 4 mL of dimethylformamide along with 4 mg of IMC. The polymer-IMC solution was then transferred to a dialysis membrane (MWCO 3500), dialyzed for 24 h against 2 L of distilled water, and freeze dried for 2 days to produce IMC-loaded micelles.

Critical micelle concentration (CMC) was determined by using a fluorescence method as previously reported.[7] Briefly, a known amount of pyrene dissolved in acetone was added to a series of vials and evaporated such that upon addition of 2 mL of polymer solution the concentration of pyrene was 6×10^{-7} M. The copolymers dissolved in water (10^3 - 10^{-3} mg/L) was added to each vial containing pyrene and before fluorescence measurement the solutions were vortexed and incubated at room temperature for 24 h. The emission wavelength λ_{em} was set at 390 nm and the λ_{ex} was scanned from 300 nm to 400 nm using a spectrofluorophotometer (RF 1501, Shimadzu, Japan) and the intensity ratio I_{338}/I_{333} against log concentration was plotted.

The particle size (nm) and size distribution were measured for all micelles by dynamic light scattering (DLS) using a Nicomp 380 Zeta Potential/Particle Sizer (Particle Sizing Systems, Santa Barbara, CA). Micelles were prepared at concentrations above their measured CMCs in distilled water, filtered through a 0.45 μm syringe filter, and vortexed briefly before each measurement.

The micellar morphology was analyzed by transmission electron microscopy (TEM, JEM-1220, JEOL Ltd., Japan). A drop of micellar suspension (0.2 mg/ml) after filtration (pore size, 0.45 μm) was placed on a 300 mesh copper grid coated with carbon. The sample was stained with a drop of 2 % phosphotungstic acid and dried at room temperature in a desiccator for 1 day. The diameters of each micelle were measured by randomly selected 10 particles from each TEM image. The average and standard deviation were calculated.

Drug release test

To determine the IMC-loading content, a small amount of IMC-loaded micelles was dissolved in 1 mL of dimethylformamide and the concentration of IMC was determined by measuring the UV-absorbance at 317 nm from a series of IMC standards in dimethylformamide. 10 mg of IMC-loaded micelles was suspended in 1 mL of PBS (pH 7.4, 0.01 M) and transferred to a dialysis bag (MWCO 3.5K). Each dialysis bag was added to 30 mL of PBS and placed into a shaking water bath (37 °C, 50 rpm). At predetermined time intervals, 10 mL of release medium was removed and replaced with fresh PBS and frozen until all samples were collected. Samples were then freeze-dried for 2 days, redissolved in 1 mL DMSO and centrifuged at 4000 rpm at room temperature. The amount of IMC released was obtained by measuring the UV-absorbance of the supernatant at 317 nm and comparing to a standard curve of IMC in DMSO. The release profile was obtained by plotting the cumulative IMC release against time.

Cytotoxicity test

KB cell line was obtained from ATCC (Manassas, VA, USA) and grown continuously as a monolayer in GIBCO RPMI 1640 medium (Invitrogen Corporation, Carlsbad, CA, USA) in a humidified incubator at 37 °C and 5% CO_2. RPMI was supplemented with penicillin (100 units/ml), streptomycin (100 mg/ml), and 10% heat-inactivated fetal bovine serum (FBS) (Invitrogen Corporation, Carlsbad, CA, USA) before use. For the assay, KB cells were seeded in 96-well plates at a density of 5×10^3 cells/well and grown in RPMI for 24 h. Cells were then treated with different concentrations of each copolymer ranging from 0.01-100 μM. After each incubation time, cells were washed and incubated for an additional 24 h. Cell viability was assessed using a CellTiter 96 AQueous One Solution (MTS) Assay (Promega, Madison, WI, USA) according to the manufacturer's protocol. The UV absorbance was measured at 490 nm using a Labsystems Multiskan Plus microplate reader (Labsystems, Finland). Mean cell viabilities were determined relative to a negative control (untreated cells) and a positive control (0.1% Triton-X, Sigma-Aldrich).

Modeling of the monomer self-assembly

We modeled by atomistic molecular dynamics (MD) simulations individual linear, PCL3.5K-mPEG2K and PCL3.5K-mPEG16K, and branched, PCL3.5K-G3-mPEG2K and PCL14K-G3-mPEG2K copolymers in water. Separately, we modeled micellar assemblies of hydrated PCL3.5K-mPEG2K, PCL3.5K-G3-mPEG2K, and PCL14K-G3-mPEGK copolymers with different aggregation numbers, N_{agg}. We used the NAMD package[8] and the CHARMM force field (CHARMM27, C35r revision for ethers, and general forcefield)[9] . In all the simulations, the Langevin damping constant of $\gamma_{Lang} = 0.01$ ps^{-1} was used to achieve a faster relaxation. Non-bonded interactions were calculated using the cut-off distance of d = 12 Å. Long-range electrostatic interactions were calculated by the PME method[10] and the MD integration timestep was set to 2 fs.

The individual copolymer molecules were solvated and equilibrated for ~ 5-7 ns in TIP3P water, using the NPT ensemble (VMD)[11], with periodic boundary conditions applied (P = 1 bar and T = 300 K). The obtained results are shown in Fig. 2b. We also studied the conformations of individual copolymers fully equilibrated in water, using the same conditions. In the equilibration of PCL3.5K-mPEG2K, PCL3.5K-mPEG16K,

PCL3.5K-G3-mPEG2K and PCL14K-G3-mPEG2K, a force of F=0.01 kcal/mol/ Å was applied to several atoms of the PEG chains, directing toward the hydrophobic PCL core. After ~ 0.2 ns, the PEG chains were collapsed on the hydrophobic cores. Then, we stopped the force and equilibrated the monomers for another 10 ns. The equilibrated conformations of the copolymers are shown in Figure S9.

In the study of micellar assemblies, the monomers were initially spherically distributed by our codes and hydrated in cells containing 30,000-530,000 atoms, with periodic boundary conditions applied. After short minimizations, the systems were heated to T = 400 K for fast reorganization, while the volume was kept constant. At the same time, the central force of $\vec{F}(\vec{r}) = k\vec{r}$ with k = 1.0 kcal/mol/Å was applied to several atoms along the PCL chains of all the copolymers, in order to accelerate aggregation of the micellar core. After 1 ns, the systems were cooled to T = 300 K, and equilibrated at P = 1 bar for ~ 4-5 ns. The obtained micelles are shown in Fig. 3b. In order to better understand the conformations of individual PCL3.5K-G3-mPEG2K molecules self-assembled in the micelle, we disintegrated the 14 PCL3.5K-G3-mPEG2K micelle using VMD[11] without changing the conformation of the individual copolymer, as shown in Figure S11.

We also estimate the approximate entropic cost in the self-assembly of linear monomers, where it is assumed that PEG chains can be described as ideal chains.[12] An ideal chain of length L is comprised of n segments of statistical length l, so that $L = ln$. When placed in a good solvent (such as water), the ideal chain swells to maximize the number of polymer-fluid contacts. Configurations of the ideal chain can be characterized by a probability distribution function that depends on the chain end-to-end (e-t-e) distance. An ideal chain has a well-defined average e-t-e distance $\langle r \rangle = ln^{1/2}$, which is associated with minimum (configuration) free energy of the polymer. When the ideal (PEG) chain becomes confined during the self-assembly process, its average e-t-e distance will increase and the free energy cost associated with this extension is purely entropic. Entropy of a freely jointed chain with a given e-t-e distance is proportional to the logarithm of the number of chain configurations for that e-t-e distance. This is in turn is proportional to the probability of the PEG having this e-t-e distance. The entropy difference between PEG chains in a given conformation with different e-t-e extensions is given by[12]

$$\Delta S = S - S_1 = k_B b^2 (r_1^2 - r^2),$$

where S and S_1 are configurational entropies associated with the e-t-e extensions r and r_1, k_B is the Boltzmann constant and $b^2 = 3/(2nl^2)$. For PCL3.5K-mPEG16K, the PEG block has n = 363 repeating unts (-CH_2CH_2O-), each of the length l is approximately 3.68 Å. In water, the average e-t-e distance of this polymer is $\langle r \rangle = ln^{1/2} \approx 70$ Å. If we assume that due to steric confinement the e-t-e distance of the chains in the micelle is extended by 50% from the above value of $\langle r \rangle$, the entropic cost for this extension is ≈ 1.11 kcal/mol ≈ 1.9 k_BT. Since every chain forming a micelle needs to pay this configurational entropy cost, the micelle formation is not favorable for polymers with long hydrophilic blocks, but in short chains attached to dendrons this entropic costs is absent. We can use the fact that the Gibbs energy associated with the monomer self-assembly is given by

$$\Delta G = \Delta H - T \Delta S,$$

where ΔH is the related enthalpy change. We can immediately see that, for the same ΔH (hydrophobic binding in the core), long linear amphiphilic molecules tend to be less stable when self-assembled (ΔS > 0) than branched dendron-based amphiphilic molecules (ΔS ~ 0).

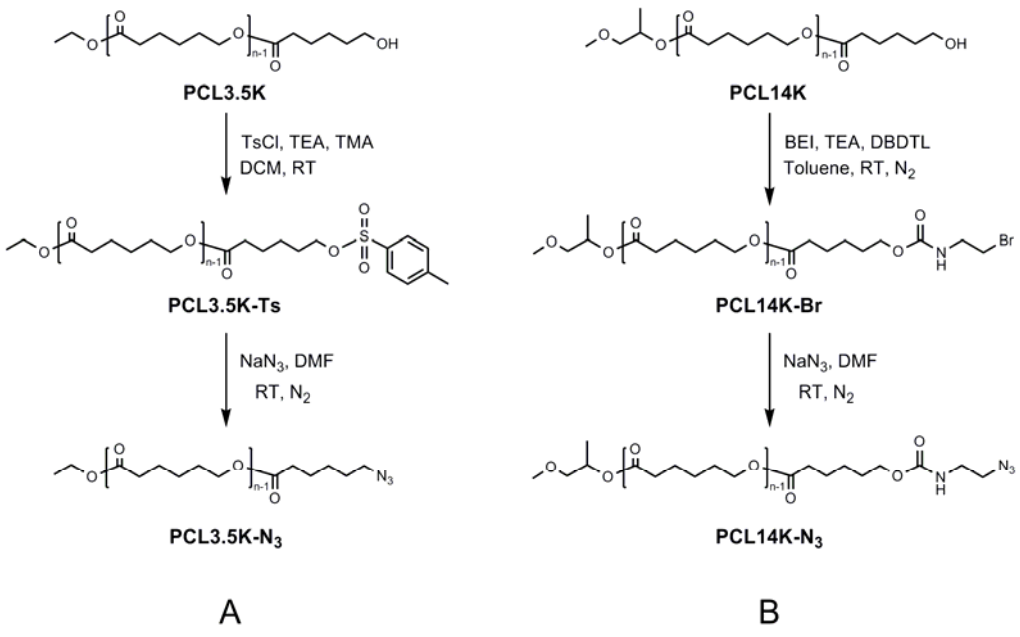


Figure S1. Azido-functionalization of PCL3.5K (A) and PCL14K (B).

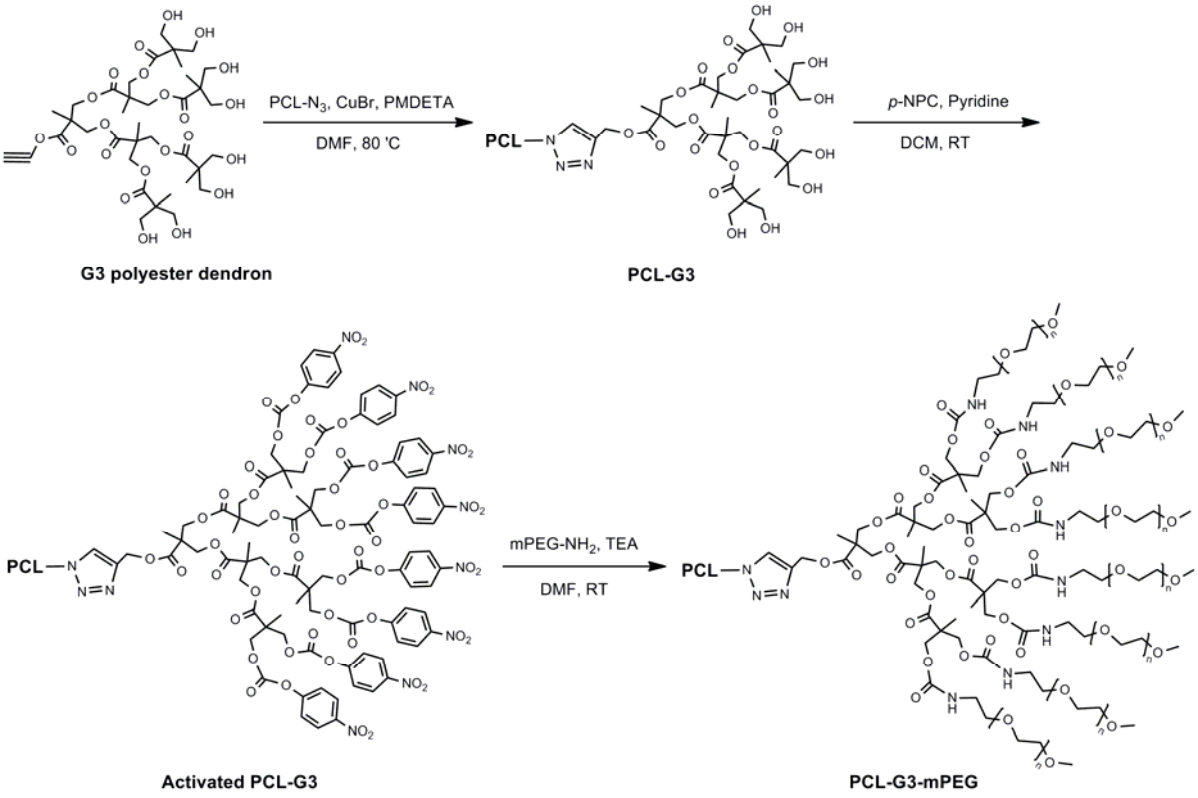
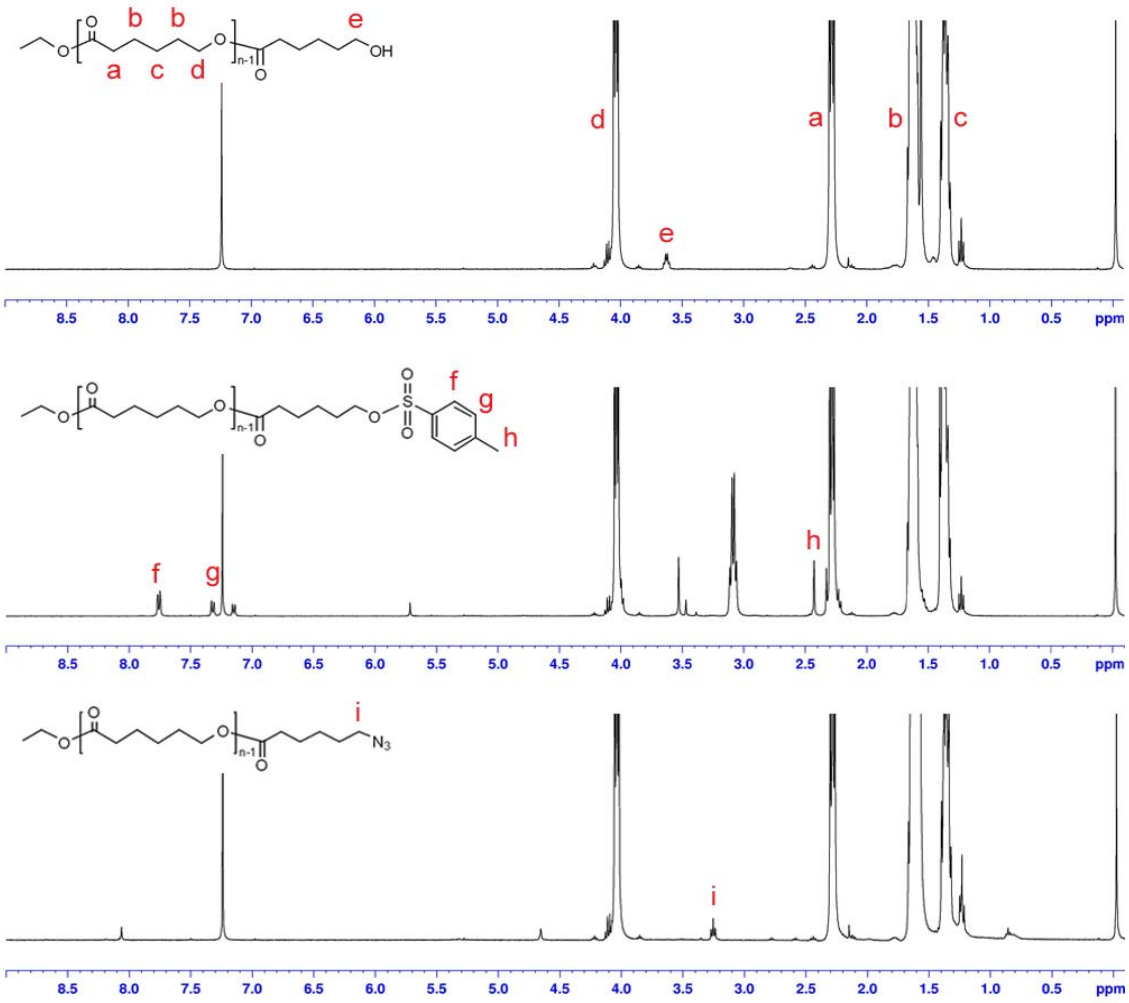


Figure S2. Synthesis of PCL-G3 via click chemistry and mPEG conjugation to PCL-G3.


a
b
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8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 ppm
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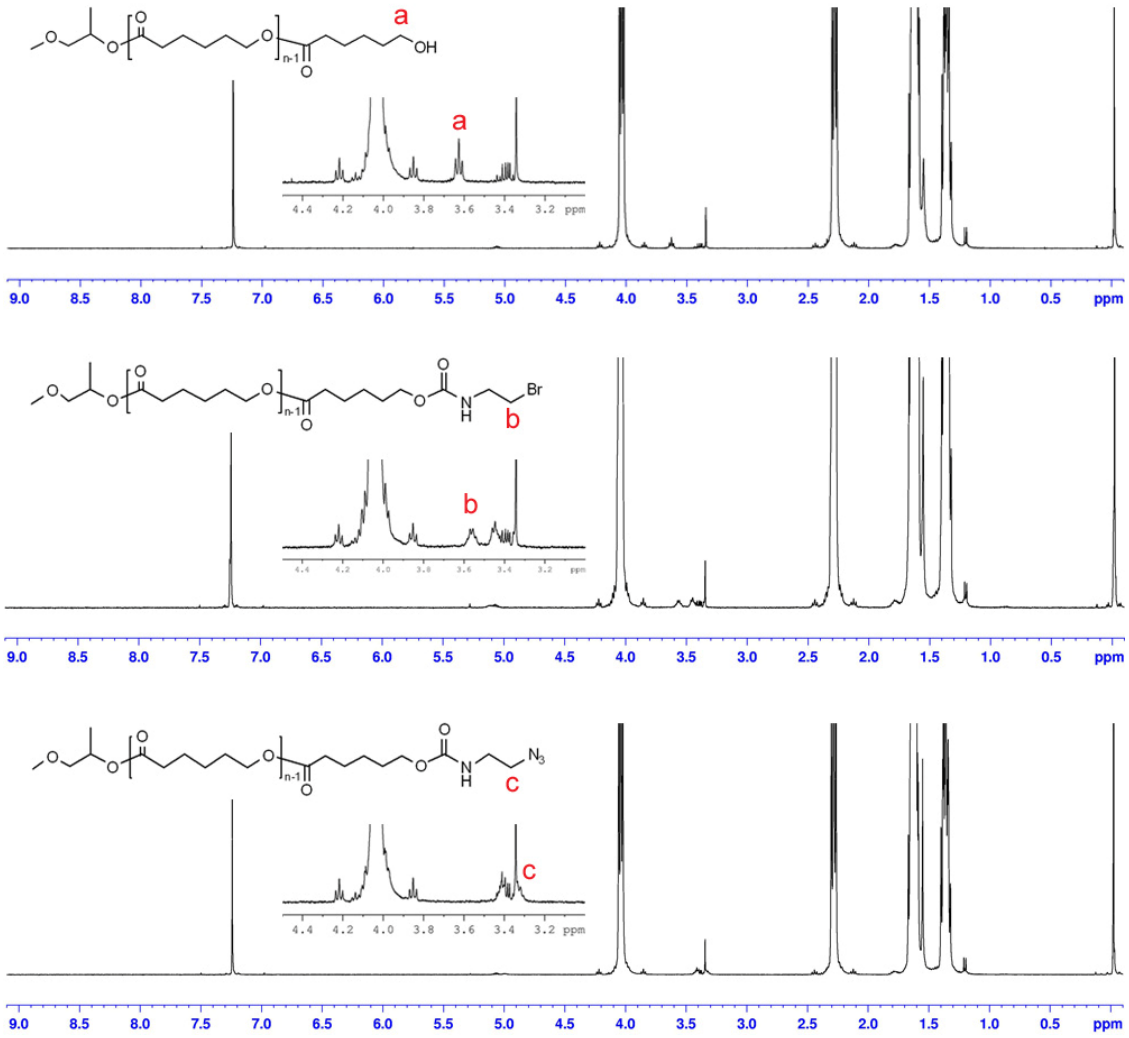
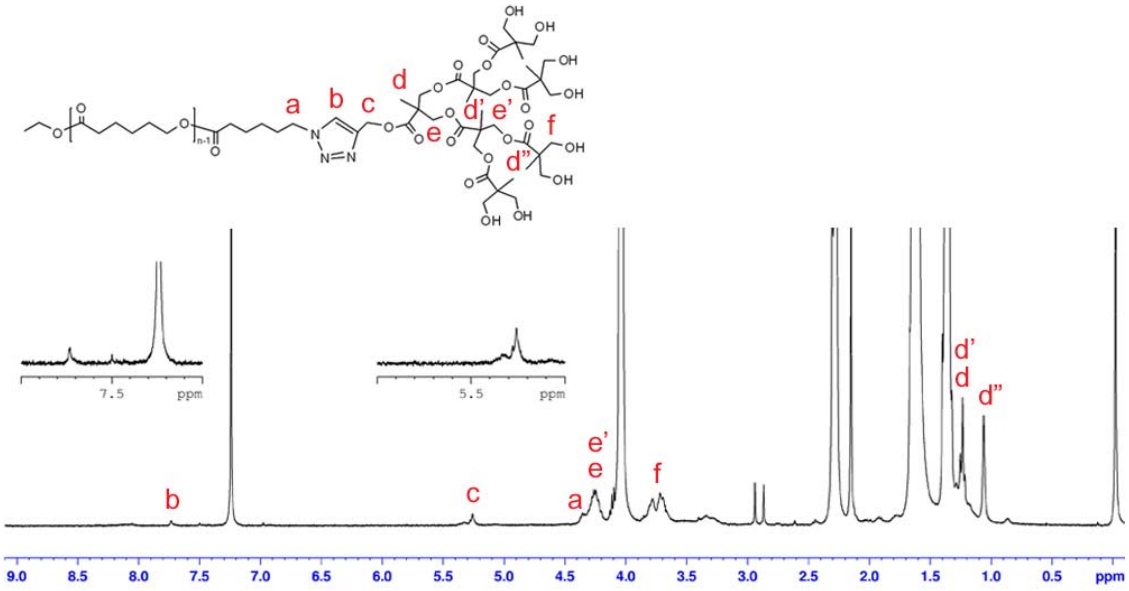
(A)

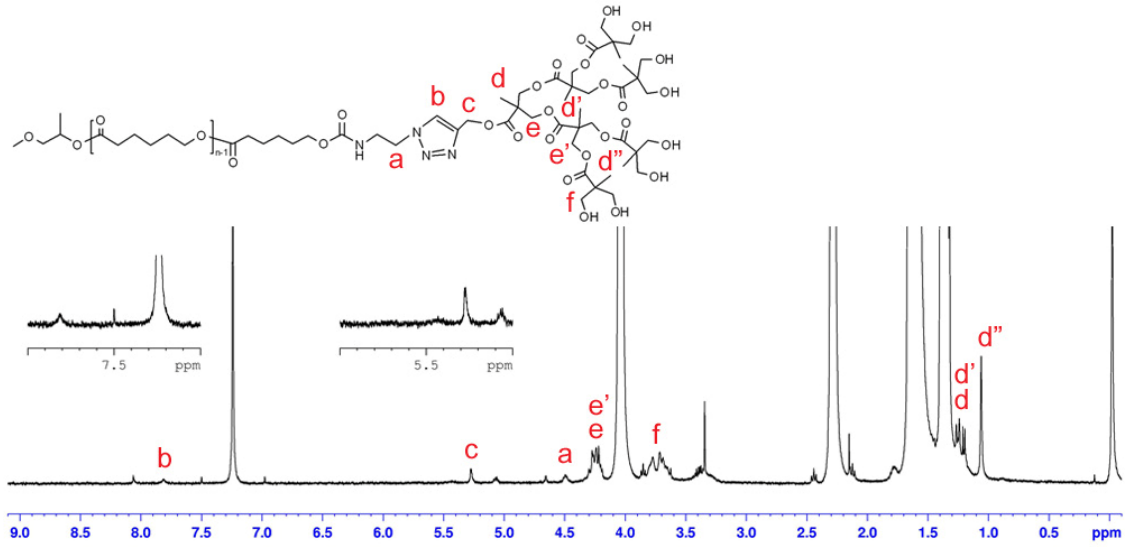


(B)

Figure S3. ^{1}H-NMR spectra of PCL3.5K (A) and PCL14K derivatives (B). The peaks corresponding to two protons adjacent to a hydroxyl group of PCL ("e" for PCL3.5K and "a" for PCL14K) completely disappeared after the reactions. The peak shifts of PCL14K between 3.7 and 3.2 were clearly observed according to each reaction step.



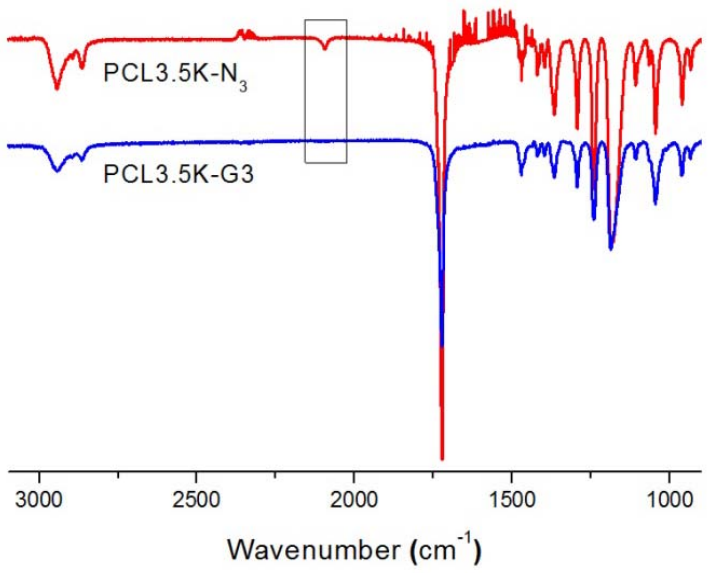
(A)



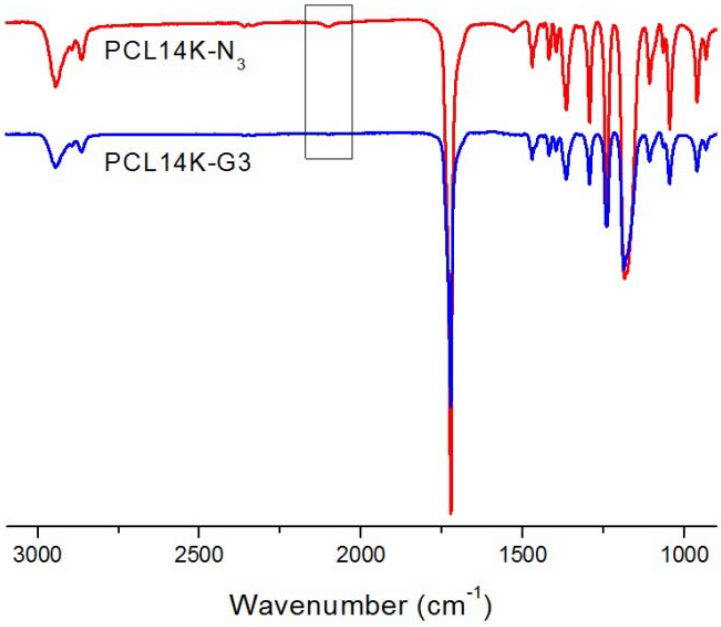
(B)

Figure S4. ^{1}H-NMR spectra of PCL3.5K-G3 (A) and PCL14K-G3 (B) prepared by click reaction. In the ^{1}H-NMR spectrum of PCL3.5K-G3, the characteristic peaks of the polyester dendron were observed at 4.40-4.18, 3.82-3.60, and 1.06. The three peaks corresponding to the triazole formation appeared at 7.73, 5.26 and the third overlapped with the G3 dendron.[3b, 13] In the case of PCL14K-G3, the peaks corresponding to the tri-

azole ring appeared at 7.80, 5.27, and 4.49.



(A)



(B)

Figure S5. FT-IR spectra of PCL3.5K-N_3 and PCL3.5K-G3 (A), PCL14K-N_3 and PCL14K-G3 (B). The disappearance of the azide peak at 2095 cm^{-1} in the FT-IR spectra supports that the click reaction was suc-

cessful.[2, 5]

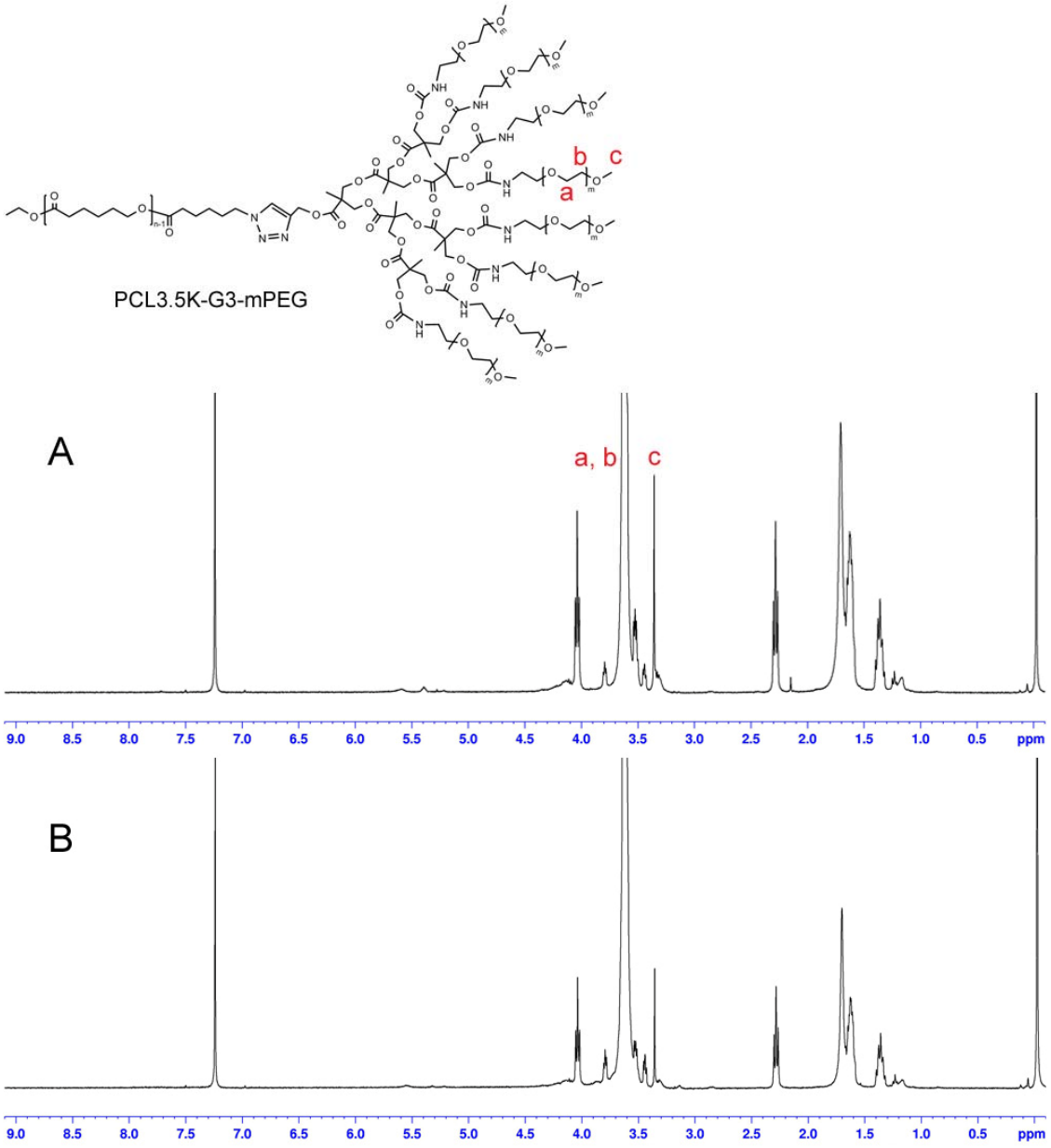


Figure S6. ^{1}H-NMR spectra of PCL3.5K-G3-mPEG2K (A) and PCL3.5K-G3-mPEG5K (B). The major peak representing the ethylene glycol repeating unit for mPEG appeared at 3.62 along with the singlet corresponding to methoxy groups at 3.36. Setting the peak integration values of PCL as a control, the conjugated mPEG integration value was close to the theoretical number of protons needed to indicate multiple mPEG molecules were successfully introduced to the periphery of the PCL3.5K-G3. In addition, the conju-

gation of mPEG5K-NH_2 was attributed to higher intensity corresponding to the number of attached mPEG molecules, compared to linear PCL-mPEG.

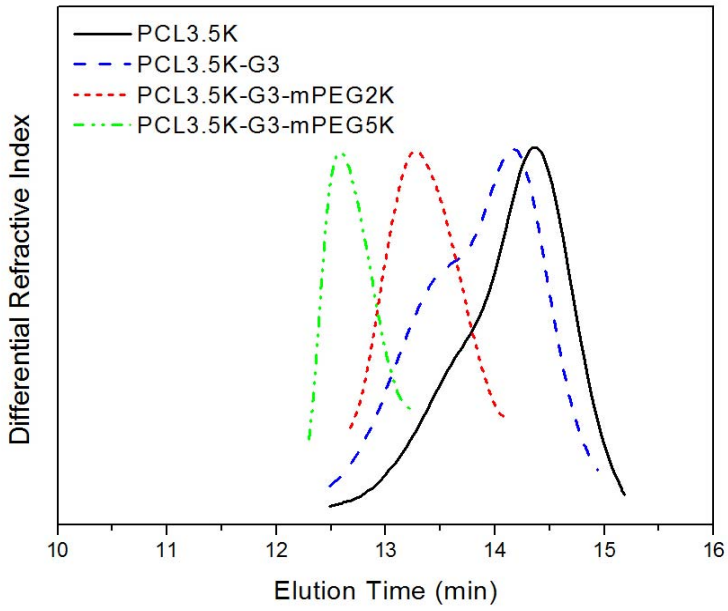


Figure S7. GPC traces of PCL3.5K, PCL3.5K-G3, PCL3.5K-G3-mPEG2K, and PCL3.5K-G3-mPEG5K. The GPC traces represent the great shift to shorter elution time as the molecular weights of the samples increase.

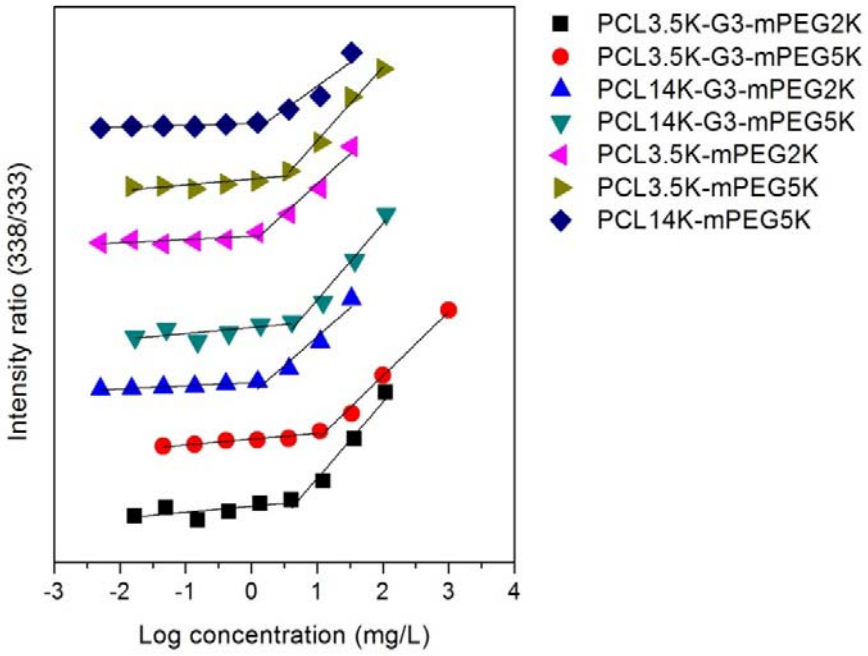


Figure S8. Plots of fluorescence intensity ratios against log concentrations of copolymers. The transition points indicate the CMC results of various PCL-G3-mPEG and PCL-mPEG copolymers.

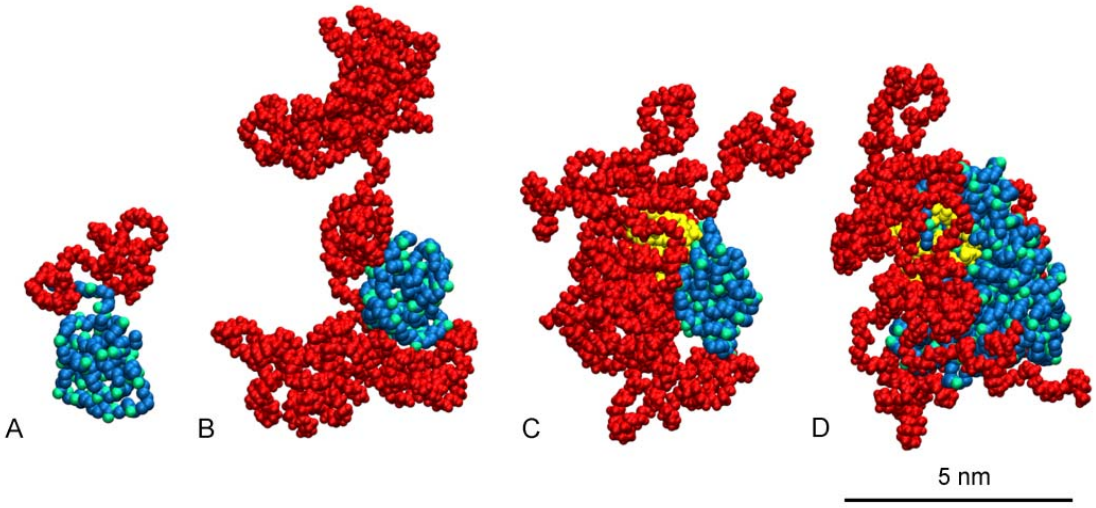


Figure S9. Equilibrated conformations of individual (A) PCL3.5K-mPEG2K, (B) PCL3.5K-mPEG16K, (C) PCL3.5K-G3-mPEG2K, and (D) PCL14K-G3-mPEG2K molecules in water (PCL: blue, G3-dendron: yellow, PEG: red). These structures represent the in-solution morphology of each individual copolymer when they are not packed into micelles. Water is not shown for clarity.

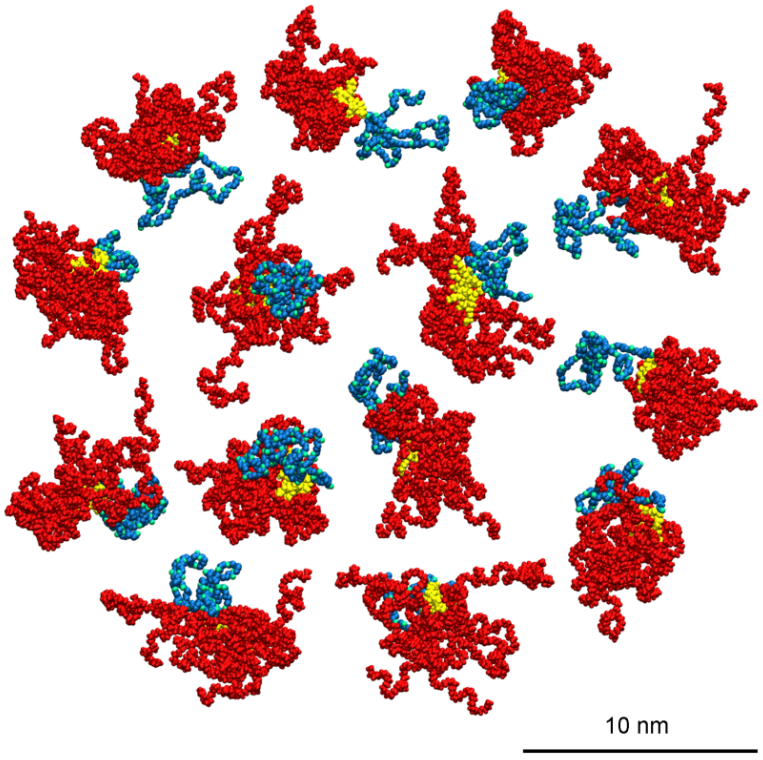


Figure S10. Equilibrated conformations of the individual PCL3.5K-G3-mPEG2K copolymers in the PCL3.5K-G3-mPEG2K micelle with 14 copolymers taken from Fig. 3b-ii. Each of the 14 copolymers that form the micelle was translated from their packed configuration and otherwise was not changed. The packed morphology of each of the PCL3.5K-G3-mPEG2K copolymers changes from the largely globular shape, as observed in Figure S10, to a significantly conical shape, resembling those in Fig. 2b-iii. This conformational change aids in the formation of spherical self-assemblies with fully PEGylated surfaces, as observed in Fig. 3b-ii. (PCL: blue, G3-dendron: yellow, PEG: red). Water is not shown for clarity.

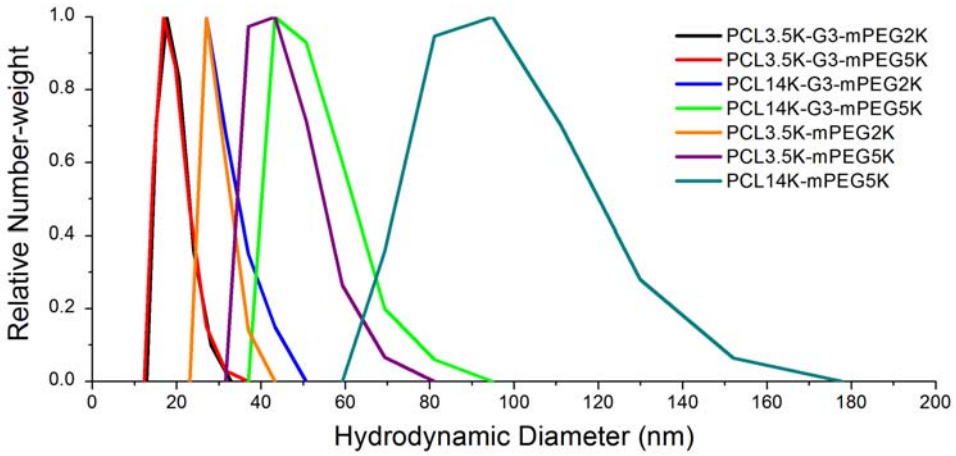


Figure S11. The measurements of hydrodynamic diameter using dynamic light scattering (DLS): PCL3.5K-G3-mPEG2K (18.6 ± 3.5 nm, black); PCL3.5K-G3-mPEG5K (18.3 ± 3.7 nm, red); PCL14K-G3-mPEG2K (30.3 ± 4.9 nm, blue); PCL14K-G3-mPEG5K (49.8 ± 8.3 nm, green); PCL3.5K-mPEG2K (29.4 ± 3.2 nm, orange); PCL3.5K-mPEG5K (44.4 ± 9.4 nm, purple); PCL14K-mPEG5K (94.6 ± 16.3 nm, dark cyan).

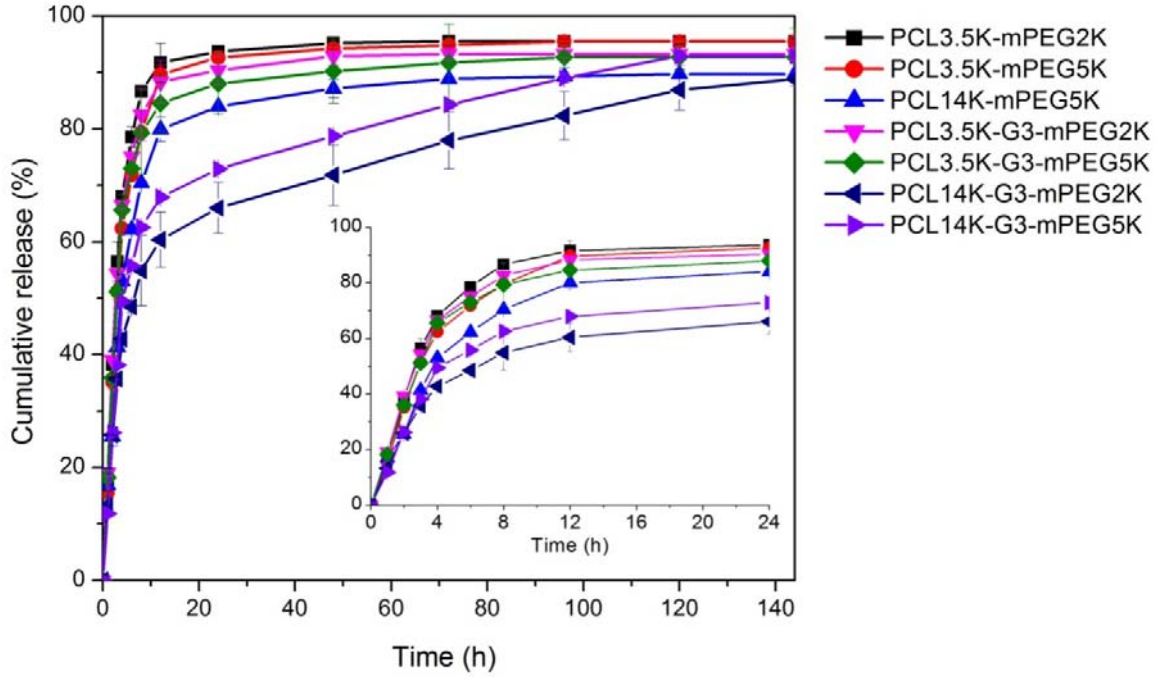


Figure S12. Release profiles of IMC from various micelles for 6 days. Inset: release profiles of various micelles over the first 24 h.

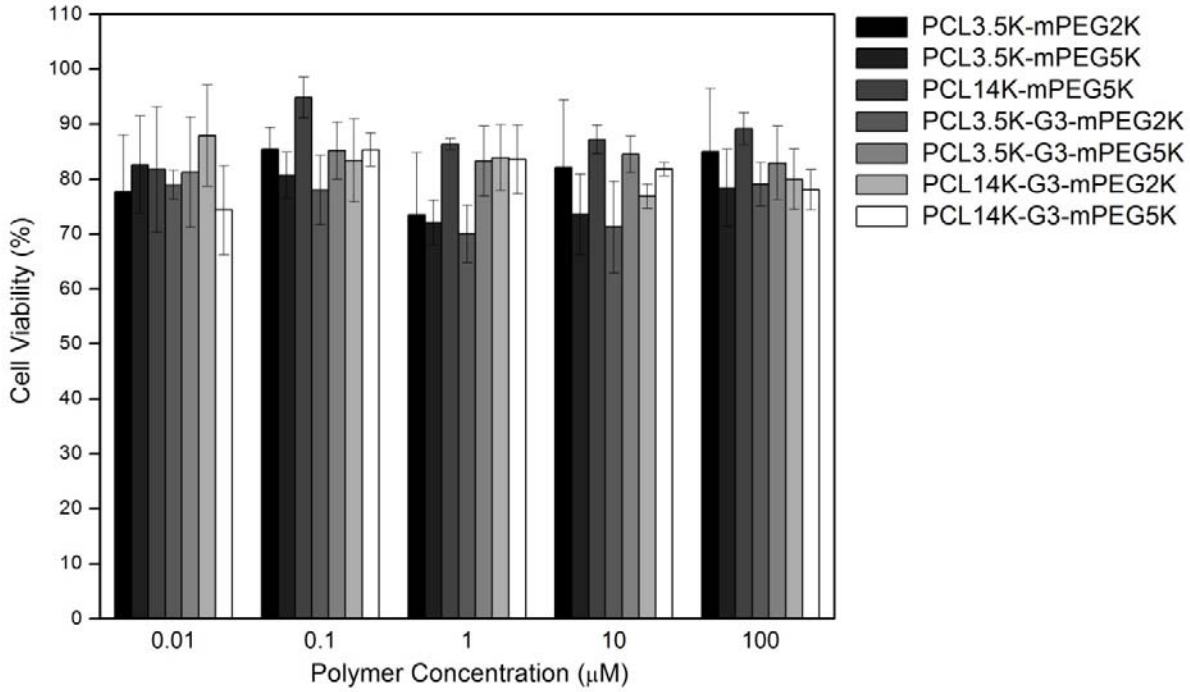


Figure S13. MTS assay results for cell viability of KB cells after 24 h incubation with various block copolymers. All data were expressed as the mean of triplicate cultures. All the polymers do not exhibit significant toxicity in the range of concentrations as high as 100 μM.

Table S1. Molecular weight and polydispersity index (PDI) of polymers used in this study. The molecular weights of PCL-G3-mPEG increased significantly due to multiple conjugations of mPEG molecules to the periphery of the G3 dendron and all copolymers showed relatively narrow polydispersity around 1.07–1.38.

Samples	Theoretical Mw	M_n[a]	M_n[b]	PDI[c]
PCL3.5K	3,500	-	3,500	1.03
PCL3.5K-G3	4,370	4,020	3,630	1.27
PCL3.5K-G3-mPEG2K	21,990	26,280	24,290	1.07
PCL3.5K-G3-mPEG5K	44,720	48,090	38,900	1.06
PCL14K	14,000	-	13,370	1.20
PCL14K-G3	14,870	14,780	16,370	1.27
PCL14K-G3-mPEG2K	32,490	32,000	27,710	1.16
PCL14K-G3-mPEG5K	55,220	58,780	54,140	1.38

[a] Number-averaged molecular weight, M_n, estimated by ^{1}H-NMR.

[b], [c] Measured by GPC using triple angle laser light scattering.

Table S2. Dependence of the total micelle diameter (d_{total}), core diameter (d_{core}) and PEG corona diameter (d_{PEG}) based on aggregation number (N_{agg}) and monomer type. These values are obtained by angular averaging (2-5 ns) of the radial extensions of the PEG chains with respect to the micelle center of mass.

Samples	N_{agg}	d_{total} *(nm)*	d_{core} *(nm)*	d_{PEG} *(nm)*
PCL3.5K-mPEG2K	86	12.94	11.44	0.75
PCL3.5K-mPEG2K	128	15.22	13.54	0.84
PCL3.5K-G3-mPEG2K	14	13.42	7.68	2.87
PCL14K-G3-mPEG2K	10	12.98	9.18	1.91

Table S3. IMC loading and encapsulation of various micelles (n = 3).

Samples	Loading (%)	Encapsulation (%)	IMC/PCL ratio
PCL3.5K- mPEG2K	6.7 ± 0.3	73.9 ± 3.3	1.62
PCL3.5K- mPEG5K	7.8 ± 0.7	85.7 ± 7.6	4.50
PCL14K- mPEG5K	8.4 ± 0.2	92.7 ± 2.4	6.05
PCL3.5K-G3-mPEG2K	8.3 ± 0.5	90.8 ± 5.6	32.05
PCL3.5K-G3-mPEG5K	5.0 ± 0.4	54.9 ± 3.9	79.83
PCL14K-G3-mPEG2K	9.3 ± 2.4	102.7 ± 25.8	19.60
PCL14K-G3-mPEG5K	6.2 ± 2.8	68.4 ± 31.2	37.75

Loading (%) = (Measured IMC amount / Mass of IMC loaded micelle) × 100 (%)

Encapsulation (%) = (Measured IMC amount/Theoretical IMC loading amount) × 100 (%)

The encapsulation efficiencies (%) were significantly different between PCL3.5K-G3-mPEG2K and PCL3.5K-G3-mPEG5K IMC loaded micelles based on a 1-way ANOVA followed by Tukey's post-hoc test at $p<0.05$. The differences among all other samples were not statistically significant. IMC/PCL ratio represents the moles of IMC encapsulated per mole of PCL present in the copolymer, demonstrating that the amount of IMC encapsulated per amount of PCL was one order of magnitude greater than that of the linear-block copolymer micelles. The IMC/PCL ratios were calculated based on following. For the example of PCL3.5K-G3-mPEG2K, assuming 1 mg of drug encapsulated micelle, (1 mg/21990 mg/mmol) × (3500 mg PCL/21990 mg micelle) = 7.238×10^{-6} mmol PCL. Calculating the mass of IMC contained within 1 mg of micelle (loading) and converting to moles IMC: (0.083 mg IMC) × (1 mmol/ 357.79 mg IMC) = 2.320×10^{-4} mmol IMC. Therefore, IMC/PCL ratio = 2.320×10^{-4} mmol IMC/7.238×10^{-6} mmol PCL = 32.05.

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