Supporting information

PDMAEMA-graft-PCL prepared by ring-opening polymerization and click chemistry

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Materials.

ε-caprolactone (Aldrich), toluene (Fluka) were dried over calcium hydride for 24 hours at room temperature and distilled under reduced pressure. Tetrahydrofuran (THF, Fluka) was dried by refluxing over a benzophenone-sodium mixture and distilled. 2-(dimethylamino)ethyl methacrylate (DMAEMA, Aldrich, 98%) was passed through a column of alumina to remove inhibitor. All other materials were obtained from Aldrich and were used without any further purification.

Instrumentation

^{1}H NMR and ^{13}C NMR spectra were recorded on a Bruker spectrometer (AMX300) operating at 300 MHz and 75 MHz respectively. Deuterated chloroform or deuterated N,N-Dimethylformamide were used as solvent, and chemical shifts were expressed in ppm with respect to tetramethylsilane (TMS). Infrared spectra were recorded with a Perkin–Elmer Spectrum 100 FT-IR spectrometer. Size exclusion chromatography (SEC) was performed at room temperature using a Waters system equipped with a guard column and a 600 mm PLgel 5 mm Mixed C (Polymer Laboratories), with a Waters 410 refractometric detector, and a Waters PDA 2996 photodiode array detector. Calibration was established with poly(styrene)

standards from Polymer Laboratories. THF was used as solvent with a flow rate of 1 mL.min^{-1}.

Synthesis of α–propargyl-δ-caprolactone (1).

To a solution of 2.0 M lithium diisopropyl amide (12 mL, 24.1 mmol) in anhydrous tetrahydrofuran (80 mL) at –78 °C was added dropwise under inert atmosphere a solution of ε-caprolactone (2.5 g, 21.9 mmol) in anhydrous tetrahydrofuran (10 mL). After one hour at -78 °C, propargyl bromide (2.92 mL, 26.3 mmol) and hexamethylphosphoramide (5 mL) were added to the lactone enolate. After being stirred for 3 hours at –30 °C, the reaction mixture was quenched with a saturated ammonium chloride solution and thoroughly washed successively with a saturated ammonium chloride solution and saturated NaCl aqueous solution. The organic layer was dried over magnesium sulfate and evaporated under reduced pressure. Purification of the crude product by silica gel chromatography (heptane/EtOAc, 7/3) afforded **1** as a colorless, viscous liquid (2,01 g, 13.2 mmol, 60% yield). ^{1}H NMR (300 MHz, CDCl3) δ (ppm)= 4.21 (m, 2H, CH_2O), 2.73 (m, 1H, COC*H*CH_2), 2.57 (m, 1H, C*H*$_2$-C≡CH), 2.29 (m, 1H, C*H*$_2$-C≡CH), 1.99 (m, 2H, C*H*$_2$$CH_2O$), 1.95 (t-d, 1H, C≡C*H*), 1.65 (m, 1H, C*H*$_2$$CH_2$ CH_2O), 1.4 (m, 2H, COCHC*H*$_2$). ^{13}C NMR (75 MHz, $CDCl_3$) δ (ppm)= 176.2 (*C*=O), 82.2 (*C*≡CH), 69.75 (*C*H_2O), 68.81 (C≡*C*H), 42.60 (CO*C*HCH_2), 28.96, 28.89, 28.38, 22.04 (*C*H_2-C≡CH). IR (ATR, cm^{-1}): 3280 (C≡CH), 1720 (C=O). MS (ES, %): *m*/*z*= 153.1 (40) [M^++1], 305.2 (100) [2M^++H].

Synthesis of poly(α-propargyl-ε-caprolactone-*co*-ε-caprolactone) (2)

Polymerization was carried out using standard Schlenk technique under an inert atmosphere of argon. εCL (2.66 g, 29.6 mmol, 90 equiv), lactone **1** (0.5 g, 3.29 mmol, 10 equiv), $Sn(OTf)_2$ (68.5 mg, 0.164 mol, 0.5 equiv), isopropanol (25 µL, 0.328 mmol, 1 equiv) and toluene (3 mL) were placed in an oven dried Schlenk tube. The tube was fitted with a rubber septum. The solution was further degassed by three freeze-pump-thaw cycle. The resulting mixture was stirred at room temperature for 48 hours. To stop the reaction, polymerization was quenched by addition of an excess of 1 N HCl. The reaction mixture was poured into cold methanol. The precipated polymer was collected by filtration and dried *in vacuo*. ^{1}H NMR (300 MHz, CDCl3) δ (ppm)= 4.96 (m, $(CH_3)_2CH$), 4.02 (t, CH_2O), 3.60 (m, CH_2OH), 2.50 (m, COC*H*CH2), 2.26 (t, $COCH_2$), 1.96 (m, C≡C*H*), 1.49-1.70 (m, CH_2-CH_2-CH_2-CH_2-O-), 1.29-1.42 (m, $COCH_2CH_2$), 1.18 (d, $(CH_3)_2CH$). IR (ATR, cm^{-1}): 3280 (C≡CH), 1720 (C=O).

$M_{n,\,SEC}$ = 6000 $g.mol^{-1}$

M_w/M_n = 1.2

Synthesis of α-azido PDMAEMA (3)

Synthesis of 3-azidopropanol: In a round-bottom flask were combined 3-chloropropanol (5.1 mL, 5.77 g, 61 mmol), sodium azide (7.94 g, 122 mmol), tetrabutylammonium hydrogen sulfate (40 mg), and water (5 mL). The reaction mixture was stirred at 80 °C for 24 hours, and then at room temperature for 12 hours. The mixture was extracted with three 100 mL portions of diethyl ether. The organic phase was dried over $MgSO_4$ and the solvent was removed *in vacuo*. After distillation, 4.8 g of 3-azidopropanol was obtained (78% yield). ^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm)= 3.69 (t, 2H, CH_2O), 3.40 (t, 2H, CH_2N_3), 1.78 (m, 2H, $CH_2CH_2CH_2$).

Synthesis of (3-Azidopropyl)bromoisobutyrate (APBIB, α-functionalized ATRP initiator): 3-azidopropanol (2 g, 19.8 mmol), triethylamine (4.1 mL, 29.7 mmol), and dry CH_2Cl_2 (50 ml)

were placed in a round bottom flask. Bromoisobutyryl bromide (3.67 mL, 29.7 mmol) was then added slowly at 0 °C, and the mixture stirred at ambient temperature for 24 h. The solution was filtered to remove the triethylammonium bromide and the solvent removed *in vacuo*. The viscous residue was dissolved in dichloromethane and washed with saturated $NaHCO_3$ solution. The product was dried over $MgSO_4$ and the volatiles removed *in vacuo*. The crude product was purified by column chromatography on silica using heptane–ethyl acetate (9:1) as eluent. ^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm)= 4.24 (t, 2H, C*H*$_2$OC=O), 3.41 (t, 2H, C*H*$_2$N$_3$), 1.92 (m, 2H, CH$_2$C*H*$_2$CH$_2$), 1.91 (s, 6H, Br(C*H*$_3$)$_2$CC=O).

*Synthesis of α-azido PDMAEMA (**3**):* Polymerization was carried out using standard Schlenk technique under an argon atmosphere. CuBr (118 mg, 0.82 mmol, 1 equiv) and the initiator (206 mg, 0.82 mmol, 1 equiv) were placed in an oven dried Schlenk tube. The tube was fitted with a rubber septum. Degassed toluene (7 mL) and DMAEMA (7 mL, 41.5 mmol, 50 equiv) were transferred to the tube *via* degassed syringe. The solution was further degassed by three freeze-thaw-pump cycles. The mixture was stirred rapidly under argon and *N,N,N',N',N''*-pentamethyldiethylenetriamine (PMDETA, 171 μL, 0.82 mmol, 1 equiv) was added. The resulting mixture was placed in a thermostatically controlled oil bath at 60 °C for 20 minutes. The solution was stopped with liquid nitrogen, and the catalyst was removed from the samples by passing through a column of activated basic alumina prior to molecular weight analysis. Conversion was measured using ^{1}H NMR. ^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm)= 3.98 (C*H*$_2$OC=O), 3.33 (C*H*$_2$N$_3$), 2.49 (C*H*$_2$N), 2.21 (N(C*H*$_3$)$_2$), 1.6-2.0 (C*H*$_2$C(CH$_3$)C=O), 0.65-1.11 (CH$_2$C(C*H*$_3$)C=O).

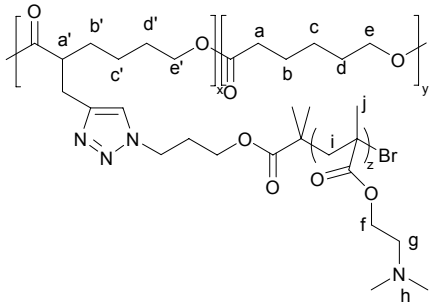
$M_{n,SEC}$ = 13600 g.mol-1

M_w/M_n = 1.11

$M_{n,NMR}$ = 6500 g.mol1

Synthesis of PDMAEMA-graft-PCL graft copolyester (4) by Huisgen 1,3-Dipolar Cycloaddition

In schlenk tube A were placed polyester **2** (50 mg, F_I= 0.08, 3.5×10^{-2} mmol of lactone **1** unit), PMDETA (15 µL, 7×10^{-2} mmol, 2 equiv), and THF (5 mL). In schlenk tube B was placed CuBr (10 mg, 7×10^{-2} mmol, 2 equiv). Both tubes A and B were fitted with a rubber septum. Tube A was further degassed by three freeze-pump-thaw cycle, and was transferred by cannula to tube B (previously placed under vacuum). The tube was stirred at room temperature for 24 hours. The solution was then passed through a basic alumina column to remove copper salt. The reaction mixture was poured into cold heptane. The precipated copolymer was recovered by filtration and dried *in vacuo*. ^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm)= 4.01 (e+e'+f), 2.51 (g), 2.23 (a+a'+h), 2.04-1.67 (i), 1.65-1.46 (d+c+d'+c'), 1.44-1.22 (b+b'), 1.15-0.64 (j).



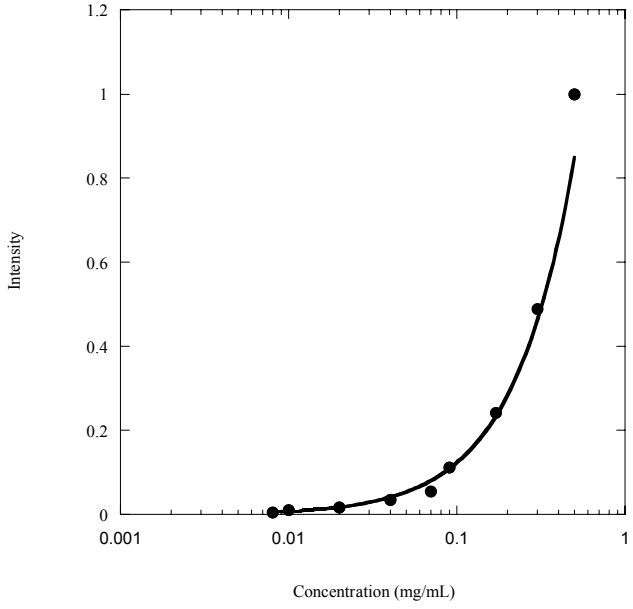
Quaternization of PDMAEMA-graft-PCL (4)

The copolymer **4** (500 mg) was dissolved in THF (100 mL) in a round-bottom flask. The mixture was stirred, and an excess of methyl iodide was added (500 µL). The solution was stirred at room temperature overnight. The crude copolymer was then purified by dialysis

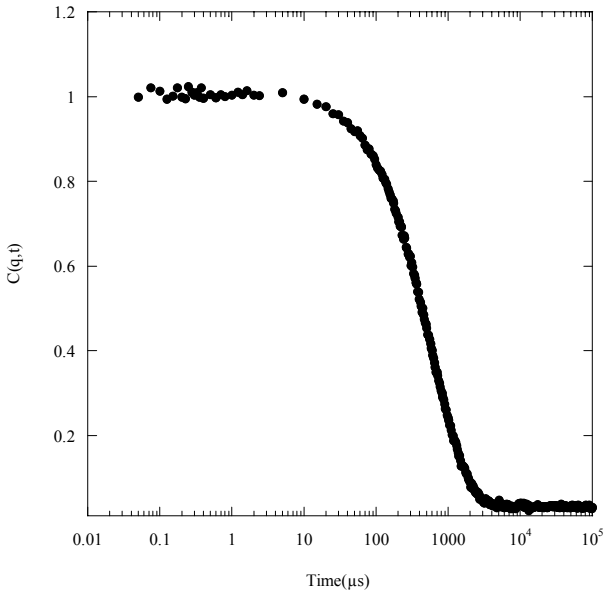
against deionized water using dialysis tubing (cut-off 10000 D). Quaternized copolyesters were obtained by lyophilization of final aqueous solution.

Preparation of aqueous micellar solutions and light scattering measurements.

The copolymer was directly dissolved in pure Millipore water. The aqueous copolymer solutions were stirred for 2 h and left to rest overnight before any light scattering measurements. The critical micelle concentration was determined by static light scattering. The mean hydrodynamic radius (Rh) and the radius distribution of the copolymer aqueous solutions aggregates were measured at 90 scattering angle using a Brookhaven BI-200 goniometer with vertically polarized incident light of wavelength λ = 488 nm supplied by an argon laser operating at 200 mW and a Brookhaven BI-9000 AT digital autocorrelator. All the measurements were made at 25° C. The distribution of relaxation times and then the radius distribution of the copolymer aggregates were obtained by using CONTIN analysis of the autocorrelation function, C(q, t).



Static light scattering variation of the graft-copolymer aqueous solutions with the concentration.



Representative autocorrelation function C(q,t) at scattering angle of 90.

Drug loading process of micelles.

The copolymer **4** (15 mg, 3 $g.L^{-1}$) was dissolved in pure Millipore water (5 mL). Clofazimine (20 mg) was added in the aqueous copolymer solution, and the solution was stirred overnight using a magnetic stirrer.