Towards smart polymeric drug carriers: Self-assembling γ-substituted polycaprolactones with highly tunable thermoresponsive behavior

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Electronic Supplementary Information

Experimental

Materials

All commercial chemicals were purchased from Aldrich Chemical Co., Inc. and were used without further purification unless otherwise noted. Benzyl alcohol and stannous (II) 2-ethylhexanoate were purified by vacuum distillation prior to use. All polymerization reactions were conducted under purified nitrogen. The polymerization glassware and syringes were dried at 120 °C for at least 24 hours before use and cooled under a nitrogen atmosphere.

*Synthesis of 2-methoxyethyl 4-methylbenzenesulfonate (**1**)*

In a 500 mL round bottom flask, 2-methoxyethanol (16.0 g, 0.21 mol), triethylamine (39 mL, 0.28 mol), and dichloromethane (DCM, 40 mL) were combined. In a separate container, p-toluenesulfonyl chloride (26.7 g, 0.14 mol) was dissolved in 250 mL DCM. The tosyl chloride solution was added by pipet slowly to the round bottomed flask over an ice bath. After complete addition, the reaction was allowed to warm to room temperature and stir overnight. A white precipitate formed. The mixture was washed with 200 mL 3M HCl, 200 mL 5 wt% $NaHCO_3$, and 200 mL water. The organic phase was dried over anhydrous magnesium sulfate and concentrated in vacuo to obtain product (**1**). **Fig. S1**. ^{1}H NMR (270 MHz, $CDCl_3$): δ_H 2.42 (s, 3H), 3.28 (s, 3H), 3.55 (t, 2H), 4.13 (t, 2H), 7.32 (d, 2H), 7.77 (d, 2H).

*Synthesis of 4-(2-methoxyethoxy)cyclohexanol (**2**)*

A suspension of dry NaH (3.90 g, 0.163 mol) in 50 mL N,N-dimethylformamide (DMF) was prepared in a round bottomed flask. To the suspension, a solution of 1,4-cyclohexane diol (16.3 g, 0.140 mol) in 100 mL DMF was added. The vessel was outfitted with a bubbler and the reaction was allowed to proceed at room temperature for one hour, followed by 5 hours of heating at 50 °C. Methoxyethoxy-tosylate (29.3 g, 0.127 mol) was added in bulk by pipet, and the mixture was allowed to react overnight at 40 °C. It was heated at 50 °C for two hours before quenching with 3M HCl. DMF solvent was removed by vacuum distillation, and the compound was extracted with diethyl ether (4 x 150 mL). The salts were filtered and the filtrate was concentrated in vacuo. The residue was purified by flash chromatography (R_f = 0.29 in

100% ethyl acetate) to obtain product (**2**). **Fig. S2**. ^{1}H NMR (500 MHz, $CDCl_3$): δ_H 1.34 (dd, 2H), 1.67 (m, 3H), 1.84 (m, 1H), 1.98 (dd, 2H), 3.28 (m, 0.5H), 3.38 (s, 3H), 3.54 (m, 4H), 3.74 (m, 0.5H).

*Synthesis of 4-(2-methoxyethoxy)cyclohexanone (**3**)*

To a 250 mL round bottom flask, compound **2** (3.05 g, 0.0175 mol) was added in bulk and was stirred over an ice water bath for 10 min. To a solution of $K_2Cr_2O_7$ (10.3 g, 0.035 mol) in 10 mL water, H_2SO_4 (3.7 mL, 0.07 mol) was added slowly (~ 1 mL / min) by pipet to form chromic acid. This was diluted with 30 mL water and poured into the product from the previous step, over an ice bath. A dark brown/orange color appears immediately upon addition of chromic acid. The reaction mixture stirred overnight at room temperature, turning a dark green color. The mixture was extracted with DCM (4 x 100 mL); then the organic phase was washed with 10 mL DIW. The organic phase was dried over $MgSO_4$, filtered, and concentrated in vacuo. The product **3** was purified by column chromatography. **Fig. S3**, ^{1}H NMR (270 MHz, $CDCl_3$): δ_H 1.96 (m, 2H), 2.06 (m, 2H), 2.26 (m, 2H), 2.57 (m, 2H), 3.38 (s, 3H), 3.56 (m, 5H).

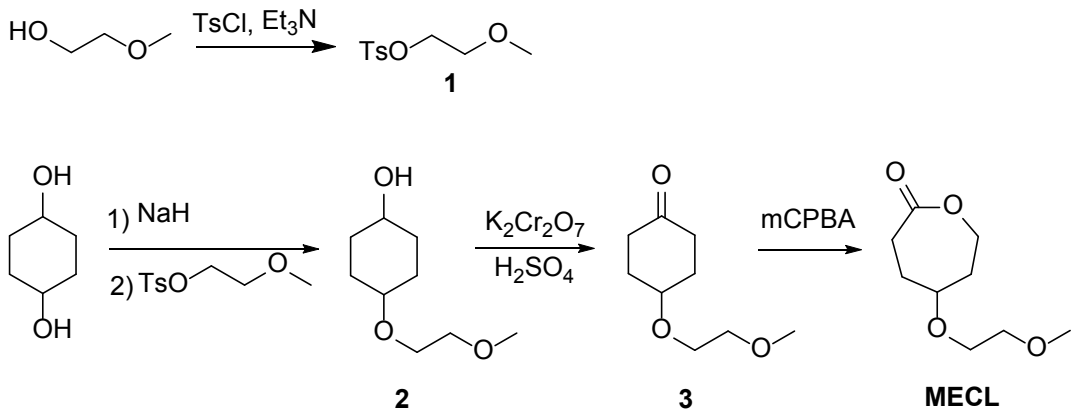
*Synthesis of γ-(2-methoxyethyoxy)-ε-caprolactone (**MECL**)*

In a clean 250 mL round bottom flask, m-chloroperoxybenzoic acid (77%, 3.07 g, 0.014 mol) was dissolved in 100 mL of DCM. While stirring over an ice bath, compound **3** (1.39 g, 0.008 mol) was added. After an hour, the reaction was allowed to stir overnight at room temperature. Approximately 2 g of potassium bicarbonate was added to the reaction vessel, along with 5 mL of DI water. This mixture was stirred vigorously for a minimum of 2 hours. The organic phase was collected, and the aqueous phase was washed twice with 20 mL of dichloromethane. The organic phase was dried, filtered and concentrated. A small amount of mCPBA remained, but was removed by flash chromatography. The acid was eluted in ~10% ethyl acetate/ 90% hexane, then the column was flushed with 100% ethyl acetate to elute the product **MECL**. Overall yield of the four steps as high as 10% has been achieved. **Fig. S4**, ^{1}H NMR (500 MHz, $CDCl_3$): δ_H 1.85 (t, 1H), 2.02 (m, 3H), 2.39 (dd, 1H), 2.97 (dd, 1H), 3.37 (s, 3H), 3.58 (m, 4H), 3.70 (m, 1H), 4.05 (dd, 1H), 4.51 (dd, 1H). ^{13}C NMR (500 MHz, $CDCl_3$): δ_C 27.19, 27.75, 33.86, 58.99, 63.33, 67.53, 72.06, 74.38, 175.92. Elemental analysis (**Table S1**) calculated for $C_9H_{16}O_4$: C, 57.43%; H, 8.57%. Found: C, 56.94%; H, 8.30%.

Analysis

^{1}H NMR spectra of the synthesized monomer and polymers were recorded on a Bruker AVANCE III 500 MHz NMR instrument at 25 °C in $CDCl_3$. ^{1}H NMR data are reported in parts per million as chemical shift relative to tetramethylsilane (TMS) as the internal standard. GC/MS was performed on an Agilent 6890-5973 GC/MS workstation. The following conditions were used for all GC/MS analyses: injector and

detector temperature, 250 °C; initial temperature, 70 °C; temperature ramp, 10 °C/min; final temperature, 80 °C. Molecular weights of the synthesized polymers were measured by size exclusion chromatography (SEC) analysis on a Viscotek VE 3580 system equipped with ViscoGEL columns (GMHHR-M), connected to a refractive index (RI) detectors. GPC solvent/sample module (GPCmax) was used with HPLC grade THF as the eluent, and calibration was based on polystyrene standards. Running conditions for SEC analysis were flow rate = 1.0 mL/min, injector volume = 100 µL, detector temperature = 30°C, and column temperature = 35°C. All the polymers samples were dissolved in THF, and the solutions were filtered through PTFE filters (0.45 µm) prior to injection.



Scheme S1. Synthesis of γ-(2-methoxyethoxy)-ε-caprolactone (**MECL**).

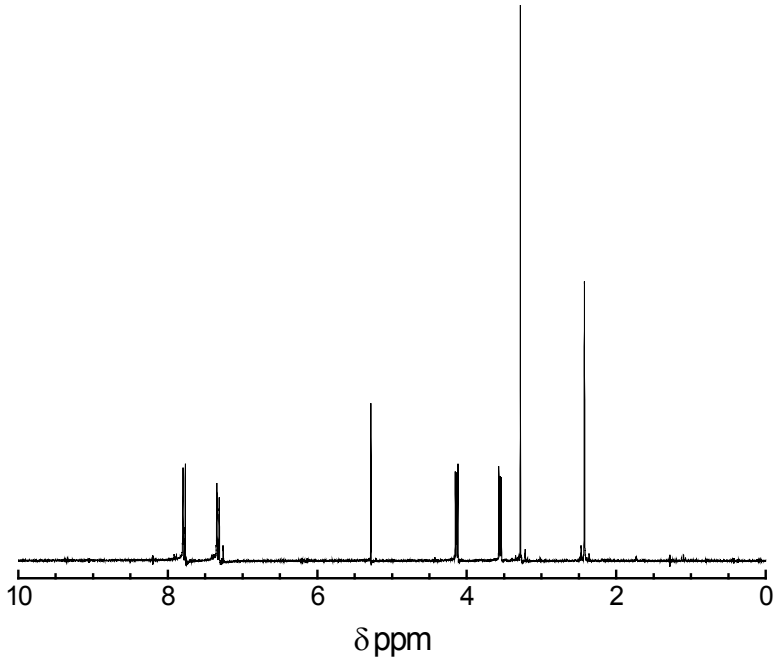


Fig. S1 ^{1}H NMR spectrum of 2-methoxyethyl 4-methylbenzenesulfonate (**1**); residual DCM solvent peak at 5.30 ppm, chloroform peak at 7.26 ppm.

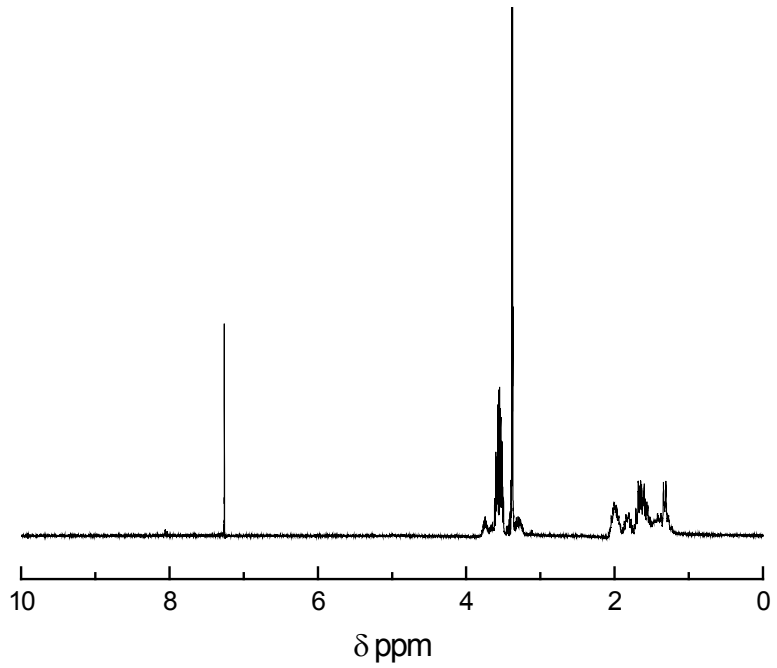


Fig. S2 ^{1}H NMR spectrum of 4-(2-methoxyethoxy)cyclohexanol (**2**); residual chloroform peak at 7.26 ppm.

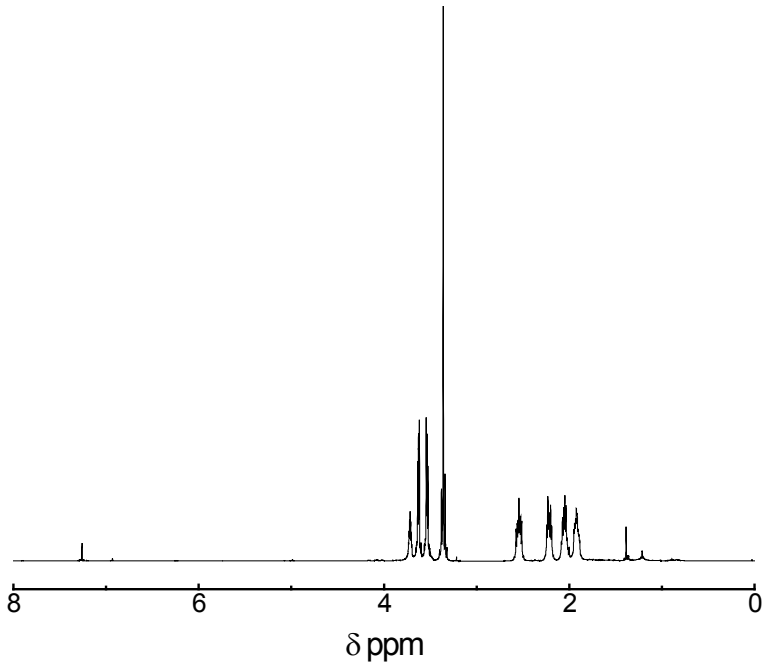


Fig. S3 ^{1}H NMR spectrum of 4-(2-methoxyethoxy)-cyclohexanone (**3**); residual water peak at 1.55 ppm, chloroform peak at 7.26 ppm.

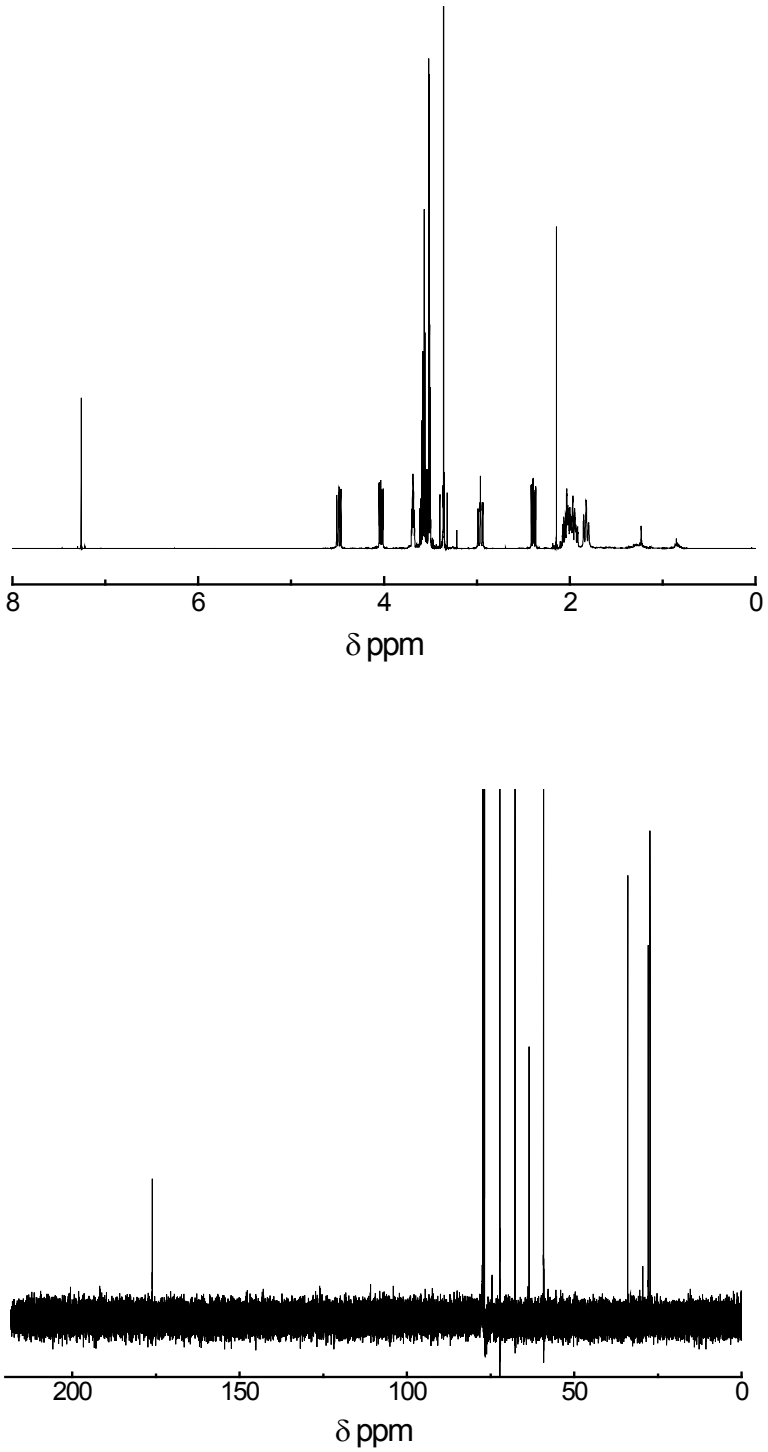
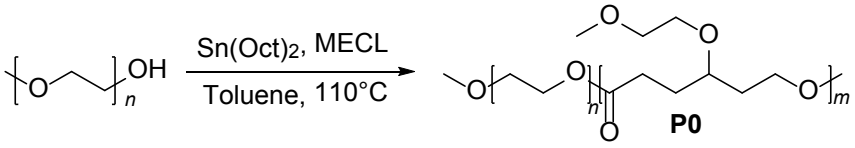


Fig. S4 ^{1}H NMR spectrum of γ-(2-methoxyethyoxy)-ε-caprolactone (top); residual grease peak 0.9 ppm, water peak at 1.55 ppm, acetone peak at 2.05 ppm, chloroform peak at 7.26 ppm. ^{13}C NMR spectrum of γ-(2-methoxyethyoxy)-ε-caprolactone (bottom); residual chloroform peak at 77 ppm.

Table S1. Elemental analysis of monomer γ-(2-methoxyethyoxy)-ε-caprolactone.

Element	Theoretical	Experimental
C	57.43%	56.94%
H	8.57%	8.30%



Scheme S2. Synthesis of PEG-*b*-PMECL (**P0**).

Table S2. Summary of **P0** properties.

Polymer	M_n[1] ($gmol^{-1}$)	PDI[1] (M_w/M_n)	PEG[2] (mol%)	MECL[2] (mol%)	CMC[3] (g/L)	D_h[4] (nm)
P0	4000	2.1	57	43	9.04×10^{-4}	33.0 ± 0.3

[1]Determined by SEC using polystyrene calibration. [2]Block content mol% determined by ^{1}H NMR. [3]Determined by fluorescence measurements with pyrene. [4]Micelle hydrodynamic diameter and at 25°C determined using dynamic light scatteing.

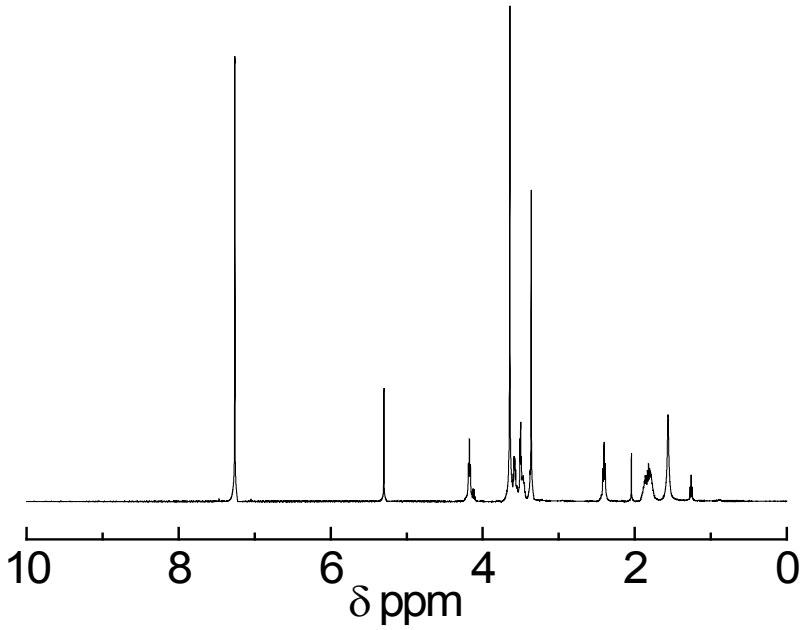


Fig. S5 ^{1}H NMR spectrum of polymer **P0**; residual water peak at 1.55 ppm, acetone peak at 2.05 ppm, DCM peak at 5.30 ppm, chloroform peak at 7.26 ppm.

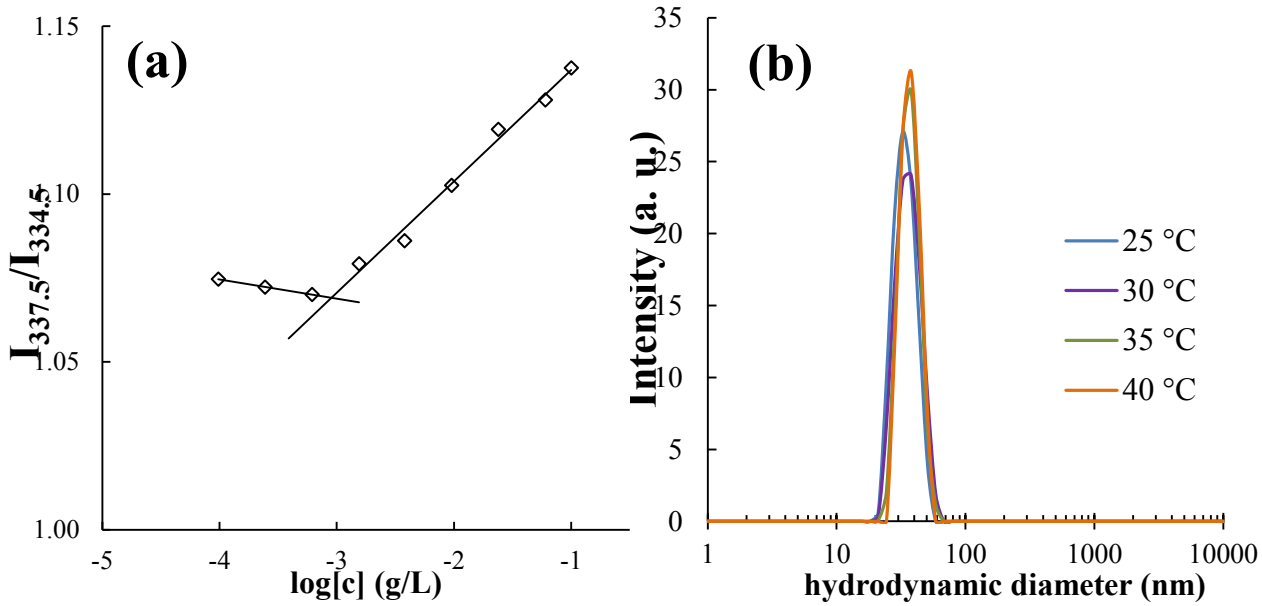
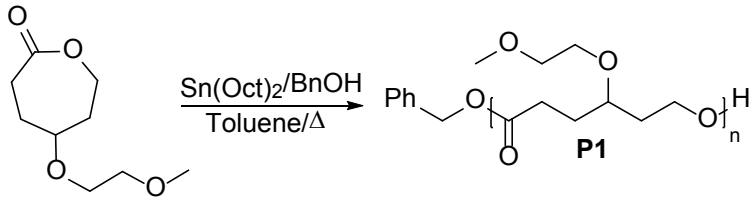


Fig. S6 (a) CMC determination of **P0** using pyrene as fluorescent probe; **(b)** DLS size determination of **P0** from 25 °C to 40 °C.



Scheme S3. Ring opening polymerization of MECL to generate homopolymer **P1**.

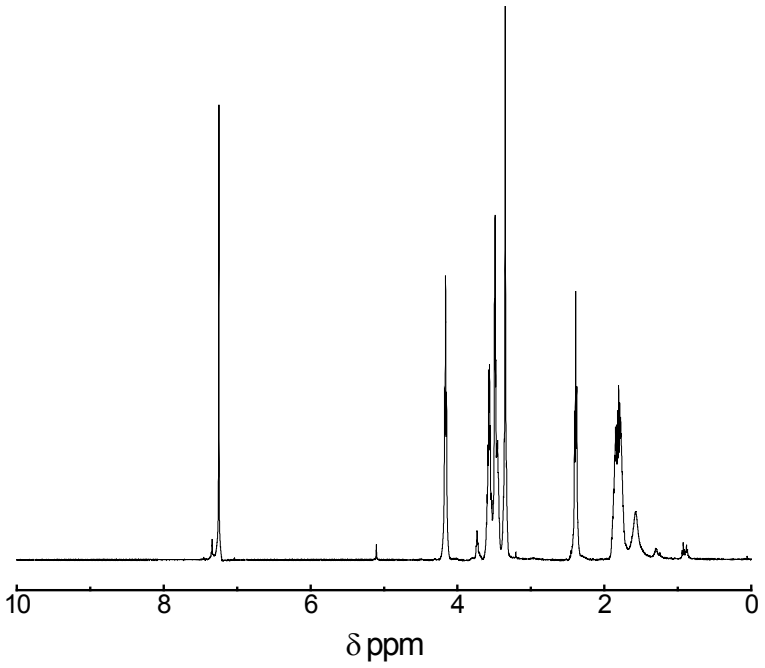


Fig. S7 ^{1}H NMR spectrum of polymer **P1**; residual chloroform peak at 7.26 ppm.

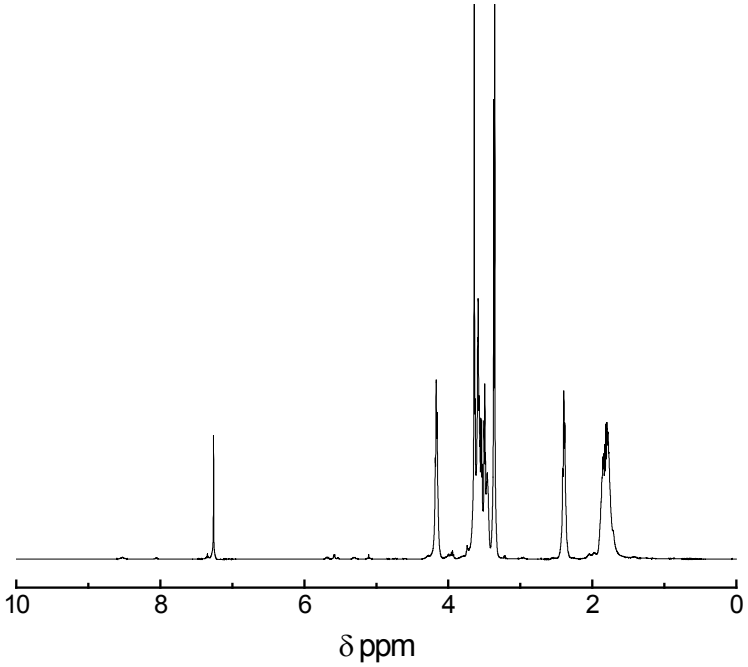
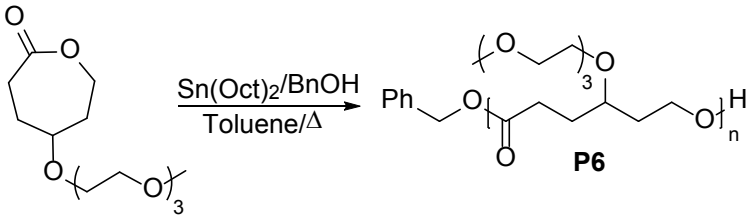


Fig. S8 Typical ^{1}H NMR spectrum of copolymer PMEEECL-*b*-PMECL (**P2** shown); residual chloroform peak at 7.26 ppm..



Scheme S4. Ring opening polymerization of MEEECL to generate homopolymer **P6**.

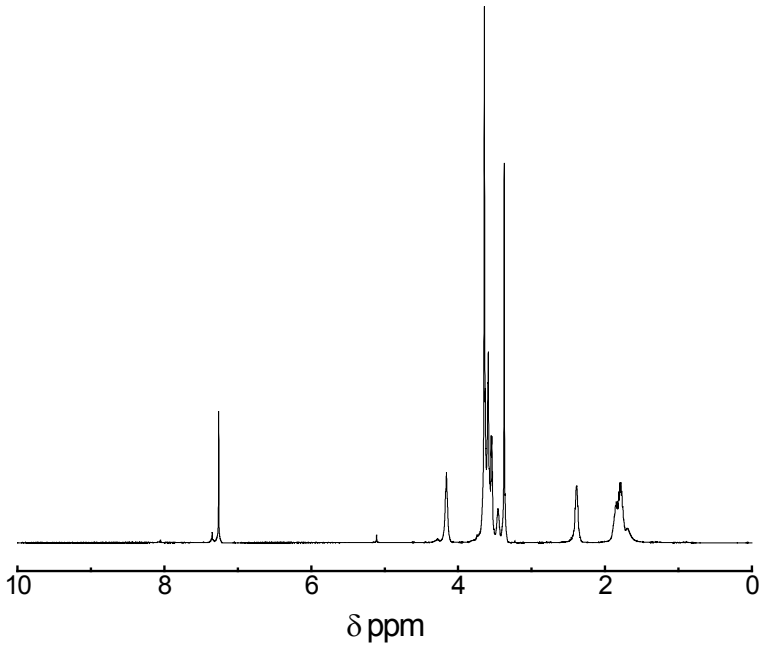


Fig. S9 ^{1}H NMR spectrum of polymer **P6**; residual chloroform peak at 7.26 ppm.

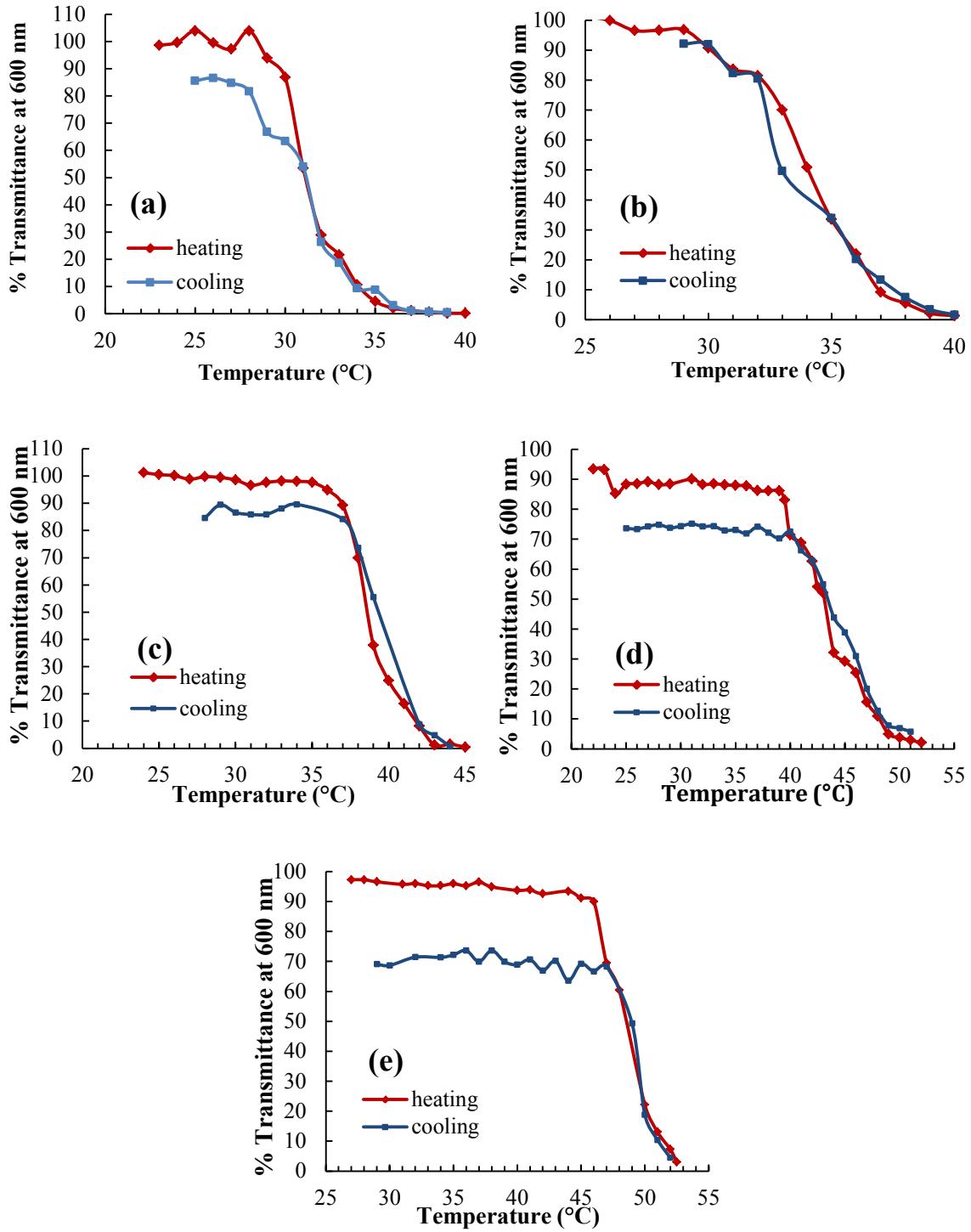


Fig. S10 LCST determination of polymers **P2** (**a**); **P3** (**b**); **P4** (**c**); **P5** (**d**); and **P6** (**e**).

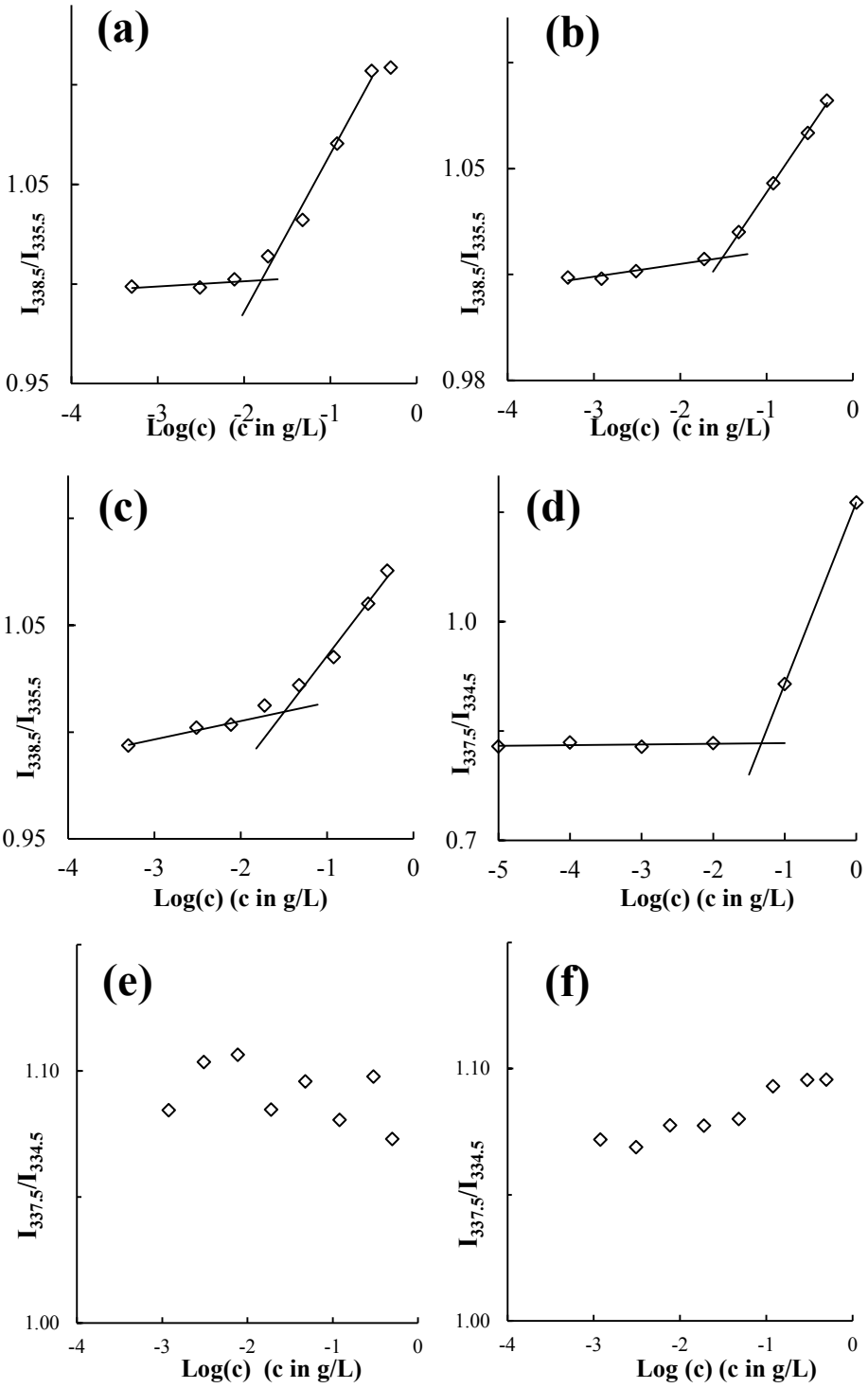


Fig. S11 CMC determination by plotting the ratio of pyrene absorption intensities at 337.5 nm to 334.5 nm vs. log of polymer concentration in g/L: **(a)** copolymer **P2**; **(b)** copolymer **P3**; **(c)** copolymer **P4**; **(d)** copolymer **P5**; **(e)** homopolymer control **P1**; **(f)** homopolymer control **P6**.

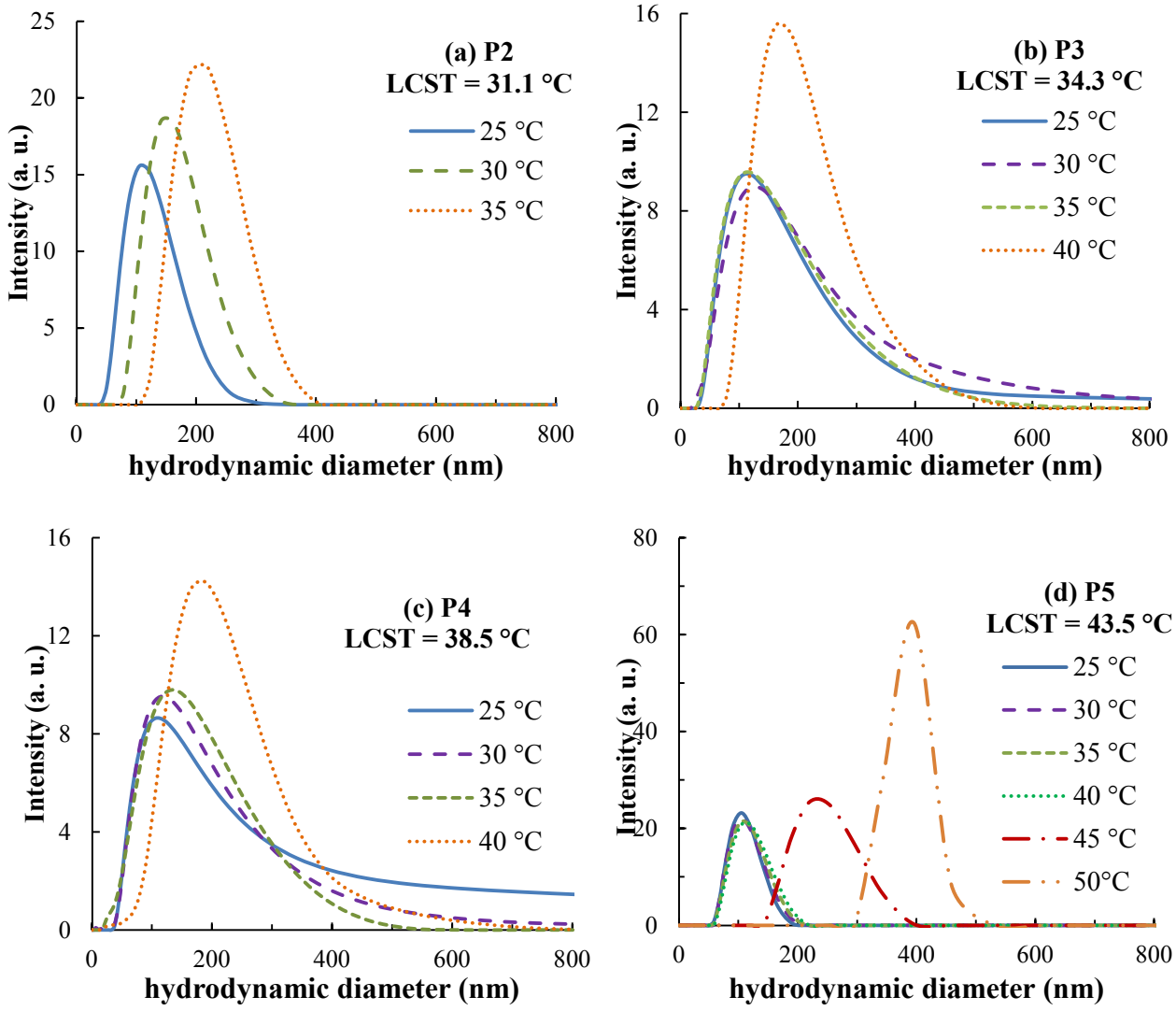


Fig. S12 DLS analysis of polymer hydrodynamic diameter from room temperature to above their LCST: **(a)** copolymer **P2; (b)** copolymer **P3; (c)** copolymer **P4; (d)** copolymer **P5.**