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RESEARCH ARTICLE

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An amphiphilic polymer for the synthesis of diverse polymer libraries exemplified using conjugates with lower critical solution temperatures that span water's liquid state

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Abstract

The discovery of new polymer functions is intrinsically tied to the synthesis of diverse structures. Herein we report the synthesis of a new polymer library precursor, poly[N-(12-carboxyl-3,6,9-trioxado) methacrylamide] (pCTMAAm), that is functionalizable with hydrophilic or hydrophobic ligands in both protic and aprotic solvents. The polymer is made through RAFT polymerization with narrow dispersities. Carboxylic acid groups terminate the pCTMAAm side chains, which allows conjugation of both hydrophobic and hydrophilic groups. As a demonstration, three very different ligands, agmantine (cation), galactosamine (polyol) and hexylamine (hydrophobic), were conjugated to pCTMAAm using DMTTM as a condensing agent with greater than 80% conjugation efficiency. To demonstrate the potential utility of the amphiphilic polymer, a polymer library containing side chains with a series of alkanes and varying degrees of substitution was synthesized showing lower critical solution temperature (LCST) profiles that span a temperature range of 7 °C to above 90 °C in water. Multivariate linear regression analysis of the polymer library was used to understand how polymer composition correlates to LCST, using molecular weight, alkane length and degree of substitution as continuous variables. The outcome showed that alkane length has the greatest influence on LCST and that LCST can be estimated using these material composition inputs.

KEYWORDS

lower critical solution temperature, polymer-analogous conjugation, polymer libraries

1 | INTRODUCTION

The design of new functional biomaterials is enhanced through a fundamental understanding of their structure/function relationships. Synthetic strategies to create polymer libraries have been generated for specific functions, such as DNA delivery and implantable biomaterials.[1–7] One challenge to the synthesis of diverse polymer libraries is the incorporation of hydrophobic and hydrophilic groups on the same polymer. As an example, polymers designed to deliver plasmid DNA generally integrate both hydrophobic and hydrophilic moieties to better mimic the structural functionality of viruses, but side reactions can make the synthesis of those compositions challenging.[8–16]

In this work, we report the synthesis of a new monomer and polymer with the ability to tightly control the backbone molecular weight and to incorporate side groups that are both hydrophobic and hydrophilic. The design criteria employed to guide the development of this new material were: (1) a monomer composition amenable

to controlled polymerization (e.g., RAFT),[17] (2) a polymer composition that allows solvent-accessible chain conformation in both organic and aqueous solvents, and (3) side chains terminated in functional groups that are amenable to conjugation in both organic and aqueous solutions.

These design criteria were employed to create a new monomer consisting of a methacrylamide headgroup combined with an oligomeric ethylene glycol side chain terminated in a carboxyl group. RAFT polymerization of this monomer creates a functionalizable polymer that meets the aforementioned design criteria. Specifically, we describe the synthesis of the new monomer, N-(12-carboxyl-3,6,9-trioxado) methacrylamide (CTMAAm), its polymerization to well-defined molecular weights via RAFT polymerization (pCTMAAm), and document its solvent-accessible side chains through the conjugation of small molecules in both organic and aqueous solvents.

As a demonstration of pCTMAAm's utility for the discovery of functional polymer compositions, a library containing side chains with a series of alkanes and varying degrees of substitution was synthesized. Owing to the composition of these polymers, they exhibit lower critical solution temperature behaviors (LCST) that range from 7 °C to above 90 °C in water. Although the variation of LCST based on polymer chemical composition is used as an example of the utility of polymer libraries to understand structure/function relationships, polymers that exhibit LCST profiles have functional uses. For example, polymers that exhibit LCST profiles have been formulated as hydrogels, micelles, polymersomes, nanoparticles, and microparticles for applications as diverse as drug delivery, gene delivery, tissue engineering, artificial muscles and chromatography columns. To further understand how polymer structure and composition influenced LCST, a multiple linear regression analysis was conducted using polymer molecular weight, alkane chain length and alkane mol% substitution as independent variables. For this class of polymer, alkane chain length was most strongly correlated to changes in LCST.

2 | EXPERIMENTAL SECTION

2.1 | Materials

Tert-butyl 12-amino-4,7,10-trioxadodecanoate (80%), methacryloyl chloride (97%), 4,4′-azobis(4-cyanopentanoic acid) (A-CPA) (98.0%), propylamine, butylamine, hexylamine, DMTMM, were purchased from Sigma-Aldrich and used as received. Triethylamine (99%), anhydrous trifluoroacetic acid (99%), anhydrous dichloromethane, anhydrous methanol, thin layer chromatography sheets (silica gel, 200 μm fluorescent indicator), Amberlyst A-21 exchange resin, silica gel powder (Whatman) were purchased from VWR International Co. Poly(methacrylic acid), sodium salt standards were purchased from Polysciences, Inc. (Warrington, PA) and Polymer Standard Services (Mainz, Germany). 4-cyanopentanoic acid dithiobenzoate (CPA-DB) was synthesized as reported by other groups[18] and characterized as previously described by our group.[19] Unless otherwise stated, all other chemicals were purchased from Fisher Scientific and VWR International Co. at the highest purity available.

2.2 | Characterization

^{1}H NMR was performed using a 400 MHz spectrometer with dimethyl sulfoxide-d_6 (DMSO-d_6) and deuterium oxide as the solvents. Elemental analysis was performed by Quantitative Technologies Inc. (Whitehouse, NJ). Number average molecular weight (M_n) and polymer dispersity ($Đ$) for pCTMAAm were obtained using a Waters Gel Permeation Chromatography (GPC) system equipped with two Ultrahydrogel™ columns (Waters) in series (500 and 250 Å), 1515 isocratic HPLC pump and 2414 refractive index detector. Temperature throughout the system was controlled at 30 °C. The mobile phase employed was phosphate buffer saline (pH = 7.4) at a rate of 0.8 mL min^{-1} calibrated with six individual poly(methacrylic acid), sodium salt standards with peak molecular weights ranging from 1670 to 110,000 Daltons and $Đ_M$ from 1.02 to 1.11.

2.3 | Synthesis of N-(*tert*-butyl 3,6,9-trioxado-12-decanoate) methacrylamide (ii)

Tert-butyl 12-amino-4,7,10-trioxadodecanoate (i) (300 mg, 0.865 mmol) and triethylamine (0.15 mL, 1.08 mmol) were dissolved in anhydrous CH_2Cl_2 (10 mL) at 0 °C under N_2 for 15 min. Methacryloyl chloride (0.11 mL, 1.08 mmol) in 5 mL of anhydrous CH_2Cl_2 was added dropwise in the mixture and stirred for 1 h at 0 °C and another 2 h at room temperature. The reaction mixture was diluted with aqueous $NaHCO_3$ and extracted with CH_2Cl_2. The organic phase was washed by water 3 times and dried over $MgSO_4$. The solution was filtered and concentrated at reduced pressure to produce a viscous yellow oil. Purification of the residue by flash chromatography (hexane/ethyl acetate 1:5) afforded a colorless oil N-(*tert*-butyl 3,6,9-trioxado-12-decanoate) methacrylamide (ii), (292 mg, 80%). ^{1}H NMR (DMSO-d_6, spectra in SI): δ 1.40 (s, 9H), δ 1.84 (s, 3H), δ 2.41 (m, 2H), δ 3.26 (m, 2H), δ 3.44 (m, 2H), δ 3.50 (m, 8H), δ 3.58 (s, 2H), δ 5.32 (s, 1H), δ 5.65 (s, 1H), δ 7.94 (s, 2H). Elemental analysis: Calcd

($C_{17}H_{31}NO_6$): C, 59.11%; H, 9.05%; N, 4.05%. Found: C, 58.79%; H, 8.97%; N, 4.14%.

2.4 | Synthesis of N-(12-carboxyl-3,6,9-trioxado) methacrylamide (CTMAAm) (iii)

(cleavage of *tert*-butyl): A mixture of CH_2Cl_2 (1 mL) and trifluoroacetic acid (TFA, 1 mL) was added to 200 mg of N-(*tert*-butyl 3,6,9-trioxado-12-decanoate) methacrylamide (ii) in a 50 mL round-bottom flask. After stirring at room temperature for 30 min, the volatiles were removed in vacuo. The oil residue was dissolved in 30 mL of anhydrous CH_2Cl_2 and treated with 1 g of Amberlyst A-21 resin. After stirring at room temperature for 1 h, the solid was removed by filtration and the solvent was removed in vacuo. Purification of the residue by flash chromatography (hexane/methanol/ethyl acetate 1:0.5:20) afforded N-(12-carboxyl-3,6,9-trioxado) methacrylamide (iii, 113 mg, 57%). ^{1}H NMR (DMSO-d_6, spectra in SI): δ 1.83 (s, 3H), δ 2.43 (m, 2H), δ 3.26 (m, 2H), δ 3.43 (m, 2H), δ 3.50 (m, 8H), δ 3.58 (s, 2H), δ 5.32 (s, 1H), δ 5.64 (s, 1H), δ 7.94 (s, 2H).

2.5 | RAFT polymerization of CTMAAm

Prior to each experiment, all liquid reagents were purged under nitrogen for at least 10 min. Individual stock solutions of the radical initiator (A-CPA) (iv) and CTA (CPA-DB) (v) were prepared with the respective solvent to ensure accurate reactant ratios. Specific reaction conditions for ~60 individual polymerizations are tabulated for the readers reference in the SI. Of note, monomer iii is highly reactive and will spontaneously polymerize. It should be used soon after its isolation, or stabilized with inhibitor if it is to be stored. A representative example for polymerization is as follows: iii (56.38 mg, 0.244 mmol) and CPA-DB (0.247 mg, 8.9×10^{-4} mmol in 122 μL of methanol) were transferred into a 1 mL glass ampule equipped with a magnetic stir bar and purged under nitrogen for 5 min. Then A-CPA (0.062 mg, 2.2×10^{-4} mmol in 30 μL of methanol) was added into the ampule and purged under nitrogen for another 2 min. The ampule was sealed with oxygen flame and immersed in a 60 °C oil bath under continuous stirring. The reaction was stopped at 48 h by immersing the ampule in an ice bath and then exposing the solution to air. The polymer, poly[N-(12-carboxyl-3,-6,9-trioxado) methacrylamide] (pCTMAAm) (vi), was obtained by precipitation in a generous amount of stirring diethyl ether, filtered and dried under vacuum overnight. The polymer was further purified by dialysis using Spectra/Pro regenerated cellulose dialysis tubing (3.5 kDa MWCO) against deionized-water for 3 days and lyophilized for 2 days. M_n and $Đ_M$ calculated by GPC for this sample were 46,100 Da and 1.07, respectively, and the percent conversion, estimated by gravimetric analysis, was 87%. ^{1}H NMR (400 MHz, D2O, ppm): δ 0.92 (br s, 3H), δ 1.65 (br s, 2H), δ 2.67 (m, 2H), δ 3.32 (m, 2H), δ 3.58 (m, 2H), δ 3.68 (m, 8H), δ 3.80 (s, 2H), δ 7.71 (s, 1H).

2.6 | Conjugation of pCTMAAm with model ligands

Agmatine (cationic), galactosamine (polyol) and hexylamine (hydrophobic) were selected as model ligands to conjugate with pCTMAAm. With a target substitution of 100% (through the use of two equivalents of ligand and DMTMM), pCTMAAm, ligands, and 4-(4,6-dimethoxy-1,3,5-triazin-2-yl)-4-methylmorpholinium chloride (DMTMM) were combined in the following ratios, [COOH in CTMAAm]:[NH_2 in ligands]:[DMTMM] = 1:2:2. The concentration of [pCTMAAm] was 20 mg/mL. Deionized water was the solvent for both the agmatine and galactosamine conjugations, whereas methanol and DMSO were used for hexylamine conjugation. Percent substitution was measured and calculated by ^{1}H NMR using the following formula: substitution yield (%) = [Ia/ (Ia + I_b)] × 100%, where I_a corresponds to the integrated peak area of the methylene protons adjacent to the amide groups and I_b corresponds to the integrated peak area of the methylene protons adjacent to the free unsubstituted carboxyl groups.

2.7 | Conjugation of agmatine to pCTMAAm (M_n 43,000 and $Đ_M$ 1.07)

Prior to conjugation, pCTMAAm was dialyzed against sodium hydroxide (pH = 11) for 2 days to form the sodium salt, and then lyophilized for 2 days. In a 5 mL pear-shaped flask, pCTMAAm (13.7 mg, 0.044 mmol of COOH groups) was dissolved in deionized water (0.69 mL) and stirred for 10 min, whereupon agmatine sulfate salt (20.1 mg, 0.088 mmol) was added. After 5 min stirring, DMTMM (24.3 mg, 0.088 mmol) was added. The flask was capped and sealed by parafilm and the reaction stirred overnight at room temperature. The product was purified by dialysis using Spectra/Pro regenerated cellulose dialysis tubing (3.5 kDa MWCO) against deionized water for 3 days and lyophilized for 2 days. ^{1}H NMR (400 MHz, D_2O, ppm): δ 0.93 (br s, 3H), δ 1.58 (s, 4H), δ 1.71 (br s, 2H), δ 2.51 (m, 2H), δ 2.57 (m, 2H) δ 2.67 (m, 2H), δ 3.30 (m, 2H), δ 3.58 (m, 2H), δ 3.60 ~ 3.72 (m, 8H), δ 3.76 (s, 2H), δ 7.67

(s, 1H). The calculated substitution yield ([$I_{\delta\ 2.57}$/($I_{\delta\ 2.57} + I_{\delta\ 2.67}$)] × 100%) was 83%.

2.8 | Conjugation of galactosamine to pCTMAAm (M_n 43,000 and $Đ_M$ 1.07)

The same conditions used for the conjugation of agmatine (above) were used for the conjugation of galactosamine. ^{1}H NMR (400 MHz, D_2O, ppm): δ 0.92 (br s, 3H), δ 1.64 (br s, 2H), δ 2.58 (m, 2H) δ 2.67 (m, 2H), δ 2.99 (m, 1H), δ 3.20 (m, 1H), δ 3.57 (m, 2H), δ 3.66 (m, 8H), δ 3.78 (m, 2H), δ 3.85 ~ 3.91 (m, 3H), δ 3.97 (m, 1H), 4.07 (m, 1H), 4.15 (m, 1H), 4.63 (m, 1H), 5.20 (m, 1H), δ 7.68 (s, 2H). The calculated substitution yield ([$I_{\delta\ 2.58}$/($I_{\delta\ 2.58} + I_{\delta\ 2.67}$)] × 100%) was 80%.

2.9 | Conjugation of hexylamine to pCTMAAm (M_n 43,000 and $Đ_M$ 1.07)

Methanol as solvent: In a 5 mL of pear-shaped flask, 19.4 mg of pCTMAAm polymer was dissolved in 0.97 mL of methanol and stirred for 10 min, then 17.7 μL of hexylamine was added. After stirring for another 5 min, 37.1 mg of DMTMM was added. The flask was capped then sealed by parafilm and the reaction allowed to proceed overnight at room temperature. The product was purified by dialysis using Spectra/Pro regenerated cellulose dialysis tubing (3.5 kDa MWCO) against deionized-water for 3 days and lyophilized for 2 days. Conjugation efficiency was calculated by ^{1}H NMR (94%). ^{1}H NMR (400 MHz, CD_3OD, ppm): δ 0.90 (s, 3H) δ 0.94 (br s, 3H), δ 1.31 (s, 4H), δ 1.49 (s, 2H), δ 1.71 (br s, 2H), δ 2.43 (s, 2H) δ 2.58 (m, 2H) δ 3.16 (m, 2H), δ 3.28 (m, 2H), δ 3.61 (m, 8H), δ 3.71 (m, 2H), δ 7.42 (s, 1H). The calculated substitution yield ([$I_{\delta\ 2.58}$/($I_{\delta\ 2.58} + I_{\delta\ 2.67}$)] × 100%) was 94%.

DMSO as solvent: In a 5 mL of pear-shaped flask, 19.8 mg of pCTMAAm polymer was dissolved in 0.99 mL of DMSO and stirred for 30 min, then 18.3 μL of hexylamine was added. After stirring for another 10 min, 38.3 mg of DMTMM was added. The flask was capped and sealed by parafilm and the reaction allowed to progress overnight at room temperature. The product was purified by dialysis using Spectra/Pro regenerated cellulose dialysis tubing (3.5 kDa MWCO) against deionized-water for 3 days and lyophilized for 2 days. Conjugation efficiency was calculated by ^{1}H NMR (82%). ^{1}H NMR (400 MHz, CD_3OD, ppm): δ 0.91 (s, 3H) δ 0.95 (br s, 3H), δ 1.31 (s, 4H), δ 1.49 (s, 2H), δ 1.71 (br s, 2H), δ 2.44 (s, 2H) δ 2.58 (m, 2H) δ 3.16 (m, 2H), δ 3.28 (m, 2H), δ 3.61 (m, 8H), δ 3.72 (m, 2H), δ 7.42 (s, 1H). The calculated substitution yield ([$I_{\delta\ 2.58}$/($I_{\delta\ 2.58} + I_{\delta\ 2.67}$)] × 100%) was 82%.

2.10 | Conjugation to poly(methacrylic acid)

Poly(methacrylic acid) (M_n 13,000; $Đ_M$ 1.13) was synthesized in methanol by RAFT polymerization as previously described by our group[19] and conjugated to each ligand in an identical fashion as described for the pCTMAAm conjugation. The lower molecular weight was used to make the total number of carboxylic acids equivalent between the pCTMAAm and poly(methacrylic acid) conjugations.

2.11 | Synthesis of polymer library

Prior to conjugation, three pCTMAAm polymers with different molecular weights were prepared by RAFT polymerization. The conjugation was conducted as follows. pCTMAAm and 4-(4,6-dimethoxy[1,3,5]triazin-2-yl)-4-methylmorpholiniumchloride (DMTMM) were dissolved in methanol prior to the addition of alkyl amines, with a predetermined molar ratio of [COOH in pCTMAAm]:[DMTMM]: [NH_2 in alkyl amines] with the concentration of pCTMAAm held constant at 20 mg/mL. After 18 h stirring at room temperature, the polymers were obtained by the removal of methanol in vacuo, redissolution in water, followed by dialysis against deionized water for 3 days (three changes daily), then lyophilized to dryness over 2 days. The [DMTMM]:[NH_2 in alkyl amines] ratio was kept at 1:1. The polymers with alkyl substitution ranging from ~23% to ~90% were obtained by adjusting the reaction ratio of [COOH in pCTMAAm]:[NH_2 in alkyl amines].

2.12 | Measurement of polymer LCST characteristics

Polymers were dissolved in deionized water (3 mg/mL, pH 6.8), 200 μL of which was transferred to a polystyrene 96-well plate. The plate containing the samples was placed at sequential and escalating temperatures within a laboratory oven (from 2 to 90 °C), and the turbidity of each well evaluated at each temperature using a UV/Vis microplate spectrophotometer at 500 nm. The LCST was defined as the midpoint of the temperature-transmission curve.

2.13 | Multiple linear regression analysis

The polymers that exhibit LCST profiles (Table 2) were used to develop a predictive model to correlate the variable aspects of polymer composition to the resulting LCST. The multiple linear regression equation included:

polymer molecuar weight (M_n), alkane carbon number (i.e., 3, 4, and 6) and the degree of alkane substitution (mol%) as continuous predictor variables. The resulting equation has the form: LCST = $\beta_0 + (\beta_1)^*(M_n) + (\beta_2)^*(\#$ of carbons) + $(\beta_3)^*$(mol%), where "β_0" denotes the y-intercept, and the sequential β_i values denote the variable coefficients that are fit by least squares. The β_i values indicate the degree to which each variable helps to predict the LCST, while controlling for the effects of the other variables in the model. The data analysis package in Excel was applied to the data in Table 2 to calculate the values of β_0, β_1, β_2, and β_3.

3 | RESULTS AND DISCUSSION

Synthesis of the CTMAAm monomer is shown in Figure 1. First, *tert*-butyl 12-amino-4,7,10-trioxadodecanoate (i) was reacted with methacryloyl chloride in the presence of base to form N-(*tert*-butyl 3,6,9-trioxado-12-decanoate) methacrylamide (ii). Second, CTMAAm (iii) was formed by deprotection of ii using trifluoroacetic acid (TFA), followed by treatment with Amberlyst A-21 to remove remaining TFA.[20] Finally, RAFT polymerization of CTMAAm was conducted at 60 °C in methanol, using 4-cyanopentanoic acid dithiobenzoate (iv) as the chain transfer agent (CTA) and 4,4′-azobis(4-cyanopentanoic acid) (v) as the radical initiator (I). The final product, poly[N-(12-carboxyl-3,-6,9-trioxado) methacrylamide] (pCTMAAm) (vi), contains a side chain comprised of a carboxyl-terminated oligomer of ethylene glycol, and the polymer is soluble in a wide range of solvents. Given the solubility profile of this polymer (Appendix S1), we considered whether pCTMAAm would fulfill the design criteria for a polymer precursor to build polymer libraries that contain a diversity of side chains (i.e., charged, hydrophilic, hydrophobic).

Several pCTMAAm polymers were prepared using different initial concentrations of monomer ($[M]_0$) and different ratios of $[M]_0/[CTA]_0$ whereas the $[CTA]_0/[I]_0$ ratio was held constant (1:0.25), as shown in Table 1. pCTMAAm polymers were obtained with molecular weights consistent with theory,[21] and with very narrow dispersity ($Đ = 1.05–1.09$). By adjusting the $[M]_0/[CTA]_0$ ratio, several number average molecular weights (M_n) of pCTMAAm polymers were obtained ranging from 10,100 up to 84,300 (entries 1–7 in Table 1) in accordance with the theoretical M_n at the same $[M]_0$ of 1.5 mol L^{-1}. These results are consistent with controlled RAFT polymerization.[22,23] The polymerization yields were high (~80%–90%) with the exception of the low yield (53%) at extremely low $[CTA]_0$ (entry 7 in Table 1) $[M]_0$ had no obvious effect on $Đ_M$ comparing three different $[M]_0$ (1.0, 1.5 and 2.0 mol L^{-1}, corresponding to Table 1 entries 8, 5, and 9, respectively), and the highest conversion and M_n were obtained at $[M]_0$ of 2.0 mol L^{-1}.

To characterize the polymerization of CTMAAm by RAFT, the relationships between monomer conversion versus M_n and $Đ_M$ of were determined. A series of RAFT polymerizations ($[M]_0/[CTA]_0/[I]_0$ = 200:1:0.25) were conducted for 4, 8, 12, 18, 24, 36, and 48 h in methanol with $[M]_0$ = 1.5 mol L^{-1}. As shown in Figure 2A, M_n (GPC) linearly agreed with the M_n (theory) over the course of the polymerization.[21]

At low conversion, the $Đ_M$ is relatively high, but decreased from 1.31 to 1.06 as M_n increased owing to eventual chain transfer equilibrium. Varying the reactant ratios ($[M]_0/[CTA]_0/[I]_0$) to compare 200:1:0.25, 150:1:0.25, and 200:1:0.1 (Figure 2B), the three polymerizations developed

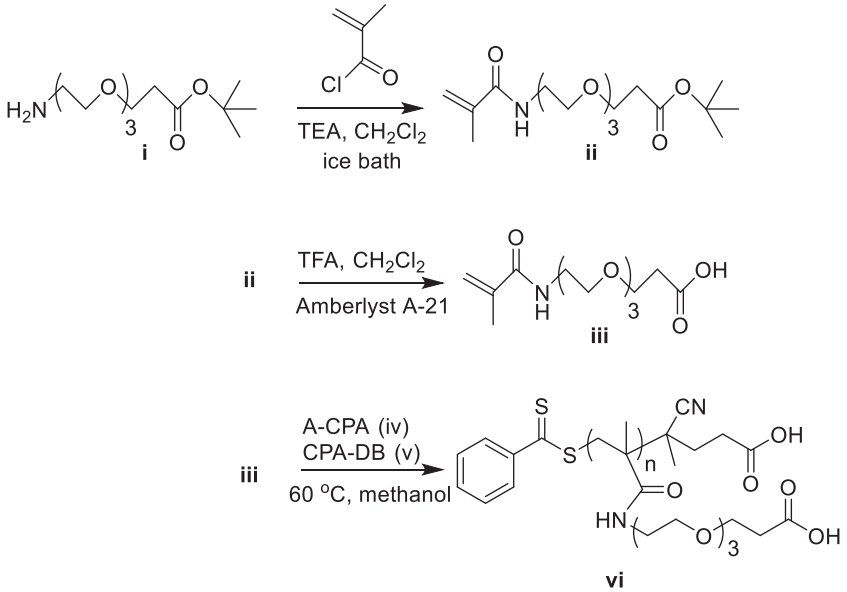

FIGURE 1 Synthesis of monomer CTMAAm (iii) and polymer pCTMAAm (vi) by RAFT polymerization end groups not shown for clarity

TABLE 1 RAFT polymerization characteristics of pCTMAAm under different reaction conditions

Entry	$[M]_0/[CTA]_0$	$[M]_0$mol L^{-1}	M_n[a] g mol^{-1}	$Đ$[a]	Yield%[b]	M_n[c] Theory g mol^{-1}
1	45	1.5	10,100	1.09	89	10,700
2	70	1.5	14,400	1.08	82	16,400
3	120	1.5	29,600	1.06	81	28,300
4	170	1.5	41,300	1.05	80	39,500
5	200	1.5	47,400	1.07	82	47,700
6	300	1.5	52,700	1.06	79	68,700
7	600	1.5	84,300	1.06	53	91,900
8	200	1.0	42,300	1.07	78	45,100
9	200	2.0	50,800	1.07	80	46,500

[a]GPC relative to poly(methacrylic acid).
[b]By gravimetric analysis.
[c]M_n (theory) calculated as previously described.[21]

TABLE 2 Molecular composition and LCST profiles of 45 unique polymers made in a library format using the pCTMAAm backbone

Sample[a,b]	Substitution (%)	LCST (°C)	Sample[a,b]	Substitution (%)	LCST (°C)	Sample[a,b]	Substitution (%)	LCST (°C)
P-56-a	79	46	P-38-a	90	40	P-15-a	88	41
P-56-b	70	52	P-38-b	68	56	P-15-b	70	57
P-56-c	53	60	P-38-c	53	60	P-15-c	56	68
P-56-d	29	72	P-38-d	29	80	P-15-d	28	85
B-56-a	79	29	B-38-a	87	23	B-15-a	90	24
B-56-b	75	34	B-38-b	76	39	B-15-b	87	29
B-56-c	62	43	B-38-c	67	45	B-15-c	70	48
B-56-d	56	49	B-38-d	57	50	B-15-d	58	60
B-56-e	50	55	B-38-e	53	60	B-15-e	37	>90
H-56-a	69	Insol	H-38-a	65	7	H-15-a	63	Insol
H-56-b	59	11	H-38-b	62	13	H-15-b	59	Insol
H-56-c	52	18	H-38-c	50	21	H-15-c	53	19
H-56-d	44	32	H-38-d	45	33	H-15-d	46	45
H-56-e	39	39	H-38-e	43	39	H-15-e	43	54
H-56-f	29	57	H-38-f	23	62	H-15-f	25	70

[a]P-propyl; B-butyl; H-hexyl.
[b]56−M_n = 56,000, $Đ_M$ = 1.08; 38−M_n = 38,000, $Đ$ = 1.07; 15−M_n = 15,000, $Đ$ = 1.14.

linearly with time. Pseudo-first-order kinetics were observed for the RAFT polymerizations with a slight induction time (about 2 h) for all the polymerization conditions. At the same $[CTA]_0/[I]_0$ ratio, decreasing $[M]_0/[CTA]_0$ led to an increase in polymerization rate, possibly from a higher relative concentration of active CTA. However, decreasing the radical initiator concentration 2.5-fold did not have a significant impact on polymerization rate.

pCTMAAm is soluble in a range of solvents (defined as >20 g/L) including water, methanol, ethanol, DMSO and DMF, suggesting that the side chain carboxyl termini will be solvent- accessible and available for substitution. To assess the solvent-accessibility of the carboxy groups, the functionalization characteristics of pCTMAAm was assessed using three model ligands, agmantine (cationic), galactosamine (polyol) and hexylamine (alkane), using DMTMM as the condensation agent (Figure 3). pCTMAAm was amenable to conjugation with each model ligand. With a target substitution of 100%, the substitution yield for each ligand exceeded 80%. Specifically, the conjugation yield of agmantine was 83% (reaction in water), galactosamine was 80% (reaction in water), and

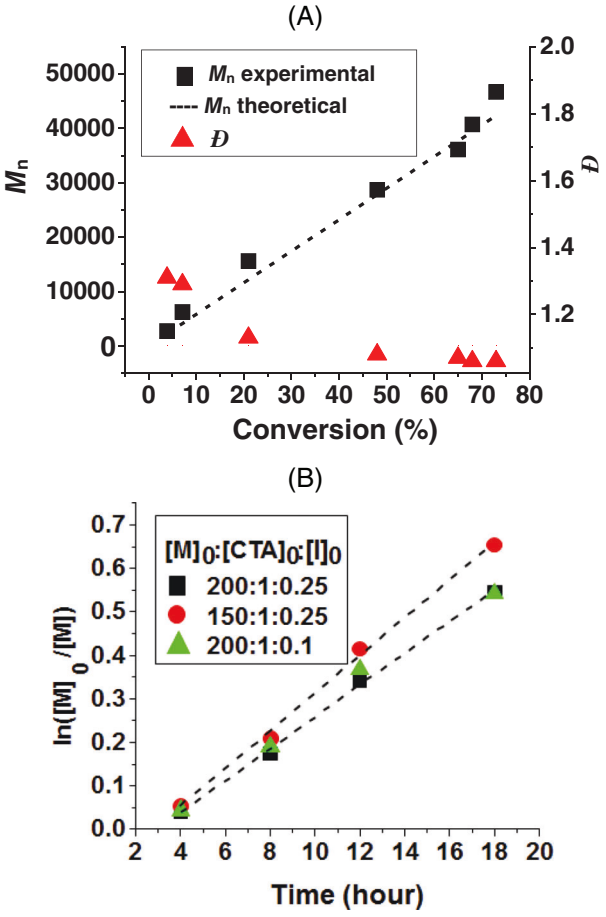

FIGURE 2 (A) Relationship of M_n experimental (GPC), M_n theoretical, and $Đ_M$ (GPC) with conversion of CTMAAm RAFT polymerization at $[M]_0/[CTA]_0/[I]_0 = 200:1:0.25$. (B) Pseudo-first-order kinetic plot with various ratios of $[M]_0/[CTA]_0/[I]_0$. Dash lines represent linear regressions calculated based on short times only where chain growth is linear with time

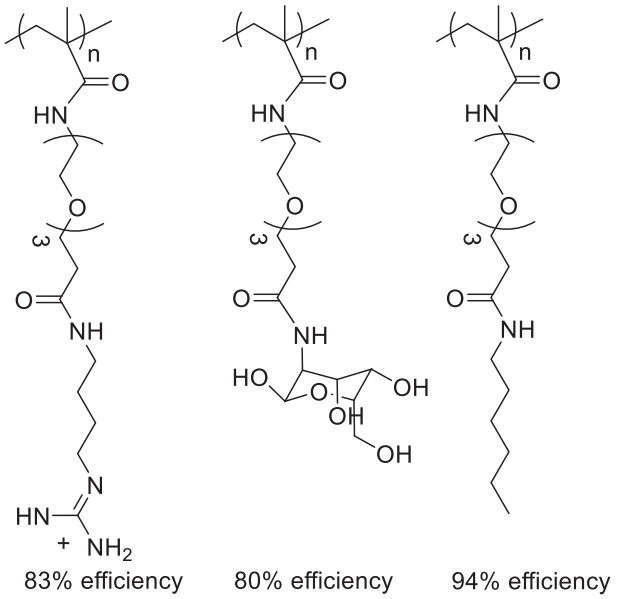

FIGURE 3 Composition and conjugation efficiencies of diverse structures condensed to pCTMAAm to demonstrate the synthetic versatility of the polymer precursor (polymer end groups not shown for clarity)

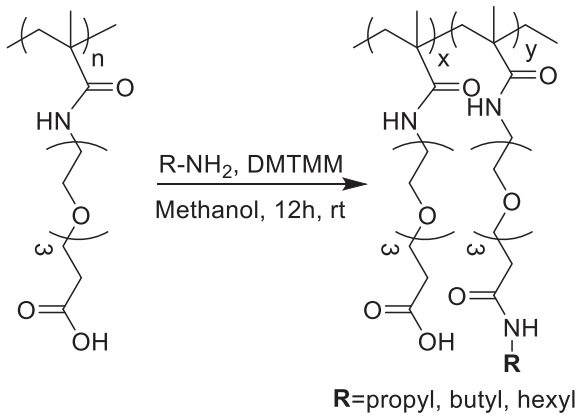

FIGURE 4 Synthesis and molecular composition of the polymers in the LCST library listed in Table 2 (polymer end groups not shown for clarity)

hexylamine was 94% (reaction in methanol) and 82% (reaction in DMSO). As a comparison, the substitution of agmantine, galactosamine and hexylamine to poly(methacrylic acid) with the same number of carboxylic acid function groups in solution resulted in insoluble products under all reaction conditions indicating that the oligomer ethylene oxide side chains of pCTMAAm help to keep the conjugates in solution throughout the reaction.

As a demonstration of the potential utility of pCTMAAm to build polymer libraries, a series of polymers with serial hydrophobic modifications was synthesized to make polymers with a wide range of lower critical solution temperature (LCST). The LCST behavior of polymers with side chains of oligo ethylene glycol side chains is known and recently reviewed.[24] The phase transition temperatures for this polymer class depends on the relative balance of hydrophobic and hydrophilic components as well as the polymer molecular weight. To this end, we synthesized a library of 45 unique polymers using pCTMAAm as a polymer precursor. The library was populated with polymers of three different molecular weights (M_n 15,000; 38,000; 56,000), three different alkyl chain lengths (propyl, butyl, hexyl) and different degrees of alkyl substitution. Figure 4 shows the synthesis and relevant molecular compositions of the polymers that comprise the LCST library. Table 2 shows the specific polymer compositions that make up the library, and the LCST of each polymer.

The LCST profiles for the polymers in the library were quite sharp, with the phase transitions occurring

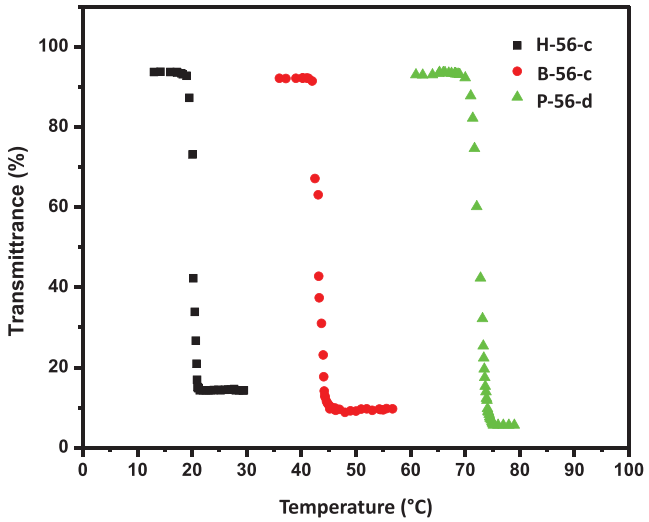

FIGURE 5 A representative set of three polymers in Table 2 that demonstrate their rapid temperature dependence as the temperatures pass through the LCST (3 mg/mL), as measured by percent transmittance at 500 nm

over ~3 °C (Figure 5). Increasing the hydrophobic character of the polymer, by increasing the degree of alkyl substitution, leads to lower LCST values for all polymers in each series in the library, with the effect more enhanced as the length (and therefore hydrophobicity) of the alkyl chain increases (Figure 6). Also, with the same length of alkyl chain at similar percent substitutions, the higher the molecular weight of the polymer backbone the lower the LCST. These results are consistent with the known LCST literature and serve as an initial validation of the pCTMAAm polymer as a building block for polymer libraries.

The data in Table 2 can be organized into an equation consisting of one dependent variable (LCST) and three independent variables (M_n, # of alkane carbons, alkane mol% substitution). With this type of relationships, multiple linear regression can be employed to empirically derive a predictive equation for LCST for a given polymer composition. In other words, the LCST can be predicted to a first approximation given the molecular weight, the number of carbons in a substituted alkane, and the alkane degree of substitution on the polymer. The general equation for this relationship is

$$\mathrm{LCST} = \beta_0 + (\beta_1) * (M_n) + (\beta_2) * (\#\text{of carbons}) + (\beta_3) * (\mathrm{mol}\%). \quad (1)$$

Using the data analysis package in Excel, the values of β_0, β_1, β_2, and β_3 were calculated, giving

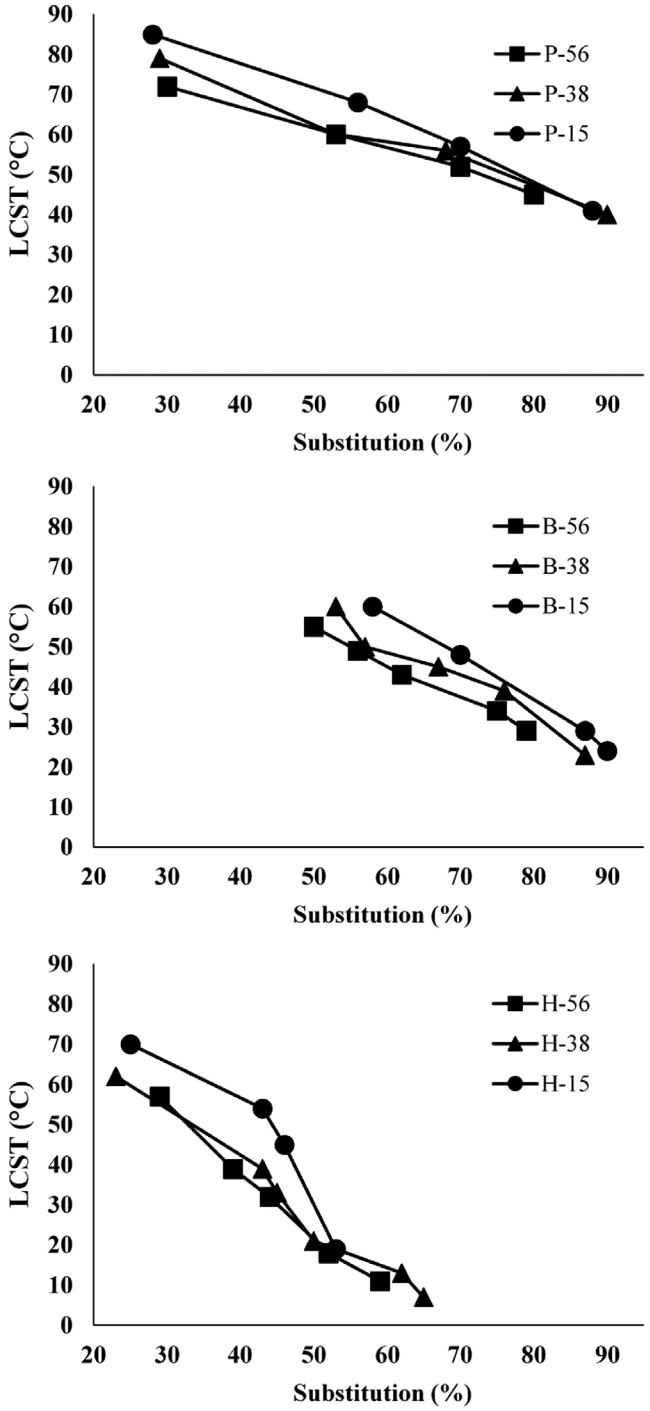

FIGURE 6 The influence of percent alkyl substitution (top), alkyl chain length (middle) and polymer molecular weight (bottom) on the LCST in water. P-propyl; B-butyl; H-hexyl. 56—M_n = 56,000, $Đ$ = 1.08; 38—M_n = 38,000, $Đ$ = 1.07; 15—M_n = 15,000, $Đ$ = 1.14

$$\mathrm{LCST} = 146.69 + (-0.00017) * (M_n) + (-11.24) * (\#\text{of carbons}) + (-0.78) * (\mathrm{mol}\%). \quad (2)$$

The full output table for the regression analysis is provided in Appendix S1. Interpretation of the coefficients for each variable suggests that each variable influences

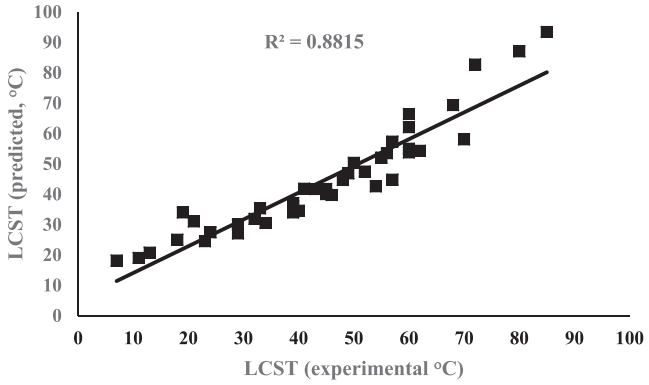

FIGURE 7 The predictive capacity of Equation (2). The R^2 value of 0.8815 is not perfect, but does give a good first approximation for an LCST within this class of polymers when given the molecular weight, number of carbons that comprise the substituted alkanes, and the mol% substitution of the alkanes onto the polymer

the LCST, with the greatest contribution imparted by the length of the alkane groups, followed by the degree of substitution, and least influenced by the polymer molecular weight. The predictive capability of the resulting equation is reasonable with an R^2 of 0.8815. as shown in Figure 7.

Collectively, a new polymer backbone for the synthesis of functional biomaterial libraries is reported. The monomer is polymerized by RAFT and yields polymers with narrow $Đ_M$ and molecular weights that closely correlate to theory. The polymer, pCTMAAm, has carboxy-terminated oligomeric ethylene glycol side chains, which are amenable to functionalization in both protic and aprotic solvents, which allows the substitution of a range of functional groups. These polymers exhibit and LCST profile that depends on the polymer molecular weight, the number of carbons in a substituted alkane and the degree of alkane substitution, which is consistent with the LCST literature (for review see[25]). The new polymer is designed to enable investigators to create functionally diverse polymer libraries.

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DATA AVAILABILITY STATEMENT

Data available on request from the authors.

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REFERENCES

[1] D.M Lynn, D.G. Anderson, D. Putnam, R. Langer. . *J. Am. Chem. Soc.* **2001**, *123*, 8155.
[2] S. Brocchini, K. James, V. Tangpasuthadol, J. Kohn, *J. Biomed. Mater. Res.* **1998**, *42*, 66.
[3] S. Y. Wong, N. Sood, D. Putnam, *Mol. Ther.* **2009**, *17*, 480.
[4] C. N. Lv, C. Z. He, X. C. Pan, *Angew. Chem., Int. Ed.* **2018**, *57*, 9430.
[5] Y.B. Zhong, B.J. Zeberl, X. Wang, J.T Luo. . *Acta Biomater.* **2018**, *73*, 21–37.
[6] V. Kardelis, R. C. Chadwick, A. Adronov, *Angew. Chem., Int. Ed.* **2016**, *55*, 945.
[7] G. Delaittre, N. K. Guimard, C. Barner-Kowollik, *Acct. Chem. Res.* **2015**, *48*, 1296.
[8] S. Y. Wong, D. Putnam, *Bioconjugate Chem.* **2007**, *18*, 970.
[9] M. Lee, S. W. Kim, *Pharm. Res.* **2005**, *22*, 1.
[10] D. Fisher, T. Bieber, Y. Li, H.-P. Elsasser, T. Kissel, *Pharm. Res.* **1999**, *16*, 1273.
[11] E. Wagner, J. Kloeckner, *Adv. Polym. Sci.* **2006**, *192*, 135.
[12] F. Richter, L. Martin, K. Leer, E. Moek, F. Hausig, J. C. Brendel, A. Traeger, *J. Mat. Chem. B.* **2020**, *8*, 5026.
[13] N. Zavradashvili, C. Sarisozen, G. Titvinidze, G. Otinashvili, T. Kantaria, D. Tugushi, J. Puiggali, V. P. Torchilin, R. Katsarava, *ACS Omega* **2019**, *4*, 2090.
[14] C. H. Jones, A. Gollakota, M. F. Chen, T. C. Chung, A. Ravikrishnam, G. J. Zhang, B. A. Pfeifer, *Biomaterials* **2015**, *58*, 103.
[15] M. R. Molla, A. Boser, A. Rana, K. Schwarz, P. A. Levkin, *Bioconjugate Chem.* **2018**, *29*, 992.
[16] A. Akinc, D. M. Lynn, D. G. Anderson, R. Langer, *JACS* **2003**, *125*, 5316.
[17] A. E. Smith, X. Xua, C. L. McCormick, *Prog. Poly. Sci.* **2010**, *35*, 45.
[18] S. H. Thang, B. Y. K. Chong, R. T. A. Mayadunne, G. Moad, E. Rizzardo, *Tetrahedron Lett.* **1999**, *40*, 2435.
[19] J. M. Pelet, D. Putnam, *Macromolecules* **2009**, *42*, 1494.
[20] A. Warnecke, F. Kratz, *Bioconjugate Chem.* **2003**, *14*, 377.
[21] H. D. Brouwer, M. A. J. Schellekens, B. Klumperman, M. J. Monteiro, A. L. German, *J. Polym. Sci. Part A: Polym. Chem.* **2000**, *38*, 3596.
[22] K. Matyjaszewski, J. Xia, *J. Chem. Rev.* **2001**, *101*, 2921.
[23] A. B. Lowe, C. L. McCormick, *Prog. Polym. Sci.* **2007**, *32*, 283.
[24] N. Badi, *Prog. Poly. Sci.* **2017**, *66*, 54.
[25] Q. Zhang, C. Weber, U. S. Schubert, R. Hoogenboom, *Mater. Horiz.* **2017**, *4*, 109.

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