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Synthesis and Thermoresponsive Solution Properties of Poly[oligo(ethylene glycol) (meth)acrylamide]s: Biocompatible PEG Analogues

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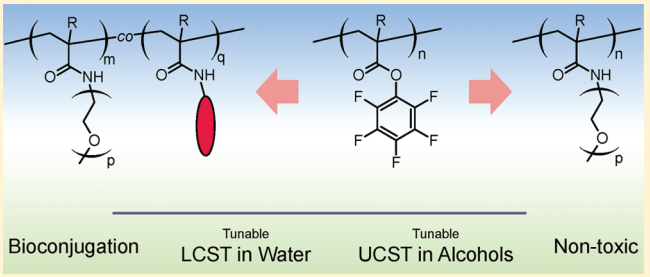
Supporting Information

ABSTRACT: A library of (meth)acrylamido (co)polymers was prepared by reacting poly(pentafluorophenyl (meth)-acrylate) with α-amino, ω-methoxy functionalized di(ethylene glycol), tri(ethylene glycol), and poly(ethylene glycol) (PEG)-350, PEG-750, and PEG-5k, in combination with hexylamine or thyroxine. The resulting copolymers showed an improved solubility in water (higher or absent LCST values) and in alcohols (lower or absent UCST values) than the analogous common series of poly[oligo(ethylene glycol) methyl ether (meth)acrylates]. The polyacrylamido species showed a better solubility than the corresponding polymethacrylamido derivatives of similar molecular weight with all polyacrylamides investigated being water-soluble at temperatures exceeding 90.0 °C. Tunable thermosensitive behavior could be effected by the incorporation of the hydrophobic hexylamide comonomer. Similarly, an acrylamido backbone with grafted oligo(propylene glycol 600) amides exhibited a sharp LCST-type transition around 22.0 °C. The UCST-type transitions of the (meth)acrylamido homopolymers were evaluated in 2-propanol and 1-octanol and were found to increase with an increasing ethylene glycol side chain length, but were essentially independent of the alcohol chain length with polymers exhibiting higher UCST transitions in 2-propanol vs 1-octanol. Cytotoxicity tests on MRC5 fibroblast cells of the di- and tri(ethylene glycol) methyl ether acrylamido homopolymers revealed no toxicity up to concentrations of 10.0 g/L. By employing mixtures of di(ethylene glycol) methyl ether amine and the prohormone thyroxine (T_4), water-soluble copolymers containing varying amounts of T_4 could be easily synthesized. Because of enhanced solubility, low toxicity, and higher hydrolytic stability of amides versus ester linkages, activated ester polymers in combination with amino-functionalized ethylene glycol based side chains are presented as a versatile platform for highly soluble, biocompatible, bioconjugated materials.



INTRODUCTION

The ability of certain (co)polymers in solution (aqueous or nonaqueous) to undergo conformational or phase transitions in response to a change in solution temperature, i.e., thermoresponsive polymers, are of interest as so-called "smart" materials. The response to a change in temperature can be exploited to induce self-directed assembly (micellization or vesiculation in block copolymers for example) or to promote the uptake or release of a particular molecular species and as such thermal 'triggers' have attracted significant attention.[1,2] The primary transition temperatures of interest to researchers are the lower critical solution temperature (LCST) in which a (co)polymer undergoes a coil-to-globule transition upon heating and is commonly associated with nonionic water-soluble species, and the upper critical solution temperature (UCST) whereby phase separation is induced by cooling. Examples of (co)polymers that exhibit LCST or UCST behavior are those containing, either wholly or partially, *N*-isopropylacrylamide (NIPAM, $LCST_{H2O}$ ~ 32 °C), *N*,*N*-diethylacrylamide (DEAM, $LCST_{H2O}$ ~ 32 °C),[3−5] *N*-vinylcaprolactam ($LCST_{H2O}$ ~ 32.5 °C),[6] 2-(dimethylamino)ethyl methacrylate (DMAEMA, $LCST_{H2O}$ ~ 50 °C), poly/oligo(ethylene glycol) (meth)-acrylates (OEGMA, $LCST_{H2O}$ varies depending on the degree of polymerization of the ethylene oxide side chain), methyl vinyl ether (MVE, $LCST_{H2O}$ ~ 36 °C),[7] pyrrolidone containing polymethacrylates ($LCST_{H2O}$~29−34 °C),[8] oligo(ethylene glycol) methyl ether methacrylate (OEGMA, $UCST_{ROH}$ varies from ca. 75−20 °C depending on the alcohol) and examples of polysulfopropylbetaines that can exhibit both UCST and LCST behavior.[9,10]

Of these thermoresponsive materials the PEG analogues, and especially the acrylic and methacrylic species, have attracted significant attention due in part to their LCST and UCST behaviors but also because of their well-established biocompatibility.[11−20] For example, Bebis et al.[20] recently described the effect of polymer concentration and added solutes on the LCST behavior of a series of well-defined, RAFT-synthesized, methacrylic-based oligo(ethylene glycol) species. The authors

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reported that the cloud point of such materials can increase by as much as 6 °C when polymer concentrations are decreased from 5.0 to 0.5 g/L and that the same measurements performed in bovine blood plasma indicated an additional further decrease of the cloud point (compared to water or PBS). The results, collectively, indicate that researchers should apply appropriate testing protocols for such materials when being examined for potential biomedical applications such as drug delivery. Roth, Jochum, and Theato recently detailed the behavior of poly-OEGMA (POEGMA) in a wide range of alcohols.[12] POEGMA solutions showed sharp, reversible transitions with low hystereses. The cloud points increased with an increasing alcohol chain length and were further tunable through end group structure and molecular weight. This UCST behavior in alcohols is promising because it occurs in both hydrophilic solvents such as ethanol as well as in the hydrophobic longer chain alcohols.

Reversible addition−fragmentation chain transfer (RAFT) radical polymerization is an example of a reversible deactivation radical polymerization (RDRP) process that is mediated by thiocarbonylthio compounds.[7,21−26] Since its discovery, RAFT has evolved into arguably the most robust and versatile of the RDRP systems and is now employed routinely in research laboratories worldwide. In recent years there has been significant interest in combining RDRP with a range of click and other highly efficient coupling chemistries. These include Cu-catalyzed alkyne−azide,[27−30] Diels−Alder,[31−34] and a range of thiol-based chemistries[35−41] with the aim of developing routes for the preparation of complex macromolecular architectures or facilitating the efficient postpolymerization modification of preformed (co)polymers. Indeed, RAFT has lent itself perfectly to such click and click-like chemistries in part due to the availability of a protected thiol, in the form of the thiocarbonylthio group, at the polymer chain end(s) and also due to its inherently high functional group tolerance allowing the synthesis of parent (co)polymers containing an impressive array of functionality.[42,43] Of the efficient coupling chemistries, the use of pentafluorophenyl (PFP) activated (meth)acrylic esters has proven to be particularly useful for the introduction of amine-containing species via the formation of amides.[11,44−49] For example, Gibson, Fröhlich, and Klok detailed the RAFT synthesis of three poly(pentafluorophenyl methacrylate)s with measured average molecular weights in the range 13 750−36 800 g/mol.[49] These precursor activated polymers were reacted with a range of hydrophilic primary amines including glycine, glucosamine, 2-hydroxypropylamine, and taurine yielding the corresponding functional, hydrophilic amides in generally high yields under facile conditions. The authors also demonstrated that the degree of modification was readily controlled by functional group stoichiometry (pentafluorophenyl ester:primary amine). In the case of precursor polymers modified with hydroxypropylamine cytotoxicity tests indicated no difference in the inherent toxicity of the materials via the postpolymerization route vs, for example, polymers obtained by the direct polymerization of 2-hydroxypropyl methacrylamide.

Employing a combination of RAFT radical polymerization with PFP activated ester chemistry herein we report our findings regarding the synthesis and thermal solution properties of a library of poly[oligo(ethylene glycol) *(meth)acrylamide*]s which serve as PEG analogues. While a few examples of such materials have been previously prepared there has not, to the best of our knowledge, been a systematic detailed investigation of their thermoresponsive phase behavior in water (LCST) or alcohols (UCST). We highlight how these (meth)-acrylamido-based materials represent an important addition to the family of PEG-analogues possessing fundamentally different solution properties from the corresponding (meth)acrylic species and as such may find application in areas not accessible to such (meth)acrylic derivatives.

EXPERIMENTAL PART

Instrumentation. Nuclear magnetic resonance (NMR) spectroscopic measurements were performed in $CDCl_3$ solutions on a Bruker DPX 300 instrument and evaluated using MestReC 4.7.0.0 software. The internal solvent signal was used as the reference signal (7.26 ppm). Size exclusion chromatography (SEC) was performed on a Shimadzu system with four phenogel columns in dimethylacetamide (DMAc) as eluent at a flow rate of 1.0 mL/min. Chromatograms were analyzed with Cirrus SEC software (version 3.0). The system was calibrated with a series of narrow molecular weight distribution polystyrene (PS) standards. Turbidity measurements were performed on a Varian Cary 300 Scan spectrophotometer equipped with a Cary temperature controller and a Peltier heating element in quartz cuvettes of 10 mm path length at a wavelength of 520 nm. Heating rates were 1.0 °C/min for all measurements. For clear solutions (cold water; warm alcohols), the baseline was set to zero absorbance, *A*. Transmittance, $T = 10^{-A}$, was plotted against temperature and cloud points were determined at 50% transmittance. The maximum temperature evaluated for aqueous solutions was 93.0 °C. Fourier transform infrared spectroscopy (FTIR) was performed on a Bruker IFS 66/S instrument under attenuated total reflectance (ATR) and was analyzed on OPUS software version 4.0. Differential scanning calorimetry measurements were performed with a Perkin-Elmer DSC7 instrument at a heating rate of 2.0 °C/min on 40 μL of 20 g/L solutions and were analyzed with PE Pyris software.

Synthesis. All reagents were purchased from the Aldrich Chemical Co. and used as received unless noted otherwise. 4-Cyano-4-((phenylcarbonothioyl)thio)pentanoic acid (CPADB) was prepared according to the literature.[22] 2,2′-Azobis(2-methylpropionitrile) (AIBN) was recrystallized from methanol.

Synthesis of Pentafluorophenyl (Meth)acrylate Monomers. Pentafluorophenyl acrylate and pentafluorophenyl methacrylate were synthesized according to the general procedure described by Eberhardt et al.[50]

Synthesis of Ethylene Glycol Methyl Ether Amines. Di- and triethylene glycol methyl ether amine were prepared according to the method of Clerc et al.[51] Diethylene glycol methyl ether amine: 65% overall yield; ^{1}H NMR, 300 MHz, $CDCl_3$, δ (ppm) = 3.55 (m, 2 H), 3.50−3.40 (m, 4 H), 3.32 (m, 3 H, $-OCH_3$), 2.80 (m, 2 H, $-CH_2NH_2$), 1.73 (bs, $-NH_2$). Triethylene glycol methyl ether amine: 64% overall yield; ^{1}H NMR, 300 MHz, $CDCl_3$, δ (ppm) = 3.52 (m, 6 H, PEG), 3.43/3.38 (m, 4 H, $-CH_2-$), 3.24 (m, 3 H, $-OCH_3$), 2.73 (m, 2 H, $-CH_2NH_2$), 1.50 (bs, $-NH_2$).

Poly(ethylene glycol) methyl ether amines with average molecular weights of 350, 750 and 5000 were prepared following the procedure of Mongondry and co-workers.[52] PEG-350 amine: 50% overall yield; ^{1}H NMR, 300 MHz, $CDCl_3$, δ (ppm) = 3.53 (bs, ∼ 29 H, PEG), 3.44−3.38 (m, 4 H, $-CH_2-$), 3.36 (s, 3 H, $-OCH_3$), 2.75 (bt, 2 H, $-CH_2NH_2$), 2.12 (bs, $-NH_2$). PEG-750 amine: 86% overall yield; ^{1}H NMR, 300 MHz, $CDCl_3$, δ (ppm) = 3.71−3.44 (bs, ∼ 83 H, PEG), 3.31 (bs, 3 H, $-OCH_3$), 2.81 (m, 2 H, $-CH_2NH_2$), 2.49 (bs, $-NH_2$). PEG-5000 amine: 73% overall yield; ^{1}H NMR, 300 MHz, $CDCl_3$, δ/ppm =3.75−3.40 (bs, PEG), 3.33 (bs, $-OCH_3$), 2.80 (m,$-CH_2NH_2$).

All PEG amines were of sufficient purity for the conversions of polymeric activated esters without further purification.

RAFT Homopolymerization of PFP Ester Monomers. Below is given a typical procedure for the RAFT homopolymerization of pentafluorophenyl acrylate (**PFPA**). The same general procedure was employed for the homopolymerization of pentafluorophenyl methacrylate (**PFPMA**).

PFPA (5.0 g, 21.0 mmol), CPADB (78 mg, 0.28 mmol), AIBN (9.2 mg, 0.056 mmol), and 1,4-dioxane (8.0 mL) were combined in a

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round bottomed flask equipped with a magnetic stir bar. The flask was sealed with a rubber septum and nitrogen was bubbled through the solution for 20 min. The reaction vessel was subsequently immersed in a preheated oil bath set to 70 °C. Polymerization was allowed to proceed for 12 h. The resulting **polyPFPA** was isolated via two precipitations into methanol and subsequently dried under vacuum. **PolyPFPA**: SEC 26.6 kg/mol, PDI 1.19. ^{1}H NMR, 300 MHz, $CDCl_3$, δ (ppm) = 3.40, 2.52, 2.15 (m, backbone). ^{19}F NMR, 282 MHz, $CDCl_3$, δ (ppm) = −153.1 (bs, 2 F, *ortho*), −153.7 (bs, 1 F, *para*), −162.1 (2 F, *meta*). IR, ν (cm^{-1}) = 2931 (w), 1780 (m), 1513 (s), 1218 (w), 1076 (m), 985 (s), 860 (w), 852 (w). **PolyPFPMA**: SEC 15.4 kg/mol, PDI 1.21. ^{1}H NMR, 300 MHz, $CDCl_3$, δ (ppm) = 2.44, 2.19, 1.46, 1.40 (m, backbone). ^{19}F NMR, 282 MHz, $CDCl_3$, δ (ppm) = −150.9, −151.9 (2 m, 2 F, *ortho*), −157.4 (bs, 1 F, *para*), −162.5 (2 F, *meta*). IR, ν (cm^{-1}) = 2967 (w), 1758 (m), 1515 (s), 1240 (w), 1043 (s), 989 (s), 854 (w).

Reaction of Pentafluorophenyl (Meth)acrylate Polymers with Primary Amines in the Presence of Methyl Methanethiosulfonate. Below is a typical example for the reaction of a pentafluorophenyl (meth)acrylate (**PFPMA**) polymer with an oligo(ethylene glycol) methyl ether amine.

To a glass vial equipped with a magnetic stir bar was added **polyPFPA** (214 mg, 0.9 mmol of pentafluorophenyl esters, 1 equiv) and DMF (2.0 mL). To a separate flask was added diethylene glycol methyl ether amine (273.2 mg, 2.25 mmol, 2.5 equiv), DBU (202 μL, 1.35 mmol), methyl methanethiosulfonate (22 μL, 10 equiv. with respect to polymer end groups) and DMF (0.9 mL). After complete dissolution the solutions were combined and the reaction allowed to proceed overnight at room temperature. For poly(pentafluorophenyl methacrylate) the excess of amines was increased to 5 equiv. and THF and triethylamine were used as solvent and auxiliary base, respectively. IR, ν (cm^{-1}) = 3457 (w), 3300 (m), 2865 (m), 1644 (s), 1539 (s), 1452 (m), 1093 (s), 1022 (m), 844 (w). Reaction of **polyPFPMA** with diethylene glycol methyl ether amine: IR, ν (cm^{-1}) = 3505 (w), 3352 (m), 2868 (m), 1637 (s), 1518 (s), 1452 (m), 1198 (m), 1095 (s), 845 (w).

Copolymer Synthesis. Reaction of **PFPA** with two different primary amines in the presence of methyl methanethiosulfonate. Below is a typical example for the reaction of a **polyPFPA** with an oligo(ethylene glycol) methyl ether amine (85 mol %) and hexylamine (15 mol %).

To a glass vial equipped with a magnetic stir bar was added **polyPFPA** (119 mg, 0.5 mmol of PFP esters, 1 equiv) and DMF (1.5 mL). To a separate flask was added di(ethylene glycol) methyl ether amine (60.8 mg, 0.51 mmol, 1.02 equiv, 20% excess), hexylamine (9.1 mg, 0.09 mmol, 0.18 equiv, 20% excess), methyl methanethiosulfonate (10 μL) and DMF (1.0 mL). The amine solution was quickly added to the polymer solution under vigorous stirring and allowed to react at room temperature overnight. IR, ν (cm^{-1}) = 3469 (w), 3284 (m), 2918 (m), 1641 (s), 1540 (s), 1440 (m), 1095 (s), 845 (m).

The same protocol was employed for the preparation of thyroxine (T_4) containing copolymers where ratios of 9:1 and 8:2 of di(ethylene glycol) methyl ether amine to thyroxine with an excess of 20 mol % of amines toward PFP esters were used.

General Purification Protocols. After stirring overnight, 0.2 mL of reaction mixture was withdrawn, 0.45 mL of $CDCl_3$ was added and a ^{19}F NMR measurement was made (example poly[di(ethylene glycol) methyl ether acrylamide]: ^{19}F NMR, 282 MHz, $CDCl_3$, δ (ppm) = −170.7 (m, 2 F, *ortho*), −171.7 (m, 2 F, *meta*), −189.5 (m, 1 F, *para*), pentafluorophenol). The NMR sample was combined with the remaining reaction mixture which was then placed into a dialysis membrane (regenerated cellulose, 3500 g/mol MWCO for di- and triethylene glycol amine and PEG-350 amine; MWCO 6−8 kg/mol for PEG-750 amine; MWCO 12−14 k for PEG-5000 amine) and dialyzed against methanol for 3 days with solvent changes twice per day. The solvent was removed by blowing in air; the residue was then dissolved in a small amount of water and lyophilized to obtain the final products.

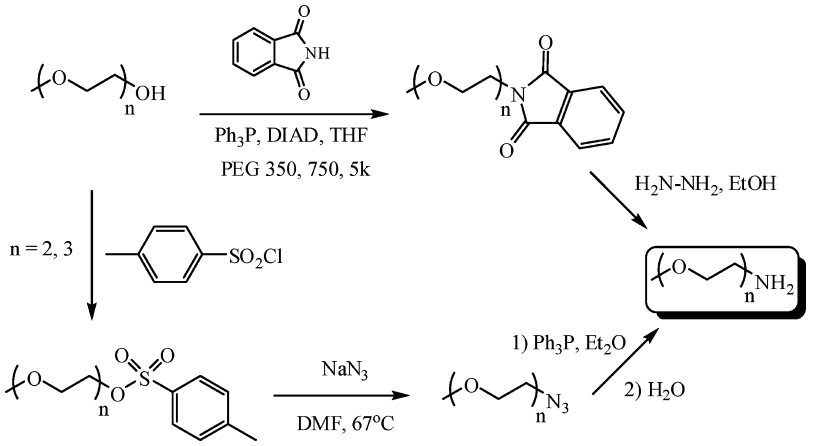
Cytotoxicity Testing. The toxicity of the prepared polymers was tested on a MRC5 fibroblast cell line. Cell culture: MRC5 fibroblast cells were cultured in growth media consisting of Dulbecco's modified Eagle's medium: Nutrient Mix F-12 (DMEM) supplemented with 10% (v/v) foetal bovine serum (FBS) in a ventilated tissue culture flask T-75 and passaged every 2−3 days when the monolayer reached around 80% confluence. The cells were used only when stable cell growth was obtained (approximately 3−4 passages). The cells were incubated at 37 °C in a 5% CO_2 humidified atmosphere. The cell density was determined by counting the number of viable cells using a trypan blue dye exclusion test. The cells were detached using 0.05% trypsin-EDTA (Invitrogen), stained using trypan blue dye, and loaded on a hemocytometer. One day prior to the treatment, the cells were seeded at required cell densities on a 96-well plate. Cell viability: The cytotoxicity of the (meth)acrylamido-based polymers was tested *in vitro* by a standard Alamar Blue assay which provides a homogeneous, fluorescent method for monitoring cell viability. The assay is based on the ability of living cells to convert a redox dye (blue resazurin) into a fluorescent end product (red resorufin). Nonviable cells rapidly lose metabolic capacity and thus do not generate a fluorescent signal. The cells were seeded in a tissue culture treated 96-well plate in 100 μL medium per well at a density of 5000 cells/well and incubated for 24 h. The medium was then replaced with fresh medium containing the polymer samples and incubated for 72 h. The final concentration of polymer in the wells was adjusted to the desired concentrations ranging from 0.005 to 10.0 g/L in DMEM media containing 10% v/v FBS. Alamar Blue dye (20 μL) was added to each well and the cells were incubated for 6 h. After the incubation step, data was recorded using a fluorescence plate reader (λ_{ex} = 540 nm; λ_{em} = 595 nm).

RESULTS AND DISCUSSION

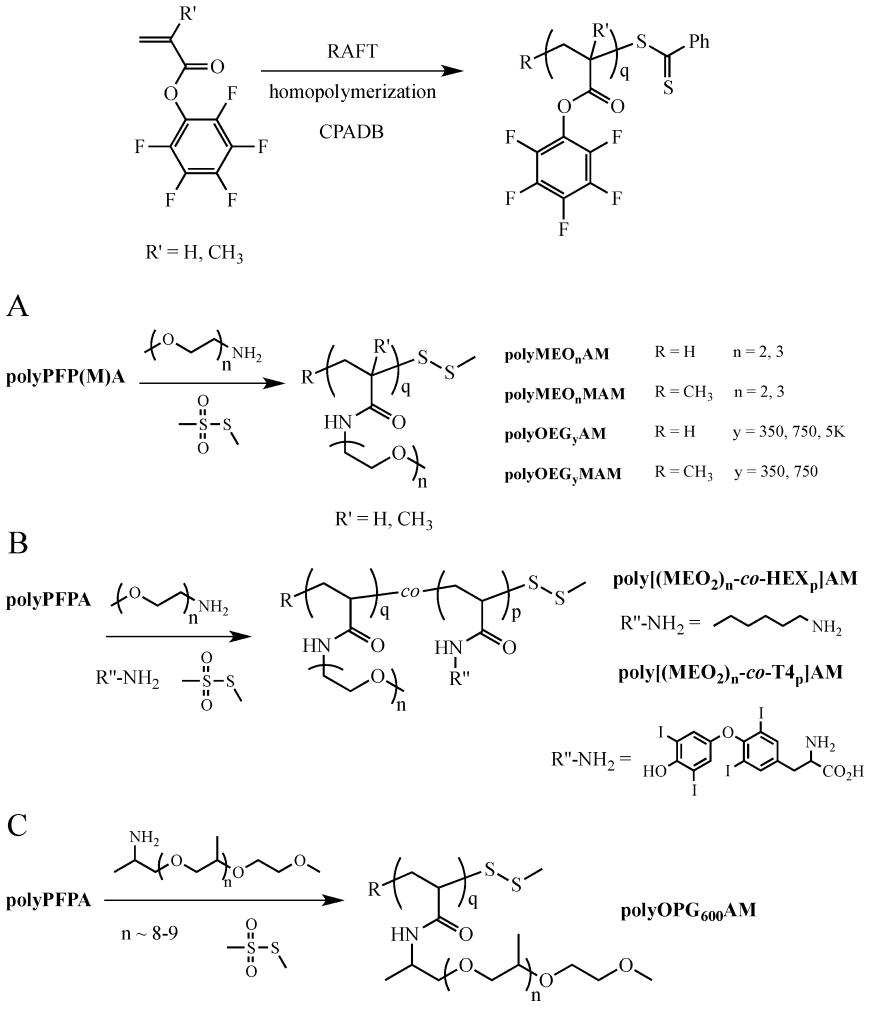
Synthesis. The desired oligo(ethylene glycol) methyl ether amines (OEGMEAs) were obtained via two different routes the choice of which was dictated by the molecular weight of the precursor oligo(ethylene glycol) methyl ether (OEGME). The di- and tri(ethylene glycol) methyl ether amines (MEO_2 and MEO_3) were prepared from the corresponding OEGME via a three step procedure involving tosylation, nucleophilic substitution with azide followed by Staudinger reduction. The higher molecular weight target OEGMEAs with average precursor molecular weights of 350, 750, and 5000 g/mol were prepared via a Mitsunobu reaction with phthalimide followed by a Gabriel synthesis-like reaction of the intermediate functional phthalimide via hydrazinolysis, Scheme 1. In all instances the target OEGMEAs were obtained in moderate to high overall yield and purity.

With the OEGMEAs in hand two different pentafluorophenyl- (PFP-) containing homopolymers (one derived from PFP-acrylate (**PFPA**) and the other from PFP-methacrylate (**PFPMA**)) were prepared under standard RAFT conditions employing 4-cyano-4-((phenylcarbonothioyl)thio)pentanoic acid (CPADB) as the mediating agent and AIBN as the source of primary radicals, Scheme 2. The RAFT (co)polymerization of **PFPA** and **PFPMA** is well-documented[46,50] and the polymerizations were allowed to proceed for a predetermined period of time yielding two homopolymers with measured number-average molecular weights (M_n) of 26 600 g/mol (**polyPFPA**) and 15 400 g/mol (**polyPFPMA**) and relatively low polydispersity indices (M_w/M_n = 1.19 and 1.21 respectively). With the **polyPFPA** and **polyPFPMA** parent homopolymers in hand a library of oligo(ethylene glycol)-based (meth)acrylamido (co)polymers were prepared via the reaction of the OEGMEAs prepared above (as well as with some commercially available primary amine species, vide infra) with the **PFPA** and **PFPMA** homopolymers, Scheme 2. The use of two simple precursor homopolymers as activated substrates thus allowed the preparation of a library of amide-based PEG analogues with identical average main-chain degrees of polymerization. It should be noted that the acyl substitution

Scheme 1. Synthetic Approaches for the Preparation of Oligo(ethylene glycol) Methyl Ether Amines



Scheme 2. Outline for the RAFT Homopolymerization of Pentafluorophenyl (Meth)acrylate Monomers and Procedures for the Subsequent Post-Polymerization Acyl Substitution Reactions with OEGMEAs



reactions were performed in the presence of *S*-methyl methanethiosulfonate (MMTS). Thiocarbonylthio functional groups (found at the ω-chain end of the RAFT-prepared **PFPA** and **PFPMA** homopolymers in the form of dithiobenzoate species) are extremely reactive towards primary and secondary amines with reactions resulting in the cleavage of the thiocarbonylthio end groups yielding the corresponding macromolecular thiol. Indeed, this represents the most commonly employed route for the removal of such end-groups in RAFT-synthesized (co)polymers with the liberated thiol being able to undergo a range of further chemical reactions.[36,43,53] However, one common problem with the formation of macromolecular thiols is their propensity to undergo oxidative coupling forming polymeric disulfides. Such

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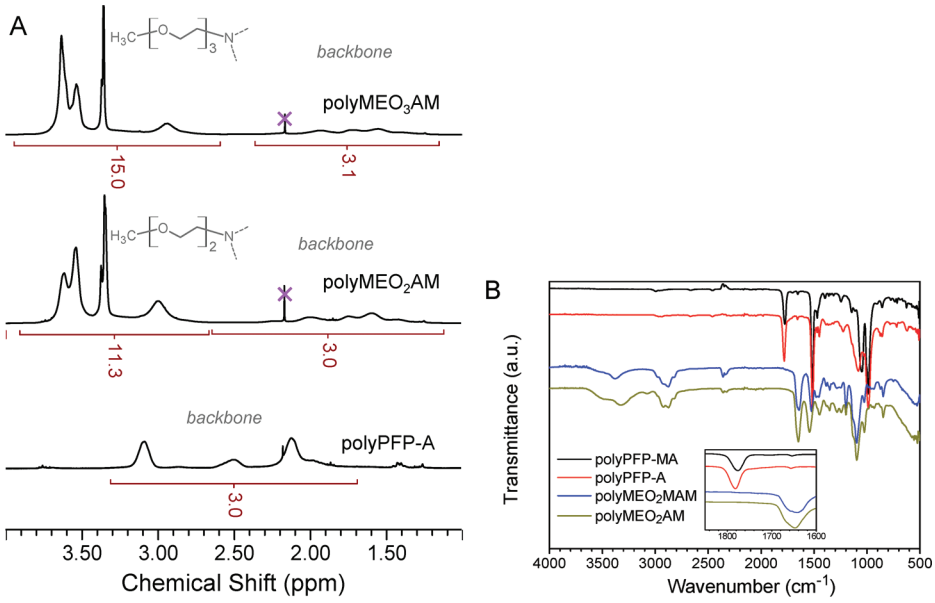

Figure 1. Monitoring the conversion of PFP esters with OEGMEAs. (A) ^{1}H NMR spectra of parent **polyPFPA** (bottom), **polyMEO$_2$AM** (middle), and **polyMEO$_3$AM** (top) showing quantitative formation (within NMR accuracy) of the PEG-amides. (B) FTIR spectra of the activated polymers **polyPFPA** and **PFPMA** and after conversion of each with di(ethylene glycol) amine, with the inset highlighting the shift of the carbonyl band.

reactions, while reversible, consume the thiol, which is clearly problematic if further modification is the goal but also results in an effective doubling of the average molecular weight or degree of polymerization. Since molecular weight is an important parameter affecting the solution phase behavior of thermoresponsive (co)polymers this precautionary step of capturing the macromolecular thiol was adopted. Indeed, it has previously been shown that MMTS, and functional derivatives thereof, are extremely effective reagents for 'trapping' liberated thiols produced during the aminolysis of end-groups in RAFT-prepared (co)polymers[12,54−57] thus negating any undesirable polymer−polymer disulfide formation.

Initially, both **polyPFPMA** and **polyPFPA** were reacted with four OEGMEAs: di(ethylene glycol) methyl ether amine, tri(ethylene glycol) methyl ether amine and two OEGMEAs with average M_n's of 350 and 750 g/mol, Scheme 2A. The resulting acrylamido (**AM**) and methacrylamido (**MAM**) analogues are denoted **polyMEO$_n$AM** and **polyMEO$_n$MAM** for the dimer and trimer ethylene glycol species (n = 2 and 3) and **polyOEG$_y$AM** and **polyOEG$_y$MAM** for those derived from the OEGMEAs with y = 350 and 750 g/mol. Conversion of the parent **polyPFPA** and **polyPFPMA** homopolymers to the corresponding PEG-amides was quantified employing a combination of ^{1}H and ^{19}F NMR spectroscopy, SEC and FTIR spectroscopy. For the acrylic activated esters quantitative conversion was achieved with 2.5 equiv of amine with regards to PFP esters. This procedure did not, however, give a full conversion of the analogous methacrylic esters to the desired amides. In case of **polyPFPMA**, ^{1}H NMR spectroscopy showed less than quantitative formation of the PEG amides and FTIR spectroscopy revealed the presence of acid groups (~1710 cm^{-1}). The nonquantitative conversion of methacrylic PFP esters with 2 equiv of amine, followed by hydrolysis of unreacted esters during work-up, has been reported.[49] Similarly, nonquantitative amidation of surface tethered poly(methacrylic acid) during the activation step with EDC/NHS (*N*-ethyl-*N*′-(3-(dimethylamino)-propyl)carbodiimide/*N*-hydroxysuccinimide) was recently described.[58] The authors detailed the formation of methacrylic acid anhydrides, as opposed to the desired activated esters, between neighboring −CO_2H groups, which subsequently formed one amide and one acid group upon exposure with amines. On the other hand, poly(acrylic acid) gave a high amide yield. While different to the current research, where the activation step took place prior to polymerization, the low amidation of methacrylic PFP derivatives and the fundamental difference compared to the polyacrylate should be noted. Here, the ratio of amine with respect to methacrylic PFP esters was increased to 5 to minimize the formation of hydrolysis byproducts. For the acrylate derivative on the other hand, as little as 1.2 equiv of amines (as used in the production of copolymers) was sufficient to achieve a quantitative conversion to the target amides as evidenced by ^{1}H, ^{19}F NMR and FTIR spectroscopies.

For example, Figure 1A shows the ^{1}H NMR spectra, plotted between 1.0 and 4.0 ppm, for the **polyPFPA** homopolymer (bottom) and the corresponding amides obtained after reaction with di(ethylene glycol) methyl ether amine (**polyMEO$_2$AM**) (middle) and tri(ethylene glycol) methyl ether amine (**polyMEO$_3$AM**) (top). The spectrum associated with **polyPFPA** is relatively simple with only backbone hydrogens visible which span the range of ~3.2−2.0 ppm. After modification with the di- and tri(ethylene glycol) amino species peaks appear in the range ~3.8−2.8 ppm which correspond to the side-chain hydrogens associated with the ethylene glycol methyl ether segments. Analysis of the integrals associated with these new peaks indicated quantitative formation of the PEG-acrylamides when compared to the integrals associated with the backbone hydrogens. Figure 1B shows the FTIR spectra for **polyPFPA**, **polyPFPMA**, **polyMEO$_2$MAM**, and **polyMEO$_2$AM**. The most distinctive feature when comparing the parent (meth)acrylic homopolymers and the resulting PEG-(meth)acrylamides, and qualitatively verifying complete reaction, relates to the C═O peak associated with the parent esters vs the product amides. In the parent homopolymers the ester C═O peak appears at ca. 1780 cm^{-1} while in the product (meth)acrylamides the amide C═O

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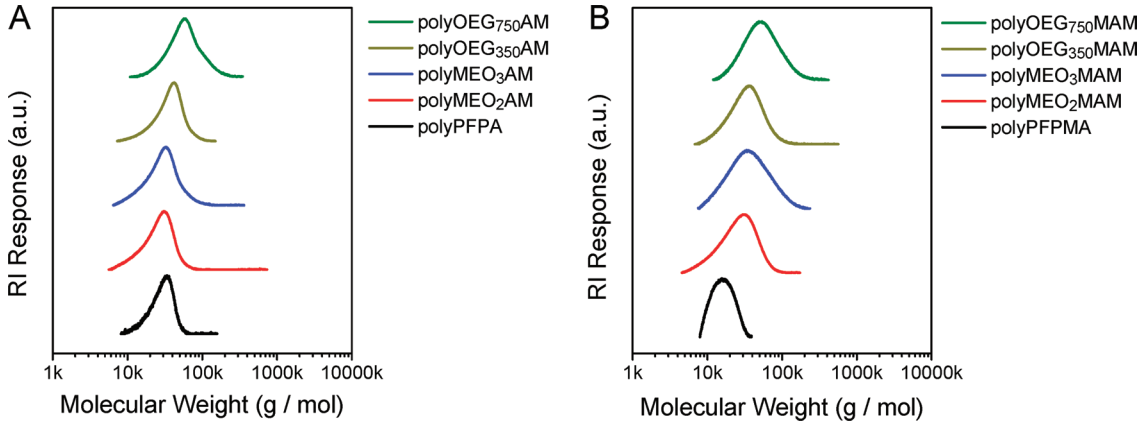

Figure 2. SEC traces of (A) the acrylamido series including the precursor **polyPFPA** and (B) the methacrylamide series with its parent polymer **polyPFPMA**.

Table 1. Summary of Prepared OEG Amide Homopolymers and Statistical Copolymers, SEC-Measured Molecular Weights, Polydispersities, and Cloud Points in Water

(meth)acrylamido (co)polymers						reference (meth)acrylates		
				cloud point (5 g/L)[b]				
entry	polymer	M_n[a] (kg/mol)	PDI[a]	heating (°C)	cooling (°C)	polymer	cloud point[b] (°C)	reference
1	**polyMEO$_2$AM**	22.5	1.22	s[c]	s	polyMEO$_2$A	38	59
2	**polyMEO$_3$AM**	24.7	1.25	s	s	polyMEO$_3$A	58	59
3	**polyOEG$_{350}$AM**	31.1	1.2	s	s	polyOEG$_{370}$A[e]	s	60
4	**polyOEG$_{750}$AM**	47.0	1.25	s	s			
5	**polyOEG$_{5k}$AM**	177.4	1.74	s	s			
6	**poly[(MEO$_2$)$_{49}$-*co*-Hex$_{51}$]AM**	19.7	1.26	ins[d]	ins			
7	**poly[(MEO$_2$)$_{76}$-*co*-Hex$_{24}$]AM**	20.7	1.24	26.0	21.1			
8	**poly[(MEO$_2$)$_{85}$-*co*-Hex$_{15}$]AM**	21.3	1.24	46.7	43.0			
9	**poly[(MEO$_2$)$_{93}$-*co*-Hex$_7$]AM**	20.7	1.25	77.7	75.9			
10	**poly[(MEO$_2$)$_{91}$-*co*-T4$_9$]AM**	42.5	1.21	s	s			
11	**poly[(MEO$_2$)$_{87}$-*co*-T4$_{13}$]AM**	49.7	1.18	s	s			
12	**polyOPG$_{600}$AM**	18.0	1.26	22.1	16.7			
13	**polyMEO$_2$MAM**	21.3	1.32	55.8	52.7	polyMEO$_2$MA	26	19
14	**polyMEO$_3$MAM**	28.2	1.38	69.3	63.2	polyMEO$_3$MA	52	19
15	**polyOEG$_{350}$MAM**	27.8	1.26	s	s	polyOEG$_{375}$MA[f]	90	17
16	**polyOEG$_{750}$MAM**	42.5	1.29	s	s			

[a]Data obtained by SEC. [b]Cloud points depend on concentration, molecular weight and polymer end groups. [c]Soluble between 0 and 93 °C. [d]Insoluble between 0 and 93 °C. [e][Poly(ethylene glycol) monomethyl ether acrylate], $M_{monomer}$ = 454 g/mol. [f][Poly(ethylene glycol) monomethyl ether methacrylate], $M_{monomer}$ = 475 g/mol.

peak appears at ca.1640 cm^{-1} with no evidence of residual ester C═O species. Additionally, we note the appearance of bands at ca. 2850 cm^{-1} that may be assigned to the C−H stretches in the CH_2 and CH_3 groups associated with the newly introduced ethylene glycol side chains and the broad bands between 3500 and 3100 cm^{-1} due to the N−H stretch of the newly introduced secondary amide functionality. Representative ^{19}F NMR spectra demonstrating the complete cleavage of the activated esters are given in Figure S1 of the Supporting Information.

Figure 2 shows the measured molecular weight distributions for the parent **polyPFPA** (Figure 2A black line) and **polyPFPMA** (Figure 2B black line) as well as the resulting (meth)acrylamides derived from reaction with the OEGMEAs. The experimentally determined molecular weight and polydispersity data is summarized in Table 1 (entries 1−4 for the acrylamides and 13−16 for the methacrylamides). In the case of the acrylamido derivatives we observe an increase in the M_n as the length of the oligo(ethylene glycol) side chain increases, as expected. In all instances the measured polydispersity indices increase slightly compared to the parent **polyPFPA** but are all between 1.20 and 1.25 with the molecular weight distributions being unimodal and essentially symmetrical. This also indicates that the macromolecular thiols were successfully trapped with MMTS since there is no evidence of high molecular weight (coupled) species in the chromatograms. The same general trends are observed in the case of the methacrylamide derivatives, with an increase in the measured M_n as side-chain degree of polymerization increases and polydispersity indices in the range 1.26−1.38.

Aqueous Solution Properties. With a library of acrylamido and methacrylamido-based PEG analogues prepared the aqueous phase behavior of the polymers was investigated at a concentration of 5.0 g/L (0.5 wt %). Consider first the range of acrylamido derivatives (entries 1−4 Table 1). In all cases, at the concentration evaluated, the PEG-based acrylamido homopolymers did not exhibit any LCST behavior in pure water and were completely soluble between 0 and 93 °C. This is in sharp contrast to acrylate derivatives in which polymers

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with di- and tri(ethylene glycol) side chains have reported LCSTs of 38 and 58 °C respectively.[59] This difference in solubility is, presumably, due to the enhanced hydrophilicity of the amide-based materials due to the H-bond donor and acceptor properties of the amide functional group and highlights how subtle functional group changes can have a marked effect on the aqueous solution properties of such PEG-containing polymers. The absence of LCST behavior for the acrylamido-based PEGs with the longer side chains is consistent with the enhanced aqueous solubility reported for analogous acrylic derivatives.[60]

It is well documented that the LCST behavior of thermoresponsive polymers may be tuned via the incorporation of a hydrophobic comonomer[61,62] or, in the case of suitably low molecular weight species, via variation of the hydrophilicity/hydrophobicity of polymer end-groups.[5,63] In an effort to induce LCST behavior the **polyPFPA** parent homopolymer was reacted with varying ratios of di(ethylene glycol) methyl ether amine and hexylamine, Scheme 2 route B, yielding, presumably, random amphiphilic copolymers containing 51, 24, 15, and 7 mol % hydrophobic hexylamide residues. Compositions were determined by ^{1}H NMR spectroscopy by a comparison of the hexyl $-CH_3$ group with the ethylene glycol $-OCH_3$ group. Both amines exhibited a similar reactivity toward the activated esters yielding polymers with essentially the same composition as the feed ratio of amines (see Figure S2, and Table S1 in Supporting Information). Molar compositions, molecular weights and polydispersity indices for these copolymers are listed in Table 1 (entries 6–9). SEC traces are shown in Figure S3. It should be noted that this yields statistical copolymers with the same degree of polymerization whose individual components are either wholly soluble in water (di(ethylene glycol) methyl ether amide residues) or completely insoluble, i.e. hydrophobic (hexylamide residues) and thus should allow for a tuning of the LCST behavior.[61,62] The LCST characteristics of these amphiphilic copolymers were evaluated under the same conditions as noted above for the acrylamide homopolymers. Two additional amphiphilic statistical copolymers were also prepared by substituting hexylamine for the prohormone thyroxine (Table 1 entries 10 and 11) and these will be discussed in the final part of this contribution.

Figure 3 shows the cloud-point curves for the **poly**-$[(\mathbf{MEO_2})_{1\text{-}x}\text{-}co\text{-}\mathbf{Hex_x}]$**AM** statistical copolymers as well as the **polyMEO$_2$AM** homopolymer. As noted above, **polyMEO$_2$AM** is completely soluble and does not exhibit a phase transition upon heating (solid blue line). With the introduction of as little as 7 mol % hexylamide residues the amphiphilic copolymer undergoes a phase transition upon heating with a measured cloud point of 77.7 °C (taken at 50% transmittance, solid red line) although the transition is not particularly sharp. Increasing the hexylamide content to 15 and 24 mol % results in a systematic lowering of the cloud point to 46.7 °C (solid black line) and 26.0 °C (solid green line) respectively and also appears to result in sharper, more distinct transitions. Increasing the hexylamide content to just above equimolar (51 mol %) yields a copolymer that is completely insoluble in water even when cooled to temperatures approaching 0 °C. Also shown in Figure 3 are the corresponding cooling curves for the three thermoresponsive copolymers, and the corresponding phase transition temperatures are listed in Table 1. Little hysteresis is observed for all three samples and in all instances the redissolution temperatures are only several degrees lower than the cloud points found for heating. This is consistent with the well-known kinetic effect and the process of rehydration of the highly compact globules and aggregates thereof. Shown inset in Figure 3 is a plot of the cloud point vs mol % hexylamide residues. It can be seen that this plot is essentially linear and indicates that the synthetic route described herein offers a simple and convenient approach to thermoresponsive copolymers with tunable, and essentially predetermined, LCST behavior simply by varying the molar content of hexylamide residues.

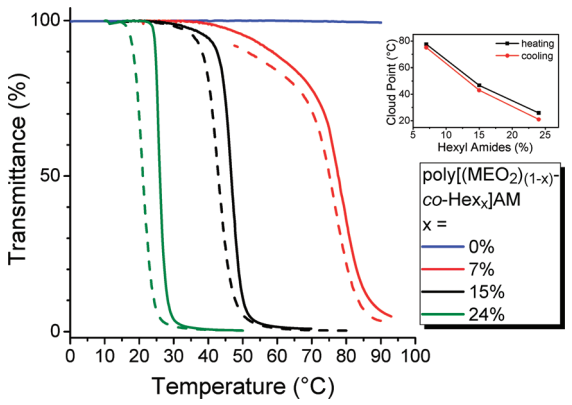

Figure 3. Turbidity curves of the **poly**$[(\mathbf{MEO_2})_{1\text{-}x}\text{-}co\text{-}\mathbf{Hex_x}]$**AM** series demonstrating tunable LCST behavior: heating curves (solid lines); cooling curves (dashed lines).

Another strategy was also investigated to induce LCST behavior into the acrylamido polymers, this time by a "hydrophobic modification" of the ethylene glycol side chains. Poly(propylene glycol) (PPG) is a well documented thermoresponsive polymer that exhibits a marked dependence of its LCST on the polymer molecular weight. For example, increasing the MW from 1000 to 2000 to 3000 results in a decrease in the LCST from 42.0 to 23.0 to 15.5 °C.[64] PPG, while not perhaps as currently widely evaluated or employed in polymer synthesis as PEG, due in part to such low thermal transitions, finds wide use as the hydrophobic middle section of the commercially important Pluronic series of surfactants.[65] To evaluate the effect of relative hydrophobicity of the oligoether side chains the **polyPFPA** homopolymer was also reacted with a commercially available oligo(propylene glycol) amine (Jeffamine) with an average molecular weight of ca. 600 g/mol. Consistent with the previous postpolymerization conjugations the reaction of the Jeffamine proceeded smoothly, quantitatively yielding a well-defined oligo(propylene glycol) methyl ether acrylamido homopolymer, **polyOPG$_{600}$AM**, entry 12 in Table 1, Figure S4 in the Supporting Information. Evaluation of the cloud point behavior of this PPG-acrylamido derivative, Figure 4, indicates a sharp phase transition upon heating at 22.1 °C with the cooling curve also indicating moderate hysteresis. Thus, the combination of PPG side-chains linked to a backbone via a hydrophilic acrylamido functional group allows for the preparation of PPG-containing polymers that exhibit distinctly different aqueous phase behavior compared to simple linear PPG materials and facilitates the preparation of water-soluble PPG-containing substrates with much higher effective molecular weights than the linear species.

The thermal phase transitions of two samples, **polyOPG$_{600}$AM** and **poly**$[(\mathbf{MEO_2})_{85}\text{-}co\text{-}\mathbf{Hex_{15}}]$**AM**, were also briefly examined by differential scanning calorimetry (DSC). Experiments were performed at 20.0 g/L, four times higher than the

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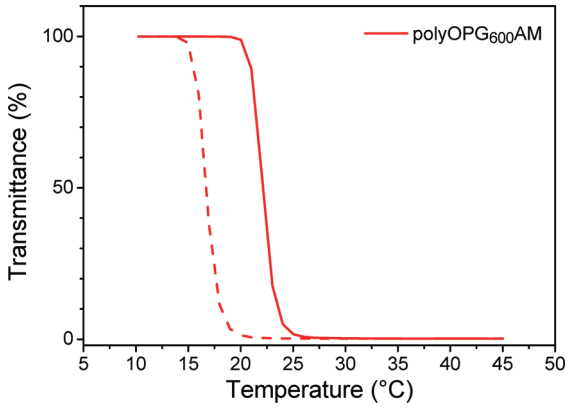

Figure 4. Turbidity measurement (dashed line cooling curve) of the Jeffamide derivative showing a sharp LCST transition.

concentration examined above in cloud point measurements. The measured transition temperatures were in agreement with turbidity measurements performed at the same concentration (Supporting Information, Figure S5). The endotherms of both polymers do however show distinct differences. **PolyOPG$_{600}$AM** shows a very broad transition occurring over a range ~10 °C with a heat of 22.94 kJ/mol monomer units calculated from the peak area, Figure 5. On the other hand, the

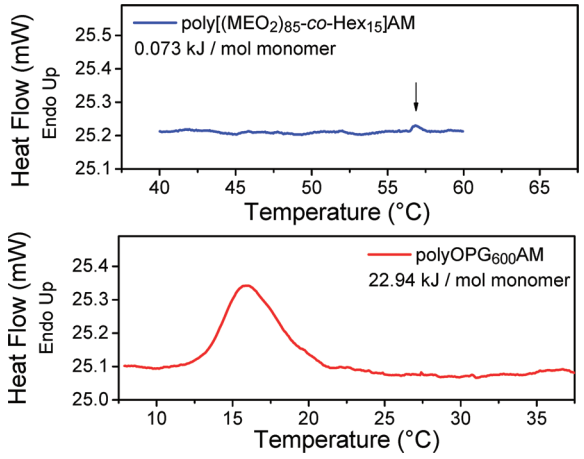

Figure 5. Heating endotherms of two samples obtained by differential scanning calorimetry showing the significant difference of the transitions of a copolymer with few hydrophobic comonomer units (blue curve, top) and a polymer with thermally responsive side chains (red curve, bottom).

poly[(MEO$_2$)$_{85}$-*co*-Hex$_{15}$]AM copolymer produced only a very small narrow peak spanning ~2 °C and an area of 0.073 kJ/mol average monomer units. The endotherms represent molecular events occurring during the phase transition and account for the loss of solvation (e.g., through hydrogen bonding) when the polymer undergoes a coil-to-globule transition. The large difference between the two observed polymers thus suggests a much larger number of hydrogen bonds are breaking during the phase transition of the OPG derivative. This is not surprising as the OPG graft homopolymer carries stronger hydrophobic side chains that are in fact thermally responsive themselves—in contrast to the **poly[(MEO$_2$)$_{85}$-*co*-Hex$_{15}$]AM** copolymer.

Next, the thermal behavior of the methacrylamido polymers, Table 1 entries 13–16, was investigated. In contrast to the **polyMEO$_2$AM** and **polyMEO$_3$AM** acrylamido homopolymers both the **polyMEO$_2$MAM** and **polyMEO$_3$MAM** species exhibit LCST behavior in water with measured cloud points of 55.8 and 69.3 °C respectively, Figure 6. Both of these values are

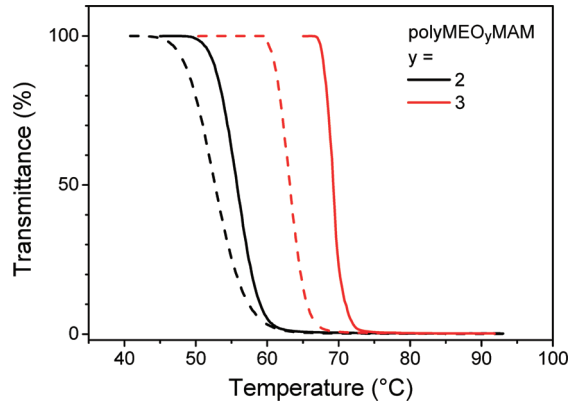

Figure 6. Turbidity curves (dashed curve cooling) for the methacrylamido polymers with 2 and 3 ethylene glycol units showing sharp transmittance decreases and low to moderate hystereses.

higher than identical methacrylic derivatives whose reported cloud points are 26.0 and 52.0 °C[19] again highlighting the enhanced hydrophilicity associated with these (meth)-acrylamido derivatives vs the corresponding (meth)acrylates. Interestingly, the observed phase transition behavior of the methacrylamido vs acrylamido species is opposite to that which has been reported for poly(*N*-isopropylacrylamide) (PNIPAM) vs poly(*N*-isopropylmethacrylamide) species (PNIPMAM).[66,67] In the case of the latter two polymers, PNIPAM has a well-documented LCST of ca. 32.0 °C whereas the methacrylamido species, PNIPMAM, has a reported higher LCST of ~47.0 °C even though a cursory examination of the structure, with the extra hydrophobic methyl group, would suggest that PNIPMAM should undergo a temperature induced phase transition at a temperature below that of PNIPAM. This interesting behavior has been attributed to the methyl group hindering the coil-to-globule transition and subsequent aggregation and phase transition. Such an effect is not, apparently, at play in the case of the ethylene glycol-based methacrylamido polymers described here. In agreement with the enhanced water solubility of amides vs esters and with an increase of OEG side chain length, **polyOEG$_{350}$MAM** and **polyOEG$_{750}$MAM** (Table 1, entries 15, 16) did not show a phase transition between 0 and 93 °C.

The effect of (co)polymer concentration on the cloud point was determined for two samples: **poly[(MEO$_2$)$_{76}$-*co*-Hex$_{24}$]AM** and **polyMEO$_2$MAM** which have cloud points of 26.0 and 55.8 °C respectively at the concentration of 5.0 g/L examined previously. The (co)polymers were also examined at concentrations of 0.5, 1.0, 2.0, and 10.0 g/L, Figure 7. Consider first the concentration effect on **poly[(MEO$_2$)$_{76}$-*co*-Hex$_{24}$]AM**. At the lowest concentration evaluated, 0.5 g/L, this amphiphilic copolymer exhibits a measured cloud point of 37.2 °C. Doubling this concentration results in a decrease of the cloud point of nearly seven degrees to 30.4 °C while increasing the concentration further to 2.0 g/L results in a further, but less pronounced decrease to 26.7 °C. Beyond this concentration

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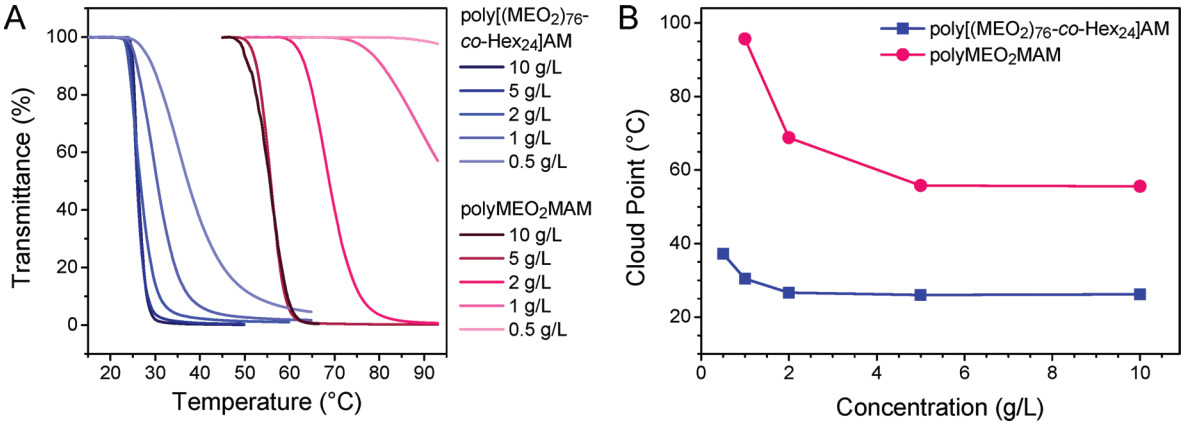

Figure 7. Influence of (co)polymer concentration on the cloud point for an acrylamido copolymer (blue curves) and a methacrylamido polymer (red curves). (A) Turbidity curves. (B) Plot of cloud point versus concentration showing the stronger increase of phase separation temperature with decreasing temperature for the methacrylamido derivative.

there appears to be little, if any, concentration dependence on the LCST with phase transitions occurring at 26.0 and 26.2 °C at concentrations of 5.0 and 10.0 g/L. The concentration dependence of the cloud point for **polyMEO$_2$MAM** is significantly more pronounced. Recall that the cloud point at a concentration of 5.0 g/L for this homopolymer was determined to be 55.8 °C. A similar value of 55.6 °C was found for a 10.0 g/L solution. At 2.0 g/L, however, the cloud point jumps by 13 to 68.8 °C, while at the a concentration of 1.0 g/L the cloud point jumps an additional ~30 to 95.7 °C (determined by extrapolation of the measured cloud point curve to 50% transmittance). Below this concentration the polymer was soluble over the entire observed temperature range. These results indicate that the acrylamido copolymer would be the better choice for applications where a larger range of, essentially, concentration independent responsiveness is desired.

Urea is a well-documented H-bond breaking molecule that can have a dramatic effect upon the solution behavior of (co)polymers in aqueous media.[68−70] For example, Sagle et al.[68] noted that the LCST of PNIPAM gradually decreases from ~32.0 to ca. 23.0 °C upon increasing the urea concentration to 7.0 M; an observation rationalized in terms of urea facilitating the hydrophobic collapse via intramolecular H-bonding to the PNIPAM amide residues. Similarly, the addition of urea can induce insoluble-to-soluble transitions upon heating (UCST-type behavior) whereby urea disrupts the inter- or intramolecular H-bonding interactions responsible for initial insolubility.[70] The effect of added urea upon the aqueous phase transitions of **polyMEO$_2$MAM** and **poly[(MEO$_2$)$_{85}$-*co*-Hex$_{15}$]** was examined with the expectation that these (meth)acrylamido-PEG species would behave in a fashion similar to that reported for PNIPAM, i.e., urea addition would result in a decrease in the LCST. As described above, **polyMEO$_2$MAM** has a measured cloud point, in pure water, of 55.8 °C at 5.0 g/L. At a urea concentration of 26.7 mM, corresponding to one molecule of urea per repeat unit, a small *increase* in the LCST is observed with the phase transition occurring at 56.5 °C. Increasing the concentration to 0.5 M urea results in a more significant increase in the LCST to 63.1 °C and finally at concentrations ≥2.0 M the LCST behavior completely vanishes and the polymer remains soluble up to temperatures in excess of 93.0 °C. This would suggest that inter- and intramolecular H-bonding associated with the amide residues is a very important factor governing the phase behavior of these materials and has a pronounced effect on the LCST. Disruption of these H-bonding associations raises the LCST by eliminating an important physical pathway by which such polymers can self-aggregate and undergo the coil-to-globule phase transition. A similar increase in the LCST was also observed for **poly[(MEO$_2$)$_{85}$-*co*-Hex$_{15}$]** upon addition of 0.5 M urea (46.7 to 48.8 °C), Figure S6 in Supporting Information.

Solution Properties in Alcohols. In addition to an evaluation of the LCST behavior in aqueous media of the (meth)acrylamido PEG derivatives their UCST behavior in 2-propanol and 1-octanol was also investigated at a concentration of 5.0 g/L. The experimentally determined UCST values are given in Table 2 and are plotted in Figure 8A. Turbidity curves measured in 2-propanol are shown in Figure 8B, curves measured in 1-octanol may be found the Supporting Information, Figure S7.

Table 2. Cloud Points (50% Transmittance) Found for (Meth)acrylamido Homopolymers in 2-Propanol and 1-Octanol

		cloud point 2-propanol (5.0 g/L)[a]		cloud point 1-octanol (5.0 g/L)[a]	
entry	polymer	cooling (°C)	heating (°C)	cooling (°C)	heating (°C)
1	**polyMEO$_2$AM**	s[b]	s	s	s
2	**polyMEO$_3$AM**	s	s	s	s
3	**polyOEG$_{350}$AM**	s	s	s	s
4	**polyOEG$_{750}$AM**	9.3	16.0	7.5	18.9
5	**pOEG$_{5k}$AM**	30.2	38.5	25.5	45.5
6	**polyMEO$_2$MAM**	s	s	s	s
7	**polyMEO$_3$MAM**	n.d.	−3[c]	n.d.	2[c]
8	**polyOEG$_{350}$MAM**	4.4	8.1	4.0	8.8
9	**polyOEG$_{750}$MAM**	13.9	17.4	9.0	18.6
10	**PEG 6k**	15.8	41.5	16.5	49.2
11	**polyMEO$_{4/5}$MA**[d]	18.7[e]			

[a]Cloud points depend on concentration, molecular weight and polymer end groups. [b]Measurements in alcohols were carried out between 0 and 70.0 °C. [c]Determined visually with a thermometer. [d][Poly(ethylene glycol) monomethyl ether methacrylate], $M_{monomer}$ = 300 g/mol. [e]Found for a 10.3 kg/mol sample at 5.0 g/L, value taken from reference[12]

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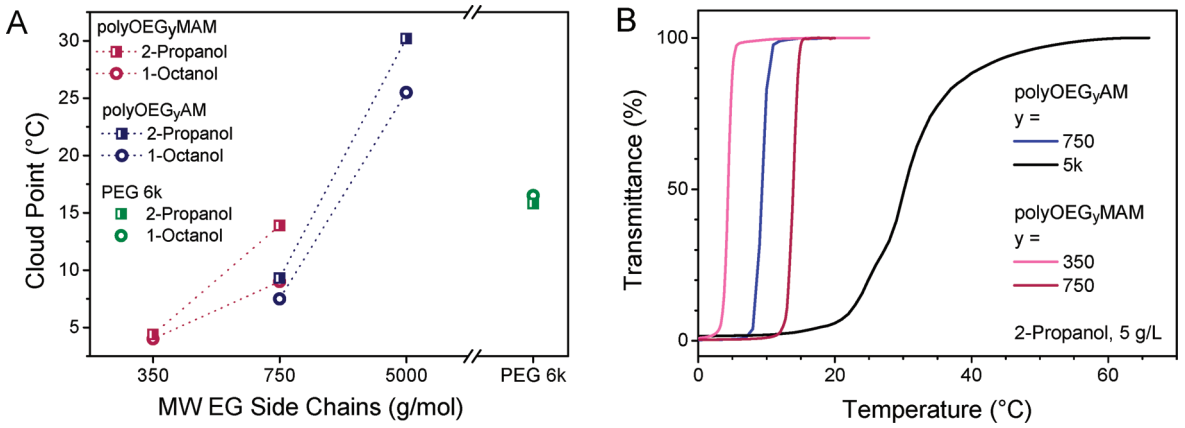

Figure 8. UCST-type behavior of OEG amide homopolymers in alcohols. (A) Cloud point values of polyacrylamides (blue), polymethacrylamides (purple), and reference PEG 6k in 2-propanol (squares) and 1-octanol (circles) in dependence of OEG side chain length (dotted lines are added to guide the eye). (B) Cooling curves of homopolymers in 2-propanol.

Several important observations were made. First, both diethylene glycol (meth)acrylamido polymers **polyMEO$_2$AM** and **polyMEO$_2$MAM** exhibit high solubility in both 2-propanol and 1-octanol with no clouding occurring when kept at −25.0 °C for several days (Table 2, entries 1 and 6). For longer EG side chains, the acrylamido derivatives showed a better solubility in both tested alcohols compared to the methacrylamido versions, very similar to the observations made on aqueous solutions. Whereas **polyMEO$_3$MAM** and **polyOEG$_{350}$MAM** (Table 2, entries 7 and 8) were found to cloud in 2-propanol at low temperatures (below −3.0 °C and at 4.4 °C, respectively), the solutions of the corresponding acrylamido polymers **polyMEO$_3$AM** and **polyOEG$_{350}$AM** (Table 2, entries 2 and 3) in both alcohols remained transparent down to −25.0 °C. In the same fashion, but significantly less pronounced, the cloud points of **polyOEG$_{750}$MAM** (Table 2, entry 9, purple symbols in Figure 8A) were found to be slightly higher (4.6 °C higher in 2-propanol, 1.5 °C higher in 1-octanol) than those of **polyOEG$_{750}$AM** (Table 2, entry 4, blue symbols in Figure 8A) indicating a decreased solubility. Second, with an increasing length of the pendant EG chains the UCST-type cloud point increases, as indicated by the dotted lines in Figure 8A. For example, in 2-propanol, the phase separation of **polyOEG$_{750}$MAM** (13.9 °C) occurred at a temperature 9.5 °C higher than that of **polyOEG$_{350}$MAM** (4.4 °C) (dotted purple lines in Figure 8A). In order to evaluate whether this was also true for acrylamido polymers and to determine the properties of a graft-copolymer with much longer PEG side chains, **polyPFPA** was reacted with PEG 5000 amine. As with the lower molecular weight amines, ^{19}F NMR of the reaction mixture indicated a complete cleavage of the activated esters. However, SEC analysis revealed a bimodal distribution with a (PS standard) molecular weight of 177.4 kg/mol and a PDI of 1.74 (Table 1, entry 5, Figure S4 in Supporting Information). This very broad distribution may be due to an incomplete conversion involving hydrolysis reactions and suggesting that this sterically challenging graft reaction poses a limitation to the PFP ester approach. It is also plausible that the PEG 5000 amine contained some diamine impurities that caused cross-linking. In spite of its broad molecular weight distribution, **polyOEG$_{5K}$AM** represented a significantly larger PEG amide analogue. As anticipated, it was found to have increased cloud points (i.e., decreased solubility) in both alcohols compared to the acrylamido polymer with shorter OEG side chains, with transitions occurring at 30.2 °C (2-propanol) and 25.5 °C (1-octanol), respectively (Table 2, entry 5, dotted blue lines in Figure 8A). It was, however, noted that the increase of transition temperature with increasing OEG side chain length also caused an increase in the hysteresis. Heating and cooling transition temperatures highlighting the hysteresis are plotted in Figure S8A, B in the Supporting Information. This effect was especially pronounced in 1-octanol, where the temperature difference between cooling and heating cycles was found to increase from 4.8 to 9.6 °C when going from **polyOEG$_{350}$MAM** to **polyOEG$_{750}$MAM** and from 11.4 to 20.0 °C when increasing the PEG chain length from **polyOEG$_{750}$AM** to **polyOEG$_{5K}$AM**. For comparison, a standard PEG 6K, with OH end groups, was measured in 2-propanol and 1-octanol giving cloud points of 15.8 and 16.5 °C, respectively, values in between those of the acrylamido OEG$_{750}$ and OEG$_{5K}$ graft polymers (Table 2, entry 10). The hystereses of PEG 6K in 2-propanol and 1-octanol were found to be very large with 25.7 and 32.7 °C, respectively, suggesting that in terms of retarded redissolution characteristics, the OEG amide polymers approach the behavior of pure PEG with an increasing OEG side chain length. Similar to the case in aqueous solutions, the amide species exhibited a better solubility than the corresponding ester analogues. As a reference, the cloud point of the polymethacrylate **polyMEO$_{4/5}$MA** in 2-propanol was found to be 18.7 °C[12] which is higher than the measured transition temperatures even for the methacrylamido polymers with longer OEG side chains, **polyOEG$_{350}$MAM** (4.4 °C) and **polyOEG$_{750}$MAM** (13.9 °C). A surprising observation was the very little difference between 2-propanol and 1-octanol, with all transition temperatures (except PEG 6K) in 2-propanol being somewhat higher than those in 1-octanol. This is in complete contrast to the behavior of the methacrylate derivatives for which a transition temperature increase of roughly 5.0 °C per additional methylene group in *n*-alcohols was found.[12] It therefore seems that the ester group plays an important role in the unfavorable interactions between an OEG-based polymer and the alcoholic solvent, that cause the polymer to lose solvation during the chain collapse. The amide group on the other hand seems to be capable of forming hydrogen bonds of similar thermal stability independent of the chain length of the alcohol. Upon exchanging the ester for an amide group, there is no decreased solubility with an increased alcohol chain length. The amide analogues therefore present an interesting

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class of materials with predictable thermal properties independent of alcohol polarity that can range from hydrophilic (2-propanol, miscible with water in all proportions) to hydrophobic (1-octanol, essentially insoluble in water).

Biocompatibility and Bioconjugation. As noted earlier, besides the interesting phase behavior exhibited by (meth)-acrylic PEG analogues, and also demonstrated herein for (meth)acrylamido species, PEG and its derivatives have attracted significant attention in the biomaterials arena due to the nontoxic, antifouling and so-called 'stealth' properties exhibited by such materials. To demonstrate the potential of these (meth)acrylamido (co)polymers in biomedical applications the cytotoxicity of two samples, **polyMEO$_2$AM** and **polyMEO$_3$AM**, were tested on MRC5 fibroblast cells at concentrations ranging from 0.005 to 10.0 g/L. These two samples were chosen since both are completely soluble in aqueous media up to temperatures in excess of 93.0 °C, in contrast to the acrylic analogues, and highlights how the (meth)acrylamido species may offer advantages in biomedical applications over the (meth)acrylic species by virtue of both enhanced solubility (higher or absent LCSTs) and significantly improved hydrolytic stability of the amide vs ester functional groups. Cell viability was determined via a fluorescence method by which living, metabolically active, MRC5 cells convert a redox dye (blue resazurin) into a fluorescent species (red resorufin). Thus, measuring the fluorescence affords a straightforward method for determining cell concentration and thus, indirectly, the cytotoxicity of the acrylamido-PEG polymers. Figure 9 shows a plot of cell viability vs polymer concentration after incubation of the MRC5 cells in the presence of **polyMEO$_2$AM** and **polyMEO$_3$AM** for 72 h. In both instances these acrylamido-based PEG analogues were found to be noncytotoxic with measured cell viability at ≥85% after 3 days even at high concentrations. These results confirm that these acrylamido-PEG derivatives exhibit desirable "biocompatible" properties which when taken in conjunction with the enhanced solubility and hydrolytic stability (vs the (meth)acrylic species) suggests that these materials may find potential application in areas not accessible to the ester-analogues.

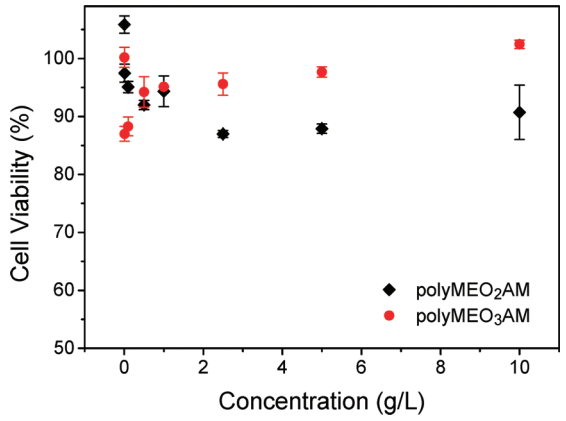

Figure 9. Cell viability after incubation of MRC5 cells with varying concentrations of acrylamido PEG polymers for 72 h.

As discussed above, the aqueous phase behavior of the acrylamido-based PEG analogues can be tuned via the incorporation of a hydrophobic comonomer. In addition to statistical copolymers containing hexylamide residues, and to demonstrate the potential of the route described herein for preparing biocompatible copolymers containing biorelevant molecules, the precursor **polyPFPA** homopolymer was treated with a mixture of di(ethylene glycol) methyl ether amine and thyroxine (T_4), Table 1 entries 10 and 11. T_4 is a prohormone serving as a precursor to triiodothyronine (T_3) secreted by the thyroid gland and is involved in controlling the rate of metabolic processes and also influences physical development.[71,72] The relevant region of T_4, where recognition and metabolic conversion into T_3 takes place, is the outer diiodophenyl ring. T_4 has been shown to selectively recognize and bind its transport protein transthyretin even when the amino group at the opposite end has been functionalized.[11,73] The synthesis was accomplished by employing a mixture of di(ethylene glycol) methyl ether amine and T_4, the incorporation ratio was verified and quantified by ^{1}H NMR spectroscopy, which showed the distinctive broad aromatic signals at 7.81 and 7.11 ppm originating from the incorporated T_4 groups. The activated esters were quantitatively cleaved, as determined by ^{19}F NMR spectroscopy, and ^{1}H NMR of the product indicated a complete presence of MEO_2 + T_4 groups in both cases (Figure S9 in Supporting Information). SEC analyses gave unimodal narrow distributions with the apparent molecular weight increasing with increasing T_4 contents which can be attributed to a conformational influence of the bulky T_4 side groups (Figure S10 in Supporting Information). Two copolymers, **poly**[(**MEO$_2$**)$_{91}$-*co*-**T4$_9$**]**AM** and **poly**[(**MEO$_2$**)$_{87}$-*co*-**T4$_{13}$**]**AM** with 9 and 13 mol % of T_4 units respectively were prepared. This demonstrates that the PFP precursors provide a versatile platform for the preparation of biofunctionalized PEG analogues. In particular, in light of specific binding of proteins to main chain bound targets, the short di(ethylene glycol) amide side chains would be at an advantage over longer, sterically more challenging, ester-bound OEG chains that would be necessary to provide a similar thermally independent water solubility. Both copolymers with 9 and 13% of T_4 were found to be totally soluble in water up to 93 °C, again highlighting the enhanced solubility provided by the OEG amides also in the presence of the biological hydrophobic comonomer.

CONCLUSIONS

Acrylamido and methacrylamido (co)polymers with ethylene glycol (EG) side chains of varying lengths were prepared from pentafluorophenyl activated ester precursor polymers with the respective ethylene glycol amines. Quantitative formation of EG amides, as well as of amide copolymers was found for the acrylic derivatives with as low as 1.2 equiv of amines per activated ester. For the methacrylate polymers a larger excess of amines was necessary to minimize the formation of acid groups. The PEG amide analogues showed an enhanced solubility in water and alcohols compared to the more commonly used ester derivatives which is attributed to the H-bond donor/acceptor properties of the amide linkage. All acrylamido species showed a better solubility (higher LCST transitions in water, lower UCST transitions in alcohols) than the corresponding methacrylamido polymers, and in contrast to the PNIPAM/PNIPMAM pair in water. Of the examined homopolymers, only the polymethacrylamides with MEO_2 or MEO_3 side groups showed LCST behavior in water, however with an unusually strong concentration dependence of the cloud point, as determined for **polyMEO$_2$MAM**. The acrylamido polymers can be rendered thermosensitive by the incorporation of hydrophobic comonomers such as *n*-hexylamide via the

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concurrent reaction of the **PFPA** precursor with a mixture of hexylamine and diethylene glycol methyl ether amine. The reactivity of hexylamine and diethylene glycol methyl ether amine toward PFP esters was found to be very similar. The cloud points of the obtained copolymers varied in a monotonically decreasing fashion with the incorporated hexylamides, providing a simple synthetic method to tune the thermal response of a library of copolymers with essentially the same degree of polymerization. An acrylamido derivative carrying stronger hydrophobic oligopropylene glycol side chains was also found to be thermally responsive with a cloud point around 22.0 °C which also exhibited a very strong endothermic transition, as determined by DSC, compared to a MEO_2/hexylacrylamide copolymer, which was attributed to a loss of hydration along the entire OPG side chains. An important finding of the study of thermal behavior in alcohols was that the strong dependence of the UCST-type transition on the alcohol chain length which was found for the ester derivatives is lost when the ester groups are exchanged for amides. With an increasing length of EG side chains, the solubility in water increased and the solubility in alcohols decreased, however, in the latter case, it was also accompanied by a strong increase of hysteresis which was also found for a linear PEG sample. **polyMEO$_2$AM** and **polyMEO$_3$AM** were nontoxic to MRC5 fibroblast cells at concentrations up to 10.0 g/L and the acrylic activated ester backbone provided a versatile platform for the incorporation of the hydrophobic prohormone thyroxine while the MEO_2 comonomers maintained an excellent solubility. The thorough investigation of the synthesis and thermal solution properties of this class of PEG analogues has provided a valuable insight into the activated ester precursor route and the significant roles comonomers, backbone methyl groups, and amide versus ester linkages play for the properties of these biocompatible materials.

■ ASSOCIATED CONTENT

Supporting Information

Representative ^{19}F NMR spectra, ^{1}H NMR spectra of hexylamide copolymers, list of feed ratios and obtained ratio for hexylamide copolymers, SEC traces of hexylamide copolymers and of **polyOPG$_{600}$AM** and **polyOEG$_{5k}$AM**, turbidity measurements of DSC samples, turbidity measurements of samples containing urea, turbidity curves of (meth)acrylamido homopolymers in 1-octanol, plot of UCST cloud points found in 2-propanol and 1-octanol showing hysteresis values, ^{1}H NMR spectra and SEC chromatograms of copolymers containing thyroxine. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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