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Thermo-Responsive Allyl-Functionalized 2-(2-Methoxyethoxy)ethyl Methacrylate-Based Polymers as Versatile Precursors for Smart Polymer Conjugates and Conetworks

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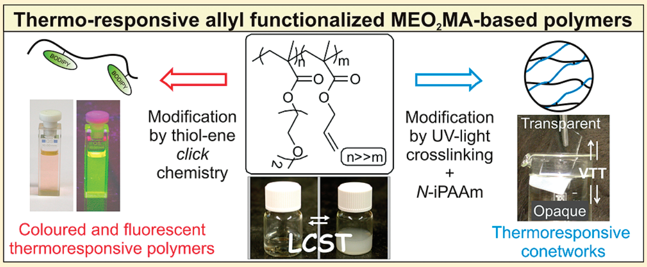
S *Supporting Information*

ABSTRACT: Several thermo-responsive polymers functionalized with allyl pendant groups were synthesized by copolymerizing 2-(2-methoxyethoxy)ethyl methacrylate (MEO_2MA) and a low ratio of allyl methacrylate (AMA) via atom transfer radical polymerization (ATRP). The thermo-responsive behavior in aqueous solution of these P(MEO_2MA-*co*-AMA) copolymers was analyzed, observing that the cloud point temperature (T_{cp}) decreased with the increment of the hydrophobic AMA units in the copolymer. More interestingly, these polymers bear allyl reactive groups, which can be used to form new thermo-responsive polymers. To demonstrate this synthetic versatility, on the one hand, these polymers were converted into fluorescent polymers by a thiol−ene *click* reaction between the allyl group and a thiol-functionalized BODIPY dye. On the other hand, they were also employed as cross-linkers for the UV initiated free radical polymerization of *N*-isopropylacrylamide (*N*-iPAAm) to form thermo-responsive conetworks structures, which show high swelling capacity and a sharp thermal phase transition in water solution.



INTRODUCTION

Nowadays, polymers based on oligo(ethylene glycol) (OEG) methyl ether methacrylates have revealed as a promising family of thermo-sensitive polymers.[1−13] Among these systems, the poly(2-(2-methoxyethoxy)ethyl methacrylate) [P(MEO_2MA)], has become one of the most relevant since it exhibits a lower critical solution temperature (LCST) at around 26 °C.[1] This value is close to the room temperature but, moreover, it can be easily modulated by copolymerization of MEO_2MA with longer OEG methacrylates; that is, without significant change in the chemical structure.[7−9,12,13] On the other hand, MEO_2MA-based polymers combine both a thermo-responsive behavior, generally comparable to poly(*N*-isopropylacrylamide) [P(*N*-iPAAm)],[14−16] and some properties of poly(ethylene glycol) (i.e., nontoxicity or anti-immunogenicity). In addition, they can be successfully obtained from controlled/living polymerizations techniques, such as atom transfer radical polymerization (ATRP),[7−9,17,18] that allows a control over molecular weight and molecular weight distribution.

Thermo-responsive conjugates or thermo-responsive superior topologies derived from this component MEO_2MA may be formed introducing reactive groups along the P(MEO_2MA) polymer chains. A versatile functionalization may be achieved in a very simple way by copolymerizing MEO_2MA with allyl methacrylate (AMA). AMA has a conjugated methacrylic and an unconjugated allylic group. The higher reactivity of the former group makes this monomer very interesting for the formation of functional linear (co)polymers with allyl pendant groups.[19−25] Thus, AMA-based polymers offer the possibility to undergo cross-linking reactions (e.g., by irradiation or thermal treatment)[20] or to obtain new polymeric structures by chemical modifications, such as addition, oxidation, etc.[22,26] Taking into account these previous investigations, in the present paper a series of P(MEO_2MA-*co*-AMA) copolymers were synthesized by ATRP, as it is shown in Scheme 1.

Thus, the new thermo-responsive polymers would exhibit controlled molecular weight and tuned LCST but, the most remarkable feature is that they will show allyl functional pendant groups through the thermo-responsive polymeric chains. Therefore, they would be able to be easily modified for obtaining new thermo-responsive materials. Two different approaches were made to prove that. The first one was to attach, covalently and quantitatively, a thiol-functionalized 4,4-difluoro-4-bora-3a,4a-diaza-*s*-indacene (BODIPY) dye, generated *in situ* from a thioester precursor, to each allylic position by thiol−ene *click* chemistry[27] (see Schemes 2 and 3). Presumably, this strategy would allow forming fluorescent thermometers in water, as it was previously shown for similar systems.[28] These systems have received significant attention for the development of sensory materials.[29,30] On the other side, the attachment of BODIPY dye

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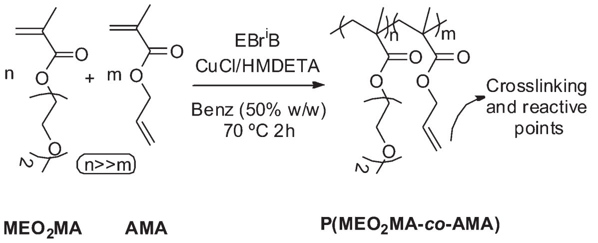
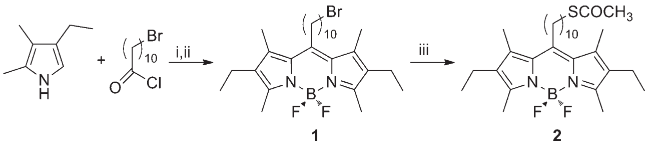
Scheme 1. Synthetic Conditions of the P(MEO$_2$MA-*co*-AMA) Copolymers

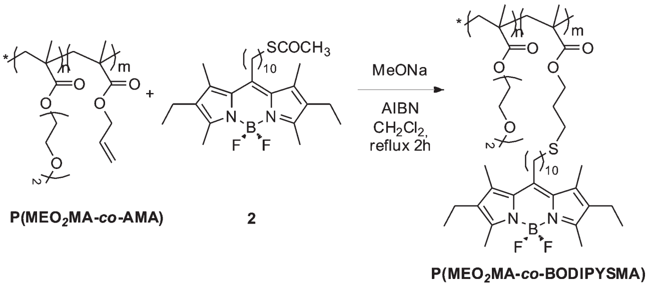


Scheme 2. Synthetic Conditions of the Thioester−BODIPY Dye

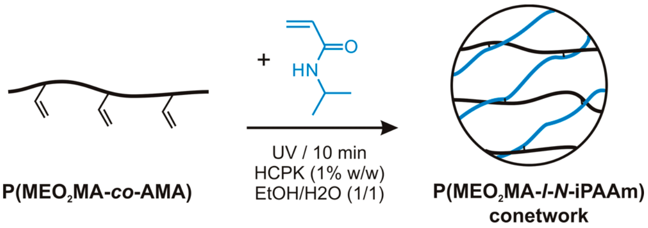


i) CH$_2$Cl$_2$, reflux ; ii) Et$_3$N, BF$_3$OEt$_2$, CH$_2$Cl$_2$; iii) KSCOCH$_3$, acetone, reflux

Scheme 3. Synthetic Conditions of the P(MEO$_2$MA-*co*-BODIPYSMA) Copolymers



Scheme 4. Synthetic Conditions of the P(MEO$_2$MA-*l*-*N*-iPAAm) Conetworks



could be used to quantify the proportion of allyl groups by absorbance measurements.

The second procedure was to use the P(MEO$_2$MA-*co*-AMA) copolymers as "macro-cross-linkers" for the UV-free radical polymerization of *N*-iPAAm leading to conetwork topologies (Scheme 4). We have shown recently, for poly(methyl methacrylate-*linked*-*N*-iPAAm) conetworks, P(MMA-*l*-*N*-iPAAm), that this particular monomeric distribution play an important effect over the final properties.[31] In this case, the result after the polymerization is a conetwork of P(MEO$_2$MA) and P(*N*-iPAAm), two different thermo-responsive chains, linked by cross-linking points derived from the AMA units. Therefore, these new structures, which combine both thermo-responsive components in a unique conetwork topology, may lead to systems with transition temperatures at around body temperature and specific swelling properties. Thus, they could be interesting for biomedical applications, such as drug delivery,[32] tissue engineering,[33] and other biorelated applications.[34−38]

■ EXPERIMENTAL PART

Materials. The monomers 2-(2-methoxyethoxy)ethyl methacrylate (MEO$_2$MA, Aldrich 95%) and allyl methacrylate (AMA, Fluka 98%) were purified by passing them by neutral alumina column to remove the antioxidant inhibitor. The ligand 1,1,4,7,10,10-hexamethyltriethylenetetramine (HMDETA, Aldrich, 97%) was purified by vacuum distillation and the initiator ethyl 2-bromoisobutyrate (EBr^{i}B, Aldrich, 99%), the catalyst CuCl (Aldrich, 99.99%) and the solvents benzonitrile (Fluka, 98%), *n*-hexane (Panreac, 98%), and chloroform (Sds, 99.9%) were used as received. For the BODIPY-thiol synthesis, model reaction, and hydrolysis/thiol−ene reaction the precursors 3-ethyl-2,4-dimethylpyrrol (Aldrich, 97%) and triethylamine (Fluka, 99%) were purified by distillation, and 11-bromoundecanoic acid (Aldrich, 99%), allyl acetate (98%, Fluka), thionyl chloride (Aldrich, 99%), boron trifluoride diethyl etherate (Fluka), 2,2′-azobis isobutyronitrile (AIBN, Across, 98%), potassium thioacetate (98%, Aldrich), and sodium methoxide (Aldrich, 95%) were employed as received. For the conetworks formation the monomer *N*-iPAAm (Acros Organics 99%) was purified by recrystallization from *n*-hexane/toluene mixture (90/10 v/v) and the UV-initiator 1-hydroxycyclohexyl phenyl ketone (HCPK Aldrich) was used as received. Water for all reactions, solutions for swelling experiments and hydrogel purification was Milli.Q from water purification facility (Millipore Milli-U10). Phosphate buffer solutions (PBS) were prepared employing sodium dihydrogen phosphate anhydrous (Fluka ≥99%), disodium hydrogen phosphate (Panreac ≥98%) and sodium chloride (Panreac ≥99.5%) to keep constant and controlled the ionic strength.

Synthesis of P(MEO$_2$MA-*co*-AMA) by ATRP. ATRP copolymerizations (see Scheme 1) were carried out in benzonitrile solution (50% w/w) with a constant monomer/initiator/catalyst ratio of 200/1/1. A typical procedure for the synthesis of these statistical copolymers is described below for sample **P1** ($f_{MEO2MA} = 0.99$). HMDETA (0.031 g, 0.133 mmol), degassed monomers MEO$_2$MA (4.966 g, 26.4 mmol) and AMA (0.034 g, 0.270 mmol), and the solvent benzonitrile (5 g) (previously bubbled with nitrogen for at least 15 min) were all added to a dry Pyrex tube ampule with CuCl (13 mg, 0.133 mmol). After the mixture was degassed by bubbling nitrogen for 20 min, the initiator EBr^{i}B (26 mg, 0.133 mmol) was introduced into the ampule using degassed syringes in order to start the polymerization. The ampules were immediately placed in a thermostatic oil bath at 70 °C, regulated with a precision of ±0.1 °C. To stop the polymerization at a selected time (120 min), the reaction mixture was cooled and quenched with chloroform and then, it was passed through a neutral alumina column to remove the catalyst. The solution was concentrated by rotary-evaporation and the polymer precipitated by adding the solution to a large excess of *n*-hexane. The precipitated products were decanted and dried under high vacuum until a constant weight was reached. Total monomer conversions were measured gravimetrically.

Synthesis of Thioacetic Acid *S*-(10-(2′,6′-Diethyl-1′,3′,5′,7′-tetramethyl-4′,4′-difluoro-4′-bora-3′a,4′a-diaza-*s*-indacen-8′-yl)-decyl) Ester (2) . The synthetic route of this molecule is shown in Scheme 2. First, the bromine-end BODIPY precursor dye (**1**, 2,6-diethyl-1,3,5,7−8-(10′-bromo-decyl)-tetramethyl-4,4-difluoro-4-bora-3a,4a-diaza-s-indacene) was synthesized according to the procedure previously described for an analogous compound by our research group.[39] Then, it was used as reagent in a second step: **1** (215 mg, 0.41 mmol) and potassium thioacetate (52 mg, 0.45 mmol) were stirred in 25 mL of acetone for 2 h at reflux. After vacuum evaporation of the solvent, the orange solid was dissolved in dichloromethane, washed with water several times, and dried over sodium sulfate. The resulting thioacetate was obtained as a red solid and was used without purification. Yield: 99%.^{1}H NMR (300 MHz, $CDCl_3$): δ (ppm) 2.95 (m, 2 H, 1′-H), 2.84 (t, 2 H, J = 7.5 Hz, 10′-H), 2.47 (s, 6H, 2 × CH_3−Ar), 2.38 (q, 4 H, J = 7.5 Hz, CH_2CH_3), 2.31 (s, 3 H, CH_3COS), 2.30 (s, 6 H, 2 × CH_3−Ar), 2.26 (m, 2 H, 9′-H), 1.59−1.49 (m, 14 H, 2′-H to 8′-H), 1.03 (t, J = 7.5 Hz, 6 H, CH_3CH_2). ^{13}C NMR (75 MHz, $CDCl_3$): δ (ppm) 195.89 (COS), 151.86 (C3/C5), 144.92 (C7a/C8a), 135.55 (C1/C7), 132.40 (C2/C6), 130.85 (C8), 34.29 (C1′), 31.74 (C2′), 30.53 (C10′), 30.24, 29.41, 29.31, 29.13, 29.03, 28.70, 28.46 (7 × CH_2), 24.89 (CH_3S), 17.10 (CH_2CH_3), 14.76 (CH_3−Ar), 13.24 (CH_3−Ar), 12.29 (CH_3CH_2). MS (EI): m/z (%): 518 [nominal mass, M^+] (100), 498 (31), 455 (30), 318 (15), 301 (21), 287 (32), 43 (18). ESI−HRMS monoisotopic molecular weight: found M^+ 517.335 (0.02 ppm from calcd mass of $C_{29}H_{45}BF_2N_4O$ S); (M − F^-) 499.3334. IR, ν (cm^{-1}): 1696, 1545, 1479, 1196, 980.

Synthesis of P(MEO$_2$MA-*co*-thioetherBODIPY methacrylate), P(MEO$_2$MA-*co*-BODIPYSMA), by Hydrolysis and Thiol−Ene *Click* Chemistry *in Situ* Reaction. The former allyl-functionalized P(MEO$_2$MA-*co*-AMA) polymers (0.01 mmol), sodium methoxide (1.3 equiv), 2,2′-azobis(2-methylpropionitrile) (AIBN) (1 equiv) and the thioester−BODIPY compound (**2**) (1.3 equiv) were stirred in anhydrous dichloromethane (25 mL) and refluxed overnight. The crude of reaction was twice precipitated into cold *n*-hexane (250 mL) and the solid was filtered and washed with the same solvent. The resulting fluorescent P(MEO$_2$MA-*co*-BODIPYSMA) copolymers were quantitatively obtained as pink solids (see Scheme 3).

Synthesis of P(MEO$_2$MA-*l*-*N*-iPAAm) Conetworks. These hydrogels were synthesized by UV-initiated free-radical cross-linking random polymerization of *N*-iPAAm with the P(MEO$_2$MA-*co*-AMA) precursors (see Scheme 4) employing a [MEO$_2$MA]/[*N*-iPAAm] ratio of 1 in all cases. A monomer/solvent ratio of 0.5 g mL^{-1}, being the solvent a water−ethanol (50% v/v) mixture, was used. HCPK (1% w/w) was employed as UV light-sensitive initiator. The procedure to obtain disk shaped gels was as follows: the mixture solution was cast on glass plates enclosed by a rubber framework-spacer with 1 mm thickness and sealed off with other glass plate in order to avoid air contact during the polymerization. Then, the polymerization took place irradiating the samples with UV light at 365 nm during 10 min. It employed a CL-1000 L ultraviolet cross-linker UVP that shows in its emission spectra from 350 to 400 nm a total light of 2.2 W m^{-2}. This value was determined by a Luzchem SPR-01 spectroradiometer. Afterward, the gel sheets were removed from the glass plate and uniform disks with a diameter of 6 mm were punched out the gel sheet using a stainless steel cork borer. Then, the hydrogel was immersed in freshwater for at least 3 days to remove the unreacted chemicals. During this time the water was replaced several times. After that, the hydrogels were dried at room temperature until constant weight.

Characterization. ^{1}H NMR and ^{13}C NMR spectra were recorded on a Bruker 300 MHz spectrometer in $CDCl_3$ at room temperature. Chemical shifts were assigned using the solvent signal as internal standard. Fourier transform infrared (FTIR) spectra were recorded on a Perkin-Elmer Spectrum One spectrophotometer with the attenuated total reflectance technique (FTIR-ATR) and with a resolution of 4 cm^{-1}. Low mass resolution spectra were recorded in an AutoSpecEQ EI apparatus by electron impact (EI, 70 eV). High resolution mass spectrum (HRMS) was recorded in an Agilent 6520 Q-TOF instrument with an ESI source. The number-average molecular weight (M_n) and molecular weight distribution (MWD) of polymers were determined by SEC with a GPC Perkin-Elmer using DMF containing LiBr (0.1% w/w) as eluent at 70 °C and a flow rate of 0.3 mL min^{-1}. Poly(methyl methacrylate) [P(MMA)] standards were employed for the calibration. The thermal stability was determined from thermogravimetric analysis (TGA) with a TA TGAQ500 (10 °C min^{-1} under 20 mL min^{-1} of dry nitrogen). The temperature at which the weight loss rate is maximum (T_{max}) was determined from the maximum of the peak of the first derivative of the weight lost. The glass transition temperatures (T_g)s were measured by means of differential scanning calorimetry (DSC) using a TA DSC Q100 connected to a cooling system to work at low temperatures (samples were scanned at 20 °C min^{-1} under 20 mL min^{-1} of dry nitrogen from −75 to +30 °C). T_g values, the width of the transition (ΔT) and the specific heat increment (ΔC_p) were determined as it was previously described in the second heating run cycle.[40] The cloud point was studied by determining the optical transmittance at 600 nm as a function of temperature of aqueous solution of the copolymers (1 mg mL^{-1}). The analysis was made using a Cary 3 BIO-Varian UV−visible spectrophotometer. Temperature was raised from 10 to 80 °C at a rate of 1 °C min^{-1}. The cloud point temperature (T_{cp}) was defined as the temperature at the inflection point of the absorbance versus temperature curve. UV−visible absorption and fluorescence spectra were recorded on a Perkin-ElmerLambda-16 and on a Perkin-Elmer LS5OB spectrophotometer, respectively. Volume transition temperatures (VTT) of the hydrogels were calculated in the same way than the T_{cp} from the hydrogels in the swollen state. The chemical composition of the hydrogels was estimated by organic elemental analysis in a Heraeus CHN-O Rapid Analysis. Equilibrium swelling values (Q_∞) were determined gravimetrically. Dried disks were left to swell in PBS or distilled water at different experimental condition during 24 h. Then, samples were taken out, wiped superficially with blotting paper and weighed. The Q_∞ was calculated in grams of water per grams of dry gel using the following expression:

$$Q_\infty = (m_\infty - m_0)/m_0 = W_\infty/m_0 \qquad (1)$$

where m_∞ is the weight of the swollen gel at equilibrium, m_0 is the weight of the dry polymer gel, and W_∞ is the weight of the solvent uptake at equilibrium.

RESULTS AND DISCUSSION

Synthesis and Characterization of P(MEO$_2$MA-*co*-AMA) Copolymers. Several allyl-functionalized MEO$_2$MA-based polymers, P(MEO$_2$MA-*co*-AMA) copolymers, were synthesized as it is shown in Scheme 1. The experimental conditions for the preparation by ATRP and the characterization of these copolymers are collected in Table 1. These experimental conditions were chosen according to our previous investigations for the ATRP of MEO$_2$MA[28,41] and AMA.[21,25,42] Therefore, polymerizations were carried out in benzonitrile using the halogen exchange technique, to avoid secondary reactions due to the allyl functional group of AMA. It should be noted that, in all cases, a high feed molar ratio of MEO$_2$MA (f_{MEO2MA}) was used to obtain polymers with low proportion of allyl pendant groups. These polymers are mainly constituted by MEO$_2$MA but they present allyl reactive groups along the macromolecular chains, which can be used as cross-linker/reactive points in a later step.

The chemical structure of the obtained copolymers was analyzed by ^{1}H and ^{13}C NMR and FTIR-ATR (see Supporting Information). As an example, Figure 1 shows the ^{1}H NMR spectra, where the signals corresponding to both comonomers are observed. Thus, the experimental molar ratio of MEO$_2$MA in the copolymers (F_{MEO2MA}) were determined from the ratio between the signals corresponding to the allyl protons (signals 6, 7, and 8) and the signals corresponding to the protons of the side chains of MEO$_2$MA units (signals 3, 4, and 5). As it can be observed, the signals associated with the allyl protons increase

Table 1. Experimental Synthetic Conditions and Characterization of P(MEO$_2$MA-*co*-AMA) Copolymers Synthesized by ATRP at 70 °C in Benzonitrile (50% w/w) during 120 min and Catalyzed by CuCl/HMDETA with a Monomer/Initiator (EBr^{i}B)/Catalyst Ratio of 200/1/1

entry	f_{MEO2MA}	F_{MEO2MA}^{a}	convn (%)	$M_{n,cal}$ (g mol^{-1})	$M_{n,SEC}$ (g mol^{-1})	M_w/M_n	$T_{max.}$^{b} (°C)	T_g^{c} (°C)	T_{cp}^{d} (°C) PBS pH 7 (μ = 0.1M)	T_{cp}^{d} (°C) water
P1	0.99	0.992^e	71.4	26 950	36 900	1.18	243	−35.3	23.8	27.0
P2	0.95	0.958, 0.943^e	71.8	26 817	31 100	1.18	240	−30.3	21.3	25.0
P3	0.90	0.915	62.5	23 036	30 980	1.23	296	−33.6	14.9	18.2
P4	0.85	0.857	69.6	25 136	39 528	1.46	295	−35.9	12.0^f	13.6^f

[a] Determined by ^{1}H NMR. [b] Determined by TGA. [c] Determined by DSC. [d] Determined by UV−visible. [e] Determined by absorbance at 524 nm of the BODIPY-modified samples. [f] Not totally soluble at low temperature.

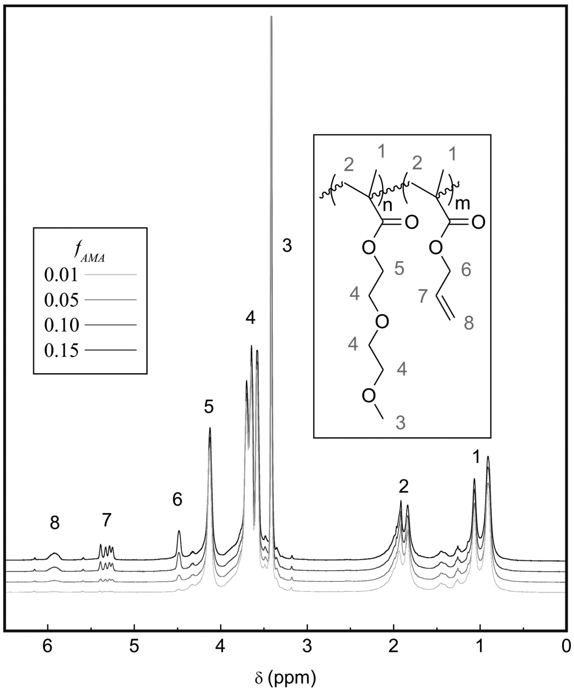


Figure 1. ^{1}H NMR spectra in CDCl$_3$ at 25 °C corresponding to the P(MEO$_2$MA-*co*-AMA) copolymers.

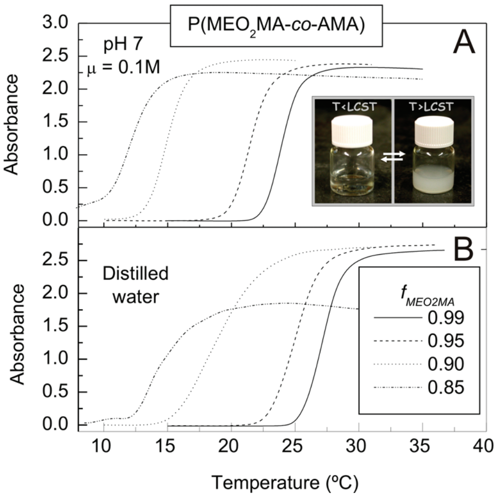


Figure 2. Absorbance at 600 nm versus temperature of solutions of P(MEO$_2$MA-*co*-AMA) copolymers as a function of composition (a) in PBS at pH 7 and (b) in distilled water. Photographs taken at temperature below T_{cp} (left part) and above T_{cp} (right part) of sample **P3** are included.

when increasing the feed molar fraction of AMA. Thus, as it can be seen in Table 1, F_{MEO2MA} is close to f_{MEO2MA} for almost the whole range of copolymer composition, which suggests that, as it could be expected, both methacrylic monomers show similar reactivity coefficients under these experimental conditions. This fact is also supported from the monomer conversions (Table 1), since they do not depend on the feed monomeric composition, being very similar in all cases.

The SEC analysis of the copolymers indicated that the polymerizations were well controlled. The molecular weight distributions were narrow and, therefore, polydispersity indices lower than 1.5 were observed in all cases. However, it seems that these indices slightly increase as higher is the AMA feed composition, observing a small shoulder at high molecular weights in the SEC traces, which could be attributed to secondary reactions caused by the allyl double bound.[19−25] The number-average molecular weights determined by GPC ($M_{n,SEC}$) varied in the same way than those predicted theoretically ($M_{n,cal}$) from the conversion and the monomeric composition. The thermal behavior of these copolymers was investigated by TGA and DSC. The TGA analyses showed a unique decomposition process. It seems that as higher the AMA composition higher is T_{max}. It is important to point out that the T_{max} of MEO$_2$MA and AMA are around 369[41] and 430 °C,[43] respectively. Therefore, the experimental values are in agreement with those expected. On the other hand, copolymers were also analyzed by DSC. Since the polymers are amorphous, the glass transition (T_g) is the unique relevant event detected in the thermograms. The experimental values of T_g are also included in Table 1. In the range of composition investigated, both types of copolymers showed only one T_g near the transition of the P(MEO$_2$MA) parent homopolymers, −35.5 °C,[41] although the T_g of P(AMA) is 54 °C.[40]

The thermal responsiveness of these copolymers in aqueous solution was evaluated by turbidimetry. The analysis is plotted in Figure 2. Samples are completely soluble (transparent solution) at low temperatures. However, when the temperature increases above the T_{cp}, the polymer solution becomes opaque as consequence of the collapse, which depends on the hydrophilic/hydrophobic balance and therefore, on the monomeric composition. Thus, as higher the percentage of the hydrophopic AMA units as lower is the T_{cp}. This fact is reflected in the numeric values of T_{cp} obtained from the inflection points of these curves (Table 1) and also from the low solubility of sample **P4** at low

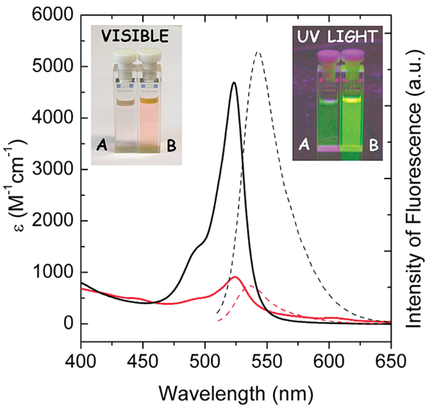


Figure 3. UV−visible absorption spectra in terms of molar absorptivity (solid lines) and fluorescence (dashed lines) of sample P(MEO$_2$MA-*co*-BODIPYSMA) with f_{MEO2MA} = 0.99 (red line, A in inset) and P(MEO$_2$MA-*co*-BODIPYSMA) with f_{MEO2MA} = 0.95 (black line, B in inset) in chloroform (0.1 mM) at room temperature. Photographs taken under visible and UV irradiation are included for both samples.

Table 2. Comonomeric Composition and Aqueous Solution Properties of the P(MEO$_2$MA-*l*-*N*-iPAAm) Conetworks Synthesized with [MEO$_2$MA Units]$_0$/[*N*-iPAAm]$_0$ = 1

entry	P(MEO$_2$MA-*co*-AMA) precursor	AMA units (% w/w)	F_{NiPAAm}[a]	Q_∞ at 5 °C	VTT (°C)
BH1	P2	2.0	0.495	33.1	36.0
BH2	P3	4.4	0.505	8.3	35.0
BH3	P4	7.7	0.499	4.7	38.1
BH4[b]	P4	7.7	0	4.1	24.1

[a] Determined by elemental analysis. [b] Synthesized using MEO$_2$MA instead of *N*-iPAAm.

temperature. Other interesting aspect is that the calculated values depend on the type of water solution. Thus, in distilled water the T_{cp} values are higher than at pH 7 (μ = 0.1M). This difference can be attributed to the effect of the ionic strength.[44] It is well know that, in this kind of cross-linked structures, as higher is this strength in a PBS solution the lower is the T_{cp}.[45]

Synthesis of P(MEO$_2$MA-*co*-thioetherBODIPY methacrylate), P(MEO$_2$MA-*co*-BODIPYSMA), by Hydrolysis and Thiol−Ene *Click in Situ* Reaction. To show the versatility of the allyl pendant groups to react by thiol−ene *click* chemistry and also to quantify these groups in P(MEO$_2$MA-*co*-AMA) copolymers with very low content of AMA (values lower than the detection limit of NMR spectroscopy), a thiol-functionalized BODIPY-based dye was prepared and incorporated to the macromolecular structure using this kind of *click* chemistry.[27] Thus, the thiol-ester-functionalized precursor compound (**2**) was obtained in a two-step reaction using the methodology previously described (Scheme 2).[46] In fact, the thiolated BODIPY, which is the result of the hydrolysis of **2** with sodium methoxide, reacts with the former P(MEO$_2$MA-*co*-AMA) in presence of AIBN and heating in an *in situ* hydrolysis/thiol−ene *click* reaction obtaining the corresponding P(MEO$_2$MA-*co*-BODIPYSMA) copolymers (Scheme 3). An analogous procedure was recently described by our research group but using an allyl-functionalized BODIPY dye and thiol-end-functionalized P(MMA).[47] In addition, a model reaction was done using allyl acetate as model compound. The reaction scheme and the characterization of the formed product are included in the Supporting Information, proving the success of this synthetic methodology to modify allyl groups by *in situ* hydrolysis/thiol−ene *click* chemistry.

The proposed polymer modification was successful. As it can be observed in Figure 3, colored and fluorescent P(MEO$_2$MA-*co*-BODIPYSMA)s were obtained. In fact, the photophysical properties of the chromophore (BODIPY dye) are not affected by the surrounding polymer, showing a maximum absorption wavelength of 524 nm and an emission wavelength of 542 nm (exciting at 490 nm), regardless of content of dye in the polymer (typical values for a BODIPY dye). Thus, the content of allyl groups in P(MEO$_2$MA-*co*-AMA) polymers with lower AMA (**P1** and **P2**) was determined by absorbance measurements taking into account the molar extinction coefficient of the chromophore (ca. 80000 M^{-1} cm^{-1} in chloroform).[28] Therefore, the amount of allyl groups determinated in **P1** and **P2** copolymers (see Table 1) were very close to the feed monomeric compositions. In addition, the cloud point temperatures of samples **P1** and **P2** modified with BODIPY dye were also determined by turbidimetry, obtaining values of 23.8 and 22.5 °C, respectively. As it was expected, the T_{cp} values practically do not change with this chemical modification since the comonomer content is very low and the hydrophobic character of the new BODIPY-monomeric units should be similar to this of AMA monomeric units.

Synthesis and Characterization of P(MEO$_2$MA-*l*-*N*-iPAAm) Conetworks. As it was previously explained, the allyl-functionalized MEO$_2$MA-based copolymers were also used as macrocroslinkers for the preparation of P(MEO$_2$MA-*l*-*N*-iPAAm) conetworks by means of a photopolymerization reaction in the presence of *N*-iPAAm (see Scheme 4). In all cases an equimolar amount of MEO$_2$MA monomeric units and *N*-iPAAm (monomer) was used, while the nominal cross-linking degree is related to the AMA fraction in the precursor. The experimental synthetic conditions and the properties of these novel hydrogels are shown in Table 2. The first interesting result is that no hydrogels were obtained with sample **P1**. We think that the low proportion of AMA units in this sample do not let to obtain a cross-linked structure. For the same reason, as higher the AMA proportion higher is the cross-linking density and therefore, lower equilibrium swelling values at 5 °C were obtained.

The swelling thermo-responsiveness of the conetworks in PBS (pH 7) and distilled water are shown in Figure 4. The three samples **BH1**, **BH2**, and **BH3** exhibit swelling equilibrium values depending on the cross-linking degree but moreover, similar VTT values, which are collected in Table 2. This indicates that the thermal transition is driven by composition since the proportion of both thermo-responsive monomers is constant in the three systems. In Figure 4, it was included for comparative purposes the experimental results obtained for a random distributed P(MEO$_2$MA-*co*-*N*-iPAAm) hydrogel synthesized under similar experimental conditions (photopolymerization and [MEO$_2$MA]$_0$/[*N*-iPAAm]$_0$ = 1) and using a standard cross-linker (0.5%), such as tetraethylene glycol dimethacrylate (TEGMEMA). It showed a less sharp transition compared to the conetworks and a higher VTT (46.1 °C). It seems that the presence of the *N*-iPAAm below its collapse temperature increases the global hydrophilicity in the conetworks and therefore, keeps soluble the MEO$_2$MA chains.

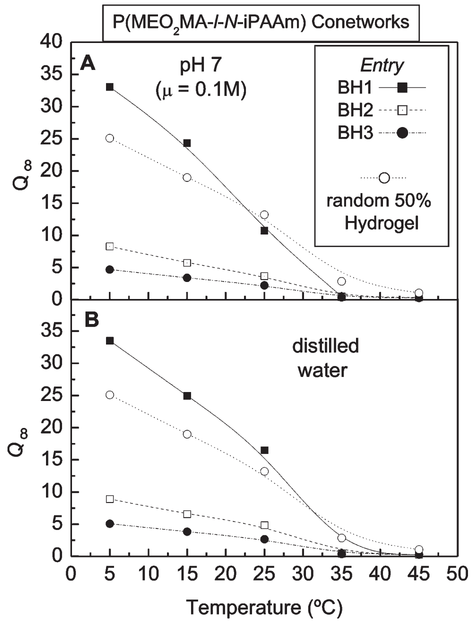


Figure 4. Equilibrium swelling for the P(MEO$_2$MA-l-*N*-iPAAm) conetworks hydrogels and for a randomly distributed P(MEO$_2$MA-*co*-*N*-iPAAm) hydrogel as a function of temperature (a) in PBS at pH 7 and (b) in distilled water.

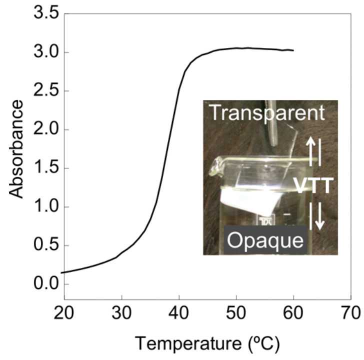


Figure 5. Absorbance at 600 nm versus temperature of solutions of P(MEO$_2$MA-l-*N*-iPAAm) conetwork (sample **BH3**). Photograph shows that the hydrogel becomes opaque very fast in a hot water solution.

On increasing temperature beyond the LCST of the P(*N*-iPAAm) chains, their collapse produces an abrupt increasing of the hydrophobic balance and the cooperative collapse of the MEO$_2$MA network. This behavior results in a sharp VTT transition. Moreover, as it can be observed in Figure 5 for sample **BH3**, the transition from transparent to opaque with increasing temperature takes place very sharply. In this figure it is also shown a picture illustrating how a hydrogel in the swollen state becomes quickly opaque when it was introduced in hot-water. Other aspect is that at low temperature the absorbance is not negligible, which may be related to the formation of microphase separated MEO$_2$MA domains. It is also important to point out that the VTT values of the conetworks samples are very close to the body temperature, which means that these hydrogels could be interesting for biomedical applications.

Finally and in order to analyze the monomeric composition effect, a new network was synthesized in the same way that sample **BH3** but using MEO$_2$MA in stead of *N*-iPAAm (sample **BH4**). As was expected, the equilibrium swelling value is almost the same than that one found for sample **BH3** whereas the VTT decreases (Table 2). These experimental results support the hypothesis that the equilibrium swelling mainly depends on the cross-linking degree, but the temperature of the transition is clearly function of the monomeric composition, and therefore, it may be modulated by varying the feed *N*-iPAAm composition.

■ CONCLUSIONS

Several allyl-functionalized MEO$_2$MA-based copolymers were synthesized in a controlled way by ATRP. The analysis of their thermo-responsiveness in aqueous solutions revealed a clear dependence of the T_{cp} values on the monomeric composition. However, the main interest of these copolymers lies in the unreacted double bonds decorating the thermo-sensitive polymer, which offer a versatile route for further modifications. Thus, a thioester BODIPY dye was quantitatively incorporated to each pendant allylic group by *in situ* hydrolysis/thiol−ene *click* reaction. This procedure establishes the formation of fluorescent thermo-responsive polymers in water but also, let us to determinate the real content of allylic groups in the MEO$_2$MA-based polymers precursors by absorbance measurements. In addition and also to show the versatility of the synthesized P(MEO$_2$MA-*co*-AMA) copolymers, they were successfully used as precursors for the UV-initiated free radical polymerization of *N*-iPAAm to obtain P(MEO$_2$MA-*l*-*N*-iPAAm) conetworks, which show sharp volume thermal transitions (VTT) at around body temperature. The methodology proposed may be easily extended to the preparation of other conjugates keeping the thermo-sensitivity of the MEO$_2$MA-based chains.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information. Figure S1 (^{13}C NMR spectrum of P(MEO$_2$MA-*co*-AMA) (sample **P4**)), Figure S2 (FTIR spectrum of P(MEO$_2$MA-*co*-AMA) (sample **P4**)), and model reaction of allyl acetate with **2** by *in situ* hydrolysis/thiol−ene reaction. This material is available free of charge via the Internet at http://pubs.acs.org.

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■ REFERENCES

(1) Han, S.; Hagiwara, M.; Ishizone, T. *Macromolecules* **2003**, *36*, 8312–8319.

(2) Aoshima, S.; Oda, H.; Kobayashi, E. *J. Polym. Sci., Polym. Chem.* **1992**, *30*, 2407–2413.

(3) Aoshima, S.; Sugihara, S. *J. Polym. Sci., Polym. Chem.* **2000**, *38*, 3962–3965.

(4) Kitano, H.; Hirabayashi, T.; Gemmei-Ide, M.; Kyogoku, M. *Macromol. Chem. Phys.* **2004**, *205*, 1651–1659.

(5) Sugihara, S.; Kanaoka, S.; Aoshima, S. *Macromolecules* **2005**, *38*, 1919–1927.

(6) Zhang, D.; Macias, C.; Ortiz, C. *Macromolecules* **2005**, *38*, 2530–2534.

(7) Lutz, J. F. *J. Polym. Sci., Polym. Chem.* **2008**, *46*, 3459–3470.

(8) Lutz, J. F.; Hoth, A. *Macromolecules* **2006**, *39*, 893–896.

(9) Lutz, J. F.; Weichenhan, K.; Akdemir, O. *Macromolecules* **2007**, *40*, 2503–2508.

(10) Fechler, N.; Badi, N.; Schade, K.; Pfeifer, S.; Lutz, J. F. *Macromolecules* **2009**, *42*, 33–36.

(11) Muñoz-Bonilla, A.; Haddleton, D. M.; Cerrada, M. L.; Fernández-García, M. *Macromol. Chem. Phys.* **2008**, *209*, 184–194.

(12) Yamamoto, S. I.; Pietrasik, J.; Matyjaszewski, K. *J. Polym. Sci., Polym. Chem.* **2008**, *46*, 194–202.

(13) Cai, T.; Marquez, M.; Hu, Z. *Langmuir* **2007**, *23*, 8663–8666.

(14) Lutz, J. F.; Akdemir, O.; Hoth, A. *J. Am. Chem. Soc.* **2006**, *128*, 13046–13047.

(15) Bignotti, F.; Sartore, L.; Penco, M.; Ramorino, G.; Peroni, I. *J. Appl. Polym. Sci.* **2004**, *93*, 1964–1971.

(16) Tanaka, T. *Phys. Rev. Lett.* **1978**, *40*, 820–823.

(17) Yamamoto, S. I.; Pietrasik, J.; Matyjaszewski, K. *Macromolecules* **2008**, *41*, 7013–7020.

(18) Jiang, X.; Zhao, B. *Macromolecules* **2008**, *41*, 9366–9375.

(19) París, R.; Mosquera, B.; de la Fuente, J. L. *Eur. Polym. J.* **2008**, *44*, 2920–2926.

(20) París, R.; de la Fuente, J. L. *Eur. Polym. J.* **2008**, *44*, 1403–1413.

(21) París, R.; de la Fuente, J. L. *React. Funct. Polym.* **2007**, *67*, 264–273.

(22) París, R.; de la Fuente, J. L. *J. Polym. Sci., Polym. Chem.* **2007**, *45*, 3538–3549.

(23) París, R.; de la Fuente, J. L. *J. Polym. Sci., Polym. Chem.* **2006**, *44*, 5304–5315.

(24) París, R.; de la Fuente, J. L. *J. Polym. Sci., Polym. Chem.* **2005**, *43*, 2395–2406.

(25) París, R.; de la Fuente, J. L. *J. Polym. Sci., Polym. Chem.* **2005**, *43*, 6247–6261.

(26) Zhang, H.; Ruckenstein, E. *Macromolecules* **2000**, *33*, 4738–4744.

(27) Iha, R. K.; Wooley, K. L.; Nyström, A. M.; Burked, D. J.; Kade, M. J.; Hawker, C. J. *Chem. Rev.* **2009**, *109*, 5620–5686.

(28) París, R.; Quijada-Garrido, I.; García, O.; Liras, M. *Macromolecules* **2011**, *44*, 80–86.

(29) Gota, C.; Okabe, K.; Funatsu, T.; Harada, Y.; Uchiyama, S. *J. Am. Chem. Soc.* **2009**, *131*, 2766–2767.

(30) Pietsch, C.; Vollrath, A.; Hoogenboom, R.; Schubert, U. S. *Sensors* **2010**, *10*, 7979–7990.

(31) Aranaz, I.; Carrasco, S.; Tardajos, M. G.; Elvira, C.; Reinecke, H.; Lopez, D.; Gallardo, A. *Polym. Chem.* **2011**, *2*, 709–713.

(32) Gutowska, A.; Bae, Y. H.; Feijen, J.; Kim, S. W. *J. Controlled Release* **1992**, *22*, 95–104.

(33) Stile, R. A.; Healy, K. E. *Biomacromolecules* **2002**, *3*, 591–600.

(34) Ali, M.; Horikawa, S.; Venkatesh, S.; Saha, J.; Hong, J. W.; Byrne, M. E. *J. Controlled Release* **2007**, *124*, 154–162.

(35) Iveković, D.; Milardović, S.; Grabarić, B. S. *Biosens. Bioelectron.* **2004**, *20*, 871–877.

(36) Takahara, J.; Takayama, K.; Isowa, K.; Nagai, T. *Int. J. Pharm.* **1997**, *158*, 203–210.

(37) Jiang, H.; Campbell, G.; Boughner, D.; Wan, W. K.; Quantz, M. *Med. Eng. Phys.* **2004**, *26*, 269–277.

(38) Mao, L.; Hu, Y.; Piao, Y.; Chen, X.; Xian, W.; Piao, D. *Curr. Appl. Phys.* **2005**, *5*, 426–428.

(39) Amat-Guerri, F.; Liras, M.; Carrascoso, M. L.; Sastre, R. *Photochem. Photobiol.* **2003**, *77*, 577–584.

(40) París, R.; de la Fuente, J. L. *J. Polym. Sci., Polym. Phys.* **2007**, *45*, 1845–1855.

(41) Medel, S.; Manuel García, J.; Garrido, L.; Quijada-Garrido, I.; París, R. *J. Polym. Sci.,Polym. Chem.* **2011**, *49*, 690–700.

(42) París, R.; de la Fuente, J. L. *React. Funct. Polym.* **2008**, *68*, 1004–1012.

(43) Zulfiqar, S.; Piracha, A.; Masud, K. *Polym. Degrad. Stab.* **1996**, *52*, 89–93.

(44) Magnusson, J. P.; Khan, A.; Pasparakis, G.; Saeed, A. O.; Wang, W.; Alexander, C. *J. Am. Chem. Soc.* **2008**, *130*, 10852–10853.

(45) París, R.; Quijada-Garrido, I. *Eur. Polym. J.* **2010**, *46*, 2156–2163.

(46) Shepherd, J. L.; Kell, A.; Chung, E.; Sinclar, C. W.; Workentin, M. S.; Bizzotto, D. *J. Am. Chem. Soc.* **2004**, *126*, 8329–8335.

(47) Liras, M.; García, O.; Quijada-Garrido, I.; París, R. *Macromolecules* **2011**, *44*, 1335–1339.