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Tunable, Temperature-Responsive Polynorbornenes with Side Chains Based on an Elastin Peptide Sequence

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Natural mammalian elastin fibers are crosslinked networks of the protein tropoelastin, which functions as the primary component of human blood vessels. Extensive physical and theoretical studies on this protein have shed light on the mechanism behind its unique elasticity.^[1] Tropoelastin is comprised of hydrophobic domains of the repeating amino acid sequence $-(VPGVG)_n-$ and domains rich in alanine and lysine residues for intermolecular crosslinking. The hydrophobic domains are conformationally dynamic and transition between random coils and tightly wound β -sheets, resulting in large changes in the hydration sphere of the protein. This process has been determined to be fundamental to the elasticity of the crosslinked networks.^[2] In the absence of chain crosslinking, the conformation change is manifested by a temperature-dependent phase transition known as a lower critical solution temperature (LCST) below which the protein is soluble and above which it is insoluble. In order to take advantage of the physical properties of tropoelastin, elastin-like polypeptides (ELPs) have been synthesized by microbial expression systems^[3] and have been studied for use as biomaterials.^[4] The promise presented by ELPs has inspired us to search for readily accessible synthetic derivatives of these proteins for the development of new materials that promote endothelial cell growth. We hoped to incorporate the elastin amino acid sequence, $-(VPGVG)-$, as the side chain on biomimetic polynorbornenes to obtain a synthetic polymer that exhibits the phase transition behaviour of its polypeptide model.

The van Hest and Cameron groups have demonstrated that polymers with the $-(VPGVG)-$ elastin sequence as a side chain exhibit LCSTs that are dependent on concentration, degree of polymerization and pH.^[5–6] The polymers were synthesized using controlled radical polymerization methods to form either ABA block copolymers with low degrees of polymerization ($DP < 12$)^[5] or homopolymers with higher DPs.^[6] Recently, the Setton group has shown that dimeric repeat units of $-(VPGVG)-$ attached to norbornene monomers could be polymerized using ring-opening metathesis polymerization (ROMP) with $(H_2IMes)(PCy_3)Cl_2Ru=CHPh$ as an initiator. The oligomers ($DP < 12$) produced exhibited temperature dependent phase transitions.^[7]

The LCSTs in most synthetic elastin-based materials are strongly dependent on the overall molecular weight of the polymer. Due to limitations in the synthetic methods used for their assembly, high molecular weight elastin-based polymers have not been investigated. Our

goal was to develop a robust method to synthesize elastin-based polymers in which the LCST was not molecular weight dependent and could be tuned for any targeted application. We anticipated that by making random copolymers using ROMP of an elastin-based monomer and a hydrophilic polyethylene glycol (PEG)-based monomer, the LCST could be manipulated based on the ratio of the comonomers in the feed. Due to its high level of tolerance towards polar functional groups, ruthenium catalyzed ROMP is an ideal method for the assembly of materials incorporating peptidic side chains,^[8] and use of (H₂IMes)(pyr)₂Cl₂Ru=CHPh (**3**) has been shown to produce low polydispersity (PDI) materials through fast initiation.^[9]

Our studies commenced with the polymerization of norbornene monomer **1**, which was synthesized using standard Fmoc-based solid phase synthesis procedures (see the supporting information for details). As shown in Scheme 1, treatment of monomer **1** with initiator **3** in a CH₂Cl₂/MeOH solvent mixture resulted in rapid polymerization. MeOH was required as a cosolvent in order to maintain a homogenous solution throughout the polymerization reaction. Other solvent systems or additives that have been reported for ROMP with peptides such as CF₃CH₂OH, AcOH or LiCl^[10] resulted in either precipitation of the polymeric products or decreased reaction rate.

The homopolymer of **1** did not exhibit an LCST and was insoluble in aqueous phosphate buffer at pH 2 and 0 °C.^[11] Upon inclusion of a PEG₅ comonomer to reduce aggregation^[12] of the VPGVG side chains and increase the hydrophilicity of the polymers, we found that random copolymerization of **1** and **2** led to complete monomer consumption and polymers with narrow PDIs (Table 1). Gel permeation chromatography (GPC) of the polymers with a multiangle laser light scattering (MALLS) detector showed narrow and monomodal molecular weight distributions. Furthermore, it was observed that the molecular weight of the polymer increased linearly with an increasing ratio of monomer to initiator, indicating a living polymerization process (polymers **4–9**). The molar ratio of **1:2** could also be varied to produce polymers with differing amounts of the elastin peptide sequence (polymers **9–12**).

In order to determine whether the ROMP polymers produced in Table 1 were random copolymers or possessed gradient composition, the disappearance of each of the monomers during the copolymerization was monitored by ¹H NMR spectroscopy. First order kinetics were observed and the results are depicted in Figure 1 in which the slope of the line [*k*_{obs} (min⁻¹)] is determined to be the polymerization rate of each of the monomers. The similar rate of incorporation of **1** and **2** throughout the course of the polymerization strongly indicates a random copolymerization.

Each of the copolymers shown in Table 1 exhibited the temperature-responsive phase behaviour characteristic of ELPs. As anticipated, the ratio of the peptidic to nonpeptidic monomers in the feed could be used to control the LCST of the resulting polymer. The temperature transitions were measured by UV-Vis spectroscopy in aqueous phosphate buffer at pH 2. Turbidity measurements for polymers in which the molar ratio of **1:2** was varied reveal a large dependence of the LCST on the elastin content (Figure 2a). For polymers of similar molecular weight, the LCSTs increased from 17–44 °C (Table 1, polymers **6, 10–12**) as elastin content was decreased from 60 to 33%. It should be noted that the homopolymer of **2** exhibited an LCST at 95 °C. The temperature responsive behaviour of polymers containing ethylene glycol units is well-documented and has been shown to be dependent on the number of ethylene glycol units on the side chain and the nature of the backbone of the polymeric material.^[13]

A relatively small dependence of the LCST (27–21 °C) on molecular weight (15–64 kDa) is observed for polymers with the same elastin content as is shown in Figure 2b. This minor

correlation of LCST to molecular weight is in contrast to results observed for ELPs,^[14] block^[7] and homopolymers^[8] with elastin-based side chains. In these reports, the LCST was strongly correlated to the degree of polymerization. The relatively small molecular weight dependence in our elastin-based polynorbornenes can be rationalized by the random composition of the comonomers in the polymer (Figure 1). Given a specific ratio of 1:2, the repeat frequency of the -(VPGVG)- monomer within the polymer chain should be constant, regardless of the overall degree of polymerization. The independence of LCST on molecular weight may be a desired attribute for use of elastin-based synthetic polymers as biomedical materials because it leads to greater consistency between batches.

A significant concentration effect was observed for all of the random copolymers synthesized. An example of the concentration dependence is depicted in Figure 3 in which the LCST of polymer 9 decreases from 30 to 16 °C with increasing polymer concentration in aqueous buffer. A similar dependence on concentration has been observed for ELPs as well as synthetic polymers with elastin side chains and is thought to originate from the increased intermolecular interactions promoting the transition from random coils to tightly wound β -sheets in more concentrated solutions.^[8, 14–15]

In conclusion, temperature-responsive, elastin-based polynorbornene materials were synthesized using ROMP. A PEG₅ comonomer was incorporated randomly into the polymer backbone to control the LCST. The polymerization reaction showed living character that was exemplified by a linear dependence of the molecular weight on the ratio of the monomer to initiator. Similar rates of incorporation of each of the monomers were observed in kinetic studies implicating a statistical distribution of the two monomers in the copolymer. The LCSTs were found to be highly dependent on the ratio of the peptidic to PEG₅ containing monomers and on concentration but showed only a small dependence on the molecular weight. The polymerization conditions developed in this communication are well suited for further studies that will incorporate monomers containing cell-binding motifs in addition to the elastin-based peptide side chains.

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11. The polymers remained soluble in phosphate buffer from 0 to 95 °C at neutral pH. An LCST for the homopolymer of **1** was also not observed when the ionic strength (salt concentration) or pH was varied.
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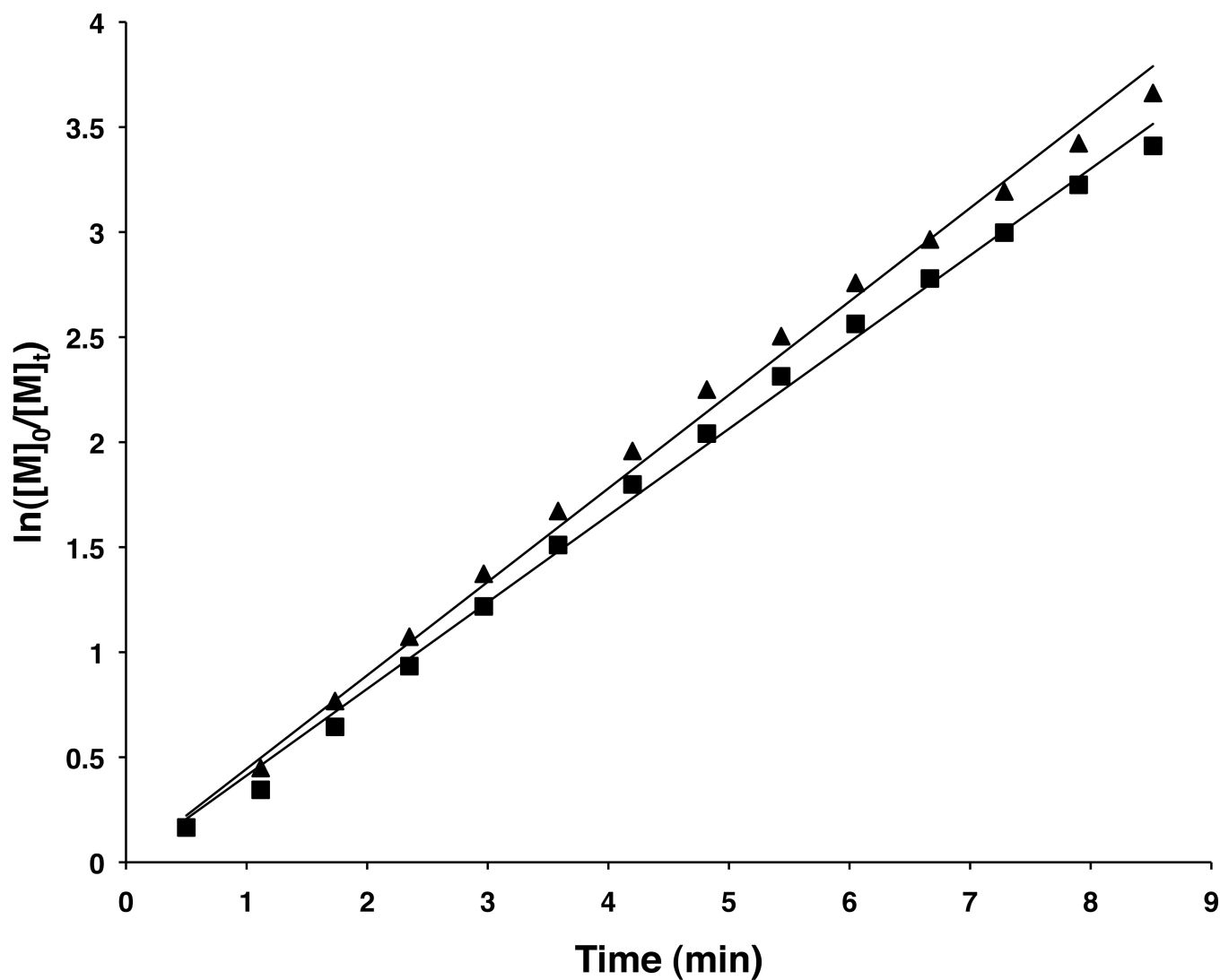
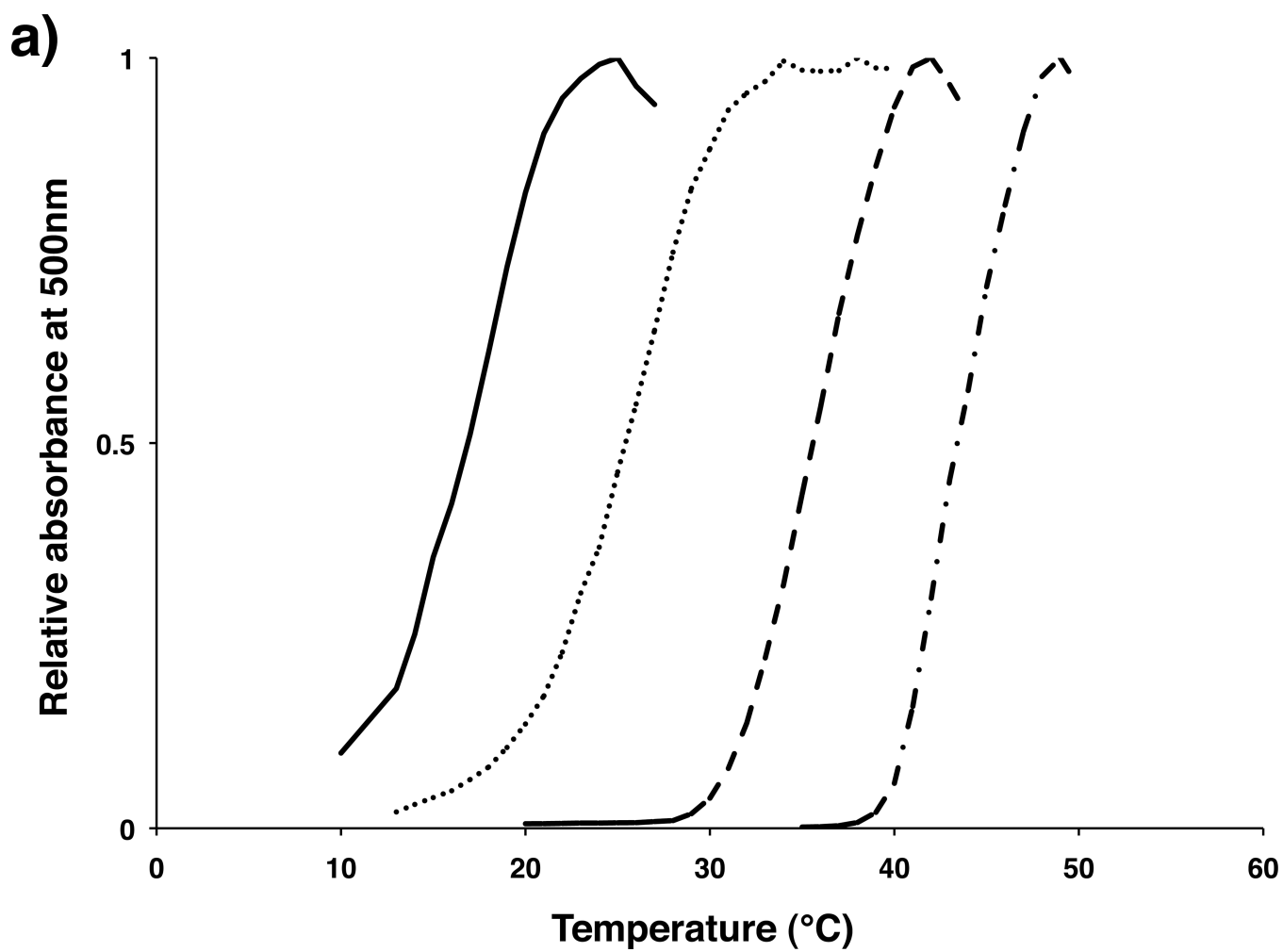


Figure 1. Log plot of the random copolymerization of monomers **1** (triangles) and **2** (squares) with $[1]_0 = [2]_0 = 0.01$ M and $[1 + 2]_0/[3]_0 = 40$ in 4:1 CD_2Cl_2/CD_3OD . Linear least-squares fitting gave slope and R^2 values, respectively, of the following: monomer **1**, (0.45, 0.996) and monomer **2**, (0.42, 0.997).



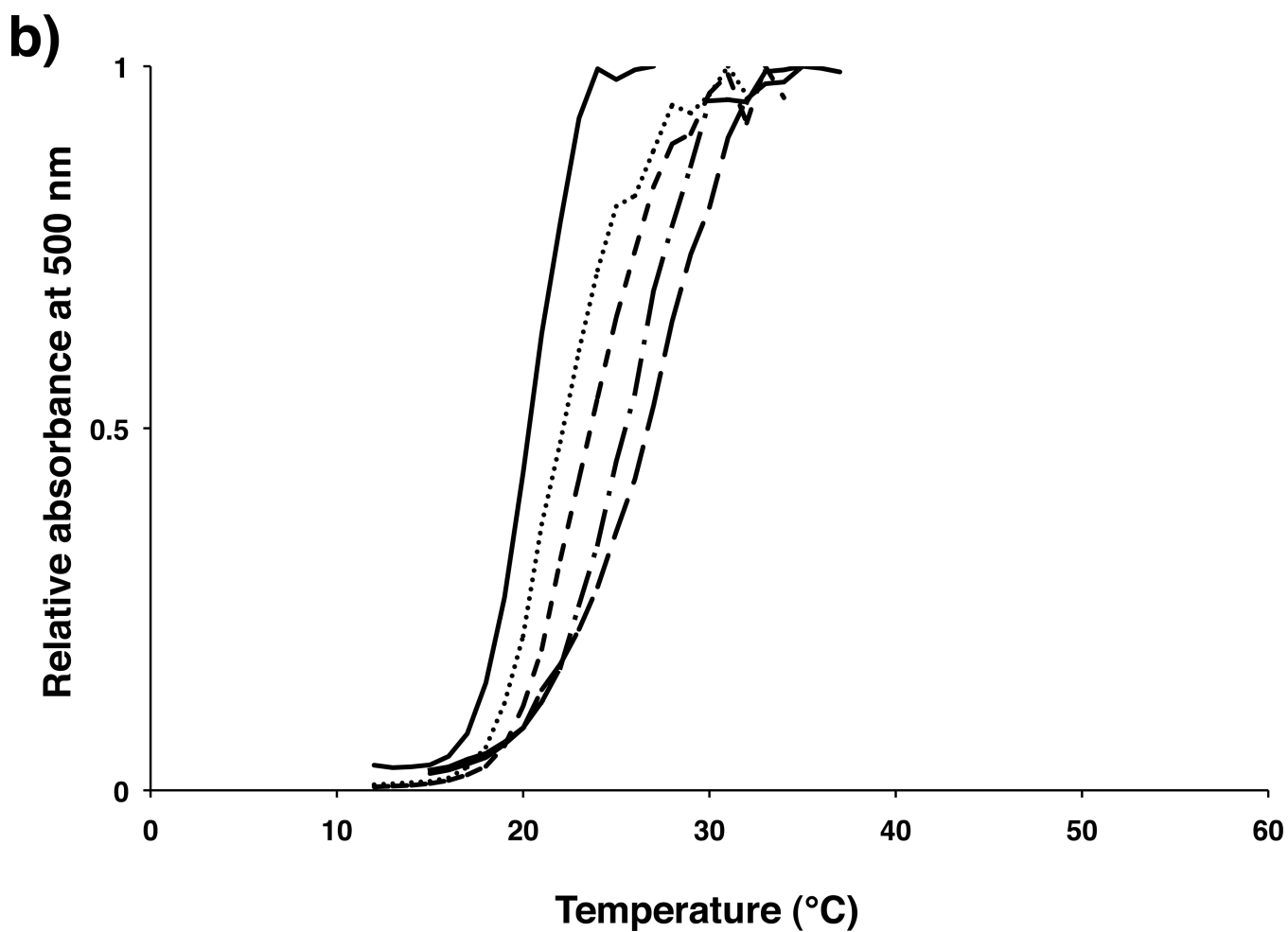


Figure 2.

Turbidity measurements for polymers of a) varying elastin content: solid line, 60%; dotted line, 50 %; short dash, 40% elastin; dash-dot, 33%; and b) varying molecular weight (as determined by GPC): solid line, 64 kDa; dotted line, 47 kDa; short dash, 40 kDa; dash-dot, 29 kDa; long dash, 15 kDa. Measurements were taken at pH 2, 0.5 mg/mL and heating at a rate of 0.5 °C/min. Absorbance values have been normalized to 1.

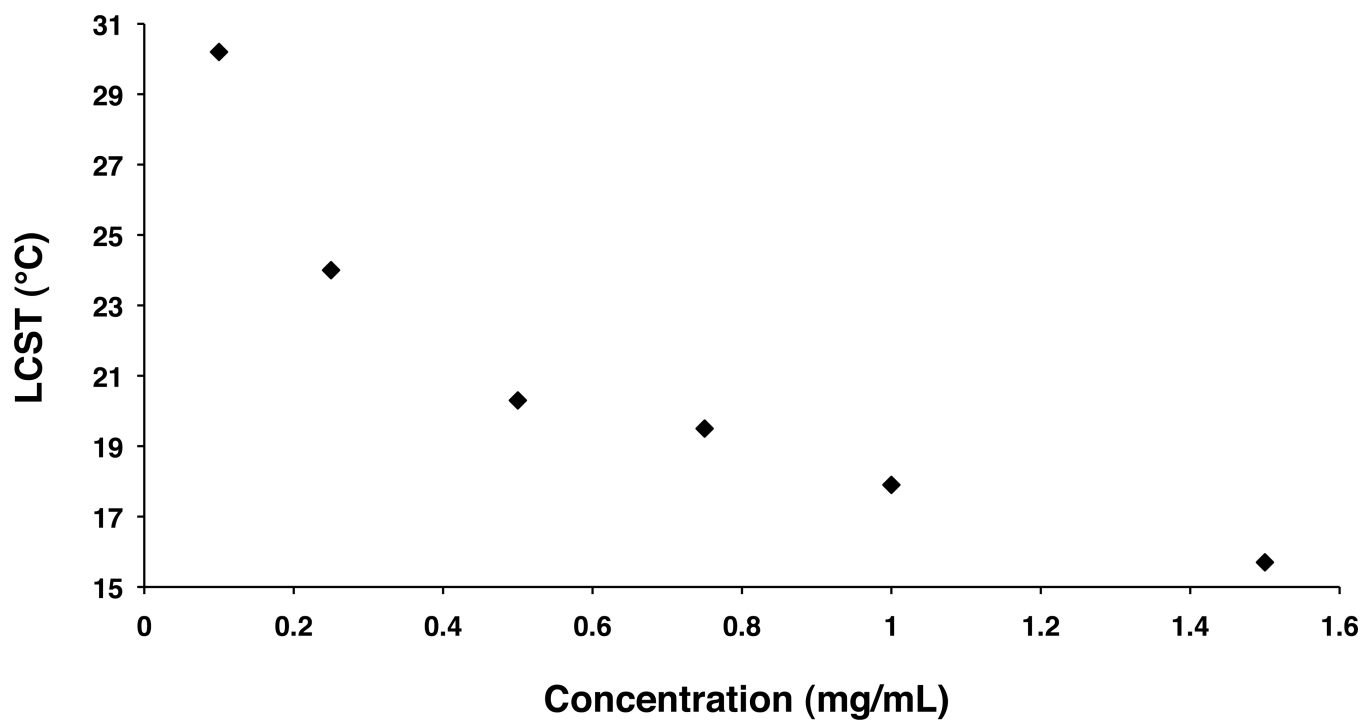
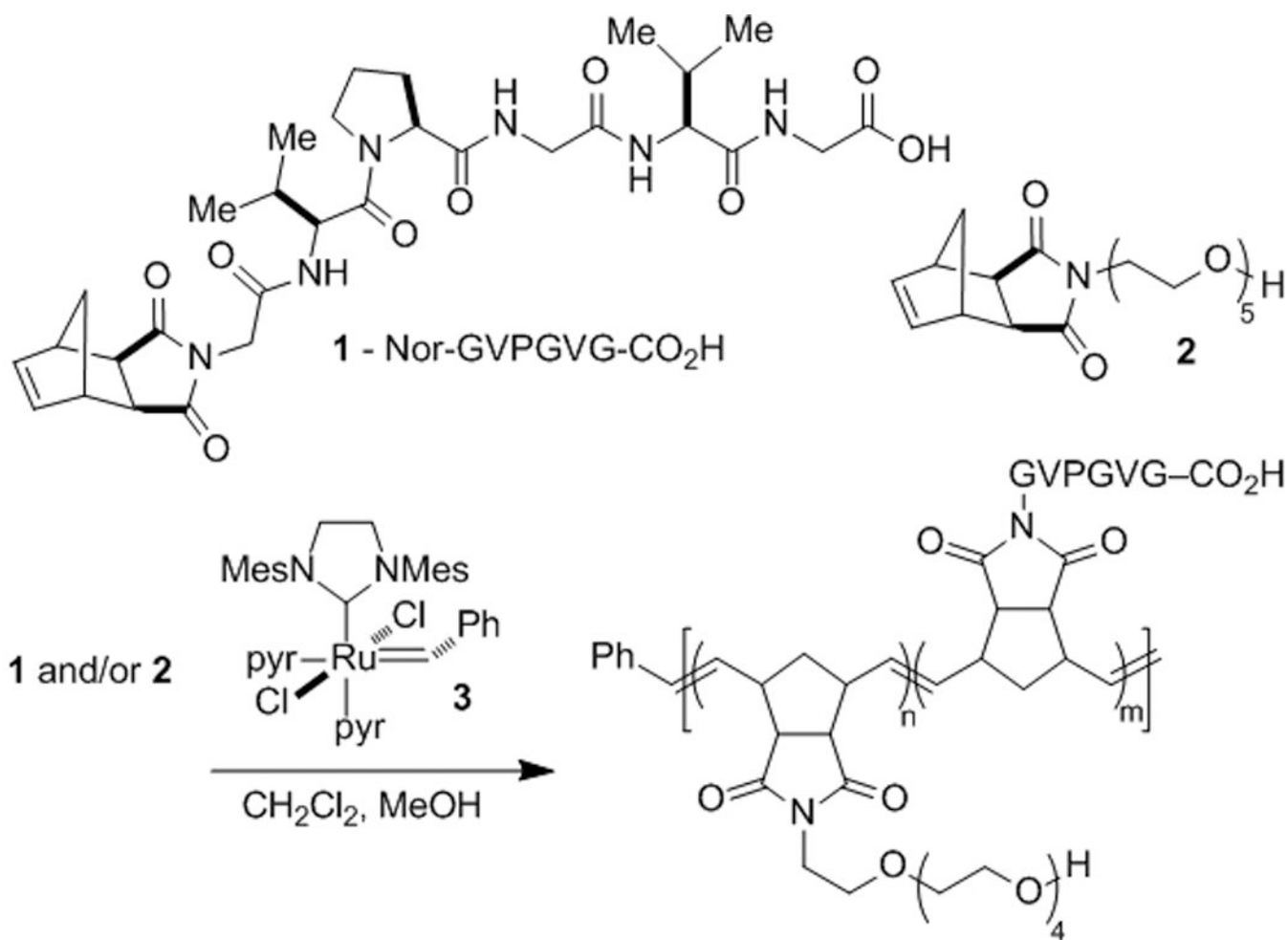


Figure 3.
Concentration dependence of LCST of polymer **9** at pH 2.



Scheme 1.
ROMP of elastin-like monomers with PEG₅ comonomers.

Table 1

GPC and LCST data for random copolymers from monomers **1** and **2** with the -(VPGVG)- elastin-based sequence and PEG₅.

Polymer	1:2	[M] ₀ /[3] ₀ [^a]	M _n (theo, kDa)	M _n (GPC, kDa)	PDI	LCST (°C)[^b]	T _{onset} (°C)[^c]
4	1.0	20	10	15	1.04	27	17
5	1.0	40	20	29	1.03	26	17
6	1.0	50	25	37	1.05	26	16
7	1.0	60	30	40	1.04	24	16
8	1.0	80	41	47	1.03	22	16
9	1.0	100	51	64	1.05	21	14
10	1.5	50	27	35	1.03	17	10
11	0.7	50	24	32	1.04	37	29
12	0.5	50	23	37	1.02	44	39

[^a][M]₀: total monomer concentration = [**1** + **2**]₀;

[^b]_{LCST} is taken as the midpoint on the turbidity profile at 0.5 mg/mL;

[^c] temperature at which the polymer begins to precipitate upon heating.