# Novel Photoresponsive *p*-Phenylazobenzene Derivative of an Elastin-like Polymer with Enhanced Control of Azobenzene Content and without pH Sensitiveness

**M. Alonso,[†] V. Reboto,[†] L. Guiscardo,[†] V. Maté,[†] and J. C. Rodríguez-Cabello*,[‡]**

*Dpto. Química Analítica, E.U.P., Universidad de Valladolid, Francisco Mendizabal 1, 47014 Valladolid, Spain, and Dpto. Física de la Materia Condensada, E.T.S.I.I., Universidad de Valladolid, Paseo del Cauce s/n, 47011 Valladolid, Spain*

*Received March 14, 2001; Revised Manuscript Received August 13, 2001*

ABSTRACT: In the past years, photochromic derivatives of elastin-like polymers have proved to be an attractive alternative to other polypeptide and non-polypeptide photoresponsive polymers because they show high efficiency and display their properties in water solutions. Up to date, the chromophores have been conjugated to elastin-like polymers exclusively by amidation of carboxylic groups present in the polymer chains. However, the control of the number of attached chromophores and their intrinsic high sensibility to pH caused by unreacted COOH groups remain as main drawbacks of these polymers. A novel approach has been used in this work to synthesize an azobenzene derivative of poly(VPGVG), the copolymer poly[$f_V$(VPGVG), $f_X$(VPGXG)] (X = L-*p*-(phenylazo)phenylalanine; $f_V$ and $f_X$ = mole fractions), by introducing the azobenzene moiety by direct synthesis and not by conjugation in one of the two comonomers prior to polymerization. In this way, the azobenzene content was reasonably controlled by the comonomer ratio in the polymerization reaction. In addition, the resulting copolymer has not pH-sensitive groups such as carboxylic or amino groups in the amino acid side chains. Different compositions were assayed. Those above $f_X = 0.20$ were water-insoluble, and those below $f_X = 0.15$ showed poor photoresponsiveness. The optimum was established at $f_X = 0.15$ in our experimental setup, which corresponds to 3 azobenzene groups per 100 amino acid residues in the polymer chain.

## Introduction

Photoresponsive polymers are able to react to light, giving reversible variations of their structure and conformation. Those changes are accompanied by variations of their physical properties.[1−3] Those modifications in their properties could be exploited for potential applications in chemical or mechanical engineering systems such as photoresponsive valves, shape memory materials, optical recording, photomechanical transducers and actuators, etc, as well as biomedical applications.[1−3]

One of the most important groups of photoresponsive polymers is poly(α-amino acids) conjugated with photochromic side chains.[1−3] These polymers respond to light, giving reversible coil−α-helix (or *β*-sheet) transitions.[1−3] Thus, photostimulated changes in viscosity[4,5] and solubility,[6] photocontrol of membrane functions,[7] and photomechanical effects in photochromic gels[7] and thin films[8,9] have been reported.

These fascinating properties suggest that photoresponsive polymers have many future opportunities as suitable materials for designing photomodulated sensors and devices. However, these materials display these properties under highly specific environments of suitable solvents (or a mix of them) that, in addition, invariably have a bad environmental consideration. Furthermore, their efficiency is intrinsically low, since the number of attached photochromic moieties needed to yield a significant coil−α-helix transition is always high.[1−3] The conventional number of chromophores attached to yield photomodulated polymers falls between 40 and 90 per 100 amino acid residues in the polymer backbone.[1−3]

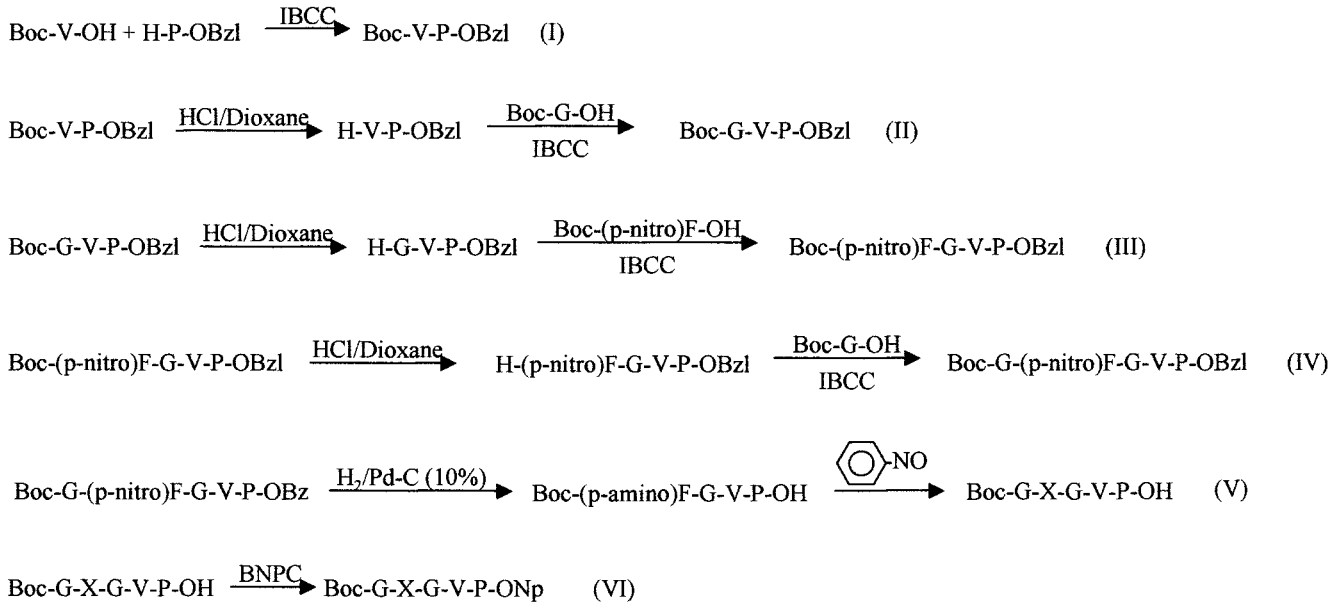
Recently, these two main drawbacks could be overcome by the use of a new family of synthetic polypeptides, the bioelastic elastin-like polymers as substitutes for the conventional poly(α-amino acids). This has been demonstrated in a pioneer work by Strzegowski et al. on an azobenzene derivative of this kind of polypeptide[10] and further expanded to sunlight-modulated polymers by the design and synthesis of a spiropyrane derivative of a related bioelastic polymer by Alonso et al.[11]

The family of polymers used in this work, elastin-like polypeptides, was designed and first synthesized according to the repetitive sequences found in the natural elastin. Poly(VPGVG) can be considered as the head of this group. All elastin-like polymers remain in solution below a given temperature, the transition temperature ($T_t$), and segregate from the solution when the temperature rises above that critical temperature. At the molecular level, this phase segregation is the macroscopical consequence of a molecular transition from a relatively extended and disordered chain below $T_t$ to a regularly folded *β*-spiral.[12] In the *β*-spiral state, the polymer chain is in good situation to suffer extensive intra- and interchain hydrophobic contacts that, eventually, lead to formation of aggregates and segregation from the solution.[12] $T_t$ is affected by the mean polarity of the polymer. Thus, in derivatives of the base polymer, $T_t$ increases as hydrophobicity decreases and decreases as hydrophobicity increases.[12] This property is also the base of the so-called "$\Delta T_t$ mechanism". By this method, properly designed modifications of the basic sequence have produced a series of related polypeptides that undergo isothermal phase transitions in response to

[†] Dpto. Química Analítica.
[‡] Dpto. Física de la Materia Condensada.
* To whom correspondence should be addressed: telephone: +34 983 423680; Fax +34 983 423544; e-mail cabello@wfisic.eis.uva.es.

10.1021/ma010455o CCC: $20.00 
Published on Web 10/05/2001

**Scheme 1. Chemical Pathway Followed in This Work To Synthesize the Monomer Boc-GXGVP-ONp**




changes in pH, ionic strength, pressure, oxidation/reduction, and others.[12,13] The photosensitive bioelastic polymers described in refs 10 and 11 exploit also this $\Delta T_t$ mechanism. Both polymers were able to show photomodulated phase separations in water solutions and with an extremely high efficiency, since only 3.3 azobenzene chromophores per 100 amino acid residues were needed for the polymer described in ref 10, while only 2.3 sipropyrane chromophores per 100 amino acids were attached in the polymer described in ref 11.

In both cases the photochromic compound was conjugated by amidation to the $\gamma$-carboxylic residue of a L-glutamic acid (E) in copolymers of the kind poly[$f_V$-(VPGVG), $f_E$(VPGEG)], where $f_E$ is around 0.3. However, despite the huge advancement in the use of water as only solvent and their high efficiency, there are still some obstacles in the practical use of this kind of polymer. First, since the amidation to the $\gamma$-carboxylic residue is always far from being complete, there always exist a considerable number of free carboxyl groups susceptible to suffer protonation−deprotonation reactions. Thus, the existence of charged carboxylate species at neutral and basic pHs interferes with the $\Delta T_t$ mechanism exploited by the chromophore and could eventually lead to the malfunctioning of the system. In fact, the characterization of these two photoresponsive bioelastic polymers has been carried out in controlled conditions of acid pHs. Second, the yield of the amidation reaction is difficult to control. In practice, the number of attached chromophores cannot be chosen as desired, which limits the systematic study of their properties.

With the aim of overcoming these two drawbacks of the photoresponsive bioelastic polymers based on amidation of carboxylic residues, a novel azobenzene derivative has been designed, synthesized, and characterized in this work.

## Experimental Section

**Materials.** All protected amino acids and other reagents described below were purchased from Sigma Chemicals. With the exception of glycine, all the amino acids were the L-isomer. Stoichiometry and purity for all the intermediate products and final polymers were routinely checked by $^{13}C$ and $^{1}H$ NMR, elemental and amino acid analysis, and chromatographic methods, similarly to those described in ref 14, which is mainly dedicated to describe the synthesis of bioelastic polymers.

**Synthesis of the Prereactive Monomer Boc-GVGVP-ONp.** Synthesis of the pentamer Boc-GVGVP-ONp has been carried out following the method described by Gowda et al.[14] Detailed data on the synthesis and molecular characterization of the intermediate oligopeptides used in this work to obtain Boc-GVGVP-ONp can be found in a previous work from our group,[15] but in short, the pentamer was synthesized by a 3 + 2 approach (GVG + VP). The *tert*-butyloxycarbonyl (Boc) group was used for α-amino protection and was removed with HCl/dioxane. The carboxyl group was masked by benzyl ester (OBzl) and removed by Pd-catalyzed hydrogenolysis. The coupling reaction was done by the mixed anhydrides method with isobutyl chlorocarbonate (IBCC). The activation of the carboxyl residue in the final pentapeptide of the monomer was achieved by the use of bis(4-nitrophenyl) carbonate (bis-PNPC) to obtain the active *p*-nitrophenyl ester (ONp).

**Synthesis of the Prereactive Monomer Boc-GXGVP-ONp.** Synthesis of this pentamer was designed from a modification of one of the methods described by Gowda et al. in ref 14 and the method described by Goodman et al.[16] for the synthesis of L-*p*-(phenylazo)phenylalanine from l-*p*-nitrophenylalanine. X represents L-(*p*-phenylazo)phenilalanine. The synthesis of the prereactive monomer corresponding to this pentamer has been depicted in Scheme 1. *Boc-V-P-OBzl (**I**).* Boc-V-OH (2.17 g, 0.01 mol) was dissolved in acetonitrile and cooled to 0 °C. Then *N*-methylmorpholine (NMM, 1.1 mL) was added. The solution was cooled to −15 ± 1 °C, and IBCC (1.3 mL) was added slowly under stirring while maintaining the temperature at −15 °C. After stirring the reaction mixture for 10 min at this temperature, 1-hydroxybenzotriazole (HOBt, 1.53 g, 0.01 mol) was added. After stirring for an additional 10 min at this temperature, a precooled solution of HCl·H-P-OBzl (2.40 g, 0.01 mol) and NMM (1.1 mL) in dimethylformamide (DMF, 10 mL) was added slowly. After 20 min at −15 °C, pH was adjusted to 7.5 with additional NMM, and the reaction mixture was stirred overnight at room temperature. The reaction mixture was subjected to reduced pressure until 10−15 mL of the reaction mixture remained, and then it was poured into about 200 mL of ice-cold 90% saturated $NaHCO_3$ solution. The precipitate was filtered, triturated, washed with water, 10% citric acid, and water, and dried to obtain 3.885 g of Boc-V-P-OBzl (yield 96.04%). *Boc-G-V-P-OBzl (**II**).* Compound **I** (4.045 g, 0.01 mol) was deblocked with 5.0 N HCl/dioxane for 1.5 h. Excess HCl and dioxane were removed under reduced pressure, triturated with ether, filtered, washed with

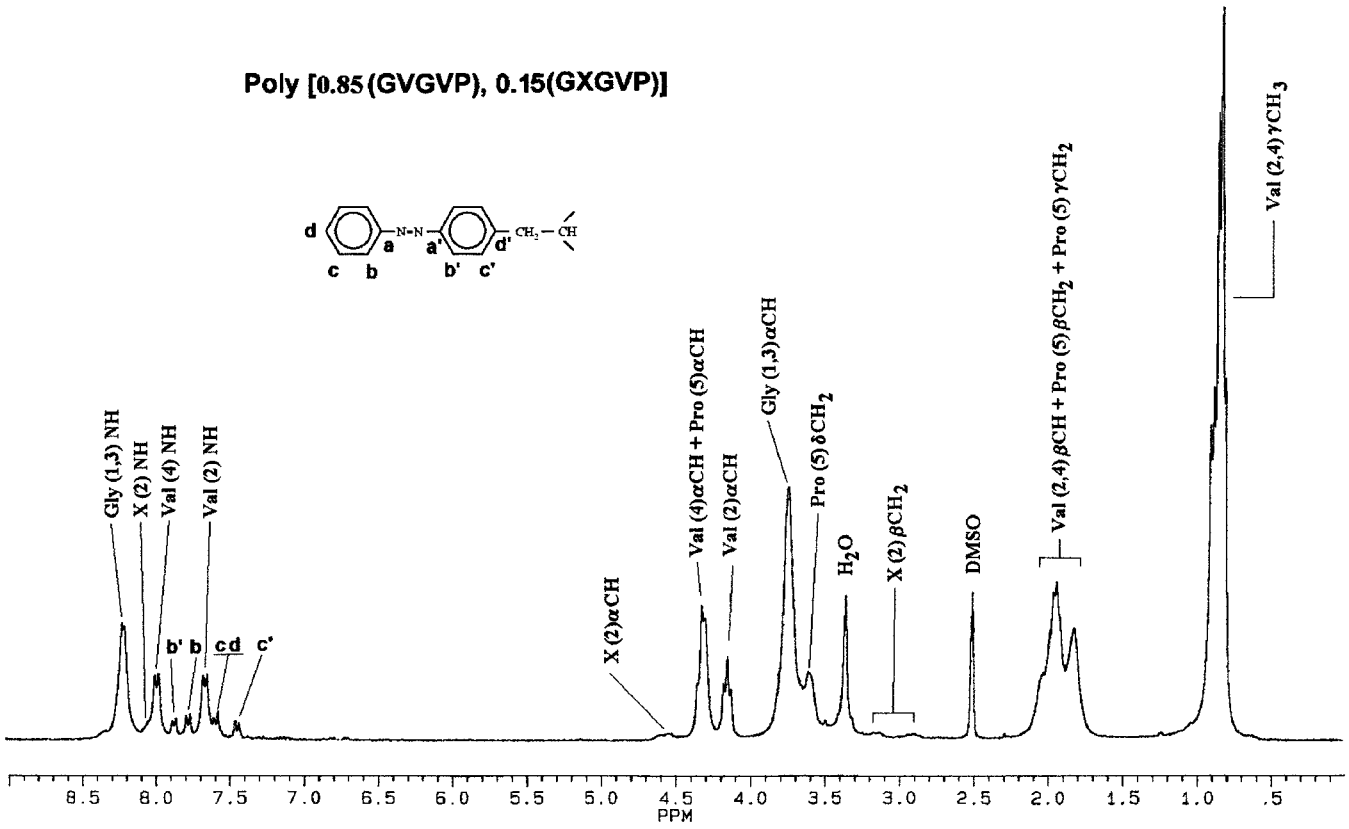



**Figure 1.** $^1$H NMR spectra of copolymer **VIIC** in hexadeuterated DMSO. Peak assignation is given in the plot.

ether, and dried (yield 100%). Boc-G-OH (1.75 g, 0.01 mol) was dissolved in 30 mL of acetonitrile and cooled to −15 °C. NMM (1.1 mL), IBCC (1.3 mL), and HOBt (1.531 g, 0.01 mol) were added as described for preparation of **I**. A precooled solution of HCl·H-V-P-OBzl (3.41 g, 0.01 mol) and NMM (1.1 mL) in DMF (10 mL) was added. The reaction mixture was worked as described for preparation of **I** and 4.40 g of **II** were obtained (yield 95.4%). *Boc-(p-nitro)F-G-V-P-OBzl (**III**).* Compound **II** (4.615 g, 0.01 mol) was deblocked with 5.0 N HCl/dioxane for 1.5 h. Excess HCl and dioxane were removed under reduced pressure, triturated with ether, filtered, washed with ether, and dried (yield 100%). Boc-(*p*-nitro)F-OH (3.10 g, 0.01 mol) was dissolved in 30 mL of acetonitrile and cooled to −15 °C. NMM (1.1 mL), IBCC (1.3 mL), and HOBt (1.531 g, 0.01 mol) were added as described for preparation of **I**. A precooled solution of HCl·H-G-V-P-OBzl (3.97 g, 0.01 mol) and NMM (1.1 mL) in DMF (10 mL) was added. The reaction mixture was worked as described for preparation of **I** and 6.12 g of **III** were obtained (yield 93.5%). *Boc-G-(p-nitro)F-G-V-P-OBzl (**IV**).* Compound **III** (6.54 g, 0.01 mol) was deblocked with 5.0 N HCl/dioxane for 1.5 h. Excess HCl and dioxane were removed under reduced pressure, triturated with ether, filtered, washed with ether, and dried (yield 100%). Boc-G-OH (1.75 g, 0.01 mol) was dissolved in 30 mL of acetonitrile and cooled to −15 °C. NMM (1.1 mL), IBCC (1.3 mL), and HOBt (1.531 g, 0.01 mol) were added as described for preparation of **I**. A precooled solution of HCl·H-(*p*-nitro)F-G-V-P-OBzl (5.89 g, 0.01 mol) and NMM (1.1 mL) in DMF (10 mL) was added. The reaction mixture was worked as described for preparation of **I** and 6.75 g of **IV** was obtained (yield 94.9%). *Boc-G−X-G-V-P-OH (**V**).* Compound **IV** (7.11 g, 0.01 mol) in glacial acetic acid (25 mL) was hydrogenated in the presence of 10% palladized charcoal catalyst at 40 psi. This reaction removed the OBzl protection and reduced the *p*-nitro residue of the *p*-nitrophenylalanine to the *p*-amino derivative.[16] The catalyst was filtered off and the solvent removed under reduced pressure. The residue was triturated with ether, filtered, washed with ether, and dried to obtain 5.45 g of Boc-G-(*p*-amino)F-G-V-P-OH (yield 92.3%). Thin-layer chromatography (TLC) and IR and UV−vis spectroscopy revealed no contamination by the pentapeptide containing unreacted *p*-nitrophenylalanine. A stirred solution of Boc-G-(*p*-amino)F-G-V-P-OH (5.90 g, 0.01 mol) at 16−18 °C in glacial acetic acid (25 mL) was added during a 40 min period a solution of nitrosobenzene (1.61 g, 0.015 mol) in glacial acetic acid (10 mL). The reaction mixture was stirred overnight at room temperature. The reaction mixture was subjected to reduced pressure, and the residue was triturated in ether, filtered, washed with ether, and dried to obtain 5.56 g of Boc-G-X-G-V-P-OH (yield 82.0%). Again, IR and UV−vis spectroscopy revealed no contamination by the pentapeptide containing unreacted l-*p*-aminophenylalanine. *Boc-G-X-G-V-P-ONp (**VI**).* Compound **V** (6.79 g, 0.01 mol) reacted with bis-PNPC (4.56 g, 0.015 mol) in pyridine (50 mL). The reaction mixture is stirred at room temperature for 32 h. After this time, an additional amount of bis-PNPC (1.51 g, 0.05 mol) was added, and the reaction was maintained with stirring at room temperature until the reaction was complete, as determined by TLC. The solvent was removed under reduced pressure, and the residue was taken into chloroform. The chloroform was washed with water, 10% citric acid, 5% $NaHCO_3$, and water and dried over $Na_2SO_4$. The solvent was removed under reduced pressure, triturated with ether, filtered, washed with ether, and dried to obtain 6.07 g of **VI** (yield 75.86%).

**Polymerization.** *Poly[$f_V$(GVGVP), $f_X$(GXGVP)] (**VII**).* Compound **VI** and Boc-GVGVP-ONp were deblocked together using trifluoroacetic acid. Six different comonomer compositions, $f_X$ = 0.05, 0.1, 0.15, 0.2, 0.3, and 0.4, were used. In the six cases, the reaction was maintained with stirring at room temperature for 0.5 h. Excess TFA was removed under reduced pressure. The residue was triturated with ether, filtered, washed with ether, and dried (yield 100%). A 1 M solution of the TFA salt in dimethyl sulfoxide (DMSO) was polymerized for the six compositions during 30 days using 1.6 equiv of NMM as base. The polymer was dissolved in water, dialyzed using 15 000 molecular weight cutoff dialysis tubing for 20 days at 5 °C, and lyophilized to obtain the final copolymers. As an example, $^1$H NMR and $^{13}$C NMR spectra for the copolymer with $f_X$ = 0.15 (copolymer **VIIC**) can be seen in Figures 1 and 2. Amino

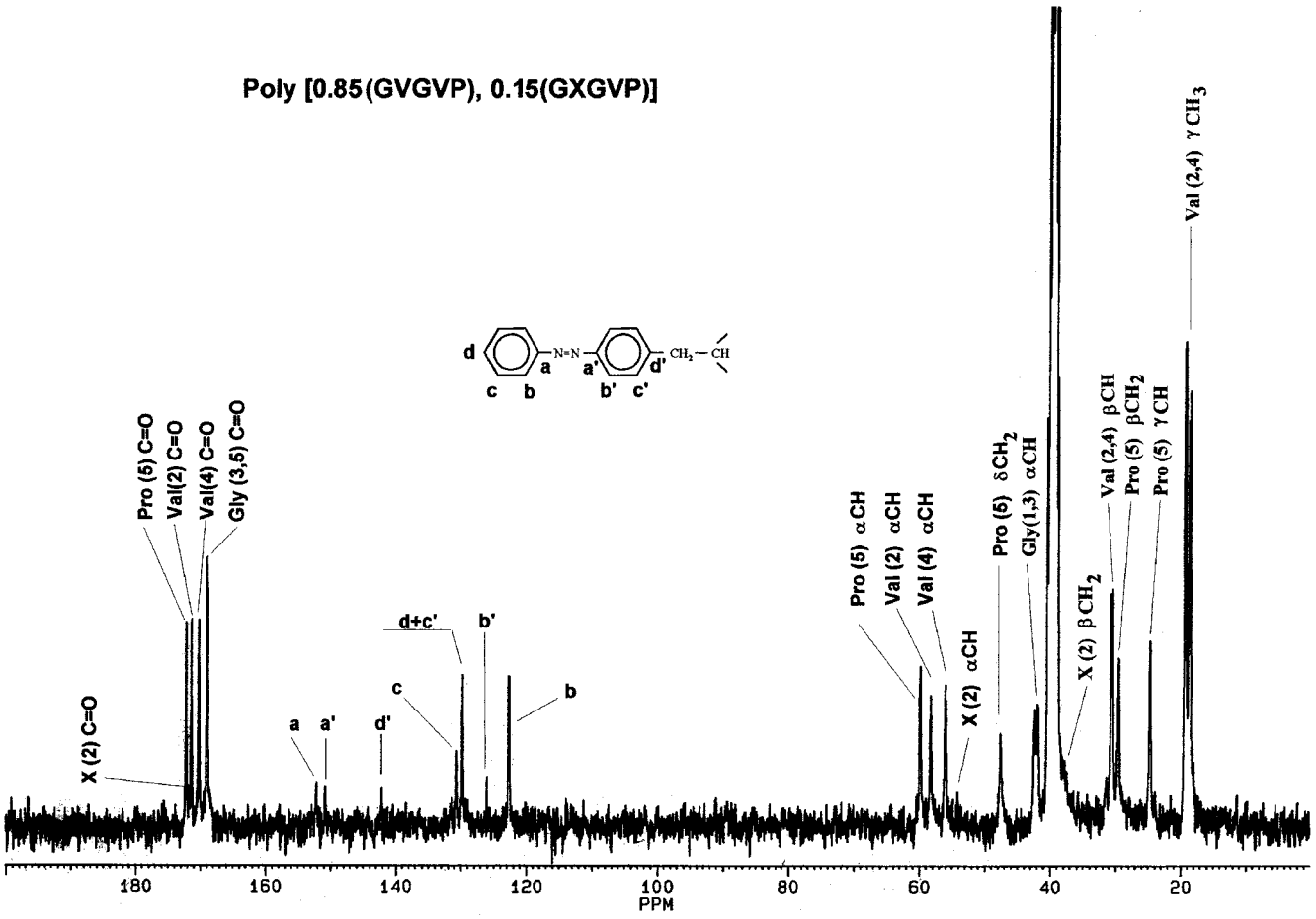



**Figure 2.** $^{13}C$ NMR spectra of copolymer **VIIC** in hexadeuterated DMSO. Peak assignation is given in the plot.

acid analysis for these copolymers revealed that the actual composition after polymerization matches well the comonomer ratio employed in the polymerization reaction with an error lower than 8% in all cases.

**UV and Visible Irradiation.** 10 mg $mL^{-1}$ polymer solutions in ultrapure water were irradiated in a standard spectrophotometer quartz cuvette with light from a 500 W Hg arc lamp (model 6285, Oriel Corp.) mounted on a lamp housing with a F/1.5 UV grade fused silica condenser and rear reflector (model 66041, Oriel Corp.). UV irradiation was achieved by the use of a band-pass filter ($200 < \lambda < 400$ nm, UG11 Schott glass) from CVI Laser Corp. Visible radiation was obtained by the use of a longwave pass filter ($\lambda > 400$ nm, SUG-11-1.00 Schott glass) from the same supplier. The exposure energy irradiation was ca. 6 mW $cm^{-2}$ for UV irradiation and ca. 8 mW $cm^{-2}$ for visible. Lamp-to-sample path length was typically 10 cm. This experimental setup was placed in a cold-storage room at a temperature of 4−5 °C. All the irradiations were made at this temperature to avoid the formation of the turbid phase of the polymer solution during UV or visible irradiation.

**Turbidity Measurements.** Turbidity experiments were conducted in a Varian Cary 50 UV−vis spectrophotometer with a thermostatized sample chamber. Turbidity was assessed by the change in absorbance at 600 nm of the polymer solutions (see the Results and Discussion section). The transition temperature is considered as the temperature at which the relative turbidity reaches 50%.

## Results and Discussion

Figure 3 shows the changes in the electronic absorption spectrum that occur upon irradiation of a 10 mg $mL^{-1}$ water solution of the copolymer **VIIC**. The dark-adapted copolymer exhibits the expected absorption spectrum of the *trans*-azobenzene chromophore, with

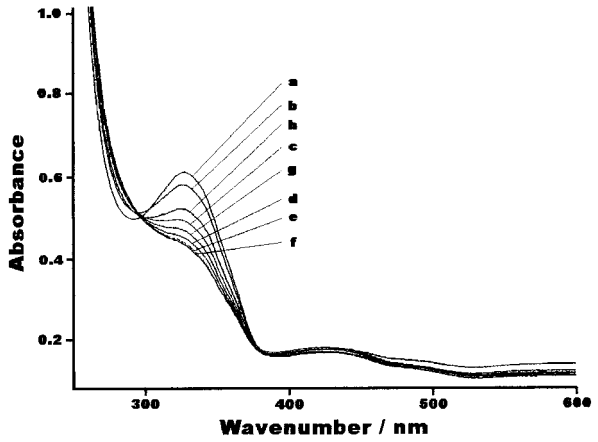



**Figure 3.** UV−vis absorption spectra of the copolymer **VIIC**. Curve a corresponds to the dark-adapted sample. Curves b−f correspond to respectively 10, 15, 20, 25, and 30 s UV-irradiated samples. Curves g and h correspond to visible radiation (30 s and 1 min, respectively) of 30 s UV-irradiated samples.

absorption maxima at 348 and 428 nm (curve a). Irradiation with UV light results in a reduction in the intensity of the 348 nm absorption band, with the photostationary state being reached in 30 s under the conditions of this experiment (curves b−f). Further irradiation with visible light restores the 348 nm absorption via partial photoreversion to the trans form of the chromophore (curves g and h). The state represented by curve h does not change upon further irradiation and is estimated to consist of ca. 50% trans and 50% cis chromophore.[10] This value is in good agree-

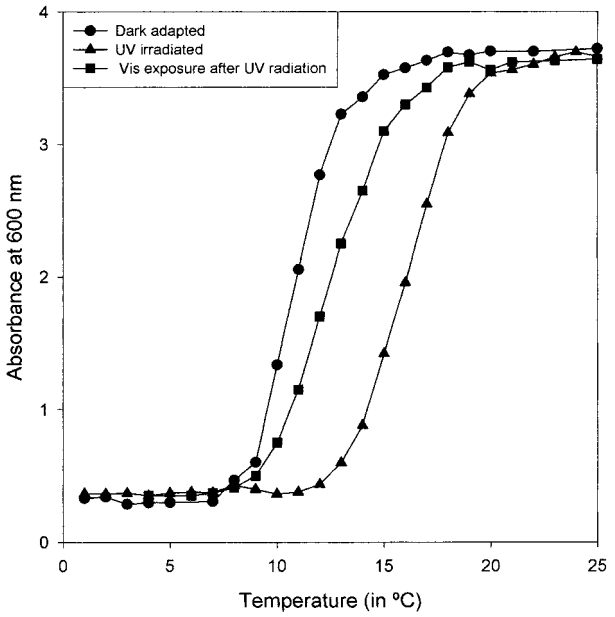



**Figure 4.** Temperature profiles of aggregation of 10 mg $mL^{-1}$ water solutions of the photoresponsive polymer **VIIC** under different illumination regimens. The correspondence between each profile and its illumination condition is indicated in the plot. The transition temperature ($T_t$) is considered as the temperature at which the change in absorbance reaches a value of 50%.

ment with that found by Strzegowski[10] et al. in the visible photostationary state of an azobenzene elastin-like derivative based on amidation of glutamic acid side chains working in water solutions. This comparatively low recovery as compared to other azobencene systems irradiated in similar conditions could be caused by the polar nature of water, which would not favor the transition to the more apolar trans isomer, and also likely by the low irradiation temperature (4−5 °C) used in this work, as described in the Experimental Section.

Figure 4 shows the temperature-dependent turbidity profile of a water solution (10 mg $mL^{-1}$) of dark-adapted polymer **VIIC**. $T_t$ for the dark-adapted polymer solution corresponds to 11.0 °C as deduced from the turbidity profile curve. This value shows a substantial decrease in $T_t$ as compared to the value obtained for poly(VPGVG), which is about 27−32 °C.[16] This decrease is in agreement with the expected behavior for a polymer modification that involve highly apolar moieties such as *p*-(phenylazo)benzene.[12] Furthermore, the magnitude of this decrease is reasonable taking into account the $T_t$ values reported in the literature for aromatic analogues of poly(VPGVG) with similar composition to polymer **VIIC**.[17]

The temperature-dependent turbidity profile of the UV-irradiated VIIC solution (30 s of irradiation) is plotted in Figure 5. $T_t$ reaches a value of 16.0 °C for this irradiated polymer. These values represent an increase in about 5 °C. This increase is in agreement with the expected behavior for this family of polymers, where a decrease in the mean hydrophobicty of the polymer chain is always accompanied by an increase in $T_t$.[12] This hydrophobicity decrease is caused by the UV-induced transformation of the trans isomer of the azobenzene moiety to the more polar cis isomer.

From the molecular point of view, the shift in $T_t$ must not be interpreted in terms of changes in the molecular

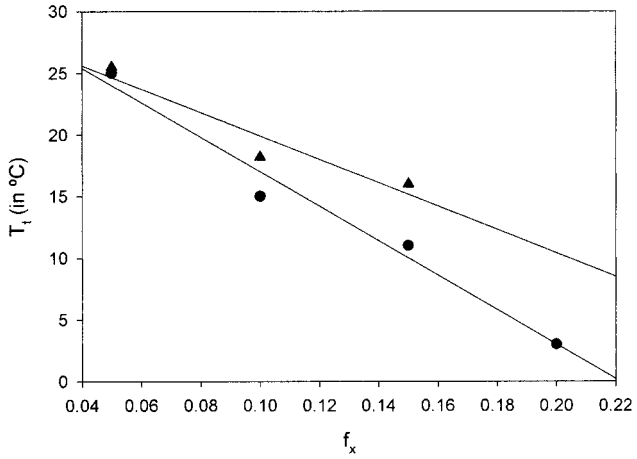



**Figure 5.** Plot of the dependence of $T_t$ on $f_X$: (▲) UV-irradiated sample; (●) dark-adapted sample. The straight lines represent the linear regressions of both plots.

**Table 1. Properties of the Copolymers Tested in This Work**

| polymer | $f_X$ | $T_t$ | $\Delta T_t$ |
|---|---|---|---|
| **VIIA** | 0.05 | 25 | 0.5 |
| **VIIB** | 0.10 | 15 | 3.2 |
| **VIIC** | 0.15 | 11 | 5.0 |
| **VIID** | 0.20 | ≈3 | ND[b] |
| **VIIE** | 0.30 | WI[a] | |
| **VIIF** | 0.40 | WI[a] | |

[a] WI = water insoluble. [b] ND = not determined. $T_t$ in °C.

structure of the extended or folded state of the polymer. It is well established that all elastin-like peptides with general formula poly[$f_V$(VPGVG), $f_U$(VPGUG)], being U any natural of chemically modified amino acid, shared the same structural pattern at the molecular level; i.e., an unordered and extended chain in solution at temperatures below $T_t$ that changes, on exceeding this temperature, to a regularly folded $\beta$-spiral formed by concatenated type II $\beta$-turns (one per pentamer), having about 3 pentamers per turn in the spiral and stabilized by hydrophobic interturn contacts (see, for example, review in ref 12). Thus, the change in polarity of the azobencene group upon photoisomerization would modify the behavior of the polymer without modifying the structure of the extended or folded state of the polymer. On the contrary, the changes in azobenzene polarity would play its role via its influence on the organization of water of hydrophobic hydration around the polymer. This has been profusely described and demonstrated for other elastin-like derivatives with substitutions able to show different polarities in response to changes in pH, redox state, and others.[12] Therefore, this effect on the structure of water of hydrophobic hydration shifts the temperature at which the transition takes place but without disturbing the structure of the extended and folded state of the polymer.[12]

The properties of the other copolymers tested in these work have been summarized in Table 1. As deduced for the data presented in this table, compositions below $f_X = 0.15$ tend to show a weak change in $T_t$. Meanwhile, compositions above $f_X = 0.20$ yield water-insoluble polymers that cannot be used for this purpose. Copolymer **VIID** ($f_X = 0.20$) is in the limit of solubility, but its low $T_t$ makes the manipulation of this polymer very problematic. For example, it was impossible in our experimental setup to irradiate the polymer without getting the turbid phase before the UV or vis illumina-

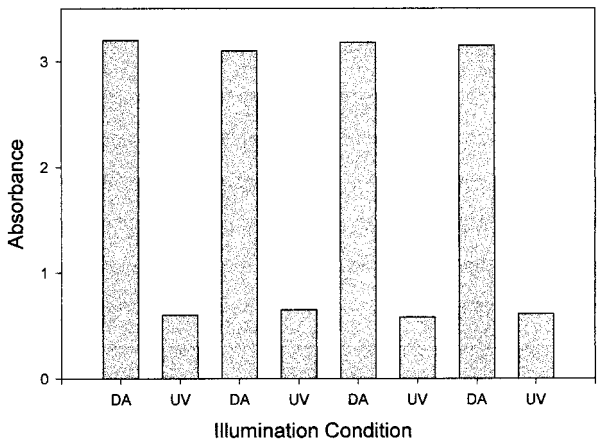



**Figure 6.** Photomodulation of phase separation of 10 mg $mL^{-1}$aqueous samples of the photochromic polymer **VIIC** at 13 °C. The illumination condition prior measurement are indicated in the horizontal axis. DA, dark adaptation; UV, UV irradiation.

tion ended. Therefore, the composition $f_X = 0.15$ showed to be the optimum composition for these kind of polymers. This composition corresponds to 3 L−*p*-(phenylazo)phenylalanine per 100 amino acid residues in the polymer chain.

The dependence of $T_t$ on the copolymer composition has been plotted in Figure 5 for the UV-irradiated (cis) and the dark-adapted (trans) sample. Urry has proposed an amino acid hydrophobicity scale by using this type of plot.[12] This scale is based on the capacity of a given amino acid to modify $T_t$ in copolymers with general formula poly[$f_V$(GVGVP), $f_U$(GUGVP)], where U is any naturally occurring or chemically modified amino acid.[12] In this sense, the value of $T_t$ obtained by extrapolation to $f_U = 1$ ($T_t^1$) gives a comparative value of the hydrophobicity for the corresponding amino acid. We have found a value of $T_t^1 = -119$ °C for the *trans*-L-(*p*-phenylazo)phenylalanine from the data presented in Figure 5. This value is lower, which means more hydrophobic, than the highest hydrophobic natural amino acid in Urry's scale, L-tryptophan ($T_t^1 = -90$ °C).[12] This low value of $T_t^1$ is in agreement with the strong decrease in $T_t$ observed for all the different polymer compositions studied here, even for those with very low content of L-(*p*-phenylazo)phenylalanine, as compared with the value found for the unsubstituted poly(VPGVG) (around 32 °C[12,16]). *cis*-L-(*p*-Phenylazo)-phenylalanine showed a $T_t^1 = -65.5$ °C, which, although higher, is still a value corresponding to a highly hydrophobic amino acid (in between L-tyrosine and L-tryptophan[12]). The determination of both values of $T_t^1$ for the *cis*- and *trans*-azobenzene forms, made possible by the control of the L-(*p*-phenylazo)phenylalanine content given by this way of polymer synthesis, will facilitate the design of adequate compositions with the desired $T_t$ shift in future work.

In the last set of experiences, the isothermal photomodulation of this kind of polymers has been studied on polymer **VIIC** as the most representative of the polymers used here. The observed increase in $T_t$ for **VIIC** after UV irradiation opens a window at a temperature around 13−14 °C (see Figure 4) where the process phase separation−redissolution of the polymer can be isothermally photomodulated by changing the illumination state between dark adaptation and UV irradiation. This is shown in Figure 6 for a 10 mg $mL^{-1}$ water solution of **VIIC** at a temperature of 13 °C subjected to repeated consecutive cycles of dark adaptation (48 h) and UV irradiation (30 s). Thus, the solubility of the polymer **VIIC** has proven to be reversibly photocontrolled at 13 °C with a polymer essentially insoluble in water after dark adaptation and almost completely soluble after UV exposition.

According to literature, the *cis*-azobenzene form of this chromophore can be rapidly converted to the trans isomer by exposing the chromophore to 420−450 nm light. This photoreversion is much faster than dark adaptation but, however, is never complete. The temperature-dependent turbidity profile of a **VIIC** water solution (10 mg $mL^{-1}$) after UV irradiation and subsequent exposition to 420−450 nm light during 1 min (photostationary state) is shown in Figure 4. As expected, $T_t$ was lower after this treatment than the one showed by the UV-irradiated sample, but $T_t$ was still above the value displayed by the dark-adapted. However, the observed value of $\Delta T_t$ between the UV-irradiated and the photoreversed sample is still high enough to open a window around 14.5 °C where substantial changes in the solubility of **VIIC** can be achieved by changing the system establishing two steady states of the trans/cis photoisomerization, which depends on the excitation wavelength.

**Acknowledgment.** This work was supported by the CICYT (Program MAT 98-0713) and by the "Junta de Castilla y León" (Program VA30/00B).

**Supporting Information Available:** Molecular characterization of oligopeptides: $R_f$ values of thin-layer chromatography, elemental analysis, 300 MHz $^1$H NMR spectra with peak assignations, and UV/vis spectra of the oligos containing chromophores. This material is available free of charge via the Internet at http://pubs.acs.org.

## References and Notes

(1) Pieroni, O.; Ciardelli, F. *Trends Polym. Sci.* **1995**, *3*, 282−287.
(2) Cooper, T. M.; Natarajan, L. V.; Crane, R. L. *Trends Polym. Sci.* **1993**, *1*, 400−412.
(3) Pieroni, O.; Fissi, A.; Popova, G. *J. Prog. Polym. Sci.* **1998**, *23*, 81−123.
(4) Irie, M.; Hirano, K.; Hashimoto, S.; Hayashi, K. *Macromolecules* **1981**, *14*, 262−266.
(5) Menju, A.; Hayashi, K.; Irie, M. *Macromolecules* **1981**, *14*, 755−759.
(6) Irie, M.; Iwayanagi, T.; Taniguchi, Y. *Macromolecules* **1985**, *18*, 2418−2420.
(7) Anzai, J.; Osa, T. *Tetrahedron* **1994**, *50*, 4039−4044.
(8) Irie, M. *Adv. Polym. Sci.* **1993**, *110*, 50−54.
(9) Menzel, H.; Hallensleben, M. L.; Schmidt, A.; Knoll, W.; Fischer, T.; Stumpe, J. *Macromolecules* **1993**, *26*, 3644−3646.
(10) Strzegowski, L. A.; Bueno Martinez, M.; Gowda, D. C.; Urry, D. W.; Tirrell, D. A. *J. Am.Chem. Soc.* **1994**, *116*, 813−814.
(11) Alonso, M.; Reboto, V.; Guiscardo, L.; San Martín, A.; Rodríguez-Cabello, J. C. *Macromolecules* **2000**, *33*, 9480−9482.
(12) Urry, D. W. *Angew. Chem., Int. Ed. Engl.* **1993**, *32*, 819−841.
(13) Urry, D. W. *J. Phys. Chem. B* **1997**, *101*, 1007−1028.
(14) Gowda, D. C.; Parker, M. T.; Harris, R. D.; Urry, D. W. *Peptides: Design, Synthesis and Biological Activity*; Basava, C., Anantharamaiah, G. M., Eds.; Birkhäuser: Boston, 1994.
(15) Rodríguez-Cabello, J. C.; Alonso, M.; Pérez, T.; Herguedas, M. M. *Biopolymers* **2000**, *54*, 282−288.
(16) Goodman, M.; Kossoy, A. *J. Am. Chem. Soc.* **1966**, *88*, 5010−5015.
(17) Luan, C. H.; Parker, T. M.; Gowda, D. C.; Urry, D. W. *Biopolymers* **1992**, *32*, 1251−1262.

MA010455O