Macromolecular Stereochemistry of Poly(*p*-biphenylmethyl L-glutamate): Linkage between Biphenyl Twist Sense and Polypeptide Conformation and Observation of Microaggregation-Driven, Sudden, Temperature-Dependent Chiral Optical Changes[1]

Michael P. Reidy and Mark M. Green*

Department of Chemistry and Polymer Research Institute, Polytechnic University, 333 Jay Street, Brooklyn, New York 11201

Received October 11, 1989

ABSTRACT: In the expectation that the normally equally populated left- (*M*) and right-handed (*P*) twist senses of a biphenyl moiety would, in the side chain of a polypeptide, be perturbed by the helical conformation of the main chain and therefore potentially reveal characteristics of the side-chain conformation, we have carried out the syntheses of the ortho- and para-substituted biphenylmethyl esters of poly(L-glutamic acid) and poly(L-aspartic acid). In the aspartate series the appropriate *N*-carboxyanhydrides yielded polymer for both the ortho- and para-substituted biphenylmethyl esters. The para isomer was not soluble in solvents appropriate to ultraviolet circular dichroism measurements while the ortho isomer indicated the absence of the α-helical conformation. In the glutamate series although we could not obtain a crystalline *N*-carboxyanhydride from the ortho isomer, in the para isomer we were able to obtain a high polymer, which, in a variety of solvents, both adopts the α-helical conformation and displays a strong positive circular dichroism band associated with the biphenyl chromophore. This chiral optical characteristic is almost entirely lost on dichloroacetic acid denaturation of the polypeptide. As a complete surprise, dilute solutions of poly(*p*-biphenylmethyl L-glutamate) in several helicogenic solvents, with the notable exception of chloroform, exhibit a sudden, large, almost concentration-independent, reversible change in their optical activity properties just below 0 °C. Intrinsic viscosity and light-scattering measurements point to aggregation associated with this change while ultraviolet, infrared, and excimer fluorescence measurements offer no evidence for significant conformational changes of the polypeptide chain or of the relationships among the biphenyl groups.

Introduction

The side-chain conformations of polypeptides have been the source of considerable interest for many years. It is well-known that different amino acid residues have differing main-chain conformational preferences[2] and together determine the overall folding of proteins.[3] Although the precise structural source of these side-chain differences remains to be completely defined,[4] enough is nevertheless known to allow design of polypeptides of defined shape.[5]

The intense effort to define these complex side-chain interactions both within a single residue and between various side chains, which eventually determine the structure of the whole macromolecule, was greatly accelerated by the study of poly(γ-benzyl L-glutamate) (PBLG)[6] and other synthetic poly(α-amino acids). Indeed, the experimental confirmation of the predicted α-helical conformation[7] of polypeptides in solution was confirmed in PBLG.[8]

Through a wide variety of experimental techniques and computational procedures much has been discovered about the factors contributing to the stability of the α-helix and other peptide conformations. Much of this effort has focused on the role of the side chain.[2-5]

Theoretical force-field calculations are consistent with the structure and conformation of the side chains in polypeptides as a key to determining the conformational properties of the backbone.[2,3,9] These computations in the case of the α-helical main chain of polyaspartate and polyglutamate esters converge on a few distinct side-chain conformations. Such calculations were notably successful in accounting for the experimentally determined conformational sensitivity of the α-helix sense to slight structural changes as seen especially in the substituted aromatic esters of the polyaspartates.[10,11] In the polyglutamates these calculations indicate[9] that nonbonded forces can favor an extended, so-called transverse, side-chain conformation while electrostatic factors favor a compact, i.e., longitudinal, side-chain conformation. The competition between these factors can be expected to be solvent sensitive, and this may, at least in part, account for the fact that, although the calculations point to the importance of longitudinal conformations in PBLG, various experiments, necessarily in organic solvents, are often at variance with this prediction. The conclusions drawn from experiments in this area are not always certain as exemplified by recent work on the ^{2}H NMR of isotopically substituted PBLG in the lyotropic state[12] where conflicting views of the side-chain conformation, perhaps associated with different sample conditions, arise from interpretation of similar experiments.

In general, the experimental work to elucidate side-chain conformation of synthetic polypeptides in solution can be broken down into three general categories: dielectric experiments,[13] NMR studies,[14] and chiral optical studies. The experiments based on dielectric measurements and NMR spectra directed to the side-chain conformation in substituted esters of poly(glutamic acid) are often in disagreement, with no clear picture of the precise disposition of the side chain and in some cases, claims that the side-chain structure is disordered.[15]

Chiral optical studies, i.e., circular dichroism (CD) and optical rotatory dispersion (ORD), long known to be sensitive to the conformational characteristics of the main chain,[2,16] also show exceptional sensitivity to side-chain conformation when appropriate chromophores are present. This could first be seen in substituted benzyl esters of poly(aspartic acid) and poly(glutamic acid)[11] and even for poly-

0024-9297/90/2223-4225$02.50/0 © 1990 American Chemical Society

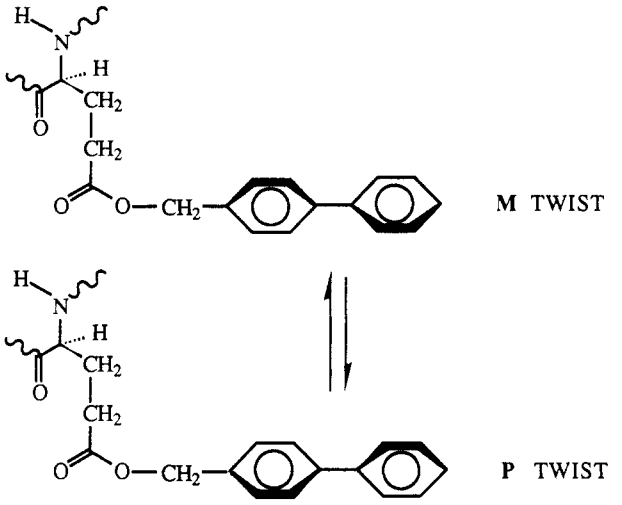

Figure 1. Conformational equilibrium of biphenyl twist sense.

(β-benzyl L-aspartate) itself, where a weak dichroism associated with the phenyl ring is observed.[17] Chiral optical measurements in both systems have demonstrated the strong relationship between side-chain structure and helix conformation, a relationship dramatically evident in the polyaspartates, where helix sense depends on the aromatic substituent.[18] Other aspartate and glutamate side-chain chromophores subject to chiral optical studies, such as poly(β-1-naphthylmethyl L-aspartate),[19a] poly(β-9-anthrylmethyl L-aspartate),[19b] poly(γ-phenacyl L-glutamate),[19c] poly(γ-1-naphthylmethyl L-glutamate),[19d] poly(γ-*N*-carboxyethyl D-glutamate),[19e] poly(*p*-(phenylazo)benzyl L-aspartate),[19f] and poly(*p*-(phenylazo)benzyl L-glutamate)[19g] and recently spiropyran containing poly(L-glutamic acid)[19h] and poly(β-phenethyl L-aspartate),[19i] often have led to interesting structural characteristics for these polypeptides, but in no case has there been possible a theoretical basis for the understanding of the side-chain chromophore chiral optical property. This is reiterated in side-chain chromophores based on the polylysine structure[20] and as well in proteins, where although chiral optical observations have been made associated with various side chains, conformational conclusions could not be drawn.[21] When polypeptide side chains are shortened so that large aromatic groups are held close to the helical main chain, extremely large circular dichroism effects are observed for the aromatic chromophores.[22] In this situation where the rigid relationships reduce the number of possible conformational states, consistent with large effects.[22]

Study of the literature cited above[16-22] demonstrates an exceptional sensitivity of circular dichroism to the side-chain conformation of spectroscopically accessible chromophores in polypeptides while, at the same time, theory is apparently not entirely adequate to deal with the complexity of the factors responsible for many of the effects observed.[23] With this in mind and with an awareness of the fact that different glutamate ester groups may affect side-chain conformation, we decided to initiate studies using the biphenyl esters, which could offer advantages over other chromophores because their chiral optical characteristics are both well understood[24] and because there is a specific structural factor, i.e., the twist about the central biphenyl bond, responsible for the chiral optical behavior near 250 nm.[24a,b,d] We could reasonably expect that a preference for one twist sense, *M* or *P* (see Figure 1), would depend on the side-chain conformation and the resulting relationship of the biphenyl group to the helical main chain.[25] This structural relationship, i.e., the spatial disposition of the side chain and backbone, could be a subject for force-field calculations,[26] which have been extremely valuable in conformational studies of other PBLG derivatives;[9,12] thus, one could be in a position in which a proposed side-chain conformation would be predicted to result in a preferred biphenyl twist sense, the latter experimentally accessible from chiral optical information.[24] This idea stimulated the syntheses of a series of polypeptides bearing the, as described, biphenyl "reporter groups" exhibited in Figure 2.

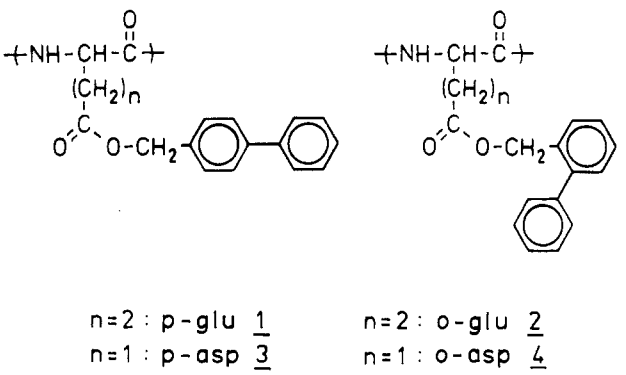

Figure 2. Poly(*p*-biphenylmethyl L-glutamate) (1); poly(*o*-biphenylmethyl L-glutamate) (2); poly(*p*-biphenylmethyl L-aspartate) (3); poly(*o*-biphenylmethyl L-aspartate) (4).

Results

Synthesis. To ensure a uniform side-chain ester structure, the sought polypeptide esters (1–4; Figure 2) were prepared from the *N*-carboxyanhydrides rather than by ester interchange. L-Aspartic and L-glutamic acids were monoesterified with the appropriate biphenylmethyl bromide at room temperature following a method based on copper chelate chemistry, the latter to protect the α-carboxyl group.[28,29] The esters were converted to the *N*-carboxyanhydrides following Goodman[30] prior to polymerization using triethylamine.[31] Combination of infrared spectrometry and intrinsic viscosity measurements demonstrated polymer formation in the case of the *N*-carboxyanhydrides, leading to **1**, **3**, and **4** (Figure 2). In the case of **2** we could not obtain a crystalline anhydride, and attempts to form the derived polypeptide of high molecular weight failed. See the Experimental Section for further details.

Ultraviolet and Circular Dichroism Spectra. The ultraviolet (UV) spectra and circular dichroism (CD) spectra for polypeptides **1**, **3**, and **4** are shown in Figures 3–5, respectively. Inspection of Figures 3–5 shows the expected blue shift and a decrease in the extinction coefficient of the *o*-biphenyl ester **4** (Figure 5) compared to the *p*-biphenyl esters **1** and **3**. This is associated with the expected larger twist angle arising from increased steric interactions in the ortho biphenyl in **4**.[32] The CD spectrum of **4** (Figure 5) exhibits a large positive band centered near 220 nm in *p*-dioxane, which could be associated with a left-handed helical conformation, as is known for poly(α-benzyl L-aspartate.[33] The spectral region associated with the biphenyl chromophore near 240 nm in the CD spectrum does show a small negative dichroism, which might indicate a twist preference in the biphenyl group. Although the spectral information in the poly(*p*-biphenyl aspartate) (**3**) is limited by the insolubility of this polypeptide, forcing use of the marginally transparent *N*-methylpyrrolidinone, nevertheless one sees little evidence of dichroism in the region of the biphenyl band (Figure 4).

The UV and CD spectra (Figure 3) of **1** show a strong circular dichroism of molar extinction near 10 000 deg·cm^2/

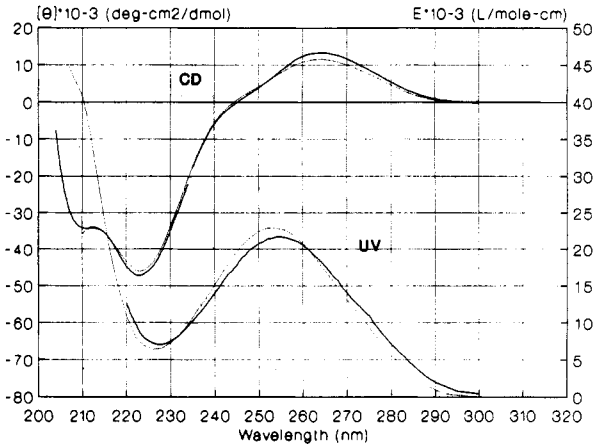

Figure 3. UV and CD spectra of poly(*p*-biphenylmethyl L-glutamate) (1) at a concentration of 2 g/L in 1,4-dioxane (—) and tetrahydrofuran (. . .).

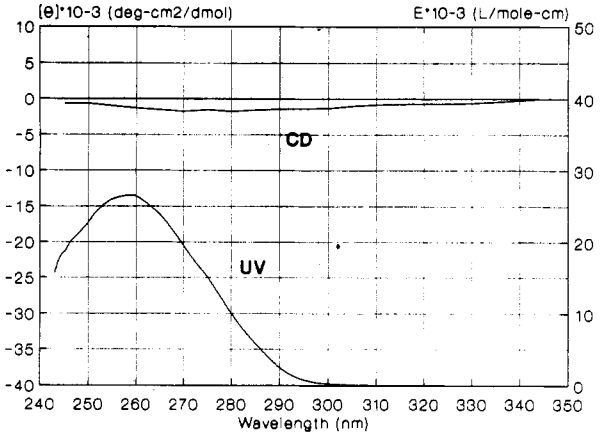

Figure 4. UV and CD spectra of poly(*p*-biphenylmethyl L-aspartate) (3) at a concentration of 0.4 g/L in *N*-methylpyrrolidinone.

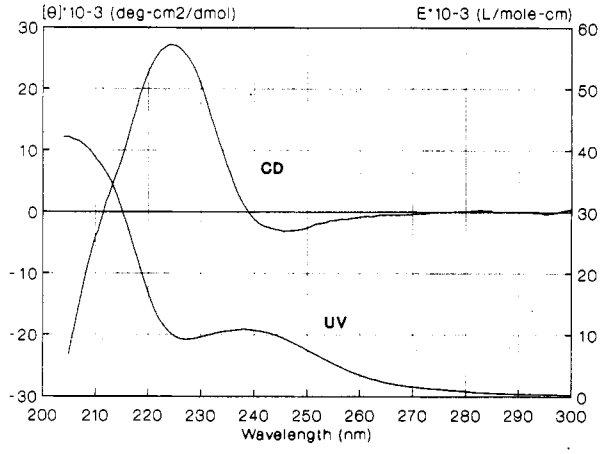

Figure 5. UV and CD spectra of poly(*o*-biphenylmethyl L-aspartate) (4) at a concentration of 1 g/L in 1,4-dioxane.

dmol for a band centered at 263 nm, which is clearly associated with the biphenyl chromophore at 255 nm in the UV spectrum. The CD spectrum also shows the known features, beginning at about 220 nm, associated with the α-helix as, for example, observed in poly(γ-benzyl L-glutamate) (PBLG) and other α-helix-forming polypeptides.[21] The same features as observed in the CD spectrum in Figure 3 in tetrahydrofuran (THF) are also seen in chloroform, 1,2-dichloroethane (DCE), and *p*-dioxane (*p*-D) within their accessible spectral windows.

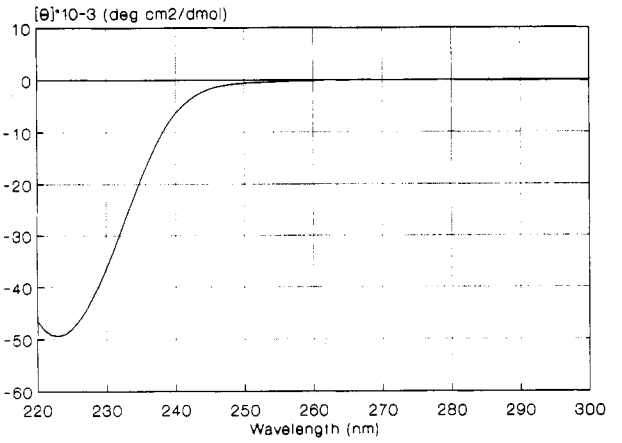

Figure 6. CD spectrum of poly(γ-benzyl L-glutamate) (PBLG) at a concentration of 1 g/L in 1,2-dichloroethane at −11.8 °C.

Our interest in the circular dichroism feature near 260 nm in 1 (Figure 3) is increased by the absence of any circular dichroism in the aromatic region in poly(γ-benzyl L-glutamate), as evidenced in the literature, for dilute solutions, and confirmed by a spectrum taken here under identical conditions (Figure 6). In addition, the naphthyl ester of poly(glutamic acid), an aromatic chromophore with no internal twisted conformational possibilities, shows a much smaller (strongly concentration-dependent) ellipticity in the aromatic region[19d] while poly(γ-phenacyl L-glutamate),[19c] which could be preferentially twisted in analogy to the biphenyl (a factor not previously considered[19c]), shows a larger ellipticity than that for 1. This is also seen in poly(glutamic acid) bearing azobenzene groups, where again a potentially internally twisted chromophore gives rise to large ellipticities in the trans azo aromatic chromophore.[19g] It could reasonably follow that assignment of the circular dichroism band at 263 nm in 1 arises from an internal conformational feature of the biphenyl group, namely, a distortion of the normally equally populated *M* and *P* twist senses about the biphenyl bond (Figure 1). Although the intensively studied[24] circular dichroism character of optically active bridged biphenyls is consistent with this conclusion and suggests an assignment of an excess of the *M* twist sense[24b] about the biphenyl group in 1, the magnitude of the excess is less clear. The UV maximum in 1 occurs at 255 nm, suggesting an analogous twist angle to a biphenyl with a bridge of between three and four members.[24a,d] Such bridged biphenyls exhibit molar ellipticities of near 50 000 deg·cm²/dmol[24a,d] while the comparable intensity in 1 is only about 20% of that value. These is, however, a difference of uncertain significance between the CD spectral characteristic of 1 and that of bridged biphenyls with similar UV spectra. The CD band in 1 centered near 260 nm is unusually broad and red-shifted compared to that of its UV spectrum (Figure 3), a feature not found in the bridged biphenyls.[24a,d]

A preferential sense of twist about the biphenyl bond in the ester group in 1, as suggested by the CD data discussed above, could likely rest on the diastereomeric relationship between the *M* and *P* conformational states of the biphenyl group and the helical character of the main chain rather than on relationships centered in the amino acid residues' stereogenic centers. It would follow that disruption of the main-chain helix, i.e., via the helix–coil transition, must substantially decrease the excess population of one twist sense about the biphenyl bond. In agreement with earlier workers,[34] we find, by viscosity measurements, that 1 undergoes the helix–coil transition

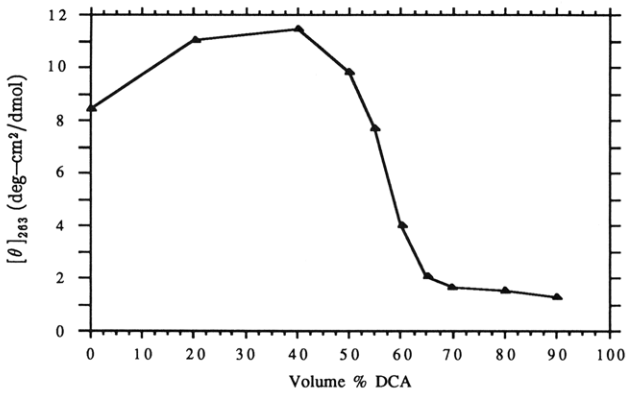

Figure 7. Molar ellipticity at 263 nm versus the volume percent of dichloroacetic acid (DCA) in 1,2-dichloroethane (DCE) for poly(*p*-biphenylmethyl L-glutamate) (1) at 25 °C; M_v = 112 000.

in the range of 55–60% dichloroacetic acid (DCA) in 1,2-dichloroethane (DCE). Following this transition, by monitoring the molar ellipticity at 263 nm, shows a sudden and substantial decrease parallel to the viscosity change, consistent with a reduced preference for one of the biphenyl twist senses in 1 (Figure 7) as the polypeptide forms the coil. This transition was shown to be fully reversible. It is interesting that the substantially reduced molar ellipticity does not go to zero, perhaps suggesting the presence of helical regions even at high DCA concentrations. This could be consistent with the formation of a liquid crystal lyotropic state for high concentrations of PBLG in DCA.[6] Although its meaning is uncertain, it is interesting also to note that, in the same solvent, 1,2-dichloroethane (DCE), the helix–coil transition in 1 requires a smaller percentage of DCA than PBLG,[35] i.e., 55% vs 76%, for comparable molecular weights.

With the likelihood, based on the helix–coil transition properties of 1, that the senses of twist (*M* and *P*) about the biphenyl bond are diastereomerically related as a consequence of the interaction with the α-helical main chain, and not from the α-carbon stereogenic center, we sought to increase the difference in relative population of *M* and *P*, and therefore the molar ellipticity at 263 nm, by lowering the temperature. This experiment, conducted by observing the D-line optical rotation as a function of temperature, was at first disappointing in that hardly any temperature dependence was found. Nevertheless, we were astonished when, near −3 °C, the optical activity suddenly changed steeply to large negative values. This is shown in Figure 8 for two molecular weights of 1 in 1,2-dichloroethane. Figure 9 exhibits the temperature dependence of the $[\alpha]_D$ of 1 in 1,2-dichloroethane, tetrahydrofuran, chloroform, 10:1 1,2-dichloroethane/dichloroacetic acid, and 10:1 1,2-dichloroethane/dimethylformamide (v/v). Also shown in Figure 9 are the comparable data for poly(γ-benzyl L-glutamate) in 1,2-dichloroethane. In Figure 10 is shown for 1 the plot for $[\alpha]_D$ vs temperature (°C) at several concentrations in 1,2-dichloroethane.

To uncover the spectral transitions responsible for the changes seen in Figures 8–10, we studied the temperature-dependent circular dichroism (CD) spectra in 1,2-dichloroethane, tetrahydrofuran, and chloroform of the two molecular weights of 1 reported in Figure 8. This spectral work corroborates the $[\alpha]_D$ changes in showing a sudden shift in the CD spectrum in the region of the 255-nm ultraviolet band associated with the biphenyl group. The data for one of the molecular weights of 1 in tetrahydrofuran are shown in Figure 11. The observed changes in 1,2-dichloroethane are identical with those in THF, while the CD spectrum of 1 in chloroform changes only slightly

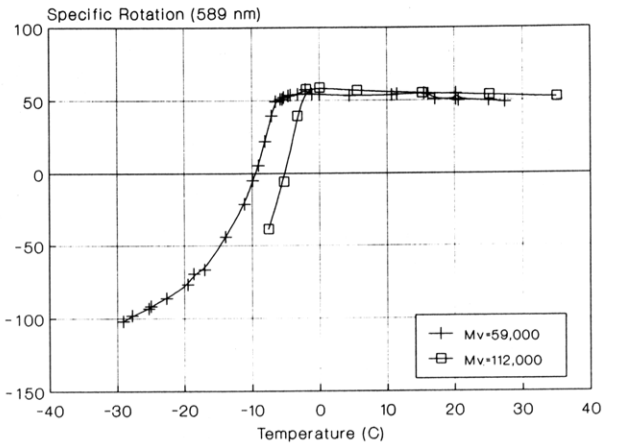

Figure 8. Specific rotation versus temperature of poly(*p*-biphenylmethyl L-glutamate) (1) at a concentration of 1 g/L in tetrahydrafuran: M_v = 58 000 (+), M_v = 112 000 (□).

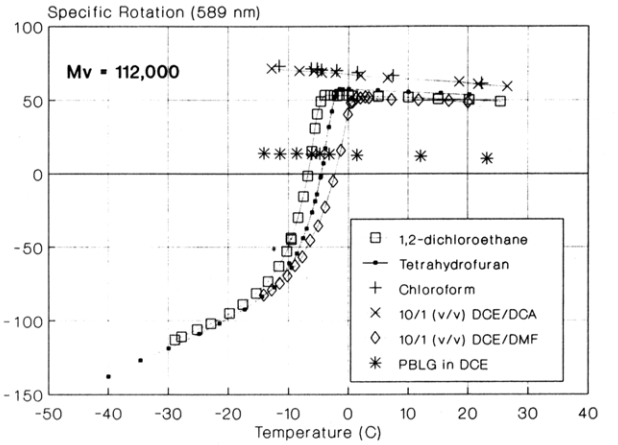

Figure 9. Specific rotation versus temperature of poly(*p*-biphenylmethyl L-glutamate) (1) at a concentration of 3 g/L in various solvents; M_v = 112 000.

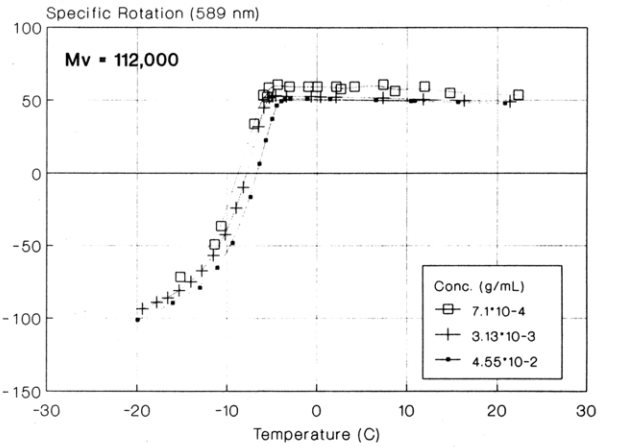

Figure 10. Specific rotation versus temperature of poly(*p*-biphenylmethyl L-glutamate) (1) 1,2-dichloroethane (DCE), at various concentrations; M_v = 112 000.

in intensity through the same temperature region as we would expect from the $[\alpha]_D$ results (Figure 9). As seen in Figure 11, the broad CD band in the region of 260 nm at room temperature splits, over a narrow temperature range, into a series of minima and maxima while the negative dichroism at 222 nm increases greatly in intensity. Most of the changes with temperature in the CD spectra (Figure 11) are associated with the biphenyl chromophore, although the changes at 222 nm could involve at least in part the backbone amide chromophore associated with the α-helix.

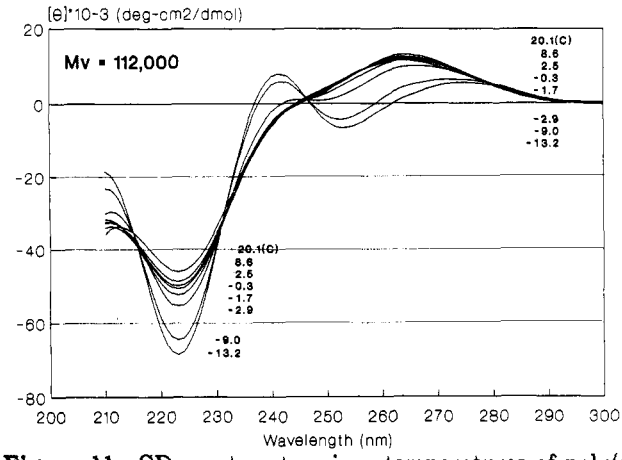

Figure 11. CD spectra at various temperatures of poly(*p*-biphenylmethyl L-glutamate) (1) at a concentration of 2 g/L in tetrahydrofuran; M_v = 112 000.

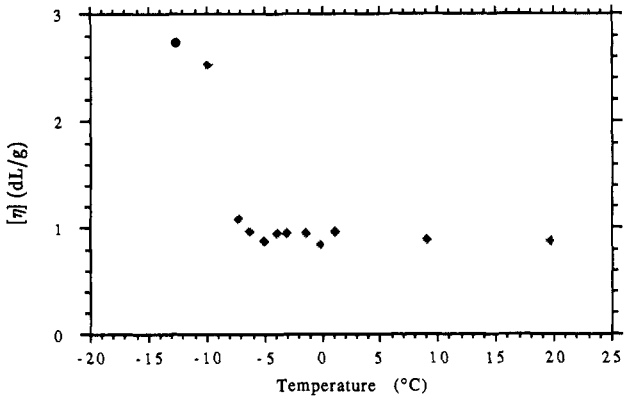

Figure 12. Intrinsic viscosity versus temperature of poly(*p*-biphenylmethyl L-glutamate) (1) in 1,2-dichloroethane (DCE); M_v = 112 000.

Changes from α-helical peptide conformation to β-forms or random coils, however, are known to lead to a decrease in intensity for the amide bands at 222 nm and below.[16,21]

Intrinsic Viscosity in DCE. Figure 12 presents the temperature dependence of the intrinsic viscosity, in DCE for **1**, M_v = 112 000, through the region where the sudden chiral optical changes take place (Figures 8–11). There is an obvious change in the viscosity near the critical temperature in Figures 8–11, pointing to an increase in particle weight, i.e., to aggregation. Aggregation is well-known in polypeptides, and in polyglutamates in particular.[36] Certainly the optical activity and intrinsic viscosity characteristics are correlated, although from the information presented above one could not assign cause and effect. Let us look further.

Ultraviolet Spectral Temperature Dependence. Although the CD spectrum is strongly temperature dependent we find that the UV spectrum of **1**, shown in Figure 13, is essentially unaffected by temperature. In detail, variation between +20 and −20 °C causes no change in shape and a slight increase in extinction coefficient, less than 5% at 253 nm and less than 3% at 210 nm. Significantly, though, this slight change in extinction coefficient occurs almost entirely in the region of the temperature of the chiral optical changes. Since the UV absorption characteristics of polypeptides, in the region of the amide chromophore below 215 nm, are known to be highly sensitive to backbone conformation,[37] one might conclude that, from the small effects here, there is little change in main-chain conformation associated with the CD changes (Figure 11). Although this view may be correct, the

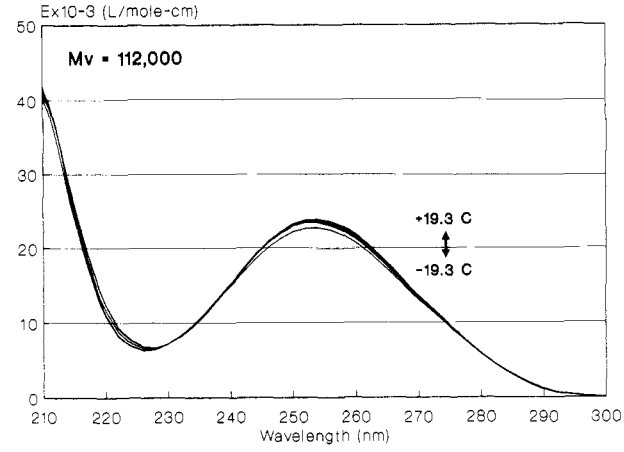

Figure 13. UV spectra at various temperatures of poly(*p*-biphenylmethyl L-glutamate) (1) at a concentration of 1 g/L in tetrahydrofuran; M_v = 112 000.

argument is weakened by our inability to penetrate to lower wavelengths where the strong hypochroism effect found in the α-helical state is important and by the intensity of the absorption at 210 nm in **1**. The latter, 40 800 L/mol·cm (THF; Figure 13) is far larger than either poly(γ-benzyl L-glutamate) or other polypeptides without side-chain chromophores, demonstrating therefore the strong contribution of the biphenyl group in this wavelength region. This was confirmed by the fact, determined here, that the model nonpeptide *p*-biphenylmethyl acetate is essentially indistinguishable in its UV characteristics from **1**. This raises an interesting point, since the CD pattern at room temperature, as seen in Figure 3, shows the typical intensity and shape of the polypeptide α-helix,[21] suggesting only small biphenyl side-chain contribution in this wavelength region. In contrast, in the spectra taken at low temperatures, where the biphenyl band near 260 nm changes greatly (Figure 11), there is a strong increase in the circular dichroism intensity at 222 nm. It is certainly reasonable that this intensity change in the CD spectrum at 222 nm is derived from a contribution to the chiral optical characteristics by the biphenyl group, which comes into play only at the lower temperatures.

Infrared Spectra. Since the UV spectral characteristics offered no information concerning a conformational source for the sudden temperature-dependent chiral optical changes, we looked further, i.e., into the infrared spectrum. The amide I and II bands in the regions of 1600 and 1500 cm^{-1}, respectively, are known to strongly shift for differing main-chain conformational states.[38] In **1** these bands occur at 1651 and 1551 cm^{-1}, respectively, in tetrahydrofuran at 20 °C, while the side-chain ester carbonyl appears at 1737 cm^{-1}. At −20 °C the amide I and II bands hardly change, to 1650 and 1553 cm^{-1}, while the ester carbonyl changes less than 0.5 cm^{-1}. In chloroform, where the sudden chiral optical shifts are not seen (Figure 9), the positions of the amide bands at both temperatures (+20 or −20 °C) are within 1 cm^{-1} of those reported above. In chloroform, the ester carbonyl is shifted from its position in tetrahydrofuran, i.e., to 1732 cm^{-1}, and is similarly unaffected by temperature.

Excimer Fluorescence. Since neither UV nor IR spectrometry revealed any conformational basis for the temperature-dependent chiral optical shifts, we decided to take another approach using a fluorescence-based technique. Although an early report[39] indicated that PBLG did not exhibit excimer emission in its fluorescence spectrum,[40] Samulski and Chapin[41] reported significant excimer formation for PBLG in methylene chloride. This

Table I
I_e/I_m (T, °C) for Poly(*p*-biphenylmethyl L-glutamate) (1)[a] at Various Concentrations (g/mL)

3.341×10^{-3}	9.206×10^{-4}	3.330×10^{-4}	9.206×10^{-5}
0.60 (25.0)	0.27 (21.0)	0.23 (19.6)	0.19 (19.0)
0.63 (21.5)	0.27 (22.4)	0.28 (1.0)	0.22 (3.4)
0.64 (13.5)	0.30 (9.0)	0.29 (−11.0)	0.25 (−15.5)
0.66 (3.5)	0.34 (−11.0)		
0.68 (−6.2)	0.33 (−14.0)		
0.68 (−12)			
0.68 (−19)			
0.61 (20.0)			

[a] In tetrahydrofuran. Solutions were filtered through 0.45-μm PTFE, purged with dried argon for 1 h. All measurements were conducted in a N_2 atmosphere. Excitation wavelength, λ_{ex} = 292 nm. I_e at 375 nm; I_m at 315 nm.

was attributed to intramolecular side-chain stacking of the phenyl groups. We have corroborated this observation for PBLG in THF,[42] suggesting that comparable measurements on 1 as a function of temperature might reveal changes in the interrelationships of the biphenyl groups responsible for the chiral optical effects seen (Figures 8–11). Table I shows the ratio of excimer to monomer fluorescence (I_e/I_m) at various concentrations as a function of temperature.[42] The ratios were characterized from the emission peak maximum at 315 nm and the shoulder at 375 nm, corresponding to monomer and excimer fluorescence, respectively. The excitation wavelength was 292 nm following the literature on biphenyl groups.[43] An inspection of the data in Table I reveals that the ratio I_e/I_m has a significant concentration dependence, which can be shown to be linear at all temperatures over the wide concentration range shown (Table I). Extrapolation to infinite dilution yields a ratio (I_e/I_m) of 0.20 at 20 °C. This suggests that the observed excimer formation is contributed to by intermolecular factors with perhaps a residual intramolecular component.[36,44] However, as seen in Table I, only a small temperature dependence, significantly, with an inflection near the transition temperature (~−5 °C), is observed over the region where the chiral optical shifts are seen (Figures 8–11). Since the conformational relationships among the biphenyl groups could be expected to strongly affect the ratio I_e/I_m, we are inclined to conclude that the sudden change in chiral optical properties (Figures 8–11) does not derive from a significant change in short-range biphenyl interactions.

Discussion

(1) Our original intent in preparing the various biphenyl esters 1–4 was to probe the side-chain conformation of the synthesized polypeptides, thereby addressing a problem of longstanding and continuing interest.[2c] Reasonably, the sought relationship between the biphenyl sense of twist and the main-chain conformation is found in 1, as evidenced in Figure 3, and discussed in the Results. The fact that there is any relationship at all between the biphenyl twist sense and the main-chain α-helix is alone enough to point to certain probable side-chain conformations in 1. The biphenyl group is a full six bonds removed from the helical backbone with the additional length of a benzene ring before reaching the critical biphenyl central bond. The nature of the α-helix of the polyglutamates also precludes close spatial relationships among the many biphenyl groups on different side chains. Indeed only pairwise close interactions are structurally possible. The possibility that chiral information is transferred among different chains by interpenetration of side chains cannot be excluded as the cause of the effects observed here (Figure 3), although change of concentration by a factor of 2×10^4 at room temperature causes almost no change in the molar optical activity intensity of the biphenyl group. This is shown in the polarimeter data (Figure 10) and also in CD spectra taken down to a concentration of 4×10^{-6} g/mL.[45] Although this weak concentration dependence argues against, but does not preclude, a significant intermacromolecular source of the preferential sense of biphenyl twist found in 1, an intermolecular source is made more unlikely by the fact that DCA up to about 30 vol % does not largely affect the $[\theta]_{263}$ intensity (Figure 7). DCA is well-known to break up polypeptide intermolecular interactions, leading to the observation of intramolecular properties.[36]

The data and arguments above leave a close spatial relationship between the biphenyl group and the α-helix as a reasonable source of the connection between the α-helix and the biphenyl twist sense in 1, as discussed in the Results (Figure 3). A force-field calculation[26] to determine the preferred sense of twist about the biphenyl bond as a function of side-chain conformation, which the CD spectrum of 1 indicates is M, must be the next step in helping to evaluate this model. This required aspect of the work is not yet accomplished. Although there is no requirement that the side-chain conformation be unaffected by substitution of biphenyl for phenyl, nevertheless, the qualitative conclusion of a side-chain conformation in 1, which places the biphenyl group adjacent to the backbone, is consistent with longstanding arguments centered on the analogous PBLG. This work[9] points to compact longitudinal orientation of the side chains in PBLG, at least in vacuum, and has recently been enforced by the conclusions of one group on ^2H NMR observations made in liquid-crystal states of PBLG.[12]

(2) The great surprise in the work reported here was the temperature- and solvent-dependent chiral optical changes for 1 (Figures 8–11). At first we sought to understand this phenomenon through single macromolecular conformational effects, a point of view stimulated by the very weak concentration dependence of the sudden chiral optical changes (Figure 10).

As discussed in the Results, the changes in the CD spectra are dominated by the biphenyl chromophore, and there is no evidence for conformational changes in the backbone, since conformations other than the α-helix are known to reduce the CD intensity at 222 nm.[16,21] This is reinforced by the infrared spectra taken in THF at various temperatures. The amide I and II bands can be classically assigned to the α-helix and as well there is little change in their character through the temperature range of the chiral optical transition. This was also found for the ester carbonyl.

The UV spectrum of 1 as a function of temperature changed only slightly, but the demonstration that its features are entirely dominated by the biphenyl group (Figure 13) restricted conformational conclusions about the backbone. Nevertheless, there is no evidence from these UV data, for conformational changes, such as changes in twist angle in the biphenyl group. The excimer fluorescence data (see Results), which could be expected to be sensitive to changes in close interactions between biphenyl groups, were also only weakly sensitive to temperature, again offering no evidence for significant conformational changes. What then is the cause of the temperature-dependent chiral optical changes (Figures 8–11)?[46]

The intrinsic viscosity data as a function of temperature for 1 in DCE, as discussed in the Results (Figure 12), could be associated with a sudden increase in particle dimension,

which could arise from local conformational changes leading to a stiffening of the polypeptide chains. This is not likely here since at the higher temperature, i.e., above 0 °C, the chain is already in the extended α-helical state as evidenced by the CD spectrum (Figure 3). Collapse of chain dimension must arise from changes in local conformation, changes for which we can find no corroboration from the UV, IR, and fluorescence data. We are therefore left with no choice but the conclusion that the chiral optical changes and the associated viscosity changes occur in or nearly in concert with an increase in particle weight,[47] i.e., with aggregation or clustering.

We have looked for kinetic and hysteresis effects involved with the temperature-dependent chiral optical changes. Near the transition temperature at about −3 °C (Figures 8–10), we could not find any time dependency for the polarimeter response. In detail, as quickly as the temperature changed, the polarimeter apparently instantly followed. At lower temperatures, near and below −10 °C, this was not the case.

The $[\alpha]_D$ of a solution brought relatively rapidly (30 min) to this temperature range from ambient temperatures was measurably smaller ($[\alpha]_D = -38°$) than that of a solution brought to these temperatures over a period of several hours ($[\alpha]_D = -45°$). The solution, which was rapidly changed, then slowly attained the full value of the $[\alpha]_D$ over the course of about 1 h held at the low temperature.

It would be desirable to understand how such strong and sudden, i.e., phase-transition-like, chiral optical changes in dilute solution can be so weakly connected to concentration[48] (Figure 10), much more strongly dependent on molecular weight (Figure 8), and yet be associated in some manner with aggregation.

Reasonably, as the temperature is lowered in these studies the solvent quality decreases and in fact an excellent solvent like chloroform is incompatible with the phenomenon (Figure 9). An aggregation breaking additive like DCA, which improves the solvation qualities of the medium,[49] also causes the loss of the effect (Figure 9), while addition of nonsolvents for 1, DMF or ethanol,[45] causes the transition to occur at a higher temperature (Figure 9).

Such observations might be reconciled by allowing the possibility that aggregates, reasonably of various sizes and perhaps in equilibrium with individual chains, already exist above the temperature of the chiral optical transition (Figure 9). This view is supported by precedent for such aggregation in a variety of poly(glutamate) esters[36] and by our own preliminary light-scattering studies.[47] Further evidence is our observation that very small percentages of DCA, well below that necessary to cause transition to the coil, cause a sharp change in the optical activity from that in the pure solvent. This is also seen in PBLG where it has been associated with breakup of aggregation.[35] This is significant since small proportions of DCA also affect loss of the sudden chiral optical changes (Figure 9). Parallel to this, we have observed that small proportions of DCA at temperatures above the transition cause a decrease in intrinsic viscosity. Determination of the Huggins constant from the intrinsic viscosity measurements, a parameter known to be affected by intermolecular phenomena, shows interesting changes further supporting this view. Thus, at temperatures above the transition, the Huggins constants are large and erratic in the range of 1.4–4.4, indicative of aggregation. Further, in the temperature region of the transition the Huggins constants decrease steadily from 3.2 at −6.2 °C to 0.85 at −9.9 °C and then rise again as the temperature drops further. This is consistent with the hysteresis discussed above found not at the temperature of the optical activity change but below it.

By exclusion of solvent from the aggregate one could reach a point where the local polymer concentration within the aggregate is high enough to cause a transition to an ordered, for example, liquid-crystal-like, state. This proposal finds precedent in the conclusions of recent light-scattering studies on poly(1,4-phenylene-2,6-benzobisthiazole). In this work Wei-Beck and Berry demonstrate,[50] in dilute solution, a transition within a microaggregate from a protonated to a neutral state as increasingly poor solvent quality drives the aggregate to higher local density by exclusion of solvent. In the parallel proposal here the solution studied, although dilute, would, below the transition temperature, contain microscopic aggregates in which the aggregated rods (worms), as is the situation for the chiral 1, may be twisted, in the manner of a chloresteric.[51] Such a picture might help us in understanding our observations, since the aggregates with such an organized structure could be expected to grow larger for the same reasons causing the higher concentrations of anisotropic states in equilibrium with isotropic states in polymer liquid-crystal lyotropes.[52] This is consistent, as discussed above, with the absence of a time dependency on the $[\alpha]_D$ data at the transition, where according to these ideas[50] changes are occurring within the aggregates. At lower temperatures where the aggregates grow in size, we do observe hysteresis effects. In addition, since aggregates of chiral molecules are well-known to lead to important changes in chiral optical power for added chromophores or chromophores within the polymer chains,[53] the change in the nature of the aggregate proposed above would be consistent with the CD and $[\alpha]_D$ changes we are seeing, changes that would derive from the chiral relationships among the worms within the aggregate,[51] in addition to or for that matter exclusive of any conformational changes within the biphenyl moieties of the individual macromolecules. This idea suggests a comparison of the CD spectrum of the planar texture of the high-concentration lyotrope of 1 with the low-temperature CD spectra in Figure 11. PBLG shows unusual optical activity in the side-chain benzene chromophore in its cholesteric state.[17,51]

Certain soluble polydiacetylenes and poly(dialkylsilanes) exhibit strong and sudden changes in their electronic spectral characteristics associated with known conformational changes of the chains and aggregated states but with, as above, very weak concentration dependencies.[54,55] This has led to counterproposals that the aggregation is preceded by and driven by intramolecular conformational changes or that the conformational changes are preceded by and driven by the aggregation.[54,55] The polypeptide under study here, 1, shows that apparently parallel experimental observations can be made in a wormlike macromolecule where conformational changes within the polymer are not obvious and perhaps therefore not a prerequisite for the aggregation phenomena. This may therefore argue on the side of the view in the other systems[54,55] that the aggregation is a necessary attendant to the conformational changes, the latter in the polydiacetylenes and poly(dialkylsilanes) the ultimate prerequisite to the chromophoric changes.[54,55] A recent finding in a chiral soluble polydiacetylene may support this in demonstrating the presence of yellow aggregates in "good" solvent conditions before the transition to the red phase.[56]

Another example of a stiff polymer in which aggregation may drive conformational and subsequent sudden chiral optical changes has been encountered in our work on the wormlike polyisocyanates in dilute solution. In that case,

and in contrast to 1, thermally reversible gels form at moderate concentrations.[57] The formation of gels is well-known in PBLG in the broad biphasic region of the Flory phase diagram for stiff chain polymers.[58] The observations for 1 may also be associated with crossing the phase boundary into this biphasic region, but without gel formation, or precipitation, under the conditions studied here.

As a historical note, Tobolsky, 25 years ago,[59] argued for the existence of metastable microscopic crystalline aggregates which could resist precipitation. Chiral optical methods, now underutilized, likely offer unusual opportunities for insight into microscopic aggregates, a material state of increasing interest.[60]

Experimental Section

Routine infrared and ^1H and ^{13}C NMR data were taken on a Shimadzu IR-435 spectrometer, a Varian EMU-390 spectrometer, and a JEOL FX-90Q spectrometer, respectively. Elemental analyses were carried out by Schwarzkopf Microanalytical Laboratory (Woodside, NY). Melting points were determined on a Thomas–Hoover apparatus. Viscosities were measured on a single-bulb Cannon-Ubbelohde type viscometer uncorrected for shear with flow times greater than 100 s. Polarimetric measurements were made on a Perkin-Elmer 141 spectropolarimeter using a 1-dm jacketed cell with temperature control using a Haake circulating bath with ethanol as the cooling fluid. For temperatures below −15 °C an auxiliary Neslab Cryo-cool CC-60 of the immersion coil type was inserted into the Haake bath. All cooling lines and the polarimeter cell were insulated, and the temperature was measured both in the cooling fluid at the cell and at the surface of the cell window by an immersion and a contact thermocouple, respectively (Type T-Omega thermocouple connected to a Model 450 ATT thermocouple thermometer). The polarimeter was kept in a glovebag filled with nitrogen, obtained by impinging nitrogen from a liquid-nitrogen source on one of the cell windows. The flow of nitrogen was adjusted to keep the cell window within a fraction of a degree of the temperature of the cooling fluid in the cell. The measured rotations are accurate to ±0.003°, and the temperature accuracy is estimated as ±0.5 °C.

UV spectra were measured on a Varian Cary 2300 spectrometer using a 0.01-cm jacketed quartz cell. CD measurements were made on an AVIV 60 DS or on an AVIV 62 DS computerized spectrometer with jacketed quartz cells of various lengths using a built in Neslab constant-temperature cooling bath capable of 0.1 °C precision. We checked the temperature of the cell by an independent thermocouple and found them to be within 2 °C at 0 °C of the block temperature used to control the instrument cooling. Fourier transform infrared measurements (FTIR) were made on a Mattson Inc. Sirious 100 spectrophotometer using a liquid-nitrogen Dewar from Beckmann Instruments. Fluorescence measurements were made under a dry-nitrogen atmosphere on a Perkin-Elmer MPF-44B fluorescence spectrophotometer using a xenon lamp.[42] All solvents used for physical measurements were purchased from Aldrich Chemical Co. at the highest purity and further purified in all glass apparatuses without stopcock grease. 1,2-Dichloroethane was dried and distilled from CaH_2, dichloroacetic acid from $MgSO_4$ under reduced nitrogen pressure, THF from $LiAlH_4$ directly before each use, dioxane from sodium benzophenone ketyl directly before each use, chloroform from P_2O_5 and shaken with $CaCO_3$ directly before use, and DMF from P_2O_5 under reduced nitrogen pressure.

Synthetic Procedures. L-Glutamic and L-aspartic acids were purchased from Aldrich Chemical Co., as was the *o*-biphenylcarboxylic acid. The $[\alpha]_D$ values of the amino acids matched known values (Merck Index). The *p*-biphenylcarboxylic acid, purchased from Fluka Chemical Co. (mp 217–222 °C), was reduced with lithium aluminum hydride (LAH) in THF, and the resulting alcohol was recrystallized from $CHCl_3$: hexane, mp 98–100 °C (lit. mp 99–101 °C); yield 74%. The *o*-biphenylmethanol similarly produced was purified by distillation, 105–106 °C (0.04 mm), to a low-melting solid. The derived bromides were synthesized from the stereoisomeric biphenylmethanols (about 20 g of reactant) using a 3-fold excess of 48% HBr. The reaction was apparently over at 100 °C in 20 min for the para isomer and in about 2 h for the ortho isomer (as followed by TLC). *p*-Biphenylmethyl bromide (yield 90%) was purified by flash chromatography in 9:1 hexanes/methylene chloride, mp 85–86 °C. The *o*-biphenylmethyl bromide (yield 87%) was distilled at 94 °C (0.2 mm). ^1H NMR spectra fit the structures exactly.

γ-(4-Biphenylmethyl) L-Glutamate.[28] In a 1-L flask, 14.7 g (0.10 mol) of L-glutamic acid was dissolved in 375 mL of distilled water at 70 °C. A solution of 20 g of $Cu(C_2H_3O_2)\cdot H_2O$ (0.103 mol) (Aldrich, 98%) in 300 mL of water was added over a 2-h period with an additional 75 mL of water. The deep violet solution crystallized at room temperature to yield a blue precipitate (21.99 g, 90% yield) of Cu_2(L-glutamate)$_2\cdot 4H_2O$. A total of 2.74 g (5.6 mmol) of this salt and 1.66 g (11.2 mmol) of L-glutamic acid were finely ground and transferred to a flask with overhead stirring, and to this were added 10 mL of DMF and 1.6 mL of H_2O. A total of 2.8 mL (22.4 mmol) of 1,1,3,3-tetramethylguanidine (distilled from CaH_2) was added dropwise over a 30-min period, causing the formation of a clear deep blue solution (over a period of 90 min). DMF (8 mL) was slowly added. A total of 5.8 g (23.5 mmol) of 4-biphenylmethyl bromide (see above) was added all at once, and the mixture was heated to 35 °C; 5 mL of DMF, 0.5 mL of water, and 5 mL of acetone were added to improve mixing. After the mixture was stirred overnight at room temperature, the contents of the flask was transferred to a rapidly stirred solution of 5.1 g (13.7 mmol) of ethylenediaminetetraacetic acid disodium salt dihydrate (EDTA, Aldrich Chemical Co.) in 30 mL of water. Heat was applied (40 °C) with stirring, and a colorless solid precipitatee. After cooling in ice the mixture was filtered and the solid was triturated with cold water. After the solid was dried over P_2O_5, a 70% yield of the subject monoester was obtained: mp 189–190 °C (dec) (lit. mp 192.5–193.5 °C);[28] $[\alpha]_D$ +16.1° (c = 1, 1:1 DMF/formic acid) [lit.[28] $[\alpha]_D$ +9.4° (same conditions)].

γ-(2-Biphenylmethyl) L-Glutamate. This ester was prepared exactly parallel to the procedure described above: yield 51%; mp 147–148.5 °C (dec); $[\alpha]_D$ +14.7° (c = 1, 1:1 DMF/formic acid).

β-(4-Biphenylmethyl) L-Aspartate. The Cu_2(L-aspartate)$_2\cdot 8H_2O$[28] was prepared exactly as above and converted to the title monoester in the same manner: yield 62%; mp 232–233.5 °C (dec); $[\alpha]_D$ +30.0° (c = 0.6, 1:1 DMF/formic acid).

β-(2-Biphenylmethyl) L-Aspartate. This monomer was prepared exactly as described above: yield 54%, mp 182–183 °C (dec); $[\alpha]_D$ +17.1° (c = 0.65, 1:1 DMF/formic acid). In all of the monoesters the infrared carbonyl bands were consistent with the structure.[58]

α-[γ-(4-Biphenylmethyl)-L-glutamic acid] *N*-Carboxyanhydride. The procedure generally followed that of Goodman and co-workers[30] with the following modification. Toluene was used in place of benzene to form a 3 M solution of phosgene. Care was taken periodically to blow off reactant gases, i.e., HCl, which built up at the reflux head. This follows recommendations of Block:[30] yield 75% after three recrystallizations from THF; mp 177–178 °C (dec) (lit.[28] mp 174 °C); $[\alpha]_D$ −13.9° (c = 0.5, dioxane) [lit.[34] $[\alpha]_D$ −12.9° (same conditions)]. The infrared spectrum was consistent with the structure.

α-[γ-(2-Biphenylmethyl)-L-glutamic acid] *N*-Carboxyanhydride. This procedure followed that of Goodman and co-workers.[30] A crystalline product could not be obtained. The HCl blow-off procedure was not used here and might have helped.

α-[β-(4-Biphenylmethyl)-L-aspartic acid] *N*-Carboxyanhydride. The procedure followed that of Goodman and co-workers[30] as discussed above: yield 92%; mp 169–174 °C (dec); recrystallized two times from ethyl acetate/hexane (dry).

α-[β-(2-Biphenylmethyl)-L-aspartic acid] *N*-Carboxyanhydride. The procedure followed that of Goodman and co-workers[30] as discussed above: yield 78%; mp 130–132 °C (dec); recrystallized from ethyl acetate/hexane.

Poly[γ-(4-biphenylmethyl) L-glutamate] (1). The *N*-carboxyanhydride described above was dissolved in *p*-dioxane sealed under N_2 (see purification note above) with difficulty at 0.02 g/mL. Triethylamine, distilled from sodium, was injected at a ratio of 100:1 ([M] [I]). The reaction was allowed to continue for up to 15 days and was followed by the infrared spectrum. The solution was poured into rapidly stirred 60:40 ethyl acetate/

hexanes, producing a fibrous white material. This was precipitated from solution in p-dioxane into ethyl acetate/hexane: yield 65%; for $[\alpha]_D$, see Figures 8–10. The intrinsic viscosity, $[\eta]$, in DCA was 0.53 dL/g corresponding to a PBLG equivalent M_v of 112 000. A parallel polymerization with an initiator ratio of 150:1 ([M]/[I]) gave a polypeptide of $[\eta]$ in DCA = 0.3 dL/g, M_v (PBLG equivalent) = 58 000. For the $[\eta]$ in DCA the data had to be taken within 4 h to preempt decomposition. This was judged by TLC. Elem Anal. Calcd: C, 73.21; H, 5.80; N, 4.74. Found: C, 72.87; H, 5.69; N, 4.54. Infrared features are discussed in the text.

Poly[β-(4-biphenylmethyl) L-aspartate] (3). The polymerization procedure paralleled that above using an [M]/[I] ratio = 50 and p-dioxane as the solvent. The polymer was obtained by precipitation in ethanol as a white fibrous solid: yield 62%; $[\eta]$ 0.22 dL/g in N-methylpyrrolidinone; IR 1655 and 1520 (amide bands I and II), 3295 (amide A), 1730 (ester carbonyl), 1720 cm^{-1} (sh). Elem Anal. Calcd: C, 72.58; H, 5.37; N, 4.98. Found: C, 72.08; H, 5.50; N, 4.86.

Poly[β-(2-biphenylmethyl) L-aspartate] (4). The polymerization was carried out as discussed above except in chloroform: yield 69%; $[\eta]$ 0.22 dL/g in chloroform; IR 1662 (amide I), 1555 and 1535 (amide II), 1735 (ester carbonyl), 3300 cm^{-1} (amide A). Elem Anal. Calcd: C, 72.58; H, 5.37; N, 4.98. Found: C, 72.64; H, 5.42; N, 4.95.

Poly[γ-(2-biphenylmethyl) L-glutamate] (2). The N-carboxyanhydride precursor here as discussed above could not be crystallized. Purification was attempted by evacuation of volatile impurities, e.g., phosgene, and the remaining liquid (off color) was treated as above with triethylamine in p-dioxane; [M]/[I] = 50. After 15 days precipitation was effected by pouring the reaction mixture into an excess of 95% ethanol, slowly yielding a fine white powder: yield 12%; IR 1625 (str, amide I) and 1690 (w, amide I) 1520 (str, amide II), and 1550 (m, amide II), 3295 (amide A), 1735 cm^{-1} (ester carbonyl).

Acknowledgment. We are grateful to the National Science Foundation (Grant CHE-8615872) and the donors of the Petroleum Research Fund, administered by the American Chemical Society, for their generous financial support for this work. We also thank Professor G. C. Berry for many helpful discussions and Professor G. Wegner for making us aware of the work in ref 55. The FTIR measurements were carried out by Dr. M. Davies in the laboratory of Professor R. Mendelsohn at Rutgers University, and for this we extend our gratitude. We are also indebted to Professor W. Agosta for allowing us to take CD measurements at Rockefeller University (instrument purchased with support of the National Institute of Health) and to Mr. Gerald Schultz for undertaking important preliminary experiments that led to the development of this work.

References and Notes

(1) Taken from: Reidy, M. P. Ph.D. Thesis, Polytechnic University, 1990.
(2) For the best leading reference to this literature, see: (a) *Poly-α-Amino Acids*; Fasman, G. D., Ed.; Marcel Dekker: New York, 1967. (b) Fasman, G. D. *Biopolymers* **1987**, *26*, 559. (c) Piela, L.; Nemethy, G.; Scheraga, H. A. *Biopolymers* **1987**, *26*, 1273.
(3) Cantor, C. R.; Schimmel, P. R. *Biophysical Chemistry*; Freeman: New York, 1980; Chapter 2, Part 1.
(4) For one recent example of this work, see: Burley, S. K.; Petsko, G. A. *Science* **1985**, *229*, 23. See also: Holtzer, A. *Bipolymers* **1989**, *28*, 901.
(5) DeGrado, W. F.; Kezdy, E. J.; Kaiser, E. T. *J. Am. Chem. Soc.* **1981**, *103*, 679. For more recent work see: DeGrado, W. F.; Wasserman, Z. R.; Lear, J. D. *Science* **1989**, *243*, 622.
(6) Block, H. *Poly(γ-Benzyl-L-Glutamate) and Other Glutamic Acids Containing Polymers*; Gordon and Breach: New York, 1983.
(7) Pauling, L.; Corey, R. B. *J. Am. Chem. Soc.* **1950**, *72*, 5349.
(8) Perutz, M. F. *Nature* **1951**, *167*, 1053.
(9) Yan, J. F.; Vanderkooi, G.; Scheraga, H. A. *J. Chem. Phys.* **1968**, *49*, 2713. Ooi, T.; Scott, R. A.; Vanderkooi, G.; and Scheraga, H. A. *J. Chem. Phys.* **1967**, *46*, 4410. See also: Reference 2c. Piela, L.; Scheraga, H. A. *Biopolymers* **1987**, *26*, S33.
(10) Yan, J. F.; Momany, F. A.; Scheraga, H. A. *J. Am. Chem. Soc.* **1970**, *92*, 1109. Erenrich, E. H.; Andreatta, R. H.; Scheraga, H. A. *J. Am. Chem. Soc.* **1970**, *92*, 1116.
(11) Bradley, D. F.; Goodman, M.; Felix, A.; Records, R. *Biopolymers* **1966**, *4*, 607, Hashimoto, M.; Aritomi, J. *Bull. Chem. Soc. Jpn.* **1966**, *39*, 2707. Konishi, Y.; Hatano, M. *J. Polym. Sci., Polym. Lett.* **1976**, *14*, 351.
(12) Meirovitch, E.; Samulski, E. T.; Leed, A.; Scheraga, H. A.; Rananavare, S.; Nemethy, G.; Freed, J. H. *J. Phys. Chem.* **1987**, *91*, 4840. Abe, A.; Yamazaki, T. *Macromolecules* **1989**, *22*, 2138. See also: Yamazaki, T.; Abe, A.; Ono, H.; Toriumi, H. *Biopolymers* **1989**, *28*, 1959.
(13) For efforts involving dielectric properties, see the following and references therein: Wada, A. In *Poly-α-Amino Acids*; Fasman, G. D., Ed.; Marcel Dekker: New York, 1967; Chapter 9. Sharp, M. *J. Chem. Soc. A* **1970**, 1596. Erenrich, E. H.; Scheraga, H. A. *Macromolecules* **1972**, *5*, 746. Nishioka, N.; Mishima, H.; Teramoto, A. *Polym. J.* **1977**, *9*, 641. Pyzuk, W. *Polym. J.* **1978**, *19*, 673. For a related experiment, see: Charney, E.; Milstien, J. B.; Yamaoka, K. *J. Am. Chem. Soc.* **1970**, *92*, 2657.
(14) NMR studies directed to side-chain conformation can be found in the following leading references: Silverman, D. N.; Scheraga, H. A. *Biochemistry* **1971**, *10*, 1340. Silverman, D. N.; Taylor, G. T.; Scheraga, H. A. *Arch. Biochem. Biophys.* **1971**, *146*, 587. Bradbury, E. M.; Carpenter, B. G.; Crane-Robinson, C.; Goldman, H. *Macromolecules* **1971**, *4*, 557. Many NMR studies have focused on the role of the side chain in the helix–coil transition. See: Bovey, F. A. *Pure Appl. Chem.* **1968**, *16*, 417. Chien, M.; Samulski, E. T.; Wade, C. G. *Macromolecules* **1973**, *6*, 638. Sase, S.; Suzuki, Y.; Inoue, Y.; Chujo, R. *Biopolymers* **1977**, *16*, 95. Pivoca, H.; Doskocilova, D.; Bradbury, E. M. *Polymer* **1979**, *20*, 139. Modern multidimensional NMR techniques have been applied to side-chain conformations in peptides: Kessler, H.; Griesinger, C.; Wagner, K.; *J. Am. Chem. Soc.* **1987**, *109*, 6927. Kessler, H.; Bats, J. W.; Griesinger, C.; Koll, S.; Will, M.; Wagner, K. *Ibid.* **1988**, *110*, 1033. Solid-state NMR methods have also been used: Shoji, A.; Osaki, T.; Saito, H.; Tabeta, R.; Ando, I. *Macromolecules* **1984**, *17*, 1472.
(15) See Erenrich and Scheraga compared to Nishioka, Mishima, and Teramoto in ref 13 above and also: Egusa, S.; Sisido, M.; Imanishi, Y. *Polym. J.* **1986**, *18*, 403. Sisido, M.; Egusa, S.; Imanishi, Y. *J. Am. Chem. Soc.* **1983**, *105*, 1041.
(16) Cantor, C. R.; Schimmel, P. R. *Biophysical Chemistry*; Freeman: New York, 1980; Chapter 8, Part II.
(17) Nomori, H.; Tsuchihashi, N.; Tokagi, S.; Hatano, M. *Bull. Chem. Soc. Jpn.* **1975**, *48*, 2522.
(18) Hashimoto, M. *Bull. Chem. Soc. Jpn.* **1966**, *39*, 2713. Hashimoto, M.; Arakawa, S. *Ibid.* **1967**, *40*, 1698. Bradley, D. F.; Goodman, M.; Felix, A.; Records, R. *Biopolymers* **1966**, *4*, 607. LeBarny, P.; Loucheux-Lefebvre, M. H. *Ibid.* **1973**, *12*, 2831. Fraser, R. D. B.; Harrap, B. S.; Ledger, R.; Macrae, T. P.; Stewart, F. H. C.; Suzuki, E. *Ibid.* **1967**, *5*, 797. Bradbury, E. M.; Carpenter, B. G.; Goldman, H. *Ibid.* **1968**, *6*, 837. For related work, see: Matsuoka, M.; Norisuye, T.; Termaoto, A.; Fujita, H. *Ibid.* **1973**, *12*, 1515.
(19) The leading articles in each polymer type with references therein to earlier literature are as follows: (a) Ueno, A.; Ishiguro, Y.; Toda, F.; Uno, K., Iwakura, Y.; *Biopolymers* **1975**, *14*, 353. (b) Sisido, M.; Okamoto, A.; Egusa, S.; Imanishi, Y. *Polym. J.* **1985**, *17*, 1253. (c) Heitz, F.; Marchal, E.; Spach, G. *Macromolecules* **1975**, *3*, 145. (d) Enomoto, T.; Nomori, H.; Hatano, M. *Chem. Lett.* **1974**, 1289. Ueno, A.; Toda, F.; Iwakura, Y. *Biopolymers* **1974**, *13*, 1213. (e) Yoshikawa, M.; Nomori, H.; Hatano, M. *Makromol. Chem.* **1978**, *179*, 2397. (f) Ueno, A.; Takahashi, K.; Anzai, J.; Osa, T.; *Macromolecules* **1980**, *13*, 459. (g) Fissi, A.; Pieroni, O. *Macromolecules* **1989**, *22*, 1115 and references to earlier literature in this area. (h) Ciardelli, F.; Fabbri, D.; Pieroni, O.; Fissi, A. *J. Am. Chem. Soc.* **1989**, *111*, 3470. (i) Toriumi, H.; Saso, N.; Yasumoto, Y.; Sasaki, S.; Uematsu, I. *Polym. J.* **1979**, *11*, 977.
(20) Matsuoka, M.; Norisuye, T.; Teramoto, A.; Fujita, H. *Biopolymers* **1973**, *12*, 1515. Yamamoto, H.; Nishida, A.; Hayakawa, T.; Nishi, N.; Yamamoto, R. *Bull. Chem. Soc. Jpn.* **1986**, *59*, 1641. Fissi, A.; Pieroni, O.; Ciardelli, F. *Biopolymers* **1987**, *26*, 1993.
(21) Beychok, S. In *Poly(α-Amino Acids)*; Fasman, G. D., Ed. Marcel Dekker: New York, 1967; Chapter 7, p 312 ff. Polypeptides with side-chain aromatic groups offer difficulty in the interpretation of main-chain circular dichroism. For a review in this area see: Tinoco, I., Jr.; Williams, A. L. Jr. *Annu. Rev. Phys. Chem.* **1984**, *35*, 329. See also: Peggion, E.; Cosani, A.; Terbojevich, M.; Palumbo, M. In *Optically Active Polymers*; Selegny, E., Ed.; Reidel: Boston 1979; pp 231–252.

(22) See Sisido and co-workers in ref 15 above. See also: Sisido, M.; Imanishi, Y. *Macromolecules* **1986**, *19*, 2187. Sisido, M.; Egusa, S.; Imanishi, Y. *J. Am. Chem. Soc.* **1983**, *105*, 4077.
(23) In vinyl copolymers with side-chain aromatic chromophores the reduced spectroscopic complexity allows greater success. See: Hug, W.; Ciardelli, F.; Tinoco, I., Jr. *J. Am. Chem. Soc.* **1974**, *96*, 3407. For leading references to later work by Ciardelli and co-workers on CD studies of synthetic, optically active polymers, see: Farina, M. In *Topics in Stereochemistry*; Wiley and Sons: New York, 1987; Vol. 17, p 85.
(24) (a) Mislow, K. *Ann. N.Y. Acad. Sci.* **1962**, *93*, 457. (b) Mislow, K.; Glass, M. A. W.; O'Brien, R. E.; Rutkin, P.; Steinberg, D. H.; Weiss, J.; Djerassi, C. *J. Am. Chem. Soc.* **1962**, *84*, 1455. (c) Bunnenberg, E.; Djerassi, C.; Mislow, K.; Moskowitz, A. *J. Am. Chem. Soc.* **1962**, *84*, 2823. (d) Mislow, K.; Bunnenberg, E.; Records, R.; Wellman, K.; Djerassi, C. *J. Am. Chem. Soc.* **1963**, *85*, 1342.
(25) In biphenyl the *M* and *P* twist senses are related as enantiomers but the energetic degeneracy of this state is broken, in principle, as a consequence of the polypeptide chirality. The observation of this energy difference will depend on the magnitude, which will be related to structural and conformational factors as discussed in the text. For a discussion of twisted biphenyls see: Brock, C. P.; Minton, R. P. *J. Am. Chem. Soc.* **1986**, *111*, 4586.
(26) Burkert, A.; Allinger, N. L. *Molecular* Mechanics; ACS Monograph 177; American Chemical Society: Washington, DC, 1982; p 274 ff and reference therein.
(27) Bradbury, E. M.; Carpenter, B. G.; Goldman, H. *Bipolymers* **1968**, *6*, 837. Konishi, Y.; Hatano, M. *J. Polym. Sci., Polym. Chem. Ed.* **1976**, *14*, 2329. Watanabe, J.; Goto, M.; Nagase, T. *Macromolecules* **1987**, *20*, 298.
(28) Van Heeswijik, W. A. R.; Eeniuk, M. J. D.; Feijen, J. *Synth. Commun.* 744 (Sept 1982).
(29) Ledger, R.; Stewart, F. H. C. *Aust. J. Chem.* **1965**, *18*, 1477.
(30) Fuller, W. D.; Verlander, M. S.; Goodman, M. *Biopolymers* **1976**, *15*, 1869. See also: Block, L. H. Reference 6; Chapter 2 and p 30 in particular.
(31) Blout, E. R.; Karlson, R. H. *J. Am. Chem. Soc.* **1956**, *78*, 941.
(32) For analogous biphenyl spectral changes, see: Jaffé, H. H.; Orchin, M. *Theory and Applications of Ultraviolet Spectroscopy*; Wiley and Sons: New York, 1962; p 406.
(33) See: Bovey, F. A. Reference 14 above.
(34) Feijen, J.; Sederel, W. L.; deGroot, K.; deVisser, A. C.; Bantjes, A. *Makromol. Chem.* **1974**, *175*, 3193.
(35) Doty, P.; Yang, J. T. *J. Am. Chem. Soc.* **1956**, *78*, 498.
(36) See ref 6, 81 ff. For the original observations see: Doty, P.; Bradbury, J. H.; Holtzer, A. M. *J. Am. Chem. Soc.* **1956**, *78*, 947. See also: Jennings, B. R.; Spach, G.; Schuster, T. M. *Bipolymers* **1968**, *6*, 635. Elias, H. G.; Gerber, J. *Makromol. Chem.* **1968**, *112*, 122. Powers, J. C., Jr.; Peticolas, W. L. *Bipolymers* **1970**, *9*, 195. Gupta, A. K.; Dufour, C.; Marchal, E. *Biopolymers* **1974**, *13*, 1293. Gupta, A. K. *Bipolymers* **1976**, *15*, 1543. Pyzuk, W.; Krupkowski, T. *Makromol. Chem.* **1977**, *178*, 817.
(37) See ref 2a, Chapter 5.
(38) See ref 2a, Chapter 2 and references therein. For more recent references in this area, see ref 6.
(39) Longworth, J. W. *Bipolymers* **1966**, *4*, 1131.
(40) For a helpful discussion and leading references to excimer fluorescence, see: Morawetz, H. *Macromolecules in Solution*, 2nd ed.; Wiley and Sons: New York, 1974; pp 241–242.
(41) Samulski, E. T.; Chapin, J. T. *Polym. Prepr. (Am. Chem. Soc., Div. Polym. Chem.)* **1980**, *21*, 67.
(42) We have observed in PBLG that degassing with an inert gas (argon) is essential to this observation. In our work see, for a sample M_v = 85 000, I_e/I_m = 0.7 for 2 × 10^{-4} g/mL in THF (filtered 0.45 μm) at 20 °C. Our maxima are 280 and 315 nm (shoulder) with an excitation wavelength of 258 nm. The wavelengths closely match those reported in the literature for polystyrene.
(43) Goldenberg, M.; Emert, J.; Morawetz, H. *J. Am. Chem. Soc.* **1978**, *100*, 7171.
(44) A significant concentration dependence was also observed for PBLG in THF (see ref 42 above). In addition, only a small change in I_e/I_m with temperature was observed.
(45) Unpublished observation in this laboratory by J. Nanos.
(46) For possible parallels in other polypeptides see: Heitz, F.; Marchal, E.; Spach, G. *Macromolecules* **1975**, *8*, 145. Fissi, A.; Pieroni, O. *Ibid.* **1989**, *22*, 1115. See also: Itou, T.; Teramoto, A.; Matsuo, T.; Suga, H. *Ibid.* **1986**, *19*, 1234.
(47) Preliminary static wide-angle light-scattering measurements on 1 in THF, taken by MPR in Professor G. C. Berry's laboratory at Carnegie-Mellon University, confirm this increase in particle weight at temperatures below the chiral optical transition point. The data also indicate aggregation both at and below room temperature. Quasi-elastic light-scattering results show in THF and DCE, but not in $CHCl_3$, a sudden decrease in translational diffusion constant near the temperature of the chiral optical transition.
(48) Precipitation, the ultimate consequence of aggregation, in a system in equilibrium, is strongly concentration and molecular weight dependent. See: Flory, P. J. *Principles of Polymer Chemistry*; Cornell University Press: Ithaca, NY, 1953; p 546 ff.
(49) See numerous observations of the effect of added DCA and also trifluoroacetic acid to depress aggregation in polypeptides in ref 36 and reference therein.
(50) Berry, G. C.; Wei, C. C.; Furukawa, R. *Polym. Prepr. (Am. Chem. Soc., Div. Polym. Chem.)* **1986**, *27*, (No. 1), 228. Wei-Beck, C.; Berry, G. C., submitted to *J. Appl. Polym. Sci., Appl. Polym. Symp.*
(51) We have attempted, without success, in dilute solution near the transition temperature to look for visible particles by microscopy. This would require the proposed aggregated states to be small in comparison to the wavelength of visible light and therefore would not allow a conventional cholesteric twisted state with a fully formed pitch if the pitch is comparable to polypeptide lyotropes, which are normally in the infrared dimension. See: Uematsu, I. *Advances in Polymer Science*; Springer-Verlag: Berlin, 1984; Vol. 59, pp 37–73 and references therein. For the most recent paper in this area, see: Itou, S. *Mol. Cryst. Liq. Cryst.* **1989**, *172*, 201. Polypeptides in thermotropic states show reduced pitch dimensions. See: Watanabe, J.; Nagase, J. *Macromolecules* **1988**, *21*, 171.
(52) For a general treatment of polymer liquid crystals, see: *Polymer Liquid Crystals*; Ciferri, A., Krigbaum, W. R., Meyer, R. B., Eds.; Academic Press: New York, 1982; for this reference Chapter 3 in particular. For a good example of this concentration effect, see: Conio, G.; Bianchi, E.; Ciferri, A.; Krigbaum, W. R. *Macromolecules* **1984**, *17*, 856.
(53) See: Saeva, F. D. In *Liquid Crystals, The Fourth State of Matter*; Saeva, F. D., Ed.; Marcel Dekker: New York, 1979; Chapter 6, p 249 ff. Aggregated states of chiral polymers show special CD properties including scattering phenomenon. See: Bustamante, C.; Maestre, M. F.; Tinoco, I., Jr. *J. Chem. Phys.* **1980**, *73*, 4273 and subsequent papers in that series.
(54) For the poly(dialkylsilanes), see: Trefonas, P.; Damewood, J. R.; West, R.; Miller, R. D. *Organometallics* **1985**, *4*, 1318. For the most recent paper in this area, see: Schilling, F. C.; Lovinger, A. J.; Ziegler, J. M.; Davis, D. D.; Bovey, F. A. *Macromolecules* **1989**, *22*, 3055. See also: Miller, R. D.; Michl, J. *Chem. Rev.* **1989**, *89*, 1359 and in particular pp 1385 ff.
(55) Leading references to the large literature of the polydiacetylenes are as follows: Lim, K. C.; Heeger, A. J. *J. Chem. Phys.* **1985**, *82*, 522. Rawiso, M.; Aime, J. P.; Fave, J. L.; Schott, M.; Muller, M. A.; Schmidt, M.; Baumgartl, H.; Wegner, G. *J. Phys. Fr.* **1988**, *49*, 861. For recent papers; see: Xu, R.; Chu, B. *Macromolecules* **1989**, *22*, 3153; Nava, A. D.; Thakur, M.; Tonelli, A. E. *Macromolecules* **1990**, *23*, 3055.
(56) Drake, A. F.; Udvarhelyi, P.; Ando, D. J.; Bloor, D.; Obhi, J. S.; Mann, S. *Polymer* **1989**, *30*, 1063.
(57) Green, M. M.; Reidy, M. P.; Johnson, R. D.; Darling, G.; O'Leary, D. J.; Willson, G. *J. Am. Chem. Soc.* **1989**, *111*, 6452.
(58) Russo, P. S.; Magestro, P.; Miller, W. G. In *Reversible Polymeric Gels and Related Systems*; ACS Symposium Series 350; Russo, P. S., Ed. American Chemical Society: Washington, DC, 1987; Chapter 11.
(59) Tobolsky, A. V. *J. Phys. Chem.* **1964**, *68*, 2267.
(60) Antonetti, M. *Angew. Chem., Int. Ed. Engl.* **1988**, *27*, 1743. P. S. Russo in ref 58, Chapter 1.

Registry No. 1, 53972-73-9; 1 (SRU), 53987-22-7; 1 carboxyanhydride, 128842-65-9; 2, 128823-14-3; 2 (SRU), 128823-18-7; 2 carboxyanhydride, 128842-66-0; 3, 125658-70-0; 3 (SRU), 128823-19-8; 3 carboxyanhydride, 128842-67-1; 4, 128823-15-4; 4 (SRU), 128823-20-1; 4 carboxyanhydride, 128842-68-2.