Experimental Verification of Predicted Helix Sense of Two Polyamino Acids[1]

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Abstract: In order to check theoretical predictions of the helix senses of poly-β-*o*- and *m*-chlorobenzyl-L-aspartate, these polymers, together with the *para* derivative, were synthesized, and their respective screw senses determined by ORD and CD in various solvents. In all helix-supporting solvents studied, poly-β-*p*-chlorobenzyl-L-aspartate was observed to have a right-handed and the *ortho* and *meta* isomers had a left-handed helix sense, in agreement with predictions.

The screw senses of approximately 25 homopolyamino acid α-helices have been computed[4-6] and are in agreement with experimental data, where available, in all cases but one (poly-β-ethyl-L-aspartate[6]). These calculations thus provide an understanding of the factors determining the (known) helix senses of these polymers. In two cases (poly-L-valine[5] and poly-γ-*p*-chlorobenzyl-L-glutamate[6]) experiments on the helix senses were subsequently carried out,[7,8] and the predicted helix senses were verified.[9]

In order to extend the theoretical calculations to unknown systems, thereby enabling further predictions to be made, the computations in the previous paper[10] were carried out. In particular, it has been predicted[10] that the left-handed α-helical forms of poly-β-*o*- and -*m*-chlorobenzyl-L-aspartate should be more stable than the right-handed ones. Therefore, these polymers were synthesized and their ORD and CD spectra measured in various solvents. The *para* isomer, known to form a right-handed α-helix, both according to experiment[11-14] and theory,[6] was also synthesized and studied.

Experimental Section

Materials. In the syntheses, the solvents used for recrystallization of N-carboxy anhydrides (NCA) and for polymerizations were purified shortly before use, dioxane and hexane by distillation over sodium, and triethylamine by drying and distillation over KOH. Dimethylformamide (DMF) was Fisher spectral grade, and was used without purification. *o*-, *m*-, and *p*-chlorobenzyl chlorides were from Aldrich Chemical Co. Inc., and lithium copper L-aspartate was prepared by the procedure of Ledger and Stewart.[15]

For the measurement of optical properties, spectral grade DMF from Fisher and dioxane from Matheson Coleman and Bell were used without further purification. Matheson Coleman and Bell ethylene dichloride (EDC) was dried over P_2O_5 and fractionally distilled. Fisher dichloroacetic acid was used without purification for viscosity measurements.

General Synthetic Procedure. For the synthesis of poly-β-*o*-, -*m*-, and -*p*-chlorobenzyl-L-aspartates (*o*-ClPBLA, *m*-ClPBLA, and *p*-ClPBLA), the procedure of Ledger and Stewart[15] was followed. Treatment of lithium copper L-aspartate[15] with *o*-, *m*-, and *p*-chlorobenzyl chloride in the presence of potassium iodide in aqueous DMF yielded copper β-*o*-, -*m*-, and -*p*-chlorobenzyl-L-aspartates. These copper complexes were converted, without purification, into the free β-esters (β-*o*-chlorobenzyl-, β-*m*-chlorobenzyl-, and β-*p*-chlorobenzyl-L-aspartate; I, II, and III) with EDTA in an overall yield of 8.5–19%. Treatment with phosgene in the usual manner gave the three isomeric β-chlorobenzyl-L-aspartate NCA's IV, V, and VI. The polymerization of the NCA's was initiated with triethylamine in dioxane (A/I = 25), and the polymers VII, VIII, and IX were obtained as white powders by addition of the reaction mixture to a large amount of vigorously stirred ether.

Of the three β-chlorobenzyl-L-aspartate polymers prepared for this study, the *para* isomer IX has been described previously in the literature.[11-14] For the synthesis of the NCA-monomer VI, Hashimoto and Aritomi[11] carried out a selective β-esterification of L-aspartic acid with *p*-chlorobenzyl alcohol, using *p*-toluenesulfonic acid as catalyst. They obtained a preparation of III in a 58.5% yield whose physical characteristics (mp and rotation at the sodium D-line) (see below) differ markedly from the ones of the product obtained here, prepared by the copper-complex method. However, conversion of their β-ester preparation into the NCA VI yielded a product whose physical characteristics, after recrystallization, correspond to the ones of our NCA. Conversion of such aspartic acid monoesters into their NCA's by treatment with phosgene proceeds in high yields. The fact that Hashimoto and Aritomi obtained only 35% yield, together with the fact that the method of esterification used in their study is known to yield mixtures of α- and β-esters,[11,16,17] suggests that their β-ester preparation must have been highly contaminated with α-ester. The method of esterification used in our study precludes the formation of α-esters.

Melting points are uncorrected. The values reported for $[\alpha]_D$ for all intermediates in the syntheses were measured on a Cary Model 60 spectropolarimeter. Elemental analyses were performed by Galbraith Laboratories, Inc., Knoxville, Tenn. Prior to analysis, the compounds were dried *in vacuo* over P_2O_5 at 60°. Using Merck's silica gel G plates, thin layer chromatograms were obtained with the following solvent systems: $R_f{}^1$, 1-butanol–acetic acid–

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(9) In the case of poly-γ-*p*-chlorobenzyl-L-glutamate, the predicted[6] energy difference between the right- and left-handed α-helical forms was very small (and within the error of the calculations); hence, the verification[8] of the prediction in this case is not, in itself, an establishment of the validity of the calculations.

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(17) T. Hayakawa, J. Noguchi, H. Nishi, S. Ikeda, T. Yamashita, and T. Isemura, *ibid.*, **82**, 601 (1961).

water, 60:20:20; R_f^2, 1-butanol–pyridine–acetic acid–water. 30:20:6:24. Neutralization equivalents were determined by titration of the compounds in absolute ethanol with standard sodium methoxide, using thymol blue as indicator.

β-*o*-Chlorobenzyl-L-aspartate (I). A mixture of lithium copper L-aspartate[15] (17.0 g), *o*-chlorobenzyl chloride (16.1 g), and potassium iodide (8.3 g) in water (100 ml) and DMF (100 ml) was stirred at 40° for 48 hr. The mixture was cooled to room temperature, acetone (250 ml) was added, and the solid copper complex of I was collected and washed with acetone (300 ml) and water (300 ml).

The material was dissolved in boiling 0.1 *M* EDTA disodium salt (pH 4.5, adjusted with acetic acid) (450 ml). A very small amount of insoluble material was removed by filtration while still hot. The desired product crystallized upon cooling: white needles, which were washed with water; 4.86 g (19%); mp 211–212° dec; $[\alpha]^{20}D$ +10.2° (*c* 2, 0.1 *N* HCl); $[\alpha]^{20}D$ +8.6° (*c* 1, glacial acetic acid); R_f^1 0.53; R_f^2 0.50; single ninhydrin-positive spot; neutralization equivalent 259.4 (theor 257.7).

Anal. Calcd for $C_{11}H_{12}NO_4Cl$: C, 51.27; H, 4.69; N, 5.44; Cl, 13.76. Found: C, 51.18; H, 4.74; N, 5.28; Cl, 13.97.

β-*m*-Chlorobenzyl-L-aspartate (II). The *m*-chlorobenzyl ester was prepared, as described above, from lithium copper-L-aspartate (17.0 g) and *m*-chlorobenzyl chloride (16.1 g): white plates; 2.18 g (8.5%); mp 217–218° dec; $[\alpha]^{20}D$ +6.6° (*c* 1, glacial acetic acid); $[\alpha]^{20}D$ +13.8° (*c* 2, 0.1 *N* HCl); R_f^1 0.53; R_f^2 0.49; single ninhydrin-positive spot; neutralization equivalent 249.6 (theor 257.7).

Anal. Calcd for $C_{11}H_{12}NO_4Cl$: C, 51.27; H, 4.69; N, 5.44; Cl, 13.76. Found: C, 51.57; H, 4.67; N, 5.27; Cl, 13.54.

β-*p*-Chlorobenzyl-L-aspartate (III). The β-*p*-chlorobenzyl ester was prepared, as described above, from lithium copper L-aspartate (17.0 g) and *p*-chlorobenzyl chloride (16.1 g): white plates; 3.575 g (14%); mp 225–226° dec; $[\alpha]^{20}D$ +9.5° (*c* 2, glacial acetic acid); R_f^1 0.53; R_f^2 0.53; single ninhydrin-positive spot; neutralization equivalent 254.8 (theor 257.7).

Anal. Calcd for $C_{11}H_{12}NO_4Cl$: C, 51.27; H, 4.69; N, 5.44; Cl, 13.76. Found: C, 51.54; H, 4.78; N, 5.42; Cl, 13.78.

Hashimoto and Aritomi[11] prepared the same compound in 58.5% yield from L-aspartic acid by selective β-esterification with *p*-chlorobenzyl alcohol: mp 208° dec; $[\alpha]^{23}D$ +25.6° (*c* 1, glacial acetic acid).

β-*o*-Chlorobenzyl-L-aspartate-NCA (IV). A slow stream of phosgene was passed through a suspension of I (2.58 g) in dioxane (100 ml). Simultaneously, the temperature was raised by means of a water bath up to 50°. When the reaction mixture became clear (after approximately 1 hr) the flow of phosgene was stopped and replaced by a stream of dry nitrogen. Heating was also discontinued at this point. After another 1 hr, the reaction mixture was concentrated to dryness and the residue dried *in vacuo* over P_2O_5. The crude solid was triturated with hexane, collected, and washed with hexane. Recrystallization from dioxane–hexane gave white needles: 2.12 g (75%); mp 127–128° dec; $[\alpha]^{20}D$ −22.1° (*c* 2, ethyl acetate).

Anal. Calcd for $C_{12}H_{10}NO_5Cl$: C, 50.81; H, 3.55; N, 4.94; Cl, 12.50. Found: C, 50.69; H, 3.62; N, 4.85; Cl, 12.37.

β-*m*-Chlorobenzyl-L-aspartate-NCA (V). Ester II (5.16 g) was treated with phosgene, as described above, to give the NCA V which crystallized from dioxane–hexane in white needles: 5.13 g (90%); mp 104–105° dec; $[\alpha]^{20}D$ −26.6° (*c* 2, ethyl acetate).

Anal. Calcd for $C_{12}H_{10}NO_5Cl$: C, 50.81; H, 3.55; N, 4.94; Cl, 12.50. Found: C, 50.64; H, 3.52; N, 4.96; Cl, 12.44.

β-*p*-Chlorobenzyl-L-aspartate-NCA (VI). Ester III (5.16 g) was converted to its NCA by treatment with phosgene, as described above: white needles from dioxane–hexane; 5.49 g (97%); mp 161–163° dec; $[\alpha]^{20}D$ −28.0° (*c* 2, ethyl acetate).

Anal. Calcd for $C_{12}H_{10}NO_5Cl$: C, 50.81; H, 3.55; N, 4.94; Cl, 12.50. Found: C, 50.67; H, 3.65; N, 4.80; Cl, 12.32.

Hashimoto and Aritomi[11] prepared the same compound in 35% yield from a β-*p*-chlorobenzyl-L-aspartate preparation: mp 162°; $[\alpha]^{24}D$ −30.1° (*c* 2, ethyl acetate).

Polymerization of NCA's. The following standard procedure was used in all cases. An NCA (0.01 mol) was dissolved in dioxane (30 ml), and a 0.4 *M* solution of triethylamine in dioxane (1.0 ml; A/I = 25) added. The mixture was kept in a desiccator over calcium chloride for 4 days and then added to vigorously stirred ether (300 ml). The precipitate was collected on a filter, washed with several portions of ether and dried *in vacuo* over P_2O_5. Data on the polyamino acids prepared are collected in Table I.

Molecular Weights. The intrinsic viscosity, [η], of each polymer was measured, as previously described.[8] The degree of polymerization (DP) was then estimated roughly by converting these data to a molar basis and using the calibration curve of Mitchell, *et al.*,[18] for poly-γ-benzyl-L-glutamate (PBLG), assuming that this curve was applicable to the substituted PBLA's. The data are shown in Table II.

Table I. Analytical Data for Polyamino Acids

Polyamino acid	Yield, %[a]	Microanalytical data, % found[b] C	H	N	Cl
o-ClPBLA (VII)	74	55.46	4.29	5.77	14.63
m-ClPBLA (VIII)	85	55.00	4.01	5.58	14.70
p-ClPBLA (IX)	38	55.08	4.25	5.60	14.70

[a] Based on NCA used. [b] Calculated for $(C_{11}H_{10}NO_3Cl)_n$: C, 55.12; H, 4.21; N, 5.84; Cl, 14.79.

Table II. Rough Estimates of DP from [η]

Polyamino acid	[η], (g/100 ml)$^{-1}$	DP
o-ClPBLA	0.15	~70
m-ClPBLA	0.11	~45
p-ClPBLA	0.23	~150

ORD and CD Measurements. ORD measurements were carried out and the data treated as described previously.[8] Circular dichroism measurements were made with the Model 6001 attachment to the Cary 60 spectropolarimeter. The molar ellipticity, $[\theta]_\lambda$, in deg cm^2/dmol was calculated from the equation

$$[\theta]_\lambda = \frac{\theta_\lambda M_0}{100cl}$$

where θ_λ = observed ellipticity in degrees, M_0 = residue molecular weight, c = concentration in g/cm^3, and l = pathlength in decimeters.

Determination of Concentration. The concentration of each solution was calculated on the basis of dry weight of polymer. This concentration was verified in most cases by either Kjeldahl analysis for nitrogen or by measurement of the optical absorption and use of an extinction coefficient calculated for a solution whose concentration had been determined by nitrogen analysis. In all cases where the concentration was measured by more than one method, the results were consistent to within a few per cent.

Results and Discussion

The values of b_0 in various solvents are shown in Table III. Assuming that b_0 = −630 and +630[19] for fully helical right- and left-handed α-helices, respectively, it appears that the *ortho* and *meta* isomers form left-handed α-helices and that the *para* isomer forms a right-handed α-helix in EDC and in dioxane. The data for the *para* isomer agree with those of Hashimoto, *et al.*[11-14] In addition, Hashimoto and Aritomi[11] obtained b_0 data on copolymers of the *para* isomer with β-benzyl-L-aspartate, confirming the conclusion from the homopolymer that the helix of the *para* isomer is right-handed in chloroform.

The b_0 values for the *ortho* and *meta* isomers in DMF indicate that the polymers are random coils in this solvent. While this conclusion is not proven, it is in accord with arguments[12] suggesting that PBLA is a random coil in this solvent.

The ORD data at short wavelengths for the three isomeric polymers in dioxane are shown in Figure 1; the spectra in EDC were similar. The minimum for the *para* isomer and the maxima for the *ortho* and *meta*

(18) J. C. Mitchell, A. E. Woodward, and P. Doty, *J. Amer. Chem. Soc.*, 79, 3955 (1957).

(19) J. T. Yang in "Poly-α-Amino Acids," G. D. Fasman, Ed., Marcel Dekker, New York, N. Y., 1967, p 239.

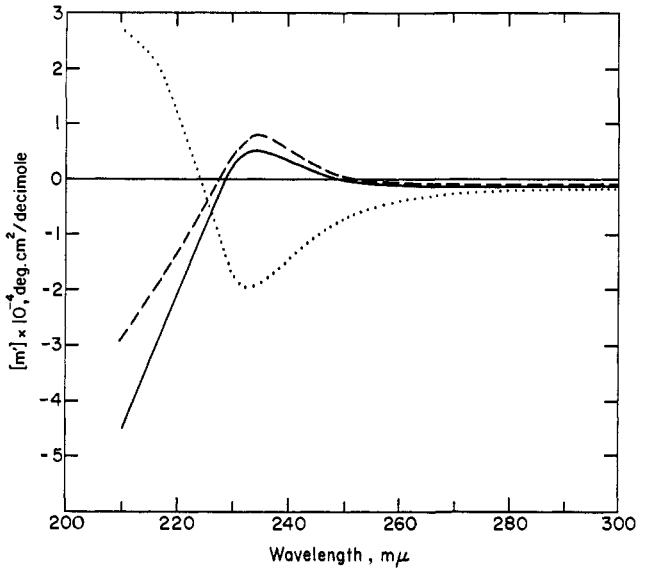

Figure 1. ORD of substituted poly-β-benzyl-L-aspartates in dioxane: ——, *o*-ClPBLA at 40°; - - - - -, *m*-ClPBLA at 25°; · · · ·, *p*-ClPBLA at 25°.

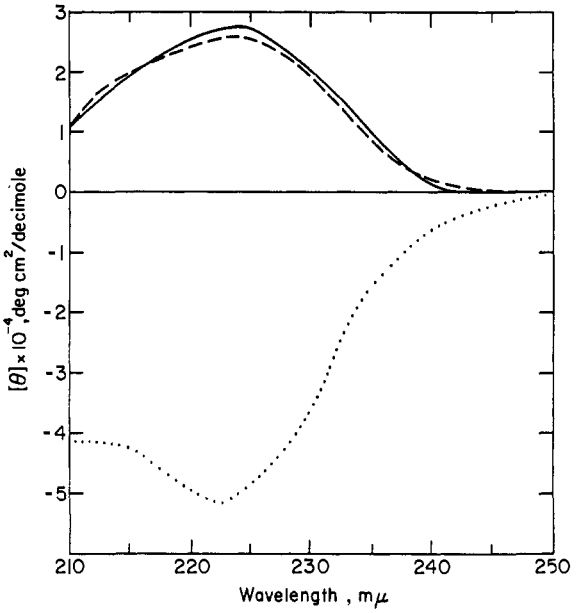

Figure 2. CD of substituted poly-β-benzyl-L-aspartates in dioxane at 25°: ——, *o*-ClPBLA (the measurement being obtained rapidly before turbidity appeared at this temperature); - - - - -, *m*-ClPBLA; · · · · ·, *p*-ClPBLA.

isomers at 233 mμ support the conclusion from the b_0 data that the former is a right-handed α-helix and the latter are left-handed ones.

Table III. Moffitt-Yang b_0 Parameters

Solvent	b_0 at 25° *o*-ClPBLA	*m*-ClPBLA	*p*-ClPBLA	PBLA
EDC	+510	+590	−640	
Dioxane	+520[a]	+560	−680	
DMF	−170	−170	−500	−315[c,d] (at 30°C)
			−554[b]	
$CHCl_3$			−686[b]	+600[e] (at 20°C)
				+630[f] (at 20°C)
DCA				−250[e] (at 20°C)
				−250[f] (at 20°C)

[a] This measurement was made at 40°C (where the solution was clear) to avoid the turbidity which appeared on standing at 25°. [b] η_{sp}/c = 0.21, for a 0.5% solution in DCA.[11] [c] Goodman, *et al.* (M. Goodman, A. M. Felix, C. M. Deber, A. R. Brause, and G. Schwartz, *Biopolymers*, **1**, 371 (1963)), report that this compound is insoluble in DMF; also see ref 11. [d] η_{sp}/c = 0.3, for a 0.5% solution in DCA.[11] [e] η_{sp}/c = 3.0, for a 0.5% solution in DCA: E. M. Bradbury, A. R. Downie, A. Elliott, and W. E. Hanby, *Proc. Roy. Soc., London*, **A259**, 110 (1960). [f] η_{sp}/c = 0.26, for a 0.2% solution in DCA: R. H. Karlson, K. S. Norland, G. D. Fasman, and E. R. Blout, *J. Amer. Chem. Soc.*, **82**, 2268 (1960).

The CD data in dioxane, shown in Figure 2, lead to the same conclusions about helix sense. Similar trends are evident in EDC (not shown), but the entire peak cannot be seen because of the high absorption by this solvent. The *ortho* and *meta* isomers display a maximum and the *para* isomer a minimum at ~224 mμ. Although the familiar "double extremum" spectrum normally observed for the α-helix[20] is not seen for the *ortho* and *meta* isomers, the spectra of these compounds as shown in Figure 2 are very similar to that for poly-β-methyl-L-aspartate (PMLA),[21,22] which is thought to form a left-handed α-helix. The CD spectrum of the *para*-isomer is qualitatively similar to that expected for a right-handed α-helix.[20]

We believe that the results reported above confirm the predictions[10] that the *ortho* and *meta* isomers form left-handed α-helices, and agree with previous results[6,11-14] that the *para* isomer forms a right-handed α-helix. In the remainder of this discussion, some other features of the ORD and CD spectra of these polyamino acids are considered.

The ORD and CD data of Figures 1 and 2 differ somewhat from the spectra usually associated with the α-helix. In part, the differences are probably due to the fact that the polymers studied here are not fully helical. However, the data of Figures 1 and 2 cannot be accounted for solely in terms of calculated ORD[23] and CD[24] curves based on mixtures of α-helix with random coil or β-structures. Also, theoretical calculations for both the parallel and antiparallel β-structures[25] indicate that neither should have positive circular dichroism near 224 mμ. The observed differences probably reside either in effects from the side-chain chromophores, in slight variations in the geometry of these compounds from that of a standard α-helix, or in differences (other than an inversion in sign) in the optical properties of left- and right-handed α-helices of L-amino acids.

In the case of *p*-ClPBLA, the differences in spectral properties between this compound and other typical right-handed α-helices are relatively small. The b_0 value of −680 is quite close to the value of −630[19] for

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(21) D. W. Urry, *Ann. Rev. Phys. Chem.*, **19**, 477 (1968).

(22) Urry[21] reports that this CD was measured in $CHCl_3$ and was able to obtain data down to ~190 mμ even though $CHCl_3$ begins to absorb strongly at ~225 mμ.

(23) N. Greenfield, B. Davidson, and G. D. Fasman, *Biochemistry*, **6**, 1630 (1967).

(24) N. Greenfield and G. D. Fasman, *ibid.*, **8**, 4108 (1969).

(25) E. S. Pysh, *Proc. Nat. Acad. Sci. U. S.*, **56**, 825 (1966).

such right-handed α-helices as PBLG, and the value of $[m']_{233} = -19{,}000$ is slightly greater than previously reported values, which lie in the range of $-13{,}000$ to $-18{,}500$ and tend to cluster about $-15{,}000$.[19] Also, the value of $[\theta]_{224} = -52{,}000$ is slightly greater than the value of $\sim -40{,}000$ reported for several other polymers.[20] Although there is some indication of the familiar double trough in the CD spectrum of the *para* isomer, the values of $[\theta]$ below 215 mμ are uncertain because of the high absorption in this region. These relatively small deviations from the optical properties usually associated with the right-handed α-helix are not nearly as large as those reported by Goodman, *et al.* (ref *c*, Table III), for *p*-NO_2PBLA and by Fraser, *et al.*,[26] for certain NO_2-substituted PBLG's, and probably arise because of slight differences in the backbone conformation between various right-handed α-helical polymers[27] or because of the influence of the side chain.

On the other hand, the *ortho* and *meta* isomers show deviations of a more fundamental nature. These same features appear in the spectra of PBLA[28] and PMLA[21] which are thought to be left-handed but also contain the side-chain ester chromophore. One example of this behavior is that the ORD is positive at high wavelengths and changes in sign between about 350 and 500 mμ for right-handed α-helices; on the other hand, the data for (left-handed) PBLA, PMLA, *o*-ClPBLA, and *m*-ClPBLA display negative rotations at high wavelengths whose signs do not change until 255 mμ. Another example of such behavior is the slight red shift ($\sim$1 to 2 mμ) of the extremum in $[m']$ near 233 mμ and a reduction in the absolute value of $[m']$ for all of the above-cited left-handed α-helices. While the decrease in the absolute value of $[m']$ may be due to partial helicity, the red shift in the position of the extremum (accompanied by a red shift in the crossing point below 233 mμ; for example, right-handed α-helices[29] and *p*-ClPBLA have a crossing point at $\sim$224 mμ, while the *ortho* and *meta* isomers cross over at $\sim$227–228 mμ) is probably due to structural features of the left-handed α-helix. Similarly, the values of $[\theta]$ at the extrema $\sim$225 mμ are smaller for the left-handed α-helices (probably partially due again to lower helicity), but the crossing point of the single peak below 225 mμ appears as if it would be red shifted compared to that of the familiar double trough in the CD spectra of right-handed α-helices. These deviations from the optical properties of the normal right-handed helix can be due to any of the causes outlined above. It is possible that the ester chromophore has an optically active transition which is perturbing the ORD and CD spectra of these compounds. Alternatively, it is clear that the left-handed α-helix of an L-amino acid is *not* the mirror image of the right-handed α-helix of an L-amino acid. Hence, one would not expect the ORD and CD properties of the two helices (aside from the inversion of sign) to be exactly the same. Furthermore, there is even the possibility, though not in accord with theoretical calculations for these polymers,[10] that the backbones (excluding the H on the C^α as part of the backbone) of the left- and right-handed helices are not mirror images of each other.

One aspect of the CD spectrum, which has not yet been related to helix sense, is the presence of small bands in the benzyl absorption region between 250 mμ and 280 mμ (not shown in Figure 2). While it is difficult to determine the exact number and ellipticity of these peaks, because of the high absorbance (compared to ellipticity), it seems that there are about three or four such peaks for each compound in dioxane, with molar ellipticities of $\sim$50 to 200. For *p*-ClPBLA, all of these extrema are negative, as also observed by Hashimoto and Aritomi.[11] However, the *meta* isomer exhibits both positive and negative CD bands (no observations were carried out for the *ortho* isomer); this behavior is similar to that described by Urry for poly-L-phenylalanine.[21] However, without further work, it is difficult to determine how these extremely weak bands are affected by the helical conformation of the polymer, since optically active bands in this region have been shown to exist in free amino acids with aromatic side chains.[30,31]

In spite of the side-chain chromophore effects, and the qualitative differences in the spectra of left- and right-handed α-helices, it seems valid to conclude that *p*-ClPBLA exists as a right-handed α-helix, and *o*-ClPBLA and *m*-ClPBLA exist as left-handed α-helices, in the solvents used here. This assignment is facilitated by the absence of the gross side-chain chromophoric effects which are observed for poly-L-tyrosine[32,33] and poly-L-tryptophan.[34] The argument is strengthened by the fact that the ORD and CD properties of the *ortho* and *meta* isomers are the same as those of PBLA and PMLA. The former of these has been shown to exist in the left-handed helical form by fiber X-ray diffraction[35] and by ORD measurements on a series of copolymers with an amino acid which forms a helix of known screw sense (ref *e* and *f* of Table III). The PMLA has been shown by this copolymer technique to form left-handed helices.[28] Thus, even though the copolymer method has not been used, we believe that the evidence presented here establishes the helix senses of the polymers studied.

Finally, the confirmation of the predicted[10] helix senses provides additional confidence in the validity of the conformational energy calculations.[4–6,10]

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