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Optically Active Polyampholytes Derived from L- and D-Carbylalanyl-L-histidine[1,2]

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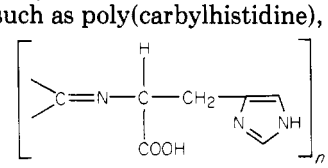
ABSTRACT: Two optically active polymers with the general structure $[>C{=}NCH(CH_3)C(O)NHCH(COOH)CH_2Im]_n$ were prepared by the polymerization of L- and D-carbylalanyl-L-histidine with catalytic amounts of nickel(II) chloride. The monomers were synthesized from the dipeptides L- and D-alanyl-L-histidine by converting the amino group of these compounds into an isocyano function. The polymer from D-alanyl-L-histidine has a $pK_a(ImH^+)$ of 8.4. It shows a polyelectrolyte viscosity behavior. Its molar rotation is $[M]^{20}_{578} \simeq 0°$. Its CD spectrum has a couplet indicative of a right-handed helix. The other polymer has a $pK_a(ImH^+)$ of 9.4 and a nonpolyelectrolyte viscosity behavior. Its $[M]^{20}_{578}$ is +1025°. The CD spectrum contains a single positive band, which does not provide information about the screw sense.

Enzymes combine high catalytic activity with high selectivity.[3] Certain polymeric model systems, called synzymes, have equal or even greater activity.[4] Striking examples are the ethylenimine-based systems developed by Klotz et al.[5] and very recently by Kunitake et al.[6] The success in this field contrasts sharply with the little progress that has been made in imitating the enantioselectivity of enzymes.[4,7] In our opinion there is a lack of appropriate chiral polymers which can be tested as enantioselective catalysts.[4] We are currently investigating whether poly(isocyanides), more properly called poly(iminomethylenes), $[RN{=}C<]_n$, are useful as such polymers. These compounds are attractive for two reasons. First, they are readily obtainable from the monomeric isocyanides, RN=C, with nickel chloride or a nickel(II) complex as catalyst.[8] These monomers in turn can be prepared in great variety from the corresponding amines.[9] Second, poly(iminomethylenes) have a stable helical structure.[8] Left-handed and right-handed helices of poly(*tert*-butyliminomethylene) have been separated by resolution.[10,11] Preferential formation of one screw occurs in the polymerization of one enantiomer of a chiral monomer.[12]

In previous papers[13,14] we reported on the synthesis and catalytic activity of imidazole-containing poly(iminomethylenes) such as poly(carbylhistidine), **1**, which could



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be models for hydrolytic enzymes. We were unable to get **1** in optically active form because its carboxyl- and imidazole-protected monomer suffered from a rapid racemization. This was due to the presence of both a basic imidazole function in the molecule and two electron-withdrawing substituents at the chiral center, viz., the isocyano and carboxyl ester function.

We have now found that racemization problems can be avoided if one starts from isocyanides of dipeptides of histidine and other amino acids. The synthesis and physical properties of two polymers derived from such isocyanides, viz., poly(L-carbylalanyl-L-histidine) and poly(D-carbylalanyl-L-histidine), are described in this paper. Their catalytic activity in the hydrolysis of chiral esters will be described elsewhere.[15]

Results and Discussion

Synthesis. As far as we are aware, isocyanides derived from dipeptides containing histidine have not been described in the literature.[9,16] We have synthesized protected L- and D-carbylalanyl-L-histidine, compounds **6a** and **6b**, according to Scheme I.

Prior to the coupling of the amino acids, the carboxyl group of histidine and the amino group of alanine were protected as the methyl ester and the formamide group, respectively. The latter protecting group was chosen because it can be transformed directly into an isocyano function.[9] Dicyclohexylcarbodiimide in acetonitrile/*N*,*N*-dimethylformamide was used as coupling agent.[17] From the synthesis of **1** we learned[13] that it is necessary to protect the imidazole nucleus before converting the *N*-formyl function into an isocyano group. We chose the *p*-toluenesulfonyl (Tos) group as an imidazole protecting group in **4**. This group is easily introduced and can be removed under mild conditions, as we found recently.[18] Moreover, a *p*-toluenesulfonyl group lowers the basicity of the imidazole nucleus, which reduces the chance of

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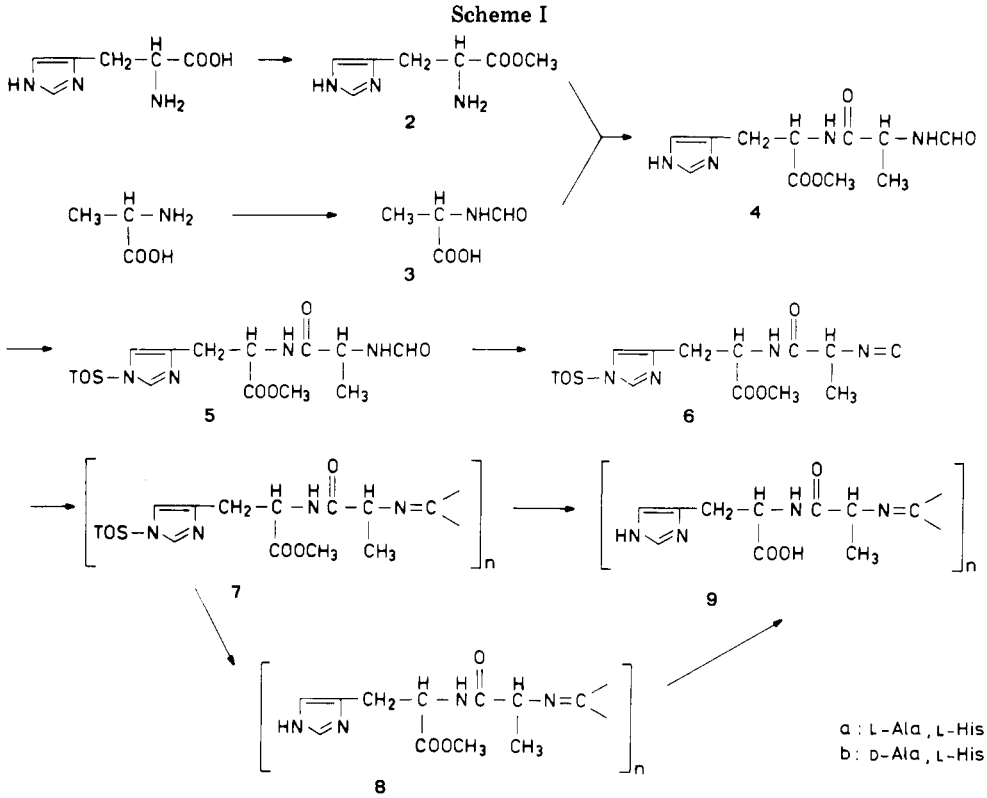
Scheme I



racemization during step **5** → **6**.

Isocyanides **6** were obtained by dehydration of formamides **5** with diphosgene and *N*-methylmorpholine.[16] We used this combination of dehydrating agent and base because it is effective even at low temperature. This latter condition is a prerequisite for preventing racemization. To a small extent the formation of isocyanide was accompanied by detosylation of the imidazole moiety. This side reaction is probably subsequent to acylation of the imidazole ring by diphosgene.[18] Nevertheless, the yields were satisfactory. After purification, **6a** and **6b** were obtained in 75% yield as odorless, white solids. The monomers were soluble in halogenated hydrocarbons, sparingly soluble in alcohols, and insoluble in diethyl ether, water, benzene, and the lower straight-chain hydrocarbons. Their infrared absorption spectra showed characteristic isocyanide stretching vibrations at 2147 ± 1 (**6a**) and 2149 ± 1 cm^{-1} (**6b**). The structures were further established by ^1H NMR and mass spectroscopy.

We checked separately whether racemization had occurred at the chiral centers during step **5** → **6**. If racemization had occurred each of the compounds **6a** and **6b** would have been contaminated to a certain extent by the other diastereomer. In the ^1H NMR spectra of **6a** and **6b** the signals of the various corresponding protons differ sufficiently to make this check possible, even without the help of a shift reagent. It appeared that within the limits of detection of the NMR technique no racemization had occurred. This result is in line with the melting behavior of the two diastereomers. Compound **6a** melted sharply at 137.0 ± 0.2 °C and compound **6b** at 134.5 ± 0.2 °C, whereas a 1:1 mixture of **6a** and **6b** had a melting range from 116 to 118 °C. Moreover, we observed that methyl 2-isocyanoalaninate, a compound which is even more sensitive[16] to racemization than **6a** and **6b**, is obtained as an optically pure product under the same reaction conditions.[19]

Monomers **6a** and **6b** were polymerized by treatment with 0.2 mol % of nickel(II) chloride in 1:1 chloroform-methanol (v/v). Under these conditions reaction was complete within a few hours at room temperature. This rate of polymerization is rapid compared to that of the monomer leading to polymer **1**.[13] In the latter case we had indications that imidazole residues block coordination sites on nickel, which are required for polymerization. Apparently this does not occur with compounds **6**.

Poly(iminomethylenes) **7** were isolated in good yields as light yellow solids. The N^{Im}-tosyl group in **7a** was removed by acetic anhydride and pyridine[18] to give polymer **8a**. The latter polymer was insoluble in most common solvents. It was treated with 0.5 mol/L aqueous NaOH for 2 days at room temperature to give a light reddish brown solid. After ultrafiltration and freeze-drying of the acidified solution the purified product was analyzed and found to be the HCl salt of polymer **9a**, containing 0.5 molecule of HCl and two molecules of crystal water per repeating unit. Acetic anhydride and pyridine were ineffective in the removal of the N^{Im}-tosyl group of **7b**, due to complete insolubility of the latter compound in this solvent mixture. We succeeded in removing the N^{Im}-tosyl and ester methyl group at the same time by treating this compound with 0.5 mol/L aqueous NaOH at 40 °C for 2 days. Ultrafiltration and freeze-drying of the dark red acidified solution afforded **9b** as a dark reddish brown solid, containing 0.5 molecule of HCl and two molecules of crystal water per polymer repeating unit.

Physical Properties. The protected polymers **7a** and **7b** were insoluble in water, ethers, benzene, and the lower straight-chain hydrocarbons. Compound **7a** was slightly soluble in methanol and soluble in chloroform. Compound **7b** was very sparingly soluble in chloroform only. Polymers **7a** and **7b** showed high optical rotations of opposite sign (Table I).

The intrinsic viscosity of **7a** was found to be $[\eta] = 1.04$ dL/g (chloroform, 30.00 °C). The intrinsic viscosity of **7b** could not be measured due to its low solubility. Applying the Mark–Houwink equation as determined for poly[(1-methylheptyl)iminomethylene],[1] $[\eta] = 1.4 \times 10^{-9} \bar{M}_w^{1.75}$, a

Table I
Optical Rotation Data of Monomers RN=C and Polymers [RN=C<]$_n$

compd	$[M]^{20}_{578}$,[a] deg	compd	$[M]^{20}_{578}$,[a] deg
6a	+164	7b	−700[b]
6b	+75	9a	+1025
7a	+580	9b	0[c]

[a] Solvent (concentration in g/dL): 6a, $CHCl_3$ (2); 6b, $CHCl_3$ (2); 7a, $CHCl_3$ (0.35); 7b, $CHCl_3$ (0.05); 9a, H_2O (0.05); 9b, H_2O (0.005). The molar optical rotation of the polymers is expressed per polymer unit. [b] This compound is sparingly soluble in chloroform only. Estimated error 10%. [c] Solutions of this compound in water are darkly colored. At the highest measurable concentration no optical rotation was observed. Estimated error ±45°.

molecular weight of 120 000 can be estimated for **7a**.

The deprotected polymers **9a** and **9b** were insoluble in common organic solvents but soluble in water with a pH lower than 5 and higher than 7. In the pH range 5–7 the polymers precipitated since they have their isoelectric points in this region. These isoelectric points, as determined by the isoelectric focusing technique,[20] were found to be 6.1 and 6.3 for **9a** and **9b**, respectively.

The ionization state of the imidazole and carboxylic acid functions in polymers **9** as a function of pH was determined by potentiometric titration. In accordance with the modified Henderson–Hasselbach equation[21]

$$\mathrm{pH} = \mathrm{p}K_a - n \log [(1 - \alpha)/\alpha]$$

where α stands for the fraction of unprotonated imidazole or carboxylate ions, plots of log $[(1 - \alpha)/\alpha]$ vs. pH were linear. In Table II the values calculated for n and for $pK_a(ImH^+)$ and $pK_a(COOH)$ are presented as well as the pK_a's of model compounds. The pK_a of the carboxylic acid group in **9a** and in **9b** lies in the range which can be expected for a peptide, but its value is lower than the value of the corresponding group in **1**. However, in polymer **1** the $pK_a(COOH)$ is relatively high due to the fact that the carboxylic functions are situated close to the polymer main chain. The latter situation results in short distances between the carboxylic anions and strong interaction between them.

The pK_a value of the imidazole function in polymers **9** has increased considerably compared to the pK_a values of the model compounds in Table II. This increase suggests that the imidazole residues in **9** are strongly affected by the negative charge of the carboxylate ions. The increase is larger for **9a** than for **9b**. One can imagine that such a difference occurs if the distance between the imidazole rings and the carboxylate ions is larger in **9b** than in **9a**. Molecular models of the two polymers show that such a difference in conformation is not unlikely.

The viscosity behavior of **9a** and **9b** is illustrated in Figure 1. Compound **9b** behaves like a normal polyelectrolyte; that is, with increasing concentration of polymer the reduced viscosity decreases, due to the effect of

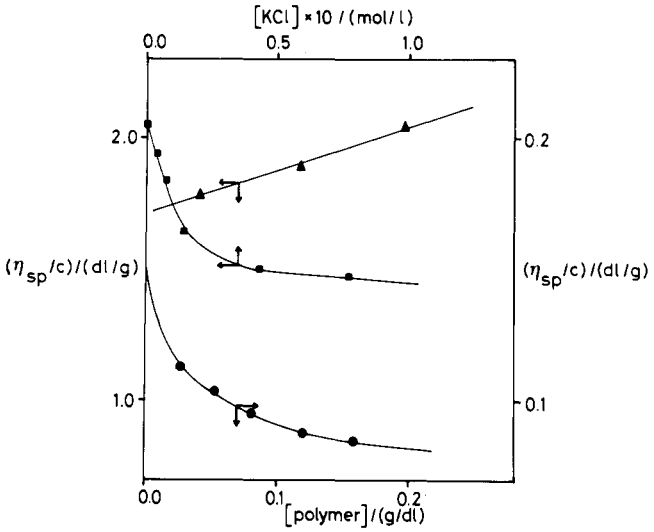


Figure 1. Viscosity behavior of poly(iminomethylenes) in aqueous solution. Reduced viscosity of **9a** vs. polymer concentration at pH 2.9 (▲) and of **9b** vs. polymer concentration at pH 3.3 (●) (in these solutions neither buffer nor additional salt was present; at the polymer concentration of 0.2 g/dL the ionic strength is 0.005 mol/L) and reduced viscosity of **9a** vs. concentration of added KCl at the polymer concentration of 0.20 g/dL at pH 2.9 (■).

counterions. The curve follows the relationship of Fuoss and Strauss[22] for polyelectrolytes:

$$\frac{c}{\eta_{sp}} = c^{1/2}\frac{C}{Y} + \frac{1}{Y}$$

where C is a constant and Y equals the intrinsic viscosity, $[\eta]$, of the polyelectrolyte. For **9b** the latter quantity was calculated to be 0.15 ± 0.01 dL/g.

A plot of reduced viscosity vs. concentration is linear for **9a**. From the intercept and slope the intrinsic viscosity and Huggins constant were calculated to be $[\eta] = 1.72$ dL/g and $k' = 0.57$, respectively. Addition of increasing amounts of KCl to **9a** lowered the reduced viscosity to about 70% of the value in the salt-free solution (Figure 1).

The difference in the hydrodynamic behavior of **9a** and **9b** is probably related to the width of the double layers surrounding these molecules. This width is determined by the net charge ($ImH^+ + COO^-$) and by the distribution of these charges over the molecules. At the pH of the viscosity measurement (pH ≃3) both ampholytes have an approximately equal net positive charge. In **9b** the positive charges are further away from the negative ones than they are in **9a** (see above). Consequently, in **9b** the positive charges will be located more at the outer side of the chain and the Cl^- ions will be pulled closer to the chain, giving rise to real polyelectrolyte behavior. Molecules of **9a** have their surrounding Cl^- ions at a larger distance from the chain and their behavior is hardly affected by an increase in this Cl^- concentration. A polyelectrolyte effect is noted

Table II
pK Values[a] of Polymers [RN=C<]$_n$ and of Model Compounds

compd	$pK_a(COOH)$	n_{COOH}	$pK_a(ImH^+)$	n_{ImH^+}
9a	2.85 ± 0.1	1.25 ± 0.1	9.4 ± 0.1	2.4 ± 0.05
9b	2.3 ± 0.2	3.0 ± 0.1	8.4 ± 0.2	2.2 ± 0.1
1[b]	5.85	1.4	9.3	1.5
poly(His)[c]			5.9	
copoly(His-Asp)[c]			7.0	
β-Ala-His[d]	2.64		6.0	
His[d]	1.82		6.0	

[a] In water at 25.0 °C. [b] In 29% v/v EtOH/H_2O (see ref 14). [c] See ref 28. [d] See ref 29.

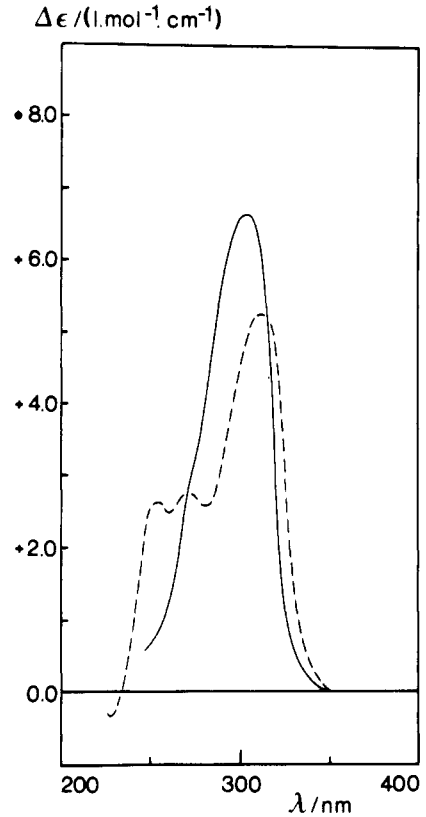


Figure 2. CD spectra of protected (solid line) and deprotected (broken line) poly(L-carbylalanyl-L-histidine), compounds **7a** and **9a** [compound (solvent, concentration in mol/L): **7a** ($CHCl_3$, 2.2 × 10^{-3}); **9a** (water, 3.6 × 10^{-4})].

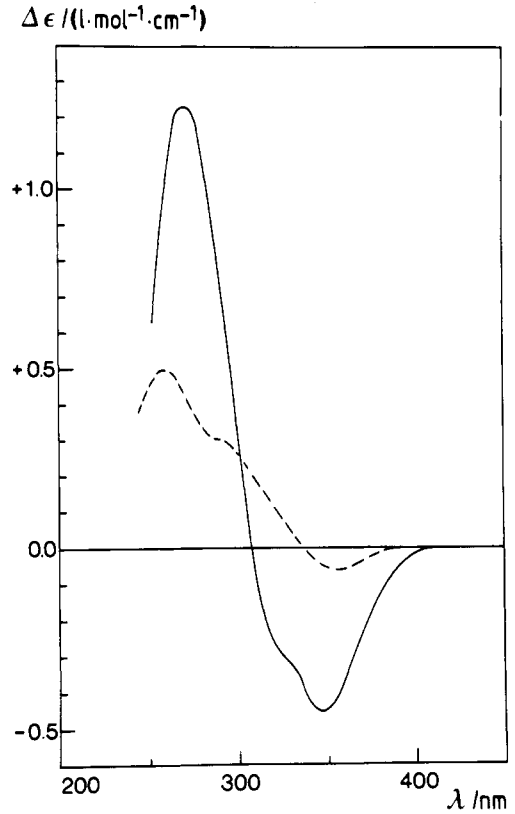


Figure 3. CD spectra of protected (solid line) and deprotected (broken line) poly(D-carbylalanyl-L-histidine), compounds **7b** and **9b** [compound (solvent, concentration in mol/L): **7b** ($CHCl_3$, 8.5 × 10^{-4}); **9b** (water, 3.7 × 10^{-3})].

only when the much higher KCl concentration is present.

Although prepared under the same reaction conditions, polymers **9a** and **9b** show a striking difference in the magnitude of their intrinsic viscosities ($[\eta]_{9a}/[\eta]_{9b}$ = 11.5) and, therefore, their molecular weights may differ. This difference may well be connected with a difference in the propagation rate of the two diastereomeric monomers **6**. If so, the side chain of **6a** would fit better into the growing helix than the side chain of **6b**.

The UV spectra of the protected polymers **7a** and **7b** in chloroform showed a shoulder at about 310 nm on the onset of a much larger band in the far-UV region. This shoulder can be attributed to the n–π^* transition of the N=C chromophore of the polymer main chain.[23] In poly(alkyliminomethylenes) this transition has been found at the same wavelength position.[1,23] The n–π^* transition is also responsible for the CD spectra of **7a** and **7b** in the region from 250–400 nm (Figures 2 and 3). In this region polymer **7a** shows a relatively strong positive Cotton effect, whereas polymer **7b** exhibits the typical "couplet" of a helical polymer.[24] The latter couplet is negative indicating that polymer **7b** is predominantly in the right-handed *P*-helical configuration. This configuration should make a negative contribution to the molar optical rotation[23] in the high-wavelength region. The optical rotation data of Table I are in line with this result: going from monomer **6b** to polymer **7b**, $[M]^{20}_{578}$ changes from +75 to −700°. In the CD spectrum of **7a** no couplet is visible, either because the polymer consists of equal amounts of left- and right-handed helices or because its helical configuration does not give rise to a couplet pattern. For the moment we are not able to decide which of these two reasons is the correct one. However, it is worth mentioning here the behavior of a similar polymer derived from D-carbylalanyl-L-histidinol. Its CD spectrum shows no couplet when the polymer is protected but does show one when the polymer is deprotected,[25] although the deprotection procedure has no effect on the number of left- and right-handed helices.

We ascribe the strong positive band in the CD spectrum of **7a** primarily to the side-chain induction in the N=C chromophore. It is remarkable that this side-chain induction is almost absent in the CD spectrum of **7b**. Apparently, in **7a** the CD contributions of the two chiral centers in the side chain reinforce each other, whereas in **7b** they compensate each other.

The UV spectra of the deprotected polymers **9a** and **9b** in water are presented in Figures 4 and 5. The n–π^* transition of the N=C chromophore at 310 nm is not clearly visible in these spectra. An additional band is present at about 370 nm, which was not visible in the spectra of the protected polymers. The intensity of this band reversibly decreases with decreasing pH. Simultaneously, the intensity increases at higher wavelengths. In the case of **9a** this behavior gives rise to an isosbestic point at 470 nm. We ascribe these absorptions to conjugation between neighboring N=C units in the polymers. This conjugation can occur if the angle between these units deviates to some extent from 90° due to electrostatic interactions of the charged imidazolyl and carboxylate groups in the side chain.

The CD spectra of the deprotected polymers **9** in water are similar to those of the protected ones in chloroform (Figures 2 and 3). These spectra reveal that the UV and visible absorptions at higher wavelengths are not CD active. In addition to the band at 310 nm the CD spectrum of **9a** shows two weak bands, at 250 and 270 nm, which probably arise from the other chromophores in the polymer. The intensity of the couplet in the spectrum of **9b** is lower than in the spectrum of **7b** by a factor of about 5. Moreover, it was found that the intensity of **9b** varies

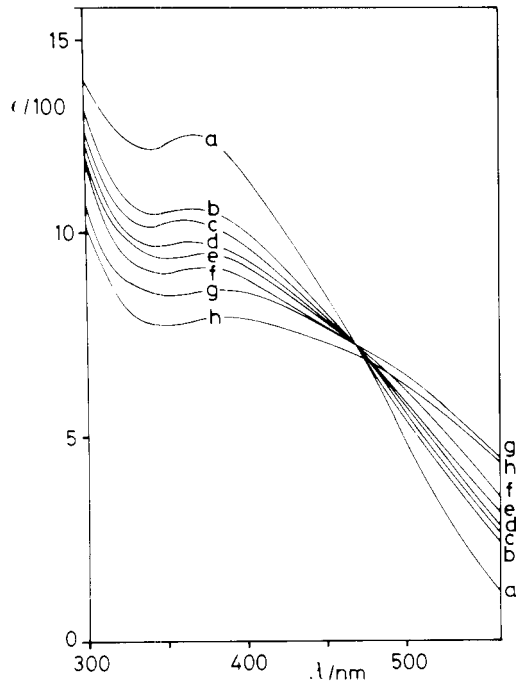


Figure 4. UV–vis spectra of poly(L-carbylalanyl-L-histidine), **9a**: a, pH >13; b, pH 11.4; c, pH 10.8; d, pH 10.0; e, pH 8.9; f, pH 7.0; g, pH 3.6; h, pH 2.0.

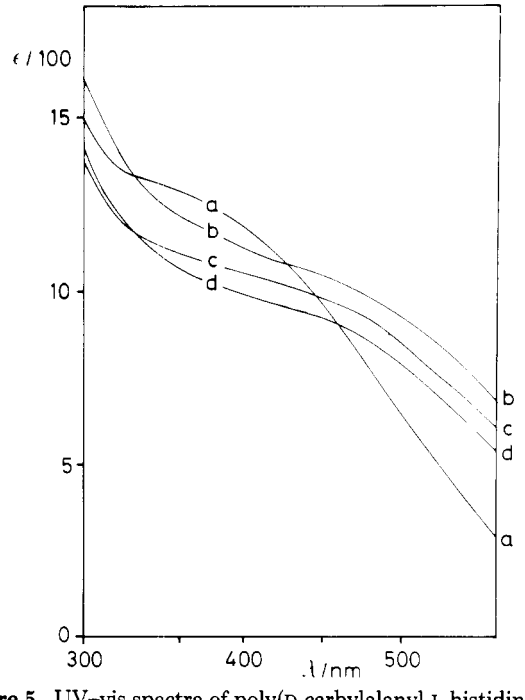


Figure 5. UV–vis spectra of poly(D-carbylalanyl-L-histidine), **9b**: a, pH 12.3; b, pH 1.5; c, pH 7.0; d, pH 3.0.

with pH. At pHs of 0.2, 7.0, and 12.9, $\Delta\epsilon$ at 350 nm is 0.12, 0.09, and 0.065, respectively. The origin of the intensity differences is not yet clear. Racemization of the helical main chain during the deprotection procedure might be an explanation. However, this is not likely, as the helix of poly(iminomethylenes) is stable under various conditions.[1,25] In our opinion the following explanations for the observed effects are more plausible. Firstly, the electronic transition moment of the N=C chromophore of **7b** and **9b** may differ. A lower value of this amount for **9b** will give rise to a lower $\Delta\epsilon$ value for this polymer.[26] Secondly, it is possible that, besides purely electrostatic interaction between neighboring N=C groups, which is a condition in exciton theory, there is also some conjugative interaction between these groups. Evidence for such conjugation in **9b** comes from the UV–vis spectrum of this polymer, as outlined above (compare Pino et al.[27]).

Experimental Section

Melting points were determined on a Mettler FP5/FP51 photoelectric melting point apparatus. Rotations were measured on a Perkin-Elmer 241 polarimeter. Infrared (IR) spectra were recorded on Perkin-Elmer 297 and 283 spectrophotometers. CD spectra were recorded on a home-built apparatus. The sensitivity of this instrument is better than 1×10^{-6} in absorbance units. Ultraviolet (UV) spectra were recorded on a Cary 15 spectrophotometer. ^{1}H NMR spectra were obtained on a Varian EM390 instrument. Chemical shifts (δ) are given in parts per million downfield from internal tetramethylsilane. Abbreviations used are as follows: s = singlet, d = doublet, t = triplet, q = quartet, dist d = distorted doublet, br = broad. Mass spectra (MS) were recorded on an AEI MS-902 mass spectrometer. Elemental analyses were carried out by the Element Analytical Section of the Institute for Organic Chemistry TNO, Utrecht, The Netherlands, under the supervision of W. J. Buis. Solution viscosity data were obtained with a Cannon-Ubbelohde viscometer. TLC was performed on silica (Schleicher and Schüll TLC Ready Plastic Foil FR-1500) and detection was effected by UV and/or iodine. Column chromatography was performed on silica (Merck Kieselgel 60/230–400 mesh).

Starting Materials. L-Histidine monohydrochloride [$[\alpha]^{20}_D$ +9.3° (*c* 5, 5 mol/L HCl)], L-alanine [$[\alpha]^{20}_{546}$ +17.1 ± 1° (*c* 5, 5 mol/L HCl)], and D-alanine [$[\alpha]^{20}_{546}$ −17.5 ± 1° (*c* 5, 5 mol/L HCl)] were purchased from Fluka.

L-Histidine Methyl Ester Dihydrochloride (2). This compound was obtained from L-histidine monohydrochloride through esterification with hydrogen chloride in methanol. It was used without further purification for the synthesis of **4**: mp 197–199 °C (lit.[30] mp 200–201 °C); $[\alpha]^{20}_D$ +15.1° (*c* 1, methanol) [lit.[31] $[\alpha]^{20}_D$ +16.0° (*c* 1, methanol)].

***N*-Formyl-L-alanine (3a) and *N*-Formyl-D-alanine (3b).** These compounds were prepared by formylation of the starting materials with acetic anhydride in formic acid. Recrystallization from acetone gave pure white crystals. **3a**: mp 131 °C; $[\alpha]^{20}_D$ −65° (*c* 2, 1 mol/L NaOH) [lit.:[32] mp 132 °C; $[\alpha]^{20}_D$ +57° (*c* 2, 1 mol/L NaOH) (in our opinion the sign of the optical rotation is incorrect)]. **3b**: mp 131 °C; $[\alpha]^{20}_D$ +63° (*c* 2, 1 mol/L NaOH) [lit.:[32] mp 130 °C; $[\alpha]^{20}_D$ −56° (*c* 2, 1 mol/L NaOH) (in our opinion the sign of the optical rotation is incorrect)].

***N*-Formyl-L-alanyl-L-histidine Methyl Ester (4a).** A suspension of 14.3 g (59 mmol) of finely powdered **2** in 150 mL of 9:1 chloroform/methanol (v/v) was treated at 0 °C with dry ammonia gas for 30 min. The reaction mixture was filtered and concentrated in vacuo at 30 °C. Chloroform was added and the solution was once again concentrated in order to remove traces of ammonia. The resulting oil was dissolved in 300 mL of 3:1 acetonitrile/dimethylformamide (v/v), and 6.9 g (59 mmol) of **3a** was added. The solution was cooled in ice, 12.4 g (60 mmol) of dicyclohexylcarbodiimide was added, and the reaction mixture was stirred for 2 h at 0 °C. After the mixture was allowed to stand overnight at room temperature, the urea was filtered off and the solution was concentrated under an oil pump vacuum at a temperature lower than 40 °C. Acetone (300 mL) was added to the obtained light brown semisolid, whereafter **4a** solidified. The white solid was filtered off and dried in vacuo over P_2O_5 to give 8.5 g of **4a**.

The mother liquid was concentrated and the residue dissolved in 100 mL of water in order to get another crop of **4a**. The latter solution was extracted three times with 100-mL portions of dichloromethane. The pH of the water layer was adjusted to pH 8 with solid sodium carbonate. After the addition of sodium sulfate the solution was extracted with 50-mL portions of 1-butanol until TLC [6:4 $CHCl_3$/EtOH (v/v), R_f(**4a**) 0.35] showed only traces of **4a** persisting in the water layer. The combined 1-butanol fractions were concentrated to yield 5 g of **4a**. The product was used without further purification for the synthesis of **5a**: yield 13.5 g (51 mmol, 85%); mp 127–128 °C; IR (KBr) 3310 (NH), 1740 ($COOCH_3$), 1650 (NHCO) cm^{-1}; ^{1}H NMR (CD_3OD) δ 8.05 (s, 1 H, CHO), 7.65 and 6.90 (2 × s, 2 H, imidazole), 4.55 (2 × m, 2

H, CH), 3.70 (s, 3 H, OCH_3), 3.05 (dist d, 2 H, CH_2), 1.30 (d, 3 H, CH_3).

***N*-Formyl-D-alanyl-L-histidine Methyl Ester (4b).** Synthesis was performed as described for **4a**. A white precipitate was obtained after the semisolid had been stirred with 400 mL of 3:1 acetone/ethyl acetate (v/v). An additional small amount of impure **4b** was obtained from the 1-butanol extractions. The compound was used without purification for the synthesis of **5b**: yield 80–85%; mp 147–148 °C; IR and ^1H NMR data are identical with those found for **4a** within 10 cm^{-1} and 0.05 ppm, respectively.

***N*-Formyl-L-alanyl-N^{Im}-tosyl-L-histidine Methyl Ester (5a).** To a solution of 12 g (44.8 mmol) of **4a** in 200 mL of 85:15 chloroform/methanol (v/v) were added 5 g of anhydrous sodium carbonate and 9.4 g (49.5 mmol) of *p*-toluenesulfonyl chloride. After being stirred overnight at room temperature, the reaction mixture was filtered and subsequently extracted twice with water. The organic layer was dried over sodium sulfate and concentrated in vacuo. Spontaneous crystallization occurred. The crystals were stirred with 150 mL of ether for 0.5 h. The solid was collected on a filter, carefully washed with ether, and dried in vacuo over P_2O_5; yield 16.7 g (39.6 mmol, 88%) of **5a**. TLC analysis [9:1 $CHCl_3$/MeOH (v/v)] showed that the solid consisted of almost pure **5a** (R_f 0.30). The compound was used without further purification for the synthesis of **6a**. Recrystallization from chloroform/ethyl acetate afforded a purified sample: mp 133–134 °C; $[\alpha]^{22}_{578}$ +7.6° (*c* 2, chloroform); IR (KBr) 3300 (NH), 1740 ($COOCH_3$), 1650 (NHCO), 1600, 1380 and 1180 (tosyl) cm^{-1}; ^1H NMR ($CDCl_3$) δ 8.15 (s, 1 H, CHO), 7.95 and 7.10 (2 × s, 2 H, imidazole), 7.80 and 7.35 (2 × d, 4 H, tosyl), 7.35 (br, 1 H, NH), 6.80 (br d, 1 H, NH), 4.75 (d of t, J_d = 6 Hz, J_t = 8 Hz, 1 H, $CHCH_2$), 4.60 (d of q, J_d = 8 Hz, J_q = 7 Hz, 1 H, $CHCH_3$), 3.65 (s, 3 H, OCH_3), 3.00 (d, 2 H, CH_2), 2.45 (s, 3 H, tosyl), 1.35 (d, 3 H, CH_3).

***N*-Formyl-D-alanyl-N^{Im}-tosyl-L-histidine Methyl Ester (5b).** Synthesis was performed as described for **5a**. The tosylated dipeptide **5b** was obtained as an oil. TLC analysis showed that the product was contaminated with some impurities. After crystallization from dichloromethane/ether almost pure **5b** was isolated: yield 80%; white needles from methanol/water; mp 113–115 °C (weakening at 95 °C); $[\alpha]^{22}_{578}$ +44.1° (*c* 2, chloroform); IR (KBr) data as for **5a** within 10 cm^{-1}; ^1H NMR ($CDCl_3$) δ 8.15 (s, 1 H, CHO), 7.95 and 7.10 (2 × s, 2 H, imidazole), 7.80 and 7.35 (2 × d, 4 H, tosyl), 7.35 (br, 1 H, NH), 6.80 (br d, 1 H, NH), 4.75 (d of t, J_d = 6 Hz, J_t = 8 Hz, 1 H, $CHCH_2$), 4.60 (d of q, J_d = 8 Hz, J_q = 7 Hz, 1 H, $CHCH_3$), 3.65 (s, 3 H, OCH_3), 3.00 (d, 2 H, CH_2), 2.45 (s, 3 H, tosyl), 1.20 (d, 3 H, CH_3).

L-Carbylalanyl-N^{Im}-tosyl-L-histidine Methyl Ester (6a). The formamide **5a** was converted to the corresponding isocyanide by a modification of the method of Skorna and Ugi.[16] Into a round-bottomed flask, equipped with a magnetic stirrer and a CO_2/acetone reflux condenser kept at −30 °C, were put 9.5 g (22.5 mmol) of **5a**, 5.5 mL (49.5 mmol) of dry *N*-methylmorpholine, and 100 mL of alcohol-free chloroform as a solvent. At −35 °C (CO_2/acetone bath) 1.40 mL (11.6 mmol) of diphosgene in 15 mL of alcohol-free chloroform was introduced into the stirred reaction mixture over a period of 2 h under a nitrogen atmosphere. The reaction was followed by TLC [1:1 $CHCl_3$/acetone (v/v), R_f(**5a**) 0.17; R_f(**6a**) 0.50]. When necessary a small additional amount of diphosgene and base were added to drive the reaction to completeness. At the end of the reaction TLC analyses showed the appearance of *p*-toluenesulfonyl chloride (R_f 0.98) resulting from a small-scale detosylation of the imidazolyl moiety by the acylating agent diphosgene.[18] The acetone–carbon dioxide bath was replaced by an ice bath and immediately thereafter 100 mL of 7.5% aqueous sodium bicarbonate was introduced in one portion into the reaction mixture. After the mixture was stirred for 15 min, the organic layer was separated and extracted twice with 50-mL portions of water. The organic layer was dried over sodium sulfate and the chloroform was evaporated in vacuo at 30 °C. *N*-Methylmorpholine was removed under an oil pump vacuum. A light brown solid was obtained, 8.8 g. This solid was dissolved in 50 mL of 4:1 chloroform/methanol (v/v), whereafter 150 mL of methanol was added. After storage overnight in the refrigerator, the white solid was collected on a filter and washed twice with cold methanol to give 5.3 g (13.1 mmol) of almost pure **6a**. The mother liquid was subjected to column chromatography [eluent 4:1 $CHCl_3$/acetone (v/v)]. Subsequent crystallization from methanol gave an additional amount of 1.6 g (4.0 mmol) of **6a**: total yield 6.9 g (75%); white needles from methanol; mp 137.0–137.2 °C; $[\alpha]^{22}_{578}$ +40.6° (*c* 2, chloroform); MS *m/e* 404 (M^+), 350 (M^+ − $CH(CH_3)NC$), 345 (M^+ − $COOCH_3$), 249 (M^+ − tosyl), 235 (M^+ − $CH(COOCH_3)NHC(O)CH(CH_3)NC$); IR (KBr) 3300 (NH); 2147 ± 1 (NC, internal calibration), 1735 ($COOCH_3$), 1665 (NHCO), 1600, 1375, and 1170 (tosyl) cm^{-1}; ^1H NMR ($CDCl_3$) δ 8.0 and 7.1 (2 × s, 2 H, imidazole), 7.80 and 7.35 (2 × s, 4 H, tosyl), 7.9 (br d, 1 H, NH), 4.80 (d of t, J_d = 7 Hz, J_t = 5 Hz, 1 H, $CHCH_2$), 4.25 (q, 1 H, $CHCH_3$), 3.65 (s, 3 H, OCH_3), 3.10 (dist d, 2 H, CH_2), 2.40 (s, 3 H, tosyl), 1.55 (d, 3 H, CH_3).

D-Carbylalanyl-N^{Im}-tosyl-L-histidine Methyl Ester (6b). Synthesis was performed as described for **6a**: yield 75%; white needles from methanol; mp 134.5–134.6 °C; $[\alpha]^{22}_{578}$ +18.6° (*c* 2, chloroform); MS data same as those for **6a**; IR (KBr) 3315 (NH), 2149 ± 1 (NC, internal calibration), 1740 ($COOCH_3$), 1670 (NHCO), 1600, 1370, and 1170 (tosyl) cm^{-1}; ^1H NMR ($CDCl_3$) δ 8.0 and 7.1 (2 × s, 2 H, imidazole), 7.85 and 7.40 (2 × d, 4 H, tosyl), 8.05 (br d, 1 H, NH), 4.80 (d of t, J_d = 7 Hz, J_t = 5 Hz, 1 H, $CHCH_2$), 4.25 (q, 1 H, $CHCH_3$), 3.65 (s, 3 H, OCH_3), 3.10 and 3.075 (2 × d, J_d = 5.3 Hz, 2 H, CH_2), 2.45 (s, 3 H, tosyl), 1.60 (d, 3 H, CH_3). A finely powdered mixture of 15 mg of **6a** and 15 mg of **6b** had a melting range from 116 to 118 °C.

Poly(L-carbylalanyl-N^{Im}-tosyl-L-histidine Methyl Ester) (7a). An amount of 5.7 mg (0.024 mmol) of $NiCl_2 \cdot 6H_2O$ was added to a solution of 5 g (12.2 mmol) of **6a** in 30 mL of 1:1 chloroform/methanol (v/v). After a short time the reaction mixture became cloudy and a paste was obtained after the mixture had been left standing overnight at room temperature. The solvent was removed in vacuo to give the polymer in quantitative yield as a light brown film. This film was used directly for the synthesis of the deprotected polymer. A sample was dissolved in a small amount of warm chloroform and this solution was added dropwise to a 30-fold excess of stirred 3:1 methanol/water (v/v). The precipitate was filtered off, washed with 1:1 methanol/water (v/v) and ether, and dried in vacuo to yield pale yellow **7a**: $[\alpha]^{22}_{578}$ +144° (*c* 0.35, chloroform); $[\eta]$ = 1.044 dL/g (chloroform, 30.00 °C); IR (KBr) 3260 (NH), 1740 ($COOCH_3$), 1660 (NHCO), 1600, 1375, and 1180 (tosyl) cm^{-1}. Anal. Calcd for $C_{18}H_{20}N_4S_2O_5$: C, 53.46; H, 4.95; N, 13.85; S, 7.92; O, 19.80. Found: C, 53.2; H, 5.1; N, 13.8; S, 7.7; O, 20.2.

Poly(D-carbylalanyl-N^{Im}-tosyl-L-histidine Methyl Ester) (7b). Isocyanide **6b** was polymerized quantitatively as described for **6a**. After removal of the solvent a light brown solid was isolated in quantitative yield, which was used directly in the preparation of **9b**. A sample of **7b** was purified by stirring it in ethanol for 1 h, whereupon the polymer was filtered off and dried in vacuo. For optical rotation measurement a sample of the purified polymer was refluxed in chloroform for 1 h. The undissolved material was filtered off and from the resulting clear solution the optical rotation was measured. The concentration of the solution was estimated from its volume and from the weight of residual **7b** after evaporation of the solvent; $[\alpha]^{22}_{578}$ −170° (*c* 0.05, chloroform); IR data as for **7a**. Anal. Calcd for $C_{18}H_{20}N_4SO_5$: C, 53.46; H, 4.95; N, 13.85; S, 7.92; O, 19.80. Found: C, 53.4; H, 5.1; N, 13.6; S, 7.7; O, 20.4.

Poly(L-carbylalanyl-L-histidine Methyl Ester) (8a). Detosylation of **7a** was performed by stirring a sample of 5 g of this compound in 200 mL of 1:1 pyridine/acetic anhydride (v/v) overnight at room temperature. After removal of the solvent in vacuo the residue was dissolved in 20 mL of methanol, and this solution was added dropwise to slightly basic water (pH 8). The resulting precipitate was filtered off and dried over P_2O_5: yield 3.1 g (100%); IR (KBr) 3250 (NH), 3500–2500 (imidazole), 1740 ($COOCH_3$), 1670 (NHCO), 1210 ($COOCH_3$) cm^{-1}.

Poly(L-carbylalanyl-L-histidine) (9a). Three grams of polymer **8a** was treated with 100 mL of 0.5 mol/L aqueous NaOH for 2 days at room temperature. After this period the pH of the light red solution was adjusted to pH 6, whereupon the polymer precipitated. Ethanol (50 mL) was added and the polymer was collected by centrifugation. The obtained paste was dissolved in 0.1 mol/L aqueous HCl and subjected to ultrafiltration (Diaflo Ultra-Filter, UM-2). Freeze-drying of the resulting slightly acidified solution afforded **9a** as a very voluminous and spongy reddish brown solid: yield 2.9 g (85%); $[\alpha]^{22}_{578}$ +350° (*c* 0.05,

water); $[\eta]$ = 1.72 dL/g (water, 30.00 °C); IR (KBr) 3700–2000 (COOH, protonated imidazole, NH, H_2O), 1730 (COOH), 1650 (NHCO), 1620 (N=C<) cm^{-1}. Anal. Calcd for $C_{10}H_{12}N_4O_3 \cdot (HCl)_{0.56} \cdot (H_2O)_{1.7}$: C, 41.7; H, 5.7; N, 19.4; O, 26.2; Cl, 6.9. Found: C, 42.3; H, 5.6; N, 18.6; O, 26.6; Cl, 6.9.

Poly(D-carbylalanyl-L-histidine) (9b). Five grams of **7b** was suspended in 200 mL of 0.5 mol/L aqueous NaOH and stirred for 2 days at 40 °C. During this period the starting material dissolved to give a reddish solution. Following the procedure described for **9a**, the polymer **9b** was obtained as a dark reddish brown powder: yield 2.8 g (80%); $[\alpha]^{22}_{578}$ 0 ± 15° (*c* 0.005, water) [polymer **9b** has a relatively high extinction coefficient at 578 nm (ϵ_{578} 435 L mol^{-1} cm^{-1}), preventing optical rotation measurements at higher concentrations of polymer]; $[\eta]$ = 0.15 dL/g (water, 30.00 °C); IR (KBr) 3700–2200 (COOH, protonated imidazole, NH, H_2O), 1730 (COOH), 1660 (NHCO) cm^{-1} (the vibration of the azomethine group is partly masked by the NHCO vibration). Anal. Calcd for $C_{10}H_{12}N_4O_3 \cdot (HCl)_{0.58} \cdot (H_2O)_{1.85}$: C, 41.3; H, 5.6; N, 19.3; O, 26.7; Cl, 7.1. Found: C, 41.7; H, 5.6; N, 18.5; O, 27.0; Cl, 7.2.

Potentiometric Titrations. Polymers **9** were dissolved in double-distilled water until a concentration of 3.5×10^{-3} mol/L was obtained. To 20-mL samples were added 0.5 mL of 0.1 mol/L aqueous HCl and an amount of KCl such that at the end point of titration μ = 0.02 mol/L. The solutions were titrated with 0.1 mol/L aqueous NaOH under a nitrogen atmosphere while being stirred. Blank titration curves were obtained by titrating 20-mL aliquots of double-distilled water acidified to the same pH and adjusted to the same ionic strength. Differential titration curves were derived graphically,[33] and from these curves the degrees of dissociation were evaluated. The inflection points between the dissociation of the imidazolium ion and the carboxylic acid group, obtained from the differential titration curves, were in good agreement with the isoelectric points found with the isoelectric focusing technique.

Isoelectric Focusing Technique. Polymers **9** in a concentration of 2.5×10^{-4} mol/L were subjected to isoelectric focusing in a 7% acrylamide gel containing 0.1% of Ampholine (pH 3–10, LKB), using a Pharmacia electrophoresis apparatus.

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