Synthesis of Polymers of Isocyanides Derived from Tripeptides Containing Imidazolyl, Carboxyl, and Hydroxymethyl Groups[1]

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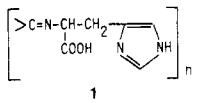
Three optically active polymers of isocyanides, $[RN{=}C<]_n$, which contain imidazolyl, carboxyl, and hydroxymethyl functions in their side chains R, are described. The polymers are derived from the following diastereomeric tripeptides: L-Ala-L-His-L-Ser, L-Ala-L-His-D-Ser, and D-Ala-L-His-L-Ser. The terminal amino groups of these tripeptides are converted into isocyano functions, which are subsequently polymerized with catalytic amounts of nickel(II) chloride. The molecular weights of the polymers are in the $\bar{M}_v$ range 20 000–35 000. The CD spectra reveal that the polymers derived from L-Ala-L-His-L-Ser and L-Ala-L-His-D-Ser have right-handed helical configurations. The pK_a values of the imidazolyl and carboxyl groups in the polymers have increased as compared to model compounds. This suggests that strong electrostatic interactions exist between these groups.

Polymers of isocyanides, $[RN{=}C<]_n$, called poly(iminomethylenes) or poly(carbonimidoyls), have a helical rigid rod configuration.[3,4] Their chirality and rigidity, with all side chains R in an almost equal environment, make them attractive as enzyme models. In earlier papers[5] we reported on the synthesis and esterolytic activity of imidazole containing poly(iminomethylenes), e.g., **1** derived from L-histidine and **2** derived from D-alanyl-L-histidinol. The



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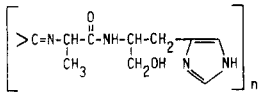

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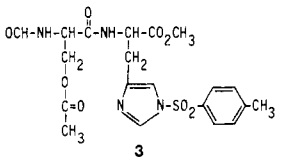
catalytic activity of these polymers was determined in homogeneous aqueous solution in the hydrolysis of 4-nitrophenyl and 2,4-dinitrophenyl esters. The activity of **1** and **2** is enhanced with respect to low molecular weight imidazole containing compounds by a factor of 5–500. A cooperative action of the imidazole groups and in particular of imidazole and carboxylic acid groups is held to be responsible for this activity increase. Moreover, a moderate enantioselectivity was observed (k_L/k_D = 1.1) in the hydrolysis of 4-nitrophenyl esters of optically active amino acids catalyzed by polymer **2**.[5d]

The esterolytic enzyme chymotrypsin possesses a so-called charge relay system in its active site.[6] This system consists of an imidazolyl, a carboxyl, and hydroxymethyl group. Therefore, it would be worthwhile to synthesize polymers that combine these three groups. We approached this problem in two ways: (i) the homopolymerization of isocyanides derived from tripeptides of alanine, histidine, and serine; (ii) the copolymerization of isocyanides derived from dipeptides of alanine and serine and of alanine and histidine. In the present paper we describe the former approach, while the latter approach is the subject of the following article.[7] The esterolytic activity and enantioselectivity will be published separately.[8] Preliminary communications describing the effect of added surfactants on these properties have appeared.[9]

Results and Discussion

Isocyanides are generally prepared by dehydration of the corresponding formamides.[10] In order to combine imidazolyl, carboxyl and hydroxymethyl groups, we initially synthesized the protected dipeptide **3**. This dipeptide



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could be converted into its isocyanide, which, however, was not stable, probably because of β-elimination of acetic acid. The reverse approach, the isocyano function at the histidine side is not attractive because during the synthesis of the isocyanide $ImCH_2CH(COOCH_3)N{=}C$ from L-histidine we observed complete racemization.[5a] Elimination and racemization could be avoided by applying an alanine residue as a spacer. The synthesis sequence is depicted in Scheme I. Three polymers, **15a–c**, were prepared in this way. Although the histidine is in the middle of the side chains, space filling (CPK) models reveal that it can be approached by a substrate molecule.

Dipeptides **7a** and **7b** were obtained after coupling $N(Im),N(\alpha)$-ditrityl-L-histidine **5**, and serine methyl esters, **6a** and **6b**, by using the dicyclohexyl carbodiimide (DCC) method.[11] These dipeptides were subsequently detritylated with hydrochloric acid to obtain compounds **8a** and **8b**. Tripeptides **11a**, **11b**, and **11c** were synthesized from the active 4-nitrophenyl esters of *N*-formylalanines **10a** and **10c** and dipeptides **8a** and **8b**. The imidazolyl and hydroxymethyl functions of **11** were protected with *p*-toluenesulfonyl and acetyl groups, respectively. Compounds **12a**, **12b**, and **12c** were isolated in rather good yields (70% from **8**). Isocyanides **13a**, **13b**, and **13c** were obtained by the phosphorus oxychloride–triethylamine procedure[10] at low temperature in about 75% yield.

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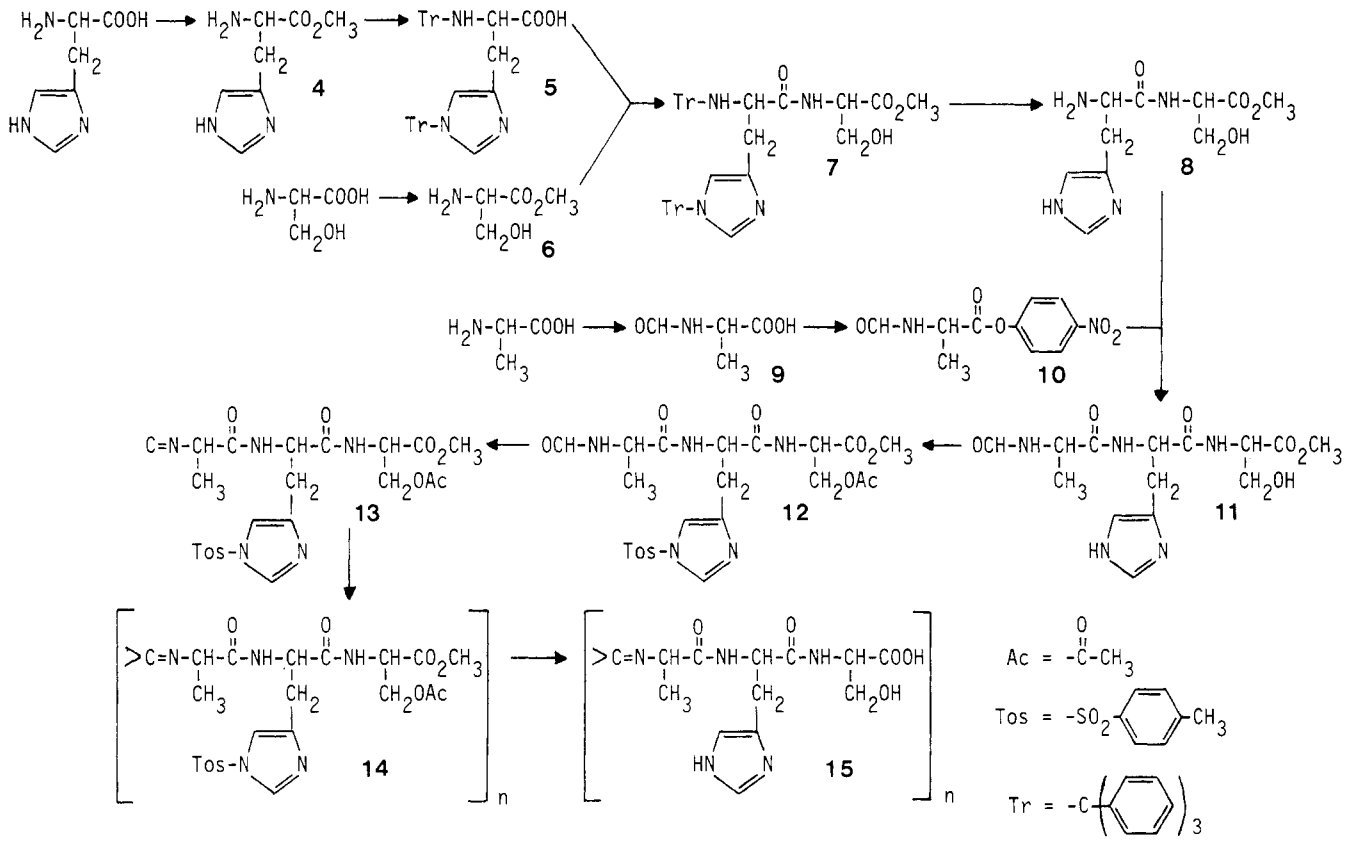
Scheme I



Configuration

a : L-Ala, L-His, L-Ser

b : L-Ala, L-His, D-Ser

c : D-Ala, L-His, L-Ser

Starting from **7** the overall yields of compounds **13a**, **13b**, and **13c** are approximately 55%.

The structures of the formamides and the isocyanides were confirmed by spectroscopic techniques. We checked separately whether racemization had occurred at the chiral centers during step **12** → **13**. If racemization had occurred, each of the compounds **13a**, **13b**, and **13c** would have been contaminated to a certain extent by the other diastereomers. In the ^1H NMR spectra of **13a**, **13b**, and **13c**, 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 (±5%) no racemization had occurred. Thin-layer chromatography confirmed this observation. The infrared absorption spectra of compounds **13** showed characteristic isocyanide stretching vibrations at 2142–2146 cm^{-1}.

Polymerization was achieved by adding 1 mol% of nickel(II) chloride to a solution of the isocyanide in chloroform–methanol, 4:1 v/v. The polymerizations were followed by observing the disappearance of the isocyanide stretching vibration in the infrared absorption spectrum. The polymerizations of isocyanides **13a**, **13b**, and **13c** were completed within two days at ambient temperature. The polymers **14** are soluble in chloroform and methanol and insoluble in water, ether, benzene, and the lower straight chain hydrocarbons. The *N*(Im)-tosyl, acetyl, and methyl groups were removed by treatment with 0.5 M aqueous NaOH for two days at 40 °C. During this reaction no hydrolysis of the imino functions of the polymer main chain occurred, as we checked separately. After acidification with hydrochloric acid, ultrafiltration, and freeze-drying, the purified products were analyzed as the polymers **15a**, **15b**, and **15c**, containing various amounts of hydrogen chloride and of water of crystallization. These polymers are soluble in water and methanol and insoluble in nonpolar solvents.

From the intrinsic viscosities of the protected polymers **14** the average molecular weights were estimated by applying the Mark–Houwink equation as determined for poly(2-octyliminomethylene): $[\eta] = 1.4 \times 10^{-9} \bar{M}_w^{1.75}$.[12]

Table I. Intrinsic Viscosity Data of Poly(iminomethylenes) and Estimated Molecular Weights ($\bar{M}_v$)[a]

no.	$[\eta]$, dL g^{-1} [b]	no.	$[\eta]$, dL g^{-1} [c]	$\bar{M}_v$
15a	0.106	**14a**	0.054	24 000
15b	0.082	**14b**	0.039	20 000
15c	0.030	**14c**	0.085	35 000

[a] $\bar{M}_v$ was obtained from intrinsic viscosity data of polymers **14** (see text). [b] In 0.02 M acetic acid–sodium acetate buffer pH 4.2. [c] In chloroform–methanol 5:2, v/v at 30.00 °C.

Table II. Optical Rotations of Monomers RN=C, 13, and of Polymers (RN=C<)$_n$, 14 and 15

no.	$[\alpha]^{20}_D$,[a] deg	no.	$[\alpha]^{20}$,[d,b] deg	no.	$[\alpha]^{20}_D$,[c] deg
13a	−13.5	**14a**	−58.9	**15a**	−8.0
13b	−2.1	**14b**	−29.3	**15b**	+8.0
13c	+9.2	**14c**	−8.0	**15c**	+30.2

[a] In chloroform, *c* 1–2. [b] In chloroform–methanol 5:2 v/v, *c* 0.2. [c] In 0.02 M acetic acid–sodium acetate buffer pH 4.2, *c* 0.05–0.1.

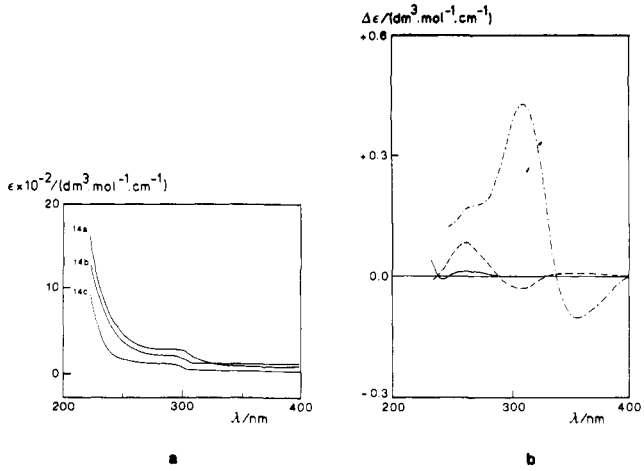


Figure 1. (a) UV spectra of polymers **14a**, **14b**, and **14c** in chloroform. (b) CD spectra of polymers **14a**, **14b** and **14c** in chloroform–methanol, 5:2 v/v.

Values in the range from $\bar{M}_v$ 20 000–35 000 were obtained (Table I). The optical rotations of the polymers and corresponding monomers are given in Table II.

The ultraviolet (UV) spectra of the protected polymers **14a**, **14b**, and **14c** in chloroform showed a shoulder at about 310 nm on the onset of a much larger band in the far UV region (Figure 1, part a). This shoulder can be attributed to the n–π^* transition of the N=C chromophore.[2,13]

Circular dichroism (CD) can be of great help to determine which screw sense of a poly(iminomethylene) is present in excess.[2,13] The CD spectra of polymers **14** in the region from 240–400 nm are given in Figure 1, part b. The spectrum of **14b** shows a negative couplet, indicating the polymer to be predominantly in the right handed (*P*) helical configuration.[2,13] The shoulder in the spectrum of polymer **14a** at 270 nm suggests the same configuration for this polymer. In the CD spectrum of **14c**, no clear 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. We are not able to decide which of these two reasons is the correct one.

The CD spectra of the deprotected polymers **15a–c** in water could not be measured accurately due to an unfavorable $\Delta\epsilon/\epsilon$ ratio (the solutions have dark colors). No clear-cut couplets are found in the region around 300 nm, probably because they are of low intensity and thus outside the limit of detection.

In reactions catalyzed by imidazole its unprotonated form appears to be the catalytically active species.[14] Knowledge about the state of ionization of the carboxylic acid and imidazole groups in the polymer is required for the elucidation of carboxylic acid–imidazole interactions during the catalysis. The relation between pH and degree of dissociation of imidazolyl and carboxyl residues in polymers **15a**, **15b**, and **15c** was determined by potentiometric titration. The titration curves were very similar (an example is given in Figure 2). From these titrations the fraction of unprotonated imidazole, α_{Im}, and carboxylate, α_{COO^-}, can be calculated at each pH. From the modified Henderson–Hasselbach equation,[15] $pH = pK_a - n \log ((1 - \alpha)/\alpha)$, $pK_a(ImH^+)$, $pK_a(COOH)$, $n(ImH^+)$, and $n(COOH)$ can be calculated (Table III). The pK_a values of the carboxylic acid groups are appreciably higher in polymers **15** than in L-histidine and L-serine. Also the $pK_a(ImH^+)$ values of the polymers have increased considerably as compared to the pK_a values of the model compounds. This increase reveals that the imidazole residues in the polymers are affected by the negative charge of the carboxylate ions. The effect is larger for **15c** than for **15a** and **15b**, suggesting that in the former polymer the interaction between the oppositely charged groups is stronger. The titration experiments and UV–vis data indicate that the imino functions of the polymer main chain remain unprotonated even in relatively strong acidic media (pH <2). The reason for this probably is that protonation causes unfavorable electrostatic interactions along the helical main chain.

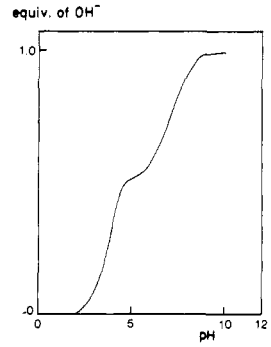


Figure 2. Titration curve of polymer **15b**.

Table III. pK_a Values of Poly(iminomethylenes) and of Model Compounds[a]

compd	pK_a (COOH)	n(COOH)	pK_a (ImH^+)	$n(ImH^+)$
15a	3.8	1.8	7.1	2.0
15b	4.7	2.2	8.4	0.9
15c	5.8	2.0	8.7	1.6
His	2.0 (1.8[b])	1.0	6.3 (6.0[b])	1.0
Ser[b]	2.2	1.0		
poly(His)[c]			5.9	
Copoly(His-Asp)[c]			7.0	
β-Ala-His[b]	2.6		6.0	
OCH-Ala-His-Ser	2.1	1.0	6.3	1.1

[a] In 30% v/v EtOH–H_2O at ambient temperature and ionic strength 0.2 M; estimated error in pK_a is ±0.1, estimated error in n value is ±0.05–0.1. [b] In water, see ref 20. [c] In water, see ref 21.

Experimental Section

Melting points were determined on a Mettler FP5/FP51 photoelectric melting point apparatus. Optical rotations were measured on a Perkin-Elmer 241 polarimeter. Infrared (IR) spectra were recorded on Perkin-Elmer 297 and 283 spectrophotometers. Ultraviolet spectra were recorded on a Perkin-Elmer 254 UV–vis spectrophotometer. ^1H NMR spectra were obtained on a Varian EM 390 instrument. Chemical shifts (δ) are given in ppm downfield from internal tetramethylsilane or sodium 2,2,3,3-tetradeuterio-3-(trimethylsilyl)propionate. Abbreviations used: s = singlet, d = doublet, q = quartet, m = multiplet, b = broad. Elemental analyses were carried out by the Elemental Analytical Section of the Institute of Chemistry TNO, Utrecht, The Netherlands. TLC was performed on silica (Schleicher and Schüll TLC Ready Plastic Foil FR-1500) and detection was effected by UV and/or iodide vapor. Column chromatography was performed on silica (Merck Kieselgel 60, 230–400 mesh). CD spectra were recorded on a home built apparatus. This instrument measures the differential absorbance (ΔA) with a sensitivity better than 1×10^{-6}. Solution viscosities were obtained with a Cannon-Ubbelohde viscometer. Intrinsic viscosities, optical rotation and CD data for solutions of the deprotected polymers were obtained in 0.02 M acetic acid–sodium acetate buffer at pH 4.2. Titrations were performed on Mettler automatic titrator devices

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types DV 10, DK 12, DK 14, and DK 25.

L-Histidine monohydrochloride, $[\alpha]^{20}_D$ +9.2° (c 5, 5 M HCl), was purchased from Fluka; L-alanine, $[\alpha]^{20}_D$ +9.7°, and D-alanine, $[\alpha]^{20}_D$ −9.4° (c 2, 1 M HCl), were purchased from BDH; L-serine, $[\alpha]^{20}_D$ −6.8°, and D-serine $[\alpha]^{20}_D$ +6.6° (c, 2, water), were purchased from Aldrich.

L-Histidine Methyl Ester Dihydrochloride (4). This compound was obtained from L-histidine monohydrochloride by treatment with hydrogen chloride gas in methanol. It was used without further purification for the synthesis of compound **5**: mp 199–200.5 °C (lit.[16] mp 200–201 °C); $[\alpha]^{20}_D$ +15.6° (c 1, methanol) (lit.[17] $[\alpha]^{20}_D$ +16° (c 1, methanol).

***N*(Im),*N*(α)-Ditrityl-L-histidine (5).** This compound was prepared by suspending **4** in chloroform at 0 °C. Triethylamine (2 equiv) was added followed by 2.1 equiv of trityl chloride. After stirring for 16 h the chloroform was evaporated and a 20% w/w KOH solution in propylene glycol was added to the residue. This mixture was refluxed for 15 min and cooled to room temperature. Water and acetic acid were introduced followed by ethanol. The crude product was collected by filtration and dissolved in hot ethanol. After refluxing for 1 h the solution was cooled to room temperature, the crystallized product filtered off and recrystallized from dichloromethane–ether. The product was washed with ether and dried over KOH: yield 65%; mp 182–184 °C (lit.[18] mp 184–185 °C); $[\alpha]^{20}_D$ +9.0° (c 1, chloroform) (lit.[18] $[\alpha]^{20}_D$ +9.6° (c 1, chloroform)).

L-Serine methyl ester hydrochloride (6a) and D-serine methyl ester hydrochloride (6b) were synthesized from L-serine and D-serine through esterification with thionyl chloride in methanol. Recrystallization from methanol–ether gave pure white crystalline products. **6a**: mp 165–176 °C; $[\alpha]^{20}_D$ +3.5° (c 2, methanol) (lit.[19] mp 165 °C; $[\alpha]^{20}_D$ +5.5° (c 1.8, methanol)). **6b**: mp 164.5 °C; $[\alpha]^{20}_D$ −3.3° (c 2, methanol).

***N*(Im),*N*(α)-Ditrityl-L-histidyl-L-serine Methyl Ester (7a).** **5** (30 g, 46 mmol) was dissolved in 150 mL of dichloromethane. To this solution was added 7.2 g (46 mmol) of **6a**. The mixture was cooled to 0 °C and 6.5 mL of triethylamine was added. Thereupon 10 g (48 mmol) of *N,N'*-dicyclohexylcarbodiimide was introduced at once. After 5 min, formation of a precipitate was observed. The reaction mixture was stirred for 2 h at 0 °C. After stirring overnight at room temperature the *N,N'*-dicyclohexylurea was filtered off and the solution concentrated in vacuum to yield a slightly yellow oil. The product was dissolved in ethanol; it crystallized upon addition of ether: yield 25 g (73%); mp 227–229 °C (lit.[18] mp 227–228 °C); $[\alpha]^{20}_D$ +23.4° (c 0.5, chloroform); IR (KBr) 3410, 3300 and 3230 (OH and NH), 3000–2800 (CH), 1745 ($COOCH_3$) and 1665 cm^{-1} (NHCO); ^1H NMR ($CDCl_3$) δ 8.7 (d, 1 H, NH(His)), 7.3 (s, 1 H, imidazole), 7.2 (m, 32 H, ArH, imidazole and NH), 6.0 (1 H, OH), 4.8 (m, 1 H, CH(Ser)), 4.4 (1 H, CH(His)), 3.9 (s, 3 H, OCH_3), 3.8 (m, 2 H, CH_2(Ser)), 3.4 and 2.9 (2 d, 2 H, CH_2(His)) and 1.9 (d, 1 H, NH).

***N*(Im),*N*(α)-Ditrityl-L-histidyl-D-serine Methyl Ester (7b).** This compound was prepared from **5** and **6b** in the same way as described for **7a**: yield 15.8 g (88%); mp 204–205 °C; $[\alpha]^{20}_D$ +13.4° (c 0.5, chloroform); IR (KBr) data as for **7a**; ^1H NMR ($CDCl_3$) δ 6.2 (1 H, OH) and data as for **7a** within 0.1 ppm.

L-Histidyl-L-serine Methyl Ester Dihydrochloride (8a). An amount of 24 g (32 mmol) of **7a** in 75 mL of an 80% aqueous ethanol solution 0.9 N in HCl was refluxed for 30 min. After cooling the solvent was evaporated and the residue dissolved in 100 mL of water. The solution was extracted twice with 25-mL portions of chloroform. The organic layer was evaporated. The resulting syrup could not be crystallized. For this reason the product was dissolved in 25 mL of water and freeze-dried. This resulted in a hygroscopic white amorphous solid: yield 9.4 g (89%); $[\alpha]^{20}_D$ +45.3° (c 0.5, water); IR (KBr) 3500–2800 (NH, COOH, OH), 2800–2200 (HCl salt), 1740 ($COOCH_3$) and 1680 cm^{-1} (CONH); ^1H NMR (D_2O) δ 9.1 and 7.7 (2 × s, 2 H, imidazole), 4.5–4.9 (m, 2 H, CH(Ser) and CH(His)), 4.2 (d, 2 H, CH_2(Ser)), 3.9 (s, 3 H, OCH_3) and 3.7 (m, CH_2(His)).

L-Histidyl-D-serine Methyl Ester Dihydrochloride (8b). This product was obtained from **7b** in the same way as described for **8a**. Yield 4.3 g (92%) of white amorphous **8b**: $[\alpha]^{20}_D$ +34.9° (c 0.5, water); IR (KBr) data as for **8a**; ^1H NMR (D_2O) data as for **8a** within 0.1 ppm.

***N*-Formyl-L-alanine (9a) and *N*-formyl-D-alanine (9c)** were prepared by formylation of L-alanine and D-alanine with a mixture of formic acid and acetic acid anhydride.[2] Recrystallization from acetone gave pure white crystals of **9a** and **9c**. **9a**: mp 130–131.5 °C; $[\alpha]^{20}_D$ +63.1° (c 2, 1 N NaOH) (lit.[4c] mp 131 °C; $[\alpha]^{20}_D$ +65°). **9c**: mp 131.5–132.0 °C; $[\alpha]^{20}_D$ −52° (c 2, 1 N NaOH) (lit.[4c] mp 130 °C; $[\alpha]^{20}_D$ −56° (c 2, 1 N NaOH)).

***N*-Formyl-L-analine 4-Nitrophenyl Ester (10a).** **9a** (5.0 g, 42.7 mmol) was dissolved in 50 mL of pyridine and cooled to 0 °C. 4-Nitrophenol (7.0 g, 50.4 mmol) was added and thereupon 9.0 g (43.7 mmol) of *N,N'*-dicyclohexylcarbodiimide. The reaction mixture was stirred for 2 h at 0 °C. During this period dicyclohexylurea precipitated. The reaction mixture was stirred for an additional 16 h at room temperature. The urea was filtered off and washed with pyridine (25 mL). The combined pyridine layers were evaporated at room temperature under diminished pressure (oil pump). The residue was dissolved in 50 mL of chloroform, cooled to 0 °C, and extracted with five portions of 5 mL of ice cold 1 N NaOH. The organic layer was dried (Na_2SO_4), filtered, and concentrated to a volume of approximately 10 mL. This solution was then subjected to column chromatography (eluent diethyl ether). The product was obtained as a pure white crystalline solid: yield 8.3 g (82%): mp 118–120 °C; $[\alpha]^{20}_D$ −17.1° (c 1, chloroform); ^1H NMR ($CDCl_3$) δ 8.0 (s, 1 H, CHO), 8.1 and 7.1 (2 d, 4 H, ArH), 7.2 (d, 1 H, NH), 4.6 (m, 1 H, CH) and 1.3 (d, 3 H, CH_3).

***N*-Formyl-D-alanine 4-Nitrophenyl Ester (10c).** This compound was obtained from **9c** as described for **10a**: yield 3.9 g (73%); mp 116.4–118.0 °C; $[\alpha]^{20}_D$ +17.0 (c 1, chloroform); ^1H NMR ($CDCl_3$) data as for **10a** within 0.1 ppm.

***N*-Formyl-L-alanyl-*N*(Im)-tosyl-L-histidyl-*O*-acetyl-L-serine Methyl Ester (12a).** An amount of 3.1 g (9.4 mmol) of **8a** was dissolved in 50 mL of acetonitrile, cooled to 0 °C, and stirred for 3 h with 15 g of molecular sieve (**4a**). Hereafter, 2.6 mL (18.8 mmol) of triethylamine was added and the mixture was stirred for 1 h at 0 °C. This solution was added dropwise over a 1-h period to 2.3 g (9.7 mmol) of **10a** dissolved in 19 mL of acetonitrile. The vigorously stirred reaction mixture was kept for 1 h at 0 °C and for one night at room temperature. The molecular sieve was removed and the solvent was evaporated. The residue **11a** was dissolved in 75 mL of acetic anhydride and 1 mL of pyridine. After standing for one day the solvent was evaporated at 40 °C in an oil pump vacuum. The remaining solid was crushed and washed three times with 50-mL portions of diethyl ether. The slightly brown colored solid was dissolved in 75 mL of chloroform, and 8 g of sodium carbonate was added. Subsequently, 2.0 g (10.5 mmol) of tosyl chloride in 10 mL of chloroform was introduced dropwise. After the reaction had stirred overnight at room temperature, the sodium carbonate was filtered off and the solution was extracted with 50 mL of 1 N acetic acid, 50 mL of 1 N sodium carbonate, and with two portions of water. The organic layer was dried (Na_2SO_4) and evaporated. The residue was subjected to column chromatography (eluent chloroform–methanol, 10:1 v/v) to give pure, white crystalline **12a**: yield 3.6 g (72%); mp 161–164 °C; $[\alpha]^{20}_D$ −10.0° (c 0.5, chloroform); IR (KBr) 3310 (NH), 1730 ($OCOCH_3$, $COOCH_3$), 1640 (NHCO), 1600, 1370 and 1180 cm^{-1} (tosyl); ^1H NMR ($CDCl_3$ + a trace of CD_3OD) δ 8.1 (s, 1 H, CHO), 8.0 and 7.2 (2 s, 2 H, imidazole), 7.9 and 7.4 (2 d, 4 H, tosyl), 4.7 (m, 3 H, CH (Ala, His, and Ser)), 4.4 (d, 2 H, CH_2(Ser)), 3.8 (s, 3 H, OCH_3), 3.0 (d, 2 H, CH_2(His)), 2.4 (s, 3 H, CH_3 tosyl), 2.0 (s, 3 H, $COCH_3$) and 1.3 (d, 3 H, $CHCH_3$).

***N*-Formyl-L-alanyl-*N*(Im)-tosyl-L-histidyl-*O*-acetyl-D-serine Methyl Ester (12b).** This tripeptide was synthesized from **10a** and **8b** as described for **12a**. A pure, white, crystalline product, **12b**, was obtained after column chromatography: yield 2.6 g (68%); mp 160–162 °C; $[\alpha]^{20}_D$ −18.2° (c 0.5, chloroform); IR (KBr) and ^1H NMR ($CDCl_3$ + a trace of CD_3OD) data as for **12a** within 5 cm^{-1} and 0.1 ppm, respectively.

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***N*-Formyl-D-alanyl-*N*(Im)-tosyl-L-histidyl-*O*-acetyl-D-serine Methyl Ester (12c).** This tripeptide was obtained from **10c** and **8a** in essentially the same way as described for **12a**. After column chromatography, pure **9c** was obtained in the form of white crystals: yield 2.9 g (75%); mp 154–157 °C; $[\alpha]^{20}_D$ +10.8° (*c* 0.5, chloroform); IR (KBr) and ^{1}H NMR ($CDCl_3$ + a trace of CD_3OD) data as for **9a** within 5 cm^{-1} and 0.1 ppm, respectively.

L-Carbylalanyl-*N*(Im)-tosyl-L-histidyl-*O*-acetyl-L-serine Methyl Ester (13a). In a round-bottomed vessel, equipped with a magnetic stirrer and a CO_2/acetone reflux condensor (kept at −50 °C) an amount of 3.2 g (5.7 mmol) of **12a** was dissolved in 50 mL of dichloromethane. The solution was brought under a nitrogen atmosphere and cooled to −40 °C. After the reaction had stirred for 30 min, 2.8 mL of triethylamine was added. An amount of 1.7 g (11 mmol) of phosphorus oxychloride in 10 mL of dichloromethane was introduced into the stirred reaction mixture over a period of 1.5 h. The reaction was followed by TLC (chloroform–methanol, 10:1 v/v, R_f **13a** 0.35 and R_f **12a** 0.05–0.10). The temperature was raised to −10 °C and 25 mL of 10% aqueous sodium bicarbonate (0 °C) was introduced at once. After stirring for 5 min the organic layer was separated and extracted twice with 25-mL portions of water. The organic layer was dried (Na_2SO_4) and the solvent was evaporated under reduced pressure at room temperature. The residual yellow-brown oil was subjected to column chromatography (eluent chloroform–methanol 10:1 v/v) to give a clear colorless oil of pure **13a**: yield 2.2 g (72%); $[\alpha]^{20}_D$ −13.5° (*c* 2, chloroform); IR (neat) 3310 (NH), 2142 (NC), 1740 ($OCOCH_3$, $COOCH_3$), 1660 (NHCO), 1600, 1370 and 1180 cm^{-1} (tosyl); ^{1}H NMR ($CDCl_3$ + a trace of CD_3OD) δ 8.0 and 7.3 (2 s, 2 H, imidazole), 7.8 and 7.4 (2 d, 4 H, tosyl), 4.8 (q, 1 H, CH(Ala)), 4.7 (m, 2 H, CH(Ser and His)), 3.0 (d, 2 H, CH_2(His)), 3.7 (s, 3 H, OCH_3), 3.0 (d, 2 H, CH_2(His)), 2.4 (s, 3 H, CH_3 tosyl), 2.0 (s, 3 H, CH_3 acetyl) and 1.3 (d, 3 H, CH_3).

L-Carbylalanyl-*N*(Im)-tosyl-L-histidyl-*O*-acetyl-D-serine Methyl Ester (13b). This compound was synthesized from **12b** as described for **13a**: yield 1.5 g (77%) of a clear colorless oil; TLC (chloroform–methanol, 10:1 v/v) R_f **12b** 0.05–0.10, R_f **13b** 0.37; $[\alpha]^{20}_D$ −2.1° (*c* 2, chloroform); IR (neat) 2143 cm^{-1} (NC); ^{1}H NMR ($CDCl_3$ + a trace of CD_3OD) data as for **13a** within 0.1 ppm.

D-Carbylalanyl-*N*(Im)-tosyl-L-histidyl-*O*-acetyl-L-serine Methyl Ester (13c). This compound was obtained from **12c** as described for **13a**: yield 1.8 g (77%). Isocyanide **13c** was obtained as a white crystalline material after column chromatography and recrystallization from methanol and ether: TLC (chloroform–methanol 10:1 v/v) R_f **12c** 0.0–0.15, R_f **13c** 0.35; mp 148–152 °C; $[\alpha]^{20}_D$ +9.2° (*c* 1, chloroform); IR (KBr) 2146 cm^{-1} (NC); ^{1}H NMR ($CDCl_3$ + a trace of CD_3OD) δ 4.7 (m, 3 H, CH(Ala, His and Ser)), other data as for **13a** within 0.1 ppm.

Poly(L-carbylalanyl-*N*(Im)-tosyl-L-histidyl-*O*-acetyl-L-serine Methyl Ester) (14a). This polymer was obtained by addition of 1 mol% of $NiCl_2 \cdot 6H_2O$ (1 mg dissolved in 0.5 mL of methanol) to a solution of 1.5 g (4.45 mmol) of **13a** in 2 mL of chloroform. After two days, the solvent was evaporated and the residue (a dark brown syrup) was poured into a vigorously stirred mixture of 50 mL of methanol–water (1:5 v/v). The precipitate was filtered off and dried: yield 1.1 g (73%) of a yellow brown solid; $[\alpha]^{20}_D$ −58.9° (*c* 0.2, chloroform); [η] 0.054 dL/g (chloroform–methanol 5:2 v/v, 30.00 °C); IR (KBr) 3300 (NH), 1749 ($OCOCH_3$, $COOCH_3$), 1670 (NHCO), 1600, 1380 and 1170 cm^{-1} (tosyl). The N=C vibration is masked by the strong absorption band at 1670 cm^{-1}.

Poly(L-carbylalanyl-*N*(Im)-tosyl-L-histidyl-*O*-acetyl-D-serine Methyl Ester) (14b). This polymer was synthesized from **13b** as described for **14a**. It was obtained as a light brown solid: yield 1.03 g (79%); $[\alpha]^{20}_D$ −29.3° (*c* 0.2, chloroform); [η] 0.039 dL/g (chloroform–methanol 5:2 v/v, 30.00 °C); IR (KBr) data as for **14a**.

Poly(D-carbylalanyl-*N*(Im)-tosyl-L-histidyl-*O*-acetyl-L-serine Methyl Ester) (14c). This polymer was synthesized from **13c** as described for **14a**. The polymer was a light brown solid: yield 1.2 g (82%); $[\alpha]^{20}_D$ −8.0° (*c* 0.2, chloroform); [η] 0.085 dL/g (chloroform–methanol, 5:2 v/v, 30.00 °C); IR (KBr) data as for **14a**.

Poly(L-carbylalanyl-L-histidyl-L-serine) (15a). Polymer **14a** was deprotected by treatment with 25 mL of 0.5 N NaOH for two days at 40 °C. The reddish-brown solution was acidified to pH 2 with 1 N HCl. The solution was subsequently submitted to ultrafiltration (Diaflo Ultrafilter UM-2) and freeze-dried. The polymer was obtained as a creamish-caramel colored spongy solid: yield 0.55 g (83%); $[\alpha]^{20}_D$ −8.0° (*c* 0.1, buffer); [η] 0.106 dL/g (buffer, 30.00 °C). Anal. Calcd for $C_{13}H_{17}N_5O_5(HCl)_{0.28}(H_2O)_{0.2}$: C, 44.0; H, 5.6; N, 19.7; O, 27.9; Cl, 2.8. Found: C, 43.9; H, 5.3; N, 19.8; O, 28.2; Cl, 2.8. IR (KBr) 3700–2800 (NH_3^+, COOH, OH), 1720–1600 (COOH, NHCO, and N=C). The N=C stretching absorption band is partly masked by the amide and acid carbonyl bands. When varying the deprotection reaction time, polymer samples with the same optical rotation values were obtained, indicating that racemization of the product under the basic conditions employed does not noticeably take place.

Poly(L-carbylalanyl-L-histidyl-D-serine) (15b). This polymer was obtained as a yellowish-brown glassy solid by deprotection of **14b** as described for **15a**: yield 0.32 g (80%); $[\alpha]^{20}_D$ +8.0° (*c* 0.05, buffer); [η] 0.082 dL/g (buffer, 30.00 °C). Anal. Calcd for $C_{13}H_{17}N_5O_5(HCl)_{0.8}(H_2O)_{2.1}$: C, 40.0; H, 5.7; N, 17.9; O, 29.1; Cl, 7.3. Found: C, 39.8; H, 5.6; N, 18.1; O, 29.3; Cl, 7.2. IR (KBr) data as for **15a** within 5 cm^{-1}.

Poly(D-carbylalanyl-L-histidyl-L-serine) (15c). This polymer was synthesized from **14c** as described for **15a**. It was obtained as a yellowish-brown powder: yield 0.57 g (81%); $[\alpha]^{20}_D$ +30.2° (*c* 0.1 buffer); [η] 0.030 dL/g (buffer, 30.00 °C). Anal. Calcd for $C_{13}H_{17}N_5O_5(HCl)_{0.6}(H_2O)_{1.8}$: C, 41.2; H, 5.7; N, 18.5; O, 28.7; Cl, 5.9. Found: C, 41.0; H, 5.6; N, 18.5; O, 28.9; Cl, 6.0. IR (KBr) data as for **15a** within 5 cm^{-1}.

Potentiometric Titrations. Polymers **15a**, **15b**, and **15c** were dissolved in ethanol–water (30% v/v) until a concentration of 10 mg/mL was obtained. These solutions were adjusted to pH 2 by adding 1 N aqueous HCl. An amount of KCl was added, such that at the end point of titration μ = 0.2 M. The solutions were titrated with 0.1 M NaOH in ethanol–water (30% v/v) while being stirred. The solution was protected from carbon dioxide by solid KOH. Blank titration curves were obtained by titrating 20-mL aliquots of ethanol–water (30% v/v) adjusted to the same pH value and ionic strength. Differential titration curves were derived graphically and from these curves the degrees of ionization were evaluated.

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Registry No. **4**, 7389-87-9; **5**, 74853-62-6; **6a**, 5680-80-8; **6b**, 5874-57-7; **7a**, 97102-77-7; **7b**, 97102-78-8; **8a**, 81040-72-4; **8b**, 97102-79-9; **10a**, 61167-49-5; **10c**, 61167-50-8; **12a**, 97102-80-2; **12b**, 97102-81-3; **12c**, 97102-82-4; **13a**, 97134-74-2; **13b**, 97102-83-5; **13c**, 97134-73-1; **14a**, 97134-75-3; **14b**, 97134-76-4; **14c**, 97169-12-5; $NiCl_2$, 7718-54-9; trityl chloride, 76-83-5; 4-nitrophenol, 100-02-7; tosyl chloride, 98-59-9.