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

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Three optically active polymers of isocyanides, \([\text{RN} = \text{C}]_n\), which contain imidazolyl, carboxyl, and hydroxymethyl functions in their side chains, 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 \(M_n\) range 20,000–25,000. 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.

Results and Discussion

Isocyanides are generally prepared by dehydration of the corresponding formamides. In order to combine imidazolyl, carboxyl and hydroxymethyl groups, we initially synthesized the protected dipeptide 3. This dipeptide could be converted into its isocyanide, which, however, was not stable, probably because of \(\beta\)-elimination of acetic acid. The reverse approach, the isocyanate function at the histidine side is not attractive because during the synthesis of the isocyanide \(\text{ImCH}_2\text{CH}({\text{COOCCH}})_3\text{N} = \text{C} - \text{L-histidine}\) we observed complete racemization. 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 \(N(\text{Im}), N(\text{\alpha})-\text{drityl-}\text{L-histidine}\) and serine methyl esters, 6a and 6b, by using the dicyclohexyl carbodiimide (DCC) method. 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–tritylamine procedure at low temperature in about 75% yield.

Polymers of isocyanides, \([\text{RN} = \text{C}]_n\), called poly(iminomethylene)s or poly(carbonimidoys), have a helical rigid rod configuration. Their chirality and rigidity, with all side chains \(R\) in an almost equal environment, make them attractive as enzyme models. In earlier papers we reported on the synthesis and esterolytic activity of imidazole containing poly(iminomethylene)s, e.g., 1 derived from \(L\)-histidine and 2 derived from \(D\)-alanyl-L-histidinol. The 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 (\(k_H/k_D = 1.1\)) in the hydrolysis of 4-nitrophenyl esters of optically active amino acids catalyzed by polymer 2.

The esterolytic enzyme chymotrypsin possesses a so-called charge relay system in its active site. 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. The esterolytic activity and enantioselectivity will be published separately. Preliminary communications describing the effect of added surfactants on these properties have appeared.

(1) Taken in part from the thesis of H. G. J. Visser, Utrecht, 1983. Part 20 in the series Poly(iminomethylene)s. For part 19, see ref 5a and part 18, ref 2.
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 $^1$H 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 ($\pm$5%) no racemization occurred, as we checked separately. 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 hydrochloride 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 (see text). $^a$In 0.02 M acetic acid-sodium acetate buffer pH 4.2. $^b$In chloroform–methanol 5:2, v/v at 30.00 °C.

Table I. Intrinsic Viscosity Data of Poly(iminomethylene) and Estimated Molecular Weights ($M_v$)$^a$

<table>
<thead>
<tr>
<th>no.</th>
<th>$[\eta]$, dL g$^{-1}$</th>
<th>$[\eta]$, dL g$^{-1}$</th>
<th>$M_v$</th>
</tr>
</thead>
<tbody>
<tr>
<td>15a</td>
<td>0.106</td>
<td>0.054</td>
<td>24000</td>
</tr>
<tr>
<td>15b</td>
<td>0.082</td>
<td>0.039</td>
<td>20000</td>
</tr>
<tr>
<td>15c</td>
<td>0.085</td>
<td>0.085</td>
<td>35000</td>
</tr>
</tbody>
</table>

$^a$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.

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

<table>
<thead>
<tr>
<th>no.</th>
<th>$[\alpha]_{D}^{20}$ deg</th>
<th>$[\alpha]_{D}^{20}$ deg</th>
<th>$[\alpha]_{D}^{20}$ deg</th>
</tr>
</thead>
<tbody>
<tr>
<td>13a</td>
<td>-13.5</td>
<td>15a</td>
<td>-30.2</td>
</tr>
<tr>
<td>13b</td>
<td>-2.1</td>
<td>15b</td>
<td>+30.2</td>
</tr>
<tr>
<td>13c</td>
<td>+3.2</td>
<td>15c</td>
<td>+30.2</td>
</tr>
</tbody>
</table>

$^a$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.

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Values in the range from $\bar{M}_r$ 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 $\text{14a}$, $\text{14b}$, and $\text{14c}$ 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 $n\rightarrow\pi^*$ transition of the $\text{N} \equiv \text{C}$ chromophore.\textsuperscript{1,2}\textsuperscript{13}

Circular dichroism (CD) can be of great help to determine which screw sense of a poly(iminomethylene) is present in excess.\textsuperscript{1,2}\textsuperscript{13} The CD spectra of polymers $\text{14}$ in the region from 240–400 nm are given in Figure 1, part b. The spectrum of $\text{14b}$ shows a negative couplet, indicating the polymer to be predominantly in the right handed (P) helical configuration.\textsuperscript{1,2}\textsuperscript{13} The shoulder in the spectrum of polymer $\text{14a}$ at 270 nm suggests the same configuration for this polymer. In the CD spectrum of $\text{14c}$, 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 $\text{15a–c}$ in water could not be measured accurately due to an unfavorable $\Delta r/\Delta t$ 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.\textsuperscript{1,4} 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 $\text{Henderson-Hasselbach equation,}$\textsuperscript{1,5} $\log K_a = \log K_a - \frac{n(\text{ImH}^+)}{n(\text{COOH})}$ can be calculated (Table III). The $pK_a$ values of the carboxylic acid groups are appreciably higher in polymers 15 than in $\text{L-histidine}$ and $\text{L-serine}$. Also the $pK_a(\text{ImH}^+)$ 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 $\text{15c}$ than for $\text{15a}$ and $\text{15b}$, 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.

### 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 Varian EM 390 instrument. Chemical shifts ($\delta$) are given in ppm downfield from internal tetrachloride or sodium 2,2,3,3-tetradecyl-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, 220–400 mesh). CD spectra were recorded on a home built apparatus. This instrument measures the differential absorbance ($\Delta A$) with a sensitivity better than $1 \times 10^{-6}$. 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.
L-Histidine methyl ester dihydrochloride (4). This compound was prepared 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); [α]D +15.6° (c 1, methanol) (lit.17 [α]D +16°, c 1, methanol).

\[ \text{L-(+) -Histidine} \rightarrow \text{L-Histidine methyl ester hydrochloride (4).} \]

**L-(−) -Histidine methyl ester hydrochloride (5).** This compound was prepared by formylation of L-alanine and D-alanine with a mixture of formaldehyde and acetic anhydride. 2 Recrystallization from acetonitrile gave pure white crystals of 9a and 9c. 9a: mp 130–131.5 °C; [α]D +63.1° (c 2, 1 N NaOH) (lit. mp 133.1 °C; [α]D +65°). 9c: mp 131.5–132.0 °C; [α]D +52° (c 2, 1 N NaOH) (lit. mp 130 °C; [α]D +56° (c 2, 1 N NaOH)).

**L-(−) -Histidyl-L-serine methyl ester (7a).** An amount of 24 g (32 mmol) of L-serine, (2 equiv) was added followed by 2.1 equiv of trityl chloride. After stirring for 3 h with 15 g of molecular sieve (4 Å), the mixture was filtered off and the residue was dissolved in ethanol; it was stirred for 1 h at 0 °C. After washing with ether and dried over KOH: yield 15.8 g (43.7 mmol) of 7a; [α]D +23.4° (c 0.5, chloroform); IR (KBr) 3410, 3300 and 3230 (OH and NH), 3000-2800 (CH); 1745 (C=O of tosyl), 1665 cm−1 (NH); \[ ^1H \] NMR (DCl3) δ 3.3 (s, 3 H, OCH3); 3.9 (s, 3 H, CH(Ser)), 4.4 (d, 2 H, CHO), 7.4 (d, 2 H, tosyl). 7a: mp 164.5 °C; [α]D +3.3°, (c 2, methanol).

**N(α)-Ditrityl-L-histidyl-L-serine methyl ester (7b).** An amount of 2.1 g (9.7 mmol) of 7a; [α]D +9.2° (c 1, chloroform); IR (KBr) 3410, 3300 and 3230 (OH and NH), 3000-2800 (CH); 1745 (C=O of tosyl), 1665 cm−1 (NH); \[ ^1H \] NMR (DCl3) δ 2.6 (s, 18 H, trityl), 4.4 (d, 2 H, CHO), 7.4 (d, 2 H, tosyl). 7b: mp 164.5 °C; [α]D +17.1° (c 1, chloroform); \[ ^1H \] NMR (CDCl3) 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, CH3).

**N-Formyl-L-alanyl-N(Im)-tosyl-L-histidyl-0-acetyl-D-serine methyl ester (10a).** 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 (4 Å). 0.2 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 crude reaction product was dissolved in a volume of acetonitrile and 10 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 (Na2SO4) 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; [α]D +10° (c 0.5, chloroform); IR (KBr) 3310 (NH), 1730 (COOCH3, COOH), 1640 (NHCO), 1600, 1370 and 1180 cm−1 (tosyl); \[ ^1H \] NMR (CDCl3) δ 8.1 (s, 1 H, CHO), 7.2 (t, 2 H, CHO), 7.1 (d, 1 H, CHO), 7.4 (d, 2 H, tosyl), 7.3 (d, 2 H, CH3), 5.3 (m, 3 H, CH2), 3.9 (m, 2 H, CH2CO), 1.9 (s, 6 H, CHO). 12a: yield 1.4 g (25%); mp 156–158 °C; [α]D +22° (c 0.5, chloroform); IR (KBr) 3410, 3300, 3230 (OH and NH), 3000-2800 (CH); 1745 (C=O of tosyl), 1665 cm−1 (NH); \[ ^1H \] NMR (DCl3) δ 3.3 (s, 3 H, OCH3); 3.8 (s, 3 H, OCH2); 3.8 (s, 3 H, CH3); 2.4 (s, 3 H, CH2tosyl); 2.0 (s, 3 H, COCH3) and 1.3 (d, 3 H, CH2).
N-Formyl-d-alanyl-N-(im)-tosyl-L-histidyl-O-acetyl-L-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; [α]D 20 +10.8° (c 0.5, chloroform); IR (KBr) and 1H NMR (CDCl3 + a trace of CD3OD) data as for 9a within 5 cm−1 and 0.1 ppm, respectively.

c-L-carballylalanyl-N-(im)-tosyl-L-histidyl-O-acetyl-L-serine Methyl Ester (13a). In a round-bottomed vessel, equipped with a magnetic stirrer and a CO2/acetone reflux condenser (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, Rf 13a 0.35 and Rf 12a 0.05-0.10). This compound was obtained as a light brown solid: yield 0.55 g (83%); [α]D 20 +30.2° (c 0.1, buffer); [γ] 0.030 dL/g (buffer, 30.00 °C). Anal. Calcd for C31H19N2O7: C, 40.0; H, 5.7; N, 12.9; O, 18.4; Cl, 7.2. IR (KBr) data as for 15a within 5 cm−1.

Poly(c-carballylalanyl-N-(im)-tosyl-L-histidyl-O-acetyl-L-serine Methyl Ester) (14a). This polymer was obtained 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 (Dialco Ultrafilter UM-2) and freeze-dried. The polymer was obtained as a cream-coloured colored spongy solid: yield 0.55 g (83%); [α]D 20 +8.0° (c 0.1, buffer); [γ] 0.106 dL/g (buffer, 30.00 °C). Anal. Calcd for C31H19N2O7: C, 40.0; H, 5.7; N, 17.9; O, 29.1; Cl, 7.3. Found: C, 43.9; H, 5.3; N, 19.8; O, 28.2; Cl, 2.8. IR (KBr) 3700-2900 (NH, 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(c-carballylalanyl-L-histidyl-L-serine) (15b). This polymer was obtained as a yellow-brown glassy solid by deprotection of 14b as described for 15a: yield 0.32 g (80%); [α]D 20 +8.0° (c 0.05, buffer); [γ] 0.082 dL/g (buffer, 30.00 °C). Anal. Calcd for C31H19N2O7: C, 40.0; H, 5.7; N, 18.5; O, 29.1; Cl, 7.3. Found: C, 41.8; 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 ju = 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. 91702-77-7; 7b. 91702-78-8; 8a. 81040-72-4; 8b. 91702-79-9; 10a. 61167-49-5; 10c. 61167-50-8; 12a. 91702-80-2; 12b. 91702-81-3; 12c. 91702-82-4; 13a. 91734-74-2; 13b. 91734-85-3; 13c. 91734-73-1; 14a. 91734-75-5; 14b. 91734-76-4; 14c. 91719-12-5; NCH3, 7718-54-9; trityl chloride, 76-83-5; 4-nitrophenol, 100-02-7; trityl chloride, 39-59-9.