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

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Three optically active polymers of isocyanides, \([\text{RN=CH}_n\text{]}\) 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 \(M_w\) 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, \([\text{RN=CH}_n\text{]}\), called poly(ylimonomethylenes) or poly(carbimidoyl), 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(ylimonomethylenes), e.g., derived from L-histidine and 2 derived from D-alanyl-L-histidinol. The catalytic activity of these polymers was determined 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(\(\alpha\))-ditrotyl-L-histidine 5, and serine methyl esters, 6a and 6b, by using the dicyclohexyl carbodiimide (DCC) method. These dipeptides were subsequently dehydrochloridated with hydrochloric acid to obtain compounds 8a and 8b. Tripeptides 11a, 11b, and 11c were synthesized from the active 4-nitrophenyl esters of N-formylnalanes 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–trichloroalene procedure at low temperature in about 75% yield.

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 isocyano function at the histidine side is not attractive because during the synthesis of the isocyanide Im\(\text{CH}_2\text{CH(OCOC}H_3)\text{N=C}\) from 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.
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 (±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): $[\eta] = 1.4 \times 10^9 M_w^{1.75}$.
Synthesis of Polymers of Isocyanides

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 $\bar{M}$ 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(aminomethylene) 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–e in water could not be measured accurately due to an unfavorable Δε/ε 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, $\alpha_{\text{Im}^+}$, and carboxylate, $\alpha_{\text{COO}^-}$, can be calculated at each pH. From the modified Henderson–Hasselbach equation,15 pH = $pK_a - n \log (1 + (10^{-pH} / (10^{-pK_a} + 1)))$.

Figure 2. Titration curve of polymer 15b.

Table III. $pK_a$ Values of Poly(iminomethylene) and of Model Compounds

<table>
<thead>
<tr>
<th>compd</th>
<th>$pK_a(\text{COOH})$</th>
<th>$n(\text{COOH})$</th>
<th>$pK_a(\text{Im}^+)$</th>
<th>$n(\text{Im}^+)$</th>
</tr>
</thead>
<tbody>
<tr>
<td>15a</td>
<td>3.8</td>
<td>1.8</td>
<td>7.1</td>
<td>2.0</td>
</tr>
<tr>
<td>15b</td>
<td>4.7</td>
<td>2.2</td>
<td>8.4</td>
<td>0.9</td>
</tr>
<tr>
<td>15c</td>
<td>5.8</td>
<td>2.0</td>
<td>8.7</td>
<td>1.6</td>
</tr>
<tr>
<td>His</td>
<td>2.0 (1.8b)</td>
<td>1.0</td>
<td>6.3 (6.0b)</td>
<td>1.0</td>
</tr>
<tr>
<td>Ser</td>
<td>2.2</td>
<td>1.0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>poly(His) &amp; n(COOH)</td>
<td></td>
<td></td>
<td>5.9</td>
<td></td>
</tr>
<tr>
<td>Copoly(His-Asp) &amp; n(ImH+)</td>
<td></td>
<td></td>
<td>7.0</td>
<td></td>
</tr>
<tr>
<td>β-Ala-His &amp; n(ImH+)</td>
<td></td>
<td></td>
<td>6.0</td>
<td></td>
</tr>
<tr>
<td>OCH-Ala-His-Ser &amp; n(ImH+)</td>
<td></td>
<td></td>
<td>6.3</td>
<td>1.1</td>
</tr>
</tbody>
</table>

*In 30% v/v EtOH–H$_2$O 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. In water, see ref 20. In water, see ref 21.

- $\alpha/a$, $pK_a(\text{Im}^+)$, $pK_a(\text{COOH})$, $n(\text{Im}^+)$, and $n(\text{COOH})$ can be calculated (Table III). The $pK_a$ values of the carboxylic acid groups are appreciably higher in polymers 15 than in L-histidine and L-serine. Also the $pK_a(\text{Im}^+)$ 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 15e than for 15a and 15b, suggesting that in the 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/FPS1 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 309 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 (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 x 10⁻⁶. Solution viscosities were obtained with a Cannon-Ubbelohde viscometer. Intrinsinc 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 D10, DK 12, DK 14, and DK 25.
1-Histidine monohydrochloride, [α]D 20 +9.2° (c 5, 5 M HCl), was purchased from Fluka; L-alanine, [α]D 20 +9.7°, and D-alanine, [α]D 20 −9.4° (c 2, 1 M HCl), were purchased from BDH; L-serine, [α]D 20 +16.6° (c 1, methanol), and d-serine, [α]D 20 +6.8° (c 2, water), were purchased from Merck.

1-Histidyl Methyl Ester Dihydrochloride (4). This compound was obtained from 1-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.18 mp 200–201°C); [α]D 20 +16.6° (c 1, methanol).

N(IIm),N(α)-Ditiryl-1-histidyl-1-serine Methyl Ester (7a). This compound was prepared by formylation of L-alanine and D-alanine with a mixture of formic acid and acetic acid anhydride. Recrystallization from acetonitrile gave pure white crystals of 9a and 9c. 9a: mp 130–131.5°C; [α]D 20 +63.1° (c 2, 1 N NaOH) (lit. mp 131°C; [α]D 20 +65°). 9c: mp 131.5–132.0°C; [α]D 20 +52° (c 2, 1 N NaOH) (lit. mp 130°C; [α]D 20 −56° (c 2, 1 N NaOH).

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 acetonitrile gave pure white crystals of 9a and 9c. 9a: mp 130–131.5°C; [α]D 20 +63.1° (c 2, 1 N NaOH) (lit. mp 131°C; [α]D 20 +65°). 9c: mp 131.5–132.0°C; [α]D 20 +52° (c 2, 1 N NaOH).

1-Histidyl-1-alanly-N(Im)-tosyl-L-histidyl-0-acetyl-1-serine Methyl Ester (12b). This tripeptide was synthesized as described for 10a and 8b as described for 12a. Pure, white, crystalline product, 12b, was obtained after column chromatography: yield 2.6 g (68%); mp 180–182°C; [α]D 20 −18.2° (c 0.5, chloroform); IR (KBr) and 1H NMR (CDCl3) δ trace of CD3OD.

1-Histidyl-1-alanly-N(Im)-tosyl-L-histidyl-0-acetyl-1-serine Methyl Ester (12b). This tripeptide was synthesized as described for 10a and 8b as described for 12a. Pure, white, crystalline product, 12b, was obtained after column chromatography: yield 2.6 g (68%); mp 180–182°C; [α]D 20 −18.2° (c 0.5, chloroform); IR (KBr) and 1H NMR (CDCl3) δ trace of CD3OD as for 12a within 0.1 ppm.

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Polycarbarylalanyl-N(Im)-tosyl-1-histidyl-0-acetyl-d-serine Methyl Ester (13a).

A round-bottomed vessel, equipped with a magnetic stirrer and a CO₂/aceton 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 stirred 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 phosphorous 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) to a solution of 1.5 g (4.45 mmol) of 11b. Methyl Ester (13b). This compound was obtained from methanol and ether: TLC (chloroform–methanol, 10:1 v/v, Rf 13a 0.35 and Rf 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 (Na₂SO₄) 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%); [α]D° −13.5° (c 2, chloroform); IR (neat) 3300 NIH, 2142 (NC), 1740 (OCOC₃, COOCH₃), 1660 (NHCNO), 1600, 1370 and 1180 cm⁻¹ (tosyl), 1H NMR (CDCl₃ + a trace of CD₃OD) 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, CH₂), 4.7 (m, 2 H, CH₂(Ser and His)), 3.0 (d, 6 H, CH₃), 2.8 (s, 3 H, CH₃ tosyl), 2.0 (s, 3 H, CH₃ acetyl) and 1.3 (d, 3 H, CH₃).

Polycarbarylalanyl-N(Im)-tosyl-1-histidyl-0-acetyl-d-serine Methyl Ester (13c). This compound was obtained from 12c as described for 13a: yield 1.5 g (77%). Isocyanide 13c was obtained as a light brown solid: yield 1.03 g (79%); [α]D° −29.3° (c 0.2, chloroform); [γ] 0.039 D/g (chloroform–methanol 5:2 v/v, 30.0 °C); IR (KBr) data as for 14a.

Polycarbarylalanyl-N(Im)-tosyl-1-histidyl-0-acetyl-d-serine Methyl Ester (14a).

This polymer was synthesized from 13b as described for 14a. It was obtained as a light brown solid:

yield 1.03 g (79%); [α]D° −29.3° (c 0.2, chloroform); [γ] 0.039 D/g (chloroform–methanol 5:2 v/v, 30.0 °C); IR (KBr) data as for 14a.

Polycarbarylalanyl-N(Im)-tosyl-1-histidyl-0-acetyl-d-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%); [α]D° −8.0° (c 0.2, chloroform); [γ] 0.085 D/g (chloroform–methanol, 5:2 v/v, 30.0 °C); IR (KBr) data as for 14a.

Polycarbarylalanyl-N(Im)-tosyl-1-histidyl-0-acetyl-d-serine Methyl Ester (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%); [α]D° −8.0° (c 0.1, buffer); [γ] 0.106 D/g (buffer, 30.0 °C). Anal. Calcd for C₁₃H₉N₂O₂(CH₃)₂SO₃H: 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) 3700−2900 (NH₃, 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.

Polycarbarylalanyl-N(Im)-histidyl-0-acetyl-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%); [α]D° +8.0° (c 0.05, buffer); [γ] 0.082 D/g (buffer, 30.0 °C). Anal. Calcd for C₁₃H₉N₂O₂(CH₃)₂SO₃H: 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.

Polycarbarylalanyl-N(Im)-histidyl-0-acetyl-d-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%); [α]D° +30.2° (c 0.1 buffer); [γ] 0.030 D/g (buffer, 30.0 °C). Anal. Calcd for C₁₃H₉N₂O₂(CH₃)₂SO₃H: 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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