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Polyisocyanides. Synthesis and polymerization of carbylhistidine and carbylhistamine

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Abstract. Polymer anchored imidazole functions were obtained by polymerization of carbylhistidine and carbylhistamine. These isocyanides were synthesized from L-histidine and histamine, respectively. In order to obtain the former isocyanide in optically active form, it was necessary to work at low basicity and to prevent temperatures higher than ambient. Polymerization occurred with 1 mol % nickel chloride and a small amount of trifluoroacetic acid in methanol. The poly(carbylhistidine) showed no optical activity. The apparent $pK_a$ of the imidazole function in poly(carbylhistamine) has decreased to 5.2 ± 0.1 compared with free imidazole (7.2), whereas it rises to 9.4 ± 0.2 in poly(carbylhistidine).

Our first results as to the activity of these systems in the hydrolysis of esters are reported elsewhere.

Results and discussion

Masked carbylhistidine (7a) and carbylhistamine (7b) were synthesized from L-histidine and histamine, respectively, in accordance with Scheme 1. Initial experiments with compounds having unprotected imidazole groups gave very low yields in step 6 → 7. It appeared that for the synthesis of 7a the imidazole nucleus of L-histidine could effectively be protected by a benzyl group (Bn). The latter group was not suitable for protecting the imidazole of histamine because of problems in isolating the N(Bn)-benzylhistamine after reaction. A tosyl group (Tos) gave better results; it was introduced after conversion of the amine 4 to the formamide 5. The isocyanides 7 were obtained by dehydration of the N-substituted formamides 6 using thionyl chloride in.

References

2. According to IUPAC nomenclature rules the compounds are named 3-[4(5)-imidazolyl]-2-isocyanopropanoic acid and 2-[4(5)-imidazolyl]-1-isocyanopropanoic acid, respectively.
Scheme 1

$N,N$-dimethylformamide as the dehydrating agent$^{10}$. A five-fold excess of dehydrating agent, appreciably more than the literature value, was found to be necessary in order to obtain any isocyanide. Other dehydrating reagents like phosphorus oxychloride$^{11}$, triphenylphosphine/carbon tetrachloride$^{12}$ and phosgene$^{7}$ gave unsatisfactory results. After purification, compounds 7 were obtained as odourless and colourless solids. They were soluble in methanol, ethyl acetate, acetone and halogenated hydrocarbons, moderately soluble in ethers and insoluble in water, benzene and the lower straight-chain hydrocarbons. The infrared absorption spectra of the solids showed characteristic isocyanide stretching vibrations at approximately 2150 cm$^{-1}$. The structure of the compounds was further established by elemental analysis, NMR and mass spectroscopy. In order to isolate compound 7a in its optically active form it was necessary to work at low basicity and to prevent temperatures higher than ambient. Without these precautions the racemized product was obtained. The ease of racemization is probably due to the presence of two electron withdrawing groups on the chiral centre. The enhanced acidity of the methine proton in 7a was confirmed by proton-deuterium exchange. The sign of rotation of 7a appeared to be opposite to the sign of its precursors. The highest optical rotation, measured on solutions of clear and colourless crystals, amounted to $[\alpha]_{D}^{20} = -4.8^\circ$. Polymerization of racemic carbylhistidine and of carbylhistamine was attempted by 1 mol % of nickel chloride in methanol. Under these conditions no polymerization was observed. However, addition of a small amount of acid started the polymerization. Probably, without acid, imidazole residues block free coordination sites on nickel, which are necessary for polymerization$^{13}$. By protonation of imidazole this coordination is prevented.

Optically active carbylhistidine is expected to give on polymerization a polymer with predominantly one screw sense$^{1-14}$. However, so far no optical activity of the polymer could be detected, because of either a low value of its specific rotation or the low enantiomeric stability of the monomer. The optical activity of poly(carbylhistidine) as well as the synthesis of other optically active imidazole-containing polyisocyanides, like poly(carbylhistidinol) and poly(z-methylcarbylhistamine) are currently under investigation. Polymers 8 were isolated as creamish brown solids. They were soluble in chlorinated hydrocarbons, acetone, methanol and acidified water. Their spectroscopic data are in agreement with the structures given. Removal of the protecting groups in 8 gave light-brown solids. After ultrafiltration and freeze-drying the purified products were analyzed as the monohydrochlorides of polymers 9, containing a varying amount of water of crystallization. The polymers 9 were soluble in the lower alcohols and in water. In most reactions catalyzed by imidazole, its unprotonated form appeared to be the catalytically active species$^{15}$. Therefore, it was of interest to determine the state of ionization of our polyisocyanide anchored imidazole. The relation between pH and degree of dissociation of imidazolium groups on proteins, Wiley, New York, 1971.

$$\text{pH} = \text{pK}_{\text{im}} - n \log \left[ \frac{[1 - x]/x}{x} \right]$$  \hspace{1cm} [1]

plots of log [(1 − 2x)/x] versus pH were linear. In Table 1 the values calculated for \( P_{\text{K}_{\text{im}}} \) and \( n \) are presented as well as the \( P_{\text{K}_{\text{im}}} \)’s of model compounds. The \( n \) values calculated from ultraviolet titration plots at different wavelengths, showed a wavelength dependency and are omitted from Table 1.

<table>
<thead>
<tr>
<th>Table 1</th>
<th>( P_{\text{K}_{\text{im}}} ) values for polyisocyanide anchored imidazoles and model compounds.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Compound</td>
<td>( P_{\text{K}_{\text{im}}} )</td>
</tr>
<tr>
<td>9c</td>
<td>9.4 ± 0.2({}^b)</td>
</tr>
<tr>
<td>9d</td>
<td>9.3 ± 0.15({}^c)</td>
</tr>
<tr>
<td>1-Histidine({}^d)</td>
<td>5.2 ± 0.2({}^b)</td>
</tr>
<tr>
<td>Histamine({}^e)</td>
<td>5.2 ± 0.1({}^c)</td>
</tr>
<tr>
<td>Imidazole({}^f)</td>
<td>6.0</td>
</tr>
<tr>
<td>Poly(t-His)({}^g)</td>
<td>6.0</td>
</tr>
<tr>
<td>Copoly(t-His, t-Asp)({}^h)</td>
<td>7.2</td>
</tr>
<tr>
<td></td>
<td>5.9</td>
</tr>
<tr>
<td></td>
<td>7.0</td>
</tr>
</tbody>
</table>

\(^{a}\) In 29% \( v/v \) EtOH/H\(_2\)O at 25°; for the polymers the \( P_{\text{K}_{\text{im}}} \) is the apparent \( P_{\text{K}_{\text{im}}} \) of the imidazole in the polymer.

\(^{b}\) Ultraviolet titration.

\(^{c}\) Potentiometric titration.

\(^{d}\) Ultraviolet titration.

\(^{e}\) In water; ref. 26.

\(^{f}\) Ref. 27.

This wavelength dependency of \( n \) is probably caused by interferences with absorption bands of the polymer backbone.

The potentiometrically determined \( n \) values of both polymers are equal within experimental error. Their magnitude suggests that more than one imidazole group participates in the reversible binding of a proton. Apparently, our \( n \) value is independent of the presence of a carboxylic group in 9. It is remarked that in polyvinyl systems the \( n \) value of the imidazole residues is affected by the introduction of a carboxylic function. Table 1 shows that the carboxylic function appreciatively increases the apparent dissociation constant of an imidazole residue in 9 but not in 1. This behaviour suggests the presence of a strong anionic field in polymer 9c.

**Experimental part**

Chemical shifts (\( \delta \)) in the \(^1\)H-NMR spectra are given in ppm downfield from internal tetramethylsilane or sodium 2,2,3,3-tetradeutero-\( \text{CDCl}_3 \). Abbreviations used are: \( s \) = singlet, \( d \) = doublet, \( t \) = triplet, \( br \) = broad, \( dist \) = distorted.

**Starting materials**

1-Histidine monohydrochloride, [\( \delta \)] \( 220^\circ + 9.3^\circ \) (c 5.5 mol/l HCl), and histamine dihydrochloride were purchased from Fluka and Aldrich, respectively.

**N(Im)–Benzyl-L-histidine (2)**

This compound was synthesized by the method of Vignaud\({}^{19}\) from 1 and benzyl chloride in liquid ammonia. M.p. 241–242\({}^\circ \), [\( \delta \)] \( 220^\circ + 10.0^\circ \) (c 2, water, 1 eq. HCl); lit.\(^{20}\) m.p. 248–249\({}^\circ \), [\( \delta \)] \( 220^\circ + 20.5^\circ \) (c 2, water, 1 eq. HCl); lit.\(^{21}\) [\( \delta \)] \( 220^\circ + 10.1^\circ \) ± 0.7° (c 2, water, 1 eq. HCl); the latter is more reliable.

**N(Im)–Benzyl-L-histidine methyl ester hydrochloride (3)**

This compound was obtained as an oil from 2 through esterification with hydrogen chloride to 3-(trimethylsilyl)propionate. Abbreviations used are: \( s \) = singlet, \( d \) = doublet, \( t \) = triplet, \( br \) = broad, \( dist \) = distorted.

A suspension of 10 g (34 mmol) of 3 in 50 ml chloroform/methanol (9:1 \( v/v \)) was treated at 0\({}^\circ \) with dry ammonia gas for 30 min. After evaporation of the ammonia at 0\({}^\circ \) the precipitated ammonium chloride was removed by filtration and the resulting clear solution concentrated in \( \text{vacuo} \) at 30°. The oily residue was dissolved in 100 ml formic acid and treated with 35 ml acetic anhydride while cooling in an ice-salt mixture. After stirring for 1 h at room temperature and removal of the excess of reagents by prolonged evaporation at 50°/0.1 mm, compound 6a was obtained in quantitative yield as a yellow brown syrup, which was sufficiently pure for the synthesis of 7a. Upon repeated stirring with aceton the syrup partly solidified. Filtration of the solid and recrystallization from acetone afforded white crystals. M.p. 120° (dec.), [\( \delta \)] \( 220^\circ + 17.4^\circ \) (c 4.8, methanol); IR (KBr): 3180 (NH), 1730 (COOCH\(_3\)), 1670 (CHO), 730 and 700 (benzyl) cm\(^{-1}\); \(^1\)H-NMR (CD\(_2\)OD): 6.80 and 7.35 (2H, \( 2 \times s \), imidazole), 8.05 (1H, s, CHO), 7.40 (5H, s, benzyl), 5.35 (2H, s, benzyl), 4.80 (1H, t, CH), 3.70 (3H, s, OCH\(_3\)), 3.20 (2H, \( 2 \times \text{H} \), dist CH\(_2\)).

**Poly(benzyl carbylhistidine methyl ester) (8a)**

To a stirred solution of 5 g (17.4 mmol) of 6a in 150 ml of N,N-dimethylformamide (DMF) was added, under a nitrogen atmosphere, a solution of 7.5 ml (100 mmol) of thionyl chloride dissolved in 30 ml of DMF, at such a rate that the temperature was kept at about −60°. After addition, the cooling bath was temporarily removed to allow the temperature to rise to −38°; then it was replaced and 23 g (210 mmol) of anhydrous sodium carbonate were slowly added, maintaining a temperature of −45°. The mixture was subsequently stirred at this temperature for 10 min. The cooling bath was then removed and the temperature allowed to rise to 0°. After addition of 50 ml of DMF, stirring was continued for 20 h while cooling in ice. To the reaction mixture 250 ml of methylene chloride was added followed by 450 ml of ice cold water. The aqueous layer was separated and rapidly extracted with three 100 ml portions of methylene chloride. The combined extracts were washed, dried over MgSO\(_4\) and concentrated in \( \text{vacuo} \). Addition of ethyl acetate to the resulting yellow-red syrup gave 150 ml of a solution of 8a in methanol. It was evaporated at 50°/0.1 mm, and the resulting oil was chromatographed on a silica gel column (acetone as eluent) to afford two fractions of compound 8a. Further purification by chromatography on a silica gel column (acetone as eluent) afforded two fractions. M.p. 238, M + -COOCH\(_3\) cm\(^{-1}\); IR (KBr): 2152 + 1 (N=C; CO was used for calibration), 1750 (COOCH\(_3\)) cm\(^{-1}\); \(^1\)H-NMR (CD\(_2\)OD): 2.65 and 2.70 (2H, \( 2 \times \text{H} \), dist CH\(_2\)).

**Poly[benzyl carbylhistidine methyl ester]**

A solution of 1.5 g (5.6 mmol) of 7a, 0.015 g (0.06 mmol) of nickel chloride hexahydrate and 5 drops of trifluoroacetic acid in 5 ml of methanol was stirred for 2 days at 25°. After evaporation of the solvent, 8a was isolated in quantitative yield and used without further purification for the synthesis of 9c. Dropwise addition of a chloroform solution of 8a into a hundredfold excess of ether afforded a

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\(^{21}\) For more details see the forthcoming thesis by J. M. van der Eijk.
purified sample. IR (KBr): 1740 (COOCH₃), 1650 (C=O), br, cm⁻¹; 1H-NMR (CD₂OD): δ 7.2 (7H, br, imidazolyl and benzyl), 4.9 (3H, br, CH and benzyl), 3.5 (3H, br, CH₂ and OCH₃).

Poly(carbonylhistidine) (9c)

An amount of 1.0 g (3.72 mmol) of finely powdered 8a was suspended in 125 ml of dry liquid ammonia. Finely divided metallic sodium was added over a period of 5 h until a blue colour persisted. The excess of sodium was then discharged with ammonium chloride and the ammonia was allowed to evaporate spontaneously. The residue was extracted with ether, dissolved in 60 ml of 1 mol/l HCl and subjected to ultrafiltration (Diaflo Ultra-Filter, UM-2). Freeze-drying of the resulting solution afforded a light-brown powder, which was dried over P₂O₅ at 40°/12 mm. Yield 0.65 g (24.3 mmol, 65%) of 9c; [C₆H₅N₂O₃(HCl)(H₂O)]ₙ (246.5); calcd. C 34.0, H 5.8, N 26.0, O 8.4; found C 34.1, H 5.7, N 25.5, O 8.2; IR (KBr): 3300 (NH), 1675 ± 1 (C=N, polystyrene was used for calibration) cm⁻¹; 1H-NMR (CD₂OD): δ 8.70 and 7.40 (2H, 2 × CH₃), 7.80 and 7.35 (4H, 2 × CH), 6.7 (IH, br, NH), 3.50 and 2.70 (4H, 2 × CH₂), 2.45 (3H, s, CH₃).

N(Im)-Tosyl-N(α)-formylhistamine (6b)

To a solution of 7.5 g (54 mmol) of 5 and 15 g of Na₂CO₃ in 100 ml of water was added 15 g (78 mmol) of π-toluene sulfonyl chloride and acetic anhydride (p-toluene sulfonyl chloride and acetic anhydride) was added 15 g (78 mmol) of π-toluene sulfonyl chloride and acetic anhydride was added 15 g (78 mmol) of π-toluene sulfonyl chloride and acetic anhydride was added 15 g (78 mmol) of π-toluene sulfonyl chloride and acetic anhydride was added 15 g (78 mmol) of π-toluene sulfonyl chloride and acetic anhydride was added 15 g (78 mmol) of π-toluene sulfonyl chloride and acetic anhydride was added 15 g (78 mmol) of π-toluene sulfonyl chloride and acetic anhydride was added 15 g (78 mmol) of π-toluene sulfonyl chloride and acetic anhydride 12.5°. In the region from 300 to 350 nm both polymers 9 showed a shoulder on the onset of a strong absorption band in the far ultraviolet. This shoulder shifted to longer wavelength on increasing the pH. The pH₀, value of the imidazole unit in 9c was determined by using its absorbance difference at 330 nm: e 2170 at pH > 11.00 and 1311 at pH < 5.20; for 9d the absorbance difference at 300 nm was used: e 1838 at pH > 12.00 and 1221 at pH < 2.20.

Poly(carbonylhistamine) (9d)

The above polymer (8b) was suspended in 50 ml of 96% ethanol, containing 1.5 g of KOH, and refluxed for 8 h. After evaporating the mixture to dryness in vacuo, 50 ml of water was added and a precipitate removed by filtration. The resulting solution was made slightly acidic with hydrochloric acid, submitted to ultrafiltration (Diaflo Ultra-Filter, UM 2) and freeze-dried. Yield 0.062 g (3.6 mmol, 52%) of 9d. As indicated by elemental analysis 9d still contained 4% of tosylated imidazole residues and some water of crystallization or N-oxide groups: [C₂₅H₂₈N₅O₃Cl₂]₀, [C₂₅H₂₈N₅O₃SO₃H]₀, (168.4); calc. C 48.5, H 5.8, N 12.6, O 14.4, S 0.8; found C 48.3, H 6.0, N 12.4, O 14.2, S 0.8; IR (KBr): 3600–2200 (NH₂, H₂O), 1615 ± 1 (C=N, polystyrene was used for calibration) cm⁻¹.

Ultraviolet titrations

Solutions (3.5 × 10⁻³ mol/l) of polymers 9 in 29% v/v ethanol/water, acidified (pH 2) by adding a small amount of 1 mol/l hydrochloric acid and adjusted to anionic strength of 0.02 mol/l by adding KCl, were titrated with 0.1 mol/l of sodium hydroxide. After each addition of hydroxide the pH of the solution was measured and the ultraviolet spectrum recorded at 25°. In the region from 300 to 350 nm both polymers 9 showed a shoulder on the onset of a strong absorption band in the far ultraviolet. This shoulder shifted to longer wavelength on increasing the pH. For the pH₀, value of the imidazole unit in 9c the pH value was determined by using its absorbance difference at 330 nm: e 2170 at pH > 11.00 and 1311 at pH < 5.20; for 9d the absorbance difference at 300 nm was used: e 1838 at pH > 12.00 and 1221 at pH < 2.20.

Potentiometric titrations

Polymers 9 were dissolved in 29% v/v ethanol/water to a concentration of 3.4 × 10⁻³ mol/l. Samples of 20 ml were acidified (pH 2–3) by adding 1 mol/l HCl. An amount of KCl was added, such that at the end point of titration μ = 0.02 mol/l. The solutions were titrated with 0.1 mol/l NaOH in 29% v/v ethanol/water at 25° under a nitrogen atmosphere while stirring. Blank titration curves were obtained by titrating 20 ml aliquots of 29% ethanol/water acidified to the same pH values and adjusted to the same ionic strength. Differential titration curves were derived graphically, from which the degrees of dissociation were evaluated.

In the titration curve of 9c the inflection point between the dissociation of the imidazolium ion and the carboxyl group was not clearly seen. However, the pH₀, value was calculated from the value of half-neutralization of the imidazolium group. The n value was evaluated by applying eq. [1] to the pH values for which pH > pH₀,.

Acknowledgement

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