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Polyisocyanides \(^5\). 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 \(5.2 \pm 0.1\) compared with free imidazole (7.2), whereas it rises to \(9.4 \pm 0.2\) in poly(carbylhistidine).

Introduction

Anchoring of homogeneous catalysts to polymers is receiving much attention\(^3\). The advantages are an easier recovery and sometimes an activity and selectivity higher than of the original species. To some extent the system approaches an enzyme in which the active site is also attached to a polymer. Therefore, the anchored catalyst systems have been used as enzyme models, e.g. imidazole anchored to vinyl and ethylenimine polymers as catalysts for the hydrolysis of activated esters\(^4\). However, these systems did not show enantioselectivity in the hydrolysis of asymmetric esters\(^5\). The reason might be that the structural characteristics of the supports so far studied, are too much different from those of enzymes. We are investigating the use of polyisocyanides, \((R-N=C\equiv C)\), as supports for homogeneous catalysts. In earlier papers\(^1,6\), we showed that these polymers probably have the configuration of a tightly coiled helix. Helices are chiral molecules. Poly(tert-butyl isocyanide) could even be resolved partly into its enantiomers\(^6\) and its screw sense derived from steric considerations as well as from CD spectra\(^1\).

Polyisocyanides are obtained\(^7\) from the corresponding isocyanides, which in turn are synthesized from the amines\(^8\). By starting from naturally occurring amino acids it might be possible to prepare polymers and copolymers of isocyanides which have a highly chiral structure and contain substituents which are catalytically active in enzymes. These polymers could be attractive model systems for the enantiospecific action of enzymes.

We decided to start from histidine; its imidazole group is a weakly basic catalyst and thus, ample material for comparison is available. In this paper we describe the synthesis of carbylhistidine as well as of carbylhistamine and their polymerization. Both isocyanides were as yet unknown.

Our first results as to the activity of these systems in the hydrolysis of esters are reported elsewhere\(^9\).

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 less suitable for protecting the imidazole of histamine because of problems in isolating the N(Bn)-benzylimidazole 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...
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Scheme 1

$N,N$-dimethylformamidino 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$^{13}$ 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 hydrogen-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 $\left[\alpha\right]_{D}^{25} = -4.8^\circ$. 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(1-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 ions is given by the Henderson–Hasselbach equation:

$$\text{pH} = \text{pK}_\text{im} - n \log \left(1 - \frac{a}{x}\right)$$

where $a$ is the amount of acid per mole of imidazole and $x$ is the fraction of ions. In accordance with the modified Henderson–Hasselbach equation$^{16}$

$$\text{pH} = \text{pK}_\text{im} - n \log \left(1 - \frac{a}{x}\right)$$

plots of log \( [1 - \alpha]/\alpha \) versus pH were linear. In Table 1 the values calculated for \( \phi_{\text{K}_a} \) and \( n \) are presented as well as the \( \phi_K \)'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 I. \( pK \) values of polysocyanide anchored imidazoles and of model compounds. 

<table>
<thead>
<tr>
<th>Compound</th>
<th>( \phi_{\text{K}_a} ) (n)</th>
</tr>
</thead>
<tbody>
<tr>
<td>9c</td>
<td>9.4 ± 0.2 (n)</td>
</tr>
<tr>
<td>9d</td>
<td>9.3 ± 0.15 (n)</td>
</tr>
<tr>
<td>6.0</td>
<td>5.2 ± 0.2 (n)</td>
</tr>
<tr>
<td>1.5 ± 0.1 (n)</td>
<td></td>
</tr>
<tr>
<td>5.2 ± 0.1 (n)</td>
<td></td>
</tr>
<tr>
<td>6.0</td>
<td></td>
</tr>
<tr>
<td>7.2</td>
<td></td>
</tr>
<tr>
<td>5.9</td>
<td></td>
</tr>
<tr>
<td>7.0</td>
<td></td>
</tr>
</tbody>
</table>

* In 29.5% v/v EtOH/H2O at 25°; for the polymers the \( \phi_{\text{K}_a} \) is the apparent \( \phi \) of the imidazole in the polymer.

The spectrophotometrically determined \( n \) values of both polymers 9 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 I shows that the carboxylic function appreciably 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-tetradeuteroacetone. Abbreviations used are: s = singlet, d = doublet, t = triplet, br = broad, dd = distorted. Elemental analyses were carried out by the Element Analytical Section of the Institute for Organic Chemistry TNO, Utrecht, The Netherlands, under supervision of W. J. Buis.

### Starting materials

1-Histidine monohydrochloride, \([x]_D^{20} + 3.9^\circ\) (c 5.5 mol/l HCl), and histamine dihydrochloride were purchased from Fluka and Aldrich, respectively.

N(Im)-Benzy1-L-histidine (2)

This compound was synthesized by the method of Vignaud from 1 and benzy1 chloride in liquid ammonia. \( \delta \) 241-242 \( [x]_D^{20} + 10.0^\circ\) (c 2, water, 1 eq. HCl); lit. \( \delta \) 248-249 \( [x]_D^{20} + 20.5^\circ\) (c 2, water, 1 eq. HCl); lit. \( \delta \) 241-242 \( [x]_D^{20} + 10.1^\circ\) (c 2, water, 1 eq. HCl); the latter is more reliable.

N(Im)-Benzy1-L-histidine methyl ester hydrochloride (3)

This compound was obtained as oil 2 through esterification with hydrogen chloride or thionyl chloride in methanol. It was used without further purification for the synthesis of 6a; \([x]_D^{20} + 9.9^\circ\) (c 2.5, methanol).

N(Im)-Benzy1-N(\(\alpha\))-formyl-L-histidine methyl ester (6a)

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 vacuo at 25°. The oily residue was dissolved in 100 ml formic acid and treated with 35 ml acetic acid 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 evacuation 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 acetone the syrup partly solidified. Filtration of the solid and recrystallization from acetone afforded white crystals. M.p. 120° (dec.), \([x]_D^{20} + 17.4^\circ\) (c 4.8, methanol); IR (KBr): 3180 (NH), 1730 (COOHCH3), 1670 (CHO), 730 and 700 (benzyl) cm\(^{-1}\). \(^1\)H-NMR (CD2OD): 6.80 and 7.53 (2H, 2 × s, imidazole), 8.05 (1H, s, CHO), 7.40 (5H, s, benzyl), 5.55 (2H, s, benzyl), 4.80 (1H, t, CH), 3.70 (3H, s, OCH3), 3.20 (2H, dist. s, CH2).

### Benzyl-L-carboxylhistidine methyl ester (7a)

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 in 30 ml of DMF, at such a rate that the temperature was kept at about \( -60^\circ \). After addition, the cooling bath was temporarily removed to allow the temperature to rise to \( -38^\circ \); then it was replaced and 23 g (210 mmol) of anhydrous sodium carbonate were slowly added, maintaining a temperature of \(-45^\circ\). The mixture was subsequently stirred at this temperature for 10 min. The cooling bath was then removed and the temperature allowed to rise to \( 0^\circ \). 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 were 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 vacuo. Addition of ethyl acetate to the resulting yellow-red syrup gave 3.3 g (70%) of light yellow crystals of 7a. Further purification by chromatography on a silica gel column (acetone as eluent) afforded transparant, colourless crystals. M.p. 77.5-78.5°, \([x]_D^{20} + 4.8^\circ\) (c 5, acetone); C\(_8\)H\(_{17}\)N\(_2\)O\(_2\) (269.1); calcd. C 66.4, H 5.7, N 15.4, O 12.4; MS: M+ 269, M+ -OCH3 238, M+ -COOCH3 210, M+ -CH(NCCOOCH3) 171, tropanyl ion 91; IR (KBr): 2152 + 1° (NC; CO was used for calibration), 1750 (COOCH3) cm\(^{-1}\). \(^1\)H-NMR (CD2OD, CDCl3): 0.765 and 7.00 (2H, 2 × s, imidazole), 7.30 (5H, s, benzyl), 5.25 (2H, s, benzyl), 4.85 (1H, t, CH), 3.75 (3H, s, OCH3), 3.15 (2H, dist. s, CH2).

### Isocyanide 7a can be recognized on thin-layer plates as red-brown spots by spraying with a solution of nickel chloride in ethanol.

Poly(benzyl carboxylhistidine methyl ester) (8a)

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 purification for the synthesis of 9c. Dropywise addition of a chloroform solution of 8a into a hundredfold excess of ether afforded a...
This compound was prepared from 3 g (10 mmol) of affording 1.55 g (55%) of pale yellow, crystalline 7b; m.p. 110° subsequent treatment of the residue with carbon tetrachloride (4H, 2\(\mathrm{H}^2\)), 2\(\mathrm{H}^2\) (2H, 2\(\mathrm{H}^2\)).

Ether; after purification of the mother liquid by column chromatography without further purification for the synthesis of 9d.

Poly(carbylhistidine) (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 (7b) was used for the polymerization of 7a. After isolation the polymer 8b was used without further purification for the synthesis of 9d.

Poly(carbylhistidine) (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:

\[
\left[\text{C}_9\text{H}_7\text{N}_2\text{HCl}, \text{O}_6\text{S}\right]_{\text{molar ionic strength of 0.10 mol/1 HCI. An amount of KCl was added, such that at pH = 12.00 and 1221 at pH \leq 2.50.; for 9d the adsorption difference at 300 nm was used: \(\varepsilon = 1838\) at pH \(\geq 12.00\) and 1221 at pH \(\leq 2.20\).}

Potentiometric titrations

Polymers 9 were dissolved in 29% v/v ethanol/water to a concentration of 3.4 \(\times 10^{-3}\) mol/l. Samples of 20 ml were acidified (pH 2) by adding 1 mol/l HCl. An amount of KCl was added, such that at the end point of titration \(\mu = 0.02\) mol/l. The solutions were titrated with 0.10 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\(^{25}\), from which the degrees of dissociation were evaluated. In the titration curve of 9e the inflection point between the dissociation of the imidazolium ion and the carboxyl group was not clearly observed. Therefore, the pKw 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 \(\geq pK_{im}\).

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