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New Polymer Syntheses

5. Poly(L-ornithine), Poly(L-lysine) and Isopoly(l-lysine) with Pending 2-Thiothymine Groups

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SUMMARY

1-(2-Carboxyethyl)-2-thiothymine was activated in the form of a N-hydroxysuccinimide ester. The free amino groups of poly (L-ornithine), poly(L-lysine) and isopoly(L-lysine) were acylated with this activated ester, and the resulting poly(nucleoamino acids) were characterized by means of elemental analyses, optical rotation, viscosities ^{1}H- and ^{13}C-NMR spectra. IR-spectra suggest that the poly(L-ornithine) and poly(L-lysine) derivatives adopt the α-helix structure in the solid state.

INTRODUCTION

Since the discovery of Willardiine, a pyrimidine derivative of L-alanine, in various plants$^{1)}$ the interest of polymer chemists and biochemists in nucleoamino acids and polypeptides with pending pyrimidine or purine bases has steadily increased. Various routes for the synthesis of polypeptides with pending nucleobases or nucleosides were explored in the course of the last two decades. One route starts with the preparation of suitable monomers containing the nucleobase in the side chain, while the polymerization is the last step $^{2-7)}$. Another strategy starts with the preparation of the basic polypeptides followed by a conversion with activated electrophilic nucleobase or nucleoside derivatives $^{8-10)}$. The latter procedure seems to be more versatile and we have used this strategy in the present work to prepare a couple of polypeptides with pending 2-thiothymine groups.

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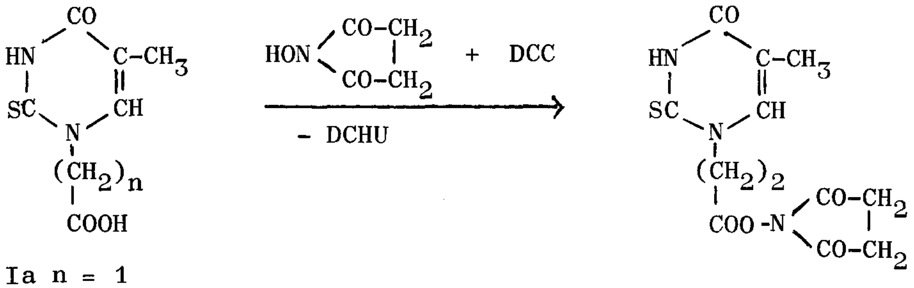
RESULTS and DISCUSSION

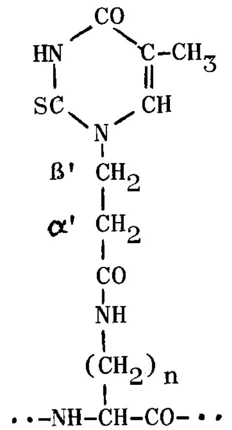
In the preceding paper of this series[10] we have described the synthesis of 1-(2-carboxyethyl)-2-thiothymine (Ib) from 3-methoxy methacryloyl isothiocyanate and ß-alanine. The corresponding glycine derivative Ia was now prepared in the same way and both compounds were activated at the carboxyl group. However, regardless of the activating reagent used e.g. thionylchloride, isobutylchloroformate, carbonyldiimidazole and dicyclohexylcarbodiimide, a black tar was the only reaction product, when Ia was used as starting material. In contrast, Ib gave a satisfactory yield of a crystalline N-hydroxysuccinimide ester (II) when treated with dicyclohexyl carbodiimide and N-hydroxysuccinimide. Thus, only the activated ester II was used for the polymer analogous acylation of basic polypeptides.



Ia n = 1
Ib n = 2

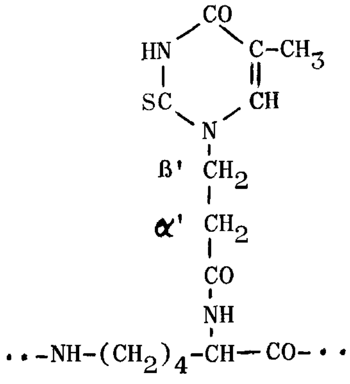
II

As reaction partners of II poly(L-ornithine),poly(L-lysine) and isopoly(L-lysine) were used. The synthesis of the latter polymer has been described by us in a previous paper[11]. The primary amino groups of these polymers were set free from their hydrobromides by means of triethylamine and reacted with II in a "one pot procedure". A slight excess was used to ensure a complete conversion of the basic polypeptides. The primary structure of the resulting poly(nucleoamino acids) III a, b and IV was checked by means of C-, H-, N-elemental analyses (Tab. 1), ^{1}H- (Tab.2) and ^{13}C-NMR spectra (Tab. 3). A comparison of the ^{1}H NMR signal intensities of the δ- or ε-CH_2 protons and of the β'-CH_2 protons allows one to estimate whether the polymer analogous acylation was complete or not.



IIIa : n = 3

IIIb : n = 4



IV

The ^{1}H-NMR results indicate a 95-100 % conversion and the ^{13}C-NMR spectra (Tab. 3 and Fig. 1) agree also with the expected structure. IR spectra of the solid poly(L-ornithine) and poly (L-lysine) derivatives III a and b suggest a helical secondary structure. The Amide I band at 1665 cm^{-1} is the most intensive amide band. However, because the amide bands of the side chain carbonyl groups overlap with the Amide I band of the main chain, the IR spectra are in this case not reliable indicators of the secondary structure.

EXPERIMENTAL

1-(2-Carboxyethyl)-2-thiothymine N-hydroxysuccinimide ester (II):

1-(2-Carboxyethyl)-2-thiothymine (21.4 g = 0.1 Mol) and N-hydroxysuccinimide (12.6 g = 0.11 mol) were dissolved in a mixture of 150 ml dry THF and 150 ml dry DMF and mixed at -10^{o}C with a solution of N,N'-dicyclohexylcarbodiimide (22.6 g = 0.11 mol) in 50 ml dry THF. The reaction mixture was stored at 0^{o}C for ca. 10 h, the precipitated N,N'-dicyclohexylurea removed by filtration and the filtrate treated with char coal. After filtration the THF solution was concentrated in vacuo and the product crystallized by portionwise addition of ethylacetate under cooling with ice. Yield: 22 g (71%); m. p. 139-142°C

ANAL: Calcd. for $C_{12}H_{13}N_3O_5S$: C, 46.30; H, 4.21; N, 13.50;
Found C, 45.86; H, 4.58; N, 13.20

Acylation of basic polypeptides (gen. procedure):

A polypeptide hydrobromide (10 mmol) was dissolved in 50 ml dry DMSO and triethylamine (2.2 g = 20 mmol) and 1-(2-carboxyethyl)-2-thiothymine N-hydroxysuccineimide ester (4.7 g = 15 mmol) were added. The reaction mixture was stirred at room temperature for 4 d and the polymer precipitated from 1 l ethanol. The product isolated by filtration, reprecipitated from DMSO/ethanol, washed with methanol and dried at 80°C/10^{-2} mbar.

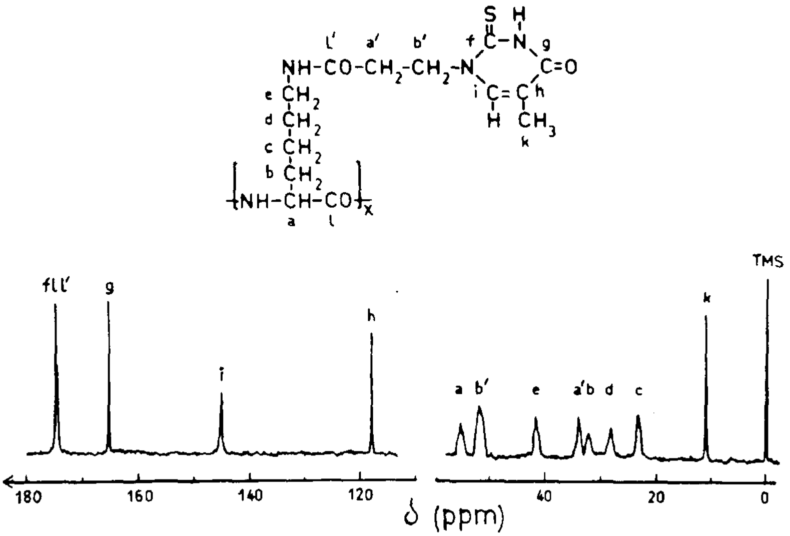


Fig. 1 22.63 MHz ^{13}C NMR spectrum of poly(nucleoamino acid) III b in trifluoroacetic acid solution.

Measurements:

The 22.63 MHz ^{13}C NMR spectra and the 90 MHz ^{1}H NMR spectra were obtained on a Bruker WH-90 FT-spectrometer. The ^{13}C NMR spectra were measured with solutions of 400 mg polypeptide in 2 ml trifluoroacetic acid using 10 mm od. sample tubes with a coaxial 4 mm od. sample tube containing dioxane-d_8 and TMS.

Tab. 1 Yields and properties of the poly(nucleoamino acids) IIIa, b and IV

Formula No.	Yield (%)	$[\alpha]_D^{20}$ a)	$\frac{\eta_{sp}}{c}$ a) cm^3g^{-1}	Elemental Formula (mole weight)		Elemental Analyses		
						C	H	N
	97	+ 42.0	18.1	$C_{18}H_{18}N_4O_3S$ (310.4)	Calcd.	50.31	5.85	18.05
					Found	48.79	6.33	16.87
	92	- 5.8	9.2	$C_{14}H_{20}N_4O_3S$ (324.4)	Calcd.	51.83	6.21	17.27
					Found	50.40	6.00	16.12
	81	- 25.0	6.5	$C_{14}H_{20}N_4O_3S$ (324.4)	Calcd.	51.83	6.21	17.27
					Found	50.68	6.04	15.20

a) c = 10g/l in dimethylformamide

Tab. 2 90 MHz ^1H NMR data of the poly(nucleoamino acids)

Formula No.	δ of Aminoacyl Unit					δ of Nucleobase Residue			
	H-α	H-β	H-γ	H-δ	H-ε	H-α'	H-β'	H-6	CH_3
III a	4.65 (1 H)	1.9 (2 H)	1.9 (2 H)	3.42 (2 H)		4.65 (2 H)	3.10 (2 H)	7.69 (1 H)	2.09 (3 H)
III b	4.65 (1 H)	1.8 (2 H)	1.8 (2 H)	1.8 (2 H)	3.42 (2 H)	4.65 (2 H)	3.15 (2 H)	7.67 (1 H)	2.09 (3 H)
IV	4.59 (1 H)	1.8 (2 H)	1.8 (2 H)	1.8 (2 H)	3.36 (2 H)	4.59 (2 H)	3.11 (2 H)	7.69 (1 H)	2.07 (3 H)

Tab. 3 22.63 MHz ^{13}C NMR data of the poly(nucleoamino acids)

Formula No.	δ of Aminoacyl Unit						δ of Nucleobase				
	CO CO'	C-α C-α'	C-β C-β'	C-γ	C-δ	C-ε	C-2	C-4	C-5	C-6	CH_3
III a	174.22 174.22	53.85 33.89	29.47 51.75	25.05	40.59		174.22	165.01	117.64	144.75	10.94
III b	174.70 174.70	55.03 33.74	28.07 51.64	31.91	22.95	41.55	174.60	165.15	117.83	145.13	11.01
IV	174.43 174.43	55.25 33.92	28.18 51.86	31.67	23.03	40.88	174.43	165.32	118.04	145.56	11.17

The following acquisition parameters wre used: pulse width 6 μs (40^o); 8 K data points/ 5000 Hz spectral width; exp. line-broadening 1.0 Hz; ca. 4000 transients. The ^{1}H NMT spectra were mea- with 50 mg polypeptide in 0.5 ml trifluoroacetic acid containing TMS in 5 od. sample tubes. A pulse width of 1.5 μs (50^o) and 8 K data points on a spectral width of 1500 Hz were used.

The viscosities were measured in an Ostwaldt viscosimeter thermostated at 20°C. The optical rotations were measured on a Zeiss "LEP Nr. 370 750". For both series of measurements solutions of 100 mg polypeptide in 10 ml dry dimethylformamide were used.

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