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Synthesis and Polymerization of Nucleic Acid Base Substituted L-Lysine

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Introduction

In order to prepare nucleic acid models with optically active structure, carboxyethyl derivatives of nucleic acid base have been grafted onto poly-L-lysine by the activated ester method (ISHIKAWA et al. 1978). The thymine and uracil derivatives were quantitatively grafted onto poly-L-lysine as the side groups, while the adenine derivative could not be introduced over 74 mol %. The present report concerns the synthesis of the nucleic acid base substituted L-lysine derivatives and the polymerization of these amino acid derivatives by the N-carboxyamino acid anhydride (NCA) method.

Experimental

p-Nitrophenyl 3(6-aminopurin-9-yl)propionate (Ade-PNP) (2a); *p*-nitrophenyl 3(2,4-dioxo-5-methyl 1,2-dihydropyrimidin-1-yl)propionate (Thy-PNP) (2b); *p*-nitrophenyl 3(2,4-dioxo-1,2-dihydropyrimidin-1-yl)propionate (Ura-PNP) (2c)

These *p*-nitrophenyl esters were prepared from *p*-nitrophenyl trifluoroacetate and the corresponding carboxyethyl derivatives in pyridine solution according to the method reported earlier (OVERBERGER, INAKI 1978; ISHIKAWA et al. 1978).

p-Nitrophenyl 3(theophyllin-7-yl)propionate (The-PNP) (2d)

The activated ester (2d) was prepared from *p*-nitrophenyl trifluoroacetate and 3(theophyllin-7-yl)propionic acid according to the similar procedure described in the preparation of Thy-PNP. Recrystallization from benzene-ethanol gave needles in 75 % yield. mp 153 - 155°C. ANAL. Calcd. for $C_{16}H_{15}O_6N_5$: C, 51.47 %; H, 4.05 %; N, 18.76 %. Found: C, 51.33 %; H, 3.89 %; N, 18.89 %.

α,N-Benzyloxycarbonyl-ε,N-3(6-aminopurin-9-yl)propionyl-L-lysine (α,N-Cbz-ε,N-Ade-L-lysine) (3a)

To the suspension of α,N-benzoyloxycarbonyl-L-lysine (α,N-Cbz-L-lysine) (1) (2.5 g, 8.9 mmol) in dimethyl sulfoxide (DMSO) solution (20 ml), Ade-PNP (2a) (3.3 g, 10 mmol) was added at 25°C with stirring. The solution became clear after few hours. After stirring for

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additional 2 days, the solvent was distilled off under reduced pressure. Addition of excess amount of ethyl acetate to the oily residue gave colorless crystals. Recrystallization from ethanol-ethyl acetate gave 3a in 70 % yield (2.9 g).

α,N-Cbz-ε,N-Thy-L-lysine (3b); α,N-Cbz-ε,N-Ura-L-lysine (3c); and α,N-Cbz-ε,N-The-L-lysine (3d)

These compounds were prepared in a similar way as the case of 3a (see TABLE 1 and 2).

Poly(ε,N-Ade-L-lysine) (5a)

To α,N-Cbz-ε,N-Ade-L-lysine (3a) (0.6 g, 1.3 mmol) was added thionyl chloride (1.0 ml, 14 mmol) at 0°C, and the reaction mixture was gradually heated to 40°C. After the evolution of gas ceased, the mixture was heated again to 60°C for 15 min, and excess amount of thionyl chloride was distilled off. The residue was washed repeatedly with dry ethyl ether to afford NCA of ε,N-Ade-L-lysine (4a). The product was dried thoroughly in vacuo overnight.

The NCA (4a) was then dissolved in DMSO (10 ml), and to the solution triethylamine (0.25 ml, 1.8 mmol) was added as an initiator. The polymerization was carried out at 25°C for 48 hr with stirring. After the solvent was distilled off in vacuo, excess ethanol was added to the oily residue to precipitate the polymer. Reprecipitation from DMSO-ethanol gave poly(ε,N-Ade-L-lysine) (5a) (0.29 g, 73 % yield).

Poly(ε,N-Thy-L-lysine) (5b), poly(ε,N-Ura-L-lysine) (5c) and poly(ε,N-The-L-lysine) (5d) were prepared using the similar procedure described for preparing 5a. The results were tabulated in TABLE 3.

Results and Discussion

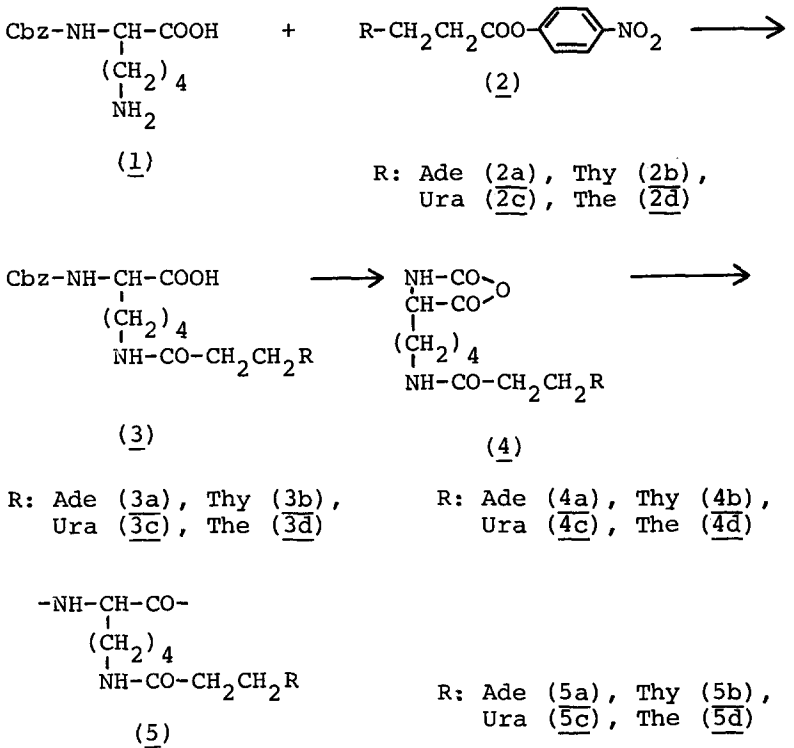
Polylysine derivatives containing nucleic acid bases were prepared as shown in SCHEME 1. In order to incorporate them exclusively at ε-position, α-amino group of L-lysine was blocked with carbobenzoxy chloride to give α,N-Cbz-L-lysine (1) (COSTOPANAGIOTIS et al. 1968). The *p*-nitrophenyl esters (2) were prepared from the carboxyethyl derivatives of nucleic acid bases and *p*-nitrophenyl trifluoroacetate according to the method of Overberger and Inaki (OVERBERGER, INAKI 1978).

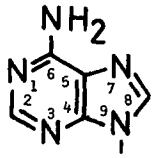
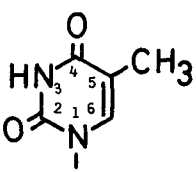
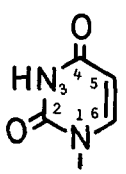
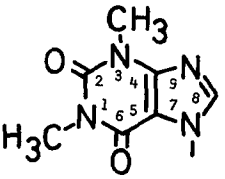
The reaction of α,N-Cbz-L-lysine (1) with *p*-nitrophenyl esters (2) was carried out in DMSO or N,N-dimethylformamide (DMF) solution at 25°C for 2 days. The reaction was found to be complete from thin-layer chromatography, however, the yield was about 70 to 90 % because of low crystallinity of the products. The products were assigned by elemental analysis, IR, UV and NMR spectroscopies. The results were listed in TABLE 1, 2 and 4. From the data, amino group of adenine at 6-position was

concluded to remain unreacted by the attack of the activated ester under the condition used.

As the solubility of NCAs (4) in common organic solvents was low, preparation of the NCAs by the Fuchs method was unsuccessful, which had been used for the preparation of alanine derivatives having pendant nucleic acid bases (TAKEMOTO et al. 1973). Consequently, the NCAs were prepared by the Leuchs method using thionyl chloride (LEUCHS 1906). As the NCAs (4) were soluble in DMSO and DMF, but insoluble in common organic solvents, purification of them by recrystallization was not preferable. They were identified from IR spectra of 1850 and 1780 cm^{-1} assigned to NCA structure. The NCAs thus prepared were washed thoroughly with dry ethyl ether, and allowed to polymerize in DMSO solution by using triethylamine as an initiator.

SCHEME 1



Adenine(Ade) Thymine(Thy) Uracil(Ura) Theophylline(The)

TABLE 1. Synthesis of α,N-Cbz-ε,N-R-L-lysines

No.	R	Solvent[1]	Yield (%)	m.p. (°C)	UV[2] $\lambda_{max,nm}$	ε_{max}	$[\alpha]_D$[3]
(3a)	Ade	DMSO	70	125-127	263	13,300	-2.4°
(3b)	Thy	DMF	87	159-161	271	9,500	-3.2°
(3c)	Ura	DMF	87	169-171	266	10,000	-2.2°
(3d)	The	DMSO	69	141-143	276	8,400	-1.0°

1) Solvents used for the reaction.
2) In ethanol, at 25°C.
3) In DMSO (c=2), at 21°C.

TABLE 2. Analytical data of α,N-Cbz-ε,N-R-L-lysines

No.	R	Calcd.for	Calcd. % C	H	N	Found % C	H	N
(3a)	Ade	$C_{22}H_{27}O_5N_7$	55.85*	5.84*	20.73*	55.90	5.75	20.58
(3b)	Thy	$C_{22}H_{28}O_7N_4$	57.38	6.13	12.17	57.37	5.95	12.21
(3c)	Ura	$C_{21}H_{26}O_7N_4$	56.49	5.87	12.55	56.32	5.81	12.55
(3d)	The	$C_{24}H_{30}O_7N_6$	56.02	5.88	16.33	56.25	5.66	16.15

* Containing 0.2 H_2O

Results of the polymerization were listed in TABLE 3. In the case of adenine derivative, triethylamine was consumed more than equimolar amount to the adenine derivative for initiating the polymerization. This fact suggests that NCA of the adenine derivative (4a) was present as a salt form with hydrogen chloride. Degree of polymerization of the polymers was calculated from terminal amino units which were determined by the trinitrobenzene sulfonate method (FIELDS 1972).

TABLE 3. Poly(ε,N-R-L-lysine)

No.	R	[I]/[M][1] mol %	Yield %	UV[2] λ_{max},nm	UV[2] ε_{max}	$[\alpha]_D$[3]	DP[4]
(5a)	Ade	1.4	73	266	11,800	+0.8°	14.5
(5b)	Thy	0.4	79	274	8,800	-5.9°	10.4
(5c)	Ura	0.4	74	269	8,300	-3.3°	9.5
(5d)	The	0.4	82	278	8,000	-0.6°	7.4

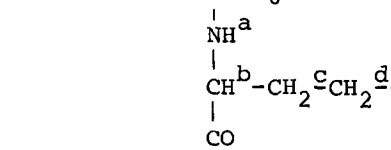
1) I and M denote initiator and monomer, respectively.
2) In DMSO solution, at 25°C.
3) In DMSO (c=1), at 22°C.
4) Calcd. from terminal amino units.

TABLE 4. NMR spectral data of poly(ε,N-R-L-lysine)[1]

No.	CH_2 c, d, e	CH_2 f	CH b	NH a	NH g	CH_2 h	CH_2 i	CH_2 Cbz	Ph
(3a)	1.32, 1.64	3.02	3.95	7.56	7.92	2.68	4.36	5.04	7.34
(5a)	1.30, 1.6	3.00	4.3		7.94	2.70	4.37		
(3b)	1.34, 1.6	3.00	3.9	7.51	7.93	2.44	3.80	5.01	7.33
(5b)	1.32, 1.6	3.00	4.18		7.96	2.50	3.80		
(3c)	1.35, 1.64	3.01	3.9	7.48	7.93	2.45	3.83	5.01	7.32
(5c)	1.33, 1.6	3.00	4.21		7.98	2.48	3.83		
(3d)	1.30, 1.60	2.96	3.90	7.49	7.84	2.66	4.40	5.01	7.33
(5d)	1.29, 1.6	2.96	4.2		7.95	2.67	4.40		

No.	8-H	2-H	6-NH_2	5-H(CH_3)	6-H	3-NH	1-CH_3	3-CH_3
(3a)	8.02	8.18	7.32					
(5a)	8.04	8.16	7.37					
(3b)				(1.72)	7.35	11.22		
(5b)				(1.72)	7.36	11.22		
(3c)				5.48	7.48	11.24		
(5c)				5.50	7.52	10.74		
(3d)	7.87						3.38	3.21
(5d)	7.88						3.36	3.18

1) In d_6-DMSO at room temperature, ppm from TMS



The spectral data of the polymers obtained were tabulated in TABLE 3 and 4. NMR Spectra of the polymers were found to be essentially coincided with those of the polymers obtained by the polymer reactions (ISHIKAWA et al. 1978). However, some peaks particularly based on α-CH in the main chain, were shifted to the lower magnetic field. As the degree of polymerization of these polymers was low, they were not in a helical conformation, and the peak of α-CH shifted to the lower magnetic field (ANAND et al. 1971).

Summary

Poly-L-lysines containing nucleic acid bases, that is, adenine, thymine, uracil and theophylline were synthesized. The nucleic acid base substituted L-lysine derivatives were prepared from α,N-Cbz-L-lysine and the *p*-nitrophenyl esters of the carboxyethyl derivatives of the nucleic acid bases. The polymers were obtained from these L-lysine derivatives by the NCA method.

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