SYNTHESIS OF REGULAR POLYPEPTIDES INCLUDING LYSINE, PROLINE, AND ALANINE RESIDUES

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Polypeptides of regular structure including lysine, proline, and alanine residues are of interest for modeling histones, since the latter contain them in considerable amount [1]. We have previously reported the preparation of polypeptides of such a structure with residues of basic amino acids [2].

The present paper describes the synthesis of regular polypeptides with the sequences -Lys-Ala-, -Lys-Pro-, -Lys-Lys-Pro-, -Lys-Lys-Lys-Pro-, and -Lys-Pro-Pro-, which were obtained by the polymerization of the 2,4,5-trichlorophenyl and pentachlorophenyl esters of the corresponding peptides. To protect the N^{α}-amino groups we selected the tert-butoxycarbonyl (BOC) group, which is easily removed in the presence of the benzyloxycarbonyl (Cbo) group. The completeness of the removal of the Cbo group from the polypeptides was checked spectrophotometrically. To obtain polypeptides more homogeneous in molecular weight, they were passed through a column of Sephadex G-25. The molecular weights of the polypeptides were determined by Van Slyke's method. When pentachlorophenol was used to activate the C-end, the molecular weights of the products increased considerably.

EXPERIMENTAL

The work was carried out with amino acids of the L form; thin-layer chromatography was performed in a fixed layer (250 mesh, plates 75 × 25 mm); the solvent systems were: 1) water–acetic acid–butan-1-ol (30 : 10 : 100), 2) 3% ammonia–sec-butanol (100 : 44), 3) toluene–dioxane–heptane–acetic acid (5 : 3 : 1.5 : 0.5), and 4) chloroform – methanol (63.7 : 6.33). THF – tetrahydrofuran; TEA – triethylamine; IBCF – isobutyl chloroformate; $OPhCl_3$ – 2,4,5-trichlorophenyl group; $OPhCl_5$ – pentachlorophenyl group; DCHCD – dicyclohexylcarbodiimide; BOC – tert-butoxycarbonyl group; Cbo – benzyloxycarbonyl group.

Preparation of the 2,4,5-Trichlorophenyl Ester of N^{α}-tert-Butoxycarbonyl-N^{ξ}-benzyloxycarbonyllysyl-N^{ξ}-benzyloxycarbonyllysylproline (I). To a solution of 2.5 g of BOC-(N^{ξ}-Cbo)Lys-(N^{ξ}-Cbo)Lys-OH in 7 ml of THF containing 0.54 ml of TEA at −15°C was added 1 g of DCHCD and, after 20 min, 1.55 g of $HBr \cdot H$-Pro-$OPhCl_3$ in 5 ml of THF and 0.54 ml of TEA. The mixture was left at 0°C for 4 h and at 20°C for 24 h, and then 0.5 ml of glacial acetic acid was added (to destroy the residual DCHCD). The THF was evaporated off, the residue was dissolved in ethyl acetate, and the solution was filtered from dicyclohexylurea and was treated with 10% citric acid (3 × 5 ml), with water, with a cooled 0.5 N solution of $NaHCO_3$ (3 × 5 ml), and with water again. The ethyl acetate solution was dried over calcined sodium sulfate and evaporated. The yield of (I) was 2.5 g (70%). After reprecipitation from ethyl acetate with absolute ether, $[\alpha]_D^{25}$ −32.8° (c 1.16; acetic acid), R_f 0.98 (1), 0.96 (2), and 0.74 (4).

Preparation of the Pentachlorophenyl Ester of N^{α}-tert-Butoxycarbonyl-N^{ξ}-benzyloxycarbonyllysylalanine (II). To a solution of 1.85 g of BOC-N^{ξ}-Cbo-Lys-OH in 10 ml of THF containing 0.7 ml of TEA and cooled to −15°C was added 0.69 ml of IBCF, and, after 20 min, 2.1 g of $HBr \cdot$Ala-$OPhCl_5$ in 10 ml of THF containing 0.7 ml of TEA. Then the reaction mixture was kept at 0°C for 2 h and at 20°C for 16 h, after which the solvent was evaporated off in vacuum at 30°C and the residue was dissolved in 70 ml of ethyl acetate. The ethyl acetate solution was extracted with cooled 10% citric acid (3 × 10 ml), 0.5 N

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TABLE 1. 2,4,5-Trichlorophenyl and Pentachlorophenyl Esters of Substituted Peptides and Their Characteristics

Esters of N-acylpeptides	Type of linkage	Method*	$[\alpha]_D^{25}$, deg (CH_3OH)	Mp, °C	R_f in system				Yield, %
					1	2	3	4	
BOC-[Lys(Cbo)]$_2$-Pro-OPhCl$_3$	2+1	2	−32,8; c 1,16	Amorph.	0,98	0,96	—	0,69	80
BOC-[Lys(Cbo)]$_2$-Pro-OPhCl$_5$	2+1	2	−27,7; c 0,94		0,97	0,98	—	0,71	76,5
BOC-[Lys(Cbo)]$_3$-Pro-OPhCl$_3$	3+1	2	−29,; c 1,31		0,98	0,96	−0,7	0,7	79,8
BOC-[Lys(Cbo)]$_3$Pro-OPhCl$_5$	3+1	2	−30; c 1,18		0,99	0,97	—	0,71	72,5
BOC-Lys(Cbo)-Pro-OPhCl$_3$	1+1	1	−40; c 0,9	Oil	0,98	0,98	0,92	0,76	70
BOC-Lys(Cbo)-Pro-Pro-OPhCl$_5$	2+1	2	−41,2; c 1,25	184—185°	0,98	0,98	—	0,65	90
BOC-Lys(Cbo)-Pro-Pro-OPhCl$_3$	1+2	2	−37,7; c 1,7	Amorph.	0,97	0,95	0,89	0,73	58
Cbo-Pro-Pro-OPhCl$_3$	1+1	1	—	Oil	0,98	0,87	0,9	—	60
BOC-Lys(Cbo)-Pro-OCH$_3$	1+1	1	−40; c 2,0		0,96	0,94	—	—	50
BOC-Lys(Cbo)-Ala-OPhCl$_5$	1+1	1	c 0,92—21,7; DMFA	150—152	0,93	0,95	—	0,73	50

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*Obtained by the mixed-anhydride method (1) or the carbodiimide method (2).

TABLE 2. Monomers for Polymerization

Peptide hydrohalides and trifluoroacetates	Method*	Reaction time, min	$[\alpha]_D^{25}$, deg (CH_3OH)	R_f (1)	Yield, %	Purification
HCl·H-[Lys (Cbo)]$_2$-Pro-OPhCl$_3$	2	40	−46,6; c 2,1	0,72	60	Ethanol/ether
CF$_3$COOH·H-[Lys (Cbo)]$_2$-Pro-OPhCl$_5$	1	60	−10,7; c 0,37	0,73	67	
CF$_3$COOH·H-[Lys (Cbo)]$_3$-Pro-OPhCl$_3$	1	40	−23; c 0,87	0,73	68	
CF$_3$COOH·H-[Lys (Cbo)]$_3$-Pro-OPhCl$_5$	1	60	−15,1; c 1,85	0,71	72	
HCl·H-Lys (Cbo)-Pro-OPhCl$_3$	2	60	−23,8; c 0,6	0,75	60	CHCl$_3$/hexane
CF$_3$COOH·H-Lys (Cbo)-Pro-Pro-OPhCl$_5$	1	60	−29; c 1,3	0,69	50	Ether/hexane
HCOOH·H-Lys (CbO)-Pro--PrO-OPhCl$_3$	3	180	—	0,71	53	Ethanol/ether
HBr·H-Pro-Pro-OPhCl$_3$	4	20	—	0,75	60	
HCl·H-Lys (Cbo)-Ala-OPhCl$_5$	2	60	−8,2; c 1,2	0,71	73	

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*Methods: 1) in absolute CF_3COOH; 2) in 2.94 N HCl in ethyl acetate; 3) in absolute HCOOH; and 4) in 38% HBr in CH_3COOH.

$NaHCO_3$ (3 × 10 ml), and water. The organic layer was dried over calcined sodium sulfate and evaporated in vacuum. The crystalline residue was recrystallized from ethanol. Yield of (II), 1.7 g (50%), mp 150-152°C, $[\alpha]_D^{25}$ −21.7° (c 0.92; dimethylformamide), R_f 0.96 (1), 0.73 (5).

The methods of obtaining the other esters of N^{α}-BOC- peptides and their characteristics are given in Table 1.

Polymerization. The required monomer was obtained from the corresponding ester of a N^{α}-BOC-peptide by the method indicated in Table 2, 1 g of the monomer being dissolved in dimethylformamide at 20°C. When a 50-60% solution of the monomer was reached, one equivalent of TEA was added to it. After ten days, methanol was added to the solution, and the precipitate that deposited was filtered off and was washed with methanol and then with ether. The yield of polymerization product was 30-60%. The N^{ε}-benzyloxycarbonyl group was eliminated from the polymer by the passage of dry HBr through a solution of the protected polypeptide in glacial acetic acid.

Fractionation of the Polypeptides on Sephadex G-25. A column 2 cm in diameter and 100 cm long was used for fractionation. The loading of the polymer was 20 mg/ml, the rate of elution 12 ml/h, and the size of the fractions collected 5 ml. Elution was performed with water, and the issuance of the polypeptides was monitored from the absorption in UV light at 235 nm.

The molecular weights of the polypeptides obtained by the polymerization of the 2,4,5-trichlorophenyl and pentachlorophenyl esters of peptides were, respectively, for H-(Lys-Pro)$_n$-OH 1500 and 5850; H-(Lys-Lys-Pro)$_n$-OH 1000 and 5000; H-(Lys-Lys-Lys-Pro)$_n$-OH 5000 and 14,000; H-(Lys-Pro-Pro)$_n$-OH 2000 and 4500; and H-(Lys-Ala)$_n$-OH 3500.

CONCLUSIONS

Polypeptides of the following sequences have been obtained: -Lys-Pro-, -Lys$_2$-Pro-, -Lys$_3$-Pro-, -Lys-Pro$_2$-, and -Lys-Ala-, with molecular weights of from 1000 to 14,000. It has been established that the use of pentachlorophenyl esters for polymerization in place of 2,4,5-trichlorophenyl esters leads to a considerable increase in the molecular weights of the polypeptides.

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