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SYNTHESIS OF PROTECTED OLIGOPEPTIDES REPRESENTING FRAGEMENTS OF HISTONE FRACTION HI

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The functional role of the primary structure of histones may be shown both in interaction with such specific enzymes as, for example, phosphokinase [1, 2] and also in the process of forming complexes with DNA [3]. Interesting information can be obtained in the investigation of individual fragments of histones. We have previously reported the isolation of compounds of this type [4]. The present paper describes the synthesis of a number of analogous oligopeptides forming segments of the histone fraction HI of calf and rabbit thymus. It must be noted that there are some differences between these histone fractions. Thus, in the HI of rabbit thymus the Ala-38 residue is replaced by Ser-38. This determined the type of compounds obtained: the fragment of the HI of rabbit thymus (31-40) contained Ala-38 and the fragment of the calf thymus HI (31-41) contained Ser-38. In addition, we synthesized two analogs in which L-Ala-37 was replaced by D-Ala-37, and the Pro-Pro fragment by Ala-Ala. In contrast to the natural sequence, in a number of oligopeptides a Lys residue was present in place of Arg-35.

The synthesis of the oligopeptides was effected in stages from the C-end by blocks consisting of 2-3 amino-acid residues using the method of mixed anhydrides and the carbodiimide method according to Schemes I and II. It must be observed that during the synthesis of the peptides the possibility of partial racemization is not excluded. In view of this, we performed the synthesis in such a way that, as far as possible, the C-terminal residue was a glycine residue. In this way, we obtained the peptide with sequence (31-40) by a (2 + 9) scheme. If the C-terminal residue was an optically active amino acid, we used the carbodimide method with tetrahydrofuran as solvent. The reaction mixture was maintained at 0 to −6°C for 1 h and at 22°C for 20 h. Under these conditions, according to the literature [5], racemization does not exceed 0.1%.

The N^{ε}-amino group of glycine was protected by a benzyloxycarbonyl grouping (Z), and the N-α-amino groups of the acids by a tert-butoxycarbonyl (Boc) grouping. The guanidine groups of the arginine residues were protected by nitro groups (NO_2).

The compounds obtained are stable and are being used as the starting materials for the preparation of the methyl esters of these peptides.

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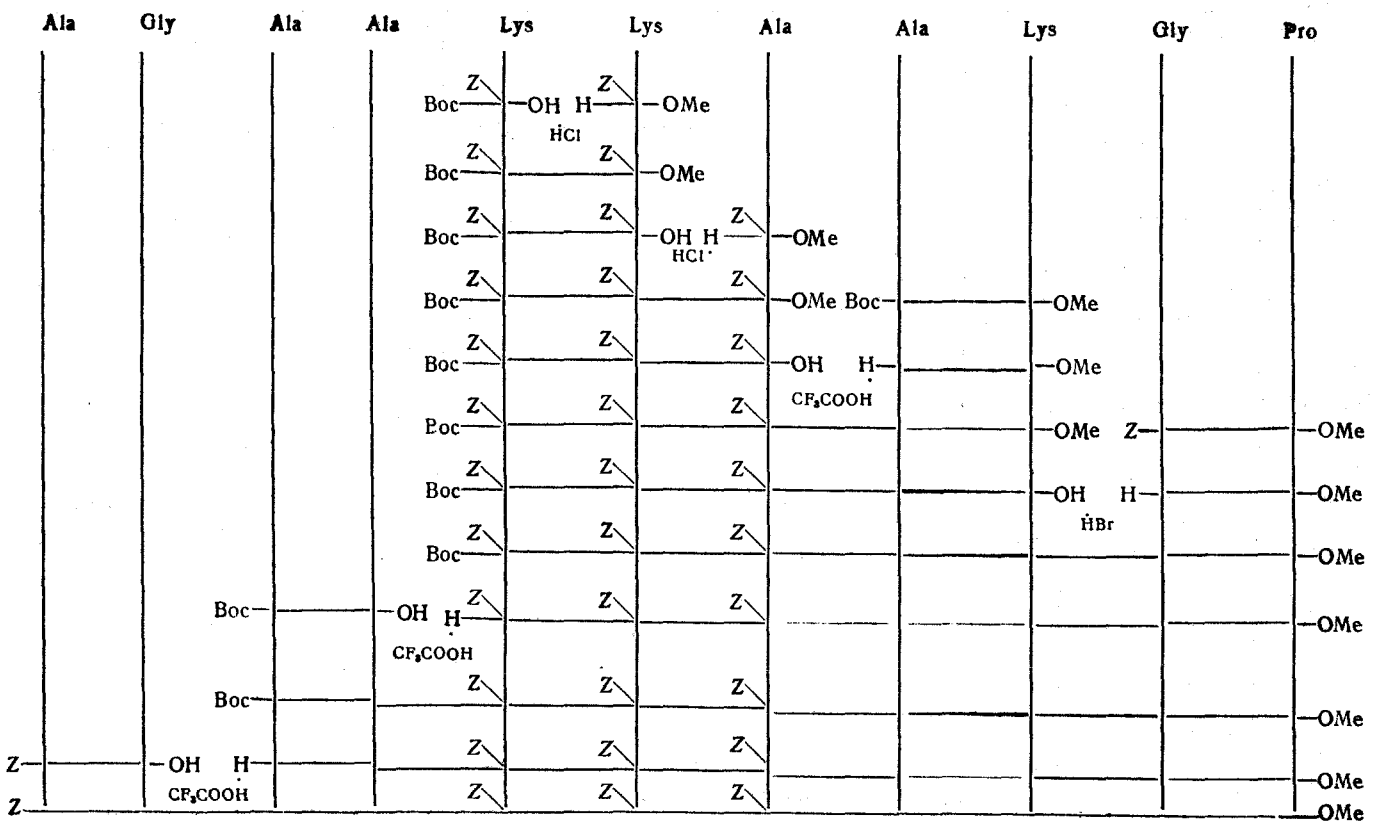
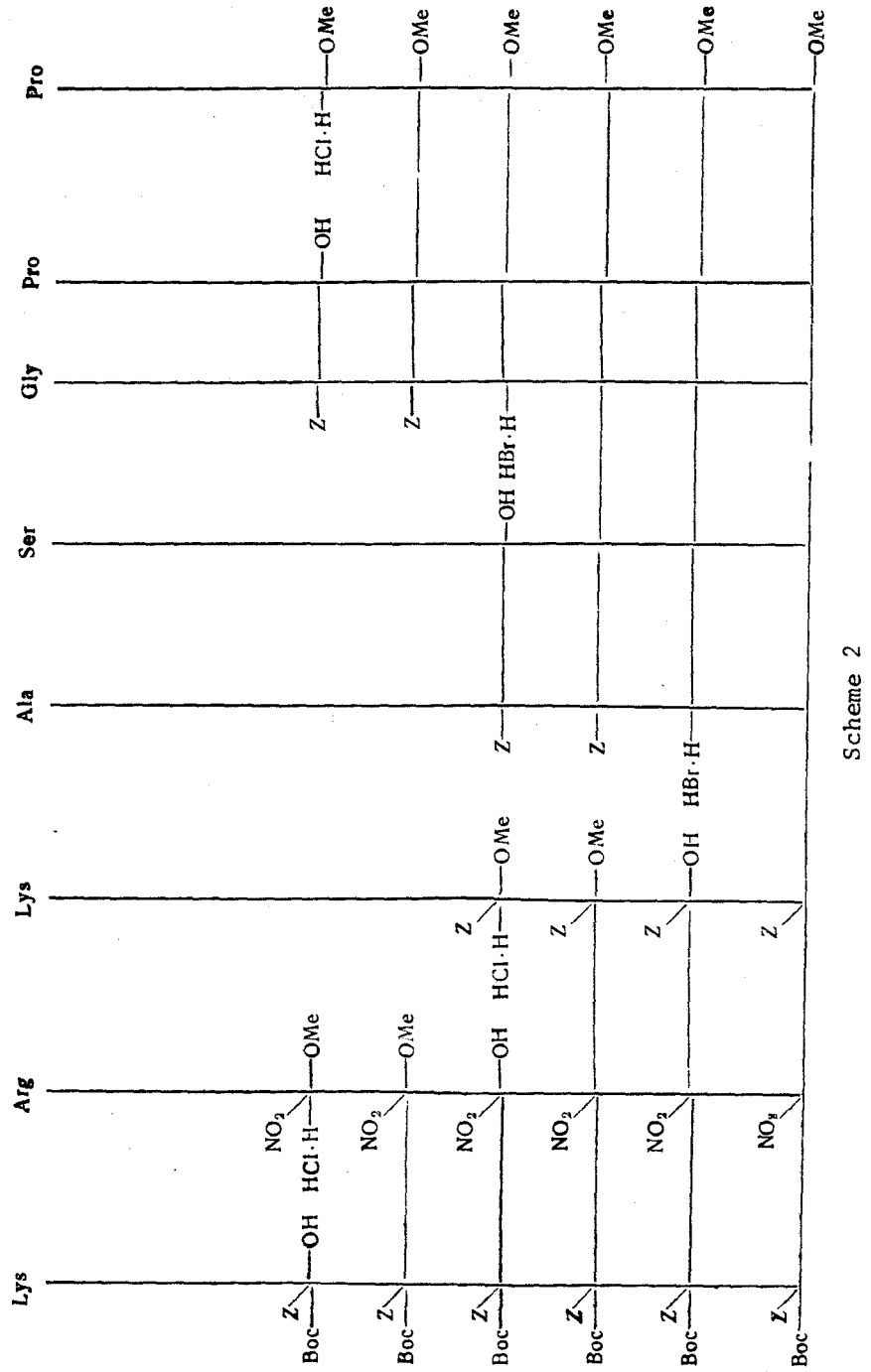

Ala
Gly
Ala
Ala
Lys
Lys
Ala
Ala
Lys
Gly
Pro
Z
Boc
OH H
HCl
OMe
CF₃COOH
HBr

Scheme 1



Scheme 2

TABLE 1

Esters of protected peptides	Type of linkage	Meth. of synthesis	yield %	mp, °C	$[\alpha]_D^t$	R_f in system				
						1	2	3	4	5
3. Boc-[(N^{ε}-Z)Lys]$_3$-Ala_2-OMe	3+2	2	68	64–66 ethanol – ether	−20,08 (*c* 0,65; ethanol)	—	—	0,75	—	—
4. Boc-Ala_2-[(N^{ε}-Z)Lys]$_3$-Ala_2-OMe	2+5	2	76	138–140 ethanol – ether	−38,64 (*c* 0,65; methanol)	—	0,80	0,50	—	—
5. Boc-Ala_2-[N^{ε}-Z)Lys]$_3$-Ala_2-Gly-Pro-OMe	7+2	2	60	185 with decomp. ethyl acetate	−42,6 (*c* 0,60; methanol)	0,91	—	—	0,70	0,96
6. Boc-(N^{ε}-Z)Lys-(N^{G}-NO_2)Arg-OMe	1+1	2	92	Amorphous	−13,72 (*c* 0,90; methanol)	0,98	0,96	0,95	—	—
7. Boc-(N^{ε}-Z)Lys-(N^{G}-NO_2)Arg-(N^{ε}-Z)Lys-OMe	2+1	2	84	75–77 ethyl acetate –	−6,06 (*c* 0,66; methanol)	0,95	0,96	0,98	—	—
8. Boc-(N^{ε}-Z)Lys-(N^{G}-NO_2)Arg-(N^{ε}-Z)Lys-Ala_2-Gly-Pro_2-OMe	3+5	2	70	ether 70–72 methanol – ether	−44,12 (*c* 0,47; methanol)	0,82	0,80	—	—	—
9. Boc-[(N^{ε}-Z)Lys]$_3$-Ala_2-Gly-Pro_2-OMe	3+5	2	80	108–110 ethanol – ether	−54,05 (*c* 0,40; methanol)	0,83	0,80	—	—	—
10. Boc-[(N^{ε}-Z)Lys]$_3$-Ala-Ser-Gly-Pro_2-OMe	3+5	2	67	Amorphous	−4,12 (*c* 0,97; CH_2Cl_2	0,66	0,71	—	—	0,90
11. Boc-[(N^{ε}-Z)Lys]$_3$-(*d*)Ala-Ser-Gly-Pro-OMe	3+5	2	79	Amorphous	−8,16 (*c* 0,98; $CHCl_3$)	0,85	0,51	—	—	0,74
12. Z-Ala-Ala-Gly-Pro-Pro_2-OMe	2+3	2	69	71–73 chloroform- ether	−27,3 (*c* 0,50; $CHCl_3$)	—	0,80	0,78	—	—
13. Boc-(N^{ξ}-Z)Lys-Ala-Ala-OMe	1+2	2	70	61–62 ethanol	−41,43 (*c* 0,90; methanol)	—	0,89	0,90	—	—
14. Boc-[(N^{ξ}-Z)Lys-Ala-Ala]$_2$-OMe	3+3	2	73	209–210 ethanol	−36,36 (*c* 0,20; methanol)	0,85	0,90	—	—	0,93
15. Z-Ala-Ser-Gly-Ala-Ala-OMe	2+3	2	58	Amorphous	−19,20 (*c* 1,04; methanol)	0,78	0,79	—	—	—
16. Z-(*d*)Ala-Ser-Gly-Pro-Pro-OMe	2+3	2	62	Amorphous	−58,30 (*c* 0,96; methanol)	0,55	0,43	—	—	—
17. Boc-[(N^{ξ}-Z)Lys-Ala-Ala]$_4$-OMe	6+6	2	80	220–225 decomp., ethanol	−18,31 (*c* 0,34; methanol)	—	0,97	—	0,61	—

TABLE 2

N-Acylpeptide	Saponification conditions	Time, min	Yield, %	mp, °C	R_f in system	
					1	3
19. Boc-Ala-Ala-[(N^{ε}-Z)Lys]$_3$-Ala-Ala-OH	Acetone + H_2O (1:1) 1 N. NaOH	60	65	Amorph.	—	0,60
20. Boc-(N^{ε}-Z)Lys-Ala-Ala-OH	Acetone 0,2N NaOH	60	70	Amorph.	—	0,51
21. Boc-(N^{ε}-Z)Lys-(N^{G}-NO_2)Arg-OH	Acetone 0,1N NaOH	100	79	Amorph.	—	0,63
22. Boc-[(N^{ε}-Z)Lys]-[(N^{G}-NO_2)Arg]-(N-Z)Lys-OH	Acetone 0,1N NaOH	80	97	65–67	0,66	0,52

EXPERIMENTAL

The L forms of the amino acids were used. The chromatographic analysis of the peptides synthesized was performed on standard plates of the "Silufol" type (Czechoslovakia) in the following solvent systems: 1) butan-1-ol–water–acetic acid (100:30:10); 2) butan-2-ol–3% aqueous NH_3 (100:44); 3) methanol–chloroform–acetic acid (1:8:2); 4) benzene–ethanol (3:20); 5) ethanol–pyridine–water–acetic acid (5:5:3:1). The electrophoretic investigation of the substances with a free amino group was performed on "Whatmann 2" paper at a potential gradient of 38 V/cm in 6% acetic acid. Ninhydrin solution and iodine vapor were used as the revealing agents. THF) tetrahydrofuran; DMFA) dimethylformamide; TEA) triethylamine; IBCF) isobutylchloroformate; DCHCD) dicyclohexyl carbodimide.

Methyl Ester of Benzyloxycarbonylalanylglycylalanylalanyl(N^{ε}-benzyloxycarbonyl)lysyl-(N^{ε}-benzyloxycarbonyl)lysyl(N^{ε}-benzyloxycarbonyl)lysylalanylalanylglycylproline (1). A solution of 0.05 g of benzyloxycarbonylalanylglycine [4] in 2 ml of DMFA was cooled to –15°C, and 0.022 ml of TEA and 0.024 ml of IBCF were added. After 15 min, a cooled solution of 0.25 g of the trifluoroacetate of the methyl ester of alanylalanyl[(N^{ε}-benzyloxycarbonyl)-lysyl]$_3$alanylalanylglycylproline containing 0.26 ml of TEA was added and the mixture was stirred at –2°C for 2 h and at room temperature for 24 h. Then it was evaporated in vacuum, the residue was dissolved in chloroform, and the solution was washed successively with water, 1 N HCl, water, 0.5 N $NaHCO_3$, and water again, and it was dried and evaporated in vacuum. This gave 0.1 g (48%) of the crystalline (I) with mp 207°C (decomp.), R_f 0.4 and 0.7 in systems 2 and 4, respectively, $[\alpha]_D^{22}$ –40.1° (c 0.51; DMFA).

Methyl Ester of tert-butyloxycarbonyl(N^{ε}-benzyloxycarbonyl)lysyl(N^{G}-nitro)arginyl(N^{ε}-benzyloxycarbonyl)lysylalanylserylglycylprolylproline (2). At –10°C, 0.2 g of DCHCD and a solution of 0.5 g of the hydrobromide of the methyl ester of alanylserylglycylprolylproline in 2 ml of THF containing 0.19 ml of TEA was added to a solution of 0.8 g of tert-butyloxycarbonyl(N^{ε}-benzyloxycarbonyl)lysine in 3 ml of THF. The mixture was stirred at –5°C for 1 h and at room temperature for 24 h. The dicyclohexylurea that deposited was filtered off, the filtrate was evaporated, the residue was dissolved in chloroform, and the solution was washed successively with water, 10% citric acid, 0.5 N $NaHCO_3$, and water again, and was dried over Na_2SO_4. After the elimination of the solvent in vacuum, 0.56 g (70%) of the amorphous product (2) was obtained with R_f 0.57 and 0.79 in systems 1 and 2, respectively, $[\alpha]_D^{22}$ –17.2° (c 0.93; methanol).

Compounds 3–17 were obtained similarly (Table 1).

tert-Butyloxycarbonyl(N^{ε}-benzyloxycarbonyl)lysylalanylalanyl(N^{ε}-benzyloxycarbonyl)-lysylalanylalanine. A solution of 0.6 g of the methyl ester of tert-butyloxycarbonyl(N^{ε}-benzyloxycarbonyl)lysylalanylalanyl(N^{ε}-benzyloxycarbonyl)lysylalanylalanine in 5 ml of acetone and 5 ml of dioxane was treated with 5 ml of 0.1 N NaOH. After 60 min, the solution was evaporated in vacuum, and the residue was diluted with water and acidified with 0.1 N HCl to pH 1–2. The precipitated oil was extracted with ethyl acetate and reprecipitated with ether. This gave 0.4 g (68%) of an amorphous product (R_f 0.7 and 0.73 in systems 1 an 2, respectively, $[\alpha]_D^{22}$ –22.7° (c 0.89; ethanol).

Compounds (19–23) were obtained similarly (Table 2).

Methyl Ester of the Trifluoroacetate of Alanylalanyl(N^{ε}-benzyloxycarbonyl)lysyl(N^{ε}-benzyloxycarbonyl)lysyl(N^{ε}-benzyloxycarbonyl)lysylalanylglycylproline (23). A solution of 0.5 g of the methyl ester of tert-butyloxycarbonylalanylalanyl[(N^{ε}-benzyloxycarbonyl)lysyl]

TABLE 3

Hydrohalides and trifluoroacetates of peptides	Meth.	Reaction time, min	Yield, %	mp, °C	R_f in system	
					1	2
24. $CF_3COOH \cdot H$-Ala-Ala-OMe	1	20	80	Amorph.	0,80	0,63
25. $CF_3COOH \cdot H$-[(N^{ε}-Z)Lys]$_3$-Ala-Ala-OMe	1	60	75	Amorph.	0,58	0,65
26. $CF_3COOH \cdot H$-[(N^{ε}-Z)Lys]-Ala-Ala-OMe	1	60	70	163—164	—	0,70
27. $CF_3COOH \cdot H$-[(N^{ε}-Z)Lys-Ala-Ala]$_2$-OMe	1	60	45	112—114	0,81	—
28. HBr·H-Ala-Ser-Gly-Ala-Ala-OMe	2*	20	79	Amorph.	—	0,41
29. HBr·H-(*d*)Ala-Ser-Gly-Pro-Pro-OMe	2	20	62	Amorph.	—	0,48
30. 5HBr·H-[Lys-Ala-Ala]$_4$-OMe†	2	50	40	Amorph.	—	—

*Obtained by the action of HBr in nitromethane [4].
†Individuality confirmed electrophoretically.

alanylalanylglycylproline in 5 ml of absolute trifluoroacetic acid was kept at room temperature for 75 min. Then 2 ml of absolute benzene was added and the mixture was evaporated in vacuum (the operation was repeated three times). The residue was dissolved in methanol and precipitated with absolute ether. This gave 0.4 g (80%) of the amorphous product (23) with R_f 0.15 and 0.2 in systems 1 and 2, respectively.

Compounds (24-27) were obtained similarly (Table 3).

SUMMARY

Protected oligopeptides corresponding to sequences (31-40) of histone fraction HI of rabbit thymus and (34-41) of histone fraction HI of calf thymus and a number of their analogs have been synthesized.

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