SYNTHESIS OF TRIFLUOROACETYL DERIVATIVES OF OLIGOPEPTIDES FORMING ANALOGS OF THE PHOSPHORYLATABLE SECTION 33-40 OF HISTONE H1

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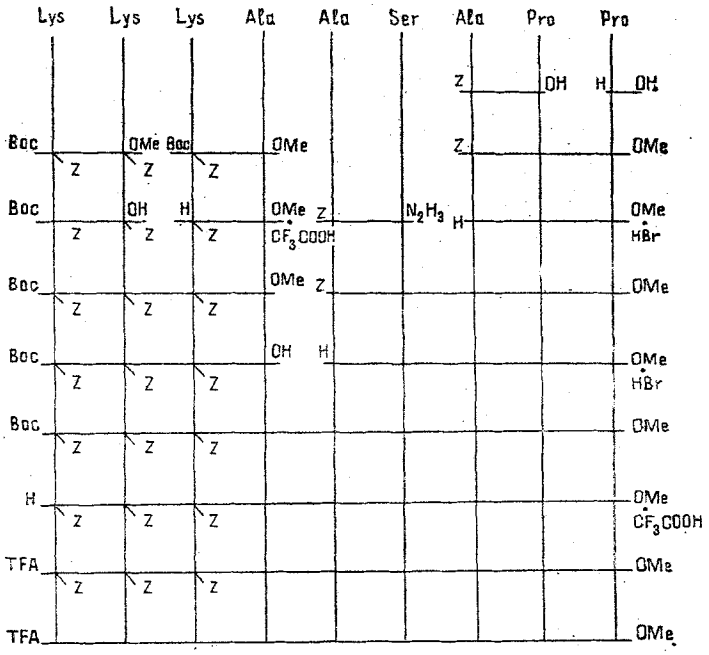
A number of peptide fragments forming analogs of section 33-40 of histone H1, including the phosphorylatable serine-37 residue have been synthesized by the carbodiimide and modified azide methods. The specific introduction of trifluoroacetyl labels at the free α-amino groups of the peptides has been effected under conditions in which the other side chains were blocked by benzyloxycarbonyl (Z) protection. Thioethyl trifluoroacetate was used as the trifluoroacetylating reagent. The Z groups were removed from the final products by the action of HBr in absolute CF_3COOH.

Recently, trifluoroacetyl derivatives of bioorganic compounds have found wide use, being used as so-called fluorine labels in the study of model biologically important systems by the ^{19}F NMR method [1, 2]. This method may be an extremely sensitive instrument for the study of the conformational changes of various histones and, in particular, histone H1 in the process of complex-formation with DNA. Furthermore, the investigation of the behavior of a DNP complex in various enzymatic modifications is extremely interesting.

It is known that the phosphorylation of histone H1 at the serine-37 residue leads to a weakening of the rigidity of the binding of the central, globular, segment of the H1 molecule with the DNA or to dissociation [3, 4]. At the same time, the question of the influence of the process of phosphorylation on the conformational properties of the section of the histone including the serine-37 residue is insufficiently clear. With this aim, we have synthesized a number of peptide fragments including analogs of the phosphorylatable section 33-40 of histone H1 protected at their α-amino groups by trifluoroacetyl groupings. We have shown previously that peptides with such a sequence are capable of undergoing enzymatic phosphorylation [5].



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The peptides were synthesized in stages from the C-end by means of blocks consisting of 2-5 amino acid residues, which were linked by the carbodiimide and azide methods according to the general scheme. It must be mentioned that in the peptide-synthesizing process the hydroxy group of the serine was left unprotected. We deliberately selected this route, since for the subsequent investigations of phosphorylation processes we needed to have compounds with a free OH group. Consequently, it appeared desirable to use the azide method as modified by Rudinger, employing butyl nitrate, which excludes side reactions connected with the free OH group of serine. To block the α-amino groups of alanine and glycine and the ε-amino group of lysine we used the benzyloxycarbonyl (Z) and for the α-amino group of lysine the tert-butoxycarbonyl (Boc) protective groupings. The side-chain protective groups were eliminated by the action of halogen hydrides in absolute trifluoroacetic acid in the presence of anisole. The final trifluoroacetyl (TFA) derivatives of the peptides were purified on a column of silica gel. The yields and constants of the compounds obtained are given in Table 1.

EXPERIMENTAL

The initial amino acids were of the L form. The individuality of the products synthesized was checked by TLC on plates with a fixed layer of silica gel (25 × 75 mm, 250 mesh), and on Silufol UV-254 -lates in the following systems: 1) butan-1-ol—water—acetic acid (100:30:10); 2) butan-2-ol—aqueous NH_3 (100:44); 3) benzene—ethanol (6:40); 4) butan-1-ol—acetic acid—pyridine—water (15:3:10:12); and 5) chloroform—methanol—acetic acid (90:9:2). Electrophoretic analysis of compounds with a free amino group was carried out on Wattman No. 2 paper at pH 2.7 in 0.2 M CH_3COOH at a voltage gradient of 38 V/cm. Angles of rotation were determined on an Al-EPL automatic polarimeter. The protected amino acids and also their methyl esters and hydrazides were obtained by standard methods [8].

Preparation of Compounds (I) and (II). A solution of 10 mmole of the hydrazide of benzyloxycarbonylalanylserine in 2 ml of dimethylformamide was acidified with 4 N HCl/tetrahydrofuran to pH 2.5. Then it was cooled to −20°C and, with stirring, 20 mmole of N-butyl nitrite [9] was added. The mixture was kept at −20°C for 25 min, and then triethylamine was added to pH 7.5 followed by a cooled solution of the hydrobromide of the methyl ester of glycylprolylproline (10 mmole) in chloroform containing 10 mmole of triethylamine. The reaction mixture was kept at room temperature for 72 h and then the solvent was evaporated off in vacuum. The residue was dissolved in chloroform and the solution was treated successively with 1 N NCl, 0.5 N $NaHCO_3$, and water and was dried over $MgSO_4$ and evaporated. The residue was twice reprecipitated from chloroform with ether.

Preparation of Compounds (X)-(XIV). A solution of 1 g of a N^{α}-protected peptide (VII) or (VIII) in 5 ml of absolute CF_3COOH was kept at room temperature for the time shown in Table 1. Then the reaction mixture was distilled three times with absolute benzene, the residue was washed with chloroform and ethyl acetate and dissolved in acetone, the solution was filtered, then ether was added for the complete precipitation of the product.

Preparation of (VIII). A solution of 1 mmole of compound (IV) in dimethylformamide was cooled to −10°C and 1 mmole of dicyclohexylcarbodiimide and, after 5 min, 1 mmole of compound (VI) in DMFA contained 1 mmole of N-methylmorpholine were added. The reaction mixture was stirred at −10°C for 5 h and was left at room temperature for 2 days. Then, in the cold, 50 ml of 10% citric acid was added. The precipitate that deposited was filtered off, dissolved in methylene chloride, and again filtered from dicyclohexylurea, and the filtrate was washed with 0.5 N $NaCHO_3$ (3 × 10 ml) and with water and was dried over $MgSO_4$ and evaporated. The product was crystallized from ethanol.

Compounds (VII) and (IX) was obtained by a similar method. The syntheses of Boc-Lys(Z)-Lys(Z)-OH and Boc-Lys(Z)-Lys(Z)-Ala-OH have been described previously [10].

Preparation of Compounds (XV-XIX). A solution of 1 mmole of one of compounds (X-XIV) in 5 ml of DMFA was brought to pH 9 with triethylamine. Then 10 mmole of thioethyl trifluoroacetate was added and the mixture was stirred with a magnetic stirrer at room temperature for from 6 to 10 h. The reaction was monitored by TLC. After its completion, the mixture was acidified with 1 N HCl to pH 4 and was extracted with chloroform (3 × 20 ml). The chloroform extract was washed with water to a neutral pH and evaporated. The residue was reprecipitated from chloroform with ether. After repeated decantation with ether, the residue was crystallized from ethanol.

TABLE 1

Compound	Method	Yield, %	mp, °C	$[\alpha]_D^{22}$, deg	R_f and system
I. Z-Ala-Ser-Gly-Pro-Pro-OMe	Azide	80	105—108	—44.3, *c* 0,98, CH_3OH	0,63 (1); 0,51 (2)
II. Z-Ala-Ser-Ala-Pro-Pro-OMe	Azide	83	134—136	—108,1, *c* 2,2; $CHCl_3$	0,65 (1); 0,30 (2)
III. Boc-Lys $(Z)_3$-Ala-OMe	Carbodiimide	55	122—124	—16,9, *c* 1,85; C_2H_5OH	0,95 (1); 0,95 (2)
IV. Boc-Lys $(Z)_3$-Ala-OH	1 N NaOH—C_2H_5OH, 105 min	94	88—89	—	0,74 (2)
V. HBr·H-Ala-Ser-Gly-Pro_2-OMe	HBr/CF_3—COOH, 25 min	80	172—174	—67,4, *c* 1,1; CH_3OH	0,55 (1); 0,48 (2)
VI. HBr·H-Ala-Ser-Ala-Pro_2-OMe	HBr/CF_3—COOH, 30	95	149—152	—	0,40 (1); 0,23 (2)
VII. Boc-Lys $(Z)_3$-Ala-Ser-Ala-Pro_2-OMe	Carbodiimide	57	120—122	—48,8, *c* 1,6 $CHCl_3$	0,69 (1); 0,82 (2)
VIII. Boc-Lys $(Z)_3$-Ala_2-Ser-Ala-Pro_2-OMe	Carbodiimide	60	158—160	—54,9, *c* 1,64; $CHCl_3$	0,80 (2); 0,88 (3)
IX. Boc-Lys $(Z)_2$-Ala_2-Ser-Gly-Pro_2-OMe	Carbodiimide	63	155—156	—70, *c* 1,0; $CHCl_3$	
X. CF_3COOH·H-Lys $(Z)_2$-Ala-Ser-Gly-Pro_2-OMe*	45 min	78	Amorph.	—	0,54 (2); 0,77 (4)
XI. CF_3COOH·H-Lys $(Z)_3$-Ala-Ser-Gly-Pro_2-OMe*	60 min	89	"	—	0,57 (2); 0,81 (4)
XII. CF_3COOH·H-Lys $(Z)_3$-Ala-Ser-Ala-Pro_2-OMe	55 min	77	"	—	0,73 (1); 0,45 (2)
XIII. CF_3COOH·H-Lys $(Z)_3$-Ala_2-Ser-Ala-Pro_2-OMe	55 min	64	"	—	0,58 (2); 0,30 (5)
XIV. CF_3COOH·H-Lys $(Z)_2$-Ala_2-Ser-Gly-Pro_2-OMe	45 min	85	128—130	—	0,45 (2)
XV. TFA-Lys $(Z)_2$-Ala-Ser-Gly-Pro_2-OMe		60	129—131	—80, *c* 1,0; $CHCl_3$	0,64 (2); 0,80 (4)
XVI. TFA-Lys $(Z)_3$-Ala-Ser-Gly-Pro_2-OMe		80	136—137	—45, *c* 1,0; $CHCl_3$	0,75 (2); 0,91 (4)
XVII. TFA-Lys $(Z)_3$-Ala-Ser-Ala-Pro_2-OMe		72	144—146	—50, *c* 1,0; $CHCl_3$	0,4 (2)
XVIII. TFA-Lys $(Z)_2$-Ala_2-Ser-Gly-Pro_2-OMe		56	182—184	—56,5, *c* 1,0; $CHCl_3$	0,79 (2); 0,93 (4)
XIX. TFA-Lys $(Z)_3$-Ala_2-Ser-Ala-Pro_2-OMe		67	153—156	—61, *c* 1,0; $CHCl_3$	0,81 (2); 0,92 (4)
XX. TFA-Lys_2-Ala-Ser-Gly-Pro_2-OMe	HBr/CF_3COOH	52	174—176	—110,5, *c* 1,0; H_2O	0,32 (2); 0,75 (4)
XXI. TFA-Lys_3-Ala-Ser-Gly-Pro_2-OMe	HBr/CF_3COOH	57	179—180	—126,4 *c* 1,0; H_2O	0,40 (2); 0,82 (4)
XXII. TFA-Lys_2-Ala_2-Ser-Gly-Pro_2-OMe	HBr/CF_3COOH	72	162—164	—117,2, *c* 1,0; H_2O	0,71 (4)
XXIII. TFA-Lys_3-Ala_2-Ser-Ala-Pro_2-OMe	HBr/CF_3COOH	67	169—170	—138,7, *c* 1,0; H_2O	0,75 (4)
XXIV. TFA-Lys_3-Ala-Ser-Ala-Pro_2-OMe	HBr/CF_3COOH	80	219—220	—135,3, *c* 1,0; H_2O	0,79 (4)

*We have described the synthesis of the Boc derivatives of Compound (X) and (XI) previously [7].

Preparation of Compounds (XX-XXIV). A current of dry HBr was passed for 70-90 min through a solution of 0.5 mmole of the methyl ester of one of compounds (XV-XXIX) for 70-90 min. Then 5 ml of absolute benzene was added and the mixture was evaporated in vacuum at 30°C. The residue was washed with acetone to eliminate traces of anisole and was reprecipitated from ethanol with acetone. The completeness of the elimination of the benzyloxycarbonyl group was checked spectrophotometrically at λ 235-280 nm. The products obtained were passed through a column of silica gel (L 40/100 μ). Elution was performed with butan-1-ol—acetic acid—pyridine—water (15:3:10:12) buffer solution. The rate of eolution was 12 ml/h. The selected fraction was freeze-dried. The homogeneity of the methyl esters of the TFA derivatives of the peptides obtained was checked by ^{19}F NMR spectroscopy.

SUMMARY

A number of methyl esters of trifluoroacetyl derivatives of peptides forming analogs of the amino acid sequence 33-40 of histone fraction H1 of calf thymus have been synthesized.

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SYNTHESIS AND STUDY OF THE STRUCTURE OF NEW N-SUBSTITUTED 2-METHYL-5-(1-METHYLETHYL)CYCLOHEXYLAMINES

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Original methods of synthesizing N-substituted 2-methyl-5-(1-methylethyl)cyclohexylamines by hydroamination reactions of (+)-S-carvone with aliphatic nitriles and the hydroamination of some aldehydes and ketones with (+)-S-carvone oxime have been developed. The optimum conditions for performing these processes has been selected. It has been established by ^{13}C NMR that the reactions studied form a mixture of N-substituted carvo-, isocarvo-, neocarvo-, and neoisocarvomenthylamines in a ratio of 65:20:10:5. As a result of the investigation, 11 secondary amines of the p-menthane series not previously described in the literature have been isolated and characterized. The absolute configurations of the compounds synthesized have been determined.

Amino derivatives of the p-menthane series are compounds with a pronounced pesticidal activity [1, 2]. However, N-substituted 2-methyl-5-(1-methylethyl)cyclohexylamines, which are potentially physiologically active substances, have been studied inadequately. This is apparently connected with the fact that the known methods [3, 4] of obtaining these compounds

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