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SYNTHESIS OF A HEPTAPEPTIDE FORMING A MODIFIED ACTH 4-10 FRAGMENT

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UDC 547.953:665.37

A scheme is given for the synthesis of a heptapeptide representing a modified ACTH 4-10 fragment on the basis of which it is possible to create a preparation that is an effective adaptogen of peptide nature. A proposed variant of the synthesis permits a peptide with an adequate degree of purity to be obtained comparatively simply on a larger scale. The intermediate compounds and the final products were obtained with good yields and were distinguished by chromatographic homogeneity. The heptapeptide synthesized did not differ with respect to its physicochemical characteristics and biological action from the analogous compound obtained previously. Some physicochemical characteristics of the compound obtained (angles of optical rotation, chromatographic mobilities) are given.

One of the promising directions in the field of peptide chemistry is the synthesis of a new medicinal preparation of peptide nature fulfilling the role of regulators of the functions of the central nervous system (CNS). In particular, it has been shown that fragments of ACTH in small doses act on the CNS and affect learning processes in animals and Man [1]. The main disadvantage of these compounds is the briefness of their action which is apparently due to their rapid degradation by enzymes and makes it difficult to use them as drugs.

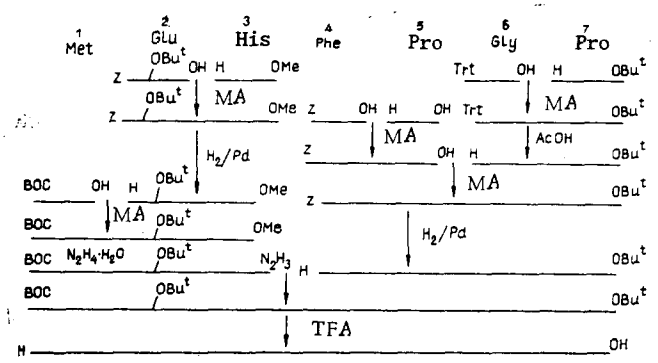
A heptapeptide consisting of a modified ACTH 4-10 fragment has recently been obtained in the Institute of Molecular Genetics of the USSR Academy of Sciences from which a preparation has been created which is an effective adaptogen of peptide nature [2]. The laboratory scheme of the synthesis of this compound has a number of disadvantages making it difficult to prepare the heptapeptide in large quantities. In the first place, it presupposes the use in the main stages of N,N'-dicyclohexylcarbodiimide as condensing agent, which is a powerful allergen and is being produced in limited amounts. In the second place, in a number of stages in the course of synthesis the intermediate products are treated repeatedly with trifluoroacetic acid to remove protective BOC groups. Nevertheless, according to the literature [3] and to the results of our own investigations, under these conditions the tripeptide (5-7) present

All-Union Scientific-Research Institute of the Technology of Blood Substitutes and Hormonal Preparations, Moscow. Translated from Khimiya Prirodnykh Soedinenii, No. 6, pp. 759-763, November-December, 1986. Original article submitted July 2, 1986.

 0009-3130/86/2206-0706$12.50

as a component of the molecule of the compound being synthesized is inadequately stable and must undergo partial cleavage, which will lead to an impairment of the quality of the intermediate and final products. We have developed a new scheme for the synthesis of the heptapeptide free from the disadvantages mentioned (all the amino acids are in the L-form; Z denotes a benzyloxycarbonyl group; OBu^t a tert-butyl ester; TFA — trifluoroacetic acid; AcOH — acetic acid; MA — the mixed-anhydride method).

As the main method of condensation we selected the mixed anhydride method which is technologically the simplest and most economical and, as a rule gives satisfactory yields. The heptapeptide was synthesized by combining the (1-3) and (4-7) fragments by the azide method. The tetrapeptide (4-7) was obtained by condensing the dipeptides (4-5) and (6-7) by the MA method. To prepare the (4-5) fragment the silylation method was used for the temporary protection of the carboxy group of proline, which made it possible to use unprotected proline in the synthesis. The dipeptide (6-7) was obtained by condensing tritylglycine with proline tert-butyl ester. The trityl group was eliminated by brief treatment with acetic acid. The tripeptide (1-3) was synthesized by the 1 + 2 scheme. The dipeptide (2-3) was obtained by the NA method, and then N^{α}-tert-butoxycarbonylmethionine was added to it. Z groups were eliminated from glutamic acid and phenylalanine residues at the intermediate stages in the process of hydrogenolysis over palladium black. The final deblocking of the heptapeptide synthesized was carried out by its brief treatment with trifluoroacetic acid in the presence of dimethyl sulfide and anisole under controlled conditions. The deblocked product was then freed from trifluoroacetic acid residues on ÉDÉ-10p anion-exchange resin and was subjected to lyophilization. The intermediate and final compounds were obtained in high yields.



The substances were chromatographically homogeneous and characterized by a fairly high degree of purity. The heptapeptide obtained did not differ with respect to its physicochemic81 characteristics (angle of optical rotation, results of high-performance liquid chromatography) and biological properties from the compound synthesized by the laboratory scheme in the Institute of Molecular Genetics Academy of Sciences of the USSR.

EXPERIMENTAL

Angles of optical rotation were determined on a VNIIÉKIPRODMASh instrument (Moscow). Chromatographic purity and mobility were determined on Silufol UV-254 plates marketed by the Kavalier enterprise (Czechoslovakia). The following solvent systems were used: 1) pyridine—AcOH—H_2O (20:6:11)—ethyl acetate (4.1:30); 2) (9.25:30); 3) 18.5:30).

1. Preparation of Z-PheProOH. A suspension of 1.7 g (5 mmole) of proline in 18 ml of the methylene chloride—dimethylformamide (5:1, ratio by volume) system was treated with 2.3 ml (18 mmole) of chlorotrimethylsilane and the reaction mixture was stirred at room temperature in a closed system until the proline had dissolved (20-30 min). Then the reaction mixture was cooled to —10 to —15°C, 3.85 ml of triethylamine was added, and coupling was performed with the mixed anhydride (MA) prepared by the following procedure: 3 g (10 mmole) of N^{α}-benzyloxycarbonyl-β-phenylalanine was dissolved in 15 ml of methylene chloride with 1.35 ml of triethylamine, the solution was cooled to —20°C, 1.3 ml (10.5 mmole) of pivaloyl chloride was added, and the mixture was kept at —10 to —15°C for 15 minutes.

The reaction mixture was kept at —5°C for 1 h and at 0°C for 12 h, and then 30 ml of chloroform was added and it was washed with 1 N HCl and with water. The organic solution was dried with anhydrous sodium sulfate, the solvent was evaporated off in vacuum, and the residue

was crystallized from diethyl ether. The yield of product was 2.5 g (63% of theory), R_f 0.49-0.50 (system 1), $[\alpha]_D^{20}$ −38.5° (s 1; MeOH).

2. Preparation of TrtGlyProOBu^t. By the method of paragraph 1, 5.5 g (26.4 mmole) of pr-line tert-butyl ester hydrochloride, 7.6 g (24 mmole) of tritylglycine, and 3.6 g (26.3 mmole) of butyl chloroformate in the presence of triethylamine gave 11.2 g (~100% of theory) of the dipeptide, R_f 0.90-0.91 (system 1), $[\alpha]_D^{20}$ −38.0° (s 1; MeOH).

3. Preparation of HGlyProOBu^t. A solution of 11.2 g (23.8 mmole) of the dipeptide (para. 2) in 60 ml of 80% AcOH was kept at 40°C for 1 h (with monitoring by TLC). The resulting precipitate was separated off and washed with water. The combined aqueous extracts were washed with diethyl ether, and the aqueous layer was cooled to 0°C and was made alkaline, pH 9, with aqueous ammonia. Then it was saturated with NaCl, and the product was extracted with the chloroform–isopropyl alcohol (1:1, ratio by volume) system. The organic layer was dried with sodium sulfate, and the solvent was driven off in vacuum. The yield of product was 4.8 g (88% of theory), R_f 0.35-0.36 (system 3), $[\alpha]_D^{20}$ −88.0 (s 1; MeOH).

4. Preparation of Z-PheProGlyProOBu^t. By the method of para. 1, 4.49 g (12.4 mmole) of N^{α}-benzyloxycarbonyl-β-phenylalanylproline, 3.2 g (14 mmole) of glycylproline tert-butyl ester, and 2.2 g (16 mmole) of butyl chloroformate yielded 6.4 g (85% of theory) of the tetrapeptide, with R_f 0.54-0.55, $[\alpha]_D^{20}$ −81° (s 1; MeOH).

5. Preparation of H-PheProGlyProOBu^t. In a current of hydrogen over palladium black, 7.1 g (12 mmole) of the product of para. 4 in 70 ml of methanol was reduced for 3-4 h (monitoring by TLC). The catalyst was filtered off, half the volume of solvent was driven off in vacuum, and the reaction products in the MeOH–H_2O (1:1) system were washed several times with the diethyl ether–heptane (1:1) system, the product was extracted from the aqueous methanolic solution into methylene chloride, and the solvent was evaporated off in vacuum. Yield 4.0 g (73% of theory), R_f 0.17-0.18 (system 3), $[\alpha]_D^{20}$ −98.0° (s 1; MeOH).

6. Preparation of Z-Glu(OBu^t)HisOMe. By the procedure of para. 1, using ethyl chloroform, 13.5 g (40 mmole) of N^{α}-benzyloxycarbonylglutamic acid γ-tert-butyl ester and 12.1 g (50 mmole) of histidine methyl ester dihydrochloride in methylene chloride yielded 17.6 g (90% of theory) of product with R_f 0.7-0.71 (system 2), $[\alpha]_D^{20}$ −10.0° (s 1; MeOH).

7. Preparation of BOC-MetGlu(OBu^t)HisOMe. Using the procedure of para. 5, 4.88 g (10 mmole) of N^{α}-benzyloxycarbonyl(γ-O-tert-butyl)glutamylhistidine methyl ester in 40 ml of DMFA with 2 ml of 10 N HCl gave a solution of (γ-O-tert-butyl)glutamylhistidine methyl ester dihydrochloride, which was cooled to −20°C and treated with 2.8 ml (20 mmole) of triethylamine.

The corresponding MA was prepared from 2.75 g (11 mmole) of N^{α}-tert-butoxycarbonylmethionine, 1.54 ml (11 mmole) of triethylamine, and 1.35 ml (11 mmole) of pivaloyl chloride by the procedure of para. 1. Another 1.54 ml (11 mmole) of triethylamine was added to the reaction mixture, this was poured into the cooled dipeptide solution, and the mixture was left at 0 to −5°C for 12 h. Then it was diluted with 50 ml of methylene chloride and was washed successively with 1 N HCl–saturated NaCl solution (1:1), H_2O–saturated NaCl solution (1:1), and 5% $NaHCO_3$ solution and was dried with anhydrous sodium sulfate and evaporated. The residue was dissolved in 100 ml of ether, the solution was cooled, and 3.6 g of the white powder that had crystallized out was filtered off. Yield 61.5% (calculated on the N^{α}-benzyloxycarbonyl(γ-O-tert-butyl)glutamylhistidine methyl ester, R_f 0.27-0.28 (system 1); 0.53-0.55 (system 2); $[\alpha]_D^{20}$ 23° (s 1; MeOH).

8. Preparation of BOC-MetGlu(OBu^t)HisN$_2$H$_3$. A solution of 1.8 g (5 mmole) of the tripeptide of para. 7 in 6 ml of methanol was treated with 1.3 g (25 mmole) of hydrazine hydrate and the reaction mixture was kept at room temperature for 10-12 h. The solvent was driven off in vacuum, the residue was dissolved in 30 ml of methylene chloride, the solution was washed with 10% aqueous NaCl and was dried with anhydrous sodium sulfate, and the solvent was evaporated off in vacuum. The yield of product was 1.6 g (89% of theory), R_f 0.25-0.26 (system 2), $[\alpha]_D^{20}$ −31.0° (s 1; MeOH).

9. Preparation of BOC-MetGlu(OBu^t)HisPheProGlyProOBu^t. Successively, with stirring, 11 ml of 5 N HCl in dioxane, 1.7 ml of isoamyl nitrite and, after 15 min, 7.7 ml of triethylamine and 2.7 g (5.7 mmole) of phenylalanylprolylglycylproline tert-butyl ester in 30 ml of chloroform were added to a solution of 6.6 g (11.3 mmole) of the tripeptide hydrazide of para. 8 in 30 ml of DMFA cooled to −40°C. The reaction mixture was kept at 0-4°C for 10-12 h and at 20-25°C for 5 h and was then diluted with 30 ml of chloroform and washed successively with water, 0.05 N HCl, and 2% aqueous $NaHCO_3$. After this, the organic layer was dried with anhydrous sodium sulfate and the solvent was evaporated off in vacuum. The yield of product was 5.85 g (~100% of theory).

A solution of 4.1 g of the heptapeptide in 11 ml of chloroform was deposited on a column (3 × 35 cm) containing Kieselgel 60 (Merck, 230-400 mesh) and was eluted with the chloroform–methanol (9.5:0.5) system. Fractions containing the chromatographically homogeneous substance (monitoring by TLC) were collected. The solvents were driven off in vacuum. The yield of product was 3.1 g (76% of theory), R_f 0.31-0.35 (system 2), $[\alpha]_D^{20}$ −72.0° (s 1; MeOH).

10. Preparation of H-MetGluHisPheProGlyProOH. A solution of 3.0 g (2.9 mmole) of the protected heptapeptide of para. 9 in 24 ml of dimethyl sulfide and 12 ml of anisole was treated with 36 ml of trifluoroacetic acid and the mixture was kept at 30°C for 3 h. Then 100 ml of diethyl ether was added and the resulting precipitate was filtered off, washed with 50 ml of ether, and dried in vacuum. The product was dissolved in 30 ml of water and the solution was passed through a column (1 × 50 cm) with EDE-10p resin in the AcO^- form. The column was washed with 20 ml of water and the combined eluates were lyophilized. The yield of product was 2.1 g (88% of theory), $[\alpha]_D^{20}$ −68° (s 0.5; AcOH).

SUMMARY

A procedure has been developed for obtaining a modified ACTH 4-10 heptapeptide which is convenient for the preparation of the substance in relatively large amounts.

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INTERACTION OF THE CHOLINESTERASES OF WARM-BLOODED ANIMALS WITH ESTERS OF N-(β-HYDROXYPROPYL)PIPERIDINES AND THE METHIODIDES

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UDC 542.91:547.1

The influence of esters of N-(β-hydroxypropyl)pyridines (alkyl radicals from CH_3 to C_4H_9) on the enzymatic activity of acetylcholinesterase (ACE) from human blood erythrocytes and on that of butyrylcholinesterase (BCE) from horse blood serum has been studied. At pH 7.5 and 25°C the majority of the piperidine derivatives inhibit reversibly (by the competitive type of inhibition) both ACE and BCE, their inhibiting properties depending only slightly on the presence of the corresponding alkyl radicals in the acid part of the molecule.*

The main types of cholinesterases — acetylcholinesterase (ACE) and butyrylcholinesterase (BCE) — differ from one another not only with respect to their localization in the organism but also with respect to their substrate specificity [1-3].

The substrate specificities of these enzymes have been studied with the use both of natural substrates and of synthetic analogs with different structures [3-5]. Specific substrates of ACE have been detected previously among synthetic analogs of acetylcholine [6, 7]: N-(β-acetoxyethyl)-N-methylmorpholinium and N-(β-acetoxyethyl)-N-methylpiperidinium. Out of the whole multiplicity of esters investigated, only acetyl-β-methylcholine is a specific substrate of ACE, and this is used as one of the main substrates in the study of the catalytic properties and identification of the cholinesterases [1, 8].

*There is considerable confusion in the original between alkyl and acyl radicals, and also in other respects, which it has been possible to resolve with certainty in only one or two cases — Translator.

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Translated from Khimiya Prirodnykh Soedinenii, No. 6, pp. 763-766, November-December, 1986. Original article submitted June 16, 1986.

0009-3130/86/2206-0709$12.50