SYNTHESIS OF A MODIFIED FRAGMENT 13-19 OF THE SEQUENCE OF ACTH USING SILYL PROTECTION

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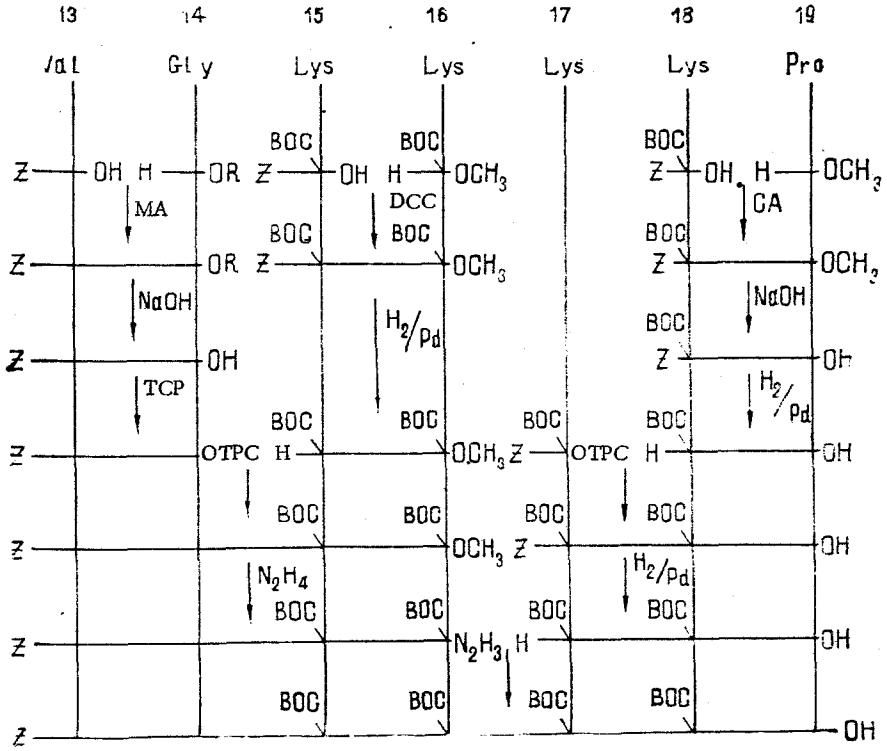
A new scheme for the synthesis of a peptide representing a modified analog of fragments 13-19 of the sequence of the adrenocorticotropic hormone has been described, which uses a number of stages the trimethylsilyl grouping for the protection of the carboxy groups of amino acids and peptides. The final and intermediate products were obtained with high yields and were distinguished by chromatographic homogeneity. ^{13}C NMR was used as a supplementary method for the identification of the compounds. The physicochemical characteristics of the compounds synthesized are given.

One of the common modifications of synthetic adrenocorticotropic hormone ($ACTH_{1-24}$), having the full biological activity of the natural polypeptide, is a product in which the arginine residues in positions 17 and 18 have been replaced by lysine [1, 2]. The synthesis of one of the immediate fragments of this sequence, namely the heptapeptide 13-19, is usually effected by the following scheme (R, CH_3, C_2H_5; BOC, tert-butoxycarbonyl; Z benzyloxycarbonyl; TCP, trichlorophenol; MA, mixed-anhydride method; DCC, dicyclohexylcarbodiimide):



We have developed a more convenient and simple scheme for obtaining such a heptapeptide, which is based on the wide use of the trimethylsilyl group for the temporary protection of

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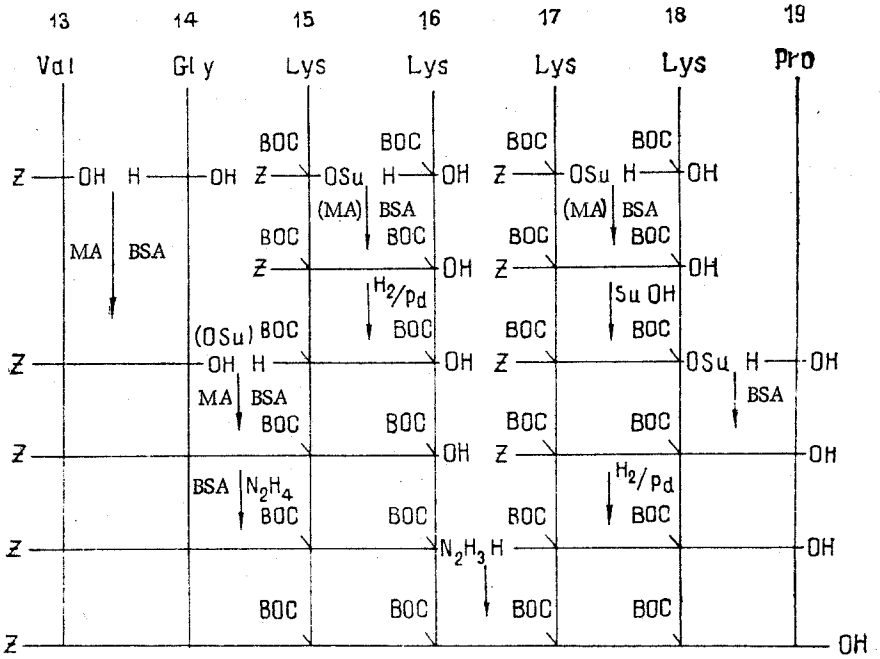
the carboxyl groups of amino acids and peptides during the peptide synthesis (all amino acids in the L- form; BSA, bis(trimethylsilyl)acetamide; SuOH, hydroxysuccinimide):



The silylation of amino acids and peptides was carried out under mild conditions (at room temperature) using bis(trimethylsilyl)acetamide as silylating agent [3]. During the reaction, the initial amino acid or peptide passed into solution. The corresponding silyl derivative obtained was used in peptide condensation without isolation. Of the condensation methods, we used the mixed-anhydride (MA) method, the activated-ester method, and azide condensation. The condensing agent in the case of the MA method was butyl chloroformate. The activated esters were obtained by the usual method with hydroxysuccinimide using dicyclohexylcarbodiimide [4].

In practically all cases of the use of activated esters it is possible to carry out the process by the MA method, but the products obtained are of poorer quality (lowering of the level of chromatographic homogeneity, decrease in the magnitude of the angle of optical rotation). The benzyloxycarbonyl group was removed from the N^{α} atom of lysine by reducing the product with hydrogen over palladium black. The hydrazide of N^{α}-benzyloxycarbonylvalylglycyl-N^{ε}-tert-butoxycarbonyllysyl-N^{ε}-tert-butoxycarbonyllysine was obtained by the hydrazinolysis of the corresponding trimethylsilyl ester of the tetrapeptide with anhydrous hydrazine, by a method that we have developed previously [5]. The heptapeptide and all the intermediate compounds were obtained with high yields and were characterized by chromatographic homogeneity.

Below we give some physicochemical characteristics of the peptides synthesized (the constants of the products obtained did not differ from those of similar compounds described in the literature):

Compound	mp, °C	$[\alpha]_D^{20}$, deg
ZValGlyOH	—	−24*
ZLys(BOC)Lys(BOC)OH	73–75	−2*
HLys(BOC)Lys(BOC)OH	191–195	+17*
ZLys(BOC)Lys(BOC)ProOH	125–127	−23
HLys(BOC)Lys(BOC)ProOH	160–165	−24
ZValGlyLys(BOC)Lys(BOC)OH	125–130	−14.5*
ZValGlyLys(BOC)Lys(BOC)$NHNH_2$	202–205	−14
ZValGlyLys(BOC)Lys(BOC)Lys(BOC)Lys(BOC)ProOH	155–157	−27*

*1% solution in methanol; the others in DMFA.

To confirm the structures and to check the purity of the compounds obtained we used the ^{13}C NMR method. The values of the chemical shifts in the ^{13}C NMR spectra of a solution of

the heptapeptide in hexadeuterodimethyl sulfoxide are as follows (in ppm relative to $(CH_3)_4Si$; the pairs of signals marked by a—a and b—b are alternative assignments):

Amino acid residue	C_0	C_α	C_β	C_γ	C_δ	C_ε
Val-1	171,61[a]	60,52	30,04	18,96 17,90		
Gly-2	168,67 171,43[a]	42,23 52,67				
Lys-3-5	171,27 171,16	52,42	29,11	22,42	31,56[b]	39,88
Lys-6	169,88	50,19	29,11	22,00	30,82[b]	39,88
Pro-7	173,02	58,49	28,47	24,35	46,46	

The values of the chemical shifts of the signals of the carbon nuclei and their assigment in the spectra of the peptides N^α-benzyloxycarbonylvalylglycine and N^α-benzoloxycarbonylvalylglycyl-N^ε-tert-butoxycarbonyllysyl-N^ε-tert-butoxycarbonyllysine have been given previously [6]. The assignment of the signals in the spectrum of the heptapeptide was made on the basis of this paper, [6], taking into account the upfield shift by 1-2 ppm of the signals of the C_o and C_α nuclei of amino acid residues preceding proline in this amino acid sequence.

On comparing the scheme for synthesizing the heptapeptide that has been developed with that known from the literature, it must be observed that the first of the four stages is shorter. Further, in our case there is no necessity for isolating special stages of obtaining the methyl (ethyl) esters and their subsequent hydrolysis. When a tert-butoxycarbonyl or a similar acid-labile grouping is present in the N^ε positon of lysine, the synthesis of the methyl ester is associated with the use of diazomethane, which is a poisonous and explosive substance [7].

EXPERIMENTAL

We used dry freshly-distilled solvents. The melting points were determined in open capillaries without correction, and the angles of optical rotation in a polarimeter. Chromatographic purity and mobility were determined by the TLC method on Silufol plates in the chloroform—methanol system and electrophoretic homogeneity and mobility by paper electrophoresis in a laboratory apparatus in the pyridine—acetic acid—water (1.2:1.0:100) system. The samples for elementary analysis were dried for 10-12 h in a vacuum drying chest over phosphorus pentoxide and potassium hydroxide. The ^{13}C NMR spectra of solutions in hexadeuterodimethyl sulfoxide (c 100 mg/ml) were recorded on a WP-80DS spectrometer with a working frequency of 20.115 MHz. The conditions for recording the spectra and the calculations of the chemical shifts were similar to those given in our previous paper [6].

1. Preparation of ZValGlyOH. Using a method described previously [8], 3.2 g (42.6 mmole) of glycine and 10 g (39.8 mmole) of N^α-benzyloxycarbonylvaline gave 9.8 g of the corresponding dipeptide. Yield 80%; chromatographically homogeneous, R_f 0.24-0.25 (9:1) system.

2. Preparation of ZLys(BOC)Lys(BOC)OH. A. Using the method of Anderson et al. [4], 5.7 g (15 mmole) of N^α-benzyloxycarbonyl-N^ε-tert-butoxycarbonyllysine gave 5 g of the corresponding hydroxysuccinimide ester. Yield 70%; chromatographically homogeneous, R_f 0.68-0.70, (9:1) system.

The ester obtained (4.8 g, 10 mmole) was dissolved in 5 ml of methylene chloride and the solution was added to the trimethylsilyl ester of N^ε-tert-butoxycarbonyllysine obtained from 2.7 g (11 mmole) of the corresponding lysine derivative and 5.7 g (28 mmole) of BSA [8]. The reaction mixture was kept at room temperature for 48 h. Then the reaction products were treated with a 0.1 N solution of hydrochloric acid and with water, and after the organic layer had been dried with sodium sulfate the solvent was evaporated off in vacuum. The residue was triturated with petroleum and was dried in vacuum.

This gave 6 g of the corresponding dipeptide. Yield 98%; chromatographically homogeneous, R_f 0.35-0.36 (9.5:0.5) system.

B. Using butyl chloroformate and the MA method [8], 9 g (23.7 mmole) of N^α-benzyloxycarbonyl-N^ε-tert-butoxycarbonyllysine and 6.7 g (27.2 mmole) of N^ε-tert-butoxycarbonyllysine gave 13.8 g of the corresponding dipeptide. Yield 96%; chromatographically homogeneous, R_f of the main spot 0.44-0.45, R_f of the secondary spot 0.31-0.33, (9:1) system.

3. Preparation of HLys(BOC)Lys(BOC)OH. At 40-45°C, 6.1 g (10 mmole) of N^{α}-benzyloxycarbonyl-N^{ε}-tert-butoxycarbonyllysyl-N^{ε}-tert-butoxycarbonyllysine in 80 ml of ethanol was reduced with hydrogen over palladium black (about 0.5 g), while monitoring by the TLC method. After the reaction, the catalyst was filtered off, the solvent was distilled off in vacuum, and the residue was treated with hot ethyl acetate. This gave 3.9 g of the corresponding product. Yield 83%; electrophoretically homogeneous, E = 111 mm, τ = 2 h, σ = 15 V/cm.

4. Preparation of Zlys(BOC)Lys(BOC)ProOH. By the method of Anderson et al. [4], 1.45 g (2.4 mmole) of N^{α}-benzyloxycarbonyl-N^{ε}-tert-butoxycarbonyllysyl-N^{ε}-tert-butoxycarbonyllysine gave 1 g of the corresponding hydroxysuccinimide ester. Yield 60%; chromatographically homogeneous, R_f 0.54-0.55, (9:1) system.

By the method of paragraph 2, 3.2 g (4.5 mmole) of the hydroxysuccinimide ester and proline trimethylsilyl ether prepared from 0.6 g (5.2 mmole) of proline and 2.6 g (13 mmole) of BSA gave 3.1 g of the corresponding tripeptide. Yield 97%; chromatographically homogeneous, R_f 0.31-0.32, (9:1) system.

5. Preparation of HLys(BOC)Lys(BOC)ProOH. By reduction over palladium black using the method of paragraph 3, 3.1 g (4.4 mmole) of N^{α}-benzyloxycarbonyl-N^{ε}-tert-butoxycarbonyllysyl-N^{ε}-tert-butoxycarbonyllysylproline was converted into 2 g of the corresponding tripeptide. Yield 80%; electrophoretically homogeneous, E=55 mm, τ = 100 min, σ = 15V/cm.

6. Preparation of ZValGlyLys(BOC)Lys(BOC)OH. A. By the method of paragraph 2A, 2.16 g (7 mmole) of N^{α}-benzyloxycarbonylvalylglycine and 3.48 g (7.35 mmole) of N^{ε}-tert-butoxycarbonyllysyl-N^{ε}-tert-butoxycarbonyllysine gave 4.7 g of the corresponding tetrapeptide. Yield 90%; chromatographically homogeneous, R_f 0.66, (4:1) system.

B. By the MA method [8], 0.3 g (1 mmole) of N^{α}-benzyloxycarbonylvalylglycine and 0.5 g (1.1 mmole) of N^{ε}-tert-butoxycarbonyllysyl-N^{ε}-tert-butoxycarbonyllysine gave 0.5 g of the corresponding tetrapeptide. Yield 68%, chromatographically homogeneous, R_f 0.65-0.66, (4:1) system.

7. Preparation of ZValGlyLys(BOC)Lys(BOC)$NHNH_2$. Using a method described previously [5], 1.0 g (1.31 mmole) of N^{α}-benzyloxycarbonylvalylglycyl-N^{ε}-tert-butoxycarbonyllysyl-N^{ε}-tert-butoxycarbonyllysine was converted into the hydrazide of the corresponding tetrapeptide. Yield 98%; chromatographically homogeneous, R_f 0.55, (7:3) system.

8. Preparation of ZValGlyLys(BOC)Lys(BOC)Lys(BOC)Lys(BOC)ProOH. With stirring, 4 ml of 4 N hydrochloric acid and 1.6 ml of a 5 N aqueous solution of sodium nitrite were added to a solution of 3.1 g (4 mmole) of the hydrazide of N^{α}-benzyloxycarbonylvalylglycyl-N^{ε}-tert-butoxycarbonyllysyl-N^{ε}-tert-butoxycarbonyllysine in 35 ml of a mixture of DMFA and isopropanol (4:1) cooled to −(10-15)°C. After being stirred for 30 min, the reaction mixture was treated with 2.4 ml of triethylamine and was poured into water (70 ml) cooled to 0°C. The azide that deposited, after being washed with cold water, was combined with a solution prepared from 2 g (3.5 mmole) of N^{ε}-tert-butoxycarbonyllysyl-N^{ε}-tert-butoxycarbonyllysylproline, 14 ml of DMFA, and 1 ml of triethylamine. The reaction mixture was stirred at 0 to −5°C until the azide had dissolved, it was then kept in the refrigerator for 10-12 h and at room temperature for 12 h. The reaction products were diluted with ethyl acetate and washed with 15% acetic acid and with water. The organic layer, after being dried over sodium sulfate, was evaporated in vacuum to a volume of 5-10 ml and the product was precipitated with an excess of diethyl ether. This gave 3.2 g of the corresponding heptapeptide. Yield 69%; chromatographically homogeneous.

SUMMARY

1. A convenient scheme for the synthesis of a heptapeptide representing fragment 13-19 of a modified analog of the sequence of ACTH has been developed.

2. With this heptapeptide as an example, the efficacy of the use of the silyl protection of the carboxy group in peptide synthesis has been demonstrated.

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N^{α},N^{im}-DI-tert-ALKOXYCARBONYL DERIVATIVES OF HISTIDINE

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The acylation of histidine with di-tert-butyl and di-tert-amyl pyrocarbonates has given the corresponding N^{α},N^{im}-di-tert-alkoxycarbonyl derivatives. The N^{α},N^{im}-di-tert-butoxycarbonyl derivative was obtained in the crystalline form by crystallization from benzene or carbon tetrachloride, or in the form of salts with cyclohexylamine, dicyclohexylamine, and diethylamine. N^{α},N^{im}-di-tert-amyloxycarbonylhistidine was characterized in the form of the salt with dicyclohexylamine.

In recent years, N^{α},N^{im}-di-tert-butoxycarbonylhistidine (di-Boc-histidine) has been finding ever wider use in the synthesis of histidine-containing peptides. It has been shown that this histidine derivative can be used successfully both in the classical variants of peptide synthesis [1-6] and in synthesis on a polymeric support [7-10]. The well-known methods of the selective elimination of N^{α}- or N^{im}-protective groupings are expanding the possibilities of the use of this compound even further [2, 11-13].

Di-Boc-L-histidine was first obtained by E. Schnabel et al. in 1968 [1] by acylating histidine with Boc fluoride. Later, Boc azide [2, 7, 12-14] and Boc chloride [8] were used with less satisfactory results for obtaining this compound. A variant of the synthesis of di-Boc-histidine starting from the benzyl ester of histidine, which was aclyated with Boc azide followed by the removal of the benzyl group by hydrogenation, has also been studied [8]. But, apparently, the most effective reagent for obtaining di-Boc-histidine has proved to be di-tert-butyl pyrocarbonate [15], since under mild conditions, with the minimum consumption of time and of the tert-butoxycarbonylating reagent, the desired product can be obtained in practically quantitative yield. However, in all the investigations mentioned the di-Boc-histidine was obtained either in the form of an oil or in the form of a dry film and was characterized by a low stability on storage.

In the process of studying the conditions for the synthesis of Boc derivatives of trifunctional amino acids using Boc_2O [16] we observed a capacity of di-Boc-histidine for crystallizing from benzene and carbon tetrachloride [17, 18]. Approximately 5% solutions of di-Boc-histidine are the most convenient for obtaining crystalline products. In this case, crystallization sometimes begins at room temperature, but sometimes this requires the solutions to be kept in the refrigerator. On studying the composition of the crystalline products, it was found that, even after being washed with petroleum ether and dried in vacuum, they continued to retain considerable amounts of benzene or carbon tetrachloride. Freshly-prepared samples of crystalline di-Boc-histidine obtained by crystallization from benzene, washed on the filter with petroleum ether, and dried in a vacuum desiccator over $CaCl_2$ for 16 h consist, according to PMR spectroscopy (solutions in deuterochloroform) of a solvate with benzene in a ratio of 1:1. In the IR spectrum, the benzene of crystallization is revealed only by an absorption band at 690 cm^{-1}, while the remaining bands do not appear or are masked. The solvent in the solvated crystal can be detected easily and reliably by gas chromatography (see the experimental part). To determine the amount of the main substance in the solvates it is possible also to use UV spectroscopy, since N^{im}-acylated histidine

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