USE OF PENTAFLUOROPHENYL ESTERS IN THE SYNTHESIS OF INTERMEDIATE FRAGMENTS OF ACTH

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The results are given of the synthesis of the heptapeptide 13-19 of the natural sequence of ACTH using, in a number of stages, the pentafluorophenyl esters of amino acids and peptides in combination with the temporary silyl protection of the carboxy group of the amino components. The intermediate compounds were obtained with good yields (70-90%) in chromatographically homogeneous form and their physicochemical characteristics did not differ from those of similar products obtained by other methods and described in the literature. The identification and checking of the purity of the compounds synthesized was carried out not only by traditional methods but also by the ^{13}C NMR method and by high-pressure liquid chromatography. Some physicochemical characteristics of the compounds synthesized are given.

The linking of amino acids by means of activated esters is one of the widespread methods of synthesis in peptide chemistry [1]. Among the various types of such compounds in recent years ever-increasing attention has been devoted to the pentafluorophenyl esters, which are obtained with the aid of dicyclohexylcarbodiimide, mixed anhydrides, or a complex of pentafluorophenol with dicyclohexylcarbodiimide [2].

It has been shown previously [2] that the hydroxysuccinimide esters, in combination with the temporary silyl protection of the carboxy group of the amino component, can be used extremely effectively in peptide synthesis. The present paper gives the results of the synthesis of heptapeptide 13-19 of the natural sequence of ACTH using pentafluorophenyl esters and temporary silyl protection of the carboxyl of the amino component in a number of stages. The synthesis was performed by the scheme on the next page (all amino acids in the L form; BSA — bis(trimethylsilyl)acetamide; PFP — pentafluorophenyl; DCC — dicyclohexylcarbodiimide; BOC — tert-butoxycarbonyl; Z — benzyloxycarbonyl; TFA — trifluoroacetic acid).

The silylation of the amino acids and peptides was carried out, as previously, under mild conditions using bis(trimethylsilyl)acetamide as silylating agent. The silyl derivative was used without isolation in the peptide condensation.

The pentafluorophenyl esters were obtained by a known method [2]. In view of the process of complex-formation between pentafluorophenol and a carbodiimide, the first was taken in threefold excess in relation to the DCC. The benzyloxycarbonyl group was eliminated from the N^{α} atom of lysine by reduction of the product with hydrogen over palladium black.

Trifluoroacetic acid and a solution of hydrogen chloride in ethyl acetate (EA) were used to eliminate the tert-butoxycarbonyl group from arginine. The methoxy group was eliminated from the peptide by alkaline hydrolysis in methanol.

The intermediate compounds, obtained as a rule, with good yields (70-90% of theory) were chromatographically homogeneous and with respect to their physicochemical characteristics did not differ from similar products described in the literature. The dipeptide arginylproline obtained via the pentafluorophenyl ester proved to be even purer than its analog prepared by the mixed-anhydride method [4].

We must dwell in somewhat more detail only on the last stage — the linkage of the tetrapeptide 13-16 with the tripeptide 17-19. Previously, azide condensation has usually been

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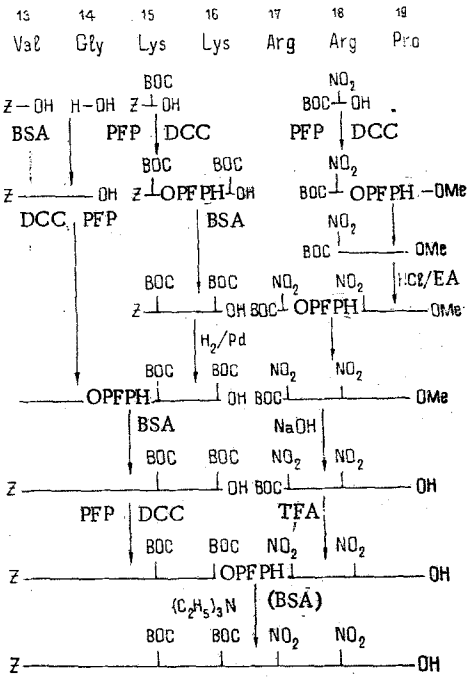

used at this stage by ourselves [3] and others. Under these conditions the product was obtained with a yield of 50-60% and with a comparatively high degree of purity. An attempt to use the pentafluorophenyl ester of the tetrapeptide 13-16 in this stage showed that with the silylated tripeptide 17-19 the corresponding heptapeptide was obtained in very low yield (10-15%). When these fragments were condensed in the presence of triethylamine, the yield of heptapeptide rose to 60-70%, but in this case the product had a relatively low angle of optical rotation (−22.5° as compared with −38° in methanol, according to the literature) and was characterized by chromatographic inhomogeneity. Analysis showed that it contained a considerable amount of unchanged tetrapeptide, even though the second component, the tripeptide 17-19, had been used in the reaction in excess. The investigation performed showed that the azide method is still the best at this stage of condensation. A negative result was also obtained in an attempt to link the pentafluorophenyl ester of N^{α}-nitroarginine with silylated proline. Apparently the inadequate activity of the activated arginine ester and the lowering to some extent of the reactivity of the α-amino group of proline that takes place when the molecule of this amino acid is silylated make it impossible to obtain the corresponding dipeptide. In the case of the methyl ester of proline, as the results presented show, the linking process takes place fairly smoothly.

The investigation performed confirmed that pentafluorophenyl esters, just like hydroxysuccinimide esters, can be used effectively in peptide condensation in association with the temporary silyl protection of the amino component. Below we give some physicochemical characteristics of the products synthesized.

Compound	mp, °C	$[\alpha]_D^{20}$, deg (*c* 1; *MeOH*)
ZLys (BOC) OPFP	70—73	−9,5*
BOCArg (NO_2) OPFP	120—122	−21,0
ZValGly OPFP	179—181	−29,0
ZValGlyLys (BOC) Lys (BOC) OPFP	133—135	− 22,0
HLys (BOC) Lys (BOC) OH	191 195	+17,0
ZVal GlyLys (BOC) Lys (BOC) OH	125—130	−12,0
BOCArg (NO_2) ProOME	68—71	—49,0
HCl·HArg (NO_2) ProOME	162—165	—44,0
BOCArg (NO_2) Arg (NO_2) ProOMe	120—124	—45,0
BOCArg (NO_2) Arg (NO_2) ProOH	120—124	—37,0
TФy·Arg (NO_2) Arg (NO_2) ProOH	245—248	—16,0
ZValGlyLys (BOC) Lys (BOC) Arg (NO_2)—Arg (NO_2) ProOH	—	−22,5

*In ethyl acetate.

The identification of the products obtained and the checking of their purity was carried out not only by TLC and electrophoresis but also by the ^{13}C NMR method and by high-pressure liquid chromatography.

EXPERIMENTAL

Melting points were determined in open capillaries without correction, and angles of optical rotation on a polarimeter. Chromatographic homogeneity was established by the TLC method on Silufol plates in the following solvent systems: 1) chloroform–methanol; 2) pyridine–acetic acid–water–ethyl acetate. Electrophoretic homogeneities and mobilities were determined by paper electrophoresis in a laboratory apparatus in the pyridine–acetic acid–water (1.2:1.0:100) system. The ^{13}C NMR spectra of solutions of the products in hexadeuterodimethyl sulfoxide (c 100 mg/ml) were recorded on a WP-80 DS spectrophotometer 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 previously [5]. The chromatographic analysis of the compounds by high-pressure liquid chromatography was carried out on a Spectra Physics 3500 B instrument under the conditions given elsewhere [6].

1. Preparation of Lys(BOC)OPFP. With stirring, 14.5 g (78.9 mmole of pentafluorophenol and 6 g (28.9 mmole) of DCC were added to a solution of 10 g (26.3 mmole) of N^{α}-benzyloxycarbonyl-N^{ε}-tert-butoxycarbonyllysine in 60 ml of the DMFA–methylene chloride (1:3) system cooled to –5 to 9°C. The reaction mixture was stirred for 2 h and was left in the refrigerator at 0°C for 10-12 h. The urea that had deposited was filtered off on a glass filter, and the solvent was eliminated in vacuum. The crude product was recrystallized from ethyl acetate–hexane (1:3). Yield 90%, chromatographically homogeneous, R_f 0.85-0.90, (1.9:1) system.

2. Preparation of BOCArg(NO)OPFP. By the procedure of paragraph 1, 10 g (30.1 mmole) of N^{α}-tert-butoxycarbonyl-N^{ω}-nitroarginine in DMFA yielded 13.8 g of the pentafluorophenyl ester. The crude product after the elimination of the urea and the solvent was extracted from the reaction mixture with methanol and the solution was treated with ether. Yield 91%. Chromatographically homogeneous; R_{f_1} 0.77-0.78 [(system 1 (8:2)], R_{f_2} (system 2, 18.5 (20:6:11):30).

3. Preparation of ZValGlyOPFP. By the procedure of paragraph 1, 4 g (12.9 mmole) of N-benzyloxycarbonylglycine in the DMFA–methylene chloride (1:1) system yielded 4.2 g (69%) of the corresponding ester. Chromatographically homogeneous, R_{f_1} 0.80-0.81 [system 1 (8.2)]. R_{f_2} 0.95-0.96 (system 2, 9.25 (20:6:11):30).

4. Preparation of ZValGlyLys(BOC)OPFP. By the procedure of paragraph 1, 2 g (2.6 mmole) of N-benzyloxycarbonylvalylglycyl-N^{ε}-tert-butoxycarbonyllysyl-N^{ε}-tert-butoxycarbonyllysine in DMFA yielded 1.8 g of the corresponding ester. The product was purified by recrystallization from EA. Yield 75%; chromatographically homogeneous; R_f 0.55-0.56 [system (9:1)].

5. Preparation of ZValGlyOH. This was prepared from glycine and N-benzyloxycarbonylvaline by the mixed-anhydride method using silyl protection [7]. Yield 85%. Chromatographically homogeneous; R_f 0.24-0.25 [system 1 (9:1)].

6. Preparation of ZLys(BOC)Lys(BOC)OH. A solution of the pentafluorophenyl ester of N^{α}-benzyloxycarbonyl-N^{ε}-tert-butoxycarbonyllysine in methyl chloride was added to the silyl derivative of N^{ε}-tert-butoxycarbonyllysine prepared from 7.25 g (29.5 mmole) of the corresponding lysine derivative and 15.6 g of BSA in methylene chloride [3], and the reaction mixture was kept in a sealed system at room temperature (20-25°C) for 24 h. The reaction products were treated with 60% citric acid and with water, and after the organic layer had been dried with sodium sulfate the solvent was driven off in vacuum. The residue was dissolved in EA and the product was reprecipitated by hexane or petroleum ether. This gave 10.7 g (76%) of the corresponding dipeptide. Chromatographically homogeneous, R_f 0.44-0.45 [system 1 (9:1)].

7. Preparation of HLys(BOC)Lys(BOC)OH. This was prepared from N^{α}-benzyloxycarbonyl-N^{ε}-tert-butoxycarbonyllysyl-N^{ε}-tert-butoxycarbonyllysine by reduction over palladium black [3]. Yield 85%; electrophoretically homogeneous (E = 110 mm, 2 h, σ = 15 V/cm).

8. Preparation of ZValGlyLys(BOC)Lys(BOC)OH. By the procedure of paragraph 6, 3.2 g (6.7 mmole) of the pentafluorophenyl ester of N-benzyloxycarbonylvalylglycine and 3.5 g (7.38 mmole) of N^{ε}-tert-butoxycarbonyllysyl-N^{ε}-tert-butoxycarbonyllysine yielded 3.8 g of the cor-

responding tetrapeptide. The product was purified by trituration with ether. Yield 74%; chromatographically homogeneous; R_f 0.66-0.67 [system 2, 9.25 (20:6:11):20].

9. Preparation of BOCArg(NO_2)ProOMe. A solution of 2.2 g (13.3 mmole) of the hydrochloride of the methyl ester of proline in 15 ml of methylene chloride cooled to −10°C was treated with 1.93 ml of triethylamine and the mixture was stirred for 30 min. The salt that had deposited was separated off on a glass filter and the filtrate was combined with a solution of 5 g (10 mmole) of the pentafluorophenyl ester of N^{α}-tert-butoxycarbonyl-N^{ω}-nitroarginine in 50 ml of DMFA. The reaction mixture was kept at room temperature for 2 h and then the solvent was driven off in vacuum and the residue was dissolved in methylene chloride. This solution was washed successively with 16% citric acid and with water. The organic layer was dried with sodium sulfate and the product was precipitated with an excess of petroleum ether. This gave 3.2 g (75%) of the dipeptide. Chromatographically homogeneous; R_{f1} 0.63-0.65 [system 1 (8:2)], R_{f2} 0.65-0.70 [system 2, 9.25 (20:6:11):30].

10. Preparation of HCl·HArg(NO_2)ProOMe. This was prepared from the methyl ester of N^{α}-tert-butoxycarbonyl-N^{ω}-nitroarginylproline by treating it with a solution of hydrogen chloride in EA [4]. Yield 90%; electrophoretically homogeneous (E = 120 mm, V = 400 volts, I = 10 mA, 4 h, σ = 13 V/cm).

11. Preparation of BOCArg(NO_2)Arg(NO_2)ProOMe. This compound was obtained by the procedure of paragraph 9 from 2.5 g (5.19 mmole) of the pentafluorophenyl ester of N^{α}-tert-butoxycarbonyl-N^{ω}-nitroarginine and 2.1 g (5.17 mmole) of the hydrochloride of the methyl ester of N^{ω}-nitroarginylproline in DMFA. The crude tetrapeptide was precipitated from DMFA with an excess of ether. The final purification of the product was carried out by dissolving it in the butanol—chloroform (1:1) system followed by the washing of the organic layer with 16% citric acid and with water. Then the solution of the product, after drying with sodium sulfate, was concentrated in vacuum and reprecipitated with ether. Yield 70%; chromatographically homogeneous, R_{f_1} 0.52-0.53 [system 1 (8:2); R_{f_2} 0.33-0.34 [system 2, 9.25 (20:6:11): 30].

12. Preparation of BOCArg(NO_2)Arg(NO_2)ProOh. This was prepared from the methyl ester of N^{α}-tert-butoxycarbonyl-N^{ω}-nitroarginyl-N^{ω}-nitroarginylproline by alkaline hydrolysis [4]. Yield 88%, chromatographically homogeneous, R_{f1} [system (1:1)], R_{f2} 0.67-0.68 [system 2, 37 (20:6:11):30].

13. Preparation of TFA·HArg(NO_2)Arg(NO_2)ProOH. This was prepared from N^{α}-tert-butoxycarbonyl-N^{ω}-nitroarginyl-N^{ω}-nitroarginylproline by treating this compound with trifluoroacetic acid [4]. Yield 98%. Electrophoretically homogeneous (E = 110 mm, 4.5 h, σ = 15 V/cm).

14. Preparation of ZValGlyLys(BOC)Lys(BOC)Arg(NO_2)ProOh. A solution of 1.1 g (1.42 mmole) of the trifluoroacetate of N^{ω}-nitroarginyl-N^{ω}-nitroarginylproline and 0.69 ml of triethylamine in 10 ml of DMFA was added to a solution of 1.1 g (1.18 mmole) of the pentafluorophenyl ester of N^{α}-benzyloxycarbonylvalylglycyl-N^{ε}-tert-butoxycarbonyllysyl-N^{ε}-tert-butoxycarbonyllysine in 10 ml of DMFA. The reaction mixture was kept at room temperature for 48 h. The solvent was driven off in vacuum, the residue was dissolved in the butanol-EA (1:4) system, and the solution was washed with 16% citric acid and with water. The organic layer after drying with sodium sulfate was concentrated in vacuum and the product was precipitated with an excess of ether. Yield 60%; chromatographically homogeneous: R_{f1} 0.45-0.46 [system 1 (8:2)]; R_{f2} 0.15-0.16 [system 2, 9.25 (20:6:11):30].

CONCLUSION

1. The heptapeptide 13-19 of the natural sequence of ACTH has been synthesized using the pentafluorophenyl esters of amino acids and peptides in a number of the stages.

2. With the synthesis of the heptapeptide as an example, the possibility has been shown of using pentafluorophenyl esters in combination with the temporary silyl protection of the carboxyl of the amino component in peptide chemistry.

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SYNTHESIS OF FRAGMENT 8-12 OF THE NATURAL SEQUENCE OF ACTH

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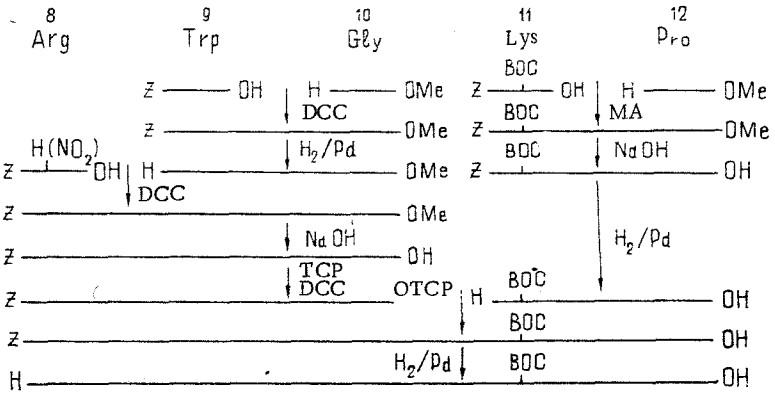
Two new variants of the synthesis of the pentapeptide 8-12 of the natural sequence of ACTH are presented. In some stages, the trimethylsilyl group was used as temporary protection of the carboxy group of the amino component. The final and intermediate compounds were obtained with good yields, were distinguished by chromatographic homogeneity, and were characterized by their angles of optical rotation, melting points, and electrophoretic behavior. Their purity was checked by TLC and by high-pressure liquid chromatography. Some physicochemical characteristics (angles of optical rotation, melting points, chromatographic mobilities) of the compounds obtained are given.

Pentapeptide 8-12 of the natural sequence of ACTH [1] is one of the widely used intermediate fragments in the synthesis of the whole moiety of the adrenocorticotropic hormone.

The total synthesis of this compound was carried out by the following scheme (BOC — tert-butoxycarbonyl; Z — benzyloxycarbonyl; TCP — trichlorophenol; MA — mixed anhydride method; DCC — dicyclohexylcarbodiimide).



We have developed a simpler (three stages shorter) scheme of synthesis of this compound in which it has been possible to avoid the use of DCC — a strong allergen. As the condensing agents we used N-ethoxycarbonyl-1,2-ethoxydihydroquinoline (EEDQ) and ethyl chloroformate. The synthesis was carried out by the scheme* on the next page.

The trityl protection was smoothly removed from the N atom of glycine by acetic acid, and the benzyloxycarbonyl and benzyl groups were eliminated by reduction with hydrogen over palladium black. In addition, we have developed another original variant of the synthesis of a similar compound which predicates the wide use in a number of stages of the trimethylsilyl

*All amino acids in the L form.

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