USE OF 2-ETHOXY-1-ETHOXYCARBONYL-1,2-DIHYDROQUINOLINE AS CONDENSING AGENT IN THE SYNTHESIS OF FRAGMENT 20—24 OF THE SEQUENCE OF ACTH

A. A. Antonov, E. P. Krysin,
V. N. Karel'skii, I. V. Lukashina,
and I. A. Kashirin

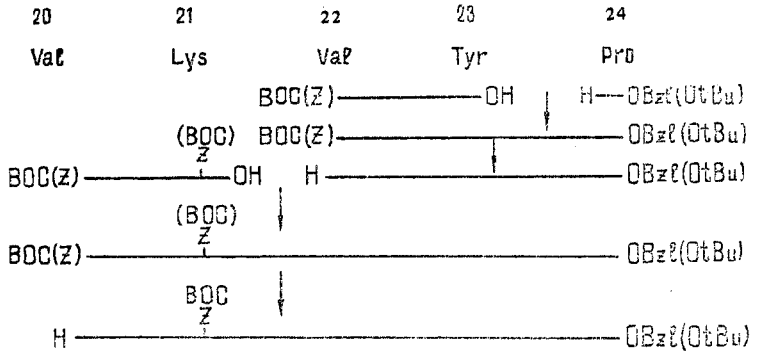
UDC 577.17:546/547.07

Using 2-ethoxy-1-ethoxycarbonyl-1,2-dihydroquinoline as condensing agent, synthesis of intermediate compounds and of the whole fragment 20—24 of the sequence of ACTH has been successfully performed. The products synthesized were obtained with good yields and in fairly high purity. They were characterized by their chromatographic mobilities, angles of optical rotation, melting points, and ^{13}C NMR spectra. The angles of optical rotation, melting points, and chromatographic mobilities of the fragments synthesized are given.

One of the methods of condensation known from the literature but which has not yet found wide use is the preparation of peptides using 2-ethoxy-1-ethoxycarbonyl-1,2-dihydroquinoline [1]. The advantage of this compound in comparison with other condensing agents is, as has been reported by a number of authors [2], the practically complete absence of racemization in the range of temperatures up to 35°C. In addition to this, there is no necessity, as there is, for example, in the azide method of condensation or where mixed anhydrides are used, to isolated the intermediate products.

We have considered the possibility of using 2-ethoxy-1-ethoxycarbonyl-1,2-dihydroquinoline (EEDQ) as a condensing agent in the synthesis of the sequence 20—24 of ACTH. N,N'-Dicyclohexylcarbodiimide (DCC) or the azide method of condensation is usually used for this purpose [3].

The synthesis of fragment 20—24 of the ACTH sequence was effected by the scheme given below (Z represents a benzyloxycarbonyl group, BOC a tert-butoxycarbonyl group, Bzl a benzyl group, and OtBu a tert-butyl group).



As can be seen from the scheme, both the benzyl and the tert-butyl esters of the corresponding pentapeptides were obtained by the successive condensation of the benzyl or tert-butyl ester of proline with the corresponding dipeptides.

The first stage of condensation with the formation of the benzyl ester of a tripeptide took place with a yield of about 40%. However, a considerable amount of the desired product remained in the mother liquor after the recrystallization of the peptide from ethyl acetate and/or acetonitrile that is usually used in this case [4]. When DCC was used, the quality of

All-Union Scientific-Research Institute of the Technology of Blood Substitutes and Hormone Preparations, Moscow. Translated from Khimiya Prirodnykh Soedinenii, No. 1, pp. 78-82, January-February, 1983. Original article submitted March 10, 1982.

0009-3130/83/1901-0074$07.50

the tripeptide obtained was worse (the specific angle of optical rotation was 10° less and the melting point 2-4°C lower). A check of literature figures for [α] showed that the azide method of condensation leads to a pure product in approximately the same yield.

In the preparation of the tert-butyl ester of the tripeptide, the yields of product using the EEDQ and the azide methods of condensation were somewhat higher (45-60%), but as in the case of the benzyl ester of the tripeptide the use of DCC led to a product of inferior quality (specific angle of optical rotation 5-6° lower) and in considerably smaller yield (about 25%).

When EEDQ was used, the stage of condensation with the formation of the benzyl ester of the pentapeptide took place with a high yield (75%) but the yield of the tert-butyl ester of the pentapeptide obtained by the azide method was 20-25% lower than the EEDQ. In all cases of condensation, the quality of the products was the same and corresponded to literature figures.

The results of the investigation performed show that the use of EEDQ in the synthesis of intermediates for fragment 20—24 of the ACTH sequence led to products of the same quality as with the use of the azide method of condensation. The yields of all the compounds with the exception of the tert-butyl ester of the pentapeptide were the same in the two cases. Thus, the use of the method of condensation under consideration for the synthesis of the compounds is preferable, since under otherwise identical conditions it is considerably simpler (there is no necessity for the isolation of an intermediate compound, and the synthesis can be performed at room temperature).

All the peptides synthesized were characterized by their angles of optical rotation, chromatographic mobilities, and melting points. Below, we give the physicochemical characteristics of the compounds synthesized:

Compound	*mp., °C*	$[\alpha]_D^{20}$, *deg*	R_f; *system*
1. BOCValTyrProOBzl	123—125	—64	0,75; 9 : 1,2 twice
2. HValTyrProOBzl	110—115	—36	0,35; 9 : 1
3. BOCValLys(Z)ValTyrProOBzl	—	—68	0,71; 9 : 1,2 twice
4. HValLys(Z)ValTyrProOBzl	90—92	—59	0,52; 8 : 2,2 twice
5. ZValTyrProOtBu	175—176	—69	0,62; 9 : 1,2 twice
6. HValTyrProOtBu	—	—48	0,41; 8 : 2
7. ZValLys(BOC)ValTyrProOtBu	165—170	—70	0,67; 9 : 1,2 twice
8. HValLys(BOC)VAlTyrProOtBu	124—130	—67	0,48; 8 : 2

A method of monitoring peptide synthesis for the products of sequence 20—24 of ACTH using ^{13}C NMR spectroscopy has been developed [5].

EXPERIMENTAL

We used dry freshly-distilled solvents. Melting points were determined in open capillaries without correction, and angles of optical rotation on a polarimeter. Chromatographic mobilities were determined by the TLC method on Silufol plates in chloroform—methanol systems (ratios by volume). Solutions of peptides in organic solvents were dried by filtration through a layer of anhydrous Na_2SO_4 and absorbent cotton in a glass funnel. Solvents were evaporated in vacuum in rotary evaporators.

1. Preparation of BOC-ValTyrPro-OBzl. A. A solution of 3.8 g (10 mmole) of N^{α}-tert-butoxycarbonylvalyltyrosine [5], 2.7 g (11 mmole) of proline benzyl ester hydrochloride [6], and 2.7 g (11 mole) of EEDQ in 50 ml of methylene chloride and 1.7 ml (12 mmole) of triethylamine (TEA) was stirred at 17-20°C for 42 h. Then the reaction mixture was diluted with 20 ml of methylene chloride and was washed with citric acid solution, H_2O, $NaHCO_3$ solution, and H_2O again, and was dried and evaporated. The residue was recrystallized from hot ethyl acetate and it was washed with ethyl acetate and ether and dried in vacuum. The yield of white crystalline substance was 2.3 g (40% of theory), $[\alpha]_D^{20}$ —64° (c 1; CH_3OH); mp 123-125°C.

B. An aqueous solution of 0.724 g of $NaNO_2$ was added to a solution of 3.9 g (10 mmole) of the hydrazide of N^{α}-tert-butoxycarbonylvalyltyrosine [5] in 25 ml of methanol and 1.3 ml of concentrated HCl cooled to —10°C. The mixture was kept at —5°C for 20 min, and then

30 ml of cooled methylene chloride was added and it was washed with H_2O, with Na_2CO_3 solution, and again with H_2O. The organic solution was dried and was added to a solution, cooled to 0°C, of 2.5 g (10 mmole) of proline benzyl ester hydrochloride in 10 ml of methylene chloride and 2 ml (14 mmole) of TEA. The mixture was kept at 0°C for 64 h and at 15-17°C for 24 h. Then it was worked up by the procedure of 1A. The yield, after recrystallization, of white crystalline powder was 2.2 g (39% of theory) $[\alpha]_D^{20} - 63°$ (c 1; CH_3OH), mp 122-125°C.

C. A solution of 2.5 g (10 mmole) of proline benzyl ester hydrochloride cooled to −10°C was treated with 1.4 ml (10 mmole) of TEA, and the precipitate was filtered off. To the resulting solution was added a solution of 3.8 g (10 mmole) of N^{α}-tert-butoxycarbonylvalyltyrosine in 30 ml of methylene chloride and 1.6 g (12 mmole) of hydroxybenzotriazole cooled to −5-0°C, and then 2.1 g (10 mmole) of DCC. The mixture was stirred at 0°C for 17 h and then at 17-20°C for 30 h. The precipitate formed was filtered off and washed with methylene chloride. The solution was treated by the procedure of paragraph 1 A. The yield of white crystalline powder after recrystallization was 2.3 g (40% of theory) $[\alpha]_D^{20} - 56°$ (c 1; CH_3OH); mp 119-122°C.

2. Preparation of H-ValTyrPro-OBzl. A solution of 5.2 g (9 mmole) of the benzyl ester of N^{α}-tert-butoxycarbonylvalyltyrosylproline in 20 ml of trifluoroacetic acid was kept at 15-17°C for 80 min and was then evaporated. The residue was washed with diethyl ether and was dissolved in 20 ml of H_2O. With cooling and stirring, 20 ml of methylene chloride and 3 ml of a 25% solution of NH_4OH to give pH 9 was added. The aqueous solution was additionally extracted with methylene chloride. The organic solution was washed with H_2O, dried, and evaporated. The yield of grayish substance was 3.6 g (85% of theory).

3. Preparation of BOC-ValLys(Z)ValTyrPro-OBzl. With stirring, 1.9 g (7.7 mmole) of EEDQ was added to a solution of 3.4 g (7.3 mmole) of the benzyl ester of valyltyrosylproline and 3.4 g (7.1 mmole) of tert-butoxycarbonylvalyl-N^{α}-benzyloxycarbonyllysine [5] in 15 ml of methylene chloride cooled to −10°C. The mixture was kept at −10°C for 20 h and at −3°C for 100 h. Then it was diluted with 50 ml of chloroform and washed by the procedure of paragraph 1A and then with 50% ethanol. The organic layer was dried and evaporated. After recrystallization from acetonitrile, the yield of white crystalline substance was 4.9 g (75% of theory).

4. Preparation of H-ValLys(Z)ValTyrPro-OBzl. The trifluoroacetate was obtained by the method of paragraph 2 from 3.5 g (3.8 mmole) of the benzyl ester of N^{α}-tert-butyoxycarbonylvalyl-N^{ε}-benzyloxycarbonylvalyltyrosylproline. The product was dissolved in 25 ml of ethyl acetate, 1.3 ml of TEA was added and it was washed with H_2O. The organic solution was dried and evaporated, giving 3.6 g (85% of theory) of a grayish pulverulent substance.

5. Preparation of Z-ValTyrPro-OtBu. A. By the method of paragraph 1A, 3.3 g (8.0 mmole) of N^{α}-benzyloxycarbonylvalyltyrosine [5] and 1.35 g (7.9 mmole) of proline tert-butyl ester [6] gave, after recrystallization from acetonitrile, 19.6 g (44% of theory) of a white crystalline powder, $[\alpha]_D^{20} - 64°$ (c 1; CH_3OH), mp 166−174°C.

B. By the method of paragraph 1B, with standing at 0°C for 7 h and at 17-20°C for 72 h, 1.7 g (3.9 mmole) of the hydrazide of N-benzyloxycarbonyltyrosine [5] and 0.68 g (3.9 mmole) of proline tert-butyl ester yielded a reaction mixture which was evaporated to half-bulk. On cooling to 0°C, the residue deposited a precipitate, which was washed with benzene−petroleum ether (1 : 1). The product was dried in vacuum. The yield of white crystalline powder was 1.0 g (46% of theory), $[\alpha]_D^{20} - 67°$; mp 173−175°C.

The mother liquor, after washing by the procedure of paragraph 1A and recrystallization from ethyl acetate, gave an additional yield of 0.3 g of dry substance (14% of theory), $[\alpha]_D^{20} - 66$, mp 168−174°C.

C. With stirring, 2.74 g of the complex of 1 mole of DCC with 3 moles of pentafluorophenol was added to a solution of 0.62 g (3.6 mmole) of proline tert-butyl ester and 1.5 g (3.6 mmole) of N^{α}-benzyloxycarbonylvalyltyrosine in 25 ml of dimethylformamide cooled to 0°C. The mixture was stirred at −5-0°C for 2.5 h and was then kept in the refrigerator at −0-5°C for 16 h and at 18°C for 6 h. The urea precipitate was filtered off and filtrate was evaporated in vacuum. The residue was recrystallized from ethyl acetate, washed with a mixture of benzene and petroleum ether, and dried in vacuum. The yield of dry substance was 0.5 g (25% of theory), $[\alpha]_D^{20} - 61°$, mp 165−175°C.

6. Preparation of H-ValTyrPro-OtBu. By reduction over a palladium catalyst in 75 ml of methanol 2.5 g (4.9 mmole) of the tert-butyl ester yielded 2.1 g of oily substance (100% of theory).

7. Preparation of Z-ValLys(BOC)ValTyrPro-OtBu. A. By the procedure of paragraph 3, with standing at 0°C for 18 h and at 17-20°C for 72 h, 0.58 g (1.2 mmole) of N^{α}-benzyloxycarbonyl-valyl-N^{ε}-tert-butoxycarbonyllysine [5] and 0.54 g (1.2 mmole) of the tert-butyl ester of valyltyrosylproline yielded, after recrystallization from acetonitrile, 0.42 g of a white pulverulent substance (42% of theory).

B. A solution of 2.3 g (4.7 mmole) of the hydrazide of N^{α}-benzyloxycarbonylvalyl-N^{ε}-tert-butoxycarbonyllysine [5] in 30 ml of dimethylformamide was mixed at −10°C with 3.2 ml of 5 N HCl, the mixture was cooled to −15°C, and a solution of 0.61 g of $NaNO_2$ in 6 ml of H_2O was added. The resulting mixture was kept at −10 to −15°C for 20 min and was poured into 200 ml of a cooled saturated solution of $NaHCO_3$. The precipitate of azide that deposited was dissolved in methylene chloride, and the solution was washed with H_2O, dried, and added at −20°C to a solution of 2.0 g (4.6 mmole) of the tert-butyl ester of valyltyrosylproline. The mixture was kept at −5°C for 72 h, and the precipitate was filtered off. The mother liquor was washed with H_2O, $NaHCO_3$ solution, H_2O, 16% citric acid, and H_2O again, and the organic layer was dried and evaporated. The residue was combined with the precipitate and recrystallized from acetonitrile. This gave 2.8 g (66% of theory) of a yellowish-white powder.

8. Preparation of H-ValLys(BOC)ValTyrPro-OtBu. By the method of paragraph 6, 2.3 g (2.6 mmole) of the tert-butyl ester of N^{α}-benzyloxycarbonylvalyl-N^{ε}-tert-butoxycarbonyllysyl-valyltyrosylproline yielded 1.9 g of a pulverulent substance (95% of theory).

SUMMARY

The possibility has been shown of using 2-ethoxy-1-ethoxycarbonyl-1,2-dihydroquinoline as a effective condensing agent in the synthesis of a number of peptides of sequence 20-24 of ACTH.

LITERATURE CITED

1. D. Belleau and G. Malek, J. Am. Chem. Soc., 90, 1651 (1968).
2. Y. Kiso and H. Yajima, J. Chem. Soc., Chem. Commun., 942 (1972).
3. R. Schwyzer, B. Riniker, and H. Kappeler, Helv. Chim. Acta, 46, 168, 1541 (1963); I. Ramachandran and Ch. H. Li, J. Org. Chem., 28, 173 (1963).
4. GFR Patent No. 1,214,242, cl. C07 c.
5. V. I. Svergun et al., Khim.-farm. Zh., 5, 92 (1981).
6. J. P. Greenstein and M. Winitz, Chemistry of the Amino Acids, Wiley, New York (1961).