USE OF 2-ALKOXY-1-ALKOXYCARBONYL-1,2-DIHYDROQUINOLINES AS CONDENSING AGENTS IN THE SYNTHESIS OF INTERMEDIATE FRAGMENTS OF THE 1—4 SEQUENCE OF ACTH

A. A. Antonov, E. P. Krysin,
V. N. Karel'skii, and L. N. Astashkina

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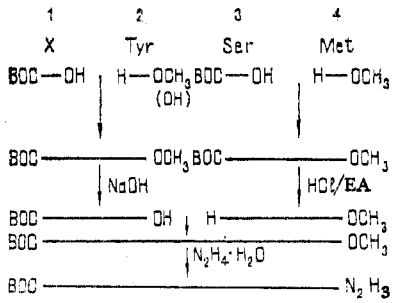
The use of 2-alkoxy-1-alkoxycarbonyl-1,2-dihydroquinolines as a condensing agent in a number of stages in the synthesis of fragments of the 1—4 sequence of ACTH is considered. In a number of cases, these compounds have successfully replaced dicyclohexylcarbodiimide, which is a strong allergen. All the compounds described were obtained in satisfactory yields and had fairly high purities. They were characterized from their angles of optical rotation, chromatographic mobilities, and melting points. The conditions for performing the reaction and some physicochemical characteristics of the compounds synthesized are presented.

One of the promising methods of condensation in the preparation of peptides is by using 2-ethoxy-1-ethoxycarbonyl-1,2-dihydroquinoline [1]. This compound, just like the corresponding isobutyl derivative, has not been used previously in the synthesis of fragments of ACTH, but it is known [2] that the use of these condensing agents makes it possible practically to eliminate racemization in the range of temperatures up to 35°C. Condensation is usually carried out in benzene, tetrahydrofuran, or ethanol, or mixtures of them. The reaction takes place through the state of the formation of an intermediate compound consisting of a carboxylic component which is then converted into a mixed anhydride that reacts with the amino component to form a peptide [1].

In the synthesis of fragments of the ACTH sequence, we have used 2-ethoxy-1-ethoxycarbonyl, 2-butoxy-1-butoxycarbonyl, and 1-butoxycarbonyl-2-ethoxy-1,2-dihydroquinolines. All the compounds were obtained by the reaction of the corresponding alcohol and chloroformic ester with quinoline in the presence of triethylamine by a method similar to that described in the literature [3]. The first compound (EEDQ), unlike the other two (BBDQ and BEDQ), is a white crystalline substance. We have studied the possibility of using these compounds in the synthesis of fragments 1—4 of the ACTH sequence. N,N'-Dicyclohexylcarbodiimide (DDC) or the azide method of condensation is usually used for these purposes [4].

The synthesis was effected by the scheme given below (BOC represents tert-butoxycarbonyl and X represents Ser or β-Ala; all amino acids in the L form).



The synthesis was carried out solely with the use of the 2-alkoxy-1-alkoxycarbonyl-1,2-dihydroquinolines, with the exception of the stage of obtaining the dipeptide 1—2, when β-Ala was present in position 1. In this case, condensation was effected by the mixed-anhy-

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TABLE 1. Influence of the Conditions of Condensation on the Yield of Dipeptide using 2-Alkoxy-1-alkoxycarbonyl-1,2-dihydroquinolines as Condensing Agents

Concentration, M					Solvent				Conditions of condensation		Yield, %	[α], deg
amino acid		condensing acid										
BOC SerOH	HCL·HMet OMe	EEDQ	BEDQ	BBDQ	CH_2Cl_2	MeOH	EtOH	BuOH	T °C	Time, h		
0,33	0,33	0,38	—	—	+	—	—	—	20	48	106	—28
0,38	0,39	0,38	—	—	+	—	—	—	20	72	90	—24
0,45	0,45	0,48	—	—	+	—	—	—	—5	1	118	—21
									20	72		
0,44	0,44	0,48	—	—	+	—	—	—	—5	1	105	—26
									20	50		
0,44	0,44	0,48	—	—	+	—	—	—	20	48	129	—21
0,48	0,48	—	0,57	—	+	+	—	—	20	48	95	—25
0,40	0,42	—	0,48	—	+	+	—	—	20	65	117	—28
0 40	0 42	—	0 48	—	+	+	—	—	20	60	106	—26
0,38	0,39	—	—	0,39	+	+	—	—	—5	1	85	—25
									20	72		
BOCSerOH	HCl·TyrOMe											
0,33	0,33	—	0,40	—	+	—	—	—	—5	0,25	92	—0,5
									20	48		
"	"	—	0,40	—	—	+	—	—	—5	"	95	—0,5
									20			
"	"	0,35	—	—	—	+	—	—	5	"	89	0
									20			
"	"	0,40	—	—	—	+	—	—	—5	"	95	—1,5
									20			
"	"	—	0,40	—	—	—	+	—	—5	"	104,6	—1,0
									20			
"	"	—	0,40	—	—	—	—	+	"	"	104,6	—2,0

dride method using bistrimethylsilylacetamide [5]. The conditions for performing the synthesis are given in Table 1.

As can be seen from Table 1, the condensation reaction took place with quantitative yield (the yields above 100% are due to the presence of impurities of nonpeptide nature in the dipeptides isolated). The choice of solvent and condensing agent had practically no influence on the quality of the product obtained and therefore we subsequently used mainly ethylene chloride as solvent and EEDQ as condensing agent.

The methyl esters of the tetrapeptides 1—4 and β-Ala^1-(1—4) of the ACTH sequence were obtained with overall yields of the order of 40%. The esters were converted into the hydrazides by the usual method. The hydrazides were used in the subsequent synthesis of ACTH.

The results of the investigation performed have shown that the use of 2-alkoxy-1-alkoxycarbonyl-1,2-dihydroquinolines and in particular EEDQ, as condensing agents in the synthesis of intermediate fragments of sequence 1—4 of ACTH has a number of advantages over other methods of condensation — the azide method or the method using DCC. (It is unnecessary to isolate the intermediate compound. The synthesis can be performed at room temperature.) The yields and quality of all the compounds were no worse than with the use of the condensation methods mentioned above.

All the peptides synthesized were characterized with respect to their angles of optical rotation, chromatographic mobilities, and melting points. The physicochemical characteristics of the compounds synthesized are given below:

Compound	mp °C	$[\alpha]_D^{20}$, deg	R_f; system
1. BOCSerTyrOCH$_3$	oil	−1.0	0.58; 9:1.2 twice
2. BOCSerTyrOH	95—100	+16.0	0.33; 8:2
3. BOCSerMetOCH$_3$	oil	−28	0.57; 8:2
4. HCl·HSerMetOCH$_3$	oil	−15	0.18; 8:2
5. BOCSerTyrSerMetOCH$_3$	125—135	−28	0.39; 9:1.2 twice
6. BOCSerTyrSerMetN$_2$H$_3$	185—187	−36	0.27; 8:2
7. BOCβ-AlaTyrOH	147—155	+16 +32	0.46; 8:2
8. BOCβ-AlaTyrSerMetOCH$_3$	120—125	−14	0.29; 9:1.2 twice
9. BOCβ-AlaTyrSerMetN$_2$H$_3$	180—188	−20	0.49; 8:2

EXPERIMENTAL

Freshly distilled solvents were used. 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 the peptides in organic solvents were dried by filtration through a layer of anhydrous Na_2SO_4 and absorbent cotton in a glass funnel. The solvents were evaporated in vacuum in rotary evaporators.

1. Preparation of BOC-SerTyr-OCH$_3$. A solution of 4.6 g (20 mmole) of the hydrochloride of tyrosine methyl ester in a mixture of methylene chloride and ethanol (2 : 1) containing 3.0 ml (22 mmole) of triethylamine (TEA) was treated with 4.1 g (20 mmole) of N^{α}-tert-butoxycarbonylserine. The solution was cooled to −5°C, and 6.6 g (24 mmole) of EEDQ was added. The mixture was stirred at −5°C for 1 h and then at 17-20°C for 140 h. The reaction mixture was diluted in 100 ml of methylene chloride and was washed with a 1 N aqueous solution of HCl, with H_2O, with a mixture of saturated $NaHCO_3$ solution, CH_3OH and H_2O in a ratio of 1 : 2 : 2, and with 50% aqueous methanol (all the aqueous solutions were saturated with NaCl). After washing, the organic solution was dried and evaporated. The yield of oily product was 7.6 g (100% of theory).

2. Preparation of BOC-SerTyr-OH. A solution of 2.4 g (60 mmole) of NaOH in 6.5 ml of H_2O was slowly added to a solution of 7.6 g (20 mmole) of the methyl ester of tert-butoxycarbonylseryltyrosine in 26 ml of methanol. The mixture was stirred at 17-20°C for 1.5 h. Then it was diluted with 12 ml of H_2O and washed with methylene chloride. The aqueous solution was acidified at 0-5°C with 1 N HCl to pH 3 and was extracted with ethyl acetate. The organic solution was washed with H_2O, dried and evaporated. The yield of white crystalline powder was 6.6 g (90% of theory).

3. Preparation of BOC-SerMet-OCH$_3$. From 4.0 g (20 mmole) of the hydrochloride of methionine methyl ester, 2.9 g (21 mmole) of TEA and 4.1 g (20 mmole) of N^{α}-tert-butoxycarbonylserine, with the addition of 5.4 g (21.8 mmole) of EEDQ, using the method of paragraph 1 with stirring at 20°C for 48 h, a reaction mixture was obtained which was washed successively with 0.1 N HCl, H_2O, 5% $NaHCO_3$ solution, 30% aqueous methanol, and H_2O. The organic solution was dried and evaporated in a rotary evaporator. The yield of yellow oily product was 7.0 g (100% of theory).

4. Preparation of HCl · HSerMet-OCH$_3$. With stirring, 40 ml (88 mmole of HCl) of a solution of HCl in ethyl acetate was added to a solution of 7.0 g (18 mmole) of the methyl ester of tert-butoxycarbonylserylmethionine in 4 ml of ethyl acetate. The mixture was kept at 17-20°C for 30 min. The ethyl acetate solution was decanted off, and residual oily product was washed with ethyl acetate. The solvent was evaporated off and the residue was dried in vacuum to constant weight. The yield of oily product was 4.6 g (95% of theory).

5. Preparation of BOC-SerTyrSerMet-OCH$_3$. A solution of 6.8 g (23.7 mmole) of the hydrochloride of the methyl ester of serylmethionine in 90 ml of ethanol was treated with 3.3 ml (24.0 mmole) of TEA and 7.7 g (20.9 mmole) of N^{α}-tert-butoxycarbonylseryltyrosine. The solution was cooled to −5°C and 5.8 g (23.4 mmole) of EEDQ was added. The reaction mixture was kept at 17-20°C for 100 h and was then diluted with 180 ml of methylene chloride and washed with H_2O, $NaHCO_3$ solution, H_2O, 16% citric acid, and H_2O again (all the aqueous solutions were saturated with NaCl). The organic solution was dried and evaporated. The dry oily residue was triturated with diethyl ether and dried in a vacuum drying chest. The yield of white or yellowish crystalline substance was 5.0 g (40% of theory).

6. Preparation of BOC-SerTyrSerMet-N$_2$H$_3$. With stirring at 5-10°C, 5 ml (100 mmole) of hydrazine hydrate was added to a solution of 5.3 g (8.8 mmole) of the methyl ester of N^{α}-tert-

butoxycarbonylseryltyrosylserylmethionine in 50 ml of methanol. The mixture was kept for 90 h. The white precipitate that had deposited was filtered off, washed with cold methanol, dried in vacuum, and treated with hot H_2O, and the mixture was cooled. The product was filtered off and dried to constant weight. The yield of white crystalline powder was 3.4 g (64% of theory).

7. Preparation of BOC-β-AlaTyr-OH. At −10 to −15°C, the silyl derivative of tyrosine obtained by the reaction of 2.0 g (11 mmole) of tyrosine in 10 ml of methylene chloride with 5.6 g (28 mmole) of bistrimethylsilylacetamide was added to the mixed anhydride obtained at −10 to −15°C by the reaction of a solution of 1.9 g (10 mmole) of N^{α}-tert-butoxycarbonyl-β-alanine in 11 ml of methylene chloride and 1.45 ml (11.5 mmole) of TEA with 1.04 ml (11 mole) of ethyl chloroformate. The reaction mixture was stirred at −10 to −15°C for 2 h and at 16°C for 48 h and was then evaporated. The oily solid substance was mixed with 25 ml of saturated $NaHCO_3$ and 20 ml of H_2O. The mixture was stirred for 1.5 h and was washed with methylene chloride. At −5 to 0°C, the pH of the aqueous solution was brought to 5 by the addition of 5 N HCl and it was washed with methylene chloride, and then the pH of the aqueous solution was brought to 2. The product was extracted with ethyl acetate. The organic solution was washed with saturated NaCl solution, dried, and evaporated. The yield of yellowish solid was 2.7 g (77% of theory).

8. Preparation of BOC-β-AlaTyrSerMet-OCH_3. By the method of paragraph 5, 3.5 g (10 mmole) of N^{α}-tert-butoxycarbonyl-β-alanyltyrosine and 2.9 g (10 mmole) of the hydrochloride of the methyl ester of serylmethionine in 22 ml of methylene chloride in the presence of 1.45 ml (10.5 mmole) of TEA and 2.7 ml (11 mmole) of EEDQ yielded 3.5 g (60% of theory) of a dry pulverulent substance.

9. Preparation of BOC-β-AlaTyrSerMet-N_2H_3. A solution of 2.9 g (5 mmole) of the methyl ester of N^{α}-tert-butoxycarbonyl-β-alanyltyrosylserylmethionine in 7 ml of methanol and 2.5 ml (50 mmole) of hydrazine hydrate was stirred at 17-20°C for 72 h. The resulting hydrazide was filtered off and washed with methanol. The product was dried in vacuum to constant weight. The weight of the white powder was 2.3 g (80% of theory).

SUMMARY

The promising nature of the use of quinoline derivatives as condensing agents in the synthesis of fragments of the 1−4 sequence of ACTH has been shown.

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