LITERATURE CITED

1. M. Funatsu, G. Funatsu, M. Ishiguro, S. Nanno, and K. Hara, Proc. Jpn. Acad., 47, 713 (1971).
2. T. Mise, G. Funatsu, M. Ishiguro, and M. Funatsu, Agr. Biol. Chem., 41, No. 10, 2041 (1977).
3. M. Ishiguro, M. Tomi, G. Funatsu, and M. Funatsu, Toxicon, 14, No. 3, 157 (1976).
4. D. A. Khasimov, Kh. G. Alimov, and P. Kh. Yuldashev, Khim. Prir. Soedin., 883 (1987).
5. G. Funatsu, T. Mise, M. Matsuda, and M. Funatsu, Agr. Biol. Chem., 42, No. 4, 851 (1978).
6. D. A. Khasimov, Kh. G. Alimov, and P. Kh. Yuldashev, Khim. Prir. Soedin., 887 (1987).
7. T. Devenyi and J. Gergely, Amino Acids, Peptides, and Proteins, Elsevier, New York (1974) [Russian translation, Moscow (1976), p. 284].
8. P. Edman, Arch. Biochem., 22, 475 (1949).
9. R. Chen, Z. Physiol. Chem., 357, No. 6, 873 (1976).
10. In: Protein Sequence Determination, Springer, New York, Vol. VIII (1970), p. 208.
11. G. F. Denisova and O. L. Polyanovskii, Biokhimiya, 39, No. 2, 401 (1974).
12. W. Konisberg and J. R. Hill, J. Biol. Chem., 237, No. 8, 2547 (1962).
13. C. H. Li and J. S. Dixon, Arch. Biochem. Biophys., 146, 233 (1971).
14. M. Wallis, FEBS Lett., 44, No. 2, 205 (1974).
15. Yu. A. Pankov and N. A. Yudaev, Biokhimiya, 37, 991 (1972).
16. A. V. Muranov, T. A. Muranova, and L. F. Markova, Bioorg, Khim., 4, No. 3, 293 (1978).
17. J. L. Bailey, Techniques in Protein Chemistry, Elsevier, Amsterdam (1962) [Russian translation, Moscow (1965), p. 27].
18. Yu. A. Ovchinnikov, A. A. Kiryushkin, I. A. Egorov, N. G. Abdullaev, A. P. Kiselev, and N. N. Modyanov, Biokhimiya, 37, No. 3, 452 (1972).
19. T. Y. Liu and V. N. Chang, J. Biol. Chem., 246, 2842 (1971).

USE OF THE SILYLATION REACTION IN SYNTHESIS OF FRAGMENT 1-4 OF THE ACTH SEQUENCE

A. K. Rabinovich and E. P. Krysin

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A new effective method of synthesizing fragment 1-4 of the ACTH sequence ensuring a high overall yield of the desired product is proposed. This result is achieved thanks to the wide use of the silylation reaction in the synthesis, which has permitted a considerable simplification of the process and the avoidance of the formation of by-products. The peptides synthesized have been characterized by their angles of optical rotation, chromatographic mobilities, and melting points. A table of chemical shifts in the ^{13}C NMR spectra of the final and intermediate compounds is given.

In recent years, the silylation reaction has found ever wider employment in organic synthesis [1]. Its use considerably simplifies the synthesis, since it permits the reaction to be performed with free amino acids. Such an approach eliminates the stage of obtaining esters of the amino acids and their hydrolysis in an alkaline medium. Furthermore, the trimethylsilyl group ensures the protection of the free hydroxy groups of amino acids [2] and, which is of no little importance, is easily eliminated during the usual acid-alkali washings.

Known methods of obtaining fragment 1-4 of the sequence of ACTH are, as a rule, either multistage, since they involve the preparation of amino acid derivatives with protected side chains [3, 4] or, where amino acid derivatives with unprotected side chains are used, lead to the desired product with a low overall yield of the order of 20% [5, 6].

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TABLE 1. Properties of the Peptides (I-VI) Obtained

Peptide	Yield, %	mp, °C	$[\alpha]_D^{20}$, deg	R_f (system)	Literature
I	92	94—97	+16	0,45 (2), 0,39 (5)	—
II	92	110—115	−3	0,72 (5), 0,65 (6), 0,58 (1; 2 times)	[8]
III	90	64—65	−30	0,57 (2), 0,54 (6)	[7]
IV	96	—	−16	0,18 (2), 0,27 (4), 0,75 (3)	—
V	90	125—130	−29	0,67 (5), 0,39 (1; 2 times), 0,43 (6)	[7]
VI	85	185—190	−36*	0,16 (4), 0,36 (5)	[8]

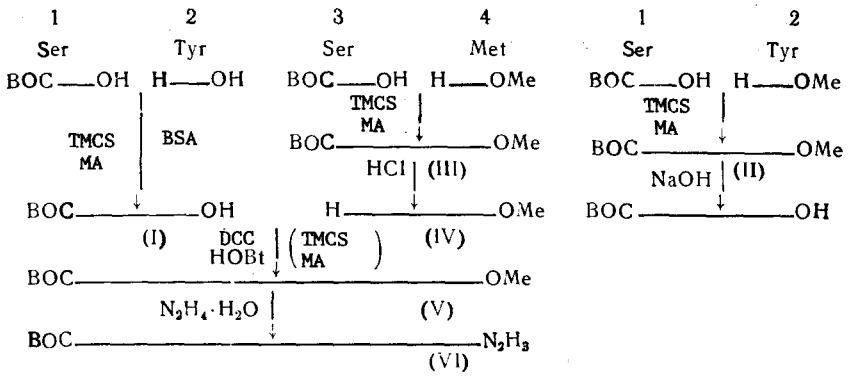
*1% solution in 50% MeOH solution; the others in MeOH.

TABLE 2. Chemical Shifts in the ^{13}C NMR Spectra of Peptides (I), (III), (V), and (VI) in DMSO-d_6 Relative to $(CH_3)_4Si$

	Nucleus	I	III	V	VI
Ser	C_0	170,19	—	170,39	170,36
	C_α	57,07	—	56,91	56,94
	C_β	61,76	—	61,85	61,88
Tyr	C_0	172,60	—	171,16	171,14
	C_α	53,49	—	54,23	54,19
	C_β	36,18	—	36,55	36,51
	C_γ	127,26	—	127,51	127,58
	$2C_\delta$	130,13	—	130,14	130,17
	$2C_\varepsilon$	115,05	—	115,00	114,95
	C_ρ	156,00	—	155,91	155,82
Ser	C_0	—	170,60	170,05	170,10
	C_α	—	56,91	55,03	55,03
	C_β	—	61,69	61,60	61,63
Met	C_0	—	171,97	171,80	169,99
	C_α	—	50,87	51,01	50,83
	C_β	—	30,81	30,80	31,79
	C_γ	—	29,38	29,47	29,44
	C_ε	—	14,48	14,50	14,46
BOC	C=O	155,20	155,11	155,40	155,34
	$-C-O$	78,37	78,35	78,44	78,75
	CH_3	28,08	28,03	28,13	28,19

A method of preparation with a high yield of fragment 1-4 using Trt protection is known [10], but because of its lability this considerably complicates the purification of the reaction products.

We have developed a simple scheme for the synthesis of fragment 1-4 of ACTh in which the silylation reaction and the method of mixed anhydrides (MAs) have been successfully employed. As the silylating agents we used bis(trimethylsilyl)acetamide (BSA) and trimethylchlorosilane (TMCS). In the case of MAs, the condensing agent was ethyl chloroformate. In the stage of obtaining the tetrapeptide (III), the DCC/HOBt method proved to be the most

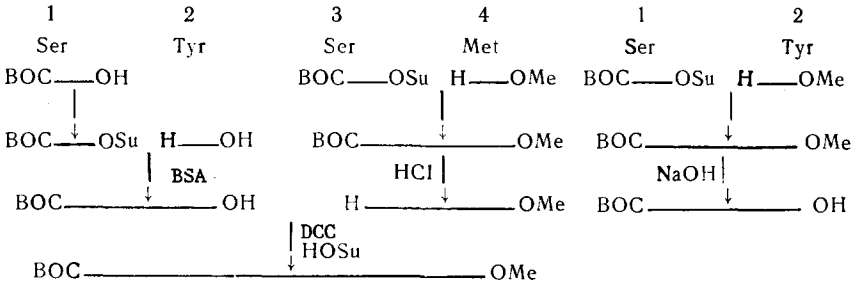


effective. It must be mentioned that the scheme of the synthesis was worked out on charges of several tens of grams, and the desired product was obtained with an overall yield of 60%.

The peptides synthesized were characterized by their angles of optical rotation, chromatographic mobilities, and melting points (Table 1) .

To identify and monitor the purity of the compounds obtained we also used the ^{13}C NMR method. It is important that the spectra of all the compounds lacked the signals corresponding to the trimethylsilyl grouping (Table 2).

In addition, the synthesis of tetrapeptide 1-4 of the ACTK sequence was also carried out by the use of the method of activated esters based on HOSu which permits the side chains of serine and tyrosine to be left unprotected during the synthesis and enables desired products of good quality with a low degree of racemization to be obtained [9]. This method has not previously been used in the synthesis of fragment 1-4 of ACTH and was of interest from a methodological point of view:



Chromatographically and optically pure dipeptides were obtained by this scheme. However, condensation of the fragments Boc-Ser-Tyr-OH and H-Ser-Met-OMe did not give the desired results. The end-product contained a number of impurities and its yield was low.

EXPERIMENTAL

Dry freshly distilled solvents were used.

Chromatographic purity and mobility were determined on silufol plates. The substances were detected on the chromatograms by treatment with a solution of p-toluidine after the exposure of the plates to chlorine in a chamber. The following solvent systems were used (ratios by volume): 1) $CHCl_3$–MeOH (9:1); 2) (8:2); 3) pyridine–AcOH–H_2O (20:6:11):EtOAc (37:30); 4) (18.5:30); 5) (9.25:30); 6) (1:30).

Melting points were determined in open capillaries without correction, and angles of optical rotation on a EPO VNIIEKIProdmash instrument (Moscow).

1. Preparation of BOC-Ser-OSu. By the procedure of [9], 4.1 g (20 mmole) of BOC-Ser-OH gave 4.8 g (16 mmole) of the corresponding N-hydroxysuccinimide ester. Yield 80%: R_f 0.41 (system 1); 0.66 (system 6).

2. Preparation of BOC-Ser-Met-OMe (III). A. At −10°C, 15.1 g (50 mmole) of BOC-Ser-OSu was added to a solution of 11 g (55 mmole) of H-Met-OMe·HCl in 60 ml of CH_2Cl_2 and 7.6 ml (55 mmole) of Et_3N, and the mixture was kept at 5°C for 5-6 h and 20°C for 12 h. The reaction products were treated with 0.1 N HCl, H_2O, 5% $NaHCO_3$ solution, and H_2O again. The organic layer was dried with Na_2SO_4 and evaporated. The residue was recrystallized from diethyl ether. This gave 11.3 g of a crystalline substance. Yield 65%, R_f 0.57 (system 2); 0.54 (system 6): $|\alpha|_D^{20}$ − 28° (c 1; MeOH). mp 80-82°C.

B. With stirring, 16.5 ml (130 mmole) of trimethylchlorosilane (TMCS) and 18 ml (130 mmole) of EtCN were added to a solution of 24 g (117 mmole) of BOC-Ser-OH in 300 ml of CH_2Cl_2 with 16.8 ml (121 mmole) of Et_3N, and after the reaction mixture had been cooled to −30°C, 12 ml (126 mmole) of ethyl chloroformate was added. The resulting mixture was kept at −20°C for 20 min, and then to it was added a solution, cooled to −20°C, of 25.2 g (126 mmole) of HCl·H-Met-OMe in 240 ml of $CHCl_3$ that had previously been treated successively with 17.4 ml (126 mmole) of Et_3N, 33 ml (260 mmole) of TMCS, and 36 ml (260 mmole) of Et_3N. The reaction mixture was stirred at 0-5°C for 16 h and was treated with 0.5 N HCl, Na_2CO_3, and H_2O. The organic layer was dried with Na_2SO_4 and evaporated. The product, which had crystallized in the form of disk-shaped crystals, was triturated with hexane. This gave 37 g of a white powder (Table 1).

3. Preparation of H-Ser-Met-OMe·HCl (IV). A solution of 29 g (83 mmole) of BOC-Ser-Met-OMe in 50 ml of ethyl acetate (EA) was treated with 63 ml of a solution of HCl in EA (c 0.189 g/ml) cooled to −30°C, and the mixture was kept at room temperature for 30 min. Then it was cooled and the product was precipitated with 150 ml of ether and was then washed with EA and dried to constant weight. Yield 22.8 g (Table 1).

4. Preparation of BOC-Ser-Tyr-OMe (II). A. At −10°C, 15.1 g (50 mmole) of BOC-Ser-OSu was added to a solution of 12.7 g (55 mmole) of H-Tyr-OMe·HCl in 60 ml of CH_2Cl_2 containing 7.6 ml (55 mmole) of Et_3N, and the reaction mixture was kept at 5°C for 3 h and at 20°C for 8 h. The reaction products were treated (all the washing solutions being prepared on a basis of saturated NaCl solution) with 0.1 N HCl, $NaHCO_3$, and saturated NaCl solution. The organic layer was dried with Na_2SO_4 and evaporated, and the residue with reprecipitated with petroleum ether from diethyl ether. This gave 12.7 g of product. Yield 67%; R_f 0.58 (system 1; twice); 0.72 (system 5); 0.65 (system 6) $[\alpha]_D^{20}$ − 3° (c 1; MeOH). mp 109-111°C.

B. To 9.2 g (40 mmole) of HCl·H-Tyr-OMe in 80 ml of $CHCl_3$ were added successively, 5.6 ml (40 mmole) of Et_3N, 10 ml (80 mmole) of TMCS, and 11.2 ml (80 mmole) of Et_3N, the mixture was stirred at −10 to −20°C for 30 min, and a solution of the corresponding mixed anhydride prepared by the method of paragraph 2B from 8 g (40 mmole) of BOC-Ser-OH, 11.2 ml (80 mmole) of Et_3N, 5 ml (40 mmole) of TMCS, and 4 ml (42 mmole) of ethyl chloroformate was added. The reaction mixture was kept at −10°C for 2 h and at +5°C for 16 h and was treated with solutions (based on NaCl) of 1 N HCl, Na_2CO_3, and 0.1 N HCl. The organic layer was evaporated and the residue was reprecipitated with hexane from ether. Yield 14 g (Table 1).

5. Preparation of BOC-Ser-Tyr-OH (I). A. By the method of Antonov et al. [6], 30 g (80 mmole) of BOC-Ser-Tyr-OMe gave 24.6 g of product. Yield 85%: R_f 0.31 (system 1; twice; 0.45 (system 2); 0.39 (system 5); $[\alpha]_D^{20}$+ 17° (c 1; MeOH). mp 94-97°C.

B. At 35°C, 1.8 g (10 mmole) of H-Tyr-OH was silyated in 5 ml of CH_2Cl_2 with 7.2 ml (30 mmole) of bis(trimethylsilyl)acetamide (BSA) for 24 h. The resulting solution was treated with 3.1 g (10 mmole) of BOC-Ser-OSu in 15 ml of CH_2Cl_2, and the mixture was kept at 20°C for 18 h. Then it was stirred with 60 ml of saturated $NaHCO_3$ solution for 2 h and was washed with diethyl ether and with ethyl acetate. The aqueous layer was treated with 1 N HCl to pH 3, the product was extracted with ethyl acetate, and the solvent was evaporated off in vacuum. This gave 3.1 g of a crystalline substance. Yield 84%; the R_f and $[\alpha]_D^{20}$ values were similar to those for the product obtained in paragraph 5A.

C. A solution of 14.5 g (80 mmole) of H-Tyr-OH in 60 ml of CH_2Cl_2 containing 60 ml of BSA, cooled to −30°C, was added to a solution, cooled to −20°C, of the mixed anhydride prepared by the method of paragraph 2B from 16 g (80 mmole) of BOC-Ser-OH, 23.2 ml (168 mmole) of Et_3N, 11 ml (88 mmole) of TMCS, and 8 ml (84 mmole) of ethyl chloroformate. The reaction mixture was kept at 0 to +5°C for 16 h, the solvent was evaporated off in vacuum, and the residue was stirred in a 4:1 (by volume) mixture of aqueous Na_2CO_3 solution and MeOH for 0.5 h, and, after being washed with CH_2Cl_2, the aqueous layer was acidified with 2 N HCl to pH 3 and the product was extracted with ethyl acetate. The solvent was eliminated in vacuum. This gave 27 g of the desired product (Table 1).

6. Preparation of BOC-Ser-Tyr-Ser-Met-OMe (V). A. A solution of 3.68 g (10 mmole) of BOC-Ser-Tyr-OH, 1.21 g (10.5 mmole) of HOSu, and 2.16 g (10.5 mmole) of dicyclohexylcarbodimide (DCC) in 15 ml of CH_2Cl_2 containing 2 ml of DMFA was kept at 5°C for 12 h, the precipitate of dicyclohexylurea was filtered off, and the filtrate was added to a solution of 3 g (10.5 mmole) of H-Ser-Met-OMe·HCl in 10 ml of CH_2Cl_2 containing 2 ml of Et_3N. The reaction mixture was kept at 20°C for 12 h and was washed with saturated NaCl solution, saturated $NaHCO_3$ solution, 0.2 N HCl to pH 3-4, and saturated NaCl solution again. The organic layer was dried with sodium sulfate and evaporated, and the residue was treated with diethyl ether. This gave 3.8 g of product. Yield 63%; R_f of the main substance 0.39 (system 1; twice); 0.43 (system 6); 0.67 (system 5); R_f of an impurity 0.44 (system 1; twice), 0.74 (system 5); 0.55 (system 6); $[\alpha]_D^{20}$ − 18° (c 1; MeOH). mp 82-86°C.

B. To 14.56 (39 mmole) of BOC-Ser-Tyr-OH in 140 ml of $CHCl_3$ were added, successively, 15.7 ml (123 mmole) of TMCS and 17.3 ml (125 mmole) of Et_3N, the mixture was cooled to −20°C, 5.6 ml (40 mmole) of Et_3N and 4 ml (42 mmole) of ethyl chloroformate were added, the new mixture was kept −15°C for 20 min, and a solution, cooled to −20°C, was added that had been prepared by the successive addition to 12.3 g (43 mmole) of H-Ser-Met-OMe·HCl in 100 ml of $CHCl_3$ (−20°C) of 5.9 ml (43 mmole) of Et_3N, 10.9 ml (86 mmole) of TMCS, and 11.9 ml (86 mmole)

of Et_3N. The reaction mixture was kept at −10°C for 1 h and at 0°C for 16 h, and, after the addition of 60 ml of CH_2Cl_2 and 60 ml of EtOH, it was treated with Na_2CO_3 (saturated)–H_2O (2:1 by volume), NaCl (saturated), 1 N HCl to pH 3, and NaCl (saturated) again. The organic layer was dried with Na_2SO_4 and evaporated in vacuum. Yield 14.1 g (60% of theory), R_f 0.67 (system 5); 0.39 (system 1; twice); 0.43 (system 6); $[\alpha]_D^{20}$ − 29° (c 1; MeOH). mp 105°C.

C. A solution of 19.5 g (68 mmole) of H-Ser-Met-OMe·HCl in 40 ml of CH_2Cl_2 containing 10 ml of DMFA was treated with Et_3N to pH 7, and another 9.4 ml (68 mmole) of Et_3N was added, the mixture was cooled, and the precipitate of Et_3N·HCl was filtered off. The filtrate was added to a solution of 25 g (68 mmole) of BOC-Ser-Tyr-OH and 9.6 g (71 mmole) of HOBt in 80 ml of CH_2Cl_2 containing 40 ml of DMFA, the mixture was cooled to −20°C, and, with stirring, 14.6 g (71 mmole) of DCC was added in portions. The reaction mixture was kept at 0°C for 18 h, the precipitate of dicyclohexylurea that had appeared was filtered off, and, after the addition of 400 ml of CH_2Cl_2, the filtrate was washed successively with 1 N HCl–NaCl (saturated) (1:1), H_2O–NaCl (saturated) (1:1), H_2O–Na_2CO_3 (saturated) (1:1), 0.5 N HCl–NaCl (saturated), and NaCl (saturated). The organic layer was dried with Na_2SO_4 and evaporated, and the residue was treated with ether. This gave 36.7 g of product (Table 1).

7. Preparation of BOC-Ser-Tyr-Ser-Met-N_2H_3 (VI). A solution of 36 g (60 mmole) of BOC-Ser-Tyr-Ser-Met-OMe in 400 ml of EtOH was treated with 35 ml of $N_2H_4·H_2O$, and the mixture was left at room temperature for 18 h. The precipitate that had deposited was filtered off, washed with EtOH, and dried in vacuum. This gave 30.6 g of product (Table 1).

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SUMMARY

A convenient method of synthesizing fragment 1-4 of ACTH with an overall yield of 60% has been developed.

The efficiency of the use of the silylation reaction in the preparation of peptides with hydroxyl-containing amino acids by the mixed-anhydride method and by the method of activated esters based on HOSu has been shown.

LITERATURE CITED

1. S. M. Andreev, V. P. Kozyukov, and N. V. Mironova, The Use of Silyl Protection in Peptide Synthesis: Review Information Series Organometallic Compounds and Their Use [in Russian], NIITEKhim [Scientific-Research Institute of Technical and Economic Investigations of the USSR Ministry of the Chemical Industry], Moscow, 1980.
2. E. Schroeder and K. Lubke, The Peptides, Academic Press, New York (1965) [Russian translation, Moscow (1967), p. 84].
3. K. Inouye and H. Otsuka, Bull. Chem. Soc. Jpn., 34, No. 1, 1 (1961).
4. A. T. Gudkov, O. V. Gudkova, and Yu. V. Mitin, Dep. VINITI [Paper deposited in the All-Union Institute of Scientific and Technical Information] December 29, 1973, No. 17534273.
5. B. Iselin and R. Schwyzer, Helv. Chim. Acta, 44, 173 (1961).
6. A. A. Antonov, E. P. Krysin, V. N. Karel'skii, and L. N. Astashkina, Khim. Prir. Soedin., 74 (1983).
7. B. Iselin, H. Kappeler, and B. Riniker, FRG Patent Application No. 1543502. Cl C07c, Published December 23, 1971.
8. H. Otsuka, K. Inouye, and K. Shinozaki, Bull. Chem. Soc. Jpn., 39, p. 6, 1171 (1966).
9. G. Anderson and J. Zimmerman, J. Am. Chem. Soc., 86, No. 9. 1839 (1964).
10. R. Boissonas, S. Guttman, and J. Pless, Belgian Patent No. 636667. Cl. C07c. Published February 27, 1964.