USE OF TRIMETYLSILYL PROTECTION IN THE SYNTHESIS OF OXYTOCIN FRAGMENTS

A. K. Ivanov, A. A. Antonov, and I. A. Donetskii

UDC 542.91:547.964.4

The possibility has been studied of obtaining silyl derivatives of amino acids forming components of oxytocin and of obtaining the corresponding peptides from them by the mixed-anhydride method.

Recently, the silylation reaction has come into greater and greater use in peptide synthesis [1, 2]. Its use permits the reaction to be performed with free amino acids, which in a number of cases, considerably simplifies the scheme of synthesis. However, judging from the literature, trimethylsilyl protection has not yet found use in the synthesis of the neurohypophyseal hormones. The advantage of its use is that, as a rule, in the silylation process the free amino acids pass into solution with the retention of the capacity of the amino group for forming the peptide bond.

In order to determine the possibility of the use of this protective group in the synthesis of oxytocin, we have, for the first time, obtained a number of fragments by the mixed-anhydride (MA) method using the silyl protection of amino acids and peptides. The silylation reaction was carried out at room temperature in methylene chloride or dimethylformamide (DMFA). The completion of transformation was judged from the dissolution of the silylated amino acid or peptide. As the silylating agent we used commercial trimethylchlorosilane (TMCS) or bis(trimethylsilyl)acetamide (BSA). Condensation by the MA method was conducted with the use of butyl chloroformate (BCF) or pivaloyl chloride (PC).

We have shown that such amino acids as tyrosine, isoleucine, proline, leucine and glycine (all amino acids of the L-series) are rapidly (in the course of a few minutes) silylated by TMCS, and, on condensation with carboxylic components, give dipeptides with good yields. On the other hand, on treatment with BSA, glutamine, and asparagine scarcely pass into solution, while with TMCS multicenter unreactive complexes soluble in aprotic solvents are formed [1], and these are decomposed when an equivalent of triethylamine, which is necessary for freeing the N^{α}-amino group from its binding with hydrogen chloride, is added to the reaction mixture.

Benzyl, benzamidomethyl, and pyrrolidonomethyl derivatives of cysteine are not silylated completely by TMS; on the other hand, methyl and benzyl derivatives pass into solution rapidly, although the yields of the dipeptides BOCAsnCys(X)OH obtained from them by the MA method do not exceed 30%. An exception is the triphenylmethyl derivative of cysteine, which is well silylated by BSA, while the yield of dipeptide of the given type from it amounts to about 75%. We have previously described the synthesis of the tripeptide BOCCys(Bzl)TyrIleOH by the MA method using temporary trimethylsilyl protection of the dipeptide HTyrIleOH [3].

All the compounds obtained were chromatographically homogeneous and were characterized by their angles of optical rotation and melting points (Table 1). We used ^{13}C NMR spectroscopy to confirm the structures of the products and to check their purity. We have successfully used products synthesized with the use of temporary trimethylsilyl protection in the synthesis of the amide of the C-terminal tetrapeptide 6-9 [4, 5] and also in the synthesis of tripeptide 1-3 of the oxytocin sequence [3]. The method permits the use, in synthesis, of free amino acids (HTyrOH, HIleOH, HProOH, and HLeuOH) and also of cysteine protected only in the mercapto function. In a number of cases it is possible to avoid the formation of by-products of the synthesis, as, for example in the preparation BocProLeuOH [5].

All-Union Scientific-Research Institute of the Technology of Blood Substitutes and Hormone Preparations, Moscow. Translated from Khimiya Prirodynykh Soedinenii, No. 1, pp. 111-114, January-February, 1993. Original article submitted February 20, 1992.

0009-3130/93/2901-0095 $12.50

TABLE 1. Physicochemical Properties of the Peptides Obtained

Peptide	Silylating agent	Yield, %	mp, °C	Specific optical rotation		Chromatographic mobility		Literature
				$[\alpha]_D^{20}$, deg	solvent	R_f	system	
BOCCys(Bzl)TyrOH	TMCS	98.0	oil	− 6.0	MeOH	0.61	S_4	3
BOCCys(Trit)TyrOH	BSA	92.0	102	+ 29.0	DMFA	0.31	S_2	− *
BOCTyrIleOH	TMCS	58.5	oil	+ 1.2	DMFA	0.66	S_4	− *
BOCAsnCys(Trit)OH	BSA	75.0	99-100	+ 5.0	DMFA	0.19	S_4	− *
BOCCys(Me)ProOH	TMCS	86.0	oil	− 29.0	DMFA	0.36	S_4	4
BOCCys(Bzm)ProOH	TMCS	78.0	oil	− 30.0	AcOH	0.35	S_4	4
BOCCys(Bzl)ProOH	TMCS	76.0	82-85	− 62.0	MeOH	0.54	S_4	5
BOCCys(Pym)ProOH	TMCS	76.0	oil	− 79.5	AcOH	0.10	S_4	4
BOCCys(Bz)ProOH	TMCS	67.5	oil	− 39.5	DMFA	0.45	S_4	4
BOCCys(Trit)ProOH	TMCS	53.0	−	+ 28.5	DMFA	0.72	S_4	4
BOCProLeuOH	TMCS	97.0	98-101	− 25.5	DMFA	0.64	S_3	5

* See the Experimental Part.

EXPERIMENTAL

In view of the low hydrolytic stability of the trimethylsilyl derivatives, the reactions were carried out under conditions excluding the access of moisture. Melting points were determined in open capillaries without correction, and angles of optical rotation on a VNIIEKI-Prodmash [All-Union Scientific-Research and Experimental Design Institute for the Construction of Food Machinery, Moscow] polarimeter.

Chromatographic purities and mobilities were determined on Silufol plates (Czechoslovakia). The substances were detected by treating the plates with a 1 N solution of acetic acid containing 0.02% of orthotoluidine and 0.1% of KI after the exposure of the plates in a chamber containing chlorine. The following eluting solutions were used (ratios by volume), pyridine–acetic acid–water–ethyl acetate (10:3:5.5:30) (S_2); (10:3:5.5:60) (S_3); and (10:3:5.5:120) (S_4).

Preparation of BOCCys(Trit)TyrOH. With stirring, a solution of 8.0 g (44 mmole) of Tyrosine and 34 ml (139 mmole) of BSA in 34 ml of methylene chloride (MC) that had previously been stirred in a thermostated cabinet at 35 ±5°C until the amino acid had dissolved completely (3-4 days) was added with stirring at 17 ±5°C over 12 min to the mixed anhydride obtained from 20.4 g (44 mmole) of BOCCys(Trit)OH, 6.15 ml (44 mmole) of triethylamine (TEA), 3.5 ml (44 mmole) of pyridine (Py), and 5.4 ml (44 mmole) of PC in 80 ml of MC.

The reaction mixture was stirred at − 5 ±2°C for 2 h and was left at 2 ±2°C for 18 h, after which 30 ml of MC was added to it and it was washed successively with 0.1 N HCl (4 × 100 ml), H_2O (3 × 100 ml), 4% $NaHCO_3$ solution (100 ml), and H_2O (2 × 100 ml) and was dried with anhydrous Na_2SO_4. The solution was evaporated in vacuum. The oily residue was reprecipitated with 150 ml of hexane from 50 ml of ether. The viscous oil was dried in vacuum at 40°C to constant weight (yield 25.3 g, see Table 1).

Preparation of BOCTyrIleOH. A cooled solution of 2.6 g (20 mmole) of isoleucine, 2.8 ml (22 mmole) of DMCS, and 3.0 ml (22 mmole) of TEA in 20 ml of DMFA was added with stirring over 12 min to the mixed anhydride obtained from 2.8 g (10 mmole) of BOCTyrIleOH, 1.5 ml (11 mmole) of TEA, and 1.4 ml (11 mmole) of DMFA. The reaction mixture was stirred at −10 ± 2°C for 1 h and was left at 2±2°C for 18 h after which 100 ml of 1 N HCl was added. The product was extracted with 50 ml of ether, which was then driven off in vacuum. The product was twice reprecipitated with hexane from ether. Yield 2.3 g (see Table 1).

Preparation of BOCAsnCys(Trit)OH. A solution cooled to −10 ±2°C of 26.1 g (72 mmole) of S-tritylcysteine and 35.1 ml (134 mmole) of BSA in 50 ml of DMFA was added at 10 ±2°C with stirring over 15-20 min to the mixed anhydride obtained from 15.3 g (66 mmole) of BOCAsnOH, 10.1 ml (72 mmole) of TEA, 5.8 ml (72 mmole) of Pyr, and 8.8 ml (72 mmole) of PC in 80 ml of DMFA. The reaction mixture was stirred at −10 ±2°C for 1 h and was left at 2°C for 18 h,

after which 400 ml of MC was added and it was washed successively with 0.1 N HCl (4 × 100 ml), and H_2O (4 × 100 ml) and was dried with anhydrous Na_2SO_4. The solution was evaporated in vacuum. The oily residue was dissolved in 100 ml of ethyl acetate, and the solution was kept at 2 ± 2°C for 48 h. The crystalline precipitate was washed with hexane and dried in vacuum at 40°C to constant weight. This gave 28.6 g of a dry white powder (see Table 1).

LITERATURE CITED

1. M. V. Kashutina, S. L. Ioffe, and V. A. Tartakovskii, Usp. Khim., 44, 1620 (1975).
2. S. M. Andreev, V. P. Kozyukov, and N. V. Mironova, The Use of Silyl Protection in Peptide Synthesis. Review Information of the Series Organometallic Compounds and Their Use [in Russian], NIITÉKhim [Scientific-Research Institute of Technical and Economic Investigations of the Ministry of the Chemical Industry], Moscow (1980).
3. A. K. Ivanov, V. N. Karel'skii, E. P. Krysin, E. É. Lavut, I. É. Zel'tser, and A. A. Antonov, Khim. Prir. Soedin., No. 1, 116 (1989).
4. A. K. Ivanov, A. A. Antonov, and I. A. Donetskii, Khim. Prir. Soedin., Nos. 3-4, 393 (1992).
5. A. K. Ivanov, E. É. Grigor'eva, A. A. Antonov, and I. A. Donetskii, Khim. Prir. Soedin., No. 304, 400 (1992).

SYNTHESIS OF THE C-TERMINAL HEXA- AND HEPTAPEPTIDE SEQUENCES OF OXYTOCIN

A. K. Ivanov, E. É. Grigor'eva, A. A. Antonov, and I. A. Donetskii

UDC 542.91:543.42.547.964.4

Three schemes for the synthesis of the heptapeptide 3-9 of the oxytocin sequence with different protective groups of the thiol function of the cysteine and the use of two main methods of condensation (the activated-ester method and the mixed-anhydride method) are considered.

Variants of the synthesis of the C-terminal tri- and tetrapeptides of the oxytocin sequence have been considered previously [1, 2]. In order to develop the optimum scheme for the synthesis of oxytocin, we have tried out variants of the synthesis of the heptapeptide starting from the C-terminal tripeptide: a 2 + (2 + 3) and a 2 + (1 + 4) scheme, and stepwise synthesis using the C-terminal tetrapeptide as the starting material.

Table 1 gives the properties of pentapeptides of the 5-9 sequence of oxytocin obtained with the use of activated pentafluorophenyl (PFPE) or paranitrophenyl (PNPE) esters, and also by the mixed-anhydride (MA) method using pivaloyl chloride by a 1 + 4 scheme. As can be seen from Table 1, the highest yield of pentapeptide was observed on the use of a PFPE and the lowest on the use of the MA method. However, if it is borne in mind that to obtain a PFPE or a PMPE a separate stage of synthesis using a strong allergen — DCHC — must be performed, the MA method at this stage has definite advantages.

Table 2 gives the properties of the 4-9 hexapeptides of the oxytocin sequence obtained by a 1 + (1 + 4) scheme. A similar situation with a change in the yield of hexapeptide on the use of the same method of condensation as in the synthesis of the peptapeptide was observed. Furthermore, with the introduction into the peptide molecule of such amino acids as asparagine and glutamine the hydrophilic properties of the compounds rose, which led to a considerable fall in the yield of certain products. Thus, when Pym protection of the thiol function of cysteine was used it was impossible to obtain a hexapeptide in satisfactory yield (∿ 15%). Thanks to its increased solubility in the majority of solvents used for the purification of peptides (water, DMSO, DMFA, THF, acetone, alcohols, ether), it was impossible to isolate this peptide in the pure form.

Attempts to obtain the 3-9 heptapeptide of the oxytocin sequence starting from the hexapeptide by the azide method of condensation and also by the use of MA (pivaloyl chloride)

All-Union Scientific-Research Institute of the Technology of Blood Substitutes and Hormone Preparations, Moscow.

Translated from Khimiya Prirodnykh Soedinenii, No. 1, pp. 114-121, January-February, 1993. Original article submitted February 2, 1992.

0009-3130/93/2901-0097 $12.50