SYNTHESIS OF THE N-TERMINAL TRIPEPTIDE OF THE OXYTOCIN SEQUENCE

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Two schemes for the synthesis of the hydrazide of the N-terminal tripeptide of the oxytocin sequence are considered. It is shown that the most rational is a 1 + 2 scheme.

The N-terminal tripeptide of the oxytocin sequence is a basic product in the synthesis of this hormone by the 3 + 6 scheme using the azide method (Az) [1] or the mixed-anhydride method (MA) [2]. In order to synthesize the nonapeptide of oxytocin by various methods, a search has been made for the optimum method of obtaining BOCCys(Bzl)Tyr-IleN$_2$H$_3$ (IV). Two schemes of synthesis were tried: 2 + 1 and 1 + 2 (Fig. 1).

The tripeptide was obtained by the 2 + 1 scheme using the azide method. The dipeptide derivative (I) was obtained by the MA method, since condensation using dicyclohexylcarbodiimide (DCHC) has no advantages at this stage, and to perform the synthesis by the activated p-nitrophenyl ester method (AE) requires an additional stage of the synthesis of the ester derivatives of cysteine, while the condensation reaction takes place to completion only with a one-and-a-half- to two-fold excess of the activated ester (Table 1).

In the 1 + 2 scheme, the tripeptide was obtained by the MA method with a yield of 93%. The benzyloxycarbonylated dipeptide (V) was obtained by the activated succinimidyl method without the isolation of the activated water, and also by the MA method with the use of 2-ethoxy-N-ethoxycarbonyl-1,2-dihydroquinoline (EEDQ). The yields of the tert-butoxycarbonyl derivatives of the dipeptide (VI) were similar (see Table 1).

The overall yield of the hydrazide (IV) obtained by the 2 + 1 scheme was about 18% on the initial tyrosine derivative, and by the 1 + 2 scheme it was about 50%. The chromatographic purities and the physicochemical properties of the dipeptide hydrazide obtained by these schemes were identical, and therefore we used the 1 + 2 scheme to develop a method for the synthesis of oxytocin.

The preparation of the tripeptide (X) with a free carboxy group by hydrolyzing the methyl ester (III) was accompanied by the formation of racemates and by-products because of the reversible β-elimination of cysteine derivatives [3] observed at alkaline pH values. We therefore performed the synthesis of the tripeptide (X) by both the 2 + 1 and 1 + 2 schemes using temporary trimethylsilyl protection of the carboxy group, but in neither case was it possible to isolate a tripeptide of adequate purity in good yield. This is connected mainly with the fact that the desired products (X) and the initial BOCCys(Bzl)TyrOH (XI) or BOCCys(Bzl)OH have practically identical solubilities in aqueous and organic media which, with yields of about 50%, interferes with their isolation by the ordinary extraction methods.

The dipeptide (XI) was obtained with quantitative yield by the MA method using the silylation reaction and was freed from unchanged initial cysteine derivative by extraction with ether–hexane (1:4) from aqueous methanolic solution. Synthesis of the tripeptide (X) from this by the 2 + 1 scheme took place with a yield of less then 50%. The desired product contained the initial dipeptide (XI) as impurity. The synthesis of the tripeptide (III) by the 2 + 1 scheme using the dipeptide (XI) (condensation methods: MA, AE, EEDQ) likewise did not lead to satisfactory results, since either the yield of product was too low, as in the MA case (less than 20%) or it was difficult to isolate the pure peptide (AE).

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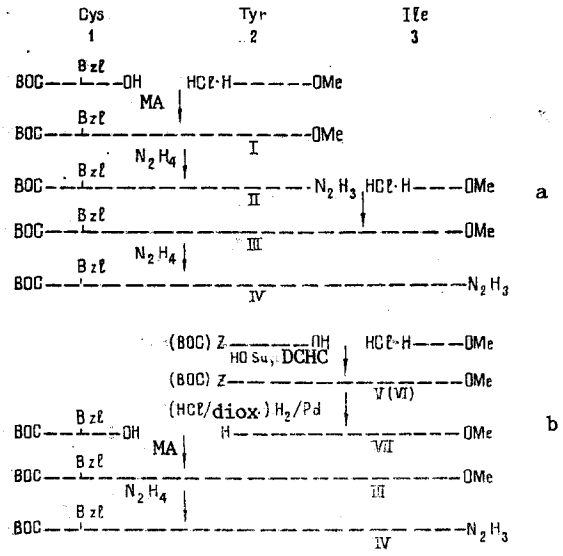

Fig. 1. Scheme of the synthesis of the hydrazide of the N-terminal tripeptide of the oxytocin sequence: a) 2 + 1 scheme; b) 1 + 2 scheme.

TABLE 1. Physicochemical Properties of the Compounds Obtained

Peptide	Method of preparation	Yield, %	mp., °C	Angle of optical rotation, deg		Chromatographic mobility	
				$[\alpha]_D^{20}$	solvent	R_f	system
I	MA	87	103—105	—18	MeOH	0,85	1
	AE	100					
II	—	74	181—184	—21	MeOH	0,73	1
III	AZ	45**	126—128	—24	MeOH	0,89	1
	MA	92*					
		72*	225—234	—25	DMFA	0,67	1
IV	—	61**					
	EEDQ	84					
V	DCHC, SuOH	87	Oil	—4,5	MeOH	0,82	1
	MA	80					
VI	DCHC, SuOH	87	Oil	—9,5	DMFA	0,87	1
VII	—	87	80—84	+1,0	DMFA	0,31	2
VIII	—	85	79—83	+1,0	DMFA	0,64	1
IX	—	87	142—144	+9,0	DMFA	0,34	3
X	MA, silyl	76	120—123	—18,0	DMFA	0,75	1
XI	MA, silyl	98	Oil	—6,0	MeOH	0,61	1
XII	—	93	142—145	—3,5	DMFA	0,62	1

*1 + 2 scheme.
**2 + 1 scheme.

It was impossible to isolate BOCTyrIleOH (VIII) or ZTyrIleOH (XII) obtained by the MA method using the silylation reaction. Peptides (VIII) and (XII) were obtained by the hydrolysis of the corresponding peptides (V) and (VI). After the deblocking of the α-amino group, the dipeptide HTyrIleOH (IX) was used for obtaining the tripeptide (X) by the 1 + 2 scheme using the MA method with temporary silyl protection of the hydroxy group of the tyrosine and of the carboxy group of the dipeptide (IX). To obtain the tripeptide (X) without traces of the initial BOCCys(Bzl)OH, a one-and-a-half-fold excess of the dipeptide (IX) was necessary. The yield of tripeptide at this stage was 76% on the cysteine derivative and about 50% on the peptide (IX). The overall yield of the tripeptide (X) with a free carboxy group by the 1 + 2 scheme (Fig. 2) was about 30%.

All the compounds obtained were chromatographically homogeneous and were characterized by their angles of optical rotation and melting points (see Table 1). To confirm the

TABLE 2. Chemical Shifts in the ^{13}C NMR Spectra of Solutions of Peptides (I), (II), (IV), (VIII), and (X) in DMSO-d_6 Relative to TMS*

Residue	Nucleus	I	II	IV	VIII	X
Cys	C_0	170,6	170,2[a]	170,4		170,4
	C_α	53,8	54,1	54,2		54,2
	C_β	33,6	33,8	33,7		33,8
Tyr	C_0	171,7	170,1[a]	170,0[b]	171,9	170,9
	C_α	53,8	53,0	53,9	56,2	53,7
	C_β	36,0	37,5	**[c]	36,6[d]	36,6[e]
	C_γ	126,8	127,5	127,5	128,2	127,5
	2 C_δ	130,0	130,2	130,3	130,2	130,3
	2 C_ε	115,1	115,0	114,9	114,9	114,9
	C_ζ	156,1	155,9	155,9	155,8	155,9
Ile	C_0			170,4[b]	172,9	172,8
	C_α			55,5	56,2	56,5
	C_β			36,8[c]	36,8[d]	36,9[e]
	C_{γ_1}			24,5	24,7	24,8
	C_{γ_2}			15,3	15,5	15,6
	C_δ			11,0	11,3	11,3
BOC	C=O	155,2	155,3	155,3	155,3	155,2
	—C—	78,4	78,6	78,5	78,2	78,5
	CH_3	28,1	28,3	28,2	28,1	28,2
Bzl	CH_2	35,4	35,4	35,4		35,5
	C_1	138,5	138,5	138,5		138,5
	C_2	128,9	129,0	129,0		128,9
	C_3	128,4	128,4	128,4		128,4
	C_4	126,8	126,9	126,9		126,9
OMe	CH_3	51,8				

*For each of the pairs of signals marked a—a, b-b, c-c, d-d, and e-e, the opposite assignment is possible.
**Signal not observed because of overlapping with a signal of the solvent.

structures and check the purities of the final and intermediate peptides we used the method of ^{13}C NMR spectroscopy. Table 2 gives the chemical shifts of compounds (I), (II), (IV), (VIII), and (X). The assignment was made on the basis of literature information [4-6] and of the nature of the splitting in the "gated decoupling" spectra, and also by comparing the values of the chemical shifts in the series of compounds under consideration.

EXPERIMENTAL

The melting points of the peptides were determined in open capillaries without correction, and the angles of optical rotation on a polarimeter.

The chromatographic purities and mobilities of the peptides obtained were determined by the TLC method on Silufol plates in the following systems (ratios by volume); 1) EtOAc—pyridine—AcOH—H_2O (60:5:1.5:2.75); 2) 30:5:1.5:2.75; 3) 30:10:3:5.5.

The ^{13}C NMR spectra of solutions of the peptides (c 100 mg/ml) in DMSO-d_6 were recorded on a WP-80 DS spectrometer (FRG) with a working frequency of 20.115 MHz. Conditions for the recording of the spectra with complete suppression of spin-spin coupling with protons: volume of the memory in the accumulation of the spectrum 8 K; in reproduction, 4 K; machine resolution 0.9 Hz (0.045 ppm); pulse length 2 μs (25°); pulse interval 1.1 s. The chemical shifts were reckoned from the signal of the solvent and converted into the δ-scale relative

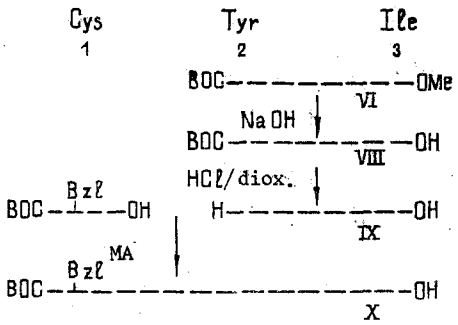

Fig. 2. Scheme of the synthesis of the N-terminal tripeptide of the oxytocin sequence with a free carboxy group.

to tetramethylsilane (PMS) by means of the formula $\delta_{TMS} = \delta_{DMSO\text{-}d_6} + 39.6$ ppm. Commercial DMSO-d_6 without the preliminary elimination of water was used to prepare the solutions.

Preparation of BOCCys(Bzl)TyrOMe (I). With stirring, a solution of HTyrOMe in 25 ml of chloroform (Chlf), obtained from 4.7 g (20.3 mmole) of HCl·HTyrOMe and 3.1 ml (22.5 mmole) of triethylamine (TEA), was added over 12 min at −10 ± 2°C to the mixed anhydride obtained from 6.2 g (19.9 mmole) of BOCCys(Bzl)OH, 2.8 ml (20.4 mmole) of TEA, and 1.9 ml (20.5 mole) of ethyl chloroformate in 25 ml of ethylene chloride (MC). The reaction mixture was stirred at −2 ± 2°C for 2 h and was then left at 0°C for 18 h, after which 30 ml of MC was added and it was washed with 1 N HCl (3 × 20 ml), H_2O (2 × 20 ml), 8% $NaHCO_3$ solution (3 × 20 ml), and H_2O (2 × 20 ml), and was dried with anhydrous Na_2SO_4. The solution was evaporated in vacuum and the oily residue was reprecipitated with hexane from ether, filtered off, washed with hexane, and dried in vacuum at 40°C. This gave 8.4 g (17.2 mole) of (I) (Tables 1 and 2).

Preparation of BOCCys(Bzl)TyrN$_2$H$_3$ (II). A solution of 8.4 g (17.2 mmole) of the dipeptide (I) in 50 ml of MeOH was treated with 50 ml of hydrazine hydrate. The solution was stirred at room temperature until a dense precipitate had formed and was then kept at 0°C for 18 h. The precipitate was filtered off and was washed with H_2O (4 × 100 ml). The residue was dried in vacuum at 40°C. This gave 6.2 h (12.8 mmole) of (II) (Tables 1 and 2).

Preparation of (Z)BOCTyrIleOMe (V, VI). A. With vigorous stirring, a solution of HIleOMe obtained after the filtration of a mixture of 4.0 g (22.0 mmole) of HCl·HIleOMe and 3.1 ml (22.5 mmole) of TEA in 20 ml of MC, was added to a suspension of 5.6 g (20.0 mmole) of BOCTyrOH and 2.0 g (17.4 mmole) of N-hydroxysuccinimide (HOSu) in 20 ml of MC. The reaction mixture was cooled to −20°C, and, with stirring, 4.1 g (20.0 mmole) of DCHC was added, after which the mixture was kept at 0°C for three days and was then filtered and extracted with 1 N HCl (3 × 10 ml), 30% MeOH (2 × 10 ml), H_2O (2 × 10 ml), 8% $NaHCO_3$ solution (3 × 10 ml), and H_2O (2 × 10 ml). The organic solution was dried with anhydrous Na_2SO_4 and evaporated in vacuum, and the residue was dried in vacuum at 40°C. This gave 7.1 g (17.4 mmole) of the oily substance (VI) (Tables 1 and 2).

Similarly, 7.7 g (17.4 mmole) of oily substance (V) (Table 1) was obtained from 6.3 g (20.0 mmole) of ZTyrOH.

B. With stirring, 2.8 ml (20.4 mmole) of TEA, 3.6 g (20.0 mole) of HCl·HIleOMe, and 6 g (24.3 mmole) of EEDQ were added to a solution of 6.3 g (20.0 mmole) of ZTyrOH in 40 ml of MC. The solution was kept at room temperature for three days and was worked up as in paragraph A. This gave 7.4 g (16.7 mmole) of (V) (Table 1).

C. With stirring, a solution of HIleOMe obtained from 3.0 g (16.5 mmole) of HCl·HIleOMe and 2.3 ml (16.5 mmole) of TEA in 20 ml of MC was added to the mixed anhydride obtained from 4.73 g (15.0 mmole) of ZTyrOH, 2.3 ml (16.5 mmole) of TEA, and 2.1 ml (16.5 mmole) of butyl chloroformate in 25 ml of ethyl acetate (EA) and 3.5 ml of DMFA. The reaction mixture was stirred at −3 ± 2°C for 2 h and was then kept at 0°C for 18 h and worked up as in paragraph A. This gave 5.3 (12.0 mmole) of (V) (Table 1).

Preparation of HTyrIleOMe (VII). A. In solution in 150 ml of MeOH, 16.7 mg (37.7 mmole) of (V) was hydrogenated at room temperature over palladium black (about 20 h). The catalyst was filtered off and was washed with MeOH (2 × 10 ml). The filtrate was treated with 100 ml of H_2O and, after being washed with 30 ml of MC, it was brought to pH 9 with 25% aqueous NH_4OH and the product was extracted with 70 ml of Chlf. The solution was dried

with Na_2SO_4 and evaporated in vacuum and the residue was dried in vacuum at 40°C. This gave 10.4 g (33.7 mmole) of (VII) (Table 1).

B. A solution of 6.6 g (16.2 mmole) of (VI) in 20 ml of EA was treated with 10.8 ml of a solution of HCl in dioxane (74 mmole of HCl). The reaction mixture was stirred vigorously for 1 h. The oily precipitate that separated out on the addition of 90 ml of ether was triturated until it formed a pulverulent substance. Then it was filtered off, washed with dry ether (2 × 10 ml), and dried over KOH in vacuum at 40°C. This gave 4.8 g (13.9 mmole) of (VII·HCl) (Table 1).

Preparation of BOCCys(Bzl)TyrIleOMe (III). A. To a suspension of 8.8 g (18.0 mmole) of (II) in 40 ml of DMFA at −50°C, with vigorous stirring, were added 20 ml (97 mmole of HCl) of a solution of HCl in dioxane, leading to the complete dissolution of the peptide, and 2.4 ml (18.0 mmole) of isoamyl nitrite. The reaction mixture was stirred at −30 ± 5°C for 45 min and then, with continued stirring, 13.0 ml (94.6 mmole) of TEA and a cooled solution of HIleOMe obtained from 2.4 h (13.2 mmole) of HCl·HIleOMe and 3.1 ml (22.6 mmole) of TEA in 20 ml of DMFA were added to it. The reaction mixture was stirred vigorously with a rise in the temperature to 0°C for 3 h with the addition, where necessary, of TEA (pH ~ 7) and was then kept at 0°C for 18 h and at room temperature for a day. After the addition of 100 ml of ether and 100 ml of H_2O, the ethereal layer was washed with 0.05 N HCl (2 × 30 ml) and with H_2O (2 × 30 ml). The solution was kept at 0°C for 18 h. The precipitate that deposited was filtered off and was dried in vacuum at 40°C. This gave 3.6 g (6.0 mmole) of (III) (Table 1).

B. With vigorous stirring and cooling to −15°C, a solution of (VII) obtained from 5.6 g (16.2 mmole) of (VII)·HCl and 2.3 ml (16.8 mmole) of TEA in 25 ml of MC at −15°C was added, still at −15°C, to the mixed anhydride obtained from 4.6 g (14.8 mmole) of BOC-Cys(Bzl)OH, 2.1 ml (15.2 mmole) of TEA, and 2.0 ml (15.4 mmole) of butyl chloroformate in 10 ml of ether. The reaction mixture was stirred at −10 ± 2°C for 30 min and at 0°C for 18 h and was then worked up as in the isolation of (VI) in paragraph A. This gave 8.19 g (13.6 mmole) of (III) (Table 1).

Preparation of BOCCys(Bzl)TyrIleN_2H_3 (IV). A solution of 8.7 g (14.4 mmole) of (III) in 40 ml of MeOH was treated with 10 ml (206 mmole) of hydrazine hydrate, and the mixture was kept at room temperature for 18 h. The precipitate that deposited was filtered off and washed with H_2O (2 × 10 ml) and with 30% MeOH (2 × 10 ml), and the residue was dried in vacuum at 40°C. This gave 6.1 g (10.1 mmole) of (IV) (Tables 1 and 2).

Preparation of BOCTyrIleOH (VIII). A solution of 4.4 g (10.8 mmole) of (VI) in 20 ml of C_2H_5OH was treated at room temperature with a solution of 1.2 g (30 mmole) of NaOH in 4 ml of H_2O. The reaction mixture was stirred for 5 h and then 40 ml of H_2O was added and it was washed with MC (3 × 15 ml), and the aqueous solution was brought to pH 3 by the addition of 5 N HCl. The product was extracted with 20 ml of MC. The extract was dried with Na_2SO_4 and evaporated in vacuum, and the residue was dried in vacuum at 40°C. This gave 3.6 g (9.1 mmole) of (VIII) (Tables 1 and 2).

Preparation of HCl·HTyrIleOH (IX). With stirring, 8 ml of a solution of HCl in dioxane (45.2 mmole of HCl) was added to a solution of 3.9 g (10.0 mmole) of (VIII) in 25 ml of EA and the mixture was stirred for 45 min, after which 150 ml of ether was added. After the formation of a pulverulent substance the solution was decanted off and the residue was dried in vacuum at 40°C. This gave 2.9 g (8.8 mmole) of (IX) (Table 1).

Preparation of BOCCys(Bzl)TyrIleOH (X). A solution of the silyl derivative of the dipeptide obtained from 2.5 g (7.6 mmole) (IX), 2.81 ml (22.5 mmole) of Me_3SiCl, and 4 ml (29.1 mmole) of TEA in 15 ml of DMFA at −20 ± 2°C over 15 min was added to the mixed anhydride obtained from 1.6 g (5.1 mmole) of BOCCys(Bzl)OH, 0.75 ml (5.5 mmole) of TEA, 0.45 ml (5.5 mmole) of pyridine, and 0.61 ml (5.5 mmole) of pivaloyl chloride in 5 ml of MC, and the mixture was stirred at −20 ± 2°C for 1 h and was then kept at 0°C for 18 h. After this, 5 ml of 1 N HCl and 50 ml of MC were added, the mixture was stirred for 30 min and was washed with H_2O (3 × 15 ml), the organic layer was dried with anhydrous Na_2SO_4, and the solvent was evaporated off in vacuum. The oily product was reprecipitated from ether with hexane and was dried in vacuum at 40°C. This gave 2.3 g (3.9 mmole) of (X) (Tables 1 and 2).

Preparation of BOCCys(Bzl)TyrOH (XI). The silylated tyrosine derivative obtained from 5.4 g (30 mmole) of tyrosine and 9.6 ml (75 mmole) of Me_3SiCl in 30 ml of DMFA–MC (1:2)

was added at 20°C over 1 h to the mixed anhydride obtained from 6.2 g (20 mmole) of BOC-Cys(Bzl), 3.04 ml (22 mmole) of TEA, and 2.8 ml (22 mmole) of butyl chloroformate in 15 ml of MC at −20 ± 2°C for 10 min, and this was followed by the addition of 10.4 ml (75 mmole) of TEA at −20°C, after which the mixture was stirred at −10 ± 2°C for 2 h and was kept at 0°C for 18 h. Then 50 ml of Chlf and 30 ml of 1 N HCl were added and this mixture was stirred for 30 min; then the organic layer was washed with 1 N HCl (3 × 25 ml) and H_2O (2 × 25 ml), and was dried with anhydrous Na_2SO_4, and the solvent was evaporated off in vacuum. The residue was dissolved in 36 ml of MeOH–H_2O (2:1.5) and the solution was washed with hexane–ether (4:1) after which the product was extracted with Chlf (3 × 15 ml). The organic solution was dried with anhydrous Na_2SO_4, the solvent was evaporated off in vacuum, and the residue was dried in vacuum at 40°C. This gave 9.3 g (19.6 mmole) of (XI) (Table 1).

SUMMARY

1. Two schemes for the synthesis of the hydrazide of the N-terminal tripeptide of the oxytocin sequence have been considered. It has been shown that the most rational is the 1 + 2 scheme of synthesis.

2. The possibility has been studied of synthesizing the tripeptide with a free carboxy group by the mixed-anhydride method using temporary trimethylsilyl protection. It has been shown that it is possible to obtain the tripeptide by this method. A method is proposed for obtaining the tripeptide by the 1 + 2 scheme using the mixed-anhydride method with the complete deblocking of BOCTyrIleOMe followed by the introduction of trimethylsilyl protection of the carboxy and hydroxy functions of the dipeptide.

3. The ^{13}C NMR spectra of the compounds obtained have been interpreted.

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