SYNTHESIS OF THE C-TERMINAL TETRAPEPTIDE OF THE OXYTOCIN SEQUENCE USING VARIOUS PROTECTIVE GROUPS FOR THE CYSTEINE THIOL FUNCTION

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The synthesis of amides of tetrapeptides with the 6–9 sequence of oxytocin containing various protective groups for the thiol function has been performed by 2+2 and 1+3 schemes. Relationships between the yield of tetrapeptide and the structures of the protective groups have been obtained. An advantage of trityl and benzyl protective groups for masking the tiol function of cysteine has been shown. The signals of the ^{13}C NMR spectra of the compounds obtained have been interpreted.

We have previously considered variants of the synthesis of the amide of the peptide having the 6–9 sequence of oxytocin using benzyl protection for the thiol function of cysteine [1] and have shown an advantage in performing the synthesis by the mixed-anhydride method and a 2+2 scheme. We have performed the synthesis of tetrapeptides by this scheme and the mixed-anhydride method, using trityl (Trit), benzamidomethyl (Bzm), benzoyl (Bz), and pyrrolidonylmethyl (Pym) protective groups for the thiol function of cysteine in comparison with methyl (Me) and benzyl (Bzl).

Table 1 gives the properties and yields of the tetrapeptides obtained, and Table 2 the yields and properties of the initial dipeptides. As follows from Table 1, in synthesis by the mixed-anhydride method the yields of the BOC derivatives of the tetrapeptides decreased in the sequence Trit → Bzl → Bz → Me = Pym → Bzm.

Tables 3 and 4 give the yields and properties of the tetrapeptides obtained by the 1+3 scheme and the mixed-anhydride method (the yields have been calculated on the tripeptide) and by the activated-ester method, without their isolation, respectively. As can be seen from Tables 3 and 4, on their synthesis by either the mixed-anhydride or the activated-ester method the yields of the BOC derivatives of the tetrapeptides decreased in the sequence Me → Bzl → Trit → Pym → Bz.

TABLE 1. Physicochemical Properties of Tetrapeptides of the Type of BOCCys(X)ProLeuGlyNH_2 Obtained by a 2+2 Scheme and the Mixed-Anhydride Method

No.	Formula	Mol. mass	mp, °C	$[\alpha]_D^{20}$, deg (c 1; DMFA	Chromat. mobility*, R_f	Yield, %
1	BOC—Cys(Trit)—NH_2	728.6	96–98	+6,5	0,63	77,6
2	BOC—Cys(Bzl)—NH_2	577,8	147–149	—53,0	0,53	74,0
3	BOC—Cys(Bz)—NH_2	591,8	182–185	—62,0**	0,40	45,0
4	BOC—Cys(Me)—NH_2	500,6	116–118	—60,5	0,54	32,0
5	BOC—Cys(Pym)—NH_2	582,6	74–76	—87,0**	0,12	32,0
6	BOC—Cys(Bzm)—NH_2	591,6	188–190	—60,0	0,49	29,0

*System: ethyl acetate–pyridine–acetic acid–water (30:2.5:0.75:1.38)
**AcOH.

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TABLE 2. Physicochemical Properties of Dipeptides of the Type of BOCCys(X)ProOH Obtained by the Mixed-Anhydride Method

Number	Formula	Mol. mass	mp., °C	$[\alpha]_D^{20}$, deg (c 1; DMFA	Chromat. mobility*, R_f	Yield, %
1	BOC—(Me)—OH	332,3	Oil	—29,0	0,36	86,0
2	BOC—(Bzm)—OH	451,4	Oil	—30,0**	0,35	78,0
3	BOC—(Bzl)—OH	407,3	82—85	—62,0***	0,54	76,0
4	BOC—(Pym)—OH	415,3	Oil	—79,5**	0,10	76,0
5	BOC—(Bz)—OH	422,3	Oil	—39,5	0,45	67,5
6	BOC—(Trit)—OH	560,3	—	+28,5	0,72	53,0
7	Form Tzl—ProOH	286,2	—	—	—	****

*System: ethyl acetate—pyridine—acetic acid—water (30:2.5:0.75:1.38).
**AcOH.
***MeOH.
****No reaction took place.

TABLE 3. Physicochemical Properties of Tetrapeptides of the Type of BOCCys(X)ProLeuGlyNH$_2$ Obtained by a 1+3 Scheme and the Mixed-Anhydride Method

Number	Formula	Mol. mass	mp, °C	$[\alpha]_D^{20}$, deg (c 1; DMFA	Chromat. mobility*, R_f	Yield, %
1	BOC—(Me)—NH$_2$	500,6	116—118	—62,5	0,47	98,0
2	BOC—(Bzl)—NH$_2$	577,8	144—146	—49,5	0,52	96,5
3	BOC—(Pym)—NH$_2$	582,6	77—79	—62,5	0,19	69,4
4	BOC—(Bz)—NH$_2$	591,6	184—185	—54,0	0,50	46,0
5	BOC—(Trit)—NH$_2$	728,9	119—123	+77,0	0,58	100,0**

*System: ethyl acetate—pyridine—acetic acid—water (30:2.5:0.75:1.38).
**The product was highly contaminated with BOCCys(Trit)OH.

TABLE 4. Physicochemical Properties of Tetrapeptides of the Type of BOCCys(X)ProLeuGlyNH$_2$ Obtained by a 1+3 Scheme and the Carbodiimide Method with the Addition of 1-Hydroxybenzothiazole

Number	Formula	Mol. mass	mp, °C	$[\alpha]_D^{20}$, deg (c 1; DMFA	Chromat. mobility*, R_f	Yield, %
1	BOC—(Me)—NH$_2$	500,6	121—123	—56,0	0,47	98,0
2	BOC—(Bzl)—NH$_2$	577,8	145—147	—48,0	0,51	79,0
3	BOC—(Trit)—NH$_2$	728,9	110—112	+8 0	0,52	75,4
4	BOC—(Pym)—NH$_2$	582,6	78—80	—71,5	0,19	66,6
5	BOC—(Bz)—NH$_2$	591,6	186—188	—58,5	0,50	53,4

*System: ethyl acetate—pyridine—acetic acid—water (30:2.5:0.75:1.38).

TABLE 5. Physicochemical Properties of Tetrapeptides of the Type of BOCCys(X)ProLeuGlyNH$_2$ Obtained by a 1+3 Scheme and the Mixed-Anhydride Method Using as Condensing Agent Pivaloyl Chloride in the Presence of Triethylamine and Pyridine

Number	Formula	Mol. mass	mp., °C	$[\alpha]_D^{20}$, deg (c 1; DMFA)	Chromat. mobility*, R_f	Yield, %
1	BOC—Me—NH$_2$	500,6	120—122	—63,5	0,45	78,0
2	BCC—Bzl—NH$_2$	577,8	147—149	—47,0	0,53	81,0
3	BOC—Bzm—NH$_2$	591,8	188—190	—52,5	0,39	60,0
4	BOC—Bz—NH$_2$	591,6	185—187	—61,0	0,47	50,0
5	BOC—Pym—NH$_2$	582,6	79—80	—68,5	0,19	28,6

*System: ethyl acetate—pyridine—acetic acid—water (30:2.5: 0.75:1.38).

On their synthesis by the mixed-anhydride method the yields of the BOC derivatives of the tetrapeptides decreased in the sequence Bzl → Me → Bzm → Bz → Pym (Table 5).

It follows from Tables 1 and 3-5 that it is undesirable to perform the synthesis of a tetrapeptide either by a 2+2 or by a 1+3 scheme using Pym and Bz groupings to mask the mercapto function of cysteine, since the yields of the tetrapeptides in these cases were, as a rule, less than 50%. The highest yields of product were observed on the use of Bzl and Trit protective groups for the thiol function of cysteine.

We used the method of ^{13}C NMR spectroscopy to confirm the structures and check the purities of the peptides obtained. The chemical shifts for the carbon nuclei of the amino acid residues and the BOC groups are given in Table 6. In some cases, tetrapeptides with the same protective group for the thiol function of cysteine obtained by different methods differed in purity. The figures given in Table 6 relate to the purest specimens. In order to assign the resonance signals, we made use of literature information [2], results for cysteine derivatives [3], and a comparison of the spectra of the tetrapeptides with different protective grous for the thiol function of cysteine and some intermediate di- and tripeptides, and also the nature of the splitting of the signals in spectra taken in the regimes of "gated decoupling" and of the selective suppression of spin—spin interaction with protons. The interpretation of the auxiliary ^{1}H NMR spectra was based on information in [4].

By comparing different spectra and taking facts given in [3] into account it was possible to assign two of the four signals in the 169-173 ppm region to the resonances of the C=O nuclei of the Gly and Cys residues. It was extremely difficult to distinguish the resonance signals of the C=O nuclei of the Pro and Lue residues. The assignment shown in Table 6 was made on the basis of the ^{13}C NMR spectra with selective suppression of spin—spin interaction with protons. However, because of the closeness of the resonance frequencies of the corresponding protons (NH of Leu and Gly, CH$_\alpha$ of Pro and Leu, and so on) the results obtained cannot be regarded as absolutely reliable.

In the analysis of the 46—80 ppm region, the main problem consisted in distinguishing the resonance signals of the C$_\alpha$ nuclei of the Leu and Cys residues. It was solved by comapring the spectra of different peptides and the result was confirmed by the spectra with selective decoupling from protons.

On the use of the WP-80 spectrometer, except for the case of the tetrapeptide (VI) the two signals corresponding to the C$_\alpha$ nucleus of Gly and the C$_\beta$ nucleus of Leu fell into the 36—43 ppm region overlapped by the powerful multiplet of DMSO-d$_6$. On passing to the WM-250 spectrometer the region occupied by the multiplet of the solvent contracted to 38—41 ppm, which enabled the signal at ~42 ppm to be assigned to the resonance of the C$_\alpha$ nucleus of the Gly residue (triplet with a constant of ~135 Hz in the gated-decoupling spectrum). In the spectrum of the tetrapeptide (VI) the signal of the nuclei of the CH$_2$ group of Bzm (δ = 40.2 ppm) fell into the same region; it could be distinguished from the C$_\alpha$ signal of Gly

TABLE 6. Chemical Shifts in the ^{13}C NMR Spectra of Tetrapeptides of the type of BOCCys(X)ProLeuGlyNH_2*

Residue	Nucleus	Compound I	II	III	IV	V	VI	Nucleus	Residue
Cys	C_o	169,8	169,0	170,0	170,0	169,8	169,2		
	C_α	52,0	52,3	52,6	51,7	52,1	51,8		
	C_β	32,8	32,7	32,5	35,1	32,4	30,3		
Pro	C_o	171,7	171,4	171,8	171,8	171,7	171,8		
	C_α	59,9	59,4	59,9	59,9	59,8	59,9		
	C_β	28,9	28,4	28,9	28,9	28,8	29,0		
	C_γ	24,5	24,4	24,5	24,5	24,5	24,5		
	C_δ	46,9	46,4	46,9	46,9	46,8	46,9		
Leu	C_o	172,2	172,1	172,1	172,1	172,0	172,2		
	C_α	51,5	51,4	51,6	51,5	51,4	51,5		
	C_β	40,1	40,3	40,1	40,1	40,1	40,1		
	C_γ	24,3	24,1	24,2	24,2	24,2	24,3		
	C_{δ_1}	23,0	22,9	23,0	23,0	23,0	23,0		
	C_{δ_2}	21,5	21,6	21,5	21,5	21,5	21,6		
Gly	C_o	171,0	170,9	171,0	170,9	170,9	171,0		
	C_α	42,1	42,0	42,0	42,1	42,0	42,1		
BOC (N)	C=O	155,4	155,1	155,3	155,4	155,4	155,4		
	C—	78,4	78,5	78,5	78,3	78,3	78,5		
	CH_3	28,2	28,1	28,2	28,2	28,1	28,1		
Bzl (S)	$-CH_2-$	35,7			15,3			CH_3-	Me (S)
	C_1	138,5				30,4		C_1	
	C_2	129,0				17,1		C_2	
	C_3	128,4				45,2		C_3	Pym (S)**
	C_4	126,9				174,7		C=O	
						44,2		$-CH_2-$	
Trit (S)	—C		66,4						
	C_1		144,4				191,4	C=O	
	C_2		129,2				136,4	C_1	
	C_3		128,0				126,9	C_2	Bz (S)
	C_4		126,8				129,1	C_3	
							134,0	C_4	
Bzm (S)	CH_2			40,2					
	C=O			166,4					
	C_1			134,0					
	C_2			127,3					
	C_3			128,4					
	C_4			131,5					

*X represents the protective group for the thiol function of cysteine: Bzl (I), Trit (II), Bzm (III), Me (IV), Pym (V), Bz (VI).

**The positions of the carbon atoms in the pyrrolidone ring are shown relative to the carbonyl group.

from its spin–spin coupling constant of 150 Hz (influence of a second electronegative substituent – the S atom).

In our analysis of the 20–36 ppm region, we used gated-decoupling spectra to identify the signals of the C_γ nuclei of Pro and Lue in the 24.1–24.5 ppm interval (triplet and doublet, respectively).

Only the resonance signals of the carbon nuclei of the protective groups for the thiol function of cysteine appeared in the other frequency intervals of the ^{13}C NMR spectra. To within ±0.3 ppm, their chemical shifts coincided with the corresponding values for cysteine derivatives [3]. The only exception was the signal of the Bz C=O group, which was shifted downfield by 0.6 ppm (δ = 191.4 ppm).

It may be mentioned that the resonance signals of the C_α and C_β nuclei of the Cys residue were shifted upfield by 1.5-2.0 and 0.5-1.0 pm, respectively, in comparison with the analogous signals of the tripeptides described previously [3]. This shift is connected with the position of the Cys residue before Pro [2].

EXPERIMENTAL

Melting points were determined in open capillaries without correction, and angles of optical rotation on a VNIÉKIPRODMASh [All-Union Scientific-Research and Experimental and Design Institute of Food Machinery] polarimeter. The chromatographic purities and mobilities of the substances obtained were determined by the TLC method on Silufol plates (Czechoslovakia) in the ethyl acetate–pyridine–acetic acid–water (30:2.5:0.75:1.38) system. The peptides were detected by treating the dried plates in an atmosphere of Cl_2, followed by spraying with a 1 N acetic acid solution containing 0.03% of o-tolidine and 0.1% of KI.

For synthesis we used BOCCys(X)OH's where X = Bzl, Me, Bzm, and Pym produced by Reakhim and those where X = Trit and Bz, and also From-TzlOH, obtained as described in [3].

The ^{13}C NMR spectra of solutions of the tetrapeptides in DMSO-d_6 (c = 100 mg/ml) were recorded on WP-80DS and WM-250 spectrometers (Bruker, Germany) with working frequencies for ^{13}C nuclei of 20.115 and 62.9 MHz, respectively. The recording conditions were similar to those given in [3]. Chemical shifts were reckoned from the signals of the solvent (δ = 39.6 ppm) and have been recalculated to the δ scale.* Commercial DMSO-d_6 was used for the preparation of the solution without the preliminary elimination of water.

The ^{13}C NMR spectra in the regimes of gated decoupling and selective suppression of spin–spin interaction with protons, and also the auxiliary ^1H NMR spectra, were taken on the WM-250 spectrometer.

Preparation of Dipeptides with the Formula BOCCys(X)ProOH. The synthesis of the dipeptides was carried out by the mixed-anhydride method using butyl chloroformate or povaloyl chloride and the chlorotrimethylsilyl derivative of proline, as in [1] (see Table 2).

Preparation of Tetrapeptides with the Formula BOCCys(X)ProLeuGlyNH$_2$. A. The synthesis of the tetrapeptides was carried out by the mixed-anhydride method starting from HLeuGlyNH$_2$ and BOCCys(X)ProOH as described in [1] (see Table 1).

B. The synthesis of the tetrapeptides was carried out by the carbodiimide method using 1-hydroxybenzotriazole and starting from BOCCys(X)OH and HProLeuGlyNH$_2$ as described in [1] (see Table 4).

C. The synthesis of the tetrapeptides by the mixed-anhydride method started from BOCCys(X)OH and HProLeuGlyNH$_2$ as described in [1] (see Table 5).

D. A solution of 5.2 g (0.022 mole) of BOCCys(Me)OH in 10 ml of methylene chloride was cooled to −6°C, and a solution of 2.5 g (0.012 mole) of dicyclohexylcarbodiimide in 15 ml of methylene chloride, cooled to 0°C, was added. The mixture was stirred with a magnetic stirrer at room temperature for 2.5 h, and the precipitate that had formed was filtered off. The solution was cooled to −6°C and to it was added a solution of 3.1 g (0.011 mole) of HProLeuGlyNH$_2$ in 10 ml of dimethylformamide cooled to −6°C. The mixture was stirred with a magnetic stirrer for 3 h. The resulting gelatinous mass was stirred with 100 ml of methylene chloride until it had dissolved completely. The solution was washed successively with 2 × 50 ml of H_2O, saturated Na_2CO_3 solution, H_2O, 1 N HCl, and H_2O, dried with anhydrous Na_2SO_4, and evaporated in vacuum to a volume of 50 ml; then 150 ml of ether was added with stirring and the mixture was left at 0°C for 18 h. The precipitate was filtered off, washed with 2 × 50 ml of ether, and dried in vacuum to constant weight. This gave 4.9 g of GOCCys-(Me)ProLeuGlyNH$_2$ in the form of a powder. The other tetrapeptides were obtained similarly (see Table 3).

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SYNTHESIS AND PROPERTIES OF PEPTIDE FRAGMENTS OF THE S-REGION OF THE SURFACE PROTEIN OF THE HEPTATITIS B VIRUS

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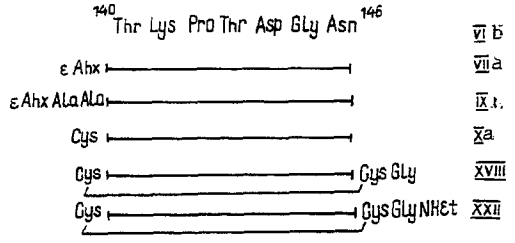
A peptide fragment with the 140–146 sequence of the main component of the surrace protein of the hepatitis B virus (HBsAG) and a number of its structural analogues have been synthesized by the classical method in solution. Conjugates of the peptides synthesized with bovine serum albumin and with a synthetic polypeptide analogue of polytuftsin have been obtained. The ability of the preparations to bind antibodies from the blood sera of hepatitis B patients has been studied. The possibility has been shown of their use for revealing antibodies to the heptatitis B virus in solid-phase enzyme-mediated immunoassay.

According to the WHO, viral hepatitis B is one of the most acute problems of public health [1]. The extremely widespread nature of this disease is connected with the absence of an effective vaccine and of a reliable system of diagnosis. And, if it is borne in mind that, because of the absence of effective systems for the replication of the virus, the main source of viral proteins still remains donor blood, the interest aroused by investigations on the synthesis of the antigenic determinants of the hepatitis B virus capable of replacing the natural material in the vaccines used and in diagnostic systems becomes understandable.

The surface antigen of the hepatitis B virus, which possesses a high antigenic and immunogenic activity, is a complex of three glycosylated proteins containing a common sequence of 226 amino acid residues (the S-protein). In addition to the main S-protein, which is the principal marker of the disease, the outer coat of the virus includes two minor proteins: medium ($S+preS_2$) and major ($S+preS_2+preS_1$).

With the aim of studying the structural-functional organization and immunochemical properties of the antigenic determinants and also of creating an effective test system for the hepatitis B virus, we have carried out the synthesis of a fragment with the sequence 140–146 of the main surface protein (VIb), which is part of the conformation-dependent group-specific determinant "a," common for all the subtypes of the virus [2, 3], and a number of its structural analogues:



where εAhx represents 6-aminohexanoic acid. The εAhx was introduced into the peptide sequence [in peptides (VIIa) and (IXb)] as a spacer for the subsequent conjugation of the haptens with

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