Mass spectrum, m/z (%): 242 (M^+; 0.5), 224(2), 136(100), 135(70), 123(16), 118(9), 107(28), 106(52), 105(11), 79(35), 78(30), 77(22).

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SYNTHESIS OF THE AMIDE OF THE C-TERMINAL TETRAPEPTIDE OF THE SEQUENCE OF OXYTOCIN

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The synthesis has been effected of the amide of the tetrapeptide forming the sequence 6—9 of oxytocin with the use of benzyl protection of the thiol function of cysteine by two main schemes 1+3 and 2+2. The advantageousness of performing the synthesis by the 2+2 scheme has been shown. The overall yield of tetrapeptide using the method of condensation with the formation of mixed anhydrides amounted to 51% by the scheme proposed.

The peptide HCys(R)ProLeuGlyNH_2 is the key fragment in the synthesis of oxytocin. In developing a method for obtaining the structure of the peptide we have studied the possibility of its synthesis by two main schemes; 1+3 and 2+2. The development of the variants of the scheme was carried out with the use of benzyl protection of the thiol function of cysteine (R = $C_6H_5CH_2$—).

In the development of the 1+3 scheme for the synthesis of the tetrapeptide we took into account the fact that in the preparation of the amide of the 7—9 tripeptide (melanostatin; MIF) in spite of information [1] on the high yields of di- and tripeptides, these compounds were obtained by the activated-ester (AE) method in far worse quality and their yields were lower than in their preparation by the mixed-anhydride (MA) method [2]. The use of the amide in place of its ester is associated with the necessity for performing the condensation reaction in an aqueous medium because of the poor solubility of glycinamide in organic solvents and also with the difficulty of isolating the amide of the dipeptide in the pure form because of its increased solubility in water. In view of this, we have performed several variants of the synthesis of the tripeptide by a 1+2 scheme (scheme A) using mixed anhydrides with ethyl and butyl chloroformates and also with pivaloyl chloride. It was found that the yield of peptides on the use of pivaloyl chloride as condensing agent was somewhat lower in the sage of obtaining the dipeptide (67%) than on the use of ethyl or butyl chloroformate (75%). At the stage of obtaining the tripeptide the best yield of product was obtained with

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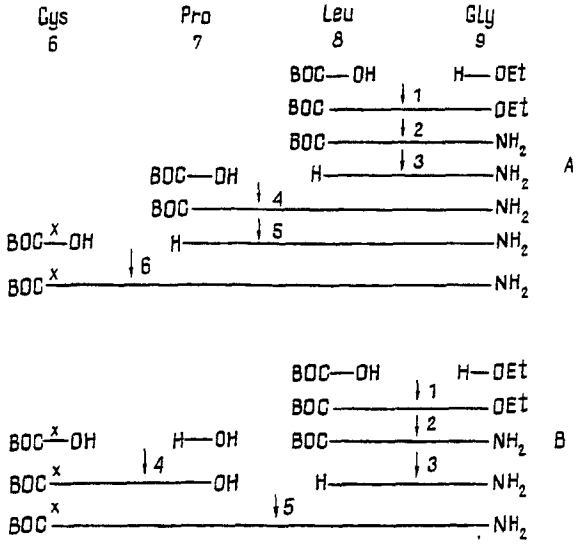
the use of ethyl chloroformate (76%) and the worst with butyl chloroformate. We have also shown that the performance of amidation at the stage of forming the dipeptide is not equivalent to that at the tripeptide stage. The successful performance of the amidation of BOCLeuGlyOEt permitted the use of benzyloxycarbonylleucine, as well, in the stage under consideration, since the amidation of the dipeptide permitted the avoidance of side reactions during the elimination of the benzyloxycarbonyl protection [1].



Scheme 1. Synthesis of a tetrapeptide of the type of BOCCys(X)ProLeuGlyNH_2, where X represents benzyl (Bzl).

In the performance of the synthesis of melanostatin by the 2+1 scheme, we tested variants of obtaining a prolylleucine derivative by the mixed-anhydride method with the methyl and trimethylsilyl esters of leucine. On the hydrolysis of the ester bond of the methyl ester, the formation of two products was observed (although the initial compound was an individual substance according to TLC and ^{13}C NMR), the separation of which was possible only by chromatographic methods. Performance of the synthesis with the mixture of products obtained on hydrolysis led to the formation of the corresponding derivative of the ethyl ester of the tripeptide with a yield of about 30%. The hoped-for results were obtained by this scheme (2+1) when the silyl ester of leucine was used. The formation of the dipeptide using the silyl derivative of leucine by the mixed-anhydride method with pivaloyl chloride or butyl chloroformate took place to the extent of 90-93%, and that of the tripeptide with a yield of 65%.

The synthesis of the tetrapeptide by the 1+3 scheme (see Table and Scheme A) was effected with the use of dicyclohexylcarbodiimide (DCHC) by the AB method without and with the isolation of the active ester, by the MA method, and also by the method of symmetrical anhydrides (sym. A).

The overall yield of the BOC derivative of the amide of the tetrapeptide (on the glycine) by the 1+3 scheme using pivaloyl chloride as the condensing agent in the last stage amounted to 41%. The maximum yield when the sym. A method was used in the key stage was 49%. However, if it is borne in mind that in the latter case the yield of tetrapeptide on the cysteine derivative was less than 50%, the advantage of the performance of this synthesis by the MA method using a mixture of triethylamine (TEA) and pyridine as the base becomes obvious. Analogous results were obtained with the use as condensing agent of DCHC with 1-hydroxybenzotriazole (HBT); however, the use of DCHC is not always desirable, since it is allergenic.

The synthesis of the tetrapeptide by the 2+2 (scheme B) was performed by the MA method (butyl chloroformate) with the use of the amide of the 8-9 dipeptide of the oxytocin sequence. The AE method without the isolation of the HBT ester had no appreciable advantages at this stage (yield 78%, product contaminated with urea).

BOCCys(Bzl)ProOH was obtianed in one stage by using the temporary trimethylsilyl protection of the proline carboxy group by the MA method with quantitative yield. The overall yield of the tetrapeptide by this scheme was 51%.

TABLE 1. Physicochemical Properties of Tetrapeptides of the Type BOCCys(Bzl)ProLeuGlyNH$_2$ Obtained by the 1+3 Scheme with Different Methods of Condensation

Number	Method of synthesis	$[\alpha]_D^{20}$ deg (c 1)		mp, °C	Chromatographic mobility,* R_f	Yield, %
		DMFA	AcOH			
1	Sym. anh.	−49,5	—	144—146	0,52	96,5
2	PNPE**	−50,0	−77,0	148—149	0,53	87,0
3	MA, Piv. chlor.	−47,0	−60,0	147—149	0,53	81,0
4	DCHC, HBT***	−48,0	−71,0	145—147	0,51	79,0
5	DCHC	−47,0	— 4,0	144—146	0,52	72,0
6	DCHC. HOST****	−46,0	−64,5	146—148	0,53	57,0
7	MA, but. chlor.	−48,0	−66,5	148—150	0,52	51,0
8	MA, 2 + 2	−47,0	—	147—149	0,53	74,0

*System: ethyl acetate—pyridine—acetic acid—water (30:2.5:0.75:1.38).
**p-Nitrophenyl ester.
***1-Hydroxybenzotriazole.
****N-hydroxysuccinimide.

Thus, the 2+2 scheme for the synthesis of the tetrapeptide has undoubted advantages over the 1+3 scheme. With an equal or higher yield of the BOC derivative of the amide of the tetrapeptide the process takes place in five, and not in six stages. The free amino acid is used instead of the BOC derivative of proline. The quality of the products obtained was the same by both schemes according to the results of ^{13}C NMR and physicochemical constants.

The only advantage of the 1+3 scheme of synthesis is the fact that the amide of the C-terminal tripeptide of the oxytocin sequences is used as an intermediate and this may be of independent interest as a final biologically active product of synthesis.

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 peptides were determined by the TLC method on Silufol plates (Czechoslovakia) in the following systems: ethyl acetate—pyridine—acetic acid—water (30:20:6:11) − S_1; (30:5:1.5:2.75) − S_3; and (30:2.5:0.75:1.38) − S_4. The peptides were detected by treating the dried plates in an atmosphere of Cl_2 followed by spraying with 1 N solution of acetic acid containing 0.03% of o-toluidine and 0.01% of KI.

Preparation of BOCLeuGlyOEt. A. With stirring, 27 ml (208 mmole) of butyl chloroformate was added to a solution of 49.8 g (200 mmole) of tert-butoxycarbonyl-L-leucine hydrate in 100 ml of CH_2Cl_2 and 29 ml (210 mmole) of triethylamine (TEA) cooled to −20°C. The reaction mixture was stirred at −15±2°C for 20 min, and a solution of 30.8 g (221 mmole) of the hydrochloride of the ethyl eseter of glycine in 100 ml of dimethylformamide (DMFA) and 32 ml (233 mmole) of TEA cooled to −20°C was added to it. The reaction mixture was stirred at −12±2°C for 1 h, and then at 0°C for 18 h. Then 150 ml of CH_2Cl_2 was added to the mixture and it was extracted successively with 3 × 50 ml of 1 N HCl, 2 × 50 ml of H_2O, 3 × 50 ml of saturated NaHCO solution, and 2 × 50 ml of H_2O. The organic layer was dried with anhydrous sodium sulfate and evaporated in vacuum. The residue was dissolved in 70 ml of hot ether, 70 ml of hexane was added, and the mixture was left at 0°C for 18 h. The precipitate that had deposited was filtered off, washed on the filter with 2 × 50 ml of hexane, and dried in vacuum to constant weight. This gave 47.4 g (75%) of BOCLeuGlyOEt, $[\alpha]_D^{20}$ − 30°, (c 1; MeOH), mp 77-79°C, R_f 0.88 (S_4).

B. With the use of 19.8 ml (208 mmole) of ethyl chloroformate, 47.4 g (75) of BOCLeuGlyOEt of similar quality to that in paragraph A was obtained.

C. With the use of 26.9 ml (208 mmole) of pivaloyl chloride in place of butyl chloroformate and the dissolution of the tert-butoxycarbonyl-L-leucine hydrate in the presence

of 59 ml (431 mmole) of TEA, we obtained 42.3 g (67%) of BOCLeuGlyOEt, $[\alpha]_D^{20}$ − 28° (c 1, MeOH), mp 76-78°C, R_f 0.87 (S_4).

Preparation of HCl·HLeuGlyOEt. A solution of 35.0 g (111 mmole) of BOCLeuGlyOEt in 90 ml of ethyl acetate was treated with 95 ml of a solution of HCl in ethyl acetate (450 mmole of HCl). The reaction mixture was stirred for 60 min and was evaporated in vacuum (without heating) to a volume of 90 ml, and 100 ml of ether was added. The solution was decanted from the oily precipitate. The latter was treated with 100 ml of ether, the mixture was cooled, the solution was decanted off, and the operation was repeated with 100 ml of hot hexane. The final residue was dried in vacuum over KOH to constant weight. This gave 28.2 g (100%) of oily HCl·HLeuGlyOEt, $[\alpha]_D^{20}$ + 18° (c 1; MeOH), R_f 0.14 (S_4), 0.80 (S_1).

Preparation of BOCLeuGlyNH$_2$. Gaseous ammonia was passed through a solution of 31.6 mg (100 mmole) of BOCLeuGlyOEt in 70 ml of MeOH in a thin-walled flask cooled to −40±2°C until the volume of the reaction mixture had increased by 50 ml. Then it was stirred until the temperature had risen to 0°C. The flask was closed with a well-ground polyethylene or Teflon stopper and left without stirring for 15±5°C for 7 days. Then the solution was filtered and evaporated in vacuum. The oily product was dried in vacuum to constant weight. The yield of oily BOCLeuGlyNH$_2$ was 28.7 g (100%), $[\alpha]_D^{20}$ − 14° (c 1; AcOH), R_f 0.55 (S_4).

Preparation of HCl·HLeuGlyNH$_2$. With vigorous stirring, 160 ml of a solution of HCL in dioxane (1.096 mole of HCl) was added to solution of 77.0 g (268 mmole) of BOCLeuGlyNH$_2$ in 200 ml of dioxane. Then the reaction mixture was stirred for 70 min, and 140 ml of ether was added to it. The solution was decanted off the precipitate, which was then treated with 140 ml of ether to form a solid powder. This powder was filtered off and was washed with 2 × 100 ml of ether. The product was dried to constant weight in vacuum over KOH. The yield of white hygroscopic powder was 60.0 g (100%): HCl·HLeuGlyNH$_2$, $[\alpha]_D^{20}$ + 33° (c 1; MeOH), mp 30°, R_f 0.50 (S_1).

Preparation of BOCProLeuOH. A suspension of 9.8 g (75 mmole) of L-leucine in 150 ml* and 19.5 ml (150 mmole) of chlorotrimethylsilane was stirred until the amino acid had dissolved. The solution was cooled to −30°C and was treated first with 21.5 ml (150 mmole) of TEA and then with the mixed anhydride obtained from 10.8 g (50 mmole) of BOCProOH and 6.6 ml (51 mole) of butyl chloroformate in 50 ml of $CHCl_3$ and 7.2 ml (51 mmole) of TEA at −20±2°C over 20 min. The reaction mixture was stirred at −7±2°C for 1 h and at 0°C for 18 h and was then extracted with 30 ml of H_2O, 30 ml of 1 N HCl, 2 × 30 ml of H_2O, 30 ml of saturated $NaHCO_3$ solution, 30% MeOH solution†, and 30 ml of H_2O. The organic solution was dried with anhydrous Na_2SO_4 and evaporated in vacuum. The oily product was reprecipitated from ether with hexane. This gave 16.0 g (97%) of BOCProLeuOH, $[\alpha]_D^{20}$ − 26° (c 1; MeOH), mp. 98-101°, R_f 0.64 (S_3).

Preparation of BOCProLeuGlyOEt. A. The mixed anhydride obtained from 21.7 g (101 mmole) of BOCProOH and 10.5 ml (110 mmole) of ehtyl chloroformate in 125 ml of ethyl acetate and 14.1 ml (103 mmole) of TEA over 10 min at −15±5°C was added to a solution of 28.0 g (111 mmole) of HCl·HLeuGlyOEt in 125 ml of ethyl acetate and 17.0 ml (124 mmole) of TEA cooled to −15°C. The mixture was stirred at −5±2°C for 2 h and at 0°C for 18 h. Then 50 ml of ethyl acetate was added to the raction mixture and it was extracted with 3 × 30 ml of 1 N HCL, 80 ml of H_2O, 3 × 40 ml of saturated $NaHCO_3$ solution, and 2 × 40 ml of H_2O, and was dried with anhydrous Na_2SO_4. The organic solution was evaporated in vacuum. The residue was recrystallized from 150 ml of ether and 150 ml of hexane at 0°C over 18 h. The precipitate was filtered off and was dried in vacuum to constant weight. This gave 29.2 g (70%) of BOCProLeuGlyOEt, $[\alpha]_D^{20}$ − 84° (c 1; MeOH), mp 100-103°, R_f 0.68 (S_4).

B. In a similar way to that described in paragraph A, 33.2 g (101 mmole) of BOCProLeuOH and 15.5 g (111 mmole) of HCl·HGlyOEt yielded 27.3 g (65%) of BOCProLeuGlyOEt, $[\alpha]_D^{20}$ −85° (c 1; MeOH), mp 105-108°, R_f 0.70 (S_4).

Preparation of BOCProLeuGlyNH$_2$. A. In a similar way to the preparation of BOCLeuGlyNH$_2$, 41.3 g (100 mmole) of BOCProLeuGlyOEt in 70 ml of MeOH yielded 37.7 g (98%) of BOCProLeuGlyNH$_2$, $[\alpha]_D^{20}$ −71° (c 1; MeOH), mp 137-139°, R_f 0.38 (S_4).

B. A solution of the mixed anhydride obtained from 4.3 g (20 mmole) of BOCProOH and 2.5 ml (20 mmole) of pivaloyl chloride in 25 ml of CH_2Cl_2 and 5.4 ml (39.3 mmole) of TEA

*Medium not given − Translator.
†Amount not given − Translator.

was added at −15±2°C to a solution of 5.0 g (22 mole) of HCl·HLeuGlyNH$_2$ in 10 ml of DMFA and 3.8 ml (27.6 mmole) of TEA, and the mixture was kept for 15 min. Then it was stirred at −8±2°C for 1 h and at 0°C for 18 h and was washed with 10 ml of 1 N HCl, 2 × 7 ml of H_2O, 2 × 7 ml of saturated $NaHCO_3$ solution, and 2 × 7 ml of H_2O. The organic solution was dried with anhydrous Na_2SO_4 and evaporated in vacuum, and the oily residue was dried in vacuum to constant weight. It was then recrystallized from ether with hexane. This gave 5.4 g (70%) of BOCProLeuGlyNH$_2$, $[\alpha]_D^{20}$ −71° (c 1; MeOH), mp 136-138°, R_f 0.64 (S_3).

Preparation of ZProLeuGlyNH$_2$. The mixed anhydride obtained from 4.6 g (18.5 mmole) of ZProOH and 2.6 ml (20 mmole) of butyl chloroformate in 10 ml of CH_2Cl_2 and 2.8 ml (20 mmole) of TEA at −15±5°C for 15 min was added to a solution of 4.2 g (18.3 mmole) of HCl·HLeuGlyNH$_2$, 10 ml of $CHCl_3$, and 2.8 ml (20 mmole) of TEA cooled to −10°C. The reaction mixture was stirred at −7±2°C for 1 h and at 0°C for 18 h, and then 10 ml of CH_2Cl_2 was added and it was extracted as in the preparation of BOCProLeuGlyNH$_2$ (paragraph B). The organic solution was dried with anhydrous Na_2SO_4 and evaporated in vacuum. The oily residue was carefully stirred with 15 ml of acetone, and the mixture was kept at 0°C for 18 h. The precipitate was filtered off, giving 5.4 g (70%) of ZProLeuGlyNH$_2$, $[\alpha]_D^{20}$ −73° (c 1; EtOH), R_f 0.59 (S_3), mp 161-163°.

Preparation of HProLeuGlyNH$_2$. A. With vigorous stirring, a current of H_2 was passed through a solution of 4.2 g (10 mmole) of ZProLeuGlyNH$_2$ in 70 ml of absolute ethanol in the presence of a Pd catalyst until hydrogenation ceased (TLC). The reaction mixture was evaporated in vacuum. The residue was dissolved in 8.1 ml of MeOH, and then 33.0 ml of ether was added and the mixture was kept at 0°C for 18 h. The precipitate that had deposited was filtered off and was dried in vacuum to constant weight. This gave 2.3 g (80%) of HProLeuGlyNH$_2$, $[\alpha]_D^{20}$ −55.5° (c 1; H_2O), mp 120-121°, R_f 0.33 (S_1).

B. A solution of HCl in ethyl acetate (241 mmole of HCl) was added to a solution of 24.4 g (63.5 mmole) of BOCProLeuGlyNH$_2$ in 60 ml of ethyl acetate and 40 ml of MeOH cooled to 0°C, The reaction mixture was stirred for 2 h, and 500 ml of ether was added. The resulting precipitate was filtered off and was dried in vacuum over KOH to constant weight. The residue was treated with a solution of 100 ml of liquid ammonia in 250 ml of $CHCl_3$ at 0°c for 1 h. The precipitate of the peptide was filtered off and was recrystallized from MeOH with ether. This gave 12.6 g (70%) of HProLeuGlyNH$_2$, $[\alpha]_D^{20}$ −50° (c 1, H_2O).

Preparation of BOCCys(Bzl)ProOH. A suspension of 15.0 g (130 mmole) of L-proline in 250 ml of CH_2Cl_2, 50 ml of DMFA, and 19.8 ml (155 mmole) of chlorotrimethylsilane was stirred until the proline had dissolved completely (3 h). Then the solution was cooled to −20±5°C and, with stirring, 35.2 ml (254 mmole) of TEA was added. With vigorous stirring at −20±2°C the mixed anhydride obtained from 31.2 g (100 mmole) of BOCCys(Bzl)OH and 12.6 ml (104 mmole) of $(CH_3)_3$ CCOCl in 200ml of $CHCl_3$ and 14.3 ml (103 mmole) of TEA at −15±5°C over 20 min was added. The reaction mixture was stirred at −10±5°C for 2 h and at 0°C for 18 h. Then it was extracted with 3 × 100 ml of 1 N HCl and 2 × 100 ml of H_2O. The organic solution was dried with Na_2SO_4 and evaporated in vacuum. The oily product was dissolved in 140 ml of ether, and 100 ml of hexane was added to give a turbid solution, which was left at 0°C for 18 h. The resulting precipitate ws filtered off and was washed with 2 × 140 ml of hexane and dried to constant weight. This gave 38.8 g (95%) of BOCCys(Bzl)ProOH, $[\alpha]_D^{20}$ −64° (c 1; MeOH), mp 82-85, R_f 0.54 (S_4).

Preparation of BOCCys(Bzl)ProLeuGlyNH$_2$. A. The mixed anhydride obtained from 24.4 g (59.7 mmole) of BOCCys(Bzl)ProOH and 8.06 ml (62.0 mmole) of butyl chloroformate in 120 ml of CH_2Cl_2 and 8.54 ml (61.7 mmole) of TEA at −20±2°C with stirring for 12 min was added to a solution fo 14.6 g (65.2 mmole) of HCl·HLeuGlyNH$_2$ in 200 ml of $CHCl_3$ and 9.06 ml (65.5 mmole) of TEA cooled to −20°C. The reaction mixture was stirred at −10±5°C for 75 min and at 0°C for 18 h. Then the mixture was extracted with 70 ml of saturated $NaHCO_3$ solution and 2 × 100 ml of H_2O. The organic layer was dried with anhydrous Na_2SO_4 and was evaporated in vacuum to a volume of 250 ml, after which 400 ml of ether was added to give a turbid solution and this was left at 0°C for 18 h. The resulting precipitate was filtered off and washed with 150 ml of ether. The product was dried to constant weight in vacuum. This gave 24.1 g (70%) of BOCCys(Bzl)ProLeuGlyNH$_2$ (see Table 1).

B. A solution of 3.1 g (10 mmole) of BOCCys(Bzl)OH and 2.6 g (9.2 mmole) of HProLeuGlyNH$_2$ in 10 ml of DMFA cooled to −20°c was treated with 2.3 g (11 mmole) of DCHC, and the mixture was stirred until the DCHC had dissolved. The resulting solution was kept at 0°C for 2.5 days and was stirred at 18±5°C for 1 h. The precipitate that had formed was filtered off.

The solution was diluted with 15 ml of CH_2Cl_2 and was extracted with 15 ml of 1 N HCl, 5 ml of 30% MeOH solution, and 15 ml of H_2O. The organic solution was dried with anhydrous Na_2SO_4 and evaporated to a volume of 10 ml. Then 100 ml of ether was added and the mixture was left at 0°C for 18 h. The precipitate that had deposited was filtered off and was washed with 2 × 30 ml of ether and dried to constant weight. This gave 3.8 g (72%) of BOCCys(Bzl)-ProLeuGlyNH$_2$ (see Table 1).

C. In a similar way to paragraph B, 3.1 g (1.0 mmole) of BOCCys(Bzl)OH, 2.6 g (9.2 mmole) of HProLeuGlyNH$_2$, 2.3 g (11 mmole) of DCHC, and 1.5 g (11 mmole) of HBT in 10 ml of DMFA yielded 4.2 g (79%) of BOCCYS(Bzl)ProLeuGlyNH$_2$ (see Table 1).

D. In a similar way to paragraph C, but with the HBT replaced by 1.3 g (11 mmole) of 1-hydroxysuccinimide (HOSI), 3.0 g (57%) of BOCCys(Bzl)ProLeuGlyNH$_2$ was obtained (see Table 1).

E. A solution of 4.0 g (9.2 mmole) of BOCCys(Bzl)-O-p-PhNO$_2$ and 2.6 g (0.2 mmole) of HProLeuGlyNH$_2$ in 10 ml of DMFA was stirred for 2 h. Then 50 ml of H_2O was added to the reaction mixture and, after vigorous stirring, the resulting precipitate was filtered off and was washed with 4 × 20 ml of H_2O, 5 × 20 ml of saturated $NaHCO_3$ solution, and 3 × 20 ml of H_2O. The precipitate was dried in vacuum to constant weight, giving 4.6 g (87%) of BOCCys(Bzl)ProLeuNH$_2$ (see Table 1).

F. A solution of 2.5 g (12 mmole) of DCHC in 15 ml of CH_2Cl_2 cooled to −5°C was added to a solution of 6.8 g (22 mmole) of BOCCYs(Bzl)OH in 10 ml of CH_2Cl_2 also cooled to −5°C. The mixture was stirred for 2.5 h. The precipitate that deposited was filtered off and the solution of the symmetrical anhydride was cooled to −5°C, after which a solution of 2.6 g (9.2 mmole) of HProLeuGlyNH$_2$ in 15 ml of DMFA cooled to −5°C was added to it. The reaction mixture was stirred at 18±5°C for 24 h and then 50 ml of CH_2Cl_2 was added to dissolve the gelatinous precipitate. The remaining insoluble precipitate of dicyclohexylurea was filtered off. The solution was extracted with 2 × 50 ml of H_2O, dried with anhydrous Na_2SO_4, and evaporated in vacuum to half-volume. This residue was treated with 150 ml of ether and the mixture was kept at 0°C for 18 h. The resulting precipitate was filtered off, washed with 2 × 30 ml of ether, and dried under vacuum to constant weight. This gave 5.1 g (96%) of BOCCys(Bzl)ProLeuGlyNH$_2$ (see Table 1).

G. A solution of the mixed anhydride obtained form 3.1 g (10 mmole) of BOCCys(Bzl)OH and 1.35 g (11 mmole) of pivaloyl chloride in 15 ml of CH_2Cl_2, 1.5 ml (11 mmole) of TEA, and 0.9 ml (11 mmole) of pyridine at −15±2°C with a holding time of 10 min was added to a solution of 2.6 g (9.2 mmole) of HProLeuGlyNH$_2$ in DMFA cooled to −15°C. The reaction mixture was stirred at −7±2°C for 1 h and at 0°C for 18 h and was worked up as in paragraph B. This gave 4.3 g (82%) of BOCCys(Bzl)ProLeuGlyNH$_2$ (see Table 1).

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