Convenient High Yielding Gram Scale Solution Synthesis of Methionine–Enkephalin[1)]

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A simple, large-scale synthesis of a cytokine, methionine–enkephalin, Tyr–Gly–Gly–Phe–Met, has been elaborated. Classical solution peptide chemistry methods without protection of amino acid side-chain functions and 1+(2+2) segment condensation were used. A nine-step synthesis from commercial amino acid derivatives was developed with yields ranging from 86% to 99%, averaging 92%. The purity of all intermediates was found to be 99.0—100% by HPLC. The process has been used to prepare greater than 150 g quantities of the pentapeptide as a monohydrate of 100% purity. Hydantoin formation was observed during saponification of Boc–Tyr–Gly–Gly–Phe–Met–OMe and minimised.

Key words methionine–enkephalin; solution peptide synthesis; large scale peptide synthesis; hydantoin formation

Methionine–enkephalin, Tyr–Gly–Gly–Phe–Met, and leucine–enkephalin, Tyr–Gly–Gly–Phe–Leu, were first isolated from pig brain[2)] and identified in 1975.[3)] They belong to the family of opioid peptides which have been associated with regulation/modulation of a large number of physiological processes.[4,5)] It is well established that the opioid system affects a number of parameters related to innate and acquired immunity. Studies *in vivo* demonstrated biphasic effects, immunostimulatory or immunoinhibitory, depending on the particular opioid agonist, dose and route of administration, opioid receptor and immune response. Treatment *in vitro* also revealed differential modulation of macrophage and lymphocyte activities.[6)] Of the two enkephalins, methionine–enkephalin proved to be the more potent immunomodulator.[6,7)] Endogenous opioids are also of great significance with regards to the stress induced growth of tumors.[4)] It has been clearly shown that they are inhibitory to oncogenesis and that the key opioid peptide playing a role in cancer at physiologically relevant concentrations is methionine–enkephalin.[8)] Clinical studies have indicated that this molecule is capable of enhancing immune function in patients suffering from multiple sclerosis,[7,9)] cancer[6)] or AIDS.[6)] Recently, it has been proposed that methionine–enkephalin be classified as a cytokine.[6)]

The requirement for substantial quantities of methionine–enkephalin for investigation of its action on the immune system and clinical evaluation in autoimmune diseases, as well as for cancer research, led us to undertake a synthesis of the hormone. However, to the best of our knowledge, the literature describes syntheses of the peptide in milligram amounts only[10)] and does not suit our need for a large scale preparation. An efficient synthetic pathway and improved methods of purification must be developed for this bioactive peptide in order to attain its full potential. We therefore decided to elaborate such a process. To this end, the methods of choice[11—16)] are solution peptide chemistry, segment condensation with conventional inexpensive methods of coupling, and the concept of minimal protection of amino acid side-chain functions. Herein, we present a convenient synthesis of methionine–enkephalin, developed following these rules, which allows the preparation of this compound in i) substantial quantities, ii) high purity and iii) good overall yield.

Results and Discussion

The general plan for forming peptide bonds consists of 1+(2+2) condensations with the methyl ester as a permanent *C*-protection group and the Boc group for temporary *N*-protection, as detailed in Chart 1. Based on initial synthetic attempts, we recognized three key problems: 1) Boc removal, 2) Boc–Gly–Gly incorporation, and 3) methyl ester hydrolysis.

1) The usual acidolytic cleavage of the Boc group can be accompanied by the *tert*-butylation of the methionine residue.[17)] Boc removal was investigated on Boc–Phe–Met–OMe, the simplest of the three Boc-peptides dealt with. Various methods were tested; Tos/dioxane advocated for selective deprotection of alkylation-prone tryptophan containing peptides,[18)] HCl/dioxane used in a small scale methionine-enkephalin synthesis,[19)] and HCl/MeOH[12,16)] as well as HCl/AcOH/EtSH[20)] verified in large scale syntheses of some peptides. The first two reagents yielded impure crude material. Although the resulting alkylated side-product decomposes during storage of crude product in the solid form,[21,22)] especially at elevated temperature,[18,23a)] we were not completely successful in removing all impurities by heating at 50—55 °C (*cf.*[23a)]). On the other hand, HCl/MeOH and HCl/AcOH/EtSH led directly to HCl·H–Phe–Met–OMe of 98.5% and >99.1% purity, respectively, as determined by HPLC. The latter reagent yielded 99% of the product and further investigation established that the EtSH additive proved dispensable. However, HCl·H–Phe–Met–OMe is known to be hygroscopic.[24)] Exchange of the chloride for tosylate anion resolved this particular problem, giving a stable salt suitable for the next step without any handling difficulties. The HCl/AcOH was successfully applied to the removal of the Boc group from Boc-tetra- and Boc-pentapeptide, and the resulting pertinent hydrochloride salts were stable compounds.

2) The most conventional prosegment for Boc–Gly–Gly incorporation would be Boc–Gly–Gly–OMe or Boc–Gly–Gly–OEt. However, we found that they did not crystallize readily, and that saponification with alkali was accompanied by extensive side-reactions and that the resulting acid Boc–Gly–Gly–OH is water-soluble, rendering work-up rather troublesome. Another standard prosegment Boc-Gly-Gly-

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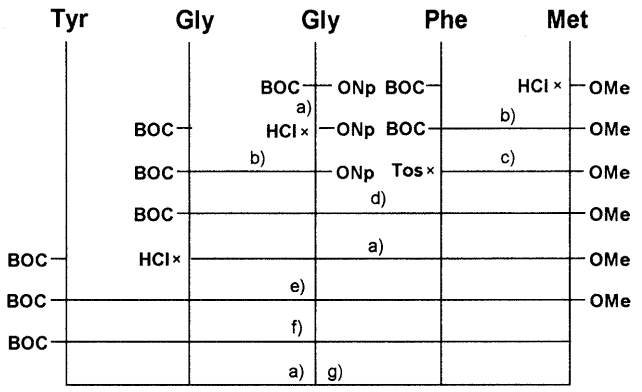

Chart 1. Synthesis of Methionine–Enkephalin

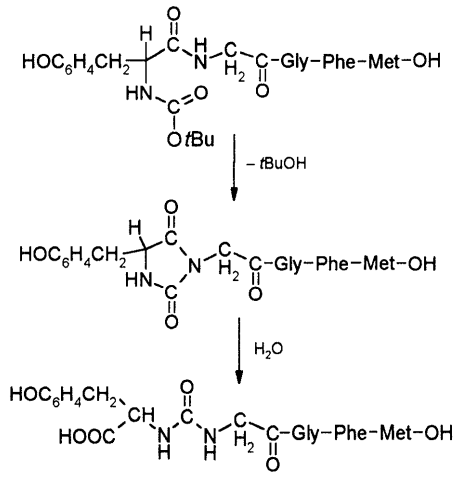

Chart 2. Formation of the Urea Derivative from Boc-Methionine–Enkephalin under the Influence of Aqueous NaOH

OBzl would require H_2/Pd–C for the Bzl group removal. The Pd catalyst is rather expensive and, moreover, the process demands investment in a stirred pressure reactor. Therefore, we turned to the so-called "backing off" approach, in which an active ester serves as a temporary *C*-protection,[23b] and chose the *p*-nitrophenyl ester. Boc–Gly–Gly–ONp was easily obtained from Boc–Gly–OH and HCl·H–Gly–ONp, in 86% yield and 99.5% purity, by means of ClCOO*iso*Bu and *N*-methylmorpholine, and proved to be very effective as a coupling agent. Aminolysis with H–Phe–Met–OMe in the presence of HOBt afforded 97% of Boc–Gly–Gly–Phe–Met–OMe of 99.2% purity as determined by HPLC.

3) Hydrolysis with aqueous alkali, the simplest and most practical method for cleaving methyl esters may be accompanied by various side-reactions.[17,23c,25] Among them, hydantoin formation at the alkoxycarbonyl protected *N*-terminus is particularly well known when glycine is the second amino acid in the sequence.[17,25—27] The hydantoin formed opens up with the production of a urea derivative.[17,25—27] Hydantoin formation is assumed not to take place,[25] and so far has not been observed,[28] if the alkyl group is the hindered, electron-donating *tert*-butyl, *i.e.* when we deal with the Boc protection. However, during hydrolysis of Boc–Tyr–Gly–Gly–Phe–Met–OMe with a standard 1 N aqueous NaOH solution, the formation of a small quantity of a side product was observed, which turned out to be the urea derivative, as presented in Chart 2. Advantageously, the amount of byproduct could be further reduced using more dilute, 0.5 N NaOH. Furthermore, the urea compound and the main product differ in acidity. This renders a mixture of their alkaline salts, formed upon saponification, separable by simple fractional acidification and an 86% isolated yield of Boc–Tyr–Gly–Gly–Phe–Met–OH of 99.0% purity was easily obtained.

The remaining problems, apart from those described above, were solved as follows. The reported syntheses of Boc–Phe–Met–OMe by conventional means using the ONp ester,[24] DCC[19,24] or DCC–HOBt[29—31] give 70[29]—86%[30] yields after standard peptide work-up. We applied the mixed anhydride method with ClCOO*iso*Bu in the presence of *N*-methylmorpholine. A 92% yield of the product with 100% purity was obtained by precipitation of the peptide with aqueous $NaHCO_3$ from the reaction mixture. Boc–Tyr–OH was best incorporated with DCC–HOBt. Contrary to the literature,[19] we did not use an excess of Boc–Tyr–OH, yet obtained a better yield, 90%, of chromatographically homogenous (99.7%) Boc–Tyr–Gly–Gly–Phe–Met–OMe. After removing both terminal protecting groups, the final product, methionine–enkephalin was liberated from its amino group salt and purified using various chromatographic processes.[10] In this synthesis, the final operation of free basing the peptide from the hydrochloride salt, associated with simultaneous purification was performed simply and inexpensively using triethylamine in an aqueous solution, from which the free peptide crystallizes easily to give a 90% yield of 100% pure product as a monohydrate.

In summary, starting from commercial amino acid derivatives the elaborated synthesis of methionine–enkephalin consists of nine steps, with yields ranging from 86% to 99% per step. All intermediates were of 99.0—100% purity as determined by HPLC. The synthesis was checked by preparing over 150 g of the pentapeptide in one batch.

Experimental

General Experimental Procedures ClCOO*iso*Bu and NMM were of 98% purity. Purified solvents were stored over drying agents. Reactions were monitored, and the preliminary homogeneity of products was checked on silica gel plates (DC Alufolien Kieselgel, 0.25 Merck #5553) using the following solvent systems (v/v): A, $CHCl_3$: acetone (1 : 1); B, $CHCl_3$: MeOH (9 : 1); C, $CHCl_3$: MeOH : NH_4 concd. (6 : 5 : 1); D, $CHCl_3$: MeOH : dioxane : NH_4 concd. (12 : 7 : 5 : 1); E, $CHCl_3$: MeOH : AcOH (95 : 5 : 3); F, $CHCl_3$: MeOH : AcOH (90 : 8 : 2); G, $CHCl_3$: MeOH : AcOH : H_2O (60 : 18 : 2 : 3); H, *n*-BuOH : AcOH : H_2O (4 : 1 : 1); I, AcOEt : *n*-BuOH : AcOH : H_2O (1 : 1 : 1 : 1); J, C_6H_6 : MeOH:acetone : pyridine : AcOH (24 : 4 : 2 : 2 : 1); K, AcOEt : pyridine: AcOH : H_2O (50 : 20 : 6 : 11); L, AcOEt : pyridine : AcOH : H_2O (30 : 20 : 6 : 11). Solvents from reaction mixtures were removed *in vacuo* on a rotary evaporator at a bath temperature not exceeding 30 °C. Mps were determined on a Böetius heating block and are uncorrected. HPLC analyses were carried out using a Beckman System Gold chromatograph, a 5 μl loop, an Alltech Alltima, C_{18}, 5 μ, 150×4.6 mm column, detection at 210 nm and a flow rate of 1 ml/min.

Methyl *N*-*tert*-Butoxycarbonyl-L-phenylalanyl-L-methioninate (Boc–Phe–Met–OMe) To a stirred solution of Boc–Phe–OH (239 g, 0.90 mol) and NMM (101.1 ml, 0.90 mol) in DMF (1.2 l), placed in a 5 l reactor and cooled to −20 °C, ClCOO*iso*Bu (120.6 ml, 0.90 mol) was added at a rate such that the temperature remained at −15—(−)20 °C. After a further 10 min stirring, HCl·H–Met–OMe (179.7 g, 0.90 mol) and NMM (101.1 ml, 0.90 mol) were introduced. Stirring was continued at −20 °C for 20 h. 1 M aqueous $NaHCO_3$ (1.0 l) was then added in portions followed by water (900 ml). The mixture was left standing for 1 h and the resultant precipitate

filtered, washed abundantly with water and dried to give Boc–Phe–Met–OMe (340 g, 92%), mp 84—85 °C (lit. mps range from 72—73 °C[30] to 93—95 °C[31]); *Rf* (C) 0.74, *Rf* (F) 0.77; t_R (0.1% TFA : acetonitrile (35 : 65), v/v) 3.79 min, 100% purity.

Methyl L-Phenylalanyl-L-methioninate Tosylate Sesquihydrate (Tos·H–Phe–Met–OMe·1.5H$_2$O) Boc–Phe–Met–OMe (308 g, 0.75 mol), placed in a 5 l reactor was dissolved in 1.5 N HCl/AcOH (2.5 l, 3.75 mol) and left standing for 1 h. Tos·H$_2$O (150 g, 0.788 mol) was then added, the solvent evaporated, Et$_2$O (3.5 l) added and the solid filtered to yield the title compound (344 g, 90%), mp 181—183 °C; *Rf* (D) 0.80, *Rf* (G) 0.67; t_R (0.1% TFA : acetonitrile (70 : 30), v/v) 3.88 min, 99.24% purity. *Anal.* Calcd for $C_{15}H_{22}N_2O_3S \cdot CH_3C_6H_4SO_3H \cdot 1.5H_2O$: C, 51.85; H, 6.52; N, 5.50. Found: C, 52.17; H, 6.25; N, 5.54.

***p*-Nitrophenyl Glycinate Hydrochloride (HCl·H–Gly–ONp)** Boc–Gly–ONp (222 g, 0.75 mol) was placed in a 5 l reactor and dissolved in 1.7 N HCl/AcOH (3.75 l, 6.38 mol) and left standing for 1 h. The solvent was then evaporated, Et$_2$O added (2.75 l) and the solid filtered to give HCl·H–Gly–ONp (173 g, 99%), mp 170—173 °C; t_R (0.1% TFA : acetonitrile (80 : 20), v/v) 4.73 min, 100% purity. *Anal.* Calcd for $C_8H_8N_2O_4 \cdot HCl$: C, 41.30; H, 3.90; N, 12.05. Found: C, 40.92; H, 3.81; N, 12.10.

***p*-Nitrophenyl *tert*-Butoxycarbonylglycylglycinate (Boc–Gly–Gly–ONp)** To a stirred solution of Boc–Gly–OH (131.4 g, 0.75 mol) and NMM (84.1 ml, 0.75 mol) in $CHCl_3$ (1.5 l) in a 3 l reactor and cooled to −15 °C, ClCOO*iso*Bu (100.5 ml, 0.75 mol) was added at a rate such that the temperature remained at −10—(−)15 °C. After a further 10 min, HCl·H–Gly–ONp (174.5 g, 0.75 mol) and NMM (84.1 ml, 0.75 mol) were introduced. Stirring was continued at −15 °C for 4 h. The mixture was then washed with 5% aqueous $KHSO_4$, brine, 5% aqueous $NaHCO_3$ and brine and dried over Na_2SO_4. The solvent was evaporated and the residue crystallized from $CHCl_3$/*n*-hexane to give Boc–Gly–Gly–ONp (227 g, 86%), mp 110—111 °C (lit.[32] mp 110—111 °C); *Rf* (A) 0.72, *Rf* (B) 0.59, *Rf* (E) 0.58; t_R (0.1% TFA : acetonitrile (60 : 40), v/v) 7.83 min, 99.50% purity.

Methyl *N*-*tert*-Butoxycarbonyldiglycyl-L-phenylalanyl-L-methioninate (Boc–Gly–Gly–Phe–Met–OMe) To a stirred solution of Tos·H–Phe–Met–OMe·1.5H$_2$O (275 g, 0.54 mol), HOBt·H$_2$O (82.7 g, 0.54 mol) and Boc–Gly–Gly–ONp (191 g, 0.54 mol) in DMF (820 ml) in a 3 l reactor, NEt_3 (74.8 ml, 0.54 mol) was added dropwise. Stirring was continued for 23 h and the DMF evaporated. To the residue, 5% aqueous $NaHCO_3$ (2.5 l) was added and the product was extracted with EtOAc. The organic layer was washed with brine, 5% aqueous $KHSO_4$ and brine, dried over Na_2SO_4 and evaporated. Et$_2$O (1.6 l) was added to the residue, and the solid filtered to give Boc–Gly–Gly–Phe–Met–OMe (275 g, 97%), mp 99—102 °C (lit.[16] mp 100—105 °C, lit[33] mp 106 °C); *Rf* (E) 0.40, *Rf* (J) 0.64; t_R (0.1% TFA : acetonitrile (60 : 40), v/v) 7.92 min, 99.20% purity.

Methyl Diglycyl-L-phenylalanyl-L-methioninate Hydrochloride (HCl·H–Gly–Gly–Phe–Met–OMe) Boc–Gly–Gly–Phe–Met–OMe (262 g, 0.50 mol) in a 5 l reactor was dissolved in 1.5 N HCl/AcOH (2.7 l, 4.1 mol) and left standing for 1 h. The solvent was then evaporated, Et$_2$O added (1.8 l) and the solid filtered to give HCl·H–Gly–Gly–Phe–Met–OMe (219 g, 95%), mp 202—206 °C (lit.[19] mp 183—187 °C); *Rf* (D) 0.57, *Rf* (G) 0.45; t_R (water : acetonitrile (70 : 30), v/v) 3.47 min, 99.10% purity.

Methyl *N*-*tert*-Butoxycarbonyl-L-tyrosyldiglycyl-L-phenylalanyl-L-methioninate (Boc–Tyr–Gly–Gly–Phe–Met–OMe) To a stirred solution of Boc–Tyr–OH (141 g, 0.50 mol), HCl·H–Gly–Gly–Phe–Met–OMe (231 g, 0.50 mol) and HOBt·H$_2$O (76.6 g, 0.50 mol) in DMF (1.0 l) in a 3 l reactor and cooled to −10 °C, NEt_3 (70 ml, 0.50 mol) was added dropwise followed by DCC (103 g, 0.5 mol) dissolved in DMF (100 ml). Stirring was continued at −5—0 °C for 1 h and at 20 °C for 24 h. Solid was then filtered and DMF evaporated. The residue, dissolved in 1 M $NaHCO_3$ was extracted with $CHCl_3$ and the organic layer washed with 1 M $NaHCO_3$, water, 5% aqueous $KHSO_4$ and water. The solvent was evaporated and the residue crystallized from acetone to give the protected pentapeptide (309 g, 90%), mp 147—148 °C (lit.[19] mp 112—114°C, lit.[34] mp 113—115 °C); *Rf* (F) 0.27, *Rf* (J) 0.49; t_R (0.1% TFA : acetonitrile (60 : 40), v/v) 8.18 min, 99.67% purity.

***N*-*tert*-Butoxycarbonyl-L-tyrosyldiglycyl-L-phenylalanyl-L-methionine (Boc–Tyr–Gly–Gly–Phe–Met–OH)** To a solution of Boc–Tyr–Gly–Gly–Phe–Met–OMe (241 g, 0.35 mol) in acetone (730 ml) in a 5 l reactor was added 0.5 M NaOH (1.4 l), and followed after 1.5 h by 1 M HCl (750 ml). The solvents were evaporated and the residue dissolved in 5% aqueous $NaHCO_3$ was extracted with EtOAc (2×500 ml). The aqueous layer was acidified with 1 M HCl to pH 4—5 and extracted with EtOAc. The organic layer was dried over Na_2SO_4 and evaporated and the product crystallized from EtOAc to give the pentapeptide (203 g, 86%), mp 153—155 °C (lit. mps range from 108—112 °C[35] to 149—151 °C[19]); *Rf* (G) 0.61; t_R (0.1% TFA : acetonitrile (60 : 40), v/v) 4.49 min, 99.02% purity.

***N*-[1-Carboxy-2-(4-hydroxyphenyl)ethylaminocarbonyl]diglycyl-L-phenylalanyl-L-methionine (4-HOC$_4$H$_4$–CH$_2$CH(COOH)NHCO–Gly–Gly–Phe–Met–OH)** The aqueous layer left after the extraction of Boc–Tyr–Gly–Gly–Phe–Met–OH was acidified to pH 3 and extracted with EtOAc. The organic layer was washed with water, dried over Na_2SO_4 and evaporated. The residue was crystallized from MeOH/EtOAc to give the title compound (18.8 g, 8.7%), mp 180—183 °C; *Rf* (G) 0.21, *Rf* (H) 0.60, *Rf* (K) 0.34. ^{13}C-NMR (Tesla BS 567, 25.142 MHz, DMSO-d_6 with TMS) δ: characteristic of the carbonyl groups 157.53 (NHCONH), 168.51, 170.23, 170.98, 172.85, 173.67 (2×COOH, 3×CONH). LSIMS(+) (AMD-604 Intectra, Germany, in a 5 : 1 mixture of dithiothreitol and dithioerythritol) *m/z*: 618 (15, $C_{28}H_{35}N_5O_9S$ requires 618, M+H$^+$), 205 (7), 182 (4, Tyr+H$^+$), 177 (7, COMet+H$^+$), 133 (21), 120 (100, PhCH$_2$CHNH+H$^+$).

L-Tyrosyldiglycyl-L-phenylalanyl-L-methionine Monohydrate (H–Tyr–Gly–Gly–Phe–Met–OH·H$_2$O) Boc–Tyr–Gly–Gly–Phe–Met (202 g, 0.30 mol), placed in a 5 l reactor was dissolved in 1.3 M HCl/AcOH (2.2 l, 2.9 mol) and after 1 h the solvent evaporated. To the residue, Et$_2$O (2.2 l) was added and the resulted precipitate filtered, dissolved in water (2.0 l) and NEt_3 (42 ml, 0.30 mol) was added to give after overnight standing crystals of the free pentapeptide in the monohydrate form (159 g, 90%), mp 199—200 °C. *Rf* (C) 0.61, *Rf* (G) 0.22, *Rf* (I) 0.69, *Rf* (L) 0.73; t_R (0.1% TFA : acetonitrile (77 : 23), v/v) 6.44 min, 100% purity. ^{1}H-NMR (Tesla BS 567, 100 MHz; DMSO-d_6 with TMS) δ: characteristic of the particular amino acid residues 2.02 (3H, s, SCH_3), 3.72 (4H, m, 2NCH_2), 6.68, 7.02 (4H, 2d, J=8.4 Hz, C_6H_4), 7,24 (5H, s, C_6H_5), 8.60 (1H, br s, Tyr(OH)). *Anal.* Calcd for $C_{27}H_{35}N_5O_7S \cdot H_2O$: C, 54.80; H, 6.30; N, 11.84. Found: C, 54.89; H, 6.38; N, 11.72.

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References and Notes

1) Abbreviations: Tyr=tyrosine, Gly=glycine, Phe=phenylalanine, Met=methionine, Boc=*tert*-butoxycarbonyl, OBzl and ONp=benzyl and *p*-nitrophenyl ester, respectively, ClCOO*iso*Bu=isobutyl chlorocarbonate, DCC=dicyclohexylcarbodiimide, EtSH=ethanethiol, HOBt=1-hydroxybenzotriazole, NMM=*N*-methylmorpholine, TFA=trifluoroacetic acid, Tos=*p*-toluenesulfonic acid.
2) Hughes J., *Brain Res.*, **88**, 195—208 (1975).
3) Hughes J. A., Smith T. W., Kosterlitz H. W., Fothergill L. A., Morgan B. R., Morris H. R., *Nature* (London), **258**, 577—579 (1975).
4) König W., "Peptide and Protein Hormones. Structure, Regulation, Activity," Verlagsgesellschaft, Weinheim, 1993, p. 72, and pertinent references therein.
5) Anonym, "Bachem Information Sheet. BA 040/01," Bachem, Bubendsdorf, 1993, pp. 1—67 and pertinent references therein.
6) Plotnikoff N. P., Faith R. E., Murgo A. J., Herberman R. B., Good R. A., *Clin. Immunol. Immunopathol.*, **82**, 93—101 (1997) and references therein.
7) Jankovič B. D., *Acta Neurol.*, **13**, 433—441 (1991) and references therein.
8) Zagon I. S., McLaughlin P. J., *Cancer J.*, **3**, 101—103 (1990) and references therein.
9) Janković B. D., Lević Z., Nikolić J., Stojsavljević N., *Int. J. Neurosci.*, **48**, 155—155 (1989).
10) There are many syntheses of methionine-enkephalin described and it is not possible to cite all of them. Therefore, we refer interested readers to the series of specialist annual reports "Amino Acids, Peptides, and Proteins," Vol. 1—27 and pertinent chapters therein, ed. by Young G. T., Sheppard R. C., Jones J. H., Davies J. S., The Royal Society of Chemistry, Cambridge, 1969—1996. Here we cite only those, which are closely related to this work (refs. 19 and 34).
11) Gorup B., *Biochem. Soc. Trans.*, **18**, 1299—1306 (1990).
12) *a*) Masiukiewicz E., Rzeszotarska B., *J. Prakt. Chem.*, **333**, 41—47 (1991); *b*) Masiukiewicz E., Rzeszotarska B., Pol. Patent, 161342 (1993) [*Chem. Abstr.*, **120**, 19231*w* (1994)].
13) Brady S. F., Sisko J. T., Ciccarone T. M., Colton C. D., Levy M. R., Witherup K. M., Duggan M. E., Payack J. F., Moreno O. A., Egbertson M. S., Hartman G. D., Halczenko W., Laswell W. L., Lee T-J., Holtz W. J., Hoffman W. F., Stokker G. E., Smith R. L., Veber D. F., Nutt R. F., "Peptides. Proc. 12th Am. Pept. Symp.," ed. by Smith J. A., Rivier J. E., Escom, Leiden, 1992, pp. 657—660.

14) Velling J., Slot L. A., Pedersen O., Andersen A. J., "Peptides. Proc. 12th Am. Pept. Symp.," ed. by Smith J. A., Rivier J. E., Escom, Leiden, 1992, pp. 664—665.
15) Friedrich A., Jäger G., Radscheit K., Uhmann R., "Peptides 1992. Proc. 22nd Eur. Pept. Symp.," ed. by Schneider C. H., Eberle A. N., Escom, Leiden, 1993, pp. 47—48.
16) *a*) Masiukiewicz E., Rzeszotarska B., Wiejak S., *Polish J. Chem.*, **69**, 674—680 (1995); *b*) Masiukiewicz E., Rzeszotarska B., Pol. Patent, 267046 (1996) [*Chem. Abstr.*, **124**, 146870*j* (1996)].
17) Bodanszky M., Martinez J., "The Peptides. Analysis, Synthesis, Biology," Vol. 5, ed. by Gross E., Meienhofer J., Academic Press, New York, 1983, pp. 111—216 and references therein.
18) Suzuki K., Endo N., Nitta K., Sasaki Y., *Chem. Pharm. Bull.*, **26**, 2198—2204 (1977).
19) Losse G., Wehrstedt K-D., *J. Prakt. Chem.*, **320**, 963—966 (1978) and references therein.
20) Henklein P., Boomgaarden M., Nieke E-M., Georgi M., Niedrich H., *Pharmazie*, **43**, 10—12 (1988).
21) Noble R. L., Yamashiro Y., Li C. H., *J. Am. Chem. Soc.*, **98**, 2324—2328 (1976).
22) Bienert M., Klauschenz E., Nikolics K., Niedrich H., *Int. J. Peptide Protein Res.*, **19**, 310—314 (1982).
23) Bodanszky M., "Principles of Peptide Synthesis," 2nd ed., Springer Verlag, Berlin, 1993; *a*) p. 296; *b*) pp. 85—86; *c*) p. 79.
24) Schattenkerk C., Voskuyl-Holtkamp I., Bokhorst R., *Rec. Trav. Chim. Pays-Bas*, **92**, 92—116 (1973).
25) Deimer K.-H., Thamm P., Stelzel P., "Methoden der Organische Chemie (Houben-Weyl)," Vol. XV/1, ed. by Wünsch E., Georg Thieme Verlag, Stuttgart, 1974, pp. 315—450.
26) Roeske R. W., "The Peptides. Analysis, Synthesis, Biology," Vol. 3, ed. by Gross E., Meienhofer J., Academic Press, New York, 1981, pp. 101—136.
27) Masiukiewicz E., Rzeszotarska B., Szczerbaniewicz J., Cierniewski C., *Polish J. Chem.*, **67**, 1035—1041 (1993).
28) Humprey J. M., Chamberlin R A., *Chem. Rev.*, **97**, 2243—2266 (1997) and references therein.
29) Kessler H., Hoelzemann G., *Liebigs Ann. Chem.*, **1981**, 2028—2044.
30) Davidovich Y. A., Il'ina A. V., Rogozhin S. V., *Izv. Akad. Nauk SSSR, Ser. Khim.*, **1981**, 422—424.
31) Lipkowski A. W., Misicka A., Drabarek S., *Polish J. Chem.*, **55**, 813—818 (1981).
32) Eberle A. N., Hübscher W., Schwyzer R., *Helv. Chim. Acta*, **60**, 2895—2910 (1977).
33) Dourtoglou V., Gross B., Lambropoulou B., Ziodrou C., *Synthesis*, **1984**, 572—574.
34) Ghosh S. K., Verma R., Ghosh U., Mamdapur V. R., *Bull. Chem. Soc. Jpn.*, **69**, 1705—1711 (1996).
35) Niu C. I., Yang S. Z., Chen Y. N., Ma A. Q., Shi P. T., Jiang Y. Q., Xu L. G., Huang W. T., "Peptides. Proc. 7th Am. Pept. Symp.," ed. by Rich D. H., Gross E., Pierce Chemical Co., Rockford, 1981, pp. 143—152.