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Synthesis of fragments of human β-lipotropin, β_h-LPH. Part VI[1]. The synthesis of des-1-tyrosine-α-endorphin and shortened peptides

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Abstract. The synthesis of des-1-tyrosine-α-endorphin and fourteen fragments of this psychostimulant type of neuropeptide is described. In these syntheses use has been made of the classical fragment condensation approach.

Introduction

In 1978 *De Wied* et al. reported on the opposite effects of α- and γ-endorphin on extinction of pole-jumping avoidance behaviour in rats[1]. Whereas γ-endorphin-derived peptides accelerated this extinction, α-endorphin, and also β-endorphin and Met-enkephalin, delayed the extinction[1,2]. Based on these and other results it was suggested by *De Wied* that des-tyrosine[1]-γ-endorphin or a closely related peptide is an endogenous neuroleptic-like neuropeptide[1]. Structure-activity studies of the γ-endorphin-type peptides have demonstrated that the fragment β-endorphin-(6–17)* is the shortest sequence with full neuroleptic-like activity[3].

α-Endorphin and its non-opioid analogue des-Tyr[1]-α--endorphin [*i.e.* β-endorphin-(2–16)] have been shown to possess effects opposite to γ-endorphin-derived peptides in a number of test situations: active[1] and passive[6,7] avoidance behaviour tests, effects on electrical self--stimulation (substantia nigra area)[8], on α-methyl-*p*--tyrosine-induced catecholamine disappearance in rat brain nuclei[9], and on the influence on dopamine-induced contraction of the rat rectum in vitro[10]. Since α-endorphin in several aspects induces effects in behavioural tests similar to those of amphetamine, it was postulated by *De Wied* that α-endorphin or a related peptide represents a psychostimulant type of neuropeptide[11].

The influence of the chain length of α-endorphin-related peptides on extinction of pole-jumping behaviour[6,12], on apomorphine-induced stereotyped sniffing in rats[13] and on the inhibition of the dopamine response in the rat rectum contraction[10] has been studied. In all of these studies as well as in metabolism studies[14,15] use was made of highly purified synthetic peptides. The synthesis by the fragment condensation approach and the purification of fifteen peptides with a primary structure contained in α-endorphin are described in this paper, part VI of our series Synthesis of fragments of human β-lipotropin.

Strategy and description of the synthesis

The strategy of the classical approach of solution synthesis, as outlined in part I of this series[16] is also applicable for the synthesis of the following fifteen peptides, β-endorphin fragments 2–5 (**I**), 2–6 (**II**), 2–7 (**III**), 2–8 (**IV**), 2–9 (**V**), 4–9 (**VI**), 5–9 (**VII**), 6–9 (**VIII**), 2–11 (**IX**), 2–13 (**X**), 2–15 (**XI**), 2–16 (**XII**), 6–16 (**XIII**), 10–16 (**XIV**) and 14–16 (**XV**). *tert*-Butyl-derived protecting groups were used for the side-chains of Glu and Lys and, incidentally, Thr, and also for the terminal NH_2 and COOH groups**. The benzyloxycarbonyl function was used for temporary blocking of the α-NH_2 group.

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[1] For part V, see This Journal **101**, 451 (1982).

* On previous occasions we have used the numbering system of β_h-LPH-(1–91). Recent changes in both length and sequence[4] (β_h-LPH is now considered to be a 89-peptide) have been reported. In order to avoid confusion by using the β-LPH numbering system we will now indicate the endorphin fragments by starting with Tyr[1] and ending with Glu[31] (in the case of β_h-endorphin). For the most recent amino acid sequence of β_h-LPH, see ref. 5.

** Standard abbreviations are used for amino acids and protecting groups [IUPAC-IUB Commission on Biochemical Nomenclature, Biochem. J. **126**, 773 (1972)]. For other abbreviations see part I of this series[16].

[1] *D. de Wied, G. L. Kovács, B. Bohus, J. M. van Ree* and *H. M. Greven*, Eur. J. Pharmacol. **49**, 427 (1978).

[2] *D. de Wied, B. Bohus, J. M. van Ree* and *I. Urban*, J. Pharmacol. Exp. Ther. **204**, 570 (1978).

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[6] *H. M. Greven* and *D. de Wied* in Hormones and the Brain, eds. *D. de Wied* and *A. van Keep*, MTP Press Ltd., Lancaster, England, 1980, p. 115.

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[8] *D. M. Dorsa, J. M. van Ree* and *D. de Wied*, Pharmacol. Biochem. & Behav. **10**, 899 (1979).

[9] *D. H. G. Versteeg, G. L. Kovács, B. Bohus, E. R. de Kloet* and *D. de Wied*, Brain Res. **231**, 343 (1982).

[10] *F. P. Nijkamp, J. M. van Ree, J. G. Nijssen, M. Versluis* and *D. de Wied*, N.-S. Arch. Pharmacol. **321**, 213 (1982).

[11] *D. de Wied* in Characteristics and Function of Opioids, eds. *J. M. van Ree* and *L. Terenius*, Elsevier/North-Holland Biomedical Press, Amsterdam, 1978, p. 113.

[12] *J. W. van Nispen* and *H. M. Greven*, Pharmacol. Ther. **16**, 67 (1982).

[13] *J. M. van Ree*, Neuropharmacol. **21**, 1103 (1982).

[14] *J. P. H. Burbach, J. G. Loeber, J. Verhoef* and *E. R. de Kloet*, Biochem. Biophys. Res. Commun. **92**, 725 (1980).

[15] *H. Schoemaker, A. Chen, T. P. Davis* and *H. I. Yamamura*, Psychopharmacol. Bull. **18**, 144 (1982).

[16] *W. A. A. J. Bijl, J. W. van Nispen* and *H. M. Greven*, Recl. Trav. Chim. Pays-Bas **98**, 571 (1979).

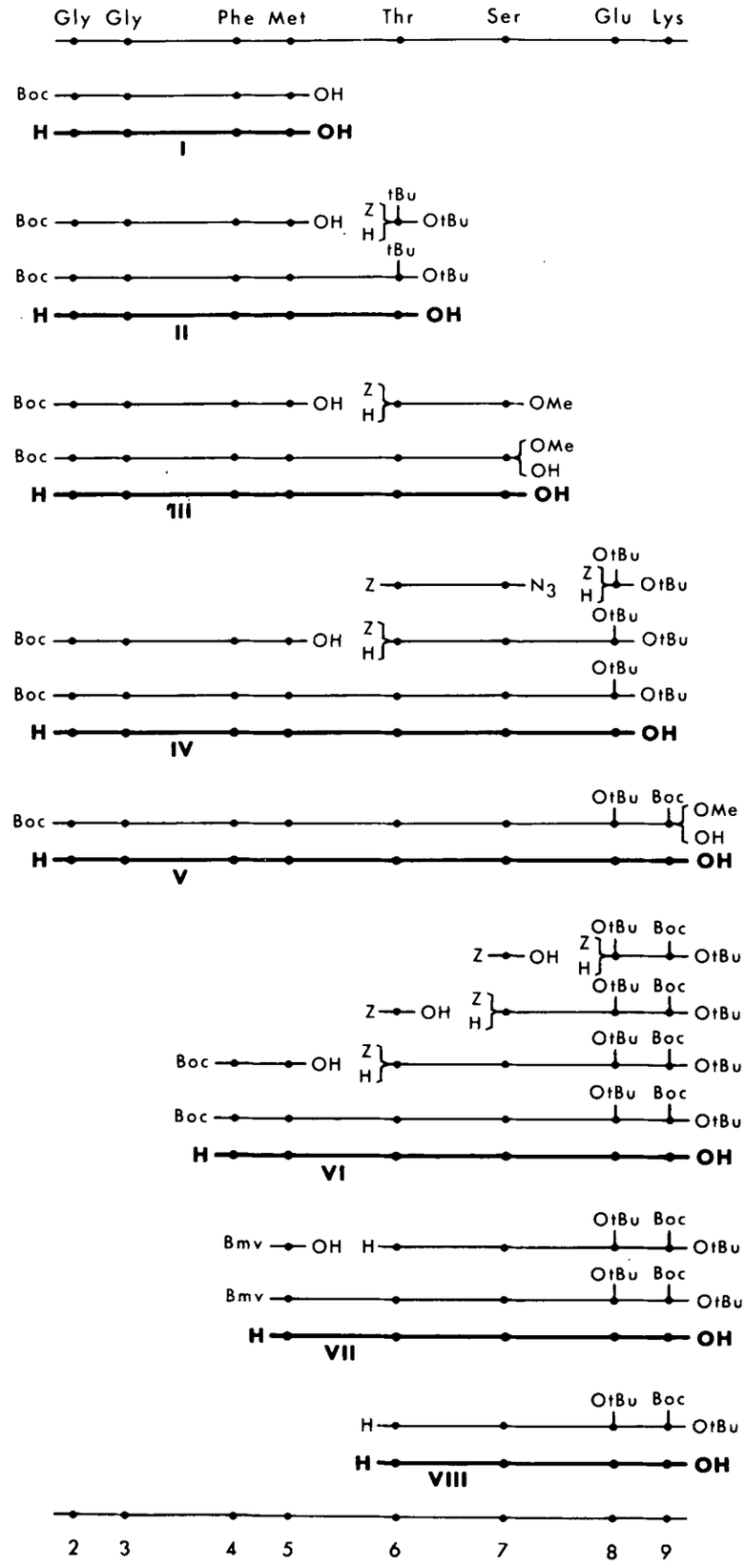

Fig. 1. Schematic representation of the synthesis of β*-endorphin-(2–9)* (**V**), *and seven smaller peptides.*

The synthesis of eight peptides related to the fragment 2–9 is outlined in Fig. 1. Stepwise shortening from the C--terminus to fragment 2–5 (*i.e.* [des-Tyr[1]]Met-enkephalin) gives Boc-Gly-Gly-Phe-Met-OH as a common intermediate in the synthesis[17]. DCC and HOBt** were used in the final coupling steps of this Boc-tetrapeptide acid with H-Thr(tBu)-OtBu, H-Thr-Ser-OMe, H-Thr-Ser-Glu-(OtBu)-OtBu and H-Thr-Ser-Glu(OtBu)-Lys(Boc)-OMe to give the protected precursor peptide for **II**, **III**, **IV** and **V**, respectively. For the synthesis of the three peptides ending with Lys[9], *i.e.* fragments 4–9, 5–9 and 6–9, H-Thr-Ser--Glu(OtBu)-Lys(Boc)-OMe or the corresponding -OtBu ester was used as the common intermediate and acylated with the relevant N-terminal peptides.

[17] *H. M. Greven, W. A. A. J. Bijl* and *J. W. van Nispen*, Recl. Trav. Chim. Pays-Bas **99**, 63 (1980).

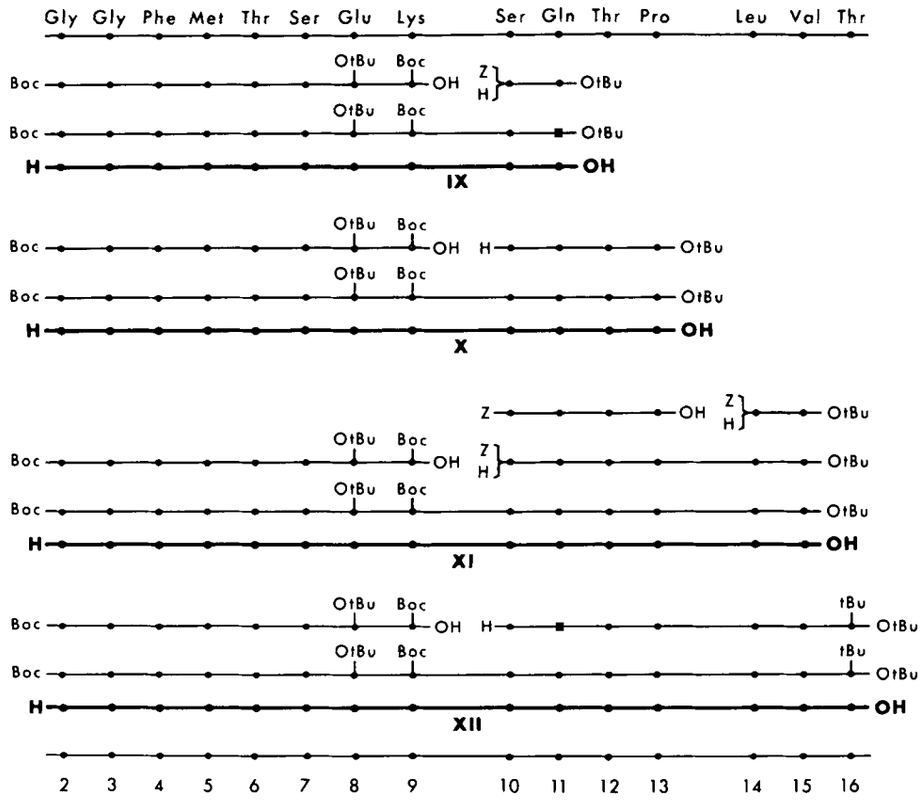

Fig. 2. Schematic representation of the synthesis of des-Tyr[1]*-α-endorphin* (**XII**)*, and three C-terminal-shortened fragments.*

The octapeptide Boc-Gly-Gly-Phe-Met-Thr-Ser-Glu-(OtBu)-Lys(Boc)-OMe[17] (see Fig. 1) was treated with NaOH in aqueous dioxane to give the Boc-peptide acid[17] which was used on the one hand to give peptide **V** and on the other hand to serve as the common intermediate in the synthesis of the longer fragments 2–11, 2–13, 2–15 and 2–16 (see Fig. 2). DCC and HOBt were again used for coupling.

Part of Z-Ser-Gln-Thr-Pro-OtBu[16] was hydrogenated followed by coupling with Boc-2–9-OH to give the protected peptide **X**, and part was treated with acid[16] and then used in the coupling step with H-Leu-Val-OtBu or H-Leu-Val-Thr(tBu)-OtBu[16].

Des-enkephalin-α-endorphin [β-endorphin-(6–16)] was obtained, as shown in Fig. 3, by elongation of H-10–16--OtBu[16] with Z-Lys(Boc)-, Z-Glu(OtBu)- and finally Z-Thr-Ser-. The C-terminal protected peptides 10–16 and 14–16 were obtained as described before[16].

Removal of the protecting groups gave the crude peptides which were purified by counter current distribution (Craig partition) or chromatography on silica gel (Merck Fertigsäule). The final products were checked for purity by TLC, HPLC, amino acid analysis, L-amino acid oxidase digestion and isotachophoresis.

Experimental

The purity of the amino acid derivatives and peptides was checked by thin-layer chromatography (TLC) on Merck silicagel plates (F. 254, 0.25 mm) using the following solvent systems (ratios are v/v);

(*a*) methylene chloride/methanol/water = 70/30/5
(*b*) methylene chloride/methanol = 4/1
(*c*) methylene chloride/methanol = 9/1
(*d*) chloroform/methanol = 4/1
(*e*) toluene/ethanol = 4/1
(*f*) toluene/ethanol = 7/3
(*g*) 1-butanol/pyridine/acetic acid/water = 8/3/1/4
(*h*) 1-butanol/pyridine/acetic acid/water = 16/3/1/4
(*i*) 1-butanol/pyridine/acetic acid/water = 4/1/1/2
(*j*) 1-butanol/pyridine/acetic acid/water = 30/50/6/25
(*k*) 1-butanol/pyridine/acetic acid/water = 40/30/5/20
(*l*) 1-butanol/acetic acid/water = 16/1/1
(*m*) 1-butanol/acetic acid/water = 2/1/1

UV light (254 nm), fluorescamine and chlorine/*o*-tolidine were used for the detection of components on TLC plates. The description of other methods is given in part I[16]. The water content of the end-products was determined by a titration with the Karl Fisher reagent according to the "dead-stop method"[18]. The

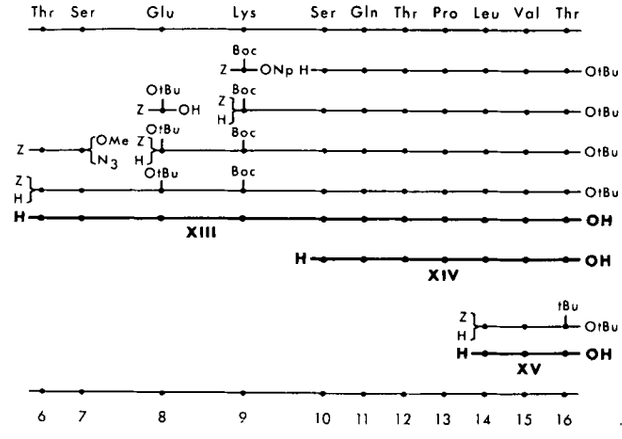

Fig. 3. Schematic representation of the synthesis of the endorphin fragments 6–16 (**XIII**)*, 10–16* (**XIV**) *and 14–16* (**XV**).

[18] *J. J. Lingane*, Electroanalytical Chemistry, Interscience Publ. Inc., New York, 1958, p. 280.

protected intermediates in the synthesis of several α-endorphin fragments (see also Fig. 1–3).

Peptide	Method; yield after purification	TLC, R_f value (system)	M.p. (°C)	$[\alpha]_D^{21}$ (c
ly-Phe-Met-OH	aqueous NaOH/dioxane; 94.3% (ref. 17)	0.65 (*h*)	116–124	−14.3°
ly-Phe-Met-Thr(tBu)-OtBu	DCC/HOBt; 36.1%	0.90 (*b*)	213–215	− 7.0°
ly-Phe-Met-Thr-Ser-OMe	DCC/HOBt; 66.0%	0.68 (*a*)	203–204 (dec)	− 7.9°
ly-Phe-Met-Thr-Ser-OH	aqueous NaOH/dioxane; 59.4%	0.43 (*a*)	130–135 (dec)	− 1.9°
Glu(OtBu)-OtBu	azide coupling; 80.0%	0.48 (*c*)	87–89	− 6.0°
ly-Phe-Met-Thr-Ser-Glu(OtBu)-OtBu	DCC/HOBt; 79.0%	0.67 (*c*)	189–191 (dec)	−12.9°
ly-Phe-Met-Thr-Ser-Glu(OtBu)-Lys(Boc)-OH	aqueous NaOH/dioxane; 78.2% (ref. 17)	0.80 (*a*)	215–218 (dec)	− 8.2°
)-OtBu	dimethylformamide di-*tert*-butyl acetal; oil; 47.5%	0.79 (*e*)		
u)-Lys(Boc)-OtBu	DCC/HOBt; oil, 92.1%	0.71 (*e*)		
OtBu)-Lys(Boc)-OtBu	DCC/HOBt; 63.4%	0.50 (*e*)	77–79	− 9.6°
Glu(OtBu)-Lys(Boc)-OtBu	DCC/HOBt; 71.9%	0.48 (*e*)	75–80	− 6.7°
et-Thr-Ser-Glu(OtBu)-Lys(Boc)-OtBu	DCC/HOBt; 86.1%	0.57 (*e*)	170–172	−10.2°
Thr-Ser-Glu(OtBu)-Lys(Boc)-OtBu	DCC/HOBt; 79.9%	0.20 (*e*)	135–145 (dec)	+30.3°
OtBu	DCC/HOBt; 40.5%	0.22 (*e*)	95–97	−12.6°
tBu	DCC/HOBt; 100%	0.50 (*b*)[a]		
tBu	DCC/HOBt; 97.9%	0.78 (*a*)[a]		
Thr-Pro-Leu-Val-OtBu	DCC/HOBt; 62.2%	0.68 (*b*)	149–152	−45.4°
tBu	DCC/HOBt; 85.6%	0.72 (*b*)[a]		
Thr(tBu)-OtBu	DCC/HOBt; 52.0% (ref. 16)	0.54 (*e*)	56–58	−37.6° (*c*
Thr-Pro-Leu-Val-Thr(tBu)-OtBu	DCC/HOBt; 65.2% (ref. 16)	0.80 (*d*)	185–187	−34.7°
tBu	DCC/HOBt; 98.0%	0.67 (*a*)	211–215 (dec)	−21.0°
Bu	−ONp ester; 88.3%	0.75 (*d*)	183–185	−32.0°
Bu	DCC/HOBt; 64.7%	0.85 (*d*)	193–195	−27.5°
Bu	azide coupling; 41.3%[b]	0.91 (*a*)		

were not determined since the compound was contaminated with residual DCU. [b] Overall yield after purification by counter current distribution in solvent system CH_3OH H_4OAc = 10/6/2/4 (v/v).

n several shortened α-endorphin fragments (see also Fig. 1–3).

Peptide	Amino acid analysis										Peptide content (%)	Content HOAc (%)	Content H_2O (%)	$[\alpha]_D^{21}$ (*c*, 1, 10% HOAc)	HPLC (main componen
	Gly	Phe	Met	Thr	Ser	Glu	Lys	Pro	Val	Leu					
-Met-OH (**I**)[a]	2.02	1.02	0.96								81.2	1.2	2.7	−18.6°	95.6%[d]
-Met-Thr-OH (**II**)[a]	2.02	1.01	0.97	1.00							96.4	0.5		−21.2°	99.6%[d]
-Met-Thr-Ser-OH (**III**)[a]	2.05	0.99	0.96	0.96	0.89						96.5	1.1	4.7	−28.3°	97.9%[e]
-Met-Thr-Ser-Glu-OH (**IV**)[a]	2.04	1.02	0.96	0.96	0.89	0.99					92.0	0.7	5.1	−41.7°	99.5%[d]
-Met-Thr-Ser-Glu-Lys-OH (**V**)[a]	2.06	1.02	0.93	0.98	0.89	1.01	0.99				89.6	6.0	3.9	−42.8°	98.1%[d]
-Ser-Glu-Lys-OH (**VI**)[b]		1.01	0.98	1.00	0.92	1.02	1.00				88.2			−34.0°	[k]
-Glu-Lys-OH (**VII**)[b]			0.96	1.02	0.99	1.04	0.86				75.0				[k]
-Lys-OH (**VIII**)[c]				0.91	0.82	0.99	1.01				90.8	2.7	5.5	−38.4°	92.7%[f]
-Met-Thr-Ser-Glu-Lys-Ser-Gln-OH (**IX**)[a]	2.03	0.95	0.98	0.98	1.68	2.01	1.05				93.2	3.3	6.2	−49.4°	94.9%[g]
-Met-Thr-Ser-Glu-Lys-Ser-Gln-Thr-	2.01	1.01	1.00	2.02	1.99	2.04	0.97	0.98			84.8	2.4	5.2	−75.6°	98.2%[h]
-Met-Thr-Ser-Glu-Lys-Ser-Gln-Thr-OH (**XI**)[a]	2.02	0.98	0.96	1.92	1.72	2.03	1.07	0.98	1.00	0.96	91.2	0.8	6.7	−86.5°	97.0%[h]
-Met-Thr-Ser-Glu-Lys-Ser-Gln-Thr-Thr-OH (**XII**)[a]	1.99	1.00	0.98	2.91	1.88	2.01	1.03	1.06	1.02	0.98	90.3	3.5	3.8	−89.7°	94.9%[h]
-Lys-Ser-Gln-Thr-Pro-Leu-Val-Thr-OH				2.87	1.68	2.00	1.03	1.00	1.03	0.95	93.8	2.0	6.0	−38.4° (*c* 0.5, DMF)	96.9%[j]
-Pro-Leu-Val-Thr-OH (**XIV**)[b]				1.91	1.02	1.04		0.98	1.04	1.00	84.4	2.7	4.6	−109.1°	98.5%[i]
-OH (**XV**)[a]				0.99					1.05	0.96	87.9	2.4	3.8	−9.7°	98.6%[f]

s by counter current distribution as described in Exp. section for peptide **II**. [b] Purification was by column chromatography on silica (Merck Fertigsäule) as described for pep ification on silica using the solvent system BuOH/HOAc/H_2O = 2/1/1 (by vol.). [d] Stationary phase: Nucleosil 10C-18. Mobile phase: system A = 0.1% H_3PO_4 (pH 2.1) an ater = 50/50 (v/v), prepared with 0.05 M TMAH and phosphoric acid to adjust the pH to 2.8. A linear gradient of 20–100% B was run in 35 min and then 5 min at this composition se: Nucleosil 10C-18. Mobile phase: system A = methanol/water = 25/75 (v/v) and B = methanol/water = 80/20 (v/v), both prepared with 0.05 M TMAH and phosphori pH to 2.8. A linear gradient of 0–70% B was run in 25 min and then 5 min at this composition. [f] Stationary and mobile phase as in d. A linear gradient of 0–100% B was run i onary phase: μ Bondapak C-18. Mobile phase: system A = methanol/water = 10/90 (v/v) and system B = methanol/water = 50/50 (v/v), both prepared with 0.05 M TMAH cid to adjust the pH to 3.0. A linear gradient of 0–100% B was run in 25 min. [h] Stationary phase: μ Bondapak C-18. Mobile phase: linear gradient run as under e. [i] Stationar phase as in g. A linear gradient of 0–100% B was run in 15 min. [j] As for g, with the exception of system A = methanol/H_2O = 5/95 (v/v). A linear gradient of 0–100% n. [k] These peptides had been synthesized before the introduction of HPLC in our laboratories.

acetic acid content of the end-products was determined isotachophoretically[19]. HPLC experiments were carried out on a Spectra Physics apparatus, model 8000. The stationary phase and details of the mobile phase are given in Table II. The area percentages of the main component and by-products present in the sample were calculated by a computer connected to the SP 8000; the total percentage of main component and by-products was set at 100%.
The synthesis and purification of a few compounds (to illustrate the used procedures) will be given in detail below. Important data of intermediates synthesized by essentially the same procedures are summarized in Table I.
In Table II we have collected data on the free peptides **I-XV**.

Synthesis of H-Gly-Gly-Phe-Met-OH, β-endorphin-(2–5), peptide **I** (Fig. 1)

H-Gly-Gly-Phe-Met-OH
1.12 g (2.2 mmol) of Boc-Gly-Gly-Phe-Met-OH[17] were treated with 12 ml of TFA/H_2O = 9/1, v/v, under N_2, in the presence of 0.12 ml of di-*tert*-butyl sulfide at room temperature for 45 min. After conversion of the trifluoroacetate salt into the acetate salt, the crude product was purified by counter current distribution in the solvent system BuOH/HOAc/H_2O = 4/1/5, by volume; yield 880 mg (88.9%). For physical constants see Table II.

Synthesis of H-Gly-Gly-Phe-Met-Thr-OH, β-endorphin-(2–6), peptide **II** (Fig. 1)

Boc-Gly-Gly-Phe-Met-Thr(tBu)-OtBu
1.50 g (2.94 mmol) of Boc-Gly-Gly-Phe-Met-OH and 0.60 g (2.68 mmol) of H-Thr(tBu)-OtBu [obtained after hydrogenation of Z-Thr(tBu)-OtBu[20] in DMF with Pd/C as the catalyst] were coupled in DMF using 1.5 equiv. of HOBt and 1.1 equiv. of DCC. After 30 min at −20°C, 2 h at 0°C and 18 h at room temperature, the solution was filtered and the filtrate was evaporated to dryness. The residue was dissolved in EtOAc and the solution extracted successively with 5% $NaHCO_3$ solution, 0.1 N HCl and saturated NaCl solution. After drying on anhydrous Na_2SO_4, the filtered solution was cooled to 5°C. The solid was filtered, washed with *n*-hexane, and dried. Yield of first crop 0.70 g (36.1%); m.p. 213–215°C; $[\alpha]_D^{21}$ −7.0° (*c* 1, DMF). TLC: R_f 0.90 (*b*).

H-Gly-Gly-Phe-Met-Thr-OH
0.60 g (0.83 mmol) of the fully protected pentapeptide was treated with 6 ml of TFA/H_2O = 9/1 (v/v), in the presence of anisole and di-*tert*-butyl sulfide, under N_2 for 45 min at room temperature. After conversion to the acetate, the crude product was purified by counter current distribution in the solvent system BuOH/HOAc/H_2O = 4/1/5, by volume. Yield 230 mg (57.5%). For physical constants see Table II.

Synthesis of H-Gly-Gly-Phe-Met-Thr-Ser-Glu-OH, β-endorphin-(2–8), peptide **IV** (Fig. 1)

Z-Thr-Ser-Glu(OtBu)-OtBu. 3.50 g (10 mmol) of Z-Thr-Ser-N_2H_3[21] in 30 ml of DMF were treated with 9 ml of 2.5 N HCl in DMF at 0°C. The hydrazide was converted into the azide by the addition of 1.5 ml (11 mmol) of isopentyl nitrite and stirring the solution for 20 min at −15/−20°C. The reaction mixture was then neutralized with *N*-ethylmorpholine (NEM), and a solution of 3.00 g (10 mmol) of H-Glu(OtBu)-OtBu·HCl (obtained after hydrogenation of 3.93 g Z-Glu(OtBu)-OtBu in DMF with Pd/C as the catalyst) in 30 ml of DMF was subsequently added. The pH of the solution was adjusted to 7.2 with NEM. After 78 h at 0°C the solvent was evaporated *in vacuo*. The concentrate was dissolved in EtOAc (125 ml) and washed successively with 5% $KHSO_4$ solution, 5% $NaHCO_3$ solution and 10% NaCl solution. After drying (Na_2SO_4) the solution was evaporated to dryness. The residue was treated with EtOAc/*n*-hexane = 1/1 (v/v). The precipitate formed was filtered, washed with *n*-hexane, and dried. Yield 4.80 g (80.0%); $[\alpha]_D^{21}$ −6.0° (*c* 1, DMF); m.p. 87–89°C. TLC: R_f 0.40 (*c*).

Boc-Gly-Gly-Phe-Met-Thr-Ser-Glu(OtBu)-OtBu. 1.00 g (2.0 mmol) of Boc-2–5-OH and 968 mg (2.0 mmol) of H-6–8-OtBu·HCl (obtained after hydrogenation of Z-6–8-OtBu in DMF, as usual) in 30 ml of DMF were coupled by the DCC/HOBt method in the same manner as described for Boc-2–6-OtBu (see above). Precipitation from EtOH/ether = 1/4, v/v, gave 1.50 g (79.0%) of Boc-2–8-OtBu; m.p. 189–191°C (dec.); $[\alpha]_D^{25}$ −12.9° (*c* 1, DMF). TLC: R_f 0.65 (*c*).

H-Gly-Gly-Phe-Met-Thr-Ser-Glu-OH. 1.40 g (1.5 mmol) of Boc-2–8-OtBu were treated with 14 ml of TFA/H_2O = 9/1, v/v, under N_2, and in the presence of anisole at room temperature for 30 min. Isolation of the crude product and purification as for peptide I gave 400 mg (50.0%) of β-endorphin-(2–8) which was homogeneous on TLC. See Table II for further data.

Synthesis of H-Phe-Met-Thr-Ser-Glu-Lys-OH, β-endorphin-(4–9), peptide **VI** (Fig. 1)

Z-Lys(Boc)-OtBu. 19.2 g (50 mmol) of Z-Lys(Boc)-OH were dissolved in CH_2Cl_2 (125 ml) and to this solution was added dimethylformamide di-*tert*-butyl acetal[22] (4 equiv., 50 ml). The reaction mixture was stirred for 42 h at room temperature and then evaporated to dryness. EtOAc (125 ml) was added and the solution successively extracted with 10% K_2CO_3 solution and H_2O. After drying (Na_2SO_4), the solution was evaporated to give Z-Lys-(Boc)-OtBu as an oil in 47.5% (10.4 g) yield. TLC: R_f 0.79 (*e*).

Z-Glu(OtBu)-Lys(Boc)-OtBu. 7.9 g (23.4 mmol) of Z-Glu-(OtBu)-OH and 7.9 g (23.4 mmol) of H-Lys(Boc)-OtBu·HCl (obtained after hydrogenation of Z-Lys(Boc)-OtBu in DMF, as usual) were coupled in DMF, in the same manner as described for Boc-2–6-OtBu (see above). Yield, after working up as usual, 92.1% (13.4 g) as an oil. TLC: R_f 0.71 (*e*).

Z-Ser-Glu(OtBu)-Lys(Boc)-OtBu. See Table I.
Z-Thr-Ser-Glu(OtBu)-Lys(Boc)-OtBu. See Table I.
Boc-Phe-Met-Thr-Ser-Glu(OtBu)-Lys(Boc)-OtBu. See Table I.

H-Phe-Met-Thr-Ser-Glu-Lys-OH. 1.5 g (1.42 mmol) of Boc-4–9-OtBu were treated with 10 ml of TFA/H_2O = 9/1, v/v, for 3 h at room temperature. After conversion of the trifluoroacetate salt into the acetate, the crude product was purified by chromatography on silica (Merck Fertigsäule) with solvent system g; yield 378 mg (36.0%). See Table II.

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