Synthesis of fragments of human β-lipotropin, β_h-LPH. Part I. The synthesis of β-LPH-(61–76) and β-LPH-(61–77), i.e. α- and γ-endorphin, respectively

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Abstract. The synthesis of β-LPH-(61–76) and β-LPH-(61–77) [i.e. α- and γ-endorphin, respectively] by means of the fragment condensation approach is described.
Several of the fragments leading to these two endorphins were also obtained as pure, free peptides.

Introduction

The hormone, or prohormone, β-lipotropin (β-LPH) is a protein consisting of 91 amino acids and can be isolated from pituitary extracts from many species[1]. It has fat-mobilizing activity, especially in the rabbit, and in addition shows melanocyte-stimulating activity[1] and according to *Thody* and *Shuster* some sebotropic activity[2].
The C-terminal fragment of β-LPH, which is released *in vitro* by mild digestion with trypsin, corresponds to β-LPH-(61–91), i.e. β-endorphin, and appears to have a high affinity for opiate binding sites in brain and smooth muscle (for a review see reference 3).
With the help of pulse-chase labelling experiments *Chrétien* et al. demonstrated that β-lipotropin may function as a prohormone for β-endorphin[4]. Fragments 61–65, i.e. [Met^5]-enkephalin, and 61–76 and 61–77, i.e. α- and γ-endorphin, respectively, also are potent opiate-like peptides.
The *N*-terminal part of β-LPH contains sequences identical to β-MSH (sequence 41–58) and ACTH 4–10 (sequence 47–53). Since these and related peptides are involved in acquisition and maintenance of conditioned behaviour in animals, it was deemed of interest to synthesize fragments of β-LPH in order to study their behavioural and biological activities. Results of these studies have been reported recently[5]. The detailed synthesis of these β-LPH fragments by the classical fragment condensation approach is the subject of this and subsequent papers.
A preliminary communication is published in the Proceedings of the Fifteenth European Peptide Symposium[6]. In the first part of this series the synthesis of α- and γ-endorphin as well as several of their partial sequences is described.

Strategy of the synthesis

Since the structure of human β-LPH has been elucidated by the studies of *Li* and *Chung*[7], we chose this sequence as the starting point for the synthesis of our β-LPH fragments. This can be useful, especially if it is decided to study the effect of such a peptide in man.
The synthesis of α-, β- and γ-endorphin has been described in the literature: camel[8] and human[9] β-endorphin by *Li* and coworkers, β_h-endorphin by *Atherton* et al.[10] and *Meienhofer* and *Chang*[11] and α- and γ-endorphin by *Ling* et al.[12]. In all these publications use has been made of the solid-phase technique*.
We, however, preferred the classical fragment condensation approach, since this method enabled us, during progress of the synthesis of larger fragments, to obtain a series of interesting shorter sequences, which could be studied pharmacologically in the meantime.
For our choice of segmentation of the sequence 1–91 of β_h-LPH, we were guided by two kinds of arguments. On the one hand we wanted to obtain as many of the natural

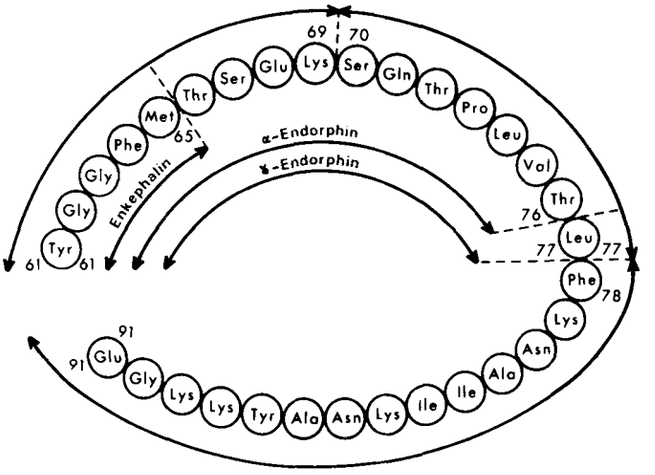

Fig. 1. Amino acid sequence of α-endorphin [β-LPH-(61–76)], γ-endorphin [β-LPH-(61–77)], and β_h-endorphin [β_h-LPH-(61–91)].

* After the preparation of this manuscript, two syntheses of β_h-endorphin via classical techniques, but using a different approach, have been described[13,14].

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2 *A. J. Thody* and *S. Shuster*, J. Endocrinol. **50**, 533 (1971).
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13 *M. Kubota, T. Hirayama, O. Nagase* and *H. Yajima*, Chem. Pharm. Bull. **26**, 2139 (1978).
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occurring fragments of LPH described in the literature as possible. On the other hand there are synthetic considerations; for example: from the point of view of racemization-safe coupling, peptides with a C-terminal prolyl or glycyl residue are to be preferred.

Figure 1 shows the amino acid sequence of α-, γ- and β_h-endorphin, and also the coupling sites in our synthetic approach, i.e. at residues 65, 69 and 77 (the last one to obtain β-endorphin).

tert-Butyl derived groups are used for the protection of lysine and glutamic acid side-chains and for α-carboxyl protection in peptides not containing Lys or Glu**. Benzyloxycarbonyl groups were used for α-amino protection with the exception of the *N*-terminal residue (i.e. tyrosine).

The side-chains of tyrosine, serine and threonine were not protected. In the final stage of the peptides synthesized, the only groups to be removed were *tert*-butyl derived. The condensation of the larger fragments was carried out with *N,N'*-dicyclohexylcarbodiimide and 1-hydroxybenzotriazole (DCC/HOBt)[15]; in the synthesis of the smaller peptides, use was also made of active esters and azide reactions.

Description and discussion of the synthesis

Figure 2 illustrates the synthesis of the protected fragments 61–65, 66–69 and 61–69. Protected enkephalin (i.e. sequence 61–65) was obtained in the following way: H-Phe-Met-OMe, obtained after removal of the α-amino protecting Boc group, was reacted with the known dipeptide Boc-Gly-Gly-OH, via a DCC/HOBt mediated reaction.

The protected tetrapeptide isolated in 89% yield was deprotected (HCl in CH_2Cl_2) and the resulting product was acylated with Boc-Tyr-OH using DCC and HOBt. Saponification of the pentapeptide (with NaOH in aqueous dioxane) gave Boc-61–65-OH in 90% yield.

In the upper part of Figure 2 is outlined our initially planned route to obtain Z-66–69-OMe. Both the alkaline hydrolysis of Z-Thr-Ser-OMe and the subsequent coupling of Z-Thr-Ser-OH with H-Glu(OtBu)-Lys(Boc)-OMe (obtained after hydrogenation of the Z-dipeptide) were not satisfactory*; therefore, we converted the dipeptide methyl ester into the hydrazide (with N_2H_4) and carried out the azide coupling reaction according to the procedure of *Honzl* and *Rudinger* using isopentyl nitrite[17]: 73% of the pure product was obtained.

Hydrogenation of Z-66–69-OMe and subsequent acylation of H-66–69-OMe with Boc-61–65-OH (using DCC and HOBt) gave 92% of the pure nonapeptide ester.

The synthesis of fragment Z-70–77-OtBu is given in Figure 3. The tetrapeptide Z-Ser-Gln-Thr-Pro-OtBu was built up stepwise, starting from H-Pro-OtBu using DCC and HOBt with the exception of Z-Gln-OH where a *p*-nitrophenyl ester reaction gave better results. The coupling of Z-Leu-Val-OH with H-Thr-Leu-OtBu (upper part of Fig. 3) using DCC and HOBt gave a crude peptide that proved difficult to purify. The purification after a mixed anhydride reaction was easier, but a LAO digestion of the tetrapeptide indicated some 40% racemization of the valine residue! Therefore, we turned again to the stepwise elongation procedure (via *p*-nitrophenyl esters or DCC/HOBt), and this proved to be very successful.

Z-Ser-Gln-Thr-Pro-OH, obtained after treatment of the corresponding –OtBu ester with trifluoroacetic acid, was coupled with H-Leu-Val-Thr-Leu-OtBu (using DCC and HOBt) to give the protected octapeptide in 72% yield.

* In a recent paper *Stabinsky* et al. have demonstrated cyclization products when similar peptides were treated with base[26].

** Standard abbreviations are used for amino acids and protecting groups [IUPAC-IUB Commission on Biochemical Nomenclature, Biochem. J. **126**, 773 (1972)]. Other abbreviations are: DCC, *N,N'*-dicyclohexylcarbodiimide; DCU, *N,N'*-dicyclohexylurea; DMF, *N,N*-dimethylformamide; EtOAc, ethyl acetate; HOAc, acetic acid; HOBt, 1-hydroxybenzotriazole; LAO, *L*-amino acid oxidase; NEM, *N*-ethylmorpholine; Pd/C, 10% palladium on charcoal; pet. ether, petroleum ether b.p. 40–60°; TLC, thin-layer chromatography; TFA, trifluoroacetic acid; TMAH, tetramethylammonium hydroxide.

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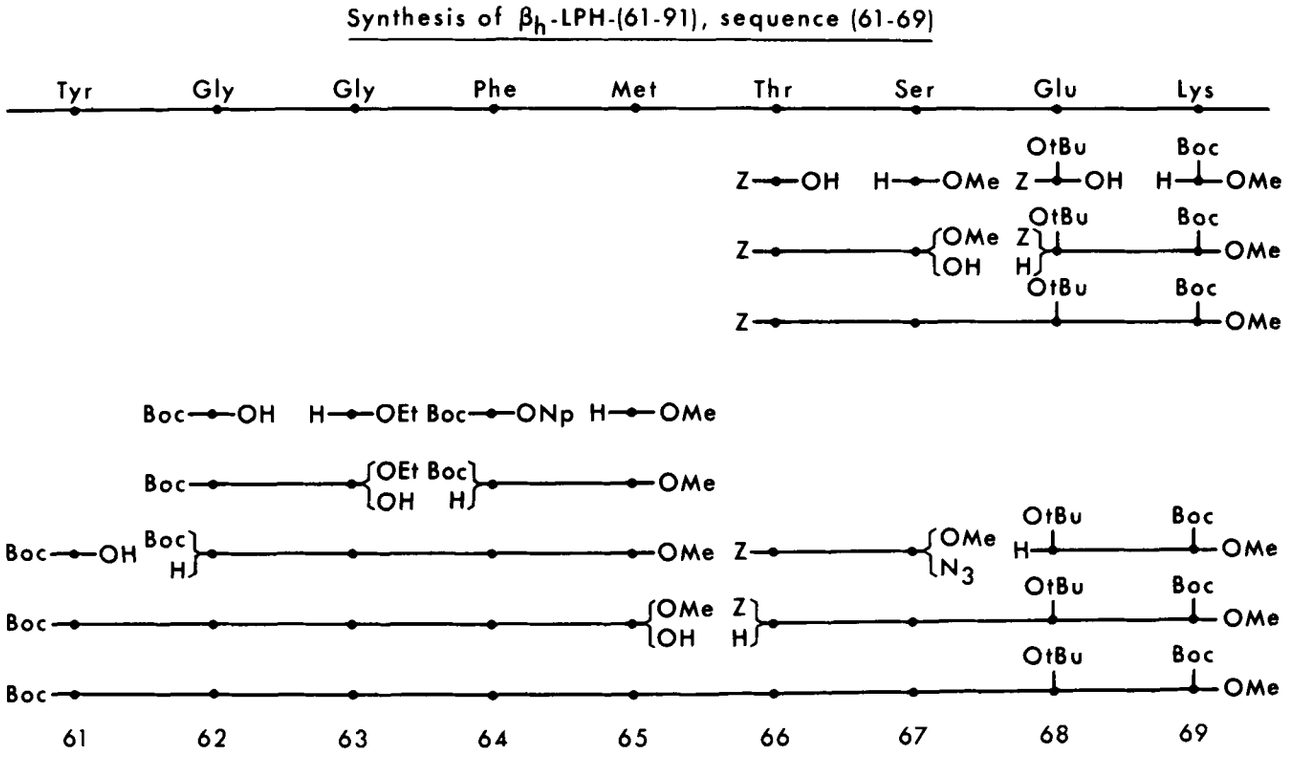

Fig. 2. Schematic representation of the synthesis of protected fragment 61–69.

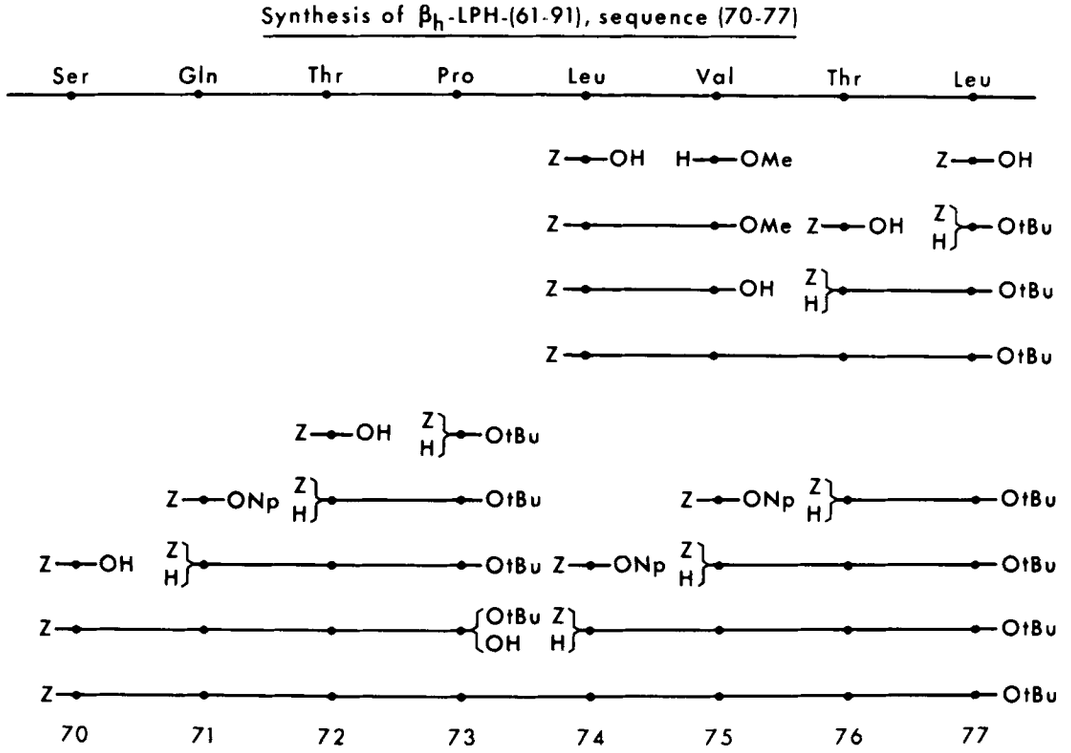

Fig. 3. Schematic representation of the synthesis of protected fragment 70–77.

In order to obtain β-LPH-(61–76) (α-endorphin) in which Thr[76] is the *C*-terminal amino acid residue, we synthesized Z-Thr(tBu)-OtBu from Z-Thr-OH according to the literature[18]. The same series of reactions leading to protected Z-70–77-OtBu was used to give protected Z-70–76-OtBu. After some preliminary experiments, careful saponification of Boc-61–69-OMe led to pure Boc-61–69-OH in 77% yield (see Fig. 4).

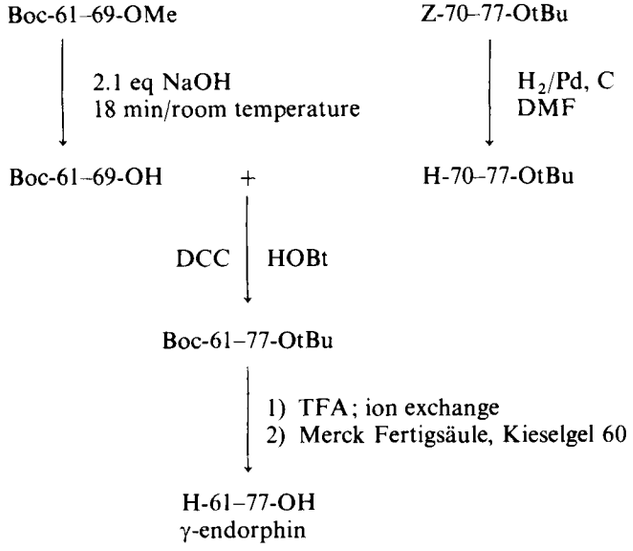

Fig. 4

Deprotection of Z-70–77-OtBu (H_2/Pd, C) and subsequent acylation of H-70–77-OtBu with the fragment Boc-61–69-OH gave the protected 17-peptide in 70.6% yield. Removal of all protecting groups was achieved with trifluoroacetic acid; purification by column chromatography (Merck Fertigsäule) gave γ-endorphin.

α-Endorphin was obtained after the same series of reactions with the exception of the purification which was achieved using counter current distribution (Craig partition). The partial sequences H-70–77-OH, H-70–76-OH, H-61–65-OH (enkephalin), and H-61–69-OH were obtained from their protected precursors in the same way and were purified by column chromatography. The final products were checked for purity by HPLC, TLC, amino acid analysis, and L-amino acid oxidase (LAO) digestion. (After acid hydrolysis of the peptide into the individual amino acids, the hydrolyzate is treated with L-amino acid oxidase; this enzyme oxidizes Tyr, Phe, Met, Lys, Leu and Val into their corresponding α-keto acids. However, the conversion of the amino acids Thr, Ser, Glu and Pro varies. The amount of unconverted amino acid, determined by amino acid analysis and corrected for the "blank" corresponds to the amount of D-amino acid in the sample).

Experimental section

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) chloroform/methanol/water = 70/30/5
b) toluene/ethanol = 4/1
c) chloroform/methanol = 4/1
d) 1-butanol/pyridine/acetic acid/water = 16/3/1/4
e) 1-butanol/pyridine/acetic acid/water = 8/3/1/4
f) toluene/ethanol = 9/1
g) 1-butanol/pyridine/ammonia (25% solution in water, m/m)/water = 20/12/3/15.

Methods used for the detection of components on TLC plates were: ultraviolet (quenching of fluorescence after exposure of the plates at 254 nm), fluorescamine reagent (free amino groups)[19] and chlorine/*o*-tolidine reagent[20] for peptide bonds.

Melting points were determined with a Totolli apparatus but not corrected. Specific rotations were measured with a Perkin-Elmer 241 polarimeter and not corrected for the peptide content.

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Amino acid analyses were carried out on samples that had been hydrolyzed in 5.7 N HCl for 24 h at 105°C.

End-products and products after alkaline saponification were routinely checked for racemization by the L-amino acid oxidase (LAO) digestion method[21].

High performance liquid chromatography (HPLC) experiments were carried out on a Spectra Physics, model 8000.

Stationary phase: μ-Bondapak C-18. Mobile phase: system A = methanol/water = 25/75 and B = methanol/water = 80/20, both with 0.05 M TMAH and phosphoric acid to adjust the pH to 3.0[22]; gradients of A and B were run at 20°C with a flow rate of 2 ml/min. Detection: 210 nm. The area percentages of the main component and by-products present in the sample were calculated by a computer connected to the SP 8000 apparatus: the total percentage of the main component and possible by-products was set at 100%.

Synthesis of Boc-Tyr-Gly-Gly-Phe-Met-Thr-Ser-Glu(OtBu)-Lys(Boc)-OH, sequence 61–69, Fig. 2

Boc-Gly-Gly-Phe-Met-OMe (62–65). Equimolar amounts of Boc-Gly-Gly-OH[23] and H-Phe-Met-OMe·HCl[24] were dissolved in DMF, followed by 1.5 equivalents of HOBt and one equivalent of NEM. The solution was cooled to approx. −20°C, and one equivalent of DCC was added. 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 oily residue was dissolved in EtOAc and the solution extracted successively with 5% $KHSO_4$ solution, H_2O, 5% $NaHCO_3$ solution, H_2O and saturated NaCl solution. After drying on anhydrous Na_2SO_4, the filtered solution was concentrated and ether was added. The precipitate was filtered, washed and dried. Yield 89%; m.p. 109–110°C; $[\alpha]_D^{21}$ −22.5° (c = 1, DMF). TLC: R_f 0.95 (a), 0.44 (b).

Boc-Tyr-Gly-Gly-Phe-Met-OMe (61–65). 4.5 g (16.0 mmol) of Boc-Tyr-OH and 6.7 g (14.5 mmol) of H-Gly-Gly-Phe-Met-OMe-HCl (obtained after treatment of the corresponding Boc compound with HCl in CH_2Cl_2) in 120 ml of DMF were coupled using the DCC/HOBt method as described above. Crystallization from EtOAc/ether/pet. ether gave 8.9 g (89.4%) of the pentapeptide derivative. M.p. 93–95°C (lit.[25] m.p. 92°C). $[\alpha]_D^{20}$ −21.4° (c = 1, DMF) [Lit.[25] $[\alpha]_D^{20}$ −20.2° (c = 2, DMF)]. TLC: R_f 0.91 (a), 0.39 (b), 0.84 (c).

Boc-Tyr-Gly-Gly-Phe-Met-OH (61–65). 5.2 g (7.6 mmol) of Boc-Tyr-Gly-Gly-Phe-Met-OMe in 120 ml of dioxane/H_2O (9/1) were treated with 8.3 ml 2.0 N NaOH for 15 min at room temperature. The pH was then adjusted to 7 with 4N HCl and the dioxane was evaporated. EtOAc (150 ml) was added and the pH of the mixture was adjusted to 1.5 with 4N HCl. The EtOAc phase was extracted with saturated sodium chloride solution. After drying (Na_2SO_4), the solution was evaporated to dryness. The residue was crystallized from EtOAc/pet. ether. Yield 4.5 g (90.0%); m.p. 123–128°C (dec.); $[\alpha]_D^{21}$ −16.4° (c = 1, DMF). TLC: R_f 0.38 (a), 0.70 (d).

Z-Glu(OtBu)-Lys(Boc)-OMe (68–69). 35.3 g (105 mmol) of Z-Glu(OtBu)-OH and 29.7 (100 mmol) of H-Lys(Boc)-OMe·HCl in 140 ml of DMF were coupled by the DCC/HOBt method and the reaction mixture was worked up as described for Boc-Gly-Gly-Phe-Met-OMe. Crystallization from ether/pet. ether (1 : 1) gave 37.8 g (65.2%) of the protected dipeptide. M.p. 54–58°C; $[\alpha]_D^{21}$ −4.7° (c = 1, DMF). TLC: R_f 0.45 (b), 0.92 (c).

Z-Thr-Ser-Glu(OtBu)-Lys(Boc)-OMe (66–69). 10.9 g (30.8 mmol) of Z-Thr-Ser-N_2H_3[26] in 75 ml of DMF were treated with 17.5 ml of 3.87 N HCl in DMF at 0°C.

The hydrazide was converted into the azide by the addition of 4.7 ml (33.9 mmol) of isopentyl nitrite and stirring the solution for 20 min at −15/−20°C. The reaction mixture was then neutralized with 8.55 ml of NEM, and a solution of H-Glu(OtBu)-Lys(Boc)-OMe (30.8 mmol) [obtained after hydrogenation of 17.8 g (30.8 mmol) of Z-Glu(OtBu)-Lys(Boc)-OMe in DMF with Pd/C as the catalyst] in 100 ml of DMF was subsequently added. The pH of the solution was adjusted to 7.2 with NEM (2 ml). After 78 h at 0°C the solvent was evaporated *in vacuo*. The concentrate was dissolved in EtOAc (120 ml) and water (20 ml) and the organic phase was extracted as usual. After drying (Na_2SO_4), the solution was evaporated to dryness. The residue was treated with MeOH/ether/pet. ether (1/2/2). The precipitate that formed was filtered, and dried. Yield 17.2 g (73.0%); m.p. 141–142°C; $[\alpha]_D^{20}$ −3.3° (c = 1, DMF). TLC: R_f 0.35 (b), 0.76 (c).

Boc-Tyr-Gly-Gly-Phe-Met-Thr-Ser-Glu(OtBu)-Lys(Boc)-OMe (61–69). To a stirred mixture of 1.88 g (2.79 mmol) of Boc-Tyr-Gly-Gly-Phe-Met-OH and 754 mg (2 equiv.) of HOBt in DMF (10 ml), a solution of 1.77 g (2.79 mmol) of H-Thr-Ser-Glu(OtBu)-Lys(Boc)-OMe [obtained after hydrogenation of 2.27 g (2.96 mmol) of Z-Thr-Ser-Glu(OtBu)-Lys(Boc)-OMe] in 10 ml of DMF was added. The solution was cooled to −22°C and 637 mg of DCC (1.1 equiv.) were added. After 10 min at −22°C and 18 h at room temperature, the solution was filtered and evaporated to dryness. The concentrate was treated with MeOH/ether/pet. ether (1/2/2) and the formed precipitate was filtered, washed with ether/pet. ether, and dried. Yield 3.32 g (92.2%); m.p. 186–187°C (dec.); $[\alpha]_D^{21}$ −10.0° (c = 1, DMF). TLC: R_f 0.77 (a), 0.90 (c).

Synthesis of Z-Ser-Gln-Thr-Pro-Leu-Val-Thr-Leu-OtBu, sequence 70–77, Fig. 3

Z-Thr-Pro-OtBu (72–73). To a stirred mixture of 84.9 g (335 mmol) of Z-Thr-OH, 57.4 g (335 mmol) of H-Pro-OtBu and 54.7 g (405 mmol, 1.2 equiv.) of HOBt in DMF (600 ml) cooled to −20°C, 69.4 g (335 mmol) of DCC were added. After 2 h at 0°C and 18 h at room temperature the filtered solution was evaporated to dryness. The oily residue was dissolved in EtOAc (750 ml) and extracted as for 62–65. After drying (Na_2SO_4), and evaporation of the organic solvent, the oily residue was triturated with pet. ether. Yield 119.6 g (87.7%); m.p. 69–73°C; $[\alpha]_D^{21}$ −51.3° (c = 1, DMF). TLC: R_f 0.40 (b).

Z-Gln-Thr-Pro-OtBu (71–73). To a solution of 19.1 g (70.0 mmol) of H-Thr-Pro-OtBu (obtained after hydrogenation of 28.5 g (70.0 mmol) of Z-Thr-Pro-OtBu in DMF with Pd/C as the catalyst) in DMF (300 ml), were added 28.1 g (70.0 mmol) of Z-Gln-ONp. The reaction mixture was stirred at room temperature for 24 h and thereafter evaporated to dryness. The residue was dissolved in EtOAc (350 ml) and the solution extracted as usual. After drying (Na_2SO_4) and evaporation of the organic solvent, the residue was triturated with ether. The obtained solid was triturated again, this time with EtOAc/ether (1/5). Yield 27.9 g (74.8%); m.p. 89–91°C; $[\alpha]_D^{20}$ −46.3° (c = 1, DMF). TLC: R_f 0.32 (b), 0.80 (c).

Z-Ser-Gln-Thr-Pro-OtBu (70–73). 11.1 g (46.2 mmol) of Z-Ser-OH and 18.5 g (46.2 mmol) of H-Gln-Thr-Pro-OtBu (obtained after hydrogenation in DMF as usual) in 250 ml of DMF, were coupled by the DCC/HOBt method. After removal of the DCU, the oily residue was dissolved in 2-BuOH/$CHCl_3$ (2/3), and extracted as described for 62–65. After isolation, the residue was treated with MeOH/ether/pet. ether. Yield 21.7 g (75.2%); m.p. 104–106°C; $[\alpha]_D^{20}$ −42.7° (c = 1, DMF). TLC: R_f 0.73 (b), 0.56 (c).

Z-Thr-Leu-OtBu (76–77). 21.5 g (84.8 mmol) of Z-Thr-OH and 15.9 g (84.8 mmol) of H-Leu-OtBu in 300 ml of CH_2Cl_2 were coupled by the DCC/HOBt method and the reaction mixture was worked up as described for Z-Thr-Pro-OtBu (72–73). The oily residue was dissolved in 25 ml of EtOAc and the solution was added to 250 ml of petroleum ether. The formed precipitate was filtered off, washed with petroleum ether, and dried. Yield 25.2 g (70.3%); m.p. 80–81°C; $[\alpha]_D^{22}$ −16.2° (c = 1, DMF). TLC: R_f 0.43 (b).

Z-Val-Thr-Leu-OtBu (75–77). To a solution of 15.0 g (50.9 mmol) of H-Thr-Leu-OtBu [obtained after hydrogenation of 21.5 g (50.9 mmol) of Z-Thr-Leu-OtBu] in 250 ml of DMF, were added 18.9 g (50.9 mmol) of Z-Val-ONp. The reaction mixture was stirred at room temperature for 18 h, and was then evaporated to dryness. The oily residue was dissolved in EtOAc, and extracted successively with 10% K_2CO_3 solution, 0.1 N HCl solution and saturated NaCl solution. After drying (Na_2SO_4) and evaporation of the organic solvent, the concentrate was crystallized from EtOAc/ether/pet.

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ether. Yield 23.1 g (87%); m.p. 123–125°C (lit.[27] m.p. 129–130°C); $[\alpha]_D^{20}$ −52.8° (*c* = 0.5, MeOH) [lit.[27] $[\alpha]_D^{20}$ −55.5° (*c* = 0.5, MeOH]. TLC: R_f 0.57 (b).
When a DCC/HOBt mediated reaction was carried out involving Z-Val-OH and H-Thr-Leu-OtBu, 76.3% of the tripeptide with the same physical constants was obtained.

Z-Leu-Val-Thr-Leu-OtBu (74–77). 16.2 g (41.9 mmol) of H-Val-Thr-Leu-OtBu (obtained after hydrogenation of Z-Val-Thr-Leu-OtBu in MeOH with Pd/C as the catalyst) and 16.2 g (41.9 mmol) of Z-Leu-ONp in 250 ml of DMF were coupled by the *p*-nitrophenyl ester method and the reaction mixture was worked up as described for Z-Val-Thr-Leu-OtBu (75–77). The concentrate was crystallized from EtOAc/pet. ether. Yield 23.8 g (89.0%); m.p. 153–155°C; $[\alpha]_D^{21}$ −21.6° (*c* = 1, DMF). TLC: R_f 0.50 (b), 0.27 (f).
A DCC/HOBt reaction gave 89.4% of peptide with the same m.p. and rotation.

Z-Ser-Gln-Thr-Pro-Leu-Val-Thr-Leu-OtBu (70–77). 9.54 g (16.9 mmol) of Z-Ser-Gln-Thr-Pro-OH (obtained after deprotection of Z-Ser-Gln-Thr-Pro-OtBu with TFA/H_2O = 9/1, v/v) and an equimolar amount of H-Leu-Val-Thr-Leu-OtBu (obtained after hydrogenation of Z-Leu-Val-Thr-Leu-OtBu in MeOH with Pd/C as the catalyst) in 130 ml of DMF were coupled by the DCC/HOBt method and the reaction mixture was worked up as described for (70–73). After drying (Na_2SO_4), the organic solvent was evaporated to dryness. The concentrate was dissolved in DMF and EtOAc was added. The precipitate was filtered, washed with EtOAc, and dried. Yield 12.0 g (71.6%); m.p. 210–212°C (dec.); $[\alpha]_D^{21}$ −40.9° (*c* = 1, DMF). TLC: R_f 0.81 (a), 0.48 (c).

Synthesis of Z-Ser-Gln-Thr-Pro-Leu-Val-Thr(tBu)-OtBu, sequence 70–76

Z-Leu-Val-Thr(tBu)-OtBu (74–76). 20.9 g (57.3 mmol) of Z-Leu-Val-OH[28] and 13.2 g (57.3 mmol) of H-Thr(tBu)-OtBu [obtained after hydrogenation of Z-Thr(tBu)-OtBu[18] in MeOH with Pd/C as the catalyst] were coupled in DMF by the DCC/HOBt method. The crude product was purified by column chromatography using silica gel and toluene/ethanol (9/1) as the eluent. Yield 17.1 g (52.0%); m.p. 56–58°C; $[\alpha]_D^{21}$ −37.6° (*c* = 1, MeOH). TLC: R_f 0.54 (b).

Z-Ser-Gln-Thr-Pro-Leu-Val-Thr(tBu)-OtBu (70–76). 2.00 g (3.54 mmol) of Z-Ser-Gln-Thr-Pro-OH and 1.68 g (3.79 mmol) of H-Leu-Val-Thr(tBu)-OtBu (obtained after hydrogenation of Z-Leu-Val-Thr(tBu)-OtBu in MeOH with Pd/C as the catalyst) were coupled by the DCC/HOBt method as described for (70–73). The working-up procedure was the same as for (70–77). Yield 2.28 g (65.2%); m.p. 185–187°C; $[\alpha]_D^{20}$ −34.7° (*c* = 1, DMF). TLC: R_f 0.80 (c).

Synthesis of H-Tyr-Gly-Gly-Phe-Met-Thr-Ser-Glu-Lys-Ser-Gln-Thr-Pro-Leu-Val-Thr-Leu-OH, γ-endorphin, Fig. 4

Boc-Tyr-Gly-Gly-Phe-Met-Thr-Ser-Glu(OtBu)-Lys(Boc)-OH (61–69). 1.29 g of Boc-61–69-OMe (1.00 mmol) were treated with 11 ml of 0.217 N NaOH in 11 ml of dioxane/H_2O (9/1) for 18 min (under N_2). Then the pH was adjusted to 2 with HCl, and water was added; Boc-61–69-OH was precipitated from the mixture. The precipitate was filtered, washed with water, and dried. Yield 990 mg (77.6%); m.p. 182–184°C (dec.), $[\alpha]_D^{20}$ −10.1° (*c* = 1, DMF). TLC: R_f 0.51 (a), 0.23 (c).

Boc-Tyr-Gly-Gly-Phe-Met-Thr-Ser-Glu(OtBu)-Lys(Boc)-Ser-Gln-Thr-Pro-Leu-Val-Thr-Leu-OtBu (61–77). To a stirred solution (cooled, approx. −22°C) of 960 mg (0.75 mmol) of Boc-61–69-OH, 760 mg (0.85 mmol) of H-70–77-OtBu (obtained after hydrogenation of 1.2 g of Z-70–77-OtBu in MeOH with Pd/C as the catalyst) and 230 mg of HOBt (2 equiv.) in 12 ml of DMF, 154 mg of DCC (0.75 mmol) were added. After stirring for 10 min at −22°C, and 18 h at +33°C under N_2, DCU was removed by filtration, 200 ml of H_2O were added and the resulting precipitate was collected by filtration. Recrystallization from DMF/EtOH/EtOAc gave 1.15 g (70.6%) of Boc-61–77-OtBu; m.p. 207–210°C; $[\alpha]_D^{20}$ −24.3° (*c* = 0.5, DMF). TLC: R_f 0.49 (c).

H-Tyr-Gly-Gly-Phe-Met-Thr-Ser-Glu-Lys-Ser-Gln-Thr-Pro-Leu-Val-Thr-Leu-OH (γ-endorphin). 1.1 g (0.51 mmol) of Boc-61–77-OtBu were treated with 20 ml of TFA/H_2O (9/1, v/v) under N_2 in the presence of anisole. After conversion of the trifluoroacetate salt into the acetate, the crude product was purified by chromatography on silica (Merck Fertigsäule) with solvent system e. Yield 440 mg; homogeneous on TLC plates: R_f 0.33 (e), 0.29 (g). $[\alpha]_D^{20}$ −70.2° (*c* = 0.5, 10% HOAc) [Lit.[12] $[\alpha]_D^{23}$ −80.5° (*c* = 1, 10% HOAc)].
Amino acid analysis: Thr 2,89, Ser 1.95, Glu 2.12, Pro 0.99, Gly 2.08, Val 1.00, Met 0.98, Leu 1.94, Tyr 0.96, Phe 1.02, Lys 1.00. Peptide content 79.5%. LAO digestion: no racemization can be detected. HPLC: a linear gradient of 0–70% B was run in 25 min; main component 97.1%.

Synthesis of H-Tyr-Gly-Gly-Phe-Met-Thr-Ser-Glu-Lys-Ser-Gln-Thr-Pro-Leu-Val-Thr-OH, α-endorphin

Boc-Tyr-Gly-Gly-Phe-Met-Thr-Ser-Glu(OtBu)-Lys(Boc)-Ser-Gln-Thr-Pro-Leu-Val-Thr(tBu)-OtBu (61–76). 1.28 g (1.00 mmol) of Boc-61–69-OH and 942 mg (1.10 mmol) of H-70–76-OtBu (obtained after hydrogenation of Z-70–76-OtBu in MeOH) were coupled by the DCC/HOBt procedure in the same way as described for Boc-61–77-OtBu. Crystallization from MeOH/H_2O (4/1, v/v) gave 1.62 g (75.6%) of Boc-61–76-OtBu; m.p. 211°C (dec.); $[\alpha]_D^{20}$ −21.6° (*c* = 0.5, DMF). TLC: R_f 0.78 (a), 0.44 (c).

H-Tyr-Gly-Gly-Phe-Met-Thr-Ser-Glu-Lys-Ser-Gln-Thr-Pro-Leu-Val-Thr-OH (α-endorphin). 1.57 g (0.74 mmol) of Boc-61–76-OtBu were treated with 30 ml of TFA/H_2O (9/1, v/v), under N_2, in the presence of anisole. After conversion of the trifluoroacetic acid salt into the acetate, the crude product was purified by counter-current distribution using the solvent system BuOH/HOAc/H_2O (4/1/5). Yield 310 mg; $[\alpha]_D^{21}$ −71.6° (*c* = 0.5, 10% HOAc) [lit.[12] $[\alpha]_D^{23}$ −76.5° (*c* = 1, 10% HOAc)]. One spot on TLC: R_f 0.25 (e).
Amino acid analysis: Thr 2.98, Ser 1.96, Glu 2.06, Pro 1.00, Gly 2.06, Val 1.00, Met 0.96, Leu 0.96, Tyr 1.01, Phe 1.00, Lys 0.98. Peptide content 91.8%. No racemization could be detected (LAO digestion). HPLC: a linear gradient of 0–70% B was run in 25 min; main component 96.1%.

H-Tyr-Gly-Gly-Phe-Met-OH, β-LPH-(61–65). 1.11 g (1.63 mmol) of Boc-Tyr-Gly-Gly-Phe-Met-OH were deprotected with 10 ml 90% aqueous TFA, under N_2, in the presence of anisole. After conversion of the trifluoroacetate into the acetate the crude product was purified by chromatography on silica (Merck Fertigsäule) using the solvent system BuOH/pyridine/HOAc/H_2O = 20/3/1/4. Yield 368 mg, $[\alpha]_D^{20}$ +19.5° (*c* = 1, 10% HOAc) [lit.[25] $[\alpha]_D^{24}$ +26.1° (*c* = 1, 95% HOAc); lit.[29] (HCl salt) $[\alpha]_D^{20}$ +18° (*c* = 1, 1 M HOAc)]. TLC: R_f 0.50 (d), 0.53 (e).
Amino acid analysis: Gly 1.97, Met 0.99, Tyr 1.00, Phe 1.04; peptide content 90.2%. No racemization was detected (LAO). HPLC: a linear gradient of 0–100% B was run in 25 min; main component 97.2%.

H-Tyr-Gly-Gly-Phe-Met-Thr-Ser-Glu-Lys-OH, β-LPH-(61–69). 1.27 g (1.00 mmol) of Boc-61–69-OH were deprotected with 15 ml of 90% aqueous TFA under N_2 in the presence of anisole. After conversion of the trifluoroacetate salt into the acetate, the crude product was purified by column chromatography on silica (Merck Fertigsäule) using solvent system e. Yield: 181 mg; $[\alpha]_D^{20}$ −18.8° (*c* = 1, 10% HOAc). TLC: R_f 0.20 (e).
Amino acid analysis: Thr 1.01, Ser 0.94, Glu 1.06, Gly 1.91, Met 1.04, Tyr 0.96, Phe 1.03, Lys 1.00. Peptide content 90.5%. No racemization could be detected. HPLC: a linear gradient of 0–100% B was run in 15 min; main component 91.0%; several traces of by-products were seen.

H-Ser-Gln-Thr-Pro-Leu-Val-Thr-OH, β-LPH-(70–76). 760 mg (0.89 mmol) of H-70–76-OtBu were treated with 10 ml of aqueous 90% TFA. After conversion of the trifluoroacetate salt into the acetate, the crude product was purified by chromatography on silica (Merck Fertigsäule) with solvent system e. Yield 540 mg; $[\alpha]_D^{20}$ −109.1° (*c* = 1, 10% HOAc). TLC: 0.35 (e).
Amino acid analysis: Thr 1.91, Ser 1.02, Glu 1.04, Pro 0.98, Val 1.04, Leu 1.00. Peptide content 84.4%. LAO digestion: no racemization was found. HPLC: a linear gradient of 0–100% B was run in 15 min; main component 98.5%.

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H-Ser-Gln-Thr-Pro-Leu-Val-Thr-Leu-OH, β-LPH-(70–77). 390 mg (0.43 mmol) of H-70–77-OtBu were treated with 90% aqueous TFA and purified in the same way as was described for 70–76. Yield 244 mg; $[\alpha]_D^{23}$ −121.3° (*c* = 0.5, 10% HOAc). Homogeneous on TLC: R_f 0.48 (e).
Amino acid analysis: Thr 1.94, Ser 0.98, Glu 1.04, Pro 1.04, Val 1.04, Leu 1.96. Peptide content 93.6%. LAO digestion: no racemization was found. HPLC: a linear gradient of 20–70% B was run in 25 min; main component: 95.3%.

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Preliminary Communication

Interpretation of vicinal proton-proton coupling constants by a generalized Karplus relation. Conformational analysis of the exocyclic C4'-C5' bond in nucleosides and nucleotides

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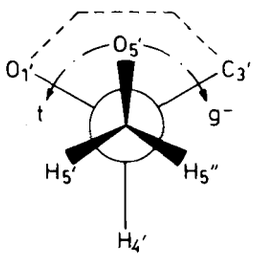
Abstract. New values for $^3J_{HH}$ along the C4'-C5' bond in nucleosides and nucleotides are proposed.

The exocyclic C4'-C5' bond in the nucleotidyl unit of nucleic acids is one of the six torsion angles which constitute the conformation of the polynucleotide sugar-phosphate backbone. The solution conformation of the 5'-CH_2OR-moiety relative to the furanose ring may comprise three types of rotamers which are denoted g^+, g^-, t (delineating the conformation of the "backbone" fragment C3'-C4'-C5'-O5', ψ-bond, Fig. 1). In aqueous solution the rapid interconversion between the three conformers yields weighted time-averaged NMR couplings that are related to the couplings of the individual conformers and their relative populations by:

Figure 1.



ψ: gauche$^+$ (g^+)

$$^3J_{4'5'} = x_{g+}J'_{g+} + x_{g-}J'_{g-} + x_tJ'_t$$

$$^3J_{4'5''} = x_{g+}J''_{g+} + x_{g-}J''_{g-} + x_tJ''_t$$

where x denotes the mole fraction of each of the conformers present ($\sum x_i = 1$). Knowledge of the individual couplings in these rotamers (J' and J'') will allow conformational analysis of the C4'-C5' bond in terms of relative rotamer populations. Several estimates[1,2,3] for these limiting coupling constants have been proposed in the literature, together with many derived simple relationships, from which the population of the major conformer, x_{g+}, may be calculated[4].

In this communication we report new values for the coupling constants in the individual rotamers as determined from solid-state knowledge of the nucleoside-moiety combined with the use of a generalized Karplus relation. The latter was recently derived in our laboratory[5] and is formulated as a standard Karplus equation extended with a correction term for the influence of electronegative substituents. The relation was empirically parametrized and yields for H-C-C-H fragments bearing *three* non-hydrogen substituents; equation (1):

$$^3J_{HH} = 13.22\cos^2\phi - 0.99\cos\phi + \sum\{0.87 - 2.46\cos^2(\xi_i\cdot\phi + 19.9\cdot|\Delta\chi_i|)\}\Delta\chi_i \qquad (1)$$

In this equation ϕ denotes the proton-proton torsion angle; ξ_i stands for +1 or -1 according