SYNTHESIS OF PEPTIDES INFLUENCING GROWTH HORMONE RELEASE*

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Received February 18, 1993
Accepted June 25, 1993

A solid phase peptide synthesis of 17 growth hormone (GH) releasing peptide analogues *Ia* – *IIIc* for their use in a pharmacological assay on GH release is described. While the linear peptide amides *Ia* – *Ij* were synthesized on *p*-methylbenzhydrylamine resin using Boc strategy, and cleaved by HF in the presence of scavengers the linear peptide amides *Ik* and *Il* were prepared on Merrifield benzyl ester type resin using Fmoc strategy and cleaved by ammonolysis. The deleted peptide amides *IIa* and *IIb* were obtained as by-products during HPLC purification of analogues *Ic* and *Id*. The linear precursors of cyclic peptides *IIIa* – *IIIc* were also prepared on Merrifield resin and cleaved under mild alkaline conditions. Their cyclization was performed in solution by diphenylphosphoryl azide.

The hexapeptide His-D-Trp-Ala-Trp-D-Phe-Lys-NH_2 *I* (GHRP-6)** was shown[2–7] to stimulate GH release both in vitro and in vivo. In man, this peptide appears to be even more efficacious than GH releasing factor as GH secretagogue.

Now, the series of the peptides *Ia* – *IIIc* derived from GHRP-6 was synthesized to test their pharmacological properties.

The peptides *Ia* – *Ij* were built on MBHA resin (Scheme 1) using HOBt esters of corresponding Boc-amino acids with side-chain protection: Lys(DCZ), Trp(For), Ser(Bzl), His(Boc), in DMF. The Boc group was cleaved by 50% TFA–DCM in the presence of EDT–indole, followed by 5% DIEA–DCM. The peptides were cleaved from the resin and side-chain protecting groups removed by treatment with HF–EDT–indole and then purified by HPLC.

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* Part CCXXXIV in the series Amino Acids and Peptides; Part CCXXXIII: Collect. Czech. Chem. Commun. *58*, 421 (1993).

** The nomenclature and symbols of amino acids and peptides obey the published recommendations[1]. In addition we use the following abbreviations: MBHA, *p*-methylbenzhydrylamine; HOBt, 1-hydroxybenzotriazol; DCC, dicyclohexylcarbodiimide; DCZ, 2,6-dichlorobenzyloxycarbonyl; DMF, dimethylformamide; DCM, dichloromethane; TFA, trifluoroacetic acid; DIEA, diisopropylethylamin; EDT, ethanedithiol; DPPA, diphenylphosphoryl azide.

R-His-X-Ala-Trp-D-Phe-Lys-NH_2
1 2 3 4 5 6

Ia, R = H; X = Tle
Ib, R = H; X = D-Tle
Ic, R = H; X = Neo
Id, R = H; X = D-Neo
Ie, R = H; X = Ser
If, R = H; X = D-Ser
Ig, R = Gly; X = D-Trp
Ih, R = Gly-Gly; X = D-Trp
Ii, R = Gly-Gly-Gly; X = D-Trp
Ij, R = Hip; X = D-Trp
Ik, R = H; X = Ser(Bu^t)
Il, R = H; X = D-Ser(Bu^t)
IIa, [Neo^2, des Ala^3]GHRP-6
IIb, [D-Neo^2, des Ala^3]GHRP-6
IIIa, c[GHRP-6]
IIIb, c[endo Gly^1GHRP-6]
IIIc, c[exo Gly^1GHRP-6]

The peptides *Ik* and *Il* were built on Merrifield type resin (Scheme 2) using HOBt esters of Boc-Lys(DCZ)-OH, Boc-D-Phe-OH, Fmoc-Trp-OH, Fmoc-Ala-OH, Fmoc-Ser(Bu^t)-OH or Fmoc-D-Ser(Bu^t)-OH and Fmoc-His-OH in DMF. The Fmoc group was removed by 20% piperidine in DMF. The hexapeptide resins were treated with ammonia–MeOH to yield the corresponding hexapeptide amides and side-chain DCZ protecting group was removed by hydrogenolysis.

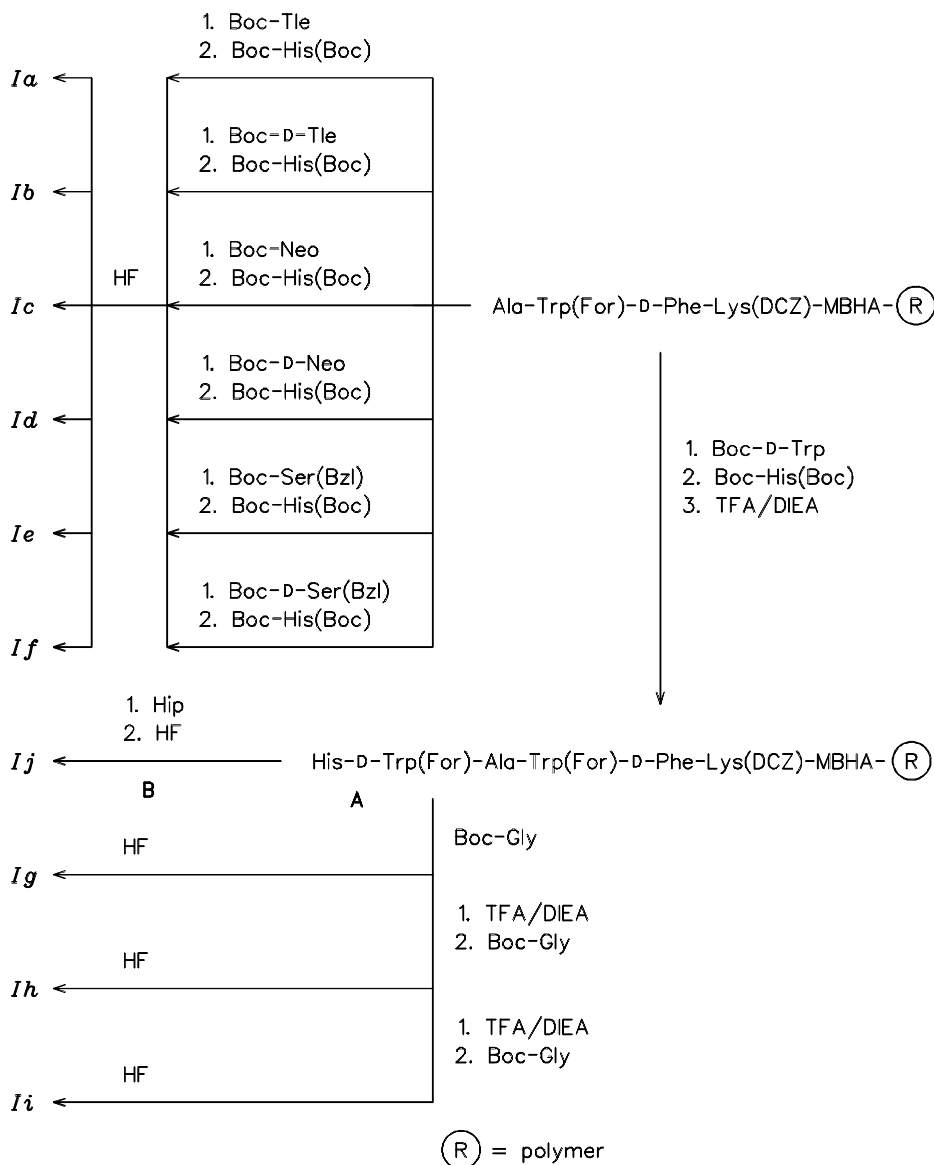
The peptides *IIa* and *IIb* with deleted Ala residue were separated from corresponding hexapeptides *Ic* and *Id* during their purification by preparative HPLC. The amount of deleted sequences in other peptides prepared was negligible.

The peptides *IIIa* – *IIIc* were built on the Merrifield chloromethyl resin (Scheme 2) starting with Boc-Lys(DCZ) and using the similar coupling protocol described for peptides *Ik* – *Il*. Then, the linear peptides were split off the resin by 0.2 M NaOH–MeOH–dioxane and cyclized by DPPA in the presence of K_2HPO_4 in DMF. The ε-amino protecting group of the Lys was removed by hydrogenation on Pd.

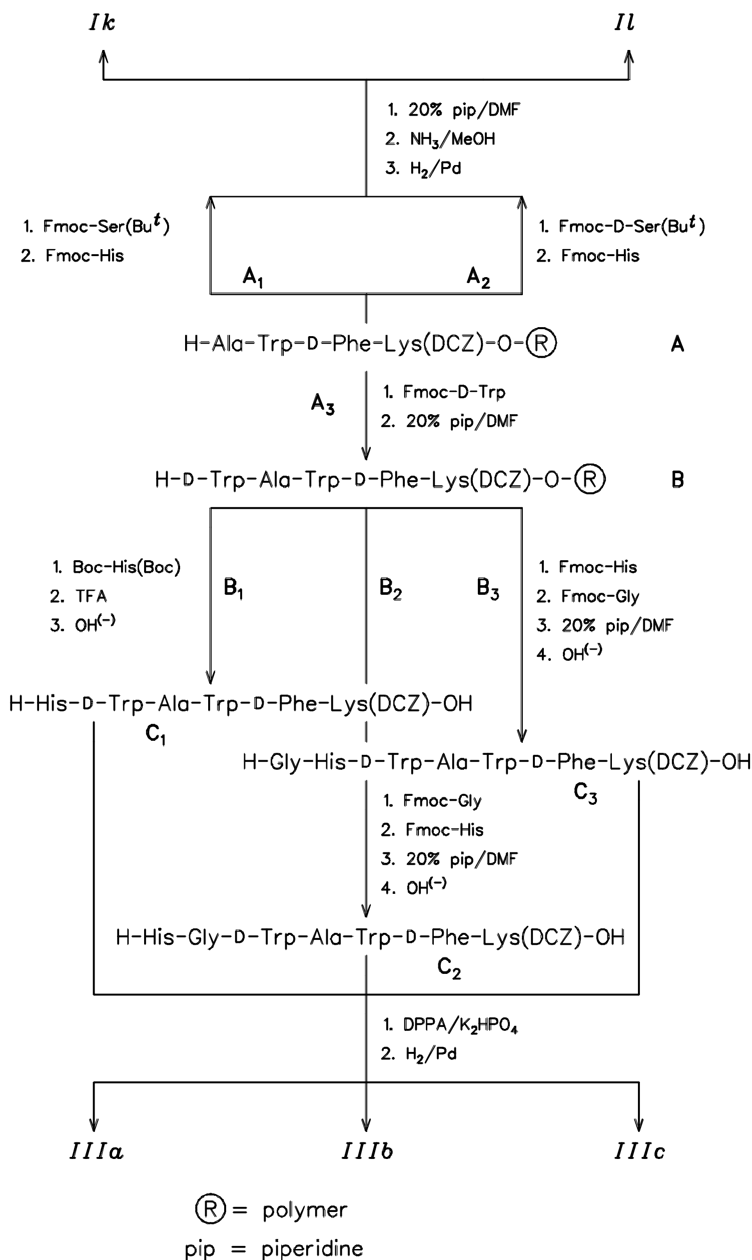
Preliminary results on effect of some GHRP analogues on GH contents in a plasma of rats and in a long term peptide therapy on the life expectancy of mice have been already described in a short communication[8]. A detailed structure–activity study on this series of the GHRP analogues will be presented elsewhere.

EXPERIMENTAL

The peptide samples for amino acid analysis were hydrolyzed by 6 M HCl at 110 °C for 20 h, Trp containing samples were hydrolyzed under the same conditions with 4% thioglycolic acid added. The amino acid analyses were performed on a Durrum D-500 (Durrum Instrum. Corp., Palo Alto, U.S.A.) or a Mikrotechna (Prague, The Czech Republic) amino acid analyzer. Mass spectroscopy with FAB technique was used for determination of M^+ of the corresponding peptides (VG Analytical, England). For HPLC a Spectra Physics instrument with an SP 8800 pump, an SP 8450 UV detector and an SP 4290 integrator was used. The analytical HPLC was carried out on a 25 × 0.4 cm Vydac column (The



SCHEME 1



SCHEME 2

Separations Group, Hesperia, U.S.A.), flow rate 60 ml/h, detection at 222 nm, mobile phase methanol with 0.05% aqueous TFA. The preparative HPLC was done on 25 × 1.0 cm column packed with the same stationary phase, flow rate 180 ml/h, mobile phase a mixture of methanol with 0.05% aqueous TFA, detection at 280 nm. Analytical electrophoresis was carried out in a moist chamber on a Whatman 3MM paper (20 V/cm) in a 6% acetic acid and in a pyridine–acetate buffer pH 5.7 for 60 min. N^{α}-Boc-protected amino acids were prepared by published methods[9 – 13], N^{α}-Fmoc-protected amino acids were from Bachem, California. Just before use, all amino acid derivatives were tested for homogeneity by thin-layer chromatography (Silufol plates, Kavalier, The Czech Republic) in the systems: 2-butanol–98% formic acid–water (75 : 13.5 : 11.5) (S1); 2-butanol–25% aqueous ammonia–water (85 : 7.5 : 7.5) (S2); 1-butanol–acetic acid–water (4 : 1 : 1) (S3); 1-butanol–pyridine–acetic acid–water (15 : 10 : 3 : 6) (S4). Detection was with iodine, ninhydrin or by the chlorination method. Solvents were evaporated on a rotary evaporator (bath temperature 30 °C) in vacuo. Progress of peptide synthesis was followed by the ninhydrin and bromophenol blue tests[14,15].

Peptides *Ia* – *Ij* and *IIa*, *IIb*

A general synthetic strategy of the synthesis of the peptides *Ia* – *Ij* is described in the Scheme 1. A mixture of the Boc-Lys(DCZ)-OH (1.34 g, 3 mmol), MBHA resin (UCB; 0.6 mmol/g, 2 g), HOBt (0.40 g, 3 mmol) and DCC (0.62 g, 3 mmol) in DMF (20 ml) was stirred for 6 h at room temperature in a reaction vessel of a home made solid phase synthesizer and the solvent was then filtered off. The Boc-Lys(DCZ)-MBHA resin was washed with DCM (3 × 40 ml), 2-propanol (3 × 40 ml) and DCM (3 × 40 ml) and residual amino groups on the resin were acetylated with a mixture acetic anhydride (3 ml)–DIEA (4.5 ml)–DCM (35 ml) for 2 h at room temperature. Then the Boc group was cleaved by stirring with a mixture 50% TFA–10% anisole in DCM (40 ml) for 5 and 30 min and TFA.H-Lys(DCZ)-MBHA resin was neutralized by 5% DIEA in DCM (2 × 40 ml), 5 min. The synthetic cycle was completed by washing the H-Lys(DCZ)-MBHA resin with DCM (5 × 40 ml) and was repeated with 3 equivalents of HOBt–DCC activated Boc-D-Phe-OH (0.78 g), Boc-Trp(For)-OH (0.98 g) and Boc-Ala-OH (0.58 g). For a cleavage of the Boc group in Trp containing sequences the mixture containing TFA (50%)–EDT (5%)–indole (2%) in DCM (40 ml) for 5 and 30 min was used.

At this stage of the synthesis the washed and dried tetrapeptide-MBHA-resin (2.7 g) was divided into several portions. While one portion of this tetrapeptide-MBHA-resin (1.5 g) was coupled consequently with HOBt–DCC activated Boc-D-Trp(For)-OH (0.55 g, 1.68 mmol) and Boc-His(Boc)-OH (0.62 g, 1.68 mmol) to obtain the sequence of the peptide *I* (in the synthesis of compounds *Ig* – *Ij*), the six other portions (6 × 0.2 g) were separately acylated with 0.34 mmol of HOBt–DCC activated: Boc-Tle-OH (0.08 g), (for *Ia*); Boc-D-Tle-OH (0.08 g), (for *Ib*); Boc-Neo-OH (0.09 g), (for *Ic*); Boc-D-Neo-OH (0.09 g), (for *Id*); Boc-Ser(Bzl)-OH (0.10 g), (for *Ie*); Boc-D-Ser(Bzl)-OH (0.10 g), (for *If*), and in the next step with Boc-His(Boc)-OH (6 × 0.12 g, 6 × 0.34 mmol).

Then, these seven hexapeptide-MBHA-resins prepared were treated with already described TFA cleaving mixture (2 × 30 ml) and neutralized with 5% DIEA in DMF (30 ml).

Each of the hexapeptide-MBHA-resins corresponding to compounds *Ia* – *If* was treated with a liquid HF (10 ml) in the presence of ethanedithiol (1 ml) and indole (0.2 g) at 0 °C for 1 h. After evaporation of HF the peptide amides *Ia* – *If* were washed out from the resin successively by 50% (3 × 20 ml) and 6% (3 × 20 ml) acetic acid. The combined acetic acid washings were extracted by ethyl acetate (3 × 150 ml), diluted with water (100 ml) and freeze dried. The crude peptide amides *Ia* – *If* were purified by gel filtration on the column 60 × 2.5 cm using Sephadex G-10 in 3 M acetic acid. The fractions containing main peak were pooled and solutions freeze dried. Finally, the peptides *Ia* – *If* were purified by reverse phase HPLC using 30 – 50% (15 min) and 50 – 70% (45 min)

gradients of methanol in 0.05 M TFA. At this purification step the peptides *IIa* and *IIb* with deleted Ala residue were separated from corresponding hexapeptides *Ic* and *Id*. The amount of deleted sequences in other peptides prepared was negligible.

The hexapeptide-MBHA-resin with a sequence of the hexapeptide *I* was dried (1.75 g) and divided into portions **A** and **B**.

The portion **A** (1.05 g) was coupled with HOBt–DCC activated Boc-Gly-OH (0.18 g, 1.02 mmol), then washed and dried as described above. After cleavage of Boc group a part of the heptapeptide-MBHA-resin (0.60 g) was separated for the preparation of the compound *Ig*. The coupling cycle was repeated with the rest of this heptapeptide-MBHA-resin which was again acylated by Boc-Gly-OH (0.12 g, 0.67 mmol) to obtain the octapeptide-MBHA-resin (0.60 g; preparation of *Ih*). Finally, Boc-Gly-OH (0.06 g, 0.34 mmol) was coupled with a part of this octapeptide-MBHA-resin and after cleavage of Boc group, washing and drying, the nonapeptide-MBHA-resin (0.61 g) for preparation of *Ii* was obtained.

The portion **B** (0.70 g) of the hexapeptide-MBHA-resin was coupled with 0.70 mmol of hippuric acid (0.14 g), (for *Ij*) following the coupling protocol described above, respectively.

After deprotection of the Boc group were corresponding peptide-MBHA-resins treated with liquid HF and purified similarly to the synthesis of the compound *Ia* – *If*. The gel filtration of the analogues *Ig* – *Ij* was carried out on the column of Sephadex G-15 in 3 M acetic acid and followed by the preparative HPLC purification using 0 – 30% (20 min) and 30 – 80% (50 min) gradients of MeOH in 0.05% TFA. The analytical data and HPLC separation conditions of the analogues *Ia* – *Ij* and *IIa*, *IIb* are shown in Table I.

Peptides *Ik*, *Il* and *IIIa* – *IIIc*

The general strategy of the synthesis of the GHRP analogues *Ik*, *Il* and *IIIa* – *IIIc* is shown on the Scheme 2. A mixture of the Boc-Lys(DCZ)-$O^{-}Cs^{+}$, prepared from Boc-Lys(DCZ)-OH (1.62 g, 3.6 mmol) according to Gissin et al.[16], and Merrifield chloromethyl resin (Lab Systems, San Mateo, U.S.A.; 0.7 mmol/g, 2.0 g) in DMF was, under anhydrous conditions, stirred for 24 h at room temperature in a reaction vessel of a home made solid phase synthesizer and the solvent was filtered off. The Boc-Lys(DCZ)-*O*-resin was washed with DMF (3 × 60 ml), DCM (3 × 60 ml), 2-propanol (3 × 60 ml), DCM (3 × 60 ml) and the Boc group was cleaved by stirring with a mixture 50% TFA–10% anisole in DCM (60 ml) for 5 and 30 min. The TFA.H-Lys(DCZ)-*O*-resin was neutralized by 5% DIEA in DCM (2 × 60 ml), 2 × 5 min, washed with DCM (5 × 60 ml) and coupled with Boc-D-Phe-OH (0.94 g, 3.6 mmol), HOBt (0.54 g, 4 mmol) and DCC (0.82 g, 4 mmol) in DMF (60 ml). After splitting off the Boc protecting group and neutralization of the TFA salt by above described procedure the Fmoc-Trp-OH (1.54 g, 3.6 mmol) and Fmoc-Ala-OH (1.13 g, 3.6 mmol) in DMF were added stepwise. After each coupling the Fmoc protecting group was removed by 20% piperidine in DMF (60 ml), 2 × 30 min. At this point the H-Ala-Trp-D-Phe-Lys(DCZ)-*O*-resin was washed with DMF (3 × 60 ml), DCM (3 × 60 ml), dried in a desiccator (2.8 g) and divided into three parts (peptide-*O*-resin **A**).

The first two parts of the peptide-*O*-resin (**A1**-**A2**; 2 × 0.56 g) were separately acylated stepwise by Fmoc-Ser(Bu^t)-OH (0.28 g, 0.72 mmol) and Fmoc-His-OH (0.27 g, 0.72 mmol) (*Ik*) or by Fmoc-D-Ser (Bu^t)-OH (0.28 g; 0.72 mmol) and Fmoc-His-OH (0.27 g, 0.72 mmol) (*Il*). Fmoc protecting group was in both cases removed by 20% piperidine in DMF (20 ml) and peptides *Ik* and *Il* were cleaved from the resin on adding hexapeptide-*O*-resins to freshly prepared anhydrous methanol (80 ml) saturated with anhydrous ammonia at −5 °C. The mixtures were stirred in pressure bottle at room temperature for 4 days. After the solvent was removed in vacuo, the cleaved peptide amides *Ij* (0.15 g) and *Ik* (0.14 g) in methanolic solutions were hydrogenated in the presence of Pd black for

TABLE I
Analytical data on GHRP analogues *Ia* – *Il* and *IIa, IIb*

Compound	Formula M.w./(M + H)$^+$	R_T [a] min	Amino acid analysis[b]					Elfo[f]	
			Lys Tle	Phe Neo	Trp Ser	Ala Gly	His	$E_{Gly}^{2.4}$	$E_{His}^{5.7}$
Ia	$C_{41}H_{57}N_{11}O_6$ 799.9/800.2	[c] 10.16	1.00 1.03	1.02 –	0.89 –	0.93 –	1.02	1.40	0.85
Ib	[d]	[c] 10.75	1.00 1.02	1.03 –	0.87 –	0.96 –	1.01		
Ic	$C_{42}H_{59}N_{11}O_6$ 814.0/814.2	[c] 11.16	1.00 –	1.02 1.01	0.90 –	0.94 –	1.03	1.38	0.84
Id	[d]	[c] 12.16	1.00 –	0.99 1.03	0.87 –	0.97 –	1.00		
Ie	$C_{38}H_{52}N_{11}O_7$ 774.9/775	[c] 9.86	1.00 –	1.04 –	0.91 1.02	1.01 –	1.05	1.42	0.86
If	[d]	[c] 10.34	1.00 –	1.02 –	0.90 1.04	0.98 –	1.04		
Ig	$C_{48}H_{59}N_{13}O_7$ 930.1/930.3	[e] 22.57	1.00 –	1.04 –	1.72 –	1.02 1.03	0.99	1.22	0.79
Ih	$C_{50}H_{62}N_{14}O_8$ 987.2/987	[e] 21.85	1.00 –	1.02 –	1.75 –	0.98 1.94	1.02	1.20	0.77

TABLE I
(*Continued*)

Compound	Formula M.w./(M + H)[+]	R_T[a] min	Amino acid analysis[b]					Elfo[f]	
			Lys Tle	Phe Neo	Trp Ser	Ala Gly	Hi	$E_{Gly}^{2.4}$	$E_{His}^{5.7}$
Ii	$C_{52}H_{65}N_{15}O_9$ 1 044.2/1 044	*e* 20.94	1.00 –	0.97 –	1.69 –	1.01 2.93	1.05	1.17	0.74
Ij	$C_{55}H_{63}N_{13}O_8$ 1 034.2/1 035	*c* 13.88	1.00 –	0.99 –	1.78 –	1.03 0.94	1.04	1.24	0.72
Ik	$C_{42}H_{59}N_{11}O_7$ 830.0/830.2	*c* 12.03	1.00 –	1.02 –	1.75 1.03	1.01 –	1.05	1.40	0.85
Il	[d]	*c* 12.46	1.00 –	1.04 –	1.77 1.05	1.03 –	1.02		
IIa	$C_{39}H_{54}N_{10}O_5$ 742.9/743.2	*c* 12.82	1.00 –	0.97 1.04	0.87 –	– –	0.98	1.49	0.85
IIb	[d]	*c* 13.89	1.00 –	0.96 1.02	0.85 –	– –	1.02		

[a] HPLC retention time. [b] Hydrolysis with thioglycolic acid. [c] Mobile phase 30 – 50% (10 min) and 50 – 100% (20 min) gradient of MeOH in 0.05% TFA, the retention time of the standard GHRP *I* was 12.71 min. [d] The same molecular weight was confirmed by mass spectroscopy. [e] Mobile phase 0 – 30% (20 min) and 30 – 80% (30 min) gradient of MeOH in 0.05% TFA. [f] Electrophoretic mobility.

6 h at room temperature. After filtration of the catalyst the solvent was evaporated and both peptides purified using HPLC as described for peptides *Ia* – *Ii*. The analytical data and HPLC separation conditions for analogues *Ij* and *Il* are presented in Table I.

The third part of the tetrapeptide-*O*-resin **A** (**A3**, 1.68 g) was acylated by Fmoc-D-Trp-OH (0.92 g, 2.16 mmol), Fmoc protecting group was cleaved as described above, the pentapeptide-*O*-resin (**B**) washed and dried (1.8 g). At this point was this peptide-*O*-resin (**B**) devided into three parts for preparation of the cyclopeptides *IIIa* – *IIIc*.

The pentapeptide-*O*-resin **B1** (0.6 g) was coupled with Boc-His(Boc)-OH (0.26 g, 0.72 mmol) using HOBt (0.11 g, 0.8 mmol) and DCC (0.26 g, 0.8 mmol) in DMF (20 ml). Boc protecting groups were cleaved by 50% TFA–DCM (30 ml) in the presence of EDT (2 ml) and indole (0.2 g), hexapeptide-*O*-resin was washed with DCM (5 × 20 ml), dried over P_2O_5 in desiccator and peptide cleaved from the resin using the method of Buis et al.[17]. The peptide resin (0.7 g) was vigorously stirred in a mixture containing dioxane (90 ml), methanol (27 ml) and 4 M NaOH (3 ml) for 2 × 3 min. Then pH was adjusted to 6 by adding acetic acid (1 ml). After filtration the resin was washed consecutively with methanol (2 × 70 ml), DCM (2 × 70 ml), DMF (50 ml), methanol (110 ml) and DCM (50 ml). The combined washings were evaporated, the residue dissolved in methanol (10 ml), the insoluble material filtered off and water was added. A precipitate of H-His-D-Trp-Ala-Trp-D-Phe-Lys(DCZ)-OH (peptide **C1** in the Scheme 2) was filtered off and dried over P_2O_5 in desiccator (0.21 g). Amino acid analysis and FAB mass spectrometry (*m/z*: 1 077 $(M + H^+)$ for $C_{54}H_{59}N_{11}O_9Cl_2$) of this crude material revealed the presence of corresponding amino acids. HPLC retention time using 50 – 90% (30 min) gradient of MeOH in 0.05% TFA was 13.93 min.

The pentapeptide-*O*-resin **B2** (0.6 g) was coupled with Fmoc-Gly-OH (0.16 g, 0.72 mmol) using HOBt (0.11 g, 0.8 mmol) and DCC (0.26 g, 0.8 mmol) in DMF (20 ml) and after cleavage of Fmoc protecting group by 20% piperidine in DCM (20 ml) the hexapeptide-*O*-resin was acylated by HOBt–DCC activated Fmoc-His-OH (0.57 g, 0.72 mmol). After cleavage of the Fmoc protecting group, the heptapeptide-*O*-resin was worked as described in the preparation of the hexapeptide **C1** to obtain the H-His-Gly-D-Trp-Ala-Trp-D-Phe-Lys(DCZ)-OH (peptide **C2** in the Scheme 2; 0.71 g). Amino acid

TABLE II
Analytical data on GHRP analogues *IIIa* – *IIIc*

Compound	Formula M.w./$(M + H)^+$	R_T[a] min	Amino acid analysis[b] Lys Ala	Phe His	Trp Gly	Elfo[d] $E^{2.4}_{Gly}$	$E^{5.7}_{His}$
IIIa	$C_{46}H_{53}N_{11}O_6$ 856.02/857	5.21	1.00 1.04	1.03 0.96	1.84 –	1.18	0.82
IIIb	$C_{48}H_{56}N_{12}O_7$ 913.06/914	5.84	1.00 1.03	1.02 0.97	1.81 0.99	1.10	0.79
IIIc	[c]	5.36	1.00 1.07	0.93 0.95	1.78 0.97	1.15	0.69

[a] HPLC retention time at mobile phase 50 – 90% (30 min) gradient of MeOH in 0.05% TFA. [b] Hydrolysis with thioglycolic acid. [c] The molecular weight as for *IIIb* was confirmed by FAB MS. [d] Electrophoretic mobility.

analysis and FAB mass spectrometry (*m/z*: 1 134 (M + H^+) for $C_{56}H_{62}N_{12}O_{10}Cl_2$) of this crude material revealed the presence of corresponding amino acids. HPLC retention time using 50 – 90% (30 min) gradient of MeOH in 0.05% TFA was 14.21 min.

The pentapeptide-*O*-resin **B3** (0.6 g) was coupled with Fmoc-His-OH (0.57 g, 0.72 mmol) and after cleavage of the protecting group by 20% piperidine in DMF the corresponding hexapeptide-*O*-resin was acylated by Fmoc-Gly-OH (0.16 g, 0.72 mmol) using HOBt (0.11 g, 0.8 mmol) and DCC (0.26 g, 0.8 mmol) in DMF (20 ml). After cleavage of the Fmoc protecting group, the H-Gly-His-D-Trp-Ala-Trp-D-Phe-Lys(DCZ)-OH (peptide **C3** in the Scheme 2) was cleaved from the resin and worked up as described in the preparation of the peptide **C1**. Amino acid analysis and FAB mass spectrometry (*m/z*: 1 134 (M + H^+) for $C_{56}H_{62}N_{12}O_{10}Cl_2$) of this crude material revealed the presence of corresponding amino acids. HPLC retention time using 50 – 90% (30 min) gradient of MeOH in 0.05% TFA was 16.01 min.

The partially protected linear hexapeptide **C1** or heptapeptides **C2** and **C3** (0.2 mmol) in DMF (70 ml) were stirred with K_2HPO_4 (0.32 g) and DPPA (0.18 ml) at 0 °C and after 12 h the second portion of DPPA (0.36 ml) was added. After 24 h at room temperature the reaction mixture was filtered and DMF evaporated. The residue was triturated with water (10 ml) and solid material filtered off and washed with ether. HPLC retention times using 50 – 90% (30 min) gradient of MeOH in 0.05% TFA were as follows: the cyclohexapeptide c(His-D-Trp-Ala-Trp-D-Phe-Lys(DCZ)) 16.39 min, the cycloheptapeptide c(His-Gly-D-Trp-Ala-Trp-D-Phe-Lys(DCZ)) 17.21 min and the cycloheptapeptide c(Gly-His-D-Trp-Ala-Trp-D-Phe-Lys(DCZ)) 19.27 min.

These cyclopeptides (50 mg) in DMF (5 ml) and MeOH (25 ml) were hydrogenated in the presence of Pd black for 6 h, the catalyst was filtered off, the solvents evaporated and peptides *IIIa* – *IIIc* were purified by semipreparative HPLC. The analytical and HPLC separation data for analogues *IIIa* – *IIIc* are given in the Table II.

The authors are indebted to Mr J. Zbrozek for the amino acid analyses. The mass spectrometry analyses were performed in the Mass Spectrometry Laboratory (Dr K. Ubik, Head) of this Institute.

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Translated by the author (J. H.).