Expedient Synthesis of Gonadotropin Releasing Hormone (GnRH) Through a Combined Chemical-Enzymatic Approach

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We have developed a chemical-enzymatic synthetic approach to gonadotropin releasing hormone Glp^1−His^2−Trp^3−Ser^4−Tyr^5−Gly^6−Leu^7−Arg^8−Pro^9−Gly^10−NH₂ (GnRH) which enables one to perform the synthesis efficiently according to minimal protection strategy with all reactive side chains of amino acids unprotected. Five peptide bonds His^3−Trp^3, Tyr^5−Gly^6, Leu^7−Arg^8 and Trp^3−Ser^4, Ser^4−Tyr^5 were formed by means of α-chymotrypsin and papain, respectively. The remaining four bonds Glp^1−His^2, Gly^6−Leu^7, Arg^8−Pro^9, Pro^9−Gly^10 were formed by chemical methods. The synthesis is simple to carry out and there are no problems in scaling up as demonstrated by the final coupling on 2 g scale affording highly pure GnRH in the yield of 84−90%.

Introduction

Gonadotropin releasing hormone (GnRH), Glp^1−His^2−Trp^3−Ser^4−Tyr^5−Gly^6−Leu^7−Arg^8−Pro^9−Gly^10−NH₂ and its analogs are increasingly used for clinical and veterinary purposes, and there is an obvious need for efficient and inexpensive methodology of their synthesis, particularly with a view to multigram preparation. So far, only a few chemical syntheses of GnRH appropriate for scaling up have been reported [1]. Enzymatic methods, with their unparalleled specificity offer clear advantages for large scale peptide synthesis when combined with a minimal protection strategy. Features such as chemoselectivity of proteolytic enzymes which react exclusively at α-carboxyl and α-amino groups, mild activation requirements, and the absence of racemization make the enzymatic coupling methodology an attractive alternative to the commonly used chemical activation. The use of enzymes is particularly appropriate for the synthesis of sequences containing side functional amino acids, the case represented by GnRH molecule. In a recent communication, Kaufmann et al. [2] demonstrated the successful utilization of α-chymotrypsin for a 3 + 7 condensation in the synthesis of the analog [D−Phe^9]GnRH. Andersen et al. [3] reported synthesis of GnRH along almost entirely enzymatic pathway. Although the experimental details were not provided, it is clear from the authors discussion that totally enzymatic approach to GnRH is not appropriate for the practical preparation because of the high cost and difficult availability of specific enzymes needed for some coupling steps.

We report here a combined chemical-enzymatic route to GnRH (Fig. 1) which features the formation of four peptide bonds by chemical couplings and five peptide bonds using α-chymotrypsin and papain as coupling catalysts [4]. This methodology provides an easy access to GnRH on a preparative scale. A preliminary communication of part of these results has appeared previously [5].

Results and Discussion

The scheme for the synthesis of GnRH described here (Fig. 1) involves the preparation of the N-terminal pentapeptide ester Glp^1−His^2−Trp^3−Ser^4−Tyr^5−OMe (6) and the C-terminal pentapeptide amide H−Gly^6−Leu^7−Arg^8−Pro^9−Gly^10−NH₂ (13) which were then joined in the final 5 + 5 coupling catalyzed by α-chymotrypsin. The 5 + 5 segment condensation follows the strategy reported by Andersen et al. [3]. We have used this approach because of its potential for increasing the scale of the synthesis. For the preparation of the N-terminal pentapeptide 6 and the C-terminal pentapeptide 13, we developed a combined chemical-enzymatic approach which enables one to perform all synthetic steps with great efficiency according to minimal protection strategy as shown in the Fig. 1.
Synthetic methodology for the N-terminal pentapeptide methyl ester

\[ \text{Glp}^{1} - \text{His}^{2} - \text{Trp}^{3} - \text{Ser}^{4} - \text{Tyr}^{5} - \text{OME} \] (6)

The preferred coupling strategy leading to N-terminal pentapeptide tert-butyl ester Glp\(^{1}\)-His\(^{2}\)-Trp\(^{3}\)-Ser\(^{4}\)-Tyr\(^{5}\)-OME (1), subsequently transformed into methyl ester 6, involves the 3 + 2 segment coupling of the tripeptide amide Glp\(^{1}\)-His\(^{2}\)-Trp\(^{3}\)-NH\(_{2}\) (2) and the dipeptide tert-butyl ester H-Ser\(^{4}\)-Tyr\(^{5}\)-OBu' (4). Based on its high efficiency in the deamination of the tripeptide amide Glp\(^{1}\)-His\(^{2}\)-Trp\(^{3}\)-NH\(_{2}\) (2), papain was chosen as a coupling catalyst. Under optimal conditions (MeOH/H\(_2\)O 3:7, pH 9.0) the tripeptide amide 2 and the dipeptide 4 in nearly equimolar amount, were joined to give pentapeptide tert-butyl ester 5 as an almost pure product precipitating from the solution in a yield of 67%. The method is simple to carry out and offers an easy access to larger amount of the pentapeptide 5 which could be used in the next step without purification. In the stepwise elongation strategy reported for the 1 – 5 segment of GnRH by Andersen et al. [3], the tripeptide amide Glp\(^{1}\)-His\(^{2}\)-Trp\(^{3}\)-NH\(_{2}\) (2) was transformed into methyl ester prior to subsequent 3 + 1 coupling. The strategy reported here is more advantageous because the straightforward use of the tripeptide amide 2 for the coupling reduces the number of synthetic steps. Dipeptide Z-Ser\(^{4}\)-Tyr\(^{5}\)-OBu' needed for 3 + 2 coupling was easily obtained in a yield of 78% from Z-Ser-OME and Tyr-OBu' in a reaction catalyzed by papain.

Based on their inherent specificity, \(\alpha\)-chymotrypsin and trypsin were chosen for the preparation of the tripeptide amide Glp\(^{1}\)-His\(^{2}\)-Trp\(^{3}\)-NH\(_{2}\) (2) from Glp-His-OME (1) and H-Trp-NH\(_{2}\). We found that the coupling by means of these enzymes in organic solvents containing a minimal amount of water is more efficient than the coupling in aqueous phase with miscible cosolvents. The best yields (78–84% by HPLC) were achieved when \(\alpha\)-chymotrypsin was used in dichloromethane or toluene containing less than 1% of water. For increasing the scale of the synthesis, dichloromethane was found to be more suitable and this can be attributed to better solubility of Trp-NH\(_{2}\). It is noteworthy that only 1.2 equivalent of the nucleophile (Trp-NH\(_{2}\)) is necessary to accomplish the synthesis in high yield. In contrast, the coupling in aqueous phase with miscible cosolvents, required a 5-fold excess of Trp-NH\(_{2}\). The N-terminal dipeptide Glp-His-OME (1) needed for the enzymatic coupling 2 + 1, was best obtained chemically according to the procedure described by Rzeszotarska et al. [1].

Attempts to use the pentapeptide tert-butyl ester 5 as an acyl donor in the \(\alpha\)-chymotrypsin-catalyzed 5 + 5 final coupling failed. The proteolytic cleavage of Trp\(^{3}\)-Ser\(^{4}\) bond was faster than the formation of Tyr\(^{5}\)-Gly\(^{6}\) bond. Therefore, the transformation of the pentapeptide tert-butyl ester 5 into the methyl ester 6 was required before the next step. It was performed by the deprotection with trifluoroacetic acid in the presence of anisole.

**Fig. 1. Synthesis of GnRH**

**Abbreviations:**
- DCC = N,N-dicyclohexylcarbodiimide;
- HOBT = 1-hydroxybenzotriazole;
- MA = mixed anhydride with isobutyl chloroformate;
- \(\alpha\)-CT = \(\alpha\)-chymotrypsin;
- P = papain.
- * Final yield after the reuse of the recovered substrates.
as a scavenger, followed by BF$_3$/MeOH esterification.

For the all intermediates and the final N-terminal pentapeptide methyl ester 6, FABMS data and amino acid analyses were consistent with the expected structures.

*Synthetic methodology for the C-terminal pentapeptide amide Gly$_6$-Leu$_7$-Arg$_8$-Pro$_9$-Gly$_{10}$-NH$_2$ (13)*

Andersen et al. [3] reported the $n + 1$ stepwise elongation strategy for the assembly of the C-terminal pentapeptide of GnRH. As discussed by the authors, this approach is not feasible for the synthesis on a preparative scale because the enzymatic formation of Arg-Pro bond occurs in a low yield and the specific enzyme chlostripain is expensive. The preferred strategy described here involves the 2 + 3 coupling catalyzed by $\alpha$-chymotrypsin, furnishing under optimal conditions (pH 9.0, 40% aqueous DMF) the pentapeptide amide 12 in the yield of 56%. The most suitable acyl donor in this condensation proved to be 2-chloroethyl ester derivative of the N-terminal dipeptide 11. In comparison with other esters such as ethyl, benzyl, $p$-nitrobenzyl, its use is profitable because the condensation can be completed over relatively shorter time at the much lower concentration of $\alpha$-chymotrypsin. The reaction is easy to carry out and the isolation of the product (purity >95% by HPLC) is straightforward by the simple extraction procedure. In addition, the relative lack of undesired by-products considerably simplifies the recovery of unreacted substrates.

The attempts to perform the coupling in organic solvents such as dichloromethane, toluene or tert-amyl alcohol were unsuccessful because of a low yield of the reaction (<5%). Limited access of the nearly insoluble Arg-Pro-Gly-NH$_2$ to the enzyme may explain the very low efficiency of the reaction. This observation indicates that non-aqueous conditions are of limited applicability for carrying out enzymatic couplings of fragments containing unprotected polar side chains.

The acyl acceptor Arg$_8$-Pro$_9$-Gly$_{10}$-NH$_2$ (10) was prepared along the four step chemical synthesis in the overall yields of 63%. The optimal synthetic route involves the preparation of Z-Pro$_9$-Gly$_{10}$-NH$_2$ (7) by mixed anhydride method and subsequent deprotection followed by N-terminus elongation (+Z-Arg(HCl)-OH) using DCC/HOBt. The strongly basic guanidino group of arginine was protected by protonation using hydrogen chloride. The advantage of this derivative lies in its easy preparation and the obvious potential for the carrying out the next synthetic steps according to minimal protection strategy. For the subsequent 2 + 3 enzymatic coupling, Arg-Pro-Gly-NH$_2$ (10) was used as a crude product. It is known that arginine is very prone to racemization when used as acyl donor in chemical coupling and therefore the preparation of the tripeptide may result in generation of D-Arg-Pro-Gly-NH$_2$. Due to the high stereospecificity of $\alpha$-chymotrypsin toward L-arginine in the $P'$ position, the diastereomer D-Arg-Pro-Gly-NH$_2$ if present as an impurity, is reaction inert and will be eliminated at the enzymatic coupling step.

For the all intermediates and the final C-terminal pentapeptide amide 12, FABMS data and amino acid analyses were consistent with the structures.

*GnRH via 5 + 5 fragment coupling*

A major concern in enzymatic peptide synthesis, particularly in a fragment coupling, is the potential cleavage of new and already existing peptide bonds due to hydrolytic activity of the protease used as catalyst. Therefore, much experimental work is directed to establish conditions under which the protease would function as “synthetase” exhibiting little or no protease activity, while the esterase activity is left intact. For example, it was reported [6] that $\alpha$-chymotrypsin in the aqueous mixture containing up to 60% of acetonitrile expressed little or no protease activity. These conditions were suggested as being ideal for peptide synthesis and therefore we have used them for the initial $\alpha$-chymotrypsin-catalyzed coupling of the pentapeptide ester 6 and the pentapeptide amide 13. By surprise, when acetonitrile/carbonate buffer system (6:4, pH 9) was used as the reaction medium, the yield of coupling was much lower than in water and the cleavage products Glp$_1$-His$_2$-Trp$_3$-Ser$_4$-Tyr$_5$-OH as well as Glp$_1$-His$_2$-Trp$_3$-OH were detected by HPLC in the reaction mixture. These results show that the use of recommended solvent system does not ensure that the protease would act as a “synthetase” and this observation supports the suggestion of Littlemore...
et al. [7] that the ratio of esterase and protease activity of enzyme depends also on other factors, for example the structure of the reactants.

The best results were obtained when the 5 + 5 coupling was performed using α-chymotrypsin at pH 8 in a aqueous mixture containing up to 50% DMF. The synthesis of GnRH was accomplished in a 84–90% yield, using a nearly equimolar amounts of the acyl donor and the nucleophile. To obtain the maximum yield, careful and rapid monitoring (HPLC) of this kinetically controlled reaction was required. The decapeptide 14 was isolated by chromatography on Sephadex LH-20 with methanol as an eluent (purity 93–96%). One more gel filtration on Sephadex G-15 in 0.2 N acetic acid furnished the product of 99% purity. The method is very simple to carry out and offers an easy access to larger amount of GnRH. We were able to repeat the synthesis without any difficulties on 2 g scale.

The obtained results indicate that the chemical-enzymatic synthesis shown in Fig. 1 represents a preparative route to multigram amounts of gonadotropin releasing hormone. We believe that the reported synthesis is optimal in terms of the number of chemical and enzymatic steps dictated by the GnRH amino acids composition, their sequence and the availability of inexpensive proteases. In comparison to enzymatic synthesis of Andersen et al. [3], its advantages are the use of inexpensive enzymes and the reduced number of synthetic steps. No comparison in terms of yields is possible because not all quantitative results are reported in the communication of the cited authors.

Materials and Methods

Melting points were determined on a Thomas-Hoover capillary melting point apparatus and are uncorrected. Optical rotations were measured in a 1 dm cell (v 1 ml) on a Perkin-Elmer polarimeter (model 241) at 589 nm (NaD line). FAB mass spectra were recorded on a Finnigan 3300 spectrometer equipped with a capillarization gas gun from Phrasor Scientific (Duarte, CA). For thin layer chromatography, 250 nm silica gel GF precoated uniplates (Analtech) were used with the solvent systems: I. BuOH/ACOH/ACOEt/H2O (1:1:1:1), II. CHCl3/MeOH/conc. ammonia/H2O (60:45:20:4), III. BuOH/ACOH/H2O (4:1:1), IV. CH3Cl/acetone (7:1), V. BuOH/ACOH/pyridine/H2O (5:1:4:1), VI. CHCl3/MeOH/ACOH/H2O (60:45:6:14). The chromatograms were developed with chlorine followed by starch/KJ or ninhydrine spray. HPLC was performed using a Vydac column C18 (4.6 × 250 mm), particle size 5 μm at a flow rate 1.0 ml/min and solvent systems: (A) 0.05% TFA in water, (B) 0.038% TFA in acetonitrile/water 90:10. Amino acids analyses were conducted on a Beckman System Gold Chromatograph instrument using DABS-amino acid derivatives. Papain (EC 3.4.22.2), α-chymotrypsin (EC 3.4.21.1) and trypsin (EC 3.4.21.4) were purchased from Sigma Co.

L-Pyroglutamyl-L-histidine methyl ester
(Glp—His—OMe) (1)

The dipeptide was obtained from Glp and His—OMe by means of DCC/HOBt according to the procedure described by Rzeszotarska et al. [1].

L-Pyroglutamyl-L-histidyl-L-tryptophane amide
(Glp—His—Trp—NH2) (2)

To Trp—NH2 (4.47 g, 22 mmol) dissolved in dichloromethane/water (99:1; 200 ml), Glp—His—OMe (I) (5.6 g, 20 mmol) and crystalline α-CT (30 mg) were added. The suspension was stirred at r.t. until no presence of 1 was detected by TLC (16 h). The reaction was terminated by the addition of MeOH (100 ml). After 1 h, the denaturated enzyme was filtered off and the filtrate was evaporated to dryness. The crude tripeptide amide 2 was isolated as a neutral product by the usual partition procedure using BuOH (4 × 50 ml). Traces of water were removed by azeotropic evaporation with AcOEt (2 × 20 ml) and the residue was triturated with hot AcOEt. The insoluble fraction was recrystallized from MeOH/AcOEt (1:10, 220 ml) to furnish 6.76 g (75%) of 2. The dipeptide Glp—His—OH was recovered by evaporation of the aqueous phase left after butanol extraction. After drying over phosphorus pentoxide, it was transformed into the methyl ester 1 using 2 N HCl-methanol solution. The nucleophile Trp—NH2 was recovered by evaporation of the ethyl acetate solution obtained after trituration of the crude 2. The reuse of recovered substrates Glp—His—OMe and Trp—NH2 under coupling conditions described above, furnished 0.91 g of tripeptide Glp—His—Trp—NH2. Final yield 85%; m.p. 220–225 °C (lit. [8] m.p. 220–230 °C dec.) [α]D20

−11.8° (c 1, MeOH), (lit. [8] [α]D20 −11.2°) (c 1, MeOH); Rf(1) 0.56, Rf(II) 0.85; HPLC: purity 97%, tR 5.5 min (gradient 15–30% B in 10 min);
N-Benzylxocarbonyl-L-seryl-L-tyrosine tert-butyl ester (Z-Ser-Tyr-OBu') (3)

Z-Ser-OMe (4.28 g, 20 mmol) and Tyr-OBu' (5.68 g, 28 mmol) was suspended in the dioxane-phosphate buffer (4:6, 80 ml) and pH was adjusted to 9.0 by 3 N NaOH. Papain (40 mg) and dithioerythritol (6 mg) were added and the reaction mixture was stirred until no presence of Z-Ser-OMe was detected by HPLC (5 h). The reaction was terminated by addition of MeOH (200 ml) and after 1 h the mixture was evaporated to dryness. The residue was dissolved in AcOEt and washed according to the usual procedure for neutral peptides. After drying, AcOEt was evaporated to give oily dipeptide 3. Yield 7.2 g (78%); Rf (IV) 0.46; HPLC: purity 97%, t_R 9.4 min (isocratic conditions 40% B); FABMS m/e 462 (M H^+). Calcd. for C_{21}H_{31}N_7O_5 461. Amino acid analysis: observed (calcd.) Glu 1.0 (1), His 1.0 (1), Ser 0.72 (1), Tyr 1.05 (1).

L-Pyroglutamyl-L-histidyl-L-tryptophyl-L-seryl-L-tyrosine methyl ester (Glp-His-Trp-Ser-Tyr-OMe) (6)

The pentapeptide tert-butyl ester (5) (3.03 g, 4 mmol) was deprotected with TFA (40 ml) in the presence of anisole (2.0 ml) at 0 °C. After 3 h the product was precipitated with diethyl ether (400 ml), filtered off and transformed into the methyl ester by esterification with 10% BF_3/MeOH solution (40 ml) at 0 °C (8 h). The pentapeptide methyl ester was precipitated with diethyl ether (400 ml), filtered off and dried over KOH under vacuum. Yield 2.36 g (83%); m.p. 220 °C decomp. [a]_D^20 -13.67° (c 1, MeOH); R_f (I) 0.55, R_f (II) 0.69. R_f (III) 0.12; HPLC: purity 94%, t_R 7.09 min (gradient 20-40% B in 25 min); FABMS m/e 717 (M H^+). Calcd. for C_{32}H_{40}N_8O_9 716.

N-Benzylxocarbonyl-L-prolyl-glycine amide (Z-Pro-Gly-NH_2) (7)

Dipeptide 7 was obtained from Z-Pro-OMe and HCl × Gly-NH_2 by mixed anhydride method, using isobutyl chloroformate according to Rzeszortarska et al. procedure [9].

N-o-Benzylxocarbonyl-L-arginyl-L-prolyl-glycine amide hydrochloride (Z-Arg(HCl) — Pro-Gly-NH_2) (9)

To a stirred and cooled at 0 °C solution of Z-Arg(HCl) — OH (14.36 g, 40 mmol) in DMF (20 ml), HOBt (5.43 g, 40 mmol) and then DCC (8.25 g, 40 mmol) were added. After addition of the cold solution of Pro-Gly-NH_2 (8) (obtained by hydrogenolysis of Z-Pro-Gly-NH_2 (7), 12.21 g, 40 mmol) in DMF (20 ml), the reaction mixture was stirred at 0 °C for 8 h and then for 24 h at r.t. The precipitated DCC was filtered off and the solvent was evaporated. The residue was suspended in water (150 ml) and stirred at 0 °C for 1 h. Solid by-products were filtered off and the aqueous phase was extracted with AcOEt (3 times). After evaporation of water the residue was dried over phosphorus pentoxide, then dissolved in methanol (60 ml) and dropped into dry AcOEt (400 ml). The precipitate was filtered off and washed with AcOEt. Yield 14.9 g (75%); m.p. 140–143 °C (lit. [10] m.p. 145–146 °C); [a]_D^20 -43.7° (c 1, MeOH) (lit. [10] [a]_D^20 -34.4°, c 1, MeOH). R_f (I) 0.47; HPLC: purity 90%, t_R 5.5 min (isocratic conditions 25% B); FABMS m/e 462 (MH^+). Calcd. for C_{21}H_{31}N_8O_9 461. Amino acid
analysis: observed (calcd.) Arg 1.10 (1), Pro 1.01 (1), Gly 1.0 (1.0).

**N-Benzoylcarbonylglycyl-L-leucine 2-chloroethyl ester (Z-Gly–Leu–OEtCl)** (11)

Dipeptide 11 was obtained from Z-Gly and HCl × Leu–OEtCl according to standard mixed anhydride procedure using isobutyl chloroformate. Yield 90%. HPLC: purity 98%, tR 20.4 min (gradient 30–50% B in 25 min); FABMS m/e 385 (MH+). Calcd. for C18H29N2O5Cl 384.5.


Z-Arg(HCl)–Pro–Gly–NH₂ (9) (9.68 g, 21 mmol) in methanol (100 ml) was hydrogenated in the presence of 10% Pd/C (0.25 g) at a pressure of 4–5 kg/cm² on a Parr apparatus for 4 h. After evaporation of the filtered solution, the glassy powder of Arg(HCl)–Pro–Gly–NH₂ (10) was dissolved in DMSO/water system (2:3; 2.5 ml). Z-Gly–Leu–OEtCl (11) (3.84 g, 10 mmol) was added, the pH was adjusted to 9.0 using 6 N NaOH and maintained by pH-stat. α-Chymotrypsin (100 mg) dissolved in water (0.5 ml) was added and after 3 h stirring at 37 °C the new portion of Z-Gly–Leu–OEtCl (11) (3.84 g, 10 mmol) was added. Stirring was continued until no presence of 11 was detected by HPLC (6 h). After dilution of the reaction mixture with water (100 ml), the pentapeptide amide 12 was isolated by the usual partition procedure using BuOH (5 × 50 ml). Traces of water were removed by azeotropic evaporation with AcOEt (3 × 20 ml). The residue was dried over phosphorus pentoxide, then dissolved in methanol (50 ml) and dropped into dry AcOEt (600 ml). The hygroscopic precipitate was quickly filtered off and washed with AcOEt. Yield 7.2 g (54%). The dipeptide Z-Gly–Leu–OH was regenerated by extraction of the acentous phase at pH 3 with ethyl acetate. After evaporation of the organic solution to dryness, the residue was dried over phosphorus pentoxide and transformed into Z-Gly–Leu–OEtCl. The unreacted nucleophile 10 was isolated by evaporation of the acentous phase to dryness. The residue of the recovered substrates Z-Gly–Leu–OEtCl and Arg(HCl)–Pro–Gly–NH₂ for coupling under conditions described above furnished 3.47 g of pentapeptide amide 12. Final yield 80%; m.p. 108–112 °C; [α]D20 −44.5° (c 1, MeOH); Rf (I) 0.61, Rf (II) 0.86, Rf (III) 0.14; HPLC: purity 95%, tR 8.60 min (gradient 20–30% B in 25 min); FABMS m/e 632 (MH+), 654 (MNa+). Calcd. for C25H45N8O₃ 631. Amino acid analysis: observed (calcd.) Gly 2.08 (2), Leu 1 (1), Arg 1.05 (1), Pro 1.01 (1).

**Glycyl-L-leucyl-L-arginyl-L-prolyl-glycine amide hydrochloride (Gly–Leu–Arg(HCl)–Pro–Gly–NH₂)** (13)

Z-Gly–Leu–Arg(HCl)–Pro–Gly–NH₂ (13) (1.34 g, 2 mmol) in methanol was hydrogenated in the presence of 10% Pd/C (0.1 g) as described above (2 h). The mixture was filtered off and the product was isolated by evaporation. The crude N-protected pentapeptide 13 was used for the subsequent enzymatic coupling. FABMS m/e 498 (MH+). Calcd. for C21H41N₅O₅ 497.


C-Terminal pentapeptide Gly–Leu–Arg(HCl)–Pro–Gly–NH₂ (13) (1.17 g, 2.2 mmol) was dissolved in DMF/0.2 M carbonate buffer system (50:50, 8 ml) and the pH was adjusted to 8.0 using 6 N NaOH. Then, N-terminal pentapeptide methyl ester Glp–His–Trp–Ser–Tyr–OMe (6) (1.43 g, 2 mmol) and α-chymotrypsin (5 mg) dissolved in minimal amount of water (0.2 ml) were added. The reaction mixture was stirred at r.t. and pH was maintained at 8.0 using pH-stat. The enzymatic reaction was monitored by HPLC at λ 254 nm and it was terminated at maximum yield of the product 14 (84%, 1 h) by addition of methanol (100 ml). After evaporation to dryness, methanol was added (10 ml), the resulting suspension centrifuged and then chromatographed on a Sephadex LH-20 column. Fractions containing GnRH were evaporated to dryness (purity 93% by HPLC), dissolved in 0.2 N acetic acid (5 ml) and then chromatographed on Sephadex G-15 in 0.2 N acetic acid. Fractions containing GnRH were combined and lyophilized. Yield 2.05 g (84%); Rf (I) 0.51, Rf (V) 0.44, Rf (VI) 0.61. FABMS m/e (MH+) 1182.76. Calcd. for C₅₅H₇₉N₁₇O₁₃ 1181.573. Amino acid analysis observed (calcd.) Glu 1.0 (1), His (0.95), Ser 0.84 (1), Tyr 1.05 (1), Gly 2.04 (2), Leu 1.0 (1), Arg 0.97 (1), Pro 1.01 (1).

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