49. Synthesis and Placental Binding Potencies of Photosensitive Analogues of Luteinizing Hormone Releasing Hormone (LHRH) with Agonistic and Antagonistic Structures[1])

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The synthesis of new photolabeling analogues of luteinizing hormone releasing hormone is described. Peptides were prepared by the solid-phase method, and the photolabeling moiety was introduced as the 4'-nitrophenylalanine precursor. This amino acid, either of the L or D configuration, was placed at different positions and was modified afterwards to 4'-azidophenylalanine. Structures with agonistic or antagonistic character were prepared, together with a peptide containing simultaneously the photolabeling moiety and a biotin substituent for future receptor isolations. Binding studies on placental and pituitary membranes indicate that these compounds represent promising candidates for receptor labeling studies.

Introduction. – The photoaffinity labeling method has gained wide acceptance in most areas of biochemistry, particularly in the area of peptide hormones and their receptors [1] [2]. Generally, two methods of photolabel introduction are utilized: either by modification of an already existing peptide with *e.g.* an active ester containing a photosensitive moiety or, second, by synthetic introduction of a photolabeling amino acid into the chosen sequence during peptide synthesis [3], an approach we propose to name 'intrinsical labeling'. Many peptide hormones have been treated with either one or even both approaches, thus helping to characterize their respective receptors. Both methods, the intrinsical and the modification pathway, have permitted to identify the receptor of angiotensin II [4] [5]. Only the intrinsical photolabel, however, achieved a receptor incorporation rate over 40%, sufficiently high to observe very significant irreversible biological effects [5] [6], and biochemical isolation of the receptor protein.

The standard procedure for the introduction of an intrinsic photolabel is the use of N^2-protected 4'-nitrophenylalanine in solid-phase or classical peptide synthesis and the conversion of this residue in the completed peptide into 4'-azidophenylalanine, the photolabeling moiety. A further advantage of the intrinsic photolabels is the relatively small structural disturbance caused by this moiety, compared to a prosthetic photolabeling group (modification pathway) which can disturb strongly the biological action and significance of a peptide.

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Several photolabeling studies with closely related, non-intrinsic photolabels of luteinizing hormone releasing hormone (LHRH) have been carried out on the pituitary [7] [8] and on gonadal tissues [9]. In all cases, a [D-Lys^6]LHRH analogue was modified with an arylazide-containing reagent, *e.g.* succinimido 4-azidobenzoate [9]. Recently, we have shown on human placenta the presence of a functional LHRH receptor which regulates in a dose-dependent manner human-chorionogonadotropin (hCG) secretion [10–12]. For yet unknown reasons, however, receptor affinity for LHRH observed on the placenta was lower than that observed on classical target organs for LHRH, such as the pituitary. In order to better characterize this placental LHRH receptor, we prepared several new intrinsical LHRH photolabels, placed the photolabel at different positions, and produced structures with agonistic and antagonistic character. We then compared the binding relevance of these new compounds by classical peptide-binding studies on the placenta and pituitary.

Design and Syntheses. – The use of agonistic and antagonistic photoaffinity labels of angiotensin II has permitted to look into the organization of the native angiotensin-II receptor [13]. The pharmacological nature of the label influenced the subunit composition of the angiotensin-II receptor, and we wondered if other closely related receptor systems present a similar behaviour.

Therefore, the proposed study on the human placental LHRH receptor was planified to include labeling experiments with agonistic labels and antagonistic labels. This need, the proposed variations of the label position for an eventual increase of labeling efficiency, and the proposed study for receptor isolation led to the peptide structures **1**–**8**.

1: LHRH (agonist), Glp-His-Trp-Ser-Tyr-Gly-Leu-Arg-Pro-Gly-NH_2

2: [D-Ala^6, Phe(N_3)^7]LHRH (agonist), Glp-His-Trp-Ser-Tyr-D-Ala-Phe(4′-N_3)-Arg-Pro-Gly-NH_2

3: [D-Phe^{2,3,6},Phe(N_3)^7]LHRH (antagonist), Glp-D-Phe-D-Phe-Ser-Tyr-D-Phe-Phe(4′-N_3)-Arg-Pro-Gly-NH_2

4: [D-Phe(N_3)^6]LHRH (agonist), Glp-His-Trp-Ser-Tyr-D-Phe(4′-N_3)-Leu-Arg-Pro-Gly-NH_2

5: [D-Phe^{2,6}, D-Phe(N_3)^3]LHRH (antagonist), Glp-D-Phe-D-Phe(4′-N_3)-Ser-Tyr-D-Phe-Leu-Arg-Pro-Gly-NH_2

6: [AcPro^1, D-Phe(N_3)^2, D-Ala(βNp)^{3,6}]LHRH (antagonist), Ac-Pro-D-(4′-N_3)-D-Ala(βNp)-Ser-Tyr-D-Ala(βNp)-Leu-Arg-Pro-Gly-NH_2

7: [D-Lys(Biot)^6, Phe(N_3)^7]LHRH (agonist), Glp-His-Trp-Ser-Tyr-D-Lys(N^6-Biot)-Phe(4′-N_3)-Arg-Pro-Gly-NH_2

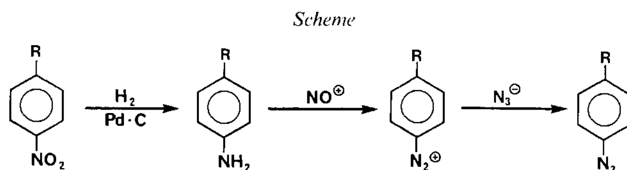
8: [D-Lys(N_3PhCO)^6]LHRH (agonist), Glp-His-Trp-Ser-Tyr-D-Lys(N^6-(4′-N_3-PhCO))-Leu-Arg-Pro-Gly-NH_2

The antagonistic structures **3**, **5**, and **6** were designed according to already described antagonist structures [14] or according to the recently published highly hydrophobic and potent antagonist [AcPro^1, D-Phe(4′-Cl)^2, D-Ala(βNp)^{3,6}]LHRH [15]. We replaced the reported 4′-chloro-D-phenylalanine modification with 4′-azido-D-phenylalanine, an alteration stereochemically so small that it should be well accepted. All syntheses were carried out by the solid-phase method using *N*-(*tert*-butoxycarbonyl)glycine (Boc-Gly) esterified to chloromethylated polystyrene resin as the anchor function and stepwise elongation of

the peptide with the Boc/CF_3COOH scheme. The photolabile function was introduced as 4′-nitro-D- or 4′-nitro-L-phenylalanine in the appropriate positions. Tryptophan *tert*-butylation during N^2-deprotection with CF_3COOH was suppressed by the use of N^2-acetyltryptophan as scavenger in the cleavage reagent. Due to the light sensitivity of 4′-nitrophenylalanine, and especially 4′-azidophenylalanine, all manipulations were carried out under UV-free illumination (incandescent), and products were stored in the dark.

Peptide couplings were performed with preformed symmetrical anhydrides, and after each step, the completeness of the couplings was controlled with a ninhydrine test. The N-terminal pyroglutamic residue was introduced with a HOBt/DCC coupling of N^2(*tert*-butoxycarbonyl)glutamine (Boc-Gln), which was to be cyclized after deprotection. The completed peptides were subjected to ammonolysis, and the recovered crude peptides were treated in liquid anhydrous HF for the removal of the residual side chain blocking groups. Finally, the N-terminus was cyclized into pyroglutamyl by incubation of the peptides in AcOH, and the products were subjected to prep. HPLC until acceptably pure (> 95%). Portions of these 4′-nitrophenylalanine-containing precursor peptides were modified to the final 4′-azidophenylalanine-containing photolabels according to the *Scheme* [22].

Scheme



After the modifications, the peptides were again purified by prep. HPLC and recovered from the pure fractions by lyophilisation. The identity of the products was assessed by amino-acid analysis and the purity by TLC and anal. HPLC. The presence of the azido function was confirmed by IR spectroscopy. In the case of the doubly labeled analogue **7**, the 4′-azidophenylalanine-containing peptide was modified with the succinimide-derived active ester of biotin at N^6 of the D-lysine in position 6.

For binding studies, the peptides were iodinated on tyrosine in position 5 by the iodogen method and purified by HPLC as already described by some of us [16].

Results and Discussion. – The obtained peptides were assayed for their binding potency on human placental membranes using rat pituitary membranes as standards of comparison. The radioactive ligand used was iodinated buserelin, ([D-Ser(*t*-Bu)^6]LHRH), and the binding parameters are expressed as K_a from scatchard analysis and the half-maximal displacement dose IC_{50}. Both the nitro precursors and the azido peptides were tested, but also the photolabel **8** which has been used for ealier studies [7] [9]. The results are presented in the *Table*. Compared to the results on the pituitary membranes, the values obtained with the human placental membranes indicate much lower affinity of all analogues, including LHRH and buserelin towards the placental LHRH receptor [10].

The peptides **5** and especially **6** displayed high nonspecific binding; this is probably due to their highly hydrophobic nature and renders these two analogues less useful for the proposed studies. All compounds, however, displaced in a dose-dependent manner iodinated buserelin and are, therefore, specific ligands for the placental LHRH receptor and potentially useful photolabels for the LHRH-receptor isolation. Peptide **8** could be

Table. *Amino-Acid Analysis and Binding Potencies of LHRH Analogues*

No.	Structure	Amino-acid analysis														
		Ser	Glu	Pro	Gly	Ala	Leu	Tyr	Phe	Phe(NO_2)	Phe(N_3)	His	Trp	D-Ala (βNp)	Lys	Arg
2	[D-Ala^6, Phe(N_3)^7]LHRH	0.95	1.09	1.03	1.10	0.99	–	0.87	–	–	pres.	0.95	pres.	–	–	0.98
3	[D-Phe^{2,3,6}, Phe(N_3)^7]LHRH	1.01	1.07	1.01	1.07	–	–	0.95	2.96	–	pres.	–	–	–	–	0.93
4	[D-Phe(N_3)^6]LHRH	0.91	1.00	0.97	1.00	–	1.00	0.95	–	–	pres.	0.95	pres.	–	–	0.99
5	[D-Phe^{2,6}, D-Phe(N_3)^3]LHRH	0.88	1.14	1.01	1.12	–	0.93	0.85	2.03	–	pres.	–	–	–	–	1.04
6	[AcPro^1, D-Phe(N_3)^2, D-Ala(βNp)^{3,6}]LHRH	0.93	–	2.03	1.15	–	1.15	0.84	–	–	pres.	–	–	1.89	–	1.03
7	[D-Lys(Biot)^6, Phe(N_3)^7]LHRH	0.92	1.10	1.07	1.00	–	–	0.93	–	–	pres.	0.96	pres.	–	0.98	1.04
8	[D-Lys(N_3PhCO)^6]LHRH	0.94	1.02	1.02	1.10	–	1.01	0.91	–	–	–	0.92	pres.	–	1.03	1.04
9	[D-Ala^6, Phe(NO_2)^7]LHRH	0.86	1.03	1.05	1.06	0.95	–	1.03	–	1.01	–	0.94	pres.	–	–	1.04
10	[D-Phe^{2,3,6}, Phe(NO_2)^7]LHRH	0.91	1.02	1.05	1.05	–	–	0.96	2.97	1.03	–	–	–	–	–	1.01
11	[D-Phe(NO_2)^6]LHRH	0.95	1.03	0.98	1.00	–	1.06	0.96	–	0.82	–	0.88	pres.	–	–	1.01
12	[D-Phe^{2,6}, D-Phe(NO_2)^3]LHRH	0.95	1.02	0.99	1.00	–	1.09	0.99	2.13	0.85	–	–	–	–	–	0.93
13	[AcPro^1, D-Phe(NO_2)^2, D-Ala(βNp)^{3,6}]LHRH	0.93	–	2.04	1.06	–	1.06	0.89	–	1.06	–	–	–	2.00	–	0.99
14	[D-Lys^6, Phe(NO_2)^7]LHRH	0.99	0.99	1.01	0.99	–	–	0.91	–	1.01	–	0.95	pres.	–	1.08	0.94
Buserelin	[D-Ser(t-Bu)^6]LHRH	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–

No.	Structure	Binding potencies			
		Human placental membranes		Rat pituitary membranes	
		log K_a [M^{-1}]	log IC_{50}	log K_a [M^{-1}]	log IC_{50}
2	[D-Ala^6, Phe(N_3)^7]LHRH	6.61	7.55	8.75	8.94
3	[D-Phe^{2,3,6}, Phe(N_3)^7]LHRH	6.94	6.67	9.00	6.97
4	[D-Phe(N_3)^6]LHRH	6.47	7.41	9.27	9.08
5	[D-Phe^{2,6}, D-Phe(N_3)^3]LHRH	6.10	7.08	7.93	8.94
6	[AcPro^1, D-Phe(N_3)^2, D-Ala(βNp)^{3,6}]LHRH	6.01	6.14	–	–
7	[D-Lys(Biot)^6, Phe(N_3)^7]LHRH	–	6.67	–	–
8	[D-Lys(N_3PhCO)^6]LHRH	6.51	7.05	–	9.40
9	[D-Ala^6, Phe(NO_2)^7]LHRH	6.24	5.91	–	–
10	[D-Phe^{2,3,6}, Phe(NO_2)^7]LHRH	6.10	6.17	–	–
11	[D-Phe(NO_2)^6]LHRH	6.39	5.77	–	–
12	[D-Phe^{2,6}, D-Phe(NO_2)^3]LHRH	4.32	4.70	–	–
13	[AcPro^1, D-Phe(NO_2)^2, D-Ala(βNp)^{3,6}]LHRH	–	–	–	–
14	[D-Lys^6, Phe(NO_2)^7]LHRH	–	–	–	–
Buserelin	[D-Ser(t-Bu)^6]LHRH	7.32	7.01	10.90	10.07

useful to purify the photoaffinity-labeled LHRH receptors by affinity chromatography on avidin-substituted supports [17]. These studies are currently under investigation and will be published elsewhere.

Experimental Part

General. N-(tert-Butoxycarbonyl)-L-amino acids, peptide reagents, and chloromethylated resin (copolystyrene/1% divinylbenzene; 0.75 mmol of Cl/g of resin) were obtained from *Bachem Fine Chemicals Inc.*, if not otherwise stated, and were used without further purification. DCC was purified by dissolving the commercial product *(Aldrich Chemicals)* in Et_2O: the insoluble material was removed by filtration and the Et_2O was evaporated. All solvents and reagents used for solid-phase synthesis were of anal.-reagent quality and were redistilled before use. All mixtures of liquids are given in *v/v*. HPLC: *Waters-M-45* system; *VYDAC 218 TP 104* column; 214 nm UV detector; gradients from 0 to 85% CH_3CN in 0.1% CF_3COOH; peptides were considered pure if the peak integral was at least 95% of the combined peptide-peak integrals. TLC: *Merck* precoated silical gel plates (type *G60-F254*); BAW = $BuOH/AcOH/H_2O$ 5:2:3, BAWP = $BuOH/AcOH/H_2O$/Pyridine 15:3:10:12; visualization with UV fluorescence, *Pauly* reagent (4-diazoniumphenylsulfonate in 2N Na_2CO_3), or a modified *Reindel-Hoppe* procedure [18]. Amino-acid analyses: *Beckmann 119C* analyzer; peptide samples were hydrolyzed for 24 h at 110° in CF_3COOH/conc. HCl soln./mercaptopropionic acid 50:49:1 in vacuum-sealed tubes; Phe(4'-NO_2) was recorded as such, Phe(4'-N_3) and Trp were partially destroyed. IR spectra (in cm^{-1}): *Perkin-Elmer-457* instrument, KBr pellets.

Peptide Synthesis. The syntheses were carried out with a *Peptomat* automatic peptide synthesizer, using procedures previously described [19]. N^2-Boc protection was used for all the amino acids, and it was removed prior to the next coupling by reaction with CF_3COOH/CH_2Cl_2 2:3 for 20 min. In the case of Trp-containing peptides, 500 mg of Ac-Trp were added to 1 l of CF_3COOH soln. in order to protect Trp against *tert*-butylation. A 5% soln. of $Et(i\text{-}Pr)_2N$ in CH_2Cl_2 was used for neutralisation of the free amino function of the growing peptide and which was then reacted with the symmetrical anhydride of the next amino acid. A 4 to 6-fold excess of the Boc-amino acid was used for each coupling. Boc-Gln was coupled with 1 equiv. of DCC and HOBt, all in 4-fold excess. The completion of every coupling was checked with a ninhydrin test [20].

The completed, CF_3COOH-deprotected peptide resins were subjected to ammonolysis in pressure bottles at 30° for 6 days in 100 ml of DMF/i-PrOH 1:1, saturated at 0° with NH_3 (gas). The bottles were cooled in ice prior to opening, the content filtered, and the residue washed with additional 100 ml of DMF. The filtrate was evaporated, redissolved in DMF, and subjected to gel filtration on *Sephadex LH20* (2 × 40 cm; elution with DMF). The peptide-containing fractions were pooled (detection by TLC), evaporated, diluted with H_2O, and directly applied onto a prep. reversed-phase liquid chromatography system (glass column *Michel-Miller* 2.2 × 30 cm (*Ace Glass*, Vineland, N.J.); *Nucleosil-C_{18}* 30 μm, *Macherey Nagel*, Dueren, Germany; gradient elution with 20–60% CH_3CN in 0.05% aq. CF_3COOH at a mean pressure of 6 atm (*FMi* lab pump, model *RP G50*, Oyster Bay, N.Y.)). Because of the photosensitivity of the nitro function [21], the peptide-containing fractions were not checked by UV absorption of the eluate but by spotting to small TLC strips, developing them without migration, and applying the peptide-containing fractions to 10 × 20 cm TLC sheets followed by migration in BAW and coloration. Pure fractions were pooled, diluted with H_2O and lyophilized twice. The remaining protecting groups were removed by exposure for 1 h at 0° to 1–2 ml of liq. anh. HF, containing *ca.* 10% anisole and 1% Et_2S as carbonium-ion scavengers. The cleavage reagent was removed by flushing with dry N_2, followed by 2× 15 min of evacuation through KOH pellets, first *in vacuo*, then under high vacuum. The peptides were extracted with 2N AcOH, lyophilized, redissolved in a few ml of 50% AcOH and left standing at r.t. overnight for glutamyl cyclisation. After the cyclisation was completed (TLC), the soln. was diluted with H_2O and directly applied to reversed-phase chromatography as indicated above. If after this chromatography the pooled peptide fractions were less pure than 95%, the purification was repeated after partial evaporation and dilution with H_2O. In refractory cases, another gradient was used: 0.25N NH_4OAc (pH 8.0), 7.5% i-PrOH with a gradient of 20–60% CH_3CN. All nitro-containing peptides **9**–**13** were obtained as AcOH salts (**14**: 2 AcOH).

Nitro-to-Azido Conversion. According to [22], 5–10 mg of the nitro-precursor peptides **9**–**14** were hydrogenated over Pd/C in 50% AcOH. The lyophilized amino derivative was redissolved in 2 ml of 0.1N HCl, stirred either at 0° for **3**, **5**, and **6** or, after addition of 1 ml of i-PrOH, at −15° (ice/MeOH bath) for the Trp-containing peptides **2**, **4**, and **7**. The peptides were diazotized by addition of 15 μl of freshly prepared 1N $NaNO_2$ which always produced, after 10 min, a positive I_2/starch reaction. Excessive $NaNO_2$ was destroyed by addition of 15 μl of 1N

sulfamic acid, and the azide was formed by addition of 10 µl of 1N NaN_3. After 10 min, the soln. was diluted to 15 ml, neutralized with solid NH_4OAc, and loaded directly onto a smaller scale prep. reversed phase column (8 × 250 mm). Further treatment was as described above for the peptide synthesis. All azido-containing peptides **2–8** were obtained as AcOH salts.

Binding Experiments. All compounds were tested on their capacity to displace iodinated buserelin from cell-membrane preparations from human term placenta [10] and from rat pituitary glands [23]. The binding experiments were carried out as published earlier [10], including the displacement of iodinated labels **2–8** with non-radioactive buserelin.

[D-Ala⁶, Phe(NO₂)⁷]LHRH (Glp-His-Trp-Ser-Tyr-D-Ala-Phe(4'-NO₂)-Arg-Pro-Gly-NH₂; **9***).* Boc-Gly was esterified to chloromethylated resin by the cesium-salt method [24] to produce a substitution of 0.41 mequiv./g resin. To 10 g of this resin were added successively Boc-Pro and Boc-Arg(N^7-Tos), and the resin was separated into 2 equal portions. The first was elongated with Boc-Phe(4'-NO_2), and ¾ of this tetrapeptidyl resin were spared for other sequences. The peptide was completed by the addition of Boc-D-Ala, Boc-Tyr(Cl_2Z), Boc-Ser(Bzl), Boc-Trp, Boc-His (N^τ-Tos), and Boc-Gln. Ammonolysis and a first purification yielded 138.3 mg, and after HF cleavage and cyclisation, 60.1 mg of crude **9**·AcOH were obtained. Final and satisfactory purification gave 29.6 mg of **9**·AcOH (4.5% overall yield from 0.492 mmol of amino-acid resin). R_f (BAW) 0.43, R_f (BAWP) 0.50.

[D-Ala⁶, Phe(N₃)⁷]LHRH (Glp-His-Trp-Ser-Tyr-D-Ala-Phe(4'-N₃)-Arg-Pro-Gly-NH₂; **2***).* As described in the general procedures, 5.0 mg of **9**·AcOH (3.76 µmol) were hydrogenated, diazotized, and converted to 4.8 mg (96%) of pure **2**·AcOH. R_f (BAW) 0.475, R_f (BAWP) 0.51. IR: 2100.

[D-Phe^{2,3,6}, Phe(NO₂)⁷]LHRH (Glp-D-Phe-Ser-Tyr-D-Phe-Phe(4'-NO₂)-Arg-Pro-Gly-NH₂; **10***).* One third of the resin ester Boc-Phe(4'-NO_2)-Arg(Tos)-Pro-Gly-OR (*ca.* 1.3 g; see under **9**) were elongated with Boc-D-Phe, Boc-Tyr(Cl_2Z), Boc-Ser(Bzl), and Boc-Gln to the desired decapeptide, cleaved, and purified as mentioned above. Ammonolysis yielded 158.1 mg, and after HF cleavage, cyclisation, and purification, 113.8 mg (16.7% with respect to the resin ester) of **10**·AcOH were obtained. R_f (BAW) 0.65, R_f (BAWP) 0.60.

[D-Phe^{2,3,6}, Phe(N₃)⁷]LHRH (Glp-D-Phe-D-Phe-Ser-Tyr-D-Phe-Phe(4'-N₃)-Arg-Pro-Gly-NH₂; **3***).* Peptide **10**·AcOH (5.0 mg) was hydrogenated, diazotized, and converted to homogenous **3**·AcOH (after purification, 2.8 mg of (56%)). R_f (BAW) 0.62, R_f (BAWP) 0.70. IR: 2110.

[D-Phe(NO₂)⁶]LHRH (Glp-His-Trp-Ser-Tyr-D-Phe(4'-NO₂)-Leu-Arg-Pro-Gly-NH₂; **11***).* To 5 g of the resin ester Boc-Arg(Tos)-Pro-Gly-OR (see under **9**) was coupled Boc-Leu, and the resulting tetrapeptide ester was separated. One fourth was used to continue this synthesis with the following amino acids: Boc-D-Phe(4'-NO_2)Boc-Tyr(Cl_2Z), Boc-Ser(Bzl), Boc-Trp, His(N^τ-Tos), and Boc-Gln. After ammonolysis, HF cleavage, cyclisation, and final purification 71.2 mg (*ca.* 14%) of **11**·AcOH were collected. R_f (BAW) 0.39, R_f (BAWP) 0.64.

[D-Phe(N₃)⁶]LHRH (Glp-His-Trp-Ser-Tyr-D-Phe(4'-N₃)-Leu-Arg-Pro-Gly-NH₂; **4***).* Peptide **11**·AcOH (5.3 mg) was treated as described under **2** and **3**. After 2 chromatographic separations, only 0.47 mg (8.9%) of **4**·AcOH were collected. R_f (BAW) 0.51, R_f (BAWP) 0.66. IR: 2105.

[D-Phe^{2,6}, D-Phe(NO₂)³]LHRH (Glp-D-Phe-D-Phe(4'-NO₂)-Ser-Tyr-D-Phe-Leu-Arg-Pro-Gly-NH₂; **12***).* The tetrapeptide resin ester described under **11** (0.3 mmol) was elongated with Boc-D-Phe, Boc-Tyr(Cl_2Z), Boc-Ser(Bzl), Boc-D-Phe(4'-NO_2), and Boc-Gln. After ammonolysis, HF cleavage, and purification 130 mg (32%) of pure **12**·AcOH were collected. R_f (BAW) 0.55, R_f (BAWP) 0.73.

[D-Phe^{2,6}, D-Phe(N₃)³]LHRH (Glp-D-Phe-D-Phe(4'-N₃)-Ser-Tyr-D-Phe-Leu-Arg-Pro-Gly-NH₂; **5***).* Peptide **12**·AcOH was converted as usual to 3.41 mg (68%) of pure **5**·AcOH. R_f (BAW) 0.56, R_f (BAWP) 0.75. IR: 1205.

[AcPro¹, D-Phe(NO₂)², D-Ala(βNp)^{3,6}]LHRH (Ac-Pro-D-Phe(4'-NO₂)-D-Ala(βNp)-Ser-Tyr-D-Ala(βNp)-Leu-Arg-Pro-Gly-NH₂; **13***).* Starting with 2 g of the peptide resin Boc-Leu-Arg(Tos)-Pro-Gly-OR (0.3 mequiv./g), prepared under **11**, Boc-D-Ala(βNp), Boc-Tyr(Cl_2Z), Boc-Ser(Bzl), Boc-D-Phe(4'-NO_2), Boc-Pro, and Ac_2O were used to complete the sequence. Ammonolysis gave 1.06 g. After HF cleavage and purification (gradients of 20–75% CH_3CN), 691 mg (80%) of pure **13**·AcOH were obtained. R_f (BAW) 0.67, R_f (BAWP) 0.82.

[AcPro¹, D-Phe(N₃)², D-Ala(βNp)^{3,6}]LHRH (Ac-Pro-D-Phe(4'-N₃)-D-Ala(βNp)-Ser-Tyr-D-Ala(βNp)-Leu-Arg-Pro-Gly-NH₂; **6***).* Peptide **13**·AcOH (8.9 mg) was converted as usual. For the reversed-phase purification, 0.1% CF_3COOH gradients of 20–75% CH_3CN were utilized, and 4.3 mg (48%) of pure **6**·AcOH were collected. R_f (BAW) 0.68, R_f (BAWP) 0.84. IR: 2110.

[D-Lys⁶, Phe(NO₂)⁷]LHRH (Glp-His-Trp-Ser-Tyr-D-Lys-Phe(4'-NO₂)-Arg-Pro-Gly-NH₂; **14***).* Resin ester Boc-Phe(4'-NO_2)-Arg(Tos)-Pro-Gly-OR (1.2 g, 0.4 mmol) from the synthesis of **9** was elongated by condensing consecutively Boc-D-Lys(Cl_2Z), Boc-Tyr(Cl_2Z), Boc-Ser(Bzl), Boc-Trp, Boc-His(N^τ-Tos), and Boc-Gln. After ammonolysis, HF cleavage, and cyclisation, 750 mg of crude **14**·2 AcOH were obtained. The purifications were performed on 210 mg of this material yielding 50 mg (30%) of **14**·2 AcOH. R_f (BAW) 0.40, R_f (BAWP) 0.43.

[D-Lys(Biot)^6,Phe(N_3)^7]LHRH (Glp-His-Trp-Ser-Tyr-D-Lys(N^6-Biot)-Phe(4'-N_3)-Arg-Pro-Gly-NH_2; 7). Peptide **14**·2 AcOH (15 mg) gave after 2 reversed-phase separations, 8.1 mg of the corresponding azide. R_f (BAW) 0.36, R_f (BAWP) 0.61. This product (5.58 µmol) was dissolved in a soln. of 1.5 ml of DMSO/H_2O/sat. $NaHCO_3$ soln. 1:1:1. To this were added 4.8 mg (2.5 equiv.) of the succinimido ester of biotine (*Pierce Chemicals*, Rockford, Ill.). The mixture was stirred overnight, diluted with H_2O, and directly loaded onto a small scale reversed phase column. Pure fractions from the eluate yielded 4.5 mg (27%) of **7**·AcOH. R_f (BAW) 0.48. IR: 2100.

*[D-Lys(N_3PhCO)^6]LHRH (Glp-His-Trp-Ser-Tyr-D-Lys(N^6-(4'-N_3PhCO))-Leu-Arg-Pro-Gly-NH_2; **8**).* [D-Lys^6]LHRH (2.5 mg, 2.1 µmol; *Bachem Inc.*) was dissolved in DMSO/H_2O/sat. $NaHCO_3$ soln. 1:1:1 and reacted with 1.5 mg (5.8 µmol) of the succinimido 4-azidobenzoate. Reaction conditions and workup as for **7** yielded 2.2 mg (72%) of pure **8**·AcOH. R_f (BAW) of educt 0.39, R_f (BAWP) of **8**·AcOH 0.51. IR: 2100.

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