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SYNTHESIS OF LUTEINIZING HORMONE RELEASING HORMONE (LH-RH); A SIMPLIFIED APPROACH TO THE SYNTHESIS OF ARGININE PEPTIDES

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A novel facilitation of peptide synthesis introduced by Young[1] involves the use of a basic 'handle' in the form of a 4-picolyl ester protecting group. p-Dimethylaminoazobenzyl esters may be used similarly[2] and the basic imidazole ring has been used as a 'handle' in the synthesis of histidine peptides[3]. We now report an approach in which the guanidino group may be used as a basic 'handle' in the synthesis of arginine peptides. This has led to a simplified synthesis of the decapeptide, luteinizing hormone releasing hormone[4,5] (LH-RH) in pure, highly active form.

The basic 'handle' is used to isolate protected peptide intermediates after each coupling step. An amino component containing arginine, protected by protonation, is coupled with an excess of the carboxyl component. The product is isolated by absorption into an acidic phase, particularly an insoluble ion-exchange resin, and the co-products removed by washing with a solvent which does not displace the product from the acidic phase. The product may be removed from the resin with a volatile buffer containing acetic acid and evaporation then gives the protected peptide as the acetate. After deprotection of the peptide, the process may be repeated until the desired peptide chain is built up. The repetitive process is somewhat similar to the solid-phase method of Merrifield[6] but has the advantages that coupling can be carried out in homogeneous solution and completion of the coupling and purity of the product can be demonstrated by t.l.c. This is particularly advantageous in fragment condensations or in sterically hindered couplings, where the standard conditions may not be sufficient to ensure 100% conversion of the amino component. The product isolated in this way is frequently chromatographically and analytically pure without further treatment. The purification of arginine peptides on ion-exchange columns is well known[7], and when the simple repetitive isolation gives impure product, use may be made of this form of column chromatography for purification.

The use of this approach in two syntheses of LH-RH is outlined in Figure I. The peptide was built up by conventional means, introducing arginine as the ω-nitro derivative. At the pentapeptide stage, hydrogenation with 10% palladium/charcoal gave derivative 1 which was

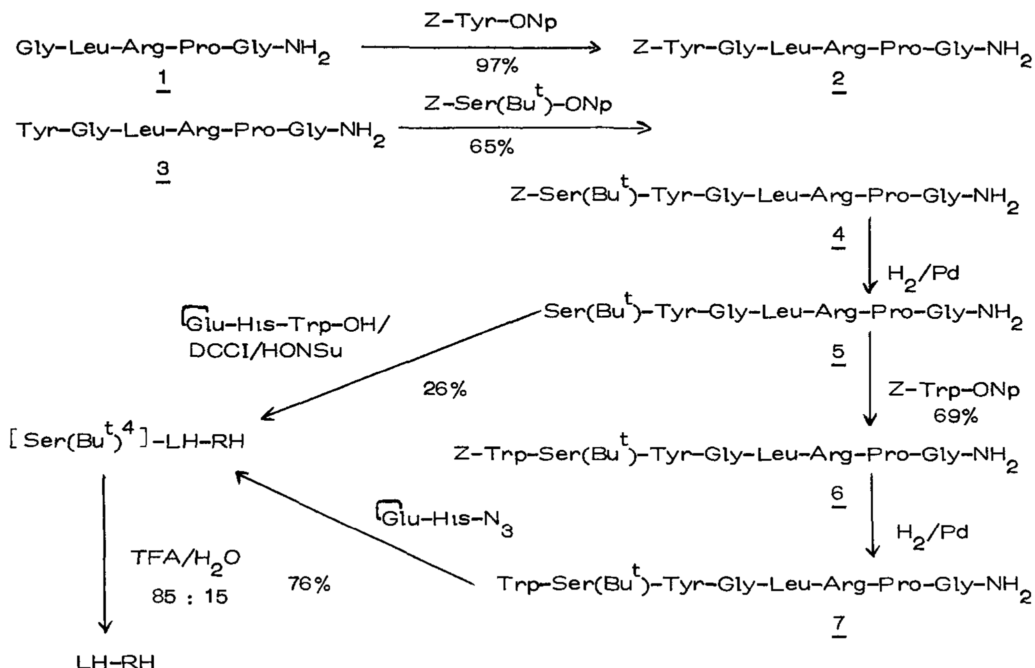

Figure 1. Synthesis of LH-RH using arginine as a basic "handle". Abbreviations follow the recommendations of the IUPAC - IUB Commission on Biochemical Nomenclature[8,9]: DCCI, dicyclohexylcarbodiimide; TFA, trifluoroacetic acid.

coupled with Z-Tyr-ONp. Isolation using carboxymethyl Sephadex C-25 resin as described below gave pure protected hexapeptide (2) and hydrogenation over 10% palladium/charcoal gave 3. Two further coupling reactions starting from 3 (prepared in this case from the N-benzyloxycarbonyl ω-nitroarginine peptide) using Z-Ser(Bu^t)-ONp and Glu-His-Trp-OH/ dicyclohexylcarbodiimide/N-hydroxysuccinimide gave [Ser (Bu^t)^4] -LH-RH. The benzyloxycarbonyl groups were removed by hydrogenation in acetic acid/water (4:1). In order to avoid the danger of acetylation during coupling, the acetate salts were converted to pivalates by filtering an aqueous solution through DEAE-Sephadex A-25 resin in the pivalate form, followed by evaporation. Removal of the t-butyl group from [Ser(Bu^t)^4] -LH-RH with trifluoroacetic acid/water (85:15 v/v) at room temperature gave essentially pure LH-RH. This material (167 mg) was chromatographed on a column of carboxymethyl Sephadex C-25 resin,

2 cm x 35 cm, eluting with a gradient from 1% pyridine, 1% acetic acid in water (v/v) to 5% pyridine, 5% acetic acid in water (v/v), pH 4.7 in each case, giving 140 mg of chromatographically pure LH-RH, with an amino acid analysis: Arg 0.99, Glu 1.04, Gly 1.94, His 0.99, Leu 1.00, Pro 1.04, Ser 0.83, Tyr 1.01, NH_3 1.33. LH Releasing activity was measured in vitro using sheep pituitary slices as described by Crighton[10], and was active at a minimum effective dose of 0.5 ng. or less per incubation. Thus with 0.5 ng. of LH-RH the amount of LH released (μg LH/mg pituitary tissue) differed significantly from the control:

0.5 ng LH-RH	1.93 (1.33 - 2.89) μg LH/mg
control	0.35 (0.16 - 0.57) μg LH/mg

(figures in parenthesis are fiducial limits of error at P = 0.95)

In the second synthesis outlined in Figure I, couplings with Z-Ser(Bu^t) ONp, Z-Trp-ONp and Glu-His-N_3 gave a similar final product in higher overall yield.

The following procedure is representative:
To a solution of Gly-Leu-Arg-Pro-Gly-NH_2 pivalate (0.53g) in dimethylformamide (4 ml) was added 0.39 g of Z-Tyr-ONp. The solution was kept at room temperature overnight, when t.l.c. failed to detect any unchanged pentapeptide. Evaporation gave a gum which was dissolved in dimethylformamide/water 3:1 (10 ml) and the solution poured through carboxymethyl Sephadex C-25 resin (H^+ form, 15g) equilibrated with dimethylformamide/water 3:1 in a 20 ml separating funnel. The resin was washed with this solvent and the product washed off with pyridine/acetic acid/dimethylformamide/water 1:1:6:2. Evaporation and trituration with ether gave the hexapeptide (2) as a chromatographically pure powder weighing 0.69 g.

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