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CHYMOTRYPSIN-CATALYZED FRAGMENT COUPLING SYNTHESIS OF D-PHE(6)-GNRH

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Summary- p-Glu-His-Trp-OEt and Ser-Tyr-D-Phe-Leu-Arg-Pro-Gly-NH_2 were coupled to obtain D-Phe(6)-GnRH using chymotrypsin-catalyzed reaction.The enzymatic reaction gave high yield without racemization.

The fragment coupling 3 + 7 is a preferred strategy for the synthesis of GnRH decapeptides[1]. The fragments p-Glu-His-Trp and Ser-Tyr-D-Phe-Leu-Arg-Pro-Gly-NH_2 can be synthesized by conventional methods without racemization and problems of alkylation of tryptophan . However, the use of chemical methods in the fragment condensation causes up to 3% racemization at the activated amino acid Trp[2] leading to a reduced peptide purity.
Enzyme-catalyzed coupling has been shown to be an excellent alternative to chemical activation in peptide synthesis[3]. Andersen et al.[4] synthesized GnRH by 5 + 5 chymotrypsin-catalyzed fragment condensation and used various enzymes for the preparation of the N-term. pentapeptide ester and the C-term. pentapeptide amide. However, the totally enzymatic peptide synthesis has some drawbacks in its application due to the high costs of some of the required enzymes and the difficult optimization of the reaction conditions. Chymotrypsin was examined as catalyst for 3 + 7 fragment condensation in the synthesis of D-Phe(6)-GnRH.
The enzymatic coupling of

p-Glu-His-Trp-OEt 1 and

Ser-Tyr-D-Phe-Leu-Arg-Pro-Gly-NH_2 2

with α-chymotrypsin (E/S= 1/800, in weight) was carried out in dimethylformamide/ water (48/52, v/v) at pH 8.0 and ambient temperature[5]. It is shown in Table 1 the dependence of the conversion rate on the reaction time.

Table 1. Dependence of conversion rate on reaction time

Reaction time (h)	2 (%)	D-Phe(6)-GnRH (%)
0	100	0
2	38.3	61.7
3	14.0	86.0
4	7.6	92.4
5	4.9	95.1
6	2.9	97.1
6.5	2.5	97.5
21	5.3	94.7
29	18.5	81.5

It is obvious that the yield of coupling product rises with increasing reaction time up to 97.5%. Besides the main product D-Phe(6)-GnRH the free acid of 1 was formed by hydrolysis of the acyl-enzyme intermediate. However, the aminolysis is much faster than the hydrolysis. With the decrease of 1 the Trp 3 - Ser 4 peptide bond in the product was cleaved after 15-20 h in competition to the aminolysis reaction, leading to an increasing amount of 2 (Table 1). The chymotryptic hydrolysis at the carboxyl group of Tyr was inhibited due to the presence of D-Phe in position 6.

The results demonstrate that chymotrypsin is very effective for the 3 + 7 fragment condensation synthesis of D-Phe(6)-GnRH. There were no problems in scaling up, thus making the method convenient for synthesis of kg quantities of the peptide.

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5. 21 g (43.7 mmol) of 1 and 20 g (23.9 mmol) of 2 were suspended in a mixture of 66 ml of DMF and 70.5 ml of water (pH 8.0, 2 N KOH). The reaction was started by addition of 50 mg of chymotrypsin. After 6.5 h 10 ml of acetic acid were added to stop the enzymatic reaction. By evaporation and final precipitation by addition of acetone there was obtained 24.4 g of the product. The crude peptide was purified by reversed-phase chromatography to give 16.2 g (53.2%) GnRH peptide and was characterized by HPLC[6] (t_R=15.5 min, 99.2%) and amino acid analysis.
6. Reversed-phase HPLC on LiChrosorb RP 8, 5 µm, 250x4 mm I.D. (Merck), mobile phase 0.05 M NaH_2PO_4, pH 2.2 / 26% acetonitrile, 1.0 ml/min, 2 t_R= 5.43 min, D-Phe(6)-GnRH t_R= 15.47 min.

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