Tetrahedron Letters No. 13, pp1135 - 1136, 1977. Pergamon Press. Printed in Great Britain.

SOLID-PHASE COUPLING OF ARGINYL-PEPTIDES

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(Received in UK 15 February 1977; accepted for publication 21 February 1977)

Coupling of arginine represents a critical step in peptide synthesis and particularly in the utilization of tryptic peptides as ready made intermediates in the chemical synthesis of polypeptides and proteins[1]. Moreover, in fragment coupling the difficulties inherent to the formation of the peptide bond involving arginine, are always accompanied by the risk of racemization.

In recent years several procedures, besides the well known azide activation[2], have been proposed to minimize racemization. Particularly promising in this respect are the dicyclohexylcarbodiimide (DCC)/ hydroxysuccinimide (HOSu)[3], N-etoxycarbonyl-2-etoxy-1,2-dihydroquinoline (EEDQ)[4], and Woodward's methods[5]. More recently, König and Geiger[6] showed that 1-hydroxybenzotriazole (HOBt) was very effective in suppressing racemization in DCC mediated couplings. Furthermore, the yields and homogeneity of the products were considerably increased.

Although several model peptides have been coupled with different results to peptide polymers[7,8], very little information is at present available on the solid-phase condensation of arginyl-peptides. We report here on the coupling between Z-Phe-Arg-OH[1] and H_2N-Leu-Leu-Leu-Val-Asp(O-Bzl)-Thr(Bzl)-Pro-Glu(O-Bzl)-Thr(Bzl)-Lys(ε-TFA)-His-Pro-O-Merrifield's polymer[9]. The resulting tetradecapeptide represents sequence 34 to 47 of a complementating fragment of S. Nuclease[10]. The results obtained with different activators are shown in the following Table:

Activation	Solvent and conditions	Yield %
DCC	CH_2Cl_2 ; 2 hrs 0°C then r.t. overnight	27
DCC/HOSu	" " " " " " "	38
DCC/HOBt	DMF ; " " " " " "	96
DCC/NAP[11]	" " " " " " "	26
Woodward K	" - - - - " "	39
Woodward L	" - - - - " "	10
EEDQ	" - - - - " "	29

In general, Z-Phe-Arg-OH (46.2 mg, 0.11 mmole) was reacted, under the conditions described in the Table, with 102 mg (0.022 mmole) of the peptide-resin in the presence of 0.11 mmole of the activating agent. After completion of the reaction the polymer was collected on a sintered filter, washed throughly with DMF,

CH_2Cl_2, MeOH, CH_2Cl_2, deblocked by a 30' treatment with 50% TFA in CH_2Cl_2, washed to neutrality with CH_2Cl_2, MeOH and finally dried under vacuum. Peptide-resins were hydrolyzed with a mixture of 2 ml of 12 N HCL, 1 ml of AcOH, and 1 ml of phenol in evacuated, sealed tubes for 22 hrs at 110°C. The values obtained for the " diagnostic " phenylalanine residue with respect to leucine residues, initially present in the dodecapeptide-resin, were used to calculate the yields reported in the Table. Each figure represents the average of at least five separate experiments.

In a typical example, the peptide obtained via DCC/HOBt, was cleaved from the resin by a 30' treatment with HF in the presence of anisole at 0°C, dissolved in 10% AcOH and lyophilized. The TFA group was removed from the side-chain of the lysine residue of the peptide with 1 M piperidine in 8 M urea. The reaction mixture was put directly onto a Sephadex G-25 SF column (60 x 3.5 cm) and eluted with 10% AcOH. The fractions collected under the peak corresponding to the peptide were pooled and lyophilized. TLC on cellulose[12] and TL-electrophoresis[13] gave one spot positive to ninhydrin and exibiting a strong fluorescence with a phenantrene-quinone reagent, specific for arginine[14]. A portion of the product, hydrolyzed in constant boiling HCl at 110°C under vacuum for 22 hrs, gave the expected amino acid composition. An L-amino acid oxidase digest of the acid hydrolysate showed no arginine, after correcting for the amount of racemization during acid hydrolysis[15].

The present results show that the solid-phase coupling of an arginyl-peptide can be performed very efficiently and without racemization with the use of the DCC/HOBt system. This is of particular interest in view of the utilization of native tryptic fragments for the synthesis of both normal and modified sequences of peptides and proteins.

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