Simplified Synthesis of Histidine Peptides

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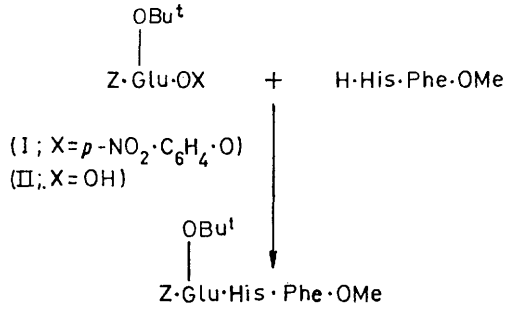
Summary The imidazole ring of histidine may be used as a basic 'handle' to simplify the isolation of products during peptide synthesis.

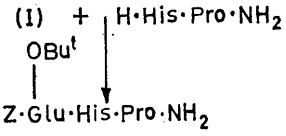
A NOVEL facilitation of peptide synthesis has been described by Young *et al.*,[1] in which a weakly basic 4-picolyl ester is



(III)

used as a 'handle' to isolate protected peptide intermediates. After each coupling stage, the protected derivatives are isolated into an acidic phase (an ion-exchange resin or aqueous acid) and the reactants and co-products are washed away with neutral solvent. An analogous method using *p*-dimethylaminoazobenzyl esters has also been reported.[2]

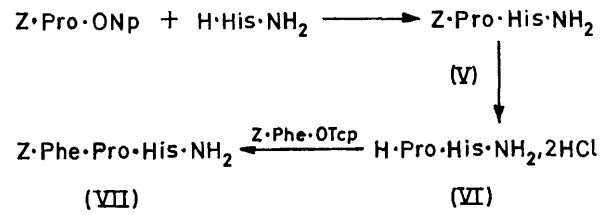
The use of the imidazole ring of an unprotected histidine residue as a 'handle' to facilitate the synthesis of histidine peptides is now reported. The method is illustrated by the synthesis of several protected peptides. The *p*-nitrophenyl ester (I) (1·25 mol. equiv.) was coupled in dimethylformamide with L-histidyl-L-phenylalanine methyl ester hydrochloride and triethylamine. The tripeptide (III) was isolated as a crystalline, chromatographically pure white solid in quantitative yield, by partitioning the reaction mixture between diethyl ether and 2N-aqueous citric acid, essentially as described for *N*-t-butoxycarbonyl-peptide 4-picolyl esters.[3] Similarly, using *NN*-'dicyclohexylcarbodi-imide and the free acid (II) for the coupling gave pure (III) (94%). In each case, no amino-component could be detected (by t.l.c.) in either the reaction mixture or the final product. Coupling of 2 mol. equiv. of (I) with L-histidyl-L-prolineamide dihydrobromide and triethylamine in dimethylformamide gave the protected tripeptide (IV) (89%) using the same isolation procedure.



(IV)

Ion-exchange resin may be used to isolate the protected peptides. Benzyloxycarbonyl-L-proline *p*-nitrophenyl ester (1·2 mol. equiv.) was coupled with L-histidineamide dihydrochloride and triethylamine in dimethylformamide. The dipeptide (V) was isolated using sulphoethyl Sephadex C-25 resin, aqueous dimethylformamide as solvent, and triethylamine for elution.[4] After catalytic hydrogenation, the resulting dipeptide amide dihydrochloride (VI) was coupled in dimethylformamide with benzyloxycarbonyl-L-phenylalanine 2,4,5-trichlorophenyl ester (1·2 mol. equiv.). A similar isolation using sulphoethyl Sephadex resin gave the protected tripeptide (VII) in an overall yield of 70% from L-histidineamide dihydrochloride. At each coupling step the absence of unchanged amino-component was demonstrated by t.l.c.

This approach makes possible a simple repetitive isolation of protected derivatives at each stage of a synthesis, but



the purity of every intermediate may be checked by the usual chemical means. In particular, the completion of coupling reactions may be demonstrated by t.l.c. at every stage. These advantages, shared by the picolyl ester approach, are absent from the 'solid-phase' method of peptide synthesis.[5] Use of the imidazole ring of histidine as a basic 'handle' eliminates the need to introduce a special protecting group and any difficulties which may occur in its subsequent removal. The approach should be applicable to the synthesis of many peptides containing histidine, which need not be at the *C*-terminal position since the peptide may be built up by conventional means until the histidine is introduced. Histidine occurs widely in biologically active peptides and the general use of this method in their synthesis is now being examined.

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[3] R. Camble, R. Garner, and G. T. Young, *J. Chem. Soc.* (*C*), 1969, 1911; D. J. Schafer, G. T. Young, D. F. Elliot, and R. Wade, *J. Chem. Soc.* (*C*), 1971, 46.
[4] R. Garner, D. J. Schafer, W. B. Watkins, and G. T. Young, in 'Peptides 1968', ed. E. Bricas, North Holland, Amsterdam, 1968, p. 145.
[5] R. B. Merrifield, *J. Amer. Chem. Soc.*, 1963, **85**, 2149.