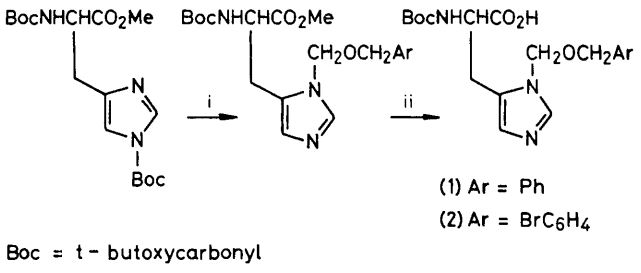
Protection of Histidine Side-chains with π-Benzyloxymethyl- or π-Bromobenzyloxymethyl-groups

BY TOM BROWN and JOHN H. JONES*
(*The Dyson Perrins Laboratory, South Parks Road, Oxford* OX1 3QY)

Summary $N(\alpha)$-t-Butoxycarbonyl-$N(\pi)$-benzyloxymethyl-L-histidine (**1**) and $N(\alpha)$-t-butoxycarbonyl-$N(\pi)$-4-bromobenzyloxymethyl-L-histidine (**2**) have been prepared and shown to be suitable derivatives for peptide synthesis with histidine; the synthetic intermediates had convenient physical properties, no side reactions were encountered, and the final deprotection proceeded smoothly under mild conditions.

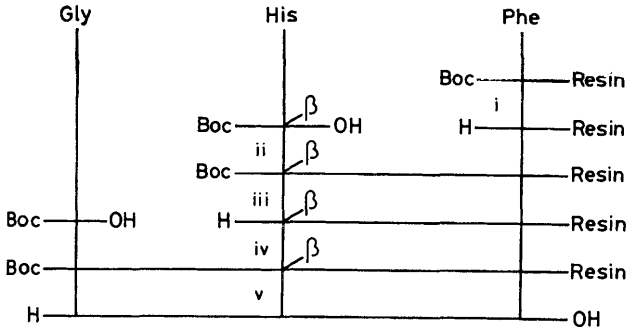
THE histidine side-chain protecting groups which have been used most often in peptide synthesis are *im*-benzyl, *im*-2,4-dinitrophenyl, *im*-toluenesulphonyl, and *im*-t-butoxycarbonyl. All have serious deficiences. Racemisation can occur on activation for coupling, at a gross level with the *im*-benzyl group, but also sometimes with the others (the literature on this point is somewhat contradictory). Reactivity towards nucleophiles is a problem with all except the *im*-benzyl group, which, in contrast, can only be removed under undesirably vigorous conditions. The solubility of the protected derivatives is at times a source of difficulty, especially with *im*-benzyl or *im*-2,4-dinitrophenyl.

We have shown that the danger of side-chain induced racemisation during the activation of histidine derivatives can be eliminated completely by location of a blocking group on the π-nitrogen, the intramolecular base action of which is the root cause of the problem. All the side-chain protecting groups which had been used prior to this were of proved or presumed τ-location. We demonstrated this point by a direct comparison of τ- and π-phenacylhistidine derivatives and went on to show that the π-phenacyl group was, in simple cases at least, a practicable histidine-protecting group for racemisation-free peptide synthesis.[1] The limitations and scope for side reactions due to the relative chemical complexity of the phenacyl group are, however, obvious. The ideal histidine side-chain protecting group should be chemically simple and should solve all the problems mentioned above simultaneously. We have therefore investigated a range of simple substituents which could, in principle, be positioned on the π-nitrogen and so prevent racemisation, but which would at the same time be likely to give easily prepared derivatives with convenient solubility properties and resistance to nucleophilic attack, combined with susceptibility to clean removal under mild conditions at the end of the synthesis.



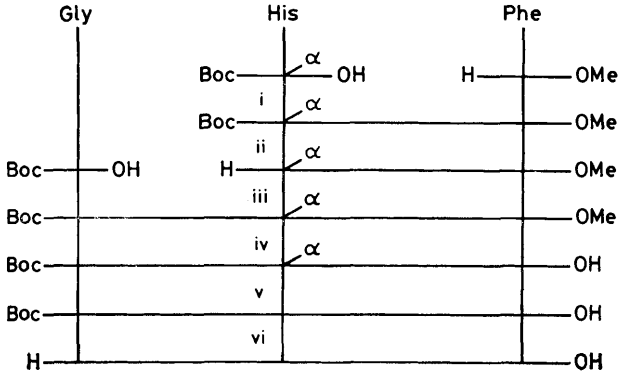
SCHEME 1. *Reagents:* i, $PhCH_2OCH_2Cl$–Et_2O, 20 °C then Et_3N–MeOH (62%); ii, aqueous NaOH (78%).

The π-benzyloxymethyl- and π-4-bromobenzyloxymethyl-groups appear to meet these requirements. $N(\alpha)$-t-Butoxycarbonyl-$N(\pi)$-benzyloxymethyl-L-histidine (**1**), m.p. 155 °C, $[\alpha]_D^{20}$ + 6·9° (*c* 0·5, MeOH), was prepared from $N(\alpha)N(\tau)$-bis-(t-butoxycarbonyl)-L-histidine methyl ester† as shown in Scheme 1. The 4-bromobenzyl analogue (**2**), m.p. 69—74 °C $[\alpha]_D^{20}$ — 9·4° (*c* 1, MeOH), can be obtained similarly.‡ The side-chain protecting groups in compounds (**1**) and (**2**) were unaffected by exposure to nucleophilic and basic reagents (in excess) for many hours at room temperature and by trifluoroacetic acid (no detectable reaction at room temperature overnight). Cleavage was slow with 6N hydrogen bromide–acetic acid (slight cleavage overnight at room temperature), but rapid with saturated hydrogen bromide–trifluoroacetic acid (quantitative cleavage in 1·5 h at room temperature). Catalytic hydrogenolysis also effected quantitative cleavage, as did hot aqueous hydrochloric acid under the standard conditions for peptide hydrolysis.



SCHEME 2. *Reagents:* i, iii, CF_3CO_2H–CH_2Cl_2, then Et_3N–CH_2Cl_2; ii, iv, protected amino-acid (4 equiv.)–dicyclohexylcarbodi-imide (DCCI) (4 equiv.)–CH_2Cl_2; v, HBr–CF_3CO_2H. $\beta = \pi$-CH_2OCH_2-C_6H_4Br-4. The resin was a 1% cross-linked Merrifield resin. The overall yield (based on BocPhe-resin) of chromatographically and analytically pure, fully characterised tripeptide was 55%.

This combination of properties is fully compatible with conventional methods and strategies in both classical and solid-phase peptide synthesis. The acids (**1**) and (**2**) were easily obtained as crystalline solids which dissolve with ease in organic solvents such as chloroform. Their use in a number of simple exercises such as those shown in Schemes 2 and 3 and in the more demanding case of a solid-phase



SCHEME 3. *Reagents:* i, iii, DCCI–HOBt (with pre-activation)–dimethylformamide; ii, vi, CF_3CO_2H; iv, aqueous NaOH; v, 10% Pd(C)–H_2. $\alpha = \pi$-CH_2OCH_2Ph. The yields of the chromatographically and analytically pure, fully characterised material were: stage i 81%, stages ii & iii together 85%, stages iv, v, and vi together 67·5%.

synthesis of 5-isoleucine-angiotensin II proved convenient and free of complications. The acid (**2**) does not offer any obvious advantage over (**1**), which is therefore preferred on grounds of simplicity; it appears to us that it offers a general solution to the long-standing problem of protecting histidine side-chains in peptide synthesis.

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† This compound has been described before by B. O. Handford, T. A. Hylton, K.-T. Wang, and B. Weinstein, *J. Org. Chem.*, 1968, **33**, 4251, but we prefer to prepare it by reaction of L-histidine methyl ester dihydrochloride with triethylamine and di-t-butyl-dicarbonate in methanol (90% yield).

‡ Using 4-bromobenzyl bromomethyl ether which was obtained by analogy with the published procedure for the preparation of benzyl bromomethyl ether (A. T. Blomquist and E. J. Moriconi, *J. Org. Chem.*, 1961, **26**, 3761) as a waxy solid and used without purification.

[1] J. H. Jones and W. I. Ramage, *J. Chem. Soc., Chem. Commun.*, 1978, 472; A. R. Fletcher, J. H. Jones, W. I. Ramage, and A. V. Stachulski, *J. Chem. Soc., Perkin Trans.* 1, 1979, 2261; J. H. Jones, W. I. Ramage, and M. J. Witty, *Int. J. Pept. Protein Res.*, 1980, **15**, 301.