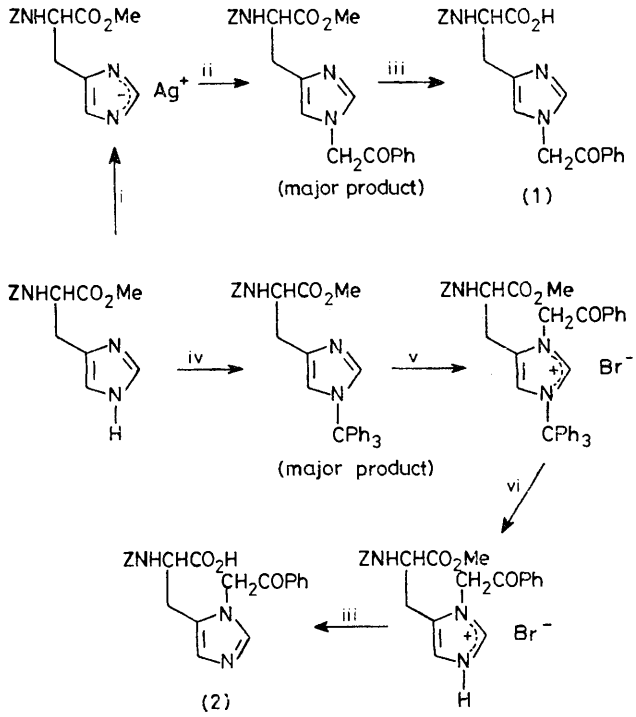
An Approach to the Prevention of Racemisation in the Synthesis of Histidine-containing Peptides

By JOHN H. JONES* and WILLIAM I. RAMAGE
(*The Dyson Perrins Laboratory, Oxford University, South Parks Road, Oxford* OX1 3QY)

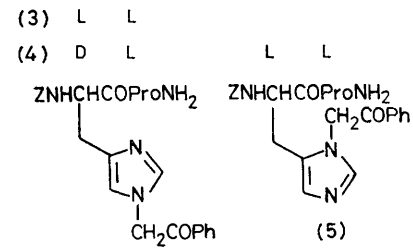
Summary $N(\alpha)$-Benzyloxycarbonyl-$N(\tau)$-phenacyl-L-histidine and $N(\alpha)$-benzyloxycarbonyl-$N(\pi)$-phenacyl-L-histidine have been prepared; extensive racemisation of the histidine residue occurs on activation of the former by means of dicyclohexylcarbodi-imide, but not with the $N(\pi)$-phenacyl acid.

EXAMPLES of the detection of substantial racemisation during the coupling of alkoxycarbonylhistidine derivatives have been reported, both in the presence[1] and absence[2] of side-chain protection. The mechanistic details are not clear, but the π-nitrogen appears to be implicated and Veber has suggested[2] that specific blockade of this position might be advantageous. The location of the side chain protecting group in the histidine derivatives in current use has in fact only been proved in one case, that of *im*-Dnp, when it was found to be the τ-nitrogen.[3] Steric considerations and analogies with the behaviour of simple 4(5)-alkylimidazoles, however, make it very probable that all the established *im*-acylation, tosylation, and benzylation protection procedures lead to τ-substitution.

We have prepared $N(\alpha)$-benzyloxycarbonyl-$N(\tau)$-phenacyl-L-histidine (**1**) and $N(\alpha)$-benzyloxycarbonyl-$N(\pi)$-phenacyl-L-histidine (**2**) as outlined in the Scheme. The locations shown for the phenacyl groups follow from the expected preference for alkylation at the least hindered nitrogen and were confirmed by application of Rapoport's n.m.r. criterion[4] for differentiation of 1,4- and 1,5-disubstituted imidazoles. Activation of the τ-phenacyl acid (**1**) by treatment with 1 equiv. of dicyclohexylcarbodi-imide in dimethylformamide (20 ml/mmol) for 1 h at 0 °C followed by addition of 1 equiv. each of prolineamide hydrochloride and triethylamine gave protected dipeptide which was seen by n.m.r. spectroscopy to be a mixture of the diastereoisomers (**3**) and (**4**): assay with L-amino acid oxidase after *im*-deprotection with zinc dust in acetic acid and hydrolysis showed that the histidine present was 35% D (corrected for racemisation during hydrolysis). In marked contrast, the isomeric π-phenacyl acid (**2**) gave under the same conditions, which were designed to exacerbate the danger of racemisation relative to that existing under the conditions normally employed for coupling, only the single diastereoisomer (**5**), and in this case enzyme assay after deprotection and hydrolysis showed a D-histidine content of $< 2\%$.†



SCHEME. i, $AgNO_3$ (92% yield); ii, $PhCOCH_2Br$, Me_2SO (major product isolated as hydrochloride in 50% yield after two recrystallisations); iii, NaOH [73% yield of (**1**); 81% yield of (**2**)]; iv, Ph_3CCl, CH_2Cl_2 (80% yield); v, $PhCOCH_2Br$, Et_2O, 3 days, 20 °C (80% yield); vi, AcOH, H_2O, 100 °C, 10 min (83% yield). All the compounds shown were obtained analytically pure and had the expected spectroscopic properties.



It is clear from this example that it is indeed advantageous to site *im*-histidine protection on the π-nitrogen and that the location as well as the chemical properties of the protecting group should be considered in developing improved methods.

Preliminary results with π-phenacyl protection in simple model peptide syntheses are encouraging. The $N(\alpha)$-t-butoxycarbonyl-$N(\pi)$-phenacyl-L-acid has also been prepared. In addition to conferring resistance to racemisation on the histidine residue, the π-phenacyl substituent (which is unaffected by hydrogen bromide in acetic acid, trifluoroacetic acid, or brief exposure to hydrogenolysis conditions) yields derivatives which have convenient solubility in organic solvents; coupling with dicyclohexylcarbodi-imide is straightforward and *im*-deprotection with zinc dust in acetic acid is rapid and clean.

We are grateful to Dr. M. Szelke for helpful discussions and information about his unpublished investigations of *im*-phenacyl protection.

(*Received*, 10*th March* 1978; *Com.* 262.)

† The uncertainty associated with the assay is *ca.* $\pm 2\%$. Under the same conditions $N(\alpha)$-benzyloxycarbonyl-N(τ)-benzyl-L-histidine gave dipeptide containing histidine which was 12% D.

[1] G. C. Windridge and E. C. Jorgensen, *J. Amer. Chem. Soc.*, 1971, **93**, 6318; H. C. Beyerman, J. Hirt, P. Kranenburg, J. L. M. Syrier, and A. van Zon, *Rec. Trav. chim.*, 1974, **93**, 256 and references there cited.
[2] D. F. Veber in 'Peptides: Chemistry Structure and Biology' (Proc. 4th American Peptide Sym.) eds. R. Walter and J. Meienhofer, Ann Arbor Science Publications Inc., Ann Arbor, 1975, p. 307.
[3] J. R. Bell and J. H. Jones, *J.C.S. Perkin I*, 1974, 2336.
[4] H. R. Matthews and H. Rapoport, *J. Amer. Chem. Soc.*, 1973, **95**, 2297.