# Acid-labile Histidine Side-chain Protection: the *N*(π)-t-Butoxymethyl Group

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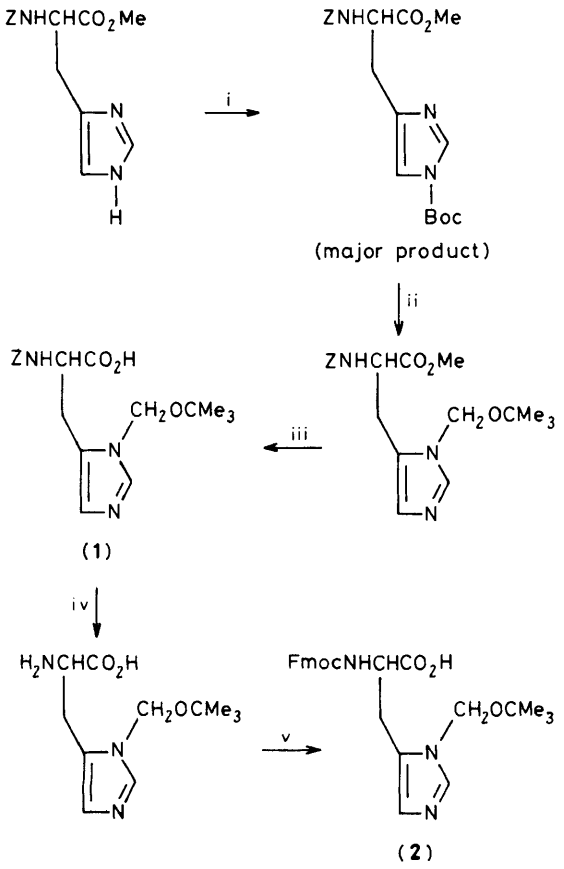
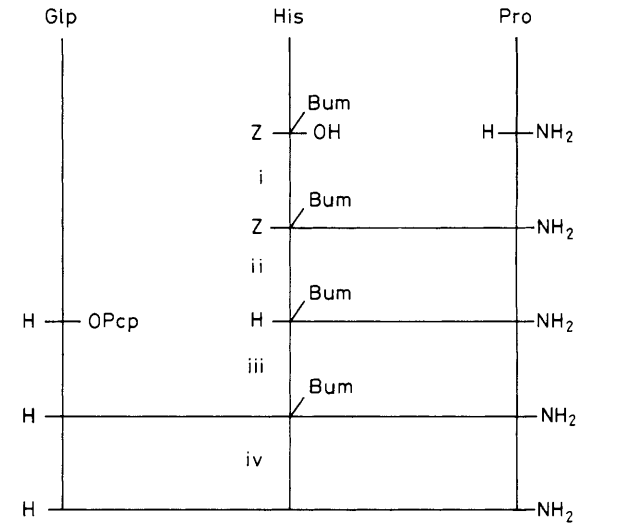
*N*(α)-Benzyloxycarbonyl-*N*(π)-t-butoxymethyl-L-histidine (**1**) and *N*(α)-fluoren-9-ylmethoxycarbonyl-*N*(π)-t-butoxymethyl-L-histidine (**2**) have been prepared and their use for the synthesis of peptides containing histidine residues has been demonstrated in two simple exercises; no difficulties were encountered, the base- and hydrogenolysis-resistant imidazole protecting group ultimately being removed by mild acidolysis.

Side-chain-induced racemization, which can occur during coupling of histidine derivatives even when *N*(α)-alkoxycarbonyl protected, can be effectively suppressed by locating a blocking group specifically on the imidazole π-nitrogen.[1] π-Phenacyl-[1] and π-benzyloxymethyl-[2] derivatives have been prepared and their effectiveness for the racemisation-free introduction of histidine residues into peptides has been demonstrated.[1-3] However, these derivatives were designed to fulfil the requirements of synthetic tactics based on N(α) acid-labile temporary protection (*e.g.* t-butoxycarbonyl), the π-phenacyl group being removable by treatment with either zinc–acetic acid or photolysis, and the π-benzyloxymethyl group being cleavable by hydrogenolysis or strong acidolysis (*e.g.* HBr–trifluoroacetic acid or HF). We have now investigated complementary π-groups which can be used with *N*(α)-benzyloxycarbonyl or *N*(α)-fluoren-9-ylmethoxycarbonyl temporary protection, *i.e.* which are stable to hydrogenolysis and base but which are removed under mild acidic conditions.

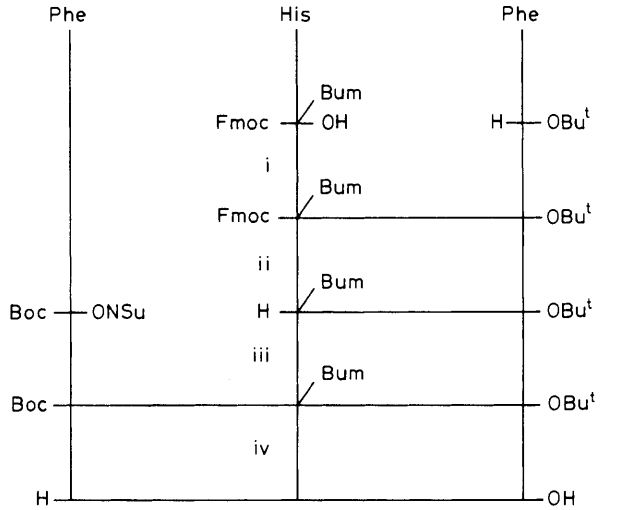
Up to this time, the most promising of these is the π-t-butoxymethyl group, which has properties comparable to those of other t-butyl-based protecting groups. *N*(α)-Benzyloxycarbonyl-*N*(π)-t-butoxymethyl-L-histidine (**1**), m.p.




**Scheme 1.** i, $Me_3COCO_2CO_2CMe_3$, MeOH (94% yield); ii, $Me_3COCH_2Cl$, $CCl_4$–$CH_2Cl_2$ (66% yield); iii, NaOH, $H_2O$–MeOH (83% yield); iv, $H_2$–10% Pd(C), AcOH (91% yield); v, fluoren-9-ylmethoxycarbonyl chloride, 10% $Na_2CO_3$, $H_2O$–dioxane (68% yield). Analytical and spectroscopic evidence in accordance with the structure of all the compounds shown was obtained.




**Scheme 2.** *Reagents:* i, DCCI–HOBt, DMF; ii, $H_2$–10% Pd(C), AcOH; iii, DMF; iv, $CF_3CO_2H$. The yields of analytically pure and fully characterized material were: stage i 75%, stages ii and iii together 81%, and stage iv 84%.




**Scheme 3.** *Reagents:* i, DCCI–HOBt, DMF; ii, $Et_2NH$, DMF; iii, DMF, iv, $CF_3CO_2H$. The yields of analytically pure and fully characterized material were: stage i 71%, stages ii and iii together 77%, and stage iv 86%.

186—187 °C, $[\alpha]_D^{18}$ − 10.7° (*c* 1, AcOH), and *N*(α)-fluoren-9-ylmethoxycarbonyl-*N*(π)-t-butoxymethyl-L-histidine (**2**), m.p. 175—176 °C, $[\alpha]_D^{18}$ − 7.5° (c 1, AcOH), were prepared from *N*(α)-benzyloxycarbonyl-L-histidine methyl ester[1] as shown in Scheme 1.† We were not able to obtain the reagent for introduction of the π-group, t-butyl chloromethyl ether, in pure form but a sufficiently stable solution for our purpose was prepared by a modification‡ of the literature method.[4] The locations shown for the N(imidazole) groups were predicted by analogy with a similar case[5] which is substantiated by crystallographic evidence: they were also confirmed by $^1$H n.m.r. spectroscopy.§ The side-chain protecting group in compound (**1**) was unaffected by exposure to nucleophilic and basic reagents (in excess) for several hours at room temperature or by catalytic hydrogenolysis at room pressure and temperature, but was cleaved cleanly and rapidly with trifluoroacetic acid or with anhydrous HCl or HBr in acetic acid.

The use of the *N*(π)-t-butoxymethyl-histidine derivatives (**1**) and (**2**) in simple exercises, such as those shown in Schemes 2 and 3, proved to be quite straightforward and free of racemization or any other problem meriting comment.

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† Abbreviations are as used and defined in 'Specialist Periodical Reports on Amino-acids, Peptides, and Proteins,' vol. 14, p. xix, with the additional use of Bum = π(−$CH_2OCMe_3$).

‡ We have found that the absence of both oxygen and traces of acid in the chloroether solution increases significantly its stability and the quality of the products obtained from it. Accordingly, the free radical halogenation of t-butyl methyl ether was performed under dry nitrogen and *N*,*N*-di-isopropyl-3-pentylamine (0.4% v/v) was added to the resulting $CCl_4$ solution of the chloroether as a proton captor.

§ For example, in (**1**) and its methyl ester, irradiation of the low field imidazole proton (the one between the heterocyclic nitrogens) gave a nuclear Overhauser enhancement of the protecting group $CH_2$ signal but irradiation of the high field more distant imidazole proton did not. In the corresponding *N*(α)-benzyloxycarbonyl-*N*(τ)-t-butoxymethyl-L-histidine methyl ester, which was isolated for comparison, a nuclear Overhauser enhancement of the protecting group $CH_2$ signal was observed on irradiation of either of the equidistant imidazole ring protons.

## References

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