The N^{im}-(2,4-dimethylpent-3-yloxycarbonyl) (Doc) group, a new nucleophile-resistant, HF-cleavable protecting group for histidine in peptide synthesis

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The 2,4-dimethylpent-3-yloxycarbonyl group as protection for the imidazole ring of histidine combines several properties that makes it ideal for solid phase peptide synthesis with Boc chemistry; it is readily cleaved by liquid HF, stable to trifluoroacetic acid, soluble in apolar organic solvents and highly resistant to nucleophiles, thereby avoiding the danger of N^{im} to N^{α} transfer and premature cleavage by other nucleophiles during solid phase synthesis.

Protection of the imidazole side chain of histidine during solid phase peptide synthesis has proved to be difficult and no completely satisfactory protecting groups exist. During activation of N^{α}- protected histidine without a side chain protecting group, the π-nitrogen can serve as a base and abstract the C^{α} hydrogen, which results in a partial loss of chirality.[1] Introduction of π-nitrogen protecting groups will prevent racemization, but the synthesis of such derivatives requires multi-step procedures. The τ-nitrogen is, for sterical reasons, significantly more reactive as a nucleophile than the π-nitrogen[2] and must therefore be protected before introduction of the π-protecting group. The Bom[3] and Bum[4] groups are examples of π-protecting groups that are currently used in peptide synthesis. The major drawback of these histidine derivatives is that during the acidic cleavage of the protecting group a number of methylated or formylated by-products are formed.[5]

The synthetic route to τ-protected histidine is more straightforward and a number of protecting groups have been suggested. Protecting groups for the τ-nitrogen that are electron-withdrawing and reduce the basicity of the π-nitrogen suppress racemization and are to be preferred. The *N*-τ-dinitrophenyl (Dnp) group[6,7] is an example of this strategy and this derivative has been widely used in Boc chemistry, but a number of drawbacks are associated with Dnp protection of histidine. The Dnp group is stable to acids and must be removed by nucleophiles under non-acidic reaction conditions. A separate deprotection step prior to final cleavage is therefore required and the success of this procedure is dependent on good solvatisation of the peptide resin, implying that cleavage might be slow for many peptides.

Urethane-type protecting groups on the τ-nitrogen are, like the Dnp group, electron-withdrawing, thereby reducing the level of racemization. A problem with both the Dnp group and the urethane-type protecting groups is that they are sensitive to nucleophiles.[8,9,10] Loss of the protecting group by the action of exogenous nucleophiles from a histidine residue incorporated into a peptide is not necessarily a serious side reaction, but when the nucleophile is an N-terminal amino group the N^{im} to N^{α} transfer will result in irreversible termination of the synthesis.

If the problem with nucleophile-sensitivity could be solved, urethane-type protecting groups would in other respects be ideal for peptide synthesis as they can be designed to be cleavable by acid along with other protecting groups, suppress racemization, reduce the nucleophilicity of the π-nitrogen and increase the solubility of the N^{α}-protected histidine derivative.

In a previous study in our laboratory of another intramolecular nucleophile-dependent side reaction, base-catalysed aspartimide formation, the β-2,4-dimethylpent-3-yl ester of aspartic acid was shown to be highly resistant to premature removal by the nitrogen at the *n*+1 peptide bond.[11] Based on this study we hypothesized that sterically hindered alkyl urethane-type protecting groups for histidine could protect the urethane carbonyl against nucleophilic attacks. We therefore synthesized the 2,4-dimethylpent-3-yloxycarbonyl (Doc) group of histidine (Fig. 1) and studied its removal by nucleophiles. A study in the same direction was reported by Nishiyama *et al.*,[12,13] who used the 2-adamantyloxycarbonyl (2-Adoc) group as a τ-protecting group. This protecting group was relatively resistant to nucleophiles, but was fully removed by piperidine within 2 hours. The 2-Adoc and the Dnp protecting groups were therefore both included in our study and compared with the new Doc protecting group.

The 2,4-dimethylpent-3-yloxycarbonyl group can be introduced selectively on the side chain of histidine by treating histidine with dichlorodimethylsilane and Doc-Cl in the presence of base, followed by the introduction of the Boc group. This procedure was worked out for the selective introduction of the N^{im} trityl group by Barlos *et al.*[14] Alternatively, the Doc group can be introduced directly to Boc-His-OH. In our hands the former procedure resulted in a product with higher purity. Boc-His(Doc)-OH† is a crystalline solid which is highly soluble in most organic solvents, such as methylene chloride and DMF, and even moderately soluble in light petroleum ether.

The Doc group is readily removed by liquid HF, but it is only partially cleaved by TFMSA (CF_3SO_3H)–thioanisole–EDT–TFA (CF_3CO_2H) under standard conditions. The acid stability of the Doc group makes it therefore well suited for use in solid phase peptide synthesis with Boc–benzyl strategy.

To study the nucleophile-sensitivity of the different protecting groups we synthesized the model peptide Ala-Pro-Lys(Boc)-Tyr(OBu^t)-NH_2 on a TFA labile resin (*p*-{(*R,S*)-α-[1-(9*H*-Fluoren-9-yl)-methoxyformamido]-2,4-dimethoxybenzyl}phenoxyacetic acid anchored to 4-methylbenzhydrylamine resin) using standard Fmoc chemistry procedures and coupled Boc-His(Doc)-OH, Boc-His(2-Adoc)-OH or Boc-His(Dnp)-OH as the terminal residue. The resin-bound peptide was treated with 20% piperidine in DMF, the peptide was

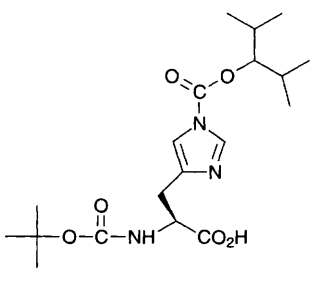

Fig. 1 Boc-His(Doc)-OH

cleaved from the resin and the *tert*-butyl protecting groups removed by trifluoroacetic acid and the relative amounts of fully deprotected peptide and peptide where the histidine protecting group was still attached were monitored by reversed phase HPLC at 215 nm. The results are shown in Fig. 2.

It is clear that although the 2-Adoc and Doc groups are both structures where the alkyl groups are branched at analogous positions, the acyclic and more flexible 2,4-dimethylpent-3-yl group provides a significantly better protection against nucleophilic attack at the alkoxycarbonyl group than the rigid, compact 2-adamantyl group. In our study we found a remarkable difference in sensitivity to nucleophiles for the two protecting groups (Table 1). When treated with 20% piperidine in DMF the 2-Adoc group was cleaved with a half-life of approximately 12 min, whereas the Doc group under the same reaction conditions was considerably more stable and removed with a half-life of 84 h. It is clear that the stability of the Doc group is sufficient for most practical purposes, including the use of piperidine for removal of orthogonal protecting groups. We therefore conclude that the greater resistance to nucleophiles of the Doc group as compared to the 2-Adoc group will dramatically decrease the danger of protecting group transfer to the N-terminal.

We also found a sensitivity to nucleophiles of the same magnitude for the Dnp group as for the 2-Adoc group (Table 1).

Despite the great stability of the Doc group to nucleophiles it can be removed by hydrazine, probably because this nucleophile is very small and can attack the urethane carbonyl despite the sterical hindrance provided by the Doc group . In our study the Doc group was found to have a half-life of 5 min in 5% hydrazine in DMF (Table 1). This means that the Doc group can rapidly be quantitatively cleaved by hydrazine, and yet is extraordinarily stable to other nucleophiles such as piperidine.

It has been shown by others[12,13,15] that urethane-type of protecting groups on the τ-nitrogen of the imidazole ring are electron-withdrawing and reduce the basicity of the π-nitrogen, thereby preventing racemization during coupling. The Doc group will therefore most likely give highly efficient protection against this side reaction.

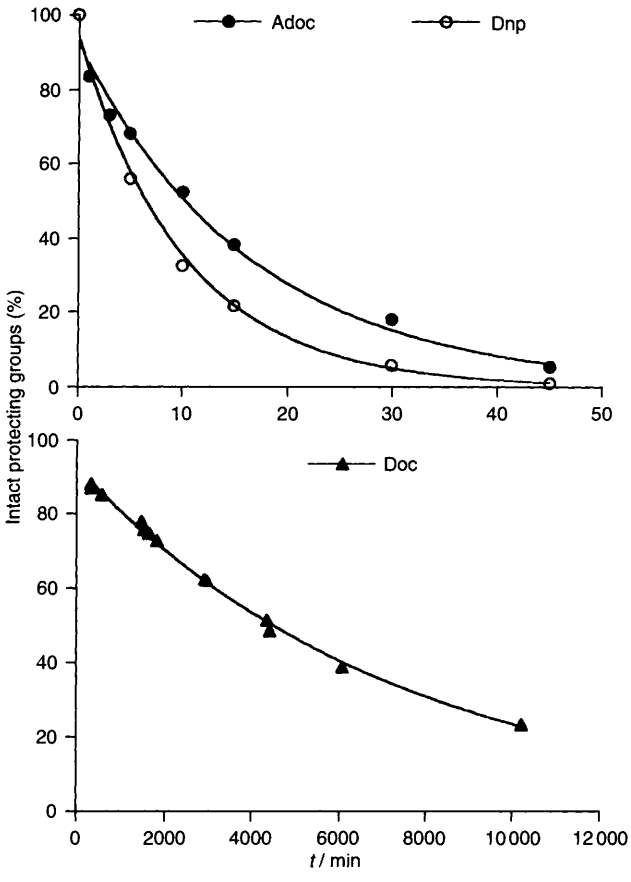

Fig. 2 The percentage of remaining imidazole-protected histidine residues in the model peptide His-Ala-Pro-Lys-Tyr-NH_2 at different time points during treatment with 20% piperidine/DMF

Table 1 Nucleophile stability of protecting groups for the imidazole side chain of histidine

Protecting group	Half-life in 20% piperidine–DMF	Half-life in 5% hydrazine–DMF
Dnp	7.3 min	n.d.[a]
2-Adoc	12 min	n.d.[a]
Doc	84 h	5.2 min

[a] n.d. = not determined.

Footnote

† *Selected data* for Boc-His(Doc)-OH: $[\alpha]_D^{20}$ +86 (*c* 1, $CHCl_3$); ^1H NMR (200 MHz; $CDCl_3$; Me_4Si) δ 8.24 (d, *J* 1.2, H-2), 7.25 (d, *J* 1.2, H-4), 5.46 (d, *J* 5.9, NH), 4.75 (t, *J* 6.1, OCH), 4.51 (m, NCH), 3.29 (m, CH_2); 2.04 (m, CH), 1.47 (Boc) and 0.97, 0.96, 0.95 and 0.94 (d, *J* 6.7, CH_3); ^{13}C NMR (50 MHz; $CDCl_3$; Me_4Si) δ 172.9 (CO_2H), 155.2 (CONH), 148.4 (NCO), 137.0 (C-2); 115.8 (C-4), 89.4 (CHO), 79.8 (Boc), 52.8 (NHCH), 29.6 (CH and CH_2); 28.4 (Boc) and 19.5, 19.5, 17.4 and 17.2 (CH_3).

References

1 J. H. Jones, W. I. Ramage and M. J. Witty, *Int. J. Peptide Protein Res.*, 1980, **15**, 301.
2 R. Colombo and F. Colombo, *J. Chem. Soc., Perkin Trans 1*, 1985, 1811.
3 T. Brown and J. H. Jones, *J. Chem. Soc., Chem. Commun.*, 1981, 648.
4 R. Colombo, F. Colombo and J. H. Jones, *J. Chem. Soc., Chem. Commun.*, 1984, 292.
5 M. A. Mitchell, T. A. Runge, W. R. Mathews, A. K. Ichhpurani, N. K. Harn, P. J. Dobrowolski and F. M. Eckenrode, *Int. J. Peptide Protein Res.*, 1990, **36**, 350.
6 F. Chillemi and R. B. Merrifield, *Biochemistry*, 1969, **8**, 4344.
7 S. Shaltiel and M. Fridkin, *Biochemistry*, 1970, **9**, 5122.
8 M. Bodanszky, *Principles of Peptide Synthesis*, Springer-Verlag, Berlin Heidelberg, 1984.
9 F. Sakiyama, K, Okawa, T. Yamakawa and S. Akabori, *Bull. Chem. Soc. Jpn.*, 1958, **31**, 926.
10 M. Fridkin and H. J. Goren, *Can. J. Chem.*, 1971, **49**, 1578.
11 A. H. Karlström and A. E. Undén, *Tetrahedron Lett.*, 1995, **36**, 3909.
12 Y. Nishiyama, N. Shintomi, Y. Kondo and Y. Okada, *J. Chem. Soc., Chem. Commun.*, 1994, 2515.
13 Y. Nishiyama, N. Shintomi, Y. Kondo, T. Izumi and Y. Okada, *J. Chem. Soc., Perkin Trans. 1*, 1995, 2309.
14 K. Barlos, D. Papaioannou and D. Theodoropoulos, *J. Org. Chem.*, 1982, **47**, 1324.
15 G. Barany and R. B. Merrifield, in *The Peptides*, ed. E. Gross and J. Meienhofer, Academic Press, New York, 1979, vol. 2, 1.

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