Protection of the indole ring of tryptophan by the nucleophile-stable, acid-cleavable N^{in}-2,4-dimethylpent-3-yloxycarbonyl (Doc) protecting group

Amelie Karlström and Anders Undén*

Department of Neurochemistry and Neurotoxicology, Stockholm University, S-106 91 Stockholm, Sweden

The N^{in}-2,4-dimethylpent-3-yloxycarbonyl (Doc) group is presented as a new protecting group for tryptophan that is stable to nucleophiles and trifluoroacetic acid, suppresses alkylation side reactions and is cleaved by strong acid along with other protecting groups used in Boc solid phase peptide synthesis.

The synthesis of peptides containing tryptophan residues is complicated by the sensitivity of the indole side chain to modifications. Two of the side reactions involving tryptophan residues that are most important in solid phase synthesis are oxidation and alkylation of the indole ring in acidic media.[1,2] In Boc/benzyl-based strategies the acidolytic cleavage of the Boc group is accompanied by significant *tert*-butylation of tryptophan. Alkylation and oxidation side reactions may be prevented by the addition of scavengers or by using indole-protected tryptophan derivatives. Thiols such as ethane-1,2-dithiol have been used as scavengers in the TFA cleavage step, but have been reported not to give full protection against *tert*-butylation.[2] The N^{in}-formyl group[3,4] is widely used as protection for tryptophan and it gives good protection against oxidation and alkylation. However, it is completely stable to HF and a separate deprotection step is required for its removal. Incomplete removal[5] and other problems associated with the removal of the formyl group under the reaction conditions normally used in Boc chemistry are known. Transfer of the formyl group to the N^{α} or N^{ε} nitrogens has been reported to occur in peptides with unprotected amino groups in aqueous ammonium hydrogen carbonate[6] or piperidine solution[5] and transformylation during solid phase synthesis can not be excluded. The formyl group can be removed by basic nucleophiles[7] (*e.g.* ethanolamine or piperidine), but this treatment may catalyse the formation of aspartimide from aspartic acid residues. In addition, the solvatisation of the peptide-resin in organic solvents at weakly basic pH is poor for many peptides and under these conditions the deprotection rate is likely to be slow. Addition of ethane-1,2-dithiol in the low HF[8] or low TFMSA[9] methods cleaves the formyl group, but a two-step procedure is required when protecting groups that are resistant to these cleavage methods are present in the peptide. In an extensive study[10,11] of the yields and purity of peptides synthesized by a large number of laboratories it was shown that a major side reaction in Boc chemistry was incomplete deprotection of formyl-Trp. Aspartimide formation was another major side reaction and, since no details were given, it can not be excluded that this side reaction to a large extent can be explained by the use of piperidine in DMF as a method to remove the formyl group of tryptophan prior to final cleavage. Clearly, since a large proportion of the side reactions in Boc chemistry can be directly or indirectly connected to tryptophan, the development of new protecting groups for this amino acid is an important issue.

An ideal protecting group for tryptophan would give good protection against oxidation and alkylation side reactions, be stable to nucleophiles and bases used during the synthesis and be cleaved by acid along with other protecting groups. In order to develop a protecting group for tryptophan that fulfills these requirements we have synthesized N^{α}-*tert*-butyloxycarbonyl-N^{in}-2,4-dimethylpent-3-yloxycarbonyl tryptophan (see Fig. 1).

The design of this N^{in}-protecting group was based on the previous successful development of new protecting groups for aspartic acid[12,13] and histidine[14] that are branched, acyclic, alkyl structures. There are a few examples of urethane-type protecting groups for tryptophan in the literature. In Fmoc-based synthesis of peptides the N^{in}-*tert*-butyloxycarbonyl group[15] has been introduced and it is reported to give good protection against side reactions during synthesis and acidolytic cleavage of peptides. In Boc chemistry the N^{in}-benzyloxycarbonyl group[16] has been reported, but this derivative is not sufficiently stable to TFA to be of practical use. However, the N^{in}-2,4-dimethylpent-3-yloxycarbonyl (Doc) group is considerably more stable to acid and due to the sterical hindrance provided by the flexible, acyclic, alkyl chains it is extremely resistant to nucleophiles.

N^{α}-*tert*-butyloxycarbonyl-N^{in}-2,4-dimethylpent-3-yloxycarbonyl tryptophan† was synthesized by reacting Boc-Trp-OBn (Bn = benzyl) with 2,4-dimethylpent-3-yl chloroformate in the presence of either 4-dimethylaminopyridine (DMAP) or potassium *tert*-butoxide, followed by cleavage of the α-benzyl ester by catalytic hydrogenation. Acylation in the presence of DMAP was very slow and the reaction was allowed to proceed for two weeks at 40 °C. On the other hand, derivatization in the presence of potassium *tert*-butoxide was very rapid, but due to the strength of the base this procedure resulted in a product with < 2% racemization, in contrast to the method using DMAP, where no racemization was detected.

The properties of the new tryptophan derivative are summarized in Table 1. It was tested by coupling Boc-Trp(Doc)-OH to the *N*-terminal of the model peptide Ala-Pro-Lys(Boc)-Tyr-

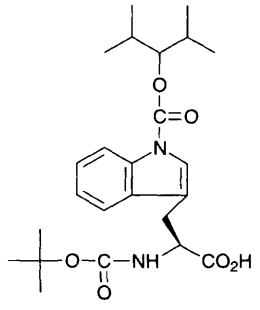

Fig. 1 Boc-Trp(Doc)-OH

Table 1 Stability of the N^{in}-2,4-dimethylpent-3-yloxycarbonyl group

Treatment	Cleavage of the N^{in}-Doc group:
20% piperidine–DMF, 3 d, room temp.	< 1%
5% hydrazine–DMF, 5 h, 50 °C	< 1%
TFMSA–thioansiole–EDT–TFA (2:2:1:20) 10 min, room temp.	100% ($t_{1/2} \leq 1$ min)
TFMSA–DMS–*m*-cresol–EDT–TFA (10:30:8:2:50), 4 h, 0 °C.	< 10%
10% *p*-cresol–HF, 1 h, 0 °C.	100%
50% TFA–CH_2Cl_2, 7 d, room temp.	*ca.* 5%

(OBu^t)-NH_2 synthesized 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). The nucleophile stability was determined by treating the resin-bound peptide with nucleophile and taking out samples at different time intervals. The successive cleavage of the Doc group was determined by analytical RP-HPLC. The results of these analyses showed that the N^{in}-Doc group is remarkably stable to nucleophiles, including hydrazine in DMF.

The N^{in}-Doc group was quantitatively cleaved by liquid HF for 1 h at 0 °C. The acid stability was further evaluated by cleaving the model peptide by standard TFMSA–thioanisole[17] and low TFMSA[9] methods. Samples were taken out at different times and the cleavage of the Doc group was determined by RP-HPLC. The N^{in}-Doc group was completely cleaved by TFMSA–thioansiole–ethane-1,2-dithiol–TFA (2:2:1:20) at room temperature for 10 min, but it was incompletely removed by TFMSA–DMS-*m*-cresol–ethane-1,2-dithiol–TFA (10:30:8:2:50) for 4 h at 0 °C. The stability to TFA was determined by treating Boc-Trp(Doc)-OH with 50% TFA–CH_2Cl_2 for one week during which time samples were taken out at different times and analysed by RP-HPLC. Extrapolation of the rate of cleavage suggests that approximately 0.01% is cleaved by a 20 min TFA–CH_2Cl_2 treatment for removal of the Boc group. In conclusion, the acid stability of the N^{in}-Doc group makes it suitable for use in Boc solid phase peptide synthesis.

The protection against *tert*-butylation was tested by coupling Boc-Trp(Doc)-OH and Boc-Trp-OH to the model peptide Ser(Bu^t)-Thr(Bu^t)-Lys(Boc)-Ser(Bu^t)-NH_2 synthesized on the TFA labile resin. The peptide and the *tert*-butyl protecting groups were cleaved by 50% TFA-DCM for 3 h. This treatment resulted in approximately 20% mono-*tert*-butylated peptide and various other by-products for the peptide synthesized with unprotected tryptophan, whereas the peptide synthesized with the N^{in}-Doc-protected tryptophan gave the expected peptide carrying Doc-protected tryptophan as a homogenous product (>98%). Thus it was concluded that the N^{in}-Doc group provides excellent protection against *tert*-butylation of tryptophan residues.

The properties of Boc-Trp(Doc)-OH makes it suitable for use in Boc solid phase peptide synthesis and it is particularly useful in that it does not require a separate deprotection step at the end of the synthesis, since the Doc group in contrast to the formyl group is cleaved by acid along with other protecting groups and the resin linkage.

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Footnote

† Boc-Trp(Doc)-OH: Mp 285 °C, $[\alpha]_D^{20}$ +31 (*c* 1, $CHCl_3$). ^1H NMR (200 MHz; $CDCl_3$; Me_4Si) δ 8.8 (b, COOH), 8.14 (d, *J* 8.3, NH), 7.61–7.14 (m, indole), 4.83 [t, *J* 6.1, C*H*(CHMe$_2$)$_2$], 4.69 (b, Hα), 3.44–2.9 (bm, Hβ), 2.18–1.92 [m, CH(C*H*Me$_2$)$_2$], 1.42 (s, Boc) and 1.02–0.96 [m, CH(CH*Me*$_2$)$_2$]; ^{13}C NMR (50 MHz; $CDCl_3$; Me_4Si) δ 175.7 (COOH), 155.6 (CONH), 151.5 (NCO), 135.6 (C-7a), 130.7 (C-3a), 124.8 (C-5), 124.1 (C-2), 122.9 (C-6), 119.1 (C-4), 115.9 (C-3), 115.4 (C-7), 86.9 [*C*H(CHMe$_2$)$_2$], 80.4 (Boc), 53.6 (Cα), 29.8 [CH(*C*HMe$_2$)$_2$], 28.2 (Boc), 19.6, 19.6, 17.6 and 17.5 [CH(CH*Me*$_2$)$_2$].

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