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The 2,4-Dimethylpent-3-yloxycarbonyl (Doc) Group As A New, Nucleophile-Resistant Protecting Group For Tyrosine In Solid Phase Peptide Synthesis

Katri Rosenthal, Amelie Karlström and Anders Undén*

Department of Neurochemistry and Neurotoxicology,
Stockholm University, S-10691 Stockholm, Sweden.

Abstract: The 2,4-dimethylpent-3-yloxycarbonyl (Doc) group is presented as a new protecting group for tyrosine. The Doc group is 1000-fold more stable towards the nucleophile piperidine than the commonly used 2-bromobenzyloxycarbonyl (2-BrZ) group is, and it is completely cleaved by hydrogen fluoride.

Several protecting groups have been suggested for protection of the hydroxyl group of tyrosine in Boc solid phase peptide synthesis[1]. Protection by substituted benzyl ethers is a now largely abandoned strategy as it results in significant migration of the benzyl group to the *ortho* position of the aromatic ring upon acidolytic cleavage[2]. The presently most commonly used protecting group for tyrosine in Boc chemistry is the 2-bromobenzyloxycarbonyl (2-BrZ) group[3]. Protection by carbamate type of protecting groups eliminates the danger of alkylation by carbocations formed upon cleavage[4], but suffers from the disadvantage of sensitivity to nucleophiles. Potent nucleophiles are generally not used in Boc chemistry, but under certain conditions the nucleophile sensitivity of a side chain protecting group can become a serious problem. Firstly, base- and/or nucleophile-cleavable protecting groups can not be employed in orthogonal protecting schemes in Boc chemistry if the standard protecting groups for aspartic acid, tryptophan, histidine or tyrosine are employed. Secondly, when nucleophile-sensitive side chain protecting groups are used, there is a danger of migration of the side chain protecting group to the free *N*-terminus during base washes and coupling. Although this reaction is in most cases not significant, it is important to note that local folding or unfavourable inter-chain aggregation can not be excluded to significantly accelerate this reaction which may therefore be difficult to predict.

In an attempt to solve these problems we therefore synthesized a new derivative of tyrosine; N^{α}-*tert*-butyloxycarbonyl-*O*-2,4-dimethylpent-3-yloxycarbonyl-L-tyrosine (Fig.1).

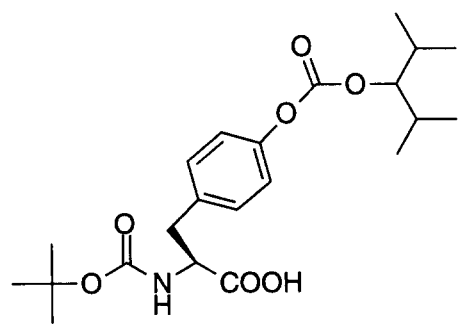
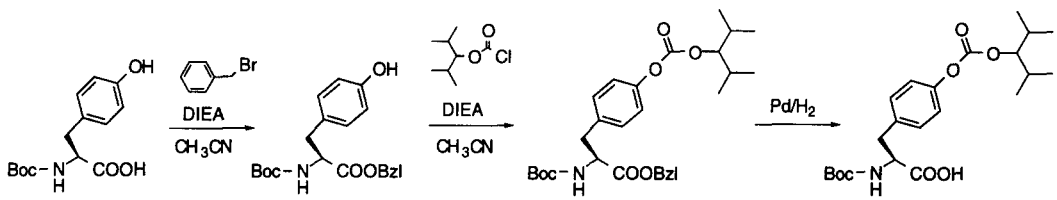


Fig.1. Boc-Tyr(Doc)-OH.

The design of the new 2,4-dimethylpent-3-yloxycarbonyl (Doc) group as protection for the hydroxyl function of tyrosine was based on the previous successful design of new protecting groups for aspartic acid[5-7] histidine[8] and tryptophan[9]. These protecting groups are all branched, acyclic, alkyl groups of the same general structure and they have been shown to be highly resistant to nucleophiles and to suppress side reactions involving bases and nucleophiles, such as base-catalyzed aspartimide formation.

N^{α}-*tert*-butyloxycarbonyl-*O*-2,4-dimethylpent-3-yloxycarbonyl-L-tyrosine[10] was synthesized according to scheme 1.



Scheme 1.

To compare the properties of the new protecting group with the 2-BrZ group the peptide Lys-*p*-nitro-Phe-Tyr(X)-Ala (where X = Doc or 2-BrZ) was synthesized on MBHA resin using 4-hydroxymethylphenoxyacetic acid as a TFA-labile linker. The linker was coupled using 1.2 eq of BOP and 1.2 eq of DIEA in DMF. The first amino acid (Fmoc-Ala-OH) was coupled using 4 eq of DCC, 3 eq of HOBt and 0.1 eq of DMAP in DMF. Deprotection of Ala was carried out with 20% piperidine in DMF for 10 min. All other amino acids were coupled using 2 eq of TBTU, 2 eq of HOBt and 4 eq of DIEA in DMF. Deprotection of the Boc group of Tyr, *p*-nitro-Phe and Lys was carried out with 50% TFA-DCM for 1+4 min.

The nucleophile stability of the Doc group and the 2-BrZ group was studied by treating the peptide resin with 20% piperidine in DMF at room temperature. Samples were taken out at different times and cleaved with 95% TFA-water for 30 min. Analysis of the cleaved peptides was performed chromatographically by reversed phase HPLC. The relative amounts of the compounds were monitored at 270 nm (absorbance maximum for *p*-nitrophenylalanine). Plasma desorption mass spectrometry analyses of the products confirmed that the molecular weights corresponded to peptides with protected or unprotected tyrosine. The half-lives of the Doc group and the 2-BrZ group in 20% piperidine-DMF were determined as 8 h and 30 sec, respectively (Table 1).

Table 1. Cleavage of Protecting Groups for the Side Chain of Tyrosine by 20% Piperidine-DMF.

Protecting group	$t_{1/2}$
Doc	8 h
2-BrZ	30 sec

It can be concluded from Table 1 that only 2 % of the Doc group will be cleaved by a 10 minute treatment with piperidine, which is the standard procedure for cleavage of the N^{α}-Fmoc group.

The rate of removal of the Doc group and the 2-BrZ group from the side chain of tyrosine by the *tert*-butyl esters of proline and glycine was also studied and the results are shown in Table 2.

Table 2. Cleavage of Protecting Groups for the Side Chain of Tyrosine by Amino Acids.

Amino acid (0.5 M in 0.5% DIEA-DMF*)	Time (h)	Loss of protection (%)	
		Doc	2-BrZ
Proline *tert*-butyl ester	4	< 0.1	42.7
Glycine *tert*-butyl ester	4	< 0.1	13.7

*0.5 % DIEA-DMF alone had no detectable effect on either 2-BrZ or Doc protection.

Although the rate of cleavage of the 2-BrZ group is low compared to the normal rate of acylation of the *N*-terminus during coupling, it is important to bear in mind that the times for complete acylation in difficult sequences can be in the range of hours.

The acid stability of the Doc group and the 2-BrZ group was studied by treating the peptides with 50% TFA-DCM followed by analysis as described above (see Table 3).

Table 3. Cleavage of Protecting Groups for the Side Chain of Tyrosine by 50% TFA-DCM.

Time (days)	Loss of protection (%)	
	Doc	2-BrZ
0.33	1.2	not detected
2	5.3	1.5
3	9.4	2.2
7	20.4	4.2
10	27.5	7.3

As can be seen in Table 3 the Doc group is more acid-labile than the 2-BrZ group. The loss of protection is 0.04% for the Doc group and 0.01% for the 2-BrZ group per 20 min TFA-DCM cycle. The acid stability of the Doc group is lower compared to most other side chain protecting groups used in Boc chemistry, but it is sufficient for most applications. For example, it can be calculated that if the Doc group is used during the synthesis of the peptide hormone Neuropetide Y, a 36 amino acid residue peptide with 5 tyrosine residues in positions 1, 20, 21, 27 and 36, only 4 % of the protecting groups are lost.

Although the 2-BrZ group is superior in terms of acid stability the relative merits of the 2-BrZ group as compared to the Doc group for protection of tyrosine in Boc chemistry can only be evaluated when more is known about two additional factors; the rate of migration of nucleophile-sensitive protecting groups to the *N*-terminus during slow couplings and the level of alkylation of the peptide by 2,4-diisoprop-3-yl cations compared to benzyl cations during the acidolytic cleavage of the protecting groups. These aspects are currently under investigation.

Acknowledgement

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10. Boc-Tyr(Doc)-OH: $[\alpha]_D^{20}$ +37° (c=1, $CHCl_3$). Crystallization from ethanol/water.
NMR spectra showed that Boc-Tyr(Doc)-OH exists in two isomeric forms with the Boc group *cis* (Z) or *trans* (E) with respect to the side chain of tyrosine. The ratio E/Z at room temperature in $CDCl_3$ was 1.75.
^1H NMR ($CDCl_3$/TMS_{int}) E-isomer: δ=7.20 (d, *J* 8.3, 2H, Tyr ring 2), 7.12 (d, *J* 8.3, 2H, Tyr ring 3), 5.02 (d, *J* 8.2, 1H, NH), 4.62 (m, 1H, α-H), 4.47 (t, *J* 2x6.4, 1H, C<u>H</u>($CH(CH_3)_2)_2$), 3.20 (m, *J* 5.6 and 14.0, 1H, β-H), 3.08 (m, *J* 6.4 and 14.0, 1H, β-H), 2.00 (m, *J* 7x7.0, 2H, CH(C<u>H</u>$(CH_3)_2)_2$), 1.43 (s, 9H, $C(CH_3)_3$), 0.97 (d, *J* 7.0, 12H, $CH(CH(C\underline{H}_3)_2)_2$).
Z-isomer: δ=7.24 (d, *J* 8.3, 2H, Tyr ring 2), 7.12 (d, *J* 8.3, 2H, Tyr ring 3), 6.69 (d, *J* 7.4, 1H, NH), 4.47 (t, *J* 2x6.4, 1H, C<u>H</u>$(CH(CH_3)_2)_2$), 4.39 (m, 1H, α-H), 3.19 (under the multiplet of the E-isomer, 1H, β-H), 2.91 (m, *J* 8.9 and 13.5, 1H, β-H), 2.00 (m, *J* 7x7.0, 2H, CH(C<u>H</u>$(CH_3)_2)_2$), 1.29 (s, 9H, $C(CH_3)_3$), 0.97 (d, *J* 7.0, 12H, $CH(CH(C\underline{H}_3)_2)_2$).
^{13}C NMR ($CDCl_3$/TMS_{int}) E-isomer: δ=175.79 (1C, COOH), 155.37 (1C, NH<u>C</u>OO), 154.34 (1C, Tyr ring 4), 150.33 (1C, O<u>C</u>OCH), 133.51 (1C, Tyr ring 1), 130.35 (2C, Tyr ring 2), 121.11 (2C, Tyr ring 3), 88.84 (1C, <u>C</u>H$(CH(CH_3)_2)_2$), 80.35 (1C, <u>C</u>$(CH_3)_3$), 54.12 (1C, α-C), 37.04 (1C, β-C), 29.46 (2C, CH(<u>C</u>H$(CH_3)_2)_2$), 28.24 (3C, C(<u>C</u>$H_3)_3$), 19.33 (2C, CH(CH(<u>C</u>$H_3)_2)_2$), 17.03 (2C, CH(CH(<u>C</u>$H_3)_2)_2$).
Z-isomer: δ=175.42(1C, COOH), 156.44 (1C, NH<u>C</u>OO), 154.29 (1C, Tyr ring 4), 150.33 (1C, O<u>C</u>OCH), 134.24 (1C, Tyr ring 1), 130.45 (2C, Tyr ring 2), 121.11 (2C, Tyr ring 3), 88.72 (1C, <u>C</u>H$(CH(CH_3)_2)_2$), 81.67 (1C, <u>C</u>$(CH_3)_3$), 55.93 (1C, α-C), 38.79 (1C, β-C), 29.46 (2C, CH(<u>C</u>H$(CH_3)_2)_2$), 27.95 (3C, C(<u>C</u>$H_3)_3$), 19.33 (2C, CH(CH(<u>C</u>$H_3)_2)_2$), 17.03 (2C, CH(CH(<u>C</u>$H_3)_2)_2$).
^1H NMR and proton decoupled ^{13}C NMR spectra were recorded on a Bruker AMX 500 spectrometer at 500 and 126 Hz, respectively.

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