Application of the 2-Adamantyloxycarbonyl (2-Adoc) Group to the Protection of the Imidazole Function of Histidine in Peptide Synthesis

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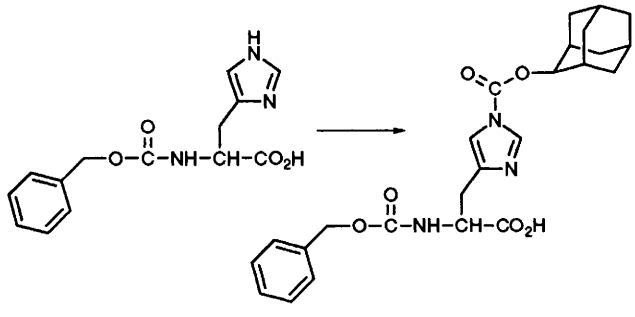
The N^{im}-2-adamantyloxycarbonyl (2-Adoc) group is suitable for the protection of the imidazole function of the histidine residue in peptide synthesis in terms of its stability to trifluoroacetic acid (TFA), tertiary amines and 1-hydroxybenzotriazole (HOBt), and its reduction of racemization rate during the coupling reaction.

Although a number of imidazole-protecting groups have been developed, the side-chain protection of histidine (His) has still been one of the most serious problems in peptide synthesis.[1] Toluene-*p*-sulfonyl (Tos)[2] is probably the most popular protecting group in *tert*-butyloxycarbonyl (Boc)-dependent peptide synthesis, but it has some disadvantages, such as susceptibility to 1-hydroxybenzotriazole (HOBt), an efficient coupling additive, and partial decomposition during removal of the N^{α}-Boc group by trifluoroacetic acid (TFA).[1,3,4] N^{im}-Dinitrophenyl(Dnp)[5] is also frequently used in Boc-chemistry, especially in solid-phase synthesis, but its main drawback is that N^{im}-Dnp is stable to anhydrous HF and trifluoromethanesulfonic acid (TFMSA), and it must be removed separately prior to final deprotection and cleavage from the resin. Jones and coworkers[6] reported that use of an N^{im} (π)-locating protecting group could completely prevent the side-chain induced racemization[7] during the coupling of His, and they recommended the use of π-benzyloxymethyl (Bom)[8] in Boc-chemistry and π-*tert*-butyloxymethyl (Bum)[9] in 9-fluorenylmethoxycarbonyl (Fmoc)-chemistry. Unfortunately, the syntheses of Boc–His(Bom)–OH and Fmoc–His(Bum)–OH are relatively complex, and these derivatives are expensive. In addition, new side reactions caused by formaldehyde from the Bom group during HF treatment were reported.[10,11] From the viewpoint of the prevention of side-chain induced racemization, some of τ-locating substituents, including Tos and Dnp, are actually effective in reducing racemization due to their electron withdrawing effect.[1,12] Therefore, the π-nitrogen of the imidazole ring does not have to be protected in many cases in the synthesis of His-containing peptides.



Scheme 1 *Reagents*: 2-Adoc-Cl, aq. NaOH–dioxane (62.2% yield)

Previously, we reported the new ε-amino protecting group, 2-adamantyloxycarbonyl (2-Adoc),[13,14] which was stable to TFA, and cleavable by HF or 1 mol dm^{-3} TFMSA–thioanisole–TFA. We report herein the introduction of the 2-Adoc group to imidazole nitrogen, and the evaluation of the N^{im}-2-Adoc group for peptide synthesis.

First, Z-His(2-Adoc)–OH† (Z, benzyloxycarbonyl) was prepared from Z-His–OH[15] and 2-adamantylchloroformate (2-Adoc–Cl) under the conditions of the Schotten–Baumann reaction as shown in Scheme 1. It is a valid assumption that the main product in the reaction of histidine side-chain with an equimolar amount of acylating reagent will be that with attachment at the less hindered nitrogen, *i.e.* N^{τ}, therefore, it is predicted that the orientation of N^{im}-2-Adoc group is τ.[16]‡ The stability and susceptibility of the N^{im}-2-Adoc group to various acidic and basic conditions were examined by the measurement of regenerated Z-His or His by HPLC or amino acid analysis, respectively. The results are summarized in Table 1 in comparison with the N^{im}-1-adamantyloxycarbonyl (1-Adoc)[18] group, the properties of which resemble those of the N^{im}-Boc group.[19] The N^{im}-2-Adoc group was stable to 7.6 mol dm^{-3} HCl–dioxane and TFA at room temp. for up to 24 h, while N^{im}-1-Adoc group was susceptible under the same conditions. N^{im}-2-Adoc group was rapidly cleaved by anhydrous HF or 1 mol dm^{-3} TFMSA–thioanisole–TFA at 0 °C. N^{im}-2-Adoc and N^{im}-1-Adoc groups were stable to 10% diisopropylethylamine (DIEA)–dimethylformamide (DMF) and 10% Et_3N–DMF at room temp. for up to 24 h, but not stable to 20% piperidine–DMF, the deprotecting reagent for the N^{α}-Fmoc group. The N^{im}-2-Adoc group was fully stable in 20% HOBt–DMF at room temp. for up to 24 h. These results indicate that the N^{im}-2-Adoc group is suitable for Boc-dependent peptide synthesis and can be employed in combination with HOBt-mediated coupling methods.

Next, the efficiency of N^{im}-2-Adoc group on the prevention of side-chain induced racemization was examined. Z-D-His(2-Adoc)-L-Phe–OMe was well separated from Z–L–His(2-Adoc)–L–Phe–OMe by HPLC,§ therefore, this sequence was employed for model study on racemization. Z–L–His(2-Adoc)–OH was coupled with H–L–Phe–OMe by DCC, DCC–HOBt, benzotriazolyl-*N*-oxytris(dimethylamino) phosphonium hexafluorophosphate (Bop),[20] *O*-(benzotriazol-1-

Table 1 Stability and susceptibility of the N^{im}-2-Adoc group

	% Cleavage[a]									
	2-Adoc					1-Adoc				
Conditions	10 min	30 min	60 min	120 min	24 h	10 min	30 min	60 min	120 min	24 h
7.6 mol dm^{-3} HCl–dioxane	0	0	0	0	0	92	100	100	100	100
TFA	0	0	0	0	0	95	100	100	100	100
10% Et_3N–H_2O	64	92	94	95	100	58	89	95	95	100
20% piperidine–DMF	45	87	94	100	100	7	11	17	33	89

[a] For 2-Adoc 100% cleavage was observed within 10 min for 25% HBr–AcOH, 1 mol dm^{-3} TFMSA–thioanisole–TFA and HF, and for 1- and 2-Adoc with 2 mol dm^{-3} NaOH aq. No cleavage was observed with 1- and 2-Adoc after 24 h with 10% $NaHCO_3$, 10% DIEA–DMF, 10% NEt_3–DMF or 20% HOBt–DMF.

Table 2 Extent of racemization during the coupling of Z-His(2-Adoc)–OH and H–Phe–OMe. % D,L is given as D,L/(L,L + D,L)

Coupling method	% D,L
DCC	1.5
DCC–HOBt	0.6
Bop	0.6
HBTU	1.2
DPPA	0.4

yl)-1,1,3,3-tetramethyl uronium hexafluorophosphate (HBTU)[21] or diphenylphosphoryl azide (DPPA),[22] and then the crude product was analysed by HPLC. The results summarized in Table 2 show that the formation of D,L peptide was particularly low on all coupling methods so far examined. The racemization rate on DCC–HOBt coupling was lower than that on DCC alone. The racemization reducing effect of HOBt was previously demonstrated using Boc–His(Boc)–OH (1.5–0.2%).[11] Aryl sulfonate-type protecting groups, including Tos, cannot be employed in combination with HOBt-mediated coupling due to their susceptibility to HOBt.

The results obtained here show that the 2-Adoc group is suitable for imidazole-protection in Boc-dependent peptide synthesis; it is stable to Boc-deprotecting conditions, and easily and rapidly removable by anhydrous HF or 1 mol dm^{-3} TFMSA–thioanisole–TFA. In addition, the N^{im}-2-Adoc group can effectively suppress the racemization of the His residue, presumably due to an electron withdrawing effect. Considering the racemization reducing effect of N^{im}-trityl group,[23] the steric hindrance of the adamantane moiety might contribute to the prevention of racemization. These results are promising for the successful application of His(2-Adoc) derivatives in the synthesis of His-containing peptides.

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Footnotes

† Z-His(2-Adoc)–OH·CHA (cyclohexylamine): mp 150–153 °C, satisfactory elemental analyses (C, H, N). Z-His(2-Adoc)–OH: amorphous powder, $[\alpha]_D$ +7.68 (*c* 1.0, CH_2Cl_2), t_R = 22.1 min [μBondasphere C_{18} (3.9 × 150 mm); flow rate, 1.0 ml min^{-1}; 0.05% aqueous TFA–0.05% TFA in MeCN 80 : 20 for 5 min, to 20 : 80 in 25 min, and 20 : 80 for 10 min], satisfactory elemental analyses (C, H, N).

‡ According to ref. 17, Z-His(2-Adoc)–OH was treated with a large excess of methyl iodide at ambient temperature for 24 h. Amino acid analysis of hydrolysate (6 mol dm^{-3} HCl, 110 °C, 24 h) of the reaction product gave histidine (*ca.* 90%) and π-methylhistidine. High recovery of unmethylated His was presumably due to the electron withdrawing effect of the 2-Adoc group. No τ-methyl isoform was detected, therefore, it was deduced that N^{im}-2-Adoc is located at the τ-nitrogen.

§ Z-L-His(2-Adoc)–L-Phe–OMe: amorphous powder, R_f ($CHCl_3$–MeOH–AcOH 90 : 8 : 2) 0.56, t_R = 25.1 min [μBondasphere C_{18} (3.9 × 150 mm); flow rate, 1.0 ml min^{-1}; 0.05% aqueous TFA–0.05% TFA in MeCN, 80 : 20 for 5 min, to 20 : 80 in 15 min, and 20 : 80 for 10 min], $[\alpha]_D$ +38.1 (*c* 1.0, CH_2Cl_2), satisfactory elemental analyses (C, H, N). Z-D-His(2-Adoc)–L-Phe–OMe: amorphous powder, R_f ($CHCl_3$–MeOH–AcOH 90 : 8 : 2) 0.56, t_R = 28.5 min (column and solvent system were same as those for L,L peptide), $[\alpha]_D$ −6.15 (*c* 1.0, CH_2Cl_2).

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