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N^{in}-Cyclohexyloxycarbonyl Group as a New Protecting Group for Tryptophan

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Abstract: Several new protecting groups were introduced at the N^{in}-position of tryptophan, and their reactivities were examined under the conditions used for peptide synthesis by Boc-strategy. Among them, the cyclohexyloxycarbonyl (Hoc) group was found to be the most suitable in terms of stability during elongation of the peptide chain and removability at the final HF reaction without resorting to the use of thiols.

In the synthesis of tryptophan (Trp)-containing peptides by the Boc strategy, the introduction of appropriate protection of the indole moiety of the Trp residue is indispensable for avoiding indole-alkylation and/or dimerization during trifluoroacetic acid (TFA), trifluoromethanesulfonic acid (TFMSA) and/or HF treatment.[1] The N^{in}-formyl (For) group has been employed for this purpose as it is stable during repetitive TFA treatment but removable by alkaline or HF in the presence of thiols, such as 1,2-ethanedithiol.[2] However, the presence of thiols in the HF reaction, which is necessary for deprotection of the N^{in}-For group, often causes handling problems such as unpleasant odors and polymerization of the thiol during the HF reaction.[3] To overcome these problems, there is a need for an N^{in}-protecting group that can be readily removed by HF without the use of thiols. Possible candidates are the mesitylenesulfonyl (Mts) group and the benzyloxycarbonyl (Z) group.[4] However, the Mts group can not be smoothly cleaved by the standard HF procedure and the Z group is slowly cleaved by TFA or base treatment during the synthesis of the protected peptides. In order to find an N^{in}-protecting group that is more stable against acids and bases than the Z group but more easily cleavable than the Mts group, we introduced cyclopentyloxycarbonyl, cyclohexyloxycarbonyl (Hoc) and cycloheptyloxycarbonyl groups into the indole moiety of the Trp residue. We expected that cleavage of a urethane-type N^{in}-protecting group would generate an N^{in}-carboxylate intermediate to reduce the

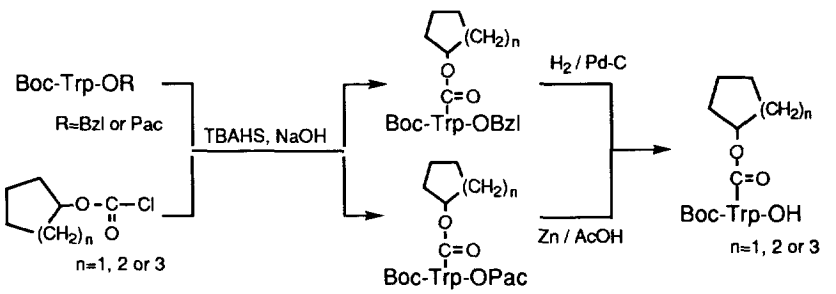


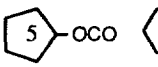
Fig. 1. Preparation of Trp derivatives.

susceptibility of the indole moiety to electrophilic attack.

Each N^{in}-protecting group was introduced by acylation of Boc-Trp-OPac (Pac: phenacyl) or Boc-Trp-OBzl using the chloroformate of the respective cycloalkylalcohols in dichloromethane (DCM) in the presence of pulverized NaOH and a catalytic amount of tetra-n-butyl ammonium hydrogensulfate (TBAHS) at room temperature for 1 h as reported previously.[5] Removal of the Pac or Bzl ester by zinc dust in AcOH or catalytic hydrogenation gave the corresponding Trp derivatives (Fig. 1).[6] The optical purity of these derivatives was confirmed to be greater than 99.5% by means of Marfey's reagent.

The stabilities of these protecting groups to various acids and bases used during the synthesis of the protected peptides by the Boc strategy were examined by measuring the amount of the regenerated Trp or Boc-Trp residue on RP-HPLC. The results are summarized in Table 1, in comparison with those of the N^{in}-Z and For groups.

Table 1. Stability of Boc-Trp(X) in acids and bases.

	Recovery of Trp[a] or Boc-Trp[b] (%)				
X=	 Hoc			Z	For
TFA	0	0	0	3.6	0
5.4 N-HCl/dioxane	0	0	1.8	3.2	0
10% DIEA/DMF	0	0	0	12	28
10% Piperidine/DMF	1.8	0	0	100	100

a) Determined by HPLC after treatment of Boc-Trp(X) under acidic (500 equiv.) conditions (rt, 18 h).
b) Determined by HPLC after treatment of Boc-Trp(X) under basic (100 equiv.) conditions (rt, 18 h).

A significant relationship was observed between the ring size of the cycloalkyloxycarbonyl groups and the stability against acids and bases, i. e., increasing the ring size decreased the stability against acids but increased that against bases. Among them, the Hoc group was found to be the most stable under both acidic and basic conditions. In the case of the Z and For groups, both were partially cleaved by 10% DIEA in DMF or DCM and completely cleaved by 10% piperidine in DMF. These reagents are usually used to neutralize or remove the Fmoc group in solid phase peptide synthesis. On the other hand, the Hoc group remained attached to the indole moiety even after several days. These results have demonstrated that the Hoc group for the Trp residue can offer permanent N^{in}-protection throughout the synthesis of the protected peptides.

Next, the removability of the Hoc group from the indole moiety of the Trp residue under the usual HF conditions was examined and compared with those of the For and Mts groups by RP-HPLC. As shown in Table 2, the Hoc group was quantitatively removable by HF treatment without use of thiols. For deprotection of the N^{in}-For group, addition of thiol such as 1,4-butanedithiol (BDT) to HF was indispensable, whereas

Table 2. Deprotection of N^{in}-protecting groups by HF treatment.

	Extent of deprotection (%)		
Condition[a]	Trp(Hoc)	Trp(For)	Trp(Mts)
HF/*p*-cresol (85:15)	100	0	>60
HF/BDT/*p*-cresol (80:15:5)	100	100	>95

a) The reactions were carried out at -5°C for 1 h.

removal of the N^{in}-Mts group was incomplete both in the presence and absence of thiols.[7] From these results, the N^{in}-Hoc group was confirmed to possess all of the chemical properties required for the Boc strategy.

The Trp residue has been known to be susceptible to dimerization during the HF reaction in the absence of thiols, particularly when it is located in the middle of the tripeptide. Therefore, the effect of the Hoc group on this modification was assessed by measuring the amount of Trp-dimer formed during the HF reaction. The model peptide, Z-Ala-Trp(Hoc)-Leu-OBzl, was treated with HF under various conditions. HPLC was used to measure the amount of dimer, in which the 2-position of one indole ring was bound with the 2'-position of the other indole ring to produce two steric isomers. The results were compared with those for a model peptide having no N^{in}-protecting group. As shown in Table 3, the data suggest that treatment of the Trp(Hoc)-containing peptides with HF results in the formation of an N^{in}-carboxylate intermediate that might help to suppress the Trp dimerization. The suppressive effect with the N^{in}-Hoc group was almost the same as that obtained by treating Z-Ala-Trp-Leu-OBzl with HF in the presence of thiol.

Table 3. Formation of Ala-Trp-Leu dimers by HF treatment.

	Formation of Trp-dimers (%)	
	N^{in}-protecting group	
Condition[a]	Hoc	None
HF	5.5	70
HF/*p*-cresol (85:15)	4.1	69
HF/*p*-cresol (85:15) at 20°C	22	74
HF/*p*-cresol/H_2O (85:15:5)	35	80
HF/BDT/*p*-cresol (85:15:5)	2.1	2.6

a) The reactions were carried out at -5°C for 1 h.

The Trp alkylation that occurred during the HF treatment is negligible but a significant amount occurs during alternative strong-acid deprotecting procedures such as that using the TFMSA or trimethylsilyl trifluoromethanesulfonate (TMSOTf)-thioanisole/TFA system, although this side reaction can be efficiently suppressed by increasing the amounts of appropriate scavengers or by the addition of indole. In particular, modification of the Trp residue by the *p*-methoxybenzyl group from the adjacent Cys residue during TMSOTf treatment was reported to be unavoidable even under the conditions described above.[8] To demonstrate the suppressive effect arising from the N^{in}-Hoc group on this modification, we synthesized [$Tyr^{5,12}$, Lys^{7}]-polyphemsin II (T22) by solid phase procedure using Boc-Trp(Hoc) or Boc-Trp(Mts), and compared the amount of [Trp(2'-MBzl)[3]]-T22 in each product after TMSOTf-thioanisole/TFA treatment of the corresponding protected peptide resin on HPLC.[9, 10]

Table 4. Formation of [Trp(2'-MBzl)[3]]-T22 by strong-acid treatment.

	T22/[Trp(2'-MBzl)[3]]-T22 ratios	
	N^{in}-protecting group	
Condition	Hoc	Mts
1 M TMSOTf-thioanisole/TFA[a]	85 : 15	60 : 40
HF/*p*-cresol (85:15)[b]	100 : 0	-[c]
HF/BDT/*p*-cresol (85:15:5)[b]	100 : 0	100 : 0

a) Protected peptide resin was treated with 1 M TMSOTf-thioanisole in TFA-*m*-cresol-EDT in ice-bath for 3 h. b) The reactions were carried out at -5℃ for 1 h. c) The Mts group was incompletely removed under this condition.

The results shown in Table 4 demonstrated that the formation of [Trp(2'-MBzl)^3]-T22 occurs only during TMSOTf treatment and not during HF treatment. Furthermore, this modification could be significantly suppressed by using the Hoc group in the case of TMSOTf treatment. These results suggested that the N^{in}-carboxylate intermediate can function as a temporary protecting group to prevent both Trp dimerization during HF treatment and Trp alkylation during TFMSA or TMSOTf-thioanisole/TFA treatment.

In conclusion, we could demonstrate that the N^{in}-Hoc group of the Trp residue is useful for synthesizing Trp-containing peptides using HF as a final deprotection procedure in the absence of thiol without dimerization of the Trp residue.

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6. Characterization of Boc-Trp(X):

N^{in}-Protecting group (X)	mp (℃)	$[\alpha]_D^{25}$(°) (c=1.0, DMF)	Formula	Calcd C	Calcd H	Calcd N	Found (%) C	Found (%) H	Found (%) N
cyclopentyloxycarbonyl	152-156	- 70.3	$C_{22}H_{28}N_2O_6$	63.45	6.78	6.73	63.25	6.92	6.70
cyclohexyloxycarbonyl (Hoc)	119-121	- 73.8	$C_{23}H_{30}N_2O_6$	64.17	7.02	6.51	64.02	7.19	6.66
cycloheptyloxycarbonyl	90-94	- 72.2	$C_{24}H_{32}N_2O_6$	64.85	7.26	6.30	64.33	7.29	6.25

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9. T22 ([Tyr^{5,12}, Lys^7]-polyphemusin II) is an 18-residue peptide amide having a sequence of Arg-Arg-Trp-Cys-Tyr-Arg-Lys-Cys-Tyr-Lys-Gly-Tyr-Cys-Tyr-Arg-Lys-Cys-Arg-NH_2. The peptide was synthesized with an automatic synthesizer (ABI 433A) using the Boc strategy on MBHA resin. The following side-chain-protected amino acids, which are cleavable by treatment with 1 M TMSOTf-thioanisole/TFA, were employed: Cys(MBzl), Lys(Z), Arg(Mts), Tyr(BrZ) and Trp(Mts) or Trp(Hoc).
10. The molecular weight of [Trp(2'-MBzl)^3]-T22 was 120 higher than that of T-22 by PD-MS. This mass difference is attributed to the MBzl group.

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