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4-Methoxybenzyloxymethyl group as an N^{π}-protecting group for histidine to eliminate side-chain-induced racemization in the Fmoc strategy

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ABSTRACT

The 4-methoxybenzyloxymethyl (MBom) group was introduced at the N^{π}-position of histidine, and its utility was examined under the conditions for peptide synthesis by Fmoc strategy. The N^{π}-MBom group proved to prevent the risk of racemization during incorporation of the His residue and to possess all of the chemical properties required for Fmoc chemistry. The side reaction associated with formaldehyde generated from the N^{π}-MBom group upon acidolysis could be effectively prevented by performing the standard TFA treatment in the presence of methoxyamine·hydrochloride ($MeONH_2$·HCl).

In peptide synthesis, side reactions sometimes occur when there is no appropriate protection of the imidazole ring of histidine (His), because the nucleophilic imidazole ring easily undergoes N-acylation. This may give rise to undesired acyl transfer reactions. In particular, His derivatives are known to be prone to racemization on activating and coupling processes involving the π-nitrogen of the imidazole ring.[1] Therefore, the regioselective protection of the π-nitrogen should enable avoidance of racemization. For this purpose, the N^{π}-benzyloxymethyl (Bom) group has been widely employed in Boc chemistry as it can effectively suppress the risk of racemization and can be readily removed by HF or TfOH.[2] Another group, the N^{τ}-Trt group, is often used in combination with N^{α}-Fmoc protection.[3] Although the steric hindrance of the Trt group may help reduce racemization to a certain extent, the N^{τ}-protecting group cannot inherently prevent racemization. To serve as the N^{π}-protecting group on His compatible with Fmoc chemistry, N^{π}-*t*-butoxymethylhistidine[4] [His(Bum)] and N^{π}-1-adamantyloxymethylhistidine[5] [His(1-Adom)] have been developed. These are ideal His derivatives for Fmoc chemistry in terms of offering N^{π}-protection and TFA lability. However, the preparation of these protected derivatives is not straightforward and also requires column chromatography on silica gel to separate the N^{τ}-isomer from the desired one. This would not only hamper the purification procedure but also large-scale production, preventing such derivatives from gaining widespread application in peptide synthesis. In order to find an N^{π}-protected His derivative applicable for Fmoc chemistry that can be easily purified by crystallization, we introduced the 4-methoxybenzyloxymethyl (MBom) group into the N^{π}-nitrogen of the imidazole ring (Fig. 1).

The synthesis of Fmoc-His(π-MBom) (**1**) is illustrated in Scheme 1.[6] The reported procedure for the His(π-Bom) and His(π-Bum) derivatives was modified for the preparation of the His(π-MBom) ones, in which the N^{α}-amino function of the intermediates was protected by the Boc group while that of the former by the Z group. This modification facilitated purification by crystallization to separate the undesired τ-isomer from the product, thus making the present synthetic procedure available for large-scale production without any obstacles. Thus, regioselective alkylation using 4-methoxybenzyloxymethyl chloride (MBom-Cl) onto Boc-His (τ-Ac)-OMe (**3**) provided Boc-His(π-MBom)-OMe, which was subsequently saponified by NaOH aq to give Boc-His(π-MBom)

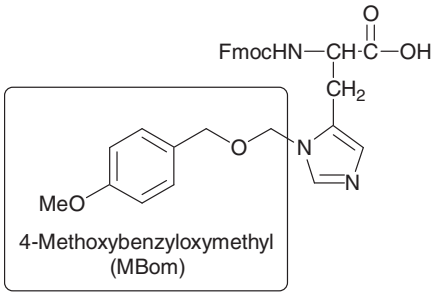

Figure 1. Structure of Fmoc-His(π-MBom) (**1**).

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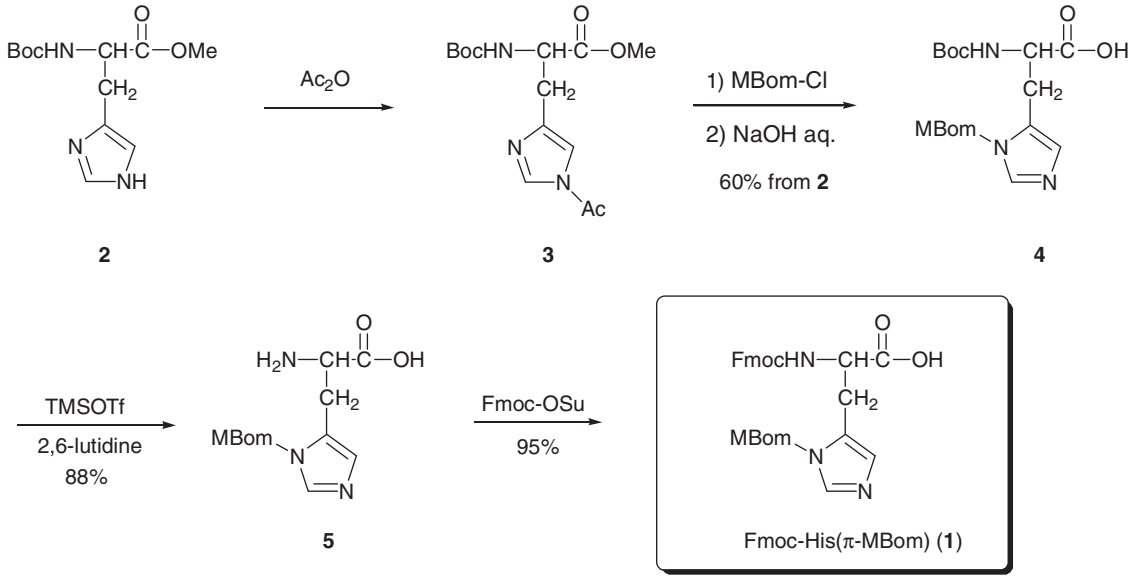

Scheme 1. Synthesis of Fmoc-His(π-MBom) (**1**).

(**4**). Recrystallization of **4** could be effectively performed to remove the contaminant, that is, the τ-isomer, without resorting to column chromatography. To convert **4** into Fmoc-His(π-MBom) (**1**), the N^{α}-Boc group on **4** was selectively cleaved by treatment with TMSOTf in the presence of 2,6-lutidine[7] with no loss of the N^{π}-MBom group and the resulting α-amino function was protected by the Fmoc group. The optical purity of **1** synthesized in this manner was >99.9% as determined by Marfey's method after removing the protected groups.[8,9]

The stability and removability of the MBom group under the standard conditions used for Fmoc-SPPS were examined by measuring the amount of recovered His(π-MBom) on RP-HPLC. The results are summarized in Table 1 in comparison with those of the τ-Trt group. The MBom group was found to be completely stable during the repetitive Fmoc deprotection reaction using 20% piperidine/DMF but readily removable by TFA in the same manner as the Trt group. On the other hand, the MBom group remained attached to the imidazole ring during the detachment of the protected peptides with a free carboxyl group from the Trt(2-Cl) resin by treatment with DCM/TFE/AcOH (v/v, 3/1/1),[10] while the Trt group is known to be susceptible to this condition. The partial loss of the Trt group on the His residue of the protected peptide segments may not only hamper their purification procedure but also be accompanied by side reactions involving the nucleophilicity of the imidazole ring. These results demonstrated that the MBom group for the His residue can offer permanent N^{im}-protection throughout the synthesis of the protected peptides. Next, the suppressive effect of N^{π}-MBom on racemization during the incorporation of His was evaluated by synthesizing a model peptide, Z-Ala-His-Pro.[11] The peptide chain was elongated onto a Pro-Trt(2-Cl) resin by the 1-min preactivation procedure of coupling with Fmoc (or Z)-amino acid/HBTU/DIEA (each of 1.5 equiv) in DMF. As shown in Table 2, Fmoc-His(π-MBom) (**1**) was found to be accompanied by virtually no racemization on the activating and coupling steps, while the regioisomeric derivative, that is, Fmoc-His(τ-MBom),[12] led to considerable racemization (16%). These results proved that the N^{π}-MBom group possessed all of the chemical properties required for the Fmoc strategy.

Table 1
Recovery of His(X) under various conditions

Conditions		Recovery of His(X) (%)[a]	
	X=	π-MBom	τ-Trt
20% Piperidine/DMF	rt, 2 h	100	100
TFA/H_2O (v/v, 95/5)	rt, 1 h	0	0
DCM/TFE/AcOH (v/v, 3/1/1)	rt, 8 h	100	64

[a] Determined by HPLC (220 nm).

Table 2
Racemization during synthesis of the model peptide, Z-Ala-His-Pro, as a function of the His protecting group

Imidazole protection	Racemization[a] (%)
τ-Trt	3.2
π-MBom	<0.2[b]

[a] Defined as (Z-Ala-D-His-Pro)/(Z-Ala-His-Pro + Z-Ala-D-His-Pro) × 100.
[b] Below detection limit.

When the His(π-Bom)- or His(π-Bum)-containing peptide is treated with HF or TFA, respectively, formaldehyde is generated from the respective N^{im}-protecting groups. This will lead to hydroxymethylated modification of α- and ε-amino groups although the extent is inconsequential. On the other hand, formaldehyde reacts almost quantitatively with a Cys residue located at the *N*-terminus to produce a thiazolidyl (Thz)-peptide during isolation from a HF or TFA cleavage mixture.[13] To circumvent this conversion, methoxyamine hydrochloride ($MeONH_2$·HCl) has been recommended as a formaldehyde scavenger in the acidolytic cleavage reaction.[11,13] Here, we applied Fmoc-His(π-MBom) to the synthesis of cystenyl-angiotensin II (Cys-Ang II: CDRVYIHPF)[14] to examine the by-product formation associated with the use of this protecting group. As was expected, Thz formation arising from the generation of formaldehyde during TFA treatment was almost completely suppressed by the addition of $MeONH_2$·HCl (5 equiv) to the reaction mixture (Table 3). Besides formaldehyde, upon acidolysis, the MBom group also generates an electrophilic alkylating species, methoxybenzyl cation, that can cause alkylation of the susceptible residues such as Cys and Trp. When cleaving the peptide from a Wang resin, resin-bound carbocations lead to reattachment of the initially cleaved peptide at Cys or Trp. The alkylation of Trp is negligible when its indole ring is protected by the Boc group, which is known to produce the N^{in}-carbamic acid compound to

Table 3
Effect of additives on Cys modification during TFA deprotection

Additive[a]	Ratio of Cys-Ang II to Cys(X)-Ang II		
	Cys-Ang II	Thz-Ang II	Cys(MeOBzl)-Ang II
None	88	5	7
$MeONH_2$·HCl (5 equiv)	91	<1	8
$MeONH_2$·HCl (5 equiv) + PhSH[b]	99	<1	<1

[a] The protected peptides were treated with TFA/TIS/H_2O (v/v, 95/2.5/2.5) in the presence of the additives listed in Table 3 at rt for 1 h.
[b] PhSH was used in the same volume as that of H_2O.

prevent such electrophilic additions during the final cleavage.[15] To avoid that of Cys, however, performing TFA cleavage in the presence of thiols is necessary. This measure using thiols could effectively prevent alkylation of Cys with methoxybenzyl cation generated from the MBom group.

In summary, the MBom group was shown to be a suitable protecting group for Fmoc chemistry to protect the π-nitrogen on the His residue in order to eliminate the side-chain-induced racemization. In particular, the MBom group proved to be indispensable for preparing the protected His-containing peptide segments on acid-sensitive linkers for the convergent synthesis.

References and notes

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9. *Fmoc-His(π-MBom) (**1**)*: ^{1}H NMR (DMSO-d_6) 2.90–3.17 (m, 2H), 3.71 (s, 3H), 4.15–4.38 (m, 6H), 5.38 (q, 2 H, J = 10.0 Hz) 6.74–6.87 (m, 3H), 7.15–7.42 (m, 6H) 7.60–7.94 (m, 6H); ^{13}C NMR (DMSO-d_6) 25.2, 46.6, 50.1, 53.2, 55.0, 63.8, 65.7, 69.0, 73.2, 113.7, 119.8, 120.1, 125.2, 126.8, 127.0, 127.1, 127.6, 127.8, 128.9, 129.5, 138.3, 140.6, 143.7, 145.2, 155.9, 158.8, 172.9; ESI MS: Calcd for $[C_{30}H_{29}N_3O_6+H]^+$ 528.21, found 528.2; $[\alpha]_D$ −8.3 (*c* 1.0, DMF); Elemental Anal. Calcd for $C_{30}H_{29}N_3O_6$; C, 68.30; H, 5.54; N, 7.96. Found C, 68.21; H, 5.58; N, 7.92; rt: 16.2 min (HPLC conditions: column, YMC-ODS AA12S05-1546WT at 40 °C; eluent, 20–70% MeCN/0.1% TFA (25 min); flow rate, 1 mL/min; detection, 220 nm.)
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12. By employing the same procedure as that for **1**, Fmoc-His(τ-MBom) was prepared from Boc-His(τ-MBom)-OMe obtained by alkylating Boc-His-OMe (**2**) with MBom-Cl. Fmoc-His(τ-MBom): ^{1}H NMR (DMSO-d_6) 2.90–3.16 (m, 2H), 3.71 (s, 3H), 4.13–4.37 (m, 6H), 5.36 (q, 2H, J = 10.0 Hz) 6.75–6.87 (m, 3H), 7.15–7.41 (m, 6H) 7.60–7.94 (m, 6H); ^{13}C NMR (DMSO-d_6) 25.1, 46.6, 50.1, 53.2, 55.2, 63.8, 65.7, 69.0, 73.2, 113.6, 119.8, 120.1, 125.2, 126.8, 127.0, 127.1, 127.7, 127.8, 128.9, 129.5, 138.3, 140.6, 143.7, 145.2, 155.8, 158.8, 172.7; Elemental Anal. Calcd for $C_{30}H_{29}N_3O_6$; C, 68.30; H, 5.54; N, 7.96. Found: C, 68.31; H, 5.62; N, 7.78; rt: 16.5 min (The same HPLC conditions described in Ref. 9).
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14. Protected Cys-Ang II was assembled with an ABI 433A (Forester, CA, USA) using Fmoc strategy on Wang resin. The peptide chain was elongated using the FastMoc® protocol of coupling with Fmoc-amino acid/HCTU/6-Cl-HOBt/DIEA (4/4/4/8 equiv) in NMP. The following side-chain-protected amino acids were employed: Cys(Trt), Asp(O^{t}Bu), His(MBom), Arg(Pbf) and Tyr(^{t}Bu).
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