DEVELOPMENT OF A NEW N^{π}-PROTECTING GROUP FOR HISTIDINE, N^{π}-1-ADAMANTYLOXYMETHYLHISTIDINE

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N^{π}-1-Adamantyloxymethylhistidine, His(N^{π}-1-Adom) was prepared, and the properties of the 1-Adom group were examined. 1-Adom group can be easily removed by TFA ; it is stable to 20% piperidine/DMF and 1*N* NaOH. His(N^{π}-1-Adom) derivatives can suppress racemization during coupling reaction. TRH was synthesized using His(N^{π}-1-Adom), successfully.

KEY WORDS histidine; protecting group; 1-adamantyloxymethyl; racemization

Various kinds of protecting groups for imidazole nitrogen of histidine residue have been developed in peptide synthesis. It is well known that protecting groups on π-nitrogen of imidazole function are more successful than those on τ-nitrogen for the prevention of racemization during peptide synthesis. Previously, N^{π}-benzyloxymethylhistidine, His(N^{π}-Bom), was developed.[3] Bom group is stable to CF_3COOH (TFA) and cleaved by hydrogenation over Pd catalyst or HF.[4] Therefore, His(N^{π}-Bom) can be applied for peptide synthesis by Boc strategy both in solution and in solid phase methods.N^{π}-*tert*-Butyloxymethylhistidine, His(N^{π}-Bum), was also developed in order to suppress racemization.[5] Bum group can be removed by TFA and is stable under alkaline conditions. Therefore, His(N^{π}-Bum) is applied for peptide synthesis by Fmoc (9-fluorenylmethyloxycarbonyl) strategy in solid phase method. However, it was reported that Fmoc-His(N^{π}-Bum)-OH had poor solubility in dichloromethane.[6] From these points of view, our studies were directed to the development of novel N^{π}-protecting groups with the objectives of suppressing side reactions, preventing racemization and increasing the solubility of His-containing peptide intermediates in organic solvents. Previously, it was reported that 1-adamantyl (Ada) ester could be removed by TFA and was stable to 20% piperidine/DMF[7] and that adamantyl ester derivatives exhibited high solubility in organic solvents.[8] These results gave us the idea of designing a new N^{π}-protecting group.

This paper deals with the synthesis of His(N^{π}-1-Adom) (**1**) (Fig. 1), its properties and its application to the synthesis of thyrotropin-releasing hormone (TRH). According to Chart 1, 1-adamantyloxymethyl chloride (1-Adom-Cl),[9] which was prepared from 1-adamantyloxymethyl methyl sulfide and sulfuryl chloride, is completely involatile and is easier to be purified than Bum-Cl. Z-His-OMe, is acetylated with acetic anhydride to give Z-His(N^{τ}-Ac)-OMe[10] (**2**), which was reacted with 1-Adom-Cl, followed by treatment with $NaHCO_3$ to afford Z-His (N^{π}-1-Adom)-OMe in

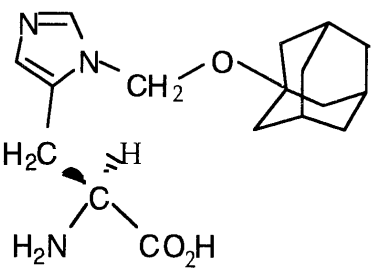

Fig. 1. Structure of **1**

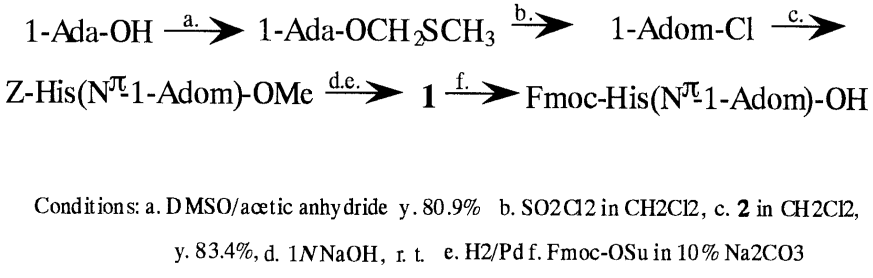

Chart 1. Synthesis of **1** and Its Derivatives

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high yield compared with Bum derivative. Z-His(N^{π}-1-Adom)-OMe was saponified with 1*N* NaOH, followed by hydrogenation over Pd catalyst to give **1** (Fig. 1).[11] The stability and susceptibility of the 1-Adom group to various acids and bases were examined, and the results are summarized in Table 1. The 1-Adom group is easily cleaved by TFA and stable to 20% piperidine/DMF at room temperature for up to 48 h. Therefore, His(N^{π}-1-Adom) can be used for peptide synthesis in combination with Fmoc as N^{α}-protecting group. Fmoc-His(N^{π}-1-Adom)-OH[12] was prepared from His(N^{π}-1-Adom) and Fmoc-OSu in a good yield, and it is more soluble in organic solvents than Fmoc-His(N^{π}-Bum)-OH.[5]

Table 1. Properties of **1** to Acids and Bases

Conditions	%Histidine regenerated						
	15min	30min	45min	60min	12h	24h	48h
TFA (200eq, 5eq anisole)	73.9	88.1	100	100			
0.1N HCl (300eq)	0	0	0	0	0	0	0
1N NaOH (100eq)	0	0	0	0	0	0	0
20% Piperidine/DMF (200eq)	0	0	0	0	0	0	0

Next, the efficiency of N^{π}-1-Adom group in the prevention of side-chain induced racemization was examined. Z-D-His(N^{π}-1-Adom)-OH was prepared by the same method as described above. Z-D-His(N^{π}-1-Adom)-L-Phe-OMe was well separated from Z-L-His(N^{π}-1-Adom)-L-Phe-OMe on HPLC.[13] Therefore, this sequence was employed for model study on racemization. Z-L-His(N^{π}-1-Adom)-OH was coupled with H-L-Phe-OMe by N,N'-dicyclohexylcarbodiimide (DCC), DCC/1-hydroxybenzotriazole (HOBt), benzotriazol-1-yloxytris (dimethylamino) phosphonium hexafluorophosphate (BOP),[14] 2-(1H-benzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate (HBTU)[15] or diphenylphosphoryl azide (DPPA),[16] and then the crude product was analyzed by HPLC. The results summarized in Table 2 show that the formation of D-L dipeptide was particularly low in all coupling methods so far examined.

Table 2. Racemization Rate During the Coupling of Z-His(N^{π}-1-Adom)-OH and H-Phe-OMe

Coupling method	D-L/(D-L+L-L) %
DCC	2.74
DCC/HOBt	0.55
BOP	0.60
HBTU	0.71
DPPA	0.50

Finally, Z-His(N^{π}-1-Adom)-OH was employed to synthesize the thyrotropin-releasing hormone (TRH). Z-His(N^{π}-1-Adom)-OH was coupled with H-Pro-NH_2 by BOP reagent to afford Z-His(N^{π}-1-Adom)-Pro-NH_2. After removal of Z group by catalytic hydrogenation, the resultant amine was coupled with *t*-butyloxycarbonylpyroglutamic acid (Boc-Pyr-OH) by BOP reagent to give Boc-Pyr-His(N^{π}-1-Adom)-Pro-NH_2. The protected tripeptide was purified by silica gel column chromatography, and the purified tripeptide was treated with TFA/thioanisole at room temperature for 1 h. After washings with ether and transformation to acetate form by treating with Amberlite IRA-93ZU resin, TRH[17] thus obtained exhibited a single peak on analytical HPLC at the same retention time as that of authentic TRH.[18]

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11) m.p.228-229°C (dec.) $[\alpha]_D^{25}$=-8.3° (*c* = 0.5 in MeOH) (*Anal.* Calcd for $C_{17}H_{25}N_3O_3$: C, 63.9; H, 7.89; N, 13.2. Found: C, 63.8; H, 7.88; N,13.2.),^{1}H-NMR spectra was measured with a Bruker ARS 500 spectrometer operating at a frequency of 500 MHz.^{1}H-NMR($CDCl_3$) δ=1.64 –1.87 (12H, m, adamantyl), 2.19 (3H, s, adamantyl), 3.09-3.45 (2H, m, $C\underline{H}_2CH$), 3.85-3.87 (1H, m, $C\underline{H}$-CH_2), 5.46 (2H, s, $NC\underline{H}_2O$), 6.93 (1H, s, 5^{im}-H), 7.68 (1H, s, 2^{im}-H).
12) m.p. 160-161°C. $[\alpha]_D^{25}$= +5.4° (*c* =0.5 in MeOH)
13) HPLC condition: Cosmosil pack 5C 18-AR (4.6x250mm); eluent: A (0.05%TFA in H_2O), B (0.05% TFA in CH_3CN),from 69/31 to 55/45 in 50 min,and to 69/31 in 5 min;flow rate:1 ml/min.
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17) $[\alpha]_D^{25}$= -62.2° (*c* =1.0 in H_2O)
18) Authentic TRH was purchased from Peptide Institute(Osaka,Japan)$[\alpha]_D^{25}$=-61.3°(*c* =1.0 in H_2O)

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