Deprotection of the Indole (N^{ind})-Formyl (For) Group on Tryptophan Employing a New Reagent, *N*,*N*′-Dimethylethylendiamine (DMEDA) in an Aqueous Solution

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The deprotection of the indole (N^{ind})-formyl (For) group on Trp was achieved in a 95% yield using *N*,*N*′-dimethylethylendiamine (DMEDA) (1.5, 2.0, 3.0 eq) in water at room temperature. A new reagent was successfully applied to the deprotection of a model peptide, H-Phe-Trp(N^{ind}-For)-Lys-Tyr-OH, to give H-Phe-Trp-Lys-Tyr-OH in a 91% yield.

Key words deprotection; *N*,*N*′-dimethylethylendiamine; indole-formyl group; peptide synthesis; tryptophan

In peptide synthesis, it is well known that tryptophan in the growing peptide chain undergoes oxidation during treatment with HCl in organic solvent necessary for the cleavage of the α-amino protected Boc (*tert*-Butoxycarbonyl) group. Furthermore, the sequential mild acid treatments to remove the α-amino protecting group often lead to electrophilic alkylation on the unprotected tryptophan residue. It is ideal to prevent these side reactions by protecting the indole moiety during the synthesis. This has been achieved by using the indole-formyl (N^{ind}-For) protecting group in Boc chemistry of peptide synthesis.

To date, a number of N^{ind}-For deprotection methods have been developed, including most well known method using low-high concentration hydrogen fluoride (HF) treatment.[1] However, this method is accompanied with treatment of large quantities of a reagent with considerable stench, such as 1,2-ethanedithiol and use of special equipment. On the other hand, the N^{ind}-For group can be removed in a weak alkaline medium, such as 1 M NH_2OH,[2] 1 M $NaHCO_3$,[3] or hydrazine hydrate (20—30 eq) in dimethylformamide (DMF).[4] However, these procedures required excess amounts of alkaline reagent and the N^{ind}-For group migration to an α-amino group of the peptide was reported.[3]

We were intrigued by a previous report showing that *N*,*N*′-diphenylethylendiamine (DPEDA) reacted with aldehyde to form a tetrahydroimidazole.[5] It hinted that *N*-alkylated diamines, such as DPEDA and *N*,*N*′-dimethylethylendiamine (DMEDA) could remove the N^{ind}-For group in aqueous medium. In light of not only commercial manufacturing practices, but also especially keeping mind "green chemistry," a new N^{ind}-For deprotecting method with equivalently controlled reactions under aqueous conditions and without using irritant reagents would be beneficial. In this communication, we describe the deprotection of the N^{ind}-formyl group on Trp employing a new reagent, DMEDA, in an aqueous solution.

Results and Discussion

We investigated N^{ind}-For in the deprotecting reaction of H-Trp(N^{ind}-For)-OH (**1**) under aqueous conditions using both DPEDA and DMEDA. Of these compounds, the former was inactive in the reaction due to its low solubility in water. In contrast, DMEDA was highly active in this regard. Therefore, our studies were directed to the deprotection of N^{ind}-For by DMEDA and we set out to evaluate the reaction conditions that allowed easy conversion of H-Trp(N^{ind}-For)-OH (**1**) to H-Trp-OH (**2**) and to optimize the conditions for preparation of the model peptide.

As shown in Fig. 1A, the treatment with 1.0 eq DMEDA did not completely remove the N^{ind}-For group even after 2 h. However, as the number of equivalents of DMEDA increased from 1.0 to 1.5, 2.0 and 3.0, the rate of the conversion became substantially faster. Complete deprotection was achieved after 1.0 h with 1.5 eq of DMEDA or in 20 min with

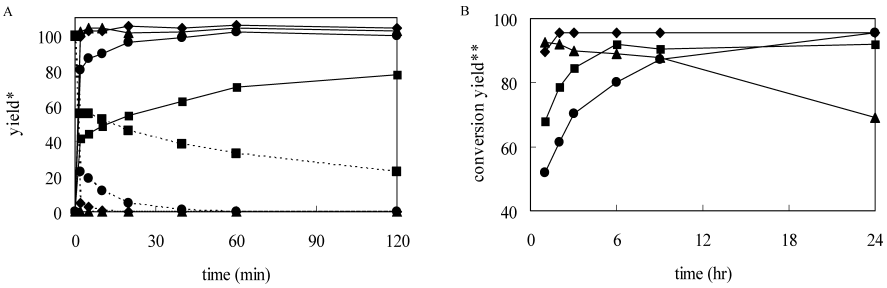

Fig. 1. The Effect of *N*,*N*′-Dimethylethylendiamine (DMEDA) on the Deprotection of Trp(N^{ind}-For) in Water

(A) The deprotecion of H-Trp(N^{ind}-For)-OH with DMEDA in water. * Both conversion yield of Trp and recovery yield of Trp(N^{ind}-For) were quantified by RP-HPLC. ■: 1.0 eq of *N*,*N*′-dimethylethylendiamine (DMEDA), ●: 1.5 eq of DMEDA, ◆: 2.0 eq of DMEDA, ▲: 3.0 eq of DMEDA, ——: H-Trp-OH, ------: H-Trp(N^{ind}-For)-OH. (B) Comparison of conversion yields for the model peptide with various reagents. ** Conversion yield was assessed by PDA integration on LC/MS/MS analysis. ◆: 3 eq of *N*,*N*′-dimethylethylendiamine (DMEDA), ▲: 30 eq of hydrazine, ●: 1 M $NaHCO_3$, ■: 1 M NH_2OH.

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2.0 eq of DMEDA, respectively; the addition of 3.0 eq of DMEDA led to a complete conversion after 2 min. Concerning side reactions, the migration of the N^{ind}-For group to α-amino group of Trp was not observed under any of the conditions investigated using RP-HPLC. LC/MS analysis revealed that the N^{ind}-For group underwent migration on one amino group of DMEDA to form **3** (Fig. 2), rather than tetrahydroimidazole. Thus, DMEDA can serve as a nucleophile for the removal of the N^{ind}-For group.

We applied this deprotecting reagent to the preparation of a model peptide. We chose a sequence of Phe-Trp-Lys-Tyr (**4**), which is a fragment of urotensin II.[6] First, we prepared a formyl derivative of **4**, namely H-Phe-Trp(N^{ind}-For)-Lys-Tyr-OH (**5**). Synthesis of **5** was carried out by solution methods using Boc chemistry, where Boc-Phe-Trp(N^{ind}-For)-Lys(N^{ε}-2-chlorobenzyloxycarbonyl)-Tyr(*O*-2,6-dichlorobenzyl)bezyl ester was treated with TMSOTf (trimethylsilyl trifluoromethanesulfonate)-thioanisole/TFA (trifluoroacetic acid)[7—9] to give H-Phe-Trp(N^{ind}-For)-Lys-Tyr-OH. The crude peptide was purified with RP-HPLC[10] to isolate H-Phe-Trp(N^{ind}-For)-Lys-Tyr-OH (**5**). Identification and purity of **5** were established by spectral data (HR-ESI-MS, LC/MS/MS). And then, **5** was subjected to deprotection using DMEDA. After treatment of **5** with DMEDA (3.0 eq) in water for 1 h, LC/MS analysis revealed a major peptide along with very few side products in the reaction mixture (Fig. 3). Characterization of the products of DMEDA treatment was performed by LC/MS/MS analysis without prior isolation: the main product was identified as the desired peptide **4** (yield=95%) and one of side products was identified as peptide **6**, arising from migration of the N^{ind}-For group to the ε-amino group of Lys residue. The migration of the N^{ind}-For group to an α-amino group was not detected in our conditions. The results obtained are summarized in Fig. 3. Those findings suggest that the ε-amino group of Lys might have higher nucleophilicity than the α-amino group and subsequently acted as a nucleophile under these conditions.

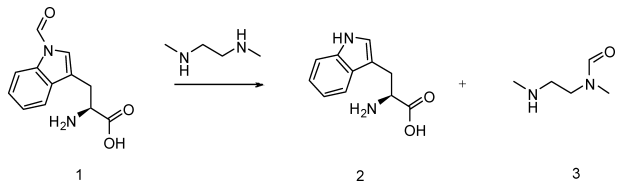

Fig. 2. Summary of the Deprotection of Trp(N^{ind}-For) with *N*,*N*′-Dimethylethylendiamine (DMEDA) in Water

We further examined the preparation of **4** by DMEDA method. Peptide **5** (5.00 mg) was treated with DMEDA (3.0 eq) in water at room temperature for 2 h, and the resulting peptide was purified with RP-HPLC[8] to give **4** (4.37 mg) in 91% yield. Identification of **4** was assessed by spectral data (HR-ESI-MS, LC/MS/MS). The deprotection of the N^{ind}-For group by DMEDA in water was successfully applied to the preparation of the peptide.

Finally, we compared the efficiency of the DMEDA deprotection method with other basic conditions using **5** as a model peptide. As shown in Fig. 1B, the conversion yield by our method using DMEDA (3.0 eq) for 2 h at room temperature was more than 95%. Treatment with 1 M $NaHCO_3$[3] (150 eq) and 1 M NH_2OH[2] (150 eq) revealed conversion yields at room temperature of 95% after 24 h and 90% after 6 h, respectively. Treatment with 1 M $NaHCO_3$[3] (150 eq) and 1 M NH_2OH[2] (150 eq) led to the migrations of N^{ind}-For group to the ε-amino group of Lys residue, and to an α-amino group of Phe residue, respectively. Hydrazine hydrate[4] treatment (30 eq) exhibited a conversion yield of 90% after 2 h at room temperature; however, **4** was found to be unstable in 30 eq hydrazine hydrate. DMEDA treatment proved to be superior to other basic reagents in terms of its quick reaction with higher purity.

In summary, a new method for the N^{ind}-For group deprotection has been developed. Trp(N^{ind}-For) was quantitatively converted to Trp in the presence of 3.0 eq of DMEDA under aqueous condition. This new reagent was successfully applied to the deprotection of a model peptide, H-Phe-Trp(N^{ind}-For)-Lys-Tyr-OH, to give H-Phe-Trp-Lys-Tyr-OH in excellent yield. The procedure would be practical and environ-

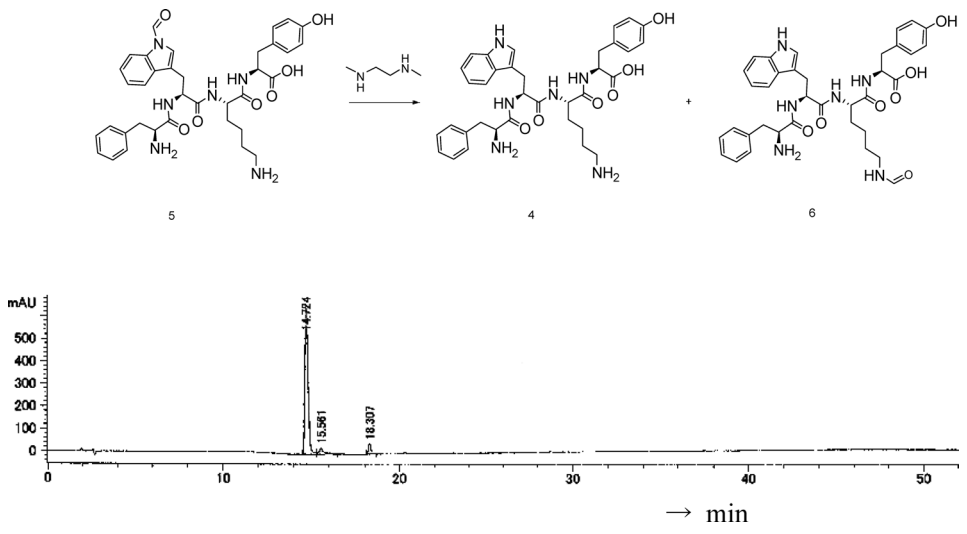

Fig. 3. Summary of the Deprotection of the Model Peptide with *N*,*N*′-Dimethylethylendiamine (DMEDA) in Water and HPLC Profile for Compounds **4**—**6**

HPLC profile for **4**—**6**. **4** (H-Phe-Trp-Lys-Tyr-OH): t_R=14.7 min, **5** [H-Phe-Trp(N^{ind}-For)-Lys-Tyr-OH]: t_R=15.4 min, **6** [H-Phe-Trp-Lys(N^{ε}-For)-Tyr-OH]: t_R=18.3 min.

mentally advantageous over the previously reported methods, especially from the current views of peptide manufacturing processes and application of the principles of green chemistry.

Experimental

General RP-HPLC Analyses for the Determination of 1—3 In a typical experiment, H-Trp(N^{ind}-For)-OH·HCl (26.8 mg) was dissolved in water (0.4 ml) and 1.0 to 3.0 eq of DMEDA was added. The mixture was stirred under ambient temperature for the appropriate time. Quantification of H-Trp-OH and H-Trp(N^{ind}-For)-OH was performed on Shimadzu CR4A HPLC system (YMCpack C18, 4.6×50 mm) in 0.1% acetic acid/water with a linear gradient from 5 to 98% acetonitrile over 5 min at a flow rate of 1.0 ml/min. Peak areas were integrated with Shimadzu SPD-6AV UV VIS detector at 280 nm.

H-Trp(N^{ind}-For)-OH (**1**): ESI-MS *m*/*z*: 233.13 $(M+1)^+$ (Calcd for $C_{12}H_{13}N_2O_3$: 233.23). t_R=2.98 min.

H-Trp-OH (**2**): ESI-MS *m*/*z*: 205.18 $(M+1)^+$ (Calcd for $C_{11}H_{13}N_2O_2$: 205.22). t_R=1.73 min.

N-Formyl-*N*,*N*′-dimethylethylendiamine (**3**): ESI-MS *m*/*z*: 117.04 $(M+1)^+$ (Calcd for $C_5H_{13}N_2O_1$: 117.16). t_R=0.35 min.

General LC/MS and LC/MS/MS Analyses for the Determination of 4—6 A 1.5 mg of H-Phe-Trp(N^{ind}-For)-Lys-Tyr-OH·2AcOH was dissolved in water (0.10 ml) and treated with various reagents including DMEDA (3.0 eq). LC/MS spectra of the reaction mixtures were obtained on Micromass ZMD (ESI) mass spectrometers and a Waters 600 HPLC system (Develosil C30-UG-5, 4.6×50 mm) in 0.1% acetic acid/water with a linear gradient from 5 to 98% acetonitrile over 4 min at 1.0 ml. Peak areas were integrated with Waters 2996 photodiode array detector. LC/MS/MS spectra of compounds were obtained by ESI (ABI, QSYAR pulsar-i) and a Hewlett Packard 1100 series (CAPCELL PAK C18, 1.5×150 mm) with a linear gradient of 5% acetonitrile containing 0.05% acetic acid/water containing 0.1% acetic acid to 100% acetoritrile containing 0.05% acetic acid over 40 min at 0.1 ml.

H-Phe-Trp-Lys-Tyr-OH (**4**): HR-ESI-MS *m*/*z*: 643.2416 $(M+1)^+$ (Calcd for $C_{35}H_{43}N_6O_6$: 643.3244). LC/MS/MS *m*/*z*: 462.3 $(M)^+$ [Calcd for $C_{26}H_{32}N_5O_3$/(H-Phe-Trp-Lys-OH)-OH: 462.5], 334.2 $(M)^+$ [Calcd for $C_{20}H_{20}N_3O_2$/(H-Phe-Trp-OH)-OH: 334.3], 310.2 $(M)^+$ [Calcd for $C_{15}H_{23}N_3O_4$/H-Lys-Tyr-OH: 310.3], 129.1 $(M)^+$ [Calcd for $C_6H_{13}N_2O_1$/(H-Lys-OH)-OH: 129.1]. t_R=14.7 min.

H-Phe-Trp(N^{ind}-For)-Lys-Tyr-OH (**5**): HR-ESI-MS *m*/*z*: 671.2835 $(M+1)^+$ (Calcd for $C_{36}H_{43}N_6O_7$: 671.3191). LC/MS/MS *m*/*z*: 490.2 $(M)^+$ [Calcd for $C_{27}H_{32}N_5O_4$/(H-Phe-Trp(N^{ind}-For)-Lys-OH)-OH: 490.5], 310.2 $(M)^+$ [Calcd for $C_{15}H_{23}N_3O_4$/H-Lys-Tyr-OH: 310.3], 129.1 $(M)^+$ [Calcd for $C_6H_{13}N_2O_1$/(H-Lys-OH)-OH: 129.1]. t_R=15.4 min.

H-Phe-Trp-Lys(N^{ε}-For)-Tyr-OH (**6**): HR-ESI-MS *m*/*z*: 671.2402 $(M+1)^+$ (Calcd for $C_{36}H_{43}N_6O_7$: 671.3191). LC/MS/MS *m*/*z*: 524.2 $(M+1)^+$ [Calcd for $C_{27}H_{34}N_5O_6$/(H-Trp-Lys(N^{ε}-For)-Tyr-OH)+H: 524.5], 490.2 $(M)^+$ [Calcd for $C_{27}H_{32}N_5O_4$/(H-Phe-Trp-Lys(N^{ε}-For)-OH)-OH: 490.5], 338.2 $(M+1)^+$ [Calcd for $C_{16}H_{24}N_3O_5$/(H-Lys(N^{ε}-For)-Tyr-OH)+H: 338.3], 334.1 $(M)^+$ [Calcd for $C_{20}H_{20}N_3O_2$/(H-Phe-Trp-OH)-OH: 334.2]. t_R=18.3 min.

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