An Efficient Synthesis of *N-tert*-Butoxycarbonyl-*O*-cyclohexyl-L-tyrosine

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A facile and efficient synthesis of *N-tert*-butoxycarbonyl-*O*-cyclohexyl-L-tyrosine [Boc-Tyr(Chx)-OH] is described. Boc-Tyr-OH was treated with NaH in dimethylformamide and then with 3-bromocyclohexene to give *N*-Boc-*O*-(cyclohex-2-enyl)-L-tyrosine [Boc-Tyr(Che)-OH] in 70% yield. Hydrogenation of Boc-Tyr(Che)-OH over PtO_2 afforded Boc-Tyr(Chx)-OH in almost quantitative yield. The highest yield was achieved when a side product in the synthesis of Boc-Tyr-OH, Boc-Tyr(Boc)-OH, was not removed, because it was also converted to Boc-Tyr(Che)-OH without any additional manipulations. The new synthetic method described here is convenient for practical use, and would facilitate the widespread use of the Chx group for the hydroxy-protection of Tyr.

Key words cyclohexyl; tyrosine; cyclohex-2-enyl; protecting group; hydroxy

In solid-phase peptide synthesis (SPPS), the phenolic hydroxy group of tyrosine (Tyr)[2)] is generally protected to prevent unwanted *O*-acylation.[3)] The 2,6-dichlorobenzyl[4)] (Cl_2Bzl) and 2-bromobenzyloxycarbonyl[5)] (BrZ) groups are being used most commonly in SPPS based on the *tert*-butoxycarbonyl (Boc)-chemistry. These protecting groups, however, are not entirely satisfactory because of two side reactions, *i.e.*, migration of the *O*-protecting group to the 3-position of the benzene ring of Tyr, and partial removal of the *O*-protecting group under basic conditions. The Cl_2Bzl group was reported to migrate to a lower degree than the classical benzyl group in the HF treatment, but the migration was not completely eliminated even in the presence of 50% anisole (5% migration at 0 °C in 10 min).[4)] The *O*-BrZ group does not cause the migration to the benzene ring, but it is partially removed even under weakly basic conditions; *ca*. 5% of the *O*-BrZ group was lost in the 24 h treatment with 10% diisopropylethylamine in dimethylformamide (DMF).[5)] Although these levels of rearrangement and removal may be tolerable to the SPPS of small peptides, they are insufficient for large peptides with many Tyr residues. Under these circumstances, Engelhard and Merrifield developed a new hydroxy-protecting group for Tyr, cyclohexyl (Chx).[6)] The Chx group was satisfactorily stable under basic and Boc-deprotecting conditions. Furthermore, no appreciable migration was detected in the HF treatment (<0.5% with HF containing 10% anisole, 0 °C, 10 min), because the intermolecular migration, which can be suppressed effectively by cation scavengers such as anisole, would take place dominantly in the removal of the Chx group, whereas the intramolecular pathway is dominant in the case of benzyl ether.[6)] Despite these advantageous features of the Chx group, it has not commonly been used to protect the hydroxy function of Tyr in peptide synthesis until now. A major obstacle to the use of the Chx protection seems to be in the complex, laborious, multi-step reactions involved in the synthesis of *N*-Boc-*O*-Chx-Tyr [Boc-Tyr(Chx)-OH]. The introduction of the Chx group to *N*-trifluoroacetyl-tyrosine methyl ester (Tfa-Tyr-OMe) with cyclohexene and BF_3 etherate was a particularly troublesome step because of the formation of the 3-alkylated product, which severely complicated the isolation of the desired product. If an easier preparative method for Boc-Tyr(Chx)-OH were available, the Chx group would be widely used to protect the hydroxy function of Tyr in SPPS. We report herein a facile and efficient synthesis of Boc-Tyr(Chx)-OH.

Recently, we have reported the synthesis of *N*-Boc-*O*-Chx-serine [Boc-Ser(Chx)-OH] and *N*-Boc-*O*-Chx-Thr [Boc-Thr(Chx)-OH], where the Chx group was readily formed by hydrogenation of the cyclohex-2-enyl (Che) moiety over a PtO_2 catalyst.[7,8)] The Che group was introduced in satisfactory yield, owing to the high reactivity of 3-bromocyclohexene (Che-Br), which has an allyl halide-like structure. The hydroxy function of Tyr, unlike that of Ser, is phenolic. This method is therefore expected to be applicable to the synthesis of Boc-Tyr(Chx)-OH, and even a higher yield than those of Boc-Ser(Chx)-OH and Boc-Thr(Chx)-OH might be achieved because of the higher acidity of the hydroxy group of Tyr than that of Ser and Thr. Therefore, we tried to apply this strategy to the synthesis of Boc-Tyr(Chx)-OH.

Boc-Tyr-OH was treated with NaH in DMF at 0 °C, then allowed to react with Che-Br at room temperature (method A in Fig. 1). Boc-Tyr(Che)-OH was obtained as its dicyclohexylamine (DCHA) salt in 70% yield, which is higher than that of Boc-Ser(Che)-OH from Boc-Ser-OH (46%).[7,8)] Troublesome side products, such as the 3-alkylated product in the original synthesis of Boc-Tyr(Chx)-OH,[6)] were not observed in this method, and therefore, the isolation of the product was very easy. The reaction with cyclohexyl bromide instead of Che-Br gave Boc-Tyr(Chx)-OH in only 2% yield, clearly proving the effectiveness of the Che strategy. Considering the higher acidity of the hydroxy function of Tyr, weaker bases than NaH, such as NaOH, may be used to synthesize Boc-Tyr(Che)-OH. When Boc-Tyr-OH was treated with Che-Br in the presence of 2 eq of NaOH in 1,4-dioxane–water (method B in Fig. 1), however, Boc-Tyr(Che)-OH was obtained only at a lower yield (28%) than that by method A. TLC analysis of the reaction mixture revealed that a large amount of the starting material remained unreacted. Furthermore, the Che ester (Boc-Tyr-OChe) was also formed (7%). Method A can therefore be concluded to be better than method B. The Che ether was then hydrogenated over PtO_2. In the synthesis of Boc-Ser(Chx)-OH, hydrogenolytic cleavage of the Che ether was observed in the Pd-catalyzed reaction, and PtO_2 was found to be a suitable catalyst for converting the Che moiety to the Chx group.[7,8)] Thus we employed PtO_2 for the hydrogenation of Boc-Tyr(Che)-OH. The desired Chx ether was obtained in 84% yield as its DCHA salt, whose mp and $[\alpha]_D$ were in agreement with the reported values.[6)] The overall

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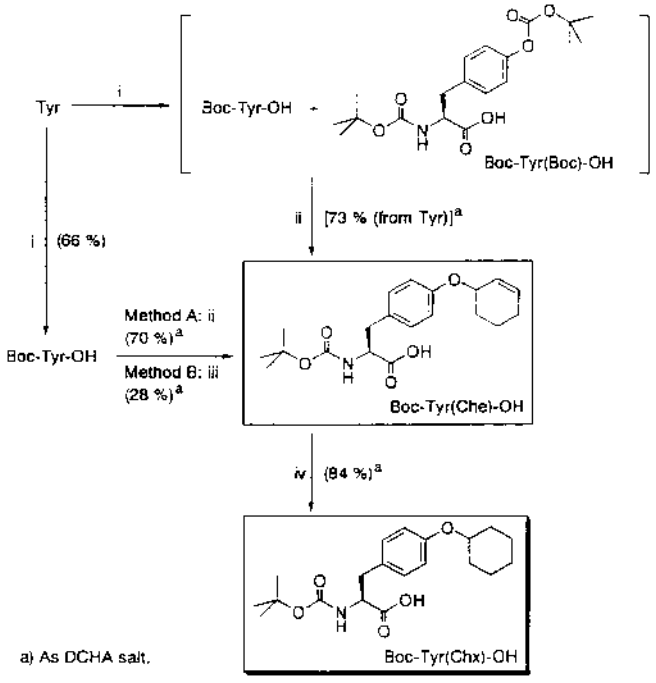

Fig. 1. Reagents and Conditions

i) $(Boc)_2O$, Et_3N, 1,4-dioxane–water (room temperature, overnight); ii) NaH, Che-Br, DMF (room temperature, overnight); iii) Che-Br, NaOH, 1,4-dioxane–water (ice-bath temperature, 2 h; room temperature, overnight); iv) H_2, PtO_2, MeOH–water (room temperature, 1 h).

yield of Boc-Tyr(Chx)-OH from Boc-Tyr-OH by method A was satisfactorily good (58%),[9] and all manipulations were very simple. Thus, the new method developed here can be regarded as an efficient synthetic route to Boc-Tyr(Chx)-OH.

The starting material in our synthesis, Boc-Tyr-OH, is commercially available, whereas that in Merrifield's synthesis, Tfa-Tyr-OMe, is not. This would be one advantage of the new method. Moreover, Boc-Tyr-OH can be readily prepared from Tyr in one step. In the usual synthesis of Boc-Tyr-OH, a small amount of Boc-Tyr(Boc)-OH is, however, known to be formed. Does this side reaction decrease the yield of Boc-Tyr(Chx)-OH from Tyr? Since the *O*-Boc group would be removed with NaH, the contaminous Boc-Tyr(Boc)-OH can also be expected to afford Boc-Tyr(Che)-OH without any additional manipulations, and a higher yield would be achieved when Boc-Tyr(Boc)-OH is not removed. To confirm this, a crude product containing Boc-Tyr(Boc)-OH besides Boc-Tyr-OH was treated directly with NaH and Che-Br. The overall yield of Boc-Tyr(Che)-OH from Tyr was 73%, whereas it was 46% when Boc-Tyr(Boc)-OH was removed [the yield of Boc-Tyr-OH from Tyr was 66% and that of Boc-Tyr(Che)-OH from Boc-Tyr-OH, 70%]. From this result, the contaminous Boc-Tyr(Boc)-OH is concluded to be an equivalent of Boc-Tyr-OH in the synthesis of Boc-Tyr(Che)-OH rather than an impediment. This route would be best in a practical aspect in terms of a high overall yield.

In conclusion, high-yield synthesis of Boc-Tyr(Chx)-OH has been accomplished by almost quantitative hydrogenation of Boc-Tyr(Che)-OH, which can be readily synthesized from Boc-Tyr-OH in good yield. Furthermore, Boc-Tyr(Boc)-OH, the contaminant in the synthesis of Boc-Tyr-OH, could also be converted to Boc-Tyr(Che)-OH without any additional reaction steps, resulting in an even higher yield from Tyr in only three simple steps. The new method developed here would be suitable for the large-scale preparation of Boc-Tyr(Chx)-OH in view of the high yield, simplicity of manipulation, and mildness of reaction conditions, and it would facilitate the widespread use of Chx as a phenolic hydroxy protection for the efficient synthesis of Tyr-containing peptides.

Experimental

For column chromatography, WAKO-gel C-200 (WAKO Pure Chemical Industries, Ltd., Japan) was used. *Rf* values in TLC (Kieselgel 60 F_{254}, Merck) refer to $CHCl_3$–MeOH–AcOH (90 : 8 : 2), and spot detection was carried out by UV light and/or staining with 0.1% ninhydrin in acetone. Optical rotations were measured with a JASCO DIP-370 polarimeter, and $[\alpha]_D$ values are in units of 10^{-1} deg cm^2 g^{-1}. ^{1}H-NMR spectra were recorded on a JEOL Lambda 400 MHz spectrometer in $CDCl_3$ solutions, tetramethylsilane being used as the internal reference.

Che-Br was purchased from Aldrich Chemical Company (U.S.A.), and was used without purification. $(Boc)_2O$ was purchased from Watanabe Chemical Industries, Co., Ltd. (Hiroshima, Japan). DMF was distilled from ninhydrin before use. Other reagents were of reagent grade and used without purification.

Boc-Tyr(Che)-OH·DCHA Method A: To a solution of Boc-Tyr-OH (1.03 g, 3.7 mmol) in DMF (50 ml) was added NaH (60% oil dispersion, 0.29 g, 7.3 mmol) portionwise at 0 °C. After the evolution of hydrogen gas ceased, Che-Br (90%, 0.65 g, 3.7 mmol) was added to the solution. The reaction mixture was stirred at room temperature overnight. After removal of the solvent under reduced pressure below 40 °C, the residue was dissolved in water (50 ml) under cooling with ice, and the solution was washed with diethyl ether (20 ml×2). The aqueous phase was acidified to pH 4 with 10% citric acid, then extracted with AcOEt (100 ml). The extract was washed successively with saturated NaCl–water and water, dried over Na_2SO_4, and then evaporated. To an ice-cooled solution of the oily residue in diethyl ether (20 ml) was added DCHA (0.66 g, 3.7 mmol) to afford a precipitate, which was collected by filtration, washed with diethyl ether, and recrystallized from AcOEt; yield 1.39 g (70%); mp 134—136 °C; $[\alpha]_D^{28}$ +32.4° (*c*=1, MeOH); *Rf* 0.50. ^{1}H-NMR (free acid form) δ (ppm) 7.08 (2H, d, *J*=8.0 Hz) 6.86 (2H, d, *J*=8.3 Hz), 5.97 (1H, dt, *J*=10, 3.4 Hz), 5.85 (1H, dd, *J*=10, 2.6 Hz), 4.93 (1H, d, *J*=8.1 Hz), 4.76 (1H, s), 4.59—4.52 (1H, m), 3.15—3.01 (2H, m), 2.18—1.80 (5H, m), 1.67—1.58 (1H, m), 1.42 (6 H, s) 1.32 (3H, s). *Anal*. Found C, 70.8; H, 9.45; N, 5.20; Calcd for $C_{32}H_{50}N_2O_5$ C, 70.8; H, 9.28; N, 5.16.

Method B: To an ice-cooled solution of Boc-Tyr-OH (1.03 g, 3.7 mmol) in 1,4-dioxane (20 ml) and water (20 ml) containing NaOH (0.29 g, 7.3 mmol) was added Che-Br (90%, 0.65 g, 3.7 mmol). The reaction mixture was stirred at ice-bath temperature for 2 h and then at room temperature overnight. Citric acid was added to the solution to adjust the pH to 7, and the resultant solution was evaporated. The residue was dissolved in 5% Na_2CO_3, then washed with ether (20 ml×2). The aqueous layer was acidified to pH 3 with 10% citric acid, then extracted with AcOEt (100 ml). The extract was washed with saturated NaCl aq. and water, dried over Na_2SO_4, and evaporated. The yellowish oil was applied to a silica gel (15 g) column, which was equilibrated with $CHCl_3$, then eluted with 1% MeOH in $CHCl_3$. The solvent of the effluent (200—500 ml) was removed by evaporation. To an ice-cooled solution of the oily residue in diethyl ether (20 ml) was added DCHA (0.66 g, 3.7 mmol) to afford a precipitate, which was collected by filtration, washed with diethyl ether, and recrystallized from AcOEt; yield 0.56 g (28%); mp 135—136 °C; $[\alpha]_D^{28}$ +31.4° (*c*=1, MeOH); *Rf* 0.50.

Removal of the solvent from the fractions moving slower than the title compound in the column chromatography gave Boc-Tyr-OChe (0.13 g, 7%) as a colorless oil; *Rf* 0.34. ^{1}H-NMR δ (ppm) 6.94—6.85 (3H, m) 6.70 (1H, d, *J*=8.0 Hz), 6.02 (1H, d-like, *J*=8.4 Hz), 5.76 (1H, t-like, *J*=11.5 Hz), 4.94 (1H, d, *J*=7.3 Hz), 4.54 (1H, d-like, *J*=5.1 Hz), 3.56 (1 H, s), 3.10—2.98 (2H, m), 2.11 (2H, s), 2.02—1.95 (1H, m), 1.77—1.72 (1H, m), 1.64—1.58 (2H, m), 1.42 (6H, s) 1.32 (3H, s). After removal of the Boc group with 4 N HCl in AcOEt, the IR spectrum (KBr) of the product showed a clear ester band at 1737 cm-1.

From Tyr [without Removal of Boc-Tyr(Boc)-OH]: To an ice-cooled solution of Tyr (2.00 g, 11 mmol) in water (20 ml) containing Et_3N (2.3 ml, 16 mmol) was added a solution of $(Boc)_2O$ (2.9 g, 13 mmol) in 1,4-dioxane (80 ml). The mixture was stirred at room temperature overnight. After evaporation of 1,4-dioxane, the residue was acidified to pH 3 with 10% citric acid, and extracted with AcOEt (200 ml). The extract was washed with saturated NaCl–water and water, dried over Na_2SO_4, and evaporated. The colorless oil

thus obtained was dissolved in DMF (80 ml) and reacted with NaH (60% oil dispersion, 0.88 g, 22 mmol) and Che-Br (90%, 2.0 g, 11 mmol) as described in method A to give Boc-Tyr(Che)-OH·DCHA (4.3 g, 73%); mp 134—135 °C; $[\alpha]_D^{28}$ +30.8° (*c*=1, MeOH); *Rf* 0.50.

Boc-Tyr(Chx)-OH·DCHA Boc-Tyr(Che)-OH [prepared from Boc-Tyr(Che)-OH·DCHA (0.98 g, 1.8 mmol) in the usual manner] in a mixture of MeOH (80 ml) and water (10 ml) was hydrogenated over PtO_2 (50 mg) for 1 h. After removal of the catalyst and the solvent, the residue was applied to a silica gel (15 g) column, which was equilibrated and eluted with $CHCl_3$. The solvent of the effluent (100—500 ml) was removed by evaporation. To an ice-cooling solution of the oily residue in diethyl ether (5 ml) was added DCHA (0.33 g, 1.8 mmol) to afford a precipitate, which was collected by filtration, washed with diethyl ether, and recrystallized from AcOEt; yield 0.84 g (84%), mp 141—142 °C [lit.[6] 142 °C (hexane)]; $[\alpha]_D^{28}$ +30.3° (*c*=1, MeOH) [lit.[6] $[\alpha]_D^{20}$ +36.5° (*c*=1, MeOH)].

References and Notes

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