Notes

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**Promoting Effect of Pentamethylbenzene on the Deprotection of
O-Benzyltyrosine and *N*^ε-Benzyloxycarbonyllysine with
Trifluoroacetic Acid¹⁾**

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O-Benzyltyrosine was rapidly deprotected without formation of 3-benzyltyrosine by treatment with trifluoroacetic acid containing pentamethylbenzene. This method was also found to be useful in the deprotection of *N*^ε-benzyloxycarbonyllysine [Lys(Z)] and *N*^G-4-methoxy-2,3,6-trimethylbenzenesulfonylarginine [Arg(Mtr)]. The new deprotecting method was successfully applied to the synthesis of kyotorphin (Tyr-Arg).

Keywords—deprotecting method; pentamethylbenzene-trifluoroacetic acid; *O*-benzyltyrosine; *N*^ε-benzyloxycarbonyllysine; *N*^G-4-methoxy-2,3,6-trimethylbenzenesulfonylarginine; kyotorphin

A prevailing strategy in peptide synthesis involves a final acidolytic cleavage of all side chain protecting groups.²⁾ Though trifluoroacetic acid (TFA) has many advantages such as high volatility, comparatively low toxicity and good solvency, it does not have sufficient acidity to remove various protecting groups.³⁾ In 1980, Kiso *et al.*⁴⁾ presented a novel method to improve the characteristics of TFA. They reported that though TFA alone deprotected *O*-benzyltyrosine [Tyr(Bzl)] slowly to yield tyrosine and 3-benzyltyrosine (by-product) in the ratio of 57 to 43,⁵⁾ a combination of thioanisole with TFA could deprotect Tyr(Bzl) rapidly without formation of 3-benzyltyrosine.⁶⁾ Moreover, this system could deprotect *N*^ε-benzyloxycarbonyllysine [Lys(Z)] quantitatively. The proposed mechanism for the deprotection of Tyr(Bzl) was addition of H⁺ to the oxygen atom of the ether bond and subsequent nucleophilic attack of the sulfur atom of thioanisole on the electron-deficient benzyl carbon atom.

On the other hand, it has been reported that in several deprotecting procedures the addition of compounds which consume a reactive species formed in the process increases the rate of the reaction or brings the reaction to completion.⁷⁾ We have now examined whether potent cation scavengers can promote the removal of benzyl-type side chain protecting groups with TFA, in order to find another deprotecting procedure suitable for large-scale peptide synthesis. The present paper describes the efficient deprotection of Tyr(Bzl), Lys(Z) and *N*^G-4-methoxy-2,3,6-trimethylbenzenesulfonylarginine [Arg(Mtr)] with TFA containing pentamethylbenzene.

Anisole, the methoxy group of which activates the benzene ring for electrophilic attack, has been widely employed as a cation scavenger, so that the promoting effect of methoxybenzenes on the deprotection of Tyr(Bzl) with TFA at 30 °C was first examined. The progress of

Fig. 1. Effect of Methoxybenzenes (2 mmol) on the Deprotection of Tyr(Bzl) (0.2 mmol) with TFA (4 ml) at 30 °C

○, no additive; ●, anisole; △, 1,3-dimethoxybenzene; ▲, 1,2,3-trimethoxybenzene.

Fig. 2. Effect of Methylbenzenes (2 mmol) on the Deprotection of Tyr(Bzl) (0.2 mmol) with TFA (4 ml) at 30 °C

○, no additive; ●, 1,3-dimethylbenzene; △, 1,2,3-trimethylbenzene; ▲, 1,2,3,4-tetramethylbenzene; □, PMB.

Fig. 3. Comparison of the Effects of Scavengers (2 mmol) on the Deprotection of Tyr(Bzl) (0.2 mmol) with TFA (4 ml) at 30 °C

○, PMB; ●, thioanisole.

the reaction was followed by high-performance liquid chromatography (HPLC). As can be seen in Fig. 1, these additives had a little ability to accelerate the cleavage of the benzyl ether. However, in the case of methylbenzenes the rate of the cleavage increased with increase of the number of methyl groups in the benzene ring, as shown in Fig. 2. Pentamethylbenzene (PMB) was the most effective among the agents tested. Complete deprotection of Tyr(Bzl) was achieved in PMB-TFA at 30 °C within 1.5 h and the rate of this cleavage was as fast as that in thioanisole-TFA, as shown in Fig. 3. Moreover, Tyr was found to be regenerated from Tyr(Bzl) almost quantitatively by the PMB-TFA method.

Though the mechanism of this cleavage reaction has not been clarified yet, except that 1-benzyl-2,3,4,5,6-pentamethylbenzene is produced, trapping of the benzyl cation by PMB presumably promotes the reaction as shown in Fig. 4. It appears to be strange that the methoxy group, which is a stronger electron donor than the methyl group,⁸⁾ did not contribute much to the promotion of the cleavage reaction. The reason may be that protonation of the methoxy group by TFA reduces its electron-releasing activity.

In addition, the deprotection of Lys(Z)⁹⁾ and Arg(Mtr)¹⁰⁾ was examined in PMB-TFA. In both cases the removal of the protecting group was accelerated, as shown in Figs. 5 and 6.

Furthermore, we examined the application of this new method to the synthesis of kyotorphin (Tyr-Arg).¹¹⁾ Boc-Tyr(Bzl)-Arg(Mtr)-OH, which was prepared by coupling

Fig. 4

Fig. 5. Effect of PMB (2 mmol) on the Deprotection of Lys(Z) (0.2 mmol) with TFA (4 ml) at 30 °C

○, no additive; ●, PMB.

Fig. 6. Effect of PMB (2 mmol) on the Deprotection of Arg(Mtr) (0.2 mmol) with TFA (4 ml) at 30 °C

○, no additive; ●, PMB.

Boc-Tyr(Bzl)-OH¹²⁾ with H-Arg(Mtr)-OH by the HOSu active ester method, was deprotected by the PMB-TFA method at room temperature overnight. The product was purified by column chromatography on YMC gel (ODS) using 0.05% HCl as an eluent. Lyophilization of the main fraction yielded the hygroscopic pure peptide, which was converted to the acetate salt by treatment with Amberlite IRA-93 (AcOH form). The homogeneous peptide was obtained in 52% overall yield from the protected peptide.

These results show that this deprotecting method using PMB, which is commercially available, might be as useful as the thioanisole-TFA method in practical peptide synthesis. Further experiments are needed to determine the scope and limitations of this new method.

Experimental

The proton nuclear magnetic resonance (¹H-NMR) spectrum was measured on a JEOL FX-90Q spectrometer using tetramethylsilane as an internal standard. Optical rotations were determined with a JASCO model DIP-140

polarimeter. Acid hydrolysis was carried out in 6N HCl at 110 °C for 22 h. Amino acid analysis was performed in a Hitachi 835 amino acid analyzer. Thin-layer chromatograms (TLC) were run on silica gel plates (precoated silica gel plates 60F₂₅₄, Merck). *R_f* values refer to the following solvent systems: *R_{f1}* CHCl₃–MeOH–AcOH (7:1:0.1), *R_{f2}* *n*-BuOH–AcOH–pyridine–H₂O (15:5:5:8). HPLC was carried out using an LC-5A pump (Shimadzu), an SDP-2A variable-wavelength UV detector (Shimadzu) and an AM-312 packed column (ODS, Yamamura Chemical Laboratory Co., Ltd.).

Measurement of Deprotection Rates of Tyr(Bzl), Lys(Z) and Arg(Mtr)—The protected amino acid (0.2 mmol), an additive (2 mmol) and benzoic acid (0.1 mmol, used as an internal standard) were dissolved in TFA (4 ml) and the solution was kept at 30 °C. The remaining amount of the protected amino acid in the solution was estimated by HPLC (detected at 210 nm) using 0.05% HCl (H₂O–CH₃CN, 78:22) as an eluent. The results are shown in Figs. 1, 2, 4 and 5.

Measurement of the Amount of Tyr Regenerated from Tyr(Bzl) by Treatment with PMB–TFA—Tyr(Bzl) (54.2 mg, 0.2 mmol) was dissolved in TFA (4 ml) containing PMB (296 mg, 2 mmol). The solution was left to stand at room temperature overnight, then 2-hydroxybenzamide (27.4 mg, 0.2 mmol, used as an internal standard) and *D*-10-camphorsulfonic acid (60 mg, 0.24 mmol) were added. The amount of Tyr in the solution was estimated by HPLC (detected at 280 nm) using 0.1% *D*-camphorsulfonic acid (H₂O–CH₃CN, 92:8) as an eluent; recovery 98%.

Isolation of 1-Benzyl-2,3,4,5,6-pentamethylbenzene—Tyr(Bzl) (2.71 g, 0.01 mol) and PMB (14.85 g, 0.1 mol) were dissolved in TFA (200 ml) and the solution was left to stand at room temperature for 7 h. After evaporation of the TFA, *n*-hexane and 1N HCl were added. The *n*-hexane layer was washed with water and both *n*-hexane and PMB were removed *in vacuo*. The oily residue was triturated with *n*-hexane to give crystals; yield 2.17 g (91%), mp 111–112 °C (uncorrected). *Anal.* Calcd for C₁₈H₂₂: C, 90.70; H, 9.30. Found: C, 90.85; H, 9.31. ¹H-NMR (CDCl₃) δ: 2.16 (6H, s, CH₃ × 2), 2.24 (9H, s, CH₃ × 3), 4.10 (2H, s, CH₂Ph), 6.90–7.30 (5H, m, CH₂Ph).

Boc-Tyr(Bzl)-Arg(Mtr)-OH—Boc-Tyr(Bzl)-OH (2.60 g, 7.0 mmol) and HOSu (0.97 g, 8.4 mmol) were dissolved in DMF (15 ml) and *N,N'*-dicyclohexylcarbodiimide (1.45 g, 7.0 mmol) was added at 0 °C. The mixture was stirred at 4 °C overnight, then a solution of H-Arg(Mtr)-OH (2.70 g, 7.0 mmol) and *N*-methylmorpholine (0.77 ml, 7.0 mmol) in DMF (16 ml) was added. The mixture was stirred at room temperature for 1 d. The solvent was evaporated, the residue was dissolved in AcOEt and the precipitate was filtered off. The filtrate was washed with 0.1N citric acid. After evaporation of the AcOEt, the residue was purified by column chromatography on silica gel (CHCl₃–MeOH, 30:1); yield 4.0 g (77%), [α]_D²⁰ +5.6° (*c*=2, MeOH), *R_{f1}* 0.49. *Anal.* Calcd for C₃₇H₄₉N₅O₉S·3/2 H₂O: C, 57.95; H, 6.83; N, 9.13. Found: C, 58.11; H, 6.48; N, 9.09.

H-Tyr-Arg-OH—Boc-Tyr(Bzl)-Arg(Mtr)-OH (1.481 g, 2 mmol) was dissolved in TFA (40 ml) containing PMB (2.96 g, 20 mmol) and the solution was left to stand overnight at room temperature. The solvent was evaporated off at 30 °C, ether and water were added to the residue and the water layer was lyophilized. The crude product was purified by column chromatography on YMC gel ODS (3 × 30 cm, 60/200, Yamamura Chemical Laboratory Co., Ltd.) using 0.05% HCl as an eluent. The main fraction was lyophilized, dissolved in water and treated with Amberlite IRA-93 (AcOH). The solvent was removed by lyophilization; yield 460 mg (52%), [α]_D²⁰ +22.5° (*c*=0.5, 0.2M AcOH), *R_{f2}* 0.48. Amino acid ratios (acid hydrolysate): Tyr 1.00, Arg 1.04 (recovery 93%). *Anal.* Calcd for C₁₅H₂₃N₅O₄·CH₃COOH·5/2H₂O: C, 46.15; H, 7.29; N, 15.83. Found: C, 46.42; H, 7.18; N, 16.02.

References and Notes

- 1) Amino acids and their derivatives in this paper are of the L-configuration. The following abbreviations are used: Bzl = benzyl, Z = benzyloxycarbonyl, Boc = *tert*-butoxycarbonyl, Mtr = 4-methoxy-2,3,6-trimethylbenzenesulfonyl, HOSu = *N*-hydroxysuccinimide, DMF = dimethylformamide.
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