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Efficient Removal of Protecting Groups by a 'Push-Pull' Mechanism. II.^{1,2)} Deprotection of *O*-Benzyltyrosine with a Thioanisole-Trifluoroacetic Acid System without *O*-to-*C* Rearrangements

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A thioanisole-trifluoroacetic acid (TFA) system was found to deprotect Tyr(Bzl) quantitatively at 25° within 3 hr by a push-pull mechanism without *O*-to-*C* rearrangements. This new deblocking system did not completely deprotect Ser(Bzl) and Thr(Bzl), but deprotected Lys(Z) quantitatively.

Keywords—deblocking method; thioanisole-trifluoroacetic acid system; dimethyl sulfide-trifluoroacetic acid system; *O*-benzyltyrosine; *O*-benzylserine; *O*-benzylthreonine; *N*^ε-benzyloxycarbonyllysine; hard soft acids bases; push-pull mechanism; peptide synthesis

In an earlier paper,⁴⁾ we reported that thioanisole had not only a suppressing effect on a side reaction, but also a promoting effect on the cleavage reaction during the acidolytic cleavage of protecting groups of tyrosine. Recently,^{1,5)} the thioanisole-trifluoromethanesulfonic acid system was found to cleave the methyl group attached at the phenolic oxygen of tyrosine by a push-pull mechanism and was conveniently applied to the synthesis of a potent enkephalin derivative. We report here that the benzyl group attached at the phenolic oxygen of tyrosine can be quantitatively cleaved by a push-pull mechanism using the thioanisole-TFA system without any side reaction.

The benzyl group used for protection of the phenolic hydroxyl group of tyrosine in peptide synthesis has been removed by strong acids such as HF,⁶⁾ methanesulfonic

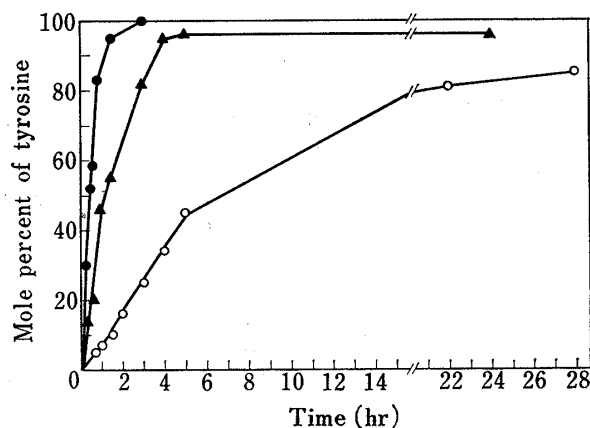


Fig. 1. Reaction of Tyr(Bzl) (0.1 mmol) with Scavenger (5 mmol) -TFA (2 ml) at 25°

● : thioanisole-TFA.
▲ : dimethyl sulfide-TFA.
○ : anisole-TFA.

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- 2) Abbreviations: TFA = trifluoroacetic acid, Tyr(Bzl) = *O*-benzyl-L-tyrosine, Ser(Bzl) = *O*-benzyl-L-serine, Thr(Bzl) = *O*-benzyl-L-threonine, Lys(Z) = *N*^ε-benzyloxycarbonyl-L-lysine.
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acid⁷⁾ or trifluoromethanesulfonic acid,⁸⁾ and is known to yield a side product, 3-benzyl-tyrosine.⁹⁾ We reasoned that the use of thioanisole as a soft nucleophile for the acidolytic cleavage of Tyr(Bzl)¹⁰⁾ might promote the cleavage reaction and therefore permit the use of a mild acid, TFA.

The deblocking reaction of Tyr(Bzl) was examined in thioanisole-TFA, dimethyl sulfide-TFA and anisole-TFA. The progress of the reaction was followed by thin-layer chromatography.

Complete cleavage of the benzyl group in Tyr(Bzl) was achieved in thioanisole-TFA at 25° within 3 hr, as shown in Fig. 1. The promoting effect on the acidolytic cleavage of examined nucleophiles was in the order thioanisole > dimethyl sulfide > anisole. A by-product, 3-benzyltyrosine, was not detected in a thioanisole-TFA system, while a side reaction occurred in both the dimethyl sulfide-TFA and anisole-TFA systems.

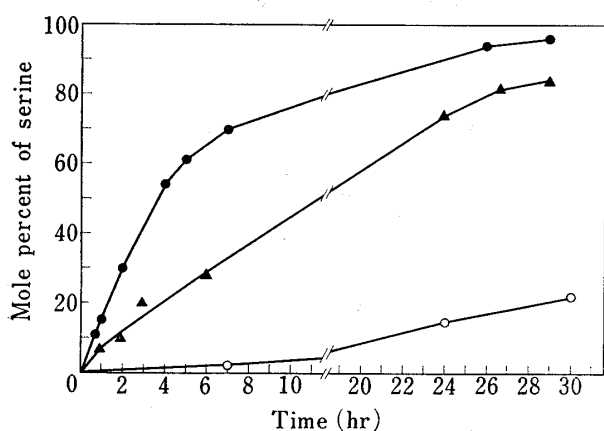


Fig. 2. Reaction of Ser(Bzl) (0.1 mmol) with Scavenger (5 mmol) -TFA (2 ml) at 25°

● : thioanisole-TFA.
▲ : dimethyl sulfide-TFA.
○ : anisole-TFA.

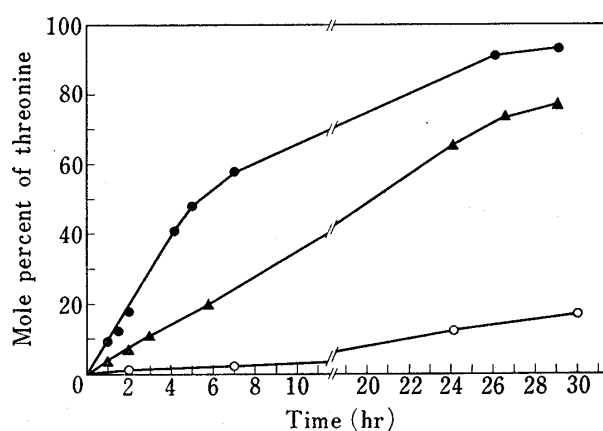


Fig. 3. Reaction of Thr(Bzl) (0.1 mmol) with Scavenger (5 mmol) -TFA (2 ml) at 25°

● : thioanisole-TFA.
▲ : dimethyl sulfide-TFA.
○ : anisole-TFA.

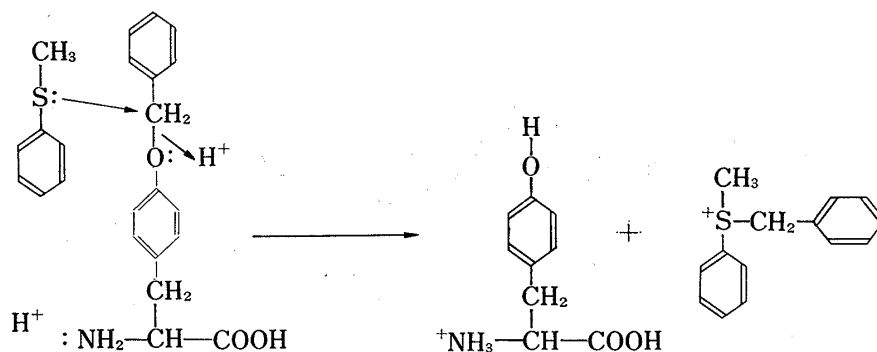


Fig. 4

Next, the deblocking reaction of aliphatic benzyl ethers, Ser(Bzl)¹¹⁾ and Thr(Bzl),¹²⁾ was examined in thioanisole-TFA, dimethyl sulfide-TFA and anisole-TFA. The cleavage reaction rates of Ser(Bzl) and Thr(Bzl) were slow compared to that of Tyr(Bzl), and the benzyl

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groups in Ser(Bzl) and Thr(Bzl) were not completely deprotected within 3 hr at 25° in thioanisole-TFA (Fig. 2 and Fig. 3). In the cases of both Ser(Bzl) and Thr(Bzl) the promoting effect on the acidolytic cleavage was in the order thioanisole > dimethyl sulfide > anisole.

In addition, we examined the role of thioanisole in this facilitated cleavage reaction of Tyr(Bzl). In the reaction of Tyr(Bzl) (1 equiv.) and thioanisole (4 equiv.) in TFA, the benzylmethylphenylsulfonium ion was obtained quantitatively. This result indicates that the role of thioanisole is to 'push' the acidolytic cleavage reaction and that this reaction occurs by the addition of H⁺ (a hard acid)¹³⁾ to the oxygen atom (a hard base) of the ether bond, and nucleophilic attack of the sulfur atom (a soft base) on the electron-deficient benzyl carbon atom (a soft acid). This reaction thus involves the cooperative action of a soft nucleophile and a hard electrophile on a substrate (push-pull mechanism) and proceeds favourably.

Thus, the use of the thioanisole-TFA system as a deblocking method permitted the *O*-benzyl group of tyrosine to be removed under mild conditions without *O*-to-*C* rearrangements. This should be valuable for the synthesis of tyrosine-containing peptides.

Very recently, Fujita *et al.*¹⁴⁾ reported the cleavage reaction of benzyl ethers based on the same considerations, using a boron trifluoride etherate-thiol system; this gave easier cleavage of aliphatic benzyl ether than aromatic benzyl ether, whereas our new deblocking system, the thioanisole-TFA system, cleaved aromatic benzyl ether [Tyr(Bzl)] more easily than aliphatic benzyl ethers [Ser(Bzl) and Thr(Bzl)]. This suggests that the nature of H⁺ as an acid is different from that of boron trifluoride, and the reasons for this interesting result are under consideration.

Finally, it was also possible to deblock Lys(Z)¹⁵⁾ quantitatively with the thioanisole-TFA system within 3 hr at room temperature. Details of this decarbobenzoylation reaction will be published in a separate paper. Application of this deblocking method to peptide synthesis is under way.

Experimental

Thin-layer chromatograms (TLC) were run on Merck silica gel 60 F-254 pre-coated TLC plates (0.25 mm) using chloroform-methanol-water (8:3:1, lower layer) and spots were visualized with ninhydrin. *R_f* values of reference samples: Tyr, 0.15; 3-BzlTyr, 0.21; Tyr(Bzl), 0.42; Ser, 0.04; Ser(Bzl), 0.38; Thr, 0.07; Thr(Bzl), 0.43; Lys, 0.03; Lys(Z), 0.41. Quantitative analysis was performed with a Shimadzu dual-wavelength TLC scanner CS-910. The ninhydrin colour intensity of spots was determined under the following operating conditions: slit 0.8 × 8.0 mm, scan speed 20 mm/min, chart speed 48 mm/min, λ₁ = 578 nm, λ₂ = 700 nm. Amounts were calculated by comparison with a reference sample. ¹H-NMR spectra were observed in TFA with a JEOL JNM-PS-100 NMR spectrometer; chemical shifts are expressed in parts per million downfield from internal tetramethylsilane.

Deprotection of Tyr(Bzl)—(a) Reaction with Thioanisole-TFA: Tyr(Bzl) (27.1 mg, 0.1 mmol) was treated with thioanisole (0.62 ml, 5 mmol)-TFA (2 ml) at 25°. The time course of the reaction was followed by TLC, scanning the plates with a TLC scanner. The results are shown in Fig. 1.

(a') Reaction with Thioanisole-TFA: A mixture of Tyr(Bzl) (678 mg, 2.5 mmol) and thioanisole (1.24 ml, 10 mmol) in TFA (2 ml) was kept at room temperature. After 4 days, the reaction mixture was subjected to ¹H-NMR spectroscopic analysis: δ 2.26 (9H, s, CH₃ in thioanisole), 2.82 (3H, s, CH₃ in benzylmethylphenylsulfonium ion), 3.25 (2H, m, C^βH₂ in tyrosine), 4.28 and 4.44 (1H each, AB-type q, *J*_{gem} = 12 Hz, CH₂ in benzylmethylphenylsulfonium ion), 4.48 (1H, m, C^αH in tyrosine). The methylene signal (δ 5.12, s) of the benzyl group in Tyr(Bzl) disappeared completely and no methylene signal (δ 4.02, s) of the benzyl group in 3-benzyltyrosine was detected.

(b) Reaction with Dimethyl Sulfide-TFA: The procedure described in (a) was repeated with dimethyl sulfide (0.35 ml, 5 mmol) in place of thioanisole.

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(c) Reaction with Anisole-TFA: The above procedure was repeated with anisole (0.54 ml, 5 mmol) in place of dimethyl sulfide.

Deprotection of Ser(Bzl)—(a) Reaction with Thioanisole-TFA: Ser(Bzl) (17.7 mg, 0.1 mmol) was treated with thioanisole (0.62 ml, 5 mmol)-TFA (2 ml) at 25°. The time course of the reaction was followed by TLC, scanning the plates with a TLC scanner. The results are shown in Fig. 2.

(b) Reaction with Dimethyl Sulfide-TFA: The above procedure was repeated with dimethyleulfide (0.35 ml, 5 mmol) in place of thioanisole.

(c) Reaction with Anisole-TFA: The above procedure was repeated with anisole (0.54 ml, 5 mmol) in place of dimethyl sulfide.

Deprotection of Thr(Bzl)—(a) Reaction with Thioanisole-TFA: Thr(Bzl) (19.1 mg, 0.1 mmol) was treated with thioanisole (0.62 ml, 5 mmol)-TFA (2 ml) at 25°. The time course of reaction was followed by TLC, scanning the plates with a TLC scanner. The results are shown in Fig. 3.

(b) Reaction with Dimethyl Sulfide-TFA: The above procedure was repeated with dimethyl sulfide (0.35 ml, 5 mmol) in place of thioanisole.

(c) Reaction with Anisole-TFA: The above procedure was repeated with anisole (0.54 ml, 5 mmol) in place of dimethyl sulfide.

Deprotection of Lys(Z) with Thioanisole-TFA—Lys(Z) (28.0 mg, 0.1 mmol) was treated with thioanisole (0.62 ml, 5 mmol)-TFA (2 ml) at 23° for 3 hr. TLC of the reaction mixture showed one spot (R_f 0.03), and Lys(Z) (R_f 0.41) was not detected.

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