Tetrafluoroboric Acid as a Useful Deprotecting Reagent in Fmoc-based Solid-phase Peptide Syntheses† (Fmoc = fluoren-9-ylmethoxycarbonyl)

Kenichi Akaji, Makoto Yoshida, Tadashi Tatsumi, Tooru Kimura, Yoichi Fujiwara, and Yoshiaki Kiso*

Department of Medicinal Chemistry, Kyoto Pharmaceutical University, Yamashina-ku, Kyoto 607, Japan

Tetrafluoroboric acid in trifluoroacetic acid, in the presence of thioanisole, has been found to cleave various protecting groups currently employed in Fmoc-based (Fmoc = fluoren-9-ylmethoxycarbonyl) solid-phase peptide synthesis without significant side reactions; this new deprotecting reagent has been successfully applied to the solid-phase synthesis of human glucagon (a 29 residue peptide) and α-MSH (a 13 residue peptide amide, acetyl-Ser-Tyr-Ser-Met-Glu-His-Phe-Arg-Trp-Gly-Lys-Pro-Val-NH_2).

Tetrafluoroboric acid (HBF_4) in trifluoroacetic acid (TFA) is a weaker acid than HBr in TFA.[1] We have found that the 4-methoxy-2,3,6-trimethylbenzenesulphonyl (Mtr)[2] group from Arg(Mtr) can be cleaved under mild conditions using HBF_4–thioanisole (1 M) in TFA within 30 min at 4 °C. This deprotecting reagent can cleave the t-butanol-based protecting groups employed in peptide synthesis. Here, we report that HBF_4–thioanisole (1 M) in TFA is a useful final deprotecting reagent in Fmoc-based[3] solid-phase peptide syntheses (Fmoc = fluoren-9-ylmethoxycarbonyl).

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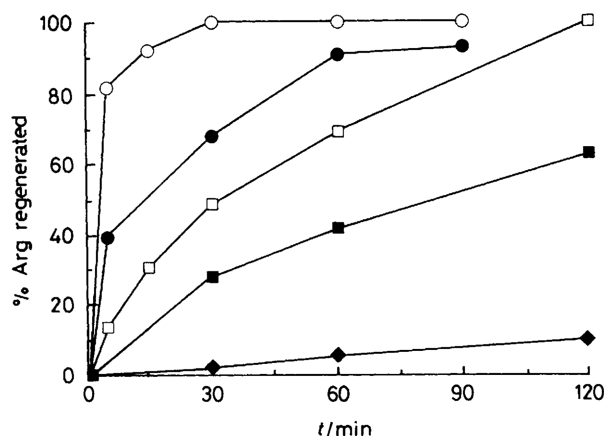

Figure 1. Cleavage of Mtr group from Arg(Mtr). ○, HBF_4–thioanisole (4 °C); ●, HBF_4–dimethylsulphide (4 °C); □, TFA–thioanisole (25 °C); ■, TFA–phenol (25 °C); ◆, TFA–dimethylsulphide (25 °C).

The cleavage rate of the Mtr group from Arg(Mtr) with HBF_4 (25 mol equiv., 4 °C) or TFA (25 °C) was examined using a Shimadzu dual wavelength TLC-scanner (Figure 1). The rate of this cleavage reaction with HBF_4 (1 M) in TFA is much faster than that using TFA–thioanisole,[4] which is a standard deprotecting reagent in Fmoc-based peptide synthesis. Thioanisole showed a more remarkable rate accelerating effect than dimethylsulphide, as in the case of the trifluoromethanesulphonic acid[5] or boron tristrifluoroacetate[6] deprotection procedures. Thus, HBF_4–thioanisole (1 M) in TFA‡ was considered to be a suitable deprotecting system and its usefulness as a deprotecting and cleavage reagent in Fmoc-based peptide synthesis was examined.

Each amino acid derivative was treated with this new reagent (25 mol equiv. per protecting group) in the presence of *m*-cresol at 4 °C and the recovery of each parent amino acid was examined quantitatively on an amino acid analyser (Table 1). Complete removal of Bu^t groups at the side chains of Ser, Thr, Tyr, Asp, and Glu, the t-butoxycarbonyl (Boc) group at Lys, the t-butoxymethyl (Bm)[7] group at His, the 4,4′-dimethylbenzhydryl (Mbh)[8] groups at Asn and Gln, and the Mtr group at Arg could be achieved within 30 min with this reagent. In the presence of ethanedithiol, quantitative regeneration of Trp from Boc-Trp-OH was obtained after 120 min treatment, but in the absence of ethanedithiol, the recovery of Trp remained nearly 80%, due to the alkylation of the indole moiety of Trp.[9] Of the various *S*-protecting groups of Cys, 4-methoxybenzyl (MBzl) and Bu^t were cleaved quantitatively within 60 min, but 4-methylbenzyl (MeBzl) was cleaved incompletely. *S*-Acm[10] cysteine was kept intact after 120 min treatment with this reagent, even in the presence of thioanisole.[11]

The cleavage of amino acid from *p*-benzyloxybenzylalcohol resin (Wang resin)[12] was examined similarly on an amino acid analyser. Amino acids including Ile, which possess a bulky side chain, could be cleaved from the resin within 30 min with this reagent. The cleavage rate of amino acid amide from *p*-methylbenzhydrylamine (MBHA) resin[13] was estimated from acid hydrolysis of the resin after the HBF_4 treatment.

‡ To prepare 10 ml of this reagent system, 10 mmol each of the HBF_4–diethyl ether complex (Aldrich Chemical Co.) and thioanisole were dissolved in TFA and the total volume was adjusted to 10 ml with TFA.

Table 1. Removal of various protecting groups by HBF_4–thioanisole (1 M) in TFA at 4 °C.

Treated amino acid derivative	% Parent amino acid regenerated 30 min	60 min	120 min
H-Thr(Bu^t)-OH	115.2		
H-Tyr(Bu^t)-OH	102.1		
H-Ser(Bu^t)-OH	95.8		
H-Glu(OBu^t)-OH	98.3		
H-Asp(OBu^t)-OH	95.8		
H-Lys(Boc)-OH	102.3		
H-His(Bm)-OH	98.9		
H-Asn(Mbh)-OH	112.4		
H-Gln(Mbh)-OH	100.0		
H-Arg(Mtr)-OH	102.6		
Boc-Trp-OH[a]		100.4	104.2
Boc-Cys(MBzl)-OH		100.0	
Boc-Cys(Bu^t)-OH		100.0	
Boc-Cys(MeBzl)-OH		86.2	93.7
Boc-Cys(Acm)-OH[b]		0	0
H-Pro-Wang resin	108.4		
H-Lys(Boc)-Wang resin	94.5		
H-Thr(Bu^t)-Wang resin	100.0		
H-Ile-Wang resin	98.7		
H-Gly-MBHA resin		100.0	
H-Ile-MBHA resin		96.7	
H-Leu-MBHA resin		96.1	
H-Phe-MBHA resin		86.9	92.0
H-Val-MBHA resin		83.5	96.6
H-Val-DMAMP resin	100.0		
H-Gly-DMAMP resin	100.0		

[a] Ethanedithiol was used as an additional scavenger. [b] Acm = acetamidomethyl.

Amino acid amides examined were cleaved from the resin within 60 min, except for Val-NH_2 and Phe-NH_2, for which more prolonged treatment (120 min) was necessary for quantitative cleavage. However, Val-NH_2 was quantitatively cleaved from acid-labile 4-(2′,4′-dimethoxyphenyl-aminomethyl)phenoxy type (DMAMP) resin[14] by treatment with this reagent for 30 min at 4 °C.

In order to demonstrate the usefulness of HBF_4–thioanisole (1 M) in TFA for Fmoc-based solid-phase peptide synthesis, we have synthesized human glucagon (H-His-Ser-Gln-Gly-Thr-Phe-Thr-Ser-Asp-Tyr-Ser-Lys-Tyr-Leu-Asp-Ser-Arg-Arg-Ala-Gln-Asp-Phe-Val-Gln-Trp-Leu-Met-Asn-Thr-OH). This peptide contains Trp, two Arg residues, and an Asp-Ser sequence, which is known easily to form succinimide.[15] The protected peptide resin [H-His(Bm)-Ser(Bu^t)-Gln-Gly-Thr(Bu^t)-Phe-Thr(Bu^t)-Ser(Bu^t)-Asp(OBu^t)-Tyr(Bu^t)-Ser(Bu^t)-Lys(Boc)-Tyr(Bu^t)-Leu-Asp(OBu^t)-Ser(Bu^t)-Arg-(Mtr)-Arg(Mtr)-Ala-Gln-Asp(OBu^t)-Phe-Val-Gln-Trp-Leu-Met-Asn-Thr(Bu^t)-$OCH_2C_6H_4OCH_2C_6H_4$-polymer], was constructed manually using Wang resin (0.71 mmol g^{-1} resin) according to the procedure proposed by Sheppard *et al.*[16] The final deprotection and purification were carried out as follows. (i) Treatment with the above reagent in the presence of *m*-cresol and ethanedithiol at 0 °C for 60 min, (ii) precipitation with dry diethyl ether, (iii) dissolving the residue in AcOH (4 M) and removal of the resin by filtration, and (iv) purification by gel filtration on Sephadex G-15, followed by fast protein liquid chromatography (FPLC, Pharmacia) on a column packed with YMC gel ODS-AQ 120A S-50 using a gradient of 60% aq. acetonitrile (0—100%) in 0.1% aq. TFA.

The homogeneous peptide§ was obtained in 33% yield (based on the starting C-terminal residue) and possessed the same retention time on HPLC as that of the commercial sample.¶

Next, this reagent was applied to the Fmoc-based solid-phase synthesis of α-MSH (acetyl-Ser-Tyr-Ser-Met-Glu-His-Phe-Arg-Trp-Gly-Lys-Pro-Val-NH_2) using MBHA resin (0.38 mmol g^{-1} resin, 400 mg). The cleavage of this acetyl peptide amide from MBHA resin is known to be difficult even with trimethylsilyl trifluoromethanesulphonate.[17] Deprotection and purification of the protected α-MSH-resin [acetyl-Ser(Bu^t)-Tyr(Bu^t)-Ser(Bu^t)-Met-Glu(OBu^t)-His(Bm)-Phe-Arg(Mtr)-Trp-Gly-Lys(Boc)-Pro-Val-MBHA resin] were carried out as follows: (i) treatment with this new reagent twice in the presence of *m*-cresol and ethanedithiol at 25 °C for 90 min, (ii) precipitation with dry diethyl ether, (iii) dissolving the residue in AcOH (4 M) and removal of the resin by filtration, (iv) purification by FPLC using the same conditions as described for glucagon synthesis. The homogeneous peptide amide ‖ (33% yield, based on the starting C-terminal resin) possessed the same retention time on HPLC as that of the commercial sample.¶

Furthermore, protected α-MSH was prepared using DMAMP resin (0.42 mmol g^{-1} resin), by the Fmoc-based solid-phase method, and deprotected with HBF_4–thioanisole (1 M) in TFA in the presence of *m*-cresol and ethanedithiol. Milder deprotecting conditions (4 °C, 120 min) than those used for the former experiment were sufficient to obtain the homogeneous peptide amide in 34% yield.

§ Amino acid ratios in HCl (6 M) hydrolysate and leucine aminopeptidase (LAP, Sigma) digestion (in parentheses); Asp 3.97 (3.00), Thr 2.82 (N.D.), Ser 3.60 (3.94), Glu 2.97 (N.D.), Gly 0.99 (1.00), Ala 1.00 (1.00), Val 0.98 (1.06), Met 1.01 (1.09), Leu 2.00 (2.04), Tyr 1.98 (1.99), Phe 1.95 (1.99), Lys 0.97 (1.00), His 0.97 (1.00), Trp 0.49 (0.98), Arg 1.97 (1.97); HPLC [YMC AM302, 4.6 × 150 mm, MeCN (20—40%, 30 min) in aq. 0.1% TFA, 0.7 ml min^{-1}], retention time 25.8 min. (N.D. = not determined.)

¶ The commercial sample was purchased from Peptide Institute, Inc., Osaka, Japan.

‖ Amino acid ratios in HCl (6 M) hydrolysate; Ser 1.85, Glu 1.06, Pro 1.07, Gly 1.06, Val 1.00, Met 0.99, Tyr 1.05, Phe 1.03, Lys 1.03, His 1.03, Trp 1.01 (determined by LAP digestion), Arg 1.04; HPLC [YMC AM302, 4.6 × 150 mm, MeCN (10—60%, 30 min) in aq. 0.1% TFA, 0.7 ml min^{-1}], retention time 16.4 min.

These excellent results show the potential of this new deprotecting procedure for peptide syntheses.

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