TETRAFLUOROBORIC ACID, A USEFUL DEPROTECTING REAGENT IN PEPTIDE SYNTHESIS[1, 2]

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We have found that tetrafluoroboric acid (HBF_4) in trifluoroacetic acid (TFA) in the presence of thioanisole cleaves various protecting groups currently used in peptide synthesis. HBF_4 in TFA cleaves an amino acid amide from 4-methylbenzhydrylamine resin more effectively than trifluoromethanesulfonic acid in TFA. Lamprey gonadotropin-releasing hormone (a 10-residue peptide amide) was synthesized using 1 M HBF_4-thioanisole in TFA by both solution-phase and solid-phase methods.

KEYWORDS tetrafluoroboric acid; final deprotecting reagent; solution-phase peptide synthesis; solid-phase peptide synthesis; peptide amide; gonadotropin-releasing hormone synthesis

Tetrafluoroboric acid (HBF_4) in trifluoroacetic acid (TFA) is a weaker acid than HBr in TFA[3]. We now report the usefulness of this mild reagent, in the presence of thioanisole, as a final deprotecting reagent in peptide synthesis.

Each amino acid derivative currently used in peptide synthesis was treated with 1 M HBF_4-thioanisole in TFA at 4°C in the presence of the additional scavenger, *m*-cresol, and the recovery of each parent amino acid was examined on an amino acid analyzer (Table I). Together with α-amino protecting

Table I. Removal of Various Protecting Groups by 1M HBF4-thioanisole in TFA (at 4°C, 60 min)

Amino acid derivatives	%a)	Amino acid derivatives	%a)
Boc-Asp(OBzl)-OH	104.7	Boc-Lys(Cl-Z)-OH	95.3
Boc-Asp(OcHex)-OH	24.3	Boc-Tyr(Cl2-Bzl)-OH	100.3
Boc-Asp(OcHex)-OHb)	106.3	Boc-His(Bom)-OH	97.5
Boc-Glu(OBzl)-OH	92.4	Boc-Trp(Ppt)-OH	107.5
Boc-Thr(Bzl)-OH	102.3	Boc-Arg(Mts)-OH	70.1
Boc-Ser(Bzl)-OH	103.4	Boc-Arg(Mts)-OHb	98.8
Boc-Lys(Z)-OH	94.3	Z(OMe)-Met(O)-OH	43.8

a) % of regenerated parent amino acid. b) treated at 25°C.

Table II. Cleavage of H-Gly-NH_2 from Boc-Gly-MBHA Resin at 4°C

Reagent	Time(h)	Gly(%)[a]	Reagent	Time(h)	Gly(%)[a]
1M HBF_4-thioanisole	2	≒0%	1M TFMSA-thioanisole	3	21%
HF-m-cresol	1	≒0%	2M TMSI-thioanisole	3	99%

a) % of Gly remained on the acid-treated MBHA resin

groups such as Boc or Z(OMe), the Z or Cl-Z group at the side chain of Lys, and the Bzl groups at Ser, Thr, Asp, and Glu were cleaved quantitatively within 60 min with this reagent. Complete removal of the Cl_2-Bzl group from Tyr, the Bom group from His, and the Ppt[4] group from Trp were also achieved within 60 min. However, a 60-min treatment with this reagent at an elevated temperature (25°C) was necessary to completely remove the cHex group from Asp and the Mts group from Arg. Met(O) was reduced back to Met in 44% yield with this reagent.

In solid-phase peptide synthesis, it has been claimed that occasionally, peptide amides could not be cleaved quantitatively from 4-methylbenzhydrylamine (MBHA) resin[5]. So, we examined the cleavage of Gly-NH_2 from Boc-Gly-MBHA resin with this new reagent as well as three other cleavage reagents:1M trifluoromethanesulfonic acid (TFMSA)[6]-thioanisole in TFA, 2M trimethylsilyl iodide (TMSI)[7]-thioanisole in TFA, and HF-*m*-cresol at 4°C, respectively. As shown in Table II, 1M HBF_4-thioanisole in TFA quantitatively cleaved Gly-NH_2 within 2 h, as did HF (1 h treatment). However, Gly-NH_2 was cleaved incompletely (ca. 80%) by the treatment of 1M TFMSA-thioanisole in TFA for 3 h, and 2M TMSI-thioanisole in TFA failed to cleave the amino acid amide from the resin. Ile-NH_2, which has a bulky side chain, was also cleaved quantitatively within 60 min with 1M HBF_4-thioanisole in TFA at 4°C. In addition, this reagent cleaved Gly, Ala, and Thr(Bzl) from the corresponding 4-(oxymethyl)-phenylacetamidomethyl (Pam) resin[8] within 120 min at 4°C.

In order to demonstrate the usefulness of 1M HBF_4-thioanisole in TFA as a deprotecting reagent for solution-phase peptide synthesis, we have synthesized lamprey gonadotropin-releasing hormone[9] (Fig.1). The protected peptide amide [mp. 181-184°C: $[\alpha]_D^{19}$-14.8° (c=0.5, DMF): Amino acid ratios in 4N MSA hydrolysate; Ser 0.83, Glu 1.87, Pro 1.00, Gly 1.00, Leu 0.99, Tyr 0.87, Lys 0.96, His 0.95, Trp 0.78 (Recovery of Gly 92%): *Anal.* Calcd. for $C_{93}H_{106}N_{15}O_{19}SP.5H_2O$: C, 58.51; H, 6.23; N, 11.00. Found: C, 58.47; H, 5.92; N, 11.01.], prepared by successive azide condensation of three fragments, was deprotected with this new reagent (60 min, at 4°C) in the presence of *m*-cresol and ethanedithiol. The product was purified by fast protein liquid chromatography (FPLC, Pharmacia) on a column packed with YMC gel ODS-AQ 300A S-50 (20 x 500

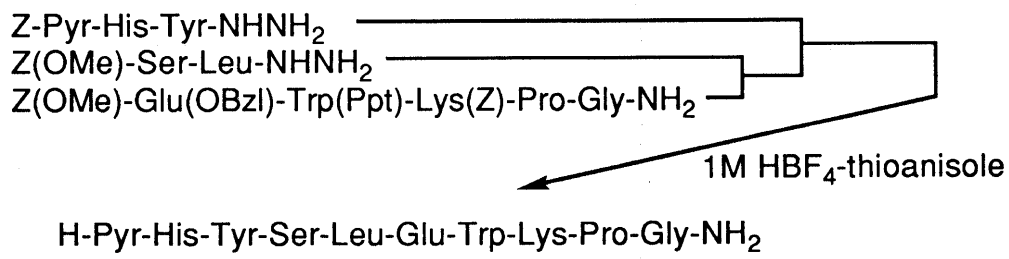

Fig.1. Synthetic Route for Lamprey Gonadotropin-Releasing Hormone

mm) using a gradient of aq. 60% MeCN (0-100%, 400 min) in aq. 0.1% TFA. The purity of the product thus obtained {$[\alpha]_D^{24}$-43.1° (c=0.6, H_2O), 39% yield from the protected peptide amide} was confirmed by analytical HPLC on a Cosmosil 5C18 ST column (4.6 x 150 mm): retention time 15.8 min, when eluted with a gradient of MeCN (10-60%, 30 min) in 0.1% aq. TFA at a flow rate of 0.7 ml/min. Acid hydrolysis with 4N MSA gave amino acids in ratios predicted by theory (numbers in parentheses): Ser 0.98 (1), Glu 1.92 (2), Pro 0.94 (1), Gly 1.00 (1), Leu 0.99 (1), Tyr 0.95 (1), His 0.94 (1), Lys 0.95 (1), Trp 0.99 (1) (recovery of Gly 90%). The observed mass value [$(M+H)^+$] on fast atom bombardment mass spectrum (FAB-MS) was 1226.641 (theoretical, 1226.596).

Next, this reagent was applied to the solid-phase synthesis of the same peptide amide using MBHA resin (0.75 mmol/g resin). Protected peptide resin [Z-Pyr-His-Tyr(BrZ)-Ser(Bzl)-Leu-Glu(OBzl)-Trp(Ppt)-Lys(Cl-Z)-Pro-Gly-MBHA resin] was prepared automatically on a Beckman System 990E Peptide Synthesizer by successive two-step cycle reactions, *i.e.*, removal of Boc group by 0.5M MSA in dioxane-DCM[10] (30 min) and condensation of the respective amino acid (2.5 eq.) by DCC in DMF. For final deprotection and cleavage, the peptide resin was treated with 1M HBF_4-thioanisole in TFA in the presence of *m*-cresol and ethanedithiol for 3 h at 4°C. Subsequent purification was carried out by the same procedure described for previous solution-phase synthesis. The homogeneous peptide amide was obtained in 28% yield (based on the starting C-terminal residue). It had the same retention time on HPLC as the previous sample.

From these experimental results, we conclude that 1 M HBF_4-thioanisole in TFA is a useful final deprotecting reagent in peptide synthesis.

REFERENCES AND NOTES

1) Presented in part at the 38th Annual Meeting of Kinki Branch, Pharmaceutical Society of Japan, Nov. 6, 1988, Higashiosaka, Japan: Abstracts, p31.
2) Abbreviations: Boc=tert-butoxycarbonyl, Z(OMe)=4-methoxybenzyloxycarbonyl, Bzl=benzyl, Cl_2-Bzl=2,6-dichlorobenzyl, Z=benzyloxycarbonyl, Cl-Z=2-chlorobenzyloxycarbonyl, Br-Z=2-bromobenzyloxycarbonyl, cHex=cyclohexyl, Mts=mesitylene-2-sulfonyl, Ppt=diphenylphosphinothioyl, Bom=benzyloxymethyl, MSA =methanesulfonic acid, DCM=dichrolomethane.
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