Pyridinium Polyhydrogen Fluoride, a Deprotecting Reagent in Peptide Chemistry

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Summary Pyridinium polyhydrogen fluoride was found to cleave efficiently a number of protecting groups currently employed in peptide chemistry without significant side reactions.

ANHYDROUS hydrogen fluoride is widely used for the final step of removing various protecting groups currently employed in peptide chemistry.[1] It is also widely used to remove the completed peptide from the resin in Merrifield solid-phase peptide synthesis.[2]

Up to 70% HF can be dissolved in pyridine, giving a stable solution which contains some free HF in equilibrium.[3] Thus, it would seem to be acting as a reservoir for HF in a convenient liquid medium. This stable reagent allows typical HF reactions to be carried out conveniently in a simple polyethylene tube.

Recently we have found that pyridinium polyhydrogen fluoride (70% HF–pyridine) can be used to remove various protecting groups currently employed in peptide synthesis, as well as the completed peptide from the Merrifield resin. In the presence of anisole (0·2 ml) as a scavenger, each amino-acid derivative (100 μmol) was treated with the reagent (2 ml) at room temperature for 1 h. After removal of HF and pyridine, 0·2N acetic acid was added. An aliquot portion of the solution was subjected to quantitative amino-acid analysis and t.l.c. Pyridinium polyhydrogen fluoride removed most of the protecting groups listed in the Table almost quantitatively, except for the benzyl and nitro groups attached to the side chain functions of His and Arg, respectively. Regeneration of Arg from Boc-Arg(NO_2)-OH was not quantitative (36%). Somewhat low recovery of Tyr from Boc-Tyr(Bzl)-OH also resulted, possibly from the formation of 3-benzyltyrosine.[4] Regeneration of Arg and His from Aoc-Arg(Tos)-OH and Boc-His(Tos)-OH was achieved in yields of 92 and 95%, respectively.

TABLE. Removal of various protecting groups by pyridinium polyhydrogen fluoride

Treated amino-acid derivatives	Amino acid regenerated (%) Room. temp., 60 min
Boc-Asp(OBzl)-OH	98[a]
Boc-Glu(OBzl)-OH	100
Boc-Ser(Bzl)-OH	93
Boc-Thr(Bzl)-OH	95
Boc-Arg(NO_2)-OH	36
Aoc-Arg(Tos)-OH	95
H-Lys(Z)-OH	100
Boc-Lys[Z(O-Cl)]	90
Z-Val-OH	100
Z-Ser-OH	93
Boc-Tyr(Bzl)-OH	68
Boc-His(Bzl)-OH	0
Boc-His(Tos)-OH	95
Boc-Cys-S-[Bzl(*p*-OMe)]-OH	93
Boc-Met-OH	92
Nps-Trp-OH	90
H-Phe-OBzl	94
H-Lys(Boc)-OBu^t	97
Boc-Leu-resin	96
Boc-Pro-resin	92
Boc-Pyroglu-Trp-Gly-Benzhydrylamine resin	57[b]

[a] 12% after 30 min at 0 °C, 32% after 60 min at 0 °C, and 61% after 30 min at room temperature. [b] After purification with Sephadex G-25 column chromatography.

This deprotecting reagent was also examined in solid phase synthesis. Free Leu (96%) and Pro (92%) were liberated from Boc-Leu-resin (Leu content, 0·36 mmol g^{-1}

resin) and Boc-Pro-resin (Pro content, 0·90 mmol g^{-1} resin), respectively after treatment with this reagent in the presence of anisole at room temperature for 1 h. The tripeptide TRH analogue, Pyroglu-Trp-Gly-NH_2, was also obtained in 57% yield (after purification with Sephadex G-25 column chromatography) from protected peptide benzhydrylamine resin[5] on treatment with this reagent under the same conditions.

From the results so far obtained, pyridinium polyhydrogen fluoride seems to be an efficient reagent in the deprotection of synthetic peptides, and is easier and safer to use than anhydrous liquid HF.

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