Trimethylsilyl Trifluoromethanesulphonate as a Useful Deprotecting Reagent in Both Solution and Solid Phase Peptide Syntheses

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Trimethylsilyl trifluoromethanesulphonate in trifluoroacetic acid has been found to cleave, in the presence of thioanisole, a number of protecting groups currently employed in peptide synthesis, without significant side reactions and with a much faster rate of reaction than trifluoromethanesulphonic acid in trifluoroacetic acid; this new deprotecting reagent has been used in solution and solid phase peptide syntheses of neuromedin U-25 (a 25-residue peptide) and a rabbit stomach peptide (an 8-residue peptide), respectively.

We have found that various protecting groups currently employed in the present peptide synthesis can be deprotected by treatment with 1 M trimethylsilyl trifluoromethanesulphonate (trimethylsilyl triflate, TMSOTf)–thioanisole (molar ratios 1:1) in trifluoroacetic acid (TFA), followed by ready hydrolysis of the resulting trimethylsilylating compounds with water or ammonium fluoride.[1] The rate of this cleaving reaction is much faster than that using 1 M trifluoromethanesulphonic acid (TFMSA)–thioanisole in TFA.[2] TMSOTf, a silyl ester, not a Brönsted acid, with a powerful silylating ability,[3] seems to play a role as a super hard acid[4] in TFA (^{29}Si n.m.r. chemical shift: δ 46.1 p.p.m., Me_4Si as an internal standard, lit.,[5] 44.6 p.p.m. in benzene). The thioanisole also seems to take part in this cleaving reaction, acting as a soft base,[6] as discussed for TFMSA deprotection[7] (Scheme 1).

Each amino acid derivative was treated with this new reagent (30 mol equiv. per protecting group) in the presence of *m*-cresol† at ice-bath temperature and periodically a part of the solution was subjected to quantitative amino acid analysis (Table 1). Together with acid-labile N^{α}-protecting groups, such as Boc and Z(OMe), the Z-group at the side chain function of Lys, and the Bzl groups at Ser, Thr, Glu, and Asp were cleaved within 10 min. The secondary alkyl Chp ester[8] was completely removed from Asp within 30 min. Treatment of Tyr(Bzl) and Tyr(Cl_2-Bzl)[9] with this reagent for 10 min regenerated Tyr in nearly quantitative yields, but in the absence of thioanisole, recovery of Tyr from Tyr(Bzl) remained at 64%, and in the absence of both thioanisole and *m*-cresol, recovery was 37%, due to the formation of a rearrangement product, 3-benzyltyrosine.[10] Complete removal of the Mts group from Trp(Mts)[11] could be achieved more readily than TFMSA–thioanisole in TFA treatment, when ethanedithiol (10 equiv. per Trp) was used as an additional scavenger. Regeneration of His from His(Tos) and His(Bom),[12] and Arg from Arg(Mts)[13] and Arg(MBS)[14] were achieved quantitatively after treatment with this reagent for 10 to 30 min. As with TFMSA treatment, the N^{G}-NO_2 group resisted the action of this reagent, but we found it was possible to remove the N^{G}-Tos group from Arg after 120 min using this thioanisole-mediated treatment. Of the various *S*-protecting groups of Cys, MBzl, Bu^t, and Ad[15] were cleaved quantitatively, but Bzl and Acm[16] remained intact. It is noteworthy that Met(O) was reduced back to Met more effectively when thioanisole was replaced by dimethylselenide.[17]

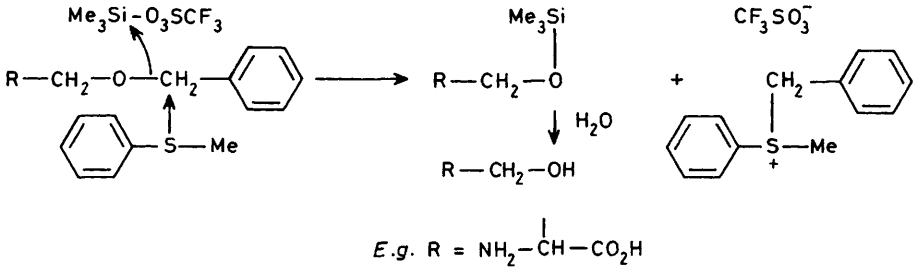
In order to examine the usefulness of 1 M TMSOTf–thioanisole in TFA as a deprotecting reagent for solution phase peptide synthesis, we synthesized a porcine spinal cord peptide, designated neuromedin U-25.[18] The protected form of neuromedin U-25 was prepared by successive azide condensations of six peptide fragments (Scheme 2), then deprotection and purification were performed as follows: (i) treatment with the above reagent in the presence of *m*-cresol at 0 °C for 60 min; (ii) precipitation with dry diethyl ether; (iii) treatment with ammonium fluoride (25 equiv.) in 5% ammonia at pH 8.0 for 30 min to hydrolyse trimethylsilylated compounds and reverse any possible N → O shift at the Ser residue; (iv) purification by gel-filtration on Sephadex G-25, followed by reversed phase high performance liquid chromatography (h.p.l.c.) on a TSK-GEL LS-410KG column with

Table 1. Removal of various protecting groups by 1 M TMSOTf–thioanisole–TFA.

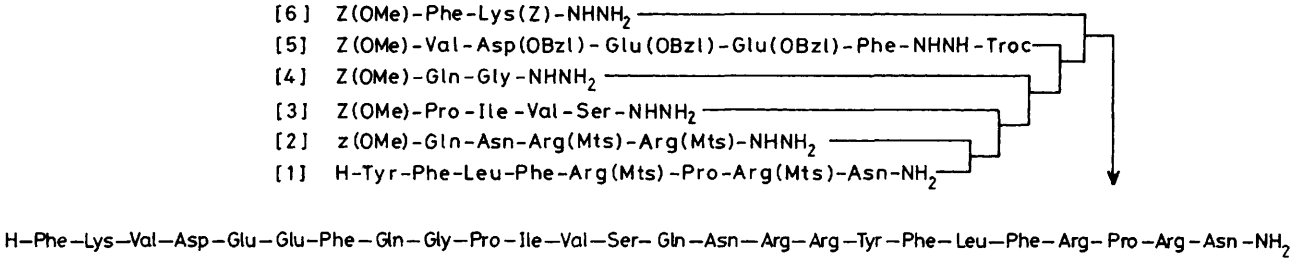
Treated amino acid derivatives	% Parent amino acid regenerated		
	10 min	30 min	60 min
Z(OMe)-Lys(Z)-OH	93.6	98.7	
Z(OMe)-Ser(Bzl)-OH	90.6	91.7	
Boc-Thr(Bzl)-OH	98.0		
Z(OMe)-Glu(OBzl)-OH	100.0		
Z(OMe)-Asp(OBzl)-OH	99.2		
Boc-Asp(OChp)-OH	95.3	100.0	
Boc-Tyr(Bzl)-OH[a]	97.8		
Boc-Tyr(Cl_2-Bzl)-OH[a]	98.1		
Boc-His(Tos)-OH	94.5		
Boc-His(Bom)-OH[a]	88.9		
Boc-Trp(Mts)-OH	100.0		
Z(OMe)-Arg(Mts)-OH	97.6		
Z(OMe)-Arg(MBS)-OH	75.5	93.5	94.2
Z-Arg(Tos)-OH	31.3	62.1	85.8
Z-Arg(NO_2)-OH	8.6	11.0	14.7
H-Cys(MBzl)-OH	95.4		
Boc-Cys(Bu^t)-OH	79.5	87.3	96.8
H-Cys(Ad)-OH	100.0		
H-Cys(Bzl)-OH	0		
Boc-Cys(Acm)-OH	0		
Z(OMe)-Met(O)-OH	17.8	27.6	44.2
Z(OMe)-Met(O)-OH[b]	88.8	90.0	90.6

[a] No other products were detected. [b] Thioanisole was replaced by Me_2Se. *Abbreviations:* Z = benzyloxycarbonyl, Z(OMe) = *p*-methoxybenzyloxycarbonyl, Boc = t-butoxycarbonyl, Bzl = benzyl, Chp = cycloheptyl, Cl_2-Bzl = 2,6-dichlorobenzyl, Tos = *p*-toluenesulphonyl, Mts = mesitylenesulphonyl, MBS = *p*-methoxybenzenesulphonyl, Bom = benzyloxymethyl, MBzl = *p*-methoxybenzyl, Ad = 1-adamantyl, Acm = acetamidomethyl.

† As a cation scavenger, thioanisole is better than anisole for Bzl-type protecting groups, while *m*-cresol is better than thioanisole for protecting Tyr (N. Fujii, S. Funakoshi, T. Sasaki, and H. Yajima, *Chem. Pharm. Bull.*, 1977, **25**, 3096; and ref. 13). We prefer to use *m*-cresol (10 mol equiv. per Tyr) as an additional cation scavenger, even though its phenolic group may be partly trimethylsilylated.



Scheme 1. Deprotection reaction by TMSOTf–thioanisole in TFA.



Scheme 2. Synthetic scheme for neuromedin U-25. Prior to each condensation, the Z(OMe) group was removed by TFA–anisole and the Troc (2,2,2-trichloroethyloxycarbonyl) group by Zn–AcOH.

isocratic elution of 26% acetonitrile in 0.1% aq. TFA. The yield was 52% from the protected peptide, while TFMSA deprotection gave 47% yield, after 150 min treatment.

Next, this reagent was applied to the solid phase synthesis of a rabbit stomach peptide,[19] Pyr-Val-Asp-Pro-Asn-Ile-Gln-Ala-OH. The protected octapeptide resin was prepared according to Merrifield's procedure,[20] then deprotection and subsequent purification were carried out as follows: (i) suspension of the peptide resin in 1 M thioanisole in TFA; (ii) addition of TMSOTf (to a final concentration of 1 M) and stirring in an ice-bath for 60 min; (iii) removal of the resin by filtration; (iv) washing of the resin with TFA; (v) concentration of the combined filtrate and washing *in vacuo* below 15 °C; (vi) precipitation of the product with dry diethyl ether; (vii) treatment with 5% ammonia containing ammonium fluoride (10 equiv.) at pH 8.0 in an ice-bath for 10 min; (viii) purification by gel-filtration on Sephadex G-10 (deprotection yield; 72%), followed by h.p.l.c. on a TSK-GEL LS-410KG column using isocratic elution of 17% acetonitrile in 0.1% aq. TFA. The overall yield, based on starting loading of Ala to resin, was 27% while TFMSA-deprotection gave 16% yield, after 120 min treatment.

This new deprotecting procedure may serve to improve the final deprotecting step for solution as well as solid phase syntheses of larger and more complex peptides.

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