

## 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.<sup>1</sup> The rate of this cleaving reaction is much faster than that using 1 M trifluoromethanesulphonic acid (TFMSA)-thioanisole in TFA.<sup>2</sup> TMSOTf, a silyl ester, not a Brønsted acid, with a powerful silylating ability,<sup>3</sup> seems to play a role as a super hard acid<sup>4</sup> in TFA (<sup>29</sup>Si n.m.r. chemical shift:  $\delta$  46.1 p.p.m., Me<sub>4</sub>Si as an internal standard, lit.,<sup>5</sup> 44.6 p.p.m. in benzene). The thioanisole also seems to take part in this cleaving reaction, acting as a soft base,<sup>6</sup> as discussed for TFMSA deprotection<sup>7</sup> (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* $\alpha$ -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<sup>8</sup> was completely removed from Asp within 30 min. Treatment of Tyr(Bzl) and Tyr(Cl<sub>2</sub>-Bzl)<sup>9</sup> 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.<sup>10</sup> Complete removal of the Mts group from Trp(Mts)<sup>11</sup> 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),<sup>12</sup> and Arg from Arg(Mts)<sup>13</sup> and Arg(MBS)<sup>14</sup> were achieved quantitatively after treatment with this reagent for 10 to 30 min. As with TFMSA treatment, the *N*<sup>G</sup>-NO<sub>2</sub> group resisted the action of this reagent, but we found it was possible to remove the *N*<sup>G</sup>-Tos group from Arg after 120 min using this thioanisole-mediated treatment. Of the various *S*-protecting groups of Cys, MBzl, Bu<sup>t</sup>, and Ad<sup>15</sup> were cleaved quantitatively, but Bzl and Ac<sup>m</sup><sup>16</sup> remained intact. It is noteworthy that Met(O) was reduced back to Met more effectively when thioanisole was replaced by dimethylselenide.<sup>17</sup>

In order to examine the usefulness of 1 M TMSOTf-

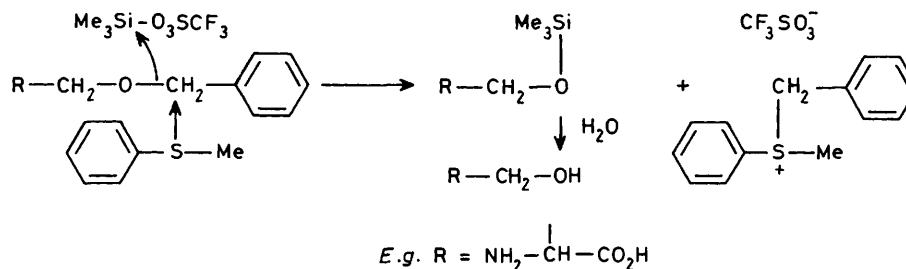
thioanisole in TFA as a deprotecting reagent for solution phase peptide synthesis, we synthesized a porcine spinal cord peptide, designated neuromedin U-25.<sup>18</sup> The protected form of neuromedin U-25 was prepared by successive azide condensations of six peptide fragments (Scheme 2), then deprotection 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  $\rightarrow$  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.

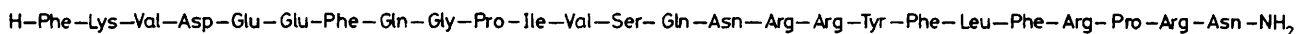
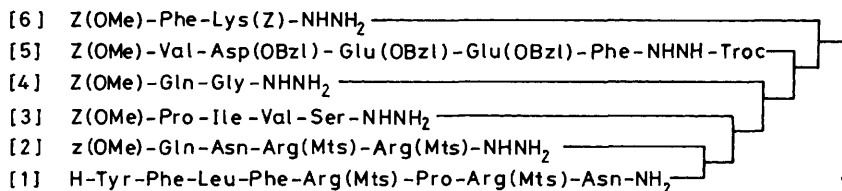
Treated amino acid derivatives	% Parent amino acid regenerated		
	10 min	30 min	60 min
Z(OMe)-Lys(Z)-OH	93.6	98.7	
Z(OMe)-Ser(Bzl)-OH	90.6	91.7	
Boc-Thr(Bzl)-OH	98.0		
Z(OMe)-Glu(OBzl)-OH	100.0		
Z(OMe)-Asp(OBzl)-OH	99.2		
Boc-Asp(OChp)-OH	95.3	100.0	
Boc-Tyr(Bzl)-OH <sup>a</sup>	97.8		
Boc-Tyr(Cl <sub>2</sub> -Bzl)-OH <sup>a</sup>	98.1		
Boc-His(Tos)-OH	94.5		
Boc-His(Bom)-OH <sup>a</sup>	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 <sub>2</sub> )-OH	8.6	11.0	14.7
H-Cys(MBzl)-OH	95.4		
Boc-Cys(Bu <sup>t</sup> )-OH	79.5	87.3	96.8
H-Cys(Ad)-OH	100.0		
H-Cys(Bzl)-OH	0		
Boc-Cys(Ac <sup>m</sup> )-OH	0		
Z(OMe)-Met(O)-OH	17.8	27.6	44.2
Z(OMe)-Met(O)-OH <sup>b</sup>	88.8	90.0	90.6

<sup>a</sup> No other products were detected. <sup>b</sup> Thioanisole was replaced by Me<sub>2</sub>Se. Abbreviations: Z = benzyloxycarbonyl, Z(OMe) = *p*-methoxybenzyloxycarbonyl, Boc = *t*-butoxycarbonyl, Bzl = benzyl, Chp = cycloheptyl, Cl<sub>2</sub>-Bzl = 2,6-dichlorobenzyl, Tos = *p*-toluenesulphonyl, Mts = mesitylenesulphonyl, MBS = *p*-methoxybenzenesulphonyl, Bom = benzyloxymethyl, MBzl = *p*-methoxybenzyl, Ad = 1-adamantyl, Ac<sup>m</sup> = 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-trichloroethoxycarbonyl) 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,<sup>19</sup> Pyr-Val-Asp-Pro-Asn-Ile-Gln-Ala-OH. The protected octapeptide resin was prepared according to Merrifield's procedure,<sup>20</sup> 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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