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IMPROVED DEPROTECTION IN SOLID PHASE PEPTIDE SYNTHESIS: QUANTITATIVE REDUCTION OF METHIONINE SULFOXIDE TO METHIONINE DURING HF CLEAVAGE

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Summary: The reduction of methionine sulfoxide residues in peptides was found to be quantitative by treatment with a low concentration of HF in dimethylsulfide (HF:dimethylsulfide, 1:3, v/v). The reduction was dependent on the acidity function of HF and was optimal with an HF concentration between 25% and 40%. The low HF concentration cleavage reagent also deprotected most of the benzyl alcohol-derived protecting groups.

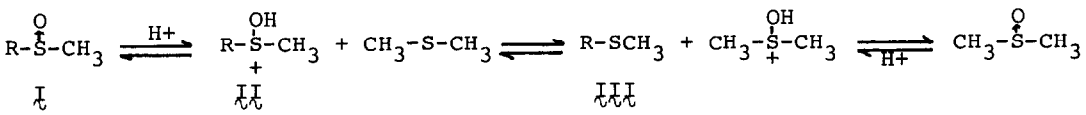
The unresolved question in the synthesis of methionine-containing peptides using a strategy of repetitive weak acid N^{α}-deprotection and final strong acid side chain deprotection and cleavage, is whether or not the thioether side chain of methionine should be protected. The use of unprotected methionine in the synthesis leads to S-alkylation to the sulfonium salt[1] and oxidation to the sulfoxide.[2,3] Since not all S-alkylation is reversible this side reaction is often left to be corrected during the purification step. Alternatively, the use of methionine sulfoxide (Met(O)) in the synthesis avoids the S-alkylation side reaction because of the reduced nucleophilicity of the thioether side chain.[2-4] However, both strategies result in the similar uncertainty of converting Met(O) to Met before the purification step.

In general, methionine sulfoxide is stable to HF cleavage conditions and requires subsequent thiolytic reduction in aqueous solution after the removal of all other protecting groups. This reduction is always slow and often accompanied by side reactions.[5] Thus, a method that will reduce methionine sulfoxide in strong acid and concommitantly deprotect side chain protecting groups in one single manipulation is highly desirable. In this paper, we wish to report a new reagent: HF/dimethylsulfide (1:3, v/v) which reduces methionine sulfoxide to methionine and deprotects most side chain protecting groups.

Oxygen exchange and S-O bond breakage of sulfoxides, as measured by the rate of racemization and reduction, are both known to occur in strong acid although the mechanism (either S_N1 or S_N2) depends on the acidity function and the nucleophilicity of the acid anion.[6] As shown in Table 1, Met(O) was found to be stable to the usual strong acid deprotecting step with HF-anisole or HF-p-cresol. The results indicate that neither the aromatic solvent nor the fluoride anion was nucleophilic enough to reduce Met(O). However, in the presence of a halide acceptor such as acetone[2] or indole[7] both HCl and HBr become efficient reducing agents of Met(O) by an S_N2 mechanism. Recently,

several reports have described an oxygen-exchange reaction between sulfoxides and sulfides via the sulfonium ion using concentrated halo acids or trifluoroacetic anhydride.[6,8] A similar oxygen-exchange reaction between thioanisole and Met(O) in trifluoromethanesulfonic acid-trifluoroacetic acid has been reported.[9] Thus, it is reasonable to assume that reduction of Met(O) can occur in anhydrous HF if a suitable reductant is used. When Met(O) was treated with HF-dimethylsulfide (9:1, v/v) for 1 h at 0° C, reduction of Met(O) to Met was clearly observed but only in 10-15% yield (Fig I). Replacement of dimethylsulfide with the more nucleophilic thioanisole did not significantly improve the reduction yield. These results were consistent whether Met(O) was free or incorporated into a peptide chain.

Our lack of success in the reduction of Met(O) to Met using a high HF concentration in dimethylsulfide led us to examine the mechanism of the reduction. Since the reduction is effected by the dimethylsulfide, it requires the sulfoxide to be protonated by HF to form the oxysulfonium salt (II) and the reductant, dimethylsulfide, to be unprotonated. That is, the acidity function (H_o) of the HF-DMS mixture required for the reduction of Met(O) is defined by

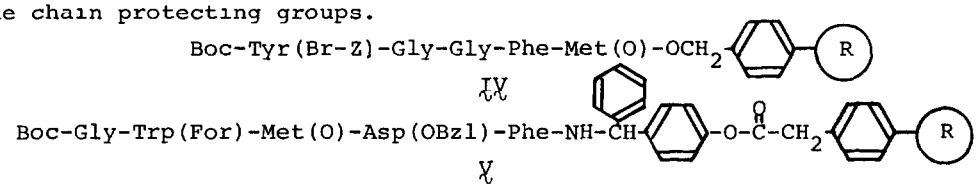


the pKa's of the sulfoxide (I) and the sulfide (III). To test this mechanism, Met(O) was treated at 0° C for 1 h in DMS containing various concentrations of HF (10-90%) which provided a range of acidity functions. As shown in Fig I, the rate of reduction reached a maximum at 25-40% of HF in dimethylsulfide. This finding agreed well with the basicities of sulfoxide and sulfide which have pKa's of -2 and -6, respectively, since the observed H_o value of 25% HF in DMS was found to be between -4.6 to -5.2 using Hammett indicators.[10] Thus, when HF was lower than 25%, the H_o of the HF-DMS solution fell and the sulfoxide was not protonated strongly enough to allow an effective rate of reduction. When HF was higher than 40% the H_o was too high, and both the sulfoxide and sulfide were strongly protonated, inhibiting the reduction. From these results, it is apparent that the rate of the oxygen-exchange reaction between sulfoxide and sulfide is dependent on the acidity function and is optimal when the sulfoxide is strongly protonated while the sulfide is largely free.

We have also examined other sulfides and thiols for use in the HF/sulfide reduction of Met(O). Thioanisole was as effective as dimethylsulfide in the reaction (Table I). However, we found that at low concentrations of HF in thioanisole, the reduced Met was alkylated to S-methylmethionine by thioanisole at a rate of 70%/h. This side reaction was not found in HF/DMS. The more basic thiacyclopentane was also found to be effective but the 6-member oxa-ring

1,4-thioxane was found to be ineffective (Table I). Since HF/dimethylsulfide could be removed in vacuo effectively while other HF-sulfide mixtures complicated work-up because of low volatility, HF-dimethylsulfide (1:3, v/v) was chosen to be the reagent for solid phase peptide synthesis.

Recently in our effort to improve the HF deprotection step in solid phase peptide synthesis, we have used this reagent to remove protecting groups by an S_N2 mechanism.[10-12] Since, in the usual application, HF is maintained at high concentration (90%) and operates by an S_N1 cleavage mechanism, it is often associated with several serious side reactions. These include alkylation of amino acids containing nucleophilic side chains by carbocations and the dehydration of aspartic and glutamic acid side chains. The idea of using the S_N2 cleavage mechanism is to prevent the generation of free carbocations and, by operating with a low acidity function, to prevent the dehydration reaction. The low concentration of HF in DMS (1·3, v/v) meets our criteria for the S_N2 cleavage reagent. Based on the studies in this paper, the significance of this reagent is enhanced by reducing Met(O) while concomitantly removing other side chain protecting groups.



To evaluate the efficacy of the low concentration HF-DMS (1:3, v/v) deprotection agent in solid phase peptide synthesis, Met(O)-containing peptides (IV and V) were synthesized on two different supports. Since we have found that HF/dimethylsulfide is a poor swelling solvent for the resin, 10% of p-cresol was added to this mixture to give HF-dimethylsulfide-p-cresol(25:65:10, v/v/v). This mixture was as effective in total reduction of Met(O) to Met as HF-DMS (25:75, v/v) (Table I). The peptide-resin was first treated with the HF-DMS-p-cresol mixture at 0° C for 1 h and then most of the HF and DMS was evaporated in vacuo. This was followed by the addition of HF to make the solution up to 9:1 HF-p-cresol and the cleavage was completed at 0° C in 0.5 to 1.0 h. This high HF step is needed for the removal of more acid-stable groups such as Arg(Tos) and for complete cleavage from the resin.[10]

Met-enkephalin (H-Tyr-Gly-Gly-Phe-Met-OH) was obtained from resin IV in 92% yield. The purity of the crude product, as assessed by HPLC, was greater than 95%. After enzymatic digestion of the crude product, ion-exchange chromatography revealed that all Met(O) had been quantitatively converted to Met. HPLC comparison with authentic samples of Met(O)-enkephalin and Met-enkephalin also revealed the absence of a Met(O)-enkephalin peak. The pentagastrin peptide (H-Gly-Trp-Met-Asp-Phe-NH_2) obtained from resin V gave

similar results after deformylation and work-up. Thus, HF cleavage under the new conditions in the presence of dimethylsulfide reduced methionine sulfoxide, removed other protecting groups to give products in good yields, and is recommended as an improvement for the synthesis of methionine-containing peptides.

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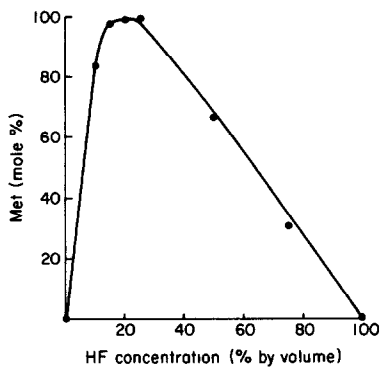
Table I. Reduction of Met(O) to Met by HF and Reductant

Reagent (% by volume)	Methionine Yield (mol%)
HF-anisole (90:10)	0
HF-p-cresol (90:10)	0
HF-p-thiocresol (90:10)	3
HF-dimethylsulfide (20:80)	100
HF-thiacyclopentane (20:80)	100
HF-1,4-thioxane (20:80)	0
HF-diphenylsulfide (20·80)	0
HF-thioanisole (25:75)	96[1]
HF-DMS-p-cresol (25:65:10)	100

[1]70 mole percent isolated as S-methyl-methionine sulfonium salt

Figure I. Reduction of Met(O) in different concentrations of HF in DMS at 0° C, 1 hour.



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