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IMPROVED DEPROTECTION IN SOLID PHASE PEPTIDE SYNTHESIS: REMOVAL OF PROTECTING GROUPS FROM SYNTHETIC PEPTIDES BY AN S_N2 MECHANISM WITH LOW CONCENTRATIONS OF HF IN DIMETHYLSULFIDE

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Summary: The use of a low-concentration HF in dimethylsulfide (1:3, v/v) for the removal of protecting groups from synthetic peptides has been found to be efficient and to eliminate several side reactions associated with the high-concentration HF in anisole (9:1, v/v) normally employed in peptide synthesis. The mechanism of the low-concentration HF cleavage is S_N2, in contrast to the S_N1 mechanism of the high-concentration HF cleavage, and consequently the reaction proceeds without generation of carbocation intermediates.

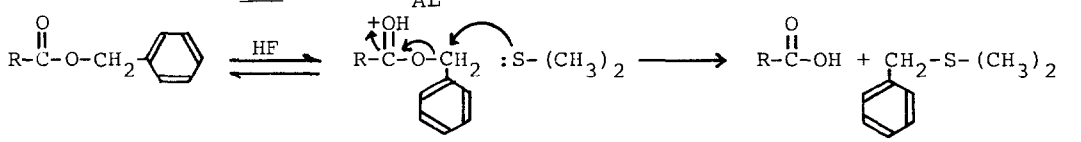
The chemical synthesis of peptides, either in solution or solid phase, often culminates in a final strong-acid step in which all protecting groups and the polymeric support are removed.[1] For this purpose, acids with strong protonating properties such as HF, HBr or sulfonic acids have been used. However, several serious side reactions are known to be associated with these strong acids; (a) alkylation of nucleophilic side chains of tyrosine, methionine, tryptophan and cysteine by the carbocations generated from the alcohol component of the protecting groups (Bzl, Bu^t, etc.)[2] and (b) dehydration of the protonated side chain carboxyl groups of aspartic and glutamic acids followed by acylation reactions of the resulting acylium ion.[3]

These side reactions resulting from an S_N1 or $A_{AL}1$ type of strong-acid cleavage mechanism can be reduced by decreasing the nucleophilicity of the side chains toward alkylation and by lowering the acidity function[3] of the strong acid to avoid the dehydration side reaction. However, they can be avoided completely by a change of the S_N1 acid cleavage mechanism to the S_N2 or $A_{AL}2$ type, in which carbenium and acylium ions are not generated. In this paper we describe the use of low HF concentration in dimethylsulfide (DMS) as a deprotecting agent that participates in an S_N2 cleavage mechanism and avoids these side reactions. The new low HF-DMS reagent concomitantly reduces methionine sulfoxide and in the presence of thiols removes N^i-formyl tryptophan protecting groups.

Anhydrous HF, with an acidity function (H_o) of -10.8,[4] is usually maintained in a high volume ratio (~90%) to an aromatic scavenger such as anisole for the removal of all protecting groups in peptide synthesis. Because an increase in diluent lowers the acidity function of the HF mixture[4] and decreases its ability to remove the protecting groups, studies have until now focused on the high concentration of HF. Our studies in the past four years have aimed at finding a concentration of HF in the presence of a weak organic base in which

a change-over in mechanism can be observed so that the cleavage mechanism is S_N2. According to the studies by Yates and McClelland,[5] proteolysis by strong acid proceeds with a distinct mechanistic acid-rate profile in which an S_N2 type of cleavage occurs at low acid concentration while an S_N1 type is found at high acid concentration. Thus we have sought a diluent that can offer nucleophilic assistance and an S_N2-like mechanism in HF. We have tested many weak organic bases that can either hydrogen-bond to or weakly complex with HF for such purposes.[6] Dimethylsulfide, a weak base with a pKa of -5.3,[7] is stable in HF and highly volatile and satisfies our requirements as the diluent.

We have studied the kinetic response of the deprotection of Ser(Bzl) to changes in concentration of HF in DMS. The deprotection mechanism of HF-DMS mixture can best be accomodated by two distinct acid-rate profiles. At high HF concentration (>50% of HF) the initial rate of deprotection of Ser(Bzl) increased rapidly with the rising concentration of HF. The rate of deprotection of Ser(Bzl) at 50% HF was found to be 1.35×10^{-3} sec^{-1} but increased to 3.01×10^{-3} sec^{-1} at 60% (Fig 1). The initial rate was too fast to be measured accurately at concentrations above 60% but the average rate was calculated to be 23×10^{-3} sec^{-1} at 75%, 42×10^{-3} sec^{-1} at 80% and 60×10^{-3} at 90% HF. Thus, under the usual high HF conditions, the deprotection of Ser(Bzl) is complete within 2 min at 0° C. However, when HF was below 50% deprotection was much slower and the change with concentration was much less dramatic (slope 0.013×10^{-3} sec^{-1}/% by volume). Thus, the rate increased only three-fold from 10% to 40% of HF, whereas an increase in HF concentration from 60% to 90% caused an increase in rate of 20-fold (slope 1.9×10^{-3} sec^{-1}/% by volume). Such a sudden break in the rate-HF concentration profile is strongly suggestive of a changeover from an S_N2 to an S_N1 mechanism, and is consistent with the results from the sulfuric acid catalyzed hydrolysis of benzyl esters.[5] Typically, the rate increases slowly at low acidity function, where the cleavage mechanism is S_N2 but fast at high acidity function where the S_N1 mechanism dominates. The role of dimethylsulfide in our studies is to serve not only as diluent but also as a nucleophile. The potential sources of carbocations never give rise to these dangerous ions because the groups are removed, not by an S_N1 mechanism, but by an S_N2 mechanism in which the DMS nucleophile (II) attacks the protonated intermediate (I) and produces the corresponding alkyldimethyl-sulfonium product (III) by an $A_{AL}2$ cleavage of the ester.



(I) (II) (III)

The change-over in mechanism of HF-dimethylsulfide can also be explained by the acidity function of the HF-dimethylsulfide mixture at various concentra-

tions of HF. Since dimethylsulfide has a pKa of -5.3, the effective acidity function of this binary mixture should be slightly below that value so that dimethylsulfide is still largely unprotonated and available for the nucleophilic participation required by the S_N2 cleavage mechanism. We found that at 25% by volume of HF in dimethylsulfide the H_o of the solution as estimated by Hammett indicators was between -4.6 and -5.2. Furthermore, at this range of acidity function most protecting groups such as esters, ethers and carbamates are protonated.[7] As the HF concentration increased, the acidity function also increased and at 90% of HF the H_o was found to be between -8.1 and -9.3. At this concentration DMS becomes fully protonated and is not an effective nucleophile. The reaction thus follows an S_N1 mechanism largely due to HF itself. At HF concentrations lower than 25% the H_o decreases to such an extent that the solution loses the protonating capacity required to remove these protecting groups efficiently.

It is clear from our results (Fig 1) that a wide range of HF concentrations (below 50%) in dimethylsulfide can be used to remove protecting groups and can still lie within the range of an S_N2 mechanism. We have found that a binary mixture of HF:dimethylsulfide of 1:3, v/v (~1:1 molar ratio) is satisfactory for all our purposes. We find that the HF-DMS mixture removes many benzyl alcohol-derived protecting groups at a reasonable rate, gives rise to very little alkylation side products during the cleavage of Tyr(Bzl),[8] reduces methionine sulfoxide completely to methionine without alkylation,[9] deprotects the N^i-formyl group of Trp(For) in the presence of a thiol,[10] and prevents dehydration of aspartic and glutamic acid side chains.

To test the new cleavage conditions, HF-DMS (1:3, v/v) was compared with HF-anisole (9:1, v/v) as a deprotecting agent. Both removed benzyl alcohol-derived protecting groups efficiently in two hours at 0° C as shown in Table I. Very acid-stable protecting groups which are known to be deprotected by an S_N1 mechanism were stable to the HF-DMS or were removed only slowly. Thus, the low HF-DMS mixture did not deprotect Arg(Tos), Arg(NO_2), Cys(4-MeBzl) or Asp(OcHex) and only partially deprotected Tyr(2,6-Cl_2-Bzl) (65%). The HF-DMS mixture was found to be a poor swelling solvent for the cleavage of peptidyl-resins but could be improved by the addition of 10% p-cresol.[6] The following new mixture: HF-DMS-p-cresol (25:65:10, v/v/v) gave excellent cleavage results.

For the cleavage of very acid-stable protecting groups such as Arg(Tos) or of peptidyl-resin bonds such as the amide in benzhydryl-amine-resin, a two stage low-high HF concentration cleavage method was instituted.[9] The advantage of the S_N2 low HF deprotecting procedure is manifested in the minimization of several side reactions associated with the high HF-anisole mixture (Table I). For example, Tyr(Bzl) which gave 20% of 3-alkyltyrosine in high HF-anisole (9:1, v/v) or HF-DMS (9:1, v/v) mixture[8] provided less than 0.5% of alkylated

tyrosine product in the low HF-DMS mixture, a greater than 40-fold improvement and a result comparable to the best HBr cleavage condition for Tyr(Bzl). Similarly, glutamic acid dehydration in the presence of aromatic scavengers (e.g. anisole) gave no ketonic adduct.[3] These results indicate that the cleavage mechanism in low HF-DMS is different than in high HF-anisole. Of particular interest were the observations that the low concentration of HF-DMS quantitatively reduced Met(O) to Met[9] and in the presence of thiols deprotected Trp(For) to Trp.[10] Both of these protecting groups are stable in HF-anisole at all concentrations and require subsequent steps for their removal. Furthermore, we have recently found conditions in which both groups are quantitatively removed in a single manipulation in an HF-DMS reagent.[8-10]

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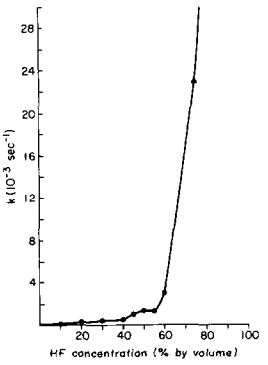
Table I Side Chain Protecting Group Deprotection by HF

Amino Acid Derivative	Free Amino Acid products (mol %) High HF[1]	Low HF[2]
Boc-Lys(Z)	100	100
Boc-Ser(Bzl)	100	100
Boc-Glu(OBzl)	87(13)[3]	100
Boc-Tyr(Bzl)	81(19)	99.5(0.5)
Boc-Trp	95(5)	100
Boc-Met(O)	0(100)[4]	100

[1]HF:anisole (9:1, v/v), 0°C, 1 h; [2]HF: DMS (1:3, v/v), 0° C, 2 h; [3]Side products in parentheses; [4]As Met(O).

Fig. 1. Plot for the apparent first order deprotection of Ser(Bzl) in different concentrations of HF in DMS: (o) experimental points; (Δ) calculated from best estimates (see text).



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