The deprotection of S-4-methylbenzyl-cysteine in HF was studied as a function of acidity and scavengers employed and a highly effective procedure was developed. Under conditions of low acidity which promote SN$_2$ deprotection of benzyl alcohol-derived protecting groups of amino acid side-chain functionalities, i.e. low HF (HF:DMS, 1:3, v/v), little or no deprotection of S-4-methylbenzyl cysteine was observed. Under conditions of high acidity (90% HF), recovery of cysteine was only 79% using the conventional scavenger, anisole. However a combination of the scavengers p-cresol and p-thiocresol provided nearly quantitative recovery of the cysteine thiol, with the preferred deprotection conditions being HF;p-cresol:p-thiocresol (90:5:5, v/v), 0°C, 1 h. The possibility that the sulfoxide of the protected cysteine thioether was involved in cysteine side reactions was investigated. The formation of the sulfoxide was found to be low (0.15% per residue added) under standard solid-phase peptide synthesis conditions. Since the sulfoxide derivative was resistant to deprotection in high HF, two methods for the reduction of the molecule to the thioether were developed. One method involved the reduction of the sulfoxide in HF at intermediate acidity levels (HF:DMS, 40:60, v/v), 0°C, 4 h. The other method involved a mixture of TFA, DMS and CH$_2$Cl$_2$ (45:10:45, v/v) containing (Et)$_4$NCl ($\times$ 50 equiv. per cysteine residue) which efficiently reduced both free and resin-bound S-4-methylbenzyl cysteine sulfoxide ($t_{1/2} = 35$ min). Both methods also reduced methionine sulfoxide to methionine.

Key words: deprotection; cysteine; S$_N$2 deprotection; S-4-methylbenzyl cysteine

Recent studies in this laboratory on the deprotection of protected peptides by HF have shown that the cleavage of protecting groups can proceed by two mechanisms, S$_N$1 and S$_N$2(1). Such studies revealed that a low concentration of HF in dimethyl sulfide removes most benzyl type side-chain protecting groups by an S$_N$2 mechanism in which the precursors of harmful carbonium ions are removed to form relatively unreactive sulfonium salts. On the other hand, deprotection of cysteine and arginine side-chain protecting groups occurs only at high HF concentrations via an S$_N$1 mechanism, which produces highly reactive...
In this paper, we describe the deprotection of the commonly used S-4-methylbenzyl derivative of cysteine, Cys(4-MeBzl)(1), using the model peptide Leu-Cys-Ala under both the S_N1 and S_N2 deprotection conditions. At low HF concentrations (<50% HF) in which S_N2 deprotection occurs, little or no deprotection of Cys(4-MeBzl) was observed. At higher HF concentrations and S_N1 deprotection, loss of cysteine was effectively eliminated using a mixture of p-cresol and p-thiocresol as scavengers in the reaction. The possible involvement of the sulfoxide of the protected cysteine thioether, Cys(4-MeBzl)[O] (2), in cysteine loss during acid deprotection was also studied. While experiments showed that the formation of Cys(4-MeBzl)[O] was very low under standard solid phase peptide synthesis conditions, any sulfoxide formed was highly resistant to cleavage at the cysteine thioether C-S bond and, in some cases, formed irreversible side products. However, two methods for the reduction of the sulfoxide of Cys(4-MeBzl) prior to acid deprotection were developed, eliminating this problem as a possible cause of cysteine loss.

![Figure 1](image-url)

carbonium ions that must be trapped by scavengers to prevent alkylation of nucleophilic amino acid side chains. One of the chief problems in high acidity HF deprotection of cysteine-containing peptides is the apparent loss of cysteine residues (2). The losses have been variously attributed to such side reactions as alkylation and irreversible polymer formation (3,4) although documentation for such side reactions is limited. Attempts to circumvent these side reactions by change in protecting groups and deprotection strategies have been partially successful, but no truly satisfactory strategy has been developed (2).
Results

Deprotection of Cys(4-MeBzl). Dependence on HF concentration

The model peptide Boc-Leu-Cys(4-MeBzl)-Ala-OBzl was used in the study of the dependence of deprotection of Cys(4-MeBzl) on HF concentration. The HF-treated peptides were analyzed using reverse phase HPLC. As seen in Table 1, no deprotection of Cys(4-MeBzl) was seen at 25% HF, the standard low HF concentration, and very little at 50% HF. However, Cys(4-MeBzl) was fully deprotected at 80% HF and above. These results are not surprising given the nature of the protecting group. Since the thioether functionality of protected cysteine has a pKa of -7.5 or below (5), measurable protonation to form the conjugate acid in low HF (25% HF, H<sub>0</sub> = -5.0) did not occur and no S<sub>N</sub>2 deprotection took place. However in high HF (H<sub>0</sub> = -8.5 to -10), protonation did take place with subsequent alkyl-sulfur fission via an S<sub>N</sub>1 mechanism (Fig. 1).

Effect of scavenger on cysteine recovery from high HF

The model peptide Boc-Leu-Cys(4-MeBzl)-Ala anchored to the standard benzyl ester resin was used in a series of experiments to determine what effect scavengers have on cysteine recovery from high HF deprotection. Resin samples were treated with 90% HF, 10% scavenger for 1 hour at 0°C. After HF was distilled off, the residue was extracted with ethyl acetate and the crude peptide solubilized in dilute aqueous acetic acid. Aliquots were oxidized with performic acid, hydrolyzed with HCl and analyzed by ion exchange chromatography. Cysteic acid recovery from various HF deprotections is shown in Table 2. Preliminary experiments determined that HF does not oxidize cysteine to cysteic acid. The results in Table 2 show that the aromatic ethers, anisole and particularly thioanisole, gave poor cysteine recovery. When the crude peptide from HF/thioanisole was examined on HPLC, very little Leu-Cys-Ala was seen. This was not due to low cleavage from the resin since similar experiments using Boc-Leu-Cys(4-MeBzl)-Ala-OBzl gave identical results. The poor recovery of Leu-Cys-Ala appeared to be due to methylation of cysteine by thioanisole, especially as the HF concentration lowered during evaporation. Experiments showed that cysteine became S-methyl cysteine when exposed to thioanisole in low HF. Anisole, on the other hand, was not only a poor scavenger but also an alkylating

---

**Table 1**

Extent of deprotection of Cys(4-MeBzl) in HF at 0°C

<table>
<thead>
<tr>
<th>Reagents (vol%)</th>
<th>Reaction time (h)</th>
<th>% cleaved&lt;sup&gt;a&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>HF 25 DMS 65 p-cresol 10</td>
<td>2.0</td>
<td>&lt;1</td>
</tr>
<tr>
<td>50</td>
<td>2.0</td>
<td>&lt;5</td>
</tr>
<tr>
<td>80</td>
<td>1.5</td>
<td>100</td>
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<tr>
<td>90</td>
<td>1.0</td>
<td>100</td>
</tr>
<tr>
<td>95</td>
<td>1.0</td>
<td>100</td>
</tr>
</tbody>
</table>

<sup>a</sup>Determined by reverse phase HPLC (see text).

---

**Table 2**

Effect of scavenger on cysteine recovery from Boc-Leu-Cys(4-MeBzl)-Ala-OCH<sub>3</sub>-resin in HF

<table>
<thead>
<tr>
<th>Scavenger composition (vol %)</th>
<th>% Cysteine recovery&lt;sup&gt;b&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>10% Anisole</td>
<td>79</td>
</tr>
<tr>
<td>10% p-Cresol</td>
<td>93</td>
</tr>
<tr>
<td>10% p-Thiocresol</td>
<td>96</td>
</tr>
<tr>
<td>10% Thioanisole</td>
<td>34</td>
</tr>
<tr>
<td>7% Anisole, 3% ethanedithiol</td>
<td>95</td>
</tr>
<tr>
<td>5% p-Cresol, 5% p-thiocresol</td>
<td>100</td>
</tr>
<tr>
<td>5% p-Cresol, 5% ethanethiol</td>
<td>92</td>
</tr>
</tbody>
</table>

<sup>a</sup>Reaction conditions were 90% HF, 10% scavenger, 0°C, 1 h.
<sup>b</sup>Determined by amino acid analysis after performic acid oxidation and subsequent HCl hydrolysis.
Deprotection of cysteine-containing peptides

**TABLE 3**

Extent of formation of the sulfoxide of Boc-Cys(4-MeBzI)-OH under synthesis conditions

<table>
<thead>
<tr>
<th>Solvent</th>
<th>% sulfoxide/44 h&lt;sup&gt;b&lt;/sup&gt;</th>
<th>average rate of sulfoxide/h&lt;sup&gt;b&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>CH&lt;sub&gt;2&lt;/sub&gt;Cl&lt;sub&gt;2&lt;/sub&gt;</td>
<td>0.90</td>
<td>0.020</td>
</tr>
<tr>
<td>DMF</td>
<td>1.90</td>
<td>0.043</td>
</tr>
<tr>
<td>TFA:CH&lt;sub&gt;2&lt;/sub&gt;Cl&lt;sub&gt;2&lt;/sub&gt; (1:1)</td>
<td>&lt;0.10</td>
<td>&lt;0.002</td>
</tr>
<tr>
<td>DIEA:CH&lt;sub&gt;2&lt;/sub&gt;Cl&lt;sub&gt;2&lt;/sub&gt; (1:19)</td>
<td>2.40</td>
<td>0.055</td>
</tr>
</tbody>
</table>

<sup>a</sup>See text for experimental details. <sup>b</sup> Determined by reverse phase HPLC.

agent in HF. The S-methylation of methionine by anisole under strongly acidic conditions has been reported (6). Therefore, it is possible that both anisole and thioanisole methylated the protected cysteine thioether to form the sulfoxonium salt which failed to deprotect in HF. The addition of 1,2-ethanedithiol to anisole provided a marked improvement in cysteine recovery. However, 1,2-ethanedithiol polymerized extensively in high HF and was therefore unsuitable. Other alkyl thiols such as ethanethiol were ineffective since alkyl thiols are totally protonated in high HF (7).

Failure of the alkyl-sulfur bond to cleave and thus not give cysteine from Cys(4-MeBzl) in these experiments was ruled out for the following reasons: earlier experiments have shown that deprotection of Cys(4-MeBzl) is quantitative in 90% HF; the peptides from the HF deprotection reactions were analyzed by HPLC where any Leu-Cys(4-MeBzl)-Ala could be detected; and performic acid oxidizes Cys(4-MeBzl) to the sulfone which is inert to HCl hydrolysis and is easily detected on the amino acid analyzer.

Excellent recoveries were obtained when either p-cresol or p-thiocresol were used, with a mixture of the two providing the best results. This was not unexpected since both compounds have been shown to be more efficient carbonation traps in high HF than more traditional scavengers such as anisole (8). Since p-thiocresol is only partially soluble in high HF, the addition of p-cresol as an additional carbonium ion trap is helpful. The aromatic thiols clearly provided the best protection for cysteine in high HF, due to their related chemical nature to the cysteine thiol and their relatively low pKas (−8.5 to −9.0) (7) which leaves them partially unprotonated in high HF (90% HF, H<sub>0</sub> = −8.5 to −10). Therefore, by proper choice of scavengers, nearly quantitative recovery of cysteine from Cys(4-MeBzl) was possible after HF deprotection.

**Formation of the sulfoxide of S-4-methylbenzyl cysteine**

To test for the formation of the sulfoxide of S-4-methylbenzyl cysteine under synthesis conditions, Boc-Cys(4-MeBzl)-OH was placed in various synthesis solutions which were saturated with O<sub>2</sub> gas and shaken. Aliquots were removed at intervals and analyzed using reverse phase HPLC. The results are shown in Table 3. The findings indicated that the polarity and acidity of the solvent influenced sulfoxide formation. Acidic conditions gave the least amount of sulfoxide (below the detection limit, 0.002%) while basic conditions gave the most. For a typical synthesis cycle with two couplings, one in CH<sub>2</sub>Cl<sub>2</sub> and one in DMF, the amount of sulfoxide formation was calculated to be 0.15% per residue added. This is not a serious problem in the synthesis of short peptides but could pose difficulties for longer syntheses where methods are needed to either prevent sulfoxide formation (synthesis under inert atmosphere) or for the reduction of the sulfoxide back to the sulfide.

**Behavior of S-4-methylbenzyl-L-cysteine sulfoxide in HF**

In order to study the behavior of Cys(4-MeBzl)[O], pure Boc-Cys(4-MeBzl)[O]-OH was prepared using the sodium perborate method (9). This compound was then subjected to various HF deprotection reactions and the results analyzed using ion exchange chromatography.
### TABLE 4

Reaction of Boc-Cys(4-MeBzl)/OH in HF at 0°C

<table>
<thead>
<tr>
<th>Reagents (vol%)</th>
<th>Reaction time (h)</th>
<th>Product composition&lt;sup&gt;a&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Cys(4-MeBzl)/OH</td>
<td>Cys(4-MeBzl) 1/2Cys side product</td>
</tr>
<tr>
<td>HF scavenger</td>
<td></td>
<td></td>
</tr>
<tr>
<td>100 0 anisole</td>
<td>1.0</td>
<td>94 0 6&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>90 10 p-cresol</td>
<td>1.0</td>
<td>97 1 0</td>
</tr>
<tr>
<td>90 10 p-thiocresol</td>
<td>1.0</td>
<td>92 0 0</td>
</tr>
<tr>
<td>100 0 anisole</td>
<td>1.0</td>
<td>57 39 8&lt;sup&gt;e&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>HF DMS p-cresol p-thiocresol</th>
<th>Reaction time (h)</th>
<th>Cys(4-MeBzl)/OH</th>
<th>Cys(4-MeBzl) 1/2Cys</th>
<th>side product</th>
</tr>
</thead>
<tbody>
<tr>
<td>25 75 0 0</td>
<td>1.0</td>
<td>71 29 0</td>
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<tr>
<td>25 65 5 5 6</td>
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<td>68 32 0</td>
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<td>0</td>
</tr>
<tr>
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<td>32 68 0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>50 40 5 5 5</td>
<td>1.0</td>
<td>49 51 0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>40 60 0 0 0</td>
<td>4.0</td>
<td>8 92 0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>25&lt;sup&gt;d&lt;/sup&gt; 65 5 5 5</td>
<td>2.0</td>
<td>67 5 9</td>
<td>19&lt;sup&gt;e&lt;/sup&gt;</td>
<td></td>
</tr>
</tbody>
</table>

<sup>a</sup>Determined by ion exchange chromatography.  
<sup>b</sup>Side peak at 38.5 min on PA-35 column.  
<sup>c</sup>Side peak at 39.5 min on PA-35 column.  
<sup>d</sup>Low HF followed by high HF (90%), 5% p-cresol, 5% p-thiocresol, 0°C, 1 h.  
<sup>e</sup>Side peak at 165 min on AA-15 column.
Deprotection of cysteine-containing peptides

\[
\begin{align*}
\text{NH}_3\text{-CH-COOH} & \quad \text{CH}_2\text{-S-CH}_2\text{-CH}_3 \\
\text{+OH} \quad \text{GCH}_3 & \quad \text{+I} \\
\longrightarrow & \quad \text{+NH}_3\text{-CH-COOH} \quad \text{+NH}_3\text{-CH-COOH} \\
\text{HF} & \quad \text{TRAPPED} \\
\text{CH}_2\text{-S-OH}_2 & \quad \text{CH}_2\text{-S} \\
\text{NH}_3\text{-CH-COOH} & \quad \text{H}_2\text{O} \quad \text{3}
\end{align*}
\]

**Figure 2**

As seen in Table 4, the sulfoxide was refractory to the conventional deprotection conditions using 90% HF and 10% aromatic scavengers. Side products of the sulfoxide in high HF were observed in the 100% HF and HF/p-cresol reactions. The ninhydrin-positive side product in the HF/p-cresol reaction was probably the p-cresol adduct (3) formed via the path indicated in Fig. 2. The side product generated in the 100% HF reaction was of unknown structure. In contrast to earlier work with S-4-methoxybenzyl-L-cysteine sulfoxide (9), anisole did not give a side product in high HF.

Under standard conditions, thiocresol in high HF was able to partially reduce Cys(4-MeBzl)[O]. This was most likely due to reduction by thiocresol during the HF evaporation step at intermediate acidity levels (50–65% HF). If reduction had occurred in high HF, the resulting Cys(4-MeBzl) would deprotect to yield cysteine, while reduction of the sulfoxide in HF by thiocresol at intermediate acidity levels would yield the thioether as found. The reactions of oxygen exchange which lead to the reduction of sulfoxides by thiols in strong acid are known. Recently, 2-mercaptopyridine in 93% HF was found to reduce methionine sulfoxide to methionine (10). Thus, it was not unexpected that partial reduction of Cys(4-MeBzl)[O] occurred using 10% p-thiocresol in HF.

The sulfoxide of Cys(4-MeBzl) is less basic than that of Met(O) because of the presence of the aromatic ring and is therefore more resistant to protonation, a step which is necessary for the reduction of sulfoxides in HF(1). Steric factors are also important in the reduction of sulfoxides of cysteine thioethers as shown by experiments with N\(^\text{α}\)-fluorenlymethylxycarbonyl-L-cysteine(t-buty1)sulfoxide (synthesized using the sodium perborate method) in which it was extremely resistant to reduction under a variety of conditions (Table 5). Thus it was expected that Cys(4-MeBzl)[O] would be only partially converted to Cys(4-MeBzl) in low HF (HF:DMS, 1:3, v/v), under conditions which completely reduced methionine sulfoxide to
methionine. Cys(4-MeBzl)[O] could be reduced in HF by the use of higher HF concentrations and longer reaction times (40% HF, 4 h). However, HF:DMS mixtures in the range of 35 to 60% HF were very difficult to evaporate due to the tight molecular complex formed between HF and DMS, making work-up difficult. Therefore, an alternative method for the reduction of the sulfoxide prior to the acid deprotection step was developed.

Reduction of the sulfoxide of S-4-methyl benzyl-L-cysteine

Studies were undertaken to develop a method for the reduction of Cys(4-MeBzl)[O] prior to the HF cleavage. Attempts to reduce the sulfoxide using thiophenol or thiophenol/DMF at room temperature gave little or no reaction (Table 6). While reduction of protected cysteine sulfoxide does occur at elevated temperatures using thiophenol (9), this is a very harsh treatment. Therefore, alternative methods were investigated. The reduction of Boc-Cys(4-MeBzl)[O]-OH in 9:1 TFA:DMS proceeded very slowly (Table 6) but the addition of chloride ion in the form of 12 N HCl accelerated the reaction 40-fold. This rate acceleration was not due to the added acidity of the HCl because the substitution of (Et)4NCl·H2O gave comparable results. The reaction of oxygen exchange between methionine sulfoxide and dimethyl sulfide in the presence of chloride ion is known (11, 12). As seen in Table 6, a variety of TFA/DMS/CH2Cl2/(Et)4NCl·H2O compositions can be used to affect reduction of Cys(4-MeBzl)[O]. As a practical matter, TFA:DMS:CH2Cl2 (45:10:45, v/v) + 50 equiv. (Et)4NCl·H2O per cysteine residue were used on large resin-bound peptides (20 mL solvent per g resin) with no apparent difficulties. This reagent concomitantly reduced any methionine sulfoxide to methionine.

DISCUSSION

The S-4-methylbenzyl derivative of cysteine, Cys(4-MeBzl), is commonly used in peptide synthesis, especially solid phase peptide synthesis. Experiments showed that the 4-methylbenzyl protecting group is stable to the low HF condition (HF:DMS, 1:3, v/v) which cleaves most benzyl alcohol-derived protecting groups via an S_N2 mechanism (1). Cys(4-MeBzl)
is deprotected in quantitative yield in high HF (90% HF) which is known to deprotect via an $S_N$1 mechanism. The inability to deprotect Cys(4-MeBzl) in low HF rules out a one step deprotection reaction but can be used to advantage in a synthetic strategy involving cleavage of the peptide from the resin support and deprotection of most side chain protecting groups while maintaining cysteine in the protected form.

The cysteine thiol functionality is potentially vulnerable to a number of damaging side reactions in high HF (2–4). However, judicious choice of scavengers can help alleviate this problem on the basis of the acid-base properties of the molecules involved. The traditional carbocation trap, anisole, is a relatively poor scavenger for cysteine in high HF. This is understandable in view of its relatively high pKa ($-6.5$) which allows substantial protonation of the molecule and renders it ineffective as a trap in HF. However, like the more nucleophilic thioanisole, both were shown to be very destructive to cysteine in high HF. This is understandable in view of its relatively high pKa ($-6.5$) which allows substantial protonation of the molecule and renders it ineffective as a trap in HF. However, like the more nucleophilic thioanisole, both were shown to be very destructive to cysteine in high HF. This is understandable in view of its relatively high pKa ($-6.5$) which allows substantial protonation of the molecule and renders it ineffective as a trap in HF. However, like the more nucleophilic thioanisole, both were shown to be very destructive to cysteine in high HF. This is understandable in view of its relatively high pKa ($-6.5$) which allows substantial protonation of the molecule and renders it ineffective as a trap in HF. However, like the more nucleophilic thioanisole, both were shown to be very destructive to cysteine in high HF. This is understandable in view of its relatively high pKa ($-6.5$) which allows substantial protonation of the molecule and renders it ineffective as a trap in HF. However, like the more nucleophilic thioanisole, both were shown to be very destructive to cysteine in high HF. This is understandable in view of its relatively high pKa ($-6.5$) which allows substantial protonation of the molecule and renders it ineffective as a trap in HF. However, like the more nucleophilic thioanisole, both were shown to be very destructive to cysteine in high HF. This is understandable in view of its relatively high pKa ($-6.5$) which allows substantial protonation of the molecule and renders it ineffective as a trap in HF. However, like the more nucleophilic thioanisole, both were shown to be very destructive to cysteine in high HF. This is understandable in view of its relatively high pKa ($-6.5$) which allows substantial protonation of the molecule and renders it ineffective as a trap in HF. However, like the more nucleophilic thioanisole, both were shown to be very destructive to cysteine in high HF. This is understandable in view of its relatively high pKa ($-6.5$) which allows substantial protonation of the molecule and renders it ineffective as a trap in HF. However, like the more nucleophilic thioanisole, both were shown to be very destructive to cysteine in high HF. This is understandable in view of its relatively high pKa ($-6.5$) which allows substantial protonation of the molecule and renders it ineffective as a trap in HF. However, like the more nucleophilic thioanisole, both were shown to be very destructive to cysteine in high HF. This is understandable in view of its relatively high pKa ($-6.5$) which allows substantial protonation of the molecule and renders it ineffective as a trap in HF. However, like the more nucleophilic thioanisole, both were shown to be very destructive to cysteine in high HF. This is understandable in view of its relatively high pKa ($-6.5$) which allows substantial protonation of the molecule and renders it ineffective as a trap in HF. However, like the more nucleophilic thioanisole, both were shown to be very destructive to cysteine in high HF. This is understandable in view of its relatively high pKa ($-6.5$) which allows substantial protonation of the molecule and renders it ineffective as a trap in HF. However, like the more nucleophilic thioanisole, both were shown to be very destructive to cysteine in high HF. This is understood

The study of the sulfoxide of S-4-methylbenzyl cysteine showed that formation of this compound is very low under normal synthesis conditions (0.15% per residue added). Polar solvents such as DMF gave the most sulfoxide while acidic solutions such as TFA/CH$_2$Cl$_2$ totally suppressed the reaction. In light of the information on reduction of sulfoxide in Table 6, small amounts of thiols or sulfides in the TFA solution should reverse any small amount of sulfoxide formed during the other steps of the synthesis.

The sulfoxide of Cys(4-MeBzl) proved to be almost totally inert to high HF although some conditions caused a small amount of side reaction. This sulfoxide was incompletely reduced in the recommended low HF cleavage condition of 25% HF and 75% DMS for 2 h, in contrast to methionine sulfoxide (1). However, nearly complete conversion could be achieved in 40% HF in 4 h.

Further studies on the reduction of Cys(4-MeBzl)[O] to Cys(4-MeBzl) showed that the addition of chloride ion to TFA/DMS markedly accelerated the reaction. The chloride ion could be introduced either via a mineral acid such as aqueous 12 N HCl or via a salt such as (Et)$_4$NCl $\cdot$ H$_2$O. A mixture of TFA/DMS/CH$_2$Cl$_2$ (45:10:45, v/v) + 50 equiv. (Et)$_4$NCl $\cdot$ H$_2$O per cysteine residue reduced the sulfoxide at a reasonable rate ($t_{1/2} = 35$ min), while causing no apparent side reaction. This reagent also reduced methionine sulfoxide to methionine.

The results of this study show that low levels of the sulfoxide of 4-methylbenzyl cysteine are formed during the synthesis of peptides, but that the resulting sulfoxide in the final peptide can be readily reduced back to the starting 4-methylbenzyl cysteine by acidic mixtures of dimethyl sulfide and chloride ion before the

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**TABLE 6**

*Reduction of Boc-Cys(4-MeBzl)[O]·OH. Rate constants $^a$; reagents, vol%*

<table>
<thead>
<tr>
<th>CF$_3$COOH</th>
<th>DMS</th>
<th>CH$_2$Cl$_2$</th>
<th>12 N HCl</th>
<th>n(Et)$_4$Cl (equiv.)</th>
<th>$10^4$ k, s$^{-1}$</th>
<th>$t_{1/2}$, min</th>
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<tbody>
<tr>
<td>85</td>
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$^a$For determination of rate constants see Experimental Procedures.
final quantitative deprotection in HF/p-cresol/p-thiocresol. We conclude that the synthesis of cysteine-containing peptides by these methods does not present serious problems of stability, yield, or purity when proper care is exercised.

EXPERIMENTAL PROCEDURES

Materials
Commercial protected amino acids were obtained from Peninsula Laboratories (San Carlos, CA). Other reagents were trifluoroacetic acid (Halocarbon) and HF (Matheson). All other chemicals were the purest grade available.

General methods
Amino acid analyses were performed on a Beckman 121 amino acid analyzer. HF reactions were carried out in a Diaflon® HF apparatus (Toho Co., Osaka, Japan). Analytical HPLC was performed on a thermostated, reverse phase μBondapak C-18 column (3.9 × 300 mm) in a Waters Associates instrument fitted with a Schoeffel variable wavelength UV photometer and an automated WISP® injector. The chromatograms were recorded on a Hewlett Packard 3380 integrator (1 mV full scale).

Reverse phase HPLC
System I: Solution A was H2O/CH3CN/H3PO4 (95:5:0.1, v/v), Solution B, H2O/CH3CN/H3PO4 (50:50:0.05, v/v). The program consisted of a linear gradient of 2 to 98% B into A in 45 min, 1 mL/min monitored at 225 nm. The elution times (min) of the peptides were H-Leu-Cys-Ala-OH (6.7), H-Leu-Cys(Me)-Ala-OH (10.5), (H-Leu-Cys-Ala-OH)2 (14.8), H-Leu-Cys(4-MeBzl)[O]-Ala-OH (18.8), H-Leu-Cys(4-MeBzl)-Ala-OH (24.8), H-Leu-Cys(4-MeBzl)-Ala-OBzl (40.9).

System II: Solution A was H2O/CH3OH/CH3COOH (90:10:0.25, v/v, adjusted to pH 4.2 with NaOH); Solution B was H2O/CH3OH/CH3COOH (10:90:0.25, v/v). The program consisted of a linear gradient from 5 to 85% B in 40 min, 2 mL/min monitored at 240 nm. The elution times (min) of the cysteine derivatives were H-Cys(4-MeBzl)[O]-OH (7.5), H-Cys(4-MeBzl)-OH (12.4), Boc-Cys(4-MeBzl)[O]-OH (24.0), Boc-Cys(4-MeBzl)-OH (32.2), Fmoc-Cys(t-Bu)[O]-OH (29.6), Fmoc-Cys(t-Bu)-OH (37.0).

Boc-Leu-Cys(4-MeBzl)-Ala-OBzl
The protected tripeptide was synthesized using classical solution phase chemistry and purified using low pressure C-8 silica liquid chromatography. The material was crystallized from CH3OH/H2O and, after drying over P2O5, melted at 107.5–110°C. TLC on silica plates using CHCl3/CH3OH (94:6) showed one spot (Rf = 0.79). Anal. calc. for C32H45N3O6S: C, 64.08; H, 7.56; N, 7.01. Found: C, 64.02; H, 7.52; N, 6.94.

Acid concentration dependence of Cys(4-MeBzl) deprotection
Boc-Leu-Cys(4-MeBzl)-Ala-OBzl (10 mg) was placed into a 50 mL fluorocarbon HF vessel and the appropriate amount of dimethyl sulfide and p-cresol were added. After cooling to −78°C, the vessel was charged to the 5 mL mark with HF and stirred magnetically at 0°C in an ice bath. The HF and DMS were distilled off under vacuum, the residue extracted with diethyl ether, the peptide solubilized in 10% aqueous acetic acid and analyzed by reverse phase HPLC using System I.

Effect of scavenger on cysteine recovery in high HF
Boc-Leu-Cys(4-MeBzl)-Ala-OCH2-resin was synthesized using standard solid phase methods (13). Resin batches (100 mg) were subjected to 5 mL 9:1 HF/scavenger at 0°C for 1 h. After the HF was removed, the residues were extracted with ethyl acetate and the crude peptides solubilized with 10% aqueous acetic acid, filtered, frozen and lyophilized. The crude peptides (0.2 mg) were oxidized with 1 mL permutic acid at 0°C for 4 h (14), hydrolyzed in 2 mL 5.7 N HCl at 110°C for 24 h in sealed, evacuated tubes and submitted to amino acid analysis. The recovery of cysteic acid was normalized to the Leu and Ala residues.

Formation of Cys(4-MeBzl)[O] under synthesis conditions
Into a 13 × 100 mm screw cap test tube, were put 10 mg Boc-Cys(4-MeBzl)-OH dissolved in 5 mL of the test solution. The solution was
Deprotection of cysteine-containing peptides

plots of $\ln\left[\frac{Q}{a - x}\right]$ versus time where $a$ was the starting concentration of the sulfoxide and $x$ the sulfide concentration at a given time.

Synthesis of Boc-Cys(4-MeBzl)[O]-OH

The sulfoxide of Boc-Cys(4-MeBzl)-OH was synthesized, using the sodium perborate method (9), in 70% yield. The material was crystallized from CH$_3$OH/petroleum ether (40–60°C fraction) and, after drying over P$_2$O$_5$, melted at 167–169°C. TLC on silica plates using CHCl$_3$/CH$_3$OH/CH$_3$COOH (85:10:5) showed one spot ($R_f = 0.60$).

Anal. calc. for C$_{16}$H$_{23}$N$_2$O$_5$S: C, 56.30; H, 6.79; N, 4.10. Found: C, 56.43; H, 6.79; N, 4.07.

Behavior of Cys(4-MeBzl)[O] in HF

Boc-Cys(4-MeBzl)[O]-OH (10 mg) was treated with HF/scavenger in a total volume of 5 mL at 0°C for the indicated time (Table 6). After the HF was removed, the residue was extracted with diethyl ether and then solubilized in 10% aqueous acetic acid. Aliquots were analyzed on the Beckman 121 amino acid analyzer and by reverse phase HPLC using System II.

Further ion exchange chromatography was performed on a Beckman 120B amino acid analyzer using a sulfonated polystyrene column (0.9 x 11 cm, PA-35 resin) thermostated at 58°C. The column was eluted with 0.35 N sodium citrate, pH 5.2 at a rate of 70 mL/h. Elution times were Cys(4-MeBzl)[O] 28.5 min, Cys(4-MeBzl) 41 min, side peak from 100% HF reaction 38.6 min and side peak from HF/p-cresol reaction 39.5 min.

Reduction studies of Cys(4-MeBzl)[O]

To 2 mL of the test solution were added 2 mg Boc-Cys(4-MeBzl)[O]-OH and magnetically stirred at 24°C. At various time intervals, 0.1 mL aliquots were removed, quenched in 1 mL 12.5% aqueous NH$_3$ and analyzed by reverse phase HPLC using System II. The apparent first order rate constants were determined from

REFERENCES


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