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Dimethyl(methylthio)sulfonium Tetrafluoroborate: A Reagent for Disulfide Bond Formation in Peptides

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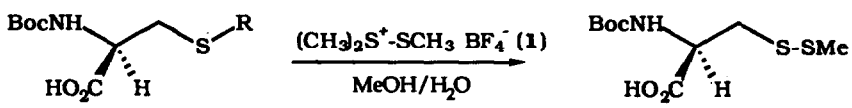
Abstract: *Dimethyl(methylthio)sulfonium tetrafluoroborate (1) has been used to deprotect a series of cysteine derivatives (BocCys-Acm, MeOBzl, Trt) with concurrent disulfide bond formation. This reagent has also been applied to the deprotection and disulfide bond formation in two peptides: a sarafotoxin A fragment and an analog of calcitonin where each peptide contains two S-protected cysteine derivatives: [Cys(Acm)]*

Disulfide linkages are a common means by which the tertiary structure of small proteins are stabilized.[1] Likewise disulfide bonds between two cysteine residues have been used to decrease the conformational flexibility of peptides in solution.[2] Although disulfide bonds have been widely used in peptide and protein chemistry, there exist only a limited number of methods available for their synthesis.[3] Many of these reagents are less than ideal due to undesired functionalization of amino acid sidechains by the reagents. In this paper we report the use of dimethyl(methylthio)sulfonium tetrafluoroborate[4] (**1**) as a selective and mild reagent used for removing cysteine S-protecting groups with concomitant disulfide bond formation in amino acid derivatives and peptides.

The ability of this reagent to remove a variety of cysteine protecting groups was initially examined using four Boc-Cys derivatives with different S-protecting groups (Acm, Bzl, MeOBzl, Trt). The product derived from the reaction of **1** with **2a**, **2c** and **2d** was the mixed disulfide **3** (Figure 1). With the benzyl derivative **2b**, only starting material was obtained after 72 hrs of reaction.



2a R = Acm
2b R = Bzl
2c R = MeOBzl
2d R = Trt

(3)

Figure 1. Deprotection and mixed disulfide formation of Cys derivatives with (1).

In a standard reaction, reagent **1** (10 eq) was added to a solution of an amino acid (**2a-d**) in MeOH/H_2O (1:1, 10 mM). After 24 hr the reaction was purified directly by reverse phase HPLC. With cysteine derivatives **2a**(Acm), **2c**(MeOBzl), and **2d**(Trt) all starting material was consumed within 24 hr with the exclusive appearance of the mixed disulfide **3** in approximately 50% yield after chromatography.[5] Derivative **2b** was recovered unchanged from the reaction mixture.

Reagent **1** was also examined for its ability to deprotect two S-protected cysteine residues within a single peptide sequence with concomitant intramolecular disulfide bond formation (Figure 2). An analog of calcitonin (**4**) was used in this study with the Acm S-protecting group due to the facile reaction of Acm with **1** in the above model studies.[6]

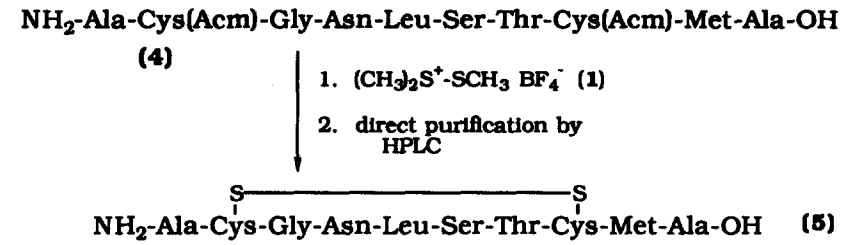


Figure 2. Deprotection and intramolecular disulfide bond formation in calcitonin analogs.

In a typical reaction a solution of peptide **4** (mixed aqueous/organic solvent systems, 0.1 or 1 mM solutions) was treated with **1** (10 eq) until complete as monitored by analytical HPLC. The reactions were purified directly by preparative HPLC. All reported yields of **5** are isolated yields after preparative HPLC (Table 1).[7,8]

Table 1. Conditions and Yields for Intramolecular Disulfide Bond Formation

Peptide	Reagent	Conc.[a]	Solvent[b]	Time	Product	Yield[c]
4	1	1mM	MeOH/H_2O	24 hr	5	28%
4	1	0.1mM	MeOH/H_2O	24 hr	5	37%
4	1	0.1mM	TFE/H_2O	24 hr	5	46%
6	1	1 mM	MeOH/H_2O	24 hr	7	59%
6	1	1 mM	MeOH/H_2O	24 hr	8	35%
6	1	1 mM	H_2O	24 hr	8	43%
6	I_2	1 mM	MeOH/H_2O	24 hr	8	28%

[a]Final concentration of peptides.
[b]1:1 mixture of organic solvent in H_2O.
[c]Isolated yields of peptides after preparative HPLC.

A longer peptide sequence corresponding to a fragment of sarafotoxin A (**6**) was examined for its reactivity with reagent **1**.[6] In this case the peptide containing the mixed disulfides with methyl mercaptan (**7**) was obtained upon treatment with **1**. Peptide **7** was converted into the cyclic analog (**8**) by treatment with a Reduce-Imm™ reducing column (Pierce), followed by oxidation with $K_3Fe(CN)_6$ (Figure 3).[7,8]

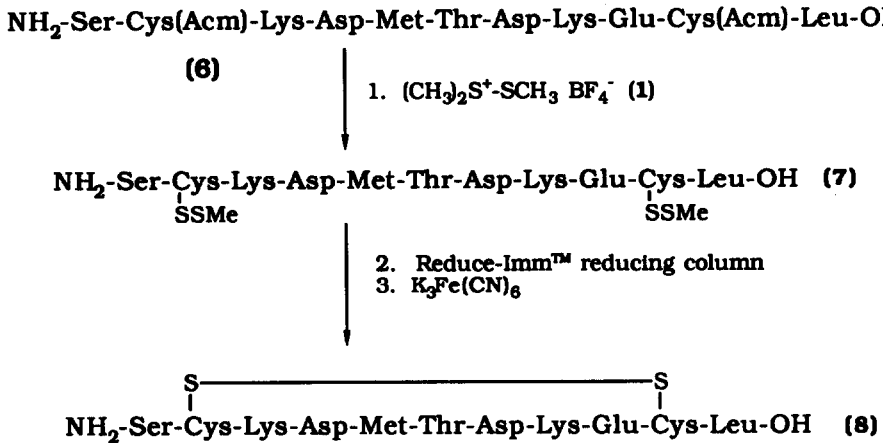


Figure 3. Deprotection and intramolecular disulfide bond formation in a fragment of sarafotoxin A.

In a typical reaction a solution of peptide **6** (aqueous or mixed aqueous/organic solvent system, 1mM solutions) and was treated with **1** (10 eq). The mixture was stirred at room temperature for 24 hr and purified by reverse phase HPLC to yield **7** (Table 1). Peptide **7** was dissolved in a 10 mM phosphate buffer (pH 8) and run through a Reduce-Imm™ column. The fractions which reacted positively with Ellman's reagent[9] were pooled and oxidized with $K_3Fe(CN)_6$ to yield the cyclic peptide **8**. Alternatively, the crude solution of **7** was concentrated to dryness *in vacuo*, the residue dissolved in a 10 mM phosphate buffer (pH 8) and reduced directly with the Reduce-Imm™ column, followed by oxidation with $K_3Fe(CN)_6$.

We have identified two different types of reaction products which are obtained from the treatment of amino acids and peptides with dimethyl(methylthio)sulfonium tetrafluoroborate (**1**). In the case of amino acids containing one S-protecting group only the mixed disulfide with methyl mercaptan is obtained. When peptides containing two S-protecting groups are treated with **1**, the cyclic peptide was obtained directly in a case where five amino acids residues intervened between the cysteine residues. In peptides with seven and eight[10] residues between the cysteines only the mixed disulfides with methyl mercaptan were obtained upon treatment with **1**, perhaps due to a lack of intramolecular disulfide exchange in the larger ring systems. In these cases the cyclic peptides were easily obtained from a DTT reduction/$K_3Fe(CN)_6$ oxidation sequence.

From an investigation of the reactivity of **1** with different S-protecting groups, we have determined that protecting groups which yield stable carbocations - such as Acm, MeOBzl, and Trt - react to give the methyl mercaptan mixed disulfide, whereas no reaction was detected with the Bzl protecting group. Reagent **1** is more versatile than other reagents used for forming disulfides in peptides, such as I_2, in that it is capable of removing the MeOBzl protecting group, can be used in a wider range of solvents (including H_2O), and gives a higher yield of deprotected peptides. In conclusion, **1** is a mild reagent for the removal of a wide range of S-protecting groups with concomitant disulfide bond formation, and as such it is a valuable addition to the methods available for disulfide bond formation in peptides.

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Abbreviations: Acm = acetamidomethyl, AcOH = acetic acid, Bzl = benzyl, DTT = dithiothreitol, MeOBzl = 4-methoxybenzyl, Trt = trityl, TFE = trifluoroethanol

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5. All reaction mixtures were purified by HPLC using a Vydac-C_8 reverse phase column (2.2 x 25 cm) with mobile phase A (100% CH_3CN/0.1% TFA) and mobile phase B (100% H_2O/0.1% TFA) with a linear gradient (5-95% A) of 60 min. All amino acid derivatives were characterized by ^1H NMR and mass spectroscopy (FAB, glycerol matrix).
6. Peptides were synthesized using solid phase methods on the *p*-alkoxybenzyl alcohol resin (Wang, S. S., *J. Am. Chem Soc.* **1973**, *95*, 1328) with the conventional fluorenylmethyloxycarbonyl-based strategy (Atherton, E.; Sheppard, R. C., in *The Peptides*; Vol. 9; Gross, E.; Meienhofer, J. Eds.; Academic Press: New York, 1987; pp. 1-38)
7. HPLC conditions: All reactions were monitored and purified by HPLC using Vydac-C_8 reverse phase columns (analytical - 1 x 25 cm, preparative - 2.2 x 25 cm) with mobile phase A (100% CH_3CN/0.1% TFA) and mobile phase B (100% H_2O/0.1% TFA) with linear gradients (5-60% A) of 30 min (analytical) or 60 min (preparative).
8. All new peptides were identified by mass spectroscopy (FAB, glycerol matrix) and amino acid analysis.
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10. Reagent **1** was also tested on a fragment of epidermal growth factor containing two Cys(Acm) derivatives (data not shown).

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