253. The Removal of *S*-Cysteine Protection by Means of 2-Pyridine Sulfenyl Chloride and the Subsequent Formation of Disulfide Bonds

Preliminary communication[1])

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Summary

2-Pyridine sulfenyl chloride (PS-Cl) is a useful reagent for simultaneously deprotecting and activating the mercapto-group of cysteine and of cysteine-peptides preliminary to disulfide-bond formation. The *S*-protecting groups that are amenable to this reaction include trityl, diphenylmethyl, acetamidomethyl, *t*-butyl, and *t*-butylsulfenyl.

In order to produce a peptide cysteine bridge, two *S*-protected cysteine residues are usually introduced during the synthesis. They are then deprotected and finally oxidized to the required disulfide bond. A wide variety of *S*-protecting groups have been described in the literature. The conditions for their removal are sometimes quite strong. For example, the cleavage of *S*-trityl cysteine with HBr or trifluoroacetic acid (TFA) affords only 60% of free cysteine because the removal of the *S*-trityl group in acid is in equilibrium with its attachment [1]; *S*-Diphenylmethyl (*S*-Dpm) requires 15–30 min of boiling in TFA and phenol [1]; HBr affords only a 50% yield at 50°. However, *S*-trityl and *S*-Dpm-cysteine can be deblocked with Ag^+- or Hg^{2+}-ions [2]. *S*-*t*-butyl-cysteine can be cleaved either by sulfitolysis [3] or with *o*-nitrophenyl-sulfenyl chloride [4]. The *S*-acetamidomethyl group [5] has also been widely used, the conditions for deprotection with Ag^+ or Hg^{2+} are very mild.

The oxidation step is also critical. While many procedures offer high yields for cysteine or cysteine derivatives [6] they sometimes fail in more complicated situations.

We wish to report here a rather general and mild method for deprotecting *S*-protected cysteine-residues which affords, after reaction, a reactive mixed disulfide which can react further with free SH-groups to provide an other disulfide.

Scheme 1 shows an outline of this *S*-deprotection by means of 2-pyridine sulfenyl chloride (PS-Cl, compound I).

PS-Cl is extremely reactive and decomposes immediately with moisture. However it is a solid and can be easily handled in water-free glacial acetic acid. The

[1]) Abbreviations are according to the IUPAC-IUB Commission on Biochemical Nomenclature.

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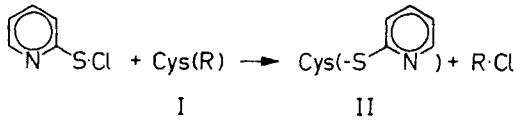
Scheme 1

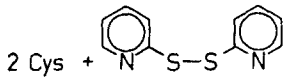
a)



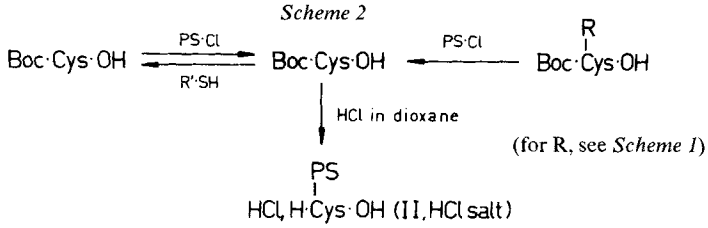
I II

R = trityl (Trt), diphenylmethyl (Dpm), acetamidomethyl (Acm), *t*-butyl, *t*-butylmercapto.

b)



Scheme 2



solution has an intense yellow colour which disappears on addition of a Cys(R)-solution. The persistence of a yellow colour shows the end point of titration. Compound II was obtained in a control reaction by reacting cysteine with di(2-pyridyl) disulfide (*Scheme 1*, reaction b).

The mixed active disulfide (II) is stable to acids with no significant decomposition. The BOC-group in BOC·Cys(PS)·OH can be eliminated in acidic medium without loss of the PS-group. BOC·Cys·OH reacts with PS-Cl to afford BOC·Cys-(PS)·OH. An equimolar amount of triethyl amine was added to avoid splitting the BOC-group by the HCl generated *'in situ' (Scheme 2).*

The mixed disulfide II is unstable towards alkali, immediately providing the symmetrical disulfides, cysteine and di(2-pyridyl) disulfide. A free SH-group can be produced quantitatively from II with an excess of thiol. The appearance of the yellow colour of 2-thiopyridone indicates the extent of the reaction.

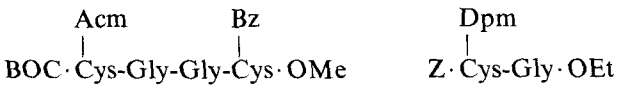
The deprotection of *S*-trityl, *S*-Dpm, *S*-Acm and *S*-*t*-butyl was instantaneous and quantitative. *S*-*t*-butylmercapto-cysteine produced, besides the desired compounds, the mixed disulfide, *t*-butyl 2-pyridyl disulfide. Attempts to use di(2-pyridyl) disulfide as a deblocking agent for I gave an equilibrium mixture with low yields of II.

The 'active' mixed disulfide II reacted immediately with free SH-groups of cysteine-derivatives in weakly acidic media (acetic acid). In HCl-solutions, the nucleophilicity of the SH-group is too low and the reaction proceeds very slowly. As a result of the reaction, the corresponding *S*, *S*-bridge is formed simultaneously with the liberation of 2-thiopyridone which can be followed spectrophotometrically. The reaction is rapid, even with larger peptides.

Compound II showed the unusual property of reacting with *S*-Acm-cysteine to give cysteine, but not with other *S*-protected groups. However, this reaction did not take place with larger peptides.

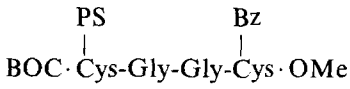
The use of PS-Cl during peptide synthesis. PS-Cl offers the unique advantage of simultaneous deprotection and activation of SH-groups. The activation enables a facile disulfide bond formation with another SH-group. PS-Cl opens the possibility of eliminating several steps during synthesis and to afford a very highly but at the same time specific active intermediate for the formation of disulfide bonds.

To illustrate the possibilities, two different peptides were synthesized:



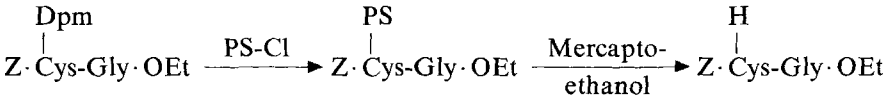
A **B**

By reacting peptide **A** with PS-Cl in AcOH in the presence of an equimolar amount of triethyl amine the pure (TLC.) peptide was obtained in quantitative yield.



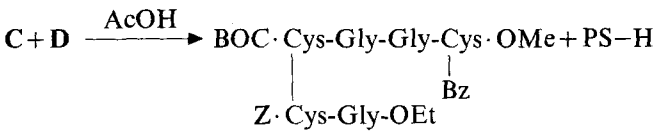
C

Peptide **B** was reacted with PS-Cl and after completion of the reaction 8 equivalents of mercaptothanol were added. The appearance of a yellow colour showed the release of thiopyridone:



D

The final step was carried out by mixing peptides **C** and **D** in acetic acid.



Deprotection, activation, and disulfide bond formation by means of PS-Cl left other protecting groups like BOC, Z, and Bz intact.

Tryptophan has been shown to react with some sulfenyl halides [7]. Thus, the possibility of complications should be kept in mind when working with tryptophan-peptides. We have not yet investigated this matter specifically.

In 1972, *Moroder et al.* [8] described a similar method for splitting the *S*-acetamidomethyl-group with another sulfenyl halide: *o*-nitrophenylsulfenyl chloride (NPS-Cl). We have tested the ability of NPS-Cl to cleave other *S*-protective groups; it is evident that NPS-Cl is able to split the same groups that PS-Cl does, but it is not as reactive. Reactions with NPS-Cl require between ten minutes (Acm) and a few hours (Dpm). The splitting of *S*-*t*-butyl with NPS-Cl was described by *Pastuszak & Chimiak* [4]. After reaction with NPS-Cl all these protected *S*-cysteine

derivatives gave the same compound, Cys(NPS), formerly described by *Phocas et al.* [9]. We have tested this compound's ability to form *S,S*-bonds and it has proven to be much less reactive that Cys(PS). Mixed disulfides have been used for the preparation of cystine bridges [10] and the preparation of an active mixed disulfide *via* di(4-pyridyl) disulfide has recently been utilized for the production of *S,S*-bonds between two different non-related proteins [11] (*cf. Scheme 1*, reaction b).

We hope that our new approach will favourably complement existing procedures.

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Experimental Part

PS-Cl is obtained by reacting a solution of di(2-pyridyl) disulfide in dry petroleum ether with dry chlorine. The reaction is complete in 1–2 h. After evaporation of the solvent, a yellow powder with pungent odour is obtained. Traces of water immediately destroy the reagent with production of the corresponding sulfenic acid. PS-Cl was isolated and identified by IR. and NMR. spectroscopy, although this is very difficult, because of the experimental conditions. The substance must be handled in an absolutely dry atmosphere. Dissolved in anhydrous acetic acid, it is stable for hours.

Deprotection of cysteine peptides. The peptide is dissolved in cold anhydrous acetic acid. A solution of 10–20% excess of PS-Cl in anhydrous acetic acid is introduced into the former solution dropwise while stirring (avoid moisture). A yellowish persistent colour (30 s) shows the end-point of the reaction. If BOC-groups are present, 10–20% excess of triethylamine should be added to neutralize the HCl that is occasionally liberated from PS-Cl whenever humidity is present. Solvents are evaporated and the residue crystallized from alcohol/water.

Regeneration of the deprotected SH-groups. The active disulfide obtained above is treated with eight equivalents of mercaptoethanol in dilute acetic acid and allowed to react for 1–2 h under nitrogen. The extent of the reaction is followed on TLC. plates. Solvents and mercaptoethanol are removed by evaporation i.HV. Instead of mercaptoethanol, three equivalents of thioacetamide can also be used [8].

Formation of disulfide bonds. The peptide with the free *SH*-group and the other peptide with the active mixed disulfide group are allowed to react in 5–10% acetic acid solution. The extent of the reaction can be followed by the appearance of the yellow colour from thiopyridone and/or by testing the reaction mixture at different time intervals by TLC.

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