CHEMISTRY LETTERS, pp. 2139-2142, 1994.

Phthalimidomethyl Group. A New Protecting Group of Thiols

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Phthalimidomethyl group is employed as a protecting group of thiols. This group can be introduced to thiols under mild reaction conditions and be removed by treatment with hydrazine hydrate followed by mercuric acetate or cupric acetate.

Development of a protecting group of thiol is an important issue in many areas of organic chemistry, especially in the field of peptide synthesis. A variety of thiol protecting groups have already been reported,[1] but with the increasing importance of peptides containing disulfide moieties such as α-neurotoxins of snake venoms[2] or conotoxins,[3] it is still desirable to devise a new thiol protecting group which can be introduced under mild reaction conditions and can be removed under specific conditions.[4] One of the most common protecting groups employed for cysteine is acetoamidomethyl(Acm) group.[5] This protecting group is highly stable to both acidic and basic conditions and can be selectively removed by treatment with heavy metal salts such as mercuric acetate[6] and silver trifluoromethanesulfonate,[7] or the protected thiol is directly oxidized to disulfide by treatment with iodine.[8] However, introduction of this protective group onto thiols necessitates strongly acidic conditions, and trifluoroacetic acid[9] or conc. hydrochloric acid[5] is usually employed as a solvent. During the study on synthesis of cyclic peptides having disulfide moiety, we encountered on the necessity of employing a new protecting group for thiols, and in this paper is described the introduction of S-phthalimidomethyl(S-Pim) group for this purpose.

Phthalimidomethyl group has been known as a protecting group of carboxyl group,[10] and also been employed as a derivatizing reagent of amino functionality.[11] Alkylthiomethylphthalimide itself was once employed as an intermediate for the synthesis of sulfinic acid and was synthesized by heating a thiol and bromomethylphthalimide without a solvent.[12] As it was obvious that this preparative method of alkylthiomethylphthalimide was not suitable for acid-sensitive thiols, we first examined the introduction of Pim group onto thiols.

In the first place, introduction of Pim group under weakly basic conditions was examined. And it was found that Pim-protected thiols were obtained in good yield by the reaction of equimolar amounts of thiols and N-chloro(or bromo)methylphthalimide[13] in the presence of triethylamine as a base in DMF at rt for several hours. Also, this protecting group can be introduced by treatment of thiols with an equimolar amount of N-hydroxymethylphthalimide in a mixture(1:19) of trifluoromethanesulfonic acid-trifluoroacetic acid at rt for a few hours. These results are summarized in Table 1. It should be noted that Pim protecting group can be introduced under very mild reaction conditions, while Acm group must be introduced under strongly acidic conditions using N-hydroxymethylacetamide due to the inaccessibility of N-halomethylacetamide.

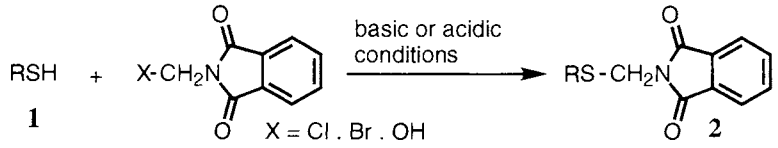


Table 1. Introduction of Pim group

Entry	Substrates	Basic conditions[a] X	Basic conditions[a] Yield/%	Acidic conditions[b] X	Acidic conditions[b] Yield/%
1	$C_8H_{17}SH$ (**1a**)	Br	57	–	–
2	$EtO_2CCH_2CH_2SH$ (**1b**)	Cl	87	–	–
3	$PhCH_2SH$ (**1c**)	Cl	>95	OH	83
4	4-Bu^t-C_6H_4SH (**1d**)	Cl	90	–	–
5	Boc-(L)-NHCHCO$_2$Me (**1e**) CH_2SH	Cl	>95	–	–
6	(L)-NH_2CHCO$_2$H (**1f**) CH_2SH	–	–	OH	80[c]

a) The reaction was carried out by mixing equimolar amounts of thiol and N-halomethylphthalimide in the presence of 1.05 equiv. of triethylamine in DMF at rt for several hours. b) The reaction was carried out by mixing equimolar amounts of thiol and N-hydroxymethylphthalimide in trifluoromethanesulfonic acid-trifluoroacetic acid (1:19) at rt for several hours. c) The product was isolated as a HCl salt.

We next examined the deprotection procedure. As it was expected that Pim group could be removed under the analogous conditions for the removal of Acm group, we first examined direct treatment of a Pim protected thiol **2e** with heavy metal salts such as mercuric acetate. However, treatment of the Pim protected thiol **2e** with mercuric acetate in methanol revealed that Pim-protected thiol is rather stable under these reaction conditions and prolonged reaction time resulted in a rather complex mixture of products. However, treatment of the Pim protected thiol **2e** with hydrazine hydrate in MeOH at 0 °C to rt for 2 h induced the cleavage of phthalimide moiety to give a hydrazide derivative, which was found to be susceptible to deprotection under the above conditions. Thus, when the disappearance of the starting material was confirmed by TLC, mercuric acetate was added to this reaction mixture at rt followed by the addition of 20%(V/V) acetic acid aqueous solution to adjust the pH of the solution to about 4. After the mixture was stirred for 2 h at rt, 2-mercaptoethanol was added to the mixture to promote the decomplexation of the resulting thiol **1e**, and then insoluble materials were filtered off and the filtrate was concentrated in vacuo. The crude product was purified by silica-gel column chromatography to give the deprotected thiol **1e** in 85% yield. More importantly, it was found that cupric acetate can also be employed instead of mercuric acetate, and deprotection occurred with the same efficiency as compared with the reaction using mercuric acetate. In Table 2 are summarized the results of the above deprotection procedure using various substrates. As clearly shown in this Table, various Pim-protected thiols having functional groups including a dipeptide **2g**[14] can be deprotected in good yield by a one-pot procedure under mild reaction conditions. Although obvious formation of hydrazides from ester functionality was not observed in the reactions of **2b**, **2e**, and **2g**, prolonged exposure to hydrazine hydrate should be avoided in these cases.[15]

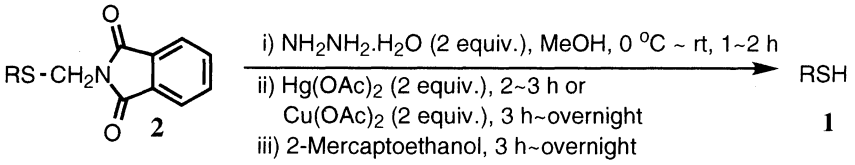


Table 2. Deprotection of S-Pim Group

Entry	Substrates	Metals	Yield / %
1	C_8H_{17}SPim (**2a**)	$Hg(OAc)_2$	83
		$Cu(OAc)_2$	79
2	$EtO_2CCH_2CH_2$SPim (**2b**)	$Hg(OAc)_2$	71
		$Cu(OAc)_2$	69
3	4-Bu^t-C_6H_4SPim (**2d**)	$Hg(OAc)_2$	92
		$Cu(OAc)_2$	88
4	Boc-(L)-NHCHCO$_2$Me (**2e**) CH_2SPim	$Hg(OAc)_2$	85
		$Cu(OAc)_2$	75
5	Boc-(L)-Cys-(L)-ValOMe (**2g**) Pim	$Hg(OAc)_2$	87
		$Cu(OAc)_2$	84

Conversion of the Pim protected thiols to disulfides was next examined. Direct conversion of a Pim protected thiol to a disulfide with iodine was not a facile process and a rather sluggish mixture of products were obtained after prolonged reaction time. However, when Pim protected thiols were treated with hydrazine hydrate and then with iodine in methanol at rt, the reaction proceeded smoothly and the corresponding disulfides were obtained in good yield.

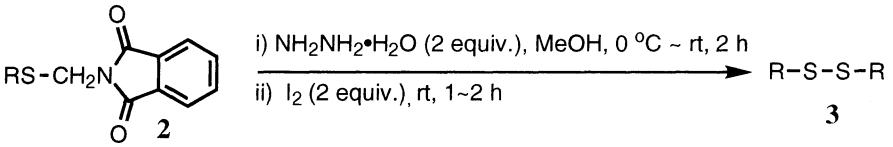
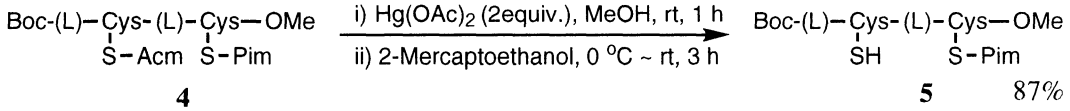


Table 3. Conversion to Disulfide

Entry	Substrates	Yield / %
1	$EtO_2CCH_2CH_2$SPim (**2b**)	82
2	$PhCH_2$SPim (**2c**)	89
3	4-Bu^t-C_6H_4SPim (**2d**)	86
4	Boc-(L)-NHCHCO$_2$Me (**2e**) CH_2SPim	79

Finally, selective cleavage of S-Acm group in the presence of S-Pim group was tried. The dipeptide composed of S-Acm protected cysteine and S-Pim protected cysteine **4** was chosen as a model substrate. When this dipeptide **4** was treated with mercuric acetate in MeOH-20% acetic acid aqueous solution (2:1) at rt for 1 h followed by the addition of 2-mercaptoethanol, the S-Acm deprotected disulfide **5** was obtained in 87% yield. Thus, S-Acm group can be selectively removed in the presence of S-Pim group.



4 **5** 87%

The authors would like to thank Professor Masaaki Ueki of Science University of Tokyo for helpful discussions on this subject and also thank Professor Koichi Narasaka of the University of Tokyo for discussions and encouragement during this work.

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13) These compounds are commercially available.
14) The dipeptide **2g** was prepared by the standard DCC-HOBt method in 91% yield. No racemization was detected by 500 MHz ^{1}H NMR spectra.
15) Standard procedure for the preparation of hydrazides from esters requires large excess of hydrazine hydrate and longer reaction time at rt. See for example; N. Fujii and H. Yajima, *J. Chem. Soc., Perkin I*, **1981**, 789. Selective removal of phthalimido group in the presence of ester functionality is reported. See for example; J. C. Sheehan and P. A. Cruickshank, *J. Am. Chem. Soc.*, **78**, 3677 (1956).

(Received August 18, 1994)