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A mild and selective protecting and reversed modification of thiols

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ABSTRACT

One selective thiol-protecting study has been investigated for a wide range of thiols including general thiols and thiols containing multiple functional groups. The reactions of bromomaleimides and thiols under the mild condition afforded the protected products in excellent yields. The thiols can be recovered very quickly using dithiothreitol (DTT) under the mild condition.

Thiols are the most important nucleophilic residues for studying peptides and proteins in chemical biology.[1] Synthesis of thiol-containing biomolecules is an important, yet challenging work which usually was puzzled by the formation of disulfide bond and unexpected acetylation or alkylation.[2,3] In the past few decades, selective chemical modification, fluorescent labeling, and detection of thiols in proteins is widely used in a range of fundamental biological and biophysical studies.[4] Significant research efforts have been realized that the optical probes for various biological thiols to achieve high sensitivity, low cost, and ease of detection have been developed.[4] Meanwhile, identification of reagents that enable blocking or labeling of protein thiols with high selectivity and conversion yields has attracted great attention.[5] Common thiol-protecting groups such as thioethers (trityl, benzyls, and *t*-butyl),[6] thioesters,[7] and disulfides[8] have limited scope of applications due to either unsatisfactory stability profiles or the harsh deprotecting conditions. The acetamidomethyl (Acm) protecting group developed by Hirschmann and co-workers has been shown to be useful in the synthesis of peptides.[9] Unfortunately, the reagents could be dimmed by the use of toxic heavy metals in deprotection process. For the purpose of protecting Cys side chain in peptides and proteins, Liu and co-workers developed a thiol protecting group called Hqm group.[10] It's a good-quality protecting group, however, several synthetic steps were required for synthesis and protecting processes. In order to protect the thiols in peptide condensation reactions, phenacyl and *N*-methylphenacyloxycarbamidomethyl were developed to improve thioether-based thiol-protecting groups by Hojo and co-workers.[11] Nevertheless, the carbonyl of these protecting groups may react with amino residues of peptides, which need to be protected before the thiol-protecting process. Other protecting groups such as *p*-toluenesulfonylacetylene[12] and quinolone[13] have limited utilization due to the instability in the presence of amino or the high protecting temperature.

Maleimides have been proved to be one of the most widely used reactive motifs for cysteine modification.[1] Bromomaleimides, developed by Baker and co-workers, react rapidly with protected cysteine to afford thiomaleimides.[14] Recently, bromomaleimides have been successfully applied in the synthesis of polymers,[15] protein labeling,[16] and peptide platforms[17] with protected cysteines. All cysteines used in these methods were protected cysteines. To the best of our knowledge, the efficient protecting and reversed modification of general thiols or thiols with active functional groups has not been studied. Herein, we wish to focus on bromomaleimides as selective thiol-protecting reagents to a wide range of thiols. Furthermore, DTT has been applied to an efficient deprotecting reagent of protected thiols under the mild condition.

The protecting group bromo-*N*-R-maleimide **1** could be easily synthesized from the corresponding *N*-R-maleimides in two steps.[18] In the initial experiment, bromo-*N*-methylmaleimide **1a**

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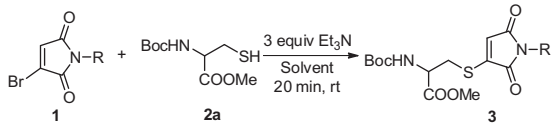
Table 1
Optimization of reaction conditions[a]



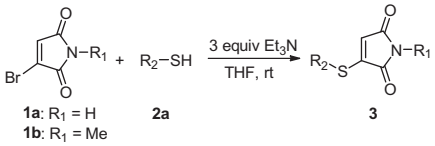
Entry	R	Solvent	Yield[b] (%)
1	Me	THF	99
2	Me	MeOH	96
3	Me	Toluene	93
4	Me	CH_2Cl_2	95
5[c]	Me	H2O	91
6	H	THF	97
7	Et	THF	92
8	Ph	THF	83
9	Bn	THF	77

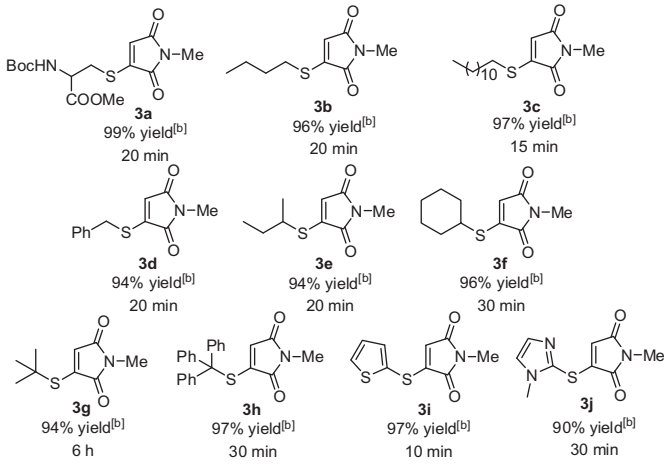
[a] Reaction condition: to a solution of **2a** (61.8 mg, 0.263 mmol) in solvents (5 mL) was added Et_3N (0.789 mmol) and **1** (0.263 mmol) and the resulting mixture was stirred for 20 min at rt.
[b] Isolated yields.
[c] 0.5 mL DMF as base and 4.5 mL H_2O was used.

and commercially available *N*-Boc-Cys-OMe **2a** were tested in the model reaction (Table 1). Treatment of **2a** (0.263 mmol) and **1a** (0.263 mmol) with 3 equiv of Et_3N in THF (5 mL) resulted in a rapid and complete reaction in 20 min with excellent yields (99%, Table 1, entry 1). Different solvents and bases were tested for the reactions. The reactions in other solvents, such as MeOH, toluene, CH_2Cl_2, and water, also gave the products in excellent yields (>93%) within 20 min (Table 1, entries 2–4). To our delight, it was convinced that the protecting process was easy to implement at room temperature, which even took place in water as solvent and DMF as base (Table 1, entry 5). Other organic or inorganic bases, such as DMAP, DIPEA and $NaHCO_3$ were also suitable for this reaction. On the basis of these exploratory studies, we probed various bromomaleimides derived thiol-protecting groups with *N*-Boc-Cys-OMe under the treatment of Et_3N in THF. It was found that the reactions with small protecting groups, such as H, Me and Et, took place very quickly to give the products in high yields (Table 1, entries 1, 6 and 7). In contrast, the bromomaleimide with bigger substitutes, such as bromo-*N*-phenylmaleimide **1d** and bromo-*N*-benzylmaleimide **1e**, afforded the corresponding products in lower yields (Table 1, entries 8 and 9). Longer reaction time could not raise the reaction yields. We envisioned that both steric and electronic effects of substituents account for the yield differences. Based on the above results, the optimized reaction condition was found: THF as solvent, Et_3N as base, bromo-*N*-methylmaleimide **1a** or bromomaleimide **1b** as the protecting reactants.

With the optimal reaction condition in hand, we turned our attention to probing the scope of different thiols (Table 2). Satisfactorily, almost all reactions finished within half an hour to give the protected products in excellent yields (Table 2). Linear thiols reacted smoothly and finished in 20 min to afford the products in excellent yields (**3b**–**3d**). Branched isobutylthiol had similar reactivity as linear thiols (**3e**). Steric effect had great effect on this reaction. Bulky cyclohexanethiol gave high yields while longer reaction time was needed (**3f**). More bulky *t*-butylthiol needed 6 h to finish the reaction (**3g**). Interestingly, triphenylmethylthiol reacted much faster than *t*-butylthiol under the same reaction condition even though its steric effect was greater than *t*-butylthiol (**3h**, 30 min). The heterocyclicthiols, such as thiophene-2-thiol and 1-methyl-1*H*-imidazole-2-thiol, afforded the corresponding products with high yields (**3i**–**3j**).

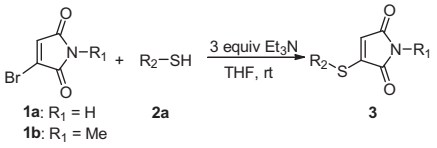
Table 2
Thiol-protecting reactions for general thiols[a]

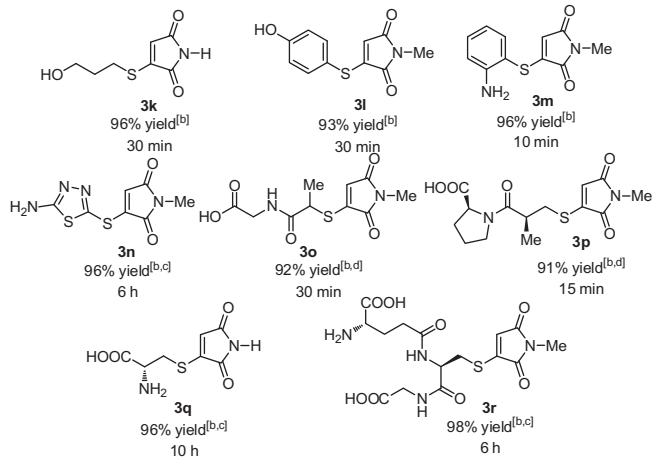




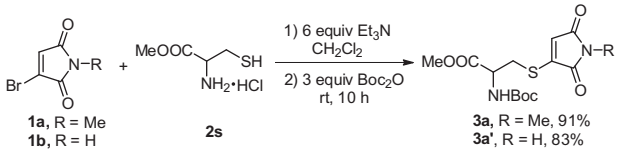
[a] Reaction condition: To a solution of **2** (0.263 mmol) in THF (5 mL) was added Et_3N (0.789 mmol) and **1** (0.263 mmol) and the mixture was stirred for the indicated time at rt.
[b] Isolated yields.

Table 3
Thiol-protecting reactions for thiols with functional groups[a]





[a] Reaction condition: To a solution of **2** (0.263 mmol) in THF (5 mL) was added Et_3N (0.789 mmol) and **1** (0.263 mmol) and the mixture was stirred for the indicated time at rt.
[b] Isolated yields.
[c] H_2O was used as solvent without the addition of Et_3N.
[d] $NaHSO_4$ (2.63 mmol) and anhydrous $MgSO_4$ (1 g) was added into the mixture.



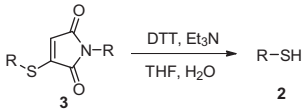
Scheme 1. Thiol-protecting with amino-protecting reactions.

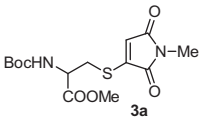
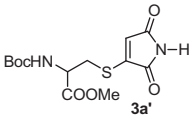
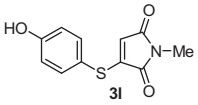
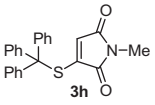
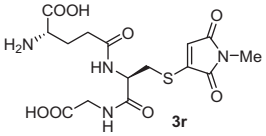
Having tested the reactivity of general thiols with bromomaleimides, next we questioned whether thiols containing functional groups also had good selectivities. Various thiols with different functional groups were investigated in the thiol protecting reactions (Table 3). Thiol substrates containing hydroxyl, phenolic hydroxyl, and phenylamino groups gave the only thiol protected products in excellent yields without any other byproduct (**3k**–**3m**). Heterocyclic 5-amino-1,3,4-thiadiazole-2-thiol afforded the mono thiol addition product **3n** in high yields. Both thiol and carboxyl are common groups in drug molecules. In order to investigate the effects of carboxyl on the thiol-protecting reactions, we selected tiopronin **2o** and captopril **2p** as models for the research. The reactions proceeded very quickly to give single thiol protected products in excellent yields (**3o**–**3p**). Encouraged by the promising results, we moved on to investigate the protecting method for water-soluble substrates, such as cysteine and glutathione. Water-soluble substrates containing mercapto, carboxy, and amino could not easy to be protected by conventional methods as a result of low solubility in organic solvents. Inspired by the result of good reaction in water in the optimization process, we mixed bromomaleimides and water-soluble thiol substrates in water under vigorous stirring. These substrates contain free amine group which can be used as base instead of Et_3N in the reaction. When the reaction completed, water was removed under vacuum and the excess protecting reagent was washed by CH_2Cl_2. To our delight, the desired thiol protected products were obtained in excellent yields although much longer reaction time was needed (**3q**–**3r**).

Next, we wanted to investigate two protecting reactions to protect thiol and amine using a 'one-pot' process. In our exploratory study, the commercially available methyl 2-amino-3-mercaptopropanoate hydrochloride **2s** was chosen as a model substrate for the proposed one-pot protecting reactions (Scheme 1). After the addition of Et_3N into the mixture of substrates in CH_2Cl_2 for 20 min, Boc_2O was added and stirred for another 10 h at rt to give the desired product. Both bromomaleimide and bromo-*N*-methylmaleimide can be used as thiol protecting groups to afford products 3a and 3a' in high yields.

In our effort on the construction and expanding scope of the synthetically useful thiol protecting method, we also wish to develop an efficient deprotecting method. In Baker and co-workers' work, expensive TCEP in buffer was applied to reverse the modification of cysteine and protein.[14] Later on, cheaper 2-mercaptoethanol (BME) and 1,2-ethanedithiol (EDT) have been applied to the cleavage strategy. However, large scale of BME (100 equiv) and longer reaction time of EDT (24 h) was needed.[16] We tested a series of potential deprotection reagents such as triphenylphosphine, tributylphosphine and DTT. The results showed that DTT in THF and water, under the treatment of Et_3N, was very efficient for cleaving C–S bond. Several selective substrates were chosen for the deprotection study and the results are listed in Table 4. In

Table 4
Deprotection of maleimide protected thiols[a]



Entry	3	DTT (equiv)	TEA (equiv)	*t* (min)	Yield[b] (%)
1	 **3a**	1.1	0.1	15	92
2	 **3a'**	3.0	1.5	15	83
3	 **3l**	3.0	3.0	30	95
4	 **3h**	1.1	3.0	15	96
5	 **3r**	3.0	3.0	30	91[c]

[a] Reaction condition: To a solution of **3** (0.2 mmol) in THF (8 mL) and H_2O (2 mL) was added dropwise DTT and Et_3N in THF (2 mL) over 5 min. Then the solution was stirred at rt for the indicated time.
[b] Isolated yields.
[c] The yield was determined by ^1H NMR using CH_2Br_2 as internal standard.

the deprotecting reaction of **3a**, the loading of DTT can be as low as 1.1 equiv and only 0.1 equiv of Et_3N was needed. For other substrates, no more than 3 equiv of DTT and Et_3N was used to give the corresponding deprotected thiols in high to excellent yields. It should be noted that all reactions completed within 30 min. With the demonstrated results, we have illustrated DTT as a synthetically useful deprotecting reagent for thiol protecting method.

In summary, bromomaleimides have been used as an efficient and selective thiol-protecting group, which is applicable for a wide range of thiols including general thiols and thiols containing multiple functional groups. The reaction proceeded very fast under the mild condition to give protected thiols in excellent yields. Furthermore, the protected group can be easily removed under the mild condition using low equivalent of DTT to release the sulfhydryl moiety.

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Supplementary data

Supplementary data (all experimental procedures, spectroscopic data) associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.tetlet.2016.05.027.

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