Disulfide formation *via* sulfenamides†‡

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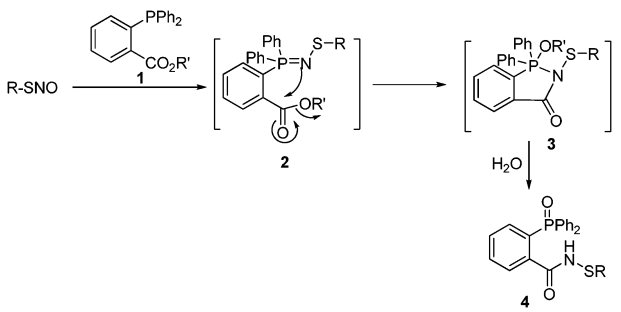
A phosphine-mediated one-step disulfide formation from *S*-nitrosothiols has been developed. This reaction can convert unstable *S*-nitrosothiols to stable disulfides *via* sulfenamide intermediates under very mild conditions. It has the potential to be used for the detection of *S*-nitrosothiols.

As an important post-translational modification, *S*-nitrosation converts protein cysteine residues (SH) to *S*-nitrosothiols (SNO). This protein modification leads to modulation of structure, inhibition of active site cysteines, disruption of allosteric interactions with other molecules, *etc.*[1] The formation of *S*-nitrosothiols in proteins may be directed by peptide sequences surrounding sensitive cysteine residues, by exogenous *versus* endogenous nitric oxide (NO) sources or by compartmentalization of nitric oxide synthases. Although the importance of *S*-nitrosation has been well recognized, the detection of *S*-nitrosation is still a challenge.[2] This is primarily due to the lability of *S*-nitrosothiols and the lack of reliable methods for their detection. From a chemistry point-of-view, SNO is a unique functional group; it might have some distinct reactivity from other biological functional groups. If specific reactions which only target SNO can be developed, such reactions may be very useful for the detection of *S*-nitrosation.

Our group recently initiated a program to study phosphine-based reactions of *S*-nitrosothiols. In 2008, a fast reductive ligation of SNO was developed (Scheme 1).[3] This reaction selectively converts unstable SNO to stable sulfenamide products *via* a Staudinger ligation-like mechanism.[3,4] However, we also noticed that sulfenamides generated from cysteine derivatives could be over-reduced by the excess of phosphine ligation reagents. This might be a problem for directly applying reductive ligation in SNO detection.



Scheme 1 Reductive ligation of SNO.

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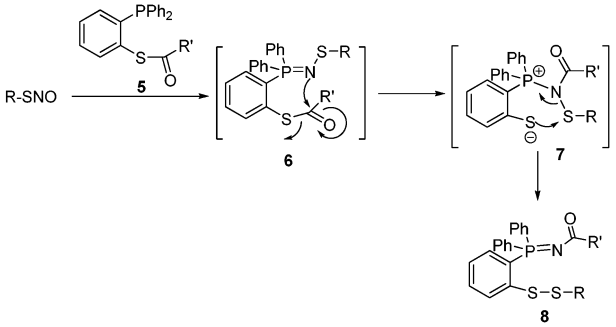


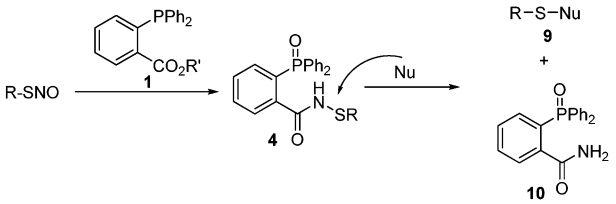
Scheme 2 Bis-ligation of SNO.

To solve this problem, we developed a bis-ligation of SNO in 2009 (Scheme 2).[5] This reaction can convert SNO to stable disulfide-iminophosphorane conjugates in one step. Previous studies have revealed that disulfides are stable towards excess triarylphosphines such as the phosphine reagents used in our reaction.[3–5]

Given the high reactivity of the sulfenamide intermediates towards the thiolate observed in bis-ligation, we propose another one-step reaction to convert unstable SNO to stable products (Scheme 3). As such, SNO will be treated with regular reductive ligation reagent **1**. As the reductive ligation is a very fast reaction, sulfenamide products should be formed in minutes. Then, if a nucleophile is added into the reaction mixture, it may convert sulfenamide **4** to a stable final product **9** and liberate the phosphine oxide **10**. The formation of simple adducts, without the bulky triarylphosphine-oxide, would be attractive for applications in protein systems. We realized that if this one-pot reaction is used in the detection of protein SNO, one critical concern is that the nucleophile should only react with sulfenamides, not with protein disulfides.

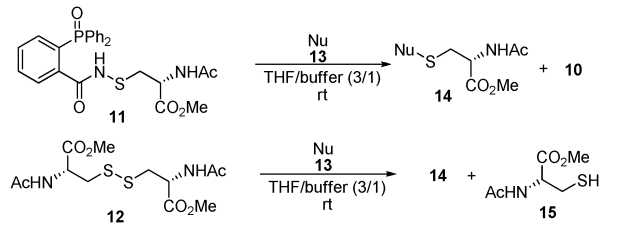
To test this idea, sulfenamide **11** was prepared and used as a model compound to test the reactivity of a series of mild nucleophiles. The reaction was carried out in a mixture of THF and PBS buffer (pH 7.4) solution. At the same time, compound **12** was used to examine the reactivity of the nucleophiles towards disulfides under the same conditions.

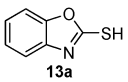
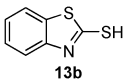
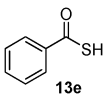
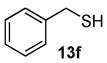
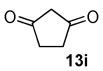
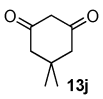
As shown in Table 1, two thiol nucleophiles (**13a** and **13b**) showed good selectivity for the sulfenamide, as no reaction



Scheme 3 Proposed one-pot reaction to convert SNO to stable products.

Table 1 Reactivity of a series of nucleophiles towards sulfenamides and disulfides

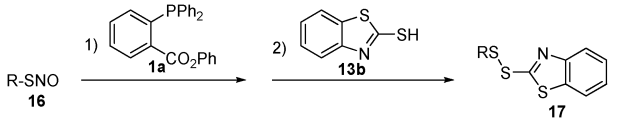


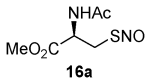
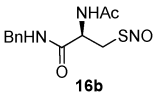
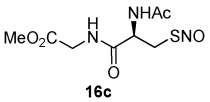
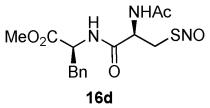
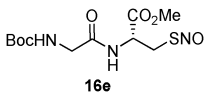
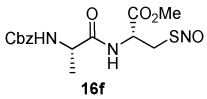
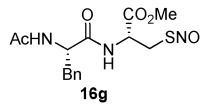
Nu	Reaction time/h	Reaction with **11**	Reaction with **12**
 **13a**	2	**14a** 78%	NR
 **13b**	0.5	**14b** 90%	NR
 **13c**	0.5	**14c** 70%	**14c** 15%
 **13d**	0.5	**14d** 92%	**14d** 14%
 **13e**	0.5	**14e** 63%	**14e** 40%
 **13f**	0.5	**14f** 85%	**14f** 90%
 **13g**	0.5	**14g** 60%	**14g** 72%
 **13h**	2	NR	NR
 **13i**	2	NR	NR
 **13j**	2	NR	NR

was observed when they were treated with disulfide **12**. Other thiols (**13c–13g**) reacted with both the sulfenamide and disulfide. Tertiary thiols like **13h**, however, showed no reactivity to either **8** or **9**, probably due to steric hindrance. We also tested carbon nucleophiles **13i** and **13j** in our reactions. **13i** was previously reported to react with cyclic sulfenamides.[6] However, both **13i** and dimedone **13j** failed to show any reactivity to sulfenamide **11** even under refluxing conditions.

With these results in hand, we turned to the one-step disulfide formation from *S*-nitrosothiols using **13b**, the best substrate found in Table 1. A series of SNO substrates were tested (Table 2). The reaction was carried out as follows: freshly prepared SNO substrates were treated with phosphine

Table 2 One-pot disulfide formation from SNO

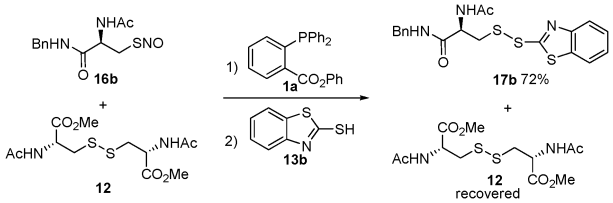


RSNO	Product	Yield (%)
 **16a**	**17a**	85
 **16b**	**17b**	86
 **16c**	**17c**	43
 **16d**	**17d**	55
 **16e**	**17e**	65
 **16f**	**17f**	82
 **16g**	**17g**	75

1a in a mixture of 3 : 1 THF/PBS buffer (pH 7.4) at room temperature. After 10 min, **13b** was added and the reaction mixture was stirred for an additional 30 min to afford the desired product. As shown in Table 2, all of the SNO substrates exhibited good reactivity in the reaction and the desired disulfide products were obtained in good yields.

In order to confirm that the reaction is specific only to SNO, while not affecting disulfides, we performed a crossover experiment. As shown in Scheme 4, the one-step disulfide formation using **16b** was carried out in the presence of disulfide **12**. In this reaction, **17b** was the only product and no crossover product was observed. Disulfide **12** was completely recovered.

In conclusion, we have developed a one-pot disulfide formation from *S*-nitrosothiols under very mild conditions. This reaction does not affect disulfide bonds. We believe this



Scheme 4 Crossover experiment.

reaction has the potential to be used in the detection of *S*-nitrosothiols.

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Notes and references

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