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One-Pot Thioether Formation from *S*-Nitrosothiols

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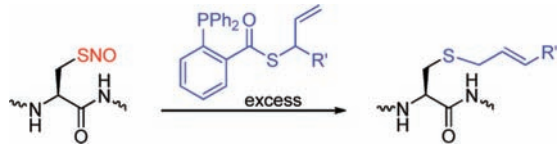
ABSTRACT



Protein *S*-nitrosation is an important post-translational modification. However, the detection of *S*-nitrosation is still problematic because *S*-nitrosation products, that is, *S*-nitrosothiols, are unstable species. Here a new reaction which can selectively convert unstable *S*-nitrosothiols to stable thioethers in one-pot under very mild conditions is reported. This reaction has the potential to be applied in the detection of protein *S*-nitrosation.

Nitric oxide (NO), an endogenous cell signaling agent, is an important mediator in biological systems. NO-mediated protein S-nitrosation is a critical post-translational modification which has strong and dynamic interactions with redox signaling.[1] However, currently the detection of S-nitrosation is still a challenge[2] because the products of *S*-nitrosation, that is, *S*-nitrosothiols (SNO), are unstable adducts and methods to capture fleeting SNO are lacking. We believe that if new bioorthogonal reactions of SNO can be developed, such reactions should hold considerable promise for SNO detection. With this idea in mind, our group has developed a series of phosphine-based reactions of SNO and proved these reactions can selectively target SNO and convert unstable SNO to stable and detectable products.[3] In a recent work, triphenylphosphine-thioester substrates were used to form disulfide conjugates with SNO in one-step and such a strategy has been successfully applied to label protein SNO in cell extracts (Scheme 1A).[3e] Although the disulfide linkage is sufficiently stable for many protein analyzing techniques, such as Western blotting, more stable conjugates than disulfides would be ideal for proteomic studies of *S*-nitrosation and for the applications in more complex biological systems, especially in the presence of free thiols such as cysteine or glutathione. Herein, we report a reaction which can selectively convert SNO in one-pot to stable thioether conjugates.

Inspired by the phosphine-mediated allyl disulfide rearrangement developed by Crich and co-workers,[4] we designed a one-pot thioether formation from SNO. As shown in Scheme 1B, the reaction between phosphine-thioester substrates like **2** and SNO should first generate an aza-ylide intermediate **3**. The reductive ligation process that follows should then provide a sulfenamide **4** and an allyl thiolate **5**.

(1) (a) Lancaster, J. R. *Nitric Oxide.* **2008**, *19*, 68. (b) Zhang, Y.; Hogg, N. *Free Radic. Biol. Med.* **2005**, *38*, 831. (c) Hess, D. T.; Matsumoto, A.; Kim, S. O.; Marshall, H. E.; Stamler, J. S. *Nat. Rev. Mol. Cell. Biol.* **2005**, *6*, 150. (d) Foster, M. W.; McMahon, T. J.; Stamler, J. S. *Trends Mol. Med.* **2003**, *9*, 160.

(2) (a) For a recent RSNO detection method, see: Bechtold, E.; Reisz, J. A.; Klomsiri, C.; Tsang, A. W.; Wright, M. W.; Poole, L. B.; Furdui, C. M.; King, S. B. *ACS Chem. Biol.* **2010**, *5*, 405. For selected reviews on RSNO detection, see: (b) Gow, A.; Doctor, A.; Mannick, J.; Gaston, B. *J. Chromatogr., B* **2007**, *851*, 140. (c) Kettenhofen, N. J.; Broniowska, K. A.; Keszler, A.; Zhang, Y.; Hogg, N. *J. Chromatogr., B* **2007**, *851*, 152. (d) MacArthur, P. H.; Shiva, S.; Gladwin, M. T. *J. Chromatogr., B* **2007**, *851*, 93. (e) Jaffrey, S. R. *Methods Enzymol.* **2005**, *396*, 105. For deficiencies of current methods, see: (f) Giustarini, D.; Milzani, A.; Dalle-Donne, I.; Rossi, *J. Chromatogr., B* **2007**, *851*, 124. (g) Gladwin, M. T.; Wang, X.; Hogg, N. *Free Radical Biol. Med.* **2006**, *41*, 557.

(3) (a) Wang, H.; Xian, M. *Angew Chem. Int. Ed.* **2008**, *47*, 6598. (b) Wang, H.; Zhang, J.; Xian, M. *J. Am. Chem. Soc.* **2009**, *131*, 13238. (c) Zhang, J.; Wang, H.; Xian, M. *Org. Lett.* **2009**, *11*, 477. (d) Zhang, J.; Wang, H.; Xian, M. *J. Am. Chem. Soc.* **2009**, *131*, 3854. (e) Zhang, J.; Li, S.; Zhang, D.; Wang, H.; Whorton, A. R.; Xian, M. *Org. Lett.* **2010**, *12*, 4208.

(4) (a) Crich, D.; Brebion, F.; Krishnamurthy, V. *Org. Lett.* **2006**, *8*, 3593. (b) Crich, D.; Krishnamurthy, V.; Brebion, F.; Karatholuvhu, M.; Subramanian, V.; Hutton, T. K. *J. Am. Chem. Soc.* **2007**, *129*, 10282.

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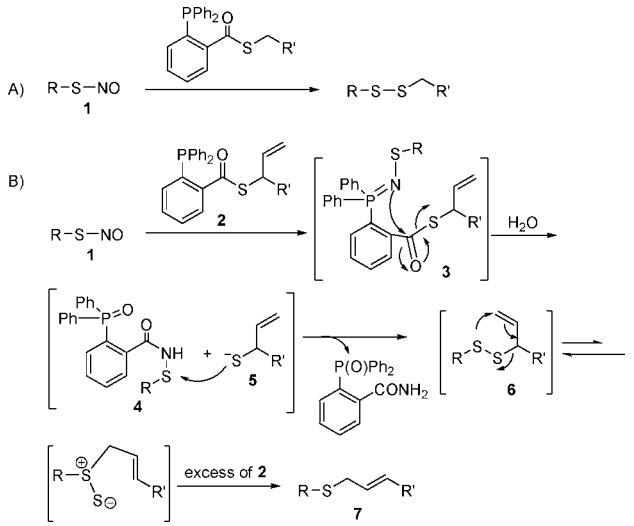
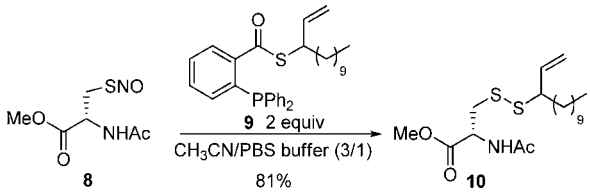
Scheme 1



A spontaneous reaction between **4** and **5** was expected to produce an allyl-disulfide **6**. If this reaction is to be applied to label protein SNO, substrate **2** will always be in large excess compared to SNO moieties in proteins. Therefore, we expected phosphine **2** should trigger the allyl disulfide rearrangement to furnish the final product **7**.

To realize this idea, we first tested the one-step disulfide formation of SNO using substrate **9** (Scheme 2). Previous

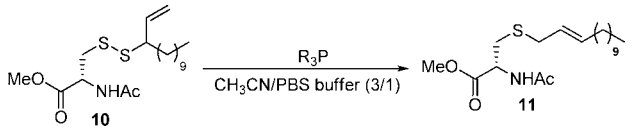
Scheme 2



results have demonstrated the feasibility of disulfide formation using phosphine-thioester substrates generated from primary thiols.[3e] However, it was not clear if substrates generated from secondary thiols like **9** could work for such process as the reaction between sulfenamides and secondary thiolates was expected to be more difficult than with primary thiolate.[3e] Nevertheless, we were pleased to find that the reaction worked nicely in CH_3CN/PBS buffer (3/1) mixtures to furnish the desired disulfide **10** in good yield.

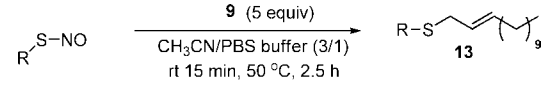
With compound **10** in hand, we tested the phosphine-mediated allyl disulfide rearrangement. Aiming the applications in protein labeling, we would like to have this rearrangement completed under mild conditions and in a short period of time. Triphenylphosphine was first tested in this study (Table 1). We found that the temperature was

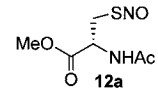
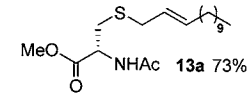
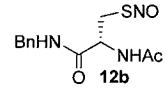
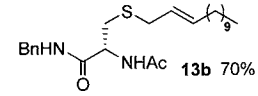
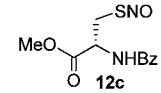
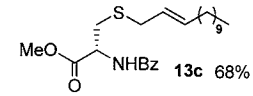
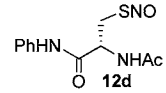
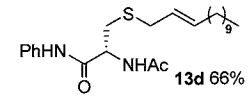
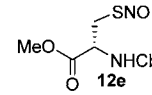
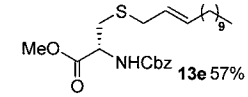
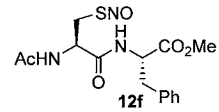
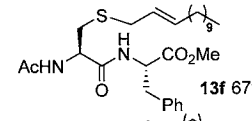
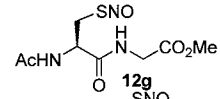
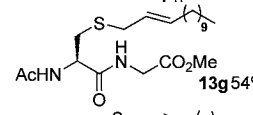
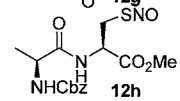
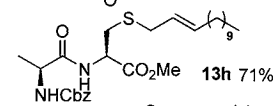
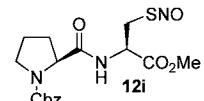
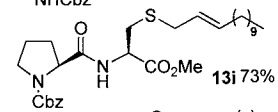
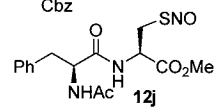
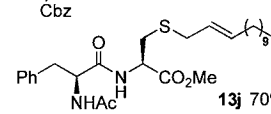
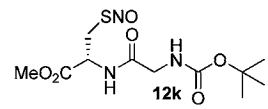
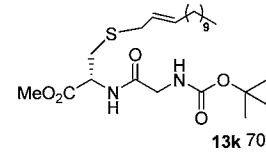
Table 1. Optimization of Allyl-Disulfide Rearrangement



R_3P	equiv	temp	reaction time	Prod(yields%)
PPh_3	2.0	rt	1.0 h	50%
PPh_3	2.0	50 °C	1.0 h	85%
PPh_3	5.0	50 °C	1.0 h	>95%
9	3.0	50 °C	1.0 h	>95%

Table 2. One-Pot Thioether Formation Scope



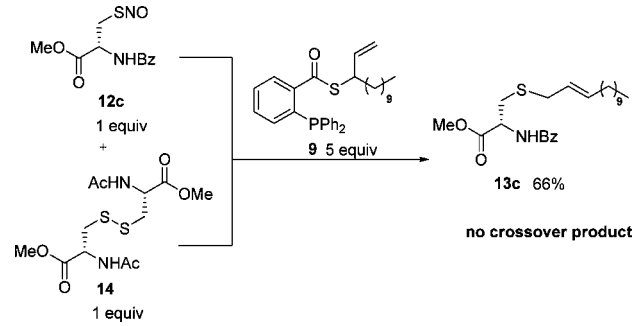
entry	RSNO	product (yield%)
1	 **12a**	 **13a** 73%
2	 **12b**	 **13b** 70%
3	 **12c**	 **13c** 68%
4	 **12d**	 **13d** 66%
5	 **12e**	 **13e** 57%
6	 **12f**	 **13f** 67%
7	 **12g**	 **13g** 54%
8	 **12h**	 **13h** 71%
9	 **12i**	 **13i** 73%
10	 **12j**	 **13j** 70%
11	 **12k**	 **13k** 70%

important for this transformation. If it was performed at rt, only ~50% transformation was achieved after 1 h. However, if the reaction was carried out at 50 °C, we obtained much higher yield. Excess of PPh_3 (5 equiv) also improved the yield. In addition, we were glad to find that compound **9**, the ligation reagent for disulfide formation, could also effectively promote the rearrangement under this optimized condition.

We then turned our attention to the proposed one-pot thioether formation from S-nitrosothiols. As shown in Table 2, a series of freshly prepared SNO were treated with compound **9** (5eq) in CH_3CN/PBS buffer. The reaction was monitored by TLC. In all cases, the reaction was able to complete within 3 h and the desired products **13a**−**k** were obtained in reasonable yields. As shown in Scheme 1, this tandem reaction included four individual steps: aza-ylide formation, ligation, disulfide formation, and allyl disulfide rearrangement. Good overall yields observed in the whole process indicated that each step proceeded very effectively. Although significant amount of organic solvent was needed in current reaction conditions, due to solubility problem, we expect this could be solved by developing water-soluble phosphine reagents in future studies.

If this reaction will be applied for labeling protein SNO, a concern is that the thiolate intermediate (i.e., **5** in Scheme 1) may react with protein disulfides to give false positive linkage. To address this concern, we tested the reaction between SNO **12c** and phosphine substrate **9** in the presence of a disulfide compound **14** (Scheme 3). As expected, only the desired product **13c** was obtained (see Supporting Information for details). No crossover product with **14** was observed.

Scheme 3



In summary, a one-pot thioether formation from *S*-nitrosothiols has been developed. This reaction can selectively convert unstable SNO to stable thioether conjugates under very mild conditions. Disulfides are not affected in the reaction. Given the stability of thioethers in biological systems, this reaction holds considerable promise in the applications for the detection of protein *S*-nitrosation.

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Supporting Information Available: Synthetic procedures, spectroscopic data, and experimental procedures. This material is available free of charge via the Internet at http://pubs.acs.org.

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