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Exploration of the "Traceless" Reductive Ligation of *S*-Nitrosothiols

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ABSTRACT

The first "traceless" reductive ligation of *S*-nitrosothiols using phosphine ester/thioester conjugates is reported. Experiments also show that stable thioimidate compounds could be formed in the reaction between *S*-nitrosothiols and some phosphine−thioester substrates.

S-Nitrosothiol (RSNO) formation on cysteine residues represents an important post-translational modification that transduces nitric oxide (NO)-dependent signals.[1] However, the detection of RSNOs in biological systems is still a challenge due to the lability of the SNO moiety.[2] The analytical deficiencies become evident when it is observed that reported values of the analysis of the same tissue or biological fluid by different research groups cover some orders of magnitude.[3] In our opinion, SNO is a unique functional group that should have distinct reactivity from other biological functional groups. If we can develop new bioorthogonal reactions specifically targeting SNO and converting unstable *S*-nitrosothiols to stable products, such a reaction would hold considerable promise in applications for RSNO detection.

Recently, our laboratory developed a reductive ligation of RSNOs which converts RSNOs to stable sulfenamide products in one step (Scheme 1).[4] We believe the reaction mechanism is similar to the Staudinger ligation of azides.[5] RSNO **1** reacts with 2 equiv of ligation substrate **2** to generate the corresponding phosphine oxide and aza-ylide **3**.[4,6] Then, an intramolecular reaction between the ester and aza-ylide may occur to provide phosphorane **5**. Finally, hydrolysis of **5** in the presence of water should furnish the final product **6**. This reductive ligation is quite selective for SNO moieties and could potentially be applied for RSNO detection.[4]

Although the ligation reaction using the phosphine−ester substrate shown in Scheme 1 has been demonstrated, a modification in which an amide bond is formed between the two coupling partners to give a product without the phosphine oxide moiety might be even more attractive. It might provide a new way to prepare useful sulfenamide derivatives. Similar to the well-studied traceless Staudinger ligation pioneered by Raines and Bertozzi,[7] we proposed that compounds like **7** should undergo a traceless reductive ligation with RSNOs (Scheme 2). As such, the nucleophilic nitrogen atom gener-

(1) For selected reviews, see: (a) Lancaster, J. R., Jr. *Nitric Oxide* **2008**, *19*, 68. (b) Foster, M. W.; McMahon, T. J.; Stamler, J. S. *Trends Mol. Med.* **2003**, *9*, 160. (c) Zhang, Y.; Hogg, N. *Free Radical Biol. Med.* **2005**, *38*, 831.

(2) For recent reviews on RSNO detection, see: (a) Gow, A.; Doctor, A.; Mannick, J.; Gaston, B. *J. Chromatogr. B* **2007**, *851*, 140. (b) Kettenhofen, N. J.; Broniowska, K. A.; Keszler, A.; Zhang, Y.; Hogg, N. *J. Chromatogr. B.* **2007**, *851*, 152. (c) MacArthur, P. H.; Shiva, S.; Galdwin, M. T. *J. Chromatogr. B* **2007**, *851*, 93. (d) Jaffrey, S. R. *Methods Enzymol.* **2005**, *396*, 105.

(3) (a) Giustarini, D.; Milzani, A.; Dalle-Donne, I.; Rossi, R. *J. Chromatogr. B* **2007**, *851*, 124. (b) Gladwin, M. T.; Wang, X.; Hogg, N. *Free Radical Biol. Med.* **2006**, *41*, 557.

(4) Wang, H.; Xian, M. *Angew Chem. Int. Ed.* **2008**, *47*, 6598.

(5) (a) Saxon, E.; Bertozzi, C. R. *Science* **2000**, *287*, 2007. (b) Lin, F. L.; Hoyt, H. M.; van Halbeek, H.; Bergman, R. G.; Bertozzi, C. R. *J. Am. Chem. Soc.* **2005**, *127*, 2686. (c) Koehn, M.; Breinbauer, R. *Angew. Chem., Int. Ed.* **2004**, *43*, 3106.

(6) Haake, M. *Tetrahedron Lett.* **1972**, *33*, 3405.

(7) (a) Nilsson, B. L.; Kiessling, L. L.; Raines, R. T. *Org. Lett.* **2000**, *2*, 1939. (b) Saxon, E.; Armstrong, J. I.; Bertozzi, C. R. *Org. Lett.* **2000**, *2*, 2141. (c) Soellner, M. B.; Tam, A.; Raines, R. T. *J. Org. Chem.* **2006**, *71*, 9824. (d) Soellner, M. B.; Nilsson, B. L.; Raines, R. T. *J. Am. Chem. Soc.* **2006**, *128*, 8820. (e) Tam, A.; Soellner, M. B.; Raines, R. T. *J. Am. Chem. Soc.* **2007**, *129*, 11421.

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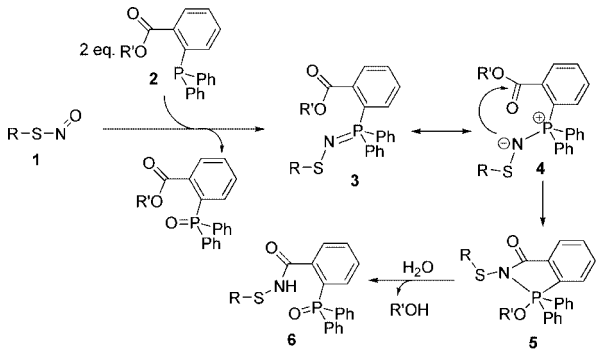
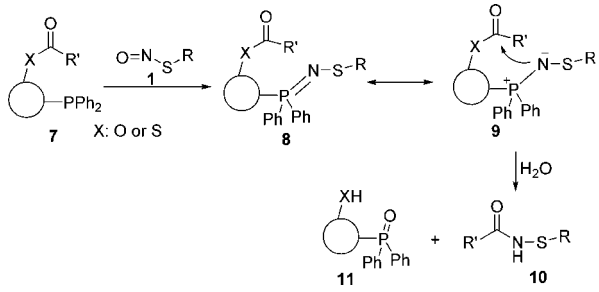
Scheme 1. Reductive Ligation of RSNOs



ated from the reaction between RSNO and the ligation substrate **7** should attack the carbonyl group. Upon hydrolysis, it should produce the sulfenamide **10**[8] and liberate the phosphine oxide **11**. Here we report the first example of the "traceless" reductive ligation.

Scheme 2. Traceless Reductive Ligation of RSNOs

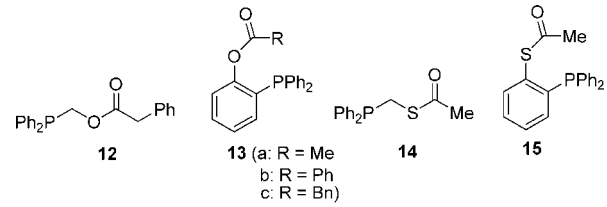


Four suitable phosphines (compounds **12**−**15**, Scheme 3) were used to examine the traceless reductive ligation of RSNOs. These compounds were prepared following the procedures reported previously.[7] In each case, intramolecular transfer of the acetyl group from the phosphine to SNO-bearing compound would indicate a successful ligation reaction.

t-Butyl SNO **16** was used as the model in this study (Table 1). Since water is necessary for the ligation process, we screened a number of water-containing solvent systems such as THF/water, dioxane/water, CH_3CN/water, DMF/water, etc. The best conditions were found to be a mixture of THF, CH_3CN, and water (1.5/1.5/1). When *tert*-butyl SNO was treated with **12**, no detectable ligation product was observed. We only isolated corresponding phosphine oxide. The flexibility of the methylene bridge in **12** may reduce the rate of cyclization such that the hydrolysis of aza-ylide intermediate predominated. In contrast, the treatment of *t*-BuSNO with ester phosphines **13a**−**c** led to the desired traceless ligation

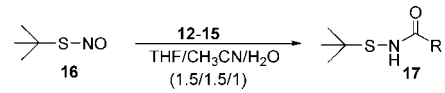
(8) For a review on the chemistry of sulfenamides, see: Craine, L.; Raban, M. *Chem. Rev.* **1989**, *89*, 689.

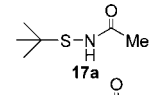
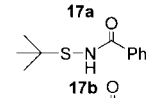
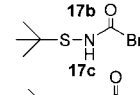
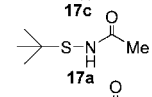
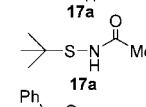
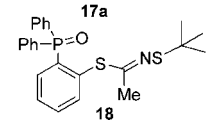
Scheme 3. Traceless Reductive Ligation Substrates



products **17a**−**c** in moderate yield (35−65%). Thioester **14** is the most efficient substrate for the traceless Staudinger ligation discovered by Raines et al.[7] However, the reaction between *t*-BuSNO and **14** only provided the ligation product in 22% yield. When substrates **13** and **14** were treated with other relatively stable tertiary RSNOs, corresponding ligation products were obtained in similar yields (see the Supporting Information for details). Under the same conditions, no ligation product was observed for unstable primary RSNOs such as BnSNO and *S*-nitrosocysteine derivatives. We noticed that these traceless ligations were much slower when compared to the original ligation.[4] It usually takes more than 12 h for the aza-ylide intermediates (or other possible intermediates such as compound **23** in Scheme 4) to be completely consumed (monitored by TLC or ^{31}P NMR). Therefore, the hydrolysis of these intermediates may predominate the reaction.

Table 1. Traceless Ligation of *t*-BuSNO

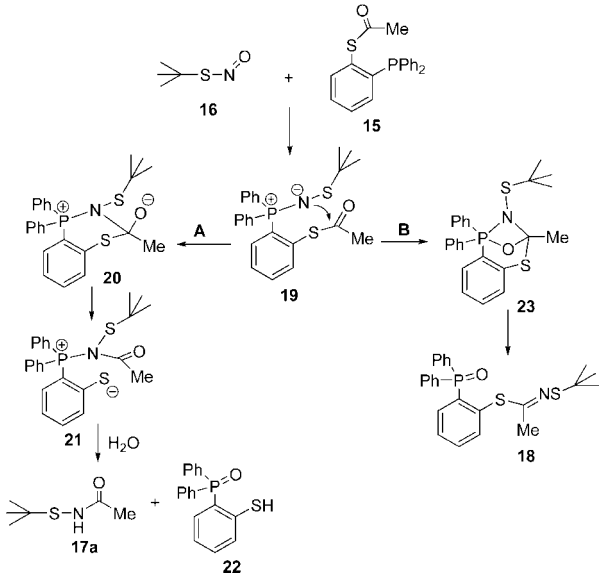


entry	substrate	product	yield (%)
1	**12**	–	–
2	**13a**	 **17a**	58
3	**13b**	 **17b**	35
4	**13c**	 **17c**	65
5	**14**	 **17a**	22
6	**15**	 **17a**	23
		 **18**	59

Interestingly, when thioester **15** was used in the reaction, we not only obtained the desired ligation product **17a** in 23%

yield but also isolated a stable thioimidate product **18** as the major product (59% yield, Table 1, entry 6). Similar imidate compounds were also observed in some Staudinger ligation processes.[9] We noticed the presence of compound **18** under the reaction conditions (THF/CH_3CN/H_2O, with or without phosphine **15**) did not lead to any detectable **17a** even after 3 days. Therefore, we suspected that sulfenamide **17a** was not the hydrolysis product of thioimidate **18**. We propose the reaction between RSNO **16** and thioester phosphine **15** involves two different pathways (Scheme 4): in pathway **A**, the aza-ylide intermediate **19** undergoes desired ligation process to form amidophosphonium salt **21**. Then, hydrolysis of the P−N bond of the amidophosphonium salt forms sulfenamide **17a** and phosphine oxide **22**. In path **B**, the aza-ylide intermediate **19** undergoes an intramolecular aza-Wittig reaction[10] to produce the stable thioimidate **18**.

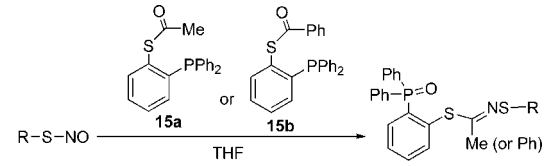
Scheme 4. Proposed Reaction Mechanism of RSNOs

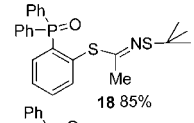
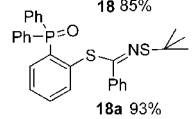
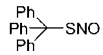
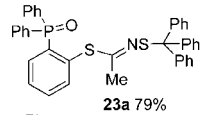
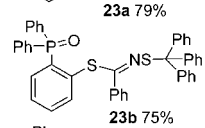
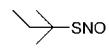
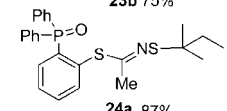
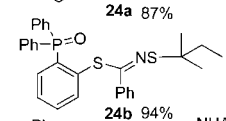
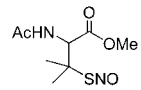
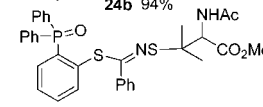


We envisioned the absence of water in the reaction medium might lead to better yield of the thioimidate products. To test this hypothesis, as well as to examine the generality of this process, we tested the reactions of a series of tertiary RSNOs with thioester phosphine **15a** and **15b** in pure THF. As shown in Table 2, in all cases, the thioimidate products were obtained in good to excellent yields. Interestingly, when these tertiary RSNOs were treated with ester phosphine substrates (**13a**−**c**) in anhydrous THF, we did not observe the formation of any *O*-imidate products.

The thioimidate products obtained here appear to be quite stable. Incubation of them in acidic (such as AcOH) or basic

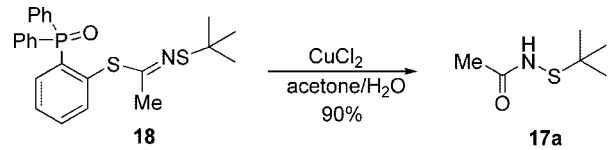
Table 2. Thioimidate Formation Results



RSNO	phosphine	product/yield
 **16**	**15a**	 **18** 85%
	15b	 **18a** 93%
	**15a**	 **23a** 79%
	15b	 **23b** 75%
	**15a**	 **24a** 87%
	15b	 **24b** 94%
	**15b**	 **25** 76%

(such as LiOH) H_2O/CH_3OH mixtures at 25 °C for 24 h does not lead to the formation of any detectable sulfenamide products, nor was any decomposition observed. However, it is possible to convert them to sulfenamides under some conditions. For example, the treatment of compound **18** with $CuCl_2$[11] in the presence of water led to sulfenamide **17a** in excellent yield (Scheme 5).

Scheme 5. Hydrolysis of Thioimidates



In conclusion, the first example of traceless reductive ligation of RSNOs has been demonstrated. We also found

(9) (a) Rose, M. W.; Xu, J.; Kale, T. A.; O'Doherty, G.; Barany, G.; Distefano, M. D. *Peptide Sci.* **2005**, *80*, 164. (b) Restituyo, J. A.; Comstock, L. R.; Petersen, S. G.; Stringfellow, T.; Rajski, S. R. *Org. Lett.* **2003**, *5*, 4357.

(10) (a) Palacios, F.; Alonso, C.; Aparicio, D.; Rubiales, G.; de los Santos, J. M. *Tetrahedron* **2007**, *63*, 523. (b) Fresneda, P. M.; Molina, P. *Synlett* **2004**, 1. (c) Molina, P.; Vilaplana, M. J. *Synthesis—Stuttgart* **1994**, 1197.

(11) Kopylova, B. V.; Bragina, I. O.; Kandror, I. I.; Freidlina, R. K. *Izvest. Akade. Nauk SSSR* **1980**, 719.

that stable thioimidate products could be obtained from the reaction between thioester ligation substrates and RSNOs. Refinements of these preliminary studies may lead to new methods for the detection of *S*-nitrosothiols.

Acknowledgment. This work has been supported by Washington State University. We thank Prof. P. Garner and Prof. R. Ronald in the Department of Chemistry for helpful discussions.

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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