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An Unexpected Bis-ligation of *S*-Nitrosothiols

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Protein S-nitrosation, the formation of *S*-nitrosothiol (RSNO) on cysteine residues, has emerged as a principle mechanism by which nitric oxide (NO) mediates a wide range of cellular functions and phenotypes.[1] However, the detection of S-nitrosation 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] SNO is a unique functional group that should have different reactivity from other biological functional groups such as thiols (−SH) or disulfides (−S−S−). If a new reaction specifically targeting SNO and converting unstable SNO to stable conjugates/products can be developed, such a reaction would hold considerable promise in applications for the detection of S-nitrosation.

With this in mind, our group initiated a program to study new reactions of RSNOs aimed at their potential applications in RSNO detection.[4,5] In 2008, we developed a fast reductive ligation of RSNOs,[4] which is believed to proceed through a Staudinger ligation- type mechanism.[6] Most recently, we studied the traceless version of the reductive ligation of RSNOs,[5] which was inspired by the well-studied traceless Staudinger ligation pioneered by Raines and Bertozzi.[7] Interestingly, when relatively stable tertiary RSNOs, such as *t*-BuSNO **1**, were treated with the thioester-based traceless ligation substrate **2**, stable thioimidates **3** were obtained as the major products (Scheme 1, eq 1).[5] An intramolecular aza-Wittig reaction is believed to be involved. Surprisingly, when we treated **2** with unstable but biologically relevant primary RSNOs such as **4a**, a stable disulfide-iminophosphorane product **5** was obtained in good yield (Scheme 1, eq 2). Given the potential of this unexpected transformation of RSNOs for applications in the detection of S-nitrosation, we studied this reaction further and report these results.

Using a primary S-nitrosothiol compound derived from cysteine, i.e., **4b**, and the thioester-phosphine **2a** as model substrates, we first studied the solvent effects on the disulfide-iminophosphorane formation. As shown in Table 1, the formation of the desired product **5a** proceeded nicely in all solvents (THF, DMSO, $CHCl_3$, DMF, etc.) in good to excellent yields. In addition, the reaction proved to be fast in most of the solvents, typically completed within 30 min at room temperature. The only exception was in toluene, which required ∼1 h for the reaction to go to completion. The best yield of **5a** (96%) was observed when a mixture of THF and CH_3CN (1/1) was used as the solvent (Table 1, entry 8).

With the optimized solvent conditions (i.e., THF/CH_3CN 1/1) in hand, we investigated the substituent effects on the reaction with a series of thioester-phosphine substrates (**2a**−**g**, Table 2), again using **4b** as the model RSNO substrate. Structural changes to the thioester portion of the substrates seemed to have little effect on their reactivity. Corresponding disulfide-iminophosphorane products were achieved in good yields with both alkyl (entries 1−3) and aryl substituents (entries 4−6). Interestingly, when **2g**, the best substrate for the traceless Staudinger ligation,[7] was employed, the desired product **5g** was also obtained, albeit with only 40% yield under these conditions (entry 7).

To examine the generality of this reaction for RSNO substrates, a series of freshly prepared primary RSNO compounds (**4a**−**f**) were employed to react with substrate **2a**. THF/CH_3CN (1/1) mixture was used as the solvent. As shown in Table 3, all primary RSNO compounds (**4a**−**e**) showed good reactivity in this reaction, furnishing corresponding disulfide-iminophosphorane products in high yields under such conditions (entries 1−5). Even with the extremely unstable *S*-nitrosothiol derived from benzyl mercaptan (**4a**), the stable product **5h** was isolated in 78% yield. To investigate if this reaction is sensitive to water, we carried out a model reaction in the presence of 25% water (entry 6), which was the highest water ratio that could be used due to substrate solubility problems. The desired product was obtained with a comparable yield to the conditions without water. In addition, *S*-nitroso-glutathione **4f**, a natural RSNO compound involved in NO

Scheme 1

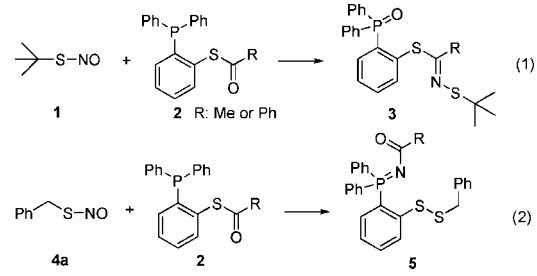
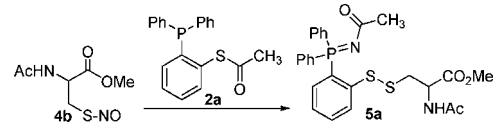
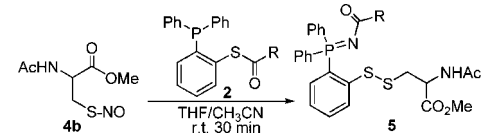


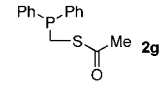
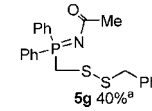
Table 1



entry	solvent	yield	entry	solvent	yield
1	THF	89%	5	toluene	92%
2	DMSO	81%	6	DMF	93%
3	$CHCl_3$	85%	7	dioxane	86%
4	acetone	79%	8	CH_3CN/THF (1/1)	96%

Table 2[a]

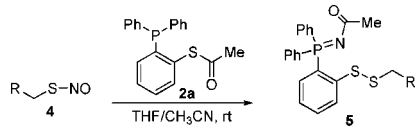


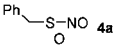
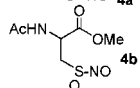
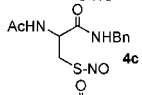
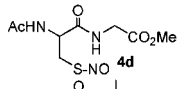
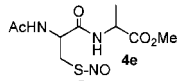
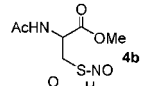
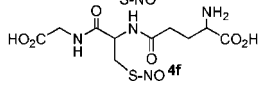
entry	phosphine substrate	product/yield
1	R: Me **2a**	**5a** 96%
2	R: *n*-propyl **2b**	**5b** 96%
3	R: *i*-propyl **2c**	**5c** 95%
4	R: Ph **2d**	**5d** 72%
5	R: *p*-MeO-phenyl **2e**	**5e** 80%
6	R: *p*-CF_3-phenyl **2f**	**5f** 87%
7	 **2g**	 **5g** 40%[a]

[a] This result was obtained using **4a**as the RSNO substrate; the reaction using **4b** gave an inseparable mixture of corresponding disulfide with unknown byproducts.

10.1021/ja900370y CCC: $40.75 © **2009 American Chemical Society**

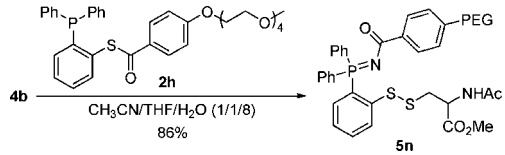
Table 3



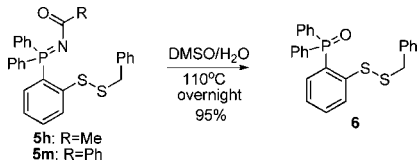
entry	RSNO	product/yield
1	 4a	**5h** 78%
2	 4b	**5a** 96%
3	 4c	**5i** 85%
4	 4d	**5j** 92%
5	 4e	**5k** 87%
6	 4b	**5a** 97%[a]
7	 4f	**5l** 60%[b]

[a] The reaction was carried out in THF/CH_3CN/H_2O (1.5/1.5/1). [b] The reaction was carried out in DMSO/H_2O (8/1) due to the solubility problem.

Scheme 2



Scheme 3



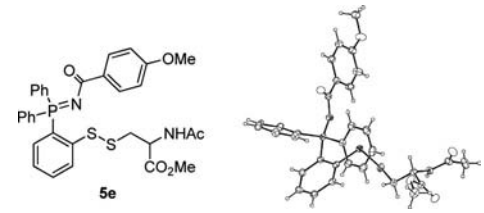
signaling, was also successfully converted to the desired product **5l** in good yield (entry 7). In all cases, the reactions were found to complete within 30 min at room temperature.

With these results in hand, we then prepared a PEG-linked phosphine thioester **2h** to further test the bis-ligation process (Scheme 2). The reaction using this reagent proceeded nicely in a solvent system containing up to 80% water. Again, the desired product was obtained in good yield.

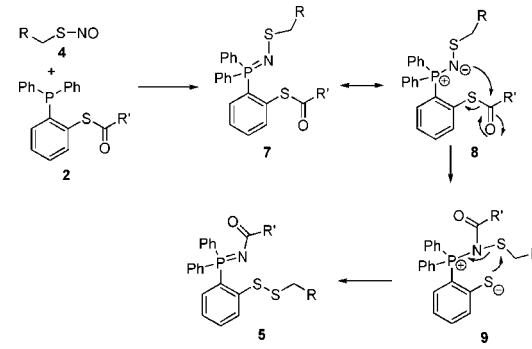
These disulfide-iminophosphorane products generated from RSNOs appear to be quite stable. Unlike the sulfenamide products from the reductive ligation of RSNOs,[4] these products proved not sensitive to phosphine reagents at all. The treatment of compounds **5** with excess phosphine substrates **2** did not lead to detectable reduction of the −S−S− bonds after 12 h.[8]

The structural assignment of this series of products was based on careful NMR (^1H, ^{13}C, and ^{31}P) and mass analysis. The formation of phosphine oxide **6** in quantitative yields from compound **5 h/5m** (DMSO/H_2O overnight at 110 °C, Scheme 3) further verified their structures. Direct evidence came from the X-ray crystal structure of **5e** (Scheme 4).

Scheme 4



Scheme 5



Based on the products observed, a bis-ligation mechanism was proposed for this reaction (Scheme 5): the treatment of primary RSNO with phosphine substrate **2** first leads to the formation of an aza-ylide intermediate **7**.[4,5,9] Then, acyl transfer from the thioester to the N-atom provides intermediate **9**. Finally, nucleophilic phenylthiolate attacks the *pseudo*-sulfenamide linkage via a fast intramolecular process to furnish the disulfide-iminophosphorane product **5**.

In summary, an unexpected bis-ligation reaction of RSNOs has been discovered. It can convert unstable but biologically relevant primary RSNOs to stable disulfide products in good yields under mild conditions. We expect this reaction can be applied to the detection of protein S-nitrosation.

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

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