J|A|C|S COMMUNICATIONS

Published on Web 08/28/2009

Facile Formation of Dehydroalanine From *S*-Nitrosocysteines

Hua Wang, Jiming Zhang, and Ming Xian*

Department of Chemistry, Washington State University, Pullman, Washington 99164

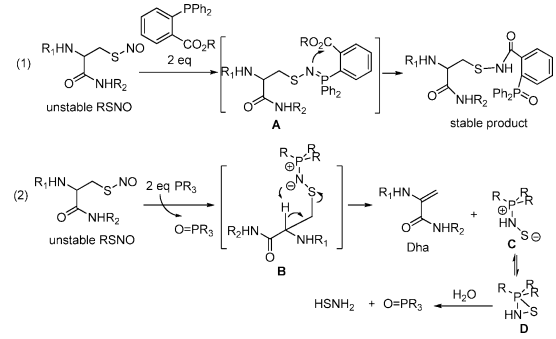
Received July 6, 2009; E-mail: mxian@wsu.edu

Nitric oxide (NO) is a signaling molecule which plays important roles in biological systems. NO exerts its actions by chemical modifications of targets, preferentially with thiol groups. *S*-Nitrosation of Cys residues in proteins is a principal reaction of NO and NO-derived species. This redox-based post-translational modification has been implicated in the cGMP-independent control of a broad spectrum of cellular functions in a variety of cell types.[1] *S*-Nitrosothiols (RSNO) are products of *S*-nitrosation that are synthesized, stored, transported, and degraded in cell systems. However, the detection of RSNO in biological samples remains a challenge because of the lability of the SNO moiety.[2] We believe if new reactions can be developed to convert unstable SNO to stable/detectable molecules, such reactions should hold considerable promise for the detection of RSNO. We recently discovered several phosphine-based reactions which can selectively convert SNO to stable conjugates such as sulfenamides and disulfide-iminophosphoranes.[3] In these reactions, an azaylide intermediate is formed upon treatment of SNO with the phosphine substrate.[3,4] Then, a cascade intramolecular ligation occurs on an electrophile (such as an ester) attaching to the phosphine, leading to the formation of stable conjugates (eq 1, Scheme 1).[3,5] In the meantime, we envisioned that if the electrophile is absent, the nucleophilic N of the azaylide intermediate could serve as a base in some cases. If an acidic proton is present, such as the α-proton in Cys derivatives (eq 2, Scheme 1), an elimination may take place to furnish dehydroalanine (Dha) derivatives. In this process, pentacoordinated P intermediates **C** or **D** might be the byproducts. Upon hydrolysis, it should provide corresponding phosphine oxide and $HSNH_2$, which is an unstable species.[6]

Dha is a potential site-specific chemical handle. It could allow straightforward chemoselective incorporation of various reporter molecules through a Michael addition. In fact, Dha formation from phosphorylated proteins has been used to enrich and analyze phosphoproteome.[7] Because *S*-nitrosocysteine (SNOC) is the basic adduct for protein *S*-nitrosation, a mild and selective transformation from SNOC to Dha would be potentially useful for the detection of protein *S*-nitrosation. Herein we report the first phosphine mediated Dha formation from SNOCs.

With the proposed mechanism (Scheme 1) in mind, we first tested the reaction between a freshly prepared SNOC derivative **2a** and triphenylphosphine (2 equiv) in THF (entry 1, Table 1). As expected, a Dha product **3a** was formed in 48% yield. The only byproduct observed was the corresponding disulfide derivative from **2a**, which is a known decomposition product from unstable *S*-nitrosothiols.[8] To optimize this reaction, a series of phosphine compounds were screened (all in 2 equiv, Table 1). With PBu_3, PEt_3, and $P(OEt)_3$, **3a** was obtained in moderate yields (entries 2−4). Interestingly, treatment of **2a** with HMPT [$P(NMe_2)_3$] led to **3a** in 83% yield (entry 5). HMPT has been reported by Davis et al. for a desulfurization of disulfides via a mechanism involving the Dha formation.[9] In a separate experiment, we also found that HMPT reacted with Cys-based disulfide, and it furnished the corresponding

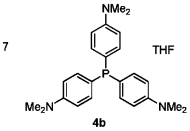
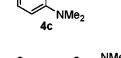
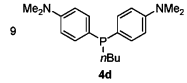
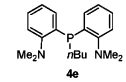
Scheme 1



Dha product (see Supporting Information). Therefore, HMPT is not a selective reagent for SNOCs. We also examined several triphenylphosphine derivatives (entries 6−10). As shown in Table 1, when an electron-deficient group was attached to P, such as **4a**, no Dha formed. With electron-donating substituents, such as **4b** and **4c**, Dha was obtained in moderate to high yields. In **4c**, the *ortho*-dimethylamino group may help to stabilize the azaylide intermediate and facilitate the intramolecular elimination process. We also considered the steric effect as smaller phosphine substrates should lead to more efficient deprotonation of the α-proton. Therefore, smaller substrates than triphenylphosphines, i.e. **4d** and **4e**, were tested, and they both gave the desired product in excellent yields. With the best substrate we have found so far, i.e. **4e**, we tested the reaction in other solvents including DMSO, DMF, dioxane, and MeOH (entries 11−14). In all cases, the formation of Dha was efficient. In addition, it should be mentioned that this phosphine mediated reductive elimination of SNOC proved to be fast (typically completed within 20 min at rt).

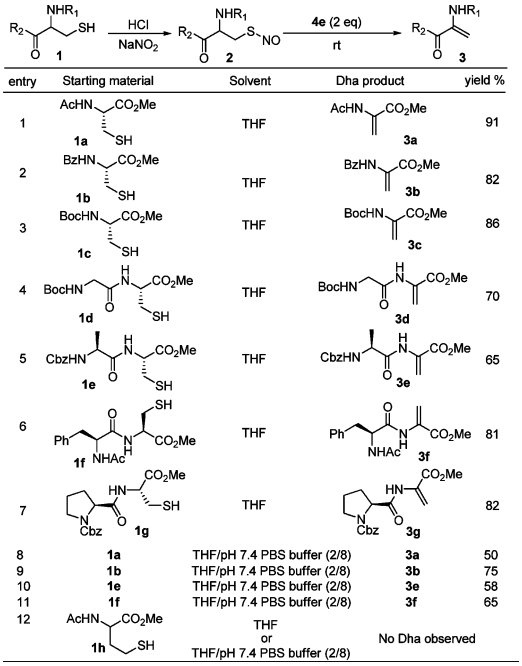
With the optimized conditions in hand, we studied the generality of this reaction using a series of SNOC derivatives (Table 2). As these

Table 1

entry	PR_3	solvent	yield (%)
1	PPh_3	THF	48
2	PBu_3	THF	57
3	PEt_3	THF	42
4	$P(OEt)_3$	THF	31
5	$P(NMe_2)_3$	THF	83
6	 **4a**	THF	0
7	 **4b**	THF	45
8	 **4c**	THF	80
9	 **4d**	THF	89
10	 **4e**	THF	91
11	**4e**	DMSO	80
12	**4e**	DMF	83
13	**4e**	Dioxane	83
14	**4e**	MeOH	79

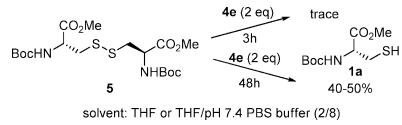
10.1021/ja905558w CCC: $40.75 © 2009 American Chemical Society

Table 2



entry	Starting material	Solvent	Dha product	yield %
1	**1a**	THF	**3a**	91
2	**1b**	THF	**3b**	82
3	**1c**	THF	**3c**	86
4	**1d**	THF	**3d**	70
5	**1e**	THF	**3e**	65
6	**1f**	THF	**3f**	81
7	**1g**	THF	**3g**	82
8	**1a**	THF/pH 7.4 PBS buffer (2/8)	**3a**	50
9	**1b**	THF/pH 7.4 PBS buffer (2/8)	**3b**	75
10	**1e**	THF/pH 7.4 PBS buffer (2/8)	**3e**	58
11	**1f**	THF/pH 7.4 PBS buffer (2/8)	**3f**	65
12	**1h**	THF or THF/pH 7.4 PBS buffer (2/8)	No Dha observed	

Scheme 2



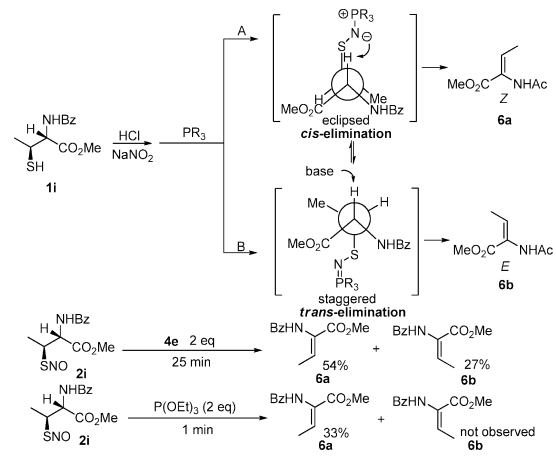
solvent: THF or THF/pH 7.4 PBS buffer (2/8)

primary RSNO compounds are unstable species, they were freshly generated from corresponding Cys starting materials and were used for the elimination without purification. The yields reported were overall yields in the two steps. As shown in Table 2 (entries 1−7), all of the SNOC derivatives provided Dha products in good yields. We have also tested this elimination in aqueous solutions, such as pH 7.4 PBS buffer. Due to a solubility problem for both SNOC compounds and phosphine **4e**, 20% of THF was needed. As shown in entries 8−11, the desired Dha products were again obtained in decent yields, albeit lower than in pure THF. In addition, a homocysteine derivative **1h** was also tested in this reaction (entry 12). As expected, no Dha formation was observed with this substrate. We believe this transformation of Cys compounds, under very mild conditions, could serve as a method to distinguish cysteine and homocysteine.

To examine the chemoselectivity of **4e**, we tested the reaction of **4e** with Cys-based disulfide **5**. Unlike HMPT, **4e** did not react with **5** to produce any detectable Dha product. In fact, disulfides were not very sensitive to **4e**. Trace amounts of −S−S− reduction products were observed after 3 h. Extending the reaction time to 48 h did lead to reduction product **1a** in 40−50% yield (Scheme 2).

To prove the intramolecular elimination mechanism proposed in Scheme 1, compound **1i** was prepared. Upon nitrosation and treatment with phosphine, an azaylide intermediate should form (Scheme 3). An intramolecular *cis*-elimination from an eclipsed conformation (path A) should give a *Z*-alkene product **6a**. In contrast, an intermolecular *trans*-elimination from the staggered conformation (path B) should provide an *E*-isomer **6b**. When **4e** was used as the phosphine reagent, the *Z*-isomer **6a** was obtained as the major product (54%); we also isolated some *E*-isomer **6b** in 27% yield. Presumably, with **4e**, a large phosphine substrate, the lower-energied staggered conformation overwhelmed the preferred *cis*-elimination to some degree, leading to the

Scheme 3



formation of *E*-isomer in a small amount. Indeed, when a smaller phosphine, $P(OEt)_3$, was employed in this reaction, only *Z*-isomer was obtained, albeit the yield was lower. In addition, the reaction with $P(OEt)_3$ (finishing in one minute) was much faster than the one with **4e** (finishing in ∼25 min).

In summary, a phosphine-mediated Dha formation from SNOCs was developed. Mechanistic study suggests that this reaction proceeds mainly via an intramolecular *cis*-elimination on the azaylide intermediate. This Dha formation procedure, under very mild conditions, holds the potential to be applied for the detection of protein *S*-nitrosation.

Acknowledgment. Financial support from the Washington State University and American Heart Association (0930120N).

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.

References

(1) (a) Lancaster, J. R. *Nitric Oxide* **2008**, *19*, 68. (b) Zhang, Y.; Hogg, N. *Free Radical 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) 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. (e) Forrester, M. T.; Foster, M. W.; Benhar, M.; Stamler, J. S. *Free Radical. Biol. Med.* **2009**, *46*, 119. For deficiencies of current method to detect RSNO, see: (f) Giustarini, D.; Milzani, A.; Dalle-Donne, I.; Rossi, R. *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) Zhang, J.; Wang, H.; Xian, M. *J. Am. Chem. Soc.* **2009**, *131*, 3854. (c) Zhang, J.; Wang, H.; Xian, M. *Org. Lett.* **2009**, *11*, 477.

(4) (a) Haake, M. *Tetrahedron Lett.* **1972**, *33*, 3405. (b) Reisz, J. A.; Klorig, E. B.; Wright, M. W.; King, S. B. *Org. Lett.* **2009**, *11*, 2719.

(5) This process is similar to the well-known Staudinger ligation: (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) Nilsson, B. L.; Kiessling, L. L.; Raines, R. T. *Org. Lett.* **2000**, *2*, 1939.

(6) (a) Lovas, F. J.; Suenram, R. D.; Stevens, W. J. *J. Mol. Spectrosc.* **1983**, *100*, 316. (b) Inagaki, Y.; Okazaki, R.; Inamoto, N. *Bull. Chem. Soc. Jpn.* **1979**, *52*, 3615. (c) Barton, D. H. R.; Ley, S. V.; Magus, P. D. *J. Chem. Soc., Chem. Commun.* **1975**, 855.

(7) (a) McLachlin, D. T.; Chait, B. T. *Anal. Chem.* **2003**, *75*, 6826. (b) Oda, Y.; Nagasu, T.; Chait, B. T. *Nat. Biotechnol.* **2001**, *19*, 379.

(8) For selected reviews, see: (a) Williams, D. L. H. *Acc. Chem. Res.* **1999**, *32*, 869. (b) Wang, P. G.; Xian, M.; Tang, X. J; Wen, Z.; Cai, T. W. *Chem. Rev.* **2002**, *102*, 1091. (c) Szacilowski, K.; Stasicka, Z. *Prog. React. Kinet. Mech.* **2001**, *26*, 1.

(9) Bernardes, G. J. L.; Grayson, E. J.; Thompson, S.; Chalker, J. M.; Errey, J. C.; El Oualid, F.; Claridge, T. D. W.; Davis, B. G. *Angew. Chem., Int. Ed.* **2008**, *47*, 2244.

JA905558W