Facile Formation of Dehydroalanine From S-Nitrosocysteines

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Introduction

S-Nitrosation represents an important post-translational modification that transduces nitric oxide-dependent signals. To date, a large group of proteins have been characterized as targets for S-nitrosation, and in many cases, S-nitrosation is believed to regulate protein activity and function. However, the detection of S-nitrosation still remains a challenge because of the liable nature of S-nitrosothiols.

Current Methods for RSNO Detection

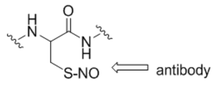
1. Chemiluminescence-based assay

R-SNO → NO $\xrightarrow{\text{ozone}}$ photon

2. Colorimetric assay

R-SNO → NO^+ → nitrite

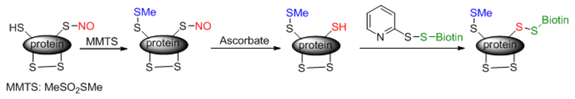
3. Antibody-based assay



4. Mass spectroscopy

R-SNO *vs* R-SH

5. Biotin-switch assay [1]



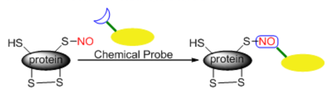
MMTS: $MeSO_2SMe$

Three Problems: [2]

1) 100% blocking of free thiols is necessary.
1) 3-steps, removal of MMTS is necessary.
2) Selective reduction of –SNO is not efficient.

Our Goal

Develop new reactions to label RSNOs

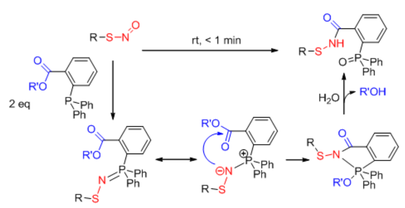


Criteria of the reactions to label RSNOs

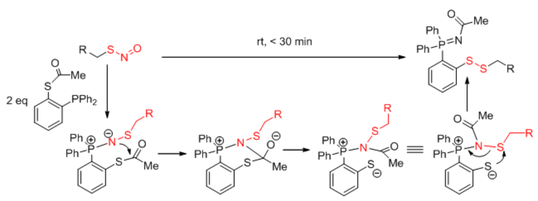
1) The reaction should be very selective for –SNO functional group.
2) The reaction products should be stable/detectable.

Previous study and hypothesis

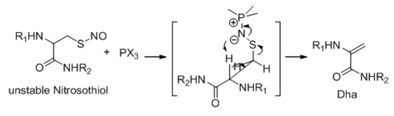
Reductive ligation: [3a]



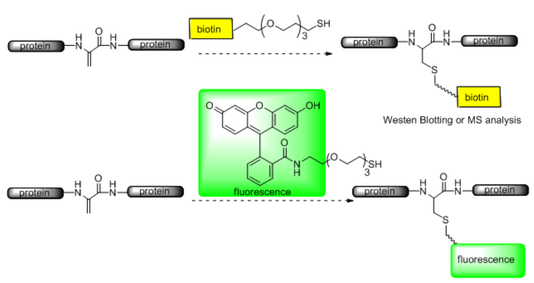
Bis-ligation: [3b]



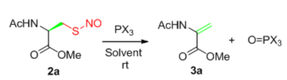
Hypothesis: If there is no electrophile present in the phosphine reagents, the reaction between S-nitrosocysteines and the phosphine will lead to a β-elimination and furnish dehydroalanine (Dha) derivatives.



Applications of Dha in protein detection



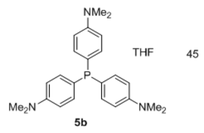
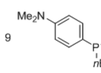
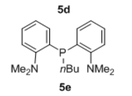
Scan of phosphine reagents



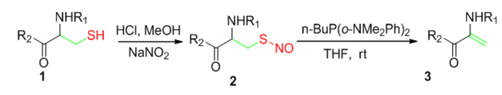
2a **3a**

entry	PX_3	solvent	yield (%)	entry	PX_3	solvent	yield (%)
1	PPh_3	THF	48	8		THF	55
2	PBu_3	THF	57				
3	PEt_3	THF	42				

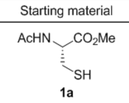
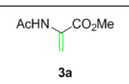
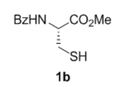
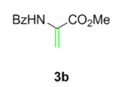
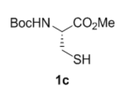
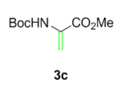
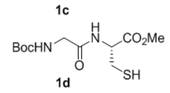
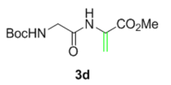
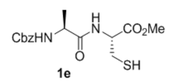
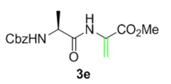
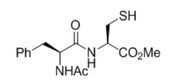
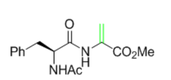
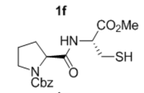
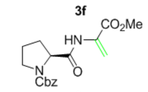
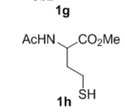
5c

4	$P(OEt)_3$	THF	31
5	$P(NMe_2)_3$	THF	83
6	 **5a**	THF	0
7	 **5b**	THF	45
9	 **5d**	THF	89
10	 **5e**	THF	91
11	**5e**	DMSO	80
12	**5e**	DMF	83
13	**5e**	Dioxane	83

Reductive elimination of S-Nitrosothiols

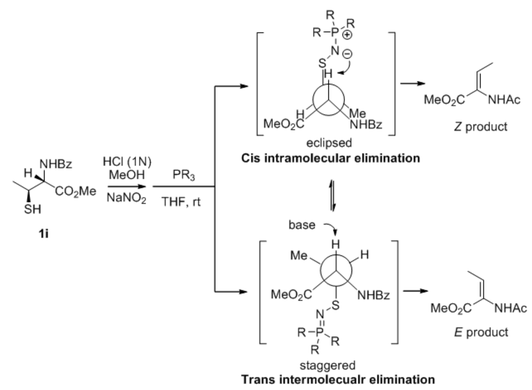


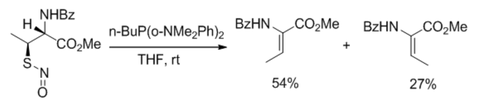
1 **2** **3**

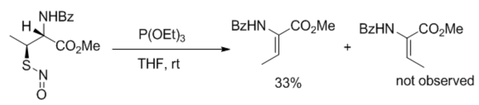
entry	Starting material	Dha product	yield %[a]
1	 **1a**	 **3a**	91
2	 **1b**	 **3b**	82
3	 **1c**	 **3c**	86
4	 **1d**	 **3d**	70
5	 **1e**	 **3e**	65
6	 **1f**	 **3f**	81
7	 **1g**	 **3g**	82
8	 **1h**	No Dha observed	

Mechanism Study

An intramolecular *cis*-elimination from a eclipsed conformation should give only a *Z*-alkene product. In contrast, an intermolecular *trans*-elimination from the staggered conformation should provide an *E*-isomer.







Summary

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

References

1. S. R. Jaffrey, et. al. *Nat. Cell Biol.* **2001**, *3*, 193-197.
2. (a) N. Hogg, et al. *J. Chrom. B.* **2007**, *851*, 152-159. (b) R. Rossi, et. al. *J. Chrom. B* **2007**, *851*, 124-139.
3. (a) H. Wang, M. Xian. *Angew. Chem. Int. Ed.* **2008**, *47*, 6598-6601. (b) J. Zhang, H. Wang, M. Xian. *J. Am. Chem. Soc.* **2009**, *131*, 3854-3855.