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Phosphite Esters: Reagents for Exploring S-Nitrosothiol Chemistry

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Abstract

The reactions between S-nitrosothiols and phosphite esters, including P(OPh)₃, P(OBn)₃, and P(OEt)₃, were studied. Two different conjugated adducts, thiophosphoramidates and phosphorothioates, were formed, depending on the structures of the S-nitrosothiol substrate (e.g., primary vs tertiary). These reactions proceeded under mild conditions, and the reaction mechanisms were studied using experiments and calculations.

Graphical Abstract

S-Nitrosylation is a nitric oxide mediated post-translational modification, which plays important regulatory roles in many biological systems.¹ Although many proteins (>3000) have been suggested to be the targets of S-nitrosylation, the detection of S-nitrosylation is still a challenge. This is primarily due to the instability of the products of S-nitrosylation, i.e., S-nitrosothiols (SNOs). Previously, chemistry studies on SNOs were focused on their preparation and degradation, while the reactivity of SNOs received less attention.² In the

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ASSOCIATED CONTENT

Supporting Information

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Experimental and characterization of each compound, as well as theoretical calculation data (PDF)

past several years, our group has been studying bioorthogonal reactions of SNOs, with the aim of developing new methods for the detection of protein S-nitrosylation. To this end, we have discovered several triarylphosphine-mediated reactions of SNOs, including (Scheme 1): (1) reductive ligation, which converts SNOs to sulfonamides; (2) bis-ligation, which converts SNOs to disulfide-iminophosphorane products; (3) one-step disulfide formation; and (4) reductive elimination, which leads to dehydroalanine products.³ Aza-ylides, which are formed from triarylphosphines and SNOs, are the key intermediates in these reactions. By manipulating the functional groups on the phosphines, different conjugates could be obtained.

It is known that aza-ylides could also be generated from phosphite substrates.⁴ Therefore, we wondered if phosphites could be used to trap SNOs. To the best of our knowledge, the reaction between SNOs and phosphites has not been reported. We predicted that the products would be different from those of the reactions with phosphine substrates. The reaction may proceed through two possible pathways (Scheme 2). In pathway a, the phosphite attacks the nitroso group (N=O) to generate the aza-phosphite ylide **I**, which should undergo hydrolysis to yield the thiophosphoramidate product **II**. Alternatively (pathway b), the phosphite attacks the sulfur atom of the S–N bond directly, similar to the reaction reported by King et al.⁵ This should afford the thiophosphonium salt **III**. Hydrolysis of **III** should produce phosphorothioate **IV**. With this hypothesis in mind, we studied the reactions between SNOs and phosphites. Herein, we report our results.

First, we screened the reactions with three phosphite substrates: P(OPh)₃, P(OBn)₃, and P(OEt)₃. A tertiary SNO (TrSNO **8**) and a primary SNO **9** were employed as the models. The reactions were carried out in a mixed MeCN/PBS buffer system. Since the formation of aza-ylides from SNOs requires at least 2 equiv of phosphites, excess phosphites (3.4 equiv) were used in our studies. Interestingly, P(OPh)₃ did not show any reactivity toward both SNOs, probably due to its low nucleophilicity. P(OBn)₃ and P(OEt)₃ showed good reactivity. Clean reactions were noticed, and isolable products **10** and **11** were obtained in good yields (Table 1). The products obtained from the TrSNO were found to be the thiophosphoramidates **10a** and **10b**, while the primary SNO **9** produced the phosphorothioates **11a** and **11b**.

Encouraged by these results, we had to decide whether P(OBn)₃ or P(OEt)₃ should be used to further understand the reactions of SNOs. Since our ultimate goal was to develop orthogonal reactions for SNOs, control reactions with the disulfide substrate **12** were then tested for P(OBn)₃ and P(OEt)₃. As shown in Scheme 3, P(OEt)₃ effectively broke the disulfide bond to produce phosphorothioate **11b** in excellent yield. In contrast, P(OBn)₃ did not react with the disulfide under the same conditions. These results suggested that P(OBn)₃ was a mild reagent and might be selective toward SNOs. Therefore, it was selected for further studies.

We then optimized the reaction conditions using P(OBn)₃. As primary SNOs are more biologically relevant, a cysteine SNO **9** was used in this experiment (see Supporting Information for Table S1). MeCN/PBS (1/1) was the optimum solvent system for this reaction (Table S1, entries 1–4). The effects of pH were studied (Table S1, entries 4–7). The

neutral to weak acidic pH levels gave similar results, while a basic pH (10) led to a slightly decreased yield. This was probably due to the increase in instability of the SNO under basic conditions. We also found that an increase in the loading of $\text{P}(\text{OBn})_3$ resulted in higher yields (Table S1, entries 8–11). Since the difference between the yields for 5 and 10 equiv was not significant, we decided to use 5 equiv of $\text{P}(\text{OBn})_3$ in our studies.

We next tested the generality of this reaction using a series of SNO derivatives. As shown in Table 2, all the tertiary SNO substrates **8**, **13**, and **14** yielded the corresponding thiophosphoramidates **10a**, **13a**, and **14a** as the major products in good yields. When the secondary and primary SNO derivatives **9**, **15–23** were used, only phosphorothioate products were obtained, all in good yields. In the case of the S-nitrosoglutathione (GSNO) **23**, an endogenous SNO, the partially hydrolyzed phosphorothioate **23a** was the isolated product. Clearly $\text{P}(\text{OBn})_3$ is an effective reagent which can convert SNOs to stable products under mild conditions.

Based on the obtained products, the mechanisms of the reactions were proposed (Scheme 4). For tertiary SNOs, since thiophosphoramidates were the major products, the phosphite preferably reacts with the nitroso group via pathway A, due to steric hindrance. In this process, 2 equiv of $\text{P}(\text{OBn})_3$ were consumed and aza-ylide **26** was formed. Hydrolysis of **26** provided thiophosphoramidate **27**. For secondary and primary SNOs, while both pathways seem possible, we feel pathway A is more feasible as the nitroso group is more accessible than the S atom. We attempted to obtain thiophosphoramidates from secondary and primary SNOs. However, all the attempts failed, even with less than 2 equiv of $\text{P}(\text{OBn})_3$. Only reduced yields of phosphorothioate products were obtained (Table S1). These results suggest that the formation of thiophosphoramidate is the rate-determining step of the process. Moreover, secondary- and primary-SNO derived thiophosphoramidates are unstable in the presence of phosphites. They are likely to react with $\text{P}(\text{OBn})_3$ to form phosphorothioate **30** and dibenzyl phosphoramidate **29**. However, we cannot completely rule out pathway B, since the direct cleavage of the S–N bond would form the same products with the release of the nitroxyl (HNO). It is known that HNO could react with phosphines to form phosphine oxide and aza-ylides.⁶ Similarly, the reaction of HNO with $\text{P}(\text{OBn})_3$ should produce **25** and **31**. Hydrolysis of **31** should yield **29**.

To better understand the mechanisms, we carried out the quantum calculations using density functional theory (DFT). All the calculations were conducted using Gaussian 09⁷ at the B3LYP/6–311+G(d,p) level,⁸ applying the PCM water solvation model⁹ (see Supporting Information for the calculated geometries). In order to save the calculation time, we selected MeSNO and t-BuSNO to represent primary- and tertiary-SNO and $\text{P}(\text{OMe})_3$ as the model of $\text{P}(\text{OBn})_3$ in calculations. We first explored the energy differences between attacking the nitroso group vs S atom by $\text{P}(\text{OMe})_3$. As we expected and discussed above, attacking the nitroso group is energetically more favorable than the S center by 3 kcal/mol (Supporting Information Figure S1). We then focused our attention toward whether thiophosphoramidate **27** could react further with $\text{P}(\text{OMe})_3$ to form **28** in both MeSNO and t-BuSNO. The two located transition states indicate that the activation energy barrier for t-BuSNO is about 24 kcal/mol, 7 kcal/mol higher than that for MeSNO (Supporting Information Figure S2). The calculated results support that (1) pathway A is more favorable than pathway B and (2) for

tBuSNO the reaction would not easily go further after **27** is formed. This explains how all tertiary SNOs gave thiophosphoramidates **27** as the main products. It should be noted that although a simplified model P(OMe)₃ was used to carry out the calculations, we do not think the results of P(OMe)₃ would give misleading information to interpret the proposed mechanisms. Even with this stronger reagent (compared to P(OBn)₃) the calculated energy difference between two pathways is as high as 7 kcal/mol. We would expect a higher energy difference between the two pathways if P(OBn)₃ was used since the steric clashes will be bigger in the TS_T structure than in the TS_P structure due to the bigger size of Bn group. In this regard, the conclusion derived from the calculations is still valid.

In conclusion, new reactions between SNO and phosphite esters were studied. Phosphorothioates were obtained as the main products from primary SNO substrates, while thiophosphoramidates were obtained as the main products from tertiary SNOs. Mechanistic studies reveal that the reaction between SNOs and phosphite esters could proceed via two reaction pathways and yields two different conjugated products depending on the structure of SNO compounds. Owing to the excellent stability of the reaction products and the very mild reaction conditions, this reaction is potentially useful for understanding SNO biology.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Scheme 1.
Reactions of SNOs with Triarylphosphines

Scheme 2.
Two Possible Reaction Pathways between SNO and Phosphites

Scheme 3. Reactions of Disulfide **12** with $\text{P}(\text{OBn})_3$ and $\text{P}(\text{OEt})_3$

Scheme 4. Mechanisms of the Reactions between SNO and $\text{P}(\text{OBn})_3$

Table 1.

Screening of Phosphites

$\text{RSNO} + \text{P(OR}^1\text{)}_3 \xrightarrow[\text{rt, 1 h}]{\text{CH}_3\text{CN/PBS (1:1)}} \text{products}$				
entry	RSNO	P(OR ¹) ₃	product	yield (%)
1		P(OPh) ₃	N.R.	N.R.
2	8	P(OBn) ₃	10a	75
3		P(OEt) ₃	10b	82
4		P(OPh) ₃	N.R.	N.R.
5	9	P(OBn) ₃	11a	61
6		P(OEt) ₃	11b	83

Table 2.

Reactions between SNOs and P(OBn)₃

RSNO + P(OBn) ₃ $\xrightarrow{\text{MeCN/PBS (1/1)}}$ product		
entry	RSNO	product (yield%)
1	8	10a (75%)
2	13	13a (71%)
3	14	14a (71%)
4	15	15a (85%)
5	16	16a (72%)
6	9	11a (82%)
7	17	17a (82%)
8	18	18a (78%)