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Proline-based Phosphoramidite Reagents for the Reductive Ligation of *S*-Nitrosothiols

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Abstract

S-Nitrosothiols have many biological implications but are rarely used in organic synthesis. In this work we report the development of proline-based phosphoramidite substrates that can effectively convert *S*-nitrosothiols to proline-based sulfenamides through a reductive ligation process. A unique property of this method is that the phosphine oxide moiety on the ligation products can be readily removed under acidic conditions. In conjugation with the facile preparation of *S*-nitrosothiols (RSNO) from the corresponding thiols (RSH), this method provides a new way to prepare proline-based sulfenamides from simple thiol starting materials.

INTRODUCTION

Sulfur-nitrogen (-S-N-) linkages are unique chemical moieties. These structures often show interesting bioactivities (Figure 1). For example, sulfonylureas are a class of important herbicides.[1,2] Sulfanilamides have been used as common drugs for infections.[3,4] *N*-Thiolated β-lactams are found to have antibacterial, antifungal, and anticancer effects.[5–12] Sulfenamides exhibit antimicrobial activities against various infectious pathogens.[13] Proline-based sulfonamides have been recognized as potential anti-proliferative and anti-infective agents.[14–16] Because of these activities, the preparation of these molecules, especially the formation of the -S-N- linkages, has become an active area in organic synthesis. So far, many methods have been developed for the construction of the S-N bonds, which include: 1) sulfenylation between sulfenyl chlorides and amines,[17,18] 2) amination of thiols with *N*-halo compounds or amines in the presence of oxidizing reagents to give unsubstituted and *N*-substituted sulfenamides,[19] 3) the treatment of disulfides with ammonia or amines in the presence of silver or mercuric salts in alkaline medium,[20,21] 4) sulfenylation of primary and secondary amines with the esters of sulfenic acids,[22] 5) derivations of *N*-chlorothio-compounds,[23,24] 6) the [2,3]-sigmatropic rearrangement of *S*-allylsulfynimines,[25] 7) reductive cleavage of sulfinimidic acids in the presence of thiophenols,[26] 8) electrolysis of 2-mercaptobenzothiazole-amine or bisbenzothiazol-2-yldisulfide-amine,[27,28] and 9) the preparation of sulfin- or sulfon-amides by the oxidation of sulfenamides in the presence of the oxidants.[29,30]

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S-Nitrosothiols are a group of -S-N- bond containing molecules. The formation of *S*-nitrosothiols in biological systems is an important post-translational modification elicited by nitric oxide (NO).[31–33] *S*-Nitrosothiols include protein-based adducts (through cysteine residues) and small molecules (such as *S*-nitrosoglutathione and *S*-nitrosocysteine). Small organic thiols (RSH) can also be easily converted to the corresponding *S*-nitrosothiols (RSNO) under mild nitrosation conditions. Normally RSNOs are unstable species, which makes the detection of *S*-nitrosothiols in biological systems very challenging. Moreover, the instability of RSNO makes these compounds unattractive for synthetic chemists. As a result, the application of RSNO in synthesis has been rarely reported. Interestingly, in our recent work on RSNO bio-orthogonal reactions (aiming at the development of novel detection methods for protein RSNO formation),[34–41] we have discovered some unusual reactivity and properties of RSNO. In our opinion, RSNO are powerful synthons that can be used to introduce S, N, and/or O atoms into molecular structures. Herein we wish to report a method to prepare proline-based sulfenamides from readily available RSH substrates via RSNO intermediates. The novel reactions and synthetic strategies introduced in this article could be applied for an effective synthesis of bioactive molecules including a proline moiety.

RESULTS AND DISCUSSION

In our previous work, we discovered that RSNO and triarylphosphines (2 equivalents) can rapidly react to generate reactive thioazaylide intermediates in high yields under mild conditions.[34] Thioazaylides are potent nucleophilic species. Upon manipulating the electrophilic groups attached to the phosphine reagents, thioazaylides can be trapped as stable products. For example, as shown in Scheme 1, when an *ortho*-ester group was attached to the phosphine reagent (compound **3**), the reactive thioazaylide **4** could be trapped via an intramolecular acyl transfer to afford sulfenamide **5**.[35–37] This reductive ligation process provides a way for capturing unstable biological RSNO as stable and detectable conjugates. In our opinion, this reaction, in conjunction with easy RSNO formation from RSH, would be also useful for the synthesis of sulfenamide derivatives from simple RSH starting materials. However, the use of phosphine substrates like **3** would lead to products like **5**, which contain an unnecessary triphenylphosphine oxide moiety. The removal of this bulky group from the final products would require harsh conditions. Therefore, seeking phosphine substrates that can undergo the reductive ligation while leading to a readily removable phosphine oxide moiety from the products is desirable. With this idea in mind, we proposed that proline-based phosphoramidites like **6** would be suitable substrates. As shown in Scheme 1, the reaction between RSNO and **6** should still follow the reductive ligation process. The resultant phosphoramidate moiety can be considered as a NH-protecting group and removable under acidic condition. As such, proline-linked sulfenamides could be prepared from simple RSH and phosphoramidite **6**.

To test this idea, three proline-based phosphoramidites (Scheme 2, **9**-**11**) were prepared. These substrates were treated with TrSNO (**12a**) to explore their reactivity. The selection of TrSNO as the RSNO model compound was due to its remarkable stability and ease of synthesis. We found the characteristic green color of TrSNO disappeared immediately when treated with all three phosphoramidites, suggesting the formation of thioazaylides were fast. For **9** and **10**, the reactions stopped at this stage as no desired ligation product was obtained even when the reaction time was extended to 24 hours. With **11**, however, we obtained the desired ligation product **16a**. Apparently this can be explained by the factor that phenyl ester is a better leaving group than the methyl and t-butyl esters. We tested a series of different solvents for this reaction. The best was found to be a mixture of THF/phosphate buffer (pH 7.4, 20 mM, 3:1), which gave **16a** in 84% yield.

As phosphoramidite **11** was found to be the most reactive substrate for this ligation, it was applied to other RSNO substrates to test the generality of the reaction. A series of primary, secondary, and tertiary RSNOs were freshly prepared from RSH and then used in this study without any purification. The results were summarized in Table 1. In all cases, the desired ligation products were obtained. For relatively stable tertiary RSNO substrates, the corresponding ligation products were isolated in modest to high yields (entries 1-4). Secondary and primary RSNO substrates gave slightly lower yields (entries 5-9). Interestingly, the purification of secondary RSNO products (entries 5 and 6) were found to be very difficult because they overlapped with the phosphine oxide byproduct. Nevertheless the formation of the products was clearly confirmed by NMR and mass spectroscopy analysis. These results demonstrated that RSH can be readily converted to proline-based sulfenamides by this two-step SNO-ligation process.

While phosphoramidite **9** did not show good reactivity to convert RSNO to the desired sulfenamides, we did observe some unique reactivity of **9**. As shown in Scheme 3, the treatment of a cysteine SNO derivative Bz-Cys[SNO]-OMe **12g** with **9** gave dehydroalanine **18** in a modest yield (37%) together with the disulfide product (46%). These results indicated that the reaction indeed proceeded to form the thioazaylide intermediate **17**. Presumably the methyl ester was not reactive enough. So the acyl transfer was slow and non-productive. Instead, the azaylide underwent an intramolecular β-elimination on the cysteine substrate to form dehydroalanine **18**.

In the proline-based sulfenamide products, the diphenylphosphoryl moiety could be considered as the protecting group of proline. We expected it could be removed under acidic conditions.[42] We then tested the deprotection of **16g** (Scheme 4). Indeed a cleavage cocktail of 10% water in TFA provided sulfenamide **19** almost quantitatively. This result revealed that the -S-N- bond on the sulfenamide compounds was quite stable under acidic conditions. Given the efficiency of this protocol, it can find applications in making water-soluble proline-based sulfenamides.

HNO is the one-electron reduced/protonated from of NO. It shows distinct physiology and pharmacology from NO.[43] The reductive ligation was also found to be effective for HNO.[44] Several specific fluorescent probes for HNO detection have been developed based on the triarylphosphine template.[45-47] We next wondered if the proline-based phosphoramidite would work for HNO and tested the reaction between **11** and HNO (generated from Angeli's salt). As shown in Scheme 5, the reaction proved to be fast and efficient. The desired ligation product **21** was obtained in a comparable yield (50%) as the one obtained from triarylphosphines,[44] suggesting the proline-based phosphoramidite may be useful for the design of novel HNO sensors.

CONCLUSIONS

In summary, we have developed a reductive ligation of *S*-nitrosothiols using *N*-diphenylphosphine proline ester substrates. This reaction was found to be effective for all small molecule *S*-nitrosothiols (primary, secondary, and tertiary), as well as for HNO. The ligation products bear a removable phosphine oxide moiety on the proline residue. In conjugation with the facile preparation of *S*-nitrosothiols (RSNO) from the corresponding thiols (RSH), this novel method provides a unique way to prepare proline-based bioactive sulfenamides from simple thiol starting materials.

MATERIALS AND METHODS

Instrumentation

^{1}H NMR spectra, ^{13}C NMR, and ^{31}P NMR were recorded at 300 MHz (Varian, VX 300) and are reported in parts per million (ppm) on the δ scale relative to residual $CHCl_3$ (δ 7.25 for ^{1}H and δ 77.0 for ^{13}C). These experiments were performed at room temperature. Mass spectra were recorded using an electrospray ionization mass spectrometry (ESI, Thermo Finnigan LCQ Advantage) or MALDI-TOF mass spectrometry. Mass data were reported in units of m/z for $[M+H]^+$ or $[M+Na]^+$.

Preparation of proline-based phosphoramidites

To a solution of the proline ester HCl salt in CH_2Cl_2 (total $c = 0.30$ M) was added freshly distilled 2.5 equiv. of triethylamine followed by PPh_2Cl (1 eq) at 0 °C. The mixture was slowly warmed to room temperature and stirred for 3~4 h. Upon completion (monitored by TLC), the reaction mixture was filtered to remove the triethylammonium salt. The filtrate was concentrated and diluted with EtOAc, and washed with sat. $NaHCO_3$, water, and brine. The organic layer was dried with anhydrous $MgSO_4$, filtered, and concentrated. The crude product was purified by flash column chromatography (with pre-neutralized silica gel by 3% TEA in hexanes).

Compound 9—^{1}H NMR (300 MHz, $CDCl_3$) δ 7.59 – 7.27 (m, 10H), 4.22 (ddd, J = 8.8, 6.0, 3.5 Hz, 1H), 3.63 (s, 3H), 3.17 – 3.06 (m, 1H), 2.88 (m, 1H), 2.22 – 1.70 (m, 4H); ^{31}P NMR (122 MHz, $CDCl_3$) δ 49.31; ^{13}C NMR (75 MHz, $CDCl_3$) δ 176.00, 138.93, 132.88, 132.61, 132.23, 131.98, 128.90, 128.40, 128.33, 128.27, 128.19, 65.28, 64.87, 51.96, 47.64, 47.57, 31.80, 31.71, 25.90; MS (ESI) *m/z* calcd for $C_{18}H_{21}NO_2P$ $[M+H]^+$ 314.1, found 314.1.

Compound 10—^{1}H NMR (300 MHz, $CDCl_3$) δ 7.49 (ddd, J = 8.1, 5.4, 1.5 Hz, 2H), 7.39 – 7.28 (m, 8H), 4.07 (ddd, J = 8.6, 6.6, 3.4 Hz, 1H), 3.17 – 3.06 (m, 1H), 2.83 (dtd, J = 9.2, 6.8, 2.7 Hz, 1H), 2.07 (dq, J = 12.0, 8.3 Hz, 1H), 1.99 – 1.82 (m, 2H), 1.76 – 1.64 (m, 1H), 1.40 (s, 9H). ^{13}C NMR (75 MHz, CDCl3) δ 174.76, 139.27, 132.93, 132.66, 132.26, 132.01, 128.81, 128.38, 128.29, 128.19, 128.12, 80.76, 66.37, 65.95, 47.72, 47.65, 31.83, 31.74, 28.24, 25.87. ^{31}P NMR (122 MHz, $CDCl_3$) δ 49.64.

Compound 11—^{1}H NMR (300 MHz, $CDCl_3$) δ 7.59 – 7.15 (m, 13H), 7.07 – 6.95 (m, 2H), 4.47 (ddd, J = 8.3, 6.3, 3.8 Hz, 1H), 3.21 (dddd, J = 8.7, 7.5, 5.1, 1.0 Hz, 1H), 2.95 (dtd,

J = 9.4, 7.0, 2.4 Hz, 1H), 2.36 – 2.14 (m, 2H), 2.09 – 1.93 (m, 1H), 1.83 (m, 1H); ^{31}P NMR (122 MHz, $CDCl_3$) δ 49.99; ^{13}C NMR (75 MHz, $CDCl_3$) δ 174.0, 150.9, 138.8, 138.7, 138.6, 132.9, 132.7, 132.2, 131.9, 129.5, 129.0, 128.5, 128.3, 128.2, 125.9, 121.6, 65.6, 65.1, 47.8, 47.7, 32.0, 31.9, 25.9; MS (ESI) *m/z* calcd for $C_{23}H_{23}NO_2P$ $[M+H]^+$ 376.1, found 376.2.

Preparation of *S*-nitrosothiols

The thiol starting material (RSH, 0.2 mmol) was dissolved in 1 mL of MeOH followed by the addition of 1 N HCl (1 mL) at room temperature. To this solution was then added freshly prepared 1 N $NaNO_2$ (1 mL) in water in dark (total c = 0.07 M). The color of the reaction was immediately turned to red (for primary and secondary RSNO) or green (for tertiary RSNO). The mixture was stirred for 10-15 min at room temperature. Upon completion (monitored by TLC), the RSNO product was directly extracted with cold diethyl ether (1 mL × 3) in dark. The organic layers were collected and dried. The solvent was removed to provide the RSNO product, which was then used for the ligation reaction without further purification.

General reductive ligation procedure

To the freshly prepared RSNO product was added a solution of 2 equiv. of **11** in 3:1 THF-aqueous buffer (pH 7.4, de-gassed by bubbling with argon). The final concentration was ~ 0.1 M. The reaction was monitored by TLC and it was usually completed within 15~30 min at room temperature. The reaction mixture was extracted with ethyl acetate. The combined organic layers were washed with water and brine, dried by anhydrous Na_2SO_4, and concentrated. The crude product was purified by flash column chromatography.

Thioazaylide 15—^{1}H NMR (300 MHz, $CDCl_3$) δ 7.81 – 7.55 (m, 4H), 7.47 – 7.17 (m, 23H), 6.94 – 6.80 (m, 3H), 4.35 (td, J = 8.8, 3.5 Hz, 1H), 3.14 (qd, J = 6.5, 2.8 Hz, 2H), 2.30 (m, 1H), 2.13 – 1.99 (m, 1H), 1.99 – 1.78 (m, 2H); ^{31}P (122 MHz, $CDCl_3$) δ 18.3; FT-IR (thin film) 3025.4, 2985.3, 1728.8 (strong, C=O, *carbonyl group*), 1601.3, 1450.0, 1372.3, 1268.0, 1247.1, 1070.2 cm^{-1} MS (ESI) *m/z* calcd for $C_{42}H_{37}N_2NaO_2PS$ $[M+Na]^+$ 687.2, found 687.1.

Compound 16a—^{1}H NMR (300 MHz, $CDCl_3$) δ 7.81 (s, 1H), 7.76 – 7.24 (m, 25H), 3.95 (dt, J = 9.2, 5.5 Hz, 1H), 3.01 (p, J = 8.1 Hz, 1H), 2.66 – 2.51 (m, 1H), 1.92 (m, 2H), 1.68 – 1.61 (m, 1H), 1.34 – 1.26 (m, 1H); ^{31}P (122 MHz, $CDCl_3$) δ 30.2; ^{13}C NMR (75 MHz, $CDCl_3$) δ 178.99, 150.71, 145.25, 133.24, 133.12, 132.87, 132.74, 132.01, 131.59, 130.54, 130.04, 129.81, 129.51, 128.59, 128.41, 128.22, 128.15, 127.56, 126.42, 126.00, 121.61, 120.73, 115.57, 63.10, 60.11, 49.03, 32.18, 25.76; MS (ESI) *m/z* calcd for $C_{36}H_{33}N_2NaO_2PS$ $[M+Na]^+$ 611.2, found 611.1.

Compound 16b—^{1}H NMR (300 MHz, $CDCl_3$) δ 9.55 (t, 5.6 Hz, 1H, N$\underline{H}$)), 9.12 (d, J = 9.5 Hz, 1H, N$\underline{H}$)), 8.88 (s, 1H, N$\underline{H}$), 8.12 – 6.82 (m, 20H), 5.18 (d, J = 9.0 Hz, 1H), 4.46 – 4.33 (m, 2H), 4.20 – 4.02 (m, 1H), 3.44 (m, 1H), 3.31 (m, 1H), 2.39 (m, 1H), 2.17 (m, 3H), 1.36 (d, J = 10.8 Hz, 6H); ^{31}P (122 MHz, $CDCl_3$) δ 30.2; ^{13}C NMR (75 MHz, $CDCl_3$) δ 173.13, 169.58, 168.43, 157.53, 150.52, 133.19, 133.06, 133.03, 132.99, 132.53, 132.39, 132.21, 132.06, 131.05, 130.90, 130.49, 129.75, 129.68, 129.62, 129.33, 129.16, 129.01, 128.93, 128.85, 128.61, 128.23, 128.12, 127.42, 126.29, 121.54, 121.42, 119.59, 115.85, 62.20, 60.24, 55.76, 47.78, 43.79, 32.61, 25.91; MS (ESI) *m/z* calcd for $C_{36}H_{39}N_4NaO_4PS$ $[M+Na]^+$ 677.2, found 677.1.

Compound 16c—^{1}H NMR (400 MHz, $CDCl_3$) δ 8.72 (s, 1H), 7.82 (m, 4H), 7.58 – 7.40 (m, 6H), 4.18 (t, *J* = 7.5 Hz, 1H), 3.19 (dd, *J* = 12.2, 6.0 Hz, 2H), 2.65 – 2.25 (m, 5H), 2.03 (t, *J* = 11.8 Hz, 2H), 1.90 (dd, *J* = 13.6, 10.1 Hz, 5H), 1.42 (d, J = 7.3 Hz, 3H), 1.25 (d, *J* = 8.4 Hz, 3H), 1.00 (d, *J* = 6.0 Hz, 2.5H), 0.93 (d, *J* = 7.1 Hz, 0.5H); ^{31}P (122 MHz, $CDCl_3$) δ 28.70; ^{13}C NMR (101 MHz, $CDCl_3$) δ 211.43, 175.80, 132.34, 132.25, 132.15, 131.96, 131.86, 128.89, 128.79, 128.66, 77.32, 77.00, 76.68, 62.25, 57.80, 52.67, 52.24, 48.20, 36.73, 34.47, 31.11, 31.05, 30.90, 29.74, 25.64, 25.16, 25.10, 22.28, 22.19; MS (Maldi) *m/z* calcd for $C_{27}H_{36}N_2O_3PS$ $[M+H]^+$ 499.2178, found 499.2201.

Compound 16d—^{1}H NMR (300 MHz, $CDCl_3$) δ 8.64 (br-s, 1H, N*H*), 7.92 – 7.72 (m, 4H), 7.52 (dddd, *J* = 12.8, 9.3, 7.3, 2.3 Hz, 6H), 4.21 (ddd, *J* = 8.5, 6.5, 2.2 Hz, 1H), 3.20 (m, 2H), 2.43 (dd, *J* = 12.7, 5.9 Hz, 1H), 2.13 – 2.02 (m, 1H), 1.91 (m, 2H), 1.28 (s, 9H); ^{31}P (122 MHz, $CDCl_3$) δ 30.0; ^{13}C NMR (75 MHz, $CDCl_3$) δ 175.98, 132.67, 132.52, 132.39, 132.32, 132.19, 129.65, 129.25, 129.13, 129.08, 128.97, 121.40, 62.58, 48.91, 48.35, 31.49, 28.92, 25.42, 25.33; MS (Maldi) *m/z* calcd for $C_{21}H_{28}N_2O_2PS$ $[M+H]^+$ 403.1609, found 403.1604.

Compound 16g—^{1}H NMR (300 MHz, $CDCl_3$) δ 8.97 (s, 1H), 8.30 (d, *J* = 8.3 Hz, 1H), 8.09 – 7.97 (m, 2H), 7.90 – 7.67 (m, 4H), 7.62 – 7.36 (m, 9H), 5.02 (ddd, *J* = 8.3, 6.3, 4.3 Hz, 1H), 4.27 – 4.02 (m, 1H), 3.74 (s, 3H), 3.32 (dd, *J* = 14.8, 6.4 Hz, 1H), 3.04 (m, 3H), 2.35 – 2.17 (m, 1H), 2.01 – 1.84 (m, 1H), 1.72 (m, 2H); ^{31}P (122 MHz, $CDCl_3$) δ 30.1; ^{13}C NMR (75 MHz, $CDCl_3$) δ 176.84, 171.32, 167.73, 133.84, 132.72, 132.50, 132.37, 132.21, 132.07, 131.97, 129.21, 129.10, 128.93, 128.65, 127.91, 61.90, 52.96, 51.28, 48.72, 42.00, 30.95, 25.32; MS (ESI) *m/z* calcd for $C_{28}H_{30}N_3NaO_5PS$ $[M+Na]^+$ 574.3, found 574.3.

Compound 16h—^{1}H NMR (300 MHz, $CDCl_3$) δ 8.92 (d, *J* = 7.6 Hz, 1H), 8.44 (s, 1H), 7.75 (tdd, *J* = 12.0, 8.3, 1.4 Hz, 4H), 7.67 – 7.38 (m, 6H), 7.01 (d, *J* = 7.2 Hz, 3H), 6.97 – 6.85 (m, 3H), 5.38 (td, *J* = 9.5, 3.2 Hz, 1H), 4.63 (td, *J* = 8.3, 5.0 Hz, 1H), 4.07 (td, *J* = 7.0, 5.0 Hz, 1H), 3.67 (s, 3H), 3.42 (m, 1H), 3.27 – 2.99 (m, 3H), 2.84 (dd, *J* = 13.9, 8.9 Hz, 1H), 2.49 (dd, *J* = 14.1, 9.9 Hz, 1H), 2.23 – 2.10 (m, 2H), 2.08 (s, 3H), 2.02 – 1.76 (m, 3H); ^{31}P (122 MHz, $CDCl_3$) δ 30.4; ^{13}C NMR (75 MHz, $CDCl_3$) δ 177.22, 171.75, 171.46, 171.04, 136.73, 132.65, 132.52, 132.33, 132.19, 129.31, 129.20, 129.10, 129.04, 128.93, 128.41, 126.81, 62.41, 54.36, 52.55, 49.93, 43.67, 37.95, 25.70, 23.40; MS (ESI) *m/z* calcd for $C_{32}H_{37}N_4NaO_6PS$ $[M+Na]^+$ 659.2, found 659.3.

Compound 16i—^{1}H NMR (300 MHz, $CDCl_3$) δ 8.21 (d, *J* = 7.4 Hz, 1H), 7.80 (m, 5H), 7.45 (m, 6H), 7.19 (d, *J* = 8.1 Hz, 1H), 4.93 (m, 1H), 4.75 (m, 1H), 4.50 (m, 1H), 4.01 (ddd, *J* = 8.2, 5.9, 2.3 Hz, 2H), 3.75 – 3.57 (m, 3H), 3.29 – 3.11 (m, 2H), 2.40 – 2.24 (m, 1H), 2.10 – 2.03 (m, 1H), 1.98 (m, 3H), 1.92 – 1.81 (m, 4H), 1.47 – 1.29 (m, 3H); ^{31}P (122 MHz, $CDCl_3$) δ 30.4; ^{13}C NMR (75 MHz, $CDCl_3$) δ 171.22, 170.56, 170.56, 170.08, 132.61, 132.57, 132.54, 132.51, 132.42, 132.37, 132.30, 132.24, 131.76, 131.52, 130.05, 129.84, 129.19, 129.10, 129.03, 128.93, 62.06, 62.02, 53.70, 52.63, 48.47, 48.06, 48.01, 42.04, 31.27, 31.19, 25.26, 25.17, 23.24, 17.97, 17.57; MS (ESI) *m/z* calcd for $C_{26}H_{35}N_4NaO_5PS$ $[M+Na]^+$ 569.2, found 569.3.

Dehydroalanine (Dha) 18—^{1}H NMR (300 MHz, $CDCl_3$) δ 8.54 (br, 1H), 7.85-7.81 (m, 2H), 7.57-7.43 (m, 3H), 6.79 (s, 1H), 5.98 (d, 1H, *J* = 1.4 Hz), 3.88 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 166.0, 165.0, 134.4, 132.3, 131.2, 129.0, 127.1, 109.1, 53.3; MS (ESI) *m/z* calcd for $C_{11}H_{12}NO_3$ $[M+H]^+$ 206.1, found 206.2.

Deprotection of the diphenylphosphoryl group

A sulfenamide product (0.05 mmol) was treated with 1 mL of cold 10% water in TFA at 0 °C (total *c* = 0.05 M). The resulted solution was stirred for 30 min at 0 °C. Upon completion, the excess TFA was removed with hexanes as the co-solvent under vacuum. To the remaining mixture was added cold diethyl ether to solidify the proline-sulfenamide TFA salt. The solid product was further washed with cold diethyl ether (2 mL × 5) and dried to provide the final product.

Compound 19—^{1}H NMR (300 MHz, $CDCl_3$) δ 10.31 (br-s, 1H), 9.70 (br-s, 1H), 9.09 (s, 1H), 7.57 – 7.35 (m, 5H), 4.91 (m, 1H), 4.64 (m, 1H), 3.74 (s, 3H), 3.38 (m, 2H), 3.23 (m, 2H), 2.32 (m, 1H), 2.01 (m, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 172.40, 171.21, 168.61, 132.96, 128.96, 128.70, 127.61, 60.54, 53.21, 51.71, 47.05, 40.52, 29.64, 24.59; MS (Maldi) *m/z* calcd for $C_{16}H_{22}N_3O_4S$ $[M+H]^+$ 352.1331, found 352.1320.

The reaction between 11 and HNO

To an argon sparged mixture of acetonitrile and water was added **11** (0.21 mmol), to this stirring mixture was added freshly prepared Angeli's salt (0.1 mmol, $Na_2N_2O_3$). The resulting solution was let stir until the reaction was completed (by TLC, or 20 minutes). The product **21** (50% yield) was isolated by extraction and flash column chromatography.

Compound 21—^{1}H NMR (300 MHz, $CDCl_3$) δ 10.04 (br-s, 1H, N*H*), 7.96 – 7.68 (m, 5H), 7.64 – 7.39 (m, 5H), 5.85 (br-s, 1H, N*H*), 4.03 (ddd, *J* = 8.1, 5.7, 2.3 Hz, 1H), 3.18 (m, 2H), 2.46 – 2.18 (m, 1H), 2.18 – 1.99 (m, 1H), 1.89 (ddt, *J* = 13.5, 8.9, 4.2 Hz, 2H); ^{31}P (122 MHz, $CDCl_3$) δ 28.6; ^{13}C NMR (75 MHz, $CDCl_3$) δ 176.32, 132.66, 132.62, 132.59, 132.55, 132.44, 132.41, 132.31, 132.28, 131.65, 131.39, 129.92, 129.71, 129.22, 129.13, 129.05, 128.96, 62.12, 62.08, 60.45, 48.07, 48.02, 31.29, 31.21, 25.27, 25.18; MS (ESI) *m/z* calcd for $C_{17}H_{19}N_2NaO_2P$ $[M+Na]^+$ 337.1, found 337.1.

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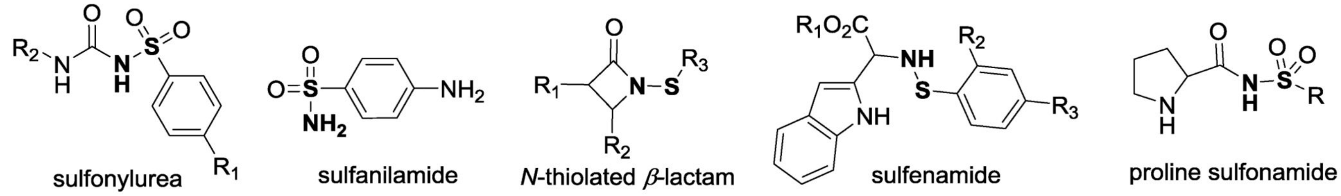
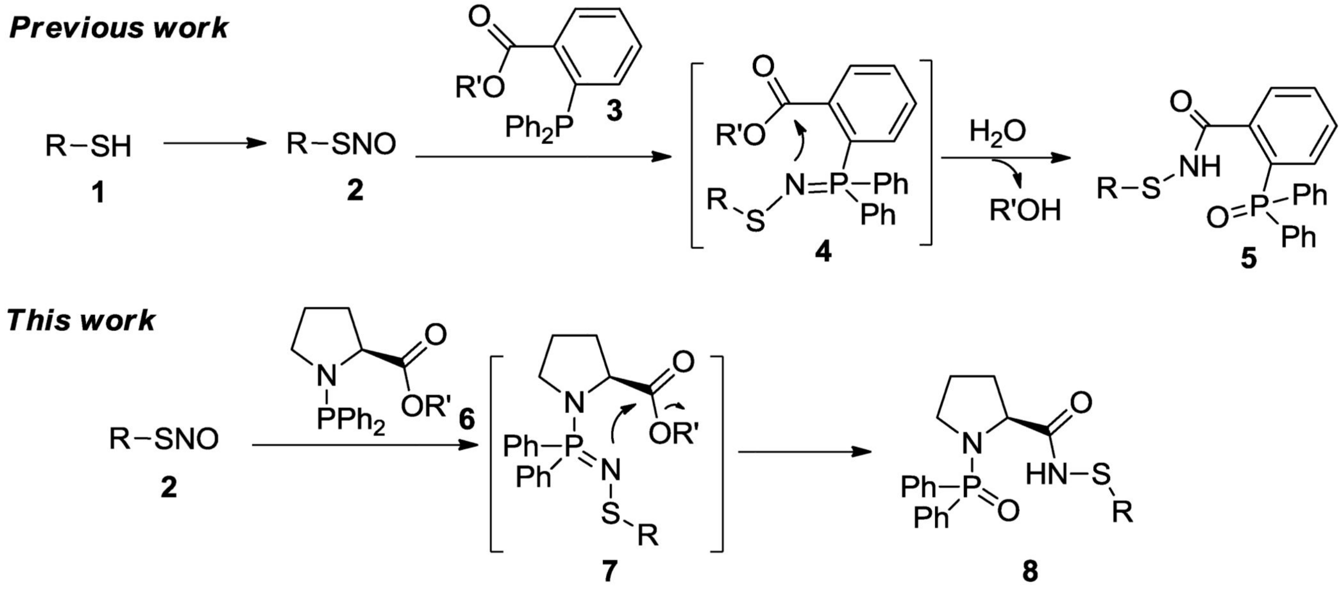


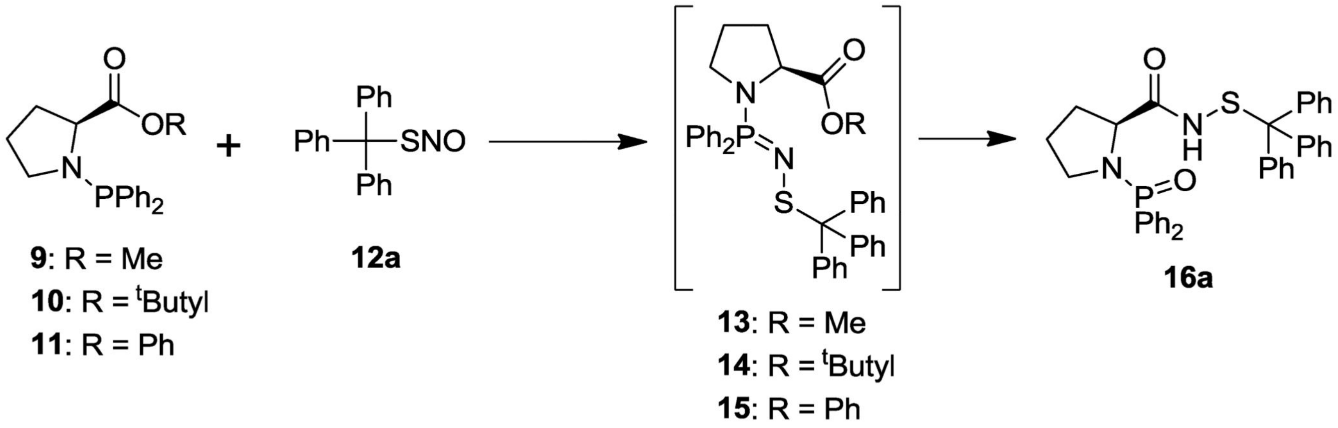
Figure 1.

Representative molecules having -S-N- linkages



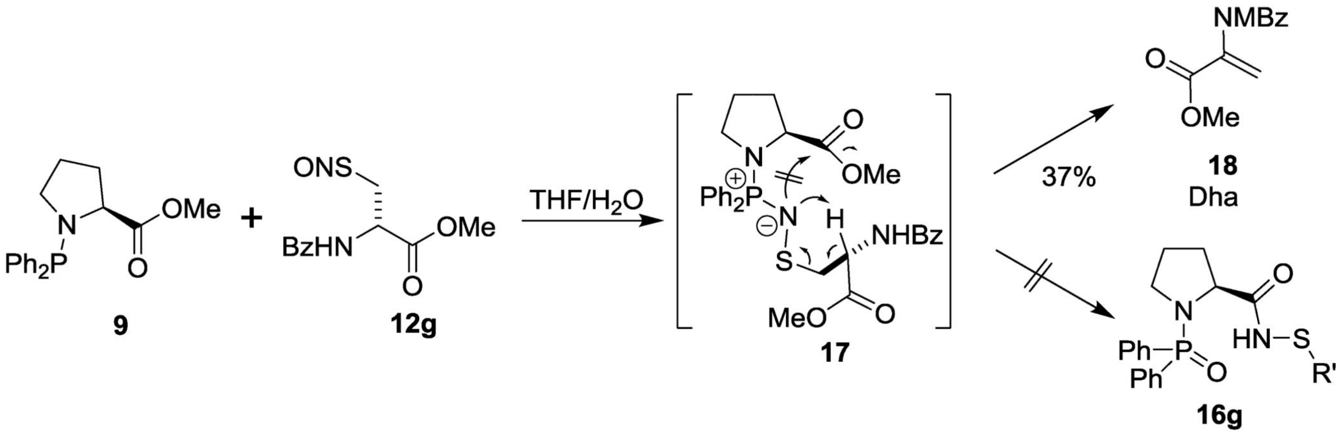
Scheme 1.

Reductive ligations of *S*-nitrosothiols



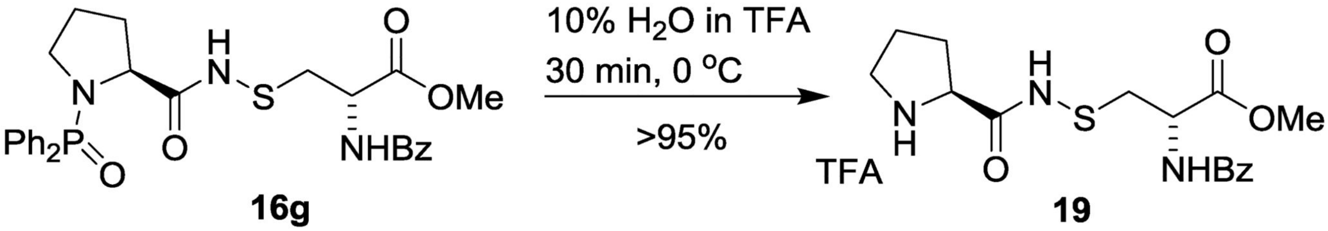
Scheme 2.

Reactions between proline-based phosphoramidites and TrSNO

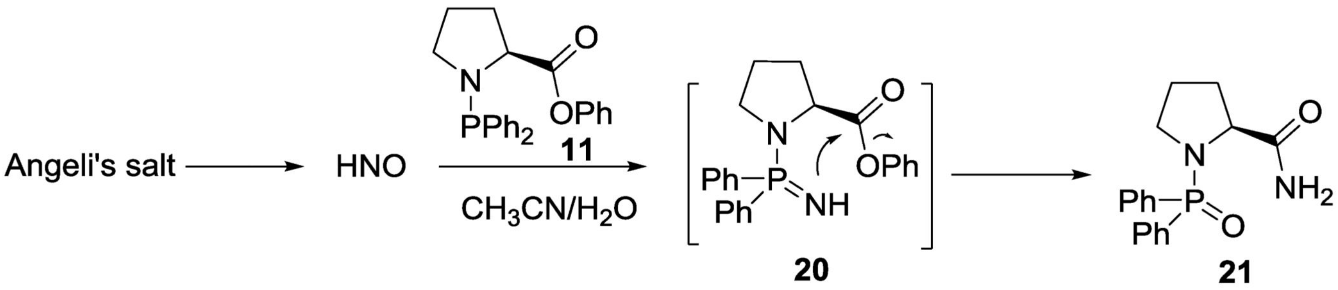


Scheme 3.

The reaction between **9** and **12g**



Scheme 4.
Deprotection of **16g**

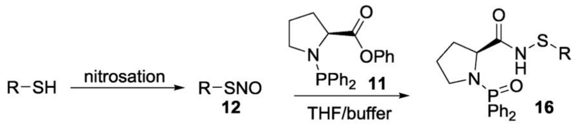


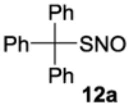
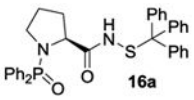
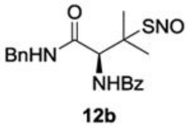
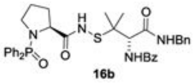
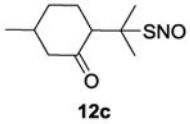
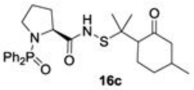
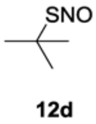
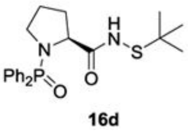
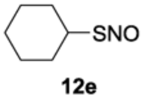
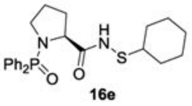
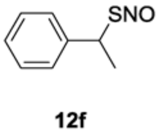
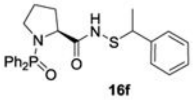
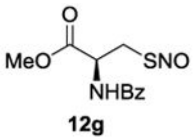
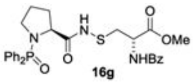
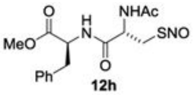
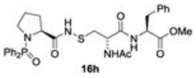
Scheme 5.

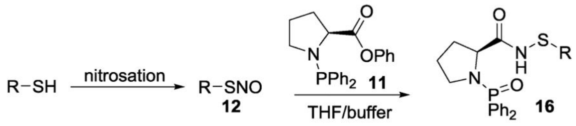
The reaction between **11** and HNO

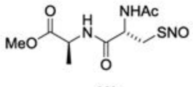
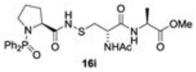
Table 1

Summary of the reactions between **11** and *S*-nitrosothiols



entry	RSNO	product	yield (%)
1	 **12a**	 **16a**	84
2	 **12b**	 **16b**	92
3	 **12c**	 **16c**	54
4	 **12d**	 **16d**	57
5	 **12e**	 **16e**	82[a]
6	 **12f**	 **16f**	40[a]
7	 **12g**	 **16g**	62
8	 **12h**	 **16h**	42



entry	RSNO	product	yield (%)
9	 **12i**	 **16i**	40

[a] Yields determined by NMR analysis