Volume 53 | Number 82 | 21 October 2017 | Pages 11257–11378

ChemComm

Chemical Communications

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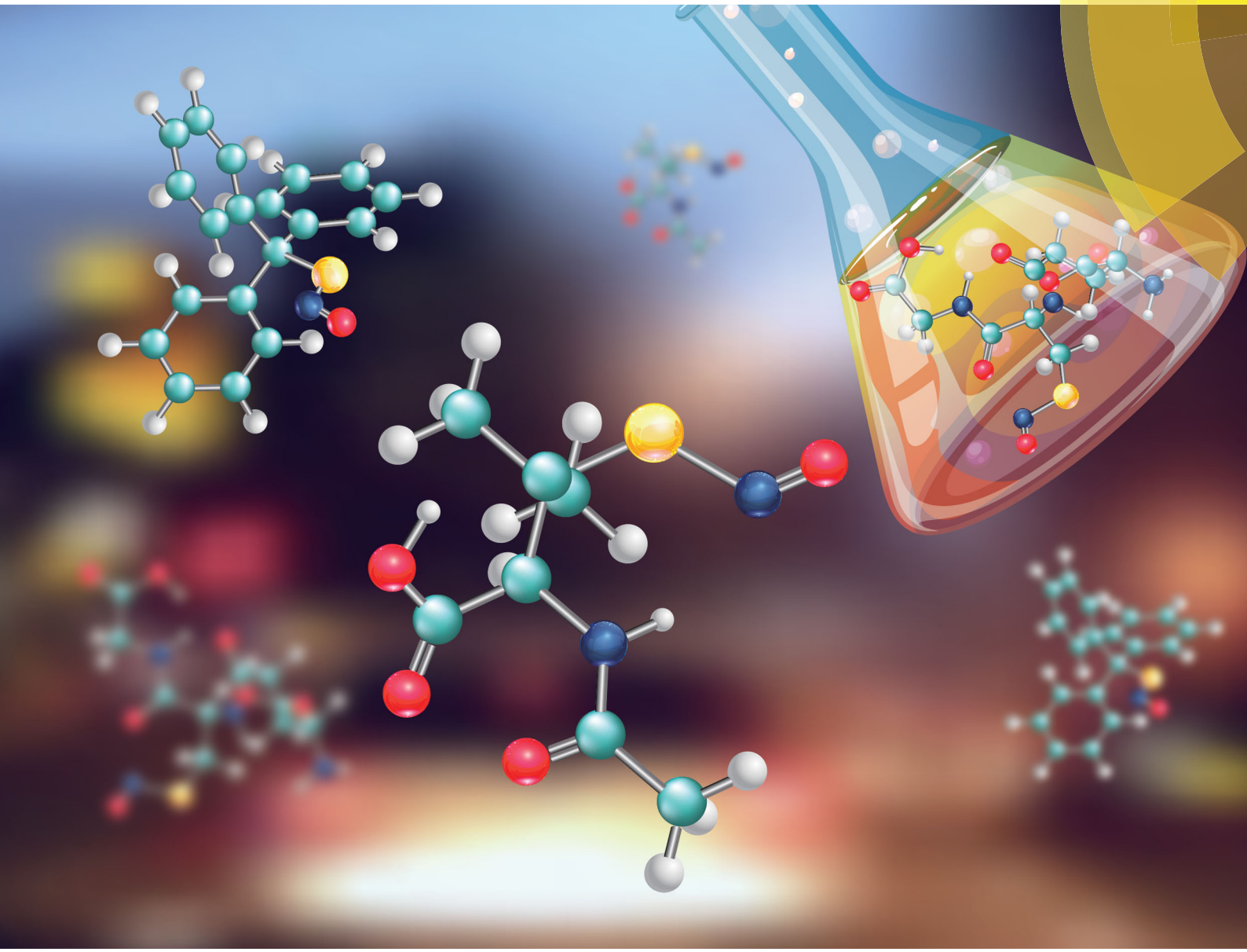

ISSN 1359-7345

ROYAL SOCIETY OF CHEMISTRY

FEATURE ARTICLE

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ChemComm

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Cite this: *Chem. Commun.*, 2017, 53, 11266

Received 21st August 2017,
Accepted 18th September 2017

DOI: 10.1039/c7cc06574d

rsc.li/chemcomm

S-Nitrosothiols: chemistry and reactions†

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The formation of *S*-nitrosothiols (SNO) in protein cysteine residues is an important post-translational modification elicited by nitric oxide (NO). This process is involved in virtually every class of cell signaling and has attracted considerable attention in redox biology. On the other hand, their unique structural characters make SNO potentially useful synthons. In this review, we summarized the fundamental chemical/physical properties of SNO. We also highlighted the reported chemical reactions of SNO, including the reactions with phosphine reagents, sulfinic acids, various nucleophiles, SNO-mediated radical additions, and the reactions of acyl SNO species.

Historical context

Interest in *S*-nitrosothiols (SNO) has surged since the identification of SNO as the key biologically relevant reaction products induced by nitric oxide (NO). NO is one of three important gaseous signaling molecules that affect many physiological and pathological processes (the other two are carbon monoxide CO and hydrogen sulfide H_2S). The formation of SNO on proteins and small molecules (such as cysteine and glutathione) is well characterized and believed to be an important signal transduction pathway of NO. In particular, SNO formation on proteins (namely *S*-nitrosation or *S*-nitrosylation) is a unique post-translational modification. So far a large number of SNO protein targets have been identified, such as protein tyrosine phosphatases, NF-κB, IκB kinase, Ras, *etc.* Specificity of SNO protein formation is directed by peptide sequences surrounding the sensitive cysteine residue, by exogenous *versus* endogenous NO sources, or by compartmentalization of NO synthases. Subsequent to SNO formation, proteins may undergo inter- or intra-molecular disulfide formation, or denitrosation to return to the basal state. These processes are highly regulated, especially during cellular responses to stress. Imbalance of SNO levels has been reported to associate with several pathologies. Nevertheless, the full nature of NO and SNO role in biological systems is under active investigation. The unstable nature of both SNO and NO complicate examination of their physiological roles. Especially the detection of SNO in biological samples is difficult and error-prone. Researchers are still looking for new methods to address these issues. In this regard, understanding the fundamental chemistry and chemical reactions of SNO is critical

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† This article is dedicated to Professor Jin-Pei Cheng on the occasion of his 70th birthday.

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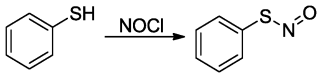
for better understanding their biological roles and for the development of better detection methods. In this review, we mainly summarize the known chemistry of SNO, particularly focusing on recent progresses on this topic. Readers interested in the biological aspects of SNO are invited to read other excellent reviews available.[1–6]

Preparation of SNO

The first preparation of SNO was reported in 1909 by Tasker and Jones,[7] which was achieved by the treatment of phenyl mercaptan with nitrosyl chloride (Scheme 1). Noted in their report is the characteristic color change (from colorless to red) indicating the formation of SNO, followed by the evolution of nitric oxide and recovery of the disulfide. Phenyl-SNO and other aryl-SNO compounds are noticeably less stable than their alkyl counterparts. So it should not be a surprise that early chemists did not think much of this transient species. SNO was then largely ignored, especially by the synthetic community, for the next 60 or so years, until it was identified as NO mediated post-translational modification products. Chemists at the time were far more interested in determining if azo-dyes could be used as antibiotics. It was common to study sulfur–nitrogen linkages in various oxidation states in an exploratory manner, and much of the initial work on SNO was done in this context.



Scheme 1

Historically a range of N-oxides (NOCl, N_2O_4, N_2O_3, NO_2, HNO_2) were used in inert solvents (*e.g.* CCl_4) to generate SNO from the corresponding thiol.[8] When these reactions are carried out at low temperatures (<263 K) SNO can be obtained in high yields. Care must be taken, as this system is sensitive to disulfide formation.

Sundquist *et al.*[9] determined that the active nitrosation reagent in these systems as N_2O_3. The mechanism of nitrosation for these N-oxides appears to be a series of disproportionation and redox reactions. More importantly, SNO are often not the

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'final' product of these reaction mixtures. Sulfonic anhydrides and other oxidation products of the starting thiol are often obtained if the buildup of nitric acid is not controlled. Oae *et al.*[10] did identify the intermediate presence of SNO in such reaction mixtures by the characteristic color change.

Under anaerobic conditions, direct reactions between thiols and nitric oxide (NO) do not produce SNO. Instead thiols are oxidized to their corresponding sulfenic acid (SOH) or disulfide.[11] Oxidation of a [R–S–N═O]$^{\bullet}$ radical by oxygen is thought to be pathway required for nitrosation to occur. Conversely, under aerobic conditions SNO are formed. Kinetic studies of such systems reveal first order oxygen concentration, and second order NO concentration dependence. Thiol concentrations show effects only at low concentrations.

The generation of SNO by NO and O_2 follows the reactions outlined below:

$$2NO + O_2 \rightarrow 2NO_2$$

$$NO_2 + NO \rightarrow N_2O_3$$

$$N_2O_3 + H_2O \rightarrow 2H^+ + 2NO_2^-$$

$$N_2O_3 + RSH \rightarrow H^+ + NO_2^- + RSNO$$

At higher concentrations of thiols, the above rate equation simplifies to: $d[RSNO]/dt = k_{NO}[NO]^2[O_2]$. These equations and empirical results obtained by Goldstein *et al.* support the equations outlined.[12] The rate-determining step of the auto-oxidation chain has been shown to be the formation of N_2O_3.

Addition of acidified nitrite cleanly and quickly generates SNO from the thiol starting material. This is a very convenient method for water soluble thiols, especially cysteine and other small alkyl thiols (*e.g. t*-BuSH). Treatment of the parent thiol in aqueous acid (typically 1 N HCl) with 5 equivalents of sodium nitrite ($NaNO_2$) generates the desired SNO in 5 to 15 minutes. Methanol can be added to the reaction mixture to assist with solubility. After completion of nitrosation the products can typically be extracted from the aqueous solution with diethyl ether or other organic solvents. The acidic conditions favor the protonated state of amines (like those in amino acids) and this helps deter unwanted *N*-nitrosation.

S-Nitrosation by alkyl nitrites is another often used method. Examination of the reaction kinetics on pH dependence reveals that this reaction is thiolate dependent. A series of papers by Williams *et al.*[13–18] explored nitrosation of small molecule thiols with a series of alkyl nitrites and the reaction rates were measured. This reaction is dependent on total thiolate concentration, and reaches a maximum rate just below a pH of 10. A series of alkyl nitrites and their nitrosation rates are shown in Table 1.

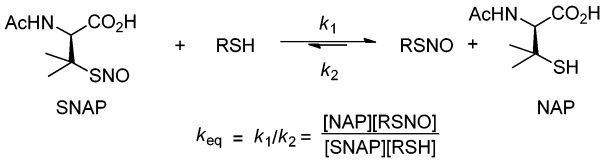
trans-Nitrosation is analogous to the alkyl nitrite transfer discussed previously. *trans*-Nitrosation between two thiols is typically an equilibrium reaction. Three forms of the thiol can be considered for a *trans*-nitrosation reaction: (1) the neutral thiol RSH, (2) the thiyl radical R–S$^{\bullet}$, (3) the anionic thiolate R–S$^-$.

Table 1 Reaction rates (mol^{-1} s^{-1}) of nitrosation of the thiolate at 25 °C

R′–S$^{\ominus}$ + RONO → R′SNO + ROH

RONO	Cys	CysOMe	CysOEt	AcNHCys	GSH
$(CH_3)_3CONO$	1.7	1.6	1.5	1.8	1.8
CH_3CH_2ONO	28	24	25	31	28
$(CH_3)_2CH(CH_2)_2ONO$	27	25	26	30	27
$C_2H_5O(CH_2)_2ONO$	169	150	165	169	159
$Cl(CH_2)_2ONO$	1045	1050	1100	1010	1070
$Br(CH_2)_2ONO$	1055	1055	1085	1030	1055
$I(CH_2)_2ONO$	1060	1060	1080	1020	1060

Table 2 Rates of *trans*-nitrosation by SNAP



$$K_{eq} = k_1/k_2 = \frac{[NAP][RSNO]}{[SNAP][RSH]}$$

RSH	pK_a	k_1 (M^{-1} s^{-1})	k_{eq}	K_{eq}^{-1}
L-Cysteine ethyl ester	6.50	6.45×10^{-1}	4.99	0.20
L-Cysteine	8.30	6.12×10^{-2}	3.63	0.28
D-Penicillamine	8.53	3.01×10^{-2}		
Glutathione	8.75	1.68×10^{-2}	3.34	0.30
N-Acetyl cysteine	9.52	8.41×10^{-3}	1.67	0.68
N-Acetyl D-penicillamine	9.90	9.90×10^{-3}		

Of these species, the neutral and anionic forms are the most predominant, while the radical concentration is typically very low. Li *et al.* used CBS-QB3 level of theory to explore possible mechanistic pathways.[19] They found the thiyl radical to have the lowest energy barrier to *trans*-nitrosation, followed by the thiolate anion and the neutral form. Since the thiolate is expected to dominate the equilibrium between the species, *trans*-nitrosation incidents are likely to occur *via* the thiolate.

S-Nitroso-*N*-acetyl penicillamine (SNAP) has a very strong tendency to donate NO, which has been attributed to steric repulsion from the two methyl groups. *trans*-Nitrosation can be monitored by UV-vis detection if the λ_{max} gap between the two species is large enough. SNAP has a UV absorption peak at 590 nm. Primary SNO have a λ_{max} around 540 nm. The UV-vis band gap between SNAP and primary SNO is large enough to allow monitoring both species simultaneously. The system was modeled as shown in Table 2. Accordingly Wang *et al.* used SNAP as a reference to gauge the *trans*-nitrosative properties of a series of thiols.[20]

Decomposition of SNO

SNO compounds are generally unstable, and can decompose through a range of processes, dependent on the experimental conditions. Condition sensitivities include pH, light, temperature, as well as the presence of heavy-metals. The study of SNO stability is difficult, as minor changes in reaction conditions yield dramatically different pathways of decomposition.

The stability of SNO varies greatly with the nature of the alkyl substituent. Some are unstable oils that decompose over

the course of minutes to hours, while others (such as trityl-SNO (TrSNO) and SNAP) can be isolated as crystal solids that can be stored for months. Early literature reports tertiary SNO compounds are more thermally stable than their primary and secondary counterparts. However, careful study has revealed that primary and secondary SNO are actually more thermally stable than their tertiary counterparts.[21] It was shown that *n*-butyl-SNO was fairly stable for 20 hours at 70 °C in a solution of deoxygenated solvent saturated with gaseous NO. An explanation comes from the reversibility of the homolytic cleavage, as shown below:

$$RSNO \rightleftharpoons RS^{\bullet} + NO^{\bullet}$$

$$2RS^{\bullet} \rightleftharpoons RSSR$$

Initial homolysis of the S–N bond gives two radical species, $RS^{\bullet}$ and $NO^{\bullet}$. Accordingly, the mostly irreversible decomposition to the disulfide then depends on the rate of disulfide formation from the thiyl radical. Tertiary radicals are more stable, and more sterically hindered to combine and to form the disulfide product. Constant argon flow, commonly used to protect reactions from oxygen, forces out the gaseous NO and drives the equilibrium to the disulfide.

Whether a given SNO decomposes in a homolytic or heterolytic fashion depends on a number of factors. Under aerobic conditions primary and secondary SNO are less stable. These observations match early reports that the rates of decomposition depend on oxygen concentration and the bulkiness of the alkyl group. An autocatalytic process may be involved in SNO decomposition. Under aerobic conditions the S–N bond scission to form $NO^{\bullet}$ occurs. $NO^{\bullet}$ then reacts with oxygen to form N_2O_3, which is a potent oxidant and is responsible for further decomposition of SNO. Addition of antioxidants inhibits this decomposition pathway, as well as the removal of endogenous NO. Additional NO accelerates the decomposition under aerobic conditions, due to the increased formation of N_2O_3.

Detailed studies on the electronic nature of the S–NO bonding motif are complicated by its instability. Cheng *et al.*[22] were the first to report experimental results of the S–NO homolytic bond dissociation energy (BDE). Both alkyl- and aryl-SNO were examined. Aryl-SNO display a BDE on the order of $\sim$20 kcal mol^{-1}. Alkyl-SNO are slightly more stable at $\sim$25–30 kcal mol^{-1}. The authors also calculated ΔH_{HOMO} using DFT at the B3LYP/6-31+G* energy level. Such calculations were in good agreement with the values obtained experimentally. SNO heterolytic cleavage energies can be compared to some analogous compounds, and a relative ranking of bond strength can be inferred: RO–NO < RS–NO < RN–NO. Calculations of the energy required for heterolytic cleavage reveal them to be higher in energy than the homolytic pathways. Heterolytic cleavage is not thought to be relevant for biological SNO.[23]

SNO decomposition can be facilitated by the presence of heavy metals like Cu^{2+} and Cu^{+}. There is often enough Cu^{2+} in distilled water to cause the decomposition of SNO. The addition of metal chelators, such as EDTA, almost completely stops the decomposition. It is thought that the ''true'' decomposition reagent is Cu^{+}, that forms from the reduction with thiolate, as shown below:

$$Cu^{2+} + 2RS^{-} \rightarrow Cu^{+} + RSSR$$

$$Cu^{+} + RSNO \rightarrow Cu^{2+} + RS^{-} + NO$$

In this process both the Cu^{2+} and RS^{-} are regenerated, and are likely present in catalytic quantities. The metal induced decomposition rates of SNO are structure dependent. The most vulnerable groups are those that can complex to the Cu^{+} bidentately. It was noted that apart from some indication of reaction with Ag^{+}, Fe^{2+} and Mg^{2+}, no other metal ions tested (Zn^{2+}, Ca^{2+}, Ni^{2+}, Co^{2+}, Mn^{2+}, Cr^{3+}, or Fe^{3+}) were effective in the decomposition of SNO.[24]

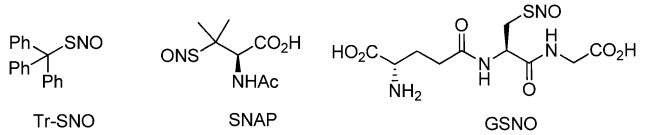
Physical and chemical properties of SNO

Small molecule SNOs are characteristically colored a vivid red or green. The red and green colors correspond to the substitution nature of the alkyl group: primary and secondary as red, and tertiary as green. Few SNO compounds are stable in a pure form. Instead they decompose thermally and photochemically to give the corresponding disulfide and nitric oxide. The rates of decomposition are substrate and condition dependent.

Some tertiary and bulky SNO like TrSNO and *S*-nitroso-*N*-acetyl-penicillamine (SNAP) (Scheme 2) can be isolated as stable solids. Solid SNAP must to be heated to *ca.* 150 °C before decomposition occurs.[25] *S*-Nitroso-glutathione (GSNO) is another notable SNO compound. While being a primary SNO, it is remarkably stable, due to its unique peptide structure that stabilizes SNO. It is also an endogenously formed SNO so it is widely used as a SNO model in biological studies. Most other small molecule SNO are unstable, and one should expect to have to prepare them freshly prior to use.

The first X-ray crystal structure of an SNO–SNAP was published in 1978. Further studies of SNO structure did not occur until 1999. This can be attributed to the use of N_2O_4 based nitrosation conditions being overly oxidative, preventing the isolation of clean SNO samples. Arulsamy[26] *et al.* used a system of aqueous acidified nitrite to generate SNO, then extracted the compound into an organic layer. This method cleanly prepares TrSNO suitable for examination by X-ray crystallography. This X-ray data is summarized in Table 3.

The dihedral angles of SNO are indicative of a double bond along the S–N axis. Typical S=N double bonds are reported to be 1.5 Å,[27] slightly shorter than those observed in SNO, which range from 1.76 Å to 1.85 Å.[28] Timerghazin *et al.* have proposed



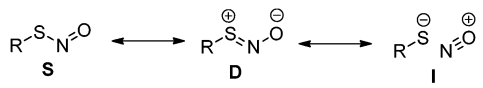
Scheme 2

Table 3 Bond lengths and angles of TrSNO and SNAP

	Bond lengths (Å)			Bond angles (deg)		
	N–O	S–N	C–S	O–N–S	N–S–C	C–S–N–O
Tr-SNO	1.177	1.792	1.867	114.0	102.1	175.7
SNAP	1.199	1.762	1.833	113.99	100.8	176.3

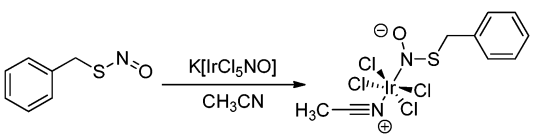
that SNOs are better understood by a combination of resonance structures.[29] PBE0/aug-pc-1 density functional theory calculations and natural resonance theory (NRT) calculations were used to generate the resonance description outlined in Scheme 3. The NRT theory predicts the structures shown and their normalized contributions. These are: (**S**) the standard RSNO Lewis structure, (**D**) a double bond along the S–N bond, and (**I**) an ionic resonance. Resonance structure **S** is dominant (70% to 80%), followed by **D** (~15–25%) and lastly **I** (~6–10%). Lewis acid interaction with at the oxygen position favors the **D** resonance. While interaction with at the nitrogen favors the standard (**S**) resonance. Lewis acid interaction with the sulfur atom favors the ionic resonance structure (**I**). These resonance structures provide satisfactory predictive explanations of some of the properties observed of SNO. The S–N bond can be significantly altered upon interactions with metals.[30] It has been shown that coordination with CuI weakens the S–N bond, both computationally[31] and experimentally.[32] Coordination with N is predicted to strengthen the S–N bond.[33] One such example was reported by Perissinotti *et al.*[34] Benzyl-SNO formed a surprisingly stable complex with $K[IrCl_5(NO)]$ in acetonitrile at room temperature (Scheme 4). The complex crystallized from solution and X-ray crystallography confirmed the structure.

The SNO motif contains some double bond character along the S–N bond. This imparts a *cis–trans* property, which is responsible for the color shift between the (*cis*-) red primary and secondary SNO, and the (*trans*-) green tertiary SNO. Bartberger *et al.* noted that the visible absorption by SNO in the 520 nm to 590 nm range corresponds to a n → π* transition.[35] The maximum absorption of the *trans* conformers are red-shifted by *ca* 30 nm. This absorption band is responsible for the change in color. The rotational energy barrier for *t*-butyl-SNO has been determined experimentally by Arulsamy *et al.* by synthesizing ^{15}N *t*Bu–SNO and performing variable temperature ^{15}N NMR.[26] The structures and value are shown in Scheme 5.

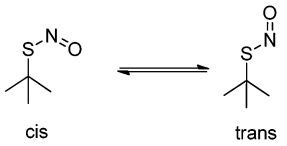


The resonance structures are designated **S**, **D**, **I**; for standard, double-bond and ionic, respectively.

Scheme 3



Scheme 4



cis-trans isomerization of t-Bu-SNO, 10.7 kcal/mol

Scheme 5

Table 4 Characteristic IR bands of RSNO

R group	V_{NO} (cm^{-1})	V_{NS} (cm^{-1})
1°, 2° alkyl	1500–1530	610–650
3° alkyl	1450–1500	650–685
Aryl	1430–1710	1000–1170

Table 5 Characteristic UV-vis bands SNO. The $n_N \rightarrow \pi^*$ transition is responsible for the red or green color of SNO

Transition	Range (nm)	ε (L mol^{-1} cm^{-1})
$\pi \rightarrow \pi^*$	255–261	10 000–20 000
$n_O \rightarrow \pi^*$	~340	1000
$n_N \rightarrow \pi^*$	550–600	10–20

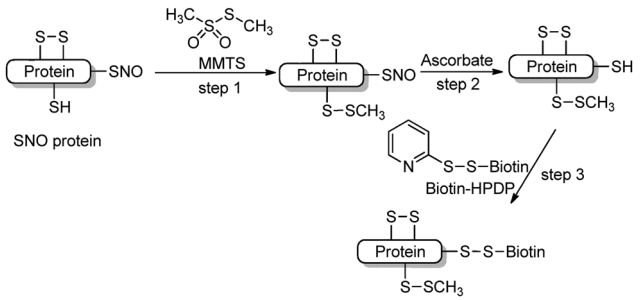
Spectra of SNO

Infrared and Raman spectra. Infrared (IR) and Raman spectra are not commonly used techniques for characterizing SNO. Some reports indicate that acquisition of these spectra can induce decomposition. In IR spectra, two shifts are attributed to SNO at ~1500 cm^{-1} and ~650 cm^{-1}. These peaks appear in the same regions in Raman spectra, they are attributed to the V_{NO} and V_{NS} vibrations. A useful feature to note is the complete loss of the V_{SH} stretching band in infra-red spectra at 2600 cm^{-1}. Upon *S*-nitrosation, the S–H vibration of GSNO is visible at up to 5% thiol contamination.[26] Typical ranges for these absorptions are given in Table 4.

UV-vis spectra. Some SNO compounds can be observed by UV-vis spectrometry in aqueous solutions. Drawbacks to this method include a required purification before measurement. The sensitivity of this method is relatively low, due to the poor molar absorptivity (ε) of SNO. UV-vis spectra of SNO usually show three bands, their assignments and typical ranges are shown in Table 5.

Detection of SNO in biological samples

Given the biological importance of SNO in NO signaling, the detection of SNO, especially in protein samples, has received considerable attention. The biotin switch assay is by far the most popular method, which can be used to detect and isolate SNO proteins from cell extracts.[36,37] The chemical principle of the biotin switch assay is shown in Scheme 6. This method utilizes three steps to selectively target and convert unstable SNO to stable biotin conjugates: (1) the free thiols in a protein or a protein mixture are blocked by reagents like MMTS (methyl



Scheme 6

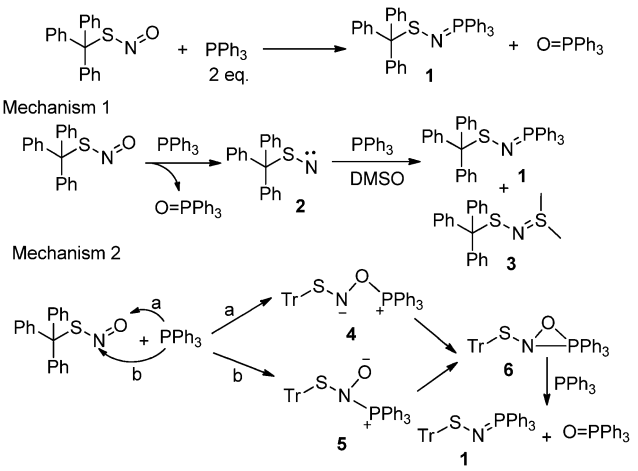
methanethiosulfonate) or NEM (*N*-ethylmaleimide). In this step proteins usually need to be denatured to ensure access of blocking reagents to all thiols. (2) The SNO residues are selectively reduced by ascorbate to form free thiols. It should be noted that the reaction mechanism of this reduction is still unclear.[38,39] (3) The newly formed thiols are treated with biotin–HPDP (*N*-[6-(biotinamido)hexyl]-3′-(2′-pyridyldithio)-propionamide), a thiol specific biotinylating reagent, to form the desired biotin conjugates.

Using this method, SNO proteins can be isolated or enriched from biological samples and subjected to mass spectrometric analysis or western blot analysis. Based on the same chemistry some improvements have been made, which include resin-assisted capture,[40] fluorescence labeling,[41–43] and microarray-based assay.[44]

Although biotin switch has been used in many studies it still suffers some problems: (1) it is a subtractive method, meaning its efficiency relies on blocking 100% of free thiols in the sample. Due to the very low concentration of SNO (at nM levels) but high concentrations of free thiols (up to mM levels) in biological systems, this is not always achievable. (2) The specificity of biotin switch is questionable. The reduction of SNO by ascorbate seems to be substrate dependent. Some SNO moieties cannot be reduced by ascorbate efficiently,[45–48] while under some circumstances ascorbate can also reduce disulfides.[49–51] (3) There is a possibility of disulfide exchange after the reduction of SNO. This could lead to incorrect identification of the nitrosated cysteine residues of a protein. These problems promoted researchers to explore new chemistry and reactions of SNO, with the goal of identifying more effective detection methods. The following sections focus on such reactions.

Phosphine-mediated reactions of SNO

In 1972 Haake reported an interesting reaction between TrSNO and PPh_3 (Scheme 7).[52] In this reaction two equivalents of phosphine were required, leading to the formation of phosphine oxide and *S*-azaylide **1**. The mechanism of this reaction is not completely clear and two possibilities exist: (1) the phosphine may directly abstract oxygen from SNO to form the thionitrene intermediate, which in turn reacts with phosphine again to form *S*-azaylide. When DMSO was used as the solvent the nitrene-trapped product **3** was obtained, albeit in very low yield (11%). (2) A second possibility is *via* initial nucleophilic attack by the phosphine on either the oxygen or nitrogen atom of SNO. In either case the formed intermediates (**4** and **5**) are expected to

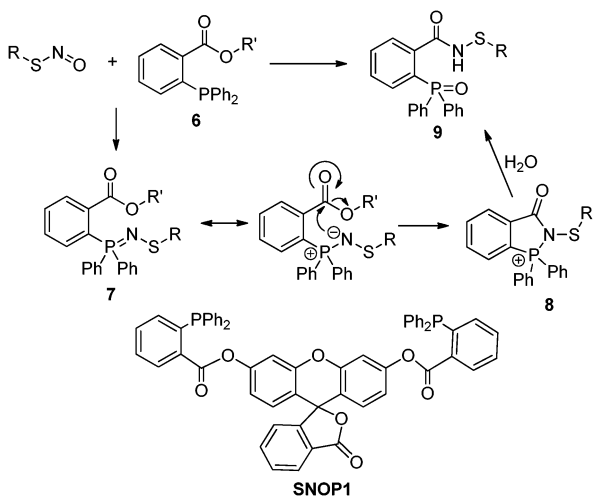


Scheme 7

collapse to the same 3-membered ring intermediate **6**. Another molecule of phosphine then abstracts the oxygen of **6** to form phosphine oxide and *S*-azaylide.

This reaction was later found to be general for SNO compounds by Xian and co-workers.[53] However, the *S*-azaylide product obtained from TrSNO was the only isolable *S*-azaylide due to its remarkable stability. Attempts to obtain other SNO-derived *S*-azaylides, especially from primary SNO, were unsuccessful as they were too unstable. Nevertheless, it was recognized that the *S*-azaylide formation is a fast process and has the potential to promote certain cascade reactions with other electrophiles attached to the phosphine site. With this idea in mind, a series of phosphine-mediated tandem reactions were developed. Similar to the well-known Staudinger ligation,[54,55] these reactions appear to be bio-orthogonal, thus holding the promise for the detection of SNO.

The first such reaction was the reductive ligation of SNO reported in 2008.[53] As shown in Scheme 8, an ester group was attached to phosphine to trap the *S*-azaylide **7**. The intramolecular acyl transfer should produce intermediate **8**. Hydrolysis of **8**

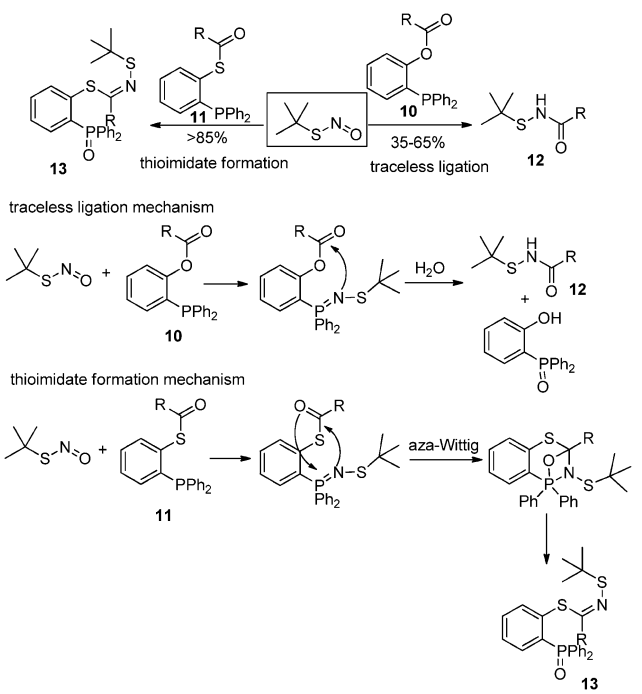


Scheme 8

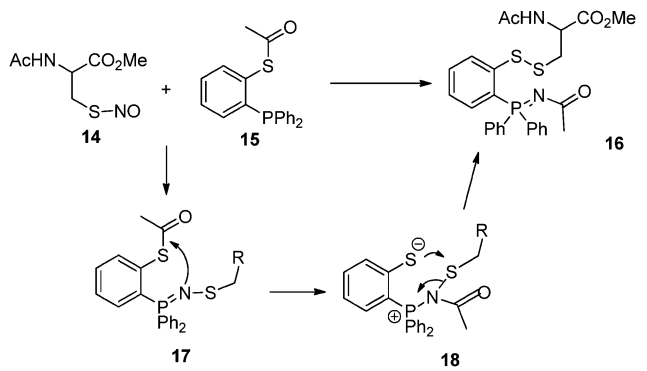
completes the reaction to give sulfenamide **9** as the final product. The reaction was found to proceed rapidly, in high yields, and under mild and aqueous conditions. A fluorescent probe-SNOP1 was designed based on this reaction.[56] It showed strong fluorescent responses to small molecule SNO like GSNO. It should be noted that King *et al.* later found this phosphine-mediated reductive ligation worked similarly toward nitroxyl (HNO), another interesting NO-related signaling molecule.[57,58] Therefore, fluorescent probes like SNOP1 were also found to respond well to HNO.[59,60]

The reductive ligation of SNO can in theory be modified to a traceless version, in which the phosphine core will not be present in the final product. This is analogous to the traceless Staudinger ligation explored by Raines and Bertozzi.[61–64] As such, Xian *et al.* tested several traceless phosphines including ester and thioester substrates (**10**, **11**, Scheme 9).[65] Interestingly they gave completely different results. When ester substrates **10** were employed, the desired traceless products **12** were obtained in modest yields. In contrast, when the thioester substrates **11** were used, the corresponding thioimidates **13** were obtained in high yields. These thioimidate compounds were found to be very stable, indicating the strong S–N bonds in these molecules. The formation of thioimidates can be attributed to an intramolecular aza-Wittig reaction of the *S*-azaylide intermediates, as shown in Scheme 9.

It is worth noting that all SNO used in the reactions shown in Scheme 9 were relatively stable tertiary SNO. When unstable but more biologically relevant primary SNO like cysteine-derived SNO substrates were tested with those "traceless" phosphines, a unique bis-ligation of SNO was discovered.[66] As shown in Scheme 10, when primary SNO **14** was treated with



Scheme 9

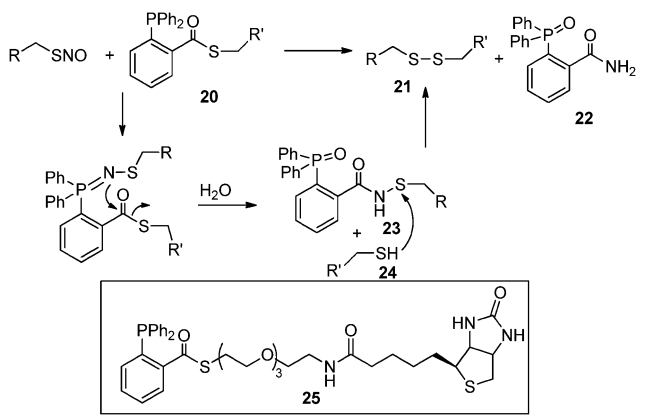


Scheme 10

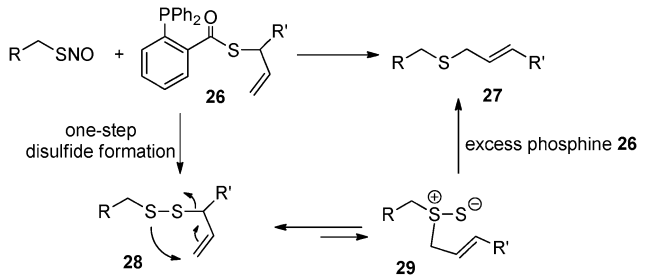
phosphine-thioester substrates like **15**, the products were very stable disulfide-iminophosphoranes **16**. The mechanism of this process was proposed as the following: the primary SNO first reacts with phosphine **15** to form an *S*-azaylide **17**. Then, acyl transfer from the thioester to the N-atom provides intermediate **18**. Finally, the nucleophilic phenylthiolate attacks the pseudo-sulfenamide linkage intramolecularly to furnish the disulfide-iminophosphorane product **16**. This reaction has been used to quantify endogenous SNO like GSNO by Tannenbaum *et al.*[67]

Inspired by the strong reactivity of thiolate toward S–N bond observed in bis-ligation, Xian *et al.* designed a simple one-step disulfide formation using phosphine-thioester substrates like **20** (Scheme 11).[68] In this reaction, the original reductive ligation occurs first to form sulfenamide **23** and thiol **24**. These two products should be in close proximity when they are formed. Then their reaction should provide the simple disulfide **21** and benzamide **22**. This is also a "traceless" version as the product **21** does not contain the phosphine oxide moiety. A biotin-conjugated phosphine **25** was prepared based on this reaction and used to label and enrich SNO proteins in cell lysates.[68]

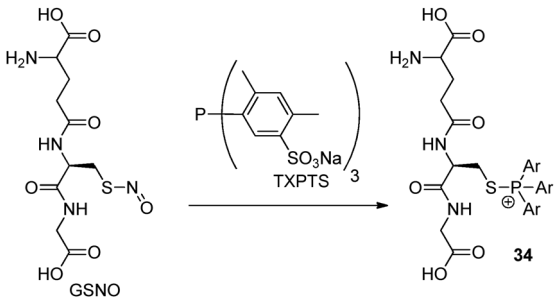
The one-step disulfide formation can be further tethered with allyl disulfide rearrangement for a "one-pot" thioether formation. The reasons of doing so are: (1) thioethers are more stable than disulfides so they are more suitable for bio-detection, and (2) the allyl disulfide rearrangement is facilitated by phosphines



Scheme 11



Scheme 12

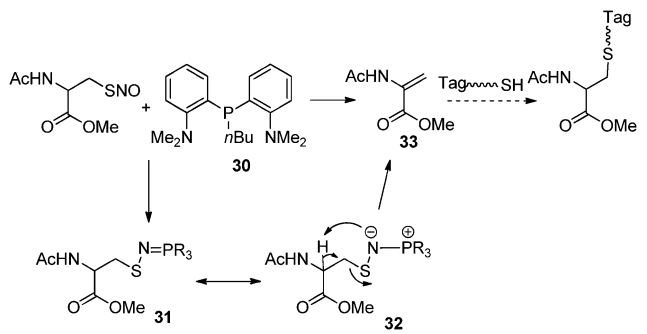


Scheme 14

so the excess of phosphine used in SNO ligation could trigger the rearrangement and no other reagent is needed. This process was achieved with allyl thioester substrates like **26** (Scheme 12).[69] The one-step disulfide formation should firstly produce product **28**. Then allyl disulfide rearrangement should be promoted by the excess phosphine **26** in the reaction mixture to give thioether **27** and the corresponding phosphine sulfoxide. This reaction was proved to work nicely with a number of cysteine SNO derivatives.[69]

The aforementioned phosphine-mediated reactions in general work well for all SNO compounds, no matter they are cysteine derivatives or not. However, cysteine SNO derivatives do have some unique reactivity due to their relatively acidic α-proton. This was observed in dehydroalanine formation (Scheme 13).[70] Phosphines like **30** do not contain electrophiles in their structures to trap the nucleophilic *S*-azaylide. Therefore, *S*-azaylide may act as a base to promote an intramolecular elimination *via* intermediate **32** to produce dehydroalanine **33**. This formation of dehydroalanine under neutral and mild conditions is significant, as the products can be further tagged with a reporting molecule through a simple Michael addition. It holds promise for being used for the detection of SNO proteins.

A limitation of these phosphine chemistry with respect to labeling biological SNO comes from their poor solubility in aqueous solutions. King *et al.* tried to address the problem by using a triaryl phosphine TXPTS with sulfonate groups to improve solubility (Scheme 14).[71] However, with this substrate the *S*-azaylide is not clearly formed. Instead this phosphine directly displaces NO to form a sulfurphosphonium adduct **34**. The displaced NO is protonated to HNO and subsequently trapped by **33**. The sulfurphosphonium products from SNO, such as GSNO, are stable enough to be detected by LC-MS.



Scheme 13

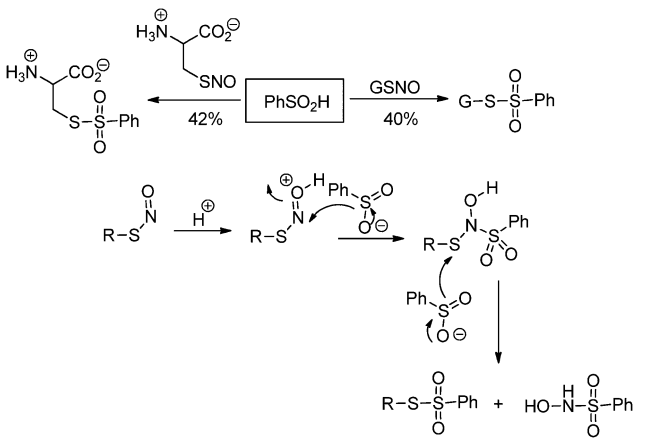
SNO reactions with sulfinic acids

In 1985 Hart reported that *S*-nitrosocysteine (CysNO) and GSNO can react with phenylsulfinic acid to form thiosulfonates (Scheme 15).[72] The reaction proceeded under highly acidic conditions (pH 0.5) and the products were formed in modest yields. This reaction was re-investigated by Grieco *et al.* recently and they found it could proceed under pH 4.0.[73,74] It was also found that the reaction needs two equivalents of sulfinic acid. The mechanism is proposed in Scheme 15. Protonation of SNO triggers the attack of sulfinate to the nitrogen atom of SNO. The second molecule of sulfinate then reacts with the sulfur atom to form thiosulfonate and $PhSO_2NHOH$. This reaction was then used to label SNO proteins like *S*-nitroso bovine serum albumin (BSA). Very recently Martin *et al.* applied this reaction as a tool for the detection of both protein *S*-sulfination (P-SO_2H) and *S*-nitrosylation (P-SNO),[75] depending on which reagent is used. In these protein labelling experiments free thiols must be blocked first, as the thiosulfonate products are vulnerable to thiol exchanges.

Analogous to aforementioned reactions, it was found that sulfite (SO_3^{2-}) can also react with both protein and small molecule SNOs to form *S*-sulfonate species (RS–SO_3^-). This reaction ($k_{sulfite}$ = 264 M^{-1} s^{-1} at pH 7.4, 37 °C) may contribute to the mechanism of sulfite allergy as it depletes SNO contents in human airways.[76]

SNO reactions for synthetic purposes

Historically SNO are not considered very useful for synthesis so only very limited reactions with potential synthetic applications



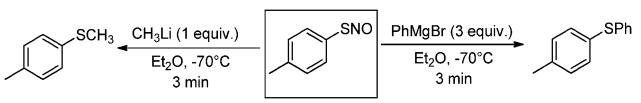
Scheme 15

are reported. Nonetheless, the unique structure of the SNO moiety has led *S*-nitrosothiols to serve as a substrate or catalyst in a handful of chemical transformation. From a reactivity standpoint, the resonance structures **D** and **I** suggest that both the S and N atoms in the SNO moiety possess electrophilic characteristics. This reactivity profile is illustrated in various nucleophilic additions with *C*-, *N*-, *S*-, *P*-nucleophilic species.

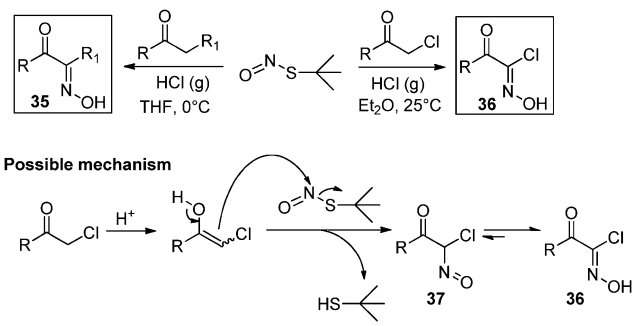
Reactions with carbon nucleophiles. In 1980 Oae and co-workers reported the reactions of various *C*-nucleophiles such as carbanionic species *i.e.* organolithiums and Grignard reagents with SNO.[77] These carbanions reacted with SNO *via* substitution resulting in the formation of sulfide products and NO^-. The model SNO substrates explored were *p*-toluene SNO and *t*-BuSNO, which reacted with both alkyl and aromatic Grignard reagents and alkyl organolithium compounds very rapidly, to produce the corresponding sulfides in moderate yields (Scheme 16). It is important to note that the *C*-nitroso compound *i.e.* RNO, which might result from the direct nucleophilic attack of the carbanions to the NO moiety of SNO, was not isolated.

In the presence of acids, enolizable ketones were shown to react with SNO. Kim *et al.* found that α-carbons of both ketones and α-haloketones underwent nitrosation to yield a *C*-nitroso intermediate such as **37**.[78,79] This intermediate subsequently tautomerized to the corresponding oxime **36** (Scheme 17). Interestingly, dimerization of the *C*-nitroso intermediate to the nitrone was not observed. From all ketone substrates tested the oxime products were obtained in low to moderate yields with concomitant formation of the disulfides derived from SNO.

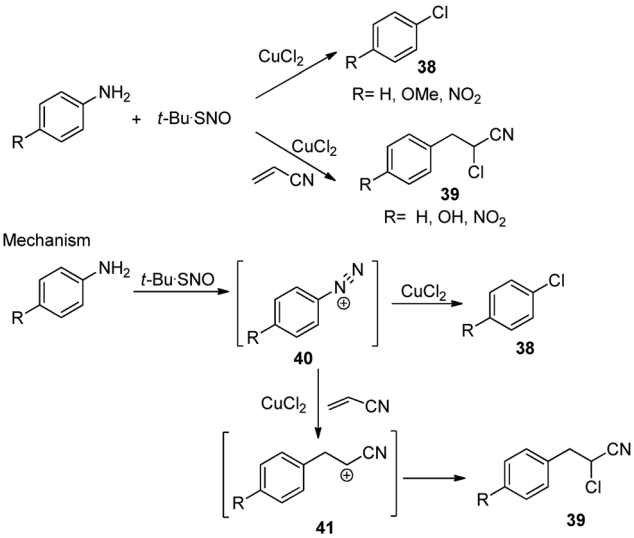
Reactions with nitrogen-based nucleophiles. *trans*-Nitrosation of amine substrates can occur in the presence of alkylnitrites and other nitrosylation agents. Oae *et al.* utilized this reaction to explore the deamination of arylamines, using SNO and Cu^{2+} salts to prepare aryl halides (Scheme 18).[80] The reaction may involve the intermediacy of the phenyl diazonium salt similar to the Sandmeyer reaction. Both electron-deficient and -rich arylamines can afford the corresponding aryl chlorides or bromides in good yields. In the same report the authors extended this reaction to examine a deamination–alkylation process. For this transformation olefins, especially electron deficient olefins, were introduced in the reaction mixture together with *t*-BuSNO, which triggered the deamination of arylamines and subsequent alkylation (Scheme 18). In addition, a cationic intermediate **41** was formed in the alkylation step, which was quenched by the halide. This transformation was amenable to a variety of electron-deficient and -rich arylamines and yields in most cases were modest.



Scheme 16



Scheme 17

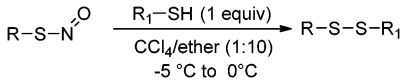


Scheme 18

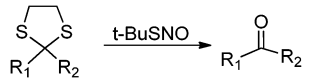
In a later report, Kim extended this reaction for the synthesis of fluorinated nucleobases.[81] The reaction did not require the use of Cu^{2+} halides to incorporate the halogen in the heterocyclic amines. Instead, sodium tetrafluoroborate ($NaBF_4$) was used as the source of fluoride to afford the corresponding fluorinated heterocycles under very mild conditions. Various heterocyclic amines, including the nucleobase adenosine, were examined and found to furnish the fluorinated products in good yields.

Reactions with sulfur-based nucleophiles. The most notorious reaction of SNO with sulfur containing nucleophiles is the reaction with thiols (RSH) to yield disulfides. This reaction is of biological significance but with limited synthetic applications. An early study by Oae *et al.* showed the use of SNO as good electrophiles for thiols.[82] Under very mild conditions (0 °C in CCl_4/ether) thiols reacted with SNO to produce mixed disulfides (Scheme 19). Both primary and aryl SNO were used as substrates to react aryl thiols and sterically hindered alkyl thiols. Various mixed disulfides were obtained in very good yields.

Sulfides can also react with SNO *via* a *trans*-nitrosation-like mechanism. Kim and co-workers reported that thioacetals and thioketals reacted with SNO to give the corresponding



Scheme 19

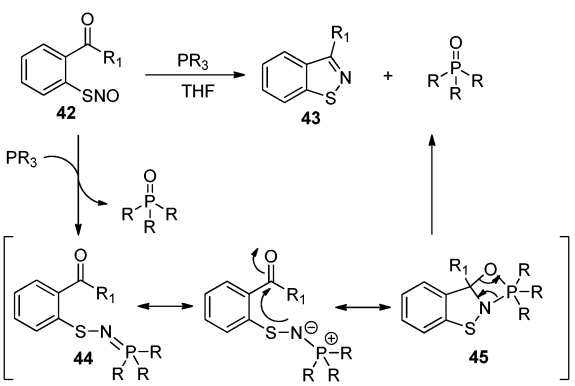


Scheme 20

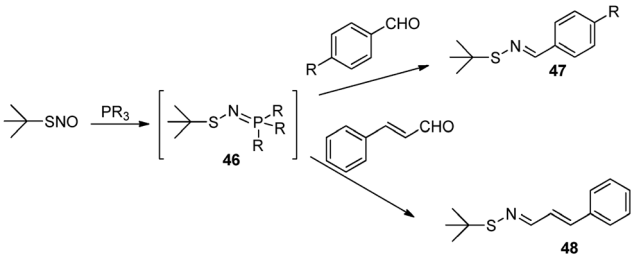
de-thioacetylation products (Scheme 20).[83] In this reaction, nitrosonium (NO^+) was transferred from SNO to the sulfur atom of the thioacetal or thioketal to activate the geminal carbon, thus triggering the de-thioacetylation process to produce the corresponding aldehydes or ketones under mild conditions. The reaction can be used to de-protect thioacetals and thioketals.

Reactions with phosphine-based nucleophiles. As discussed earlier the reactions of SNO with phosphine containing nucleophiles have been explored for the development of bio-orthogonal reactions to label biological SNO. The key intermediates in these reactions are the *S*-azaylide species. It was envisioned that *S*-azaylides could be used for synthetic applications and therefore SNO might be useful synthons. To this end, Xian *et al.* designed substrates like **42** which contain a ketone *ortho* to SNO on the benzene ring (Scheme 21). Such substrates could undergo an aza-Wittig reaction to produce benzisothioazole **43**.[84] Due to the instability of aryl-SNO derivatives **42** were prepared in a single step *via* nitrosation of the parent thiol *i.e. o*-mercaptoacylphenones with isoamylnitrite (i-pentylONO). Upon *in situ* formation of **42** the phosphine reagent was added to form the *S*-azaylide **44** and promote the aza-Wittig reaction to produce 3-substituted benzisothioazole **43**.

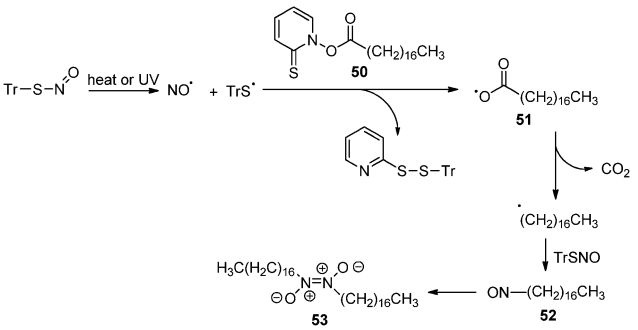
Recently this reaction was extended to an intermolecular version using aldehydes as the electrophiles for the aza-Wittig process.[85] SNO like *t*-BuSNO were found to be effective when used in excess together with excess amounts of PPh_3 (relative to the aldehyde substrates). Under these conditions the intermolecular aza-Wittig reaction took place and the corresponding *N*-thio-imine products **47**, **48** were formed (Scheme 22). Cinnamaldehyde and substituted benzaldehydes (*p*-H, *p*-OMe and *p*-CF_3) worked nicely with moderate to good yields. Not surprisingly, this transformation required extended reaction times (overnight) and higher temperatures than those established when intramolecular ketones were used as electrophiles as the reaction proceeded in an intermolecular fashion.



Scheme 21



Scheme 22

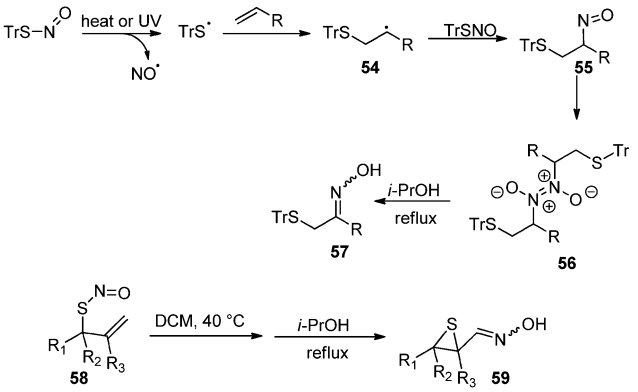


Scheme 23

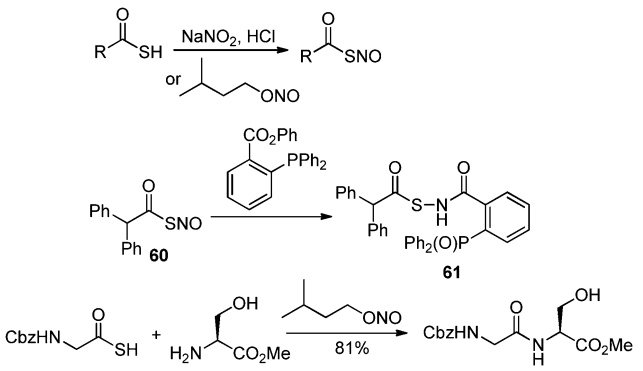
SNO mediated radical additions

SNO can easily decompose to produce a thiyl radical and a NO radical. Motherwell and co-workers demonstrated that these radicals can be trapped *in situ* with radical acceptors, which provides a new synthetic method for S- or NO-containing molecules.[86] For example, *O*-acylthiohydroxamates **50** (Barton esters) were tested as radical acceptors and found to undergo a decarboxylative chain reaction to yield *trans*-nitroso dimer **53** as the major product (Scheme 23).[86]

In subsequent studies the same group explored SNO radical reactions with alkenes (Scheme 24).[87] It was found that similar radical chain reaction could proceed to form nitroso sulfide **55**, which then dimerized to **56** (Scheme 24). Interestingly upon addition of 2-propanol, the α-nitroso dimer tautomerized to afford α-thiooxime product **57**. This reaction was later extended to an intramolecular process.[88] In this case, both the SNO



Scheme 24



Scheme 25

moiety and alkene were constructed in the same substrate. The sequential thiyl radical formation, addition, dimerization, and tautomerization produced a unique thioepoxide product **59**. Other products isolated from this transformation were the disulfide species of the parent thiol.

Acyl SNO and reactions

In addition to well-characterized alkyl and aryl SNO, acyl SNO (also named as *S*-nitrosothioacids) can also be prepared *via* simple nitrosation of thioacids by $HCl/NaNO_2$ or organonitrite (Scheme 25).[89] Acyl SNO show a deep green color and appear to be very unstable as the green color readily fade. The decomposed products are the corresponding acyl disulfides. However, some acyl SNO like **60** are stable enough to be captured by phosphine-mediated reductive ligation to form product **61**, providing a direct evidence of acyl SNO. The formation of acyl SNO can significantly activate the thioacid moiety for amidation. The typical procedure is to mix the thioacid and the amine first. Then organonitrite is added into the solution. Amidation under these conditions is fast and no additional base is needed. A number of solvents and amino acid side chain functional groups can tolerate in this procedure. One example is shown in Scheme 25. This method should be useful for peptide synthesis.

Conclusions

In this review, we summarized the known chemistry and reactions of SNO, a group of unique thiol derivatives. The importance of SNO in redox biology is well recognized and much work has been done regarding SNO's formation, distribution, and biological roles in biological systems. To this end, understanding the fundamental chemistry and properties of SNO is critical, especially when current SNO detection methods are still not ideal. More specific and convenient detection methods are needed and this is expected to be an active research area in the coming years. On the other hand, the unique structures and easy preparation of SNO make them attractive synthons. Potentially SNO can be used to introduce multiple heteroatoms (S, N, O) into molecular structures in an effective way. Some recent developments on SNO reactions have proved this hypothesis. We expect more interesting and synthetically useful reactions of SNO will emerge in the future.

Conflicts of interest

There are no conflicts to declare.

Acknowledgements

This work was supported by NSF (CHE1738305) and the Hundred Talent Program of Shanxi Province, China.

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