New Thionitrites: Synthesis, Stability, and Nitric Oxide Generation

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In order to study the influence of substitutions at the α and β carbon atoms on the stability of the S–NO bond, water-soluble thionitrites RSNO have been synthesized by nitrosation of cysteamine and mercaptoethanol derivatives and characterized. ^1H and ^{13}C NMR spectroscopies have proven to be excellent probes for the nitrosation of thiols. In water, at physiological pH, the compounds decomposed into nitric oxide NO and the corresponding disulfides. The rate at which NO was released was very sensitive to modifications at the α and β carbon atoms. Tertiary thionitrites were more stable than primary thionitrites. The β-substituents decreased the rates of decomposition in the following order: OH > $NHCOCH_3$ > NH_3^+. *S*-Nitrosocysteamine derivatives were greatly stabilized at low pH. The compounds described here might be convenient and useful as vehicles for spontaneous generation of nitric oxide in biological systems, at rates that can be finely tuned and controlled over a wide range.

Nitric oxide, NO, has recently been implicated in a variety of important bioregulatory processes, including control of blood pressure, macrophage-induced cytostasis and cytotoxicity, and neurotransmission.[1]

NO is a highly unstable gas having limited solubility in aqueous solutions. In aerobic aqueous media, NO is rapidly oxidized into nitrite.[2] It is thus difficult to introduce reliably into most biological systems without premature decomposition. Consequently, there is an increasing interest in chemical agents that might serve as vehicles for the controlled delivery of NO into biological systems. Such compounds might be useful research tools for the study of the biological effects of NO and might also display pharmacological effects as potential vasodilators or antiproliferative agents, for example.

In our opinion the major requirements for a good NO generator are the following: first, the compound has to be easy to prepare in a stable and pure form, preferably as a solid; second, it has to be unstable in water at physiological pH, preferably without the need for redox activation; third, it has to generate NO quantitatively, at a predictable rate; and fourth, the byproducts should be nontoxic. Such compounds are extremely rare.

Very recently, a number of nucleophile/nitric oxide adducts of structure $[XN(O)N{=}O]^-$, where X is a nucleophilic residue, have been studied and shown to display those properties.[3] We have been interested in the possibility that thionitrites RSNO might also serve as NO generators, since previous studies have shown that they spontaneously decompose in solution into the corresponding disulfide RSSR and nitric oxide.[4] However, most stable *S*-nitroso compounds, such as *tert*-butyl and trityl thionitrite and other alkylthionitrites, are completely insoluble in water.[5] Furthermore, water-soluble thionitrites are generally much too unstable and thus too difficult to prepare and purify. Thus, only few of these compounds, such as cysteine and glutathione derivatives, have been isolated.[6] The indefinite stability of *S*-nitroso-*N*-acetyl-D,L-penicillamine (SNAP) as green crystals allows analysis by X-ray crystallography.[7] SNAP has actually been used as a generator of NO in biological studies. Finally, the cysteine residues of a number of proteins can be nitrosated.[8] The stability of *S*-nitrosocysteines within a polypeptide chain is remarkable compared to that of free *S*-nitrosocysteine.[9]

In the present report we describe the preparation and the characterization of new thionitrites from cysteamine and mercaptoethanol derivatives. We have studied their stabilities in aqueous solutions as a function of pH and shown that they spontaneously generate nitric oxide at physiological pH. Their properties make them convenient and useful NO generators in water.

Results

Preparation of Thionitrites. Several methods are available to transform a thiol into a thionitrite. For the preparation of water soluble thionitrites from cysteamine ($H_2NCH_2CRR'SH$) or mercaptoethanol derivatives ($HOCH_2CRR'SH$), we used the reaction with *tert*-butyl nitrite in water. The reaction, performed at room temperature with a slight excess (1.1 equiv) of *t*-BuONO, was quan-

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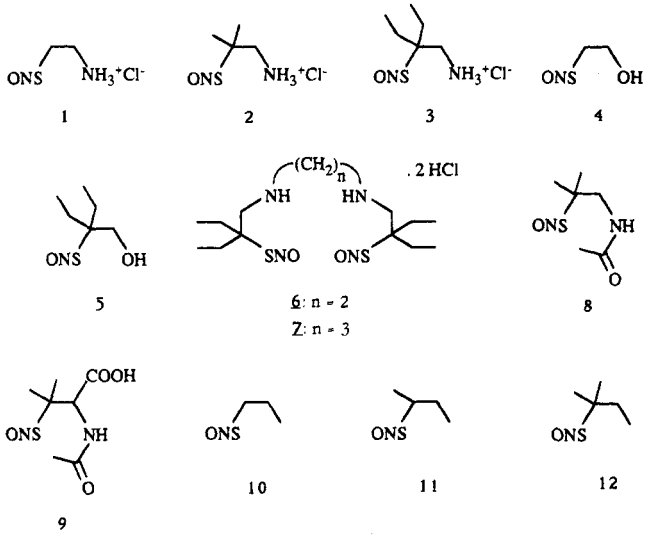

Figure 1. Structures of thionitrites **1**–**12**. The corresponding thiols and disulfides are labeled **A** and **B**, respectively.

titative. Because of their relatively low boiling points, *tert*-butyl nitrite and 2-methyl-2-propanol could be easily removed at the end of the reaction. When the amino group was acetylated, no thionitrite could be formed under these conditions; a larger excess of *tert*-butyl nitrite was required.[10] This was true with both *N*-acetyl-1-amino-2-methylpropan-2-thiol and *N*-acetyl-D,L-penicillamine. In both cases, the thionitrite could be obtained by reaction of the parent thiol with sodium nitrite in acidic solutions.[7] We also prepared propylthionitrites by reaction of the corresponding thiols with 1 equiv of *tert*-butyl nitrite in toluene at room temperature. The reaction was completed after 10 min, as shown by ^{1}H NMR spectroscopy. We did not find appropriate conditions for complete separation of the product from 2-methyl-2-propanol. Among the 12 compounds whose structures are depicted in Figure 1, only thionitrite **9** (SNAP) had been prepared and characterized previously.[7]

Thionitrites **2**, **3** (both amine hydrochloride salts), **8**, and **9** were obtained as stable green crystals in the pure form. Thionitrites **6** and **7**, also green solids, were obtained together with a small amount of disulfide because of the facilitated intramolecular reaction between the two SNO groups. Compound **1** cannot be stored as a solid. Red crystals of **1** decompose in a few minutes in air to the corresponding disulfide quantitatively with the formation of brown fumes of NO_2. Compound **1** can thus only be stored in acidic aqueous solutions as shown below. The other thionitrites shown in Figure 1 are red or green liquids.

Preparation of the Corresponding Thiols and Disulfides. Thiol precursors of compounds **2**, **3**, **5**–**7**, and **9** had never been prepared and characterized before. The common starting material was 2,2,5,5-tetraalkyl-(methyl or ethyl)-3,4-dithiahexane-1,6-dial[11] (Figure 2). Its reduction by $NaBH_4$[12] or $LiAlH_4$[13] afforded disulfide **5B** or monothiol **5A**, respectively. It should be mentioned that $NaBH_4$ is kinetically incapable of reducing the disulfide bond, probably because of steric hindrance. Reaction of the starting dialdehydes with *O*-methylhydroxylamine and subsequent reduction by BH_3/SMe_2 instead of diborane[14] afforded disulfides **2B** and **3B**. Thiols **2A** and **3A** were obtained during reduction of the bis oximes of **13** and **14** by $LiAlH_4$. For the preparation of disulfides **6B** and **7B**, the dialdehydes were transformed into cyclic diimines and reduced by $NaBH_4$.[15] Reduction to thiols by phenyltellurol, generated *in situ* by reduction of the corresponding ditelluride, in refluxing methanol/H_3PO_2 was found to be a very convenient and almost quantitative method. Diphenylselenol[16] was much less efficient and required refluxing ethanol. H_3PO_2 alone was not able to reduce the disulfides.

Characterization of Thionitrites. The presence of a SNO group in a molecule can be readily determined from both infrared and UV–vis spectroscopy.[17] The broad and strong IR band at 1480–1530 cm^{-1} has been assigned to the stretching vibration of the N=O bond of the thionitrite. N=O vibrations of tertiary thionitrites have been found at frequencies lower than those of primary thionitrites. A second absorption band at 600–730 cm^{-1} is characteristic of the vibration of the C–S bond.

The UV–vis spectra of the thionitrites display two bands at around 330–340 nm and 550–600 nm, which are responsible for their red or green color in solution. These bands are shifted to lower energies by substitution at the α carbon atom. Tertiary thionitrites are usually green compounds, whereas primary and secondary thionitrites are red.

Thionitrites have not been previously characterized by ^{1}H or ^{13}C NMR spectroscopy. Both methods allowed a very convenient and reliable proof for nitrosation of thiol compounds (Table 1). While thiols and disulfides had very similar ^{1}H NMR spectra, the α protons in the thionitrite were strongly shifted downfield (about 1 ppm). The β protons were also affected by the S-nitrosation; to an extent, that was dependent on the substitution at the α carbon. Almost no shift was observed for the primary thionitrites, about 0.1 ppm for the secondary thionitrites and 0.5–0.8 ppm for the tertiary thionitrites. It is quite remarkable that the four methylenic protons of *S*-nitrosomercaptoethanol (**4**) were magnetically equivalent, appearing in a single peak (Table 1). This equivalence was confirmed by ^{1}H–^{13}C COSY experiments and indicates that SNO and OH groups have comparable deshielding effects.

The ^{13}C NMR spectra were also changed by nitrosation of thiols. Resonances of α-carbon atoms were shifted downfield (7–13 ppm), with stronger shifts observed with tertiary thionitrites; and resonances of β-carbon atoms were shifted upfield (2–5 ppm). The ^{13}C NMR chemical shifts for thionitrites were in general found to be intermediate between those of the corresponding thiols and those of the disulfides (data not shown).

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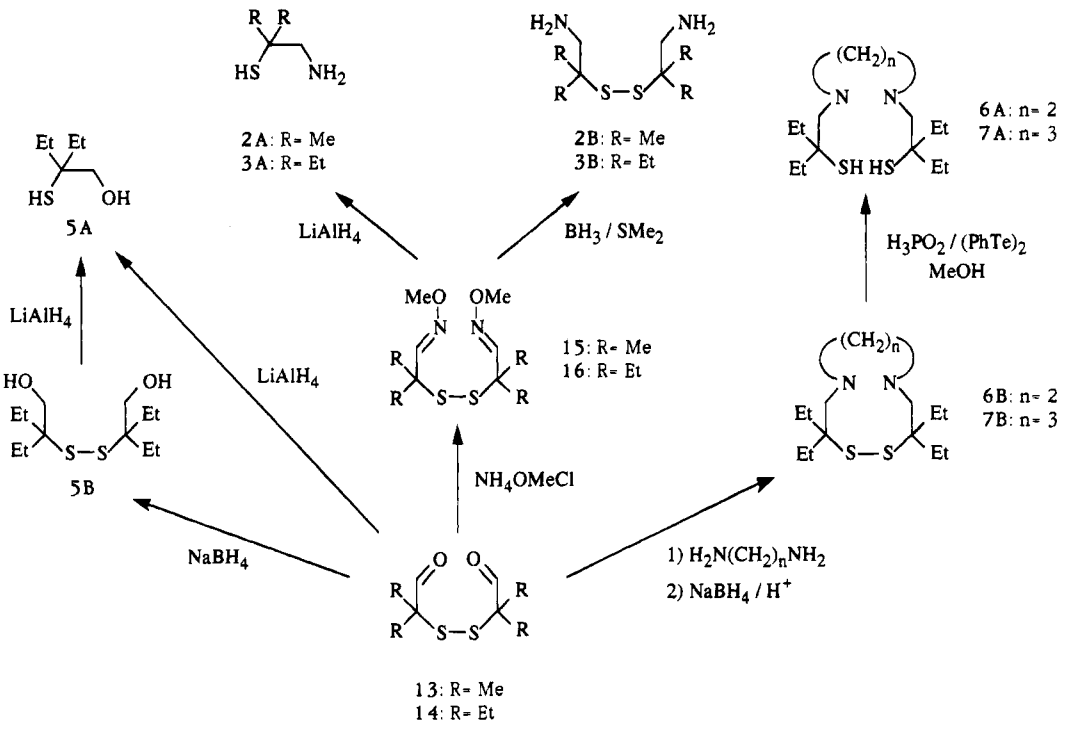
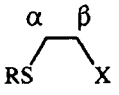


Figure 2. Synthesis of disulfides and thiols.

Table 1. ^1H and ^{13}C Chemical Shifts in ppm for Thiols and Their Corresponding Thionitrites[a]

compds	δ H_α	δ H_β	δ C_α	δ C_β
1A[c]	2.69	2.93	21.2	41.8
1[c]	3.96	2.99	30.3	37.6
3A[c]		2.91	50.0	47.1
3[c]		3.72	62.8	44.2
4A[b]	2.72	3.73	27.7	63.9
4[b]	3.71	3.71	35.6	60.5
5A[b]		3.49	54.9	69.0
5[b]		4.31	66.5	66.9
10A[b]	2.51	1.64	27.2	26.6
10[b]	3.49	1.59	34.8	22.4
11A[b]	2.87	1.57	37.3	33.8
11[b]	4.30	1.69	44.8	28.8
12A[b]		1.61	45.1	39.1
12[b]		2.19	57.2	35.9

[a] Compounds are labeled **A** for R = H and unlabeled for R = NO (see Figure 1). Protons and carbon atoms are labeled with respect to:



[b] $CDCl_3$. [c] DMSO-d_6.

Stability of the Thionitrites. Water soluble thionitrites (**1**–**4**, **6**–**9**) spontaneously decompose in aqueous buffer pH 7 at 37 °C, even in the dark. The reaction was monitored by UV–vis spectroscopy, since decomposition of thionitrites correlated with the bleaching of the solution and the disapppearence of the 330–340 and 500–550 nm bands. Results are expressed in terms of $t_{1/2}$, the half-life of the thionitrite at an initial concentration of 1.8 mM. Kinetic studies showed that the process was first order with respect to the substrates (data not shown).

Compounds found to decompose very rapidly at pH 7 could be manipulated during the initial dissolution and dilution phases of the kinetic studies by keeping the medium acidic and then mixing rapidly with excess pH 7 buffer to start the decomposition.

At neutral pH, the rate of decomposition was found to be very much affected by substitution at both the α and the β carbon atoms, as shown in Table 2. Tertiary thionitrites (**2** and **3**) were more stable than primary thionitrites (**1**). This phenomenon was also observed with water-insoluble propylthionitrites. Half-lives of 36 mM **10**, **11**, and **12** in toluene at 60 °C were 51, 96, and 303 min, respectively. Furthermore, the stability drastically increased when the ammonium group on the β carbon was changed to a hydroxyl or an acetamido group (compare **1** to **4** and **2** to **8** and **9**). SNAP, the well-known NO generator, had a $t_{1/2}$ value of 6 h and thus decomposed only very slowly, even though the extra carboxyl group on the β carbon had a destabilizing effect (compare **8** and **9**). Compounds **6** and **7** instantaneously decomposed in solution at neutral pH. It is important to note that a careful comparison of a variety of thionitrites in terms of their stability absolutely requires that experiments be run under similar conditions (temperature, aeration, buffer preparations). Thionitrite decomposition may indeed be dependent on a number of parameters, including oxygen tension,[18] the presence of metal impurities,[19] light, etc.

Table 2. Effects of Substituents at α and β Carbons on the Stability of Thionitrites, at pH 7.0[a]

thionitrite	$t_{1/2}$	thionitrite	$t_{1/2}$
1	5 min	**8**	40 h
2	12 min	**9**	5 h
4	44 h		

[a] Thionitrites (1.8 mM) were dissolved in 1.5 mL of potassium phosphate buffer 50 mM, pH 7.0, in a spectroscopic cuvette. The absorbance at 333 nm (primary thionitrites) or 339 nm (tertiary thionitrites) was recorded at time intervals. Results are expressed in terms of $t_{1/2}$, the half-life of the compound. The reaction was carried out at 37 °C.

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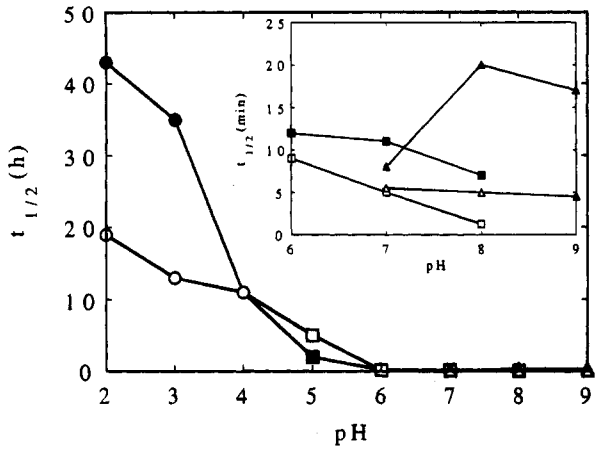

Figure 3. Dependence of thionitrite decomposition on pH. The experiments were carried out with compounds **1** (open symbols) and **3** (closed symbols) as described in Table 2, except for the buffer. Buffers were 50 mM citrate between pH = 2 and pH = 4 (○, ●), 50 mM potassium phosphate between pH = 5 and pH = 8 (□, ■), 50 mM Tris-Cl between pH = 7 and pH = 9 (△, ▲). Results are expressed in terms of half-life of the compound.

Rates of decomposition were also dependent on the pH of the aqueous solution (Figure 3). Compounds **1** and **3**, with $t_{1/2}$ values of 5 and 12 min in pH 7 phosphate buffer, respectively, were greatly stabilized at acidic pH. At pH 4, $t_{1/2}$ values were about 12 h for both compounds. This stabilization allowed the preparation of stable solutions of **1**, whose crystals are very labile. At high pH (>7), experiments are less reproducible because of the very fast decomposition of the thionitrites.

Generation of NO by Thionitrites. The reaction products were analyzed by ^{1}H NMR spectroscopy. Disulfides were the major products, with yields between 90 and 100%. The minor products were not identified. However, we did not find any evidence for the presence of the corresponding thiols. Nitrogen was recovered in the form of nitrite, assayed colorimetrically as described in the Experimental Section (data not shown). This suggested the expected intermediate formation of nitric oxide.[2]

The presence of NO was demonstrated qualitatively by low-temperature EPR spectroscopy, from the detection of HbNO, the nitrosyl–ferrohemoglobin complex, during decomposition of thionitrites in the presence of HbO_2, the oxyhemoglobin complex, an efficient trap of NO.[20] The observed three-line hyperfine EPR signal of the reaction mixture, shown in Figure 4, was identical to that of HbNO. No EPR signal could be detected when the thionitrite was replaced by the same concentration of sodium nitrite under identical conditions.

Quantitative analysis of NO formation during decomposition was made spectrophotometrically. The assay is based on the oxidation by NO of oxyhemoglobin to methemoglobin,[21] a reaction which is accompanied by a large modification of the visible spectrum. The kinetics of the reaction are shown in Figure 5: 21 μM HbO_2, i.e., 84 μM oxyheme, were almost completely oxidized by 82 μM compound **1** (80% after 1 h reaction). Controls

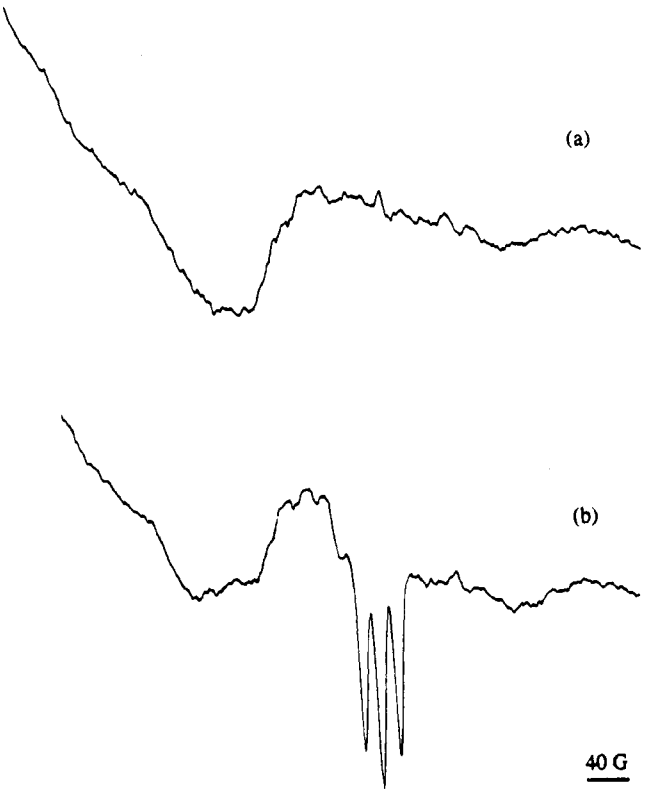

Figure 4. Trapping of NO by oxyhemoglobin during decomposition of **3**. Solutions contained 0.72 mM HbO_2 in 50 mM phosphate buffer, pH 6.5, in the absence (a) or in the presence (b) of 20 mM thionitrite **3**. After 30 s incubation the solutions were frozen in liquid nitrogen, and EPR spectra were recorded at 110 K. Instrument conditions: microwave power, 10 mW; modulation amplitude, 3.2 G.

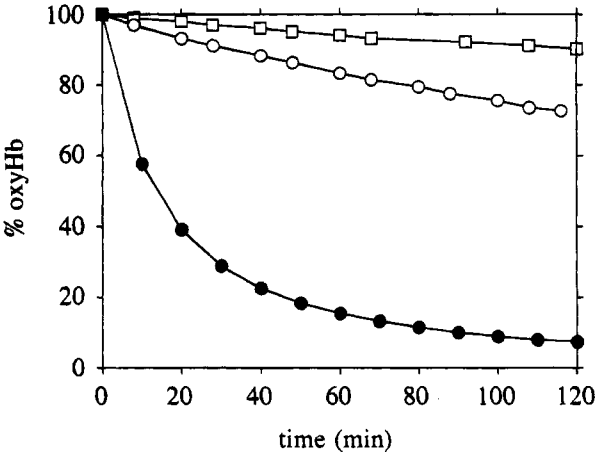

Figure 5. Oxidation of HbO_2 in the absence (□) or in the presence (●) of thionitrite **1** or in the presence of sodium nitrite (○) monitored spectrophotometrically as described in the Experimental Section. The solution contained initially HbO_2 (21 μM), thionitrite **1**, or $NaNO_2$ (82 μM) in 10 mM phosphate buffer pH 6.5 at 25 °C.

without thionitrite or with the same concentration of sodium nitrite showed a much slower oxidation of HbO_2 (15% after 1 h for the reaction with NO_2^-). This result is consistent with an almost quantitative decomposition of RSNO into NO.

The intermediate formation of NO suggested that the reaction was proceeding through the homolytic cleavage of the S–N bond, thus generating a thiyl intermediate radical RS$^\bullet$. Such radicals are very unstable and cannot

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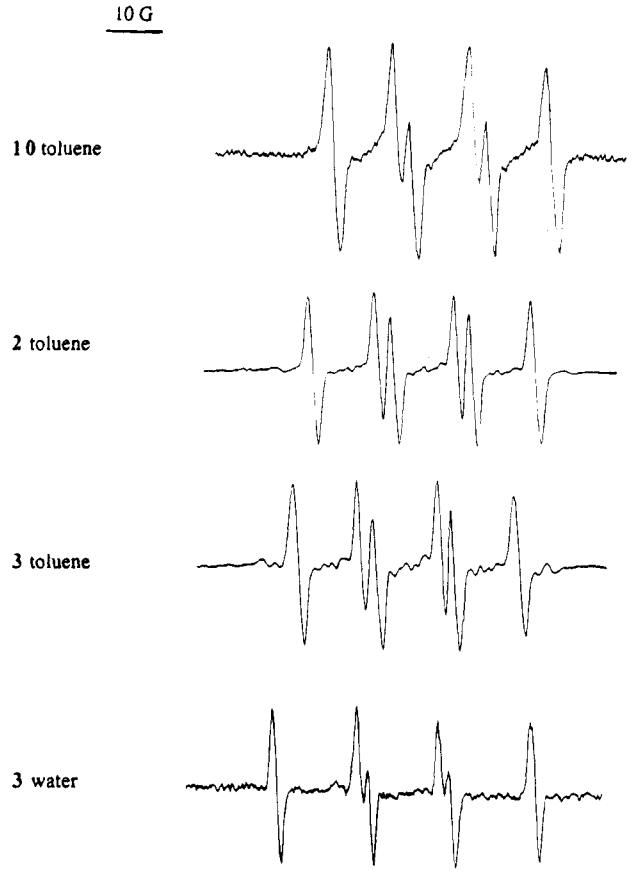


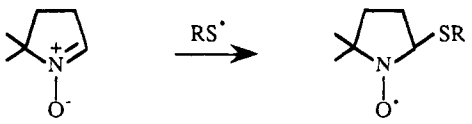
Figure 6. EPR spectra of DMPO-SR nitroxides during decomposition of compounds **2**, **3**, and **10**. The reaction mixture contained 0.2 M DMPO and 0.05–0.1 M thionitrite in 200 μL of toluene (compounds **2**, **3**, **10**) or water (compound **3**). EPR conditions: rt, microwave power 15 mW; modulation amplitude 0.5 G.

Table 3. Hyperfine Splitting Constants of Thiyl Radical DMPO Spin Adducts[a]

thionitrite	solvent	a_N (G)	a_H (G)
2	toluene	13.9	11.3
3	toluene	13.9	11.3
3	water	14.5	16.6
10	toluene	14.0	11.3

[a] Experiments were carried out as described in Figure 6.

be detected by EPR spectroscopy unless they are transformed into more stable radicals, for example, by reaction with a spin trap agent such as 5,5-dimethyl-1-pyrroline *N*-oxide (DMPO). Figure 6 shows EPR spectra of reaction mixtures recorded at room temperature during decomposition of thionitrites **2**, **3**, and **10** in the presence of a large excess of DMPO. They display signals characteristic of DMPO-SR free radicals derived from the addition of thiyl RS$^\bullet$ radicals to DMPO[22] (eq 1).



(1)

The hyperfine splitting constants a_N and a_H (Table 3) and, consequently, the shape of the signals (Figure 6) were dependent on the substitution at the α carbon atom of the thionitrite and also on the reaction medium. They were larger in water than in toluene because of the larger relative dielectric constant of the former.[23]

Discussion

Substituted cysteamines and mercaptoethanols RSH can serve as sources of rather stable, water-soluble thionitrites RSNO. Reaction with *tert*-butyl nitrite gives quantitative yields of thionitrites, without nitrosation of other reactive groups in the molecule such as amines or alcohols. Since the reaction was carried out under rather acidic conditions (with most substrates as amine hydrochlorides in unbuffered water), the mechanism, previously investigated by Williams *et al.*,[24] is better described by the following equations:

$$RONO + H_2O \rightleftharpoons ROH + HNO_2 \quad (2)$$

$$HNO_2 + H^+ \rightleftharpoons H_2NO_2^+ \quad (3)$$

$$RSH + H_2NO_2^+ \rightleftharpoons RSNO + H_2O + H^+ \quad (4)$$

The direct nucleophilic attack of thiols on *tert*-butyl nitrite is also possible but would require more basic conditions.

As solids or liquids, thionitrites can be prepared in most cases in pure and stable form. ^{1}H and ^{13}C NMR spectroscopies have proven to be excellent probes for the nitrosation of thiols, since chemical shifts of thionitrites are significantly different from those of both the corresponding thiols and disulfides. The greater electronegativity of the *S*-nitroso group, which is comparable to that of an OH group, has a significant proton deshielding effect.

In dilute, neutral, aqueous solutions, RSNO compounds spontaneously decompose, giving rise to the corresponding disulfide RSSR and nitrite ions with quantitative yields. During the reaction two intermediate unstable free radicals, nitric oxide and the thiyl radical RS$^\bullet$, have been detected by EPR spectroscopy using specific spin traps. Quantitative amounts of NO could be trapped. This result strongly suggests that the reaction mechanism involves a homolytic cleavage of the S–N bond, as depicted in eq 5. The resulting thiyl radicals then

$$2RSNO \rightarrow 2RS^\bullet + 2NO \quad (5)$$

$$2RS^\bullet \rightarrow RSSR \quad (6)$$

$$4NO + O_2 + 2H_2O \rightarrow 4H^+ + 4NO_2^- \quad (7)$$

combine to generate the disulfide, while NO is oxidized into nitrite[2] (eqs 6 and 7). Such a mechanism has been previously demonstrated for other thionitrites under different conditions.[4] Decomposition of the thionitrite through a hydrolytic pathway (reverse eq 4) may be ruled out on the basis of the great stability of thionitrites under acidic conditions.

The stability of water soluble thionitrites **1**–**4** and **6**–**9** depends very much on the substituents at the α and β

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carbon atoms and on the pH. This dependence can be rationalized in terms of the relative weight of the two resonance structures of the SNO functionality, depicted in eq 8.

$$\underset{\text{form I}}{RSN{=}O\lambda} \leftrightarrow \underset{\text{form II}}{RS^{+}{=}NO^{-}\lambda} \qquad (8)$$

Form II, with a double bond between the sulfur and the nitrogen atom, will provide strength to the S–N bond and prevent homolytic cleavage. Its contribution to the actual electronic structure of the group should increase because of the favorable increasing electron release from the methyl groups, in going from primary to tertiary thionitrites. This expectation is in agreement with the greater proton deshielding effect of S-nitrosation on the tertiary thionitrites, with the lower frequencies of their N=O vibrations, and with their higher stability. Moreover, the contribution of II should also increase with increased H^+ concentrations, explaining the much higher stabilities observed at acidic pH. Finally, it may explain why the presence of a positively charged ammonium group at the β carbon has a destabilizing effect compared to an uncharged hydroxyl or acetamido substituent, since the repulsive electrostatic interaction with the sulfonium center will lower the relative weight of form II. One may also suggest that the acetamido substituent stabilizes the molecule by forming a 6-membered cyclic structure in which the carbonyl oxygen interacts with the electron deficient sulfur atom of form II.

It should be mentioned that many other factors may affect the half-life of a given thionitrite. Oxygen tension is one of those parameters.[18] Recently, McAninly et al. also demonstrated that metal impurities dramatically accelerate thionitrite decomposition.[19]

The high lability of NO in the gaseous or aqueous phase complicates its manipulation in biological systems. The need for prodrug forms of NO is thus greatly increasing. The new compounds studied here generate quantitative amounts of NO in water at physiological pH spontaneously, without redox activation, as expected for thionitrites. Some are stable during storage in a water-soluble solid or liquid form. We show that the rate at which NO is released can be finely adjusted over a wide range by simple chemical modifications at both α an β carbon atoms. Half-lives vary from seconds to hours. It is important to note that *S*-nitroso-*N*-acetyl-D,L-penicillamine (SNAP), a thionitrite widely used as a NO donor in biological studies, is in fact very stable under physiological conditions, with a half-life of several hours. We would like to suggest that, consequently, not all effects of SNAP previously observed can be ascribed only to NO release, especially when assays were carried out over a short period of time. Moreover, that only one decomposition product, the corresponding well-characterized disulfide, is formed during the reaction limits the number of control experiments that have generally to be carried out in biological studies. All these advantages clearly make thionitrites very convenient NO generators, which will be useful in elucidating the important pharmalogical and physiological actions of NO.

Experimental Section

All melting and boiling points were uncorrected. ^{1}H NMR data were obtained at 200 and 300 MHz and ^{13}C NMR data at 50 and 75 MHz. Chemical shifts values are reported in δ (ppm) relative to TMS or to the residual peak of the solvent. ^{13}C NMR peak assignements were confirmed by DEPT experiments. EPR spectra were recorded on a Varian E102 spectrometer operating at 9.2 GHz. Elemental analyses were obtained from the Service Central de Microanalyse (CNRS, Vernaison, France). In some cases, analyses for nitrogen were unsatisfactory. Consequently, supplementary material (^{1}H and ^{13}C NMR spectra) is being made available. TLC was performed by using DC Alufolien Kieselgel $60F_{254}$ and visualized by spraying phosphomolybdic acid and Ellman's reagent for thiol compounds. All solvents were purified before use by standard procedure. *N*-Acetyl-D,L-penicillamine was obtained from Sigma. *t*-BuONO (90% or 98%), S_2Cl_2, $MeONH_3Cl$, and DMPO were purchased from Aldrich. Cysteamine hydrochloride and BH_3/SMe_2 were obtained from Fluka. DMPO was distilled under reduced pressure. The organic extracts were dried over anhyd Na_2SO_4. All reactions were performed under an efficient hood. Oxyhemoglobin solution was prepared by hemolyzing fresh human blood with 50 mM phosphate buffer, pH 6.5. The concentration was determined from the absorption coefficient: λ max (nm); ϵ ($mM^{-1}\cdot cm^{-1}$) 541 (13.5); 576 (14.6).[25]

2,2,5,5-Tetramethyl-3,4-dithiahexane-1,6-dial (13).[11a] Caution! Since poisonous CCl_4 and S_2Cl_2 are used and HCl is evolved, an efficient fumehood, proper gloves, and eye protection should be used. Fresly distilled S_2Cl_2 (135.0 g, 1.0 mol) was added dropwise to a solution of freshly distilled 2-methylpropanal (144.0 g, 2.0 mol) in 140 mL of dry CCl_4 kept at 50–55 °C. After a short lag phase, evolution of gas started (HCl). The mixture was stirred for 2 h at 60–65 °C and then for 16 h at 50 °C. The remaining HCl was then displaced by bubbling dry nitrogen into the solution (10 h). After concentration, the brown stinking oil was distilled twice to gave an oil, which crystallized in the cold (144.0 g, 0.7 mol, 72%): bp 78 °C (0.06 mmHg); ^{1}H NMR ($CDCl_3$) δ 1.37 (s, 12 H), 9.08 (s, 2H); ^{13}C ($CDCl_3$) δ 20.7, 56.5, 194.6; IR (net) 1360, 1385, 1720, 2700 cm^{-1}.

2,2,5,5-Tetraethyl-3,4-dithiahexane-1,6-dial (14).[11b] Compound **14** was prepared by means of the above procedure from 200.0 g (2.0 mol) of 2-ethylbutanal in 140 mL of CCl_4 and 135.0 g (1.0 mol) of S_2Cl_2. Crystallization from EtOH afforded 184.3 g of white crystals (0. 70 mol, 70%) mp 71–72 °C (lit.[9b] mp 72 °C); ^{1}H NMR (Me_2SO-d_6) δ 0.91 (t, J = 7.4 Hz, 12H), 1.73 and 1.78 (m, ABX_3 pattern, J^2_{AB} = 15 Hz, $J^3_{AX} = J^3_{BX}$ = 7.4 Hz, 8H), 9.09 (s, 2H); ^{13}C NMR (Me_2SO-d_6) δ 8.1, 21.7, 65.5, 194.8; IR (net) 1360, 1385, 1720 cm^{-1}. Anal. Calcd for $C_{12}H_{22}O_2S_2$: C, 54.91; H, 8.45; S, 24.43. Found: C, 54.89; H, 8.40; S, 24.21.

2,2,5,5-Tetraethyl-3,4-dithiahexane-1,6-diol (5B). A solution of dialdehyde **14** (10.48 g, 40 mmol) in 100 mL of absolute EtOH was cooled to 0 °C, and $NaBH_4$ (6.08 g, 160 mmol) was added portionwise. After 1 h of stirring, the mixture was carefully treated with cooled 6 N HCl and then poured into 500 mL of chilled water. After extraction with Et_2O (6 × 30 mL), the combined extracts were washed with brine (3 × 20 mL), dried, and concentrated to gave the crude diol, which was purified by recrystallization from hexane with traces of ethanol to afford a white microcrystalline solid (9.85 g, 37 mmol, 92%): mp 54–55 °C; R_f(ether) 0.60; ^{1}H NMR (Me_2SO-d_6) δ 0.82 (t, J = 7.3 Hz, 12H), 1.43 (q, J = 7.3 Hz, 8H), 3.32 (d, J = 5.3 Hz, 4H), 4.69 (t, J = 5.3 Hz, 2H); ^{13}C NMR (Me_2SO-d_6) δ 8.0, 25.3, 57.9, 63.3. Anal. Calcd for $C_{12}H_{26}O_2S_2$: C, 54.09; H, 9.83. Found: C, 54.09; H, 9.86.

2-Ethyl-1-hydroxybutane-2-thiol (5A). To a cooled suspension of $LiAlH_4$ (6.0 g, 157 mmol) in 80 mL of dry Et_2O was added over a period of 1 h a solution of dialdehyde **14** (13.0 g, 50 mmol) in 100 mL of dry Et_2O. The mixture was slowly warmed to rt and then refluxed for 24 h. The mixture was cooled to 0 °C, and then AcOEt (10 mL) and MeOH (20 mL) were added. The mixture was poured on crushed ice, and HCl (12 N) was added to reach pH 1. After extraction with Et_2O (5 × 50 mL), the combined extracts were washed successively with water (2 × 10 mL) and brine (3 × 20 mL) and then dried. After removal of Et_2O, 12.78 g of almost pure oily thiol **5A**

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was obtained (47 mmol, 95%): R_f (ether) 0.85; ^{1}H NMR (Me_2SO-d_6) δ 0.85 (t, J = 7.3 Hz, 6H), 1.51 (q, J = 7.3 Hz, 4H), 3.32 (s, 2H); ^{13}C NMR (Me_2SO-d_6) δ 8.4, 29.9, 53.2, 66.9; IR 2550, 2900–3400 cm^{-1}. Anal. Calcd for $C_6H_{14}OS$: C, 53.68; H, 10.51. Found: C, 54.19; H, 10.50.

General Procedure for the Preparation of Tetraalkyldiamino Disulfides. To a solution of the appropriate dialdehyde (10 mmol) and $MeONH_3Cl$ (3.34 g, 40 mmol) in absolute EtOH (50 mL) was added an aqueous solution of NaOH (2.6 N, 15 mL). The mixture was refluxed for 6 h, cooled in a ice bath, and then poured in 200 mL of water. The product was extracted with Et_2O (4 × 20 mL), dried, and concentrated to afford the crude bis-*O*-methyloxime as an oil, which was carefully dried.

To a solution of the bis-*O*-methyloxime derivative (1 mmol) in dry THF (50 mL) was added a solution of BH_3/SMe_2 in THF (2 M, 20 mL). The mixture was refluxed for 15 h, and MeOH (10 mL) was carefully added. Then a solution of MeOH saturated with HCl (20 mL) was added. After 1 h reflux, the solvents were removed. The residue was dissolved in an aqueous solution of HCl (5%, 50 mL) and extracted with Et_2O (3 × 10 mL); ammonia (30% in water) was then added (pH = 12.5–13), and the aqueous layer was extracted with CH_2Cl_2 (3 × 15 mL). The CH_2Cl_2 extract was dried and concentrated. The oily product was converted to the hydrochloric salt by dissolving it in a solution of EtOH (50 mL) and HCl (12 N, 8 mL) and evaporating it to dryness. The product was crystallized from the minimum amount of ethanol (yield 85–90%).

2,2,5,5-Tetramethyl-3,4-dithiahexane-1,6-dial *O*-methyloxime (15): ^{1}H NMR ($CDCl_3$) δ 1.15 (s, 12H), 3.7 (s, 6H), 7.15 (s, 2H); IR (film) 1060, 1720, 2800 cm^{-1}.

2,2,5,5-Tetramethyl-3,4-dithiahexane-1,6-diamine dihydrochloride (2B): mp 269–274 °C dec; ^{1}H NMR (Me_2SO-d_6) δ 1.33 (s, 12H), 2.87 (s, 4H), 8.38 (brs, 6H); ^{13}C NMR (Me_2SO-d_6) δ 25.4, 47.7, 50.1.

2,2,5,5-Tetraethyl-3,4-dithiahexane-1,6-dial *O*-methyloxime (16): ^{1}H NMR ($CDCl_3$) δ 0.89 (t, J = 7.4 Hz, 12H), 1.70 (q, J = 7.4 Hz, 8H), 3.86 (s, 6H), 7.14 (s, 2H); ^{13}C NMR ($CDCl_3$) δ 8.3, 25.9, 57.9, 61.6, 152.9; IR (film) 1060, 1720, 2860, 2880 cm^{-1}.

2,2,5,5-Tetraethyl-3,4-dithiahexane-1,6-diamine dihydrochloride (3B): mp 192–200 °C dec; ^{1}H NMR (Me_2SO-d_6) δ 0.85 (t, J = 7.3 Hz, 12H), 1.63 (m, J = 7.3 Hz, 8H), 2.88 (brs, 4H), 8.37 (brs, 8H); ^{13}C NMR (Me_2SO-d_6) δ 7.7, 25.4, 43.0, 56.1.

General Procedure for the Preparation of (Dialkylamino)ethanethiols. To a solution of the appropriate dialdehyde (0.10 mol) and NH_3OCl (20.85 g, 0.30 mol) in 100 mL of absolute EtOH was added an aqueous solution of NaOH (15 N, 20 mL). The mixture was refluxed for 3 h, cooled in a ice bath, and then poured in 500 mL of distilled water. The product was extracted with Et_2O (4 × 30 mL) and then dried. The solvents were evaporated to afford the crude bisoxime which was carefully dried over P_2O_5.

To a suspension of $LiAlH_4$ (6.00 g, 0.15 mol) in dry THF (150 mL) was added a solution of the appropriate bis-oxime (0.05 mol) in 100 mL of dry THF. After 15 h reflux under argon, the mixture was cooled to 0 °C. The excess of $LiAlH_4$ was destroyed carefully by adding 10 mL of water and 38.00 g (0.71 mol) of dry NH_4Cl. The clear suspension obtained was filtered over a pad of Celite and dried. After concentration, the oil was dissolved in 50 mL of chilled EtOH and acidified with concentrated HCl. After evaporation to dryness the white solid was crystallized in EtOH–AcOEt to give the pure thiol as a colorless solid (yield 82–85%).

1-Amino-2-methylpropane-2-thiol hydrochloride (2A): ^{1}H NMR (Me_2SO-d_6) δ 1.35 (s, 6H), 2.92 (s, 2H), 8.23 (brs, 3H); ^{13}C NMR (Me_2SO-d_6) δ 29.4, 42.0, 51.6; MSCI (NH_3) m/e 122 (M + NH_3), 105 (M), 71 (M − H_2S); IR (KBr pellet) 1480, 2500, 2560, 2800–3200 cm^{-1}. Anal. Calcd for $C_4H_{12}NSCl$: C, 33.92; H, 8.48; N, 9.89; S, 22.60. Found: C, 33.89; H, 8.41; N, 9.79; S, 23.

1-Amino-2-ethylbutane-2-thiol hydrochloride (3A): mp 191–200 °C dec; ^{1}H NMR (Me_2SO-d_6) δ 0.86 (t, 6H), 1.58 (q, 4H), 2.91 (s, 2H), 3.34 (s, 1H), 8.06 (brs, 3H); ^{13}C NMR (Me_2SO-d_6) δ 8.2, 29.7, 47.1, 50.0; MS m/e 134 (M^+ + 1 − HCl), 117 (M^+ + 1 − HCl − NH_3), 101 (M^+ + 1 − HCl − NH_3 − SH); IR (KBr pellet) 1480, 2580, 2800–3000 cm^{-1}.

***N*-Acetyl-1-amino-2-methylpropane-2-thiol (8A).** To a suspension of thiol **2A** (1.41 g, 1.0 mmol) in dry AcOEt (45 mL) were successively added 2.95 mL of Et_3N and 0.96 mL (1.02 g, 1.0 mmol) of Ac_2O under argon. The mixture was left at rt for 1 h and then poured into HCl (1%, 100 mL). The organic layer was dried and the residue chromatographed over silica gel with CH_2Cl_2–EtOH as eluant to afford 1.15 g (0.78 mmol, 78%) of pure thiol **8A**: ^{1}H NMR ($CDCl_3$) δ 1.19 (s, 6H), 1.61 (s, 1H), 2.07 (s, 3H), 3.32 (d, J = 6.1 Hz, 2H), 5.81 (NH, brt, J = 6.1 Hz, 1H); ^{13}C NMR ($CDCl_3$) δ 23.3, 25.8, 48.0, 50.1. Anal. Calcd for $C_6H_{13}NOS$: C, 48.97; H, 8.84; N, 9.52. Found: C, 48.95; H, 8.79; N, 9.45.

General Procedure for the Cyclic Diamino Disulfides. To a vigorously stirred solution of tetraalkyl dialdehyde (40 mmol) in 200 mL of absolute EtOH was rapidly added an ethanolic solution (50 mL) of the appropriate diamine (40 mmol). The reaction mixture was refluxed for 4 h and then cooled to 0 °C. $NaBH_4$ (10.0 g) was added, and the solution was kept at rt for 2 h. After the solution was cooled to 0 °C, concentrated HCl was added dropwise until the pH reached 1. The mixture was diluted with water (600 mL) and extracted with Et_2O (3 × 50 mL). Then the aqueous layer was basified with ammonia (30% in water) to pH 12.5. This solution was extracted with CH_2Cl_2 (3 × 50 mL). The CH_2Cl_2 layer was washed with brine, dried, and evaporated to give the almost pure disulfide.

5,8-Diaza-3,3,10,10-tetraethyl-1,2-dithiacyclodecane (6B). A 10.48 g (40 mmol) portion of dialdehyde **14** and 2.4 g (40 mmol) of ethylenediamine afforded 10.44 g (36 mmol, 90%) of the diamine disulfide as a colorless oil: R_f (CH_2Cl_2/hexane/33% Me_2NH in ethanol, 18/3/1) 0.33; ^{1}H NMR ($CDCl_3$) δ 0.89 (t, J = 7.3 Hz, 12H), 1.46–1.66 (m, 8H), 1.84 (brs, 2H), 2.57 and 2.96 (2 d, J = 12.6 Hz, AB pattern, 2 × 2H), 2.76 (s, 4H); ^{13}C NMR ($CDCl_3$) δ 7.8, 8.0, 26.2, 27.6, 46.6, 54.8, 59.3; FABMS (glycerol) m/e 291 (M^+ + 1); IR (film) 1380, 1460, 3300 cm^{-1}.

5,9-Diaza-3,3,11,11-tetraethyl-1,2-dithiacycloundecan Dihydrochloride (7B). A 10.48 g (40 mmol) portion of dialdehyde **14** and 2.96 g (40 mmol) of 1,3-diaminopropane afforded 10.70 g (35 mmol, 87%) of the diaminodisulfide as a colorless oil. The hydrochloric salt was obtained by dissolving the oil in 50 mL of EtOH, adding 10 mL of concentrated HCl, and evaporating to dryness: mp 222–236 °C dec; ^{1}H NMR (D_2O) δ 0.88 (t, J = 7.4 Hz, 12H), 1.43–1.81 (m, 10H), 2.64–2.89 (m, 8H); ^{13}C NMR (D_2O) δ 7.8, 8.0, 23.7, 26.5, 27.5, 50.8, 55.4, 57.6; FABMS (glycerol) m/e 305 (M^+ + 1); IR (free amine, film) 2800, 2950, 3300 cm^{-1}.

General Procedure for the Reduction of the Cyclic Diamino Disulfides to the Corresponding Diamino Dithiols. To a solution of cyclic disulfide (7.00 mmol) and diphenyl ditelluride (5 mg, 0.01 mmol) in 50 mL of MeOH under argon was injected H_3PO_2 (50%in water, 5 mL). After 10 h of reflux, the mixture was cooled in an ice bath, acidified with HCl (8 N, 2 mL), and then diluted with 200 mL of chilled water. This solution was extracted with Et_2O (3 × 30 mL). The aqueous layer was basified to pH 13 with an ammonia solution (28%) and then extracted with Et_2O (3 × 50 mL). These last combined extracts were dried and concentrated to give an oil. The hydrochloric salt was obtained by dissolving the oil in 10 mL of EtOH, adding 1 mL of concentrated HCl, and evaporating to dryness.

5,8-Diaza-3,10-diethyldodecane-3,10-dithiol Dihydrochloride (6A). From 2.03 g (7.00 mmol) of 5,8-diaza-3,3,10,10-tetraethyl-1,2-dithiacyclodecane was obtained 2.31 g (6.33 mmol, 90%) of dithiol. Crystallization in EtOH afforded beautiful shiny crystals of **6A**: mp 191–197 °C dec; ^{1}H NMR (free amine, $CDCl_3$) δ 0.93 (t, J = 7.0 Hz, 12H), 1.67 (q, J = 7.0 Hz, 8H), 2.63 (s, 4H), 2.75 (s, 4H), 1.5 (brs, 4H); FABMS (glycerol) m/e 293 (M^+ + 1−2HCl); IR (free amine, film) 2540, 3300 cm^{-1}. Anal. Calcd for $C_{14}H_{34}N_2S_2Cl_2$: C, 46.00; H, 9.37; N, 7.66; S, 17.54; Cl, 19.40. Found: C,45.90; H, 9.66; N, 7.55; S, 17.64; Cl, 19.54.

5,9-Diaza-3,11-diethyltridecane-3,11-dithiol Dihydrochloride (7A). From 2.13 g (7.00 mmol) of 5,9-diaza-3,3,11,11-tetraethyl-1,2-dithiacycloundecane was obtained 2.52 g

(6.60 mmol, 94%) of a microcrystalline white powder. Crystallization of **7A** was achieved in a minimum amount of 2-propanol: ^{1}H NMR (D_2O) δ 0.94 (t, J = 7.4 Hz, 12H), 1.69 (q, J = 7.4 Hz, 8H), 2.22–2.31 (m, 2H), 3.25 (t + s, J = 8.3 Hz, 2 × 4H); FABMS (glycerol) m/e 307 (M^+ + 1−2HCl); IR (free amine, film) 2540, 3300 cm^{-1}. Anal. Calcd for $C_{15}H_{36}N_2S_2Cl_2$: C, 47.49; H, 9.50; N, 7.40; S, 16.88; Cl, 18.73. Found: C, 47.38; H, 9.51; N, 7.37; S, 16.95; Cl, 18.78.

General Procedure for S-Nitrosation of Thiols. Method A. Nitrosation with *t*-BuONO. **Method B.** Nitrosation with $NaNO_2$ in acidic medium as previously described by Field.[7]

Thionitrite 1. To an aqueous solution (1 mL) of cysteamine hydrochloride (12.50 mg, 0.11 mmol) was added *t*-BuONO (90%, 15 μL, 0.11 mmol) under an argon atmosphere. After 5 min, water (10 mL) was added to the red mixture (pH 4), and then the solution was concentrated to a final volume of 1 mL to remove *t*-BuOH and excess *t*-BuONO: UV max (H_2O) 333, 546 nm (ϵ 793, 15); ^{1}H NMR (Me_2SO-d_6) δ 2.99 (t, J = 7.0 Hz, 2H), 3.96 (t, J = 7.0 Hz, 2H), 8.43 (brs, 3H); ^{13}C NMR (Me_2SO-d_6) δ 30.3, 37.6; FABMS (glycerol) m/e 107 (M^+ + 1 − HCl).

Thionitrite 2. *t*-BuONO (90%, 309 μL, 2.33 mmol) was added to an aqueous solution (8 mL) of thiol **2A** (300 mg, 2.11 mmol) at rt under argon. The solution became green-red immediately. After 5 min of vigorous stirring, the mixture was concentrated (T < 35 °C) and Et_2O was added to the resulting green powder. The precipitate was filtrated, washed with ether, and dried *in vacuo* to afford pure **2** (336 mg, 1.96 mmol, 93%): UV max (MeOH) 342, 597 nm (ϵ 549, 12); ^{1}H NMR (Me_2SO-d_6) δ 1.93 (s, 6H), 3.64 (s, 2H), 8.54 (brs, 3H); ^{13}C NMR (Me_2SO-d_6) δ 26.3, 48.4, 54.6; FABMS (glycerol) m/e 135 (M^+ + 1 − HCl), 118 (M^+ + 1 − HCl − NH_3), 105 (M^+ + 1 − HCl − NO); IR (KBr pellet) 630, 665, 675, 1500 cm^{-1}. Anal. Calcd for $C_4ClH_{11}N_2OS$: C, 28.15; H, 6.50. Found: C, 28.43; H, 6.63.

Thionitrite 3. By means of method A, from 300 mg (1.76 mmol) of thiol **3A** and 244 μL (90%, 1.84 mmol) of *t*-BuONO was obtained 284 mg (1.43 mmol, 81%) of thionitrite **3** as a green powder: UV max (MeOH) 344, 599 nm (ϵ 540, 11); ^{1}H NMR (Me_2SO-d_6) δ 0.98 (t, J = 7.4 Hz, 6H), 2.26 (q, J = 7.4 Hz, 4H), 3.72 (s, 2H), 8.43 (brs, 3H); ^{13}C NMR (Me_2SO-d_6) δ 7.7, 27.0, 44.2, 62.8; FABMS (glycerol) m/e 163 (M^+ + 1 − HCl), 146 (M^+ + 1 − HCl − NH_3), 133 (M^+ + 1 − HCl − NO); IR (KBr pellet) 655, 665, 1500 cm^{-1}. Anal. Calcd for $C_6ClH_{15}SN_2O$: C, 36.27; H, 7.61. Found: C, 36.17; H, 7.81.

Thionitrite 6. The procedure was method A, except that 2.2 equiv of *t*-BuONO was used. Thionitrite **6** was obtained as a green powder contaminated with disulfide **6B** (10%): ^{1}H NMR (Me_2SO-d_6) δ 1.00 (t, J = 7.4 Hz, 12H), 2.34 (q, J = 7.4 Hz, 8H), 3.49 (brs, 4H), 3.92 (brs, 4H), 9.47 (brs, 4H).

Thionitrite 7. The procedure was the same as that described for compound **6**. Thionitrite **7** was obtained as a green powder with a small amount of disulfide **7B** (5%): ^{1}H NMR (Me_2SO-d_6) δ 0.99 (t, J = 7.3 Hz, 12H), 2.35 (q, J = 7.3 Hz, 8H), 3.07 (brs, 4H), 3.87 (brs, 4H), 4.04 (brs, 2H), 9.27 (brs, 2H).

Thionitrite 8. By means of method B, from 50 mg (0.34 mmol) of thiol compound **8A** and 35 mg (0.51 mmol) of $NaNO_2$ was obtained 45 mg (0.26 mmol, 75%) of thionitrite **8** as a green powder: ^{1}H NMR (Me_2SO-d_6) δ 1.81 (brs, 9H), 3.79 (d, J = 6.3 Hz, 2H), 8.18 (brs, 1H); ^{13}C NMR (Me_2SO-d_6) δ 22.4, 26.3, 48.1, 58.1, 169.4; FABMS (glycerol) m/e 177 (M^+ + 1); IR (KBr pellet) 670, 1485 cm^{-1}.

Thionitrite 9. Compound **9** was prepared by means of method B (77%): mp 150–151 °C (lit.[7] mp 153–154 °C); UV max (MeOH) 342, 595 nm (ϵ 825, 16); ^{1}H NMR (Me_2SO-d_6) δ 1.86 (s, 3H), 1.93 (s, 3H), 1.95 (s, 3H), 5.15 (q, J = 9.5 Hz, 1H), 8.49 (d, J = 9.5 Hz, 1H), 13.11 (s, 1H); ^{13}C NMR (Me_2SO-d_6) δ 22.2, 25.1, 26.2, 58.2, 59.1, 169.5, 170.7; FABMS (glycerol) m/e 219 (M^- − 1), 189 (M^- − 1 − NO); IR (KBr pellet) 665, 670, 1480 cm^{-1}. Anal. Calcd for $C_7H_{12}SN_2O_4$: C, 38.17; H, 5.50. Found: C, 38.46; H, 5.57.

Thionitrites 4, 5, and 10–12. For NMR experiments, thiol compounds (0.18 mmol) were dissolved in 1 mL of $CDCl_3$ and were allowed to react with *t*-BuONO (98%; 0.18 mmol) at rt. After 10 min of stirring, ^{1}H and ^{13}C NMR spectra were recorded.

Thionitrite 4: ^{1}H NMR δ 3.71 (brs, 4H); ^{13}C NMR δ 35.6, 60.5.

Thionitrite 5: ^{1}H NMR δ 1.06 (t, J = 7.4 Hz, 6H), 1.90 (s, 1H), 2.27 (q, J = 7.4 Hz, 4H), 4.31 (s, 2H); ^{13}C NMR δ 8.0, 26.9, 66.5, 66.9.

Thionitrite 10: ^{1}H NMR δ 0.94 (t, J = 7.3 Hz, 3H), 1.59 (m, 2H), 3.49 (t, J = 6.8 Hz, 2H); ^{13}C NMR δ 13.2, 22.4, 34.8.

Thionitrite 11: ^{1}H NMR δ 0.92 (t, J = 7.3 Hz, 3H), 1.37 (d, J = 7.3 Hz, 3H), 1.69 (m, 2H), 4.30 (m, 1H); ^{13}C NMR δ 11.4, 19.6, 28.8, 44.8.

Thionitrite 12: ^{1}H NMR δ 1.04 (t, J = 7.4 Hz, 3H), 1.87 (s, 6H), 2.19 (q, J = 7.4 Hz, 2H); ^{13}C NMR δ 9.1, 28.4, 35.9, 57.2.

Nitric Oxide Titration. Nitric oxide concentrations were determined spectrophotometrically by monitoring the oxidation of oxyhemoglobin to methemoglobin in phosphate buffer pH 6.5 at 25 °C. In a typical reaction, thionitrite **1** (82 μM) was incubated in the 21 μM oxyhemoglobin solution, and OD_{578} and OD_{525}, the absorbances at 578 nm (maximum for HbO_2) and at 525 nm (isobestic point) were recorded over a period of 2 h. The percentage of remaining oxyhemoglobin was obtained from $R = OD_{578}/OD_{525}$. R references values corresponding to 100% and 0% HbO_2 were obtained from pure solutions of 21 μM oxyhemoglobin and methemoglobin, respectively.

Nitrite Assay. Nitrite concentrations were measured via diazotation of sulfanilamide and subsequent coupling with *N*-(1-naphthyl)ethylenediamine dihydrochloride to form the azo dye.[26] In a typical experiment, the thionitrite (25 mmol) was allowed to stand in a phosphate buffer 50 mM pH 7 (25 mL). After total decomposition of thionitrite, as judged by its UV–vis spectrum, the solution was diluted 10 times and nitrite was assayed.

Abbreviations: DMPO, 5,5-dimethyl-1-pyrroline *N*-oxide; EPR, electron paramagnetic resonance; HbO_2, oxyhemoglobin; HbNO, nitrosohemoglobin; metHb, methemoglobin; SNAP, *S*-nitroso-*N*-acetyl-D,L-penicillamine.

Supplementary Material Available: NMR spectra for obtained compounds (29 pages). This material is contained in libraries on microfiche, immediately follows this article in the microfilm version of the journal, and can be ordered from the ACS; see any current masthead page for ordering information.

(26) Bratton, A. C.; Marshall, E. K. *J. Biol. Chem.* **1939**, *128*, 537–550.