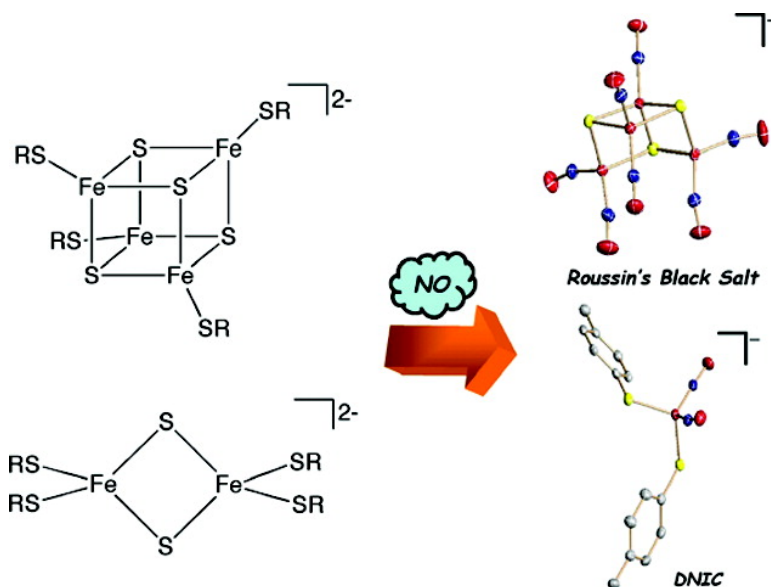


## Reactions of Synthetic [2Fe-2S] and [4Fe-4S] Clusters with Nitric Oxide and Nitrosothiols

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## Reactions of Synthetic [2Fe-2S] and [4Fe-4S] Clusters with Nitric Oxide and Nitrosothiols

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**Abstract:** The interaction of nitric oxide (NO) with iron–sulfur cluster proteins results in degradation and breakdown of the cluster to generate dinitrosyl iron complexes (DNICs). In some cases the formation of DNICs from such cluster systems can lead to activation of a regulatory pathway or the loss of enzyme activity. In order to understand the basic chemistry underlying these processes, we have investigated the reactions of NO with synthetic [2Fe-2S] and [4Fe-4S] clusters. Reaction of excess NO(g) with solutions of  $[\text{Fe}_2\text{S}_2(\text{SR})_4]^{2-}$  (R = Ph, *p*-tolyl (4-MeC<sub>6</sub>H<sub>4</sub>), or  $1/2$  (CH<sub>2</sub>)<sub>2</sub>-*o*-C<sub>6</sub>H<sub>4</sub>) cleanly affords the respective DNIC,  $[\text{Fe}(\text{NO})_2(\text{SR})_2]^-$ , with concomitant reductive elimination of the bridging sulfide ligands as elemental sulfur. The structure of  $(\text{Et}_4\text{N})[\text{Fe}(\text{NO})_2(\text{S-}i>p\text{-tolyl})_2]$  was verified by X-ray crystallography. Reactions of the [4Fe-4S] clusters,  $[\text{Fe}_4\text{S}_4(\text{SR})_4]^{2-}$  (R = Ph, CH<sub>2</sub>Ph, <sup>t</sup>Bu, or  $1/2$  (CH<sub>2</sub>)-*m*-C<sub>6</sub>H<sub>4</sub>) proceed in the absence of added thiolate to yield Roussin's black salt,  $[\text{Fe}_4\text{S}_3(\text{NO})_7]^-$ . In contrast,  $(\text{Et}_4\text{N})_2[\text{Fe}_4\text{S}_4(\text{SPh})_4]$  reacts with NO(g) in the presence of 4 equiv of (Et<sub>4</sub>N)(SPh) to yield the expected DNIC. For all reactions, we could reproduce the chemistry effected by NO(g) with the use of trityl-*S*-nitrosothiol (Ph<sub>3</sub>CSNO) as the nitric oxide source. These results demonstrate possible pathways for the reaction of iron–sulfur clusters with nitric oxide in biological systems and highlight the importance of thiolate-to-iron ratios in stabilizing DNICs.

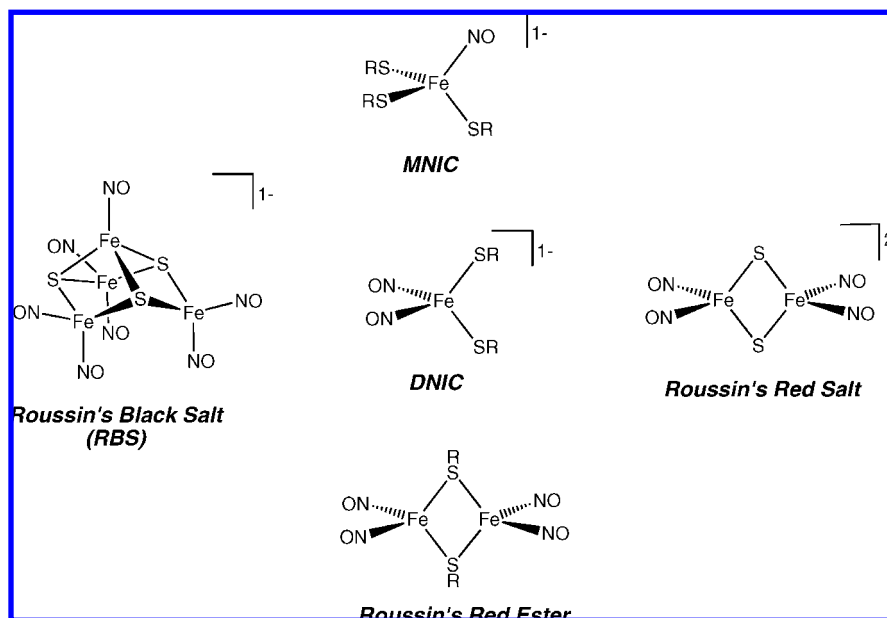
### Introduction

The physiological roles played by nitric oxide (NO) encompass a variety of important biological functions from smooth muscle relaxation<sup>1–3</sup> to neurotransmission<sup>4</sup> and the immune response.<sup>5</sup> Much of the biological chemistry of NO involves transition metal centers in the active sites of proteins.<sup>6,7</sup> Heme-iron, in particular,<sup>8,9</sup> has elicited a great deal of attention since its interactions with NO were first identified as playing a central role in the activation of guanylyl cyclase to form cGMP.<sup>10–12</sup> Recently, non-heme targets have been identified as potential NO reaction sites in biology.<sup>13</sup> For instance, NO may play a role in regulating intracellular iron levels by interaction with

ferric uptake regulatory protein (Fur)<sup>14,15</sup> and the NO-responsive transcription factor (NorR),<sup>16,17</sup> both of which contain non-heme iron centers. Nitric oxide has also been implicated in the disassembly of a variety of iron–sulfur proteins leading to the formation of dinitrosyl iron complexes (DNICs).<sup>18–20</sup> These DNICs are believed to take the form of tetrahedral  $[\text{Fe}(\text{NO})_2(\text{SR})_2]^-$  species, where  $\text{RS}^-$  may represent cysteinyl residues along the protein backbone<sup>21–23</sup> or mobile ligands such as glutathione.<sup>24</sup> The identity and assignment of these nitrosylated species are based on their observed  $g = 2.03$  electron paramagnetic resonance (EPR) signals, which are characteristic of sulfur-ligated dinitrosyl iron species. The potential for DNICs to serve as carriers of nitric oxide in vivo has generated renewed interest in these compounds, and several groups have demon-

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Chart 1. Common Iron–Sulfur Nitrosyl Compounds



strated the ability of synthetic DNICs to transfer their NO equivalents to other metal centers.<sup>25,26</sup>

Although the reactivity of NO with iron–sulfur proteins suggests that these sites represent pathological targets, recent work has also demonstrated a possible physiological role. The transcriptional regulator, SoxR, becomes activated when its [2Fe-2S] cluster component is degraded in the presence of NO to form a protein-bound DNIC.<sup>27</sup> Subsequent work demonstrated in vitro repair of [2Fe-2S] clusters from DNIC by treatment with L-cysteine in the presence of cysteine desulfurase.<sup>28</sup> Both NO and nitrosothiols inhibit cellular respiration by degradation of iron–sulfur clusters along the electron-transfer chain in mitochondria.<sup>29</sup> In certain instances this inhibition can be reversed by light, suggesting again the possibility for repair of nitrosylated clusters.<sup>30</sup>

Despite the growing body of literature demonstrating the reaction of NO with iron–sulfur cluster proteins, there remain few studies exploring the reactivity of NO with synthetic cluster analogues. Early work demonstrated that a variety of nitrosylated iron products form upon reaction of synthetic [2Fe-2S] and [4Fe-4S] systems with NO(g) and nitrite.<sup>31</sup> For example, reaction of an unspecified amount of NO(g) with a *N,N*-dimethylformamide (DMF) solution of the [4Fe-4S] analogue,  $[\text{Fe}_4\text{S}_4(\text{SR})_4]^{2-}$ , resulted in *no reaction* at room temperature. Brief heating of this reaction mixture led to the formation of a *mixture of paramagnetic mononuclear iron species* that were not completely characterized. The identity of R was not specified, further

complicating the interpretation of the reported results. Among the species proposed and identified solely on the basis of EPR *g*-values and coupling constants were DNICs containing DMF (the reaction solvent) ligands and  $[\text{Fe}(\text{NO})_2(\text{DMF})(\text{SR})]$ . Formation of the nitrosylated [4Fe-3S] cluster known as Roussin's black salt (RBS) resulted when these clusters were allowed to react with nitrite in aqueous alkaline medium followed by an acidic workup (Chart 1). More recent work reveals the formation of *S*-bound DNICs upon continuous purging of NO(g) through a heterogeneous tetrahydrofuran (THF) solution of a synthetic cluster containing polysulfide ligands,  $[(\text{S}_5)\text{Fe}(\mu\text{-S})_2\text{Fe}(\text{S}_5)]^{2-}$ .<sup>25</sup> The mechanism of DNIC formation and any intermediates along the reaction pathway, however, remain to be elucidated. Moreover, of the reported synthetic DNICs, few are produced by direct reaction of an iron–sulfur cluster precursor with NO. As illustrated by these examples, no systematic examination of the reaction of stoichiometric and controlled quantities of nitric oxide with both [2Fe-2S] and [4Fe-4S] clusters has appeared. Such information is necessary to provide a foundation for understanding the chemistry in biological systems when protein-bound iron–sulfur clusters encounter NO.

In order to address this need, we recently initiated a study of the reaction pathways of synthetic iron–sulfur compounds with nitric oxide. We began our investigations by examining the reactions of NO with mononuclear homoleptic iron–sulfur complexes of composition  $[\text{Fe}(\text{SR})_4]^{2-}$ .<sup>32,33</sup> The results of this work provided the first structurally and spectroscopically characterized mononitrosyl iron complexes (MNICs) of the type  $[\text{Fe}(\text{SR})_3(\text{NO})]^-$  as well as their analogous DNICs following stoichiometric addition of NO to  $[\text{Fe}(\text{SR})_4]^{2-}$  complexes. Subsequently, we have been investigating the reactions of several synthetic [2Fe-2S] and [4Fe-4S] clusters with NO and *S*-nitrosothiols. Here we report results of a systematic study that demonstrate the formation of DNICs as the predominant iron-containing products of nitrosylation

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in all cases and that reveal the ratio of thiolate to iron to be critical in trapping and stabilizing these species. These observations establish a general reactivity pattern of NO with such cluster systems, a necessary step toward elucidating the underlying mechanism of the cluster-to-DNIC transformation. The availability of reaction model studies from synthetic systems of this kind aids in the identification of reactive species and mechanistic pathways for nitrosylation of biological iron–sulfur cluster sites. Collectively, the experiments described here report the first detailed attempt to define such reaction pathways. The use of trityl-*S*-nitrosothiol as an organic-soluble, stoichiometric reagent for NO is also highlighted.

## Experimental Section

**General Considerations.** Manipulations of air- and moisture-sensitive materials were performed under an atmosphere of argon gas using standard Schlenk techniques, or in an MBraun glovebox under an atmosphere of purified nitrogen. All reagents were purchased from commercial suppliers and used as received unless otherwise noted. Acetonitrile, tetrahydrofuran, dichloromethane, diethyl ether, pentane, and toluene were purified by passage through activated alumina.<sup>34</sup> Methyl alcohol was distilled from Mg(OMe)<sub>2</sub> under N<sub>2</sub> and stored over 4 Å molecular sieves prior to use. Acetonitrile-*d*<sub>3</sub> was dried over CaH<sub>2</sub> and vacuum-distilled prior to use. Nitric oxide (Matheson, 99%) was purified by a method adapted from the literature.<sup>35</sup> The NO stream was passed through an Ascarite column (NaOH fused on silica gel) and a 6 ft coil filled with silica gel cooled to –78 °C. NO gas was stored and transferred under an inert atmosphere using standard gas storage bulbs and gas-tight syringes, respectively. For reactions involving NO or nitrosothiol, care was taken to prevent light exposure by covering reaction glassware in aluminum foil or by performing experiments in a darkened room.

**Materials.** The iron–sulfur clusters (Et<sub>4</sub>N)<sub>2</sub>[Fe<sub>2</sub>S<sub>2</sub>(SPh)<sub>4</sub>], (“Bu<sub>4</sub>N)<sub>2</sub>[Fe<sub>2</sub>S<sub>2</sub>(SPh)<sub>4</sub>], (Et<sub>4</sub>N)<sub>2</sub>[Fe<sub>2</sub>S<sub>2</sub>(*S-p*-tolyl)<sub>4</sub>], (“Bu<sub>4</sub>N)<sub>2</sub>[Fe<sub>2</sub>S<sub>2</sub>(*S-2-o*-xyl)<sub>2</sub>], (Et<sub>4</sub>N)<sub>2</sub>[Fe<sub>2</sub>S<sub>2</sub>(*S-2,4,6-Me<sub>3</sub>C<sub>6</sub>H<sub>2</sub>)<sub>4</sub>], (Et<sub>4</sub>N)<sub>2</sub>[Fe<sub>4</sub>S<sub>4</sub>(SPh)<sub>4</sub>], (Et<sub>4</sub>N)<sub>2</sub>[Fe<sub>4</sub>S<sub>4</sub>(SCH<sub>2</sub>Ph)<sub>4</sub>], (Et<sub>4</sub>N)<sub>2</sub>[Fe<sub>4</sub>S<sub>4</sub>(*S-Bu*)<sub>4</sub>], and (“Bu<sub>4</sub>N)<sub>2</sub>[Fe<sub>4</sub>S<sub>4</sub>(*S-2-m-xyl*)<sub>2</sub>] were prepared by literature procedures or slight modifications thereof.<sup>36–38</sup> (PPN)[Fe(NO)<sub>2</sub>I<sub>2</sub>] was prepared as described.<sup>39</sup> Trityl-*S*-nitrosothiol (Ph<sub>3</sub>CSNO) was prepared by a modified literature procedure described below.<sup>40</sup>*

**Physical Measurements.** IR spectra were recorded with a ThermoNicolet Avatar 360 spectrophotometer running the OMNIC software; solid samples were pressed into KBr disks and solution samples were prepared in an air-tight Graseby-Specac solution cell with CaF<sub>2</sub> windows and 0.1 mm spacers. In situ IR spectra were recorded on a ReactIR 1000 instrument from ASI equipped with a 1 in. diameter, 30-reflection silicon ATR (SiComp) probe. In a typical experiment the instrument was blanked with MeCN and the sample concentrations ranged from 15 to 30 mM. NO(g) was added to the anaerobic sample compartment through a rubber septum with a gas-tight syringe. UV–vis spectra were recorded on a Cary-50 diode-array spectrophotometer in air-tight Teflon-capped quartz cells. Samples for <sup>57</sup>Fe Mössbauer studies were

prepared by grinding a solid sample and mixing it with Apiezon-N grease. Samples were placed in a 4.2 K cryostat during measurement. A <sup>57</sup>Co/Rh source was moved at a constant acceleration at room temperature against the absorber sample. All isomer shift (δ) and quadrupole splitting (ΔE<sub>Q</sub>) values are reported with respect to <sup>57</sup>Fe-enriched metallic iron foil that was used for velocity calibration. The displayed spectrum was folded to enhance the signal-to-noise ratio. Fits of the data were calculated by the WMOSS version 2.5 plot and fit program.<sup>41</sup> X-band EPR spectra were recorded on a Bruker EMX EPR spectrometer (9.38 GHz). Temperature control was maintained with an Oxford Instruments ESR900 liquid-He cryostat. Spectra were recorded in 4 mm o.d. quartz EPR tubes capped with a tight-fitting rubber septum.

**Preparation of Trityl-*S*-nitrosothiol (Ph<sub>3</sub>CSNO).** A 100 mL round-bottom flask was charged with 2.763 g (10.0 mmol) of Ph<sub>3</sub>CSH and 15 mL of methylene chloride and capped with a rubber septum. No attempt was made to exclude ambient moisture or dioxygen from the reaction. Once the thiol had dissolved, 2.0 mL (15 mmol) of isoamyl nitrite was added via syringe, after which the solution took on a dark green color. The flask was wrapped in aluminum foil to exclude light and stirred at room temperature for 60 min. Methyl alcohol (30 mL) was added and the mixture allowed to stand at –25 °C for 2 h. During this time, 2.293 g of the compound precipitated as dark green needles. The needles were isolated by filtration, washed with methyl alcohol, and dried in vacuo. Yield: 75%. FTIR (KBr pellet): ν<sub>NO</sub> = 1514 cm<sup>-1</sup>. UV–vis (CH<sub>2</sub>Cl<sub>2</sub>) (ε, M<sup>-1</sup> cm<sup>-1</sup>): 348 nm (740), 556 (sh), 602 (40). Spectra (see Supporting Information) were identical to those previously reported for Ph<sub>3</sub>CSNO.<sup>42</sup>

**Reaction of [2Fe-2S] and [4Fe-4S] Clusters with NO(g).** In a typical reaction, a 100 mL Schlenk flask was charged with 60–80 μmol of the cluster and 15–20 mL of acetonitrile. The reaction vessel was capped with a rubber septum, and 5–10 mL (depending on the anticipated volume of added NO) of headspace was removed from the flask via syringe. NO gas was then introduced into the headspace of the flask by syringe. The mixture was allowed to stir at ambient temperature (295–298 K) in a darkened glovebox for 2 h. All volatiles were removed in vacuo and the resulting dark red residue was extracted successively with diethyl ether (3 × 5 mL), THF (3 × 5 mL), and finally MeCN (3 × 5 mL) if any insoluble material remained. Evaporation of each extract then afforded different reaction products that were identified by IR, UV–vis, and EPR spectroscopy. For reactions followed by their UV–vis spectra, excess NO(g), typically 50–100 μL, was injected directly into the headspace of a cuvette containing 3 mL of a 40–60 μM solution of cluster. For reactions involving added (Et<sub>4</sub>N)(SPh), thiolate solutions in MeCN were prepared by mixing equal molar amounts of anhydrous Et<sub>4</sub>NCl and NaSPh followed by filtration through glass filter paper into the reaction mixture. Reactions employing Ph<sub>3</sub>CSNO were conducted in an identical fashion to those using NO(g) except that nitrosothiol was introduced as a solution in THF or MeCN.

**(Et<sub>4</sub>N)[Fe(NO)<sub>2</sub>(SPh)<sub>2</sub>].** To a 15 mL MeCN solution containing 104.9 mg (120 μmol) of (Et<sub>4</sub>N)<sub>2</sub>[Fe<sub>2</sub>S<sub>2</sub>(SPh)<sub>4</sub>] was added 15.0 mL (500 μmol) of NO(g) through a gas-tight syringe. The solution was allowed to stir at ambient temperature for 2.5 h, during which time the color changed from dark purple to dark red. All volatiles were removed in vacuo, and the residue was washed with Et<sub>2</sub>O. FTIR analysis of the residue indicated (Et<sub>4</sub>N)[Fe(NO)<sub>2</sub>(SPh)<sub>2</sub>] as the only NO-containing species. Recrystallization of the crude product from MeCN at –25 °C afforded 73.3 mg (66%) of dark red needles, the

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spectral features of which matched those of previously published benzenethiolate DNICs.<sup>25,43</sup>

**(Et<sub>4</sub>N)[Fe(NO)<sub>2</sub>(S-*p*-tolyl)<sub>2</sub>].** To a 5 mL MeCN solution containing 26.0 mg (28.1 μmol) of (Et<sub>4</sub>N)<sub>2</sub>[Fe<sub>2</sub>S<sub>2</sub>(S-*p*-tolyl)<sub>4</sub>] was added 4.0 mL (178.6 μmol) of NO(g) through a gas-tight syringe, resulting in a rapid color change from violet to red. After the solution was stirred under an NO atmosphere for 4 h at room temperature, a very small amount of a pale yellow precipitate was observed and filtered off. The MeCN was then evaporated to afford a dark, oily residue. This residue was washed several times with Et<sub>2</sub>O to aid in solidifying the material, affording 23.7 mg (86% yield) of product. FTIR (KBr, cm<sup>-1</sup>): 3056 (w), 2975 (w), 1732 (vs, ν<sub>NO</sub>), 1691 (vs, ν<sub>NO</sub>), 1486 (m), 1434 (w), 1392 (w), 1364 (w), 1170 (w), 1085 (m), 1018 (w), 997 (w), 809 (w), 782 (w), 630 (w), 492 (w). FTIR (THF, cm<sup>-1</sup>): 1738, (s, ν<sub>NO</sub>), 1694 (s, ν<sub>NO</sub>). UV-vis (THF) (ε, M<sup>-1</sup> cm<sup>-1</sup>): 483 (3100), 795 (560). Anal. Calcd for C<sub>22</sub>H<sub>34</sub>N<sub>3</sub>O<sub>2</sub>S<sub>2</sub>Fe: C, 53.65; H, 6.96; N, 8.53. Found: C, 54.03; H, 6.87; N, 8.62.

**(PPN)[Fe(NO)<sub>2</sub>{(SCH<sub>2</sub>)<sub>2</sub>-*o*-C<sub>6</sub>H<sub>4</sub>}]**. A flask was charged with 0.327 g (0.36 mmol) of (PPN)[Fe(NO)<sub>2</sub>I<sub>2</sub>] and 15 mL of MeCN. To the brown solution was added 0.127 g (0.59 mmol) of Na<sub>2</sub>[(SCH<sub>2</sub>)<sub>2</sub>-*o*-C<sub>6</sub>H<sub>4</sub>] as a solid in one portion. The resulting mixture was allowed to stir at ambient temperature for 2 h. All volatiles were removed in vacuo, and the remaining residue was extracted into 20 mL of THF and filtered through a plug of Celite. The THF solution was concentrated to 5 mL in vacuo and layered with several volumes of diethyl ether. After the solution was left to stand at -25 °C for 24 h, the DNIC precipitated as 0.115 g (39%) of brown needles. FTIR (KBr, cm<sup>-1</sup>): 2955 (w), 2933 (m), 2854 (w), 1734 (s, ν<sub>NO</sub>), 1688 (s, ν<sub>NO</sub>), 1588 (w), 1574 (w), 1484 (w), 1438 (m), 1383 (w), 1285 (m), 1266 (m), 1184 (w), 1115 (m), 1070 (w), 1046 (w), 1027 (w), 998 (w), 768 (w), 746 (w), 724 (m), 692 (m). FTIR (MeCN, cm<sup>-1</sup>): 1725 (ν<sub>NO</sub>), 1682 (ν<sub>NO</sub>). UV-vis (MeCN) (ε, M<sup>-1</sup> cm<sup>-1</sup>): 379 (3650), 425 (sh), 584 (950), 868 (450). EPR (10 K, 2-MeTHF glass): g<sub>av</sub> = 2.029. Anal. Calcd for C<sub>44</sub>H<sub>38</sub>N<sub>3</sub>O<sub>2</sub>P<sub>2</sub>S<sub>2</sub>Fe: C, 64.24; H, 4.66; N, 5.11. Found: C, 63.73; H, 4.71; N, 5.29.

**Synthesis of Roussin's Black Salt (RBS, (Et<sub>4</sub>N)[Fe<sub>4</sub>S<sub>3</sub>(NO)<sub>7</sub>]) from (Et<sub>4</sub>N)<sub>2</sub>[Fe<sub>4</sub>S<sub>4</sub>(SPh)<sub>4</sub>].** **a. From NO(g).** To a 5 mL MeCN solution containing 51.2 mg (48.8 μmol) of (Et<sub>4</sub>N)<sub>2</sub>[Fe<sub>4</sub>S<sub>4</sub>(SPh)<sub>4</sub>] was added 9.0 mL (401.8 μmol) of NO(g) to the headspace of the reaction flask. The reaction mixture gradually changed from a deep dark red-brown to a brown solution. After 2 h of stirring at ambient temperature in the dark, the solution was filtered to remove a small amount of a yellow precipitate (sulfur), after which the filtrate was stripped to dryness. The brown residue was washed with several portions of Et<sub>2</sub>O, which were found by GC-MS to contain PhSSPh, and the residue was dissolved in 5 mL of THF and filtered. To the filtrate was added an equivalent volume of pentane to precipitate 30.2 mg (93%) of black needles, the spectral features of which were identical to those reported for RBS.<sup>44</sup>

**b. From Ph<sub>3</sub>CSNO.** To a solution containing 70.0 mg (66.7 μmol) of (Et<sub>4</sub>N)<sub>2</sub>[Fe<sub>4</sub>S<sub>4</sub>(SPh)<sub>4</sub>] in 10 mL of MeCN was added a solution of 143.2 mg (360 μmol) of Ph<sub>3</sub>CSNO dissolved in 10 mL of MeCN. The reaction mixture was allowed to stir in the dark at ambient temperature for 3 h, after which time all volatiles were removed in vacuo. The residue was washed with 10 mL of Et<sub>2</sub>O and extracted into 10 mL of THF. Evaporation of the THF afforded 86.3 mg of black solid that was found by IR spectroscopy to be a mixture of RBS, (Et<sub>4</sub>N)(SPh), and some trityl-containing species. The RBS could subsequently be purified by crystallization from THF/pentane as described above.

**Synthesis of Dinitrosyl Iron Complex (DNIC) (Et<sub>4</sub>N)-[Fe(NO)<sub>2</sub>(SPh)<sub>2</sub>] from (Et<sub>4</sub>N)<sub>2</sub>[Fe<sub>4</sub>S<sub>4</sub>(SPh)<sub>4</sub>].** To a 10 mL MeCN solution containing 72.5 mg (69.1 μmol) of (Et<sub>4</sub>N)<sub>2</sub>[Fe<sub>4</sub>S<sub>4</sub>(SPh)<sub>4</sub>] and 276 μmol of (Et<sub>4</sub>N)(SPh) was added 16.0 mL (563 μmol) of NO(g) to the headspace of the reaction flask. The reaction was stirred at ambient temperature in the dark for 2 h, during which time the color turned from brown-red to dark red. All volatiles were removed in vacuo, and the residue was treated with Et<sub>2</sub>O to remove elemental sulfur. Analysis of the crude product by FTIR and UV-vis spectroscopy indicated (Et<sub>4</sub>N)[Fe(NO)<sub>2</sub>(SPh)<sub>2</sub>] to be the sole NO-containing product. The crude product was recrystallized from a minimal amount of MeCN at -25 °C to afford 69.4 mg of the DNIC (54%) as dark red needles.

#### X-ray Data Collection and Structure Solution Refinement.

Black blocks of (Et<sub>4</sub>N)[Fe(S-*p*-tolyl)<sub>2</sub>(NO)<sub>2</sub>] were grown anaerobically at -25 °C by slow diffusion of pentane into a solution of the compound dissolved in THF. A suitable crystal was mounted in Paratone N oil on the tip of a glass capillary and frozen under a 110 K nitrogen cold stream maintained by a KRYO-FLEX low-temperature apparatus. Data were collected on a Bruker APEX CCD X-ray diffractometer with Mo Kα radiation (λ 0.71073 Å) controlled by the SMART software package.<sup>45</sup> Empirical absorption corrections were calculated with SADABS,<sup>46</sup> and the structures were checked for higher symmetry by the PLATON software.<sup>47</sup> The structure was solved by direct methods with refinement by full-matrix least-squares based on *F*<sup>2</sup> using the SHELXTL-97<sup>48</sup> software incorporated in the SHELXTL software package.<sup>49,50</sup> All non-hydrogen atoms were located and their positions refined anisotropically. Hydrogen atoms were assigned to idealized positions and given thermal parameters equal to either 1.5 (methyl hydrogen atoms) or 1.2 (non-methyl hydrogen atoms) times the thermal parameters of the atoms to which they were attached.

## Results and Discussion

**[2Fe-2S] Clusters.** Reaction of the synthetic [2Fe-2S] cluster, (Et<sub>4</sub>N)<sub>2</sub>[Fe<sub>2</sub>S<sub>2</sub>(SPh)<sub>4</sub>], with 4 equiv of NO(g) in the absence of light and dioxygen afforded the previously described DNIC, (Et<sub>4</sub>N)[Fe(NO)<sub>2</sub>(SPh)<sub>2</sub>], as the sole iron-containing product. Verification of the DNIC was provided by IR, UV-vis, and EPR spectroscopy.<sup>33</sup> The only detectable byproduct was a white solid that precipitated from the acetonitrile reaction solution. This ether-soluble solid displayed no IR spectral features. Subsequent treatment of the white material with triphenylphosphine in CD<sub>3</sub>CN at ambient temperature gave rise to a new <sup>31</sup>P NMR resonance at 43.9 ppm after 24 h, consistent with formation of S=PPh<sub>3</sub>.<sup>51</sup> The solid therefore appears to be an allotrope of elemental sulfur.

In all reactions employing gaseous nitric oxide, identical results were obtained when trityl-*S*-nitrosothiol<sup>42</sup> (Ph<sub>3</sub>CSNO) was used in place of the NO(g). We find that Ph<sub>3</sub>CSNO, for which we provide an improved synthesis, can deliver a stoichiometric quantity of NO(g) equivalents in reactions with iron-sulfur compounds. Such reactivity is important, considering that nitrosothiols are

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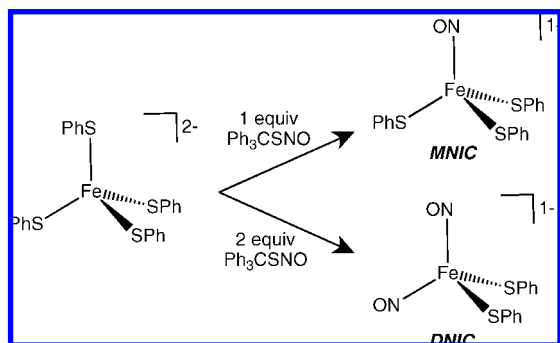
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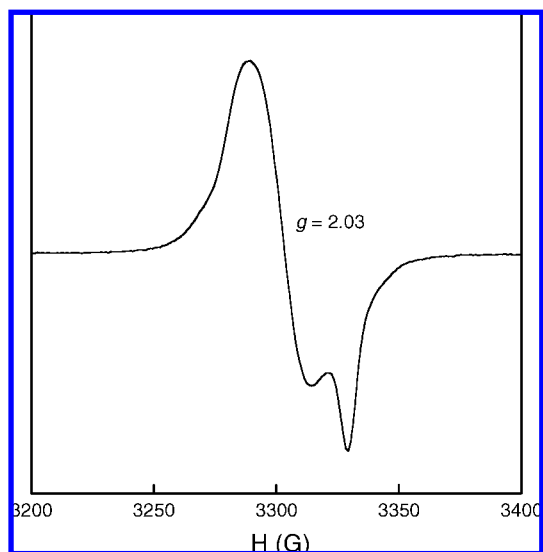
## Scheme 1



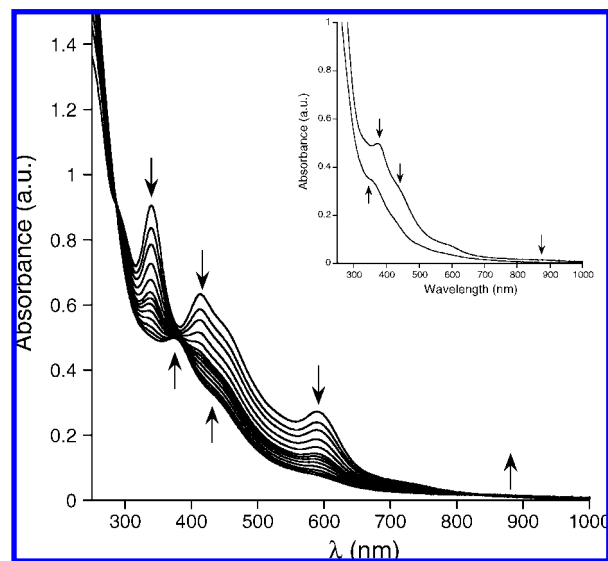
believed to be carriers of NO in living systems.<sup>52</sup> Solid  $\text{Ph}_3\text{CSNO}$  can be isolated in greater than 75% yield from the reaction of  $\text{Ph}_3\text{CSH}$  and isoamyl nitrite (see Experimental Section for details). The resulting green crystals are stable for months when stored cold ( $-25^\circ\text{C}$ ) in the absence of light. The relatively high molecular weight (305 g/mol) and good solubility of this nitrosothiol render it an ideal source of NO in nonaqueous systems. As a test of the nitrosylating ability of  $\text{Ph}_3\text{CSNO}$ , the homoleptic thiolate complex  $[\text{Fe}(\text{SPh})_4]^{2-}$  was treated with 1 and 2 equiv of the nitrosothiol. Examination of the reaction products by UV–vis spectroscopy (see Supporting Information, Figure S1) demonstrated clean formation of the previously reported<sup>53</sup> MNIC (1 equiv) and DNIC (2 equiv) species, confirming that the compound delivers stoichiometric NO equivalents to the iron–thiolate complexes (Scheme 1).

Reaction of  $(\text{Et}_4\text{N})_2[\text{Fe}_2\text{S}_2(\text{SPh})_4]$  with fewer than 4 equiv of  $\text{NO}(\text{g})$  or  $\text{Ph}_3\text{CSNO}$  resulted in formation of the DNIC and recovery of the unreacted cluster. Examination of the reaction by IR or EPR spectroscopy demonstrated immediate formation of  $[\text{Fe}(\text{NO})_2(\text{SPh})_2]^-$  with no observable intermediates. For example, treatment of a 300  $\mu\text{M}$  solution of  $(\text{Et}_4\text{N})_2[\text{Fe}_2\text{S}_2(\text{SPh})_4]$  with an excess of  $\text{NO}(\text{g})$  followed by immediate freezing in liquid nitrogen gave rise to the EPR spectrum shown in Figure 1, characteristic of a DNIC. Similarly, addition of increasing equivalents of the *S*-nitrosothiol,  $\text{Ph}_3\text{CSNO}$ , to a 380  $\mu\text{M}$  solution of  $(^t\text{Bu}_4\text{N})_2[\text{Fe}_2\text{S}_2(\text{SPh})_4]$  in THF at 295 K yielded an increasingly intense signal derived from  $(^t\text{Bu}_4\text{N})[\text{Fe}(\text{NO})_2(\text{SPh})_2]$ , as judged by EPR spectroscopy (see Supporting Information, Figure S2). Similar results were obtained when the reaction was monitored by IR spectroscopy.

Under more dilute concentrations ( $<100\ \mu\text{M}$ ),  $[2\text{Fe}-2\text{S}]$  clusters were found to react with  $\text{NO}(\text{g})$  to yield RBS instead of DNIC. For example, when a 47  $\mu\text{M}$  solution of  $(\text{Et}_4\text{N})_2[\text{Fe}_2\text{S}_2(\text{SPh})_4]$  was treated with  $\text{NO}(\text{g})$  with the reaction monitored by UV–vis spectroscopy, a featureless spectrum resulted following the disappearance of the optical bands of the  $[2\text{Fe}-2\text{S}]$  cluster. Nearly identical results were obtained with the bulkier mesityl thiolate cluster,  $(\text{Et}_4\text{N})_2[\text{Fe}_2\text{S}_2(\text{S}-2,4,6\text{-Me}_3\text{C}_6\text{H}_2)_4]$ . When the reaction with  $(\text{Et}_4\text{N})_2[\text{Fe}_2\text{S}_2(\text{SPh})_4]$  was repeated in the presence of additional equivalents of  $(\text{Et}_4\text{N})(\text{SPh})$ , optical bands for the DNIC were observed to form (see Supporting Information, Figure S3). In contrast to these results for the aryl thiolate clusters, examination by UV–vis spectroscopy of the reaction of  $\text{NO}(\text{g})$  with the  $[2\text{Fe}-2\text{S}]$  cluster containing the chelating  $[(\text{SCH}_2)_2\text{-}o\text{-C}_6\text{H}_4]^{2-}$  (*S*-*o*-xyl) ligand in the absence of free thiolate initially revealed



**Figure 1.** X-band EPR spectrum obtained upon introduction of excess  $\text{NO}(\text{g})$  to a 300  $\mu\text{M}$  solution of  $(\text{Et}_4\text{N})_2[\text{Fe}_2\text{S}_2(\text{SPh})_4]$  in 1:1 MeCN/toluene followed by immediate freezing in liquid nitrogen. The spectrum was recorded at 10 K (microwave frequency, 9.385 GHz; microwave power, 2.0 mW; modulation amplitude, 4.00 G).

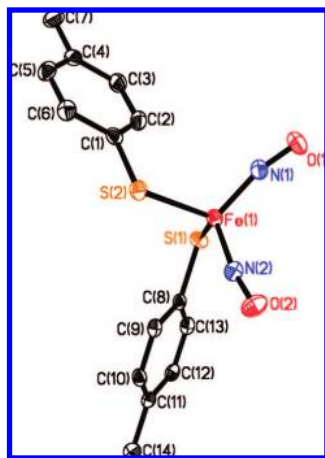


**Figure 2.** UV–vis spectrum of the reaction of  $(^t\text{Bu}_4\text{N})_2[\text{Fe}_2\text{S}_2(\text{S}_2\text{-}o\text{-xyl})_2]$  (64  $\mu\text{M}$ ) with excess ( $\sim 30$  equiv)  $\text{NO}(\text{g})$  at 298 K in MeCN. Spectra were recorded over 45 min. Inset shows the spectral change associated with transformation of the DNIC to RBS.

the appearance of optical bands consistent with a DNIC (vide infra) over the course of 30 min. At longer times (1 h), optical bands for the DNIC diminished, giving rise to a less-featured spectrum similar to that observed in the reaction of  $[\text{Fe}_2\text{S}_2(\text{SPh})_4]^{2-}$  with  $\text{NO}$  (Figure 2). The featureless spectrum is consistent with the formation of RBS,  $[\text{Fe}_4\text{S}_3(\text{NO})_7]^-$ , the formation of which requires oxidation of the thiolate ligands. Elemental sulfur formed during the course of the reaction could serve as an oxidant, as might  $\text{NO}$  or one of its disproportionation products, such as  $\text{NO}_2$ . RBS could be isolated from reactions of  $(\text{Et}_4\text{N})_2[\text{Fe}_2\text{S}_2(\text{SPh})_4]$  with excess  $\text{NO}(\text{g})$  when the reaction was allowed to stir for longer than 5 h. In this instance, the disulfide  $\text{PhSSPh}$  was identified in the reaction mixture, consistent with oxidation of the thiolate ligands. When  $(\text{Et}_4\text{N})_2[\text{Fe}_2\text{S}_2(\text{S}-2,4,6\text{-Me}_3\text{C}_6\text{H}_2)_4]$  was allowed to react with excess  $\text{NO}(\text{g})$ , consumption of the cluster was much slower, and no DNICs were

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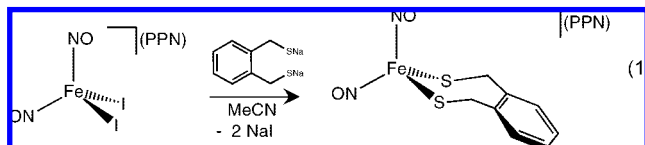
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**Figure 3.** Thermal ellipsoid (50%) drawing of the anion of  $(\text{Et}_4\text{N})[\text{Fe}(\text{S-}p\text{-tolyl})_2(\text{NO})_2] \cdot 0.5\text{THF}$ . Hydrogen atoms and cocrystallized tetrahydrofuran are omitted for clarity. See Supporting Information for complete list of metric parameters (Tables S1 and S2).

identified by IR spectroscopy. Dimesityl disulfide was isolated from the reaction mixture, indicating oxidation of the thiolate ligands.

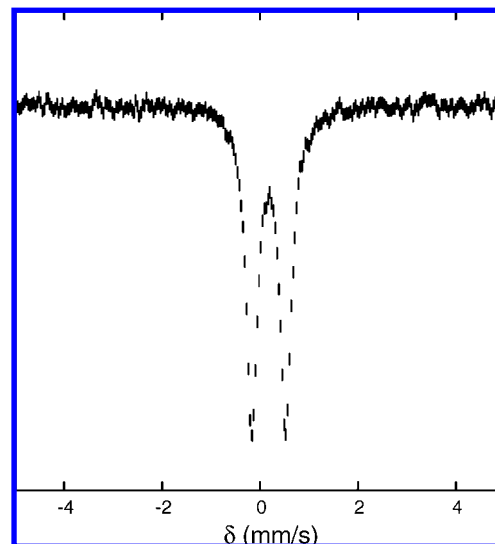
Unfortunately, attempts to isolate the previously unreported DNIC,  $[\text{Fe}(\text{NO})_2(\text{S}_2\text{-}o\text{-xyl})]^-$ , as the  $\text{Et}_4\text{N}^+$  or  $^n\text{Bu}_4\text{N}^+$  salt were unsuccessful. The compound could be obtained, however, as the  $\text{PPN}^+$  ( $\text{PPN} = \mu\text{-nitrido-bistriphenylphosphonium}$ ) salt by a direct salt metathesis reaction using  $(\text{PPN})[\text{Fe}(\text{NO})_2\text{I}_2]$ , as shown in eq 1.



The DNIC thus obtained crystallized as thin brown needles from a mixture of THF and diethyl ether. The molecule displays  $\nu_{\text{NO}}$  values in KBr at 1734 and 1688  $\text{cm}^{-1}$ , compared to 1743 and 1683  $\text{cm}^{-1}$  for the benzenethiolate derivative. The electronic absorption spectrum shows two relatively intense maxima at 379 and 420 nm in MeCN that are identical to those observed in reactions with the [2Fe-2S] cluster and NO(g) (Figure 2).

In order to verify the reaction stoichiometry observed with  $(\text{Et}_4\text{N})_2[\text{Fe}_2\text{S}_2(\text{SPh})_4]$  and  $(^n\text{Bu}_4\text{N})_2[\text{Fe}_2\text{S}_2(\text{S}_2\text{-}o\text{-xyl})_2]$ , mass-balance analysis of the analogous reaction with  $(\text{Et}_4\text{N})_2[\text{Fe}_2\text{S}_2(\text{S-}p\text{-tolyl})_4]$  was carried out in the presence of a slight excess of NO(g) (6 mol equiv). Reaction of  $(\text{Et}_4\text{N})_2[\text{Fe}_2\text{S}_2(\text{S-}p\text{-tolyl})_4]$  at 295 K with excess NO(g) in MeCN afforded the corresponding DNIC,  $(\text{Et}_4\text{N})[\text{Fe}(\text{S-}p\text{-tolyl})_2(\text{NO})_2]$ , in 86% yield. As expected, the IR spectrum of the DNIC displayed two strong  $\nu_{\text{NO}}$  bands at 1732 and 1691  $\text{cm}^{-1}$  (KBr), similar to the spectra of other known DNICs. Workup of the byproducts of the reaction of NO(g) with  $(\text{Et}_4\text{N})_2[\text{Fe}_2\text{S}_2(\text{S-}p\text{-tolyl})_4]$  afforded an off-white solid that we assign as elemental sulfur by analogy to the observations with the benzenethiolate cluster. There was very little to no evidence of disulfide formation in this or any of the NO reactions with the [2Fe-2S] systems when the reactions were stopped after 1–2 h. Thus, formation of the two DNICs upon reaction of NO with [2Fe-2S] clusters results in reductive elimination of 2 mol equiv of elemental sulfur.

X-ray crystallographic analysis further confirmed the proposed DNIC structure (Figure 3). The structure of the anion contains a four-coordinate iron center in a distorted tetrahedral geometry. The distortions at the metal center are most readily apparent from the



**Figure 4.** Zero-field Mössbauer (4.2 K) spectrum of  $(\text{Et}_4\text{N})[\text{Fe}(\text{NO})_2(\text{SPh})_2]$ . See Supporting Information (Figure S5) for the theoretical fit from which the isomer shift and quadrupole splitting parameters were obtained.

bond angles at iron, which deviate considerably from the 109.5° values of a perfect tetrahedron (viz. N1–Fe1–N2, 115.68(7)°; N2–Fe1–S2, 102.15(5)°; Supporting Information, Table S2). The Fe–S distances (Fe1–S1, 2.3329(7) Å; Fe1–S2, 2.3343(7) Å) are nearly identical and slightly longer than those found in other structurally characterized DNICs containing aromatic thiolate donors.<sup>25,43</sup> The Fe–N bond lengths are slightly variable (Fe1–N1, 1.7210(15) Å; Fe1–N2, 1.7096(15) Å), as are the N–O distances (N1–O1, 1.1992(19) Å; N2–O2, 1.1958(19) Å). The nitrosyl ligands are slightly bent (Fe1–N1–O1, 166.04(14)°; Fe1–N2–O2, 170.05(14)°) and flared toward each other in the typical attracto conformation observed in DNICs.<sup>54</sup>

Despite the large number of reports of DNIC species, very few studies of their Mössbauer spectra have been undertaken. The Mössbauer spectrum of a “dimeric DNIC” prepared by in situ generation from aqueous  $\text{FeSO}_4$ , NO(g), and cysteine displays an isomer shift of 0.14 mm/s and a quadrupole splitting of 1.1 mm/s.<sup>55</sup> A 296 K Mössbauer spectrum of  $(\text{Et}_4\text{N})[\text{Fe}(\text{NO})_2(\text{SPh})_2]$ , prepared by reaction of RBS with molten diphenyl disulfide in the presence of KOH and  $\text{Et}_4\text{NCl}$ , has  $\delta = 0.08$  mm/s and  $\Delta E_Q = 0.78$  mm/s.<sup>43</sup> In the present work we recorded the Mössbauer spectrum of  $(\text{Et}_4\text{N})[\text{Fe}(\text{NO})_2(\text{SPh})_2]$  isolated from the reaction of NO with iron–sulfur clusters. The 4.2 K zero-field spectrum is shown in Figure 4. The isomer shift of 0.18(2) mm/s (versus iron foil at 296 K) and quadrupole splitting of 0.69(2) mm/s may be compared with those just cited, bearing in mind the disparity in recording temperature.<sup>56</sup>

The NO chemistry of the [2Fe-2S] clusters discovered here is somewhat surprising in the context of recent work demonstrating that homoleptic Fe(III) thiolates react via thiolate oxidation to afford mononitrosyl iron complexes.<sup>53</sup> Since  $\{\text{Fe}_2\text{S}_2\}^{2+}$  cores formally contain Fe(III), we might expect similar reactivity. Our observations indicate, however, that thiolate oxidation is only a minor side

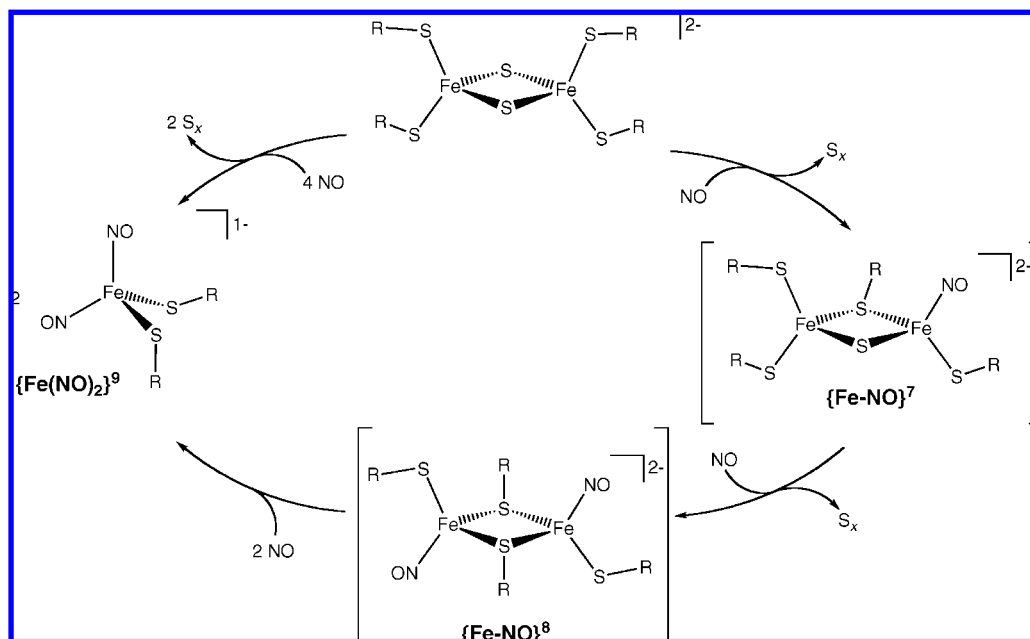
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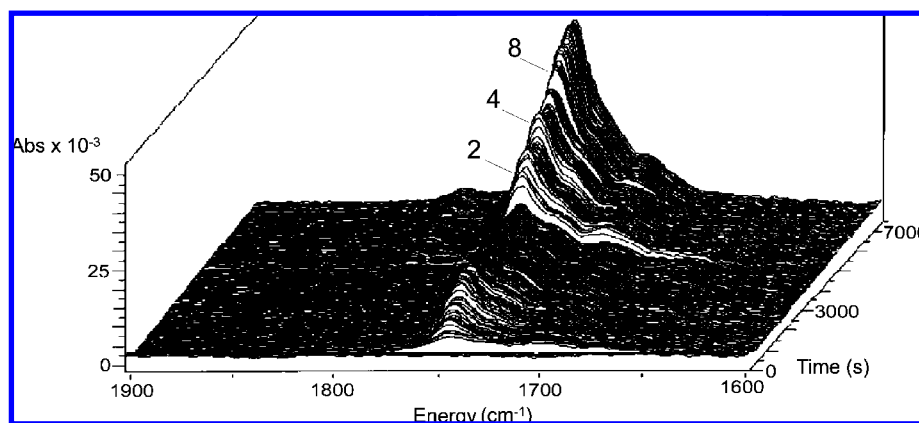
Scheme 2. Possible Reaction Pathway for [2Fe-2S] Clusters with NO



reaction of [2Fe-2S] clusters with NO, leading to formation of nitrosylated species other than DNICs. Instead, inorganic sulfide provides all of the necessary reducing equivalents to transform the cluster to 2 equiv of the DNIC. A possible pathway for this transformation is depicted in Scheme 2. If addition of NO occurs concomitantly with elimination of sulfur, then reaction of the first NO equivalent will lead to elimination of 1 mol equiv of sulfur and formation of a {Fe-NO}<sup>7</sup> mononitrosyl iron complex (Scheme 2). Addition of the second equivalent of NO results in elimination of a second mol-equiv of elemental sulfur and formation of a putative dimeric {Fe-NO}<sup>8</sup> species. Break-up of the dimer by addition of another 2 equiv of NO affords the corresponding DNICs. In support of this proposal, the nickel analogue of (Et<sub>4</sub>N)<sub>2</sub>[Fe<sub>2</sub>(μ-SPh)<sub>2</sub>(NO)<sub>2</sub>(SPh)<sub>2</sub>] has recently been isolated and crystallographically characterized by our laboratory.<sup>57</sup>

**[4Fe-4S] Clusters.** The reaction of the [4Fe-4S] cluster, (Et<sub>4</sub>N)<sub>2</sub>[Fe<sub>4</sub>S<sub>4</sub>(SPh)<sub>4</sub>], with NO(g) was performed in a similar fashion to that described above for the [2Fe-2S] systems. We initially chose to monitor the reaction by in situ ReactIR spectroscopy, which facilitates characterization of all

iron–nitrosyl species formed after addition of NO. IR spectra were acquired every 30 s after introduction of NO to the headspace of a reaction flask containing a MeCN solution of (Et<sub>4</sub>N)<sub>2</sub>[Fe<sub>4</sub>S<sub>4</sub>(SPh)<sub>4</sub>] for a total time of 2 h. Introduction of 1 mol-equiv of NO(g) resulted in an immediate color change from deep red-brown to a slightly lighter brown and an IR spectrum with two broad ν<sub>NO</sub> bands at 1745 and 1708 cm<sup>-1</sup> and a small peak at 1800 cm<sup>-1</sup>. These bands grow in intensity until 20 min after NO addition. These values match identically the IR spectrum of the tetraphenylarsonium salt of the iron–sulfide–nitrosyl cluster (Ph<sub>4</sub>As)[Fe<sub>4</sub>S<sub>3</sub>(NO)<sub>7</sub>] (RBS).<sup>58</sup> To determine whether any other iron–nitrosyl complexes might form during this process, additional equivalents of NO(g) were added to the MeCN solution of [Fe<sub>4</sub>S<sub>4</sub>(SPh)<sub>4</sub>]<sup>2-</sup>. As revealed by ReactIR, no other complexes could be detected up to the addition of 8 mol-equiv of NO, and each stoichiometric addition resulted only in growth of IR peaks associated with RBS and no other nitrosyl species (Figure 5). The formation of RBS from [Fe<sub>4</sub>S<sub>4</sub>(SPh)<sub>4</sub>]<sup>2-</sup> and excess NO(g) in MeCN was also monitored by UV–vis

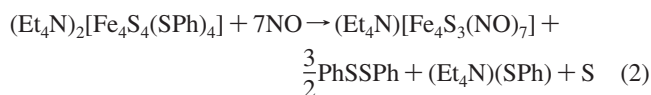


**Figure 5.** In situ ReactIR spectra following the addition of 1 (0 s), 2, 4, and 8 (indicated) mol-equiv of NO(g) into the headspace of a flask containing (Et<sub>4</sub>N)<sub>2</sub>[Fe<sub>4</sub>S<sub>4</sub>(SPh)<sub>4</sub>] (21.0 mM) in MeCN at 298 K. The bands at 1800, 1745, and 1708 cm<sup>-1</sup> increase in intensity as more NO equivalents are added. The reaction was monitored over the course of 2 h. Each acquisition represents eight scans collected at 30 s intervals.



spectroscopy. The characteristic intense 450 nm band of this cluster immediately diminished and a notably featureless spectrum resulted, having several small absorptions in the 320–380 nm range. A shoulder occurs at 425 nm with an additional broad peak at 580 nm that matched the published spectrum of RBS. Similar results were obtained for other [4Fe-4S] clusters containing  $\text{PhCH}_2\text{S}^-$ ,  $^t\text{Bu-S}^-$ , and the chelating  $(-\text{SCH}_2)_2\text{-}m\text{-C}_6\text{H}_4$  ( $\text{S}_2\text{-}m\text{-xyl}$ ) thiolates, as well as reactions employing trityl-*S*-nitrosothiol. Although we have not performed detailed kinetic studies on these reactions, the rate of appearance of RBS qualitatively appears to correlate with the steric bulk of the thiolate ligand. The least sterically demanding cluster containing the chelating  $\text{S}_2\text{-}m\text{-xyl}$  thiolate reacts in less than 5 min to yield RBS according to UV–vis spectroscopy. In contrast, the bulkiest cluster,  $[\text{Fe}_4\text{S}_4(\text{S-}^t\text{Bu})_4]^{2-}$ , required nearly 2 h to react completely with NO(g).

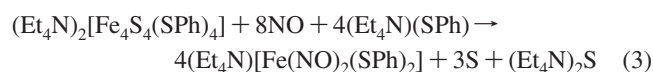
Additional evidence that RBS is the sole product of this chemistry was obtained by isolation of the cluster from a reaction of  $(\text{Et}_4\text{N})_2[\text{Fe}_4\text{S}_4(\text{SPh})_4]$  with NO(g) in MeCN. After removal of the volatile components, PhSSPh was obtained as a white solid by extraction of the reaction mixture with diethyl ether. The black salt could be isolated by subsequent extraction with THF and crystallization. Examination of the crude THF extract by IR spectroscopy also revealed vibrations due to  $\text{PhS}^-$ , suggesting the presence of  $(\text{Et}_4\text{N})(\text{SPh})$ . Taken together, these observations are consistent with the chemistry given in eq 2,



where the tetranuclear 2Fe(II)–2Fe(III) cluster is transformed into RBS, 1Fe(II)–3Fe(I), assuming the nitrosyl ligands to be formally neutral, with three electrons coming from the coordinated thiolates and two electrons from one of the bridging sulfides. FTIR analysis of the crystalline product of this reaction revealed  $\nu_{\text{NO}}$  peaks at 1799, 1733, 1711, and 1693  $\text{cm}^{-1}$ , in agreement with RBS formation. An X-ray crystallographic analysis of the product confirmed it to be the tetraethylammonium salt of RBS,  $(\text{Et}_4\text{N})[\text{Fe}_4\text{S}_3(\text{NO})_7]$ . The geometric parameters and unit cell dimensions match those of a previously published structure of the  $\text{Et}_4\text{N}^+$  salt of RBS.<sup>44</sup>

The formation of RBS from reactions of the [4Fe-4S] clusters with NO may be contrasted with related results with bacterial HiPIPs, in which treatment of the cluster with DEANO, diethylamino NONOate, produced an EPR spectrum characteristic of a DNIC.<sup>22</sup> From this comparison we form the working hypothesis that formation of  $[\text{Fe}_4\text{S}_3(\text{NO})_7]^-$  from  $[\text{Fe}_4\text{S}_4(\text{SPh})_4]^{2-}$  outside of a protein environment most likely proceeds by disassembly of the cluster and formation of mononuclear iron–nitrosyl species that reassemble to generate RBS. Such reactivity has previously been proposed for transformations involving RBS, Roussin's red salt, and the tetranitrosyl cluster  $[\text{Fe}_4\text{S}_4(\text{NO})_4]$ .<sup>59</sup> Reaction of synthetic [4Fe-4S] iron–sulfur clusters with NO to form RBS via mononuclear DNIC species that are too unstable to observe might proceed in the following manner. At low thiolate-to-iron (1:1) ratios, DNICs containing solvent ligands<sup>31</sup> would

react rapidly in the presence of the elemental sulfur generated to form RBS. To test this hypothesis, we investigated the reaction of  $(\text{Et}_4\text{N})_2[\text{Fe}_4\text{S}_4(\text{SPh})_4]$  with 8 mol-equiv of NO(g) in the presence of 4 equiv of  $(\text{Et}_4\text{N})(\text{SPh})$ . After workup of the reaction, the only nitrosyl-containing species detected by IR, UV–vis, and EPR spectroscopy was  $(\text{Et}_4\text{N})[\text{Fe}(\text{NO})_2(\text{SPh})_2]$ . Importantly, there was only a very small amount of disulfide in the reaction mixture, less than 1 equiv per cluster, indicating that very little thiolate oxidation had taken place. The formation of DNICs in these reactions could occur through reaction of RBS with thiolate.<sup>60,61</sup> This scenario is unlikely, however, since significant quantities of disulfide would still be formed. These results support the conclusion that the reactivity of [4Fe-4S] clusters is similar to that of the [2Fe-2S] clusters, with the sulfide ligands providing the electrons necessary for reduction of the iron centers to form DNICs. A balanced equation for  $[\text{Fe}_4\text{S}_4(\text{SPh})_4]^{2-}$  reacting with NO and excess thiolate to generate 4 equiv of DNIC is presented in eq 3. We have not been able to identify the sulfur byproducts in any of the reactions of this type thus far investigated.



## Conclusions

The reactions of gaseous NO and trityl-*S*-nitrosothiol with synthetic [2Fe-2S] and [4Fe-4S] clusters afford  $\{\text{Fe}(\text{NO})_2\}^9$  DNIC species or Roussin's black salt as the nitrosylated iron products. The nature of the thiolate ligand and the ratio of thiolate to iron appear to be critical in determining which products are formed. With synthetic [2Fe-2S] systems, a 2:1 ratio of thiolate to iron is sufficient to allow formation and isolation of the corresponding DNICs, with  $(\text{Et}_4\text{N})[\text{Fe}(\text{S-}p\text{-tolyl})_2(\text{NO})_2]$  being structurally characterized by X-ray crystallography. The mechanism of [2Fe-2S] cluster nitrosylation appears to involve inorganic sulfide ligands as the reducing agents, which provide all the necessary reducing equivalents for the reaction to form elemental sulfur and the DNIC. In contrast, reaction of stoichiometric and excess amounts of NO(g) with [4Fe-4S] clusters in the absence of added thiolate affords RBS. When additional equivalents of thiolate are present, the corresponding DNIC is observed as the sole nitrosylated product. Taken together, these results demonstrate that the end products in iron–sulfur degradation by NO gas or nitrosothiols are  $\{\text{Fe}(\text{NO})_2\}^9$  dinitrosyl iron complexes. Disassembly of the iron–sulfur core appears to take place readily with both two and four iron systems. Furthermore, sufficient equivalents of thiolate are necessary to trap and stabilize the DNIC formed during nitrosylation. When the thiolate-to-iron ratio is too low (<2), subsequent reactivity with sulfur occurs to afford RBS. These results suggest that, biologically, the breakdown of iron–sulfur clusters must occur in tandem with the sequestration of inorganic sulfur (or sulfide) to prevent formation of thermodynamically stable iron–sulfur–nitrosyl clusters. Protection of the clusters in a folded protein may divert this chemistry,

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a possibility currently under investigation with the use of site-differentiated constructs.<sup>62–64</sup>

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**Supporting Information Available:** Additional spectra, crystallographic data, refinement details, and a fully labeled ORTEP diagram for  $(\text{Et}_4\text{N})[\text{Fe}(\text{NO})_2(\text{S-}p\text{-tolyl})_2]$ , as well as the corresponding CIF file. This material is available free of charge via the Internet at <http://pubs.acs.org>.

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