Published in final edited form as:

J Am Chem Soc. 2011 February 9; 133(5): 1419–1427. doi:10.1021/ja107551u.

Influence of Thiolate Ligands on Reductive N–O Bond Activation. Probing the O₂^{•−} Binding Site of a Biomimetic SOR Analogue, and Examining the Proton-Dependent Reduction of Nitrite

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Abstract

Nitric oxide (NO) is frequently used to probe the substrate-binding site of “spectroscopically silent” non-heme Fe²⁺ sites of metalloenzymes, such as superoxide reductase (SOR). Herein we use NO to probe the superoxide binding site of our thiolate-ligated biomimetic SOR model [Fe^{II}(S^{Me}₂N₄(tren))]⁺ (**1**). Like NO-bound *trans* cysteinate-ligated SOR (SOR-NO), the rhombic S = 3/2 EPR signal of NO-bound *cis* thiolate-ligated [Fe(S^{Me}₂N₄(tren)(NO))]⁺ (**2**; *g* = 4.44, 3.54, 1.97), isotopically sensitive $\nu_{\text{NO}}(\nu_{15\text{NO}})$ stretching frequency (1685(1640) cm^{−1}), and 0.05 Å decrease in Fe–S bond length are shown to be consistent with the oxidative addition of NO to Fe(II) to afford an Fe(III)–NO[−] {FeNO}⁷ species containing high-spin (S = 5/2) Fe(III) antiferromagnetically coupled to NO[−] (S = 1). The *cis* versus *trans* positioning of the thiolate does not appear to influence these properties. Although it has yet to be crystallographically characterized, SOR-NO is presumed to possess a bent Fe–NO similar to that of **2** (Fe–N–O = 151.7(4)°). The N–O bond is shown to be more activated in **2** relative to N- and O-ligated {FeNO}⁷ complexes, and this is attributed to the electron donating properties of the thiolate ligand. Hydrogen bonding to the cysteinate sulfur attenuates N–O bond activation in SOR as shown by its higher ν_{NO} frequency (1721 cm^{−1}). In contrast, the $\nu_{\text{O–O}}$ frequency of SOR peroxo intermediate and its analogues is not affected by H-bonds to the cysteinate sulfur, or other factors influencing the Fe–SR bond strength. These only influence the $\nu_{\text{Fe–O}}$ frequency. Reactions between **1** and NO₂[−] are shown to result in the proton-dependent heterolytic cleavage of an N–O bond. The mechanism of this reaction is proposed to involve both Fe^{II}–NO₂[−] and {FeNO}⁶ intermediates similar to those implicated in the mechanism of NiR-promoted NO₂[−] reduction.

Introduction

Superoxide reductases (SORs) are cysteinate-ligated non-heme iron enzymes that selectively reduce superoxide (O₂^{•−}) to hydrogen peroxide (H₂O₂) via a putative Fe(III)–OOH intermediate. Synthetic analogues of this peroxo intermediate have been reported by several groups.^{1–6} Exogenous ligands, such as azide, nitric oxide, and cyanide, have been shown to bind to the high spin (S = 2) N₄SFe²⁺ SOR site,⁴ *trans* to an apical cysteine,⁷ consistent with an inner sphere mechanism of superoxide reduction. Detailed spectroscopic

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Supporting Information. Contains experimental, and crystallographic data for complexes **2** and **7**, including an ESI mass spec, cyclic voltammogram, and IR spectrum of **2**. An electronic absorption spectrum of **7**. Magnetic data for **2** and **7**. And, quantitative titrations and/or reactions between **1** and NO₂[−], and **2** and O₂, monitored by electronic absorption spectroscopy. This material is available free of charge via the Internet at <http://pubs.acs.org>.

data is available for NO-bound SOR (SOR-NO),⁸ however, this form of the enzyme has yet to be crystallographically characterized. Five-coordinate, thiolate-ligated $[\text{Fe}^{\text{II}}(\text{S}^{\text{Me}_2}\text{N}_4(\text{tren}))]^+$ (**1**)⁹ was shown previously by our group to reduce superoxide (O_2^-) via a metastable hydroperoxo intermediate $[\text{Fe}^{\text{III}}(\text{S}^{\text{Me}_2}\text{N}_4(\text{tren})(\text{OOH}))]^+$ --the first reported example of a synthetic thiolate-ligated iron-peroxo.¹ This biomimetic SOR analogue also reacts with dioxygen (O_2)¹⁰ to afford a metastable intermediate that converts to μ -oxo dimer $[\text{Fe}^{\text{III}}(\text{S}^{\text{Me}_2}\text{N}_4(\text{tren}))_2(\mu^2\text{-O})(\text{PF}_6)_2]$ upon warming.¹⁰ Given the architecture of our $(\text{tren})\text{N}_4^{\text{Me}_2}\text{S}^-$ ligand, O_2 and O_2^- are assumed to bind *cis* with respect to the thiolate, however in the absence of crystallographic evidence this is difficult to prove. Nitric oxide is frequently used to probe O_2 binding sites,^{8,11–14} and form analogues of key metastable Fe- O_2 intermediates, since its reduced derivative (NO^-) is isoelectronic with O_2 , but affords a more stable, highly covalent Fe-NO bond.¹⁵ Since it is a radical, NO is also frequently used as a tag to probe “spectroscopically silent” Fe^{2+} enzyme substrate-binding sites such as that of isopenicillin N-synthase (IPNS),^{14,16} and superoxide reductase (SOR).⁸ The substrate-derived thiolate ligand of the IPNS iron active site has been shown to stabilize both the key Fe- O_2^- superoxo intermediate and Fe-NO derivative.¹⁶ The thiolate ligand of the synthetic SOR peroxo intermediate analogue $[(\text{L}^8\text{py}_2)\text{Fe}(\text{III})(\text{SR})(\text{OOR})]^+$ was shown to increase its lifetime,⁴ whereas that of $[\text{Fe}(\text{III})([15]\text{aneN}_4)\text{SAr}(\text{OOR})]^+$ and $[\text{Fe}(\text{III})(\text{cyclam-PrS})(\text{OOH})]^+$ was shown to weaken the Fe-OOR(H) bond, without affecting the O-O bond.^{2,3}

In order to gain insight regarding the mechanism of O_2^- reduction by our thiolate-ligated SOR analogue $[\text{Fe}^{\text{II}}(\text{S}^{\text{Me}_2}\text{N}_4(\text{tren}))]^+$ (**1**), and probe its superoxide and dioxygen binding site, we describe herein the synthesis, and geometric and electronic structure properties of a more stable NO-bound analogue. Comparison of the properties of this SOR-NO analogue with that of SOR-NO¹⁷ should allow us to determine how the superoxide binding sites differ, and determine whether this influences properties critical to promoting superoxide reduction. Upon binding to transition-metals, the N-O bond of nitric oxide can, in some cases, be activated via π -back donation into a $\pi^*(\text{NO})$ orbital.¹⁸ Electron-rich thiolate ligands have also been shown to enhance N-O bond activation by donating electron density to a metal ion orbital that is involved in σ -overlap with a $\sigma^*(\text{NO})$ orbital.^{8,19} A similar mechanism may be involved in the activation of thiolate-ligated peroxo O-O bonds. Thus information regarding N-O bond strength-dependence on the presence of thiolate donors, and their *cis* vs *trans* orientation, could provide insight regarding the influence of thiolates on peroxo bond cleavage. The ability of reduced **1** to activate and cleave the N-O bond of additional substrates such as NO_2^- will also be explored.

Experimental Section

General Methods

All reactions were performed under an atmosphere of dinitrogen in a glove box or using standard Schlenk techniques, or using a custom-made solution cell equipped with a threaded glass connector sized to fit a dip probe. Reagents purchased from commercial vendors were of the highest purity available and used without further purification. 3-Methyl-3-mercapto-2-butanone,²⁰ and $[\text{Fe}^{\text{III}}(\text{S}^{\text{Me}_2}\text{N}_5(\text{tren})(\text{MeCN}))][\text{BPh}_4]_2$ (**8**)¹ were synthesized as previously described. The triflate salt of $[\text{Fe}^{\text{II}}(\text{S}^{\text{Me}_2}\text{N}_4(\text{tren}))](\text{OTf})$ (**1**), was synthesized as previously described,⁹ using NaOTf in place of NaPF₆. Toluene, tetrahydrofuran (THF), diethyl ether (Et_2O), and acetonitrile (MeCN) and pentane were rigorously degassed and purified using solvent purification columns, housed in a custom stainless steel cabinet, and dispensed via a stainless steel schlenk-line (GlassContour). Methanol (MeOH) and ethanol (EtOH) were distilled from magnesium methoxide and degassed prior to use. Methylene chloride (DCM) was distilled from CaH_2 and degassed prior to use. ¹H NMR spectra were recorded on Bruker AV 300 or Bruker AV 301 FT-NMR spectrometers and are referenced to an external standard of TMS (paramagnetic compounds) or to residual protio-solvent (diamagnetic

compounds). Chemical shifts are reported in ppm and coupling constants (J) are in Hz. EPR spectra were recorded on a Bruker EPX CW-EPR spectrometer operating at X-band frequency at ~ 7 K. IR spectra were recorded on a Perkin-Elmer 1700 FT-IR spectrometer as KBr pellets. Cyclic voltammograms were recorded in MeCN (100 mM Buⁿ₄N(PF₆) solutions) on a PAR 273 potentiostat utilizing a glassy carbon working electrode, platinum auxiliary electrode, and an SCE reference electrode. Magnetic moments (solution state) were obtained using the Evans' method²¹ as modified for super-conducting solenoids.²² Temperatures were obtained using Van Geet's method.²³ Solid state magnetic measurements were obtained with polycrystalline samples in gel-caps using a Quantum Design MPMS S5 SQUID magnetometer. Ambient temperature electronic absorption spectra were recorded on a Hewlett-Packard Model 8450 spectrometer, interfaced to an IBM PC. Low temperature electronic absorption spectra were recorded using a Varian Cary 50 spectrophotometer equipped with a fiber optic cable connected to a "dip" ATR probe (C-technologies), with a custom-built two-neck solution sample holder equipped with a threaded glass connector (sized to fit the dip probe). Elemental Analyses were performed by Galbraith Atlantic Microlabs, Norcross, GA.

Synthesis of [Fe(S^{Me2}N₄(tren)(NO)](OTf) (2)

Reduced [Fe^{II}(S^{Me2}N₄(tren))](OTf) (1) (200 mg, 0.448 mmol) was dissolved in acetonitrile, placed in a thick-walled bomb, equipped with a Teflon stopcock and a side arm joint. On a high vacuum line, the solution was frozen (by placing the flask in liquid nitrogen), and the container was evacuated. Nitric oxide gas (420 Torr, 1.2 equiv) (Scotts Specialty Gases) was then added to the frozen solution. The container was sealed, and the contents were allowed to thaw. The solution was stirred overnight under nitrogen, and then concentrated under vacuum to ~ 5 mL. Pentane (5 mL) and Et₂O (25 mL) were layered on top of this solution, and the layers were allowed to diffuse together. After two days, a brown powder formed (159 mg, 70% yield). ESI-MS calcd for [FeC₁₁H₂₅N₅OS]⁺: 331.269, found 331.2. Electronic absorption (CH₃CN): λ_{max} (ϵ) = 440 (2560), nm; (MeOH): λ_{max} (ϵ) = 438 (2560) nm; IR (KBr pellet) $\nu(\text{cm}^{-1})$: 1605 (imine), 1685 (ν_{NO}). Solution magnetic moment (303 K; MeCN) μ_{eff} = 4.12 BM. $E_{1/2}$ (MeCN) = + 450 mV vs. SCE. EPR (MeCN/Toluene glass (1:1), 7 K): g_1 = 4.41, g_2 = 3.60, g_3 = 1.98. Anal. calcd for FeC₃₅H₄₃BN₅O₁S₁: C, 64.74 %, H, 6.62 %, N, 10.79 %. Found: C, 64.67 %, H, 6.53 %, N, 10.29 %.

Synthesis of [Fe^{III}(S^{Me2}N₄(tren))(NO₂)](PF₆) (7)

Acetonitrile-bound¹ [Fe^{III}(S^{Me2}N₄(tren))MeCN](PF₆)₂ (500 mg, 0.789 mmol) and n-Bu₄NNO₂ (227 mg, 0.789 mmol, Fluka) were dissolved in MeCN (25 mL) and allowed to stir for 24h under a nitrogen atmosphere in a glovebox. The solution was filtered through a fine glass frit, MeCN was removed under reduced pressure, and the solution was concentrated to ~ 1 mL. Diethyl ether (25 mL) was then layered on top of the solution, and the two layers were allowed to diffuse together at -30°C . After 24h, a purple solid (356 mg, 92 %) was isolated. ESI-MS calcd for [FeC₁₁H₂₅N₅O₂S]⁺: 348.26, found 347.5. Electronic absorption spectrum (MeOH): λ_{max} = 565(1460) nm). IR (KBr pellet) $\nu(\text{cm}^{-1})$: 1608 (imine), $\nu_{\text{asym}}(\text{NO}_2)$ = 1478 cm^{-1} , and $\nu_{\text{sym}}(\text{NO}_2)$ = 1362 cm^{-1} . Solution magnetic moment (303 K; MeCN) μ_{eff} = 1.76 BM. $E_{1/2}$ (MeCN) = -0.480 V vs. SCE. EPR (MeOH/EtOH glass (9:1), 7 K): g_1 = 2.17, g_2 = 2.13, g_3 = 1.98. Anal. calcd for FeC₁₁H₂₅PN₅O₂F₆S: C, 26.84 %, H, 5.12 %, N, 14.23 %. Found: C, 27.67 %, H, 5.53 %, N, 14.59 %.

Formation of [Fe^{III}(S^{Me2}N₄(tren))(NO₂)](BPh₄) (7) via O₂ Oxidation of [Fe(S^{Me2}N₄(tren)(NO)] [BPh₄] (2)

A solution of [Fe(S^{Me2}N₄(tren)(NO)] [BPh₄] (200 mg, 0.308 mmol) in 30 mL of acetonitrile was stirred in air for 30 min, and then stirred under N₂ for an additional 5 h. The solution was then filtered, concentrate to ~ 2 mL, layered with ~ 25 mL of ether and placed at -30°C .

The two layers diffuse together overnight to afford purple crystals (172 mg, 86 %). ESI-MS calcd for $[\text{FeC}_{11}\text{H}_{25}\text{N}_5\text{O}_2\text{S}]^+$: 348.26, found 347.5. Electronic absorption (CH_3CN): λ_{max} (ϵ) = 565 (1460) nm; IR (KBr pellet) $\nu(\text{cm}^{-1})$: 1682 (imine), 1478 cm^{-1} ($\nu_{\text{asym}}(\text{NO}_2)$), and 1362 cm^{-1} ($\nu_{\text{sym}}(\text{NO}_2)$). Solution magnetic moment (303 K; MeCN) $\mu_{\text{eff}} = 1.76$ BM. $E_{1/2}$ (MeCN) = -0.480 V vs. SCE. EPR (MeOH/EtOH glass (9:1), 7 K): $g_1 = 2.18$, $g_2 = 2.14$, $g_3 = 1.99$.

X-ray Crystallographic Structure Determination

A brown prism of **2** ($0.26 \times 0.24 \times 0.14$ mm ($0.30 \times 0.21 \times 0.18$ mm)) was mounted on a glass capillary with oil. Data was collected at -143°C . The crystal-to-detector distance was set to 30 mm and exposure time was 60 seconds per degree for all data sets with a scan width of 1.0° . The data collection was 99% complete to 25° in θ . A total of 34240 partial and complete reflections were collected covering the indices, $h = -19$ to 18, $k = -11$ to 11, $l = -20$ to 20. 2541 reflections were symmetry independent and the $R_{\text{int}} = 0.0599$ indicated that the data was good (average quality = 0.07). Indexing and unit cell refinements indicated an orthorhombic P lattice in the space group Pnma (No. 62). The data was integrated and scaled using hkl-SCALEPACK, and an absorption correction was performed using SORTAV. Solution by direct methods (SIR97) produced a complete heavy atom phasing model consistent with the proposed structure. All non-hydrogen atoms were refined anisotropically by full-matrix least-squares methods, while all hydrogen atoms were then located using a riding model. The structure showed disorder of the nitric oxide oxygen O(1), and one of the methylene groups in the amine/imine chelate ring (N(1)(CH₂)₂N(2) with respect to a mirror plane containing N(1), N(4), Fe, and S(1).

A purple crystal plate of **7** ($0.07 \times 0.24 \times 0.09$ mm) was mounted on a glass capillary with oil. Data was collected at -143°C . The crystal-to-detector distance was 30 mm and exposure time was 30 seconds per degree for all sets. The scan width was 2.0° . Data collection was 92.7% complete to 27.09° and 97.4% complete to 25° in θ . A total of 26490 partial and complete reflections were collected covering the indices, $h = -17$ to 17, $k = -10$ to 10, $l = -19$ to 19. 2023 reflections were symmetry independent and the $R_{\text{int}} = 0.1474$ indicated that the data was of less than average quality (0.07). Indexing and unit cell refinement indicated an orthorhombic P lattice. The space group was found to be P n m a (No.62). The data was integrated and scaled using hkl-SCALEPACK. Solution by direct methods (SIR97) produced a complete heavy atom phasing model consistent with the proposed structure. All hydrogen atoms were located using a riding model. All non-hydrogen atoms were refined anisotropically by full-matrix least-squares. Crystal data for **2** and **7** is presented in Table 1. Selected bond distances and angles are assembled in Table 2.

Results and Discussion

Reactivity of $[\text{Fe}^{\text{II}}(\text{S}^{\text{Me}_2}\text{N}_4(\text{tren}))]^+$ (**1**) with Nitric Oxide (NO)

Quantitative addition of 1.0 equiv of NO(g) to an acetonitrile solution of five-coordinate, thiolate-ligated $[\text{Fe}^{\text{II}}(\text{S}^{\text{Me}_2}\text{N}_4(\text{tren}))]^+$ (**1**) under anaerobic (Scheme 1) conditions affords a compound with a parent ion in the triple quad ESI-MS ($m/z = 330.7$; Figure S-1 (supplemental material)) that is consistent with the addition of one equivalent of NO. Nitric oxide binding to **1** was confirmed by X-ray crystallography. As shown in the ORTEP diagram of Figure 1, nitric oxide binds *cis* to the thiolate sulfur, and *trans* to the imine nitrogen (N(1)). This *cis* orientation is similar to that of NO-bound IPNS,¹⁶ but contrasts with the presumed *trans* orientation in SOR-NO.⁸ Selected bond distances ($\text{\AA}$) and angles (deg) of **2** are compared with those of **1**, as well as previously reported $[\text{Fe}^{\text{III}}(\text{S}^{\text{Me}_2}\text{N}_4(\text{tren}))(\text{OAc})]^+$ (**3**)²⁴, and $[\text{Fe}^{\text{III}}(\text{S}^{\text{Me}_2}\text{N}_4(\text{tren}))(\text{N}_3)]^+$ (**4**),²⁴ in Table 2. As is true for the majority of $\{\text{FeNO}\}^7$ systems (*vide infra*),^{11,18,25} the Fe–N(4)–O(1) bond angle is bent ($151.7(4)^\circ$). This

angle is also presumed to be bent in SOR-NO,⁸ however crystallographic data is not yet available to support this. In **2**, this angle is bent towards the *cis* thiolate sulfur (Figure 1), suggesting that if the hydroperoxo's orientation in $[\text{Fe}^{\text{III}}(\text{S}^{\text{Me}_2}\text{N}_4(\text{tren})(\text{OOH})]^+$ is the same, it would be capable of H-bonding to the sulfur. A hydrogen-bonded ring structure may, in fact, provide a driving force for the formation of hydroperoxide-ligated $[\text{Fe}^{\text{III}}(\text{S}^{\text{Me}_2}\text{N}_4(\text{tren})(\text{OOH})]^+$.¹ Most significant in the structure of **2** is the fact that the Fe–S bond decreases in length upon NO binding (from 2.3281(9) Å in **1** to 2.278(1) Å in **2**), consistent with the *oxidative addition* of NO to the metal ion. This contrasts with the Fe–S bond lengthening (by 0.11 Å) that occurs upon NO binding to our previously reported ferric complex $[\text{Fe}^{\text{III}}(\text{S}_2^{\text{Me}_2}\text{N}_3(\text{Pr},\text{Pr}))]^+$ (**5**)²⁶ to afford *trans/cis* bis-thiolate ligated $[\text{Fe}(\text{S}_2^{\text{Me}_2}\text{N}_3(\text{Pr},\text{Pr}))(\text{NO})]^+$ (**6**, Scheme 2). As shown by the considerably shorter Fe–S distances in the ferric complexes **3** and **4** (Table 2),²⁴ NO binding to **1** induces only a fractional increase in oxidation state (*vide infra*).

With transition-metal nitric oxide complexes, the assignment of oxidation states is somewhat ambiguous due to the extensive delocalization of electrons within the highly covalent M–NO bond. For this reason, it is preferable to describe complexes of this type using the Enemark and Feltham notation $\{\text{MNO}\}^n$,²⁷ where *n* is the total number of *d* + $\pi^*(\text{NO})$ electrons. Complex **2** and SOR would thus both be described as $\{\text{FeNO}\}^7$ compounds, wherein the charge distribution could fall anywhere within the range Fe(I)–NO⁺, to Fe(II)–NO[•], to Fe(III)–NO[−].^{18,27} In order to accurately assess the most appropriate electronic description detailed spectroscopic (Mossbauer, MCD, EPR, X-ray absorption spectroscopy (XAS), IR, and/or resonance Raman) and theoretical (DFT) studies would be required.^{8,11,14,28–33} Complex **6**,²⁶ on the other hand, would be described as an $\{\text{FeNO}\}^6$ compound, wherein the charge distribution could fall anywhere within the range Fe(II)–NO⁺, to Fe(III)–NO[•], to Fe(IV)–NO[−]. Complex **2** is irreversibly oxidized at a potential of $E_{\text{pa}} = +450$ mV (vs. SCE) in MeCN solution (supplemental Figure S-2), implying that its $\{\text{FeNO}\}^6$ derivative is unstable in this solvent.

Nitric oxide induced bond length changes in **2** are caused by several factors, including an increase in both coordination number and oxidation state (*vide infra*), and the introduction of a strong-field ligand. The Fe–N(3, 3') bonds elongate (by 0.074 Å), whereas the Fe–S(1), and Fe–N(2) bond lengths decrease (by 0.050 Å, and 0.057 Å, respectively). The increase in Fe–N(3, 3') bond lengths is most likely caused by the increase in coordination number, and steric crowding in the FeN(1)N(3)N(3')N(4) plane which results from the introduction of the NO ligand. For the ligating atoms, S(1) and N(2), orthogonal to this plane, bond length decreases are observed, most likely due to an increase in Z_{eff} (*vide infra*),³¹ and oxidation state, resulting from the oxidative addition of NO, and the absence of the steric factors present in the FeN(1)N(3)N(3')N(4) plane. Although one would expect the *trans* influence of the NO ligand to elongate the Fe–N(1) bond, there is very little change to this bond length (less than 3 esds), most likely because the metal ion oxidation state increase (*vide infra*) offsets the *trans* influence. Being *trans* to the NO ligand the imine nitrogen N(1) is less affected by steric crowding. The extremely short Fe–X (X= NO) bond of **2** (Table 2) reflects the highly covalent nature of the Fe–NO bond.^{18,27} This bond length is comparable to the DFT calculated distance in IPNS Fe-NO,¹⁶ is slightly above the range typical for $\{\text{FeNO}\}^7$ species (1.68–1.76 Å), and well above that of $\{\text{FeNO}\}^6$ species (1.63–1.67 Å),^{18,28,32,34–40} including $[\text{Fe}(\text{S}_2^{\text{Me}_2}\text{N}_3(\text{Pr},\text{Pr}))(\text{NO})]^+$ (**6**, Fe–NO= 1.676(3) Å).²⁶ The N–O bond length (1.118(6) Å) in **2** is closer to that of free NO (1.15 Å) than to NO[−] (1.26 Å), and is unexpectedly shorter than that of **6** (1.161(4) Å), despite the presence of an additional electron in the π^* -manifold of $\{\text{FeNO}\}^7$ **2** versus $\{\text{FeNO}\}^6$ **6**. Although the *trans*-thiolate (present in **6**, but not in **2**) could be responsible for the longer N–O bond in **6**,¹⁹ it might also simply be an artifact of disorder. The nitric oxide oxygen (O(1)) is disordered over two

positions in structure **2** (but not in **6**), making the N-O bond distance a less reliable parameter (in **2**).

Vibrational Data

The solid state (KBr pellet) IR spectrum of the NO-bound product, $[\text{Fe}(\text{S}^{\text{Me}_2}\text{N}_4(\text{tren})(\text{NO}))]^+$ (**2**), displays an ^{15}NO isotope sensitive stretch at $\nu_{\text{NO}}(\nu_{^{15}\text{NO}}) = 1685(1640) \text{ cm}^{-1}$ (supplemental Figure S-3). This frequency is substantially shifted below that of free $\text{NO}^\bullet$ ($\nu_{\text{NO}} = 1875 \text{ cm}^{-1}$), and closer to that of free NO^- ($\nu_{\text{NO}} = 1470 \text{ cm}^{-1}$),¹⁸ consistent with the *oxidative addition* of NO to the metal ion, and activation of the N–O bond. Spectroscopic and theoretical data indicates that NO is also reduced and activated upon binding to the Fe^{2+} sites of SOR,⁸ isopenicillin N-synthase (IPNS),¹⁶ and superoxide dismutase (SOD).³⁰ The ν_{NO} frequency of **2**, is however, significantly lower than that of NO-bound SOR ($\nu_{\text{NO}}(\nu_{^{15}\text{NO}}) = 1721(1690) \text{ cm}^{-1}$),⁸ indicating that more electron-density shifts from the Fe^{2+} ion to the NO in **2**. Vibrational data is not available for NO-bound IPNS and SOD. One would expect the ν_{NO} stretching frequency to reflect the amount of electron density in the $\pi^*(\text{NO})$ and/or $\sigma^*(\text{NO})$ orbitals, which would be affected by the total number of d + $\pi^*(\text{NO})$ electrons, the ligand field of the metal ion, and charge distribution within the Fe–NO bond. The ν_{NO} stretch of **2** falls in the range ($1607\text{--}1812 \text{ cm}^{-1}$)^{18,28} typically observed for non-heme $\{\text{FeNO}\}^7$ compounds,²⁷ and is closest to those shown to possess an $\text{Fe(II)-NO}^\bullet$ ($\nu_{\text{NO}} = 1607\text{--}1682 \text{ cm}^{-1}$),^{18,28,35,37,41–43} as opposed to an Fe(III)-NO^- ($\nu_{\text{NO}} = 1710\text{--}1812 \text{ cm}^{-1}$),^{11,18,29,44} electronic structure. Compounds with the Fe(III)-NO^- electron distribution are $S = 3/2$, and have been shown, via detailed spectroscopic and theoretical calculations, to contain high-spin ($S = 5/2$) Fe(III) antiferromagnetically coupled to $S = 1 \text{ NO}^-$.^{8,11,30,33} Whereas compounds with the $\text{Fe(II)-NO}^\bullet$ electron distribution are typically $S = 1/2$, and are usually described as containing a low-spin ($S = 0$) Fe(II) ligated by ($S = 1/2$.) $\text{NO}^\bullet$,^{28,29,32,45} although the electronic description of these compounds is more controversial.³¹ Compound **2** is intermediate spin $S = 3/2$ (*vide infra*), suggesting that the former description might be more accurate, despite the fact that the ν_{NO} frequency falls below that of an Fe(III)-NO^- compound. Although counterintuitive, the higher ν_{NO} frequency for Fe(III)-NO^- compounds is a result of the shift of π^* electron density away from the N–O bond and into the Fe–NO bond via constructive bonding overlap with the half-filled Fe d_{xz} and d_{yz} orbitals.⁴⁶ As described previously,⁸ one of these metal ion orbitals is filled in low-spin Fe(II) causing electron density to shift away from the Fe–NO bond and into the π^* N–O orbital in $\text{Fe(II)-NO}^\bullet$ compounds. DFT calculations show³¹ that the electron-donating properties of the thiolate ligand of **2** has a similar effect by pushing electron density onto the metal ion, and thereby preventing the shift of electron density away from the π^* N–O orbital and into the Fe–NO bond. This would imply that the thiolate ligand is responsible for the unusually low ν_{NO} frequency of **2** (1685 cm^{-1}). In support of this, nitrogen and oxygen-ligated $S = 3/2 \text{ Fe(III)-NO}^-$ compounds, such as $(\text{TACN})\text{Fe}(\text{NO})(\text{N}_3)_2$,¹¹ $(\text{EDTA})\text{Fe}(\text{NO})$,¹¹ $[\text{Fe}(\text{NO})(i\text{Pr}_3\text{tcmba})]^-$,⁴⁴ and $[\text{Fe}_2(\text{NO})_2(\text{Et-HPTB})(\text{O}_2\text{CPh})]^{+2}$,⁴⁷ for example, display ν_{NO} stretches at 1712 cm^{-1} , 1776 cm^{-1} , 1729 cm^{-1} , and 1785 cm^{-1} , respectively. Whereas the ν_{NO} stretch (1682 cm^{-1}) for thiolate-ligated $(\text{TACN}-(\text{BzS})_2)\text{Fe-NO}$,³⁷ lies much closer to that of **2**. Given that the molecular charge and, in some cases, the coordination numbers of these compounds differ from **2**, these comparisons are not necessarily ideal. However, density functional calculations show that the ligand field, and electron donating properties of the thiolate ligand of **2** raise the energy of the d-orbitals relative to the π^* N–O making it more energetically favorable for electrons to reside on the NO ligand in an antibonding orbital.³¹ Together these data imply that thiolate ligands promote more N–O bond activation in nitric oxide than nitrogen-, or oxygen-containing ligands due to their electron-rich nature. Hydrogen-bonds attenuate the electron donating properties of the SOR cysteinate ligand,^{48,49} and this diminishes the extent of N–O bond activation by SOR, as is reflected in the higher ν_{NO} stretch (1721 cm^{-1}) for SOR-NO .⁸ In contrast, $\text{RS}\cdots\text{H}$ -bonds have been

shown to strengthen the SOR Fe–O(peroxo) bond without influencing the peroxo O–O bond.⁵⁰ Most likely this is because a peroxo has two additional antibonding $\pi^*(\text{O}=\text{O})$ electrons relative to an NO ligand, making it a poorer electron-acceptor. This prevents the delocalization of electrons, at least via a mechanism involving π -overlap, and decreases the covalency of the Fe–OOH bond relative to an Fe–NO bond.⁸

Electronic and Magnetic Properties of $[\text{Fe}(\text{S}^{\text{Me}_2}\text{N}_4(\text{tren}))(\text{NO})]^+$ (**2**)

Both temperature-dependent magnetic susceptibility curves for solid samples of **2** (Figure S–4, ($\mu_{\text{eff}} = 4.12 \mu_{\text{B}}$), and the near ambient temperature (290 K) solution state magnetic moment of **2** ($\mu_{\text{eff}} = 3.95 \mu_{\text{B}}$ in MeCN), are consistent with an $S=3/2$ ground state that is maintained over a wide-temperature range (5 – 290 K). The low-temperature (6.3 K) X-band EPR spectrum of **2** is rhombic with features at $g_x = 4.44$, $g_y = 3.54$ and $g_z = 1.97$ (Figure 2), also consistent with an $S=3/2$ spin-state. Shoulders on the $g = 4.44$, 3.54 features could either be attributed to superhyperfine interaction between the unpaired electron spin and the ^{14}N ($I = 1$) nucleus of the NO ligand,³² or to slightly different conformations of the molecule. The anisotropic nature of this spectrum indicates that the metal ion has been oxidized as a result of NO addition. Nitric oxide-bound SOR¹⁷ and IPNS¹⁴ are also intermediate-spin $S = 3/2$, and display rhombic EPR signals with features at $g = 4.34$, 3.76, 2.00 and $g = 4.09$, 3.95, 2.0, respectively, also consistent with oxidative NO addition. The former contains a thiolate sulfur *trans* to the NO, whereas the latter contains a thiolate sulfur *cis* to the NO, showing that the $S = 3/2$ ground state is favored regardless of the orientation of the thiolate ligand.

As mentioned earlier, nitric oxide-bound complexes and enzymes belonging to the $\{\text{FeNO}\}^7$ class can be either low-spin ($S = 1/2$),^{28,35,37,41–43} or intermediate-spin ($S = 3/2$),^{11,12,29,44,51} depending on the ligand environment,¹⁸ and the ν_{NO} frequency for $S = 3/2$ Fe(III)–NO[–] compounds tends to be higher than those of $S = 1/2$ Fe(II)–NO• compounds. Although the ν_{NO} stretching frequency of **2** falls in the range typically seen with Fe(II)–NO• compounds, the $S = 3/2$ spin-state would be inconsistent with this electronic structure, and suggests that it is better described as containing a high-spin ($S = 5/2$) Fe^{III} antiferromagnetically coupled to NO[–] ($S = 1$).¹¹ DFT calculations support this description.³¹ The electronic absorption spectrum of **2**, which displays an intense band at $\lambda = 440(2560)$ nm, in MeCN (Figure 3), is also more consistent with this electronic description. Intense bands in this energy region are a characteristic feature of $S=3/2$ Fe^{III}–NO[–] systems, have been assigned as NO[–] (π^*) $\rightarrow \text{Fe}^{3+}(\text{d}\pi)$ charge transfer transitions,^{11,16,33,44} and reflect the highly covalent nature of the Fe–NO bond. An analogous, albeit weaker, band is seen in the red-brown NO-bound form of SOR at 475(530) nm.¹⁷

In addition to the EPR and electronic absorption spectral data described above, recently obtained sulfur K-edge X-ray absorption spectral (XAS) data, and DFT calculations,³¹ also support an Fe^{III}–NO[–] electronic description for **2**. Two intense pre-edge features (at 2470.1 and 2571.0 eV)³¹ are seen in the sulfur K-edge XAS spectrum of **2**, which can be assigned as sulfur 1s to metal ion 3d transitions. These transitions are in the region typically observed for thiolate-ligated Fe(III) complexes, and reflect the effective nuclear charge (Z_{eff}) at the metal ion, and thus the oxidation state. Based on the energy of these transitions, the iron oxidation state in complex **2** is estimated (using Slater-Zener rules)³¹ to be Fe^{+2.75}. The minimized DFT structure, and calculated MO diagram, shows that the ligand-field of **2** favors an $S = 5/2$ spin-state, and calculated Mulliken spin-densities show that the $S = 5/2$ Fe(III) and $S = 1$ NO[–] are antiferromagnetically coupled.³¹ Delocalization of electrons from the NO[–] to the Fe d-orbitals slightly decreases the Z_{eff} , and thus the oxidation state from Fe⁺³ to Fe^{+2.75}.³¹ Thus, sulfur K-edge XAS data and DFT calculations for **2** are most consistent with an electronic structure consisting of a high-spin ($S = 5/2$) Fe^{III}

antiferromagnetically coupled to NO^- ($S = 1$).³¹ The electronic structure of nitric oxide-bound SOR has also been shown to be best described in the same manner.¹⁷

Relevance to SOR

Nitric oxide binding to **1** probes the superoxide binding site of our biomimetic SOR analogue.^{1,9} The associated structural changes, as well as vibrational ν_{NO} parameter, electronic absorption data, DFT calculations,³¹ and sulfur K-edge XAS data³¹ for NO-bound **2**, are consistent with a mechanism involving the *oxidative addition* of NO (and thus superoxide) *cis* to the thiolate. A similar oxidative mechanism is involved in NO binding to SOR, except that NO binds *trans*, as opposed to *cis*, to the thiolate.¹⁷ To our knowledge, **2** is the first reported synthetic analogue of SOR-NO. Additional parallels can be drawn between NO-bound **2** and SOR-NO, including an identical $S = 3/2$ spin-state, and similar EPR and electronic absorption spectra. Given the *trans* influence of thiolates, and the push/pull effect of combining a π -donating thiolate with a π -accepting NO, one might expect *trans* cysteinylated SOR-NO ($\nu_{\text{NO}} = 1721 \text{ cm}^{-1}$)⁸ to have a lower, rather than higher ν_{NO} frequency relative to *cis* thiolate-ligated **2** ($\nu_{\text{NO}} = 1685 \text{ cm}^{-1}$). However, hydrogen-bonding between the cysteinyl sulfur and two highly conserved SOR residues (Scheme 3), causes the thiolate sulfur to donate less electron density to the NO (via the metal ion) in SOR, thereby increasing its ν_{NO} stretching frequency.⁴⁸ Similar effects have been noted with synthetic H-bonded thiolate/porphyrin-ligated ferric compounds.¹⁹ Hydrogen-bonding noticeably lengthens the SOR Fe-S^{cys} bond (to 2.46 Å),⁴⁸ and has also been suggested to modulate reactivity.^{49,50} Together these data would imply that electron-density at the thiolate sulfur, and its overlap with a metal ion orbital that is involved in bonding to NO, are more important than the thiolate's *trans* influence in determining the extent of N–O bond activation. With P450, a *trans* orientation of the cysteinyl ligand is proposed to promote O–O, as opposed to Fe–O bond cleavage, due to its *trans* effect (the “push effect”), and the low spin-state induced strengthening of the Fe–O bond.^{52,53} In contrast, Goldberg and Niviere have each concluded that *trans* thiolate donors weaken peroxo Fe–O bonds (in SOR⁵⁰ and related model complexes)³ without affecting O–O bond strengths, even with a low-spin peroxo. Recent DFT calculations suggest that a spin-crossing barrier would prevent H_2O_2 release from the low-spin Fe-OOH of P450.⁴⁹ However, low-spin ($S = 1/2$) $[\text{Fe}^{\text{III}}(\text{S}^{\text{Me}_2}\text{N}_4(\text{tren}))(\text{OOH})]^+$ has been shown to release H_2O_2 via a proton-induced mechanism.^{1,54} NO-bound $\{\text{FeNO}\}^7$ **2**, described herein, provides a less reduced analogue of metastable $\{\text{FeOOH}\}^9$ $[\text{Fe}^{\text{III}}(\text{S}^{\text{Me}_2}\text{N}_4(\text{tren}))(\text{OOH})]^+$. Given the π -accepting properties of NO (and not HOO^-), it's not surprising that the factors which affect thiolate-induced Fe–NO vs N–O bond activation are different from those which would affect Fe–O vs O–O bond activation in iron peroxos. With **2**, we were not able to detect any $\nu_{\text{Fe-NO}}$ stretches for **2**, and thereby assess anything about its Fe–NO bond strength relative to other $\{\text{FeNO}\}^7$ complexes lacking a thiolate ligand.

Proton-Dependent Reduction of Nitrite

In order to determine whether thiolate-ligated $[\text{Fe}^{\text{II}}(\text{S}^{\text{Me}_2}\text{N}_4(\text{tren}))]^+$ (**1**) is capable of reducing substrates other than superoxide and nitric oxide, we also examined its reactivity with nitrite (NO_2^-). Nitrite is a biologically-relevant^{55–57} π -acceptor ligand, which has been shown to form strong bonds to heme and non-heme Fe(II).^{58,59} If one equivalent of Bu_4NNO_2 is added to colorless **1** in THF, then no observable reaction occurs (as monitored by electronic absorption spectroscopy), until an external proton donor (such as NH_4^+) is added, indicating that the reaction is proton-dependent. Two equivalents of proton donor are required per equivalent of NO_2^- (Figure 4), and two products are observed in a 1:1 ratio (Scheme 4), each of which was identified via independent synthesis and structural characterization. One of the products, nitrite-bound $[\text{Fe}^{\text{III}}(\text{S}^{\text{Me}_2}\text{N}_4(\text{tren}))(\text{NO}_2)]^+$ (**7**) ($\lambda_{\text{max}} = 565(1458) \text{ nm}$; Figure S-5), can be synthesized directly (*vide infra*) via the addition of one

equiv of NO_2^- to $[\text{Fe}^{\text{III}}(\text{S}^{\text{Me}_2}\text{N}_4(\text{tren}))(\text{MeCN})]^{2+}$ (**8**). The other product, $[\text{Fe}(\text{S}^{\text{Me}_2}\text{N}_4(\text{tren})(\text{NO}))^+]^+$ (**2**) (*vide supra*, $\lambda_{\text{max}} = 440(2560)$ nm) contains a two electron reduced NO^- ligand (Scheme 5, eqn (1)). The rate of this reaction was found to depend on the pK_a of the proton donor, and takes 3 hrs (with 0.75 mM of **1** and 1.02 mM Bu_4NNO_2) to go to completion in MeOH, versus 3 min (with 0.56 mM of **1** and 0.78 mM Bu_4NNO_2) in THF with added NH_4^+ . Quantitative titrations monitored via electronic absorption spectroscopy (Figure S-6) establish that two equivalents of Fe(II) are consumed per equivalent of NO_2^- reduced (to afford Fe(III)- NO^- (**2**)). The other equiv of NO_2^- binds to the oxidized $[\text{Fe}^{\text{II}}(\text{S}^{\text{Me}_2}\text{N}_4(\text{tren}))]^+$ electron donor to afford **7** (Scheme 4). If a sacrificial reductant, such as NaBH_4 is added, then only one equiv of Fe(II) is required per equiv of NO_2^- reduced, and only one Fe-containing product $[\text{Fe}(\text{S}^{\text{Me}_2}\text{N}_4(\text{tren})(\text{NO}))^+]^+$ (**2**), forms (Figure S-7). Although no intermediates are detected in this reaction, even at temperatures as low as -78°C (Figure S-7), likely intermediates would include nitrite-bound ferrous $[\text{Fe}^{\text{II}}(\text{S}^{\text{Me}_2}\text{N}_4(\text{tren}))(\text{NO}_2)]$ (**9**; step (1) of Scheme 5), if the reaction occurs via an inner-sphere mechanism similar to that of **1**-promoted O_2^- reduction.^{1,9,54} An $\text{Fe}^{\text{II}}\text{-NO}_2^-$ intermediate is implicated in the mechanism of heme iron-containing nitrite reductase (NiR)-promoted NO_2^- reduction,⁵⁵ and related synthetic porphyrin-ligated $\text{Fe}^{\text{II}}\text{-NO}_2^-$ compounds have been isolated.⁵⁸ Extensive π -back bonding within the $\text{Fe}^{\text{II}}\text{-NO}_2^-$ fragment is proposed to facilitate heterolytic N-O bond cleavage by NiR.⁵⁵ Proton-induced heterolytic cleavage of the N-O bond (step (2) of Scheme 5) would initially convert proposed nitrite-bound intermediate $[\text{Fe}^{\text{II}}(\text{S}^{\text{Me}_2}\text{N}_4(\text{tren}))(\text{NO}_2)]$ (**9**) to $[\text{Fe}(\text{S}^{\text{Me}_2}\text{N}_4(\text{tren})(\text{NO}))]^{2+}$ (**10**) -- the less stable, oxidized $\{\text{FeNO}\}^6$ derivative of **2** (*vide supra*). An $\{\text{FeNO}\}^6$ species would contain nitrogen in either a +3 ($\text{Fe}(\text{II})\text{-NO}^+$) or +2 ($\text{Fe}(\text{III})\text{-NO}^\bullet$) oxidation state, meaning that N-O bond cleavage may or may not be reductive in this first N-O bond cleaving step (step (2) of Scheme 5). The necessity for two, as opposed to one proton implies that H_2O , as opposed to OH^- , is the preferred leaving group, as one would expect in THF solvent. A thiolate would help promote this step either by activating the N-O bond via the transfer of electron-density into the $\pi^*(\text{N-O})$ and/or $\sigma^*(\text{N-O})$ orbital(s), or by favoring the formation of a highly covalent Fe(III)-SR bond. It would also facilitate proton-induced heterolytic N-O bond cleavage by making the nitrite oxygen more basic. Thiols have previously been shown to reduce Fe(III)- NO_2^- species to afford an $\{\text{FeNO}\}^7$ product.⁶⁰ Both $\{\text{FeNO}\}^6$ and $\{\text{FeNO}\}^7$ species have been implicated as intermediates in NiR-promoted NO_2^- reduction, although these have proven difficult to detect.^{55,61} Consistent with its limited stability (*vide supra*), an intermediate $\{\text{FeNO}\}^6$ species,

$[\text{Fe}(\text{S}^{\text{Me}_2}\text{N}_4(\text{tren})(\text{NO}))]^{2+}$ (**10**), is not observed in NO_2^- reduction by **1**. Instead, a second equivalent of Fe(II) is consumed (step (3) of Scheme 5) in order to afford the more stable $\{\text{FeNO}\}^7$ species **2**. Thus, the net reaction promoted by **1** is the two electron, two proton reduction of nitrite to the nitric oxide anion NO^- (eqn (1)). The proton-dependence of NO_2^- reduction by **1** is similar that of superoxide reduction by **1**. Recent kinetics studies showed that initial protonation of the superoxide anion (O_2^-) is necessary in order to generate a more potent HO_2 oxidant.⁵⁴ With NO_2^- reduction (Scheme 5), protons are most likely required in order to promote heterolytic (as opposed to homolytic) cleavage of the N-O bond.⁵⁵ Given the instability of O_2^- , heterolytic N-O bond cleavage is unlikely to occur in the absence of protons. The pH-dependent redox potential of NO_2^- ($E_{1/2}(\text{pH}=14) = -0.46$ V; $E_{1/2}(\text{pH}=0) = +0.996$ V vs NHE), and its relative position on the basic, versus acidic, Frost diagrams,⁶² support this, and show that protons are essential in order to make NO_2^- reduction feasible under mild conditions.

Nitrite-bound $[\text{Fe}^{\text{III}}(\text{S}^{\text{Me}_2}\text{N}_4(\text{tren}))(\text{NO}_2)]^+$ (**7**; 1478 cm^{-1} ($\nu_{\text{asym}}(\text{NO}_2)$), 1362 cm^{-1} ($\nu_{\text{sym}}(\text{NO}_2)$) was independently synthesized via the addition of one equiv of NO_2^- to $[\text{Fe}^{\text{III}}(\text{S}^{\text{Me}_2}\text{N}_4(\text{tren}))(\text{MeCN})]^{2+}$ (**8**; Scheme 6), and crystallographically characterized. In protic solvents (e.g., MeOH), NO_2^- -bound **7** can also be generated via O_2 oxidation of NO-bound **2** (Figure S–8). In contrast to high-spin **2**, nitrite-bound **7**, which contains a more highly oxidized Fe^{3+} ion, is low-spin ($g = 2.17, 2.13, 1.98$ (Figure 5); $\mu_{\text{eff}}(\text{MeCN}, 303\text{ K}) = 1.76\text{ BM}$; $\mu_{\text{eff}}(\text{solid state}) = 1.72\text{ BM}$ (Figure S–9)), most likely due to the stronger, more covalent, Fe(III)–SR bond (Table 2). As shown in the ORTEP diagram of Figure 6, nitrite coordinates to **7** as the more commonly observed $\eta^1\text{-N}$ (nitro) (as opposed to $\eta^1\text{-O}$ (nitrito)) linkage isomer,⁶³ with NO_2^- *trans* to the imine, and *cis* to the thiolate. The Fe–S(1) bond of **7** is $0.1\text{ \AA}$ shorter than that of nitric oxide-ligated **2** (Table 2), and is closer to that of authentic ferric complexes **3** and **4**, indicating that Z_{eff} , ie, the metal ion oxidation state, is higher in **7** (+3) than in **2** (+2.75).³¹ Due to the less covalent nature of the iron-nitrogen bond, the Fe–N(4) distance is $0.193\text{ \AA}$ longer in NO_2^- -bound **7** versus NO-bound **2**. The Fe–N(4)–O bond angle is closer to that of an idealized sp^2 hybridized nitrogen N(4) in **7** ($123.9(9)^\circ$) versus **2** ($151.7(4)^\circ$).

Summary and Conclusions

In order to probe the superoxide (O_2^-) and dioxygen (O_2) binding site of our thiolate-ligated biomimetic SOR model $[\text{Fe}^{\text{II}}(\text{S}^{\text{Me}_2}\text{N}_4(\text{tren}))]^+$ (**1**), and gain insight regarding the mechanism of N–O (and thus O–O) bond activation, a nitric oxide– (NO) bound derivative $[\text{Fe}(\text{S}^{\text{Me}_2}\text{N}_4(\text{tren})(\text{NO}))]^+$ (**2**) was synthesized and spectroscopically characterized. The *electron-rich thiolate ligand was found to enhance N–O bond activation* resulting in an unusually low ν_{NO} frequency. Structural, magnetic, and spectroscopic (UV/vis, IR) data were all found to be consistent with a *mechanism* involving the *oxidative addition of NO* to **1**, and an electronic description for $\text{S}=3/2\{\text{FeNO}\}^7$ **2** consisting of a high-spin ($\text{S}=5/2$) Fe(III) antiferromagnetically coupled to NO^- ($\text{S}=1$). Recent sulfur K-edge XAS data and DFT calculations support this.³¹ Comparison of the properties of NO-bound **2** with those of NO-bound SOR shows that while the $\text{S}=3/2$ spin-state is identical, and the EPR and electronic absorption spectra are similar, the ν_{NO} frequency is considerably lower in **2** despite the presence of a *cis*, as opposed to *trans*, thiolate. Hydrogen bonding to the cysteinate is suggested to be responsible for attenuating the extent of N–O bond activation in SOR by decreasing the sulfur ligand's electron donating properties. In contrast, the $\nu_{\text{O-O}}$ frequency of SOR peroxo intermediate, and its analogues,³ is not affected by H-bonds to the cysteinate sulfur,⁵⁰ or other factors influencing the Fe–SR bond strength. These only influence the $\nu_{\text{Fe-O}}$ frequency. Given that a peroxo ligand has two additional antibonding $\pi^*(\text{O-O})$ electrons relative to an NO ligand, it can not π -accept electron density, and therefore has a less covalent Fe–X ($\text{X} = \text{OOH}, \text{NO}$) bond relative to an Fe–NO compound. Thiolate-ligated **1** was also shown to *promote the proton-dependent heterolytic cleavage of an N–O bond of nitrite* (NO_2^-) to afford NO^- -bound **2**, the overall reaction of which was shown to involve the $2\text{e}^-/2\text{H}^+$ reduction of NO_2^- . The mechanism of this reaction is proposed to involve both nitrite-bound ferrous $[\text{Fe}^{\text{II}}(\text{S}^{\text{Me}_2}\text{N}_4(\text{tren}))(\text{NO}_2)]$ and $\{\text{FeNO}\}^6$ intermediates similar to those implicated in the mechanism of nitrite reductase (NiR)-promoted NO_2^- reduction.⁵⁵

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

This work was supported by NIH (#GM 45881). We wish to thank Morgan Gleaves and Jessica Pikul for experimental assistance.

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Figure 1. ORTEP plot of the cation of [Fe(S^{Me}₂N₄(tren)(NO))][OTf] (**2**). All H-atoms have been omitted for clarity.

Figure 2. Low temperature (6.5 K) X-Band EPR of $[\text{Fe}(\text{S}^{\text{Me}_2}\text{N}_4(\text{tren})(\text{NO}))][\text{OTf}]$ (**2**) in MeCN/toluene (1:1) glass.

Figure 3.
Electronic absorption spectrum of **2** in MeCN (298 K).

Figure 4.

Reaction between colorless $[\text{Fe}(\text{S}^{\text{Me}_2}\text{N}_4(\text{tren}))][\text{OTf}]$ (**1**) and one equivalent of NO_2^- in THF (298 K) requires two equivalents of proton donor (NH_4^+), and affords two intensely colored products, **2** and **7**.

Figure 5. Low temperature (7 K) X-Band EPR spectrum of $[\text{Fe}^{\text{III}}(\text{S}^{\text{Me}_2}\text{N}_4(\text{tren}))(\text{NO}_2)]^+$ (7) in MeCN/toluene (1:1) glass.

Figure 6. ORTEP plot of the cation of [Fe^{III}(SMe₂N₄(tren)(NO₂)](PF₆) (**7**). All H-atoms have been omitted for clarity.

Scheme 1.
Synthesis of $[\text{Fe}(\text{S}^{\text{Me}_2}\text{N}_4(\text{tren})(\text{NO}))]^+$ (**2**).

Scheme 2.

Scheme 3.

Scheme 4.

Scheme 6.

Table 1

Crystal Data, Intensity Collections^a and Structure Refinement Parameters for [Fe(S^{Me}₂N₄(tren))(NO)](OTf) (**2**), and [Fe(III)(S^{Me}₂N₄(tren))(NO₂)](PF₆) (**7**).

	2	7
formula	FeC ₁₂ H ₂₅ F ₃ N ₅ O ₄ S ₂	FeC ₁₁ H ₂₅ F ₆ N ₅ O ₂ PS
MW	480.34	492.24
T, K	130(2)	130(2)
unit cell	orthorhombic	orthorhombic
a, Å	14.4552(2)	13.6260(5)
b, Å	8.5295(5)	8.5790(9)
c, Å	15.6713(4)	15.8870(10)
V, Å ³	1932.2(3)	1857.1(2)
Z	4	4
d(calc), g/cm ³	1.651	1.761
space group	<i>P n m a</i>	<i>P n m a</i>
R	0.0514 ^b	0.0707 ^b
R _w	0.1413 ^c	0.1865 ^d
GOF	1.025	1.018

^a Mo K α ($\lambda = 0.7107$ Å) radiation; graphite monochromator; -90 °C.

^b $R = \Sigma ||F_o| - |F_c|| / \Sigma |F_o|$.

^c $R_w = [\Sigma w(|F_o| - |F_c|)^2 / \Sigma w F_o^2]^{1/2}$, where $w^{-1} = [\sigma^2_{\text{count}} + (0.05 F^2)^2 / 4 F^2]$.

^d $R_w = \{\Sigma [w(F_o^2 - F_c^2)^2 / \Sigma w(F_o^2)^2]\}^{1/2}$; $w = 1/[\sigma^2(F_o^2) + (0.0620P)^2 + 0.000P]$, where $P = [F_o^2 + 2F_c^2]/3$.

Table 2

Selected Bond Distances (Å) and Bond Angles (deg) for the cations of $[\text{Fe}^{\text{II}}(\text{S}^{\text{Me2}}\text{N}_4(\text{tren}))]^+$ (PF_6^-) (**1**), $[\text{Fe}(\text{S}^{\text{Me2}}\text{N}_4(\text{tren}))(\text{NO})](\text{OTf})^-$ (**2**), $[\text{Fe}^{\text{III}}(\text{S}^{\text{Me2}}\text{N}_4(\text{tren}))(\text{OAc})]^+$ (BPh_4^-) (**3**)²⁴, $[\text{Fe}^{\text{III}}(\text{S}^{\text{Me2}}\text{N}_4(\text{tren}))(\text{N}_3)]^+$ (PF_6^-) (**4**)²⁴, and $[\text{Fe}^{\text{III}}(\text{S}^{\text{Me2}}\text{N}_4(\text{tren}))(\text{NO}_2)](\text{PF}_6^-)$ (**7**).

	1	2	3	4	7
Fe-S(1)	2.328(1)	2.278(1)	2.168(2)	2.176(2)	2.177(3)
Fe-N(1)	2.091(3)	2.099(3)	1.910(6)	1.917(6)	1.951(9)
Fe-N(2)	2.268(3)	2.211(3)	2.050(6)	2.002(6)	2.053(8)
Fe-N(3)	2.131(3)	2.198(3)	2.003(6)	2.011(5)	2.036(6)&
Fe-N(4)	2.117(3)	2.198(3)	2.000(5)	2.002(5)	2.036(6)&
Fe-X	N/A	1.770(3)	1.972(5)	1.999(6)	1.963(10)
N-O(1)	N/A	1.118(6)	N/A	N/A	1.218(13)
N-O(2)	N/A	N/A	N/A	N/A	1.171(13)
Fe-N-O	N/A	151.7(4)	N/A	N/A	123.9(9)
S(1)-Fe-N(1)	84.02(8)	82.57(8)	87.0(2)	86.8(2)	85.4(2)
S(1)-Fe-N(2)	163.02(7)	162.45(9)	172.1(2)	172.1(2)	170.0(3)
N(1)-Fe-X	N/A	177.5(1)	174.9(2)	176.7(2)	176.3(4)
X-Fe-S(1)	N/A	95.0(1)	94.96(16)	95.5(2)	90.9(3)
N(3)-Fe-N(4)	115.2(1)	154.3(1)	163.4(2)	165.6(2)	163.5(4)

X= NO (**2**), NO₂⁻ (**7**), OAc⁻ (**3**), and N₃⁻ (**4**).

& Because **2** and **7** each lie on a crystallographic mirror plane that relates N(3) and N(3'), there are only four, as opposed to five, symmetry-independent nitrogen atoms. For the purposes of comparison N(3') for structures **2** and **7** is listed as N(4) in the Table above, and the nitric oxide and nitrite nitrogen for structures **2** and **7** is listed as X.