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Phenol Nitration Induced by an $\{Fe(NO)_2\}^{10}$ Dinitrosyl Iron Complex

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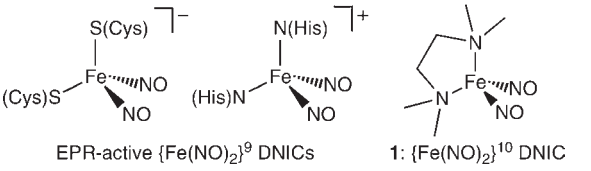
Supporting Information

ABSTRACT: Cellular dinitrosyl iron complexes (DNICs) have long been considered NO carriers. Although other physiological roles of DNICs have been postulated, their chemical functionality outside of NO transfer has not been demonstrated thus far. Here we report the unprecedented dioxygen reactivity of a N-bound $\{Fe(NO)_2\}^{10}$ DNIC, [Fe(TMEDA)$(NO)_2$] (**1**). In the presence of O_2, **1** becomes a nitrating agent that converts 2,4,-di-*tert*-butylphenol to 2,4-di-*tert*-butyl-6-nitrophenol via formation of a putative iron-peroxynitrite [Fe(TMEDA)(NO)(ONOO)] (**2**) that is stable below −80 °C. Iron K-edge X-ray absorption spectroscopy on **2** supports a five-coordinated metal center with a bound peroxynitrite in a cyclic bidentate fashion. The peroxynitrite ligand of **2** readily decays at increased temperature or under illumination. These results suggest that DNICs could have multiple physiological or deleterious roles, including that of cellular nitrating agents.

Dinitrosyl iron complexes (DNICs), Chart 1, are naturally occurring iron species that are generated from the reactions of nitric oxide (NO) with cellular nonheme iron species such as iron−sulfur clusters.[1,2] A series of S- or N-bound DNICs have been reported[2−6] since the initial discovery[7] of a cysteine-bound DNIC formulated as $[Fe(NO)_2(SR)_2]^-$, an $\{Fe(NO)_2\}^9$ species in the Enemark−Feltham notation.[8] Although EPR-active S-bound $\{Fe(NO)_2\}^9$ DNICs are more common, N- or O-bound DNICs have been also observed.[9]

Several physiological roles of DNICs have been suggested, including storage and transfer of NO.[1,10] However, the nature of the chemistry that allows DNICs to play these physiological roles is not well understood. A notable recent report from the Lippard group suggests that the NO-donating ability of a N-bound DNIC might be $\{Fe(NO)_2\}^9/\{Fe(NO)_2\}^{10}$ redox-dependent.[6] While such a proposal offers important chemical insights, we were intrigued by the highly reducing nature of $\{Fe(NO)_2\}^{10}$ DNICs, which may suggest a rich chemistry between these motifs and oxidants such as O_2. Such reactivity could shed light on undiscovered physiological or deleterious roles of DNICs. Herein, we describe the unprecedented dioxygen reactivity of an N-bound $\{Fe(NO)_2\}^{10}$ DNIC, [Fe(TMEDA)$(NO)_2$] (**1**), where TMEDA = *N,N,N′,N′*-tetramethylethylenediamine (Chart 1), and we demonstrate the formation of an intermediate that leads to the nitration of phenol.

Chart 1. Dinitrosyl Iron Complexes (DNICs)



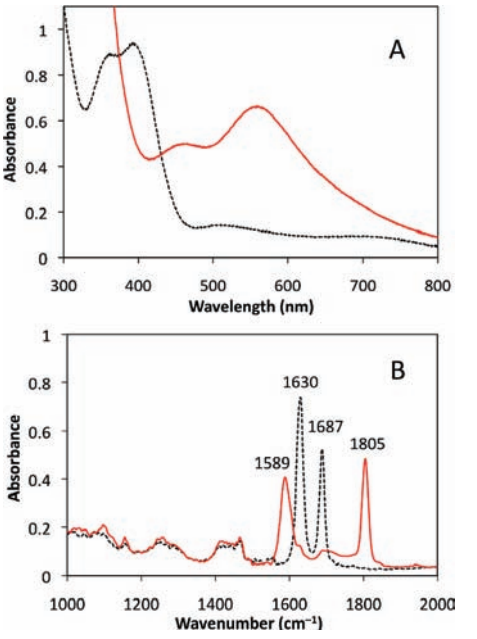


Figure 1. UV−vis (A) and IR (B) spectra of [Fe(TMEDA)$(NO)_2$] (**1**) (black dashed line) and of the putative intermediate [Fe(TMEDA)(NO)(ONOO)] (**2**) (red) at −80 °C in dichloromethane.

Compound **1** was synthesized as previously reported by Hung et al.[5d] Bubbling O_2 through a solution of **1** in dichloromethane at −80 °C leads to the formation of an EPR-silent, dark purple complex with absorption bands at 460 (ε = 420 M^{-1} cm^{-1}) and 560 nm (ε = 580 M^{-1} cm^{-1}), Figure 1A. This purple complex is stable below −80 °C but decomposes to an orange, insoluble

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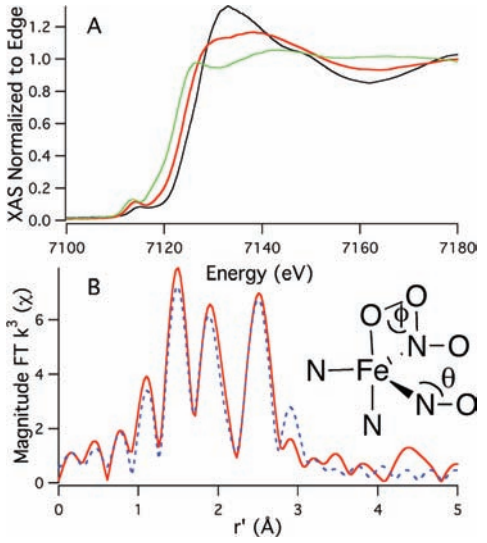


Figure 2. (A) XANES region of the XAS for **1** (green), **2** (red), and the thermal decomposition product (black). (B) Experimental (red solid line) and simulated (blue dashed line) magnitude FT k^3 EXAFS data for **2**. Best fit includes: shell #1: 2 N scatterers, $r = 2.14(1)$ Å, $\sigma^2 = 0.003(2)$ Å^2; O_2NO shell: 1 O_2NO scatterer, $r_1 = 1.91(1)$ Å, $r_2 = 1.91$ Å (restrained), $\sigma^2 = 0.002(1)$ Å^2, $\phi = 98(7)°$; NO shell: 1 NO scatterer, $r = 1.67(1)$ Å; $\sigma^2 = 0.008(2)$ Å^2; $\theta = 172(2)°$. $E_o = 7120.4$ eV. $\varepsilon^2 = 0.57$.

precipitate upon warming.[11,12] IR monitoring of this O_2-reaction also shows the generation of a new quasi-stable species with the appearance of two new ν_{NO} peaks at 1589 and 1805 cm^{-1} concomitant with the disappearance of the ν_{NO} of **1** at 1630 and 1687 cm^{-1} (Figure 1B).[13] No other significant change occurs in the midfrequency region of the IR spectra. This transformation is very different from what was observed by Tonzetich et al.[6] in the case of another N-bound $\{Fe(NO)_2\}^{10}$ DNIC with a monoanionic bidentate β-diketiminate ligand. In that study, air exposure led to the one-electron oxidation of the $\{Fe(NO)_2\}^{10}$ DNIC to an EPR-active $\{Fe(NO)_2\}^9$ DNIC, which was also signified by >130 cm^{-1} upshifts of the two IR-active NO stretching frequencies. Here, the intermediate generated from **1**/O_2 is EPR silent and exhibits an up- (1805 cm^{-1}) and a downshifted (1589 cm^{-1}) NO band. These data are inconsistent with the simple one-electron oxidation of **1**.[14] The possibility of generating a five-coordinate superoxide (O_2^-) adduct, [Fe(TMEDA)-$(NO)_2(O_2)$], has been considered. Although such a species is not known, an iodide (I^-) analogue, [Fe(TMEDA)$(NO)_2(I)$], has been reported to have two upshifted ν_{NO} frequencies higher than 1700 cm^{-1},[15] which is again different from what we observe in the **1**/O_2 reaction.[16] Interestingly, the ν_{NO} at 1589 cm^{-1} closely matches those from known *trans*-peroxynitrite species, O=NOOM, where M = Li, Na, K.[17] These IR and EPR characteristics, along with the EXAFS data (*vide infra*), led us to consider the intermediate to be a peroxynitrite bound iron mononitrosyl species, [Fe(TMEDA)-(NO)(ONOO)] (**2**).[18]

Iron K-edge X-ray absorption spectroscopy was used to further probe complex **2**. Comparison of the edge energies of **1** and **2** shows a shift by +1.8(4) eV upon O_2 exposure to cold solutions of **1** (Figure 2A), which is consistent with the formal oxidation of **1** by one electron. The Fe 1(s) → 3(d) transition found in the pre-edge region of **2** has a peak area slightly larger than that corresponding to **1** (35(1) vs 36(1) eV × % relative to the edge height). This is most consistent with **2** having an Fe-center contained in a noncentrosymmetric coordination environment. The EXAFS region for **2** is best

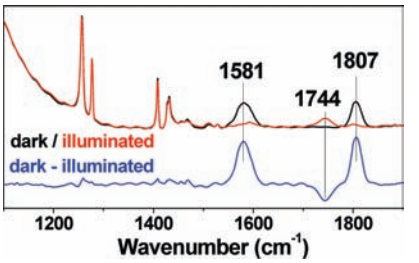
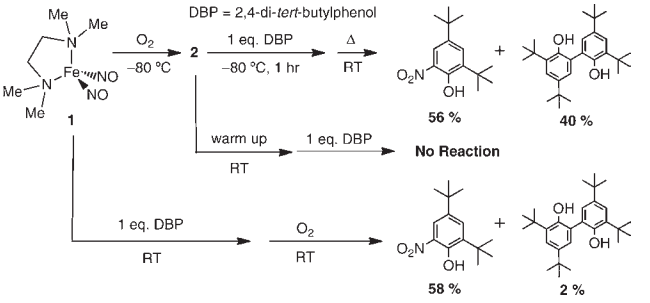


Figure 3. IR spectra of a [Fe(TMEDA)(NO)(ONOO)] (**2**) in CH_2Cl_2 at 11 K before (black) and after illumination (red). The dark minus illuminated difference spectrum is also shown (blue). Sharp IR bands are from dichloromethane.

Scheme 1



modeled as a five-coordinate Fe-species with a coordinated bidentate O_2NO^- moiety (Figure 2B). We find strong multiple-scattering (MS) pathways originating from this cyclic O_2NO^- ligand (average N/O distance 1.91(1) Å; see inset Figure 2B). In addition, strong MS pathways are also found originating from the NO ligand (1.67(1) Å). Two additional N-scatters derived from the TMEDA ligand are also observed. The thermal decomposition product resulting from warming of **2** to room temperature yields a further shift in the edge energy of +2.6(3) eV, consistent with the production of an $Fe^{2+/3+}$ species (Figure 2A).

Attempts at characterization of **2** by resonance Raman spectroscopy were hampered by photosensitivity. The decomposition of **2** with white light was monitored at −90 °C by UV−vis spectroscopy but failed to reveal new absorption features (Figure S5). In the IR, continuous white-light illumination of **2** leads to the progressive and irreversible bleaching of the 1581 and 1807 cm^{-1} bands with the concomitant appearance of a single band at 1744 cm^{-1} (Figure 3). The loss of the IR band at 1581 cm^{-1} and appearance of a new ν_{NO} at 1744 cm^{-1} suggest that illumination of **2** might lead to the formation of a new iron-mononitrosyl species after photochemistry at the peroxynitrite ligand. Like **2**, the photoproduct is EPR silent at 10 K (data not shown). While the nature of the photoproduct remains in doubt,[19] the photoactive behavior of **2** is reminiscent of $[Co(CN)_5(ONOO)]^{3-}$, where photolysis destroys the coordinated $ONOO^-$.[20]

The presence of peroxynitrite is often indicated by oxidation and/or nitration chemistry especially with phenolic substrates.[21] When 1 equiv of 2,4-di-*tert*-butylphenol (DBP) is added to **2** and the reaction mixture is subsequently warmed to room temperature, 2,4-di-*tert*-butyl-6-nitrophenol (NO_2-DBP) is observed along with the oxidative coupling product 2,2′-dihydroxy-3,3′,5,5′-tetra-*tert*-butyl-1,1′-biphenyl (Scheme 1).[22] These reaction products do not form when DBP is added *after* warming the

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solution of **2** to room temperature, signifying that intermediate **2** is a crucial species in phenol nitration and oxidation. At room temperature, **2** is too unstable to be monitored by regular UV−vis spectroscopy, although a glimpse of purple color can be seen momentarily. Despite the short lifetime of **2** at room temperature, when O_2 is added to a mixture of **1** and DBP (1 equiv) at room temperature nitration chemistry still occurs, yielding NO_2-DBP, while only a small amount (2%) of the oxidative coupling product is generated. This indicates that **1**/O_2 induces nitration much more efficiently than oxidation at room temperature.[23] When $^{18}O_2$ is used, approximately 50% of the ^{18}O atom incorporates into the substrate. GC-MS analysis of NO_2-DBP displays a distribution of $^{18}O_2$N-DBP, $^{18/16}O_2$N-DBP, and $^{16}O_2$N-DBP in a ratio of 30(2)%, 48(1)%, and 22(2)%.[12] Although the mechanistic investigation of phenol nitration by **2** is beyond the scope of this report, the O atom isotope distribution in NO_2−BDP warrants future mechanistic studies and likely involves the cleavage of peroxynitrite and participation of the other NO.[24]

The nitration of biological phenols, such as seen in protein tyrosine nitration (PTN), is an important posttranslational modification associated with various pathological conditions including inflammatory, neurodegenerative, and cardiovascular diseases.[25] Although elusive, the current view[25d] of PTN suggests that different types of cellular nitrating agents could be responsible for its specificity at various sites. Two major ways to generate PTN are known.[25] One is through peroxynitrite ($ONOO^-$) that is formed from nitric oxide (NO) and superoxide (O_2^-). The other involves reactions of heme peroxidases with hydrogen peroxide and nitrite (NO_2^-). The reactivity of the $\{Fe(NO)_2\}^{10}$ DNIC we report herein suggests that cellular DNICs could provide a new route to generate PTN; the DNIC derived peroxynitrite moiety in **2** may directly nitrate the phenol or biological tyrosine (via homolytic cleavage to •O(H) + $•NO_2$) or act as a $•NO_2$ generator (where 2 equiv may lead to ArOH nitration).[21,25] The results also agree with several literature examples of the oxidation chemistry of metal-nitrosyls,[26] though observation of metal-peroxynitrite is rare.[26d,26e] It is conceivable that small molecule metal species such as DNICs act as mobile nitrating agents in cells.

In summary, we have described the unprecedented O_2 reactivity of an $\{Fe(NO)_2\}^{10}$ iron-dinitrosyl complex [Fe(TMEDA)-$(NO)_2$] (**1**). In the presence of O_2, **1** becomes a potent nitrating agent via formation of a putative iron-peroxynitrite species. The O_2 reactivity of **1** demonstrated here suggests that the physiological functions of DNICs are not limited to NO storage and transfer and deserve further study.

■ ASSOCIATED CONTENT

Supporting Information. Experimental details concerning spectroscopy, reaction product characterization and quantification, isotope labeling, electrochemistry, and nitrite detection. This material is available free of charge via the Internet at http://pubs.acs.org.

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(11) The presence of nitrite (NO_2^-), Fe, and TMEDA in the precipitate has been qualitatively confirmed.[12] Further speculation concerning the nature of decomposition product(s) is not warranted.
(12) See Supporting Information.
(13) Synthesis of the intermediate using ^{15}NO shows a clear IR shift to lower energy supporting the ν_{NO} assignments and formation of putative [Fe(TMEDA)(NO)(ONOO)] (**2**) (vide infra).[12]
(14) The reversible $\{Fe(NO)_2\}^{9/10}$ redox behavior of **1** is observed in cyclic voltammetry, with $E_{1/2} = -0.527$ V (vs ferrocene/ferrocenium). Chemical oxidation of **1** by I_2 has been shown to yield a five-coordinate compound [Fe(TMEDA)$(NO)_2$I].[15] Attempts to isolate a four-coordinate counterpart, $[Fe(TMEDA)(NO)_2]^+$, were not successful probably due to its strong preference to be five-coordinate, as was previously discussed.[15]
(15) ν_{NO} = 1773 and 1719 cm^{-1} in diethyl ether. Chen, C.-H.; Ho, Y.-C.; Lee, G.-H. *J. Organomet. Chem.* **2009**, *694*, 3395–3400.
(16) Even more evidence to disfavor a superoxide adduct formation includes the lack of an isotope sensitive ν_{O-O} in the region of ~1100 cm^{-1} (Figure 1b) and the inconsistent EXAFS data for such a model. See Supporting Information' for alternative EXAFS fitting results.

(17) ν_{NO} = 1528−1581 cm^{-1} for *trans*-MOONO, where M = K, Na, and Li. Lo, W. J.; Lee, Y. P.; Tsai, J. H. M.; Tsai, H. H.; Hamilton, T. P.; Harrison, J. G.; Beckman, J. S. *J. Chem. Phys.* **1995**, *103*, 4026–4034.

(18) The two ν_{NO} of **2** at 1589 and 1805 cm^{-1} remain unchanged upon $^{18}O_2$ substitution. Characterization of **2** by ESI-MS was attempted without success due to the insufficient stability of **2**.

(19) The EPR silent character of the photoproduct suggests a diamagnetic or integer spin species. This electronic structure may reflect the iron-mononitrosyl complex or the overall spin of this complex coupled with the nearby product of the photolyzed peroxynitrite. For an analogous situation in a metalloprotein, see the EPR studies of $\{FeNO\}^6$ myoglobin complexes, where the photolyzed NO (S =1/2) remains spin-coupled with the high-spin iron(III) (S = 5/2). Hori, H.; Ikeda-Saito, M.; Lang, G.; Yonetani, T. *J. Biol. Chem.* **1990**, *265*, 15028–15033. Hori, H.; Masuya, F.; Dou, Y.; Ikeda-Saito, M. *J. Inorg. Biochem.* **2000**, *82*, 181–187.

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(22) Future investigations include the reaction with Tyr as the substrate using water-soluble analogs of **1**.

(23) This may also indicate that the oxidative coupling product can be generated from other low temperature stable species besides **2**.

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