Reactivity of Dinitrosyl

Iron Complexes with Oxygen


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**Abstract**

Several dinitrosyl iron complexes were synthesized and characterized by cyclic voltammetry and IR, NMR, and UV-Vis spectroscopy. The reactivity of these compounds with oxygen was then explored. Several of the reactions went through an unstable, UV-Vis featureless first intermediate that could only be observed at low temperature (-80°C) before reaching a second, low-temperature-stable intermediate. The lifetimes of the first intermediates were determined, and the trends observed. In general, the lower the $E_{1/2}$ of the DNIC in question, the more stable the first intermediate.

**Introduction**

Dinitrosyl iron complexes (DNICs) are a class of compounds defined by an iron center and two nitric oxide (NO) ligands. DNICs occur in biological systems, where they are primarily used for storage and transportation of NO.[1-2] Due to the ambiguous electronic structure of the NO ligand, DNICs are usually described using the Enemark-Feltham notation, which has the form $\{Fe(NO)_x\}^n$, where x is the total number of NO ligands, and n is the sum of iron d-electrons and NO $\pi^*$ electrons.

Physiological roles, such as transferring and storing nitric oxide, have been suggested for DNICs, but the chemistry behind their reactivity is not well understood.[3-4] Although the most common DNICs have S-bound ligands, there are many O- and N-bound DNICs known.[5-17] The specific DNICs examined here are neutral, N-bound $\{Fe(NO)_2\}^{10}$ species. Previous research from our group has shown that these complexes can perform phenol nitration in the presence of oxygen.[18] This reaction was indicated to proceed via a low-temperature-stable peroxynitrite intermediate. Peroxynitrite is a generator of protein tyrosine nitration, a biologically significant reaction associated with pathological conditions such as cardiovascular and neurodegenerative diseases.[18,19]

The primary purpose of this project has been to elucidate more information on the reactivity of neutral, N-bound $\{Fe(NO)_2\}^{10}$ DNICs with dioxygen. We have synthesized many of these DNICs and we were only able to spectroscopically observe the peroxynitrite intermediate with [Fe(TMEDA)(NO)$_2$]. Thus, we wondered whether different ligands might stabilize other, previously unobserved low-temperature stable intermediates. Specifically, we want to determine the effects of different bite angles and electronics of various N-donor ligands on this reactivity. To this end, we synthesized and characterized several new DNICs. We then explored the correlation between the properties of different ligands and the stability of low temperature intermediates resulting from reactions between these DNICs and O$_2$.

## Experimental Section

### Materials and Methods

All reactions were performed under an inert atmosphere, either in an MBraun glovebox or using standard Schlenk line techniques. Solvents were purified by passing through alumina columns under an Ar atmosphere (MBraun solvent purification system), with the exception of propionitrile, which was dried over calcium hydride, distilled, and degassed. All solvents were stored over 4 Å molecular sieves prior to use. 2,4-Di-*tert*-butylphenol, sodium hydroxide, sodium nitrite, iron pentacarbonyl, calcium chloride, phosphorus pentoxide, 4-dimethylaminopyridine, 4-methoxypyridine, 1-methylimidazole, picoline, and 4-methyl-nitropyridine were purchased from Sigma Aldrich and used as received. UV-Vis spectroscopy was carried out using a Varian Cary 50 Bio spectrometer equipped with a 2 mm Hellma All-Quartz Immersion probe. IR spectroscopy was carried out using a Bruker Tensor 27 FT-IR spectrometer. GC-MS data were recorded using a Hewlett-Packard (Agilent) GCD 1800C GC-

MS spectrometer. Electrochemical data was collected with a CV-50W, Version 2.3 from

Bioanalytical Systems, Inc. A 3-electrode system composed of a glassy carbon working electrode

(circular, 3 mm diameter), a $Ag/Ag^+$ reference electrode, and a platinum wire auxiliary electrode

was employed. All cyclic voltammetry experiments were conducted with 0.10 M $Bu_4NPF_6$ as

supporting electrolyte at room temperature under $N_2$ atmosphere, and the data were referred to

ferrocene/ferrocenium couple at 0.00 V. CV data was collected for DNICs at .01 M

concentration. $[Fe(CO)_2(NO)_2]$[20] and all DNICS were synthesized by variations on published
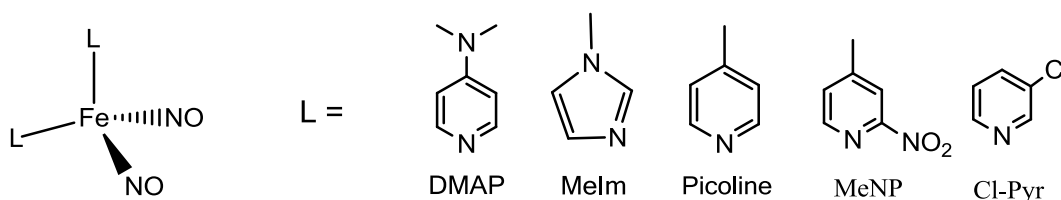
procedure.[2]



**Chart 1.** DNICs studied. The ligands correspond to compounds **1**, **2**, **3**, **4**, and **5** respectively.

## Synthesis of [Fe(DMAP)₂(NO)₂] (1)

71 mg (.58 mmol) of 4-dimethylaminopyridine (DMAP) was first dissolved in

acetonitrile and added to solution of 50 mg (.29 mmol) $Fe(CO)_2(NO)_2$. Within seconds of the

DMAP addition, the solution changed color from dark orange to dark green. The solution was

left to stir for 20 minutes before being pipetted into a vial and dried. The solid left behind was a

green, grainy powder. IR spectroscopy of the compound indicated characteristic $\nu_{NO}$ at 1624 and

1671 $cm^{-1}$. UV-Vis spectroscopy showed a peak at 372 nm. An NMR spectrum taken in

deuterated acetonitrile showed peaks at 8.01 (m), 6.55 (m), and 3.00 (s) ppm.

## Synthesis of [Fe(MeIm)₂(NO)₂] (2)

46 μL (47 mg, .58 mmol) of 1-methylimidazole (MeIm) was added to solution of 50 mg (.29 mmol) $Fe(CO)_2(NO)_2$ in acetonitrile. After addition of MeIm, the solution slowly changed color from dark orange to dark, muddy green. The solution was left to stir for 1 hour before being pipetted into a vial and dried. The solid left behind was a green, grainy powder. IR spectroscopy of the compound indicated characteristic $\nu_{NO}$ at 1618 and 1671 cm$^{-1}$. UV-Vis spectroscopy showed a shoulder at approximately 400 nm. An NMR spectrum taken in deuterated acetonitrile showed peaks at 7.52 (m), 7.08 (m), 6.85 (m), and 3.68 (s) ppm.

## Synthesis of [Fe(Me-Pyr)$_2$(NO)$_2$] (3)

57 μL (54 mg, .58 mmol) of 4-methylpyridine (Me-Pyr, picoline) was added to solution of 50 mg (.29 mmol) $Fe(CO)_2(NO)_2$ in acetonitrile. After addition of picoline, the solution rapidly changed color from dark orange to dark green. The solution was left to stir for 30 minutes before being pipetted into a vial and dried. The solid left behind was a green, grainy powder. IR spectroscopy of the compound indicated characteristic $\nu_{NO}$ at 1647 and 1689 cm$^{-1}$. UV-Vis spectroscopy showed a peak at 420 nm. An NMR spectrum taken in deuterated acetonitrile showed peaks at 8.46 (m), 7.21 (m), and 2.36 (s) ppm.

## Synthesis of [Fe(MeNP)$_2$(NO)$_2$] (4)

80 mg (.58 mmol) of 4-methyl-2-nitropyridine (MeNP) was dissolved in acetonitrile and added to solution of 50 mg (.29 mmol) $Fe(CO)_2(NO)_2$. After addition of MeNP, the solution slowly changed color from dark orange to light brownish-green. The solution was left to stir for 1 hour before being pipetted into a vial and dried. The solid left behind was a brownish-green, grainy powder. IR spectroscopy of the compound indicated characteristic $\nu_{NO}$ at 1786 and 1718

cm$^{-1}$. UV-Vis spectroscopy showed a peak at 298 nm. An NMR spectrum taken in deuterated acetonitrile showed peaks at 8.81 (d), 7.96 (d), 7.87 (m), and 2.70 (s) ppm.

## Synthesis of [Fe(Cl-Pyr)$_2$(NO)$_2$] (5)

55 μL (65 mg, .58 mmol) of 3-Chloropyradine (Cl-Pyr) was added to solution of 50 mg (.29 mmol) Fe(CO)$_2$(NO)$_2$ in acetonitrile. After addition of Cl-Pyr, the solution changed color from dark orange to a darker brown. The solution was left to stir for 1 hour and then dried in the Schlenk flask. IR spectroscopy of the compound indicated characteristic $\nu_{NO}$ at 1691 and 1637 cm$^{-1}$. UV-Vis spectroscopy showed a peak at 262 nm. An NMR spectrum taken in deuterated acetonitrile showed peaks at 8.62 (m), 8.53 (m), 7.82 (m) and 7.39 (m) ppm.

## UV-Vis Spectroscopy

Low temperature UV-Vis monitoring was carried out as follows: First, a background was taken of 5 mL propionitrile under argon at -80$^{o}$C. A solution of the DNIC being studied was prepared from 2-4 mg DNIC and 3 mL propionitrile. The solution was added to the flask (under positive argon pressure), and a scan was taken. O$_2$ gas was bubbled through the solution for 5 seconds, and the solution was scanned every 2-3 minutes until no change was detected, after which it was left to sit for 5 minutes before being scanned again. More O$_2$ was then added to the flask, and the process repeated until no change was detected whatsoever. From this point, the solution was warmed to room temperature and then cooled back down to -80$^{o}$C, and was scanned one final time. Using the same procedure, except that O$_2$ gas was only added once, the spectrometer was set to automatically scan every 2 minutes, allowing for a more accurate estimate of the duration of the observed intermediate species' stability. All DNICs were tested for their ability to perform phenol nitration.
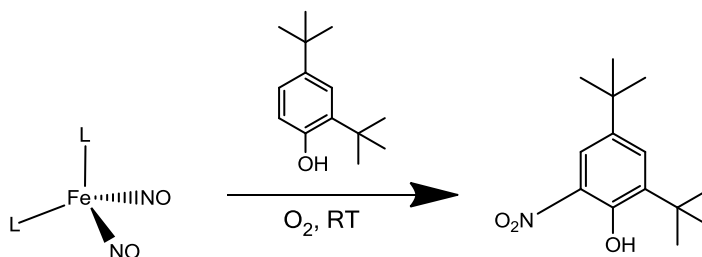
**Scheme 1.** Phenol nitration reaction

This was done by weighing out 5 mg of the complex and dissolving it in acetonitrile with one equivalent (3.1-3.5 mg, .015-.017 mmol depending on DNIC) of 2,4-di-*tert*-butylphenol (DBP). Oxygen was added, and the solution was left to sit for 30 minutes. It was then filtered through silica, and the resulting solution was analyzed by GC-MS, looking specifically for evidence of phenol nitration of DBP.

## Results and Discussion

### [Fe(DMAP)$_2$(NO)$_2$] (1)

A phenol nitration experiment indicated that the compound was capable of phenol nitration in the presence of oxygen. This suggests that it forms a peroxynitrite intermediate, even though the intermediate has not been observed spectroscopically. Cyclic voltammetry indicated $E_{1/2}$ = -725 mV in acetonitrile (Table 1). In order to further explore the O$_2$ reactivity of **1**, a room temperature UV-Vis was taken of a solution of the compound in acetonitrile. Additional of oxygen resulted in the loss of the initial peak at 372 nm, and the formation of a peak at 256 nm, so a low-temperature UV-Vis experiment was carried out following the procedures in the Materials and Methods section.
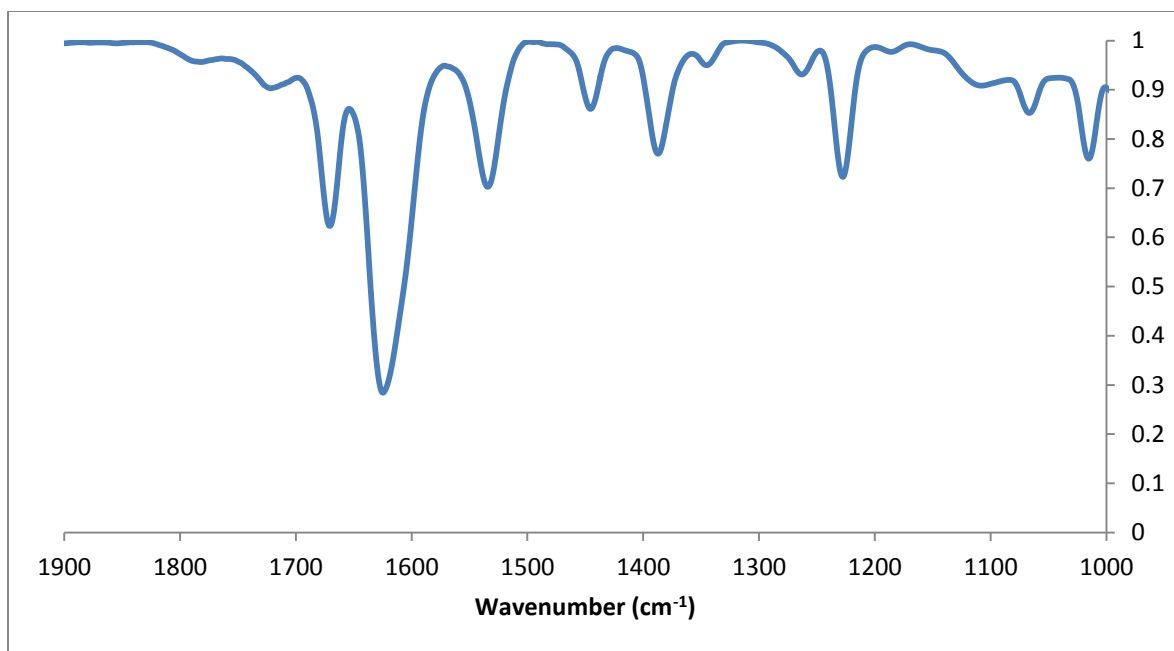
**Figure 1.** IR spectrum of **1**, showing characteristic $v_{NO}$ at 1624 and 1671 cm$^{-1}$.

The low-temperature UV-Vis experiment indicated the presence of two intermediates in the reaction between **1** and $O_2$. Immediately after the addition of oxygen, the spectrum became effectively featureless. After 2 minutes, a UV-Vis feature developed at 398 nm and remained stable at -80°C. When the solution showed no change for 15 minutes, it was warmed to room temperature and cooled back to -80°C, at which point an entirely different spectrum was recorded. This indicates that observed low temperature species were not the final decomposition products, but possible intermediates in the DNIC's reactivity with dioxygen.

In order to more accurately estimate the reaction time of the DNIC and oxygen, a kinetic experiment was carried out using the same setup as the low temperature UV-Vis, but scanning exactly every two minutes to remove human error.
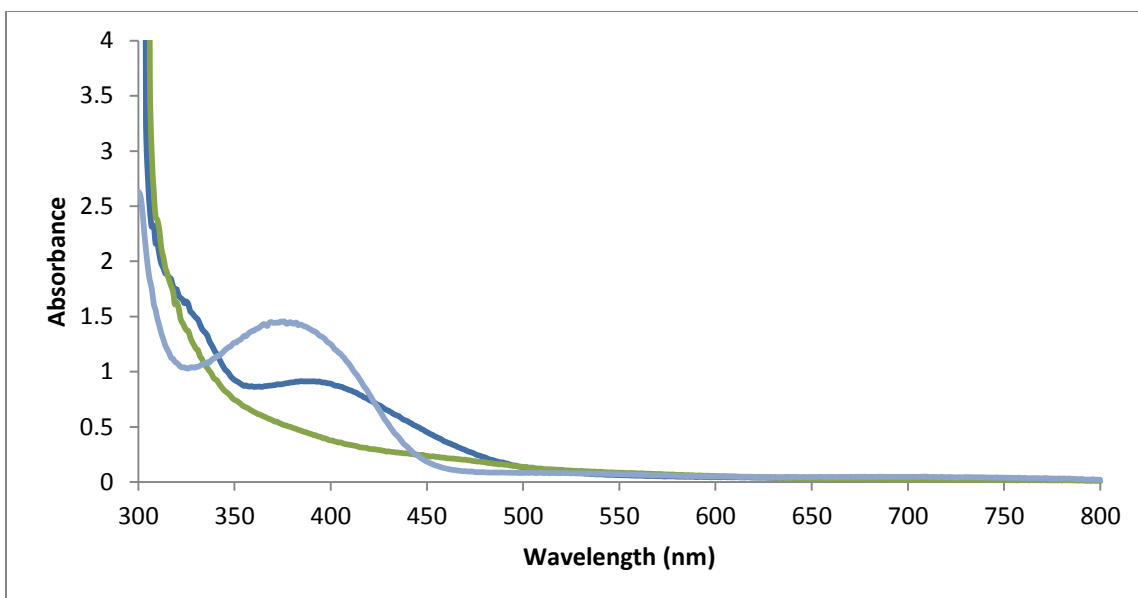
**Figure 2.** UV-Vis spectra of reaction of **1** with oxygen at -80°C in propionitrile. Light blue is initial spectrum (peak at 372 nm), green is 30 seconds after addition of $O_2$, dark blue is spectrum at 2 hours (peak at 398 nm). Not pictured: final decomposition product (peak at 256 nm).

From these results it was determined that the first intermediate was present for 3-5 minutes.

## [Fe(MeIm)₂(NO)₂] (2)

Phenol nitration was determined by GC-MS to occur when the compound was exposed to air in the presence of DBP. Cyclic voltammetry showed $E_{1/2}$ = -734 mV in acetonitrile (Table 1).

As the RT UV-Vis showed a change in spectrum with the loss of the shoulder at 400 nm and the formation of a peak at 315 nm, a low-temperature UV-Vis experiment was carried out using the same procedure as before. Once again, the reaction showed evidence of two intermediates, the first effectively featureless. Again, the second intermediate was effectively stable at -80°C but decomposed at room temperature. This second intermediate was indicated by the formation of a shoulder at 364 nm. Unlike in previous experiments, however, the first

intermediate was very short-lived, only being present for a single scan of the device. A kinetic study, undertaken with the same procedures as above, indicated that the lifespan of the first intermediate was less than one minute.
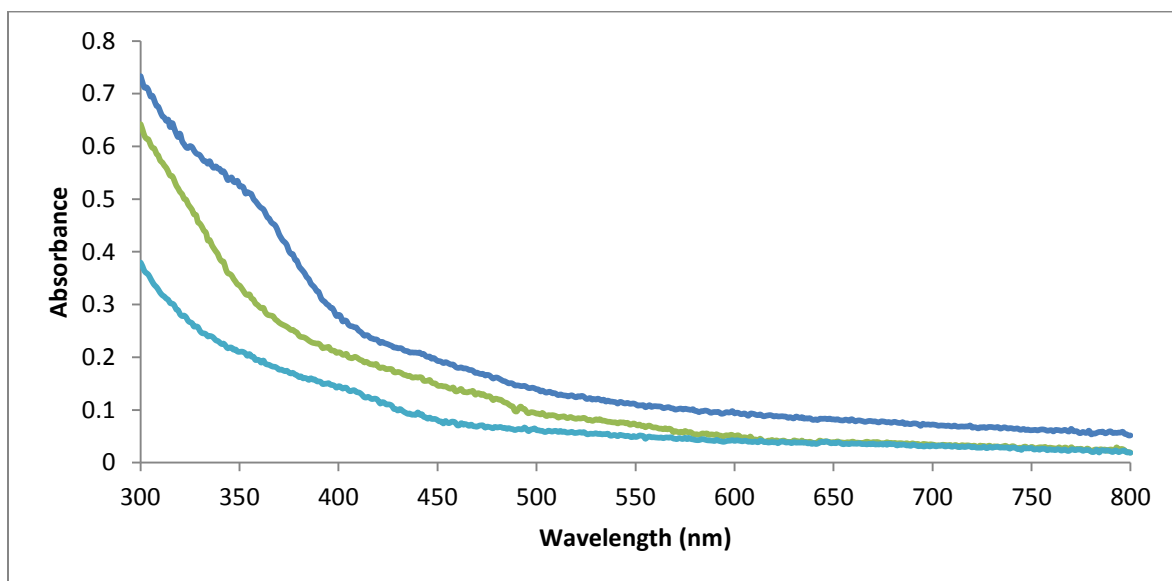


**Figure 3.** Kinetic experiment of **2** reaction with oxygen. Light blue is initial spectrum (shoulder at 400 nm), green is 30 seconds after addition of $O_2$, dark blue is spectrum at 2 hours (shoulder at 364 nm). Not pictured: final decomposition product (peak at 315 nm).

## [Fe(4-MePyr)$_2$(NO)$_2$] (3)

Phenol nitration occurred when **3** reacted with DBP in the presence of $O_2$, indicating a peroxynitrite intermediate. Cyclic voltammetry indicated $E_{1/2} = -499$ mV in acetonitrile (Table 1).

As with the previous DNICs, a UV-Vis experiment showed a change in the spectrum upon the addition of oxygen, losing the peak at 420 nm and forming a peak at 254 nm, so a low-temperature UV-Vis experiment was performed. Again, a short-lived featureless intermediate gave way to a second, more stable intermediate (shoulder at 356 nm) that decomposed at room

temperature. A kinetic experiment carried out under the usual conditions indicated that the first

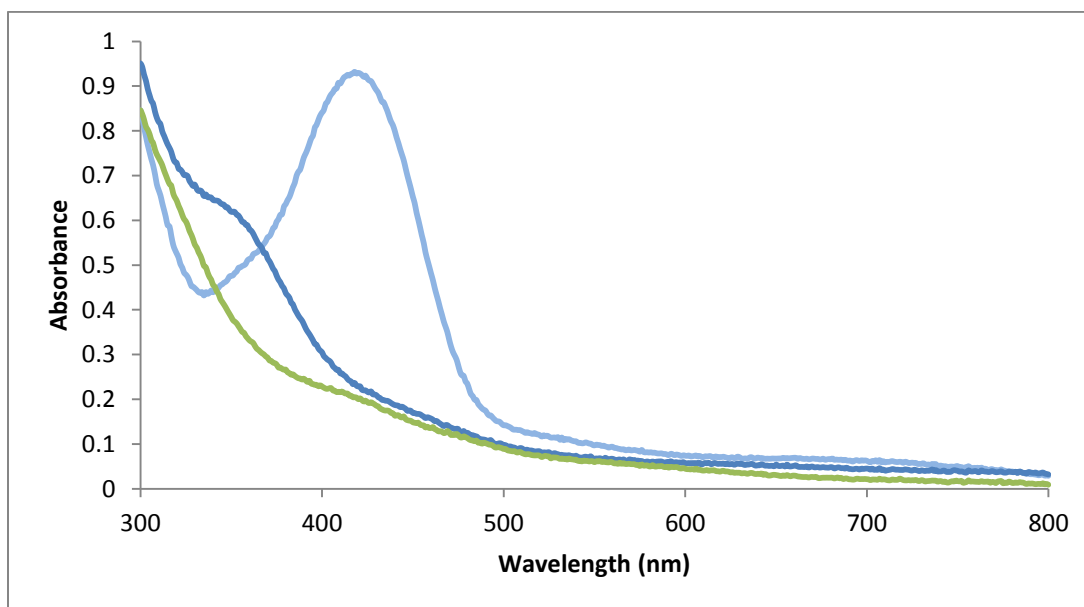intermediate has a lifespan of approximately 2 minutes.



**Figure 4.** Kinetic experiment of **3** reaction with oxygen. Light blue is initial spectrum (peak at 420 nm), green is 30 seconds after addition of $O_2$, dark blue is spectrum at 2 hours (shoulder at 356 nm). Not pictured: final decomposition product (peak at 254 nm).

## $[Fe(MeNP)_2(NO)_2]$ (4)

As with all DNICs tested, **4** was determined to be capable of phenol nitration by GC-MS. A UV-Vis experiment showed a slight change in the spectrum when oxygen was added, losing the peak at 298 nm and forming a shoulder at 291 nm, and thus a low-temperature UV-Vis experiment was performed. Unlike in previous experiments, addition of $O_2$ resulted in no immediate change of the spectrum. A UV-Vis experiment was carried out at -60$^o$C, instead of -80$^o$C, in order to see if there was a change detected. Again, no change was observed. A third UV-Vis experiment, this time at -30$^o$C, again showed no change upon addition of $O_2$. As stated before, the IR spectrum of **4** showed that its NO peaks were very weak, indicating that some

decomposition had occurred. From these observations it was concluded that **4** may have decomposed prior to oxygen addition.

**[Fe(Cl-Pyr)$_2$(NO)$_2$] (5)**

Once again, GC-MS confirmed that **5** was capable of phenol nitration. Cyclic voltammetry in acetonitrile showed $E_{1/2}$ = -391 mV (Table 1).

UV-Vis at room temperature showed a change in the spectrum when oxygen was added, losing the peak at 262 nm and forming a peak at 250 nm and a slight shoulder near 450 nm. Thus, a low-temperature study was conducted. However, the limitations of the probe used made observing the 250-300 nm range impossible. As a result, the initial scan of the DNIC, before addition of oxygen, showed what appeared to be a featureless spectrum, which did not change immediately when oxygen was added. The spectrum slowly developed a slight shoulder at 460 nm, starting about 3 minutes after addition of oxygen. After approximately 5 minutes, the shoulder was fully formed and the spectrum did not change from that point onwards. Due to the featureless initial spectrum, it is difficult to determine whether a featureless intermediate even formed, let alone how long it was present for. This unusual initial observation could be due to instability of the DNIC, either by reaction with oxygen or simply decomposing. The very small change observed during the experiment indicates that it had likely decomposed prior to starting.
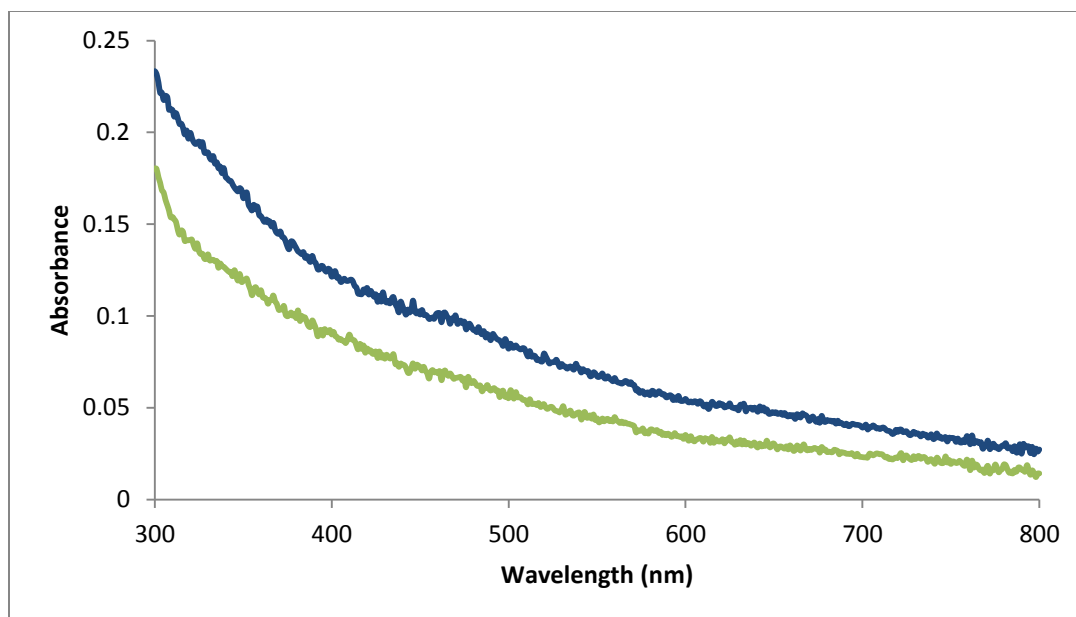
**Figure 5.** Kinetic experiment of **5** reaction with oxygen. Green is initial spectrum, dark blue is spectrum 2 hours after adding $O_2$ (shoulder at 460 nm). Not pictured: final decomposition product (shoulder at 450 nm).

## Discussion

The stability of the first intermediate in all DNIC reactions seemed to be influenced by ligand electronics. In general, a broad trend can be observed: DNICs with lower $E_{1/2}$ have a more stable first intermediate.

**Table 1.** Comparison of $E_{1/2}$ and first intermediate lifetime for various DNICs

| Ligand | $E_{1/2}$ of DNIC (mV) | Lifetime of first intermediate (min) |
|---|---|---|
| DMAP | -725 | 3-5 |
| MeIm | -734 | <1 |
| Picoline | -499 | 2 |
| Cl-Pyr | -391 | Unknown |
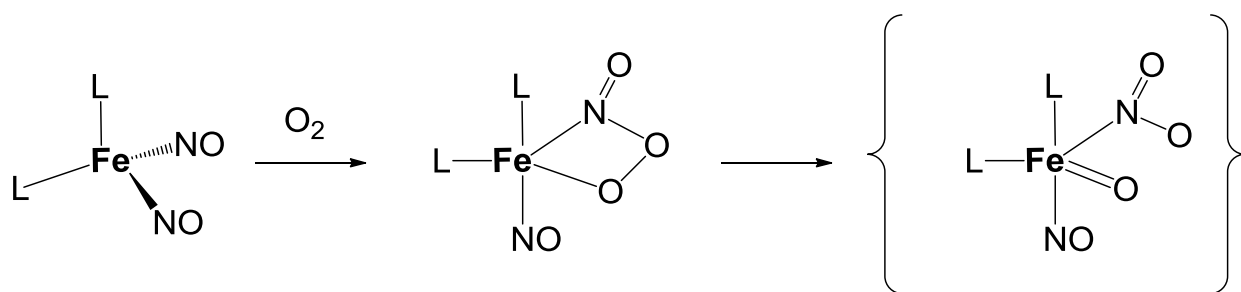
**Scheme 2.** Possible pathway for reaction of DNICs with oxygen

Above is a possible reaction scheme for the studied DNICs and oxygen. An iron(IV)-oxo species could form after cleavage of peroxynitrite's O-O bond. This is a strong oxidizing species and would be stabilized by electron donating ligands. Strong electron-donating ligands seem to be necessary for the appearance of a featureless intermediate, as our previous studies with less electron-donating ligands demonstrated.[18,19] and this would stabilize the iron-oxo moiety. Because the correlation between ligand electronics and intermediate stability is not exact, we believe that the sterics of the ligands play a role and warrant future study.

## Future Work

Despite our focus on the stability and observation of the first intermediate in the DNICs studied, the actual structure remains unknown. Solution IR would be a valuable tool to examine it, but unfortunately low stability of the intermediate prevents this. By continuing to find compounds with more stable first intermediates, eventually one may be found that is long-lasting enough for a successful solution IR observation, which would help elucidate structural information. Other potential spectroscopic methods that were not used in this work that could be used to characterize the first intermediate include Mössbauer spectroscopy, X-ray absorption spectroscopy (XAS), and electron paramagnetic resonance spectroscopy (EPR). Another possible avenue of study is the use of bidentate ligands. Previous work from our group using TMEDA[18]

or di-methyl-phenanthroline[19] as a ligand resulted in very different behavior than was observed with the ligands used in this work. No featureless intermediate was observed with these ligands, which were weaker electron-donators than the ones used in this study. Furthermore, these previously explored DNICs had bidentate ligands, while the ones studied here were all monodentate. Thus, determining the exact effects of denticity on reaction behavior would certainly broaden our understanding of these compounds and their reactivity. In addition, steps are currently underway to identify the second low temperature intermediate.

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