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Lewis Acid Activation of the Ferrous Heme–NO Fragment Towards the N–N Coupling Reaction with NO to Generate N_2O

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Supporting Information Placeholder

ABSTRACT: Bacterial NO reductase (bacNOR) enzymes utilize a heme-nonheme active site to couple two NO molecules to N_2O. We show that BF_3 coordination to the nitrosyl O-atom in (OEP)Fe(NO) activates it towards N–N bond formation with NO to generate N_2O. ^{15}N-isotopic labeling reveals a reversible nitrosyl exchange reaction and follow-up N–O bond cleavage in the N_2O formation step. Other Lewis acids ($B(C_6F_5)_3$ and K^+) also promote the NO coupling reaction with (OEP)Fe(NO). These results, complemented by DFT calculations, provide experimental support for the *cis*:b_3 mechanism in bacNOR.

The conversion of nitric oxide (NO) to the greenhouse gas nitrous oxide (N_2O) is an important component of the global N cycle.[1] Elucidating the N–N bond forming reaction between two NO molecules to generate N_2O remains one of the high impact research goals in NO bioinorganic chemistry. Bacterial NO reductases (bacNOR) utilize a di-Fe heme/non-heme active site (heme b_3:non-heme Fe_B) to couple two NO molecules to generate N_2O and the μ-oxo (heme)Fe–O–Fe_B that is reused in the reaction cycle.[2-3] X-ray crystal structures of bacNOR enzymes are available,[2-4] and provide a framework for the elucidation of the NO coupling reaction.

Three mechanisms for the NO coupling reaction catalyzed by bacNOR have been proposed (Figure 1), and all involve the formation of a new N–N bond and a hyponitrite intermediate. In the *cis*:b_3 mechanism, the second (external) NO molecule attacks the heme-nitrosyl N atom to form the hyponitrite intermediate. In the *trans* mechanism, both active site Fe atoms first bind one NO each prior to N–N bond formation. In the *cis*:Fe_B mechanism, both NO molecules bind at the non-heme Fe_B site prior to the coupling reaction.

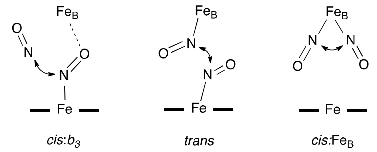

Figure 1. The three common proposed mechanisms for NO coupling by bacterial NO reductases.

Detailed computational investigations on the bacNOR active site by Blomberg are in support of the *cis*:b_3 pathway, where the heme-NO moiety is stabilized by an O atom interaction with the Fe_B center.[5-6] Definitive experimental evidence for this *cis*:b_3 mechanism is, however, lacking. Spectroscopic studies on heme models have suggested the *trans* mechanism as a viable mechanism for NO coupling by bacNOR.[7] The operative mechanism for NO reduction by the di-Fe bacNOR enzyme is, however, still under active debate.[3,8-9] Importantly, outstanding work by Lu[8, 10] and Karlin[11] on engineered myoglobins and on synthetic dinitrosyl (por)$Fe(NO)_2$/Cu^I/acid systems, respectively, have established the requirement for the second non-heme metal to enable N_2O formation.

It is interesting to note that two of the three proposed mechanisms for NO reduction by bacNOR (the *cis*:b_3 and *trans* mechanisms) involve the ferrous (por)Fe(NO) fragment, whose participation is central to both these mechanisms. Curiously, however, the (por)Fe(NO) compounds in isolation are unreactive towards external NO for N_2O generation.[11-14] We thus sought to probe experimental factors that could activate the isolated (por)Fe(NO) fragment towards N–N bond formation with external NO to generate N_2O. In this article, we demonstrate experimentally, complemented by DFT calculations, that Lewis acid addition to a synthetic (por)Fe(NO) compound activates the FeNO moiety for engagement in a three-way synergistic reaction with external NO for the critical N–N bond formation step to generate N_2O.

Addition of 2.5 equiv. of the Lewis acid BF_3•OEt_2 to an anaerobic CH_2Cl_2 solution of (OEP)Fe(NO) (υ_{NO} 1664 cm^{-1}) at 0 °C results in a ~32–35% decrease in υ_{NO} intensity and the appearance of a new IR band at 1425 cm^{-1} (sh) that shifts to 1406 cm^{-1} when (OEP)Fe(^{15}NO) is used (Figure 2). It is intriguing to note that the large υ_{NO} shift of –241 cm^{-1} that occurs when the Lewis acid is added to the $\{FeNO\}^7$ precursor (OEP)Fe(NO) to form the (OEP)Fe(NO)•BF_3 adduct is similar to those observed for the 1-electron reduced $\{FeNO\}^8$ anions (Table 1) whose υ_{NO}s are also of weak-to-medium intensities.[15-18] This large υ_{NO} shift in (OEP)Fe(NO)•BF_3 is suggestive of a significant increase in backdonation of electron density from the Fe center to the nitrosyl ligand as a result of Lewis acid coordination to the terminal nitrosyl O atom.[19-21] A smaller yet still significant negative $\Delta\upsilon_{NO}$ shift of ~50 cm^{-1} has been documented for the engineered mononitrosyl di-Fe myoglobin mutant, Fe^{II}-Fe_BMb(NO).[22]

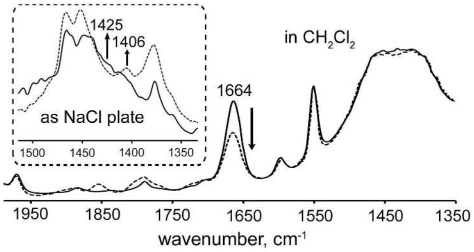

Figure 2. IR spectra showing the reduction in intensity of the υ_{NO} band of the starting (OEP)Fe(NO) (υ_{NO} 1664 cm^{-1}) in CH_2Cl_2 upon reaction with BF_3•Et_2O. *Inset*: IR spectrum of the product mixture (as a NaCl plate) when (OEP)Fe(NO) (solid line) is reacted with BF_3•OEt_2. The corresponding IR spectrum for the (OEP)Fe(^{15}NO) reaction is shown as a dashed line. The new bands are obscured in the reaction solvent (CH_2Cl_2) mixture.

Table 1. NO stretching frequencies (cm^{-1}) for (OEP)Fe(NO)•BF_3 and anionic $\{FeNO\}^8$ iron porphyrins.

system	υ_{NO}	$\Delta\upsilon_{NO}$[a]	ref
(OEP)Fe(NO)•BF_3	1425	–241	t.w.
$[(OEP)Fe(NO)]^-$	1445	–228	15
$[(TPP)Fe(NO)]^-$	1496	–185	16
$[(TFPPBr_8)Fe(NO)]^-$	~1450	–165	17
$[$(3,5-Me-BAFP)Fe(NO)$]^-$	~1466	–218	18
$[$(T*o*-F_2PP)Fe(NO)$]^-$	1473	–214	18

[a] $\Delta\upsilon_{NO}$ with respect to the neutral $\{FeNO\}^7$ precursors.

We then proceeded to evaluate the effect of the Lewis acid addition on the reaction of (OEP)Fe(NO) with external NO. When the ^{15}N-labeled (OEP)Fe(^{15}NO) is reacted with ^{14}NO in the absence of Lewis acid, a nitrosyl exchange reaction occurs (eq 1) as determined by IR spectroscopy, consistent with an earlier report.[23] Further,

$$(OEP)Fe(^{15}NO) + NO \rightleftharpoons (OEP)Fe(NO) + {}^{15}NO \quad (1)$$

and consistent with previous results for related (por)Fe(NO) compounds,[11, 13-14] we find that (OEP)Fe(NO) does not react with NO to generate N_2O.[12]

When (OEP)Fe(NO) is reacted with NO *in the presence* of BF_3•OEt_2, however, we observed the ready formation of the N–N coupled product N_2O (υ_{N_2O} 2237/2212 cm^{-1}; Figure 3A top) in 55-63% yields as determined by IR spectroscopy.[24] When ^{15}N-labeled (OEP)Fe(^{15}NO) is reacted with unlabeled ^{14}NO in the presence of BF_3•Et_2O, the mixed labeled $^{15}N^{14}NO$ and $^{14}N^{15}NO$ products form in addition to $^{14}N_2O$ and $^{15}N_2O$ (Figure 3A bottom),[25] due to the exchange process in eq. 1. In this reaction, [(OEP)Fe(NO)]BF_3OH (υ_{NO} 1839 cm^{-1}) is isolated as the byproduct in 59-64% unoptimized isolated yield.[26]

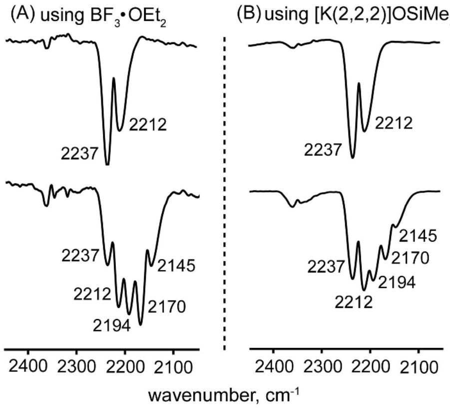

Figure 3. (A) *Top:* IR spectrum of the headspace from the reaction of (OEP)Fe(NO) with BF_3•OEt_2 and NO, showing the formation of N_2O (2237/2212 cm^{-1}). *Bottom*: Headspace IR spectrum when (OEP)Fe(^{15}NO) is reacted with BF_3•Et_2O and unlabeled ^{14}NO, to give the mixed labeled ^{15}N^{14}NO (~2212/2194 cm^{-1}) and ^{14}N^{15}NO (~2194/2170 cm^{-1}) products as well as $^{14}N_2O$ and $^{15}N_2O$ (~2167/2145 cm^{-1}). These products also form when (OEP)Fe(^{14}NO) is reacted with ^{15}NO. (B) Corresponding spectra when [K(2.2.2)]OSiMe$_3$ was used instead of BF_3•Et_2O. NO_2 (υ_{NO_2} ~1618 cm^{-1}) was not formed in these reactions. The isotopic N_2O products were identified by their characteristic IR spectra.[25]

The requirement for BF_3 in the reaction suggests that the Lewis acid plays an important role in activating (OEP)Fe(NO) towards reactivity with NO, most likely via the pathway shown in Figure 4.

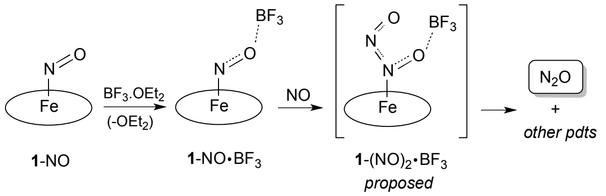

Figure 4. Proposed pathway for the N–N coupling reaction enabled by the Lewis acid BF_3.

We employed density functional theory calculations using an unsubstituted porphine macrocycle (P; see SI for details) to probe the proposed involvement of **1**-NO•BF_3 and **1**-(NO)$_2$•BF_3 in the N–N coupling reaction depicted in Figure 4. The optimized structures are shown in Figure 5 (*right*), and are compared with those for the non-BF_3 containing analogs (*left*).

The interaction of BF_3 with the terminal O atom in **1**-NO•BF_3 results in a lengthening of both the Fe–NO (from 1.718 Å to 1.911 Å) and FeN–O (1.157 Å to 1.225 Å) bonds. Interestingly, the NO moiety in **1**-NO•BF_3 becomes partially anionic (–0.239e), compared with a partially cationic charge (+0.131e) in the precursor **1**-NO. This helps explain the significant lowering of the experimental υ_{NO} by –241 cm^{-1} (Table 1) upon BF_3 coordination to the FeNO moiety in (OEP)Fe(NO)•BF_3 with substantial Fe^{III} and nitroxyl character (Tables S1 and S2). We note that a related $\Delta\upsilon_{NO}$ of –228 cm^{-1} was observed for the 1-electron reduced {FeNO}^{8} anion [(OEP)Fe(NO)]$^-$ (as its K$^+$(2.2.2) salt), whose N–O bond length increased by ~0.02-0.04 Å from that of the neutral precursor.[15] A similar nitroxyl character was proposed for the protein model Fe^{II}-Fe$_B$Mb(NO).[22] Importantly, the spin density on the nitrosyl N atom of **1**-NO•BF_3 is –1.108e, which is substantially increased from that of its precursor **1**-NO (+0.401e) (Table S2), rendering it susceptible to attack by external NO.

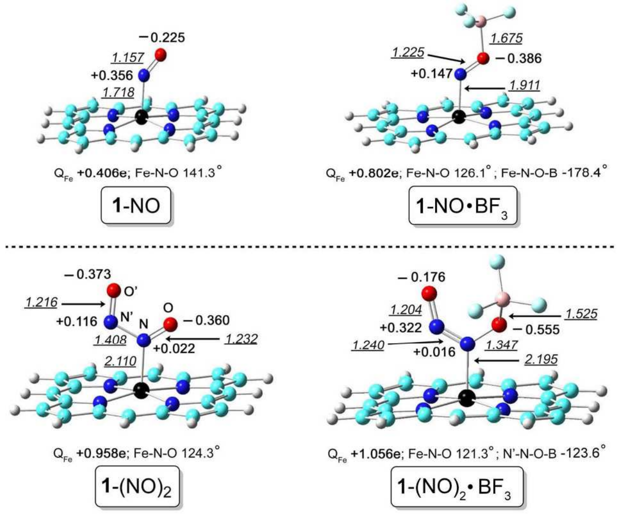

Figure 5. Calculated geometries (in Å and deg) and selected NPA charges (in e units) of the optimized precursor (P)Fe(NO) (**1**-NO) and products from its reactions with NO and/or BF_3. Bond distances are in italics-underline, and atomic charges in bold font.

As mentioned above, (OEP)Fe(NO) in the absence of the Lewis acid BF_3 does not engage in the N–N coupling reaction with external NO to generate N_2O. Likely structures of **1**-(NO)$_2$ (bottom left of Figure 5) were probed. The reaction ΔG of 11.90 kcal/mol (Table S4) for NO coupling essentially shows no catalytic role of the (P)Fe unit alone for NO coupling.[27-28]

Inclusion of BF_3 in the reaction of (OEP)Fe(NO) with external NO leads to the successful generation of N_2O which necessitates the consideration of the N–N bond-forming step. The optimized structure of **1**-(NO)$_2$•BF$_3$ is shown in Figure 5 (bottom right). In the presence of BF$_3$, the N–N coupling reaction has a large decrease in ΔG from ~12 kcal/mol (without BF$_3$ present) to –0.54 kcal/mol (with BF$_3$ present). The reductions in other energy terms are also pronounced and result in –13.53, –10.60, and –11.62 for reaction ΔE, zero-point-energy-corrected ΔE, and ΔH, respectively. The data clearly show a strong enthalpic driving force to help overcome the big entropy drop due to NO coupling. Both the Fe–N and (Fe)N–O(B) distances become longer in **1**-(NO)$_2$•BF$_3$ (by 0.284 and 0.122 Å, respectively) than those in the precursor **1**-NO•BF$_3$, thus weakening these interactions upon binding of the second NO. The effect of BF$_3$ addition on the nature of the NO-coupled intermediate is even more striking when one considers that without BF$_3$ (i.e., in **1**-(NO)$_2$), both the proximal NO (–0.338e charge; directly bound to Fe) and distal NO (–0.257e charge) groups are negatively charged, which hinders NO coupling due to Coulombic repulsion and explains the lack of NO coupling determined experimentally. In contrast, however, when BF$_3$ is attached to the proximal O atom in **1**-(NO)$_2$•BF$_3$, it induces a large negative charge of –0.539e to this proximal NO moiety, whereas the distal NO moiety changes to be positively charged (+0.146e), which results in a strong favorable Coulombic attraction to facilitate the NO coupling reaction as observed experimentally. The resulting short N–N bond distance of 1.240 Å in **1**-(NO)$_2$•BF$_3$ is indicative of a strong N–N bond typical of that determined in hyponitrite [O–N=N–O]-salts.[29-30] Additional results in the SI show that BF$_3$ alone does not enable NO coupling (nor does NO by itself),[31] so it is the *synergistic effect* of both iron porphyrin and Lewis acid that is critical for NO coupling, which may help understand the biological NOR mechanism in systems where both heme and nearby Lewis acid are available.

Our results provide experimental support for the *cis:b$_3$* mechanism for the NO-coupling reaction, where it is the original heme nitrosyl FeN–O(Lewis acid) bond in the **1**-(NO)$_2$•BF$_3$ intermediate that, is cleaved to give N$_2$O. The observed N$_2$O may derive from two isomers of the *cis*-hyponitrite intermediate, with the second (*O*-coordinated) form (Figure 6; *middle*) having a slightly lower energy (by ~1 kcal/mol; Table S1 footnote) but a clearly longer proximal N–O bond (by 0.081 Å). This latter intermediate can explain both the facile N–O cleavage and formation of the (OEP)Fe(μ-OH)B(C$_6$F$_5$)$_3$ product (isolated in ~55% yield when B(C$_6$F$_5$)$_3$ was used as the Lewis acid) whose X-ray structure was obtained. Such a *cis*-hyponitrite isomerization has recently been proposed for bacNOR based on DFT calculations.[6]

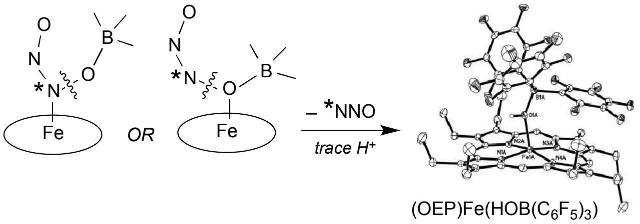

Figure 6. Proposed pathway for the formation of N$_2$O.

In summary, our experimental results, supported by DFT calculations, demonstrate for the first time that Lewis acid activation of a synthetic monoheme-NO model, via coordination of the Lewis acid to the terminal nitrosyl O-atom, renders the FeNO moiety susceptible to attack by external NO to generate a new N–N bond en route to N$_2$O formation. Importantly, we show that it is *the synergistic and not the separate* effects of (por)Fe and Lewis acid BF$_3$ that is critical for the NO coupling reaction. Our preliminary extensions to other Lewis acids such as [K(2.2.2)]OSiMe$_3$ (Figure 3B) and B(C$_6$F$_5$)$_3$ (Figure S2) show that they also enable the stoichiometric {heme-^{15}NO + ^{14}NO} coupling reaction. Indeed, our results suggest that a redox-active (second) metal is not necessarily required for the *first but critical* stoichiometric N–N bond formation step for NO coupling with the (por)Fe(NO) fragment. However, in bacNOR catalysis, a second redox center in the active site would be required to complete the reaction to generate both N$_2$O and water, and be catalytically competent while maintaining strict control of this biological heme-NO activation. These results lend the first chemical support for the proposed *cis-b$_3$* mechanism as a viable, but not necessarily exclusive, pathway for NO coupling by the bacterial NO reductase enzymes and related systems.

ASSOCIATED CONTENT

Supporting Information.
The Supporting Information is available free of charge via the internet at http://pubs.acs.org.

Experimental, crystallography details (CCDC 1813020), additional figures, tables, and computational details (PDF)

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Notes

The authors declare no competing financial interest.

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REFERENCES

1. Wasser, I. M.; de Vries, S.; Moenne-Loccoz, P.; Schroder, I.; Karlin, K. D., *Chem. Rev.* **2002,** *102*, 1201-1234.
2. Tosha, T.; Shiro, Y., *IUBMB Life* **2013,** *65*, 217-226.
3. Shiro, Y.; Sugimoto, H.; Tosha, T.; Nagano, S.; Hino, T., *Phil. Trans. R. Soc. B.* **2012,** *367*, 1195-1203.
4. Sato, N.; Ishii, S.; Sugimoto, H.; Hino, T.; Fukumori, Y.; Sako, Y.; Shiro, Y.; Tosha, T., *Proteins* **2014,** *82*, 1258-1271.
5. Blomberg, L. M.; Blomberg, M. R. A.; Siegbahn, P. E. M., *Biochim. Biophys. Acta* **2006,** *1757*, 240-252.
6. Blomberg, M. R. A.; Siegbahn, P. E. M., *J. Comput. Chem.* **2016,** *37*, 1810-1818.
7. Collman, J. P.; Dey, A.; Yang, Y.; Decreau, R. A.; Ohta, T.; Solomon, E. I., *J. Am. Chem. Soc.* **2008,** *130*, 16498-16499.
8. Chakraborty, S.; Reed, J.; Sage, J. T.; Branagan, N. C.; Petrik, I. D.; Miner, K. D.; Hu, M. Y.; Zhao, J. Y.; Alp, E. E.; Lu, Y., *Inorg. Chem.* **2015,** *54*, 9317-9329.
9. Blomberg, M. R. A., *Biochemistry* **2017,** *56*, 120-131.
10. Lin, Y.-W.; Yeung, N.; Gao, Y.-G.; Miner, K. D.; Tian, S.; Robinson, H.; Lu, Y., *Proc. Natl. Acad. Sci. USA* **2010,** *107*, 8581-8586.
11. Wang, J.; Schopfer, M. P.; Pulu, S. C.; Sarjeant, A. A. N.; Karlin, K. D., *Inorg. Chem.* **2010,** *49*, 1404-1419.
12. Wayland,[13] Ford,[14] and Karlin[11] have shown that (por)Fe(NO) compounds react with NO to generate the *trans* dinitrosyls (por)Fe$(NO)_2$.
13. Wayland, B. B.; Olson, L. W., *J. Am. Chem. Soc.* **1974,** *96*, 6037-6041.
14. Lorkovic, I.; Ford, P. C., *J. Am. Chem. Soc.* **2000,** *122*, 6516-6517.
15. Kundakarla, N.; Lindeman, S.; Rahman, M. H.; Ryan, M. D., *Inorg. Chem.* **2016,** *55*, 2070-2075.
16. Wei, Z. C.; Ryan, M. D., *Inorg Chem* **2010,** *49*, 6948-6954.
17. Pellegrino, J.; Hubner, R.; Doctorovich, F.; Kaim, W., *Chem. Eur. J.* **2011,** *17*, 7868-7874.
18. Goodrich, L. E.; Roy, S.; Alp, E. E.; Zhao, J. Y.; Hu, M. Y.; Lehnert, N., *Inorg. Chem.* **2013,** *52*, 7766-7780.
19. Similar ν_{NO} shifts of –118 to –261 cm^{-1} have been noted for crystallographically characterized coordination compounds with metal-NO-BX_3 linkages.[20-21]
20. Lee, K. E.; Arif, A. M.; Gladysz, J. A., *Chem Ber* **1991,** *124*, 309-320.
21. Choualeb, A.; Blacque, O.; Schmalle, H. W.; Fox, T.; Hiltebrand, T.; Berke, H., *Eur. J. Inorg. Chem.* **2007,** 5246-5261, and refs therein.
22. Hayashi, T.; Miner, K. D.; Yeung, N.; Lin, Y. W.; Lu, Y.; Moenne-Loccoz, P., *Biochemistry* **2011,** *50*, 5939-5947.
23. Yoshimura, T., *Inorg. Chim. Acta* **1984,** *83*, 17-21.
24. Saier, E. L.; Pozefsky, A., *Anal. Chem.* **1954,** *26*, 1079-1080.
25. Dubowski, Y.; Harush, D.; Shaviv, A.; Stone, L.; Linker, R., *Soil Sci. Soc. Am. J.* **2014,** *78*, 61-69.
26. This [(OEP)Fe(^{14}NO)]BF_3OH product likely forms from the attack of excess added ^{14}NO on an [(OEP)Fe](O(H)BF_3) intermediate after N_2O loss. The related ferric nitrosyl [(OEP)Fe(NO)](B(C_6F_5)OH•B(C_6F_5))), obtained from the analogous B$(C_6F_5)_3$ reaction has been characterized by X-ray crystallography (Figure S5).
27. Yi, J.; Morrow, B. H.; Campbell, A. L. O. C.; Shen, J. K.; Richter-Addo, G. B., *Chem. Commun.* **2012,** *48*, 9041-9043.
28. Heinecke, J. L.; Khin, C.; Iretskii, A. V.; Ford, P. C., Poster presentation INOR-465 at the 237th National Meeting of the American Chemical Society, Salt Lake City, UT, USA, March 2009.
29. Arulsamy, N.; Bohle, D. S.; Imonigie, J. A.; Sagan, E. S., *Inorg. Chem.* **1999,** *38*, 2716-2725.
30. Feldmann, C.; Jansen, M., *Z. Kristallogr.* **2000,** *215*, 343-345.
31. Dimerization of NO has been discussed extensively in the literature: Urban, B.; Strobel, A.; Bondybey, V. E., *J. Chem. Phys.* **1999,** *111*, 8939-8949, and references therein.

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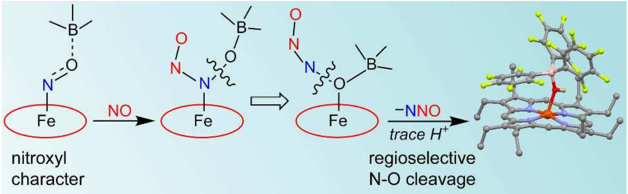
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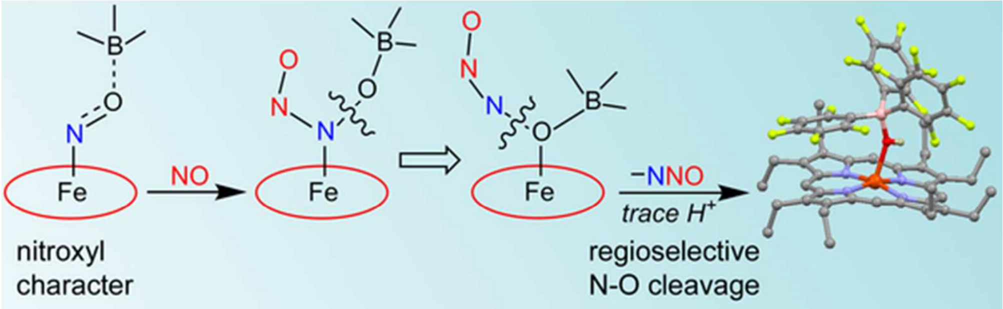

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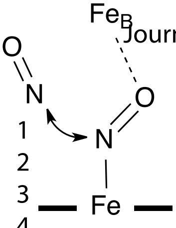

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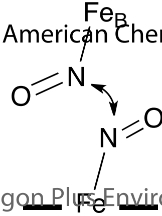

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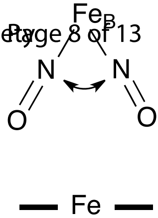

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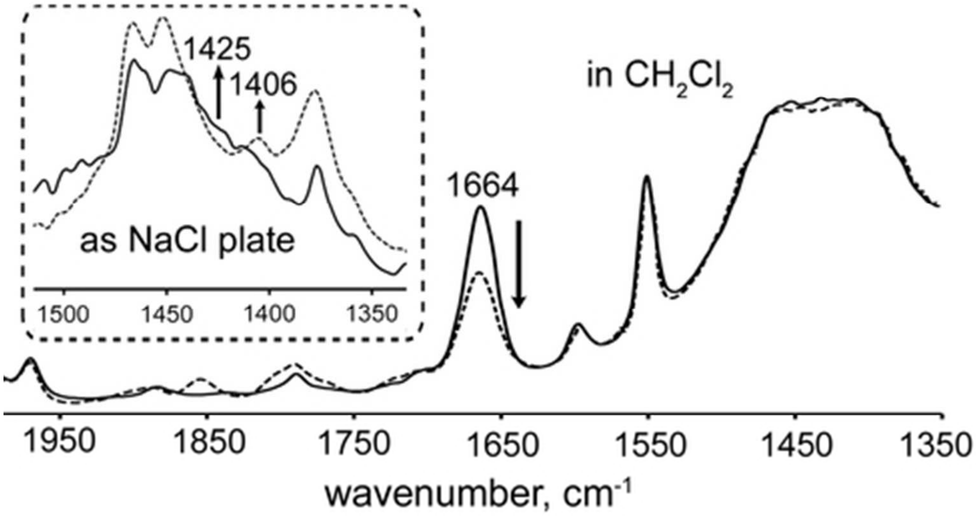

Figure 2

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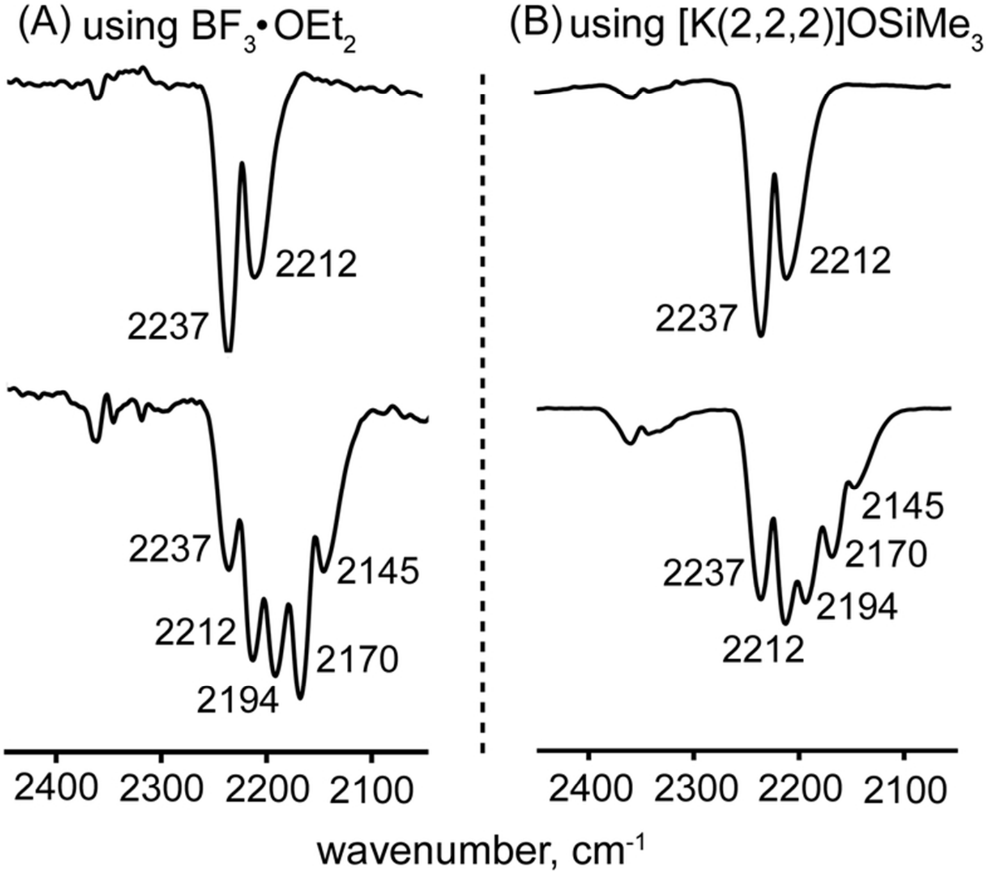
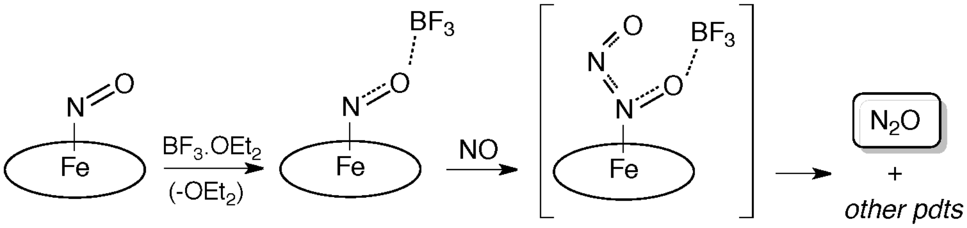


Figure 3

67x59mm (300 x 300 DPI)



1-NO **1**-NO•BF_3 **1**-$(NO)_2$•BF_3 *proposed*

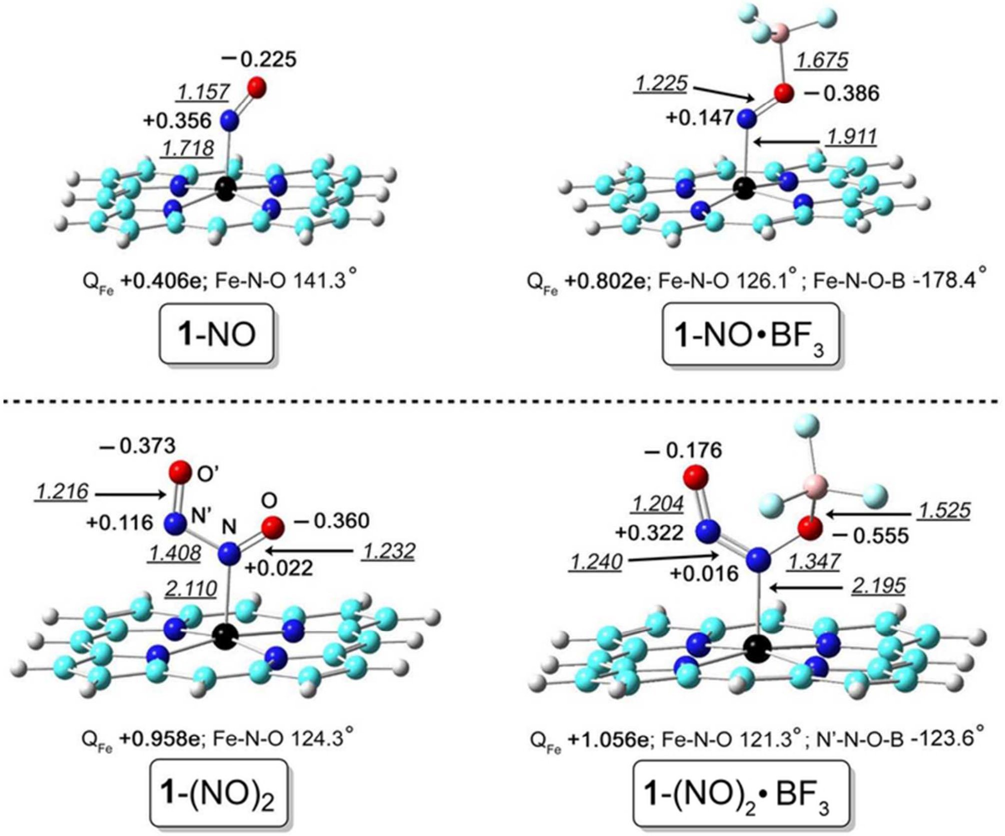
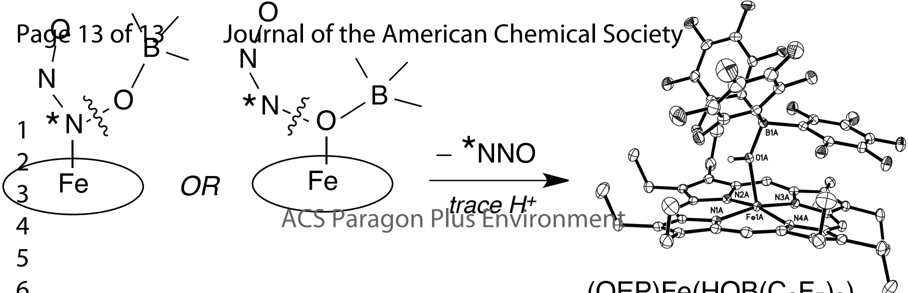


Figure 5

64x54mm (300 x 300 DPI)



(OEP)Fe(HOB(C_6F_5)$_3$)