Published in final edited form as:

J Am Chem Soc. 2012 May 30; 134(21): 8758–8761. doi:10.1021/ja302112y.

Addition of Dioxygen to an N₄S(thiolate) Iron(II) Cysteine Dioxygenase Model Gives a Structurally Characterized Sulfinato-Iron(II) Complex

Alison C. McQuilken, Yunbo Jiang, Maxime A. Siegler, and David P. Goldberg*

Department of Chemistry, The Johns Hopkins University, Baltimore, Maryland, 21218, United States

Abstract

The non-heme iron enzyme cysteine dioxygenase (CDO) catalyzes the *S*-oxygenation of cysteine by O₂ to cysteine sulfinic acid. The synthesis of a new structural and functional model of the cysteine-bound CDO active site, [Fe^{II}(N3PyS)(CH₃CN)]BF₄ (**1**) is reported. This complex is prepared with a new facially chelating 4N/1S(thiolate) pentadentate ligand. Reaction of **1** with O₂ results in oxygenation of the thiolate donor to afford the doubly-oxygenated sulfinate product [Fe^{II}(N3PySO₂)(NCS)] (**2**), which was crystallographically characterized. The thiolate donor provided by the new N3PyS ligand has a dramatic influence on the redox potential and O₂ reactivity of this iron(II) model complex.

Metalloenzymes containing mononuclear non-heme iron centers are implicated in a variety of biological roles, including the activation of O₂ for substrate oxidation. These non-heme iron oxygenases form a class of enzymes that utilize dioxygen for catalytic oxidations and are typically comprised of a 2-His-1-carboxylate donor set bound to the iron center. Cysteine dioxygenase (CDO), on the other hand, differs from the norm in that its active site consists of a mononuclear high-spin Fe^{II} center with three His residues bound in a “facial triad” (Scheme 1).¹ Upon substrate binding,² CDO utilizes O₂ to catalyze the *S*-oxygenation of cysteine to cysteine sulfinic acid (Cys-SO₂H), which is essential for the biosynthesis of pyruvate and taurine. This enzyme is also vital for the maintenance of healthy levels of cysteine in the body. While information on the mechanism of O₂ activation in CDO is just emerging,^{3a,b} several key X-ray crystal structures of CDO have been determined, including a substrate-bound form where the Cys is coordinated bidentate through the S and NH₂ donors.^{1e,3c}

Mononuclear non-heme iron model complexes have proven beneficial for addressing questions regarding enzyme mechanism, but the use of biologically relevant O₂ as oxidant with these models remains quite rare.⁴ Previously, we described the syntheses of two N₃S(thiolate)Fe^{II} model complexes of CDO, which react with O₂ to yield sulfonato products.⁵ In one case, the thiolate donor was covalently tethered to the N₃ platform (Figure 1a), whereas in the other case an exogenous arylthiolate ligand was employed. In each of these models, a high-spin Fe^{II} center was ligated by three planar N donors from a bis(imino)pyridine (BIP) framework in a distorted square pyramidal geometry with a triflate

*Corresponding Author: dpg@jhu.edu.

The authors declare no competing financial interest.

ASSOCIATED CONTENT

Supporting Information. Experimental details, spectra, electrochemical data, and X-ray crystallographic files (CIF). This material is available free of charge via the Internet at <http://pubs.acs.org>.

anion occupying the axial position. One finding to result from these studies was that there appears to be a requirement for the S donor to be positioned *cis* to the potential binding site for O₂ to obtain *S*-oxygenation. These analogs contain only three N donors bound to the iron(II) center, unlike the four N donors ligated in the Cys-bound form of the enzyme.^{1e} The three nitrogen ligands are also constricted to one plane by the BIP framework, leading to a meridional configuration, unlike the facial arrangement of the 3 His donors found in CDO. Furthermore, while there was some indirect evidence for *S*-oxygenation giving sulfinato intermediates in these model systems, the final, isolated *S*-oxygenated iron complexes were found to be triply-oxygenated sulfonato products, reaching a level of oxygenation beyond that observed in the biochemical reaction.

To improve our model design, we set out to prepare a pentadentate N₄S ligand that would be constrained to provide three neutral N donors in a *facial* arrangement, as well as a fourth N donor to mimic the amino coordination from Cys, and a single S donor held *cis* to the putative O₂ binding site at the metal center. The N₄Py ligand established previously for non-heme iron models appeared to be an ideal scaffold for building the desired N₄S system (Figure 1b).⁶ The pentadentate N₄Py ligand provides a rigid structure that binds iron(II) in a square-pyramidal fashion, and has allowed for the characterization and isolation of several key iron-oxygen (e.g. Fe=O, Fe-OO(H)) intermediates. These beneficial properties have provided motivation for the synthesis of derivatives of the N₄Py ligand by several groups, but thus far none of these efforts have led to the incorporation of a thiolato donor.⁷ Herein we report the synthesis of a new pentadentate ligand, N₃PySH, and its corresponding iron(II) complex [Fe^{II}(N₃PyS)(CH₃CN)]BF₄ (**1**) (Scheme 2). It is shown that **1** reacts with O₂ via sulfur oxygenation to generate the doubly-oxygenated sulfinato complex, [Fe^{II}(N₃PySO₂)]⁺ (Scheme 3). Thus **1** is a close structural and functional model of CDO, and provides a rare example of an iron(II)-thiolate complex reacting with O₂ to give a sulfinato complex. This product was characterized by X-ray crystallography, and is, to our knowledge, the first example of a structurally characterized mononuclear Fe^{II}-sulfinate complex.

Reaction of the protected phenylthiolate 3-(2-bromomethylphenylsulfanyl)propionitrile (BrCH₂PhSEtCN)₈ with *N*-[di(2-pyridinyl)methyl]-*N*-(2-pyridinylmethyl)amine^{7c} in the presence of K₂CO₃ at 60 °C in acetonitrile affords the protected pentadentate ligand N₃PySEtCN (Scheme 2). Addition of ^tBuOK in THF yields after workup the deprotected ligand, N₃PySH, in moderate yield (43%). Addition of Fe^{II}(BF₄)₂ to N₃PySH in CH₃CN in the presence of 1 equiv Et₃N affords the dark red Fe^{II} complex [Fe^{II}(N₃PyS)(CH₃CN)]BF₄ (**1**). X-ray quality crystals were grown from vapor diffusion of diethyl ether into an acetonitrile solution of **1**. The X-ray structure of **1** reveals the pentadentate ligand bound in a pseudo-square pyramidal geometry about the Fe^{II} center as designed, with the phenylthiolate donor held *cis* to the sixth site occupied by a labile acetonitrile ligand (Figure 2). The four neutral nitrogen and one thiolate donor motif corresponds closely to the cysteine-bound active site of CDO.

The Fe-N bond distances (average Fe-N_{py} = 1.9594(13) Å) in **1** are consistent with low-spin iron(II) (ls-Fe^{II}) complexes utilizing pyridyl ligands.^{6a,9} Likewise, the Fe-S distance is consistent with other Fe^{II}-thiolate complexes (Fe-S = 2.3018(4) Å).^{5a,b,10} The diamagnetic ¹H NMR spectrum in CD₃CN is characteristic of a low-spin iron(II) ion. When dissolved in acetonitrile, intense UV-vis features at 325, 418, and 493 nm (ϵ = 4475 M⁻¹ cm⁻¹, 4550 M⁻¹ cm⁻¹, and 4820 M⁻¹ cm⁻¹, respectively) are observed (Figure S1a). However, upon dissolution in methanol, **1** converts to a high-spin Fe^{II} complex, as indicated by a solution state magnetic moment (μ_{eff} = 4.5 μ_{B} , Evan's method) and less intense UV-vis bands (Figure S1b). It is concluded that CH₃OH, a relatively weak-field ligand, likely

displaces CH₃CN in the labile site of **1**, providing a convenient switch to access the biologically relevant hs-iron(II) state that mimics resting CDO.

Addition of excess O₂ to a methanolic solution of high-spin **1** results in an immediate color change from the dark brown of **1** (470 nm, 1000 M⁻¹ cm⁻¹) to a new deep green (674 nm, 1300 M⁻¹ cm⁻¹, Figure S3). Efforts to obtain X-ray quality crystals of this green species for characterization have thus far been unsuccessful. However, preliminary experiments suggest this species may be an *S*-oxygenated intermediate (*vide infra*). To isolate a crystalline product, SCN⁻ was added as a potentially good ligand for the labile site on the iron product. Immediately upon addition of one equivalent of KSCN, the green solution turns brown. Vapor diffusion of diisopropyl ether results in the isolation of orange-red lath-like crystals of **2** in 6 days. Structural determination of **2** by X-ray crystallography (Figure 2) revealed a low-spin iron(II) complex wherein the chelated S atom has been doubly oxygenated to give the sulfinate product, as well as a thiocyanate anion bound in place of the acetonitrile ligand of **1**.

The average Fe-N distance is consistent with other ls-Fe^{II} complexes and is only slightly larger than that of complex **1** (average Fe-N_{py} for **2** = 1.971(3) Å). The Fe-S bond distance decreases upon *S*-oxygenation (Fe-S for **2** = 2.1812(9) Å), which is consistent with the decrease in other metal-sulfur (M-S) bond lengths observed upon *S*-oxygenation.^{11a-e} The reduction in M-S bond length going from the thiolate to the sulfinate donor corresponds to both a contraction in the size of the sulfur atom in the sulfinate due to the increase in formal oxidation state, and an elimination of the repulsive interaction between the metal's filled d-orbitals and the sulfur lone pairs on the thiolate donor. The S-O distances (S1-O1 = 1.488(2) Å, S1-O2 = 1.476(2) Å) are consistent with other sulfinate bond lengths.¹¹ Spectroscopic characterization of crystalline **2** shows two intense UV-vis bands at 380 and 451 nm (ϵ = 4600 M⁻¹ cm⁻¹ and 4200 M⁻¹ cm⁻¹, respectively, Figure S2) in CH₃CN, similar to the spectrum for the ls-Fe^{II} starting material **1** in CH₃CN. The attenuated total reflectance infrared (ATR-IR) spectrum of neat **2** reveals two prominent peaks at 1129 and 1012 cm⁻¹. These peaks fall in the observed range for metal-sulfinate complexes,¹¹ and therefore we assign these bands to the asymmetric and symmetric S-O stretching modes. Thus addition of O₂ to the CDO model **1** leads to the isolation and characterization of an Fe^{II}-sulfinate complex, wherein the thiolate donor has been doubly oxygenated in a manner parallel with the enzymatic chemistry. In addition, although there are a few examples of structurally characterized Fe^{III}-sulfates,^{11f,12} to our knowledge **2** is the first example of a crystallographically characterized mononuclear Fe^{II}-sulfinate complex.

The influence of the thiolate donor on non-heme iron active sites is of fundamental interest, and the N3PySH ligand provides us with an ideal system to address this issue by comparison with the all-nitrogen N4Py analog. Complex **1** and [Fe^{II}(N4Py)]²⁺ show a dramatic difference in O₂ reactivity. Complex **1** reacts with O₂ without the use of added reductants, whereas [Fe^{II}(N4Py)]²⁺ reacts with O₂ only in the presence of BNAH (BNAH = 1-benzyl-1,4-dihydronicotinamide) to generate [(N4Py)Fe^{III}(OOH)]²⁺.¹³ As we have reported, a prerequisite for O₂ reactivity is $E_{1/2}(\text{Fe}^{\text{III/II}}) < -0.1$ V vs Fc⁺/Fc. 5b Cyclic voltammetry of **1** in CH₃CN showed a single quasi-reversible wave at -0.226 V vs Fc⁺/Fc (Figure S4a), which we assign to the Fe^{III/II} couple. This potential is shifted significantly more negative compared to the same couple for the non-thiolate analog [Fe^{II}(N4Py)]²⁺ ($E_{1/2}$ = +0.61 V vs Fc⁺/Fc in CH₃CN).¹⁴ These results show that replacement of a pyridyl donor with a single thiolate ligand causes a dramatic cathodic shift of over 800 mV compared to N4Py. The redox potential of **1** was also measured in CH₃OH, revealing two quasireversible waves at $E_{1/2}$ = -233 mV and -583 mV vs Fc⁺/Fc. The wave at $E_{1/2}$ = -233 mV is assigned to some CH₃CNbound **1** that remains in equilibrium with the methanol complex. The Fe^{III/II} couple of the high-spin CH₃OHbound **1** is assigned to the more negative potential, which clearly

falls well within the previously noted range for O₂ reactivity. As expected, the redox potential for **2** (−0.001 V, Figure S4b) is shifted more positive than **1** by 225 mV, consistent with less electron donation from the S-bound sulfinate group. However, the $E_{1/2}$ for S-bound **2** remains significantly more negative than that of [Fe^{II}(N4Py)]²⁺.

Isotope labeling studies with ¹⁸O₂ were conducted to gain insight into the mechanism of the reaction of **1** with O₂. Analysis of reaction mixtures with natural abundance O₂ by electrospray ionization mass spectrometry (ESI-MS) revealed a prominent isotopic cluster centered at m/z 581.7 which can be assigned to [(K)(Fe^{II}(N3PyS₁₆O₂)(NCS))]⁺ ([M+K]⁺, Figure 3). Upon running the reaction with ¹⁸O₂ (98% isotope-enriched), a shift in the isotopic pattern is observed. Simulations of this pattern indicate that 14% of the sulfinate-iron(II) complex contains one labeled oxygen atom (S₁₆O₁₈O), while approximately 2% of the product is labeled in both oxygen positions (S₁₈O₂). These results suggest that O₂ is a source of O atoms for the *S*-oxygenation, but there is likely significant exchange with exogenous H₂O during the course of the reaction. To test this idea, the reaction was carried out with the addition of H₂ ¹⁸O (50 μL) followed by ESI-MS analysis, which revealed 70% S₁₈O₂ and 28% S₁₆O₁₈O. This experiment suggests that the O atoms in the sulfinate complex are susceptible to exchange during *S*-oxygenation. However, independent experiments with pure **2** and H₂ ¹⁸O revealed that the O atoms of the lowspin Fe^{II}-sulfinate were *not exchangeable* with H₂ ¹⁸O under the same conditions (data not shown). Thus the isotope-labeling results implicate one or more intermediates in the *S*-oxygenation pathway that undergo facile O exchange with H₂O. One such intermediate could include a mono-oxygenated sulfenato-iron species. Sulfenato-metal (RS(O)-M) complexes are rare, but have been shown to undergo facile O-atom exchange with H₂O.¹⁵

Given these results, we speculated that the green intermediate (674 nm) may contain a singly-oxygenated sulfenato donor. In previous studies, it has been shown that dimedone reacts selectively with sulfenic acids, but not thiols or sulfinic acids.¹⁶ Addition of dimedone (50 equiv) caused the complete decay of the band at 674 nm over 2 h. Mass spectral analysis revealed a major peak at m/z 591.4, corresponding to [Fe-N3PyS-dimedone – H]⁺, the expected product from reaction of dimedone with a sulfenic acid group (Scheme 4). Metal-sulfenates are also known to react with PR₃.¹⁷ Anaerobic addition of PPh₃ (2 equiv) to the green intermediate resulted in a color change to brown and the production of OPPh₃ (50%, ³¹P NMR, based on total Fe). Addition of H₂ ¹⁸O to the green intermediate followed by reaction with PPh₃ led to 49% ¹⁸O-labeled OPPh₃. In contrast, the sulfinato product **2** does not react with either dimedone or PPh₃ under the same conditions. These data are consistent with the presence of a sulfenato-iron intermediate that contains an exchangeable O atom. However, these experiments do not rule out other intermediates being present at this stage, including doubly-oxygenated sulfinato species.

In summary, we report the synthesis of a novel N₄S(thiolate) ligand, which functions as designed to give [Fe^{II}(N3PyS)]⁺, a structural analog of substrate-bound CDO. A single thiolate donor is incorporated into the coordination sphere to mimic the Cys sulfur ligation in the enzyme. This substitution is critical for the reactivity of the iron(II) complex with dioxygen, in contrast to the all-nitrogen N4Py analog, which is completely inert to O₂ in the absence of co-reductants. The findings presented here, in comparison with our previous CDO model complexes, suggest that a *facial* arrangement of the N donors about the iron(II) center biases the O₂ reactivity toward production of the biologically relevant sulfinato product. Although more work needs to be done to understand the mechanism of *S*-oxygenation for **1**, the preliminary data suggest that the O₂ addition proceeds through monooxygenase-like steps, as indicated by calculations for the CDO mechanism.^{3b}

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

The NIH (GM62309) is gratefully acknowledged for financial support and Y.J. is grateful for the Ernest M. Marks Fellowship.

References

1. (a) Joseph CA, Maroney MJ. *Chem Commun.* 2007:3338–3349. (b) McCoy JG, Bailey LJ, Bitto E, Bingman CA, Aceti DJ, Fox BG, Phillips GN Jr. *Proc Natl Acad Sci USA.* 2006; 103:3084–3089. [PubMed: 16492780] (c) Simmons CR, Liu Q, Huang QQ, Hao Q, Begley TP, Karplus PA, Stipanuk MH. *J Biol Chem.* 2006; 281:18723–18733. [PubMed: 16611640] (d) Stipanuk MH, Ueki I, Dominy JE Jr, Simmons CR, Hirschberger LL. *Amino Acids.* 2009; 37:55–63. [PubMed: 19011731] (e) Ye S, Wu X, Wei L, Tang DM, Sun P, Bartlam M, Rao ZH. *J Biol Chem.* 2007; 282:3391–3402. [PubMed: 17135237]
2. (a) Gardner JD, Pierce BS, Fox BG, Brunold TC. *Biochemistry.* 2010; 49:6033–6041. [PubMed: 20397631] (b) Tchesnokov EP, Wilbanks SM, Jameson GNL. *Biochemistry.* 2012; 51:257–264. [PubMed: 22122511]
3. (a) Crawford JA, Li W, Pierce BS. *Biochemistry.* 2011; 50:10241–10253. [PubMed: 21992268] (b) Kumar D, Thiel W, de Visser SP. *J Am Chem Soc.* 2011; 133:3869–3882. [PubMed: 21344861] (c) Simmons CR, Krishnamoorthy K, Granett SL, Schuller DJ, Dominy JE Jr, Begley TP, Stipanuk MH, Karplus PA. *Biochemistry.* 2008; 47:11390–11392. [PubMed: 18847220]
4. Kim SO, Sastri CV, Seo MS, Kim J, Nam W. *J Am Chem Soc.* 2005; 127:4178–4179. [PubMed: 15783193] Lee YM, Hong S, Morimoto Y, Shin W, Fukuzumi S, Nam W. *J Am Chem Soc.* 2010; 132:10668–10670. [PubMed: 20681694] MacBeth CE, Golombek AP, Young VG Jr, Yang C, Kuczera K, Hendrich MP, Borovik AS. *Science.* 2000; 289:938–941. [PubMed: 10937994] Thibon A, England J, Martinho M, Young VG Jr, Frisch JR, Guillot R, Girerd JJ, Münck E, Que L Jr, Banse F. *Angew Chem, Int Ed.* 2008; 47:7064–7067. A CDO model study in which O₂ was utilized was recently described. See Sallmann M, Siewert I, Fohlmeister L, Limberg C, Knispel C. *Angew Chem, Int Ed.* 2012; 51:2234–2237.
5. (a) Jiang YB, Widger LR, Kasper GD, Siegler MA, Goldberg DP. *J Am Chem Soc.* 2010; 132:12214–12215. [PubMed: 20712312] (b) Badieli YM, Siegler MA, Goldberg DP. *J Am Chem Soc.* 2011; 133:1274–1277. [PubMed: 21207980] (c) Kumar D, Sastry GN, Goldberg DP, de Visser SP. *J Phys Chem A.* 2012; 116:582–591. [PubMed: 22091701]
6. (a) Lubben M, Meetsma A, Wilkinson EC, Feringa B, Que L Jr. *Angew Chem, Int Ed.* 1995; 34:1512–1514. (b) Ho RYN, Roelfes G, Hermant R, Hage R, Feringa BL, Que L Jr. *Chem Commun.* 1999:2161–2162. (c) Klinker EJ, Kaizer J, Brennessel WW, Woodrum NL, Cramer CJ, Que L Jr. *Angew Chem, Int Ed.* 2005; 44:3690–3694.
7. (a) Roelfes G, Vrajmasu V, Chen K, Ho RYN, Rohde JU, Zondervan C, la Crois RM, Schudde EP, Lutz M, Spek AL, Hage R, Feringa BL, Münck E, Que L Jr. *Inorg Chem.* 2003; 42:2639–2653. [PubMed: 12691572] (b) Ligtenbarg AGJ, Oosting P, Roelfes G, La Crois RM, Lutz M, Spek AL, Hage R, Feringa BL. *Chem Commun.* 2001:385–386. (c) Roelfes G, Branum ME, Wang L, Que L Jr, Feringa BL. *J Am Chem Soc.* 2000; 122:11517–11518. (d) Sen Soo H, Komor AC, Iavarone AT, Chang CJ. *Inorg Chem.* 2009; 48:10024–10035. [PubMed: 19780564] (e) van den Heuvel M, van den Berg TA, Kellogg RM, Choma CT, Feringa BL. *J Org Chem.* 2004; 69:250–262. [PubMed: 14725436]
8. Thapper A, Behrens A, Fryxelius J, Johansson MH, Prestopino F, Czaun M, Rehder D, Nordlander E. *Dalton Trans.* 2005:3566–3571. [PubMed: 16234939]
9. Zang Y, Kim J, Dong YH, Wilkinson EC, Appelman EH, Que L Jr. *J Am Chem Soc.* 1997; 119:4197–4205.
10. (a) Namuswe F, Kasper GD, Sarjeant AAN, Hayashi T, Krest CM, Green MT, Moënné-Loccoz P, Goldberg DP. *J Am Chem Soc.* 2008; 130:14189–14200. [PubMed: 18837497] (b) Kitagawa T,

- Dey A, Lugo-Mas P, Benedict JB, Kaminsky W, Solomon E, Kovacs JA. *J Am Chem Soc.* 2006; 128:14448–14449. [PubMed: 17090014]
11. (a) Buonomo RM, Font I, Maguire MJ, Reibenspies JH, Tuntulani T, Darensbourg MY. *J Am Chem Soc.* 1995; 117:963–973. (b) Mullins CS, Grapperhaus CA, Frye BC, Wood LH, Hay AJ, Buchanan RM, Mashuta MS. *Inorg Chem.* 2009; 48:9974–9976. [PubMed: 19795870] (c) Grapperhaus CA, Darensbourg MY. *Acc Chem Res.* 1998; 31:451–459. (d) Masitas CA, Kumar M, Mashuta MS, Kozlowski PM, Grapperhaus CA. *Inorg Chem.* 2010; 49:10875–10881. [PubMed: 20973591] (e) Masitas CA, Mashuta MS, Grapperhaus CA. *Inorg Chem.* 2010; 49:5344–5346. [PubMed: 20486695] (f) Galardon E, Giorgi M, Artaud I. *Chem Commun.* 2004:286–287. (g) Lee CM, Hsieh CH, Dutta A, Lee GH, Liaw WF. *J Am Chem Soc.* 2003; 125:11492–11493. [PubMed: 13129340]
12. (a) Heinrich L, Li Y, Vaissermann J, Chottard G, Chottard JC. *Angew Chem, Int Ed.* 1999; 38:3526–3528. (b) Lugo-Mas P, Taylor W, Schweitzer D, Theisen RM, Xu L, Shearer J, Swartz RD, Gleaves MC, DiPasquale A, Kaminsky W, Kovacs JA. *Inorg Chem.* 2008; 47:11228–11236. [PubMed: 18989922] (c) Noveron JC, Olmstead MM, Mascharak PK. *J Am Chem Soc.* 2001; 123:3247–3259. [PubMed: 11457060] (d) Tyler LA, Noveron JC, Olmstead MM, Mascharak PK. *Inorg Chem.* 1999; 38:616–617.
13. Hong S, Lee YM, Shin W, Fukuzumi S, Nam W. *J Am Chem Soc.* 2009; 131:13910–13911. [PubMed: 19746912]
14. Collins MJ, Ray K, Que L Jr. *Inorg Chem.* 2006; 45:8009–8011. [PubMed: 16999397]
15. Heinrich L, Mary-Verla A, Li Y, Vaissermann J, Chottard JC. *Eur J Inorg Chem.* 2001:2203–2206.
16. (a) Heinecke J, Ford PC. *J Am Chem Soc.* 2010; 132:9240–9243. [PubMed: 20565124] (b) Seo YH, Carroll KS. *Angew Chem, Int Ed.* 2011; 50:1342–1345.
17. (a) Farmer PJ, Verpeaux JN, Amatore C, Darensbourg MY, Musie G. *J Am Chem Soc.* 1994; 116:9355–9356. (b) Lugo-Mas P, Dey A, Xu L, Davin SD, Benedict J, Kaminsky W, Hodgson KO, Hedman B, Solomon EI, Kovacs JA. *J Am Chem Soc.* 2006; 128:11211–11221. [PubMed: 16925440]

Figure 1.

(a) *S*-oxygenation reaction of a previous CDO model with a covalently tethered phenylthiolate donor and (b) N3PyS ligand design.

Figure 2. Displacement ellipsoid plots (50% probability level) of the cation of **1** and complex **2**. The H atoms are omitted for clarity.

Figure 3. ESI-MS spectra after the reaction of **1** with O_2 (top left), $^{18}\text{O}_2$ (top right), and $\text{O}_2/\text{H}_2\ ^{18}\text{O}$ (bottom).

Scheme 1.
Sulfur Oxygenation of Cysteine by CDO with O_2

Scheme 2. Synthesis of N3PySH and $[\text{Fe}^{\text{II}}(\text{N3PyS})(\text{CH}_3\text{CN})]\text{BF}_4$ (1)

Scheme 3.

O₂ Reactivity of [Fe^{II}(N3PyS)(CH₃CN)]⁺ in CH₃OH

Scheme 4.
Reaction of Dimedone with the Green Species