Biomimetic Modeling of the Active Site of Soluble Methane Monooxygenase Hydroxylase (sMMOH)

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Biomimetic Modeling of the Active Site of Soluble Methane Monooxygenase Hydroxylase (sMMOH)

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© 2012, Chan Myae Myae Soe, Dr. Yang Li, and Dr. Stephen J. Lippard
Abstract

Soluble methane monooxygenase (sMMO) belongs to a family of metalloproteins called bacterial multicomponent monooxygenases (BMMs), which contain carboxylate-bridged non-heme diiron cores. These sMMO enzymes are of high interest because they utilize readily available molecular oxygen in their energy conversion of methane to methanol in the metabolic system of methanotrophic bacteria. Methane is abundant in natural gas, and if it can be converted to methanol, a liquid form, under mild conditions as in the enzyme, transportation of this energy source to remote areas will be safer and more convenient. Our research group has a long-term interest in developing small molecule synthetic analogs that can mimic both the structure and function of the active site of the hydroxylase component of sMMO (sMMOH). Unfortunately, no ligand system designed to date has been able to achieve this goal. In our further attempt, synthesis of a triptycene-based bis(benzimidazole) diester ligand L3 is discussed in this paper along with its coordination with iron(II) salt and an external carboxylate. Characterization of the diiron(II) complexes was achieved using UV-vis spectrophometric titrations, X-ray diffraction studies, Mössbauer spectroscopy, and IR spectroscopy. Preliminary oxygenation studies of the diiron(II) complexes with molecular oxygen is also included.
Acknowledgements

This thesis would not have been possible without the support of my dear professors, family, and friends. I am very fortunate to have learned from each and every one of you throughout my life journey.

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To my mentor, Dr. Yang Li: I owe my deepest gratitude to you for all your help on this thesis. Thank you for the generous donation of your time to discuss the project with me. I truly appreciate that you took time out of your busy schedule and made several trips to Wellesley to attend my thesis committee meetings.

To Professor Christopher Arumainayagam and Professor Mala Radhakrishnan: You are my role models in chemistry, whom I have admired since my first year at Wellesley. I feel privileged to have you on my thesis committee. Your critical comments, suggestions, and insightful advice have furthered my understanding of the project. I would especially like to thank Chris for being a continuing source of support for anything all these four years: chemistry, courses, research, thesis, graduate school, life. Thank you so much for introducing me to the world of research and convincing me how fun it can be. Thank you for all your contributions to my growth as a person and as a scientist.

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Last, but not least, I would like to thank my family for being supportive in everything I do and having faith in me. Without your unending love and support, I would not have been able to complete this thesis.
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Introduction

Thousands of chemical reactions taking place in living cells at any given instant time are made possible by virtue of enzymes in the living organisms. Enzymes lower the energy barrier in metabolic processes and thus accelerate the rates of chemical reactions. Proteins containing metal centers at their active sites are called metalloenzymes. Their importance in transformations, such as nitrogen fixation, water splitting, and hydrogen production has led to a tremendous surge of research interest aimed at studying metalloenzymes.

Carboxylate-bridged Non-heme Diiron Proteins

Among iron-containing proteins, an important class of metalloenzymes has carboxylate-bridged non-heme diiron active sites. Examples include hemerythrin (Hr), ribonucleotide reductase (RNR), and bacterial multicomponent monooxygenases (BMMs). Although these metalloproteins perform different functions in nature, they share the same initiation step in their respective reaction mechanisms. This first step involves binding and/or activation of O₂ at the diiron(II) active sites. In hemerythrin (Hr), the reversible coordination of dioxygen to a single iron site enables the O₂ transport in marine invertebrates such as sipunculids, annelids, priapulids and brachiopods. In RNR-R2 and BMMs, dioxygen is consumed by the diiron(II) active sites in the catalytic cycle, forming Fe(III)/Fe(IV) intermediates that act as oxidases in chemical reactions. The intermediate in RNR-R2 is important in conversion of ribo- to deoxyribonucleotides while the C—H bond activation is performed by the intermediate in the hydroxylase protein of some BMMs.
Figure 1. Active site structures in the hydroxylase proteins of certain members of bacterial multicomponent monooxygenases (BMMs): a) soluble methane monooxygenase (sMMO); b) toluene/o-xylene monooxygenase (ToMO); c) phenol hydroxylase (PH). Their active site structures are analogous although the proteins perform different functions in nature.

Soluble Methane Monooxygenase (sMMO)

Of all the remarkable carboxylate-bridged non-heme diiron proteins, our laboratory has a long-term interest in studying bacterial multicomponent monooxygenases (BMMs). They include soluble methane monooxygenase (sMMO), toluene/o-xylene monooxygenase (ToMO), phenol hydroxylase (PH), and alkene monooxygenase (AMO) among others. Despite their diverse functions, these proteins share similar active site structures, which include diiron(II) cores coordinated by terminal and bridging carboxylate ligands, water and/or OH groups, and two histidine residues (Figure 1). Among the BMM family members, soluble MMOs have been widely investigated because they can utilize earth-abundant molecular oxygen to oxidize methane to methanol under physiological conditions (eq 1).

\[
\text{CH}_4 + \text{O}_2 + \text{H}^+ + \text{NAD(P)H} \rightarrow \text{CH}_3\text{OH} + \text{NAD(P)}^+ + \text{H}_2\text{O} \quad (1)
\]

This chemical reaction is significant because breaking the strong C—H bond in methane chemically (104 kcal/mol, the highest C—H bond energy among hydrocarbons) is very
challenging. The ability of sMMO to selectively oxidize methane to methanol under ambient conditions is quite impressive. Understanding how the enzyme performs will demystify this marvelous natural phenomenon.

Soluble MMO enzymes are typically found in the metabolic system of methanotrophic bacteria, which consume methane as their sole source of carbon and energy. Methane is generated from carbon dioxide by methanogenic bacteria under anaerobic conditions such as lakes, oceans, and wetlands. Since both methane and dioxygen are essential for methanotrophs, these bacteria thrive in the borderline region of the aerobic and anaerobic environments. Expression of sMMO is favored at low copper concentration in the environment whereas virtually all methanotrophic bacteria express a membrane-bound, particulate form of methane monooxygenase (pMMO). Although sMMO contains a non-heme diiron unit, recent studies on pMMO have revealed the presence of two distinct copper-containing active sites. The particulate form is unstable, difficult to purify, and capable of accommodating methane only. The soluble form, on the other hand, has been widely studied due to its high stability, ease of purification, and ability to oxidize a broad range of organic substrates such as saturated and unsaturated, linear, branched, and cyclic hydrocarbons; aromatic, heterocyclic, and chlorinated compounds; sulfides; and many others. This versatile function of soluble methane monooxygenase makes methanotrophs important in bioremediation of the environment. Practical applications of the bacteria include purifying drinking water from chlorinated hydrocarbons and cleaning beaches that are contaminated with petroleum oil.

Catalytic Cycle of sMMOH

Extensive studies of sMMO have led to the delineation of its structural and electronic properties to a large extent. As shown in Figure 2a, sMMO is composed of three components: the
hydroxylase (MMOH), the reductase (MMOR), and a regulatory protein (MMOB). Although all three components of sMMO play important roles in the catalytic cycle, MMOH alone is sufficient for both dioxygen activation and substrate hydroxylation.\textsuperscript{24} Figures 2b and 2c depict the diiron core structures of sMMOH in different oxidation states in the catalytic cycle.\textsuperscript{33} Both diiron coordination spheres contain oxygen rich environments with (i) four glutamate carboxylates, (ii) two hisidine $N$-donors with syn stereochemistry (on the same side) to the Fe-Fe vector, (iii) OH/H$_2$O bridge(s), (iv) a water molecule, and (v) a carboxylate bridge in the orthogonal position to the diiron-dinitrogen coordination plane.

**Figure 2.** a) Overall fold of the three components of sMMO\textsuperscript{24} : the reductase component (MMOR), the regulatory protein (MMOB) and the hydroxylase component (MMOH); b) Active site structure of sMMOH in its diiron(III) resting state; c) in its diiron(II) reduced state. In both states, the diiron core is coordinated by two histidine residues (nitrogen atoms shown in blue) and four glutamic acid residues (oxygen atoms shown in red).\textsuperscript{33}
The catalytic cycle of sMMO for dioxygen activation and methane hydroxylation is shown in Scheme 1. At the beginning of the cycle, the coordination core is in its resting diiron(III) state ($H_{ox}$) (Figure 2b). Upon the gain of two electrons from MMOR, $H_{ox}$ gets reduced to a diiron(II) state ($H_{red}$), which readily reacts with dioxygen to form a diiron(III) peroxo intermediate. The peroxo adduct can further decay to a high-valent di($\mu$-oxo)diiron(IV) species called intermediate Q. Intermediate Q is responsible for the C—H bond activation in methane by abstracting the hydrogen atom via a radical mechanism. When $H_3C\cdot$ radical recombines with the OH/H$_2$O ligand at the diiron core, methanol is extruded from the active site. Efficient catalysis is made possible by the regulatory protein MMOB.$^{33}$

Scheme 1. Proposed catalytic cycle of sMMOH, showing the diiron core transformation.$^{33}$

Biomimetic Modeling of the Active Site of sMMOH

All the knowledge accumulated from the enzymology studies of sMMOH has inspired chemists to construct synthetic models that can faithfully replicate its active site structure and, more importantly, its catalytic activity. Small molecule synthetic modeling has been employed as a useful tool for chemists to further our understanding of the biological system.$^{34}$ For instance,
by comparing the spectroscopic properties of the synthetic diiron complexes with those of the enzyme, the structural information of the transient state of the enzyme in the catalytic cycle can be elucidated to some extent.\textsuperscript{33}

There are two main approaches in synthetic modeling of the active sites of metalloenzymes.\textsuperscript{35,36} A \textit{biomimetic} approach places an emphasis on faithfully replicating the geometry and coordination environment around the metallic core whereas a \textit{bio-inspired} approach imposes less restriction on the identity and orientation of the coordinating ligands relative to the metal centers. The bio-inspired approach focuses more on simulating the function of the target metalloenzyme. Because biomimetic model compounds display structural characteristics of the active site of the metalloenzyme while reserving their potential implication on the catalytic activity of the target enzyme, the first approach is usually more favored in modeling studies for bioinorganic chemists. Biomimetic modeling of the active sites of metalloenzymes will not only enrich our knowledge of the structures and mechanistic functions of the enzymes but also advance our attempts to make functional catalysts.

As described earlier, the catalytic cycle of sMMOH involves multiple enzyme components and various chemicals such as NADH, O\textsubscript{2}, methane, proton/water, etc. Modeling the structure and chemistry of the active site of sMMOH is certainly quite challenging. Over the past three decades, our group and several other research groups have developed many types of ligand systems that could construct diiron complexes with various similar properties to the sMMOH active site. The evolutional progress of this work has led to the attention of dinucleating ligand systems and \textit{syn} N-donor character.\textsuperscript{36} In the past decade, our group has focused on the design and synthesis of a series of dinucleating ligands with several criteria that need to be addressed to best mimic the natural protein.\textsuperscript{33} (i) To be a good structural and functional mimic, the ligand needs to
provide an oxygen-rich environment with all donor arms approaching the diiron core at specific orientations. (ii) The ligand should allow carboxylates to bridge between the diiron centers. (iii) Steric effects in the ligand framework should be carefully tuned to obtain a dinucleating ligand platform. Mononuclear iron species tend to form if the ligands are too bulky whereas undesired polynuclear clusters can be conceived if the framework does not have sufficient steric repulsion. (iv) Since oxygenation of sMMOH involves transient diiron(III) and (IV) intermediates, the synthetic models are expected to accommodate different oxidation states of the diiron core. In other words, the coordination sphere of the diiron(II) core should not be fully saturated or should have readily replaceable ligands such as water or organic solvent molecules. (vi) Flexibility of the model compound structure should also be taken into consideration as dioxygen activation can result in carboxylate shifts in the active site of sMMOH.

Some Prior Ligand Designs for sMMOH

Over the past three decades, much effort has been made in small molecule synthetic modeling of the active site structure of sMMOH. Although there has not yet been any model compound that can faithfully replicate both the structure and function of its active site, the information obtained from the different ligand systems has enriched our understanding of the enzyme and advanced our mimicking attempts. Some remarkable prior achievements are listed in Table 1.

Dicarboxylate Ligands

Failure of simple benzoates to mimic the carboxylate-bridged non-heme diiron core of sMMOH led to the investigation of better candidates with both high structural integrity and flexibility. Dicarboxylates were found to be promising dinucleating platforms that have bridging potential between the two metal centers. A good example is the doubly deprotonated form of m-
xylenediamine bis(Kemp’s triacid imide) (XDK$^{2-}$, Table 1).$^{36,38,39}$ The preorganized nature of the ligand framework and a definite cleft formed by the two carboxylate units in XDK$^{2-}$ afforded a carboxylate-bridged dinuclear complex [Fe$^{II}_2$(XDK)(μ-PhCyCO$_2$)(PhCyCO$_2$(py)$_2$)] (1, where PhCy = 1-phenylcyclohexylcarboxylate and py = pyridine).$^{36,40}$ The employment of a basic N-donor (py) and a sterically hindered external carboxylate (PhCy) allowed 1 to maintain its dinuclear structure upon exposure to dioxygen and also form a stable peroxo intermediate at low temperature. Unfortunately, there was no conversion of the peroxo adduct to a di(μ-O) Q-intermediate upon warming. This shortcoming could be attributed to the geometric restriction at the diiron core imposed by the doubly bridging nature of XDK$^{2-}$.$^{36,40}$

**Scheme 2.** A proposed mechanism for the O$_2$ reactivity of [Fe$^{II}_2$(Ar$^{Tol}$CO$_2$)$_4$(4-^t^Bupy)$_2$] (2A)$^{36}$

**Terphenylcarboxylate Ligands**

In the pursuit of better modeling platforms, reducing the steric constraints of the ligand framework was desired, and terphenylcarboxylates were discovered to be ideal building blocks. One significant model derived from a terphenylcarboxylate ligand is a quadruply bridged “paddlewheel” compound [Fe$^{II}_2$(Ar$^{Tol}$CO$_2$)$_4$(4-^t^Bupy)$_2$] (2A, Table 1) where Ar$^{Tol}$CO$_2$ is
Table 1. Some prior ligand designs in biomimetic modeling of the active site of sMMOH.36

<table>
<thead>
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<tbody>
<tr>
<td>XDK2-</td>
<td>-Enables assembly of [FeIII]O units that could not be accessed using simple carboxylates</td>
<td>-Does not enforce dinuclearity</td>
<td>36, 40</td>
</tr>
<tr>
<td>ArTolCO2-</td>
<td>-Forms diiron complexes in the presence of Fe(II) salts and an appropriate base</td>
<td>-The steric encumbrance of the 2,6-aryl groups restricts access to the diiron core</td>
<td>36,44</td>
</tr>
<tr>
<td>EtLBCQEBEt</td>
<td>-Stabilizes high-valent iron species</td>
<td>-Does not prevent formation of polyiron species</td>
<td>-Easy to synthesize</td>
</tr>
<tr>
<td>[Fe2ArTolCO2]2(Bup)2</td>
<td>-Enforces the syn stereochemistry of nitrogen donors relative to the Fe-Fe vector</td>
<td>-Neutral oxygen donors, rather than anionic</td>
<td>-Accommodates binding of external carboxylates to the diiron core</td>
</tr>
<tr>
<td>[Fe2ArTolCO2]2(EtLBCQEBEt)5</td>
<td>-Supports a carboxylate-bridged diiron(II) unit</td>
<td>-Does not prevent formation of tetrarion species</td>
<td>-Maintains a dinuclear core upon reaction with O2</td>
</tr>
<tr>
<td>[Fe2ArTolCO2]2(PIM)6</td>
<td>-Can be sterically tuned without obstructing access to the metal binding pocket</td>
<td></td>
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</tr>
</tbody>
</table>

Table 1. (Continued)  
Ligand/ Example of Iron Complex* Desirable Characteristics | Undesirable Characteristics | Ref. No. |
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<tbody>
<tr>
<td>[Fe2(XDK)(CyPhCO2)2(Py)3] 1</td>
<td>-Enables assembly of [FeIII]O units that could not be accessed using simple carboxylates</td>
<td>-Does not enforce dinuclearity</td>
</tr>
<tr>
<td>[Fe2(ArTolCO2)2(4-Bupy)2]2A</td>
<td>-Forms diiron complexes in the presence of Fe(II) salts and an appropriate base</td>
<td>-The steric encumbrance of the 2,6-aryl groups restricts access to the diiron core</td>
</tr>
<tr>
<td>[Fe2(ArTolCO2)2(EtLBCQEBEt)5] 5</td>
<td>-Stabilizes high-valent iron species</td>
<td>-Does not prevent formation of polyiron species</td>
</tr>
<tr>
<td>[Fe2(ArTolCO2)2(PIM)6] 6</td>
<td>-Enforces the syn stereochemistry of nitrogen donors relative to the Fe-Fe vector</td>
<td>-Neutral oxygen donors, rather than anionic</td>
</tr>
<tr>
<td>[Fe2(ArTolCO2)2(PIM)6] 6</td>
<td>-Can be sterically tuned without obstructing access to the metal binding pocket</td>
<td>-Does not prevent formation of tetrarion species</td>
</tr>
</tbody>
</table>
terphenylcarboxylate and 4-t-Bupy is 4-tert-butylpyridine.\textsuperscript{36,44} Reaction of 2A with O\textsubscript{2} irreversibly gave a deep green intermediate, comprised of equimolar amounts of a diiron(II,III) (3) and a diiron(III,IV) (4) species. Scheme 2 shows the proposed mechanism of the formation of the two intermediate forms. Worthy of notice is the fact that 2A represents the first synthetic model compound, which upon oxygenation afforded an iron(IV) intermediate as in the enzymatic mechanism. Despite these advances, the oxygenated intermediates exhibited low reactivity with hydrocarbons because the intermediates participated in intermolecular electron transfer reactions and were rapidly deactivated.\textsuperscript{36,44}

**Syn N-Donor Ligands**

One of the main challenges in biomimetic modeling of the active site structure of sMMOH is to reproduce the syn stereochemistry of the nitrogen donors from the histidine residues relative to the diiron vector.\textsuperscript{33} A continued search for potential syn N-donor ligands led to the investigation of the iron chemistry of 1,2-bis(3-ethynyl-8-carboxylatequinoline)-4,5-diethynylbenzene ethyl ester (Et\textsubscript{2}BCQEB\textsubscript{Et}, Table 1).\textsuperscript{36,45} Treating Et\textsubscript{2}BCQEB\textsubscript{Et} with iron(II) triflate and Ar\textsuperscript{Tol}CO\textsubscript{2} afforded [Fe\textsuperscript{II}\textsubscript{2}(Et\textsubscript{2}BCQEB\textsubscript{Et})(\mu-\text{Ar}^\text{Tol}CO\textsubscript{2})\textsubscript{3}]\textsuperscript{+} (5), a diiron(II) complex with three bridging carboxylates and syn arrangement of N-donors from quinoline moieties. Although 5 closely mimicked the diiron core of sMMOH, the neutral oxygen donors from ethyl esters were not capable of stabilizing the diiron centers.\textsuperscript{36,45}

**Macrocyclic Ligands**

Macrocyclic ligands show an unprecedented property in our efforts to develop sophisticated but well-defined diiron complexes that can accurately replicate the coordination environment in our targeted metalloenzyme. One such macrocyclic compound is H\textsubscript{2}PIM, comprised of two phenoxylimine metal binding units linked by diphenylsulfone and
Upon the reaction of H$_3$PIM with [Fe$_2$(Mes)$_4$] (Mes = 2,4,6-trimethylphenyl) and terphenyl carboxylic acid, Ar$^{\text{Tol}}$CO$_2$H, a diiron complex [Fe$^{\text{II}}_2$(μ-Ar$^{\text{Tol}}$CO$_2$)$_2$(PIM)] (6) was obtained (Table 1). X-ray single crystal analysis of 6 revealed its ability to closely resemble the sMMOH$_{\text{red}}$ active site motif based on the characteristics such as the syn N-donor conformation and an asymmetric μ-η$^1$,$\eta^1$ and μ-η$^1$,$\eta^2$ binding mode of carboxylates. Exposure of 6 to dioxygen afforded a mixture of (μ-oxo)diiron(III) [Fe$^{\text{III}}_2$(μ-O)(Ar$^{\text{Tol}}$CO$_2$)$_2$(PIM)] (7) and di(μ-hydroxo)diiron(III) [Fe$^{\text{III}}_2$(μ-OH)$_2$(Ar$^{\text{Tol}}$CO$_2$)$_2$(PIM)] (8) compounds. However, both 7 and 8 were found to transform to tetranuclear [Fe$^{\text{III}}_4$(μ-OH)$_6$(PIM)$_2$(Ar$^{\text{Tol}}$CO$_2$)$_2$] species when treated with excess water. Formation of this polynuclear compound may be prevented by modifying the PIM$^{2-}$ ligand platform with bulkier groups and employing less sterically demanding external carboxylates. Although phenolate and imine groups in PIM$^{2-}$ are not biologically relevant donors in sMMOH and are susceptible to either oxidation or hydrolysis, this ligand design is noteworthy because of its syn N-donors and anionic O-donors as well as its ability to accommodate multiple oxidation states and external carboxylate bridges between the diiron centers.

**Proposed Ligand Design**

As mentioned above, our goal is to design novel dinucleating pre-organized ligand frameworks that contain a hydrophobic pocket and a syn N-donor character to the diiron vector in an oxygen-rich environment. In our further attempt to fulfill the described requirements, Dr. Yang Li, my mentor, has proposed the design of a triptycene-based bis(benzoazole) ligand with three carboxylic acids (9, Figure 3a). In contrast to the external carboxylate bridges in all prior synthetic models, an internal carboxylate bridge is incorporated in the ligand design to examine its unique property in tuning the stability and reactivity of the diiron core. The two carboxylic
acids on the benzimidazole rings are intended to represent the two terminal glutamic acids while the rigid biphenyl hanging down from the third wing of the triptycene backbone is designed to deliver an internal carboxylic bridge close enough to the potential diiron core. The expected diiron complex derived from 9 is illustrated in its SPARTAN model (Figure 3b).

Figure 3. a) Initial proposed ligand design, triptycene-based bis(benzoxazole) triacid ligand 9 to mimic the active site structure of sMMOH. b) A SPARTAN model of the expected diiron complex derived from 9. Each iron site is coordinated by a benzoxazole nitrogen atom and a carboxylic acid oxygen atom. The diiron core is linked by an internal carboxylate bridge and two hydroxo bridges between the diiron centers, as in the enzyme.

Because the construction of this three-arm ligand 9 is synthetically challenging, and the coordination behavior of the two N,O-donor arms with iron is unknown, exploring the iron chemistry of the two-armed benzoxazole diester ligand L1 (Figure 5) became our first priority. The synthesis of L1 has been accomplished in 11 steps. Coordination of L1 with iron(II) salt achieved a dinuclear iron complex [Fe2L1(μ-OH)(μ-O2CarTol)(OTf)2] (Figure 4a), which closely mimics the core of sMMOH. Furthermore, its diacid analog, H2L2Ph4 (Figure 4b) with sterically encumbered group on the ortho-position of the carboxylic acid was prepared. Unexpectedly, treatment of this diacid ligand, H2L2Ph4, with iron(II) salt and an external carboxylate afforded a
triiron complex $[\text{NaFe}_3(L^2\text{Ph}_4)_2(\mu_3-O)(\mu-O_2\text{CCPh}_3)_2(H_2O)_3](\text{OTf})_2$, revealing the inert nature of the benzoxazole $N$-donors in the triiron complex.

![Figure 4](image)

**Figure 4.** a) X-ray crystal structure of $[\text{Fe}_2L^1(\mu-\text{OH})(\mu-O_2\text{CAr}_\text{Tol})(\text{OTf})_2]$ 24; b) SPARTAN model of the triptycene-based bis(benzoxazole) diacid ligand, $\text{H}_2L^2\text{Ph}_4$, with sterically hindered groups on the ortho-position of the carboxylic acids.

This weak electron donating nature of the nitrogen to the metal center is rationalized by the low basicity of the benzoxazole nitrogen ($pK_b = 13.2$). In contrast, the $pK_b$ of a benzimidazole nitrogen ($pK_b = 8.2$) is lower than that of a benzoxazole nitrogen by five orders of magnitude (Figure 5). The close resemblance of the former with the $pK_b$ of the histidine residues ($pK_b \sim 8.0$) at the diiron core of sMMOH suggests that this benzimidazole diester ligand $L^3$ (Figure 5) is more biologically relevant to the sMMOH active site. To avoid ambiguity between the two $N$-donors of a benzimidazole ring in the future iron coordination chemistry, the *meta*-nitrogen to the carboxylate has to be protected by a methyl group. Since I joined this project last summer, I have been involved in several aspects of this work: i) scaled-up synthesis of ligand $L^3$; ii) UV-Vis titration experiments to determine the stoichiometry of $L^3$, iron(II) salt, and an external carboxylate; iii) iron coordination chemistry of $L^3$; iv) reactivity studies of the resulting diiron complexes with $O_2$. 

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Figure 5. **Top.** Comparison of the basicity of a benzoxazole nitrogen (left) and a benzimidazole nitrogen (right). **Bottom.** Because the $pK_b$ of benzimidazole is much lower than that of benzoxazole, $L_3$, instead of $L_1$, became our next target to tune the electron-donating ability of the N-donors.
Experimental Section

Materials and Methods

Unless otherwise noted, all reagents were obtained from TCI, Aldrich Chemical Co., and Alfa Aesar, and used as received. Column chromatography was performed using Silicycle 60 Å, ultrapure silica gel. All solvents were degassed by passage over two columns of activated alumina ($\text{Al}_2\text{O}_3$) under an argon atmosphere. Air-sensitive manipulations were carried out by standard schlenk techniques as well as in a MBraun nitrogen double glovebox. IR spectra were acquired on a ThermoNicolet Avatar 360 spectrophotometer with OMNIC software. Spectroscopic measurements were taken on a Cary 50 spectrophotometer using 6Q Spectrosil quartz cuvettes with 1 cm path lengths.

X-ray Collection and Data Refinement (by Justin J. Wilson)

Samples for X-ray analysis were prepared by mounting single crystals in Paratone-N oil on a cryoloop and freezing them under a 110K KRYO-FLEX nitrogen cold stream. Data collection was performed by measuring diffraction intensities on a Bruker APEX CCD X-ray diffractometer with Mo-K$\alpha$ radiation ($\lambda = 0.71073$ Å) at 100(2) K using the APEX2 software. Absorption corrections were performed using SADABS. The structures were solved by direct methods with SHELXS and refined by full-matrix least squares based on $F^2$ using SHELXL.

$^{57}\text{Fe}$ Mössbauer Spectroscopy (by Dr. Ulf-Peter Apfel)

Solid samples were suspended in Apiezon M grease and placed in nylon sample holder. Mössbauer data were acquired at 90 K on a MSI spectrometer (WEB Research Co.) with a $^{57}\text{Co}$ source in a Rh matrix maintained at room temperature. The software reported isomer shift values
relative to metallic iron used for velocity calibration at room temperature. The WMOSS plot and fit program (WEB Research Co.) was used to fit the spectra with Lorentzian line shapes.

**Scaled-up Synthesis**

When I started this project, the reaction conditions for the synthesis of the L3 ligand have been optimized by Dr. Li. Described below are the six reactions (highlighted in blue in Scheme 3) that I ran for the scaled-up synthesis of L3. The rest were completed by Dr. Li.

**1,8-Dihydroxytriptycene dipropargyl ether (11)**

Solid 1,8-dihydroxytriptycene (10) (14.0 g, 49.0 mmol) was combined with propargyl bromide (80% w/w in toluene, 7.28 g, 49.0 mmol, 1 equiv), and K2CO3 (20.3 g, 147 mmol, 3 equiv) in 200 mL of acetone. The reaction mixture was refluxed, and over the course of 24 h, two additional portions of propargyl bromide (1 equiv per portion) were added at 12-h intervals. The reaction was quenched by cooling it to room temperature and then adding 200 mL of water. The organic phase was extracted with DCM (200 mL × 3). The combined organic extracts were washed with brine, dried over Na2SO4, passed through a filter paper, and concentrated under vacuum. The resulting material was purified by silica gel column chromatography (acetone: hexanes, 1:2) to obtain 16.5g of yellow solid (11) (93%). IR (KBr) 3446, 3293, 3284, 3059, 2959, 2930, 2865, 2122, 1591, 1478, 1455, 1377, 1274, 1264, 1234, 1106, 1067, 1052, 788, 743, 688, 667, 638 cm⁻¹; Melting point: 214–216°C.

**Diacid (15)**

Dialdehyde (14) (400 mg, 1.02 mmol), 30% H2O2 (697 mg, 6.14 mmol, 6.0 equiv), and NaH2PO4·H2O (848 mg, 6.14 mmol, 6.0 equiv) were combined and suspended in a stirred mixture of acetone and H2O (200 mL and 60 mL respectively) at 10–15°C in a water bath. A 50
mL aqueous solution of NaClO₂ (556 mg, 6.14 mmol, 6.0 equiv) was added dropwise to the stirred suspension via a dropping funnel over 2 h. The resulting suspension was stirred at room temperature overnight, and a clear light yellow solution was formed upon the complete consumption of the insoluble starting material, dialdehyde. Acetone was evaporated under reduced pressure. Acidification of the resulting solution with 10% aqueous HCl to pH < 1 afforded a yellow precipitate. The precipitate was filtrated, redissolved in THF (100 mL), and washed with water (10 mL) and brine (10 mL). The solution was dried over Na₂SO₄, filtered, and dried in vacuo to give 413 mg (95%) of diacid (15). IR (KBr) 3400, 3117, 3069, 2955, 2470, 1908, 1710, 1631, 1564, 1459, 1406, 1333, 1289, 1185, 936, 853, 767, 751, 680, 645 cm⁻¹; Melting point: 341–344°C.

*Methyl 3-chloro-2-nitrobenzoate (17)*

Solid 3-chloro-2-nitrobenzoic acid (16) (10.0 g, 50 mmol) was suspended in 250 mL Et₂O under nitrogen gas. Into the stirred suspension was bubbled diazomethane, CH₂N₂, generated from slow addition of 10% NaOH to a 50 mL EtOH solution of Diazald until the TLC analysis indicated the complete consumption of the starting material 16. The product solution was purified by silica gel column chromatography (Acetone), and the resulting acetone eluate was concentrated by rotary evaporation to give 10.6 g (99%) of methyl 3-chloro-2-nitrobenzoate (17). IR (KBr) 3107, 3066, 2957, 2906, 2850, 1732, 1552, 1444, 1384, 1288, 1216, 1172, 978, 853, 765, 718 cm⁻¹; Melting point: 105–106 °C.

*3-(Methylamino)-2-nitrobenzoic acid*

A reaction mixture of methyl 3-(N-methylacetamido)-2-nitrobenzoate (18) (8.0 g, 31.7 mmol) in 520 mL of 10% NaOH and 200 mL EtOH was allowed to stir at 80–85°C for 10 h. The solution was then cooled to 0°C before its pH was adjusted to 1.5–2.0 with concentrated HCl.
The organic phase, from extraction with EtOAc (4 × 150 mL), was washed with brine (30 mL), dried over Na₂SO₄, passed through a filter paper, and evaporated to dryness. The resulting red crude material (7.2 g) was employed in the next reaction step without further purification. IR (KBr) 3392, 2911, 1700, 1615, 1575, 1512, 1448, 1253, 1180, 1078, 1053, 799, 765 cm⁻¹.

*Methyl 3-(methylamino)-2-nitrobenzoate (19)*

Slow addition of small aliquots of 10% aqueous NaOH via a syringe to an EtOH solution of Diazald produced diazomethane, which was bubbled into a suspension of a 1-g portion of the crude 3-(methylamino)-2-nitrobenzoic acid in 150 mL Et₂O under a N₂ environment. To convert all the starting material, the described 1-gram scale reaction was repeated seven times under the same conditions. Completion of each reaction was monitored by TLC. Purification of the resulting red oil by silica gel column chromatography (Hexanes: EtOAc = 1:0, 10:1–4:1) gave 6.0 g (90% in two steps) of methyl 3-(methylamino)-2-nitrobenzoate (19) as red oil. IR (KBr) 3398, 3002, 2952, 2839, 1732, 1613, 1573, 1511, 1446, 1358, 1280, 1208, 1119, 1079, 1054, 983, 853, 792, 754, 704, 547, 482 cm⁻¹.

*Triptycene-based Bis(Benzimidazole) Diester Ligand L3*

Solid diacid (15) (84.4 mg, 0.2 mmol) was suspended in 80 mL of anhydrous DCM under a N₂ atmosphere. Pyridine (646 μL, 8 mmol, 40 equiv) and thionyl chloride (584 μL, 8 mmol, 40 equiv) were added in order to the suspension. After stirring at room temperature for 60 h, the resulting solution was concentrated in vacuo, followed by high-vacuum drying for 8 h. The resulting solid was dissolved in 40 mL of anhydrous DCM. The solution was treated with pyridine (646 μL, 8 mmol, 40 equiv) before methyl 2-amino-3-(methylamino)benzoate (20) (72 mg, 0.4 mmol, 2 equiv) was added. The resulting light brown reaction mixture was allowed to
stir at room temperature for 30 h. The crude solid 21, obtained upon rotary evaporation of all the volatiles, was used immediately in the subsequent reaction.

The crude solid 21 was dissolved in 20 mL of HOAc. The reaction mixture was refluxed for 1 h, after which HOAc was removed. The residue, neutralized with Et₃N on silica gel, was purified by flash column chromatography (DCM: EtOAc = 20:1–6:1) to give ligand L3 (68 mg, 48% in 3 steps) as a pale yellow solid. IR (KBr) 3127, 3065, 2947, 2839, 1711, 1636, 1606, 1449, 1384, 1294, 1254, 1207, 1118, 1061, 836, 752, 713, 680 cm⁻¹; Melting point: 333–335 °C.

\[
\text{[Fe}_2\text{L3(μ-OH)(μ-O}_{2}\text{CAr}_{\text{Tol}})(\text{OTf})_2]\text{](22)}
\]

Inside a nitrogen box, a solution of Fe(OTf)₂(MeCN)₂ (OTf = OSO₂CF₃) (36.9 mg, 0.085 mmol, 2 equiv relative to L3) in 2 mL of MeCN was prepared, to which 2,6-di(p-tolyl)benzoic acid (HO₂CAr₆Tol) (12.8 mg, 0.042 mmol, 1.0 equiv relative to L3) in THF (2 mL) and wet Et₃N (11.7 μL, 0.085 mmol, 2.0 equiv relative to L3) were added in order. The mixture was stirred at room temperature for 5 min. Addition of a solution of ligand L3 (30 mg, 0.042 mmol) in DCM (2 mL) to the mixture instantaneously gave a red solution. The reaction suspension was stirred for 30 min, after which the solvent was removed under vacuum. Dissolving the resulting orange solids in DCM/MeOH (2 mL/0.5 mL) afforded a clear red solution. Impurities were removed by filtration with glass wool. By slowly diffusing Et₂O into the purified solution of the compound over a few days, red needle-like crystals were obtained. The crystals were washed with Et₂O twice, and upon drying under vacuum, 22.4 mg (34.8%) orange red crystals were yielded. IR (KBr) 3491, 3132, 3023, 2961, 2920, 1672, 1620, 1582, 1449, 1409, 1327, 1298, 1237, 1158, 1027, 756, 637 cm⁻¹.
Inside a nitrogen box, a solution of Fe(OTf)_2(MeCN)_2 (36.9 mg, 0.085 mmol, 2.0 equiv relative to L3) in 2 mL of MeCN was prepared and combined with a solution of NaO_2CCPh_3 (sodium 2,2,2-triphenylacetate) (13.1 mg, 0.042 mmol, 1 equiv relative to L3) in THF (2 mL). The mixture was stirred at room temperature for 5 min. Slow addition of a solution of ligand L3 (30 mg, 0.042 mmol) in DCM (2 mL) to the mixture instantaneously gave a red solution. The reaction suspension was stirred for 30 min, after which the solvent was removed under vacuum. Dissolving the resulting orange solids in DCM/MeOH (2 mL/0.5 mL) afforded a dark red solution. Impurities were removed by filtration with glass wool. By slowly diffusing Et_2O into the purified solution of the compound over a few days, red needle-like crystals were obtained. The crystals were washed with Et_2O twice, and upon drying under vacuum, 28 mg (43%) orange red crystals were yielded. IR (KBr) 3514, 3134, 3055, 2963, 2860, 1664, 1597, 1446, 1347, 1305, 1238, 1161, 1030, 877, 754, 637 cm^{-1}.

UV-Vis Spectrophotometric Studies

Inside an anaerobic box, the following four stock solutions were prepared: L3 (10 μM in DCM), Fe(OTf)_2 (4.0 mM in MeCN), Ph_3CCO_2Na (4.0 mM in THF), and Et_3NH^+Ar^{Tol}CO_2^- (4.0 mM, prepared by equimolar amounts of Ar^{Tol}CO_2H and Et_3N in THF).

Reaction of L3 with Fe(OTf)_2

Inside a nitrogen drybox, a sealed UV-Vis quartz cuvette holding 4 mL of L3 stock solution and a 50 μL airtight syringe loaded with Fe(OTf)_2 stock solution were prepared and brought outside the box. Small aliquots of the anaerobic Fe(OTf)_2 solution (5 μL, 0.5 equiv
relative to L3) were titrated to the sample in the cuvette, and their absorption spectra were recorded.

*Reaction of Ph$_3$CCO$_2$Na or Et$_3$NH$^+$Ar$^{Tol}$CO$_2^-$ with a mixture of L3 and 2 equiv of Fe(OTf)$_2$*

Inside a nitrogen box, a sealed UV-vis quartz cuvette that contained a mixture of 4 mL of L3 stock solution and a 20 μL portion of the Fe(OTf)$_2$ solution (2 equiv relative to L3) was prepared and brought outside the box. To avoid air contact, a 50 μL airtight syringe loaded with a stock solution of Ph$_3$CCO$_2$Na or Et$_3$NH$^+$Ar$^{Tol}$CO$_2^-$ was inserted into a septum before being brought outside the nitrogen box. Small aliquots of the anaerobic Ph$_3$CCO$_2$Na or Et$_3$NH$^+$Ar$^{Tol}$CO$_2^-$ solution (5 μL, 1 equiv relative to L3) were titrated to the sample in the cuvette, and their absorption spectra were recorded.

*Reaction of Fe(OTf)$_2$ with a mixture of L3 and 1 equiv of Ph$_3$CCO$_2$Na or Et$_3$NH$^+$Ar$^{Tol}$CO$_2^-$*

Inside a nitrogen box, a sealed UV-vis quartz cuvette that contained a mixture of 4 mL of L3 stock solution and a 10 μL portion of the Ph$_3$CCO$_2$Na or Et$_3$NH$^+$Ar$^{Tol}$CO$_2^-$ solution (1 equiv relative to L3) was prepared and brought outside the box. To avoid air contact, a 50 μL airtight syringe loaded with the Fe(OTf)$_2$ stock solution was inserted into a septum before being brought outside the nitrogen box. Small aliquots of the anaerobic Ph$_3$CCO$_2$Na or Et$_3$NH$^+$Ar$^{Tol}$CO$_2^-$ solution (5 μL, 0.5 equiv relative to L3) were titrated to the sample in the cuvette, and their absorption spectra were recorded.

*Reaction of [Fe$_2$L3(μ-OH)(μ-O$_2$CAr$^{Tol}$)(OTf)$_2$] with O$_2$*

A piece of [Fe$_2$L3(μ-OH)(μ-O$_2$CAr$^{Tol}$)(OTf)$_2$] red crystal was dissolved in 4 mL of degassed DCM in a nitrogen box. The solution was placed in a UV-vis dewar to maintain low temperature. The cell was capped with a septum, sealed with electronic tape, and taken out of the
glovebox. The DCM solution was cooled to -78 °C in a dry ice/acetone bath. Excess dry dioxygen (5 mL) was then bubbled into the cryogenic solution. Temperature of the solution was gradually raised to room temperature over the course of 10 h, and absorption spectra were recorded at different temperatures.
Results and Discussion

Ligand Synthesis

Scheme 3: Synthesis of benzimidazole diester ligand L3
A synthetic pathway to benzimidazole diester ligand \textbf{L3} is shown in Scheme 3. Optimal reaction conditions had been established by Dr. Li when I started the project, and I mainly worked on the scaled-up synthesis of \textbf{L3} to obtain materials for coordination studies. The reactions I have personally conducted are highlighted in blue. (I) Using propargyl bromide in acetone enabled allylation of 1,8-dihydroxytriptycene 1 to give 2 in 93% yield. (II) Oxidation of dialdehyde 5 to diacid 6 was achieved by using NaClO₂ and 30% H₂O₂, buffered with NaH₂PO₄. Acidification with concentrated HCl gave a high yield of diacid. (III) The starting material 3-chloro-2-nitro-benzoic acid 7 was reacted with diazomethane, produced from the reaction of diazald and sodium hydroxide in ethanol. Methyl 3-chloro-2-nitrobenzoate 8 was obtained as a product in 99% yield. (IV) The reaction of methyl 3-(N-methylacetamido)-2-nitrobenzoate 9 with an excess amount of 10% NaOH in ethanol at 80°C gave hydrolysis of ester and amide group, resulting in 3-(methylamino)-2-nitrobenzoic acid. This crude product when reacted with diazomethane underwent esterification to give a 95% yield of methyl 3-(methylamino)-2-nitrobenzoate 10 over two steps. (V) Using HATU as a reagent, amide coupling of the previously synthesized diacid intermediate 6 with diamine 11 gave 12. In this one-pot two-reaction step, cyclization of 12 was achieved by using acetic acid to give the desired \textbf{L3}.

**UV-Vis Spectrophotometric Titrations**

Upon the successful synthesis of the \textbf{L3} ligand, our next step is to explore its coordination with iron as well as its ability to form an external carboxylate bridge. The coordination sphere of sMMOH\textsubscript{red} includes a diiron(II) core coordinated by two histidine \textit{N}-donor residues syn with respect to Fe-Fe vector and four carboxylate amino acid residues (two bridging and two terminal). To closely imitate the active site structure of the enzyme, the ligand \textbf{L3} would need to bind with two iron(II) atoms and allow external carboxylate bridge(s) in the
system displayed intense optical features. To examine the ligand-metal-carboxylate interaction in the system that displayed prominent optical responses, a series of UV-vis spectrophotometric titrations were conducted.

Figure 6. UV-vis absorption spectra acquired by titration of Fe(OTf)$_2$ (0.5 equiv relative to L3 per aliquot) to a DCM solution of L3 (10 μM). The apo L3 ligand is shown by the black dotted trace whereas the colored solid lines represent the spectra obtained upon the addition of iron(II) to L3. Maximal optical changes were observed at 386 nm, and a plot of the absorbance change at 386 nm against the titrated amount of Fe(OTf)$_2$ to L3 is illustrated in the top right corner.

The experimental design was such that the metal-to-ligand stoichiometry in the absence of ancillary carboxylates was first explored. Each set of titration was repeated three times under the same conditions to obtain an averaged trend. As shown in Figure 6, free L3 ligand displayed a strong absorption at 345 nm. Successive addition of Fe(OTf)$_2$ to the L3 solution resulted in a gradual decrease in the intensity of the 345 nm band. When > 1 equiv of iron(II) was present in the solution, the band at 345 nm shifted hypsochromically to 351 nm, concomitant with an
increased absorption at 365 and 386 nm. A plot of the optical changes at 386 nm, characteristic of an Fe-O(H)-Fe species, suggests that \( \text{L3} \) is capable of binding with at least two iron atoms although determination of the exact metal-to-ligand ratio was prevented by the large fluctuation of the data, regardless of multiple repetitions (Figure 6). The presence of an isosbestic point at 308 nm demonstrates a conversion from A to B.

Next, in order to investigate the stoichiometry of carboxylate, small aliquots of \( \text{Et}_3\text{NH}^+\text{ArTolCO}_2^- \) (terphenylcarboxylate for simplicity) were titrated to a premixed solution containing 2 equiv of \( \text{Fe(OTf)}_2 \) and 1 equiv of \( \text{L3} \). This 2:1 metal-to-ligand ratio was chosen to construct dinuclear complexes as in the enzyme. In Figure 7a is shown a plot of the optical change at 386 nm versus the titrated amount of \( \text{Et}_3\text{NH}^+\text{ArTolCO}_2^- \) to the solution. Absorption at 386 nm grew upon the addition of terphenylcarboxylate and reached an optimal value at 1 equiv of carboxylate. The presence of excess carboxylate resulted in a dramatic decrease in the band at 386 nm as well as a spectral growth at 345 nm, a characteristic absorption of the free \( \text{L3} \) ligand. This behavior may therefore be indicative of the labile nature of the resulting diiron complex system with an external carboxylate. An excess amount of bulky carboxylates seems to disrupt the coordination sphere by extracting iron(II) from the complex, forming iron carboxylates and free \( \text{L3} \) ligands. It can be concluded from this set of experiment that a 1:2:1 iron(II)-to-\( \text{L3} \)-to-\( \text{Et}_3\text{NH}^+\text{ArTolCO}_2^- \) complex is formed favorably in the solution.

In the last set of titrations, a premixed solution containing \( \text{L3} \) and \( \text{Et}_3\text{NH}^+\text{ArTolCO}_2^- \) (1 equiv relative to \( \text{L3} \) based on the previous titration) was prepared, to which various aliquots of \( \text{Fe(OTf)}_2 \) were added. A plot of the absorbance changes versus the titrated amount of iron(II) is given in Figure 7c. The spectral band at 386 nm gradually increased and reached a maximum at 2.0 equiv of the Fe(II) solution. A slight decrease in the absorption at 386 nm in the
Figure 7: Plots of the spectral change at 386 nm (at which maximum absorbance change occurs) against the titrated amount of a) Fe(OTf)$_2$ to a solution containing L3 (10 μM in DCM) and Et$_3$NH$^+$/ArTol$^-$CO$_2^-$ (1 equiv rel to L3); b) Fe(OTf)$_2$ to a solution containing L3 (10 μM in DCM) and NaO$_2$/CCPh$_3$ (1 equiv rel to L3); c) Et$_3$NH$^+$/ArTol$^-$CO$_2^-$ to a solution containing L3 (10 μM in DCM) and Fe(OTf)$_2$ (2 equiv rel to L3); d) NaO$_2$/CCPh$_3$ to a solution containing L3 (10 μM in DCM) and Fe(OTf)$_2$ (2 equiv rel to L3). The error bars represent the standard deviations from three repeating experiments.
presence of excess iron(II) could be a result of the free Fe(OTf)$_2$ extruding carboxylate from the diiron complex system.

Interaction of L3 with Fe(OTf)$_2$ and a different external carboxylate with similar steric effects as terphenylcarboxylate, Ph$_3$CCO$_2$Na (triphenylacetate for simplicity), was also examined with similar UV-Vis spectrophotometric studies. Comparable results were obtained, suggesting the 1:2:1 stoichiometry among the ligand, iron(II), and triphenylacetate. Based on these data, L3 can support a diiron structure with an external carboxylate. The effect of a structural difference between Ph$_3$CCO$_2$Na and Et$_3$NH$^+$Ar$^{\text{Tol}}$CO$_2$ on the coordination chemistry appears to be minimal.

### Assembly and Characterization of Diiron(II) Complexes

#### X-ray Diffraction Studies

To examine the structures of the diiron complexes derived from L3, single red crystals were grown by slow vapor diffusion of diethyl ether into a DCM/MeOH solution of the 1:2:1 reaction mixture of L3, Fe(OTf)$_2$ and an external carboxylate (Et$_3$NH$^+$Ar$^{\text{Tol}}$CO$_2$ or Ph$_3$CCO$_2$Na). Single crystals suitable for X-ray diffraction analysis were obtained from both carboxylates, and their molecular formula are [Fe$_2$L$_3$(μ-OH)(μ-O$_2$CR)(OTf)$_2$], where R = Ar$^{\text{Tol}}$ (22, Figure 8, Table 2) or Ph$_3$C (23, Figure 9, Table 2). By comparison of 22 and 23, the two structures have similar five-coordinated metal coordination geometries. In addition to a bridging hydroxide and a bridging carboxylate between the two iron centers, each iron unit is coordinated by a terminal triflate (OTf) and chelated by a nitrogen atom and an ester carbonyl oxygen atom from one benzimidazole arm of L3 ligand. Although the triflate ligands from iron(II) salt were expected to be replaced by external carboxylates, they remained coordinated to the iron centers in both complexes. This observation could be partly due to the large size of both carboxylates, which could not fit into the position of the terminal triflate ligands. One of these triflate ligands
interacts with the bridging hydroxide via hydrogen bonding. As shown in Table 3, bond metrics in both complexes are analogous. The Fe—Fe distances in both complexes (3.489 Å in 22 and 3.444 Å in 23) resemble the Fe—Fe distance in sMMOH (3.43 Å). The average Fe—O distances (carboxylate) in 22 and 23 are 2.071 Å and 2.068 Å respectively. The average distances between iron and ester carbonyl oxygen atoms are also similar (2.107 Å in 22 and 2.131 Å in 23). The Fe—O distances of the triflate ligands vary from 2.184 and 2.284 Å, which are longer than other Fe—O distances in the structures.

The effect of employing a more basic benzimidazole ligand L3 rather than L1 was explored by comparing the structures of 22 and 23 with a previously reported X-ray structure of a diiron complex derived from L1 [Fe2L1(μ-OH)(μ-O2CArTol)2(OTf)2] (24, Figure 4a). Table 3 lists relevant parameters in their respective structures. Although the nitrogen donors in 22 and 23 are much more basic than those in 24, the two structures show similarity in Fe—N distances as well as other Fe—L distances. Interestingly, however, there was a significant change in the Fe⋯O (benzofuran) distances between complexes derived from L1 and L3. The average Fe⋯O (benzofuran) distances in 22 and 23 are 2.69 Å and 2.74 Å, respectively (Table 3) whereas the distance in 24 is much longer (3.02 Å, Table 3). These shorter distances in 22 and 23 could be an outcome of intrinsic different electronic properties of benzimidazole-based ligands. In addition, the coordination geometries of the three complexes 22-24 were compared based on the parameter, τ, described in Table 3. This parameter (eq 2) describes the distortion of a five-coordinate structure between idealized trigonal-bipyramidal (τ = 1) and square-pyramidal (τ = 0) geometries.

$$\tau = (\beta - \alpha)/60, \beta > \alpha \quad (2)$$
In 22, the $\tau$ values of the two irons are 0.05 and 0.08 (both close to 0), which correspond to a nearly perfect square-pyramidal coordination geometry. On the other hand, the $\tau$ values for 23 are 0.20 and 0.30, and those for 24 are 0.30 and 0.41. These values indicate that the iron atoms in these structures attain a higher degree of distortion towards trigonal-bipyramidal geometries.

**Figure 8.** X-ray crystal structure of $[\text{Fe}_2\text{L}_3(\mu-O\text{H})(\mu-O_2\text{CAr}^{\text{Tol}})(\text{OTf})_2]$ 22 with 50% probability thermal ellipsoid and a partial numbering scheme. Hydrogen atoms and solvent molecules are omitted for clarity. Iron, green; carbon, gray; oxygen, red; nitrogen, blue; sulfur, yellow; fluorine, yellow green. Selected bond lengths (Å) and angles (deg): $\text{Fe}_1$-$\text{N}_1$, 2.084(6); $\text{Fe}_2$-$\text{N}_2$, 2.100(6); $\text{Fe}_1$-$\text{O}_5$, 2.091(6); $\text{Fe}_2$-$\text{O}_7$, 2.122(5); $\text{Fe}_1$-$\text{O}_10$, 2.074(5); $\text{Fe}_2$-$\text{O}_11$, 2.068(5); $\text{Fe}_1$-$\text{O}_9$, 1.943(5); $\text{Fe}_2$-$\text{O}_9$, 1.962(5); $\text{Fe}_1$-$\text{O}_12$, 2.284(3); $\text{Fe}_2$-$\text{O}_15$, 2.212(6); $\text{Fe}_1$-$\text{O}_9$-$\text{Fe}_2$, 126.5(3); $\text{O}_9$-$\text{Fe}_1$-$\text{O}_10$, 97.9(2); $\text{O}_9$-$\text{Fe}_2$-$\text{O}_11$, 95.0(2); $\text{O}_9$-$\text{Fe}_1$-$\text{N}_1$, 167.3(3); $\text{O}_9$-$\text{Fe}_2$-$\text{N}_2$, 168.1(2); $\text{O}_{10}$-$\text{Fe}_1$-$\text{O}_{12}$, 170.50(16); $\text{O}_{11}$-$\text{Fe}_2$-$\text{O}_{15}$, 163.6(2).
Figure 9. X-ray crystal structure of [Fe$_2$L$_3$(μ-OH)(μ-O$_2$CCPh$_3$)(OTf)$_2$] 23 with 50% probability thermal ellipsoid and a partial numbering scheme. Hydrogen atoms and solvent molecules are omitted for clarity. Iron, green; carbon, gray; oxygen, red; nitrogen, blue; sulfur, yellow; fluorine, yellow green. Selected bond lengths (Å) and angles (deg): Fe1-N1, 2.106(4); Fe2-N2, 2.113(4); Fe1-O5, 2.133(4); Fe2-O7, 2.128(4); Fe1-O10, 2.073(4); Fe2-O11, 2.062(4); Fe1-O9, 1.961(4); Fe2-O9, 1.953(4); Fe1-O12, 2.230(4); Fe2-O15, 2.184(4); Fe1-O9-Fe2, 123.3(2); O9-Fe1-O10, 97.04(15); O9-Fe2-O11, 93.23(15); O9-Fe1-N1, 158.25(17); O9-Fe2-N2, 177.68(17); O10-Fe1-O12, 170.52(15); O11-Fe2-O15, 159.51(16).
Table 2. Crystal data and structure refinement for complexes 22 and 23.

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<td>\begin{align*}a &amp;= 16.0657(9) \text{ Å} \ b &amp;= 19.7408(11) \text{ Å} \ c &amp;= 19.9989(11) \text{ Å} \end{align*}</td>
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<td>Theta range for data collection</td>
<td>1.55 to 23.38°</td>
<td>1.63 to 25.14°</td>
</tr>
<tr>
<td>Index ranges</td>
<td>\begin{align*}-22 &amp;\leq h \leq 22 \ -23 &amp;\leq k \leq 23 \ -22 &amp;\leq l \leq 22 \end{align*}</td>
<td>\begin{align*}-18 &amp;\leq h \leq 19 \ -23 &amp;\leq k \leq 23 \ -23 &amp;\leq l \leq 23 \end{align*}</td>
</tr>
<tr>
<td>Reflections collected</td>
<td>97291</td>
<td>101906</td>
</tr>
<tr>
<td>Independent reflections</td>
<td>10347 [R(int) = 0.0640]</td>
<td>11325 [R(int) = 0.1103]</td>
</tr>
<tr>
<td>Completeness to theta=24.83°</td>
<td>99.6 %</td>
<td>99.8 %</td>
</tr>
<tr>
<td>Absorption correction</td>
<td>Semi-empirical from equivalents</td>
<td>Semi-empirical from equivalents</td>
</tr>
<tr>
<td>Max. and min. transmission</td>
<td>0.9400 and 0.7876</td>
<td>0.9593 and 0.8729</td>
</tr>
<tr>
<td>Refinement method</td>
<td>Full-matrix least-squares on ( F^2 )</td>
<td>Full-matrix least-squares on ( F^2 )</td>
</tr>
<tr>
<td>Data / restraints / parameters</td>
<td>10347 / 244 / 946</td>
<td>11325 / 56 / 913</td>
</tr>
<tr>
<td>Goodness-of-fit on ( F^2 )</td>
<td>1.054</td>
<td>1.047</td>
</tr>
<tr>
<td>Final R indices [I&gt;2(\sigma(I))]</td>
<td>( R_1 = 0.0917, ) ( wR_2 = 0.2417 )</td>
<td>( R_1 = 0.0569, ) ( wR_2 = 0.1190 )</td>
</tr>
<tr>
<td>R indices (all data)</td>
<td>( R_1 = 0.1204, ) ( wR_2 = 0.2682 )</td>
<td>( R_1 = 0.0860, ) ( wR_2 = 0.1314 )</td>
</tr>
<tr>
<td>Largest diff. peak and hole</td>
<td>4.375 and -0.698 e.Å⁻³</td>
<td>1.029 and -0.435 e.Å⁻³</td>
</tr>
</tbody>
</table>

\( ^a \) The residual electron density and hole are in the region of disordered/partially occupied solvent molecules.

Table 3. Selected distances, angles, and geometric parameter (\( \tau \)) in complexes 22-24.

<table>
<thead>
<tr>
<th></th>
<th>22</th>
<th>23</th>
<th>24</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fe1···Fe2</td>
<td>3.487</td>
<td>3.444</td>
<td>3.448</td>
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<tr>
<td>N1···N2</td>
<td>7.335</td>
<td>7.233</td>
<td>7.320</td>
</tr>
<tr>
<td>Fe1···O1</td>
<td>2.690</td>
<td>2.860</td>
<td>3.060</td>
</tr>
<tr>
<td>Fe2···O2</td>
<td>2.695</td>
<td>2.610</td>
<td>2.987</td>
</tr>
<tr>
<td>O1···O2</td>
<td>5.135</td>
<td>5.169</td>
<td>5.076</td>
</tr>
<tr>
<td>Fe–OH–Fe</td>
<td>126.5</td>
<td>123.3</td>
<td>124.3</td>
</tr>
<tr>
<td>( \tau ) (Fe1)</td>
<td>0.05</td>
<td>0.20</td>
<td>0.30</td>
</tr>
<tr>
<td>( \tau ) (Fe2)</td>
<td>0.08</td>
<td>0.30</td>
<td>0.41</td>
</tr>
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</table>
Mössbauer Spectroscopy

The zero-field $^{57}$Fe Mössbauer spectra of the complexes 22 and 23 were acquired at 77 K to examine the oxidation state of iron atoms in the complexes. As shown in Figure 10, the Mössbauer spectrum of 22 shows a single quadrupole doublet, indicating that the two iron units in the complex are chemically equivalent. This complex has an isomer shift ($\delta$) value of 1.218 mm·s$^{-1}$ and a quadrupole splitting ($\Delta E_Q$) is 1.960 mm·s$^{-1}$ (Table 4). In addition, the complex 23 shows an isomer shift ($\delta$) value of 1.224 mm·s$^{-1}$ and a quadrupole splitting ($\Delta E_Q$) is 2.064 mm·s$^{-1}$ (Table 4). These parameters are characteristic of our desired high spin diiron(II) complexes. Mössbauer data thus suggested that we have obtained diiron(II) complexes derived from L3.

Table 4. Mössbauer fits for complexes 22 and 23.

<table>
<thead>
<tr>
<th></th>
<th>22</th>
<th>23</th>
</tr>
</thead>
<tbody>
<tr>
<td>isomer shift ($\delta$) (mm·s$^{-1}$)</td>
<td>1.218</td>
<td>1.224</td>
</tr>
<tr>
<td>quadruple splitting ($\Delta E_Q$) (mm·s$^{-1}$)</td>
<td>1.960</td>
<td>2.064</td>
</tr>
</tbody>
</table>

Figure 10. The zero-field $^{57}$Fe Mössbauer data of [Fe$_2$L3($\mu$-OH)($\mu$-O$_2$Car$_{Tol}$)(OTf)$_2$] (22)
IR Spectroscopy

To obtain the IR spectra of the diiron complexes 22 and 23, the KBr pallets containing the samples were prepared in a nitrogen glove box to avoid air contact. The obtained spectra were compared with the spectrum of the free L3 ligand. As shown in Figures 11 and 12, the carbonyl stretch shown in L3 (1711 cm\(^{-1}\), red) was shifted to a lower frequency in the diiron complexes, 1672 cm\(^{-1}\) (blue) in 22 and 1664 cm\(^{-1}\) (magenta) in 23. This frequency shift suggests that the carbonyl oxygen atoms from the L3 esters are coordinated to the iron centers in the complexes.

Figure 11. Overlaid IR spectra of the free L3 ligand (red) and the diiron complex [Fe\(_2\)L3(μ-OH)(μ-O\(_2\)CAr\(^\text{Tol}\))(OTf)\(_2\)] (22, blue). The carbonyl stretch in L3 (1711 cm\(^{-1}\)) is shifted to a lower wavenumber (1672 cm\(^{-1}\)) in 22.
Figure 12. Overlaid IR spectra of the apo L3 ligand (red) and the diiron complex of [Fe$_2$L$_3$(µ-OH)(µ-O$_2$CCPh$_3$)(OTf)$_2$] (23, magneta). The carbonyl stretch in L3 (1711 cm$^{-1}$) is shifted to a lower wavenumber (1664 cm$^{-1}$) in 23.

Reactivity of [Fe$_2$L$_3$(µ-OH)(µ-O$_2$CAR$_{Tol}$)(OTf)$_2$] (22) with O$_2$

With the diiron(II) complex 22 in hand, we were interested in studying its reactivity with molecular oxygen. Figure 13 shows the absorption spectra of the DCM solution of 22 acquired at room temperature, both before and after the introduction of O$_2$. A comparison of the two spectra shows a change in the optical profile after O$_2$ exposure of 22 (Figure 13). This new spectrum looks very similar to that obtained from the apo L3 ligand (Figure 6, black line), suggesting that the diiron complex of 22 was decomposed to the free ligand upon the O$_2$ reaction at room temperature.

Although reaction of 22 with molecular oxygen at rt led to decomposition of the diiron complex, an oxygenated species could form at lower temperature. To explore this possibility, a
solution of 22 in DCM was prepared in a dewar to maintain low temperature. As shown in Figure 14, absorption spectra were taken at various temperatures, from -78°C to rt. Ideally, we expected to see a peroxo intermediate, as in the sMMOH catalytic cycle, which corresponds to a broad absorption band from 600-800 nm. Unfortunately, no spectral change was observed at -78°C after 22 was exposed to dry O₂. There was also no optical change when the temperature was gradually raised till it reached rt when the diiron complex decayed to a free L₃ ligand.

Figure 13. Absorption spectra of 22 acquired at rt, both before and after the introduction of molecular oxygen

Figure 14. Absorption spectra of 22 acquired at different temperatures to examine its reactivity with O₂
Conclusion

In our attempt to design a dinucleating ligand platform to afford diiron complexes that mimic the active site structure of sMMOH, our L3 ligand system has shown promise as a pre-organized precursor. Its interaction with iron(II) salt and an external carboxylate was examined by UV-vis spectroscopic titration experiments, which revealed the ideal stoichiometry of L3, Fe(II), and carboxylate to be 1:2:1. Single crystals of the diiron complexes derived from L3 could be grown in a few days, and X-ray single crystal analysis of the structures 22 and 23 showed similarities between their coordination cores and the active sites of sMMOH. The diiron cores in the model compounds have an oxygen-rich environment with syn N-donor characters. This ligand platform allows the bridging of a bulky external carboxylate between the two metal centers. When compared with the similar diiron complex derived from the previously reported benzoxazole ligand L1, complexes 22 and 23 have much shorter Fe···O (benzofuran) distances. The coordination of carbonyl ester oxygen atoms to the iron centers is confirmed by the shift of carbonyl stretch in L3 to a lower frequency in the complexes 22 and 23. In addition, Mössbauer spectroscopy has revealed the presence of high-spin iron(II) units in 22. Upon oxygenation at rt, the complex 22 was found to decay to a free L3 ligand. Although the desired peroxo species was not observed when 22 was reacted with O2 at -78°C, information from the coordination chemistry of L3 has led to ongoing work with an emphasis on modifying the L3 system to its dicarboxylic acid analog to achieve a better mimic of the active site of sMMOH.
References


