DALTON PERSPECTIVE

# Sterically hindered benzoates: a synthetic strategy for modeling dioxygen activation at diiron active sites in proteins †

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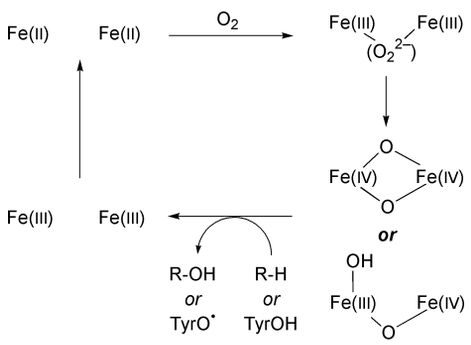
**Bulky terphenyl carboxylates and related benzyl-substituted benzoates have been used to assemble a variety of new diiron complexes analogous to nonheme diiron protein active sites. Through this conceptually simple approach, novel biomimetic structures have been accessed and biologically relevant oxidized intermediates and products have been isolated and characterized by both spectroscopic and X-ray crystallographic methods. Interesting similarities and differences in the observed chemistry of the iron(II) complexes, including ligand substitution and dioxygen activation reactions, result from variation of the specific carboxylate and accompanying N-donor ligand structures. As a result, new insights have been obtained into structure/function relationships in carboxylate-rich nonheme diiron proteins.**

## 1 Introduction

Among the ubiquitous multimetallic arrays that play wide-ranging roles in biology,[1] those with substantial carboxylate ligation are of special interest due to the significance of the reactions they perform and the complexity of their structures and mechanisms of action.[2,3] The carboxylate-rich nonheme diiron active sites of enzymes that bind and activate dioxygen represent a particularly important subclass that has been studied extensively.[3] X-Ray crystallographic, spectroscopic, and theoretical investigations have revealed details of the active site structures of many proteins in this subclass, of which the diiron(II,II) and diiron(III,III) forms of methane monooxygenase (MMO), ribonucleotide reductase (RNR), and $\Delta^9$ stearoyl-acyl carrier protein $\Delta^9$ desaturase shown in Fig. 1 are representative examples.[4–8] Although the structures are remarkably similar insofar as each contains two iron atoms ligated by two histidine imidazoles and four carboxylates, differences are apparent in the metal ion coordination numbers, carboxylate binding modes, and water/hydroxide ligation. Understanding how these structural disparities impact the reaction paths traversed by the various sites is an important goal of current research.

Through a combination of biochemical and synthetic modeling studies,[3c–e,9] a general mechanism has been developed for dioxygen activation by these enzymes (Scheme 1). According to this scheme, the reduced, diiron(II,II) form of the protein reacts with dioxygen to yield a (peroxo)diiron(III,III) species. Subsequent O–O bond scission yields an oxo-bridged intermediate,




**Scheme 1** General mechanism for dioxygen activation by nonheme diiron enzymes.

† Based on the presentation given at Dalton Discussion No. 4, 10–13th January 2002, Kloster Banz, Germany.

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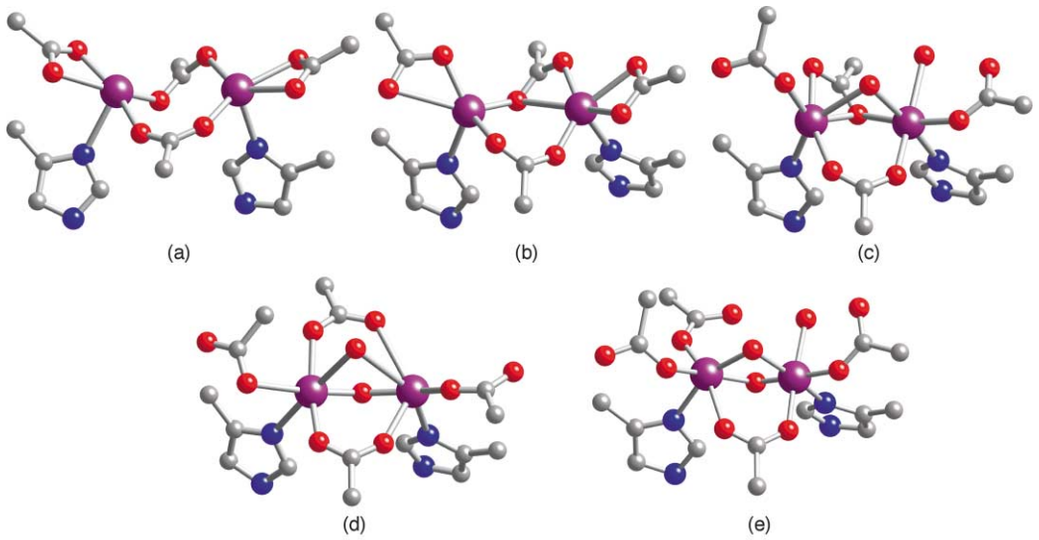


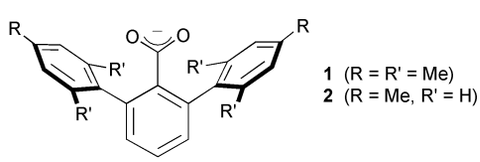

**Fig. 1** Selected structures of diiron active sites of nonheme diiron proteins: (a) reduced diiron(II,II) form of $\Delta^9$D (pdb 1AFR),[4] (b) reduced diiron(II,II) form of RNR from *Escherichia coli* (pdb 1PFR),[5] (c) reduced diiron(II,II) form of MMO from *Methylococcus capsulatus (Bath)* (pdb 1FYZ),[6] (d) oxidized diiron(III,III) form of RNR from *Salmonella typhimurium* (pdb 2R2F),[7] and (e) oxidized diiron(III,III) form of MMO from *Methylosinus trichosporium OB3B* (pdb 1MHY).[8] Key: purple = Fe, red = O, blue = N, gray = C. Single red spheres indicate coordinated hydroxide or water molecules.

with one (RNR) or two (MMO) Fe(IV) sites, that is responsible for attacking substrate (hydrocarbons in MMO, a nearby tyrosine in RNR). Despite consensus support for this overall mechanism, many important issues remain unresolved. For example, while detailed structures of the key peroxo and oxo-bridged intermediates have been suggested (*e.g.*, on the basis of theoretical calculations[10]), these notions have yet to be verified by experiment. In addition, the underlying structural reasons for the divergent functions of the proteins need to be elucidated; possible important factors may include metal coordination number, carboxylate flexibility and/or binding modes, and hydrogen bonding to ligands and/or substrates.

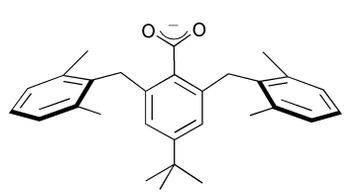
The synthesis, characterization, and study of the reactivity of complexes that model important aspects of the nonheme diiron active sites has been a useful method for gleaning fundamental chemical information relevant to the aforementioned mechanistic issues.[3c,d,9] Moreover, novel and potentially catalytically useful molecules may be developed as an offshoot of such an approach. This tactic presents numerous challenges, however, that derive from the geometric complexity of the active sites (Fig. 1), the flexibility of the carboxylate ligands that may shift binding modes readily (*e.g.* among bidentate bridging, monodentate bridging, and terminal monodentate or chelating),[11] and the inherently high reactivity of the desired models of the key peroxo and high valent intermediates. A successful synthetic strategy must incorporate the appropriate ligand set (two N-donors and four carboxylates) organized to control complex nuclearity so that the desired diiron species are formed, while at the same time providing appropriate steric shielding to stabilize oxidized intermediates and inhibit undesired intermolecular processes. In principle, by using suitably designed supporting ligands the challenge of constructing diiron(II,II) complexes that are structurally analogous to the protein active sites and that react with $O_2$ to yield metastable intermediates amenable to characterization and comparison to their protein congeners may be met.

Many supporting ligands intended for this purpose have been used, a common strategy being the incorporation of N- or carboxylate-donors into complicated organic scaffolds designed to "pre-organize" them, such that the multiple donor ligation (number and geometry) of the active sites may be favored.[12] While successful applications of such ligands have been reported, cumbersome and time-consuming protocols for preparing such intricate molecules sometimes are problematic. The pre-organized ligand geometry may also restrict the reactivity surface available to the dimetal unit, thereby limiting the types of intermediates that can be formed. A complementary tactic that has roots in the organometallic chemistry field[13] entails the use of simpler, less pre-organized ligands that have bulky substituents. In this approach, interligand steric interactions are relied upon to induce low coordination numbers and nuclearities in derived complexes, with hydrophobic shielding effects of the large organic substituents acting to stabilize reactive species and influence redox properties.

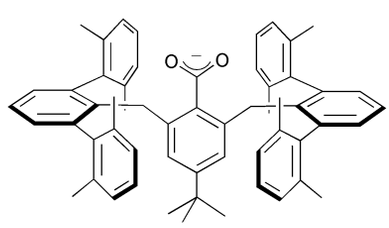
Of the plethora of bulky ligands used with these ideas in mind, those derived from terphenyl have been exploited with particular success for the construction of many types of organometallic and coordination compounds.[14] Inspired by these achievements, we hypothesized that terphenyl carboxylate **1**[15] and related benzyl-substituted benzoates **3**[16] and **4**[17] would enable the construction of novel, low-coordination number model complexes of diiron(II,II) metalloprotein active sites that might be potentially useful for $O_2$ reactivity studies. A similar approach focusing on the 2,6-ditolylbenzoate **2** was developed independently and essentially simultaneously by Lippard and Lee.[18] In this article we survey the fruitful and complementary results of research efforts by our two groups using ligands **1**–**4**,




**1** (R = R' = Me)
**2** (R = Me, R' = H)




**3**




**4**

with a particular view toward comparing the effects of variations in the specific ligand structure on the observed iron chemistry. Importantly, this chemistry has provided fundamentally new insights into diiron protein active site structure and function.

## 2 Ligands and iron(II) complexes

### 2.1 Carboxylate ligands

The terphenyl-carboxylic acid precursors of **1** and **2** may be prepared in good yield and large scale by treatment of 2,6-dichloroiodobenzene[19] with the appropriate substituted phenyl Grignard followed by quenching with $CO_2$.[20] Crystalline forms of the carboxylate salts ($Li^+$ for **1**,[15] $Tl^+$ for **2**[18]) were used directly in the preparations of iron(II) complexes. The carboxylic acid precursors to ligands **3** and **4** were prepared[16,17] by functionalization of 1-bromo-2,6-dibromomethyl-4-*tert*-butylbenzene.[21]

Compared to **2**, terphenyl ligand **1** appears to be more sterically hindered because of the *ortho* methyl groups that enforce an orthogonal relationship between the substituent arenes and the benzoate ring. This ring orthogonality and the resulting concave shape of **1** are illustrated in the X-ray crystal structure of its $Li^+$–$Et_2O$ salt (Fig. 2a).[15] The benzyl linkages in **3** engender greater flexibility and decreased steric demand relative to **1** and **2**. In contrast, the highly elaborated **4** provides a large, bowl-like enclosure (Fig. 2b)[17] that in other derivatives has enabled the isolation of highly reactive species.[22] In sum, one may construct an approximate working scale of steric bulk (**4** > **1** > **2** > **3**) that is useful for understanding the coordination chemistry of these ligands.

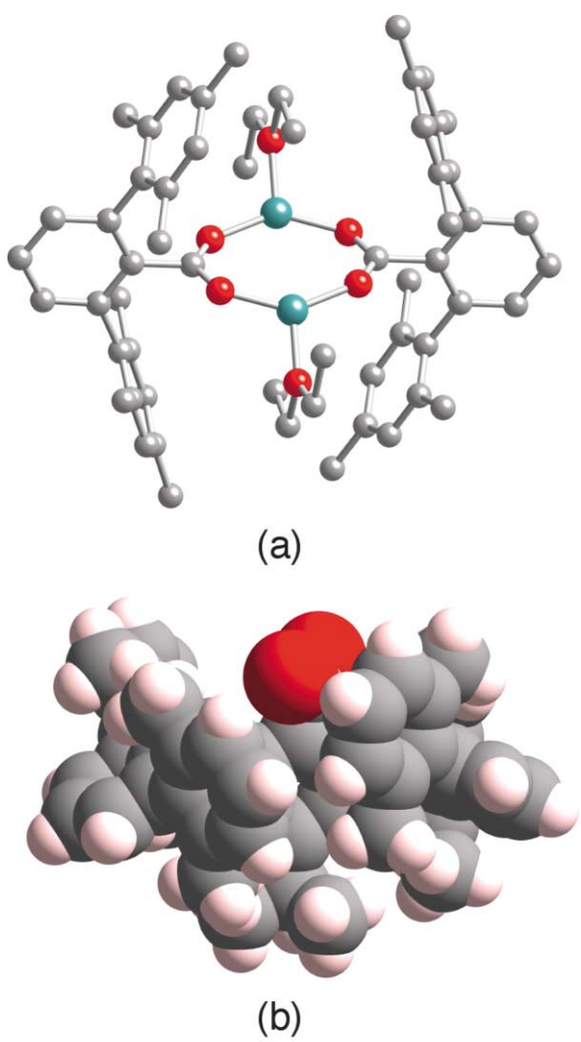



**Fig. 2** (a) Ball and stick representation of the X-ray structure of [Li(**1**)($Et_2O$)]$_2$. (b) Space-filling representation of the X-ray structure of the carboxylic acid precursor of **4** (acidic proton not shown). Key: red = O, turquoise = Li, gray = C.

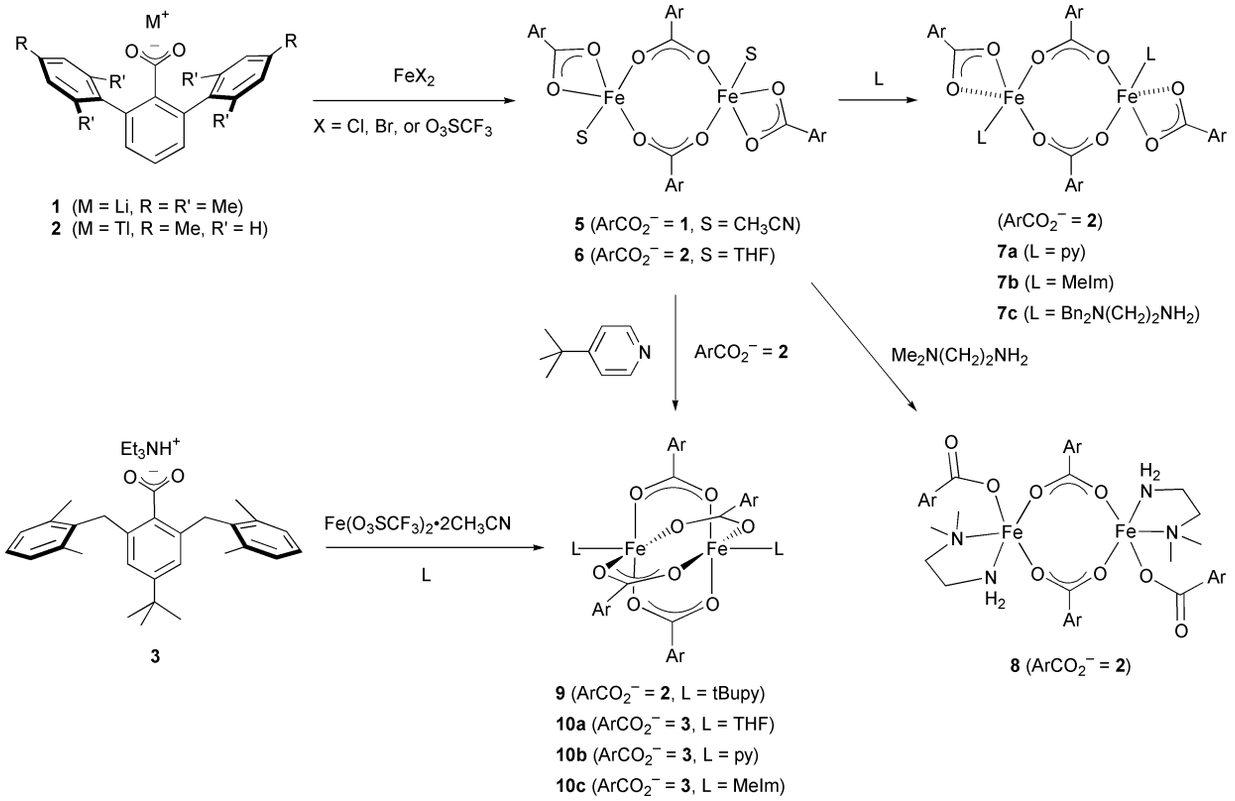
### 2.2 Iron(II) complexes

**2.2.1 Syntheses.** Mixing of iron(II) salts and the carboxylate anions **1** or **2** in the presence of the donor solvents $CH_3CN$ or THF yielded diiron(II,II) complexes **5** and **6**, respectively (Scheme 2).[15,18] Although structurally analogous, the reactivities of these complexes differ, because of the divergent steric demands of their respective terphenyl units. Thus, the carboxylate ligation in **6** is essentially unperturbed upon substitution of the THF ligand with the N-donors py,[18] MeIm,[18] or $Bn_2N(CH_2)_2NH_2$,[23] which affords compounds **7a**–**c**. Interestingly, in **7b** shifting of the bidentate terminal carboxylates to monodentate was observed in one of the independent molecules of the asymmetric unit in its X-ray structure. Coordination of *N,N*-dimethylethylenediamine to **6** induces a similar terminal carboxylate shift to yield **8**.[23] With 4-*tert*-butylpyridine (tBupy), **6** converts to **9**,[24] which exhibits a precedented tetracarboxylate-bridged "paddlewheel" topology.[25] Similar dinuclear paddlewheels **10a**–**c** form with the less-hindered carboxylate **3**.[16] Isolation of dibridged **7a** with py as N-donor and of paddlewheel **9** with tBupy (with the same carboxylate **2**) suggests that the approximately isomeric yet topologically distinct structures for the complexes have similar thermodynamic stabilities. Evidently, interligand steric interactions relatively far-removed from the metal coordination sphere are sufficient to determine which form predominates.

In contrast to the substitution reactions of **6** that generally yield compounds which retain their dinuclear structure, addition of a variety of N-donors to **5** induces fragmentation to afford monoiron(II) compounds (**11**–**13**, Fig. 3).[23,26] In a more convenient procedure, **11**–**13** may be generated directly by mixing $Fe(OTf)_2{\cdot}2CH_3CN$, **1**, and the N-donor in the appropriate stoichiometry. Similar monomeric complexes **14a**,**b** derive from both **5** and **6** in reactions with tetramethylethylenediamine (TMEDA). Presumably, the greater steric demand of the carboxylates in **5** compared to those in **6** is responsible for the inability to isolate dinuclear analogs of **5** with N-donors larger than $CH_3CN$. Consistent with this notion, monomer **15** results with the extremely bulky carboxylate **4**.[17]

**2.2.2 Structures.** Extensive X-ray crystal structural data has verified the structures of the complexes shown in Scheme 3 and Fig. 3. The data also have provided support for the aforementioned ideas about the steric influences of the various carboxylate ligands on the nature of the isolated iron(II) complexes. Comparison of the structures of **5** and **6** is particularly illuminating (Fig. 4). Both complexes closely model the diiron(II,II) forms of the active sites of $\Delta^9$D and RNR (Fig. 1a,b) with respect to carboxylate ligation and Fe ··· Fe separation (**5**: 4.12 Å, **6**: 4.28 Å, $\Delta^9$D: 4.2 Å, RNR: 3.9 Å). In a contrast with the biosites in which the imidazole ligands are arranged *syn*, the S-donors in **5** and **6** (and the N-donors L in **7**) adopt an *anti* disposition. Encapsulation of the diiron cores in both **5** and **6** by the terphenyl groups is apparent, but to a noticeably greater degree in **5**, where orthogonality of the substituent mesityl and central benzoate rings in its carboxylates is enforced. Indeed, the $CH_3CN$ ligands in **5** are completely covered by the hydrophobic ligand sheath. Greater rotational flexibility of the tolyl groups in the carboxylates of **6** allows greater access to its metal centers.

Comparison of the X-ray structures of the series **11**–**13** that comprise identical carboxylates (**1**) reveals interesting effects of the different N-donors. The geometry of the complex correlates




**Scheme 2** Syntheses of diiron(II,II) complexes using carboxylate ligands **1**–**3**.

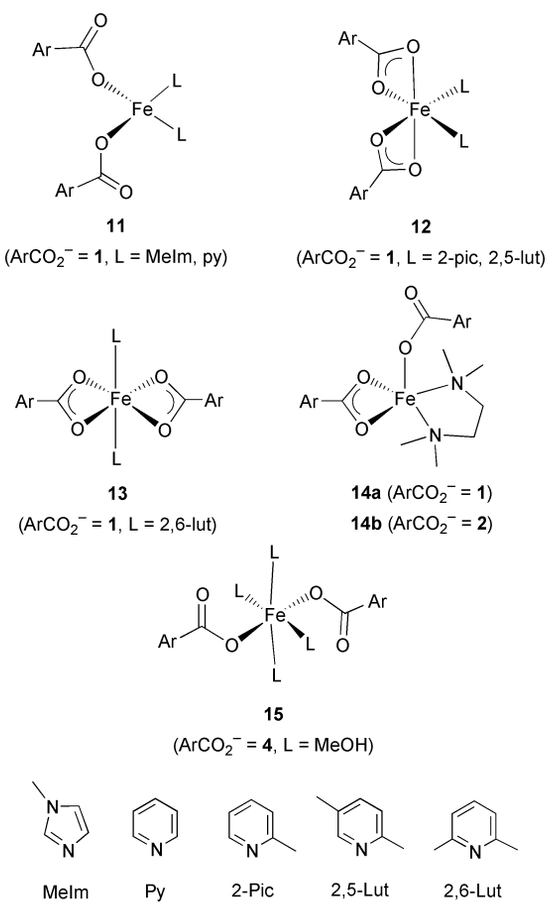



**Fig. 3** Monoiron(II) complexes prepared using carboxylates **1** and **2**.

with the degree of steric bulk at the α position of the N-donor ligand. Thus, as the α substitution increases py < 2-pic/2,5-lut, the coordination number increases from 4 to 6 (**11** and **12**). This counterintuitive trend may be rationalized by positing that α substitution causes weakening of the Fe–N-donor interaction, thus increasing the Lewis acidity of the iron(II) ion and inducing a compensating shift in the carboxylate binding mode. In **13**, steric clashes between the 2,6-lut ligands rationalizes observation of their *trans* positions. The apparent variation of carboxylate ligation mode as a function of Fe–N-donor interaction in the series of complexes suggests that tuning of the latter in the proteins (*e.g.* by a subtle conformational change) may be important in controlling carboxylate coordination during catalysis.

## 3 Reactivity of diiron(II,II) complexes with oxidants

Just as the ligand substitution reactions of **5** and **6** follow paths that vary because of their different carboxylate ligand structures, these ligand disparities also influence the reactivity of their diiron(II,II) complexes with oxidants, including $O_2$. These reactions may be broadly divided into two types: (a) "outer-sphere" one-electron processes whereby mixed-valent diiron(II,III) complexes are formed, and (b) "inner-sphere" reactions involving the binding and activation of $O_2$, yielding higher oxidation state diiron species.

### 3.1 Diiron(II,III) compounds from one-electron oxidation

Several types of mixed valent diiron(II,III) complexes have been prepared from the diiron(II,II) precursors supported by carboxylates **1** and **2** (Scheme 3).[24,27,28] These compounds are of interest both for fundamental reasons[29] and because of their relevance to numerous such mixed-valent sites found in proteins, studies of which have focused on understanding how their structural features relate to their functionally important magnetic and redox behavior.[30] Oxygenation of **5** in the presence of $^{i}$PrOH yielded a stable blue complex, **16**, formulated as a mixed-valent diiron(II,III) complex on the basis of X-ray crystallographic and spectroscopic/physical data.[27] In solution, **16** exhibits a low energy absorption with $\lambda_{max}$ = 780 nm ($\varepsilon \approx 2000$ $M^{-1}$ $cm^{-1}$) ascribed to an intervalence transition. Interestingly, the complex crystallizes in two structurally distinct forms that

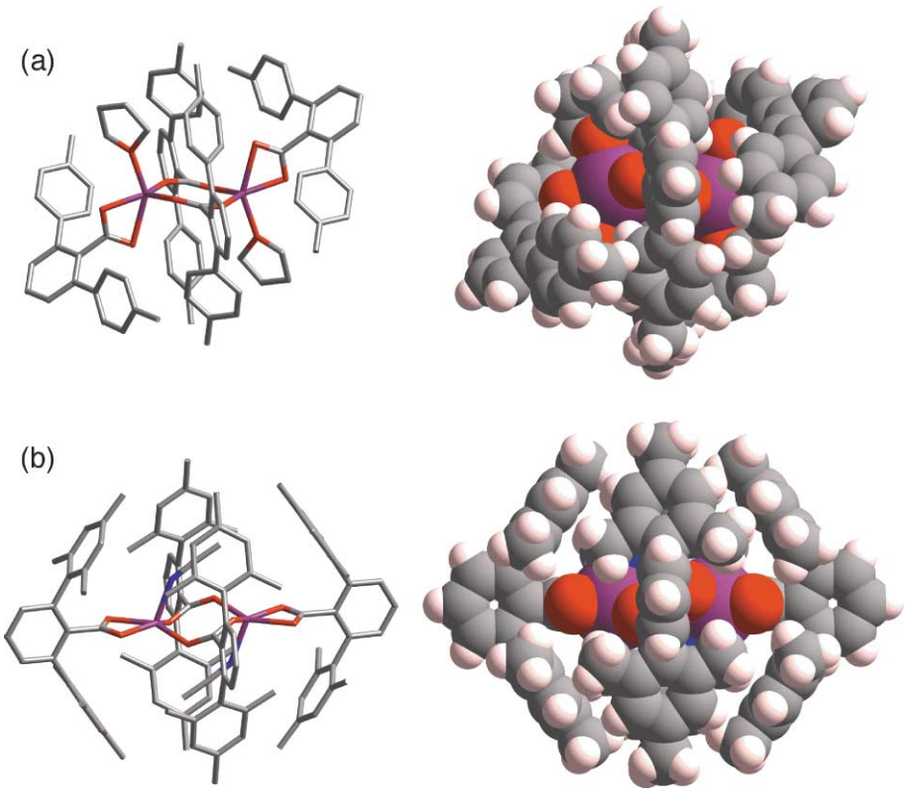
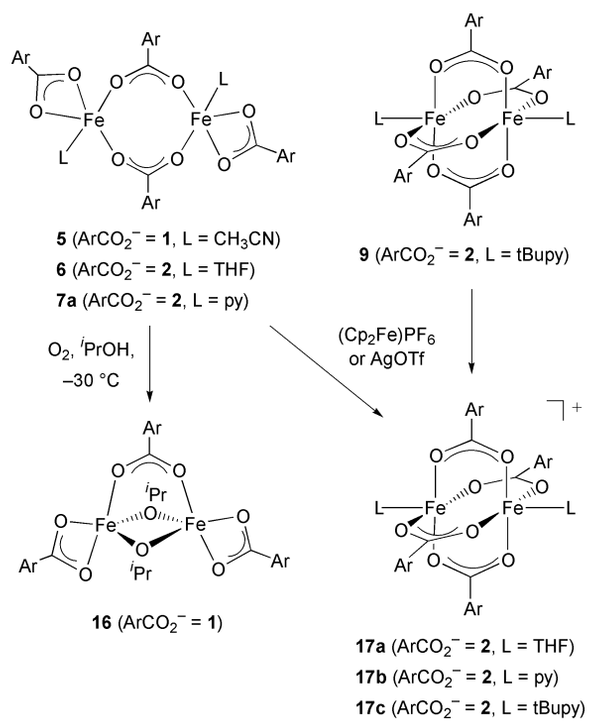



**Fig. 4** X-Ray structures of the diiron(II,II) complexes **6** (a) and **5** (b) as stick (left) and space-filling drawings (right).




**Scheme 3** Synthesis of diiron(II,III) complexes.

differ principally with respect to the Fe–Fe separation and the conformation of the bridging isopropoxide groups (Fig. 5). In form A that includes $Et_2O$ solvate molecules in the crystal lattice, the Fe–Fe distance is short [2.6241(9) Å] and the isopropoxide methyl groups reside on an axis that is approximately perpendicular to the Fe–Fe vector. In form B (obtained under different crystallization conditions and lacking $Et_2O$ solvate), the Fe–Fe distance is 0.13 Å longer and the isopropoxide methyl groups are rotated by ≈90° so that they lie on an axis parallel to the Fe–Fe vector. Interactions between these methyl groups and the carboxylate substituents appear to underly the

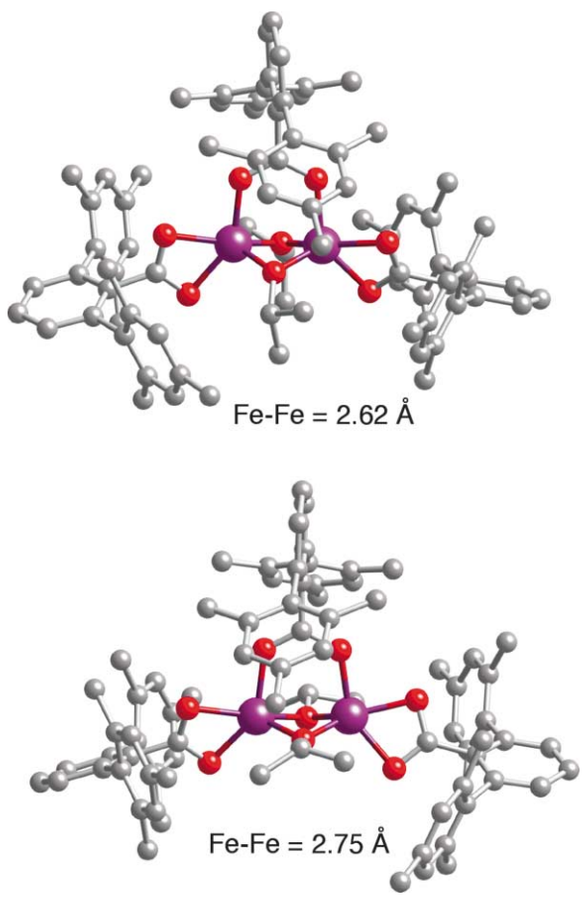



**Fig. 5** X-Ray structures of forms A (top) and B (bottom) of **16**.

expanded Fe–Fe distance observed in this form. Thus, the isopropoxide conformation is a primary influence on the intermetal separation in the two forms.

Physicochemical data show that the two isomeric forms are rare examples of $S = 9/2$ species with parallel coupling of

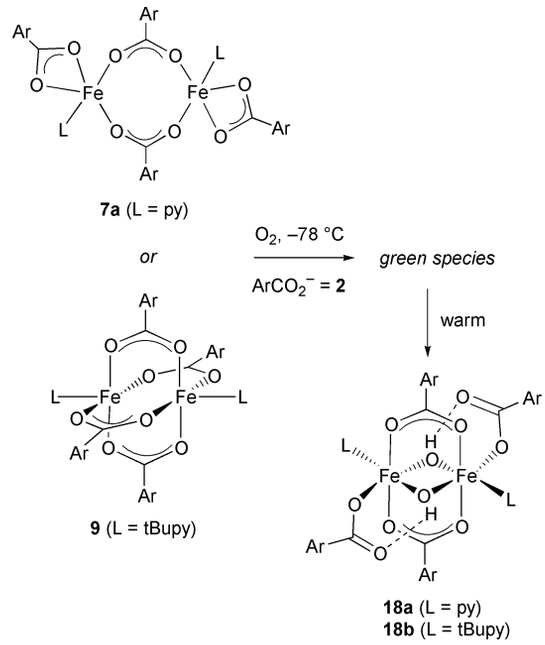
electronic spins;[24,28,31] in most diiron(II,III) compounds the high spin ions couple antiferromagnetically to yield an $S$ = 1/2 ground state.[32] Most interestingly, the two forms of **16** exhibit different electronic ground states due to differing valence delocalization. Both Fe atoms in form A are similarly distant from their ligand donors [2.001(3) Å for Fe1, 2.008(3) Å for Fe2], consistent with complete valence delocalization, whereas form B appears partially localized on the basis of different average Fe–O bond lengths [Fe1–O = 2.039(3) Å, Fe2–O = 1.998(3) Å]. More convincing evidence comes from Mössbauer spectra acquired on polycrystalline samples at 4.2 K in weak magnetic field. Form A exhibits a six-line pattern consistent with full delocalization (equivalent Fe sites in a class III system).[33] In contrast, two six-line patterns are observed for form B, indicative of different Fe atoms. It appears that dominant double exchange interactions[34] in form A, which has a shorter Fe–Fe distance, are sufficiently powerful to yield a fully delocalized $S$ = 9/2 ground state. The same spin state is found in form B, but the greater intermetal separation accompanying the isopropoxide conformational change results in weaker magnetic interactions and, thus, localization at low temperature (class II behavior). Thus, **16** represents a unique system in which ground-state electronic structural differences (*i.e.* valence delocalization) correlate with well-defined conformational alterations.

Another set of complexes **17a**–**c** with fully delocalized $S$ = 9/2 ground states was prepared by one-electron oxidation of **6**, **7a**, or **9**.[28] On the basis of spectroscopic similarities, species **17c** also is believed to be a coproduct of the reaction of **9** with $O_2$ (Section 3.2).[24] Support for the electronic structure assignment for the series of deep green diiron(II,III) molecules came from data for **17c**, specifically absorption spectroscopy ($\lambda_{max}$ = 670 nm, $\varepsilon$ = 3200 $M^{-1}$ $cm^{-1}$), magnetic susceptibility measurements ($\mu_{eff}$ = 11.0 $\mu_B$), EPR spectroscopy ($g$ = 10), and Mössbauer data (six-line spectrum at 4.2 K and >3 tesla). The lack of single atom bridges between the magnetically coupled metal ions in the paddlewheels **17a**–**c** is a notable feature that distinguishes these mixed valent complexes from other $S$ = 9/2 systems. Shortening of the Fe–Fe distance by ≈0.11 Å upon oxidation of **9** to yield **17c** was observed, suggestive of direct Fe–Fe interactions in the latter that may be important in mediating the double exchange interaction.

### 3.2 Dioxygen binding and activation reactions

Both the nature of the carboxylate ligands and the N-donors dramatically affect the reactivity of the diiron(II,II) complexes with $O_2$. As noted above, treatment of complex **5** with $O_2$ in the presence of $^i$PrOH yields mixed-valent **16**. In noncoordinating solvents, the reaction instead gives a purple solution with $\lambda_{max}$ = 540 nm ($\varepsilon$ = 2300 $M^{-1}$ $cm^{-1}$).[15] This absorption feature resembles those of previously reported (μ-peroxo)diiron(III,III) complexes.[35] Consistent with the postulate of the purple species as a peroxo complex, the Raman spectrum obtained with $\lambda_{ex}$ = 514.5 nm contains a peak attributable to an O–O vibration at 885 $cm^{-1}$. This peak shifts by only 14 $cm^{-1}$ upon $^{18}O_2$ substitution, however, an amount less than predicted for a pure O–O stretch (≈50 $cm^{-1}$). Further characterization of this species is thus required to substantiate the proposed formulation.

Different behavior from that exhibited by **5** is found with the diiron(II,II) complexes supported by carboxylate **2**. While oxygenation of **6** has not led to identifiable products, its congeners with N-donors **7a**–**c** and **9** yield interesting oxidized species.[18,23,24] The py and tBupy adducts **7a** and **9**, respectively, form deep green solutions upon treatment with $O_2$ at −78 °C, which upon warming yield diiron(III,III) products **18a**,**b** (Scheme 4). The bis(μ-hydroxo)bis(μ-carboxylato)diiron(III,III) core of these structurally characterized products is novel, key features being hydrogen bonds between the hydroxides and the terminal carboxylates and a Fe–Fe separation significantly shorter than



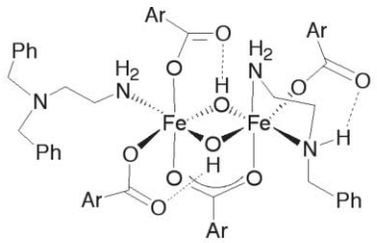

**Scheme 4** Dioxygen reactivity of diiron(II,II) complexes supported by carboxylate **2**.

in previously reported bis(μ-hydroxo)diiron compounds as a result of the additional carboxylate bridges (*cf.* Fe–Fe = 2.88 Å for **18a**). Such a "quadruply-bridged" core provides precedence for similar structures in the catalytic cycles of the diiron enzymes, for which even shorter intermetal separations have been identified (*e.g.* 2.46 Å in MMO compound "Q").[36]

Mössbauer and EPR spectroscopic data acquired on the initially formed deep green solution derived from reaction of **9** with $O_2$ indicate that it is a mixture of three species.[24] These are (a) an antiferromagnetically coupled diiron(III,III) compound (30%), (b) an $S$ = 9/2 diiron(II,III) species subsequently identified as **17c** (34%),[28] and (c) an $S$ = 1/2 species (36%) identified as a diiron(III,IV) compound primarily on the basis of its Mössbauer spectral properties ($\delta$ = 0.12 and 0.55 mm $s^{-1}$). The spectral data for this diiron(III,IV) species compare favorably to those reported for intermediate X in RNR,[37] a one-electron reduced form of intermediate Q in MMO,[38] and a well-characterized synthetic complex.[39] The finding of an approximately 1 : 1 ratio of the diiron-(II,III) and -(III,IV) species, in conjunction with $O_2$ uptake data, was interpreted to suggest the overall eqn. (1). Accordingly, the pathway for the formation of the $S$ = 9/2 species was postulated to involve oxidation of the starting diiron(II,II) complex by a putative diiron(IV,IV) intermediate as it was formed. The oxidative power of the diiron(III,IV) product was demonstrated by the generation of a phenoxyl radical from a phenol, a reaction that models tyrosyl radical formation by intermediate X in RNR.[3]

$$2Fe(II)Fe(II) + O_2 \longrightarrow Fe(III)Fe(IV)(O^{2-})_2 + Fe(II)Fe(III) \quad (1)$$

An intramolecular oxidation of a coordinated ligand was observed upon oxygenation of **7c**, to yield a new type of diiron(III,III) complex (**19**) with a bis(μ-hydroxo)(μ-carboxylato) core (Fig. 6).[23] This core structure is analogous to that of the oxidized form of MMO (*cf.* Fig. 1e).[8,40] Oxidative N-dealkylation of $Bn_2N(CH_2)_2NH_2$ was indicated by the observation of $BnHN(CH_2)_2NH_2$ as a ligand in **19** and of the coproduct PhCHO. Incorporation of $^{18}O$ into the latter when $^{18}O_2$ was used implicates an oxygenated intermediate as the reactive species responsible for the N-dealkylation. While such a process has precedent in copper[41] and cytochrome P450 chemistry,[42] it had not been seen in previous efforts to model nonheme diiron biosites.



19

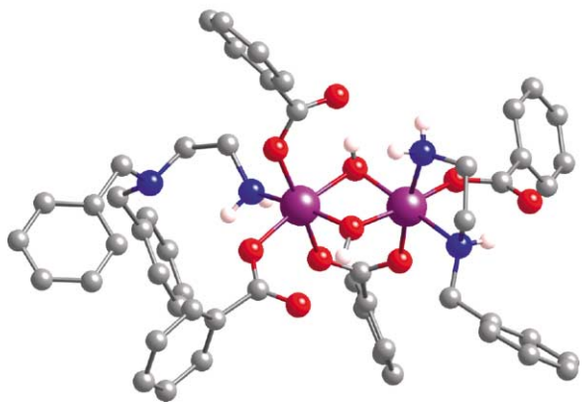
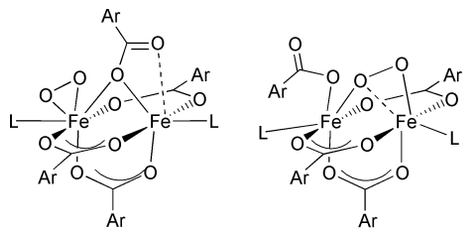


**Fig. 6** Drawing (top) and X-ray structure (bottom) of **19**, the product of oxygenation and N-dealkylation of **7c**.

In a contrast to the $O_2$ chemistry outlined in Scheme 4 for the diiron(II,II) complexes supported by carboxylate **2**, oxygenation of **10a–c** comprising the less hindered, more flexible carboxylate **3** yielded (peroxo)diiron(III,III) species with unusual properties.[16] Cryogenic stopped-flow kinetics studies of the oxygenation reactions revealed a second-order rate-law [eqn. (2)] for the formation of the peroxo species characterized by $\lambda_{max}$ 500–550 nm ($\varepsilon$ 1000–1200 $M^{-1}$ $cm^{-1}$). A similar mechanism for each derivative was indicated by activation parameters that were linearly correlated (isokinetic temperature 216 K).

$$\text{rate} = k[\text{diiron(II,II)}][O_2] \quad (2)$$

The presence of a peroxide ligand was confirmed by a $\nu$(O–O) at 822 $cm^{-1}$ ($\Delta^{18}O_2$ = 43 $cm^{-1}$) in the resonance Raman spectrum of the species derived from **10b** ($\lambda_{ex}$ = 615 nm). This peroxide stretching frequency is notably lower than most[43] others reported previously for (1,2-peroxo)diiron(III,III) compounds (848–910 $cm^{-1}$),[35,44] but falls within the range for ($\eta^2$-$O_2$)iron(III) species (range 816–827 $cm^{-1}$).[35,45] Divergent Fe(III) environments were indicated by the Mössbauer spectrum, which was fit to two doublets in a 1 : 1 ratio, with $\Delta E_Q(1)$ = 1.27(3), $\delta(1)$ = 0.65(2) mm $s^{-1}$ and $\Delta E_Q(2)$ = 0.71(2), $\delta(2)$ = 0.52(2) mm $s^{-1}$. The intermediate is EPR silent at 4.2 K, and from fits to Mössbauer spectra acquired in high magnetic fields an $S = 0$ ground state with $J \approx 30(5)$ $cm^{-1}$ ($J \cdot S_1 \cdot S_2$) was estimated. This coupling interaction is weak compared to previously reported data for (1,2-peroxo)diiron(III,III) species (66–200 $cm^{-1}$).[43,46] Taken together, the physicochemical data suggest a novel structure for the peroxo species, and in order to rationalize the observed low $\nu$(O–O), different Fe(III) sites, and weak antiferromagnetic coupling, **20** or **21** was postulated (Fig. 7). These structures are speculative, and the detailed nature of the peroxo species will remain unclear until X-ray crystallographic data are available. Nonetheless, it is important to note that the clean formation of the peroxo compounds from the oxygenation of **10a–c** distinctly contrasts with the generation of the mixture of oxidized intermediates from **9** (Scheme 4). This entirely different reactivity of structurally analogous diiron(II,II) complexes points to significant effects of the carboxylate ligand structures on the stability of intermediates derived from $O_2$, with potential relevance to similarly important



20 21

**Fig. 7** Possible structures for the (peroxo)diiron(III,III) species resulting from low temperature oxygenation of **10a–c**.

"second-sphere" influences that dictate the course of the oxygenation reactions of the different nonheme diiron enzymes.

## 4 Summary

The bulky benzoate ligands **1–4** have been found to be useful ligands for the construction of iron(II) complexes with structural features and reactivity patterns that vary as a result of differences in the carboxylate ligand steric properties. Most importantly, diiron complexes that model various forms of the nonheme diiron protein active sites have been accessed using combinations of these ligands and additional N-donors. Diiron(II,II) models of the reduced active sites react with oxidants or dioxygen to yield novel mixed valence species, diiron(III,III) compounds, and/or a peroxo intermediate, with the pathway observed depending on the specific nature of the supporting ligands. By implication, these results highlight how subtle influences beyond the primary metal coordination sphere may have important effects on metalloenzyme mechanism, particularly for the nonheme diiron class of proteins. Thus, as a general strategy for modeling the chemistry of multimetal active sites in proteins, the use of sterically hindered carboxylates like **1–4** shows much promise, and exciting new discoveries are sure to follow as applications toward a wider array of biological systems are pursued.

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