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# Self-Assembly of the 2-His-1-carboxylate Facial Triad in Mononuclear Iron(II) and Zinc(II) Models of Metalloenzyme Active Sites

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A synthetic strategy involving the use of sterically hindered N-donor and terphenylcarboxylate ligands has been used to prepare complexes of iron(II) and zinc(II) that feature $N_2$(carboxylate) donors. X-ray crystallographic and NMR data show that the 2-His-1-carboxylate facial triad found in metalloenzyme active sites is closely modeled by the mononuclear complexes. In addition, by virtue of the flexibility of the ligands used, the geometries and coordination environments of the complexes display carboxylate binding mode differences such as those seen in the enzymes.

A ubiquitous structural motif in metalloenzyme active sites features facial coordination of a pair of histidine imidazoles and a carboxylate ligand from Asp or Glu to a divalent metal ion (referred to as the "2-His-1-carboxylate facial triad").[1] Representative examples from the large class with iron(II) centers[2] and those in zinc(II) enzymes, such as thermolysin[3a] and carboxypeptidase,[3b] are sketched in Figure 1. Although structural comparisons of the various active sites reveal certain similarities, there are subtle differences in the carboxylate binding mode. Such "carboxylate shifts"[4] that have been proposed to be important in nonheme dimetal enzyme function may also play a role in catalysis by the mononuclear sites with the 2-His-1-carboxylate facial triad.

Efforts to date to prepare synthetic models of this motif have focused on using "preorganized" tridentate chelates such as bis(pyrazolyl- or imidazolyl)acetates or -propionates and analogues.[1d,5] However, because of the tethering of the carboxylate in these ligands, variability and flexibility of the binding modes found in the enzymes are difficult to achieve. A synthetic strategy that has met with considerable success for generating active site models of nonheme diiron active sites involves the use of terphenylcarboxylate ligands, which through steric and hydrophobic effects limit metal coordination numbers and enable isolation of reactive intermediates.[6] Herein we report the use of a related approach in which sterically hindered carboxylate and N-donor ligands are combined to assemble mononuclear complexes of iron(II) and zinc(II). Structural data show that they replicate the geometry and ligand donor set of the 2-His-1-carboxylate

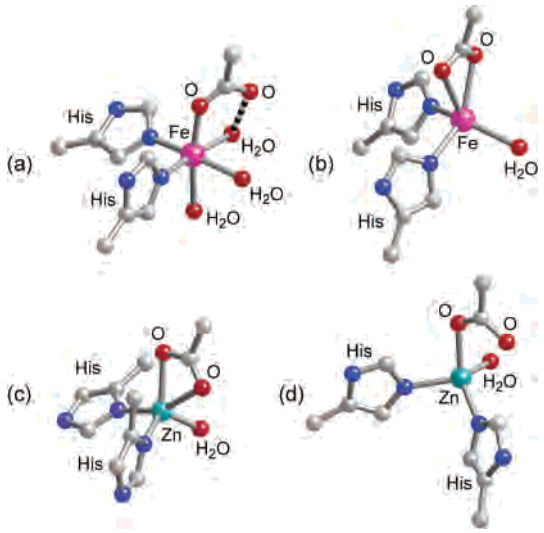


**Figure 1.** Illustrative examples of metalloenzyme active sites with the 2-His-1-carboxylate motif: (a) phenylalanine hydroxylase binary enzyme−tetrahydrobiopterin complex (1J8U);[2a] (b) phenylalanine hydroxylase ternary enzyme−tetrahydrobiopterin−substrate complex (1KW0);[2b] (c) carboxypeptidase (1YME);[3b] (d) thermolysin (3TMN).[3a]

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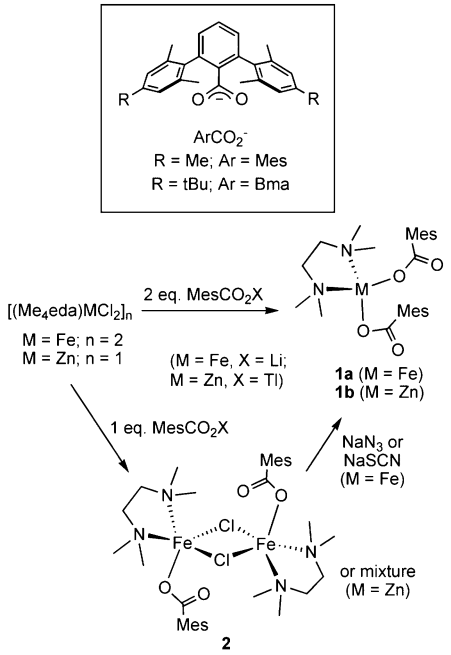
**Scheme 1**




facial triad and demonstrate biomimetic carboxylate binding mode variation.

Initial synthetic efforts focused on the treatment of $CH_2Cl_2$ solutions of $[(Me_4eda)MCl_2]_n$ ($Me_4eda$ = *N*,*N*′,*N*′,*N*′-tetramethylethylenediamine; M = Fe, $n = 2$;[7] M = Zn, $n = 1$[8]) with either 1 or 2 equiv (per metal) of $MesCO_2X$ (X = Li[9] or Tl;[10] Scheme 1). Chloride metathesis was facile for M = Fe and X = Li, but thallium carboxylate was required for M = Zn. With 2 equiv of carboxylate, **1a** (previously reported[11]) and **1b** (new; X-ray structure shown in Figure S1 in the Supporting Information) were isolated.[12] With 1 equiv of $MesCO_2Tl$ for M = Zn, a complex mixture of products formed (NMR). However, under similar reaction conditions for M = Fe, dinuclear **2** was obtained.

The X-ray structure of **2** (Figure 2) shows five-coordinate iron(II) ions ($\tau = 0.44$) with the carboxylate ligand bound in a monodentate fashion [Fe1−O2 = 2.575(2) Å]. Each metal contains $N_2$(carboxylate) ligation that mimics the 2-His-1-carboxylate motif, but dimerization via chloride bridges mitigates the biological relevance [Fe−Fe = 3.6929(5) Å]. Furthermore, attempts to displace the chlorides via metathesis with azide or thiocyanate in $CD_3CN$ resulted in disproportionation and the formation of **1a**.

Reasoning that greater steric hindrance was needed to disfavor the formation of dimers such as **2** and binding of two carboxylates as in **1**, we pursued analogous syntheses

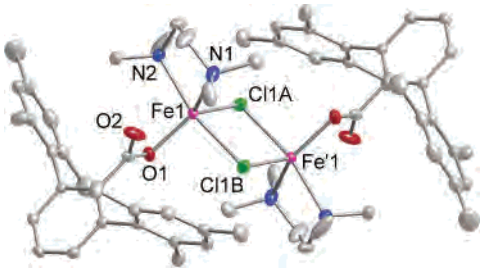



**Figure 2.** X-ray structural representations of **2** with all atoms shown as 50% thermal ellipsoids. Hydrogen atoms are omitted for clarity. Selected bond distances (Å) are as follows: Fe1−N1, 2.3002(16); Fe1−N2, 2.1964(17); Fe1−O1, 2.0277(14); Fe1−O2, 2.575(3); Fe1−Cl1, 2.4086(5); Fe1−Cl′1, 2.5279(5); Fe1−Fe′1, 3.6929(5).

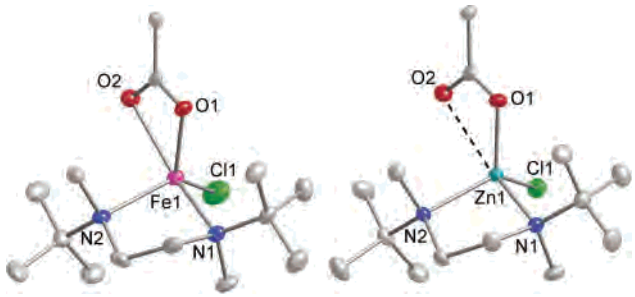
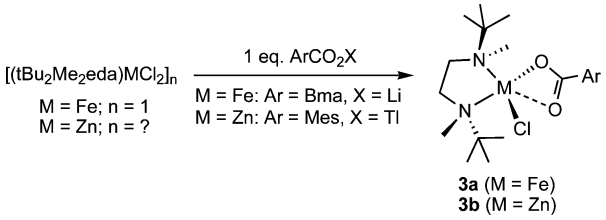



**Figure 3.** X-ray structural representations of **3a** (left) and **3b** (right), with all atoms shown as 50% thermal ellipsoids. Hydrogen atoms and the terphenyl portion of the carboxylate ligands are omitted for clarity. Selected bond distances (Å) are as follows. **3a**: Fe1−N1, 2.226(3); Fe1−N2, 2.221(3); Fe1−O1, 2.058(2); Fe1−O2, 2.360(3); Fe1−Cl1, 2.2577(11). **3b**: Zn1−N1, 2.149(3); Zn1−N2, 2.153(3); Zn1−O1, 1.976(3); Zn1−O2, 2.647(3); Zn1−Cl1, 2.2306(12).

**Scheme 2**




using the bulkier N-donor ligand *N*,*N*′-di-*tert*-butyl-*N*,*N*′-dimethylethylenediamine ($tBu_2Me_2eda$; Scheme 2).[13] The desired mononuclear complexes **3a** and **3b** were isolated in high yield (~90%), by using a *p*-*tert*-butyl-substituted carboxylate $BmaCO_2Li$ (Scheme 1) in the former case and by using $MesCO_2Tl$ in the latter. X-ray crystal structures reveal donor sets analogous to the 2-His-1-carboxylate motif (Figure 3), but the carboxylate binding differs for iron(II) and zinc(II). In **3a**, asymmetric bidentate coordination [Fe−O = 2.058(2) and 2.360(3) Å] results in a five-coordinate geometry ($\tau = 0.49$). In **3b**, however, one carboxylate oxygen does not bind (Zn−O > 2.6 Å) and the zinc(II) ion adopts a distorted tetrahedral geometry. The overall geometries and, notably, the disposition of the $N_2$(carboxylate) donor sets in **3a** and **3b** closely model these features of the active sites of phenylalanine hydroxylase and thermolysin, respectively (Figure 4). $^1$H NMR data of the complexes are consistent with retention of the structures in solution ($CD_3CN$). For

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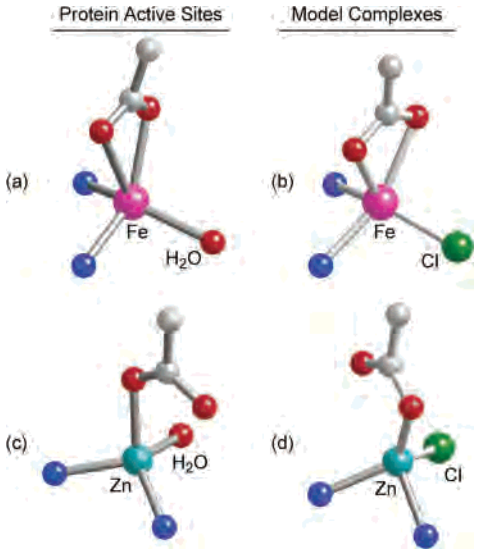


**Figure 4.** Comparison of the cores of the X-ray crystal structures of (a) the phenylalanine hydroxylase ternary enzyme−tetrahydrobiopterin−substrate complex (1KW0),[2b] (b) complex **3a**, (c) thermolysin (3TMN),[3a] and (d) **3b**.

example, four well-separated resonances are observed in the paramagnetically shifted spectrum of **3a** for the ethylene-backbone hydrogen atoms of the $tBu_2Me_2$eda ligand at $\delta$ 54, 68, 127, and 192 ppm (Figure S2 in the Supporting Information). This indicates $C_1$ symmetry for the high-spin iron(II) complex. In contrast, these hydrogen atoms give rise to two peaks at $\delta$ 103 and 218 ppm in the spectrum of the $C_2$-symmetric precursor $(tBu_2Me_2eda)FeCl_2$ (NMR, Figure S3 in the Supporting Information; X-ray structure, Figure S4 in the Supporting Information).

Importantly for future functional modeling studies, preliminary attempts to metathesize the chloride ligands in **3a** yielded monomeric derivatives without disproportionation or other deleterious rearrangements. Thus, the reaction of **3a** with NaY ($Y = N_3^-$ or $SCN^-$) in $CD_3CN$ showed clean conversion to single species, identified as $[(tBu_2Me_2eda)(BmaCO_2)FeY]$ by $^1H$ NMR and Fourier transform IR spectroscopy [$\nu(N_3) = 2082$ cm$^{-1}$; $\nu(SCN) = 2056$ cm$^{-1}$], as well as CHN analysis of the isolated solid for $Y = SCN^-$.

In conclusion, through a synthetic approach different from those used previously,[1d,5] we have obtained $N_2$(carboxylate) ligation to mononuclear iron(II) and zinc(II) metal centers, effectively modeling the 2-His-1-carboxylate facial triad of metalloenzyme active sites. The observed variation of the carboxylate binding in these synthetic complexes mimics the flexible coordination of the carboxylate ligand observed in the biological systems. Successful metathesis of the chloride ligand suggests that other ligand-exchange reactions with biologically relevant cosubstrates are feasible, opening the door to future functional modeling studies.

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**Supporting Information Available:** Synthetic procedures and characterization data for all new compounds (PDF) and crystallographic information (CIF). This material is available free of charge via the Internet at http://pubs.acs.org.

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