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New diamino-diheterophenol ligands coordinate iron(III) to make structural and functional models of protocatechuate 3,4-dioxygenase†

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Three diamino, dihetero-phenol ligands were synthesized by sequential Mannich condensations. These ligands were combined with $FeCl_3$ to produce three five-coordinate Fe(III) complexes that are structural models for the enzyme 3,4-PCD. The three Fe(III) complexes were characterized by elemental analysis, single crystal X-ray diffraction studies, UV-vis spectroscopy, and cyclic voltammetry. Combining the Fe(III) complexes with 3,5-di-*t*-butylcatechol and O_2 resulted in oxidative cleavage similar to the function of 3,4-PCD.

Protocatechuate 3,4-dioxygenase (3,4-PCD) is a well-studied example of an intradiol dioxygenase whose role in nature is to catalyze the ring cleavage of 3,4-dihydroxybenzoate, resulting in β-carboxy-*cis*,*cis*-muconate.[1–7] As a dioxygenase, 3,4-PCD incorporates both atoms from molecular oxygen between the vicinal hydroxyl groups of a catechol substrate. X-ray crystallographic data has shown that the active-site of 3,4-PCD consists of a high-spin five-coordinate ferric ion bound by tyrosine and histidine in the two axial positions and tyrosine, histidine, and hydroxide ligands in the equatorial plane.[8,9] Upon binding of the catechol substrate, the active-site shifts to a square-pyramidal geometry where the axial tyrosine and the hydroxide ligands have been replaced by the double deprotonated catecholate.

Many iron(III) model complexes of the 3,4-PCD active-site have been synthesized and characterized.[10–19] A majority of the early examples utilized nitrogen based ligand systems, but more recently mixed nitrogen/phenolate ligands have been used to try and model the structure and function of the enzyme.[13,16–18] Various groups have been able to either synthesize good structural models (*i.e.* produce five-coordinate iron(III) complexes with two nitrogen and two phenolate donors)[13,14,19,20] or produce monomeric iron(III) complexes that oxidize molecules like 3,5-di-*t*-butylcatechol to match the function of 3,4-PCD,[16,17,20] but to our knowledge no group has been able to combine these two roles in a single complex. Palaniandavar and coworkers reported a series of iron(III) complexes prepared from bis(phenolate)diamine ligands (**1–4**, Chart 1).[20] Use of ligand **3** led to the isolation of a monomeric trigonal bipyramidal iron(III) complex, which unfortunately did not exhibit any intradiol cleavage of catechol. The other three ligands (**1**, **2**, and **4**) were used to make monomeric octahedral iron(III) complexes that did exhibit oxidation chemistry similar to 3,4-PCD. Our group has recently been preparing a series of multiphenolate/amine ligands, which led us to wonder if we could prepare mixed phenolate ligands to better model bioinorganic systems.[21–23] Using stepwise Mannich condensations we have prepared and characterized three heterophenolate/diamine ligands **6–8** and used them to form the first monomeric trigonal bipyramidal iron(III) complexes that carry out the intradiol cleavage of catechols.

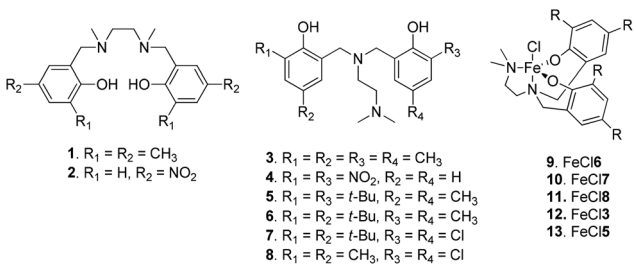

Chart 1 Ligands and complexes in this study.

The Mannich condensation is a multicomponent reaction (MCR) between a non-enolizable aldehyde (in this case, formaldehyde), a 1° or 2° amine, and an enolizable carbonyl compound. Our group and many others have used 2,4-disubstituted phenols as the enolizable carbonyl compound (which limits reactivity to the 6-position of the phenol) to make amino-diphenol complexes where both phenols are the same, as in **3** and **4** in Chart 1.[24–26] Having prepared a series of

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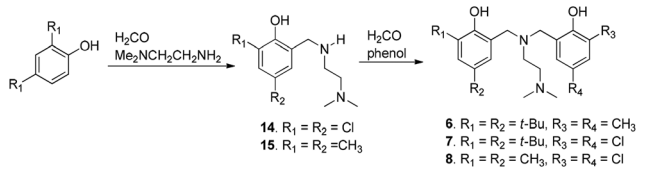
†Electronic supplementary information (ESI) available: X-ray crystallographic file (CIF) for **8–11**, additional experimental, structural, and electrochemical details. CCDC 961571, 961572, 961573 and 961574. For ESI and crystallographic data in CIF or other electronic format see DOI: 10.1039/c3dt53431f



Scheme 1 Synthetic approach for ligands **6–8**.

tetrahydrosalens using Mannich condensations, instead of the more common reductive amination, led us wonder if we could prepare complexes such as **6–8** by a two-step Mannich condensation to prepare hetero-diphenols. A single example of this type of compound appears in the literature and it was prepared by a statistical reaction where one equivalent of 2,4-dimethylphenol and 2,4-di-*t*-butyl phenol were combined with *N*,*N*-dimethylethylene diamine and formaldehyde resulting in three products (**3**, **6**, and a compound similar to **3** where the methyl groups on the phenols are *t*-butyl groups).[25] These three compounds were then separated by a series of recrystallizations and column chromatography. Our strategy was to synthesize one-armed intermediates such as **14** and **15** (Scheme 1) that could then be used to make a series of heterophenolate diamines such as **6–8**.

One armed diaminophenols **14** and **15** were prepared by combining one equivalent of formaldehyde and *N*,*N*-dimethylethylenediamine in refluxing methanol for an hour followed by addition of the appropriate phenol and refluxing overnight. Compounds **14** and **15** are susceptible to further Mannich condensations. Side-product formation was minimized by the pre-reaction of amine with formaldehyde, but **14** and **15** were isolated in 55% and 38% yields respectively. The decreased solubility of **14** compared to **15** likely leads to its formation in higher yield as the product falls out of solution prior to undergoing deleterious further reactions. Compounds **14** and **15** were used as precursors for ligands **6–8**, by reaction of the one-armed diaminophenol with one equivalent of formaldehyde and one equivalent of the appropriate second phenol in refluxing methanol. Compounds **14** and **15** are prone to retro-Mannich reactions, which can lead to scrambling of the phenols. Phenol scrambling was minimized in the lower temperature methanol reflux and becomes more problematic under higher temperature conditions. Final compounds were isolated by precipitation from methanol solutions. No chromatography was required at any time during the synthesis. Only three examples were prepared for this study, but **14** and **15** could serve as starting materials for a large number of diamino heterodiphenol ligands similar to **6–8**. This synthetic process provides greater control over the steric and electronic properties of this family of ligands and also introduces chirality into any four-coordinate metal complexes. A single crystal X-ray diffraction study confirmed the solid state structure of **8** and a thermal ellipsoid diagram and the crystallographic details can be found in the ESI.†

Mononuclear trigonal bipyramidal iron(III) complexes **9–11** were prepared by combining $FeCl_3$, triethylamine, and the appropriate ligands **6**, **7**, or **8** in methanol. After refluxing for two hours, the methanol was removed *in vacuo*. The resulting purple solids were combined with acetonitrile, filtered, recrystallized, and isolated in yields ranging from 67 to 82%. Compounds **9–11** were isolated as FeClL complexes where L = **6**, **7**, or **8** respectively. Single crystal X-ray structures indicate that **9–11** exist as distorted trigonal bipyramidal complexes, Fig. 1 and Table 1. Bond lengths, angles, and τ values for complexes **9–13** are compiled in Table S1 found in the ESI.† For each complex the chloride and the internal nitrogen heteroatom (N1 in Fig. 1) act as the axial ligands and the two phenols and the substituted dimethylamine (N2 in Fig. 1) act as the equatorial ligands. Complexes **9–11** have similar structures to **12**[20] and **13**,[19] which contain either two 2,4-dimethylphenol or two 2-*t*-butyl-4-methylphenol moieties, respectively. The difference between the two Fe–O bond lengths in **12** and **13** (which have two identical phenols coordinated to iron) are 0.013–0.020 Å. Complex **9**, in which both phenol moieties have two alkyl substituents, exhibits similar behavior, but **10** and **11** both have differences in Fe–O bond lengths of 0.27–0.36 Å, where the

Table 1 Crystal data and structural refinement parameters for **8–11**

Compound	**8**	**9**	**10**	**11**
Chemical formula	$C_{20}H_{26}Cl_2N_2O_2$	$C_{28}H_{42}ClFeN_2O_2$	$C_{26}H_{36}Cl_3FeN_2O_2$	$C_{20}H_{24}Cl_3FeN_2O_2$
FW	397.33	529.94	570.77	486.61
Crystal system	Orthorhombic	Monoclinic	Monoclinic	Monoclinic
Space group	$P2_12_12_1$	$P2_1/c$	$P2_1/c$	$P2_1/c$
a/Å	8.377(2)	11.477(4)	11.359(15)	8.471(6)
b/Å	13.897(3)	17.017(6)	16.93(2)	24.118(16)
c/Å	16.716(3)	14.198(5)	14.284(18)	12.526(9)
α/°	90.00	90.00	90.00	90.00
β/°	90.00	96.999(6)	96.78(2)	109.195(13)
χ/°	90.00	90.00	90.00	90.00
V/Å^3	1946.2(7)	2752.2(17)	2728(6)	2417(3)
Z	4	4	4	4
D_c/g cm^{-3}	1.356	1.279	1.390	1.337
μ/mm^{-1}	0.351	.672	.872	1.085
R_1 (on F_o^2, $I > 2\sigma(I)$)	0.0461	0.0379	0.0478	0.0634
wR_2 (on F_o^2, $I > 2\sigma(I)$)	0.0985	0.0933	0.1082	0.1561

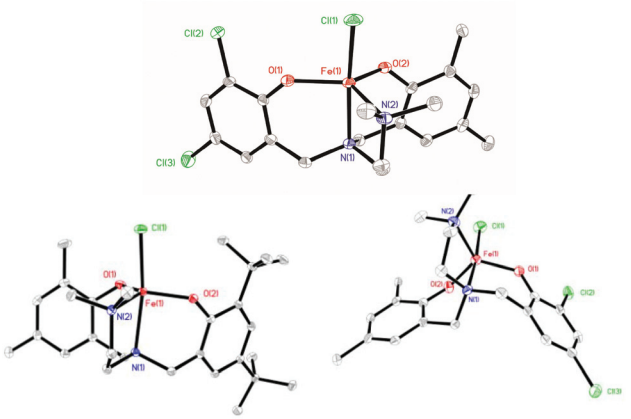
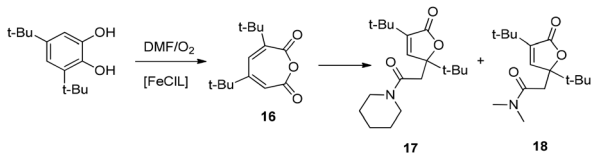


Fig. 1 Thermal ellipsoid plots of the single crystal X-ray crystallographic structures of **9–11**. Ellipsoids are drawn at 50% and hydrogen atoms are omitted for clarity.

Fe–O bond length for the 2,4-dichlorophenol is longer (~1.88 Å) than the 2,4-dialkylphenol bond length (1.84–1.85 Å). This differentiation in phenol binding relates to the enzyme, in that the proposed first step for the oxidation of catechols is loss of one of the tyrosine phenols. All five complexes **9–13** have similar trigonality indexes[27] (τ values range from 0.76–0.79) which make these structures closer to a trigonal bipyramid than the enzyme active site which has a τ value of 0.44. Other complexes using salen ligand sets have come closer to the τ value of the native enzyme,[13,14] but lack the matching reactivity.

UV-visible spectroscopy was used to look at the electronic spectra of the free ligands (**3** and **6–8**), the iron(III) complexes (**9–12**), as well as the iron(III) complexes in the presence of 3,5-di-*t*-butylcatechol, Table 2. For **9–12** there were two higher intensity bands in the near UV and two bands in the visible region of the spectra. The highest intensity band is associated with the π–π* transition of the phenol/phenolate groups (ranging from 281–287 nm) and there is a small red-shift when chloro-substituents are present (compared to alkyl substituents) and a small blue shift upon binding of iron(III). The lower energy bands are associated with ligand to metal charge-transfer transitions from phenolate groups to the iron(III) center. The lower energy charge-transfer bands are broad, presumably from having two different phenol ligands contributing



Scheme 2 Catechol oxidation products.

to these peaks. Significant changes in the UV-visible spectra are observed upon addition of 3,5-di-*t*-butylcatecholate to **9–12**. There is a pronounced difference between those complexes that have a 2,4-dichlorophenolate group (**10** and **11**) with a large red shift in the lower energy charge-transfer bands.

Cyclic voltammetry was used to examine the Fe(III/II) redox potential of acetonitrile solutions of complexes **9–12**. Each complex exhibited one reversible cathodic peak and the Fe(III/II) redox potentials followed the trend of decreasing Lewis acidity about the iron(III) center with **10** > **11** > **9** > **12** (−0.561 V, −0.575 V, −0.624 V, and −0.640 V respectively *vs.* Fc/Fc^{+}). This data shows that the replacement of methyl substituents by *t*-butyl substituents on the phenolate groups destabilizes Fe(III) compared to Fe(II) by ~15 mV. Similarly replacement of an alkyl substituted phenolate by a chloro substituted phenolate is worth ~64 mV of destabilization.

Compounds **9–11** were combined with 1 equivalent of 3,5-di-*t*-butylcatechol and 2 equivalents of piperidine in DMF and exposed to molecular oxygen for 24 hours. After an acidic workup, the products were investigated by GCMS analysis. As opposed to **12**,[20] which was found to be non-reactive as an intradiol dioxygenase, **9–11** all exhibited intradiol dioxygenase reactivity as shown by the formation of compounds **16**, **17** and **18**. Compounds **17** and **18** are the products formed when either piperidine or dimethylamine (an impurity found in DMF) react with the acid anhydride product (**16**) of the reaction. Compounds **16–18** typically made up 75–85% of the product mixture and were the three most prevalent products (Scheme 2).

Conclusions

We have elucidated a new route to diamino/heterophenol complexes that provide a means to differentiate the binding

Table 2 Electronic spectral data for iron(III) complexes in CH_3CN

	λ_{max} (nm) (ε, M^{-1} cm^{-1})			
	11	**10**	**9**	**12**
Free ligand	287 (4730)	285 (4370)	284 (4460)	283 (4740)
FeClL complex	471 (2120)	470 (2390) sh	478 (2220)	473 (3920)
	390 (2030) br	400 (2540)	390 (2030)	390 (3580)
	320 (4260) sh	324 (4880)	320 (3710) sh	321 (6560)
	284 (8190)	280 (8710)	281 (7400)	281 (13 600)
FeClL + 3,5-di-*t*-butylcatechol	620 (1630)	619 (1350)	478 (4500)	477 (2700)
	427 (2880)	422 (2240)	340 sh	340 sh
	296 (10 000)	296 (8940)	282 (15 900)	283 (9820)

strength of the two phenols to a metal center by varying the electronic and steric properties of those moieties. These new ligands provide asymmetry at the metal center that could be useful for new catalysts and have been shown to be useful in modeling the active-sites of biological systems in this study. When complexes **6–8** are bound to iron(III), all three form trigonal bipyramidal complexes that are a close match to the structure of the active-site of 3,4-PCD and exhibit dioxygenase reactivity with a catechol substrate. Our lab is investigating a wider series of ligands and the reactivity of the resulting Fe(III) complexes. Those results will be reported soon.

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