Chapter 2

Reactivity of Biomimetic Iron(II)-2-Aminophenolate Complexes toward Dioxygen: Mechanistic Investigations on the Oxidative C-C Bond Cleavage of 2-Aminophenols
Chapter 2
2.1 Abstract

The isolation and characterization of a series of iron(II)-2-aminophenolate complexes [(6-Me$_3$-TPA)Fe$^{II}$($X$)]$^+$ ($X = 2$-amino-4-nitrophenolate (4-NO$_2$-HAP), 1; $X = 2$-aminophenolate (2-HAP), 2; $X = 2$-amino-3-methylphenolate (3-Me-HAP), 3; $X = 2$-amino-4-methylphenolate (4-Me-HAP), 4; $X = 2$-amino-5-methylphenolate (5-Me-HAP), 5; $X = 2$-amino-4-tert-butylphenolate (4-tBu-HAP), 6 and $X = 2$-amino-4,6-di-tert-butylphenolate (4,6-di-tBu-HAP), 7) and iron(III)-2-amidophenolate complexes [(6-Me$_3$-TPA)Fe$^{III}$(4-tBu-AP)]$^+$ (6$^{\text{Ox}}$) and [(6-Me$_3$-TPA)Fe$^{III}$(4,6-di-tBu-AP)]$^+$ (7$^{\text{Ox}}$) supported by a tripodal nitrogen ligand (6-Me$_3$-TPA = tris(6-methyl-2-pyridylmethyl)amine) are reported. Substituted 2-aminophenols were used to prepare the biomimetic iron(II) complexes either as perchlorate or tetraphenylborate salts to understand the effect of electronic and structural properties of aminophenolate rings on the dioxygen reactivity and on the selectivity of C-C bond cleavage reactions. Crystal structures of the cationic parts of 5, 6 and 7 show six-coordinate iron(II) centers ligated by a neutral tetradentate ligand and a monoanionic 2-aminophenolate in a bidentate fashion. While 1 does not react with oxygen, other complexes undergo oxidative transformation in the presence of dioxygen. Initially, the iron(II)-2-aminophenolates rapidly convert to the corresponding iron(III)-2-amidophenolate species which further react slowly to affect the oxidation/oxygenation of the coordinated aminophenolates. The reaction of 2 with dioxygen affords 2-amino-3H-phenoxazine-3-one, the auto-oxidation product of 2-aminophenol, whereas complexes 3, 4, 5 and 6 form the corresponding aromatic C-C bond cleavage products. On the other hand, both 7 and 7$^{\text{Ox}}$ produce a mixture of 4,6-di-tert-butyl-2H-pyran-2-imine and 4,6-di-tert-butyl-2-picolinic acid. Labeling experiments with $^{18}$O$_2$ establish incorporation of one oxygen atom from dioxygen into the cleavage products. The reactivity (and stability) of the intermediate, which directs the course of aromatic ring cleavage reaction, is found to be dependent on the nature of ring substituents. The presence of two tert-butyl groups on the aminophenolate ring in 7 makes the latter slow to cleave the C-C bond of 4,6-di-tBu-HAP, whereas 4 containing 4-Me-HAP displays fastest reactivity. DFT calculations were performed on complex [(6-Me$_3$-TPA)Fe$^{III}$(4-tBu-AP)]$^+$ (6$^{\text{Ox}}$) in order to gain a mechanistic insight into the regioselective C-C bond cleavage reaction. Based on the
experimental and computational studies, a five-coordinate iron(II)-2-iminobenzosemiquinone intermediate is proposed to react with dioxygen resulting in oxidative C-C bond cleavage of the coordinated 2-aminophenolates.

2.2 Introduction

Aromatic C-C bond cleavage is a crucial step in the biodegradation of organic compounds such as catechols, aminophenols, hydroquinones, salicylic acid, gentisic acid etc. by aerobic microorganism.[1-7] A variety of nonheme iron oxygenases are involved in catalyzing the C-C bond cleavage of aromatic compounds in the biodegradation pathway.[8-17] The ring-cleaving oxygenases belong to the cupin superfamily of nonheme enzymes with the 3His or 2/3His-1-Glu ligand environment around the iron(II) centre.[9, 18-19] Catechol dioxygenases are most familiar in this class of enzymes which is categorized as extradiol and intradiol dioxygenases depending upon the position of ring cleavage of catechol.[9, 20-22] Similarly, 2-aminophenol dioxygenases catalyze the ring cleavage at meta position of 2-aminophenols in the biodegradation of nitroaromatics.[23-25] 2-Aminophenol-1,6-dioxygenase (APD), the nonheme iron enzyme isolated and purified from Pseudomonas Pseudoalcaligenes, catalyze the oxygenolytic ring cleavage of 2-aminophenols under aerobic conditions. [11, 26-27] The reaction occurs via an oxidative C1-C6 bond cleavage to form 2-aminomuconic acid semialdehyde which spontaneously cyclizes with loss of a water molecule to form 2-picolinic acid (Scheme 2.2.1). Very recently, the crystal structures of APD from Comamonas sp. strain CNB-1 as the apoenzyme, the holoenzyme and as complexes with the lactone intermediate (4Z,6Z)-3-iminooxepin-2(3H)-one, and the product 2-aminomuconic acid-6-semialdehyde with the suicide inhibitor 4-nitrocatechol have been reported.[28] The active site structure in the resting state describes the asymmetric binding of aminophenolate to the ferrous ion and the other structures provide insight into the reaction mechanism. Another enzyme 3-hydroxyanthranilate-3,4-dioxygenase (HAD), isolated from Saccharomyces cerevisiae, catalyze the C3-C4 bond cleavage of 3-hydroxyanthranilate to quinolinic acid in the tryptophan catabolism pathway.[29-32] A similar pathway is followed in the degradation of other nitroaromatic compounds.[27, 33-36] Both HAD and APD, exhibit a common “2-His-1-carboxylate facial structural motif” and show functional similarity with extradiol dioxygenases.
Functional Models of 2-Aminophenol Dioxygenases

Scheme 2.2.1 Reaction catalyzed by 2-aminophenol dioxygenases.

On the basis of structural and biochemical studies on HAD and APD, a mechanistic proposal similar to that of extradiol catechol dioxygenases has been reported. The substrate binds to the ferrous ion and the enzyme-substrate complex activates dioxygen to form an iron(III)-superoxide species. An iron(II)-peroxide intermediate is subsequently generated via an iron(II)-2-iminobenzosemiquinonato radical upon electron transfer from the aminophenolate ring to the metal center. A lactone intermediate is formed via O-O bond heterolysis of the peroxide intermediate with incorporation of one oxygen atom into the lactone ring. Hydrolysis of the lactone affords the cleavage product. The conversion 2-amino muconic acid semialdehyde to 2-picolinic acid then takes place through a nonenzymatic pathway. Interestingly, most of the intermediates have been successfully characterized crystallographically in APD similar to the structures isolated with different enzyme stains of homoprotocatechuate 2,3-dioxygenase (HPCD).

Scheme 2.2.2 Redox non-innocence of 2-aminophenol.

The ring cleavage reactivity of 2-aminophenol dioxygenases (HAD and APD) has fuelled the interest in studying metal-coordinated 2-aminophenols. Moreover, the “redox non-innocent” nature of aminophenols (Scheme 2.2.2), the key electronic feature responsible for dioxygen reactivity in the enzymes, has prompted to investigate the coordination chemistry of aminophenolates. Several iron(II) complexes of 2-aminophenolates have been reported by Wieghardt et al.
and others where the coordinated aminophenolates exhibited different redox levels.\textsuperscript{[38-44]} In biomimetic chemistry, reactivity of model iron(II)-2-aminophenolate is less explored. Fiedler \textit{et al.} have recently reported an iron(II)-2-aminophenolate complex with a facial N\textsubscript{3} donor ligand. Reaction of the iron(II) complex with an oxidant led to the isolation of an iron(II)-2-iminobenzosemiquinone and iron(III)-2-iminobenzosemiquinone complexes.\textsuperscript{[45-46]}

In this chapter, we report the synthesis and characterization of seven iron(II)-2-aminophenolate complexes with the general composition [(6-Me\textsubscript{3}-TPA)Fe\textsuperscript{II}(X)]\textsuperscript{+} (X = 2-amino-4-nitrophenolate (4-NO\textsubscript{2}-HAP), 2-aminophenolate (HAP), 2-amino-3-methylphenolate (3-Me-HAP), 2-amino-4-methylphenolate (4-Me-HAP), 2-amino-5-methylphenolate (5-Me-HAP), 2-amino-4-\textit{tert}-butyphenolate (4-\textit{t}Bu-HAP), 2-amino-4,6-di-\textit{tert}-butylphenolate (4,6-di-\textit{t}Bu-HAP), and 6-Me\textsubscript{3}-TPA = tris(6-methyl-2-pyridylmethyl)amine) as biomimetic models of 2-aminophenol dioxygenases (Scheme 2.3.1). The iron(II)-2-aminophenolate complexes react with dioxygen to undergo oxidative transformation. The influences of structural and electronic properties of substituents on the nature of metal-based intermediate and on the C-C bond cleavage reactivity of the coordinated aminophenolates are discussed. On the basis of experimental and computational studies, a mechanism of aromatic C-C bond cleavage reaction of 2-aminophenols on the iron(II) complex is presented.

2.3 Results and Discussion

2.3.1 Synthesis and Characterization

The iron(II) complexes were isolated from the reactions of equimolar amounts of ligand and iron(II) perchlorate hydrate with respective 2-aminophenols in the presence of triethylamine under nitrogen atmosphere (Scheme 2.3.1.1). The complexes are air-stable in solid state but are extremely sensitive in solution. IR spectra of the complexes in solid state exhibit strong and broad $\tilde{\nu}_{\text{NH}}$ band at around 3400 cm$^{-1}$. Additionally, a strong band is observed at around 1600 cm$^{-1}$ for all the complexes characteristic of metal-coordinated monoanionic aminophenolates. The presence of bands due to countercations (perchlorate or tetraphenylborate) in the IR spectra suggests monocationic nature of the complexes. Complex 1 in acetonitrile exhibits an absorbance band...
at 378 nm in the optical spectrum, whereas other complexes show intense absorption band at around 400 nm. The complexes exhibit room temperature magnetic moment values in the range of 4.8–5.2 $\mu_B$, consistent with high-spin iron(II) complex. In the $^1$H NMR spectra, the complexes display paramagnetically shifted resonances of the protons in the region between -40 ppm and 60 ppm (A-2-1 to A-2-7, Appendices). A comparison of $^1$H NMR spectra of the iron(II) complexes suggests that the broad signal appeared in the upfield region between -25 ppm and -35 ppm is attributable to the resonance of $\alpha$-CH$_3$ protons of 6-Me$_3$-TPA ligand.$^{[47-48]}$ The pyridyl protons of the ligand are observed in the downfield region as sharp singlets. The methylene protons are too broad to be observed in the spectra. The aromatic protons of 2-aminophenols appear in between 7 ppm and 9 ppm. The $^1$H NMR spectra along with the magnetic moment values strongly support the high-spin nature of the iron(II) complexes.

Scheme 2.3.1.1 Synthesis of Fe(II)-2-aminophenolate complexes.

2.3.2 Crystal Structures

Complexes 5, 6 and 7 were further characterized by single-crystal X-ray diffraction. For 6 and 7, the tetraphenylborate salts of the complexes were used
to grow crystals. Single crystals of 5, 6·CH₃OH and 7·CH₃OH were isolated from a solvent mixture of dichloromethane, methanol and diethyl ether at room temperature. Complex 5 and 7 crystallizes in the monoclinic system with space group \( P2_1/n \) and \( P2_1/c \), whereas 6 crystallizes in the triclinic system with space group \( P-1 \). F

The structure of the mononuclear complex cation of 5 (Figure 2.3.2.1) shows a six coordinate iron centre coordinated by four nitrogen donors from the N₄ ligand and a bidentate monoanionic aminophenolate. The aminophenolate ring coordinates through one phenolate oxygen (O1) and one amine nitrogen (N5) with the Fe1-O1 and Fe1-N5 distances of 1.954(5) Å and 2.299(7) Å, respectively. The coordination geometry of the iron centre is distorted octahedron where the axial positions are occupied by one of the pyridine nitrogens (N4) of the tetradentate ligand and the amine nitrogen (N5) of aminophenolate with the O4-Fe1-N5 angle of 171.4 (2)°. The equatorial plane is comprised of O1, N1, N2 and N3 donors. The C-C bond distances in the coordinated aminophenolate moiety indicate the retention of aromatic character without any quinonoid distortion.

![Figure 2.3.2.1 X-ray single crystal structure of the cationic part of 5. All hydrogen atoms except those on N5 and counter anion have been omitted for clarity.](image)

42
The cationic part of complex 6·CH$_3$OH and 7·CH$_3$OH are iso-structural and iso-electronic with the iron(II)-catecholate complex, [(6-Me$_3$-TPA)Fe$^{III}$(DBCH)]$^+$ with comparable metal-ligand bond distances and angles.\cite{50} A bidentate binding motif of 2-amino-4-tert-butylphenolate (4-tertBu-HAP) and 2-amino-4,6-di-tert-butylphenolate (4,6-di-tertBu-HAP) (Figure 2.3.2.2 and 2.3.2.3), similar to that in 5, is observed. The steric interaction between the tert-butyl group on the aminophenolate ring and the methyl groups on the pyridine rings of the ligand forces the metal ion to adopt a distorted octahedral coordination geometry with the axial O1-Fe1-N2 angle of 171.56(7)$^\circ$ in 7·CH$_3$OH. It is worth mentioning here that the monoanionic aminophenolates bind asymmetrically to the iron centre in all the structurally characterized iron(II)-aminophenolate complexes in which the phenolate oxygens coordinate trans to the amine nitrogen of 6-Me$_3$-TPA ligand.

**Figure 2.3.2.2** X-ray crystal structure of 6·CH$_3$OH. All hydrogen atoms except those attached to N5 and counter anion have been omitted for clarity.
Chapter 2

Figure 2.3.2.3 Solid state structure of the cationic complex 7·CH₃OH. All hydrogen atoms except those attached to N5 and counter anion have been omitted for clarity.

Table 2.3.2.1 Selected bond length (Å) and angles (°) of 5, 6·CH₃OH and 7·CH₃OH.

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<th>Bond distance/angles</th>
<th>5</th>
<th>6·CH₃OH</th>
<th>7·CH₃OH</th>
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<tr>
<td>Fe(1)–N(2)</td>
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<td>2.232(2)</td>
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<td>2.282(3)</td>
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<td>1.934(3)</td>
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Functional Models of 2-Aminophenol Dioxygenases

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<th>N(1)–Fe(1)–N(5)</th>
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<td>168.43(7)</td>
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The crystal structure of the substrate bound APD reveals an asymmetric binding of a suicide inhibitor, monoanionic 4-nitrocatechol, consistent with the crystal structure of homoprotocatechuate 2,3-dioxygenase. The asymmetric binding of monoanionic 2-aminophenolate to the iron(II) in biomimetic complexes structurally mimics the enzyme-substrate adduct of 2-aminophenol dioxygenases. Although the X-ray diffraction quality single crystals could not be isolated for 1, 2, 3 and 4 after several attempts, the spectroscopic and analytical data suggest that the cationic part of theses complexes are mononuclear with six-coordinate geometry at iron(II) centre similar to that in 5, 6 and 7.

2.3.3 Dioxygen Reactivity

All the iron(II)-2-aminophenolate complexes, except 1, are extremely sensitive towards oxygen in solution. Exposure of an acetonitrile solution of 2 to dioxygen results in a color change from yellow to deep green. Over a period of 15 min, the CT band at 402 nm disappears and three new bands at around 365 nm, 560 nm and 920 nm appears (Figure 2.3.3.1). The new bands, are assigned to as 2-amidophenolate to iron(III) charge transfer transition. The solution exhibits a predominant molecular ion peak at \( m/z = 494.03 \) with the isotopic distribution pattern calculated for \([ (6-\text{Me}_3\text{-TPA})\text{Fe}(\text{AP})]^+ \). X-band EPR spectrum of the solution at 77K displays two signals at \( g = 4.2 \) and 1.99 (A-2-8, Appendices). The spectral data of the reaction solution after 15 min thus support the formation of an iron(III)-2-amidophenolate species, \([ (6-\text{Me}_3\text{-}...\]
Chapter 2

TPA)Fe^{III}(AP)(ClO_4). Further exposure of the green solution to dioxygen slowly yields an orange solution during which time the 920 nm band diminishes with concomitant formation of a relatively sharp band at 360 nm. The final oxidized solution of 2 exhibits an intense rhombic EPR signal at \( g = 4.2 \) typical of high-spin iron(III) species.

Figure 2.3.3.1 Time-dependent optical spectral changes during the reaction of 2 with dioxygen at 298 K (concentration: 0.5 mM in acetonitrile). Inset: Plot of absorbance versus time.

\(^1\)H NMR spectrum of the organic product derived from 2-aminophenol after acidic work-up of the final oxidized solution of 2 shows proton resonances in between 6-8 ppm attributable to aromatic protons (A-2-9, Appendices). The positions of the proton resonances match well with those of 2-amino-3H-phenoxazin-3-one, the oxidized product of 2-aminophenol.\(^{[52]}\) It is worth mentioning here that 2-picolinic acid, the C-C bond cleavage product formed in the catalytic cycle of APD, is not observed in the reaction (A-2-9b, Appendices). Interestingly, reaction of an equimolar mixture of iron(II) perchlorate and 2-aminophenol affords the same product (A-2-9c, Appendices). It can therefore be concluded that the reaction of 2 with dioxygen forms 2-amino-3H-phenoxazin-3-one via an auto-oxidation pathway (Scheme 2.3.3.1). The weak signals in the
Functional Models of 2-Aminophenol Dioxygenases

$^1$H NMR spectrum of the reaction mixture may arise from other auto oxidation products from 2-aminophenol. The auto-oxidative formation of 2-amino-3$H$-phenoxazin-3-one from 2-aminophenol on iron(II) complex is reminiscent of the reactions catalyzed by 2-aminophenol oxidase and tyrosinase.[53]

Scheme 2.3.3.1 Reaction of [(6-Me$_3$-TPA)Fe$^{II}$(HAP)]$^+$ with dioxygen.

The reactions of complexes 3, 4 and 5 with dioxygen, on the contrary, involve three consecutive steps. In the first step, the yellow solutions of the complexes turn dark green immediately upon exposure to dioxygen. For [(6-Me$_3$-TPA)Fe$^{II}$(3-Me-HAP)](BPh$_4$) (3), the charge transfer band at around 401 nm disappears with the formation of two intense bands at around 620 nm and 935 nm within 5 min (Figure 2.3.3.2a). X-band EPR spectrum of the green solution exhibits a signal at $g = 4.2$ supports the formation of rhombic iron(III) species in the first step (A-2-10, Appendices). A molecular ion peak at $m/z = 509.21$ with the isotope distribution pattern calculated for [(6-Me$_3$-TPA)Fe(3-Me-AP)$]^+$ is found in the ESI-MS spectrum of the green solution (Figure 2.3.3.3a). The first step thus involves the generation of an iron(III)-2-amidophenolate species.
Figure 2.3.3.2 Optical spectral changes with time during the reaction of 3 (0.5 mM in acetonitrile) with dioxygen at 298 K for (a) 5 min (b) 10 min and (c) for 14 h. Inset: Plot of absorbance versus time.

Further exposure of the dark green solution to dioxygen leads to a faint green solution. The sharp band at 935 nm disappears within 12 min with concomitant formation of a distinct band at 645 nm (Figure 2.3.3.2b). During the reaction a signal is observed at $g = 8.4$ in the EPR spectrum. The band at 645 nm then slowly decays to form a light orange solution over a period of 16 h (Figure 2.3.3.2c). The final reaction solution displays a rhombic signal at $g = 4.2$ along with a weak signal at $g = 1.98$ in the EPR spectrum (A-2-10c, Appendices). Similar changes in the optical and EPR spectra are observed for complexes 4 and 5 (A-2-11 to A-2-13, Appendices) Of note, the total time required for the reaction of the iron(II)-2-aminophenolate species depends on the position of methyl group on the aromatic ring. While complex 4 requires about 1 h 50 min,
complexes 3 and 5 take about 16.5 and 8.5 h, respectively. ESI–MS spectrum of the final oxidized solution of 3 show ion peak at \( m/z = 524.28 \) with the isotope distribution calculated for \([(6-\text{Me}_3\text{-TPA})\text{Fe}(3-\text{Me}-2\text{-picolinate})]^+ \) (Figure 2.3.3.3b). Final oxidized solutions of 4 and 5 also display similar ESI-mass spectra.

![Figure 2.3.3.3](image)

**Figure 2.3.3.3** ESI-MS spectra of the species formed (a) after 5 minutes and (b) at the end of the reaction of 3 with dioxygen at 298 K.

\(^1\text{H} \) NMR spectra of organic products obtained from the oxidized solutions of 3, 4 and 5 show three proton resonances in the aromatic region between 7.5 ppm and 8.6 ppm along with one sharp singlet at around 2.25 ppm (Experimental). Although the peaks from organic products in all three cases appear almost at the identical positions, the multiplicity and the peak area of the proton resonances strongly support the formation of 5-methyl-2-picolinic acid from 5 (Figure 2.3.3.4), 3-methyl-2-picolinic acid from 3 and 4-methyl-2-picolinic acid 4 (A-2-14, Appendices and Scheme 2.3.3.3).
Complex (6) reacts with molecular oxygen in acetonitrile under ambient conditions. A light yellow solution rapidly turns to deep green within 2 min. During the reaction, the absorption band at 404 nm decays and three new bands at 366 nm, 600 nm, and 934 nm grow rapidly (Figure 2.3.3.5a). The dark green solution formed after 2 min shows EPR signal at $g = 4.21$ typical of high-spin iron(III) species with an $S = 5/2$ spin state (A-2-15, Appendices). To prove the formation of an iron(III) species in the first step, attempts were made to independently synthesize the iron(III) complex. Unfortunately, no iron(III) complex could be isolated via direct synthesis by mixing the ligand, iron(III) salt and 2-amino-4-tert-butylphenol in the presence of a base. However, controlled one-electron oxidation of 6 with a stoichiometric amount of KMnO$_4$ results in the isolation of a dark green complex, $6^{Ox}$ (see experimental). The optical spectral features and the EPR data of $6^{Ox}$ (A-2-16, Appendices) bear resemblance to those of the deep green species observed in the reaction of 6 with dioxygen. Thus the initial change in the optical spectrum of 6 during the reaction with
oxygen is associated with the formation of \( [(6\text{-Me}_3\text{-TPA})\text{Fe}^{\text{III}}(4\text{-tBu-AP})](\text{ClO}_4) \) \( (6^{\text{Os}}) \).

**Figure 2.3.3.5** Optical spectral changes with time during the reaction of 6 (1 mM solution in acetonitrile, path length = 0.5 cm) with dioxygen at 298 K: (a) reaction during the first 2 minutes, (b) slow reaction for 6 h. Inset: Plot of absorbance vs time.

In the next step of the reaction with \( \text{O}_2 \), the charge transfer bands at 934 nm and 600 nm decay immediately with the formation of new band at 660 nm which decays further slowly over a period of 6 h to form a light orange solution (Figure 2.3.3.5b). A similar spectral change is observed upon exposure of an acetonitrile solution of \( 6^{\text{Os}} \) to dioxygen for 6 h. During the time period, the EPR signal at \( g = 1.99 \) slowly diminishes leaving the high-spin iron(III) signal at \( g = 4.21 \) after 6 h (A-2-15, Appendices). ESI-MS spectrum of the oxidized solution shows an ion peak at \( m/z = 566.2 \) with the isotope distribution pattern calculated for \( [(6\text{-Me}_3\text{-TPA})\text{Fe}(4\text{-tBu-2-picolinate})]^+ \). The formation of picolinate was further confirmed by labelling experiment. ESI-MS of the oxidized solution after reaction of 6 with \( ^{18}\text{O}_2 \) displays an ion peak at \( m/z = 568.2 \), suggesting incorporation of one \( ^{18}\text{O} \) atom into 4-tert-butyl-2-picolinate (Figure 2.3.3.6). Furthermore, addition of \( \text{H}_2^{16}\text{O} \) in the labelling experiment with \( ^{16}\text{O}_2 \) shows about 30% incorporation of one \( ^{18}\text{O} \) into the product picolinate (A-2-17, Appendices)
Figure 2.3.3.6 ESI-MS (positive ion mode in acetonitrile) of the solution after reaction of 6 with (a) $^{16}$O$_2$ and (b) $^{18}$O$_2$. The solid bars indicate the corresponding computer simulated spectra.

$^1$H NMR spectrum of the organic product derived from 2-amino-4-tert-butylphenolate reveals four distinct and sharp proton resonances; two doublets at 8.6 ppm ($J = 5$ Hz) and 7.4 ppm ($J = 5$ Hz) and, two singlet at 8.2 ppm and 1.36 ppm (Figure 2.3.3.7c). The coupling constant values of the doublets suggest that the peaks are due to aromatic protons. Of note, a singlet at 6.7 ppm and a multiplet signal at 6.6 ppm are observed in the $^1$H NMR spectrum of the substrate, 2-amino-4-tert-butylphenol (Figure 2.3.3.7a). The position of three proton resonances and the coupling constant of the doublet peaks suggest the formation of 4-tert-butyl-2-picolinate during the reaction of 6 with dioxygen. The absence of any peak of aminophenol substrate in the NMR spectrum confirms a stoichiometric conversion of 2-amino-4-tert-butylphenol to 4-tert-butyl-2-picolinic acid in 6 h.
**Figure 2.3.3.7** $^1$H NMR (500 MHz, CDCl$_3$ at 298 K) spectrum of (a) 2-amino-4-tert-butylphenol, (b) 2-picolinic acid and (c) the organic product obtained after removal of metal ion from the oxidized solution of 6. Peaks assigned with asterisk (*) are for aromatic protons of pyridine ring of 6-Me$_3$TPA derived fragment came in the organic products during acidic work-up at pH 2-3 and # assigned as the residual CHCl$_3$ in CDCl$_3$.

To confirm it further, the organic product was analysed by GC-MS. GC-MS of methyl ester of the organic product shows an ion peak at $m/z = 193.2$ along with expected fragmentation patterns of methyl-4-tert-butyl-2-picolinate (Figure 2.3.3.4). When the reaction is carried out in the presence of $^{18}$O$_2$, the ion peak at $m/z = 193.2$ is shifted to 195.2 and around 40% incorporation of $^{18}$O into methyl-4-tert-butyl-2-picolinate is observed (Figure 2.3.3.8).
Chapter 2

Figure 2.3.3.8 GC-mass spectrum of methyl ester of 4-tert-butyl-2-picolinic acid derived from the reaction of 6 with (a) $^{16}$O$_2$ (b) $^{18}$O$_2$.

All these results firmly establish that the reaction of 6 with dioxygen leads to the formation of 4-tert-butyl-2-picolinic acid via C–C bond cleavage of 2-amino-4-tert-butylphenol (Scheme 2.3.3.2) and functionally mimics the reaction catalyzed by 2-aminophenol-1,6-dioxygenase (APD) and 3-hydroxyanthranilate-3,4-dioxygenase (HAD). To understand the role of ligand in the oxidative C–C bond cleavage of aminophenolate on the model complex, an equimolar mixture of iron(II) perchlorate, 2-amino-4-tert-butylphenol and Et$_3$N in acetonitrile was reacted with dioxygen for 6 h. No C–C bond cleavage of 2-amino-4-tert-butylphenolate was observed.

Scheme 2.3.3.2 Reaction of the iron(II)-aminophenolate complex (6) with dioxygen.

The spectral change during the reaction of 7 with oxygen is completely different than that observed with other complexes discussed above. In the
reaction, the CT band at 402 nm decays and three new bands at 325 nm, 520 nm and 665 nm are formed over a period of 10 min in acetonitrile at 298 K (Figure 2.3.3.9a). The ESI-MS spectrum of the solution exhibits a molecular ion peak at $m/z = 565.23$ with the isotopic distribution calculated for [(6-Me$_3$-TPA)Fe(4,6-di-tBu-AP)]$^+$. The green solution displays a rhombic signal at $g = 4.2$ in the X-band EPR spectrum typical of a high-spin iron(III) species. Additionally a sharp signal at $g = 1.99$ is observed possibly due to the presence of some non-coordinated radical impurity in the intermediate species (A-2-18, Appendices).

Figure 2.3.3.9 Optical spectral changes during the reaction of 7 (0.5 mM solution in acetonitrile) with dioxygen at 298 K (a) over a time period of 10 min. (b) over the next 22 h.

The green solution further reacts with dioxygen during which time the bands at 520 nm and 665 nm decay (Figure 2.3.3.9b). Although the final oxidized solution could not be analyzed by ESI-MS, the X-band EPR spectrum suggests formation of a high-spin iron(III) species (A-2-18, Appendices).
The organic products from the oxidized solution of 7 were isolated by acidic work-up and subsequent extraction with diethyl ether. The $^1$H NMR spectrum of organic products shows three sharp singlets at 8.07 ppm, 7.59 ppm and 6.05 ppm (Figure 2.3.3.10). The sharp signals observed in the range of 1.44-1.16 ppm represent the aliphatic protons of the tert-butyl groups. The absence of aromatic protons at 6.81 ppm and 6.91 ppm strongly suggests that no unreacted 4,6-di-tert-Bu-HAP is present in the oxidized solution. The protons of 4,6-di-tert-butyl-2H-pyrane-2-one, an extradiol cleavage product from 3,5-di-tert-butylcatechol, exhibit a singlet resonance at 6.05 ppm in the $^1$H NMR spectrum. These results unambiguously support the formation of 4,6-di-tert-butyl-2H-pyran-2-imine via C1-C6 cleavage of 4,6-di-tert-Bu-HAP. Comparing the $^1$H NMR spectrum of 4-tert-butylpicolinic acid derived from 4-tert-butylaminophenol,[49] the sharp singlets at 8.07 ppm and 7.59 ppm are assigned to the aromatic protons of 4,6-di-tert-butylpicolinic acid (Scheme 2.3.3.3). The organic products, 4,6-di-tert-butyl-2H-pyran-2-imine (35%) and 4,6-di-tert-butyl-2-picolinic acid (65%), were quantified by calculating the relative ratio of the aromatic protons. For further confirmation, 4,6-di-tert-butyl-2-picolinic acid was esterified with a
freshly prepared diazomethane solution for analysis by $^1$H NMR and GC-MS. Two singlets are observed at 7.92 ppm and 7.49 ppm in addition to a singlet at 4.00 ppm in the $^1$H NMR spectrum confirming the formation of methyl-4,6-di-tert-butyl-2-picolinate in the reaction (A-2-19, Appendices). The GC-MS analysis of the organic products after esterification shows two distinct ion peaks at $m/z = 208$ and 249 with the expected fragmentation patterns of 4,6-di-tert-butyl-2H-pyran-2-one and methyl-4,6-di-tert-butyl-2-picolinate (Figure 2.3.3.11a and Figure 2.3.3.11b). The imine product gets hydrolyzed during the acidic work-up to form 4,6-di-tert-butyl-2H-pyran-2-one. ESI-MS spectrum also supports the formation of these two products (A-2-20, Appendices).

![Figure 2.3.3.11](image)

Figure 2.3.3.11 GC-MS spectra of organic products obtained after removal of metal ion from the oxidized solution of 7 and subsequent treatment of a freshly prepared diazomethane after reaction of 7 with $^{16}$O$_2$ (a and b) and with $^{18}$O$_2$ (c and d).

The reaction of 7 with $^{18}$O$_2$ was carried out to establish the source of oxygen atoms into the cleavage products. The organic phase was treated with
Chapter 2

diazomethane and was analyzed by GC-MS. The ion peaks are shifted two mass unit higher for 4,6-di-tert-butyl-2H-pyran-2-one and methyl-4,6-di-tert-butyl-2-picolinate. The distribution patterns for 4,6-di-tert-butyl-2H-pyran-2-one and methyl-4,6-di-tert-butyl-2-picolinate observed in the spectra (Figure 2.3.3.11c and 2.3.3.11d) support incorporation of one $^{18}$O atom into each product. While 80% incorporation of labelled oxygen into pyrone is observed, only 40% incorporation of one oxygen atom into picolinate takes place. A lower percentage of incorporation of $^{18}$O may be explained possibly due to exchange with water during the workup of organic products from the oxidized solution prior to analysis.$^{[54]}$ ESI-MS spectrum also support incorporation of one oxygen atom into each organic product derived from 4,6-di-tertBu-HAP (A-2-21, Appendices).

Scheme 2.3.3.3 Reactivity of the iron(II)-2-aminophenolate complexes with dioxygen.

To get a better understanding about the reaction of 7 with oxygen, controlled one-electron chemical oxidation of 7 using a stoichiometric amount of KMnO$_4$ or 2,4,6-tri-tert-butylphenoxy radical under nitrogen environment was carried out to isolate 7$^{\text{Ox}}$. Unfortunately, all attempts to isolate the single crystal of 7$^{\text{Ox}}$ were failed. The acetonitrile solution of 7$^{\text{Ox}}$ is dark green in color and exhibits sharp bands at 366 nm ($\varepsilon = 3000$ cm$^{-1}$M$^{-1}$), 618 nm ($\varepsilon = 1900$ cm$^{-1}$M$^{-1}$) and 925 nm ($\varepsilon = 2700$ cm$^{-1}$M$^{-1}$) under nitrogen environment (Figure
Functional Models of 2-Aminophenol Dioxygenases

2.3.3.12. Three prominent signals at around $g = 8.6$, 5.07 and 3.71 are seen in the EPR spectrum at 77K describing an axial $S = 5/2$ spin system in solution (A-2-22, Appendices). In addition, a sharp signal at $g = 1.99$ due to some non-coordinated radical species is also observed. Therefore, the controlled chemical oxidation of 7 leads to isolation of an iron(III) species, $7^{\text{Ox}}$, [(6-Me$_3$-TPA)Fe$^{\text{III}}$(4,6-di-tBu-AP)](BPh$_4$), which decays readily upon exposure to dioxygen. Furthermore, the ESI-MS spectrum of the solution exhibits a molecular ion peak at $m/z = 565.21$ as observed for the solution obtained from the reaction of 7 with oxygen after 10 min. Interestingly, an instantaneous decay of the band at 925 nm followed by a concomitant shift of the band at 618 nm to 665 nm is observed upon exposure of the solution of $7^{\text{Ox}}$ to dioxygen (Figure 2.3.3.12). Moreover the spectrum matches perfectly with the spectrum obtained after exposure of 7 to oxygen for 10 min. Of note, the spectrum of $7^{\text{Ox}}$ bears resemblance with the spectra obtained in the first step of the reactions of 3, 4, 5, and 6 with oxygen.

![Figure 2.3.3.12](image)

**Figure 2.3.3.12** Optical spectra of $7^{\text{Ox}}$ (0.5 mM in acetonitrile). under nitrogen atmosphere (pink line) and after exposure to dioxygen (green line).

The optical spectral features during the reaction of 2-5 and 7 with dioxygen at low temperature are not much different from those observed at ambient temperature. Exposure of an acetonitrile solution of 6 to dioxygen at 248 K results in the formation of a green chromophore with three distinct band
at 325 nm, 520 nm and 665 nm (Figure 2.3.3.13). The spectral pattern obtained from the solution of 6 in acetonitrile at low temperature differs from that generated at room temperature. Interestingly, the optical spectral changes during the reaction of 6 with dioxygen at 248 K matches with the change observed for the reaction of 7 with dioxygen at room temperature. At room temperature, 6 reacts with dioxygen to yield a dark green solution which exhibits three bands at 366 nm, 600 nm and 934 nm.\cite{49} The final reaction solution of 6 obtained at 248K was subjected to X-band EPR which showed a rhombic signal at $g = 4.2$ (Figure 2.3.3.13). Reaction of 7 with dioxygen at 248K reveals an almost identical optical spectrum along with a similar X-band EPR signals. The green chromophore formed at 248K is stable for several hours at this temperature. Furthermore, the decay of the green species is not observed in the presence of organic acid like (Et$_3$NH)(ClO$_4$) or (PyH)(ClO$_4$) and external substrate like aromatic phenols, thioanisole or at reduced oxygen pressure. All these studies support that the species is neither an oxygen derived iron-oxygen adduct nor a high-valent iron-oxo species.

![Figure 2.3.3.13](image)

*Figure 2.3.3.13 Change in optical spectra during the reaction of 6 (0.5 mM in acetonitrile) with dioxygen at 248 K. Inset: X-band EPR spectrum of the oxidized solution at 77K.*
From the foregoing discussion, it is clear that two different iron (III) species are formed in the reaction pathway and are in equilibrium in solution. The position of the equilibrium and hence the percentage of iron(III) species depends on the nature of substituents and also on the presence of dioxygen. The presence of two bulky tert-butyl groups on the AP ring in 7 forces the equilibrium to shift more towards a rhombic iron(III) species by changing the coordination mode of the N₄ ligand from κ⁴ to κ³ via dissociation of one of the pyridyl donors from the metal center. A five-coordinate species is resulted for further reaction with dioxygen. This hypothesis supports the reactivity of 7Oₓ with dioxygen to form the same aromatic ring cleavage products. For other complexes both the iron(III) species are present and only the five-coordinate iron(III) species undergo reactions with oxygen. In case of 6, the equilibrium is shifted towards the five-coordinate iron(III) complex only at low temperature.

2.3.4 DFT Calculations

DFT calculations have been conducted on [(6-Me₃-TPA)Fe³⁺(4-tBu-HAP)]⁺ (6Oₓ) to unravel the probable reaction pathways of regioselective C-C bond cleavage of 2-aminophenols. As observed experimentally, an iron(II)-2-aminophenolate complex (A) reacts rapidly with dioxygen to form an iron(III)-2-amidophenolate (B) via an outer sphere one electron oxidation process. The plausible pathway for the formation of iron(III)-peroxo species from a six-coordinate iron(III)-2-amidophenolate (B) has been investigated (Figure 2.3.41). The free energy difference between the sextet and quartet spin state of B is predicted to be 11.07 kcal/mol, where the former is more stable with the Fe-Namide and Fe-Ophenolate distances of 2.156 Å and 1.991 Å, respectively (A-2-23 and A-2-24, Appendices). The low energy difference between the two spin states opens up the possibility that both of them can possibly activate oxygen. However, B being a six-coordinate iron centre, dioxygen activation at the metal centre is unfavorable. By analogy to the mechanism proposed for the oxidative decarboxylation of a six-coordinate iron(II)-α-hydroxy acid complex, [(6-Me₃-TPA)Fe²⁺(mandelate)](BPh₄) reported by Paine et al. it could be proposed that the dissociation of one of the pyridyl arms from the metal centre results in a five-coordinate iron(II)-2-iminobenzoquinoninate (C) species and provides a vacant site on the metal for dioxygen activation to form an iron(III)-peroxo species (D). The dissociation of the iron imide bond could also be another
possibility for the formation of iron(III)-peroxo species (D). Optimization of D was performed considering both the possibilities. However, calculations suggest that there exists a stable intermediate after dissociation of one pyridyl arm in both the sextet and the quartet surface, but no minima could be located for iron-imide bond dissociation. This result strongly indicates that the initial oxygen activation by B involves opening of a pyridyl arm from the metal centre. Formation of a five-coordinate species (C) is 4.9 kcal/mol higher in energy on the sextet surface, whereas it is 14.5 kcal/mol higher on the quartet surface. As reported earlier by Georgiev and coworkers the peroxy species is formed on a quartet surface.\[55\] Although, a stable iron(III)-peroxo species could not be obtained in the sextet state, a peroxy species (D) is formed with an free energy of 26.2 kcal/mol with respect to the ground state. In the optimized geometry of the peroxy species (D) the Fe-O, O-O and C-O distances are found to be 1.796 Å, 1.448 Å and 1.448 Å, respectively (A-2-23, appendices). The homolytic cleavage of O-O bond of the peroxy species (D) on the quartet surface was studied. The free energy activation barrier involved in this process is a staggering 41.9 kcal/mol. A large O-O dissociation energy on the quartet state indicates that the C-C bond cleavage does not take place via homolytic O-O bond dissociation route. It was suggested earlier that the loss of a proton occurs during the formation of iron(III)-2-amidophenolate (B) from the corresponding iron(II)-2-aminophenolate complex. This proton plays a crucial role in the O-O bond cleavage step. A protonated peroxy species may form in the reaction pathway as a reactive intermediate. The role of proton in the regioselective C-C bond cleavage of catechol has recently been implicated.\[56\] The solvent acetonitrile is not a good proton acceptor. However, like any other solvents it is likely to contain trace amounts of water. The highly polar water molecules are expected to cluster around charged ionic species in acetonitrile medium. Water molecules can accept protons and release those protons to facilitate reactions. To mimic such a condition a H₃O⁺ was included in the computational model, which takes into account the lost proton in the first oxidation step of conversion of A to B. It was found that a hydronium can act as a proton source and can protonate one of the peroxy oxygens favorably in E. Optimized structures of E in both the sextet and the quartet states show that the hydroperoxide group ligated to the iron(III) centre is positioned axially with respect to the amidophenolate ring (Figure 2.3.4.2). In such geometry, the O–O bond is aligned in antiperiplanar geometry with the C1–C6 bond, which is an essential requirement for obtaining
selective cleavage product.\cite{22} The heterolytic O-O bond dissociation free energy barrier of the protonated peroxo species (E) to form C1-C6 cleaved product (F) was calculated. In case of the sextet state the required free energy activation barrier for O-O bond cleavage is 20.3 kcal/mol (24.1 kcal/mol in quartet surface), which indicates that the reaction pathway is feasible under the aforementioned experimental conditions. Formation of the C1-C6 cleavage product, a seven member lactone ring, is highly exothermic (calculated 56.6 kcal/mol) in nature. Computational studies reveal that one of the pyridine rings cis to the aminophenolate ring gets dissociated from the coordination site for dioxygen activation and a proton lost in the first step of the reaction is essential for heterolytic O-O bond cleavage. The antiperiplanar arrangement of C1-C6 bond with the O-O bond of protonated-peroxide results in selective C-C bond cleavage product.

![Energy profile of the reaction of 6Ox with dioxygen.](image)

Figure 2.3.4.1 Energy profile of the reaction of 6Ox with dioxygen.

The presence of an electron withdrawing nitro group at the 4-position on the aminophenolate ring in 1 makes it unreactive towards dioxygen. Two-electron oxidation of 2-aminophenol to iminoquinone followed by coupling with
another molecule of 2-aminophenol is observed in the reaction of 2 resulting in phenoxazine product. However, incorporation of electron donating groups like methyl (3-Me-HAP; 3 / 4-Me-HAP; 4 / 5-Me-HAP; 5), tert-butyl (4-tBu-HAP; 6 and 4,6-di-tBu-HAP; 7) results in oxygenolytic aromatic ring cleavage reaction (Scheme 2.3.3.3). The substitutions on the aminophenolate ring not only control the formation of five-coordinate iron(III) species in solution, but also stabilize the O$_2$-activating iron(II)-2-iminobenzosemiquinone intermediate. The intermediate gets stabilized by two tert-butyl groups on the AP ring and as a result complex 7 exhibits slow reactivity compared to other iron(II)-aminophenolate complexes. A subtle balance between the electronic and steric properties of the substituents allows the complexes to exhibit C-C bond cleavage reactivity.

![Figure 2.3.4.2 Transition state geometry of the protonated peroxo species in sextet state](image)

Although, the iron(II)-2-iminobenzosemiquionone intermediate could not be isolated for spectroscopic characterization, a mechanism is proposed on the basis of the experimental and theoretical calculations (Scheme 2.3.4.1). The iron(II)-2-aminophenolate complex (A) initially reacts with oxygen to form an iron(III)-2-amidophenolate (B) species as depicted for the reported iron(II)-catecholate and iron(II)-aminophenol complexes.[50, 51, 57] The redox isomer of (B), an iron(II)-2-iminobenzosemiquinonato radical species (B$^\cdot$) then reacts further with oxygen to form iron(III)-peroxide (D). Theoretical studies with the extradiol catechol dioxygenases suggest that initially oxygen activation occurs at the metal centre followed by an attack of iron(III)-superoxide to the substrate to
form the peroxo intermediate.\textsuperscript{[58]} The protonation of iron(III)-peroxide (D) species preferably at the oxygen attached to the metal centre results in a protonated peroxo species E. Heterolytic O-O bond cleavage of the protonated peroxo species (E) through alkenyl migration leads to the formation of a lactone intermediate (F). Muconic acid semialdehyde is formed via hydrolysis of the lactone species by the metal-coordinated hydroxide. A subsequent rearrangement followed by loss of a water molecule forms picolinic acid. Loss of a CO molecule from the lactone yields pyranimine product observed for 7.

\textbf{Scheme 2.3.4.1 Proposed mechanism for the C–C bond cleavage of 2-aminophenols in biomimetic iron(II) complexes.}

2.4 Experimental Section

All reagents and solvents were purchased from commercial sources and were used without further purification. Solvents were distilled and dried before use. Preparation and handling of air-sensitive materials were carried out under an inert atmosphere in a glove box. Ligand 6-Me\textsubscript{3}-TPA was prepared according
to a literature procedure.\textsuperscript{[59]} Fourier transform infrared spectroscopy on KBr pellets was performed on a Shimadzu FT-IR 8400S instrument. Elemental analyses were performed on a Perkin Elmer 2400 series II CHN analyzer. Electro-spray ionization (ESI) mass spectra were recorded with a Waters QTOF Micro YA263 instrument. Solution electronic spectra (single and time-dependent) were measured on an Agilent 8453 diode array spectrophotometer. All room temperature NMR spectra were collected on a Bruker Avance 500 MHz spectrometer. Room temperature magnetic data were collected on Gouy balance (Sherwood Scientific, Cambridge, UK). Diamagnetic contributions were calculated for each compound using Pascal’s constants. GC-MS measurements were carried out with a Perkin Elmer Clarus 680 GC and SQ8T MS, using Elite 5 MS (30 m x 0.25 mm x 0.25 µm) column with maximum temperature 300ºC. X-band EPR measurements were performed on a JEOL JES-FA 200 instrument. Labeling experiments were carried out with $^{18}$O$_2$ gas (99 atom %) or H$_2$O$^{18}$ (98 atom %) from Icon Services Inc., USA.

2.4.1 General Method for the Syntheses of Iron(II)-2-Aminophenolate Complexes

Iron(II) perchlorate hydrate (0.36 g, 1 mmol) was added to a solution of the ligand (1 mmol) in methanol (5 mL). To the resulting mixture was added a solution of aminophenol (1 mmol) and 1 equiv Et$_3$N in methanol (5 mL). The solution was then allowed to stir at room temperature for 4 h. The solution was concentrated and diethyl ether was added. The resulting mixture was stirred further for 4-5 h to precipitate a solid. The solid was isolated by filtration, washed 2-3 times with diethyl ether and dried. The tetraphenylborate salts of the complexes were isolated from the reaction of perchlorate salt of the complexes with 1 equivalent of sodium tetraphenylborate in methanol.

2.4.2 [(6-Me$_3$-TPA)Fe$^{II}$(4-NO$_2$-HAP)](BPh$_4$) (1)

Red solid. Yield: 0.61 g (71%). Anal. calcd for C$_{51}$H$_{49}$BFeN$_6$O$_3$ (860.63 g/mol): C, 71.17; H, 5.74; N, 9.76. Found: C, 70.69; H, 5.61; N, 9.9%. IR (KBr, cm$^{-1}$): 3443, 3292, 3055(m), 1601(s), 1578(m), 1499(s), 1456(m), 1286(s), 1086(m), 787(s), 735(s), 708(s). UV-vis in CH$_3$CN: 378 nm ($\varepsilon = 21300$ M$^{-1}$cm$^{-1}$). Magnetic moment $\mu_{\text{eff}}$ (298K): 4.8 $\mu_B$. 

66
2.4.3 [(6-Me₃-TPA)FeⅡ(HAP)](BPh₄) (2)

Yellow solid. Yield: 0.31 g (53%). Anal. calcd for C₂₇H₃₀ClFeN₅O₅ (595.86 g/mol): C, 54.42; H, 5.07; N, 11.75. Found: C, 53.79; H, 4.84; N, 11.57 %. IR (KBr, cm⁻¹): 3452(br), 3331(m), 1605(s), 1578(m), 1487(s), 1456(s), 1294(s), 1117(m), 1094(s), 781(m). UV-vis in CH₃CN: 401 nm (ε = 950 M⁻¹cm⁻¹). Magnetic moment μₑff (298 K): 5.1 μB.

2.4.4 [(6-Me₃-TPA)FeⅡ(3-Me-HAP)](BPh₄) (3)

Yellow solid. Yield: 0.63 g (76%). Anal. calcd for C₅₂H₅₂BFeN₅O (829.66 g/mol): C, 75.28; H, 6.32; N, 8.44. Found: C, 75.3; H, 6.4; N, 8.2 %. IR (KBr, cm⁻¹): 3431(br), 3055(m), 2926(m), 1605(s), 1578(s), 1466(s), 1429(m), 1310(m), 785(m), 735(s), 706(s). UV-vis in CH₃CN: 401 nm (ε = 1900 M⁻¹cm⁻¹). Magnetic moment μₑff (298 K): 4.7 μB.

2.4.5 [(6-Me₃-TPA)FeⅡ(4-Me-HAP)](BPh₄) (4)

Yellow solid. Yield: 0.62 g (75%). Anal. calcd for C₅₂H₅₂BFeN₅O (829.66 g/mol): C, 75.28; H, 6.32; N, 8.44. Found: C, 74.7; H, 6.5; N, 8.3 %. IR (KBr, cm⁻¹): 3431(br), 3053(m), 2922(m), 1605(s), 1578(s), 1491(m), 1458(s), 1290(m), 1010(w), 787(m), 739(s), 706(s). UV-vis in CH₃CN: 404 nm (ε = 2100 M⁻¹cm⁻¹). Magnetic moment μₑff (298 K): 4.8 μB.

2.4.6 [(6-Me₃-TPA)FeⅡ(5-Me-HAP)](ClO₄) (5)

Yellow solid. Yield: 0.29 g (48%). Anal. calcd for C₂₈H₃₂ClFeN₅O₅ (609.88 g/mol): C, 55.14; H, 5.29; N, 11.48. Found: C, 55.17; H, 5.18; N, 11.44 %. IR (KBr, cm⁻¹): 3433(br), 3028(m), 2922(m), 1603(s), 1580(m), 1497(s), 1456(m), 1302(s), 1157(m), 1120(s), 1090(s), 783(m), 627(m). UV-vis in CH₃CN: 401 nm (ε = 2200 M⁻¹cm⁻¹). Magnetic moment μₑff (298 K): 5.1 μB.

2.4.7 [(6-Me₃-TPA)FeⅡ(4-tBu-HAP)](ClO₄) (6)

Yield: 0.34 g (52 %). Elemental analysis calcd (%) for C₃₁H₃₈ClFeN₅O₅ (651.96 g/mol): C 57.11, H 5.87, N 10.74; Found: C 57.3, H 6.0, N 10.3. IR (KBr, cm⁻¹): 3323(br), 3291(m), 2862(m), 1605(s), 1580(m), 1497(s), 1460(s), 1302(m), 1117(s), 1094(s), 1009(m), 791(m), 623(m). UV-vis in CH₃CN: 404 nm (ε = 2500 M⁻¹cm⁻¹). Magnetic moment μₑff (298 K): 5.1 μB. Single crystals of
the tetraphenylborate salt of 6 were grown from a solvent mixture of dichloromethane, methanol and diethyl ether at 273 K.

2.4.8 [(6-Me₃-TPA)Fe²⁺(4,6-di-tBu-HAP)](ClO₄) (7)

Yellow solid. Yield: 0.31 g (44 %). Anal. calcd for C₃₅H₄₆ClFeN₅O₅ (708.07 g/mol): C, 59.37; H, 6.55; N, 9.89. Found: C, 58.4; H, 6.5; N, 9.9 %. IR (KBr, cm⁻¹): 3425(m), 2950(m), 2864(m), 1605(s), 1578(m), 1460(s), 1443(m), 1302(m), 1277(m), 1115(s), 1094(s). Single crystals of the tetraphenylborate salt of 7 were grown from a solvent mixture of dichloromethane, methanol and diethyl ether at 273 K.

2.4.9 [(6-Me₃-TPA)Fe³⁺(4-tBu-HAP)](ClO₄) (6⁰x)

Controlled one-electron chemical oxidation of 6 was carried out using KMnO₄ as the oxidant. Solid KMnO₄ (15 mg, 0.1 mmol) was added to a solution of 6 (65 mg, 0.1 mmol) in 10 mL dichloromethane under nitrogen environment. The mixture was allowed to stir for 2h during which time the yellow solution turned deep green. The mixture was passed through a sintered glass funnel to remove the dark particles. The deep green filtrate was concentrated and diethyl ether was added to it. The solid residue was filtered off and washed with diethyl ether for 2-3 times and dried. Yield: 58 mg (89%). Elemental analysis calcd (%) for C₃₁H₃₇ClFeN₅O₅ (650.95 g/mol): C 57.20, H 5.73, N 10.76; Found: C 57.4, H 5.5, N 10.3. IR (KBr, cm⁻¹): 3427(br), 2926(m), 2858(m), 1605(s), 1495(s), 1485(s), 1304(m), 1117, 1090(s), 787(m), 623(m). ESI-MS in CH₃CN (in positive ion mode): m/z = 551.23 (20%, [(6-Me₃-TPA)Fe(4-tBu-AP)]⁺) UV-vis in CH₃CN: 370 nm (ε = 2750 M⁻¹cm⁻¹), 590 nm (ε = 1300 M⁻¹cm⁻¹) and 935 nm (ε = 2600 M⁻¹cm⁻¹). Complex 6⁰x can also be isolated by controlled aerial oxidation of the iron(II)-2-aminophenolate complex. Complex 6 (65 mg, 0.1 mmol) was dissolved in 10 mL dry and degassed acetonitrile under nitrogen environment and dry oxygen was allowed to pass through the solution for 1 min. The solution was stirred further for 1 min under oxygen environment. The yellow solution turned deep green during the time period. The solution was then concentrated in vacuum and the green mass was taken into glove box. The dry mass was washed thoroughly with diethyl ether to isolate a deep green solid of 6⁰x. Yield: 61 mg (94%).
2.4.10 \([(6-\text{Me}_3\text{-TPA})\text{Fe}^{\text{III}}\text{(4,6-di-\text{-}tBu-HAP)}](\text{BPh}_4)\) (7\text{Ox})

Tetraphenylborate salt of complex 7 (37 mg, 0.04 mmol) was dissolved in dichloromethane and 0.04 mmol of KMnO$_4$ (6.3 mg, 0.04 mmol) or 2,4,6-tri-\text{tert}-butylphenoxy radical (10.4 mg, 0.04 mmol) was added to it. The reaction mixture was stirred for 3 h and filtered to remove the solid particles. The filtrate was then concentrated and washed thoroughly with diethyl ether for 2-3 times. The dark green solid was then isolated by filtration and dried in vacuum. Yield: 0.23 g (64 %). Anal. calcd for C$_{59}$H$_{66}$BFeN$_5$O (926.84 g/mol): C, 76.46; H, 7.07; N, 7.56. Found: C, 75.6; H, 7.1; N, 7.6 %. Magnetic moment $\mu_{\text{eff}}$ (298K): 5.2 $\mu_B$.

2.4.11 Reaction of the Complexes with Dioxygen and Analysis of Organic Product

In a typical reaction, dry oxygen gas was bubbled through a solution of iron(II)-aminophenol complex (0.02 mmol) in 10 mL dry acetonitrile for 2 min. The solution was kept for stirring at room temperature under oxygen environment. The yellow solution immediately turned deep green and then slowly to orange. The solvent was evaporated to dryness and the residue was treated with 2 $M$ HCl solution (10 mL) to maintain the pH at 2-3. The organic products were then extracted with diethyl ether (3×15mL), the combined organic layer was washed with a saturated brine solution, and dried over anhydrous Na$_2$SO$_4$. After removal of the solvent the solid mass was analyzed by $^1$H NMR and GC-MS without further purification. $^1$H NMR (500 MHz, CDCl$_3$, 298 K): 3-Methyl-2-picolinic acid: $\delta$(ppm) 8.49 (d, 1H, $J$ = 4 Hz), 7.95 (d, 1H, $J$ = 4 Hz), 7.46 (t, 1H, $J$ = 5 Hz), 2.12 (s, 3H). 4-Methyl-2-picolinic acid: $\delta$(ppm) 8.60 (d, 1H, $J$ = 4 Hz), 7.98 (s, 1H), 7.53 (d, 1H, $J$ = 5 Hz), 1.66 (s, 3H). 5-Methyl-2-picolinic acid: $\delta$(ppm) 8.57 (s, 1H), 8.04 (d, 1H, $J$ = 6 Hz), 7.64 (d, 1H, $J$ = 5 Hz), 1.67 (s, 3H). 4-\text{-}t\text{er}t\text{-}butyl-2-picolinic acid: $\delta$ (ppm) 8.64 (d, 1H, $J$ = 5 Hz), 8.15 (s, 1H), 7.46 (d, 1H, $J$ = 5 Hz), 1.36 (s, 9H). 4,6-Di-\text{-}t\text{er}t\text{-}butyl-2-picolinic acid: $\delta$(ppm) 8.07 (s, 1H), 7.59 (s, 1H), 1.27 (s, 9H), 1.40 (s, 9H). 4,6-Di-\text{-}t\text{er}t\text{-}butylpyran-2-imine: $\delta$(ppm) 6.05 (s, 1H), 1.21 (s, 9H), 1.36 (s, 9H).

2.4.12 Preparation of Methyl-4-\text{-}t\text{er}t\text{-}butyl-2-picolinate and Methyl-4,6-di-\text{-}t\text{er}t\text{-}butyl-2-picolinate

The organic product, isolated from the reaction solution according to the procedure mentioned above, was reacted with excess diazomethane in dry
Chapter 2

diethyl ether (5 mL) at 0°C. The reaction solution was stirred for 5 min. After separating the insoluble part, the clear ether layer was analyzed by GC-MS. \(^1\)H NMR (500 MHz, CDCl\(_3\), 298 K): Methyl-4-tert-butyl-2-picolinate: \(\delta\) (ppm) 8.61 (d, 1H, \(J = 5\) Hz), 8.0 (s, 1H), 7.35 (d, 1H, \(J = 5\) Hz), 3.98 (s, 3H), 1.36 (s, 9H). Methyl-4,6-di-tert-butyl-2-picolinate: \(\delta\) (ppm) 7.92 (s, 1H), 7.49 (s, 1H), 3.97 (s, 3H), 1.26 (s, 9H), 1.40 (s, 9H).

Table 2.4.13.1. Crystallographic data for complexes 5, 6·CH\(_3\)OH and 7·CH\(_3\)OH

<table>
<thead>
<tr>
<th></th>
<th>5</th>
<th>6·CH(_3)OH</th>
<th>7·CH(_3)OH</th>
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<tr>
<td><strong>Empirical formula</strong></td>
<td>C(<em>{28})H(</em>{32})ClFeN(_5)O(_5)</td>
<td>C(<em>{56})H(</em>{70})BFeN(_5)O(_5)</td>
<td>C(<em>{56})H(</em>{70})BFeN(_5)O(_2)</td>
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<tr>
<td><strong>Formula wt</strong></td>
<td>609.89</td>
<td>890.66</td>
<td>959.87</td>
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<tr>
<td><strong>Crystal system</strong></td>
<td>Monoclinic</td>
<td>Triclinic</td>
<td>Monoclinic</td>
</tr>
<tr>
<td><strong>Space group</strong></td>
<td>(P2(1)/n)</td>
<td>(P-I)</td>
<td>(P2(1)/c)</td>
</tr>
<tr>
<td>(a) (Å)</td>
<td>17.034(7)</td>
<td>11.023(2)</td>
<td>22.532(3)</td>
</tr>
<tr>
<td>(b) (Å)</td>
<td>9.392(4)</td>
<td>11.421(2)</td>
<td>10.964(1)</td>
</tr>
<tr>
<td>(c) (Å)</td>
<td>19.457(11)</td>
<td>20.581(4)</td>
<td>22.470(3)</td>
</tr>
<tr>
<td>(\alpha) (deg.)</td>
<td>90</td>
<td>83.596(4)</td>
<td>90.00</td>
</tr>
<tr>
<td>(\beta) (deg.)</td>
<td>114.958(10)</td>
<td>82.442(4)</td>
<td>108.980(4)</td>
</tr>
<tr>
<td>(\gamma) (deg.)</td>
<td>90</td>
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<td>90.00</td>
</tr>
<tr>
<td><strong>Volume (Å(^3))</strong></td>
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<td>2392.4(8)</td>
<td>5249.0(12)</td>
</tr>
<tr>
<td><strong>Z</strong></td>
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<td>2</td>
<td>4</td>
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<tr>
<td><strong>(D_{calcd.}) (mg/m(^3))</strong></td>
<td>1.435</td>
<td>1.236</td>
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<tr>
<td>(\mu) Mo-K(_\alpha) (mm(^{-1}))</td>
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<td>(F(000))</td>
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<td>934</td>
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<tr>
<td><strong>(\theta) range (deg.)</strong></td>
<td>1.34–22.03</td>
<td>1.00 – 24.45</td>
<td>0.96–26.00</td>
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<tr>
<td><strong>Reflections</strong></td>
<td>18825</td>
<td>21456</td>
<td>63203</td>
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<td><strong>Reflections unique</strong></td>
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<td>7845</td>
<td>10244</td>
</tr>
<tr>
<td><strong>(R)(\text{int}))</strong></td>
<td>0.1519</td>
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<td>0.0486</td>
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<tr>
<td><strong>Data ((I&gt;2\sigma(I)))</strong></td>
<td>2260</td>
<td>5812</td>
<td>8781</td>
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<tr>
<td><strong>Parameters refined</strong></td>
<td>365</td>
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<td><strong>Goodness-of-fit on</strong></td>
<td>1.079</td>
<td>1.279</td>
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<td><strong>(R1) ([I&gt;2\sigma(I)])</strong></td>
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<tr>
<td><strong>w(R2)</strong></td>
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<td>0.1948</td>
<td>0.1368</td>
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</tbody>
</table>
2.4.13 X-ray Crystal Structure Determination

Diffraction data for 5, 6·CH$_3$OH and 7·CH$_3$OH were collected on a Bruker Smart APEX II (Mo-Ka radiation, $\lambda = 0.71073$ Å). Cell refinement, indexing and scaling of the data set were carried out using the APEX2 v2.1-0 software.$^{[60]}$ The structures were solved by direct methods and subsequent Fourier analyses and refined by the full-matrix least-squares method based on $F^2$ with all observed reflections.$^{[61]}$ In all the complexes the hydrogen atoms were fixed. The non-hydrogen atoms were treated anisotropically.

2.4.14 DFT Calculations

All intermediates and transition state geometries were optimized in vacuum using the hybrid density functional, B3LYP$^{[62-66]}$ along with 6-31G(d) basis functions for N, C, H, O and effective core potential LANL2 along with LANL2DZ basis set on Fe atom.$^{[67-68]}$ All computations were conducted using the Gaussian 09 quantum chemistry suite.$^{[69]}$ Gas phase enthalpy and free energy values were computed for 1 atmospheric pressure and 298K temperature. Using the CPCM solvent model$^{[70-72]}$ for acetonitrile relative free energy activation barriers were computed with 6-31+G(d, p) basis sets on N, C, H, O atoms and effective core potential LANL2 along with LANL2DZ on Fe atom are reported in the ensuing discussion.$^{[68, 73]}$ For solvent phase free energy changes entropic corrections, i.e. empirically scaled gas phase entropies were incorporated. For solution phase entities, the entropies used for estimating solution phase free energies were derived by scaling the corresponding gas phase entropies computed using the ideal gas model by a factor of 0.5.$^{[74-75]}$ This is a standard approximation which has been used in other quantum chemical studies.$^{[76-78]}$

2.5 References

Chapter 2

Functional Models of 2-Aminophenol Dioxygenases

[37] E. G. Kovaleva, J. D. Lipscomb, Science 2007, 316, 453
Chapter 2


