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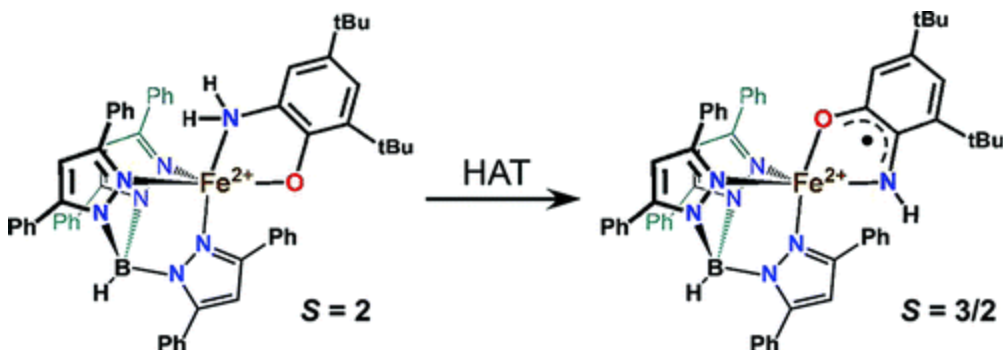
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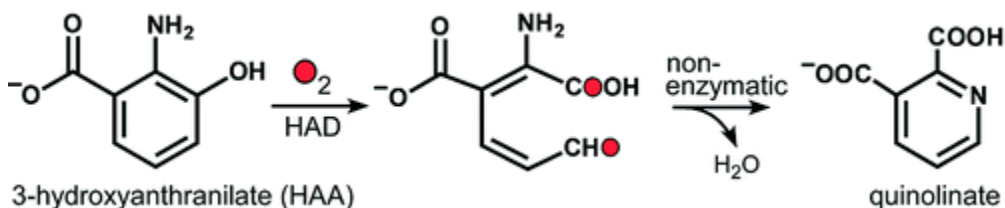
Abstract



The oxidative ring cleavage of aromatic substrates by nonheme Fe dioxygenases is thought to involve formation of a ferrous–(substrate radical) intermediate. Here we describe the synthesis of the trigonal-bipyramidal complex $\text{Fe}^{\text{(Ph}_2\text{Tp)}}(\text{ISQ}^{\text{tBu}})$ (**2**), the first synthetic example of an iron(II) center bound to an iminobenzosemiquinonate (ISQ) radical. The unique electronic structure of this $S = 3/2$ complex and its one-electron oxidized derivative (**[3]**⁺) have been established on the basis of crystallographic, spectroscopic, and computational analyses. These findings further demonstrate the viability of Fe^{2+} –ISQ intermediates in the catalytic cycles of *o*-aminophenol dioxygenases.

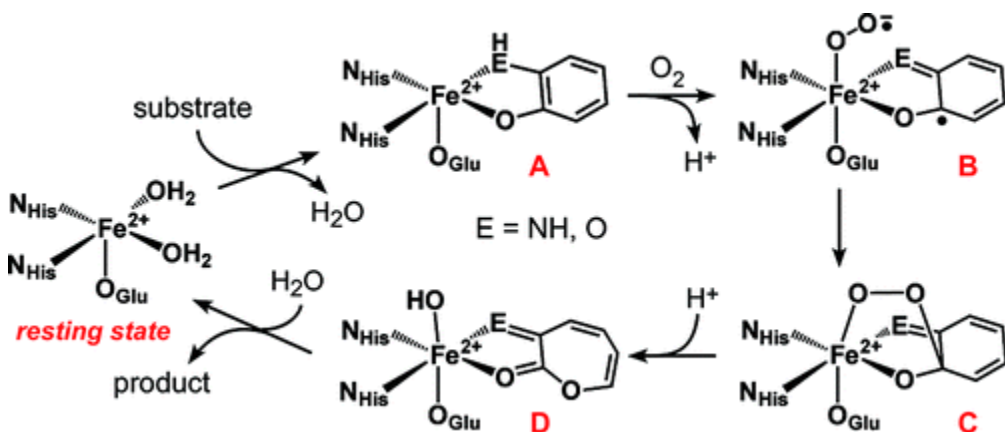
In biochemical pathways, the oxidative ring cleavage of substituted aromatic compounds, such as catechols and *o*-aminophenols, is generally performed by mononuclear nonheme iron dioxygenases.¹ While these enzymes are usually found in bacteria, some play important roles in human metabolism: for instance, a key step in tryptophan degradation involves the O_2 -mediated ring cleavage of 3-hydroxyanthranilate (HAA) by HAA-3,4-dioxygenase (HAD; Scheme 1).² With the exception of the intradiol catechol dioxygenases, the ring-cleaving dioxygenases share a common O_2 -activation mechanism, illustrated in Scheme 2.¹ A notable feature of this proposed mechanism is the superoxo- Fe^{2+} -(iminobenzo)semiquinonate intermediate (**B**) that is thought to form after O_2 binding to the enzyme–substrate complex (**A**). The development of radical character on the substrate ligand presumably facilitates reaction with the bound superoxide, yielding the key Fe^{2+} -alkylperoxo intermediate (**C**).³ Although the electronic structure of **B** remains somewhat controversial,⁴ evidence in favor of substrate radical character has been provided by radical-trap experiments⁵ and DFT calculations,³ as well as a remarkable X-ray structure of the Fe/O_2 adduct of an

extradiol dioxygenase in which the radical character of the bound substrate was inferred from its nonplanar geometry.⁶



Scheme 1. Reaction Catalyzed by HAA Dioxygenase (HAD)

Despite these biological precedents, synthetic analogues of intermediate **B** in which a ferrous center is coordinated to an (iminobenzo)semiquinone radical, (I)SQ, have been lacking in the literature, even though numerous ferric complexes with such ligands exist.⁷⁻¹¹ Herein, we report the synthesis and detailed characterization of an Fe^{2+} -ISQ complex, **2**, that represents the first synthetic model of this important type of enzyme intermediate. We also examine the geometric and electronic structures of the species [**3**]⁺ generated via one-electron oxidation of **2**.



Scheme 2. Catalytic Cycle of Ring-Cleaving Dioxygenases

In our efforts to generate synthetic models of HAD, we have used the tris(pyrazolyl)borate ligand, Ph^2Tp ,¹² to mimic the facial His_2Glu coordination environment of the enzyme active site. The reaction of $[(\text{Ph}^2\text{Tp})\text{Fe}(\text{OBz})]$ ¹³ with 2-amino-4,6-di-*tert*-butylphenol ($^{\text{tBu}}\text{APH}_2$) in the presence of base provided the light yellow complex $[(\text{Ph}^2\text{Tp})\text{Fe}^{2+}(\text{tBuAPH})]$ (**1**) in 71% yield. The X-ray crystal structure of **1** reveals a five-coordinate (5C) Fe^{2+} center in which the $^{\text{tBu}}\text{APH}^-$ ligand binds in a bidentate fashion (Figure 1; crystallographic details are

summarized in Table S1 in the Supporting Information). The average Fe1–N_{TP} bond length of 2.15 Å is typical of high-spin Fe²⁺ complexes with Tp ligands,^{13,14} while the short Fe1–O1 distance of 1.931(1) Å is consistent with coordination by an aminophenolate anion (Table 1). The complex adopts a distorted trigonal-bipyramidal geometry ($\tau = 0.61$ ¹⁵) with the amino group of ^tBuAPH[−] in an axial position *trans* to N5. To the best of our knowledge, **1** represents the first synthetic model of an aminophenol dioxygenase.

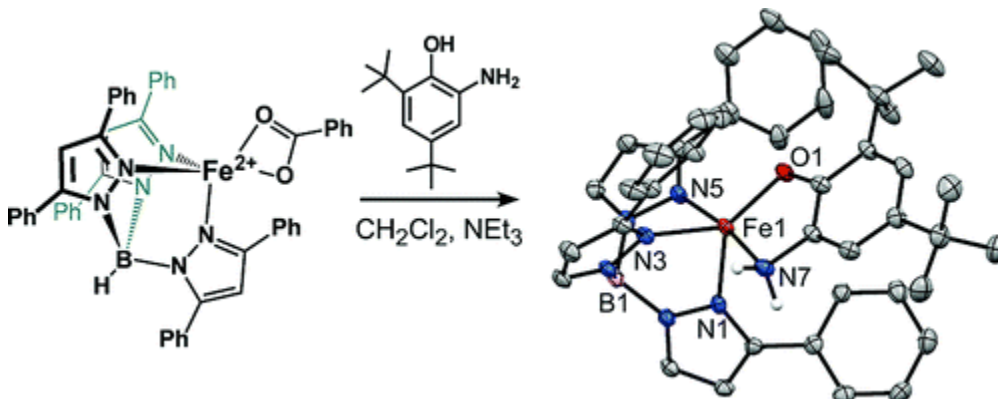


Figure 1. Synthesis and thermal ellipsoid diagram of complex **1**. For the sake of simplicity, the 5-Ph substituents of the ^{Ph}2Tp ligand have been omitted and only the amino hydrogens are shown. Selected bond lengths are provided in Table 1.

Reaction of **1** with 1 equiv of 2,4,6-tri-*tert*-butylphenoxy radical (TTBP[•]) at RT in CH₂Cl₂ gives rise to a distinct chromophore, **2**, with a broad absorption manifold centered at 715 nm ($\epsilon_{\text{max}} = 0.76 \text{ mM}^{-1} \text{ cm}^{-1}$; see Figure 2). Addition of MeCN, followed by cooling to $-30 \text{ }^\circ\text{C}$, provides pale green crystals of **2** suitable for crystallographic analysis. As with **1**, the X-ray structure of **2** features a neutral 5C Fe complex with a distorted trigonal-bipyramidal geometry ($\tau = 0.58$), although O1 now occupies an axial position instead of N7 (Figure S1). The N7 atom in **2** is monoprotonated, confirming that **2** is generated via abstraction of a H-atom from the $-\text{NH}_2$ group of **1**.

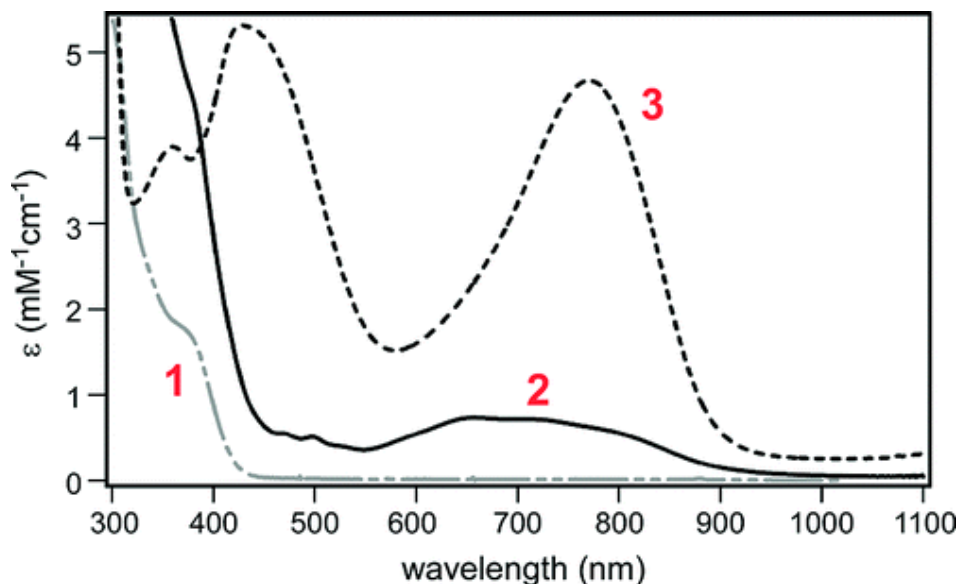


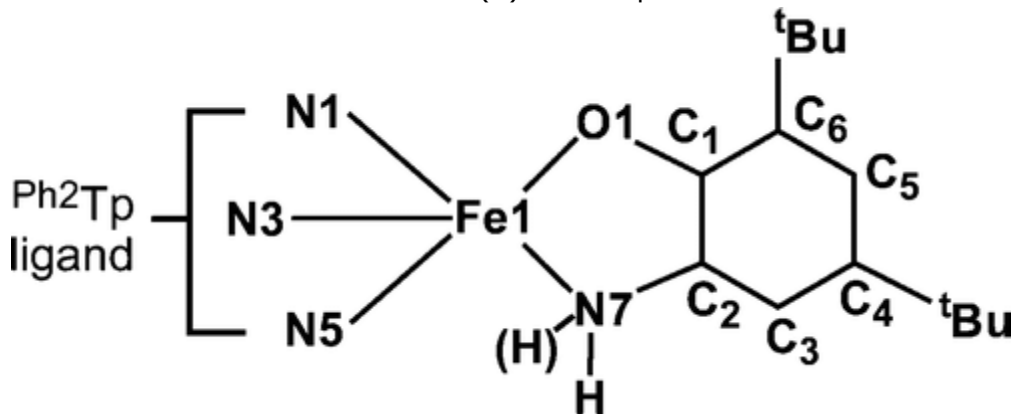
Figure 2. Electronic absorption spectra of **1** (---), **2** (—), and **[3]SbF₆** (- · -) measured in CH₂Cl₂ at RT.

Interestingly, the average Fe1–N_{TP} bond distance observed for **2** (2.136 Å) is nearly identical to the value found for **1** (2.150 Å), suggesting minimal change in Fe charge. Metric parameters for the *O,N*-coordinated ligand, however, are dramatically different in the two structures. In the structure of **1**, the six C–C bonds of the ^tBuAPH[−] ring are approximately equidistant (1.40 ± 0.02 Å), reflecting its closed-shell, aromatic nature. In contrast, the corresponding C–C bond distances in **2** exhibit the “four long/two short” distortion commonly observed for quinoid moieties (Table 1).^{7–11} The short O1–C1 and N7–C2 distances of 1.285(3) and 1.328(4) Å, respectively, are also characteristic of ISQ[−] ligands, as amply demonstrated by Wieghardt^{8–10} and others.⁷ Thus, the X-ray crystallographic data strongly support the formulation of **2** as [(Ph²Tp)Fe²⁺(^tBuISQ)]. This assignment rationalizes the absorption spectrum of **2**, which closely resembles those reported for Co³⁺ and Ni²⁺ complexes with a lone ISQ[−] ligand.^{9a}

The X-band EPR spectrum of **2** displays an intense peak at $g = 6.5$, along with a broad derivative-shaped feature centered near $g = 1.8$ (Figure 3). Such spectra are typical of $S = 3/2$ systems with large and rhombic zero-field splitting parameters.^{9,16} The simulated spectrum in Figure 3 assumed a negative D -value (with $|D| \gg h\nu$), an E/D -ratio of 0.24, and g -values of 2.36, 2.30, and 2.17. Significant E/D strain was incorporated to adequately account for the broadness

of the higher-field features. The combined experimental results therefore indicate that **2** contains a high-spin Fe²⁺ center ($S = 2$) antiferromagnetically coupled to a ^tBuISQ radical anion.

Table 1. Selected Bond Distances (Å) for Complexes **1–3**



	1	2	[3] ^{SbF₆} ^a
Fe1–N1	2.101(1)	2.108(2)	2.071(7)
Fe1–N3	2.127(1)	2.087(2)	2.038(7)
Fe1–N5	2.223(1)	2.216(2)	2.134(6)
Fe1–O1	1.931(1)	2.095(2)	2.082(6)
Fe1–N7	2.214(1)	1.982(2)	2.017(8)
O1–C1	1.345(2)	1.285(3)	1.26(1)
N7–C2	1.451(2)	1.328(4)	1.33(1)
C1–C2	1.398(2)	1.469(5)	1.47(1)
C2–C3	1.388(2)	1.413(4)	1.42(1)
C3–C4	1.388(2)	1.363(4)	1.35(2)
C4–C5	1.403(2)	1.427(4)	1.43(2)
C5–C6	1.394(2)	1.375(4)	1.37(2)
C1–C6	1.420(2)	1.440(4)	1.44(1)

^aThe bond distances listed here represent the average distance in the two independent units of [**3**]⁺, while the uncertainty is taken to be the larger of the two σ -values.

Further evidence in favor of a ligand-based radical was obtained from density functional theory (DFT) calculations. Two geometry-optimized models of **2** with $S = 3/2$ were computed that differ with respect to their electronic configurations. Analysis of the geometric and electronic structure of the first model (**2_A**) indicates that it contains an intermediate-spin Fe³⁺ center coordinated to a closed-shell imidophenolate ligand, ^tBuAP²⁻. The optimized structure of **2_A** features a square-pyramidal geometry ($\tau = 0.18$) with very short Fe–O1 and Fe–

N7 distances of $\sim 1.87 \text{ \AA}$, in poor agreement with the experimental structure (Table S2). Furthermore, the computed bond distances for the $t^{\text{Bu}}\text{AP}^{2-}$ ligand deviate sharply from the distances found experimentally for **2**, with nearly all such differences being significantly greater than the estimated error (3σ) in the crystallographic data. The second model (**2_B**) was generated via a broken-symmetry calculation in order to obtain the $[(^{\text{Ph}}_2\text{Tp})\text{Fe}^{2+}(t^{\text{Bu}}\text{ISQ})]$ electronic configuration described above. The resulting structure accurately reproduces the overall trigonal-bipyramidal geometry of **2** and provides reasonably consistent Fe–ligand distances. Most importantly, the computed and experimental $t^{\text{Bu}}\text{ISQ}^-$ bond distances exhibit remarkable agreement, with an rms deviation of merely 0.007 \AA (Table S2). Model **2_B** is also 9 kcal/mol more stable than **2_A**, indicating an energetic preference for the $\text{Fe}^{2+}-t^{\text{Bu}}\text{ISQ}$ form.

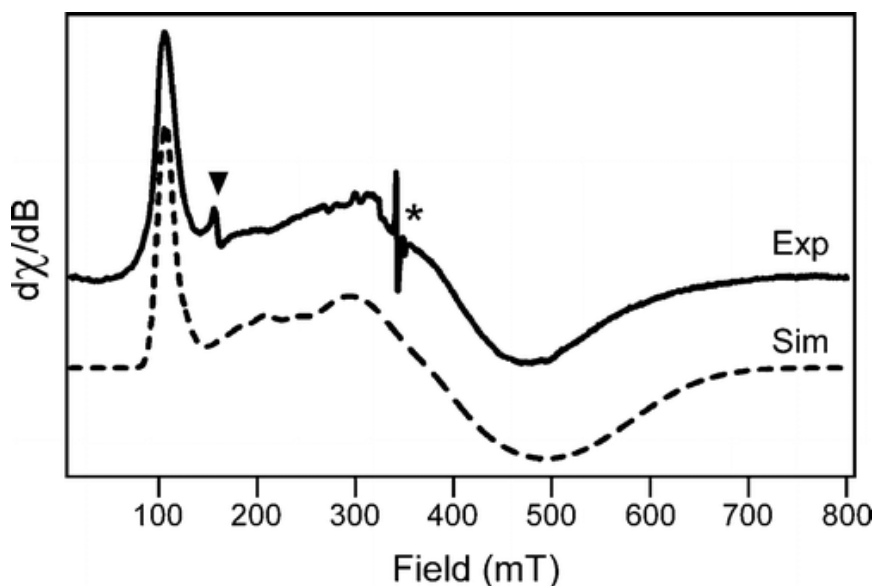


Figure 3. X-band EPR spectrum of **2** at 20 K. The derivative-shaped feature at $g = 4.3$ (▼) arises from a minor ferric impurity, while the feature at $g = 2.0$ (*) is due to a residual TTBP radical. Parameters used to generate the simulated spectrum are provided in the text.

To the best of our knowledge, the electronic structure of **2** has no precedent among synthetic complexes. While $\text{Fe}^{2+}\text{-SQ}$ intermediates are often invoked in the mechanisms of catechol dioxygenases, all relevant models to date feature unambiguous $[\text{Fe}^{3+}\text{-catecholate}]^+$ units.^{17,18} Similarly, the $\text{Fe}^{3+}\text{-ISQ}$ complexes generated by Wieghardt and co-workers exclusively undergo ligand-based reductions to give the corresponding $\text{Fe}^{3+}\text{-AP}$ species.^{8,9} The unique

Fe²⁺-ISQ configuration of **2** is likely due to the presence of a high-spin, 5C Fe ion, whereas related complexes prepared by Wieghardt (such as [(L)Fe³⁺(^RISQ)]⁺, where L = *cis*-cyclam and R = H or ^tBu) generally feature low-spin, 6C Fe centers.⁸ Thus, changes in spin state and coordination geometry are capable of shifting the delicate balance between the Fe²⁺-ISQ and Fe³⁺-AP valence tautomers.

Reaction of **2** with 1 equiv of an acetylferrocenium salt in CH₂Cl₂ provides a dark green species, [**3**]⁺, with intense absorption features at 770 and 430 nm (Figure 2). Treatment of [**3**]⁺ with 1 equiv of reductant (such as Fe(Cp*)₂) fully regenerates **2** (Figure S2), indicating that the two species are related by a reversible one-electron process. EPR experiments with frozen solutions of [**3**]⁺ failed to detect a signal in either perpendicular or parallel mode, indicative of an integer-spin system. Indeed, the magnetic moment of [**3**]⁺ was found to be 5.0(1) μ_B at RT, close to the spin-only value for an S = 2 paramagnet.

X-ray quality crystals of [**3**]SbF₆ were prepared by vapor diffusion of pentane into a concentrated dichloroethane solution. The resulting structure (Figure S3) contains two symmetrically independent Fe units, each featuring a distorted square-pyramidal geometry (τ = 0.42 and 0.38). Despite the difference in charge, complexes [**3**]⁺ and **2** have identical atomic compositions. Yet the average Fe-N_{TP} bond distance shortens from 2.132 to 2.081 Å upon conversion of **2** to [**3**]⁺, suggesting an increase in Fe-based charge. While the structural parameters of the bidentate *O,N*-donor ligand of [**3**]⁺ are consistent with a ^tBuISQ⁻ radical, it was not possible to rule out a neutral iminobenzoquinonate ligand (^tBuIBQ) due to sizable uncertainties in the bond distances.

We therefore turned to DFT calculations to further explore the electronic structure of [**3**]⁺. The resulting geometry-optimized model, [**3**_{DFT}]⁺, exhibits good agreement with the crystallographic data, although the DFT structure is more distorted toward the trigonal-bipyramidal limit (τ = 0.64; Table S3). The computed Fe-ligand bond distances nicely match the experimental values (rms deviation = 0.022 Å), indicating that the calculation converges to the correct S = 2 electronic configuration. Comparison of [**3**_{DFT}]⁺ and **2**_B reveals more pronounced "quinoid" character in the *O,N*-donor ligand of the former.

Using the experimentally derived correlations of bond distances and ligand oxidation states recently published by Brown, the *O,N*-donor ligand of [**3**_{DFT}]⁺ has an oxidation state of $-0.35(5)$ (i.e., partway between ISQ¹⁻ and IBQ⁰).¹⁹ Moreover, the highest-occupied spin-down MO (β -HOMO) of [**3**]⁺ contains roughly equal Fe and ligand character (47 and 42%, respectively), and the β -LUMO is evenly delocalized over the two units (Figure S4). Thus, the DFT results suggest that the electronic structure of [**3**]⁺ lies between the Fe³⁺-^tBuISQ and Fe²⁺-^tBuIBQ limits. Detailed spectroscopic studies are currently underway to better understand the electronic structure of [**3**]⁺.

Complexes **1–3** replicate key structural and electronic aspects of the proposed *o*-aminophenol dioxygenase mechanism. In particular, the conversion of **1**→**2** mimics the transformation of the enzyme-substrate complex (**A**) into a ferrous-ISQ species (**B**) via coupled proton and electron transfers. Our results therefore provide a synthetic precedent for the existence of Fe²⁺-ISQ intermediates in enzymatic catalysis. Of course, complex **2** is an imperfect model of intermediate **B**, since it lacks the coordinated superoxo ligand. Attempts are currently in progress to characterize species formed during the reaction of **1** and **2** with O₂ (and its surrogate, NO). These studies will yield further insights into the role of noninnocent ligands in ring-cleaving dioxygenase mechanisms.

Supporting Information

Experimental details, computational methods and models, crystallographic structures and data (CIFs), and absorption spectra of the interconversion of **2** and [**3**]⁺. This material is available free of charge via the Internet at <http://pubs.acs.org>.

The authors declare no competing financial interest.

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- ¹²Abbreviations: ^{Ph}2Tp = hydrotris(3,5-diphenylpyrazol-1-yl)borate(1–). ^RAP = *o*-imidophenolate(2–) anion with R-groups at the 4- and 6-positions. ^RISQ = *o*-iminobenzosemiquinone(1–) with R-groups at the 4- and 6-positions.
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