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Functional models of nonheme diiron enzymes: kinetic and computational evidence for the formation of oxoiron(iv) species from peroxodiiron(iii) complexes, and their reactivity towards phenols and H₂O₂†

The reactivity of the previously reported peroxo adducts $[Fe_2(\mu-O_2)(L^1)_4(CH_3CN)_2]^{2+}$, and $[Fe_2(\mu-O_2)(L^2)_4(CH_3CN)_2]^{2+}$, $(L^1 = 2-(2'-pyridyl)benzimidazole$ and $L^2 = 2-(2'-pyridyl)-N$ -methylbenzimidazole)

towards H₂O₂ as catalase mimics, and towards various phenols as functional RNR-R2 mimics, is described.

Kinetic, mechanistic and computational studies gave direct evidence for the involvement of the $(\mu-1,2-peroxo)$ diiron(μ) intermediate in the O–H activation process *via* formation of low-spin oxoiron(ν) species.

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Introduction

Nonheme diiron enzymes ribonucleotide reductases (RNR-R2), steraoyl-ACP Δ^9 -desaturase (Δ^9 D), soluble methane monooxygenase (sMMO), membrane bound alkane monooxygenases, and deoxyhypusine hydroxylase (hDOHH) catalyse a large variety of dioxygen-dependent transformations such as oxidation, hydroxylation and oxygenation.¹ RNR-R2 is responsible for the formation of an essential tyrosyl radical from tyrosine,² Δ^9 D for the dehydrogenation of fatty acid alkyl chains,³ sMMO for the hydroxylation of methane and alkanes,⁴ and hDOHH for the hydroxylation of deoxyhypusine to form hypusine.⁵ For such enzymes, catalytic (µ-1,2-peroxo)diiron(III) (P) intermediates have been postulated, which subsequently undergo O-O bond scission leading to the formation of mixed valent iron(m)iron(w)(X), or diiron(iv) (Q) intermediates to react via electrophilic hydrogen atom transfer (HAT) reaction. The formation of $(\mu$ -1,2-peroxo) diiron(III) species in these enzymes can be proved by their characteristic ligand to metal charge transfer absorptions between 600 and 750 nm, and the stretching mode of the (µ-1,2peroxo)diiron(m) core in their resonance Raman spectra (Table 1). Furthermore, the generated and trapped hDOHH_{peroxo}

species was fully characterized based on XAS (r(Fe-Fe) = 3.44/ 3.48 Å) and single-crystal X-ray diffraction data.⁵ Similar spectroscopic and kinetic evidence was found for a biomimetic dinuclear diiron peroxides,6 including the three crystallographically characterized derivatives however, the reactivity of these species has not been thoroughly explored.⁷ We have previously reported the synthesis and structure of iron(II) precursor complexes $[Fe^{II}(L^1)_3](CF_3SO_3)_2$ (1) and $[Fe^{II}(L^2)_3](CF_3SO_3)_2$ (2) based on the bidentate ligands L_1 ($L_1 = 2$ -(2'-pyridyl)benzimidazole) and L_2 $(L_2 = 2-(2'-pyridyl)-N-methylbenzimidazole)$ (Scheme 1), and the UV/Vis (ν_{max} = 720 nm (ε = 1360 M⁻¹ cm⁻¹) and 685 nm (ε = 1400 M^{-1} cm⁻¹), respectively), EPR, and resonance Raman ν (O-O) = 876 cm⁻¹; (ν (Fe–O) = 463 cm⁻¹) spectroscopic characterization of a transient green species with a $Fe^{III}(\mu-1,2-O_2)Fe^{III}$ (3,4) core derived from the reaction of 1 and 2 with H₂O₂.⁸ Based on the similarities on the structural and spectroscopic behaviours of the enzymatic and synthetic (µ-1,2-peroxo)diiron(III) species, compound 3 and 4 are good structural models for the RNR-R2 enzymes. Herein, we present the reactivity of 3 and 4 towards H₂O₂ as catalase-mimics, and towards various phenols as functional RNR-R2 mimics, while also seeking direct evidence for the involvement of the (µ-1,2-peroxo)diiron(III) intermediate in the O-H activation process.

Experimental

Materials and methods

All manipulations were performed under a pure argon atmosphere using standard Schlenk-type techniques unless other-

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 Table 1
 Properties of (µ-1,2-peroxo)diiron(ııı) units in enzymes and their models

Complex	UV-vis, $\lambda_{max}/nm (\varepsilon, M^{-1} cm^{-1})$	Raman ν (O–O)/cm ⁻¹	Ref.	
hDOHH	630 (2800)	855	5 <i>b</i>	
sMMO	725 (1800)	_	4b,c	
Δ9D	700 (1100)	898	3 <i>a</i> , <i>b</i>	
RNR-R2	700 (1800)	868	2d	
$[Fe_2(\mu-O)(\mu-O_2)(6-Me_3-TPA)]^{2+}$	490 (1100), 640 (1100)	847	6k	
$[Fe_2(\mu-O)(\mu-O_2)(BQPA)]^{2+}$	480 (1000), 620 (1000)	844	6 <i>i</i>	
$[Fe_2(\mu-O)(\mu-O_2)(6-Me-BQPA)]^{2+}$	480 (1000), 620 (1000)	853	6 <i>i</i>	
$[Fe_2(\mu-O)(\mu-O_2)(BnBQA)(CH_3CN)_2]^{2+}$	505 (1300), 650 (1300)	854	6 <i>i</i> ,h	
$[Fe_2(\mu-OH)(\mu-O_2)(BnBQA)(CH_3CN)_2]^{3+}$	730 (2400)	925	6 <i>i</i>	
$[Fe_2(\mu-O)(\mu-O_2)(IndH)]^{2+}$	690 (1500)	874	7 <i>d</i>	
$[Fe_2(\mu-O_2)(L^1)_4(CH_3CN)_2]^{2+}$	720 (1360)	876	8	
$[Fe_2(\mu-O_2)(L^2)_4(CH_3CN)_2]^{2+}$	685 (1400)	876	8	
$[Fe_2(HB(3,5-iPr_2pz)_3)_2)(\mu-O_2)(\mu-O_2CCH_2C_6H_5)_2]$	694 (1725)	885	6f	
$[Fe_2(N-Et-HPTB)(\mu-O_2)(OPPh_3)_2]$	588 (1500)	900	6b,d	
$[Fe_2(6-Me_2-BPP)_2(\mu-O_2)(\mu-OH)]^+$	644 (3000)	908	6 <i>l</i>	
$[Fe_2(6-Me_2-BPP)_2(\mu-O_2)(\mu-O)]$	577 (1500)	847	6 <i>l</i>	
$[Fe_2(Ph-bimp)(\mu-O_2)(\mu-O_2CC_6H_5)]^{2+}$	700 (br) (1700)	884	6 <i>e</i>	

 $6-Me_3$ -TPA = tris(6-methyl-2-pyridylmethyl)amine; BQPA = bis(2-quinolylmethyl)(2-pyridylmethyl)amine; 6-Me-BQPA = bis(2-quinolylmethyl)(6-methylpyridyl-2-methyl)amine; BnBQA = *N*-benzyl-*N*,*N*-bis(2-quinolylmethyl)amine; IndH = l,3-bis(2'-pyridilimino)isoindoline); pz = pyrazole; *N*-Et-HPTB = anion of *N*,*N*,*N'*,*N'*-tetrakis(1'-ethylbenzimidazolyl-2'-methyl)-2-hydroxy-1,3-diaminopropane); $6-Me_2$ -BPP = *N*,*N*-bis(6-methyl-2-pyridylmethyl)-3-aminopropionate); Ph-bimp = 2,6-bis[bis{2-(1-methyl-4,5-diphenylimidazolyl)-methyl}aminomethyl]-4-methylphenolate).



wise stated. Solvents used for the reactions were purified by standard methods and stored under argon. Iron(II) triflate was purchased from commercial sources. The ligands L_1 and L_2 , and complexes **1** and **2** were prepared according to published procedures.⁸ Infrared spectra were recorded with an Avatar 330 FTIR Thermo Nicolet instrument. UV/Vis spectra were recorded with an Agilent 8453 diode-array spectrophotometer with quartz cells. Microanalyses were performed by the Microanalytical Service of the University of Pannonia and Atlantic Microlab.

Catalase-like activities

All reactions were carried out in a 50 mL reactor containing a stirring bar under air. Acetonitrile (30 mL) was added to the complex and the flask was closed with a rubber septum. Hydrogen peroxide was injected through the septum with a Hamilton syringe. The reactor was connected to a graduated burette filled with mercury and dioxygen evolution was measured volumetrically at time intervals of 30 or 60 s. The reaction rates were calculated using the initial rate method.

Oxidation reactions

All reactions were carried out under thermostated conditions at different temperatures indicated in text, in 1 cm quartz cuvettes with stirring under argon. The $(\mu$ -1,2-peroxo)diiron(m)

intermediates 3 and 4 were generated in situ with stoichiometric amounts of hydrogen peroxide before the addition of the corresponding substrate. The oxidation reactions were followed with UV-Vis spectroscopy between 400 and 1000 nm. The reaction rates were calculated using the initial rate method $(V_{\text{ox}} = V_{\text{i}} - V_0 = k_{\text{ox}}[S][3 \text{ or } 4]^{0.5})$. The oxidation reaction rate (V_i) were extracted from plots of concentration [(μ -1,2peroxo)diiron(III)] intermediate versus time, and were corrected with the decomposition reaction rate (V_0) . The $k_{\rm rel}$ values for the Hammett plot were calculated as $k_{\rm X}/k_{\rm H}$ for different paraand meta-substituted substrates. For the determination of the KIE (SIE), separate reactions were carried out for 2,6-di-tertbutylphenol and 2-chloro-1,4-hydroquinone in the presence of 100 equivalents of D₂O or H₂O (based on 3 and 4 concentration), and the kinetic isotope effect was calculated from $k_{\rm H}/k_{\rm D}$. For product analysis, the samples were filtered through alumina to remove the complex. The products were identified by GC-MS and confirmed by comparison with authentic samples. The products were quantified by GC relative to biphenyl as an internal standard.

Computational details

All calculations were started from the crystal structure of $Fe(\pi)$ (L²)₃ (CCDC 955599),⁸ where one ligand was substituted with acetonitrile and oxygenic ligands so as to obtain [Fe(π) (L₂)₂(MeCN)O]²⁺ (given as Fe(π)O from now) and [(L₂)₂(MeCN) Fe(π)–O–O–Fe(π)(L₂)₂(MeCN)]⁴⁺ (given as Fe₂O₂ from now). The barriers for oxidations and O–O bond breaking were obtained by determining the transition states (TS) for the processes. All calculations were carried out with the Gaussian 09 suite.⁹ To determine the different broken symmetry solutions for the dimeric peroxo complexes, the fragment editor from Gaussview 5 was employed: fragments were defined to force the desired spin solution, then a guess for the resulting wave-

function was made followed by the determination of the accurate wavefunction and subsequent geometry optimisation. A total of 12 different broken symmetry solutions were computed and they are denoted with the following notation: S =n(X, Y), where n is the spin multiplicity, X the number of unpaired electrons on the first iron atom, Y the number of unpaired electrons on the second iron atom, and positive/ negative values signify spin up/down electrons. All geometry optimisations were carried out with the B3LYP functional and the 6-31G(d,p) basis set. Final energies are Gibbs free energies and contain solvent corrections (acetonitrile, conductor-like polarized continuum model), dispersion energies computed with Grimme's D3 method with BJ damping,^{10,11} and single point calculations using the higher 6-311++G(d,p) basis set for all atoms except for iron for which one extra set of d and f functions was added. All wavefunctions were confirmed to be stable. Intrinsic reaction coordinate calculations were used for confirming the TS of the monoiron complexes, but not for the diiron complexes due the very high computational cost.

Results and discussion

Catalase-like activity of 3 and 4

1.4

1.2

1

The reactivity of complex **1** and **2** towards hydrogen peroxide was investigated in MeCN by measuring changes in UV-Vis, and by volumetric determination of evolved dioxygen. Upon formation, the peroxo-diiron(III) complexes **3** and **4** decompose to form dioxygen and regenerate the precursor Fe(II) complexes, suggesting a catalase-like reactivity (Fig. 1 and 2).

The thermal stability of the (μ -peroxo)-bridged species shows considerable dependence on both the substitution (NH *versus* NMe) in the ligand as well as on the temperature (Table 2). The half-life ($t_{1/2}$'s) for complex **3** is 11 min, while for complex **4** it is 54 min at 20 °C, demonstrating that complex **3** is thermally much less stable compared to complex **4**. The catalase-like activity of the *in situ* formed peroxodiiron(m) complexes **3** and **4** to disproportionate H₂O₂ into



Fig. 1 Formation and temperature-dependent stability of 3. $[1]_0 = 2 \times 10^{-3}$ M, $[H_2O_2]_0 = 8 \times 10^{-3}$ M in MeCN at 720 nm.



Fig. 2 Formation and temperature-dependent stability of 4. [2]₀ = 2 \times 10⁻³ M, [H₂O₂]₀ = 8 \times 10⁻³ M in MeCN at 685 nm.

Table 2 Temperature-dependent stability of (μ -1,2-peroxo)diiron(III) complexes (3, 4)

3 $t_{1/2}$ (s)	4 $t_{1/2}$ (s)		
1650	n.a.		
1200	4740		
645	3210		
390	1770		
n.a.	870		
	3 t _{1/2} (s) 1650 1200 645 390 n.a.		

H₂O and O₂ was examined in MeCN at 25 °C using initial rates method monitoring the increase of the evolved dioxygen, and it was found that both complexes have such activity. Fig. 3 shows typical dioxygen evolution *versus* time curves for the disproportionation process catalyzed by **3** and **4**. Based on the observed initial rates, compound **4** was more reactive than **3**. The estimated initial rates (under same conditions) were $V_0 =$ 2.95 × 10⁻⁵ M s⁻¹ (TOF = 0.65 min⁻¹), and $V_0 = 5.10 \times 10^{-5}$ M s⁻¹ (TOF = 1.43 min⁻¹), respectively to the compound **3** and **4**



Fig. 3 Disproportionation of H_2O_2 catalyzed by the *in situ* formed 3 and 4. [1 or 2]₀ = 1 × 10⁻³ M, [H₂O₂]₀ = 0.2 M at 25 °C in MeCN.



Fig. 4 Dependence of the initial reaction rate (V_0) on the catalyst (**3** or **4**) concentration for the disproportionation of H_2O_2 catalyzed by the *in situ* formed **3** and **4**. [H_2O_2]_0 = 0.2 M at 25 °C in MeCN.

at 25 °C. In order to determine the rate dependence on the various reactants, disproportionation runs were performed at different substrate and catalyst concentrations (Table S1, ESI†). At constant $[H_2O_2]_0$, the initial rate of H_2O_2 disproportionation varies linearly with the *in situ*-formed [catalyst 3 or 4]^{0.5}, meaning that both reactions are half-order in catalyst, and suggesting a dissociation process *via* homolytic cleavage of the O–O bond (Fig. 4). At low H_2O_2 concentrations, the reactions are first-order in peroxide concentration as shown in Fig. S1 (ESI†), to establish a rate law of $-d[H_2O_2]/dt = k_{cat}[H_2O_2][3 \text{ or } 4]^{0.5}$ with $k_{cat} = 2.81 \times 10^{-3} \text{ M}^{-1/2} \text{ s}^{-1}$ for 3 and $k_{cat} = 5.06 \times 10^{-3} \text{ M}^{-1/2} \text{ s}^{-1}$ for 4 at 25 °C.

Substrate oxidation studies

Stoichiometric O-H activation of 2,6-di-tert-butylphenols mediated by complex 3 and 4. Firstly, we have investigated the properties of 3 and 4 as oxidants against various organic compounds, in order to obtain an initial assessment of their oxidizing power. To get direct evidence for the involvement of the ${\rm Fe}^{\rm III}(\mu\text{-}1,2\text{-}O_2){\rm Fe}^{\rm III}$ species in the O–H activation processes, reactions of 3 and 4 with various substrates, such as 2,6-di-tertbutylphenol, and 1,4-hydroquinones were investigated. The distinction between nucleophilic versus radical peroxo character can be proved with phenols since deprotonation leads to phenoxide coordination, whereas HAT provides a phenoxyl radical, which can lead to an easily-identifiable 2,2'-biphenolcoupled product in case of 2,6-di-tert-butylphenols or 1,4benzoquinones after the disproportionation of the forming semiquinones (Scheme 2). Peroxo O-O bond cleavage resulting in oxoiron(iv) intermediate may also occur prior to HAT in these reactions. Complexes 3 and 4 are capable of performing HAT reactions with 2,6-di-tert-butylphenol 3,3',5,5'-tetra-tertbutyl-4,4'-diphenoquinone (~80% based on 1 and 2) formed via the phenoxyl intermediate, and 1,4-hydroquinone transformed to 1,4-benzoquinone (~90%). These results raise the question of which active species is involved in the mechanism.



Scheme 2 $(\mu$ -1,2-Peroxo)diiron(μ)-mediated oxidation of phenols and hydroquinones.

The (μ -1,2-peroxo)diiron(m) complexes 3 and 4, which are well-characterized iron-based oxidants with an absorbance band in the visible region ($\nu_{max} = 720 \text{ nm} (1360 \text{ M}^{-1} \text{ cm}^{-1})$ and $\nu_{max} = 685 \text{ nm} (1400 \text{ M}^{-1} \text{ cm}^{-1})$, respectively) were generated by reaction of 1 and 2 with H₂O₂ in MeCN, and the rate of the decay of the absorption band at 720 nm and 685 nm was measured as a function of the concentration of added substrates (Fig. 5 and 6).

The reaction of **3** and **4** was investigated in detail. To generate complexes **3** and **4**, **1** and **2** (0.5 mM) were dissolved in MeCN, and treated with 4 equivalent of aqueous H₂O₂, which led to the maximum generation of **3** and **4** (<320 and 700 s), as judged by UV-vis spectroscopy, followed by a slower decomposition of the green species, and the formation of **3**,3',5,5'-tetra*tert*-butyl-4,4'-diphenoquinone. No shifts have been observed for the 2,6-di-*tert*-butylphenols, excluding their complexation with the oxidant. Plots of reaction rates ($V_{\text{ox}} = V_{\text{i}} - V_0 = k_{\text{ox}}$ [S] [**3** or **4**]^{0.5}) *versus* 2,6-di-*tert*-butylphenol concentrations, as shown for **3** and **4** in Fig. 7, state that the reaction is first order with respect to the substrate concentration in both cases (Tables S2 and S3, ESI[†]), which in turn indicates that the rate-determining step of the stoichiometric reaction involves sub-



Fig. 5 UV-Vis spectral change during the 3-mediated oxidation of phenols monitored at 720 nm. Reaction conditions: [3] = 0.5×10^{-3} M, [2.6-DTBPh] = 0.1 M, in MeCN at 20 °C. Inset shows time course of the decay of 3, without substrate (a), and with 0.1 M 2.6-DTBPh (b), $\Delta t = 45$ s.



Fig. 6 UV-Vis spectral change during the **4**-mediated oxidation of phenols monitored at 685 nm. Reaction conditions: [4] = 0.5×10^{-3} M, [2.6-DTBPh] = 0.06 M, in MeCN, at 20 °C Inset shows time course of the decay of **4**, without substrate (a), and with 0.06 M 2.6-DTBPh (b), Δt = 150 s.



Fig. 7 Dependence of the reaction rate (V_{ox}) on the substrate concentrations for the oxidation of 2.6-di-*tert*-butylphenol mediated by 3 (\Box) or 4 (\blacksquare) at 20 °C in MeCN. [3 or 4]₀ = 0.5 × 10⁻³ M.

strate oxidation. At constant [substrate]₀, the reaction rates (V_i and V_{ox}) of the decay of 3 and 4 vary linearly with the *in situ* formed [catalyst 3 or 4]^{0.5}, meaning that both reactions show a half-order dependence in catalyst, suggesting a dissociation process *via* a homolytic cleavage of the O–O bond in a fast equilibrium (Fig. 8 and Scheme 3).

The reaction kinetic parameters for complexes 3 and 4 are $k_{\rm ox} = 20.2 \times 10^{-5} \text{ M}^{-1/2} \text{ s}^{-1}$, $\Delta H^{\#} = 43 \text{ kJ mol}^{-1}$, $\Delta S^{\#} = -171 \text{ J mol}^{-1} \text{ K}^{-1}$, and $k_{\rm ox} = 5.75 \times 10^{-5} \text{ M}^{-1/2} \text{ s}^{-1}$, $\Delta H^{\#} = 64 \text{ kJ mol}^{-1}$, $\Delta S^{\#} = -108 \text{ J mol}^{-1} \text{ K}^{-1}$ at 293 K, respectively (Fig. 9). As shown in Fig. 10, there is a linear correlation between log $k_{\rm ox}$ and O–H bond dissociation energy (BDE) for 3, which suggests that the oxidizing species involved is selective in nature. This may be taken as evidence for a HAT including a concerted hydrogen abstraction (Table S6, ESI[†]).



Fig. 8 Dependence of the reaction rate (V_{ox}) on the $(\mu$ -1,2-peroxo)diiron(III) (3 or 4) concentrations for the oxidation of 2.6-di-*tert*-butylphenol mediated by 3 (\Box) or 4 (\blacksquare) at 20 °C in MeCN. [2.6-DTBPh]₀ = 0.1 M.







Fig. 9 Eyring plots for the oxidation of 2.6-di-*tert*-butyl-phenols mediated by $3 (\Box)$ or $4 (\blacksquare)$ in MeCN.

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Fig. 10 Plot of rate constants *versus* O–H BDE for the oxidation of *para*-substituted 2.6-di-*tert*-butylphenols (0.06 M) by 3 (\Box) or 4 (\blacksquare) in MeCN at 20 °C.

This is supported by a kinetic isotope effect (KIE) of 1.78 for the 3-mediated oxidation of 2.6-di-*tert*-butylphenol. A similar plot is observed for 4, but with slower rates, in line with its observed stability. The introduction of substituents in the *para* position of the phenyl ring of phenol affected the rate appreciably, the electron-releasing substituents accelerated the rate significantly, suggesting the electrophilic character of the key oxidant in both cases ($\rho = -0.67$ and -0.71 for 3 and 4, respectively) (Table 3, and Fig. 11).

Stoichiometric O–H activation of 1,4-hydroquinones mediated by complex 3 and 4. The reactivities of complex 3 and 4 with 1,4-hydroquinones in HAT show that both complexes exhibit enhanced reactivities when compared to the 2.6di-*tert*-butylphenol oxidation. Kinetic experiments, similarly to the latter substrate, revealed 1st-order dependence on the 2-chloro-1,4-hydroquinone, and half-order dependence on the (μ -1,2-peroxo)diiron(m) 3 with $k_{ox} = 0.38 \text{ M}^{-1/2} \text{ s}^{-1}$, $\Delta H^{\#} =$ 36 kJ mol⁻¹ and $\Delta S^{\#} = -121 \text{ J mol}^{-1} \text{ K}^{-1}$ at 5 °C (Table 3, Fig. 12 and 13, and Tables S4 and S5, Fig. S2, ESI†). A slower rate, $k_{ox} = 0.06 \text{ M}^{-1/2} \text{ s}^{-1}$, was observed with respect to complex

Table 3 Comparison of kinetic data obtained by 3 and 4 complexes at 20 $^\circ\text{C}^a$ and 5 $^\circ\text{C}^b$

Substrate	Complex				
_	3	4			
$t_{1/2}$ (s)	645	3210			
2.6-DTBPh ^a					
$k_{\rm ox} (10^{-5} {\rm M}^{-1/2} {\rm s}^{-1})$	20.22	5.75			
$\Delta H^{\#}$ (kJ mol ⁻¹)	43	64			
$\Delta S^{\#}$ (\tilde{J} mol ⁻¹ K^{-1})	-171	-108			
ρ	-0.67	-0.71			
KIE	1.78				
2-Cl-H ₂ Q ^b					
$k_{\rm ox} ({\rm M}^{-1/2} {\rm s}^{-1})$	0.62	0.059			
$\Delta H^{\#}$ (kJ mol ⁻¹)	36	51			
$\Delta S^{\#} (J \text{ mol}^{-1} \text{ K}^{-1})$	-121	-83			
KIE	2.9	2.3			



Fig. 11 Hammett plot for the oxidation of *para*-substituted 2.6-di-*tert*butylphenols mediated by **3** (\Box) or **4** (**a**) at 20 °C. Reaction conditions: [**3** or **4**]₀ = 0.5 × 10⁻³ M; [4R-2.6-DTBPh]₀ = 0.06 M.



Fig. 12 Dependence of the oxidation reaction rates (V_{ox}) on the substrates concentrations for the oxidation of 2-chloro-1,4-hydroquinone with **3** (\Box) or **4** (\blacksquare) in MeCN at 5 °C.



Fig. 13 Dependence of the reaction rates on the (μ -1.2-peroxo) diiron(μ) intermediate concentrations for the oxidation of 2-chloro-1.4-hydroquinone with 3 (\Box) or 4 (\blacksquare) in MeCN at 5 °C.



Fig. 14 Plot of rate constants versus O–H BDE for the oxidation of substituted hydroquinones (0.01 M) by 3 (\Box) (-0.147) or 4 (\blacksquare) (-0.253) in MeCN at 5 °C.



Fig. 15 Hammett plot for the oxidation of hydroquinones (H_2Q-X , X: H, Br, Cl, Me, ^tBu). Reaction conditions: 0.5 mM 3 (\Box) or 4 (**\blacksquare**), 0.01 M substituted H_2Q in MeCN at 5 °C.

4. The different reactivities can be explained by the introduction of the methyl-substituent on the NH group of the benzimidazole which increases the electron density on the iron centre, and decreases its electrophilic character. Upon using meta-substituted 1,4-hydoquinones with electron donating groups the rate of the decay processes were increased remarkably (Fig. 14 and 15, and Table S7, ESI[†]), suggesting that the metal-based oxidant is electrophilic. The non-linear nature of the Hammett plot can be explained by the two available O-H groups in different positions. A similar trend was reported for the [(TMC)Fe^{IV}(O)]-mediated (TMC = 1,4,8,11-tetramethyl-1,4,8,11-tetraazacyclotetradecane) oxidation of substituted hydroquinones. A kinetic isotope effect (KIE) value of 2.9 was obtained by determining the rate constants (k_{ox}) corresponding to separate reactions of 2-chloro-1,4-hydroquinone with 3, using the same conditions as described above, demonstrating that the H-atom abstraction is involved in the rate-determining step. This value is comparable to that was observed for the [(TMC)Fe^{IV}(O)]-mediated oxidation above (2.7).¹²

Computational characterisation of peroxo-diiron(m) complexes and O-O homolysis

Our first computational step was the geometry optimisation of the ground state structures of the 12 possible broken symmetry



Fig. 16 Potential energy surfaces for hemolytic bond breaking (the 6 lowest energy processes are shown).

Table 4	Energies (kcal mol ⁻¹	¹), selected bond lengths (Å), spin densities for the 12 or	otimised spin solutions
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Spin solution	ΔG (vaccum)	ΔG (MeCN)	Selected bond lengths				Spin densities			
			Fe1–O1	Fe2-O2	0-0	Fe-Fe	Fe1	Fe2	01	O2
S = 0(1, -1)	0.0	0.0	1.80	1.80	1.41	4.40	0.81	-0.81	0.15	-0.15
S = 1(1,1)	0.4	0.7	1.80	1.80	1.42	4.40	0.84	0.84	0.20	0.20
S = 2(3,1)	-2.0	0.6	1.80	1.80	1.42	4.39	0.86	2.84	0.17	0.07
S = 3(5,1)	-4.4	0.7	1.81	1.87	1.40	4.55	0.90	4.18	0.23	0.39
S = 4(5,3)	-8.0	-0.2	1.86	1.82	1.39	4.56	4.17	2.92	0.35	0.08
S = 5(5,5)	-10.9	-0.8	1.89	1.89	1.39	4.71	4.19	4.19	0.44	0.44
S = 0(5, -5)	-11.3	-0.8	1.86	1.86	1.37	4.72	4.17	-4.17	0.25	-0.25
S = 1(3, -1)	-2.5	0.3	1.80	1.80	1.41	4.40	-0.83	2.82	-0.17	0.03
S = 2(5, -1)	-7.2	-2.2	1.80	1.87	1.39	4.55	-0.86	4.15	-0.03	0.35
S = 1(5, -3)	-8.2	-0.3	1.87	1.81	1.38	4.57	4.16	-2.85	0.38	0.08
S = 0(3, -3)	-0.1	5.6	1.81	1.80	1.41	4.44	2.81	-2.80	0.05	-0.07
S = 3(3,3)	-1.9	2.6	1.81	1.79	1.41	4.35	2.85	2.91	0.02	-0.02

Spin solution	ΔG (vaccum)	ΔG (MeCN)	Selected bond lengths				Spin densities			
			Fe1–O1	Fe2-O2	0-0	Fe-Fe	Fe1	Fe2	01	O2
S = 0(1, -1)	15.3	17.5	1.69	1.69	1.86	4.57	0.95	-0.95	0.71	-0.71
S = 2(3,1)	17.5	22.3	1.71	1.68	1.91	4.61	0.73	2.93	-0.36	0.58
S = 3(5,1)	12.6	20.0	1.66	1.72	1.84	4.82	1.10	3.98	0.59	0.09
S = 4(5,3)	10.9	20.8	1.71	1.67	1.82	4.82	3.95	3.03	0.09	0.45
S = 0(5, -5)	12.2	25.8	1.69	1.69	1.78	5.14	3.81	-3.81	0.16	-0.16
S = 1(3, -1)	13.1	17.5	1.70	1.69	1.85	4.56	-0.96	2.90	-0.69	0.56

Table 5 Energies (kcal mol⁻¹), selected bond lengths (Å), spin densities for the transition states of the O–O homolytic bond breaking in Fe_2O_2 models (the 6 lowest energy processes are shown)

spin solutions for the Fe₂O₂ complexes. In vaccuum, the lowest energy solution was the antiferromagnetically coupled singlet S = 0(5,-5) (containing 5 spin-up electrons on the first iron atom and 5 spin-down electrons on the second), closely followed by the antiferromagnetically coupled triplet S = 1(5, -3)and the S = 5(5,5) solutions. After inclusion of solvation energies using acetonitrile as solvent, all 12 spin solutions were found to be degenerate in energy, with approximately 6.5 kcal mol^{-1} between the lowest energy solution S = 0(5,-5) and highest energy S = 0(3, -3). Selected geometrical parameters are described in Table 4. Fe-O bonds vary between 1.79-1.89 Å and are typical of Fe(III)-peroxo ligands, while Fe-Fe distances are similar to those derived from resonance Raman spectroscopy.⁸ The O-O homolytic bond cleavage processes were then explored: Fig. 16 (see Table 5 and S9 for all spin states, ESI[†]) show the that the potential energy surfaces (PESs) intersect for different spin solutions, suggesting that spin cross-over is possible during homolysis. The TSs occur early along the O-O PES and are degenerate in energy, with values suggesting a feasible process. All these data indicate that O-O homolysis is facile and will occur on multiple spin PESs simultaneously.

Computational characterisation of Fe(IV)O catalase and phenol reactivity

The DFT estimates on the catalase reaction and the HAT reaction of DTBPh are shown in Fig. 17 and 18 respectively; both processes are predicted to occur through a concerted HAT reaction, similarly to what was determined experimentally. Although ³Fe(IV)O and ⁵Fe(IV)O are essentially degenerate in energy, the reaction is preferred along the lower spin state surface for both cases. For the catalase reaction, the S = 2 transition state is 9 kcal mol⁻¹ higher in energy that the *S* = 1 transition state, indicating that the preferred reaction path is along S = 1, although there is a clear similarity in geometrical parameters for Fe-O and FeO-H and substrate-H (Fig. 17). Both processes occur at a late stage along the O-H PES (Fig. S6, ESI^{\dagger}), with only the *S* = 1 reaction being exergonic (Fig. 17). In the case of the HAT reaction for DTBPh, no hydrogen bound adducts could be obtained between the substrate and the oxidant for any of the spin states, and no TS could be optimised for the S = 2 spin state (consistent with the B3LYP PES given in Fig. S7, ESI[†]). All energies shown in Fig. 18 indicate a preferred S = 1 spin state for this reaction.



Fig. 17 Reaction barriers for the catalase reaction (kcal mol⁻¹ in acetonitrile), together with relevant bond lengths (in Å), spin densities and partial charges (in parenthesis). Models are simplified for ease of view (L^2 cropped to N-C-C-N, for full models see coordinates in ESI†).



Fig. 18 Reaction barriers for the hydrogen atom abstraction from 2.6-DTBPh (kcal mol⁻¹ in acetonitrile), together with relevant bond lengths (in Å), spin densities and partial charges (in parenthesis). Models are simplified for ease of view (L^2 cropped to N-C-C-N and 2.6-DTBPh cut, for full models see coordinates in ESI†).

Conclusions

The reactivity of two peroxo-diiron(m) complexes (3 and 4) has been investigated in O–H activation processes as a structural and functional model of RNR-R2 enzyme. The decay of the peroxo species was affected by 2,6-di-*tert*-butylphenol and 1,4hydroquinone, leading to a 2,2'-biphenol-coupled product and 1,4-benzoquinones, respectively. Based on detailed kinetic (half-order dependence for 3 and 4), mechanistic (KIE = 1.78, $\rho = -0.7$ for 2,6-DTBPh), and computational studies, an electrophilic oxoiron(IV) species with S = 1 spin state, was suggested as reactive species responsible for the HAT processes. Based on our preliminary results complex 3 is capable of oxidizing both oxygen-atom transfer and hydrogen-atom abstraction, that is a further evidence for the presence of electrophilic oxoiron(IV) species as key oxidant (Scheme 3). From benzyl alcohol benzaldehyde (34% based on 3) was formed and triphenylphosphine gave triphenylphosphine oxide (93% based on 3) at 20 °C in MeCN (Fig. S3–S5, ESI†). Detailed kinetic studies on these two systems are in progress.

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