Supporting Information

for

Modeling Iron Halogenases: Synthesis and Reactivity of Halide-Iron(IV)-oxo Compounds

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TABLE OF CONTENTS

1. Instrumentation	
2. Materials	2
3. Experimental	
3.1 Preparation and characterization of 1-Cl and 1-Br	
3.2 Oxidation of activated C-H bonds and sulfides	5
3.2.1 Oxidation of sulfides	6
3.2.2. Oxidation of activated C-H bonds	
3.3. ESI-MS of the final iron species	
4. References	

1. Instrumentation

UV-vis spectroscopy was performed using an Agilent 8453 UV-vis spectrophotometer. Absorption of 1 mM solutions of the complexes in acetonitrile/water (100:1) was measured from 200 nm to 1100 nm with a resolution of 1 nm in a 1 cm UV quartz cells. The low temperature control was performed with a cryostat from Unisoku Scientific Instruments (Japan). High resolution mass spectra were recorded on a BrukerMicrOTOF-Q IITM instrument using electrospray (ESI) or cryospray (CSI) ionization sources at Serveis Tècnics of the University of Girona. ⁵⁷Fe Mössbauer experiments were performed at 80 K on a zero-field Mössbauer spectrometer equipped with a Janis SVT-400 cryostat or at 4.2 K on a strong field Mössbauer spectrometer equipped with an Oxford Instruments Spectromag 4000 cryostat operating with a field of 7 T applied parallel to the γ beam. Analysis of the data was performed with the program WMOSS (WEB Research, Edina, MN, USA). GC/FID analysis was performed with an Agilent 7820A chromatograph fitted with a flame ionization detector provided by Agilent (Santa Clara, CA, USA), interfaced to a computer running EZChrom Elite software. GC-MS analysis was performed with an Agilent 7820A chromatograph fitted with an Agilent 5975C quadrupole mass spectrometer provided by Agilent (Santa Clara, CA, USA), interfaced to a computer running Enhaced Data Analysis software. Analytes were separated in a TR-5MS column (30 x 0.25mm,0.25µm film thickness) coated with 5% phenylmethylpolisiloxane stationary phase (Thermo Fischer Scientific). The mass spectrometer was operated in electron impact (EI) ionization mode.

2. Materials

Reagents and solvents were of commercially available reagent quality unless otherwise stated. $H_2^{18}O$ (95% ¹⁸O-enriched) was received from ICON isotopes. Anhydrous acetonitrile was purchased from Scharlau. Compound $[Fe^{IV}(O)(Pytacn)(NCMe)]^{2+}$ (1-NCMe) was prepared following the reported procedure.¹

3. Experimental

3.1 Preparation and characterization of 1-Cl and 1-Br

In a typical experiment, to a 2.5 mL solution of **1-NCMe** (1 mM, 2.5 μ mols) in acetonitrile at -30 °C, 2 equiv of the appropriate tetraalkylammonium salt (NBu₄Cl or NBu₄Br) (5.0 μ mols dissolved in 80 μ L acetonitrile) were added at once under stirring. The ligand exchange reaction was monitored by UV-vis spectroscopy. In particular, the shift of the band initially situated at 750 nm (corresponding to **1-NCMe**) was followed. The exchange occurred within 9 min for **1-Cl** and 5 min for **1-Br**. ⁵⁷Fe-enriched samples of **1-Cl** and **1-Br** for Mössbauer spectroscopy were prepared following the same procedure as described above but starting from [⁵⁷Fe(OTf)₂(Pytacn)] to generate **1-NCMe**.¹



Figure S1. UV-vis monitoring corresponding to the generation of **1-Br** by reaction of **1**-**NCMe** with 2 equiv NBu₄Br in acetonitrile at -30°C. Inset: kinetic profile of this process at 750 and 823 nm.



Figure S2. Left: CSI-MS-TOF spectrum (at 243 K) of **1-Cl**, which exhibited a clear signal at m/z = 355.0993. It should be noted that the slight mismatch between the experimental and calculated isotopic patterns is due to the contamination with the mass peak of the iron(III)-hydroxo species $[Fe^{III}(OH)(Cl)(Pytacn)]^+$ with a mass-to-charge ratio one unit higher than $[Fe^{IV}(O)(Cl)(Pytacn)]^+$ (m/z = 356.1074). Right: CSI-MS-TOF spectrum (at 243 K) of ¹⁸O-labeled **1-Cl**, which exhibited a clear signal at m/z = 357.1003, slightly contaminated by the mass peak of the iron(III)-hydroxo species (m/z = 358.1074) and non-labeled **1-Cl** (m/z = 355.1001).



Figure S3. Mössbauer spectrum of **1-Br** in acetonitrile recorded at 80 K. Solid lines correspond to spectral simulations. The black sub-spectrum corresponds to the Fe^{IV} ion (see text) and the gray sub-spectrum to a (μ -oxo)diferric impurity¹ (11 %).

compound		$\delta (\text{mm} \cdot \text{s}^{-1})$	$\Delta E_Q (mm \cdot s^{-1})$	$\Gamma (\mathbf{mm} \cdot \mathbf{s}^{-1})$	%
1-Cl	Fe ^{IV}	0.06	0.89	0.30	88
	$Fe^{III}_{2}(\mu-oxo)$	0.54	1.49	0.35	12
		0.45	1.11	0.35	
1-Br	Fe ^{IV}	0.07	0.89	0.33	89
	$Fe^{III}_{2}(\mu$ -oxo)	0.58	1.26	0.35	11
		0.40	1.22	0.35	

Table S1. Mössbauer parameters for 1-Cl and 1-Br.

3.2 Oxidation of activated C-H bonds and sulfides

The appropriate amounts of substrates (diluted in acetonitrile) were added to a solution of **1-NCMe**, **1-Cl** or **1-Br** (1 mM) and the subsequent decay of the characteristic absorption band of the iron(IV)-oxo (750 nm for **1-NCMe**, 803 nm for **1-Cl** and 823 nm for **1-Br**) was directly monitored by UV-vis spectroscopy. Kinetic studies were performed at 243 K. Under conditions of excess substrate, reactions showed pseudo-first-order behaviour, so that the observed rate constants (k_{obs}) could be obtained by fitting the decay of the characteristic absorption band of the iron(IV)-oxo species to a single exponential function.

Product analyses for the oxidation of thioanisole derivatives were performed by gas chromatography. Prior to injection, an internal standard (biphenyl) was added to the solution, which was further filtered through silica and washed with ethyl acetate. Calibration curves with standard products enabled the quantitative determination of the oxidized compounds.

3.2.1 Oxidation of sulfides



Figure S4. UV-vis spectral changes observed upon reaction of **1-Cl** with 20 equiv thioanisole at 243 K. Inset: kinetic trace at 803 nm for this transformation.



Figure S5. Plot of the observed reaction rates (k_{obs}) of 1-Cl, 1-Br and 1-NCMe versus concentration of thioanisole at 243 K.

Table S2. Amounts of methyl phenyl sulfoxide (PhS(O)Me) and methyl phenyl sulfone (PhS(O)₂Me) obtained in the oxidation of thioanisole (PhSMe) by 1-X.^a

	Yield PhS(O)Me (%)	Yield PhS(O) ₂ Me (%)
1-NCMe	76	5
1-Cl	88	5
1-Br	92	-

^aReactions were run in acetonitrile at 243 K with 10 equiv of methyl phenyl sulfide.

Table S3. Logarithm of the second-order rate constants $(k_{\rm Y}, {\rm M}^{-1}{\rm s}^{-1})$ determined in the reaction of **1-X** with *para*-substituted thioanisoles (*p*-Y-thioanisoles).^a

			1-NCMe	1-Cl	1-Br
Y	E_{ox} vs. SCE (V) ^b	σ _p ^c	$\log(k_{\rm Y})^{\rm d}$	$\log(k_{\rm Y})^{\rm d}$	$\log(k_{\rm Y})^{\rm d}$
OCH ₃	1.13	-0.27	-3.5133	-3.2785	-3.2981
CH ₃	1.24	-0.17	-3.7447	-3.3632	-3.5850
Н	1.34	0.00	-3.9720	-3.5740	-3.6709
Cl	1.37	0.23	-4.2218	-3.7063	-3.9456
CN	1.61	0.66	-4.8751	-4.1761	-4.2730

^aReactions were run in acetonitrile at 243 K with 20 equiv of the selected *para*-substituted methyl phenyl sulfide (*p*-Y-thioanisole). Second order rate constants ($k_{\rm Y}$) were determined by dividing the observed rate constant ($k_{\rm obs}$) by the concentration of substrate. ^bOne-electron oxidation potentials for *p*-Y-thioanisoles were obtained from the literature. ²Hammett parameters. ^dLogarithm of the second-order rate constants ($k_{\rm Y}$, M⁻¹s⁻¹) in the reaction of **1-X** with *p*-Y-thioanisoles.



Figure S6. Hammett plot corresponding to the reaction of **1-Br** (red) and **1-NCMe** (green) with different *para*-substituted methyl phenyl sulphides (determined at 243 K).



Figure S7. Plot of $log(k_Y)$ against the one electron oxidation potentials of *p*-Y-thioanisoles (determined at 243 K).

3.2.2.Oxidation of activated C-H bonds



Figure S8. UV-vis spectral changes observed upon reaction of **1-Br** with 20 equiv DHA at 243 K. Inset: kinetic trace at 823 nm for this transformation.



Figure S9. Plot of the observed reaction rates (k_{obs}) (determined at 243 K) of **1-Cl**, **1-Br** and **1-NCMe** versus concentration of 9,10-dihydroanthracene (DHA).



Figure S10. Plot of $log(k_2)$ (determined at 243 K) against the C-H bond dissociation energy (BDE) of the different substrates for **1-Br** (red) and **1-NCMe** (green).

Measurement of the kinetic isotope effect (KIE) for hydrogen atom abstraction was determined by dividing the observed reaction rate (k_{obs}) of **1-X** towards DHA and d₄-9,10-dihydroanthracene under the same experimental conditions. 20 equiv of DHA or d₄-DHA (40 µmols diluted in 200 µL acetonitrile) were added at once uder stirring to 2 mL of a 1 mM solution of **1-X** (2 µmols) in acetonitrile at -30°C. Reactions were monitored by UV-vis spectroscopy and the decay of the absorption band at 750 nm (**1-NCMe**), 803 nm (**1-Cl**) and 823 nm (**1-Br**) was fitted to a single exponential function giving the k_{obs} values detailed in Table S4.

Table S4. k_{obs} values for the reaction of **1-X** with DHA and d₄-DHA (40 equiv) at 243K. KIE values were obtained by dividing the observed rate constants.

1-X	$k_{\rm obs}$ (DHA) (s ⁻¹)	$k_{\rm obs} ({\rm d}_4\text{-}{\rm DHA}) ({\rm s}^{-1})$	KIE
Cl	0.042	0.0017	25
Br	0.038	0.0015	25
NCMe	0.016	0.0006	27

3.3. ESI-MS of the final iron species



Figure S11. ESI-MS spectrum of the final reaction mixture obtained by reaction of **1-Cl** with 300 equiv triphenylmethane under N₂.



Figure S12. ESI-MS spectrum of the final reaction mixture obtained by reaction of **1-Cl** with 300 equiv triphenylmethane under air.

4. References

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