Ortho-hydroxylation of benzoic acids with hydrogen peroxide at a non-heme iron center†

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Experimental.

All reagents were obtained from commercially available sources and used without purification, unless otherwise noted. Complex **1** was synthesized according to a published procedure.[1] ^{18}O-labeled hydrogen peroxide (2% in water, 90% atom) was purchased from Icon Isotopes (Summit, NJ). Recrystallized *m*-CPBA was a gift from Dr. Jacob D. Soper (MIT). NMR spectra were recorded on a Bruker DPX-300 spectrometer and UV-vis spectra were acquired on a JASCO V-570 spectrophotometer. *In situ* electrospray mass spectra (ESI-MS) were obtained on a Finnigan LTQ mass spectrometer in positive ion detection mode and X-band EPR spectra were recorded on a Bruker EMX spectrometer.

Elemental analyses were performed by Schwarzkopf Microanalytical Laboratory, inc. (Woodside, NY). The kinetic measurements were performed using a High-Tech Scientific (Salisbury, Wiltshire, UK) SF-43 cryogenic stopped-flow instrument. X-ray diffraction analyses were performed on a Bruker APEX CCD based diffractometer at Harvard University (Cambridge, MA) for complex **2** and on an Enraf-Nonius CAD4 diffractometer at Brandeis University (Waltham, MA) for complex **3a**.

CAUTION: Although no problems were encountered in this study, perchlorate salts of metal complexes with organic ligands are potentially explosive and should be handled with care!

[Fe(BPMEN)(Sal)](ClO$_4$) (**2**). BPMEN[2] (271 mg, 1.00 mmol) was dissolved in 10 mL ethanol and added to a solution of $Fe(ClO_4)_3 \cdot 6H_2O$ (515 mg, 1.00 mmol) also dissolved in 10 mL ethanol. The solution turned brown and was stirred for 10 minutes. Salicylic acid (139 mg, 1.01mmol) was dissolved in 20 mL ethanol in presence of two equivalents of triethylamine (280 μL, 2.01 mmol). The salicylate solution was added dropwise to the iron containing solution and the mixture turned dark blue. The solution was filtered and concentrated under vacuum then redissolved in 10 mL of ethanol and left for slow evaporation of solvent. Large blue crystals were obtained after 5 days. Yield: 264 mg (47%). UV-vis (300-800 nm, CH_3CN: λ_{max}, nm (ε, L mol^{-1} cm^{-1}): 590 (2298). ES-MS(+) (CH_3CN): *m/z* 462.1 ($\{[Fe(BPMEN)(OOC(O)C_6H_4)]\}^+$, 100). Anal. Calcd (found) for $FeC_{23}H_{27}N_4O_{7.5}Cl$: C, 48.40 (48.24); H, 4.77 (4.74); N, 9.82 (9.67); Fe 9.78 (9.78). Crystals suitable for x-ray analysis were obtained after redissolution in water and slow evaporation of the solvent.

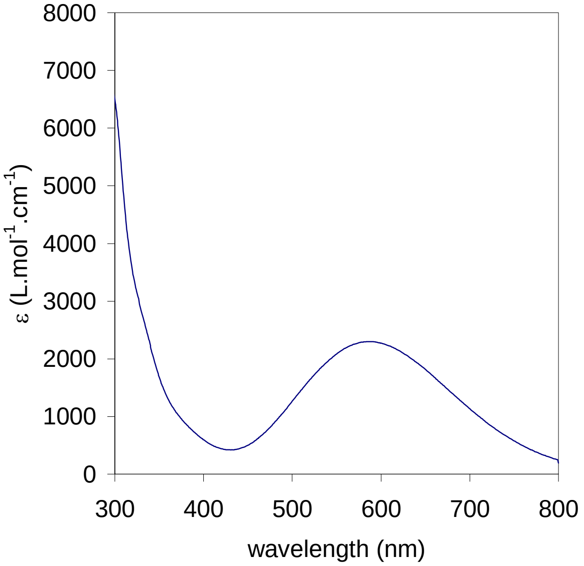


Fig. S1. Electronic spectrum of [Fe(BPMEN)(Sal)](ClO$_4$) (**2**) prepared from BPMEN, Salicylate and Fe(ClO$_4$)$_3$ in acetonitrile.

[Fe$_2$(μ-O)(μ-OOC(C$_6$H$_5$))(BPMEN)$_2$](ClO$_4$)$_3$ (**3**). BPMEN^2 (54 mg, 0.20 mmol) was dissolved in 1 mL acetonitrile and added to a solution of Fe(ClO$_4$)$_3$·6H$_2$O (104 mg, 0.20 mmol) also dissolved in 1 mL acetonitrile. Benzoic acid (26 mg, 0.21 mmol) was dissolved in 2 mL acetonitrile in presence of one equivalents of triethylamine (28 μL, 0.20 mmol). The benzoate solution was added to the iron containing solution and the mixture turned dark orange/brown. Crystals were obtained after vapor diffusion of diethyl. Yield: 72 mg (66%). UV-vis (400-800 nm, CH$_3$CH): λ_{max}, nm (ε, L mol^{-1} cm^{-1}): 422 (sh) (1005), 465 (1161), 495 (sh) (783), 512 (684), 543 (sh) (209), 671 (152). ES MS(+) (CH$_3$CN): *m/z* 987.0 ({[Fe$_2$(O)(OOC(C$_6$H$_5$))(BPMEN)$_2$](ClO$_4$)}$^+$, 20), 717.0 ({[Fe$_2$(O)(OOC(C$_6$H$_5$))(BPMEN)](ClO$_4$)}$^+$, 6), 447.3 ({[Fe(OOC(C$_6$H$_5$))(BPMEN)]}$^+$, 100). Anal. Calcd (found) for $Fe_2C_{39}H_{53}N_8O_{17}Cl_3$: C, 41.68 (41.72); H, 4.75 (4.50); N, 9.97 (9.90).

$[Fe_2(\mu\text{-}O)(\mu\text{-}OOC(C_6H_5))(BPMEN)_2](Ce^{III}(NO_3)_6)$ (**3a**). Benzoic acid dissolved in dry acetonitrile (3 mL, 0.03 mmol, 10 mM) was added to a solution of complex **1** (3 mL, 0.03 mmol, 10 mM) under argon atmosphere. An equimolar amount of $Ce(NH_4)_2(NO_3)_6$ was added to the previous mixture and the solution turned yellow. Dark green crystals suitable for X-ray diffraction analysis were obtained after five days by slow evaporation of the solvent. Yield: 11 mg (50%). UV-vis (400-800 nm, CH_3OH/CH_3CN): same than **3**. ES MS(+) (CH_3OH/CH_3CN): *m/z* 912.6 ($\{[Fe_2(O)(OOC(C_6H_5))(BPMEN)_2](NO_3)_2\}^+$, 6), 643.1 ($\{[Fe_2(O)(OOC(C_6H_5))(BPMEN)](NO_3)_2\}^+$, 14), 581.1 ($\{[Fe_2(O)(OOC(C_6H_5))(BPMEN)](NO_3)\}^+$, 14), 447.2 ($\{[Fe(OOC(C_6H_5))(BPMEN)]\}^+$, 56), 388.0 ($\{[Fe(BPMEN)(NO_3)]\}^+$, 46), 425.5 ($\{[Fe_2(O)(OOC(C_6H_5))(BPMEN)_2](NO_3)\}^{2+}$, 38), 263.2 ($\{[Fe_2(O)(OOC(C_6H_5))(BPMEN)_2]\}^{3+}$, 100).

Isolation of salicylic acid and m-chlorosalicylic acid for NMR studies. . The reaction between complex **1** (20 mg, 0.033 mmol), the appropriate substituted benzoic acid (1 mL, 0.033 mmol, 33 mM) and hydrogen peroxide (1 mL, 0.050 mmol, 50 mM) was run at room temperature in 7 mL acetonitrile (final iron complex concentration: 4.7 mM). As a control, a solution of complex **1** and the substituted benzoic acid without hydrogen peroxide was prepared and subject to the same following extraction procedure.

After 20 minutes, concentrated HCl (~200 μL) was added until the solution turns yellow. Iron residues were precipitated out by adding a large volume of ether (~20 mL) and leaving the mixture overnight in the freezer. After filtration and drying over sodium sulfate, the ether was removed yielding a crude yellow to brown product which was analyzed by ^{1}H NMR.

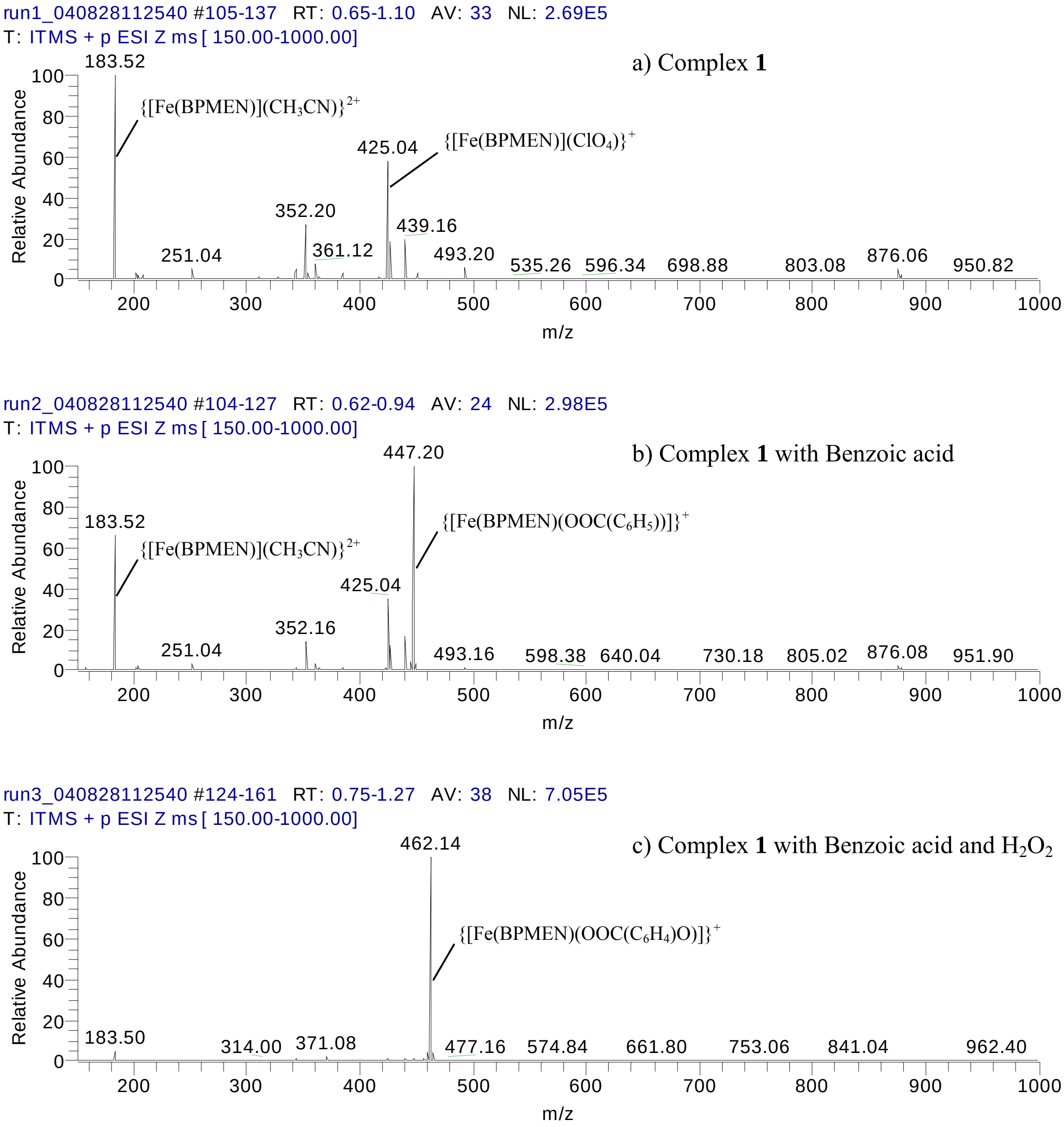


Fig. S2. Electrospray mass spectra of a) Complex **1**, b) Complex **1** with benzoic acid, c) Complex **1** with benzoic acid and hydrogen peroxide.

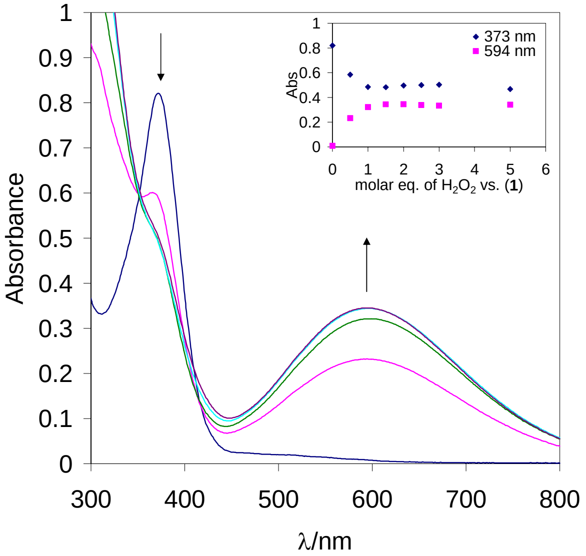


Fig. S3 Spectral change upon addition of different amounts of hydrogen peroxide to **1**/ *m*Cl-benzoic acid solution in acetonitrile (0 to 1.5 molar equivalents of H_2O_2 versus **1**, with 0.2 mM of **1** and 0.4 mM of *m*Cl-benzoic acid). Absorbancies were adjusted for dilution. Insert shows the cross sections at 373 nm and 594 nm with 0 to 5 molar equivalents of H_2O_2 versus **1**

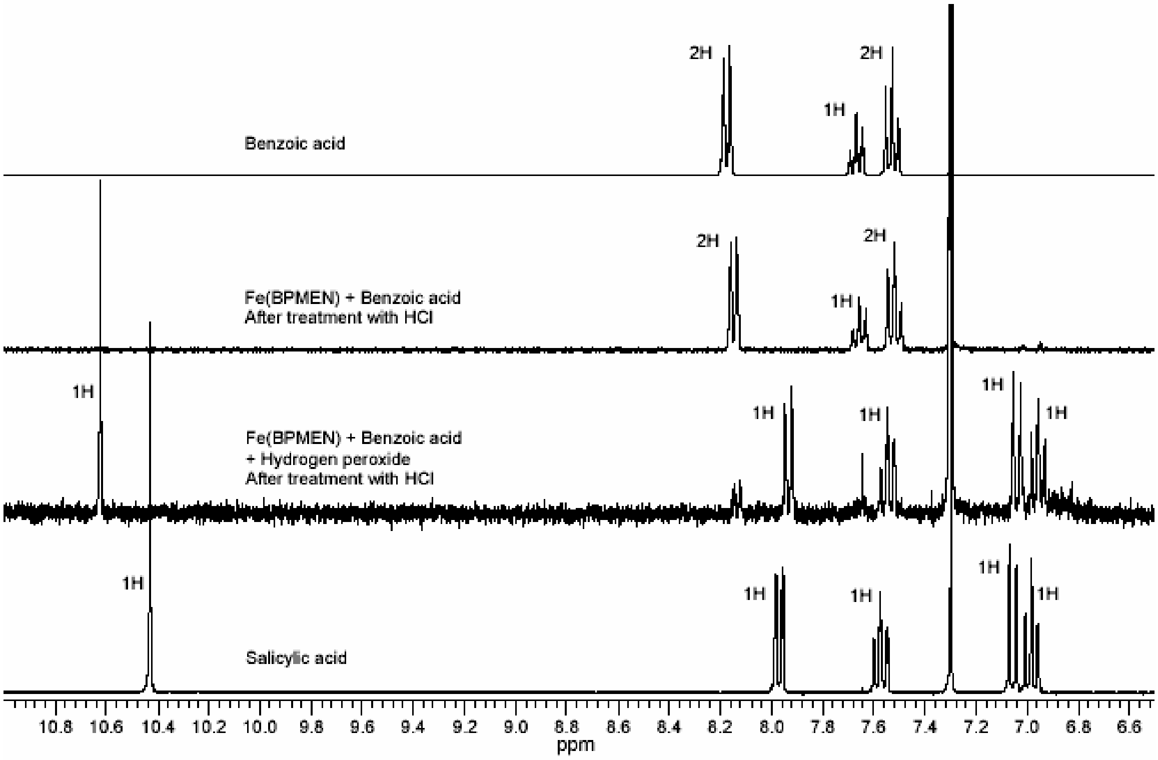


Fig. S4. ^{1}H NMR spectra in $CDCl_3$ comparing pure benzoic acid and pure salicylic acid to that obtained from reacting with **1** in presence and in absence of hydrogen peroxide. Conversion = 86%, selectivity = 100%.

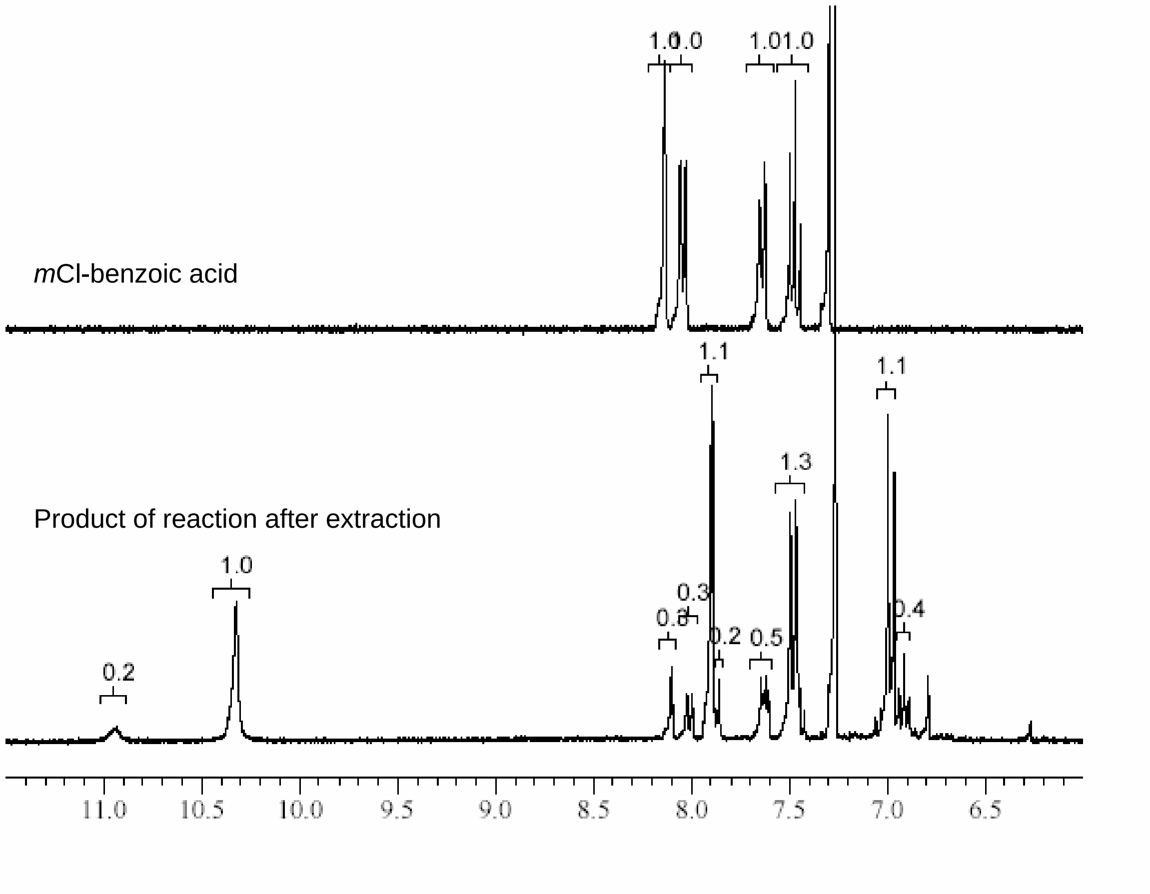


Fig. S5. ^{1}H NMR spectra in $CDCl_3$ comparing pure *m*Cl-benzoic acid and its product of reaction with **1**/hydrogen peroxide.

Conversion = 80%, selectivity for the *para* isomer = 83%.

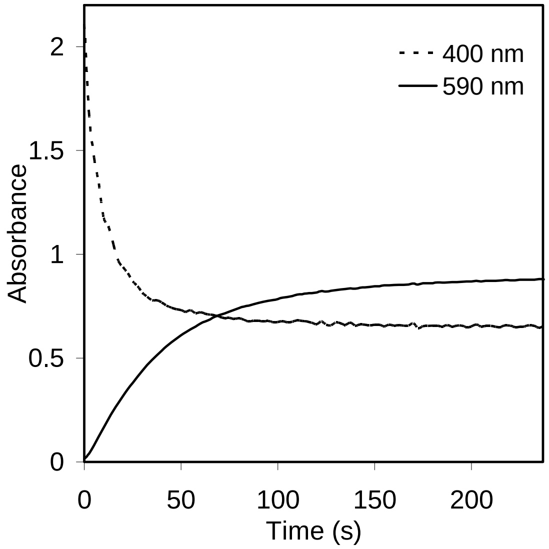


Figure S6. Kinetic traces at 400 nm and 590 nm acquired in the diode array mode using stopped-flow techniques (0.50 mM of **1**, 0.75 mM of benzoic acid and 12.0 mM of hydrogen peroxide after mixing at -15 ºC in acetonitrile). Two exponential global fit using Specfit/32 gave $k_{1obs} = 0.10\ s^{-1}$ and $k_{2obs} = 0.024\ s^{-1}$ under these conditions

$[H_2O_2]$, M	k_1(obs), s^{-1}	k_2(obs), s^{-1}
0.012	0.090	0.022
0.017	0.124	0.024
0.025	0.163	0.037

Table S1 Observed rate constants obtained from one-exponential single wavelength fits performed with IS-2 Rapid Kinetic software from High-Tech Scientific at λ = 410 nm for k_1(obs) and λ = 594 nm for k_2(obs) (with 0.50 mM of **1**, 0.75 mM of benzoic acid after mixing at -15 ˚C in acetonitrile).

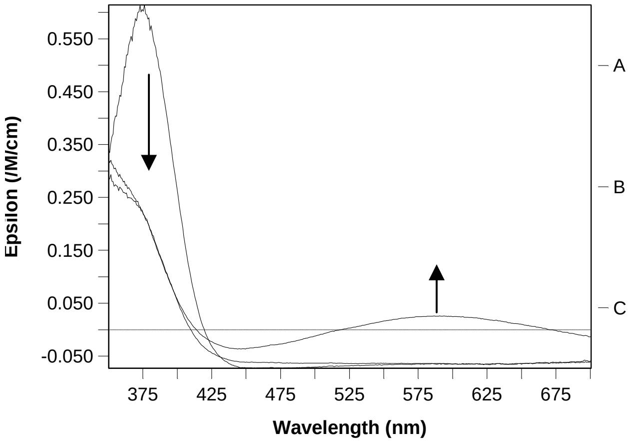


Fig. S7. Spectra of starting complex **1**, final product **2** and intermediate **1a** generated by Specfit/32 using a two exponential global fit model (with 0.125 mM of **1**, 0.375 mM of benzoic acid and 17 mM of hydrogen peroxide after mixing at -15 ˚C in acetonitrile).

EPR experiments.

All measurements were performed at 120 K. Solutions of complex **1** premixed with benzoic acid were prepared under argon atmosphere with a final concentration of 4 mM in complex **1**. Hydrogen peroxide solutions were prepared under air atmosphere. In a typical experiment, 0.5 to 6 equivalents of hydrogen peroxide were added to the iron containing solution at -42 °C (acetonitrile/CO_2 bath). The reaction was stopped by quickly immersing the EPR tube in liquid nitrogen (1 to 2 minute reaction).

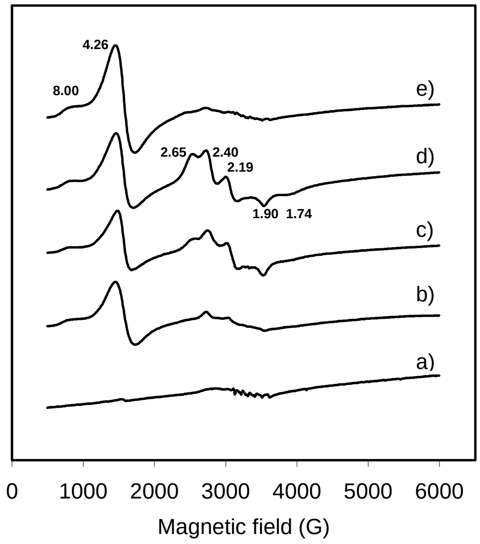


Fig. S8. EPR spectra before (a) and immediately after addition of hydrogen peroxide at -42 °C. b) 0.5 molar equivalents of H_2O_2 added. c) 1 molar equivalents of H_2O_2 added. d) 6 molar equivalents of H_2O_2 added. e) Same as d) after warming up to room temperature. Spectrometer parameters: radiation power 10.082 mW, radiation frequency 9.410 GHz, modulation frequency 100.00 kHz, modulation amplitude 10.00G.

Ce(IV) experiments.

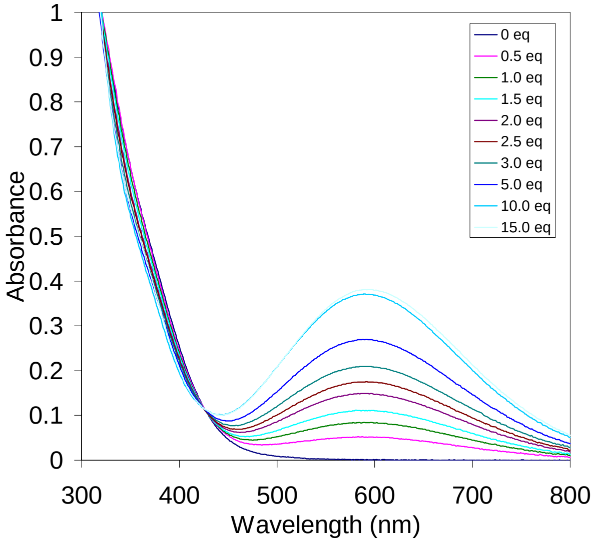


Fig. S9. Spectral change upon addition of benzoic acid pre-mixed with different amounts of H_2O_2 to **1**/ $[Ce^{IV}(NO_3)_6](NH_4)_2$ solution in acetonitrile (0.2 mM of **1**, 0.2 mM of Ce(IV) and 0.4 mM of benzoic acid after mixing). Absorbancies were adjusted for dilution.

^{18}O Isotope labeling experiments.

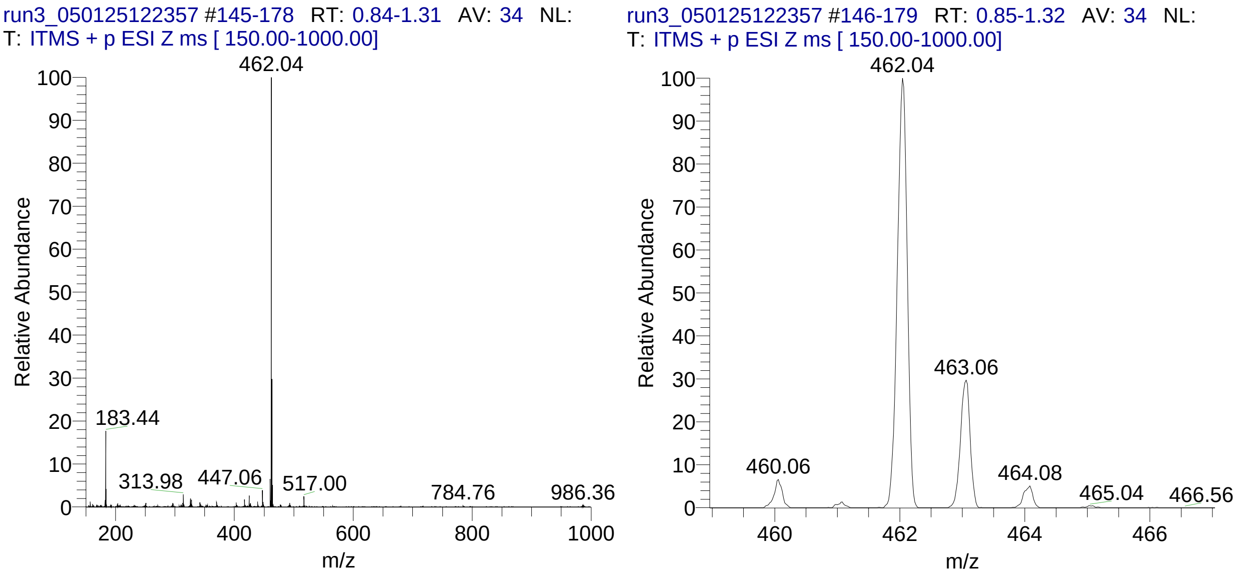


Fig. S10. ESI-MS after mixing **1**/benzoic acid and 3 molar equivalents of ^{16}O hydrogen peroxide in presence of 300 equivalents of ^{16}O water.

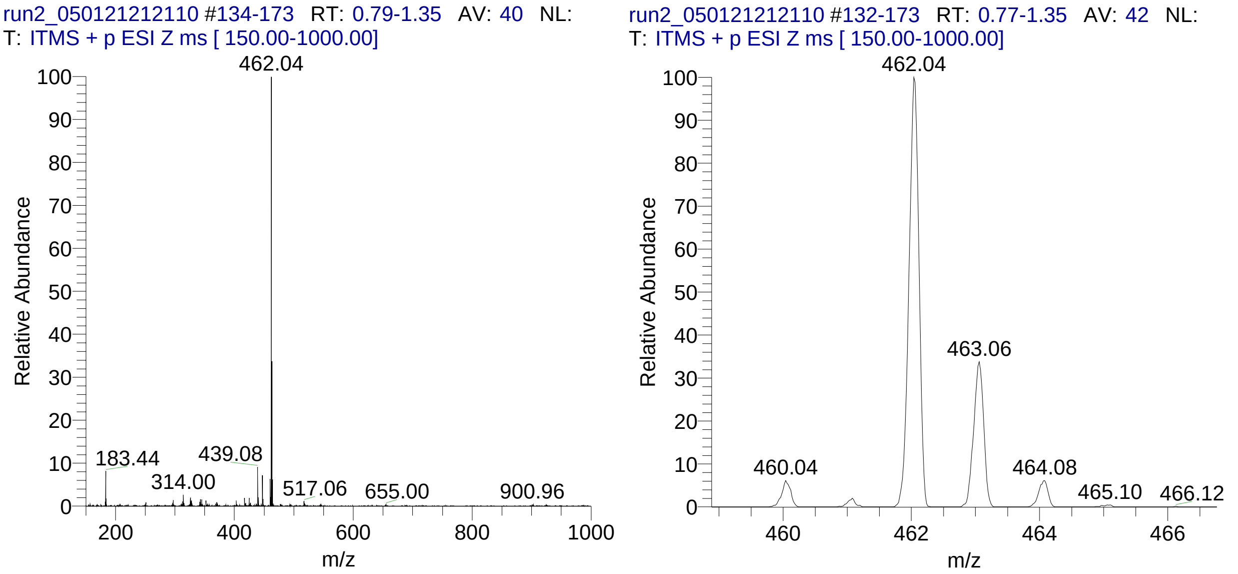


Fig. S11. ESI-MS after mixing **1**/benzoic acid and 3 molar equivalents of ^{16}O hydrogen peroxide in presence of 300 equivalents of ^{18}O water (95% atom). No ^{18}O incorporation observed.

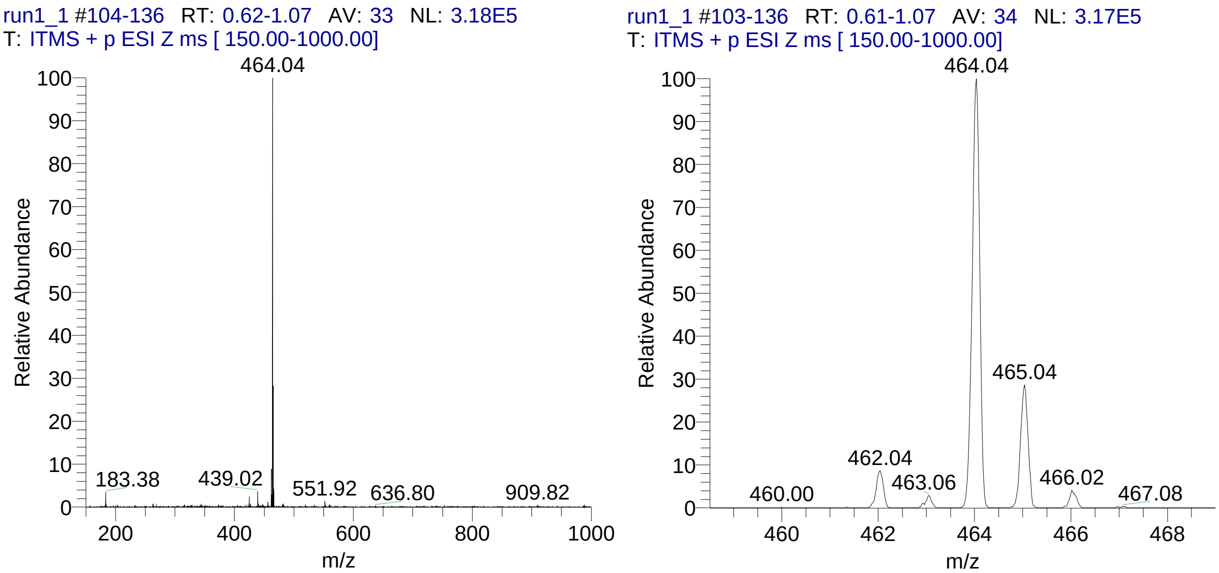
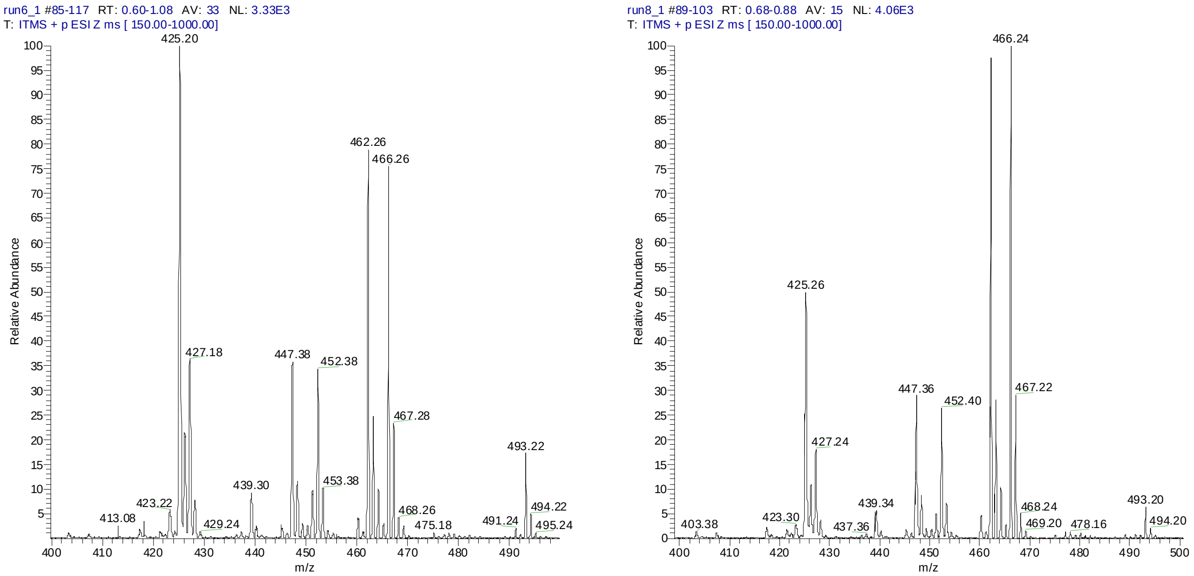


Fig. S12. ESI-MS after mixing **1**/benzoic acid and 3 molar equivalents of ^{18}O hydrogen peroxide (90% atom) in presence of ^{16}O water. Full ^{18}O incorporation observed.

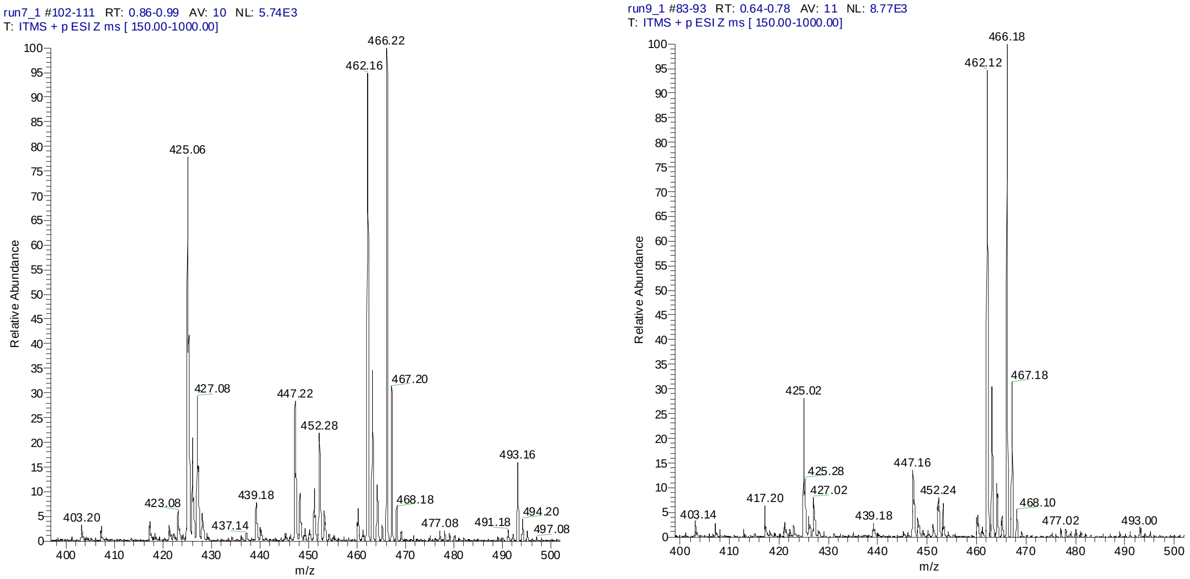
d_5 **Isotope labeling experiments.**

Kinetic isotope effect: for the second step, average KIE = $k_H / k_D = 0.91 \pm 0.07$, KIE = 0.92 ± 0.07 for the first step.

ESI-MS experiments: Competition experiments were performed in order to observe the isotope effect on the product of the reaction. Complex **1** was mixed with a 1:1 solution of ^{H}PhCOOH and ^{D}PhCOOH with 0.5 (MIX1) or 2 (MIX2) equivalents in each isotopomer versus **1** and 0.25 to 1 equivalents of hydrogen peroxide was added. No isotope effect was observed and peaks corresponding to both species were detected with the same intensity for both isomers (peaks present: 425.3 from **1**, 447.4 from (BPMEN)Fe^{II}(^{H}PhCOO), 452.4 from (BPMEN)Fe^{II}(^{D}PhCOO), 462.3 from (BPMEN)Fe^{III}(C_6H_4(O)COO) and 466.3 from (BPMEN)Fe^{III}(C_6D_4(O)COO)).



MIX1 - 0.25eq. H_2O_2 MIX2 - 0.5eq. H_2O_2



MIX1 - 0.5eq. H_2O_2 MIX2 – 1.0 H_2O_2

Fig. S13. Competition experiments.

X-ray diffraction.

For complex **2**, data were collected using a Bruker APEX CCD (charge coupled device) based diffractometer equipped with an Oxford Cyrosystream 700 series low-temperature apparatus operating at 193 K. A suitable crystal was chosen and mounted on a glass fiber using paratone oil. A total of 1305 frames were collected with a maximum resolution of 0.75 Å. Cell parameters were retrieved using SMART[3] software and refined using SAINT on all observed reflections. Data reduction was performed using the SAINT[4] software which corrects for Lorentzian polarization. No absorption corrections were applied and there were 0 systematic absence violations and 8 bad equivalents. The structure was solved by the direct method using the SHELXS-97[5] program and refined by least squares method on F^2, SHELXL-97,[6] incorporated in SHELXTL V5.10.[7] The structure was solved in the space group P2(1)/c (#14) by analysis of systematic absences. All non-hydrogen atoms were refined anisotropically. Hydrogen atoms were calculated by geometrical methods and refined as a riding model. The weighting parameters were 0.067100 and 0.000000. Successful convergence was indicated by the maximum shift/esd of 0.000 for the last cycle of least square refinement. The largest peak in the final Fourier difference map (0.92 $eÅ^{-3}$) was located at 2.21 Å from H34B. Two crystallographically unique but chemically identical complex cations of **2** were found per asymmetric unit. One perchlorate molecule was found to be disordered and was modeled in two positions with similarity restraints.

For complex **3a**, data were collected using an Enraf-Nonius CAD4 diffractometer equipped with Mo Kα radiation and operating at 294 K. A suitable crystal was chosen

and mounted on a glass fiber using epoxy. Data were collected using the Enraf–Nonius EXPRESS program,[8] and processed using the Enraf–Nonius MolEN package.[9] Crystals of this substance were typically of below-average diffraction quality; little data with I/sigmaI > 3.0 was available at theta values > 24 degrees. Neither a CCD instrument nor low temperature apparatus were available for data collection. Nonetheless the structure is quite well-defined and the results are unambiguous. Of the 9218 reflections measured, 8946 reflections were used in structure solution and refinement. Azimutal absorption corrections[10] were applied and there were 0 systematic absence violations and 0 bad equivalents. The structure was solved using direct methods (SIR92).[11] Full-matrix least-squares refinement was carried out using the Oxford University CRYSTALS-PC system[12] and SHELXL-97,[6] incorporated in SHELXTL V5.10.[7] The structure was solved in the space group P-1 (#2). All non-hydrogen atoms were refined anisotropically. Hydrogen atoms were calculated by geometrical methods and refined as a riding model. The weighting parameters were 0.028600. Successful convergence was indicated by the maximum shift/esd of 0.000 for the last cycle of least square refinement. The largest peak in the final Fourier difference map (1.33 $eÅ^{-3}$) was located at 2.16 Å from H15. Two half molecules of the counter ion cerium nitrate were found in the asymmetric unit.
Pertinent crystallography data and experimental conditions are summarized in Table S1.
A full report on structures **2** and **3a** is available from the authors as a CIF file. All drawings are done at 50% ellipsoids (Fig. 2 and S12).

	2·1.5(H_2O)	**3a·3(CH_3CN)(H_2O)**
empirical formula	C_{46} H_{58} C_{l2} Fe_2 N_8 O_{17}	C_{45} H_{58} Ce Fe_2 N_{17} O_{21}
formula weight (amu)	1177.60	1424.90
crystal habit, color	plate, blue	block, green
crystal system	monoclinic	triclinic
space group	*P2(1)/c*	*P-1*
a (Å)	15.874(5)	11.741(2)
b (Å)	11.407(4)	12.072(2)
c (Å)	29.333(10)	21.838(4)
α (deg)	90	83.52(3)
β (deg)	101.599(7)	82.33(3)
γ (deg)	90	74.75(3)
V (Å^3)	5203(3)	2949.8(10)
Z	4	2
D_c (g cm^{-3})	1.503	1.604
T (K)	193(2)	294
crystal size (mm)	0.18 x 0.18 x 0.04	0.27 x 0.22 x 0.12
R [I>2sigma]	0.0838	0.0385
R_w [I>2sigma]	0.1504	0.0807

Table S2. Crystallography data.

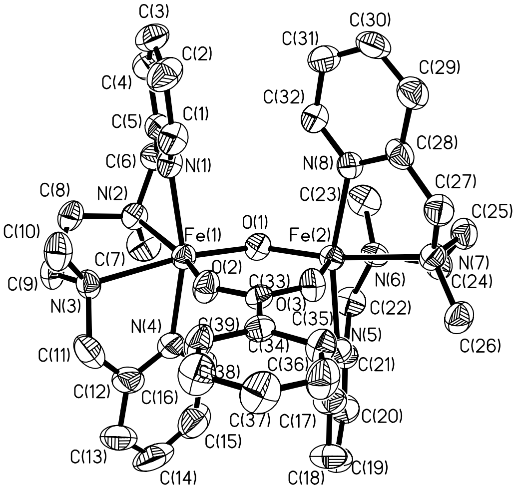


Fig. S14. X-ray structure of the complex cation in **3a**, showing 50% probability thermal ellipsoids. Hydrogen atoms are omitted for clarity.

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