# Electronic Supplementary Information

# for

# An isoelectronic NO dioxygenase reaction using a nonheme iron(III)-peroxo complex and nitrosonium ion

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## Experimental Section

**Materials and Instrumentation**. All chemicals obtained from Aldrich Chemical Co. were of the best available purity and used without further purification unless otherwise indicated. Solvents were dried according to published procedures and distilled under Ar prior to use.[S1] The [Fe(14-TMC)$(OTf)_2$] (14-TMC = 1,4,8,11-tetramethyl-1,4,8,11-tetraazacyclotetradecane) complex was prepared in a dry box according to the literature methods.[S2] Iodosylbenzene (PhIO) was prepared from iodobenzene diacetate according to a literature procedure.[S3] Air-sensitive reactions with iron complexes were performed using either a dry box or standard Schlenk techniques. UV-vis spectra were recorded on a Hewlett-Packard 8453 diode array spectrophotometer equipped with a UNISOKU Scientific Instruments for low-temperature experiments. Electrospray ionization mass spectra (ESI-MS) were collected on a Thermo Finnigan (San Jose, CA, USA) LCQ$^{TM}$ Advantage MAX quadrupole ion trap instrument, by infusing samples directly into the source using a manual method. The spray voltage was set at 4.2 kV and the capillary temperature at 80 °C. EPR spectra were taken at 5 K using an X-band Bruker EMX-plus spectrometer equipped with a dual mode cavity (ER 4116DM). Low temperatures were achieved and controlled using an Oxford Instruments ESR900 liquid He quartz cryostat with an Oxford Instruments ITC503 temperature and gas flow controller. Product analysis was performed with an Agilent Technologies 6890N gas chromatograph (GC). Cyclic voltammetry (CV) measurements were performed on an ALS 630B electrochemical analyzer, and voltammograms were measured in deaerated $CH_3CN$ containing 0.1 M $TBAPF_6$ as a supporting electrolyte at –10 ºC, with use of Pt electrode as working electrode, $Ag/AgNO_3$ as a reference electrode and a Pt wire as a counter electrode. The Pt working electrode was routinely polished with BAS polishing

alumina suspension and rinsed with $CH_3CN$ before use. The potentials were measured with respect to the $Ag/AgNO_3$ (10 mM) reference electrode and were converted to values vs SCE by adding 0.29 V.[S4] All electrochemical measurements were carried out under an atmospheric pressure of Ar. The yield of nitrate was determined by using QUANTOFIX® Nitrate/Nitrite testing tips (MACHEREY-NAGEL, Germany) and sodium nitrate solution was used as authentic samples; 1.0, 2.0, 3.0, 4.0, 5.0 and 6.0 mM aqueous solutions of $NaNO_3$ were prepared and the color change of the testing tips were recorded in each solution as a prior analysis. After the reaction, the solvent was removed and dried under vacuo. The resulting compound was redissolved in dichloromethane and extracted with known amount of sodium chloride solution (i.e., 0.5 mL) and checked the color of the testing tips (Note to use 6 mM as a maximum concentration.). The color comparison of the tips of resulting solution with that of $NaNO_3$ solutions gives the concentration of nitrate ion obtained, which then is converted to the yield of nitrate ion.

**Purification of Nitrogen Dioxide.** Nitrogen dioxide gas ($NO_2$) was obtained from Dong-A Specialty Gases and purified as follows: $NO_2$ was trapped into a Schlenk flask using liquid $N_2$ and the frozen $NO_2$ (as crystalline $N_2O_4$) was warmed with an acetone/dry-ice bath (–80 ºC) to eliminate the impurity (e.g., nitric oxide). $CH_3CN$ (15 mM) was added to the purified $NO_2$ and kept for 30 min in a closed Schlenk flask at room temperature. The concentration of the $NO_2$-$CH_3CN$ was determined by the spectroscopic absorptivity value for $NO_2$ in $CH_3CN$ ($\varepsilon$ = 64 $M^{-1}$ $cm^{-1}$ at 372 nm at –40 ºC). The $\varepsilon$ value of $NO_2$ in $CH_3CN$ was determined from the reaction of purified nitric oxide (NO)[S5] and dioxygen gas ($O_2$(g)) in

$CH_3CN$ at –40 ºC. Then, the appropriate amount of $NO_2$-$CH_3CN$ was added to reactant by gas-tight syringe.

**Synthesis of [Fe(14-TMC)($O_2$)]OTf (1).**[S6] Treatment of [Fe(14-TMC)$(OTf)_2$] (69.3 mg, 0.1 mmol) with 2 equiv. of tetraethylammonium hydroxide (28 $\mu$L, 0.2 mmol) and 5 equiv. of $H_2O_2$ (51 $\mu$L, 0.5 mmol) in 2 mL of $CH_3CN$ at –20 °C afforded the formation of a bluish-green solution. Cold $Et_2O$ (40 mL) was added to the solution at –40 °C to precipitate purple product. The solvent was removed and the purple product was washed with cold $Et_2O$ several times. Then, the purple product was dried in vacuo. Yield: 80% (3.95 mg, 0.08 mmol). UV-vis ($\varepsilon$, $M^{-1}$ $cm^{-1}$) in $CH_3CN$ at –10 °C: 868 nm (600).

**Synthesis of [Fe(14-TMC)(O)($CH_3CN$)]$(OTf)_2$ (5).**[S6] [$Fe^{II}$(14-TMC)$(OTf)_2$] (50mg, 78 $\mu$mol) was dissolved in $CH_3CN$ (1 mL) at room temperature, and iodosylbenzene (20 mg, 227 $\mu$mol) was added. The solution was stirred for 1 h at room temperature and filtered. To this filtrate, cold $Et_2O$ (50 mL) was added at –40 ºC to precipitate green product, and the solvent was removed. After washing with cold $Et_2O$ (50 mL) several times, the green product was dried under vacuum at –40 ºC. Yield: 58% (30 mg, 45 $\mu$mol). UV-vis ($\varepsilon$, $M^{-1}$ $cm^{-1}$) in $CH_3CN$ at –10 °C: 820 nm (400).

**Synthesis of [Fe(14-TMC)(O)(F)](OTf) (7).** The Complex **5** (30mg, 45 $\mu$mol) was dissolved in 0.5 mL of $CH_3CN$ at –30 ºC, and tetrabuylammonium fluoride (TBAF) (15 mg, 54 $\mu$mol) in 0.5 mL of $CH_3CN$ was added. After addition of TBAF, the color of the solution changed from green to orange. Addition of cold $Et_2O$ (20 mL) to the reaction mixture resulted in an orange precipitate, and the solvent was then removed. The resulted orange solid was

washed with $Et_2O$ several times, and dried under vacuum. Yield: 90% (20 mg, 40 $\mu$mol). UV-vis ($\varepsilon$, $M^{-1}$ $cm^{-1}$) in $CH_3CN$ at –10 °C: 812 nm (53), 1015 (69) (Figure S7).

**Reactivity Study.** All reactions were run in an UV cuvette and monitored using UV-vis spectroscopy on the reaction solutions. Reactions were run at least in triplicate, and the data reported represents the average results observed for these reactions. Preparation of samples and all reaction experiments with nitrosonium hexafluorophosphate ($NOPF_6$) were performed under an inert gas in a dry box. The purity of 2,4-di-*tert*-butylphenol (DTBP) was checked with GC prior to use. Products were analyzed by injecting the reaction mixture directly into GC. Products were identified by comparing with authentic samples, and product yields were determined by comparison against standard curves prepared with authentic samples and using decane as an internal standard.

**Reaction of Fe(III)-Peroxo Complex (1) and Nitrosonium Ion ($NO^+$).** [Fe(14-TMC)($O_2$)](OTf) (**1**) was dissolved in $CH_3CN$ (1 mM) at –10 °C under Ar, and 1.2 equiv. of $NOPF_6$ (1 mM) was added under Ar. UV-vis spectroscopy was used to monitor the reaction (Figure 1a). The yield of nitrate was estimated to be 67%.

**Reaction of Fe(IV)-Oxo Complex (4) and $NO_2$.** To the complex **4** (1 mM, 2 mL) in $CH_3CN$ at –10 ºC under Ar in the presence and absence of 1 mM $TBAPF_6$, 5 equiv. of $NO_2$ was added and stirred for 30 min. UV-vis spectral changes were monitored and depicted in Figure S4. To this solution, 1.2 equiv. of TBAF (tetrabutylammonium fluoride) was added and stirred for 30 min. The yield of nitrate was estimated as 67%.

**Reaction of Fe(IV)-Oxo Complex Bonding Fluoride Axial Ligand (7) and $NO_2$.** $NO_2$ (5 equiv.) was added to the solution of **7** (1 mM, 2 mL) in $CH_3CN$ at –10 ºC under Ar, and its

UV-vis spectral changes were monitored (Figure S8). The yield of nitrate was estimated to be 67%.

**Nitration of 2,4-Di-*t*-Butylphenol (DTBP)**. The reaction was carried out in a closed system. The solution of $NOPF_6$ (1.2 mM) was prepared in an UV cuvette fitted with a septum at –10 °C under Ar. To this solution, $[Fe(14\text{-}TMC)(O_2)]^+$ (**1**) (1 mM) in $CH_3CN$ was added by a gas tight syringe. Subsequent to the formation of the Fe(IV)-oxo complex, $[Fe(14\text{-}TMC)(O)]^{2+}$ (**2**), 2 equiv. of DTBP in $CH_3CN$ were added by a gas tight syringe and stirred for 20 min**.** Product analysis was performed by GC, and it was found that 2,4-di-*t*-butyl-6-nitrophenol (nitro-DTBP) and 3,3',5,5'-tetra-*t*-butyl-(1,1'-biphenyl)-2,2'-diol (DTBP-dimer) formed in 65% and 3% yield, respectively (Figure S2). When the above reaction was carried out in an open UV cuvette, nitro-DTBP and DTBP-dimer were found to be formed in 38% and 0% yield, respectively.

## References

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S3. H. Sahzman, J. G. Sharefkin, *Organic Synthesis*, Collect. 5th ed., Wiley: New York, 1973.

S4. Mann, C. K.; Barnes, K. K. *Electrochemical Reactions in Nonaqueous Systems*; Marcel Dekker: New York, 1990.

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S6. (a) J. Cho, S. Jeon, S. A. Wilson, L. V. Liu, E. A. Kang, J. J. Braymer, M. H. Lim, B. Hedman, K. O. Hodgson, J. S. Valentine, E. I. Solomon, W. Nam, *Nature* 2011, **478**, 502. (b) J. Annaraj, Y. Suh, M. S. Seo, S. O. Kim, W. Nam, *Chem. Commun.* 2005, 4529. (c) M. S. Seo, J. Y. Kim, J. Annaraj, Y. Kim, Y.-M. Lee, S.-J. Kim, J. Kim, W. Nam, *Angew. Chem. Int. Ed*. 2007, **46**, 377. (d) W. L. F. Armarego, C. L. L. Chai, *Purification of Laboratory Chemicals*, 5th ed., Butterworth-Heiemann, Oxford, 2003.

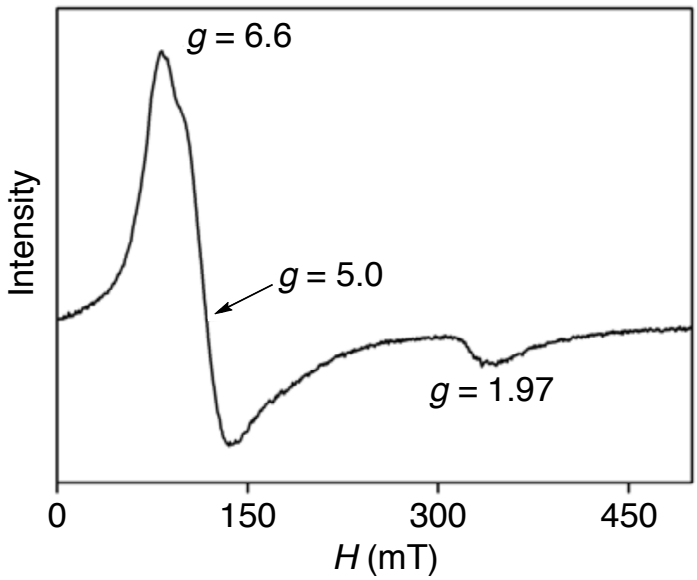


**Fig. S1.** EPR spectrum of the reaction solution of $[Fe(14\text{-}TMC)(O_2)]^+$ (**1**) (1 mM) and 1.2 equiv. of $NOPF_6$ in $CH_3CN$ at 5 K. Experimental parameters: Power, 1.0 mW; Frequency, 9.647 GHz; Receive Gain, $1.0 \times 10^4$; Modulation Frequency, 100 kHz; Modulation Amplitude, 10 G.

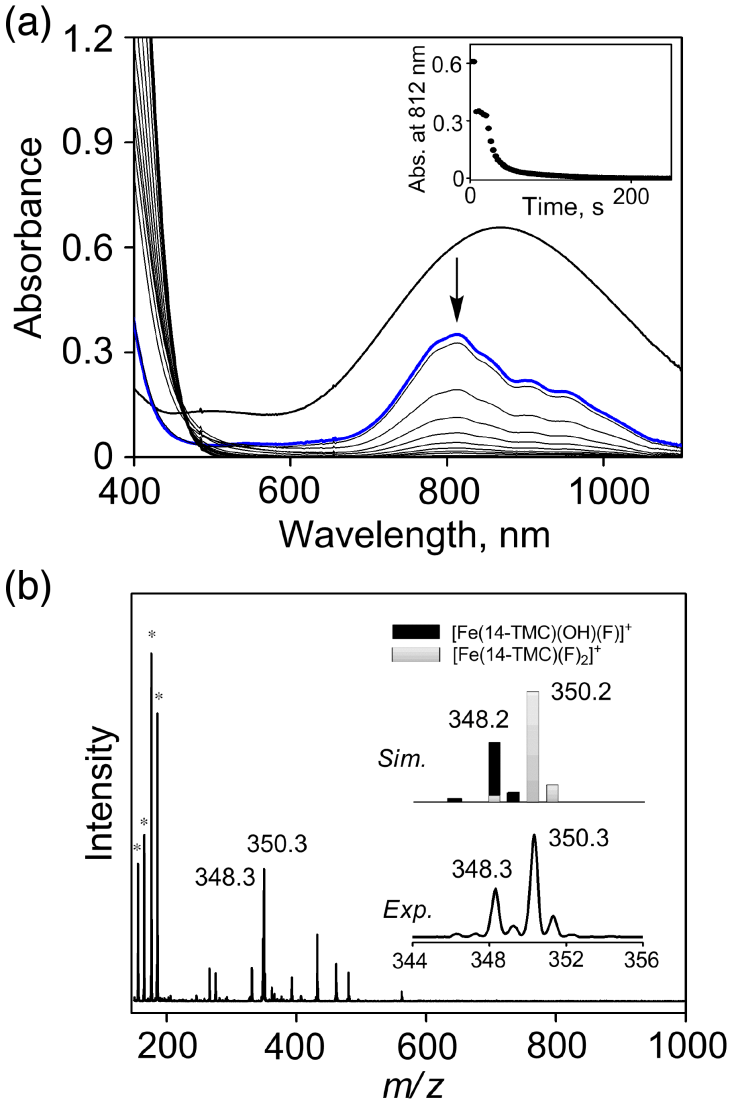


**Fig. S2**. (a) UV-vis spectral changes of the reaction of $[Fe^{III}(14\text{-TMC})(O_2)]^+$ (**1**) (1 mM) and 1.2 equiv. of $NOPF_6$, followed by the addition of 2 equiv. of DTBP in $CH_3CN$ at –10 °C under Ar. Inset shows the time course of the absorbance at 812 nm. (b) ESI-MS spectrum of the resulting solution. Insets show the experimental (lower) and simulated (upper) spectra of the resulting solution in the region of *m/z* 344 – 356. The ion peaks at *m/z* of 348.3 and 350.3 correspond to $[Fe^{III}(14\text{-TMC})(OH)(F)]^+$ (**8**) and $[Fe^{III}(14\text{-TMC})(F)_2]^+$, respectively. The peaks at *m/z* of 156.2, 165.6, 176.5, and 185.9 with an asterisk were assigned to $[Fe^{II}(14\text{-TMC})]^{2+}$, $[Fe^{III}(14\text{-TMC})(F)]^{2+}$, $[Fe^{II}(14\text{-TMC})(CH_3CN)]^{2+}$, and $[Fe^{III}(14\text{-TMC})(CH_3CN)(F)]^{2+}$, respectively.

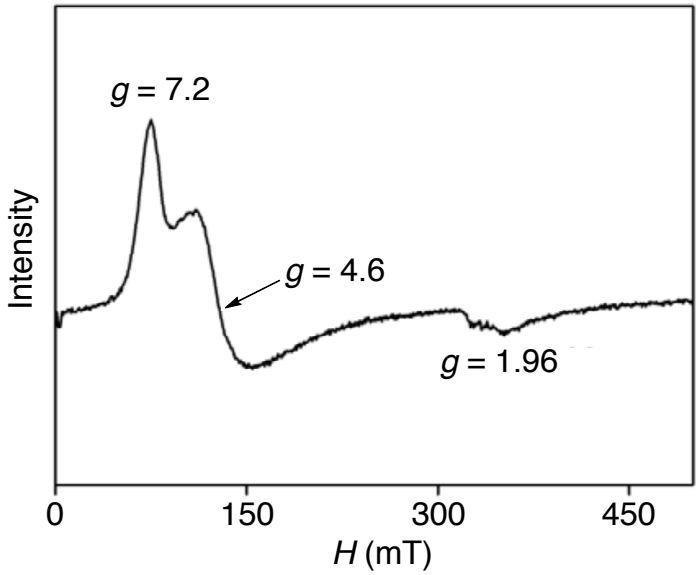


**Fig. S3.** EPR spectrum of the reaction solution of $[Fe^{III}(14\text{-TMC})(O_2)]^+$ (**1**) (1 mM) and 1.2 equiv. of $NOPF_6$, followed by the addition of 2 equiv. of DTBP in frozen $CH_3CN$ at 5 K. Experimental parameters: Power, 1.0 mW; Frequency, 9.647 GHz; Receive Gain, $1.0 \times 10^4$; Modulation Frequency, 100 kHz; Modulation Amplitude, 10 G.

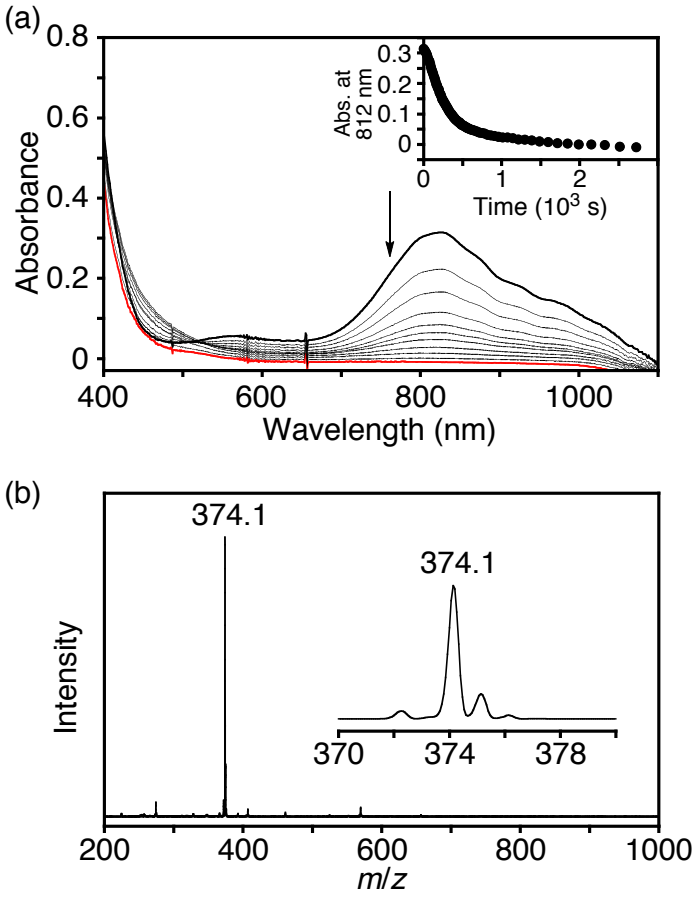


**Fig. S4.** (a) UV-vis spectral changes of $[Fe^{IV}(14\text{-TMC})(O)(CH_3CN)]^{2+}$ (1 mM) upon addition of $NO_2$ (5 equiv.) in $CH_3CN$ at –10 ºC. (b) ESI-MS spectrum of the resulting solution. Inset shows the region of *m/z* 370 – 380. The ion peak at *m/z* of 374.1 corresponds to $[Fe^{II}(14\text{-TMC})(NO_3)]^+$ (calcd. *m/z* of 374.3).

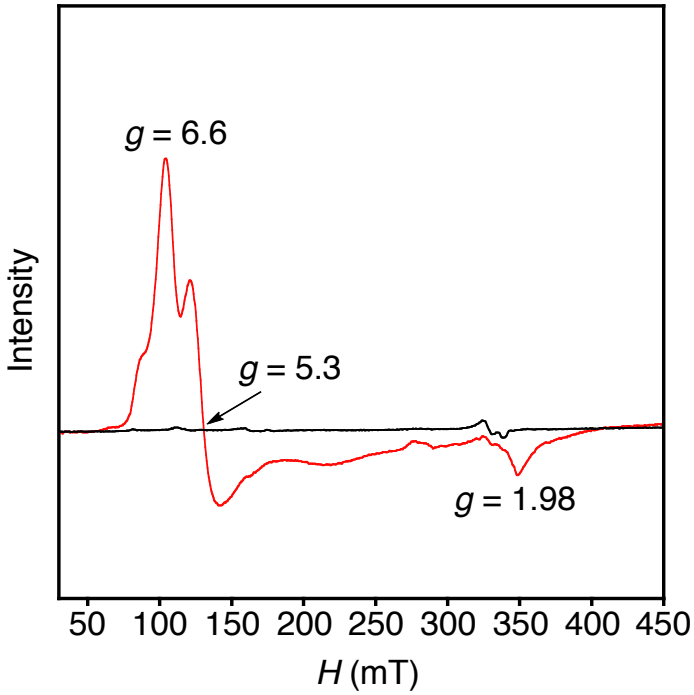


**Fig. S5.** EPR spectra of the reaction solution of $[Fe^{IV}(14\text{-TMC})(O)(CH_3CN)]^+$ (**5**) and 5 equiv. of $NO_2$ (black) and $[Fe^{II}(14\text{-TMC})(NO_3)]^+$ (**6**) with 1.2 equiv. of TBAF (red) in frozen $CH_3CN$ at 5 K. Experimental parameters; Power, 1.0 mW; Frequency, 9.647 GHz; Receive Gain, $1.0 \times 10^4$; Modulation Frequency, 100 kHz; Modulation Amplitude, 10 G.

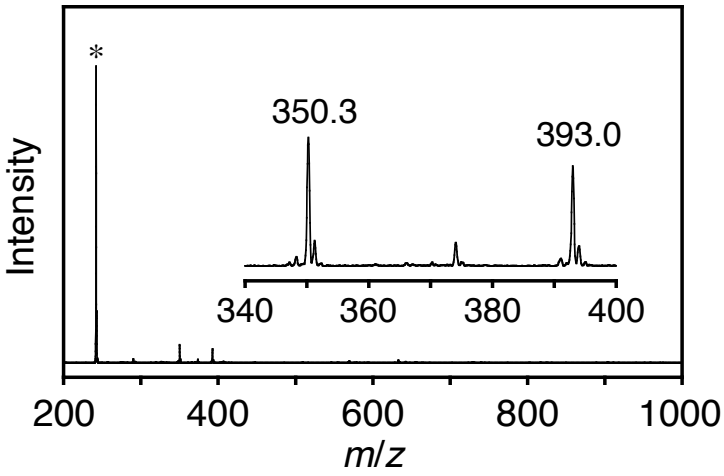


**Fig. S6**. ESI-MS spectrum of the reaction solution of $[Fe^{II}(14\text{-TMC})(NO_3)]^+$ (**5**) (1 mM) with TBAF (1.2 mM) in $CH_3CN$ at –10 ºC. Inset shows the region of *m/z* 340 – 400. The ion peaks at *m/z* of 350.3 and 393.0 correspond to $[Fe^{III}(14\text{-TMC})(F)_2]^+$ (calcd. *m/z* of 350.3) and $[Fe^{III}(14\text{-TMC})(NO_3)(F)]^+$ (calcd. *m/z* of 393.3), respectively. The peak at *m/z* of 242.3 with asterisk was assigned to $TBA^+$ (tetrabutylammonium ion).

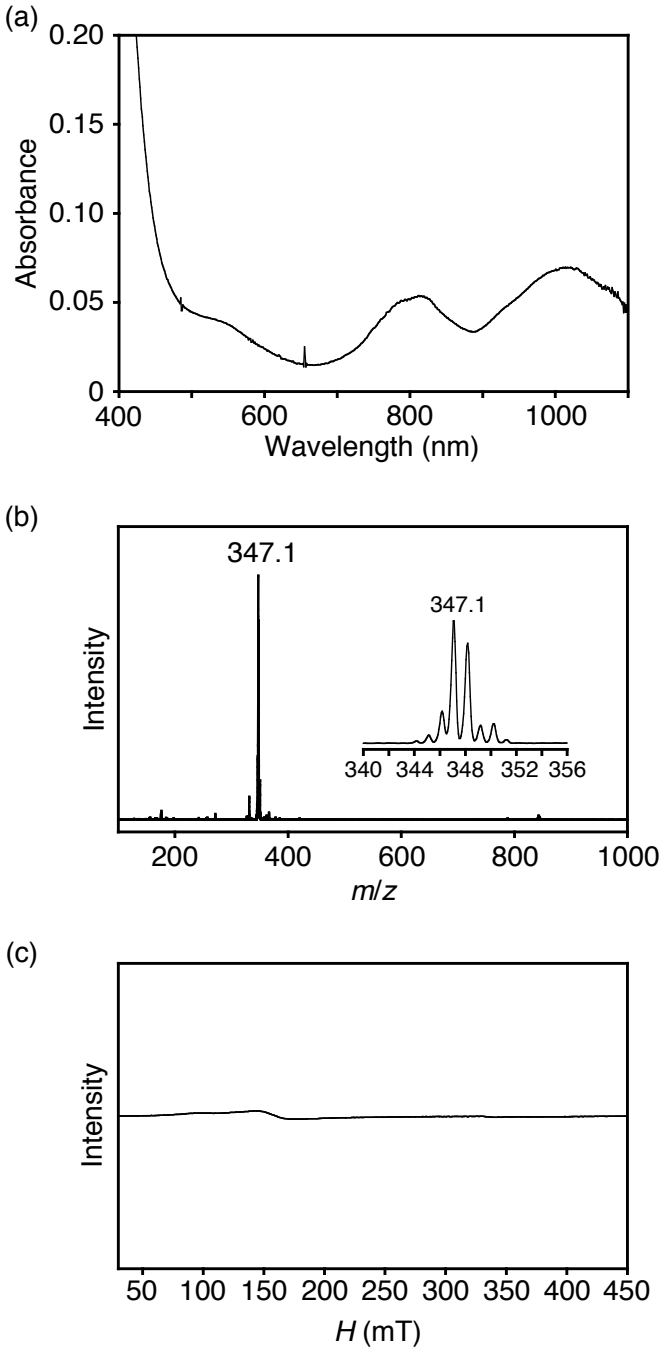


**Fig. S7.** (a) UV-vis spectrum of $[Fe^{IV}(14\text{-TMC})(O)(F)]^+$ (**7**) in $CH_3CN$ (1 mM) at –10 ºC under Ar. (b) ESI-MS spectrum of **7** (calcd. *m/z* of 347.2). Inset shows isotope distribution pattern for **7** at *m/z* of 347.1. (c) EPR spectrum of **7** in frozen $CH_3CN$ at 5 K. Experimental parameters; Power, 1.0 mW; Frequency, 9.647 GHz; Receive Gain, $1.0 \times 10^4$; Modulation Frequency, 100 kHz; Modulation Amplitude, 10 G.

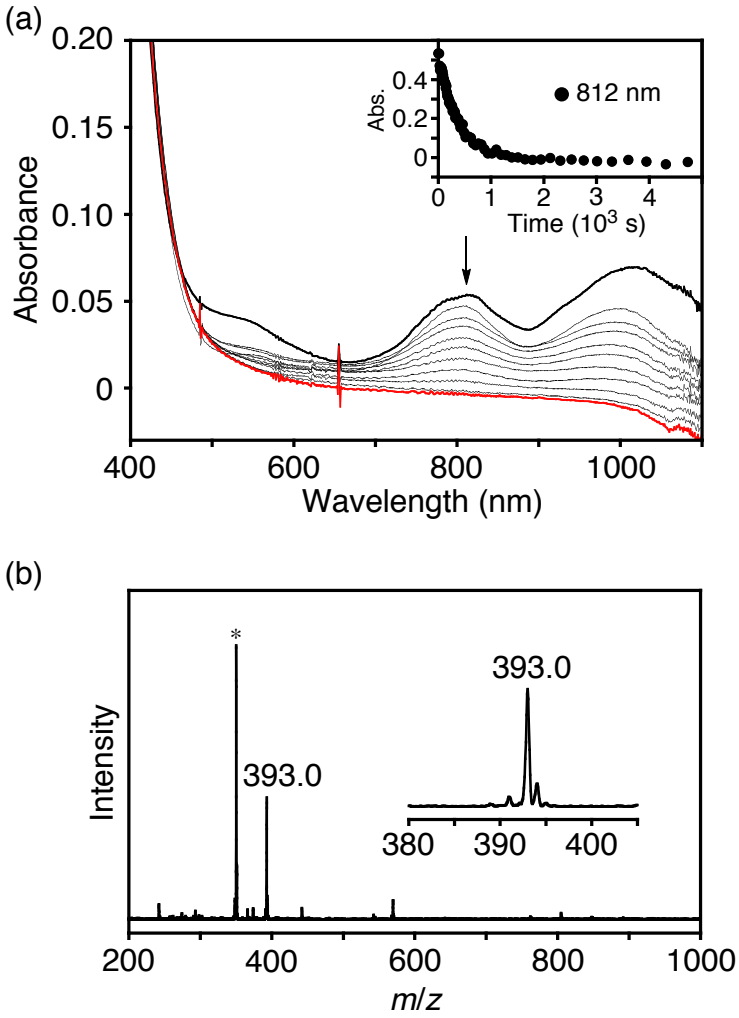


**Fig. S8.** (a) UV-vis spectral changes of $[Fe^{IV}(14\text{-TMC})(O)(F)]^+$ (**7**) (1 mM) upon addition of 5 equiv. of $NO_2$ in $CH_3CN$ at –10 ºC under Ar. Inset shows time course of the absorbance change at 812 nm. (b) ESI-MS spectrum of the resulting solution. Inset shows the region of *m/z* 380 – 405. The ion peak at *m/z* of 393.0 corresponds to $[Fe^{III}(14\text{-TMC})(NO_3)(F)]^+$ (calcd. *m/z* of 393.2). The peak at *m/z* of 350.2 with asterisk was assigned to $[Fe(14\text{-TMC})(F)_2]^+$ (calcd. *m/z* of 350.2).

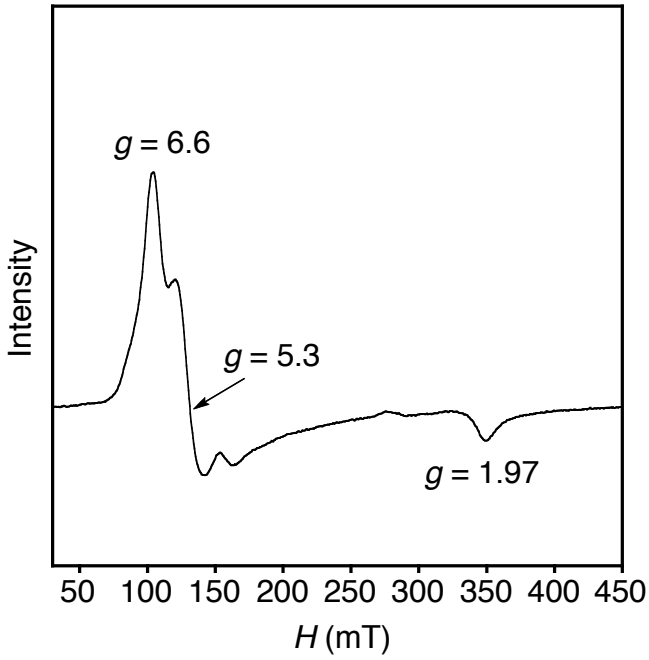


**Fig. S9.** EPR spectrum of the reaction solution of $[Fe^{IV}(14\text{-TMC})(O)(F)]^+$ (**7**) (1 mM) with 5 equiv. of $NO_2$ in frozen $CH_3CN$ at 5 K. Experimental parameters; Power, 1.0 mW; Frequency, 9.647 GHz; Receive Gain, $1.0 \times 10^4$; Modulation Frequency, 100 kHz; Modulation Amplitude, 10 G.

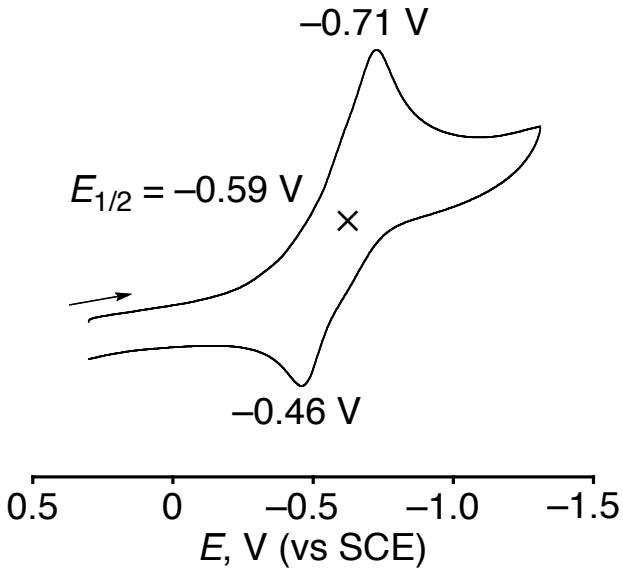


**Figure S10.** Cyclic voltammogram of [$Fe^{IV}$(14-TMC)(O)(F)](OTf) (**7**) in $CH_3CN$ at –10 ºC under Ar in the presence of 0.1 M $TBAPF_6$.