Electronic Supplementary Information for:

Spin Crossover Iron(II) Complexes as PARACEST MRI Thermometers

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Chemical Science

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Experimental Section

General Considerations. The manipulations described below were performed under a dinitrogen atmosphere in a Vacuum Atmospheres Nexus II glovebox or using a standard Schlenk line. Diethyl ether was dried using a commercial solvent purification system from Pure Process Technology. Ethanol, nitromethane, and acetone were deoxygenated by three successive freeze-pump-thaw cycles. Deionized water was deoxygenated by vigorous stirring under reduced pressure for 3 hours. The compounds 2,6-di(pyrazol-3-yl)pyridine (3-bpp), 4-dimethylamino-2,6-*bis*(1,1-*bis*(2-pyridyl)ethyl)pyridine (Me₂NPY5Me₂), and [Fe(3-bpp)₂](BF₄)₂·3Et₂O (1) were prepared as reported previously.^{1,23} All other reagents were purchased from commercial vendors and used without further purification.

[(Me₂NPY5Me₂)Fe(H₂O)](BF₄)₂·H₂O (2). Fe(BF₄)₂·6H₂O (68 mg, 0.20 mmol) and Me₂NPY5Me₂ (97 mg, 0.20 mmol) were added to a 9:1 mixture of acetone/water (10 mL). The resulting brown solution was stirred for 3 hours at ambient temperature and then then dried under reduced pressure. The olive green solid residue was extracted into a mixture of 9:1 acetone/water (2 mL). Diffusion of diethyl ether vapor into the resulting solution yielded dark green block-shaped crystals of $2 \cdot 3H_2O$ suitable for X-ray analysis. These crystals were then collected on a nylon membrane filter (0.22 μm), washed with successive aliquots of Et₂O (2 × 2 mL), and then allowed to dry on the filter for 30 minutes to yield 95 mg (63%) of **2**. FT-IR (ATR, cm⁻¹): 3426(w); 1619(m); 1601(m); 1525(w); 1467(m); 1440(m); 1375(w); 1051(s, br); 861(w); 817(w); 797(w); 755(m); 664(m); 631(w). Anal. Calcd. for C₃₁H₃₄F₈FeN₆O₂B₂ C 49.5, H 4.56, N 11.2 %.

X-ray Structure Determination. A single crystal of 2 suitable for X-ray analysis was directly coated with Paratone-N oil and mounted on a MicroMountsTM rod. The crystallographic data were collected at 100 K on a Bruker APEX II diffractometer equipped with MoKa sealed tube source. Raw data were integrated and corrected for Lorentz and polarization effects using Bruker APEX2 v. 2009.1.⁴ Absorption corrections were applied using SADABS.⁵ Space group assignments were determined by examination of systematic absences, E-statistics, and successive refinement of the structures. Structures were solved with SIR-92⁶ and further refined with SHELXL⁷ operated with the WIN-GX interface.⁸ The hydrogen atoms for the water ligand were located in the difference map and refined, and hydrogen atoms for the interstitial water molecules were not placed but are considered in the final formula. Disordered lattice water molecules are also present in the void space, but the exact localization could not be determined based on the difference Fourier map. Therefore, the program SQUEEZE⁹, part of the PLATON¹⁰ package, was used to calculate the solvent disorder area. Two additional water molecules per unit cell were estimated based on 34 electrons in a void volume of 87 Å³, which were treated as a diffuse contribution to the overall scattering without specific atom positions. These molecules were included in the final molecular formula directly. All other hydrogen atoms were placed at calculated positions using suitable riding models and refined using isotropic displacement parameters derived from their parent atoms. Thermal parameters were refined anisotropically for all non-hydrogen atoms. Crystallographic data and the details of data collection are listed in Table S1.

CEST experiments. Variable temperature z-spectra were recorded on a Varian Inova 400 MHz (9.4 T) spectrometer at different temperatures. Samples were prepared and maintained under a dinitrogen atmosphere to prevent decomposition over time. After temperature stabilization for at lest 5 minutes at each temperature, NMR spectra were acquired using the presaturation pulse applied for 2 seconds at a power level of 6 μ T for **1** and 21 μ T for **2**. The saturation frequency offsets were screened with a step

increase of 0.4 ppm for **1** and 1 ppm for **2**, respectively. The obtained NMR spectra at one temperature were plotted as normalized water intensity against frequency offset to produce a Z-spectrum. Direct saturation of the water signal was set to 0 ppm. D_2O was placed in an inner capillary to lock the sample. Exchange rate constants were calculated by using a previously reported procedure.¹¹ The NMR spectra were acquired at different presaturation pulse powers between 4.3 and 24 μ T applied for 6 seconds. Samples of **1** and **2** contained 10 mM of complex in H₂O and 1.6 mM of complex in 3 mM MES pH 7 buffer, respectively.

Imaging experiments. Temperature imaging was performed on a Bruker Biospec 9.4T MRI scanner, using a 40-mm Bruker volume RF coil. A Rapid Acquisition with Relaxation Enhancement (RARE, spin echo) sequence was modified by Bruker to add a constant saturation pulse, prior to the imaging pulses. The phantom containing 1.6 mM of **2** in 3 mM MES pH 7 buffer solution was heated by a hot water blanket connected to a circulating bath. Temperature was monitored using a MRI-compatible thermocouple with the animal monitoring system (SA Instruments, Inc., Stony Brook, NY). The temperature was allowed to stabilize for at least 5 minutes at each temperature. The images were acquired with the following parameters: TR = 2100 ms, TE = 17 ms, FOV = 30 mm, matrix = 128 × 128, slice thickness = 2 mm with a transaxial orientation, saturation irradiation power of 10 μ T, saturation time of 2 s. Saturation offset frequencies from -8 kHz to 30 kHz were probed using 35 images. Both pixel-by-pixel and region of interest analysis for the temperature images was performed using MATLAB v8.0 (The Mathworks Inc., Natick, MA).

Magnetic Measurements. Magnetic measurements of **2** in solid state were performed on a microcrystalline sample sealed in a polyethylene bag under a dinitrogen atmosphere. All data were collected using a Quantum Design MPMS-XL SQUID magnetometer. For solution samples of **1** and **2**, Evans method¹² was used to determine the magnetic moment in H₂O by collecting variable temperature ¹H NMR spectra using a on a Varian Inova 400 MHz (9.4 T) spectrometer. The compounds **1** and **2** were dissolved in H₂O containing dimethyl sulfoxide (DMSO) as a reference and the resulting solution was placed in a NMR tube with a sealed capillary including a solution of H₂O and DMSO (1.7 v/v % of DMSO). Two measurements were averaged to afford the resulting data. All dc susceptibility data were corrected for diamagnetic contributions from the sample holder and for the core diamagnetism of each sample (estimated using Pascal's constants¹³).

Other Physical Measurements. Elemental analyses of **2** were performed by the Midwest Microlab (Indianapolis, IN). Infrared spectra were recorded on a Bruker Alpha FTIR spectrometer equipped with an attenuated total reflectance accessory. UV/Visible spectra were obtained using a Varian Cary 5000 spectrophotometer. Thermogravimetric analysis was performed on a TA instrument Discovery TGA from 28 to 400 °C with a scan rate of 2 °C/min.

 Table S1 | Crystallographic data of 2.

	2
Empirical formula	$C_{31}H_{42}B_2F_8FeN_6O_6$
Formula weight, g mol ⁻¹	824.18
Crystal system	Triclinic
Space group	<i>P</i> -1
Wavelength, Å	0.71073
Temperature, K	100
<i>a</i> , Å	11.904(7)
b, Å	12.224(7)
<i>c</i> , Å	13.994(8)
<i>α</i> , [°]	107.153(1)
β, °	110.035(1)
γ, [°]	103.564(1)
V, Å ³	1695.5(17)
Ζ	2
$ ho_{ m calcd},{ m Mg}\;{ m m}^{-3}$	1.579
μ , mm ⁻¹	0.540
Refections coll./unique	31343/9869
R(int)	0.0304
${}^{a}R_{1} (I > 2\sigma(I))$	0.0444
$^{\mathrm{b}}\mathrm{w}R_{2}$ (all)	0.1148
GoF	1.065

 ${}^{a}R_{1} = \Sigma ||F_{0}| - |F_{C}|| \Sigma |F_{0}|, \text{ and } {}^{b}wR_{2} = [\Sigma w (F_{0}^{2} - F_{C}^{2})^{2} / \Sigma w (F_{0}^{2})^{2}]^{1/2}$



Figure S1 | Crystal structure of $[(Me_2NPY5Me_2)Fe(H_2O)]^{2+}$, as observed in 2·2H₂O, recorded at 100 K. Thermal ellipsoids are shown at the 50% probability level. Orange, red, blue, and dark gray ellipsoids correspond to Fe, O, N, and C atoms, respectively. Hydrogen atoms are omitted for clarity.



Figure S2 | Variable-temperature dc magnetic susceptibility data for a solid sample of **2**, collected under an applied field of 1 T. The solid black line is a fit to the data using the ideal solution model.¹⁴



Figure S3 | ¹H-NMR spectra of **2** in D₂O, obtained at 35 $^{\circ}$ C (top) and 60 $^{\circ}$ C (bottom).



Figure S4 | Omega plot of $M_Z/(M_0-M_Z)$ versus $1/\omega^2$, recorded at 9.4 T for 10 mM of **1** (red) and 1.6 mM of **2** (blue) at 25 °C, using a 6 s pre-saturation pulse.



Figure S5 | Variable-temperature exchange rate (k_{ex}), obtained from Omega plot method, for 10 mM of **1** (red) and 1.6 mM of **2** (blue) using a 6 s pre-saturation pulse.



Figure S6 | Correlation of temperature of phantom containing 1.6 mM of **2** obtained from imaging experiment versus temperature obtained independently using thermocouple.



Figure S7 | UV-visible spectra of 1 in aerobic H₂O (top) and 2 in pH 7 MES buffer (bottom) at 298 K.



Figure S8 | Thermogravimetric analysis of 2, consistent with loss of one H_2O molecule.

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