

Supporting Information

A pair of highly biotolerated diamagnetic and paramagnetic iron(II) complexes displaying electroneutrality

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Synthetic protocols

Experimental

Available reagents and solvents were purchased from Aldrich, Acros, and Alfa Aesar and used without further purification. All solvents used for complexation were dried by activated molecular sieves and degassed with the method "freeze-pump-thaw". Column chromatography was performed using Merck silicagel Si-60 (40~63 μm) or Acros activated neutral Alumina (60 \AA , 50~200 μm). ^1H and ^{13}C NMR spectra were acquired on a Bruker DPX 300 or 500 instrument (300 or 500, and 75 or 100 MHz, respectively) at 298 K. Chemical shifts (δ) are reported in ppm (s = singlet, d = doublet, t = triplet, m = multiplet, br = broad) and referenced to the solvent peak. NMR coupling constants (J) are reported in Hertz. HRMS spectra were acquired by the "Centre Commun de Spectrométrie de Masse" of Université Claude Bernard Lyon 1 (France).

1-picolyl-1,4,7-triazacyclononane (7) Picolyl chloride hydrochloride (3.2 g, 19.51 mmol) was neutralized with 40 mL NaOH (1 M) and extracted three times with diethylether. The combined ethereal phases were washed with brine, dried over anhydrous sodium sulfate, and evaporated to dryness to give 2.4 g of a pink oil as picolyl chloride (98%). 1.81 g (14.37 mmol) of this material was dissolved in 4 mL THF and added dropwise to a solution of orthoamide **6** (2.0 g, 14.37 mmol) in 5 mL THF at 0 $^\circ\text{C}$ under argon causing a colorless precipitate to form. The mixture was stirred for another 4 days at room temperature. The precipitate was filtered off and washed with dry THF to give a colorless powder which was subsequently dissolved in 15 mL H_2O and heated to 100 $^\circ\text{C}$ overnight. The resulting mixture was evaporated to dryness and the residue dissolved in 15 mL EtOH. KOH (2.0 g) was added and the suspension refluxed overnight. The mixture was evaporated to dryness before being separated between 15 mL water and Et_2O . The combined organic phases were washed with brine, dried over anhydrous sodium sulfate and evaporated to dryness. The obtained light yellow oil was dissolved in 20 mL anhydrous EtOH and treated with 6 mL conc. HCl to give a brown solution. This solution was again evaporated to dryness before 40 mL EtOH were added to produce a precipitate which was subsequently filtered and washed with Et_2O to give **7** in the form of a pink powder (4.14 g; 94 %). ^1H NMR (300 MHz, D_2O) δ 8.62 (1H, dd, J = 5.4, 0.8 Hz), 8.42 (1H, t, J = 7.9 Hz), 7.92 (1H, d, J = 8.0 Hz), 7.84 (1H, t, J = 6.8 Hz), 4.17 (2H, s), 3.57 (4H, s), 3.25 (4H, t, J = 5.6 Hz), 2.98 (4H, t, J = 5.6 Hz); ^{13}C NMR (75 MHz, D_2O) δ 150.56, 146.94, 142.22, 127.99, 126.42, 55.32, 48.06, 43.87, 42.52; ESI-MS: calcd. for $\text{C}_{12}\text{H}_{21}\text{N}_4$ [M + H] 221.1761; found 221.1762.

1-benzyl-1,4,7-triazacyclononane hydrochloride (8). A solution of **6** (1.70 g, 12.2 mmol) in anhydrous THF (8 mL) at 0 $^\circ\text{C}$ was treated dropwise with a solution of benzyl bromide (2.0 g, 12.2 mmol) in 5 mL THF. The mixture was stirred at room temperature for one day before the formed colorless precipitate was filtered off and washed with anhydrous THF. The precipitate was dissolved in 15 mL H_2O and 2.1 g KOH (s) were added. The resulting solution was stirred at 100 $^\circ\text{C}$ overnight before being extracted three times with Et_2O . The combined ethereal phases were then dried over anhydrous sodium sulfate before being evaporated to dryness. The oily residue was dissolved in 20 mL anhydrous EtOH and treated with 5 mL concentrated HCl. Evaporation of all volatiles gave a colorless powder which was washed with 30 mL EtOH and Et_2O to give product **8** (2.52 g; 63 %). ^1H NMR (300 MHz, D_2O) δ 7.34 (5H, s), 3.82 (2H, s), 3.51 (4H, s), 3.10

(4H, t, $J = 5.8$ Hz), 2.92 (4H, t, $J = 5.8$ Hz); ^{13}C NMR (75 MHz, D_2O) δ 135.25, 130.23, 128.83, 128.33, 59.08, 47.68, 43.61, 42.20; ESI-MS: calcd. for $\text{C}_{13}\text{H}_{22}\text{N}_3$ [M + H] 220.1808; found 220.1804.

Ethyl 6-Chloromethyl-pyrid-3-yl-carboxylate (9)

Thionyl chloride (66 mL, 0.91 mol) was added to a suspension of 2,5-pyridinedicarboxylic acid (20 g, 0.12 mol) in 200 mL anhydrous EtOH in a 500 mL flask at room temperature. The resulting mixture was refluxed at 80 °C for 16 h. Excess SOCl_2 was distilled off before the remainder was poured on ice and extracted with DCM three times. The combined organic phases were washed with saturated NaHCO_3 twice and with brine once, then dried over anhydrous Na_2SO_4 and finally concentrated under vacuum. The resulting oil was superposed by a layer of petroleum ether and vigorously stirred to result in a light-yellow precipitate which was filtered. Yield of the intermediate diester : 25 g, 93.6%. ^1H NMR (300 MHz, CDCl_3) δ 9.33 (1H, $J = 1.7$ Hz), 8.44 (1H, dd, $J = 8.1, 2.0$ Hz), 8.20 (1H, d, $J = 8.1$ Hz), 4.56-4.43 (4H, m), 1.50-1.42 (6H, m); ^{13}C NMR (75 MHz, CDCl_3) δ 164.54, 164.46, 151.13, 150.82, 138.19, 128.78, 124.60, 62.40, 61.93, 14.30, 14.24

To a solution of diethyl pyridine-2,5-dicarboxylate (5.0 g, 0.02 mol) in 50 mL anhydrous EtOH was added NaBH_4 (0.5 g, 0.013 mol) and anhydrous CaCl_2 (2.49 g, 0.02 mol). The suspension was stirred at room temperature overnight then evaporated to dryness. The residue was purified by chromatography on silica gel to give ethyl 6-(hydroxymethyl)-pyrid-3-yl-carboxylate (3.36 g, 82.7%). ^1H NMR (300 MHz, CDCl_3) δ 9.15 (1H, d, $J = 1.5$ Hz), 8.28 (1H, dd, $J = 8.1, 2.0$ Hz), 7.38 (1H, d, $J = 8.1$ Hz), 4.84 (2H, s), 4.38 (2H, q, $J = 7.1$ Hz), 3.90 (1H, s), 1.40 (3H, t, $J = 7.1$ Hz); ^{13}C NMR (75 MHz, CDCl_3) δ 165.15, 163.59, 149.91, 137.76, 125.18, 119.99, 64.30, 61.45, 14.27

To a suspension of ethyl 6-(hydroxy-methyl)-pyrid-3-yl-carboxylate (1.75 g, 9.65 mmol) in 15 mL CCl_4 , was added dropwise $\text{P}(\text{nBu})_3$ (3.60 mL, 14.48 mmol) During further stirring at room temperature for 30 min the mixture became clear. It was then concentrated to dryness and subjected to chromatography on silica gel to give target **9** as a light yellow oily (1.82 g, 94.3%). ^1H NMR (200 MHz, CDCl_3) δ 9.03 (1H, s), 8.17 (1H, dd, $J = 8.2, 2.0$ Hz), 7.44 (1H, d, $J = 8.2$ Hz), 4.60 (2H, s), 4.24 (2H, q, $J = 7.1$ Hz), 1.26 (3H, t, $J = 7.1$ Hz); ^{13}C NMR (50 MHz, CDCl_3) δ 164.34, 160.10, 150.05, 137.69, 125.16, 121.79, 61.07, 45.74, 13.82

1,4-Bis(5-ethoxycarbonyl-2-picolyl)-7-picolyl-1,4,7-triazacyclononane (10a). Compound **7** (0.6 g, 1.82 mmol) was dissolved in 10 mL NaOH (1 M) before being extracted three times with dichloromethane. The combined organic phases were washed with brine, dried over anhydrous sodium sulfate and evaporated to dryness to give 0.4 g of a light yellow oil. A solution of this oil (0.4 g, 1.82 mmol) in 16 mL CH_3CN and 4 mL H_2O was treated with ethyl chloromethylpyridylcarboxylate **9** (0.54 g, 2.73 mmol) and K_2CO_3 (0.5 g, 3.64 mmol). The mixture was heated to 50 °C for 2h before being quenched with 10 mL H_2O and extracted with dichloromethane (20 mL \times 3). The combined organic phases were washed with brine, dried over anhydrous sodium sulfate and evaporated to dryness. The crude product was purified by column chromatography on neutral alumina (EA : MeOH = 100 : 5) to give target **10a** as a yellow oil (0.65 g; 88 % over 2 steps). ^1H NMR (300 MHz, CDCl_3) δ 9.02 (2H, s), 8.42 (1H, d, $J = 4.1$ Hz), 7.57 (2H, d, $J = 8.0$ Hz), 7.65-7.57 (3H, m), 7.52 (1H, s), 7.13 (1H, t, $J = 5.8$ Hz), 4.31 (4H, q, $J = 7.1$ Hz), 3.98 (6H, s), 2.91 (12H, br), 1.33 (6H, t, $J = 7.1$ Hz) ^{13}C NMR (75MHz, CDCl_3) δ 165.15, 150.26, 149.07, 137.43, 136.69, 128.62, 128.25, 124.92, 122.90, 61.34, 53.46, 14.23; ESI-MS: calcd. for $\text{C}_{30}\text{H}_{39}\text{N}_6\text{O}_6$ [M + H] 547.3027; found 547.3029.

1,4-Bis(5-carboxyl-2-picolyl)-7-picolyl-1,4,7-triazacyclononane hydrochloride (10b). **10a** (0.17 g, 0.31 mmol) was treated with 7 mL HCl (2 N) and heated to 70 °C. The hydrolysis reaction

was monitored by mass spectrometry, stopped at 34 h and brought to room temperature. All volatiles were evaporated and residual water was removed by azeotropic assistance of toluene to give **10b** as a pink powder (0.15 g; 68.2%). ¹H NMR (300 MHz, D₂O) δ 9.11 (2H, d, *J* = 1.5 Hz), 8.59 (1H, d, *J* = 2.0 Hz), 8.57 (1H, d, *J* = 2.0 Hz), 8.53 (1H, dd, *J* = 5.9, 1.0 Hz), 8.36 (1H, td, *J* = 7.9, 1.5 Hz), 8.06 (1H, d, *J* = 8.0 Hz), 7.85-7.80 (3H, m), 4.44 (4H, s), 4.23 (2H, s), 3.19 (4H, s), 3.13-3.09 (4H, m), 2.92-2.91 (4H, m); ¹³C NMR (75 MHz, D₂O) δ 166.32, 155.27, 152.14, 147.08, 146.95, 143.17, 141.34, 128.03, 127.45, 126.24, 125.74, 56.91, 55.54, 50.74, 48.50, 47.58; ESI-MS: calcd. for C₂₆H₃₁N₆O₄ [M + H] 491.2401; found 491.2377.

1,4-Bis(5-ethoxycarbonyl-2-picolyl)-7-benzyl-1,4,7-triazacyclononane (11a). This compound was prepared from compound **8** according to the protocol for **10a** (55 %). ¹H NMR (300 MHz, CDCl₃) δ 9.09 (2H, s), 8.22 (2H, d, *J* = 8.1 Hz), 7.57 (2H, d, *J* = 8.2 Hz), 7.27-7.29 (5H, m), 4.34 (4H, qd, *J* = 7.1, 1.1 Hz), 3.86 (4H, s), 3.59 (2H, s), 2.92-2.71 (12H, m), 1.35 (6H, td, *J* = 7.1, 1.1 Hz); ¹³C NMR (75 MHz, CDCl₃) δ 165.36, 165.18, 150.20, 140.05, 137.27, 129.02, 128.13, 128.09, 126.83, 124.54, 122.57, 64.48, 63.42, 61.21, 55.90, 55.82, 55.78, 14.30; ESI-MS: calcd. for C₃₁H₄₀N₅O₄ [M + H] 546.3075; found 547.3067

1,4-Bis(5-carboxyl-2-picolyl)-7-benzyl-1,4,7-triazacyclononane hydrochloride (11b). This compound was prepared from compound **11a** according to the protocol for **10b** (80 %). ¹H NMR (300 MHz, D₂O) δ 8.91 (2H, d, *J* = 2.0 Hz), 8.58 (2H, dd, *J* = 8.2, 2.0 Hz), 7.58 (2H, d, *J* = 8.2 Hz), 7.44-7.32 (5H, m), 4.41 (2H, s), 4.21 (4H, s), 3.48 (4H, s), 3.17 (4H, br), 2.91 (4H, s); ¹³C NMR (75 MHz, D₂O) δ 166.13, 156.49, 146.43, 143.69, 130.94, 130.63, 129.69, 129.30, 128.20, 125.88, 60.52, 57.59, 51.16, 49.78, 47.97; ESI-MS: calcd. for C₂₇H₃₂N₅O₄ [M + H] 490.2449; found 490.2437.

Complex 1. The hydrochloride **10b** (0.38 g) was dissolved in 3.0 mL degassed anhydrous EtOH under argon before being treated dropwise with a solution of Fe(BF₄)₂·6H₂O (0.18 g) in 1.0 mL EtOH. A white precipitate formed temporarily but disappeared again upon completion of the addition where the solution is colored deep red. Dropwise addition of Et₃N (0.45 mL) again causes a substantial amount of precipitate to form. Mass analysis of the supernatant indicates complete disappearance while a signal for the target complex cannot be detected. The precipitate was filtered off after 30 minutes stirring and washed with isopropanol and Et₂O to give 0.20 g of a dark-red powder. This material was recrystallized in acetonitrile containing 5 % water by ether diffusion and characterized by ¹H NMR, HRMS and X-ray diffraction. ¹H NMR (500 MHz, D₂O) δ 8.30 (2H, s), 7.85 (1H, s), 7.71 (4H, s), 7.62 (1H, s), 7.31 (1H, s), 7.24 (1H, s), 4.23 (4H, s), 3.58 (4H, s), 3.35 (2H, s), 3.07 (4H, s), 2.28 (4H, s); ESI-MS: calcd. for C₂₆H₃₀N₆O₄Fe [M]/2 273.0833; found 273.0839.

Complex 2. The hydrochloride **11b** (0.25 g) was dissolved in a degassed mixture of 1.0 mL MeOH and 2.0 mL CH₃CN under argon before being treated with Et₃N (0.36 mL). The resulting light-brown solution was treated dropwise with a solution of Fe(BF₄)₂ (0.125 g) in 2.0 mL MeOH resulting in substantial darkening. This mixture was kept at room temperature overnight without however causing any precipitate to form. Addition of 15 mL diethylether produced an oily precipitate. Decanting of the supernatant and repeated treatment with fresh diethylether turned the oil into a powder which was subsequently purified by flash chromatography on a reversed-phase cartridge (C₁₈; H₂O/CH₃CN = 100 :10) to give 110 mg of a dark-yellow to brown powder. 30 mg of this powder were dissolved in 3 mL MeOH and 5 % water and submitted to slow ether diffusion from a reservoir. After two days, a precipitate formed which was filtered off. Concentration of the filtrate resulted in the formation of dark-yellow needles which were collected (20 mg). HRMS: calcd. for C₂₇H₃₁N₅O₄Fe [M]/2 272.5857; found 272.5857.

Spectral Characterization

Figure S01. ¹H NMR spectrum of 1 (dissolved crystals in D₂O, 500MHz)

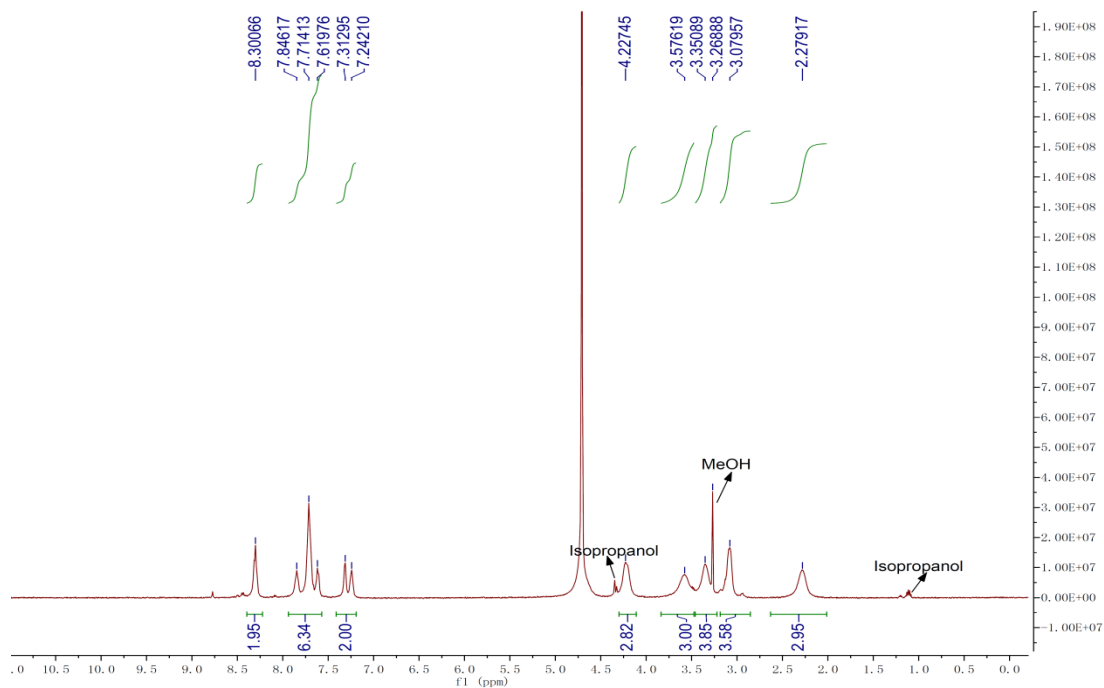


Table S01 Crystal, collection and refinement data of 1

empirical formula	C ₂₆ H ₃₀ FeN ₆ O ₄ ·BF ₄ ·Cl·H ₂ O
M / g.mol	686.68
Crystal system	Monoclinic
Space group	<i>P2₁/n</i>
a / Å	13.014 (1)
b / Å	12.449 (1)
c / Å	17.724 (2)
β / deg	96.55 (1)°
V / Å ³	2852.7 (5)
Z	4
T / K	160
λ / Å	0.7107
D _{cal} / mg. cm ³	1.599
M (Mo Ka) / mm ⁻¹	0.70
No. of data measured	26543
Unique data (R _{int})	(0.072)
Observed data [I > 2(σ)I]	4994
R[F ² > 2σ(F ²)] ^a	0.064
wR(F ²) ^b	0.168
Δρ _{min} , Δρ _{max} / e. Å ³	-1.56, 1.05

Figure S02 High resolution mass analysis of 1

The peak 273.0839 is attributed to $[\text{Fe} + \text{L}]^{2+}/2$.

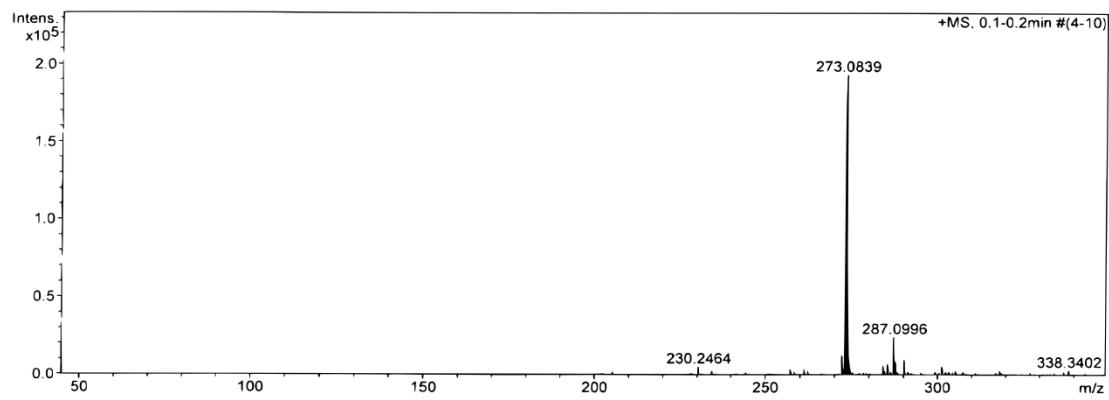


Figure S03 High resolution mass analysis of 2

The peaks 272.5867, 544.1653 and 590.1675 are respectively attributed to $[\text{Fe} + \text{L}]^{2+}/2$, $[\text{Fe} + \text{L}-\text{H}]^+$, $[\text{Fe} + \text{L} + \text{HCOO}]^+$

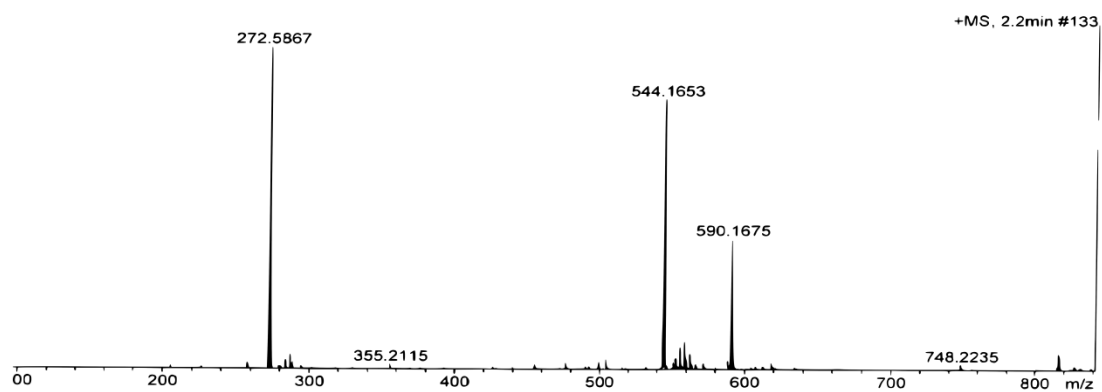


Figure S04 UV-vis spectrum of 1 (crystals in water, 0.02 mM, 298K)

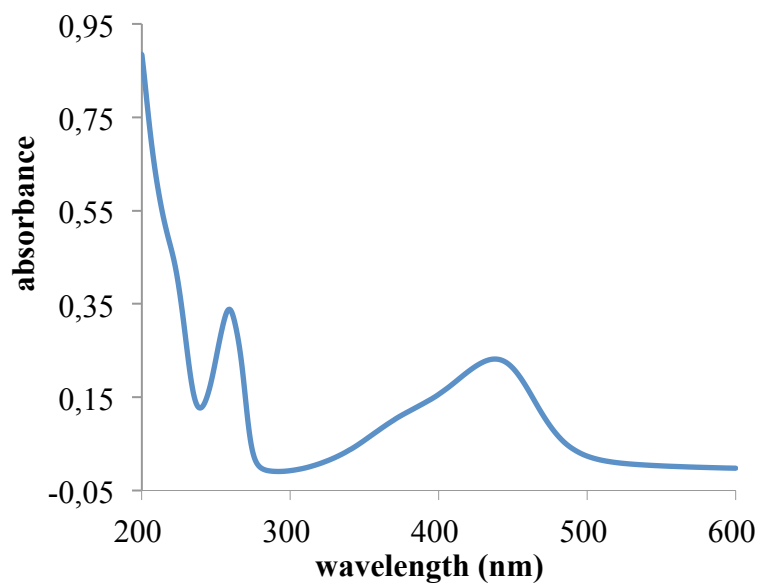


Figure S05 UV-vis spectrum of 2 (crystals in water, 0.02 mM, 298K)

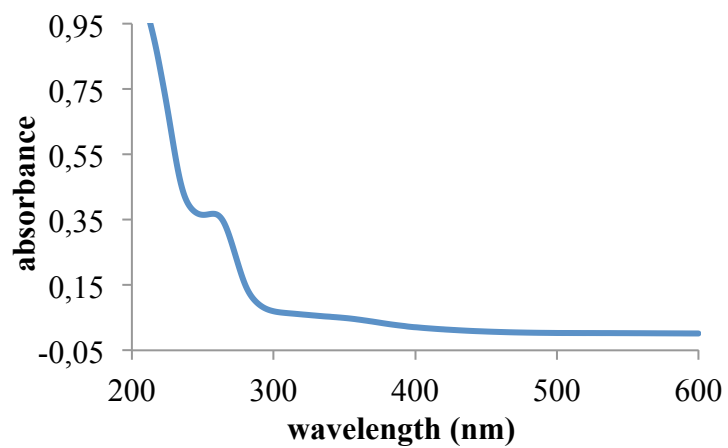


Figure S06 UV-Vis spectra of 1 at different pH values

Crystals of **1** in water at 0.02 mM were titrated with 2 mM NaOH at the beginning and then 4 mM to increase pH; crystals of **1** in water at 0.02 mM were titrated with 4 mM HCl in the beginning and then 6 mM to decrease pH.

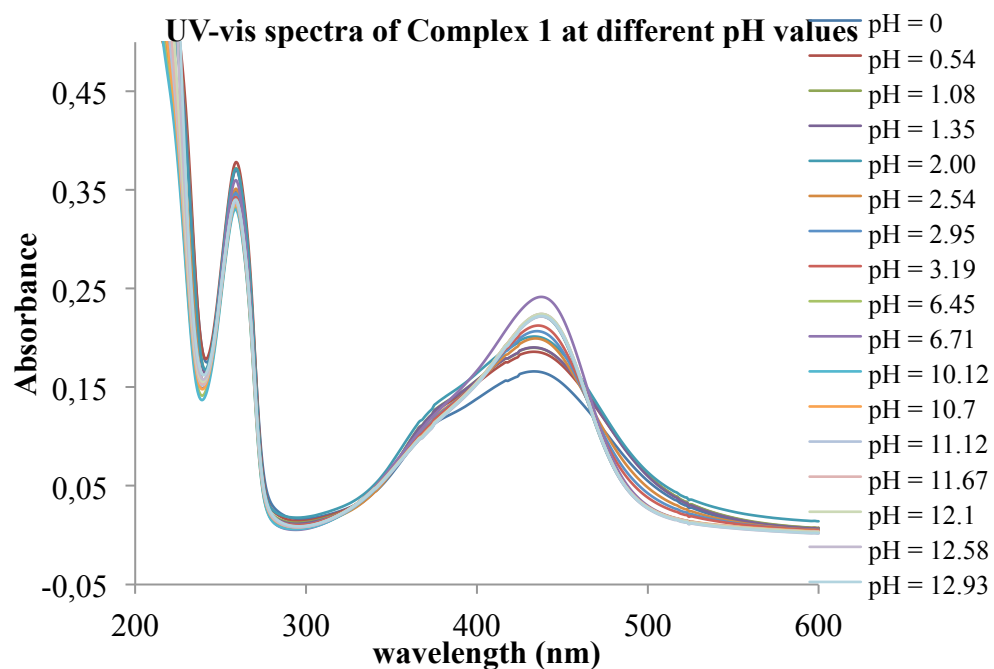
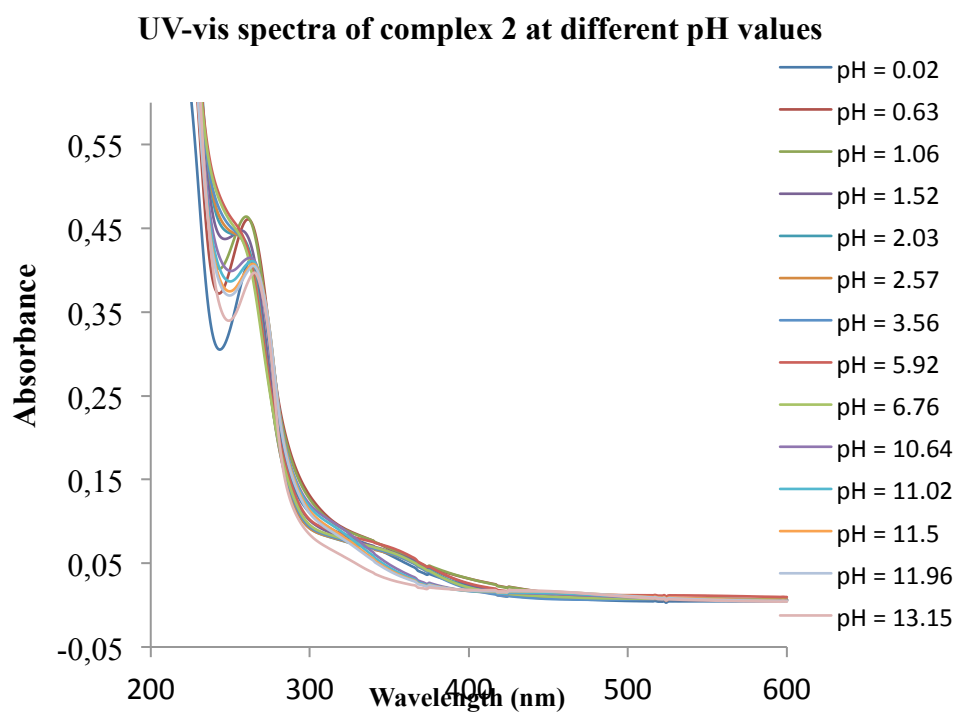


Figure S07 UV-Vis spectra of 2 at different pH values.

Powder of **2** in water at 0.02 mM was titrated with 2 mM NaOH in the beginning and then with 4 mM to increase pH; powder of **2** in water at 0.02 mM was titrated with 4 mM HCl in the beginning and then 6 mM to decrease pH)

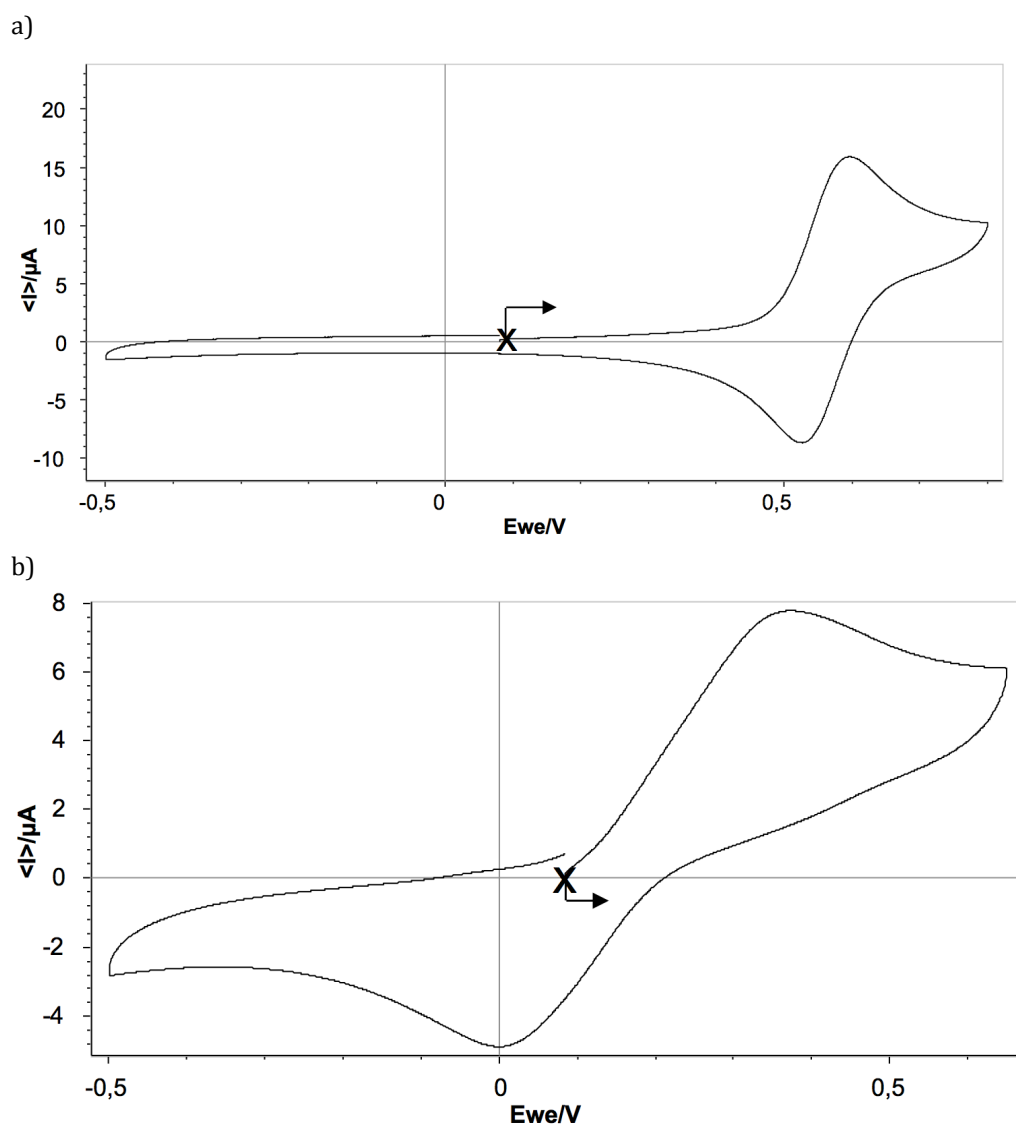


Cyclic Voltammetry protocol

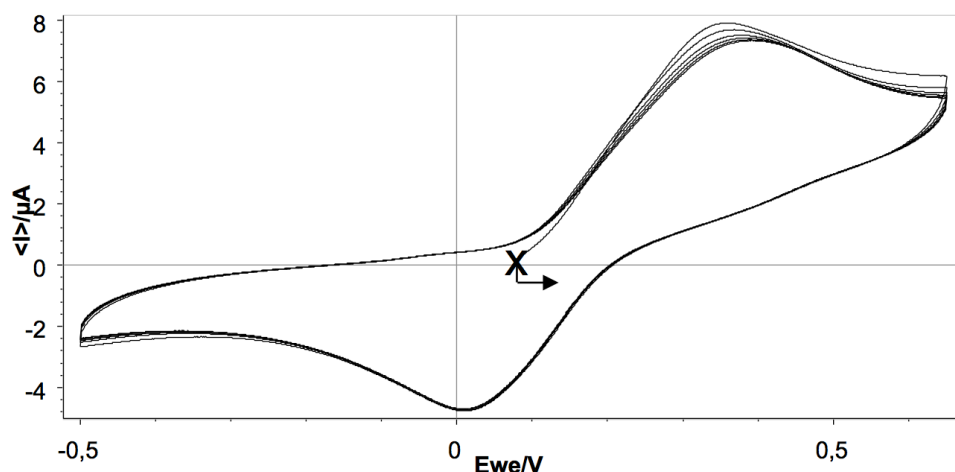
Cyclic voltammetry (CV) experiments were conducted in a standard one-compartment, three-electrode electrochemical cell with a biologic ESP300 potentiostat. Phosphate Buffer Saline (Dulbecco's PBS, Invitrogen Corp.) was used as solvent with no additional supporting electrolyte. A vitreous carbon working electrode ($\varnothing = 3$ mm, ALS Co.) was polished with 1 mm diamond paste before each recording. The counter electrode was a Pt wire. Electrode potentials are referred to a Saturated Calomel reference electrode (ALS Co.). The concentrations of the complexes were established around 1 mM and the measurements were performed at a 100 mV/s scan rate. For complex **1**, crystalline sample was used.

Figure S08. Cyclovoltammograms of **1** and **2**.

In PBS (1 mM; a)), **2** (1 mM ; single wave; b)), and **2** (1 mM ; five cycles ; c)). 3 mm carbon glass working electrode. SCE reference. Pt counter electrode. Scan rate 100 mV/sec. Ri drop correction.



c)



Protocol for Magnetic moment determination

The following equation is used by the Evans' method to determine the number of unpaired electrons in a given paramagnetic compound by first calculating the magnetic moment μ_{eff} and then applying it to the spin-only formula. This formula is only valid for transition elements of the first row with low Z (spin-orbit coupling negligible) and for the 1A_1 ground state. This is the case for the low-spin and high-spin iron(II) compounds studied herein.

$$2.828 \sqrt{\frac{T \times \delta_\nu}{\nu_0 \times S_f \times C}} = \mu_{eff} = 2\sqrt{S(S+1)}$$

Where T is the temperature (K), S_f is the symmetry factor of the coil ($4\pi/3$ for a cylindrical sample in a superconducting magnet with sample axis parallel to the magnetic field), C is the molar concentration ($\text{mol}\cdot\text{cm}^{-3}$), ν_0 is the ^1H NMR spectrometer frequency and δ_ν is the frequency shift (Hz) compared to the reference. No diamagnetic correction was introduced into the calculations for paramagnetic compounds. However, they can be evaluated either 1) by using this same procedure to also determine the diamagnetic contribution for the corresponding diamagnetic complex with a different metal ion.¹ or 2) by use of Pascal's constants.

Herein we dissolved crystals of **1** or **2** in 90% H_2O and 10% D_2O which contained 2% *t*-BuOH to prepare 5 mM solution.

Figure S09 Frequency shift of the signal from *t*-BuOH in presence of **1**

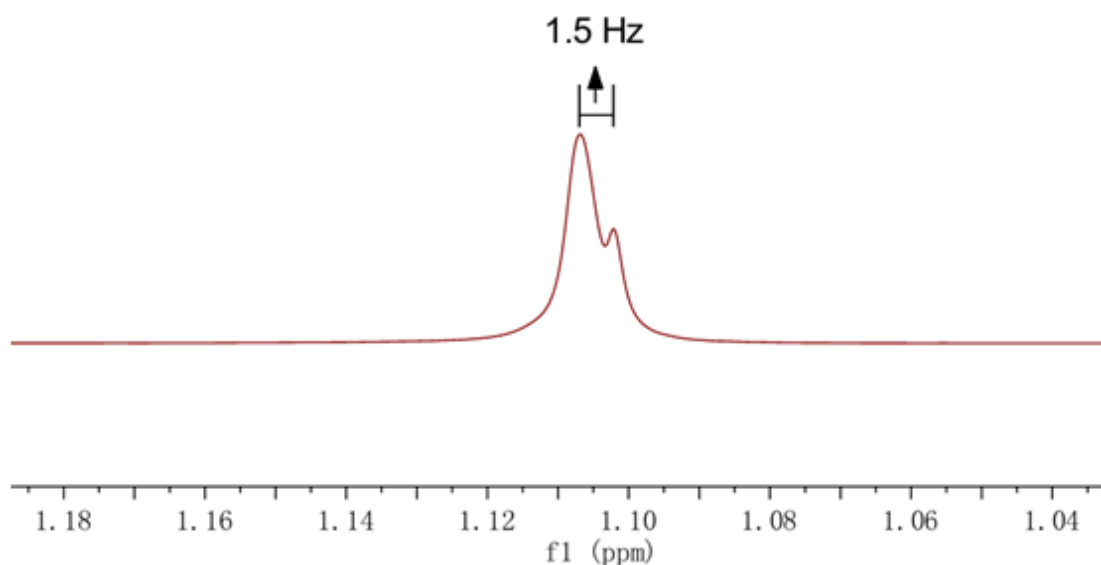
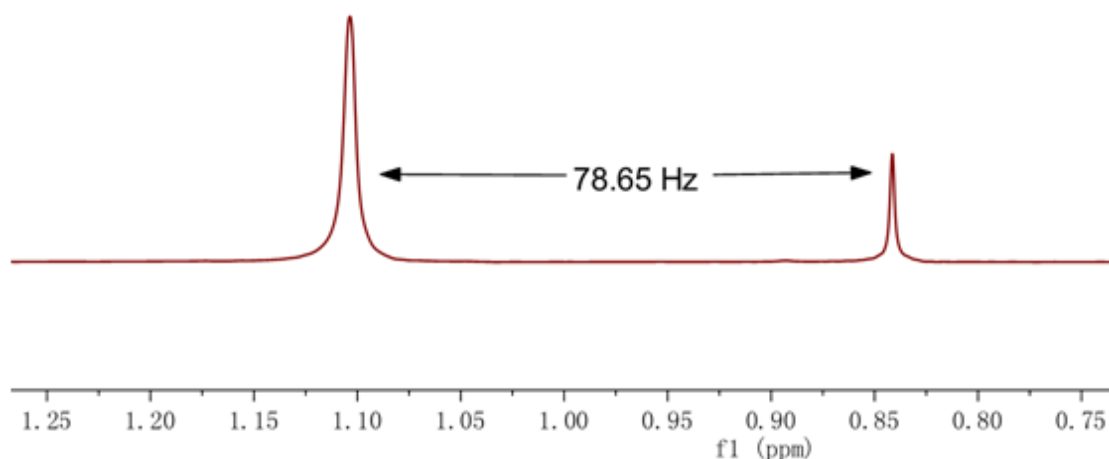


Figure S10 Frequency shift of the signal from *t*-BuOH in the presence of **2**.



Experimental parameters: $T = 298 \text{ K}$; $\nu_0 = 300 \text{ MHz}$.

Considering that for the HS complex a water molecule may slowly replace acetonitrile, the magnetic moment was measured three times after preparation of the solution.

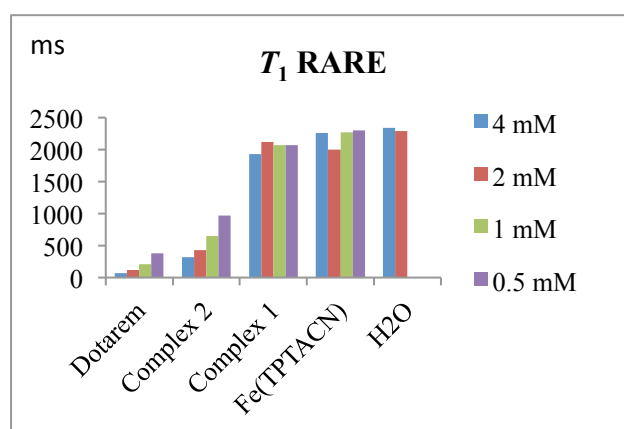
Table S02 Calculation of magnetic moments of **1** and **2**.

Compound	1	2		
concentration (mM)	5.0	5.0		
Time (h)	0	0	0.5	336
frequency shift (Hz)	1.5	78.65	77.78	77.76
Magnetic susceptibility $\chi_m(T)$ [emu]	0.07	3.62	3.58	3.58
Magnetic Moment (BM)	0.76	5.38	5.35	5.35

Table S03 T_1 determination of 1 and 2 (both crystals) in water (300 MHz)

Compound	1		2		
concentration (mM)	4.0		4.0		
Time (min)	0	0	30	60	90
T_1 (s)	2.168	0.55	0.50	0.49	0.48

Figure S11 T_1 measurement of 1 and 2 (both crystals) in PBS.



Cell culture protocol

HeLa cells were cultured in Dulbecco's modified Eagle's medium (DMEM) (Invitrogen Corp.) supplemented with 10 % (v/v) fetal bovine serum (Invitrogen Corp.), 50 U.mL⁻¹ penicillin, and 50 µg.mL⁻¹ streptomycin (Invitrogen Corp.) in a humidified incubator containing 5 % CO₂ in air at 37°C.

Viability tests :

10.10³ HeLa cells were seeded in 100 µL of supplemented DMEM in a clear 96-well plate (Corning Costar). After 24 h of incubation the medium was removed, cells were washed with PBS and medium was replaced by an appropriate volume of DMEM (Invitrogen Corp.). A desired volume of stock solutions of complexes **1** or **2** at 40 mM (in PBS) was added to create a range of concentrations between 0 µM and 4 mM in 150 µL final volume. Cells were then incubated for 24 h. The supernatant was subsequently removed, cells were washed with PBS; 100 µL of DMEM and 20 µL of the MTS reagent (CellTiter 96® AQueous NonRadioactive Cell Proliferation Assay, Promega) were added. Cells were incubated for another 4 h period and absorbance at 490 nm was measured with a microplate reader (Multiscan GO Microplate Spectrophotometer, Thermo Scientific). The percentage of viable cells is calculated by dividing the absorbance at a given concentration by the absorbance at 0 µM. The given results are the mean of 2 independent experiments performed in triplicate.

References

- (1) C. Piguet, *J. Chem. Educ.* 1997, 74, 815-816.