Electronic Supplementary Information for:

pH-Dependent Spin State Population and ^{19}F NMR Chemical Shift via Remote Ligand Protonation in an Iron(II) Complex

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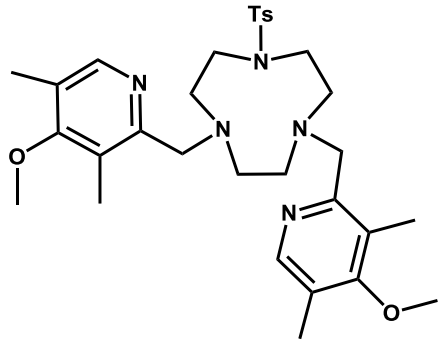
Chemical Communications

Table of Contents

Experimental Section

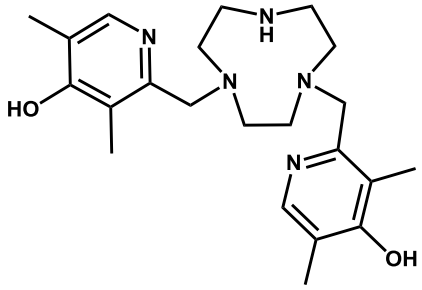
General Considerations. Unless otherwise specified, the manipulations described below were carried out under a dinitrogen atmosphere in a glovebox or using standard Schlenk line techniques. For water-free manipulations, glassware was oven-dried at 150 °C for at least 4 h and allowed to cool under vacuum prior to use. Acetonitrile (MeCN), dichloromethane (CH_2Cl_2), diethyl ether (Et_2O), diisopropylethylamine (DIPEA) and methanol (MeOH) were dried using a commercial solvent purification system from Pure Process Technology and stored over 3 or 4 Å molecular sieves prior to use. Water was obtained from a purification system from EMD Millipore. Elemental analysis was conducted by Midwest Microlab Inc. Deuterated solvents were purchased from Cambridge Isotope Laboratories. The compounds H_2Ts-TACN and 3-fluoro-2-formyl-6-methylpyridine were synthesized following literature procedures.[1] All other compounds were purchased from commercial vendors and used without further purification.

Synthesis of *N*, *N′*-bis(4-methoxy-3,5-dimethyl-2-picolyl)-*N″*-monotosyl-1,4,7-triazacyclononane.



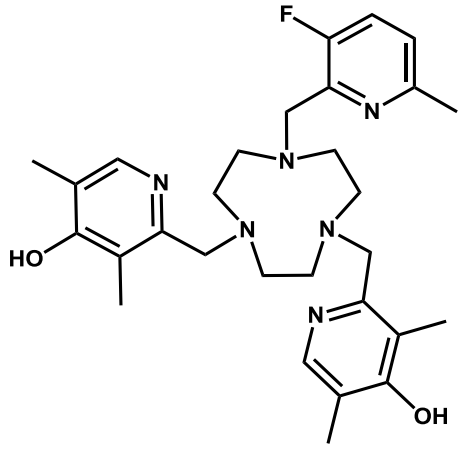
Ts-TACN (1.8 g, 6.4 mmol) and 2-(chloromethyl)-4-methoxy-3,5-dimethylpyridine hydrochloride (2.8 g, 13 mmol) were dried for 12 h under vacuum before being added to a flame-dried 200 mL Schlenk flask along with dry DIPEA (8.4 g, 65 mmol) and dry MeCN (50 mL). The reaction mixture was stirred and heated at 60 °C under a dinitrogen atmosphere. The reaction was followed by ESI-MS, which indicated a complete reaction after 16 h. The resulting white suspension was cooled to room temperature and evaporated to dryness to give an orange oil and white crystalline solid. The crude mixture was dissolved in 1M aqueous NaOH solution (30 mL) and extracted with CH_2Cl_2 (4 × 50 mL). The organic layer was dried with $MgSO_4$(s), filtered and evaporated to dryness under reduced pressure to give an orange oil. The crude product was purified by column chromatography using silica gel. The column was loaded with 5% triethylamine (TEA) in hexanes and initially run with 1.5% TEA in 1:1 EtOAc/hexanes to remove excess 2-(chloromethyl)-4-methoxy-3,5-dimethylpyridine starting material. Then the solvent mixture was changed to 2% TEA in EtOAc to elute the title compound, which was obtained as a white oily solid after removing the solvent under reduced pressure (2.2 g, 3.8 mmol, 58%). ESI-MS (*m*/*z*): Calcd. for $C_{31}H_{42}N_5O_4S$ $(M+H)^+$: 582.30, found 582.30. ^{1}H NMR (500 MHz, $CDCl_3$, 25 °C): δ 8.10 (s, 2H), 7.54 (d, J = 8.2 Hz, 2H), 7.22 (d, J = 7.9 Hz, 2H), 3.70 (s, 4H), 3.70 (s, 6H), 3.01 (d, J = 8.4 Hz, 8H), 2.62 (s, 4H), 2.38 (s, 3H), 2.28 (s, 6H), 2.21 (s, 6H).

Synthesis of *N, N′*-bis(4-hydroxy-3,5-dimethyl-2-picolyl)- 1,4,7-triazacyclononane.



N, N′-bis(4-methoxy-3,5-dimethyl-2-picolyl)-*N′′*-monotosyl-1,4,7-triazacyclononane (1.9 g, 3.3 mmol) and a magnetic stir bar were introduced into a 25 mL round bottom flask. To this, concentrated H_2SO_4 solution (10 mL) was slowly added and the resulting orange-yellow mixture was placed under vacuum for 1 h to remove all traces of oxygen. Afterwards, the reaction flask was connected to a reflux condenser and the homogeneous orange solution was heated to 160 °C under a dinitrogen atmosphere with stirring for 16 h. The resulting black reaction mixture was cooled to room temperature and then added to stirring EtOH (60 mL) at −20 °C. Subsequent addition of Et_2O (300 mL) resulted in the formation of a pale brown suspension, which was filtered to give a black hygroscopic solid. The solid was dissolved in saturated aqueous K_2CO_3 solution (10 mL) and EtOH (200 mL) was added to create a biphasic mixture, which was stirred at 25 °C for 30 min. The EtOH layer was collected and filtered to give an orange solution which was evaporated to dryness to give an orange oil. The crude product was dissolved in MeCN (50 mL) and concentrated AcOH solution (1 mL) was added to generate the neutral species. The resulting solution was evaporated to dryness to give a mixture of a brown oil and a white crystalline solid. This mixture was triturated with CH_2Cl_2 (100 mL) and the supernatant was filtered and evaporated to dryness to give the product as an orange oil (0.75 g, 1.9 mmol, 56%). ESI-MS (*m*/*z*): Calcd. for $C_{22}H_{33}N_5O_2$ $(M+H)^+$: 400.27, found 400.28. ^{1}H NMR (500 MHz, CD_3OD, 25 °C): δ 7.49 (s, 2H), 3.65 (s, 4H), 2.91–2.76 (m, 8H), 2.47 (broad s, 4H), 1.97 (s, 6H), 1.96 (s, 6H).

Synthesis of *N, N′*-bis(4-hydroxy-3,5-dimethyl-2-picolyl)-*N′′*-mono(3-fluoro-6-methyl-2-picolyl)-1,4,7-triazacyclononane (H_2L).



N, N′-bis(4-hydroxy-3,5-dimethyl-2-picolyl)-1,4,7-triazacyclononane (0.75 g, 1.9 mmol) and 3-fluoro-2-formyl-6-methylpyridine (0.28 g, 2.0 mmol) were added to a 100 mL Schlenk flask in a dinitrogen atmosphere glovebox. To this, dry CH_2Cl_2 (40 mL) was added and the flask sealed with a septum. This yellow solution was stirred at 40 °C for 1 h in the glovebox. Next, solid $NaHB(OAc)_3$ (1.3 g) was added and the reaction was followed by ESI-MS. After stirring for 1 h, the reaction was 85% complete and did not improve upon further heating. Therefore, the reaction mixture was cooled to room temperature, brought out of the glovebox, and quenched with concentrated aqueous K_2CO_3 solution (20 mL). The resulting solution was extracted with CH_2Cl_2 (3 × 50 mL). The organic layers were collected, dried with $MgSO_4$(s), and filtered. The solvent was removed under reduced pressure to give a pale yellow powder. This powder was dissolved in MeCN (4 mL) and concentrated AcOH solution (1 mL) was added. This solution

was evaporated to dryness to give an orange oil which was triturated with CH_2Cl_2 (5 mL) and filtered through diatomaceous earth. The filtrate was evaporated to dryness to give the crude product as a yellow solid. The ligand was purified by column chromatography using aluminum oxide basic and 0–10% H_2O in MeCN as eluent. The ligand was obtained as a yellow solid after removing the solvent under reduced pressure (0.12g, 0.22 mmol, 12%). ESI-MS (*m*/*z*): Calcd. for $C_{29}H_{39}FN_6O_2$ $(M+H)^+$: 523.32, found 523.33. ^{1}H NMR (500 MHz, $CDCl_3$, 25 °C): δ 7.29 (s, 2H), 7.24–7.13 (m, 1H), 6.99 (dd, J = 8.5, 3.6 Hz, 1H), 3.83 (s, 2H), 3.64 (s, 4H), 2.87–2.76 (m, 4H), 2.72–2.65 (m, 4H), 2.62 (s, 4H), 2.34 (s, 3H), 1.95 (s, 6H), 1.94 (s, 6H). ^{19}F NMR (376 MHz, $CDCl_3$, 25 °C): δ −129.91 (d, J = 7.2 Hz).

Synthesis of $[(H_2L)Fe](BF_4)_2$ (1). Under an inert atmosphere of dinitrogen, a pale pink suspension of $[Fe(H_2O)_6](BF_4)_2$ (0.097 g, 0.29 mmol) in MeCN (2 mL) was added dropwise to a stirring light orange solution of H_2L (0.15 g, 0.29 mmol) in MeCN (6 mL). The resulting dark green solution was stirred at ambient temperature for 3 h and then filtered. The dark green filtrate was concentrated under reduced pressure to give a dark green-brown film. This film was dissolved in deionized H_2O at 60 °C and upon cooling to room temperature, light yellow-green crystalline solid formed. This solid was washed with cold deionized H_2O (1 mL) and dried under reduced pressure for 16 h to give **1** (0.11 g, 0.15 mmol, 52%). Anal. Calcd. for $C_{29}H_{39}B_2F_9FeN_6O_2$: C, 46.31%; H, 5.23%; N, 11.17%. Found: C, 45.79%; H, 5.33%; N, 11.17%. Yellow-green block-shaped crystals of **1** suitable for X-ray diffraction analysis were grown by slow cooling of a concentrated H_2O solution of the compound.

X-ray Structure Determination. Single crystals of **1** were directly coated with deoxygenated Paratone-N oil, mounted on a MicroMounts rod and frozen under a stream of dinitrogen during data collection. Crystallographic data were collected at 100 K using a Bruker Kappa Apex II diffractometer equipped with a Triumph detector, a MoKα source, and MX Optics. Raw data were integrated and corrected for Lorentz and polarization effects with SAINT v8.27B.[2] Absorption corrections were applied using SADABS.[3] Space group assignments were determined by examination of systematic absences, E-statistics, and successive refinement of the structures. Structures were solved with SHELXT[4] and further refined with SHELXL[5] operated within the OLEX2 interface.[6] Positional disorder of the $(BF_4)^-$ anions was modeled with partial occupancies. All non-acidic hydrogen atoms were placed at calculated positions using suitable riding models and refined using isotropic displacement parameters derived from their parent atoms. The hydroxylpyridine protons were found in the Fourier difference map and were freely refined. Thermal parameters were refined anisotropically for all non-hydrogen atoms. Crystallographic data and the details of data collection are listed in Table S1.

Solution Magnetic Measurements. The solution magnetic moments of **1** were determined using the Evans method,[7] by collecting variable-temperature ^{1}H NMR spectra using an Agilent DD2 500 MHz (11.7 T) spectrometer. Samples for measurements were prepared according to the following protocol. A 50 μL aliquot of a 14.0 mM stock solution of **1** in deoxygenated H_2O was dissolved in 0.45 mL of deoxygenated 100 mM phosphate buffer solutions containing 2% (v/v) dimethyl sulfoxide (DMSO) as a reference. The pH of the phosphate buffer solutions ranged from 5.4 to 7.8. Blank samples containing 50 μL water and 0.45 mL of the same

DMSO/phosphate buffer solutions were also created for each pH value. The samples were added to NMR tubes equipped with flame-sealed capillaries containing 2% (v/v) trifluoroacetic acid (TFA) and 5% (v/v) DMSO in D_2O reference solutions. The frequency differences of the DMSO resonance in the sample and reference solutions from the blank samples were subtracted from the analogous DMSO frequency differences of the compound samples. The average of three measurements afforded the resulting data. All data were corrected for diamagnetic contributions (determined from elemental analysis) from the core diamagnetism of each sample (estimated using Pascal's constants).[8] The paramagnetic molar susceptibility χ_M^{para} (cm^3 mol^{-1}) was calculated using the following equation: [7]

$$\chi_M^{para} = (3\Delta\nu M_w)/(4\pi\nu_0 m) - \chi_M^{dia} \quad \text{(S1)}$$

In this equation $\Delta\nu$ is the frequency difference (Hz) of the DMSO resonance as described above, M_w is the molecular mass of the paramagnetic compound (g mol^{-1}), ν_0 is the operating frequency of the NMR spectrometer (Hz), m is the concentration of the paramagnetic compound (g cm^{-3}), and χ_M^{dia} is the diamagnetic contribution to the molar susceptibility (cm^3 mol^{-1}).

NMR Spectroscopy. ^1H and ^{19}F NMR spectra of ligand H_2L and ligand precursors were collected at 25 °C on either an Agilent DD2 500 MHz (11.7 T) system, at 500 and 470 MHz frequencies respectively, or on an automated Agilent DD MR 400 MHz (9.40 T) system equipped with Agilent 7600 96-sample autosampler, at 400 and 376 MHz frequencies respectively. Variable-temperature ^{19}F NMR spectra of **1** were collected on an Agilent DD2 500 MHz (11.7 T) system at 470 MHz. NMR spectra of samples in 100 mM phosphate buffer solutions containing 2% (v/v) DMSO at various pH values were acquired using 1% (v/v) TFA in D_2O in an inner capillary to lock the sample. Chemical shift values (δ) are reported in ppm and referenced to residual proton signals from the deuterated solvents for all ^1H NMR spectra (7.26 ppm for $CDCl_3$ and 3.31 ppm for CD_3OD). ^{19}F NMR chemical shift values for spectra recorded in $CDCl_3$ were referenced to trichlorofluoromethane ($CFCl_3$) at 0 ppm. For variable-pH NMR spectra of **1** in 100 mM phosphate buffer solutions, the ^{19}F NMR chemical shift values are referenced to TFA at 0 ppm. All coupling constants (J) were measured in Hertz (Hz). The MestReNova 10.0 NMR data processing software was used to analyze and process all recorded NMR spectra.

UV-Visible Absorption Measurements. UV-Visible absorption experiments were carried out on an Agilent Cary 5000 UV-Visible-NIR spectrometer. Spectra were collected in the 200–800 nm range for 70 μM samples of $[(LH_x)Fe]^{x+}$ in 100 mM phosphate buffer solutions containing 2% (v/v) DMSO at various pH values. The molar absorptivities at 271 and 380 nm as a function of pH were fitted to a Boltzmann sigmoidal function[9] according to the following equation, to estimate the average pK_a value of the hydroxylpyridine protons of **1**:

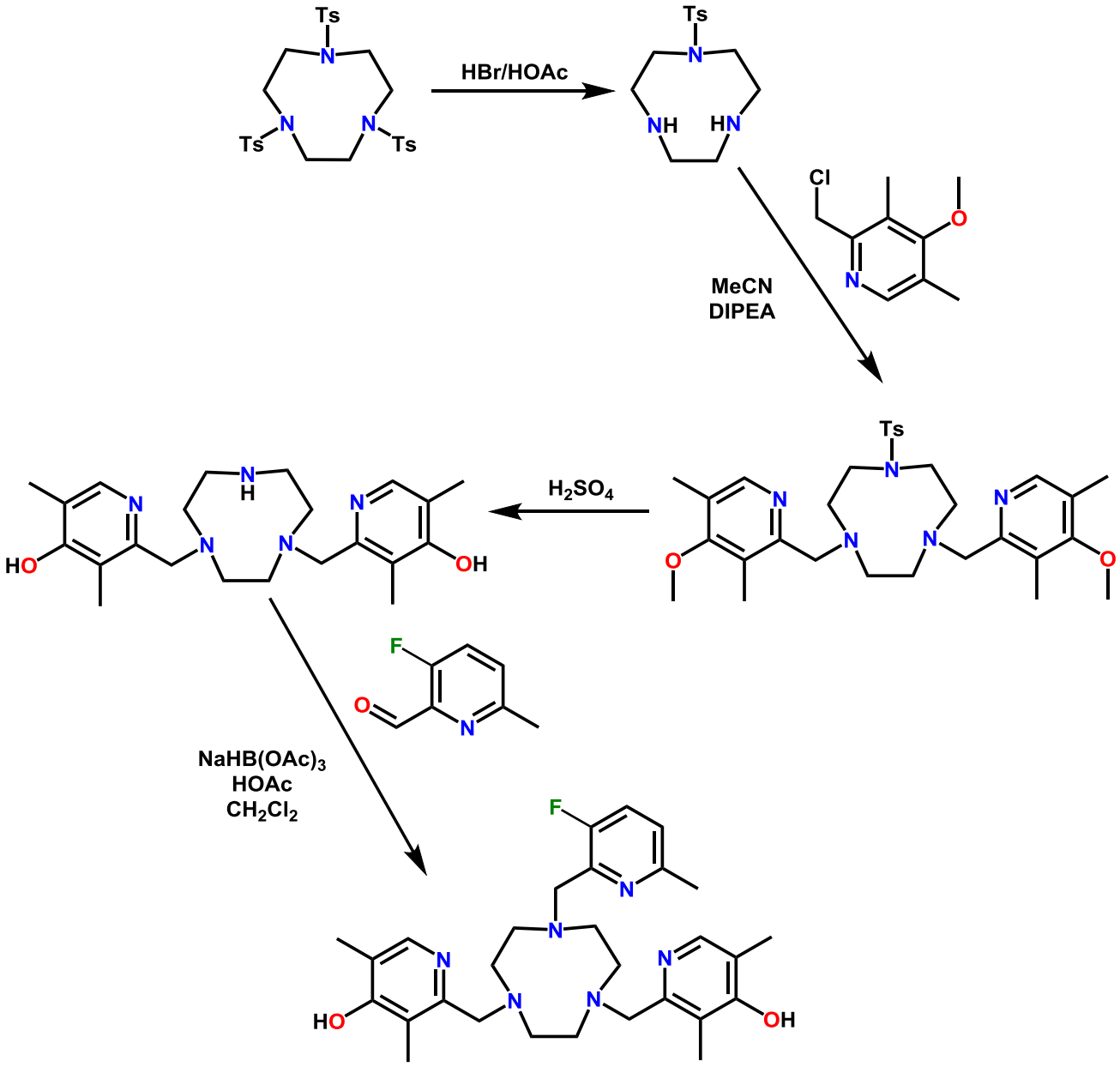
$$\varepsilon_{obs} = \varepsilon_2 + (\varepsilon_1 - \varepsilon_2)/(1 + \exp((pH - pK_a)/dx)) \quad \text{(S2)}$$

In this equation ε_{obs} is the observed molar absorptivity at a single wavelength (271, 380 nm), ε_2 is the theoretical molar absorptivity of the fully deprotonated species, ε_1 is the theoretical molar

absorptivity of the fully protonated species, *dx* is a parameter describing the steepness of the curve, and pK_a is obtained as the inflection point of the curve.

Other Physical Measurements. Electrospray ionization mass spectrometry (ESI-MS) measurements were performed on a LC−MS Bruker AmaZon SL quadrupole ion trap instrument equipped with a Compass software version 1.3 SR2. Inductively coupled plasma optical emission spectroscopy (ICP-OES) was performed on a Thermo iCAP 7600 dual view ICP-OES instrument equipped with a CETAC ASX520 240-position autosampler. Samples were dissolved in a 3% aqueous nitric acid solution and the emissions for Fe were compared to standard solutions. All pH measurements were carried out at ambient temperature using a Thermo Scientific Orion 9110DJWP double junction pH electrode connected to a VWR sympHony B10P pH meter. The pH meter was calibrated using standardized pH buffer solutions at 4.01, 7.00, and 10.00 purchased from LaMotte Company. 100 mM aqueous phosphate buffer solutions containing 2% (v/v) DMSO were prepared inside the glovebox according to the following procedure. Stock solutions of 0.10 M Na_2HPO_4(aq) and 0.10 M NaH_2PO_4(aq) were prepared in deoxygenated water and these solutions were mixed in appropriate amounts according to the Henderson-Hasselbach equation shown below (equation S3) to give 100 mM phosphate buffer solutions with selected pH values ranging from 4.6 to 7.8. Note that DMSO was added to each solution to give 2% (v/v). These buffer solutions were used to prepare samples of compound **1** for NMR and UV-Visible measurements. An aliquot of a concentrated stock solution of **1** in deoxygenated H_2O was added to each phosphate buffer solution to prepare the samples. The exact pH values of these sample solutions were measured after NMR and UV-Visible measurements were performed by exposing the sample to air and immediately measure the pH of the solution. Note that for ^{19}F NMR and solution magnetic measurements, the pH of each sample was assumed to vary insignificantly with temperature between 5 and 60 °C.

$$pH = 7.21 + \log([HPO_4^{2-}]/[H_2PO_4^{-}]) \quad \text{(S3)}$$



Scheme S1 | Synthesis of H_2L.

Table S1 | Crystallographic data for **1**.

Empirical formula	$C_{29}H_{39}B_2F_9FeN_6O_2$
Formula weight, g mol^{-1}	752.13
Temperature, K	100.0
Crystal system	Orthorhombic
Space group	*Pbca*
a, Å	15.049(11)
b, Å	14.809(10)
c, Å	29.11(2)
α, °	90
β, °	90
γ, °	90
V, Å^3	6486(8)
Z	8
ρ_{calc}, g cm^{-3}	1.540
μ, mm^{-1}	0.556
F(000)	3104.0
Crystal size, mm^3	0.256 × 0.103 × 0.076
Radiation	MoKα (λ = 0.71073 Å)
Reflections collected	51003
Independent reflections	5963 (R_{int} = 0.0720, R_{sigma} = 0.0415)
Goodness-of-fit on F^2	1.032
Final R indexes ($I > 2\sigma(I)$)	R_1 = 0.0786,[a] wR_2 = 0.1887 [b]
Final R indexes (all)	R_1 = 0.1199,[a] wR_2 = 0.2141[b]
Largest diff. peak/hole, e$^-$ Å^{-3}	1.36 / −0.61

[a] $R_1 = \Sigma||F_0| - |F_C||/\Sigma|F_0|$. [b] $wR_2 = [\Sigma w(F_0^2 - F_C^2)^2/\Sigma w(F_0^2)^2]^{1/2}$.

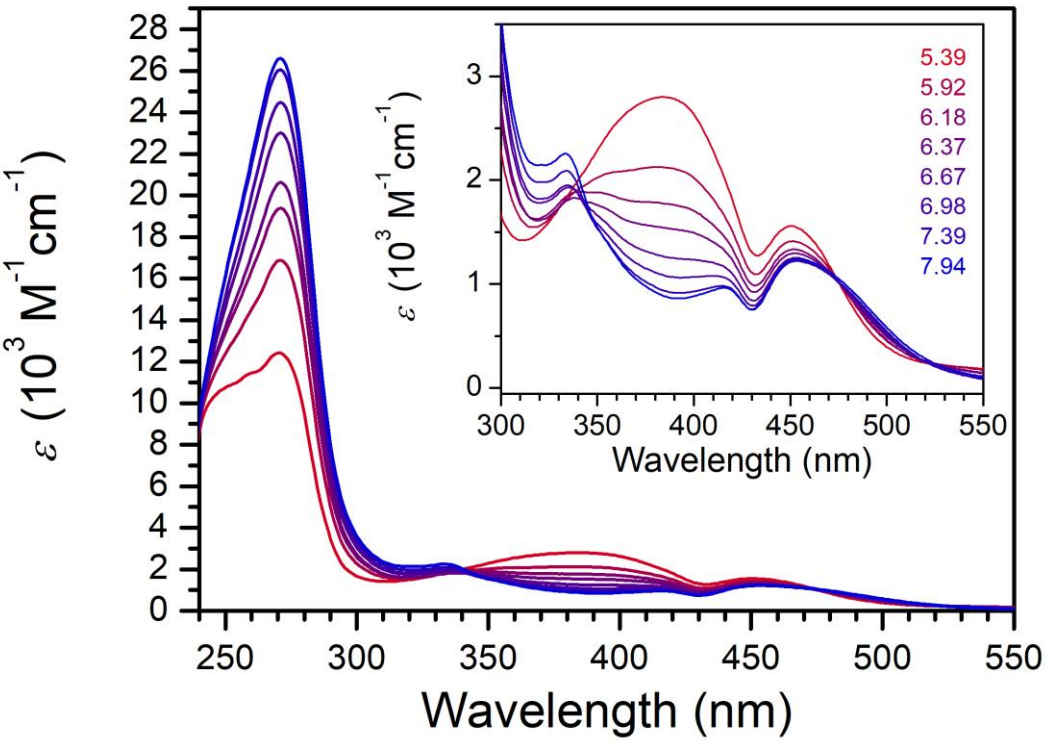


Figure S1 | Variable-pH UV-Visible spectra for **1** in 100 mM phosphate buffer solutions containing 2% (v/v) DMSO. Spectra were measured at 25 °C in the pH range 5.39–7.94. Colored numbers in the legend denote the pH of the solutions measured with a pH electrode.

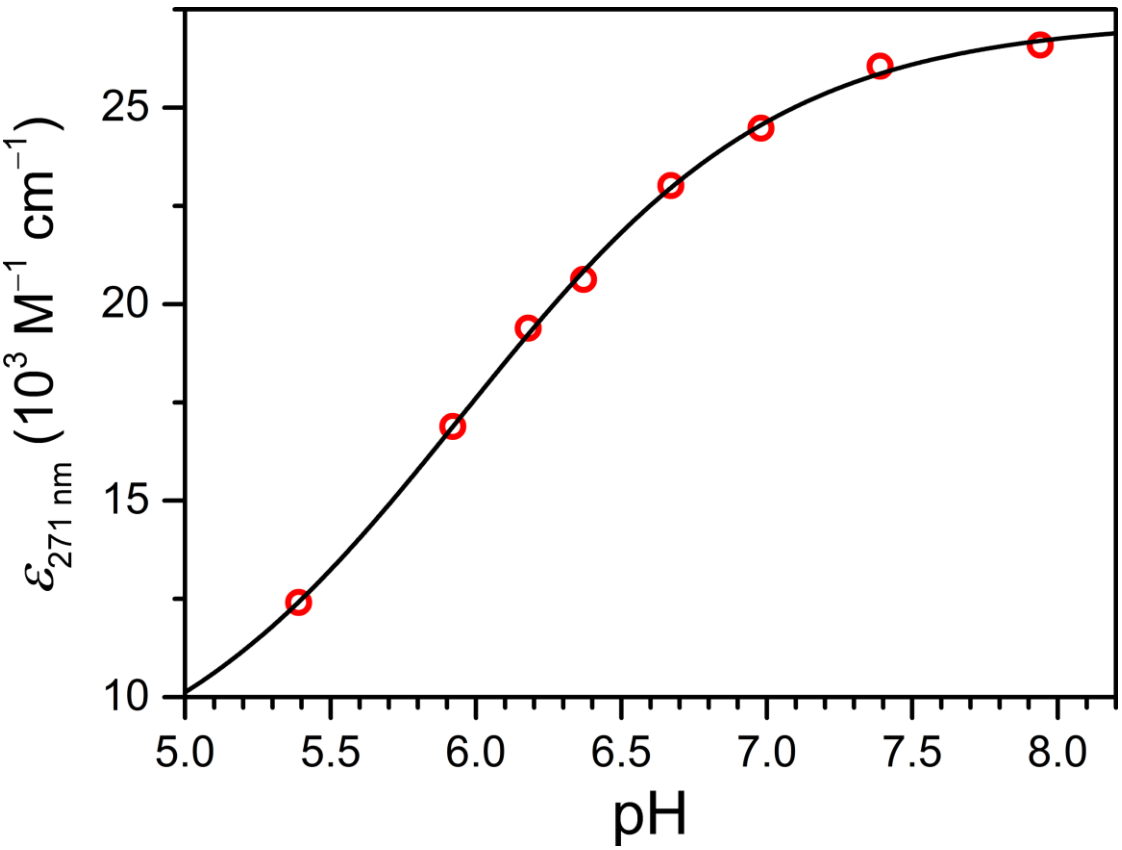


Figure S2 | pH dependence of the molar absorptivity at 271 nm for **1** in 100 mM phosphate buffer solutions containing 2% (v/v) DMSO. Data was obtained at 25 °C in the pH range 5.39–7.94. Circles represent experimental data and the black solid line denotes a sigmoidal fit of the data using equation S2, giving pK_a = 5.95(7) (see Table S2).

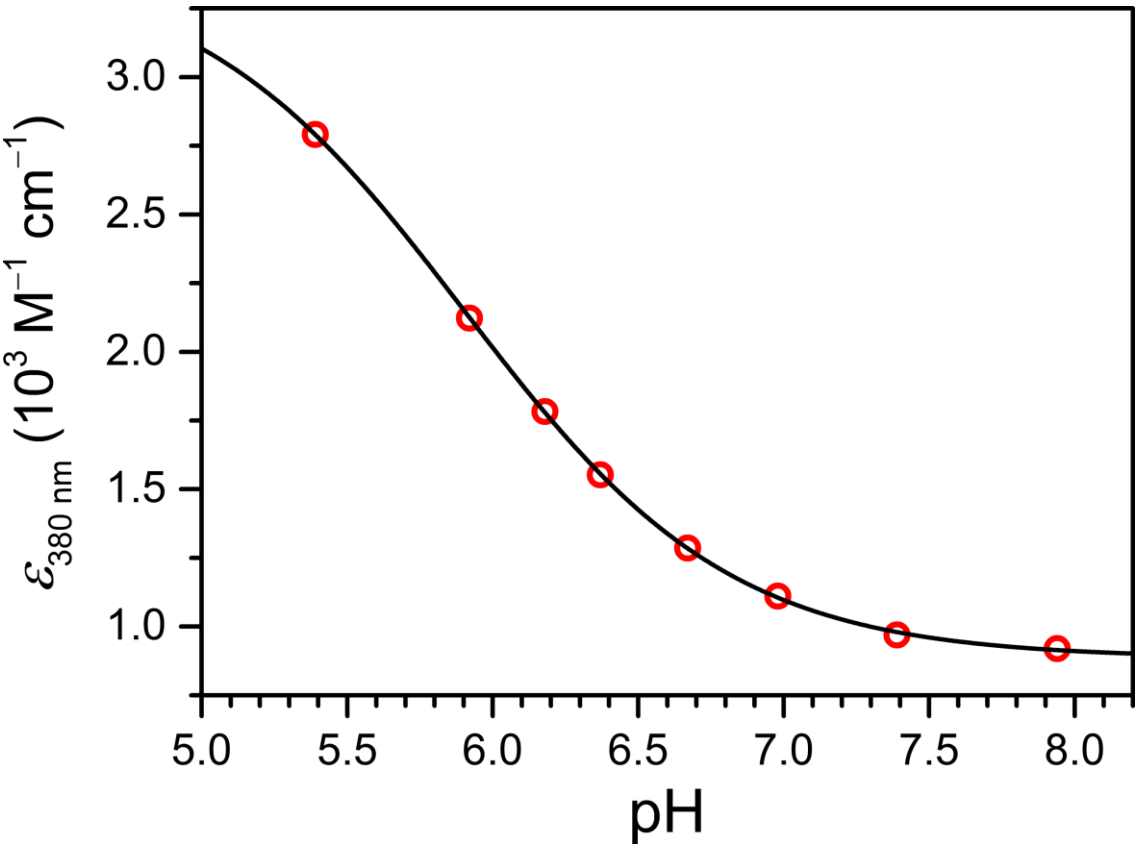


Figure S3 | pH dependence of the molar absorptivity at 380 nm for **1** in 100 mM phosphate buffer solutions containing 2% (v/v) DMSO. Data was obtained at 25 °C in the pH range 5.39–7.94. Circles represent experimental data and the black solid line denotes a sigmoidal fit of the data using equation S2, giving pK_a = 5.90(2) (see Table S2).

Table S2 | Parameters obtained from sigmoidal fits of the molar absorptivity vs pH data for **1** to equation S2.

	271 nm	380 nm
Adj. R^2	0.999	0.999
ε_1	7(1)	3.41(5)
ε_2	27(1)	0.88(1)
pK_a	5.95(7)	5.90(2)
dx	0.55(5)	0.46(1)

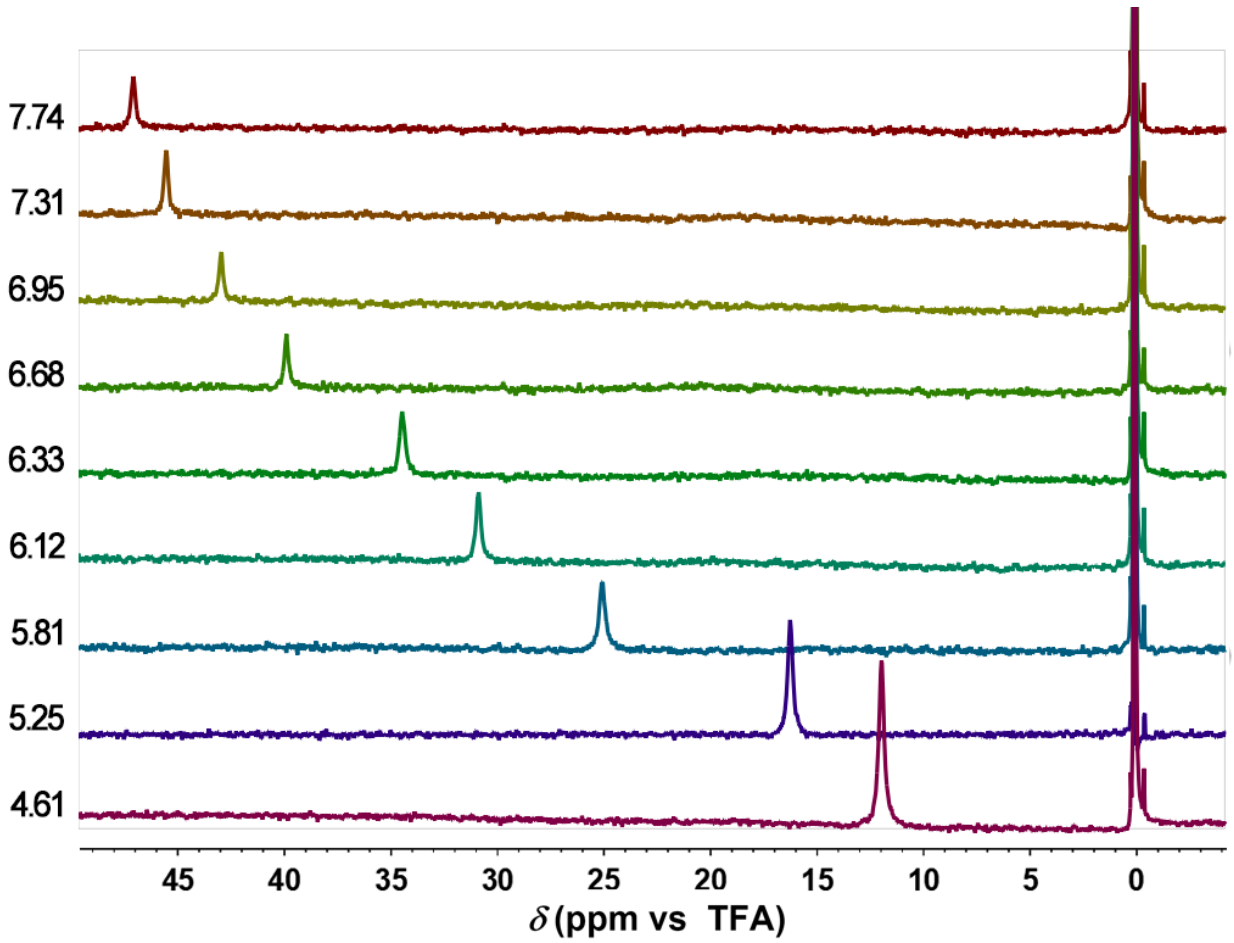


Figure S4 | Variable-pH ^{19}F NMR spectra of **1** in 100 mM phosphate buffer solutions containing 2% (v/v) DMSO at 25 °C. The ^{19}F chemical shift is referenced to TFA at 0 ppm for all spectra. Black numbers on the left denote the pH of the solutions measured with a pH electrode.

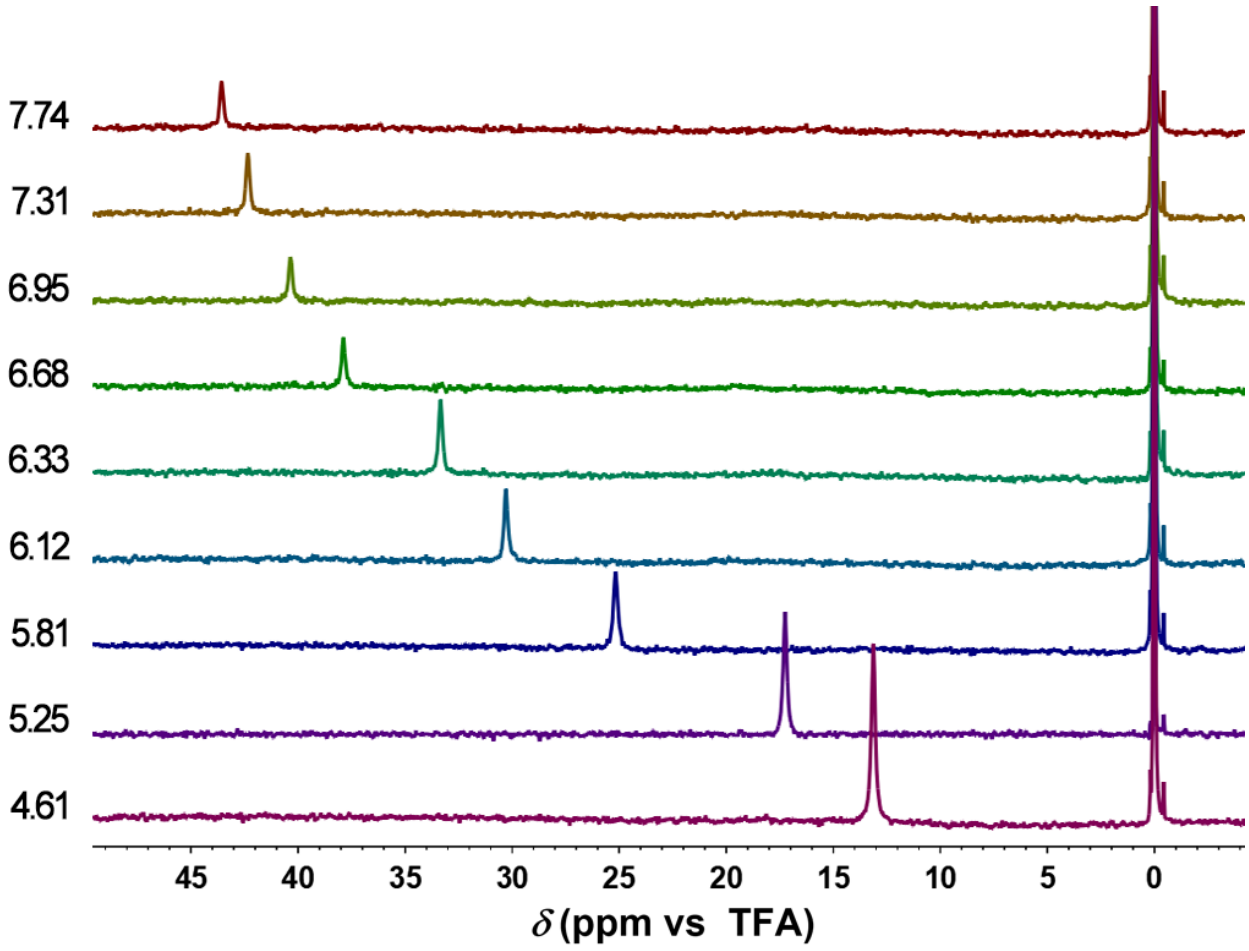


Figure S5 | Variable-pH ^{19}F NMR spectra of **1** in 100 mM phosphate buffer solutions containing 2% (v/v) DMSO at 37 °C. The ^{19}F chemical shift is referenced to TFA at 0 ppm for all spectra. Black numbers on the left denote the pH of the solutions measured with a pH electrode.

Table S3 | Parameters obtained from sigmoidal fits of the ^{19}F chemical shift vs pH data for **1** to equation 1 (see manuscript).

	25 °C	37 °C
Adj. R^2	0.999	0.999
A_1	9.5(3)	11.0(2)
A_2	48.3(2)	44.5(1)
pK_a	6.03(1)	5.97(1)
dx	0.51(1)	0.50(1)

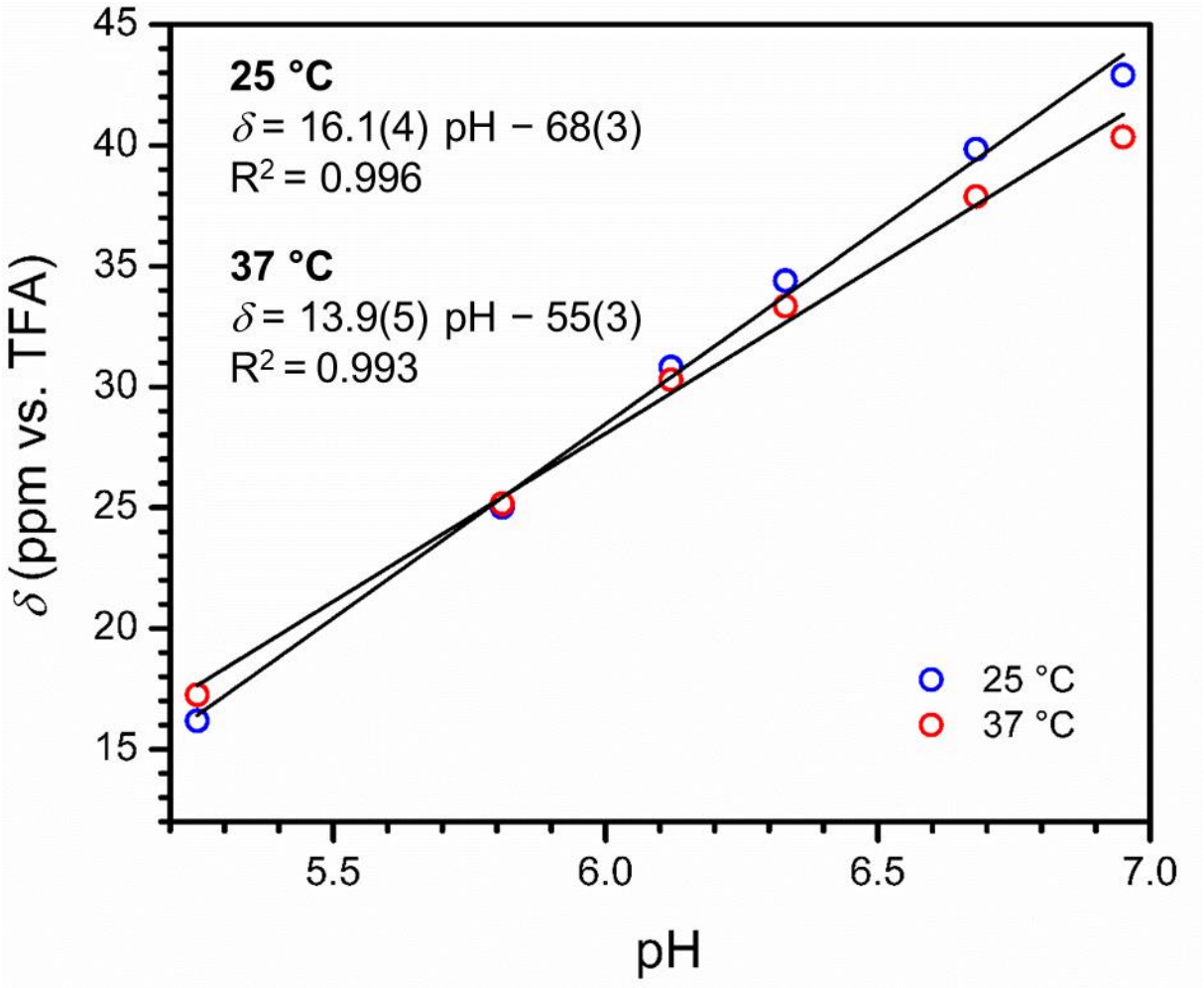


Figure S6 | pH dependence of the ^{19}F chemical shift of **1** vs TFA in 100 mM phosphate buffer solutions containing 2% (v/v) DMSO at 25 °C (blue) and 37 °C (red) between pH 5.25 and 6.95. Circles represent experimental data and black solid lines denote linear fits to the data (see equations on the graph).

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