SUPPORTING INFORMATION

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Bis[squaramido]ferrocenes as Electrochemical Sulfate Receptors

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1. General Information

Procedures employing oxygen-sensitive materials were performed with degassed solvents (*vide infra*) using standard inert-atmosphere techniques (atmosphere of anhydrous dinitrogen).

NMR spectra were recorded at 25 °C unless stated otherwise. ^1H, $^{13}C\{^1H\}$ and ^{19}F NMR spectra were recorded at 500 MHz, 125 MHz and 470 MHz, respectively, on a Bruker Avance 500 spectrometer. ^1H and ^{13}C NMR chemical shifts are reported in ppm relative to tetramethylsilane, and are referenced to the appropriate residual solvent peaks:

- DMSO-d_6: δ_H = 2.50 ppm, δ_C = 39.52 ppm
- MeCN-d_3: δ_H = 1.94 ppm, δ_C = 1.32 ppm (*C*H$_3$) & 118.26 ppm (*C*N)

^{19}F NMR chemical shifts are reported in ppm relative to an external standard of CCl$_3$F (0.00 ppm). Coupling constants, *J*, are reported in Hz and are uncorrected for digitization. The following abbreviations (and their combinations) are used to label the multiplicities: s (singlet), d (doublet), t (triplet), q (quartet), quint (quintet), sept (septet), m (multiplet) and br (broad).

High resolution mass spectra were recorded on a Bruker Apex II Fourier Transform Ion Cyclotron Resonance (FTICR) mass spectrometer with a 7.0 T magnet, fitted with an off-axis.

Infrared (IR) absorption spectra were recorded on a Bruker Alpha-E FT-IR spectrometer using attenuated total reflection (ATR) of solid samples. Notable vibrational wavenumbers are recorded in cm^{-1}.

2. Chemicals

Reagent grade solvents (Fisher Technical) were employed. With the following exceptions, all other reagents were obtained from commercial sources and were used as received:

- Degassed ethanol and DMSO were prepared via three freeze-pump-thaw cycles and then stored under dinitrogen atmosphere.

- DMSO-d_6 used for the characterization of FcSq$_2$ was degassed via three freeze-pump-thaw cycles, dried by passage through a column of 4Å molecular sieves and then stored under dinitrogen atmosphere.

- Anhydrous toluene was purchased from Sigma Aldrich.

- Phenylsquaramate and 3,5-bis[trifluoromethyl]phenylsquaramate were prepared following literature methods. [1]

- diaminoferrocene was prepared following literature methods (see below). The method of Tamm[2] was followed to access the Boc-protected precursor from which point the method of Hierso[3] was used to access diaminoferrocene.
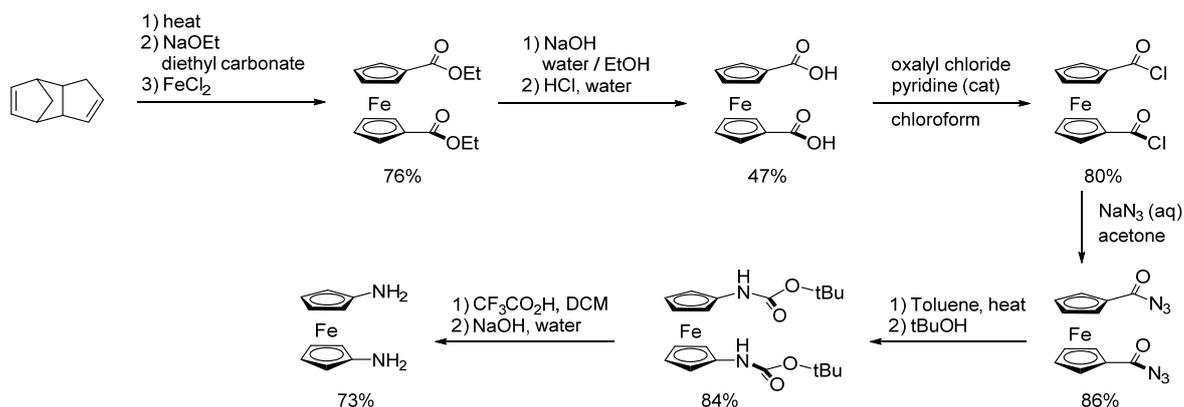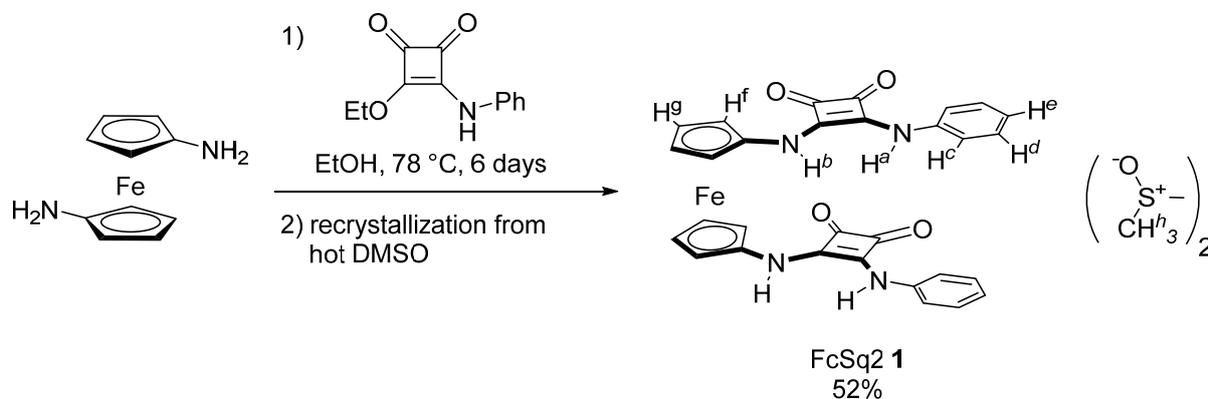

- Aminoferrocene was prepared *via* literature methods[4] with modification of conditions used for the Curtius rearrangement and deprotection steps. See the scheme shown below. Detailed experimental procedures starting from ferroceneacyl azide are described in section **S4**.
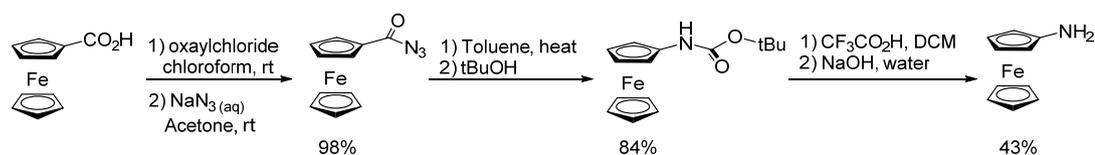
3. Synthesis and Characterization

FcSq₂ 1

FcSq2 **1**
52%

Synthesis

Within a glovebox, diaminoferrocene (34.4 mg, 1.59×10^{-4} mols, 1 equiv.) was added to a 10 mL Schlenk flask containing a small magnetic stir bar. A septum was inserted into the Schlenk flask and the reaction vessel was then removed from the glovebox and connected to a double manifold schlenk line under dinitrogen atmosphere. Degassed ethanol (1.75 mL) was added and the mixture was stirred, slowly effecting full dissolution. A solution of Phenylsquaramate (70.0 mg, 3.22×10^{-4} mols, 2 equiv.) in degassed ethanol (3.5 mL), prepared under dinitrogen atmosphere, was added to the solution of diaminoferrocene via syringe. Upon addition of the squarate solution, the initially yellow solution of diaminoferrocene darkened to a red/brown color over several minutes. The reaction was stirred and immersed in an oil bath heated to 78 °C where the temperature was held for 6 days during which time a light brown precipitate formed in the reaction mixture. The reaction was cooled to rt and disconnected from dinitrogen atmosphere. Under air, the precipitate was collected by filtration and washed with ethanol (30 mL) to yield the crude product (79 mg) as a light brown powder.

Purification

The crude product was placed in a 100 mL schlenk tube and the atmosphere was exchanged for dinitrogen. Degassed DMSO (18 mL) was added and the resultant suspension was swirled by hand while heating evenly by heat gun until full dissolution occurred. *Note that this requires heating to near boiling temperature; the system should have an appropriate vent installed (the bubbler attached to the Schlenk line was sufficient at this scale). Note also that the recrystallization must be performed under oxygen-free conditions. Performing the recrystallization under air atmosphere results in total decomposition of the product.* The resultant solution is dark and almost black in color. The solution was allowed to cool under dinitrogen atmosphere and stand at rt for 14 hours. After this time, small red crystals have formed. Under air, the crystals are isolated by filtration, washed with DMSO (3 × 3

mL), acetone (2 × 5 mL) and then dried under high vacuum to yield the pure product as a red microcrystalline powder (59 mg, 52% yield).

^{1}H NMR (500 MHz, DMSO-d_6): δ 9.56 (broad s, 2H, H^a), 9.18 (broad s, 2H, H^b), 7.16 (d, J = 7.7 Hz, 4H, H^c), 7.04 (t, J = 7.6 Hz, 4H, H^d), 6.85 (t, J = 7.2 Hz, 2H, H^e), 4.85 - 4.80 (broad m, 4H, H^f), 4.11 (broad t, J = 1.9 Hz, 4H, H^g), 2.54 (s, 12 H, 2 × DMSO from recrystallization as solvate).[5]

^{13}C{^{1}H} NMR: Acquiring a ^{13}C-NMR spectrum was not possible due to low solubility of FcSq$_2$ **1**. See Figure S3 for the ^{13}C-NMR spectrum of the related **1**•SO$_4^{2-}$ complex.

IR v_{max} (solid)/cm^{-1}: 3282, 3176, 3133, 3054, 2990, 2912, 1779, 1674, 1634, 1593, 1556, 1502, 1487, 1436, 1388, 1294, 1271, 1224, 1154, 1104, 1079, 1056, 1010, 957, 933, 820, 800, 759, 742.

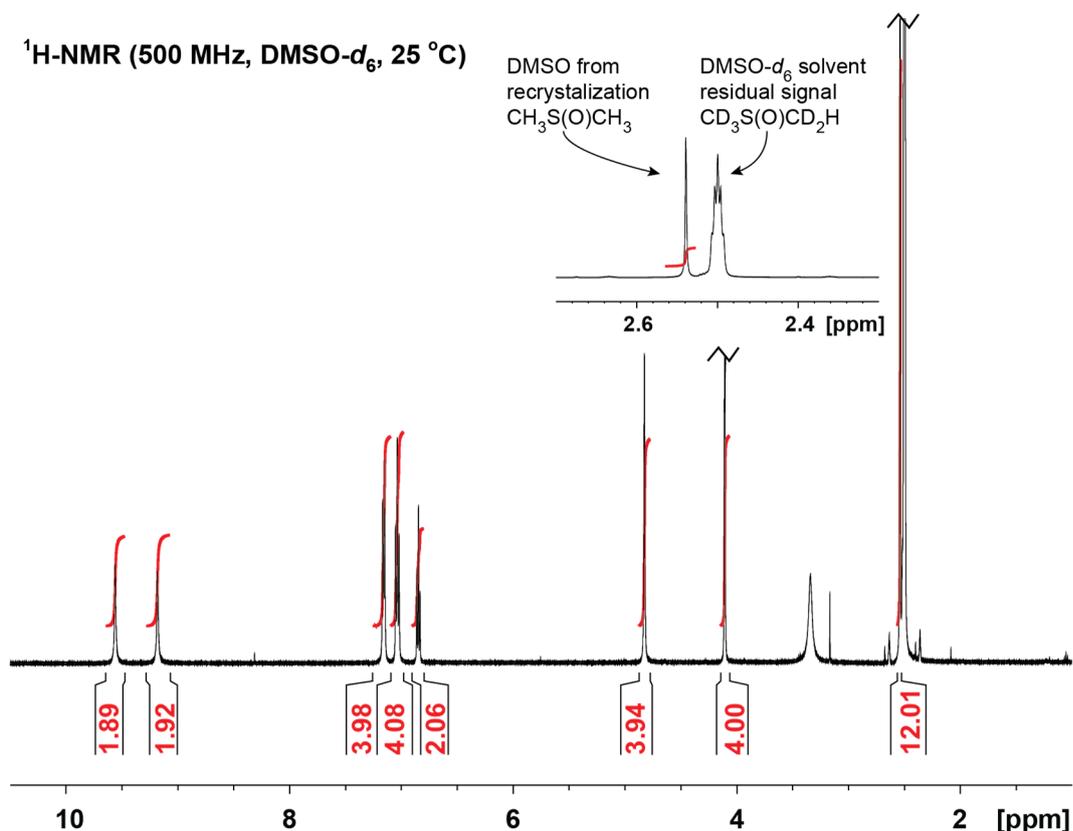**HRMS (ESI$^+$)** *m/z* calcd for C$_{30}$H$_{22}$FeN$_4$O$_4$ ([M]$^+$) 558.0990, found 558.0985.

Figure S1: ^{1}H-NMR spectrum of FcSq$_2$ **1**.

Assignment of the product as the 1•(DMSO)$_2$ complex

Upon dissolution of the product in DMSO-d_6 for the purpose of ^{1}H-NMR spectroscopic analysis, co-crystalized DMSO molecules (which are non-deuterated) are presumably released into the bulk medium. The ^{1}H-NMR signal corresponding to the CH_3 protons belonging to the released DMSO can be observed as a singlet, approximately 0.04 ppm downfield shifted with respect to the DMSO-d_6 solvent residual signal (CD$_2$H) which appears as a pentet at 2.50 ppm. Comparing the Integral value of the signal corresponding to the CH_3 group belonging to released DMSO with the integral values of the signals corresponding to protons belonging to FcSq2 **1** reveals a 2:1 DMSO:**1** ratio, suggesting that after recrystallization, **1** is isolated as the solvate, coordinated to two DMSO molecules.

FcSq₂ 2

FcSq₂ 2
42%
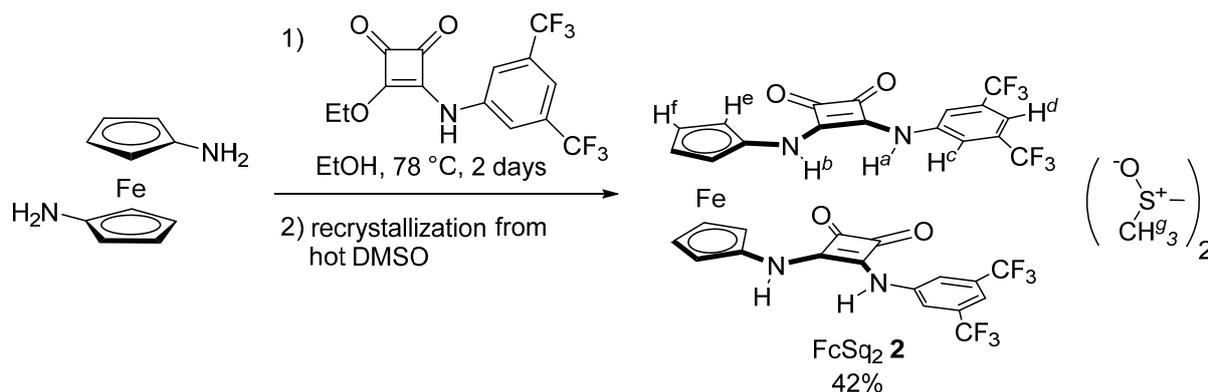
Synthesis

Within a glovebox, diaminoferrocene (35.5 mg, 1.64×10^{-4} mols, 1 equiv.) was added to a 10 mL Schlenk flask containing a small magnetic stir bar. A septum was inserted into the Schlenk flask. The reaction vessel was removed from the glovebox and connected to a double manifold schlenk line under dinitrogen atmosphere. Degassed ethanol (1.75 mL) was added and the mixture was stirred, slowly effecting full dissolution. A solution of 3,5-bis[trifluoromethyl]phenylsquaramate (125.0 mg, 3.53×10^{-4} mols, 2.15 equiv.) in degassed ethanol (6 mL), prepared under dinitrogen atmosphere, was added to the solution of diaminoferrocene via syringe. Upon addition of the squarate solution, the initially yellow solution of diaminoferrocene darkened to a red/brown color over several minutes. The reaction was stirred and immersed in an oil bath heated to 78 °C where the temperature was held for 2 days during which time a light brown precipitate formed in the reaction mixture. The reaction was cooled to rt and disconnected from dinitrogen atmosphere. Under air, the precipitate was collected by filtration and washed with ethanol (30 mL) to yield the crude product (80 mg) as a light brown powder.

Purification

The crude product was placed in a 100 mL schlenk tube and the atmosphere was exchanged for dinitrogen. Degassed DMSO (10 mL) was added and the resultant suspension was swirled by hand while heating evenly by heat gun until full dissolution occurred. *Note that this requires heating to near boiling temperature; the system should have an appropriate vent installed (the bubbler attached to the Schlenk line was sufficient at this scale). Note also that the recrystallization must be performed under oxygen-free conditions. Performing the recrystallization under air atmosphere results in total decomposition of the product.* The resultant solution was dark and almost black in color. The solution was allowed to cool under dinitrogen atmosphere and stand at rt for 14 hours. After this time, small red crystals had formed. Under air, the crystals were isolated by filtration, washed with DMSO (3 × 3

mL), acetone (2 × 5 mL) and then dried under high vacuum to yield the pure product as a red microcrystalline powder (68 mg, 42% yield).

^{1}H NMR (500 MHz, DMSO-d_6): δ 10.16 (broad s, 2H, H^a), 9.25 (broad s, 2H, H^b), 7.72 – 7.68 (broad m, *4*H, H^c), 7.37 – 7.32 (broad m, *2*H, H^d), 4.91 - 4.84 (broad m, 4H, H^e), 4.18 - 4.11 (broad m, 4H, H^f), 2.54 (s, 12 H, 2 × DMSO from recrystallization as solvate).[6]

^{13}C{^{1}H} NMR: Acquiring a ^{13}C-NMR spectrum was not possible due to low solubility of FcSq$_2$ **2.** See Figure S4 for the ^{13}C-NMR spectrum of the related **2**•SO$_4$$^{2−}$ complex.

^{19}F NMR (470 MHz, DMSO-d_6): −62.19.

IR ν$_{max}$ (solid)/cm^{-1}: 3264, 3183, 3062, 3011, 2917, 1789, 1693, 1639, 1614, 1577, 1512, 1472, 1447, 1380, 1328, 1278, 1210, 1181, 1122, 1054, 1005, 956, 921, 883, 876, 861, 842, 826, 804.

HRMS (ESI$^+$) *m/z* calcd for C$_{34}$H$_{18}$F$_{12}$FeN$_4$O$_4$Na ([M+Na]$^+$) 853.0378, found 853.0378.

¹H-NMR (500 MHz, DMSO-d_6, 25 °C)

DMSO from recrystalization CH$_3$S(O)CH$_3$

DMSO-d_6 solvent residual signal CD$_3$S(O)CD$_2$H

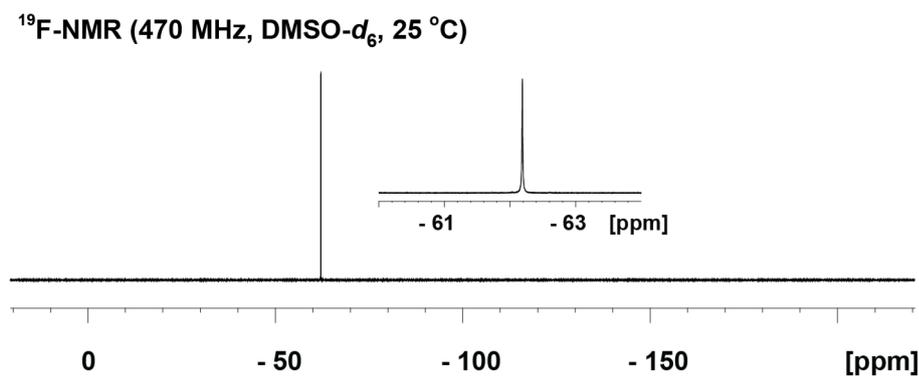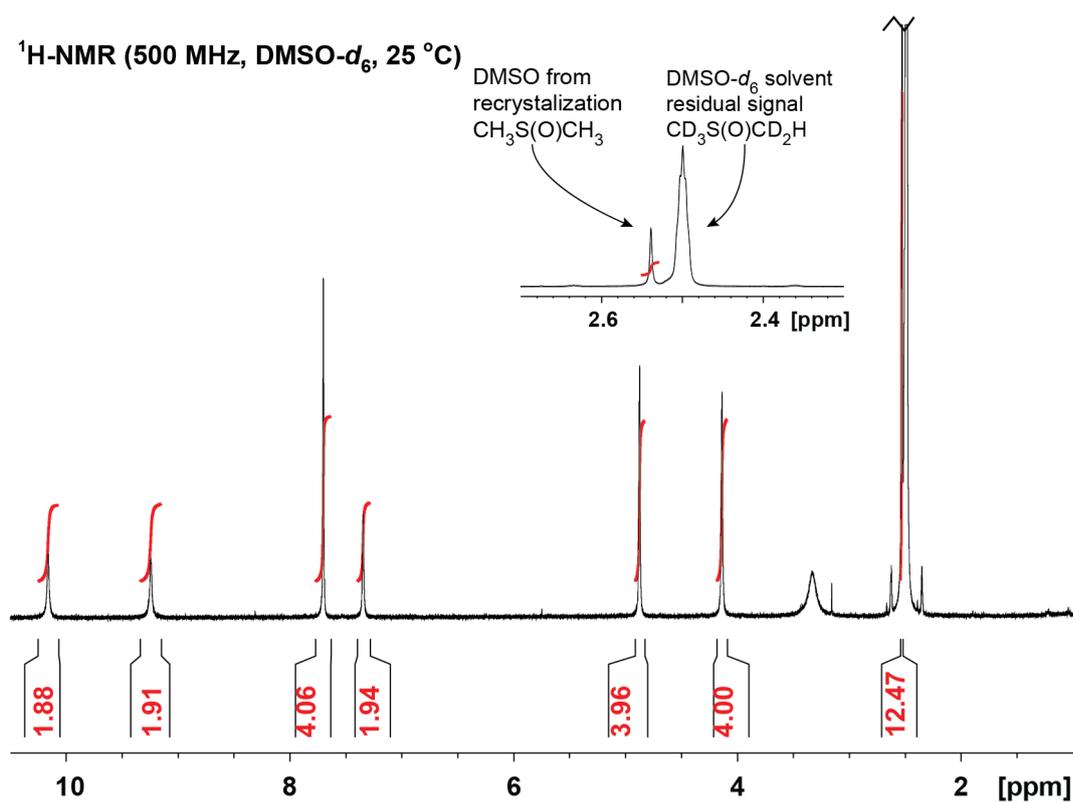**¹⁹F-NMR (470 MHz, DMSO-d_6, 25 °C)**

Figure S2: NMR spectra of FcSq$_2$ **2**.

Assignment of the product as the 2•(DMSO)₂ complex

Upon dissolution of the product in DMSO-d_6 for the purpose of ¹H-NMR spectroscopic analysis, co-crystalized DMSO molecules (which are non-deuterated) are presumably released into the bulk medium. The ¹H-NMR signal corresponding to the CH_3 protons belonging to the released DMSO can be observed as a singlet, approximately 0.04 ppm downfield shifted with respect to the DMSO-d_6 solvent residual signal (CD$_2$H) which appears as a pentet at 2.50 ppm. Comparing the Integral value of the signal corresponding to the CH_3 group belonging to released DMSO with the integral values of the signals corresponding to protons belonging to FcSq2 **2** reveals a 2:1 DMSO:**2** ratio, suggesting that after recrystallization, **2** is isolated as the solvate, coordinated to two DMSO molecules.

FcSq₁ 3

FcSq₁ **3**
72%
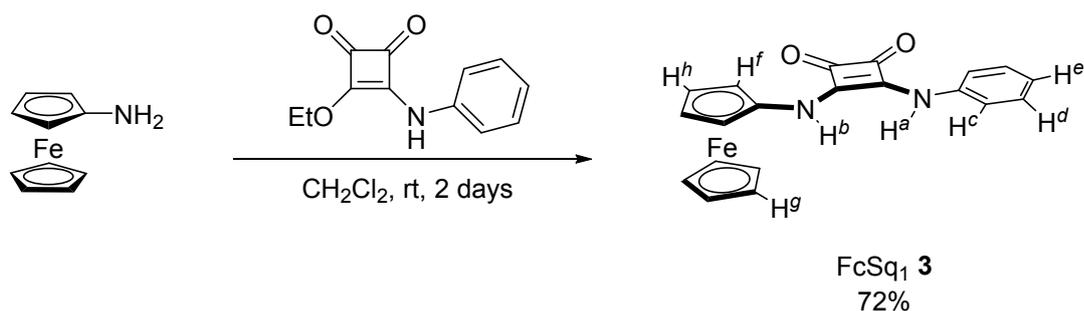
Synthesis

Under air, dichloromethane (2mL) was added to a 10 ml reaction tube charged with aminoferrocene (20.0 mg, 9.95×10^{-5} mols, 1 equiv.) and phenylsquaramate (21.5 mg, 9.90×10^{-5} mols, 1 equiv.) to give a yellow colored solution. The reaction tube was sealed by addition of a glass stopper and the reaction was stirred at rt for 2 days during which time the reaction changed to a pale red colored solution. The volatiles were removed under reduced vacuum to give the crude product as a red solid.

Purification

Methanol (2 mL) was added to the crude product and the reaction tube was immersed in a sonic bath producing a yellow precipitate that was isolated by centrifugation and decantation of the red-colored liquid phase. The resultant yellow solid was washed with acetone (2 × 1 mL) and then dried under high vacuum to yield the pure product **3** as a light yellow solid (27 mg, 72% yield).

NMR spectroscopic data is in agreement with that reported in the literature.[7]

¹H NMR (500 MHz, DMSO-d_6): δ 9.68 (broad s, 1H, H^a), 9.27 (broad s, 1H, H^b), 7.50 (d, J = 7.9 Hz, 2H, H^c), 7.38 (t, J = 7.6 Hz, 2H, H^d), 7.07 (t, J = 7.5 Hz, 1H, H^e), 4.75 (broad t, J = 1.7 Hz, 2H, H^f), 4.21 (s, 5H, H^g); 4.09 (broad t, J = 1.7 Hz, 2H, H^h).

¹³C{¹H} NMR (125 MHz, DMSO-d_6): 181.7, 180.7, 166.4, 165.1, 138.8, 129.4, 123.0, 118.2, 96.5, 69.2, 64.8, 60.8.

IR ν$_{max}$ (solid)/cm^{-1}: 3253, 3195, 3155, 3056, 1791, 1667, 1618, 1608, 1587, 1542, 1500, 1450, 1388, 1348, 1268, 1253, 1154, 1105, 1084, 1014, 999, 930, 808.

**HRMS (ESI⁺)** *m/z* calcd for $C_{20}H_{16}FeN_2O_2$ ([M]⁺) 372.0561, found 372.0555.

1•(TMA)₂SO₄ Solution In MeCN-d₃

FcSq₂ **1** → **1**•(TMA)₂SO₄
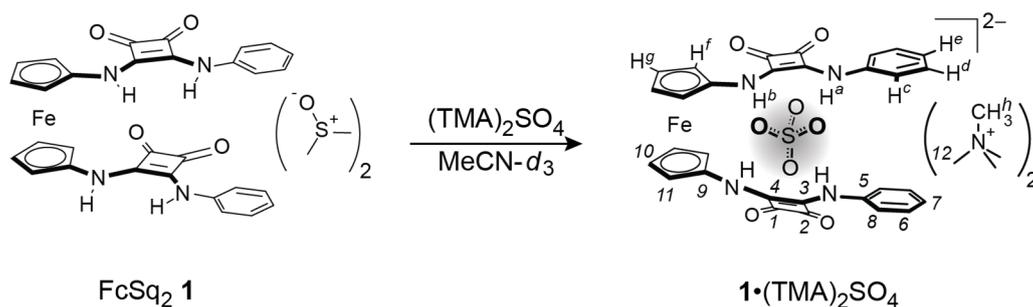
Synthesis

MeCN-d_3 (0.55 mL) was added to a 1 mL microcentrifuge tube charged with FcSq₂ **1** (3.0 mg, 4.20×10^{-6} mols, 1 equiv.) and tetramethylammonium sulfate (10.1 mg, 4.13×10^{-5} mols, 10 equiv.). The microcentifuge tube was closed, shaken by hand to homogenize the mixture, and immersed in a sonic bath for 5 min during which time the supernatant solution took on a deep orange color while the residual solid became a pure white. The tube was placed in a centrifuge and spun at 13K RPM for 5 min. The microcentrifuge tube was opened, the supernatant was carefully removed and transferred to a NMR tube via Pasteur pipette and NMR spectroscopic analysis was performed.

¹H NMR (500 MHz, MeCN-d_3): δ 11.20 (broad s, 2H, H^a), 11.01 (broad s, 2H, H^b), 7.49 (broad d, J = 7.8 Hz, 4H, H^c), 7.04 (broad t, J = 8.0 Hz, 4H, H^d), 6.80 (tt, J = 7.4, 1.1 Hz, 2H, H^e), 4.91 (t, J = 2.0 Hz, 4H, H^f), 4.02 (t, J = 1.9 Hz, 4H, H^g); 3.09 (broad s, 24H, H^h).
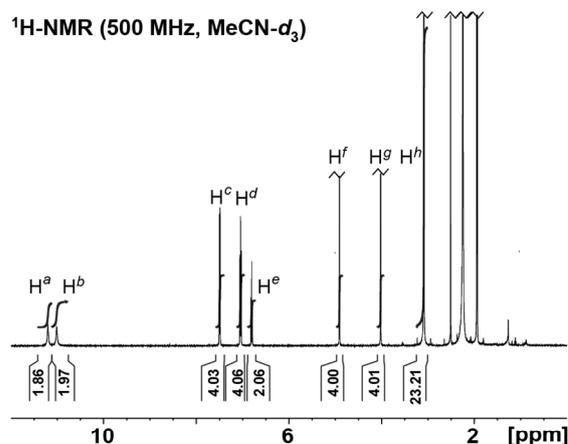
¹³C{¹H} NMR (125 MHz, MeCN-d_3): 183.1 (C^1 or C^2), 182.2 (C^1 or C^2), 167.9 (C^3 or C^4), 166.8 (C^3 or C^4), 140.7 (C^5), 129.5 (C^6), 123.1 (C^7), 119.9 (C^8), 99.1 (C^9), 66.0 (C^{10}), 63.3 (C^{11}), 56.2 (t, J = 4.1 Hz, C^{12}).
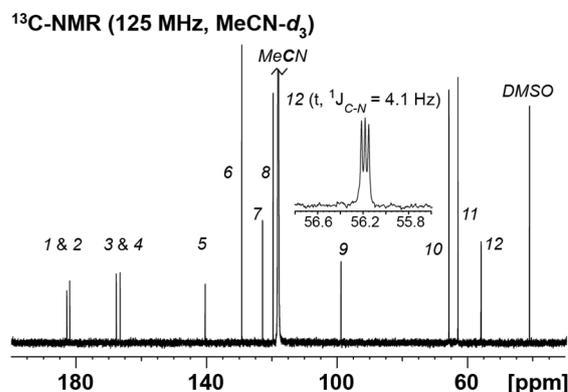
Figure S3: NMR spectra of **1**•(TMA)₂SO₄.

2•(TMA)$_2$SO$_4$ Solution In MeCN-d_3

FcSq$_2$ **2**   **2**•(TMA)$_2$SO$_4$
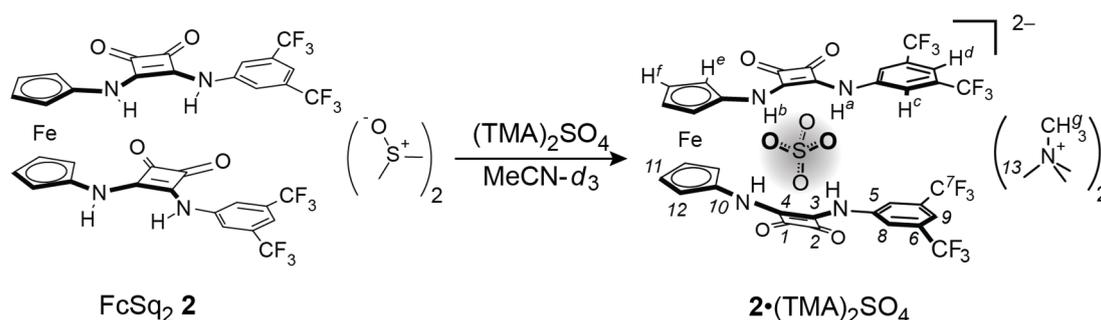
Synthesis

MeCN-d_3 (0.60 mL) was added to a 1 mL microcentrifuge tube charged with FcSq$_2$ **2** (3.6 mg, 3.65 × 10^{-6} mols, 1 equiv.) and tetramethylammonium sulfate (9.6 mg, 3.93 × 10^{-5} mols, 11 equiv.). The microcentifuge tube was closed, shaken by hand to homogenize the mixture, and immersed in a sonic bath for 5 min during which time the supernatant solution took on a deep orange color while the residual solid became a pure white. The tube was placed in a centrifuge and spun at 13K RPM for 5 min. The microcentrifuge tube was opened, the supernatant was carefully removed and transferred to a NMR tube via Pasteur pipette and NMR spectroscopic analysis was performed.

^{1}H NMR (500 MHz, MeCN-d_3): δ 11.97 (broad s, 2H, H^a), 11.12 (broad s, 2H, H^b), 8.09 (d, J = 1.2 Hz, 4H, H^c), 7.22 (t, J = 1.3 Hz, 2H, H^d), 4.94 (t, J = 1.9 Hz, 4H, H^e), 4.05 (t, J = 2.0 Hz, 4H, H^f); 3.09 (broad s, 24H, H^g).
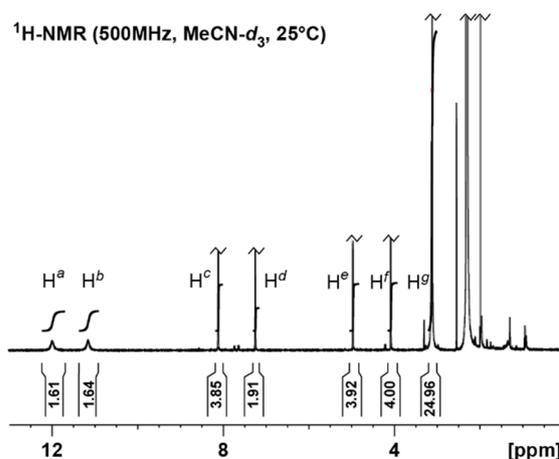
^{13}C{^{1}H} NMR (125 MHz, MeCN-d_3): 183.6 (C^1 or C^2), 181.9 (C^1 or C^2), 168.3 (C^3 or C^4), 165.8 (C^3 or C^4), 142.8 (C^5), 132.3 (q, J = 33 Hz, C^6), 124.4 (q, J = 272 Hz, C^7), 119.6 (C^8), 115.4 (C^9), 98.6 (C^{10}), 66.4 (C^{11}), 63.1 (C^{12}), 56.2 (t, J = 4.1 Hz, C^{13}).
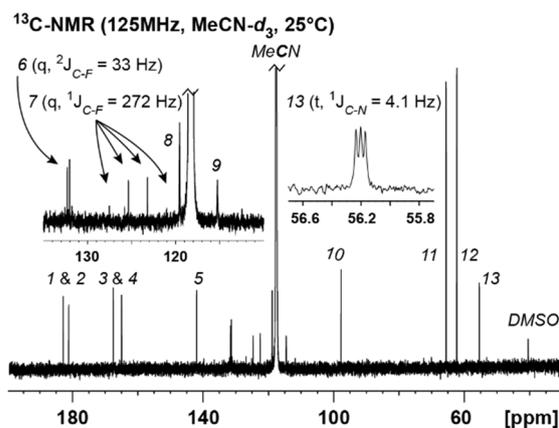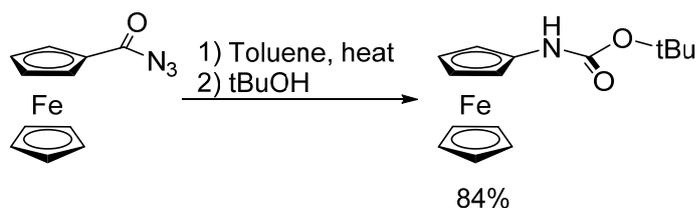

Figure S4: NMR spectra of **2**•(TMA)$_2$SO$_4$.

Boc-protected aminoferrocene

84%

Synthesis

Under nitrogen atmosphere, anhydrous toluene (40 mL) was added to a 2-neck round bottom flask fitted with a reflux condenser and charged with ferroceneacyl azide (5.00 g, 1.96×10^{-2} mols, 1 equiv.). The reaction was stirred and immersed in an oil bath heated to 110 °C. After 5 minutes heating, bubbling started, increasing to a rapid rate after 10 minutes and then ceasing after 30 min. The temperature setting of the heating bath was adjusted to 100 °C. The reaction was removed from the heating bath and allowed to cool for 5 min after which time *tert*-butanol was added (3.5 mL, 3.66×10^{-2} mols, 1.87 equiv.). The reaction was returned to the heating bath and stirred for 45 min. The reaction was allowed to cool to rt, during which time, the product crystalizes out of solution as fine needles.

Purification

The volume of toluene in the reaction vessel is brought to 100 mL. The system stirred and heated to reflux which effects complete dissolution of the crystalline material. The reaction is allowed to cool to rt and stand, unstirred, for 14 h after which time fine needles have crystalized. The crystals are collected by filtration and washed with cold toluene (70 mL). The crystals are dried by passage of air through the filter and then under high vacuum to yield the pure boc-protected aminoferrocene as fine yellow needles (5.00 g, 84% yield)

^{1}H-NMR spectroscopic data is in agreement with that reported in the literature.[4]

Rf = 0.3 (toluene, silica plate)

^{1}H NMR (500 MHz, DMSO-d_6): δ 8.49 (broad s, 1H), 4.44 (broad s, 2H), 4.09 (s, 5H), 3.89 (t, J = 2Hz, 2H), 1.45 (s, 9H).

^{13}C{^{1}H} NMR (125 MHz, DMSO-d_6): 153.0, 97.2, 78.3, 68.6, 63.4, 59.8, 28.1.

Aminoferrocene

Synthesis

Under air, a suspension of boc-protected aminoferrocene (2.00 g, 6.6×10^{-3} mols, 1 equiv.) in dichloromethane (12 mL) in a 100 mL round bottom flask was immersed in a cold bath at 35 °C. Once the temperature within the reaction mixture had equilibrated, trifluoroacetic acid (2.54 mL, 3.3×10^{-2} mols, 5 equiv.) was added dropwise to the reaction over 1.5 minutes, during which time the reaction changed from a pale yellow turbid solution to a dark brown and completely clear solution. The reaction was allowed to stir while coming to rt for 30 min. All volatiles were removed under reduced pressure and the resultant residue converted to the free base by addition of 1M NaOH$_{(aq)}$ (50 mL). The reaction mixture was extracted with ethyl acetate (3×25 mL), dried over Na_2SO_4, filtered and the ethyl acetate solution dried under reduced pressure and then high vacuum to give the crude product as a black solid.

Purification

The crude product was purified on silica gel (ethyl acetate) to give the pure aminoferrocene as a brown solid (0.575 g, 43% yield)

^{1}H-NMR spectroscopic data in C_6D_6 is similar to that reported in the literature obtained in $CDCl_3$.[8]

^{1}H NMR (500 MHz, C_6D_6): δ 3.97 (s, 5H), 3.73 (t, J = 1.9 Hz, 2H), 3.69 (t, J = 1. Hz, 2H), 1.85 (broad s, 2H).

^{1}H NMR (500 MHz, DMSO-d_6): δ 4.02 (s, 5H), 3.84 (t, J = 1.8 Hz, 2H), 3.70 (t, J = 1.8 Hz, 2H), 3.59 (broad s, 2H).

^{13}C{^{1}H} NMR (125 MHz, DMSO-d_6): 108.6, 68.4, 62.1, 56.6.

4. Binding Studies

1H-NMR titrations

All titrations were carried out on a Bruker 500 MHz or 400 MHz spectrometer, at 298 K. The association constants between $FcSq_2$ **1** or $FcSq_1$ **3** and the different anions were determined by monitoring the chemical shift changes of the proton signals corresponding to the receptors in the ^1H NMR spectrum as incremental amounts of the guest were added. As many proton signals as possible were monitored but sometimes excessive broadening or peak overlap rendered a specific signal impossible to follow. The value of the association constant was calculated using the BindFit (v0.5) package, available online at http://supramolecular.org.[9] In all cases the data fit well to a simple 1:1 binding model.

Specifically, the association constants were determined using 2.0 – 2.5 mM solutions of **1** and **3**, and adding aliquots of a solution of $(TBA)_xA^{x-}$, approximately 10 times more concentrated, in the same solvent. In this manner, by using the afore mentioned 2.0 – 2.5 mM solutions of **1** and **3** to prepare the solutions of $(TBA)_xA^{x-}$ the concentration of the receptor was maintained constant throughout the titration. Experimental error is estimated to be <15% for each K_a value obtained. Titrations of **3** with $(TMA)_2SO_4$ and $TMAH_2PO_4$ were run in duplicate to give K_a values.

UV-Vis Titrations

All titrations were carried out on a Cary 400 UV-Vis spectrophotometer equipped with stirring, at 298 K. The association constants between $FcSq_2$ **1** or $FcSq_1$ **3** and the different anions were determined by monitoring the change in absorbance over the range of wavelengths 300 – 400 nm as incremental amounts of the guest were added, to allow for a global fit. The value of the association constant was calculated using the BindFit (v0.5) package, available online at http://supramolecular.org.[9] In all cases the data fit well to a simple 1:1 binding model.

For UV-Vis titrations, the association constants were determined using 15 – 25 μM solutions of **1** and **3**, and adding aliquots of a solution of $(TBA)_xA^{x-}$, approximately 100 times more concentrated, in the same solvent. In this manner, by using the afore mentioned 15 – 25 μM solutions of **1** and **3** to prepare the solutions of $(TBA)_xA^{x-}$ the concentration of the receptor was maintained constant throughout the titration. Experimental error is estimated to be <15% for each K_a value obtained. Titrations of **1** with $(TMA)_2SO_4$ and with $TMAH_2PO_4$ were run in duplicate to give K_a values.

^{1}H-NMR analysis of interaction between FcSq$_2$ 1 and Cl$^-$, HSO$_4^-$, AcO$^-$ and F$^-$

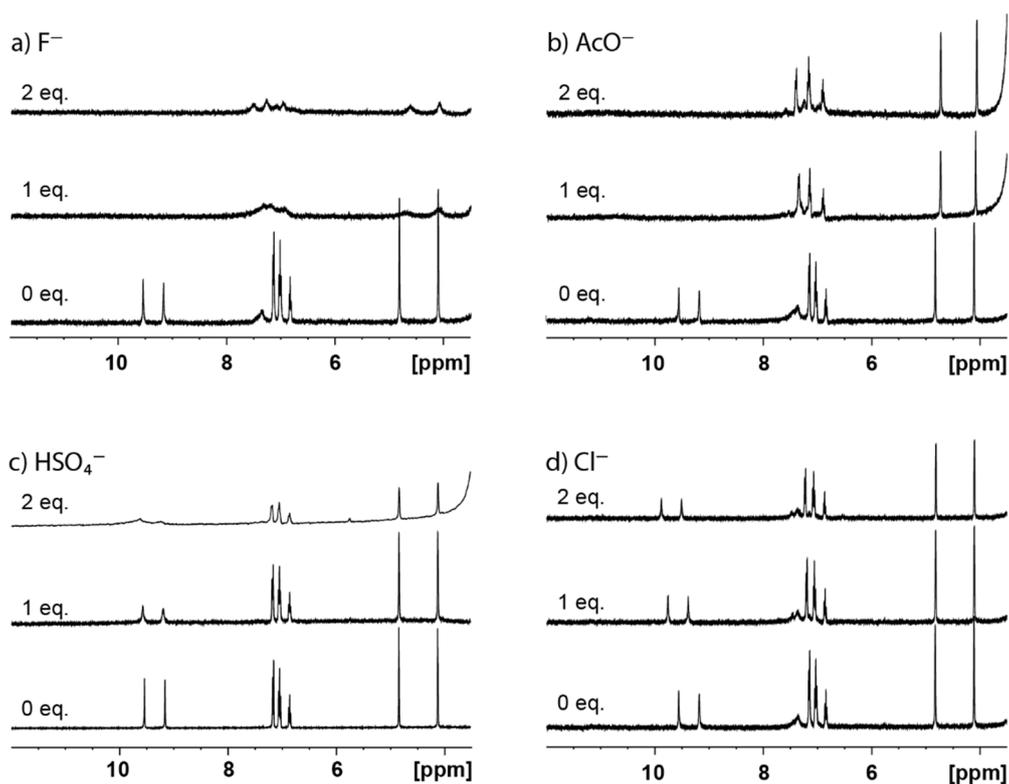**Figure S5**: ^{1}H NMR spectra of free **1** (2.5 mM in 1% water in DMSO-d_6) and after addition of one and two equivalents of a) TBAF, b) TBAOAc, c) TBAHSO$_4$, d) TBACl

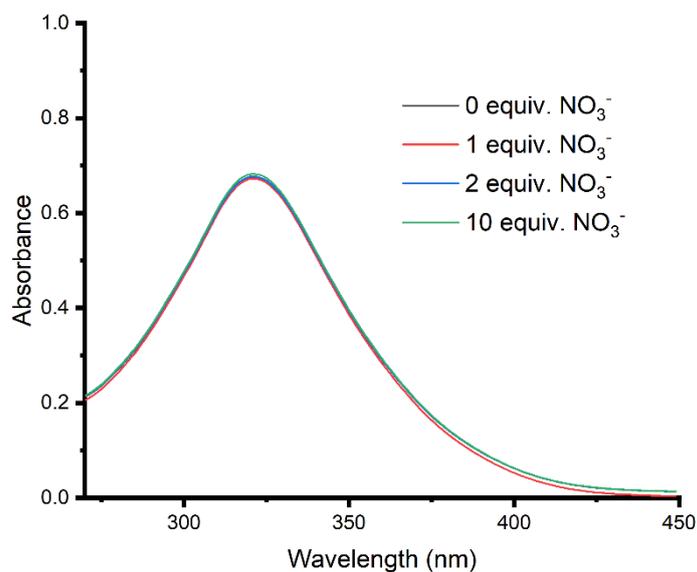**UV-Vis analysis of interaction between FcSq$_2$ 1 and NO$_3^-$**

Figure S6: ^{1}H NMR spectra of free **1** (20 µM, 1% water in DMSO) and after addition of one, two and 10 equivalents of TBANO$_3$.
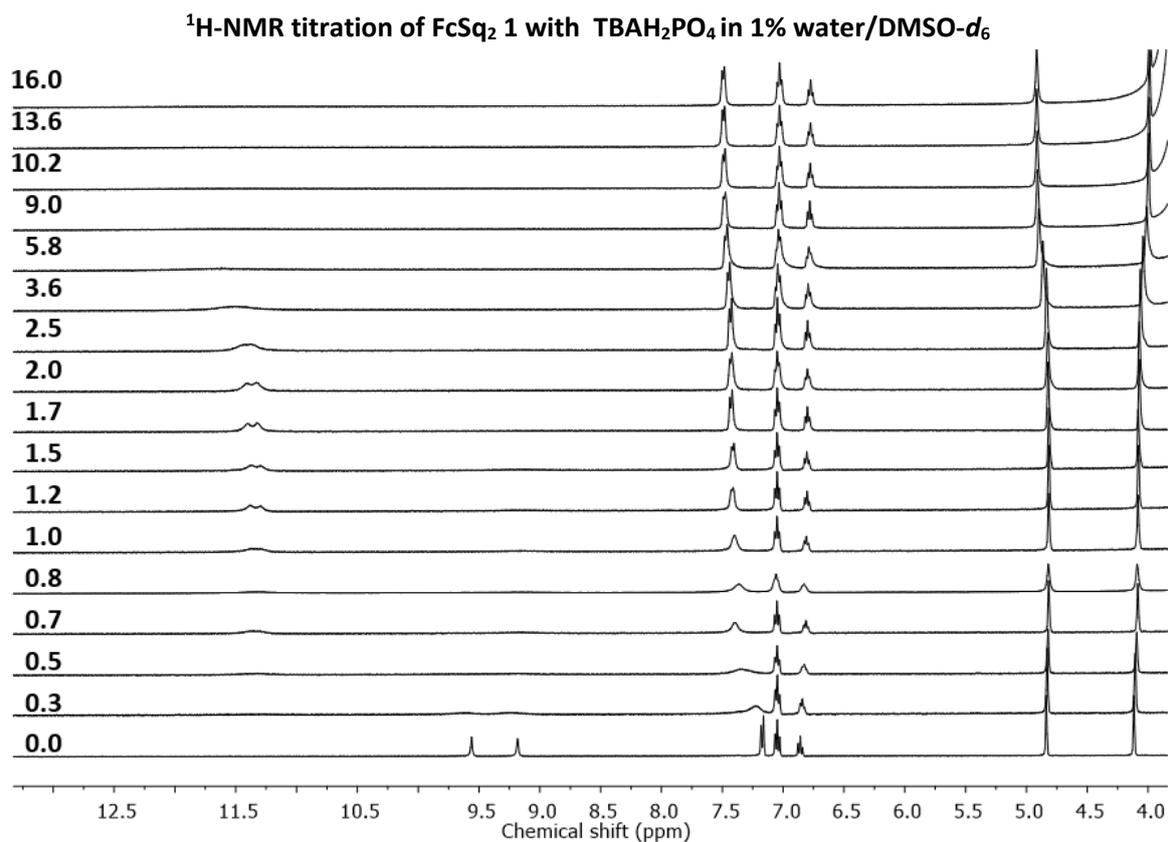
^{1}H-NMR titration of FcSq$_2$ 1 with TBAH$_2$PO$_4$ in 1% water/DMSO-d_6
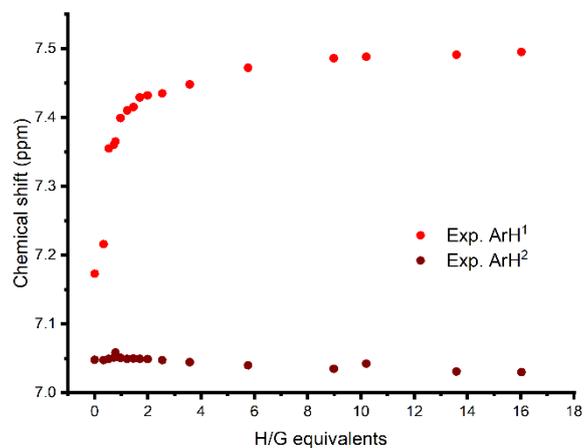
Figure S7: ^{1}H NMR (400 MHz, 1% H$_2$O/DMSO-d_6) spectroscopic binding titration of **1** (2.0 mM) TBAH$_2$PO$_4$ at 298 K. 0 – 16 equiv. NH protons appear to exhibit slow exchange binding kinetics. Aromatic proton changes in chemical shift over course of titration could not be fit to a 1:1 binding model.

^{1}H-NMR titration of FcSq$_1$ 3 with TBAH$_2$PO$_4$ in 1% water/DMSO-d_6

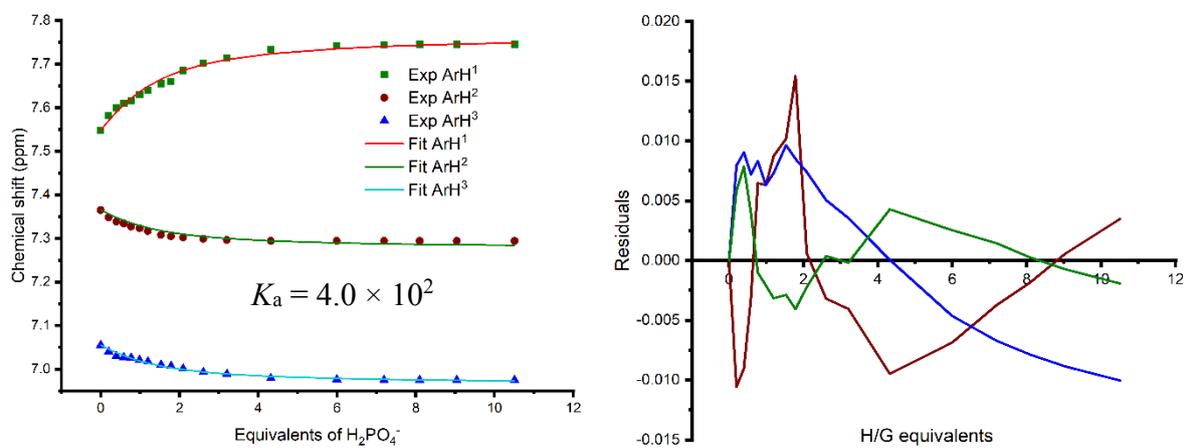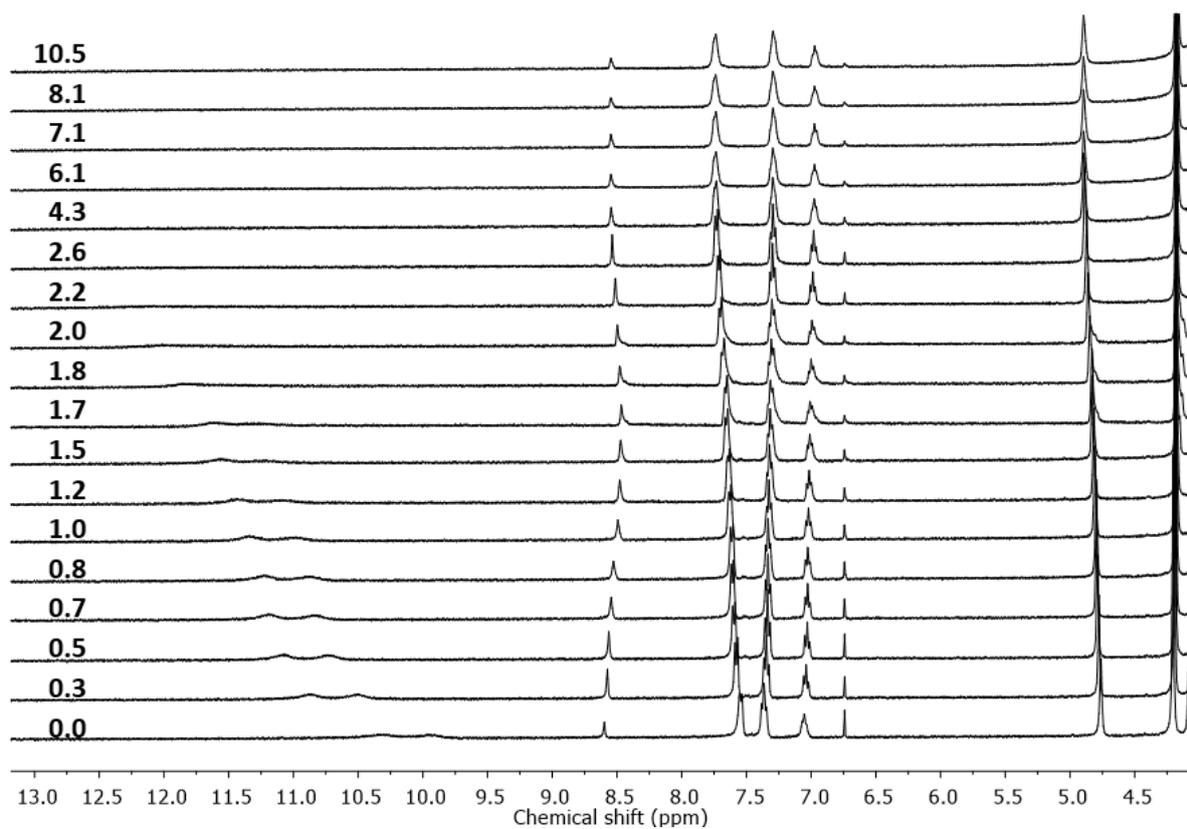**Figure S8**: ^{1}H NMR (400 MHz, 1% H$_2$O/DMSO-d_6) spectroscopic binding titration of **3** (2.5 mM) TBAH$_2$PO$_4$ at 298 K. 0 – 10 equiv. ArH protons fit to a 1:1 binding model and residuals (8% fitting error).

^{1}H-NMR titration of FcSq$_1$ 3 with (TBA)$_2$SO$_4$ in 1% water/DMSO-d_6
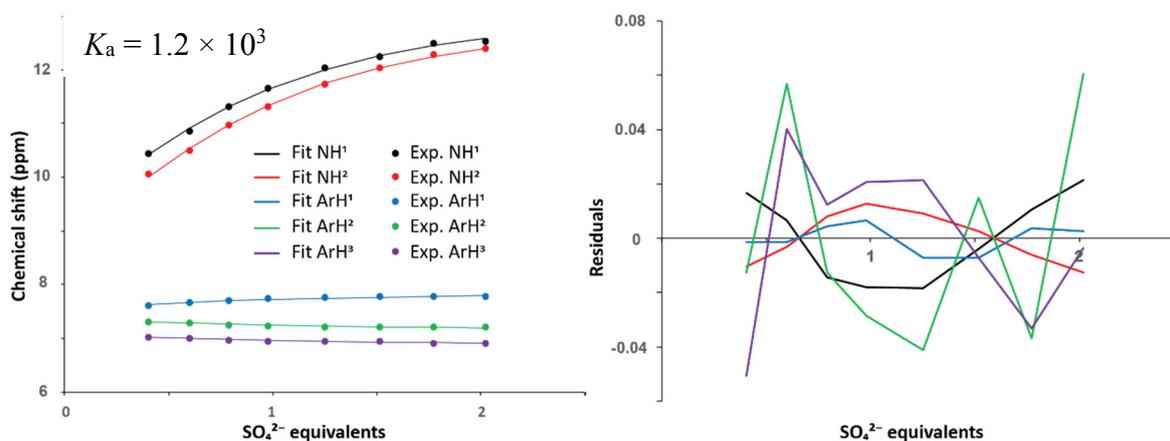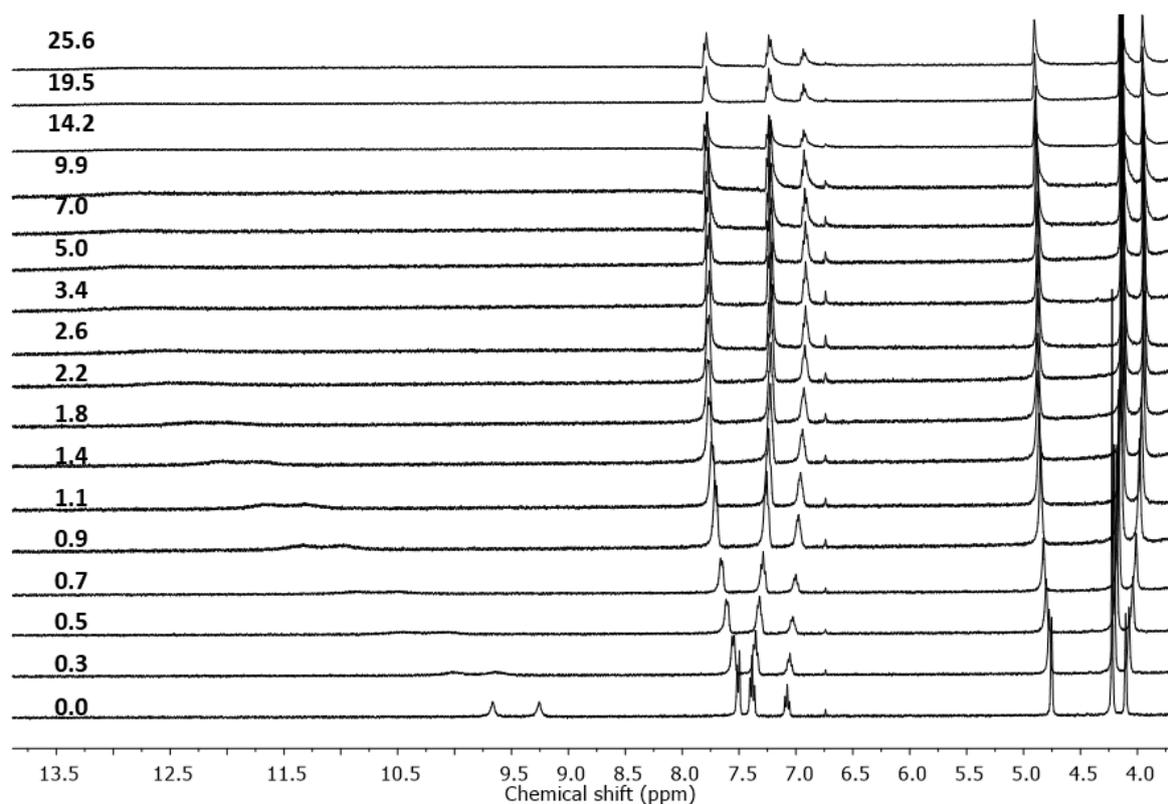
$K_a = 1.2 \times 10^3$

Figure S9: ^{1}H NMR (400 MHz, 1% H$_2$O/DMSO-d_6) spectroscopic binding titration of **3** (2.5 mM) TBA$_2$SO$_4$ at 298 K. 0 – 25 equiv. NH and ArH protons fit to a 1:1 binding model and residuals (4% fitting error).

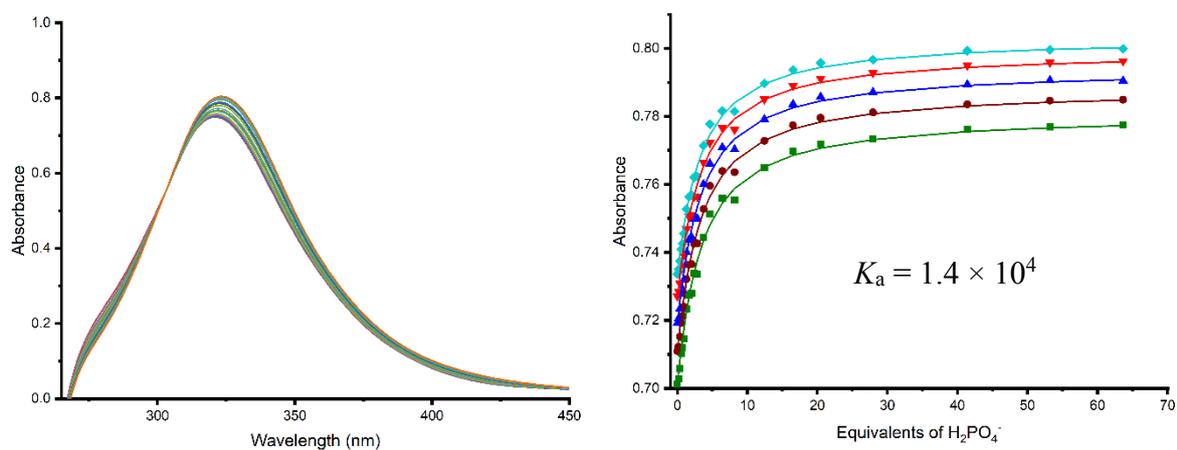**UV-Vis titration of FcSq₂ 1 with TBAH₂PO₄ in 20% water/DMSO-*d*₆**

Figure S10: UV-Vis Spectroscopic titration of **1**. (Left) UV-Vis spectra recorded over the course of a titration of Fc5 (24 μM) with TBAH₂PO₄ in DMSO (20% water) at 298 K. 0 – 63 equiv. (Right) Example fitting of wavelengths 330 nm – 326 nm to a 1:1 binding model (fitting error 1%)

UV-Vis titration of FcSq₂ 1 with (TBA)₂SO₄ in 20% water/DMSO
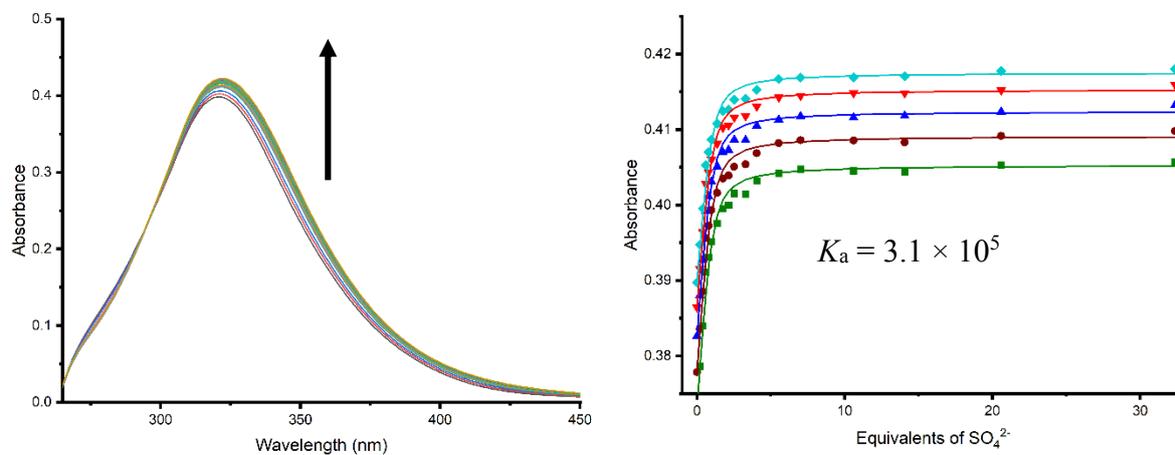

$K_a = 3.1 \times 10^5$

Figure S11: UV-Vis Spectroscopic titration of **1**. (Left) UV-Vis spectra recorded over the course of a titration of Fc5 (15 μM) with TBA₂SO₄ in DMSO (20% water) at 298 K. 0 – 32 equiv. (Right) Example fitting of wavelengths 330 nm – 326 nm to a 1:1 binding model (fitting error 4%).

5. Electrochemistry

Cyclic wave voltammetry was performed on an Autolab Potentiostat/Galvanostat model PGSTAT204, controlled by Nova 2.1.5 software (Metrohm Autolab). A one-compartment three-electrode electrochemical cell was used with a glassy carbon solid disc working electrode, platinum wire auxiliary electrode, and a silver/silver chloride reference electrode. The experiments were typically carried out with a 0.25 mM solution of sample in 20% water/DMSO containing 0.1 M $TBAClO_4$ as supporting electrolyte at 80 °C. *Note, potential formation of highly explosive perchlorate salts or compounds*. A 0.25 mM ferrocene sample was used to check the reference electrode and internal resistance of the equipment. Under these conditions, ferrocene displays $E_{1/2}$ = 0.474 V vs Ag/Ag^+, $I_{pa}/I_{pc} \approx 1$, and an anodic peak-cathodic peak separation of 69 mV.

All of the potential values reported for **1** are relative to the Fc/Fc^+ redox couple at 80 °C. Deoxygenation of the cell and solutions was achieved by passage of dinitrogen gas for at least 10 min and the working electrode was cleaned after each run. The cyclic voltammograms were recorded with a scan rate of 100 mV·s^{-1}.

A typical procedure is as follows.

A suspension of FcSq$_2$ **1** (1.05 mg, 1.5×10^{-6} mol) stirring in a solution of $TBAClO_4$ (205 mg, 6×10^{-4} mol) in DMSO (4.8 mL) was degassed by bubbling gaseous dinitrogen through the suspension for 10 minutes. The degassed suspension was then stirred and heated by heat gun to effect full dissolution of **1**. *Note, that failure to degas the suspension prior to heating results in extensive decomposition of the sample*. The solution of **1** and electrolyte in DMSO was allowed to stir while cooling to ≈ rt, at which point water (1.2 mL) was added. Rapidly, the full sample (which presented as a yellow solution) was taken into a 10 mL syringe *via* wide-bore needle. 5 mL of the solution were transferred to the electrochemical cell which had been fitted with an already revolving magnetic stir bar, was pre-purged with dinitrogen gas and had been placed in a heat block adjusted to the appropriate setting to ensure that, upon thermal equilibrium, the temperature of the sample within the cell was held at 80 °C. A further 0.5 mL of the remaining solution were transferred to a vial containing the relevant TBA salt (≈ 1.85×10^{-5} mol) designated for titration. During the aforementioned liquid transfers, precipitation of **1** sometimes occurred. However, upon stirring and heating to 80 °C, solution was reestablished in the electrochemical cell, while upon addition to TBACl, TBANO$_3$, TBAH$_2$PO$_4$ or (TBA)$_2$SO$_4$ the solution of **1** was stable even at rt.

Upon thermal equilibrium of the sample in the electrochemical cell, stirring was halted and cyclic wave voltammetry experiments were run. Then stirring was re-established and the relevant solution of TBA salt was added *via* microsyringe. After addition, stirring was stopped and the cyclic wave voltammetric properties of the solution were monitored.

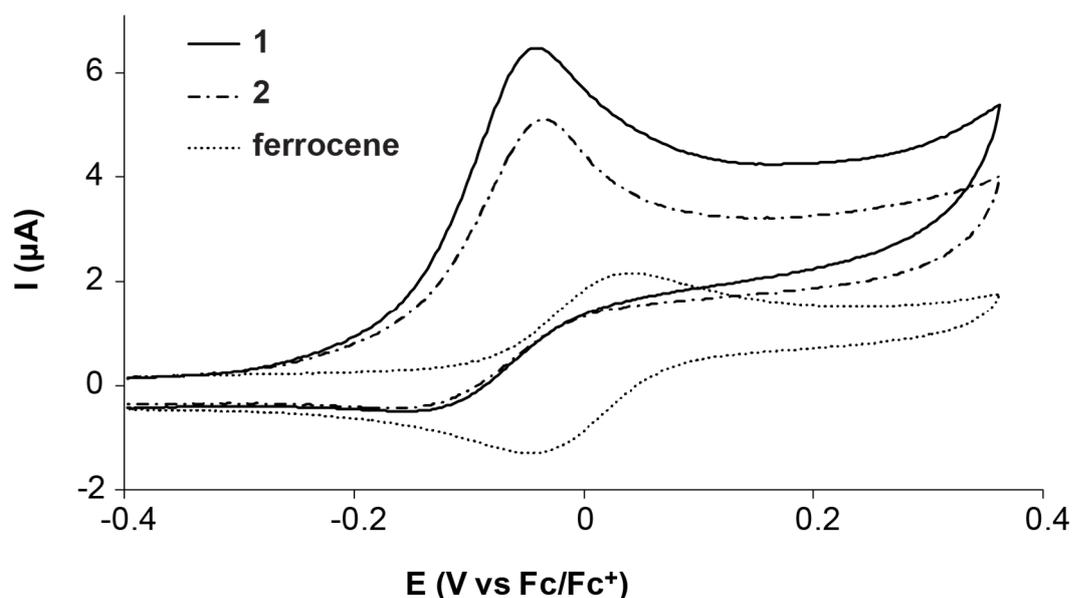## Cyclic Wave Voltammetry of FcSq₂ 1 & 2, and Ferrocene

Figure S12: CV response of FcSq₂ **1**, FcSq₂ **2** and ferrocene (0.25 mM) in 20% water/DMSO; supporting electrolyte, 0.1M TBAClO₄; scan rate, 100 mV s⁻¹; T = 80 °C.
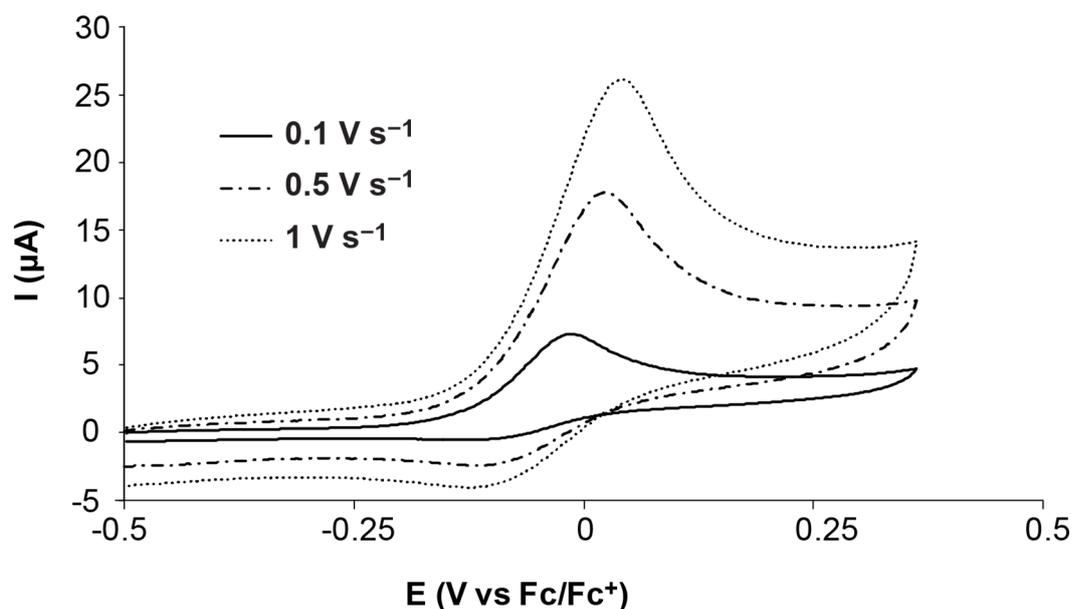
Cyclic Wave Voltammetry of FcSq₂ 1 at Varied Scan Rates

Figure S13: CV response of **1** (0.25 mM) in 20% water/DMSO at varied scan rates; supporting electrolyte, 0.1M TBAClO₄; scan rates of 100, 500 and 1000 mV s⁻¹; T = 80 °C.

Scan Rate (V/s)	ΔE (V)	E_{pa} (V)	E_{pc} (V)
0.1	0.088	-0.019	-0.107
0.5	0.127	0.023	-0.104
1.0	0.156	0.037	-0.119

Table S1: ΔE, E_{pa}, E_{pc} for FcSq₂ **1** at various scan rates; supporting electrolyte, 0.1M TBAClO₄; T = 80 °C. Potential compared to Fc/Fc⁺.

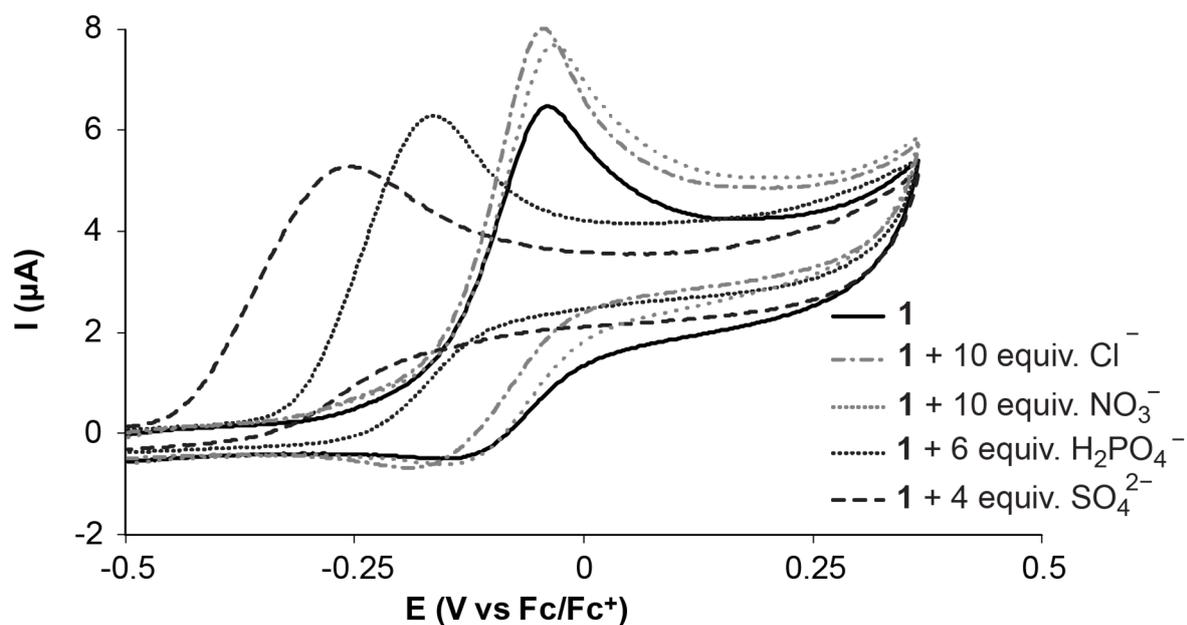## Cyclic Wave Voltammetry of FcSq$_2$ 1 in presence of various anions

Figure S14: CV response of **1** (0.25 mM) in 20% water/DMSO before and after addition of noted equivalents of TBACl, TBANO$_3$, (TBA)$_2$SO$_4$ and TBAH$_2$PO$_4$; supporting electrolyte, 0.1M TBAClO$_4$; scan rate, 100 mV s^{-1}; T = 80 °C.

Titration with TBAH$_2$PO$_4$

Figure S15: CV response of **1** (0.25 mM) in 20% water/DMSO before and after addition of noted equivalents of TBAH$_2$PO$_4$; supporting electrolyte, 0.1M TBAClO$_4$; scan rate, 100 mV s^{-1}; T = 80 °C.
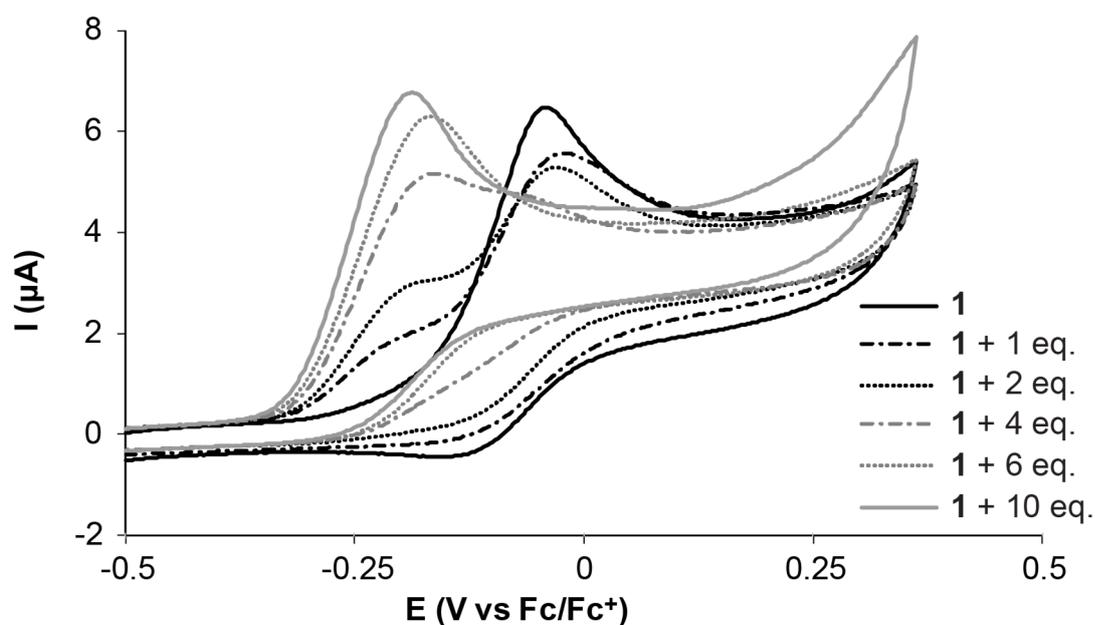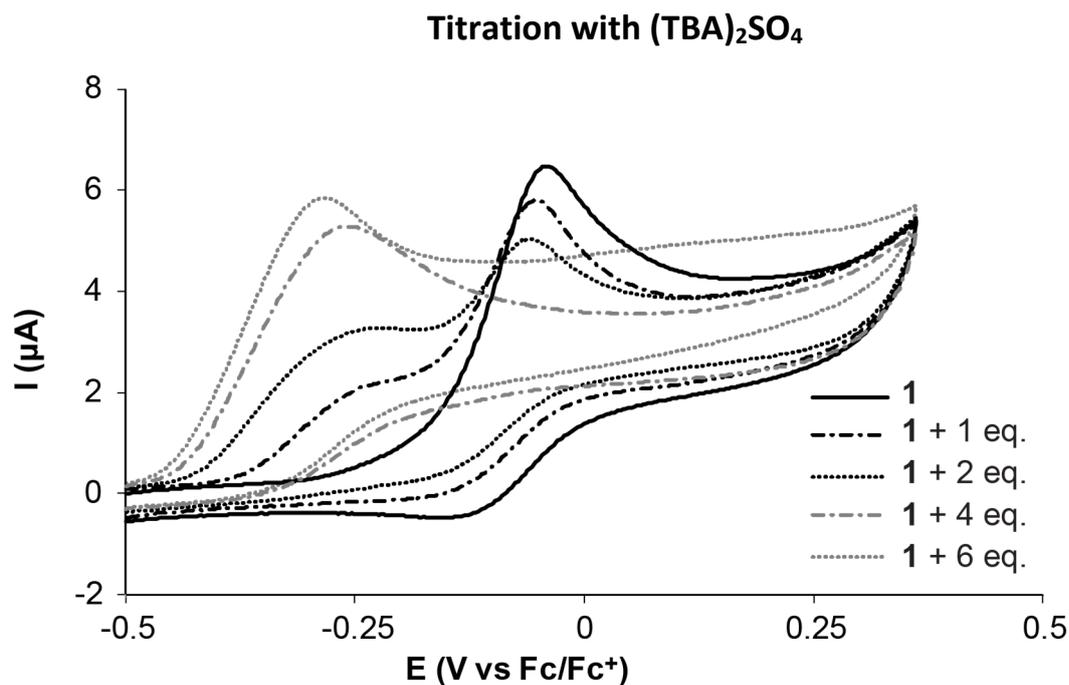
Titration with $(TBA)_2SO_4$

Figure S16: CV response of **1** (0.25 mM) in 20% water/DMSO before and after addition of noted equivalents of $(TBA)_2SO_4$; supporting electrolyte, 0.1M $TBAClO_4$; scan rate, 100 mV s^{-1}; T = 80 °C.

Discussion of Irreversible Electrochemical Manifold of $FcSq_2$

Irreversibility of **1** is indicated by the following: 1) the difference between anodic and cathodic peaks (ΔE) is significantly greater than 60 mV, 2) The ratio of anodic current (I_{pa}) to cathodic current (I_{pc}) is ≈ 3 whereas a value of 1 is expected for reversible systems, 3) The potentials at which anodic and cathodic peaks (E_{pa} and E_{pc}, respectively) are observed is highly dependent on the scan rate whereas these values should be independent of scan rate for reversible systems.

The redox behavior of ferrocenes, including both the amide- and urea-analogues of $FcSq_2$,[10,11] is known to be robust. Therefore, it is somewhat surprising that $FcSq_2$ **1** and **2** both show irreversible oxidation waves.

We note that when comparing cyclic wave voltamograms of ferrocene itself with $FcSq_2$ **1** & **2**, the cathodic current (the current during the reduction process, I_{pc}) for all three compounds is similar (Figure S12). However, the anodic current (the current during the oxidation process, I_{pa}) is approximately three times larger for both $FcSq_2$ **1** & **2** than for ferrocene itself. A possible explanation for this observation is that the oxidation wave observed for $FcSq_2$ is comprised of: 1) a component that corresponds to reversible one-electron oxidation of the ferrocene core of $FcSq_2$ and 2) a

component that corresponds to different and irreversible oxidation process(es) involving two electrons in total. Given the 2 : 1 ratio of irreversible : reversible components of overall oxidation matches the 2 : 1 ratio of 'arms' : ferrocene within the structure of FcSq$_2$, a reasonable presumption may be that the squaramide 'arms' are each sites of irreversible one-electron oxidation processes (1 × reversible 1e⁻ oxidation for Fc core + 2 × 1e⁻ irreversible oxidation for the two arms).

To the best of our knowledge, the redox chemistry of squaramides has not been investigated. Therefore, to briefly probe the hypothesis that the squaramide 'arms' are responsible for the irreversible component of FcSq$_2$ oxidation, we engaged diphenylsquaramide (Ph$_2$Sq) as a model 'arm' (Figure S17). Cyclic wave voltammetry of Ph$_2$Sq revealed: **1)** an irreversible oxidation process at relatively high potential (E_{pa} = 0.41 V vs Fc/Fc⁺), **2)** that signs of the reduction process expected to partner this oxidation were almost completely absent, **3)** that the anodic current (I_{pa}) for Ph$_2$Sq is equal in magnitude to that observed for ferrocene under the same conditions, suggesting that the oxidation process involving Ph$_2$Sq is a one-electron process. These three observations tentatively suggest that the squaramide arms of FcSq$_2$ may not be electrochemically inert and may be responsible for the observed irreversible electrochemical behavior of FcSq$_2$.
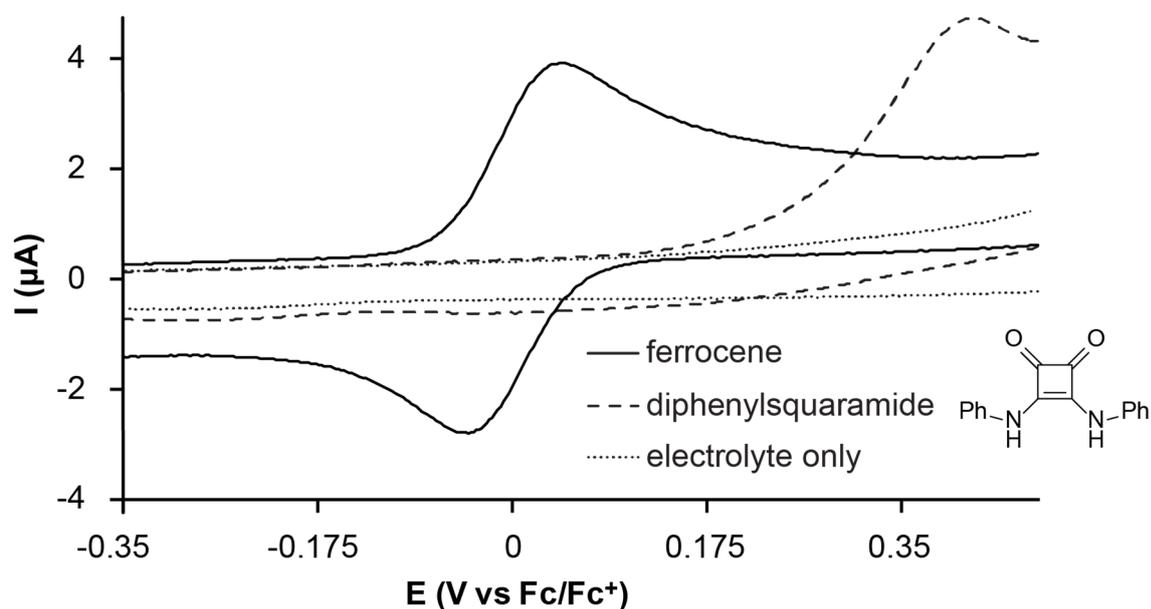

Figure S17: CV response of ferrocene (0.5 mM), dihenylsqaramide (0.5 mM), or simply supporting electrolyte in DMSO; supporting electrolyte, 0.1M TBAClO$_4$; scan rate, 100 mV s⁻¹; T = 20 °C.

6. X-Ray Crystallographic Data

Experimental

Single crystals of $C_{38}H_{26}F_{12}FeN_4O_8$ **2**•(AcOH)$_2$ were obtained from acetic acid solution. A suitable crystal was analysed on a SuperNova, Dual, Cu at near, Atlas diffractometer. The crystal was kept at 100(2) K during data collection. Using Olex2,[12] the structure was solved with the olex2.solve[13] structure solution program using Charge Flipping and refined with the SHELXL[14] refinement package using Least Squares minimisation.

Crystal structure determination of 2

Crystal Data for $C_{43}H_{36}F_{11.995}FeN_4O_{13}$ (M =1100.51 g/mol): triclinic, space group P-1 (no. 2), a = 13.1283(3) Å, b =   13.3090(3) Å, c =   14.7500(3) Å, α =   110.216(2)°, β =   104.1886(19)°, γ = 99.8556(19)°, V = 2250.00(9) Å^3, Z = 2, T = 100(2) K, μ(Cu Kα) = 3.778 mm^{-1}, $Dcalc$ = 1.624 g/cm^3, 32455 reflections measured (7.238° ≤ 2Θ ≤ 145.314°), 8834 unique (R_{int} = 0.0214, R$_{sigma}$ = 0.0186) which were used in all calculations. The final R_1 was 0.0376 (I > 2σ(I)) and wR_2 was 0.1038 (all data).

```
Bond precision:   C-C = 0.0032 A          Wavelength=1.54184

Cell:        a=13.1283(3)       b=13.3090(3)        c=14.7500(3)
             alpha=110.216(2)   beta=104.1886(19)   gamma=99.8556(19)
Temperature:  100 K

                  Calculated                  Reported
Volume          2250.01(10)                   2250.00(9)
Space group     P -1                          P -1
Hall group      -P 1                          -P 1
Moiety formula  C34 H18 F12 Fe N4 O4,         C34 H18 F11.996 Fe N4 O4,
                4.5(C2 H4 O2)                 4.5(C2 H4 O2)
Sum formula     C43 H36 F12 Fe N4 O13         C43 H36 F11.99 Fe N4 O13
Mr              1100.55                       1100.51
Dx,g cm-3       1.625                         1.624
Z               2                             2
Mu (mm-1)       3.778                         3.778
F000            1119.9                        1120.0
F000'           1122.38
h,k,lmax        16,16,18                      16,16,18
Nref            8951                          8834
Tmin,Tmax       0.540,0.725                   0.642,1.000
Tmin'           0.490

Correction method= # Reported T Limits: Tmin=0.642 Tmax=1.000
AbsCorr = GAUSSIAN

Data completeness= 0.987          Theta(max)= 72.657

R(reflections)= 0.0376( 8073)     wR2(reflections)= 0.1038( 8834)

S = 1.061                   Npar= 814
```
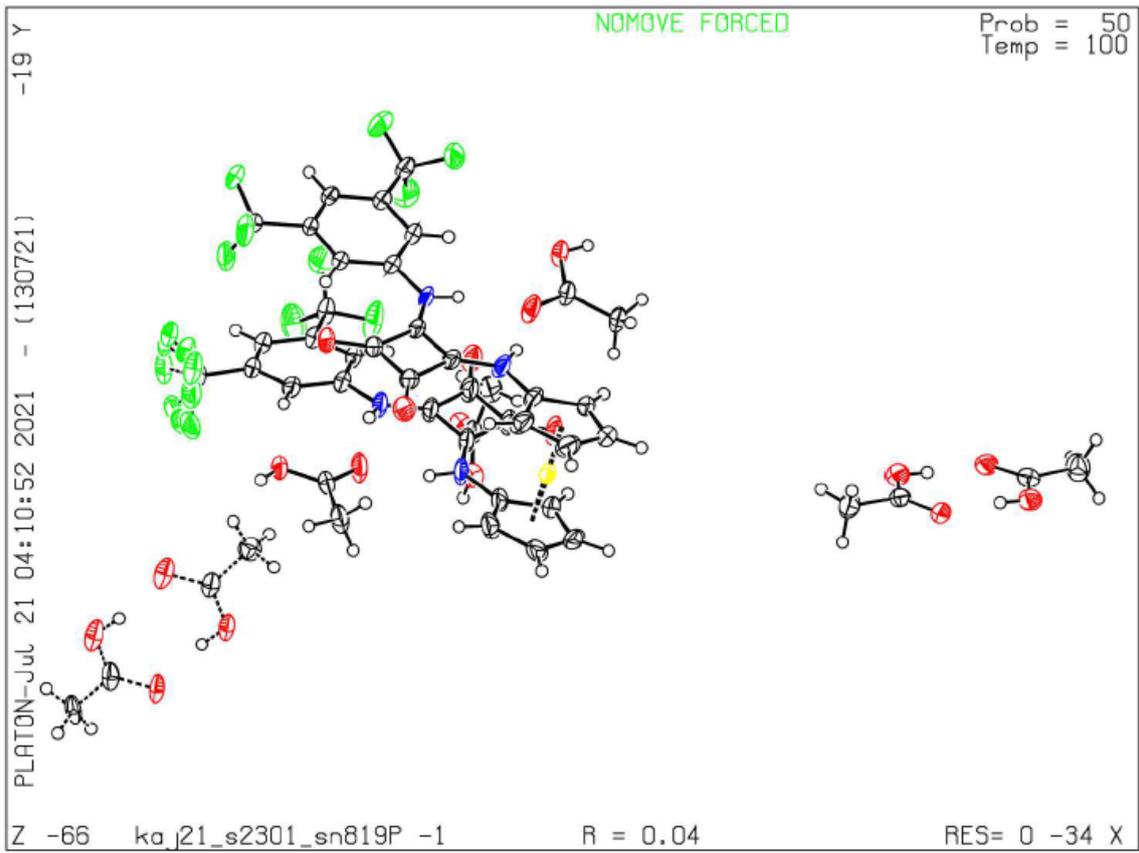
7. References

(1) A. Rostami, A. Colin, X. Y. Li, M. G. Chudzinski, A. J. Lough, M. S. Taylor, *J. Org. Chem.* 2010, **75**, 3983-3992

(2) A. R. Petrov, K. Jess, M. Freytag, P. G. Jones and M. Tamm, *Organometallics*, 2013, **32**, 5946-5954.

(3) A. Marchenko, A. Hurieva, H. Koidan, V. Rampazzi, H. Cattey, N. Pirio, A. N. Kostyuk and J.-C. Hierso, *Organometallics*, 2012, **31**, 5986-5989.

(4) L. Barišić, M. Čakić, K. A. Mahmoud, Y.-n. Liu, H.-B. Kraatz, H. Pritzkow, S. I. Kirin, N. Metzler-Nolte and V. Rapić, Chemistry – A European Journal, 2006, 12, 4965-4980.

(5) Proton assignments are made by analogy to the spectrum of the sulfate complex **1**•SO$_4$ and FcSq$_1$ **3**, the superior solubilities of which permit more complete NMR spectroscopic analysis.

(6) Proton assignments are made by analogy to the spectrum of the sulfate complex **2**•SO$_4$, the superior solubility of which permits more complete NMR spectroscopic analysis.

(7) M. Zaleskaya, D. Jagleniec, M. Karbarz, Ł. Dobrzycki and J. Romański, Inorganic Chemistry Frontiers, 2020, 7, 972-983.

(8) D. C. D. Butler and C. J. Richards, Organometallics, 2002, 21, 5433-5436.

(9) P. Thordarson, *Chemical Society Reviews*, 2011, **40**, 1305-1323

(10) P. D. Beer, J. J. Davis, D. A. Drillsma-Milgrom and F. Szemes, *Chemical Communications*, 2002, 1716-1717

(11) F. Oton, A. Tarraga, A. Espinosa, M. D. Velasco and P. Molina, Journal of Organic Chemistry, 2006, 71, 4590-4598.

(12) O.V. Dolomanov, L. J. Bourhis, R. J. Gildea, J. A. K. Howard, H. Puschmann, H. *J. Appl. Cryst.* 2009, **42**, 339-341.

(13) L. J. Bourhis, O. V. Dolomanov, R. J. Gildea, J. A. K. Howard and H. Puschmann, *Acta Cryst. Sect. A*, 2015, **71**, 59-75.

(14) G. Sheldrick, *Acta Cryst. Sect. C*, 2015, **71**, 3-8.