Supporting Information

Chemical Communications

A Lab-on-a-Molecule with an Enhanced Fluorescent Readout on Detection of Three Chemical Species

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Chemicals

Chemicals were used as received. 4-bromo-1,8-naphthalic anhydride (95%, Sigma-Aldrich), ferrocenecarboxaldehyde (98%, Acros), 1-methylpiperazine (98+%, Alfa-Aesar), hydroxylamine (50 wt% in H_2O, Sigma-Aldrich), iron (III)chloride hexahydrate (+98%, BDH), lithium aluminium hydride (97%, Alfa-Aesar), sodium methanesulfonate (98%, Sigma-Aldrich), magnesium sulfate anhydrous (99.5%, Alfa-Aesar), methanesulfonic acid (≥99.5%, Sigma-Aldrich), *N*,*N*-dimethylethylenediamine (≥ 98.0%, Sigma-Aldrich), sodium chloride (≥ 99%, Merck), sodium triacetoxyborohydride (95%, Sigma-Aldrich), triethylamine (HPLC Grade, Fisher Chemicals), *o*-anisidine (≥ 99% Sigma-Aldrich), phosphorus (V) oxychloride (99%, Sigma-Aldrich), 2-chloroethanol (99%, Sigma-Aldrich), 1,2-bis(chloroacetoxy)ethane (≥ 98%, TCI), quinine hemisulfate salt monohydrate (≥98%, Sigma-Aldrich).

Silica gel 60 (70-230 mesh, Fluka Analytical) and alumina were used for column chromatography (type WN 3, Aldrich). Sand (extra pure, low iron, 40-100 mesh, Fisher Chemical) was used for column chromatography. TLC aluminium foils (Silica gel coated with fluorescent indicator 254 nm, Fluka analytical) were used for thin-layer chromatography. 4Å molecular cylinder sieves (Aldrich) were dried in a furnace at 330 °C for 48 hours before use and stored in an oven at ~100°C.

Chloroform (HPLC grade, Sigma-Aldrich**,** tetrahydrofuran (HPLC grade Sigma-Aldrich), methanol (HPLC grade , Sigma-Aldrich), dichloromethane (HPLC grade; Fisher/ Sigma-Aldrich), 1,2-dichloroethane (> 99%, Fisher), acetone (GPR grade, LEVO lab services), diethyl ether (≥ 98, Sigma-Aldrich), chloroform-*d* (99.8 atom %, deuterated, Sigma-Aldrich), glacial acetic acid (> 99.9% , Carlo Erba), hexanes 40-60 °C(≥ 95%, mixture of isomers, Sigma Aldrich), petroleum ether (AR, bp 60-80°C, Fisher), *N*,*N*-dimethylformamide (HPLC grade, Sigma-Aldrich), hydrochloric acid (37% w/w, Fisher), 1,4-dioxane (99+%, Sigma-Aldrich).

Instrumentation

Melting points were recorded using a Stuart SMP40 automatic melting point apparatus. Fourier transform infrared (FTIR) spectra were recorded using a Shimadzu IR–Affinity-1 spectrophotometer as thin films on NaCl discs dropcast from chloroform. Nuclear Magnetic Resonance (NMR) spectra were recorded at 298 K on a Bruker Avance III HD NMR equipped with an Ascend 500 11.75 Tesla superconducting magnet and 5 mm PABBO probe at operating frequencies of 500.13 MHz and 125.76 MH for the ^{1}H and ^{13}C nuclei, respectively. Spectra were processed using TOPSPIN® V3.5pl7 software. Chemical shifts were calibrated against tetramethylsilane (TMS) (δ = 0.00 ppm) and $CDCl_3$ (δ =77.00 ppm) for ^{1}H and ^{13}C NMR, respectively. UV-visible absorption spectra were measured using a Jasco V650 spectrophotometer connected to a PC running Windows XP and the spectra analysis software used was the Spectra Manager Suite® through the spectrum analysis option. The instrumentation parameters were set to medium response, a bandwidth of 1 nm and a scan speed of 200 nm min^{-1}. Fluorescence measurements were recorded with a Jasco FP-8300 spectrofluorimeter analysed with Spectra Manager Suite®. The instrument parameters were set at 2.5 nm for both the excitation and emission slits, respectively. The scan rate was set at 200 cm^{-1}. Spectra were background subtracted for solvent. Quartz SUPRASIL cuvettes (101-10-40) with 10 mm pathlength with transparent windows on all four sides were used. Curve fitting of fluorimetric titration data were performed with SigmaPlot 13.0. pH/mV measurements were taken using a ProMinent® GmbH DULCOTEST® Portable handheld meter coupled to parallel temperature sensing. The temperature probe was an in-house modified pt 1000 sensor attached to the handheld meter by a coaxial cable and SN6 standard plugs calibrated with Merck® pH standards at pH 7.0 and pH 4.0. The pH sensor was a pH-reference combination probe from ProMinent® GmbH PHES 112 SE® double junction sensor. Redox readings in mV were taken using a Redox-Combination Probe from ProMinent® GmbH RHER-Pt-SE®. HRMS was performed by Medac Ltd UK.

Synthesis

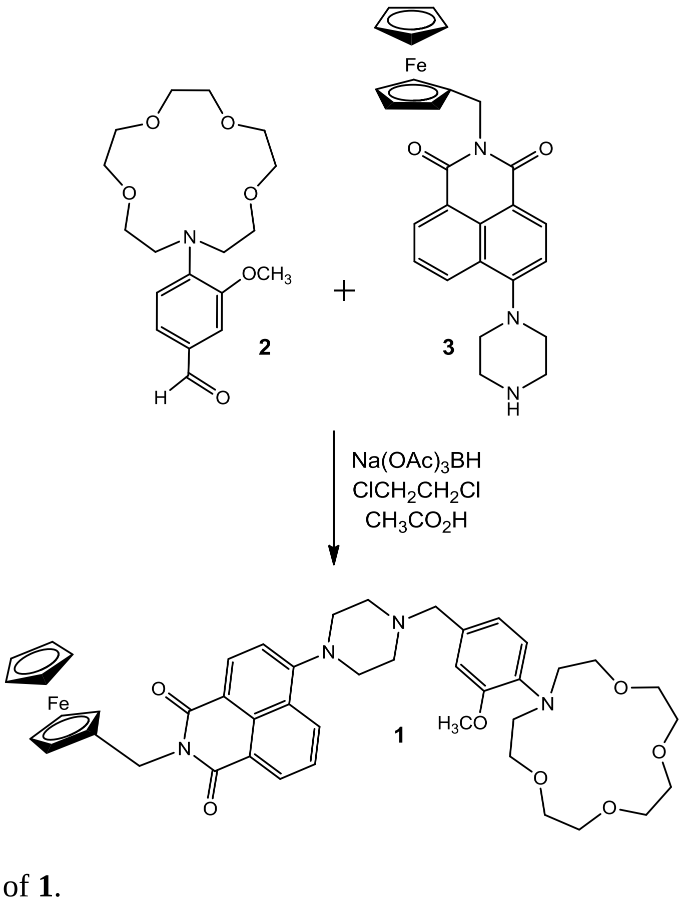
Reactions were carried out in round-bottomed flasks connected to Liebig condensers with ground glass joints and heated in a mineral oil bath on an IKA C-MAG HS 7 hotplate fitted with an IKA ETS-D5 temperature probe. For reaction temperatures above 120 °C silica oil was used. Solvent removal was performed using a Stuart rotary evaporator with a temperature water bath and low pressure vacuum pump with vapour traps. Trace solvent was removed using an electric Roots vacuum pump and glass manifold by freeze drying with dry ice and subsequent slow heating under high vacuum. Reactions were monitored by thin-layer chromatography (TLC) using Sigma-Aldrich® TLC plates 60 with fluorescent indicator visualised with a handheld lamp using 254 nm and 365 nm UV light.

Synthesis of 6-(4-(3-methoxy-4-(1,4,7,10-tetraoxa-13-azacyclopentadecan-13-yl)benzyl)piperazin-1-yl)-2-methyl-1H-benzo[de]isoquinoline-1,3(2H)-dione (**1**)

2-Methoxy-4-formylbenzo-aza-15-crown-5 ether **2** (0.38 g, 0.50 mmol) and *N*-ferrocenylmethyl-4-methylpiperazine-1,8-naphthalimide **3** (0.25 g, 0.52 mmol) were reacted with sodium triacetoxyborohydride (0.19 g , 8.8 mmol) in 25 ml of dry 1,2-dichloroethane and 5 drops of acetic acid and stirred at room temperature under N_2 for 5 days. The mixture was filtered, washed with 500 ml of distilled water and extracted with dichloromethane and dried over anhydrous $MgSO_4$. Column chromatography was done using a 45 cm × 3.5 cm column packed with 100 g of alumina slurry in hexane. The crude product was eluted with a gradient solvent from 1:99 ethyl acetate/hexane up to 100 % ethyl acetate, and gradually substituted with methanol. Removal of the solvent by rotary evaporation gave 0.12 g of yellow-orange powder in 31% yield.

Characterisation Data

m. p. 82-83°C; R_f = 0.18 (95:5(v/v) $CHCl_3$: CH_3OH); ^{1}H NMR ($CDCl_3$, ppm): δ_H 8.54 (d, 1H, *J* = 7.0 Hz, Ar-*H*), 8.48 (d, 1H, *J* = 8.1 Hz, Ar-*H*), 8.37 (d, 1H, *J* = 8.3 Hz, Ar-*H*), 7.63 (dd, 1H, *J* = 8.0 Hz, Ar-*H*), 7.17 (d, 1H, *J* = 8.0 Hz, Ar-*H*), 7.04 (d, 1H, *J* = 8.0 Hz, Ar-*H*), 6.85-6.87 (m, 2H, Ar-*H*), 5.11 (s, 2H, Ar-*H*), 4.50 (s, 2H, -CH_2- spacer), 4.20 (s, 5H, Ar-*H*), 4.06 (s, 2H, Ar-*H*), 3.85 (s, 3H, O-CH_3), 3.70-3.60 (m, 20H, crown and piperazine), 3.60 (s, 2H, -CH_2-), 3.46 (m, 4H), 3.27 (s, broad, 4H) and 2.74 (s, broad, 3H); ^{13}C NMR ($CDCl_3$, ppm): δ_C 164.25 (–), 163.78(–), 156.08(–), 152.68(–), 139.30(–), 132.60(↑), 131.61(↑), 131.10(↑), 130.33(↑), 129.88(–), 126.12(–), 125.51(↑), 123.33(–), 121.45(↑), 120.31(↑), 116.66(–), 114.83(↑), 112.60(↑), 83.38(–), 70.98(↓), 70.57(↓), 70.40(↑), 70.35(–), 70.19(↓), 68.59(↑), 68.02(–), 67.97(↑), 62.87(↓), 55.50(↑), 53.42(↓), 53.11(↓), 53.02(↓), 39.06(↓), 30.93(↑),29.70(↓); IR (KBr, cm^{-1}): 3050, 2858, 2849, 2831, 1695, 1652, 1645, 1587, 1575, 1447, 1382, 1372, 1330, 1312, 1290, 1247, 1133, 1121, 1117, 1105, 1080, 820, 728; HRMS Calculated for $C_{45}H_{53}N_4O_7Fe$ 817.3264; found 817.3280.



Scheme S1 Synthesis of **1**.

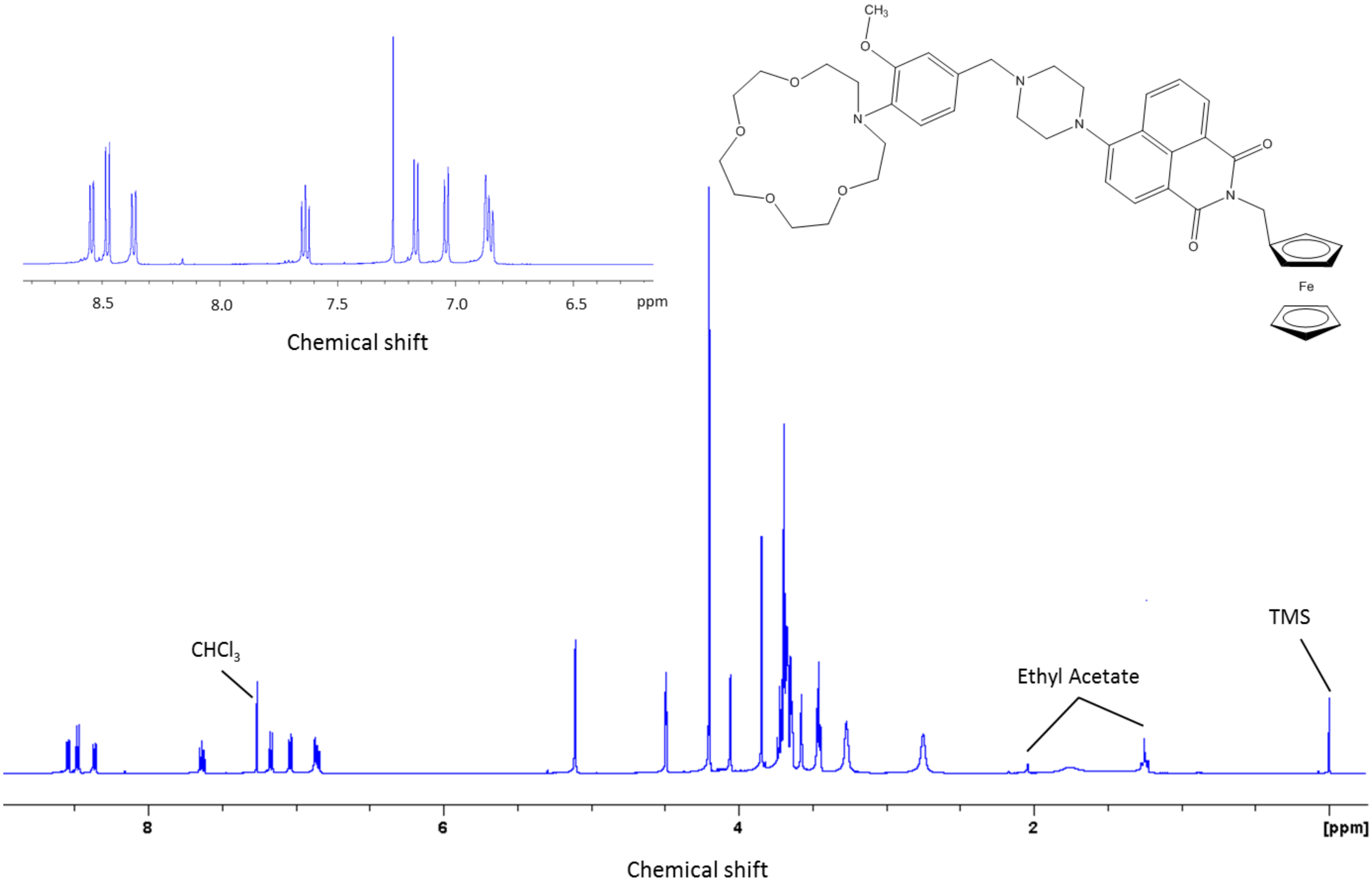


Fig. S1 ^{1}H NMR spectrum of **1** in $CDCl_3$ at 500 MHz.

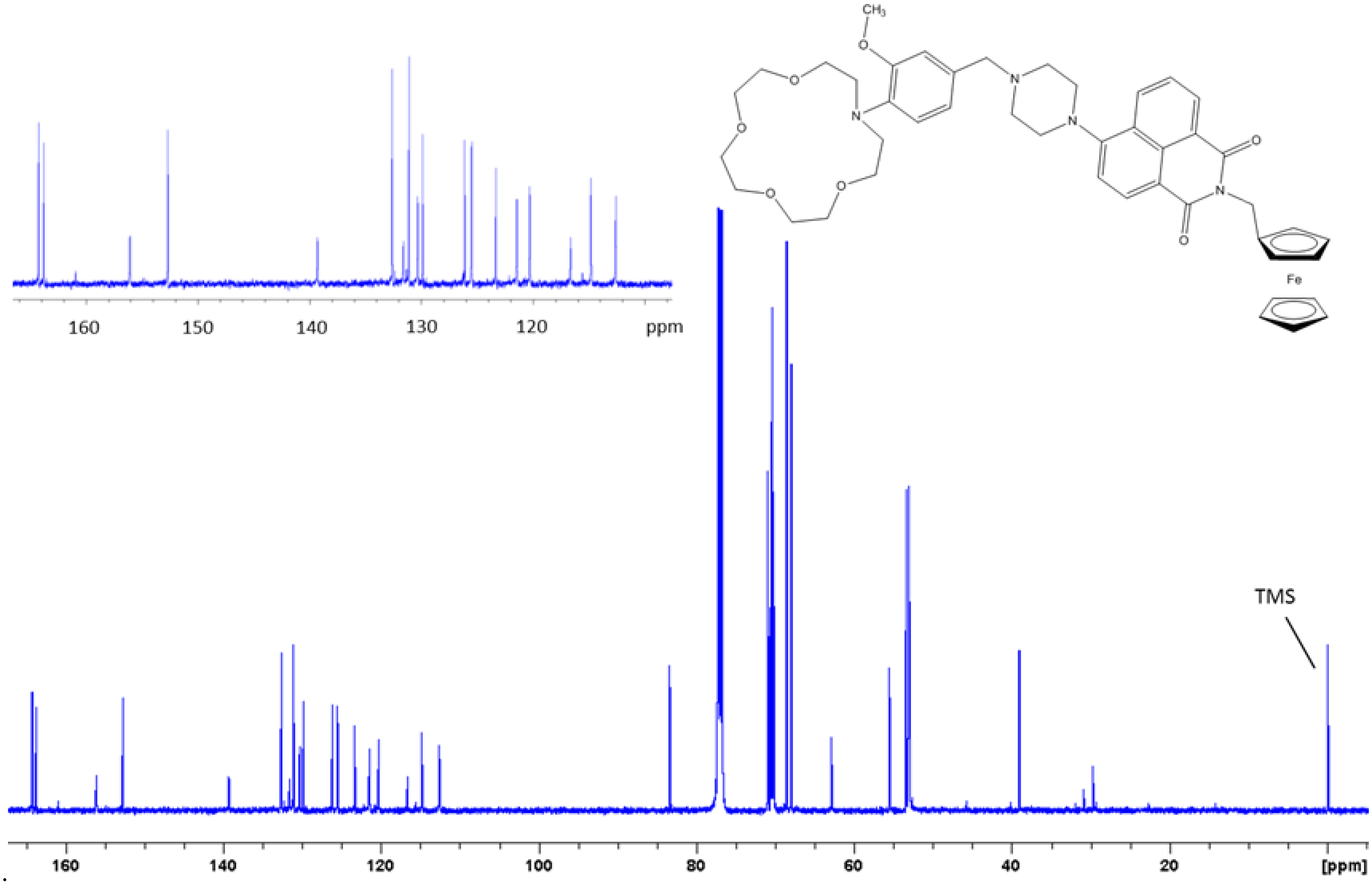


Fig. S2 ^{13}C NMR spectrum of **1** in $CDCl_3$ at 126 MHz.

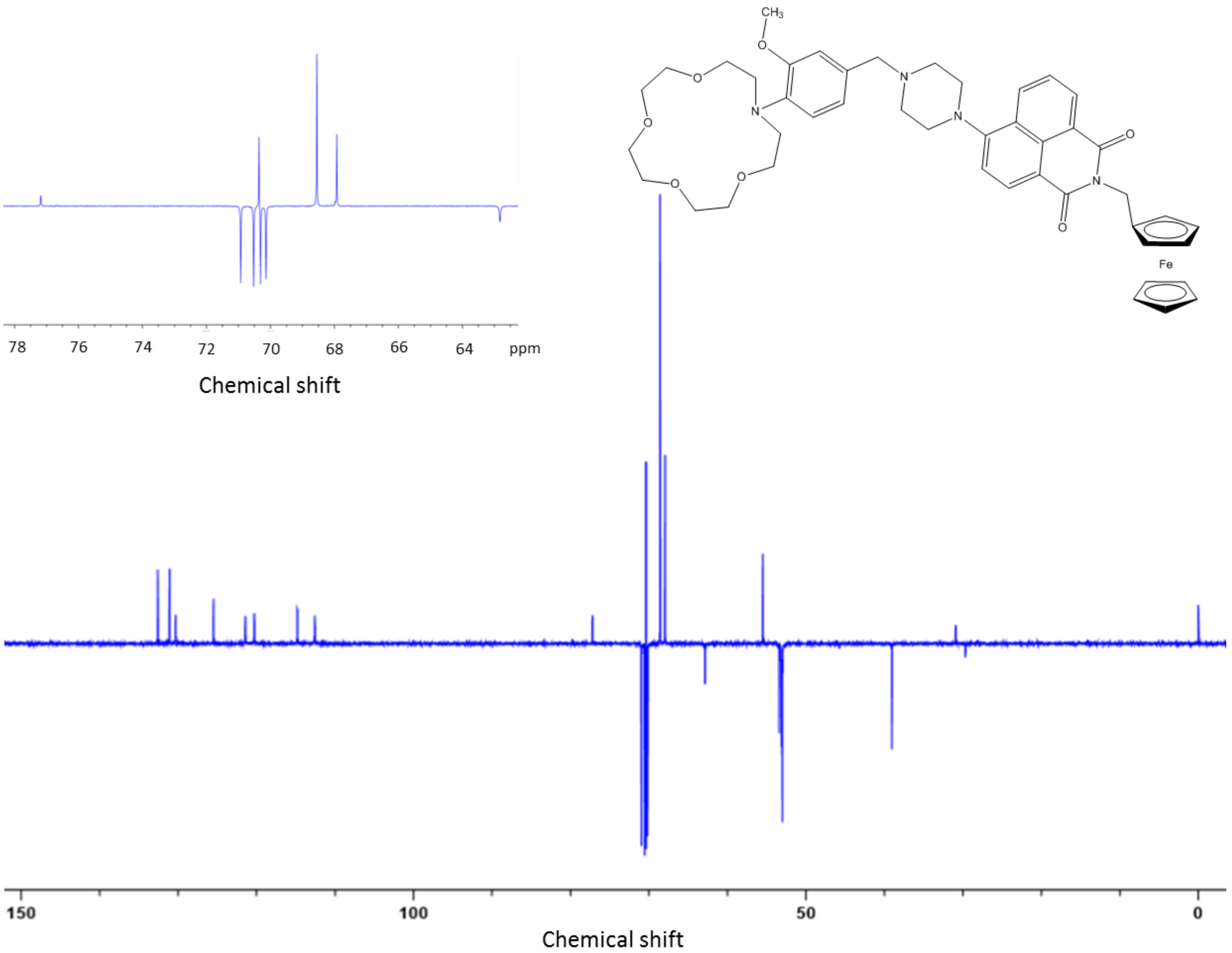


Fig. S3 ^{13}C DEPT NMR spectrum of **1** in $CDCl_3$ at 126 MHz.

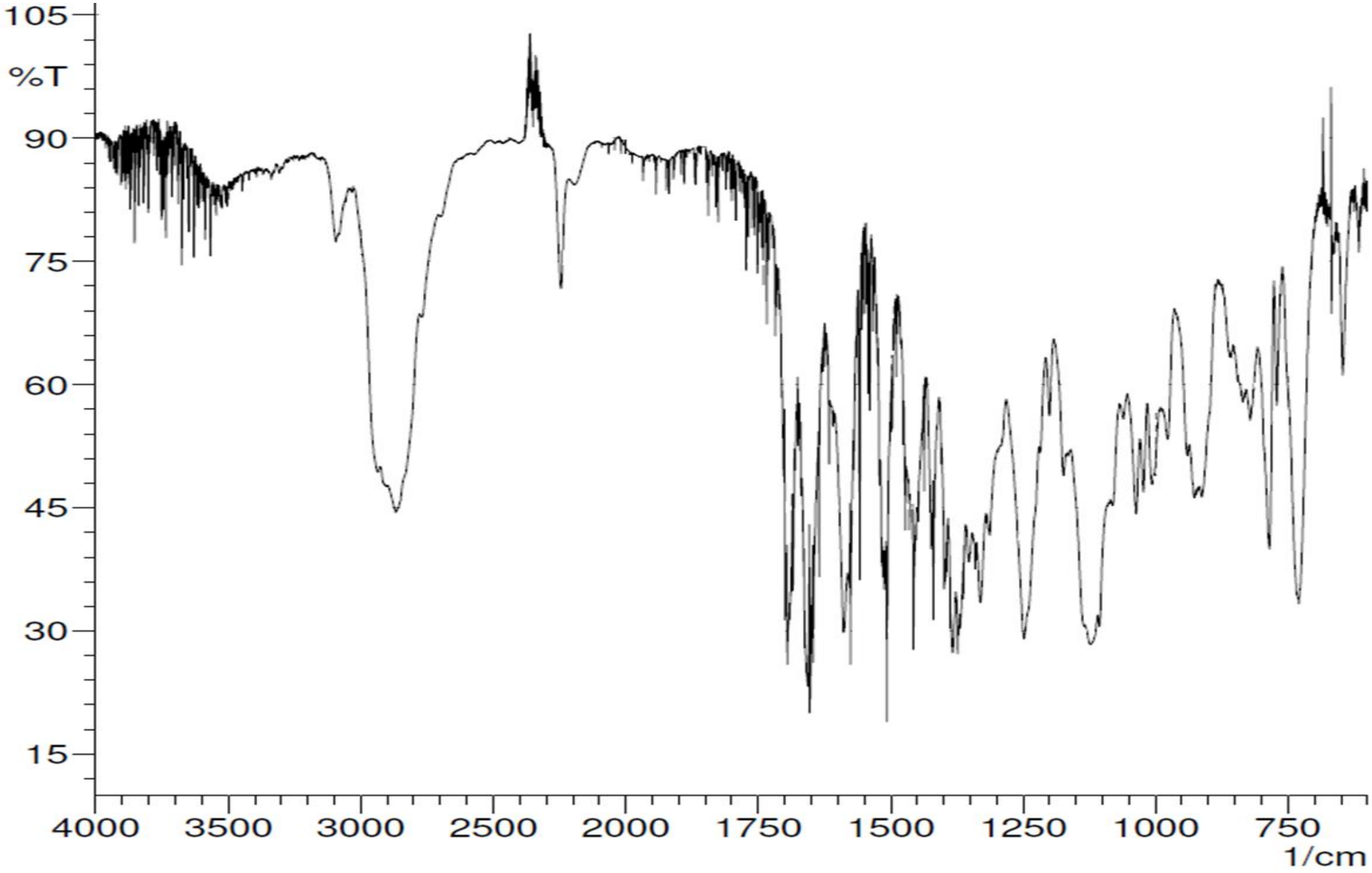


Fig. S4 IR spectrum of **1** (NaCl thin film from chloroform).

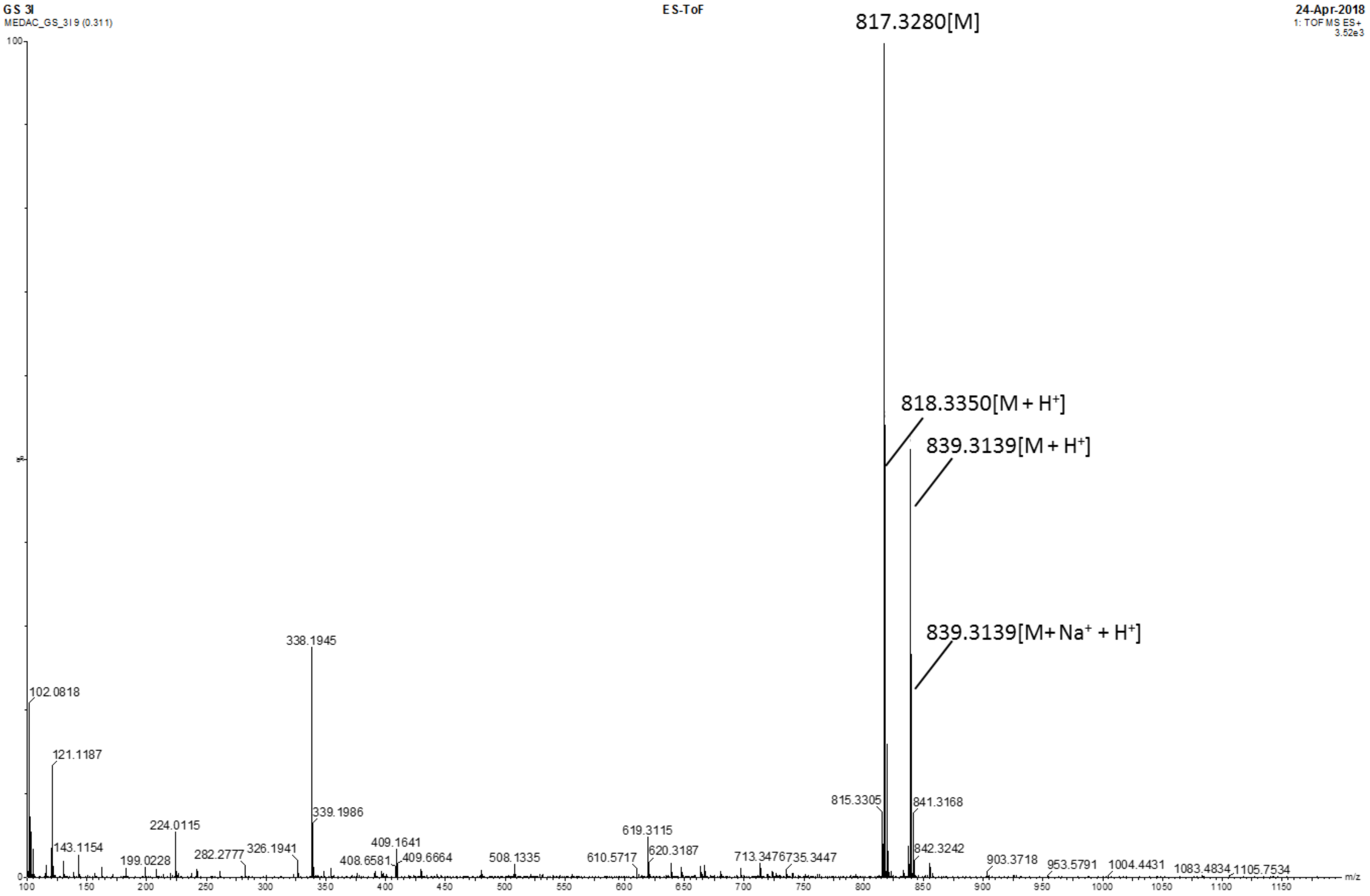


Fig. S5 High resolution mass spectrum of **1**.

Elemental Composition Report

Single Mass Analysis

Tolerance = 10.0 PPM / DBE: min = -1.5, max = 80.0

Number of isotope peaks used for i-FIT = 3

Monoisotopic Mass, Even Electron Ions

477 formula(e) evaluated with 2 results within limits (all results (up to 1000) for each mass)

Elements Used:

C: 45-45 H: 0-200 N: 0-10 O: 0-10 Na: 0-1 56Fe: 0-1

Minimum:				-1.5			
Maximum:		5.0	10.0	80.0			
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	i-FIT (Norm)	Formula
817.3280	817.3264	1.6	2.0	21.5	72.7	0.4	C45 H53 N4 O7 56Fe

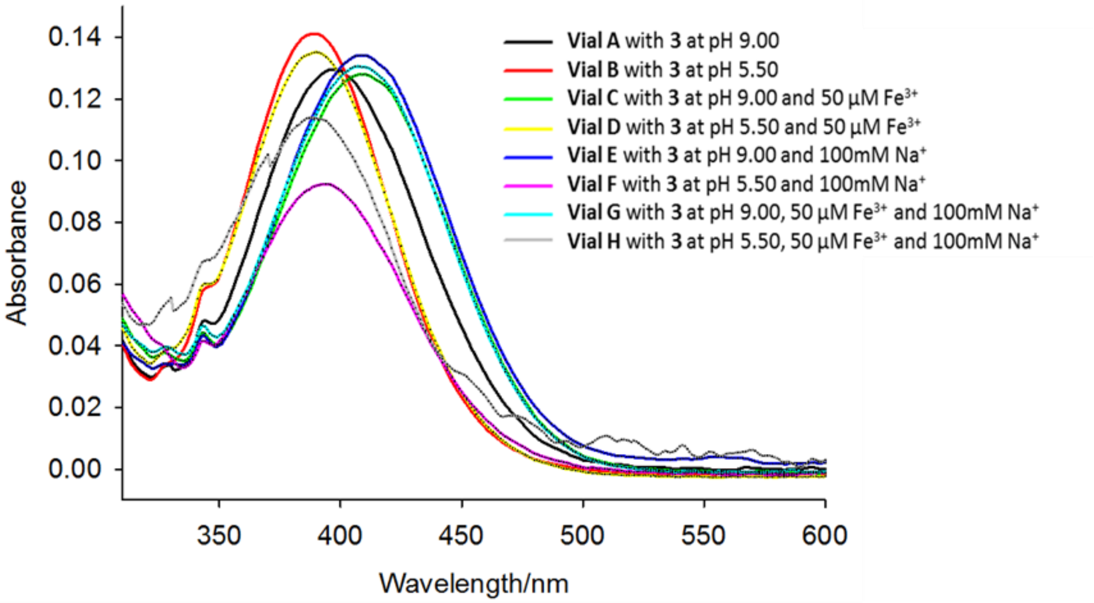


Fig. S6 UV-visible absorption spectra of 6 µM **1** in 1:1 (*v/v*) MeOH/H_2O for the eight input combinations of H^+, Fe^{3+} and Na^+. Chemicals inputs were 0.10 M HCl or 0.10 M KOH, 50 µM $FeCl_3 \cdot 6H_2O$ and 100 mM CH_3SO_3Na.

Table S1 Truth table and fluorescence quantum yields (Φ_F) of **1** in 1:9 (*v/v*) MeOH/H_2O by steady-state fluorescence spectroscopy.[a]

Label	$Input_1$ (H^+)[b]	$Input_2$ (Fe^{3+})[c]	$Input_3$ (Na^+)[d]	Output (Φ_F)[e]
A	0 (low)	0 (low)	0 (low)	0 (low, 0.001)
B	1 (high)	0 (low)	0 (low)	0 (low, 0.002)
C	0 (low)	1 (high)	0 (low)	0 (low, 0.004)
D	1 (high)	1 (high)	0 (low)	0 (low, 0.005)
E	0 (low)	0 (low)	1 (high)	0 (low, 0.002)
F	1 (high)	0 (low)	1 (high)	0 (low, 0.003)
G	0 (low)	1 (high)	1 (high)	0 (low, 0.001)
H	1 (high)	1 (high)	1 (high)	1 (high, 0.028)

[a] 6 μM. [b] High and low conditions adjusted with 0.10 M HCl and 0.10 M KOH solutions. High level $10^{-4.5}$ M H^+, low level $10^{-9.0}$ M H^+. [c] High level 50 μM $FeCl_3 \cdot 6H_2O$, low level no $FeCl_3 \cdot 6H_2O$ added. [d] High level 100 mM CH_3SO_3Na, low level no CH_3SO_3Na added. [e] The threshold limit set at Φ_{Flu} = 0.014. Quinine hemisulfate monohydrate in 0.1 M H_2SO_4 (Φ_F = 0.58) was used as the primary standard.