Electronic Supplementary Material (ESI) for Organic & Biomolecular Chemistry.

Electronic Supporting Information

for

Ratiometric Electrochemical Detection of Hydrogen Peroxide and Glucose

by

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Contents

General Information

Instruments and Analysis

Proton (^{1}H), Boron (^{11}B) and Carbon (^{13}C) nuclear magnetic resonance (NMR) spectra were recorded on a Bruker Avance 300 MHz spectrometer (^{1}H NMR at 300 MHz, ^{11}B NMR at 96 MHz and ^{13}C NMR at 75.5 MHz). Chemical shifts for protons are reported in parts per million (ppm) down field from tetramethylsilane (TMS) and are referenced,[1] to residual protium in the solvent (^{1}H NMR: $CHCl_3$ at 7.26 ppm, C_6D_5H at 7.16 ppm, $CD_3S(O)CD_2H$ at 2.50 ppm). Chemical shifts for carbons are reported in ppm downfield from TMS are referenced,[2] to the carbon resonances of the solvent peak (^{13}C NMR: $CDCl_3$ at 77.0 ppm, C_6D_6 at 128.1 ppm, d_6-DMSO at 39.5 ppm). Chemical shifts for borons are reported in ppm referenced to $BF_3{\cdot}OEt_2$ and are uncorrected. NMR data are represented as follows: chemical shift (integration, multiplicity [s = singlet, br s = broad singlet, d = doublet, dd = doublet of doublets, dt = doublet of triplets, ddt = doublet of doublet of triplets, m = multiplet, app = apparent], coupling constant(s) [in Hz]). Infrared (IR) spectra were recorded on a Perkin-Elmer 1600 FTIR spectrophotometer with absorbencies quoted as ν in cm^{-1}. High resolution mass spectrometry was performed on a Bruker Daltonik μTOF electrospray time-of-flight (ESI–TOF) mass spectrometer. Melting points were obtained on a Bibby–Sterilin SMP10 melting point machine and are uncorrected. pH was measured using a Hanna Instruments HI 9321 pH meter and was calibrated prior to use.

Materials

Thin-layer chromatography (TLC) was performed using aluminium-backed plates coated with Alugram® SIL G/UV_{254} purchased from Macherey–Nagel and were visualised by UV light (254 nm) and/or $KMnO_4$ staining if required. Silica gel column chromatography was carried out using 60 Å, 200–400 mesh particle size silica gel purchased from Sigma–Aldrich.

Chemicals

All reactions were performed in oven-dried glassware under an atmosphere of nitrogen unless otherwise stated. Water was purified through a Merck Millipore reverse osmosis purification system prior to use. Anhydrous dichloromethane (CH_2Cl_2), tetrahydrofuran (THF), and toluene were dried and degassed by passing through anhydrous alumina columns using an Innovative Technology Inc. PS–400–7 solvent purification system (SPS). Petrol refers to the petroleum ether distillate fraction that has boiling point range of 40–60 which was purchased from VWR and used as received. Hexane refers to high-pressure liquid chromatography (HPLC) grade hexane which was purchased from Sigma–Aldrich and used as received. Anhydrous dimethylsulfoxide (DMSO) and anhydrous dimethylformamide (DMF) were purchased from Sigma–Aldrich and used as received. Ferrocenecarboxylic acid **7** was purchased from Fluorochem (UK) and used as received. 4-(hydroxymethyl)phenylboronic acid **9** was purchased from Frontier Scientific and used as received. Benzyl alcohol, potassium acetate and pinacol were purchased from Alfa Aesar and used as received. Schwartz's reagent ($Zr(cp^*)_2HCl$) and $PdCl_2{\cdot}dppf$ were purchased from Strem Chemicals and used as received. All other chemicals and reagents were purchased from Sigma–Aldrich and used as received.

Electrochemistry

All electrochemical analysis was conducted through the direct application of a 25 µL assay sample to a screen-printed electrochemical cell (GM Nameplate, Seattle) comprised of a carbon graphite working electrode ($A \approx 2.36$ mm^2), a carbon graphite counter electrode ($A \approx 10.15$ mm^2) and a silver (pseudo Ag/AgCl) reference electrode (0.85 mm^2). The potential across the cell was controlled by a Metrohm Autolab PGSTAT30 potentiostat (Metrohm AG, Herisau) operated via a personal computer running General Purpose Electrochemical System (GPES) (Eco Chemie B.V., Utrecht) software in differential pulse mode (modulation = 0.04 s, interval = 0.1 s, initial voltage = −475 mV, end voltage = 500 mV, step potential = 3 mV, modulation amplitude = 49.95 mV. Peak integrals were obtained using the 'peak search' function and ratiometric electrochemical conversions were calculated using the equation:

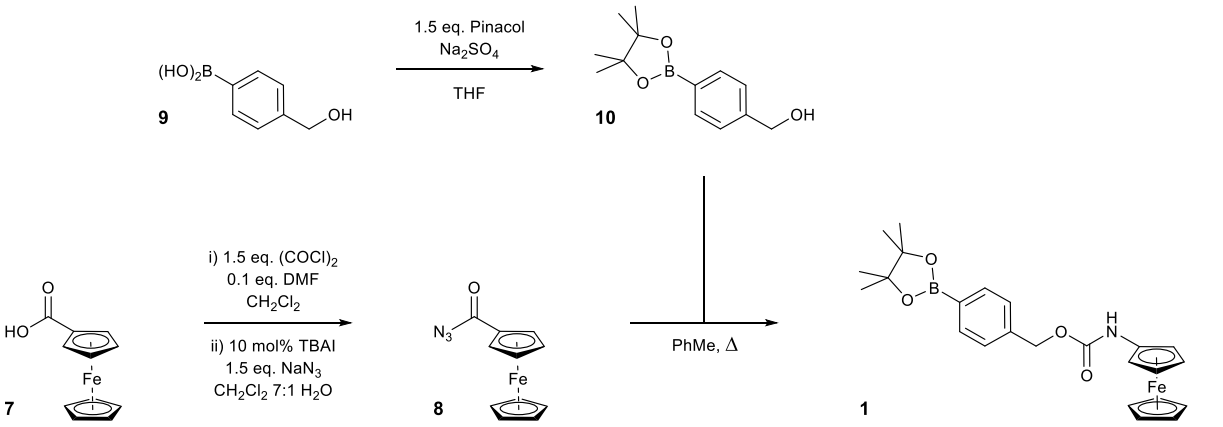
$$Conversion\ (\%) = \left(\frac{\int \mathbf{6}}{(\int \mathbf{6} + \int \mathbf{1}\ or\ \mathbf{2})}\right) \times 100$$

Diagnostic Assays

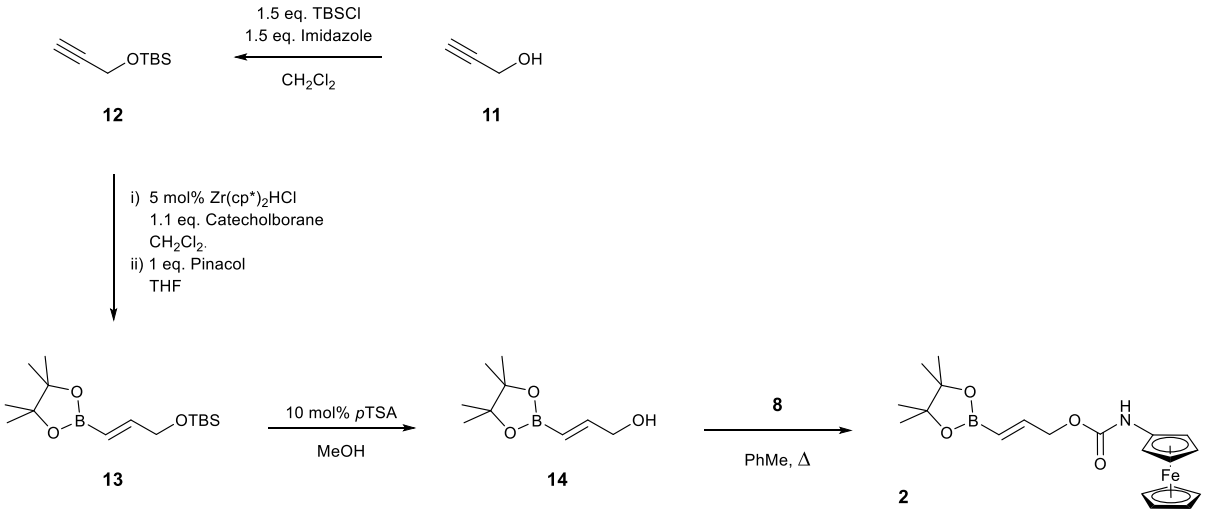
Standard 50 mM buffer recipes comprising the appropriate weights of the buffer's conjugate acid and base per 100 mL deionised water were used to make buffers to an approximate pH as desired. The exact pH was then carefully adjusted using 1M $NaOH_{(aq.)}$ and/or 1M $HCl_{(aq.)}$ accordingly using a pH meter. 10 mM ferrocene probe master solutions were made by weighing 0.01 mmol of the probe and dissolving the solid in 1 mL dimethylsulfoxide (DMSO). From this master solution, 1 mM stock solutions of ferrocene probe in DMSO 1:9 buffer were made by diluting 100 µL into 900 µL buffer. 1 M H_2O_2 master solutions were made by diluting 57 µL of a commercially available 50% wt. H_2O_2 in water (17.6 M) into water. From this master solution, serial dilutions were performed to obtain 10x solutions prior to testing in the diagnostic assay. 100 mM glucose master solutions were made by dissolving 0.1 mmol of α-D-glucose (18 mg) into 1 mL of water. 10x glucose stock solutions were then diluted into water as desired prior to testing in the diagnostic assay. 1000 UmL^{-1} glucose oxidase (GOx) master solutions were made by dissolving 47 mg of 21.2 Umg^{-1} GOx into pH 9 Tris buffer. 10x GOx stock solution were then diluted into pH 9 Tris buffer and stored at 4 °C. Buffers and master ferrocene solutions were made fresh weekly, glucose solutions were made fresh daily and stock ferrocene solutions were made fresh prior to use. GOx and H_2O_2 solutions were made fresh daily and stored at 4 °C until immediate use.

Synthetic Routes

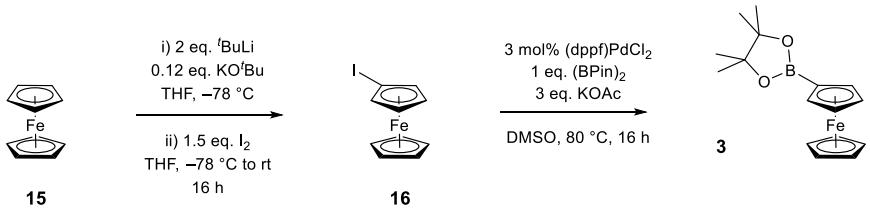
Synthetic Route to Probe **1**



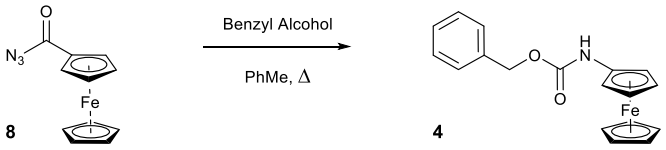
Synthetic Route to Probe **2**



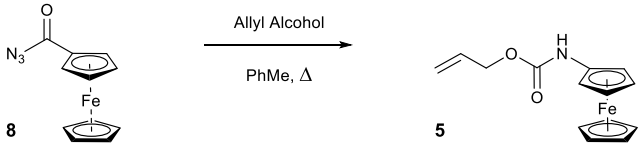
Synthetic Route to Compound **3**



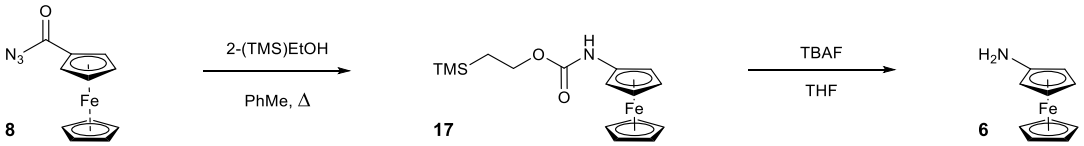
Synthetic Route to Compound **4**



Synthetic Route to Compound **5**



Synthetic Route to Aminoferrocene **6**



General Procedures

A: *Curtius rearrangement of ferrocenoyl azide* **8** *to ferrocenylcarbamates*

To an oven-dried carousel tube charged purged with argon was added ferrocenoyl azide **8** (1 eq.). The solids were dissolved in anhydrous toluene (3 mL/mmol) and to this stirring solution was added the alcohol (2 eq.). The reaction mixture was then heated to reflux and stirred for 2 hours. Upon allowing to cool to room temperature, the reaction mixture was transferred to a round-bottom flask and concentrated under reduced pressure to afford the crude product.

B: *Ratiometric electrochemical detection of H_2O_2 using probes* **1**–**2**

To an Eppendorf tube equipped with a magnetic triangular stirrer bar, was added 800 µL pH 8.1 Tris buffer followed by 100 µL of a 1 mM stock solution of probe **1** or **2** in DMSO 1:9 buffer. 100 µL of 10x H_2O_2 concentration in water was then added to give a 1 mL assay mixture containing 0.1 mM ferrocene probe and X mM H_2O_2. Immediately after addition of the H_2O_2 solution, the Eppendorf tube was then sealed and place into sand held in an aluminium DrySyn® block upon a stirrer hotplate set at the desired temperature. A timer was started along with vigorous stirring of the assay mixture at 1000 rpm. Every 2 minutes thereafter, a 25 µL sample was taken from the assay and subjected to ratiometric electrochemical analysis as described previously.

C: *Ratiometric electrochemical detection of H_2O_2 at different temperatures*

To an Eppendorf tube equipped with a magnetic triangular stirrer bar, was added 800 µL pH 9 Tris buffer followed by 100 µL of a 1 mM stock solution of probe **1** in DMSO 1:9 buffer. 100 µL of 2.5 mM H_2O_2 concentration in water was then added to give a 1 mL assay mixture containing 0.1 mM probe **1** and 0.25 mM H_2O_2. Immediately after addition of the H_2O_2 solution, the Eppendorf tube was then sealed and place into sand held in an aluminium DrySyn® block upon a stirrer hotplate set at the desired temperature. A timer was started along with vigorous stirring of the assay mixture at 1000 rpm. Every 2 minutes thereafter, a 25 µL sample was taken from the assay and subjected to ratiometric electrochemical analysis as described previously.

D: *Ratiometric electrochemical detection of different peroxides*

To an Eppendorf tube equipped with a magnetic triangular stirrer bar, was added 800 µL pH 9 Tris buffer followed by 100 µL of a 1 mM stock solution of probe **1** in DMSO 1:9 buffer. 100 µL of 5 mM [peroxide] concentration in water was then added to give a 1 mL assay mixture containing 0.1 mM probe **1** and 0.5 mM [peroxide]. Immediately after addition of the peroxide solution, the Eppendorf tube was then sealed and placed within a sample vial held in an aluminium DrySyn® block upon a stirrer hotplate. A timer was started along with vigorous stirring of the assay mixture at 1000 rpm. Every 2 minutes thereafter, a 25 µL sample was taken from the assay and subjected to ratiometric electrochemical analysis as described previously.

E: *Ratiometric electrochemical detection of glucose at different GOx concentrations*

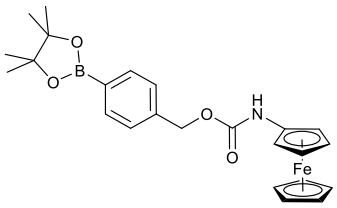
To an Eppendorf tube equipped with a magnetic triangular stirrer bar, was added 700 µL pH 9 Tris buffer followed by 100 µL of a 1 mM stock solution of probe **1** in DMSO 1:9 buffer. 100 µL of a 50 mM α-D-glucose solution was then added followed by 100 µL of a 10x GOx solution to give a 1 mL assay mixture containing 0.1 mM probe **1**, 5 mM glucose and X mM GOx. Immediately after the addition of GOx, the Eppendorf tube was left unsealed and placed within a sample vial held in an aluminium DrySyn® block upon a stirrer hotplate. A timer was started along with vigorous stirring of the assay mixture at 1000 rpm. Every 2 minutes thereafter, a 25 µL sample was taken from the assay and subjected to ratiometric electrochemical analysis as described previously.

F: *Ratiometric electrochemical detection of glucose*

To an Eppendorf tube equipped with a magnetic triangular stirrer bar, was added 700 µL pH 9 Tris buffer followed by 100 µL of a 1 mM stock solution of probe **1** in DMSO 1:9 buffer. 100 µL of a 1000 UmL^{-1} GOx solution was then added followed by 100 µL of a 10x mM α-D-glucose solution to give a 1 mL assay mixture containing 0.1 mM probe **1**, 100 UmL^{-1} GOx and X mM glucose. Immediately after the addition of glucose, the Eppendorf tube was left unsealed and placed within a sample vial held in an aluminium DrySyn® block upon a stirrer hotplate. A timer was started along with vigorous stirring of the assay mixture at 1000 rpm. Every 2 minutes thereafter, a 25 µL sample was taken from the assay and subjected to ratiometric electrochemical analysis as described previously.

Compound Data

4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)benzyl ferrocenylcarbamate **1**



Ferrocenoyl azide **8** (255 mg, 1 mmol, 1 eq.) and 4-(hydroxymethyl)phenylboronic acid pinacol ester **10** (468 mg, 2 mmol, 2 eq.) were reacted together according to general procedure **A**. Purification by silica gel column chromatography (hexane 9:1 EtOAc, R_f = 0.15) gave the title compound as a yellow solid (333 mg, 72%).

MP: 148–150 °C (decomp.).

IR (solid, cm^{-1}); ν 3376 (N–H), 2994 (Ar–H), 1724 (C=O), 1534 (C=N), 1355 (B–O).

^{1}H NMR (300 MHz, $CDCl_3$); δ_H 7.84 (2H, d, *J* = 7.4 Hz), 7.38 (2H, d, *J* = 7.4 Hz), 6.23 (1H, br s), 5.18 (2H, s), 4.53 (2H, s), 4.17 (5H, s), 4.00 (2H, s), 1.35 (12H, s).

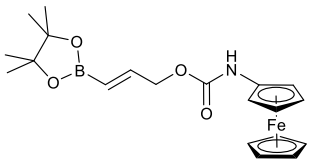
^{11}B NMR (96 MHz, $CDCl_3$); δ_B 33.8.

^{13}C NMR (75.5 MHz, $CDCl_3$); δ_C 153.7, 139.3, 135.1, 129.0, 127.2, 96.1, 83.9, 69.3, 66.7, 64.6, 60.8, 24.9.

HRMS (ESI): $C_{24}H_{28}BFeNNaO_4$ $[M+Na]^+$ – theoretical *m/z* 484.135, measured *m/z* 484.139.

***E*$_{ox}$** (DPV, 100 µM, pH 9.0 Tris buffer): 48 mV (±8 mV) vs Ag/Ag^+

(E)- 3-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)allyl ferrocenylcarbamate **2**



Ferrocenoyl azide **8** (84 mg, 0.33 mmol, 1 eq.) and (*E*)-3-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)prop-2-en-1-ol **14** (121 mg, 0.66 mmol, 2 eq.) were reacted together according to general procedure **A**. Purification by silica gel column chromatography (hexane 8:2 EtOAc, R_f = 0.60) gave the title compound as a yellow solid (59 mg, 43%).

MP: 119–124 °C

IR (solid, cm^{-1}); ν 3287 (N–H), 3085 (N–H), 2974 (C–H), 1702 (C=O), 1648 (C=C), 1553 (C=O, amide II), 1320 (B–O), 1098 (C–O).

^{1}H NMR (300 MHz, $CDCl_3$); δ_H 6.64 (1H, dt, *J* = 18.1, 4.5 Hz), 6.09 (1H, br s), 5.70 (1H, dt, *J* = 18.1, 1.8 Hz), 4.70 (2H, d, *J* = 4.5 Hz), 4.47 (2H, s), 4.15 (5H, s), 3.96 (2H, s), 1.26 (12H, s).

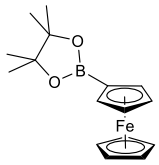
^{11}B NMR (96 MHz, $CDCl_3$); δ_B 33.2.

^{13}C NMR (75.5 MHz, $CDCl_3$); δ_C 153.5, 146.6, 95.5, 83.5, 69.2, 66.1, 64.5, 61.0, 24.9.

HRMS (ESI): $C_{20}H_{26}BFeNNaO_4$ $[M+Na]^+$ – theoretical *m/z* 434.120, measured *m/z* 434.123.

***E*$_{ox}$** (DPV, 100 µM, pH 9.0 Tris buffer): 30 mV (±3 mV) vs Ag/Ag^+.

Ferroceneboronic acid pinacol ester **3**



To an oven-dried carousel tube purged with argon was added [1,1'-bis(diphenylphosphino)ferrocene]dichloropalladium(II) (25 mg, 0.03 mmol, 3 mol%). The solids were dissolved in anhydrous DMSO (3 mL) before the addition of bis(pinacolatoboron) (254 mg, 1 mmol, 1 eq.), iodoferrocene **16** (312 mg, 1 mmol, 1 eq.) and potassium acetate (294 mg, 3 mmol, 3 eq.). The reaction mixture was then heated to 80 °C and left to stir for 16 hours. Upon allowing to cool to room temperature, the reaction mixture was transferred to a round-bottom flask and concentrated under reduced pressure. The residue was then partitioned between H_2O (20 mL) and Et_2O (20 mL) and the organics were then separated. The aqueous layer was extracted with Et_2O (20 mL) twice further and the combined organics were washed with water (50 mL), dried over $MgSO_4$ and concentrated under reduced pressure to afford the crude compound. Purification by silica gel column chromatography (hexane 19:1 Et_2O, R_f = 0.15) gave the title compound as an orange solid (147 mg, 47%).

MP: 119–121 °C.

^{1}H NMR (300 MHz, $CDCl_3$); δ_H 4.44 (2H, app t, *J* = 1.8 Hz), 4.39 (2H, app t, *J* = 1.8 Hz), 4.16 (5H, s), 1.35 (12H, s).

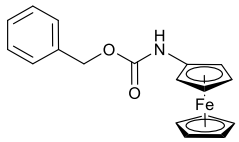
^{11}B NMR (96 MHz, $CDCl_3$); δ_B 36.1.

^{13}C NMR (75.5 MHz, $CDCl_3$); δ_C 83.2, 73.8, 72.1, 68.6, 25.0.

***E*$_{ox}$** (DPV, 100 µM, pH 9.0 Tris buffer): 14 mV (±1 mV) vs Ag/Ag^+

NMR data in accordance with literature precedent.[2]

Benzyl ferrocenylcarbamate **4**



Ferrocenoyl azide **8** (255 mg, 1 mmol, 1 eq.) and benzyl alcohol (0.2 mL, 2 mmol, 2 eq.) were reacted together according to general procedure **A**. Purification by silica gel column chromatography (hexane 9:1 EtOAc, R_f = 0.35) gave the title compound as a dark orange crystalline solid (323 mg, 96%).

MP: 110–113 °C (decomp.)

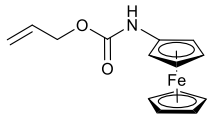
^{1}H NMR (300 MHz, C_6D_6); δ_H 7.35–7.33 (2H, m), 7.26–7.16 (3H, m), 5.66 (1H, br s), 5.18 (2H, s), 4.56 (2H, s), 4.13 (5H, s), 3.86 (2H, s).

^{13}C NMR (75.5 MHz, C_6D_6); δ_C 153.8, 137.1, 128.7, 128.6, 96.4, 69.5, 67.0, 64.5, 60.9.

***E*$_{ox}$** (DPV, 100 µM, pH 9.0 Tris buffer): 96 mV (±6 mV) vs Ag/Ag^+.

NMR data in accordance with literature precedent.[3]

Allyl ferrocenylcarbamate **5**



Ferrocenoyl azide **8** (539 mg, 2.11 mmol, 1 eq.) and allyl alcohol (0.3 mL, 4.22 mmol, 2 eq.) were reacted together according to general procedure **A**. Purification by silica gel column chromatography (hexane 19:1 EtOAc, R_f = 0.15) gave the title compound as an orange solid (491 mg, 82%).

MP: 81–83 °C

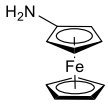
^{1}H NMR (300 MHz, C_6D_6): δ_H 5.76 (1H, ddt, *J* = 17.2, 10.6, 5.6 Hz), 5.34 (1H, br s), 5.14 (1H, dd, *J* = 17.2, 1.6 Hz), 4.99 (1H, dd, *J* = 10.6, 1.6 Hz), 4.51 (2H, d, *J* = 5.6 Hz), 4.41 (2H, br s), 4.03 (5H, s), 3.75 (2H, t, *J* = 2.0 Hz).

^{13}C NMR (75 MHz, C_6D_6): δ_C 153.7, 133.3, 117.5, 96.4, 69.5, 65.7, 64.5, 60.9.

***E*$_{ox}$** (DPV, 100 µM, pH 9.0 Tris buffer): 47 mV (±1 mV) vs Ag/Ag^+

NMR data in accordance with literature precedent.[4]

Aminoferrocene **6**



2-(trimethylsilyl)ethyl ferrocenecarbamate **17** (1.04 g, 3 mmol, 1 eq.) was dissolved in tetrabutylammonium fluoride (TBAF) solution [1M in THF] (12 mL, 12 mmol, 4 eq.) and the reaction mixture was heated to 50 °C for 2 hours. After allowing to cool to room temperature, the reaction mixture was concentrated under reduced pressure and the residue was quenched with water (20 mL), then extracted with CH_2Cl_2 (3 × 20 mL). The combined organics were dried over $MgSO_4$ and concentrated under reduced pressure to afford the crude product. Purification by silica gel column chromatography (hexane 8:2 EtOAc, R_f = 0.20) gave the title compound as an orange crystalline solid (0.51 g, 85%).

MP: 151–153 °C (decomp.). **Lit:** 155 °C (decomp.).[5]

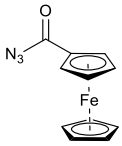
^{1}H NMR (300 MHz, C_6D_6); δ_H 3.98 (5H, s), 3.73 (2H, s), 3.69 (2H, s), 1.86 (2H, br s).

^{13}C NMR (75.5 MHz, C_6D_6); δ_C 106.7, 69.1, 63.5, 58.3.

***E*$_{ox}$** (DPV, 100 µM, pH 9.0 Tris buffer): −199 mV (±1 mV) vs Ag/Ag^+.

NMR data in accordance with literature precedent.[6]

Ferrocenoyl Azide **8**



To a stirring suspension of ferrocenecarboxylic acid **7** (2.00 g, 8.7 mmol, 1 eq.) in anhydrous CH_2Cl_2 (20 mL) at 0 °C was added oxalyl chloride (1.5 mL, 17.4 mmol, 2 eq.) followed by a drop of dimethylformamide (DMF). The reaction mixture was allowed to warm to room temperature and left to stir for 3 hours before being concentrated under reduced pressure. The residue was then taken up in anhydrous CH_2Cl_2 (20 mL) and cooled to 0 °C. Tetrabutylammonium bromide (0.03 g, 0.09 mmol, 1 mol%) was then added followed by a solution of sodium azide (0.85 g, 13.1 mmol, 1.5 eq.) in water (4 mL). The reaction mixture was then allowed to warm to room temperature and left to stir for 16 hours. The reaction was then quenched with water (20 mL) and the organics separated. The aqueous layer was then extracted twice further with CH_2Cl_2 (20 mL). The combined organics were then dried over $MgSO_4$ and concentrated under reduced pressure to afford the crude product. Purification by silica gel column chromatography (hexane 1:1 CH_2Cl_2, Rf = 0.45) gave the title compound as a crystalline orange solid (1.75 g, 79%).

MP: 86–87 °C (decomp.). **Lit:** 84–86 °C.[7]

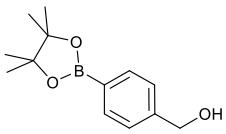
IR (solid, cm^{-1}); ν 2149 (N_3), 1671 (C=O).

^{1}H NMR (300 MHz, C_6D_6); δ_H 4.74 (2H, s), 4.02 (2H, s), 3.91 (5H, s), 1.86 (2H, br s).

^{13}C NMR (75.5 MHz, C_6D_6); δ_C 176.3, 97.7, 72.7, 70.7, 70.4.

NMR data in accordance with literature precedent.[7]

4-(Hydroxymethyl)phenylboronic acid pinacol ester **10**



To a stirring solution of 4-(hydroxymethyl)phenylboronic acid **9** (1.52 g, 10 mmol, 1 eq.) and pinacol (1.77 g, 15 mmol, 1.5 eq.) in anhydrous THF (20 mL) was added sodium sulfate (≈ 1.5 g). The reaction mixture was then stirred at room temperature for 2 hours before being gravity filtered and the filtrate concentrated to afford the crude product. Purification by silica gel column chromatography (hexane 9:1 EtOAc, R_f = 0.10) followed by recrystallisation (hexane) gave the title compound as colourless needles (1.25 g, 53%).

MP: 75–77 °C. **Lit:** 75–77 °C.[8]

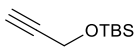
^{1}H NMR (300 MHz, $CDCl_3$); δ_H 7.75 (2H, d, *J* = 7.8 Hz), 7.27 (2H, d, *J* = 7.8 Hz), 4.56 (2H, s), 3.87 (1H, br s), 1.31 (12H, s).

^{11}B NMR (96 MHz, $CDCl_3$); δ_B 34.8.

^{13}C NMR (75.5 MHz, $CDCl_3$); δ_C 144.2, 134.8, 127.4, 83.7, 64.4, 24.7.

NMR data in accordance with literature precedent.[8]

tert-butyldimethyl(prop-2-yn-1-yloxy)silane **12**



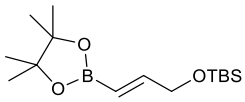
To a stirring solution of propargyl alcohol **11** (1.16 mL, 20 mmol, 1 eq.) in anhydrous CH_2Cl_2 (50 mL) at 0 °C, was added imidazole (2.04 g, 30 mmol, 1.5 eq.) followed by portion-wise addition of *tert*-butyldimethylsilyl chloride (4.52 g, 30 mmol, 1.5 eq.). The reaction mixture was allowed to slowly warm to room temperature and the suspension was left to stir for 16 hours. The reaction was then quenched with $NH_4Cl_{(sat.)}$ (50 mL) and the organics separated. The aqueous layer was then extracted twice with Et_2O (50 mL). The combined organics were then washed with brine (50 mL), dried over $MgSO_4$ and concentrated under reduced pressure to afford the crude product. Purification by silica gel column chromatography (2.5% EtOAc in Petrol, R_f = 0.80) gave the title compound as a colourless liquid (2.56 g, 75%).

^{1}H NMR (300 MHz, $CDCl_3$); δ_H 4.30 (2H, d, *J* = 2.4 Hz), 2.38 (1H, t, *J* = 2.4 Hz), 0.90 (9H, s), 0.12 (6H, s).

^{13}C NMR (75.5 MHz, $CDCl_3$); δ_C 82.5, 73.0, 51.6, 25.9, 18.4, −5.1.

NMR data in accordance with literature precedence.[9]

(E)-tert-butyldimethyl((3-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)allyl)oxy)silane **13**



To a flame-dried Schlenk tube under argon was added Schwartz's reagent (130 mg, 0.5 mmol, 5 mol%) followed by anhydrous CH_2Cl_2 (5 mL). The resulting stirring suspension was then cooled down to 0 °C before the dropwise addition of a solution of *tert*-butyldimethyl(prop-2-yn-1-yloxy)silane **12** (1.70 g, 10 mmol, 1 eq.) and catecholborane (1.2 mL, 11 mmol, 1.1 eq.). The reaction mixture was then allowed to warm to room temperature before being allowed to stir for 24 hours. The reaction was then quenched with water (50 mL) and extracted with Et_2O (3 × 50 mL). The combined organics were then dried over Na_2SO_4 and concentrated under reduced pressure. The residue was then taken up in anhydrous THF (10 mL) before the addition of pinacol (1.18 g, 10 mmol, 1 eq.). The reaction mixture was then allowed to stir at room temperature for 2 hours before being concentrated under reduced pressure. Hexane (50 mL) was then added and the resulting suspension was gravity filtered. The filtrate was then dried over Na_2SO_4 and concentrated under reduced pressure to afford the crude product. Purification by silica gel column chromatography (5% EtOAc in Petrol, R_f = 0.55) gave the title compound as a colourless liquid (1.30 g, 44%).

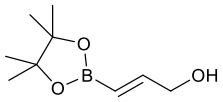
^{1}H NMR (300 MHz, $CDCl_3$); δ_H 6.67 (1H, dt, *J* = 17.9, 3.5 Hz), 5.74 (1H, dt, *J* = 17.9, 2.1 Hz), 4.24 (2H, d, *J* = 3.5, 2.1 Hz), 1.26 (12H, s), 0.90 (9H, s), 0.05 (6H, s).

^{11}B NMR (96 MHz, $CDCl_3$); δ_B 33.3.

^{13}C NMR (75.5 MHz, $CDCl_3$); δ_C 152.3, 83.3, 64.6, 26.1, 24.9, 18.5, −5.2.

NMR data in accordance with literature precedence.[10]

(E)- 3-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)prop-2-en-ol **14**



To a stirring solution of (*E*)-*tert*-butyldimethyl((3-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)allyl)oxy)silane **13** (1.25 g, 4.2 mmol, 1 eq.) in MeOH (3 mL), was added *p*-toluenesulfonic acid (72 mg, 0.4 mmol, 10 mol%). The reaction mixture was allowed to stir at room temperature for 30 minutes before being concentrated under reduced pressure. The residue was then taken up into EtOAc (40 mL) and washed with water (40 mL). The organics were then separated, dried over $MgSO_4$ and concentrated under reduced pressure to afford the crude product. Purification by silica gel column chromatography (20% EtOAc in Petrol, R_f = 0.20) gave the title compound as a colourless oil (0.53 g, 69%).

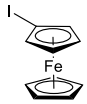
^{1}H NMR (300 MHz, $CDCl_3$); δ_H 6.61 (1H, dt, *J* = 18.1, 4.14 Hz), 5.59 (1H, dt, *J* = 18.1, 1.94 Hz), 4.09 (2H, br s), 3.19 (1H, br s), 1.16 (12H, s).

^{11}B NMR (96 MHz, $CDCl_3$); δ_B 32.9.

^{13}C NMR (75.5 MHz, $CDCl_3$); δ_C 152.2, 116.7 (br), 83.2, 64.1, 24.7.

NMR data in accordance with literature precedence.[11]

Iodoferrocene **16**



To an oven-dried, two-necked round-bottom flask equipped with an addition funnel was added ferrocene (2.79 g, 15 mmol, 1 eq.) and potassium *tert*-butoxide (0.21 g, 1.875 mmol, 0.125 eq.). The solids were then suspended in anhydrous THF (160 mL) and cooled to −78 °C. *tert*-Butyl lithium [1.7 M *in pentane*] (1.13 mL, 30 mmol, 2 eq.) was then added dropwise and the reaction mixture was then left to stir at −78 °C for 1 hour. A solution of iodine (5.71 g, 22.5 mmol, 1.5 eq.) in anhydrous THF (40 mL) was then added dropwise and after complete addition, the reaction mixture was allowed to slowly warm to room temperature and left to stir for 16 hours. The reaction was then quenched with water (20 mL) and stirred for 10 minutes. The solvent was removed under reduced pressure and the residue taken up in Et_2O (100 mL). The organics were washed with $NaS_2O_{3\ (sat.\ aq.)}$ (100 mL) before being dried over $MgSO_4$ and concentrated under reduced pressure. The residue was then taken up in hexane (150 mL), filtered, and washed with 0.2 M $FeCl_{3(aq.)}$ (100 mL). The organics were then washed with water until washings were colourless, dried over $MgSO_4$, and passed through a plug of silica, eluting with further hexane. The filtrate was then concentrated under reduced pressure to afford the title compound as a dark orange solid (2.36 g, 50%).

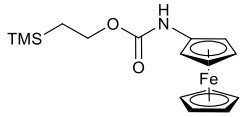
MP: 47–51 °C. **Lit:** 49–50 °C.[12]

^{1}H NMR (300 MHz, C_6D_6); δ_H 4.32 (2H, app t, *J* = 1.8 Hz), 4.05 (5H, s), 3.86 (2H, app t, *J* = 1.8 Hz).

^{13}C NMR (75.5 MHz, C_6D_6); δ_C 74.8, 71.3, 69.1, 40.1.

NMR data in accordance with literature precedent.[13]

2-(trimethylsilyl)ethyl ferrocenylcarbamate **17**



Ferrocenoyl azide **8** (1.02 g, 4 mmol, 1 eq.) and 2-(trimethylsilyl)ethanol (1.14 mL, 8 mmol, 2 eq.) were reacted together according to general procedure **A**. Purification by silica gel column chromatography (hexane 1:1 EtOAc, R_f = 0.80) gave the title compound as a pale orange solid (1.09 g, 79%).

MP: 87–89 °C.

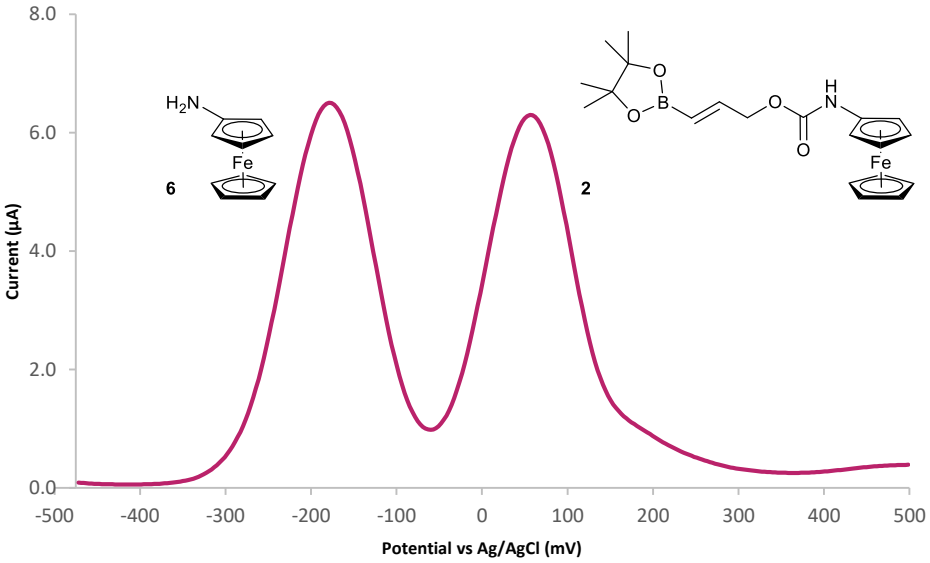
IR (solid, cm^{-1}); ν 3095 (N–H), 2954 (C–H), 2899 (C–H), 1698 (C=O), 1241 (Si–CH_3).

^{1}H NMR (300 MHz, d_6-DMSO); δ_H 8.73 (1H, br s), 4.46 (2H, s), 4.14–4.09 (7H, m), 3.91 (2H, s), 1.00 (2H, t, *J* = 8.1 Hz), 0.05 (9H, s).

^{13}C NMR (75.5 MHz, d_6-DMSO); δ_C 153.8, 97.0, 68.7, 63.5, 61.9, 59.8, 17.3, −1.3.

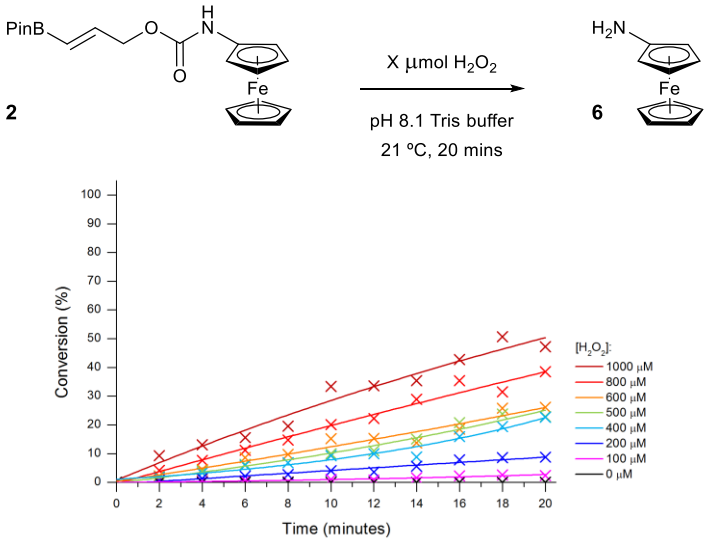
HRMS (ESI): $C_{16}H_{23}FeNNaO_2Si$ $[M+Na]^+$ – theoretical *m/z* 368.074, measured *m/z* 368.075.

Voltammogram of Probe 2 and Aminoferrocene 6



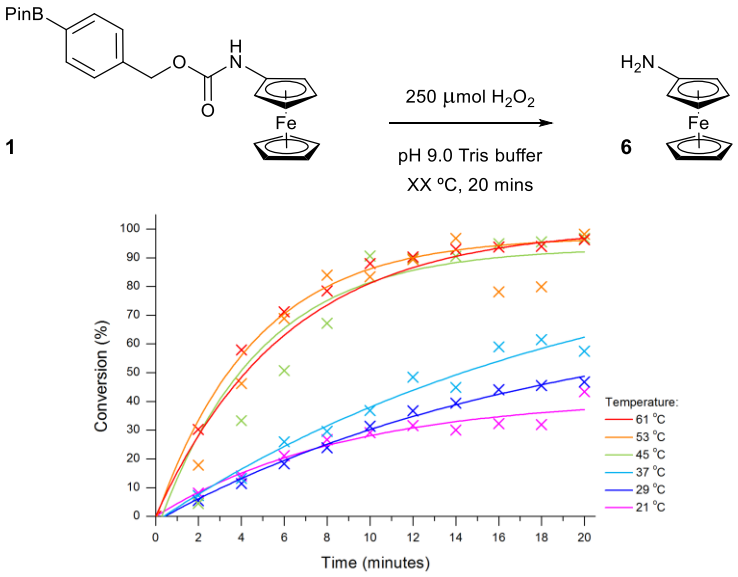
DPV of probe **2** (50 µM) and aminoferrocene **6** (50 µM) in 50 mM pH 8.1 Tris buffer.

Conversion of Probe 2 to Aminoferrocene 6



Conversion of probe **2** (100 μM) to aminoferrocene **6** in 50 mM pH 8.1 Tris buffer in the presence of various concentrations of H_2O_2.

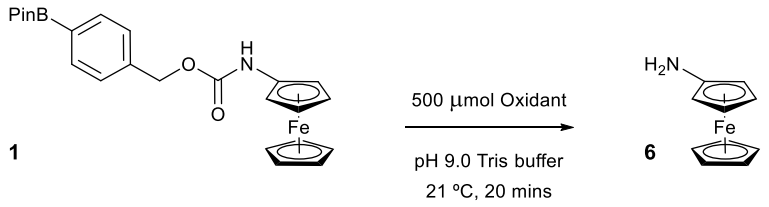
Temperature Screen of Probe 1 with H_2O_2

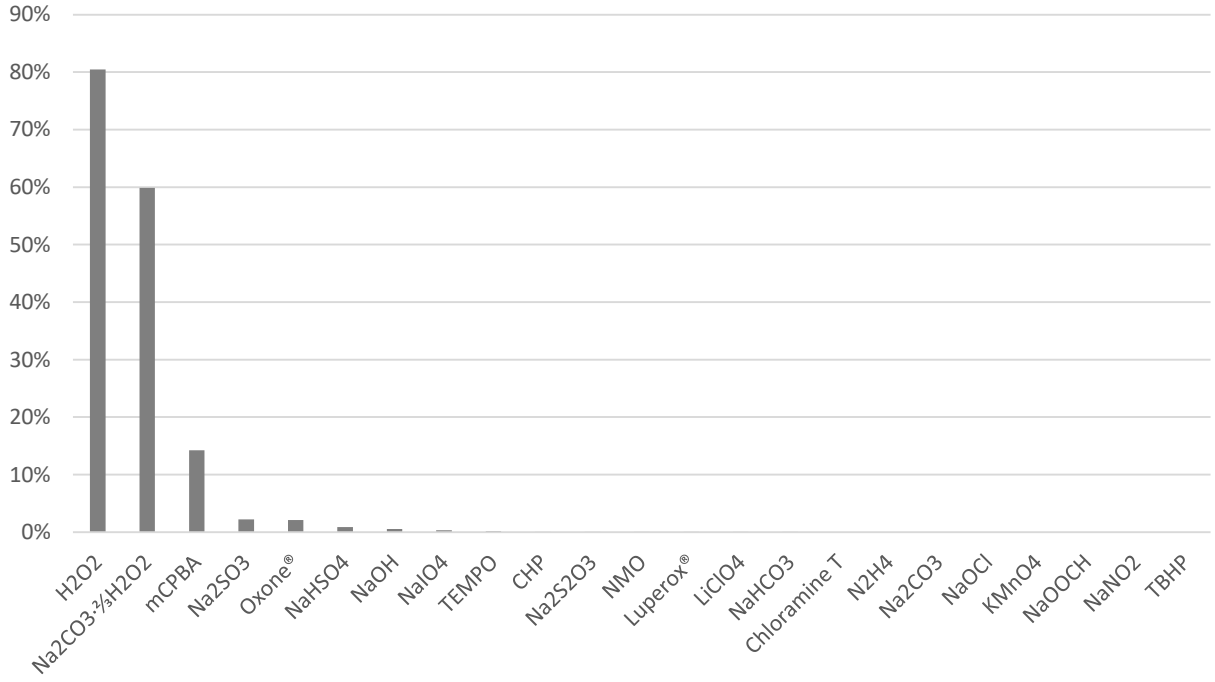


Conversion of probe **1** (100 µM) to aminoferrocene **6** in 50 mM pH 9.0 Tris buffer in the presence of 250 µM of H_2O_2 at varying temperatures.

Full Peroxide Selectivity Study

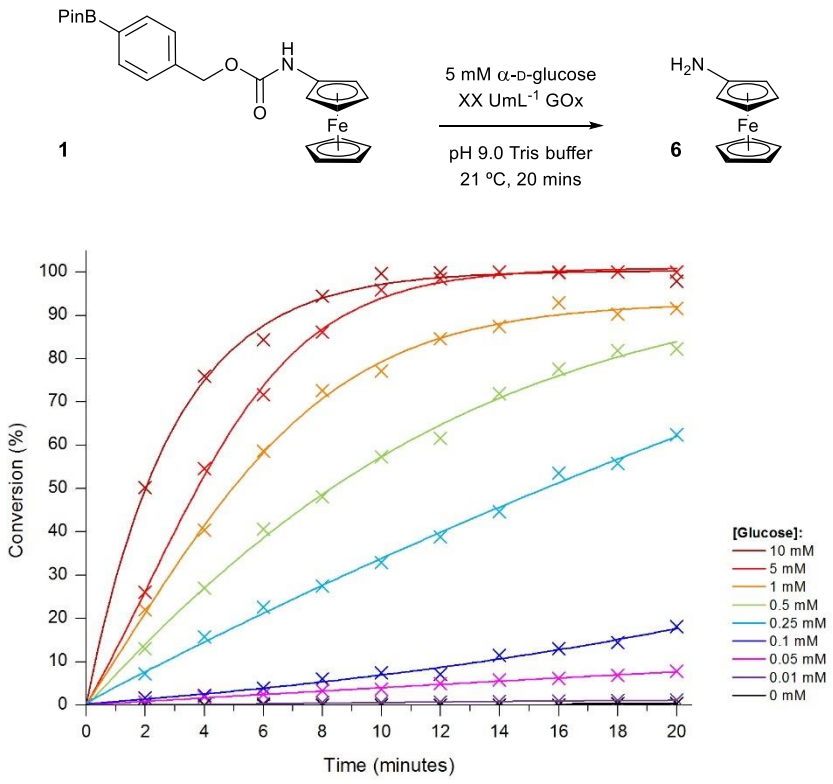
Peroxides (also oxidants and salts) were screened according to general procedure **D**. For solids, a 5 mM stock solution was made by first dissolving 0.05 mmol into 1 mL H_2O (except for mCPBA, which was dissolved in MeOH, and TEMPO and NMO, which was dissolved in MeCN) and then serial diluting by 10. For liquids, a 5 mM stock solution was made by dissolving 0.5 mmol into 1 mL H_2O (except for cumene hydroperoxide (CHP) which was dissolved in MeOH) and then serial diluting by 100.





Benzoyl peroxide (BPO), sodium persulfate ($Na_2S_2O_8$) and sodium hydrosulfite ($Na_2S_2O_4$) were found to be incompatible with the probe under the assay conditions as substantial decomposition of the probe, possibly through oxidation of the iron(II) centre, was observed.

Glucose Oxidase Assay



Conversion of probe **1** (100 µM) to aminoferrocene **6** in 50 mM pH 9.0 Tris buffer in the presence of 5 mM α-D-glucose with varying units of GOx.

References

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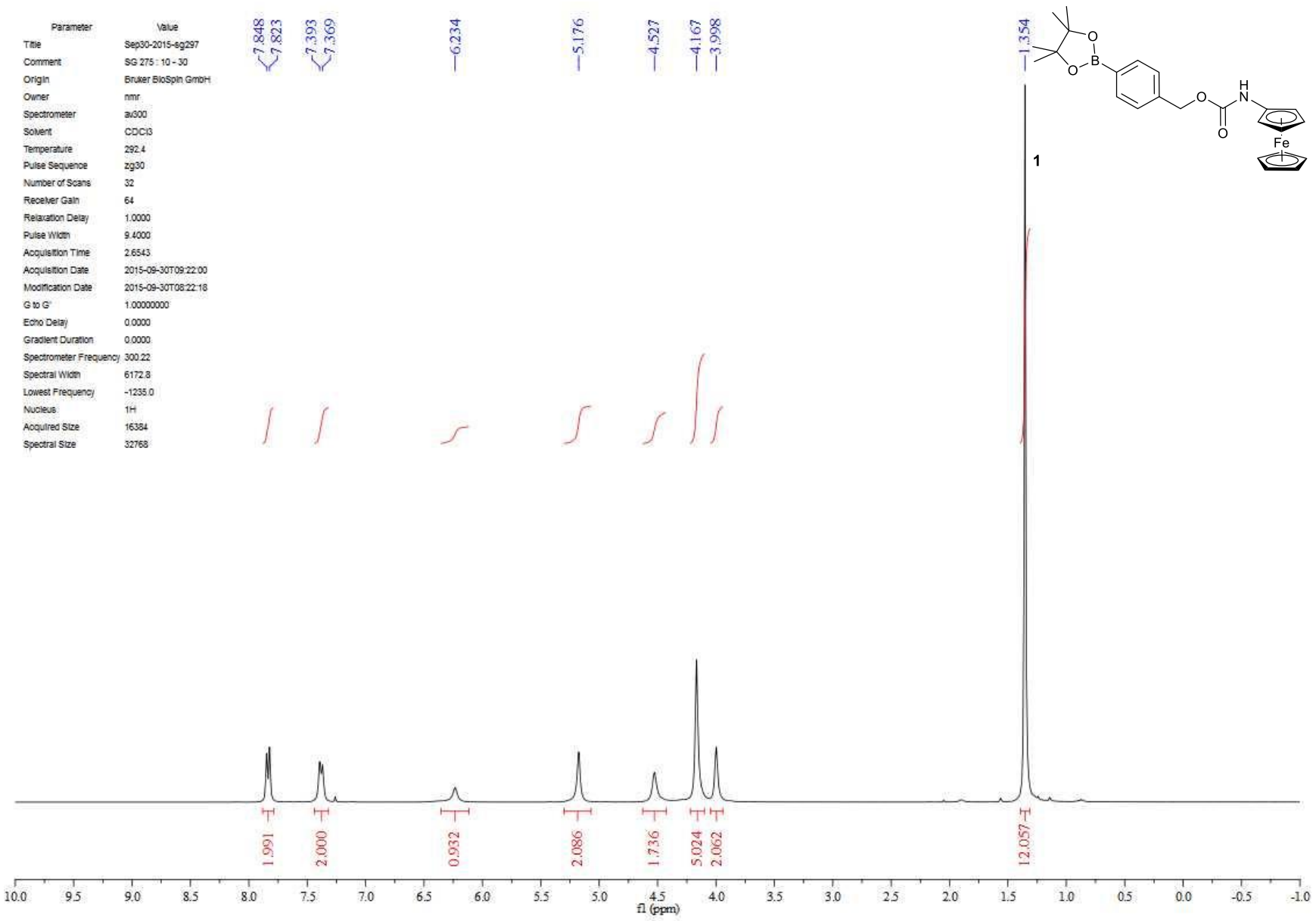
NMR Spectra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Acquisition Date 2015-09-30T09:22:00
Modification Date 2015-09-30T08:22:18
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Nucleus 1H
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f1 (ppm)

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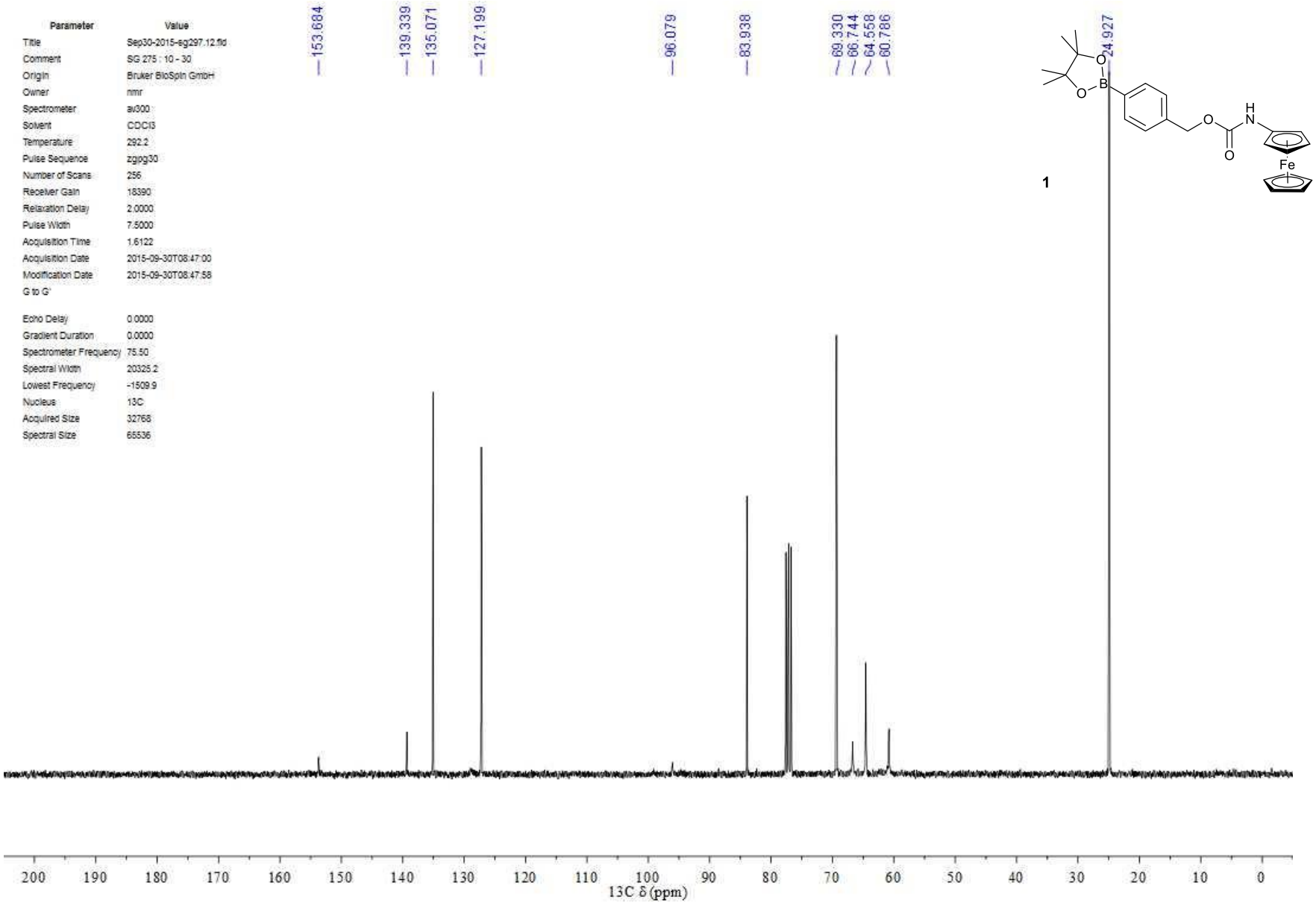
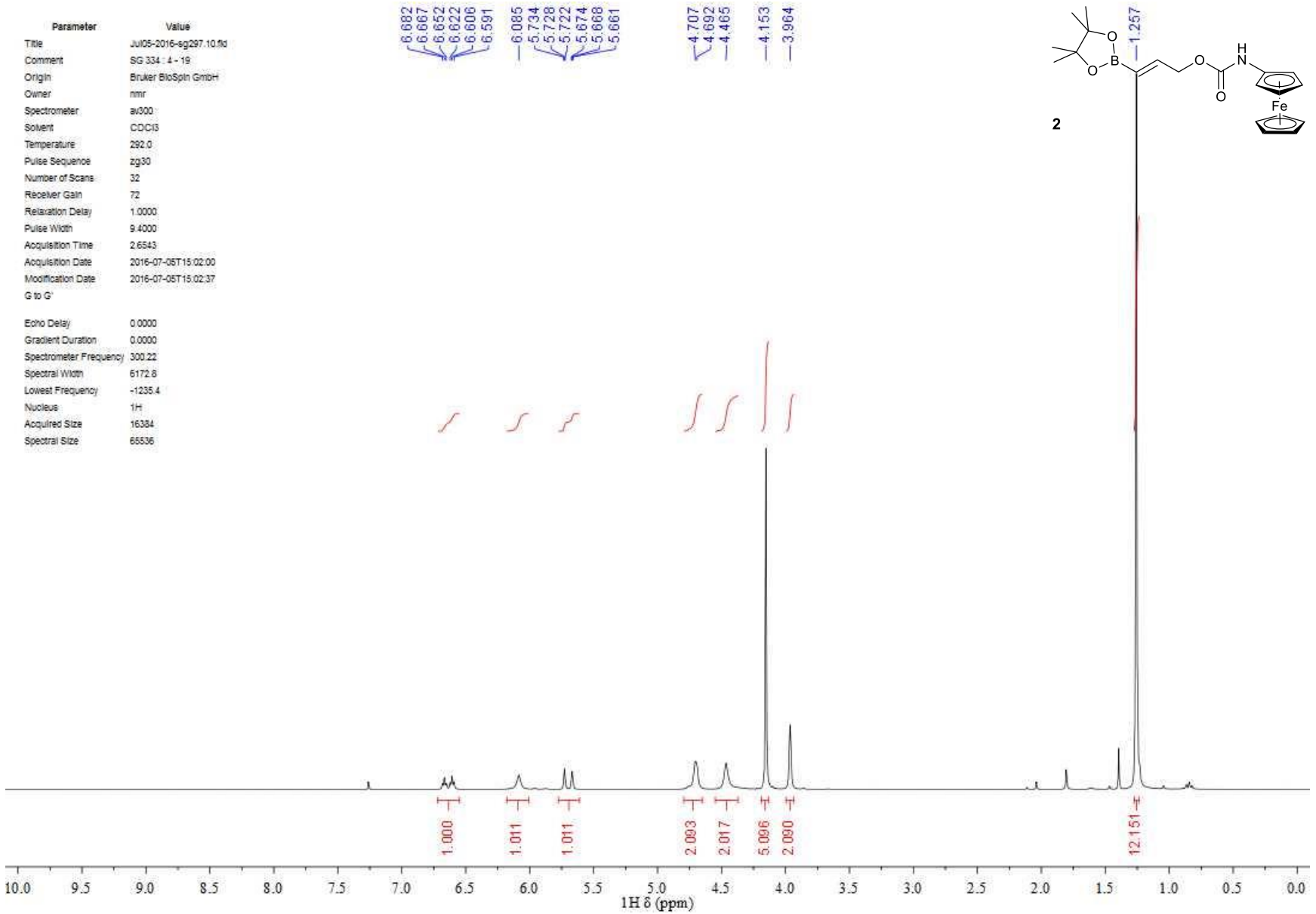


1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1H δ (ppm)

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