Redox-tagged carbon monoxide-releasing molecules (CORMs): Ferrocene-containing [Mn(C^N)(CO)4] complexes as a promising new CORM class

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This study describes the synthesis and characterization of a new class of ferrocene-containing carbon monoxide-releasing molecules (CORMs, **1**-**3**). The ferrocenyl group is both a recognized therapeutically viable co-ligand and a handle for informative infrared spectroelectrochemistry. Deoxy-myoglobin CO-release assays and *in situ* infrared spectroscopy confirm compounds **2** and **3** as photoCORMs and **1** as a thermal CORM, attributed to the increased sensitivity of the Mn-ferrocenyl bond to protonation in **1**. Electrochemical and infrared spectroelectrochemical experiments confirm a single reversible redox couple associated with the ferrocenyl moiety with the Mn tetracarbonyl center showing no redox activity up to +590 mV vs Fc/Fc+, though no concomitant CO release was observed in association with the redox activity. The effects of linker length on communication between the Fe and Mn centers suggest the incorporation of redox-active ligands into CORMs focus on the first coordination sphere of the CORM. Redox-tagged CORMs could prove to be a useful mechanistic probe; our findings could be developed to use redox changes to trigger CO-release.

Introduction

Carbon monoxide (CO) is produced endogenously by mammals. The largest source of CO generation is the heme oxygenase (HO) family of enzymes, involved in the catabolism of heme.1 Two heme oxygenase isoforms are known to catalyze this reaction: the inducible HO1 and the constitutive HO2.1 A third, HO3, is has been characterized to a lesser extent.2 Studies of the ubiquitous HO1 show that this enzyme is upregulated during stress and plays a vital role in the biochemical response to many forms of stress.3,4

The status of CO as a biochemical signaling molecule, and the beneficial effects of exogenous CO, are becoming established,5,6 including antimicrobial,7,8 antimalarial,9 and vasodilatory10 effects. Thus, the use of therapeutic CO represents an intriguing area of study in drug discovery. The administration of CO gas presents challenges in terms of localization and selectivity, in addition to engineering difficulties. An alternative approach is to employ carbon monoxide-releasing molecules (CORMs).11 These compounds, predominantly metal carbonyl complexes, are designed to achieve controlled CO delivery, to specific tissue and cellular targets, for therapeutic prodrug applications.12 The incorporation of redox-active ligands into CORMs could provide an alternative trigger for CO-release. In addition to providing a conceivable way of modulating CO release rates, redox-active groups could also provide an additional mechanistic handle for the CO-release mechanism, which is often difficult to pin down.

Various methods have been used to promote CO-release including thermal, enzymatic and photochemical triggers. Early work by Motterlini and co-workers involved simple structures such as the DMSO-soluble complexes [RuCl2(CO)3]2, Fe(CO)5 and Mn2(CO)10, of which the latter two complexes require photolysis to deliver CO to myoglobin.13 Crucially, this work demonstrated that the vasodilatory and anti-hypertensive effects of HO1 could also be conferred by CO delivery from CORMs. The first water-soluble CORM, *fac*-[Ru(CO)3Cl(glycinate)], CORM-3,14 has since become widely used to study the effects of CO release on biological systems.

In recent years, there has been considerable interest in photochemical CO release from so-called photoCORMs (Figure 1). A diverse array of metals and co-ligands have been employed, as comprehensively discussed in several recent reviews.15–18



Figure 1: Selected Mn-containing photoCORMS.

Many photoCORMs employ Mn(I) chemistry due to the thermal stability associated with this oxidation state, relative ease of synthesis and handling and typically low toxicity of these compounds.19,20 This has allowed for further functionalization to improve selectivity and alter the CO release properties. Schatzschneider and co-workers synthesized a versatile photoCORM based on a [Mn(CO)3(tpm)]+ scaffold (where tpm = tris(pyrazolyl)methane). The tpm backbone allowed installation of an alkynyl group for further functionalization with peptides.21 Zobi and co-workers took a comparable approach in their synthesis of CORM conjugates with cobalamin.22 Mascharak and co-workers have combined theoretical and experimental approaches to better understand the photochemical properties of some Mn(I) tricarbonyl photoCORMs, leading to the development of one class of visible-light-triggered photoCORMs.23 Soluble, water-stable and non-toxic Mn(I) tricarbonyl complexes of amino acids19,24 and recently dinuclear complexes with cysteamine20 have also been developed as photo-CORMs with desirable properties. Fairlamb and co-workers additionally reported Mn(I) tetracarbonyl species bearing a readily functionalizable cyclomanganated 2-phenylpyridine ligand, which can be functionalized to improve water solubility25 and enable further conjugation *via* Suzuki couplings.26

Complementary to the advances in photoCORMs is the drive to develop other mechanisms for triggering CO release. One such possibility is redox-triggered CO-release. Many changes of state of both healthy and diseased cells are accompanied by a change in cellular redox balance.27 It is therefore desirable to produce CORMs sensitive to redox environment. Depending on the target, both oxidative and reductive mechanisms may be desirable. For example, reductive activation of other classes of anti-cancer compounds is already established,28 whilst some ferricenium salts have been shown to have increased anti-tumour activity compared to ferrocene.29

Taking forward related structures to those tested by Lynam and co-workers,30 Pryce and co-workers demonstrated that a Cr(CO)5(aminocarbene) complex slowly releases one equivalent of CO upon electrochemical oxidation.31 Further examples and mechanistic investigation are required to inform the design of future redox-triggered CORMs.

In designing a CORM, there is inherently a compromise between the inclusion of multiple equivalents of CO, and the tendency of CO ligands to stabilize low oxidation states, shifting the redox potential. Controllable redox activity at the metal carbonyl center at a biologically relevant redox potential is challenging as a result of this.

In this work, three ferrocenyl-substituted CORMs **1-3**, based on the parent structure of the air and water stable photoCORM Mn(CO)4(2-phenylpyridine), are synthesized (Figure 2). The linker length connecting the Fe and Mn centers is varied and the electrochemical, photochemical and CO release properties are assessed. The reporter properties of both the metal carbonyl center and ferrocenyl tag enable the effect of linker length on communication between the Fe and Mn centers to be studied. The ferrocenyl moiety can also be considered as a therapeutically active co-ligand complementary to CO; derivatives of ferrocene are validated as therapeutics, including anti-cancer32 and antimalarial33 agents.



Figure 2: Ferrocenyl CORMs synthesized in this work. Note: complex 3 is racemic (planar chirality).

Experimental Section

**Reagents and Solvents.** Reactions in O2 free or anhydrous conditions were performed with dry, deoxygenated solvents under an inert atmosphere, using either standard Schlenk techniques (under N2)or a balloon (of argon), as specified in the procedures. Commercial chemicals were purchased from either Acros Organics, Alfa Aesar, Fluorochem or Sigma-Aldrich and were used without further purification unless otherwise stated. Dry Et2O, THF, toluene, dichloromethane and hexane were dispensed from a Pure Solv MD-7 solvent machine and stored in oven-dried ampoules under N2. Dry THF and Et2O were then deoxygenated by bubbling with N2 and sonication. Water, EtOH and *t*BuOH were deoxygenated by vigorous bubbling of N2 through the solution for at least 1 h. “Petrol” refers to petroleum ether 40‑60 ᵒC.

**Nuclear Magnetic Resonance Spectroscopy.** Solution 1H and 13C NMR analysis was carried out at 298 K on Jeol ESC400/ESX400 and Bruker AV500/AV700 spectrometers. The spectra were processed in MNova software. All coupling constants are quoted to the nearest 0.5 Hz. All chemical shifts are reported in ppm and are referenced to the residual NMR solvent: 1H: CDCl3: 7.26 ppm, DMSO-*d*6: 2.50 ppm, MeOH-*d*4: 3.31 ppm, CD2Cl2: 5.32 ppm; 13C: CDCl3: 77.36 ppm, DMSO-*d*6: 39.52 ppm, MeOH-*d*4: 49.00 ppm, CD2Cl2: 53.49 ppm). The 13C NMR spectra of some tetracarbonyl manganese(I) complexes did not show the metal carbonyl resonances due to the long relaxation times of metal carbonyls. In such cases, infrared spectroscopic analysis confirmed the presence of these metal carbonyls.

**Infrared** **Spectroscopy.** Spectra were recorded on a Bruker Alpha IR spectrometer, usually at a resolution of 4 cm-1. Details of the method (ATR, transmission cell, solvent etc.) are given next to the data. Peak intensities are grouped qualitatively as very strong (vs), strong (s), medium (m) and weak (w), according to common convention.

**Mass Spectrometry.** Mass spectrometry was carried out using a Bruker microTOF spectrometer in positive ion mode using Electrospray Ionization (ESI) or Liquid Injection Field Desorption Ionization (LIFDI) as the ionization methods. High resolution ESI data is within 5 ppm error or the theoretical value unless otherwise stated. All LIFDI data is within 120 ppm error.

**Elemental Analysis.** Elemental analyses were performed on an Exeter Analytical CE-440 Elemental Analyzer. The quoted percentages composition of C, H and N are the average of two experiments.

**UV-Visible Spectroscopy.** All UV-visible spectra were recorded with a Jasco V-560 spectrometer using standard methods. Extinction coefficients (ε) were calculated by fitting measured absorbance to the Beer-Lambert law using at least five different concentrations.

**Melting Point Determination.** Melting points were measured on a Stuart SMP30 apparatus (ramp rates of 2 or 3 ᵒC min-1) or a using Differential Scanning Calorimetry (DSC) on a Perkin Elmer DSC7 machine calibrated with an indium standard. The DSC experiments were run at a ramp rate of 10 ᵒC min-1 and the melting point was taken as the onset of the observed endothermic peak.

**Chromatography.** Thin layer chromatography (TLC) was carried out using aluminum-backed TLC plates (5554, Merck). Visualization was by quenching of fluorescence using a lamp with *λ*max = 254 nm. Flash column chromatography was performed using silica gel 60 (Fluorochem, particle size 40-63 µm).

X-ray crystallography

Single crystals were grown in ambient conditions using dichloromethane/pentane layering or slow evaporation. A suitable crystal was selected and mounted on an Oxford DiffractionSuperNova, Dual source, Cu at zero (orientation of the Cu source relative to the goniometer axes in the dual-source SuperNova X-ray diffractometer – the molybdenum source is offset relative to the goniometer axes), Eos diffractometer. The crystal was kept at 110 K during data collection. Using Olex234, the structures were solved with the ShelXS35 structure solution program using Patterson Method or Superflip using Charge Flipping. The structures were refined with the ShelXL36 refinement package using Least Squares minimization.

**Myoglobin CO-release assay**

Experiments and data analysis were performed according to the procedures described previously.37

**Electrochemistry**

Cyclic voltammograms were recorded in a three-electrode cell comprising a Pt disc working electrode, a Pt wire counter electrode and an Ag wire *pseudo*-reference electrode. Each analyte was calibrated to the FeCp2/FeCp2+ redox couple by the addition of ferrocene or acetylferrocene (Fe(η-C5H4COMe)Cp/[Fe(η-C5H4COMe)Cp]+ measured to be +276 mV vs FeCp2/FeCp2+ to enable potentials to be quotes against FeCp2/FeCp2+. All voltammograms were performed in the presence of NBu4PF6, at a concentration of 1 M, with a scan rate of 100 mV s-1.

Synthetic Procedures and Characterization

This general procedure for cyclomanganation with BnMn(CO)5 is referred to elsewhere as “General Procedure 1”.38 A typical reaction was conducted on 0.5 mmol scale. To an oven-dried Schlenk tube containing a stirrer bar, BnMn(CO)5 (1 eq.) and ‘CH^N’ ligand (1 eq.) was added dry, deoxygenated hexane (16 mL per mmol of BnMn(CO)5) was added *via* syringe. The was solution heated to reflux for between 6-24 h (covered in aluminum foil to exclude ambient light). Reaction progress was monitored by IR spectroscopic analysis by taking aliquots directly from the reaction mixture. On reaction completion, the mixture was allowed to cool to room temperature, then filtered through a pipette packed with cotton wool. Any solid product that precipitated out of solution was dissolved in a small amount of CH2Cl2. The solvent was removed *in vacuo* to afford the product. Where required, further purification was performed using flash column chromatography.

**2-(4'-[(3''-Triisopropylsilyl-prop-2-ynyl-oxy)methyl]phenyl)pyridine (4).** To an oven-dried Schlenk tube containing a solution of 2-(4-[(prop-2-ynyloxy)methyl]phenyl)pyridine (1.0 eq., 3.87 mmol, 0.865 g ) in dry, deoxygenated THF (40 mL) at -78 ᵒC was added a solution of LDA (1.0 eq., 3.87 mmol, 9.6 mL) in THF *via* syringe over five minutes. The LDA was freshly prepared by lithiation of freshly distilled diisopropylamine with *n*-BuLi (titrated against *n*-benzylbenzamide before use).39 After the addition of LDA was complete, the Schlenk tube was placed in an ice/water bath at 0 ᵒC for 10 minutes before being cooled back to ‑78 ᵒC for the addition of TIPS-Cl (1.0 eq., 3.87 mmol, 0.84 mL). The reaction mixture was then allowed to warm (to RT) and left for 19 h, at which point TLC analysis indicated reaction completion. The reaction was quenched using saturated ammonium chloride (30 mL) and extracted with Et2O (3 × 50 mL). The combined organic layers were washed with water (30 mL) and brine (30 mL), before being dried (MgSO4) and filtered. The solvent was removed *in vacuo* to give the crude product, which was purified by flash column chromatography on silica, eluting with petrol and then petrol/Et2O 4:1 *v/v*. The solvent was removed *in vacuo* to give the *title compound* as a yellow oil (1.25 g, 85% yield). 1H NMR (400 MHz, CDCl3): *δ* (ppm) = 8.69 (ddd, 5.0, 2.0, 1.0 Hz, 1H, Ar-H), 7.99 (d, 8.5 Hz, 2H, Ar-H), 7.77‑7.71 (m, 2H, Ar-H), 7.47 (d, 8.0 Hz, 2H, Ar-H), 7.22 (ddd, 5.5, 5.0, 2.5 Hz, 1H, Ar-H), 4.70 (s, 2H. CH2), 4.23 (s, 2H, CH2), 1.09 (m, 21 H, CH and CH3 from TIPS); 13C NMR (101 MHz, CDCl3) *δ* (ppm) = 157.7, 150.2, 139.4, 138.8, 137.2, 129.0, 127.4, 122.5, 120.9 (Ar); 103.4 (C≡C-Si); 88.4 (C≡C-Si); 70.7 (benzylic CH2); 57.8 (propargylic CH2) , 18.4 (TIPS CH3); 11.0 (TIPS CH); HRMS (ESI+): *m/z* = 380.2391 [MH]+ (C24H34NOSi requires 380.2410); IR (CH2Cl2, cm‑1): 2170 (w), 1703 (w), 1603 (w), 1589 (w), 1580 (w), 1565 (w), 1468 (s), 1436 (m), 1384 (w), 1351 (m), 1210 (w), 1153 (w), 1079 (s), 1029 (w), 1017 (w), 998 (m), 991 (m).

**Tetracarbonyl 2-(4'-[(3''-triisopropylsilyl-prop-2-ynyloxy)methyl]phenyl) κ,C2-pyridine- κ,N) manganese(I) (5).** Using General Procedure 1, BnMn(CO)5, (1 eq., 2.93 mmol, 840 mg) and 2-(4-[(3-triisopropylsilyl-prop-2-ynyl-oxy)methyl]phenyl)pyridine (0.96 eq., 2.82 mmol, 1.07 g) were reacted in hexane (40 mL). The *title compound* was isolated as a yellow solid (1.35 g, 88% yield) with no additional purification required. M.P.: 82 ᵒC (DSC); 1H NMR (400 MHz, CDCl3): *δ* (ppm) = 8.72 (d, 5.5 Hz, 1H, Ar-H), 7.92 (s, 1H, Ar-H), 7.87 (d, 8.0 Hz, 1H, Ar-H), 7.82‑7.75 (m, 2H, Ar-H), 7.20 (dd, 8.0, 1.5 Hz, 1H, Ar-H), 7.11 (ddd, 7.5, 5.0, 1.5 Hz, 1H, Ar-H), 4.70 (s, 2H, CH2), 4.30 (s, 2H, CH2), 1.11 (m, 21H, CH and CH3 from TIPS); 13C NMR (101 MHz, DMSO-*d*6): *δ* (ppm) = 220.0, 214.5, 213.4 (Mn-C≡O); 172.5, 164.7, 154.4, 145.9, 140.9, 139.1, 139.0, 124.5, 124.0, 123.7, 120.0 (Ar); 104.3 (C≡C-Si); 86.8 (C≡C-Si); 70.5 (benzylic CH2); 57.3 (propargylic CH2); 18.4 (TIPS CH3); 10.6 (TIPS CH); HRMS (ESI+): *m/z* = 568.1327 [MNa]+ (C28H32MnNNaO5Si requires 568.1328); Elemental analysis (CHN): C: 61.48%, H: 5.91%, N: 2.51% (C28H33NO5SiMn requires C: 61.64%, H: 5.91%, N: 2.57%); IR (CH2Cl2, cm‑1): 3685 (w), 3157 (w), 2961 (m), 2946 (m), 2927 (m), 2893 (m), 2866 (m), 2076 (s), 1991 (vs), 1976 (vs), 1933 (vs), 1605 (m), 1587 (m), 1567 (m), 1479 (m), 1467 (m), 1351 (s).

**Tetracarbonyl 2-(4'-[(prop-2''-ynyloxy)benzyl) κ,C2-pyridine- κ,N) manganese(I) (6).** To a solution of tetracarbonyl 2-(4'-[(3''-triisopropylsilyl-prop-2'-ynyloxy)methyl]phenyl) κ,C2-pyridine-κ,N) manganese(I) (1 eq., 1.10 mmol, 599 mg) in CH2Cl2 (2 mL) was added a solution of TBAF∙3H2­O (1.2 eq., 1.32 mmol, 417 mg) in CH3CN (10 mL). The brown solution was stirred under air for 25 min, at which time the reaction was judged complete (TLC analysis). Water (10 mL) was added and mixture extracted with CH2Cl2 (3 × 15 mL). The combined organic layers were, dried (MgSO4) and filtered. The solvent was removed *in vacuo* to yield the crude product, which was purified by flash column chromatography on silica gel, starting with petrol/EtOAc 10:1 *v/v*, increasing the gradient slightly to 10:1.5 *v/v*, to afford the product. The solvent was removed *in vacuo* to give the *title compound* as a light brown solid (0.253 g, 59% yield). M.P.: 107 ᵒC (decomp.); 1H NMR (400 MHz, DMSO-*d*­6): *δ* (ppm) = 8.76 (d, 5.5 Hz, 1H, Ar-H), 8.24 (d, 8.0 Hz, 1H, Ar-H), 8.07‑8.00 (m, 2H, Ar-H), 7.78 (s, 1H, Ar-H), 7.37 (m, 1H, Ar-H), 7.12 (dd, 1.0, 8.0 Hz, 1H, Ar-H), 4.57 (s, 2H, CH2), 4.25 (t, 2.5 Hz, 2H CH2), 3.52 (d, 2.5 Hz, 1H, C≡C-H); 13C NMR (101 MHz, CDCl3): *δ* (ppm) = 175.4, 166.4, 154.2, 146.1, 141.4, 139.3, 138.2, 124.3, 124.3, 122.7, 119.7 (Ar); 80.1, 75.0, 72.3, 57.8 (propargylic CH2); IR (CH2Cl2, cm-1): 3664 (w), 3302 (w), 2964 (m), 2945 (m), 2929 (w), 2893 (m), 2866 (m), 2075 (s), 1991 (vs), 1976 (vs), 1932 (vs), 1605 (m), 1588 (m), 1566 (w), 1478 (m), 1468 (m); HRMS (ESI+): *m/z* = 411.9992 [MNa]+ (C19H12MnNNaO5 requires 411.9994); Elemental analysis (CHN): C: 58.58%, H: 3.23%, N:3.48 % (C19H13NO5Mn requires C: 58.62%, H: 3.11%, N: 3.60%).

**Tetracarbonyl (2-[4-(([1-(ferrocenyl)-1H-1,2,3-triazol-4-yl]methoxy)methyl)phenyl-κ,C2]pyridine-κ,N) manganese(I) (1).** To a solution of tetracarbonyl 2-(4'-[(prop-2-ynyloxy)methyl]phenyl) κ,C2-pyridine- κ,N) manganese(I) (1.0 eq., 0.2 mmol, 78 mg) in N2-saturated *t*BuOH (3 mL) was added azidoferrocene (1.0 eq., 0.2 mmol, 46 mg) and water (2.7 mL). Copper(II) sulfate (0.3 eq., 0.06 mmol, 200 μL of 300 mM aqueous solution) and sodium ascorbate (0.6 eq., 0.12 mmol, 120 μL of 500 mM aqueous solution) were added and the reaction mixture was stirred at room temperature in the dark under an argon balloon.After 24 h, the reaction mixture was diluted with water (20 mL). The product was extracted with EtOAc (4 × 20 mL). The combined organic layers were dried with MgSO4 and filtered, before removal of the solvent yielded the crude product. The crude product was purified by flash column chromatography on silica, using petrol/EtOAc, starting with 3:2 *v/v* and moving to 1:1 *v/v* to elute the product. The solvent was removed to give the *title compound* (74 mg, 60% yield) as a yellow crystalline solid. M.P.: 143 ᵒC (decomp.); 1H NMR (700 MHz, CDCl3): *δ* (ppm) = 8.72 (d, 5.5 Hz, 1H, Ar-H), 7.97 (d, 1.5 Hz, 1H, Ar‑H), 7.89‑7.86 (m, 1H, Ar-H), 7.84 (s, 1H, Ar-H), 7.81-7.76 (m, 2H, Ar-H), 7.21 (dd, 8.0, 1.5 Hz, 1H, Ar-H), 7.12 (ddd, 7.0, 5.5, 1.5 Hz, 1H, Ar-H), 4.83 (t, 2.0 Hz, 2H, Cp‑H), 4.82 (s, 2H, CH2), 4.71 (s, 2H, CH2), 4.26 (t, 2.0 Hz, 2H, Cp-H), 4.22 (s, 5H, Cp-H); 13C NMR (176 MHz, CDCl3) *δ* (ppm) = 220.3, 214.1, 214.0 (M-C≡O); 175.1, 166.1, 154.0, 145.9, 145.5, 140.7, 139.6, 138.0, 124.2, 123.9, 122.5, 122.3, 119.5 (Ar); 94.0 (Cp); 73.0 (CH2); 70.3 (Cp); 66.8 (Cp); 64.2 (CH2); 62.3 (Cp); HRMS (ESI+): *m/z* = 617.0292 [MH]+ (C29H22N4O5MnFe requires 617.0315); IR (CH2Cl2, cm-1): 3048 (w), 2864 (w), 2076 (s), 1991 (vs), 1977 (vs), 1931 (s), 1606 (m), 1587 (m), 1567 (w), 1519 (w), 1472 (m), 1432 (w), 1312 (w), 1108 (m); Elemental analysis (CHN): C: 56.28%, H: 3.48%, N: 8.69% (C29H21N4O5MnFe requires C: 56.52%, H: 3.44%, N: 9.09%); UV-visible: λmax = 270 nm, ε(270 nm) = 28 500 dm3 mol-1 cm-1; *R*f: 0.40 (petrol/EtOAc 3:2 *v/v*).

**[4-(2'-Pyridinyl)phenyl]-ferrocene (8).** To an oven-dried Schlenk tube with stirrer bar were added 2-(4-bromophenyl)pyridine (1.0 eq., 1.0 mmol, 234 mg), ferroceneboronic acid (2.0 eq., 2.0 mmol, 461 mg), Pd(OAc)2 (0.053 eq., 0.053 mmol, 11.9 mg), PPh3 (0.21 eq., 0.21 mmol, 55.5 mg) and K2CO3 (4.0 eq., 4.0 mmol, 555 mg). Anhydrous, deoxygenated toluene (10 mL) was added in one portion *via* syringe. The red reaction mixture was refluxed for 20 h, at which point TLC analysis showed complete consumption of the starting material and the appearance of a product spot. The reaction mixture was allowed to cool and the toluene was removed *in vacuo*. The mixture was re-suspended in CHCl3 (100 mL) and washed with water (2 × 100 mL). The combined organic extract was dried (MgSO4) and filtered. The solvent was removed *in vacuo* and the crude product was purified by flash column chromatography on silica gel using petrol/EtOAc, starting with 9:1 *v/v*, increasing the gradient to 8:2 *v/v*. Removal of the solvent *in vacuo* gave the *title compound* as an orange powder (173 mg, 55 % yield). M.P.:122 ᵒC; 1H NMR (400 MHz, CDCl3): *δ* (ppm) = 8.69 (d, 4.5 Hz 1H, Ar-H), 7.93 (d, 8.0 Hz, 2H, Ar‑H), 7.73 (d, 4.0 Hz, 2H, Ar‑H), 7.58 (d, 8.0 Hz 2H, Ar‑H), 7.21 (dd, 9.0, 4.0 Hz 1H, Ar‑H), 4.71 (t, 2.0 Hz, 2H, Cp-H), 4.36 (t, 2.0 Hz, 2H, Cp-H), 4.05 (s, 5H, Cp-H); 13C NMR (101 MHz, CDCl3) *δ* (ppm) = 157.4, 149.8, 140.5, 136.9, 136.8, 127.0, 126.4, 121.0, 120.3 (Ar); 84.6, 69.8, 69.3, 66.7 (Cp); HRMS (ESI+): *m/z* = 340.0770 [M-H]+ (C21H18FeN requires 340.0789)

**Tetracarbonyl (2-[4-ferrocenyl]phenyl-κ,C2-pyridine-κ,N) manganese(I) (2).** This reaction was performed using General Procedure 1 with BnMn(CO)5 (1.0 eq., 0.25 mmol, 72.5 mg), [4-​(2'-​pyridinyl)​phenyl]​ferrocene (1.0 eq., 0.25 mmol, 84.5 mg) and hexane (4 mL). Infrared spectroscopic analysis showed that the reaction was complete after 6 h. The *title compound* was purified by flash column chromatography on silica, using petrol and then petrol/EtOAc 9:1 *v/v*. The solvent was removed *in vacuo* to afford a red microcrystalline solid (79 mg, 59% yield). M.P.:138 ᵒC (decomp.); 1H NMR (700 MHz, CDCl3): *δ* (ppm) = 8.70 (d, 5.0 Hz, 1H, Ar‑H), 8.12 (s, 1H, Ar‑H), 7.84 (d, 8.0 Hz, 1H, Ar‑H), 7.77 (apr. t, 1H, Ar‑H), 7.68 (d, 8.0 Hz, 1H, Ar‑H), 7.28 (s, 1H, Ar‑H), 7.08 (dd, 6.5, 6.0 Hz, 1H, Ar‑H), 4.77 (t, 2.0 Hz, 2H, Cp-H), 4.37 (t, 2.0 Hz, 2H, Cp-H), 4.09 (s, 5H, Cp‑H); 13C NMR (176 MHz, CDCl3) *δ* (ppm) = 220.4, 214.2, 214.1 (Mn-C≡O); 174.2, 166.2, 153.8, 144.0, 141.4, 139.0, 137.7, 123.9, 122.2, 121.8, 119.0 (Ar); 85.4, 69.8, 69.2, 67.1 (Cp); MS (LIFDI+): *m/z* = 505.04 [M+] C29H21N4O5MnFe requires 504.9809; Elemental analysis (CHN): C: 59.69%, H: 3.27%, N: 2.73% (C29H21N4O5MnFe requires C: 59.44%, H: 3.19%, N: 2.77%); IR (CH2Cl2, cm-1): 2967 (w), 2074 (s), 1990 (s), 1975 (s), 1931 (s), 1733 (w), 1606 (m), 1583 (m), 1563 (m), 1474 (m), 1425 (w);

2-(Ferrocenyl)pyridine (9). Bromoferrocene (1.0 eq., 1,9 mmol, 513 mg) was stirred in dry ethyl acetate (10 mL) at -78 ᵒC. After the dropwise addition of n-BuLi (1.1 eq., 2.0 mmol, 800 μL) over 15 min, the solution was allowed to warm to room temperature and stirred for a further 10 min when a bright orange precipitate had formed. The reaction mixture was cooled back to -78 ᵒC and transferred via cannula onto dry ZnCl2 (2.0 eq., 3.8 mmol, 518 mg) at -78 ᵒC. After the reaction mixture had been stirred at room tempaerature for 1 hour, it was transferred via cannula to a Schlenk tube containing Pd(PPh3)4 (10 mol %, 0.19 mmol, 220 mg) and 2-bromopyridine (2.5 eq., 4.75 mmol, 450 μL) was added dropwise. The reaction mixture was stirred at room temperature for 19 hours then quenched with sat. aq. NH4Cl (15 mL). The aqueous layer was extracted with chloroform (3 × 10 mL) and the combined organic layers dried with MgSO­4­ and filtered. Removal of the solvent in vacuo gave an orange powder. Further purification by silica gel flash column chromatography using petrol/EtOAc 9:1 v/v afforded an analytically pure sample of the title compound as an orange powder (371 mg, 74 % yield). M.P. 90-92 ᵒC; 1H NMR (700 MHz, CD2Cl2): *δ* (ppm) = 8.51 (ddd, 5.0, 2.0, 1.0 Hz, 1H, Ar-H), 7.57 (ddd, 8.0, 7.5, 1.0 Hz, 1H, Ar‑H), 7.41 (ddd, 8.0, 1.0, 1.0 Hz, 1H, Ar‑H), 7.06 (ddd, 7.5, 5.0, 1.0 Hz 1H, Ar‑H), 4.92 (dd, 2.0, 2.0 Hz, 2H, Cp-H), 4.40 (dd, 2.0, 2.0 Hz, 2H, Cp-H), 4.05 (s, 5H, Cp-H); 13C NMR (176 MHz, CD2Cl2) *δ* (ppm) = 159.3, 149.4, 136.0, 120.6, 120.2 (Ar); 83.7, 70.0, 69.7, 67.3 (Cp); HRMS (ESI+): *m/z* = 264.0473 [M-H]+ (C15H14NFe requires 264.0470); IR (CH2Cl2, cm-1): 1609 (m), 1588 (w), 1574 (w), 1469 (m), 1434 (m), 1277 (w); ); UV-visible: λmax = 314 nm, ε(314 nm) = 22 900 dm3 mol-1 cm-1; 378 nm, ε(378 nm) = 8 000 dm3mol‑1 cm-1; 460 nm, ε(460 nm) = 2 040 dm3mol‑1 cm-1.

**(±)-Tetracarbonyl (2-ferrocenyl-κ,C2-pyridine-κ,N) manganese(I) (3)**

This reaction was performed using General Procedure 1 with BnMn(CO)5 (1.0 eq., 2.3 mmol, 65.2 mg), 2-(ferrocenyl)pyridine **(**1.0 eq., 0.23 mmol, 60.0 mg**)** and hexane (6 mL). Infrared spectroscopic analysis showed that the reaction was complete after 6 h. The *title compound* was purified by flash column chromatography on silica using petrol/Et2O/NEt3 94:5:1 *v*/*v* to afford a dark red solid (67.2 mg, 71 % yield). M.P. 109-111 ᵒC; 1H NMR (700 MHz, CD2Cl2): *δ* (ppm) = 8.50 (d, 5.0 Hz, 1H, Ar-H), 7.65 (ddd, 8.0, 7.5, 1.0 Hz, 1H, Ar‑H), 7.41 (d, 8.0 Hz, 1H, Ar‑H), 7.02 (ddd, 7.5, 5.0, 1.0 Hz 1H, Ar‑H), 4.93 (d, 2.0 Hz, 1H, Cp-H), 4.66 (dd, 2.0, 2.0 Hz, 1H, Cp-H), 4.56 (d, 2.0 Hz, 1H, Cp-H), 4.07 (s, 5H); 13C NMR (176 MHz, CD2Cl2): *δ* (ppm) = 221.1, 213.2, 212.3, 211.7 (Mn-C≡O); 169.0, 154.4, 137.5, 120.2, 119.5 (Ar); 103.1, 90.5, 77.4, 72.4, 69.6, 65.2 (Cp); MS (LIFDI+): *m/z* = 426.9565 [M+] C19H12NO4MnFe requires 426.9543; Elemental analysis (CHN): C: 53.00%, H: 2.91%, N: 3.20% (C19H12NO4MnFe requires C: 53.18%, H: 2.82%, N: 3.26%); IR (CH2Cl2, cm-1): 2077 (s), 1988 (vs), 1976 (vs), 1932 (vs), 1607 (m), 1498 (s); UV-visible: λmax = 292 nm, ε(292 nm) = 18 300 dm3 mol-1 cm-1; 486 nm, ε(486 nm) = 1 230 dm3mol‑1 cm-1; *R*f: 0.28 (petrol/Et2O 9:1 *v*/*v*).

Results and Discussion

Compound **1**, which accommodates the ferrocenyl group in the most remote location relative to the Mn(I) center, was synthesized employing versatile alkynyl building block **6** (Scheme 1).

Complex **6** could not be synthesized directly by cyclomanganation of the corresponding terminal alkyne. Based on work by Nicholson *et al.*,40 we hypothesized that addition of the alkyne into the Mn(I) center could compete with coordination of the pyridyl nitrogen directing group. The poor functional group tolerance of such a side-reaction to bulky silyl substituents on the alkyne was exploited as a protecting group strategy, affording TIPS analogue **5** in 88% yield. Deprotection affords alkyne **6**,areadily functionalisable building block for the synthesis of remotely conjugated modular CORMs complementary to the current libraries. Alkyne **6** was shown to be a viable reagent in a copper-catalysed azide-alkyne cycloaddition (CuAAC) reaction. Reaction with azidoferrocene afforded the triazole product **1** in 61% yield after flash column chromatography. An alternative route may be envisaged whereby the Click conjugation takes place before the cyclomanganation, although we anticipate that this would present a challenge for the inclusion of more water-soluble derivatives, due to the non-polar solvents typically employed in the cyclomanganation step. Compound **2** (Scheme 2), in which the ferrocenyl group is conjugated to the 2-phenylpyridyl ligand, was accessed via a Suzuki–Miyaura coupling of aryl bromide **7** to afford ferrocenyl ligand **8** in an acceptable yield of 55%.

The yield represents a slight improvement on the previously reported Grignard-based synthesis of this molecule, where the reported yield was 38%.41 Late-stage Suzuki coupling of the cyclomanganated derivative of **7**, using ferroceneboronic acid with the conditions of Ward and co-workers,23 was unsuccessful due to degradation of the starting complex at the high reaction temperatures required. With the desired ligand in hand, cyclomanganation with BnMn(CO)5 proceeded in good yield to afford target **2**.

Finally, compound **3**, incorporating the redox-active moiety in the first coordination sphere of the metal, was synthesized via a Negishi coupling to afford ligand **9** in 74% yield before cyclomanganation with BnMn(CO)5 to give **3**.

Single crystals of **1**, **2** and **8** suitable for X-ray diffraction were obtained, either by layering pentane on a concentrated dichloromethane solution of the product (**1**), or slow evaporation of a chloroform solution (**2** and **8**). Thermal ellipsoid plots are shown for target complexes **1** and **2** in Figure 3; a thermal ellipsoid plot for **8** is provided in the Supporting Information file along with summary data tables for all three structures.

Comparison of **1** and **2** with other Mn(I) phenylpyridine complexes shows the same slight distortion from the idealized octahedral structure at manganese, due to the rigidity of the 2‑phenylpyridine system.38 The structures also suggest that the cyclomanganation of **8** forces the pyridyl and phenyl rings to become planar in complex **2**, having been twisted slightly in **8** – the torsion angle increasing from -8.9(6) in **8** to -20.3(2) in **2**.



Scheme 1: Synthesis of alkynyl building block 6 and ferrocenyl complex 1.

Scheme 2: Synthesis of complexes 2 (panel A) and 3 (panel B).



Complexation of the ligands to manganese was also evidenced by NMR and infrared spectroscopies. The 1H NMR spectra of **3** and **9** in CD2Cl2 (Figure 4) showed a significant change in the region of the ferrocenyl protons on the substituted Cp ring. The two triplets at *δ*=4.92 and 4.40 ppm were replaced by three resonances each corresponding to a single proton, reflecting the deprotonation and desymmetrization of this ring upon cyclomanganation. Smaller changes in chemical shifts were also observed for the protons on the unsubstituted Cp ring and the pyridyl ring.

The infrared spectra of the three complexes all show the expected four carbonyl stretching bands for a *CS* or pseudo-*C2v* symmetry (*vide infra,* Table 2). The carbonyl stretches for the three complexes reflect the similarity of the first coordination sphere of the manganese in each of the compounds.

Electrochemical characterization of the three Mn complexes along with starting materials **8** and **9**, was carried out to understand their redox properties. Cyclic voltammetry was performed on solutions of each complex in dichloromethane. An example voltammogram using **1** is shown in Figure 5.

Figure 3: X-ray crystal structures of compounds 1 (top) and 2 (bottom), with hydrogen atoms omitted. Atoms are displayed as thermal ellipsoids at 50% probability.



Figure 4: A stack of 700 MHz 1H NMR spectra showing cyclomanganated complex 3 against ligand 9. Spectra were recorded at 298 K in CD2Cl2 (the proton integrals are displayed as integers)



Figure 5: Cyclic voltammogram of a dichloromethane solution of 1 mM 1. The electrolyte was tetra-*n*-butylammonium hexafluorophosphate, present at a concentration of 1 M, and the scan rate was 100 mV s‑1.

For each compound, repeated scans showed a single reversible one-electron redox reaction which were attributed to the ferrocenyl portions of the molecules. In each voltammogram, the separation between the anodic and cathodic peaks was comparable to that of the ferrocene/ferricenium calibration run, although solution resistance led to these values being higher than the ideal 59 mV, up to 170 mV.42

The midpoint potentials, *E*1/2, are compiled in Table 1. Triazole **1** displays a relatively high redox potential of +220 mV vs Fc/Fc+, though this is consistent with other ferrocenyl triazoles and is a result of the electron-withdrawing nature of the triazole, which destabilizes the cation relative to the neutral molecule.43 The midpoint potentials for **2** and **8** show a very slight decrease in *E*1/2 of 17 mV upon cyclomanganation. This suggests that the effect of cyclomanganation on the redox properties of ferrocene in this position is small. A similar trend is seen upon the reaction of **9** to form **3**, but the incorporation of the ferrocenyl group into the first coordination sphere of the Mn makes this effect much more pronounced with a lowering of *E*­1/2 from +76 mV to -138 mV, both vs Fc/Fc+. The change in *E*1/2 implies that the effect of cyclomanganation is to stabilize the cation more than the neutral molecule, so that **2** and **3** are more easily oxidized than their counterparts **8** and **9**. It can therefore be concluded that the electron density on the ferrocenyl moiety increases as a result of cyclomanganation.

Table 1: Midpoint potentials and peak current ratios of ferrocenyl Mn complexes 1-3 and two uncomplexed derivatives 8 and 9 in dichloromethane. Peak currents were calculated by linearly extrapolating the baseline current. The electrolyte was 1 M tetra-*n*-butylammonium hexafluorophosphate. Potentials are referenced against the Fc/Fc+ redox couple.

|  |  |  |
| --- | --- | --- |
| Compound | *E*1/2 (mV) | *i*a/*i*c |
| 1 | +220 | 1.05 |
| 2 | +31 | 0.94 |
| 3 | -138 | 1.04 |
| 8 | +48 | 1.03 |
| 9 | +76 | 0.97 |

Repeated scans of each complex were performed, but no new peaks were observed, and the peaks due to the complexes remained, suggesting that **1**, **2** and **3** are all stable to repeated oxidation and reduction.

The response of the infrared stretches of the carbonyl stretches to oxidation of the ferrocenyl moiety were assessed by infrared spectroelectrochemistry. The results, shown in Figure 6, demonstrate the importance of the linker length in determining the extent of communication between the iron and manganese centers. The carbonyl bands in complex **1** (Figure 6, panel A), showed no change upon oxidation, confirming that the oxidation is that of the iron center. Complex **2**, with the less remote reporter group (Figure 6, panel B) shows a small increase of around 5 cm-1 in each carbonyl band when oxidized. Complex **3** (Figure 6, panel C) shows much larger shifts of the bands, ranging from 12-32 cm‑1. Overlaying a series of spectra during the oxidation of **3** (Figure 6, panel D), shows that the oxidized and reduced species share isosbestic points at 2081, 1994, 1968 and 1949 cm-1, suggesting that no other metal carbonyl-containing species are present during this conversion. This remains the case for the reduction back to the neutral species, the spectra for which have been omitted for clarity.

The observed increase in stretching frequency is consistent with the removal of electron density from the manganese, resulting in weaker π-backbonding to the carbonyl groups. This is consistent with a through-bond communication between the two metal centers and suggests that redox-active ligands should be incorporated into the first coordination sphere of the manganese carbonyl. Consistent with the reversibility of the single redox process in the voltammetric results, the spectra show that the oxidized ferricenium cation of each complex can be reduced back to the neutral form. There is no electrochemically triggered CO-release upon oxidation of the iron center. Despite the lack of redox-triggered CO release, these results demonstrate the communication between the iron redox state and the bonding properties of the metal carbonyls. The infrared stretches of the reduced and oxidized forms of the three complexes are tabulated below (Table 2).

As related Mn(I) tetracarbonyl complexes bearing 2-phenylpyridine-derived ligands have previously been shown to release CO photochemically,25 the light-triggered CO release properties of ferrocenyl complexes **1-3** were assessed.

The results in Figure 7 show that **1** is stable in DMSO solution in ambient light for > 1 hour, but degradation occurs with a half-life of 21 minutes upon irradiation, as shown by the bleaching of the peaks at 2076, 1990, and 1975 cm-1. The peak at 1931 cm-1 changes only slightly during the irradiation, suggesting that another peak grows in near this wavenumber from the photoproduct. New peaks rapidly grow in at 2012 and 1904 cm-1, indicating that a metal-carbonyl containing photoproduct accumulates. The three bands are consistent with the formation of a *pseudo*‑*C3v* symmetric tricarbonyl species in which a DMSO solvent molecule has substituted one of the *trans* carbonyl ligands. The sulfur-oxygen stretching mode for a bound DMSO was not observed at the concentrations studied. This intermediate then degrades over a longer timescale as another peak grows in at 1862 cm-1. A very similar result was obtained for the degradation of complex **2**, suggesting that the triazole moiety of **1** does not coordinate to the Mn as part of the degradation process.

Table 2: Infrared stretching frequencies (recorded in a dichloromethane solution) for the neutral and oxidized forms of complexes 1-3.

|  |  |
| --- | --- |
| Compound | ν(CO) (cm-1) |
| **1** | 2076 (s), 1991 (vs), 1977 (vs), 1931 (s) |
| [**1**]+ | 2076 (s), 1991 (vs), 1977 (vs), 1931 (s) |
| **2** | 2074 (s), 1990 (vs), 1975 (vs), 1931 (s) |
| [**2**]+ | 2078 (s), 1995 (vs), 1980 (vs), 1936 (s) |
| **3** | 2077 (s), 1988 (vs), 1976 (vs), 1932 (s) |
| [**3**]+ | 2089 (s), 2010 (vs), 2000 (vs), 1964 (s) |



Figure 6: Infrared spectroelectrochemistry of complexes 1-3 (panels A-C respectively). Experiments were performed in dichloromethane at 298 K, at a concentration of 5.0 mM in the presence of 1.0 M tetra-*n*-butylammonium hexafluorophosphate as the electrolyte. Spectra were recorded 240 s after the potential was applied to allow complete electrolysis of the solution to occur. All spectra are subject to a single-point baseline correction at 2050 cm-1. Panel D: Selected spectra from the oxidation step of panel C showing the isosbestic points.



Figure 7: *In situ* infrared spectroscopic monitoring of compounds 1 and 2 in DMSO. The start of continuous irradiation with a 365 nm LED drawing a power of 5.0 W is indicated on the graphs. Panel A: Selected spectra of a 12 mM solution of compound 2 after irradiation began. Panel B: Peak height, fitted to a two-point baseline, of selected peaks over time of compound 2. Panel C: Selected spectra of a 20 mM solution of compound 1 after irradiation began. Panel B: Peak height, fitted to a two-point baseline, of selected peaks over time of compound 1.

Direct observation of CO release from **2** was carried out in water using the well-established UV-visible myoglobin CO release assay (Figure 8). Complex **2** is stable in water in the absence of light, with CO release photochemically triggered by 400 nm LED irradiation. An assay using 40 μM **2** and *ca.* 45 μM deoxymyoglobin approached saturation of the myoglobin after 240 min. The slow timescale of CO release may be due to the poor water solubility of **2**. This must be accounted for when comparing the CO release kinetics of this complex with analogous CORMs. The relatively poor water solubility of the ferrocenyl group could be overcome by further functionalization of the 2-phenylpyridine co-ligand or oxidation to the ferricenium salt with a water-soluble anion. However, the concentrations of CORM used in the myoglobin assay (tens of micromolar) are still very high for practical therapeutic use and such a high concentration of CORM in solution is unlikely to be required.



Figure 8: Myoglobin CO release assays. Irradiation from a 400 nm LED drawing 5.0 W power began where indicated. Irradiation was performed in cycles of 2 min on, 3 min off. Left: A 40 μM assay of 2. Right: A 10 μM assay of 3.

Complex **1**, in which the ferrocenyl group is closest to the manganese, was also assessed using the myoglobin assay (Figure 8). In this case, slow conversion of myoglobin to carboxymyoglobin was observed in the dark. Irradiation of a solution of **1** with a 400 nm LED accelerated CO release, but the complex also displays significant thermal release.

It was hypothesized that the thermal CO release behavior of **1** was due to the susceptibility of the Mn-Cp bond to protonation in protic solvents. To test this, a solution of **1** was prepared in dichloromethane and aliquots of the non-coordinating proton source [HNMe2Ph]BF4 were added via syringe. The resulting decomposition of **1** was monitored using *in situ* infrared spectroscopy (Figure 9). The electrochemical and infrared spectroelectrochemical results have demonstrated that **3** does not degrade upon oxidation on the same timescale as it does during addition of [HNMe2Ph]BF4, suggesting that protonation rather than oxidation by H+ is responsible for the decomposition observed. The results are consistent with NMR spectroscopic studies that clearly show formation of protonated ligand **9**. This result demonstrates that **1** is sensitive to protons and its CO release rate could therefore be modulated based on pH.

Figure 9: The effect of successive additions of the proton source [HNMe2Ph]BF4 to a 20 mM solution of 3 in dichloromethane solution. Left: Changes in the infrared bands with the total [HNMe2Ph]BF4 stoichiometry. Right: Evolution of selected bands over time.

# Conclusions

Redox-tagged ferrocenyl-CORMs have been synthesized and fully characterized. The ferrocenyl moiety is a validated isostere of the phenyl group in drug discovery and for the first time this has been incorporated into the structure of a CORM class. Compounds **1** and **2** display photochemical degradation at the same wavelengths as analogous Mn(I) tetracarbonyl complexes,26 whilst compound **3** additionally releases CO in water in the dark; a process which appears to be triggered by protonation.

The versatility of alkynyl building-block **6** has been showcased in a CuAAC functionalization reaction, enabling a series of compounds to be made analogously to other modular CORM classes.

Each redox-tagged CORM exhibited a single-electron reversible redox couple due to oxidation of the ferrocenyl moiety, whilst the Mn(I) tetracarbonyl moiety was redox silent within the electrochemical window assessed (up to a potential of + 590 mV vs Fc/Fc+). However, previous studies on Mn(I) tricarbonyl systems have shown that an irreversible oxidation of the Mn(I) center does occur at very strongly oxidizing potentials.44,45

Infrared spectroelectrochemistry revealed the effect of ferrocene oxidation upon the Mn center as being dependent on the linker length between the two metal centers. Comparison of CORMs **1-3** showed that a substantial shift in the metal carbonyl stretching frequencies took place only when the ferrocenyl moiety was in the first coordination sphere of the Mn(I) center. Although the perturbation to the structure of the complexes on oxidation was not sufficient to promote CO-release, these observations provide motivation for further incorporation of redox-active groups into the first coordination sphere of CORMs with the ultimate goals of triggering and monitoring CO-release electrochemically in a tunable and predictable manner.

ASSOCIATED CONTENT

Supporting information for this paper includes general synthetic procedures to known compounds, representative NMR spectra, UV-visible spectra, electrochemical results and summary tables of X-ray data. This material is available free of charge via the Internet at http://pubs.acs.org.

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ABBREVIATIONS

CORM, carbon monoxide-releasing molecule; DSC, differential scanning calorimetry; HO, heme oxygenase; Mb, myoglobin; Mb-CO, carbonmonoxymyoglobin; OCP, open-circuit potential

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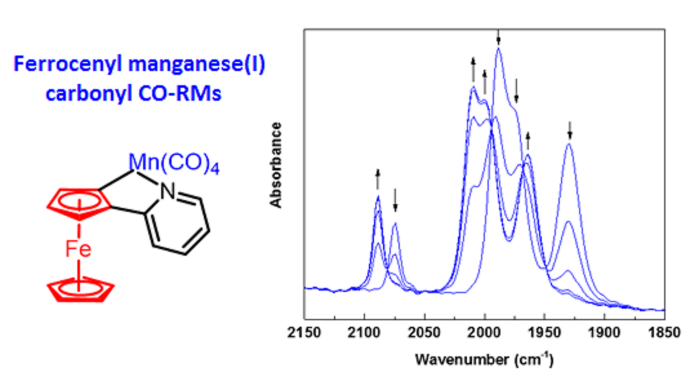
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**TOC**



[Mn(C^N)(CO)4] complexes, with a therapeutically-accepted ferrocene moiety, exhibit CO-release when exposed to photochemical irradiation (or thermal stimulus for a proximal ferrocenyl-Mn system).