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COMMUNICATION

A therapeutically viable photo-activated manganese-based CO-releasing molecule (photo-CO-RM)†

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A new class of photochemically-activated CO-releasing molecule (photo-CO-RM), based on a $Mn(CO)_4(C^N)$ system, is reported in this study. Three CO molecules are released per CO-RM molecule. Complex 3 is a fast releaser, thermally stable in the dark and a viable therapeutic agent.

Carbon monoxide (CO) is a well-established biological effector molecule within the human body, *e.g.* CO binds and affects the activity of proteins and receptors.[1] Using it in a therapeutic sense requires the safe and controlled delivery of CO *via* a suitable molecular vehicle. Therapeutic metal-carbonyl complexes have thus been designed as CO-releasing molecules (CO-RMs), receiving significant attention since about 2003.[2,3] There are three classes of CO-RMs – those which: (i) thermally liberate CO (thermo-CO-RMs); (ii) liberate CO by a trigger, that is by hydrolytic (solvent) processes or other species, *e.g.* proteins *inter alia* (triggered-CO-RMs);[4] (iii) release CO from dark-and-thermally-stable metal-carbonyl complex prodrugs under photochemical conditions (photo-CO-RMs).[5,6] Each class has their advantages and disadvantages. Our research group has spent considerable effort in developing several CO-RM classes that release CO under physiological conditions, for which their cell viability has been demonstrated.[4c–e,4g,5i,10]

In 2007/8 we initiated studies on a $Mn(CO)_4(C^N)$ system (C^N = *ortho*-metallated 2-phenylpyridine or benzoquinoline),[7] which was found to be photochemically active in the presence of a 2-electron donor ligand (*e.g.* DMSO and PPh_3). Worthy of note is Mann's CO-RM, namely $Mn(CO)_4(S_2CNMeCH_2CO_2H)$,[8] which releases CO on exposure to myoglobin (Mb) under thermal conditions. By contrast, the related $Mn(CO)_4(C^N)$ systems are thermally stable both in the dark and presence of myoglobin/other strong 2-electron donors. In this paper we detail the synthesis of water compatible complexes **2** and **3** (Scheme 1), their CO-releasing ability and potential as therapeutic photo-CO-RMs. Crucially we have determined the cell viability of the photo-CO-RMs prior to and following photoirradiation.

Complex **2** was prepared as shown in Scheme 1 in 81% yield (see ESI† for synthetic and characterisation details).[7b] Complex **2** crystallises from CH_2Cl_2/pentane (vapour diffusion), allowing its structure to be determined by single crystal X-ray diffraction (Fig. 1).[9] Complex **2** was hydrolysed under mild conditions to afford water compatible complex **3** in 84% yield.

CO-release from CO-RMs can be measured by a UV-vis spectrometric deoxy-Mb → Mb-CO assay,[10] with irradiation of

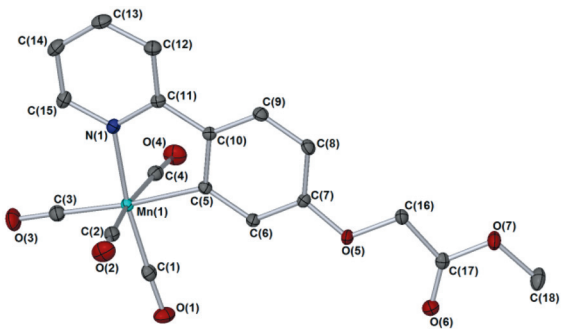
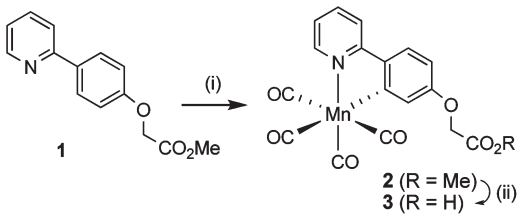


Fig. 1 X-ray structure of complex **2**. Thermal ellipsoids set at 50% and H-atoms omitted for clarity. Selected bond distances (Å): C1–Mn1 1.8148(14), C2–Mn1 1.8660(14), C3–Mn1 1.8263(14), C4–Mn1 1.8527(14), C5–Mn1 2.0528(13), C1–O1 1.1443(18), C2–O2 1.1330(17), C3–O3 1.1500(17), C4–O4 1.1381(17), Mn1–N1 2.0672(11). Selected bond angles (°): C1–Mn1–N1 172.00(5), C3–Mn1–N1 95.30(5), C5–Mn1–N1 80.08(5). Selected torsion angles (°): Mn1–C5–C10–C11 3.62(14), C5–C10–C11–N1 −2.70(16), C10–C11–N1–Mn1 0.43(14).



Scheme 1 Photo-CO-RMs developed in this study; (i) $Mn(CO)_5(Bn)$, toluene, 75 °C, (ii) Et_3N (3 eqv.), LiBr (10 eqv.), CH_3CN/H_2O (98 : 2, v/v), 2 h, 25 °C.

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†Electronic supplementary information (ESI) available: Details of all experimental procedures, characterisation data, kinetic experiments and biological assays. CCDC reference number 887264. For ESI and crystallographic data in CIF or other electronic format see DOI: 10.1039/c2dt31588b

Table 1 CO-release ability of complexes **2** and **3** under photoirradiation

Complex	Irradiation source	
	hv (365 nm), $t_{1/2}$ (s); conc. (μM)[a]	*hv* (400 nm), $t_{1/2}$ (s); conc. (μM)[b]
2	1740; 40	—
		—
3	960; 40	300; 10
		360; 40

[a] UV Lamp (6 W; the effective power is significantly less). [b] LED (2.4 W).

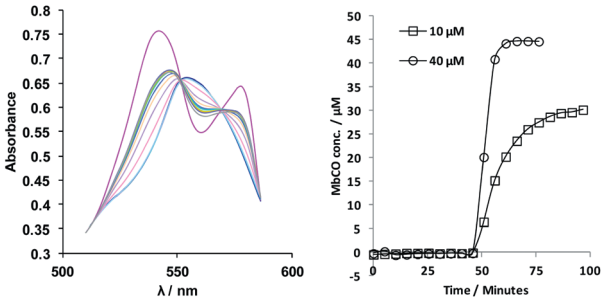

Fig. 2 Deoxy-Mb → Mb-CO conversion monitored (Q-bands) by UV-vis spectroscopy over time – photochemical induced release of CO using an LED (at 400 nm, 2.4 W) from complex **3** (c = 10 and 40 μM). Photochemical activation was at t = 45 min, the LED cycle was on for 2 min and off for 3 min.

the photo-CO-RMs using a UV-lamp (365 nm). The $t_{1/2}$ values for the release of CO from **2** and **3** are listed in Table 1. An alternative experimental setup was also developed in this study allowing UV cuvettes to be directly irradiated with an LED system (at 400 nm; see ESI† for details). The advantage of this setup is that it is more controlled and convenient to use than a UV-lamp, typically used in TLC analyses (*e.g.* power and time settings can be controlled, and the system is compatible with 96 well plates and Falcon tubes).

The Deoxy-Mb → Mb-CO conversion cleanly occurs on irradiation of **3** (Fig. 2).[11]

The CO-release profiles at **3** concentrations of 10 and 40 μM indicate that approximately three CO ligands are released from **3**. This demonstrates that the degraded species could contain one remaining CO ligand *vide infra*. Irradiation of **3** in a stepwise manner revealed that CO release could be switched on and off. Again, three CO ligands are released per molecule of **3**. In the absence of protein, irradiation of **3** in CH_3CN led to decomposition, showing that CO release is not protein assisted (Fig. 3). A similar trend is seen in H_2O/DMSO (see ESI†).

Investigation of the species produced by photoirradiation of **3** in H_2O/CH_3CN[12] by ESI-MS[13] showed that the starting complex was rapidly converted to **I** and one equivalent of CO (not quantified) within the first few seconds of irradiation (Fig. 4). The disappearance of **I** is noted after 2 min total irradiation time. The formation of **II** (and **4**) is noted as an intermediate, which accumulates and then degrades to give uncharacterised Mn species and CO. A peak at *m/z* 274 accumulates over time,

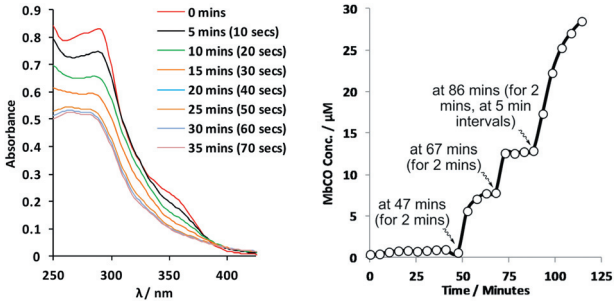

Fig. 3 Left: Photochemical-induced release of CO from complex **3** (c = 4.81 × 10^{-5} mol dm^{-3}) in CH_3CN using an LED (at 400 nm, 2.4 W) system monitored by UV-vis spectroscopy over time (total irradiation time in brackets). Right: Deoxy-Mb → Mb-CO conversion monitored (Q-bands) by UV-vis spectroscopy over time, with stepwise induced photochemical irradiation of complex **3** (c = 10 μM) using a LED (400 nm, 2.4 W).

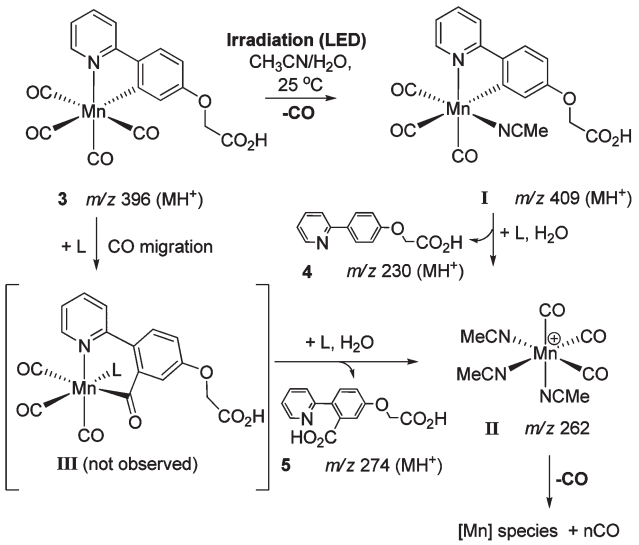

Fig. 4 Decomposition of **3** in H_2O/CH_3CN, followed by ESI-MS analysis (see ESI† for mass spectra over time).

and is most likely diacid **5**, formed by a CO migration/insertion process from **3** *via* proposed intermediate **III**. Hydrolysis of **III** would be expected to afford **II** and **5**. Interestingly, the CO insertion to give **III** would explain in part the measurement of three liberated molecules of CO. It is interesting to note that **II** is isostructural with the known $Mn(CO)_3$(scorpionate) photo-CO-RMs.[5b] Recent studies by Continuous Wave EPR spectroscopy have demonstrated that such complexes decompose to give paramagnetic Mn^{II} species.[14] The appearance of a yellow colour, which occurs on exposure of **3** to light indicates that paramagnetic species are formed (*cf.* signal broadening is observed by ^{1}H NMR spectroscopic analysis).

Having comprehensively established the CO-release behaviour of **3**, its cell viability against RAW 264.7 murine macrophages (Alamar Blue assay) and its effect on cell lysis (lactate dehydrogenase (LDH) activity) was determined (Fig. 5). The first assay measures the ability of living cells to reduce Resazurin, whereas the latter provides quantification of cell lysis based on the

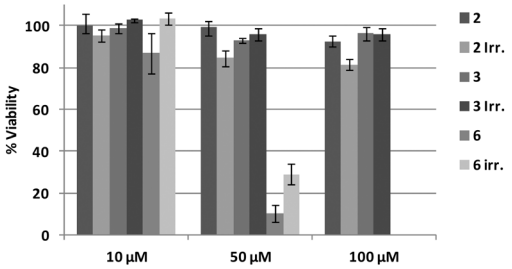

Fig. 5 Cell viability of RAW 264.7 murine macrophages, as measured by Alamar blue assay, in the presence of photo-CO-RMs **2**, **3** and **6**, prior to and following photoirradiation at 400 nm (LED) (8 min, 2.4 W in DMSO before addition to cells). Experiments were run in triplicate against a positive control (100% cell viability) and 1% Triton control (0% cell viability). Error bars are included (average standard deviation values).

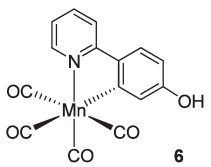

Fig. 6 Complex **6**, a potential degradation product of **2–b**.

measurement of LDH activity released from the cytosol of dead cells. Complex **2** and related hydroxy-derivative **6** are included for comparison (see ESI† for the synthesis and characterisation of **6**). Complex **6** was considered a potential biodegradation product from either **2** or **3**, which could form on cleavage of the ether bond in biological systems (Fig. 6).

In keeping with previous studies, **2**, **3** and **6** were tested over three concentrations (10, 50 and 100 µM).[4] We deemed it appropriate to test cell viability prior to and following photoirradiation, which allows the toxicity of the degradation species to also be determined.

Complex **3** exhibits excellent viability at all the concentrations tested, no difference was noted with photoirradiation, showing that the degradation species (formed within 8 min irradiation) resulting from **3** are viable in this assay. Complex **2** is viable at 10 µM; however following photoirradiation at higher concentrations we note that cell viability is reduced (to *ca.* 80%). Finally, complex **6** is more toxic at 50 µM < 10% of the cells are viable. Interestingly, at both 10 and 50 µM the cells are more viable following photoirradiation of **6**.

The results from the LDH assay show that **2** and **3** do not induce release of LDH at 10, 50 and 100 µM concentrations. Complex **6** was found to induce the release of LDH at 50 µM (*ca.* 63% compared to the Triton control). The data is consistent with the cell viability assay results. There is no evidence for formation of **6** from either **2** or **3**, in the assays tested or ESI-MS study.

In conclusion‡ we have identified two biologically compatible photo-CO-RMs **2** and **3**. Their CO-release profiles compare well against other reported photo-CO-RMs. Studies by ESI-MS indicate that an intermediate, $Mn(CO)_3(NCCH_3)_3^+$, is formed on photoirradiation of **3**, which is an isostructural variant of scorpionate-derived,§ and related photo-CO-RMs.[5b,f,6] The cell viability and LDH activity measurements on photo-CO-RM **3** were excellent in the assays used. The finding that the photo-irradiated species derived from **6** were less toxic than non-activated **6** was quite remarkable, and moreover, surprising. Further studies employing the LED photoirradiation equipment are on-going, as are time-resolved infrared spectroscopic experiments on the manganese(I) carbonyl complexes reported in this paper.

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Notes and references

‡ Findings detailed in this paper were presented by I.J.S.F. at the 6th International Symposium on Bioorganometallic Chemistry (ISBOMC), held in Toronto, Canada, July 8th–12th 2012.
§ Whilst it is unlikely that the chelating-scorpionate ligand is displaced by other 2-electron donor ligands, to generate similar Mn^I species, we cannot rule it out.

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9 Crystal Data for **2**. $C_{18}H_{12}NO_7Mn$, M = 409.23, triclinic, a = 7.0000(4) Å, b = 11.2458(6) Å, c = 12.1356(8) Å, α = 67.181(6)°, β = 83.042(5)°, γ = 86.381(4)°, V = 873.93(9) Å^3, T = 110.00(10), space group $P\bar{1}$ (no. 2), Z = 2, μ(Mo Kα) = 0.797, 9348 reflections measured, 5500 unique (R_{int} = 0.0247) which were used in all calculations. The final wR_2 was 0.0831 (all data) and R_1 was 0.0322 (>2sigma(I)).

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11 The photo-CO-RMs do not release CO in the presence of myoglobin and sodium dithionite until photoirradiation is initiated. A recent paper indicates that sodium dithionite can initiate CO-release from some CO-RMs, see: S. McLean, B. E. Mann and R. K. Poole, *Anal. Biochem.*, 2012, **427**, 36.

12 It was necessary to substitute DMSO with CH_3CN (*i.e.* a similar 2-electron donor ligand), as the former was found to be incompatible with ESI-MS analysis with $(DMSO)_2H^+$ saturating the detector).

13 It was necessary to reduce the skimmer voltage which diminishes M-CO fragmentation (see ESI† for mass spectra and optimisation). In all the MS experiments conducted the ion peak at *m*/*z* 230 appears, which could be formed by an ionisation process, in addition to degradation induced by photoirradiation. The ion peaks at *m*/*z* 274 and *m*/*z* 409 are real intermediate species (in solution).

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