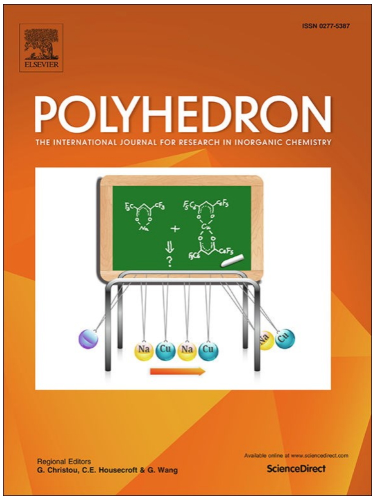

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Twelve CO molecules for the price of one. A simple water-soluble organometallic CORM, $[Mn(CO)_3(\mu_3\text{-}OH)]_4$

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ABSTRACT

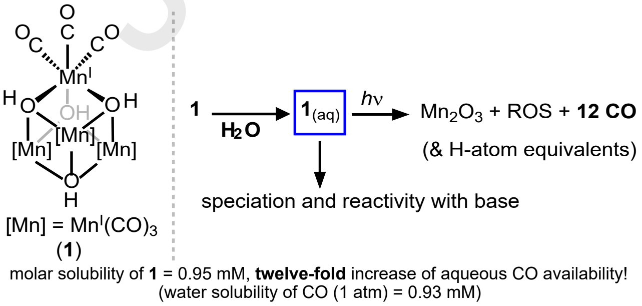
The tetramer $[Mn(CO)_3(\mu_3\text{-}OH)]_4$ (**1**) forms hydrogen bonded adducts with a variety of molecules. Most commonly, these are **1**···aromatic hydrocarbon adducts obtained from synthesis and purification, and these adducts are insoluble in water. We hypothesized that the adduct-free tetramer is water-soluble and tested this by developing a gram-scale synthesis of the adduct-free tetramer (**1**) and measuring its molar solubility, which we determined to be 0.95 ± 0.06 mM at 20 °C. This success allowed us to study the characterization and aqueous properties of **1** for the first time. For example, ambient light exposure causes loss of all twelve CO ligands, accompanied by formation of reactive oxygen species (ROS) and manganese oxides. Since CO solubility in water is 0.93 mM at 20 °C and 1 atm, a solution of **1** effectively increases the aqueous availability of CO twelve-fold from atmospheric pressure CO. This prompted our exploration of **1** as a water-soluble CO releasing molecule (CORM) and its ability to elicit cell antiproliferation was tested with four cell lines. Indeed, **1** induces dose-dependent cell antiprolifer-ation properties, albeit the cytotoxicity of CO cannot be decoupled from manganese oxide or ROS toxicity and the role of light was not explicitly controlled for. This point is magnified because of recent revelations that biological studies of CORMs often neglect these other factors. Nevertheless, **1** is an excellent CORM in water and does not carry ancillary ligands that may interfere with applications that use the released CO molecules.

Keywords: manganese carbonyl, CO photolysis, water soluble CORM

1. Introduction

The molecular tetramer $[Mn(CO)_3(\mu_3\text{-}OH)]_4$ (**1**) has distinctive photochemical properties. Specifically, photolysis of a CO ligand induces O–H homolysis that manifests as either H_2 evolution or chemically trapped H-atom equivalents.[1,2] The O–H homolysis results in oxidation of the Mn and initiates catastrophic cluster decomposition with release of all twelve CO ligands and formation of manganese oxides and ROS (ROS = Reactive Oxygen Species, derived from the H_2O_2 formed as a product of photolysis).[1-3] Granted, previous studies were performed in organic solvent or toluene/water emulsions. In the latter, **1** is dissolved in the organic phase, and it has been noted in the literature that **1** is insoluble in water.[1,2,4,5] We suspect that the water-insolubility is due to the fact that **1** forms hydrogen bonded (H-bonded) adducts with water-insoluble aromatic hydrocarbons and is not an intrinsic property of **1**. Thus, we hypothesized that the adduct-free **1** is soluble in water. This hypothesis was additionally rationalized on the basis that when a chloroform solution of **1** is exposed to air, **1** crystalizes as the water adduct **1**•$4H_2O$.[6] Although isolation of the adduct free tetramer has been accomplished,[7] its solubility in water is unknown and its aqueous chemical and physical properties have not been studied. Moreover, in our hands, the literature procedures are irreproducible or low yielding.

Scheme 1. Summary of this work



Therefore, this study begins with development of a new, highly reproducible, and simple gram-scale procedure for adduct-free **1**. With the adduct-free tetramer in hand, we determined the molar solubility of **1** in water and then used this opportunity to explore its aqueous chemistry for the first time (Scheme 1). This included a cell antiproliferation study since manganese-based CO releasing molecules (CORMs) have been used within this context.[8] However, the mass balance resulting from CO photolysis in water includes more than just CO. Accompanying CO photolysis are manganese oxides and reactive oxygen species (ROS) meaning that cytotoxicity from CO cannot be decoupled from other effects. Failure to recognize this fact has recently been highlighted,[9] and our findings provide the empirical basis for these other factors (oxides and ROS) and demonstrated through phenomenological cell antiproliferation. Nevertheless, $\mathbf{1}_{(aq)}$ effectively increases the availability of CO twelve-fold compared to atmospheric pressure of CO, which may have applications where CO is needed in aqueous environments.

2. Synthesis of adduct-free $[Mn(CO)_3(\mu_3\text{-}OH)]_4$ (1)

The first preparation of **1** was performed by Hieber and Stanner in 1969,[10] but they assigned the $Mn(CO)_3(OH)$ unit as part of a monomeric molecule rather than being part of a larger tetramer. Later, Herberhold and Wehrmann correctly deduced the tetrameric structure.[11] These early preparations involved treatment of $[Mn(CO)_3(OH_2)_3]^+$ (**2**) with base. Later, **1** was rediscovered by Zaworotko using a more convenient synthetic procedure that involves treatment of $Mn_2(CO)_{10}$ with excess $Me_3NO{\bullet}2H_2O$ in THF followed by work up with benzene or toluene to afford $\mathbf{1}{\bullet}(\text{arene})_2$.[12] We studied this reaction in detail under rigorously dry conditions to discover that **1** does not form directly from $Mn_2(CO)_{10}$ and Me_3NO.[13] In fact, both water and Me_3NO serve as the source of O-atoms for the hydroxide ligands in **1**, as deduced by careful isotopic labeling and mass balance from reactions of a tetranuclear intermediate with rare Me_3NO and carbonate coordination.

As noted, the tetramer forms adducts with H-bonded acceptors, $\mathbf{1}{\bullet}\boldsymbol{a}_{\mathbf{n}}$ (***a*** = H-bond acceptor; *n* = 1-4) (Figure 1).[2,6,7,12,14] Notably, the O···X (X = acceptor) distances are consistent with strong to moderate H-bonding interactions, although our solution-based studies noted below indicate that the H-bonded adduct is broken up by the solvent. For

Table 1. FTIR and ^1H NMR spectroscopic features of 1

[redacted]	[redacted]	[redacted]	[redacted]
crude **1**	-	3563	2, [c]
$\mathbf{1}{\bullet}(H_2O)_4$	not determined	3613(s) & 3400(b)[d]	7
$\mathbf{1}{\bullet}(C_6H_6)_2$	1.1 (CD_3CN)	3570	13
$\mathbf{1}{\bullet}(C_7H_8)_2$	1.1 (CD_3CN)	3563(s)	13, [c]
	-0.8 ($CDCl_3$)		
	-3.0 (toluene-d_8)		2
1	1.1 (CD_3CN)	3633(s) & 3617(s)	[c]
	-0.8 ($CDCl_3$)		
	-3.0 (toluene-d_8)		

Table notes [a] ^1H NMR shifts are reported to the nearest tenth (0.1 ppm) as they drift slightly depending on variable trace water content. [b] ATR-FTIR unless otherwise noted. [c] this work. [d] method of IR not described.

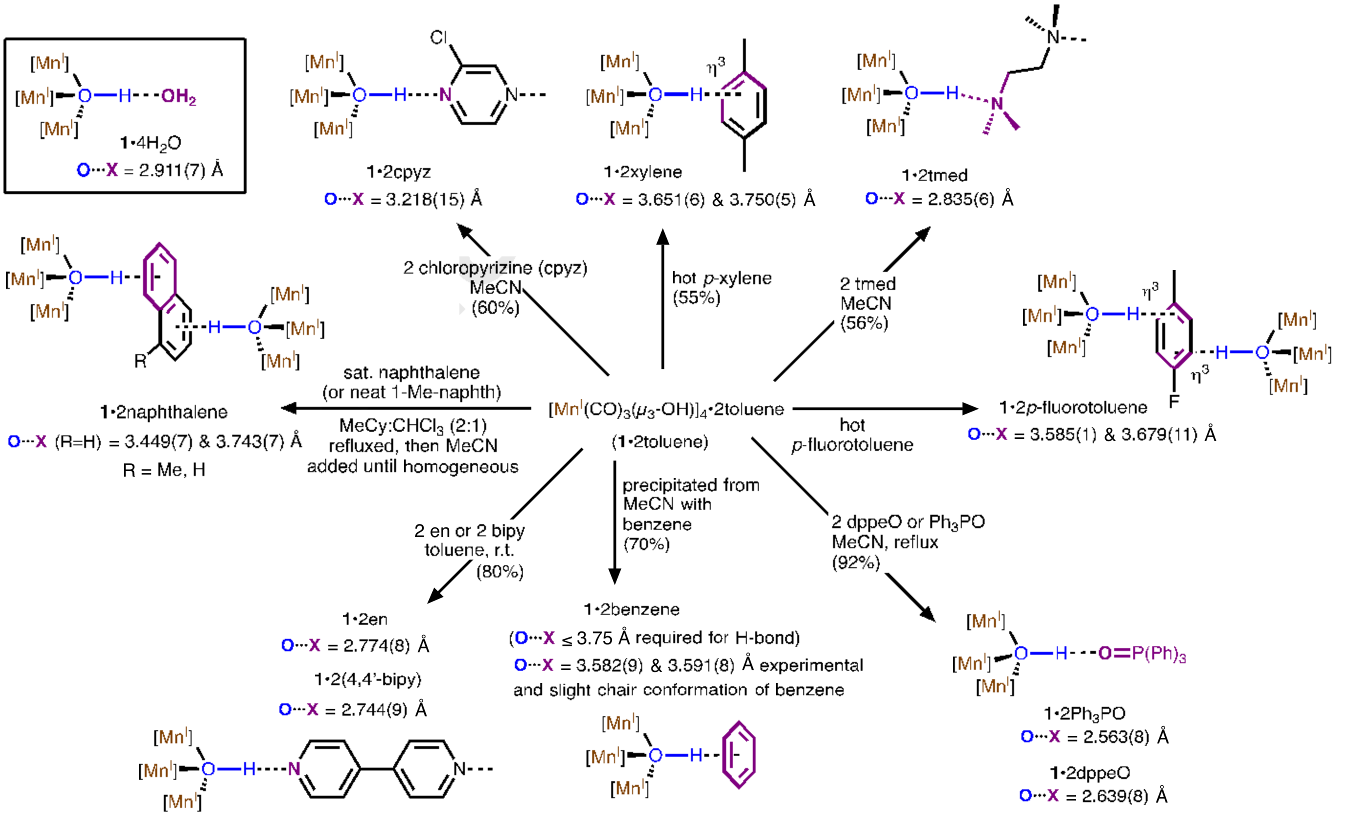
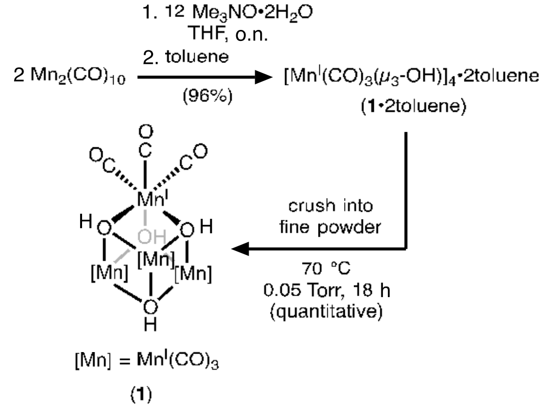


Figure 1. Summary of H-bonded adducts with **1** in the literature (see text for citations). The O···X distance is the distance from the O-atom of the μ_3-OH ligand to the H-bond acceptor group (shown in purple) as determined from X-ray crystallog-raphy.

Scheme 2. General procedure to prepare 1.



instance, a solution of **1** in MeCN-*d*$_3$ prepared from **1**•2toluene, **1**•$(C_6H_6)_2$, or adduct free **1** have identical δ_{OH} shifts (Table 1). Similarly, in $CDCl_3$, the NMR spectra are identical except for the free residual toluene or benzene solvent. However, the H-bonding persists in the solid state as evidenced from the variable ν_{OH} depending on the H-bond acceptor (Table 1). There is no strong correlation between O···X and observed ν_{OH} ($R^2 \leq 0.5$), which is to be expected considering the variability in acceptor type on display in Figure 1 (arene vs. amine vs. phosphine oxide, etc.), H-bonding angles, and other factors.

While procedures for preparing adduct-free **1** or **1**•(arene)$_2$ are available in the literature, we have found that synthesis of and handling bulk **1** (and its adducts) is a nuanced art, fraught with challenges that we suspect stem from the light sensitivity of the tetramer and its initially perplexing solubility properties. Obtaining a crystal of a H-bonded adduct for **1** is one thing – synthesizing bulk quantities for chemical and physical property studies is another. We emphasize here that we are the only group to have successfully reported on the chemical properties of **1**, whereas prior studies involving **1** have exclusively been the analysis of XRD data. We have found the literature procedures for preparing adducts of **1**, including those from our own group, are difficult to reproduce with acceptable yields. This is not a reflection on previous researchers but is simply a statement of mere fact. For this reason, we have carefully carried out a new synthetic study.

For this work, we have provided a procedure that is reproducible by several researchers including undergraduate students to obtain gram-quantities of analytically pure **1** (Scheme 2). In our experience, we have found it best to isolate **1**•(toluene)$_2$ or **1**•$(C_6H_6)_2$ as a way of preparing clean tetramer since the crude material is a mixture of adducts, including THF and Me_3N. Purified **1**•$(C_7H_8)_2$ can be liberated from toluene by pulverizing it into a fine powder and heating to 70 °C under vacuum (≤ 50 mTorr); higher temperatures induce decomposition. Although **1** is stable open to air, light must be rigorously excluded to avoid decomposition. The NMR and FTIR spectroscopic data for **1** and important adducts are in Table 1 (Figures S1-S9); the spectroscopic information for **1** is reported for the first time here.

3. Aqueous chemistry of [Mn(CO)$_3$(μ$_3$-OH)]$_4$ (1)

As noted, the literature states that **1** is insoluble in water. However, we have found that adduct free **1** is in fact water-soluble, albeit sparingly, with a solubility of 0.59 ± 0.04 mg·mL^{-1} (molar solubility = 0.95 ± 0.06 mM). A Beer's law plot furnished an extinction coefficient of 4100 M^{-1} cm^{-1} for λ_{max} = 384 nm (slope = 4056.7 ± 159.8 M^{-1} cm^{-1}, Figure S16). The UV-vis spectrum of **1** in water is essentially identical in shape to **1**•$(C_6H_6)_2$ dissolved in MeCN, THF, DMSO, and toluene indicating that the electronic structure of the Mn(I) ions is the same, and by extension the tetramer's overall structure is unaltered in water. The UV-vis spectrum for $[Mn(CO)_3(OH_2)_3]^+$ (**2**) in water is known and has a similar feature at 385 nm,[15] but a mass spectrum of **1** in water does not contain **2** and instead has an m/z = 622.6959 associated with mono-deprotonated **1** (Figure S10). UV-vis spectra of **1** in water remained virtually unchanged for over two years when stored in the dark.

The solubility of **1** in water can be enhanced by addition of salts. For instance, the solubility of **1** follows a logarithmic dependence on $[NaCl]_{aq}$ and buffers (phosphate buffer and borate buffer, Table S1), asymptotically approaching a maximum solubility for **1** of 1.70 mg·mL^{-1} in $[NaCl]_{aq}$, 1.29 mg·mL^{-1} in phosphate buffer and 1.14 mg·mL^{-1} in borate buffer (Figure S17). The logarithmic relationship can be interpretated as evidence for ionization of **1** according to the Debye-Hückel equation. Elucidation of this ionization was explored next.

Attempts to measure the pK_a were not possible since we were unable to isolate/verify the solution state deprotonated anion or the protonated cation of **1**. Saturated solutions made from **1** in deionized and unbuffered water raised the pH from 7.00 to 7.62 ± 0.05. Interpretation of this pH increase in water seems to implicate that **1** is a base, perhaps indicating protonation of the cluster (i.e. forming [**1**+H]OH) or dissociation of OH (i.e. forming $[Mn_4(\mu\text{-}OH)(CO)_{12}]^+$). However, the only species observed in water using mass spectroscopy is [**1**-H]$^-$ (Figure S10); positive mode did not contain [**1**+H]$^+$ or the known compound **2**. Furthermore, we noted in an ear-lier publication that deprotonation required strong bases (e.g., $KN(TMS)_2$) and the product was not stable (suggesting to us that [**1**-H]$^-$ forms as a result of ionization during MS analysis, not as the major species present in water).[2] Collectively, these data are consistent with the intact cuboidal structure of **1** in water, which is the same premise deduced from the UV-vis spectra discussed earlier.

In the absence of quantitative aqueous thermodynamics, we can compare the aqueous chemistry of **1** and **2** and other comparable ions. Deprotonation of **2** by basification of aqueous media is said to induce formation of a trinuclear complex $[Mn_3(CO)_9(OH)_4]^-$ (**3**, Chart 1) and precipitation of the neutral tetramer **1**.[4] We attempted various reactions to isolate **3** by forming it in solution and precipitating with [PPN]Cl or

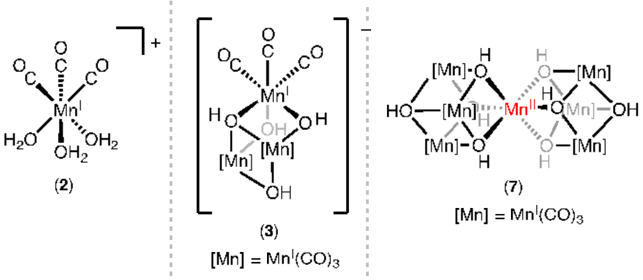
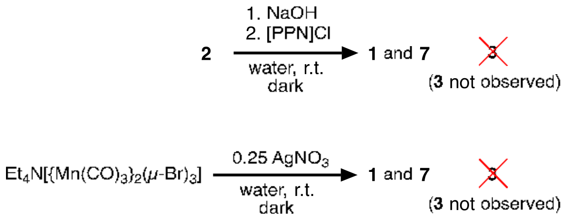


Chart 1. Other water-soluble Mn carbonyl complexes discussed in this report.

other cations, akin to what was attempted with [$AsPh_4$]Cl.[4] Our attempts always resulted in formation of a heptanuclear species (denoted "**7**", Chart 1 and Scheme 3). The complex **7**, first characterized by Zaworotko,[12] is a heptanuclear complex where two tetramers are fused at a central Mn(II) node. Formation of this neutral complex was confirmed with HRMS, FTIR, and crystallized as the salt PPN$_2$[**7**$\cdots$2Cl] (connectivity only, Figure S11).

Scheme 3. Attempted synthesis of 3



Another attempt to prepare **3** followed a procedure that was used to form the known Re analog.[16] For this, we treated $Et_4N[\{Mn(CO)_3\}_2(\mu\text{-}Br)_3]$ with $AgNO_3$, but this likewise resulted in formation of halide adducts of **7** (Scheme 3). Since rigorous care to remove and avoid generation of Mn(II) ions was not made in this study,[5] it may be that the trinuclear species **3** eluded our detection. However, we note that exclusion of Mn(II) ions is nearly impossible for studies with **1** because it readily decomposes via CO photolysis induced H-atom transfer (loss of H_2) in the presence of ambient laboratory lighting, and this process results in formation of Mn(II) ions.[1-3] Albeit the reactions reported here for the preparation of **7** are the first strategic procedures for its formation. Previous iterations used benzophenone as a sacrificial oxidant,[12] but we failed to reproduce these methods to yield **7**.[17]

4. CO photolysis in water

Carbon monoxide is an essential gasotransmitter with applications in therapy and various disease treatments.[18] Although at this stage **1** is not relevant for CO therapeutic applications, we were intrigued by the possibility of demonstrating **1** as a photochemical CO releasing molecule (photoCORM). Our intrigue stems from the fact that **1** has twelve CO ligands on a single molecule, contains only H, C, O, and Mn elements, and has no ancillary ligands. In this regard, **1** has an increased CO payload compared to other Mn-containing photoCORMs. Furthermore, **1** is also water soluble, as we have demonstrated here. Therefore, an increased CO payload *and* water-solubility are unique properties amongst photoCORMs studied so far.

Note that previous photochemical studies with **1** were not conducted in water. Rather, in our previous studies, we showed that **1** decomposes with loss of CO when irradiated in organic solvents or biphasic organic/water mixtures.[1,2] In this study, the photochemical decomposition is repeated in pure water. When **1** is irradiated in water using a 26 W CFL lamp or 100 W Xe-arc lamp with a 420 nm cutoff filter it loses all 12 CO ligands (Figure 2). Under inert atmosphere conditions, gelatinous white Mn(II)(OH)$_2$ forms,[19] which rapidly turns brown when exposed to air. The brown material appears to be a mixture of Mn_2O_3, MnO_2, and $MnCO_3$ from its pXRD pattern (Figure S14) and featureless ATR-FTIR spectrum. H_2 and H_2O_2 are also products of photolysis with the 100 W Xe-arc lamp,[1] but with the compact fluorescence lamp (CFL) these products appear to be near or less than the detection limit of our GC corresponding to <1% yield and iodometric techniques were only just sufficient to detect the fleeting presence of H_2O_2. Note, these low concentrations only indicate that the steady state concentration is low, not that they do not form. Rather, the CO photolysis event was shown to elicit H• chemistry and the observation of H_2 and H_2O_2 in water corroborate these earlier findings.[1-3]

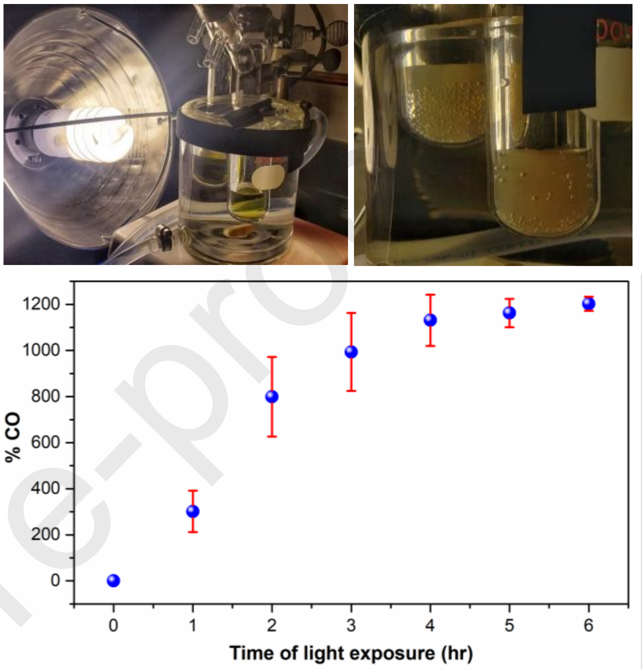


Figure 2. Top: 26 W CFL irradiation of 0.15 mM **1** in water, r.t. with CO bubble formation shown after two hours. Bottom: CFL light induced loss of CO with plot showing percent yield of CO loss relative to **1**.

To determine if **1** is also a thermal CORM, we treated solutions of deoxymyoglobin (deoxy-Mb) with **1** and monitored the speciation with UV-vis spectroscopy. In the dark, no CO was transferred to deoxy-Mb and instead required 420 nm irradiation (Figure S18-S19).

5. Cell antiproliferation study with 1

After having demonstrated the water solubility of **1** and its efficacy toward loss of CO, we sought to determine if **1** could induce cell cytotoxicity *in vitro*. It is critical to note that CO-photolysis does not occur in isolation of free-radical and Mn-oxide formation, all of which exhibit cytotoxicity.[20,21] Thus, it is impossible to distinguish CO toxicity from other effects using only the studies performed here. We state at the onset: the cell antiproliferation studies that follow are phenomenological and the individual chemical properties of **1** are not decoupled from the other. This is important to note because inferences made about the biological effects of CO from CORMs in biological studies have been called into serious question.[9]

We rationalized that cells cultured in suspension were best suited to assess the dose-dependent impact of **1** on cellular proliferation with resazurin. We chose the well-studied HeLa cell line in addition to murine pro-B Ba/F3 cell line expressing diverse oncogenic EGFR mutants: exon 19 deletion E746-A750, the drug resistant triple-point mutation L858R/T790M/C797S, and the extracellular vIII deletion.[22] Cells were dosed with **1** for 5 minutes in the dark before being exposed to laboratory fluorescent lighting for 30 minutes to promote CO gas release. After incubation for 72 hours, we find that **1** induces dose-dependent antiproliferative effects on EGFR expressing Ba/F3 delE746-A750, L858R/T790M/C797S, vIII and HeLa cells showing effective concentration at 50% survival (EC_{50}) values of 37 ± 10 µM, 20 ± 2 µM, 20 ± 5 µM, and 73 ± 10 µM, respectively (Figure 3). These findings confirm that **1** is capable of eliciting antiproliferative effects in cell line models; the multi-day nature of these experiments did not allow for a sufficient non-illuminated (dark) control. Other photoCORMS with the $Mn(CO)_3$ have IC_{50} values ranging from 4 µM to >100 µM,[8] although, as noted it is difficult to deduce if CO or other byproducts of CO photolysis are responsible. For instance, the toxicity of nanoparticulate Mn_2O_3 is not so different from that of **1**.[21] Furthermore, although outside the scope of this proof-of-concept study, precise experiments including the use of variable duration of light exposure period and dark controls are needed to assess the role of light in the cytotoxicity of **1**.

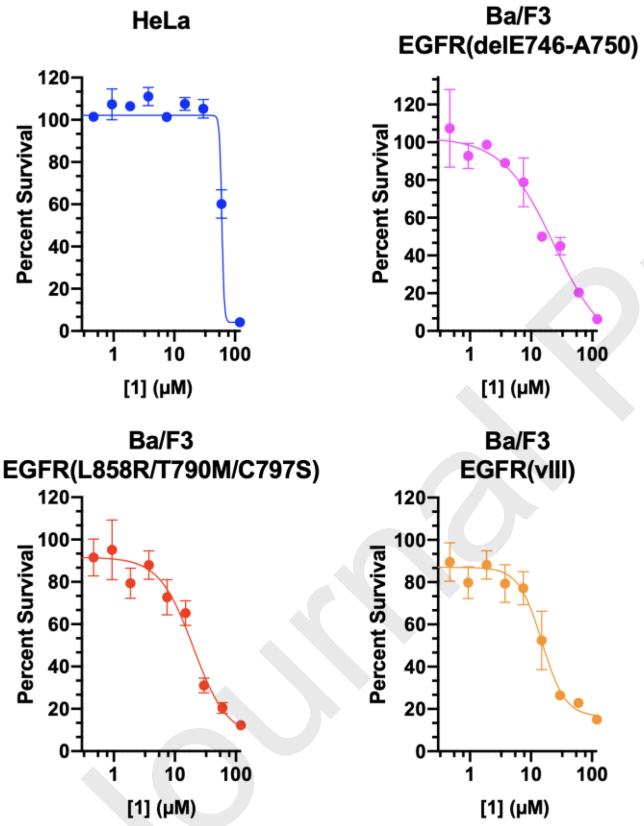


Figure 3. Percent survival of HeLa and EGFR expressing Ba/F3 cells dosed with **1** for 72 hours. Dose-response curves are representative of at least three independent experiments.

6. Conclusion

To conclude, the aqueous chemistry associated with **1** was studied for the first time here. This study required a convenient gram-scale synthesis for the adduct free tetramer, and we have furnished an improved synthesis that undergraduates can successfully carry out. We showed that **1** has a defined water solubility of 0.95 mM. Given that $\mathbf{1}_{(aq)}$ increases the pH of water to 7.62, it is a weak base in water, but UV-vis and mass spectral analysis indicates that **1** remains intact. Attempts to deprotonate **1** led to **7** instead of well-defined $[\mathbf{1}\text{-H}]^-$, and other chemical manipulations tended to give similar results.

The salient features of **1** in water is that it effectively increases the availability of soluble CO twelve-fold compared to $CO_{(g)}$ over water. For instance, the solubility of $CO_{(g)}$ in water at room temperature is 0.93 mM (20 °C),[23] and the solubility of **1** in water is essentially the same (0.95 mM). Hence, a saturated solution of $\mathbf{1}_{(aq)}$ increases the effective CO concentration by 12-fold in pure water, or 35-fold in saline. By effective CO concentration, we mean that all twelve CO ligands in **1** are released by simple exposure to ambient room lighting, and the only byproducts are ROS, $MnCO_3$, Mn_2O_3, and MnO_2. Collectively, the products resulting from photolysis of $\mathbf{1}_{(aq)}$ were shown to elicit cellular antiproliferative effects. Although, the source of the effects were not decoupled and so it could be any one or combination of the products and the role of light was not controlled for.

7. Experimental

Synthesis of $[Mn(CO)_3(OH)]_4\bullet(C_7H_8)_2$ (**1**•$(C_7H_8)_2$)*:*

All glassware used in the synthesis were covered with aluminum foil to minimize light exposure. Manipulations were conducted in a dark room and a red headlamp or flashlight was used for lighting. Open to air, a round bottom flask was charged with 1.02 g of $Mn_2(CO)_{10}$ (2.62 mmol), and 20 mL of tetrahydrofuran (THF) was added to dissolve all the material. To the reaction mixture, 1.74 g of $Me_3NO\bullet2H_2O$ (15.7 mmol, 6 equiv.) was added and blanketed with argon. The resulting dark orange solution was stirred overnight in the dark during which it turned yellow and formed a precipitate. Open to air, the solution was filtered through celite and rinsed with 10 mL of THF. The THF from the combined filtrates and washings were removed in vacuo. Thereafter, the yellow/orange solid was dissolved in the minimal amount of chloroform (typically about 3 mL, volume varied), passed through a 0.5-1.0-inch silica gel plug in a 15 mL frit and washed with chloroform (final total volume 10 mL) into a scintillation vial. The volume was reduced to a yellow/orange paste on a rotary evaporator (laboratory lights were off). Hot toluene (about 10 mL) was used to dissolve the material in the vial resulting in homogeneous solution (before cooling to r.t., if any material remained it was removed by filtration). All solvent was removed in vacuo to yield $[Mn(CO)_3(OH)]_4{\bullet}(C_7H_8)_2$ (**1**$\bullet(C_7H_8)_2$, 1.02 g, 96%) as a yellow-orange microcrystalline solid. The synthesized **1**$\bullet(C_7H_8)_2$ was characterized by the CHN, FTIR, NMR, and ESI-mass spectroscopy (see SI).

HRMS (ESI)

- In DCM: [**1**H]$^-$ ([M-H]$^-$) calculated mass: 622.6943 and found mass: 622.6959, [**1**•Cl]$^-$ ([M•Cl]$^-$) calculated mass: 658.6710 and found mass: 658.6726.
- In CH_3OH **1**$\bullet(C_7H_8)_2$ formed major species [**1**H]$^-$ ([M-H]$^-$) calculated mass: 622.6943 and found mass: 622.6955.
- In ((50:50) % v/v) toluene:DCM **1**$\bullet(C_7H_8)_2$ formed major species [**1**H]$^-$ ([M-H]$^-$) calculated mass: 622.6943 and found mass: 622.6948 and [**1**•Cl]$^-$ ([M•Cl]$^-$) calculated mass: 658.6710 and found mass: 658.6717.

CHN analysis [calculated (found)]: %C: 38.64 (38.42), %H: 2.49 (2.38), %N; 0 (0).

ATR-FTIR: $\nu_{Mn\text{-}CO}$ 2025(m), 1918(s); ν_{OH} 3563(m) cm^{-1}.

^{1}H NMR

(400 MHz, CD_3CN-*d_3*) δ = 1.08 (s, 4H relative to toluene);

(500 MHz, $CDCl_3$-*d_1*) δ = -0.83 (s, 4H relative to toluene);

(400 MHz, toluene-*d_8*) δ = -2.96 (s, 4H relative to toluene).

Synthesis of adduct-free $[Mn(CO)_3(OH)]_4$ (1):

1 gram of synthesized **1**$\bullet(C_7H_8)_2$ (1.238 mmol) was ground into a fine powder and introduced to a scintillation vial connected with a vacuum adaptor. The solid was heated to 70 °C in vacuo (≤50 mTorr) for 24 h to yield **1** as a yellow microcrystalline solid (771.0 mg, 1.235 mmol, 100% yield). The synthesized **1** was characterized by CHN, FTIR, NMR and mass spectroscopy (FTIR, NMR, and ESI-mass spectrum shown in figures S6-S10). The purity of three batches of **1** prepared this way was confirmed to be greater than 99% by HPLC (see SI).

HRMS (ESI): in water, [**1**H]$^-$ ([M-H]$^-$) calculated mass: 622.6943 and found mass: 622.6946.

CHN analysis [calculated (found)]: %C: 23.10 (23.37), %H: 0.65 (0.77), %N; 0 (0).

ATR-FTIR: $\nu_{Mn\text{-}CO}$ 2032(m), 1941(m), 1915(s); ν_{OH} 3633(m), 3617(m) cm^{-1}.

^{1}H NMR: (400 MHz, CD_3CN-*d_3*) δ = 1.08 (s, 4H); (500 MHz, $CDCl_3$-*d_1*) δ = -0.80(s, 4H); (400 MHz, toluene-*d_8*) δ = -2.96 (s, 4 H).

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Notes

There are no conflicts to declare.

Appendix A. Supplementary data

Supplementary data including spectra and additional experimental details for this article can be found online.

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10. References

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1. Kadassery, K. J.; Dey, S. K.; Cannella, A. F.; Surendhran, R.; Lacy, D. C. Photochemical Water-Splitting with Organomanganese Complexes. *Inorg. Chem.* **2017**, *56*, 9954-9965.
2. Kadassery, K. J.; Sethi, K.; Fanara, P. M.; Lacy, D. C. CO-Photolysis-Induced H-Atom Transfer from Mn^{I}O–H Bonds *Inorg. Chem.* **2019**, *58*, 4679-4685.
3. Marchi, R. C.; de Aguiar, I.; Camilo, M. R.; Braga, A. H.; Do Nascimento, E. S. P.; Santana, V. T.; Nascimento, O. R.; Carlos, R. M. Photochemical Properties of a Mononuclear Mn(I) Triscarbonyl Complex in Water: An Insight into Different Oxidation States. *ChemistrySelect*, **2021**, *6*, 8746-8753.
4. Mann, B. E. CO-Releasing Molecules: A Personal View. *Organometallics,* **2012**, *31*, 5728-5735.
5. Prinz, U.; Koelle, U.; Ulrich, S.; Merbach, A. E.; Maas, O.; Hegetschweiler., K. The Organometallic *fac*-$[(CO)_3Mn(H_2O)_3]^+$ Aquaion: Base-Hydrolysis and Kinetics of H_2O-Substitution. *Inorg. Chem.* **2004**, *43*, 2387-2391.

6. Clerk, M. D.; Copp., S. B.; Subramanian, S.; Zaworotko, M. J. Supramolecular properties of $[Mn(CO)_3(\mu_3\text{-}OH)]_4$, a neutral organometallic molecule that is capable of binding a variety of small and large guest molecules *Supramolecular Chemistry* **1992**, *1*, 7-9.
7. Holman, K. T.; Zaworotko, M. J. Crystal and molecular structure of $[Mn(CO)_3(\mu_3\text{-}OH)]_4$ *J. Chem. Cryst.* **1995**, *25*, 93-95.
8. Some selected recent examples and reviews: (a) Ward, J. S.; De Palo, A.; Aucott, B. J.; Moir, J. W. B.; Lynam, J. M.; Fairlamb, I. J. S. A biotin-conjugated photo-activated CO-releasing molecule (biotinCORM): efficient CO-release from an avidin–biotinCORM protein adduct. *Dalton Trans.* **2019**, *48*, 16233-16241. (b) Rossier, J.; Delasoie, J.; Haeni, L.; Hauser, D.; Rothen-Rutishauser, B.; Zobi. F. Cytotoxicity of Mn-based photoCORMs of ethynyl-α-diimine ligands against different cancer cell lines: The key role of CO-depleted metal fragments. *J. Inorg. Biochem.* **2020**, *209*, 111122. (c) Hu, M. ; Zhu, B.; Zhou, H.; Qiao, L.; Fan, J.; Du, Y.; Chang, F.; Yu., S. Water-soluble UV/visible light activated Mn-CO-releasing molecules: Synthesis, structure, CO releasing and biological activities evaluation. *Inorg. Chem. Commun.* **2020**, *119*, 108093. (d) Stenger-Smith, J.; Chakraborty, I.; Ouattara, R.; Sameera, W. M. C.; Rue, K.; Mascharak, P. CO release from Mn(i)-based photoCORMs with single photons in the phototherapeutic region. *Chem. Commun.* **2021**, *57*, 1101-1104. (e) Ward, J. S. Manganese-Based Carbon Monoxide-Releasing Molecules: A Multitude of Organometallic Pharmaceutical Candidates Primed for Further Biological Analysis *Comprehensive Organometallic Chemistry IV*, **2022**, *15*, 314-330.
9. Bauer, N.; Yuan, Z.; Yang, X.; Wang, B. Plight of CORMs: The unreliability of four commercially available CO-releasing molecules, CORM-2, CORM-3, CORM-A1, and CORM-401, in studying CO biology. *Biochemical Pharmacology.* **2023**, *214*, 115642. https://doi.org/10.1016/j.bcp.2023.115642
10. Hieber, W.; Stanner, F. Organochalkogenid-substituierte Mangan(I)-carbonylhalogenide und ihr Verhalten gegenüber sauerstoffhaltigen Liganden sowie S- und N-Basen. *Chem. Ber.* **1969**, *102*, 2930.
11. Herberhold, M.; Wehrmann, R.; Neugebauer, D. Huttner, G. Die photo-induzierte reaktion von dekacarbonyl-dimangan mit ammoniak, kristall- und molekülstrusktur des produkts [*fac*-$Mn(CO)_3(NH_3)_3][Mn(CO)_5]$ *J. Organomet. Chem.* **1978**, *152*, 329-336.
12. Clerk, M. D.; Zaworotko, M. J. High-nuclearity manganese carbonyl complexes: structures of $[\{Mn(\mu_3\text{–}OH)(CO)_3\}_4]$ and $[Mn_7(\mu_3\text{–}OH)_8(CO)_{18}]$ *J. Chem. Soc., Chem. Commun.* **1991**, 1607.
13. Kadassery, K. J.; Dey, S. K.; Friedman, A. E.; Lacy, D. C. Exploring the Role of Carbonate in the Formation of an Organomanganese Tetramer. *Inorg. Chem.* **2017**, *56*, 8748-8751.
14. Copp, S. B.; Holman, K. T.; Sangster, J. O. S. Subramanian, S.; Zaworotko, M. J. Supramolecular chemistry of $[\{M(CO)_3(\mu_3\text{-}OH)\}_4]$(M = Mn or Re): a modular approach to crystal engineering of superdiamondoid networks *J. Chem. Soc. Dalton Trans.* **1995**, 2233-2243.
15. (a) The UV-vis spectrum for $[Mn(CO)_3(H_2O)_3]^+$ is different from **1**. (b) Bamford, C. H.; Coldbeck, M. Evidence for the formation of the triaquatricarbonylmanganese(I) cation and related derivatives from pentacarbonylchloromanganese *J. Chem. Soc., Dalton Trans.,* **1978**, 4-8.
16. Alberto, R.; Egli, A.; Abram, U.; Hegetschweiler, K.; Gramlich, V.; Schubiger, P. A. Synthesis and reactivity of $[NEt_4]_2[ReBr_3(CO)_3]$. Formation and structural characterization of the clusters $[NEt_4][Re_3(\mu_3\text{-}OH)(\mu\text{-}OH)_3(CO)_9]$ and $[NEt_4][Re_2(\mu\text{-}OH)_3(CO)_6]$ by alkaline titration. *J. Chem. Soc., Dalton Trans.* **1994**, 2815-2820.
17. The original procedure to produce **7** used benzophenone and the parent conditions to form **1** as outlined in Scheme 2. However, the authors of that study also noted that products associated with reduced benzophenone were not observed, and likely that **7** was a byproduct of some other oxidant. We suspect that it formed from Mn(II) impurities during the formation of the clusters leading to **1**.
18. Selected reviews: (a) Ryter, S. W.; Alam, J.; Choi, A. M. K. Heme Oxygenase-1/Carbon Monoxide: From Basic Science to Therapeutic Applications. *Physiol. Rev.* **2006**, *86*, 583–650. (b) Kim, H. P.; Ryter, S. W.; Choi, A. M. CO as a cellular signaling molecule *Annu. Rev. Pharmacol. Toxicol.* **2006**, *46*, 411–449. (c) Mann, B. E.; Motterlini, R. CO and NO in medicine. *Chem. Commun*. **2007**, 4197-4208. (d) Motterlini, R.; Otterbein.; L. E. The therapeutic potential of carbon monoxide. *Nat. Rev. Drug Discov.* **2010**, *9*, 728-743. (d) Gullotta, F.; di Masi, A.; Ascenzi, P. Carbon monoxide: An unusual drug *IUBMB Life* **2012**, *64*, 378-386.
19. Bricker, O. Some stability relations in the system MnO_2-H_2O at 25° and one atmosphere total pressure *Am. Min*., **1965**, **50**, 1296-1354.
20. Yuan, Z.; Yang, X.; Wang, B. Redox and catalase-like activities of four widely used carbon monoxide releasing molecules (CO-RMs) *Chem. Sci.* **2021**, *12*, 13013-13020.
21. Tolliver, L. M.; Holl, N. J.; Hou, F. Y. S.; Lee, H.-J.; Cambre, M.; Huang, Y.-W. Differential Cytotoxicity Induced by Transition Metal Oxide Nanoparticles is a Function of Cell Killing and Suppression of Cell Proliferation. *Int. J. Mol. Sci.* **2020**, *21*, 1731. https://doi.org/10.3390/ijms21051731
22. Warmuth, M.; Kim, S.; Gu, X.-J.; Gang, X.; Adrián, F. Ba/F3 cells and their use in kinase drug discovery. *Current Opinion in Oncology*. **2007**, *19*, 55-60.
23. Romão, C. C.; Bättler, W. A.; Seixas, J. D.; Bernardes, G. J. L. Developing drug molecules for therapy with carbon monoxide. *Chem. Soc. Rev.* **2012**, *41*, 3571-3583.

TOC Synopsis: The “adduct-free” tetramer $[Mn(CO)_3(\mu_3\text{-}OH)]_4$ (**1**) is soluble in water and releases all twelve CO ligands when exposed to ambient laboratory lighting. Such a system is well-suited to release CO for a variety of applications. This study explores the properties of **1** that make it an effective photoCORM including a demonstration of dose-dependent cell antiproliferations.

TOC Graphic