# Photoinduced CO release, cellular uptake and cytotoxicity of a tris(pyrazolyl)methane (tpm) manganese tricarbonyl complex†

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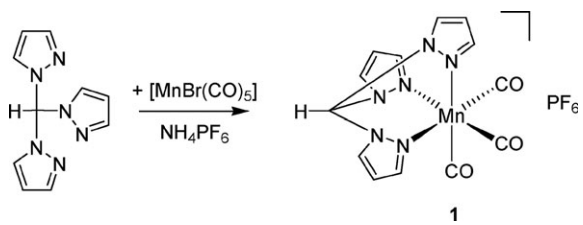
**Cell viability studies of HT29 colon cancer cells treated with the CO-releasing compound $[Mn(CO)_3(tpm)]PF_6$ revealed a significant photoinduced cytotoxicity comparable to that of established agent 5-fluorouracil (5-FU), while controls kept in the dark were unaffected at up to 100 μM.**

A major challenge in the chemotherapy of malignant diseases is the selective destruction of cancer cells, while leaving normal tissue unaffected. An attractive approach is the utilization of light to initiate the cytotoxic action of inactive prodrugs with precise spatial and temporal control. Conventional agents for photodynamic therapy (PDT) in clinical use, such as aminolevulinic acid or derivatives of hematoporphyrin, act as photosensitizers to generate reactive singlet oxygen to destroy cancer cells.[1] A drawback of these systems is the catalytic nature of the photosensitization process, which leads to long term light sensitivity of the treated body area, as well as to the non-specific action of singlet oxygen. Additionally, a number of tumor environments are hypoxic. Recently, several new classes of transition metal-based systems have been evaluated as PDT agents with more specific cellular targets. Bednarski, Sadler and co-workers investigated kinetically-inert octahedral platinum(IV) complexes that undergo photoinitiated reduction to square-planar platinum(II) species, with a mode of action similar to that of the well-established anti-cancer drug Cisplatin, *cis*-$[PtCl_2(NH_3)_2]$.[2] Turro *et al*. reported on the photoactivation of octahedral mononuclear ruthenium and dinuclear rhodium complexes, with some of the latter showing a significantly increased cytotoxicity upon irradiation, in some cases even surpassing the effect observed for the commercial PDT agent Photofrin.[3] Half-sandwich complexes of the type $CpM(CO)_3R$ and $CpM(CO)_2R$ (with M = Cr, Mo, W as well as Fe, and R = alkyl, aryl) are among the few organometallic compounds that show photoinduced action on biological targets. DNA cleavage by these systems was investigated with a plasmid relaxation assay and other methods, but no cytotoxicity data has been reported so far.[4] Very recently, it was demonstrated that bioorganometallic dinuclear piano stool arene ruthenium complexes also show an increased frequency of DNA cross-linking upon irradiation in the 300 to 400 nm range.[5] Also, some metal carbonyl complexes like $Mn_2(CO)_{10}$ and $[RuCl_2(CO)_3]_2$ have been studied with regard to the physiological action of CO ligands liberated upon irradiation, but there are only a few recent reports on the chemopreventive effects of such compounds against carcinogenesis.[6]

In this Communication, we report on the photoinduced CO release, cellular uptake and cytotoxicity of the manganese complex $[Mn(CO)_3(tpm)]PF_6$ (tpm = tris(pyrazolyl)methane) (**1**) in the presence and absence of UV light to explore its potential as a novel PDT agent. It is the parent compound of a family of metal tricarbonyl complexes with functionalized tpm ligands, allowing the preparation of bioconjugates for targeted delivery to cells, which are currently under study in our laboratory.

Already briefly mentioned in the original work of Trofimenko on poly(pyrazolyl) ligands,[7] the preparation of **1** is facilitated by easy access to multigram amounts of tpm by following the procedure of Reger *et al*., which also allows the introduction of various functional groups by substitution of the acidic methine proton.[8] The manganese tricarbonyl moiety was introduced by the reaction of tpm with $MnBr(CO)_5$ in anhydrous acetone under heating, leading to **1** (Scheme 1); the original procedure involving reflux in dimethylformamide did not yield the desired product in our hands.[7] Both the IR and $^1H$ NMR spectrum are in accordance with a facial coordination of the tpm ligand to the manganese tricarbonyl unit.

The solid state structure of **1**, crystallized by cooling a solution of the compound in methanol to −20 °C, was determined by X-ray crystallography and is shown in Fig. 1.‡ The Mn(I) center is in a pseudo-octahedral coordination environment bound to three CO ligands and three nitrogen donor atoms from the facially-coordinating tpm ligand.




**1**

**Scheme 1** Synthesis of manganese tricarbonyl complex **1**.

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† Electronic supplementary information (ESI) available: Experimental procedures, and analytical and spectroscopic data for compound **1**. Crystallographic data for **1**. Details of the myoglobin CO release assay, cytotoxicity measurements and cellular uptake studies. See DOI: 10.1039/b719075a

 

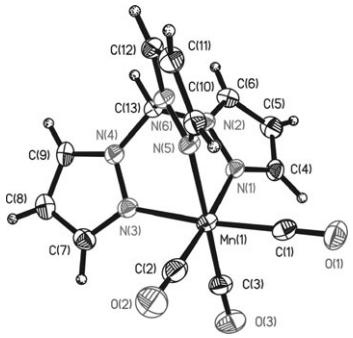


**Fig. 1** The molecular structure of $[Mn(CO)_3(tpm)]^+$, the cation of **1**. Ellipsoids are drawn at the 30% probability level. Selected bond length [Å] and angles [°]: Mn–C1 1.804(5), Mn–C2 1.803(6), Mn–C3 1.812(5), Mn–N1 2.025(4), Mn–N3 2.045(3), Mn–N5 2.029(3), C1–O1 1.131(5), C2–O2 1.133(6), C3–O3 1.119(5); C1–Mn–C2 92.9(2), C1–Mn–C3 88.5(2), C2–Mn–C3 91.6(2), N1–Mn–N3 84.06(14), N1–Mn–N5 84.74(14), N3–Mn–N5 84.38(14), N1–Mn–C1 90.28(17), N1–Mn–C2 175.13(17), N1–Mn–C3 92.11(18), N3–Mn–C1 174.20(17), N3–Mn–C2 92.62(19), N3–Mn–C3 93.01(18), N5–Mn–C1 93.85(17), N5–Mn–C2 91.40(18), N5–Mn–C3 176.09(18).

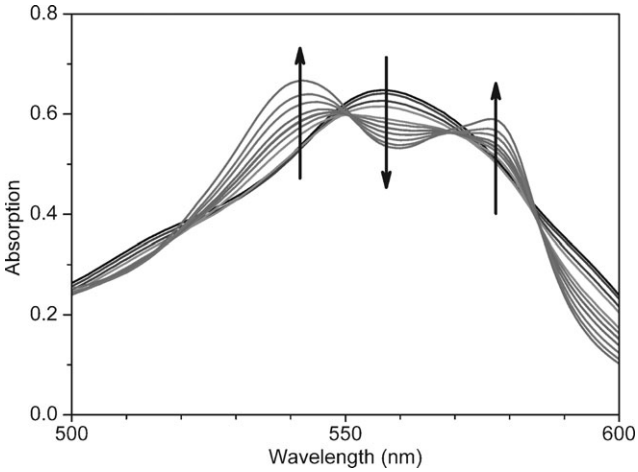


**Fig. 2** UV/Vis spectral changes in the Q band region of a solution of reduced horse skeletal muscle myoglobin (75 μmol $l^{-1}$) and $[Mn(CO)_3(tpm)]PF_6$ (**1**) (20 μmol $l^{-1}$) in 0.1 M phosphate buffer upon irradiation at 365 nm ($t$ = 0 to 100 min).

The metrical parameters are comparable to those of other $[Mn(CO)_3(tpm)]^+$ complexes characterized by Reger *et al.*[8,9]

In order to study its suitability as a novel light-activatable cytotoxic agent and establish the number of photolabile CO ligands, **1**, dissolved in dimethylsulfoxide due to solubility limitations, was added to a buffered aqueous solution of horse skeletal muscle myoglobin (MbFe(II)), freshly reduced with excess sodium dithionite under nitrogen. The spectral changes in the Q band region of MbFe(II) upon irradiation of the mixture at 365 nm are shown in Fig. 2. The intensity of the band at 557 nm slowly decreases, while two new features at 542 and 577 nm, assigned to the MbFeCO complex, appear. This is paralleled by changes in the Soret band region, where the maximum at 423 nm also decreases in intensity, while a broad shoulder between 430 and 450 nm grows in. These data demonstrate that some of the carbonyl ligands in this tpm–metal tricarbonyl complex are photolabile. When the experiment is repeated under conditions of a limiting amount of metal complex, taking into account the molar extinction coefficient of MbFeCO,[10] it was found that approximately 1.96 moles of CO were liberated per mole of **1**. Thus, two out of the three CO ligands are apparently lost from the manganese tricarbonyl unit, which contrasts with only one in the case of $[Mn(Cp)(CO)_3]$, which is isoelectronic to **1**.[11] Irradiation of MbFe(II) under the same conditions without the manganese tricarbonyl complex or with prolonged stirring of MbFe(II) and **1** in the dark did not lead to any spectral changes.

In order to study the bioavailability of **1**, we then quantified the cellular Mn uptake by HT29 human colon cancer cells at various complex concentrations using graphite furnace atomic absorption spectroscopy. Cellular Mn (expressed as nmol Mn per mg of cellular protein) increased in an almost linear fashion ($r^2 > 0.97$) with increasing incubation concentration and did not show saturation effects up to 100 μM, which indicates an uptake by passive diffusion rather than active transport. Based on an estimation of the molar cellular Mn concentration,[12] and after dividing by the exposure concentration, the mean cellular accumulation of **1** was found to be 3.1 ± 1.1. That is, the intracellular concentration exceeded the extracellular concentration approximately three-fold. According to this, and provided that two CO equivalents are quantitatively released from **1**, the intracellular CO concentration after incubation with 100 μM of **1** can be estimated to be approximately 0.6 mM. Thus, the cellular uptake experiments clearly demonstrate that **1** is accumulated inside certain cancer cells and so might be a useful tool for the cellular delivery of CO equivalents.

To study the differential cytotoxicity of the complex with and without irradiation, cell cultures of HT29 were incubated with **1** at 100 μM in the dark for 24 h, then irradiated for 10 min at 365 nm, and further grown for another 24 h in the dark before the cell biomass present was quantified with a crystal violet assay.[13] An additional experiment was conducted in the presence of **1**, but without irradiation. Further controls were run with (a) pure medium, (b) dimethylsulfoxide at the same concentration as used for the solubilization of **1** and (c) with 5-fluorouracil (5-FU) as an established cytotoxic agent,[14] all in the presence and absence of UV light (Fig. 3). While 5-FU at 20 μM efficiently reduced the amount of cell biomass in the dark to about 25% of the reference samples without a marked effect of irradiation, **1** is inactive in the absence of light at a concentration of 100 μM. Upon irradiation, the action on cell growth, however, becomes comparable to that of known cytotoxic agent 5-FU, with a reduction of cell biomass to about 30% of the control's. It is clear from Fig. 3 that this is solely due to the effect of UV light on complex **1**, since neither irradiation of cells in the medium alone or in the presence of dimethylsulfoxide used as a solubilizer shows any marked effect.

Thus, we have established that tpm manganese tricarbonyl complex **1** undergoes photoinduced CO release upon irradiation with UV light in aqueous buffer, with two out of three CO ligands being lost under these conditions. The compound shows photoinitiated cytotoxicity against the human colon cancer cell line HT29, with a reduction in cell biomass

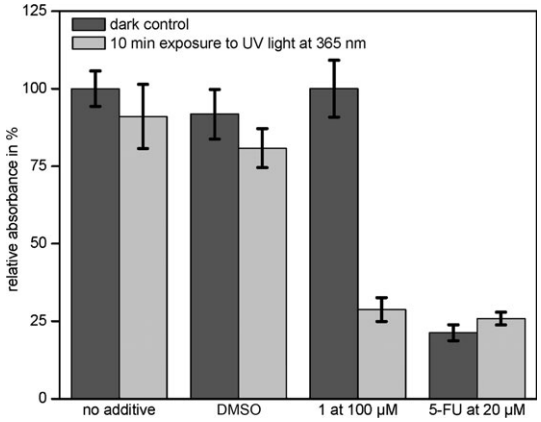


**Fig. 3** The cytotoxicity of **1** on HT29 human colon cancer cells. Cells were grown in the dark for 24 h in the presence of the indicated additive, then kept in the dark or irradiated for 10 min at 365 nm, and afterwards further grown for 24 h in the absence of light before the cell biomass was measured with a crystal violet assay, here indicated as relative absorbance at 570 nm compared to a standard control. The black bars indicate the standard deviation of six individual runs.

comparable to that of established agent 5-FU, but is inactive in the dark at concentrations up to 100 μM. Further investigations will help to clarify whether this is due to the action of the CO released, follow-up reactions of the coordinatively unsaturated or solvated metal complex fragment generated, or a combination of both. The ease of functionalization of the tpm ligand will allow the preparation of water-soluble bioconjugates. Experiments on the preparation and targeted delivery of such photoactivatable cytotoxic bioconjugates to different cellular targets, as well as the incorporation of other metal tricarbonyl units, are under way in our group.

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## Notes and references

‡ Crystal data for **1**: $C_{13}H_{10}F_6MnN_6O_3P$, $M_r$ = 498.18, monoclinic, $C2/c$, $a$ = 26.669(5), $b$ = 11.855(3), $c$ = 12.869(3) Å, $\beta$ = 118.006(5)°, $V$ = 3592.3(13) Å$^3$, $T$ = 213(2) K, $Z$ = 8, $\rho_{calc}$ = 1.842 g cm$^{-3}$, $\mu$(Mo-K$_\alpha$) = 0.71073 mm$^{-1}$, $F(000)$ = 1984; a total of 9772 reflections up to $h(-27/31)$, $k(-14/11)$, $l(-15/15)$ in the range 1.92 < $\theta$ <25.27 were collected, of which 3203 were unique ($R_{int}$ = 0.0676); final $R$ indices ($I > 2\sigma(I)$) $R_1$ = 0.0557, w$R2$ = 0.1336, GOF = 1.077. CCDC 670201. For crystallographic data in CIF or other electronic format see DOI: 10.1039/b719075a

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