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PhotoCORMs: CO release moves into the visible

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The potential of carbon monoxide to act as a therapeutic agent is now well-established. Controlled delivery of CO is best achieved using 'CORMs': molecules which release known amounts of carbon monoxide in response to a stimulus. Metal carbonyl complexes will release CO if irradiated with ultraviolet light, but it is only in the past five years that development of true 'photoCORMs' has been explored. Recent exciting developments in this area now show that design of photoCORMs operating well into the visible region is achievable. In this Perspective, we examine the growth of photoCORMs from their origins in the photophysics of metal carbonyls to the latest visible-light agents.

1 Introduction

The toxic nature of carbon monoxide is well known. As an excellent π-acceptor ligand, CO reacts with haemoglobin to form carboxyhaemoglobin (COHb), reducing the oxygen-carrying capacity of the blood and thus impairing respiration. This donor ability is also implicated in other aspects of the dangers of CO: COHb formation alone cannot fully account for the acute toxicity seen.[1]

This same ability to bind strongly to metal centres provides CO with an additional, beneficial role. Studies on the biological role of CO have established that it functions as a signalling molecule,[2–5] reminiscent of the discovery that NO plays a similar role in cell processes.[6] Controlled amounts of carbon monoxide show a positive role in controlling cardiovascular and inflammatory impairment, in promoting wound healing, and bactericidal action.[7–15] Notably, the mode of action of CO in these beneficial roles is as-yet unresolved. However, that does not prevent exploitation of the positive behaviour of CO in medical applications.

The key challenge in using carbon monoxide as a therapeutic is that it must be delivered in a strictly-controlled manner. Delivery of gaseous carbon monoxide is possible but attaining fine control of volumes is non-trivial. Delivery as a gas is also tied to absorption by the lungs, limiting the potential for topical or targeted application. There has therefore been significant effort to produce molecules which can release CO, so-called CORMs (carbon monoxide releasing molecules).[16–20] The majority of these systems are based around metal carbonyl fragments, which offer a direct route to the release of CO. Such organometallic therapeutics are unusual, and efforts have been made to exploit alternative CO sources. Non-organometallic systems are in the main limited by low rates and/or harsh conditions for CO release.[1]

CORMs may release carbon monoxide in response to a range of stimuli. Dissolution of many CORM systems in aqueous media leads to release of CO at physiological temperatures: thermodynamic CORMs. Whilst this provides for the ability to readily control the amount of CO delivered, it does not allow control of when and where the CO is released. To do that, triggered CORMs are required. Enzyme-triggered CORMs (ET-CORMs) make use of the presence of esterases in cells to bring about CO release at metal centres.[21–24] Triggering using small molecules is also possible, with the combination of a CORM with a second chemical agent[25–27] allowing temporal control of release. More exotic approaches have also been explored, for example release triggered by electromagnetic heating.[28]

Most CORMs are metal carbonyls, which are well known as photosensitive systems. An attractive strategy is therefore to trigger release using light: so-called photoCORMs.[29] The development of photoCORMs has accelerated rapidly over the past decade. Early work in this area built on known photochemistry of carbonyl molecules in the hard ultraviolet (UV), moving to molecules which release on the boundaries of the visible spectrum and most recently systems which work well under ambient light. In this Perspective we will trace development of photoCORMs from the earliest reports to the present day, with a particular focus on the use of visible light to effect CO release.

2 Ideal behaviours

First and foremost, any putative CORM must release CO in a clearly-defined way. For a photoCORM this means releasing CO in response to stimulation by light. It should not release any CO, or indeed react in any way, *until* exposed to light, so should be both water and oxygen tolerant. These conditions are far removed

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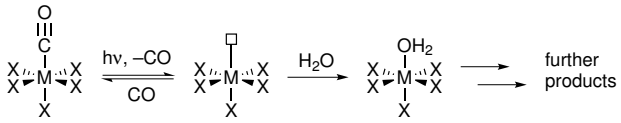

Fig. 1 Generalised reactivity of a photoCORM

from the typical organometallic chemistry environment and so provide a challenging set of requirements. The CORM must react only when required, not with the surrounding chemical environment or by enzymatic attack.

Both the photoCORM itself and the breakdown products must be non-toxic and excretable. For the by-products formed after activation, a challenge is that they are likely to be reactive, as at least one site will have been vacated by CO. Typically, water adducts are expected to form initially, although this may be followed by further chemistry (Fig. 1).

To allow delivery as a solution, the photoCORM should be soluble in water or failing that in a mixture of water with dimethylsulfoxide (DMSO). Facilitating this solution may involve modification of the core molecule architecture with groups to promote this, such as alcohol functionality.[25] Such modification may lead to an increased mass of the pro-drug, which reduces the overall active content available.

Whilst the requirement to release CO to constitute a photoCORM seems trivial, a more nuanced concern is the nature of the light required to achieve meaningful activity. Given a light source emitting far enough into the UV, most carbonyl complexes will decompose with loss of CO. Many potential photoCORMs rely on light in the UV to activate, but this is both potentially damaging to cells and has poor penetration depth into tissue.[30,31] Indeed, the ideal wavelength for issue penetration is in the near infra-red (IR) region (700 nm to 1100 nm).[32]

3 Assessing photoCORMs

By definition, a photoCORM must give out carbon monoxide when exposed to light. As such, any assessment of the activity of the molecule needs to quantify the CO release and relate this to the light exposure experienced by the target. Carbon monoxide release is typically monitored using the carboxymyoglobin (COMb) assay.[33] As noted earlier, CO binds strongly to metals, and its reactivity with haemoglobin is at least in part responsible for its toxicity. The COMb assay exploits this reactivity, with the irreversible binding of CO to myoglobin (Mb) leading to a clear change in the visible spectrum (Fig. 2). Monitoring the strong absorbance at 540 nm can thus be used to quantify CO release, provided appropriate controls are used.

Separating out the contribution to the UV spectrum of the photoCORM (and breakdown products) from the Mb/COMb elements is not without issue.[34] Myoglobin is also reactive toward oxygen, and the dithionite used in the assay may itself react with CORMs.[35] This has driven efforts to develop alternative assays for (photo)CORMs.[17,31] Direct measurement of CO concentration is possible by gas chromatography,[36] electrochemical means[37] or by using a suitably-designed integrated IR cell.[38] Carbon monoxide will also react with rhodium complexes[39,40] and

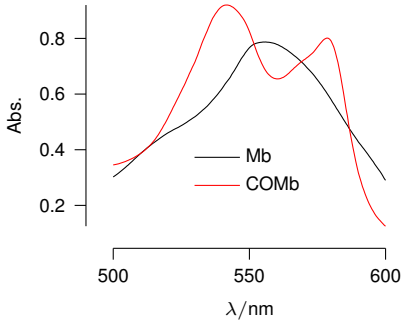

Fig. 2 Visible spectra for myoglobin and carboxymyoglobin

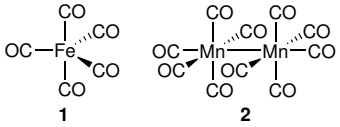

Fig. 3 First photoCORMs reported by Motterlini *et al.*[33]

fluorescence precursors.[41–44] However, to date it is common to compare (photo)CORMs based on their performance using the COMb assay even if one of these alternative methods is also employed.

Converting rates of CO release into photoreactivity requires quantification of light input. Typically, this is assessed by calculating the quantum yield (Φ_{CO}) for CO release at a given wavelength (λ_{ex}). This can then be related to the more useful therapeutic measure, the rate of CO release, by using the intensity of the light source used, with the necessary correction for the difference between light given out by the source and that absorbed by the photoCORM. It is notable that direct comparison of photoCORMs can be difficult as they have varying λ_{ex} values and thus different rates of practical CO release under similar conditions. In particular, whilst 'half-life' values are sometimes given in the literature, the rate of release of CO is directly dependent on the intensity of the incident light and the absorption of the solution.

4 Early studies

Studies on the photochemistry of metal carbonyls have a long history,[45] and it is therefore unsurprising that early work in this area overlaps with more fundamental physical chemistry research into the nature of the complexes.

The first explicit use of light to trigger release of CO from a CORM was reported by Motterlini *et al.* in 2002 (Fig. 3).[33] In a study of the behaviour of simple metal carbonyls as CORMs, **1** and **2** were found to be inert when mixed with water but did release CO when irradiated with 'cold light' over approximately one hour. These simple metal carbonyls are not amenable to modification, for example to control light sensitivity or water solubility, so as platforms for further development were somewhat limited. This is particularly true for $Fe(CO)_5$, which in addition to low water solubility is also significantly toxic.

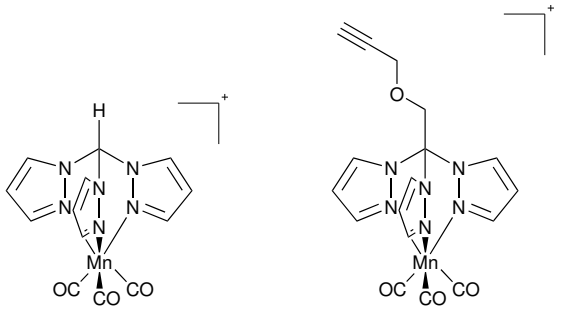

Fig. 4 First manganese-based photoCORM and functionalised derivative[46,48]

5 Ultraviolet photoCORMs

The first system developed specifically to target photoCORM behaviour (though before the term was coined) was reported by Schatzschneider and co-workers (**3**, Fig. 4).[46] This manganese-based system features a tris(pyrazolyl)methane (tpm) supporting ligand, leaving three sites on the metal filled by CO. Whilst this might be expected to allow the complex to deliver three equivalents of carbon monoxide, photolysis of **3** at 365 nm yielded only two photolabile CO molecules per metal centre. Nuernberger and co-workers later established using ultrafast laser spectroscopy that only one of the CO groups is liberated from the metal centre by the primary photochemical step.[47]

The cationic nature of complex **3** means that it is water-soluble, and was shown to have efficacy in reducing the viability of cancer cells.[47] Complex **3** was subsequently used in bioimaging applications,[49] although in this context the bound CO molecules were not released but rather used as markers based on the strong and distinct C≡O IR band.

The readily-accessible nature of **3** along with the synthetic flexibility of the tpm architecture has been exploited by to develop a range of photoCORM systems building upon this. Schatzschneider and co-workers functionalised the 'rear' of the ligand with a triple bond, allowing the organometallic part to be coupled using Sonogashira or 'click' chemistry (**4**).[48] The alkyne group could be successfully reacted to introduce peptide functionality into the molecule under both of the target reactions without affecting the photoCORM core. Schatzschneider and co-workers later reported the use of 'click' chemistry to bind complex **4** to the surface of SiO_2 particles.[50] The immobilised complex shows very similar CO release properties to the parent, with qualitative evidence for photolability at 365 nm.

Work by the Kunz group exploited tripodal nitrogen ligands featuring a central phosphorus atom (Fig. 5).[51] The resulting complexes have similar structures and CO-release properties to **3**, with photolability of between one and two equivalents of CO at 365 nm.

In 2010, Ford and co-workers reported the tungsten-containing complex **7** (Fig. 6), at the same time introducing the term 'photoCORM'. Complex **7** releases CO when irradiated at a number of wavelengths in the UV (between 305 nm and 405 nm). Examination of the carbon monoxide release behaviour using a range

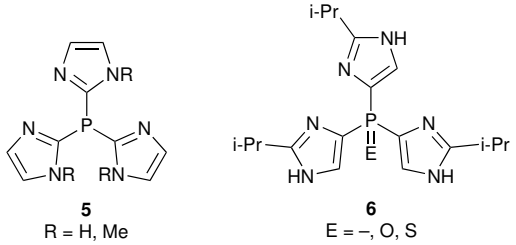

Fig. 5 Tripodal nitrogen ligands featuring a phosphorus core[51]

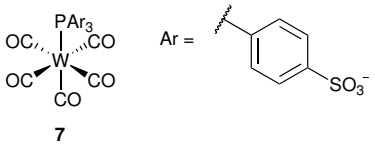

Fig. 6 Tungsten-based photoCORM reported by Ford and co-workers[36]

of different detection techniques established that only one equivalent of CO is released by photolysis: subsequent degradation to release further CO equivalents was attributed to the action of dioxygen on the photoactivated intermediate.

The Kunz group extended the range of tripodal manganese carbonyls studied by exploiting bis(pyridylmethyl)amine ligands (Fig. 7). These materials release CO when irritated with a hand-held UV lamp (365 nm). Complex **9b** is a more effective photoCORM than the **9a** or **9c**; the latter complexes release only two equivalents of CO while **9b** releases all three available COs when irradiated. The ligand architecture was incorporated into two related 2-hydroxypropyl methacrylamide copolymer (formed before addition of the manganese source), using the side chains in **9b** and **9c** as linkers to the polymer backbone. These polymer-bound photoCORMs showed release activity under the myoglobin assays, although solution turbidity did affect the quantification.[34]

Several iron-containing metal complexes were examined in 2011 for CO release activity, although not necessarily directly for use as photoCORMs. In a study focussed on ^{11}C sources for positron emission tomography, the Long group disclosed the reversible CO-release behaviour of complex **10** (Fig. 8).[53] Carbon monoxide is released by this system when irradiated by a high-pressure mercury lamp operating at 365 nm.

Works and co-workers examined the behaviour of the [FeFe]-hydrogenase mimic **11** (Fig. 9) when irradiated using flash photolysis. In accord with related work by Hunt and co-workers,[54,55]

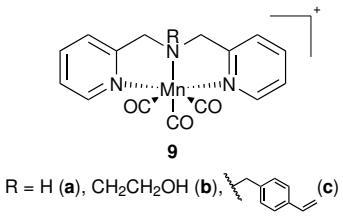

Fig. 7 Bis(pyridylmethyl)amine-supported manganese complexes[52]

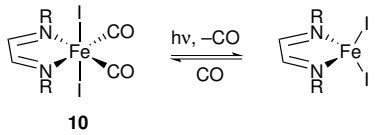

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Fig. 8 Simple iron carbonyl[53]

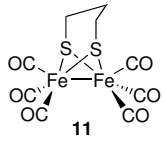

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Fig. 9 [FeFe]-hydrogenase mimic[56]

photolysis at UV wavelengths (365 nm) gave signals consistent with loss of a single CO and formation of a *solvato* intermediate. This CO loss is reversible.

The dicationic monocarbonyl **12** (Fig. 10, R = H) is both air stable and water soluble, and was reported by Kodanko and co-workers as a rapid CO releaser when irradiated at 365 nm.[57] Complete release of the available CO was observed within 10 minutes of the start of irradiation with a yield of recovered CO of 92 % from the myoglobin assay. A peptide-bearing derivative was reported (R = Ac-Ala-Gly-OBn) but no quantitative release data was included.

The cyclopentadienyl-molybdenum complex **13** (Fig. 11) was reported to release CO but only when irradiated with 'hard' UV light (325 nm).[58] This water-soluble complex releases CO slowly in the dark (indeed, other complexes in the same study were examined as thermal CORMs by virtue of reaction with water alone), but showed significantly enhanced release properties when irradiated. Myoglobin data showed that at least two equivalents of CO were released when the complex was irradiated with a hand-held lamp.

Berends and Kurz examined the detailed behaviour of complex **3** along with a second related neutral manganese complex **14** (Fig. 12).[59] Using a combination of UV, IR and electron paramagnetic (EPR) spectroscopies, they established that CO loss is a stepwise process and postulated a mechanism involving formation of *solvato* intermediate adducts before oxidation of the metal centre [Mn(I) to Mn(II)] leads to the formation of Mn–O–Mn units with retention of the supporting ligand. Complex **14** is more active in loss of CO than **3**, although this still requires the use of 365 nm UV illumination.

Mascharak and co-workers also examined cationic tripodal

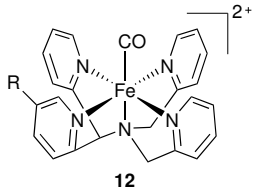

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Fig. 10 Monocarbonyl reported by Kodanko an co-workers (R = H, Ac-Ala-Gly-OBn)[57]

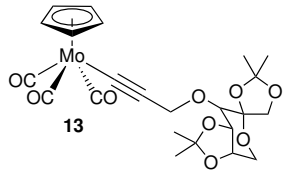

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Fig. 11 Mo-photoCORM[58]

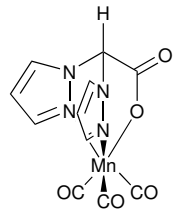

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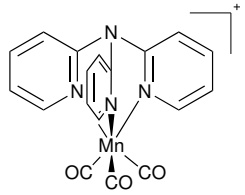
Fig. 12 Manganese photoCORM examined in detail by Berends and Kurz[59]

manganese complexes related to **3** but featuring one or more pyridyl rings (Fig. 13).[60] Replacement of one pyridyl by a simple amine donor (**15** to **16**) and extension of the π-system (**16** to **17**) are both beneficial in terms of the position of UV maxima, moving from between 240 nm to 260 nm for **15** to 350 nm for **16** and 360 nm for **17**. The latter also shows a greater degree of absorption over a wider range. Irradiation above 350 nm with a low power light (120 mW) resulted in quantum yields at 358 nm of around 0.07.

Schatzschneider and co-workers explored the behaviour of a range of ruthenium-containing structures based around 2,2′-bypryidine (bpy) ligands (and related structures) (Fig. 14). The $Ru(bpy)_n$ core has been extensively studied,[61] and these systems are known to exhibit strong MLCT (metal-to-ligand charge transfer) transitions from the metal to low-lying π^* orbitals. Complexes **18**–**20** show strong absorption maxima in the region approx. 300 nm to 325 nm, with variation in the aromatic architecture (*e.g.* **18c** *versus* **18d**) leading to shifts of the exact position of the bands. The complexes were largely stable in the absence



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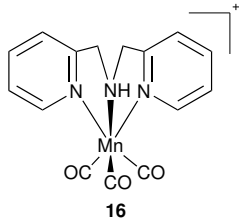

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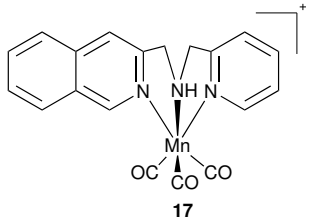

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Fig. 13 Tripodal pyridyl-based photoCORMs[60]

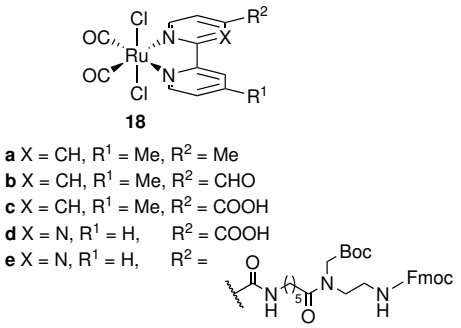


Fig. 14 Ruthenium bi- and ter-pyridine-based photoCORMs (Boc = *tert*-butyloxycarbonyl, Fmoc = 9-fluorenylmethyloxycarbonyl)[62]

of light (though **18e** and **20** did show some decomposition in the dark in solution) but released up to one equivalent of CO when irradiated at 365 nm. Half-lives for this process ranged between 20 minutes (**18c**) to 66 minutes (**18d**). Notably, there was no correlation between the total amount of CO released and the strength of the UV absorption bands (*vide infra*). Quantum yield were reported but were described as best taken as a *relative* measure of the efficiency of reaction due to the internal shielding effect of myoglobin in the assays.

Extending their earlier work with symmetrical tripodal manganese systems, Schatzschneider and co-workers have recently reported a family of complexes based around the tridentate bis(pyrazoyl)ethylamine core (Fig. 15).[44] This architecture has been chosen to allow derivatization to improve potential delivery routes. These new structures retained photolability under irradiation at 365 nm with quantum yields of the order of 10^{-3}. Carbon monoxide release was also reported to occur at 410 nm from complex **22a**: the total amount of photolabile CO remained roughly two equivalents whilst the time taken to release CO was (under identical conditions) increased by a factor of roughly four.

6 Visible light photoCORMs

As detailed earlier, an ideal photoCORM will release CO when irradiated in the visible region, with the challenge then being to find molecules stable in the dark but photoactive in the

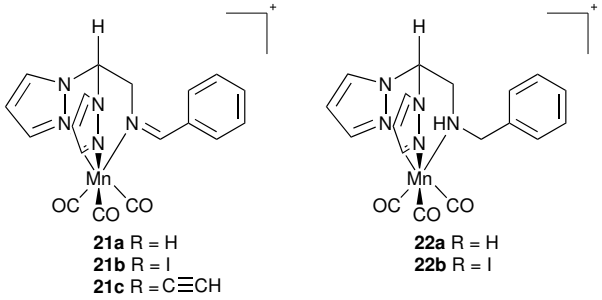


Fig. 15 Tripodal manganese complexes featuring a bis(pyrazoyl) core[44]

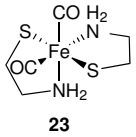


Fig. 16 CORM-S1[64]

light. Over the past half-decade this challenge has begun to be addressed. A particularly fruitful avenue has been the design of complex which facilitate electron transfer from electron-rich metals to π^* orbitals of the ligand *via* strong metal-to-ligand charge transfer (MLCT) transitions in the visible/near-IR region.[30] These transfers tend to favour CO release, particularly at d^6 metal centres [Mn(I), Re(I), Fe(II), Ru(II), *etc.*] where overall stability is high. This strategy has been strongly-supported by computational (density functional theory) calculation, and has recently been reviewed in detail.[30]

Cleanly delineating the line between a photoCORM active in the UV and one active in the visible is not straight-forward. Electronic transitions are broad and may have appreciable extension from the UV to the visible. Thus molecules with absorption maxima in the UV ($\lambda < 400$ nm) can release CO when irradiated with visible light. Here, we have taken the division based on the light used to bring about CO release rather than the absorption maxima involved: for practical applications, slow release in the visible is entirely reasonable even if faster release would be possible in the UV.

Westerhausen and co-workers examined the CO release properties of the known complex dicarbonylbis(cysteamine)iron(II) (**23**, Fig. 16)[63] in 2011.[64] Complex **23** was irradiated at 470 nm it released CO over a period of several minutes. Exposure to broadband white light led to rapid release of CO (full release within minutes). This system was the first to be reported as releasing CO in the visible region, though the ligand architecture did not offer obvious handles for further development and modification. Notably, this system bears no organic chromaphores.

Carbon monoxide release from [FeFe]-hydrogenase mimics has been extended into the visible region. A system bearing water-soluble side chains was examined by Fan and co-workers for CO release at the edge of the visible spectrum.[65] Complex **24** (Fig. 17) is water soluble due to the carboxylate groups, and adopts a significantly more 'open' geometry than complex **11**. Complex **24** releases all of the bound CO under broadband visible

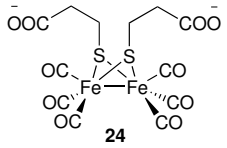


Fig. 17 [FeFe]-hydrogenase mimic[65]

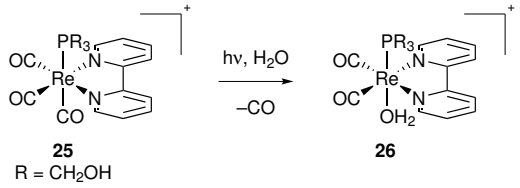


Fig. 18 Luminescent rhenium-based photoCORM[67]

irradiation within a few minutes, though detailed data are reported for irradiation at 390 nm. Complexes similar in structure to **24** have been studied by Liu and co-workers as chemically-triggered CORMs.[25–27]

Complexes of the form *fac*-$[Re(bpy)(CO)_3(X)]^+$ (X = halide) are well-known as CO oxidation catalysts and luminescence molecules.[66] Ford and co-workers used this known behaviour as the basis to develop a water-soluble and luminescent photoCORM which releases CO at the edge of the visible range.[67] By incorporating a hydroxylated ligand, $P(CH_2OH)_3$, into the complex (Fig. 18), Ford was able to solubilise the target rhenium systems in water whilst leaving the overall charge on the system unchanged. Complex **25** was shown to release one CO when irradiated at 405 nm, with a quantum yield of 0.11 (a higher quantum yield, 0.21, was found at 365 nm). The complex was also strongly luminescent in aqueous solution, exhibiting a band at 515 nm, whilst the photoproduct **26** is also active with a band at 585 nm. These luminescent properties allowed cell uptake of the photoCORM to be examined, showing that cells remain viable in the presence of this system. The luminescent properties of both the photoCORM and breakdown product allowed qualitative demonstration of CO release in cells, with the shift of emission detectable *in vitro*.

As in the UV, systems based around supporting manganese tricarbonyl cores have been widely used as visible-light photoCORMs. Smith and co-workers have exploited the bypyridyl ligand bound to manganese to develop dendritic photoCORMs.[68] These systems were prepared in two dendrimer generations, bearing four and eight metal centres, respectively. Both showed stability in the dark with CO release at 410 nm and comparable total CO quantities available. The dendritic systems and a monometallic model **28** showed appreciable extinction coefficients in the visible, $\varepsilon_{410} = 3527\,M^{-1}\,cm^{-1}$ for the latter. As would be expected, this value scaled roughly with the total metal complex loading. Release of CO at 410 nm was found to take around twice as long for the dendrimer as for the monomeric system under otherwise identical conditions. Quantum yields for all three was similar at approx. 3×10^{-3}: as with other values from myoglobin assay conditions, this may be suppressed by the presence of the protein

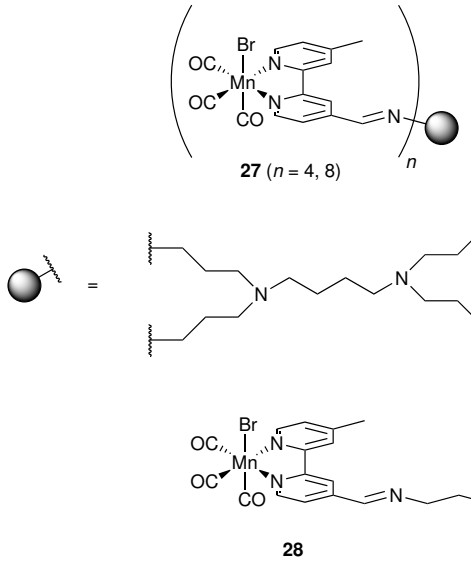


Fig. 19 Dendritic photoCORMs and model system[68]

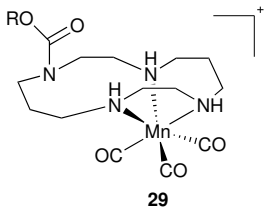


Fig. 20 Combined photoCORM/IR imagining complex (R = Cyanocobalamin/B_{12} derivative)[69]

complex.

Zobi *et al.* have supported the *fac*-$Mn(CO)_3$ fragment using a tetra-azacyclotetradecane ligand (Fig. 20).[69] Complex **29** features a vitamin B_{12}-derived side-chain which confers water solubility and cellular targeting whilst being remote from the photoCORM centre. Whilst **29** shows an absorption maximum at 388 nm, irradiation at 470 nm (blue LED source) gave CO release over a period of around three hours. Illumination using a green Ar laser (several discrete bands in the 450 nm to 550 nm range) also gave CO release though at a reduced rate. Experiments under limiting conditions showed that all three equivalents of CO were released from the photoCORM during irradiation, though no intermediates were observed. Cellular uptake of the photoCORM and a protective effect of light-activated CO release under conditions of hypoxia was demonstrated.

Bengali and co-workers used a di-imine ligand in the construction of *fac* complexes (Fig. 21), giving a system with an absorption maximum as 582 nm.[70] This system exhibits CO release at 560 nm as indicated by IR spectroscopy of the complex itself. The photolability of the Mn–CO bond was assigned to a metal-ligand charge-transfer (MLCT) band to the di-imine π^* orbitals from a combination of the Mn–CO π orbital the bromide p orbital. This change would be expected to disrupt back-bonding from CO to the metal and thus promote ligand loss. This analysis was supported by time-dependent density function theory (TD-DFT) cal-

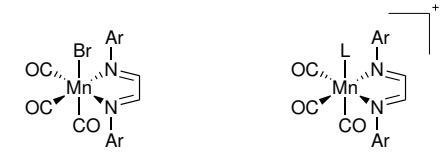


Fig. 21 Di-imine system with rapid CO release at 560 nm[70]

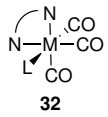


Fig. 22 General complex structure explored by Mascharak group (L = Br^-, PPh_3, solvent, chelating ligand; M = Mn, Re)

culations.

Conversion of the neutral system to a series of cationic complex (**31**, L = CO, THF, MeCN) could be achieved using a halide abstractor. These systems lack the halide p orbital present in **30**, displacing the absorption maximum to 420 nm and significantly increasing light stability. For example, in the case L = CO, the time required for CO release is raised by around six times under identical conditions.

The most significant contribution to date to the development of visible light photoCORMs has been made by the Mascharak group.[30] Their approach has been focussed on a *fac*-$Mn(CO)_3$ core featuring a bidentate (or potentially tridentate) nitrogen ligand and one additional supporting ligand (typically Br^- or PPh_3) (Fig. 22). In this approach, the ability of the CO to dissociate is increased by providing low-lying orbitals on the ligand system. This then allows electron density to transfer from the M–CO bonding orbitals on photo-activation and thus enhances photolability.

Following early work with release primarily in the UV,[60] this approach was followed making use of both bidentate and potentially tridentate Schiff base ligand architectures (Fig. 23).[71] When complexed to the target manganese core, only two donors (highlighted in blue) bind to the metal, with the third remaining uncoordinated. These systems show significant absorptions in the visible, with the sulfur-containing ligand **34** giving the most desirable outcome. Notably, there was a marked difference between complexes bearing halide ligands and those featuring other donors: *fac*-Mn(**34**)$(CO)_3$Br as an absorbption maximum of 535 nm which is shifted to 435 nm in *fac*-[Mn(**34**)$(CO)_3$(MeCN)][ClO_4]. Illumination with low power (5 mW) visible light (broad band with a 400 nm cutoff) led to CO release with quantum yields in the range 0.12 to 0.37 at 509 nm. The complex *fac*-[Mn(**32**)$(CO)_3$(MeCN)][ClO_4] was later supported electrostatically on mesoporous Al-MCM-41 nanoparticles,[72] where broadband illumination leads to the anticipated CO release.

Ligand **34** was later reacted with ruthenium to give a series of complexes featuring a meridional architecture (Fig. 24):[73] in contrast to manganese, all three donor atoms bind to ruthenium.

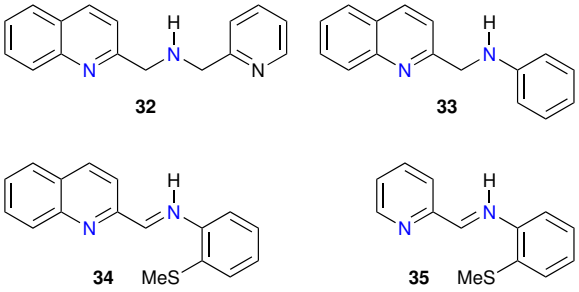


Fig. 23 Tridentate ligands explored by the Mascharak group: nitrogen atoms which chelate in a bidentate fashion with Mn are highlighted in blue

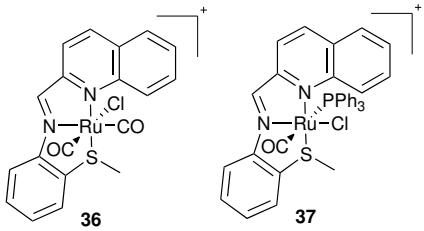


Fig. 24 Meridional ruthenium complexes[73]

Both **36** and **37** release CO when illuminated in the UV (30 mW, 300 nm to 350 nm), but only **37** does so in visible light. The rate of CO release is dependent on wavelength: k_{CO} for **37** is 0.031(1) with light cut off at 380 nm but only 0.0032(1) when the filter is at 440 nm. Thus the best CO release behaviour is seen with the *fewest* CO ligands, a consequence of the need to control MLCT bands.

Building on this work, Mascharak described the behaviour of *fac*-$Mn(CO)_3$ supported by bidentate ligands **38** and **39** (Fig. 25).[74,75] These systems offer good CO release behaviour in the visible, with *fac*-[Mn(**38**)$(CO)_3$Br] showing an absorption maximum at 586 nm, and a rate for CO release of 5 min^{-1} when irradiated with broadband light (cutoff 520 nm). Carbon monoxide release from *fac*-[Mn(**39**)$(CO)_3$Br] can be traced *in vitro* by fluorescence measurement and was shown to lead to cell apoptosis.

The comparison between Mn and Re complexes of ligand **38** was used to explore the detailed requirements for CO release.[76] Whilst both complexes of formula *fac*-M(**38**)$(CO)_3$Br (M = Mn, Re) have absorption maxima between 500 nm and 600 nm, only the manganese complex is effective as a visible light photoCORM. Similar results were obtained for *fac*-[M(**38**)$(CO)_3(PPh_3)$][ClO_4] (though here there is greater difference in the λ_{max} values). This

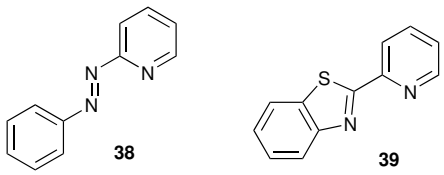
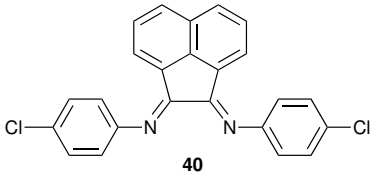


Fig. 25 Bidentate *N,N*-ligands for *fac*-$Mn(CO)_3$ photoCORMs[74,75]



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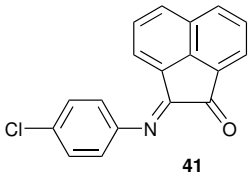

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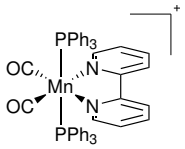
Fig. 26 Ligands for extremely active manganese photoCORMs[77]



42

Fig. 27 PhotoCORM for use with a nanoparticle up-converter system.[78]

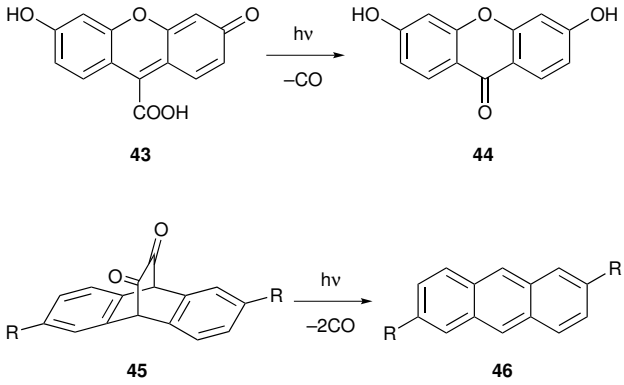
experimental data was combined with TD-DFT results to show that UV-visible spectra alone are not predictive of good CO release: it is the nature of the absorbing orbitals which is important, as energy must be transferred to CO-bond cleavage.

Most recently, this work has been extended to the ligands **40** and **41** (Fig. 26).[77] These more extended π-systems lead to *fac*-Mn(L)$(CO)_3$Br complexes with strong bands well into the visible (above 550 nm) and which are light sensitive in the solid state under ambient light. Complex *fac*-Mn(**40**)$(CO)_3$Br is currently the lead example of a visible light photoCORM, with a quantum yield of $0.7(2)$ at 545 nm. The rate of CO release from these systems is also very high, with complete CO release achieved in seconds.

Complementing this approach of ligand-based tuning, Ford and co-workers have recently reported an approach making use of nanoparticle up-converters to enhance CO release.[78] Complex **42** (Fig. 27) will release roughly two equivalents of CO when irradiated at 470 nm. This complex could then be entrapped in a a phospholipid-functionalised poly(ethylene glycol) layer surrounding lanthanide ion doped upconversion nanoparticles. The polymer renders the ensemble water-soluble whilst the nanoparticle system will allow useful CO release using infra-red light. Irradiation of the combined photoCORM/nanoparticle construct at 980 nm resulted in CO release mirroring that seen when using UV light (365 nm). This approach thus offers the opportunity to create very sensitive release systems by bypassing elaborate ligand/complex design and synthesis.

7 Metal-free photoCORMs

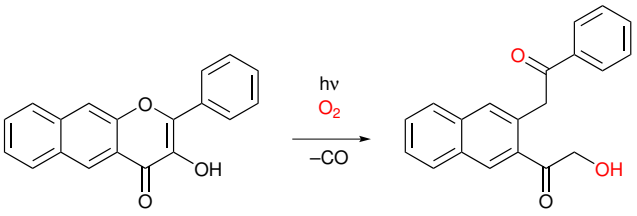
Whilst most photoCORMs are based around metal carbonyl complexes, a small number of systems have been reported which do not feature a metal centre . Klán and co-workers have reported a system based on the fluorescein analogue **43** (Fig. 28).[79] The behaviour of this system is pH-dependent, with the compound giving off CO under visible irradiation (500 nm) in phosphate buffer (pH = 7.4) with a small quantum yield, $7(3) \times 10^{-4}$. Under neutral and acidic conditions the photoproduct could be identified as **44**. Published almost at the same time, Liao and co-workers described CO release from the anthracene derivative **45** (Fig. 28).[80] This material is formed *via* Diels–Alder chemistry, and then undergoes a known photoreaction to release two equivalents of CO under irradiation at 470 nm. To prevent hydration of the diketone unit interfering in this process, the molecules were encapsulated in hydrophobic micelle structures.



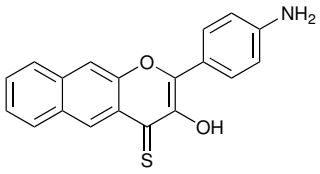
43 **44**

45 **46**

Fig. 28 Metal-free photoCORMs (R = H, $(OC_2H_4)_2OMe$, OC_8H_{17})[79,80]



47 **48**



49

Fig. 29 Metal-free photoCORM requiring O_2[81]

Very recently, Berreau and co-workers have reported the light-driven release of CO from a series of compounds related to **47** (Fig. 29).[81] Carbon monoxide is released by this system when irradiated at 419 nm in the presence of oxygen, which labelling studies demonstrated is incorporated into the photoproduct. By adjusting the framework to give **49**, CO release could be affected using light with wavelength >546 nm.

8 Biological testing

Whilst testing photoCORM systems for CO release is well understood in terms of myoglobin assay and quantum yield measure-

ments, there has been much less attention paid to testing the same systems *in vitro*.

Testing for cytotoxicity is the most widely examined criterion, with cell viability assessed either in presence or absence of light in a number of reports.[65,67,75,80] Whilst these studies show that the compounds tests are not toxic, the potential of photoCORMs in active toxicity toward cancer cells has also been considered.[46,74] The latter studies focus on the possible 'turn on' of activity when irradiated.

Beyond toxicity, there are a small number of examples of testing for other biological effects. Mascharack and co-workers have tested for vascular dilatation in rat aorta cells,[60,72] whilst Quaroni and co-workers have shown a protective effect in hypoxia conditions.[69] Activation of Ca^{2+} potassium channels expressed has been demonstrated by Westerhausen and co-workers.[64] Notably, whilst anti-inflammatory effects are known for CO, and have been demonstrated in other CORMs,[82] to date there are no reports of photoCORMs showing this activity in whole-cell assays.

9 Summary and outlook

Developments in photoCORMs has progressed rapidly in the past decade. Early structures based around simple metal carbonyls have been supplanted by more sophisticated approaches in which manipulation of MLCT bands allows tuning the photosensitivity well into the visible range.[30] Achieving CO release with visible light in combination with stable, water-soluble materials is beginning to be realised, but we are only now mapping out the key requirements for this chemistry to be achieved. New systems are likely to address both the fundamental and practical challenges that remain in the coming years, particularly in combination with biomaterials for intracellular delivery.[83]

Whilst the underlying chemistry is now becoming better-understood, it is clear that for application more attention must begin to be focussed on charactering the performance of putative photoCORMs *in vitro*. Currently, data on the cytotoxicity of the new materials is sparse, with activity in for example anti-inflammatory assays thus-far unreported. Understanding the behaviours of the new systems in these real-world tests is vital.

New visible-light photoCORMs pose their own questions: just how much light sensitivity is 'best'? By expanding the range of molecules available such that we can now being to test this, current developments are pathing the way to examine this question. It can only be fully understood outside of the laboratory context: the complexities of real use cases are often a long way from the idealised conditions of the academic laboratory.

Biographies

Mark A. Wright obtained a first class Masters in Chemistry from the University of East Anglia in 2013, writing his Masters dissertation on amine adducts of mono(pentafluorophenyl)borane and dehydrocoupling intermediates using group four metallocenes. He then began his PhD developing carbon monoxide releasing molecules as potential therapeutic agents.

Joseph Wright obtained his PhD degree from the University of Cambridge in 2003 working on the mechanism of the Wacker reaction under the supervision of Dr Jonathan Spencer. He then undertook postdoctoral positions at the University of Southampton (2003–2004 in the group of Dr Andreas Danopoulos) and at UEA (2005–2008 with Professor Manfred Bochmann and 2008–2012 with Professor Christopher Pickett). In 2012 he was appointed as Lecture in Energy Materials at the University of East Anglia, and has interests in organometallic chemistry, reaction mechanism and DFT modelling.

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