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CO Release from Norbornadiene Iron(0) Tricarbonyl Complexes: Importance of Ligand Dissociation

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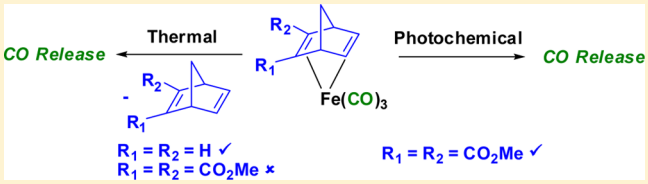
Supporting Information

ABSTRACT: An investigation into the CO-releasing properties of a range of iron tricarbonyl and chromium and molybdenum tetracarbonyl complexes is presented. Iron tricarbonyl complexes containing the 2,5-bicyclo[2.2.1]-heptene (norbornadiene) ligand are shown to be effective CO-releasing molecules, in which the rate and extent of CO release may be modulated by modification of the norbornadiene framework. Species containing the parent norbornadiene and those with a substituent at the 7-position of the organic ligand exhibit CO release; those containing ester substituents at the 2- and/or 3-positions do not. A mechanism for CO release in this species is proposed which involves initial norbornadiene dissociation, a suggestion which is supported by the spectroscopic data and the observation that the addition of excess substituted norbornadiene retards the rate of CO release. CO release from the diester-containing norbornadiene complex may be promoted photochemically, and cell viability studies indicate that in the absence of light this complex is nontoxic, making it an excellent candidate for further study as a photo-CO-RM. Both the chromium and molybdenum tetracarbonyl complexes release CO, which in the case of the molybdenum analogue is rapid.



INTRODUCTION

In apparent contrast to its well-established toxicity, carbon monoxide (CO) has been shown to have a significant physiological function.[1] In mammals, the majority of endogenously produced CO arises from the catabolism of heme. Following the discovery that CO plays a well-defined physiological role, its ability to act as a potential beneficial therapeutic agent has been extensively investigated. Indeed, CO gas has been shown to be anti-inflammatory,[2] to protect ischemic damage and reperfusion injury,[3] to regulate blood pressure,[4] and to act as an antibacterial agent.[5] CO may also suppress organ graft rejection[6] and assist with the treatment of cerebral malaria.[4,7]

Although the beneficial effects of carbon monoxide have been explicitly demonstrated, the gaseous form does not represent a long-term therapeutic solution, principally due to its inherent toxicity and lack of selectivity. Furthermore, the precise dosing of the flammable gas is problematic. In order to circumvent these potential drawbacks, carbon monoxide-releasing molecules (CO-RMs) have been developed as therapeutic agents that will act as sources of CO in vivo.[1a,8] The vast majority of CO-RMs take advantage of the well-known affinity of transition-metal compounds for CO, although a boron-based CO-RM has also been employed[9] and it has been shown that organic compounds such as CH_2Cl_2 may also act as sources of carbon monoxide.[1a]

Due to the diverse nature of the applications, the optimum release rate for CO release is not known; thus, the development of a variety of CO-RMs with different activities is desirable. One successful strategy to develop a new CO-RM has focused on the incorporation of biologically compatible ligands such as amino acids,[10] sugars,[11] and the 2-pyrone motif,[12] with a view to ensuring that potential toxic side effects are minimized. In addition, much of the development of new CO-RMs has focused on the use of metals with a defined biological role such as iron,[10d,e,12,13] manganese,[14] and molybdenum.[10c,11,12b,13c] However, this has not precluded the use of a wide range of metals which have no, or little, intrinsic biological role, notably with ruthenium in CO-RM-3[10a,b] as well as chromium-,[10c,13c] tungsten-,[10c,13c,15] rhenium-,[14h,16] cobalt-,[17] and iridium-based[18] systems being developed.

The mechanistic processes which control CO from a potential therapeutic agent have been investigated. In some instances, the rate of release of CO has been compared with various spectroscopic parameters involving the carbonyl ligand, such as the CO stretching frequency in the infrared spectrum,[13b,d] and in some cases a higher CO stretching frequency correlates with a more rapid rate of release. These data have been interpreted in terms of reduced metal−carbon back-bonding, resulting therefore in a more labile CO ligand (q.v.) and a mechanistic picture in which initial CO dissociation is dominant.[13d,14f] However, in some instances, the infrared stretching frequency of the metal carbonyl groups does not correlate with the rate of CO release and a number of alternative mechanistic processes in which release is triggered by, for example, hydrolysis[13c] or dissociation[10c] of a coligand or interaction with the delivery medium[19] have been established. Other methods for the activation of metal carbonyls have

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Scheme 1. Preparation of Iron Norbornadiene Complexes According to Pettit[22a] (4a), Watson[22b] (4b), and Landesberg[23] (4d)

3a $R_1 = R_2 = R_3 = H$
3b $R_1 = R_2 = CO_2Me$ $R_3 = H$
3c $R_1 = CO_2Me$ $R_2 = Ph$ $R_3 = H$
3d $R_1 = R_2 = H$ $R_3 = O_2CPh$

4a $R_1 = R_2 = R_3 = H$ 5 % yield
4b $R_1 = R_2 = CO_2Me$ $R_3 = H$ 9 % yield
4c $R_1 = CO_2Me$ $R_2 = Ph$ $R_3 = H$ 5 % yield
4d $R_1 = R_2 = H$ $R_3 = O_2CPh$ 25 % yield

employed the use of open-shell metal carbonyl complexes,[16] species in which CO release is initiated by interaction with an enzyme[13f] (enzyme-triggered or ET-CORMs), and the use of photochemical methods to promote carbonyl dissociation.[10e,11,13h,14b,c,f,g,i,j,15,20]

A clear understanding of the mechanism of CO release for any given structural type is required in order for structure–activity relationships to be developed. A distinct advantage of using transition-metal complexes as CO-RMs is that subtle ligand alterations may be used to modulate the steric and electronic properties of the metal center and ultimately the rate and extent of CO release. However, the modification of a given ligand framework must be informed by the fundamental steps that govern the release process. Indeed, the parallels between designing transition metals for catalytic and therapeutic applications have recently been discussed.[21]

As our previous investigations into the chemistry of iron tricarbonyl complexes containing 2-pyrone ligands[12] have demonstrated that substitution of the ligand backbone may be used to modulate CO release and that the complexes exhibited low toxicity, we have explored other tunable ligand frameworks bound to this metal fragment. We now report the CO-releasing properties of a number of iron tricarbonyl complexes containing substituted norbornadiene ligands as well as their chromium and molybdenum tetracarbonyl analogues.

RESULTS AND DISCUSSION

The aim of this study was to investigate the CO-releasing properties of Fe(0), Cr(0), and Mo(0) complexes containing norbornadiene and related ligands. A particular goal was to establish the factors controlling the CO release from the metal. As shown in Scheme 1, the preparation of the iron complexes is generally conveniently performed from the reaction of the free ligand with either $Fe(CO)_5$ or $Fe_2(CO)_9$:[22,23] the chromium and molybdenum tetracarbonyl analogues may be prepared from an analogous reaction using the corresponding hexacarbonyl complexes as precursors.[24]

The CO-releasing properties of the complexes described in this study were assessed with the aid of an assay based on myoglobin. Treatment of an aqueous solution of deoxygenated myoglobin (deoxy-Mb) with the complex in question results in the subsequent capture of any CO released by the complex as carbonmonoxymyoglobin (Mb-CO), and this process may be quantified using the changes to the Q bands of the protein in the UV/vis spectra. This allows for the rate and extent of CO release to be determined. It is convenient to quote the half-life for CO-release, $t_{1/2}$, defined as the time taken for a solution with CO-RM concentration of X μM to produces a concentration of Mb-CO of $X/2$ μM, as a mechanism-independent measure of the release rate.

Thermal CO-Release Studies. Neither (η^4-1,3-cyclohexadiene)iron(0) tricarbonyl (**1**) nor [(η^5-cyclohexadienyl)iron(II)tricarbonyl][BF_4] (**2**) exhibited any CO release over the course of 2 h. This is consistent with the (diene)iron tricarbonyl based ET-CORMs, which do not release CO unless in the presence of an enzyme.[13f] In contrast, (η^4-norbornadiene)iron(0) tricarbonyl (**4a**) (Scheme 1) was able to convert deoxy-Mb to Mb-CO with $t_{1/2}$ = 49.8, 49.8, and 59.5 min for concentrations of **4a** of 20, 40, and 60 μM, respectively (Figure 1). The final concentration of Mb-CO formed by the two lower concentrations of CO-RM indicates that only one CO is liberated from the complex.

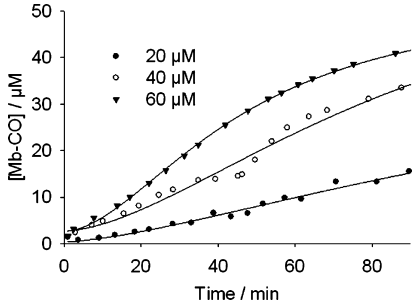

Figure 1. CO-release profile of **4a** showing [Mb-CO] versus time. DMSO was used as the addition solvent, and the CO-RM concentration is indicated in the legend.

The well-controlled CO-release profiles exhibited by **4a** prompted the exploration of the effects of different substituents within the norbornadiene framework in order to modulate the release rate and provide mechanistic insight into the release process. Previous work has shown the importance of electron-withdrawing substituents in accelerating CO-release rates, which is consistent with a reduction in π back-bonding from the metal to the carbonyl ligand and thus weaker M–C bonds.[13d] Therefore, the inclusion of ester substituents onto the norbornadiene backbone would be expected to increase π back-donation to this ligand at the expense of the metal carbonyls, thus weakening the metal–carbon bond. Indeed, the π-acidic nature of a diester-substituted norbornadiene has been shown to enhance catalytic activity in palladium-catalyzed cross-coupling reactions.[25] Two strategies were employed to achieve this goal: first, ester groups were incorporated at the 2- and 3-positions of the norbornadiene ligands, and second, ester and alcohol groups were introduced at the 7-position of the ligand.

The complex containing ester substituents at both the 2- and 3-positions of the norbornadiene framework, **4b**, was obtained from the reaction of $Fe_2(CO)_9$ with diester **3b** (Scheme 1). The monoester-substituted norbornadiene ligand **3c** was prepared as a racemic mixture via a Suzuki coupling from the corresponding bromo-substituted precursor using a palladium-

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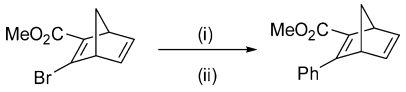
(II) precatalyst (Scheme 2) developed by Fairlamb and Taylor.[26] Reaction of **3c** with $Fe_2(CO)_9$ results in the formation of **4c**, also as a racemic mixture (Scheme 1).

Scheme 2. [a]



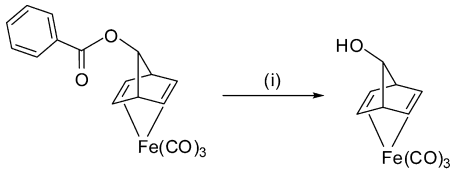
3f **3c**

[a]Legend: (i) $PhB(OH)_2$, [PdBr(*N*-succinimidyl)$(PPh_3)_2$] (1 mol %), THF, 60 °C; (ii) 2 M Na_2CO_3 80 °C, 2 h.

An examination of the IR spectra of **4b,c** reveals that the introduction of the electron-withdrawing substituents on the norbornadiene ligand resulted in a shift in the highest frequency CO band in comparison with the parent complex **4a** (**4a**, 2028 cm^{-1}; **4b**, 2058 cm^{-1}; **4c**, 2046 cm^{-1}). However, no CO release was observed from either **4b** or **4c** in the myoglobin-based assay.

The behavior of the complexes in which a substituent had been incorporated at the 7-position of the norbornadiene framework was further examined. The reaction of 7-benzoyloxynorbornadiene (**3d**) with $Fe(CO)_5$ allowed for the formation of **4d**.[23] Reduction of **4d** with PhMgCl led to the formation of the alcohol derivative **4e** (Scheme 3). In contrast

Scheme 3. Preparation of 4e according to Landesberg[23] [a]



4d **4e**

[a]Legend: (i) PhMgCl, THF, 20 °C, 18 h.

to **4b,c** **4d,e** both released CO in the myoglobin-based assay. However, as shown in Figure 2, the CO-release profiles for both of these complexes exhibited some unusual behavior. Taking complex **4e**, for example, the release rate for the 60 μM concentration of CO-RM release is *slower* than the rate when the assay was performed with a CO-RM concentration of 40 μM. In addition, the 40 μM profile has a plateau in the evolution of CO at approximately 7−17 min, followed by resumption of CO release.

Mechanistic Considerations. The nontrivial behavior exhibited by the iron norbornadiene complexes prompted a series of studies to gain insight into the fundamental process underpinning the CO-release mechanism. A consideration of the spectroscopic data for complexes **4** provided some insight into the ground-state properties of these species. Selected IR and ^{13}C NMR spectroscopic data for the complexes are presented in Table 1. As detailed above, the IR spectroscopic data for ester-containing complexes **4b,c** are at a higher frequency than the substituted derivatives. A traditional interpretation of these data would be that there is less metal−carbon back-bonding to these carbonyl ligands, due to the electron-withdrawing nature of the ester-containing norbornadiene ligand. However, recent studies on metal carbonyl complexes have indicated that high CO stretching frequencies may not necessarily be indicative of weak metal−

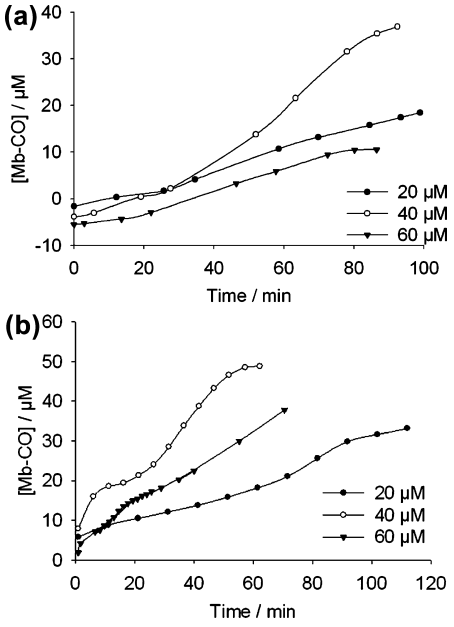


Figure 2. CO-release profile for complexes **4d** (a) and **4e** (b) showing [Mb-CO] versus time. DMSO was used as the addition solvent, and the CO-RM concentration is indicated in the legend.

carbon bonds, as σ bonding and changes to the polarization of the C−O bond are important factors.[27] In any case, the complexes with the highest C−O stretching frequencies do not release CO thermally. An examination of the ^{13}C NMR spectroscopic data for **4a,b** provides insight into the nature of the bonding between the metal and the norbornadiene ligand. In the case of complex **4a** the coordination shift of the norbornadiene ligand (defined as the difference in chemical shift between the free and coordinated ligands) is 105.8 ppm. In the case of **4b**, however, the ester-containing alkene has a far greater coordination shift (116.9 ppm), although the shift for the unsubstituted alkene is not as great.[28]

These data indicate that the ester-substituted alkene in the norbornadiene ligands is more strongly bound to the metal than in the equivalent unsubstituted complex, which points to a mechanism of CO release that is triggered by the initial loss of the norbornadiene ligand rather than CO under thermal conditions.[29]

A further series of experiments were undertaken to probe the unusual CO-release profile exhibited by these complexes. The CO release exhibited by complex **4d** was reassessed using a modified myoglobin assay with added **3d**. To this end, 0, 5, 10, and 20 μM of ligand **3d** were added to four different myoglobin assay solutions prior to the addition of **4d** (40 μM). The four assays were run in parallel, and the amount of Mb-CO formed was calculated in the standard manner. The results of this experiment (Figure 3) show that the release rate of the CO-RM is retarded in the presence of the free ligand, supporting the supposition that the release of CO from this CO-RM is controlled by dissociation of the norbornadiene ligand from the complex.

It is therefore proposed that the key mechanistic step in the thermal CO release from complexes **4** is dissociation of the norbornadiene ligand. This is supported by the spectroscopic data, which suggested that when norbornadiene is more strongly bound, then CO release is slow, and vice versa. In addition, this mechanistic proposal provides an explanation for

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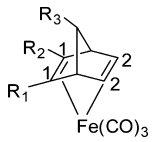
Table 1. [a]



	IR data ν(C≡O)/cm^{-1}			^{13}C NMR data/ppm					
complex	ν^1	ν^2	ν^3	δ_f^1	δ_f^2	δ_c^1	δ_c^2	$\Delta\delta^1$	$\Delta\delta^2$
4a	2028	1954		143.4		37.6		105.8	
4b	2058	2003	1976	153.3	143.1	36.4	60.5	116.9	82.6
4c	2046	1992	1962						
4d	2034	1963							
4e	2032	1959							

[a]Definitions: δ_f = frequency of uncoordinated alkene; δ_c = frequency of coordinated alkene; $\Delta\delta^1 = \delta_f^1 - \delta_c^1$; $\Delta\delta^2 = \delta_f^2 - \delta_c^2$.

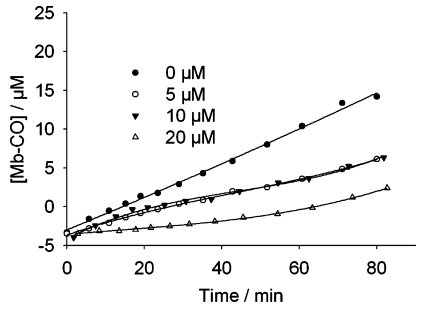


Figure 3. CO-release profile of **4d** (40 μM) with added free norbornadiene ligand (**3d**, concentration of norbornadiene indicated in legend) showing [Mb-CO] versus time. DMSO was used as the addition solvent.

the unusual CO-release behavior exhibited by these complexes. For example, in the case of **4d**,**e** the most rapid release is when the concentration of CO-RM is 40 μM and the rate and extent of CO release by **4d** is the slowest for the most concentrated solution. If the CO-release process is controlled by the loss of the norbornadiene, then as complete substitution at the metal by a suitable donor such as DMSO occurs, the concentration of the free ligand in solution will increase with time. This liberated ligand would then be expected to inhibit further loss of coordinated norbornadiene. Inhibition will be more rapid for higher concentrations of CO-RMs, which explains the unusually slow release of the more concentrated solutions of **4d**,**e**. Although a full kinetic analysis of the CO-release profiles has not been performed, the presence of free ligand inhibiting norbornadiene dissociation may explain the stepwise nature of the release profile of a number of these species. No evidence for interaction of the free ligands **3a**,**d** (40 μmol) with either deoxy-Mb or Mb-CO was obtained under the conditions of the myoglobin-based assay. Finally, it is important to note that the CO-release behavior exhibited by the parent compound **4a** shows fewer deviations from the release profiles typically observed than do either **4d** or **4e**. This may simply reflect the lower solubility of unsubstituted norbornadiene in the aqueous medium in comparison to the ester- or alcohol-substituted compounds; thus, inhibition in the case of **4a** will not be as pronounced. It should also be noted that the half-life for CO release in the case of **4a** is slowest for the most concentrated solution.

Photochemical CO Release and Toxicity Study on 4b. Given that no CO release was observed from **4b** under thermal conditions, the possibility of using photochemical methods to activate the complex was explored. A solution of **4b** (final concentration 40 μM) was prepared and administered to the Mb assay: as expected, only a trace amount of Mb-CO was formed over the course of ca. 40 min. After this time the sample was exposed to irradiation from an LED light source (2.4 W, 400 nm, irradiation for 2 min during each 5 min period), which promoted the release of CO (Figure 4a) and saturation of the

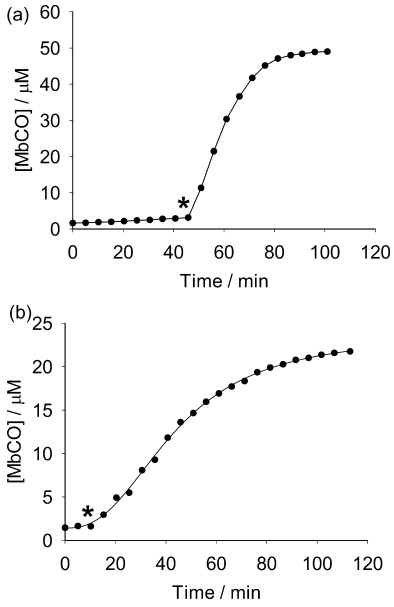


Figure 4. CO-release tests on **4b** showing [Mb-CO] versus time: (a) 2.4 W, 400 nm irradiation, 2 min on, 3 min off, [**4b**] = 40 μM; (b) 2.4 W, 400 nm irradiation, 2 min on, 3 min off, [**4b**] = 10 μM. The asterisk marks the start of irradiation.

assay within ca. 1 h of irradiation. An identical assay performed on a solution of CO-RM (10 μM) resulted in a final Mb-CO concentration of ca. 20 μM, indicating that each molecule was releasing on average 2 equiv of CO (Figure 4b). These data illustrate that **4b** is a photochemically activated CO-releasing molecule (photo-CO-RM), and the fact that two molecules of CO are released as opposed to one for the thermally activated analogues may point to a different mechanistic process (i.e., direct CO expulsion from the metal) occurring in this case.

The cytotoxicity of **4b** in the absence of irradiation was assessed with the aid of two assays. Both lactate dehydrogenase (LDH) and Alamar Blue cell viability assays were performed on RAW264.7 macrophages.[12b,13a,b,d,14f,19] The LDH assays allow

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for an evaluation of the damage to a cell membrane, whereas the Alamar Blue assay is an index of cell metabolism. The results of both assays demonstrate that under the conditions employed **4b** is essentially nontoxic to the cells. No LDH is released from the cells up to a **4b** concentration of 140 μM (Figure 5), indicating that no detectable damage to the cell wall

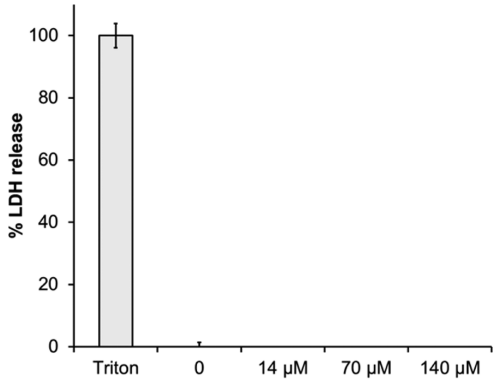


Figure 5. LDH release from RAW264.7 cells treated for 24 h with increasing concentrations of **4b**. LDH is expressed as a percentage of the total LDH released from Triton-treated cells.

was occurring. Furthermore, the addition of **4b** up to concentrations of 140 μM did not significantly affect cell viability (Figure 6). The inherently low toxicity of **4b** makes it an attractive photo-CO-RM, as it is predicted that no adverse side effects will occur in areas which are not exposed to irradiation.

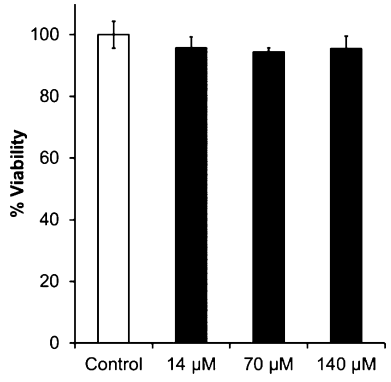
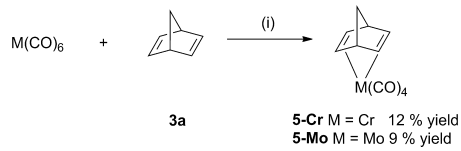


Figure 6. Cell viability of RAW264.7 macrophages exposed to increasing concentrations of **4b** for 24 h. Viability is expressed as a percentage relative to the control.

Chromium and Molybdenum Norbornadiene Complexes. Given the diversity of CO-release profiles that are exhibited by the iron complexes **4**, the related chromium- and molybdenum-containing complexes **5-Cr** and **5-Mo** were prepared from the reaction of the corresponding metal hexacarbonyl and norbornadiene (Scheme 4). Both **5-Cr** and **5-Mo** were found to convert deoxy-Mb to Mb-CO when added as either EtOH or DMSO solutions to the assay, indicating that both complexes are active CO-RMs. The half-lives of CO release for **5-Cr** were found to be 3.2, 4.2, and 2.8 min at CO-RM concentrations of 20, 40, and 60 μM, respectively, in EtOH and 0.8, 1.6, and 0.7 min at the same concentrations, respectively, in DMSO. The molybdenum analogue was found to release CO extremely rapidly: in EtOH, $t_{1/2}$ values

Scheme 4. [a]



[a]Legend: (i) $^{n}Bu_2O$, hexane, reflux (**5**-Cr, 2 days; **5**-Mo, 20 h).

of 0, 2.3, and 12.0 min at CO-RM concentrations of 20, 40, and 60 μM, and in DMSO, $t_{1/2}$ values of <1 min at 20 and 40 μM and 0.7 min at 60 μM.

An examination of the behavior exhibited by **5-Mo** demonstrated an important feature that must be considered when evaluating fast-releasing CO-RMs. The stock solution of CO-RM was prepared in EtOH and then added to six different solutions of deoxy-Mb at intervals of ca. 10 s; the evolution of Mb-CO was then monitored over the course of ca. 30 min. As shown in Figure 7, the incubation time in the solvent before

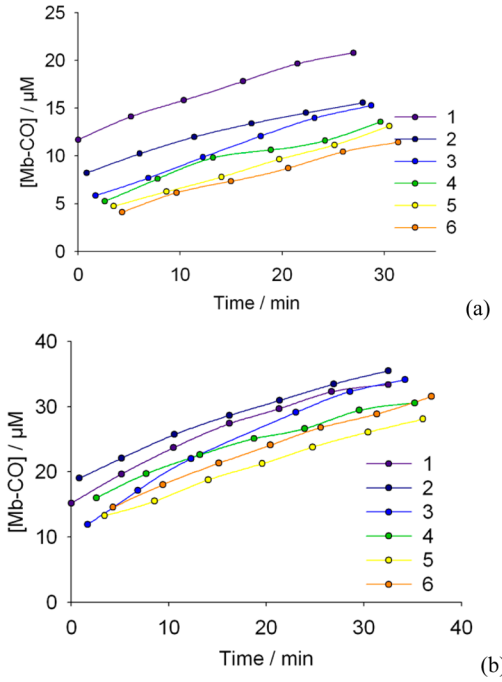


Figure 7. CO-release profile of **5-Mo** (20 μM) showing [Mb-CO] versus time: (a) EtOH as the addition solvent; (b) DMSO as the addition solvent. The order of addition of the CO-RM to deoxy-Mb is indicated in the legend and occurred at 10 s intervals.

addition to the protein had a profound effect on the release profile. Longer incubation times resulted in lower conversion to Mb-CO—similar observations were made when DMSO was used as the delivery solvent, although the effect was not as pronounced. These data demonstrate that **5-Mo** undergoes a rapid reaction on dissolution in EtOH or DMSO solutions which triggers CO release. Hence, the longer the residence time in the solvent before addition to deoxy-Mb, the more CO is released to the surroundings and the less is sequestered as Mb-CO. Similar incubation effects have been observed in the case of the $[Co_2(CO)_6(\mu\text{-alkyne})]$ complexes.[19] In the case of these cobalt complexes differences in CO-release profiles were observed on incubation over a period of minutes, rather than seconds. The results from both sets of complexes illustrate the

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importance of establishing the nature of any incubation effects exhibited by any given CO-RM and that consistent residence times before administration to deoxy-Mb are used.

The behavior of **5-Cr** and **5-Mo** in EtOH and DMSO solutions was probed by in situ IR analysis using a ReactIR instrument. IR spectra of **5-Cr** recorded immediately on dissolution in DMSO exhibited three bands at 2022, 1923, and 1882 cm^{-1}, similar to those observed in CH_2Cl_2 (2027, 1932, 1893 cm^{-1}). Over the period of ca. 40 min these bands decreased in intensity, to be replaced by a weak peak at 2077 cm^{-1} and a strong band at 1951 cm^{-1} (Figure 8). After addition

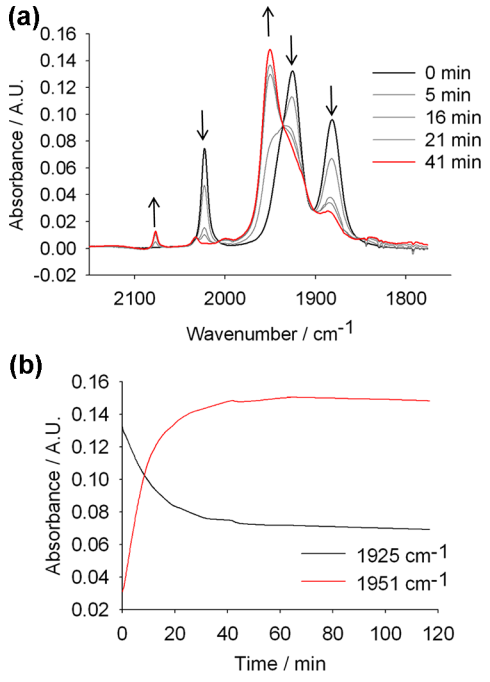

Figure 8. (a) IR spectrum of **5-Cr** in DMSO over time. (b) Time dependence of the bands at 1951 and 1925 cm^{-1}.

of CH_2Cl_2 to this solution and washing with water, the resulting organic layer was dried with $MgSO_4$. Removal of the solvent in vacuo afforded a yellow solid which exhibited a strong band at 1953 cm^{-1} and weak peaks at 2004 and 2080 cm^{-1}. This species has been observed previously in the reaction of $[NEt_4][CrBr(CO)_5]$ with DMSO and was assigned to $[Cr(CO)_5(DMSO)]$.[13c] Unfortunately, the behavior of the molybdenum analogue was far more complicated and meaningful information could not be obtained. Immediately on addition of DMSO a number of new bands were observed (Figure 9) which could not be assigned; given that bands were observed at frequencies as low as 1750 cm^{-1}, it is proposed that a number of CO ligands were substituted by DMSO and complexes containing both terminal and bridging CO ligands were formed. However, mechanistic information on this process could not be obtained on this rapid reaction.

The IR spectrum of **5-Cr** recorded on immediate dissociation in EtOH exhibited three bands at ν 2029, 1936, and 1902 cm^{-1}, which are similar to those observed in CH_2Cl_2 solution. Over a period of time the bands at 2029 and 1902 cm^{-1} decreased in intensity and a new peak for $Cr(CO)_6$ was observed at 1983 cm^{-1}: the band at 1936 cm^{-1} does not alter in intensity, suggesting that an intermediate species with a band coincident with that of **5-Cr** has been formed (Figure 10a). The

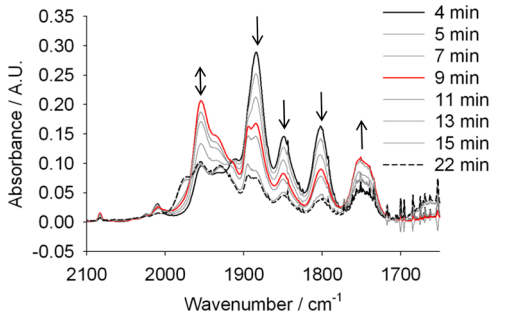

Figure 9. IR spectrum of **5-Mo** in DMSO over time.

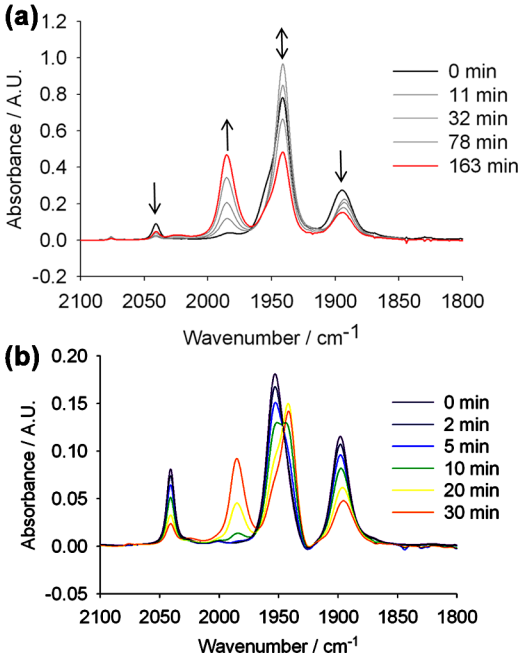

Figure 10. IR spectra of (a) **5-Cr** and (b) **5-Mo** in EtOH recorded over time.

corresponding experiment with **5-Mo** showed different behavior, as the spectrum recorded on initial dissolution in EtOH was markedly different from that observed in CH_2Cl_2 solution (EtOH, 1942 cm^{-1}; CH_2Cl_2, 2041, 1952, 1888 cm^{-1})—however, on standing, this band decreased in intensity to be replaced by a band due to $Mo(CO)_6$ at 1985 cm^{-1} (Figure 10b). Repeating the experiment with **5-Mo** in the presence of 100 equiv of free norbornadiene resulted in a far slower degradation. In this case, the initial IR spectra recorded in EtOH closely matched those acquired in CH_2Cl_2 solution and a slower conversion to the intermediate with a band at 1942 cm^{-1} and subsequently $Mo(CO)_6$ occurred.

These in situ IR spectroscopic studies demonstrate that both **5-Cr** and **5-Mo** are intrinsically unstable in both EtOH and DMSO and, as would be expected on the basis of the CO-release profiles obtained from the myoglobin assay, the molybdenum complex degrades more rapidly than its chromium congener. As is the case with related studies on other group 6 metal carbonyls such as $[NEt_4][CrCl(CO)_5]$, the formation of the corresponding hexacarbonyl species and $[Cr(CO)_5(DMSO)]$ is observed,[13c] indicating that in the absence of myoglobin the group 6 metal acts as an effective CO scavenger. Given that Mb-CO is formed in the appropriate assays, the binding constant between the group 6 metal and the

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liberated CO must be lower than that of the iron in myoglobin (ca. 10^7 M^{-1}).[30] Although norbornadiene dissociation does evidently occur in these group 6 systems, it is less clear if this is the rate-controlling step, as appears to be the case for the iron-containing analogues.

CONCLUSIONS

The nature of the norbornadiene ligand may dramatically modulate the nature of the CO-release process in a series of Fe(0) complexes. Indeed, the incorporation of ester substituents at the 2- and/or 3-positions of the norbornadiene framework provides a sufficient perturbation to inhibit thermal CO release. The spectroscopic and mechanistic data indicate that CO release is controlled by dissociation of norbornadiene rather than direct CO loss from the metal center. The inhibition of thermal release in the ester-substituted compounds provided the opportunity to develop a well-controlled photo-CO-RM. A particularly beneficial feature is that the parent CO-RM does not display any toxicity, ensuring that side effects in areas which are not irradiated could potentially be minimized. Therefore, the ability to modulate the CO-release behavior of this series of molecules is an attractive feature, indicating that higher-yielding synthetic routes should be pursued.

The behavior of **5-Mo** demonstrates that considerable care must be taken when assessing complexes which undergo extremely rapid active solvent-induced CO release. In these cases the incubation time in the delivery solvent may result in artifacts in the myoglobin-based assay being obtained, mirroring previous observations on $[Co_2(CO)_6(\mu\text{-alkyne})]$ complexes.[19] In the most extreme case, no CO release would be detected if the residence time in the delivery solvent is too long in comparison to the rate of release. It is suggested that for complexes which do undergo such rapid release the residence time should be minimized and varied to establish the effects of this variable.

Under thermal conditions for both complexes **4** (Fe) and **5** (Cr and Mo) the active CO-releasing molecules are generated in the reaction medium through either loss of norbornadiene and/or interaction with the solvent. This is an important factor to consider when deriving structure−activity relationships for CO-RMs and indeed other metallo-drugs and may be compared to activation of a precatalyst, which is frequently required in homogeneous catalysis.

EXPERIMENTAL SECTION

General Details. THF, benzene, and diethyl ether were dried over sodium-benzophenone ketyl and were distilled prior to use. DCM was purified with aid of an Innovative Technology anhydrous solvent engineering system. TLC analysis was carried out on Merck TLC aluminum sheets (silica gel 60 F254), and flash chromatography was run on silica gel (Fluka). Compounds were visualized using UV light (254 nm) and a basic aqueous solution of potassium permanganate. Solution cell infrared spectroscopy was undertaken using a Thermo Nicolet Avatar 370 FT-IR spectrometer (2 cm^{-1} resolution). Reactions were monitored using a Mettler Toledo ReactIR ic10 instrument with K6 Conduit, SiComp (silicon) probe, and MCT detector: resolution 4 cm^{-1}, range 4000−650 cm^{-1}, and gain adjustment at 1×. Ultraviolet−visible spectra were recorded using a JASCO V-560 (bandwidth 1 nm). ^1H and ^{13}C NMR spectra were obtained, in the appropriate deuterated solvent indicated, using Bruker AMX500, JEOL EXC400, and JEOL EX270 spectrometers at 500, 400, and 270 MHz for ^1H and 126, 100, and 68 MHz for ^{13}C, respectively. Chemical shifts are reported in parts per million (δ) downfield from an internal tetramethylsilane reference. Coupling constants are reported in hertz (Hz), and spin multiplicities are indicated by the following symbols: s (singlet), d (doublet), t (triplet), q (quartet), m (multiplet), br (broad). ^{13}C spectra were recorded with full proton decoupling. Photoinitiated carbon monoxide release was carried out using a 5 W 400 nm LED attached to the top of a UV cuvette directly above the solution, drawing 2.4 W of power.

Tricarbonyl(η^4-1,3-cyclohexadiene)iron(0) (**1**),[31] tricarbonyl(η^4-1,3-cyclohexadiene)iron(II) tetrafluoroborate (**2**),[31] methyl 3-bromobicyclo[2.2.1]hepta-2,5-diene-2-carboxylate (**3f**),[32] tricarbonyl(η^4-bicyclo[2.2.1]hepta-2,5-diene)iron(0) (**4a**),[22a] tricarbonyl(η^4-dimethylbicyclo[2.2.1]hepta-2,5-diene-2,3-dicarboxylate)iron(0) (**4b**),[22b] tricarbonyl(η^4-7-benzoyloxybicyclo[2.2.1]hepta-2,5-diene)-iron(0) (**4d**),[23] Tricarbonyl(η^4-7-bicyclo[2.2.1]hepta-2,5-dieneol)iron-(0) (**4e**),[23] tetracarbonyl(η^4-bicyclo[2.2.1]hepta-2,5-diene)chromium-(0) (**5-Cr**),[24] and tetracarbonyl(η^4-bicyclo[2.2.1]hepta-2,5-diene)-molybdenum(0) (**5-Mo**)[24] were prepared by literature methods. Additional preparative, spectroscopic, and analytical data for these known compounds and organic precursors are provided in the Supporting Information.

***rac*-Methyl 4-Phenylbicyclo[2.2.1]hepta-2,5-diene-2-carboxylate (3c).** Compound **3f** (0.5 mmol, 115 mg), phenylboronic acid (0.5 mmol, 61 mg), and bromo(*N*-succinimidyl)bis-(triphenylphosphine)palladium(II) (5 μmol, 1 mol %, 4 mg)[26] were added to a Schlenk tube under N_2, dissolved in THF (2 mL), and heated to 60 °C. Aqueous Na_2CO_3 (2 M, 1 mL) was added, and the reaction mixture was heated at 80 °C for a further 2 h. The reaction mixture was cooled to room temperature, extracted with Et_2O, and concentrated in vacuo. The residue was purified by chromatography on silica gel using hexane/Et_2O (9/1 v/v), giving **3c** as a colorless oil (67 mg, 59%). ^1H NMR (270 MHz, $CDCl_3$): δ 2.07 (app dt, J = 6.6, 1.6 Hz, 1H), 2.26 (app dt, J = 6.6, 1.6 Hz, 1H), 3.68 (s, 3H), 3.86 (m, 1H), 4.07 (m, 1H), 6.92 (ddd, J = 5.0, 3.0, 0.8, 1H), 6.99 (ddd, J = 5.0, 3.0, 0.7, 1H), 7.31−7.41 (m, 3H), 7.52−7.55 (m, 1H). ^{13}C NMR (68 MHz, $CDCl_3$): δ 51.2, 58.5, 70.6, 127.7, 128.6, 135.6, 138.9, 140.8, 143.6, 165.9, 166.8. HR (ESI-MS): m/z calcd for $C_{15}H_{15}O_2$ 227.1067, found 227.1068 $[M + H]^+$.

***rac*-Tricarbonyl(η^4-methyl 4-phenylbicyclo[2.2.1]hepta-2,5-diene-2-carboxylate)iron(0) (4c).** To a solution of $Fe_2(CO)_9$ (1.39 mmol, 507 mg) in THF (15 mL) under N_2 was added **3c** (0.93 mmol, 210 mg). The reaction mixture was heated at reflux for 18 h. The solution was concentrated in vacuo and the residues were purified by chromatography on silica gel using hexane/Et_2O (19/1 v/v) to give **4c** as a yellow solid (16.1 mg, 5%). Mp: 53−54 °C. ^1H NMR (500 MHz, CD_2Cl_2): δ 1.32 (app dt, J = 9.6, 1.6 Hz, 1H), 1.49 (app dt, J = 9.6, 1.6 Hz, 1H), 3.60 (m, 1H), 3.65 (s, 3H), 3.83 (m, 1H), 3.95 (app t, J = 3.7 Hz, 1H), 4.11 (app t, J = 3.7 Hz, 1H), 7.22 (t, J = 7.4 Hz, 1H), 7.30 (t, J = 7.4 Hz, 2H), 7.57 (d, J = 7.4 Hz, 2H). ^{13}C NMR (126 MHz, CD_2Cl_2): δ 34.7, 45.5, 51.0, 53.3, 55.0, 55.1, 55.2, 56.1, 126.3, 127.5, 130.7, 141.5, 173.6, 212.9. IR (CH_2Cl_2): ν 2046 (s), 1992 (s), 1962 (s) cm^{-1}. HR(ESI-MS): m/z calculated for $C_{18}H_{15}FeO_5$ 367.0257; found 367.0257 $[M + H]^+$.

CO-release tests were performed as described previously.[10c,13c,17,19] Details of the Almar blue and LDH assays are provided in the Supporting Information.

ASSOCIATED CONTENT

Supporting Information

Text and figures giving preparative and spectroscopic details for known compounds and experimental details for the Alamar blue and LDH assays. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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(29) In a recent publication it has been shown that the sulfur compounds in the myoglobin assay may trigger CO release from CO-RM-3. In the case of the photochemically activated CO-RMs this is clearly not the case, as no CO is released in the dark and the group 6 norbornadiene complexes release CO in pure EtOH or DMSO solution, again indicating that the process is not intitiated by the conditions of the assay. See: McLean, S.; Mann, B. E.; Poole, R. K. *Anal. Biochem.* **2012**, *427*, 36−40.

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