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Synthesis, Characterization and CO-Releasing Properties of Linked Alkyne-Bridging Cobalt Carbonyl Clusters

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Abstract. A new group of CO-releasing molecules (CORMs), linked alkyne-bridging tetrahedral carbonyl clusters, $[CO_2(CH_2C{\equiv}CH\text{-}\mu)_2][Co_2(CO)_6]_2$ (**I**), $[(CH_2)_8(CO_2CH_2C{\equiv}CH\text{-}\mu)_2][Co_2(CO)_6]_2$ (**II**) and $[(CH_2)_8(CO_2\text{-}CH_2C{\equiv}CH\text{-}\mu)_2][Co_4(CO)_8](PPh_3)_4]$ (**III**) have been prepared by reaction of $Co_2(CO)_8$ with two dipropargyl esters $(HC{\equiv}CCH_2OOC)_2$, $(CH_2)_8(CO_2CH_2C{\equiv}CH)_2$ and PPh_3, respectively. All clusters were characterized by elemental analysis, ^{1}HNMR and IR spectroscopy. Cluster III was also characterized by ^{13}CNMR, ^{31}PNMR and MS spectroscopy. The properties of CO releasing from the three clusters was investigated spectro-photometrically by measuring the conversion of deoxymyoglobin (deoxy-Mb) to carbonmonoxy myoglobin (MbCO) in phosphate buffered saline (PBS) solution (PH=7.4) at 37℃. The cluster II shows the best con-trollable CO-Releasing result among these clusters.

Keywords: CO-releasing molecules, Cobalt carbonyl clusters, Dipropargyl ester, Releasing properties

1. Introduction

In the last decade, carbon monoxide (CO) as an important signaling molecule in mammals has received increasing attention in medicine due to its documented beneficial therapeutic effects.[1-5] There are three main areas where CO is being considered as a valuable medical agent: 1) inflammation, 2) cardiovascular diseases and 3) organ preservation and transplantation.[6,7] Although it has been evaluated as an efficient therapic agent in clinical medicine, there are still several problems using gaseous CO in respect of safe administration and delivery to specific target sites in a controlled and measurable manner. As a consequence, a number of metal carbonyl complexes have been designed as prodrugs to release CO *in vivo,* which are known as CO-releasing molecules (CORMs).

Transition metal carbonyl complexes have been predominantly evaluated as CORMs. The initial paper of Mann and Motterlini reported the use of $[Mn_2(CO)_{10}]$, $[Ru(CO)_3Cl_2]_2$, $[Fe(CO)_5]$and $[RuCl(glycinate)(CO)_3]^-$ to act as CORMs. It was shown that they could promote the CO-dependent pharmacological activities, such as producing vasodilatation, attenuating coronary vasoconstriction and reducing acute hypertension, and the $[RuCl(glycinate)(CO)_3]^-$ appears to be excellent from the biological and therapeutic viewpoint.[8,9] Since then, numerous examples based on V, Cr, Mo, W, Mn, Re, Fe, Ru and Co, and a handful of water-soluble CORMs have been reported to act as fast CO donors in the literatures (see a recent review).[1,10]

In previous studies, a series of μ_2-alkyne dicobalt tetrahedron hexacarbonyl clusters have also been evaluated as CORMs,[11] because they possess labile CO ligands[12] and the alkyne ligand which allows the electronic properties of the cobalt centre to be modulated to control over the release rate. Furthermore, this class of alkyne-bridging cobalt carbonyl clusters have been proved to exhibit cytotoxicity towards leukaemia and tumour cells. [13] Basing on the current interest in CORMs, we synthesized and characterized three linked alkyne-bridging cobalt carbonyl clusters $[CO_2CH_2C{\equiv}CH\text{-}\mu)_2][Co_2(CO)_6]_2$ (**I**), $[(CH_2)_8(CO_2CH_2C{\equiv}CH$-

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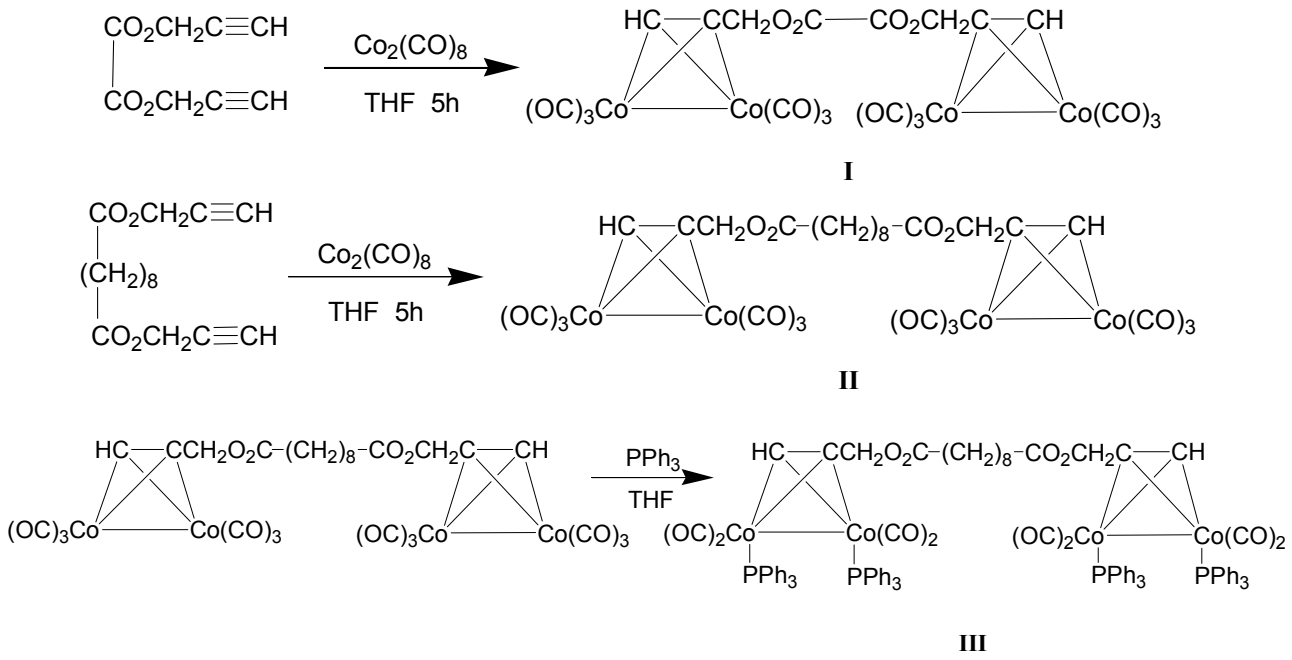
μ)$_2$][$Co_2(CO)_6$]$_2$ (**II**) and [$(CH_2)_8(CO_2CH_2C{\equiv}CH$-μ)$_2$][$Co_4(CO)_8$]($PPh_3$)$_4$] (**III**), and investigated their CO-release behaviours in order to learn the effect of different ligand, the chain's length between two alkyne units on the CO-releasing properties.

2. Experimental

2.1. General details

All reactions were carried out under a nitrogen atmosphere using Schlenk and vacuum line techniques. The solvents were treated using the usual methods for preparing anhydrous and deoxygenated solvents. Chromatographic separations were performed on silica gel columns (160-200 mesh) of varying length. Thin-layer chromatography (TLC) was performed on commercial Merck plates coated with a 0.20mm layer of silica gel. Infrared spectra were recorded on a Nicolet NEXUS 670 FT-IR spectrophotometer. $^1H(^{13}C/^{31}P)$ NMR spectra were measured on a Bruker Avance DPX-400MHz spectrometer in chloroform-d[1]. Chemical shifts are quoted in parts per million (ppm) and referenced to tetramethylsilane (TMS). ESI-MS positive ion spectra were obtained on an Agilent 1100 LC-MSD-Trap-XCT instrument. Elemental analyses (C, H) were performed on a Perkin-Elmer 2400. UV–vis spectra were recorded on a Tu-1901. $(HC{\equiv}CCH_2OOC)_2$ and $(HC{\equiv}CCH_2OOC)_2(CH_2)_8$ were prepared by literature methods[14,17,20]. PPh_3 and $Co_2(CO)_8$ were used as received from a commercial source.



Scheme 1: Synthesis of cluster **I- III**

2.2. Synthesis of cluster I and II

According to the literatures' methods [14, 20], a solution of $Co_2(CO)_8$ (857.7 mg, 2.5 mol) in 15mL of THF was added dropwise into a solution of $(HC{\equiv}CCH_2OOC)_2$ (169.4mg, 1.0 mmol) in 20mL of THF at room temperature. The mixture was stirred for 5h and monitored by TLC. Then, THF was removed under reduced pressure. The residue was s dissolved with 5mL CH_2Cl_2 and eparated by column chromatography on silica gel. Elution with petroleum ether: CH_2Cl_2(1:1) and (3:4) gave the orange-red [$CO_2CH_2C{\equiv}CH$-μ)$_2$][$Co_2(CO)_6$]$_2$ (**I**) (200.5mg, 19.5%) based on $Co_2(CO)_8$. Anal. Found: C, 32.56%; H, 1.04%. Calcd for $C_{20}H_6O_{16}Co_4$: C, 32.55%; H, 0.82%. IR(KBr cm^{-1}): ν(C≡O) 2098.1(m), 2055.7(vs), 2023.1(vs) cm^{-1}, ν(C=O)1659.4 (m) cm^{-1}. ^1H NMR ($CDCl_3$, 400 MHz): δ 6.09 (s, 2H, 2≡CH), 5.58 (s, 4H, 2OCH_2) ppm.

The similar method afforded [$(CH_2)_8(CO_2CH_2C{\equiv}CH$-μ)$_2$][$Co_2(CO)_6$]$_2$ (**II**) (539.6mg, 66.7%). m.p. 132^{o}C; Anal. Found: C, 40.1%; H, 3.01%. Calcd for $C_{28}H_{22}O_{16}Co_4$: C, 39.56%; H, 2.61%. IR(KBr cm^{-1}):

ν(C≡O) 2097.9 (m), 2059.1 (vs), 2025.5 (vs) cm^{-1}, ν(C=O) 1741.7 (m) cm^{-1}. ^1H NMR ($CDCl_3$, 400 MHz): δ 6.06 (s, 2H, 2≡CH), 4.68 (s, 4H, $2OCH_2$), 2.37 (s, 4H, $2CH_2$), 1.64 (s, 4H, $2CH_2$), 1.31 (s, 8H, $4CH_2$) ppm.

2.3. Reaction of cluster II with PPh_3 to give cluster III

Cluster **II** (0.2711g, 0.3189mmol) was treated with PPh_3 (1.6712 g, 6.38 mmol) in 25mL THF at 38℃. The mixture was stirred for 20h and monitored by TLC. Then, the solvent was removed and the residue was separated on silica gel chromatography. Elution with petroleum ether: CH_2Cl_2 (1:2) gave the red $[(CH_2)_8(CO_2CH_2C{\equiv}CH\text{-}\mu)_2][Co_4(CO)_8](PPh_3)_4]$ (**III**) (212.3mg, 10.9%). Anal. Found: C, 64.25%; H, 5.13%. Calcd for $C_{96}H_{82}Co_4P_4O_{12}$: C, 64.51%; H, 4.62%. IR: ν (Ar-H), 3053.47(w) cm^{-1}. ν (C≡O), 2015.9(m), 1987.00(ms), 1957.7 (s) cm^{-1}. ^{1}HNMR($CDCl_3$, 400MHz): δ =7.69-7.27 (60H, $12C_6H_5$), 4.31-3.97 (s, 4H, OCH_2), 1.44-1.29 (s, 16H, $8CH_2$) ppm; ^{13}CNMR ($CDCl_3$): δ =128.28-131.80 ($-C_6H_5$), 77.08 (-C≡C-) ppm; ^{31}P NMR ($CDCl_3$): δ=29.77ppm. MS (m/z):1694.57 (M^+-3CO-8H).

2.4. Determination of CO release using the myoglobin assay

The release of CO from CORMs was measured by using electronic spectrophotometrically to monitor the formation of carboxymyoglobin (COMb) from deoxymyoglobin (deoxy-Mb) at 37 ^oC[8,15,16]. A stock solution of myoglobin (lyophilised horse heart) (*ca.* 60 mM final concentration) was prepared fresh by dissolving the protein in phosphate buffered saline (PBS) (0.01M, pH=7.4). Sodium dithionite (0.1%) was added to convert the myoglobin stock to deoxy-Mb. A 2 mL quantity of this solution was measured to obtain the deoxy-Mb spectrum and then bubbled with CO to get the COMb spectrum. CORMs were dissolved in an appropriate solvent (DMSO or EtOH) and added to deoxy-Mb in the cuvette mixed using a pipette and then overlaid with a little light mineral oil to prevent CO escaping or the myoglobin being oxygenated. [19] The electronic spectra were measured between 500 and 600 nm and the amount of formed COMb was quantified by measuring the absorbance of the heme Q-bands at 540 nm. [18]

3. Results and discussion

3.1. Characterization of cluster I, II and III

The IR spectra of all clusters show a large number of strong terminal carbonyl absorption bands located at 2100–1900 cm^{-1}. The IR spectra of clusters $[CO_2CH_2C{\equiv}CH\text{-}\mu)_2][Co_2(CO)_6]_2$ (**I**) and $[(CH_2)_8(CO_2CH_2C{\equiv}CH\text{-}\mu)_2][Co_2(CO)_6]_2$ (**II**) are very similar, and the vibration frequencies at 2100, 2060 and 2030 cm^{-1} are characteristic of $Co(CO)_3$ unit [14, 20]. Comparing with the parent cluster **II,** the IR spectra of cluster $[(CH_2)_8(CO_2CH_2C{\equiv}CH\text{-}\mu)_2][Co_4(CO)_8](PPh_3)_4]$ (**III**) shows that the highest value of terminal CO stretching frequencies shift from 2097 cm^{-1} toward the lower value 2063 cm^{-1}, resulting from the carbonyl ligands are replaced by PPh_3 groups. This is consistent with our knowledge that the PPh_3 group behaves as a stronger σ-donor and a weaker π-acceptor, when compared to the CO ligand, increasing the electron density on the metals in the cluster. Besides the terminal carbonyl and bridged carbonyl absorption bands, there is an absorption band at 3053 cm^{-1} and 1593 cm^{-1}, which is caused by the C-H vibration of benzene and P-C_6H_5 respectively.

The ^{1}H-NMR spectra of all of clusters **I-III** are consistent with the alkyne-bridged linked tetrahedron structure and show the presence of hydrogen atoms in their corresponding organic groups. For example, in the ^{1}H-NMR spectra of cluster **III,** the broad singlet at aboutδ7.27-7.69 ppm is caused by the protons of the benzene ring. The signals atδ4.07-4.59 ppm and δ2.17-3.97 ppm can be assigned to the protons of the -OCH_2 groups and -CH_2. To further confirm the structure of the cluster **III**, the ^{31}P-NMR spectra also were determined. In the ^{31}P-NMR spectra, it can be seen only one singlet atδ29.77ppm, which reveals that the four P atoms lie in the same chemical environment.

3.2. Delivery of CO to Myoglobin.

As seen in Fig. 1, all of the three clusters can readily release the CO group and reduced deoxy-myoglobin (Deoxy-Mb) to COMb under physiological conditions in 90 minutes, but the cluster **III** seems less controlled due to its speedy CO-releasing behaviour when it was rapidly dissolved and data collected. From Fig.2, it can be seen that cluster **II** is faster and more quantitative as a CO donor than cluster **I** (Fig. 2), this CO-releasing behaviour may be caused by the longer alkyl chain -$(CH_2)_8$- between two alkynes units in

cluster **II**. In contrast with cluster **II**, cluster **III** contains four PPh_3 ligands besides the same alkyl chain between two alkynes units. This less controlled CO-releasing behaviour of cluster **III** perhaps arise from the presence of PPh_3 ligand. Therefore, it maybe not a good method of introducing PPh_3 group into the alkyne-bridging cobalt carbonyl cluster to act as CORMs.

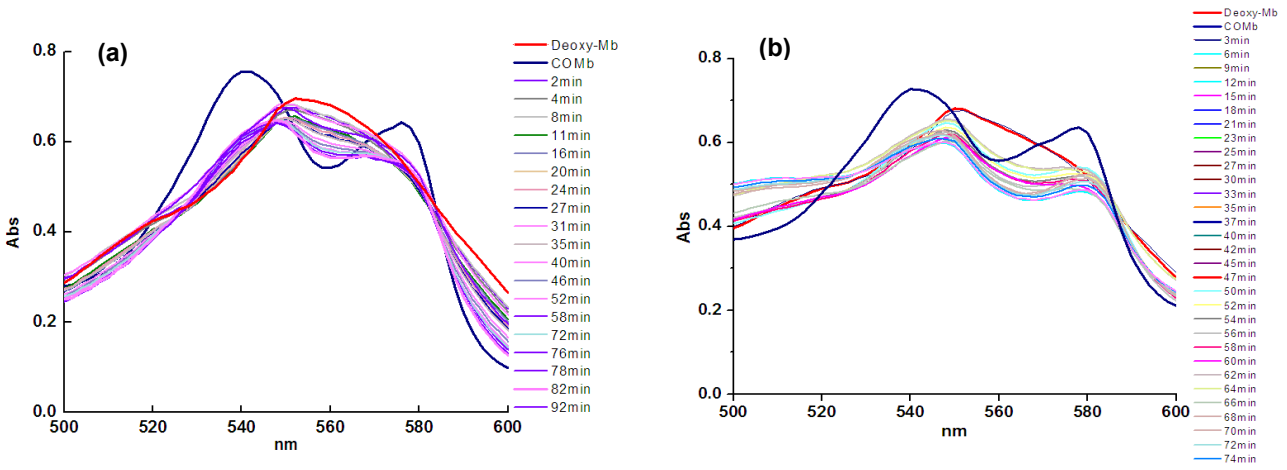


Fig. 1: Absorption spectra of MbCO formed over time after reaction of cluster **I** (a), cluster **II** (b) and cluster **III**(c) with deoxy-Mb in phosphate buffer, pH 7.4 at 37 ◦C

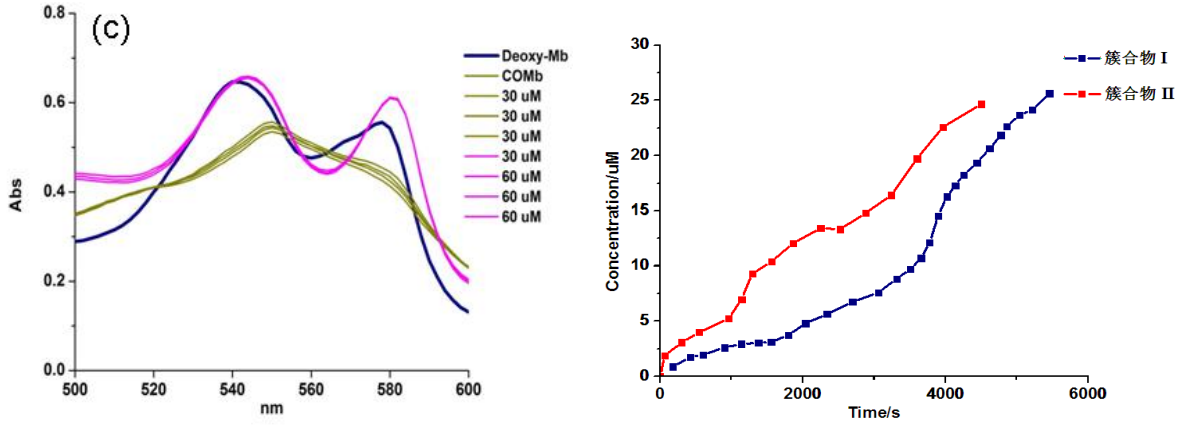


Fig. 2: A plot of CO released from 60 uM cluster **I** and cluster **II** to myoglobin at 37 ^{o}C as a function of time.

4. Acknowledgements

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[18] D. Scapens, H. Adams, T. R. Johnson, et al. $[(\eta\text{-}C_5H_4R)Fe(CO)_2X]$, X = Cl, Br, I, NO_3, CO_2Me and $[(\eta\text{-}C_5H_4R)Fe(CO)_3]^+$, R = $(CH_2)nCO_2Me$ (n = 0-2), and $CO_2CH_2CH_2OH$: a new group of CO-releasing molecules. Dalton Trans., 2007, 4962-4973.

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