

Modified biovectors for the tuneable activation of anti-platelet carbon monoxide release†

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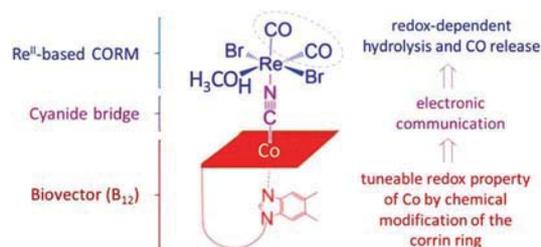
This communication describes the anti-platelet effects of a new class of *cis*-rhenium(II)-dicarbonyl-vitamin B₁₂ complexes (B₁₂-ReCORMs) with tuneable CO releasing properties.

Carbon-monoxide releasing molecules (CORMs) represent an innovative class of compounds which attract interest due to their potential therapeutic utility. Unlike most common drugs whose pharmacological action is dependent on their interaction with a macromolecular target and whose potency is dictated by the stability of the drug-target complex, CORMs exert their therapeutic action *via* the liberated CO molecules.^{1–5} However, apart from the common scientific consensus that CORM-based therapy should not lead to significant carboxy-hemoglobin (COHb) formation and to the inhibition of respiratory enzymes that are sensitive to CO, it is questionable whether CORMs should release CO slowly or rapidly and what kinetics of CO release is most advantageous for therapeutic applications. There are only few reports clearly showing the advantages of CORMs slowly releasing CO over those releasing CO instantly^{6,7} and they relate to the anti-platelet effects of CORMs. Furthermore, it has proved chemically challenging to fine-tune the activation and the rate of CO release within a family of structurally similar CORM compounds. For all of these reasons, versatile classes of CORMs with tuneable release properties affording anti-platelet activity are highly desired for tackling these open questions in systematic structure–activity relationship studies. Such studies will facilitate the development of CORMs with optimal anti-platelet activity.^{6–8}

Inspired by our groups' efforts to develop (a) versatile CORMs for biomedical applications^{9–12} and (b) vitamin B₁₂ derivatives with tuneable coordination and redox properties,^{13–15} we envisaged to design and study *cis*-rhenium(II)-dicarbonyl-vitamin B₁₂ complexes with adjustable CO releasing properties (Scheme 1) within the range for kinetics of CO release of CORM A1 (Scheme 2a).¹⁶ This molecule exhibits strong anti-platelet and anti-thrombotic activities without a hypotensive effect.^{6,7} In particular, for the present prodrug strategy, we started from prototype B₁₂-ReCORM-2¹⁰ (compound **8** in the present study) in which the CORM *cis*-[Re(CO)₂Br₄]^{2–} (**1**) is attached to the axial cyano group of vitamin B₁₂. We speculated whether modifications of the Co^{III}/Co^I redox properties of the B₁₂ ligand would translate into control of the CO-releasing properties at the opposite Re^{II}-(CO)₂ fragment.

This approach seemed to be reasonable because small variations in the coordination sphere of rhenium complexes have profound consequences on the electrochemistry, water stability and CO releasing properties of the dicarbonyl core.¹⁷ B₁₂ appeared to be attractive as ligand for the rhenium-based CORM entity mainly because of two reasons: (a) its cellular uptake properties can be exploited to deliver therapeutic agents specifically at disease sites;^{18–20} (b) the electronic properties at the cobalt center can be selectively modified by introducing structural modifications at the corrin- π -system.^{21–24}

Having the general design of the B₁₂-ReCORMs derivatives in mind (Scheme 1), we synthesized and studied first a series of



Scheme 1 General design concept for the tuneable activation of CORM-biovectors conjugates.

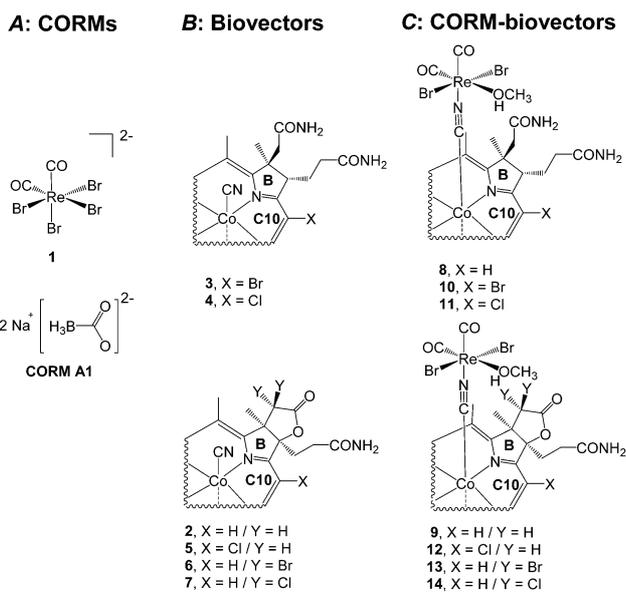
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Scheme 2 (A) Re^{II}-Based CORM **1** and CORM A1; (B) modified B₁₂-biovectors **2–7**; (C) CORM-B₁₂ complexes **8–14**.

Table 1 Electrochemical and spectroscopic data of vitamin B₁₂ derivatives (**2–7**)

Compound	E_{red}^a (mV)	γ band, nm (log ϵ)	β/α ($\pi-\pi^*$) bands, nm (log ϵ)
B ₁₂	-1010	361 (4.4)	519 (3.9)/550 (3.9)
2	-929	359 (4.3)	523 (3.7)/551 (3.7)
3	-798	365 (4.3)	550 (3.7)/576 (3.7)
4	-810	364 (4.2)	551 (3.6)/574 (3.7)
5	-784	363 (4.6)	551 (3.6)/577 (3.6)
6	-710	363 (4.2)	531 (3.7)/559 (3.7)
7	-715	363 (4.2)	531 (3.7)/554 (3.7)

^a E_{red} data refer to Co^{III} → Co^I reduction (in 0.1 M Tris buffer at pH 8).

six cobalamins for coordinating them later to **1** (Scheme 2A). In particular, the cobalamin ligands contain structural modifications at C10 and/or at the B ring of the corrin macrocycle (Scheme 2B) and were synthesized according to literature procedures or modifications thereof (see ESI[†]).^{25–28} We examined the electronic properties of compounds **2–7** (Table 1) by spectroscopic (UV-vis) and electrochemical means (cyclic voltammetry). As a general trend, we observed an increase in E_{red} (Co^{III}/Co^I) (cathodic reduction potential for the Co^{III} to Co^I transition) when electronic withdrawing groups (Br or Cl) and/or a *c*-lactone moiety at the B-ring were introduced in the cobalamin structure. For example, E_{red} (Co^{III}/Co^I) was increased by 212 mV compared to B₁₂ when one Br is introduced at C10 (**3**) and this effect was further enhanced by 88 mV when an additional dibromolactone moiety was attached to the periphery of the B ring (**6**).

Having demonstrated that chemical modifications of the corrin macrocycle change the overall electronic properties of the cobalamin ligands **2–7**, these biovectors were coordinated to CORM **1** through their axial cyano group (Scheme 2).²⁹ The formation of a Co–CN–Re bond to afford CORM-biovectors **8–14** (Scheme 2C) was achieved in one single step and was confirmed

Table 2 Half-life ($t_{1/2}$) of stability of **8–14** in water and their CO release

Compound	$t_{1/2}$ hypochromic shift ^a	$t_{1/2}$ of CO release ^{a,b}
8	60.1 ± 2.9	33.6 ± 2.3
9	40.2 ± 1.6	24.5 ± 1.8
10	29.5 ± 2.6	25.3 ± 1.8
11	29.6 ± 2.9	22.6 ± 0.7
12	27.3 ± 1.7	24.3 ± 3.0
13	19.1 ± 1.4	20.2 ± 2.9
14	24.9 ± 2.2	16.2 ± 1.2

^a In H₂O (minutes). ^b Measured from the first order exponential increase of MbCO solet band.

by IR spectroscopy and inductively coupled plasma/optical emission spectrometry (ICP/OES) (see ESI[†]). With these compounds in hand, we investigated the stability and therefore the CO releasing properties of the Re^{II}-based CORM within the CORM-biovector scaffolds (**8–14**) by UV-vis kinetic experiments. The main difference in the spectra of the biovectors (Scheme 2B, **2–7**) and the CORM-biovectors (Scheme 2C, **8–14**) is the presence of a band at *ca.* 410 nm for the latter compounds. This band is hypsochromically shifted when the bromide ions of the Re^{II} complex undergo aquation. This ligand exchange is the first step towards the aerobic degradation of the Re^{II} core leading to CO release.¹⁰ Therefore, changes in this band in DMSO (see ESI[†], Fig. S21) and aqueous solutions (Table 2) over time were monitored.

CORM-biovectors **8–14** show a half-life ($t_{1/2}$) stability in DMSO varying from 1 to > 3 hours (see ESI[†], Table S1). When this experiment was performed in an aqueous solution, a first order decay behavior was observed. We were delighted to find that $t_{1/2}$ of aquation of the *cis*-[Re(CO)₂Br₂]⁰ core steadily decreased as E_{red} (Co^{III}/Co^I) of the B₁₂-biovector increased (Fig. 1), varying from 60 (**8**) to *ca.* 20 min (**13**). The observed trend may be rationalized considering that an electron deficient Co^{III} ion obtains an enhanced σ -donation from the axial cyanide bridge. As a consequence, the 17-electron Re^{II} complex receives weaker electronic stabilization from the bridging ligand, rendering it more labile towards ligand substitution. Thus, within CORM-biovectors **8–14**, electronic properties of the modified biovectors appear to be the

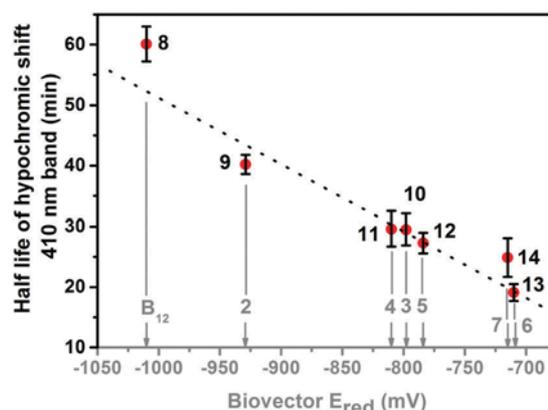


Fig. 1 Plot of $t_{1/2}$ (min) of hypochromic shift of 410 nm band of compounds **8–14** vs. E_{red} (Co^{III}/Co^I) of the same species.

main effect regarding the rate of aquation of the Re^{II} -based CORM. Nonetheless, a slight deviation from linearity ($R^2 = 93\%$) suggests that additional factors might also play a minor role.

Knowing that aquation is the prerequisite for CO release, the relative CO releasing rates of CORM-biovectors **8–14** were determined using the well-known myoglobin (Mb) assay. The apparent rate and amount of CO release were calculated by UV-vis spectroscopy: the formation of carboxy-myoglobin (MbCO) is monitored by an increase in the Soret band of the protein ($\lambda_{\text{max}} = 424 \text{ nm}$). Consequently, the $t_{1/2}$ of Soret band shift was taken as a direct measure of $t_{1/2}$ of CO release (Table 2). The $t_{1/2}$ of CO release decreases as a function of E_{red} ($\text{Co}^{\text{III}}/\text{Co}^{\text{I}}$), matching the results obtained for the $t_{1/2}$ of aquation of the Re^{II} CORM within CORM-biovectors **8–14**. Our results

point to three defined areas of CO-release within the range of 15–35 min: a $t_{1/2}$ of CO release in a 30–35 min range for cobalamin derivatives with an $E_{\text{red}} \leq -1 \text{ V}$; a 20–25 min range between -0.95 and -0.75 V ; a 15–20 min range for Co $E_{\text{red}} > -0.75 \text{ V}$ (Fig. 2).

These results are important since the modulation of the $t_{1/2}$ of CO release allowed us to study the biological effects on the inhibition of platelet aggregation of various CO-releasing rates within a family of structurally similar CORM compounds in comparison with CORM A1 that has a $t_{1/2}$ of CO release of approximately 20 min.¹⁶ As shown in Fig. 3 and Table 3, all B_{12} -ReCORMs (compounds **8–14**) inhibited platelet aggregation in the concentration range of 10 to 300 μM . In fact, the anti-aggregatory effects of **8** ($\text{IC}_{50} = 79.64 \pm 1.21$), **10** ($\text{IC}_{50} = 87.64 \pm 1.13$), **11** ($\text{IC}_{50} = 80.87 \pm 1.17$) and **14** ($\text{IC}_{50} = 48.20 \pm 1.21$) were quite similar to each other and in the same range as observed for CORM A1 ($\text{IC}_{50} = 77.13 \pm 1.17$).^{6,7} Only B_{12} -CORMs **9** ($\text{IC}_{50} = 206.2 \pm 1.22$) and **13** ($\text{IC}_{50} = 29.39 \pm 1.21$) deviated significantly from these values (see Table 3). Compounds **8–11**, **14** and CORM A1 inhibited platelet aggregation by more than 80% at 300 μM , while **13** achieved the same effect already at 100 μM . Accordingly, all the presented CORM-biovectors have anti-aggregatory effects relatively close to CORM A1. Only **13** displays a slightly more potent anti-platelet activity than CORM A1 and the other tested B_{12} -ReCORMs.^{6–8}

In summary, we have presented a series of B_{12} -ReCORMs with tuneable CO releasing properties. These CORM-biovectors were synthesized by connecting a Re^{II} -dicarbonyl CORM and modified B_{12} derivatives through a cyanide bridge. In this proof-of-concept study it was demonstrated that alterations of the electronic properties at the Co^{III} center of the B_{12} -biovectors translate directly into variations of the CO release kinetics at the Re^{II} metal ion. Although differentiation of the CO releasing kinetics between the different complexes is still relatively small, we demonstrated already that B_{12} -ReCORMs displayed pronounced anti-platelet activity similar or even slightly higher than CORM A1, a compound that affords anti-platelet and anti-thrombotic activities *in vivo* without a hypotensive effect.⁷ These findings are encouraging for our future efforts to develop tuneable B_{12} -ReCORMs with further optimized CO-releasing properties to achieve optimal anti-platelet effects for therapeutic applications.

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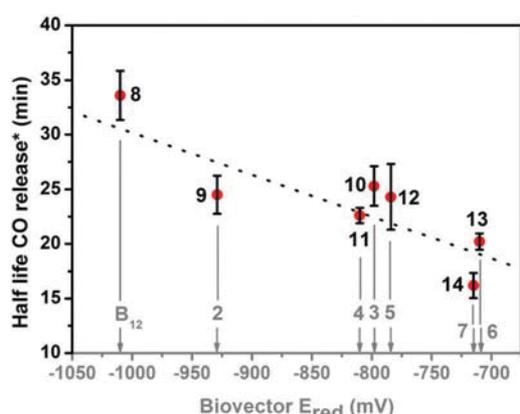


Fig. 2 Plot of $t_{1/2}$ (min) of hyperchromic shift in the MbCO Soret band ($\lambda_{\text{max}} = 424 \text{ nm}$) of a Mb solution exposed to compounds **8–14** vs. reduction potential of Co^{III} to Co^{I} of the same species. * The $t_{1/2}$ of hyperchromic shift of the 424 nm soret band is taken as a direct measure of $t_{1/2}$ of CO release.

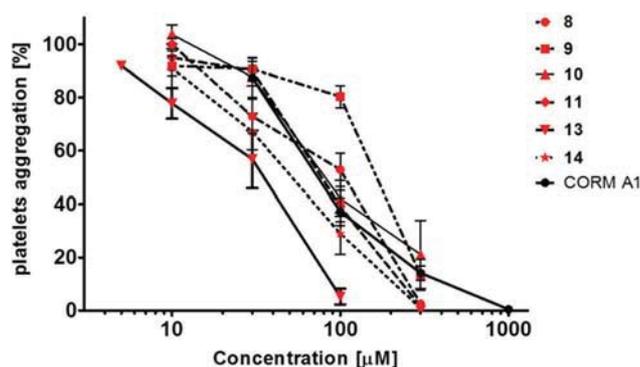


Fig. 3 Inhibition of platelet aggregation by B_{12} -ReCORMs and CORM A1. Results presented as a mean \pm SEM ($n = 4–22$).

Table 3 IC_{50} values for anti-aggregatory effects of B_{12} -ReCORMs and CORM A1

COMP.	8	9	10	11	13	14	CORMA1
$\text{IC}_{50} \pm \text{SEM}$	79.64 ± 1.21	206.20 ± 1.22	87.64 ± 1.13	80.87 ± 1.17	29.39 ± 1.21	48.20 ± 1.21	77.13 ± 1.17

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