Electronic Supplementary/Supporting Information (ESI) Synthesis of oxime-based CO-releasing molecules, CORMs and their immobilization on maghemite nanoparticles for magnetic-field induced CO release

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Synthesis of oxime-CORMs

In a first step following the literature,¹ imidazole-2-carboxaldehyde (4) was transformed to imidazole-2-carbaldehyde-oxime (20) with hydroxylamine hydrochloride and sodium carbonate in water. The reaction time was extended to 24 h at 70 °C to form product 2 with a yield of 41 %. A ¹H-NMR spectrum of imidazole-2-carbaldehyde-oxime (20) could not be recorded, not even in DMSO-d₆ due to very low solubility. Instead, a mixture of NaOD-D₂O was used to deprotonate the oxime in order to achieve solubility.



Scheme S1 Synthesis of the oxime-based CORMs Ru(imidazole-2-carbaldehyde-oxime)(methoxycarbonyl)(CO)₂Cl (1a) and Ru(imidazole-2-carbaldehyde-oxime)(ethoxycarbonyl)-(CO)₂Cl (2a) starting from imidazole-2-carboxaldehyde (4) over imidazole-2-carbaldehyde-oxime (20). a) NH₂OH·HCl, Na₂CO₃, H₂O, 24 h, 70 °C; b) MeOH, 5 d, 30 °C; c) EtOH, 5 d, 30 °C.

The tricarbonyldichloridoruthenium dimer (12) was synthesized in a steel autoclave with glass-inlett according to a known procedure of Mantovani.² To form the oxime-based CORMs 1a and 2a the reaction conditions, as described by Oresmaa,¹ where modified to 30 °C and 5 d. Otherwise, only a very small amount of product was formed for both (1a and 2a). We were able to receive 31 % yield for 1a and 45 % yield for 2a, which is only slightly, less than the literature values (41 % and 53 %, respectively).

Synthesis of imidazole-2-carbaldehyde-oxime (20): 1.44 g (20.7 mmol) NH₂OH·HCl were dissolved in 8 mL of d.d. water and neutralized with 1.1 g (10.4 mmol) Na₂CO₃. Afterwards 1.0 g (10.4 mmol) imidazole-2-carboxaldehyde (4) was added and the solution was stirred for 24 h at 70 °C. After cooling with ice a colorless solid was sedimented, filtered and washed with d.d. water. The solid was dried under vacuum. Yield: 478 mg (41 %, Lit.: 44 % ¹). IR (KBr): $\tilde{\nu}$ [cm⁻¹] = 3193, 3034, 2949, 930, 984. ESI-MS: m/z = 112.05 [M+H]⁺. ¹H-NMR (300 MHz, NaOD/D₂O): δ [ppm] = 6.78 (s, 1H, C-CH=N), 6.39 (s, 2H, imi-CH=CH).

¹H and ¹³C NMR Spectra



Fig. S1a ¹³C NMR spectrum (75 MHz, DMSO-d₆) of 1-(2-(3,4-dihydroxyphenyl)-2-oxoethyl)-1H-imidazole-2-carbaldehyde, **5**.



yl)ethanone (6)



Fig. S1c ¹H NMR (300 MHz, acetone- d_6) of 1-(2-(2,2-dimethylbenzo[d][1,3]dioxol-5-yl)-2-oxoethyl)-1H-imidazole-2-carbaldehyde (7).







Fig. S1e ¹H NMR spectrum (300 MHz, DMSO-d₆) of (E)-1-(2-(2,2-dimethylbenzo[d][1,3]dioxol-5-yl)-2-oxoethyl)-1H-imidazol-2-carbaldehyde-oxime (**8**).

-1.70





Fig. S1f ¹H NMR spectrum (600 MHz, DMSO-d₆) of 1-(2-(3,4-dihydroxyphenyl)-2-oxoethyl)-1H-imidazole-2-carbaldehyde-oxime (**9**).





Fig. S1i ¹H NMR (300 MHz, acetone-d₆) of $[Ru(\eta^2-8)(ethoxycarbonyl)(CO)_2Cl]$ (11).

IR spectra



Fig. S2-1 Carbonyl region in IR spectra of compounds **1a** and **2a** (a) and the modified compounds **10** and **11** (b) in KBr disks. The bands at 2143 cm⁻¹ correspond to liberated free CO.







Fig. S2-3 IR spectra of the composite material **17** as KBr disk. The OH-vibration of the dextran is present at 3600-3000 cm⁻¹. The enlarged carbonyl region (right) shows three strong absorptions at 2064, 1995 and 1961 cm⁻¹.

Electron spray ionization mass spectra (ESI-MS)



Fig. S3a Experimental (black) and calculated (red) electron spray ionization (ESI) mass spectrum of compound 11 in water/acetonitrile. $m/z = 527.0 [M - OEt^- - Cl^- - H^+ + CH_3CN]^+$, 499.1 $[M - CO/OEt^- - Cl^- - H^+ + CH_3CN]^+$.



Fig. S3b Experimental (black) and calculated (red) electron spray ionization (ESI) mass spectrum of compound **14** in water/acetonitrile. $m/z = 487.0 [M - OEt^- - Cl^- - H^+ + CH_3CN]^+$, 459.2 [M - CO/OEt⁻ - Cl⁻ - H⁺ -CO + CH_3CN]⁺.



High-resolution electron spray ionization mass spectra (HR-ESI MS)

Fig. S4a Experimental electron spray ionization (ESI) mass spectrum of compound 9 in water/acetonitrile. m/z = 262.0822 fits very well to the calculated mass for the composition $[M + H]^+$ with m/z = 262.8023.

The absence of significant other peaks (besides the isotope pattern) confirms the purity of compound 9.



Fig. S4b HR-ESI MS with experimental (top-blue) and calculated (bottom) pattern around m/z = 487 of compound **14** in water/acetonitrile.

The experimental pattern at m/z = 486.9828 matches very wee with the calculated pattern at m/z = 486.9822 for the composition $[M - OEt^- - Cl^- - H^+ + CH_3CN]^+$.



Fig. S4c HR-ESI MS with experimental (top-blue) and calculated (bottom) pattern around m/z = 459 of compound 14 in water/acetonitrile. m/z = 458.9877 fits very well to the calculated mass for the composition $[M - CO/OEt^- Cl^- - H^+ + M^-]$

m/z = 458.9877 fits very well to the calculated mass for the composition $[M - CO/OEt - CI - H^+ + CH_3CN]^+$ with m/z = 458.9873.



Fig. S4d HR-ESI MS with experimental (top-blue) and calculated (bottom) pattern around m/z = 420 of compound 14 in water/acetonitrile.

m/z = 419.9768 fits very well to the calculated mass for the composition $[M - CO/OEt^- - Cl^+ + H]^+$ with m/z = 419.9764.

In a methanol (MeOH)/acetonitrile mixture the HR-ESI-MS spectra of 14 show an additional mass of $m/z = 477.9823 [M - OEt^- - Cl^- - CO + OMe^-]^+$ (calculated m/z = 477.98244) and $m/z = 449.9872 [M - OEt^- - Cl^- - CO + OMe^-]^+$ (calculated m/z = 449.98752).

CO release from compounds 1a and 2a in the myoglobin assay at various temperatures

The determination of the half-lifes was performed by time-dependent visible absorption spectra and a plot of intensity changes at the wavelengths 541, 556 and 578 nm against time. By using an approximated exponential fit

$$y = y_o + Ae^{R_o^2}$$

the half-life could be calculated for a first order kinetic with the following formula:

 $t_{1/2} = ln2 / - R_o$



Fig. S5: Time-dependent visible absorption spectra (left) and plot of the intensity changes of selected wavelengths (right) of Ru(imidazole-2-carbaldehyde-oxime)(methoxycarbonyl)(CO)₂Cl (**1a**) with myoglobin assay at 20 °C to yield an averaged half-life for **1a** of $t_{1/2} = 220\pm23$ min.



Fig. S6: Time-dependent visible absorption spectra (left) and plot of the intensity changes of selected wavelengths (right) of Ru(imidazole-2-carbaldehyde-oxime)(methoxycarbonyl)(CO)₂Cl (**1a**) with myoglobin assay at 37 °C to yield an averaged half-life for **1a** of $t_{1/2} = 21\pm 2$ min.



Fig. S7: Time-dependent visible absorption spectra (left) and plot of the intensity changes of selected wavelengths (right) of Ru(imidazole-2-carbaldehyde-oxime)(methoxycarbonyl)(CO)₂Cl (**1a**) with myoglobin assay at 50 °C to yield an averaged half-life for **1a** of $t_{1/2} = 5\pm 1$ min.



Fig. S8: Time-dependent visible absorption spectra (left) and plot of the intensity changes of selected wavelengths (right) of Ru(imidazole-2-carbaldehyde-oxime)(ethoxycarbonyl)(CO)₂Cl (**2a**) with myoglobin assay at 20 °C to yield an averaged half-life for **2a** of $t_{1/2} = 226\pm9$ min.



Fig. S9: Time-dependent visible absorption spectra (left) and plot of the intensity changes of selected wavelengths (right) of Ru(imidazole-2-carbaldehyde-oxime)(ethoxycarbonyl)(CO)₂Cl (**2a**) with myoglobin assay at 37 °C to yield an averaged half-life for **2a** of $t_{1/2} = 15\pm1$ min.



Fig. S10: Time-dependent visible absorption spectra (left) and plot of the intensity changes of selected wavelengths (right) of Ru(imidazole-2-carbaldehyde-oxime)(ethoxycarbonyl)(CO)₂Cl (**2a**) with myoglobin assay at 50 °C to yield an averaged half-life for **2a** of $t_{1/2} = 6\pm 1$ min.



Fig. S11: Bar diagram for the half-lifes from Ru(imidazole-2-carbaldehyde-oxime)- (methoxycarbonyl)(CO)₂Cl (1a, left bars) and Ru(imidazole-2-carbaldehyde-oxime)(ethoxy-carbonyl)(CO)₂Cl (2a, right bars) at 20 °C, 37 °C and 50 °C (with standard deviations) in the myoglobin assay.

CO release from compounds 10 and 11 in the myoglobin assay at various temperatures



Fig. S12: Time-dependent visible absorption spectra (left) and plot of the intensity changes of selected wavelengths (right) of Ru(η^2 -8)(methoxycarbonyl)(CO)₂Cl (10) with myoglobin assay at 20 °C to yield an averaged half-life for 10 of t_{1/2} = 172±23 min.



Fig. S13: Time-dependent visible absorption spectra (left) and plot of the intensity changes of selected wavelengths (right) of Ru(η^2 -8)(methoxycarbonyl)(CO)₂Cl (10) with myoglobin assay at 37 °C to yield an averaged half-life for 10 of t_{1/2} = 18±1 min.



Fig. S14: Time-dependent visible absorption spectra (left) and plot of the intensity changes of selected wavelengths (right) of Ru(η^2 -8)(methoxycarbonyl)(CO)₂Cl (10) with myoglobin assay at 50 °C to yield an averaged half-life for 10 of t_{1/2} = 4±1 min.



Fig. S15: Time-dependent visible absorption spectra (left) and plot of the intensity changes of selected wavelengths (right) of Ru(η^2 -8)(ethoxycarbonyl)(CO)₂Cl (11) with myoglobin assay at 20 °C to yield an averaged half-life for 11 of t_{1/2} = 155±13 min.



Fig. S16: Time-dependent visible absorption spectra (left) and plot of the intensity changes of selected wavelengths (right) of Ru(η^2 -8)(ethoxycarbonyl)(CO)₂Cl (11) with myoglobin assay at 37 °C to yield an averaged half-life for 11 of $t_{1/2} = 18\pm 1$ min.



Fig. S17: Time-dependent visible absorption spectra (left) and plot of the intensity changes of selected wavelengths (right) of Ru(η^2 -8)(ethoxycarbonyl)(CO)₂Cl (11) with myoglobin assay at 50 °C to yield an averaged half-life for 11 of $t_{1/2} = 4\pm 1$ min.



Fig. S18: Bar diagram for the half-lifes from $Ru(\eta^2-8)(COOMe)(CO)_2Cl$ (10) and $Ru(\eta^2-8)(ethoxycarbonyl)(CO)_2Cl$ (11) at 20 °C, 37 °C and 50 °C (with standard deviations) in the myoglobin assay.



CO release from compound 14 in the myoglobin assay at various temperatures

Fig. S19: Time-dependent visible absorption spectra (left) and plot of the intensity changes of selected wavelengths (right) of Ru(η^2 -9)(ethoxycarbonyl)(CO)₂Cl (14) with myoglobin assay at 20 °C to yield an averaged half-life for 14 of t_{1/2} = 207±6 min. (Spectra were collected every 10 min.)



Fig. S20: Time-dependent visible absorption spectra (left) and plot of the intensity changes of selected wavelengths (right) of Ru(η^2 -9)(ethoxycarbonyl)(CO)₂Cl (14) with myoglobin assay at 37 °C to yield an averaged half-life for 14 of t_{1/2} = 16±1 min.



Fig. S21: Time-dependent visible absorption spectra (left) and plot of the intensity changes of selected wavelengths (right) of Ru(η^2 -9)(ethoxycarbonyl)(CO)₂Cl (14) with myoglobin assay at 50 °C to yield an averaged half-life for 14 of t_{1/2} = 3±1 min.



Fig. S22: Half-lifes from $Ru(\eta^2-9)(ethoxycarbonyl)(CO)_2Cl$ (14) at 20 °C, 37 °C and 50 °C (with standard deviations) in the myoglobin assay.

CO release from the composite material alginate@dextran@oximeCORM@IONP (18) in the myoglobin assay at various temperatures



Fig. S23: Time-dependent visible absorption spectra (left) and plot of the intensity changes of selected wavelengths (right) of alginate@dextran@oximeCORM@IONP (**18**) with myoglobin assay at 20 °C to yield an averaged half-life for **18** of $t_{1/2} = 814\pm23$ min. (Spectra were collected every 10 min.)



Fig. S24: Time-dependent visible absorption spectra (left) and plot of the intensity changes of selected wavelengths (right) of alginate@dextran@oximeCORM@IONP (**18**) with myoglobin assay at 37 °C to yield an averaged half-life for **18** of $t_{1/2} = 346\pm83$ min.



Fig. S25: Time-dependent visible absorption spectra (left) and plot of the intensity changes of selected wavelengths (right) of alginate@dextran@oximeCORM@IONP (**18**) with myoglobin assay at 50 °C to yield an averaged half-life for **18** of $t_{1/2} = 73\pm1$ min.



Fig. S26: Picture of the alginate spheres on the bottom of a temperature controllable UV/VIS cell with myoglobin solution.

CO release from compound 18 with applied alternating (AC) magnetic field



Fig. S27: Time-dependent visible absorption spectra (left) and plot of the intensity changes of selected wavelengths (right) of alginate@dextran@oximeCORM@IONP (**18**) with myoglobin assay at 37 °C with the applied alternating (AC) magnetic field (31.7 kAm⁻¹, 247 kHz, 39.9 mTesla) to yield an averaged half-life for **18** of $t_{1/2} = 153\pm27$ min.



Fig. S28: IR spectra of the composite material alginate@dextran@oximeCORM@IONP (**18**) in KBr disks before and after the CO release in the alternating (AC) magnetic field. The carbonyl region (enlarged at right) showed two strong absorptions at 2061, 1991 cm⁻¹ before the CO release (black) and absorptions shifted to 2071, 2015 and 1992 cm⁻¹ (red) after the CO release.

Determined amount of released CO

We calculated the amount of released CO with equation (1):

$$c(MyoCO) = \left(\frac{A(t)}{l} - \frac{A(t=0)}{l}\right) \cdot \frac{1}{\varepsilon_{540 nm}(MyoCO) - \frac{A(t=0)}{c_0(MyO) \cdot l}}$$
Eq. (1)

with A(t) = absorption at time t,

A(t = 0) = absorption at time t = 0,

l = path length of the cuvette,

 $\mathcal{E}_{540\text{nm}}(\text{MyoCO}) = 15.4 \text{ L} \cdot \text{mmol}^{-1} \cdot \text{cm}^{-1}$, and $c_0(Myo) = \text{concentration of myoglobin from first}$ absorption spectrum (here volume of cuvette V = 0.0014 L).

The correction of the spectra was done by the method of ATKIN *et al.*³ The amount of CO was determined with the Lambert-Beer-law. The results are shown in Table S1.

Table S1	Amount of	f carbon	monoxide	released	from	the o	compounds	10,	11	and	14	in mol	carbon
monoxide p	er mol Ru.												

compund	mol carbon monoxide per mol Ru
10	0.9(1)
11	0.8(1)
14	0.9(1)
18	0.2(1)

It is now known and quantified from recent literature that CORM-2 will lose 1.8 CO as CO₂ in water within 24 h.⁴

Leaching experiments

Three time-dependent leaching experiments were prepared by dispersing 20 mg each of dextran@oximeCORM@IONP (17) in 1 mL MOPS buffer (pH 7.4) and mixing this solution with 2.4 mL alginate solution according to the synthetic procedure of alginate@dextran@oximeCORM@IONP (18). After the formation of the alginate spheres with calcium chloride solution and fully crosslinking of the alginate network, all of the formed spheres were washed as described. Two samples were stored in 5 mL MOPS buffer (pH 7.4) for 12 h and 24 h and washed twice with 5 mL deionized water afterwards. The ruthenium quantity in the alginate composite directly after the preparation of 18 (t₀)

and after the storage in buffered solution for 12 h (t_{12h}) and 24 h (t_{24h}), was determined by AAS and yielded in: t_0 = 0.60wt%, t_{12h} = 0.17wt% and t_{24h} = 0.13wt% in the 20 mg sample of dextran@oximeCORM@IONP (17). This corresponds to a leaching of 72% after 12 h and 78% after 24 h of the initial amount of ruthenium into the surrounding solution. These results suggest a weaker ruthenium metal-oxime bond than the metal-amino-alkoxide bond in CORM-3 analogous system which we described earlier.⁵

Dynamic light scattering, DLS and transmission electron microscopy, TEM measurements of functionalized maghemite nanoparticles



Fig. S29: Synthesis of functionalized maghemite nanoparticles with the ligands **9**, **11** and **15**. a) d.d. water, NaOH (pH = 10), **9**; b) d.d. water, NaOH (pH = 10), **11**; a) d.d. water, NaOH (pH = 10), **15**.

Immobilization of 1-(2-(3,4-dihydroxyphenyl)-2-oxoethyl)-1H-imidazole-2-carbaldehyde (5) on maghemite nanoparticles (21): A suspension of 100 mg of 5 in 6 mL of d.d. water was mixed with sodium hydroxide solution until pH = 10 was reached. Maghemite nanoparticles (3) were added (2 mL, 10 mg/mL) and the solution was stirred for 15 min. After neutralizing with hydrochloric acid the solution was combined with 100 mL acetone and the formed solid separated with centrifugation. The solid was washed with 3x 5mL of acetone and dried under vacuum. Yield: 25 mg, IR (KBr): $\tilde{\nu} = 1686$, 1488 cm⁻¹.

Immobilization of 1-(2-(3,4-dihydroxyphenyl)-2-oxoethyl)-1H-imidazol-2-carbaldehyde-oxime (9) on maghemite nanoparticles (22): A suspension of 5 mg of 9 in 2 mL of d.d. water was mixed with a few drops of 0.1 mol/L sodium hydroxide solution to form a clear solution. Maghemite nanoparticles (3) were added (1 mL, 10 mg/mL) and the solution was stirred for 15 min. After neutralizing with hydrochloric acid the solution was combined with 50 mL of acetone and the formed solid separated by centrifugation. The solid was washed with 3x 5mL of acetone and dried under vacuum. Yield: 7 mg, IR (KBr): $\tilde{\nu} = 1638$, 1490 cm⁻¹.



Fig. S30: Size distribution of the hydrodynamic diameter of particles of 3 (black), 21 (red), 22 (blue) and 16 (green) in water (c = 1 mg/mL) from dynamic light scattering (DLS) investigations. The broad distribution of 16 (green) is due to sedimentation processes during the measurement. See Table S2 for the diameter values.

compound		medium hydrodynamic				
		diameter [nm] ^a				
	maghemite (IONP, 3)	8±2				
	maghemite + aldehyde (21)	23±4				
	maghemite + oxime (22)	32±10				
	maghemite + CORM (23) ^b	133±31				

Table S2: Average hydrodynamic diameters of functionalized maghemite nanoparticles.

^a Measured as aqueous dispersions with a minimum concentration of 1 mg/mL in doubly deionized water. With standard deviation σ in nm.

^b The large hydrodynamic diameter is due to low water solubility of the maghemite+CORM nanoparticles so that larger sedimentated aggregates exist in the dispersion; cf. Fig. S34.



S31: TEM image of maghemite Fig. nanoparticles (3)



Fig. functionalized maghemite nanoparticles 22.



Fig. S32: TEM image of the aldehydefunctionalized maghemite nanoparticles 21.



S33: TEM image of the oxime- Fig. S34: TEM image of the CORMfunctionalized nanoparticles 16.

Toxicity Tests



Fig. S35: Toxicity test of CORM-2 in different concentrations, sodium chloride (0.9%), DMSO (1% and 0.1% as solvent), and cDDP (10⁻⁴ mol/L Cisplatin) with the cells A2780 (left), CalSens (middle) and HEK293 (right).



Fig. S36: Toxicity test of CORM-3 in different concentrations, sodium chloride (0.9%), DMSO (1% and 0.1% as solvent), and cDDP (10⁻⁴ mol/L Cisplatin) with the cells A2780 (left), CalSens (middle) and HEK293 (right).



Fig. S37: Toxicity test of $\text{Ru}(\eta^2-9)(\text{COOEt})(\text{CO})_2\text{Cl}$ (14) (here labeled Oxim-CORM) in different concentrations, sodium chloride (0.9%), DMSO (1% and 0.1% as solvent), and cDDP (10⁻⁴mol/L Cisplatin) with the cells A2780 (left), CalSens (middle) and HEK293 (right).



Fig. S38: Toxicity test of dextran@oximeCORM@IONP (**18**) (here labeled Dextran@Oxim-CORM@IONP) in different concentrations, sodium chloride (0.9%), DMSO (1% and 0.1% as solvent), and cDDP (10-⁴mol/L Cisplatin) with the cells A2780 (left), CalSens (middle) and HEK293 (right).



Determination of the activation energy for the CO release from oximeCORMs

Fig. S39: Arrhenius plot of $\ln k = \ln((\ln 2)/(t_{1/2}))$ versus $1/T_{Kelvin}$ to determine the activation energy from the slope $(-E_A/R, R = 8.314 \text{ J mol}^{-1} \text{ K}^{-1})$ of the graph. a) **1a** b) **2a** c) **10** d) **11** and e) **14**.



Fig. S40 Setup of the **CO release measurements in alternating current (AC) field** with a Hüttinger HF generator AXIO T5 and the thermostated quartz cell placed inside the water-cooled copper induction coil.

Literature

1 L. Oresmaa, H. Kotikoski, M. Haukka, J. Salminen, O. Oksala, E. Pohjala, E. Moilanen, H. Vapaatalo, P. Vainiotalo, P. Aulaskari, *J. Med. Chem.* 2005, **48**, 4231.

2 A. Mantovani, S. Cenini, Inorg. Synth. 1976, 16, 51.

3 A. J. Atkin, J. M. Lynam, B. E. Moulton, P. Sawle, R. Motterlini, N. M. Boyle, N. M. Dalton Trans. 2011, 40, 5755–5761.

4 E. Neuse, Met.-Based Drugs, 2008, 1-19.

5 H. Meyer, F. Winkler, P. Kunz, A. M. Schmidt, A. Hamacher, M. U. Kassack and C. Janiak, *Inorg. Chem.*, **2015**, 54 (23), 11236–11246.