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Polymer Conjugates of Photoinducible CO-Releasing Molecules

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CO-releasing molecules (CORMs) were coordinated to polymeric carrier systems for passive transport to tumour tissue or sites of inflammation, as described by the enhanced permeability and retention (EPR) effect. The developed conjugates consist of an organometallic *fac*-$Mn(CO)_3$ fragment, which is bound to a methacrylate or methacrylamide polymer backbone by bis(pyridylmethyl)amine-type ligands. The resulting $Mn(CO)_3$–polymer conjugates were investigated as photoinducible CO-releasing molecules (photoCORMs). Furthermore, three model complexes $[LMn(CO)_3]O_2SCF_3$ containing *N*,*N*,*N*-ligands L = bis(pyridylmethyl)amine (bpma), bis(pyridylmethyl)ethanolamine (bpmea) and bis(pyridylmethyl)-*p*-vinylbenzylamine (bpmvba) were also investigated. The solid-state structure of the bromide complex $[(bpmea)Mn(CO)_3]Br·(CH_3)_2CHOH$ (**4**·$(CH_3)_2CHOH$) was determined by X-ray analysis. The complexes and the polymer conjugates show photoCORM activity with polymer molecular weights and size distributions suitable for passive drug delivery.

Introduction

Carbon monoxide is potentially a very useful therapeutic as it possesses critical signalling functions, which are relevant in inflammation processes, cellular proliferation, apoptotic cell death and neural transmission.[1–5] The majority of endogenously produced CO is as a result of the oxidation of heme, catalysed by the heme oxygenase (HO) enzymes. The potential therapeutic effects of CO are widely recognised in vasodilation, inflammation, hypertension, organ transplantation and cerebral malaria.[6–8] There is a pharmacological requirement, however, to deliver precise amounts of CO to specific locations under physiological conditions, which makes the development of CO-releasing molecules (CORMs) as benign CO sources highly desirable.[9–14]

Both the cytoprotective and cytotoxic properties of CO can be exploited for therapeutic purposes.[1,15,16] At low concentrations the cytoprotective activity is dominant, whereas at very high systemic concentrations of free CO, for example, by inhalation of CO, toxicity to the whole organism occurs due to inference with heme proteins involved in oxygen transport and storage, as well as respiratory functions. If a high concentration of CO can be achieved in a locally restricted tissue volume, for example, in tumour tissue, an overall beneficial cytotoxic effect for the organism should occur.

In the treatment of cancerous diseases, conventional chemotherapy usually entails a series of injections of highly toxic drugs, which owe their selectivity for cancer cells to proliferation rates that are much higher than those in healthy tissue. Conventional chemotherapeutics are small enough to leave the vascular system by passing through pores in the blood vessel walls. Therefore, they are distributed throughout the body, which can lead to increased toxicities against healthy tissues that also show enhanced proliferative rates, such as in the bone marrow, gastrointestinal tract and hair follicles. The resulting severe side effects often restrict the frequency and size of dosages, much to the detriment of tumour inhibition.[17,18]

The selective toxicity of an anticancer drug can be increased either by increasing the dose of the drug that reaches the diseased tissue or by decreasing the dose that reaches healthy tissues. Several approaches for improving the selective toxicity of anticancer therapeutics are being pursued at present; one of the most promising is the conjugation of anticancer drugs to macromolecular carrier systems. Conjugated to polymers, drugs are limited to the vascular system and can be transported directly to the area where the drug is required to take effect. This is possible because macromolecular systems selectively accumulate in tumour tissue as described by the enhanced permeability and retention (EPR) effect.[19–22]

The concept of polymer–anticancer conjugates was first proposed in 1975 by Helmut Ringsdorf. These macromolecular prodrugs consist of a minimum of three components:

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the polymeric carrier, the biodegradable polymer-drug linker and the antitumour agent.[23,24]

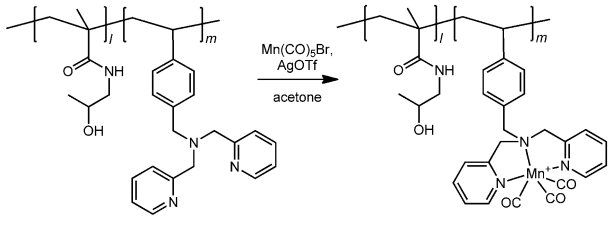
The choice of an appropriate polymeric carrier is crucial for systemic administration and 2-hydroxypropyl methacrylamide (HPMA)-based polymers have drawn special attention.[25,26] The linear or branched polymer chain usually serves as the structural component of a conjugate. Most of the clinically tested polymer conjugates have the typical structure consisting of a polymer backbone, a linker and the bioactive unit. Modern polymer chemistry is developing increasingly intricate polymer carriers, such as multivalent, branched or graft polymers as well as micelles, dendrimers and the like. Their potential advantages include a more defined chemical composition, tailored surface multivalency and creation of defined 3D architectures.[27–30]

The combination of CO-releasing agents and nano-sized carriers was first used in a micellar system and in functionalised SiO_2 nano-particles.[31,32]

We are interested in metal-based compounds for diagnostic and therapeutic applications.[33–38] Herein we present the concept of linking photoinducible CO-releasing molecules (photoCORMs) to HPMA-based copolymers thus generating nanometer-sized functionalised copolymers ("nanoCORMs" or "polyCORMs"), which can be used for the passive delivery of the CO-releasing metallodrugs to tumour tissue or sites of inflammation.

Results and Discussion

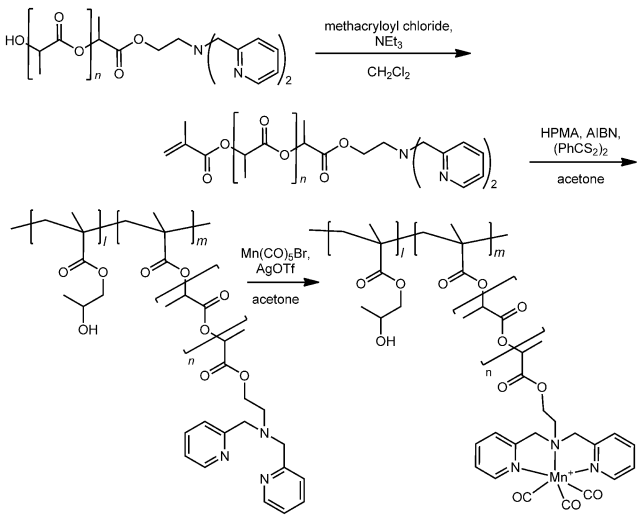
The bis(pyridylmethyl)amine (bpma)-functionalised HPMA copolymer **P1** (Scheme 1) was synthesised according to a reported procedure.[38] Instead of the conventional radical polymerisation conditions, we used RAFT conditions and added bis(thiobenzoyl) disulfide as the chain transfer agent to obtain polymers with low polydispersity.[39] For the preparation of the HPMA copolymer with ligand-functionalised biodegradable lactide side chains, we first prepared the ligand-functionalised polylactide (PLA)–bpmea [bpmea = bis(pyridylmethyl)ethanolamine] according to the procedure of Häfeli.[40] Attaching lactide side chains to polymer carriers can be achieved by turning them into macromonomers, introducing polymerisable end groups. Methacrylate (MA) groups open up the possibility of polymerising the lactide with a library of co-monomers through radical polymerisation. In this way, lactide side chains can be incorporated into large carrier systems with additional functionalities and properties.



Scheme 1. Preparation of the $Mn(CO)_3$ complex of the functionalised HPMA copolymer **P1**.

The synthesis of MA–PLA–bpmea was done by esterification of the hydroxy end group with methacryloyl chloride under basic conditions. The bpmea lactide and triethylamine were reacted in dichloromethane with a slight excess of methacryloyl chloride. After work-up, the product was precipitated with diethyl ether in yields of close to 90%. It is important to note that complete conversion to the methacrylated lactides could only be achieved with short chain lengths of less than ten repeat units.

Integration of the bpmea-functionalised macromonomer into a larger carrier system was realised using HPMA as a co-monomer. A monomer ratio (HPMA to MA–PLA–bpmea) of 5:1 was used in the experiments; the macromonomers had an average number of four repeat units. AIBN was used as radical starter and 2-cyanoisopropyl dithiobenzoate as the chain transfer agent. The polymer **P2** was isolated in 73% yield (Scheme 2). The molecular weight is dependent on the initiator to monomer ratio; an increase in the chain length is achieved with a decrease in initiator concentration. Molecular weights of approximately 50 kDa translate to a hydrodynamic diameter of approximately 10 nm as determined by gel permeation chromatography (GPC) and dynamic light scattering (DLS).



Scheme 2. Preparation of the functionalised HPMA–PLA copolymer **P2** and corresponding $Mn(CO)_3$ complex.

Polymers carrying bpma-type ligands can easily be labelled with the *fac*-$M(CO)_3$ cores as has been shown using $Re(CO)_3$.[34,38] This makes the labelling procedure more convenient than with, for example, functionalised nanoparticles, which have to be labelled using functionalised $Mn(CO)_3$ complexes.

The $Mn(CO)_3$ cores were coordinated to the carrier by the addition of an acetone solution of $[Mn(CO)_3(acetone)_3]OTf$ [prepared from $Mn(CO)_5Br$ and silver triflate] to methanolic solutions of **P1** or **P2**. The corresponding $Mn(CO)_3$–polymer conjugates precipitated upon addition of diethyl ether. The manganese content was determined by atomic absorption spectroscopy (AAS) [Mn-

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$(CO)_3$@P1: 8.9%; $Mn(CO)_3$@P2: 2.8%]. This load is within the range also found in the micellar systems of Hubbell or the functionalised SiO_2 nanoparticles of Schatzschneider.[31,32]

For comparison, three molecular $Mn(CO)_3$ complexes **1**–**3** of bpma-type ligands were prepared. The complexes **1**–**3** serve as models for the coordination environment of the $Mn(CO)_3$ moiety in the polymers. All bpma-type ligands form complexes of the composition $[LMn(CO)_3]OTf$ [L = bpma, bpmea and bis(pyridyl-methyl)-*p*-vinylbenzylamine (bpmvba)] when Mn(CO)5Br, AgOTf and the ligand are reacted in equimolar quantities in refluxing methanol (Figure 1).

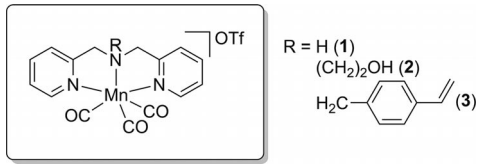

Figure 1. $Mn(CO)_3$ complexes **1**–**3** of bpma-type ligands.

Upon coordination, the methylene protons of the 2-pyridylmethyl groups in the ^1H NMR spectra at δ = 4.7 to 4.9 ppm are no longer chemically equivalent due to the C_s symmetry of complexes **1**–**3** and form an AB spin system. The IR spectra of the complexes show the characteristic bands of the ligands and the two bands of a nearly C_{3v}-symmetrical $Mn(CO)_3$ fragment, with the doubly degenerate E band split (Table 1). This splitting has also been observed in the corresponding $Re(CO)_3$ and $Mo^I(CO)_3$ complexes of a similar bpma-type ligand.[38,41]

Table 1. IR bands (ATP), half lives for CO release[a] and moles of CO released[b] for **P1**, **P2** and complexes **1**–**3**.

Compound	ν_{CO} [cm^{-1}]	$t_{1/2}$ [min]	*n*(CO)
$Mn(CO)_3$@P1	2037, 1948, 1934	20	–[c]
$Mn(CO)_3$@P2	2036, 1945, 1929	20	–[c]
1	2038, 1949, 1933	20	1.59
2	2038, 1949, 1937	20	2.65
3	2038, 1950, 1937	20	1.65

[a] Half life time of CO release after irradiation. [b] Equivalents of CO released per mole of complex as determined by the myoglobin assay. [c] Not determined (see text).

The FAB^+ mass spectra of complexes **1**–**3** exhibit peaks for the ions $[LMn(CO)_3]^+$ at the expected *m*/*z* values. The IR spectroscopic data of the $Mn(CO)_3$ compounds as well as the results from the CO-release study (see below) are summarised in Table 1.

For a better understanding of the $Mn(CO)_3$ binding in the functionalised polymers, we synthesised and determined the molecular structure of the model complex [(bpmea)-$Mn(CO)_3$]Br (**4**), which generated crystals of better quality than the triflate congener **2**. Bpma-type ligands usually coordinate facially, but they can also coordinate meridionally.[42–45] Additionally, in complexes **2** and **4** the OH functionality of the ligand could serve as a donor. Using X-ray analysis we evidenced that the $Mn(CO)_3$ core was facially coordinated by the bpma ligand.

Crystals of **4**·0.5$(CH_3)_2$CHOH were grown from a saturated solution of **4** in hot 2-propanol. The manganese centre is in a slightly distorted octahedral coordination environment, with the *N*,*N*,*N* ligand and the three carbonyl ligands coordinating facially (Figure 2). The metric parameters found in the structure of **4**·$(CH_3)_2$CHOH are within the range of these found in manganese complexes of bpma-type ligands and $Mn(CO)_3$ complexes with *N*,*N*,*N* ligands.[46,47] To the best of our knowledge, this is the first example of a single-crystal structure of a bpma ligand in combination with the $Mn(CO)_3$ core.

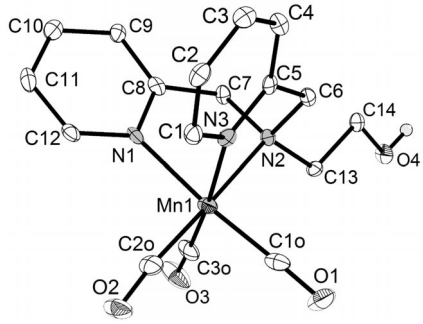

Figure 2. Molecular structure of **4**·0.5$(CH_3)_2$CHOH. Only one of the two crystallographic independent $Mn(CO)_3$ complexes of the asymmetric unit is shown. The co-crystallised solvent molecule, the bromide counter anions and the H atoms have been omitted for clarity and displacement ellipsoids are drawn at 50% level. Selected bond lengths [Å] and angles [°]: Mn1–C1O 1.807(4), Mn1–C3O 1.813(4), Mn1–C2O 1.820(4), Mn1–N1 2.040(3), Mn1–N3 2.047(3), Mn1–N2 2.108(3); C1O–Mn1–C3O 93.61(19), C1O–Mn1–C2O 86.46(18), C3O–Mn1–C2O 88.72(18), C1O–Mn1–N1 174.33(15), C3O–Mn1–N1 91.13(16), C2O–Mn1–N1 96.77(16), C1O–Mn1–N3 93.18(15), C3O–Mn1–N3 171.28(16), C2O–Mn1–N3 97.15(16), C1O–Mn1–N2 94.71(15), C3O–Mn1–N2 92.08(15), C2O–Mn1–N2 178.54(15), N1–Mn1–N2 82.00(12), N1–Mn1–N3 81.81(12), N3–Mn1–N2 81.92(12).

CO-Release Study

To study the suitability of the compounds to act as novel CO-releasing molecules (CORMs), the $Mn(CO)_3$-labelled copolymers $Mn(CO)_3$@P1/2 as well as the manganese complexes **1**–**3** were investigated by using the UV/Vis-based myoglobin (Mb) assay.[48,49] Upon irradiation of complexes **1**–**3**, the corresponding UV/Vis spectra showed the evolution of the myoglobin–CO adduct, and, in the Q-band region (500–600 nm), isosbestic points at 504, 517, 550, 570 and 586 nm were observed (Figure 3).

As the $Mn(CO)_3$ load of the $Mn(CO)_3$-labelled polymers $Mn(CO)_3$@P1/2 is only 8.9 and 2.8%, respectively, a larger amount (56.1 and 55.5 mg/5 mL) of polymer was used in the solutions for the myoglobin assay. Photoinduced CO release was observed for both polymers, but the solutions turned slightly turbid over the course of the reactions. This

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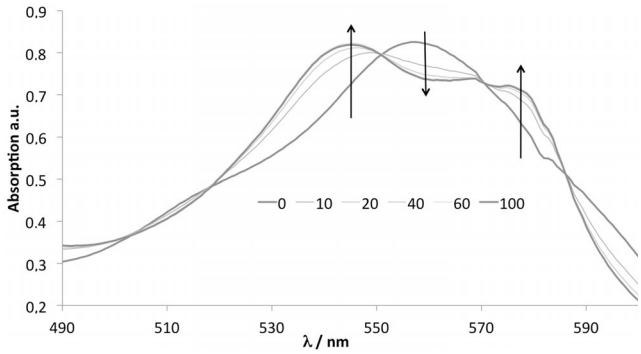

Figure 3. UV/Vis spectral changes in the Q-band region of a solution of reduced horse skeletal muscle myoglobin (75 μM) and **3** (20 μM) in 0.1 M phosphate buffer upon irradiation at 365 nm (t = 0–100 min).

led to offsets in the UV/Vis spectra (see the Supporting Information), which were corrected by applying the method recently described by Fairlamb et al. (Figure 4).[50]

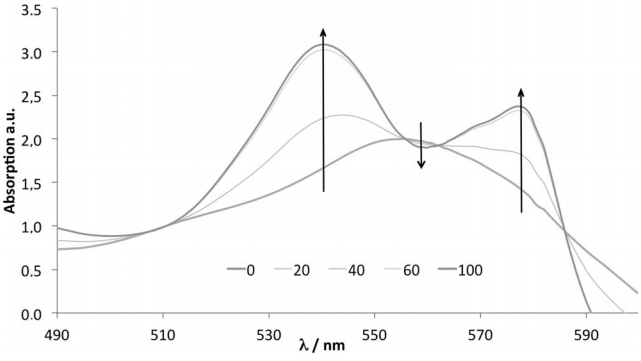

Figure 4. UV/Vis spectral changes in the Q-band region of a solution of reduced horse skeletal muscle myoglobin (75 μM) and $Mn(CO)_3$@P1 (20 μM Mn) in 0.1 M phosphate buffer upon irradiation at 365 nm (t = 0–100 min).

The $Mn(CO)_3$ complexes of bpma-type ligands release half of the CO load upon irradiation within a period of 20 min. For the calculation of the number of moles of CO released per mole of complex **1**, **2**, or **3**, we determined the starting concentration of myoglobin from the absorption data by using ε_{560nm} = 13.8 mmol L^{-1} cm^{-1} and applying Lambert–Beer's law.[51] By this method we observe a mean amount of about two moles of CO released per mole of **1** and **3**, and three moles CO per mole of **2**.

Cytotoxicity

It is known that the conjugation of non-toxic organometallic species to a non-toxic peptide can still lead to cytotoxic conjugates.[52–54] Therefore, we tested the cytotoxicity of the compounds **1**–**3**, **P1**, **P2** and $Mn(CO)_3$@P1/2 in Hct116 human colon carcinoma and HepG2 human hepatoma cells and compared them to the cytotoxicity of tricarbonyldichloridoruthenium(II) dimer ($[Ru_2(CO)_6Cl_4]$, CORM-2). The compounds **1**, **2** and $Mn(CO)_3$@P1 do not show any significant cytotoxicity in the dark or when irradiated. The reference compound CORM-2, a molecule, which spontaneously releases CO when dissolved in DMSO, also did not show any significant toxic effect on the two cell lines. Complex **3** shows some cytotoxicity as does $Mn(CO)_3$@P2. For these two compounds, the cytotoxicity does not appear to be due to the CO-releasing effect, as the effect is observed upon irradiation as well as in the dark (see the Supporting Information). The IC_{50} values for $Mn(CO)_3$@P2 upon irradiation and in the dark were determined to be (50 ± 21) and (41 ± 6) μg/mL in Hct116 and (60 ± 13) and (61 ± 10) μg/mL in HepG2 cell lines, respectively.

The cytotoxic effect of **3** can be rationalised on the basis that **3** is a styrene derivative; styrene derivatives are potent mutagens, and are hepatotoxic and pneumotoxic in mammals.[55,56] More interestingly, the polymer conjugate $Mn(CO)_3$@P2 shows cytotoxic activity, whereas none of the bpma-type ligands nor the polymer **P2** shows marked toxicity. Similar behaviour has been observed in conjugates of cymantrene, $CpMn(CO)_3$, with cell-penetrating peptides. The peptide and the cymantrene compound themselves are not toxic, however, the conjugates show significant cytotoxicity, accompanied by differences in the cellular uptake and intracellular distribution.[53,54]

Conclusion

We prepared two HPMA copolymers with suitable pendant ligands for the coordination of $Mn(CO)_3$-based CORMs. The polymers consist of either HPMA backbones with incorporated ligating units or HPMA backbones with biodegradable oligolacide side chains decorated with ligand moieties. The size and molecular weight of the HPMA–oligolactid copolymers can be designed to (1) suit the EPR effect for passive accumulation and drug delivery and (2) be degraded after delivery for renal clearance.

The bpma-type complexes **1**–**3** are effective CO-releasing molecules and release about two moles of CO per mole complex upon irradiation. The same still applies when the $Mn(CO)_3$ complexes are conjugated to polymers ("polyCORMs"). Compounds **1**, **2** and $Mn(CO)_3$@P1 do not show any significant cytotoxicity. Complex **3** and the polymer conjugate $Mn(CO)_3$@P2 show cytotoxic effects with IC_{50} values of 50–70 μM and 50–60 μg/mL, respectively.

In this paper, we have demonstrated the use of ligand-functionalised polymers as carrier systems for $Mn(CO)_3$-based CORMs. The carrier systems must be chosen carefully because a non-toxic carrier in conjugation with a non-toxic $Mn(CO)_3$ complex may result in a polymer conjugate with a distinct toxicity.

Experimental Section

General: The ligands bis(pyridylmethyl)amine (bpma), bis(pyridylmethyl)ethanolamine (bpmea) and bis(pyridylmethyl)-*p*-vinylbenzylamine (bpmvba) were prepared according published pro-

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cedures.[38,57,58] All chemicals were used as purchased from Aldrich and Fluka unless otherwise stated. Reactions carried out under inert conditions were done using standard Schlenk techniques. ^{1}H NMR spectra were recorded with a Bruker AM 200 and Bruker DRX 500 spectrometer. The spectra were calibrated against the residual proton signals of the solvents as internal references. The ESI mass spectra were recorded with an Ion-Trap-API mass spectrometer Finnigan LCQ Deca. MALDI-TOF mass spectra were recorded with a Bruker Ultraflex TOF mass spectrometer. IR spectra were recorded with a Bruker IFS 66 FT-IR spectrometer. The manganese content was determined using a Perkin–Elmer atomic absorption spectrometer 3100. Dynamic light scattering (DLS) experiments were carried out with a Malvern HPPS-ET apparatus at 20 °C. The particle size distribution was derived from a deconvolution of the measured intensity autocorrelation function of the sample by the general-purpose mode algorithm included in the DTS software. Each experiment was performed five times to obtain statistical information.

MA–PLA–bpmea: A solution of methacryloyl chloride (0.42 g, 4.0 mmol) in dichloromethane was added dropwise to a solution of PLA–bpmea (1.93 g, 3.6 mmol, average of four lactide units) and triethylamine (0.41 g, 4.0 mmol) in dichloromethane at 45 °C. The reaction mixture was stirred at this temperature for 3 h. The volume was reduced to 10 mL and extracted with hydrochloric acid (10 mL, 0.1 M), brine and bidest. water. The combined aqueous phase was extracted with dichloromethane and the combined organic phase dried with Na_2SO_4. The solvent was removed in vacuo after filtration and the resulting oily residue dried under high vacuum; yield 1.7 g (78%). ^{1}H NMR ($CDCl_3$): δ = 1.58 (m, PLA-CH_3), 2.01 (m, 3 H MA-CH_3), 2.95 (m, 2 H, NCH_2CH$_2$O), 3.98 (d, 4 H, pyCH_2), 5.07–5.33 (m, PLA-CH), 5.67 (s, 1 H, vinyl*H-cis*), 6.26 (s, 1 H, vinyl*H-trans*), 7.18–7.75 (m, 6 H, py), 8.57 (m, 2 H, py) ppm. MALDI-TOF: *m*/*z* (%): 388 (1250) [1 (dimer) PLA–bpmea], 456 (5050) [1 (dimer) MA–PLA–bpmea], 532 (5660) [3 (tetramer) PLA–bpmea], 600 (5800) [3 (tetramer) MA–PLA–bpmea], 676 (2260) [5 (hexamer) PLA–bpmea], 744 (1600) [5 (hexamer) MA–PLA–bpmea], 820 (650) [7 (octamer) PLA–bpmea]. Average of four lactide units.

P1: The synthesis described here is an improved procedure compared with the one previously described.[36]

HPMA (4.33 g, 30.0 mmol) and bis(pyridiylmethyl)-*p*-vinylbenzylamine (0.96 g, 3.0 mol) were dissolved in acetone (10 mL) and AIBN (32.1 mg) and bis(thiobenzoyl) disulfide (85.6 mg) added. The reaction was stirred for 24 h at 60 °C and concentrated to a viscous oil. The oil was then poured into a mixture of acetone and diethyl ether (1:4, 200 mL). The precipitated was collected by filtration, washed with diethyl ether and dried in vacuo. The ratio of the two monomers in the copolymer was determined from the ^{1}H NMR spectroscopic analysis by comparing the area under the curves (AUCs) of the respective proton signals. The ratio of L to HPMA was approximately 1:9; yield 3.5 g (66%). ^{1}H NMR (CD_3OD): δ = 1.01 (CH_2 backbone, HPMA-H^1), 1.98 (m, HPMA-H^4), 3.51 (m, HPMA-H^3), 3.89 (s, 2 H, NCH_2Ph), 3.98 (s, pyCH_2), 4.05 (m, HPMA-H^2), 7.12–7.87 (m, py), 8.68 (m, py) ppm. IR (ATP): $\tilde{\nu}$ = 2980–2800, 1720, 1597, 1572, 743 cm^{-1}.

P2: AIBN (6.75 mg) and bis(thiobenzoyl) disulfide (18 mg) as the chain transfer agent were added to a solution of HPMA (0.90 g, 6.3 mmol) and MA–PLA–bpmea (0.75 g, 1.3 mmol) in acetone (10 mL). The solution was heated at reflux for 1 h and concentrated in vacuo. The viscous solution was poured into a mixture of acetone and diethyl ether (1:3, 200 mL). The product precipitated as an off-white solid, which was collected by filtration, washed with diethyl ether and dried in vacuo; yield 1.00 g (61%). The ratio of the two monomers in the copolymer was determined by ^{1}H NMR spectroscopic analysis by comparing the AUCs of the respective proton signals. The ratio L to HPMA to lactide was approximately 1:4:4. ^{1}H NMR ($CDCl_3$): δ = 1.22 (m, HPMA-CH_3), 1.63 (m, PLA-CH_3), 1.98 (s, 3 H MA-CH_3), 2.14 (s, HPMA-CH_3), 2.98 (m, 2 H, NCH_2CH$_2$O), 3.98 (d, 4 H, pyCH_2), 4.00–4.11 (m, HPMA-CH_2, HPMA-CH), 5.1 (m, 4 H, PLA-CH), 7.46–7.70 (m, 6 H, py), 8.54 (m, 2 H, py). IR (ATP): $\tilde{\nu}$ = 2991, 2953, 1723, 1138, 1609, 1259 cm^{-1}.

Synthesis of [LMn(CO)$_3$]OTf Complexes 1–3: In a Schlenk tube, pentacarbonyl manganese bromide (100 mg, 0.36 mmol) and silver triflate (93.5 mg, 0.36 mmol) were dissolved in anhydrous acetone (20 mL). After heating at reflux for 1.5 h, the precipitated silver bromide was filtered off and the clear yellow solution added to an acetone solution of the respective ligand (0.36 mmol). Further heating at reflux for 1.5 h led to formation of the product, which precipitated after reducing the volume of the reaction solution to 5 mL and adding diethyl ether. After filtration, the solid product was washed with diethyl ether and dried in vacuo.

[(bpma)Mn(CO)$_3$]OTf (1): Yield 79%. ^{1}H NMR (CD_3OD): δ = 4.79 (s, 4 H, pyCH_2), 7.45 (m, 2 H, pyH^2), 7.52 (m, 2 H, pyH^4), 7.92 (m, 2 H, pyH^3), 9.03 (d, 2 H, pyH^1) ppm. ^{13}C{^{1}H} NMR (CD_3OD): δ = 62.9, 124.0, 126.78, 140.8, 153.83, 160.5 ppm. IR (methanol): $\tilde{\nu}$ = 2038, 1949, 1933 cm^{-1}. FAB$^+$: *m*/*z* (%) = 338 (22) [{(pyCH$_2$)$_2$NH}Mn(CO)$_3$]$^+$, 254 (10) [{(pyCH$_2$)$_2$NH}Mn]$^+$, 154 (37) [py(CH$_2$)$_2$NCH$_2$NH$_2$]$^+$, 136 (50) [py(CH$_2$)$_2$NH]$^+$, 107 (33) [py(CH)$_2$NH]$^+$, 89 (77) [PyC]$^+$ cm^{-1}. $C_{16}H_{13}F_3MnN_3O_6S$ (486.99): calcd. C 39.44, H 2.69, N 8.62; found C 39.22, H 2.60, N 8.39.

[(bpmea)Mn(CO)$_3$]OTf (2a): Yield 77%. ^{1}H NMR (CD_3OD): δ = 3.98 (t, $^3J_{HH}$ = 5 Hz, 2 H, CH_2CH$_2$OH) 4.12 (t, $^3J_{HH}$ = 5 Hz, 2 H, NCH$_2$CH_2OH), 4.72–4.89 (m, 4 H, pyCH_2), 7.46 (m, 4 H, py$H^{4,2}$), 7.94 (m, 2 H, pyH^3), 8.98 (m, 2 H, pyH^1) ppm. ^{13}C{^{1}H} NMR (DMSO): δ = 57.7, 67.5, 70.5, 122.9, 125.7, 139.9, 152.4, 161 ppm. IR (methanol): $\tilde{\nu}$ = 2038, 1948, 1937 cm^{-1}. FAB$^+$: *m*/*z* (%) = 382 (35) [{(pyCH$_2$)$_2$NCH$_2$CH$_2$OH}Mn(CO)$_3$]$^+$, 298 (15) [{(pyCH$_2$)$_2$NCH$_2$CH$_2$}Mn]$^+$, 154 (40) [(pyCH$_2$)$_2$NCH$_3$]$^+$, 136 (61) [py(CH$_2$)$_2$N]$^+$, 77 (88) [py]$^+$. $C_{18}H_{17}F_3MnN_3O_7S$ (531.01): calcd. C 40.67, H 3.23, N 7.91; found C 40.67, H 3.48, N 8.02.

[(bpmvba)Mn(CO)$_3$]OTf (3): Yield 82%. ^{1}H NMR (CD_3OD): δ = 4.17 (s, 2 H, PhCH_2), 4.94 (m, 4 H, [AB]$_2$-system, pyCH_2), 5.39 (d, $^3J_{HH}$ = 12 Hz, 1 H, *cis*-CH_2CH), 5.41 (d, $^3J_{HH}$ = 16 Hz, 1 H, *trans*-CH_2CH), 6.84 (dd, 1 H, CHCH$_2$), 7.39–7.83 (m, 10 H, pyH^{3-5}, Ph), 8.99 (d, pyH^1) ppm. ^{13}C{^{1}H} NMR (CD_3OD): δ = 68.2, 73.1, 116.6, 124.2, 126.1 (Ph), 128.1 (Ph), 132.8, 137.7, 140.7, 141.0, 153.8, 162.0 ppm. IR (methanol): $\tilde{\nu}$ = 2039, 1950, 1937 cm^{-1}. FAB$^+$: *m*/*z* (%) = 454 (60) {[(pyCH$_2$)$_2$N(Ph(CH$_2$)C$_2$H$_3$]Mn(CO)$_3$}$^+$, 370 (34) {[(pyCH$_2$)$_2$N(Ph(CH$_2$)C$_2$H$_3$]Mn}$^+$, 253 (40) [(pyCH$_2$)$_2$-NC$_4$H$_6$]$^+$, 154 (21) [py(CH$_2$)NC$_2$H$_4$NH$_2$]$^+$, 136 (35) [py(CH$_2$)$_2$-NH]$^+$, 107 (28) [py(CH$_2$)N]$^+$, 89 (66) [pyC]$^+$, 77 (75) [py]$^+$. $C_{25}H_{21}F_3MnN_3O_6S{\cdot}CH_2Cl_2$ (690.03): calcd. C 45.36, H 3.37, N 6.10; found C 44.97, H 3.48, N 6.14.

[(bpmea)Mn(CO)$_3$]Br (4): In a Schlenk tube, bromide (100 mg, 0.36 mmol) in anhydrous acetone (20 mL) was heated at reflux for 1.5 h and the clear yellow solution added to an acetone solution of the bpmea ligand (88 mg, 0.36 mmol). After further heating at reflux for 1.5 h, the product precipitated after reducing the volume of the reaction solution to 5 mL and adding diethyl ether. After filtration, the solid product was washed with diethyl ether and dried in vacuo; yield 133 mg (80%). ^{1}H NMR (CD_3OD): δ = 3.98 (t, $^3J_{HH}$ = 5 Hz, 2 H, CH_2CH$_2$OH) 4.12 (t, $^3J_{HH}$ = 5 Hz, 2 H, NCH$_2$CH_2OH), 4.72–4.89 (m, 4 H, pyCH_2), 7.46 (m, 4 H, py$H^{4,2}$),

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7.94 (m, 2 H, pyH^3), 8.98 (m, 2 H, pyH^1) ppm. IR (methanol): $\tilde{\nu}$ = 2038, 1948, 1937 cm^{-1}.

$Mn(CO)_3$-Labelling of Copolymers P1 and P2: In a Schlenk flask, bromide (50 mg, 0.18 mmol) and silver triflate (49 mg, 0.18 mmol) were dissolved in anhydrous acetone (15 mL). After heating at reflux for 1.5 h, the precipitated silver bromide was filtered off and a methanolic solution of the respective copolymer was added (**P1** 56 mg, **P2** 106 mg) to the clear yellow solution. Further heating at reflux for 1.5 h led to formation of the product, which precipitated after reducing the volume of the reaction solution to 30 mL and adding diethyl ether. After filtration, the solid product was washed with diethyl ether and dried in vacuo.

$Mn(CO)_3$@P1: Yield 294 mg (92%). ^1H NMR (CD_3OD): δ = 1.05 (CH_2, HPMA-H^1), 2.01 (m, HPMA-H^4), 3.51 (m, HPMA-H^3), 3.89 (s, NCH_2Ph), 4.01 (m, HPMA-H^2), 7.12–7.90 (m, py$H$), 8.68 (m, py$H$) ppm. IR (methanol): $\tilde{\nu}$ = 2980–2800, 2943, 2037, 1948, 1934, 1695, 1612 cm^{-1}. Mn content (AAS): 8.9%.

$Mn(CO)_3$@P2: Yield 270 mg (93%). ^1H NMR (CD_3OD): δ = 1.23 (m, HPMA-CH_3), 1.63 (m, PLA-CH_3),1.99 (s, MA-CH_3), 2.14 (s, HPMA-CH_3), 2.96 {m, [$(pyCH_2)_2NCH_2CH_2O$]}, 4.00–4.12 (m, HPMA-CH_2, HPMA-CH), 5.15 (m, PLA-CH), 7.11–7.70 (m, py), 8.55 (m, py) ppm. IR (ATP): $\tilde{\nu}$ = 2991, 2943, 2036, 1954, 1940, 1754, 1609 cm^{-1}. Mn content (AAS): 2.8%.

X-ray Crystallography: Single-crystal X-ray diffraction data of $C_{37}H_{42}Br_2Mn_2N_6O_9$ (**4**·0.5C_3H_7OH) were collected at 100 K on an Oxford Xcalibur diffractometer equipped with an EOS detector (Oxford Diffraction, Oxford) with Mo-K_α radiation (λ = 0.71073 Å, graphite monochromator). The structure was solved by direct methods after empirical absorption correction using spherical harmonics, implemented in SCALE3 ABSPACK scaling algorithm.[59] For the structure solution and refinement, the program package SHELX97 was used.[60] A Table with the crystallographic data of **4**·0.5C_3H_7OH can be found in the Supporting Information.

CCDC-826758 contains the supplementary crystallographic data for this paper. These data can be obtained free of charge from The Cambridge Crystallographic Data Centre via www.ccdc.cam.ac.uk/data_request/cif.

CO-Release Studies, Myoglobin Assay: All UV/Vis spectroscopic measurements were performed with a AnalyticJena Specord 300 spectrophotometer at room temperature in a quartz cuvette (d = 1 cm). Horse skeletal muscle myoglobin (Fluka) was dissolved in 0.1 M phosphate buffer pH = 7.3 and degassed by bubbling with nitrogen. It was then reduced by the addition of an excess of sodium dithionite in the same solvent, and finally buffer was added to the cuvette to give a total volume of 749 μL. The complex (1 μL) dissolved in DMSO was added to this solution to give a final concentration of 20 μmol L^{-1} of metal complex and 75 μmol L^{-1} of myoglobin with A_{557nm} = 1. Solutions were then either kept in the dark or irradiated for given time intervals under nitrogen at 365 nm with a UV hand lamp positioned perpendicular to the cuvette at a distance of 6 cm. Irradiations were interrupted at regular intervals to measure UV/Vis spectra. All measurements were carried out in triplicate to analyse the reproducibility of the CO release.

Cell Culture: Human colon carcinoma cells (Hct116) and human hepatoma cells (HepG2) were grown in Dulbecco's modified Eagle's medium (DMEM, GIBCO; Germany) containing 10% foetal calf serum (PAA Laboratories; Austria), penicillin (100 U/mL) and streptomycin (100 μg/mL) at 5% CO_2 and 37 °C.

Determination of Cytotoxicity: The rate of cell survival under the action of substances was evaluated by an improved MTT assay. The assay is based on the ability of viable cells to metabolise yellow 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT, Sigma, Germany) to violet formazane crystals that can be detected spectrophotometrically. In brief, cells were plated on 96-multiwell plates with 10.000 cells/well, allowed to attach for 24 h and then treated with different concentrations of the substances for 24 h. After this time, the treatment medium was changed and cells were incubated for 3 h under cell culture conditions with 5 mg/mL MTT. Then the cells were lysed with DMSO. The concentration of reduced MTT as marker for cell viability was measured photometrically (560 nm) using a Wallace Victor^2 1420 multilabel counter (Perkin–Elmer). The absorbance of untreated control cells was taken as 100% viability. All tests were performed in triplicate.

Supporting Information (see footnote on the first page of this article): Uncorrected data for the myoglobin assay of $Mn(CO)_3$@P1 (Figure ESI1), crystallographic data of **4**·0.5C_3H_7OH (Table ESI1) and data of the cytotoxicity assays.

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