A Single-Pot Template Reaction Towards a Manganese-Based T₁ Contrast Agent

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**Abstract:** Manganese-based contrast agents (MnCAs) emerged as suitable alternatives to gadolinium-based contrast agents (GdCAs). However, due to their kinetic lability and laborious synthetic procedures, only a few MnCAs have found clinical MRI application. In this work, we have employed a highly innovative single pot template synthetic strategy to develop a MnCA, \( \text{MnL}^{\text{Me}} \), and studied the most important physicochemical properties in vitro. \( \text{MnL}^{\text{Me}} \) displays optimized, \( r_1 \) relaxivities at both medium (20 and 64 MHz) and high magnetic fields (300 and 400 MHz) and an enhanced \( r_1 \) \( = 21.1 \) mM\(^{-1}\)s\(^{-1}\) (20 MHz, 298 K, pH 7.4) upon binding to BSA \( (K_a = 4.2 \times 10^3 \text{ M}^{-1}) \). *In vivo* studies show that \( \text{MnL}^{\text{Me}} \) is cleared intact into the bladder through hepatobiliary excretion and a prolonged blood half-life compared to the commercial GdCA Magnevist. \( \text{MnL}^{\text{Me}} \) shows great promise as a novel MRI contrast agent.

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Figure S20. Biodistribution of Magnevist and MnLMe: (A) Representative images of Magnevist-induced contrast enhancement in vivo. (B) Total signal intensity over time of MnLMe represented as the area under the curve (AUC±SEM (ΔSI/min)) was comparable to Magnevist in all analyzed organs (mean ± SEM, n = 3 for MnLMe, n = 3 for Magnevist). Percentage of signal intensity enhancement normalized to pre-injection over time for all organs of interest is given in (C) for MnLMe and (D) for Magnevist.

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Experimental Procedures

Materials and Measurements. All of the materials and reagents for the synthesis of monoanuclear Mn(II) and Zn(II) complexes ([MnL\textsubscript{Me}] and [ZnL\textsubscript{Me}]) were purchased from Sigma-Aldrich (Dorset, UK) and used without further purification. Solvents were purchased from VWR (Leicestershire, UK). IR spectra were recorded on a Perkin Elmer RX FTIR x2 with diamond ATR, DRIFT attachment. \textsuperscript{1}H-NMR and \textsuperscript{13}C-NMR measurements were carried out on a JEOL JNM-LA400 Spectrometer (400 MHz). Electronic absorption spectral titration experiments were done using a ThermoFisher Scientific Evolution 300 UV-Vis spectrophotometer, and the fluorescence emission studies were carried out on a Horiba Fluoromax-4P spectrofluorometer. High resolution mass spectrometry (HRMS) spectra of the compounds were recorded on Advion MS SOP electrospray ionization (ESI) spectrometer. The pH measurements were carried out by using a Jenway 3520 digital pH meter with a Mettler-Toledo 51343160 glass electrode.

Synthesis of Mn(II) complex [MnL\textsubscript{Me}]. (1). 2,6-diacetylpyridine (0.163 g, 1.0 mmol) was added to 60 mL of methanol (MeOH), and solid MnCl\textsubscript{2} (0.121 g, 0.96 mmol) was added and stirred for an hour at 60 °C. To this mixture, a methanolic solution (20 mL) of acetylhydrazide (0.148 g, 2.0 mmol) was added dropwise. The mixture turned slowly from colorless to pale yellow and was refluxed overnight, and hot filtered. The solvent was evaporated in air, and the resultant bright yellow solid was washed with little amounts of isopropanol/diethyl ether mixture and dried. The obtained water-soluble yellow solid was recrystallized from water and MeOH (2:1, v/v) mixture. Isolated yield: bright yellow block crystals, 0.32g (91%). Anal. Calcd for [Mn(L\textsubscript{Me})Cl(H\textsubscript{2}O)]•Cl.2H\textsubscript{2}O: C, 34.30; H, 5.09; N, 15.39. Found: C, 34.31, H, 5.07, N, 15.35. FTIR (neat, cm\textsuperscript{-1}): 3428b (N=H), 1637s (C=N), 1609s (C=O), 1026s (−N−N). MS (ESI\textsuperscript{+}) in CH\textsubscript{3}OH: m/z 365.04 ([Mn(L\textsubscript{Me})Cl(H\textsubscript{2}O)])\textsuperscript{+}, 329.07 07 ([Mn(L\textsubscript{Me}−H)\textsuperscript{+}]\textsuperscript{+})\textsuperscript{+}. HRMS: calcd. for C\textsubscript{13}H\textsubscript{17}Cl:N2O2Mn, 365.0446 found 365.0451.

Synthesis of Zn(II) complex [ZnL\textsubscript{Me}]. (2). The Zn(II) complex was synthesized by following the above procedure with 24 h refluxing using ZnCl\textsubscript{2} (0.126 g, 0.96 mmol) instead of MnCl\textsubscript{2}. Isolated yield: Pale yellow crystals, 0.18 g (49%). Anal. Calcd for [Zn(L\textsubscript{Me})Cl(H\textsubscript{2}O)]•Cl.2H\textsubscript{2}O: C, 33.53; H, 4.90; N, 15.04. Found: C, 33.30, H, 4.91, N, 14.84. \textsuperscript{1}H NMR (D\textsubscript{2}O, 400 MHz) δ (ppm): 8.2 (br, 1H, H−Pyridine), 8.00 (d, 2H, J = 10.0 Hz, H−Pyridine), 2.44 (br, 6H, H−methyl) and 2.23 (br, 2H, 6H−methyl). \textsuperscript{13}C NMR (D\textsubscript{2}O, 100 MHz) δ (ppm): 12.60, 19.69, 124.50, 143.13, 147.60, 148.09 and 173.33. FTIR (neat, cm\textsuperscript{-1}): 3453s (N=H), 1642s (C=N), 1614s (C=O), 1037s (−N−N). MS (ESI\textsuperscript{+}) in CH\textsubscript{3}OH: m/z 374.18 ([Zn(L\textsubscript{Me})Cl(H\textsubscript{2}O)])\textsuperscript{+}, 339.14 ([Zn(L\textsubscript{Me}−H)\textsuperscript{+}]\textsuperscript{+}). ES-MS: calcd. for C\textsubscript{13}H\textsubscript{17}Cl:N2O2Zn, 374.04 found 374.18.

X-Ray Data Collection and Reduction. Single crystal X-ray diffraction data were collected in series of ω-scans using a Stoe IPS2 image plate diffractometer utilizing monochromated Mo radiation (λ = 0.71073 Å). Standard procedures were employed for the integration and processing of the data using X-RED.\textsuperscript{[1]} Samples were coated in a thin film of perfluoropolyether oil and mounted at the tip of a glass fiber located on a goniometer. Data were collected from crystals held at 150 K in an Oxford Cryosystems nitrogen gas cryostream.

Crystal structures were solved using dual space methods implemented within SHELXT.\textsuperscript{[2]} Completion of structures was achieved by performing least squares refinement against all unique F2 values using SHELXL-2018.\textsuperscript{[3]} All non H atoms were refined with anisotropic displacement parameters. Hydrogen atoms were placed using a riding model. Where the location of hydrogen atoms was obvious from interatomic distances, C−H and O−H bond lengths were refined subject to chemically sensible restraints.

Pseudosymmetry in the crystal structures of MnL\textsubscript{Me} and ZnL\textsubscript{Me}. Both structures crystallize in P2\textsubscript{1}/c but are close to being body centred. Each structure solves and refines very satisfactorily in this space group and the refinement is stable. In neither case is there convincing evidence for a body-centring translation although the symmetry of the primitive cell generates a pseudo-body-centred array of the complexes. However, the strict bodycentring translation is not obeyed; complexes related by the pseudo translation are rotated by approximately 180 degrees about the Zn1−N1 bond.

The diffraction data below demonstrate that the true symmetry is P ̅n l.

Observed diffraction data for MnL\textsubscript{Me}:

Reflections with h+k+l=2n:

5688 refs, mean ⟨l/σl⟩ = 10.4 and of these 2483 have l> 4σl

Reflections with h+k+l=2n+1: 5426 refs, mean ⟨l/σl⟩ = 4.9 and of these 1676 have l> 4σl.

The data consistent with bodycentring are thus weak but not systematically absent.

Observed diffraction data for ZnL\textsubscript{Me}
The longitudinal relaxation times ($T_1$) and water proton relaxation rates ($r_1 = 1/T_1$) of the MnL$^{106}$ were measured on a JEOL JNM-LA 400 Spectrometer (400 MHz). The $T_1$ values were obtained by the inversion-recovery method (180° − r − 90°). The relaxivity of the complex was determined by different concentrations of MnL$^{106}$ (1.0 to 5.0 mM) at pH = 7.3 (50 mM HEPES buffer, 0.15 M NaCl, 298 ± 0.2 K). The water proton relaxivity $r_1$ of the MnL$^{106}$ was determined from the slope of the plot of 1/$T_1$ vs. [MnL$^{106}$]. Stock solutions of MnL$^{106}$ (10 mM) and BSA were prepared in MilliQ water. The concentration of BSA was determined spectroscopically based on the tryptophan absorbance at 280 nm ($ε_{280} = 43824$ M$^{-1}$ cm$^{-1}$) in water. The hydration state of the metal was empirically calculated by applying the following equations (1) and (2):\(^{(1)}\)
\[ y = 9.10 \left(1 - \exp\left(-2.97 \times 10^{-3} \times F_W \times 10^{-11}\right)\right) \quad \ldots(1) \]
\[ q_{\text{calc}} = \frac{r_1}{y} \quad \ldots(2) \]
where, $F_W = 366.28$ g/mol; $y = 6.05$; $r_1 = 13.9$ mM$^{-1}$ s$^{-1}$ and $q = 2.3$.

\(^1^H\) Nuclear Magnetic Relaxation Dispersion (NMRD) profiles were measured on a Fast-Field Cycling (FFC) Stelar Smart Tracer Relaxometer over a continuum of magnetic field strengths from 0.00024 to 0.25 T (corresponding to 0.01-10 MHz proton Larmor Frequencies). Additional data points in the range 20-120 MHz were obtained with a High Field Relaxometer (Stelar) equipped with the HTS-110 3T Metrology Cryogen-free Superconducting Magnet. The measurements were performed using the standard inversion recovery sequence (20 experiments, 2 scans) with a typical 90° pulse width of 3.5 μs and the reproducibility of the data was within ± 0.5%. The temperature was con-trolled with a Stelar VTC-91 heater airflow equipped with a copper-constantan thermocouple (uncertainty of ± 0.1 K). The Mn(II) concentration was estimated by \(^1^H\)-NMR (Bruker Advance III Spectrometer equipped with a wide bore 11.7 T magnet) measurements using Evans’s method.\(^{(2)}\) The hydration number $q$ was adjusted to 2; the distance between the metal ion and the protons of the bound, $f_{\text{prox}}$, and outer sphere, $a_{\text{prox}}$, water molecule were set to 2.74 and 3.6 Å, respectively. The value of the distance $f_{\text{prox}}$ is consistent with the average distances obtained ($f_{\text{prox}} = 2.7373$ Å) from X-ray crystal structure data of MnL$^{106}$.

\(^{1^7}\)O NMR

MnL$^{106}$: \(^{1^7}\)O NMR spectra were acquired on a Bruker Avance III spectrometer (11.7 T) using a 5 mm probe under temperature control. An aqueous solution of the Mn(II) chelate (2.3 mM) in HEPES buffer (pH = 7.4) containing 2.0\% \(^{1^7}\)O-enriched water (Cambridge Isotope) was prepared and analysed. The transverse relaxation rates were measured from the signal width at half-height, as a function of temperature in the 278-350 K range. A diamagnetic blank solution containing acidified water (pH = 3.0) enriched with H$_2^{1^7}$O (2.0\%) was used as reference. Notably, the observed transverse relaxation rates $R_2$ depend on the rate of water exchange at 298 K, $k_{\text{ex}}$, and its activation enthalpy $\Delta H_{\text{A}}$, the electronic relaxation time $\tau_2$, and the \(^{1^7}\)O hyperfine coupling constant $A_{\text{hf}}/\hbar$.

MnL$^{106}$/BSA: the same procedure was applied to the BSA-bound complex. A 0.12 mM solution of the complex and 1.43 mM of BSA in HEPES containing 2.0\% \(^{1^7}\)O-enriched water (Cambridge Isotope) was prepared. The diamagnetic blank solution was prepared by dissolving BSA (1.43 mM) in HEPES containing 2.0\% of the \(^{17}\)O isotope (Cambridge Isotope).

The hydration state of Mn(II) ($q$) was calculated by using the empirical equation\(^{(6)}\) reported below:

\[ q = \frac{r_2^{\text{max}} [H_2O]}{\left(\frac{2}{\sqrt{3}}\right) A_{\text{hf}}/\hbar} \approx \frac{r_2^{\text{max}}}{510} \]

where, $r_2^{\text{max}}$ is the maximum \(^{1^7}\)O transverse relativity and $A_{\text{hf}}/\hbar$ is the hyperfine coupling constant.

MR Imaging. Phantoms of 0.1-0.5 mM MnL$^{106}$ in 50 mM HEPES buffer (pH 7.4) and 0.1-0.5 mM MnL$^{106}$ with 0.6 mM BSA in 50 mM HEPES buffer (pH 7.4) were prepared in a customized 5 × 5 well plate (0.25 mL capacity). Phantom and in vivo MR imaging were acquired on a 7 T preclinical MR scanner (Bruker BioSpec 70/30, Bruker BioSpin, Ettlingen, Germany) using an 86-mm diameter \(^1^H\) transceiver volume coil (Bruker). The sample temperature was regulated with a heated pad system (Medes medical research GmbH, Cologne, Germany) and monitored with corresponding temperature probe and by \(^1^H\) spectroscopy (PRESS) on ethylene glycol. $T_1$-weighted MR images of MnL$^{106}$ were acquired with a standard 3D T1-FLASH sequences and the following parameters: TE = 2.64 ms, TR = 8.78 ms, flip angle 30°, FOV 60×40×21.21 mm$^3$, image size 272×136×96, slice thickness 21.12 mm, resolution 0.220×0.220×0.220 mm$^3$, 1 average with a total acquisition time of 1 min 54 s. For comparison of MnL$^{106}$ to Magnevist in $T_2$-weighted imaging, the flip angle was reduced to 10°. $T_2$ maps were acquired with a 2D RARETR sequence (TE 6.705 ms, FOV 50×50 mm$^2$, image size 128×128, resolution 0.390×0.390 mm$^2$, total scan time: 12 min 52 s) with 15 TR values of 50, 100, 200, 300, 400, 500, 600, 700, 800, 900, 1000, 1500, 2000, 2500 and 3000 ms. $T_2$-weighted images were acquired with a standard 2D spin-echo sequence and the following parameters: TE 8.993 ms, TR 4253.613 ms, FOV 60×40 mm$^2$, image size 200×134, resolution...
0.300×0.298 mm², 1 average with a total acquisition time of 1 min 8 s. T₂-maps were acquired with a standard MSME sequence, variable TEs of 8-200ms in 8 ms steps and the following parameters: TR 2000 ms, FOV 50×50 mm², image size 128×128, resolution 0.300×0.298 mm², total scan time 4 min 16 s.

For in vivo imaging, healthy female C56BL/6 mice were anesthetized with 1.5 % isoflurane in pure oxygen. For the application of the MR contrast agents, a catheter was placed in a lateral tail vein. T₁-weighted pre- and post-contrast in vivo MR images were acquired with a 3D FLASH-sequence and the following parameters: TE = 2.64 ms, TR = 8.78 ms, flip angle 10°, FOV 75×35×21.21 mm³, image size 272×136×96, slice thickness 21.12 mm, resolution 0.276×0.257×0.220 mm³, total acquisition time 1 min 54 s. After the pre-contrast acquisition, MnLMe₄ (n = 3) or Magnevist (n = 3) were applied at a final dose of 0.1 mmol/Kg body weight and allowed to distribute for 5 min before 16 post-contrast images were acquired back-to-back. For data analysis in PMOD (PMOD Technologies LCC, Bruker), fixed volumes of interest were placed in the kidneys, liver, heart, bladder, and thigh muscle. Post-contrast values were normalized to pre-contrast, and the resulting percent signal change represented over time. The area under the curve was measured over a period of 1 – 40 minutes post-contrast administration and plotted using GraphPad Prism 7. Statistical significance was evaluated using unpaired two-tailed t-tests, p-values < 0.05 were considered significant. All animal procedures were conducted following German federal regulations on the use and care of experimental animals and approved by the local authorities (Regierungspräsidium Tübingen).

HPLC strategies. Liquid chromatography-mass spectrometry (LC-MS) was performed using an Agilent LC-MSD system (LC: 1200 series, MS: 6120 quadrupole) and Daly conversion dynode detector with UV detection at 220, 254 and 280 nm and used the following method to analyse the samples. Luna C18 column (250 × 2 mm), eluent A: CH₃CN, eluent B: NH₄OAc buffer (pH 7.0, 298 K), gradient (0–95% CH₃CN over 18 min, flow rate 0.3 mL/min. Urine samples were preprocessed with a 10 kDa molecular weight cut off filter before injection onto the column.

NMR titrations. NMR spectra for transmetalation of MnLMe₄ and [Gd(DTPA)(H₂O)]²⁻ (Magnevist®) with 1 and 25 molar equivalents Zn²⁺ were acquired on a 600 MHz Bruker Avance III spectrometer at 298 K. T₁ relaxation was measured via an inversion recovery experiment using 8 inversion times (0.05, 0.075, 0.1, 0.25, 0.35, 0.5, 0.75 and 2 s) before and after addition of Zn²⁺. T₂ relaxation was measured using a Carl-Purcell-Meiboom-Gill spin-echo experiment. Relaxivity values (r₁, r₂) were calculated for each transmetalation experiment from the previously determined concentration. The concentration of paramagnetic complexes in solution was determined from bulk magnetic susceptibility shift in NMR spectra.[5]

Results and Discussion
Figure S1. $^1$H-NMR spectrum of Zn$^{2+}$ complex ZnLMe recorded in D$_2$O.

Figure S2. $^{13}$C-NMR spectrum of Zn$^{2+}$ complex ZnLMe recorded in D$_2$O.
Figure S3. Absorption spectra of MnLMe, MnLMe$^{2+}$ + 10 eq. Zn$^{2+}$ and ZnLMe (50 mM) recorded in 50 mM HEPES buffer at pH = 7.3.
Figure S4. HRMS (ESI$^+$) spectrum of MnL$^{Me}$ recorded in CH$_3$OH.
Figure S5. ESI\(^+\) spectrum of ZnL\(^{Me}\) recorded in CH\(_3\)OH.

Figure S6. ORTEP diagram of ZnL\(^{Me}\) drawn at 50\% ellipsoid level. Bound water (O3) forms two hydrogen bonds to unbound water and chloride. The N-H functions of the ligand form hydrogen bonds to unbound water and chloride. The bound chloride acts as a hydrogen bond acceptor to unbound water, and O1 also acts as a hydrogen bond acceptor to unbound water. Both complexes are arranged in layers parallel to (101). Within and between the layers, these hydrogen bonds assemble the complexes and unbound species into a 3-D hydrogen-bonded network.

Figure S7. Crystal packing diagram of MnL\(^{Me}\).
Figure S8. The plot of $1/T_1$ vs. $[\text{MnL}^\text{Me}]$ where the $T_1$ values determined at 9.4 T, 298 K and 1.5 T, 298 K (Top) and $T_1$-weighted MRI phantom images (1.5 T, 298 K) as a function of $\text{MnL}^\text{Me}$ concentration (1 to 5 mM) and clinical standard Magnevist (M) (1 mM) (Bottom).
**Figure S9.** Transmetallation of 2 mM Mn$^{2+}$ complex MnL$^{Me}$ by 10 mM Zn$^{2+}$ monitored by relaxation rate; R1 (1/T$_1$, s$^{-1}$) change as a function of time in water, 400 MHz (9.4 T), 298K, (A: MnL$^{Me}$ alone).

**Figure S10.** Time-dependent relaxivity and Zn$^{2+}$ (5 and 10 mM) transmetallation of MnL$^{Me}$ (1 mM) in water, 400 MHz (9.4 T), 298 K. (5 and 10 mM aqueous solutions of Zn$^{2+}$ were added to the stock solution of MnL$^{Me}$ (10 mM) and made up 5 and 10 equivalent solutions on day 8).
Figure S11. Time-dependent relaxivity and Zn\(^{2+}\) (25 mM) transmetallation of 1 mM solutions of MnL\(^{3+}\), [Mn(EDTA)(H\(_2\)O)]\(^{2-}\), [Mn(DTPA)]\(^{3-}\) and [Gd(DTPA)]\(^{3-}\) in 50 mM MES (2-(N-morpholino)ethanesulfonic acid) buffer at pH 6.0, 400 MHz (9.4 T), 298 K.
Figure S12. Chelating stability of MnLMe was monitored by change in the absorption spectral intensity as a function of time. Absorption spectra of MnLMe (50 µM) obtained over 10 days (A), Change in the absorption intensities at 213 and 275 nm (B), and the ratio of Abs275/Abs213 as the function of time (C). To reduce the errors during measurement, the ratio of absorption maxima λmax values was plotted with a function of time.
Figure S13. Change in relaxivity of $\text{MnL}^{\text{Me}}$ (1 mM) in 50 mM aqueous (phosphate/phosphoric acid (pH 3.0), MES (pH 5.2 and 6.0), HEPES (pH 7.3), Potassium phosphate/KOH (pH 8.5), NH$_4$Cl/NH$_3$ (pH 9.3 and 10.0) buffer solutions, 400 MHz (9.4 T), 25 °C.

Figure S14. Transchelation of $\text{MnL}^{\text{Me}}$. No transchelation was observed in competition with 25 molar equivalents of DTPA (b), nor with 25 molar equivalents EDTA (c) in comparison to pure $\text{MnL}^{\text{Me}}$ (a) Solutions were incubated for 30 mins with the competing ligands. The asterisk (*) marks a signal from the eluent system.
Figure S15. LC-MS of mouse urine samples. LC-MS analysis of pure MnL\textsubscript{Me}(a) and a urine sample from a healthy mouse after intravenous administration of a clinical dose of MnL\textsubscript{Me} (b). A control urine sample without prior MnL\textsubscript{Me} administration (c) and a urine sample spiked with MnL\textsubscript{Me} (d) on an Agilent LC-MS system (LC: 1200 series, MS: 6120 quadrupole). MS chromatogram (e) of the pure MnL\textsubscript{Me} extracted at m/z + 329.1 and the ESI-MS spectra at 520 sec (8.6 min). At 40 min post intravenous MnL\textsubscript{Me} (0.1 mmol/kg bodyweight, 2.5x10\textsuperscript{-6} M) injection, 24 % of the contrast agent was excreted intact into the bladder. The asterisk (*) marks a signal from the eluent system.
Figure S16. $^1$H NMRD profiles of MnL$^{Me}$ at pH 6 and three different temperatures (283 K (•), 298 K (○) and 310 K (●). [Mn$^{2+}$] = 4.0 mM. The curves through the data points were calculated with the parameters in Table S11.

Figure S17. Temperature dependence of the proton relaxivity of MnL$^{Me}$ (22 MHz).
**Figure S18.** Fluorescence spectral profile of 50 μM BSA protein in 50 mM HEPES buffer (pH 7.4, 298 K) with increasing quantity of aq. Mn²⁺ solution (0–250 μM, λ<sub>ex</sub> = 280 nm).

**Figure S19.** T₁ and T₂-weighted phantom imaging of MnL<sub>Me</sub> and Magnevist. (A) T₁-weighted imaging of 0.1–0.5 mM MnL<sub>Me</sub> without and with 0.6 mM BSA in 50 mM HEPES (first and second row, respectively) illustrates highly enhanced contrast enhancement in MnL<sub>Me</sub> samples with BSA in comparison to MnL<sub>Me</sub> samples without BSA. Compared to 0.1–0.5 mM Magnevist without and with 0.6 mM BSA in 50 mM HEPES (third and fourth row, respectively), overall contrast enhancement of MnL<sub>Me</sub> samples is lower. Images were recorded at 7 T and 298 K with a flip angle of 10°. (B) T₂-weighted imaging of 0.1–0.5 mM MnL<sub>Me</sub> without and with 0.6 mM BSA in 50 mM HEPES (first and second row, respectively) shows superior T₂ contrast modulation of MnL<sub>Me</sub> in comparison to 0.1–0.5 mM Magnevist without and with 0.6 mM BSA in 50 mM HEPES (third and fourth row, respectively).
Figure S20: Biodistribution of Magnevist and MnL\textsuperscript{Me}: (A) Representative images of Magnevist\textsuperscript{®}-induced contrast enhancement in vivo. (B) Total signal intensity over time of MnL\textsuperscript{Me} represented as the area under the curve (AUC\textsuperscript{0-40 min} (ΔSI*min)) was comparable to Magnevist in all analyzed organs (mean ± SEM, n = 3 for MnL\textsuperscript{Me}, n = 3 for Magnevist\textsuperscript{®}). Percentage of signal intensity enhancement normalized to pre-injection over time for all organs of interest is given in (C) for MnL\textsuperscript{Me} and (D) for Magnevist\textsuperscript{®}. Bars represent standard error of the mean; *p-value < 0.05, **p-value < 0.01.

Scheme S1. MnL\textsuperscript{Me} binds with bovine serum albumin (BSA), resulting in an enhanced relaxivity due to the decreased molecular rotation.
### Table S1. Crystallographic data for MnL°° and ZnL°°

| Parameters                      | MnL°°                          | ZnL°°                          |
|--------------------------------|--------------------------------|--------------------------------|
| Formula unit                   | C₁₃H₂₃ClMnN₅O₅                 | C₁₃H₂₃ClN₅O₅Zn                 |
| Formula weight                 | 455.20                         | 465.63                         |
| T (K)                          | 150(2)                         | 150(2)                         |
| Crystal system                 | Monoclinic                     | Monoclinic                     |
| Space group                    | P 2₁/c                         | P 2₁/c                         |
| a (Å)                          | 6.0344(7)                      | 7.9265(5)                      |
| b (Å)                          | 13.1800(7)                     | 13.2381(10)                    |
| c (Å)                          | 19.0413(14)                    | 18.7719(11)                    |
| β (°)                          | 99.852(6)                      | 99.377(5)                      |
| Volume (Å³)                    | 1986.6(3)                      | 1943.4(2)                      |
| Z                               | 4                              | 4                              |
| D<br>calc (Mg m⁻³) and μ (mm⁻¹) | 1.522, 0.967                   | 1.591, 1.573                   |
| F(000), Crystal size           | 940,                           | 960,                           |
|                                | 960, 0.150 x 0.110 x 0.070 mm³ |                                |
| Theta range for data collection| 1.888 to 26.371°               | 1.891 to 26.372°               |
| Index ranges                   | -7 ≤ h ≤ 10, -14 ≤ k ≤ 16, -23 ≤ l ≤ 23 | -6 ≤ h ≤ 9, -16 ≤ k ≤ 15, -23 ≤ l ≤ 23 |
| Reflections collected          | 8383                           | 9904                           |
| Independent reflections        | 4057                           | 3911 [R(int) = 0.0987]          |
| Completeness to theta = 98.3%  | 26.371°                        | 25.242°                        |
| Absorption correction          | multi-scan                     | Semi-empirical from equivalents |
| Max. and min. transmission     | 0.901 and 0.910                | 0.942 and 0.918                |
| Refinement method              | Full-matrix least-squares on F² | Full-matrix least-squares on F² |
| Data / restraints / parameters | 4027/9/269                     | 3911 / 9 / 259                 |
| Goodness-of-fit on F²          | 0.850                          | 0.856                          |
| Final R indices [R=2sigma(I)]  | R₁ = 0.0322, wR₂ = 0.0678       | R₁ = 0.0515, wR₂ = 0.1088       |
| R indices (all data)           | R₁ = 0.0686, wR₂ = 0.0611       | R₁ = 0.1124, wR₂ = 0.1248       |
Largest diff. peak and hole 0.287 and -0.277 eÅ⁻³ 0.529 and -0.415 eÅ⁻³

\[ R_1 = \frac{\sum |F_o| - |F_c|}{|F_o|}, \quad wR_2 = \left[ \frac{\sum w(F_o^2 - F_c^2)^2}{\sum w(F_o^2)} \right]^{1/2} \]

Table S2. Details of all of the unique classical hydrogen bonds in MnLMe.

| D–H    | H...A   | D...A   | 〈DHA〉 | Symmetry  |
|--------|--------|--------|--------|-----------|
| 0.95   | 2.82   | 3.761(3)| 171.2  | C2–H2...Cl2_Si |
| 0.95   | 2.79   | 3.516(3)| 133.6  | C4–H4...Cl1_Si |
| 0.98   | 2.94   | 3.898(3)| 164.5  | C7–H7B...Cl2_Si |
| 0.98   | 2.69   | 3.592(3)| 153.5  | C9–H9B...Cl2_Sii |
| 0.98   | 2.58   | 3.549(4)| 168.7  | C9–H9C...O2_Siv |
| 0.98   | 2.61   | 3.405(4)| 138.3  | C11–H11C...O1W |
| 0.98   | 2.49   | 3.349(4)| 145.6  | C13–H13B...O1W |
| 0.82   | 2.00   | 2.788(3)| 161.8  | N5–H5...O1W |
| 0.83   | 2.39   | 3.194(2)| 163.3  | N3–H3A...Cl2_Siii |
| 0.80(3)| 2.30(3)| 3.0905(18)| 168(3) | O3–H3B...Cl2 |
| 0.80(3)| 1.86(3)| 2.654(3)| 173(3) | O3–H3C...O2W |
| 0.80(2)| 2.40(3)| 3.153(2)| 156(4) | O2W–H2A...Cl1_Siv |
| 0.79(2)| 2.36(2)| 3.124(2)| 161(3) | O1W–H1B...Cl1_Svi |
| 0.81(2)| 2.10(2)| 2.833(3)| 151(3) | O2W–H2B...O1_Siv |
| 0.80(2)| 2.33(2)| 3.131(3)| 177(3) | O1W–H1A...Cl2_Svii |

Symmetry equivalent atoms are generated by the following operators: i = –x+1, –y+1, –z+1, ii = –x, –y+1, –z+1, iii = x, –y+3/2, z–1/2, iv = –x+1, –y+2, –z+1, v = x+1, y, zvi = x, –y+3/2, z+1/2, vii = x–1, y, z

Table S3. Atomic coordinates (x 10⁴) and equivalent isotropic displacement parameters (Å² x 10³) for MnLMe. U(eq) is defined as one third of the trace of the orthogonalized Uij tensor.

|      |      |      |      |      |
|------|------|------|------|------|
| C(1) | 3124(4) | 5663(2) | 4383(1) | 24(1) |
| C(2) | 3180(4) | 4610(2) | 4341(1) | 30(1) |
Table S4. Bond lengths [Å] and angles [*] for MnLMe.

| Bond                  | Length/Angle |
|-----------------------|--------------|
| C(1)-N(1)             | 1.342(3)     |
| C(1)-C(2)             | 1.392(3)     |
| C(1)-C(6)             | 1.497(3)     |
| C(2)-C(3)             | 1.380(4)     |
| C(2)-H(2)             | 0.9500       |
| C(3)-C(4)             | 1.381(4)     |
| C(3)-H(3)             | 0.9500       |
| C(4)-C(5)             | 1.394(4)     |
| C(4)-H(4)             | 0.9500       |
| C(5)-N(1)             | 1.341(3)     |
| C(5)-C(10)            | 1.486(4)     |
| C(6)-N(2)             | 1.284(3)     |
C(6)-C(7) 1.494(3)
C(7)-H(7A) 0.9800
C(7)-H(7B) 0.9800
C(7)-H(7C) 0.9800
C(8)-O(1) 1.238(3)
C(8)-N(3) 1.358(3)
C(8)-C(9) 1.483(3)
C(9)-H(9A) 0.9800
C(9)-H(9B) 0.9800
C(9)-H(9C) 0.9800
C(10)-N(4) 1.279(3)
C(10)-C(11) 1.504(3)
C(11)-H(11A) 0.9800
C(11)-H(11B) 0.9800
C(11)-H(11C) 0.9800
C(12)-O(2) 1.230(3)
C(12)-N(5) 1.362(3)
C(12)-C(13) 1.497(3)
C(13)-H(13A) 0.9800
C(13)-H(13B) 0.9800
C(13)-H(13C) 0.9800
N(1)-Mn(1) 2.2922(19)
N(4)-N(5) 1.369(3)
N(4)-Mn(1) 2.2872(18)
N(5)-H(5) 0.82(3)
N(2)-N(3) 1.365(3)
N(2)-Mn(1) 2.2979(19)
N(3)-H(3A) 0.83(3)
O(1)-Mn(1) 2.2446(16)
O(2)-Mn(1) 2.2418(16)
O(3)-Mn(1) 2.173(2)
O(3)-H(3B) 0.80(3)
O(3)-H(3C) 0.80(3)
Mn(1)-Cl(1) 2.4990(8)
O(1W)-H(1B) 0.79(2)
O(1W)-H(1A) 0.80(2)
O(2W)-H(2A) 0.80(2)
O(2W)-H(2B) 0.81(2)
N(1)-C(1)-C(2) 121.3(2)
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N(1)-C(1)-C(6) 114.9(2)
C(2)-C(1)-C(6) 123.8(2)
C(3)-C(2)-C(1) 118.5(2)
C(3)-C(2)-H(2) 120.7
C(1)-C(2)-H(2) 120.7
C(2)-C(3)-C(4) 120.1(2)
C(2)-C(3)-H(3) 120.0
C(4)-C(3)-H(3) 120.0
C(3)-C(4)-C(5) 118.7(2)
C(3)-C(4)-H(4) 120.6
C(5)-C(4)-H(4) 120.6
N(1)-C(5)-C(4) 120.9(2)
N(1)-C(5)-C(10) 115.2(2)
C(4)-C(5)-C(10) 123.8(2)
N(2)-C(6)-C(7) 124.9(2)
N(2)-C(6)-C(1) 113.3(2)
C(7)-C(6)-C(1) 121.8(2)
C(6)-C(7)-H(7A) 109.5
C(6)-C(7)-H(7B) 109.5
H(7A)-C(7)-H(7B) 109.5
C(6)-C(7)-H(7C) 109.5
H(7A)-C(7)-H(7C) 109.5
H(7B)-C(7)-H(7C) 109.5
O(1)-C(8)-N(3) 120.5(2)
N(3)-C(8)-N(3) 117.4(2)
N(3)-C(8)-C(9) 122.2(2)
C(8)-C(9)-H(9A) 109.5
C(8)-C(9)-H(9B) 109.5
H(9A)-C(9)-H(9B) 109.5
C(8)-C(9)-H(9C) 109.5
H(9A)-C(9)-H(9C) 109.5
H(9B)-C(9)-H(9C) 109.5
N(4)-C(10)-C(5) 113.8(2)
N(4)-C(10)-C(11) 125.3(2)
C(5)-C(10)-C(11) 120.9(2)
C(10)-C(11)-H(11A) 109.5
C(10)-C(11)-H(11B) 109.5
H(11A)-C(11)-H(11B) 109.5
C(10)-C(11)-H(11C) 109.5
| Bond                        | Angle (°) |
|-----------------------------|-----------|
| H(11A)-C(11)-H(11C)        | 109.5     |
| H(11B)-C(11)-H(11C)        | 109.5     |
| O(2)-C(12)-N(5)            | 121.4(2)  |
| O(2)-C(12)-C(13)           | 121.8(2)  |
| N(5)-C(12)-C(13)           | 116.8(2)  |
| C(12)-C(13)-H(13A)         | 109.5     |
| C(12)-C(13)-H(13B)         | 109.5     |
| H(13A)-C(13)-H(13B)        | 109.5     |
| C(12)-C(13)-H(13C)         | 109.5     |
| H(13A)-C(13)-H(13C)        | 109.5     |
| H(13B)-C(13)-H(13C)        | 109.5     |
| C(5)-N(1)-C(1)             | 120.4(2)  |
| C(5)-N(1)-Mn(1)            | 119.42(16)|
| C(1)-N(1)-Mn(1)            | 120.15(15)|
| C(10)-N(4)-N(5)            | 122.63(19)|
| C(10)-N(4)-Mn(1)           | 122.89(16)|
| N(5)-N(4)-Mn(1)            | 114.24(14)|
| C(12)-N(5)-N(4)            | 115.78(19)|
| C(12)-N(5)-H(5)            | 122.1     |
| C(6)-N(2)-N(3)             | 121.6(2)  |
| C(6)-N(2)-Mn(1)            | 123.13(16)|
| N(3)-N(2)-Mn(1)            | 115.09(13)|
| C(8)-N(3)-N(2)             | 115.56(18)|
| C(8)-N(3)-H(3A)            | 122.2     |
| N(2)-N(3)-H(3A)            | 122.2     |
| C(8)-O(1)-Mn(1)            | 119.48(15)|
| C(12)-O(2)-Mn(1)           | 118.27(16)|
| Mn(1)-O(3)-H(3B)           | 128(3)    |
| Mn(1)-O(3)-H(3C)           | 127(2)    |
| H(3B)-O(3)-H(3C)           | 103(3)    |
| O(3)-Mn(1)-O(2)            | 88.32(7)  |
| O(3)-Mn(1)-O(1)            | 85.73(7)  |
| O(2)-Mn(1)-O(1)            | 83.58(6)  |
| O(3)-Mn(1)-N(4)            | 87.07(7)  |
| O(2)-Mn(1)-N(4)            | 70.24(7)  |
| O(1)-Mn(1)-N(4)            | 153.01(7) |
| O(3)-Mn(1)-N(1)            | 87.43(8)  |
| O(2)-Mn(1)-N(1)            | 138.62(7) |
| O(1)-Mn(1)-N(1)            | 136.98(7) |
Symmetry transformations used to generate equivalent atoms:

Table S5. Anisotropic displacement parameters (Å² x 10³) for MnL₆. The anisotropic displacement factor exponent takes the form: -2π²[ h²a*²U₁₁ + ... + 2 h k a* b* U₁₂ ]

|      | u¹¹ | u²² | u³³ | u²³ | u¹² | u¹³ |
|------|-----|-----|-----|-----|-----|-----|
| C(1) | 25(2)| 19(1)| 27(1)| -2(1)| -3(1)| -1(1)|
| C(2) | 29(2)| 21(1)| 38(1)| -4(1)| -1(1)| 1(1) |
| C(3) | 31(2)| 20(1)| 53(2)| 2(1) | -1(1)| -2(1)|
| C(4) | 26(2)| 24(1)| 42(1)| 8(1) | 0(1) | -5(1)|
| C(5) | 21(2)| 23(1)| 32(1)| 7(1) | 1(1) | -3(1)|
| C(6) | 24(2)| 23(1)| 26(1)| -3(1)| 2(1) | 3(1) |
| C(7) | 52(2)| 30(2)| 30(1)| -7(1)| 12(1)| 7(1) |
| C(8) | 26(2)| 23(1)| 21(1)| 0(1) | 6(1) | -3(1)|
| C(9) | 47(2)| 24(1)| 29(1)| -1(1)| 16(1)| -11(1)|
| C(10)| 22(2)| 28(1)| 29(1)| 10(1)| 3(1) | -5(1)|
| C(11)| 41(2)| 44(2)| 37(1)| 18(1)| 11(1)| -5(2)|
| C(12)| 26(2)| 33(2)| 22(1)| 0(1) | 6(1) | 2(1) |
| C(13)| 43(2)| 43(2)| 29(1)| -2(1)| 15(1)| 10(2)|
| N(1) | 23(1)| 19(1)| 27(1)| 0(1) | 4(1) | -2(1)|
| N(4) | 21(1)| 29(1)| 23(1)| 2(1) | 5(1) | -1(1)|
| N(5) | 30(1)| 35(1)| 21(1)| 6(1) | 12(1)| 3(1)|
| N(2) | 26(1)| 20(1)| 23(1)| 0(1) | 6(1) | -3(1)|
Table S6. Hydrogen coordinates (x 10^4) and isotropic displacement parameters (Å^2 x 10^3) for MnLMe.

|    | x     | y     | z     | U(eq) |
|----|-------|-------|-------|-------|
| H(2) | 3596  | 4286  | 3961  | 36    |
| H(3) | 2623  | 3327  | 4842  | 43    |
| H(4) | 1663  | 4146  | 5787  | 38    |
| H(7A) | 3700  | 6266  | 2785  | 56    |
| H(7B) | 4238  | 5226  | 3196  | 56    |
| H(7C) | 5549  | 6148  | 3248  | 56    |
| H(9A) | 3695  | 10340 | 3280  | 48    |
| H(9B) | 4976  | 9564  | 3006  | 48    |
| H(9C) | 5546  | 10177 | 3732  | 48    |
| H(11A) | 1984  | 5772  | 7063  | 60    |
| H(11B) | 844   | 4960  | 6573  | 60    |
| H(11C) | 22    | 5994  | 6792  | 60    |
| H(13A) | 1439  | 10001 | 6889  | 56    |
| H(13B) | -6    | 9239  | 7043  | 56    |
| H(13C) | -391  | 10032 | 6400  | 56    |
| H(5) | 58112 | 76546 | 672212| 33    |
| H(3A) | 452113 | 79224 | 323712 | 29    |
| H(3B) | 512040 | 769030 | 599514 | 69    |
| H(3C) | 568040 | 816030 | 551317 | 69    |
| H(2A) | 779050 | 889020 | 485215 | 69    |
| H(1B) | -54040 | 758030 | 792312 | 69    |
| H(2B) | 741050 | 944917 | 521317 | 69    |
| H(1A) | -176030 | 741030 | 741417 | 69    |
Table S7. Atomic coordinates (x 10^4) and equivalent isotropic displacement parameters (Å^2 x 10^3) for ZnL_Me. U(eq) is defined as one third of the trace of the orthogonalized U^ij tensor.

|    | x        | y        | z        | U(eq) |
|----|----------|----------|----------|-------|
| C(1)| 3123(7)  | 5670(4)  | 4412(2)  | 24(1) |
| C(2)| 3138(8)  | 4628(4)  | 4377(3)  | 30(1) |
| C(3)| 2562(9)  | 4079(4)  | 4916(3)  | 36(1) |
| C(4)| 2001(7)  | 4572(4)  | 5481(3)  | 30(1) |
| C(5)| 2027(7)  | 5625(4)  | 5491(2)  | 26(1) |
| C(6)| 3686(8)  | 6350(4)  | 3870(2)  | 27(1) |
| C(7)| 4299(9)  | 5957(4)  | 3204(3)  | 36(2) |
| C(8)| 3908(7)  | 8983(4)  | 3856(2)  | 25(1) |
| C(9)| 4538(7)  | 9839(4)  | 3439(3)  | 33(1) |
| C(10)| 1459(7) | 6254(4)  | 6061(2)  | 28(1) |
| C(11)| 976(9)  | 5792(5)  | 6726(3)  | 37(2) |
| C(12)| 1105(7) | 8889(4)  | 6167(2)  | 27(1) |
| C(13)| 532(8)  | 9677(4)  | 6643(3)  | 35(1) |
| N(1)| 2563(6)  | 6145(3)  | 4958(2)  | 24(1) |
| N(4)| 1449(6)  | 7197(3)  | 5924(2)  | 25(1) |
| N(5)| 969(6)   | 7906(3)  | 6379(2)  | 27(1) |
| N(2)| 3609(6)  | 7292(3)  | 4031(2)  | 24(1) |
| N(3)| 4119(5)  | 8044(3)  | 3614(2)  | 24(1) |
| O(1)| 3297(5)  | 9135(3)  | 4412(2)  | 30(1) |
| O(2)| 1704(5)  | 9095(3)  | 5619(2)  | 30(1) |
| O(3)| 4889(5)  | 7835(3)  | 5597(2)  | 33(1) |
| Zn(1)| 2428(1) | 7811(1)  | 4971(1)  | 23(1) |
| Cl(1)| -311(2)  | 7817(1)  | 4205(1)  | 32(1) |
| Cl(2)| 5539(2) | 6928(1)  | 7120(1)  | 39(1) |
| O(1W)| -725(6) | 7779(5)  | 7583(2)  | 56(1) |
| O(2W)| 7465(7) | 8673(3)  | 5231(2)  | 49(1) |

Table S8. Bond lengths [Å] and angles [°] for ZnL_Me.

| Bond     | Length [Å] | Angle [°] |
|----------|------------|-----------|
| C(1)-N(1)| 1.338(7)   |           |
| C(1)-C(2)| 1.382(7)   |           |
| C(1)-C(6)| 1.480(7)   |           |
| Bond          | Distance (Å) |
|--------------|--------------|
| C(2)-C(3)    | 1.382(8)     |
| C(2)-H(2)    | 0.9500       |
| C(3)-C(4)    | 1.379(8)     |
| C(3)-H(3)    | 0.9500       |
| C(4)-C(5)    | 1.394(8)     |
| C(4)-H(4)    | 0.9500       |
| C(5)-N(1)    | 1.339(6)     |
| C(5)-C(10)   | 1.484(8)     |
| C(6)-N(2)    | 1.287(6)     |
| C(6)-C(7)    | 1.506(7)     |
| C(7)-H(7A)   | 0.9800       |
| C(7)-H(7B)   | 0.9800       |
| C(7)-H(7C)   | 0.9800       |
| C(8)-O(1)    | 1.236(6)     |
| C(8)-N(3)    | 1.344(7)     |
| C(8)-C(9)    | 1.508(7)     |
| C(9)-H(9A)   | 0.9800       |
| C(9)-H(9B)   | 0.9800       |
| C(9)-H(9C)   | 0.9800       |
| C(10)-N(4)   | 1.274(7)     |
| C(10)-C(11)  | 1.494(7)     |
| C(11)-H(11A) | 0.9800       |
| C(11)-H(11B) | 0.9800       |
| C(11)-H(11C) | 0.9800       |
| C(12)-O(2)   | 1.231(6)     |
| C(12)-N(5)   | 1.370(7)     |
| C(12)-C(13)  | 1.491(8)     |
| C(13)-H(13A) | 0.9800       |
| C(13)-H(13B) | 0.9800       |
| C(13)-H(13C) | 0.9800       |
| N(1)-Zn(1)   | 2.208(4)     |
| N(4)-N(5)    | 1.365(6)     |
| N(4)-Zn(1)   | 2.218(4)     |
| N(5)-H(5)    | 0.8800       |
| N(2)-N(3)    | 1.367(6)     |
| N(2)-Zn(1)   | 2.237(4)     |
| N(3)-H(3A)   | 0.8800       |
| O(1)-Zn(1)   | 2.210(4)     |
| O(2)-Zn(1)   | 2.220(4)     |
| Bond                  | Distance  |
|----------------------|-----------|
| O(3)-Zn(1)           | 2.107(4)  |
| O(3)-H(3B)           | 0.771(19) |
| O(3)-H(3C)           | 0.78(2)   |
| Zn(1)-Cl(1)          | 2.4006(15)|
| O(1W)-H(1B)          | 0.74(4)   |
| O(1W)-H(1A)          | 0.73(4)   |
| O(2W)-H(2A)          | 0.80(4)   |
| O(2W)-H(2B)          | 0.77(4)   |
| N(1)-C(1)-C(2)       | 120.8(5)  |
| N(1)-C(1)-C(6)       | 114.6(4)  |
| C(2)-C(1)-C(6)       | 124.6(5)  |
| C(3)-C(2)-C(1)       | 118.9(5)  |
| C(3)-C(2)-H(2)       | 120.5     |
| C(1)-C(2)-H(2)       | 120.5     |
| C(4)-C(3)-C(2)       | 120.1(5)  |
| C(4)-C(3)-H(3)       | 120.0     |
| C(2)-C(3)-H(3)       | 120.0     |
| C(3)-C(4)-C(5)       | 118.5(5)  |
| C(3)-C(4)-H(4)       | 120.8     |
| C(5)-C(4)-H(4)       | 120.8     |
| N(1)-C(5)-C(4)       | 120.7(5)  |
| N(1)-C(5)-C(10)      | 114.9(5)  |
| C(4)-C(5)-C(10)      | 124.4(5)  |
| N(2)-C(6)-C(1)       | 113.4(4)  |
| N(2)-C(6)-C(7)       | 124.2(5)  |
| C(1)-C(6)-C(7)       | 122.4(5)  |
| C(8)-C(7)-H(7A)      | 109.5     |
| C(8)-C(7)-H(7B)      | 109.5     |
| H(7A)-C(7)-H(7B)     | 109.5     |
| C(6)-C(7)-H(7C)      | 109.5     |
| H(7A)-C(7)-H(7C)     | 109.5     |
| H(7B)-C(7)-H(7C)     | 109.5     |
| O(1)-C(8)-N(3)       | 121.6(5)  |
| O(1)-C(8)-C(9)       | 121.7(5)  |
| N(3)-C(8)-C(9)       | 116.7(4)  |
| C(8)-C(9)-H(9A)      | 109.5     |
| C(8)-C(9)-H(9B)      | 109.5     |
| H(9A)-C(9)-H(9B)     | 109.5     |
| C(8)-C(9)-H(9C)      | 109.5     |
| Bond/Angle | Value  |
|------------|--------|
| H(9A)-C(9)-H(9C) | 109.5  |
| H(9B)-C(9)-H(9C) | 109.5  |
| N(4)-C(10)-C(5) | 113.4(4) |
| N(4)-C(10)-C(11) | 125.2(5) |
| C(5)-C(10)-C(11) | 121.4(5) |
| C(10)-C(11)-H(11A) | 109.5  |
| C(10)-C(11)-H(11B) | 109.5  |
| H(11A)-C(11)-H(11B) | 109.5  |
| C(10)-C(11)-H(11C) | 109.5  |
| H(11A)-C(11)-H(11C) | 109.5  |
| H(11B)-C(11)-H(11C) | 109.5  |
| O(2)-C(12)-N(5) | 120.9(5) |
| O(2)-C(12)-C(13) | 122.8(5) |
| N(5)-C(12)-C(13) | 116.3(4) |
| C(12)-C(13)-H(13A) | 109.5  |
| C(12)-C(13)-H(13B) | 109.5  |
| H(13A)-C(13)-H(13B) | 109.5  |
| C(12)-C(13)-H(13C) | 109.5  |
| H(13A)-C(13)-H(13C) | 109.5  |
| C(12)-C(13)-H(13C) | 109.5  |
| C(1)-N(1)-C(5) | 121.0(4) |
| C(1)-N(1)-Zn(1) | 120.0(3) |
| C(5)-N(1)-Zn(1) | 119.0(3) |
| C(10)-N(4)-N(5) | 122.8(4) |
| C(10)-N(4)-Zn(1) | 122.0(4) |
| N(5)-N(4)-Zn(1) | 115.0(3) |
| N(4)-N(5)-C(12) | 115.3(4) |
| N(4)-N(5)-H(5) | 122.3  |
| C(12)-N(5)-H(5) | 122.3  |
| C(6)-N(2)-N(3) | 122.8(4) |
| C(6)-N(2)-Zn(1) | 121.6(3) |
| N(3)-N(2)-Zn(1) | 115.4(3) |
| C(8)-N(3)-N(2) | 114.5(4) |
| C(8)-N(3)-H(3A) | 122.7  |
| N(2)-N(3)-H(3A) | 122.7  |
| C(8)-O(1)-Zn(1) | 117.9(3) |
| C(12)-O(2)-Zn(1) | 117.2(4) |
| Zn(1)-O(3)-H(3B) | 119(5)  |
| Zn(1)-O(3)-H(3C) | 127(5)  |
H(3B)-O(3)-H(3C)      110(4)  
O(3)-Zn(1)-N(1)        88.78(17) 
O(3)-Zn(1)-O(1)        85.46(14)  
N(1)-Zn(1)-O(1)        140.13(16) 
O(3)-Zn(1)-N(4)        88.24(14)  
N(1)-Zn(1)-N(4)        70.51(16)  
O(1)-Zn(1)-N(4)        148.34(14) 
O(3)-Zn(1)-O(2)        88.93(15)  
N(1)-Zn(1)-O(2)        142.00(15) 
O(1)-Zn(1)-O(2)        77.38(12)  
N(4)-Zn(1)-O(2)        71.51(14)  
O(3)-Zn(1)-N(2)        88.62(15)  
N(1)-Zn(1)-N(2)        69.97(16)  
O(1)-Zn(1)-N(2)        70.48(14)  
N(4)-Zn(1)-N(2)        140.40(14) 
O(2)-Zn(1)-N(2)        147.86(14) 
O(3)-Zn(1)-Cl(1)       176.97(11) 
N(1)-Zn(1)-Cl(1)       92.13(13)  
O(1)-Zn(1)-Cl(1)       91.99(10)  
N(4)-Zn(1)-Cl(1)       94.80(11)  
O(2)-Zn(1)-Cl(1)       92.12(11)  
N(2)-Zn(1)-Cl(1)       88.97(11)  
H(1B)-O(1W)-H(1A)      107(4)     
H(2A)-O(2W)-H(2B)      97(3)      

Symmetry transformations used to generate equivalent atoms:

|    | u11 | u22 | u33 | u23 | u13 | u12 |
|----|-----|-----|-----|-----|-----|-----|
| C(1)| 22(3)| 25(3)| 26(2)|-4(2)| 2(2)| 2(2)|
| C(2)| 37(4)| 20(3)| 33(3)|-2(2)| 3(2)| 4(2)|
| C(3)| 38(3)| 17(2)| 49(3)| 3(2)|-2(3)|-1(3)|
| C(4)| 29(3)| 24(3)| 36(3)| 4(2)| 5(2)|-6(2)|
| C(5)| 26(3)| 24(3)| 28(2)| 3(2)| 4(2)|-2(2)|
| C(6)| 37(4)| 25(3)| 21(2)|-2(2)| 6(2)| 7(3)|
| C(7)| 52(4)| 27(3)| 32(3)|-5(2)|13(3)| 5(3)|

Table S9. Anisotropic displacement parameters (\(\AA^2\times 10^3\)) for ZnL\textsuperscript{Me}. The anisotropic displacement factor exponent takes the form: \(-2\pi^2 u^2 a^2 u_{11} + ... + 2 h k a^* b^* U_{12}\)
Table S10. Hydrogen coordinates (x $10^4$) and isotropic displacement parameters ($\AA^2 \times 10^3$) for ZnL.<br>

|     | x     | y     | z     | U(eq) |
|-----|-------|-------|-------|-------|
| H(2) | 3539  | 4294  | 3989  | 36    |
| H(3) | 2552  | 3362  | 4897  | 43    |
| H(4) | 1605  | 4202  | 5855  | 36    |
| H(7A) | 3549  | 6208  | 2773  | 54    |
| H(7B) | 4277  | 5217  | 3206  | 54    |
| H(7C) | 5470  | 6191  | 3199  | 54    |
| H(9A) | 3588  | 10298 | 3269  | 50    |
| H(9B) | 4995  | 9566  | 3024  | 50    |
| H(9C) | 5441  | 10208 | 3753  | 50    |
| H(11A) | 1803  | 5995  | 7148  | 56    |
| H(11B) | 978   | 5054  | 6681  | 56    |
| H(11C) | -168  | 6022  | 6784  | 56    |
| H(13A) | 1514  | 10088 | 6855  | 52    |
| H(13B) | 33    | 9350  | 7028  | 52    |
| H(13C) | -328  | 10109 | 6357  | 52    |
Table S11. Parameters from the analysis of $^1$H NMRD data.

| Parameters | $[\text{MnL}^\text{Me}]^{2+}$ (MW = 366 g/mol) | $[\text{Mn(dpama)]}^{\text{Ref [7]}}$ (MW = 390 g/mol) | $[\text{Mn(EDTA)]}^{2-}$ Ref [8] (MW = 361 g/mol) | $^{1}[\text{Mn(H}_2\text{O)}_6]^2+\text{Ref [7]}$ (MW = 163 g/mol) |
|------------|---------------------------------|---------------------------------|---------------------------------|---------------------------------|
| $\Delta H_\text{s}$ / 10$^{-2}$ s$^{-1}$ | 5.7 | 5.3 | 3.3 | 7.7 |
| $\Delta E_\text{s}$ / kJ mol$^{-1}$ | 5.3 | 2.38 | 6.9 | 0.6 |
| $\Delta E_\text{v}$ / kJ mol$^{-1}$ | 48.0±1.1 | 39.2 | 27.9 | 10.0 |
| $\Delta E_\text{r}$ / ps | 29.7±1.2 | 28.1 | 33.5 | 45.6 |
| $\Delta E_\text{r}$ / ns | 191±9 | 3.3 | 2.1 | 35.5 |
| $\Delta E_\text{r}$ / ps | 28±1 | 45.8 | 40.5 | 34.6 |
| $\Delta E_\text{r}$ / ps | 16.0±0.1 | 47.8 | 57 | 30 |
| $\Delta E_\text{r}$ / ps | 28±1 | 45.8 | 40.5 | 34.6 |
| $\Delta E_\text{r}$ / ps | 16.0±0.1 | 47.8 | 57 | 30 |
| $\Delta E_\text{r}$ / ps | 16.0±0.1 | 47.8 | 57 | 30 |
| $\Delta E_\text{r}$ / ps | 16.0±0.1 | 47.8 | 57 | 30 |
| $\Delta E_\text{r}$ / ps | 16.0±0.1 | 47.8 | 57 | 30 |
| $\Delta E_\text{r}$ / ps | 16.0±0.1 | 47.8 | 57 | 30 |

*Fixed in the fitting procedure.

Table S12. Parameters from the analysis of $^1$H NMRD and $^{17}$O NMR data of MnL$^{\text{Me}}$ / BSA.

| Parameters | MnL$^{\text{Me}}$ / BSA$^a$ |
|------------|-------------------------------|
| $\Delta H_\text{s}$ / 10$^{-2}$ s$^{-1}$ | 2.1±0.3 |
\[ \tau / \text{ps} \quad 3.7 \pm 0.6 \]
\[ \tau / \text{ns} \quad 378 \pm 11 \]
\[ \tau_{\text{RG}} / \text{ps} \quad 50000^a \]
\[ \tau_{\text{RL}} / \text{ps} \quad 408 \pm 45 \]
\[ S^2 \quad 0.28 \pm 0.01 \]
\[ A_0 / \hbar / \text{rad s}^{-1} \quad 30 \pm 2 \]
\[ q \quad 1 \]
\[ r / \lambda \quad 2.74^a \]
\[ a / \lambda \quad 3.6^a \]
\[ D / 10^6 \text{cm}^2 \text{s}^{-1} \quad 2.30^a \]

\(^a\)fixed in the fitting procedure

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Author Contributions

S.A. synthesised and characterized the complexes \( \text{MnL}^{\text{Me}} \) and \( \text{ZnL}^{\text{Me}} \), and carried out the relaxometry, transmetallation, pH-dependent relaxivity, PRE and BSA binding experiments (Figures 3CD, S1-S5, S8-S13 and S18). T. R. P. collected the single crystal X-ray data and obtained the crystal structures (Figures 1, S6 and S7). F.C. and M.B. recorded and analyzed the \(^{1}H\)-NMRD profiles, BSA relaxometric titration and \(^{17}O\)-NMR data (Figures 3AB, S16-S17). L.K. acquired the \( T_1 \)-weighted phantom images (1-5 mM) from 1.5 T clinical MRI (Figure S8). TWP perform LCMS transchelation experiments. S. H. L. H and A. F.M. performed and analysed the transmetallation kinetics, \( \text{in vivo} \) MRI and \( T_1 \)-weighted phantom imaging studies (Figures 2-4, S14, S15, S19 and S20). While all authors contributed to the text, G. J. S. and AFM coordinated the project. G.J.S. supervised and conceptualised the scientific work.