High relaxivity Mn^{2+}-based MRI contrast agents

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Abstract

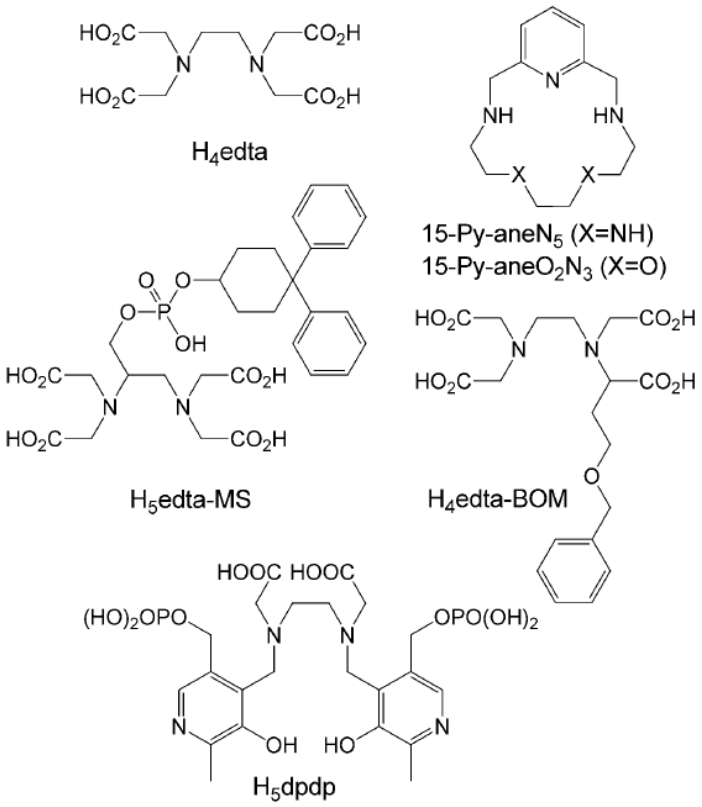
Stable Mn^{2+} mono- and binuclear complexes containing pentadentate 6,6′-((methylazanediyl)bis(methylene)) dipicolinic acid coordinating units give remarkably high relaxivities due to the presence of two inner-sphere water molecules. The mononuclear derivative binds human serum albumin (HSA) with an association constant of 3372 M^{-1}, which results in the replacement of the coordinated water molecules by donor atoms of protein residues. The dinuclear analogue also binds HSA while leaving one of the Mn^{2+} centres exposed to the solvent with two coordinated water molecules. Thus, this complex shows remarkably high relaxivities upon protein binding (39.0 mM^{-1} s^{-1} per Mn, at 20 MHz and 37 °C).

Keywords: contrast agents; coordination compounds; human serum albumin; manganese; NMR imaging

Introduction

Stable high-spin Mn^{2+} complexes represent an attractive alternative to the classical Gd^{3+}-based contrast agents (CAs) for application in magnetic resonance imaging (MRI).[1, 2] CAs are paramagnetic species that enhance the contrast between a specific tissue or organ of interest and the surrounding tissues of the body, thus increasing the diagnostic accuracy of the technique and shortening the examination time. All Gd^{3+}-based CAs that have entered clinical practice are complexes with poly(aminocarboxylate) ligands with high thermodynamic and often kinetic stability. These agents present a single water molecule coordinated to the paramagnetic ion that exchanges sufficiently rapidly with the bulk water, thereby accelerating the longitudinal and/or transverse relaxation rates of water molecules in the proximities of the paramagnetic centre.[3]

CAs based on Mn^{2+} complexes present certain potential advantages over their Gd^{3+} counterparts. Indeed, Mn^{2+} is an essential element present in the human body, and thus humans have developed efficient mechanisms to manage an excess of this ion in organs and tissues. On the contrary, Gd^{3+} is extremely toxic, and the release of the metal ion after the administration of certain Gd^{3+}-based CAs to patients suffering from severe renal failure has been shown to trigger a potentially fatal disease called nephrogenic systemic fibrosis (NSF).[4] Mn intoxication is known to promote motor and psychiatric disturbances, although the doses and exposure times that produce such effects are not precisely known.[5] The well-known CA mangafodipir trisodium (Na_3[Mn(dpdp)], TESLASCAN, see Scheme 1), has been used as a hepatocyte specific MRI contrast agent. This CA releases Mn^{2+}, which is sequestered by the liver, where it is taken up and decomplexed by hepatocytes, allowing an enhanced contrast for diagnostic imaging of lesions in the liver and pancreas.[6] Thus, new Mn^{2+} MRI probes sufficiently stable to minimize the release of the metal ion are expected to expand the potential applications of these agents. For instance, these new agents could replace the traditional CAs in those cases where administration of Gd^{III}-based agents represents a risk for the patient.[7]



Scheme 1. Ligands discussed in this paper.

The efficiency of CAs in vitro is normally expressed in terms of their relaxivity, r_{1p}, which is defined as the relaxation enhancement of water protons promoted by a 1 mM concentration of the paramagnetic metal ion.[1] The main drawback of Mn^{2+}-based CAs is their lower effective magnetic moments, which often results in lower relaxivities. Indeed, the relaxivities of small Mn^{2+} complexes containing one inner-sphere water molecule are typically 2.4–3.7 $mM^{-1}\,s^{-1}$ (0.47 T, 25 °C),[8] while those of the commercially available Gd^{3+} CAs fall within the range 4.4–5.2 $mM^{-1}\,s^{-1}$ at identical temperature and magnetic field.[1, 2]

An obvious strategy to improve the relaxivity of Mn^{2+}-based contrast agents is to increase the number of water molecules coordinated to the metal ion. This approach has been widely exploited to improve the relaxation properties of Gd^{3+}-based agents, often with the use of hexa- or heptadentate ligands that leave two coordination positions for water ligands and still ensure a high thermodynamic stability and enough kinetic inertness.[9] However, in some cases the relaxivity gain obtained by increasing the hydration number may be partially quenched by the coordination of endogenous anions to the metal ion displacing the inner-sphere water molecules.[10]

The number of Mn^{2+}-based complexes containing two coordinated water molecules so far reported as potential MRI CAs is scarce. Recently, Tóth et al.[11] reported a series of Mn^{2+} complexes with pentadentate macrocyclic ligands such as 15-Py-aneN_5 and 15-Py-aneO_2N_3 (Scheme 1) that form pentagonal bipyramidal Mn^{2+} complexes in solution with two water molecules occupying the axial positions. However, the relaxivity gain associated with the presence of two inner-sphere water molecules is quite modest, and furthermore the stability of the complexes was found to be relatively low.

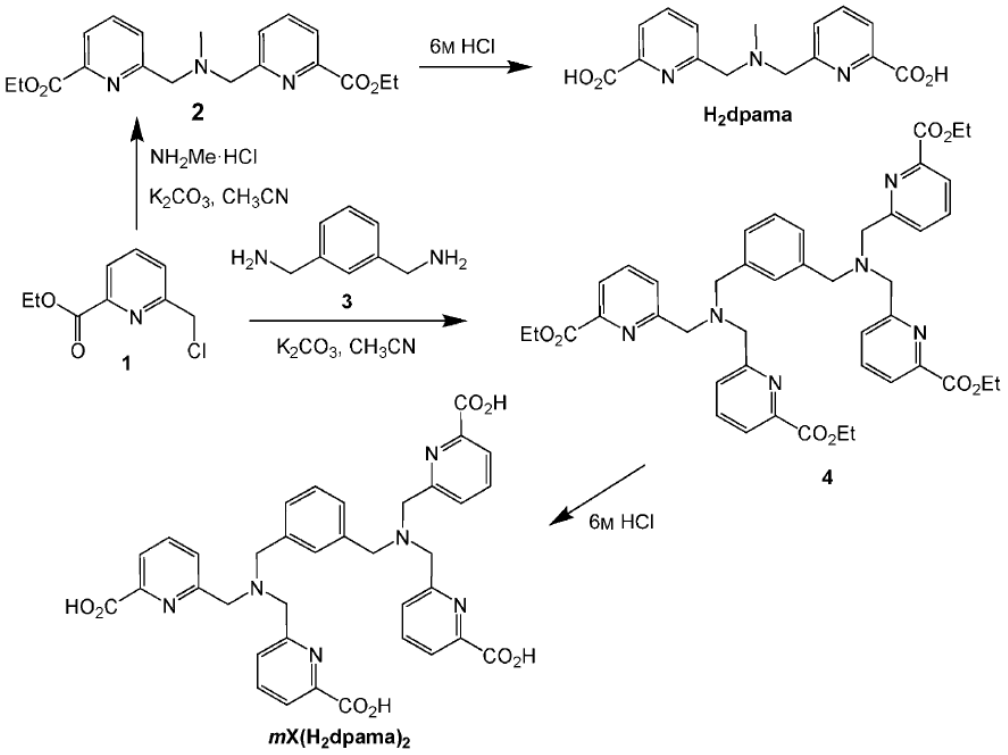
A second approach that can be used to attain high relaxivities consists in decreasing the tumbling rate of the complex, which can be achieved by covalent binding of the chelate unit to a slowly tumbling substrate such as a macromolecule or a nanoparticle.[12] Non-covalent binding of the paramagnetic chelate to proteins such as human serum albumin (HSA) has been also exploited to improve the relaxation properties of the paramagnetic probe.[13] This approach was first applied to Gd^{3+}-based CAs[14] and then extended to Mn^{2+} complexes such as the edta-derivatives bearing hydrophobic pendant groups able to promote strong non-covalent binding interactions with HSA ($[Mn(edta\text{-}BOM)]^{2-}$and $[Mn(edta\text{-}MS)]^{3-}$; Scheme 1).[15]

Herein, we investigate the potential of the Mn^{2+} complex of $dpama^{2-}$ as a CA (Scheme 2). The ligand is potentially pentadentate and then is expected to form mono- or bisaqua complexes with Mn^{2+}. We discovered that this complex binds rather strongly to HSA, which results in the displacement of the water molecules coordinated to Mn^{2+} by donor atoms of the protein. Thus, we also prepared the binucleating analogue based on a *meta*-xylene core, *m*X(H_2dpama)$_2$, which was designed to target HSA with one of the $dpama^{2-}$ units while leaving the second one exposed to the solvent.

The synthesis of H_2dpama was achieved in two steps by treatment of 6-chloromethylpyridine-2-carboxylic acid ethyl ester (**1**)[16] with methylamine chlorohydrate in the presence of K_2CO_3, followed by hydrolysis of the ethyl ester groups in 6 M HCl (Scheme 2). The desired ligand was isolated as the chlorohydrate salt with a yield of 66 % over the two steps. This represents a 2.6-fold increase with respect to the yield reported previously for the analogous ligand derived from ethylamine.[17] Ligand *m*X(H_2dpama)$_2$ was prepared in 76 % yield following a similar procedure by treatment of **1** with 1,3-phenylenedimethanamine (**3**) followed by acid hydrolysis of the ester groups. Treatment of H_2dpama and *m*X(H_2dpama)$_2$ with $Mn(ClO_4)_2{\cdot}6\,H_2O$ in the presence of triethylamine resulted in the formation of the charge neutral complexes $[Mn(dpama)(H_2O)_2]{\cdot}2\,H_2O$ and $[m\text{X}(Mn(dpama)(H_2O)_2)_2]{\cdot}6\,H_2O$, respectively, which were isolated in 65 % yield. The high-resolution mass spectra (ESI^+) and analytical data confirm the formation of the desired neutral complexes (see the Supporting Information).

The relaxivity (r_{1p}) measured for [Mn(dpama)] at 20 MHz and 298 K (pH 7.3) amounts to 5.32 $mM^{-1}\,s^{-1}$, a value that is about 60 % higher than those measured under the same conditions for small Mn^{2+}complexes containing one coordinated water molecule (i.e., r_{1p}=3.3 $mM^{-1}\,s^{-1}$ for $[Mn(edta)]^{2-}$, Figure 1).[8] Interestingly, the relaxivity measured for [Mn(dpama)] is also higher than those determined for bis(aquated) seven-coordinate Mn^{2+} complexes with neutral pentadentate macrocyclic ligands (r_{1p}=3.5–4.5 $mM^{-1}\,s^{-1}$, 20 MHz, 25 °C),[11] or most commercially available Gd^{3+}-based contrast agents (r_{1p}=4–5 $mM^{-1}\,s^{-1}$, 20 MHz, 25 °C).[1] A further improvement of the relaxivity is observed for [*m*X(Mndpama)$_2$], which presents a r_{1p} value (8.63 $mM^{-1}\,s^{-1}$, 20 MHz, 25 °C) higher than those typically observed for small Gd^{3+} bisaqua complexes such as Gd(do3a) and $Gd(aazta)^-$.[9f] The high relaxivity values determined for [Mn(dpama)] and

[*m*X(Mndpama)$_2$] can only be explained by the presence of two water molecules in the inner coordination sphere of the metal ion.



Scheme 2. Synthesis of the ligands.

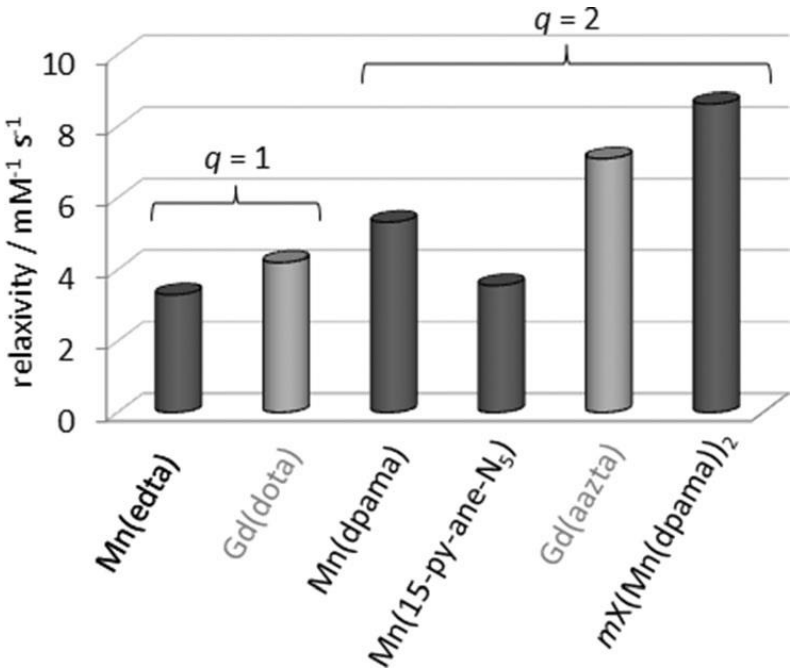


Figure 1. Plot of the relaxivity, r_{1p}, for selected Gd^{3+} and Mn^{2+} complexes at 20 MHz and 298 K.

Aiming to obtain information on the solution structure of the [Mn(dpama)(H_2O)$_2$] complex we turned our attention to theory. In previous works we have shown that an adequate treatment of the solvation effects in Mn^{2+} and Gd^{3+} complexes containing coordinated water molecules can be achieved by using DFT calculations and a mixed cluster/continuum approach treating explicitly the most important hydrogen-bonding interaction between the coordinated water molecule(s) and second-sphere water molecules.[8] Thus, we performed geometry optimizations of the [Mn(dpama)(H_2O)$_2$]·4 H_2O system in aqueous solution at the TPSSh/SVP level. The optimized structure (Figure 2) shows that the dpama ligand provides five donor atoms arranged in a rather planar fashion (rms deviation from planarity 0.056 Å), which leaves two coordination positions for water molecules above and below the ligand mean plane. The metal coordination environment can be described as pentagonal bipyramidal, where the five donor atoms of the ligand describe the equatorial plane and water molecules occupy the apical positions. The calculated Mn–O_{water} lengths (2.21 Å) are close to those observed for a related Mn^{2+}complexes.[8]

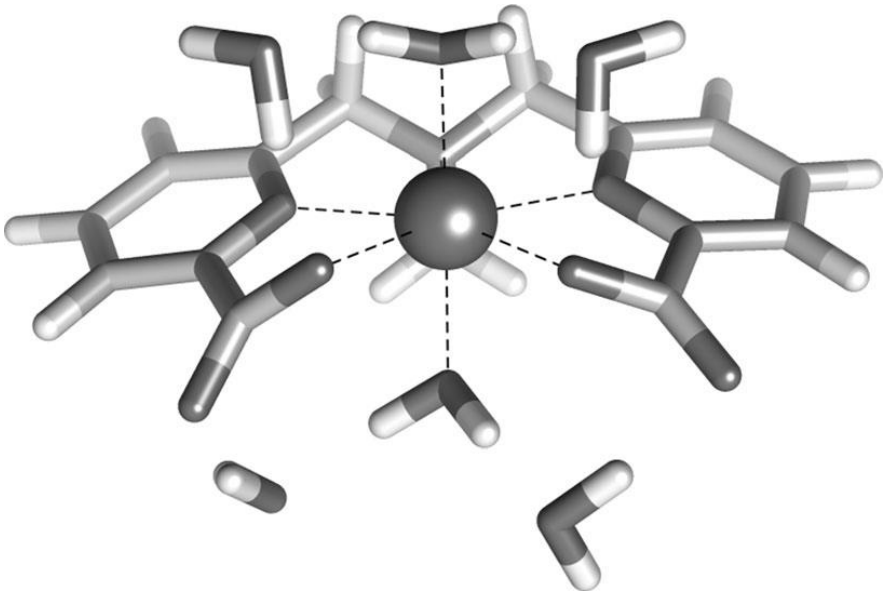


Figure 2. Optimized geometry of the [Mn(dpama)(H_2O)$_2$]·4 H_2O complex obtained with DFT calculations performed in aqueous solution at the TPSSh/SVP level. Average bond lengths [Å] of the Mn^{II} coordination environment: Mn–O_{water} 2.205(1); Mn–O_{COO} 2.335(64); Mn–N_{amine} 2.569; Mn–N_{PY} 2.287(9).

The stability of the [Mn(dpama)] complex was first assessed by measuring its proton relaxivity (25 °C, 20 MHz) as a function of pH. The relaxivity remains constant within a rather wide pH range from 10.3 to 5.5, while below pH~5.5, r_{1p} increases due to the stepwise dissociation of the complex and gradual Mn^{2+} release (see the Supporting Information). The stability over time of [Mn(dpama)] was assessed by relaxometric measurement (0.47 T, 37 °C) of a 0.98 mM solution of the complex in a lyophilized serum of human origin (Seronorm), which is normal human serum without added preservatives and therefore contains endogenous levels of the different serum components. The complex proved to be stable at least over 140 h (see the Supporting Information), as only very small and negligible fluctuations in the relaxation rate data were detected, well within the experimental error (±3–4 %).

The proton relaxivity measured in Seronorm at 0.47 T and 37 °C (11.14 mM^{-1} s^{-1}) was found to be considerably higher than that observed in pure water (4.17 mM^{-1} s^{-1}). This prompted us to investigate the interaction of the complex with HSA, which is the most common protein present in human blood plasma, through the well-established proton relaxation enhancement technique (see the Supporting Information).[14a] Addition of HSA to an aqueous solution of [Mn(dpama)] (0.285 mM, pH 7.2, 310 K)

induces a significant increase of the observed longitudinal relaxation rate of water proton nuclei of the solution (Figure 3).

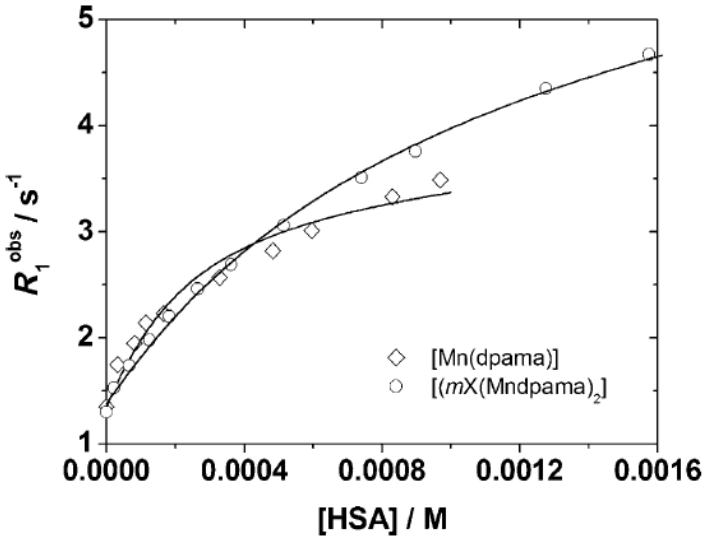


Figure 3. Changes in the observed longitudinal relaxation rates of water protons detected upon addition of HSA to solutions of the [Mn(dpama)] (0.285 mM) and [*m*X(Mndpama)$_2$] (0.151 mM) complexes. The solid lines represent the least-squares fits of the data according to a 1:1 binding isotherm.

The least-squares fit of the relaxometric titration data provide an association constant of 3372(±138) M^{-1}, with a calculated relaxivity for the fully bound form of 12.2(±0.8) $mM^{-1}\,s^{-1}$. The ^1H nuclear magnetic relaxation dispersion (NMRD) obtained for the fully bound form presents a peak in the region 8–60 MHz, which is characteristic of slowly tumbling systems with long rotational correlation times (Figure 4). This confirms that [Mn(dpama)] binds to HSA, which slows down the rotation of the complex in solution. However, the overall relaxivity determined for the fully bound form is relatively low when compared to other Mn^{2+} complexes that bind HSA,[15] with a rather small relaxivity gain with respect to that of the complex in pure water. Moreover, the peak in the NMRD profile is rather broad and significantly lower than that typical of paramagnetic adducts with HSA. Rather, the shape of the profile and the value of r_{1p} corresponding to the maximum of the peak are more similar to those of q=0 Gd^{3+} and Mn^{2+} chelates bound to HSA.[14b, 15a] This suggests that protein binding results in the replacement of the two water molecules coordinated to Mn^{2+} by donor groups contained in residues of the protein.[18] In light of the results obtained for [Mn(dpama)], we hypothesized that a dimeric analogue of this complex could bind HSA while leaving a complex unit exposed to the solvent, which should result in a sizeable inner-sphere contribution to ^1H relaxivity.

DFT calculations performed on the [(*m*X(Mndpama)(H_2O)$_2$·4 H_2O)$_2$] system provide bond lengths of the metal coordination environment very similar to those of the mononuclear analogue, the Mn···Mn distance being about 10.8 Å. The ^1H NMRD profile of [*m*X(Mndpama)$_2$] is characteristic of a Mn^{2+}complex with a low molecular weight, with a single dispersion at 1–10 MHz. Titration of 0.151 mMsolution of [*m*X(Mndpama)$_2$] with HSA (pH 7.2, 37 °C) indeed confirmed the binding of the complex to the protein with an association constant of 1125(±35) M^{-1} (Figure 3). This association constant is somewhat lower than that obtained for [Mn(dpama)], which indicates that the association with the protein is hindered by the presence of the *m*X(Mndpama) unit. However, the relaxivity of the fully bound form at 20 MHz and 37 °C (39.0 (±1.3) $mM^{-1}\,s^{-1}$) is very high, confirming that one of the (Mndpama) moieties is exposed to the solvent

providing a significant response in terms of relaxivity. Furthermore, the NMRD profile obtained for the fully bound form (Figure 4) is characteristic of a slowly tumbling species with sizeable inner-sphere contribution to relaxivity. It should be noted that this value of r_{1p} represents an average between that of a Mn^{2+} ion with q=0, which contributes to the relaxivity with only the outer- and second-sphere mechanisms, and that of a q=2 Mn^{2+} ion, the contribution of which (inner-sphere) is largely dominant. Assuming in first approximation that the contribution of the q=0 Mn^{2+} is similar to that of [Mn(dpama)]–HSA (12.2 $mM^{-1}\,s^{-1}$), then we can estimate a relaxivity for the second Mn^{2+} of about 66 $mM^{-1}\,s^{-1}$.

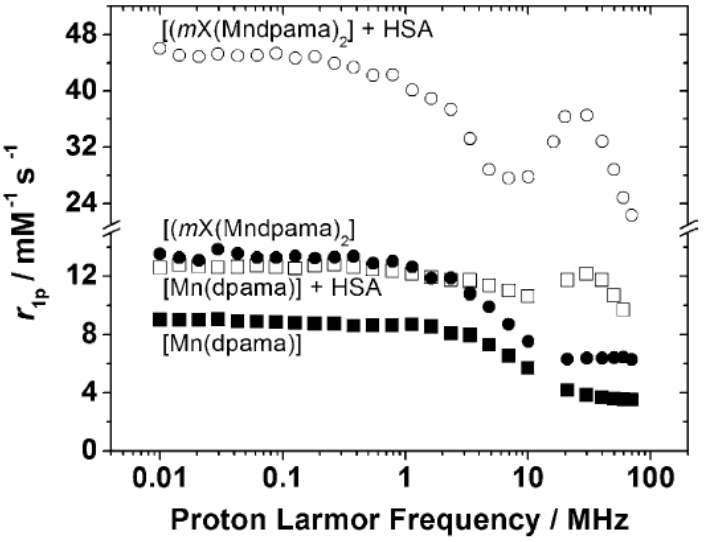


Figure 4. NMRD profiles recorded at 37 °C for the [Mn(dpama)] and [mX(Mndpama)$_2$] complexes and their fully bound forms to HSA. The break in the ordinate axis was introduced for better visualization.

In conclusion, we have designed a pentadentate ligand for stable Mn^{2+} complexation that leaves two coordination positions for water molecules and shows a relatively high relaxivity when compared to other small Mn^{2+} and Gd^{3+} chelates. This complex interacts with HSA, which results in the displacement of the coordinated water molecules and therefore in a limited relaxivity gain. This prompted us to prepare a binucleating analogue that shows an improved relaxivity in aqueous media. The binuclear complex also binds HSA while retaining the coordinated water molecules in one of the Mn^{2+} centres, which results in high relaxivities. This ditopic complex shows that it is possible to develop low molecular weight Mn^{2+} chelates that may represent viable alternatives to Gd-based MRI probes. Its relaxometric properties both in the free form and bound to HSA are fully comparable or better than those of Gd^{3+} complexes commonly used in the clinical practice. The synthesis of new derivatives with improved relaxation properties as well as a detailed physicochemical characterization of these systems is currently in progress.

Experimental Section

General

Chemicals were purchased from commercial sources and used without further purification. SiO_2 (Fluka, pore size 60 Å, 70–230 mesh) was used for preparative column chromatography. ^1H and ^{13}C NMR spectra were recorded at 25 °C on a Bruker Avance 500 MHz spectrometer. High resolution ESI-TOF mass spectra were recorded using a LC-Q-q-TOF Applied Biosystems QSTAR Elite spectrometer in the positive mode.

Elemental analyses were carried out on a ThermoQuest Flash EA 1112 elemental analyser. IR spectra were recorded using a Bruker Vector 22 spectrophotometer equipped with a Golden Gate attenuated total reflectance (ATR) accessory (Specac).

^{1}H relaxivity

The water proton longitudinal relaxation rates as a function of temperature (20 MHz) were measured with a Stelar Spinmaster spectrometer FFC-2000 (Mede, PV, Italy) on about 0.6–2.0 mM aqueous solutions in nondeuterated water. The exact concentrations of Mn^{II} ions were determined by measurement of bulk magnetic susceptibility shifts of a *t*BuOH signal on a Bruker Avance III spectrometer (11.7 T). The ^{1}H T_1 relaxation times were acquired by the standard inversion recovery method with typical 90° pulse width of 3.5 μs, 16 experiments of four scans. The reproducibility of the T_1 data was ±5 %. The temperature was controlled with a Stelar VTC-91 airflow heater equipped with a calibrated copper-constantan thermocouple (uncertainty of ±0.1 °C). The proton $1/T_1$ NMRD profiles were measured on a fast field-cycling Stelar SmartTracer relaxometer over a continuum of magnetic field strengths from 0.00024–0.25 T (corresponding to 0.01–10 MHz proton Larmor frequencies). The relaxometer operates under computer control with an absolute uncertainty in $1/T_1$ of ±1 %. Additional data points in the range 15–70 MHz were obtained on a Bruker WP80 NMR electromagnet adapted to variable-field measurements (15–80 MHz proton Larmor frequency) using a Stelar console.

Diethyl 6,6′-((methylazanediyl)bis(methylene))dipicolinate (2)

6-Chloromethylpyridine-2-carboxylic acid ethyl esther[16] (6.40 g, 32.1 mmol) and K_2CO_3 (6.77 g, 49.0 mmol) were added to a solution of $MeNH_2$·HCl (1.00 g, 14.8 mmol) in acetonitrile (50 mL). The mixture was stirred at 45 °C for a period of 4 days and after this, was heated to reflux for 1 day. The excess of K_2CO_3 was filtered off, the filtrate was concentrated to dryness, and the orange residue was extracted with a mixture of H_2O and $CHCl_3$ (1:3; 200 mL). The organic phase was evaporated to dryness to give an oily residue that was purified by column chromatography on SiO_2 with a CH_2Cl_2/MeOH 5 % mixture as the eluent to give 4.85 g of **2** as an orange oil. Yield: 74 %; elemental analysis calcd (%) for $C_{19}H_{23}N_3O_4$·CH_2Cl_2: C 54.31, H 5.70, N 9.50; found: C 54.52, H 5.54, N 9.36; HR-MS (ESI^+, MeOH/CH_3CN/H_2O 9:1:1): *m*/*z* calcd for $[C_{19}H_{24}N_3O_4]^+$: 358.1761; found: 358.1757; IR (ATR): $\tilde{\nu}$=1736, 1714 cm^{-1} (C=O); ^{1}H NMR ($CDCl_3$, 500 MHz, 25 °C, TMS): δ=7.99 (dd, 2 H, ^{3}J=6.7 Hz, ^{4}J=2.0 Hz), 7.81 (m, 4 H), 4.47 (c, 4 H, ^{3}J=7.2 Hz), 3.91 (s, 4 H), 2.34 (s, 3 H), 1.43 ppm (t, 6 H, ^{3}J=7.2 Hz); ^{13}C NMR ($CDCl_3$, 125.8 MHz, 25 °C, TMS): δ=165.3, 160.1, 147.7, 137.3, 125.9, 123.5, 63.2, 61.8, 42.7, 14.3 ppm.

6,6′-((Methylazanediyl)bis(methylene))dipicolinic acid (H_2dpama·2 HCl)

A solution of compound **2** (1.10 g, 2.49 mmol) in 6 M HCl (50 mL) was heated to reflux for 24 h, and then the solvent was removed in a rotary evaporator to give a yellow oil. A small amount of H_2O was added (~20 mL) and the mixture evaporated to dryness. This process was repeated once with addition of water and twice with addition of diethyl ether (~20 mL) to give 0.825 g of the desired ligand as a dark-yellow solid. Yield: 89 %; elemental analysis calcd (%) for $C_{15}H_{15}N_3O_4$·2 HCl: C 48.14, H 4.58, N 11.23; found: C 47.86, H 4.58, N 10.92; MS (ESI^+, MeOH/CH_3CN/H_2O 9:1:1): *m*/*z*: 340 $[C_{15}H_{15}KN_3O_4]^+$; IR (ATR): $\tilde{\nu}$=1726 cm^{-1} (C=O); ^{1}H NMR (D_2O, pD 1.8, 500 MHz, 25 °C, TMS): δ=7.93 (d, 2 H, ^{3}J=7.8 Hz), 7.87 (t, 2 H, ^{3}J=7.8 Hz), 7.53 (d, 2 H, ^{3}J=7.8 Hz), 4.62 (s, 4 H), 3.09 ppm (s, 3 H); ^{13}C NMR (D_2O, pD 1.8, 125.8 MHz, 25 °C, TMS): δ=167.2, 149.7, 146.8, 139.7, 128.3, 125.3, 60.3, 43.0 ppm.

[Mn(dpama)(H_2O)$_2$]·(H_2O)$_2$

A solution of H_2dpama·2 HCl (0.100 g, 0.267 mmol), triethylamine (0.108 g, 1.07 mmol) and $Mn(ClO_4)_2$·6 H_2O (0.097 g, 0.267 mmol) in 2-propanol (10 mL) under argon atmosphere was stirred at room temperature for 24 h, which resulted in the formation of a white solid. This was isolated by filtration, washed

with 2-propanol and diethyl ether, and dried under vacuum. Yield 0.074 g, 65 %; elemental analysis calcd. (%) for $C_{15}H_{13}MnN_3O_4 \cdot 4\,H_2O$: C 42.26, H 4.97, N 9.86; found: C 41.84, H 5.22, N 9.98; HR-MS (ESI^+, MeOH/CH_3CN/H_2O 9:1:1): *m*/*z* calcd for $[C_{15}H_{14}MnN_3O_4]^+$: 355.0359; found: 355.0359; IR (ATR): $\tilde{\nu}$=1620, 1586 cm^{-1} (C═O).

Tetraethyl 6,6′,6″,6‴-(((1,3-phenylenebis(methylene))bis(azanetriyl))tetrakis(methylene))tetrapicolinate (4)

6-Chloromethylpyridine-2-carboxylic acid ethyl ester **1** (6.16 g, 30.8 mmol) and K_2CO_3 (6.70 g, 48.5 mmol) were added to a solution of 1,3-phenylenedimethanamine (1.00 g, 7.34 mmol) in acetonitrile (100 mL). The mixture was stirred for a period of 4 days at 45 °C and after this was heated to reflux for 1 day. The excess of K_2CO_3 was filtered off, the filtrate was concentrated to dryness, and the yellow residue was extracted with a mixture of H_2O and $CHCl_3$ (1:3; 200 mL). The organic phase was evaporated to dryness to give an oily residue that was purified by column chromatography on $SiO_2$with a CH_2Cl_2/MeOH 5 % mixture as the eluent to give 6.27 g of **2** as a yellow oil. Yield: 82 %; elemental analysis calcd (%) for $C_{44}H_{48}N_6O_8 \cdot 3CH_2Cl_2$: C 54.09, H 5.22, N 8.05; found: C 54.32, H 5.44, N 7.82; HR-MS (ESI^+, MeOH/CH_3CN/H_2O 9:1:1): *m*/*z* calcd for $[C_{44}H_{49}N_6O_8]^+$: 789.3606; found: 789.3607; IR (ATR): $\tilde{\nu}$=1714 cm^{-1} (C═O); ^1H NMR ($CDCl_3$, 500 MHz, 25 °C, TMS): δ=7.96 (m, 4 H), 7.80 (m, 8 H), 7.34 (m, 4 H), 4.45 (c, 8 H, ^3J=7.1 Hz), 3.92 (s, 8 H), 3.70 (s, 4 H), 1.41 ppm (t, 12 H, ^3J=7.1 Hz); ^{13}C NMR ($CDCl_3$, 125.8 MHz, 25 °C, TMS): δ=165.2, 160.3, 147.7, 138.8, 137.3, 129.3, 128.4, 127.8, 125.7, 123.4, 61.8, 59.6, 58.6, 14.3 ppm.

6,6′,6″,6‴-(((1,3-Phenylenebis(methylene))bis(azanetriyl))tetrakis(methylene))tetrapicolinic acid (*m*X(H_2dpama)$_2$·7 HCl·H_2O)

A solution of compound **2** (1.00 g, 0.958 mmol) in 6 M HCl (50 mL) was heated to reflux for 24 h and then the solvent was removed in a rotary evaporator to give a yellow oil. A small amount of H_2O was added (~20 mL) and the mixture evaporated to dryness. This process was repeated once with addition of another portion of H_2O and twice with addition of diethyl ether (~20 mL) to give 0.846 g of the desired ligand as a light-yellow solid. Yield: 93 %; elemental analysis calcd (%) for $C_{36}H_{32}N_6O_8 \cdot 7\,HCl \cdot H_2O$: C 45.52, H 4.35, N 8.85; found: C 45.22, H 4.54, N 8.91; MS (ESI^+, MeOH/CH_3CN/H_2O 9:1:1): *m*/*z*: 677 $[C_{36}H_{33}N_6O_8]^+$; IR (ATR): $\tilde{\nu}$ =1730, 1707 cm^{-1} (C═O); ^1H NMR (D_2O, pD 7.0, 500 MHz, 25 °C, TMS): δ=7.72–7.19 (m, 16 H), 4.24 (s, 8 H), 4.20 ppm (s, 4 H); ^{13}C NMR (D_2O, pD 7.0, 125.8 MHz, 25 °C, TMS): δ=171.8, 152.4, 151.9, 138.8, 138.7, 132.4, 131.5, 129.4, 125.9, 123.3, 59.5, 58.8 ppm.

[*m*X(Mn(dpama)(H_2O)$_2$)$_2$]·(H_2O)$_6$

A solution of *m*X(H_2dpama)$_2$·7 HCl·H_2O (0.100 g, 0.105 mmol), triethylamine (0.117 g, 1.16 mmol) and $Mn(ClO_4)_2 \cdot 6\,H_2O$ (0.076 g, 0.210 mmol) in 2-propanol (10 mL) was stirred at room temperature for 24 h under argon atmosphere; this resulted in the formation of a white solid which was isolated by filtration. The solid was then suspended in acetonitrile (10 mL) and stirred at room temperature for 24 h under argon. The solid was isolated by filtration, washed with acetonitrile and diethyl ether, and dried under vacuum. Yield 0.066 g, 65 %; elemental analysis calcd. (%) for $C_{36}H_{28}Mn_2N_6O_8 \cdot 10\,H_2O$: C 44.92, H 5.03, N 8.73; found: C 44.91, H 4.82, N 8.79; HR-MS (ESI^+, MeOH/CH_3CN/H_2O 9:1:1): *m*/*z* calcd for $[C_{36}H_{28}Mn_2N_6NaO_8]^+$: 805.0621; found: 805.0627; IR (ATR): $\tilde{\nu}$=1622, 1587 cm^{-1} (C═O).

DFT calculations

Full geometry optimizations of the [Mn(dpama)(H_2O)$_2$]·4 H_2O and [(*m*X(Mndpama)(H_2O)$_2$·4 H_2O)$_2$] systems were performed in aqueous solution at the TPSSh/SVP[19, 20] level employing the Gaussian 09 package (Revision B.01).[21] The stationary points found on the potential energy surfaces as a result of geometry optimizations were tested to represent energy minima rather than saddle points via frequency analysis. Solvent effects were included by using the polarizable continuum model (PCM), in which the solute cavity is

built as an envelope of spheres centred on atoms or atomic groups with appropriate radii. In particular, the integral equation formalism (IEFPCM) variant as implemented in Gaussian 09 was used.[22]

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