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PAPER

$[Gd(Try\text{-}TTDA)(H_2O)]^{2-}$: A new MRI contrast agent for copper ion sensing†

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In this study, we have developed two new L-tryptophan based contrast agents $[Gd(Try\text{-}TTDA)(H_2O)]^{2-}$ and $[Gd(Try\text{-}ac\text{-}DOTA)(H_2O)]^{-}$. Upon addition of Cu(II) to $[Gd(Try\text{-}TTDA)(H_2O)]^{2-}$, significant increases in the relaxivity (r_1) and hydration number of $[Gd(Try\text{-}TTDA)(H_2O)]^{2-}$ were observed. However, it only induced a minute increase in the relaxivity (r_1) in the case of $[Gd(Try\text{-}ac\text{-}DOTA)(H_2O)]^{-}$. Furthermore, the interaction of Cu(II) with the indole ring of Gd(III) complexes was explored by measuring the intrinsic fluorescence of the tryptophan of the Gd(III) complex. With the addition of one equivalent of Cu(II) to $[Gd(Try\text{-}TTDA)(H_2O)]^{2-}$ the indole fluorescence was completely quenched. Moreover, the $[Gd(Try\text{-}TTDA)(H_2O)]^{2-}$ complex shows excellent selectivity towards Cu(II) over other metal ions (Cu(II) > La(III) > Mg(II)). Importantly, the significant signal intensity (2073 ± 67) for *in vitro* MR imaging using $[Gd(Try\text{-}TTDA)(H_2O)]^{2-}$ in the presence of Cu(II) implicates that this new smart contrast agent ($[Gd(Try\text{-}TTDA)(H_2O)]^{2-}$) can serve as a Cu(II) sensor for MR imaging.

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Introduction

Magnetic resonance imaging (MRI) offers several advantages over other clinical diagnostic techniques in molecular imaging, including high spatial resolution, noninvasiveness, high anatomical contrast and lack of harmful radiation.[1–3] On the other hand, the sensitivity of MRI in depicting a small molecule is constrained by the ubiquitous protons in the body, resulting in a high background and low signal to noise ratio (SNR). To enhance the MR image signal intensity, gadolinium ion based contrast agents have been developed. This metal ion, with the high electron spin and a symmetric electronic ground state ($^8S_{7/2}$), couples to a large magnetic moment with a long electron spin relaxation time (~10^{-9} s at the magnetic field strengths of interest for MRI applications), these properties ensure an optimum efficiency for the nuclear spin relaxation of the interacting nuclei.[4] Currently, most of the MRI contrast agents approved in clinical use are Gd(III)-poly(aminocarboxylate) complexes.[1,2] However, these contrast agents are non-specific and provide only anatomical information.

The MR signal enhancement depends on the relaxation rate of the protons in water molecules interacting with the metal centre. The relaxation rate is influenced by a variety of factors such as the number of inner-sphere water molecules (q), the rotational correlation time (τ_R), and the mean residence lifetime of the Gd(III) coordinated/bound water molecule (τ_M). Recently, several smart contrast agents, which have abilities to respond to a micro-environment such as pH,[5] partial oxygen pressure,[6] Ca(II),[7] Zn(II),[8] K(I),[9] Cu(I)/Cu(II),[10] and Fe(II)[11] metal ions, enzyme activity[12] and sugars[13] have been reported. These smart MR contrast agents furnish structural changes upon target interaction, which significantly change their signal properties.

Among all the bio-relevant metal ions, copper plays an important role in various biological processes.[14,15] An imbalance of Cu(II) ions within the human body can cause many diseases including Menkes disease,[16,17] occipital horn syndrome[18] and Wilson's disease.[19,20] The normal concentration of copper in normal blood is 100–150 μg/dL (15.7–23.6 μM).[17] The deciphering of the relationship between copper regulation and its physiological consequences is interesting, however, understanding remains elusive at the molecular level.

Over the past few decades, several fluorescence-based sensors for the detection of Cu(II) ions have been reported.[21,22] In contrast,

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† Electronic supplementary information (ESI) available: Procedures for ^{17}O NMR and dissociation constant (K_d), ESI mass spectra (S1, S2, S4, S6, S8), HPLC chromatograms (S3, S5), Job's plot (S7), determination of dissociation constant (S9), emission spectra (S10), and quenching plot (S11). See DOI: 10.1039/c1dt10033e

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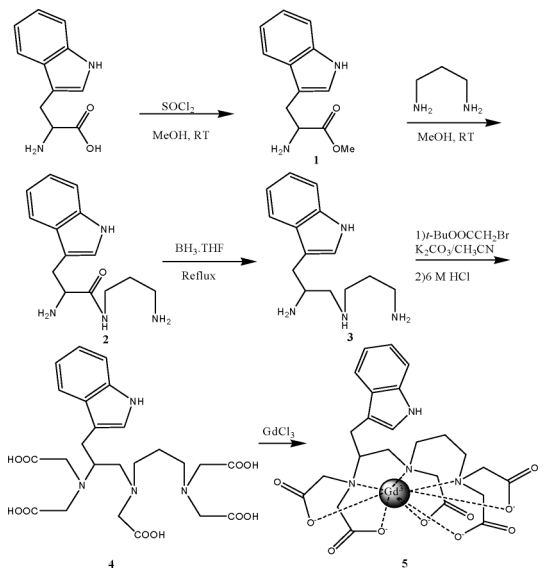
the reported MR-based copper ion sensors are limited. Given that noncovalent binding forces, such as interactions between a cation and an aromatic ring, play important functional and structural roles in biological molecules,[23,24] the bio-related indole side chain bearing a poly(aminocarboxylate) such as EDTA-bis(L-tryptophan methyl ester) has been demonstrated to interact significantly with the metal ion (Cu(II)), exhibiting the strongest interaction.[25] Therefore, the development of an optical and MRI dual mode Cu(II) sensing molecule with an indole group incorporated in the Gd(III) complex is fascinating.

In this report, we designed and synthesized (4*S*)-4-indolyl-3,6,10-tri(carboxymethyl)-3,6,10-triazadodecanedioic acid (Try-TTDA) and *N*-L-tryptophan-(4,7,10-tris(carboxymethyl)-4,7,10-tetraaza-cyclododec-1-yl)-acetamide (Try-ac-DOTA) and their Gd(III) complexes. The synthetic strategy of $[Gd(Try\text{-}TTDA)(H_2O)]^{2-}$ and $[Gd(Try\text{-}ac\text{-}DOTA)(H_2O)]^{-}$ was depicted in Schemes 1 and 2. Subsequently, the T_1 relaxivity was determined in the presence and absence of Cu(II) at 37.0 ± 0.1 °C and 20 MHz. Furthermore, the quenching of the intrinsic fluorescence of the indole with various biologically relevant metal ions was studied. In addition, *in vitro* T_1 weighted MR imaging studies were also investigated to demonstrate a smart Cu(II) sensing contrast agent.

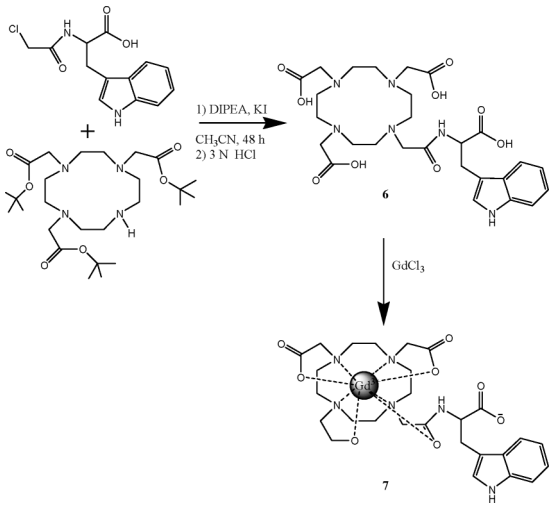


Scheme 1 Synthetic scheme of $[Gd(Try\text{-}TTDA)(H_2O)]^{2-}$.

Experimental

Materials and instrumentation

L-Tryptophan and bromoacetylbromide were purchased from Alfa Aesar. Thionyl chloride, BH_3·THF, gadolinium(III) chloride hexahydrate, europium chloride, copper chloride, magnesium chloride, *N*-chloroacetyl-L-tryptophan and phosphate buffered saline were purchased from Aldrich. Cyclen was purchased from Strem chemicals. 4,7,10-(Tris-*tert*-butyl carboxymethyl)-(1,4,7,10-tetraazacyclodecane) (DO3A-tris-*tert*-butyl ester) was synthesized using the previously reported procedure.[26,27] *tert*-Butylbromoacetate was synthesized according to the reported procedure.[28] The $[Gd(TTDA)(H_2O)]^{2-}$ complex was prepared



Scheme 2 Synthetic scheme of $[Gd(Try\text{-}ac\text{-}DOTA)(H_2O)]^{-}$.

by using our previously reported procedure.[29] All commercial grade chemicals were used without further purification. Fluorescence and luminescence were measured by using a Varian Cary Eclipse fluorescence spectrophotometer and ^1H (400 MHz), ^{13}C (100 MHz), and ^{17}O (54.2 MHz) NMR spectra were recorded on a Varian Gemini-400 spectrometer. The concentration of the Gd(III) complex was determined by ICP-MS with a Perkin–Elmer OPTIMA 2000. The relaxivity measurements were performed using a relaxometer operating at 20 MHz and 37.0 ± 0.1 °C (NMR-120 Minispec, Bruker). MR imaging was performed with a 3.0 T MR scanner (Sigma; GE Medical Systems, Milwaukee, WI) using a knee coil. The HPLC experiments were performed on an Amersham ÄKTAbasic 10 instrument equipped with an Amersham UV-900 detector and Amersham Frac-920 fraction collector. LC- mass spectral analyses were performed with a Waters Micromass-ZQ mass spectrometer.

Preparations

Synthesis of methyl ester-L-tryptophan hydrochloride (1). L-Tryptophan (10.0 g, 49.0 mmol) was dissolved in methanol (200 mL) and then thionyl chloride (4.29 mL, 58.8 mmol) was added slowly. Upon completion of the addition, the reaction mixture was stirred at room temperature for 9 h. The reaction mixture was concentrated under reduced pressure and then diethyl ether was added, which resulted in a white precipitate. The resulting product was filtered and dried under vacuum for 48 h. The white powder **1** was obtained (8.0 g, 67.0%). ^1H NMR (DMSO-d_6, 400 MHz): δ (ppm) = 6.96 –7.60 (m, 5H, indolyl protons), 4.2 (m, C*H*), 3.64 (s,OCH_3) 3.15 (m, CH_2). ^{13}C NMR (DMSO-d_6, 100 MHz): δ (ppm) = 169.77, 136.21, 126.90, 125.01, 121.16, 118.6, 118.00, 115.88, 106.32, 52.65, 26.09.

Synthesis of 2-amino-*N*-(3-aminopropyl)-3-(1*H*-indol-2-yl)propanamide trihydrochloride (2). L-Tryptophan methyl ester hydrochloride (8.23 g, 32.4 mmol) was dissolved in 100 mL of methanol and then 1,3-diaminopropane (4.29 mL, 162.0 mmol) was added. The resulting reaction mixture was stirred at room temperature for 24 h. The reaction mixture was concentrated under reduced pressure to obtain a yellow viscous oil. Then, 7 M NH_4OH (100 mL) solution was added to this oil and the pH

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was adjusted to 10–11.5. The aqueous phase was extracted with chloroform (five portions of 500 mL). The organic layer was dried over anhydrous $MgSO_4$ and filtered. The filtrate was concentrated under reduced pressure and the crude amide was purified using cationic exchange (AG 50 W × 8 column (200–400 mesh, H^+ form, 100 mL resin), 4.2 cm column diameter). The product was eluted with 4.0–4.5 M HCl. The solution was evaporated to dryness to obtain **2** as a hygroscopic powder (2.4 g, 20.2%). ^{1}H NMR (D_2O, 400 MHz): δ (ppm) = 7.1–7.60 (m, 5H, indolyl protons), 4.1 (t, indolyl-CH_2–C*H*-), 3.31–2.93 (m, NHC$H_2$$CH_2CH_2$$NH_2$), 2.4 (m, indolyl-C$H_2$-CH), 1.42 (quint, $NHCH_2CH_2CH_2NH_2$). ^{13}C NMR (D_2O, 100 MHz): δ (ppm) = 169.69, 136.25, 126.73, 125.23, 122.29, 119.70, 118.35, 112.12, 106.78, 54.11, 48.99, 36.79, 36.44, 26.92, 26.17.

Synthesis of *N*-(2-amino-3-(1*H*-indol-2-yl)propyl)propane-1,3-diamine trihydrochloride (3). Amide (**2**) (2.4 g, 6.55 mmol) was placed in a 250 mL two necked round bottom flask, fitted with a reflux condenser. After purging with nitrogen, the flask was cooled to 0–10 °C. The 1 M BH_3·THF (30 mL) was cannulated into the reaction flask. The reaction mixture was warmed up to room temperature and brought to reflux for 24 h. The solution was cooled to 0–10 °C and the excess borane was quenched by a slow and dropwise addition of water until the evolution of gas ceased. The reaction mixture was evaporated and the residue was dissolved in 6 M HCl (100 mL). The resulting solution was refluxed for 3 h, followed by stirring at room temperature for 24 h. The acidic reaction mixture was loaded into a cationic exchange column (AG 50 W × 8 column (200–400 mesh, H^+ form, 100 mL resin); 4.2 cm column diameter). The product was eluted with 5.5–6.0 M HCl. The solution was evaporated to dryness. A pale yellow compound was obtained (**3**) (2.02 g, 86.6%). ^{1}H NMR (D_2O, 400 MHz): δ (ppm) = 7.1–7.60 (m, 5H, indolyl protons), 3.95 (m, indolyl-CH_2–C*H*), 3.4 (m, indolyl-CH_2-CH), 3.28–2.94 (m,NH_2C$H_2$$CH_2CH_2$$NH_2$), 2.03 (quint, $NH_2CH_2CH_2CH_2NH_2$). ^{13}C NMR (D_2O, 100 MHz): δ (ppm) = 136.25, 126.73, 125.63, 122.52, 119.88, 118.20, 112.34, 106.40, 49.01, 45.48, 36.52, 26.78, 23.73.

Synthesis of (4*S*)-4-indolyl-3,6,10-tri(carboxymethyl)-3,6,10-triazadodecanedioic acid (Try-TTDA) (4). To a solution of amine (**3**) (2.02 g, 5.74 mmol) and anhydrous K_2CO_3 (7.12 g, 51.6 mmol) in CH_3CN (200 mL), *tert*-butyl bromoacetate (4.64 mL, 31.35 mmol) was added. The resulting reaction mixture was refluxed for 24 h. The K_2CO_3 was removed by filtration through a Buchner funnel and the solvent was removed under reduced pressure. Then, 6 M HCl (100 mL) was added to the residue, and the mixture was stirred at room temperature for 4 h. The solution was evaporated by rotary evaporation. The acid treatment was repeated three times. The residue was dissolved in distilled water and adjusted to pH 11.0 by adding aqueous ammonia and loaded to the anion exchange column (AG1 × 8 column (200–400 mesh, HCO_2^- form, 100 mL resin); 4.2 cm column diameter). The product was eluted with 500 mL of water and a formic acid gradient. The trace of formic acid was removed by co-evaporation with water (3 × 100 mL), and the product was dried under vacuum to give a pale yellow powder of Try-TTDA (**4**) (1.0 g, 31.0%). MS ESI$^+$ for $[C_{24}H_{32}N_4O_{10}]^+$ calculated 536.21, found 536.77, (see Fig. S1†). ^{1}H NMR (DMSO-d_6, 400 MHz): δ (ppm) = 7.1–7.60 (m, 5H, indolyl protons), 3.81 (s, 8H, terminal, NCH_2COOH), 3.4 (s, central, NCH_2COOH), 3.08–2.94 (m, NH_2C$H_2$$CH_2CH_2$$NH_2$), 2.5 (quint, NH_2CH_2C$H_2$$CH_2NH_2$). ^{13}C NMR (DMSO-$d_6$, 100 MHz): δ (ppm) = 178.00, 176.00, 174.60, 171.55, 140.25, 130.96, 126.73, 125.16, 122.52, 122.33, 115.61, 113.34, 49.11, 45.48, 36.52, 27.48, 23.73.

Synthesis of *N*-L-tryptophan-(4,7,10-tris(carboxymethyl)-4,7,10-tetraaza-cyclododec-1-yl)-acetamide(Try-ac-DOTA)(6). 4,7,10-(Tris-*tert*-butylcarboxymethyl)-(1,4,7,10 tetraazacyclodecane) (DO3A-tris-*tert*-butyl ester) (1.22 g, 2.38 mmol), KI (0.078 g, 0.48 mmol) and *N*,*N*-diisopropylamine (DIPEA) (1.36 mL, 7.8 mmol) were dissolved in acetonitrile (150 mL) and then the mixture was stirred for 2 h. Subsequently, the *N*-chloroacetyl-L-tryptophan (0.7 g, 2.5 mmol) was added to the solution. The reaction mixture was heated to 60–70 °C and stirred for 48 h. The solvent was removed by rotary evaporation. Then, 6 M HCl (100 mL) was added to the residue and the mixture was stirred at room temperature for 4 h. The solution was evaporated by rotary evaporation. The acid treatment was repeated three times. The residue was dissolved in water and adjusted to pH 11 with ammonium hydroxide, and subjected to anion exchange (AG1 × 8 column (200–400 mesh, HCO_2^- form, 100 mL resin); 4.2-cm column diameter). The product was eluted with 2.0 L of water and a formic acid gradient. The trace amount of formic acid was removed by co-evaporation with water (3× 100 mL), yielding a yellow viscous oil of Try-ac-DOTA (**6**) (0.70 g, 50%) MS ESI$^+$ for $[C_{27}H_{38}N_6O_9]^+$: calculated 590.27, found 591.10, (see Fig. S2†) ^{1}H NMR (D_2O, 400 MHz): δ (ppm) = 7.0–7.60 (m, 5H, indolyl protons), 2.6–3.81(m, 27H, cycle CH_2, indolyl-CH, CH_2), ^{13}C NMR (D_2O, 100 MHz): δ (ppm) = 176.40, 175.0, 170.55, 169.89, 135.82, 127.06, 124.36, 121.73, 119.34, 118.57, 111.70, 110.13, 47.5–56.14 (12C, cycle CH_2), 42.25, 27.15.

Complexation. The Gd(III) and Eu(III) complexes were prepared by dissolving the ligand (Try-TTDA or Try-ac-DOTA) (0.05 mmol) in H_2O (8 mL) and adjusting the pH of the solution to 6.5 with 1 N NaOH. To these solutions, 2.0 mL of an aqueous $LnCl_3$ solution (0.05 mmol) was added dropwise, while maintaining the pH at 5.5–6.0 with 1 N NaOH (aq). The mixture solution was stirred at room temperature. The absence of free lanthanide ions in the solutions was verified by the xylenol orange test. The solutions were evaporated under reduced pressure. The purity of the Gd(III) complexes was determined by HPLC and identified by a mass spectrometer. MS ESI$^+$ for $[Gd(Try\text{-}TTDA)(H_2O)]^{2-}$ $[C_{24}H_{29}GdN_4O_{10}]^+$: calculated 689.10, found 689.69, (see Fig. S3 and S4†). MS ESI$^+$ for $[Gd(Try\text{-}ac\text{-}DOTA)(H_2O)]$ $[C_{27}H_{35}GdN_6O_9]^{3+}$: calculated 745.15, found 748.10 (see Figs. S5 and S6†)

Relaxation time measurement (r_1)

Relaxation times (T_1) of aqueous solutions of Gd(III) complexes were measured to determine relaxivity (r_1). All measurements were made using a relaxometer operating at 20 MHz and 37.0 ± 0.1 °C (NMS-120 Minispec, Bruker). Before each measurement, the relaxometer was tuned and calibrated. The values of r_1 were determined from six data points generated by an inversion-recovery pulse sequence. The proton spin–lattice relaxation (T_1) was measured by using various concentrations of $[Gd(Try\text{-}TTDA)(H_2O)]^{2-}$ or $[Gd(Try\text{-}ac\text{-}DOTA)(H_2O)]^-$ at 37.0 ± 0.1 °C

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in pH 7.4 PBS buffer. The interaction with metal ions was confirmed by titration method. The different concentrations of metal ions were added with a fixed concentration of the [Gd(Try-TTDA)(H_2O)]$^{2-}$ or [Gd(Try-ac-DOTA)(H_2O)]$^-$ complex in acetate buffer at pH 5.5, and 37.0 ± 0.1 °C and their relaxivity values were measured.

Luminescence method for establishing solution hydration state

Luminescence lifetime data has been obtained for the Eu(III) complex to determine the number of inner-sphere water molecules in the aqueous solution.[30] The luminescence lifetime (τ) has been determined in both H_2O and D_2O. The number of inner-sphere water molecules contained in the complex was calculated by eqn (1):

$$q = ([1/\tau_{H2O} - 1/\tau_{D2O}] - 0.25) \times 1.2 \quad (1)$$

where q is the number of water molecules bound to metal ions, τ_{H_2O} is the luminescence half-life in water solution and τ_{D_2O} is the luminescence half-life in deuterium oxide solution, respectively.

Fluorescence study of [Gd(Try-TTDA)(H_2O)]$^{2-}$ and [Gd(Try-ac-DOTA)(H_2O)]$^-$ for metal ion interaction

To examine the metal ion-indole ring interaction, the fluorescence quenching studies of [Gd(Try-TTDA)(H_2O)]$^{2-}$ and [Gd(Try-ac-DOTA)(H_2O)]$^-$ with various metal ions were performed. The metal ions were titrated into a solution of 0.1 mM Gd(III) complex in acetate buffer at pH 5.5. The excitation wavelength was fixed at 281 nm, and the emission intensity at 360 nm was recorded.

In vitro MR imaging study

The *in vitro* T_1-weighted MR imaging of [Gd(Try-TTDA)(H_2O)]$^{2-}$ was performed by the concentration-dependent imaging. The different concentrations (0.01, 0.05, 0.10 and 0.20 mM) of [Gd(Try-TTDA)(H_2O)]$^{2-}$ and metal ions in the molar ratio of 1 : 1 were mixed at room temperature. The MR imaging was performed with a 3.0 T MR imaging scanner (Sigma, GE Medical Systems, Milwaukee, WI) using a knee coil. The T_1-weighted scanning was performed under the following condition: fast gradient echo, TR = 500 ms, TE = 1.5 ms, coronal view, and section thickness = mm, FOV = 10 cm.

Results and discussion

Synthesis

In this study, we reported two L-tryptophan moiety-bearing ligands (Try-TTDA and Try-ac-DOTA), as shown in Fig. 1. Synthesis of L-tryptophan-based TTDA ligand and its Gd(III) complex was shown in Scheme 1. Synthesis of Try-TTDA was achieved by the following steps: the reaction of L-tryptophan with thionyl chloride in methanol at room temperature led to the formation of compound **1**, followed by the addition of 1,3-diaminopropane in methanol, which yielded compound **2**. The mixed solution of **2** and BH_3·THF was stirred for 15 h at 60 °C, followed by the addition of 6 M HCl (aq) solution, which results in the formation of compound **3**. The compound **3** was treated with *tert*-butylbromoacetate in the presence of anhydrous K_2CO_3 in CH_3CN at 80 °C to provide the *tert*-butyl ester of Try-TTDA and, subsequently, the hydrolysis of the *tert*-butyl ester using 6 M HCl gave Try-TTDA (**4**). Finally, the [Gd(Try-TTDA)(H_2O)]$^{2-}$ (**5**) complex was achieved by the complexation of the Gd(III) ion with the Try-TTDA ligand in water at room temperature. The resulting complex was identified by MS (ESI†).

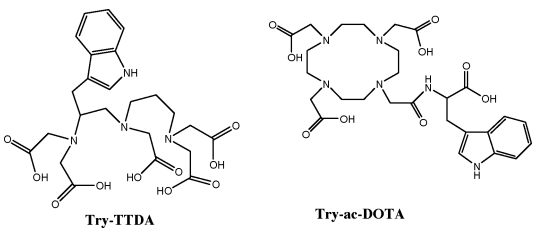

Fig. 1 Structural formula of L- tryptophan based ligands.

The synthesis of the L-tryptophan-based DOTA ligand and its Gd(III) complex was shown in Scheme 2. Preparation of Try-ac-DOTA was accomplished by the following steps: the reaction of *N*-chloroacetyl-L-tryptophan with DO3A-tris-*tert*-butyl ester in the presence of a catalytic amount of KI in acetonitrile, at 80 °C, led to the formation of the *tert*-butyl ester of Try-ac-DOTA and, subsequently, the hydrolysis of the *tert*-butyl ester using 3 N HCl(aq) at room temperature was completed to obtain Try-ac-DOTA (**6**). Furthermore, the ligand was treated with $GdCl_3$·$6H_2O$ in water at room temperature to afford the complex [Gd(Try-ac-DOTA)(H_2O)]$^-$ (**7**). The resulting complex was identified by MS (ESI†).

Luminescence study

To determine the number of inner-sphere water molecules before and after Cu(II) interaction with [Gd(Try-TTDA)(H_2O)]$^{2-}$ or [Gd(Try-ac-DOTA)(H_2O)]$^-$, the luminescence method was used. We conducted the luminescence lifetime (τ) measurements of the equivalent Eu(III) complexes in both H_2O and D_2O,[30] as shown in Table S1†. The number of inner sphere molecules (q) was calculated by using eqn (1). The estimated number of inner sphere molecules for [Eu(Try-TTDA)(H_2O)]$^{2-}$ is 1.23 in the absence of Cu(II) and 1.94 in the presence of Cu(II). These results indicate that the interaction of Cu(II) with the indole ring changes the chelating mode for [Eu(Try-TTDA)(H_2O)]$^{2-}$. Presumably, a carboxylate arm switches to bind with Cu(II), leaving space for the coordination of a second water molecule to the lanthanide, which leads to an increase in the hydration number (Δq = 0.7). On the other hand, in the presence or absence of Cu(II), the hydration number remains the same for [Eu(Try-ac-DOTA)(H_2O)]$^-$, since no change in the chelation is necessary to accommodate the Cu(II). These results support the proposed structures of the addition of Cu(II) to [Gd(Try-TTDA)(H_2O)]$^{2-}$ or [Gd(Try-ac-DOTA)(H_2O)]$^-$, as shown in Scheme 3.

Stoichiometry analysis

In order to investigate the binding between Cu(II) and [Gd(Try-TTDA)(H_2O)]$^{2-}$, the fluorescence based Job's method was performed.[31] A plot of the fluorescence intensity as a function of [Cu(II)]/([Cu(II)] + [Gd(Try-TTDA)(H_2O)]$^{2-}$) is shown in Fig. S7.† The inflection point at 0.5 indicates that the [Gd(Try-TTDA)(H_2O)]$^{2-}$–Cu(II) complex exists with 1 : 1 stoichiometry.

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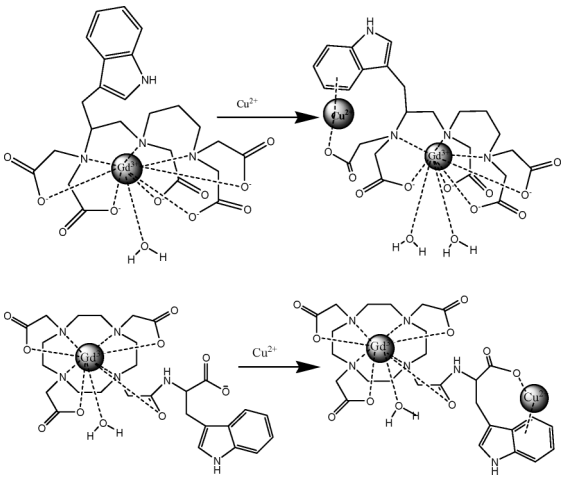

Scheme 3 Proposed structures of Cu(II) ion interaction with $[Gd(Try\text{-}TTDA)(H_2O)]^{2-}$ and $[Gd(Try\text{-}ac\text{-}DOTA)(H_2O)]^{-}$.

Table 1 The relaxivity of $[Gd(Try\text{-}TTDA)(H_2O)]^{2-}$ and $[Gd(Try\text{-}ac\text{-}DOTA)(H_2O)]^{-}$ in the presence and absence of Cu(II), $[Gd(TTDA)(H_2O)]^{2-}$, $[Gd(DTPA)(H_2O)]^{2-}$, and $[Gd(DOTA)(H_2O)]^{-}$ at 37.0 ± 0.1 °C and 20 MHz

Complexes	r_1/mM^{-1} s^{-1}
$[Gd(Try\text{-}TTDA)(H_2O)]^{2-}$	4.22 ± 0.03
$[Gd(Try\text{-}TTDA)(H_2O)]^{2-}$+Cu(II)	7.42 ± 0.03
$[Gd(Try\text{-}DOTA)(H_2O)]^{2-}$	4.02 ± 0.05
$[Gd(Try\text{-}DOTA)(H_2O)]^{2-}$+Cu(II)	4.65 ± 0.04
$[Gd(TTDA)(H_2O)]^{2-}$ [a]	3.85 ± 0.03
$[Gd(DTPA)(H_2O)]^{2-}$ [b]	3.89 ± 0.03
$[Gd(DOTA)(H_2O)]^{-}$ [c]	3.56

[a] Data was obtained from ref. 29 [b] ref. 32 [c] ref. 33

These results also support the proposed structure of $[Gd(Try\text{-}TTDA)(H_2O)]^{2-}$ in the presence of Cu(II), as shown in Scheme 3.

Relaxivity measurements

The relaxivity (r_1) values of $[Gd(Try\text{-}TTDA)(H_2O)]^{2-}$ and $[Gd(Try\text{-}ac\text{-}DOTA)(H_2O)]^{-}$ in the presence and absence of Cu(II), $[Gd(TTDA)(H_2O)]^{2-}$, $[Gd(DTPA)(H_2O)]^{2-}$, and $[Gd(DOTA)(H_2O)]^{-}$ at 37.0 ± 0.1 °C and 20 MHz are shown in Table 1. The relaxivity values of $[Gd(Try\text{-}TTDA)(H_2O)]^{2-}$ and $[Gd(Try\text{-}ac\text{-}DOTA)(H_2O)]^{-}$ are higher than those of $[Gd(TTDA)(H_2O)]^{2-}$,[29] $[Gd(DTPA)(H_2O)]^{2-}$,[32] and $[Gd(DOTA)(H_2O)]^{-}$.[33] Fig. 2 represents the relaxivity of $[Gd(Try\text{-}TTDA)(H_2O)]^{2-}$ titrated by a variable concentration of metal ions at 37.0 ± 0.1 °C and 20 MHz in pH 5.5 acetate buffer solution.[34]

Upon the addition of one equivalent of Cu(II) to $[Gd(Try\text{-}TTDA)(H_2O)]^{2-}$, the relaxivity significantly increases, owing to an increase in the number of inner sphere water molecules. Moreover, Cu(II) itself is paramagnetic and has a relaxivity (r_1) value of 1.0 mM^{-1} s^{-1}, measured at 10 MHz in water.[35] This relaxivity increase compares favourably with other reported Cu(II) sensors, such as the Copper-Gad (CG) family.[10] The addition of more Cu(II) has no effect on the relaxivity. This result is in good agreement with the fluorescence based Job's method for the 1 : 1 molar ratio binding of $[Gd(Try\text{-}TTDA)(H_2O)]^{2-}$ to Cu(II). The Cu(II) interaction with $[Gd(Try\text{-}TTDA)(H_2O)]^{2-}$ was also

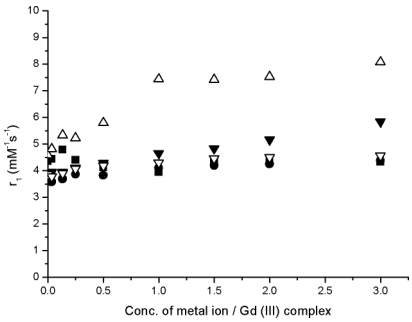

Fig. 2 Dependence of the proton spin-lattice relaxivity (r_1) on metal ion concentration for $[Gd(Try\text{-}TTDA)(H_2O)]^{2-}$ {Cu(II) (△), Mg(II) (■), La(III) (●)}, $[Gd(Try\text{-}ac\text{-}DOTA)(H_2O)]^{-}$ {Cu(II) (▼)}, and $[Gd(TTDA)(H_2O)]^{2-}$ {Cu(II) (▽)} at 37.0 ± 0.1 °C and 20 MHz in pH 5.5 acetate buffer solution.

confirmed by mass spectroscopic analysis. The expected mass was observed at m/z 752, corresponding to ($[Gd(Try\text{-}TTDA)]^{2-}$ + [Cu(II)]), as shown in Fig. S8.† Moreover, the dissociation constant (K_d) is estimated to be 2.52 mM (Fig. S9†). On the other hand, after addition of Cu(II) to $[Gd(Try\text{-}ac\text{-}DOTA)(H_2O)]^{-}$ and $[Gd(TTDA)(H_2O)]^{2-}$, only a minor increase in the relaxivity was observed because there is no increase in the number of inner sphere water molecules, as shown in Table 1. In addition, we measured the relaxivity of $[Gd(Try\text{-}TTDA)(H_2O)]^{2-}$ titrated by a variable concentration of different metal ions at 37.0 ± 0.1 °C and 20 MHz in pH 5.5 acetate buffer solution, as shown in Fig. 2. The result showed that there was no remarkable increase in the relaxivity for Mg(II) and La(III). These relaxivity studies also indicate that the interaction of the indole ring with Cu(II) is significantly stronger than those of the indole ring with La(III) and Mg(II).

^{17}O NMR Study

The kinetic parameters were determined for $[Gd(Try\text{-}TTDA)(H_2O)]^{2-}$ by variable-temperature ^{17}O NMR studies. The chemical shifts ($\Delta\omega_r$), the longitudinal ($1/T_{1r}$) and transverse ($1/T_{2r}$) relaxation rates were simultaneously analyzed (see ESI†). The data for $[Gd(Try\text{-}TTDA)(H_2O)]^{2-}$ are plotted in Fig. 3, with the corresponding curves representing the results of the best fitting of the data according to equations (1S–9S). The kinetic parameters obtained were illustrated in Table 2, the large number of parameters influence the data obtained by the different techniques. Nevertheless, the ^{17}O NMR technique has an advantage that the outer-sphere contributions to the relaxation rates are negligibly small, which is a result of the oxygen nucleus being closer to the paramagnetic centre when it is bound in the inner sphere.[36] The scalar coupling constant ($A/\hbar$) of $[Gd(Try\text{-}TTDA)(H_2O)]^{2-}$ is very similar to those obtained for other Gd(III) complexes (–3.2 ± 0.3 and –3.8 ± 0.2 × 10^6 rad s^{-1} for $[Gd(TTDA)(H_2O)]^{2-}$ and $[Gd(DTPA)(H_2O)]^{2-}$, respectively) with one inner-sphere water molecule. In the overall temperature range, the transverse ^{17}O relaxation rates ($1/T_{2r}$) decrease with an increasing temperature, indicating that this system is in the fast exchange regime.[37] Consequently, $1/T_{2r}$ is determined by the relaxation rate of the coordinated water molecule ($1/T_{2m}$), which is influenced by the water residence lifetime ($\tau_M = 1/k_{ex}$), the longitudinal electronic relaxation rate ($1/T_{1e}$), and the scalar coupling constant ($A/\hbar$). The water-exchange rate (k_{ex}^{298}) for

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Table 2 Kinetic and NMR parameters of $[Gd(Try\text{-}TTDA)(H_2O)]^{2-}$, $[Gd(TTDA)(H_2O)]^{2-}$, $[Gd(DTPA)(H_2O)]^{2-}$, and $[Gd(ETPTA)(H_2O)]^{2-}$ as obtained from the simultaneous fit of ^{17}O-NMR data[a]

Parameter	$[Gd(TryTTDA)(H_2O]^{2-}$	$[Gd(TTDA)(H_2O]^{2-a}$	$[Gd(DTPA)(H_2O]^{2-b}$	$[Gd(ETPTA)(H_2O]^{2-c}$
k_{ex}^{298} (10^6 s^{-1})	40.9 ± 2.7	146 ± 17	4.1 ± 0.3	330 ± 40
$\Delta H^{\neq}$ (kJ mol^{-1})	28.7 ± 0.2	23.1 ± 0.5	52.0 ± 1.4	27.9 ± 11
$\Delta S^{\neq}$ ($Jmol^{-1}$ k^{-1})	−2.7 ± 0.1	−11.1 ± 3.1	56.2 ± 5.0	11.0 ± 3.0
$A/\hbar$(10^6rad s^{-1})	−3.4 ± 0.1	−3.2 ± 0.3	−3.8 ± 0.2	−3.9 ± 0.2
τ_R^{298} (ps)	*562 ± 2*	104 ± 12	103 ± 10	75 ± 6
C_{os}	0	0	0.13 ± 0.06	0.1
E_R (kJ mol^{-1})	16.9 ± 1.6	24.8 ± 1.5	18 ± 2	17.7 ± 1.0
Method	^{17}O	^{17}O	^{17}O	^{17}O, EPR, NMRD

[a] Data was obtained from ref. 29 [b] ref. 32. [c] ref. 38.

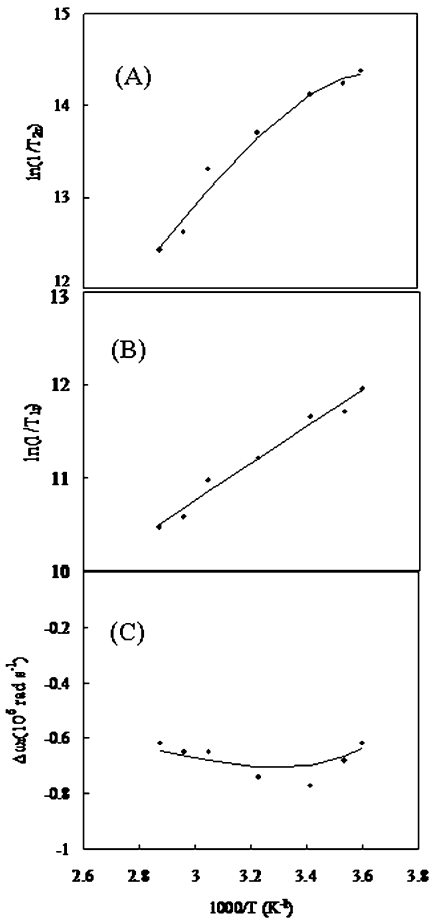

Fig. 3 Temperature dependence of (a) transverse and (b) longitudinal ^{17}O relaxation rates and (c) ^{17}O chemical shifts at B = 9.4 T for $[Gd(Try\text{-}TTDA)(H_2O)]^{2-}$. The lines represent simultaneous least-squares fits to all data points displayed.

$[Gd(Try\text{-}TTDA)(H_2O)]^{2-}$ ($40.9 \pm 2.7 \times 10^6$ s^{-1}) is lower than those of other $[Gd(TTDA)(H_2O)]^{2-}$ derivatives, but is significantly higher than that of $[Gd(DTPA)(H_2O)]^{2-}$ ($4.1 \pm 0.3 \times 10^6$ s^{-1}). Additionally, the value of correlation time (τ_R) for the Gd(III) complex with Try-TTDA was obtained at 298 K from the fitting of the ^{17}O NMR. The τ_R^{298} value of $[Gd(Try\text{-}TTDA)(H_2O)]^{2-}$ (562 ps) is higher than those of $[Gd(TTDA)(H_2O)]^{2-}$ (104 ps),[29] $[Gd(DTPA)(H_2O)]^{2-}$ (103 ps),[32] and $[Gd(EPTPA)(H_2O)]^{2-}$ (75 ps).[38] Therefore, the relaxivity of $[Gd(Try\text{-}TTDA)(H_2O)]^{2-}$ is higher than those of $[Gd(TTDA)(H_2O)]^{2-}$, $[Gd(DTPA)(H_2O)]^{2-}$, and $[Gd(DOTA)(H_2O)]^{-}$ as shown in Table 1.

Fluorescence quenching study

The interaction between the metal ions and the side chain indole of $[Gd(Try\text{-}TTDA)(H_2O)]^{2-}$ and $[Gd(Try\text{-}ac\text{-}DOTA)(H_2O)]^{-}$ was investigated by the quenching of the intrinsic tryptophan fluorescence using fluorescence spectroscopy. The $[Gd(Try\text{-}TTDA)(H_2O)]^{2-}$ was titrated with various biologically relevant metal ions, such as Na(I), Ca(II), Mg(II), Zn(II), Cu(II), and La(III) in acetate buffer at pH = 5.5, and the quenching of the intrinsic tryptophan fluorescence was measured with excitation wavelength at 281 nm and emission wavelength at 360 nm. As shown in Fig. 4a, the fluorescence of $[Gd(Try\text{-}TTDA)(H_2O)]^{2-}$ is not quenched by the addition of Na(I), Ca(II), Mg(II), Zn(II) or La(III) (in molar ratio 1 : 1). However, under the same mol ratio conditions, the fluorescence emission intensity dramatically decreases after adding Cu(II). We further studied the titration of $[Gd(Try\text{-}TTDA)(H_2O)]^{2-}$ with various amounts of Cu(II). The fluorescence intensity of $[Gd(Try\text{-}TTDA)(H_2O)]^{2-}$ is completely quenched when one equivalent of Cu(II) is added, as shown in Fig. 4b. The consequence is consistent with stoichiometry analysis and mass spectroscopic studies. These results indicate that the interaction between the indole ring of $[Gd(Try\text{-}TTDA)(H_2O)]^{2-}$ and Cu(II) is stable in solution state and quenches more strongly than those of other metal ions. Furthermore, we studied the titration of $[Gd(Try\text{-}ac\text{-}DOTA)(H_2O)]^{-}$ with various amounts of Cu(II), as shown in Fig. S10.† However, the fluorescence quenching plot is nonlinear with a downward curve (Fig. S11†). Therefore, the fluorescence quenching of $[Gd(Try\text{-}ac\text{-}DOTA)(H_2O)]^{-}$ after binding with Cu(II) is significantly lower than that of $[Gd(Try\text{-}TTDA)(H_2O)]^{2-}$.

In vitro MR imaging study

To demonstrate the potential utility of this new MRI contrast agent ($[Gd(Try\text{-}TTDA)(H_2O)]^{2-}$), MR imaging studies were performed using a 3.0 T MR imaging scanner. The different concentrations of $[Gd(Try\text{-}TTDA)(H_2O)]^{2-}$(0.01, 0.05, 0.10 and 0.20 mM) and metal ions (Cu(II), La(III) and Mg(II)) in the molar ratio of 1 : 1 were mixed at room temperature and T_1 weighted MR images were performed. As shown in Fig. 5, the signal intensity values for the 0.2 mM concentration of Cu(II), La(III) and Mg(II) are 2073 ± 67, 1101 ± 16 and 1106 ± 24, respectively. The signal intensity of *in vitro* MR imaging increases as the concentration

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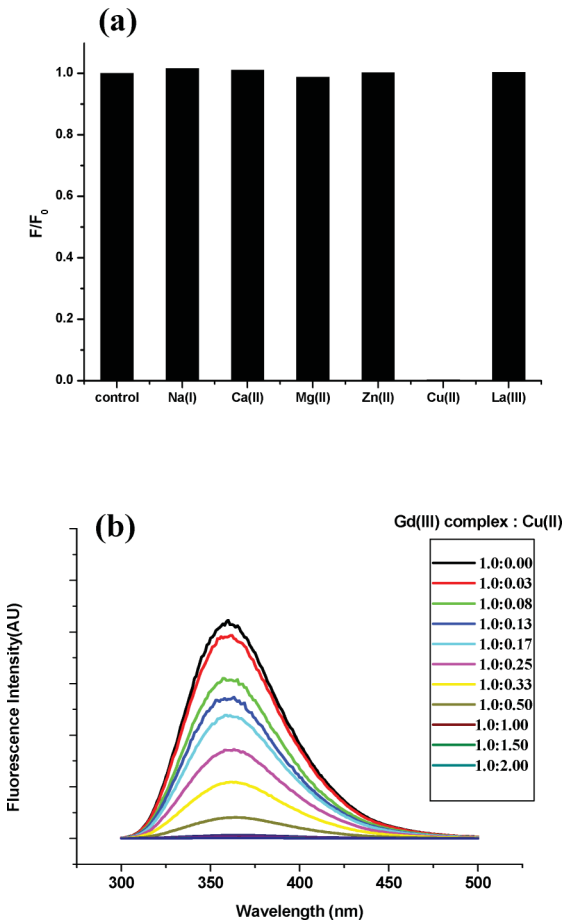

Fig. 4 (a) Quenching plots of the fluorescence of [Gd(Try-TTDA)-$(H_2O)]^{2-}$ against 1 : 1 mol equivalents of the metal ions added. (b) Fluorescence emission spectra of 100 μM [Gd(Try-TTDA)$(H_2O)]^{2-}$ interaction with Cu(II) at room temperature and pH 5.5 acetate buffer solution.

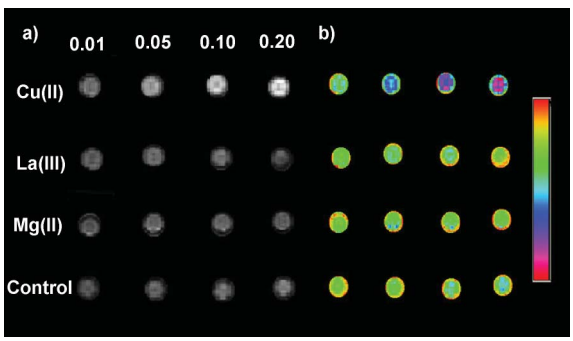

Fig. 5 a) T_1-weighted MR images of [Gd(Try-TTDA)$(H_2O)]^{2-}$ and metal ions in the molar ratio = 1 : 1, pH 5.5 acetate buffer solution at 3.0 T MR imaging scanner. b) Colour mapping of (a).

of the Cu(II) ion and the Gd(III) complex increases in molar ratio 1 : 1, as shown in Table S3.† In contrast, the Mg(II) and La(III) ions do not exhibit signal enhancement. These results implicate that the high relaxivity of [Gd(Try-TTDA)$(H_2O)]^{2-}$ in the presence of Cu(II) is reflected in the signal intensity of MR images. Hence, the [Gd(Try-TTDA)$(H_2O)]^{2-}$ can be potentially used as a contrast agent to detect Cu(II) by using MR imaging.

Conclusion

In summary, two new L-tryptophan based contrast agents, [Gd(Try-TTDA)$(H_2O)]^{2-}$ and [Gd(Try-ac-DOTA)$(H_2O)]^{-}$, were successfully synthesized. The [Gd(Try-TTDA)$(H_2O)]^{2-}$ effectively recognizes Cu(II) among the metal ions examined at pH 5.5 acetate buffer solution using fluorescence measurements. This Gd(III) complex displays high selectivity for Cu(II) *via* quenching of intrinsic tryptophan fluorescence. In particular, the relaxivity studies show a significant increase in relaxivity upon Cu(II) detection. In addition, the sensing of Cu(II) by [Gd(Try-TTDA)$(H_2O)]^{2-}$ was proven by *in vitro* T_1-weighted MR imaging. Hence, [Gd(Try-TTDA)$(H_2O)]^{2-}$ may serve as a smart contrast agent for the detection of Cu(II) ions.

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References

1 P. Caravan, J. J. Ellison, T. J. McMurry and R. B. Lauffer, *Chem. Rev.*, 1999, **99**, 2293–2352.
2 S. Aime, M. Fasano and M. Terreno, *Chem. Soc. Rev.*, 1998, **27**, 19–29.
3 P. Caravan, *Chem. Soc. Rev.*, 2006, **35**, 512–523.
4 R. S. Ranganathan, N. Raju, H. Fan, X. Zhang, M. F. Tweedle, J. F. Desreux and V. Jacques, *Inorg. Chem.*, 2002, **41**, 6856–6866.
5 (*a*) M. P. Lowe, D. Parker, O. Reany, S. Aime, M. Botta, G. Castellano, E. Gianolio and R. Pagliarin, *J. Am. Chem. Soc.*, 2001, **123**, 7601–7609; (*b*) S. Aime, A. Barge, M. Botta, D. Parker and A. S. DeSousa, *J. Am. Chem. Soc.*, 1997, **119**, 4767–4768; (*c*) M. P. Lowe and D. Parker, *Chem. Commun.*, 2000, 707–708; (*d*) J. Hall, R. Haner, S. Aime, M. Botta, S. Faulkner, D. Parker and A. S. De Sousa, *New J. Chem.*, 1998, **22**, 627–631; (*e*) S. R. Zhang, K. C. Wu and A. D. Sherry, *Angew. Chem., Int. Ed.*, 1999, **38**, 3192–3194.
6 (*a*) S. Aime, M. Botta, E. Gianolio and E. Terreno, *Angew. Chem., Int. Ed.*, 2000, **39**, 747–750; (*b*) P. Z. Sun, Z. B. Schoening and A. Jasanoff, *Magn. Reson. Med.*, 2003, **49**, 609–614.
7 (*a*) W. H. Li, S. E. Fraser and T. J. Meade, *J. Am. Chem. Soc.*, 1999, **121**, 1413–1414; (*b*) W. H. Li, G. Parigi, M. Fragai, C. Luchinat and T. J. Meade, *Inorg. Chem.*, 2002, **41**, 4018–4024; (*c*) K. Dhingra, P. Fouskova, G. Angelovski, M. E. Maier, N. K. Logothetis and E. Toth, *JBIC, J. Biol. Inorg. Chem.*, 2008, **13**, 35–46.
8 (*a*) K. Hanaoka, K. Kikuchi, Y. Urano and T. Nagano, *J. Chem. Soc., Perkin Trans. 2*, 2001, 1840–1843; (*b*) R. Trokowski, J. M. Ren, F. K. Kalman and A. D. Sherry, *Angew. Chem., Int. Ed.*, 2005, **44**, 6920–6923; (*c*) A. C. Esqueda, J. A. Lopez, G. Andreu-de-riquer, J. C. Alvarado-Monzon, J. Ratnakar, A. J. M. Lubag, A. D. Sherry and L. M. De Leon-Rodriguez, *J. Am. Chem. Soc.*, 2009, **131**, 11387–11391.
9 H. Hifumi, A. Tanimoto, D. Citterio, H. Komatsu and K. Suzuki, *Analyst*, 2007, **132**, 1153–1160.
10 (*a*) E. L. Que and C. J. Chang, *J. Am. Chem. Soc.*, 2006, **128**, 15942–15943; (*b*) E. L. Que, E. Gianolio, S. L. Baker, A. P. Wong, S. Aime and C. J. Chang, *J. Am. Chem. Soc.*, 2009, **131**, 8527–8536; (*c*) E. L. Que, E. Gianolio, S. L. Baker, S. Aime and C. J. Chang, *Dalton Trans.*, 2010, **39**, 469–476.
11 (*a*) J. B. Livramento, E. Toth, A. Sour, A. Borel, A. E. Merbach and R. Ruloff, *Angew. Chem., Int. Ed.*, 2005, **44**, 1480–1484; (*b*) V. Comblin, D. Gilsoul, M. Hermann, V. Humblet, V. Jacques, M. Mesbahi, C. Sauvage and J. F. Desreux, *Coord. Chem. Rev.*, 1999, **185–186**, 451–470; (*c*) R. Ruloff, G. V. Koten and A. E. Merbach, *Chem. Commun.*, 2004, 842–843.
12 (*a*) A. Y. Louie, M. M. Huber, E. T. Ahrens, U. Rothbacher, R. A. Moats, R. E. Jacobs, S. E. Fraser and T. J. Meade, *Nat. Biotechnol.*, 2000, **18**, 321–325; (*b*) R. A. Moats, S. E. Fraser and T. J. Meade, *Angew. Chem., Int. Ed. Engl.*, 1997, **36**, 726–728; (*c*) M. M. Alauddin, A. Y.

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Louie, A. Shahinian, T. J. Meade and P. S. Conti, *Nucl. Med. Biol.*, 2003, **30**, 261–265.
13 (*a*) R. Trokowski, S. R. Zhang and A. D. Sherry, *Bioconjugate Chem.*, 2004, **15**, 1431–1440; (*b*) S. R. Zhang, R. Trokowski and A. D. Sherry, *J. Am. Chem. Soc.*, 2003, **125**, 15288–15289; (*c*) L. Frullano, J. Rohovec, S. Aime, T. Maschmeyer, M. I. Prata, J. J. P. De Lima, C. F. G. C. Geraldes and J. A. Peters, *Chem.–Eur. J.*, 2004, **10**, 5205–5217; (*d*) S. Aime, D. Delli Castelli, F. Fedeli and E. Terreno, *J. Am. Chem. Soc.*, 2002, **124**, 9364–9365.
14 E. L. Que, D. W. Daimalle and C. J. Chang, *Chem. Rev.*, 2008, **108**, 1517–1549.
15 H. Kodama and C. Fujisawa, *Metallomics*, 2009, **1**, 42–52.
16 D. Strusak, J. F. B. Mercer, H. H. Dieter, W. Stremmel and G. Multhaup, *Brain Res. Bull.*, 2001, **55**, 175–185.
17 I. Voskoboinik and J. Camakaris, *J. Bioenerg. Biomembr.*, 2002, **34**, 363–371.
18 S. F. Lazoff, J. J. Rybak, B. R. Parker and L. Luzzatti, *Birth Defects, Orig. Artic. Ser.*, 1975, **11**, 71–74.
19 M. M. O. Penna, J. Lee and D. J. Thiele, *J. Nutr.*, 1999, **129**, 1251–1260.
20 V. Desai and S. G. Kaler, *Am. J. Clin. Nutr.*, 2008, **88**, 855S–8S.
21 K. Rurack, M. Kollmannsberger, U. Resch-Genger and J. Daub, *J. Am. Chem. Soc.*, 2000, **122**, 968–969.
22 S. Y. Moon, N. R. Cha, Y. H. Kim and S.-K. Chang, *J. Org. Chem.*, 2004, **69**, 181–183.
23 J. Hu, L. J. Barbour and G. W. Gokel, *J. Am. Chem. Soc.*, 2002, **124**, 10940–10941.
24 S. Yun, Y. O. Kim, D. Kim, H. G. Kim, H. Ihm, J. K. Kim, C.-W. Lee, W. J. Lee, J. Yoon, K. S. Oh, S,-M. Park and K. S. Kim, *Org. Lett.*, 2003, **5**, 471–474.
25 (*a*) Y. Li and C. M. Yang, *Chem. Commun.*, 2003, 2884–2885; (*b*) Y. Li and C. M. Yang, *J. Am. Chem. Soc.*, 2005, **127**, 3527–3530.
26 H. Z. Gai and T. A. Kaden, *Helv. Chim. Acta*, 1994, **77**, 383–398.
27 K. Kumar, C. A. Chang and M. F. Tweedle, *Inorg. Chem.*, 1993, **32**, 587–593.
28 A. Vollmar and M. S. Dunn, *J. Org. Chem.*, 1960, **25**, 387–390.
29 Y. M. Wang, C. H. Lee, G. C. Liu and R. S. Sheu, *J. Chem. Soc., Dalton Trans.*, 1998, 4113–4118.
30 (*a*) A. Beeby, I. M. Clarkson, R. S. Dickins, S. Faulkner, D. Parker, L. Royle, A. S. D. Sousa, J. A. G. Williams and M. Woods, *J. Chem. Soc., Perkin Trans. 2*, 1999, 493–504; (*b*) W. D. Horrocks, Jr. and D. R. Sudnick, *J. Am. Chem. Soc.*, 1979, **101**, 334–340; (*c*) W. D. Horrocks, Jr. and D. R. Sudnick, *Acc. Chem. Res.*, 1981, **14**, 384–392.
31 Y. Yang, X. Gou, J. Blecha and H. Cao, *Tetrahedron Lett.*, 2010, **51**, 3422–3425.
32 K. Micskei, L. Helm, E. Brucher and A. E. Merbach, *Inorg. Chem.*, 1993, **32**, 3844–3850.
33 S. Aime, P. L. Anelli, M. Botta, F. Fedeli, M. Grandi, P. Paoli and F. Uggeri, *Inorg. Chem.*, 1992, **31**, 2422–2428.
34 J. Paris, C. Gameiro, C. Humblet, P. K. Mohapatra, V. Jacaques and J. F. Desreux, *Inorg. Chem.*, 2006, **45**, 5092–5102.
35 J. Vymazal, J. W. M. Bulte, J. A. Frank and R. A. Brook, *J. Magn. Reson. Imaging*, 1993, **3**, 637–640.
36 M. H. Ou, C. H. Tu, S. C. Tsai, W. T. Lee, G. C. Liu and Y. M. Wang, *Inorg. Chem.*, 2006, **45**, 244–254.
37 K. Micskei, D. H. Powell, L. Helm, E. Brucher and A. E. Merbach, *Magn. Reson. Chem.*, 1993, **31**, 1011–1020.
38 S. Laus, R. Ruloff, E. Toth and A. E. Merbach, *Chem.–Eur. J.*, 2003, **9**, 3555–3566.

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