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BRIEF ARTICLE

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An Electroneutral Macrocyclic Iron(II) Complex That Enhances MRI Contrast in Vivo

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Supporting Information

ABSTRACT: The first example of a macrocyclic ferrous complex, where two tetrazolyl pendent arms compensate the charge of the metal center, is synthesized and examined for its capacity to enhance MRI contrast in vitro and in vivo in the mouse.

INTRODUCTION

We recently proposed a new approach to the design of responsive MRI contrast agents that act in the off/on mode. Contrary to established strategies based on the permanently paramagnetic lanthanide ion gadolinium, our tactic makes use of macrocyclic complexes based on iron(II) as the paramagnetic center influencing the nuclear spins that surround the probe.[1,2] Iron is a homeostatically managed metal ion in all live organisms on earth[3] that can adopt various spin states and, in its oxidation state II, can exist in a paramagnetic (high-spin) or a diamagnetic (low-spin) state. The macrocycle ensures high complex stability and thus minimum leaching of the central metal ion,[4,5] and evidence has also been accumulated for its favoring the attainment of the low-spin state.[6] By contrast, iron(III) complexes have already been evaluated for their capacity to act as MRI contrast agents,[7] but they cannot be made diamagnetic and thus invisible by MRI, such as is possible for iron(II)-based agents.

We had previously identified two structurally similar iron(II) complexes (Scheme 1, complexes **2**[8,9] and **3**[10,11]) based on the trivalent ligand 1,4,7-triazacyclononane (TACN).[1] Complex **2** has been found to be paramagnetic in aqueous solution. It therefore increases relaxation of surrounding water protons and generates significant contrast in T_1-weighted MR images. An aqueous sample of complex **3** was found to be diamagnetic, and its MR image did not show any contrast modification compared to that of pure water. Consequently, an MRI-silent (off) or -active (on) complex can be obtained from the base structure, depending on the presence or absence of a third pendent arm.

Our first-generation paramagnetic model complex **2** (Scheme 1), while generating significant relaxivity, has the fundamental disadvantage of carrying two positive charges. Its thermodynamic stability is thus necessarily not optimal (lack of Coulombic charge compensation).[12] It possesses an elevated Lewis acid character and undoubtedly an unfavorable osmolalilty. In this context it is not surprising that commercial MRI contrast agents based on the lanthanide ion Gd(III) are all electroneutral or negatively charged.[13,14] We set out to address these limitations by rendering our MRI-active complex electroneutral (Scheme 1, complex **1**) while ensuring the presence of two imine-type nitrogen atoms as found in the original pyridyl groups (**2**). Groups displaying these imine-type nitrogens have to exhibit a pK_a compatible with physiological pH (i.e., lower than 7.4) to prevent weakening of the complex thermodynamically and kinetically by protonation.[12] Herein, we present the synthesis of a novel macrocyclic chelator and its corresponding electroneutral iron(II) complex (**1**). We then report on its evaluation as a contrast agent for T_1-weighted MR imaging in vitro and in the live animal in comparison with reference complexes **2** and **3**.

RESULTS AND DISCUSSION

We used our very recently established strategy of introducing the tetrazole in the form of a preformed, preprotected synthon rather than establishing the azole in situ as is widely practiced in the literature (see refs 15 and 16, references therein, and refs 17 and 18). This gave very satisfactory results (Scheme 2), in contrast to those reported for a comparable synthetic target.[19] We apply benzylated tetrazolylmethyl chloride[15,16] (**7**, Scheme 2) here for the introduction of two pendent arms by double alkylation of formylated 1,4,7-triazacyclonane (**6**)[20] that can be obtained reliably from the N/N/N orthoacetal **5** by acidic hydrolysis if great care is applied. Key intermediate **8** is purified to homogeneity by silica gel chromatography to give satisfactory yields (62−78%). While hydrolysis of the formamide group of **8** is straightforward (95%), the removal of the two benzyl groups by catalytic hydrogenation requires amounts of palladium on carbon exceeding the weight of the introduced starting material (3.5 equiv w/w) but then succeeds efficiently in the course of 48 h at 1 atm of hydrogen. The reason for the high amounts of catalyst is attributed to the presence of the tertiary amine groups,[21] and our own experiences appear to indicate that bidentate chelating motifs may also reduce the catalytic power of palladium on carbon.[15,16] We eventually ran the reaction at the gram scale,

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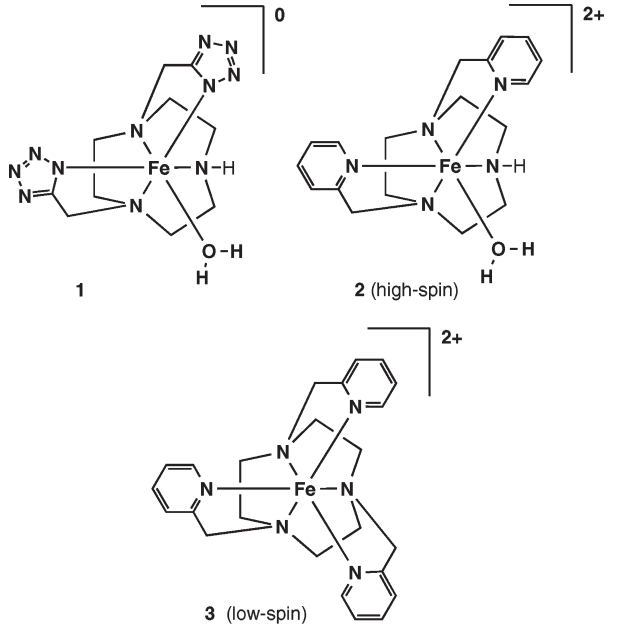
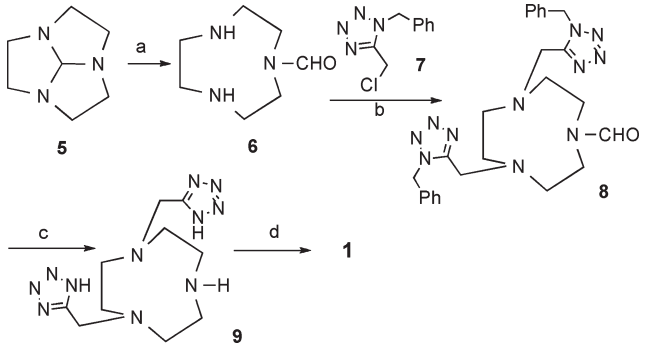
Scheme 1. Macrocyclic Ferrous Complexes



Scheme 2[a]



[a] (a) (1) HCl, (2) NaOH, pH 11.4; 0 °C, 63−75%; (b) MeCN, TEA, 62−78%; (c) (1) NaOH, EtOH, reflux, 95%, (2) H_2, 0.8 equiv of Pd/C, 60−78%; (d) $Fe(BF_4)_2 \cdot 6H_2O$, MeOH.

and subsequent purification of **9** by chromatography on a reversed-phase cartridge led to final yields of 60−78%.

Complex **1** was synthesized by reacting ligand **9** with $Fe(BF_4)_2 \cdot 6H_2O$ under argon. Elaboration of a stringent isolation/purification protocol allowed for the reproducible isolation of pure samples of **1** as a pale pink solid. We have singled out the tetrazolylmethyl group as a pendent arm with an N-acidic moiety to ensure electroneutrality of our target complex **1** (Scheme 1).[22] It exhibits a very attractive aqueous pK_a of ~5,[23] comparable to that of acetic acid, thus offering a full negative charge at pH 7.4. Electroneutral complex **1** is highly soluble in water and HEPES buffer and insoluble in common organic solvents (MeOH, $CHCl_3$, CH_2Cl_2, CH_3CN, THF).

T_1 values (7 T, 300 MHz magnetic field strength, proton resonance frequency) of aqueous solutions of **1** and **3** were determined at four different concentrations (Figure 1). For comparison, the values for a commercial sample of the clinically

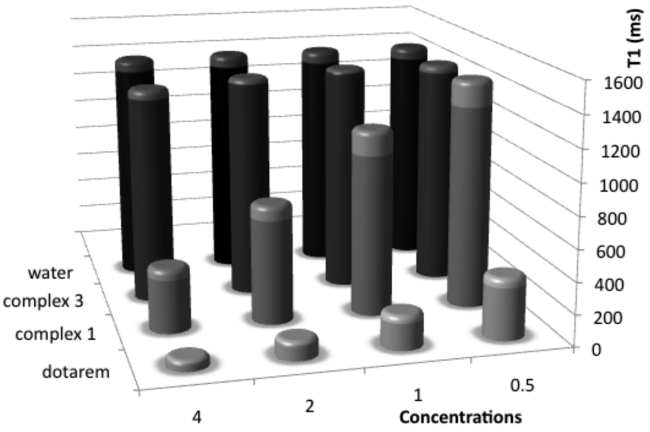


Figure 1. Average T_1 values (at 7 T) of several independently synthesized samples (standard deviations at column tip) at four different concentrations (mM).

applied contrast agent $[Gd(DOTA)]^{-1}$ (Dotarem, Guerbet, France) were included. These values correspond to relaxivities of r_1 of 0.57 mM^{-1} s^{-1} (**1**) and 3.75 mM^{-1} s^{-1} (Dotarem). The electronic correlation relaxation time T_{1e} of iron(II) centers is recognized not to be optimal for lowering T_1 of the surrounding water hydrogen spins at lower clinical field strengths (e.g., 1.5 T).[1] However, at higher field strengths, T_{1e} for iron(II) progressively loses its role as a relevant parameter for the modulation of relaxivity as it decreases with the square of the field.[24,25] However, the water exchange rate (a relevant parameter at high fields) of the ferrous ion is known to be high (10^{-10} to 10^{-7} s) compared to that of the ferric ion (>10^{-5} s) and in the same range as that of lanthanide ions,[26] thus contributing to satisfactory relaxivity. As expected, low-spin complex **3** modifies neither T_1 nor T_2 beyond a negligible factor consistent with the temperature-independent residual paramagnetism for this complex in aqueous solution (0.9 μ_B).[1]

To initially avoid the intrinsic problem of molecule delivery through cell membranes, we have developed an in vivo injection−electroporation procedure into the tibialis muscle to test molecule candidates directly in mice. The protocol was developed at the example of the commercial contrast agent Dotarem. Mice were treated under anesthesia (intraperitoneal injection of ketamin (100 mg/kg) and xylazin (10 mg/kg)). The contrast agent (50 μL, 50 mM) or pure water as negative control was injected into the tibialis muscle and electroporated (8×, 200 V/cm, 20 ms with 500 ms of intervals) to allow diffusion into muscle cells. MRI scans were performed 24 h postinjection. We determined the accuracy of this internalization protocol by verifying the degree of correlation of the MRI signal intensity with the amount of injected gadolinium agent. The mice were imaged using a 32 mm imaging probe and a T_1-weighted spin echo sequence in coronal and transverse orientations. The images were acquired with TR/TE 500/12 ms and 4 NEX. For all the scans, FOV = 30 × 30 mm^2, slice thickness = 0.8 mm, and acquisition matrix size = 256 × 192. Selected slices clearly depicted the presence of contrast agent entrapped in muscle cells. By use of homemade software, the mean value of signal intensity within the tibialis muscle was calculated, corrected to the experimentally determined noise standard deviation, and normalized to an internal reference as shown in Figure 2A. Our results prove that signal intensity is directly proportional to the quantity of injected gadolinium agent.

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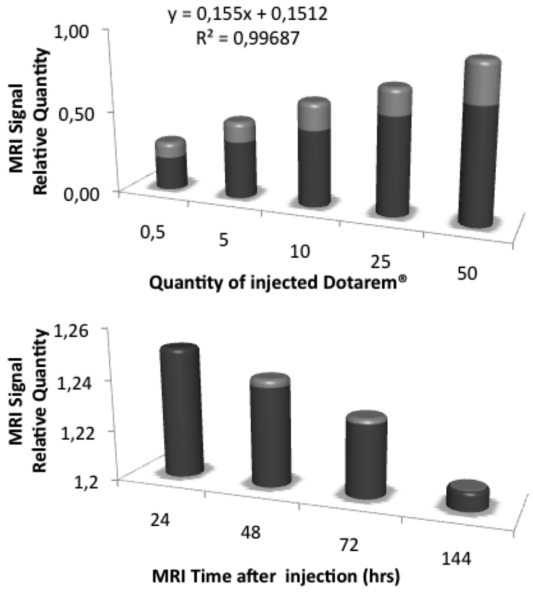

Figure 2. MRI signals (mean ± SEM) averaged over at least three independent acquisitions: as a function of Dotarem concentration (mM) injected into the mouse tibialis, average value from at least three animals (top); as a function of a constant Dotarem concentration (50 μL, 0.5 mM) (bottom).

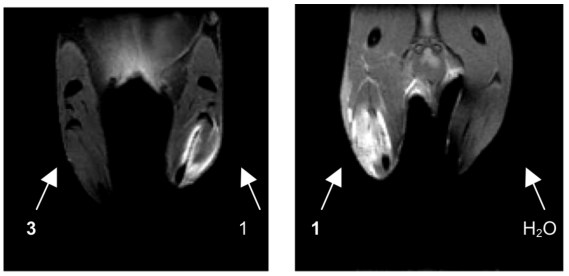

Figure 3. T_1-weighted images 24 h postinjection of a mouse (left) that received **1** and **3** (25 μL of 25 mM in pure water) into its right and left legs, respectively, by an injection−electroporation procedure into the respective tibialis muscle, and another mouse (right) that was treated with **1** (50 μL of 50 mM) in the left leg and pure water into the right leg.

In another experiment, we determined the kinetics of signal disappearance (and the associated residence time) of injected Dotarem at 24, 48, and 72 h postinjection (Figure 2B). In fact, contrast above background can be detected up to 144 h postinjection. The kinetics of MRI signal decrease are very small compared to those habitually observed for intravenously administered MRI contrast agents. This result gives strong support to the notion that our in vivo injection−electroporation protocol internalizes molecular contrast agents and that it is a valid method for testing our molecule candidates directly in mice, even in the absence of any chemical/molecular delivery strategy.

We then applied this administration protocol to our test candidates **1** and **3** and the imaging of the thus treated mice (Figure 3). Samples of **1** (varying from 25 μL of 25 mM to 50 μL of 50 mM) raised T_1 contrast to a significant degree (2.7-fold over background), while the low-spin and diamagnetic ferrous complex **3** did not modify contrast to any extent. Several repetitions of the above experiments with independently prepared samples of complex **1** attest to the in vivo tolerance observed for this macrocyclic ferrous complex with an N_3(amine)N_2-(tetrazol)O(H_2O) coordination motif and demonstrate the efficiency of **1** in raising contrast.

CONCLUSION

Our results support the notion of biocompatibility of our binary and macrocyclic ferrous complexes at the chosen doses, whether displaying an open or closed coordination shell. They also demonstrate that the principal two objections to the use of iron-based MRI agents in oxidation state II, that of an unfavorable electronic relaxation time and only four unpaired electrons, do not rule out their consideration for niche applications in molecular and functional imaging, at least at elevated field strengths. The here-developed synthetic methodology and the in vivo imaging results constitute an important step toward our goal to discover an iron(II)-based contrast agent that passes from the low-spin (off) to the high-spin (on) state upon the encounter of, and transformation by, a specific target enzyme in the live animal.

EXPERIMENTAL SECTION

Reagents and solvents were purchased from Aldrich, Acros, and Alfa Aesar and used without further purification. THF was degassed and dried of solvent columns on neutral alumina (glassware system). Column chromatography was performed using Merck silica gel Si 60 (40−63 μm). ^{1}H and ^{13}C NMR spectra were recorded on a Varian Unity 500 spectrometer (499.83 and 126.7 MHz, respectively) or a Bruker DPX 200 instrument (200.13 and 50.13 MHz, respectively). Chemical shifts (δ) are reported in ppm (s = singlet, d = doublet, t = triplet, m = multiplet, br = broad) and referenced to solvent. NMR coupling constants (J) are reported in hertz. The purity of target **1** and its preceding ligand **9** was proved to be >95% by way of HPLC analysis. HRMS were recorded by the "Centre Commun de Spectrométrie de Masse" of the University Claude Bernard in Lyon (France) and the Service Central d'Analyse of the CNRS in Solaize (France).

1-Formyl-4,7-bis[1-benzyltetrazol-5-yl]methyl-1,4,7-triazacyclonane (8). A mixture of 700 mg of 1-benzyltetrazol-5-ylchloromethane **7** (3.4 mmol, 2.1 equiv) dissolved in acetonitrile (3 mL) and distilled triethylamine (1 mL) is added to a stirred solution of 1-carboxaldehyde-1,4,7-triazacyclononane **6** (250 mg, 1.6 mmol, 1 equiv) in 12 mL of dry acetonitrile. After 3 days a white precipitate is formed, the solvent is removed under reduced pressure, water (5 mL) is added, and the resulting solution is extracted with chloroform (3 × 15 mL), dried over Na_2SO_4, filtered, and concentrated. The crude product is purified on a silica gel column to yield 500 mg (62%) of **8** as a white solid. ^{1}H NMR ($CDCl_3$, 300 K, 200 MHz) δ: 7.93 (s, 1H); 7.32 (m, 6H); 7.13 (m, 4H); 5.57 (s, 4H); 3.82 (s, 4H); 3.29 (m, 2H); 3.01 (m, 4H); 2.85 (m, 2H); 2.59 (m, 2H); 2.44 (m, 2H). ^{13}C NMR ($CDCl_3$, 300 K, 50 MHz) δ: 163.6 (C_4, tetrazole); 152.9 (C_4, Ar); 152.6 (C_4, Ar); 133.4 (CH, Ar); 133.4 (CH, Ar); 129.4 (CH, Ar); 129.2 (CH, Ar); 129.1 (CH, Ar); 127.5 (CH, Ar); 127.4 (CH, Ar); 56.3 (CH_2); 54,9 (CH_2); 54.2 (CH_2); 53.3 (CH_2); 51.1 (CH_2); 51.1 (CH_2); 50.1 (CH_2); 50.0 (CH_2); 49.9 (CH_2); 46.6 (CH_2).

1,4-Bis(1-benzyltetrazol-5-yl)methyl-1,4,7-triazacyclonane. Sodium hydroxide pellets (1.4 g, 29 equiv) is added to a stirred solution of **8** (500 mg, 0.99 mol, 1 equiv) in 10 mL of ethanol. After 24 h, 10 mL of water is added and the aqueous phase is extracted with chloroform (3 × 15 mL), dried over Na_2SO_4, filtered, and concentrated. 1,4-Bis(1-benzyltetrazol-5-yl)methyl-1,4,7-triazacyclonane is obtained as a yellowish oil (440 mg, 94%) of sufficient purity to be used in the next step as is. ^{1}H NMR ($CDCl_3$, 300 K, 200 MHz) δ: 7.23 (m, 6H); 7.08 (m, 4H); 5.54 (s, 4H); 3.76 (s, 4H); 2.56 (m, 4); 2.50 (m, 8H). ^{13}C NMR ($CDCl_3$,

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300 K, 50 MHz) δ: 152.9 (C_4, tetrazole); 133.6 (C_4, Ar); 129.2 (CH, Ar); 128.9 (CH, Ar); 127.5 (CH, Ar); 53.2 (CH_2); 53.1 (CH_2); 51.0 (CH_2); 49.5 (CH_2); 47.3 (CH_2).

1,4-(Bis(tetrazol-5-yl)methyl)-1,4,7-triazacyclonane (9). In our hands, optimization of the hydrogenation reaction was possible only by use of LCMS. It is particularly important to advance the reaction as much as possible in no more than 48 h and by using a new commercial batch of Pd/C to avoid the presence of residual, partially deprotected polyamines that are difficult to separate.

To a solution of 1,4-bis(1-benzyltetrazol-5-yl)methyl-1,4,7-triazacyclonane (0.4 g, 0.84 mmol) in ethanol (85 mL) is added 1.43 g of 5% Pd/C (Degussa quality). The flask is sealed with a septum, purged with hydrogen, and positive hydrogen pressure is maintained with a balloon regularly refilled with hydrogen. The mixture is stirred at room temperature for 48 h before being filtered over Celite, washed with ethanol, and concentrated to give the crude product as a yellow-white oil that solidifies spontaneously. The resulting solid is dissolved in a minimal volume (a few milliliters) of a mixture of water and acetonitrile (9:1) to achieve complete solubilization and is purified via a reversed-phase cartridge (C18) that has been preconditioned with pure water (eluant, water). The fractions containing the product (slightly yellow) are evaporated on a rotatory evaporator, and **9** is obtained as a white resin (195 mg, 0.66 mmol 78%) that slowly crystallizes. ^{1}H NMR (D_2O, 300 K, 200 MHz) δ: 3.95 (s, 4H); 2.95 (m, 4H); 2.80 (m, 4H); 2.47 (s, 4H). ^{13}C NMR (D_2O, 300 K, 50 MHz) δ: 159.9 (C_4, tetrazole); 49.4 (CH_2); 48.5 (CH_2); 46.2 (CH_2); 43.6 (CH_2). The purity of ligand **9** has been determined to be in excess of 95%; refer to the Supporting Information for the HPLC results.

Iron(II) 1,4-Bis(tetrazol-5-yl)methyl-1,4,7-triazacyclonane (1, (Fe 9)·H_2O). A solution of ligand **9** (215 mg, 0.733 mmol) in water at approximately pH 7 was degassed properly and treated with solid $Fe(BF_4)_2 \cdot 6H_2O$ (248 mg, 0.733 mmol) under inert atmosphere (Ar). The color of the solution immediately turns to pale pink/purple, and the mixture was stirred for 4−5 h. Then the volume was reduced to ∼2 mL, which caused a partial precipitation of the target complex **1**. Then an amount of 5 mL of degassed acetonitrile was added to help in the purification of **1**. The mixture was stirred for an additional $^1/_2$ h. The volume was reduced to 2 mL, the pale pink solid filtered under Ar, and the filtrate discarded. The microcrystalline precipitate was washed twice with degassed methanol and dried under vacuum (yield, 83%). HRMS $[M + H]^+$ calcd, 348.1096; found 348.1094. The purity of complex **1** has been determined to be in excess of 95%; refer to the Supporting Information for HPLC results.

■ ASSOCIATED CONTENT

Supporting Information. NMR spectra of synthetic intermediates and tested complex **1**; HPLC results and mass spectra of **9** and **1**; MRI protocol. This material is available free of charge via the Internet at http://pubs.acs.org.

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■ ABBREVIATIONS USED

MRI, magnetic resonance imaging; TACN, 1,4,7-triazacyclononane; HEPES, (4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid); DOTA, 1,4,7,10-tetraazacyclododecane; TR/TE, repetition time and echo time; NEX, number of excitations; FOV, field of view

■ REFERENCES

(1) Stavila, V.; Allali, M.; Canaple, L.; Stortz, Y.; Franc, C.; Maurin, P.; Beuf, O.; Dufay, O.; Samarut, J.; Janier, M.; Hasserodt, J. Significant relaxivity gap between a low-spin and a high-spin iron(II) complex of structural similarity: an attractive off/on system for the potential design of responsive MRI probes. *New J. Chem.* **2008**, *32*, 428–435.

(2) Stavila, V.; Stortz, Y.; Franc, C.; Pitrat, D.; Maurin, P.; Hasserodt, J. Effective repression of the fragmentation of a hexadentate ligand bearing an auto-immolable pendant arm by iron coordination. *Eur. J. Inorg. Chem.* **2008**, 3943–3947.

(3) Marchetti, A.; Parker, M. S.; Moccia, L. P.; Lin, E. O.; Arrieta, A. L.; Ribalet, F.; Murphy, M. E. P.; Maldonado, M. T.; Armbrust, E. V. Ferritin is used for iron storage in bloom-forming marine pennate diatoms. *Nature* **2009**, *457*, 467–470.

(4) Cacheris, W. P.; Quay, S. C.; Rocklage, S. M. The relationship between thermodynamics and the toxicity of gadolinium complexes. *Magn. Reson. Imaging* **1990**, *8*, 467–481.

(5) Port, M.; Idee, J. M.; Medina, C.; Robic, C.; Sabatou, M.; Corot, C. Efficiency, thermodynamic and kinetic stability of marketed gadolinium chelates and their possible clinical consequences: a critical review. *BioMetals* **2008**, *21*, 469–490.

(6) Toftlund, H. Spin equilibria in iron(II) complexes. *Coord. Chem. Rev.* **1989**, *94*, 67–108.

(7) Schwert, D. D.; Richardson, N.; Ji, G. J.; Raduchel, B.; Ebert, W.; Heffner, P. E.; Keck, R.; Davies, J. A. Synthesis of two 3,5-disubstituted sulfonamide catechol ligands and evaluation of their iron(III) complexes for use as MRI contrast agents. *J. Med. Chem.* **2005**, *48*, 7482–7485.

(8) Spiccia, L.; Fallon, G. D.; Grannas, M. J.; Nichols, P. J.; Tiekink, E. R. T. Synthesis and characterisation of mononuclear and binuclear iron(II) complexes of pentadentate and bis(pentadentate) ligands derived from 1,4,7-triazacyclononane. *Inorg. Chim. Acta* **1998**, *279*, 192–199.

(9) Gasser, G.; Tjioe, L.; Graham, B.; Belousoff, M. J.; Juran, S.; Walther, M.; Kunstler, J. U.; Bergmann, R.; Stephan, H.; Spiccia, L. Synthesis, copper(II) complexation, Cu-64-labeling, and bioconjugation of a new bis(2-pyridylmethyl) derivative of 1,4,7-triazacyclononane. *Bioconjugate Chem.* **2008**, *19*, 719–730.

(10) Wieghardt, K.; Schoffmann, E.; Nuber, B.; Weiss, J. Syntheses, properties, and electrochemistry of transition-metal complexes of the macrocycle 1,4,7-tris(2-pyridylmethyl)-1,4,7-triazacyclononane (L). Crystal structures of $[NiL](ClO_4)_2$, $[MnL](ClO_4)_2$, and $[PdL](PF_6)_2$ containing a distorted-square-base-pyramidal $Pd^{II}N_5$ core. *Inorg. Chem.* **1986**, *25*, 4877–4883.

(11) Christiansen, L.; Hendrickson, D. N.; Toftlund, H.; Wilson, S. R.; Xie, C. L. Synthesis and structure of metal-complexes of triaza macrocycles with 3 pendant pyridylmethyl arms. *Inorg. Chem.* **1986**, *25*, 2813–2818.

(12) Martell, A. E.; Hancock, R. D. *Metal Complexes in Aqueous Solutions*; Plenum Press: New York, 1996.

(13) Aime, S.; Crich, S. G.; Gianolio, E.; Giovenzana, G. B.; Tei, L.; Terreno, E. High sensitivity lanthanide(III) based probes for MR-medical imaging. *Coord. Chem. Rev.* **2006**, *250*, 1562–1579.

(14) Hermann, P.; Kotek, J.; Kubicek, V.; Lukes, I. Gadolinium(III) complexes as MRI contrast agents: ligand design and properties of the complexes. *Dalton Trans.* **2008**, 3027–3047.

(15) Touti, F.; Maurin, P.; Hasserodt, J. A tetrakis(tetrazolyl) analogue of EDTA. *Eur. J. Org. Chem.* **2009**, 1495–1498.

(16) Touti, F.; Avenier, F.; Lefebvre, Q.; Maurin, P.; Hasserodt, J. A synthon for the convenient and efficient introduction of tetrazolylmethyl

groups into nucleophile-bearing compounds. *Eur. J. Org. Chem.* **2010**, 1928–1933.

(17) Giraud, M.; Andreiadis, E. S.; Fisyuk, A. S.; Demadrille, R.; Pecaut, J.; Imbert, D.; Mazzanti, M. Efficient sensitization of lanthanide luminescence by tetrazole-based polydentate ligands. *Inorg. Chem.* **2008**, *47*, 3952–3954.

(18) Facchetti, A.; Abbotto, A.; Beverina, L.; Bradamante, S.; Mariani, P.; Stern, C. L.; Marks, T. J.; Vacca, A.; Pagani, G. A. Novel coordinating motifs for lanthanide(III) ions based on 5-(2-pyridyl)tetrazole and 5-(2-pyridyl-1-oxide)tetrazole. Potential new contrast agents. *Chem. Commun.* **2004**, 1770–1771.

(19) Aime, S.; Cravotto, G.; Crich, S. G.; Giovenzana, G. B.; Ferrari, M.; Palmisano, G.; Sisti, M. Synthesis of the Gd(III) complex with a tetrazole-armed macrocyclic ligand as a potential MRI contrast agent. *Tetrahedron Lett.* **2002**, *43*, 783–786.

(20) Blake, A. J.; Danks, J. P.; Li, W. S.; Lippolis, V.; Schroder, M. Synthesis and characterisation of pendant-arm amino derivatives of 1,4,7-triazacyclononane and alkyl-bridged bis(1,4,7-triazacyclononane) macrocycles and complexation to Cu(II). *J. Chem. Soc., Dalton Trans.* **2000**, 3034–3040.

(21) Sajiki, H.; Hirota, K. A novel type of Pd/C-catalyzed hydrogenation using a catalyst poison: chemoselective inhibition of the hydrogenolysis for *O*-benzyl protective group by the addition of a nitrogen-containing base. *Tetrahedron* **1998**, *54*, 13981–13996.

(22) Shikhova, I. A.; Sinitsyna, T. A.; Latosh, N. I. Aminoalkyltetrazoles. 7. Synthesis and properties of tri[5-tetrazolyl(1*H*)alkylene]-amines. *Zh. Obshch. Khim.* **1990**, *60*, 2135–2140.

(23) Trifonov, R. E.; Ostrovskii, V. A. Protolytic equilibria in tetrazoles. *Russ. J. Org. Chem.* **2006**, *42*, 1585–1605.

(24) Lauffer, R. B. Paramagnetic metal-complexes as water proton relaxation agents for NMR imaging: theory and design. *Chem. Rev.* **1987**, *87*, 901–927.

(25) Caravan, P.; Ellison, J. J.; McMurry, T. J.; Lauffer, R. B. Gadolinium(III) chelates as MRI contrast agents: structure, dynamics, and applications. *Chem. Rev.* **1999**, *99*, 2293–2352.

(26) Frausto Da Silva, J. J. R.; Williams, R. J. P. *The Biological Chemistry of the Elements*, 2nd ed.; Oxford University Press: New York, 2001; Chapter 9.