Inorg. Chem. **2006**, *45*, 5092–5102

Auto-Assembling of Ditopic Macrocyclic Lanthanide Chelates with Transition-Metal Ions. Rigid Multimetallic High Relaxivity Contrast Agents for Magnetic Resonance Imaging

Jérôme Paris, Cristiana Gameiro,[§] Valérie Humblet, Prasanta K. Mohapatra,[†] Vincent Jacques, and Jean F. Desreux*

Coordination and Radiochemistry, University of Liège, Sart Tilman B16, B-4000 Liège, Belgium

Received February 22, 2006

PhenHDO3A is a ditopic ligand featuring a tetraazacyclododecane unit substituted by three acetate arms and one 6-hydroxy-5,6-dihydro-1,10-phenanthroline group (PhenHDO3A = *rel*-10-[(5R,6R)-5,6-dihydro-6-hydroxy-1,10-phenantholin-5-yl)-1,4,7,10-tetraazacyclododecane-1,4,7-triacetic acid). This ligand was specially designed so as to obtain highly stable heteropolymetallic assemblies. PhenHDO3A has been prepared starting from phenanthroline epoxide and either a triprotected tetraazacyclododecane or *tert*-butyl triester of *N*,*N*′,*N*″-tetraazacyclododecane-triacetic acid. The latter yields PhenHDO3A in a single step. PhenHDO3A forms kinetically stable lanthanide complexes (acid-catalyzed kinetic constant $k_H = (1.2 \pm 0.2) \times 10^{-3}$ s^{-1} M^{-1}) whose solution structure has been deduced from a quantitative analysis of the paramagnetic shifts and the longitudinal relaxation times of the proton nuclei of YbPhenHDO3A. The alcohol group of the dihydro-phenanthroline unit remains coordinated to the encapsulated metal ion despite the steric crowding brought about by this group. Furthermore, the complexes are monohydrated, as shown by luminescence lifetime measurements on EuPhenHDO3A solutions. Relaxivity titrations at 20 MHz clearly indicate that the phenanthroline unit of GdPhenHDO3A is available for the spontaneous formation of highly stable tris complexes with the Fe^{2+} and Ni^{2+} ions. The water-exchange times and the rotational correlation times of GdPhenHDO3A and $Fe(GdPhenHDO3A)_3^{2+}$ have been deduced from variable temperature ^{17}O NMR studies and from nuclear relaxation dispersion curves. Despite rather slow water-exchange rates ($\tau_m^0 = 1.0–1.2 \times 10^{-6}$ s), relaxivity gains of 90% have been observed upon the formation of the heterometallic tris complexes. The latter rotate about four times more slowly ($\tau_r^0 = 398$ ps) than the monomeric unit ($\tau_r^0 = 105$ ps) and their relaxivity is, accordingly, twice as high. The relaxivity of the tris complexes between 10 and 50 MHz is comparable to relaxivities reported for Gd^{3+}-containing dendrimers of much higher molecular weights. The high relaxivity of the tris-PhenHDO3A lanthanide complexes is attributed to their internal rigidity.

Introduction

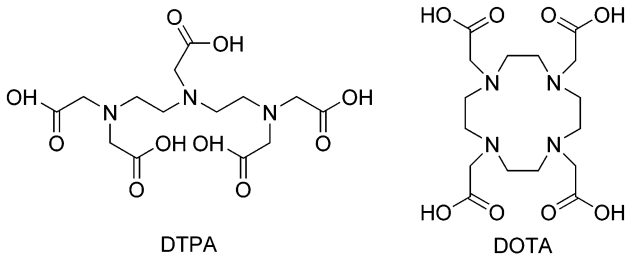
Magnetic resonance imaging (MRI) with gadolinium-containing contrast agents has rapidly evolved into an important clinical modality that is now used every day in hospitals.[1,2] The contrast of MRI images is improved by the injection of Gd(III) complexes, which are able to drastically increase the longitudinal relaxation rate $1/T_1$ of water protons in the extracellular space. The first generation of contrast agents consisted of nonspecific contrast agents that are carried by blood circulation out to tumors. New paramagnetic lanthanide chelates are constantly being synthesized in hopes of reaching the largest possible reduction of the longitudinal proton relaxation time T_1 in vivo and thus the best contrast in magnetic resonance images.[1,3,4] Contrast agents must fulfill strict requirements concerning toxicity, biodistribution, and relaxivity (relaxation rate $1/T_1$ in s^{-1} and per mmol of Gd^{3+}). The toxicity of the Gd^{3+} ion itself has been drastically

* To whom correspondence should be addressed. E-mail: jf.desreux@ulg.ac.be.
[§] On leave from the Departamento de Quimica Fundamental, Universidade Federal de Pernambuco, Recife, PE, Brazil.
[†] On leave from the Bhabha Atomic Research Center, Trombay, India.

(1) Merbach, A. E., Toth, E., Eds. *The Chemistry of Contrast Agents in Medical Magnetic Resonance Imaging*; Wiley: Chichester, U.K., 2001.
(2) Caravan, P.; Ellison, J. J.; McMurry, T. J.; Lauffer, R. B. *Chem. Rev.* **1999**, *99*, 2293−2352.
(3) Jacques, V.; Desreux, J. F. *Top. Curr. Chem.* **2002**, *221*, 123−164.
(4) Aime, S.; Cabella, C.; Colombatto, S.; Geninatti Crich, S.; Gianolio, E.; Maggioni, F. *J. Magn. Reson. Imaging* **2002**, *16*, 394−406.

10.1021/ic0603050 CCC: $33.50
Published on Web 06/03/2006

Scheme 1



reduced by encapsulating this ion into polyamino polycarboxylic cages, most of which are derived from DTPA (diethylenetriaminepentaacetic acid) or DOTA (1,4,7,10-tetraazacyclododecane-*N*,*N*′,*N*″,*N*‴-tetraacetic acid) skeletons (see Scheme 1).

The DOTA structure ensures that a kinetically inert complex is formed because its macrocyclic structure hampers the protonation of the coordinated nitrogen atoms.[5] These small chelates are rapidly tumbling in water, and their relaxivity essentially depends on their short rotational correlation time. A sizable increase in relaxivity is achieved if Gd^{3+} chelates are covalently or noncovalently linked to macromolecules, because the rotational correlation time becomes comparable to or larger than the electronic and water-exchange correlation times. The latter are then the major factors that govern the relaxivity of these slowly rotating species.[6] Dendrimeric polymers featuring a large number of metal chelates have thus been synthesized; however, the achieved relaxivity gains, although large, are not as high as expected, presumably because the movements of the Gd^{3+} chelates are partially independent from those of the macromolecular units.[7] Rigidity is a factor of utmost importance for reaching very high relaxivities. It is with this requirement in mind that we decided to synthesize very compact multimetallic structures that are formed by the self-association process illustrated in Scheme 2 (where Mn^{n+} is a transition-metal ion).

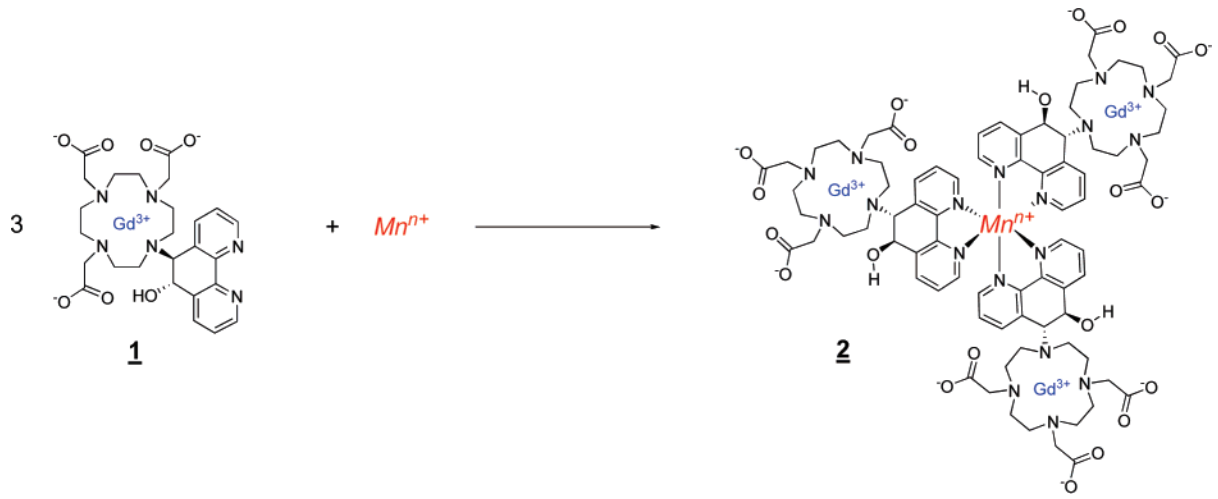
The PhenHDO3A ligand, **1**, features two tightly connected complexing units: first, a DOTA-like unit that is specifically designed to form kinetically inert lanthanide complexes, as already reported for triacetic monoalcoholic ligands such as HP-DO3A,[8] and second, a 5,6-dihydro-phenanthroline group that is well-known to form very stable complexes with Fe^{2+} or Ni^{2+} (PhenHDO3A = *rel*-10-[(5R,6R)-5,6-dihydro-6-hydroxy-1,10-phenantholin-5-yl)-1,4,7,10-tetraazacyclododecane-1,4,7-triacetic acid, HPDO3A = 10-(2-hydroxypropyl)-1,4,7,10-tetraazacyclododecane-1,4,7-triacetic acid, ProHance). It is also quite possible that the encapsulation of a metal ion in a complexing subunit could lead to a cooperative rigidification in another coordinating entity. In addition to being able to form tightly packed supramolecular structures that are interesting in their own right, PhenHDO3A is also an appealing addition to the list of MRI contrast agents that are responsive to a given stimulus, whether the oxygen content,[9] pH,[10,11] a biological molecule,[2,3] or biologically relevant ions such as Ca(II).[12] We already published a preliminary account on PhenHDO3A and showed that the approach illustrated in Scheme 2 indeed leads to a sizable increase in relaxivity.[13] This account was the source of inspiration of recent reports by Livramento et al.,[14,15] who investigated a similar system featuring phenanthroline and a DTPA analogue. A full description of the syntheses[16] and some complexing properties of PhenHDO3A is presented here. NMR spectroscopy and the dispersion of the nuclear magnetic relaxation are used to unravel the behavior of PhenHDO3A, **1**, and its complexes.

Experimental Section

General. All commercial reagents were used as received unless otherwise noted. All solvents were dried using common techniques. ^1H and ^{13}C NMR spectra were recorded on a Bruker DRX 400 spectrometer, and chemical shifts are reported in parts per million relative to tetramethylsilane. The longitudinal relaxation times T_1 were measured by the inversion recovery pulse sequence. Two-dimensional NMR spectra of paramagnetic samples were recorded as reported elsewhere.[17,18] Water ^{17}O transverse relaxation times of GdPhenHDO3A samples (4.5 mM) enriched with $H_2{}^{17}O$ (1.3%) were determined on a Bruker AM250 spectrometer using the standard Carr−Purcell−Meiboom−Gill spin−echo pulse sequence. Nuclear magnetic relaxation dispersion data were collected on a Stelar Spinmaster FFC2000 relaxometer (Stelar, Mede, PV, Italy) to which a Bruker 2 T permanent magnet had been added. The Stelar electronic equipment and the Stelar software were used to record T_1 values with both the pulsed and permanent magnets. Time-resolved luminescence spectra were obtained in water and in D_2O with a Photon Technology International (Birmingham, NJ) spectrometer equipped with a N_2 laser. Quantitative interpretations of the ^{17}O, NMRD, and luminescence data were performed with the Scientist[19] and Mathcad[20] software programs and with internally produced computer programs. Melting or decomposition points were

(5) Toth, E.; Brücher, E.; Lazar, I.; Toth, I. *Inorg. Chem.* **1994**, *33*, 4070−4076.
(6) Aime, S.; Chiaussa, M.; Digilio, G.; Gianolo, E.; Terreno, E. *J. Biol. Inorg. Chem.* **1999**, *4*, 766−774.
(7) Nicolle, G. M.; Toth, E.; Schmitt-Willich, H.; Raduchel, B. E.; Merbach, A. E. *Chem.—Eur. J.* **2002**, *8*, 1040−1048.
(8) Kumar, K.; Jin, T. Z.; Wang, X. Y.; Desreux, J. F.; Tweedle, M. F. *Inorg. Chem.* **1994**, *33*, 3823−3829.
(9) Aime, S.; Ascenzi, P.; Comoglio, E.; Fasano, M.; Paoletti, S. *J. Am. Chem. Soc.* **1995**, *117*, 9365−9366.
(10) Aime, S.; Botta, M.; Fasano, M.; Terreno, E. *Acc. Chem. Res.* **1999**, *32*, 941−949.
(11) Zhang, S.; Wu, K.; Sherry, A. D. *Angew. Chem., Int. Ed.* **1999**, *38*, 3192−3194.
(12) Li, W.; Parigi, G.; Fragai, M.; Luchinat, C.; Meade, T. J. *Inorg. Chem.* **2002**, *41*, 4018−4024.
(13) Comblin, V.; Gilsoul, D.; Hermann, M.; Humblet, V.; Jacques, V.; Mesbahi, M.; Sauvage, C.; Desreux, J. F. *Coord. Chem. Rev.* **1999**, *185−186*, 451−470.
(14) Livramento, J. B.; Toth, E.; Sour, A.; Borel, A.; Merbach, A. E.; Ruloff, R. *Angew. Chem., Int. Ed.* **2005**, *44*, 1480−1484.
(15) (a) Livramento, J. B.; Sour, A.; Borel, A.; Merbach, A. E.; Toth, E. *Chem.—Eur. J.* **2006**, *12*, 989−1003, (b) Livramento, J. B.; Weidensteiner, C.; Prata, M. I. M.; Allegrini, P. R.; Geraldes, C. F. G. C.; Helm, L.; Kneuer, R.; Merbach, A. E.; Santos, A. C.; Schmidt, P.; Toth, E. *Contrast Media Mol. Imaging* **2006**, *1*, 30−39.
(16) Desreux, J. F.; Jacques, V.; Humblet, V.; Hermann, M.; Comblin, V.; Tweedle, M. F. U.S. Patent 6 056 939, 2000.
(17) Ranganathan, R. S.; Raju, N.; Fan, H.; Zhang, X.; Tweedle, M. F.; Desreux, J. F.; Jacques, V. *Inorg. Chem.* **2002**, *41*, 6856−6866.
(18) Jacques, V.; Desreux, J. F. *Inorg. Chem.* **1994**, *33*, 4048−4053.
(19) *Scientist 3.0*; Micromath: St. Louis, MO; http://www.micromath.com/scientist.htm.
(20) *Mathcad*, version 13; Mathsoft: Cambridge, MA; http://www.mathsoft.com/.

Scheme 2



measured on an Electrothermal IA 9100 apparatus. Electrospray mass spectra were obtained on a Fisons VG platform and on Bruker Daltonics micrOTOF spectrometers. Ytterbium(III) trifluoromethanesulfonate (99.99%) was purchased from Aldrich. The radiochemical tracer ^{153}Gd was obtained from Perkin−Elmer Life and Analytical Sciences as a chloride salt in dilute HCl. The γ activities were measured with a Canberra Packard γ detector equipped with a Ge−Li detector. All pH measurements were performed with a Schott cg840 pH meter and a Schott N5900A electrode.

1a,9b-Dihydro-oxireno[f][1,10]phenanthroline, 3. Na_2HPO_4 (800 g, 5.6 mol) was dissolved in water (1.2 L) under vigorous stirring and gentle heating. This solution was poured into 4.5 L of commercial bleach, and the pH was carefully adjusted to 8.6 with 6 M HCl. This mixture was added to a solution of 1,10-phenanthroline (40 g, 0.22 mol) in CH_2Cl_2 (2 L). Tetrabutylammonium hydrogensulfate (12 g, 35.3 mmol) was finally added, and the mixture was stirred for 18 h at room temperature while the pH of the aqueous phase was strictly maintained at 8.6 with 3 M NaOH. The organic phase was separated, washed with 10% aq $Na_2S_2O_3$ (1.6 L) and then water (3 × 1 L), and finally dried over $MgSO_4$. After evaporation under reduced pressure, the obtained brown solid was suspended in acetone, filtered, and dried in vacuo to give 35.5 g (0.181 mol, 82% yield) of a light beige solid (mp: 162−163 °C dec (lit.[21] 163−165 °C)). ESI−TOF−MS: calcd for $C_{12}H_9N_2O$ ($M + H^+$) 197.0709, found 197.0710**.** ^{1}H NMR (DMSO-d_6): δ 8.79 (dd, 2H, J = 1.4 Hz, 4.4 Hz), 8.24 (dd, 2H, J = 1.4 Hz, 7.6 Hz), 7.52 (dd, 2H, J = 7.6 Hz, 4.4 Hz,), 4.82 (s, 2H). ^{13}C NMR (DMSO-d_6): δ 150, 148.8, 138.4, 129.6, 123.8, 54.5.

6-(1,4,7,10-Tetraazacyclodec-1-yl)-5,6-trans-dihydro-1,10-phenanthroline-5-ol, 6. Anhydrous $LiClO_4$ (4.99 g, 46.9 mmol in 9 mL of dried CH_3CN) was added to a solution of 1a,9b-dihydro-oxireno[f][1,10]phenanthroline, **3** (4.6 g, 23.46 mmol), in dried CH_3CN (80 mL). A solution of octahydro-5H,9bH-2a,4a,7,9a-tetraazacycloocta[cd]pentalene, **4**[22] (4.7 g, 25.7 mmol), in CH_3CN (35 mL) was added, and the reaction mixture was refluxed under stirring for 32 h under nitrogen. The solvent was removed under vacuum to yield a brown oil. This crude material was dissolved in a solution of HCl in methanol (40 mL of fuming HCl in 400 mL of methanol). The mixture was stirred at reflux for 24 h. After being cooled to room temperature, the reaction vessel was kept in an ice−water bath until a white solid precipitated. The precipitate was collected by filtration, and the filtrate was concentrated until a second crop of precipitate was formed. These fractions were combined and dried in vacuo before being recrystallized in boiling water. Polyamine **6** (5.80 g, 12.1 mmol, 51% yield) was obtained in tris-hydrochloride form after drying (mp: 258−259 °C). ESI−TOF−MS of the neutralized amine: calcd for $C_{20}H_{29}N_6O$ ($M + H^+$) 369.2397, found 369.2392. ^{1}H NMR (D_2O): δ 8.75 (m, 2H), 8.48 (d, 1H, J = 8 Hz), 8.32 (d, 1H, J = 8 Hz), 7.92 (m, 1H), 7.84 (m, 1H), 5.24 (d, 1H, J = 11.6 Hz), 4.7 (d, 1H, J = 11.6 Hz), 3.05−3.58 (m, 16H). ^{13}C NMR (D_2O): δ 148.4, 147.5, 146.9, 144.8, 142.5, 142.3, 139.6, 136.3, 130.3, 129.9, 69.9, 64.6, 49.4, 47.4, 45.9, 44.1.

***rel*-10-[(5R,6R)-5,6-dihydro-6-hydroxy-1,10-phenanthrolin-5-yl)-1,4,7,10-tetraazacyclododecane-1,4,7-triacetic acid, PhenHDO3A, 7. Synthesis from Polyamine 6.** Anhydrous K_2CO_3 (4.98 g, 36 mmol) was added to a suspension of compound **6** (3.82 g, 8 mmol) in dry CH_3CN (80 mL). The mixture was refluxed overnight under nitrogen. The hot suspension was filtered, and the filtrate was evaporated to yield amine **6** in neutral form as a yellow solid (2.98 g). This compound was dissolved in methanol (75 mL); a solution of potassium bromoacetate (26.4 mmol; 3.8 g of bromoacetic acid neutralized with 1.48 g of KOH) in methanol (60 mL) was added, along with 3.65 g of anhydrous K_2CO_3 (26.4 mmol). The mixture was heated at 45 °C overnight. After a second addition of K_2CO_3 (3.65 g, 26.4 mmol), the temperature was raised to 65 °C. After 6 h, the inorganic salts were filtered off and washed with 10 mL of methanol. The filtrate was acidified to pH 2.5−3 with 6 M HCl and was left standing until a precipitate appeared. This precipitate was collected by filtration and recrystallized in water to give 2.21 g of product. The resulting filtrate was evaporated under reduced pressure, and the residue was suspended in 30 mL of boiling methanol. The inorganic salts were filtered off, and the filtrate was brought to dryness in vacuo to give 2.08 g of product. The two fractions were combined, dissolved in 5 mL of water, and loaded onto a Dowex 50 × 2−200 (H^+ form) cation-exchange resin (70 mL of wet resin). The column was washed with water (350 mL), and the product was recovered by elution with 0.5 M aqueous NH_3 (400 mL). The eluate was evaporated under reduced pressure, and the remaining light yellow solid (3.02 g) was dissolved in decarbonated water (5 mL). The pH was brought to 10 with aqueous NH_3, and the solution was transferred onto a Dowex 1 × 2−200 (OH^- form) anion-exchange resin (65 mL of wet resin). The column was washed with decarbonated water (350 mL), and the product was eluted with aqueous formic acid (0.0075 M, 1 L). The eluate was evaporated to dryness under reduced pressure. Repetitive

(21) Krishnan, S.; Kuhn, D. G.; Hamilton, G. A. *J. Am. Chem. Soc.* **1977**, *99*, 8121−8123.
(22) Atkins, T. J. *J. Am. Chem. Soc.* **1980**, *102*, 6364−6365.

evaporations after the addition of water were performed in order to remove formic acid completely. Yield: 2.76 g of PhenHDO3A (5.08 mmol, 63% yield) (mp: 193−195 °C dec). ESI−TOF−MS: calcd for $C_{26}H_{35}N_6O_7$ ($M + H^+$) 543.2562, found 543.2568. MS (ES+): *m*/*z* 543.1 ($M + H^+$); 272.0 ($[(MH_2)/2]^+$). ^{1}H NMR (D_2O): δ 8.80 (d, 1H, $J = 4.8$ Hz), 8.75 (d, 1H, $J = 4.8$ Hz), 8.57 (broad s, 1H), 8.15 (d, 1H, $J = 7.6$ Hz), 7.75−7.6 (m, 2H), 5.41 (s, 1H), 4.97 (s, 1H), 2.2−4.2 (m, 22H). ^{13}C NMR (D_2O, 275 K): δ 177.9, 171.7, 170.2, 149.1, 148.6, 147.6, 145.6, 145.4, 144.5, 137.1, 134.7, 131.6, 130.8, 64.0, 60.8, 58.0, 56.7, 55.7, 55.3, 54.3, 54.0, 52.5, 51.6, 51.2, 48.2, 45.8.

Synthesis from Epoxide 3 and Tris(*tert*-butyl)-1,4,7,10-tetraazacyclododecane-1,4,7-triacetate, 8. Tris-*tert*-butyl-1,4,7,10-tetraazacyclododecane-1,4,7-tris-acetate hydrobromide was prepared according to a published procedure.[23] The neutral form of this ester was obtained by percolation of a THF solution of the hydrobromide salt through a column of A-21 resin (ACROS), followed by evaporation under reduced pressure. A solution of 1a,9b-dihydrooxireno[f][1,10]phenanthroline, **3** (0.55 g, 2.8 mmol), and tris(*tert*-butyl)-1,4,7,10-tetraazacyclododecane-1,4,7-triacetate, **8** (2 g, 3.88 mmol), in CH_3CN (30 mL) was added dropwise to a stirred solution of Yb(III) trifluoromethanesulfonate (0.2 g, 0.32 mmol) in CH_3CN (25 mL). The mixture was stirred for 7 days at 65 °C under nitrogen. The solvent was evaporated in vacuo, and the remaining oil was dissolved in 250 mL of ethyl acetate. This solution was washed with water (4 × 100 mL). Drying of the organic layer over $MgSO_4$ and removal of the solvent in vacuo gave 2.7 g of a light brown oil containing a mixture of ester **8** and compound **9** (MS (ES+): *m*/*z* 711.4 ($M + H^+$); 356.1 ($M + 2H$)/2^+). This oil was dissolved in 20 mL of trifluoroacetic acid. The solution was stirred at room temperature for 48 h and then brought to dryness to obtain 3 g of a light brown oil. This oil was dissolved in water (5 mL, pH ∼2) and loaded onto a Dowex 50 × 2−200 (H^+ form) cation-exchange resin (4 cm × 8 cm). The column was washed with water (1.3 L), and the sought product was recovered by elution with 0.25 M aqueous NH_3 (500 mL). The eluate was evaporated under reduced pressure, and the light brown solid residue was dissolved in decarbonated water (5 mL). The pH was brought to 10 with aqueous NH_3, and the solution was transferred onto a Dowex 1 × 2−200 (formiate form) anion-exchange resin (4 cm × 8 cm). The column was washed with decarbonated water (1.5 L) and the sought product was eluted with aqueous formic acid (0.002 M, 2.5 L). The fractions containing the desired product were combined and evaporated to dryness under reduced pressure. Repetitive evaporations after the addition of water were done in order to remove formic acid. The remaining colorless, glassy solid was dissolved in methanol (30 mL) and left for 1 day at −20 °C. The resulting white solid was collected by filtration, washed with cold methanol and acetone, and dried in a vacuum to obtain 624 mg of a white powder. (yield: 41% from epoxide **3**).

Preparation of Metal Complexes. Typically, PhenHDO3A, **7** (30 mg, 0.055 mmol), was dissolved in water (7 mL), and a 5% stoichiometric excess of a lanthanide trichloride solution (∼0.1 M) was added dropwise. The solution was stirred at 50 °C while the pH was continuously maintained at pH 5.5 by adding 1 M KOH until no more pH variation was observed. The solution was passed through a column of Chelex 100 resin (Sigma) and eluted with water to remove the metal ions in excess. Evaporation under reduced pressure yielded the complex as a glassy, colorless material. LaPhenHDO3A, ESI−TOF−MS: calcd for $C_{26}H_{32}N_6O_7LaNa$ ($M + Na^+$) 701.1210, found 701.1208. GdPhenHDO3A, **1**, ESI−TOF−MS: calcd for $C_{26}H_{32}N_6O_7Gd$ ($M + H^+$) 698.1568, found 698.1573. Anal. Calcd for $C_{26}H_{31}GdN_6O_7 \cdot 1.4H_2O$ (water and Gd content determined by Karl Fisher titration and inductively coupled plasma analysis, respectively): C, 43.25; H, 4.72; Gd, 21.78; N, 11.64. Found: C, 43.10; H, 4.74; Gd, 21.73; N, 11.60. YbPhenHDO3A, ESI−TOF−MS: calcd for $C_{26}H_{32}N_6O_7Yb$ ($M + H^+$) 714.1716, found 714.1705.

Results and Discussion

Syntheses. Two synthetic pathways to PhenHDO3A, **7**, have been tested. The first synthetic approach is presented in Scheme 3.

Both pathways start with the epoxidation of [1,10]-phenanthroline. The synthesis of epoxide **3** has been reported by several authors with yields ranging from 50 to 98%.[21,24−26] This reaction has systematically been carried out by treating a chloroform solution of [1,10]phenanthroline with an aqueous solution of sodium hypochlorite, to which tetrabutylammonium hydrogensulfate is added as a phase-transfer catalyst. In our hands, this reaction led to high yields, provided that chloroform was replaced by dichloromethane, a large excess of tetrabutylammonium hydrogensulfate was used, and the pH was strictly maintained at 8.6 during the reaction. If these conditions were not met, we obtained mixtures of compounds containing the desired **3** along with *trans*-5-chloro-6-hydroxy-5,6-dihydro-1,10-phenanthroline and unreacted 1,10-phenanthroline in variable proportions. We needed very large quantities of epoxide **4**, and its synthesis on a large scale is reported in the experimental part. Small samples of this compound recently became commercially available.[27] Epoxide **3** was reacted with triprotected tetraaza macrocycle **4**[28] using $LiClO_4$ as catalyst. The resulting condensation product **5** was not isolated but was directly treated with HCl in order to obtain the unprotected tetraazacycle **6**. The ligand PhenHDO3A, **7**, was finally obtained by reacting **6** with an excess of bromoacetic acid and was purified by ion-exchange chromatography.

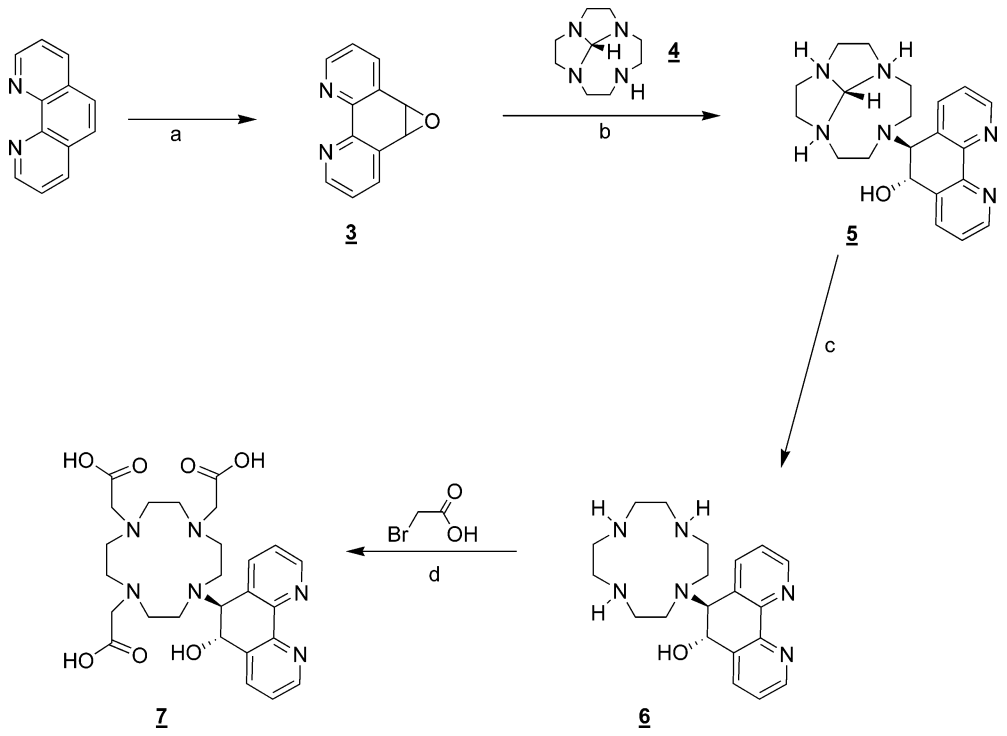
The second approach to the synthesis of PhenHDO3A is depicted in Scheme 4.

In this approach, phenanthroline epoxide **3** was reacted with a small excess of triester **8** to yield PhenHDO3A, **7**, after treatment with trifluoroacetic acid and purification by ion-exchange. The hydrobromide salt of triester **8** is readily obtained from 1,4,7,10-tetraazacyclododecane (cyclen) and 3 equiv of *tert*-butyl bromoacetate in *N*,*N*-dimethylacetamide in the presence of 3 equiv of sodium acetate.[23,29] This compound is also commercially available.[30] The first step in the reaction of **3** with **8** was a neutralization of the hydrobromide salt of **8** with an Amberlyst A-21 resin. This step provides the free amine whose enhanced nucleophilicity

(23) Schultze, L. M.; Bulls, A. R. U.S. Patent 5 631 368, 1997.

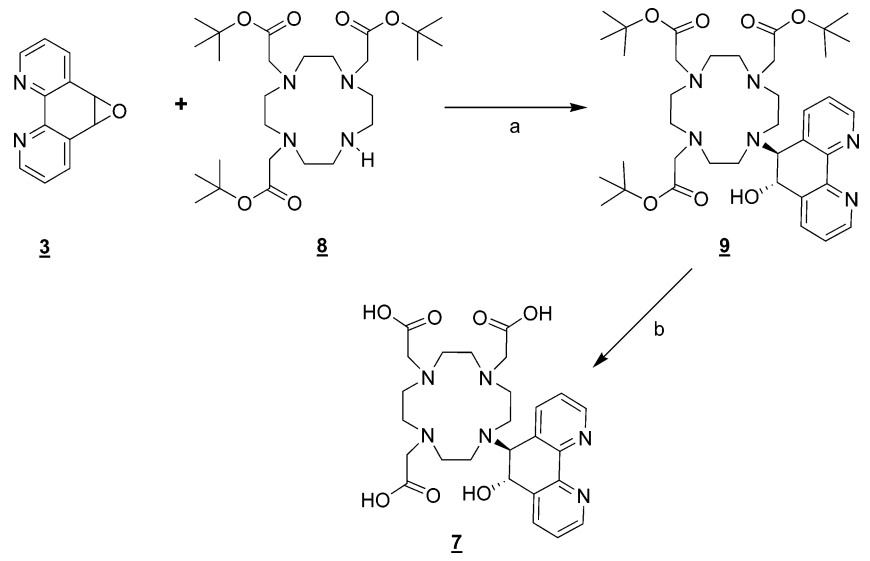
(24) Abu-Shqara, E.; Blum, J. *Heterocycles* **1990**, *27*, 1197−1200.
(25) Moody, C. J.; Rees, C. W.; Thomas, R. *Tetrahedron* **1992**, *48*, 3589−3602.
(26) Shen, Y.; Sullivan, B. P. *Inorg. Chem.* **1995**, *34*, 6235−6236.
(27) Sample available from Aldrich Chemical Co., Milwaukee, WI.
(28) Tweedle, M. F.; Hagan, J. J.; Gaughan, G. T. U.S. Patent EP 292 689, 1998.
(29) Berg, A.; Almen, T.; Klaveness, J.; Rongved, P.; Thomassen, T. U.S. Patent EP 299 795, 1989.
(30) Compound available from Macrocyclics, Dallas, TX.

Scheme 3 [a]



[a] a = NaOCl, Na_2HPO_4, $(n\text{-butyl})_4N^+HSO_4^-$, 82%; b = $LiClO_4$; c = HCl; d = K_2CO_3, 63%.

Scheme 4 [a]



[a] a = $Yb(OTf)_3$; b = TFA, 41% from 3.

is required for the subsequent ring opening of epoxide **3**. A range of activators such as Lewis acids or lithium salts[31−33] was then tested to carry out the aminolysis of **3** by **8**. We first evaluated $LiClO_4$ as a catalyst, because this compound had already been used successfully in the first approach to the synthesis of PhenHDO3A (Scheme 3). Unfortunately, all attempts using this catalyst failed and we then examined the effectiveness of Yb^{3+} trifluoromethanesulfonate in tetrahydrofuran or dichloromethane as proposed by Meguro[34] and Chini.[35] The aminolysis reaction was first carried out in dry tetrahydrofuran under reflux as suggested by Meguro,[34] with a 2:1 ratio of **8** and **3**. After 48 h, the sought product **9** was barely detected by ES−MS with no evolution even after more than a week. Moreover, performing the reaction with a 1:2 ratio of **8** and **3**, i.e., an excess of epoxide in acetonitrile or dimethylformamide, also led to very low yields. These

(31) Schoffers, E.; Tran, S. D.; Mace, K. *Heterocycles* **2003**, *60*, 769−771.
(32) Chini, M.; Crotti, P.; Macchia, F. *Tetrahedron Lett.* **1990**, *31*, 4661−4664.
(33) Chini, M.; Crotti, P.; Macchia, F. *J. Org. Chem.* **1991**, *56*, 5939−5942.
(34) Meguro, M.; Asao, N.; Yamamoto, Y. *J. Chem. Soc., Perkin Trans. 1* **1994**, 2597−2601.
(35) Chini, M.; Crotti, P.; Favero, L.; Macchia, F.; Pineschi, M. *Tetrahedron Lett.* **1994**, *35*, 433−436.

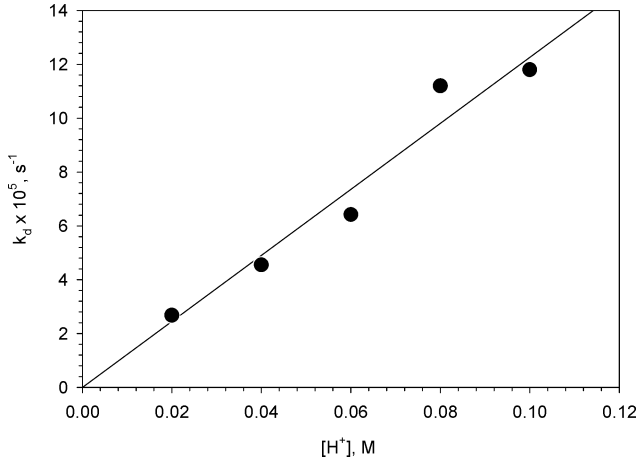


Figure 1. Acid dependence of the first-order rate constant k_d for the dissociation of GdPhenHDO3A.

conditions were selected because **3** and **9** are easily separated from each other by chromatography, whereas the separation of triesters **8** and **9** proved more difficult by chromatography either on alumina or on silica gel under diverse elution conditions. During the tests of various experimental conditions, it appears that acetonitrile was the most-suitable solvent for obtaining **9** along with a small excess of nucleophile **8**. The aminolysis step was thus finally conducted with a 1.4:1 ratio of **8** and **3** in acetonitrile, and the resulting amino-alcohol **9** was used as such in the cleavage of the *tert*-butyl ester groups. Pure PhenHDO3A ligand **7** was isolated in 41% yield from **3** after purification on ion-exchange resins (Dowex 50 × 2−200 and Dowex 1 × 2−200) followed by recrystallization in cold methanol.

Kinetic Stability. The motivation of this study was to design a highly stable gadolinium complex with a ditopic ligand able to spontaneously form a supramolecular entity around a transition-metal ion. This structure had to be extremely compact so that it would rotate as a single entity without any internal rotations that would make it susceptible to a lowered relaxivity. One obvious risk of this approach was that the structure could be too crowded and thus insufficiently stable. The kinetic stability and the solution structure of the lanthanide PhenHDO3A complexes were thus investigated in order to ascertain whether these complexes are indeed stable and conformationally rigid.

The dissociation of GdPhenHDO3A was investigated in acidic media by a method already reported.[36] The dissociation was followed at regular time intervals by measuring the activity of acidic solutions of GdPhenHDO3A (ionic strength 1 M NaCl, 25 °C) prepared from $GdCl_3$ spiked with ^{153}Gd. The Gd^{3+} liberated during the dissociation of the complex was completely taken up by a cationic resin previously conditioned to the same ionic strength and acidity, and the activity of the solutions was measured by γ spectrometry. As shown in Figure 1, the dissociation constant k_d follows the simple law

$$k_d = k_0 + k_H[H^+] \quad (1)$$

for acidities ranging from 0.02 to 0.12 M with $k_H = (1.2 \pm 0.2) \times 10^{-3}$ s^{-1} M^{-1}, and no spontaneous dissociation was observed within the limits of the errors.

The acid-assisted dissociation of GdPhenHDO3A is thus about 100 times faster than that in the case of GdDOTA ($k_H = 8.4 \times 10^{-6}$ M^{-1} s^{-1}).[36] The dissociation is also five times faster than in the case of GdHP-DO3A ($k_H = 2.6 \times 10^{-4}$ M^{-1} s^{-1}), the Gd^{3+} complex of a triacetic monoalcoholic derivative of tetraazacyclododecane[8,37] currently used in hospitals for magnetic resonance imaging under the name ProHance. However, GdPhenHDO3A is still kinetically quite stable, with a $t_{1/2}$ value for the dissociation of 65 days at pH 4. The dissociation probably proceeds by the protonation of one of the carboxylic groups followed by a proton transfer to one of the cyclic nitrogen atoms, as suggested by earlier kinetic studies.[8,37] The stability of GdPhenHDO3A is also borne out by relaxation-rate measurements at different pH levels. The relaxivity is essentially independent of acidity between pH 3 and 11. At lower pH, a slow dissociation is taking place and the relaxivity is increasing toward the value of the free Gd^{3+} ion, as already observed for GdHP-DO3A.[38]

Solution Structure. NMR spectroscopy yields information on the structure and rigidity of the lanthanide complexes, as shown in a number of studies of dia- and paramagnetic species.[17,39−42] Various NMR techniques are applied here to the PhenHDO3A chelates. All the resonances exhibited by an aqueous solution of PhenHDO3A at pD 5.4 are shifted by the addition of diamagnetic La^{3+} (see the Supporting Information, Figure S1). Furthermore, the peaks at 5.36 and 4.90 ppm due to the dihydrophenanthroline (N)C*H*−C*H*(OH) group become much better resolved, and the general appearance of the broad resonances between 4 and 2 ppm assigned to the ethylenic and acetic protons is altered. The spectrum of LaPhenHDO3A is insufficiently resolved to be amenable to a determination of the solution structure from the values of the J coupling constants,[43] but a quantitative structural study can be performed in the presence of paramagnetic ions.

The paramagnetic lanthanide ions induce large NMR shifts δ_i that are essentially of dipolar origin and a direct function of the structure of their chelates in solution, as shown in the eq 2

$$\delta_i = D_{ax}\left\langle \frac{3\cos^2\theta_i - 1}{r_i^3} \right\rangle + D_{rh}\left\langle \frac{\sin^2\theta_i \cos 2\psi_i}{r_i^3} \right\rangle \quad (2)$$

(36) Wang, X. Y.; Jin, T. Z.; Comblin, V.; Lopez-Mut, A.; Merciny, E.; Desreux, J. F. *Inorg. Chem.* **1992**, *31*, 1095−1098.
(37) Toth, E.; Kiraly, R.; Platzek, J.; Raduchel, B. E.; Brücher, E. *Inorg. Chim. Acta* **1996**, *249*, 191−199.
(38) Zhang, X.; Chang, C. A.; Brittain, H. G.; Garrison, J. M.; Telser, J.; Tweedle, M. F. *Inorg. Chem.* **1992**, *31*, 5597−5600.
(39) Desreux, J. F. *Inorg. Chem.* **1980**, *19*, 1319−1324.
(40) Forsberg, J. H.; Delaney, R. M.; Zhao, Q.; Harakas, G.; Chandran, R. *Inorg. Chem.* **1995**, *34*, 3705−3715.
(41) Aime, S.; Barbero, L.; Botta, M.; Ermondi, G. *J. Chem. Soc., Dalton Trans.* **1992**, 225−228.
(42) Zhang, S.; Kovacs, Z.; Burgess, S.; Aime, S.; Terreno, E.; Sherry, A. D. *Chem.—Eur. J.* **2001**, *7*, 288−296.
(43) Jacques, V.; Desreux, J. F. *Inorg. Chem.* **1996**, *35*, 7205−7210.

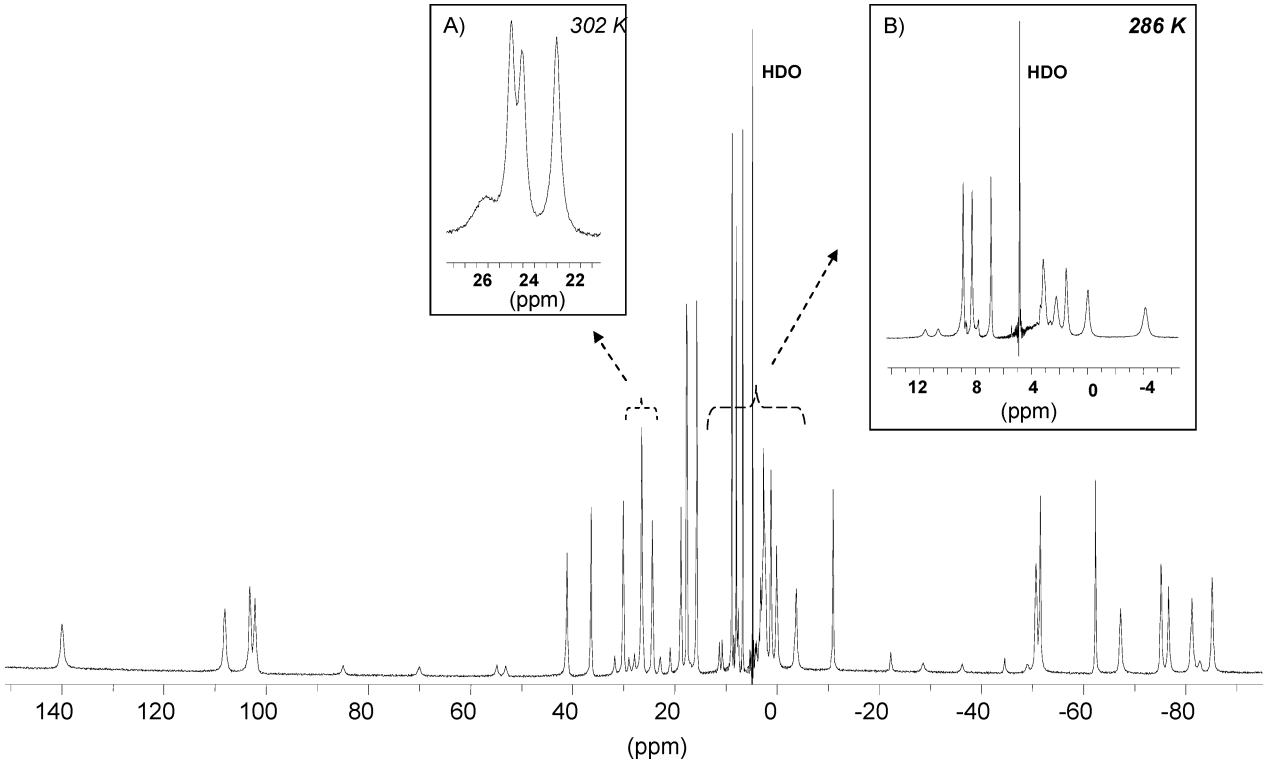


Figure 2. ^{1}H NMR spectrum of YbPhenHDO3A at 279 K, pD 2.4.

where r_i, θ_i, and ψ_i are the polar coordinates of nucleus i relative to the principal axes of the magnetic susceptibility tensor; D_{ax} and D_{rh} are the axial and rhombic anisotropy terms of the susceptibility tensor, respectively, and are identical for all nuclei in a complex. This equation yields reliable quantitative information on the structure of a lanthanide complex, provided the solution species are slowly exchanging rigid structures. If this condition is not met, eq 2 is of little use because rapid exchanges between different labile structures lead to paramagnetic shifts that are no longer directly related to single geometric and anisotropic factors. The complexes derived from DOTA are usually very rigid because of the steric requirements of this macrocycle. Equation 2 has thus been applied successfully to several DOTA derivatives.[17,39,42] However, a contact contribution to paramagnetic shifts δ_i also has to be taken into account, but its magnitude is difficult to assess.[44,45] Quantitative studies are thus easier to perform with lanthanide ions for which the contact contributions to the paramagnetic shifts are essentially negligible in comparison to large dipolar contributions, as in the case of the Yb^{3+} ion. Figure 2 presents the ^{1}H NMR spectrum of YbPhenHDO3A at 279 K. This complex displays 30 NMR peaks of equal area, covering about 240 ppm. Each proton thus gives rise to a separate resonance. In addition, the spectrum features several less-shifted minor peaks.

YbPhenHDO3A is thus present in solution in the form of two rigid, slowly exchanging species, as already observed for YbDOTA and its derivatives.[18,46] In analogy with the latter, it is assumed that the intense much-shifted peaks are due to a square antiprismatic species in which the metal ion forms five-membered rings with the N−CH_2−C(O)−O groups and with the tetraaza N−CH_2−CH_2−N units in a $\Delta(\lambda\lambda\lambda\lambda)$ or $\Lambda(\delta\delta\delta\delta)$ arrangement, whereas the minor peaks arise from a twisted square antiprism species with $\Delta(\delta\delta\delta\delta)$ or $\Lambda(\lambda\lambda\lambda\lambda)$ geometries.[47] YbPhenHDO3A is thus a highly rigid structure despite the bulkiness of the phenanthroline group. The proof that this group is firmly coordinated to the central metal ion through its alcohol function is derived from a quantitative analysis of the relative magnitude of the paramagnetic shifts of the major isomer on the basis of eq 2.

A geometric model of GdPhenHDO3A was generated at the MM2 level using the parameters specifically computed for lanthanide complexes by Hay[48] and Cundari.[49] These parameters allowed these authors to predict the geometries of Gd complexes within 3% of the bond lengths and bond angles determined by crystallography. They have already been successfully used for creating models of macrocyclic lanthanide complexes that were in very good agreement with NMR studies.[17] The minimum-energy structure of GdPhenHDO3A in a square antiprismatic arrangement is reproduced in Figure 3. The Gd−N and Gd−O distances were not restricted during minimization, and the computed

(44) Bryden, C. C.; Reilley, C. N.; Desreux, J. F. *Anal. Chem.* **1981**, *53*, 1418−1425.
(45) Piguet, C.; Edder, C.; Rigault, S.; Bernardinelli, G.; Bünzli, J.-C. G.; Hopfgartner, G. *J. Chem. Soc., Dalton Trans*. **2000**, 3999−4006.
(46) Benetollo, F.; Bombieri, G.; Calabi, L.; Aime, S.; Botta, M. *Inorg. Chem.* **2003**, *42*, 148−157.
(47) Howard, J. A. K.; Kenwright, A. M.; Moloney, J. M.; Parker, D.; Port, M.; Navet, M.; Rousseau, O.; Woods, M. *J. Chem. Soc., Chem. Commun.* **1998**, 1381−1382.
(48) Hay, B. P. *Inorg. Chem.* **1991**, *30*, 2876−2884.
(49) Cundari, T. R.; Moody, E. W.; Sommerer, S. O. *Inorg. Chem.* **1995**, *34*, 5989−5999.

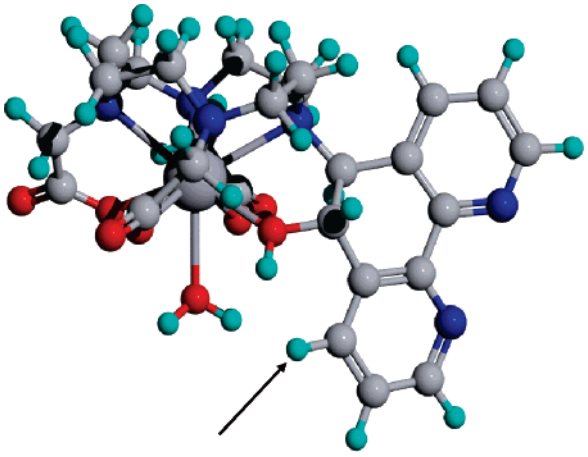


Figure 3. Molecular structure of GdPhenHDO3A calculated at the MM2 level using Hay[48] and Cundari[49] parameters.

values are in good agreement with the values reported in the Cambridge Structural Database[50] (Gd−N = 2.55−2.65 Å, Gd−O = 2.35−2.48 Å). The twist angle between the two square planes formed, respectively, by the four coordinated O and N atoms is 36.6°, as expected for a square antiprismatic structure.

The NMR analysis of the solution structure of YbPhenHDO3A was thwarted in part by difficulties in assigning the featureless NMR peaks and in orienting the magnetic susceptibility tensor. The NMR resonances of YbPhenHDO3A were partly assigned by comparison with the spectrum of YbDOTA and on the basis of the COSY spectrum that displayed a few cross-peaks. However, we were unable to obtain COSY cross-peaks for all resonances, and EXSY cross-peaks were observed only between the minor and the major species. Longitudinal relaxation times T_1 were thus measured for all resonances in order to make more-reliable assignments (see below). The susceptibility tensor (eq 2) was oriented so as to obtain the best agreement between the calculated and experimental paramagnetic shifts. It was also verified that the peak assignments were in keeping with both the $1/T_1$ values and the paramagnetic shifts measurements. All solutions that were not in agreement with the COSY patterns were discarded. Solutions that gave peak assignments that were too different from the peak ordering of symmetric Yb^{3+} macrocyclic complexes such as YbDOTA were also discarded, as they systematically gave very poor agreements.

The relationship between the calculated and experimental paramagnetic shifts is presented in Figure 4 (slope = 0.97, $r^2 = 0.97$). The Z axis of the susceptibility tensor was found to be nearly perpendicular to the mean plane of the nitrogen atoms (angle = −1°), and the magnetic susceptibility terms D_{ax} and D_{rh} were calculated to be 3748 ± 128 and 696 ± 275 ppm, respectively. A structural analysis on the basis of the relative magnitude of induced paramagnetic shifts is not without flaws, for instance, because of contact contributions to the chemical shifts or because several protons have very similar geometric factors.[17] In the present study, we could not obtain an R factor better than 13.6%, whereas lower values have been systematically obtained for simpler tetraaza

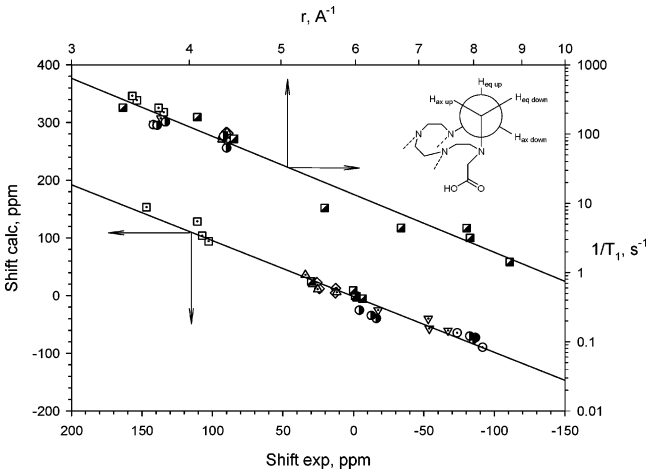


Figure 4. Correlation between the calculated and experimental paramagnetic dipolar shifts of YbPhenHDO3A (bottom) and between the longitudinal relaxation rate $1/T_1$ of the protons and the H-metal distance (top) (279 K, pD 5.4) Symbols: axial up (⊡), equatorial up (◈), axial down (▽), equatorial down (△), acetate (◓), dihydrophenanthroline (◪).[17]

macrocyclic ligands ($R = [\sum(\delta_{calcd} - \delta_{obs})^2/\sum\delta_{obs}^2]$, where δ is a paramagnetic shift).[17,42] Obtaining reliable parameters for predicting the solution geometry of lanthanide complexes remains a challenge despite recent progresses,[51] and the parameters we used might not be entirely appropriate for a chelate featuring a bulky dihydrophenanthroline group. A quantitative analysis of the longitudinal relaxation times T_1 was thus performed to support the paramagnetic shift study. The electron−nucleus dipolar interaction and the Curie spin−relaxation mechanism both contribute to the relaxation rate $1/T_1$ and are both functions of a $1/r_i^6$ term. A $\log(1/T_1)$ vs $\log r_i$ plot with a slope of −6 must thus be obtained if a reliable geometric model has been selected. Figure 4 shows the correlation between the $1/T_1$ values and the metal−proton distances in a log−log plot for which the slope is −5.6 ($r^2 = 0.97$) (similar levels of agreement have been reported in the literature[40,41,52] for lanthanide complexes). A particularly noteworthy aspect of these measurements is that only five poorly shifted resonances are found with relaxation rates much shorter than those of all other peaks (Figure 4). These resonances are due to five of the six aromatic protons in the dihydrophenanthroline moiety. The sixth proton, indicated by an arrow in Figure 3, is much more shifted and its relaxation rate is much higher, as it is closer to the paramagnetic center.

The YbPhenHDO3A thus appears to be a compact structure featuring a dihydrophenanthroline unit that is coordinated to the central metal ion with two nitrogen atoms that are pointing away from the DOTA-like unit and are available for bonding with a transition-metal ion.

Relaxivity Properties. The gadolinium(III) ion induces an increase in the relaxation rate $1/T_{1M}$ of the water protons through a dipolar interaction that can be accounted for by

(50) *Cambridge Structural Database*; Cambridge Crystallographic Data Centre: Cambridge, U.K.; http://www.ccdc.cam.ac.uk/.
(51) Rocha, G. B.; Freire, R. O.; da Costa, N. B., Jr.; de Sa, G. F.; Simas, A. M. *Inorg. Chem.* **2004**, *43*, 2346−2354.
(52) Dubost, J. P.; Leger, J. M.; Langlois, M. H.; Meyer, D.; Schaefer, M. *C. R. Acad. Sci., Ser. IIb: Mec., Phys., Astron.* **1991**, *312*, 349−354.

the Solomon−Bloembergen−Morgan equations. The SBM equations and their limitations have been reported in several textbooks and papers.[1,53,54] A copy of the Mathcad, version 13,[55] SBM equations used here for the analysis of relaxation measurements is included in the Supporting Information (Figure S2a−c). The longitudinal water relaxation rate $1/T_1$ per mmol of Gd^{3+} ion, also called relaxivity, depends on a number of factors. Among these are q_{H_2O}, the hydration number of the metal ion; τ_r and τ_m, the rotational and solvent exchange correlation times; T_{1e}, the electronic longitudinal relaxation time; τ_v, the correlation time of the modulation of the zero-field splitting; Δ^2, the mean square zero-field splitting energy; $A/\hbar$, the hyperfine coupling constant; and r, the metal−proton distance. The solvent molecules in the outer coordination sphere around the metal complex also make a contribution to the relaxivity. This contribution depends on the metal−proton distance r_{outer} and on the diffusion coefficient D_{diff}.[1,53,54] In SBM theory, the experimental relaxation rate $1/T_{1M}$ depends on a correlation time τ_c that is itself a function of the times τ_r, τ_m, and T_{1e} according to

$$\frac{1}{\tau_c} = \frac{1}{\tau_r} + \frac{1}{\tau_m} + \frac{1}{T_{1e}} \qquad (3)$$

Small complexes such as GdPhenHDO3A are tumbling rapidly in solution. One can safely assume that τ_r is then the smallest term in eq 3 and predominates the relaxivity at all frequencies, because the correlation time τ_c is then essentially a function of τ_r only. In these conditions, the dispersion of the relaxivity with frequency (NMRD) is a simple S-shape curve between 0.01 and 80 MHz. Larger species rotate more slowly and the influence of the τ_m and T_{1e} factors is likely to become more significant. It has been shown that this leads to a relaxivity increase at all frequencies. The NMRD plot then takes the form of an S-shape curve followed by a maximum at 20−60 MHz. A slower rotation is expected to lead to a sizable relaxivity increase in this frequency range.

Figure 5 presents a 20 MHz relaxivity titration of GdPhenHDO3A by $FeCl_2$ at 25 °C and pH 5.5. The relaxivity increases linearly until a $[Fe^{2+}]/[GdPhenHDO3A]$ concentration ratio of ~0.33 is reached. A plateau with a small negative slope is observed at higher concentration ratios. This titration curve is entirely in agreement with the self-assembly process illustrated in Scheme 2.

A highly stable tris complex is obviously formed between Fe^{2+} and GdPhenHDO3A. The stability constant of this complex is unknown but is expected to be quite high in view of the sudden relaxivity break at the expected stoichiometry and in reference to the strong preference for the formation of a highly stable tris complex between Fe^{2+} and phenanthroline ($\log \beta_n = (5.85), (11.15), 21.1$ for $n = 1$, 2, and 3,

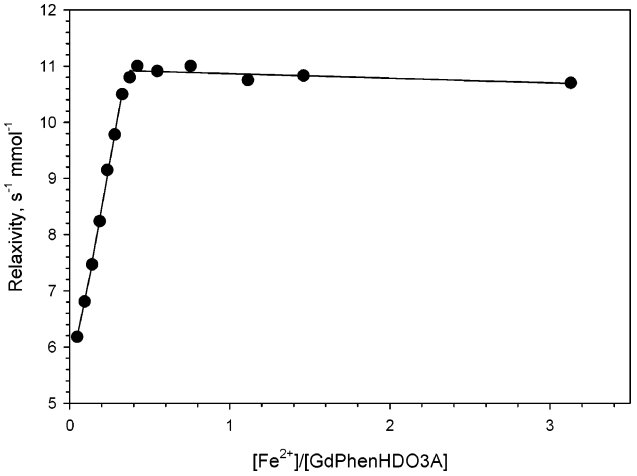


Figure 5. Relaxivity titration of Fe^{2+} by GdPhenHDO3A (pH 5.5, 25 °C).

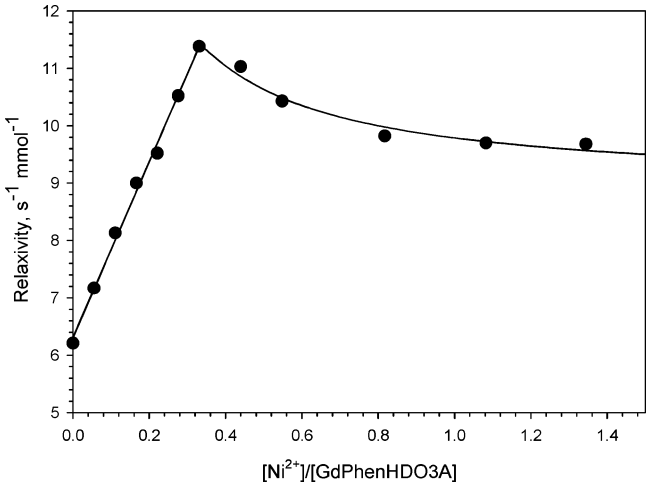


Figure 6. Relaxivity titration of Ni^{2+} by GdPhenHDO3A (pH 5.5, 25 °C).

respectively).[56] The high stability of the Fe^{2+} complex is also supported by the ease with which GdPhenHDO3A extracts iron from the metal tubing of HPLC equipment (see the Supporting Information, Figure S3). The small negative slope observed in the presence of an excess of Fe^{2+} in Figure 5 is assigned to the formation of a small proportion of the mono complex, in agreement with computations of the distribution of the different forms on the basis of the stability constants of the Fe^{2+}−phenanthroline complexes. The relaxivity gain is 90%, a sizable increase obtained by a simple self-assembly process. A similar relaxivity increase is observed when GdPhenHDO3A forms a tris complex with the Ni^{2+} ion, as shown in Figure 6.

A linear relaxivity increase is again observed until the $[Ni^{2+}]/[GdPhenHDO3A]$ ratio reaches 0.33 but is followed by a marked relaxivity decrease. The preference for the formation of a tris complex with phenanthroline is much less pronounced for Ni^{2+} than for Fe^{2+}. The relaxivity decrease in Figure 6 is thus assigned to the formation of the mono and bis complexes with Ni^{2+}. The titration curve

(53) Banci, L., Bertini, I., Luchinat, C., Eds. *Nuclear and Electron Relaxation*; VCH: Weinheim, Germany, 1991; pp 1−208.
(54) *Relaxometry of Water−Metal Ion Interactions*; van Eldik, R., Bertini, I., Eds; Advances in Inorganic Chemistry, Vol. 57; Elsevier: Amsterdam, 2005.
(55) *Mathcad*, version 13; Mathsoft: Cambridge, MA; http://www.mathsoft.com/.
(56) Smith, R. M.; Martell, A. E., Eds. *NIST Critically Selected Stability Constants of Metal Complexes Database*, version 4.0; National Institute of Standards and Technology: Gaithersburg, Md, 1997.

was simulated using the stability constants reported for the Ni^{2+}−phenanthroline system (log β_n = 8.9, 16.8, 24.4 for n = 1, 2, and 3, respectively)[56] and calculated relaxivities of 8.9, 10.3, and 11.4 s^{-1} $mmol^{-1}$ were computed for the 1:1, 1:2, and 1:3 Ni^{2+}:GdPhenHDO3A complexes, respectively. As expected, the relaxivity increases with the size of the complexes.

Many of the parameters in the SBM equations are unknown, and assumptions on their values must be made or, preferably, have to be measured independently. The metal−proton distance in the inner and outer coordination spheres r and r_{outer} have been set at (3.05 ± 0.5) Å and 3.6 Å, respectively, by reference with literature data.[57] The diffusion coefficient of the complexes has been fixed at (2.2 ± 0.2) × 10^{-9} m^2 s^{-1} and/or was computed by Hindeman's approach.[58,59] The hydration number q_{H_2O} of EuPhenHDO3A was deduced from the measurement of the luminescence lifetimes τ_{H_2O} and τ_{D_2O} of a EuPhenHDO3A solution in water and in D_2O using the corrected Horrocks equation[60]

$$q_{H_2O} = 1.2\left[\left(\frac{1}{\tau_{H_2O}} - \frac{1}{\tau_{D_2O}}\right) - 0.25\right] \qquad (3)$$

At pH 3.89, the q_{H_2O} value was 1.41. Both the water protons and the hydroxyl group on the dihydrophenanthroline group contribute to the quenching of the luminescence of the Eu^{3+} ion. A similar situation has been reported in the case of TbHP-DO3A.[38] It is thus assumed that q_{H_2O} is equal to 1.

In most cases, the main parameters of the SBM equations are deduced by fitting simultaneously the temperature dependence of the ^{17}O nuclear transverse relaxation rate and the nuclear relaxation dispersion curves (NMRD). Applying this procedure to both GdPhenHDO3A and $Fe(GdPhenHDOA)_3^{2+}$ did not lead to a sufficiently good fit, and the ^{17}O and NMRD data were thus interpreted separately. This difficulty has already been encountered by other authors,[61−63] including a recent study of a self-assembling process similar to the one reported here.[15] The water-exchange times τ_m were deduced from variable-temperature measurements of the ^{17}O transverse relaxation times, as these data essentially depend on this factor. Following the same line of reasoning, the rotational correlation times τ_r were deduced from the NMRD curves whose shape is essentially a function of the rotation rate. The water-exchange times deduced from the ^{17}O measurements were introduced as fixed parameters in the fitting of the NMRD curves. The hyperfine coupling constant was fixed to −3.5 × 10^6 rad s^{-1}, in keeping with values found for most gadolinium polyaza polycarboxylic complexes.[57] Moreover, the activation energy E_v of the modulation of the zero-field splitting was kept constant at 1 kJ/mol. The water-exchange correlation times τ_m of GdPhenHDO3A and its tris complex with Fe(II) were deduced from a fitting of the data presented in Figure 7 using the Swift and Connick equations.[57,64] These equations are reproduced in the Mathcad 13 file included in the Supporting Information. The best agreement between the experimental and the computed transverse relaxation times of GdPhenHDO3A and $Fe(GdPhenHDO3A)_3^{2+}$ were found for Δ^2 = 4.7 × 10^{19} s^{-2}, ΔH_m = 69 kJ/mol, τ_v^0 = 6 ps, τ_m^0 = (1.2 ± 0.1) × 10^{-6} s; and Δ^2 = 8.5 × 10^{19} s^{-2}, ΔH_m = 62 kJ/mol, τ_v^0 = 6 ps, τ_m^0 = (1.0 ± 0.1) × 10^{-6} s, respectively. The errors on the water-exchange times were estimated by taking into account the influence of the parameters $A/\hbar$, Δ^2, ΔH_m, and τ_v^0. The rate of water exchange is rather slow, as already observed for a dimeric Gd(III) complex with two triacetic DOTA ligands featuring an alcohol arm substituted with an aliphatic ether chain rather than a phenanthroline-alcohol group.[65] Within the error limits, the self-assembling process around Fe(II) has no or little influence on the water-exchange time. However, a rigidification of the dihydrophenanthroline ring due to the complexation of Fe(II) could bring about a small change in the water-exchange time.

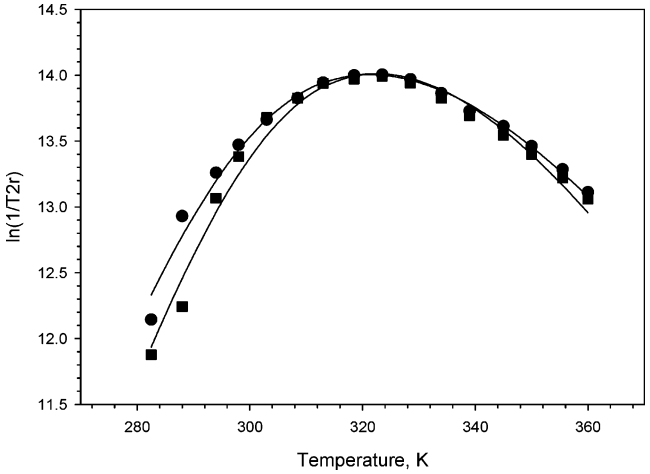


Figure 7. Temperature dependence of the reduced ^{17}O transverse relaxation rate $1/T_{2r}$ of water in a solution of GdPhenHDO3A (■) and Fe-[GdPhenHDO3A]$_3$ (●), pD 5.0.

The NMRD curve of GdPhenHDO3A and its tris complex with Fe^{2+} and Ni^{2+} are reproduced in Figure 8. The curves of the tris complexes exhibit small maxima at 20−50 MHz, in agreement with Solomon−Bloembergen equations. Simulations of the NMRD data for GdPhenHDO3A and $Fe(GdPhenHDO3A)_3^{2+}$ yielded Δ^2 = 3.1 × 10^{19} s^{-2}, τ_v^0 = 24 ps, τ_r^0 = 105 ± 20 ps; and Δ^2 = 1.5 × 10^{19} s^{-2}, τ_v^0 = 35 ps, τ_r° = 398 ± 40 ps. The formation of the tris complex brings about a 4-fold increase in the rotational correlation time, a clear indication that the self-assembling process is indeed taking place. No quantitative interpretation of the

(57) Powell, D. H.; Ni Dhubhghaill, O. N.; Pubanz, D.; Helm, L.; Lebedev, Y. S.; Schlaepfer, W.; Merbach, A. E. *J. Am. Chem. Soc.* **1996**, *118* (8), 9333−9346.
(58) Hindman, J. C. *J. Chem. Phys.* **1974**, *60*, 4488−4495.
(59) Rudovsky, J.; Kotek, J.; Hermann, P.; Lukes, I.; Mainero, V.; Aime, S. *Org. Biomol. Chem.* **2005**, *3*, 112−117.
(60) Beeby, A.; Clarkson, I. M.; Dickins, R. S.; Faulkner, S.; Parker, D.; Royle, L.; de Sousa, A. S.; Williams, J. A. G.; Woods, M. *J. Chem. Soc., Perkin Trans. 2* **1999**, 493−503.
(61) Botta, M.; Quici, S.; Pozzi, G.; Marzanni, G.; Pagliarin, R.; Barra, S.; Crich, S. G. *Org. Biol. Chem.* **2004**, *2*, 570−577.
(62) Muller, R. N.; Radüchel, B.; Laurent, S.; Platzek, J.; Piérart, C.; Mareski, P.; Vander Elst, L. *Eur. J. Inorg. Chem.* **1999**, 1949−1955.
(63) Laus, S.; Sour, A.; Ruloff, R.; Toth, E.; Merbach, A. E. *Chem.−Eur. J.* **2005**, *11*, 3064−3076.
(64) Swift, T. J.; Connick, R. E. *J. Chem. Phys.* **1962**, *37*, 307−320.
(65) Toth, E.; Vauthey, S.; Pubanz, D.; Merbach, A. E. *Inorg. Chem.* **1996**, *35*, 3375−3379.

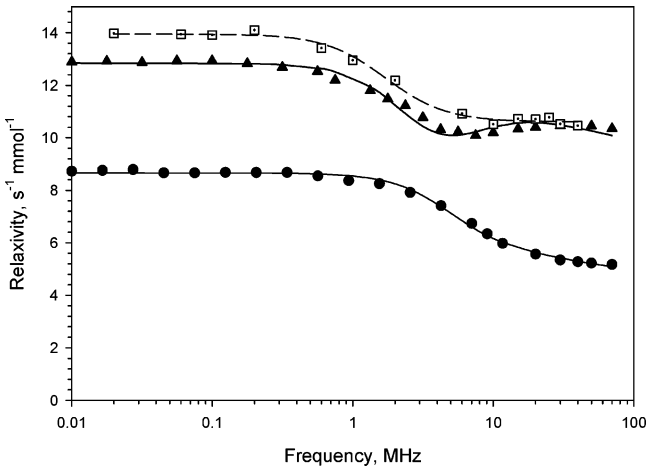


Figure 8. Nuclear magnetic relaxation curves (NMRD) of GdPhenHDO3A (●), Fe(GdPhenHDO3A)$_3^{2+}$ ([Fe^{2+}]/[GdPhenHDO3A] = 0.33) (▲), and Ni(GdPhenHDO3A)$_3^{2+}$ ([Ni^{2+}]/[GdPhenHDO3A] = 0.33) (⊡) at pH 5.5, 25 °C. Calculated NMRD curves are shown for GdPhenHDO3A and Fe(GdPhenHDO3A)$_3^{2+}$ (−). A simple Cole−Cole[70] curve (- - -) is shown for Ni(GdPhenHDO3A]$_3^{2+}$ (see text).

NMRD curve of Ni(GdPhenHDO3A)$_3$ was performed, as this species could not be obtained alone and the influence of the Ni(II) paramagnetism is not easily taken into account even if only an outer sphere effect is expected. However, the NMRD curves of the tris complexes with Fe(II) and Ni(II) are similar. In keeping with the rather large τ_m values, a study of the dependence of the relaxivity of Fe(GdPhenHDO3A)$_3^{2+}$ upon temperature (see the Supporting Information, Figure S4) indicates that the relaxivity is essentially independent of temperature between 5 and 30 °C and decreases between 30 and 50 °C. This behavior is expected if τ_m is close to T_{1M} at low temperatures and less than T_{1M} at high temperatures, as observed for several DTPA and DOTA derivatives.[66] It should be stressed here that the correlation times deduced from the NMRD curves should be considered with caution, as several parameters are strongly correlated. However, the τ_m^0 and τ_r^0 factors are considered to be reliable, as the influence of the other parameters on these factors is rather limited. It should also be noted that the Ni(II)- and Fe(II)-centered assemblies are most probably mixtures of isomers.

Conclusions

The aim of the work reported in this paper was to prepare stable multimetallic Gd(III) complexes of high relaxivity. It was anticipated that this goal could be achieved only if all Gd(III)-containing units would rotate as a single entity. Thus, all internal movements had to be eliminated. The selected approach consisted in preparing a ditopic ligand with two different complexing units firmly connected to each other that could form rigid polymetallic assemblies around transition-metal ions. As reported in a preliminary account,[13] ligand PhenHDO3A exhibits the sought characteristics and is able to form a stable Gd(III) complex that easily self-assembles around Fe(II). The formation of tris complexes around Fe(II) or Ni(II) results in a 90% relaxivity increase, and the achieved relaxation enhancements are close to those obtained with dendrimers of much higher molecular weights but much lower rigidity.[67] Merbach et al. recently[15] reported on a polymetallic complex whose design was built on the same idea of self-assembling we proposed earlier. The elegant approach adopted by these authors led to a tetra-anionic hexagadolinium complex that exhibits a very high relaxivity between 30 and 60 MHz (33.6 mmol^{-1} s^{-1} at 25 °C). This remarkable achievement was possible because the two ligands *N*,*N*-bis(2-aminoethyl)ethylamine-*N*′,*N*′,*N*″,*N*″-tetraacetic acid attached by these authors on bipyridine form a dihydrated Gd(III) complex with a high water-exchange rate. This rate is achieved at the cost of a low thermodynamic stability that is comparable to that of GdEDTA (pGd = 14.9 vs pGd = 16.1 for EDTA at pH 7.4, [Gd^{3+}] = 1 μM, [ligand] = 10μM).[15,68] However, the bis complex is rapidly cleared by renal elimination in mice.[15] The GdPhenHDO3A complex proposed herein essentially keeps the kinetic inertness of GdDOTA, but its water-exchange rate is slower. It is as water-soluble, electronically neutral a species as the commercially available GdHPDO3A (ProHance) and GdDTPA-BMA (5,8-bis(carboxymethyl)-11-[2-(methylamino)-2-oxoethyl]-3-oxo-2,5,8,11-tetraazatridecan-13-oic acid, Omniscan) that were developed because of the low osmolality of their solutions. Finally, GdPhenHDO3A is only monohydrated but does not suffer from the competition from endogenous ions such as CO_3^{2-}, lactate, or amino acids, which are able to completely remove the inner-sphere water molecules from complexes in which Gd(III) is only heptacoordinated.[10,69]

Acknowledgment. The authors gratefully acknowledge the Fonds National de la Recherche Scientifique and the Institut Interuniversitaire des Sciences Nucléaires of Belgium for their financial support. The authors also thank the EC COST Action D18 "Lanthanide Chemistry for Diagnosis and Therapy". C.G. is grateful for support from the Conselho Nacional de Desenvolvimento Cientifico e Tecnológico of Brazil for a postdoctoral fellowship and thanks Prof. Severino Alves Jr. for help and advice.

Supporting Information Available: ^{1}H NMR spectra of PhenHDO3A and LaPhenHDO3A, equations used for interpreting ^{17}O and NMRD data in a Mathcad program, HPLC chromatograms of PhenHDO3A and mixtures with increasing proportions of Fe(II), and dependence of the relaxivity of Fe(GdPhenHDO3A)$_3^{2+}$ on temperature at 20 MHz. This material is available free of charge via the Internet at http://pubs.acs.org.

IC0603050

(66) Aime, S.; Barge, A.; Bruce, J. I.; Botta, M.; Howards, J. A. K.; Moloney, J. M.; Parker, D.; de Sousa, A. S.; Woods, M. *J. Am. Chem. Soc.* **1999**, *121*, 5762−5771.

(67) Margerum, L. D.; Campion, B. K.; Koo, M.; Shargill, N.; Lai, J.-J.; Marumoto, A.; Sontum, P. C. *J. Alloys Compd.* **1997**, *249*, 185−190.

(68) Paul-Roth, C.; Raymond, K. N. *Inorg. Chem.* **1995**, *34*, 1408−1412.

(69) Dickins, R. S.; Aime, S.; Batsanov, A. S.; Beeby, A.; Botta, M.; Bruce, J. I.; Howard, J. A. K.; Love, C. S.; Parker, D.; Peacock, R. D.; Puschmann, H. *J. Am. Chem. Soc.* **2002**, *124*, 12697−12705.

(70) Hallenga, K.; Koenig, S. H. *Biochemistry* **1976**, *15*, 4255−4263.