Synthesis and Relaxivity Studies of a Tetranuclear Gadolinium(III) Complex of DO3A as a Contrast-Enhancing Agent for MRI

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Synthesis and Relaxivity Studies of a Tetranuclear Gadolinium(III) Complex of DO3A as a Contrast-Enhancing Agent for MRI

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A tetranuclear gadolinium(III) complex, $[Gd_4(\mathbf{4})(H_2O)_8]$, of DO3A appended onto the pentaerythrityl framework was synthesized to improve the water proton relaxivity for MRI application. The longitudinal relaxivity of $[Gd_4(\mathbf{4})(H_2O)_8]$ is 28.13 mM^{-1} s^{-1} (24 MHz, 35 ± 0.1 °C, pH 5.6) which is 5.86 times higher than that of $[Gd(DO3A)(H_2O)_2]$. The relaxivity is based on "molecular" relaxivity of the tetramer and the r_{1p} value is "7 per Gd". The high relaxivity of the tetramer is the result of the decrease in the rotational correlation (τ_R) and the presence of eight inner-sphere water molecules ($q = 8$). The complex exhibits pH-dependent longitudinal relaxivity, and the high relaxivity both at low and high pH (r_{1p} = 28.13 mM^{-1} s^{-1} at pH 5.6 and 16.52 mM^{-1} s^{-1} at pH 9.5) indicates that it could be used as a pH-responsive MRI contrast agent. The transverse relaxivity of the tetramer is 129.97 mM^{-1} s^{-1} (24 MHz, 35 ± 0.1 °C, pH 5.6), and the r_{2p}/r_{1p} ratio of 4.6 shows that it could be used as a T_2-weighted contrast agent.

Introduction

Medical magnetic resonance imaging (MRI) is the state-of-the art noninvasive imaging modality in clinical medicine for the diagnosis of various diseases and the examination of almost all organs. The image contrast obtained in MRI is a three-dimensional signal intensity map of the spatially encoded proton signal of the in vivo water molecules in a given volume element (voxel). It depends essentially on the differences in the intensity of the water proton signals in different tissues. Therefore, the contrast can be augmented by administering contrast agents (CAs)[1] which enhance the longitudinal and transverse relaxation rates of the water protons thereby contributing to the intensity of the MRI signal and thus to the contrast of the image.[1,2] Gd(III) complexes of polyazacarboxylate ligands, because of their favorable thermodynamic and kinetic properties,[3] have been extensively studied as CAs.

The relaxation of the solvent water protons by gadolinium-based contrast agents results from short-range dipolar interactions between the unpaired electron spin of the metal ion and the proton nuclei of the water molecules coordinated in the inner-sphere of the metal ion, mediated to the bulk by chemical exchange (*inner-sphere* relaxation) and by the long-range through-space dipolar interactions between the metal ion and the water molecules in the second coordination sphere (H-bonded to the complex) and the water molecules that diffuse in the proximity of the paramagnetic center (*outer-sphere* relaxation).[4] The prototropic exchange of the mobile protons of the complex may also be involved in the transmission of the magnetic interaction (*prototropic* con-

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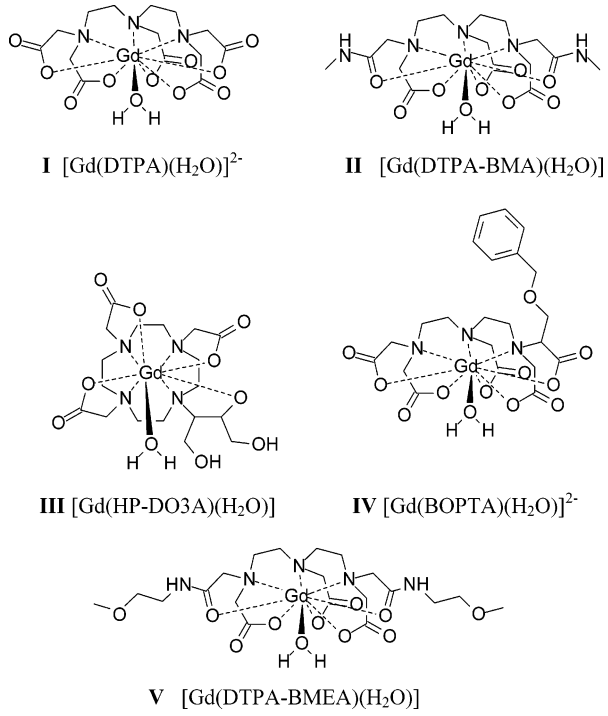
tribution).[5] The relaxivity enhancement by a paramagnetic chelate is the sum of the inner- and outer-sphere contributions and can be interpreted using the Solomon−Bloembergen−Morgan (SBM)[6] and Freed's[7] equations, respectively.

The gadolinium(III) chelates approved by the FDA for human imaging are as follows: $(NMG)_2[Gd(DTPA)(H_2O)]$[8] (gadopentetate dimeglumine, Magnevist, Schering, Germany), $[Gd(DTPA\text{-}BMA)(H_2O)]$[9] (gadodiamide, Omniscan, Amersham-Nycomed), $[Gd(HP\text{-}DO3A)(H_2O)]$[10] (gadoteridol, ProHance, Bracco Diagnostics Inc.), $[Gd(BOPTA)(H_2O)]$[11] (gadobenate dimeglumine, MultiHance, Bracco Diagnostics Inc.), and $[Gd(DTPA\text{-}BMEA)(H_2O)]$[12] (gadoversetamide, OptiMark, Tyco Healthcare) (Chart 1). New classes of MRI contrast agents[13] such as blood-pool agents,[14] targeting CAs,[13] and smart CAs, such as those that are pH-responsive,[15] oxygen-pressure (pO_2) responsive,[16] enzyme responsive,[17] and metal-ion concentration dependent,[18] are emerging. Receptor-induced magnetization-enhancement (RIME) CAs, which involve targeting Gd(III) chelates to protein binding sites,[19] chemical-exchange saturation-transfer (CEST) CAs,[20] pH-reporter CAs,[21] Gd(III) complexes of bifunctional chelates (BFCs) conjugated to biological targeting vectors thattarget and enhance the efficiency of CAs,[22]gadofullerene carboxylates,[23] gadolinium metallofullerenols,[24] and gadonanotubes as high-performance CAs[25] have also been reported.

Chart 1. FDA Approved MRI Contrast Agents



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In MRI, a reasonably large amount of contrast agent is administered intravenously to improve the image contrast because of the low sensitivity of the MRI technique (clinical dose is 0.1−0.2 mmol/kg body weight). Therefore, new contrast agents with improved in vivo performance are desirable for administering smaller amounts. In general, higher relaxivity may be achieved by improving the inner-sphere relaxivity. According to SBM theory, inner-sphere relaxivity is governed by several parameters,[4a,26] and the efficiency of the relaxation process depends primarily on the number of water molecules coordinated to the paramagnetic metal ion (q) and the correlation time τ_c, which modulates the dipole−dipole relaxation mechanism. One approach to increase relaxivity is by increasing q. The increase in the hydration number (q) increases the exchange rate of the coordinated water molecules because of a shift in the mechanism of water exchange from dissociative to associative.[27] Gd(III) forms stable enna-coordinate complexes with the octacoordinating DOTA[28] with $q = 1$ and with hepta-coordinating ligands such as DO3A,[10a,c,29] PCTA,[29b,30] functionalized PCTA,[31] PCP2A,[32] AAZTA,[33] and HOPO-derivatives[34] with $q = 2$. The number of coordinated water molecules can be further increased by employing hexaco-ordinating ligands, but their Gd(III) complexes exhibit lower thermodynamic stability.[35]

The correlation time, τ_c, depends on the rotational correlation time (τ_R) of the complex, the exchange lifetime ($k_{ex} = 1/\tau_m$) of the coordinated water molecules, and the electronic relaxation times ($T_{1e,2e}$) of the metal ion.[1a,4a] High relaxivity may be achieved by contrast agents characterized by a slow rotational correlation time and an optimal residence lifetime (τ_m) of the coordinated water molecules. Therefore, another strategy to attain high relaxivity is by slowing down the molecular rotation by increasing the molecular weight and dimensions by binding the Gd(III) chelates to systems of different dimensions,[36] such as proteins,[37] polymers,[38] or dendrimers,[39] or by polymerization of the chelate itself.[41]

The simultaneous relaxation of two Gd(III) ions in a dimer, and the possible advantage of using a multimetal paramagnetic center was envisioned by Bryant et al.[41] When two or more Gd(III) ions are held in close proximity, an additional relaxation mechanism results from the intramolecular dipole−dipole electronic relaxation between the Gd(III) electron spins, compared to a corresponding mononuclear complex.[26a] This phenomenon has been observed at high magnetic fields for the dimeric complexes [pip{Gd(DO3A)(H_2O)}$_2$] and [bisoxa{Gd(DO3A)(H_2O)}$_2$] by Powell et al.[26a] and in the trinuclear complex $[Gd_3(taci)_2(H_2O)_6]^{3+}$ by Toth et al.[42] Other polynuclear Gd(III) chelates which exhibit high longitudinal

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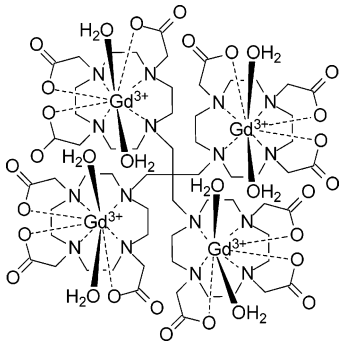
relaxivity by slow molecular rotation include the dinuclear Gd(III) complex [BO{Gd(DO3A)(H_2O)}$_2$] (r_{1p} = 4.61 mM^{-1} s^{-1}) by Merbach et al.,[43] the dinuclear Gd(III) complex containing two DTPA moieties bridged by a bisindole derivative (r_{1p} = 6.8 mM^{-1} s^{-1}) by Binnemans et al.,[44] the dinuclear Gd(III) complex of DTTA chelators bridged by *m*- and *p*-xylene (r_{1p} = 16.79 and 15.84 mM^{-1} s^{-1}) by Merbach et al.,[45] and the multinuclear Gd(III) chelates of DO3A (dimers, trimers, tetramers, hexamers, and octamers) covalently linked via either amide bonds or 2-hydroxypropylidine bonds by Ranganathan et al.[46]

The nine-coordinate Gd(III) complex of DO3A features two inner-sphere water molecules (q = 2), has a r_{1p} value of 4.8 mM^{-1} s^{-1}, and exhibits good in vivo stability. Multiple Gd(DO3A) chelates anchored symmetrically in close proximity onto a rigid structural motif would exhibit high relaxivity resulting from the slow rotation of the whole molecular assembly and intramolecular dipole−dipole electronic relaxation. We report herein the synthesis and relaxivity studies of tetranuclear Gd(DO3A) chelates covalently appended onto the pentaerythrityl moiety (Chart 2).

Experimental Section

Chemicals. Diethylenetriamine, *p*-toluenesulfonyl chloride, diethanolamine, bromoethyl acetate, pentaerythritol, benzenesulfonyl chloride, xylenol orange, Celite, and potassium carbonate (anhydrous) (Fluka) were used as received. The Amberlite IR120 (H^+, strongly acidic) (Fluka) cation-exchange resin was washed with distilled water five times before use. Sulfuric acid (98%) (Merck, India), sodium bromide, potassium hydroxide pellets (Rankem, India), and gadolinium(III) oxide (Aldrich) were used as received. The solvents were purified by the standard procedures.[47] Cyclen was synthesized by the method of Richman and Atkins,[48] and DO3A-triethyl ester was synthesized by the method of Mishra and Chatal.[49]

Chart 2. Gd(DO3A) Tetramer [Gd_4(4)(H_2O)$_8$]



Physical Measurements. Infrared spectra were recorded on a Perkin-Elmer Spectrum RX-I FTIR spectrometer in the range of 4000−400 cm^{-1} using KBr pellets. Potassium bromide (FTIR grade, Aldrich) was used to make the pellets. The electron-impact mass spectra (EIMS) were recorded using a Finningan Mat 8230 mass spectrometer. The accelerating voltage was 70 eV, and the spectra were recorded at room temperature. Electrospray-ionization mass spectra (ESIMS) were recorded using a Micromass Quattro-II Triple Quatrapole mass spectrometer. The sample was dissolved in water and introduced into the ESI capillary using a 5 μL syringe pump. The ESI capillary was set at 3.5 kV with a cone voltage of 40 V. CHN microanalyses were carried out using a Perkin-Elmer 2400 Series II CHNS/O elemental analyzer interfaced with a Perkin-Elmer AD 6 autobalance. Helium (analytical grade) was used as the carrier gas. Analytical and preparative HPLC analyses were carried out using a Varian PrepStar 218 (Varian Instruments Inc., USA) binary-gradient solvent-delivery module with an inline three-channel degasser (Model 2000) for solvent delivery. A Rheodyne injector valve (20 μL) was used for sample injection. An HPLC column (250 × 4.6 × 1/4 in., Valco, Microsorb-MV 100−5 C18, analytical) and a DYNAMAX HPLC column (250 × 10.0 mm, Microsorb 300−10 C18, preparative) were used. A Model 345 UV−vis detector operating in the range of 190−1100 nm was used. The fractions were collected using a Model 704 fraction collector. EPR spectra were recorded on a JEOL instrument at the Q band (34.5 MHz) and X band (9.4 MHz) with a scan range of 8 kg, and the field was set at 12 500 T. Magnetic susceptibility measurements were carried out on an EG&G PAR Model 155 vibrating sample magnetometer at 25 °C. ^{1}H and ^{13}C NMR spectra were recorded in D_2O and $CDCl_3$ (9.995 atom % D, Aldrich) on a JEOL GSX-400 multinuclear NMR spectrometer working at 400 MHz (for ^{1}H) and at 100 MHz (for ^{13}C) at 25 °C.

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Longitudinal Relaxivity (r_{1p}) Measurements. The longitudinal relaxivity of the Gd(III) complex was determined from the spin-lattice relaxation time, T_1. The T_1 measurements were carried out on a Maran wide-line NMR (Resonance Instruments Ltd., U.K.) operating at 24 MHz and 35 ± 0.1 °C. The temperature was controlled using a temperature console. The complex solutions in triply distilled water were taken in a 10 mm stoppered quartz tube, and the instrument parameters were optimized for each T_1 measurement. The T_1 measurements were made using the standard inversion-recovery pulse sequence (180°−τ−90°) with phase-sensitive detection[50] and varying τ values ranging from 50 μs to 6 s for each concentration of the complex. The computer program WINFIT was used to plot the time versus the signal intensity to get an exponential plot, and the T_1 values were calculated from the plot. A delay of at least 5 T_1 was maintained between successive pulses to allow the complete return of the spin system to equilibrium. The T_1 values for five different concentrations of the complex were measured. A plot of $1/T_1$ versus the concentration of the complex gave a straight line, and the slope was taken as the longitudinal relaxivity (r_{1p}). Five different concentrations of the complex (0.2, 0.5, 1.0, 1.5, and 2.5 mM) in triple-distilled water were used. The longitudinal relaxivity at pH 8.5 for five different concentrations (0.2, 0.5, 1.0, 1.5, and 2.5 mM) were measured by maintaining the pH by adding TRIS buffer.

Transverse Relaxivity (r_{2p}) Measurements. The transverse relaxivity was determined from the spin−spin relaxation time, T_2. A standard CPMG (Carl−Purcel−Meiboon−Gill) pulse sequence (90°−τ−180°)[51] with a τ value of 50 μs was used to determine T_2. The computer program WINFIT was used to plot the time versus signal intensity to get an exponential curve, and T_2 was calculated from the graph. The T_2 values for six different concentrations were measured. The transverse relaxivity was calculated from the slope of the regression line, obtained by the plot of $1/T_2$ versus the concentration of the complex by the least-squares fitting method. Five different concentrations of the complex (0.2, 0.5, 1.0, 1.5, and 2.5 mM) were prepared in triple-distilled water.

Synthesis of the Ligand and Complex. Pentaerythrityl Tetrabromide [1,3-Dibromo-2,2-bis(bromomethyl)propane] (1). Stage 1. A solution of pentaerythritol (6.528 g, 0.048 mol) in 60 mL of dry pyridine was taken in a three-neck round-bottom flask, fitted with a mechanical stirrer, a dropping funnel, and a double surface condenser, and benzenesulfonyl chloride (7.3 g, 0.212 mol) in 150 mL of pyridine was added slowly over a period of 5 h at 30−35 °C with stirring. The resulting solution was further stirred at 40 °C for 2 h, the volume of the solution was reduced to 25 mL, and transferred slowly into a one liter beaker containing 85 mL of conc hydrochloric acid in 125 mL of ice-cold water and 175 mL of methanol; then pentaerythritol benzenesulfonate separated out as white solid. The product was filtered using a G3 filter funnel, washed with ice-cold water and ethanol, and recrystallized from hot ethanol. mp: 138−142 °C.

Stage 2. Pentaerythritol benzenesulfonate (6.96 g, 10 mmol) was added to 45 mL of diethylene glycol in a 100 mL round-bottom flask; sodium bromide (6 g, 58 mmol) was added as a solid, and the mixture was heated in an oil bath at 140−150 °C with stirring for 12 h. The resulting orange solution was cooled to 90 °C; 350 mL of crushed ice was added with vigorous stirring, and the white precipitate that separated out was filtered, washed with cold ethanol, and recrystallized from hot acetone. Yield: 2.79 g (72%). mp: 154−156 °C (lit 156−158 °C).[52] FTIR (KBr, cm^{-1}): 3013 ν_a(C−H), 2959.1 ν_s(C−H), 1423.0 ν_s(C−H) (CH_2), 609.9 ν(C−Br). ^{1}H NMR ($CDCl_3$, 400 MHz): δ 2.53 (s, 8 H). ^{13}C NMR ($CDCl_3$, 100 MHz): δ 34.72 (−*C*−CH_2−Br), 43.42 (−C−*C*H_2−Br). MS (EI): *m*/*z* 389 $(M + 1)^+$, 307 $(M - Br)^+$, 212 $(C_4H_6Br_2)^+$, 149 $(C_5H_4Br)^+$, 69 $(C_5H_9)^+$, 55 $(C_4H_7)^+$ (*tert*-butyl moiety). Anal. Calcd for $C_5H_8Br_4$ (M_r = 388): C, 15.46; H, 2.06. Found: C, 15.40; H, 2.04.

{4,7-Bis(carboethoxymethyl)-10-[3-(4,7,10-tris(carboethoxymethyl)-1,4,7,10-tetraazacyclododecan-1-yl)-2,2-bis(4,7,10-tris(carboethoxymethyl)-1,4,7,10-tetraazacyclododecan-1-ylmethyl)propyl]-1,4,7,10-tetraazacyclododecan-1-yl}acetic Acid Ethyl Ester (3). DO3A-triethyl ester (**2**) (0.45 g, 1.05 mmol) and finely ground anhydrous potassium carbonate (0.145 g, 1.4 mmol) in 60 mL of dry acetonitrile was taken in a three-neck 250 mL round-bottom flask, fitted with a dropping funnel and a double-surface condenser. Nitrogen gas was purged through the solution, and pentaerythrityl tetrabromide (0.1 g, 0.262 mmol) in 30 mL of dry acetonitrile was added dropwise with stirring over a period of 18 h under a nitrogen blanket; the mixture was stirred at 70−80 °C for 72 h under a nitrogen atmosphere and then filtered through Celite. The filtrate was evaporated in a rotavapor and the yellow viscous liquid was purified by silica gel column chromatography by eluting with dichloromethane−methanol (7:3, v/v) to yield a pale yellow viscous liquid. Yield: 0.78 g (42%). FTIR (KBr, cm^{-1}): 2980.3 ν(C−H), 1731.2 ν(C═O), 1305 ν_s(C−O), 1206.7 ν(C−O), 1027.1 ν(C−N). ^{1}H NMR ($CDCl_3$, 400 MHz): δ 1.16−1.19 (t, 36 H, J = 5.9 Hz), 2.82 (t, 16 H, J = 4.9 Hz), 3.07 (s, 32 H), 3.32 (s, 8 H), 3.42 (s, 24 H), 3.74 (t, 16 H, J = 4.7 Hz), 4.05−4.11 (q, 24 H). ^{13}C NMR ($CDCl_3$, 100 MHz): δ 13.77 (12 C, −CO(O)-CH_2CH_3), 46.9 (8 C, $-C^9H_2-N-C^{11}H_2-$), 48.8 (8 C, $-C^8H_2-CH_2N-CH_2C^{12}H_2-$), 51.2 (16 C, $-C^2H_2C^3H_2-N-C^5H_2C^6H_2-$), 54.9 (12 C, α-$CH_2$), 56.6 (4 C, −C−$CH_2$−N−), 60.9 (12 C, −CO-(O)CH_2CH_3), 170.9 (12 C, *C*═O). Anal. Calcd for $C_{85}H_{156}N_{16}O_{24}$ (M_r = 1784): C, 57.16; H, 8.74; N, 12.55. Found: C, 57.08; H, 8.72; N, 12.51.

4,7-Bis(carboxymethyl)-10-[3-(4,7,10-tris(carboxymethyl)-1,4,7,10-tetraazacyclododecan-1-yl)-2,2-bis(4,7,10-tris(carboxymethyl)-1,4,7,10-tetraazacyclododecan-1-yl-methyl)propyl]-1,4,7,-10-tetraazacyclododecan-1-yl Acetic Acid (4). Ester **3** was dissolved in 10% aqueous potassium hydroxide (50 mL) in ice-cold conditions and stirred at 5 °C for 2 days. The solution was diluted with 25 mL of water and flash evaporated to dryness. This process was repeated three times to remove the base. The hydrolyzed product was dissolved in 10 mL of methanol−water (1:0.8, v/v) and purified by passing through a column packed with Amberlite IR-120 (H^+, acidic) cation-exchange resin and eluting with methanol−water (6:4, v/v). The eluent was flash evaporated, and the resulting white solid was triturated with methanol and dried in a vacuum desiccator. Yield: 0.424 g (67%). mp: 194−196 °C (dec). FTIR (KBr, cm^{-1}): 3409 ν_s(OH), 2921.9, 2851.9 ν_s(C−H), 1663.8 ν_a(COO^-), 1391.0 ν_s(OH), 1384.8 ν_s(COO^-), 1098.1. ^{1}H NMR (D_2O, 400 MHz): δ 2.08 (s, 8 H), 2.32−2.38 (t, 16 H, J = 6.16 Hz), 2.58 (s, 32 H) 2.73−2.7 (t, 16 H, J = 5.93 Hz), 2.88 (s, 24 H). ^{13}C NMR (D_2O, 100 MHz): δ 30.68 (4 C, −C−CH_2−N), 50.88 (8 C, $-C^9H_2-N-C^{11}H_2-$), 51.62 (8 C, $-C^8H_2CH_2N-CH_2C^{12}H_2-$), 58.23 (16 C, $-C^2H_2C^3H_2-N-C^5H_2C^6H_2-$), 65.61 (12 C, α-$CH_2$), 177.91 (12 C, *C*═O). Anal. Calcd for $C_{61}H_{108}N_{16}O_{24}$ (M_r = 1448): C, 50.54; H, 7.45; N, 15.46. Found: C, 50.48; H, 7.42; N, 15.42.

{4,7-Bis(carboxymethyl)-10-[3-(4,7,10-tris(carboxymethyl)-1,4,7,10-tetraazacyclododecan-1-yl)-2,2-bis(4,7,10-tris(carboxy-

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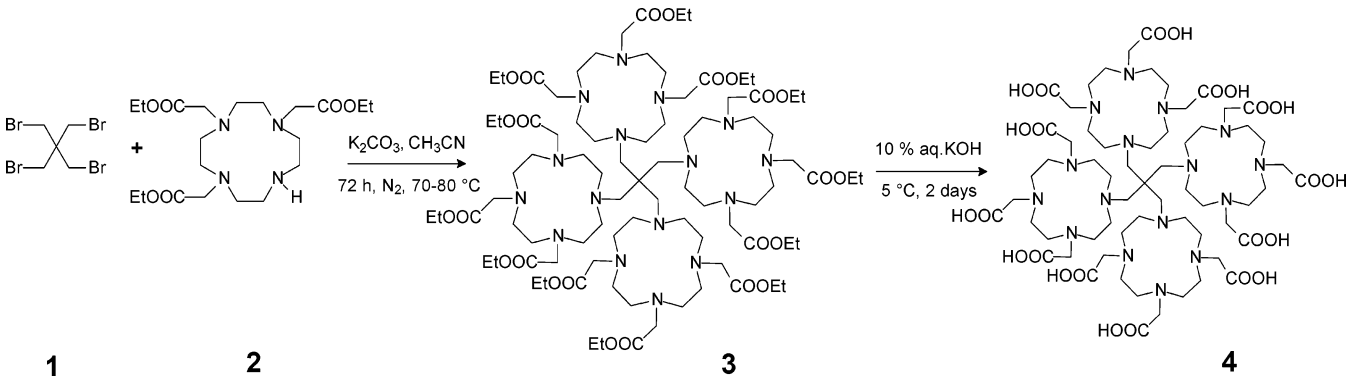
Scheme 1. Synthesis of the Tetrakis(DO3A)-Appended Ligand (**4**)



methyl)-1,4,7,10-tetraazacyclododecan-1-ylmethyl)propyl]-1,4,7,10-tetraazacyclododecan-1-yl}acetato(octaaqua)tetragadolinium(III) [Gd_4(4)$(H_2O)_8$]. Gadolinium(III) oxide (0.35 g, 0.964 mmol) was added to a solution of **4** (0.35 g, 0.241 mmol) in 25 mL of water in a 100 mL round-bottom flask, and the suspension was heated at 80−90 °C for 3 days with constant stirring. The solution was cooled to room temperature and filtered through a 0.2 μm filter funnel. The filtrate was evaporated to dryness in a rotavapor, and the white solid that separated out upon the addition of acetone−methanol (6:4 v/v) was recrystallized from triple-distilled water and stored in a refrigerator. Yield: 0.385 g (72%). mp: >213 °C (dec). FTIR (KBr, cm^{-1}): 3369.7 ν_s(OH), 2989 ν_a(OH), 1452 ν(COO^-), 620 ρ_r(OH), 509.1 ρ_w(OH), 439 ν(Gd−O). MS (ESI) *m*/*z*: 2210 M^+ $(Gd_4C_{61}H_{96}N_{16}O_{24} + 8H_2O)^+$, 2156 [M − $3H_2O$]$^+$, 2102 [M − $6H_2O$]$^+$, 2066 [M − $8H_2O$]$^+$, 2023 [Gd_4-($C_{60}H_{96}N_{16}O_{22}$)]$^+$, 1997 [Gd_4($C_{58}H_{93}N_{16}O_{22}$)]$^+$, 1968 [Gd_4($C_{57}H_{92}N_{15}$-O_{22})]$^+$, 1942 [Gd_3($C_{47}H_{72}N_{12}O_{18}$)]$^+$, 1071 [Gd_2($C_{33}H_{54}N_8O_{12}$)]$^+$, 556 [Gd(DO3A − CH_3)]$^+$, 514 [Gd(DO3A − CH_2−C(CH_3)═CH_2)]$^+$. Anal. Calcd for $C_{61}H_{112}N_{16}O_{32}Gd_4$ (M_r = 2210): C, 33.14; H, 5.07; N, 10.14. Found: C, 33.09; H, 5.04; N, 10.11.

Results and Discussion

Synthesis of the Ligand and Complex. The ligand **4**, containing four DO3A units covalently appended onto the pentaerythrityl framework through the secondary amine nitrogen of DO3A, is synthesized by the reaction of DO3A-triethyl ester (**2**) with pentaerythrityl tetrabromide (**1**) in acetonitrile in the presence of anhydrous potassium carbonate followed by deprotection of the ester by alkaline hydrolysis. Pentaerythrityl tetrabromide is synthesized by brominating the tetrabenzenesulfonyl ester derivative, prepared by the reaction of pentaerythritol with four equivalents of benzenesulfonyl chloride and sodium bromide in diethylene glycol. The reaction is depicted in Scheme 1. The tetranuclear gadolinium(III) complex [Gd_4(**4**)$(H_2O)_8$] is synthesized by the reaction of performed ligand **4** with Gd_2O_3 in a 1:4 molar ratio in deionized water at 80−90 °C for 3 days. The absence of the uncomplexed Gd(III) ions is confirmed by testing the solution with xylenol orange.[53] The purity of the complex is checked by HPLC and the chromatogram shows a single peak.

The pendant arms appended cyclens form stable complexes with lanthanides since the 12-membered ring predisposes them to form the face of the antiprism, while the pendant arms form a quadrangular conformation in the complexes. Gd(III) complexes of such ligands are mostly nine-coordinate with tricapped trigonal prism or capped-square antiprism coordination geometry, and the former geometry is more favorable when the steric effect is absent.[54] Each DO3A unit of **4** coordinates to the Gd(III) ion through the four nitrogen donors and three carboxylate oxygens. Two water molecules coordinate to each metal ion in the inner-coordination sphere.

Spectroscopic Studies. Infrared Spectra. The infrared spectrum of **4** shows a broad band at 3409 cm^{-1} from the ν_s(O−H) vibration. The sharp bands at 1663.8 and 1384.8 cm^{-1} are the result of the ν_a(C═O) and ν_s(C═O) vibrations, respectively, of the carboxylic acid pendant arms. The low energy of the ν_a(C═O) vibration is caused by intramolecular hydrogen bonding. The bands at 2921.9 and 2851.9 cm^{-1} are assigned to the ν_s(C−H) vibration of the methylene groups. The weak bands at 1391.0 and 1098.1 cm^{-1} are assigned to the ν_s(O−H) and ν_s(C−N) vibrations, respectively. The infrared spectrum of [Gd_4(**4**)$(H_2O)_8$] shows a broad band at 3369.7 cm^{-1} assignable to the ν_s(OH) vibration. The band at 2989 cm^{-1} is assigned to the ν_s(C−H) vibration. The rocking and wagging vibrational modes of coordinated water molecules appear at 620 and 509.1 cm^{-1}, respectively. The ν(Gd−O) vibration occurs at 439 cm^{-1}.[55]

^{1}H NMR Spectrum. The ^{1}H NMR spectrum of **4** shows a sharp singlet at 2.08 ppm (s, 8 H) for the methylene protons of the pentaerythrityl moiety. The resonance at 2.32−2.38 ppm (16 H, t, J = 6.16 Hz) is assigned to the methylene protons (bonded to the C_8 and C_{12} carbons), and the signal at 2.73−2.78 ppm (16 H, t, J = 5.93 Hz) is attributed to the methylene protons (bonded to the C_9 and C_{11} carbons) of the cyclen ring of DO3A. The acetate methylene protons appear as a singlet at 2.88 ppm (24 H). The ^{1}H NMR spectrum of **4** is shown in Figure 1.

^{13}C NMR Spectrum. The ^{13}C NMR spectrum of **4** shows a signal at 30.68 ppm assignable to the methylene carbon of the pentaerythrityl moiety. The resonances at 50.88, 51.62, and 58.23 ppm are assignable to the methylene carbons of cyclen. The resonance at 65.61 ppm is the result of the

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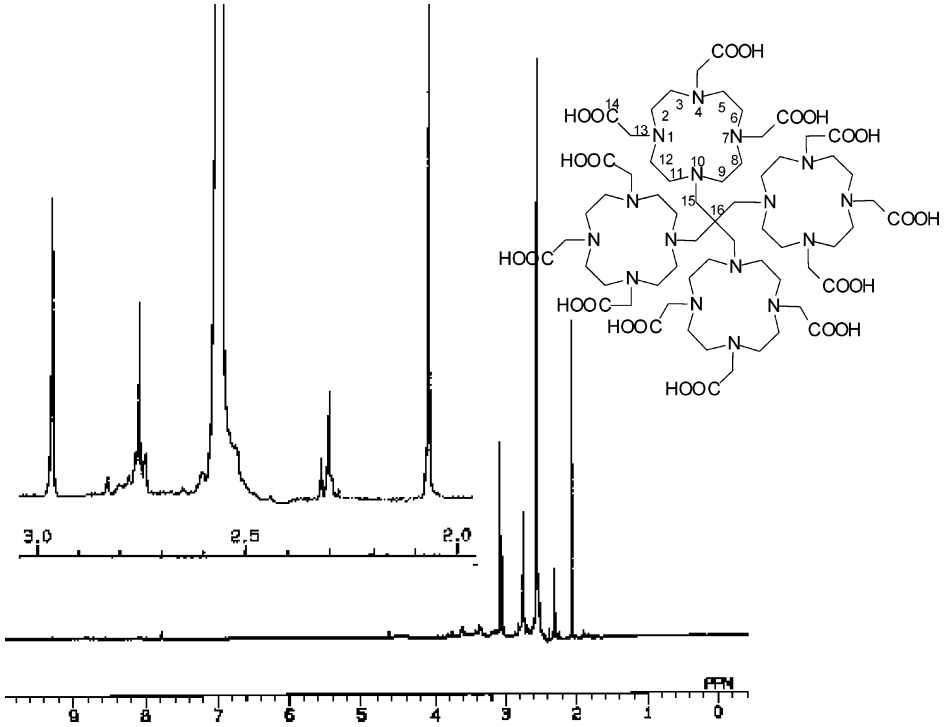

Figure 1. 400 MHz ^{1}H NMR spectrum of **4** in D_2O.

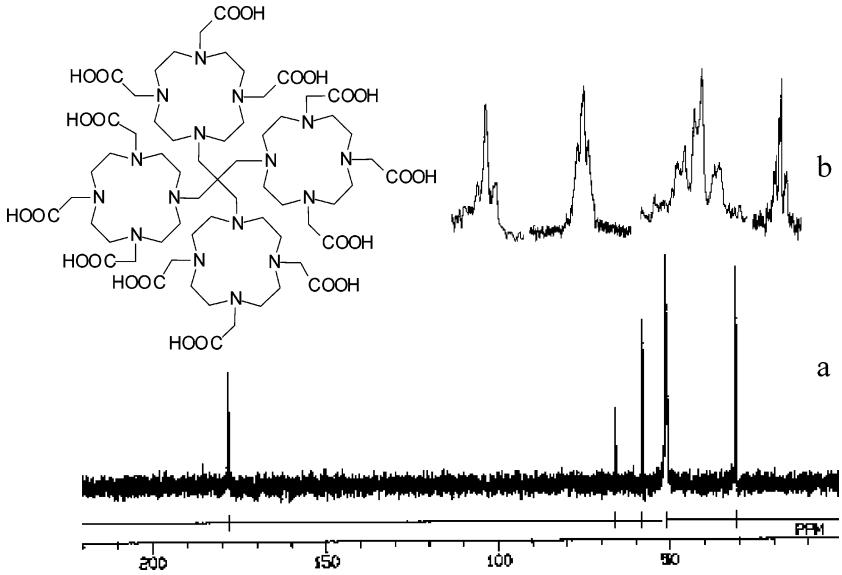

Figure 2. (a) 100 MHz ^{13}C NMR spectrum of **4** in D_2O and an (b) off-resonance decoupled ^{13}C NMR spectrum of **4**.

methylene carbon of the acetate arms. The acetate carbonyl carbons resonate at 177.91 ppm. The ^{13}C NMR spectrum of **4** is shown in Figure 2a.

Off-Resonance Decoupled ^{13}C NMR Spectrum of 4. The carbonyl carbon of the carboxylate function gives a singlet at 177.91 ppm. The methylene carbons of the pentaerythrityl moiety appear as triplet at 30.68 ppm. The cyclen methylene carbons appear as a triplet at 58.23 ppm and doublet of triplets at 51.62 and 50.88 ppm because of the distorted C_4 symmetry of the macrocyclic ring. The triplet at 65.61 ppm is from the methylene carbon of the acetate arms. The off-resonance decoupled ^{13}C NMR spectrum of **4** is shown in Figure 2b.

ESI Mass Spectrum. The ESI mass spectrum of $[Gd_4(\mathbf{4})(H_2O)_8]$ shows a peak at *m*/*z* 2210 for the molecular ion. The molecular ion undergoes fragmentation by the loss of coordinated water molecules and forms the species $[Gd_4(C_{61}H_{96}N_{16}O_{24})(H_2O)_5]^+$, $[Gd_4(C_{61}H_{96}N_{16}O_{24})(H_2O)_2]^+$, and $[Gd_4(C_{61}H_{96}N_{16}O_{24})]^+$ at *m*/*z* 2156, 2102, and 2066, respectively. The intense peak at *m*/*z* 2023 (100%) is assignable to the $[Gd_4(C_{60}H_{97}N_{16}O_{22})]^+$ fragment formed by the removal of COO^- from the acetate arm of DO3A. The peak at *m*/*z* 1997 is assignable to the $[Gd_4(C_{58}H_{93}N_{16}O_{22})]^+$ fragment formed by the removal of the acetate moiety from one of the DO3A units. The peak at *m*/*z* 1968 is attributable to the $[Gd_4(C_{57}H_{92}N_{15}O_{22})]^+$ species formed by the loss of $[C_4H_8$-

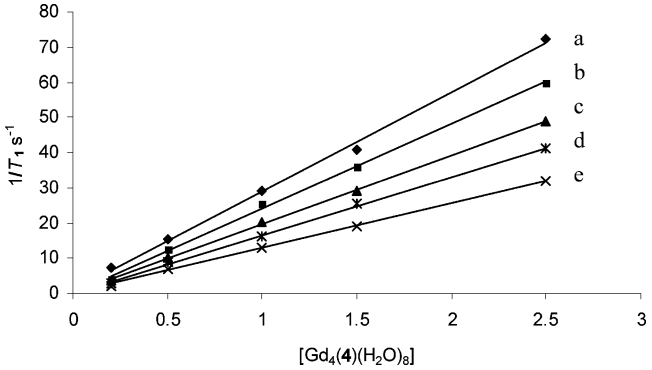

Figure 3. Plot of the concentration of $[Gd_4(\mathbf{4})(H_2O)_8]$ vs $1/T_1$ at (a) pH 5.6, (b) 6.5, (c) 7.2, (d) 9.5, and (e) 8.5.

$NO_2]^+$ caused by the α cleavage of the macrocyclic ring. The peak at *m*/*z* 1942 is assignable to the $[Gd_3(C_{47}H_{72}N_{12}O_{18})]^+$ fragment formed by the loss of one Gd(DO3A) chelate. The loss of two Gd(DO3A) chelates is characterized by the peak at *m*/*z* 1071 for the species $[Gd_2(C_{33}H_{54}N_8O_{12})]^+$. The peaks at *m*/*z* 556 and 514 are assignable to the fragments $[Gd(DO3A-CH_3)]^+$ and $[Gd(DO3A-CH_2-C(CH_3)=CH_2)]^+$, respectively.

EPR Spectrum and Magnetic Moment of $[Gd_4(4)(H_2O)_8]$. The X-band EPR spectrum consists of a broad band with no hyperfine splitting at room temperature and at liquid nitrogen temperature. The *g* value is 2.029 (X-band, LNT). The broad band shows that the dipolar interaction of Gd(III) is small, and the absence of any hyperfine splitting suggests a weak crystal-field effect. The magnetic moment is 7.69 μ_B which is close to the theoretical value for the free Gd(III) ion (7.83 μ_B). With the half-filled 4f shell, trivalent gadolinium has an $^8S_{7/2}$ ground state; since $L = 0$, the orbital contribution is almost entirely quenched, and the isotropic *g* value of 1.99 is universal. Because of the long spin-lattice relaxation times, EPR spectra may be observed at room temperature. Its magnetochemistry is, therefore, straightforward and perhaps the best known of all rare earth elements. Furthermore, the first excited state is located at some 30 000 cm^{-1} above the ground state. The zero-field splitting within this ground state is very weak, on the order of 10^{-2} cm^{-1}, and is not detectable in magnetism except in the temperature range of fractions of a Kelvin. The magnetic susceptibility is almost perfectly isotropic and follows the Curie law.[56]

Relaxivity Studies of $[Gd_4(4)(H_2O)_8]$. Longitudinal Relaxivity. The longitudinal relaxivity of $[Gd_4(\mathbf{4})(H_2O)_8]$ is 28.13 mM^{-1} s^{-1} at 24 MHz, 35 ± 0.1 °C, and pH 5.6. The plot of $1/T_1$ versus the concentration of $[Gd_4(\mathbf{4})(H_2O)_8]$ is given in Figure 3. The relaxivity is higher than that of $[Gd(DOTA)(H_2O)]^-$ (r_{1p} = 3.56 mM^{-1} s^{-1}, 20 MHz, 39 °C, pH 7.3)[57] and $[Gd(DO3A)(H_2O)_2]$ (4.8 mM^{-1} s^{-1}, 20 MHz, 40 °C).[58] The relaxivity of $[Gd_4(\mathbf{4})(H_2O)_8]$ is 5.86 times higher than that of $[Gd(DO3A)(H_2O)_2]$ which indicates that it is not merely the sum of the relaxivity of the four Gd(DO3A) chelates but that the tetramer accelerates water proton relaxivity as a single molecule wherein all the four [Gd(DO3A)] chelates function cooperatively. The relaxivity is based on the "molecular" relaxivity of the tetramer, and the r_{1p} value of 28.13 mM^{-1} s^{-1} is "7 per Gd". The higher relaxivity is the result of the increase in the molecular weight of the tetramer with a concomitant decrease in τ_R and of the presence of a large number of inner-sphere water molecules ($q = 8$). The proton residence time under physiological conditions is normally assumed to be equal to the residence time of the oxygen nucleus since, at neutral pH, the proton exchange is expected to take place primarily via the exchange of the whole water molecule. Thus, with an optimum water exchange rate, systems with large *q* values would lead to an increase in relaxivity. The longitudinal relaxivity of $[Gd_4(\mathbf{4})(H_2O)_8]$ (MW = 2210) is also higher than that of other tetranuclear Gd(DO3A) chelates bridged by a 2-hydroxypropylidine bond (MW = 2229−2501, r_{1p} = 8.4−9.8 mM^{-1} s^{-1}, 20 MHz, 40 °C),[46] dendrimer-conjugated Gd(DO3A) chelates (r_{1p} = 14−36 mM^{-1} s^{-1}, 25 MHz, 37 °C),[59] and the insulin-Gd(DO3A) conjugate $[Gd_{37}(API\text{-}(DO3ASQ)_{37})]$ (r_{1p} = 20.3 mM^{-1} s^{-1}, 20 MHz, 37 °C).[60]

The relaxivity per gadolinium(III) correlates well with the molecular weight for a series of mononuclear and polynuclear chelates.[1a,46] It has been recognized that the rigidity of the linker also plays a role in determining the relaxivity of polynuclear gadolinium(III) chelates. The rigidity of the linker, which is expected to affect the intrachelate rotational correlation time (τ_R*) that makes a contribution to the overall correlation time (τ_R), exerts a noticeable effect in polynuclear chelates.[46] The longitudinal relaxivity of the $[Gd_4(\mathbf{4})(H_2O)_8]$ tetramer and a similar tetranuclear Gd(DO3A) chelate appended onto the pentaerythrityl framework through a 2-hydroxypropylidine bond illustrates the importance of the linker in affecting the relaxivity: while $[Gd_4(\mathbf{4})(H_2O)_8]$ (MW = 2210) exhibits an r_{1p} of 28.13 mM^{-1} s^{-1}, the latter (MW = 2417) exhibits an r_{1p} of 8.4 mM^{-1} s^{-1}. The rotational motion of the tetramer $[Gd_4(\mathbf{4})(H_2O)_8]$ is limited by the linker with the bridgehead carbon of pentaerythrityl. As the rotational correlation time is reduced, the dipole−dipole interaction between the paramagnetic metal ion and the water protons is efficient, and as a result, more paramagnetic effect is transferred to the bulk solvent. In the case of polynuclear gadolinium(III) chelates, an intramolecular dipole−dipole mechanism contributes to electronic relaxation. This mechanism dominates electronic relaxation at high magnetic fields ($B > 5$ T). Its proportion to the overall relaxation decreases with decreasing magnetic field, and at fields used in magnetic resonance imaging (20 MHz), the importance of this contribution is negligible compared to the zero-field splitting mechanism, hence it cannot limit proton relaxivity.

pH Dependence of Longitudinal Relaxivity. The tetramer $[Gd_4(\mathbf{4})(H_2O)_8]$ exhibits pH-dependent longitudinal

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Table 1. Longitudinal Relaxivity of $[Gd_4(\mathbf{4})(H_2O)_8]$ at Different pH (24 MHz, 35 ± 0.1 °C)

pH[a]	r_{1p} (mM^{-1} s^{-1})
5.6	28.13
6.5	23.98
7.2	19.35
8.5	12.71
9.5	16.52

[a] pH was adjusted using TRIS buffer.

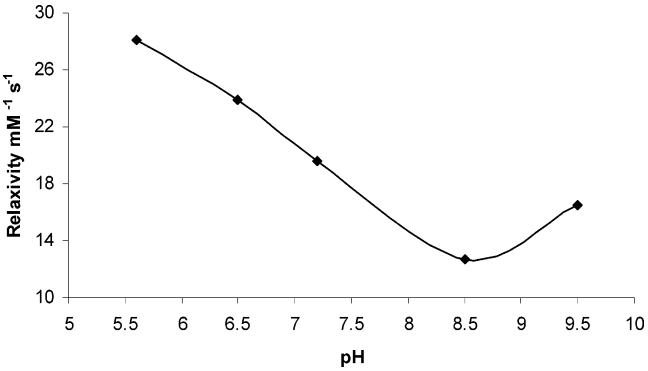

Figure 4. Plot of pH vs r_{1p} mM^{-1} s^{-1} (24 MHz, 35 ± 0.1 °C).

relaxivity. The relaxivity at pH 5.6 is 28.13 mM^{-1} s^{-1}, and it decreases with increasing pH. At pH 8.5, it reaches the low value of 12.71 mM^{-1} s^{-1} and further increases with increasing pH exhibiting a value of 16.52 mM^{-1} s^{-1} at pH 9.5. The longitudinal relaxivities at various pHs are given in Table 1. The plot of pH versus longitudinal relaxivity is shown in Figure 4. The increase of relaxivity at higher pH may be attributed to the OH^--catalyzed prototropic exchange of the bound water protons in the complex. The higher relaxivity at low pH may be caused by the protonation of the carboxylate oxygen and the nitrogen atoms of the ligand and the presence of a large number of water molecules hydrogen bonded to the periphery of the DO3A chelates with a concomitant increase in the outer-sphere contribution. The outer-sphere contribution to relaxivity depends mainly on the electronic relaxation rates and the rate at which an outer-sphere water molecule diffuses away from the gadolinium complex. The prototropic exchange is catalyzed by both $[H_3O^+]$ and $[OH^-]$ ions, and therefore, the relaxivity enhancement observed for the tetramer at pH 5.6 and 9.5 is the result of the occurrence of a fast prototropic exchange superimposed on the exchange of the whole water molecule. The higher relaxivities of $[Gd_4(\mathbf{4})(H_2O)_8]$ at both low and high pH indicate that it could be used as a pH-responsive MRI contrast agent. The pH-dependent modulation of relaxivity is useful for differentiating normal and tumor tissues because the extracellular pH of tumors is more acidic (ca. 6.8−6.9) than both tumor intracellular pH (7.2) and that of normal extracellular tissue (ca. 7.4).[61]

Transverse Relaxivity. The transverse relaxivity of the tetramer $[Gd_4(\mathbf{4})(H_2O)_8]$ is 129.97 mM^{-1} s^{-1} (24 MHz, 35 ± 0.1 °C, pH 5.6) which is 28 times higher than that of $[Gd(DOTA)(H_2O)]^-$ (r_{2p} = 4.75 mM^{-1} s^{-1}, 20 MHz, 39 °C).[57] The r_{2p}/r_{1p} ratio of 4.6 shows that it could be used as a T_2-weighted contrast agent.

Conclusions

The relaxation efficiency is higher for the tetramer $[Gd_4(\mathbf{4})(H_2O)_8]$ than that of the FDA approved mononuclear CAs and other tetranuclear chelates; this is consistent with the general unimportance of the antiferromagnetic coupling for lanthanide complexes. The molecular relaxivity of the tetramer is 7 per Gd which makes the complex versatile for in vivo applications. Polynuclear Gd(III) chelates covalently appended onto rigid structural motifs thus offer a beneficial increase in relaxivity resulting from the decrease in the rotational correlation time and the increase in the hydration number. The covalent conjugation of the Gd(DO3A) chelate onto the pentaerythrityl framework makes the complex remain intact under different experimental conditions, and it is expected to be as biocompatible and nontoxic as the parent $[Gd(DO3A)(H_2O)_2]$ chelate. Furthermore, such polynuclear chelates allow the administration of a lesser amount of CAs for intravenous injection compared to the mononuclear chelates. The study demonstrates the utility of multimetal Gd(III) chelates in accelerating water proton relaxivity.

In the case of the polynuclear Gd(III) chelates reported thus far, rotation does slow in proportion to the molecular weight and dimension, but the r_{1p} does not reach the maximum attainable value (greater than 100 mM^{-1} s^{-1} for a $q = 1$ system)[62] because either the attached chelate is too flexible (internal motions dominate τ_R) or water exchange becomes limiting ($\tau_m > T_{1M}$). In the design of new polynuclear Gd(III) complexes, to maximize relaxivity, the linking group should be sufficiently rigid to ensure that the Gd(III)−water vector does not rotate more rapidly than the whole complex (i.e., the attached chelates should not be too flexible) and the water exchange rate on the complexes is sufficiently rapid to ensure efficient transfer of relaxivity to the surrounding water. The development of CAs with an optimum water exchange rate continues to be a challenge for chemists. The pH-dependent modulation of proton relaxivity both at low and high pH makes this system versatile for tissue discrimination. Furthermore, the tetramer could be studied for evaluation of prototropic exchange mechanism. Because the intrinsic T_2 relaxation rates of the fluid surrounding tissues and organs are fast, the contrast agents are more effective in modulating T_1 and, therefore, the signal intensity of T_1-weighted images. The high transverse relaxivity of the tetramer $[Gd_4(\mathbf{4})(H_2O)_8]$ is expected to be effective in modulating T_2 and enhancing the signal intensity of the T_2-weighted images.

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Supporting Information Available: The T_1 values for different concentrations of $[Gd_4(\mathbf{4})(H_2O)_8]$ at pH 5.6, 6.5, 7.2, 8.5, and 9.5 (Table S1), the T_2 values of $[Gd_4(\mathbf{4})(H_2O)_8]$ (Table S2), the HPLC chromatogram of $[Gd_4(\mathbf{4})(H_2O)_8]$ (Figure S1), the EPR spectrum (X band) of $[Gd_4(\mathbf{4})(H_2O)_8]$ (Figure S2), and the plot of the concentration of $[Gd_4(\mathbf{4})(H_2O)_8]$ versus $1/T_2$ (Figure S3). This material is available free of charge via the Internet at http://pubs.acs.org.

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