Inorg. Chem. **2006**, *45*, 244–254

Synthesis and Physicochemical Characterization of Two Gadolinium(III) TTDA-like Complexes and Their Interaction with Human Serum Albumin

Ming-Hung Ou,[†,‡] Chien-Hung Tu,[†] Su-Ching Tsai,[†] Wei-Thung Lee,[†] Gin-Chung Liu,[§] and Yun-Ming Wang*[,†]

Faculty of Medicinal and Applied Chemistry, Graduate Institute of Pharmaceutical Sciences, Kaohsiung Medical University, and Department of Medical Imaging, Kaohsiung Medical University, 100 Shih-Chuan First Road, Kaohsiung 807, Taiwan, Republic of China

Received March 3, 2005

Two novel derivatives of TTDA (3,6,10-tri(carboxymethyl)-3,6,10-triazadodecanedioic acid), TTDA-BOM and TTDA-*N*′-BOM, each having a benzyloxymethyl group, were synthesized. ^{17}O NMR longitudinal and transverse relaxation rates and chemical shifts of aqueous solutions of their Gd(III) complexes were measured at variable temperature with a magnetic field strength of 9.4 T. The water exchange rate (k_{ex}^{298}) values for $[Gd(TTDA\text{-}BOM)(H_2O)]^{2-}$ (117×10^6 s^{-1}) and $[Gd(TTDA\text{-}N'\text{-}BOM)(H_2O)]^{2-}$ (131×10^6 s^{-1}) are significantly higher than those of $[Gd(DTPA)(H_2O)]^{2-}$ (4.1×10^6 s^{-1}) and $[Gd(BOPTA)(H_2O)]^{2-}$ (3.45×10^6 s^{-1}). The rotational correlation time (τ_R) values for $[Gd(TTDA\text{-}BOM)(H_2O)]^{2-}$ (119 ps) and $[Gd(TTDA\text{-}N'\text{-}BOM)(H_2O)]^{2-}$ (125 ps) are higher than those of $[Gd(DTPA)(H_2O)]^{2-}$ (103 ps) and $[Gd(TTDA)(H_2O)]^{2-}$ (104 ps). The stepwise stoichiometric binding constants of $[Gd(TTDA\text{-}BOM)(H_2O)]^{2-}$ and $[Gd(TTDA\text{-}N'\text{-}BOM)(H_2O)]^{2-}$ bound to HSA are obtained by ultrafiltration studies. Fluorescent probe displacement studies exhibit that $[Gd(TTDA\text{-}BOM)(H_2O)]^{2-}$ and $[Gd(TTDA\text{-}N'\text{-}BOM)(H_2O)]^{2-}$ can displace dansylsarcosine from HSA with inhibition constants (K_i) of 1900 and 1600 μM, respectively; however, they are not able to displace warfarin. These results indicate that $[Gd(TTDA\text{-}BOM)(H_2O)]^{2-}$ and $[Gd(TTDA\text{-}N'\text{-}BOM)(H_2O)]^{2-}$ have a weak binding to site II on HSA. In addition, the mean bound relaxivity ($\bar{r}_{1b}$) and bound relaxivity (r_1^b) values for the $[Gd(TTDA\text{-}BOM)(H_2O)]^{2-}$/HSA and $[Gd(TTDA\text{-}N'\text{-}BOM)(H_2O)]^{2-}$/HSA adducts are obtained by ultrafiltration and relaxivity studies, respectively. The bound relaxivity of these adducts values are significantly higher than those of $[Gd(BOPTA)(H_2O)]^{2-}$/HSA and $[Gd(DTPA\text{-}BOM_3)(H_2O)]^{2-}$/HSA. These results also suggest that bound relaxivity is site dependent. In binding sites studies of Gd(III) chelates to HSA, a significant decrease of the relaxation rates (R_{1obs}) was observed for the $[Eu(TTDA\text{-}BOM)(H_2O)]^{2-}$ complex which was added to the $[Gd(TTDA\text{-}N'\text{-}BOM)(H_2O)]^{2-}$/HSA solution, and this indicated that these Gd(III) complexes share the same HSA binding site. Finally, as measured by the Zn(II) transmetalation process, the kinetic stability of these Gd(III) complexes are significantly higher than that of $[Gd(DTPA\text{-}BMA)(H_2O)]$.

Introduction

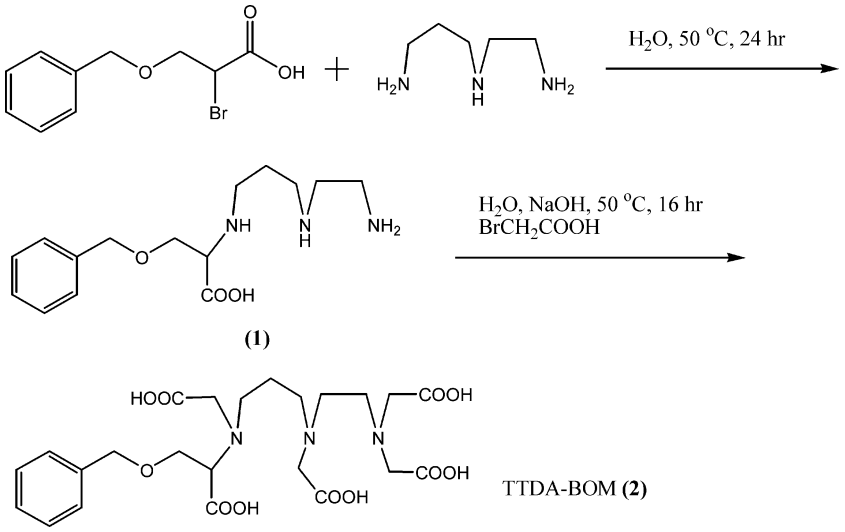
The development of novel applications of MRI contrast agents largely depends on the availability of systems which have high relaxivities or are targeted on specific organs/tissues.[1,2] With the magnetic fields commonly used in MRI (0.5−1.5 T), high relaxivities are obtained in the presence of a long molecular reorientational time and fast exchange of the water molecule coordinated to the paramagnetic metal ion. Long molecular reorientational times are pursued either through the formation of covalent conjugates between a paramagnetic complex and a macromolecular substrate[3,4] or through the formation of noncovalent adducts between suitably functionalized complexes and endogenous (e.g.,

* To whom correspondence should be addressed. Phone: 886-7-3121101 ext. 2218. Fax: 886-7-3125339. E-mail: ymwang@kmu.edu.tw.
† Faculty of Medicinal and Applied Chemistry.
‡ Graduate Institute of Pharmaceutical Sciences.
§ Department of Medical Imaging.

(1) Caravan, P.; Ellison, J. J.; McMurry, T. J.; Lauffer, R. B. *Chem. Rev.* **1999**, *99*, 2293−2352.
(2) Merbach, A. E.; Toth, E. *The Chemistry of Contrast Agents in Medical Magnetic Resonance Imaging*; Wiley: New York, 2001.
(3) Aime, S.; Botta, M.; Geninatti Crich, S.; Giovenzana, G.; Palmisano, G.; Sisti, M. *Bioconjugate Chem.* **1999**, *10*, 192−199.
(4) Lebduskova, P.; Kotek, J.; Hermann, P.; Vander Elst, L.; Muller, R. N.; Lukes, I.; Peters, J. A. *Bioconjugate Chem.* **2004**, *15*, 881−889.

10.1021/ic050329r CCC: $33.50
Published on Web 12/10/2005

Scheme 1

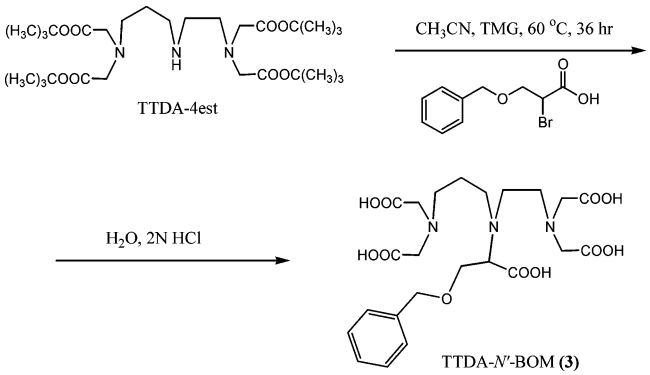


serum albumin) or exogenous (e.g., poly-β-cyclodextrin) substrates.[5,6]

Research on MRI contrast agents has been primarily focused on complexes of Gd(III) since this metal ion, with an *S* ground-state electronic structure, couples to a large magnetic moment with a long electron spin relaxation time ($\sim 10^{-9}$ s at the magnetic field strengths of interest for MRI applications), two properties that ensure an optimum efficiency for nuclear spin relaxation of the interacting nuclei. Other general requirements of contrast agents for MRI are low toxicity,[7] rapid excretion after administration, good water solubility, and low osmotic potential of the solutions clinically used. Moreover, since the free metal ions are poorly tolerated, they must be coordinated by a strongly binding ligand that occupies most of the available coordination sites. Eventually, the preferred metal complexes, in addition to showing high thermodynamic (and possibly kinetic) stability, should present at least one water molecule in their inner coordination sphere, in rapid exchange with the bulk solvent, to strongly affect the relaxation of all solvent protons. ^{17}O NMR spectroscopy is an efficient technique for the study of the water exchange rate because the oxygen of the coordinated water molecule, which is directly bound to the paramagnetic Gd(III) ion, is a more sensitive antenna than the protons.[8]

In this study, the ligands, which are the derivatives of TTDA bearing a benzyloxymethyl group, were synthesized (Schemes 1 and 2) to increase lipophilicity. Their Gd(III) complexes which have faster water exchange rates are expected to increase the binding affinity with protein, leading to increased bound relaxivity. ^{17}O NMR relaxation rates and

Scheme 2



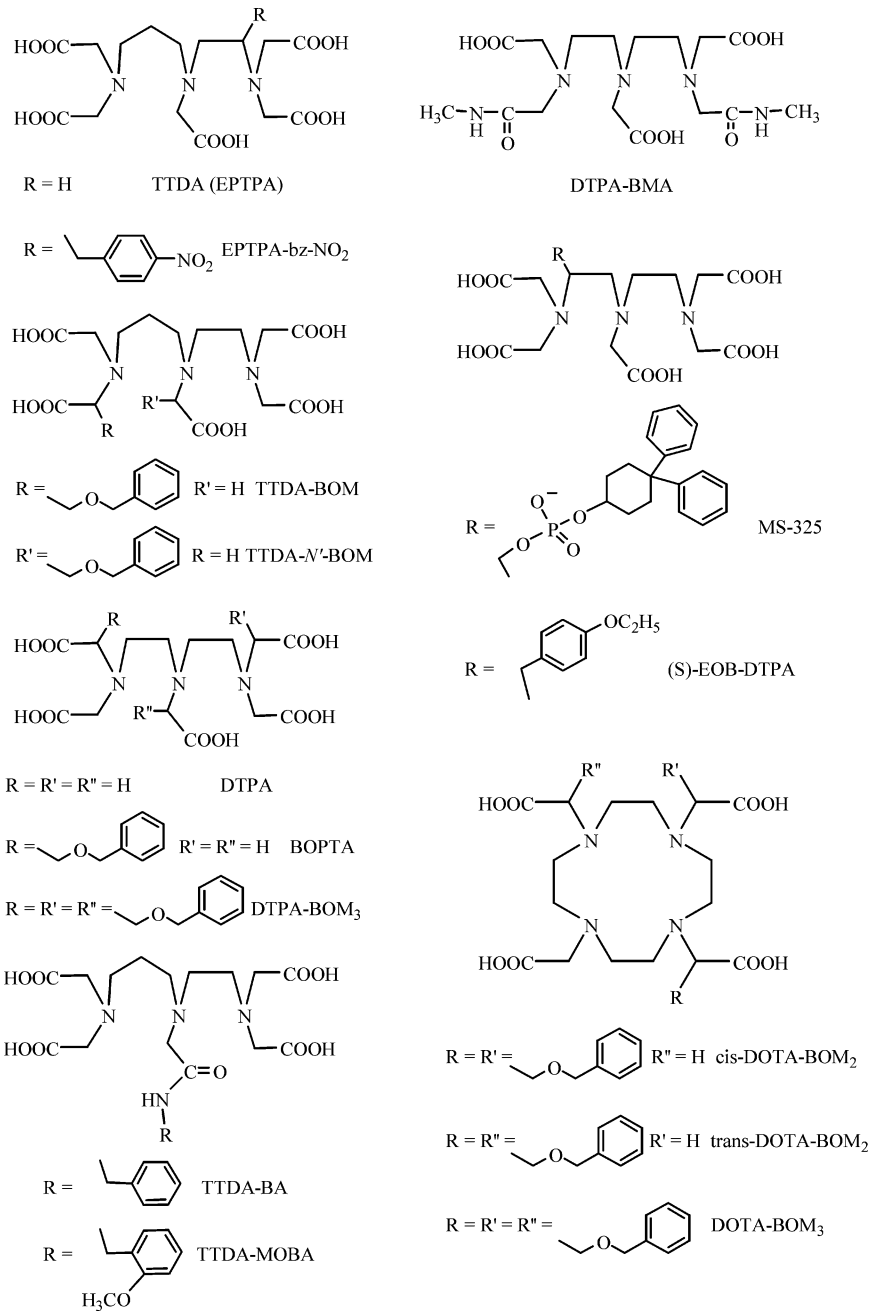
angular frequencies of the free Gd(III) complexes at variable temperature are reported. To support the protein binding studies of Gd(III) complexes, further protein−complex binding was determined by ultrafiltration and relaxivity studies, and the results are compared with the previous results for other related Gd(III) complexes.

Materials and Methods

Chemicals. 2-Bromo-3-(phenylmethoxy)propanoic acid and 3,6,-10-triazadodecane-3,10-tetraacetic acid (tetra)-*tert*-butyl ester (TTDA-4est) were prepared using a previously published method.[9,10] All other reagents used for the synthesis of the ligands were purchased from commercial sources, unless otherwise noted. ^{17}O-enriched water (20.3%) was purchased from Isotec Inc. Human serum albumin (HSA, product no. A-1653, Fraction V Powder 96−99%) was purchased from Sigma and used without any further purification.[11] The molecular weight was assumed to be 66.5 kDa. (±)-Warfarin [3-(acetonylbenzyl)-4-hydroxycoumarin] and (*S*)-(+)-ibuprofen [(*S*)-(+)-α-methyl-4-(2-methylpropyl)phenylacetic acid] were purchased from Aldrich. The 50mM phosphate buffer was used to maintain the pH of all solutions (pH 7.4) containing HSA.

(5) Caravan, P.; Cloutier, N. J.; Greenfield, M. T.; McDermid, S. A.; Dunham, S. U.; Bulte, J. W. M.; Amedio, J. C., Jr.; Looby, R. J.; Supkowski, R. M.; Horrocks, W. D., Jr.; McMurry, T. J.; Lauffer, R. B. *J. Am. Chem. Soc.* **2002**, *124*, 3152−3162.
(6) Aime, S.; Botta, M.; Fedeli, F.; Gianolio, E.; Terreno, E.; Anelli, P. *Chem.—Eur. J.* **2001**, *7*, 5261−5269.
(7) Ranganathan, R. S.; Raju, N.; Fan, H.; Zhang, X.; Tweedle, M. F.; Desreux, J. F.; Jacques, V. *Inorg. Chem.* **2002**, *41*, 6856−6866.
(8) Micskei, K.; Helm, L.; Brucher, E.; Merbach, A. E. *Inorg. Chem.* **1993**, *32*, 3844−3850.
(9) Grassman, W.; Wunsch, E.; Deufel, P.; Zwick, A. *Chem. Ber.* **1958**, *91*, 538−541.
(10) Wang, Y. M.; Li, C. R.; Huang, Y. C.; Ou, M. H.; Liu, G. C. *Inorg. Chem.* **2005**, *44*, 382−392.
(11) Cohn, E. J.; Strong, L. E.; Hughes, W. L., Jr.; Mulford, D. J.; Ashworth, J. N.; Melin, M.; Taylor, H. L. *J. Am. Chem. Soc.* **1946**, *68*, 459−475.

Chart 1



^{1}H (400 MHz), ^{13}C (100 MHz), and ^{17}O (54.2 MHz) NMR spectra were recorded on a Varian Gemini-400 spectrometer with 5 mm sample tubes. The ^{13}C NMR spectra were referenced internally relative to 3-(trimethylsilyl)-1-propanesulfonic acid for D_2O. Gadolinium concentrations were determined by ICP-MS with a Perkin Elmer OPTIMA 2000.

Synthesis of Ligands. (*R*,*S*)-2-[3-(2-Amino-ethylamino)-propylamino]-3-benzyloxy Propanoic Acid (1). 2-Bromo-3-(phenylmethoxy)propanoic acid (5.2 g, 20 mmol) was added over 10 min to a solution of *N*-(2-aminoethyl)-1,3-propanediamine (17.7 mL, 140.1 mmol) in H_2O (80 mL). The mixture was stirred at 50 °C for 24 h and then evaporated by rotary evaporation. The oil was obtained, dissolved in 100 mL of distilled water, and alkalized with ammonia water to pH 11.2, and the solution was loaded into an AG 1 × 8 anion-exchange resin column (200−400 mesh, HCO_2^- form). The column was eluted with H_2O first to remove excess *N*-(2-aminoethyl)-1,3-propanediamine, and then it was eluted with with 0.001 N formic acid. The acidic solution (2 L) was collected and evaporated to dryness. The trace of formic acid was removed by coevaporation with 200 mL of water five times, and a colorless viscous oil was obtained (2.66 g, 45.1%). MS (ESI): *m*/*z* 296.1 $[M + H]^+$. Anal. Calcd (Found) for $C_{15}H_{25}N_3O_3 \cdot 2H_2O$: C, 54.36 (54.40); H, 8.82 (8.91); N, 12.68 (12.53). ^{1}H NMR (D_2O): δ 1.74−1.90 (m, 2H, −N−CH_2−CH_2−CH_2−N−), 2.74−2.93 (m, 8H, −CH_2−), 3.24−3.29 (t, 1H, CH_2C*H*COOH), 3.52−3.69 (m, 2H, OCH_2CH), 4.43 (s, 2H, CH_2Ar), 7.25−7.35 (m, 5H, Ar*H*). ^{13}C NMR (D_2O): δ 24.7, 36.6, 43.8, 44.3, 46.2, 62.4, 70.2, 72.5, 127.8, 127.9, 128.3, 136.8, 170.4.

(*R*,*S*)-4-Carboxy-5,9,12-tris(carboxymethyl)-1-phenyl-2-oxa-5,9,12-triazatetradecan-14-oic Acid (TTDA-BOM, 2). NaOH (2 N, 50 mL) was added to a stirred solution of bromoacetic acid (13.5 g, 97.1 mmol) in H_2O (50 mL) at 0−5 °C. After the mixture was heated to 50 °C, a solution of (*R*,*S*)-2-[3-(2-amino-ethylamino)-propylamino]-3-benzyloxy propanoic acid (4.4 g, 14.9 mmol) in 2

N NaOH (30 mL) was added over 30 min, and the resulting solution was stirred at 50 °C for 16 h. During this time, a pH of 10 was maintained by continuous addition of 2 N NaOH. The reaction solution was stirred at room temperature for 12 h and loaded into an AG 1 × 8 anion exchange column (200−400 mesh, HCO_2^- form). This was eluted first with H_2O and then with a gradient of formic acid. The 0.3−0.4 N formic acid solution containing the product was evaporated to dryness, and the trace of formic acid was removed by coevaporation with 200 mL of water five times. The light yellow compound obtained was TTDA-BOM (1.7 g, 22.1%). MS (ESI): *m*/*z* 528.6 $[M + H]^+$. Anal. Calcd (Found) for $C_{23}H_{33}N_3O_{11}\cdot 2H_2O$: C, 49.02 (49.11); H, 6.62 (6.68); N, 7.45 (7.36). ^{1}H NMR (D_2O): δ 1.92−2.08 (m, 2H, −N−CH_2−CH_2−CH_2−N−), 2.83−3.31 (m, 8H, −CH_2−), 3.54−3.77 (m, 11H, OCH_2CH and NCH_2COOH), 4.37 (s, 2H, CH_2Ar), 7.18−7.27 (m, 5H, ArH). ^{13}C NMR (D_2O): δ 20.9, 43.2, 50.4, 51.9, 52.6, 55.1, 55.3, 56.4, 56.5, 61.1, 66.2, 72.8, 127.7, 128.3, 128.7, 136.9, 165.8, 170.2, 170.4, 172.2, 172.5.

(*R*,*S*)-3-Benzyloxy-2-{[2-(biscarboxymethylamino)-ethyl]-[3-(biscarboxymethylamino)-propyl]-amino}-propanoic acid (TTDA-*N*′-BOM, 3). Tetramethylguanidine (TMG) (15.2 mL, 121.8 mmol) was added to a stirred solution of TTDA-4est (10.0 g, 17.4 mmol) in CH_3CN (50 mL), and the mixture was stirred for 30 min at 10 °C. 2-Bromo-3-(phenylmethoxy)propanoic acid (6.8 mL, 26.2 mmol) was added over 10 min, and the resulting mixture was stirred, refluxed overnight, and then evaporated in a vacuum. Distilled water was added to the residue to dissolve the tetramethylguanidine. The hydrate was removed by filtering the reaction mixture with a Büchner funnel and washing it with distilled water. Then, 2 N HCl (50 mL) was added to the residue, and the mixture was stirred for 12 h at room temperature. The reaction solution was alkalized with ammonia water to pH 11.0, and the solution loaded into an AG 1 × 8 anion-exchange resin column (200−400 mesh, HCO_2^- form). The product was eluted first with 2 L of water and then with a gradient of formic acid. The 0.3−0.4 N formic acid solution containing the product was evaporated to dryness, and the trace formic acid was removed by coevaporation with 200 mL of water five times, yielding 3.4 g (25.2%) of TTDA-*N*′-BOM as a colorless viscous oil. MS (ESI): *m*/*z* 528.6 $[M + H]^+$. Anal. Calcd (Found) for $C_{23}H_{33}N_3O_{11}\cdot 2H_2O$: C, 49.02 (49.15); H, 6.62 (6.71); N, 7.45 (7.38). ^{1}H NMR (D_2O): δ 1.97−2.03 (m, 2H, −N−CH_2−CH_2−CH_2−N−), 2.91−3.34 (m, 8H, −CH_2−), 3.58−3.82 (m, 11H, OCH_2CH and NCH_2COOH), 4.40 (s, 2H, CH_2Ar), 7.18−7.25 (m, 5H, ArH). ^{13}C NMR (D_2O): δ 26.8, 42.7, 49.4, 52.1, 52.3, 54.0, 54.9, 55.0, 55.1, 59.7, 64.9, 72.5, 127.8, 127.9, 128.2, 136.2, 165.1, 165.4, 169.0, 172.6, 172.8.

Complexation. The Dy(III) and Gd(III) complexes were prepared by dissolving the ligand (0.05 mmol) in H_2O (3 mL) and adjusting the pH of the solution to 7.5 with 1 N NaOH. To these solutions, 2.5 mL of an aqueous solution of $LnCl_3$ (0.05 mmol) was added dropwise, maintaining the pH at 7.5 with 1 N NaOH. The Ln(III) chelate formations were instantaneous at room temperature. The absence of free lanthanide ions in the solutions was verified by the xylenol orange test. The solutions were then evaporated under reduced pressure. The purity of $[Gd(TTDA\text{-}BOM)(H_2O)]^{2-}$ and $[Gd(TTDA\text{-}N'\text{-}BOM)(H_2O)]^{2-}$ were determined by HPLC and mass, and two fractions of pure isomers A and B were observed. As the starting ligands are racemic mixtures of 4*R* and 4*S* enantiomers, the two peaks represent the coalition of the 4*S*9*S*/4*R*9*R* and 4*S*9*R*/4*R*9*S* enantiomers, respectively. The HPLC chromatograms of these complexes are deposited as Supporting Information (Figures 1S and 2S).

HPLC Method. All HPLC experiments were performed on an Amersham ÄKTAbasic 10 instrument equipped with an Amersham UV-900 detector and an Amersham Frac-900 fraction collector. A Supelcosil RP-C18 column (5 μm, 4.6 × 250 mm) was used. The eluent was a gradient of 0−100% MeOH using 0.1% TFA in water as the aqueous phase with a 0.5 mL/min flow rate.

Sample Preparation for ^{17}O Longitudinal and Transverse Relaxation Rate Measurement. $Gd(ClO_4)_3$ stock solutions were prepared by dissolving a slight excess of Gd_2O_3 in perchloric acid. The pH of the solution was adjusted to 4 after filtration. The lanthanide concentration was determined by chelatometric titration with an Na_2H_2EDTA solution using xylenol orange indicator. All solutions were prepared by weight. For the preparation of the $[Gd(TTDA\text{-}BOM)(H_2O)]^{2-}$ and $[Gd(TTDA\text{-}N'\text{-}BOM)(H_2O)]^{2-}$ complexes, weighed quantities of solid ligands were dissolved in double-distilled water, and then a weighed amount of $Gd(ClO_4)_3$ stock solution was added dropwise to form the chelate complexes with a ligand excess of 2−3%. The pHs of all solutions were adjusted using 1 M NaOH or $HClO_4$. The solutions were then evaporated under reduced pressure, and an equivalent weight of 5.5% ^{17}O-enriched water was added.

Relaxation Time Measurement. The relaxation times, T_1, of aqueous solutions of gadolinium complexes were measured to determine relaxivity, r_1. All measurements were made using a NMR relaxometer operating at 20 MHz and 37.0 ± 0.1 °C (NMS-120 Minispec, Bruker). Before each measurement the relaxometer was tuned and calibrated. The values of r_1 were determined from six data points generated by an inversion−recovery pulse sequence.

^{17}O NMR Measurements. The hydration numbers of $[Gd(TTDA\text{-}BOM)(H_2O)]^{2-}$ and $[Gd(TTDA\text{-}N'\text{-}BOM)(H_2O)]^{2-}$ were determined by the method of Alpoim et al.[12] The ^{17}O NMR spectra were recorded by a Varian Gemini-400 spectrometer at 25 °C. The dysprosium-induced (Dy-induced) ^{17}O shift (d.i.s.) measurements were determined using D_2O as an external standard.

For the variable-temperature ^{17}O NMR measurements, the concentrations and pH values of the solutions used were as follows: $[Gd(TTDA\text{-}BOM)(H_2O)]^{2-}$ 0.050 mol kg^{-1}, pH 5.2; $[Gd(TTDA\text{-}N'\text{-}BOM)(H_2O)]^{2-}$ 0.026 mol kg^{-1}, pH 5.5. The ^{17}O longitudinal and transverse relaxation rates and chemical shift measurements were carried out with a Varian Gemini-400 (9.4 T, 54.2 MHz) spectrometer, equipped with a 10 mm probe, using an external D_2O lock. The Varian 600 temperature control unit was used to stabilize the temperature in the range of 278−338 K. The samples were sealed in glass spheres and fitted into 10 mm NMR tubes to eliminate susceptibility corrections to the chemical shift. Solutions containing 5.5% of the ^{17}O isotope were used.

Preparation of 4.5% (w/v) Human Serum Albumin (HSA) and GdL/HSA Solutions. HSA was dissolved in a 10 mM sodium phosphate and 150 mM sodium chloride (pH 7.4) solution. Two 4.5% (w/v) HSA solutions were made: one containing the GdL chelate at a concentration of 5.3 mM and another without the GdL chelate.

Ultrafiltration Measurements of Binding of GdL Chelate to HSA. GdL/HSA solutions ranging from 0.05 to 5.3 mM GdL chelate in 4.5% (w/v) HSA were made by combining appropriate amounts of 4.5% (w/v) HSA and 5.3 mM GdL in 4.5% (w/v) HSA solution. Aliquots (400 μL) of these solutions were placed in 30 kDa ultrafiltration units, incubated at 37.0 ± 0.1 °C for 20 min, and then centrifuged at 3500 *g* for 10 min. The filtrates obtained from these ultrafiltration units were used to measure the concentration of free GdL chelate. Duplicate aliquots were operated for each

(12) Alpoim, M. C.; Urbano, A. M.; Geraldes, C. F. G. C.; Peters, J. A. *J. Chem. Soc., Dalton Trans.* **1992**, *3*, 463−467.

sample. The Gd(III) concentrations of the GdL/HSA solutions and ultrafiltrates were obtained using ICP-MS.

Determination of Site-Specific Binding Dissociation Constants for GdL. A solution containing 5 μM warfarin or dansylsarcosine and 200 μM GdL chelate in 5 μM HSA was prepared in phosphate buffer (pH 7.4). Aliquots of this solution were 2-fold diluted serially by addition of a solution that contained 5 μM warfarin or dansylsarcosine and 5 μM HSA. The fluorescence of 100 μL aliquots of each of these samples was measured in quadruplicate in a 96 well plate. Sixteen 100 μL aliquots of the solution containing 5 μM warfarin or dansylsarcosine in 5 μM HSA were also measured in the same 96 well plate, along with sixteen 100 μL aliquots of 5 μM HSA. These controls set the dynamic range for fluorescence change when the GdL chelate displaces a fluorescent probe. The inhibition constant, K_i, of the GdL chelate was determined by the method of Caravan et al.[5,13]

Transmetalation Experiments. This technique is based on measurement of the evolution of the water proton paramagnetic longitudinal relaxation rate (R_1^p) of a phosphate buffer solution containing 2.5 mM Gd(III) complex and 2.5 mM $ZnCl_2$. Relaxation rate (R_1^p) measurements were performed at 37.0 $\pm$ 0.1 °C and 20 MHz (Bruker Minispec 120).

Data Analysis. The simultaneous least-squares fitting of ^{17}O NMR data and the binding parameters to HSA were determined by fitting the experimental data using the program SCIENTIST for Windows by MICROMATH, version 2.0.

Results and Discussion

Synthesis of the Ligands. The ligand TTDA-BOM was prepared in two steps. First, *N*-(2-aminoethyl)-1,3-propanediamine was selectively monoalkylated on a primary amino group with 2-bromo-3-(phenylmethoxy)propanoic acid. The alkylation occurred on the terminal propylene nitrogen because the longer carbon backbone led to increased nitrogen basicity. Second, bromoacetic acid was chosen because of its lower steric effect. The TTDA-*N*′-BOM ligand was synthesized from TTDA-4est, whose central nitrogen is easy to alkylate with 2-bromo-3-(phenylmethoxy)propanoic acid. The purity of the TTDA-BOM and TTDA-*N*′-BOM ligands was determined by HPLC. The HPLC chromatograms of these ligands are deposited as Supporting Information (Figures 3S and 4S).

Relaxometric Studies of the Gd(III) Complex. The longitudinal water proton relaxivity (r_{1p}) results from the contribution of the water molecules in the inner and outer coordination spheres

$$r_{1p} = R_{1p}^{is} + R_{1p}^{os} \quad (1)$$

The outer-sphere term, R_{1p}^{os}, describes the contribution of the water molecules diffusing near the paramagnetic chelate and is given by[14]

$$R_{1p}^{os} = C^{os}\left(\frac{1}{aD}\right)[7J(\omega_s) + 3J(\omega_H)] \quad (2)$$

where C^{os} is a constant (5.8×10^{-13} s^{-2} M^{-1}), a is the minimum distance between the metal and the diffusing water molecules, D is the relative solute−solvent diffusion coefficient, and the dependence on the electronic relaxation times is expressed in the non-Lorentzian spectral density functions $J(\omega_i)$. For compounds of similar size, it can be assumed that the outer-sphere mechanism makes a similar contribution at high magnetic flux densities (>0.1 T). Therefore, the small differences in relaxivity can be ascribed to differences in R_{1p}^{is}. The contribution from the exchange of the water molecules directly coordinated to the paramagnetic metal ion is obtained by eq 3[15,16]

$$R_{1p}^{is} = [C]q/[55.6(T_{1M} + \tau_M)] \quad (3)$$

where [C] represents the molar concentration of the gadolinium(III) complex, q is the number of water molecules bound to metal ions, T_{1M} is the longitudinal relaxation time of the inner-sphere water protons, and τ_M is the residence lifetime of the bound water.

Table 1. Relaxivity r_1 of $[Gd(TTDA\text{-}BOM)(H_2O)]^{2-}$, $[Gd(TTDA\text{-}N'\text{-}BOM)(H_2O)]^{2-}$, $[Gd(BOPTA)(H_2O)]^{2-}$, $[Gd(DTPA)(H_2O)]^{2-}$, and $[Gd(TTDA)(H_2O)]^{2-}$ at 37.0 $\pm$ 0.1 °C and 20 MHz

complex	pH	relaxivity r_1 (mM^{-1} s^{-1})
$[Gd(TTDA\text{-}BOM)(H_2O)]^{2-}$	7.4 $\pm$ 0.1	4.42 $\pm$ 0.02
$[Gd(TTDA\text{-}N'\text{-}BOM)(H_2O)]^{2-}$	7.4 $\pm$ 0.1	4.44 $\pm$ 0.03
$[Gd(BOPTA)(H_2O)]^{2-}$[a]	7.4 $\pm$ 0.1	4.39 $\pm$ 0.01
$[Gd(DTPA)(H_2O)]^{2-}$[b]	7.6 $\pm$ 0.1	3.89 $\pm$ 0.03
$[Gd(TTDA)(H_2O)]^{2-}$[c]	7.5 $\pm$ 0.1	3.85 $\pm$ 0.03

[a] Data were obtained from ref 17. [b] Ref 18. [c] Ref 19.

The longitudinal relaxivity (r_1) values of $[Gd(TTDA\text{-}BOM)(H_2O)]^{2-}$ and $[Gd(TTDA\text{-}N'\text{-}BOM)(H_2O)]^{2-}$ compared with those of $[Gd(BOPTA)(H_2O)]^{2-}$ (BOPTA = 4-carboxy-5,8,11-tris(carboxymethyl)-1-phenyl-2-oxa-5,8,11-triazatridecan-13-oic acid, and the structural formula is shown in Chart), $[Gd(DTPA)(H_2O)]^{2-}$, and $[Gd(TTDA)(H_2O)]^{2-}$ are shown in Table 1. The values of $[Gd(TTDA\text{-}BOM)(H_2O)]^{2-}$ and $[Gd(TTDA\text{-}N'\text{-}BOM)(H_2O)]^{2-}$ are 4.42 and 4.44 mM^{-1} s^{-1}, respectively, which are similar to that of $[Gd(BOPTA)(H_2O)]^{2-}$.[17] However, the relaxivity values are higher than those of $[Gd(DTPA)(H_2O)]^{2-}$ [18] and $[Gd(TTDA)(H_2O)]^{2-}$.[19] It is quite clear that the introduction of the benzyloxymethyl group in DTPA or TTDA increases the relaxivity values.

Dy(III)-Induced ^{17}O Water NMR Shifts. The Dy(III)-induced water ^{17}O NMR shifts against the Dy(III) chelate concentration for solutions of $DyCl_3$, $[Dy(TTDA\text{-}BOM)(H_2O)]^{2-}$, and $[Dy(TTDA\text{-}N'\text{-}BOM)(H_2O)]^{2-}$ in D_2O at 25 °C are deposited as Supporting Information (Figure 5S). The slope for $DyCl_3$ is −436.4 ppm/mM, and a hydration number of eight has previously been proposed for the dysprosium(III) ion.[20−22] For $[Dy(TTDA\text{-}BOM)(H_2O)]^{2-}$ and [Dy-

(13) Caravan, P.; Greenfield, M. T.; Li, X.; Sherry, A. D. *Inorg. Chem.* **2001**, *40*, 6580−9587.
(14) Hwang, L. P.; Freed, J. H. *J. Chem. Phys.* **1975**, *63*, 4017−4025.
(15) Swift, T. J.; Connick, R. E. *J. Chem. Phys.* **1962**, *37*, 307−320.
(16) Luz, Z.; Meiboom, S. *J. Chem. Phys.* **1964**, *40*, 1058−66.
(17) Uggeri, F.; Aime, S.; Anelli, P. L.; Botta, M.; Brocchetta, M.; Haeen, C.; Ermondi, G.; Grandi, M.; Paoli, P. *Inorg. Chem.* **1995**, *34*, 633−642.
(18) Weinmann, H. J.; Brasch, R. C.; Press, W. R.; Wesbey, G. E. *Am. J. Roentgenol.* **1984**, *142*, 619−624.
(19) Wang, Y. M.; Lee, C. H.; Liu, G. C.; Sheu, R. S. *J. Chem. Soc., Dalton Trans.* **1998**, *24*, 4113−4118.
(20) Cossy, C.; Barnes, A. C.; Enderby, J. E.; Merbach, A. E. *J. Chem. Phys.* **1989**, *90*, 3254−3260.

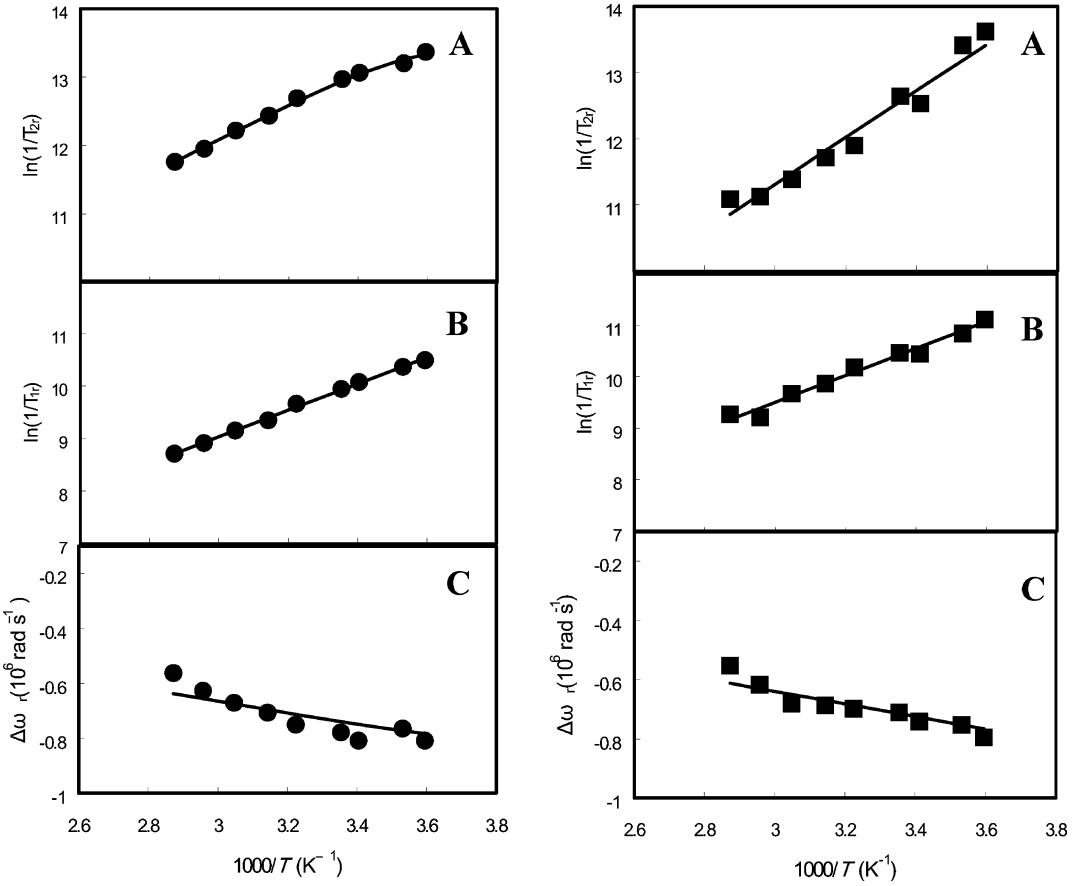


Figure 1. Temperature dependence of (**A**) transverse and (**B**) longitudinal ^{17}O relaxation rates and (**C**) ^{17}O chemical shifts at $B = 9.4$ T for [Gd(TTDA-BOM)$(H_2O)]^{2-}$ (left) and [Gd(TTDA-*N*′-BOM)$(H_2O)]^{2-}$ (right). The lines represent simultaneous least-squares fits to all data points displayed.

Table 2. Parameters Obtained from Simultaneous Fitting of ^{17}O NMR Relaxation and Chemical Shift Data[a]

parameter	[Gd(TTDA-BOM)$(H_2O)]^{2-}$	[Gd(TTDA-*N*′-BOM)$(H_2O)]^{2-}$	[Gd(TTDA)$(H_2O)]^{2-}$[b]	[Gd(ETPTA)$(H_2O)]^{2-}$[c]	[Gd(DTPA)$(H_2O)]^{2-}$[d]	[Gd(DTPA)$(H_2O)]^{2-}$[e]	[Gd(BOPTA)$(H_2O)]^{2-}$
k_{ex}^{298} (10^6 s^{-1})	117 ± 8	131 ± 12	146 ± 17	330 ± 40	4.1 ± 0.3	3.3 ± 0.2	3.45[f]
$\Delta H^{\ddagger}$ (kJ mol^{-1})	18.7 ± 0.7	19.1 ± 1.4	23.1 ± 0.5	27.9 ± 11	52.0 ± 1.4	51.6 ± 1.4	
$\Delta S^{\ddagger}$ (J mol^{-1} K^{-1})	-27.6 ± 2.3	-25.3 ± 3.6	-11.1 ± 3.1	11.0 ± 3.0	56.2 ± 5.0	53.0 ± 4.7	
A/h (10^6 rad s^{-1})	-3.1 ± 0.2	-3.1 ± 0.2	-3.2 ± 0.3	-3.9 ± 0.2	-3.8 ± 0.2	-3.8 ± 0.2	
τ_R^{298} (ps)	119 ± 8	125 ± 10	104 ± 12	75 ± 6	103 ± 10	58 ± 11	88 ± 2[g]
C_{os}	**0.1**	**0.1**	**0**	**0.1**	0.13 ± 0.06	0.18 ± 0.04	
E_R (kJ mol^{-1})	20.9 ± 2.8	20.5 ± 2.2	24.8 ± 1.5	17.7 ± 1.0	18 ± 2	17.3 ± 0.8	
method	^{17}O	^{17}O	^{17}O	^{17}O, EPR, NMRD	^{17}O	^{17}O, EPR, NMRD	NMRD

[a] The bold values were fixed in the fitting procedure. [b] Data were obtained from ref 10. [c] Ref 25. [d] Ref 8. [e] Ref 26. [f] Ref 24. [g] Ref 17.

(TTDA-*N*′-BOM)$(H_2O)]^{2-}$ at pH 5.0 the slopes are −66.2 and −72.4 ppm/mM, respectively. Therefore, the [Dy(TTDA-BOM)$(H_2O)]^{2-}$ and [Dy(TTDA-*N*′-BOM)$(H_2O)]^{2-}$ complexes at pH 5.0 contain 1.2 and 1.3 inner-sphere water molecules per Dy(III) ion, respectively. The number of Ln(III)-bound water molecules in the complexes will provide information about the coordination mode of the ligand. In general, the coordination number of the Gd(III) complex with polyamine(aminocarboxylate) is nine. The coordination site of the Gd(III) complex with a TTDA derivatives (eight sites are available for three amine nitrogen atoms and five carboxylate oxygen atoms) is occupied by one water molecule.

Water-Exchange Rate and Rotational Correlation Time Studies of Gd(III) Complexes. The experimental data for a given Gd(III) complex, that is, the ^{17}O NMR chemical shifts ($\Delta\omega_r$) and the longitudinal ($1/T_{1r}$) and transverse ($1/T_{2r}$) relaxation rates were analyzed simultaneously (eqs 1S−9S used in the Supporting Information). The results for [Gd(TTDA-BOM)$(H_2O)]^{2-}$ and [Gd(TTDA-*N*′-BOM)$(H_2O)]^{2-}$ are plotted in Figure 1 with the corresponding curve representing the results of the best fitting of the data according to the equations.

As illustrated by Table 2, there are a large number of parameters influencing the data obtained by the different techniques. The ^{17}O NMR technique has the advantage that the outer-sphere contributions to the relaxation rates are negligibly small, which is a result of the oxygen nucleus being closer to the paramagnetic center when bound in the

(21) Cossy, C.; Helm, L.; Powell, D. H.; Merbach, A. E. *New J. Chem.* **1995**, *19*, 27−35.
(22) Kowall, T.; Foglia, F.; Helm, L.; Merbach, A. E. *J. Am. Chem. Soc.* **1995**, *117*, 3790−3799.

inner sphere. From Table 2, the scalar coupling constant values of $[Gd(TTDA\text{-}BOM)(H_2O)]^{2-}$ and $[Gd(TTDA\text{-}N'\text{-}BOM)(H_2O)]^{2-}$, $A/\hbar$, are very similar to those obtained for other Gd(III) complexes (-3.8×10^6 and -3.4×10^6 rad s^{-1} for $[Gd(DTPA)(H_2O)]^{2-}$ and $[Gd(DOTA)(H_2O)]^{-}$, respectively) with one inner-sphere water molecule, and they were confirmed with the above Dy(III)-induced water ^{17}O NMR study. Over the whole temperature range, the transverse ^{17}O relaxation rates ($1/T_{2r}$) decrease with increasing temperature, indicating that these systems are in the fast-exchange regime.[23] $1/T_{2r}$ is thus determined by the relaxation rate of the coordinated water molecule ($1/T_{2m}$), which is influenced by the water residence time ($\tau_M = 1/k_{ex}$), the longitudinal electronic relaxation rate ($1/T_{1e}$), and the scalar coupling constant ($A/\hbar$). Estimates of the k_{ex}^{298} values are 117×10^6 s^{-1} and 131×10^6 s^{-1} for $[Gd(TTDA\text{-}BOM)(H_2O)]^{2-}$ and $[Gd(TTDA\text{-}N'\text{-}BOM)(H_2O)]^{2-}$, respectively. We can find that the k_{ex}^{298} values of $[Gd(TTDA\text{-}BOM)(H_2O)]^{2-}$ and $[Gd(TTDA\text{-}N'\text{-}BOM)(H_2O)]^{2-}$ are significantly higher than those of $[Gd(DTPA)(H_2O)]^{2-}$ [8] and $[Gd(BOPTA)(H_2O)]^{2-}$,[24] but they are similar to those of $[Gd(TTDA)(H_2O)]^{2-}$ [10] and $[Gd(EPTPA\text{-}bz\text{-}NO_2)(H_2O)]^{2-}$ (EPTPA-bz-NO_2 = (*p*-nitrobenzyl)ethylenepropylenetriaminepentaacetic acid).[25] The longer amine backbone of these TTDA-like series ligands is pulled tightly into the first coordination sphere, and this results in high steric constraints on the water binding site.[26] This result leads to an increase in the water exchange rate, which falls into the optimal range, and high relaxivities can be expected when the molecular reorientational time of the complexes is lengthened to the nanosecond range.[27]

Nine-coordinate monohydrated Gd(III) chelates with poly(aminocarboxylates), including all commercial Gd(III)-based MRI contrast agents, undergo a dissociative (D) or dissociative-interchange (I_d) water exchange, in contrast to the associative (A) mechanism on $[Gd(H_2O)_8]^{3+}$.[2] The activation volume ($\Delta V^{\ddagger}$) for the water exchange reaction of $[Gd(TTDA)(H_2O)]^{2-}$ has been determined (i.e., $\Delta V^{\ddagger} = +6.6$);[25] therefore, the water exchange of $[Gd(TTDA\text{-}BOM)(H_2O)]^{2-}$ and $[Gd(TTDA\text{-}N'\text{-}BOM)(H_2O)]^{2-}$ must be dissociatively activated on the basis of its analogy to $[Gd(TTDA)(H_2O)]^{2-}$. As has been observed above, the water exchange rate decreases from $[Gd(H_2O)_8]^{3+}$ (830×10^6 s^{-1}) to the complex with one inner-sphere water molecule, $[Gd(TTDA)(H_2O)]^{2-}$ (146×10^6 s^{-1}). This rate decrease is accompanied by an increase of $\Delta H^{\ddagger}$. Since the results for the Gd(III) complexes with TTDA-BOM and TTDA-*N*′-BOM obey this trend, we thus conclude that water exchange on these complexes takes place most probably via a limiting dissociative D mechanism.

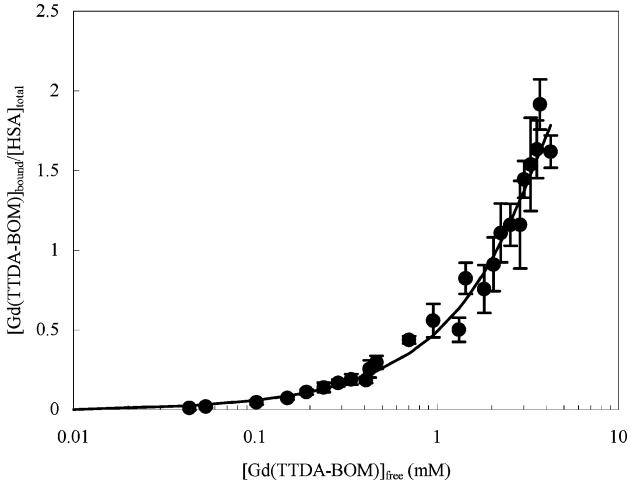


Figure 2. Plot of the ratio of bound $[Gd(TTDA\text{-}BOM)(H_2O)]^{2-}$ per HSA versus the concentration of free $[Gd(TTDA\text{-}BOM)(H_2O)]^{2-}$ (37.0 ± 0.1 °C, phosphate buffer, pH 7.4).

At 298 K, the values of rotational correlation time (τ_R) for Gd(III) complexes with TTDA-BOM and TTDA-*N*′-BOM obtained from the fitting of ^{17}O NMR data are shown in Table 2. The τ_R values of $[Gd(TTDA\text{-}BOM)(H_2O)]^{2-}$ (119 ps) and $[Gd(TTDA\text{-}N'\text{-}BOM)(H_2O)]^{2-}$ (125 ps) are higher than those of $[Gd(DTPA)(H_2O)]^{2-}$ (103 ps)[8] and $[Gd(TTDA)(H_2O)]^{2-}$ (104 ps).[10] It is indicated that the introduction of the benzyloxymethyl group in TTDA increases the τ_R value and causes the higher relaxivity of the Gd(III) complexes, as shown in Table 1. The τ_R values of $[Gd(DTPA)(H_2O)]^{2-}$ and $[Gd(BOPTA)(H_2O)]^{2-}$ obtained from the fitting of the ^{17}O NMR, EPR, and NMRD data are 58 and 88 ps, respectively.[25,16] The similar relationship between the τ_R value and relaxivity was observed in Gd(III) complexes with DTPA and BOPTA. The rotational dynamics of Gd(III) complexes as potential MRI contrast agents are a crucial point in determining proton relaxivity.

Ultrafiltration Studies of Gd(III) Chelates to HSA. HSA binding studies for $[Gd(TTDA\text{-}BOM)(H_2O)]^{2-}$ and $[Gd(TTDA\text{-}N'\text{-}BOM)(H_2O)]^{2-}$ were performed by ultrafiltration through a membrane with a 30 kDa MW cutoff. The concentration of unbound GdL chelates and the total concentration of GdL chelates were determined by ICP-MS. The results were shown in Figures 2 and 3 as a plot of the ratio of bound $[Gd(TTDA\text{-}BOM)(H_2O)]^{2-}$ and $[Gd(TTDA\text{-}N'\text{-}BOM)(H_2O)]^{2-}$ per HSA molecule ($\bar{n}$) versus the concentration of unbound $[Gd(TTDA\text{-}BOM)(H_2O)]^{2-}$ and $[Gd(TTDA\text{-}N'\text{-}BOM)(H_2O)]^{2-}$, respectively. From Figures 2 and 3, $\bar{n}$ increases as the molar concentration of the unbound GdL chelate increases. The value at which $\bar{n}$ reaches a plateau would give the number of binding sites on HSA when it is saturated with GdL chelates.[5] It is apparent that saturation does not happen at 2 mol of bound $[Gd(TTDA\text{-}BOM)(H_2O)]^{2-}$ and $[Gd(TTDA\text{-}N'\text{-}BOM)(H_2O)]^{2-}$ per HSA. Moreover, it was possible to fit the data to a stoichiometric model to estimate the stepwise binding constants.[28] Table 3 shows the stepwise stoichiometric binding constants of [Gd(TTDA-

(23) Micskei, K.; Powell, D. H.; Helm, L.; Brucher, E.; Merbach, A. E. *Magn. Reson. Chem.* **1993**, *31*, 1011−1020.
(24) Aime, S.; Botta, M.; Fasano, M.; Terreno, E. *Acc. Chem. Res.* **1999**, *32*, 941−949.
(25) Laus, S.; Ruloff, R.; Toth, E.; Merbach, A. E. *Chem.—Eur. J.* **2003**, *9*, 3555−3566.
(26) Powell, D. H.; Ni Dhubhghaill, O. M.; Pubanz, D.; Helm, L.; Lebedev, Y. S.; Schlaepfer, W.; Merbach, A. E. *J. Am. Chem. Soc.* **1996**, *118*, 9333−9346.
(27) Aime, S.; Botta, M.; Fasano, M.; Terreno, E. *Chem. Soc. Rev.* **1998**, *27*, 19−29.
(28) Klotz, I. M. *Ligand Receptor Energetics—A Guide for the Perplexed*; John Wiley & Sons: New York, 1987.

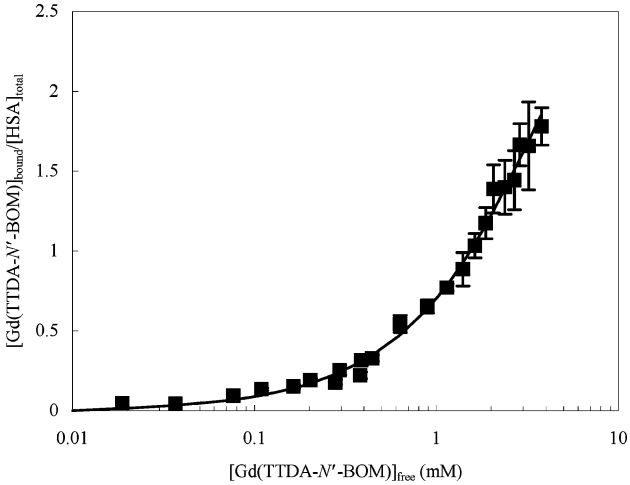


Figure 3. Plot of the ratio of bound $[Gd(TTDA\text{-}N'\text{-}BOM)(H_2O)]^{2-}$ per HSA versus the concentration of free $[Gd(TTDA\text{-}N'\text{-}BOM)(H_2O)]^{2-}$ (37.0 ± 0.1 °C, phosphate buffer, pH 7.4).

Table 3. Association Constants for the Binding of $[Gd(TTDA\text{-}BOM)(H_2O)]^{2-}$, $[Gd(TTDA\text{-}N'\text{-}BOM)(H_2O)]^{2-}$, and MS-325 to HSA in Phosphate Buffer (pH 7.4) at 37.0 ± 0.1 °C

binding constant	$[Gd(TTDA\text{-}BOM)(H_2O)]^{2-}$	$[Gd(TTDA\text{-}N'\text{-}BOM)(H_2O)]^{2-}$	MS-325[a]
K_{a1}	$5.9 \pm 1.6 \times 10^2\ M^{-1}$	$7.0 \pm 2.5 \times 10^2\ M^{-1}$	$1.1 \times 10^4\ M^{-1}$
K_{a2}	$0.9 \pm 0.3 \times 10^2\ M^{-1}$	$1.9 \pm 0.9 \times 10^2\ M^{-1}$	$8.4 \times 10^2\ M^{-1}$
K_{a3}	−	−	$2.6 \times 10^2\ M^{-1}$
K_{a4}	−	−	$4.3 \times 10^2\ M^{-1}$

[a] Data were obtained from ref 5.

$BOM)(H_2O)]^{2-}$ and $[Gd(TTDA\text{-}N'\text{-}BOM)(H_2O)]^{2-}$ bound to HSA. From Table 3, the affinities of $[Gd(TTDA\text{-}BOM)(H_2O)]^{2-}$ and $[Gd(TTDA\text{-}N'\text{-}BOM)(H_2O)]^{2-}$ to HSA are significantly lower than that of MS-325 (4-(*R*)-[(4,4-diphenyl-cyclohexyl)-phosphanooxyethyl]3,6,9-triaza-3,6,9-tris-(methoxy-carbonyl)-undecanedioato gadolinium(III)).[5] The introduction of a benzyloxymethyl group onto TTDA results in only weak binding of these GdL chelates to HSA.

Two sites on HSA bind a variety of drugs.[29,30] Site I, subdomain IIA, binds warfarin and salicylate. Site II, subdomain IIIA, is capable of binding ibuprofen and dansylsarcosine.[31] The displacement of warfarin and dansylsarcosine from HSA by $[Gd(TTDA\text{-}BOM)(H_2O)]^{2-}$ and $[Gd(TTDA\text{-}N'\text{-}BOM)(H_2O)]^{2-}$ chelates was studied. The effect of increasing the concentration of the Gd(III) chelate to solutions containing equal amounts of warfarin or dansylsarcosine and HSA was investigated by measuring the fluorescence with added Gd(III) chelate. Figure 4 shows the displacement study of $[Gd(TTDA\text{-}BOM)(H_2O)]^{2-}$. The displacement study of $[Gd(TTDA\text{-}N'\text{-}BOM)(H_2O)]^{2-}$ is in the Supporting Information (Figure 6S). The results indicate that $[Gd(TTDA\text{-}BOM)(H_2O)]^{2-}$ and $[Gd(TTDA\text{-}N'\text{-}BOM)(H_2O)]^{2-}$ are weak displacers for dansylsarcosine. Warfarin is not displaced by these Gd(III) chelates. It is clear that [Gd-

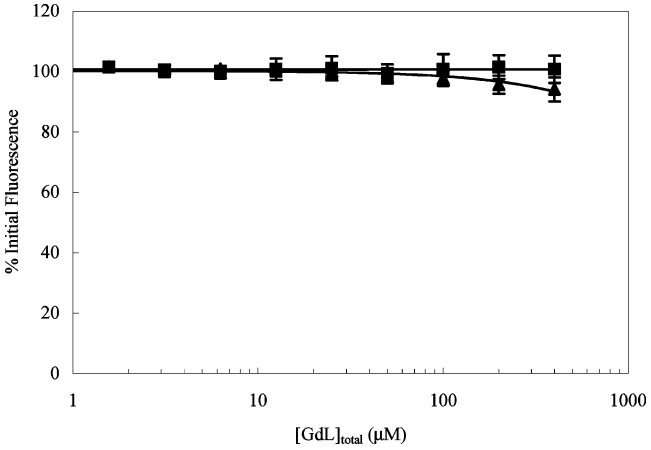


Figure 4. Displacement of fluorescent probes dansylsarcosine (▲, 5 μM) and warfarin (■, 5 μM) from equimolar HSA by $[Gd(TTDA\text{-}BOM)(H_2O)]^{2-}$ (37.0 ± 0.1 °C, phosphate buffer, pH 7.4).

Table 4. Inhibition Constants for [GdL] at 37.0 ± 0.1 °C in Phosphate Buffer at pH 7.4 for the Displacement of Various Fluorescent Probes

	K_i	
complex	dansylsarcosine	warfarin
$[Gd(TTDA\text{-}BOM)(H_2O)]^{2-}$	1900 ± 1300	no displacement
$[Gd(TTDA\text{-}N'\text{-}BOM)(H_2O)]^{2-}$	1600 ± 980	no displacement

$(TTDA\text{-}BOM)(H_2O)]^{2-}$ and $[Gd(TTDA\text{-}N'\text{-}BOM)(H_2O)]^{2-}$ have a lower affinity for site II. The inhibition constants[5,13] for these Gd(III) complexes, K_i, were obtained by fitted the displacement data shown in Table 4. The first stepwise dissociation constants ($K_d = 1/K_a$) for $[Gd(TTDA\text{-}BOM)(H_2O)]^{2-}$ and $[Gd(TTDA\text{-}N'\text{-}BOM)(H_2O)]^{2-}$ were 1695 and 1429 μM, respectively. It is interesting to find that the inhibition constant for dansylsarcosine is similar to the first stoichiometric dissociation constant for these Gd(III) chelates. It is obvious that at a low concentration of dansylsarcosine, the Gd(III) chelate is mainly bound to site II even though this is a weak binding.

Site-Dependent Relaxivity Study. To evaluate the dependence of site on relaxivity, an average bound relaxivity, $\bar{r}_{1b}$, was defined and determined as in eq 4[5,13]

$$\bar{r}_{1b} = \frac{1/T_1^{obs} - {}^1/T_1^{dia} - [GdL]_f r_1^F}{[GdL]_b} \qquad (4)$$

where $[GdL]_f$ and $[GdL]_b$ are the concentration of free GdL and bound GdL chelate, and r_1^F is the relaxivity of free GdL chelate. For the ultrafiltration experiment, relaxation times (T_1) were also determined at 20 MHz and 37.0 ± 0.1 °C. Figure 5 shows $\bar{r}_{1b}$ as a function of the concentration of $[Gd(TTDA\text{-}BOM)(H_2O)]^{2-}$ and $[Gd(TTDA\text{-}N'\text{-}BOM)(H_2O)]^{2-}$ in the presence of 4.5% (w/v) HSA. As the concentration of the GdL chelate decreases, the $\bar{r}_{1b}$ value increases. The $\bar{r}_{1b}$ values for the $[Gd(TTDA\text{-}BOM)(H_2O)]^{2-}$/HSA and $[Gd(TTDA\text{-}N'\text{-}BOM)(H_2O)]^{2-}$/HSA adducts are 64.5 and 60.2 $mM^{-1}\ s^{-1}$, respectively. It is clear that bound relaxivity is site dependent.

Relaxivity Studies of Gd(III) Chelates to HSA. Moreover, assessment of the HSA-binding capability of a given contrast agent is also conveniently carried out in vitro by

(29) Sudlow, G.; Birkett, D. J.; Wade, D. N. *Mol. Pharmacol.* **1975**, *11*, 824−832.
(30) Sudlow, G.; Birkett, D. J.; Wade, D. N. *Mol. Pharmacol.* **1976**, *12*, 1052−1061.
(31) Yamasaki, K.; Maruyama, T.; Kragh-Hansen, U.; Otagiri, M. *Biochim. Biophys. Acta* **1996**, *1259*, 147−157.

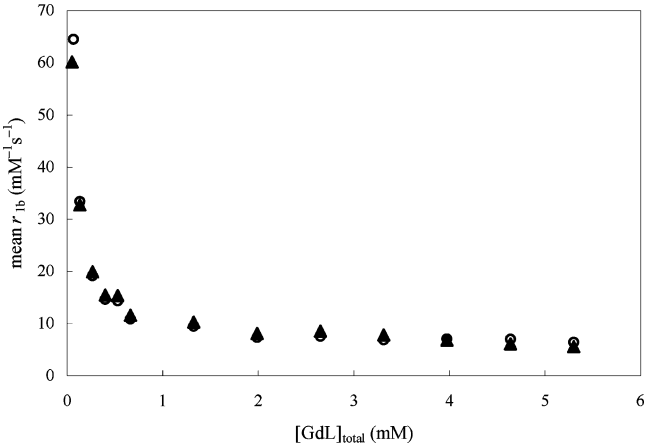


Figure 5. Mean bound relaxivity versus total concentration of [Gd(TTDA-BOM)$(H_2O)]^{2-}$ (○) and [Gd(TTDA-*N*′-BOM)$(H_2O)]^{2-}$ (▲) (20 MHz, 37.0 ± 0.1 °C, 4.5% (w/v) HSA, phosphate buffer, pH 7.4).

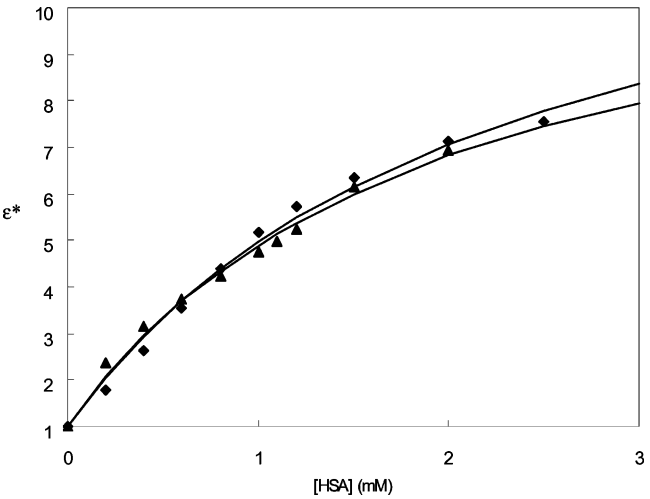


Figure 6. E titration of a 0.1 mM solution of [Gd(TTDA-BOM)$(H_2O)]^{2-}$ (◆) and [Gd(TTDA-*N*′-BOM)$(H_2O)]^{2-}$ (▲) with HSA (20 MHz, 25.0 ± 0.1 °C, 50 mM phosphate buffer, pH 7.4). The solid curves represent the best fit.

exploiting the proton relaxation enhancement (PRE) method, which allows us to obtain reliable values for the thermodynamic binding constant (K_A), the number of binding sites (n), and the relaxivity of the macromolecular adduct (r_1^b). The experimental procedure consists of carrying out two distinct titrations, called E and M titrations (see the Supporting Information).[32]

The results of the E and M titrations of the Gd(III) complexes with TTDA-BOM and TTDA-*N*′-BOM are shown in Figures 6 and 7. These complexes show an increase in the longitudinal water proton relaxation rate upon addition of HSA. The binding parameters calculated for the single binding site (n) to HSA of these Gd(III) complexes are presented in Table 5. For [Gd(TTDA-BOM)$(H_2O)]^{2-}$ and [Gd(TTDA-*N*′-BOM)$(H_2O)]^{2-}$, the K_A values are 4.6×10^2 M^{-1} and 5.4×10^2 M^{-1}, respectively. These values are lower than that of MS-325 (3.0×10^4 M^{-1}),[33] but they are similar to those of [Gd(BOPTA)$(H_2O)]^{2-}$ (4.0×10^2 M^{-1})[2] and [Gd((*S*)-EOB-DTPA)$(H_2O)]^{2-}$ (7.7×10^2 M^{-1}) ((*S*)-EOB-DTPA = (4*S*)-4-(4-ethoxybenzyl)-3,6,9-tris(carboxylatomethyl)-3,6,9-triazaundecanedioic acid).[34] The affinities of [Gd(TTDA-BOM)$(H_2O)]^{2-}$ and [Gd(TTDA-*N*′-BOM)$(H_2O)]^{2-}$ for HSA estimated from the relaxivity study are slightly lower than those estimated from the ultrafiltration experiment.

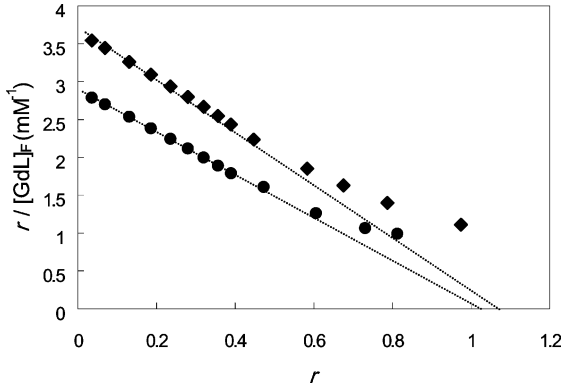


Figure 7. Scatchard plots for the binding of [Gd(TTDA-BOM)$(H_2O)]^{2-}$ (◆) and [Gd(TTDA-*N*′-BOM)$(H_2O)]^{2-}$ (●) to HSA (20 MHz, 25.0 ± 0.1 °C, phosphate buffer, pH 7.4, 0.6 mM [HSA]). $r = [GdL - HSA]/[HSA]_T$.

In addition, the values of bound relaxivity (r_1^b) can be obtained by multiplying b by r_1^F.[35] The values of r_1^b for the [Gd(TTDA-BOM)$(H_2O)]^{2-}$/HSA and [Gd(TTDA-*N*′-BOM)$(H_2O)]^{2-}$/HSA adducts are 65.8 and 61.5 mM^{-1} s^{-1}, respectively. These values are similar to that determined by the ultrafiltration experiment. The r_1^b values of [Gd(TTDA-BOM)$(H_2O)]^{2-}$ and [Gd(TTDA-*N*′-BOM)$(H_2O)]^{2-}$ are significantly higher than those of [Gd(BOPTA)$(H_2O)]^{2-}$, [Gd(DTPA-BOM_3)$(H_2O)]^{2-}$ ([4*S*-[4*R**,8(*R**),12*R**]]-4-carboxy-5,11-bis(carboxymethyl)-8-[1-carboxy-2-(phenylmethoxy)-ethyl]-1-phenyl-12-[(phenylmethoxy)methyl]-2-oxa-5,8,11-triazatridecan-13-oic acid) and [Gd((S)-EOB-DTPA)$(H_2O)]^{2-}$. One of the reasons for this is that the water exchange rate values of [Gd(TTDA-BOM)$(H_2O)]^{2-}$ and [Gd(TTDA-*N*′-BOM)$(H_2O)]^{2-}$ are significantly higher than those of [Gd(BOPTA)$(H_2O)]^{2-}$, [Gd(DTPA-BOM_3)$(H_2O)]^{2-}$, and [Gd((S)-EOB-DTPA)$(H_2O)]^{2-}$. To support this conclusion, a plot of r_1^b for a series of Gd(III) complexes versus the τ_M values as measured for the free Gd(III) complexes at 25 °C is shown in Figure 8, and a linear relationship can be observed when the τ_M values of free Gd(III) complexes fall in the range of 10−300 ns. In addition, the noncovalent interactions of these Gd(III) complexes with the HSA promote a slowing of the molecular motion, thus increasing the rotational correlation time and obtaining the high bound relaxivity.

Binding Sites Studies of Gd(III) Chelates to HSA. HSA can interact with many substrates and display quite different structural features. To investigate the binding sites of the [Gd(TTDA-BOM)$(H_2O)]^{2-}$ and [Gd(TTDA-*N*′-BOM)$(H_2O)]^{2-}$ complexes, a competition assay was carried out using

(32) Dwek, R. A. *Nuclear Magnetic Resonance in Biochemistry, Applications to Enzyme Systems*; Clarendon Press: Oxford, U.K., 1973.

(33) McMurry, T. J.; Parmelee, D. J.; Sajiki, H.; Scott, D. M.; Ouellet, H. S.; Walovitch, R. C.; Tyeklar, Z.; Dumas, S.; Bernard, P.; Nadler, S.; Midelfort, K.; Greenfield, M.; Troughton, J.; Lauffer, R. B. *J. Med. Chem.* **2002**, *45*, 3465−3474.

(34) Vander Elst, L.; Chapelle, F.; Laurent, S.; Muller, R. N. *J. Biol. Inorg. Chem.* **2001**, *6*, 196−200.

(35) Aime, S.; Botta, M.; Fasano, M.; Crich, S. G.; Terreno, E. *J. Biol. Inorg. Chem.* **1996**, *1*, 312−319.

Table 5. Binding Parameters to HSA from PRE Measurements (25 °C, 20 MHz, pH 7.4, 50 mM phosphate buffer)[a]

complexes	K_A (M^{-1})	n	b	r_1^F (mM^{-1} s^{-1})	r_1^b (mM^{-1} s^{-1})
$[Gd(TTDA\text{-}BOM)(H_2O)]^{2-}$	$4.6 \pm 0.1 \times 10^2$	1	13.7 ± 0.1	4.8 ± 0.02[b]	65.8 ± 2.7
$[Gd(TTDA\text{-}N'\text{-}BOM)(H_2O)]^{2-}$	$5.4 \pm 0.1 \times 10^2$	1	12.3 ± 0.1	5.0 ± 0.03[b]	61.5 ± 1.8
$[Gd(BOPTA)(H_2O)]^{2-}$ [c]	$4.0 \pm 0.1 \times 10^2$	–	–	–	33.0
$[Gd(DTPA\text{-}BOM_3)(H_2O)]^{2-}$ [d]	$4.0 \pm 0.3 \times 10^4$	1	–	–	44.0
MS-325[d]	$3.0 \pm 0.2 \times 10^4$	1	–	6.6 ± 0.4[b]	47.0 ± 4
$[Gd((S)\text{-}EOB\text{-}DTPA)(H_2O)]^{2-}$ [e]	$7.7 \pm 0.2 \times 10^2$	1	–	5.6 ± 0.4[b]	44.1
$[cis\text{-}Gd(DOTA\text{-}BOM_2)(H_2O)]^{-}$ [f]	$3.2 \pm 0.4 \times 10^2$	2	5.2 ± 0.1	6.8 ± 0.1[b]	35.7 ± 0.9
$[trans\text{-}Gd(DOTA\text{-}BOM_2)(H_2O)]^{-}$ [f]	$3.6 \pm 0.4 \times 10^2$	2	6.8 ± 0.1	6.5 ± 0.1[b]	44.2 ± 1.3
$[Gd(DOTA\text{-}BOM_3)(H_2O)]^{-}$ [f]	$1.7 \pm 0.1 \times 10^3$	2	7.1 ± 0.1	7.5 ± 0.1[b]	53.2 ± 1.5
$[Gd(TTDA\text{-}BA)(H_2O)]^{-}$ [g]	$1.0 \pm 0.2 \times 10^3$	1	–	6.5 ± 0.3[b]	53.9 ± 2
$[Gd(TTDA\text{-}MOBA)(H_2O)]^{-}$ [g]	$1.3 \pm 0.2 \times 10^3$	1	–	6.7 ± 0.2[b]	50.0 ± 1

[a] The relaxivity values for the bound chelates were calculated from a solution containing 0.1 mM complex and 4.5% HSA. [b] These values refer to a solution containing 0.1 mM paramagnetic complex in pH 7.4 phosphate buffer. [c] Data were obtained from ref 2. [d] Ref 38. [e] Ref 34. [f] Ref 35. [g] Ref 10.

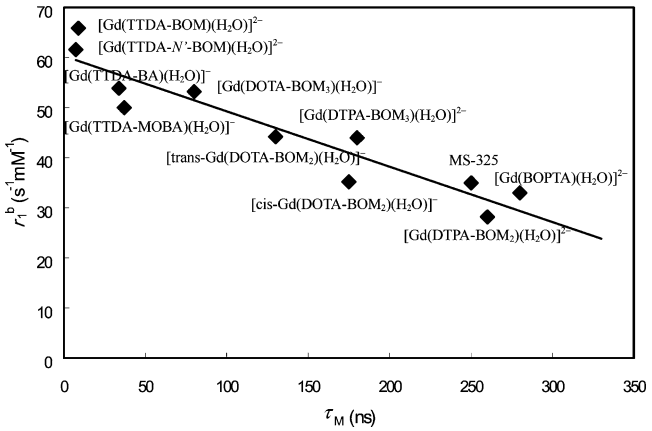


Figure 8. Correlation between the relaxivities (0.47 T and 25.0 ± 0.1 °C) of some Gd(III) complexes fully bound to HSA (r_1^b) and their τ_M values determined for the free chelates at the same temperature.

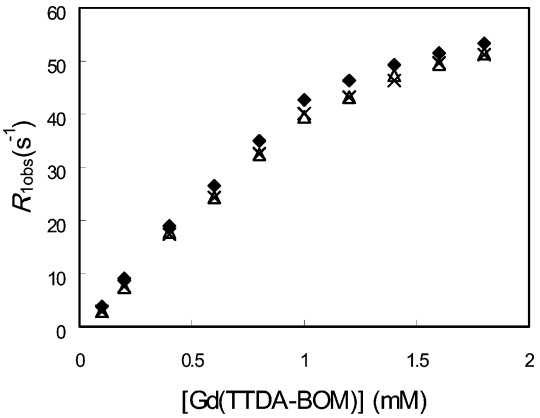


Figure 9. Water proton relaxation rates of aqueous solutions for the binding to 0.6 mM HSA of $[Gd(TTDA\text{-}BOM)(H_2O)]^{2-}$ alone (◆) or in combination with displacer drugs [ibuprofen 0.6 mM (×), warfarin 0.6 mM (△)] (20 MHz, 25.0 ± 0.1 °C, 50 mM phosphate buffer, pH 7.4).

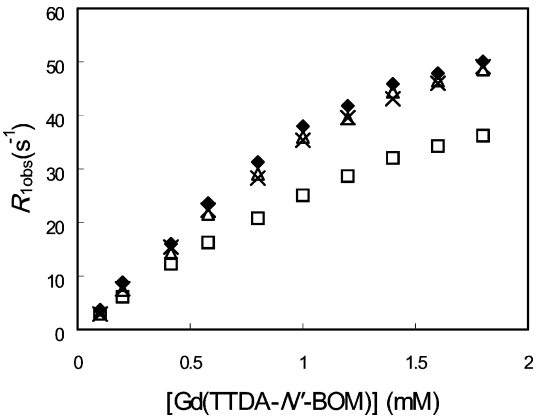


Figure 10. Water proton relaxation rates of aqueous solutions for the binding to 0.6 mM HSA of $[Gd(TTDA\text{-}N'\text{-}BOM)(H_2O)]^{2-}$ alone (◆) or in combination with displacer drugs [ibuprofen 0.6 mM (×), warfarin 0.6 mM (△)] and $[Eu(TTDA\text{-}BOM)(H_2O)]^{2-}$ (□) (20 MHz, 25.0 ± 0.1 °C, 50 mM phosphate buffer, pH 7.4).

two competitor probes, which are able to bind to different regions of the protein: warfarin and ibuprofen, which bind primarily to sites I and II of HSA,[36,37] were used in the competition assay. These substrates bind HSA more strongly than $[Gd(TTDA\text{-}BOM)(H_2O)]^{2-}$ and $[Gd(TTDA\text{-}N'\text{-}BOM)(H_2O)]^{2-}$.[35] Only a slight decrease of the relaxation rates (R_{1obs}) was observed for the competition assays of these substrates, as shown in Figures 9 and 10. This behavior is interpreted as an effect of the occurrence of conformational changes in the protein structure induced by the binding of the competitors.[38] Furthermore, to determine if $[Gd(TTDA\text{-}BOM)(H_2O)]^{2-}$ and $[Gd(TTDA\text{-}N'\text{-}BOM)(H_2O)]^{2-}$ share the same interaction site, the $[Eu(TTDA\text{-}BOM)(H_2O)]^{2-}$ complex was added to the $[Gd(TTDA\text{-}N'\text{-}BOM)(H_2O)]^{2-}$/HSA solution shown in Figure 10. The results of these measurements show that the R_{1obs} values are significantly decreased. It is clearly indicated that these Gd(III) complexes share the same binding site.

Transmetalation. The Gd(III) chelates could be sensitive to transmetalation by endogenous ions, such as Cu(II), Ca(II) and Zn(II).[39−41] Out of the three metal ions mentioned here, Zn(II) combines a rather high concentration (50 μmol/L) with a high affinity toward poly(aminocarboxylates). Therefore, this metal ion is able to replace a significant amount of Gd(III), which may result in the release of the toxic Gd(III) aqua ion in the body.[42] The kinetic stability of $[Gd(TTDA\text{-}BOM)(H_2O)]^{2-}$- and $[Gd(TTDA\text{-}N'\text{-}BOM)(H_2$-

(36) Oida, T. *J. Biochem.* **1986**, *100*, 99−113.
(37) Carter, D. C.; Ho, J. X. *Adv. Protein Chem.* **1994**, *45*, 153−203.
(38) Aime, S.; Chiaussa, M.; Digilio, G.; Gianolio, E.; Terreno, E. *J. Biol. Inorg. Chem.* **1999**, *4*, 766−774.
(39) Corot, C.; Hentsch, A. M.; Curtelin, L. *Invest. Radiol.* **1994**, *29*, S164−S167.
(40) Puttagunta, N. R.; Gibby, W. A.; Puttagunta, V. L. *Invest. Radiol.* **1996**, *31*, 619−624.
(41) Cacheris, W. P.; Quay, S. C.; Rocklage, S. M. *Magn. Reson. Imaging* **1990**, *8*, 467−481.
(42) Tweedle, M. F.; Hagan, J. J.; Kumar, K.; Mantha, S.; Chang, C. A. *Magn. Reson. Imaging* **1991**, *9*, 409−415.

Table 6. Values of R_1^p ($t = 3d$)/R_1^p ($t = 0$) for the Gd(III) Complexes after 3 Days of Transmetalation with Zn(II) at 20 MHz and 37.0 ± 0.1 °C

complexes	R_1^p ($t = 3d$)/R_1^p ($t = 0$) (%)
$[Gd(TTDA\text{-}BOM)(H_2O)]^{2-}$	43.9
$[Gd(TTDA\text{-}N'\text{-}BOM)(H_2O)]^{2-}$	43.2
$[Gd(DTPA)(H_2O)]^{2-}$	49.79
$[Gd(DTPA\text{-}BMA)(H_2O)]$[a]	9

[a] Data was obtained from ref 43.

$O)]^{2-}$-containing phosphate buffer and $ZnCl_2$ to measure the relaxation rate (R_1^p) at 37.0 ± 0.1 °C and 20 MHz was studied by transmetalation with Zn(II) (Supporting Information Figure 7S). Table 6 shows the percentage of Gd(III) complexes left in the solution after 3 days. The sequence of the kinetic stability decreases in the following order: $[Gd(DTPA)(H_2O)]^{2-} > [Gd(TTDA\text{-}BOM)(H_2O)]^{2-} \approx [Gd(TTDA\text{-}N'\text{-}BOM)(H_2O)]^{2-} \gg [Gd(DTPA\text{-}BMA)(H_2O)]$.[43] Therefore, the kinetic stability toward Zn(II) transmetalation of $[Gd(TTDA\text{-}BOM)(H_2O)]^{2-}$ and $[Gd(TTDA\text{-}N'\text{-}BOM)(H_2O)]^{2-}$ is less than that of $[Gd(DTPA)(H_2O)]^{2-}$ but is significantly higher than that of $[Gd(DTPA\text{-}BMA)(H_2O)]$. Furthermore, the pM values are considered to be a better gauge of physiologically relevant complex stability.[25] The higher pM value implies the great stability of Gd(III) complex under the given conditions. The pM values of $[Gd(TTDA)(H_2O)]^{2-}$ (15.2)[10] and $[Gd(EPTPA\text{-}bz\text{-}NO_2)(H_2O)]^{2-}$ (15.3)[25] are similar to that of $[Gd(DTPA\text{-}BMA)(H_2O)]$ (15.8).[44] The pM values of the $[Gd(TTDA\text{-}BOM)(H_2O)]^{2-}$ and $[Gd(TTDA\text{-}N'\text{-}BOM)(H_2O)]^{2-}$ complexes, which are the derivatives of $[Gd(TTDA)(H_2O)]^{2-}$, are probably similar to those of $[Gd(TTDA)(H_2O)]^{2-}$ and $[Gd(DTPA\text{-}BMA)(H_2O)]$. Therefore, the kinetic and thermodynamic stability of $[Gd(TTDA\text{-}BOM)(H_2O)]^{2-}$ and $[Gd(TTDA\text{-}N'\text{-}BOM)(H_2O)]^{2-}$ are stable enough to be used as the contrast agents for MRI.

(43) Laurent, S.; Vander Elst, L.; Copoix, F.; Muller, R. N. *Invest. Radiol.* **2001**, *36*, 115−122.
(44) Paul-Roth, C.; Raymond, K. N. *Inorg. Chem.* **1995**, *34*, 1408−1412.

Conclusion

We have synthesized and characterized two TTDA derivatives, which have a benzyloxymethyl group. Simultaneous treatment of ^{17}O NMR data is used to obtain several parameters affecting the proton relaxivity of Gd(III) complexes. It is found that the replacement of the ethylene backbone with a propylene backbone increases the steric constraints at the water binding site and thereby increases the water exchange rate of $[Gd(TTDA\text{-}BOM)(H_2O)]^{2-}$ and $[Gd(TTDA\text{-}N'\text{-}BOM)(H_2O)]^{2-}$. From the ultrafiltration studies, $[Gd(TTDA\text{-}BOM)(H_2O)]^{2-}$ and $[Gd(TTDA\text{-}N'\text{-}BOM)(H_2O)]^{2-}$ have a lower affinity for site II. In addition, the r_1^b values of these Gd(III) complexes have remarkably high values with HSA and are site dependent. The kinetic stability of these Gd(III) complexes toward transmetalation with Zn(II) is significantly higher than that of $[Gd(DTPA\text{-}BMA)(H_2O)]$. Therefore, $[Gd(TTDA\text{-}BOM)(H_2O)]^{2-}$ and $[Gd(TTDA\text{-}N'\text{-}BOM)(H_2O)]^{2-}$ may be potential MRI contrast agents.

Acknowledgment. We are grateful to the National Science Council of the Republic of China for financial support under Contracts NSC 92-2113-M-037-009 and NSC 93-NU-7-037-001. This research was also supported in part by grants from National Health Research Institutes under Contract NHRI-EX-94-9424EI.

Supporting Information Available: Figures showing HPLC data, Dy(III)-induced shifts, fluorescent probe displacement, water proton relaxation rates, tables showing the temperature dependence of the relaxation rates, methods and equations for the variable-temperature ^{17}O NMR measurements, and the PRE method. This material is available free of charge via the Internet at http://pubs.acs.org.

IC050329R