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Hetero-Tripodal Hydroxypyridonate Gadolinium Complexes: Syntheses, Relaxometric Properties, Water Exchange Dynamics, and Human Serum Albumin Binding[1]

Marlon K. Thompson,[†] Dan M. J. Doble,[†] Luke S. Tso,[†] Serena Barra,[‡] Mauro Botta,[‡] Silvio Aime,[§] and Kenneth N. Raymond*,[†]

Department of Chemistry, University of California, Berkeley, California 94720, Dipartimento di Scienze dell'Ambiente e della Vita, Università del Piemonte Orientale "Amedeo Avogadro", Piazza Ambrosoli 5, I-15100 Alessandria, Italy, and Dipartimento di Chimica I.F.M., Università di Torino, Via P. Giuria 7, I-10125 Torino, Italy

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The synthesis and relaxometric properties of hetero-tripodal hydroxypyridonate-terephthalamide gadolinium (Gd^{3+}) chelates with differing structural features for probing human serum albumin (HSA) interactions are reported. The Gd^{3+} complexes are divided into two series. The first series (**3**–**5**) features a benzyl derivative connected to the hydroxypyridonate (HOPO) moiety. The second series of complexes (**6**–**10**) has the common feature of a poly(ethylene glycol) (PEG) attached to the terephthalamide (TAM) moiety and is nonbenzylated. The water exchange of the complexes is in the fast exchange regime with rates (k_{ex}) in the range 0.45–1.11 $\times$ 10^8 s^{-1}. The complexes have a moderate interaction with HSA with association constants (K_A's) in the range 0.7–8.6 $\times$ 10^3 M^{-1}. Protein binding results in an enhancement in proton relaxivity from 7.7–10.4 mM^{-1} s^{-1} (r_{1p}) to 15–29 mM^{-1} s^{-1} (r_{1p}^b). It is concluded that the interaction of the complexes with HSA (i) is enhanced by the presence of benzyl groups, (ii) is entropically driven, and (iii) results in a lower hydration number (q).

Introduction

Magnetic resonance imaging (MRI) is a rapidly growing field in diagnostic medicine: it is noninvasive, has no radiation burden, and provides high spatial resolution and excellent tissue contrast.[2,3] An important component of the rapid progress of this diagnostic technique is the development and use of contrast agents which enhance the images acquired. Magnevist, $[Gd(DTPA)(H_2O)]^{2-}$, was the first clinically approved MRI contrast agent and is widely used in hospitals.[3] The development of MRI as a clinical modality capable of targeting receptors which are usually present in low concentrations in vivo (10^{-9}–10^{-13} mol/g of tissue)[4] hinges on improving the relatively low sensitivity of the technique. It has been estimated that visualization of receptors on the surface of cells requires 10^2–10^3 paramagnetic chelates per cell to bring about sufficient T_1 relaxation.[5] Despite the higher magnetic field strengths of current MRI scanners in clinical use (0.5–1.5 T, corresponding to proton Larmor frequencies of 20–60 MHz), there is a need for higher image enhancement capabilities, i.e., greater proton relaxivity.

Most contrast agents in development (and at the basic research stage) are based on a poly(amino-carboxylate) ligand framework. It is now evident that a serious limitation of commercial contrast agents is the intrinsically slow water exchange at the metal center (k_{ex} < 10^7 s^{-1}). The water exchange rate becomes the kinetically limiting factor in increasing proton relaxivity when the rotation of the contrast agent in solution is slow (nanosecond regime).[3] There are

* To whom correspondence should be addressed: E-mail: raymond@socrates.berkeley.edu.
[†] University of California, Berkeley.
[‡] Università del Piemonte Orientale "Amedeo Avogadro".
[§] Università di Torino.

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several methods of increasing the water exchange rate of poly(amino-carboxylate) complexes. Recently, Sherry and co-workers have demonstrated that locking out arm and macrocycle backbone isomerizations generates a DOTA analogue which has a fast water exchange rate.[6] There are several methods of slowing the tumbling (increasing the rotational correlation time, τ_R) of Gd^{3+} complexes, but the most straightforward method is via reversible, noncovalent binding of the contrast agent to a biological receptor.

The biological receptor that has previously been successfully targeted is human serum albumin (HSA), a 69 kDa blood protein present as 4.5% of plasma and the most abundant protein in serum.[3] Binding of the contrast agent to HSA increases intravascular retention, increases rotational correlation times (nanosecond regime as compared to picosecond rotation of unbound agent), and results in improved relaxivity in the blood.[3,7] It is difficult to rationally design a Gd^{3+} complex with a high affinity for HSA ($K_A \approx 10^4$ M^{-1}), although it is known that the presence of hydrophobic groups as well as negative electric charges are among the basic requirements for binding a substrate to serum albumin.[8] These observations are consistent with the structural characteristics of the primary drug binding sites of the protein (sites I and II).[9,10] The entrances of the binding sites are surrounded by positively charged residues while the inside walls of the pockets are lined by hydrophobic side chains.[9]

Several literature contributions in blood pool targeting of gadolinium chelates have been made in recent years,[4] particularly by Epix Medical, makers of MS-325, a DTPA-based complex designed for MR angiography applications.[7] This chelate possesses a strong albumin binding affinity, and the resulting relaxivity (at 20 MHz) is 9 times greater than that for the unbound chelate. The agent possesses a lipophilic diphenyl-cyclohexyl pendant group that interacts favorably with the human serum albumin, while the charged phosphodiester moiety enhances water solubility and deters hepatobiliary uptake.[7]

The Gd^{3+} complexes based on a tripodal hydroxypyridonate (HOPO) ligand scaffold (**1**) are interesting candidates for the development of second-generation MRI contrast agents due to their high thermodynamic stability[11,12] and proton relaxivity (Figure 1).[12−14] The relatively high relaxivity (9−10 mM^{-1} s^{-1}) of these eight-coordinate Gd^{3+} complexes is due to the presence of two coordinated water molecules ($q = 2$).[12,14] However, the rotational correlation times of the complexes are relatively short ($\tau_R \sim 100$ ps) due to their low molecular weight (<1000 Da). The inner-sphere water exchange rate of the complexes is fast ($k_{ex} \approx 7 \times 10^7$ s^{-1}).[14] This is due to a low activation energy barrier for the transition between eight- and nine-coordinate states.[15] The Solomon−Bloembergen−Morgan theory of inner-sphere proton relaxation[16] predicts that significantly higher relaxivities are attainable (> 200 mM^{-1} s^{-1}, at 20 MHz) if the rotation of the HOPO-based complex is slowed by 2 orders of magnitude.

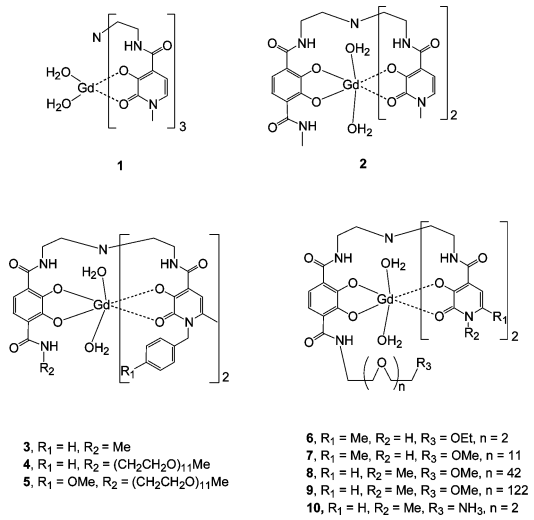

Figure 1. Complexes relevant to discussion.

Despite the appealing stability and relaxivity properties of the parent homo-tripodal Gd^{3+} complex (**1**), the original ligand design is not readily amenable to substitution on the HOPO ring nitrogen. While the substitution of a methoxyethyl group onto the ring N of the HOPO was previously reported,[13] the synthetic procedure then used was cumbersome. The parent complex, Gd[TREN-1-Me-3,2-HOPO] (**1**), is also of very low solubility in water, which prevents full relaxometric investigation. The neutral charge of the complex is less desirable than an anionic overall charge in order to facilitate interaction with the positively charged amino acid residues at the mouth of the HSA binding site.[2] Hence, a ligand design that allowed for facile derivatization to insert (i) hydrophilic groups for water solubility, (ii) aromatic groups for HSA interaction, and (iii) a net negative chelate charge was necessary. The hetero-tripodal design previously reported,[17] Gd[TREN-bis(1-Me-3,2-HOPO)-(TAM-Me)] (**2**), features two HOPO units and a terephthalamide (TAM) unit, resulting in a chelate charge of −1 at neutral pH. The TAM

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moiety also has an ortho-oxygen binding motif; hence, the favorable stability and relaxivity properties of the complex are maintained.

Of critical importance to the development of slowly tumbling and site-specific HOPO-based contrast agents is the development of synthetic methodologies that allow for facile substitution on the chelating units. The TAM moiety conveniently contains a second amide group that allows for functionalization with primary amines. It has been previously shown that the attachment of poly(ethylene glycol) (PEG) chains to the TAM significantly enhances the water solubility and also allows for a weak, noncovalent interaction with HSA.[18]

Herein, we report the synthesis of a HOPO isomer functionalized with benzyl (Bn) groups to enable noncovalent interaction of the chelate with albumin. The synthesis of a series of structurally related Gd^{3+}complexes (Figure 1), with subtle differences in ligand design, is reported and features a PEG substituted on the TAM. The relaxivity properties of these complexes are presented, highlighting the effects of both PEG and Bn groups on HSA interaction.

Experimental Details

General Considerations. Starting materials were obtained from commercial suppliers and used without further purification unless otherwise noted. Flash silica gel chromatography was performed using Merck silica gel (40−7 mesh). Microanalyses were performed by the Microanalytical Services Laboratory, and mass spectra were recorded at the Mass Spectrometry Laboratory, both of the College of Chemistry, University of California, Berkeley. Unless otherwise stated, NMR spectra were recorded at room temperature on either AMX-300, AM-400, AMX-400, or DRX-500 Brüker FT spectrometers.

Synthesis. The syntheses of complexes **3** and **10** are described as representative of the series (see Supporting Information for details).

6-Me-1-Bn-HOPO-Bn-ester (22). Compound **21** was synthesized as previously reported.[19] K_2CO_3 (10.5 g, 75.4 mmol) was added to a suspension of **21** (5.20 g, 26.4 mmol) in dry DMF (10 mL) under an atmosphere of N_2. Benzyl chloride (6.96 g, 55.0 mmol) was added and the reaction stirred at 65 °C for 30 h, during which time the progress of the reaction was monitored by TLC. The solvents were removed by rotary evaporation, and the residue was taken up in EtOAc (100 mL) and water (100 mL). The layers were separated, and the organic layer was washed with water (5 × 100 mL), dried (Na_2SO_4), filtered, and evaporated to dryness. The crude product was purified by flash silica column chromatography (eluent: CH_2Cl_2) and the solvent removed by rotary evaporation to afford a light brown oil (yield: 7.46 g, 75%). ^{1}H NMR ($CDCl_3$, 300 MHz): δ = 1.28 (t, J = 6.9 Hz, 3H, CH_3 Et ester), 2.26 (s, 3H, HOPO CH_3), 4.30 (q, J = 7.1 Hz, 2H, CH_2), 5.31 (s, 2H, Bn-CH_2), 5.36 (s, 2H, Bn-CH_2), 6.18 (s, 1H, HOPO CH), 7.10−7.51 (m, 10H, Ar H) ppm. FAB-MS (+): m/z: 378.2 $[M + H]^+$.

6-Me-1-Bn-HOPO-Bn-acid (24). Compound **22** (11.54 g, 26.4 mmol) was dissolved in a solution of KOH (2.3 g, 40 mmol) in MeOH (200 mL). The reaction mixture was stirred for 2 days, after which time TLC indicated that the reaction was complete. The solvent was removed and the residue dissolved in distilled water. The solution was acidified to pH 1 by addition of 6.0 M HCl to yield a white precipitate, which was filtered and dried (Yield: 6.27 g, 68%). ^{1}H NMR ($CDCl_3$, 400 MHz): δ = 2.30 (s, 3H, HOPO CH_3), 5.37 (s, 2H, Bn-CH_2), 5.63 (s, 2H, Bn-CH_2), 6.58 (s, 1H, HOPO CH), 7.30−7.44 (m, 10H, Ar H) ppm. Anal. Calcd (Found) for $C_{21}H_{19}NO_4 \cdot 0.4H_2O$: C, 72.19 (72.19); H, 5.48 (5.66); N, 4.01 (3.94). FAB-MS (+): m/z: 350 $[M + H]^+$.

6-Me-1-Bn-HOPO-Bn-thiaz (26). Compound **24** (5.36 g, 15.3 mmol) was dissolved in CH_2Cl_2 (100 mL) under an atmosphere of N_2. 2-Mercaptothiazoline (1.88 g, 16.1 mmol) and (dimethylamino)-pyridine (DMAP, 0.16 g, 1.0 mmol) were then added, and the solution was stirred for 1 h. Dicyclohexylcarbodiimide (DCC, 3.45 g, 16.9 mmol) was then added in small portions, and the reaction mixture was stirred for 16 h and then allowed to stand for 24 h at 0 °C. The reaction mixture was filtered, and the solvent was removed by evaporation. EtOAc (10 mL) was added, and the solution was filtered. This last step was repeated three times in order to remove dicyclohexylurea (DCU), yielding a yellow oil. The product was purified by flash column chromatography (silica, CH_2Cl_2 as eluent). The solvents were removed by rotary evaporation, yielding a viscous yellow oil, which was recrystallized from acetone to give a yellow crystalline solid (yield: 5.10 g, 74%). ^{1}H NMR ($CDCl_3$, 400 MHz): δ = 2.27 (s, 3H, HOPO CH_3), 2.90 (t, 2H, J = 7.3 Hz, thiaz CH_2), 4.59 (t, J = 7.3 Hz, 2H, thiaz CH_2), 5.34 (s, 2H, Bn-CH_2), 5.38 (s, 2H, Bn-CH_2), 5.98 (s, 1H, HOPO CH), 7.12−7.49 (m, 10H, Ar H) ppm. Anal. Calcd (Found) for $C_{24}H_{22}N_2O_3S_2$: C, 63.98 (64.04); H, 4.92 (5.02); N, 6.22 (6.03). FAB-MS (+): m/z: 451 $[M + H]^+$.

TREN-bis(6-Me-1-Bn-HOPO-Bn)$_2$ (30). A solution of compound **26** (1.125 g, 2.50 mmol) in CH_2Cl_2 (100 mL) was added dropwise, over 20 h, to a rapidly stirring solution of tris(2-aminoethyl)amine (TREN, 0.201 g, 1.37 mmol) in CH_2Cl_2 (100 mL). The reaction was allowed to continue for 30 h (TLC indicated the formation of the tris-, bis-, and mono-substituted products). The solvent was evaporated, and the bis-substituted product was purified by flash silica column chromatography (eluent: 99:1 CH_2Cl_2/NEt_3 with increasing gradient of MeOH to 5%). Evaporation of the solvents yielded a white foam (yield: 1.02 g, 92.0%). ^{1}H NMR ($CDCl_3$, 400 MHz): δ = 2.19 (s, 3H, CH_3), 2.18 (s, 3H, CH_3), 2.32−2.47 (m, 8H, TREN CH_2), 3.26 (m, 4H, TREN HN−CH_2), 5.29 (s, 4H, Bn CH_2), 5.33 (s, 4H, Bn CH_2), 6.48 (s, 1H, HOPO CH), 6.52 (s, 1H, HOPO CH), 7.02−7.36 (m, 20H, Ar H), 8.02−8.08 (t, 2H, amide NH) ppm.

TREN-bis(6-Me-1-Bn-HOPO-Bn)-(Bn$_2$-TAM-thiaz) (34). Compound **30** (1.21 g, 1.5 mmol) was dissolved in CH_2Cl_2 and added dropwise to a rapidly stirring solution of excess **19** (14.0 g, 24 mmol) in CH_2Cl_2 (100 mL) over 2 h. The solution was evaporated, and the crude product was purified twice by flash silica column chromatography (eluent: CH_2Cl_2 with increasing gradient of MeOH to 5%). The solvents were then removed to yield a yellow oil. Excess NEt_3 was removed by washing with 1.0 M KOH solution and repeatedly evaporating the residual solution with MeOH (3 × 10 mL). The final product was a yellow foam (yield: 1.39 g, 73%). ^{1}H NMR ($CDCl_3$, 400 MHz): δ = 2.25 (s, 6H, HOPO CH_3), 2.37 (m, 6H, TREN CH_2), 2.93 (t, J = 7.3 Hz, 2H, thiaz CH_2), 3.38 (m, 6H, TREN CH_2), 4.37 (t, J = 7.3 Hz, 2H, thiaz CH_2), 5.09 (s, 2H, TAM Bn CH_2), 5.12 (s, 2H, TAM Bn CH_2), 5.35 (s, 8H, HOPO Bn CH_2), 6.58 (s, 2H, HOPO CH), 7.09−7.38 (m, 31H, Ar H), 7.27 (br s, 1H, TAM NH), 7.85 (d, 1H, TAM CH), 7.94 (br s, 2H, HOPO NH), 8.30 (br s, 1H, TAM NH) ppm. Anal. Calcd (Found) for $C_{73}H_{71}N_7O_{10}S_2 \cdot 3.5H_2O$: C, 65.77 (65.52); H, 5.86 (5.56); N, 7.36 (7.04). FAB-MS(+): m/z: 1270.5 $[M + 1]^+$.

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TREN-bis(1-Bn-6-Me-HOPO-Bn)-(Bn$_2$-TAM-Me) (38). Excess methylamine (solution in H_2O) was shaken with a solution of compound **34** (1.12 g, 0.883 mmol) in CH_2Cl_2 (100 mL), resulting in an instantaneous color change from yellow to colorless. The product was purified by flash silica column chromatography (eluent: CH_2Cl_2 with increasing gradient of MeOH) to yield a white foam after removal of solvents (yield: 0.76 g, 73%). ^{1}H NMR ($CDCl_3$, 400 MHz): δ = 2.17 (s, 6H, HOPO CH_3), 2.51 (m, 6H, TREN CH_2), 2.71 (d, 3H, TAM CH_3), 3.29 (m, 6H, TREN CH_2), 5.00 (s, 2H, TAM Bn CH_2), 5.03 (s, 2H, TAM Bn CH_2), 5.18 (s, 4H, HOPO Bn CH_2), 5.28 (s, 4H, HOPO Bn CH_2), 6.18 (s, 2H, HOPO CH), 7.05−7.37 (m, 32H, Ar H), 8.20−8.25 (m, 4H, NH) ppm. Anal. Calcd (Found) for $C_{71}H_{71}N_7O_{10}\cdot 2H_2O$: C, 69.99 (69.96); H, 6.20 (6.03); N, 8.05 (7.88). FAB-MS (+): *m*/*z*: 1182.6 [M + 1]$^+$.

TREN-bis(1-Bn-6-Me-HOPO)-(TAM-Me) (44). 10% Pd on carbon (0.235 g) was added to a solution of compound **38** (0.540 g, 0.457 mmol) in AcOH (10 mL) and H_2O (5 mL) and the mixture stirred under an atmosphere of H_2 under ambient conditions for 48 h. The reaction was filtered, and the solvent was removed under reduced pressure. The remaining residue was converted to the Cl^- salt by dissolving in MeOH (10 mL) and one drop of concentrated HCl, and the solvent was removed (×3). The residue was then dissolved in MeOH (10 mL), and the solvent was evaporated (×3). The remaining residue was taken up in MeOH (2 mL) and added to a rapidly stirring solution of Et_2O (200 mL) to afford a white precipitate which was filtered and dried under vacuum (yield: 0.324 g, 79%). ^{1}H NMR (d_6-DMSO, 300 MHz): δ = 2.79 (d, 3H, CH_3), 3.46 (m, 6H, TREN CH_2), 3.72 (m, 6H, TREN CH_2), 5.27 (s, 4H, Bn CH_2), 6.36 (s, 2H, HOPO CH), 7.04−7.32 (m, 12H, Ar H), 8.68 (br s, 2H, NH), 8.90 (br s, 1H, NH), 9.11 (br s, 1H, NH) ppm. Anal. Calcd (Found) for $C_{43}H_{47}N_7O_{10}\cdot HCl\cdot 3H_2O$: C, 56.61 (56.60); H, 5.97 (5.89); N, 10.75 (10.46). FAB-MS (+): *m*/*z*: 822 [M + H]$^+$.

Gd-TREN-bis(1-Bn-6-Me-HOPO)-(TAM-Me) (3). Excess pyridine was added to a solution of $GdCl_3\cdot 6H_2O$ (0.040 g, 0.109 mmol) and compound **44** (0.100 g, 0.114 mmol) in H_2O (10 mL). The resulting pale yellow solution was heated under reflux for 2 h, during which time a cream-colored precipitate developed. The solvent was removed, and the residue was suspended in *i*-PrOH, sonicated, and filtered three times. The resulting light brown solid was dried under vacuum (yield: 0.114 g, 95%). Anal. Calcd (Found) for H**3**$\cdot 2H_2O$, $C_{43}H_{51}N_7O_{12}Gd\cdot 2H_2O$: C, 49.32 (48.95); H, 4.90 (4.90); N, 9.36 (8.91). FAB-MS (−): *m*/*z*: 975.2 [M]$^-$.

TREN-bis(1-Me-HOPO-Bn)-(Bn$_2$-TAM-thiaz) (37). Compound **33** was synthesized from **29** and TREN as reported.[19] A solution of **33** (3.00 g, 4.77 mmol) in CH_2Cl_2 (150 mL) was added dropwise to a solution of **19** (20.77 g, 47.7 mmol) in CH_2Cl_2 (250 mL) over a period of 1 h. The resulting solution was then stirred for a further 8 h before purification (twice) by silica column chromatography (eluent: CH_2Cl_2 with increasing gradient of MeOH from 0 to 4%). The solvents were removed under reduced pressure to afford a yellow foam (yield: 3.56 g, 68%). ^{1}H NMR ($CDCl_3$, 500 MHz): δ = 2.29 (m, 6H, TREN CH_2), 2.93 (t, *J* = 7.3 Hz, 2H, thiaz CH_2), 3.12 (m, 6H, TREN CH_2), 3.57 (s, 6H, CH_3), 4.38 (t, *J* = 7.3 Hz, 2H, thiaz CH_2), 5.06 (s, 2H, TAM Bn CH_2), 5.11 (s, 2H, TAM Bn CH_2), 5.30 (s, 4H, HOPO Bn CH_2), 6.65 (d, *J* = 7.2 Hz, 2H, HOPO CH), 7.08 (d, *J* = 7.2 Hz, 2H, HOPO−H), 7.18 (d, *J* = 8.2 Hz, 1H, TAM CH), 7.22−7.41 (m, 20H, Ar H), 7.78 (d, *J* = 8.2 Hz, 1H, TAM CH), 7.82 (t, 2H, HOPO amide NH) ppm. Anal. Calcd (Found) for $C_{59}H_{59}N_7O_{10}S_2$: C, 65.00 (64.68); H, 5.45 (5.30); N, 8.99 (8.73). FAB-MS (+), *m*/*z*: 1090.5 [M + H]$^+$.

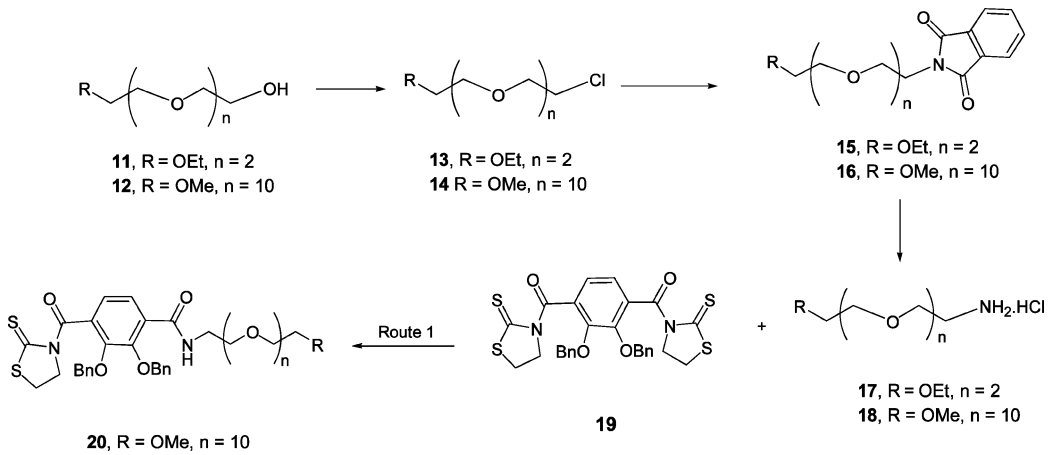
TREN-bis(1-Me-HOPO-Bn)-(Bn$_2$-TAM-PEG-NH$_2$) (43). A solution of compound **37** (1.50 g, 1.41 mmol) in CH_2Cl_2 (100 mL) was added to a rapidly stirring solution of 2-[2-(2-amino-ethoxy)-ethoxy]-ethylamine (10.46 g, 70.6 mmol) in CH_2Cl_2 (200 mL). The resulting clear solution was purified by column chromatography (eluent: 99:1 CH_2Cl_2/NEt_3, with an increasing gradient of MeOH from 0 to 10%). The solvents were removed to yield a colorless oil (yield: 0.96 g, 61%). ^{1}H NMR (d_6-DMSO, 400 MHz): δ = 3.18−3.54 (m, 18H, TREN and PEG CH_2, HOPO CH_3), 5.02 (s, 4H, Bn CH_2), 5.17 (s, 4H, Bn CH_2), 6.22 (d, 1H, *J* = 6.9 Hz, HOPO CH), 7.25−7.39 (m, 22H, Ar H), 7.46 (d, 1H, *J* = 6.8 Hz, HOPO CH), 8.14 (br s, 2H, HOPO NH), 8.18 (br s, 1H, TAM NH), 8.37 (br s, 1H, TAM NH) ppm. Anal. Calcd (Found) for $C_{62}H_{70}N_8O_{12}\cdot 2H_2O$: C, 64.46 (64.45); H, 6.46 (6.45); N, 9.70 (9.45). FAB-MS (+), *m*/*z*: 1119.7 [M + H]$^+$.

TREN-bis(1-Me-HOPO)-(TAM-PEG-NH$_2$) (49). Compound **43** (0.83 g, 0.74 mmol) was dissolved in AcOH (10 mL) and concentrated HCl (10 mL) and stirred for 3 days. The solvents were removed, and the residual solid was dissolved in MeOH (2 mL), H_2O (10 mL), and concentrated HCl (5 mL) for 10 h. The solvent was removed under reduced pressure, the residue was dissolved in MeOH (10 mL), and the solvent was evaporated. This procedure was repeated six times, with the addition of 6 M HCl (2 drops) to the first three solutions. The final residue was dissolved in MeOH (3 mL) and added to a rapidly stirring solution of Et_2O (150 mL) to afford a white powder, which was filtered and dried under vacuum (yield: 0.48 g, 73%). ^{1}H NMR (d_6-DMSO/D_2O, 500 MHz): δ = 3.43−3.63 (m, integral obscured by H_2O band, TREN and PEG CH_2), 6.35 (d, *J* = 7.4 Hz, 2H, HOPO CH), 7.06 (d, *J* = 7.4 Hz, 2H, HOPO CH), 7.24 (d, 1H, TAM CH), 7.27 (d, 1H, TAM CH) ppm. Anal. Calcd (Found) for $C_{34}H_{46}N_8O_{12}\cdot 2HCl\cdot 3H_2O$: C, 46.11 (46.48); H, 6.14 (6.16); N, 12.65 (12.21). FAB-MS (+), *m*/*z*: 759 [M + H]$^+$.

Gd-TREN-bis(1-Me-HOPO)-(TAM-PEG-NH$_2$) (10). Imidazole (0.058 g, 0.85 mmol) was added to a solution of $GdCl_3\cdot 6H_2O$ (0.060 g, 0.16 mmol) and compound **49** (0.150 g, 0.169 mmol) in H_2O (10 mL). The resulting pale yellow solution was heated under reflux for 2 h. The solvent was removed to yield a yellow residue which was suspended in *i*-PrOH, sonicated, and filtered three times. The resulting light brown solid was dried under vacuum (yield: 0.117 g, 73%). Anal. Calcd (Found) for **10**, $C_{34}H_{47}N_8O_{14}Gd$: C, 43.03 (42.87); H, 4.99 (4.61); N, 11.81 (11.63). FAB-MS (+), *m*/*z*: 913.2 [M + H]$^+$.

Water Proton Relaxivity Measurements. The water proton $1/T_1$ longitudinal relaxation rates were measured with a Stelar Spinmaster Spectrometer (Mede, Pv, Italy) on 0.1−1.0 mM aqueous solutions of the complexes, at 20 MHz and 25 °C. ^{1}H spin−lattice relaxation times T_1's were acquired by the standard inversion−recovery method with typical 90° pulse width of 3.5 ms, 16 experiments of 4 scans. The reproducibility of the T_1 data was ±1%. The temperature was controlled with a Stelar VTC-91 air-flow heater equipped with a copper−constantan thermocouple (uncertainty of ±0.1 °C). The $1/T_1$ nuclear magnetic relaxation dispersion profiles of water protons were measured over a continuum of magnetic field strength from 0.00024 to 0.28 T (corresponding to 0.01−12 MHz proton Larmor frequency) on a fast field-cycling Stelar Spinmaster FFC relaxometer. The relaxometer operates under complete computer control with an absolute uncertainty in the $1/T_1$ values of ±1%. Additional data points at 20 and 90 MHz were recorded on the Stelar Spinmaster and on JEOL EX-90 spectrometers, respectively. The concentration of the solutions used was in the range 0.1−1.0 mM. For the complexes whose solubility was lower than 0.1 mM at 25 °C (**3** and **10**), no attempts were made to analyze the data.

Scheme 1. Synthesis of PEG Monoamine and TAM-Conjugated Intermediate



The characterization of the binding parameters of the Gd^{3+} complexes (GdL) to human serum albumin (HSA) was carried out by a relaxometric method. In a typical titration experiment, several (8−10) aqueous solutions of the paramagnetic complex (0.1−0.2 mM) were prepared containing different concentrations of HSA (0−1.8 mM), and the water proton relaxation rate of each solution was measured at 25 °C. The pH of the solutions (pH = 7.0 ± 0.2) was controlled before and after each measurement. The observed relaxation rate is given by the sum of three contributions[2]

$$R_{1obs} = r_{1p}^{f}[GdL] + r_{1p}^{b}[GdL{-}HSA] + R_{1d} \quad (1)$$

where r_{1p}^{f} and r_{1p}^{b} are the relaxivity of the free complex and of the paramagnetic macromolecular (GdL−HSA) adduct, respectively, and R_{1d} is the diamagnetic contribution. For the equilibrium

$$GdL + HSA \leftrightarrow GdL{-}HSA$$

it is possible to obtain the association constant K_A (actually the product $K_A{\cdot}n$, where n is the number of independent and equivalent sites characterized by the same K_A value) and r_{1p}^{b} by combining eq 1 with the expression for the association constant K_A

$$K_A = [GdL{-}HSA]/([GdL][nHSA]) \quad (2)$$

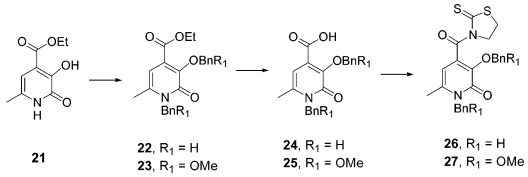
In the absence of more detailed information, in the data analysis we have assumed the presence of one equivalent binding site (n = 1). HSA (crystallized and lyophilized) was purchased from Sigma (St. Louis, MO.) and was used without any further purification. The molecular weight was assumed to be 69 kDa.

VT ^{17}O Relaxation Measurements. Variable-temperature ^{17}O NMR data were recorded on JEOL EX-90 (2.12 T, 12.2 MHz) and Bruker Avance-600 (14.09 T, 81.3 MHz) spectrometers, using 5 mm probes and standard temperature control units. Experimental settings were as follows: flip angle of 90°, acquisition time 10 ms, 1200−2400 scans, and no sample spinning. Aqueous solutions of the paramagnetic complexes (pH = 7) containing 2.6% of ^{17}O isotope (Yeda, Israel) were used. In the case of the measurement of the ^{17}O data for the complexes in the presence of HSA, a highly ^{17}O-enriched water (20%, Cambridge Isotope Laboratories, Inc., Andover, MA) was utilized. The observed transverse relaxation rates (R_2's) were calculated from the signal width at half-height.

Results and Discussion

Synthesis. The synthesis of the hetero-tripodal ligands features the amide coupling of three key intermediates: TREN, thiazolide-activated HOPO (**26**−**29**), and thiazolide-activated TAM (**19**, **20**). In order to ensure that the complex is soluble in water, PEG was conjugated to the TAM moiety (Scheme 1). The synthesis of the PEG monoamines follows general synthetic protocols starting from the alcohol (**11**, **12**). Halogenation of the alcohol with thionyl chloride to form the corresponding halide (**13**, **14**) is followed by conversion to the phthalimide (**15**, **16**). Reduction of the phthalimide (**15**, **16**) with hydrazine produces the amine, which is then converted to the HCl salt (**17**, **18**). The TAM moiety can be functionalized with the PEG either prior to (route 1) or following (route 2) connection with the TREN backbone (Scheme 3). The former route (route 1) was used in the synthesis of **4** and **7**; here, the coupling of PEG monoamine (**18**) with bis-activated TAM (**19**) (synthesized as previously reported)[19] involves a slow addition, high dilution reaction in an excess of **19** to yield the monoactivated TAM intermediate (**20**).

Scheme 2. Synthesis of Thiazolide-Activated Benzylated 6-Me-3,2-HOPO



The synthesis of the 6-Me-3,2-HOPO ester (**21**) (previously reported)[19] allows for substitution on the HOPO nitrogen, thereby facilitating derivatization of the HOPO ring (Scheme 2). The synthesis of this isomer of the parent 1-Me-3,2-HOPO is preferable to previous 3,2-HOPO syntheses[12,13] because the HOPO nitrogen remains available for further substitution. Under highly basic reaction conditions, the substitution of benzyl (Bn) and *p*-methoxybenzyl (MOB) groups onto the HOPO nitrogen are possible, giving the doubly protected intermediates (**22**, **23**). Saponification of the ester (**22**, **23**) is followed by thiazolide-activation of the acid (**24**, **25**) to form the key activated intermediates, **26** and **27**.

The synthesis of the Gd^{3+} complexes follows general synthetic protocols for HOPO-TAM amide couplings, deprotection, and complexation,[18−20] as illustrated in Scheme 3. The synthesis of Gd[TREN-bis(6-Me-3,2-HOPO-Bn)-(TAM-

Scheme 3. Synthesis of Ligands and Complexes

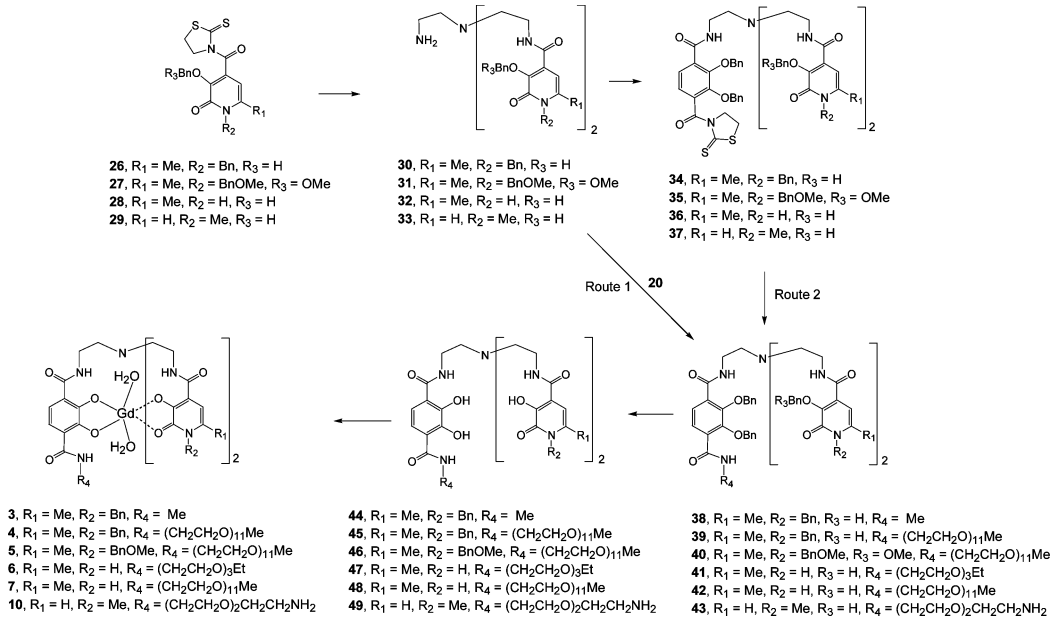


Table 1. Best Fitting Parameters Determined by Analysis of ^{17}O NMR[a] and NMRD[b] Profiles at 25 °C and pH 7.0[c]

	3[d]	**4**	**5**	**6**	**7**	**8**	**9**	**10**[d]
r_{1p} (mM^{-1} s^{-1})	10.4	8.8	9.2	8.9	7.7	9.1	9.1	9.9
$\tau_V^{e,f}$ (ps)		22 ± 3	26 ± 2	24 ± 2	20 ± 3	24 ± 3	23 ± 2	
ΔH_V^f (kJ/mol)		8 ± 3	5 ± 2	8 ± 4	5 ± 2	8 ± 3	7 ± 2	
τ_R^e (ps)		268 ± 8	243 ± 10	160 ± 8	300 ± 10	349 ± 15	370 ± 13	
$\Delta^{2\,e,f}$ (10^{19} s^{-2})		7.1 ± 0.5	8.1 ± 0.8	9.0 ± 2.0	9.5 ± 1.2	8.6 ± 0.7	9.2 ± 0.5	
k_{ex}^f (10^7 s^{-1})		11.1 ± 1.3	4.5 ± 0.4	5.3 ± 0.6	7.7 ± 0.8	5.3 ± 0.3	3.2 ± 0.2	
ΔH_M^f (kJ/mol)		24 ± 2	22 ± 3	26 ± 1	20 ± 2	20 ± 2	18 ± 3	
$q^{e,f}$	2	1	1	2	1	1	1	
r^e (Å)		2.98 ± 0.02	3.00 ± 0.05	3.10 ± 0.02	3.02 ± 0.03	3.05 ± 0.05	3.10 ± 0.03	

[a] For the hyperfine coupling constant, $A/\hbar$, a value of -3.8 rad s^{-1} was utilized. [b] For the parameters D (relative diffusion coefficient) and a (distance of closest approach of outer-sphere water molecules to the paramagnetic metal ion), the standard values of 2.24×10^{-5} cm^2 s^{-1} and 3.8 Å were used, respectively. [c] Data for compound 6 are taken from ref 15. [d] The solubility of the complex is too low for accurate measurements of ^{17}O NMR and NMRD profiles. [e] NMRD. [f] ^{17}O NMR.

PEG550)] (**4**) is described as an example of the procedure used to synthesize **3**−**7** and **10**; the synthesis of **8** and **9** was reported previously.[18] The bis-substituted TREN intermediate (**30**) is produced by a slow addition, high dilution reaction involving stoichiometric amounts of **26** and TREN. Compound **30** is then coupled to the thiazolide-activated TAM-PEG intermediate (**20**) to yield the protected ligand (**39**), route 1 amide coupling. In route 2 amide coupling, the TREN-bisHOPO amine intermediate (**30**) is coupled with the bis-activated TAM intermediate (**19**) to form the thiazolide-activated ligand (**34**). Compound **34** can then be coupled with **18** to form the protected ligand (**39**). Deprotection (removal of the Bn groups) to form the protonated ligand (**45**) occurs under acidic conditions. The syntheses of ligands **44**−**46** are made possible by the fact that the N-Bn and N-MOB groups (the primary protein binders) are not cleaved under the deprotection conditions; hence, the HOPO protecting group serves a dual role. Complexation of **45** to form **4** is performed with $GdCl_3$ in the presence of a base.

Relaxivity Studies. The ^{1}H relaxivity, r_{1p}, of **3**−**10** was measured at 20 MHz and 25 °C and is reported in Table 1. Despite significant differences in their molecular weights, the relaxivity values of the complexes are rather similar (7.7−10.4 mM^{-1} s^{-1}). Typically, under these experimental conditions the relaxivity of low molecular weight Gd^{3+} chelates (with an identical number of bound water molecules, q) is primarily determined by their rotational dynamics in terms of the correlation time (τ_R). Therefore, a linear dependence of r_{1p} on the molecular weight is often found.[17] Deviations from this behavior may arise from the occurrence of (i) slow exchange of the coordinated water molecule(s) which decreases the efficiency of the transfer of the paramagnetic effect from the metal ion to the bulk solvent, (ii) the presence of internal local motions superimposed on the global reorientation of the complex, or (iii) a change of the hydration number q among the complexes.

The parent complex (**2**) has a fast rate of water exchange ($k_{ex} = 1.3 \times 10^8$ s^{-1}),[18] which is characteristic of the HOPO series of Gd(III) complexes. Therefore, a limiting effect of k_{ex} on r_{1p} can be safely excluded. On the other hand, these complexes (other than **3**) have PEG chains which are probably endowed with a large internal motion not synchronous with the global motion of the Gd^{3+} coordination site; thus, the relaxivity does not linearly increase with molecular

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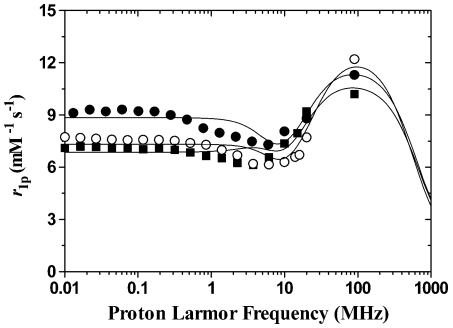

Figure 2. $1/T_1$ proton nuclear magnetic relaxation dispersion (NMRD) profiles for complexes **4** (●), **5** (■), and **7** (○) at 25 °C and pH = 7.2.

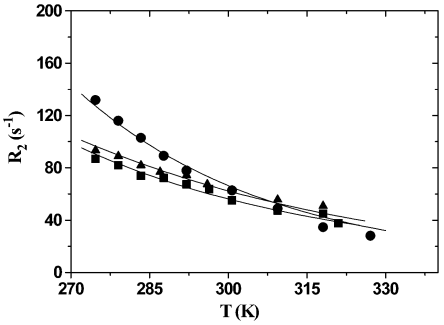

Figure 3. Temperature dependence of the paramagnetic contribution (R_2) to the transverse ^{17}O water relaxation rate of aqueous solutions of complexes **4** (9.9 mM; ●), **5** (6.6 mM; ▲) and **7** (8.8 mM; ■) at 2.1 T and pH = 7.2.

weight. On the basis of previous results, a change in the number of inner-sphere water molecules (q) for **3**−**10** must be considered.[17,18] Moreover, the different nature of the substituents may influence the hydrophobic and hydrophilic regions of the complexes, thus affecting the number, distribution, and lifetime of the water molecules in the outer hydration sphere with consequent effects on the relaxivity. Finally, water molecules in the second hydration sphere may also play some role.[18]

NMRD Profiles. Nuclear magnetic relaxation dispersion (NMRD) profiles can be used to determine the values of the parameters that contribute to the relaxivity of a Gd^{3+} complex. The experiment involves measuring the magnetic field strength (Larmor frequency) dependence of the water proton longitudinal relaxation rate in the presence of the Gd^{3+} complex. Figure 2 shows the NMRD profiles for **4**, **5**, and **7**, which are similar to NMRD profiles of similar complexes of the HOPO-TAM series.[17,18] The solubilities of **3** and **10** were too low (<0.1 mM) for accurate NMRD measurement. The data were fitted to the routinely used model of inner- and outer-sphere components of the relaxivity (see Supporting Information).[16] As previously observed for **9**, this model is not adequate when a sizable contribution from the water molecules in the second coordination sphere of Gd^{3+} is expected.[21] From the NMRD analyses (Table 1), it appears that the electronic parameters are rather similar to those previously found for the parent complexes: this implies that the coordination geometry is very similar, as expected. The differences in the Gd−water proton distance, r, are small and very likely not significant. In fact, this parameter is not directly obtained from the analysis as only the ratio τ_R/r^6 can be estimated. Moreover, the actual value of r depends on how accurately the inner-sphere contribution of the relaxivity is evaluated. Recently, the r value for the Gd^{3+} aquoion and for a series of four commercial Gd^{3+}-based contrast agents was determined using the pulsed ENDOR technique and was found to be essentially the same (3.1±0.1 Å) for all five complexes.[22] Thus, the most important factors accounting for the different relaxivities of the complexes are the parameters τ_R and q. A single water molecule is likely to be present in the inner coordination sphere of complexes **4**−**5** and **7**−**9**: by imposing $q = 2$, a reasonable fit is obtained with unrealistic values of the structural parameter τ_R (and/or r) and of the parameters describing the electron relaxation, Δ^2 and τ_V (see Supporting Information). This result is in agreement with previous results on complex **9**[18] and with recent data for macrocyclic complexes functionalized with PEG chains.[23] Although we cannot exclude the occurrence of an effective fractional q value arising from a mixture of coordination isomers with $q = 2$ (low concentration) and $q = 1$ (predominant), we assume for simplicity that these complexes are monoaquo species.

Variable-Temperature ^{17}O NMR. The water exchange rate (k_{ex}), a crucial parameter for the evaluation of the efficacy of a contrast agent, can be independently obtained by a variable-temperature (VT) ^{17}O NMR measurement of the water nuclear transverse relaxation rate (R_2).[24] The VT ^{17}O NMR curves for complexes **4**, **5**, and **7** are shown in Figure 3 while that of **6** was previously reported.[15] Complexes **3** and **10** were not sufficiently soluble for ^{17}O NMR studies. The data were recorded at 2.1 T (90 MHz for the proton and 12 MHz for ^{17}O) and pH ≈ 7. The curves were analyzed in terms of the well-established equations of Swift−Connick, rearranged in a form suitable for Gd^{3+}.[16] The shape of the profiles of Figure 3 is similar to that of the profiles of complexes **6**, **8**, and **9** and is typical of systems in the fast exchange regime.[14,17,18,25] Under these conditions, the bandwidth is largely controlled by the electron relaxation parameters. The evaluation of the water exchange rate is enhanced by a careful comparison of the ^{17}O data with the NMRD profile (from the analysis of both experiments the same set of values for the electronic parameters must be obtained) and by measuring ^{17}O data at two magnetic fields.[15]

For all the complexes, an excellent fit of the experimental data was obtained with the parameters shown in Table 1. For Gd^{3+}, the electronic relaxation rate is usually ascribed to a transient zero field splitting (ZFS) brought about by solvent collisions or molecular vibrations. τ_V is a correlation time for the modulation of this ZFS, and Δ^2 is the mean square of the ZFS energy. There is a certain covariance of k_{ex} with τ_V or Δ^2, and so, by imposing different starting

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Table 2. Binding Parameters and Relaxivities Measured with HSA at 25 °C, 20 MHz, and pH 7.0[a]

	3	4	5	6	7	8	9	10
K_A (M^{-1})	8640 ± 2000	1510 ± 300	1800 ± 200	2900 ± 540	690 ± 100	731 ± 39	186 ± 50	3040 ± 210
$r_{1p}{}^b$ (mM^{-1} s^{-1})	15.4 ± 0.3	19.3 ± 0.9	15.7 ± 0.3	29 ± 1	19.8 ± 1	17	74 ± 14	24.6 ± 0.5

[a] Data for compound **9** are taken from ref 18.

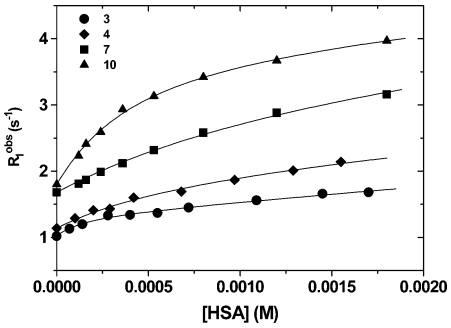

Figure 4. The water proton longitudinal relaxation rate of a solution of complexes **3** (0.06 mM), **4** (0.09 mM), **7** (0.17 mM), and **10** (0.014 mM) as a function of HSA concentration at 20 MHz, 25 °C and pH = 7.0.

values to the electronic parameters it is possible to obtain a different estimate of k_{ex}. However, only with the values of the electronic parameters reported in Table 1 is it possible to fit both the NMRD and ^{17}O profiles, and thus, the rather narrow range of the water exchange rates obtained (k_{ex} = 0.45−1.1 × 10^8 s^{-1}) can be considered reliable. These results represent further confirmation of the fact that all the complexes are octacoordinate and the water exchange process occurs through a common associative mechanism involving a low energy nonacoordinate intermediate, as recently proven for complex **6**.[15]

Relaxometric Analysis of HSA Interactions. Proton Relaxation Enhancement (PRE) Titrations. The noncovalent interaction between HSA and the Gd^{3+} complexes was investigated using the well-established proton relaxation enhancement (PRE) method[2] that allows both the binding parameters (K_A's) and the relaxivity enhancement of the (Gd^{3+} complex)−HSA adduct to be determined. In this method, the water proton longitudinal relaxation rates (r_{1p}) of solutions containing the Gd^{3+} complex and increasing concentrations of the serum protein are measured. The results of the PRE study (at 20 MHz and 25 °C) of complexes **3**−**8** and **10** with HSA are shown in Figure 4 and Table 2. Although HSA is not likely to possess a single specific binding site, we have assumed that the multiple binding sites are characterized by identical affinity constant and relaxivity. This may be a crude approximation, but it allows for an easy comparison with similar data that have been previously reported.[2,18]

The complexes analyzed (**3**−**8** and **10**) have moderate affinities for HSA with K_A values of 0.7−8.6 × 10^3 M^{-1}. Complex **3** has the highest affinity for HSA (K_A = 8.6 × 10^3 M^{-1}), significantly greater than that of **4** (K_A = 1.5 × 10^3 M^{-1}). This indicates that the PEG chain has a negative influence on protein interaction. Complexes **4** and **5** have a greater protein affinity than **7**, indicating that the benzyl groups enhance the HSA interaction, as expected. The protein affinities of complexes **4** and **5** are very similar, and it appears that the *p*-methoxy group of the MOB substituent is of minor influence on protein interactions. Complex **10** has an albumin affinity very similar to that of **6**: the major structural difference (other than isomerism) between these two complexes is that R_3 is an ethoxy group in **6** and a primary amine in **10**. The amine is protonated at neutral pH; hence, **10** is zwitterionic, while **6** remains anionic. The lack of a difference in protein binding strength between the neutral and anionic complexes indicates that chelate charge does not play a major role in the protein interaction.

The PRE study indicates that the relaxivities of the HSA adducts of complexes **3**−**8** and **10** are 15−29 mM^{-1} s^{-1}, at 20 MHz and 25 °C (Table 2). In all cases, the bound relaxivity ($r_{1p}{}^b$) is higher than that of the free complex ($r_{1p}{}^f$) but is significantly lower than anticipated. The relaxivities are also significantly lower than that of the HSA adduct of **9**.[18]

NMRD of HSA Adducts. NMRD profiles of the HSA adducts of **4** and **5** were obtained (Figure 5). The low $r_{1p}{}^b$ values at 20 MHz (from the PRE titrations) were confirmed by the NMRD profiles which closely resemble that measured for the adduct with BSA of GdDOTP, a complex with q = 0.[2,21] This result strongly suggests the formation of ternary complexes involving the displacement of the coordinated water molecule(s) by the protein.[26] The shape of the profiles at higher field strength indicates a dependence of the proton relaxivity on the rotational dynamics of the metal chelates. This has been previously observed and attributed to a contribution from (i) the exchange of the mobile protons of the protein located in the proximity of the binding site and dipolarly relaxed by the paramagnetic metal ion; (ii) second-sphere water molecules whose rotational mobility is markedly reduced in the ternary complexes.[2]

In order to probe the source of the low relaxation observed, several key experiments were performed. The first set of key experiments involved relaxivity competition titrations of the complexes against acetate, lactate, malonate, citrate, and phosphate anions, to see if they displace the coordinated water molecule. Very low, insignificant interactions were observed, and there was no detectable formation of ternary adducts under physiological conditions, as evident by the very low association constants. This is not surprising as similar results were obtained for the titration of Gd-TREN-HOPY (HOPY = hydroxypyrimidinone analogue of the parent 3,2-HOPO) with acetate, lactate, and malonate anions.[25]

Variable-Temperature Relaxivity (R_1) of HSA Adducts. An alternative explanation for the low $r_{1p}{}^b$ values could be a dramatic decrease in the water exchange rate upon binding. In that case, the low k_{ex} value could markedly limit the

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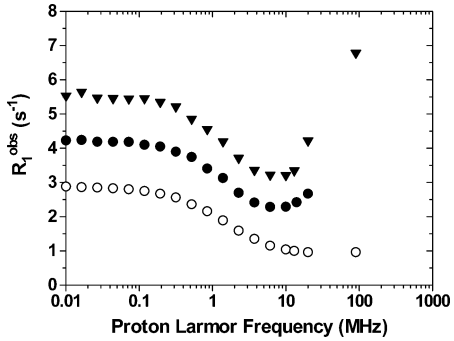

Figure 5. $1/T_1$ proton NMRD profiles of complexes **4** (0.190 mM; ▼) and **5** (0.085 mM; ●) in the presence of HSA (1.8 mM) at 25 °C and pH = 7. The open circles (○) represent the profile of a diamagnetic solution of the protein (1.8 mM).

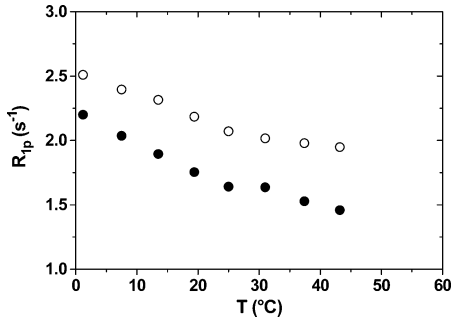

Figure 6. Variable-temperature ^{1}H relaxation rate of aqueous solutions of complexes **5** (0.12 mM; ○) and **7** (0.12; ●) containing HSA (1.8 mM), measured at 20 MHz.

increase of the relaxivity expected from the lengthening of τ_R (slow exchange condition: $\tau_M \geq T_{1M}$).[27] This hypothesis was investigated by measuring the temperature dependence of the $r_{1p}{}^b$ relaxation rate of the Gd-complex−HSA adducts at 20 MHz. Data were obtained for complexes **5** and **7** (Figure 6). The relaxation rate decreases with temperature in the range 0−45 °C, indicative of either fast water exchange or that only the second-sphere relaxation component is operating. Due to the very low $r_{1p}{}^b$ values, the latter is more likely.

Variable-Temperature ^{17}O NMR of HSA Adducts. The third set of experiments involved variable-temperature (VT) ^{17}O NMR measurement of the water nuclear transverse relaxation rate (R_2) of excess **4** and **8** in the presence of HSA. The ^{17}O R_2 data (14.1 T, pH = 7) for three solutions are shown in Figure 7. Over the entire temperature range 275−325 K, the addition of the paramagnetic complex to the solution of the protein does not induce any significant increase of the transverse relaxation rate. This unambiguously indicates the absence of water molecules bound to gadolinium whose scalar ^{17}O relaxation is transmitted via exchange to the bulk. Thus, the decrease of the hydration of the complexes in the adducts with serum albumin, already observed in the case of other complexes,[2,26] accounts for the limited relaxation enhancement observed. Verification of the hydration number (q) of the Eu^{3+} and Tb^{3+} analogues of these Gd^{3+} complexes in the presence of HSA was attempted

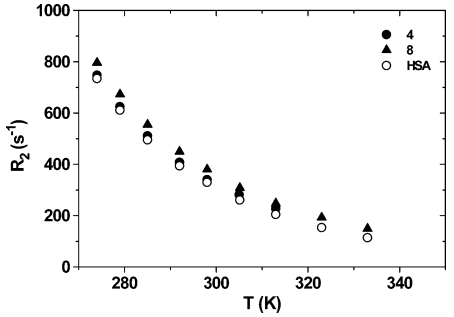

Figure 7. Temperature dependence of the paramagnetic contribution (R_2) to the transverse ^{17}O water relaxation rate of aqueous solutions of complexes **4** (3.0 mM) and **8** (4.0 mM) containing HSA (1.8 mM) at 2.1 T and pH = 7.2. The open circles (○) represent the data of a diamagnetic solution of the protein (1.8 mM).

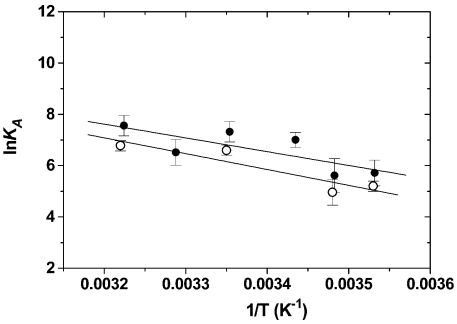

Figure 8. Plot of ln K_A vs $1/T$ for complexes **4** (●) and **8** (○).

by luminescence decay kinetics. However, the Eu^{3+} and Tb^{3+} chelates are nonluminescent.

Variable-Temperature PRE Titrations of HSA Adducts. It is clear from the PRE studies at 25 °C that ligand structural features influence the albumin association constants, the most visible effect being the negative influence of the PEG chain. PEG is known to be resistant to protein adsorption, a phenomenon attributed to steric stabilization effects and its molecular conformation in aqueous solution.[28] However, there are a few reports that indicate that PEG has a weak interaction with HSA.[29,30] The thermodynamic contributions to the binding interaction of **4** and **8** were evaluated by performing the PRE titrations (enabling the determination of K_A) over a temperature range. The van't Hoff plots are shown in Figure 8, and analysis of the data gives the following result: **4**, $\Delta H = 44.6 \pm 18.1$ kJ/mol and $\Delta S = 206.0 \pm 62.3$ J mol^{-1} K^{-1}; **8**, $\Delta H = 51.8 \pm 16.1$ kJ/mol and $\Delta S = 224.8 \pm 54.9$ J mol^{-1} K^{-1}. The results show that the enthalpy of interaction is endothermic and that the albumin interactions are entropically driven. This result is plausible and is consistent with that of a previous report;[30] the interaction of the PEG chains with HSA results in the release of water molecules from both the protein and the PEG, resulting in a positive change in entropy.

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Conclusions

The proton relaxation experiments indicate that the interaction of the Gd^{3+} complexes with HSA results in metal-bound water displacement with a marginal increase in proton relaxivity. This ligand displacement effect has been previously observed with Gd-DO3A ($q = 2$) and its analogues,[26] as well as Gd-texaphyrin ($q = 3$).[31] The increase in relaxivity in the presence of the protein can be attributed to second-sphere water proton relaxation and/or prototropic effects. The relaxivity of the HSA adducts of complexes **3**−**8** and **10** is much lower than that of the HSA adduct of **9**. The following hypothesis is presented: due to the higher protein affinity of **3**−**8** and **10**, the metal ion is much closer to the surface of the protein resulting in competition with carboxylate groups for the Gd-bound water. However, due to the lower protein affinity and the shielding of the Gd^{3+} center by the very long PEG chain, the metal ion in **9** is further from the surface of the protein and therefore retains a water molecule. The increase in the rotational correlation time therefore results in a significant enhancement in relaxivity.

The HOPO-appendaged aromatic groups appear to enhance protein affinity while the PEG groups generally appear to hinder HSA interaction. The chelate−albumin interactions are entropically driven, indicating that the release of water of hydration from the protein and the PEG chain plays a major role in this process. In conclusion, the strategy of using HOPO complexes with $q > 1$ to target HSA in order to attain higher relaxivities has to be optimized. This might require the design of a suitable rigid spacer between the recognition synthon and the metal coordination site in order to avoid the close contact with the surface of the protein and prevent water displacement.

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Supporting Information Available: Theory used in the analysis of the relaxometric data. NMRD profile of **5** and best fit parameters by assuming $q = 1$ and $q = 2$. NMRD profile of **8**. Proton relaxivities as a function of magnetic field (expressed as proton resonance frequency), ^{17}O paramagnetic transverse relaxation rates, and proton longitudinal relaxation rates as a function of HSA concentration of **4** and **5** at 25 °C. Plot of r_{1p} of as a function of HSA concentration for **5**, **6**, and **8**. $1/T_1$ proton NMRD profile of **7** in the presence of HSA. Variable-temperature ^1H relaxation rate of aqueous solution of **4** containing HSA. Synthetic details of intermediates and complexes **4**−**7**. This material is available free of charge via the Internet at http://pubs.acs.org.

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