The Effect of a Phosphodiester Linking Group on Albumin Binding, Blood Half-Life, and Relaxivity of Intravascular Diethylenetriaminepentaacetato Aquo Gadolinium(III) MRI Contrast Agents

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Received May 30, 2001

Amphiphilic gadolinium complexes were investigated as potential magnetic resonance imaging (MRI) contrast agents. A series of complexes was synthesized in order to study the effect of hydrophilic phosphodiester groups on albumin binding, relaxivity, and blood half-life in rats. Thus, compound **11a**, a diethylenetriaminepentaacetato aquo gadolinium(III) (Gd-DTPA) derivative with an octyl substituent, was synthesized and compared to **5b**, the analogous octyl derivative containing a phosphodiester linkage between the gadolinium chelate and the alkyl chain. Likewise, **11b**, a naphthyl Gd-DTPA derivative, was compared to the naphthyl phosphodiester derivative **5c**. A direct comparison is not available for **5a**, a 4,4-diphenylcyclohexyl phosphodiester Gd-DTPA derivative; however, its pharmacokinetic properties mirror those of the other phosphodiester derivatives. Although the introduction of the phosphodiester moiety decreased log *P* by approximately 1.7 units, albumin binding data obtained in 4.5% human serum albumin (HSA) indicated that derivatives containing the phosphodiester group exhibited somewhat higher albumin affinity than their alkyl analogues (54 ± 5 and 44 ± 4% for **5b** and **11a**, respectively; 40 ± 4 and 30 ± 3% for **5c** and **11b**, respectively). Both classes of agents were characterized by enhanced relaxivity in the presence of 4.5% HSA (r1 = 16−42 mM^{-1} s^{-1} at 20 MHz and 37 °C) as compared with the relaxivity values measured in phosphate-buffered saline (PBS) alone (r1 = 4.6−6.6 mM^{-1} s^{-1} at 20 MHz and 37 °C). Pharmacokinetic data indicated that compound **5b** had a half-life of 14.3 ± 1.8 min in the rat as compared with a half-life of 6.20 ± 0.04 min for the non-phosphodiester analogue **11a**. Similarly, the half-life obtained for the phosphodiester **5c** was 14.3 ± 1.7 min as compared with a half-life of 6.80 ± 0.03 min for **11b**. The percent biliary excretion was significantly lower for the phosphodiester compounds than for non-phosphodiester analogues (17.7 ± 4.0 and 66.9 ± 3.4% for **5b** and **11a**, respectively; 17.0 ± 1.6 and 64.3 ± 9.0% for **5c** and **11b**, respectively). The percent biliary excretion (15.8 ± 4.4%) and plasma half-life in the rat (23.1 ± 2.9 min) for **5a** are consistent with the extended plasma half-life of the other phosphodiester derivatives. Taken together, the enhanced relaxivity and extended blood half-life of the phosphodiester derivatives support the concept of using endogenous albumin binding to achieve blood pool-like properties for small-molecule magnetic resonance imaging (MRI) contrast agents.

Introduction

Magnetic resonance angiography (MRA) is an imaging technique in development to permit assessment of cardiovascular disease, including the evaluation of stenoses and occlusions in peripheral and coronary arteries.[1−3] Selective intravascular water proton relaxation rate enhancement using paramagnetic contrast agents is thought to be a critical factor in enabling routine T_1-weighted MRA examinations, partly because of imaging complications resulting from poor flow in blocked arteries. Gadolinium-based contrast agents are extremely effective at causing proton relaxation rate enhancement, and the resultant short blood T_1 values following intravenous injection permit rapid pulsing and enable very high resolution images. Current extracellular magnetic resonance imaging (MRI) contrast agents are limited by relatively short half-lives and are also freely extravasated into background muscle, which decreases the contrast-to-noise ratio during steady state imaging. For routine clinical use in MRA applications, it is desirable that the contrast agent be selectively retained in the vascular space and have an extended blood half-life in order to provide a sufficient plasma concentration over a 1 h imaging window. The extended blood half-life facilitates imaging of multiple body regions and acquiring long, pulse-gated scans of coronary arteries.

The design of MRI "blood pool" contrast agents that provide sufficient proton relaxation rate enhancement selectively within the vascular space, but that are also efficiently excreted, has been a difficult challenge. For example, excellent blood tracer properties may be achieved using high-molecular weight polymeric conjugates of gadolinium chelates (such as Gd-DTPA-BSA[4] or Gd-DTPA-polylysine[5]) or particulate iron oxides such

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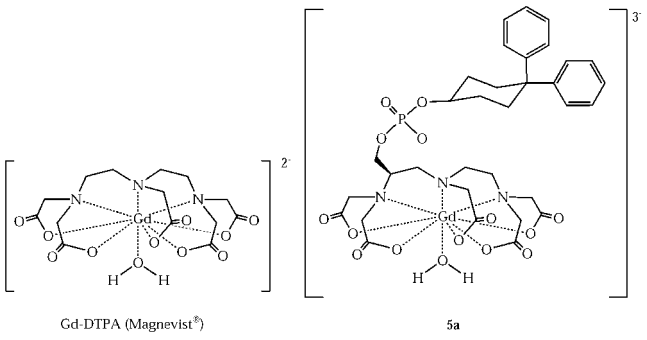
10.1021/jm0102351 CCC: $22.00
Published on Web 06/27/2002

as NC100150;[6,7] however, these materials generally have poor excretion properties. One exception to this trend is the development of a dendrimer-based macromolecular agent, Gadomer 17, which is a highly monodisperse 17 kDa compound with a relatively short terminal half-life (10 min in the rabbit).[8]

An alternative approach to creating intravascular contrast agents exploits noncovalent binding of small-molecule gadolinium chelates to endogenous serum proteins, such as human serum albumin (HSA). In principle, albumin binding can protect the contrast agent from extravasation into surrounding tissue, thus reducing background enhancement and improving contrast. An additional benefit results from the decreased rate of molecular tumbling of the protein-bound agent which enhances the efficacy of the electron−nuclear interaction between the gadolinium(III) ion and water protons, leading to very short T_1 relaxation times and bright blood vessels on an MRI image.[9,10] In theory, this approach combines the practical advantages of a small-molecule pharmaceutical with the blood tracer properties of a high-molecular weight species.

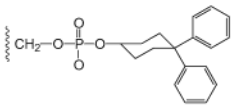
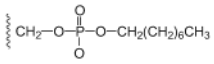
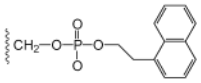
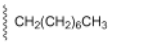
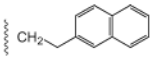
A particular challenge in the design of albumin-targeted blood pool agents is to select substituents that permit both strong albumin binding *and* long blood half-life. In the development of small-molecule blood pool MRI contrast agents, these have been conflicting goals: gadolinium chelates with hydrophobic substituents tend to be rapidly excreted through the liver. For example, the addition of the *p*-ethoxybenzyl or *p*-butylbenzyl moieties to gadolinium diethylenetriaminepentaacetate (Gd-DTPA) was shown to promote HSA binding but also to decrease plasma elimination half-life in rats due to an increased rate of hepatocellular uptake.[11,12] Recently, it has been shown that trisodium-{(2-(*R*)-[(4,4-diphenylcyclohexyl)phosphonooxymethyl]diethylenetriaminepentaacetato)(aquo)gadolinium(III)} (**5a**), a phosphodiester derivative of Gd-DTPA substituted with a hydrophobic protein binding group, has an extended blood half-life in humans, rabbits, and monkeys compared to Gd-DTPA.[13−16] While the signal intensity of blood drops rapidly after injection of Gd-DTPA, little change was noted over 1 h for **5a** in Phase I clinical trials. Compound **5a** is currently in clinical trials to assess blockages in arteries.[17]



Gd-DTPA (Magnevist®) 5a

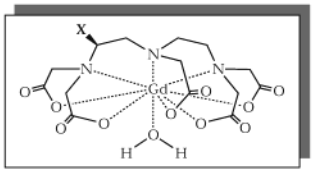
One key molecular feature that is responsible for the longer blood half-life of **5a** is the presence of the hydrophilic phosphodiester group. This group appears to decrease the rate of hepatobiliary uptake, thus increasing blood half-life in this class of amphiphilic Gd-DTPA derivatives. This paper focuses on key structure−activity relationships (SAR) in rats which provided the groundwork for the development of small-molecule blood pool agents, as exemplified by **5a**. In particular, the effect of phosphodiester insertion between Gd-DTPA and hydrophobic protein-binding groups on plasma pharmacokinetics and excretion was examined. Thus, **11a**, a Gd-DTPA derivative with an octyl substituent, was compared to **5b**. Likewise, **11b**, a naphthyl derivative, was compared to **5c**. Corresponding data for **5a** are also reported (Chart 1).

Chart 1. Chemical Structures of Gd-DTPA, **5a**, **5b**, **5c**, **11a**, and **11b**

COMPOUND	X
Gd-DTPA	H
5a	
5b	
5c	
11a*	
11b*	

*racemic

Chelate Substructure



Results

The alkyl-substituted DTPA gadolinium complexes were prepared in good yields as racemic compounds by modifications of literature methods.[20,21] The synthesis of the novel phosphodiester-linked chelates was achieved starting from 2-(*R*)-hydroxymethyl-DTPA-penta-*tert*-butyl ester (**1**).[35] A modified, scaleable synthesis of this particular intermediate has also been reported.[36] Phosphoramidite chemistry was then used to couple **1** to alcohols 4,4-diphenylcyclohexanol, 1-octanol, or 2-(1-naphthyl)ethanol. Oxidation using *tert*-butylhydroperoxide gave the cyanoethyl-protected phosphorus(V) triester which was subsequently deprotected with ammonia, methanol, and triethylamine. Complete deprotection of the *tert*-butyl esters using either TFA or HCl gave the penultimate polyaminocarboxylate ligand which was subsequently reacted with gadolinium oxide and base to prepare the corresponding chelate.

The insertion of a hydrophilic phosphodiester group between the hydrophobic side chains and the Gd-DTPA

Table 1. In Vitro Partition Coefficient (log *P*), Protein Binding, and Relaxivity Data of Contrast Agents

sample	partition coefficient log $P_{butanol/PBS}$	percent bound 4.5% HSA[b]	relaxivity[a] ($mM^{-1}\,s^{-1}$) PBS[c]	observed relaxivity[a] ($mM^{-1}\,s^{-1}$) 4.5% HSA	calculated relaxivity bound[d] ($mM^{-1}\,s^{-1}$) 4.5% HSA
Gd-DTPA	−4.3 ± 0.3	0	4.6 ± 0.3	4.6 ± 0.3	not applicable
5a	−2.11 ± 0.06	88 ± 2[e]	6.6 ± 0.4[f]	42.0 ± 2.3[f]	47 ± 4
5b	−2.34 ± 0.06	54 ± 5	6.1 ± 0.3	not measured	−
5c	−3.25 ± 0.15	40 ± 4	6.0 ± 0.3	19.5 ± 2.0	40 ± 8
11a	−0.63 ± 0.04	44 ± 4	5.9 ± 0.3	20.7 ± 2.1	40 ± 9
11b	−1.53 ± 0.03	30 ± 3	5.5 ± 0.3	16.3 ± 1.6	42 ± 10

[a] r1, 0.47 T (20 MHz), 37 °C. [b] In 0.1 mM drug, 4.5% HSA, fraction V. [c] Phosphate-buffered saline. [d] Calculated using eq 1. [e] From reference 22. [f] From reference 13.

Table 2. Comparison of Rat Pharmacology Data for Phosphodiester and Non-Phosphodiester-Based Contrast Agents (dose = 0.1 mmol/kg)

sample	percent biliary excretion[a]	Vd_{ss}[b] (L/kg)	$T_{1/2\beta}$[c] (min)	AUC_{conc}[d] (mM/min)	$AUC_{1/T1}$[e] (s^{-1} min)
Gd-DTPA	6.3 ± 3.7[g]	0.28 ± 0.04	15.4 ± 1.3	6.21 ± 0.09	46.4 ± 4.2
5a[f]	15.8 ± 4.4	0.24 ± 0.03	23.1 ± 2.9	9.35 ± 1.30	285 ± 27
5b	17.7 ± 4.0	0.31 ± 0.02	14.3 ± 1.8	5.96 ± 0.81	150 ± 17
5c	17.0 ± 1.6	0.32 ± 0.03	14.3 ± 1.7	5.57 ± 0.38	94.2 ± 5.0
11a	66.9 ± 3.4	0.62 ± 0.05	6.20 ± 0.04	3.06 ± 0.13	69.7 ± 3.1
11b	64.3 ± 9.0	0.44 ± 0.07	6.80 ± 0.03	2.58 ± 0.49	40.1 ± 0.9

[a] In 24 h. [b] Volume of distribution, steady state. [c] Elimination half-life. [d] Area under concentration versus time curve, 0−30 min. [e] Area under plasma $1/T_1$ versus time curve, 0−30 min. [f] From reference 13. [g] Values are mean ± 1 standard deviation.

chelate decreased the overall hydrophobicity of the molecules by approximately 1.7 log units as shown by butanol buffer partition coefficients (Table 1). Despite the lower hydrophobicity, the phosphodiester derivatives bound somewhat more strongly to HSA than their non-phosphodiester counterparts (54 ± 5 and 44 ± 4% for **5b** and **11a**, respectively; 40 ± 4 and 30 ± 3% for **5c** and **11b**, respectively). Under similar conditions (0.1 mM drug, 4.5% HSA, ICP detection), **5a** was found to be 88 ± 2% protein bound.[22]

In rats, the largest differences between the phosphodiester compounds and their counterparts were in the percent of the injected dose excreted by the biliary route. The phosphodiester derivatives had less than 20% biliary excretion compared to greater than 60% bilary excretion for the non-phosphodiester compounds. The reduced hepatocellular uptake for the phosphodiester agents resulted in longer elimination half-lives (14.3 ± 1.8 and 6.2 ± 0.04 min for **5b** and **11a**, respectively; 14.3 ± 1.7 and 6.8 ± 0.03 min for **5c** and **11b**, respectively) and greater area under the curve concentrations (AUC_{conc}) from 0 to 30 min (5.96 ± 0.81 and 3.06 ± 0.13 min for **5b** and **11a**, respectively; 5.57 ± 0.38 and 2.58 ± 4.9 min for **5c** and **11b**, respectively; Table 2 and Figure 1). The low percent biliary excretion observed for **5a** (15.8 ± 4.4%) in the rat combined with a higher albumin binding resulted in the longest blood half-life of the group of compounds studied (23.1 ± 2.9 min).

Relaxivity data in PBS, 4.5% HSA, and calculated relaxivity-bound values are also shown in Table 1. The relaxivity of the protein-bound agent, $r1_b$, is calculated according to eq 1 where $r1_{obs}$ is the observed longitudinal relaxivity, f_b is the fraction of bound drug, $r1_u$ is the relaxivity of the drug in the absence of protein, and f_u is the fraction of unbound drug (measured by ultrafiltration).

$$r1_{obs} = r1_b f_b + r1_u f_u \quad (1)$$

The data in Table 1 indicate that noncovalent binding of the gadolinium complexes to albumin was accompanied by an increase in observed relaxivity, the ability of the paramagnetic complexes to alter proton relaxation rate enhancement. Interestingly, when normalized for albumin binding by calculating $r1_b$, the relaxivity of the bound complexes was similar.

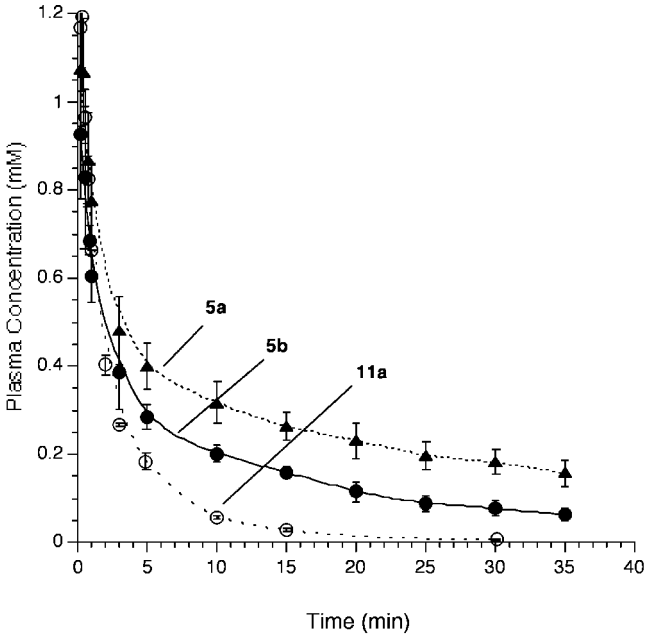


Figure 1. Comparison of the **5a**, **5b**, or **11a** plasma concentration following an intravenous injection in the rat (0.1 mmol/kg). Plots show plasma concentration as a function of time (min). The plasma concentration of **5b** is generally greater than that of **11a**, demonstrating the plasma half-life extension ability of the phosphodiester linker. The increased half-life of **5a** is reflected in the calculated area under the curve data (AUC_{conc} in mM/min) shown in Table 2.

As a measure of the potential MRI blood signal, the $1/T_1$ relaxation rate of plasma samples was also measured (Figure 2). The AUC-$1/T_1$ values for the phosphodiester derivatives were roughly twice that of the closely related non-phosphodiester analogues.

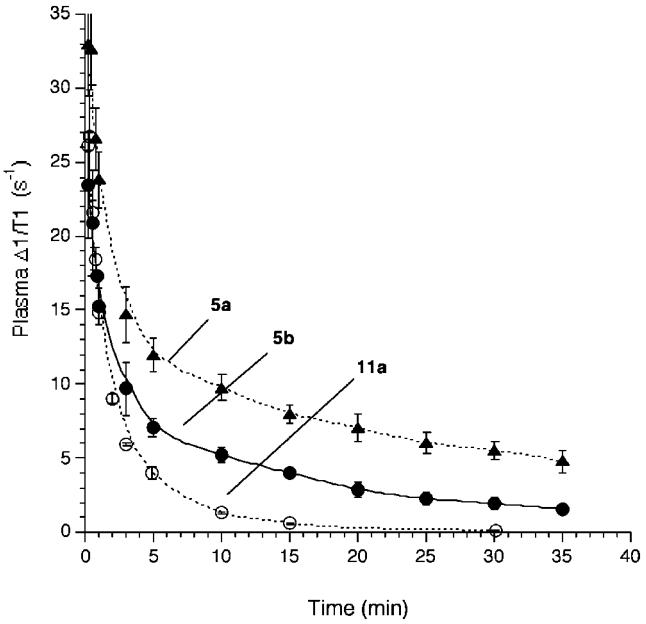


Figure 2. Comparison of relaxation rate enhancement ($\Delta 1/T_1$) measured in rat plasma following an intravenous injection of **5a**, **5b**, or **11a** (0.1 mmol/kg). Plots show plasma $\Delta 1/T_1$ as a function of time (min). The plasma relaxation rate enhancement is generally greater for **5b** than **11a** primarily due to the higher concentration of the phosphodiester agent at any given time point. The increased efficacy of **5a** relative to **5b** or **11a** or Gd-DTPA is reflected in the larger area under the curve for the plasma relaxation rate enhancement (AUC_{1/T_1}, s^{-1} min).

Discussion

Synthesis. Alkyl- and aryl-substituted chelates derived from amino acid starting materials have found wide application in both MRI and nuclear imaging applications.[10] The synthetic route chosen for the synthesis of the racemic polyaminocarboxylate ligands **11a** and **11b** (Scheme 1) is analogous to those previously reported for the synthesis of *p*-NCS-Bz-DTPA and EOB-DTPA.[20,23] The synthesis of chiral key intermediate 2-(*R*)-hydroxymethyl-DTPA-penta-*tert*-butyl ester (**1**), which contains the basic functionality necessary for metal chelation, has been reported previously in the literature.[35,36] The penta-*tert*-butyl esters were purified by flash chromatography and the polyaminocarboxylates by C_{18} Sep-Pak reversed phase chromatography. Clean transformation to the gadolinium complexes was accomplished using 0.5 equiv of Gd_2O_3 and stoichiometric quantities of sodium hydroxide or *N*-methyl-D-glucamine (NMG) base.

Phosphoramidite chemistry, the method of choice for automated polynucleotide synthesis,[24] was used to prepare the novel unsymmetrical phosphodiesters **5a**, **5b**, and **5c**. In this work, 2-(*R*)-hydroxymethyl-DTPA-penta-*tert*-butyl ester (**1**, Scheme 1) was converted to a common phosphoramidite intermediate which could then be coupled to a variety of albumin binding moieties prior to the formation of the desired gadolinium complexes. The phosphodiester linking group was found to be remarkably robust under synthetic conditions used for chelation (95 °C, pH 2−4).

Albumin Binding. The gadolinium DTPA chelates described herein are negatively charged (2^- or 3^-), extremely hydrophilic molecules. Butanol/water (PBS buffer) partition coefficients ranged from −0.63 to −3.25; butanol was selected as a substitute for the typical octanol because of the experimental difficulty in accurately determining low concentrations of chelates in the more traditional octanol/water system. Consistent with expectations, the addition of a phosphodiester moiety to the chelate backbone further increased the hydophilicity of the gadolinium chelates and reduced log *P* by about 1.7 units. In the two cases examined (Table 1, **11a** vs **5b** and **11b** vs **5c**), the increased hydrophilicity of the phosphodiester was accompanied by a slight increase in equilibrium albumin binding.

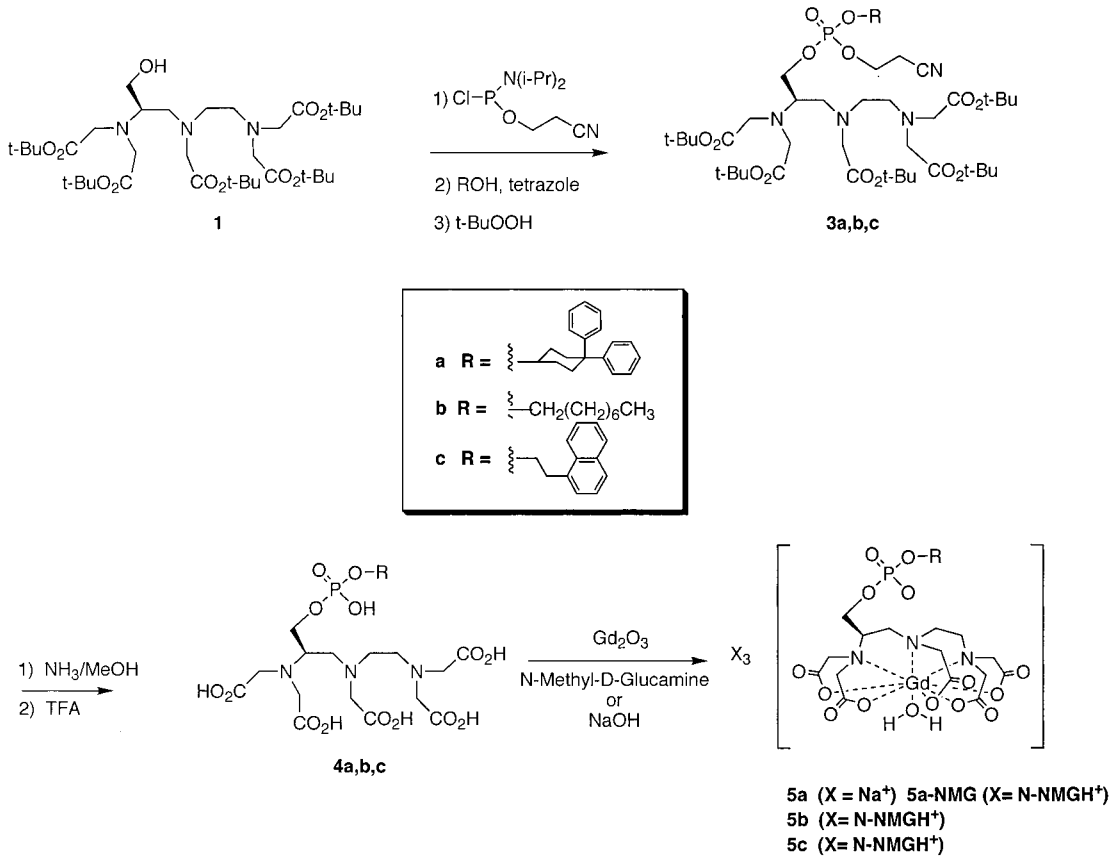
The maintenance of good, even moderately improved, HSA binding upon the addition of the very hydrophilic phosphodiester group to an already extremely hydrophilic Gd-DTPA dianion (2^-) is striking. These results are contrary to the common notion that albumin binding is simply lipophilicity driven and suggest that, in this case, the interaction with the protein more than compensates for the strong hydration of the anionic functional group. The proposed structure of HSA binding sites afforded by X-ray analysis suggests sock-shaped hydrophobic sites lined by positively charged residues near the "ankle"[25] which could provide a site for electrostatic interaction with the phosphodiester. Additional SAR studies to further elucidate the nature of the binding interaction are in progress.

Blood Half-Life. The addition of lipophilic albumin binding moieties to Gd-DTPA chelates generally results in a blood half-life shorter than that of the parent, unsubstituted DTPA chelate because of hepatobiliary extraction. This characteristic was exploited in the design of the liver imaging agent gadolinium ethoxy benzyl (EOB) DTPA.[26] Therefore, a significant challenge in the design of effective blood pool imaging agents was the combination of significant albumin binding with *extended* blood half-life.

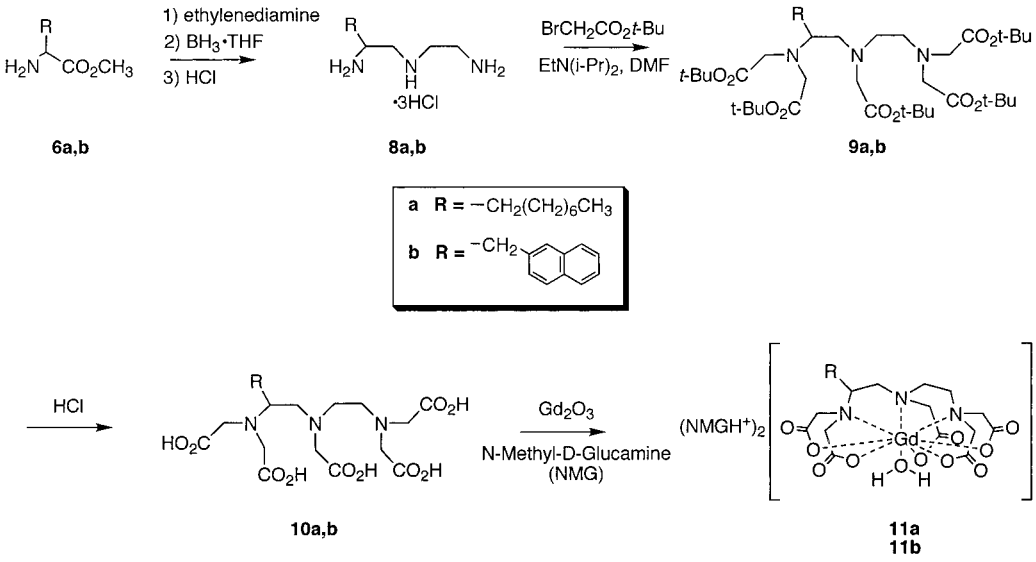
While some rational design criteria might be useful in designing HSA binders, the success of the phosphodiester in reducing biliary excretion was unexpected. Aside from the requirement of some hydrophobic residues, structure−activity relationships for hepatocellular uptake are less understood.[27] Perhaps the added hydrophilicity was sufficient to tip the balance away from rapid hepatocellular uptake while, at the same time, the phosphodiester placement maintained good HSA binding. Regardless of the mechanism, reduction of hepatobiliary extraction was critical to the improvement in blood half-life for the phosphodiester derivatives. With hepatobiliary elimination minimized, the relatively slow glomerular filtration of the unbound drug dominated the excretion pathway, thus extending the blood half-life. Other functional groups, such as carboxylate or sulfonate, may function in the same manner.[28−30]

Relaxivity. The currency of MRI contrast agent efficacy is proton relaxation enhancement, as it is this effect that translates to a brighter image during the MRI examination. The data in Table 1 indicate that the observed reversible protein binding was clearly coupled to an increase in relaxivity by the paramagnetic complexes. Relaxivity is the paramagnetic relaxation rate enhancement normalized to 1 mM and is dependent on both field strength and temperature.[9,10] A larger relaxivity indicates a greater enhancement effect and, pro-

Scheme 1. Synthetic Scheme for the Phosphodiester Gadolinium Complexes **5a**, **5b**, and **5c**



Scheme 2. Synthetic Scheme for the Non-Phosphodiester DTPA Gadolinium Complexes **11a** and **11b**



vided other pharmacokinetic factors are equivalent, a more efficacious MRI contrast agent. Relaxivity is a complex biophysical phenomenon dependent on a number of key molecular properties, including the rotational diffusion rate of the paramagnetic chelate unit, the exchange rate of the coordinated water, and the electronic properties of the gadolinium ion. For the type of Gd-DTPA chelates discussed here, theory predicts that relaxivity should increase due to slowing of the overall rotational diffusion rate of the paramagnetic chelate. The observed increase in relaxivity at 20 MHz (a clinically relevant field strength) upon binding to HSA was approximately 3 times higher for the moderately bound compounds **5b**, **11a**, **5c**, and **11b** and about 7 times higher for **5a**, the most efficacious agent of the group.

The relaxivity of the bound contrast agent was calculated from the measured percent bound at 0.1 mM drug in 4.5% HSA, the total observed relaxivity in 4.5% HSA, and the relaxivity of the free (non-protein-bound) agent in PBS alone. For these calculations, a single binding site was assumed. The bound relaxivity values reported here (Table 1) were significantly larger than values obtained for covalently labeled Gd-DTPA poly-

mers (r1 = 6−18 mM^{-1} s^{-1} at 20 MHz)[5,31−33] and superior to that reported for Gd-DTPA conjugated dendrimers (r1 = 18−36 mM^{-1} s^{-1} at 25 MHz).[34] Interestingly, the calculated bound relaxivity of the various gadolinium complexes was quite similar (40−47 mM^{-1} s^{-1}), indicating that the complexes experience similar local rotational properties and may occupy similar sites on HSA. Studies of the effect of site occupancy on relaxivity for this class of compounds are in progress.

Conclusions

The human serum albumin binding, blood half-life, and relaxivity of amphiphilic gadolinium complexes containing a phosphodiester moiety were compared with those of complexes which are lacking the negatively charged, hydrophilic linker. A modest increase in albumin binding was observed for the compounds containing the phosphodiester linker. Moreover, a dramatic shift from predominantly biliary excretion to mostly renal excretion was observed following the addition of the phosphodiester moiety. Concomitantly, an extended blood half-life was observed for the phosphodiester compounds, presumably because glomerular filtration is a relatively slow process compared to biliary excretion. Because of the extended blood half-life and pronounced relaxivity enhancement observed in albumin solution, the gadolinium chelate phosphodiester compound **5a** is now being investigated in blood vessel enhancement studies.

Experimental Section

Unless otherwise noted, all materials were obtained from commercial suppliers and used without further purification. THF was distilled from potassium benzophenone ketyl immediately prior to use. Methylene chloride was distilled over calcium hydride. All column chromatography was carried out by flash methods with silica gel (230−400 mesh, EM separation). 2-(*R*)-Hydroxymethyl-DTPA-penta-*tert*-butyl ester (**1**) was obtained from 2-(*R*)-hydroxymethyldiethylenetriamine trihydrochloride as described in ref 35. Reactions were monitored by thin-layer chromatography (TLC) performed on aluminum-backed silica gel 60 F_{254}, 0.2 mm plates (EM separation), and compounds were visualized under UV light (254 nm), Ninhydrin-Plus reagent, or Dragendorff's reagent (both Alltech) with subsequent heating. Routine proton NMR spectra were recorded at 300 MHz in $CDCl_3$ with TMS as the internal standard except for the spectra recorded in D_2O (CD_3CN ref). Coupling constants (*J*) are reported in hertz (Hz). ^{31}P NMR spectra were obtained at 121.4 MHz and referenced to H_3PO_4. Fast atom bombardment-mass spectrometry (FAB-MS) samples were run at the University of California, Berkeley, CA, and electrospray samples were obtained on a Hewlett-Packard 1100 MSD instrument operating in the negative ion mode, unless otherwise noted. Elemental analyses were performed by Galbraith Laboratories, Inc., Knoxville, TN. Solutions of the *N*-methyl-D-glucamine salt of Gd-DTPA (molecular weight = 938) were either prepared from Gd_2O_3, H_5DTPA, and *N*-methyl-D-glucamine (NMG) or obtained commercially (Magnevist, Berlex, Wayne, NJ). Analytical HPLC was performed using either a Thermohypersil C4 column (150 × 4.6 mm, 5 μm HyPURITY) or a YMC-Pack TMS column (250 × 4.6 mm, 3 μm) at a flow rate of 1.5 mL/min and a gradient of 5−20% B over 5 min, and then 20−100% B over 18 min. HPLC solvent A comprised 970 mL of phosphate buffer (0.146 M phosphate, 0.175 mM EDTA, pH 6.85) and 30 mL of ACN, while HPLC solvent B comprised 400 mL of phosphate buffer (0.146 M phosphate, 0.175 mM EDTA, pH 6.85) and 600 mL of ACN. Detection was performed at 220 nm (compounds **5c**, **11b**) or 197 nm (compound **5b**).

Phosphoramidite Intermediate (2). To a stirred solution of the penta-*tert*-butyl ester **1** (107.45 g, 0.15 mmol) and diisopropylethylamine (53 mL, 304 mmol) in distilled CH_2Cl_2 (600 mL) was added 2-cyanoethyl-*N*,*N*-diisopropylchlorophosphoramidite (40 mL, 179 mmol) at RT under N_2. The mixture was stirred at RT for 2 h. The reaction mixture was washed with ice cold 10% $NaHCO_3$ solution (700 mL), H_2O (500 mL), and brine (500 mL), and then dried over $MgSO_4$. The organic layer was evaporated to afford the crude product as a pale yellow oil (150 g). This crude oil was used for coupling reactions without further purification. R_f (ether/hexanes = 2:1) = 0.41. ^{31}P NMR (CD_3CN): δ_P 138.1, 138.2. A small portion of **2** was purified by flash chromatography on silica gel (EtOAc/hexanes/Et_3N 90:10:1 ratio) for analysis. ^1H NMR ($CDCl_3$): δ 1.16 (d, J = 16.4 Hz, 2H), 1.42 and 1.43 (2s, 45H), 2.6−2.73 (m, 3H), 2.75−2.87 (m, 5H), 2.93−3.03 (m, 1H), 3.33−3.48 (m, 6H), 3.48−3.7 (m, 7H), 3.7−3.92 (m, 3H). ^{13}C NMR ($CDCl_3$): δ 20.2, 20.3, 24.5, 24.6, 28.01, 28.03, 28.1, 42.9 (δ, J = 10.1 Hz), 43.0, 52.5, 52.7, 52.8, 53.7, 53.8, 53.9, 54.2, 55.8, 56, 56.1, 58.2 (d, J = 8.6 Hz), 58.5 (d, J = 8.1 Hz), 60.9 (2d ≈ t_{app}, J = 8.1 Hz), 63.1 (d, J = 15.1 Hz), 63.6 (d, J = 15.6 Hz), 80.2, 80.3, 80.6, 117.6, 117.7, 170.5, 171, 171.1, 171.2, 171.23. Exact mass calcd for $C_{44}H_{83}N_5O_{12}P$: 904.577587. Found: 904.574520. Anal. ($C_{44}H_{82}N_5O_{12}P$) C, H, N, P.

Cyanoethyl-(4,4-diphenylcyclohexyl)-(*R*)-phosphonooxymethyl-penta-*tert*-butyl-DTPA (3a). To a crude phosphoramidite intermediate **2** (prepared from 107.47 g (152.7 mmol) of 1-(*R*)-hydroxymethyl-DTPA-penta-*tert*-butyl ester) and 4,4-diphenylcyclohexan-1-ol (39.51 g, 0.157 mol) in distilled CH_3CN (900 mL) was added 1*H*-tetrazole (10.96 g, 0.156 mol). The reaction mixture was stirred overnight at RT, when ^{31}P NMR (CH_3CN) spectroscopy showed complete conversion of **2** (δ_p = 147.3 and 147.4) into the expected phosphite (δ_p = 134.2 and 134.0). To the latter mixture was added *tert*-butylhydroperoxide (Aldrich, 90% solution, 17.62 mL, ca. 0.158 mol), and the mixture was stirred for 1.5 h. The solution was tested with peroxide paper (EM separation) in order to indicate the absence of residual peroxide, and then concentrated in vacuo. The gummy residue was partitioned between diethyl ether (1000 mL) and H_2O (750 mL). The organic layer was washed with H_2O (500 mL), ice cold 10% $NaHCO_3$ solution (500 mL), and brine (2 times, 500 mL each), and then dried over $MgSO_4$ and evaporated. The residue was used for the next reaction without further purification. R_f ($CHCl_3$/MeOH = 10:1) = 0.47. ^{31}P NMR (CH_3CN): δ_P −8.1.

Cyanoethyl-(*n*-octyl)-(*R*)-phosphonooxymethyl-penta-*tert*-butyl-DTPA (3b). Compound **3b** was prepared from *n*-octanol (16.9 mmol) and crude phosphoramidite intermediate **2** (obtained from 1-hydroxymethyl-DTPA-penta-*tert*-butyl ester **1**, 4.40 g, 6.40 mmol) by the same procedure described for **3a**. The product was purified by silica gel column chromatography ($CHCl_3$/MeOH) to give 2.71 g (44.7% total yield) from **2** as a 1:1 mixture of diastereoisomers. R_f ($CHCl_3$/MeOH = 10:1) = 0.33. ^{31}P NMR (CD_3CN): δ_P −7.79. ^1H NMR ($CDCl_3$): δ 0.8−0.9 (m, 3H), 1.18−1.38 (m, 10H), 1.41 and 1.42 (2s, 45H), 1.6−1.64 (m, 2H), 2.53−2.68 (m, 1H), 2.72−2.82 (m, 6H), 2.82−2.93 (m, 1H), 3.02−3.13 (m, 1H), 3.27−3.56 (m, 10H), 4.0−4.11 (m, 2H), 4.14−4.33 (m, 4H). ^{13}C NMR ($CDCl_3$): δ 14.3, 19.7, 19.8, 22.8, 25.5, 28.27, 28.32, 29.27, 29.30, 30.4 (d, J = 7.1 Hz), 31.9, 52.5, 52.9, 53, 53.5, 53.6, 53.7, 53.9, 56, 56.3, 60.5 (2d ≈ t_{app}, J = 8.6 Hz), 61.9, 68.2 (2d ≈ t_{app}, J = 7.6 Hz), 68.5 (2d ≈ t_{app}, J = 4 Hz), 80.40, 80.44, 80.5, 80.6, 116.2, 116.3, 169.8, 170.10, 170.14, 170.34, 170.4. Anal. ($C_{46}H_{85}N_4O_{14}P$) C, H, N, P.

Cyanoethyl-(2-(1-naphthyl)ethyl)-(*R*)-phosphonooxymethyl-penta-*tert*-butyl-DTPA (3c). Compound **3c** was prepared from 2-(1-naphthyl)ethanol (1.6 g, 9.5 mmol), crude phosphoramidite intermediate **2** (3.5 g, 3.8 mmol), and tetrazole (0.53 g, 7.6 mmol) in CH_3CN (40 mL) as described above for compound **3a**. After the mixture was oxidized with *tert*-butylhydroperoxide (1.2 mL) for 1 h, the solution was concentrated and partitioned between EtOAc (30 mL) and water (10 mL). The organic layer was extracted with brine, dried ($MgSO_4$), and evaporated to give 4.7 g of crude product **3b** as

a 1:1 mixture of diastereoisomers which was immediately converted to compound **4c**. A portion was purified by silica gel chromatography (CH_2Cl_2/MeOH) for characterization purposes. R_f ($CHCl_3$/MeOH = 10:1) = 0.22. ^{31}P NMR (CD_3CN): δ_P −4.12. ^{1}H NMR ($CDCl_3$): δ 1.41, 1.416, and 1.418 (3s, 45H), 2.52−2.65 (m, 3H), 2.76 (br s, 4H), 2.82−2.93 (m, 1H), 2.82−2.93 (m, 1H), 3.02−3.12 (m, 1H), 3.27−3.54 (m, 12H), 4.0−4.24 (m, 4H), 4.36−4.47 (m, 2H), 7.41 (d, J = 5.3 Hz, 2H), 7.46−7.58 (m, 2H), 7.72−7.79 (m, 1H), 7.83−7.88 (m, 1H). ^{13}C NMR ($CDCl_3$): δ 19.1, 19.2, 27.9, 28, 33.4, 33.5, 52.2, 52.57, 52.60, 53.2, 53.3, 53.5, 55.7, 56, 60.1, and 60.2 (2d ≈ t_{app}, J_1 = 7.6 Hz, J_2 = 7.1 Hz), 61.5 and 61.6 (2d ≈ t_{app}, J = 5 Hz), 67.5 and 67.6 (2d ≈ t_{app}, J = 5.5 Hz), 67.9 and 68 (2d ≈ t_{app}, J = 7.1 Hz), 80.47, 80.51, 80.53, 80.6, 116.4, 116.5, 123.2, 125.3, 126.1, 127.2, 127.4, 128.7, 131.7, 132.66, 132.69, 170.4, 170.67, 170.71, 170.89, 170.94. Anal. ($C_{50}H_{79}N_4O_{14}P\cdot H_2O$) C, H, N, P. FAB-MS exact mass calcd for $C_{50}H_{79}N_4O_{14}P^+$: 991.5409. Found: 991.5402.

4,4-Diphenylcyclohexyl-phosphonooxymethyl-DTPA (4a). The solution of **3a** in 2 M NH_3-MeOH (1000 mL) was stirred at RT overnight. The solvent was evaporated. The residue was treated with Et_3N (21.3 mL, 153 mmol) and purified with silica gel column chromatography (divided into two portions of ca. 80 g each) (diameter 5.5 cm × height 40 cm, from CH_2Cl_2 only to 95:5 CH_2Cl_2/Et_3N, silica gel was treated with 5% Et_3N in CH_2Cl_2 prior to loading) to afford purified intermediate (138.8 g, 89%, 3 steps total from **5**). R_f ($CHCl_3$/MeOH = 10:1) = 0.12. ^{31}P NMR ($CDCl_3$): δ_p −7.37. Exact mass calcd for $C_{53}H_{85}N_3O_{14}P$: 1018.576919. Found: 1018.577300. A portion of the *tert*-butyl ester intermediate (135.2 g, 132 mmol) was dissolved in a mixture of concentrated HCl (Fisher Scientific trace metal grade, 150 mL) and ether (300 mL) and stirred at RT overnight. Ether was evaporated off, and the aqueous solution was treated with 8 M NaOH solution to adjust the pH to ∼1.5. The resulting white precipitate was filtered and washed with H_2O (5 times, 200 mL each) and ether (2 times, 150 mL each). The white solid product was dried under a vacuum desiccator (P_2O_5) at RT for 24 h to give the purified product **4a** (80.49 g, 77−82%). ^{31}P NMR (D_2O + NaOD, pD 13.5): δ_p −0.3. ^{1}H NMR (D_2O): δ 1.49 (m, 2H), 1.73 (m, 2H), 1.98−2.28 (m, 4H), 2.42−2.57 (m, 6H), 2.69 (d, 1H, J = 16.4 Hz), 2.78 (m, 1H), 2.92−3.07 (m, 8H), 3.12 (d, J = 16.4 Hz, 1H), 3.68 (m, 2H), 4.08 (m, 1H), 6.97−7.35 (m, 10H). ^{13}C NMR (MeOD): δ 31, 34.1 (br s), 46.6, 50.5, 53.2, 54, 55.2, 55.5, 55.8, 58.8 (br s), 63.8 (br s), 75 (br s), 126.6, 127.9, 128.3, 129.2, 129.4, 147.9, 149.4, 169.3, 174.4, 175.5. Anal. ($C_{33}H_{44}N_3O_{14}P\cdot H_2O$) C, H, N, P.

***n*-Octyl-phosphonooxymethyl-DTPA (4b).** A solution of *n*-octyloxy phosphate cyanoethyl ester **3b** (2.17 g, 2.3 mmol) in 2 M NH_3-MeOH (25 mL) was stirred at RT for 18 h. The solvent was evaporated. The residue was purified by flash chromatography on silica gel (from a 99:1:1 to a 96:4:1 CH_2Cl_2/MeOH/Et_3N ratio) to afford the penta-*tert*-butyl ester-protected *n*-octylphosphodiester product as a triethylammonium salt (1.95 g, 2.0 mmol, 87%). The foaming solid (1.27 g, 1.3 mmol) was then dissolved in dioxane (5 mL), and 10 mL of 12 N HCl was added. The solution was stirred at RT for 24 h. The dioxane was evaporated, and the pH was adjusted to ≈1.6 with 8 N NaOH. The solid was filtered and washed with a HCl solution (pH ≈ 1.6) and then with ether. The white solid was desalted on a C_{18} reverse phase silica gel column (Sep-Pak pre-packed cartridge, Waters) (from H_2O to 1:4 CH_3CN/H_2O) to give the pure product **4b** (0.44 g, 56.7%). ^{31}P NMR (D_2O): δ_P 2.3. MS: *m/e* 614.5 [(M − H)$^-$]. ^{1}H NMR (D_2O): δ 0.8−0.9 (m, 3H), 1.0−1.22 (m, 10H), 1.36−1.56 (m, 2H), 3.06−3.18 (m, 2H), 3.18−3.48 (m, 4H), 3.48−3.96 (m, 15H). ^{13}C NMR ($CDCl_3$): δ 23.7, 26.9, 30.4, 31.8 (d, J = 7.6 Hz), 33, 50.6, 53.2, 54, 55.2, 55.5, 55.7, 58.9 (d, J = 7.1 Hz), 64 (d, J = 5.1 Hz), 66.9, 67.2 (d, J = 6 Hz), 169.3, 174.3, 175.5. Anal. ($C_{23}H_{42}N_3O_{14}P\cdot 0.55NaCl$) C, H, N, P. FAB-MS exact mass calcd for $C_{23}H_{43}N_3O_{14}P^+$: 616.2483. Found: 616.2484.

2-(1-Naphthyl)ethyl-phosphonooxymethyl-DTPA (4c). A solution of 2-(1-naphthyl)ethanol phosphate cyanoethyl ester **3c** (3.21 g, 3.2 mmol) was deprotected following the same procedure described for **4b** in 2 M NH_3-MeOH (35 mL) at RT for 18 h. The phosphodiester intermediate was purified by flash chromatography on silica gel as the triethylammonium salt (1.82 g, 1.75 mmol, 54.7%). The *tert*-butyl esters were deprotected (1.71 g, 1.64 mmol) in 12 N HCl (7 mL) and dioxane (15 mL) for 22 h at RT to give the product **4c** (0.71 g, 1.1 mmol, 65.5%) after desalting on a C_{18} reverse phase silica gel column (from H_2O to 1:4 CH_3CN/H_2O). ^{31}P NMR (MeOD): δ 2.3. MS: *m/e* 656.5 [(M − H)$^-$]. ^{1}H NMR (D_2O): δ 2.86−3.56 (m, 19H), 3.95 (AB system, J = 6.7 Hz, 1H), 4.0 (AB system, J = 6.7 Hz, 1H), 4.36 (s, 2H), 7.14−7.35 (m, 4H), 7.48−7.55 (m, 1H), 7.6−7.66 (m, 1H), 7.92 (d, J = 8.2 Hz). ^{13}C NMR (MeOD): δ 35.0 (d, J = 7.1 Hz), 50.5, 53.1, 53.8, 55.1, 55.5, 55.7, 58.6 (d, J = 7.1 Hz), 63.6 (d, J = 3.8 Hz), 67.1 (d, J = 5 Hz), 124.9, 126.69, 126.74, 127.3, 128.3, 128.6, 129.8, 133.4, 135.3, 135.7, 169.3, 174.3, 175.4. Anal. ($C_{27}H_{36}N_3O_{14}P\cdot 1.1NaCl$) C, H, N, P. FAB-MS exact mass calcd for $C_{27}H_{37}N_3O_{14}P^+$: 658.2013. Found: 658.2015.

Trisodium-{(2-(*R*)-[(4,4-diphenylcyclohexyl)phosphonooxymethyl]diethylenetriaminepentaacetato)(aquo)gadolinium(III)} (5a). A test tube was charged with gadolinium oxide (Gd_2O_3) (0.95 g, 2.6 mmol), compound **4a** (purity: 98 wt %, 3.76 g, 5 mmol), and sodium hydroxide (10 mL of a 1.0 N solution, 10 mmol). The mixture was stirred at 95 °C for 6 h. The reaction mixture was cooled to room temperature, and the pH was adjusted to ∼7.5 by adding 4.5 mL of 1.0 N (4.5 mmol) NaOH solution in small portions. Half of the solvent was removed by evaporation at 95 °C. The remaining mixture was cooled to room temperature and loaded on a 10 g Sep-Pak column. Elution with water followed by freeze drying yielded 4.70 g (4.8 mmol, 96%) as a white powder. Anal. ($C_{33}H_{40}GdN_3Na_3O_{15}P$) C, Gd, H, N. FAB-MS, 981 [(M − H_2O + Na)$^+$], 959 [(M − H_2O + H)$^+$], 937 [(M − H_2O − Na + 2H)$^+$], 915 [(M − H_2O − 2Na + 3H)$^+$]. *N*-Methyl-D-glucamine (NMG) used as base instead of sodium hydroxide produces the *N*-methyl-D-glucammonium (NMG) salt, **5a-NMG**. Anal. ($C_{54}H_{95}N_6O_{30}PGd$) C, H, N, Gd. +FAB-MS: 1478 [(M − H_2O + H)$^+$], 1283 [(M − H_2O − NMG + 2H)$^+$], 1088 [(M − H_2O − 2NMG + 3H)$^+$].

Tris-[*N*-methyl-D-glucamine]-{(2-(*R*)-[octylphosphonooxymethyl]diethylenetriaminepentaacetato)(aquo)gadolinium(III)} (5b). A test tube was charged with gadolinium oxide (0.18 g, 0.5 mmol), compound **4b** (0.70 g, 1.05 mmol), *N*-methylglucamine (NMG) (0.38 g, 2 mmol), and deionized water (3.5 mL). The mixture was stirred at 95 °C for 6 h; the solution was cooled to room temperature, and the pH was adjusted to 7. The total volume was adjusted to 5.0 mL with deionized water, and the solution was heated again to 95 °C for 10 min. The cooled solution was filtered through a 0.2 μm syringe filter to give an aqueous solution of the titled compound (5.6 μg/mL Gd). MS: 767.2 [(M − H_2O + H)$^-$]. FAB-MS exact mass calcd for $C_{23}H_{40}GdN_3O_{14}P^+$: 771.1489. Found: 771.1514. HPLC purity: 94% (YMC-Pack TMS).

Tris-[*N*-methyl-D-glucamine]-{(2-(*R*)-[(2-(1-naphthyl)-ethyl) phosphonooxymethyl]diethylenetriaminepentaacetato)(aquo)gadolinium(III)} (5c). Gadolinium oxide (0.15 g, 0.4 mmol), compound **4c** (0.73 g, 0.84 mmol), and *N*-methylglucamine (0.31 g, 1.6 mmol) were reacted in deionized water (3 mL) and isolated as described above for compound **5b** to give an aqueous solution of **5c** (5.6 μg/mL Gd). MS: 809.2 [(M − H_2O + H)$^-$]. FAB-MS exact mass calcd for $C_{27}H_{34}GdN_3O_{14}P^+$: 813.1020. Found: 813.1027. HPLC purity: 99% (Thermohypersil C4).

2-Aminodecanoic Acid Ethylenediamine Amide (7a). Racemic methyl 2-aminodecanoate hydrochloride **6a** (6.94 g, 29.2 mmol) was prepared by the method of O'Donnell.[18] **6a**. ^{1}H NMR ($CDCl_3$): δ 0.8−0.9 (m, 3H), 1.2−1.4 (m, 12H), 1.42−1.6 (m, 3H), 1.62−1.76 (m, 1H), 3.42 (dd, J_1 = 5.5 Hz, J_2 = 7.4 Hz, 1H), 3.7 (s, 3H). ^{13}C NMR ($CDCl_3$): δ 13.8, 22.4, 25.3, 28.9, 29.11, 19.13, 31.6, 34.7, 51.5, 54.1, 176.4. Anal. ($C_{11}H_{23}NO_2$) C, H, N, P. Compound **6a** was converted to the free base by treatment with triethylamine (6.1 mL, 43.8 mmol) in methanol (10 mL). Diethyl ether was added to precipitate triethylammonium chloride, which was then removed by filtration. The

filtrate was concentrated. Ethylenediamine (75 mL) was added, and the mixture was stirred overnight at RT under nitrogen. Ethylenediamine was removed by evaporation at reduced pressure, and the resulting solid was dried in vacuo to give 5.94 g (25.9 mmol, 89% yield). ^{1}H NMR ($CDCl_3$): δ 0.82 (t, J = 6.6 Hz, 3H), 1.21 (br s, 12H), 1.36−1.53 (m, 1H), 1.71−1.85 (m, 1H), 2.20 (br s, 4H), 2.82 (t, J = 5.8 Hz, 2H), 3.25−3.38 (m, 3H), 7.64 (t, J = 5.4 Hz, 1H). ^{13}C NMR ($CDCl_3$): δ 13.7, 22.2, 25.5, 28.9, 29.1, 31.4, 34.9, 40.80, 40.83, 55, 175.5. FAB-MS exact mass calcd for $C_{12}H_{28}N_3O^+$: 230.2232. Found: 230.2230.

Naphthylalanine Ethylenediamine Amide (7b). Racemic 2-naphthylalanine methyl ester hydrochloride **6b** (3.53 g, 13.4 mmol) was prepared by the method of O'Donnell.[18] **6b.** ^{1}H NMR ($CDCl_3$): δ 3.0 (dd, J_1 = 7.9 Hz, J_2 = 13.4 Hz, 1H), 3.25 (dd, J_1 = 5.0 Hz, J_2 = 13.4 Hz, 1H), 3.7 (s, 3H), 3.82 (dd, J_1 = 5.3 Hz, J_2 = 7.9 Hz, 1H), 7.28−7.32 (m, 1H), 7.38−7.48 (m, 2H), 7.63 (br s, 1H), 7.72−7.83 (m, 3H). ^{13}C NMR ($CDCl_3$): δ 41.1, 51.8, 55.6, 125.4, 125.9, 127.1, 127.35, 127.43, 127.8, 128.0, 132.2, 133.2, 134.6, 175.2. Anal. ($C_{14}H_{15}NO_2$) C, H, N, P. Compound **6b** was converted to compound **7b** by the same procedure described for **7a**. The product was isolated as an off-white solid (3.39 g, 13.2 mmol, 98%). ^{1}H NMR ($CDCl_3$): δ 1.05−1.35 (br m, 4H), 2.77 (t, J = 6.1 Hz, 2H), 2.90 (dd, J_1 = 13.7 Hz, J_2 = 8.9 Hz, 1H), 3.29 (m, 2H), 3.39 (dd, J_1 = 13.7 Hz, J_2 = 4.2 Hz, 1H), 3.69 (dd, J_1 = 8.9 Hz, J_2 = 4.2 Hz, 1H), 7.36 (dd, J_1 = 8.4 Hz, J_2 = 1.8 Hz, 1H), 7.4−7.54 (m, 3H), 7.64 (br s, 1H), 7.74−7.84 (m, 3H). ^{13}C NMR ($CDCl_3$): δ 41.1, 41.3, 41.7, 56.2, 125.5, 126.0, 127.1, 127.2, 127.5, 127.8, 128.2, 132.1, 133.2, 135.2, 174.4. Anal. ($C_{15}H_{19}N_3O\cdot0.3H_2O$) C, H, N. FAB-MS exact mass calcd for $C_{15}H_{20}N_3O^+$: 258.1606. Found: 258.1602.

2-Octyldiethylenetriamine Trihydrochloride (8a). 2-Aminodecanoic acid ethylenediamine amide **7a** (5.94 g, 25.9 mmol) was suspended in 50 mL of THF. Borane·THF (130 mL, 1.0 M) was added slowly, and the mixture was then refluxed under argon for 16 h. The excess borane was quenched by careful addition of methanol (100 mL: *Caution, Exothermic!*). The reaction mixture was concentrated under reduced pressure. Dry ethanol (150 mL) was added, and the solution was saturated with HCl gas at 0 °C. The mixture was brought to reflux for 24 h, after which the solution was cooled in an ice bath. The resulting solid was filtered, washed with diethyl ether, and vacuum-dried to give 6.08 g (18.7 mmol, 72%) of a white powder. ^{1}H NMR (MeOD): δ 0.74−0.8 (m, 3H), 1.1−1.42 (m, 12H), 1.52−1.80 (m, 2H), 3.22−3.44 (m, 6H), 3.46−3.63 (m, 1H).^{13}C NMR (MeOD): δ 14.4, 23.7, 26, 30.29, 30.35, 32.1, 32.9, 37, 46.5, 50.4, 50.6. Anal. ($C_{12}H_{32}Cl_3N_3$) C, H, N. FAB-MS exact mass calcd for $C_{12}H_{30}N_3^+$: 216.2440. Found: 216.2444.

2-((2-Naphthyl)methyl)diethylenetriamine Trihydrochloride (8b). Compound **8b** was prepared from racemic naphthylalanine ethylenediamine amide **7b** (3.39 g, 13.2 mmol) by the same procedure described for **7a**. Product **8b** was obtained as an off-white solid (4.22 g, 12 mmol, 91%). ^{1}H NMR (D_2O): δ 3.02 (dd, J_1 = 8.4 Hz, J_2 = 14.2 Hz, 1H), 3.12−3.5 (m, 5H), 3.84−3.97 (m, 1H), 7.29 (d, J = 8.7 Hz, 1H), 7.36−7.46 (m, 2H), 7.68 (br s, 1H), 7.72−7.84 (m, 3H). ^{13}C NMR (MeOD): δ 37, 38, 46.5, 50.4, 51.5, 127.4, 127.6, 128, 128.7, 129.7, 130.2, 132.9, 134.2, 135. Anal. ($C_{15}H_{24}Cl_3N_3$) C, H, N. FAB-MS exact mass calcd for $C_{15}H_{22}N_3^+$: 244.1814. Found: 244.1814.

2-Octyldiethylenetriamine Penta-*tert*-butyl Acetate (9a). 2-Octyldiethylenetriamine trihydrochloride **8a** (6.08 g, 18.7 mmol) was suspended in dry DMF (100 mL). Diisopropylethylamine (47 mL, 280 mmol) was added followed by *tert*-butylbromoacetate (24.2 mL, 0.15 mmol). The mixture was stirred at room temperature for 16 h under argon. The DMF and excess reagents were removed by evaporation under reduced pressure. The residue was partitioned between concentrated aqueous $NaHCO_3$ and dichloromethane. The organic solution was washed one time each with water and saturated aqueous NaCl, and then dried over Na_2SO_4, filtered, and concentrated under reduced pressure. The residue was purified by flash chromatography (20% EtOAc/hexanes) to give **9a** as a light orange oil (0.54 g, 13.4 mmol, 72% yield). ^{1}H NMR ($CDCl_3$): δ 0.82 (m, 3H), 1.22 (m, 14H), 1.44 (s, 45H), 2.24 (m, 1H), 2.7 (m, 6H), 3.3−3.55 (m, 10H). ^{13}C NMR ($CDCl_3$): δ 14, 22.6, 26.8, 28, 28.05, 28.09, 29.3, 29.5, 29.9, 31.1, 31.8, 52.4, 53, 53.1, 56, 56.5, 60.7, 80.2, 80.4, 80.6, 170.6, 171.1, 171.5. Anal. ($C_{42}H_{79}N_3O_{10}\cdot0.12CH_2Cl_2$) C, H, N. FAB-MS exact mass calcd for $C_{42}H_{80}N_3O_{10}^+$: 786.5844. Found: 786.5823.

2-((2-Naphthyl)methyl)diethylenetriamine Penta-*tert*-butyl Acetate (9b). Compound **9b** was prepared from 2-((2-naphthyl)methyl)diethylenetriamine trihydrochloride **8b** (4.22 g, 12 mmol) by the same procedure described for **9a**. Product **9b** was purified by flash chromatography (25% EtOAc/hexanes) to give an orange oil (8.38 g, 10.3 mmol, 86%). ^{1}H NMR ($CDCl_3$): δ 1.32 (s, 9H), 1.40 (s, 36H), 2.53 (dd, J_1 = 6.5 Hz, J_2 = 13.2 Hz, 1H), 2.77−2.93 (m, 2H), 2.99 (dd, J_1 = 6 Hz, J_2 = 13.7 Hz, 1H), 3.14−3.26 (m, 1H), 3.34 (s, 2H), 3.36 (s, 4H), 3.47 (s, 4H), 7.32−7.43 (m, 3H), 7.63 (br s, 1H), 7.66−7.78 (m, 3H). ^{13}C NMR ($CDCl_3$): δ 27.8, 27.9, 37.2, 52.1, 52.6, 53.3, 55.6, 55.7, 55.9, 62.7, 80.2, 80.4, 124.8, 125.4, 127.2, 127.4, 127.7, 131.8, 133.3, 138.0, 170.4, 170.9, 171.1. Anal. ($C_{45}H_{71}N_3O_{10}$) C, H, N.

2-Octyldiethylenetriamine Pentaacetic Acid (10a). The 2-octyldiethylenetriamine penta-*tert*-butyl acetate **9a** (10.54 g, 13.4 mmol) was dissolved in dioxane and concentrated HCl (1:1 ratio, trace metal grade, 150 mL) and was stirred at room temperature for 16 h. The reaction mixture was concentrated under reduced pressure to provide 8.07 g of a tan foam. A portion of this material (4.57 g) was chromatographed on a C_{18} reversed phase silica gel column (from H_2O to 20−60% CH_3CN/H_2O) applying 1.5 g per run. Acetonitrile was evaporated under a stream of argon, and the resulting aqueous solution was lyophilized, producing a flocculent white solid (2.97 g, 5.9 mmol). ^{1}H NMR (D_2O, CD_3CN ref, pH <1): δ 0.74 (t, 3H), 1.10−1.30 (m, 12H), 1.4 (m, 1H), 1.70 (m, 1H), 2.86 (dd, 1H), 3.20−3.04 (m, 3H), 3.70−3.40 (m, 5H), 4.45 (m, 4H), 4.26 (m, 4H). ^{13}C NMR (MeOD): δ 14.5, 23.7, 27.7, 28.4, 30.4, 30.5, 30.8, 33, 52, 52.3, 53.7, 55.2, 56.5, 57.1, 60.9, 171.5, 172.9, 174. Anal. ($C_{22}H_{39}N_3O_{10}$) C, H, N. MS: *m/e* 504.5 [(M − H)$^-$].

2-((2-Naphthyl)methyl)diethylenetriamine Pentaacetic Acid (10b). Compound **10b** was prepared from 2-((2-naphthyl)methyl)diethylenetriamine penta-*tert*-butyl acetate **9b** (4.41 g, 5.42 mmol) by the same procedure described for **10a**. Product **10b** was purified by chromatography on a C_{18} reversed phase silica gel column (from H_2O to 20% CH_3CN/H_2O) and isolated as a fluffy white powder (2.3 g, 4.31 mmol, 80%). ^{1}H NMR (D_2O): δ 2.83 (dd, 1H), 3.0−3.19 (m, 3H), 3.28−3.4 (m, 2H), 3.91−3.49 (m, 9H), 4.19 (s, 4H), 7.34−7.49 (m, 3H), 7.71 (s, 1H), 7.75−7.85 (m, 3H). ^{13}C NMR (MeOD): δ 35.2, 51, 52.8, 53.7, 55.9, 56.7, 61.7, 126.9, 127.4, 128.3, 128.6, 128.7, 129, 129.7, 133.8, 135, 136.3, 170.7, 173.8, 175.1. Anal. ($C_{25}H_{31}N_3O_{10}$) C, H, N.

Bis-[*N*-methyl-D-glucamine]-{(2-(octyldiethylenetriamine)diethylenetriaminepentaacetato)(aquo)gadolinium(III)} (11a). A test tube was charged with gadolinium oxide (0.145 g, 0.4 mmol), compound **10a** (0.457 g, 0.84 mmol), *N*-methylglucamine (0.31 g, 1.6 mmol), and deionized water (3.0 mL). The mixture was stirred at 95 °C for 6 h; the solution was cooled to room temperature, and the pH was adjusted to 7.0 with 0.07 g of NMG. The volume was adjusted to 4.0 mL, and the solution of the product was filtered through a 0.2 μm filter. Gadolinium content, calcd: 200 mM. Found (ICP): 196 mM. A portion of this material was isolated by reversed phase chromatography on a C_{18} silica gel column (from H_2O to 20−50% CH_3CN) and lyophilized to give a white solid. FAB-MS exact mass calcd for $C_{22}H_{34}GdN_3Na_3O_{10}^+$: 727.1178. Found: 727.1189. Anal. ($C_{36}H_{70}GdN_5O_{21}\cdot3H_2O$) C, Gd, H, N.

Bis-[*N*-methyl-D-glucamine]-{(2-((2-naphthyl)methyl)-diethylenetriaminepentaacetato)(aquo)gadolinium-(III)} (11b). Compound **11b** was prepared from compound **10b** (0.590 g, 1.05 mmol) and gadolinium oxide (0.181 g, 0.5 mmol) by the same procedure described for **11a**. A clear solution of the gadolinium complex was obtained. MS: *m/e* 685.9 [(M − H_2O + H)$^-$]. FAB-MS exact mass calcd for $C_{25}H_{27}GdN_3$-

$Na_2O_{10}^+$: 733.0733. Found: 733.0749. HPLC purity: 99% (YMC-Pack TMS).

Radiolabeling Procedure. Radiochemical trace labeling was accomplished using ^{153}Gd chloride as described in the literature.[19] Samples of ^{153}Gd chloride of 99% radionucleotide purity and known specific activity (10−12 mCi/mg) were obtained from Dupont New England Nuclear (North Billerica, MA). Solutions of gadolinium chelates (0.1−0.25 mM) were reacted with ^{153}Gd chloride at pH 4−5 at 80 °C for 1−3 h. Specific activity of the labeled compounds was typically 200−300 μCi/mmol, with values for free gadolinium generally less than 0.2% as determined by TLC.[19] The pH of the reaction mixture was adjusted between pH 7 and 8 with *N*-methyl-D-glucamine or sodium hydroxide. HPLC experiments confirmed that this procedure resulted in no degradation of the chelate, as evidenced by a single ^{153}Gd chelate peak at a retention time consistent with that of the unlabeled material. Gamma counting was performed using a Cobra II gamma counter (Packard, Downers Grove, IL) operating with a counting window of 15−140 keV. Samples were counted for 30 min or until deviations of >1% sigma were achieved.

Butanol Buffer Partition Coefficients. Because of the overall hydrophilic nature of the gadolinium chelates, partition coefficients were measured in butanol/phosphate-buffered saline (PBS) instead of octanol/PBS. ^{153}Gd-labeled chelate ($[Gd]_T$ = 0.1 mM in 0.5 mL of PBS) was equilibrated at RT for 1−2 h with PBS-saturated butanol (0.5 mL). The vials were centrifuged at 2000*g* for 5 min to ensure that the layers were separated. Aliquots (100 μL) from each phase were removed and counted in a Packard Cobra II gamma counter. The partition coefficient (*P*) was calculated as follows (CPM = counts per minute):

$$P = \frac{\text{average CPM}/\mu\text{L of butanol}}{\text{average CPM}/\mu\text{L of PBS}}$$

Protein Binding. The protein binding studies were performed by ultrafiltration using ^{153}Gd-radiolabeled chelates **5b**, **5c**, **11a**, **11b**, and Gd-DTPA. A detailed analysis of the binding properties of **5a** is presented elsewhere.[22]

Protein binding studies were initiated in duplicate by adding 10 mM ^{153}Gd-labeled chelate solution to a vial containing 500 μL of 4.5% HSA to give a total gadolinium concentration of 0.1 mM. The contents of the vials were mixed by vortex, and a 25−50 μL aliquot from each sample was pipetted to a small vial and counted in the gamma counter. A portion of each sample (400 μL) was pipetted to an ultrafiltration unit (Ultrafree-MC 5000 Nominal Molecular Weight Limit filter unit, Millipore, Bedford, MA) and centrifuged at room temperature (22 ± 1 °C) or in an incubator after a 15 min equilibration at 37 ± 1 °C (**5a**). Filtrate (25 μL) was pipetted to a small vial and counted in the gamma counter. The percent of the agent unbound was calculated by comparing the counts per microliter in the filtrate to that in the original solution. The error in the binding data is ±10%.

Rat Pharmacology Studies. All studies involving laboratory animals were approved by the Institutional Animal Care and Use Committee (IACUC) at EPIX Medical, Inc. and were in accordance with the guidelines in the "Guide to the Care and Use of Laboratory Animals" published by the National Research Council and the Institute of Laboratory Animal Resources.

Unanesthetized male Sprague-Dawley rats (150−250 g, Harlan Sprague Dawley, Indianapolis, IN; n = 2−5) were injected via tail vein catheter with ^{153}Gd-labeled chelate at a dose of 0.1 mmol/kg. Plasma samples were obtained by centrifugation of freshly heparinized blood at 2000*g* for up to 20 min. The plasma supernatant was pipetted away from the pellet fraction and either frozen or analyzed within 24 h of collection. Concentrations were determined by inductively coupled plasma (ICP) analysis (Galbraith Laboratories), and plasma water T_1 relaxation times were obtained as described below.

Plasma concentration data were analyzed with the JMP statistical package (SAS Institute Inc., Cary, NC) using the biexponential equation

$$C = A\exp(-\alpha t) + B\exp(-\beta t)$$

where C is the plasma concentration, A is the distribution coefficient, α is the distribution rate constant, B is the elimination coefficient, β is the elimination rate constant, and t is the time. The elimination half-life ($T_{e1/2}$), distribution half-life ($T_{d1/2}$), and apparent volume of distribution (V_d) were calculated by means of the following formulas:

$$T_{e1/2} = 0.693/\beta$$

$$T_{d1/2} = 0.693/\alpha$$

$$V_d = \text{dose}/B$$

Area under the curve (AUC) was calculated for each individual animal by JMP using the trapezoidal rule

$$\text{AUC}^{0\rightarrow t_x} = \sum_{i=1}^{n}\left[\left(\frac{C_i + C_{i+1}}{2}\right)(t_{i+1} - t_i)\right]$$

where $\text{AUC}^{0\rightarrow t_x}$ is the time period desired beginning with 0, n is the number of time points, and C_i is the plasma concentration at time t_i. For excretion studies, rats were housed in metabolic cages for the collection of urine and feces and were sacrificed at 24 h. Tissue samples, urine, and feces were analyzed for ^{153}Gd content by gamma counting. The percent injected dose was determined as follows, where % ID is percent of injected dose and CPM is counts per minute.

$$\%\ \text{ID} = \frac{\text{total CPM sample}}{\text{injected dose (in CPM)}} \times 100$$

In Vitro Relaxivity. Proton T_1 relaxation times were determined at 20 MHz using a Minispec PC 120/125/VTs (Bruker Canada, Milton, ON) nuclear magnetic resonance (NMR) process analyzer. The temperature was regulated at 37 ± 0.2 °C with a circulating bath. The longitudinal (T_1) relaxation rate was determined by the use of an inversion recovery pulse sequence and the fitting of the intensity data from eight experimental time points to an exponential. Two scans were averaged for each data point with a recycle delay that was 5 times longer than the T_1 observed for the sample. The T_1 was determined in triplicate, and the average T_1 measurement was calculated.

Solutions of HSA (4.5 wt %, fraction V, 96−99% albumin, Sigma Chemical Co., St. Louis, MO) were prepared by adding HSA to phosphate-buffered saline (PBS, 10 mM sodium phosphate, 150 mM sodium chloride, pH 7.4). A relaxivity titration consisted of five sequential 1−10 μL syringe additions of a solution of gadolinium chelate (1−10 mM) to a 10 mm NMR tube containing 500−1000 μL of phosphate-buffered saline or 4.5% HSA solution. After the addition of each chelate, the sample was thoroughly mixed by manual agitation and returned to the Bruker Minispec for the T_1 determination. Concentrations of the 1−10 mM chelate solutions were determined by either UV absorbance at 220 nm or ICP analysis.

Acknowledgment. We thank Nathan Ekborg for assistance in obtaining the mass spectrometry data.

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JM0102351