CH_2CH_3). Anal. ($C_{18}H_{19}FN_2O_6$) C, H, N. Treatment of the earlier fractions (eluate from 160 mL to 185 mL) gave 0.86 g (23%) of 3′-*O*-ethyl compound **7b**.

5′-*O*- and 3′-*O*-benzyl compounds (**7c** and **7d**) of **6** were synthesized similarly.

Antitumor Test. Mice of the ICR strain (Japan Clea Inc., Tokyo, Japan) were used. Five-week-old male ICR mice were inoculated subcutaneously in the axillary region with 5×10^6 sarcoma 180 cells and given test compounds orally once a day for 7 consecutive days beginning 24 h after inoculation of the tumor cells. Groups of seven mice were used for each dose, and the test compounds were suspended in 0.5% (carboxymethyl)cellulose (CMC) solution containing 0.1% Tween 80. On day 10, the tumors were excised and weighed. The inhibitory effects of test compounds were calculated from the ratio of the tumor weight in the test group to that in the control group. The results are given in Table I.

Acknowledgment. We are grateful to Professor Tohru Ueda, Faculty of Pharmaceutical Science, Hokkaido University, for his helpful suggestions.

Registry No. **1**, 95969-47-4; **2b**, 95969-74-7; **2c**, 95969-75-8; **2e**, 95969-77-0; **2f**, 95969-78-1; **3a**, 116953-91-4; **3b**, 96141-36-5; **3c**, 96141-38-7; **3d**, 116953-92-5; **3e**, 95969-49-6; **3f**, 96141-37-6; **3g**, 95969-61-2; **3h**, 95969-62-3; **3i**, 95969-63-4; **3j**, 95969-64-5; **3k**, 95969-65-6; **3l**, 95969-66-7; **3m**, 95969-67-8; **3n**, 95969-68-9; **4**, 116953-93-6; **5**, 117069-24-6; **8a**, 95969-43-0; **8b**, 95969-42-9; **8c**, 95969-45-2; **8d**, 95969-44-1; F_3Thd, 70-00-8; FUdR, 50-91-9.

Development of Phosphonate Derivatives of Gadolinium Chelates for NMR Imaging of Calcified Soft Tissues

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We have synthesized several classes of gadolinium (Gd) complexes for use as NMR contrast agents in the detection of soft-tissue calcification. Class I was made up of strongly chelated GdDTPA complexes with one carboxylate arm coupled to a phosphonate-containing molecule through an amide link. Class II complexes were formed by Gd with several aminophosphonates and phosphono carboxylic acids. Class III were Gd complexes of weak chelates containing no phosphonate. The calcium-seeking ability of each complex was assessed by in vivo bone uptake. Tissue distribution in normal rats showed that only the complexes of GdDTPA modified with a diphosphonate group and GdEDTMP (EDTMP is ethylenediaminetetrakis(methylenephosponate)) showed adequate bone localization at the concentrations required for NMR contrast enhancement (~20% of a 100 μmol/kg dose).

Despite the high sensitivity of magnetic resonance (MR) parameters to changes in tissue water, they are not, by themselves, disease specific.[1,2] Different pathologic conditions may share identical relaxation times and spin densities. More diagnostic specificity is available from magnetic resonance imaging (MRI) with the use of paramagnetic contrast agents, but these have not been fully exploited to date. GdDTPA has found widespread application in neuroradiology, in studying abnormalities of the GI tract, liver, and kidney, and also in visualizing heart infarcts, tumors, and other edematous lesions.[3-9] The success of GdDTPA is due to its uptake into extracellular fluid spaces,[10-14] and it is not diagnostically useful for lesions that do not involve significant changes in fluid distribution.

A variety of diseases cause changes that result in abnormal calcium accumulations in soft tissue. These include cerebral infarcts, myocardial infarcts, and some tumors (mammary tumors, hepatocellular carcinoma, and bone metastases).[10-14] Most can be visualized by radioisotope scanning techniques when ^{99m}Tc-labeled phosphonate complexes are used for localization.[15-17] When large amounts of calcium are deposited in these lesions, they can also become opaque to X-rays, providing important diagnostic information. Unfortunately MRI, which relies on tissue water, provides no information on tissue calcium concentration.

Diseased cells, because they are unable to keep extracellular free calcium ions out of the phosphate-rich intracellular medium, precipitate calcium phosphates irreversibly.[18] These amorphous precipitates, like bone material, can be identified by calcium-seeking agents carrying paramagnetic probes. Modifications of Gd chelates that

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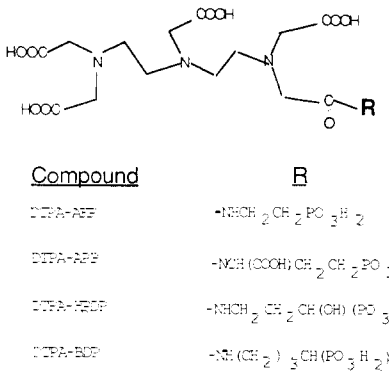

Figure 1. Phosphonate-altered DTPA ligands.

convert them to calcium seekers would result in their entrapment in calcified soft tissues, thus changing the local MRI signal. Our goal, therefore, is to synthesize a contrast agent that is taken up preferentially by a target tissue richer in calcium than normal tissues; eventually, this will prove useful in making clinical diagnoses by NMR imaging.

To enhance uptake into calcium-rich damaged tissue, we have synthesized several Gd complexes of phosphonate-altered DTPA. First we converted one of the carboxylate side chains into an amide; the starting amines were 2-aminoethylphosphonate, 2-amino-4-phosphonobutyric acid, 3-amino-1-hydroxypropane-1,1-diphosphonate, 4-aminobutanediphosphonate (AEP-DTPA, APB-DTPA, HPDP-DTPA, and BDP-DTPA, respectively). Figure 1 shows the structures of these ligands. We also synthesized Gd complexes of some bidentate and tridentate ligands containing the phosphonate group, i.e. 2-amino-4-phosphonobutyric acid (APB), and 2,3-dicarboxypropane-1,1-diphosphonic acid (DPD). We then evaluated the Gd complexes of these ligands in animals in comparison with the parent GdDTPA and other Gd complexes.

Since bone agents (radiolabeled phosphonate complexes) do localize in calcified soft tissues, bone uptake can serve as a good predictor of a contrast agent's ability to concentrate in damaged soft tissues. For this reason, we monitored normal bone uptake as a measure of an agent's suitability.

High doses of contrast agents are generally required to alter the tissue water proton relaxation parameters, T1 and T2. GdDTPA doses of approximately 0.05 mmol/kg of body weight are currently injected in animals to induce adequate changes in these parameters. It is expected that similarly high doses of the altered agents will also be required. Complexes that showed adequate bone localization at low doses were therefore tested in increasing amounts, up to these high dose levels.

Biodistribution Results

A. $GdCl_3$. The bulk of this injectate accumulated in the liver, with 15% of the no carrier added (NCA) dose going to bone via weak complexation by serum citrate or other weak systemic ligands.[20] As shown in Table IA, blood clearance was relatively slow at higher doses.

B. Gd Complexes of Weak Chelates. Ascorbate, citrate, and the N_2O_2 series depicted in Figure 2 (except I-71) all delivered approximately 50% of the Gd dose to bone at NCA concentrations, but were ineffective at higher levels of Gd. Only 28% of the injected NCA GdI-71 dose was

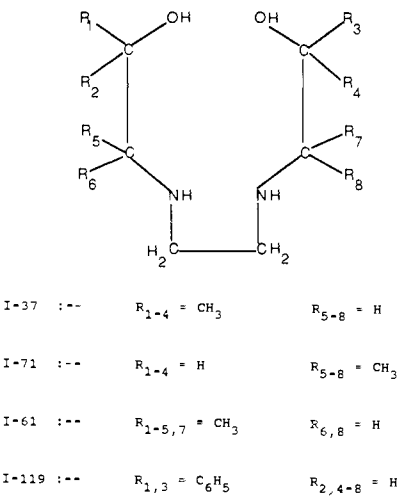

Figure 2. Structures of the N_2O_2 ligands for Gd chelation.

taken up by bone, with a significantly higher 41% accumulating in the liver, compared with 51% and 29% respectively for its structural variant (GdI-37). At the higher Gd concentrations, most of the injected dose cleared through the liver. Table IB–D summarizes these results.

C. GdAPB. The ligand 2-amino-4-phosphonobutyric acid (APB) was effective only at NCA Gd-153 concentrations, where ~53% of the injected dose was taken up by bone. Attempts to prepare carrier-added solutions resulted in precipitation of all radioactivity at pH <6. This precipitate persisted at higher pH and on heating.

D. GdDPD. The dicarboxypropanediphosphonate (DPD) was evaluated in the hope that it would mimic or even surpass the polycarboxylates in its ability to deliver Gd to bone. Solutions of NCA Gd in 0.01 M ligand and 0.05 M Gd in 0.15 M DPD were used at pH 7.

No bone uptake was evident; instead a high liver uptake (~70% ID/organ) was observed, symptomatic of lipophilic complexes and insoluble precipitates. A pharmacokinetic study at the higher Gd dose of 50 μmol/kg indicated almost 90% accumulation in liver and spleen within 6 h, with no gut uptake. This would rule our the production of fat-soluble complexes that clear into the lower gut.

Since high liver uptake was observed even at NCA Gd levels, it was suspected that the phosphono group may have been precipitating free blood calcium (~50 mg/L or 1.25 mM). Solutions were prepared of the ligand, its complex, and the other diphosphono-altered complexes at 0.01 M. $CaCl_2$ (1–5 mM) was added to these, with negative results. From this, we concluded that a clear solution of 0.05 M Gd in the ligand persists at pH 7 in vitro but precipitates ($Gd(OH)_3$) in vivo.

Clearly, the less flexible ligand did not form a stable calcium-seeking complex with Gd^{3+}, even at NCA levels. Gadolinium citrate had a bone uptake of ~60% of dose under identical conditions. All data are summarized in Table II.

E. GdDTPA-AEP. There was virtually no bone uptake 2 h after administration of contrast agent (see Table III). Tissue uptake was minimal overall.

F. GdDTPA-APB. Injection into normal animals did not lead to bone uptake after 2 h.

G. GdHPDP-DTPA. High bone uptake was realized for this agent (60% ID at NCA Gd, 15% at 0.1 mmol/kg) in 2 h (see Table III). The results of a comparative pharmacokinetic study are summarized in Table IV.

H. GdBDP-DTPA. This agent also showed a high bone uptake. Unlike the others, however, it was also taken up by the normal muscle tissues, a consequence of the long

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Table I. Biodistribution of $GdCl_3$ and the Gd Complexes of Selected Weak Chelates in Normal Rats (% ID/Organ (1 h))

A. $GdCl_3$

	NCA	0.001 M	0.01 M
blood	16.2	18.4	51.0
liver	45.4	47.5	32.6
heart	0.21	0.18	0.37
spleen	0.28	0.4	0.73
kidney	2.41	0.73	1.78
bone	14.7	4.31	7.73
muscle	4.78	1.91	4.43

B. Gd(ascorbate)

	NCA	0.01 M Gd/0.02 M asc
blood	2.81 ± 0.05	68.8
liver	18.7 ± 0.1	33.1
heart	0.325 ± 0.025	0.44
spleen	0.245 ± 0.025	
kidney	3.96 ± 0.44	1.94
bone	45.2 ± 0.8	3.59
muscle	7.18 ± 0.77	3.55

C. Weak Chelates N_2O_2 Donors

	GdI-37	GdI-61	GdI-71	GdI-119
		NCAGd/0.01 M lig		
blood	3.12 ± 0.53	2.31 ± 0.06	7.25 ± 0.7	2.35 ± 0.01
liver	28.5 ± 7.2	21.5 ± 2.1	41.0 ± 2.8	20.9 ± 3.8
heart	0.295 ± 0.035	0.315 ± 0.035	0.28 ± 0	0.34 ± 0.05
spleen	0.44 ± 0.3	0.13 ± 0	0.19 ± 0.05	0.135 ± 0.005
kidney	2.87 ± 0.17	2.75 ± 0.50	2.85 ± 0.18	3.57 ± 0.75
bone	51.4 ± 1.2	59.6 ± 0.7	27.7 ± 0.4	56.8 ± 2.3
muscle	5.85 ± 0.05	6.25 ± 0.07	4.09 ± 0.01	6.09 ± 0.39
		0.01 M Gd/0.011 M lig		
blood	9.83		19.0	
liver	74.1		64.0	
heart	0.08		0.15	
spleen	4.95		4.86	
kidney	0.43		0.72	
bone	2.0		2.56	
muscle	1.09		1.74	

D. Gd(citrate)

	NCA Gd/0.02 M citr	0.001 M Gd/0.01 M citr	0.01 M Gd/0.01 M citr
blood	2.78 ± 0.13	58.2 ± 3.6	55.2 ± 2.1
liver	19.7 ± 1.4	20.3 ± 2.7	28.3 ± 6.2
lung	0.545 ± 0.104	1.09 ± 0.18	1.01 ± 0
heart	0.278 ± 0.023	0.455 ± 0.015	0.345 ± 0.055
spleen	0.228 ± 0.095	0.27 ± 0	0.53 ± 0.11
kidney	3.00 ± 0.48	1.95 ± 0.01	2.15 ± 0.01
bone	46.1 ± 4.1	10.2 ± 0.2	6.84 ± 0.40
muscle	5.58 ± 0.39	5.15 ± 0.16	4.14 ± 0.41

Table II. Evaluation of 2,3-Dicarboxypropane-1,1-diphosphonic Acid (DPD) in Normal Rats (% ID/Organ)

A. Biodistribution

	NCA Gd/0.01 M lig	0.05 M Gd/0.15 M lig
blood	2.62 ± 0.48	0.319
liver	67.3 ± 1.0	73.0
heart	0.099 ± 0.006	0.059
spleen	2.67 ± 0.09	
kidney	0.339 ± 0.049	0.447
bone	6.91 ± 0.90	1.18
muscle	2.38 ± 0.43	0.124

B. Pharmacokinetics (ID = 50 μmol of Gd/kg)

	15 min	2 h	6 h
blood	23.4 ± 3.5	0.260 ± 0.037	0.217 ± 0.016
liver	34.2 ± 18.4	74.3 ± 5.6	80.2 ± 1.6
spleen	11.2 ± 0.3	12.5 ± 0.2	8.21 ± 1.07
stomach	0.221 ± 0.049	0.178 ± 0.073	
s. intest	0.86 ± 0.18	1.03 ± 0.23	0.675 ± 0.059
l. intest	0.138 ± 0.001	0.334 ± 0.155	0.293 ± 0.016

blood pool retention resulting from the higher lipophilicity. The tissue uptake sites were saturated at the higher concentrations explored (see Tables III and IV).

I. GdEDTMP. This represents a typical, strongly complexed bone-seeking agent, with uptakes comparable to those of bone agents in clinical use.[24,25] The bulk of the dose ended up in the bone with slight saturation of uptake sites at higher doses. There was minimal accumulation in the liver (Table V).

Discussion

The issues addressed in this study are: (1) the denticity of the chelates required to deliver Gd to bone (or other calcium-rich tissue); (2) the minimum number of phos-

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Table III. Biodistribution of Modified-DTPA Complexes in Normal Rats (% ID/Organ, 2 h)[a,b]

	A. GdDTPA-AEP		
	0.001 M/0.008 M	0.01 M/0.01 M	0.05 M/0.05 M
blood	0.143 ± 0.069	0.503 ± 0.076	0.556 ± 0.096
liver	0.239 ± 0.061	18.3 ± 0.04	14.8 ± 0.52
heart	0.004 ± 0.001	0.012 ± 0.003	0.016 ± 0.002
kidney	0.268 ± 0.036	0.222 ± 0.009	0.264 ± 0.026
bone	0.264 ± 0.053	0.641 ± 0.064	0.483 ± 0.065

	B. GdDTPA-HPDP		
	NCA Gd/0.01 M	0.05 M/0.08 M	0.10 M/0.11 M
blood	0.260 ± 0.01	0.270 ± 0.011	0.19 ± 0.01
liver	0.082 ± 0.007	0.109 ± 0.009	
heart	0.032 ± 0.005	0.007 ± 0.001	0.006 ± 0.001
kidney	0.273 ± 0.037	0.423 ± 0.127	
bone	65.7 ± 1.4	17.6 ± 0.6	16.4 ± 0.5

	C. GdDTPA-BDP ($Meglu^+$)[c]		
	0.01 M	0.10 M	0.05 M
blood	9.49 ± 1.67	1.62 ± 0.16	2.53 ± 0.28
liver	5.21 ± 0.73	0.795 ± 0.040	1.23 ± 0.170
heart	0.129 ± 0.026	0.033 ± 0.007	0.048 ± 0.004
kidney	0.476 ± 0.060	0.290 ± 0.027	
bone	20.5 ± 1.5	13.3 ± 0.8	16.3 ± 0.8
muscle	4.38 ± 0.51	3.59 ± 0.42	4.47 ± 0.83
spleen	0.185 ± 0.071	0.013 ± 0.003	

[a] All solutions were prepared by mixing acidic solutions of $GdCl_3$ with Na^+ salts of the ligand to the final concentrations specified as [Gd]/[ligand], pH 7.0. [b] Injected dose (ID) = 0.1 mL of solution/100 g body weight. [c] This complex was available preformed as the methylglucamine ($Meglu^+$) salt buffered at pH 6.4; it was made to the indicated concentrations for use.

phonate groups required for effective bone uptake; (3) the optimal distance between the phosphonate and DTPA ends of the bifunctional chelate, and the nature of the groups in this spacer region that will induce ideal uptake and washout.

Denticity. The complexes we explored clearly indicate that the non-DTPA chelates are too weak to effectively coordinate Gd. This is reflected in the impossibility of preparing carrier-added solutions of their Gd complexes without precipitating $Gd(OH)_3$. At NCA Gd concentrations, however, APB was able to deliver Gd to bone, most likely by the same mechanism as other weak chelates, i.e., transchelation into the bone matrix.[20] The diphosphonate DPD, however, turned out to be too sterically hindered to adequately complex Gd.[19,21]

Phosphonate Requirements. Early in the development of bone-seeking radiopharmaceuticals, the diphosphonates, $RCH(PO_3H_2)_2$, were employed as easily modifiable alternatives to pyrophosphates for delivering radionuclides to bone.[22-25] Since the diphosphonate serves the double function of both chelating the metal center (with a coordination requirement of six or more donors) and seeking calcium (in bone), complexes based on such ligands invariably have two, four, six, or eight phosphonate moieties per molecule. In an attempt to delineate the phosphonate requirement of bone-seeking agents, Kung et al.[26] demonstrated that a Tc complex of the monophosphonate carbamyl phosphate (CAP), $NH_2C(O)OPO_3H_2$, was just as effective as the Tc complex of methylenediphosphonate (MDP), $CH_2(PO_3H_2)_2$. The CAP and MDP complexes have three and six phosphonates per molecule, respectively. Castronovo et al.[27] attempted a similar study, using the Tc complex of *N*-(phosphonoamidino)sarcosine (phosphocreatine), $COOHCH_2N(CH_3)C(=NH)NHPO_3H_2$, with negative results. Thus, the minimum phosphonate requirement has not yet been truly established.

In our own study, in which the chelating responsibilities were taken over by the DTPA, we needed to establish the number of phosphonate groups required on the DTPA for effective localization. The diphosphonate-modified DTPAs (HPDP-DTPA and BDP-DTPA) delivered Gd to bone, while the monophosphonate-modified DTPAs (AEP-DTPA and ABP-DTPA) did not. The differences in the bone-seeking capabilities between the di- vs monophosphonate-modified GdDTPA complexes are more than would be expected on the basis of the statistics of 1- vs 2-phosphonates interacting with the bone surface calcium atoms. A possible explanation based on the present results is that a cis-($-PO_3$, $-PO_3$) environment, i.e., a "fake" diphosphonate front, must be presented by the complex to the bone crystalline surface. This would then account for the efficiency of the $Tc(CAP)_3$ complex of Kung et al.[26] The nature of the interaction of the phosphonate(s) with the bone surface calcium(s) requires crystal structural confirmation, which is lacking at the moment. Clearly other factors predisposing an agent to bone uptake are yet to be determined.

Spacer. This is the most flexible parameter in the design of this class of bifunctional chelates, since the termini are fixed as diphosphonate and strong chelate (DTPA). Spacers that incorporate hydrophilic groups would induce fast systemic clearance, while hydrophobic groups, by virtue of their interactions with nonpolar blood components, would stay in circulation longer, thus reducing target/background contrast. Upon complexation, the ligand containing the hydroxypropyl spacer, HPDP-DTPA, yielded an agent with good bone localization and fast blood clearance. The C_4 chain of BDP-DTPA resulted in an agent with slow blood clearance, despite its high bone localization (see Table IV).

Summary

The ability of a contrast agent to deliver Gd to calcified tissues is best measured by its ability to localize in bone. Tables VI and VII summarize this efficiency for all the agents studied. With the exception of $GdCl_3$, which is in a class by itself, two general classes of bone-seeking agents were realized irrespective of phosphonate content: (1) all weakly chelated complexes; (2) complexes of strong phosphonate-altered chelates. In the first group, the precentage of injected dose of complex localizing in bone dropped from approximately 50% at NCA doses to 10% of dose at 0.001 mmol of Gd/kg. The second group, the strong chelates, accumulated in bone in significant proportions at all Gd doses, with a slight drop-off at high doses (i.e., ~20% at 200 μmol/kg).

For the weak chelates at high Gd doses, large liver and spleen uptakes were noted, indicating that a large amount of uncomplexed Gd^{3+} ends up as $Gd(OH)_3$ which is cleared through the liver. The unprecipitated fraction reaching the bone is then transchelated to the bone surface phosphates.

The strong-chelate complexes are so stable that practically all Gd^{3+} is complexed in the presence of the 10% or more excess ligand, and remains in normal circulation. Since the parent GdDTPA does not localize in bone, transchelation is assumed to contribute little to bone localization. The interaction of chelate phosphonates with the bone surface Ca^{2+} is the main mode of entrapment. The complexes formed by AEP-DTPA and APB-DTPA,

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Table IV. Pharmacokinetics of the Modified DTPA Complexes in Normal Rats (% ID/Organ, ID = 50 μmol/kg)

	A. GdDTPA-HPDP			
	15 min	30 min	1 h	4 h
blood	4.07 ± 0.34	2.46 ± 0.44	0.543 ± 0.068	0.068 ± 0.027
liver	0.745 ± 0.248	0.519 ± 0.059	0.201 ± 0.033	0.202 ± 0.090
heart	0.081 ± 0.014	0.040 ± 0.008	0.011 ± 0.002	0.003 ± 0.002
bone	15.5 ± 0.4	16.8 ± 0.04	20.0 ± 1.5	18.1 ± 0.3
muscle	6.36 ± 0.32	4.09 ± 0.85	0.889 ± 0.050	0.223 ± 0.139
	B. GdDTPA-BDP			
	20 min	1 h	2 h	4 h
blood	4.43 ± 0.31	2.74 ± 0.11	2.53 ± 0.28	2.26 ± 0.29
liver	1.02 ± 0.06	1.06 ± 0.04	1.23 ± 0.17	2.06 ± 0.07
heart	0.085 ± 0.002	0.055 ± 0.005	0.048 ± 0.004	0.037 ± 0.006
bone	11.0 ± 0.5	17.6 ± 0.9	16.3 ± 0.8	18.5 ± 1.8
muscle	8.97 ± 0.60	5.17 ± 0.16	4.47 ± 0.83	4.50 ± 0.30
	C. GdDTPA			
	20 min	1 h	2 h	4 h
blood	6.03 ± 0.47	1.02 ± 0.08	0.094 ± 0.025	0.005 ± 0.004
liver	1.59 ± 0.06	0.418 ± 0.053	0.267 ± 0.024	0.163 ± 0.001
heart	0.097 ± 0.014	0.019 ± 0.002	0.004 ± 0.002	0.002 ± 0.001
bone	3.50 ± 0.24	0.779 ± 0.081	0.386 ± 0.030	0.287 ± 0.034

Table V. Biodistribution of GdEDTMP in Normal Rats (% ID/Organ, 2 h)

	NCA Gd/ 0.01 M lig	0.005 M Gd/ 0.01 M lig	0.01 M Gd/ 0.05 M lig
blood	0.045 ± 0.007	1.70 ± 0.70	0.7 ± 0.5
liver	0.22 ± 0.01	1.6 ± 0.5	0.5 ± 0.3
lung	0.02 ± 0	0.10 ± 0.04	0.09 ± 0.08
heart	0.004 ± 0.001	0.04 ± 0.02	0.13 ± 0.009
spleen	0.004 ± 0.0	0.02 ± 0.0	0.009 ± 0.002
kidney	0.29 ± 0.03	0.40 ± 0.06	0.38 ± 0.04
bone	47.0 ± 0.8	42.9 ± 0.6	35.0 ± 2.0
muscle	0.3 ± 0.1	2.4 ± 0.8	2.0 ± 2.0

Table VI. Comparison of a Modified DTPA Complex with Other Chelates That Deliver Gd to Bone (% ID in Bone at 1 h)[a,b]

	Gd(citrate)	GdEDTMP	GdDTPA-HPDP
NCA (10^{-6})	47	54	57
0.005	~10	54	44
0.01	7	51	31
0.02		~37	29
0.05		27	22
0.10			17
0.20			13

[a] Data from rat femur assuming 10% body weight is bone. [b] All complexes are Na^+ salts at pH 7–8.

each with one phosphonate group per complex compound, also did not localize in bone. This suggests that more than one phosphonate group per complex compound is required for effective bone localization.

A further indication of an agent's suitability for the intended contrast application is the ratio of accumulated Gd in the target tissue relative to the background. The bone is the target, and blood and muscle have been used as backgrounds for the data in Table VII. Again, the weak chelates demonstrated low ratios, partly because of their low bone uptakes and high backgrounds due to the binding of free Gd^{3+} by blood and muscle proteins. Of the phosphonate-containing, strongly chelated Gd complexes that localized in bone, those with significant lipophilicity would be expected to persist in blood and muscle as a consequence of hydrophobic interactions with fatty blood and muscle components. This was the case for the GdDTPA-BDP complex.

In conclusion, various complexes of Gd have been evaluated for their potential usefulness in NMR imaging.

Table VII. Summary of Bone/Blood and Bone/Muscle Ratios at 1 h Postinjection

	[Gd]	bone	bone/ blood	bone/ muscle
$GdCl_3$	NCA	14.7%	0.907	3.07
	0.001	4.31%	0.234	2.26
	0.01	7.73%	0.152	1.75
Gd(ascorbate)	NCA	45.2%	16.1	7.04
	0.001	7.69%	0.202	1.97
	0.01	3.59%	0.052	1.01
Gd(citrate)	NCA	46.1%	16.6	8.26
	0.001	10.2%	0.175	1.98
	0.01	6.84%	0.124	1.65
GdI-37	NCA	51.4%	16.5	8.81
	0.0005	6.54%	0.234	2.19
	0.002	6.9%	0.206	1.81
	0.005	5.07%	0.167	1.67
GdI-61	NCA	59.6%	25.8	9.53
	0.01 (N)[a]	3.33%	0.18	1.55
	0.01 (A)[a]	6.88%	0.145	1.22
GdI-71	NCA	27.7%	3.82	6.78
	0.01 (N)	2.56%	0.135	1.47
	0.01 (A)	7.23%	0.143	1.61
GdI-119	NCA	56.8%	24.2	9.46
	0.01 (N)	6.36%	0.188	2.14
	0.01 (A)	6.37%	0.139	1.60
GdDTPA-HPDP	NCA	57.0%	108	70.6
	0.005	44.4%	110.4	52.7
	0.02	28.9%	34.9	20.1
	0.05	21.2%	29.8	19.6
	0.10	16.1%	25.7	12.5
	0.20	13.5%	13.9	8.08
GdDTPA-BDP	0.01	22.9%	2.12	5.39
	0.05	17.6%	6.42	3.38
	0.10	13.7%	8.56	2.76
GdEDTMP	NCA	54.8%	91.8	51.2
	0.002	53.1%	73.3	43.5
	0.01	50.1%	37.3	27.7
	0.025	35.4%	20.4	12.4

[a] N is neutral; A indicates pH 2–3.

Only strongly chelated complexes with at least two cis phosphonate groups localize at high enough levels in calcified tissue at the doses required for eventual application as NMR contrast agents. For these, structural modifications that decrease the lipophilicity are needed to induce fast clearance from nontarget tissues.

Experimental Section

Synthesis. GdDTPA-AEP. Equivalent amounts of cyclic DTPA dianhydride and 2-aminoethylphosphonic acid were re-

fluxed in 500 mL of dry chloroform over 72 h. The suspension was evaporated under vacuum (rotatory evaporated) until it was dry. The progress of the coupling was monitored by the infrared spectroscopic analysis of dried aliquots. The mixture of ligands was complexed to Gd-153-labeled $GdCl_3$ and purified by RP-HPLC on a C_{18} column, using dilute HCl pH ~2.5 as eluant, with gamma detection. At a flow rate of 1 mL/min, two peaks were eluted with retention times of 3.2 and 5 min, corresponding to GdDTPA and GdDTPA-AEP, respectively. A mixture of $GdCl_3$ and 2-aminoethylphosphonic acid elutes at the solvent front, ~2.5 min, under identical conditions. Injectable solutions were prepared at pH 7, with a Gd concentration range of carrier-free or NCA (5×10^{-6} M) to 0.05 M.

DTPA-HPDP. This ligand was synthesized by coupling an activated DTPA to 3-amino-1-hydroxypropane-1,1-diphosphonic acid as follows: 2.35 g (10 mmol) of aminodiphosphonic acid was suspended in 200 mL of water and mixed with 20 mL of 1 N NaOH to a pH of 9. N^3-[(2,6-Dioxomorpholino)ethyl]-N^6-[(ethoxycarbonyl)methyl]-3,6-diazaoctane diacid (12.10 g, 30 mmol) was added while maintaining the constant pH 9. After the solution was stirred overnight, its pH was adjusted to 12.5 and it was stirred further for 3 h. The pH was then lowered to 7 with Amberlite IR 120, the resin was filtered away, and the product was purified by silica gel chromatography (mobile phase: butanol/ammonia–ethanol/water). The combined eluates were dried in vacuo, the residue was taken up in 50 mL of water, and the solution was treated with Amberlite IR 120. The solution was then filtered off the resin and dried in vacuo to yield 1.3 g of a white powder that melted at 145 °C. Anal. ($C_{17}H_{32}N_4O_{16}P_2$) C: Calcd, 33.45; found, 33.56. H: calcd, 5.28; found, 5.50. N: calcd, 9.18; found, 9.30. P: calcd, 10.15; found: 10.02.

GdDTPA-HPDP. This ligand was complexed to Gd as described under Protocol, below.

DTPA-BDP. This ligand was also synthesized by the following coupling process: 20.17 g (50 mmol) of N^3-[(2,6-dioxomorpholino)ethyl]-N^6-[(ethoxycarbonyl)methyl]-3,6-diazaoctane diacid was dissolved in 500 mL of dimethylformamide (DMF). To this was added 25.3 g (250 mmol) of triethylamine, and then 17.27 g (50 mmol) of 4-aminobutane-1,1-bis(phosphonic acid diethyl ester) in 250 mL of DMF was dripped into this solution at ~5 °C. The solution was allowed to stand for 2 h in an ice bath, then stirred for 16 h at room temperature, and concentrated in vacuo to dryness. The residue was dissolved in methanol and purified by silica gel chromatography. The methanolic eluate was then dried to yield 18 g of resin which was redissolved in 250 mL of DMF; 48 g (240 mmol) of trimethyliodosilane was then added dropwise and the mixture was stirred overnight at room temperature. Water (180 mL) was added and the solution was heated at 50 °C for 2 h. The aqueous phase was separated after cooling and concentrated in vacuo until dry. The residue was dissolved in 200 mL of methanol and purified on silica gel. Evaporation of the methanolic eluate produced 8.5 g of a white powder that decomposed at ~120 °C (57% yield). Anal. ($C_{18}H_{34}N_4O_{15}P_2$) C: calcd, 35.53; found, 35.70. H: calcd, 5.63; found, 5.85. N: calcd, 9.21; found, 9.10. P: calcd, 10.18; found, 10.37.

GdDTPA-BDP. This ligand was complexed to Gd as described under Protocol, below.

GdAPB. The ligand 2-amino-4-phosphonobutyric acid (APB) was purchased from Sigma and complexed to NCA Gd-153 (10^{-5} M), pH 7.

GdDTPA-APB. The coupling of the amine APB to DTPA anhydride was carried out following the procedure described for PDP-DTPA. Two fractions were isolated from silica gel chromatography and used as ligands for NCA Gd-153 (10^{-5} M) at pH 7.

GdDPD. A sample of the ligand synthesized at the Behring Institute, Frankfurt, West Germany, was obtained from E. Deutsch, University of Cincinnati, and was complexed to various concentrations of Gd, at pH 7.

GdEDTMP. Ethylenediaminetetrakis(methylenephosphonate) (EDTMP), the phosphonate analogue of EDTA synthesized by Dow Chemical Co., was obtained from W. Volkert, University of Missouri, and complexed to Gd at pH 8.

Gd(N_2O_2) Complexes. A series of linear tetradendate N_2O_2 donors, Figure 2, were obtained from H. Kung, SUNY at Buffalo, and evaluated as weak Gd chelates of varying lipophilicity. These were complexed to Gd in various concentrations at pH 3 and 7.

Protocol. The biodistribution of the contrast agents was studied in male Sprague–Dawley rats, ~250 g.

Solutions of various agents were prepared at pH 7–8. At low [Gd] (10^{-2} M or less), ligands were used in at least a 10-fold excess. At [Gd] > 10^{-2} M, a 10% excess of ligands over Gd were used to minimize the toxic effects of free Gd^{3+} and the precipitation of $Gd(OH)_3$. The carrier-free (no carrier added or NCA) solutions were 10^{-5} M in Gd and had a specific activity of 50 μCi/mg Gd. All radiolabeled solutions contained 5 μCi/mL of Gd-153. Whenever cloudy solutions were obtained, the precipitates, after centrifugation, were monitored for evidence of radioactivity.

For each different solution, doses of 0.1 mL/100 g body weight were injected intravenously in each of three rats, under light ether anesthesia. The animals were sacrificed at predetermined times, and relevant tissues were excised and counted for radioactivity. The concentrations of contrast agent present in the various tissues were calculated by comparing the measured radioactivity with appropriate standards.

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