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Toward Optimized High-Relaxivity MRI Agents: Thermodynamic Selectivity of Hydroxypyridonate/Catecholate Ligands[1]

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The thermodynamic selectivity for Gd^{3+} relative to Ca^{2+}, Zn^{2+}, and Fe^{3+} of two ligands of potential interest as magnetic resonance imaging (MRI) contrast agents has been determined by NMR spectroscopy and potentiometric and spectrophotometric titration. The two hexadentate ligands TREN-6-Me-3,2-HOPO (H_3**L2**) and TREN-bisHOPO-TAM-EA (H_4**L3**) incorporate 2,3-dihydroxypyridonate and 2,3-dihydroxyterephthalamide moieties. They were chosen to span a range of basicity while maintaining a structural motif similar to that of the parent ligand, TREN-1-Me-3,2-HOPO (H_3**L1**), in order to investigate the effect of the ligand basicity on its selectivity. The 1:1 stability constants (β_{110}) at 25 °C and 0.1 M KCl are as follows. **L2**: Gd^{3+}, 20.3; Ca^{2+}, 7.4; Zn^{2+}, 11.9; Fe^{3+}, 27.9. **L3**: Gd^{3+}, 24.3; Ca^{2+}, 5.2; Zn^{2+}, 14.6; Fe^{3+}, 35.1. At physiological pH, the selectivity of the ligand for Gd^{3+} over Ca^{2+} increases with the basicity of the ligand and decreases for Gd^{3+} over Fe^{3+}. These trends are consistent with the relative acidities of the various metal ions;— more basic ligands favor harder metals with a higher charge-to-radius ratio. The stabilities of the Zn^{2+} complexes do not correlate with basicity and are thought to be more influenced by geometric factors. The selectivities of these ligands are superior to those of the octadentate poly(aminocarboxylate) ligands that are currently used as MRI contrast agents in diagnostic medicine.

Introduction

In the past two decades, magnetic resonance imaging (MRI) has evolved into one of the most powerful techniques used in diagnostic medicine and biomedical research. The increasing importance of medical MRI has prompted the development of a new class of pharmacological products called contrast agents. These agents function by shortening the relaxation rate of complexed water protons, thereby improving the ability to distinguish between different tissues. Because Gd(III) is highly paramagnetic, with seven unpaired electrons and a long electronic relaxation time, it is ideal as a relaxation agent for MRI.[2,3] However, the high toxicity of $[Gd(H_2O)_8]^{3+}$, with LD_{50} values in the range of 0.1−0.2 mmol/kg, requires that the metal be strongly complexed by a chelating agent when administered to patients.[4,5] Unfortunately, chelation always displaces coordinated water molecules and usually slows the exchange of the remaining ones, so that the resulting Gd complexes demonstrate lower relaxivities and, hence, lower image-enhancing capabilities. Injection of gram quantities of the complexes is therefore necessary in order to obtain satisfactory contrast in the resulting image. A major research goal in this field is to increase relaxivity by increasing the number of coordinated water molecules and matching their exchange rate to that of the water proton Larmor frequency while maintaining sufficient in vivo inertness. Currently, all commercial contrast agents, such as $[Gd(DTPA)(H_2O)]^{2-}$ (Magnevist), Gd(DTPA-BMA)(H_2O) (Omniscan), $[Gd(DOTA)(H_2O)]^{-}$ (Dotarem), and Gd(HP-DO3A)(H_2O) (Prohance) (see Chart 1), are poly(aminocarboxylate) ligands that feature a nine-coordinate Gd^{3+} center bound to only one slowly exchanging water molecule. Furthermore, they are all low molecular weight compounds with relatively short retention times of a few hours.[2,6] Newer target-specific and macromolecular agents such as the blood pool imaging agent MS-325,[7−9] the hepatobilary agent $[Gd(BOTPA)(H_2O)]^{-}$,[10] and the dendrim-

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(2) Caravan, P.; Ellison, J. J.; McMurry, T. J.; Lauffer, R. B. *Chem. Rev.* **1999**, *99*, 2293−2352.

(3) Merbach, A. E.; Tóth, E. *The Chemistry of Contrast Agents*; Wiley: Chichester, U.K., 2001.

(4) Cacheris, W. P.; Quay, S. C.; Rockladge, S. M. *Magn. Reson. Imaging* **1990**, *8*, 467−481.

(5) Wedeking, P.; Kumar, K.; Tweedle, M. F. *Magn. Reson. Imaging* **1992**, *10*, 641−648.

(6) Clarkson, R. B. *Top. Curr. Chem.* **2002**, *221*, 201−235.

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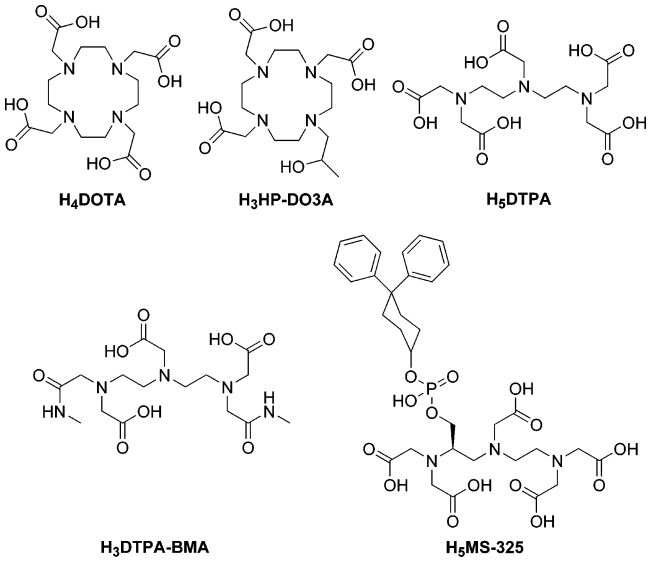
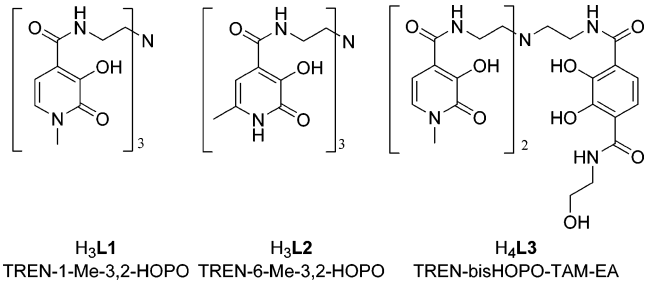
Chart 1. Ligands Used in Commercial Contrast Agents



eric Gadomer-17[11] are expected to have longer in vivo retention times. Therefore, it becomes increasingly important that the next generation of contrast agents demonstrate adequate in vivo inertness, either thermodynamically or kinetically. The general class of compounds that is the subject of this paper shows fast water exchange through an associative mechanism.[12] For such new relaxation agents with fast water exchange rates and longer in vivo retention times, it is all the more important to understand how complex selectivity can be maximized while increasing the number of coordinated water molecules *and* optimizing their exchange rate.

The importance of the selectivity of a ligand for Gd^{3+} relative to physiologically available metals was first articulated by Tweedle et al., who demonstrated that the relative stability of various chelates versus that of endogeneous metals was related to the toxicity of the corresponding Gd complex.[13] Soon after, Cacheris et al. established that the toxicity of kinetically labile complexes such as $[Gd(DTPA)(H_2O)]^{2-}$ and $Gd(DTPA\text{-}BMA)(H_2O)$, which reach thermodynamic equilibrium during their in vivo retention time, is directly related to the amount of uncomplexed Gd^{3+} released.[4] This release is a direct consequence of the transmetalation that occurs with endogenous metal cations. This prediction was supported by subchronic rodent toxicity experiments, in which the rodents demonstrated symptoms

Chart 2. Ligands Studied



similar to those caused by a Zn^{2+} deficiency. Gd^{3+} has also been shown to inhibit Ca^{2+} binding to mammalian cardiac sarcoplasmic reticulum and other Ca^{2+} binding proteins, with the mechanism of toxicity apparently involving hemodynamic disruption.[14,15] On the basis of this thermodynamic selectivity, Cacheris et al. accurately predicted that adding 5 mol % Na[CaDTPA-BMA] to the formulation of Gd(DTPA-BMA)(H_2O) would prevent transmetalation with Zn^{2+}. There was a consequent increase in the LD_{50}, thereby enhancing the safety of the contrast agent.[4] The benefits of this formulation were later supported by long-term biodistribution studies of Gd(DTPA-BMA)(H_2O) conducted by Tweedle et al.[16] It is important to note that these thermodynamic considerations do not apply to sterically rigid and more kinetically inert complexes such as $[Gd(DOTA)(H_2O)]^-$ and Gd(HP-DO3A)(H_2O), which do not attain thermodynamic equilibrium during their biological lifetimes.

We have previously reported Gd^{3+} complexes of a series of hydroxypyridonate (HOPO) ligands based on the parent TREN-1-Me-3,2-HOPO [H_3**L1** in Chart 2; TREN = tris(2-aminoethyl)amine].[17] Both the X-ray crystallography of Gd**L1** and its nuclear magnetic relaxation dispersion profile, and those of its derivatives, indicate that, in each case, the Gd^{3+} center is coordinated by two water molecules. Complexes of this family demonstrate near-optimal water exchange rates that result in exceptionally high relaxivity.[12] It becomes important to establish that this increase in the number of coordinated water molecules and in their exchange rate is achieved without sacrificing the chelates' high stability and selectivity, which ensures their low toxicity. Previous studies also indicated that these complexes have high thermodynamic stabilities, with the highest stability obtained being that for a ligand of intermediate basicity, TREN-bisHOPO-TAM-Me.[18] This complex, with a pGd of 20.1,[19] is more stable than the commercial agents $[Gd(DTPA)(H_2O)]^{2-}$

(7) Caravan, P.; Comuzzi, C.; Crooks, W.; McMurry, T. J.; Choppin, G. R.; Woulfe, S. R. *Inorg. Chem.* **2001**, *40*, 2170−2176.
(8) Parmalee, D. J.; Walowitch, R. C.; Ouellet, H. S.; Lauffer, R. B. *Invest. Radiol.* **1997**, *32*, 741−747.
(9) Lauffer, R. B.; Parmalee, D. J.; Dunham, S. U.; Ouellet, H. S.; Dolan, R. P.; Witte, S.; McMurry, T. J.; Walowitch, R. C. *Radiology (Oak Brook, Ill.)* **1998**, *207*, 529−538.
(10) Cavanga, F. M.; Maggioni, C. P. M.; Dapra, M.; Imperatori, L. G.; Lorusso, V.; Jenkins, B. G. *Invest. Radiol.* **1997**, *32*, 780−796.
(11) Nicolle, G. M.; Tóth, E.; Schmitt-Willich, H.; Raduchel, B.; Merbach, A. E. *Chem.*−*Eur. J.* **2002**, *8*, 1040−1048.
(12) Thompson, K. H.; Botta, M.; Nicolle, G. M.; Helm, L.; Aime, S.; Merbach, A. E.; Raymond, K. N. *J. Am. Chem. Soc.* **2003**, *125*, 14274−14275.
(13) Tweedle, M. F.; Gaughan, G. T.; Hagan, J.; Wedeking, P.; Sibley, P.; Wilson, L. J.; Lee, D. W. *Nucl. Med. Biol.* **1988**, *15*, 31−36.
(14) Pidcock, E.; Moore, G. R. *JBIC, J. Biol. Inorg. Chem.* **2001**, *6*, 479−481.
(15) Geraldes, C. F. G. C.; Luchinat, C. *Met. Ions Biol. Syst.* **2003**, *40*, 513−588.
(16) Tweedle, M. F.; Wedeking, P.; Kumar, K. *Invest. Radiol.* **1995**, *30*, 372−380.
(17) Xu, J.; Franklin, S. J.; Whisenhunt, J. D. W.; Raymond, K. N. *J. Am. Chem. Soc.* **1995**, *117*, 7245−7246.
(18) Doble, M. J.; Melchior, M.; O'Sullivan, B.; Siering, C.; Xu, J.; Pierre, V. C.; Raymond, K. N. *Inorg. Chem.* **2003**, *42*, 4930−4937.
(19) (a) pM is defined as pM = $-\log [M]_{free}$ and is calculated under the standard conditions $[L]_{total} = 10\ \mu M$, $[M]_{total} = 1\ \mu M$, and pH = 7.4. The more stable the complex, the lower the concentration of free or uncomplexed metal and the higher the pM value. (b) The metal association constant (β_{mlh}) is defined as $mM + lL + hH \rightleftarrows M_mL_lH_h$ and $\beta_{mlh} = [M_mL_lH_h]/([M]_m[L]_l[H]_h)$.

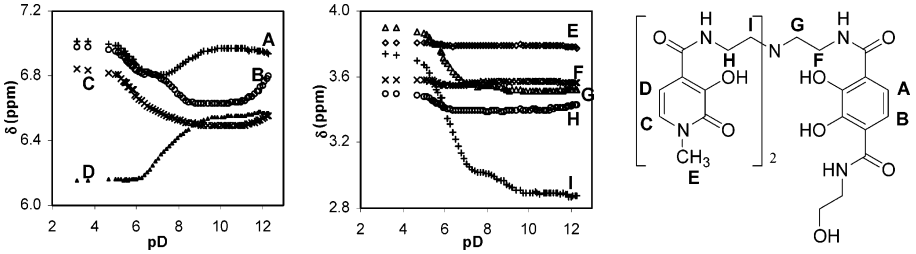


Figure 1. Assignments and determination of the protonation constants of TREN-bisHOPO-TAM-EA (H_4**L3**) by ^{1}H NMR spectroscopy. Variation of ^{1}H chemical shifts with pD are shown in (a) the aromatic region and (b) the aliphatic region. The observed chemical shifts are marked with points, whereas the calculated curves are shown with solid lines. The signals corresponding to the ethanol moiety have been removed for clarity. Experimental conditions: 25 °C; 0.1 M KCl.

(pGd = 19.4),[4] Gd(DTPA-BMA)(H_2O) (pGd = 15.8),[4] [Gd(DOTA)(H_2O)]$^-$ (pGd = 19.3),[20,21] and Gd(HP-DO3A)(H_2O) (pGd = 17.2).[2] More noteworthy, the labile parent complex, Gd-TREN-1-Me-3,2-HOPO (Gd**L1**, log K_{sel} = 10.3),[22] also showed a greater selectivity for Gd^{3+} over Ca^{2+} and Zn^{2+} than its comparative labile poly(aminocarboxylate) complexes[17] [Gd(DTPA)(H_2O)]$^{2-}$ (log K_{sel} = 7.04) and Gd(DTPA-BMA)(H_2O) (log K_{sel} = 9.04). The favorable thermodynamic stability and selectivity of Gd**L1** can be attributed to two factors. First, Gd^{3+} is highly oxophilic and will more readily bind to the six oxygen donors of **L1** than it will to the partially nitrogen-donating ligands of the commercial agents, and even more readily than will the less oxophilic Ca^{2+} and Zn^{2+}.[23,24] Second, the internal hydrogen bonding within the ligand and the Gd^{3+} complex and the preorganization of the two oxygen donors of the HOPO moiety to bind to the metal in a five-membered chelate ring increase the stability of the metal complex.[24,25] This study is concerned with evaluating the effect of a ligand's basicity on its selectivity for Gd^{3+} over physiologically available metals such as Ca^{2+}, Zn^{2+}, and Fe^{3+} for a series of hexadentate, hydropyridinonate, and terephthalamide ligands (Chart 2).

Results and Discussion

The ligands used in this study are shown in Chart 2. Ligands H_3**L1** and H_3**L2** were previously evaluated with regard to the stability of their Gd^{3+} complexes.[18] Ligand H_4**L3** is similar to TREN-bisHOPO-TAM-Me, which was found to have the highest stability in this previous study. The addition of an ethanolamine on the terephthalamide moiety increases the water solubility of the ligand and its metal complexes, thereby facilitating its thermodynamic study in water. The same tripodal backbone, TREN, is maintained throughout the series, in order to minimize the effects of ligand architecture. As demonstrated in the previous study,[18] these ligands exhibit a wide range of basicities.

Thermodynamic Evaluation. The log K_a values of ligand H_4**L3** were determined by potentiometric titration in a procedure identical to that used for H_3**L1** and H_3**L2**. As confirmation, an independent ^{1}H NMR spectroscopy titration was conducted. This experiment has the advantage of indirectly assigning a specific proton to a log K_a value. As seen in Figure 1, the chemical shift of nearby protons can drastically change as a result of the ligand deprotonation. Most importantly, the chemical shift of the methylenes corresponding to the TREN backbone, and most notably, that of protons **I** and **G**, which are adjacent to the tertiary nitrogen, are shifted most drastically at a pD $\approx$ 6. This indicates that the lowest log K_{a5} value corresponds to that of the tertiary amine of the TREN backbone, and not to any of the chelating arms. This low log K_a value, observed for a tertiary amine, is explained by the presence of stabilizing hydrogen bonding that can only be shared between the central amine and the three amides if the former is deprotonated.[17] The log K_a^D values in D_2O were calculated from a least-squares refinement of the δ = f (pD) curves after correcting the measured pH into pD according to pD = pH + 0.4.[26] The corrected log K_a^H values in water were then calculated from the log K_a^D values according to log K_a^D = 0.32 + 1.044 log K_a^H.[26]

The overall basicity of each ligand can be conveniently quantified by calculating $\sum$ log K_a according to the rule first established by Kumar et al.[27] which includes in the summation only those protonation steps that result in a neutral ligand. This definition enabled a direct comparison of diverse ligands with different backbones and coordinating arms. The $\sum$ log K_a values used for H_3**L1**, H_3**L2**, and H_4**L3** therefore do not include the last log K_a that corresponds to the tertiary amine of the TREN backbone as its protonation leads to a positively charged ligand. A comparison of the resulting $\sum$ log K_a values of H_4**L3** ($\sum$ log K_a = 32.95), shown in Table 1, to that of its parent TREN-bisHOPO-TAM-Me ($\sum$ log K_a = 32.36)[18] indicates that the addition of an ethanolamine on the terephthalamide moiety does not significantly change the basicity of the ligand. The overall basicity of the ligands

(20) Bianchi, A.; Calabi, L.; Ferrini, L.; Losi, P.; Uggeri, F.; Valtancoli, B. *Inorg. Chim. Acta* **1996**, *249*, 13−15.
(21) Clarke, E. T.; Martell, A. E. *Inorg. Chim. Acta* **1991**, *190*, 37−46.
(22) The selectivity constant K_{sel} is defined as $K_{sel} = \beta_{Gd10}(\alpha H^{-1} + \alpha Ca^{-1} + \alpha Zn^{-1})^{-1}$ where $\alpha H^{-1} = 1 + K_{a1}[H^+] + K_{a1}K_{a2}[H^+]^2 + K_{a1}K_{a2}K_{a3}[H^+]^3 + ...$, $\alpha Ca^{-1} = \beta_{Ca10}[Ca^{2+}]$, and $\alpha Zn^{-1} = \beta_{Zn10}[Zn^{2+}] + \beta_{Zn11}[Zn^{2+}][H^+] + \beta_{Zn12}[Zn^{2+}][H^+]^2$. The concentrations of Ca^{2+} and Zn^{2+} used in calculating K_{sel} are the physiological concentrations of their free metals: 2.5 mM and 50 μM, respectively. A higher selectivity constant means that the ligand is more selective for Gd^{3+} and that, in turn, the contrast agent will be less toxic.
(23) Martell, A. E.; Hancock, R. D.; Motekaitis, R. J. *Coord. Chem. Rev.* **1994**, *133*, 39−65.
(24) Hancock, R. D.; Martell, A. E. *Chem. Rev.* **1989**, *89*, 1875−1914.
(25) Hancock, R. D. *Analyst (Cambridge, U.K.)* **1997**, *122*, 51R−58R.
(26) Perrin, D. D.; Dempsey, B. *Buffers for pH and Metal Ions Control*; Chapman and Hall: London, 1974.
(27) Kumar, K.; Tweedle, M. F.; Malley, M. F.; Gougoutas, J. Z. *Inorg. Chem.* **1995**, *34*, 6472−6480.

Table 1. Thermodynamic Parameters for the Ligands **L1**, **L2**, and **L3**[a]

		potentiometry			
		L1[b]	**L2**[c]	**L3**	NMR spectroscopy
$\log K_{a1}$	$[L] + [H] \rightarrow [HL]$	8.19(2)	8.73(6)	11.42(7)	11.42(5)
$\log K_{a2}$	$[HL] + [H] \rightarrow [H_2L]$	6.97(1)	7.50(2)	8.57(4)	7.92(8)
$\log K_{a3}$	$[H_2L] + [H] \rightarrow [H_3L]$	5.83(1)	6.12(3)	7.04(3)	6.92(9)
$\log K_{a4}$	$[H_3L] + [H] \rightarrow [H_4L]$	4.97(1)	5.26(4)	5.94(3)	6.13(7)
$\log K_{a5}$	$[H_4L] + [H] \rightarrow [H_5L]$			5.08(2)	5.28(7)
$\Sigma \log K_a$[d]		20.99	22.35	32.95	32.39

[a] Values in parentheses give the uncertainty for each value in units of least significant digits. [b] See ref 17. [c] See ref 18. Experimental conditions: 25 °C; 0.1 M KCl. [d] See text for details. Charges omitted for clarity.

Table 2. Thermodynamic Parameters for the Gd^{3+}, Ca^{2+}, Zn^{2+}, and Fe^{3+} Complexes of Ligands H_3**L1**, H_3**L2**, and H_4**L3**[18,21,a]

			L1	**L2**	**L3**
Gd	β_{110}	$L^{n-} + Gd^{3+} \rightleftarrows LGd^{3-n}$	19.2(1)	20.3(1)	24.3(1)
	β_{111}	$L^{n-} + Gd^{3+} + H^+ \rightleftarrows LGdH^{4-n}$	22.7(1)	24.4(1)	29.8(1)
	β_{112}	$L^{n-} + Gd^{3+} + 2H^+ \rightleftarrows LGdH_2^{5-n}$	26.2(1)	27.6(1)	34.8(1)
	pM		19.2[b]	19.5[b]	20.3
Ca	β_{110}	$L^{n-} + Ca^{2+} \rightleftarrows LCa^{2-n}$	7.6(1)	7.4(1)	5.2(1)
	pM		7.6[c]	6.8	6.0
Zn	β_{110}	$L^{n-} + Zn^{2+} \rightleftarrows LZn^{2-n}$	13.1(5)	11.9(2)	14.6(1)
	β_{111}	$L^{n-} + Zn^{2+} + H^+ \rightleftarrows LZnH^{3-n}$	18.1(1)	19.3(1)	24.1(1)
	β_{112}	$L^{n-} + Zn^{2+} + 2H^+ \rightleftarrows LZnH_2^{4-n}$	22.5(1)	24.2(1)	30.4(1)
	pM		13.1[c]	11.4	12.3
Fe	β_{110}	$L^{n-} + Fe^{3+} \rightleftarrows LFe^{3-n}$	26.8(1)	27.9(1)	35.1(1)
	β_{111}	$L^{n-} + Fe^{3+} + H^+ \rightleftarrows LFeH^{4-n}$	30.7(3)	33.1(1)	39.3(1)
	pM		26.8[c]	27.2	30.3
$\Sigma \log K_a$			20.99	22.35	32.95
pGd − pCa			11.6	12.7	14.3
pGd − pZn			6.1	8.1	8.0
pFe − pGd			7.6	7.7	10.0
$\log K_{sel}$			10.3	12.3	11.5

[a] Values in parentheses give the uncertainty for each value in units of least significant digits. Experimental conditions: 25 °C; 0.1 M KCl. [b] See ref 18. For comparative reasons, the association constants of all metal complexes of H_4**L3** were calculated using the $\log K_a$ values obtained from the ligand's potentiometric titrations. [c] See ref 17.

follows the order H_3**L1** < H_3**L2** < H_3**L3**; for example, 1-Me-3,2-HOPO is less basic than 6-Me-3,2-HOPO, which is in turn significantly less basic than the terephthalamide moiety.

The Gd^{3+} complexation of **L3** was studied in a procedure identical to that used for **L1** and **L2**.[18] The association constants (β_{mlh}) as well as the pM values of each metal complex[18] (M = Gd, Ca, Zn, or Fe) are shown in Table 2. It is important to note that the addition of an ethanolamine tail to the terephthalamide moiety of H_4**L3** does not change the affinity of the ligand for Gd^{3+} (pM = 20.3 for Gd**L3** as opposed to 20.1 for the parent Gd-TREN-bisHOPO-TAM-Me). Therefore, functionalization of the terephthalamide moiety in this complex with, for instance, protein docking groups is predicted not to affect its stability.

The affinities of ligands H_3**L1**, H_3**L2**, and H_4**L3** for Ca^{2+}, Zn^{2+}, and Fe^{3+} are shown in Table 2. Those of H_3**L1** were previously reported.[17] The Ca^{2+} and Zn^{2+} complexations of H_3**L2** and H_4**L3** were determined by direct spectrophotometric titration in a procedure similar to that used for Gd^{3+}. The affinities of H_3**L2** and H_4**L3** for Fe^{3+} were too high to be determined by direct titration and were therefore determined by competition with ethylenediaminetetraacetic acid (EDTA) according to a previously reported procedure.[28] An example of the data acquired during a direct spectrophotometric titration showing the molar absorptivity of the Zn^{2+}/H_4**L3** system with varying pH is shown in Figure 2a. The molar absorbance of each species, as determined from nonlinear least-squares refinements, is shown in Figure 2b. [The species distribution diagrams for ligands H_3**L1**, H_3**L2**, and H_4**L3** with Gd^{3+}, Ca^{2+}, Zn^{2+}, and Fe^{3+} (pH 7.4, $[M]_{total} = 1 \times 10^{-6}$ M, and $[L]_{total} = 1 \times 10^{-5}$ M) are available in the Supporting Information.] The relative stabilities of various metal complexes at physiological pH are often compared using pM values.[19] The pM values of the Ca^{2+}, Zn^{2+}, and Fe^{3+} complexes of ligands H_3**L1**, H_3**L2**, and H_4**L3** calculated

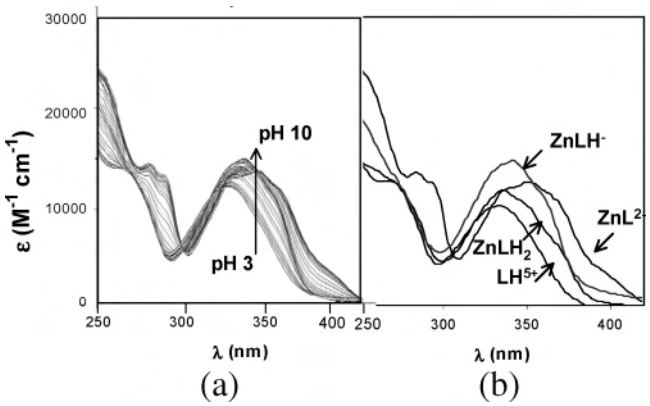


Figure 2. (a) Example of data acquired during a spectrophotometric titration showing the molar absorptivity of the Zn^{2+}/TREN-bisHOPO-TAM-EA (H_4**L3**) system with varying pH. (b) The spectra for the individual species are determined from nonlinear least-squares refinement of the formation constants. Experimental conditions: 25 °C; 0.1 M KCl.

(28) Cohen, S. M.; O'Sullivan, B.; Raymond, K. N. *Inorg. Chem.* **2000**, *39*, 4339−4346.

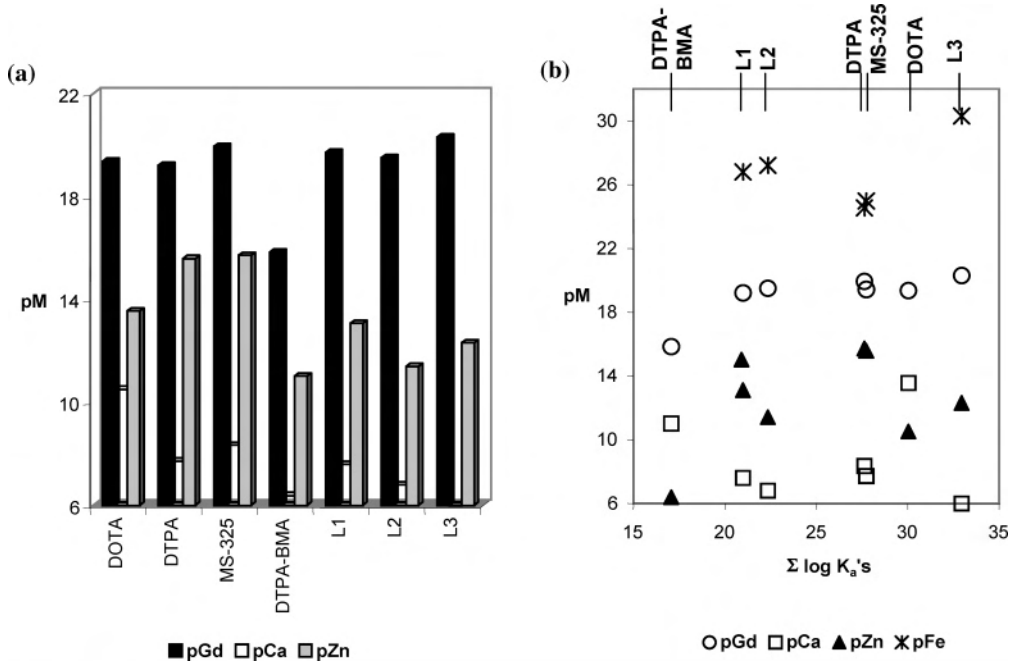


Figure 3. (a) Stabilities of the Gd^{3+}, Ca^{2+}, and Zn^{2+} complexes of ligands H_3**L1**, H_3**L2**, and H_4**L3** as compared to those of commercial contrast agents (see Chart 1). pM values are calculated using the formation constants in Table 2.[19] For DOTA, see refs 20 and 21, for DTPA and DTPA-BMA, see ref 4, and for MS-325, see ref 7. (b) Stabilities of the Gd^{3+}, Ca^{2+}, and Zn^{2+} complexes as a function of the basicity of the ligand, in terms of $\sum$ pK_a values.

in the standard conditions[19] are shown in Table 2. Under these conditions, the lowest theoretical pM is 6.0, when no complex is formed ($[M]_{total} = [M]_{free} = 1\ \mu M$). All three ligands form complexes of moderate stability with Zn^{2+} and very weak complexes with Ca^{2+}. Ca**L3** has a pM = 6.0 at physiological pH, which indicates that no Ca complex could be observed; the ligand is thus extremely selective for Gd^{3+} over Ca^{2+}.

A comparison of the pGd, pCa, and pZn values of H_3**L1**, H_3**L2**, and H_4**L3** with those of commercial agents H_4DOTA,[2] H_5DTPA,[4] H_3DTPA-BMA,[4] and H_5MS-325[8] (see Chart 1 for structure) is shown in Figure 3a. HOPO-based ligands H_3**L1**, H_3**L2**, and H_4**L3** form Gd^{3+} complexes as stable as those commercial agents. Figure 3b illustrates the pM of these seven ligands as a function of their basicity, described in terms of their $\sum$ log K_a values using the summation rule described above. It is interesting to note that despite their different scaffolds, a qualitative trend can still be observed for Gd^{3+} and Ca^{2+}. The stability of the Gd complexes at physiological pH (pGd) increases with the basicity of the ligand. This trend was first observed by Kumar et al. upon comparing K_{GdL} and $\sum$ log K_a values of all linear and macrocyclic poly(aminocarboxylates).[27,29−32] Although they have a different chelating moiety, the HOPO-based ligands H_3**L1**, H_3**L2**, and H_4**L3** still fit in this trend. Similarly, the stability of the Ca^{2+} complexes decreases with increasing ligand basicity. This trend thus follows the relative acidities of the metal ions described by Martell et al.[24] Harder ligands such as hydroxypyridinone favor harder metals such as Gd^{3+}. No trend could be observed for the stability of Zn^{2+} complexes with ligand basicity, which may reflect that ligand scaffold and cavity size are more important factors in determining the affinity of a ligand for Zn^{2+}, and such factors were not taken into consideration in a mere pZn versus $\sum$ log K_a values plot.

The selectivity of a ligand for one metal over another can be conveniently assessed on the basis of the difference in the pM values. For instance, the selectivity of a ligand for Gd^{3+} over Ca^{2+} and Zn^{2+} can be described by pGd − pCa and pGd − pZn, respectively. Figure 3a indicates that the selectivities, measured in terms of these differences, of H_3**L1**, H_3**L2**, and H_4**L3** are higher than those of commercial agents. The selectivities of H_3**L1**, H_3**L2**, and H_4**L3** as a function of their basicity are shown in Table 2. The identical scaffold of these three ligands allows for an accurate study of the effect of ligand basicity on selectivity. The selectivity for Gd^{3+} over Ca^{2+} increases with the basicity of the ligand. More basic ligands favor harder metals with a higher charge-to-radius ratio ($Gd^{3+} = 2.84\ Å^{-1}$ and $Ca^{2+} = 2.00\ Å^{-1}$).[33] The selectivity for Gd^{3+} over Zn^{2+} does increase from H_3**L1** to the more basic H_3**L2**, but not to the yet more basic H_4**L3**. This suggests that for Zn^{2+}, which has a charge-to-radius ratio similar to that of Gd^{3+} ($Zn^{2+} = 2.70\ Å^{-1}$),[33] maximum selectivity is attained by adjusting the ligand cavity size and not its basicity.

Another definition of selectivity was introduced by Cacheris et al.[4] while describing the relationship between the thermodynamic parameters of kinetically labile contrast agents and their toxicity. It was found that, for labile agents,

(29) Kumar, K.; Chang, C. A.; Tweedle, M. F. *Inorg. Chem.* **1993**, *32*, 587−593.
(30) Irving, H.; Rossotti, H. *Acta Chem. Scand.* **1956**, *10*, 72−93.
(31) Choppin, G. R. *J. Less-Common Met.* **1985**, *112*, 193−205.
(32) Huskens, J.; Torres, D. A.; Kovacs, Z.; Andre, J. P.; Geraldes, C. F. G. C.; Sherry, A. D. *Inorg. Chem.* **1997**, *36*.
(33) Shannon, R. D. *Acta Crystallogr., Sect. A* **1976**, *A32*, 751−767.

toxicity was due to the release of a specific amount of Gd^{3+} (13−15 μM) and that this amount is due to ligand exchange metathesis, and hence can be predicted from the selectivity of the ligand for Gd^{3+} over physiologically available metal ions (Ca^{2+} and Zn^{2+}) as calculated in terms of the selectivity constant, K_{sel}.[22] A higher selectivity constant means that the ligand is more selective for Gd^{3+} and that, in turn, the contrast agent will be less toxic. The selectivity constants of H_3**L1**, H_3**L2**, and H_4**L3** are shown in Table 2. All three ligands are significantly more selective than the kinetically labile commercial agents DTPA ($\log K_{sel} = 7.04$) and DTPA-BMA ($\log K_{sel} = 9.04$),[4] suggesting that their Gd complexes would be of low toxicity. We are currently investigating the in vivo toxicity and biodistribution of this family of compounds to verify their low toxicity.

Conclusion

In summary, we have demonstrated that within this family of related ligands, ligand basicity is an important factor to consider in optimizing ligands for high selectivity for Gd^{3+} over physiologically available metals such as Ca^{2+} or Zn^{2+} and, for comparison, Fe^{3+}. This is especially important as the toxicity of kinetically labile contrast agents for MRI is directly linked to the amount of metathesis occurring in vivo. As the basicity of the ligand increases, its selectivity for Gd^{3+} over Ca^{2+} increases and its selectivity for Gd^{3+} over Zn^{2+} remains high. Therefore, by adequately choosing basic oxygen donors, we have maintained a high selectivity for Gd^{3+} over endogenous cations, higher than that of commercial contrast agents, with fewer donor atoms. This provides a component of the rational design of ligands of high selectivity for a new generation of contrast agents with high relaxivity.

Experimental Section

The ligands TREN-6-Me-3,2-HOPO (H_3**L2**) and TREN-bisHOPO-TAM-EA (H_4**L3**) were synthesized as previously reported.[18,28]

Solution Thermodynamics. Experimental protocols and equipment followed closely those previously described for related ligands.[18,34] Ligand acidity was examined by potentiometric (pH vs total proton concentration) titration, while all metal coordination properties were studied by spectrophotometric (absorbance vs pH) titration, with data analysis performed using the HYPERQUAD[35,36] and pHAB[37,38] suite of computer programs, respectively. All experiments were performed at 25 °C and 0.1 M KCl. Each determination resulted from at least three independent experiments. Each experiment consisted of two titrations, first with acid and then in reverse with base, to ensure that reversibility and thermodynamic equilibrium were attained. Gd^{3+} stock solution was standardized against EDTA to the arsenazo III end-point in ammonium acetate buffer solution. Fe^{3+} stock solution was standardized with variamine blue.[39]

Protonation constants for both ligands were determined by potentiometric titrations using a ligand concentration of ca. 0.5 mM and an equilibration time of 300 s in the pH range 3−10.5 for H_3**L2** and 3−11.5 for H_4**L3**. In the case of H_4**L3**, protonation constants and their assignment were confirmed by ^{1}H NMR spectroscopy. A solution of 0.0060 M H_4**L3**, 0.10 M KCl, and 0.0040 M 3-(trimethylsilyl)-1-propanesulfonic acid (DSS) shift agent in D_2O was prepared and titrated by addition of 0.50 M NaOD. Aliquots were retrieved after each NaOD addition, and pD values for each were calculated from the measured pH according to the relationship[26] pD = 0.4 + pH. ^{1}H NMR spectra of each aliquot were generated on a Brucker DRX 500 MHz using a pulse angle of 90° and 16 scans per sample, for a total of 65 aliquots. Chemical shifts were referenced to the CH_3−Si peak of DSS (0.000 ppm). Protonation constants were detemined by nonlinear least-squares refinement using the program HypNMR[40] with an estimated uncertainty in chemical shift of 0.01 ppm (based on the observed variation between duplicate spectra) and corrected for the deuterium effect after the refinement according to the correlation $\log K^D = 0.32 + 1.044 \log K^H$.[26]

Complexation of Gd^{3+}, Ca^{2+}, and Zn^{2+} by both ligands was investigated by spectrophotometric titrations with a molar ratio of 1:1 for metal/ligand with typical ligand and metal concentrations of 50 μM. The spectral data from ca. 70 wavelengths in the range of 240−450 nm were included in each analysis. The Gd^{3+}/H_4**L3** system forms protonated complexes ($GdLH_2$ and GdLH) over the pH range 1.5−3, necessitating a strong acid titration during which the electrode was corrected for liquid−liquid junction potentials observed in the course of pH electrode calibration.[34] The resulting stability constants of these protonated species were included as fixed parameters in the subsequent determination of the constant of the neutral species, GdL.

In the case of Zn**L2** and Zn**L3**, several species ($ZnLH_2$, ZnLH, and ZnL) could be monitored over the pH range 2−10. The presence of multiple absorbing species was indicated by factor analysis. Similarly, in the case of Ca**L2** and Ca**L3**, only one metal absorbing species (CaL) was observed in the pH range 3−11, whereas several species corresponding to the stepwise deprotonation of the ligand were present.

The affinity of each ligand for Fe was too high to be studied directly and was therefore determined by batch spectrophotometric titration by competition with ethylenediaminetetraacetic acid (EDTA) according to a procedure previously reported.[28] The titration was performed between pH 3 and 6 with a ratio of ligand to Fe to EDTA of 1:1:5 and an equilibration time of 48 h for each aliquot. Factor analysis of the spectral data indicated the presence of two species, FeL and FeLH.

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Supporting Information Available: Species distribution diagrams for ligands H_3**L1**, H_3**L2**, and H_4**L3** with Gd^{3+}, Ca^{2+}, Zn^{2+}, and Fe^{3+}. This material is available free of charge via the Internet at http://pubs.acs.org.

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(34) Johnson, A. R.; O'Sullivan, B.; Raymond, K. N. *Inorg. Chem.* **2000**, *39*, 2652−2660.
(35) Gans, P.; Sabatini, A.; Vacca, A. *Talanta* **1996**, 43.
(36) *HYPERQUAD2000*, version 2.1(NT); Gans, P., Sabatini, A., Vacca, A.; Protonic Software, 1999.
(37) Gans, P.; Sabatini, A.; Vacca, A. *Ann. Chim. (Rome)* **1999**, *89*, 45−49.
(38) *pHAB*, version 2.0(NT); Gans, P., Sabatini, A., Vacca, A.; Protonic Software, 1999.
(39) Martell, A. E.; Smith, R. M.; Motekaitis, R. J. *NIST Critical Stability Constants of Metal Complexes*; NIST Standard Reference Data: Gaithersburg, MD, 1993.
(40) *HypNMR2000*, version 2.0(NT); Gans, P., Sabatini, A., Vacca, A.; Protonic Software, 1999.