Inorg. Chem. **2007**, *46*, 4796–4798

Optimized Relaxivity and Stability of [Gd(H(2,2)-1,2-HOPO)(H_2O)]$^-$ for Use as an MRI Contrast Agent[1]

Christoph J. Jocher,[†] Mauro Botta,[‡] Stefano Avedano,[‡] Evan G. Moore,[†] Jide Xu,[†] Silvio Aime,[§] and Kenneth N. Raymond*,[†]

Department of Chemistry, University of California, Berkeley, California 94720-1460, Dipartimento di Scienze dell'Ambiente e della Vita, Università del Piemonte Orientale "A. Avogadro", Via Bellini 25/G, I-15100 Alessandria, Italy, and Dipartimento di Chimica I.F.M., Università di Torino, Via P. Giuria 7, I-10125 Torino, Italy

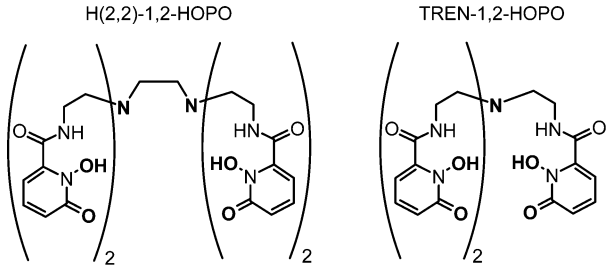
Received March 1, 2007

Relaxometry and solution thermodynamic measurements show that Gd(H(2,2)-1,2-HOPO) is a good candidate as a contrast agent for magnetic resonance imaging (MRI-CA). Acidic, octadentate H(2,2)-1,2-HOPO forms a very stable Gd(III) complex [pGd = 21.2(2)]. The coordination sphere at the Gd(III) center is completed by one water molecule that is not replaced by common physiological anions. In addition, this ligand is highly selective for Gd(III) binding in the presence of Zn(II) or Ca(II). The symmetric charge distribution of the 1,2-HOPO chelates is associated with favorably long electronic relaxation time $T_{1,2e}$ comparable to those of GdDOTA. This, in addition to the fast water exchange rate typical of HOPO chelates, improves the relaxivity to $r_{1p} = 8.2$ mM^{-1} s^{-1} (0.47 T). This remarkably high value is unprecedented for small-molecule, $q = 1$ MRI-CA.

Introduction

Recently, the Eu(III) luminescence-sensitizing properties of 1,2-HOPO chelates were discovered.[2] Ternary complexes of tetradentate 1,2-HOPO ligands support very efficient Eu(III) sensitization with quantum yields as high as $\Phi_{Eu} = 21.5\%$. However, octadentate ligands such as H(2,2)-1,2-HOPO (**1**; Chart 1), which were investigated for improved aqueous stability,[3] gave a quantum yield for [Eu(**1**)]$^-$ of $\Phi_{Eu} = 3.6\%$, almost 1 order of magnitude lower. This decrease was ascribed to the presence of one water molecule in the first coordination sphere and a change of symmetry at the Eu(III) center. While both properties are unfavorable for highly luminescent Eu(III) chelates, they increase the relaxivity of Gd(III) complexes, which can be used as a contrast agent for magnetic resonance imaging (MRI-CA). Particularly, the electronic relaxation time is one of the limiting factors for most of the commercially available MRI-CAs. The favorable electronic relaxation times of 1,2-HOPO versus 3,2-HOPO chelates have been recognized previously for hexadentate TREN-1,2-HOPO (**2**).[4] However, the relatively low selectivity of **2** for Gd(III) over Zn(II) may represent a problem in utilizing this compound as the MRI-CA.[5] For this reason, relaxometric and solution thermodynamic properties of [Gd(**1**)]$^-$ have been investigated.

Chart 1. Potential MRI-CA Based on 1,2-HOPO Chelates



Results

The $1/T_1$ nuclear magnetic relaxation dispersion (NMRD) profile of a 0.42 mM aqueous solution of [Gd(**1**)]$^-$ was recorded at pH 7.4 at 25 and 37 °C (Figure 1). Relaxivities of [Gd(**1**)]$^-$ are very high compared to those of commercial MRI-CAs.[6] The low-field relaxivity $r_{1p} = 15$ mM^{-1} s^{-1} (25 °C) is twice the corresponding value for [Gd(DTPA)(H_2O)]$^{2-}$ (DTPA = diethylenetriaminepentaacetic acid; $r_{1p} = 7.8$

* To whom correspondence should be addressed. E-mail: raymond@socrates.berkeley.edu. Fax: +1 510 486 5283. Phone: +1 510 642 7219.
† University of California, Berkeley.
‡ Università del Piemonte Orientale "A. Avogadro".
§ Università di Torino.

(1) High Relaxivity Gadolinium MRI Agents. 21. Part 20: Werner, E. J.; Avedano, S.; Botta, M.; Hay, B. P.; Moore, E. G.; Aime, S.; Raymond, K. N. *J. Am. Chem. Soc.* **2007**, *129*, 1870−1871.
(2) Moore, E. G.; Xu, J.; Jocher, C. J.; Werner, E. J.; Raymond, K. N. *J. Am. Chem. Soc.* **2006**, *128*, 10648−10649.
(3) Moore, E. G.; Jocher, C. J.; Werner, E. J.; Xu, J.; Raymond, K. N. *Inorg. Chem.* **2007**, *46*, accepted for publication.
(4) Xu, J.; Churchill, D. G.; Botta, M.; Raymond, K. N. *Inorg. Chem.* **2004**, *43*, 5492−5494.
(5) Jocher, C. J.; Raymond, K. N., unpublished results.
(6) Laurent, S.; Vander Elst, L.; Muller, R. N. *Contrast Media Mol. Imaging* **2006**, *1*, 128−137.

10.1021/ic700399p CCC: $37.00
Published on Web 05/11/2007

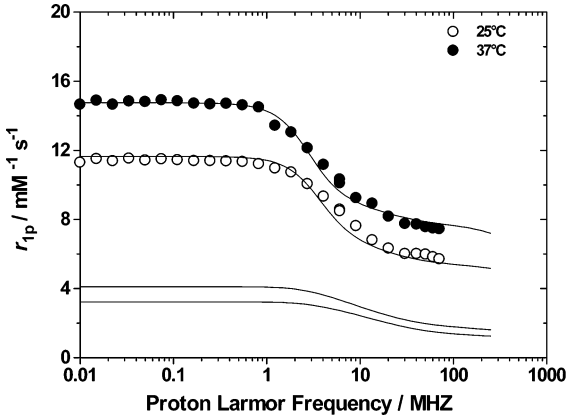


Figure 1. $1/T_1$ NMRD profile of a solution (pH 7.4) of [Gd(**1**)]$^-$ at 25 and 37 °C.

Table 1. Parameters from Refining NMRD Profiles of [Gd(**1**)]$^-$ at 25 and 37 °C[a]

parameter	[Gd(**1**)]$^-$ (298 K)	[Gd(**1**)]$^-$ (310 K)	[Gd(DOTA)(H$_2$O)] (298 K)
r_{1p} (mM^{-1} s^{-1}) 20 MHz	8.2	6.3	3.9
Δ^2 (s^{-2}; $\times 10^{19}$)	0.9(1)	1.0(2)	1.60
τ_V (ps)	51(2)	47(3)	11
τ_R (ps)	157(6)	107(2)	77
τ_M (ns)	10[b]	8[b]	473
q	1[b]	1[b]	1
r (Å)	3.0[b]	3.0[b]	3.13
a (Å)	4[b]	4[b]	
D (cm^2 s^{-1}; $\times 10^5$)	2.24[b]	3.0(1)	2.2

[a] The third column lists those for [Gd(DOTA)(H$_2$O)] as a comparison.[9] [b] These parameters were fixed during refinement.

mM^{-1} s^{-1}) and ca. 25% higher than that for [Gd(DOTA)(H$_2$O)]$^-$ (r_{1p} = 12 mM^{-1} s^{-1}). Furthermore, relaxivities at relevant field strengths r_{1p} = 8.2 mM^{-1} s^{-1} (20 MHz) and r_{1p} = 7.9 mM^{-1} s^{-1} (60 MHz) approximately double those of both [Gd(DOTA)(H$_2$O)]$^-$ and [Gd(DTPA)(H$_2$O)]$^{2-}$. These relaxivity values, very high considering [Gd(**1**)]$^-$ as a small, monohydrated (q = 1) complex, can be rationalized by fitting the NMRD profiles to equations for inner- and outer-sphere paramagnetic relaxation (Table 1).[7] The predominant contribution arises from a longer rotational correlation time, τ_R, about twice that of [Gd(DOTA)(H$_2$O)]$^-$. This implies a rather rigid structure because the increase in the size of [Gd(**1**)]$^-$ is efficiently transferred into a corresponding increase of τ_R. Compared to other MRI-CAs and Gd(III) chelates studied,[8] Δ^2 is small and τ_V long, which indicates a very favorable longitudinal electronic correlation time T_{1e} of ca. 70 ns at 60 MHz. This is also the case for [Gd(DOTA)(H$_2$O)]$^-$ (T_{1e} = 10 ns at 60 MHz), which, however, is limited by a long water residence time, τ_M = 472 ps.[8]

The relationship between the parameters Δ^2, τ_V, τ_R, τ_M, and relaxivity r_{1p} at high field has been detailed recently.[10]

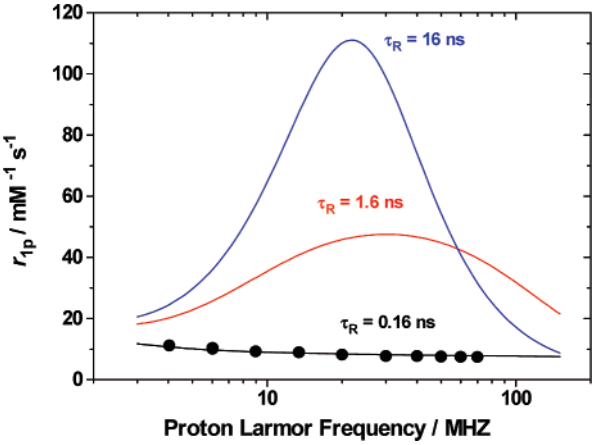


Figure 2. Simulation of relaxivity r_{1p} for [Gd(**1**)]$^-$ calculated as a function of τ_R using parameters of Table 1 (298 K).

Further improvement of the relaxivity of [Gd(**1**)]$^-$ can be achieved by increasing the rotational correlation time τ_R. T_{1e} can be estimated from Δ^2 and τ_V to be significantly longer than those for other HOPO chelates reported.[11] Thus, T_{1e} and τ_M represent optimal values for a significant improvement of r_{1p} to over 100 mM^{-1} s^{-1} by slowing rotational tumbling (Figure 2).

Attaching such a small-molecule contrast agent to a large and rigid molecule can significantly improve τ_R. For this reason, one may take advantage of the association of an MRI-CA to human serum albumin (HSA).[12] However, no interaction of [Gd(**1**)]$^-$ with HSA was observed in serum samples. Attachment of a HSA binding moiety to [Gd(**1**)]$^-$ is in progress and will be reported.

The most important parameters for evaluating the toxicity of [Gd(**1**)]$^-$ are the complex stability and selectivity of **1** for Gd(III) binding versus Zn(II) and Ca(II).[13] The protonation constants[3] illustrate the acidity of **1** compared to that of 3,2-HOPO chelates.[14] The four acidic protonation steps are associated with deprotonation of the 1,2-HOPO moieties, while the tertiary nitrogen atoms represent the two basic protonation sites.

Gd(III) binding of **1** versus the benchmark ligand DTPA (pGd = 19.11) was monitored by spectrophotometry. Batch titration techniques were employed because of slow protonation kinetics of the complex preventing variable pH titrations. Conditional stability constants were determined at pH 6.0, 7.4, and 9.0 (Table 2). From these data, two protonation constants of the Gd(III) complex and log β values could be calculated. The two basic protonation sites of [Gd(**1**)]$^-$ are located at the scaffold amines because relaxivities of GdL$^-$, GdLH, and GdLH$_2^+$ are nearly identical (Figures 3 and S5 in the Supporting Information). The high solubility of [Gd(**1**)]$^-$ (>0.5 mM) is not affected by protonation at pK_a = 9.7(5). However, the protonation of

(7) Aime, S.; Botta, M.; Terreno, E. Gd(III)-based Contrast Agents for MRI. In *Advances in Inorganic Chemistry*; van Eldik, R., Bertini, I., Eds.; Elsevier: San Diego, 2005; Vol. 57, pp 173−237.
(8) Caravan, P.; Ellison, J. J.; McMurray, T. J.; Lauffer, R. B. *Chem. Rev.* **1999**, *99*, 2293−2352.
(9) Powell, D. H.; Ni Dhubhghaill, O. M.; Pubanz, D.; Helm, L.; Lebedev, Y. S.; Schlaepfer, W.; Merbach, A. E. *J. Am. Chem. Soc.* **1996**, *118*, 9333−9346.
(10) Caravan, P. C. *Chem. Soc. Rev.* **2006**, *35*, 512−523.
(11) Raymond, K. N.; Pierre, V. C. *Bioconjugate Chem.* **2005**, *16*, 3−8.
(12) Merbach, A. E.; Tóth, E. *The Chemistry of Contrast Agents*; Wiley: Chichester, U.K., 2001.
(13) Cacheris, W. P.; Quay, S. C.; Rocklage, S. M. *Magn. Reson. Imaging* **1990**, *8*, 467−481.
(14) Doble, D. M. J.; Melchior, M.; O'Sullivan, B.; Siering, C.; Xu, J.; Pierre, V. C.; Raymond, K. N. *Inorg. Chem.* **2003**, *42*, 4930−4937.

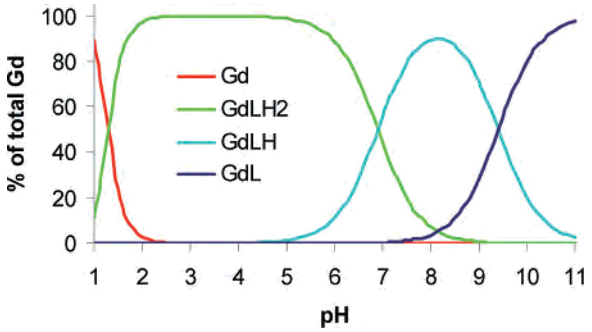


Figure 3. Calculated speciation of a 0.1 mM solution of [Gd(**1**)]$^-$.

Table 2. Metal Binding Constants of **1**[a]

$\log \beta_{GdL}$	21.5(5)	$pGd^{9.0}$	21.7(1)
$\log \beta_{GdLH}$	31.2(2)	$pGd^{7.4}$	21.2(2)
$\log \beta_{GdLH2}$	38.1(3)	$pGd^{6.0}$	20.0(2)
pZn	14.5(1)	pCa	8.3(2)

[a] $pM = -\log [M^{free}]$ in a solution with $c_L^{tot} = 10\ \mu M$ and $c_M^{tot} = 1\ \mu M$ at pH 7.4 or as specified by the superscript.

the second scaffold amine with $pK_a = 6.9(3)$ reduces the solubility to 0.1 mM. Under physiological conditions, the monoprotonated, neutral complex GdLH is dominant (75%), with one proton being shared by the two amine functions in the scaffold.

Zn(II) and Ca(II) binding of **1** is weak (Table 2) as determined by pM values obtained from competition batch titration versus DTPA. The addition of a fourth 1,2-HOPO moiety to the ligand does not improve the stability of the Zn(II) complex, resulting in a high selectivity of **1** for lanthanides versus five- or six-coordinate transition metals. The difference between pGd and pZn is 6.7(3). This is well beyond the ability of the benchmark compound DTPA (4.2) and comparable to more basic 3,2-HOPO chelates.[15]

Calcium binding of **1** is stronger than that of DTPA by almost 1 order of magnitude. However, the resulting pCa = 8.3(2) indicates that a 13 log unit excess of Ca(II) is required to remove Gd(III) from [Gd(**1**)]$^-$, although the formation of a mixed complex with Ca(II) cannot be excluded.

[Gd(H**1**)] and [Eu(H**1**)] show no affinity for carbonate, biphosphate, and fluoride as established by luminescence spectroscopy (Figure 4) and relaxometry at 60 MHz (Supporting Information), respectively, upon the addition of 1100−1600 equivalents of anion. The lack of changes in the luminescence intensity or relaxation rate observed

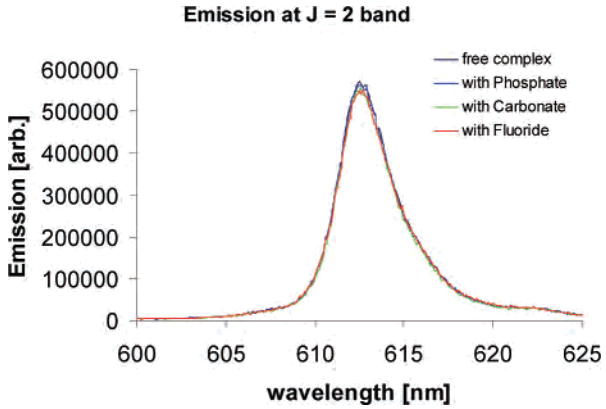


Figure 4. Obtained emission spectra between 600 and 625 nm ($J = 2$ band) of [Eu(**1**)]$^-$ (black; 17 μM) and after the addition of 1100 equiv of phosphate (blue), 1100 equiv of carbonate (green), or 1600 equiv of fluoride (blue) at pH 7.4 after 24 h of equilibration.

indicates affinities K_A for all three anions well below 10 M^{-1}. Thus, binding constants of [Eu(H**1**)] are far lower than the already uncritical affinities of Gd-DOTA, Gd-DTPA, and Gd-DTPA-BMA for biphosphate ranging from 100 to 160 M^{-1}.[11]

Summary and Outlook

[Gd(**1**)]$^-$ reveals excellent properties for utilization as an MRI-CA. The very high relaxivity is achieved by optimizing all relevant parameters for this small molecule. Solution thermodynamic assessment predicts very low toxicity based on the high stability of the Gd(III) complex and the excellent selectivity of **1** for Gd(III) over Zn(II) and Ca(II). In addition, the low affinities for phosphate, carbonate, and protein interactions are valuable for a good performance as an MRI-CA in vivo and clean excretion. Current efforts aim at exploring strategies to reduce rotational tumbling.

Acknowledgment. This work [University of California, Berkeley (UCB)] was partially supported by NIH Grant HL69832 and NATO Travel Grant PST.CLG.980380. C.J.J. thanks the German Research Foundation (DFG) for a postdoctoral fellowship. Support from MIUR (COFIN 2005) is also gratefully acknowledged (M.B. and S.A.). The authors thank Professor Chang (UCB) for use of a 60 MHz relaxometer.

Supporting Information Available: Additional stability data, figures, and tables. This material is available free of charge via the Internet at http://pubs.acs.org.

IC700399P

(15) Pierre, V. C.; Botta, M.; Aime, S.; Raymond, K. N. *Inorg. Chem.* **2006**, *45*, 8355−8364.