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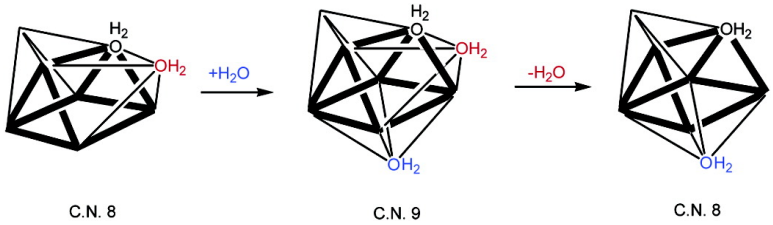

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A Highly Stable Gadolinium Complex with a Fast, Associative Mechanism of Water Exchange

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The prominence of magnetic resonance imaging (MRI) as a medical diagnostic technique has prompted intense interest in the development of contrast agents. The primary clinical contrast agents are nine-coordinate gadolinium (Gd^{III}) complexes based on a poly(amino carboxylate) ligand and function by enhancing the relaxation rate of water protons.[1−3] The image enhancement capability (proton relaxivity, r_{1p}) of current clinical contrast agents is only a few percent of that theoretically possible[2,4] due to the presence of only one inner sphere water molecule and a short rotational correlation time. When the rotational correlation time is optimized, the slow water exchange rate ($k_{ex} \approx 10^6\ s^{-1}$) becomes the limiting factor in attaining higher relaxivities.[1] Therefore, any rational design of a high-relaxivity contrast agent requires a thorough understanding of the mechanism of water exchange at the metal center.

The Gd^{III} complexes based on a hexadentate, hetero-tripodal hydroxypyridonate (HOPO) ligand, such as [Gd-TREN-bis(1-Me-HOPO)-(TAM-Me)$(H_2O)_2$] (Gd-**1**) (Figure 1), are promising candidates for the development of second-generation MRI contrast agents.[5,6] In this series of complexes, the metal ion is eight-coordinate and possesses two inner sphere water molecules.[7] The generally high stability and fast water exchange of the complexes make them highly desirable as candidates for MRI. [Gd-TREN-bis(6-Me-HOPO)-(TAM-TRI)$(H_2O)_2$] (Gd-**2**) represents a new entry into this class of complexes and is based on a hetero-tripodal ligand design involving 6-Me-3,2-HOPO chelating units, as opposed to the 1-Me-3,2-HOPO isomer in the parent complex (Gd-**1**). A tri(ethylene glycol) is conjugated to the terephthalamide (TAM) chelating unit to enhance the water solubility of the complex.

The stability of a MRI contrast agent is critical, because the toxicity of the agent has been shown to be directly related to the concentration of free Gd^{III} in vivo.[8] As contrast agent development is now oriented toward targeted imaging and longer in vivo residence times are sought, the thermodynamic stability of future agents will come under increased scrutiny. The stability of Gd-**2** was assessed using both potentiometric and spectrophotometric titration techniques. The ligand protonation constants of TREN-bis(6-Me-HOPO)-(TAM-TRI) (**2**) were determined by potentiometric titration. The experimental procedure, including instrumentation and solution preparations, is as described in detail in previous reports.[9−11] Ligand **2** is slightly more basic than **1**, in keeping with the higher basicity of the 6-Me-HOPO moiety as compared to the 1-Me-HOPO isomer.[11] Gd^{III} formation constants were determined by spectrophotometric titrations in the pH 3−9 range using procedures previously reported.[9−11]

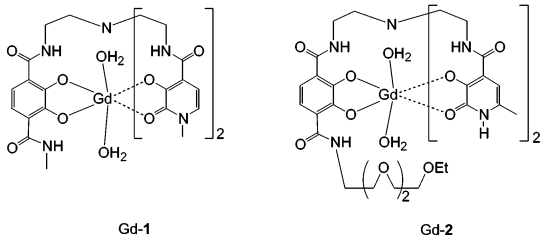

Figure 1. Gd-TREN-bis(1-Me-HOPO)-(TAM-Me)$(H_2O)_2$ (Gd-**1**) and Gd-TREN-bis(6-Me-HOPO)-(TAM-TRI)$(H_2O)_2$ (Gd-**2**).

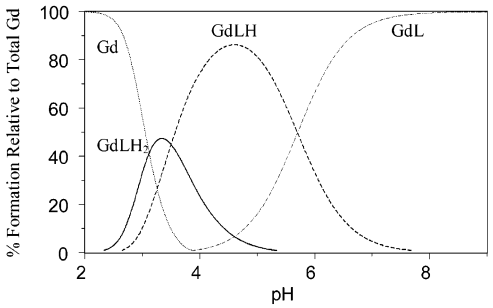

Figure 2. Species distribution diagram calculated for the Gd-**2** system for 1 μM Gd^{III} and 10 μM **2**.

The chemical model employed in the fitting of the Gd^{III} titration data closely resembles that applied in related ligand systems,[5,9−11] with the formation of a monomeric complex with stepwise addition of up to two protons before the complex dissociates below pH 2.5 (Figure 2). The formation constant (log β_{110}) of Gd-**2** is 24.9, and the calculated pM[12] is 20.6, a value slightly higher than that of Gd-**1** (pM = 20.1).[5,11] This can be attributed to the greater basicity of the 6-Me-HOPO chelator as compared to that of the 1-Me-HOPO isomer. Spectrophotometric competition titration against DTPA was used to verify the stability of Gd-**2** (Supporting Information).

The water exchange rate (k_{ex}) of Gd-**2** was assessed by variable temperature (VT), proton decoupled ^{17}O NMR measurement of the water nuclear transverse relaxation rate (R_{2p}).[4,13] The VT ^{17}O NMR curves for Gd-**2** are shown in Figure 3. The data were measured at 2.12 T (90 MHz for the proton and 12 MHz for ^{17}O) and 14.09 T at pH $\approx$ 7. The curves were analyzed in terms of the Swift−Connick equations, rearranged in a form suitable for Gd^{III}.[2] The profiles of Figure 3 have a shape typical of systems in the fast exchange regime.[5,6,14,15] Under these conditions, it is difficult to obtain a reliable evaluation of the mean residence lifetime if no direct measurement of the electron spin relaxation is available. In fact,

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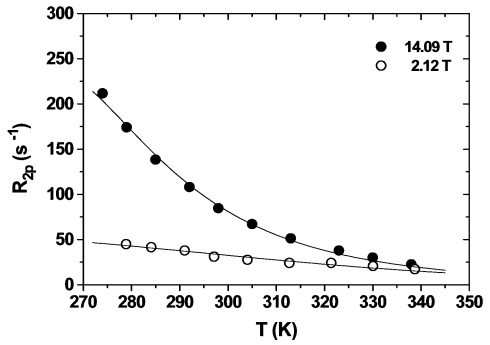

Figure 3. Temperature dependence of the paramagnetic contribution (R_{2p}) to the transverse ^{17}O water relaxation rate of Gd-**2** (1.7 mM, pH 7.3).

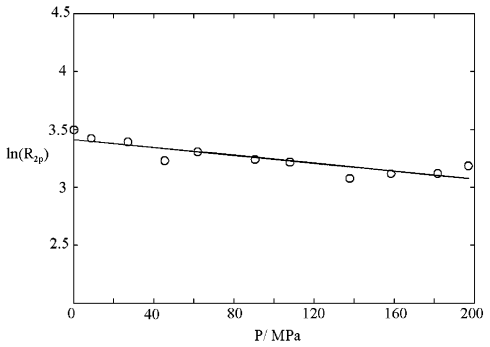

Figure 4. Pressure dependence of the increase of the ^{17}O transverse relaxation rate at 9.4 T and 319.9 K for Gd-**2**.

only a careful comparison of the ^{17}O data with the NMRD profile (from the analysis of both experiments, the same set of values for the electronic parameters must be obtained) allows for a convincing solution. An excellent fit of the experimental data was obtained with $q = 2$, $k_{ex} = 5.3\ (\pm 0.6) \times 10^7\ s^{-1}$, $\Delta H^{\ddagger} = 25.9\ (\pm 1.4)$ kJ/mol, $\Delta^2 = 0.9\ (\pm 0.2) \times 10^{20}\ s^{-1}$, $\tau_V = 24\ (\pm 2.0)$ ps, $E_V = 2.0\ (\pm 1.2)$ kJ/mol, and $A/\hbar = -3.8$ MHz. From these parameters, it can be seen that scalar relaxation of ^{17}O is governed by exchange and not by electron spin relaxation, that is, $k_{ex} \gg 1/T_{1e}$.

The water exchange mechanism can be assessed by determining the activation volume, $\Delta V^{\ddagger}$, from variable pressure ^{17}O transverse relaxation measurements.[2] The increase of the ^{17}O transverse relaxation rates (R_{2p}) due to the presence of paramagnetic Gd^{III} was investigated at variable pressure using procedures previously reported.[2,16,17] The Y^{III} analogue of Gd-**2**, [Y-TREN-bis(6-Me-HOPO)−(TAM-TRI)(H_2O)$_2$] (Y-**2**), was used as a diamagnetic reference for these studies. The pressure dependence of the relaxation rates of Gd-**2** and Y-**2** was measured at pH 8.2 and 319.9 K (Figure 4). From these studies, $\Delta V^{\ddagger}$ for water exchange on Gd-**2** is $-5\ (\pm 1)\ cm^3\ mol^{-1}$.

Both the negative sign and the magnitude of $\Delta V^{\ddagger}$ are indicative of an associative interchange (I_a) mechanism for water exchange in Gd-**2**, as seen in three other gadolinium complexes.[2] The activation volume is similar to that obtained for the eight-coordinated aqua-ion $[Gd(H_2O)_8]^{3+}$ ($\Delta V^{\ddagger} = -3.3\ cm^3\ mol^{-1}$), which also has a fast exchange rate.[18] The contrast agents in clinical use undergo water exchange via a dissociative mechanism involving an eight-coordinate intermediate. The very small value of $\Delta H^{\ddagger}$ indicates that the activation energy barrier for the formation of the transition state of Gd-**2** is low and that the eight- and nine-coordinate states of the HOPO complexes are close in energy.

In summary, this report describes a new member of the class of hydroxypyridonate-based Gd^{III} complexes targeted for future MRI contrast agent development. Gd-**2** is sufficiently stable for clinical applications and has a fast rate of water exchange, a combination of traits unique to this class of Gd^{III} complexes. The very fast water exchange rate should allow for optimization of the water proton relaxivity by slowing the molecular rotation of the complex (work in progress). While the mode of water exchange in this class of complexes was previously assumed to be associative,[5−7,9,14,15] definitive proof of this mechanism has now been presented.

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Supporting Information Available: Synthesis and characterization of complexes, table of formation constants, spectrophotometric titration spectra, variable pressure ^{17}O NMR data, NMRD profile of Gd-**2** (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) Caravan, P.; Ellison, J. J.; McMurry, T. J.; Lauffer, R. B. *Chem. Rev.* **1999**, *99*, 2293.
(2) Toth, E.; Helm, L.; Merbach, A. E. *The Chemistry of Contrast Agents in Medical Magnetic Resonance Imaging*; Wiley: New York, 2001; pp 45−119.
(3) Aime, S.; Botta, M.; Fasano, M.; Terreno, E. *Chem. Soc. Rev.* **1998**, *27*, 19.
(4) Aime, S.; Botta, M.; Fasano, M.; Crich, S. G.; Terreno, E. *Coord. Chem. Rev.* **1999**, *185*−*6*, 321.
(5) Cohen, S. M.; Xu, J.; Radkov, E.; Raymond, K. N.; Botta, M.; Barge, A.; Aime, S. *Inorg. Chem.* **2000**, *39*, 5747.
(6) Doble, D.; Wang, J.; Botta, M.; Aime, S.; Raymond, K. *J. Am. Chem. Soc.* **2001**, *123*, 10758.
(7) Xu, J.; Franklin, S. J.; Whisenhunt, D. W., Jr.; Raymond, K. N. *J. Am. Chem. Soc.* **1995**, *117*, 7245−7246.
(8) Cacheris, W. P.; Quay, S. C.; Rocklage, S. M. *Magn. Reson. Imaging* **1990**, *8*, 467.
(9) O'Sullivan, B.; Doble, D. M. J.; Thompson, M. K.; Siering, C.; Xu, J.; Botta, M.; Aime, S.; Raymond, K. N. *Inorg. Chem.* **2003**, *42*, 2577−2583.
(10) Johnson, A. R.; O'Sullivan, B.; Raymond, K. N. *Inorg. Chem.* **2000**, *39*, 2652−2660.
(11) 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.
(12) $pM = -\log[Gd]_{free}$; $[Gd]_T = 1\ \mu M$, $[L]_T = 10\ \mu M$ (pH 7.4, 25 °C, 0.1 M KCl).
(13) Aime, S.; Botta, M.; M., F.; Terreno, E. *Acc. Chem. Res.* **1999**, *32*, 941.
(14) Hajela, S.; Botta, M.; Giraudo, S.; Xu, J.; Raymond, K. N.; Aime, S. *J. Am. Chem. Soc.* **2000**, *122*, 11228.
(15) Sunderland, C. J.; Botta, M.; Aime, S.; Raymond, K. N. *Inorg. Chem.* **2001**, *40*, 6746−6756.
(16) Gonzalez, G.; Powell, D. H.; Tissieres, V.; Merbach, A. E. *J. Phys. Chem.* **1994**, *98*, 53−59.
(17) Cossy, C.; Helm, L.; Merbach, A. E. *Inorg. Chem.* **1989**, *28*, 2699−2703.
(18) Micskei, K.; Powell, D. H.; Helm, L.; Brücher, E.; Merbach, A. E. *Magn. Reson. Chem.* **1993**, *31*, 1011.

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