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Gadolinium(III) 1,2-Hydroxypyridonate-Based Complexes: Toward MRI Contrast Agents of High Relaxivity[1]

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Prospective gadolinium(III) MRI contrast agent precursors [Gd-TREN-1,2-HOPO] (**1**) {TREN-1,2-HOPO = tris[(1-hydroxy-2-oxo-1,2-dihydropyridine-6-carboxamido)ethyl]amine} and [Gd-TREN-bis(Me-3,2-HOPO)-1,2-HOPO] (**2**) have been synthesized and characterized by relaxometric measurements. The water proton relaxivity values of **1** and **2** (20 MHz and 25 °C) are 9.5 and 9.3 $mM^{-1}s^{-1}$, respectively, suggesting the presence of two coordinated water molecules. The molecular structure of [**1**·DMF]$_2$ was obtained and reveals a similar eight-coordinate geometry to [Gd-TREN-Me-3,2-HOPO·2H$_2$O] ([**3**·2H$_2$O]). A shape analysis of the coordination polyhedron of **1** reveals that this geometry is best described as a bicapped trigonal prism, poised to accommodate an additional donor atom to give a tricapped trigonal prismatic intermediate. This geometry supports the model that formation of a tris-aquo intermediate for **1** enables fast and associative water exchange.

Magnetic resonance imaging (MRI) has revolutionized diagnostic medicine, and novel gadolinium(III) contrast agents are being developed to optimize clinical imaging capabilities.[2,3a] Highly stable tripodal HOPO-based gadolinium chelates[3b] from our laboratory are centered on the exceptional nature of the metal coordination sphere, beginning with the report of [Gd-TREN-Me-3,2-HOPO·2H$_2$O] ([**3**·2H$_2$O]).[1,4–10]

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(1) Paper number 11 in the series: Thompson, M. K.; Botta, M.; Nicolle, G.; Helm, L.; Aime, S.; Merbach A. E.; Raymond, K. N. A Highly Stable Gadolinium Complex with a Fast, Associative Mechanism of Water Exchange. *J. Am. Chem. Soc.* **2003**, *125*, 14274–14275.
(2) *The Chemistry of Contrast Agents in Medical Magnetic Resonance Imaging*; Merbach, A. E., Toth, E., Eds.; John Wiley & Sons Limited: Chichester, U.K., 2001.
(3) High stability of Gd^{3+} agents is essential since free Gd^{3+} is toxic in vivo, displacing endogenous ions such as Ca^{2+}. See: (a) Caravan, P.; Ellison, J. J.; McMurry, T. J.; Lauffer, R. B. *Chem. Rev.* **1999**, *99*, 2293–2352. (b) 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.
(4) Xu, J.; Franklin, S. J.; Whisenhunt, D. W.; Raymond, K. N. *J. Am. Chem. Soc.* **1995**, *117*, 7245–7246.
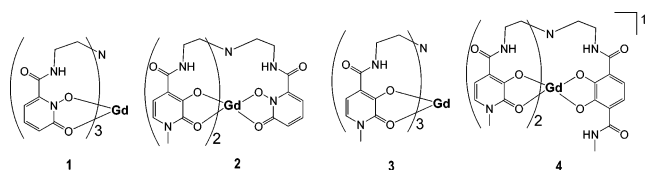
Figure 1. Gd-TREN-1,2-HOPO (**1**), Gd-TREN-bis(Me-3,2-HOPO)-1,2-HOPO (**2**), Gd-TREN-Me-3,2-HOPO (**3**), and Gd-TREN-bis(Me-3,2-HOPO)-TAM-Me (**4**).

Mean water residence lifetime (τ_m) is the limiting parameter for clinical agents (octadentate amino-carboxylate-based) with values of 100 to 1000 ns. Even if these agents possessed large rotational correlation times, proton relaxivity would still be a few percent of that theoretically possible. HOPO-class chelates, however, possess shorter lifetimes with τ_m on the order of 10 ns even when the complex is heteropodal and side-chains are present (Figure 1).[1,4,6]

Clinically used agents involve one bound water molecule ($q = 1$). The [TREN-Me-3,2-HOPO] ligand however (i) allows for $q = 2$, doubling the potential imaging capabilities of clinical agents, giving an eight-coordinate Gd^{3+} complex, and which (ii) possesses fast, associative water exchange, indicating a small activation barrier between the $q = 2$ ground state and the $q = 3$ intermediate.[4,8] Our understanding of the ground, intermediate, and transition state geometries responsible for such water exchange is improved with structural characterization of these chelates.

We report here the synthesis, X-ray crystal structure, and relaxometric data of a promising 1,2-HOPO derivative, Gd-TREN-1,2-HOPO (**1**) {TREN-1,2-HOPO = tris[(1-hydroxy-2-oxo-1,2-dihydro-pyridine-6-carboxamido)ethyl]-

(5) Pierre, V. C.; Melchior, M.; Raymond, K. N. *Inorg. Chem.*, submitted.
(6) Doble, D. M. J.; Botta, M.; Wang, J.; Aime, S.; Barge, A.; Raymond, K. N. *J. Am. Chem. Soc.* **2001**, *123*, 10758–10759.
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(8) Johnson, A. R.; O'Sullivan, B.; Raymond, K. N. *Inorg. Chem.* **2000**, *39*, 2652–2660.
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(10) 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.

Scheme 1. Synthesis of **1**[a]
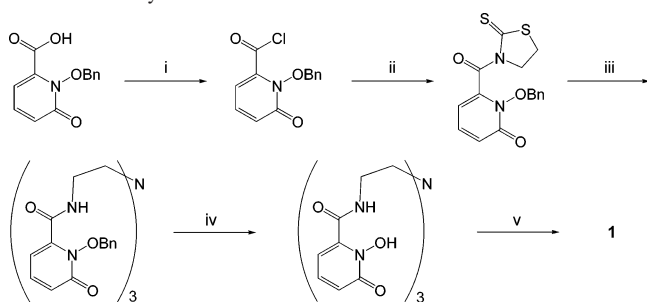
[a] (i) Oxalyl chloride/toluene, 93% yield; (ii) 2-mercaptothiazoline and triethylamine/THF, 90% yield; (iii) TREN/THF, 80% yield; (iv) HCl + AcOH, 90% yield; (v) Gd(acac)$_3$, pyridine/methanol, refluxing for 6 h, 80% yield.
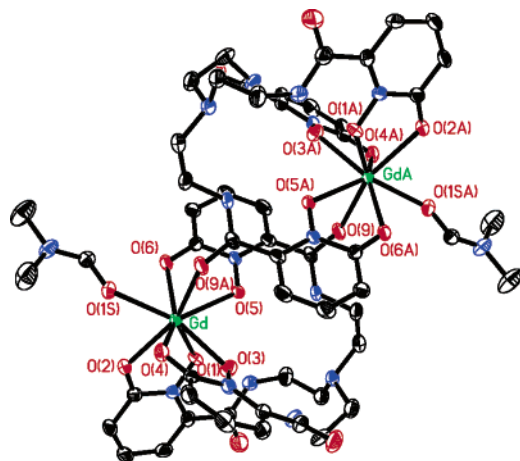

Figure 2. Molecular structure of [**1**·DMF]$_2$, illustrated with 50% probability ellipsoids. Select bond distances (Å): Gd−O(1), 2.383(4); Gd−O(3), 2.348(4); Gd−O(5), 2.400(4); Gd−O(2), 2.378(4); Gd−O(4), 2.368(4); Gd−O(6), 2.376(4); Gd−O(1S), 2.386(4); Gd−O(9A), 2.453(4). Hydrogen atoms are omitted for clarity.

amine} (Figure 1). The synthesis of **1** is illustrated in Scheme 1.[11]

The mixed Me-3,2-HOPO and 1,2-HOPO ligand in **2** was prepared via a method similar to that previously reported.[12]

In the crystal structure of [**1**·DMF]$_2$ (Figure 2), each gadolinium ion is coordinated by six HOPO oxygen atoms. To complete the eight-coordinate geometry in **1**, a DMF oxygen and amide oxygen atom of a neighboring complex are present instead of bound waters.[13,14]

The molecular structure of **1** reveals favorable hydrogen bonding, also observed in **3**, which increases complex stability. The three [$O_{py}\cdots N_{amide}$] distances in [**1**·DMF]$_2$ all have the value of 2.62 Å, close to an average of 2.68 Å for [**3**·2H$_2$O]. The average TREN [$N_{tert}\cdots N_{amide}$] distance in [**1**·DMF]$_2$ is 3.03 Å, compared to that for [**3**·2H$_2$O] of 3.08 Å.

(11) Xu, J.; Durbin, P. W.; Kullgren, B.; Ebbe, S. N.; Uhlir, L. C.; Raymond, K. N. *J. Med. Chem.* **2002**, *45*, 3963−3971.
(12) Raymond, K. N.; Xu, J. U.S. Patent 5,624,901, 1997
(13) X-ray crystallographic data for [Gd-TREN-1,2-HOPO·DMF]$_2$: T = 173(2) K, monoclinic, $P2_1/n$, a = 15.381(3) Å, b = 10.982(2) Å, c = 25.167(5) Å, β = 102.69(3)°, V = 4147.3(14) Å^3, Z = 4, R1 = 0.0555, wR2 = 0.1019.
(14) The structural information has been submitted to the Cambridge Crystallographic Data Centre, and the deposition number CCDC 214433 has been allocated.
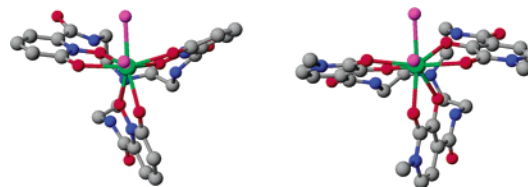
Figure 3. Comparison of [**1**·DMF]$_2$ (left) and [**3**·2H$_2$O] (right). Non-HOPO oxygen donors are in pink. Noncoordinating atoms in the DMF, bridging amide groups, and hydrogen atoms are omitted for clarity.

A general structural comparison of **1** and **3** reveals a similar tripodal ligand shape around the metal. In [**1**·DMF]$_2$ and [**3**·2H$_2$O], the HOPO rings appear on the sides and bottom of each structure as drawn in Figure 3, with two labile donor atoms present at the front and top.[15]

Trivalent lanthanide ions such as Gd^{3+} generally display variable coordination numbers of eight, nine, or higher, with the energy difference between the various coordination geometries believed to be very small. We have thus employed the shape measure S to assess these subtle differences.[16,17]

$$S = \min\left[\sqrt{\frac{1}{m}\sum_{i=1}^{m}(\delta_i - \theta_i)^2}\right]$$

We have assigned the eight-coordinate geometry derived from the X-ray structure in **1** and **3** to the closest of these high-symmetry polyhedra: the bicapped trigonal prism (C_{2v}), trigonal dodecahedron (D_{2d}), or square antiprism (D_{4d}). By this method, the dihedral angles of adjacent triangular faces of the idealized polyhedron are compared with those for the coordination polyhedron. Moreover, S is a measure of agreement in the variance of dihedral angles along all edges, between the polyhedron derived from the crystal structure and the idealized one. The lowest value of S for the three pairs represents the best fit to the nearest idealized geometry.

For [**1**·DMF]$_2$, the shape measure for the bicapped trigonal prism ($S(C_{2v})$ = 13.2°) is the lowest value ($S(D_{2d})$ = 16.9°; $S(D_{4d})$ = 22.4°) (Figure 4), differing from that of [**3**·2H$_2$O] (D_{2d}). This suggests Gd^{3+} can in theory accept an additional coordinated water (to become tricapped) with minimal disruption to the initial coordination geometry. This assignment strongly suggests that the energy difference between the nine-coordinate and eight-coordinate (plus water) structures of **1** is very small, consistent with the observed fast water exchange in related systems.

The proton relaxivity, r_{1p}, values of **1** and **2** were measured to be 9.5 and 9.3 mM^{-1} s^{-1}, respectively, at 20 MHz, 25 °C, and pH = 7.2. These values are very similar to those

(15) Because of the difference in charge separation of the aromatic resonance structures, the 1,2-HOPO isomer has a more nearly equal distribution of negative charge on the coordinating oxygen atoms than does the 3,2-HOPO isomer.
(16) Xu, J.; Radkov, E.; Ziegler, M.; Raymond, K. N. *Inorg. Chem.* **2000**, *39*, 4156−4164.
(17) In this formula, m is the number of edges of the coordination polyhedron, δ_i is the observed dihedral angle along the ith edge of δ (angle between normals of adjacent triangular faces), θ_i is the same angle of the corresponding ideal polytopal shape θ, and min is the minimum of all possible values. The calculation of shape measure has been extended to evaluate the geometries of nine-coordinate complexes and will be described elsewhere.
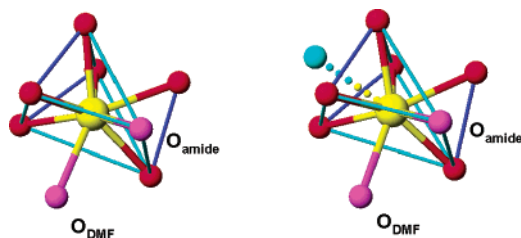
Figure 4. Diagrams of the coordination polyhedron of **1** derived from the X-ray structure. Dark blue lines represent chelation edges; DMF and amide oxygen donors are pink. Geometry **1** is depicted as a bicapped trigonal prism (left). An oxygen donor atom (light blue) binds at the open rectangular face to complete the tricapped trigonal prism (right).
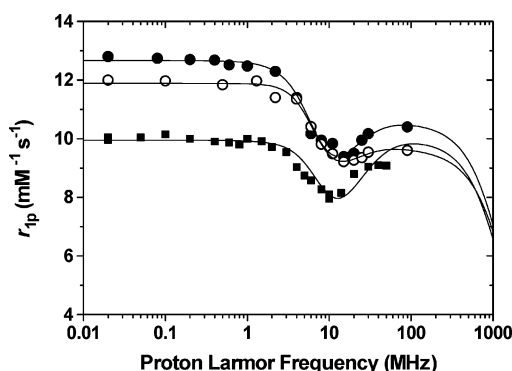

Figure 5. $1/T_1$ NMRD profiles of **1** (●), **2** (○), and **4** (■) at pH = 7.2 and 25 °C. The solid curves through the data points are calculated using the parameters reported in Table 1.

measured for related complexes, derived from modifications of the parent compound **3**. This result strongly supports the presence of two water molecules in the inner Gd^{3+} coordination sphere. A more complete characterization of the relaxometric properties of **1** and **2** was obtained by measuring and analyzing the magnetic field dependence of the relaxivity by recording the nuclear magnetic relaxation dispersion (NMRD) profiles. These profiles were obtained at 25 °C and pH = 7.2 on a fast field-cycling relaxometer operating in the range of proton Larmor frequencies from 0.01 to 40 MHz (Figure 5) and are compared with the corresponding data for **4**. The additional data points at 90 MHz were recorded on a high-resolution NMR spectrometer. The analysis of the data, according to the set of equations for inner and outer sphere paramagnetic relaxation, provides the values of important structural and dynamic parameters such as q, the [Gd···H$_{water}$] distance r, the reorientational molecular correlation time (τ_R), and the parameters describing electronic relaxation, Δ^2 and τ_V (Table 1).[2,3]

Table 1. Best-Fit Parameters Obtained from the Analysis of $1/T_1$ NMRD Profiles of **1** and **2** (25 °C and pH = 7.2)

complex[a]	q	τ_R (ps)	r (Å)	τ_V (ps)	Δ^2 (s^{-1})/10^{19}
1	2	104	3.00	23.4	6.6
2	2	116	3.00	20.6	7.1
4[b]	2	120	3.05	17.8	10.9

[a] Parameters of the outer sphere relaxivity were fixed to standard values: $D = 2.24 \times 10^{-5}$ cm^2 s^{-1}, $a = 4.0$ Å. [b] Reference 3b.

The water solubility of **1** and **2** is too low for direct assessment of the water exchange rate. However, from the relaxivity values at low fields, we may estimate an upper limit for the mean residence lifetime, τ_M, of the coordinated water molecules to be ca. 50 ns. This confirms a fast rate of water exchange for these complexes, consistent with values for **3** and **4**,[7] and supports the conclusions derived from the above structural analysis. Noteworthy are the low frequency range (Figure 5) and the r_{1p} values that decrease in the sequence **1** > **2** > **4**. Nevertheless, the best-fit parameters are similar (Table 1), supporting very close structural properties for these three complexes in aqueous solution.

In summary, a prospective 1,2-HOPO based gadolinium MRI contrast agent precursor [Gd-TREN-1,2-HOPO] (**1**) has been synthesized and characterized by X-ray crystallography and proton relaxivity techniques (9.5 mM^{-1} s^{-1}) supporting a monomeric aqueous state with two bound water molecules. Similarly, the relaxivity of [Gd-TREN-bis(Me-3,2-HOPO)-1,2-HOPO] (**2**) was also measured (9.3 mM^{-1} s^{-1}). The relaxometric values for the HOPO-type chelates **1**, **2**, and **4** are close, which is in agreement with the subtle differences in the molecular structures of [**1**·DMF]$_2$ and [Gd-TREN-Me-3,2-HOPO·2H$_2$O], pointing to an associative water exchange mechanism, promising for MRI applications.

Supporting Information Available: Single crystal X-ray diffraction data and synthetic and experimental details (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

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