

## Tuning the Coordination Number of Hydroxypyridonate-Based Gadolinium Complexes: Implications for MRI Contrast Agents<sup>1</sup>

Valérie C. Pierre,<sup>†</sup> Mauro Botta,<sup>‡</sup> Silvio Aime,<sup>§</sup> and Kenneth N. Raymond<sup>\*;†</sup>

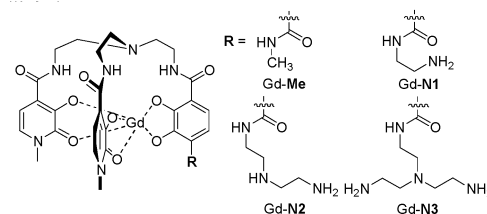
Department of Chemistry, University of California, Berkeley, California 94720-1460, Dipartimento di Scienze dell'Ambiente e della Vita, Università del Piemonte Orientale "A. Avogadro", Via Bellini 25/G, I-15100 Alessandria, Italy, and Dipartimento di Chimica I.F.M., Università di Torino, Via P. Giuria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

Received November 16, 2005; E-mail: raymond@socrates.berkeley.edu

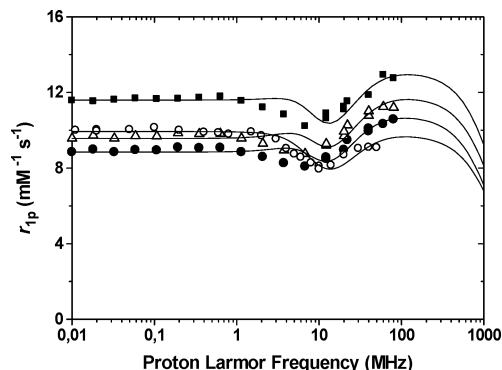
With seven unpaired electrons and a long electronic relaxation time, Gd<sup>III</sup> is ideal for use as a relaxation agent in Magnetic Resonance Imaging (MRI). Its high toxicity, however, requires that it be complexed by a strong chelator for in vivo applications. Current commercial poly(amino carboxylate)-based chelates have only one water molecule coordinated, which exchanges too slowly with the bulk solvent such that it limits the image enhancing capability (relaxivity,  $r_{1p}$ ) of macromolecular derivatives.<sup>2</sup> The development of second generation agents, for example, site-specific, requires much higher relaxivity. As predicted by the Solomon–Bloembergen–Morgan theory, this can be achieved by increasing the number of water molecules coordinated to the Gd<sup>III</sup> without destabilizing the complex.<sup>2</sup>

We have previously reported hydroxypyridonate (HOPO)-based Gd<sup>III</sup> chelates which display high relaxivity while maintaining high stability.<sup>3</sup> In these complexes, the Gd<sup>III</sup> centers are eight-coordinate with a bicapped trigonal prism geometry.<sup>4</sup> Since the ligands are hexadentate, water molecules occupy two of the coordination sites of Gd<sup>III</sup>, resulting in a relaxivity double that of commercial agents. These complexes exchange water rapidly ( $k_{ex} \sim 10^8 \text{ s}^{-1}$ ) through an associative interchange mechanism, suggesting that the eight and nine coordination states are close in energy.<sup>5</sup> This is supported by the structure of the La<sup>III</sup> analogue of TREN-1-Me-3,2-HOPO which crystallized as a dimer, with one La being eight-coordinate and the other nine.<sup>6</sup> The crystal structure of Gd-TREN-1-Me-3,2-HOPO indicates that filling the open coordination site of Gd<sup>III</sup> with a third water molecule would result in minimal distortion of the complex.<sup>4</sup> This indicates that it would be possible to stabilize the nine coordination state to achieve  $q = 3$  complexes which would still maintain high stability. One possibility to achieve this is to graft a hydrogen bond acceptor, such as an amine, on the terephthalamide moiety. A significant hydrogen bonding interaction between the amine and another water molecule close to the Gd<sup>III</sup> could facilitate its coordination.

Three derivatives of Gd-TREN-bisHOPO-TAM-Me (Gd-Me) bearing either one, two, or three pendant amines were synthesized (Figure 1). Their  $1/T_1$  NMRD profiles (Figure 2) indicate that the relaxivity of the smaller Gd-N1 is noticeably higher than that of the intermediate Gd-N2, which is, in turn, higher than that of the larger Gd-N3. The refinement parameters (Table 1) indicate that all three complexes have similar electronic relaxation times ( $\Delta^2$  and  $\tau_v$ ), and that their rotational correlation times,  $\tau_R$ , are proportional to their molecular weight. The large difference in relaxivity observed, which is inversely proportional to the size of the complexes, is therefore not due to a change in  $\tau_R$ , but rather to an increase in the number of inner sphere water molecules,  $q$ , for



**Figure 1.** Gd-TREN-bisHOPO-TAM-Me (Gd-Me) and the amine derivatives Gd-N1, Gd-N2, and Gd-N3.



**Figure 2.**  $1/T_1$  NMRD profile at 298 K and pH 7.4 of Gd-N1 (■), Gd-N2 (△), Gd-N3 (●), and Gd-Me (○).

**Table 1.** Stability and Refinement Parameters of the  $1/T_1$  NMRD Profiles of Gd-N1, Gd-N2 and Gd-N3

	Gd-Me <sup>b</sup>	Gd-N1	Gd-N2	Gd-N3
pM	20.1	19.5	20.0	18.8
${}^{298}r_{1p}$ ( $\text{mM}^{-1} \text{ s}^{-1}$ ) <sup>a</sup>	8.7	11.1	9.7	9.0
$q$	$1.94 \pm 0.09$	$2.82 \pm 0.08$	$2.39 \pm 0.07$	$1.98 \pm 0.05$
$\tau_R$ (ps)	$110 \pm 2$	$110 \pm 5$	$114 \pm 3$	$127 \pm 3$
$\Delta^2$ ( $10^{20} \text{ s}^{-2}$ )	$1.2 \pm 0.2$	$1.3 \pm 0.2$	$1.4 \pm 0.1$	$1.3 \pm 0.1$
$\tau_v$ (ps)	$17.1 \pm 0.3$	$18.7 \pm 0.9$	$19.2 \pm 0.4$	$20.0 \pm 0.9$
$\tau_m$ (ns)	$8.0 \pm 0.9^b$	$2.6 \pm 0.9^b$	$3^c$	$2.1 \pm 0.8^b$

<sup>a</sup> At 20 MHz. <sup>b</sup> Determined from the temperature dependence of the paramagnetic contribution to the water <sup>17</sup>O NMR  $r_{2p}$ . <sup>c</sup> Fixed.

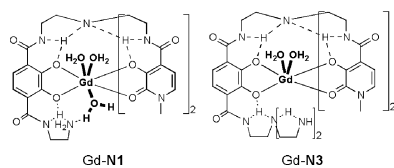
the smaller Gd-N1.<sup>2</sup> Refinements of the NMRD profiles indicate that Gd-N3, like the parent methyl derivative, Gd-Me, bears two inner sphere water molecules, whereas Gd-N1 coordinates three. The intermediate Gd-N2 displays  $q = 2.4(\pm 0.1)$ , indicating that at room temperature the eight and nine coordination states are present in almost equal proportions.<sup>7</sup>

Further evidence of the 8.5 coordination number of Gd-N2 (at 298 K) was obtained by temperature dependence of the paramagnetic contribution to the water <sup>17</sup>O NMR transverse relaxation rate (Supporting Information). Unlike Gd-N3, Gd-N2 does not display the exponential decrease of  $r_{2p}$  with temperature, typical of

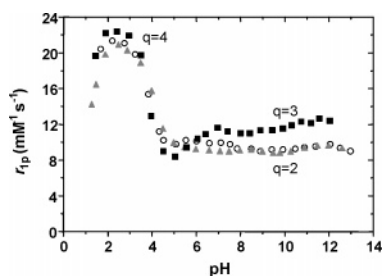
<sup>†</sup> University of California, Berkeley.

<sup>‡</sup> Università del Piemonte Orientale.

<sup>§</sup> Università di Torino.



**Figure 3.** Proposed hydrogen bonding network of Gd-N1 and Gd-N3.

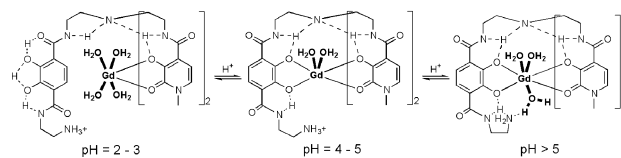


**Figure 4.** The pH dependence of the longitudinal relaxivity ( $r_{1p}$ ) of Gd-N1 (black squares), Gd-N2 (open circles), and Gd-N3 (gray triangles).

complexes with fast water exchange.<sup>2</sup> The “S” shaped profile observed, characteristic of two overlapping decays, indicates a change in coordination number with temperature. The nine-coordinate complex is predominant at high temperature, and the eight-coordinate one at low temperature.

Since no change in electronic parameters is observed, we do not believe that the difference in  $q$  is due to a change in the ligands coordinating the Gd<sup>III</sup>. The increase in coordination number from Gd-N3 to Gd-N1 may, however, be rationalized by the hydrogen bonding network created by the terminal moiety (Figure 3). The ethylene bridge to the terminal primary amine of Gd-N1 positions the nitrogen such that it may intramolecularly hydrogen bond to the proton of the amide, which is also bonded to the catechol oxygen. In our proposed model, the terminal amine hydrogen bonds a solvent water molecule, thereby bringing it close to the open coordination site of the Gd<sup>III</sup> and facilitating its coordination. In Gd-N3, however, the tertiary nitrogen is probably intramolecularly hydrogen bonded to the two primary amines. This is favored over an intermolecular bond to a water molecule, such that the complex remains eight-coordinate. Such hydrogen bonding networks have already been observed in several crystal structures of lanthanide poly(amino carboxylates) and HOPO-based complexes.<sup>4,8–10</sup>

The hydrogen bonding network and, hence, the relaxivity of the complexes are pH dependent (Figure 4) and in agreement with previous solution thermodynamic studies.<sup>11</sup> Between pH 13 and 4,  $r_{1p}$  of Gd-N2 and Gd-N3 remains constant and corresponds to  $q = 2$  complexes. At pH 3, the protonated TAM podand dissociates the Gd<sup>III</sup> and is replaced by two water molecules, resulting in a 2-fold increase in  $r_{1p}$ . The relaxivity then decreases toward pH 1 as the complex completely dissociates. The relaxivity of Gd-N1 displays different pH dependence (Figure 5). It remains constant between pH 13 and 7 corresponding to the nine-coordinate,  $q = 3$ , complex. It decreases by about a third, to presumably a  $q = 2$  complex at pH 5, corresponding to the  $pK_a$  of the terminal amine, and increases again at pH 3 to the partially dissociated  $q = 4$  form. As previously observed with the tertiary nitrogen of the TREN backbone, favorable hydrogen bonding stabilizes the deprotonated form of the pendant amine and consequently significantly decreases its  $pK_a$  to 5.<sup>8,11</sup> Our interpretation is that, at this pH, the terminal



**Figure 5.** Proposed model for the pH dependence of the hydrogen bonding network of Gd-N2.

ammonium can no longer hydrogen bond to, and stabilize, the coordination of a third water molecule. The resulting complex is only eight-coordinate, with lower relaxivity.

The stabilities of the three Gd<sup>III</sup> complexes were measured by spectrophotometric titration against DTPA. All complexes are highly stable, with  $pM$  values<sup>11</sup> comparable to those of the commercial agents Gd-DTPA (19.2) and Gd-DOTA (19.4).<sup>2</sup> In particular, the stability of Gd-N1 ( $pM = 19.5$ ) is similar to that of the parent Gd-Me (20.1).<sup>6</sup> This indicates that coordination of a third water molecule does not destabilize the Gd<sup>III</sup> complex.

In summary, this report describes the increase of the hydration number of HOPO-based Gd<sup>III</sup> complexes by substituting the TAM podand with a moiety capable of hydrogen bonding a third water molecule close to the metal center. The resulting nine-coordinate Gd<sup>III</sup> complex is the first displaying three inner sphere water molecules and high stability. The ensuing high relaxivity of this small complex renders it a promising candidate for the development of second generation contrast agents.

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**Supporting Information Available:** Detailed experimental procedures and characterization data for the synthesis of Gd-N1, Gd-N2, and Gd-N3; spectrophotometric titration data, pH dependence of the hydrogen bonding network of Gd-N3, temperature dependence of the paramagnetic contribution to the water <sup>17</sup>O NMR transverse relaxation rate ( $r_{2p}$ ) for Gd-N2, Gd-N1, and Gd-N3. This material is available free of charge via the Internet at <http://pubs.acs.org>.

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