## Communications to the Editor

## Gadolinium Complex of Tris[(3-hydroxy-1-methyl-2-oxo-1,2-didehydropyridine-4-carboxamido)ethyl]amine: A New Class of Gadolinium Magnetic **Resonance Relaxation Agents**

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Magnetic resonance imaging (MRI) has revolutionized diagnostic internal medicine in the past 20 years.<sup>1</sup> Complexes of gadolinium(III) have proven to be especially effective MRI contrast agents;  $Gd^{3+}$  ( $S = \frac{7}{2}$ ) is unique in having the highest isotropic magnetic moment. The injection of gram quantities of potentially toxic metal cations requires that the complexes be stable to in vivo transmetalation, particularly with the competing calcium(II) ion in high concentration,<sup>2</sup> while relaxivity is dependent on both the number and the rate of exchange of water molecules from the complex inner sphere. This competition between strong complexation and free water coordination sites is a challenge to MRI enhancement agent design.

We report here the synthesis and characterization of Gd(III) TREN-Me-3.2-HOPO (7) {TREN-Me-3.2-HOPO = tris[(3hydroxy-1-methyl-2-oxo-1,2-didehydropyridine-4-carboxamido)ethyl]amine}, which represents a promising new class of lanthanide compounds that meet these criteria. The hydroxypyridinonate (HOPO) monoanions are bidentate ligands that form effective multidentate sequestering agents, particularly when the HOPO ring carbon adjacent to the carbonyl or hydroxy group is functionalized and attached through an amide linkage to a suitable backbone.<sup>3</sup> The geometry thus imposed promotes strong chelation of metal ions in general, and of Gd(III) in particular.

As shown in Scheme 1, 1-methyl-3-hydroxy-2(1H)-pyridinone (1) was carboxylated under Kolbe-Schmidt conditions.<sup>4</sup> The resulting 4-carboxy-1-methyl-3-hydroxy-2(1H)-pyridinone (2) was selectively protected to produce 4-carboxy-1-methyl-3-benzyloxy-2(1H)-pyridinone (3), which was converted to the activated species 4 and coupled with the backbone tris(2aminoethyl)amine (TREN) to yield the protected ligand 5. Catalytic hydrogenation of the protected ligand gave the TREN-Me-3,2-HOPO ligand (6) in overall 41.8% yield.<sup>5</sup> The Gd(III) TREN-Me-3,2-HOPO complex (7) is obtained as white material

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Chapter 14, pp 372-437.
(2) Cacheris, W. P.; Quay, S. C.; Rocklage, S. M. Magn. Reson. Imaging Scheme 1



by treatment of GdCl<sub>3</sub> or Gd(NO<sub>3</sub>)<sub>3</sub>·5H<sub>2</sub>O with 1 equiv of TREN-Me-3.2-HOPO in methanol in the presence of a weak base.6

X-ray quality crystals of the Gd(III) complex were obtained from a solution of wet DMF diffused with ether; an ORTEP diagram of this complex is shown in Figure 1, and the coordination polyhedron is shown in Figure 2.7 The crystal structure of 7 shows that the hexadentate ligand chelates the metal ion through the hydroxypyridinone oxygens; two water molecules complete the coordination sphere.<sup>8-11</sup>

This complex is eight-coordinate, as is the aqueous ion [Gd- $(H_2O)_{8^{3^+}}$ ,<sup>12</sup> rather than nine-coordinate like the polyaminocarboxylate complexes.<sup>13-17</sup> The difference in energy between the eight- and nine-coordinate species is small: the early lanthanide aqua complexes are nine-coordinate, while the smaller mid-tolate lanthanides are eight-coordinate.<sup>12</sup> The same small difference in energy for eight- versus nine-coordinate multidentate ligand complexes for 7 is expected. This assumption is supported by the obvious hole in the coordination sphere of the structure; the coordination geometry about the metal ion can most accurately be described as a slightly distorted bicapped trigonal prism.

By attaching the 3,2-HOPO moieties through an ortho amide linkage, the ligand obtains not only the correct geometry for

(6) Gd(III) TREN-Me-3,2-HOPO (7) complex: white solid material; MS (+FAB) m/z 753.3 (M + H)<sup>+</sup>. Anal. Calcd (found) for GdC<sub>27</sub>H<sub>30</sub>. N<sub>7</sub>O<sub>9</sub>1.4H<sub>2</sub>O (779.05): C, 41.62 (41.70); H, 4.24 (4.26); N, 12.58 (12.28). (7) Colorless single crystals suitable for X-ray structure analysis were (7) Colorless single crystals surface for X-ray structure already so were obtained from a DMF solution of the complex by vapor diffusion of ether. (7)-C<sub>3</sub>H<sub>7</sub>NO:  $M_r = 862.96$ ; triclinic space group P1 (No. 2); a = 10.791-(3) Å, b = 12.901(4) Å, c = 13.566(4) Å,  $\alpha = 85.42(2)^{\circ}$ ,  $\beta = 67.38(2)^{\circ}$ ,  $\gamma = 74.58(2)^{\circ}$ , V = 1680(1) Å<sup>3</sup>; Z = 2,  $g_{calc} = 1.71$  g cm<sup>-3</sup>. An empirical absorption correction was applied on the basis of azimuthal scans; min-(unique) = 84.9%, min(av) = 86.0%. The data  $(+h,\pm k,\pm l)$  were collected at low temperature (-117 °C) on an Enraf-Nonius CAD-4 diffractometer from  $3^{\circ} \le 2\theta \le 45^{\circ}$  using Mo K $\alpha$  radiation. Intensity standards measured every 1 h of exposure time indicated no significant decomposition during data collection. The same three reflections were checked against their predicted positions every 200 reflections, and crystal orientation was redetermined if any of the three deviated by more than 0.1°. The crystal was reoriented twice during the 23.5 h of exposure. The structure was solved by Patterson methods, and the refinement was performed against F). Ligand hydrogen positions were predicted and included in the structure factor calculation, but not refined. The final residuals for 460 variables refined against 3576 data were R = 3.58% and  $R_w = 3.91\%$ , with GOF = 1.385

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<sup>(5)</sup> TREN-Me-3,2-HOPO (6): yellow crystalline solid, mp 130−132 °C dec; <sup>1</sup>H NMR (250 MHz, DMSO-*d*<sub>6</sub>) δ 2.29 (6H, t, *J* = 5.9 Hz), 3.07 (6H, a, J = 5.8 Hz), 3.45 (9H, s), 6.45 (3H, d, J = 7.2 Hz), 7.12 (3H, d, J = 7.2 Hz), 8.46 (3H, t); <sup>13</sup>C NMR (400 MHz, DMSO- $d_6$ )  $\delta$  36.80, 37.42, 52.68, 102.6, 116.9, 127.4, 147.5, 158.0, 165.3; MS (+FAB) m/z 600.3 (M + H)<sup>+</sup>, 62.2 (M + Na)<sup>+</sup>. Anal. Calcd (found) for  $C_{27}H_{33}N_7O_9^{-1}.5H_2O$ (626.634): C, 51.75 (51.84); H, 5.79 (5.54); N, 15.64 (15.59).

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**Figure 1.** Structure of Gd(III) TREN-Me-3,2-HOPO-2H<sub>2</sub>O (ORTEP). Selected bond lengths (Å): Gd-O1, 2.346(4); Gd-O2, 2.430(5); Gd-O4, 2.390(4); Gd-O5, 2.386(5); Gd-O7, 2.338(4); Gd-O8, 2.409-(5); Gd-O10, 2.446(5); Gd-O11, 2.436(4); N2-O1, 2.652(7); N3-O4, 2.654(7); N4-O7, 2.729(7).



**Figure 2.** Coordination polyhedron of Gd(III) TREN-Me-3.2-HOPO- $2H_2O$ . The polyhedron is close to a tricapped trigonal prism whose three-fold axis would run from left to right. The missing ligand site of the  $D_{3h}$  tricapped trigonal prism is directly toward the viewer.

complexation but also some degree of preorganization and stability due to internal hydrogen bonds.<sup>18,19</sup> The distances between amide nitrogens and the bound hydroxy groups are between 2.65 and 2.73 Å. The stability of an MRI agent is critical, since the toxicity of an MRI agent has been shown to be directly related to the concentration of free Gd(III) ion *in vivo*.<sup>2</sup> The most acidic protonation constant (log  $K_{a4} = 4.96$ ) is assigned to the tertiary amine of the TREN backbone on the basis of the similarity to other TREN derivatives.<sup>20</sup> The protonation constants of the 3,2-HOPO moieties are centered around 7. The formation constants of **6** with Ca(II), Zn(II), and Gd(III), and comparison formation constants for three currently approved MRI agents, DTPA, the bis-methylamide derivative of DTPA (DTPA-BMA),<sup>2</sup> and DOTA, are sum-

**Table 1.** Protonation Constants and Metal Chelate StabilityConstants $^a$ 

	ligand			
	DTPA-BMA <sup>b</sup>	DTPA <sup>c</sup>	DOTA <sup>f.g</sup>	6
$\log K_{ai}$	9.37	10.45	11.14 [11.14]	8.20(1)
$\log K_{a2}$	4.38	8.53	9.69 [9.50]	6.95(3)
$\log K_{a3}$	3.31	4.28	4.84 [4.61]	5.80(3)
$\log K_{a4}$	1.43	2.65	3.95 [4.30]	4.96(5)
$\log K_{a5}$		1.82		
$\log \beta_{110} (\text{GdL})^d$	16.85	22.46	24.0 [25.3]	20.3(2)
$\log \beta_{111}$ (GdHL)		24.85	26.8	23.8(1)
$\log \beta_{110}(CaL)$	7.17	10.75	16.37	7.6(1)
$\log \beta_{111}$ (CaHL)	11.62	16.86	19.97	13.7(1)
$\log \beta_{112}(\text{CaH}_2\text{L})$				18.9(1)
$\log \beta_{110}(ZnL)$	12.04	18.29	18.7	13.1
$\log \beta_{111}(\text{ZnHL})$	16.08	23.89	24.03	18.08
$\log \beta_{112}(\text{ZnH}_2\text{L})$		26.95	27.99	22.55
pGd <sup>e</sup>	15.47	19.09	18.92 [20.42]	20.3
-				

<sup>*a*</sup> The constants in the right-most column were determined in the this work. <sup>*b*</sup> Reference 1. <sup>*c*</sup> Reference 24. <sup>*d*</sup> The cumulative formation constant  $\beta_{mlh}$  is for the complexation reaction  $mM + lL + hH \leftrightarrow M_mL_lH_h$ . <sup>*c*</sup>  $p[Gd] = -log [Gd]_{free}$  at pH 7.4,  $[Gd]_{total} = 1 \ \mu M$ , and [ligand]<sub>total</sub> = 10  $\mu$ M. <sup>*f*</sup> References 25, 26. <sup>*s*</sup> The values in brackets are from ref 27.

marized in Table  $1.^{21-23}$  A comparison shows that **6** forms a Gd(III) complex that is much more stable than Gd(III) DTPA-BMA toward transmetalation with Zn(II) and Ca(II), respectively. Thus, the HOPO complex is more ion-discriminating than DTPA and its neutral amide derivatives and therefore is less prone to undergo transmetalation with these physiological ions.

A critical property of a contrast agent's potential is its ability to relax water protons. The relaxivity of 7 ( $R_1$ ) at 37 °C and 20 MHz is 10.5 mM<sup>-1</sup> s<sup>-1</sup>, some 2.5 times that of Gd DTPA. This is undoubtedly due in part to the higher number of inner sphere waters in 7 (two versus one for Gd DTPA) and the larger molecular size, which leads to a longer rotational correlation time. Since there is little difference in the energies of the eightversus nine-coordinate complexes, we expect a readily accessible intermediate to water exchange in 7 through an associative mechanism, which should yield more rapid exchange rates.

The hexadentate chelator **6** has recently been shown to be an effective americium(III) sequestering agent in mice,<sup>3</sup> removing 89% of total body  $^{241}$ Am in 24 h. The ligand has low acute toxicity, with no evident tissue damage even at substantial doses.<sup>3</sup> The nearly identical coordination properties of americium and gadolinium make these observations consistent with the promise shown for the development of nontoxic 3,2-HOPObased MRI contrast agents.

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