A Tris-hydroxymethyl-Substituted Derivative of Gd-TREN-Me-3,2-HOPO: An MRI Relaxation Agent with Improved Efficiency

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Gadolinium is used as a relaxation agent in magnetic resonance imaging (MRI) because of its large paramagnetic moment, high water exchange rate, and consequent high proton relaxivity. However, the ion must be complexed to avoid toxicity. The Gd(III) complex of TREN-Me-3,2-HOPO represents the net increase in the water proton nuclear magnetic relaxation rate per millimolar concentration of the paramagnetic gadolinium complex with favorable molecular reorientation rate (1/τM) and/or a shorter Gd–Owater distance. Support for this hypothesis can be gained by the analysis of the magnetic field dependence of τp most likely arises from the low solubility of the complex (less than 0.1 mM at pH 7).

To selectively modify the properties of TREN-linked podand ligands and their metal complexes, we have explored methodologies for synthesis of functionalized TREN derivatives. The gadolinium complex of homochiral tris(2-hydroxymethyl)-TREN-Me-3,2-HOPO, was chosen as the initial target and its synthesis is outlined in Scheme 1. As desired, the increased water solubility of the complex (less than 0.1 mM at pH 7) has allowed for a complete characterisation of the relaxivity behavior of these new Gd(III) complexes as a function of temperature and magnetic field strength using 1H and 17O NMR methods (vide infra).

The efficacy of paramagnetic complexes as contrast enhancement agents is given by their relaxivity, rGd (mM⁻¹ s⁻¹), which represents the net increase in the water proton magnetic relaxation rate per millimolar concentration of the paramagnetic compound.⁴ At 20 MHz and 25 °C, rGd of 2 is 90 mM⁻¹ s⁻¹, a value remarkably higher than those of the currently used monoaquo contrast agents, Gd(DTPA) (4.7 mM⁻¹ s⁻¹), Gd(DTPA-BMA) (4.4 mM⁻¹ s⁻¹). The presence of two water molecules in the Gd(III) inner coordination sphere for 2 does not fully account for this increment. Indeed, it is well-known that for small polycarboxylate-derived Gd(III) chelates, only about 2.5 to 3.0 mM⁻¹ s⁻¹ of rGd is due to the contribution of each of the metal-bound water molecules (rOwat); the inner-sphere contribution; additionally, approximately 2.0 to 2.5 mM⁻¹ s⁻¹ is attributable to the outer-sphere water molecules diffusing next to the complex (rOa, the outer-sphere contribution). For example, the nine-coordinate Gd(III) complex of the heptadentate macrocyclic ligand DO3A also features two inner-sphere waters (q) and has a τp value of 6.1 mM⁻¹ s⁻¹. In the case of 2, the approximately 3.0 mM⁻¹ s⁻¹ enhancement observed for τp most likely arises from variations in two other key parameters for relaxivity: a slower molecular reorientation rate (1/τM) and/or a shorter Gd–Owater distance. Support for this hypothesis can be gained by the analysis of the magnetic field dependence of τp most likely arises from the low solubility of the complex (less than 0.1 mM at pH 7).

(7) DTPA = diethyltriaminepentaacetic acid, DOTA = 1,4,7,10-tetraazacyclododecane-N,N,N′,N′,N″,N″-tetracetic acid, HP-DO3A = 2-hydroxypropyl-1,4,7,10-tetraazacyclododecane-N,N,N′,N″-tetracetic acid, DTPA-BMA = diethyltriamine-N,N,N′,N″-tetracetic acid-N,N′-bis(hydroxymethyl), DO3A = 1,4,7,10-tetraazacyclododecane-N,N′-tetracetic acid.

other dendrimeric and macromolecular derivatives, this prevents cleanly follows a monoexponential behavior in the range 0°–70°. This is particularly important since fast water exchange is a crucial requisite for an optimized contrast agent and determines C. The lower curves represent the outer-sphere contributions to the profiles calculated from the parameters given in the text and in Table 1.

Table 1. Fitting Parameters for NMRD Studies of 2

<table>
<thead>
<tr>
<th>parameter</th>
<th>15 °C</th>
<th>25 °C</th>
<th>39 °C</th>
</tr>
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<tbody>
<tr>
<td>τSD/ps</td>
<td>47</td>
<td>50</td>
<td>39</td>
</tr>
<tr>
<td>τW/ps</td>
<td>22</td>
<td>20</td>
<td>17</td>
</tr>
<tr>
<td>τM/ps</td>
<td>17°</td>
<td>16°</td>
<td>15°</td>
</tr>
<tr>
<td>τW/ps</td>
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<td>129</td>
<td>95</td>
</tr>
<tr>
<td>r/A</td>
<td>3.08</td>
<td>3.08</td>
<td>3.08</td>
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</table>

*From 17O NMR data.

The experimental NMRD data taken at 15, 25 (Figure 1), and 39 °C have been fitted to the SBM and Freed equations by setting g = 2, adopting standard values for d (3.8 Å) and D (1.65, 2.24 and 3.15 × 10⁻⁵ cm² s⁻¹) at 15, 25 and 39 °C, respectively, and using τSD, τW, τM, and r as adjustable parameters (see Table 1). We have assumed a fast exchange condition for 2 over the entire range of magnetic fields and temperature investigated, i.e., the water residence lifetime in the coordination site is much shorter than its longitudinal relaxation time. This assumption is validated by the observation that, in contrast to Gd(DOTA)₁⁰ and Gd(DTPA), the temperature dependence of τp of 2 at 20 MHz cleanly follows a monoexponential behavior in the range 0°–70°. This is particularly important since fast water exchange is a crucial requisite for an optimized contrast agent and determines to a large extent the enhancement of τp attainable when a Gd chelate is linked to a slowly tumbling macromolecular substrate. In fact, strong leveling effects on τp due to low water exchange rates have already been observed in several cases of noncovalent adducts of Gd-chelates with human serum albumin (HSA), and other dendrimeric and macromolecular derivatives, this prevents these systems from attaining their expected relaxation enhancements and severely limits their potential utility.

We have also performed a variable-temperature 17O NMR study at 9.4 T of the water exchange kinetics of an aqueous solution of 2 at pH 7 following established procedures. The data are reported in Figure 2, along with the curve obtained using the parameters from a fitting procedure of the data to the pertinent equations. In the analysis we used the τW and τM values obtained by NMRD fitting and adopted the standard value of –3.6 × 10⁶ rad·s⁻¹ for the hyperfine coupling constant (A/H). In confirmation of the water exchange considerations discussed above, a value of (7.0 ± 0.6) × 10⁶ s⁻¹ is obtained for kₑ (1/τₑ) at 25 °C, which is comfortably in the range of exchange rate values required for attaining maximum relaxivities. Using the parameters obtained in this study, we can predict the relaxivity that these new agents may obtain when tethered to slowly tumbling macromolecular compounds. For example, at 25 °C using a τW value of 15 ns, the predicted relaxivity for 2 is 30 mM⁻¹ s⁻¹ at 20 MHz, and 90 mM⁻¹ s⁻¹ at 60 MHz.

In summary, this report describes a new water-soluble derivative of a new class of contrast agents endowed with high thermodynamic stability and enhanced relaxivity arising from the presence of two inner-sphere water molecules and a long reorientational correlation time. The eight-coordinate ground state of 2 displays rapid associative exchange of the bound waters which is characterized by a near optimal exchange rate constant. The concomitant occurrence of these highly favorable relaxation characteristics demonstrates that complexes such as 1 and 2 are prototypes for the development of new contrast agents that promise to achieve the high relaxivity values required by new and emerging applications of MRI, such as cellular receptor targeted imaging and real-time angiography.

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Supporting Information Available: Synthentic references and characterization for the new compounds (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

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