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

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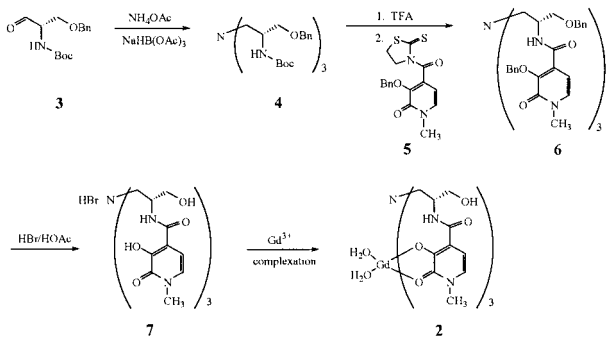
Gadolinium is used as a relaxation agent in magnetic resonance imaging (MRI) because of its large paramagnetic moment, high water exchange rate, and consequent high proton relaxivity. However, the ion must be complexed to avoid toxicity. The Gd(III) complex of TREN-Me-3,2-HOPO (**1**) {TREN-Me-3,2-HOPO = tris[(3-hydroxy-1-methyl-2-oxo-1,2-didehydropyridine-4-carboxamido)ethyl]amine} has been proposed as the basis for a promising new class of relaxation agents for magnetic resonance imaging (MRI) applications.[1] The tris-bidentate chelation of the ligand is unique among imaging agents and offers several advantageous features: (1) high stability; (2) low expected toxicity; and (3) high relaxivity, based on an initial measurement at 37 °C.[1] In particular, the high relaxivity of **1** has been attributed to the high number of water molecules (two) in the inner sphere of the eight-coordinate ground state of the complex with favorable (rapid) water exchange kinetics expected via an associative exchange mechanism through an easily accessible nine-coordinate transition state. Unfortunately, attempts to more clearly delineate the origins for this high relaxivity have been frustrated by 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.[2] The gadolinium complex of homochiral tris(2-hydroxymethyl)-TREN-Me-3,2-HOPO, **2**, was chosen as the initial target and its synthesis is outlined in Scheme 1.[3] As desired, the increased water solubility of **2** (ca. 15 mM at 25 °C, pH 7) has allowed for a complete characterization of the relaxivity behavior of these new types of gadolinium compounds as a function of temperature and magnetic field strength using ^{1}H and ^{17}O NMR methods (vide infra).

The efficacy of paramagnetic complexes as contrast enhancement agents is given by their relaxivity, r_{1p} (mM^{-1} s^{-1}), which represents the net increase in the water proton nuclear magnetic relaxation rate per millimolar concentration of the paramagnetic compound.[4−6] At 20 MHz and 25 °C, r_{1p} of **2** is 9.0 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(DOTA) (4.7 mM^{-1} s^{-1}), Gd(HP-DO3A) (4.2 mM^{-1} s^{-1}), and Gd(DTPA-BMA) (4.4 mM^{-1} s^{-1}).[7,8] 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 poly-aminocarboxylate-derived Gd^{III} chelates, only about 2.5 to 3.0 mM^{-1} $s^{-1}$of r_{1p} is due to the contribution of each of the metal-bound water molecules (r_{1is}, 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 (r_{1os}, the outer-sphere contribution).[9] For example, the nine-coordinate Gd^{III} complex of the heptadentate macrocyclic ligand DO3A also features two inner-sphere waters (q) and has a r_{1p} value of 6.1 mM^{-1} s^{-1}.[10] In the case of **2**, the approximately 3.0 mM^{-1} s^{-1} enhancement observed for r_{1p} most likely arises from variations in two other key parameters for relaxivity: a slower molecular reorientation rate ($1/\tau_R$) and/or a shorter $Gd-O_{water}$ distance. Support for this hypothesis can be gained by the analysis of the magnetic field dependence of r_{1p} over a range of proton Larmor frequencies from 0.1 to 50 MHz, the so-called nuclear magnetic relaxation dispersion (NMRD) profile.[11] Figure 1 compares the experimental NMRD profile of **2** (CN = 8, q = 2) with those of Gd(DTPA)[12] (CN = 9, q = 1) and Gd(DO3A)[8] (CN = 9, q = 2). The experimental data were analyzed in terms of the Solomon−Bloembergen−Morgan (SBM)[13,14] and Freed[15] equations for inner-sphere and outer-sphere contributions, respectively (with best fitting curves as indicated in Figure 1). The inner-sphere term depends on the number of metal-bound waters (q), their mean residence lifetime (τ_M), the water proton−gadolinium distance (r), τ_R, the electronic relaxation time at zero-field (τ_{S0}), and the correlation time for the modulation of τ_{S0} (τ_V). The outer-sphere contribution depends on τ_{S0}, τ_V, the distance of closest approach between the paramagnetic center and the

Scheme 1



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(7) DTPA = diethylenetriaminepentaacetic acid, DOTA = 1,4,7,10-tetraazacyclododecane-*N*,*N*′,*N*″,*N*‴-tetraacetic acid, HP-DO3A = 2-hydroxypropyl-1,4,7,10-tetraazacyclododecane-*N*,*N*′,*N*″-triaacetic acid, DTPA-BMA = diethylenetriamine-*N*,*N*′,*N*″-triacetic acid-*N*,*N*″-bismethylamide, DO3A = 1,4,7,10-tetraazacyclododecane-*N*,*N*′,*N*″-triaacetic acid.

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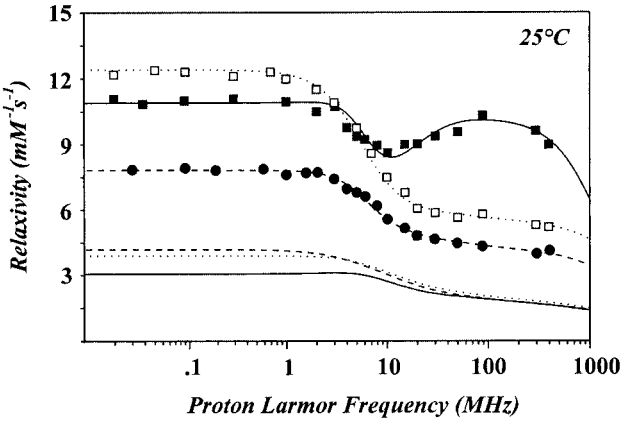

Figure 1. Comparison of the $1/T_1$ NMRD profiles of **2** (■), Gd(DTPA) (●), and Gd(DO3A) (□) at 25 °C. The lower curves represent the outer-sphere contributions to the profiles calculated from the parameters given in the text and in Table 1.

Table 1. Fitting Parameters for NMRD Studies of **2**

parameter	15 °C	25 °C	39 °C
τ_{SO}/ps	47	50	39
τ_V/ps	22	20	17
τ_M/ns	17[a]	16[a]	15[a]
τ_R/ps	171	129	95
r/Å	3.08	3.08	3.08

[a] From ^{17}O NMR data.

diffusing water protons (d), and the relative diffusion constant (D). From Figure 1 we note that the inflection point for the profile of **2** is shifted to lower field when compared with the other two complexes; this reflects a longer value of τ_R, as a consequence of the higher molecular weight of **2**. The relaxivity of **2** is significantly higher than that of Gd(DTPA) over the entire range of magnetic field strengths and also higher than that of Gd(DO3A) for Larmor frequencies above 5 MHz. In particular, at the frequencies at which most MRI scanners operate (10−60 MHz), r_{1p} of **2** increases and the NMRD profile assumes a bell shape centered on 100 MHz. This is a further consequence of the increased value of τ_R, which, in turn, renders visible the field-dependence of the electronic relaxation time. At low fields the profile of **2** is lower than that of Gd(DO3A) reflecting a substantially lower value of τ_{S0}.

The experimental NMRD data taken at 15, 25 (Figure 1), and 39 °C have been fitted to the SBM and Freed equations by setting $q = 2$, adopting standard values for d (3.8 A) and D (1.65, 2.24 and 3.15×10^{-5} cm^2 s^{-1} at 15, 25 and 39 °C, respectively), and using τ_{S0}, τ_V, τ_R, 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),[16] the temperature dependence of r_{1p} of **2** at 20 MHz cleanly follows a monoexponential behavior in the range 0−70 °C. 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 r_{1p} attainable when a Gd chelate is linked to a slowly tumbling macromolecular substrate. In fact, strong leveling effects on r_{1p} due to low water exchange rates have already been observed in several cases of noncovalent adducts of Gd-chelates with human serum albumin (HSA),[1,17] and other dendrimeric and macromolecular derivatives,[18] this prevents

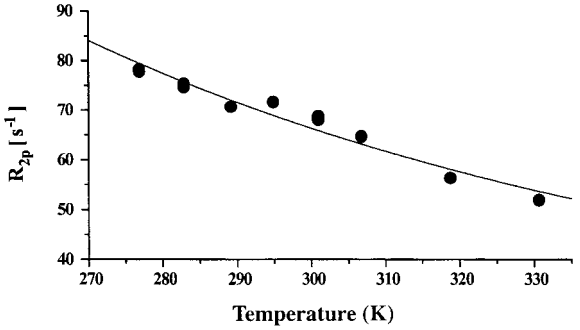

Figure 2. Temperature dependence of the paramagnetic contribution to the water ^{17}O NMR transverse relaxation rate (R_{2p}) for **2** (0.02 M) at 9.4 T. The fitting parameters are the following: Δ^2 ($s^{-2} \times 10^{19}$) = 12.4, τ_V^{298} (ps) = 20, ΔH_V (kJ mol^{-1}) = 6.0, ΔH_M (kJ mol^{-1}) = 2.6, ΔH_R (kJ mol^{-1}) = 20.

these systems from attaining their expected relaxation enhancements and severely limits their potential utility.

We have also performed a variable-temperature ^{17}O NMR study at 9.4 T of the water exchange kinetics of an aqueous solution of **2** at pH 7 following established procedures.[1,10,19] 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 τ_R and τ_V values obtained by NMRD fitting and adopted the standard value of -3.6×10^6 rad·s^{-1} for the hyperfine coupling constant ($A/\hbar$). In confirmation of the water exchange considerations discussed above, a value of $(7.0 \pm 0.6) \times 10^7$ s^{-1} is obtained for k_{ex} ($1/\tau_M$) 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 τ_R 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[20] 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[21] and real-time angiography.[22]

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Supporting Information Available: Synthetic 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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