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Maximizing T_2 -exchange in Dy^{3+} -DOTA-(amide) $_x$ chelates: Fine-tuning the water molecule exchange rate for enhanced T_2 contrast in MRI

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Abstract

Purpose—The water molecule exchange rates in a series of DyDOTA-(amide) $_x$ chelates were fine-tuned to maximize the effects of T_2 -exchange line broadening and improve T_2 contrast.

Methods—Four DyDOTA-(amide) $_x$ chelates having a variable number of glycinate side-arms were prepared and characterized as T_2 -exchange agents. The non-exchanging DyTETA chelate was also used to measure the bulk water T_2 reduction due solely to T_2^* . The total transverse relaxivity (r_{2tot}) at 22, 37, and 52 °C for each chelate was measured *in vitro* at 9.4 T (400 MHz) by fitting plots of total T_2^{-1} versus concentration. The water molecule exchange rates for each complex were measured by fitting ^{17}O line-width versus temperature data taken at 9.4 T (54.3 MHz).

Results—The measured transverse relaxivities due to water molecule exchange (r_{2ex}) and bound water lifetimes (τ_M) were in excellent agreement with Swift-Connick theory, with DyDOTA-(gly) $_3$ giving the largest $r_{2ex} = 11.8 \text{ s}^{-1} \text{ mM}^{-1}$ at 37 °C.

Conclusion—By fine-tuning the water molecule exchange rate at 37 °C, the transverse relaxivity has been increased by 2 to 30 times compared to previously studied Dy^{3+} -based chelates. Polymerization or dendrimerization of the optimal chelate could yield a highly sensitive, molecule-sized T_2 contrast agent for improved molecular imaging applications.

Keywords

MRI; T_2 contrast; T_2 -exchange; water molecule exchange; *in vitro*; Dysprosium(III)

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INTRODUCTION

Contrast in MR imaging

Magnetic resonance imaging (MRI) can be used to create a three-dimensional image representing the water proton (^1H) density within a subject (1,2). Since the human body is approximately 70% water, most tissues have a sufficiently large water proton signal to allow for high-resolution anatomical imaging. The contrast between different soft tissue types, which results in some tissues appearing brighter or darker than others, depends upon both the endogenous proton density and the T_1 and T_2 relaxation times (3). MRI tissue contrast can be further enhanced by introducing an exogenous contrast agent (4). These agents shorten the endogenous T_1 and T_2 relaxation times of tissue water to enhance the contrast and highlight specific anatomic features or dynamic processes.

The most widely used MRI contrast agents consist of various chelated forms of Gd^{3+} where the central ion is surrounded by a multidentate ligand that typically occupies eight of nine possible coordination positions (5,6). Gd^{3+} is most effective at relaxing water protons because this ion has seven unpaired electrons distributed isotropically in the 4f orbitals ($^8\text{S}_{7/2}$) and an electron relaxation rate that is about 10^6 times slower than any other lanthanide ion (7). This reduced electron relaxation rate is more in tune with the typical Larmor frequencies of ^1H protons in MRI ($\omega = \gamma B_0$) and therefore enhances the mechanism of electron to proton dipole-dipole relaxation (8). The relaxation efficiency of Gd^{3+} also depends upon the rapid chemical exchange of water molecules between a single inner-sphere coordination position of Gd^{3+} ion and bulk water. Although Gd^{3+} shortens both the T_1 and T_2 of water protons, the T_1 effects are more pronounced because the T_1 of tissue water protons are approximately an order of magnitude longer than T_2 . Hence, for most common imaging sequences, a typical clinical dose of Gd^{3+} agent (e.g., 0.1 mmol/kg) will cause tissue regions of uptake to appear brighter than the surrounding tissue. Nonetheless, the effects of T_2 shortening (image darkening) can be observed in tissues (such as the kidneys) where Gd^{3+} accumulates in high concentrations.

Other lanthanide ions such as Tb^{3+} , Dy^{3+} , and Ho^{3+} have fewer unpaired electrons distributed anisotropically in the 4f orbitals and relatively rapid electron relaxation rates compared to Gd^{3+} . These faster rates, combined with larger magnetic moments (7), make these ions more effective as T_2^* agents. This susceptibility-based T_2 relaxation mechanism is due to the magnetic moments of the Ln^{3+} ions causing local B_0 inhomogeneities in their immediate vicinity ($\approx 15 \text{ \AA}$ diameter) (7). Water molecule protons either directly bound to the Ln^{3+} ion (i.e., inner-sphere relaxation) or within the vicinity of (i.e., outer-sphere relaxation) the Ln^{3+} ion experience an altered B_0 field and precess at a different Larmor frequency ($\omega = \gamma(B_0 \pm B)$). These different Larmor frequencies result in increased spin dephasing in the plane transverse to B_0 and thus shorter effective T_2 times. In this way, certain paramagnetic chelates can act as susceptibility or T_2^* agents in a manner similar to superparamagnetic iron oxide nanoparticles (9). Furthermore, unlike Gd^{3+} , the remaining paramagnetic lanthanides ions can induce large hyperfine shifts in all chelate protons including those in exchange with water protons (i.e., $-\text{NH}$ and $-\text{OH}$ protons and any inner-sphere water molecules) (10-12). This hyperfine shift (ω) has led to the development of

two relatively new MRI contrast mechanisms: chemical exchange saturation transfer (CEST) (13) and T_2 -exchange (T_{2ex}). While the former has been recently reviewed in depth elsewhere (14,15), the latter is discussed in more detail in the following section.

T_2 -exchange theory and background

It was recently shown that the same intermediate water molecule exchange rates that facilitate paramagnetic chemical exchange saturation transfer (paraCEST) (16) between the inner-sphere of EuDOTA-(amide)₄ chelates and bulk water can also significantly reduce the bulk water T_2 through a T_{2ex} mechanism (17). The T_{2ex} contribution of these Eu³⁺-based agents can be quite substantial even though the Eu³⁺ ion is only weakly paramagnetic (18). T_{2ex} is caused by mobile proton exchange between the chelate and the bulk water. This proton exchange can occur through exchangeable -NH and -OH protons on the ligand and through water molecule exchange with the inner-sphere of the Ln³⁺ ion. The T_{2ex} mechanism, which is independent of the CEST saturation pulse, is a function of the agent concentration (mM), the number of proton or water molecule exchange sites (q), the bound proton chemical shift (ω), and most importantly the proton or water molecule exchange rates (k_{ex}). It has also been shown that the transverse relaxivity caused by T_{2ex} (i.e., r_{2ex}) reaches a peak value for a given B_0 at a specific exchange rate defined by $k_{ex} = \omega$, where ω is expressed in rad s⁻¹ (17,19,20).

Diamagnetic T_{2ex} from compounds with exchanging -NH or -OH protons (e.g., NH₄Cl) has long been used for bulk water solvent signal suppression in high-resolution NMR experiments (21,22), and has more recently been proposed as an exogenous T_2 contrast mechanism for MRI (23,24). However, diamagnetic T_{2ex} agents require high concentrations (e.g., 500 mM) to achieve significant bulk water T_2 reduction and negative contrast, making them impractical for *in vivo* applications. The T_{2ex} effect can be greatly increased by using inner-sphere water molecule exchange in chelates containing paramagnetic Ln³⁺ ions. This was originally demonstrated by Aime et al. (25) where it was shown that the amount of DyDOTA-(mono-amide) required to suppress the bulk water peak in high-resolution NMR experiments was reduced by a factor of 200 (from 500 mM to 2.5 mM). This increase in T_{2ex} sensitivity is mainly due to the increase in ω when moving from diamagnetic -NH and -OH proton exchange (where ω is typically 2 to 5 ppm) to paramagnetic Ln³⁺ inner-sphere water molecule exchange (where ω can range from 50 ppm for Eu³⁺ to 800 ppm for Dy³⁺) (11). It is important to note that the reduction in the bulk water T_2 due to T_{2ex} is *in addition* to the paramagnetic line broadening caused by the Ln³⁺ ion itself (T_{2para}). This makes some of the Ln³⁺ ions (most notably Dy³⁺) strong candidates for development into highly-sensitive T_2 contrast agents for MRI.

In fact, some chelates containing the Dy³⁺ ion have previously been pursued as T_2 contrast agents. The most well-known example being DyDTPA-BMA or Sprodiamide™ (Nycomed, U.K.) (26), the Dy³⁺ analog of the nonionic GdDTPA-BMA or gadodiamide (i.e., Omniscan™; Nycomed, U.K.). DyDTPA-BMA was first assessed for demarcation of myocardial ischemia (26-30), tumor tissue characterization (31), and even Phase I clinical trials for cerebral perfusion imaging (32). These *in vivo* examples illustrate the many possible applications for a small molecule-sized T_2 MRI contrast agent. Other previous

Dy³⁺-based chelate studies include DyDTPA-based derivatives and starch microparticles (19,33,34), DyDOTA-based monomers and dendrimers (25,33,35), and the MS-325 human serum albumin binding ligand (20). It is important to note that for these previous Dy³⁺ chelates the water molecule exchange rates at 37 °C were either too fast or too slow to maximize r_{2ex} , typically being one to two orders of magnitude away from the ideal exchange rate defined by $k_{ex} = \omega$ (19,33). Interestingly, several of the early studies on Dy³⁺ chelates did observe that their total transverse relaxivity (r_{2tot}) was indeed proportional to the water molecule exchange rate (k_{ex}) and bound water chemical shift (ω). This was demonstrated by varying the chemical structure, temperature, and B_0 field (19,20,25,33), and was elegantly explained in theoretical detail by Caravan et al. (20). Although the concept of maximizing the transverse relaxivity for a Dy³⁺-based chelate by adjusting the water molecule exchange to the ideal rate had been proposed, to the best of our knowledge it has not been previously implemented.

Hypothesis and Objectives

In this study Swift-Connick theory (17,19,20) was used to optimize the transverse relaxivity produced by a series of DyDOTA-(amide)_x systems at 37 °C. This was achieved by modulating the water molecule exchange rate with different DOTA ligand side-arm structures, and by maximizing ω with magnetic field strength ($B_0 = 9.4$ T). The DyDOTA-(amide)_x chelate structure that gave the ideal exchange rate at 400 MHz (9.4 T) and 37 °C was determined by *in vitro* T₂ measurements, and the water molecule exchange rates for each chelate were confirmed using variable temperature ¹⁷O line-width measurements. Based on these results, one can hypothesize that polymerized or dendrimerized versions of these DyDOTA-(amide)_x chelates could create a new class of highly-sensitive T₂ contrast agents for MRI.

METHODS

Chelate Synthesis

Five different Dy³⁺ chelates were synthesized (Fig. 1), each having a different water molecule exchange rate. Four of the chelates (a-d) used the DOTA ligand (1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraacetic acid) and one (e) used the TETA ligand (1,4,8,11-tetraazacyclotetradecane-1,4,8,11-tetraacetate). The four DOTA-(amide)_x ligands were synthesized according to earlier published methods (36-38), while the TETA ligand was purchased from Sigma-Aldrich (St. Louis, MO). All five Dy³⁺ complexes were prepared by reacting the corresponding free ligand with DyCl₃ in water at pH 6.0 and 25 °C for 24 hours. The absence of any free Dy³⁺ metal was confirmed using the Xylenol Orange indicator test, and the concentration of Dy³⁺ in each complex was measured using ICP-OES analysis.

The water molecule exchange rates of the four DOTA-(amide)_x chelates were inversely proportional to the number glycinate side-arm structures (NHCH₂COO⁻) attached to the DOTA ring (i.e., 0, 2, 3, or 4). Therefore, the water molecule exchange rate ranged from fast for DyDOTA, to slower with additional glycinate amide groups, to slowest for DyDOTA-(gly)₄. The DyTETA chelate, while structurally similar to DyDOTA, lacks an inner-sphere

water molecule and cannot by definition have a T_{2ex} contribution to the water line-width. Hence, this chelate served as a useful control to measure the changes in the bulk water T_2 due solely to the paramagnetic effects of the Dy^{3+} ion (i.e., outer-sphere relaxation only).

***In Vitro* Experiments**

The total transverse relaxivity (r_{2tot}) was measured as a function of temperature for each of the five Dy^{3+} chelates. This was performed by creating a concentration array for each chelate (0.125, 0.25, 0.5, and 1.0 mM) in 5 mm NMR tubes, with each sample adjusted to a pH of 7.0. The total T_2 (T_{2tot}) for each sample was then measured on an Agilent (Santa Clara, CA) 400 MHz NMR system using the Carr-Purcell-Meiboom-Gill pulse sequence. The T_{2tot} data were acquired at 22, 37, and 52 °C for each chelate to determine how the r_{2tot} varied as a function of water molecule exchange rate. The r_{2tot} values (in units of $s^{-1} mM^{-1}$) were calculated by plotting T_{2tot}^{-1} versus Dy^{3+} concentration and using the slope of the least squares fitted line. The transverse relaxivity due to water molecule exchange (r_{2ex}) for each DyDOTA-(amide)_x chelate was then calculated by subtracting the total transverse relaxivity of DyTETA from r_{2tot} (i.e., $r_{2ex} = r_{2tot} - r_{2DyTETA}$) at each temperature. The measurement error in r_{2tot} from the least squares fitting was less than 10 % and the Dy^{3+} concentration of each sample was verified by ICP-OES analysis.

The water molecule exchange rate for each Dy^{3+} chelate was calculated by measuring the variation in the ^{17}O peak line-width as a function of temperature. Aqueous samples of each chelate (18 mM [Dy^{3+}], pH 7.0, and enriched to 2% ^{17}O) were loaded into 18 μL spherical capillaries from Wilmad-LabGlass (Vineland, NJ) to remove any susceptibility effects and then placed inside thin-walled, water-filled 5 mm NMR tubes also from Wilmad-LabGlass. An Agilent 400 MHz NMR system was used to measure the ^{17}O line-width (TR = 250 ms, acquisition time = 80 ms, 128 averages) as the temperature was varied from 5 to 90 °C in 5 °C steps (18 points). The change in transverse relaxation rate due to exchange (T_{2ex}^{-1}) as a function of temperature (i.e., water molecule exchange rate) was calculated by first converting the line-width data ($T_{2tot}^{-1} = \pi \times \text{line-width}$) then subtracting the DyTETA data ($T_{2ex}^{-1} = T_{2tot}^{-1} - T_{2DyTETA}^{-1}$). The water molecule exchange rates were then calculated by fitting the temperature dependant T_{2ex}^{-1} data with a model given by Pubanz et al. (39) and the MATLAB nonlinear least squared algorithm (Natick, MA). The measurement error in τ_M ($\pm$ one standard deviation) was estimated to be less than 20 %.

In vitro images of the five Dy^{3+} chelate samples, along with a pure water standard, were acquired simultaneously on an Agilent (Santa Clara, CA) 9.4 T (400 MHz) small animal MRI system using standard 5 mm diameter HPLC glass tubes and a 38 mm diameter ^1H birdcage volume coil. Each sample was approximately 200 μL with a Dy^{3+} concentration of 20 mM and a pH of 7.0. The sample temperature was monitored with a thermocouple and held constant by a heated air system from Small Animal Instruments (Stony Brook, NY). The Fast Spin-Echo settings were: TR/TE = 2500/12.4 ms, echo train = 8, averages = 8, field of view = 64x16x5 mm, matrix = 512x128x1 pixels, with an image scan time of 5 m 25 s.

RESULTS

In Vitro Results

A plot of $T_{2\text{tot}}^{-1}$ versus Dy^{3+} concentration is shown in Fig. 2 for DyDOTA-(gly)₂ at 22, 37, and 52 °C. The slope of each least squares fitted line gives the calculated $r_{2\text{tot}}$ at that temperature. Fig. 2 shows that $r_{2\text{tot}}$ decreases with increasing temperature indicating that, within this temperature range, slower water molecule exchange rates (k_{ex}) give higher total relaxivity values for DyDOTA-(gly)₂. The similarly measured $r_{2\text{tot}}$ values for all five Dy^{3+} chelates studied are summarized in Table 1, which also shows the calculated $r_{2\text{ex}}$ values (i.e., $r_{2\text{ex}} = r_{2\text{tot}} - r_{2\text{DyTETA}}$) for each temperature. Table 1 shows that over the given temperature range, $r_{2\text{tot}}$ and $r_{2\text{ex}}$ are inversely proportional to temperature for DyDOTA and DyDOTA-(gly)₂ yet are proportional to temperature for DyDOTA-(gly)₃ and DyDOTA-(gly)₄. Note that for DyTETA, which has no water molecule exchange, the $r_{2\text{tot}}$ is relatively independent of temperature. Also included in Table 1 are the bound water lifetimes ($\tau_M = k_{\text{ex}}^{-1}$) at 22, 37, and 52 °C that were calculated from the variable-temperature ¹⁷O data. Figure 3 shows a plot of the ¹⁷O measured $T_{2\text{ex}}^{-1}$ versus temperature for DyDOTA-(gly)₄ (raw data shown as circles). The Matlab fit to these data, based on Equation 1 in Pubanz et al. (39), gave values for k_{ex}^{298} and $H^\pm$ which were used to calculate τ_M at each temperature using the equation below:

$$\tau_M^{-1} = \frac{k_{\text{ex}}^{298} T}{298.15} \exp \left[\frac{\Delta H^\pm}{R} \left(\frac{1}{298.15} - \frac{1}{T} \right) \right] \quad [1]$$

where k_{ex}^{298} is the water molecule exchange rate at 25 °C, T is temperature in Kelvin, $H^\pm$ is the enthalpy of activation, and R is the universal gas constant. Similar fits were performed for the other DyDOTA-(amide)_x chelates. A full description of the ¹⁷O methods and Matlab fit can be found in the supplemental information.

The data from Table 1 can be more easily interpreted using a Swift-Connick plot (17) for Dy^{3+} at 400 MHz (9.4 T) as shown in Fig 4. Swift-Connick theory (21) predicts that the transverse relaxivity due to water molecule exchange ($r_{2\text{ex}}$) is a function of the bound water molecule lifetime (τ_M) as given by:

$$r_{2\text{ex}} = (1.8 \times 10^{-5}) \frac{\tau_M \Delta\omega^2}{1 + \tau_M^2 \Delta\omega^2} \quad [2]$$

where ω is the paramagnetic frequency shift of the bound water molecule protons expressed in rad s⁻¹ (17,40). Equation 2 predicts that for Dy^{3+} at 9.4 T (using $\omega = -730$ ppm or 1.835×10^6 rad s⁻¹) the $r_{2\text{ex}}$ will reach a peak value of $16.5 \text{ s}^{-1} \text{ mM}^{-1}$ at a specific bound water lifetime given by $\omega^{-1} = 545$ ns. The “fast” side of the Swift-Connick plot is then defined as $\tau_M < 545$ ns, while the “slow” side is defined as $\tau_M > 545$ ns.

The 37 °C data from Table 1 was plotted in Fig. 4 for each DyDOTA-(amide)_x chelate. It can be seen that the measured $r_{2\text{ex}}$ and τ_M data are in excellent agreement with the Swift-Connick theory. For example, while the faster water molecule exchange rate of DyDOTA places it on the fast side of the Swift-Connick peak, the slower water molecule exchange

rate of DyDOTA-(gly)₄ places it on the slow side. This explains why $r_{2\text{tot}}$ decreases with increasing temperature (smaller τ_M) for DyDOTA, yet increases with increasing temperature for DyDOTA-(gly)₄. For DyDOTA-(gly)₂ and DyDOTA-(gly)₃, their intermediate water molecule exchange rates place them close to the peak maximum of the Swift-Connick plot. Figure 4 shows that at 37 °C the highest $r_{2\text{ex}}$ value is given by DyDOTA-(gly)₃ (11.8 s⁻¹ mM⁻¹ or 72% of the maximum $r_{2\text{ex}}$) making it a potential candidate for development into a T₂ contrast agent.

The effect that τ_M has on T_{2ex}, and thus the total T₂ contrast, can be qualitatively observed in the *in vitro* images shown in Fig. 5. The top row shows a fast spin-echo image of a water standard and the five Dy³⁺ chelates (each at 20 mM) taken at 37 °C. It can be seen that the signal intensities for DyDOTA (fast water molecule exchange) and DyTETA (no exchange) are similar to that of pure water, indicating little or no T₂ contrast at this TE value (12.4 ms). Yet for the remaining three chelates, their level of T₂ contrast (i.e., darkening) is proportional to their vertical position ($r_{2\text{ex}}$ value) on the Swift-Connick plot in Fig. 4, with DyDOTA-(gly)₃ having the largest $r_{2\text{ex}}$ value (11.8 s⁻¹ mM⁻¹) and thus the darkest vial. The bottom row shows a similar fast spin-echo image but now at 22 °C. The slower water molecule exchange at this temperature leads to different τ_M and thus $r_{2\text{ex}}$ values for each DyDOTA-(amide)_x chelate, with DyDOTA-(gly)₂ now having the largest $r_{2\text{ex}}$ value (18.6 s⁻¹ mM⁻¹) and the darkest vial (see Fig. 6b). Similar *in vitro* spin-echo images for two DyDTPA-based chelates at 4.7 T and 25 °C was shown by Vander Elst et al. (19) where one vial appears darker simply due to the difference in τ_M and thus T_{2ex}.

DISCUSSION

It is interesting to compare these new DyDOTA-(amide)_x chelates to previous Dy³⁺-based T₂ contrast agents that have reported τ_M and r_2 data. Fig. 6(a) displays experimental data (black symbols) for DyDOTA-4AmCE (35), DyDTPA-BMA (39), DyDTPA-BEA (19), and Dy-L1 (the Dy³⁺ analog of the Gd³⁺-based human serum albumin binding MS-325 blood pool agent) (20,41) on a Swift-Connick plot for 9.4 T ($\omega = -730$ ppm). The τ_M values at 37 °C (calculated from ¹⁷O data and Eq. [1] when not directly given) were 8 μs, 128.2 ns, 60 ns, and 16.1 ns, respectively. These τ_M values were then used to estimate the $r_{2\text{ex}}$ values at 9.4 T via Fig. 6(a), which gave 2.2, 7.0, 3.5, and 1.0 s⁻¹ mM⁻¹ respectively. These estimated $r_{2\text{ex}}$ values are in agreement with their previously reported r_2 data at 9.4 T. It can be seen that these previous Dy³⁺ chelates all had water molecule exchange rates that were either too fast or too slow to maximize $r_{2\text{ex}}$ at 9.4 T. Although DyDTPA-BMA gives an $r_{2\text{ex}}$ value of approximately 7 s⁻¹ mM⁻¹, it is still less than half of the maximum (16.5 s⁻¹ mM⁻¹). Due to their intermediate water molecule exchange rates at 37 °C, both DyDOTA-(gly)₂ and DyDOTA-(gly)₃ give higher $r_{2\text{ex}}$ values at 9.4 T. Although the τ_M values for these two chelates are not quite at the ideal time of 545 ns, their $r_{2\text{ex}}$ values are 59% and 72% the maximum respectively. These relative differences in $r_{2\text{ex}}$ values become even more pronounced at fields lower than 9.4 T, where the maximum $r_{2\text{ex}}$ value reduces proportionately and moves towards slower exchange (larger τ_M) as shown in the 3.0 T plot in Fig. 6(a). For example, at 9.4 T (and 37 °C) there is a 41% drop in $r_{2\text{ex}}$ when moving from DyDOTA-(gly)₃ to DyDTPA-BMA (11.8 to 7.0 s⁻¹ mM⁻¹), yet at 3.0 T this reduction is 85% (5.3 to 0.8 s⁻¹ mM⁻¹). Also, at 3.0 T the peak $r_{2\text{ex}}$ value of 5.3 s⁻¹ mM⁻¹ occurs at

$\omega^{-1} = (5.871 \times 10^5 \text{ rad s}^{-1})^{-1} = 1703 \text{ ns}$, which makes the DyDOTA-(gly)₃ chelate ($\tau_M = 1795 \text{ ns}$ at 37 °C) ideally suited for use at 3.0 T.

In Fig. 4, it was assumed that all of the DyDOTA-(amide)_x chelates studied here shared the same ¹H ω value of -730 ppm (11). Yet there is evidence from the $r_{2\text{ex}}$ and τ_M data that the ω values do vary slightly between chelates. This should be expected because of their different chemical structures and ligand fields surrounding the Dy³⁺ ion. Fig. 6(b) shows a Swift-Connick plot for Dy³⁺ at 9.4 T (400 MHz) where four different values for ω have been plotted (see Equation 2). It can be seen that as the absolute value of ω increases, the maximum $r_{2\text{ex}}$ value increases proportionally and moves towards faster exchange. It can also be seen that while there is little difference between the Swift-Connick plots on the slow side, where the four curves converge, there is a significant difference on the fast side and most notably in the peak maximum. By using the $r_{2\text{ex}}$ data for all three temperatures measured in Table 1 and their corresponding calculated τ_M values, one can see how the $r_{2\text{ex}}$ for each chelate varies with τ_M in Fig. 6(b). All of these data appear to be in fairly good agreement with $\omega = -730 \text{ ppm}$ except for DyDOTA-(gly)₂ at 22 °C (green circle at $\tau_M = 400 \text{ ns}$) which, due to its high $r_{2\text{ex}}$ value of $18.6 \text{ s}^{-1} \text{ mM}^{-1}$, appears to agree with a larger ω of around -810 ppm. Yet for DyDOTA-(gly)₃ at 52 °C (yellow circle at $\tau_M = 330 \text{ ns}$) the experimental $r_{2\text{ex}}$ value of $13.0 \text{ s}^{-1} \text{ mM}^{-1}$ is more consistent with a lower ω of around -650 ppm, even though it shares a similar τ_M . This could be explained by the two chelates having different chemical fields that lead to different ω values and also by the variation of ω with temperature, where the absolute value of ω is inversely proportional to temperature. It is well known that hyperfine shifts such as these are extremely sensitive to temperature (42) so the exact shape and magnitude of these Swift-Connick type plots may depend upon getting a precise determination of ω from hyperfine shift measurements of other non-exchanging protons in these paramagnetic complexes by high resolution NMR spectroscopy. These observations agree with quantitative estimates of the bound water molecule ¹H ω made by measuring the chemical shifts of the cyclen H₄ protons in the DOTA ligand as a function of temperature (see Supplemental Information) (11).

CONCLUSIONS

These data represent the first time that $T_{2\text{ex}}$ for a series of DyDOTA-(amide)_x chelates has been optimized by methodically adjusting the inner-sphere water molecule exchange rate at 9.4 T and 37 °C. In order to achieve the goal of having an $r_{2\text{ex}}$ value greater than $10 \text{ s}^{-1} \text{ mM}^{-1}$, the τ_M needed to be in the “Goldilocks Zone” (43) (i.e., not too fast, not too slow, but just right) of approximately 200 ns to 2000 ns, with DyDOTA-(gly)₃ meeting this requirement ($\tau_M = 1800 \text{ ns}$, $r_{2\text{ex}} = 11.8 \text{ s}^{-1} \text{ mM}^{-1}$) and DyDOTA-(gly)₂ coming close ($\tau_M = 190 \text{ ns}$, $r_{2\text{ex}} = 9.7 \text{ s}^{-1} \text{ mM}^{-1}$). Preliminary *in vivo* results from the DyDOTA-(gly)₂ chelate showed a promising order of magnitude improvement in dose sensitivity compared to the EuDOTA-(gly)₄ chelate (44). Polymerization or dendrimerization of these chelates could further increase the $r_{2\text{ex}}$ (per molecule) by a factor of 100 to approximately 1000 to $1200 \text{ s}^{-1} \text{ mM}^{-1}$ thereby creating highly-sensitive, molecule-sized T_2 contrast agents for MRI. These data also stress the importance of selecting the correct water molecule exchange rate (and ω) when designing lanthanide-based contrast agents as recently described by Sherry and Wu (45).

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1.

Chemical structures of the five Dy³⁺ chelates used in this study. The four DyDOTA-(amide)_X chelates (a-d) have varying inner-sphere water molecule exchange rates, while the DyTETA chelate (e) has no water molecule exchange and therefore no T_{2ex} effects on the bulk water T₂.

Figure 2.

A plot of total transverse relaxation rate ($T_{2\text{tot}}^{-1}$) versus Dy^{3+} concentration for the DyDOTA-(gly)₂ chelate at 22, 37, and 52 °C. The slope of the least square fitted lines gives the total transverse relaxivity ($r_{2\text{tot}}$) at that temperature. Measurement error for $r_{2\text{tot}}$ was less than 10%.

Figure 3. A plot of the ^{17}O measured exchange transverse relaxation rate (T_{2ex}^{-1}) versus temperature for the DyDOTA-(gly) $_4$ chelate. The least squares fit to the data (line) allows the water molecule exchange rate at 37 °C to be calculated (39).

Figure 4.

A Swift-Connick plot for Dy^{3+} at 9.4 T (400 MHz) using a ω of -730 ppm (1.835×10^6 rad s^{-1}). Data markers for the measured r_{2ex} and τ_M values at $37^\circ C$ (from Table 1) are in excellent agreement with the Swift-Connick exchange theory, with DyDOTA-(gly)₃ giving the largest r_{2ex} .

Figure 5.

Fast spin-echo images ($TE = 12.4$ ms) of the five Dy^{3+} chelates (and water standard) in 5 mm diameter vials taken at 9.4 T. By taking the same image at two different temperatures the dependence of T_{2ex} (and thus the total T_2 contrast) upon the water molecule exchange rate can be qualitatively shown. The brightness and contrast levels for each image are the same.

Figure 6.

(a) A Swift-Connick plot comparing the new DyDOTA-(amide)_x chelates (colored markers) to previous Dy^{3+} -based chelates (black markers) at 9.4 T ($\omega = -730$ ppm or 1.835×10^6 rad s^{-1}) and 37 °C. Also shown is a Swift-Connick plot for Dy^{3+} at 3.0 T ($\omega = -730$ ppm or 5.871×10^5 rad s^{-1}). (b) Swift-Connick plots for Dy^{3+} at 9.4 T using four different values for ω . Data markers for each chelate are shown (from Table 1) with temperatures given in °C. Note that the abscissa and ordinate ranges are different in (a) and (b).

Table 1

The measured $r_{2\text{tot}}$ and $r_{2\text{ex}}$ values for the five Dy^{3+} chelates taken at three different temperatures, where $r_{2\text{ex}} = r_{2\text{tot}} - r_{2\text{DyTETA}}$. Also shown are the variable-temperature ^{17}O measured bound water lifetimes. Compared to the other four chelates, the $r_{2\text{tot}}$ for DyTETA is relatively independent of temperature due to the lack of water molecule exchange. Measurement errors for $r_{2\text{tot}}$ and τ_{M} were less than 10 % and 20 % respectively.

Dy ³⁺ chelate	total transverse relaxivity $r_{2\text{tot}}$ (s ⁻¹ mM ⁻¹)			exchange transverse relaxivity $r_{2\text{ex}}$ (s ⁻¹ mM ⁻¹)			bound water molecule lifetime τ_{M} (ns)		
	52 °C	37 °C	22 °C	52 °C	37 °C	22 °C	52 °C	37 °C	22 °C
DyDOTA	0.17	0.43	1.5	0.01	0.22	1.3	0.8	3.7	22
DyDOTA-(gly) ₂	3.7	9.9	18.8	3.6	9.7	18.6	93	190	400
DyDOTA-(gly) ₃	13.2	12.0	4.2	13.0	11.8	3.9	330	1,800	7,700
DyDOTA-(gly) ₄	8.6	3.6	1.4	8.5	3.4	1.2	1,800	4,900	14,000
DyTETA	0.16	0.21	0.22	(no water molecule exchange)					