New Bifunctional Chelates with Optimal Water Residence Times for Molecular Imaging

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Introduction: The enormous versatility of magnetic resonance imaging (MRI) offers the promise of molecular and cellular imaging. No matter the MR technique molecular and cellular imaging requires either exogenous or endogenous markers. The use of exogenous stains or contrast agents requires a suitable concentration of the agent at the desired target by delivery of relatively large numbers of paramagnetic or super paramagnetic ions (such as Gd, Mn, and Fe) in the form of polymeric drug delivery systems and/or nanoparticles, or a smaller number of more highly efficient agents. We report below the synthesis and characterization of two new highly efficient agents.

For larger macromolecular systems that have long clearance times toxicity issues require kinetically stabile systems such as the clinically approved Gd(III) chelates of DOTA. Unfortunately Gd(III)-DOTA has an inner sphere water residence time that reduces the efficiency of these agents and limits its sensitivity in molecular imaging. Gd(III) complexes of DOTMA are also kinetically stabile and have the desired property of an optimal water residence time. We therefore invented two new bifunctional derivatives of DOTMA, R = CH_3, (and their DOTA, R = H, analogs as controls, chart 1) that can be covalently attached to a delivery system using either click, $R^1 = N_3$, or standard peptide coupling chemistry, $R^1 = NH_2$, while maintaining the optimal water residence time for increased molecular imaging sensitivity. Long chain biotin (LC-biotin) derivatives of the Gd(III) chelates associated with avidin were used to demonstrate higher efficiencies.

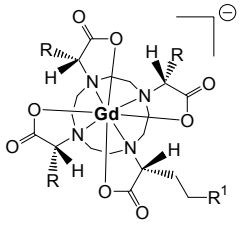

Chart 1.

Methods: ***Water Residence Time Measurements:*** Water residence times were determined using ^{17}O-NMR as described by Laurent et al.[1] Cbz-Gd-Chelate solutions were prepared in distilled water (pH 6.5-7.0) at concentrations of 23 mM. Solutions (0.35 mls) were transferred to 5 mm o.d. NMR tubes and ^{17}O NMR measurements were made at 11.7 T in a Bruker AVANCE-500 spectrometer. Data analysis and treatment was performed as described by Laurent S et al.[2]

Relaxivity Measurements: Relaxivity was determined from nuclear magnetic relaxation dispersion (NMRD) profiles. Proton NMRD measurements were made over a magnetic field range of 0.47 mT to 1.0 T on a Stelar Spin fast field cycling (FFC) NMR relaxometer. Three different solutions of each Gd(III)-chelate were prepared in 50 mM HEPES buffer, pH = 7.35, with 150 mM NaCl. Measurements were performed on 0.800 ml samples in 10 mm o.d. NMR tubes. Additional relaxation rates were measured at 20 and 60 MHz on a Bruker Minispec mq-20 and mq60 at temperatures ranging from 3 to 50 °C.

Inductively Coupled Argon Plasma (ICP) Mass Spectrometry: Gadolinium concentrations of each sample were measured by ICP mass spectrometry (University of Illinois at Urbana-Champaign {UIUC}). The samples, 0.05 ml, were added to 0.500 mls of optima grade nitric acid and heated to 80 °C in a closed vial over night, left for 7 weeks at room temperature, and shipped to UIUC.

Results: We prepared the Cbz-analog of the amine bifunctional chelates to determine if the stereochemistry was conserved. The proton NMR spectra of the Cbz-Eu(III)-DOTMA complexes demonstrated that the chelates had a majority of the fast exchanging isomer which implied that the Gd(III) derivatives should have rapid exchange. These results were supported by x-ray crystallographic data and actual measurements of the water residence time (Table 1).

Table 1. Parameters obtained by the theoretical analysis of the ^{17}O experimental data.

Complex	τ_M^{310} ns	τ_V^{298} ps	$\Delta H^{\ddagger}$ kJ mol^{-1}	B x 10^{20} s^{-2}	E_V kJ mol^{-1}	A/ ħ x 10^6 Rad s^{-1}	$\Delta S^{\ddagger}$ J mol^{-1} K^{-1}
Gd(III)-Cbz-DOTA	101 ± 5	4.9 ± 0.6	54.1 ± 0.5	2.0 ± 0.2	0 ± 20	-3.0 ± 0.2	63 ± 2
Gd(III)-Cbz-DOTMA	42 ± 5	5.2 ± 0.4	63.1 ± 0.2	6.4 ± 0.5	8 ± 4	-3.0 ± 0.1	99.7 ± 0.4

NMRD studies demonstrate the higher relaxivities of LC-biotin-Gd(III)-DOTMA relative to LC-biotin-Gd(III)-DOTA, Figure 1. The use of variable temperature NMRD indicated that while the relaxivity of the DOTA derivative was limited by the water residence time, the relaxivity of the DOTMA derivative was limited by the rotational correlation time. At 3 °C the relaxivity of the DOTMA derivative was 100% higher than that of the DOTA derivative and at 37 °C it was 50% higher.

Conclusions: We have reported four new bifunctional chelates and the characterization of the two bifunctional chelates with primary amine functionality. We have compared these two chelates and demonstrated that the Gd(III)-DOTMA core has a shorter water residence time, that when rotationally constrained has higher relaxivities, and that this relaxivity can be 40 to 100% higher than constrained conventional Gd(III)-DOTA chelates. This higher relaxivity has the potential to increase the sensitivity of molecular imaging or reduce the dose of targeted agents. The two other chelates with the azide functionality are suitable to use with click chemistry.[3]

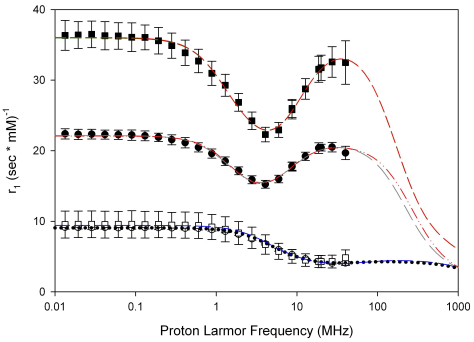

Figure 1. NMRD profiles of LC-biotin-Gd(III)-DOTA (circles) and LC-biotin-Gd(III)-DOTMA (squares) with (filled) and without (open) avidin at 37 °C.

[1] Laurent S, Vander Elst L, and Muller RN. Comparative Study of the Physiochemical Properties of Six Clinical Low Molecular Weight Gadolinium Constrast Agents. Contrast Media & Molecular Imaging 2006 1: 128-137.

[2] Laurent S, Vander Elst L, Houze S, Guerit N, Muller RN. Synthesis and characterisation of various benzyl diethylenetriaminepentaacetic acids (dtpa) and their paramagnetic complexes. Potential contrast agents for magnetic resonance imaging. *Helv. Chim. Acta* 2000; **83**: 394–406.

[3] Mastarone DJ, Harrison VS, Eckermann AL, Parigi G, Luchinat C, Meade TJ. A modular system for the synthesis of multiplexed magnetic resonance probes. J Am Chem Soc. 2011 Apr 13;133(14):5329-37. Epub 2011 Mar 17. PMCID: PMC3086647 [Available on 2012/4/13] PMID: 21413801 [PubMed - in process]