SYNTHESIS AND RELAXIVITY MEASUREMENTS OF NOVEL Gd(III)COMPLEX OF DOTVA AS MRA CONTRAST AGENTS

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Abstract.

The synthesis and relaxivity of a new gadolinium(III) complex of a polyazatricarboxylate macrocycle (DOTVA) having 2-bromoisovaleric acid pendant arm is reported. The longitudinal relaxivity (r_{1p}) of [Gd(DOTVA)(H$_2$O)] is 6.72 mM^{-1} s^{-1} (20 MHz, 37 ± 0.1 °C, pH 7) which is higher than that of [Gd(DOTA)(H$_2$O)]$^-$ (r_{1p} = 4.2 mM^{-1} s^{-1}, 20 MHz)by 1.6% than the clinically approved CAs. The r_{2p}/r_{1p} ratio for Na[Gd(DOTVA)(H$_2$O)] is 1.16 showing that the complex is a T_1-weighted contrast agent. The complexes show pH dependent relaxivity at low and high pH values. It forms adduct with HSA and shows relaxivity enhancement. The 2-bromoisovaleric acid seems to be an excellent pendant arm functionality in making new CAs for MRI and the amide core is a versatile core molecule for the creation of polynuclear gadolinium(III) chelates and dendrimeric CAs.

Key words. MRA Contrast agents, Blood Pool Agents, MRI, 2-bromoisovaleric acid, DOTVA

I. INTRODUCTION

In the world of medicine, magnetic resonance imaging (MRI) is the most important and prominent technique in biomedical research.[1] The 2003 Nobel Prize for medicine was awarded to Professor Paul C. Lauterbur (Biomedical Magnetic Resonance Laboratory, University of Illinois) and Professor Sir Peter Mansfield (University of Nottingham) for their seminal discoveries concerning the use of magnetic resonance to visualize different structures leading to the development of modern MRI.[2] Due to high spatial resolution,[3] MRI has become the clinical imaging procedure for a large number of studies involving the central nervous system, especially the brain,[4] visualizing soft tissues,[5] diagnosis of various diseases and the examination of almost all organs,[6] and imaging of physiological properties such as diffusion, perfusion, and vascularity of tissues.[3,7] MRI also provides real-time images of visualizing drug delivery,[8] monitoring biological processes, and following functional changes in vivo.[8a,9] The image contrast obtained in MRI is a three-dimensional signal intensity map of the spatially encoded proton signal of the in vivo water molecules in a given volume element (voxel).[10] Contrast in MRI images arises from variations in the relaxation times among water protons caused by differences in the local environment in tissues. For many clinical applications, it is a common practice to administer an exogenous chemical, called contrast enhancing agent, to improve the image contrast.[10b] Currently, about 30% of the MRI examinations use contrast agents.[5,11]

Contrast agents can be divided into two classes depending on whether they cause changes in longitudinal relaxation time (T_1) or transverse relaxation time (T_2) of the water protons, known as positive and negative agents, respectively.[12] The relaxivity reflect the efficiency of a contrast agent to accelerate longitudinal and transverse water proton relaxation rates $1/T_1$ and $1/T_2$, respectively, normalized to one millimole concentration of the agent.[10a,13] The signal observed in MRI tends to increase with an increase in $1/T_1$ and decrease with an increase in $1/T_2$ and it is usual for contrast agents to affect both $1/T_1$ and $1/T_2$ to varying degrees.[10a] Positive contrast agents are commonly made up of paramagnetic materials, mainly those based on metal ions with large number of unpaired electrons such as Mn(II) and Gd(III). Positive contrast agents increase the brightness of

images while the negative contrast agents give dark images. Superparamagnetic materials such as iron oxide nanoparticles are employed as negative CAs.[1]

Low molecular weight complexes of paramagnetic metal ions are administered as contrast agents prior to the acquisition of MRI images.[1] Over the last decade there has been an increasing interest in the chemistry of Gd(III) complexes because they have been used in diagnostic medicine as contrast agents for MRI. The symmetric S-state of Gd(III) is a more hospitable environment for electron spins leading to a much slower electronic relaxation rate. Due to the large magnetic moment ($\mu_{eff}^2 = g^2(S(S+1)) = 63$) with a long electron spin relaxation time (10^{-9} s) Gd(III) is widely used as a contrast agent for MRI.[14] Free gadolinium ions get accumulated as phosphate salts in lysomes of heptocytes and macrophages in spleen, bone marrow, and lungs and as a result of its high in vivo toxicity Gd(III) ion is complexed with ligands to form stable complexes.[15] The commercially used contrast agents are Gd(III) complexes of polyaminocarboxylate ligands.

The choice of a contrast enhancing agent suitable for clinical use is governed by stringent biological requirements such as (i) high solubility in water (of the order of 0.5 M), (ii) effective catalyst for relaxation of protons in the bulk water, i.e., a high water proton relaxivity, (iii) better body distribution, (iv) low in vivo toxicity, i.e., extremely inert to the loss of the metal ion, (v) selective tissue/organ localization, (vi) rapid tissue clearance, and (vii) high thermodynamic stability and kinetic inertness. A sizable increase in relaxivity is achieved if Gd(III) chelates are covalently or noncovalently linked to macromolecules, because the rotational correlation time becomes comparable to or longer than the electronic and water-exchange correlation times.[16] The conjugation of gadolinium(III)chelates to polymers, dendrimers, or biological molecules increases the rotational correlation time and improves the relaxivity "per Gd" atom. Targeting polynuclear conjugates are envisioned to provide MRI with the ability to image the low-concentration receptors by delivering a large payload of Gd(III) chelates. The present investigation could also

be extended to the development of macromolecular CAs by appending the Gd(DO3VA) chelate onto the macromolecular substrates such as polymers,[17] proteins, and dendrimers.[18]

The mononuclear Gd(III) complexes of 2-isovaleric acid functionalized cyclen derivatives and polynuclear Gd(DO3VA) complexes covalently conjugated to different amide linkers and core molecules can be subjected to detailed kinetic and pharmacokinetic studies to evaluate their suitability as contrast agents. Additional relaxivity gains may be achieved by increasing the number of the mobile protons of the amide linkers by the prototropic mechanism. The incorporation of 2-isovaleric acid groups is expected to increase the molecular weight as well as molecular dimension of the complex resulting in a longer rotational correlation time and thus leading to high proton relaxivity. The relaxivity of the mononuclear and polynuclear Gd(III) chelates of DO3VA depends on the residence time of the inner-sphere water molecules and the steric crowding and the formation of a secondary hydration sphere by the bulky 2-isovaleric acid group. Thus, there is a wide scope of studying the rotational dynamics and other relaxivity parameters for these systems. The gains made in the relaxivity of this mono- and polynuclear gadolinium complexes would shed more light on the design parameters for future MRI contrast agents.

After the first generation clinically approved extracellular contrast agents, the next largest class of applications may involve blood pool agents that serve as contrast agents for MRI angiography. Magnetic resonance angiography represents a more attractive procedure for patients and doctors for vascular imaging. The addition of lipophilic groups to the carbon backbone of polyazapoly carboxylates profoundly alters the pharma cokinetic and biodistribution properties of their gadolinium complexes. Furthermore, the interaction of the Gd(DO3VA) complexes with human serum albumin (HSA), their stability at the physiological pH, and their vascular retention time can be studied to evaluate their suitability as contrast agents for magnetic resonance angiography (MRA).

II. EXPERIMENTAL SECTION

A.   Materials.

Bis(3-aminopropylamine), benzene- sulfonyl chloride, TRIS base, Celite®, xylenol orange, and molecular sieves (4Å) (Fluka); 2-bromoisovaleric acid and gadolinium(III) chloride (Aldrich); cyclen (Strem chemicals); and 2-chloroacetyl chloride, sodium bromide, potassium hydroxide, sodium hydroxide, disodium hydrogen phosphate, magnesium turnings, iodine, and sodium carbonate (anhydrous) (Merck, India) were used as such. Charcoal (Merck, India) was used for the purification of compounds after activating by heating at 150 °C overnight. Calcium chloride (anhydrous) and sodium wire (Merck, India) were used for drying purposes. Hydrochloric acid (AR, 35.4%, d = 1.18, Merck India) was used as supplied. Silica gel (blue indicator, Fluka) was used as desiccant after activating by heating in an oven at 200 °C overnight. Amberlite IR-120 (H$^+$ form, 16-45 mesh), Amberlite IR-400 (Cl$^-$ form, 20-50 mesh), Dowex 50W x 8-200 (H$^+$-form, 8% cross linking, 100-200 mesh), and Dowex 1 x 8-400 (Cl$^-$ form, 8% cross linking, 200-400 mesh) (Aldrich) were washed with double distilled water five times before use. KBr (FT-IR grade), DMSO-d_6 (99.9 atom % D), acetone-d_6 (99.9 atom % D), D$_2$O (100 atom % D) (Aldrich), and mineral oil (for IR spectroscopy, Fluka) were used as received. Super dry ethanol, super dry methanol, and triply distilled water were prepared by the standard procedures.[15] HPLC grade water (Merck, India) was used to prepare solutions of the complexes for relaxivity measurements.

III. METHODS

A.   Physical Measurements.

Infrared spectra were recorded on a Perkin-Elmer Spectrum RX-I FT-IR Spectrometer in the range of 4000-400 cm^{-1}. Spectra for the solid samples were recorded by making transparent KBr pellets. The electrospray ionization mass spectra were recorded on a micromass Quattro II triple quadrupole mass spectrometer. The sample dissolved in methanol, acetonitrile, or water was introduced into the ESI source through a syringe pump at the rate of 5 μL per min. The ESI capillary was set at 3.5 kV and the cone voltage at 40 V. The average spectra of 6-8 scans were printed. CHN microanalyses were carried out using a Perkin-Elmer 2400 Series II CHNS/O Elemental Analyzer interfaced with a Perkin-Elmer AD 6 Autobalance. Helium was used as the carrier gas. *Caution!* All hygroscopic compounds were dried in desiccator over silica gel for 24 h prior to the analysis. ^{1}H and ^{13}C NMR spectra were recorded on a Bruker AVANCE III 500 MHz multinuclei NMR spectrometer. Broad band gradient probe head 'BBO' 5 mm probe was used for the ^{1}H NMR measurements. Inverse Quad Probe head 5mm 'QXI' was used for the ^{13}C NMR measurements. 400 MHz ^{1}H NMR spectra were recorded on a Jeol GSX-400 multinuclear NMR spectrometer working at 25 ºC. The 100 MHz ^{13}C NMR spectra were recorded using Jeol GSX-400 instrument. The standard 5 mm probe was used for the ^{1}H and ^{13}C NMR measurements. pH measurements were made using Labindia PICO$^+$ pH/Conductive meter (3P) calibrated with buffer solutions of pH 4.0, 7.0, and 9.2.

B.   Longitudinal relaxivity measurements.

The longitudinal relaxivity of the Gd(III) complexes were determined from the spin lattice relaxation time (T_1). The T_1 measurements were carried out on a Bruker minispec *mq* 20 NMR Analyzer operating at a frequency of 20 MHz and the temperature was maintained using a temperature console at 37 ± 0.1 °C. The solutions of the complexes were taken in a 180 x 10 mm stoppered glass tube. The instrument parameters were optimized for each T_1 measurement. Solutions of six concentrations (0.2, 0.5, 1.0, 1.5, 2.0, and 2.5 mM) for each complex were prepared in HPLC grade water (Merck, India) in 5 mL standard measuring flask (Vensil, Class "A"). The presence of free gadolinium(III) ion in the solution has been checked by xylenol orange test. The T_1 measurements were made using the standard inversion recovery pulse sequence (180°-τ-90°) with phase sensitive detection with τ values ranging from 50 µs to 6 s for each concentration of the complex.[19] The computer program "Excel" (version 2007) was used to plot the time versus signal intensity to get a monoexponential plot and T_1 was calculated from the plot. The T_1 values for six concentrations of each complex were measured. The T_1

curves for all the concentrations have a monoexponential decay character. The longitudinal relaxivity was calculated from the slope of the regression line, obtained by the plot of the concentration of the complex versus $1/T_1$ by least squares fitting method. The instrument was calibrated by measuring the relaxivity of $[Gd(DO3A)(H_2O)_2]$ in aqueous solution (r_{1p} = 4.63 mM^{-1} s^{-1}, 20 MHz and 37 °C; lit.[20] 4.80 mM^{-1} s^{-1}, 20 MHz, 40 °C).

C.   *Transverse relaxivity measurements.*

The transverse relaxation time (T_2) was determined by the standard Carr-Purcell-Meiboom-Gill (CPMG) pulse sequence (90°-τ-180°) with a τ value of 50 µs.[21] The computer program "Excel" (version 2007) was used to plot the time versus signal intensity to get an exponential curve and T_2 was calculated from the graph. The T_2 values for six concentrations were measured for each complex. The transverse relaxivity was calculated from the slope of the regression line, obtained by the plot of the concentration of the complex versus $1/T_2$ by least squares fitting method. All other experimental conditions are the same as that employed for the T_1 measurements.

IV.   SYNTHESIS OF LIGANDS AND COMPLEXES

A.   *1,4,7,10-Tetraazacyclododecane-1,4,7,10-tetra-2-isovaleric acid (DOTVA) (1).*

A solution of α-bromoisovaleric acid (2.17 g, 12 mmol) in 50 mL of water was added dropwise to a suspension of cyclen (0.52 g, 3 mmol) and sodium hydroxide (0.48 g, 12 mmol) in 100 mL of water at 60 °C for 1 h. The reaction mixture was further heated to 70 °C and stirred under argon atmosphere for 36 h, cooled to room temperature, filtered, flash evaporated, and dried in vacuum. The resulting pale yellow solid was washed with super dry methanol and recrystallized in water: yield (1.58 g, 92%), mp 270 °C. Anal. calcd. for $C_{28}H_{52}N_4O_8$ (M_r = 573): C, 58.62; H, 9.15; N, 9.78. Found: C, 58.62; H, 9.10; N, 9.76. IR (KBr, cm^{-1}): 3475 v(O-H) (acid), 2924 v(C-H), 1638 v(C=O) (acid), 1124 v(C-N) (tertiary amine), 617 ρ(CH$_3$) (isopropyl group). MS (ESI): m/z 569 [M-4]$^+$, 449 [M-C$_2$H$_4$O$_6$]$^+$, 389 [(M-4)-C$_6$H$_{12}$O$_6$]$^+$.

B.   *Na[Gd(DOTVA)H$_2$O] (C1).*

A solution of the ligand **1** (2.86 g, 5 mmol) in 25 mL of water was added to a solution of GdCl$_3$.6H$_2$O (1.85 g, 5 mmol) in 40 mL of water. The pH of the solution was maintained at 7 throughout the reaction by adding an aqueous solution of NaOH and heated to 60 °C under argon atmosphere for about 12 h.[212] It was cooled to room temperature, filtered through a 0.2 µm filter funnel, flash evaporated, and dried in vacuum. The resulting colorless hygroscopic solid was purified by column chromatography (Dowex ion exchange resin, cationic) using water and recrystallized in water: yield (3.01 g, 81%). Anal. calcd. for $C_{28}H_{50}N_4O_9GdNa$ (M_r = 767): C, 45.14; H, 6.72; N, 7.53. Found: C, 45.12; H, 6.68; N, 7.51. IR (KBr, cm^{-1}): 3414 v(O-H), 2924 v(C-H), 1638 v(C=O) (acid), 1115 v(C-N) (tertiary amine), 621 ρ(CH$_3$) (isopropyl group), 617 ρ(O-H), 532 ω(O-H), 482 v(Gd-O). MS (ESI): m/z 767 [M]$^+$, 725 [(M-1)-NaH$_2$O]$^+$, 551 [(M-1)-NaGdCH$_5$O$_2$]$^+$.

V.   RELAXIVITY STUDIES

A.   *Longitudinal relaxivity of Na[Gd(DOTVA)(H$_2$O)] (C1)*

The longitudinal relaxation times of water proton for the neat aqueous solutions of Na[Gd(DOTVA)(H$_2$O)] at six concentrations are given in Table 1. The plot of the concentration of the complex versus $1/T_1$ is given in Figure 1. The longitudinal relaxivity of Na[Gd(DOTVA)(H$_2$O)] is 6.72 mM^{-1} s^{-1} which is 1.60, 1.50, 1.77, 1.24, 1.24, 1.03, 1.05, and 1.01 units more than that of [Gd(DOTA)(H$_2$O)]$^-$ (r_{1p} = 4.2 mM^{-1} s^{-1}, 20 MHz). The longitudinal relaxivity of **C1** is also higher than that of the other monomeric complexes such as [Gd(*syn*-Cy$_2$-DOTA)(H$_2$O)] (r_{1p} = 4.5 mM^{-1} s^{-1}, 20 MHz), [Gd(DOTMA)(H$_2$O)] (r_{1p} = 3.8 mM^{-1} s^{-1}, 20 MHz), [Gd(DOTA-PNB)(H$_2$O)] (r_{1p} = 5.4 mM^{-1} s^{-1}, 20 MHz), [Gd(DOTA)-(BOM)(H$_2$O)] (r_{1p} = 5.4 mM^{-1} s^{-1}, 20 MHz), [Gd(*trans*-DOTA)(BOM)$_2$(H$_2$O)] (r_{1p} = 6.5 mM^{-1} s^{-1}, 20 MHz], [Gd(DNA-DOTA)] (r_{1p} = 6.4 mM^{-1} s^{-1}, 4 T), and [Gd(DOTA)(BOM)$_3$(H$_2$O)] (r_{1p} = 6.7 mM^{-1} s^{-1}, 20 MHz), respectively. The r_{1p} value is comparable to that of [Gd(*cis*-DOTA)(BOM)$_2$(H$_2$O)] (r_{1p} = 6.8 mM^{-1} s^{-1}, 20 MHz).[44] The enhancement in the relaxivity of **C1** when

compare to other monomeric complexes with acetate pendant arms is attributed to an increase in the rotational correlation time (τ_R) due to an increase in the molecular dimension of the complex by the covalent conjugation of 2-isovaleric acid to the cyclen macrocycle.
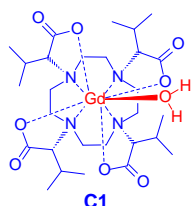

Fig.1 Na[Gd(DOTVA)(H$_2$O)] (C1)

Table 1. Longitudinal relaxation times of Na[Gd(DOTVA)(H$_2$O)]

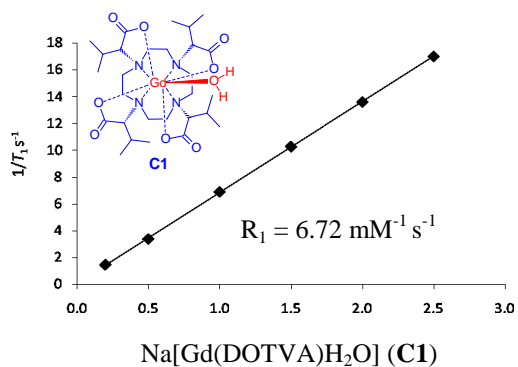| Concentration (mM)	T_1 x 10^{-3} s	1/T_1 x 10^3 s^{-1}
0.0	3425.78	00.29
0.2	0689.65	01.45
0.5	0296.73	03.37
1.0	0144.93	06.90
1.5	0097.28	10.28
2.0	0073.42	13.62
2.5	0058.79	17.01

Fig. 2. Plot of the concentration of Na[Gd(DOTVA)H$_2$O] versus 1/T_1.

B.   *Transverse relaxivity of Na[Gd(DOTVA)(H$_2$O)] (C1)*

The transverse relaxation times for six concentrations of Na[Gd(DOTVA)(H$_2$O)] are given in Table 2 and the plot of the concentration of the complex versus 1/T_2 is depicted in Figure 2. The transverse

relaxivity is 7.81 mM^{-1} s^{-1} which is 1.64 times higher than that of [Gd(DOTA)(H$_2$O)] (r_{2p} = 4.75 mM^{-1} s^{-1}, 20 MHz, 39 °C). The higher r_{2p} value is attributed to the presence of a large number of water molecules hydrogen bonded to the periphery of the hydrophilic 2-isovaleric acid groups (outer-sphere mechanism). The r_{2p}/r_{1p} ratio for Na[Gd(DOTVA)(H$_2$O)] is 1.16 showing that the complex is a T_1-weighted contrast agent.

Table 2. Transverse relaxation times of Na[Gd(DOTVA)(H$_2$O)]

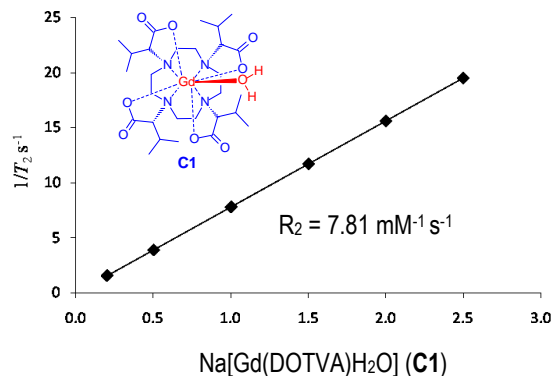| Concentration (mM)	T_2 x 10^{-3} s	1/T_2 x 10^3 s^{-1}
0.2	641.02	01.56
0.5	256.41	03.93
1.0	128.20	07.81
1.5	085.47	11.71
2.0	064.10	15.62
2.5	051.28	19.53

Fig. 3. Plot of the concentration of Na[Gd(DOTVA)H$_2$O] versus 1/T_2.

C.   *pH Dependent Relaxivity of [Gd(DOTVA)(H$_2$O)] complex.*

The complex [Gd(DOTVA)(H$_2$O)$_2$] exhibits longitudinal relaxivities (r_{1p}) of 11.05, 11.31, 12.38, and 13.16 mM^{-1} s^{-1} at pH 2.6, 3.6, 4.6, and 5.6, respectively. These values are significantly higher than that of the complex in neat solution (r_{1p} = 5.32 mM^{-1} s^{-1}, pH = 7). The relaxivity values at pH

7.2, 7.5, and 8.0 (TRIS-HCl buffer) are 4.90, 6.10, and 7.21, respectively. However, at pH 8.5, 9.6, and 10.6 the complex shows the longitudinal relaxivity of 0.90, 0.74, and 0.84, respectively. The higher relaxivity at low pH may be caused by the protonation of the carboxylate oxygen, the nitrogen atoms of the ligand, and the water molecules hydrogen bonded to the periphery of the DOTVA chelates.

D.   *Relaxivity of [Gd(DOTVA)(H$_2$O)] complex in the presence of HSA.*

The complex [Gd(DOTVA)(H$_2$O)] exhibits r_{1p} and r_{2p} values of 7.35 and 9.40 mM^{-1} s^{-1}, respectively, in the presence of HSA (1.36%) which is 1.38 and 1.22 times higher than that of the complex in neat aqueous solution. The higher relaxivity of the complexes is due to the binding of the 2-isovaleric acid pendant arm with the serum protein which results in a higher rotational correlation time for the macromolecular adduct. This promises their use as blood pool CAs for magnetic resonance angiography.

VI. CONCLUSIONS.

Improvements in MRI technology have enhanced the sensitivity of the technique. The sensitivity and selectivity of the technique is further improved by the relaxation efficiency of the CAs. Thus, the future is still bright for gadolinium(III)-based CAs. In the present investigation a convenient methodology has been developed for the synthesis of mono- and dinuclear gadolinium(III) complexes of 1,4,7,10-tetraazacyclodo- decane-1,4,7,10-tetra-2-isovaleric acid DOTVA] as contrast enhancing agents for MRI. The 2-isovaleric acid pendant arm appears to be a versatile moiety in the development of new contrast agents for MRI. A new paradigmatic approach is demonstrated in developing a complex with good relaxivity value. The pH-dependent relaxivity of the complexes could be exploited for their use as pH-responsive MRI contrast agents. Binding to HSA causes a significant increase in the relaxivity of the monomer. These complexes with the potential of binding with blood serum albumin through noncovalent interactions may offer a wide scope of evaluating their suitability as blood pool agents for MRA.

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