Synthesis of a Novel Lipophilic Gadolinium Complex as a Potential MRI Contrast Agent

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Abstract: The synthesis of a novel lipophilic diethylentriaminotetracetic(DTTA)-dodecane gadolinium complex is reported. The Gd-DTTA-dodecane complex is able to form mixed micelles with 1,2-dimyristoyl-sn-glycero-3-phosphocholine (DMPC) and a non-ionic surfactant like Tween 80. This novel multicomponent system can find potential application in magnetic resonance angiography (MRA).

Key words: contrast agent, gadolinium complex, mixed micelles, magnetic resonance imaging, magnetic resonance angiography

Magnetic resonance imaging (MRI) is a powerful and valuable non-invasive diagnostic technique providing images of selected parts of the human body.[1] A great part of MRI scans are performed employing a contrast agent, an exogenous compound able to enhance the relaxation rates of water protons and to improve the MRI images.

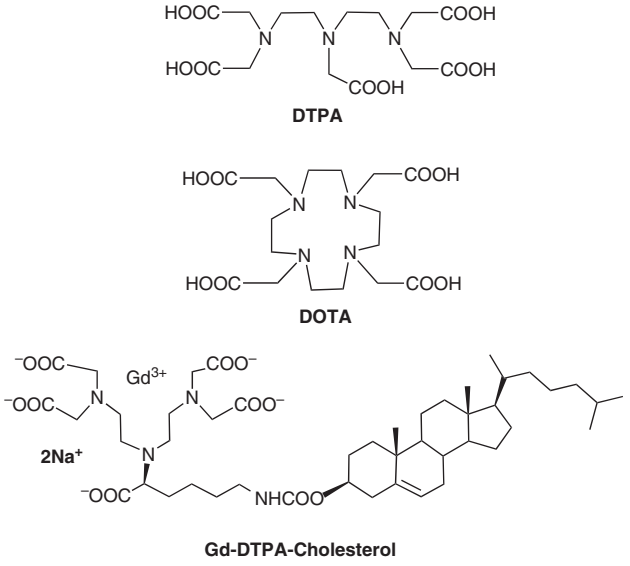
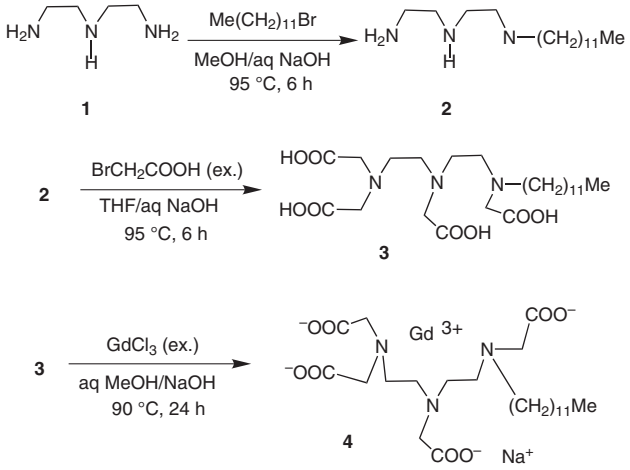


Figure 1 Chemical structure of common polyaminopolycarboxylic ligands used in MRI

Polyaminopolycarboxylic Gd(III) complexes are the most widely used contrast agents in MRI with $Gd(DTPA)^{2-}$ being the most common (Figure 1).[2–4] Several other Gd complexes have been reported in the last decades[5,6] (Figure 1) but the synthesis of novel contrast agents with improved properties are required for specific applications in magnetic resonance angiography (MRA).[7]

One of the most important aims of MRI is the imaging of the cardiovascular system.[8,9] The contrast agent must persist in the bloodstream for a long time after administration to patients and several strategies are currently employed: serum protein binders and polymeric[10] or dendrimeric[11] Gd complexes, which stay confined in the blood vessels owing to their large dimension. Moreover, liposomes[4,12,13] or micelles[14,15] have also been used. Several formulations using Gd-DTPA-cholesterol have been reported recently[7] (Figure 1) and other lipophilic Gd complexes bearing one or two aliphatic chains have been studied and have given promising results as MRA contrast agents.[15,16]

Here we report the synthesis of the novel Gd complex **4** containing a dodecyl chain as a lipophilic moiety. Due to the hydrophobic nature of dodecane, the resulting Gd(III) complex **4** is easily incorporated into liposomes or micelles.



Scheme 1 Synthesis of Gd-DTTA-dodecane complex **4**[21]

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To the best of our knowledge **4** is the first example of a Gd-DTTA complex attached to a dodecyl chain. Scheme 1 reports the synthesis of **4** starting from commercially available **1** by coupling to bromododecane in alkaline solution. The hydrophobic derivative **2** was further reacted with an excess of bromoacetic acid to give **3** as a white solid. Reaction of **3** with $GdCl_3$ afforded **4** in 46% overall yield. Complex **4** is soluble in water up to 50 mg/mL and is able to form aggregates when mixed with a phospholipid (DMPC) and a neutral surfactant like Tween 80 (in a 1:1:1 ratio). The mixture was diluted 1:10 with water and bath-sonicated for 30 minutes at 70 °C. Particles with a mean diameter of 49.8 ±5.6 nm (polyindex = 0.35 ± 0.05) were obtained and measured by dynamic light scattering (Zetasizer Nano ZS 90, Malvern, UK).

T_1 relaxation times were obtained by the inversion recovery method and r_1 relaxivity from a linear least squares regression analysis of relaxation rate ($1/T_1$) vs C_{eff} (mM) by means of a Minispec PC-120b (Bruker GmbH, Rheinstetten, Germany). Complex **4** shows a high relaxivity (r_1 = 21.7 $mM^{-1}s^{-1}$ in Tris buffer, pH 8, 20 MHz). This relaxivity value is extremely high compared to the relaxivity of Gd-DTPA (r_1 = 3.7 $mM^{-1}s^{-1}$),[17] and it is indirect evidence of a micellar self-organization of **4** in water. In fact, such high relaxivity is only achieved with multimeric Gd complexes like dendrimers or polymers.[2,3] Moreover, mixed micelles obtained with complex **4** show a high relaxivity (r_1 = 19.4 $mM^{-1}s^{-1}$) comparable to the Gd-DTPA-cholesterol derivative (r_1 = 25 $mM^{-1}s^{-1}$) recently reported in the literature.[7] No free Gd^{3+} ions were detected by assaying complex **4** with Arsenazo III dye.[18] Hence, the high values of relaxivity suggest that the Gd complex may be heptadentate with two water molecules coordinated to the metal (q = 2). Moreover, a favorable molecular reorientational time (τ_r) and a low water residence time (τ_m) may account for the high relaxivity as reported for similar compounds.[19,20] In conclusion, we have reported the synthesis and characterization of the novel lipophilic Gd complex **4**. This derivative can easily be incorporated into supramolecular systems like mixed micelles, a new and very promising class of blood pool MRI/MRA contrast agents.

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(21) **Compound 2**: A solution of 1-bromododecane (20 g, 80 mmol) in EtOH (100 mL) was added over 1 h to a stirred solution of diethylentriamine **1** (8.25 g, 80 mmol) in EtOH (100 mL) at r.t. NaOH (0.1 M) was added to the reaction mixture until the pH >7. The resulting solution was heated at 95 °C for 6 h, stirred at r.t. for 18 h, and then the solvent was evaporated under reduced pressure. The crude oil was purified by distillation collecting the fraction with a boiling point of 265–270 °C, which furnished **2** as a transparent oil (9.46 g, 41%). ^{1}H NMR (300 MHz, D_2O): δ = 0.96 (CH_3, t, 3 H), 1.37 (CH_2, br, 18 H), 1.56 (CH_2, m, 2 H), 2.51 (CH_2N, t, 2 H), 2.73 (CH_2N, t, 2 H), 2.83 (CH_2N, br, 4 H), 2.87 (CH_2N, t, 2 H). ^{13}C NMR (300 MHz, D_2O): δ = 13.41, 22.03, 26.88, 28.97, 29.19 (br m), 29.55, 31.28, 41.08, 48.73, 48.90, 49.40, 51.86. ESI-MS: m/z = 272.3 $[M + H]^+$. Anal. Calcd for $C_{16}H_{37}N_3$: C, 70.77; H, 13.74; N, 15.48. Found: C, 71.01; H, 13.93; N, 15.53. **Compound 3**: To a solution of **2** (2.7 g; 10 mmol) in THF (250 mL), was added bromoacetic acid (13.9 g, 0.1 mol, 10 equiv) in H_2O (100 mL) over 2 h; the pH of the reaction mixture was maintained at 12 by the constant addition of NaOH (0.1 M). The mixture was heated at 95 °C for 6 h, then the solvent was evaporated under reduced pressure. The milky residue was treated with HCl until the solution was pH 1 and the resulting acidic solution was filtered. The solid was washed several times with cold EtOH and dissolved again in H_2O (pH 8). The solution was treated with HCl until a precipitate formed. The white solid was filtered, washed several times with cold H_2O, and dried in a vacuum desiccator (1.1 g; 22%). ^{1}H NMR (300 MHz, D_2O): δ = 0.65 (CH_3, t, 3 H), 1.04 (CH_2, br, 20 H), 2.24 (CH_2N, m, 2 H), 2.32 (CH_2N, m, 8 H), 2.83 (CH_2COOH, br, 8 H). ^{13}C NMR (300 MHz, D_2O): δ = 14.18, 22.77, 25.22, 27.82, 29.52, 29.80 (br m), 32.06, 51.18, 52.26, 52.65, 55.10, 58.46, 59.02, 59.41, 179.34. ESI-MS: m/z = 262.96 $[M + Na]^{2+}$.

Anal. Calcd for $C_{24}H_{45}N_3O_8$: C, 57.24; H, 9.01; N, 8.34. Found: C, 57.17; H, 9.24; N, 8.33. **Compound 4**: A solution of $GdCl_3 \cdot 6H_2O$ (0.148 g, 0.4 mmol) in H_2O (20 mL) was added dropwise to a solution of **3** (0.2 g, 0.4 mmol) in H_2O (100 mL) and NaOH (2 M, 5 mL); the pH of the reaction mixture was maintained at 12 by the constant addition of NaOH (2 M). After 6 h the solution was concentrated to 25 mL and absolute EtOH (100 mL) was added. The precipitate was washed several times with EtOH, filtered, and dried in vacuo to obtain the complex **4** as a white solid (0.12 g, 46%). ESI-MS: m/z = 219.20 $[M + Gd]^{3+}$. Anal. Calcd for $C_{24}H_{41}N_3O_8Gd \cdot 4H_2O$: C, 39.55; H, 6.78; N, 5.76. Found: C, 40.01; H, 6.93; N, 5.84.