Inorganic Chemistry Communications 51 (2015) 110–113

Contents lists available at ScienceDirect

Inorganic Chemistry Communications

journal homepage: www.elsevier.com/locate/inoche

Synthesis and characterization of a new polyaminocarboxylic macrocyclic ligand and its non-ion gadolinium complex. *In vitro* relaxivity studies at 0.2 T

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ARTICLE INFO

Article history:
Received 14 October 2014
Accepted 20 November 2014
Available online 21 November 2014

Keywords:
Magnetic resonance imaging
Macrocycle
Gadolinium
Relaxivity
Contrast agent

ABSTRACT

In this study a novel ligand with three carboxylic groups (H_3L^1) was synthesized. Its non-ion complex with GdL^1 holding promise of magnetic resonance imaging (MRI) was synthesized, and the relaxivity of the complex determined. The relaxivity of GdL1 (R_1 = 4.5 $Mmol^{-1}\cdot s^{-1}$) was larger than that of $Gd(DTPA)^{2-}$(R_1 = 3.9 $Mmol^{-1}\cdot s^{-1}$). The results showed that the non-ion complex is a prospective MRI contrast agent.

Over the past two decades, magnetic resonance imaging (MRI) has become a very powerful tool of diagnostic medicine [1–3]. Paramagnetic materials have been investigated as MRI contrast agents. These materials enhance the contrast of the image indirectly by remarkably shortening the magnetic relaxation time of coordinated water protons coordinated compared with protons of the surrounding tissues [4–6]. The most frequently used contrast agents are stable gadolinium complexes with hydrophilic poly(aminocarboxylate) ligands, which result in rapid extracellular distribution and renal elimination. Gd(III) is preferred because of its favorable magnetic properties.

The acyclic complex $[Gd(DTPA)(H_2O)]^{2-}$ (Gd(DTPA) (DTPA = diethylenetriamine-N,N,N,N,N-pentaacetic acid), commercially known as Magnevist®, was the first contrast-enhancing agent to be approved for *in vivo* use in MRI [7]. However, its ionic characteristic led to some side effects associated with its hyperosmolality at clinical dose. Different $[Gd(DTPA)(H2O)]^{2-}$ derivatives were also introduced into clinical practice with the aim to improve the properties of the probe [8–10]. In addition, the contribution of cyclic structure to the stability of lanthanide complexes has been evaluated on different ligand platforms and some indications of their relative stability are now available [11,12].

In this respect, R.D. Hancock observed that the addition of neutral oxygen donors, whether as part of pendent donor groups or a macrocyclic ring, leads to an increase in complex stability for metal ions with ionic radius larger than 1 Å [13]. Different research groups have used the 18-membered crown ethers 18-crown-6, dibenzo-18-crown-6, and 1,10-diaza-4,7,13,16-tetraoxa-cyclooctadecane, as secondary ligands to improve the separation of adjacent tervalent lanthanide metals from their mixture [14–16].

With these results in mind, we reasoned that the replacement of the two CH_2COOH moieties on N-1 and N-7 (1,4,7-triazaheptane numbering) by 1,3-bis(2-ethylphenoxy)propan-2-ol groups in the DTPA structure might lead to a non-ion efficient complex formed from these ligands and the paramagnetic metal ion Gd(III) according to charge law. This may be a new type of potential MRI contrast agent with low osmotic pressure due to the non-ion complex.

The ligand was synthesized following the synthetic route shown in Scheme 1.

The ^1H and ^{13}C NMR spectral data of compounds **1** and **2** have been previously reported [17]. Compound **3** and **H_3L^1–CF_3COOH** were characterized by spectral data (Infrared (IR), uni- and bi-dimensional nuclear magnetic resonance spectroscopy, electrospray ionization-high resolution mass spectrometry (ESI-HRMS)), and elemental analysis.

1,3-Bis(2-formylphenoxy)-2-propanol (**1**): 230 mg (2.5 mmol) of epichlorohydrin and a magnetic stirrer bar were added to a solution of salicylaldehyde (610 mg, 5 mmol, 2 equiv) and anhydrous K_2CO_3 (340 mg, 2.5 mmol) in DMF (10 mL). The mixture was heated at reflux for 12 h. The reaction mixture was cooled to room temperature, the solvent was evaporated under reduced pressure and the solid residue redissolved in CH_2Cl_2 (10 mL) and extracted with water (3 × 5 mL). The organic phase was dried over anhydrous sodium sulfate. After evaporation of the solvent, the crude product was purified by recrystallization from benzene. 1,3-Bis(2-formylphenoxy)-2-propanol was finally obtained as pale yellow needles. Yield: 60% (0.449 g, 1.5 mmol). IR

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http://dx.doi.org/10.1016/j.inoche.2014.11.019
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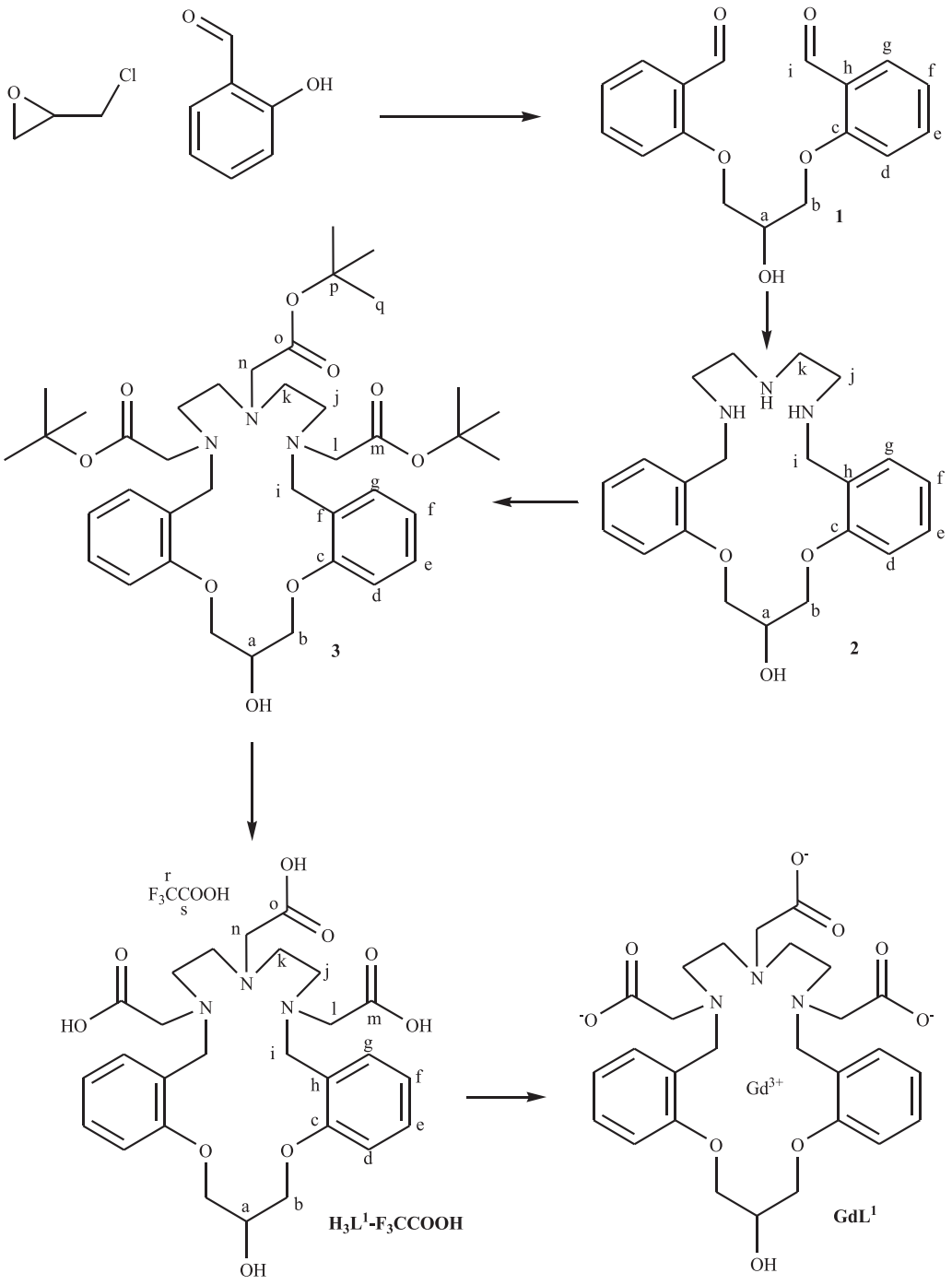

Scheme 1. Synthesis route of $\mathbf{H_3L^1}$ and the $\mathbf{L^1}$-**Gd** complex.

(cm^{-1}): ν = 757, 1030, 1241, 1691, 3452. ^{1}H NMR (200 MHz, $CDCl_3$): δ = 4.32 (d, 4H, H_b), 4.51 (m, 1H, H_a), 7.05 (m, 4H, H_d and H_f), 7.56 (m, 2H, H_e), 7.79 (m, 2H, H_g), 10.39 (s, 2H, H_i). ^{13}C NMR ($CDCl_3$): δ 64.5 (C_a), 69.3 (C_b), 112.8 (C_d), 121.3 (C_e), 124.9 (C_f), 129.592 (H_h), 160.3 (C_c), 189.7 (H_i). EI-MS: 300 Da.

1,13,16-Triaza-5,9-dioxa-7-hydroxy-3,4:l0,11-dibenzocyclooctadeca-3,10-diene (**2**): diethylene triamine (206 mg, 2 mmol) in methanol (5 mL) was added to a boiling solution of **1** (592 mg, 2 mmol) in methanol (10 mL). The resulting mixture was refluxed with stirring for 3 h and cooled over ice bath, and then solid $NaBH_4$ (1.125 g, 15 mmol) was carefully added in portion. After 2 h, the solution was concentrated to approximately 5 mL in a rotary evaporator and the volume was increased 3-fold by the addition of crushed ice. Stirring was continued at room temperature for 12 h in an open beaker. The solution was extracted with CH_2Cl_2 (3 × 10 mL), and the organic layer was dried over anhydrous sodium sulfate and then taken to dryness in a rotary evaporator. Compound **2** was obtained as a pale yellow solid (0.631 g, 1.7 mmol, Yield: 85%). IR (cm^{-1}): ν = 756, 1243, 1033, 1600, 3202, 3298, 3360. ^{1}H NMR (200 MHz, $CDCl_3$): δ 2.71 (s, 4H, H_i), 3.30 (m, 4H, H_m), 3.75 (m, 4H, H_l), 4.20 (m, 5H, H_a and H_b), 7.10 (m, 4H, H_d and H_f), 7.59 (m, 2H, H_e), 7.77 (m, 2H, H_g). ^{13}C NMR (CDCl3): δ 49.6 (C_i), 49.8 (C_k), 51.3 (C_l), 68.2 (C_a), 70.6 (C_b), 113.3 (C_d), 121.0 (C_f), 128.3 (C_e), 128.6 (C_g), 130.8 (C_h), 157.7 (C_c). EI-MS: 371 Da.

Tri *tert*-butyl 2,2′,2"-(20-hydroxy-7,8,10,11,20,21-hexahydro-5*H*,19*H*dibenzo[1,15,5,8,11]dioxa triazacyclooctadecine-6,9,12-triyl) triacetate (**3**): *tert*-butyl bromoacetate (1.088 g, 5.6 mmol) was added

to a solution of the crude macrocyclic triamine **2** (631 mg, 1.7 mmol) and di-*iso*-propylethylamine (987 mg, 7.6 mmol) in acetonitrile (15 mL). The mixture was stirred for 24 h at 70 °C. The crude mixture was concentrated, then taken up in CH_2Cl_2 (60 mL) and water (15 mL). The organic layer was further washed with water (2 × 10 mL), dried and concentrated. The residue obtained was purified by chromatography on silica gel (EtOAc/hexane, 9:1 v/v) to give the title compound **3** as a pale yellow oil (0.785 g, 1.1 mmol, Yield: 65%). TLC: EtOAc/Hexane (9:1, v/v), Rf = 0.8, irradiation with UV light, I_2, Dragendorff. Yield: 77% (0.432 g, 0.69 mmol). Anal. Calcd for $C_{39}H_{59}N_3O_9$ (H_3L^1): C, 51.40; H, 5.65; F, 8.41; N, 6.20; O, 28.33. Found: C, 51.32; H, 5.70; F, 8.40; N, 6.20; O, 28.32 IR (cm^{-1}): ν 1740. ^1H NMR (500 MHz, $CDCl_3$): δ = 1.44 (s, 27H, H_q) 2.83 (m, 8H, H_j and H_i), 3.21 (m, 4H, H_k), 3.82 (m, 8H, H_n and H_n), 4.32 (m, 5H, H_a and H_b), 6.80 (m, 4H, H_d and H_f), 7.16 (m, 2H, H_g), 7.65 (m, 2H, H_e). ^{13}C NMR ($CDCl_3$): δ = 28.2 (m, C_q) 48.5 (m, C_j); 49.5 (C_i); 51.4 and 51.5 (C_k); 54.6, 55.1, 55.4 and 56.3 (C_n); 57.4, 57.8 and 61.1 (C_l); 68.1, 68.2, 68.3, 68.4 and 68.6 (C_b); 72.2, 72.4 (C_a); 80.8 (C_p); 113.1, 113.2, 113.3 and 113.5 (C_d); 117.0, 117.3 and 117.5 (C_e); 120.6 (C_f); 127.2, 128.4 and 129.0 (C_g); 131.7 and 131.8 (C_h); 158.0 and 158.2 (C_c); 170.4 and 170.5 (C_m and C_o).

2,2′,2"-(20-hydroxy-7,8,10,11,20,21-hexahydro-5*H*,19*H*-dibenzo[b,m] [1,15,5,8,11]dioxatriazacyclooc tadecine-6,9,12-triyl)triacetic acid trifluoroacetate salt, monohydrate [**H_3L^1–F_3CCOOH**]·**H_2O**: 5 mL of trifluoroacetic acid (TFA) was added to a solution of **3** (642 mg, 0.9 mmol) in 10 mL of CH_2Cl_2 and stirred for 48 h. The reaction mixture was concentrated in a rotary evaporator, and the residue was dissolved in methanol (2 mL) and added to diethyl ether (7 mL) under vigorous stirring. Finally, it was dried in vacuum with P_4O_{10} recrystallized from H_2O–Et_2O–EtOH and [**H_3L^1–F_3CCOOH**]·**H_2O** was obtained as a white solid. Yield: 77% (0.432 g, 0.69 mmol). Anal. Calcd for $C_{29}H_{38}F_3N_3O_{12}$ ([H_3L^1–F_3CCOOH]H_2O): C, 51.40; H, 5.65; F, 8.41; N, 6.20; O, 28.33. Found: C, 51.32; H, 5.70; F, 8.40; N, 6.20; O, 28.32. IR (cm^{-1}): ν = 1732, 1671. ^1H NMR (500 MHz, D_2O): δ = 2.92 (m, 4H, H_j), 2.95 (m, 4H, H_i), 3.33 (m, 4H, H_k), 3.93 (m, 2H, H_n), 4.34 (m, 4H, H_b), 4.50 (m, 4H, H_l), 4.60 (m, 1H, H_a), 7.1 (m, 2H, H_d), 7.2 (m, 2H, H_f), 7.4 (m, 2H, H_g), 7.6 (m, 2H, H_h). ^{13}C NMR (D_2O): δ = 48.5 (m, C_j); 49.5 (C_i); 51.4 and 51.5 (C_k); 54.6, 55.1, 55.4 and 56.3 (C_n); 57.4, 57.8 and 61.1 (C_l); 68.1, 68.2, 68.3, 68.4 and 68.6 (C_b); 72.2, 72.4 (C_a); 112.5 and 112.6 (C_d); 115.4 (q, J = 291 Hz, C_r); 117.0, 117.3 and 117.5 (C_e); 121.8 and 121.9 (C_f); 128.4 and 129.0 (C_g); 132.7, 133.1 and 133.2 (C_h); 157.0, 157.2 and 158.2 (C_c); 162.732 (q, J = 35 Hz, C_s); 168.9, 169.0 and 169.1 (C_m and C_o). ESI-MS: 604.24872 Da. $[M + 2H_2O + Na]^+$.

The corresponding Gd complex **$GdL^1 \cdot H_2O$** was obtained as follows. Its structure was confirmed by elemental analyses, ESI-HRMS and FT-IR spectroscopy. Its metal content was determined by titration with EDTA in elemental analyses.

The initial pH of a solution of macrocyclic ligand **H_3L^1** (0.150 g, 0.228 mmol) in deionized water (5 mL) was adjusted to pH 5.7 with an aqueous solution of KOH 0.5 M. Gadolinium(III) chloride hexahydrate (0.085 g, 0.228 mmol) was added in one portion, which induced a decrease of pH to pH 4.2 so that pH was readjusted to 5.8 (KOH 0.5 M). The resulting mixture was warmed at 80 °C for 24 h. The pH was then increased to 8.7 (KOH 0.5 M). A white precipitate appeared after prolonged stirring at room temperature, and persisted by decreasing the pH to 8.2. The precipitate was filtered off on a 0.2 mm PES filter (Waters, WAT200539). The clear yellow aqueous filtrate was concentrated (2.0 mL) and CH_3CN (10 mL) was then added. A solid stuck to the reaction flask was formed. The filtrate was removed and a second portion of CH_3CN (15 mL) was poured. After vigorous stirring, the powder formed was filtered on a hardened ashless filter paper (Whatman, 542) to give the gadolinium complex as a white solid. Yield: 52% (0.122 g, 0.118 mmol). Anal. Calcd for $C_{27}H_{34}N_3O_{10}Gd$: C, 45.18; H, 4.77; N, 5.85; and Gd, 21.91. Found: C, 45.15; H, 4.82; N, 5.79; and Gd, 21.98. IR (cm^{-1}): ν = 1686, 1609. ESI-MS: 699.13296 Da. $[M - H]^-$.

The mass spectrum (negative mode) contained the signal corresponding to the ion $[(\mathbf{GdL^1})–H]^-$ = 699.13296 Da (theoretical m/z value: 699.13232 Da) with appropriate isotope pattern distribution characteristic of Gd^{3+} and its complexes [18].

The infrared spectrum of **GdL^1** showed a band corresponding to C═O vibration stretches with the maximum of the band at 1686 and 1610 cm^{-1}, a lower wavenumber than the C═O vibration stretch in the infrared spectrum of the ligand **H_3L^1–CF_3COOH** (1732 and 1671 cm^{-1}). This indicates that, as expected, the C═O bonds of the three carboxylate groups lengthened and weakened upon complexation. Meanwhile, the Ar – O – CH_2 vibrations in the complex were also shifted to a lower frequency, which shows the coordination effect of the oxygen atoms from the Ar – O – CH_2 and groups with the Gd^{3+} ion [19].

For measurements of the relaxivity R_1, four different concentrations of 0.18, 0.35, 0.7, and 1 mM of the **GdL^1** and Gd-DTPA were prepared in tubes using physiological solution as the solvent.

Relaxivity was measured on an NMR0.2 T relaxometer (Unit of Microanalysis and Physical Methods Applied to Organic Chemistry, Argentina) at 0.2 T. The temperature in the sample holder was held at 37 °C with an air stream. Gradient-echo sequence was used to study their R_1 relaxivities with TE = 6 ms and TR = 4, 2 s, 1 s, 200 ms, 150 ms, 100 ms, 50 ms, 25 ms and 18 ms. The background noise effect was removed by subtracting the mean intensity signal with mean intensity signal noise. A non-linear fitting was used to fit the value T_1 based on the signal form $S = S_0 * *(1 - \exp(-TR/T_1))$ [20].

The enhancement value of the relaxation rate of the complex for water protons is calculated by the equation [21].

$$(1/T_1)_p = (1/T_1)_0 - (1/T_1)_d$$
$$R_1 = (1/T_1)_p/[M]$$

where $(1/T_1)_0$ is the observed solvent relaxation rate in the presence of a paramagnetic species, $(1/T_1)_d$ is the solvent relaxation rate in the absence of a paramagnetic species, and $(1/T_1)_p$ represents the additional paramagnetic contribution. [M] is the concentration of complexes.

The relaxivity consists mainly of two components: the inner-sphere and the outer-sphere relaxivity. The high relaxivity is favorable for tissue imaging. The values of R_1 observed (3.9 Mm^{-1} s^{-1}) at 0.2 T and 37 °C for Gd(DTPA) were similar to those previously reported [20], while a value for **GdL^1** R_1 = 4.5 Mm^{-1} s^{-1} was obtained.

In conclusion, these data demonstrate the potential of the newly synthesized complex as a prospective MRI contrast agent. Since **GdL^1** is a non-ion complex, it may be preferable to Gd-DTPA in some applications, for example when crossing of lipid membranes is desired *in vivo*.

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