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MRI Contrast Agents: Macrocyclic Lanthanide(III) Complexes with Improved Relaxation Efficiency

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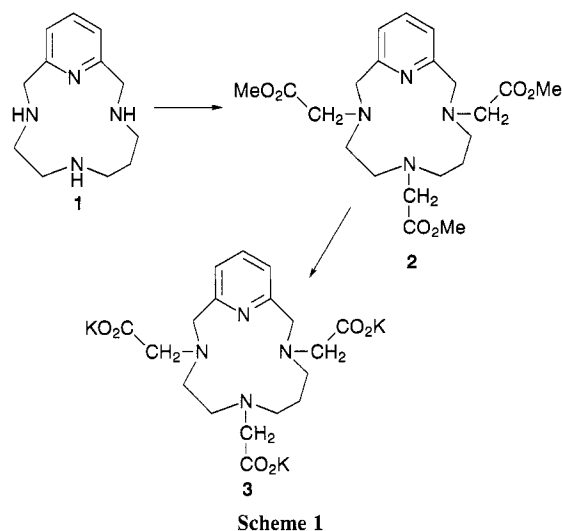
Lanthanide(III) complexes of a novel heptadentate chelating ligand display structural and dynamic properties that are particularly interesting in view of potential applications in magnetic resonance imaging (MRI).

The search for new contrast agents (CA) for MRI is currently orientated towards the synthesis of paramagnetic complexes, possibly neutral, of increasing ability to enhance the water proton relaxation rates, while maintaining the favourable complexing properties of dota and dtpa-like ligands.^{1†} We report here the synthesis and solution state NMR characterisation of a novel macrocyclic ligand **3** and its complexes with Eu^{III}, Yb^{III} and Gd^{III} ions which satisfy both requirements. Ligand **3** was synthesised as shown in Scheme 1.

3,6,10,16-Tetraazabicyclo[10.3.1]hexadeca-1(16),12,14-triene **1** was prepared according to the procedure of Costa and Delgado.² The three carboxymethyl moieties on N³, N⁶ and N¹⁰ were introduced by the reaction of compound **1** with 3 equiv. of methyl bromoacetate in the presence of silver(I) carbonate in THF at room temperature. The reaction mixture was filtered through Celite, the filtrate evaporated to dryness and the residue acidified with hydrochloric acid. After addition of sodium sulfide, the silver(I) sulfide precipitated was filtered off. The solution was made basic to pH 11 and extracted with dichloromethane. The resulting triester **2**[‡] was obtained in 80% yield.

By treating compound **2** with 3 equiv. of KOH in methanol, the potassium salt of the triacid **3**[§] was isolated in quantitative yield and used for the preparation of the Ln^{III} complexes. Eu^{III}, Yb^{III} and Gd^{III} complexes of ligand **3** were prepared by mixing stoichiometric amounts of the ligand and the lanthanoid(III) chloride at neutral pH.

The high-temperature-limit 400 MHz ¹H NMR spectrum of the Eu^{III}-**3** complex (363 K) shows 23 resonances [Fig. 1 (A)] spread over a range of ca. 30 ppm. The resonances could be assigned to the 23 different protons by use of homo- and hetero-correlated 2D experiments carried out at 2.1 T in order to minimize the magnetic-field-induced line-broadening.³ On cooling to room temperature and below, all resonances markedly broaden, indicating that a dynamic process is slowing down although it was not possible to obtain the spectrum corresponding to the 'frozen' structures.



Analogous behaviour was observed in the variable-temperature ¹³C NMR spectra. The observed exchange process may be accounted for in terms of an interconversion mechanism analogous to that reported for Ln^{III}-dota complexes⁴ (Scheme 2).

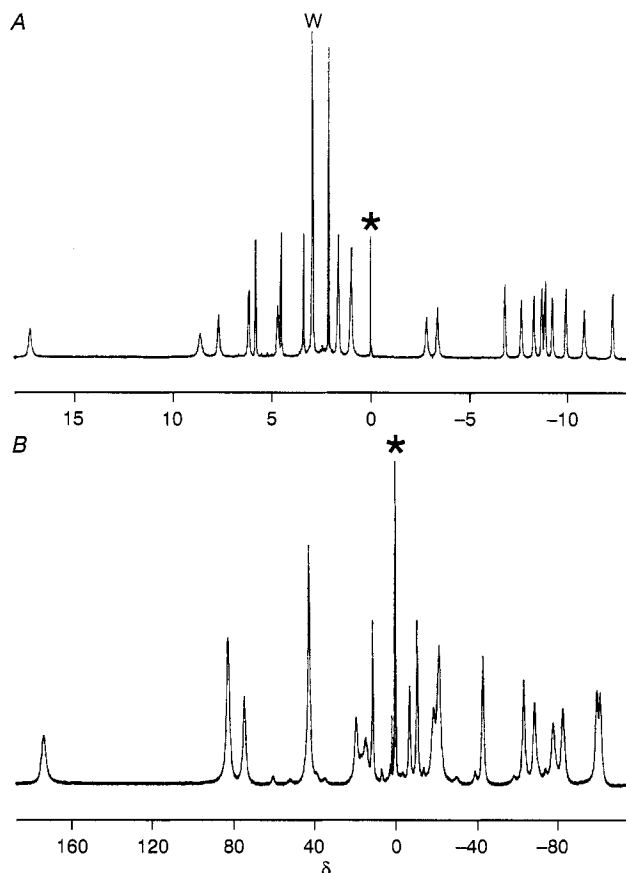
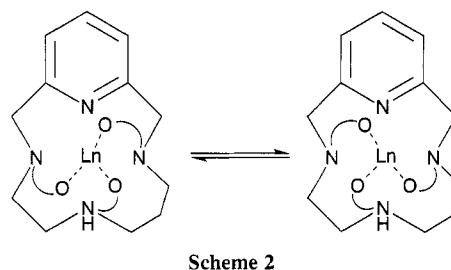


Fig. 1 (A) ¹H NMR spectrum of Eu-**3** recorded at 9.4 T (400 MHz) and 363 K in D₂O at pH 7. (B) ¹H NMR spectrum of Yb-**3** recorded at 2.1 T (90 MHz) and 266 K in D₂O, at pH 7 with presaturation of the solvent peak. In both spectra, the resonance labelled * refers to Bu¹OH (ca. 1%) added as an internal reference [δ (ppm) = 0]. In the Eu-**3** spectrum the additional peak labelled W refers to HDO.



There are, however, two main differences between the complexes with dota and those with the heptadentate ligand **3**: (i) the flexibility of the macrocycle is partially lost following the introduction of the pyridine moiety in the macrocyclic ring; (ii) the layout of the acetate arms is affected by the asymmetry in the macrocycle. This means that the two interconverting isomers may have quite different populations. This has been proved in the low-temperature-limit ^1H NMR spectrum of the Yb-**3** complex whose higher spread of chemical shifts allows the observation of the separate resonances of the two isomers [Fig. 1(B)]. The isomeric ratio is about 15:1 at 266 K. As the temperature is increased an exchange takes place between the two isomers. As expected the broadening is much more severe for the minor isomer whose resonances quickly disappear in the spectral noise. The large frequency separation between the exchanging sites in the Yb-**3** complex does not allow the condition of fast exchange to be obtained as some resonances are still rather broad at the highest attainable temperature (363 K).

The thermodynamic formation constant for the Eu^{III} complex was estimated through competition experiments between ligand **3** and Eu^{III} -cdta ($\log K = 19.5$).⁵ From the intensities in the ^1H NMR spectrum of the species **3**, Eu-**3**, cdta and Eu-cdta in several experiments carried out with different concentrations of **3** and Eu-cdta we obtained a K_f value of $3 \pm 2 \times 10^{18}$. The good thermodynamic stability calculated for this complex prompted us to investigate the relaxometric properties of the Gd^{III} -**3** complex in view of its potential application as a contrast agent for MRI. The relaxivity of Gd^{III} -**3** complex was found to be $6.3 \text{ mmol dm}^{-3} \text{ s}^{-1}$ (20 MHz; 298 K), about 35% higher than for Gd-dota and similar to that reported for Gd-do3a.^{1e} This is in good agreement with the value of 7.64 obtained in plasma at 40 °C reported in a patent dealing with a closely related Gd^{III} complex containing a 12-N-4 ring moiety.⁶

The observed relaxivity value is consistent with the presence of two exchangeable water molecules in the inner coordination sphere of the complex. More insight into the paramagnetic relaxation pathway was gained from the analysis of the NMRD (nuclear magnetic resonance dispersion) profile obtained from the measurement of the solvent proton relaxation rate over a wide range of proton Larmor frequencies (0.01–50 MHz).⁷ The fitting of the experimental data to the values calculated on the basis of the Solomon–Bloembergen–Morgan⁸ (for the inner sphere contribution) and Freed⁹ (for the outer sphere contribution) equations for the paramagnetic relaxation yields the following parameters: τ_{SO} (electronic relaxation time at zero field) = $1.41 \times 10^{-10} \text{ s}$; τ_v (correlation time responsible for the modulation of τ_{SO}) = $3.20 \times 10^{-11} \text{ s}$; τ_M (exchange lifetime of the coordinated water) = $5.00 \times 10^{-9} \text{ s}$; r (H_2O –Gd distance) = $3.20 \times 10^{-8} \text{ cm}$; a (distance of closest approach of the water molecules diffusing in the proximity of the complex) = $3.80 \times 10^{-8} \text{ cm}$; D (relative diffusion coefficient for water and complex) = $2.24 \times 10^{-5} \text{ cm}$.

The presence of two coordinated water molecules is responsible for both the enhanced relaxivity and the rather short value for τ_M thus ruling out a possible quenching effect on the relaxation efficiency caused by long exchange lifetimes as recently shown to occur in related neutral Gd^{III} complexes containing a single coordinated water molecule.¹⁰

An insight into the kinetic stability of this complex has been gained by the observation that R_{1p} does not vary in the pH range 3–9. At lower pHs, the acid-promoted dissociation occurs to a significantly lower extent than in Gd-dtpa. Furthermore, the

relaxivity in serum was constant after several hours at ambient temperature.

In conclusion we think that the structural, dynamic, thermodynamic and relaxometric properties of this novel class of lanthanoid(III) complexes make them potential candidates for the development of new, neutral contrast agents for MRI. Furthermore the inclusion of the pyridine moiety in the macrocycle will provide an anchoring site for the introduction of a number of functionalities on the surface of the complex aimed at an improved specificity in the targeting of tissue and organs.

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Footnotes

† dota = 1,4,7,10-tetraazacyclododecane-*N,N',N'',N'''*-tetraacetic acid, dtpa = diethylenetriaminepentaacetic acid, cdta = 1,2-diaminocyclohexane-*N,N,N',N'*-tetracetic acid, do3a = 1,4,7,10-tetraazacyclododecane-1,4,7-triacetic acid.

‡ MS(EI): m/z (rel. int.) 436 (M^+ , 28); ^1H NMR (200.13 MHz; CDCl_3): δ 1.69 (m, 8 H), 3.21 (s, 2 H), 3.48 (s, 2 H), 3.52 (s, 2 H), 3.65 (s, 3 H), 3.73 (s, 3 H), 3.74 (s, 3 H), 7.16 (d, 1 H, J 7.5 Hz), 7.18 (d, 1 H, J 7.4 Hz), 7.62 (t, 1 H, J 7.4 Hz); ^{13}C NMR (50.32 MHz; CDCl_3): δ 23.4 (CH_2), 47.9 (2 CH_2), 50.5 (CH_2), 50.9 (CH_2), 51.3 (CH_3), 51.5 (2 CH_3), 56.1 (CH_2), 56.9 (2 CH_2), 59.0 (2 CH_2), 122.4 (2 CH), 136.6 (CH), 157.0 (C), 157.2 (C), 171.6 (C).

§ ^1H NMR (200.13 MHz; D_2O): δ 1.21 (m, 2 H), 2.10–2.45 (m, 8 H), 2.77 (s, 2 H), 3.04 (s, 2 H), 3.08 (s, 2 H), 3.64 (s, 4 H), 7.19 (d, 2 H, J 7.6 Hz), 7.64 (t, 1 H, J 7.6 Hz); ^{13}C NMR (50.32 MHz; D_2O): δ 23.3 (CH_2), 51.2 (CH_2), 51.8 (CH_2), 53.7 (CH_2), 54.4 (CH_2), 60.6 (CH_2), 61.2 (CH_2), 61.6 (CH_2), 62.6 (CH_2), 126.2 (2 CH), 140.8 (CH), 159.2 (C), 159.4 (C), 179.5 (C), 181.1 (C), 181.2 (C)

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