Structure and relaxivity of macrocyclic gadolinium complexes incorporating pyridyl and 4-morpholinopyridyl substituents

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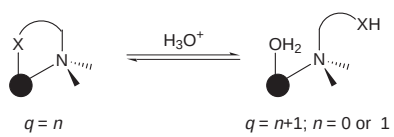
Received* (*in Cambridge, UK*) *3rd March 1999, Accepted 7th May 1999

Octadentate N_5O_3 ligands containing a coordinating pyridyl group form kinetically stable complexes with Eu and Gd that resist protonation; the structure of a representative Gd complex reveals a short Gd–N_{py} bond [2.535(1) Å], and the water exchange rate ($k_{ex}^{298} = 2.7 \times 10^6$ s^{-1}) is sufficiently fast so as not to limit the pH independent relaxivity ($R_{1p}^{298} = 5.3$ mM^{-1} s^{-1}, 20 MHz).

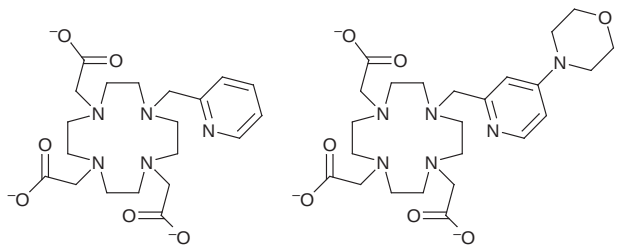
Whilst the first generation of paramagnetic contrast agents† *e.g.* $[Gd(dota)]^-$ and $[Gd(dtpa)]^{2-}$ function effectively as general-purpose extracellular agents for magnetic resonance imaging,[1] there is continued interest in the development of paramagnetic complexes whose relaxivity (the increment of the water proton relaxation rate per unit concentration) is a function of a defined biochemical variable.[2] Complexes which respond sensitively to local pH are of particular interest as they may afford a means by which the more acidic extracellular region in tumour tissue is distinguished from healthy tissue.[3] Therefore mechanisms by which the relaxivity of a given complex increase with acid concentration are potentially particularly important.[4] Among several possible means of achieving this—including the pH dependent labilisation of slow-exchanging bound water[4,5] and acid catalysis of prototropic exchange in the vicinity of the paramagnetic centre—an attractive mechanism is based upon a pH dependent change in ligand coordination number (Scheme 1). Thus protonation of a ligand donor atom (usually O or N) may lead to competitive binding of an additional water molecule giving a possible relaxivity increase of up to *ca.* 100%. In order to maintain sufficient complex stability to avoid premature Gd dissociation, the effective ligand coordination number should only change from 9 to 8 or from 8 to 7. With this background in mind, we have embarked upon the study of a series of such complexes in order to pinpoint suitable donor sites that are amenable to reversible protonation in the Gd complex over the pH range 5–7. The synthesis and relaxivity behaviour of two pyridine-containing ligands[6] have now been assessed, using pyridines of differing charge density, *i.e. a* 2-methylpyridine derivative L^1 and a subsituted 4-morpholinopyridine, L^2.

The synthesis of the target ligands involved alkylation of the tri-ester **3** with the protonated benzylic halide **4** or **5**

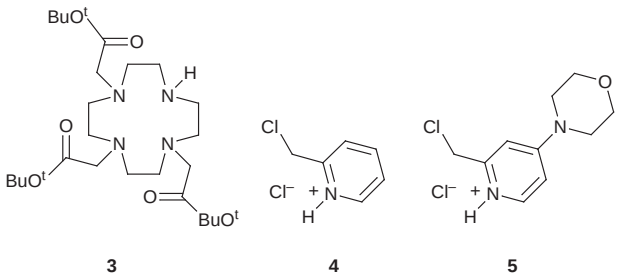


Scheme 1

(MeCN, Na_2CO_3, 18 h, 65 °C) followed by purification by chromatography on silica [CH_2Cl_2–MeOH (96 : 4)]. The resultant alkylated triester was deprotected (CF_3CO_2H, CH_2Cl_2, 18 h, 20 °C) and the lanthanide complex formed by reaction with $Ln(OAc)_3$ at pH 6 (90 °C, 6 h, Ln = Eu, Gd). The neutral complex was conveniently purified by chromatography on neutral alumina (80% CH_2Cl_2–20% MeOH) and the complexes were crystallised from cold ether. Crystallographic analysis of the Eu and Gd complexes of L^2 revealed that they were isostructural and the crystal structure of $[GdL^2(OH_2)] \cdot 9H_2O$ showed a mono-capped square antiprismatic coordination geometry (Fig. 1).‡ The Gd–OH_2 distance was 2.400(1) Å and remaining Gd–O bond lengths averaged 2.369 Å. The pyridyl N–Gd bond was quite short [2.53(1) Å] in comparison to the other Gd–N distances which averaged 2.665 Å. Torsion angles at the N–C–C–N (58.7°) and N–C–C–O bonds (−28.5°) were typical of those found in Λ(δδδδ) square antiprismatic lanthanide complexes.[7] In $[EuL^2]$, the rate of decay of the metal based emission was measured in H_2O [$k = 1.69$ $(ms)^{-1}$] and in D_2O [$k = 0.56$ $(ms)^{-1}$], allowing the corrected hydration number to be calculated[8] to be $q = 1.06$. Similar values were measured for $[EuL^1]$ confirming the crystallographic bound hydration state. The measured rates in H_2O and D_2O were the same at pD 3 and 8, suggesting that over this pH range no change in hydration number was occurring. Analysis of the proton NMR spectra[9] of $[EuL^1]$ and $[EuL^2]$ (D_2O, 293 K, 200 MHz) revealed the presence of



L^1 L^2



3 **4** **5**

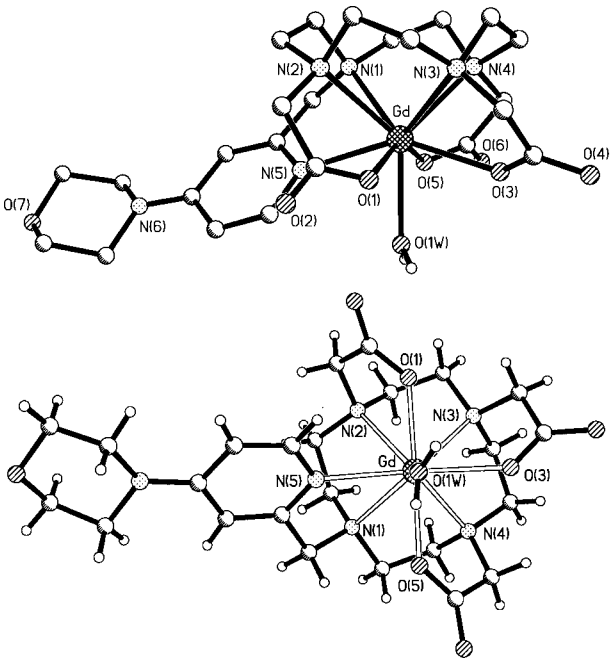


Fig. 1 Views of the structure of [GdL^2] in the crystal (120 K).

two diastereoisomeric species in ratio 2 : 1 and 2.5 : 1 respectively with characteristic shifts for the ring axial and equatorial protons corresponding to the regular square-antiprismatic and twisted square-antiprismatic (minor) complexes. The spectra were unchanged over the pD range 2.5–11.0.

Variable temperature ^{17}O NMR studies on aqueous samples of [GdL^2] (25.5 mM complex, 10% enriched $^{17}OH_2$, 12 MHz, 298 K) gave transverse relaxation rates whose variation with temperature allowed an estimation of the ^{17}O mean water exchange rate (Fig. 2) on and off the Gd paramagnetic centre.[10] The rate of exchange was estimated to be 2.7×10^6 s^{-1} and is similar to that found for related neutral complexes, for example with a *N*-2-hydroxypropyl substituent.[11] This relatively high value may be related to the relatively high proportion of the twisted square-antiprismatic complex in solution. Related complexes that possess a high proportion of the twisted square-antiprismatic isomer in solution have been reported to undergo water exchange considerably faster than those in which the diastereoisomeric square-antiprismatic complex predominates.[12,13]

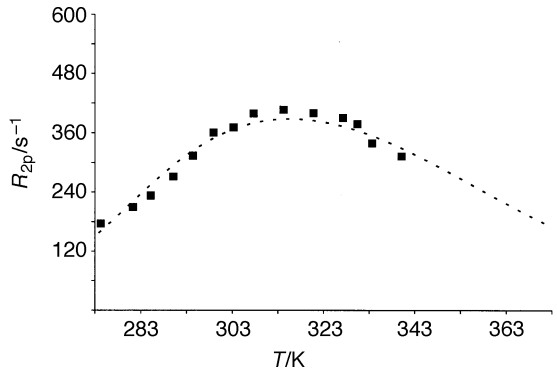


Fig. 2 Variation of the ^{17}O transverse relaxation rate (298 K, 12 MHz) with temperature showing the least-squares fitting to the experimental data ($\Delta^2 = 8.7 \times 10^{20}$ s^{-2}, $\tau_{vo} = 2.5$ ps, $\Delta H^{\#} = 45.3$ kJ mol^{-1}, $k_{ex} = 2.7 \times 10^6$ s^{-1}, $\tau_r = 86$ ps, $A/\hbar = -3.8 \times 10^6$ rad s^{-1}).

The relaxivity of [GdL^2] and [GdL^1] was measured as a function of pH and field (NMRD profile). Similar pH and field dependent behavior was observed for each complex and for [GdL^2], the measured relaxivity value, $R_{1p} = 5.3$ mM^{-1} s^{-1} (298 K, 20 MHz). This value was almost unchanged (± 0.3 mM^{-1} s^{-1}) over the pH range 2.5 to 12.5. Evidently the pyridyl nitrogen is an excellent donor for the Gd^{3+} ion in such tribasic, N_5O_3 octadentate ligands, and is not susceptible to protonation. Therefore in seeking appropriate donor groups which may serve to exemplify the process depicted in Scheme 1, less strongly ligating substituents are required, and are being studied at present.

Acknowledgements

We thank EPSRC, the EC COST-D8 action group for support and the Royal Society for a Leverhulme Trust Senior Research Fellowship (DP).

Notes and references

† dota = 1,4,7,10-tetraazacyclododecanetetraacetate; dtpa = diethylenetriaminepentaacetate.

‡ Crystal data for $C_{24}H_{55}GdN_6O_{17}$, $M = 856.99$, triclinic, space group $P\bar{1}$, $a = 8.914(1)$, $b = 9.478(1)$, $c = 21.317(1)$ Å, $\alpha = 81.65(1)$, $\beta = 80.57(1)$, $\gamma = 83.54(1)°$; $U = 1750.6(3)$ Å^3, $D_c = 1.626$ g cm^{-3}, λ(Mo-Kα) = 0.71073 Å, $Z = 2$, $\mu = 1.97$ mm^{-1}. Data were collected on a Siemens SMART at 120(2) K. Refinement of 672 parameters by full matrix, least squares on F^2 (SHELX 96) converged at $R = 0.018$, $wR_2 = 0.042$ for 8881 observed reflections with $I > 2\sigma(I)$. CCDC reference number 440/112.

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Letter 9/01704F