

^{19}F NMR based pH probes: lanthanide(III) complexes with pH-sensitive chemical shifts†

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Experimental measurements and theoretical analysis of magnetic properties, structural dynamics and acid–base equilibria for several lanthanide(III) complexes with tetraazacyclododecane derivatives as ^{19}F NMR chemical shift pH probes are presented; $\text{p}K_{\text{a}}$ values vary between 6.9 and 7.7, with 18 to 40 ppm chemical shift differences between the acidic and basic forms for Ho(III) complexes possessing T_1 values of 10 to 30 ms (4.7–9.4 T, 295 K).

^{19}F Magnetic resonance probes offer considerable scope for biological and biomedical studies.^{1–6} The absence of a background signal, the high sensitivity of ^{19}F NMR spectroscopy and the ability to acquire spectra or images on instruments with similar probes and frequencies to ^1H NMR spectroscopy, each suggest an important role for such probes. Significant progress has been made recently,^{6,7} but some difficulties remain, most notably with longitudinal ^{19}F relaxation times, which are typically of the order of 1 to 2 seconds at high magnetic fields.^{2,8} In high-field ^{19}F MRI studies in biosystems, high concentrations (~ 50 mM) are currently required in order to obtain reasonable signal intensities on the MRI experiment timescale.^{9,10}

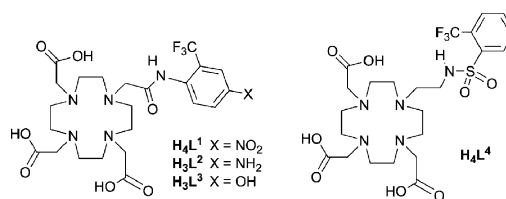
One way to surmount these difficulties involves creating a paramagnetic centre (such as an open-shell lanthanide ion) within 7 Å of the fluorine nucleus. This enhances the rate of ^{19}F relaxation, predominantly *via* dipolar mechanisms,^{11,12} by about two orders of magnitude.¹³ As an added benefit, the pseudocontact shift (PCS)¹² induced by the Ln^{3+} ion amplifies the ^{19}F chemical shift sensitivity to minor structural changes. A balance needs to be maintained between the benefits of relaxation enhancement and the reduced detection sensitivity associated with the broader line width. The signal intensity gain by using Tm, Dy or Tb complexes (compared to diamagnetic Y(III) analogues) in ^{19}F NMR spectroscopy has been estimated to be 20–50 fold, bringing the practicable use of ^{19}F imaging experiments within grasp¹³ with sub-millimolar probe concentrations.

We describe two classes of pH-sensitive paramagnetic complexes that can serve as ^{19}F chemical shift based pH probes. The ligands used in this work are shown in Scheme 1. They were prepared using previously published synthetic methodo-

logy.¹³ ^{19}F NMR chemical shifts and relaxation parameters at 9.4 T with selected lanthanide ions are listed in Table 1 (see ESI for further details on synthesis, basic properties and NMR relaxation data over the field range 4.7 to 16.5 T†).

The *para*-substituent at the aromatic ring of L^1 – L^3 ligands regulates the acidity of the amide hydrogen and therefore the pH sensitivity of the complex. Consistent with the low acidity ($\text{p}K_{\text{a}} > 9.5$) of the amide proton of L^2 and L^3 ligands (*para*- NH_2 and -OH groups, respectively), no pH dependence of the ^{19}F chemical shift of $[\text{Ln}\cdot\text{L}^2]$ and $[\text{Ln}\cdot\text{L}^3]$ was observed over the pH range of 3.5 to 9.0. The nitro-substituted L^1 ligand is significantly more acidic, and the NH group of $[\text{Ln}\cdot\text{L}^1]$ deprotonates, causing the CF_3 group of $[\text{Ho}\cdot\text{L}^1]$ to shift from -55.1 ppm to -36.8 ppm under basic conditions (Scheme 2). Because the acid–base equilibrium is fast, only one signal is observed, with the chemical shift corresponding to the weighted average of the chemical shifts of the individual forms. Fitting the acid–base equilibrium curve to the experimental chemical shift data (Fig. 1) resulted in a $\text{p}K_{\text{a}} = 7.77 \pm 0.02$, in 0.1 M NaCl solution.

While the longitudinal ^{19}F relaxation rate of $[\text{Ho}\cdot\text{L}^1]$ is nearly pH-independent, we observed an abrupt increase (by a factor of 8) in the linewidth (Table 1), which disappears upon heating, indicating the presence of an intermediate timescale chemical exchange process in the deprotonated complex. Two possible chemical exchange processes were considered: lanthanide re-coordination, from oxygen to nitrogen, or *cis*–*trans* isomerisation around the >C=N- double bond (Scheme 2). Based on DFT calculations,†lanthanide re-coordination can be ruled out, as the DFT energy difference between the two coordination isomers of $[\text{La}\cdot\text{L}^1]$ is 62 kJ mol⁻¹ in favour of the oxygen-bound isomer. However, the calculated energy difference between the *cis*- and *trans*-isomers is only 18 kJ mol⁻¹, making the *cis*–*trans* isomerisation a more likely explanation for the additional ^{19}F signals observed. Based on the saddle point energy, the forward and backward activation energies for the *cis*–*trans* isomerisation are 27 kJ mol⁻¹ and 45 kJ mol⁻¹, consistent with the exchange broadening observed in



Scheme 1

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† Electronic supplementary information (ESI) available: DFT calculations, calculated structures, relaxation analysis, synthesis and characterisation. See DOI: 10.1039/b802838a

Table 1 ^{19}F chemical shifts and relaxation parameters^a (295 K, 1 mM complex, D_2O) for lanthanide(III) complexes with ligands L^1 to L^4

Complex	$[\text{HoL}^1\text{H}]$	$[\text{HoL}^1]^-$	$[\text{HoL}^4\text{H}]$	$[\text{HoL}^4]^-$	$[\text{TbL}^2]$	$[\text{TbL}^3]^b$	$[\text{DyL}^3]^b$	$[\text{HoL}^3]^b$	$[\text{TmL}^3]^b$
$\delta_{\text{F}}/\text{ppm}$	-55.1	-36.8	-58.0	-98.1	-53.0	-52.5, -40.6	-66.7, -43.8	-57.9, -49.0	-78.1, -89.2
R_1 @ 9.4 T/s^{-1}	92 ± 9	116 ± 21	112 ± 6	128 ± 5	125 ± 6	133 ± 7	162 ± 11	124 ± 10	47 ± 7
R_2^c @ 9.4 T/s^{-1}	179	1490 ^d	210	989 ^e	272	206	355	192	89

^a Diamagnetic complexes (with La^{3+} or Y^{3+}) with the same ligands under the same conditions have $R_1 \sim 1 \text{ s}^{-1}$ and $R_2 \sim 3 \text{ s}^{-1}$; δ_{F} and R_1 values given here were independent of complex concentration over the range 0.2 to 2 mM. ^b The share of minor species (at the second chemical shift quoted): 12% (Tb), 12% (Ho), 9% (Tm) and 20% (Dy). ^c R_2 values estimated as $2\pi(\text{half-width@half-height})$. ^d Intermediate exchange regime between two *cis-trans* isomers. See text for further details. ^e Intermediate exchange regime between two coordination isomers. See text for further details.

the ^{19}F NMR spectra. The calculations highlighted the important role of the bulky CF_3 group in favouring rotamers that place the CF_3 group away from the amide oxygen.

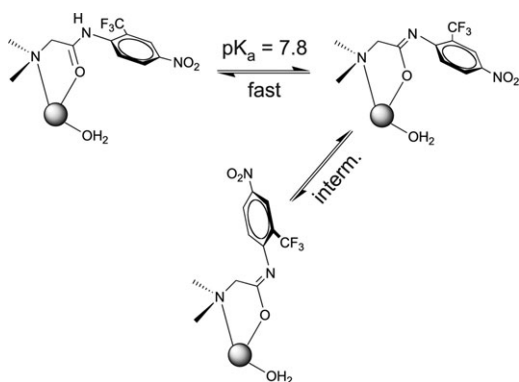
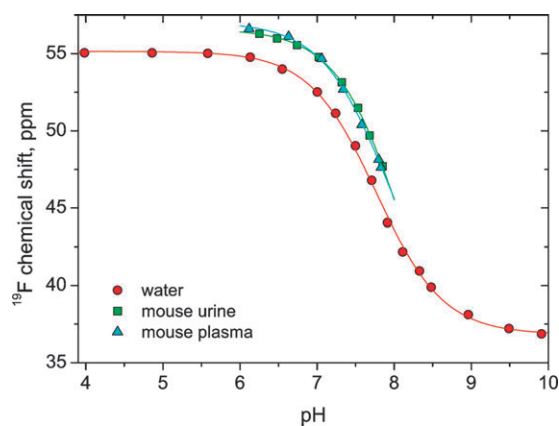
Complexes that belong to a “slow-exchange” class may also be considered, *e.g.* $[\text{Ln}\cdot\text{L}^4]$ (Scheme 1), in which chemical shifts are pH-independent, and it is relative signal intensities that are altered.^{13–15} In aqueous solution, two signals with pH-dependent intensities and 40 ppm chemical shift difference were observed for $[\text{Ho}\cdot\text{L}^4]$, corresponding to N-coordinated and diaqua species (Scheme 3). Fitting the titration data (Fig. 2) yielded $\text{p}K_{\text{a}}$ values of 5.71 ± 0.02 (water), 6.88 ± 0.02 (murine urine) and 6.92 ± 0.02 (murine plasma). The higher $\text{p}K_{\text{a}}$ values in biofluids are the result of additional stabilisation of the acidic form by carbonate coordination (Scheme 3).^{16,17} The basic form exists as a mixture of two diastereomers, defined by the *R* or *S* configuration at sulfur (Scheme 3). The experimental ratio is 6 : 1 (298 K, pH = 8), resonating at -98 and -112 ppm, respectively. A slow re-coordination process interconverts these two species: EXSY spectroscopy and a ^{19}F NMR study revealed an exchange process (T_{c} 323 K at 376 MHz), that probably occurs *via* a cooperative $\text{S}=\text{O}\text{--}\text{Ln}$ bond cleavage/reformation. DFT calculations confirmed this hypothesis—the computed energy difference between the two coordination isomers (*via* either oxygen at the stereogenic S centre) is 8.9 kJ mol^{-1} for $[\text{La}\cdot\text{L}^4]$. A first-order saddle point was found between the two structures, corresponding to forward and backward activation energies of 85 and 76 kJ mol^{-1} .

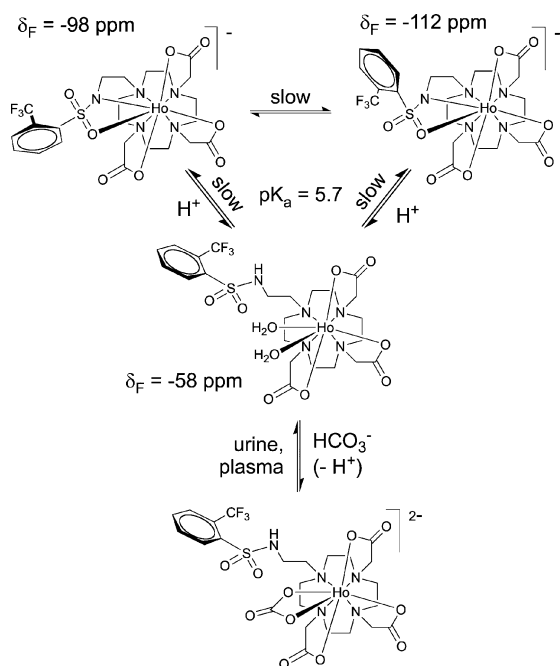
The fluorine–lanthanide distances may be obtained from the relaxation analysis,^{11,12} which was performed for the L^3 ligand complexes. The dominant relaxation mechanisms for the paramagnetic systems in question are electron–nucleus dipole–dipole (DD) and Curie processes (CSR). The total

longitudinal relaxation rate is:^{11,12}

$$R_1 = \frac{2}{15} \left(\frac{\mu_0}{4\pi} \right)^2 \frac{\gamma_{\text{F}}^2 \mu_{\text{eff}}^2}{r^6} \left[\frac{7\tau_{\text{r+e}}}{1 + \omega_{\text{c}}^2 \tau_{\text{r+e}}^2} + \frac{3\tau_{\text{r+e}}}{1 + \omega_{\text{F}}^2 \tau_{\text{r+e}}^2} \right] + \frac{2}{5} \left(\frac{\mu_0}{4\pi} \right)^2 \frac{\omega_{\text{F}}^2 \mu_{\text{eff}}^4}{(3kT)^2 r^6} \frac{3\tau_{\text{r}}}{1 + \omega_{\text{F}}^2 \tau_{\text{r}}^2}$$

where all the symbols have their usual meaning^{11,12} and $\tau_{\text{r+e}} = (\tau_{\text{r}} + 1/\tau_{\text{c}})^{-1}$ is the effective dipolar correlation time (composed of the rotational τ_{r} and the electronic τ_{c} contribution). Because a sufficiently large data set is available (four lanthanides at four different fields), we decided to avoid further approximations (extreme narrowing is sometimes assumed in one or both terms) and perform the non-linear global fit directly. Since the geometry does not change significantly between different lanthanides (ionic radius variation is minor: Tb^{3+} 1.09 Å, Dy^{3+} 1.08, Ho^{3+} 1.07 and Tm^{3+} 1.05 Å in 9 coordinate systems¹⁸) the Ln–F distance and the rotational correlation time can be set to be global between the Tb, Dy, Ho and Tm data sets (full tables are in the ESI†). The values of μ_{eff} and $\tau_{\text{r+e}}$ are kept ‘local’ for every lanthanide dataset. While each individual fit is ambiguous, the global fit (Fig. 3) has a single well-defined weighted least-squares minimum. The resulting values are $r_{\text{Ln–F}} = 6.9 \pm 0.8 \text{ Å}$, $\tau_{\text{r}} = 280 \pm 12 \text{ ps}$ (alternative fitting using fixed values of μ_{eff} : 7.6 for Tm^{3+} , 10.6 for Ho^{3+} , 9.7 for Tb^{3+} and 10.6 for Dy^{3+} results in $r_{\text{Ln–F}} = 6.24 \pm 0.01 \text{ Å}$, $\tau_{\text{r}} = 286 \pm 12 \text{ ps}$), compared with the Stokes–Einstein rotational correlation time estimated from DFT volume data $\tau_{\text{r}} = 243 \text{ ps}$ ($\langle r_{\text{mol}} \rangle = 6.18 \text{ Å}$) and the DFT Ln–F distance of $\langle r \rangle = 6.95 \text{ Å}$. Lower values of τ_{r} have usually been reported by analysing ^1H NMRD

**Scheme 2****Fig. 1** Variation of ^{19}F chemical shift for $[\text{Ho}\cdot\text{L}^1]$ with pH (295 K, 1 mM complex in 0.1 M aqueous NaCl). Solid lines: least-squares fits to the fast-exchange acid–base equilibrium for the chemical shift.



Scheme 3

profiles that implicitly examine the motion of the Gd–water proton vector.¹⁹ Few independent measurements of the overall tumbling rate of the complex have been made but studies of ¹⁷O VT NMR (water motion) or ligand ¹³C or ²H relaxation time analysis (²H labelled ligand) to derive τ_r values have been reported.²⁰ The values are in the range 160–265 ps at 298 K for complexes of similar molecular volume, and were systematically higher than those derived by examining water proton motion or relaxation.

In summary, two new classes of pH-sensitive ¹⁹F-labelled paramagnetic complexes have been defined. With holmium(III), 40 ppm ([Ho·L⁴]) and 18.3 ppm ([Ho·L¹]) changes in chemical shift were observed between acidic and basic forms. [Ho·L¹] belongs to the ‘fast-exchange’ type and changes its ¹⁹F chemical shift with pH, whereas [Ho·L⁴] is a ‘slow-exchange’ ratio-metric probe, responding to pH changes by variation of

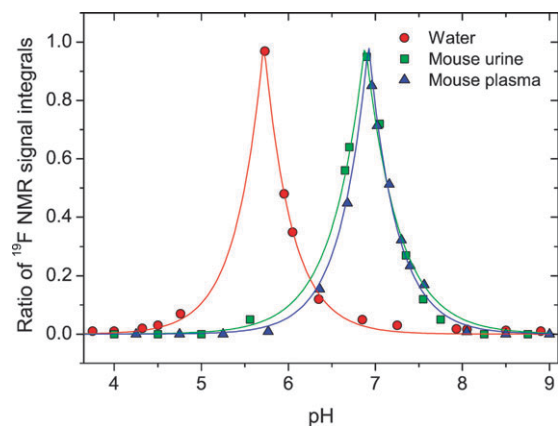


Fig. 2 Variation of the ratio (smaller/greater) of ¹⁹F signal integrals of [Ho·L⁴H] and [Ho·L⁴] with pH (295 K, 0.5 mM complex in 0.1 M aq. NaCl). Solid lines: least-squares fits to the slow-exchange acid–base equilibrium equation for the NMR intensity ratio.

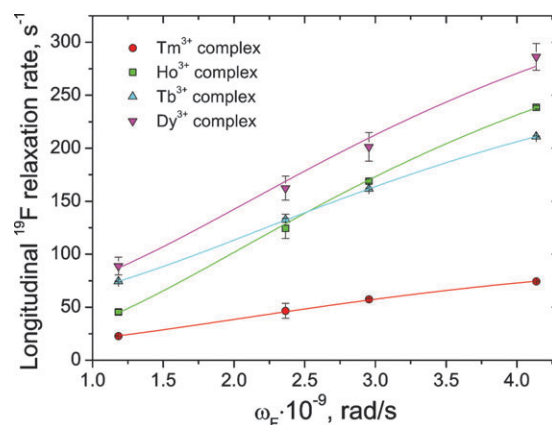


Fig. 3 Variation of the observed relaxation rate with ω_F for [Ln·L³] (Ln = Tb, Dy, Ho and Tm, 295 K, 1 mM complex, D₂O). Solid lines: global least-squares fits to relaxation theory equation.

relative signal intensities. Such behaviour augurs well for chemical shift imaging studies using such complexes.

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