Responsive fluorinated lanthanide probes for ^{19}F magnetic resonance spectroscopy†

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The introduction of CF_3 reporter groups close to the paramagnetic centre in macrocyclic lanthanide(III) complexes allows faster acquisition of ^{19}F magnetic resonance data, and amplifies chemical shift non-equivalence, as exemplified by the definition of ratiometric chemical shift probes for pH and, in principle, enzyme activity.

Fluorinated probes are of much current interest in positron emission tomography (^{18}F) and magnetic resonance imaging and spectroscopy.[1,2] The high NMR sensitivity and large chemical shift range (> 300 ppm), accompanied by a near-zero endogenous background, render ^{19}F-MRS and MRI intrinsically attractive for biological studies. Several reports have described the use of ^{19}F magnetic resonance spectroscopy in quantitative studies to track *in vivo* the metabolism of ^{19}F-labelled drugs, *e.g.* 5-fluorouracil or gemcitabine.[3] In this work, signal to noise ratios of the order of 5 : 1 have been reported for fluorinated compounds in the 0.2–0.5 mM range (310 K, 282 MHz), for 10 minute spectral acquisitions. Perfluorinated compounds have also attracted attention, *e.g.* as tags that may be conjugated to biomolecules to allow the tracking of the conjugate, as in the monitoring of labelled actin during polymerization or in its interaction with myosin.[4] More recently, CF_3 groups have been introduced to arylgalactopyranosides, that should allow the activity of the β-galactosidase enzyme to be followed by ^{19}F NMR spectroscopy.[5] Using certain trifluoromethyl arylgalactopyranosides, chemical shift imaging studies have been demonstrated *in vitro* (*ca.* 50 mM substrate), monitoring the differing shifts of the fluorinated glycoside and phenolic product ($\Delta\delta_F \leqslant 1$ ppm). Thus, a ^{19}F NMR assay for β-galactosidase activity has been advocated,[5] in principle allowing gene expression (*e.g.* for the lacZ gene) to be tracked.

A critical limiting feature of this ^{19}F NMR work relates to the slow longitudinal relaxation rate of the ^{19}F nucleus, especially in CF_3 groups where R_1 values are of the order of 0.5 to 1 s^{-1}. This determines the spectral acquisition time, owing to the need to wait for a 3 to 6 second repetition time (3 to 5 times T_1). As signal/noise ratios vary with the square root of the number of scans acquired, at least an order of magnitude increase in R_1 is desired, preferably without too much line-broadening. A solution to this problem is to place the ^{19}F nucleus close to a paramagnetic lanthanide ion, in a kinetically stable complex or conjugate, leading to much shorter T_1 (and T_2) relaxation times. The Ln ion may be permuted in its complex with a common ^{19}F-labelled ligand, allowing the introduction of either strongly relaxing ions (*e.g.* Dy, Tm, Tb, Ho; dipolar and Curie relaxation rates follow an r^{-6} dependence) or ions that lead to large dipolar shifts, but do not affect relaxation to such an extent (*e.g.* Eu, Yb; the paramagnetic dipolar shift follows an r^{-3} dependence). There is an obvious analogy with the development of magnetic resonance contrast agents (Gd), and innumerable lanthanide complexes have been studied over the past 20 years that are well tolerated *in vivo*, typically at doses in the 0.1 to 0.2 mM kg^{-1} range.[6]

There has been very little systematic work reported to date concerning the relaxation and dipolar shift effects of lanthanide ions on ^{19}F NMR spectral parameters, notwithstanding the early interest in lanthanide shift reagents, based on fluorinated β-diketonates. Indeed, examples of the interpretation of ^{19}F-NMR shifts of paramagnetic complexes are very rare, but do include some work on F-labelled iron(III) porphyrins.[7] Isolated studies have examined the T or pH dependence of ^{19}F relaxation rates for ionic systems in which an anion is ion-paired (*e.g.* $CF_3SO_3^-$ anions[8]) or reversibly bound (*e.g.* trifluorolactate[9]) to a lanthanide centre.

Three sets of fluorinated macrocyclic lanthanide(III) complexes [LnL^1]–[LnL^3] have been synthesised and their ^{19}F NMR spectral properties assessed. Ligands L^1 and L^3 are mono-amides and form charge neutral mono-aqua complexes with Ln(III) ions;[6] ligand L^2 incorporates a sulfonamide moiety that is able to bind reversibly to the Ln ion *via* the N atom, in a process that is a sensitive function of pH. The exchange between N-bound and the unbound *N*-protonated ligation modes in water is accompanied by a change in hydration of the Ln ion, and has previously been examined for certain Eu and Gd complexes, varying the protonation constant by changing the electron demand of the sulfonamide substituent.[10] The synthesis of ligands† L^1 and L^3 involved alkylation of the tri-ester **1** with the appropriate α-chloroamide **2** or **3** (CH_3CN, Cs_2CO_3, 1% KI). Following chromatographic purification on neutral alumina, treatment with CF_3CO_2H removed the protecting groups and reaction with the appropriate $LnCl_3$ salt in water at pH 5, followed by chromatography on alumina (20% MeOH–CH_2Cl_2) led to the isolation of the neutral complexes, [LnL^1] and [LnL^{3a}]. The conversion of [LnL^{3a}] to the acetate [LnL^{3b}] was achieved by reaction with acetyl chloride in aqueous dioxan. Lanthanide complexes of L^2 were prepared by stepwise alkylation of **1** with the *bis*(trifluoromethyl)-arylsulfonamide, **4**, followed by TFA de-protection and complex formation in aqueous media.

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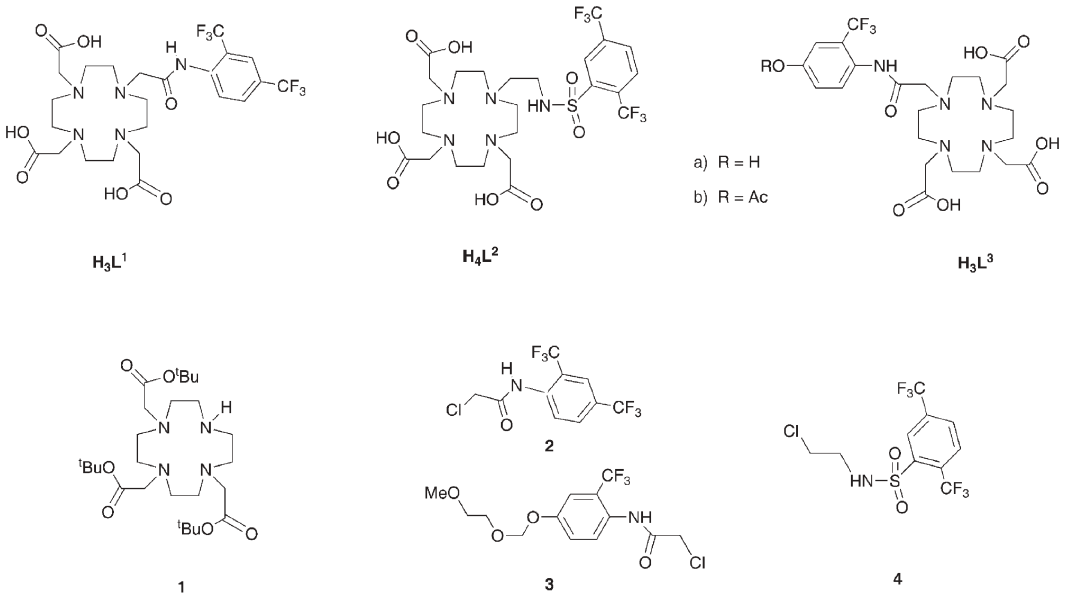
† Electronic supplementary information (ESI) available: Selected details of ligand and complex synthesis; equations defining the factors modulating the electron–nuclear interaction and their effect on R_1 and R_2 (chemical exchange, dipolar and Curie mechanisms); examples of the use of emission spectroscopy to measure the protonation constant for [LnL^2]/[$LnHL^2$] (Ln = Eu, Tb). See DOI: 10.1039/b705844f



^{19}F NMR chemical shifts and longitudinal relaxation rates for various lanthanide complexes of L^1, L^2 and L^3 were measured at 188 MHz (4.7 T, 298 K) (Table 1). Both the dipolar shifts and the relaxation rate enhancements were largest for the *ortho*-CF_3 groups: in $[TbL^1]$, the rate of relaxation was 70 s^{-1} and for $[DyL^2]^-$ the CF_3 resonance shifted 100 ppm to lower frequency (of the ligand) and the measured R_1 value was 93 s^{-1}. The behaviour was slightly more complex for the $[LnL^2]^-/[LnL^2H]$ system, as chemical exchange between these species led to some additional line broadening. Chemical exchange was generally in the slow-exchange regime on the NMR timescale; taking $[EuL^2]^-/[EuL^2H]$ as an example, separate shifted ^{19}F resonances were observed for each complex (Fig. 1), and their co-observation allowed the complex to function as a chemical shift pH probe.[11] Thus, the ratio of the integrals of the pairs of resonances at −59/−53 and −65/−64 ppm reflects the relative concentration of the conjugate base and its protonated form, allowing determination of the complex protonation constant, pK_{MLH} = 5.5 (±0.05). Independent validation of this value comes from an analysis of the variation of the Eu luminescence emission intensity ratio (λ_{exc} 397 nm; observe emission bands at 612/616 nm or 680/590 nm) with pH[8] (see ESI). A value of 5.5 for pK_{MLH} was also estimated for $[TbL^2]^-/[TbL^2H]$, monitoring the change in the overall Tb emission intensity with pH.

Table 1 Limiting ^{19}F chemical shift (ppm) and longitudinal relaxation rate (s^{-1}) data (188 MHz, 298 K, pH 5.5) for selected lanthanide complexes of L^1, L^2 and L^3

Complex	$\delta_{F\bullet}$	$\delta_{F\circ}$	$R_1^{\bullet}$	R_1°
$[TbL^1]$	−50.9	−74.7	90	11
$[YbL^1]$	−65.4	−59.6	4.6	1.7
$[TmL^1]$	−79.6	−55.0	16	5.6
$[YL^1]$	−61.3	−63.1	0.9	0.9
$[DyL^1]$	−65.0	−81.2	77	12
$[HoL^1]$	−55.0	−70.1	49	8.1
$[EuL^2]^{-a}$	−53 (−59)	−65 (−64)	2.5 (2.2)	1.5 (1.2)
$[TbL^2]^{-a,b}$	−159 (−59)	−45 (−64)	71	23
$[DyL^2]^{-a,b}$	−158 (−59)	−39 (−64)	93	27
$[TbL^{3a}]$	−52.0	—	78	—
$[TbL^{3b}]$	−62.0	—	30	—
$[TmL^{3a}]$	−77.0	—	24	—
$[TmL^{3b}]$	−71.7	—	15	—

[a] Values in parentheses refer to the *N*-protonated complex (pH 5.5, see Fig. 1). [b] For $[DyHL^2]$ and $[TbHL^2]$, the resonances of the two CF_3 groups were exchange broadened and were not distinguished at pH 5.5 (188 MHz), and mean rates of 23 s^{-1} and 27 s^{-1} were measured respectively. [c] Shifts were internally referred to $NaCF_3CO_2$ (δ_F −76.1 ppm) which typically had a relaxation rate of 0.5 s^{-1} under the stated conditions.

For $[LnL^3]$, the ^{19}F chemical shift non-equivalence of the CF_3 reporter group in $[LnL^{3b}]$ *vs.* the product of cleavage of the ester bond, $[LnL^{3a}]$ ($\Delta\delta_F$ = 10 ppm for Tb, 5.3 ppm for Tm) should allow this complex to report on the activity of any esterase that catalyses hydrolysis of the ester bond in the $[LnL^{3a}]$ complex. Similar effects are expected for related derivatives, *e.g.* arylglycosides, and, using the appropriate benzoic acid derivative, for benzoate esters and benzamides. This shift non-equivalence is encouraging for the proposed usage of such a system, and variants thereof, in monitoring enzyme activity or gene expression in appropriate transfection systems using ^{19}F MRS or ^{19}F chemical shift imaging.

The distinct advantages of introducing the Ln ion are to amplify any chemical shift non-equivalence in the reporter resonances and to enhance the rate of relaxation allowing acquisition of more scans and hence increased signal intensity in a given time period. A comparative analysis examined the *ortho*-CF_3 resonance in ^{19}F NMR spectra of the diamagnetic Y complex, $[YL^1]$ (R_1 0.93 s^{-1}, {linewidth, $\omega_{1/2}$ = 2.5 Hz}) and the terbium (R_1 90 s^{-1} {$\omega_{1/2}$ 90 Hz}) and Dy (R_1 77 s^{-1} {$\omega_{1/2}$ 35 Hz}) analogues. Using a relaxation delay of 3 times T_1, ^{19}F spectra were acquired over a common time interval of 30 minutes (2 mM complex, 188 MHz, 298 K, typical S/N ratio per mM was 70 : 1). The relative signal intensity ratios per mM (*i.e.* peak height times peak width at half height; processing used a line-broadening function equal to half of the observed linewidth, for the given complex resonance) for observation of the major *ortho*-CF_3 resonance in $[TmL^1]$, $[DyL^1]$ and $[TbL^1]$ normalised to the diamagnetic analogue, $[YL^1]$, were

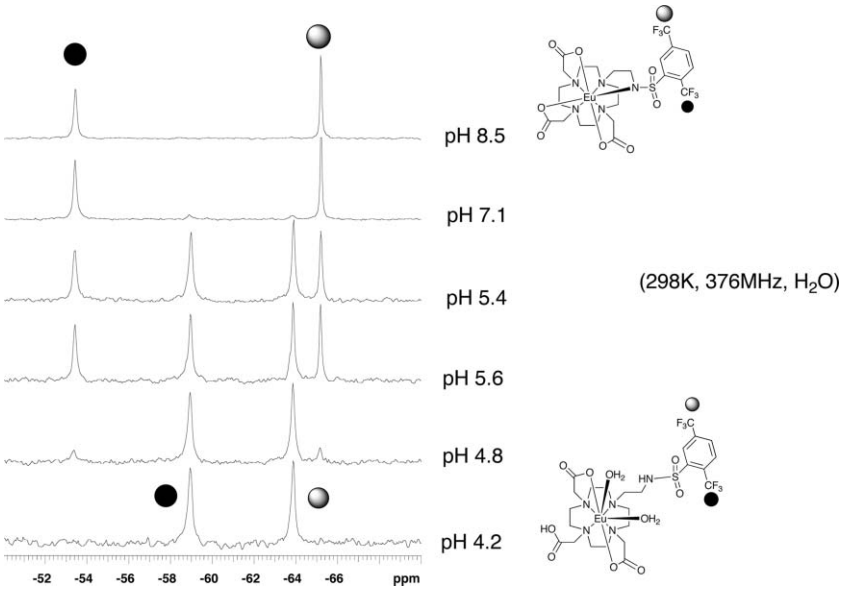


Fig. 1 ^{19}F NMR spectra (376 MHz, 298 K) for $[EuL^2]^-$ and $[EuL^2H]$ as a function of pH.

50 : 1, 22 : 1 and 41 : 1, respectively,[12] consistent with the ability to increase the number of scans acquired for the faster relaxing CF_3 resonance in the paramagnetic complexes.

In summary, this behaviour augurs well for the more widespread use of such paramagnetic ^{19}F labelled probes in biological MRS and T_1 weighted spectroscopy and imaging studies.

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12 The greater than expected normalised signal intensity gain partly reflects the differential benefit of applying the line-broadening function to the FID (half of the observed linewidth, in each case, *e.g.* 1 Hz for $[YL^1]$ and 17.5 Hz for $[DyL^1]$).