Lanthanide complexes of a cyclen derivative with phenylphosphinic pendant arms for possible ^{1}H and ^{31}P MRI temperature sensitive probes

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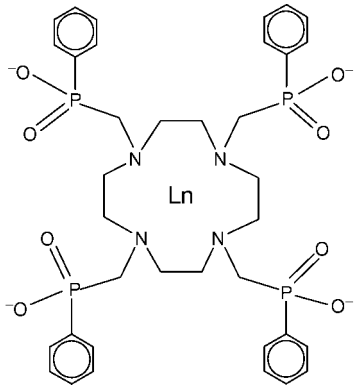
Lanthanide complexes of 1,4,7,10-tetraazacyclododecane-1,4,7,10-tetrakis[methylene(phenylphosphinic) acid] (H_4L) were found to give a linear correlation of ^{1}H and ^{31}P NMR chemical shifts to temperature in the physiologically relevant temperature range, with dependencies comparable or better than those found for existing complexes; due to the greater hydrophobic character of the ligand the complexes may exhibit a different biodistribution in the body.

Paramagnetic metal complexes are widely used as contrast agents in magnetic resonance imaging (MRI) to enhance the natural contrast between tissues. Most of these compounds are obtained by complexation of lanthanide ions with open chain or macrocyclic ligands.[1] Such complexes have also been proposed for more specialised applications in NMR spectroscopy and/or MRI. Agents have been suggested as pH sensitive NMR probes,[2] pH responsive relaxometric probes[3] and temperature probes.[4–6] The use of bifunctional agents for simultaneous MRI and fluorescence imaging,[7] determination of the activity of β-galactosidase enzyme[8] or concentrations of sodium,[9] magnesium[10] and calcium[11] ions and as agents helping to suppress the water ^{1}H NMR signal in biofluids has also been proposed.[12] Non-invasive temperature measurement is an interesting target that could help in the detection and localisation of regions of abnormal metabolic activity. Until recently, the NMR signals of fluorocarbons[13] were employed for such measurements. Improved NMR temperature sensors based on paramagnetic lanthanide complexes of macrocyclic derivatives have been suggested recently.[4–6] Probably, the best chemical shift per degree temperature change ratio, up to almost 1 ppm °C^{-1} for ^{1}H NMR and greater than 2 ppm °C^{-1} for ^{31}P NMR, was found for thulium 1,4,7,10-tetraazacyclododecane-1,4,7,10-tetrakis(methylene phosphonate) ([Tm(DOTP)]$^{5-}$).[6] However, the complex is highly charged and, despite its strong association with sodium ions, is not very convenient due to osmolality problems. On the other hand, the praseodymium complex[4] of 10-(2-methoxyethyl)-1,4,7,10-tetraazacyclododecane-1,4,7-triacetate ([Pr(MOE-DO3A)]) is non-ionic but exhibits only a small change with temperature (0.13 ppm °C^{-1}, as does the ytterbium complex[5] or 1,4,7,10-tetraazacyclododecane-1,4,7,10-tetrakis(methylacetate) ([Yb(DOTMA)]$^{-}$) with a value of 0.42 ppm °C^{-1}.

Cyclen derivatives with phosphinic acid pendant groups have been widely studied in recent years.[10,14–19] In comparison, they exhibit lower stability constants with lanthanides than their acetic or phosphonic analogues,[15] but only slightly lower or comparable kinetic inertness.[17] Unlike these derivatives the biodistributions of phosphinic ligand complexes is highly dependent on the substituents on the phosphorus atom[16] and is controlled by the hydrophobicity of these substituents. In this communication, we wish to present alternative complexes with possible utility as temperature probes based on 1,4,7,10-tetraazacyclododecane-1,4,7,10-tetrakis[methylene(phenylphosphinic) acid] (H_4L) (Scheme 1). The ligand and the complexes are relatively hydrophobic and thus, their biodistribution will be different from the complexes mentioned above. In addition, singly charged anionic lanthanide complexes may be more convenient than their phosphonate analogues.

We found that lanthanide complexes of H_4L are present in methanol or aqueous solutions as a mixture of isomers giving rise to rather complicated ^{1}H and ^{31}P NMR spectra (Fig. 1) because of the Λ/Δ isomerisation of pendant arms and *R*/*S* orientation of substituents on the phosphorus atoms.[20] The *RRRS* diastereoisomer is the species with the highest abundance followed by the *RRRR* isomer. The *RRRS* variant gives rise to 4 peaks in its NMR spectrum and the *RRRR* derivative only 1, and thus, the intensity of the peak corresponding to the *RRRR* isomer is higher than the intensity of any one of the 4 peaks of the *RRRS* compound. Hence, the *RRRR* isomer is readily distinguished from the others. The abundances of the other isomers are lower as are the intensities of the peaks in their NMR spectra. When the Λ and Δ isomerisation of the pendant arms is taken into consideration, then the Δ-*RRRR* derivative is an enantiomer to the Δ-*SSSS* variant and, analogously, this also applies to the other combination. The enantiomers are not distinguished by NMR and therefore, only one isomeric form, Λ, is considered. A detailed discussion of the solution behaviour of Li[LnL] complexes is given in ref. 20. For the reasons mentioned above, the temperature sensitivity of the ^{1}H and ^{31}P NMR shifts was investigated only for the *RRRR* isomer, although the dependences found for the other isomers with lower intensity signals are similar. The isomers do not interchange on the NMR time scale and their relative abundance is not temperature sensitive through the temperature range studied. The formation of such isomeric mixtures with one major isomer in solution is common[21] for



Scheme 1

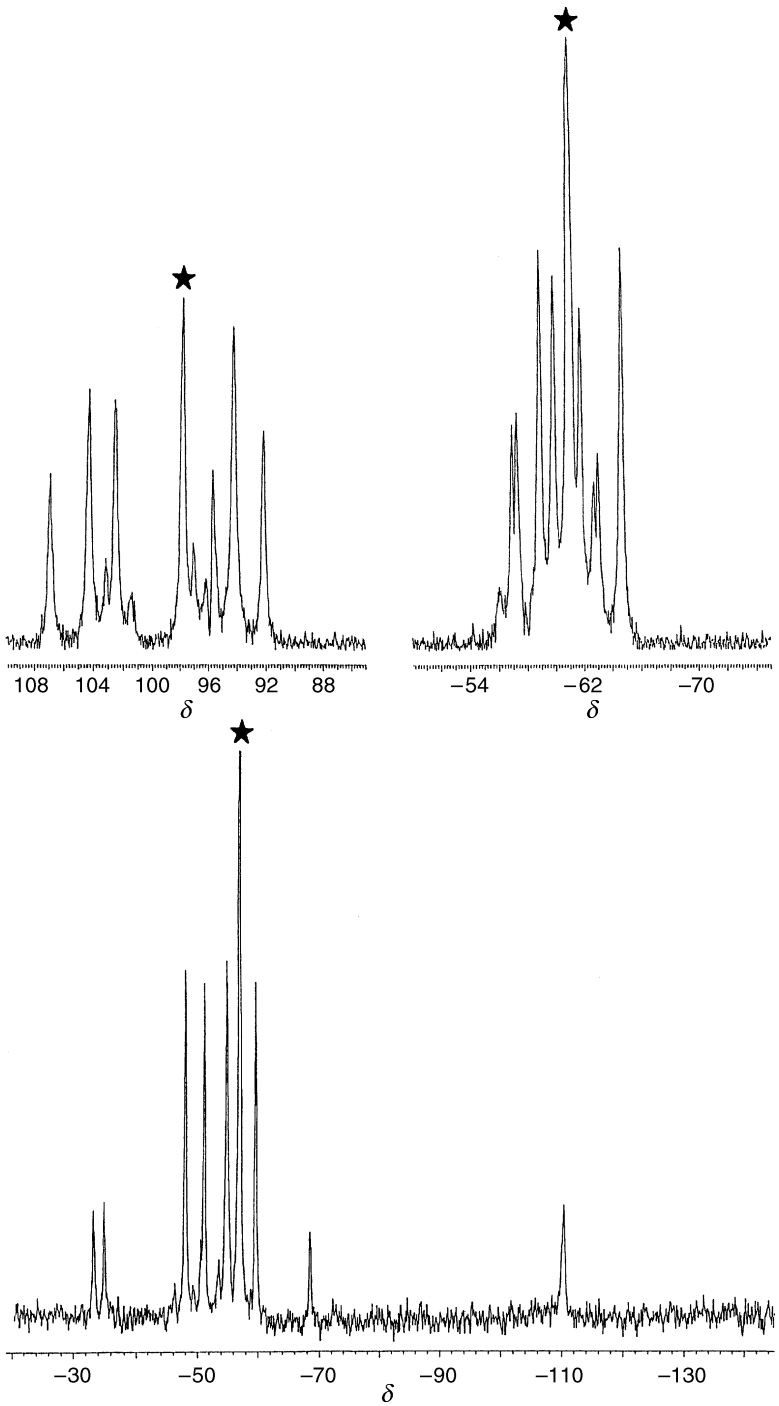
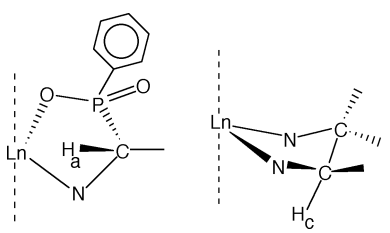


Fig. 1 Relevant parts of the ^{1}H NMR (upper: H_c left; H_a right) and ^{31}P NMR spectra (lower) of $[YbL]^-$ in D_2O at 25 °C. Resonances due to the Δ-*RRRR* isomer are denoted by an asterisk.

lanthanide complexes with macrocyclic ligands and would not limit the use of ^{1}H and ^{31}P NMR resonances as temperature probes.[1,2] Similar behaviour was described for lanthanide complexes of 1,4,7,10-tetraazacyclododecane-1,4,7,10-tetrakis[methylenephosphonate mono(2,2,2-trifluorethyl)ester] which also form isomeric mixtures.[22]

Despite the complexity of the ^{1}H and ^{31}P NMR spectra, due to the isomerisation mentioned above, we found excellent linear correlation between temperature T and chemical shifts, both $\delta(^1H)$ and $\delta(^{31}P)$, in CD_3OD for all lanthanide complexes (Table 1). Solutions in CD_3OD were used because of the better solubility of the compounds studied in this solvent. The lanthanide complexes with the steepest slopes in methanolic solution were also investigated in D_2O solutions (Table 2).

The ^{1}H NMR spectra offer a number of signals. Among the signals observed, the steepest slope of the $\delta(^1H)$–T correlation was found for the H_a and H_c atoms (Scheme 2). The unequivocal assignment of H_a and H_c was done using H,H-COSY on $[EuL]^-$, as described in ref. 20. The signals of these protons are well separated from the $\delta(^1H)$ range of protons in biological materials, and therefore, the strong water signal presented by biofluids can be effectively suppressed. The H_c



Scheme 2

Table 1 Coefficients in linear correlations of $\delta(^{31}P)$ and $\delta(^{1}H)$ *versus* temperature (T) in methanolic Li[LnL] solutions. Chemical shifts of the Δ-*RRRR* isomer only are considered

	$\delta(^{31}P) = c_d + c_T T$		$\delta(^{1}H_a) = c_d + c_T T$		$\delta(^{1}H_c) = c_d + c_T T$	
	c_d/ppm	c_T/ppm °C^{-1}	c_d/ppm	c_T/ppm °C^{-1}	c_d/ppm	c_T/ppm °C^{-1}
Ce	31.61	−0.001	20.80	−0.068	−14.68	0.057
Pr	45.43	−0.131	29.93	−0.096	−39.85	0.167
Nd	13.00	0.038	22.41	−0.091	−18.44	0.090
Sm	39.34	0.026	10.40	−0.003	−4.94	0.005
Eu	41.10	0.239	−25.03	0.123	27.31	−0.082
Tb	552.8	−2.039	148.3	−0.655	−126.0	0.498
Dy	476.4	−1.675	103.3	−0.282	−111.3	0.423
Ho	225.5	−0.648	51.17	−0.143	−52.88	0.336
Er	−198.4	1.134	−15.75	0.109	38.28	−0.184
Tm	−412.6	2.135	−103.7	0.5452	152.0	−0.863
Yb	−59.89	0.427	−74.65	0.271	109.4	−0.422

proton, bonded on the macrocyclic ring, is directed toward the Ln^{3+} ion and toward the fourfold principal magnetic axis of the complex (Scheme 2).

Thus, the H_c proton is the hydrogen atom most exposed to the magnetic influence of the paramagnetic lanthanide ion.

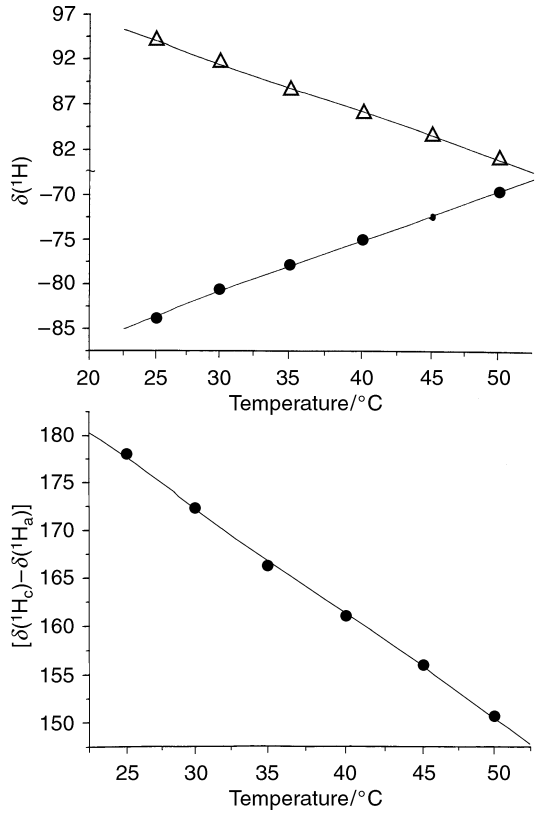


Fig. 2 Dependences of $\delta(^{1}H_a)$ and $\delta(^{1}H_c)$ on temperature (upper) and dependence of the difference $[\delta(^{1}H_a) - \delta(^{1}H_c)]$ (lower) on temperature for $[TmL]^-$ in human plasma.

From X-ray crystal structure investigation of seven Ln complexes (Ln = La, Ce, Nd, Eu, Tb, Er, Yb) with 1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraylmethyltrimethylenetris-(phenylphosinic) acid[23] and three Ln complexes (Ln = La, Eu, Yb) with 1,4,7,10-tetraazacyclododecane-1,4,7,10-tetrakis-[methylene(benzyl)phosphinic acid],[24] it is known that the structure of complexes of these ligands does not change along a series except for the inclusion of a water molecule in the first coordination sphere. Especially, the geometry of the azacycle and pendant methylenes is virtually the same. Therefore, results obtained from the X-ray crystal structure investigation[20] of $Li[CeL(H_2O)] \cdot 10H_2O$ can be applied to the other structures of the same ligand. The distance Ce–H_c is in the range 3.70 to 4.00 Å in the solid state for the *RSRS* configuration. The H_a proton belongs to the $-CH_2-$ group in the pendant arm which is situated in the space of a McConnel cone and has a different sign compared to H_c (Scheme 2). Again, the distance Ce–H_a found in the crystal structure is in the range 3.60 to 3.75 Å. The resonances of hydrogens bonded to the rest of macrocycle and to the phenyl rings are much less temperature sensitive than H_c or H_a. As was expected, the biggest differences were observed for complexes of Dy, Tm and Yb.[2,3]

The Li[LnL] complexes where Ln = Pr, Eu, Dy, Tm and Yb were also studied in D_2O solutions. From Table 2, it is clear that the steepest slopes were found for Dy and Tm complexes. The lines studied are reasonably sharp, so as to be resolved in millimolar concentrations throughout the temperature range. The temperature dependence does not vary with pD (±1.0 pD unit). A comparison of the slopes in the $\delta(^{31}P)$–T and $\delta(^{1}H)$–T correlation shows that the values determined for $[TmL]^-$ are lower than those for $[Tm(DOTP)]^{5-}$ (ref. 6). However, the difference is not large and confirms the utility of phosphorus-based cyclen derivatives for such measurements. The advantages of H_4L complexes over $[Tm(DOTP)]^{5-}$ are a much lower charge and the independence of chemical shifts on pH and concentration of sodium and/or calcium ions, due to the low basicity of phosphinic pendant arms[19] and the low ion-pairing ability of the

Table 2 Coefficients in linear correlations of $\delta(^{31}P)$ and $\delta(^{1}H)$ *versus* temperature (T) in D_2O and in human plasma (*) Li(LnL) solutions. Chemical shifts of the Δ-*RRRR* isomer only are considered

	$\delta(^{1}H_a) = c_d + c_T T$		$\delta(^{1}H_c) = c_d + c_T T$		$\delta(^{31}P) = c_d + c_T T$	
	c_d/ppm	c_T/ppm °C^{-1}	c_d/ppm	c_T/ppm °C^{-1}	c_d/ppm	c_T/ppm °C^{-1}
Pr	25.54	−0.089	−33.95	0.134	35.79	−0.058
Eu	−16.20	0.084	31.01	−0.122	66.57	0.012
Dy	88.45	−0.300	−96.49	0.504	448.05	−1.812
Tm	−94.91	0.513	107.06	−0.515	−395.36	2.122
Tm*	−97.73	0.565	107.06	−0.522	—	—
Yb	−66.96	0.238	96.92	−0.289	−45.07	0.253

complexes in aqueous solution,[20] respectively. On the other hand, a comparison with the non-ionic complex[4] [Pr(MOE-DO3A)] or the singly charged ytterbium complex[5] $[Yb(DOTMA)]^-$ shows that $[TmL]^-$ is the more efficient agent. In Table 2, it can be seen that the c_T slopes in the $\delta(^1H_a) = c_d + c_T T$ and $\delta(^1H_c) = c_d + c_T T$ equations have the opposite sign and thus, the $\delta(^1H_a) - \delta(^1H_c)$ difference is the most sensitive value from the $\delta(^1H)$–T correlations. The measurement of two peaks in a spectrum offers another advantage, no internal standard is required, only the relative values of the chemical shifts are used.

The $\delta(^1H)$–T correlation was also investigated for a Li[TmL] solution in human plasma as an *in vitro* model. In the ^1H NMR spectrum, the half-width of the peaks is high (about 300 Hz), the spectrum is not as clear as that in D_2O solution, nevertheless, the peak of the *RRRR* isomer was well distinguished and its maximum could be read with an adequate accuracy. Measurements were run under the same conditions as for the D_2O solutions with presaturation of the water signal. The shape of the spectrum was similar to those in aqueous solution and thus, we suppose, no significant interaction with proteins and other biomolecules in plasma occurs. The correlation found

$$\delta(^1H_a) - \delta(^1H_c) = 207.9 - 1.08T$$

is also depicted in Fig. 2.

In conclusion, the results obtained indicate that $[TmL]^-$ could be used as an efficient temperature probe. The complex shows lipophilic character and is stable under physiological conditions.[16]

Experimental

Detailed experimental procedure for the preparation[19] of H_4L, as well as its complexes[20] with Ln^{3+}, has been published elsewhere. Briefly, H_4L was prepared by a Mannich reaction starting from cyclen hydrochloride phenylphosphinic acid and formaldehyde. The lanthanide complexes $Li[LnL] \cdot nH_2O$ ($n = 7,8$) were obtained by reaction of H_4L and $LnCl_3 \cdot xH_2O$ in a H_2O/MeOH medium in the presence of LiOH and were purified by recrystallisation from the same solvent mixture. The purity of $Li[LnL] \cdot nH_2O$ ($n = 7,8$) complexes was checked by elemental analysis and TLC (t-BuOH : H_2O : CH_3CN = 2 : 9 : 2 (v/v), "Silufol" silica plate, made by Kavalier Votice, CZ; UV detection). The X-ray crystal structure analysis of $Li[LaL(H_2O)] \cdot 10H_2O$ and $Li[CeL(H_2O)] \cdot 10H_2O$ has been published elsewhere.[20]

NMR measurements were done on a Varian Inova 400 instrument (^1H 399.9 MHz, ^{31}P 161.9 MHz) in CD_3OD or D_2O (Aldrich, both 99.9%D) with t-BuOH as internal (^1H) and 85% H_3PO_4 as external (^{31}P) standards. Samples were made by dissolution of 15 mg of the appropriate solid complex in 1.0 mL of CD_3OD or D_2O, respectively, to give approx. 0.015 mol dm^{-3} solutions. Temperature was controlled by a VT-regulator, containing a thermocouple calibrated using MeOH and $HOCH_2CH_2OH$ according to a literature procedure.[25] The standard s2pul pulse sequence supplied by Varian was used. Measurements in human plasma (fresh frozen, type-0, Rh+, donation from the University Hospital) were under the same conditions (15 mg Li[TmL] were dissolved in 1.0 mL of plasma at 25 °C) and with presaturation of the H_2O signal. A coaxial capillary with a mixture of D_2O and ButOH was used for a lock. Each sample was measured at six different temperatures in the range 25 to 50 °C. The linear correlations found were better than 0.995 for all dependences.

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