# Lanthanoid(III) Cations as Nuclear Magnetic Resonance Conformational Probes: Studies on Cytidine 5'-Monophosphate at pH 2

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Complex formation between cytidine 5'-monophosphate (cmp) and lanthanoid(III) cations has been followed by n.m.r. spectroscopy, making use of induced shifts of the n.m.r. lines of cmp caused by the bound lanthanoid cations. Shift data have been obtained under a variety of conditions using most of the lanthanoids. These data show that the conformation of cmp is similar to that of adenosine 5'-monophosphate (amp). Relaxation studies employing Gd$^{III}$ as perturbing cation confirm the conclusions drawn from the shift data.

THE n.m.r. method used here for determination of the conformation of the molecules in solution has been described previously.[1] It involves measurement of the induced shift and relaxation perturbations which are caused by specific binding of the lanthanoid(III) cations. In most systems the shifts are known to arise from through-space (pseudo-contact) rather than through-bond (contact) effects when proton resonances are studied, although this may not be the case for resonances of other nuclei.[2] The equation for such pseudo-contact shifts can be written [3,4] as in (1), where $r$

$$\delta = A \left[ D_z \frac{(3 \cos^2 \theta - 1)}{r^3} + \frac{(D_x - D_y) \sin^2 \theta \cos 2\Omega}{r^3} \right] \quad (1)$$

is the distance of a given nucleus from the metal ion, $A$ is characteristic of each lanthanoid ion, $D_x$, $D_y$, and $D_z$ are zero-field splitting parameters, and $\theta$ and $\Omega$ are angles between a line joining the given nucleus and the metal ion, and the magnetic symmetry axes. The principle axis of symmetry lies along the $z$ direction, and $\theta$ is the angle subtended to this axis. Thus if values of $\delta$ can be determined for a number of nuclei in a molecule which forms a well defined complex with lanthanoid cations, the conformation of that molecule in the complex can be obtained from the shift data. Conformations generated in a computer search may be further tested by examination of the relaxation induced by Gd$^{III}$ which is proportional [1,5] to $1/r^6$ [equation (2)].

$$(1/T_{ip}) \propto (1/r^6) \quad (i = 1 \text{ or } 2) \quad (2)$$

In this type of search acceptable conformations are those which generate both shift and relaxation data within limits prescribed by the experimental accuracy.[6] Here we give a detailed analysis of the shift data, with a large number of lanthanoids under various conditions, and present relaxation data (both $T_1$ and $T_2$), for all the observable protons in cmp.

## EXPERIMENTAL

Lanthanoid(III) chloride solutions were prepared from the corresponding oxides. The oxides (Koch–Light, 99·9% purity) were heated to 900 °C for several hours and solutions up to 0·5M prepared by dissolving weighed quantities in DCl and D$_2$O (99·8%). Cytidine 5'-monophosphate (cmp) and cytidine were obtained from Sigma Chemical Company. After lyophilisation from D$_2$O, 30 mM solutions were prepared by dissolving the solid in D$_2$O (99·8%) or in lanthanoid(III) chloride solutions. The nominal pH of all samples was adjusted to 2·0 ± 0·1 with NaOD and DCl.

$^1$H N.m.r. spectra were recorded on a Brücker HX90 (90 MHz) or on a 270 MHz spectrometer employing an Oxford Instrument Company superconducting magnet. A Nicolet 1085 computer was employed for Fourier-transform operations. 3-(Trimethylsilyl)propanesulphonic acid, sodium salt (tss), was used as internal standard. Spin–lattice ($T_1$) relaxation times were obtained by means of a 180°–τ–90° pulse sequence, and spin–spin ($T_2$) values from linewidths. $^{31}$P Spectra were run on a Brücker HX90 spectrometer operating at 36·436 MHz.

## RESULTS AND DISCUSSION

Figure 1 shows a titration of cmp (30 mM) with one of the lanthanoids (Pr$^{III}$) obtained by measuring the induced shift on each proton at various different concentrations of Pr$^{III}$. The assignments of the resonances were obtained from previous work,[7] from analysis of the multiplet structure and from spin-decoupling experiments. Because there is fast exchange between the bound and unbound forms of cmp, the shift at any concentration of lanthanoid is a weighted average of the bound and unbound chemical shifts. The data show that a 1 : 1 complex is formed between each lanthanoid ion and cmp. Figure 2 shows Pr$^{III}$ shift data for the H$_5'$ protons at two temperatures. The binding curves were fitted by a computer program. At 25 °C, $K = 2·8 ± 0·2$ l mol$^{-1}$, and the corresponding fully bound shift ($\Delta$) is $-9·9$ p.p.m., at 85 °C, $K = 9·0 ± 1·1$

[1] C. D. Barry, J. A. Glasel, A. C. T. North, R. J. P. Williams, and A. V. Xavier, *Nature*, 1971, **232**, 236.

[2] C. M. Dobson, R. J. P. Williams, and A. V. Xavier, *J.C.S. Dalton*, 1973, 2662.

[3] B. Bleaney, *J. Mag. Resonance*, 1972, **8**, 91.

[4] B. Bleaney, C. M. Dobson, B. A. Levine, R. B. Martin, R. J. P. Williams, and A. V. Xavier, *J.C.S. Chem. Comm.*, 1972, 791.

[5] I. Solomon, *Phys. Rev.*, 1955, **99**, 559; N. J. Bloembergen, *J. Chem. Phys.*, 1957, **27**, 572; T. J. Swift and R. E. Connick, *ibid.*, 1962, **37**, 307.

[6] C. D. Barry, J. A. Glasel, R. J. P. Williams, and A. V. Xavier, *J. Mol. Biol.*, 1974, **84**, 471.

[7] C. D. Jardetzky and O. Jardetzky, *J. Amer. Chem. Soc.*, 1960, **82**, 22.

1 mol$^{-1}$ and $\Delta = -7\cdot8$ p.p.m. This decrease in the fully bound shift with increasing temperature is in agreement with theoretical predictions.[3] The binding
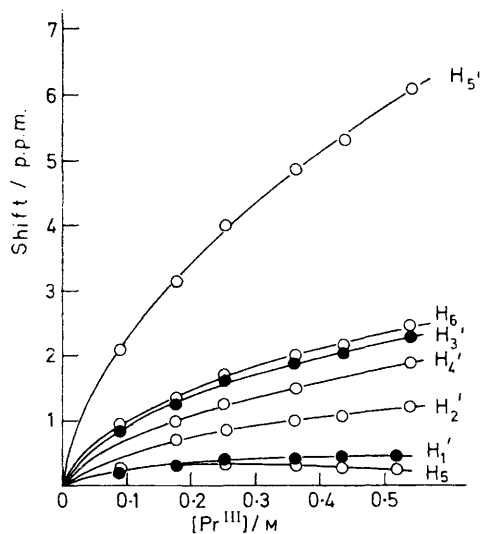


FIGURE 1 Titration curves for cmp (30 mM) with Pr$^{III}$

constants are almost independent of the lanthanoid(III) cation studied. As there were no significant induced shifts of the protons of cytidine in lanthanoid(III) solutions, we may assume that the phosphate group is the essential binding site.
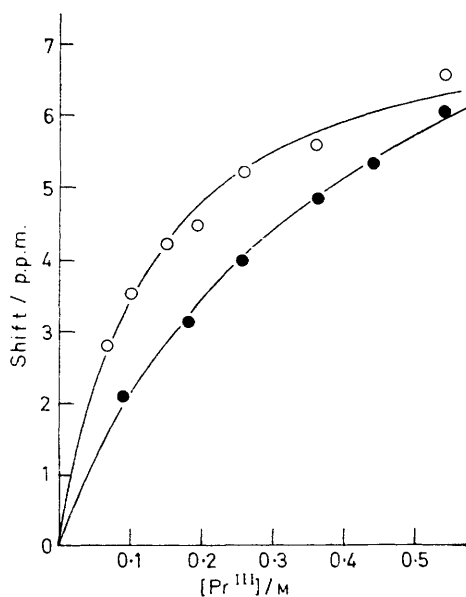


FIGURE 2 Titration curves for the H$_5$' protons of cmp with Pr$^{III}$ at 25 (●) and 85 °C (○)

Ratios of shifts of a given proton relative to that of the two H$_5$' protons (which suffer very nearly the same induced shift) are shown in Figure 3 at various concentrations of Dy$^{III}$. This Figure also shows that the ratios change slightly as the concentration of lanthanoid is increased, but this is largely due to the change in

ionic strength. This was confirmed by changing the ionic strength using KCl. To obtain ratios at 'zero ionic strength,' extrapolation to ratios at zero concentration of lanthanoid was carried out, as described previously.[1] At low temperature (25 °C) the shift ratios were the same, within experimental error, for all the lanthanoid cations, except for Tm$^{III}$ (Tables 1 and 2). At high temperature (85 °C) these ratios were little changed, again with the exception of those obtained with Tm$^{III}$ (Table 3). Details of the Tm$^{III}$ shifts will be discussed in a subsequent paper,[8] but it is clear that the shift magnitudes and directions are dependent on temperature, and that the ratios are only comparable
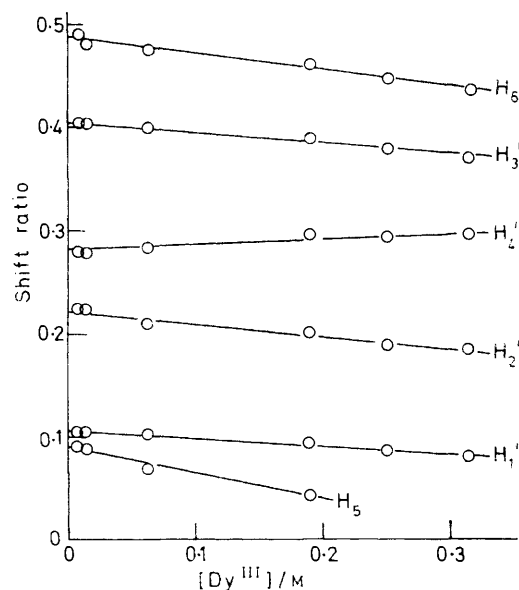


FIGURE 3 Shift ratios relative to the H$_5$' protons (1·00) at different concentrations of Dy$^{III}$

TABLE 1

Shift ratios $^a$ (relative to H$_5$') extrapolated to [M$^{III}$] = 0 at 25 °C

| M | H$_6$ | H$_5$ | H$_1$' | H$_2$' | H$_3$' | H$_4$' |
|---|---|---|---|---|---|---|
| Pr | 0·47 | 0·11 | 0·11 | 0·23 | 0·41 | 0·30 |
| Nd | 0·51 | 0·10 | 0·08 | | | |
| Eu | 0·50 | 0·07 | 0·10 | 0·18 | 0·40 | 0·33 |
| Tb | 0·45 | 0·01 | 0·09 | 0·20 | 0·39 | 0·25 |
| Dy | 0·49 | 0·10 | 0·10 | 0·23 | 0·41 | 0·28 |
| Ho | 0·51 | 0·05 | 0·08 | 0·18 | 0·42 | 0·26 |
| Er | 0·50 | 0·00 | 0·09 | 0·18 | 0·42 | 0·26 |
| Tm | 0·61 | −0·43 | −0·06 | | 0·35 | |
| Yb | 0·51 | 0·03 | 0·09 | 0·20 | 0·43 | 0·26 |
| Mean $^b$ | 0·49 | 0·06 | 0·09 | 0·20 | 0·41 | 0·28 |

$^a$ Corrected for small shifts observed on addition of the diamagnetic La$^{III}$ ion. $^b$ Not including Tm$^{III}$.

to those obtained with other lanthanoids at temperatures in excess of 50 °C.

From equation (1), the close similarity of the shift ratios with different lanthanoids means that the shifts are pseudo-contact in origin. In addition either $D_z/(D_x - D_y)$ is the same for all the lanthanoids, a

[8] C. M. Dobson, C. F. Geraldes, B. A. Levine, D. R. Martin, R. J. P. Williams, and A. V. Xavier, unpublished work.

highly improbable condition, or $D_x - D_y = 0$. The latter condition, known as axial symmetry, will be

Shift ratios [a] (relative to $H_5'$) extrapolated to $[M^{III}] = 0.5M$ at 25 °C

| M | $H_6$ | $H_5$ | $H_1'$ | $H_2'$ | $H_3'$ | $H_4'$ |
|---|---|---|---|---|---|---|
| Pr | 0.43 | 0.05 | 0.08 | 0.21 | 0.39 | 0.31 |
| Nd | 0.40 | 0.07 | 0.05 | | | |
| Eu | 0.40 | −0.04 | 0.06 | 0.15 | 0.35 | 0.29 |
| Tb | 0.35 | −0.02 | 0.04 | 0.14 | 0.33 | 0.28 |
| Ho | 0.41 | | | | 0.31 | 0.26 |
| Tm | −0.05 | −0.75 | 0.15 | | | |
| Yb | 0.43 | −0.09 | 0.04 | 0.13 | 0.34 | 0.25 |
| Mean [b] | 0.40 | −0.01 | 0.06 | 0.16 | 0.34 | 0.29 |

[a] Corrected for small shifts observed on addition of the diamagnetic $La^{III}$ ion. [b] Not including $Tm^{III}$.

assumed in what follows and equation (1) now reduces to (3). Table 4 gives the relative shift, and Table 5

$$\delta = AD_z(3\cos^2\theta - 1)/r^3 \qquad (3)$$

the relative broadening magnitudes, for the different lanthanoids. The shift directions for the different lanthanoids agree with the theory of Bleaney [3,4] and the magnitudes are close to those predicted.

Shift ratios [a] (relative to $H_5'$) extrapolated to $[M^{III}] = 0$ at 85 °C

| M | $H_6$ | $H_5$ | $H_1'$ | $H_2'$ | $H_3'$ | $H_4'$ |
|---|---|---|---|---|---|---|
| Pr | 0.40 | 0.08 | 0.11 | 0.22 | 0.39 | 0.34 |
| Nd | 0.35 | 0.05 | 0.08 | 0.22 | 0.35 | 0.31 |
| Eu | 0.48 | 0.06 | 0.13 | 0.15 | 0.40 | 0.34 |
| Tb | 0.39 | 0.04 | 0.09 | 0.20 | 0.38 | 0.30 |
| Dy | 0.41 | 0.07 | 0.10 | 0.21 | 0.39 | 0.31 |
| Ho | 0.41 | 0.04 | 0.09 | 0.21 | 0.38 | 0.30 |
| Er | 0.43 | 0.02 | 0.09 | 0.19 | 0.40 | 0.32 |
| Tm | 0.45 | −0.05 | 0.08 | 0.20 | 0.42 | 0.30 |
| Yb | 0.46 | 0.07 | 0.11 | 0.19 | 0.39 | 0.32 |
| Mean | 0.42 | 0.04 | 0.10 | 0.20 | 0.39 | 0.32 |

[a] Corrected for small shifts observed on addition of the diamagnetic $La^{III}$ ion.

$H_5'$ Shifts relative to $Dy^{III} = -100$ at 25 °C

| | Theory | Found [a] |
|---|---|---|
| La | 0 | 0 |
| Ce | −6.3 | |
| Pr | −11.0 | −7.1 |
| Nd | −4.2 | −2.6 |
| Pm | 2.0 | |
| Sm | −0.7 | −0.2 |
| Eu | 4.0 | 4.0 |
| Gd | 0 | |
| Tb | −86 | −50 |
| Dy | −100 | −100 |
| Ho | −39 | −39 |
| Er | 33 | 12 |
| Tm | 53 | 8.2 [b] |
| Yb | 22 | 10 |
| Lu | 0 | |

[a] Assumes binding constants for all lanthanoids are equal. [b] At 85 °C this value is 17.

From the shift ratios, and on the assumption of axial symmetry, the conformation of cmp was obtained

in the manner described earlier.[1] The molecular co-ordinates of cmp were obtained by using ribose phosphate co-ordinates from amp,[1] and adding the cytosine ring (co-ordinates from X-ray diffraction [9]) to replace the adenine ring. These molecular co-ordinates were given as input data into a computer

Broadening data relative to $Dy^{III} = 100$ at 25 °C

| | Relative broadening [a] | Broadening/ Shift |
|---|---|---|
| La | 0 | [b] |
| Ce | | |
| Pr | [c] | [c] |
| Nd | [c] | [c] |
| Pm | | |
| Sm | [c] | |
| Eu | [c] | [c] |
| Gd | v. large | v. large |
| Tb | 40 | 80 |
| Dy | 100 | 100 |
| Ho | 25 | 80 |
| Er | 20 | 170 |
| Tm | 30 | 250 [d] |
| Yb | 5 | 50 |
| Lu | | |

[a] Means of $H_5'$ and $H_6$ (±10 for Tb to Tm). [b] No paramagnetic shift. [c] Very small. [d] Very temperature dependent.

program which operated as follows.[1,6] From a fixed metal co-ordination scheme, rotation about each of the bonds shown in Figure 4 was carried out in turn
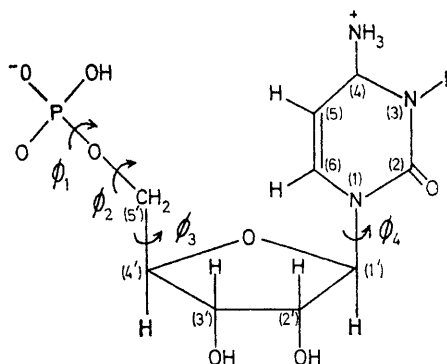


FIGURE 4  Structure and rotatable bonds of cmp

at 4° intervals. For each computer-generated conformation, after checking that no atoms were closer than the van der Waals radii allow, predicted shift ratios were compared to measured ratios. Only when this comparison was within set tolerances did the program proceed to rotation of the next bond. When calculated shift data agreed with experimental data for all the protons the conformation was accepted as a solution, to be further tested against relaxation data. For cmp the procedure was initially carried out using the ribose ring-pucker and metal co-ordination scheme used for the 'low-resolution' conformation of amp.[1] 22 Solutions, all of one closely related family, were obtained [Table 6(a)]. Wide tolerances were used in

[9] M. Sundrahingham and L. H. Jensen, J. Mol. Biol., 1965, 13, 930.

this search to ensure that no possible conformations were missed. Shift data for $H_4'$ and $H_2'$ protons gave relatively poor agreement with the computed conformations.

Shift data for ribose ring protons of amp and cmp were, however, very similar.[10] This implies that the

### TABLE 6
#### Computer data for cmp

(a) Initial run [a]

| | $H_5'$ | $H_4'$ | $H_3'$ | $H_2'$ | $H_1'$ | $H_5$ | $H_6$ |
|---|---|---|---|---|---|---|---|
| Input data | 1 000 | 287 | 415 | 195 | 83 | 88 | 496 |
| Tolerance | 30 | 150 | 150 | 100 | 30 | 50 | 30 |
| Typical solution | 1 024 976 | 363 | 405 | 149 | 63 | 110 | 494 |

Range of angles (°) $\phi_1$ 12, $\phi_2$ (fixed), $\phi_3$ 8, $\phi_4$ 16.

(b) Refined conformation [b]

| | $H_5'$ | $H_4'$ | $H_3'$ | $H_2'$ | $H_1'$ | $H_5$ | $H_6$ |
|---|---|---|---|---|---|---|---|
| Input data [c] | 1 000 | 280 | 410 | 225 | 105 | 95 | 490 |
| Tolerance | 30 | 20 | 20 | 20 | 15 | 20 | 15 |
| Typical solution | 1 024 975 | 280 | 408 | 222 | 103 | 106 | 487 |

[a] cf. Ref. 1.   [b] cf. Ref. 6.   [c] From Dy[III] data.

conformations of the ribose rings in amp and cmp are similar. The procedure for a change in ring pucker has been described in detail for amp [6] and an exactly parallel analysis was carried out for cmp. The derived ring puckers for amp and cmp were very similar. In particular, change of pucker of the ribose ring had negligible effect on the conformation of the base relative to the rest of the molecule and, as Table 6(b) shows, conformations of the ribose ring were obtained which fit the n.m.r. data extremely well. One of the family of conformations obtained for cmp is shown in Figure 5. We appreciate that some form of averaging
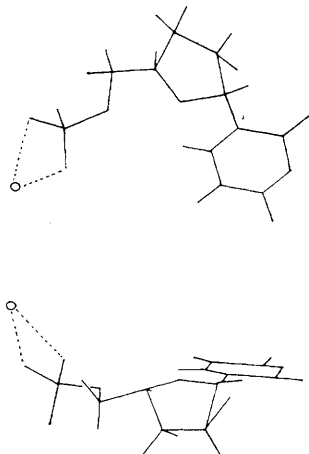


FIGURE 5   Two views of conformation of cmp derived from n.m.r. data

of the ring pucker may be occurring, but stress that, within the experimental errors, both the cmp and amp shift data can be generated by the same ring pucker (distorted 2'-endo [6]). A more general discussion

of this point is given in the previous paper. Clearly the conformation of the base of cmp relative to the ribose ring is anti, as in amp.

Gd[III] relaxation studies were carried out using line-width measurements $(T_2)$ on solutions where the resonances were well separated by Dy[III], and spin–lattice $(T_1)$ measurements on solutions where the peaks
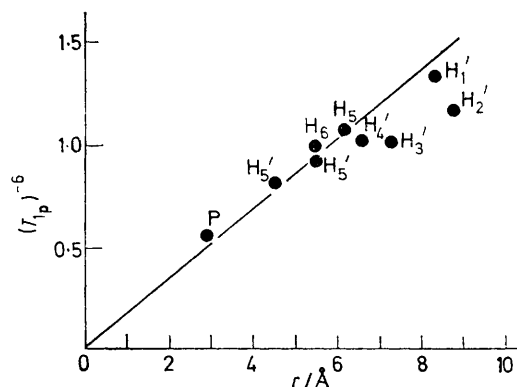


FIGURE 6   Plot of relative values of $(T_{1p})^{-6}$ against $r$ for the conformation shown in Figure 5

were separated by Eu[III]. This information is summarised in Table 7, where data for the ^{31}P resonance of

### TABLE 7
Relaxation data relative to the $H_6$ proton of cmp

| | | $H_6$ | $H_5$ | $H_1'$ | $H_2'$ | $H_3'$ | $H_4'$ | $H_5'$ [a] |
|---|---|---|---|---|---|---|---|---|
| Dy | $1/T_{2p}$ | 1·00 | 0·46 | 0·32 | 0·60 | 1·00 | 0·78 | 3·0 |
| Gd | $1/T_{2p}$ [b] | 1·00 | 0·52 | 0·22 | 0·44 | 1·00 | 0·80 | 3·0 |
| Gd | $1/T_{1p}$ [c] | 1·00 | 0·68 | 0·32 | 0·50 | 0·90 | 0·90 | 1·5 3·0 |
| Gd | $1/T_{1p}$ [d] | 1·00 | 0·67 | 0·18 | 0·42 | | | 1·6 3·2 |

[a] The two $H_5'$ resonances are well enough separated at 270 MHz for $T_1$ measurements to be carried out. The $T_2$ data are for the two peaks together.   [b] In the presence of 0·05M-Dy[III].   [c] In the presence of 0·2M-Eu[III]. The ^{31}P value is 33·5, corrected for the difference in frequency of the measurements.   [d] Corrected for the effect on cytidine.

cmp are also given. [31]P $T_{2p}$ Data could not be used because of the scalar contribution to relaxation. The agreement, within experimental error, between $T_{1p}$ and $T_{2p}$ ratios for the protons shows that for relaxation, as well as shift, the conditions for fast exchange apply.[5] It is also interesting to note that broadening of lines caused by Dy[III] is very similar to that caused by Gd[III], suggesting that isotropic relaxation is occurring here also. Figure 6 is a plot of relative values of $(T_{1p})^{-6}$ (corrected for the small effect of Gd[III] on cytidine) against values of $r$ for one of the family of solutions (that shown in Figure 5) from the computer. This shows that relative distances from the metal atom to the protons and to the phosphorus atom obtained experimentally fit the computed structure well. It is important to note that relative distances obtained for

[10] C. M. Dobson, R. J. P. Williams, and A. V. Xavier, previous paper.

the $H_5$ and $H_6$ protons of cmp are quite similar (6·2 and 5·5 Å respectively). The shift ratios however are quite different (0·06 and 0·49 from Table 1). This emphasises the importance of the angular term in the shift equation. The good agreement between the computed conformation, the relaxation data, and the shift data implies that averaging over widely differing conformations cannot be occurring.

*Conclusions.*—Overall the agreement between data from the two independent probe methods (shift and relaxation) gives confidence that averaging of the molecular conformation in solution, which must occur to some extent, is small. The observed conformation of cmp closely resembles that of amp.[1] In addition, the small change in shift ratios with increase in temperature strengthens the impression of a relatively well defined

conformation in solution, even over a range of temperature. This stability of the limited family of conformations is consistent with an *increased* stability constant of the lanthanoid complex on raising the temperature. It could be that the structure of water plays a very important role in the control of conformation in solution, for it is known that complex formation favoured by a positive entropy change, as in this case, is largely due to the entropy changes of bound and free water.