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Polyhedron 22 (2003) 711–724

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Synthesis, structural and biological evaluation of GlyAla based lanthanide macrocyclic conjugates as supramolecular ribonuclease mimics

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Received 2 October 2002; accepted 3 December 2002

Abstract

The glycine–alanine conjugated ligands of cyclen (1,4,7,10,-tetraazacyclododecane) **1** and **2**, possessing methyl and benzyl alanine esters respectively, and the corresponding lanthanide complexes **1La**, **1Eu**, **1Tb**, **1Yb**, **2La**, **2Eu**, and **2Tb** were designed with the aim of mimicking the nature of the hydrophobic cavity of ribonucleases. X-ray crystallographic investigations showed that **2Tb** has a typical monocapped square antiprism geometry, where the Tb(III) ion is central, coordinating to the four amino moieties of the cyclen ring and four of the oxygens of amide carbonyl groups of the glycine residues of the four pendant arms, with the ninth coordinated site being occupied by a water molecule. All the complexes were shown to promote the hydrolysis of the phosphodiester bond of 2-hydroxypropyl *p*-nitrophenyl phosphate (HPNP, $\tau_{1/2} = 5.78 \times 10^3$ h) with **1Tb** being the most efficient in promoting such hydrolysis at pH 7.4 and at 37 °C for the **1Ln** family with $\tau_{1/2} = 4.9$ h. For the **2Ln** family, **2La** was most effective in promoting hydrolysis of HPNP, with $\tau_{1/2} = 3.7$ h. The rate of hydrolysis was also investigated for **1La** and **2La** as function of pH, with both complexes displaying bell-shaped pH dependence within the physiological pH range. For **1Ln** the highest activity was observed at pH 7.0, with $\tau_{1/2} = 4.6$ h, whereas for **2La** it occurred at pH 7.4. Beyond pH 8, the rate of both complexes was shown to be almost linearly increased. The ability of **1Eu** and **2Eu** to cleave a 23-mer sequence from the mRNA of the GAG–HIV gene was also investigated. It was found that both gave rise to cleavage of the sequence at every nucleotide residue after 4 h of incubations at pH 7.4 and 37 °C.
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Keywords: 2-Hydroxypropyl *p*-nitrophenyl phosphate; Glycine–alanine; Benzyl alanine esters; Lanthanide complexes

1. Introduction

There is much current interest in the design and development of small or medium size supramolecular compounds that can mimic important enzymatic reactions under physiological conditions [1,2]. Ribonucleases (and ribozymes) are biological systems that can achieve rapid and catalytic cleavage of the phosphodiester bonds of nucleic acids such as mRNA [3]. This is of great importance since the genetic information is stored by DNA and manifested as proteins, with messenger RNA (mRNA) serving as the conduit. Hence, research into ways of developing small molecules or molecular assemblies that can selectively prevent protein expression by hindering DNA transcription or by specific interactions with mRNA (with subsequent destruction of the mRNA) could in principle yield artificial enzymes

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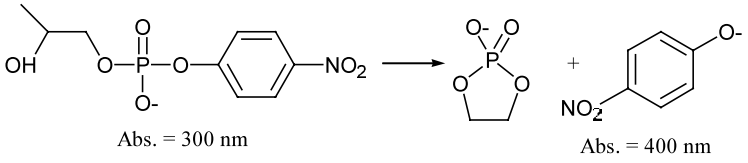
0277-5387/02/$ - see front matter © 2002 Elsevier Science Ltd. All rights reserved.
doi:10.1016/S0277-5387(02)01402-X

or 'tailor-made drugs' such as antisense based ribozyme mimics [4]. Investigations have shown that the 2′-hydroxy function on the RNA ribose makes hydrolytic strand cleavage or transesterfication much faster than in DNA, which lacks this functionality [5]. Furthermore, many ribonucleases have been shown to carry out phosphodiester hydrolysis through the synergistic action of several metal ions [6]. For instance, nuclease P1 has three zinc ions surrounded by several histidine amino acid residues as well as two metal bound water molecules in its active site [3,6]. In general, there are thought to be three direct, or *inner sphere*, modes of activation that the metal ion itself can provide in the hydrolysis, and a further three indirect modes, or *outer sphere*, activations provided by metal-bound water and hydroxy ions [5]. Recently, transition [7] and lanthanide ion complexes [8] have been designed as ribonuclease mimics and their catalytic ability investigated by employing phosphodiester models such as 2-hydroxypropyl *p*-nitrophenyl phosphate (HPNP; the mechanism for the hydrolysis of HPNP is shown in Scheme 1), dinucleotides and mRNA itself. Ideally, such a system should also provide a structural platform for incorporating cofactors such as amino moieties, quaternised amines, etc., that can participate in the hydrolytic process, but only a few such examples have been reported to date [9].

We have been interested in the development of supramolecular devices by employing carboxyamide functionalised lanthanide ion cyclen complexes as luminescent switches and sensors, and for mimicking the function of logic gates in silicon based computing [10,11]. Such complexes are known to be both kinetically and thermodynamically stable in respect of metal ion dissociation. Consequently, we have recently initiated a research programme into the use of such functionalised carboxyamide cyclen complexes as ribonuclease mimics for phosphodiester hydrolysis of mRNA [11]. There are several advantages in developing such lanthanide based complexes for this purpose. For instance, Ln(III) have high charge density, La(III) being 2.8 $Å^{-1}$ (Z/r) while Eu(III) is 3.2 $Å^{-1}$, and high ionisation potentials, being 9.18 eV for La(III) and 24.94 eV for Eu(III) [12]. These are thus hard Lewis acids, which are needed for both binding and activation of phosphodiesters as discussed above. However, these ions are known to precipitate in water as their hydroxides, and are thought to be potentially toxic. It is thus important to embed them in a coordination environment that preserves their Lewis acid character at the same time as making them both kinetically and thermodynamically stable. Our aim was to develop cyclen complexes that would mimic the nature of the active site of ribonuclease by incorporating various cofactors (such as amino acids, amines, etc.) covalently into the cyclen structure as pendant arms. It is well established that tetra-substituted cyclen lanthanide complexes give rise to a concave structure consisting of either square antiprism or twisted square antiprism geometry in solution [13]. Furthermore, due to the high coordination requirement of lanthanide ions, which is usually not fulfilled by macrocyclic ligands, the complexes can take up several water (or other solvent) molecules to become coordinatively saturated [14]. We thus anticipated that the synergetic action of these features, i.e. cofactors, concaved structure, Lewis acid, and metal bound water molecules, would aid in the hydrolytic process either directly, such as in general acid base catalysis or by direct nucleophilic activation, or indirectly, such as providing a hydrophobic environment around the metal ion. Ideally, a combination of both would be preferred. Our initial approach was to use pseudo 'dipeptides' that could extend the size of the concave structure of cyclen. We hoped that such modification would give rise to the formation of a hydrophobic cavity around the central lanthanide ion, which would mimic that seen in the active sites of ribonucleases. With this in mind we incorporated four glycine–alanine (GlyAla) residues into the cyclen structure. This design gave the two ligands **1**, using L-alanine methyl ester, and **2**, using L-alanine benzyl ester respectively, where the Gly amino acid had its amino terminus as a part of the cyclen ring structure. For **2** we predicted that the benzyl esters would extend the size of the arms substantially compared to that of **1**, giving the complexes larger, or deeper cavities. In this paper we give a full account of our work, describing the synthesis and structural characterisation of **1** and **2** and several of their cationic Ln(III) complexes **1Ln** and **2Ln** where Ln = La(III), Eu(III), Tb(III) and Yb(III) for **1Ln** and La(III), Eu(III) and Tb(III) for **2Ln**. We also describe the evaluation of these systems in cleaving HPNP at pH 7.4, and the effect of pH upon the rate of hydrolysis of HPNP by **1La** and **2La**, together with an investigation into the effect of **1Eu** and **2Eu** on a 23-mer mRNA sequence derived from the GAG gene of HIV [15].



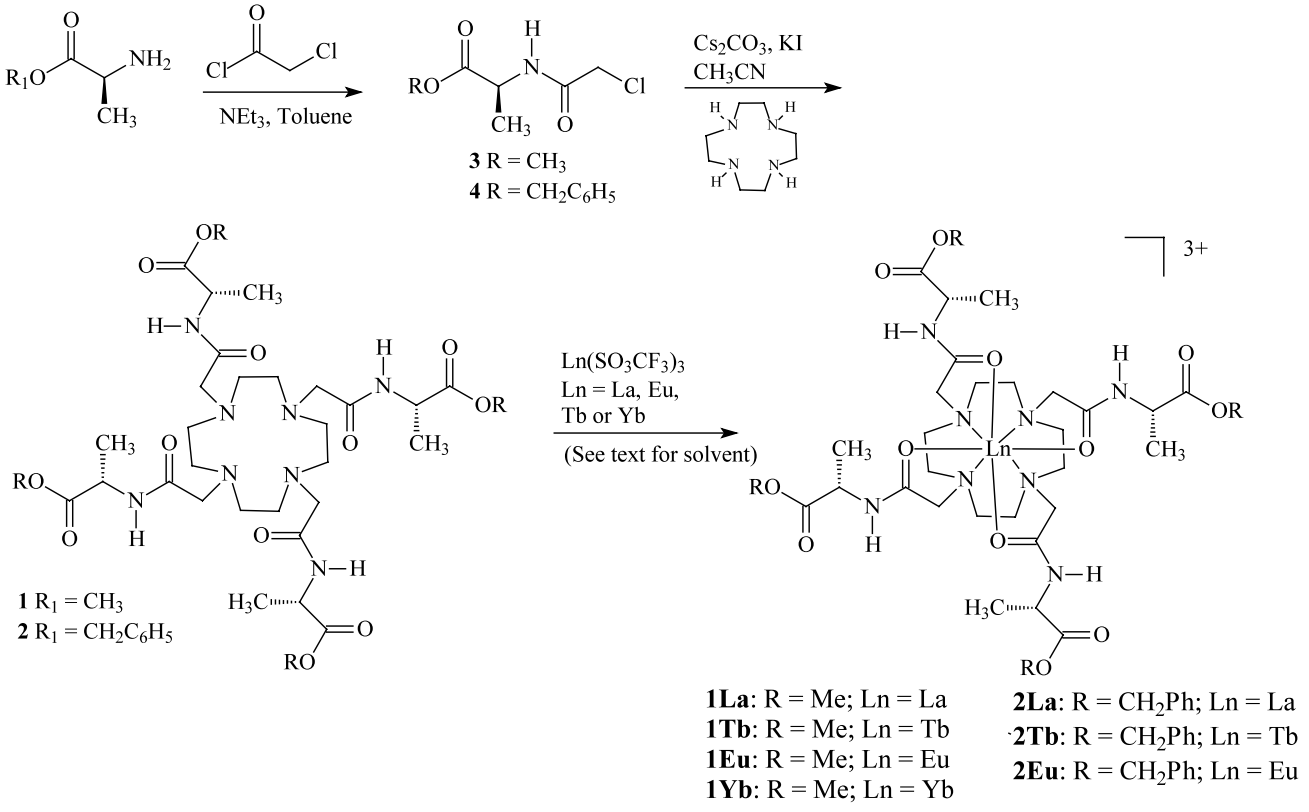
Scheme 1. The mechanism for the hydrolysis of HPNP.

2. Results and discussion

2.1. Synthesis of 1 and 2 and their lanthanide ion complexes

The synthesis of the two ligands **1** and **2** and their corresponding lanthanide ion complexes is shown in Scheme 2. The two α-chloroamides **3** and **4** were made by a using modified literature procedure [16] by reacting L-alanine methyl ester hydrochloride and L-alanine benzyl ester hydrochloride respectively with chloroacetylchloride (in CH_2Cl_2 for the methyl ester and in toluene for the benzyl ester) in the presence of 3 equivalents of triethylamine at −20 °C for 2 h. The resulting solution was allowed to warm to room temperature and stirred for a further 24 h before filtering off the triethylammoniumchloride, and removing the solvent under reduced pressure. The resulting oily residues were taken up into $CHCl_3$, washed several times with 0.1 M HCl and dried over K_2CO_3. Removing the organic solvent under reduced pressure gave the two products in 65 and 98% yield respectively as oils that solidified upon standing. No further purification was necessary. The two ligands **1** and **2** were formed by reacting 4.1 equivalents of **3** or **4** with cyclen in refluxing acetonitrile in the presence of 4.1 equivalents of KI and Cs_2CO_3 for 48 h. Upon cooling to room temperature the inorganic solids were filtered off under reduced pressure and the filtrate reduced to dryness to give oils. These were taken up into $CHCl_3$ and thoroughly washed with saturated KCl solution. The resulting organic phase was dried over K_2CO_3 and the solvent removed under reduced pressure to give the two ligands in 49 and 74% yield respectively. No further purification was needed. The two ligands were characterised using conventional methods. The ^1H NMR for both ligands showed that **1** and **2** had C_4 symmetry, where the ring protons, and the protons of the adjacent α-methyl group (the glycine unit of the pendant arms) were all chemically inequivalent. For **1**, the ring protons appeared as AB systems at 2.91 and 2.69 ppm with $J = 10$ Hz, whereas the glycine methyl protons appeared at 3.27 and 3.12 ppm with $J = 16$ Hz. Similar results were observed for **2**.

The lanthanide ion complexes **1Ln** and **2Ln** were made from the corresponding ligands by refluxing the ligands in dry CH_3CN, with the exception of **1La** and **2La** complexes which were made in MeOH and EtOH respectively, in the presence of 1.1 equivalents of the corresponding lanthanide triflate salts [$LnSO_3CF_3$, where Ln = La(III), Eu(III), Tb(III) and Yb(III)] for 24 h. After cooling to room temperature, the resulting complexes were isolated by precipitation in diethylether, giving **1La**, **1Eu**, **1Tb** and **1Yb** complexes of **1** and **2La**, **2Eu** and **2Tb** complexes of **2** respectively as hygroscopic salts. These complexes were analysed by accurate mass



Scheme 2. Synthesis of the ligands **1** and **2** and the lanthanide complexes **1Ln** and **2Ln**.

analysis obtained by Electrospray MS, ^{1}H NMR, IR and m.p. determination. The ^{1}H NMR of the **1Eu**, **1Tb**, **1Yb**, and **2Eu**, **2Tb** **2Yb** complexes showed the expected shifts and line broadening in their ^{1}H NMR. For instance the ^{1}H NMR spectra when recorded in CD_3OD, of **1Eu** and **2Eu** showed well spread out resonances for the equatorial and the axial protons of the azamacrocycle and the methyl protons of the glycine moiety. For **1Eu** these appeared at 24.1, −0.4, −2.5, −4.7, −8.2 and −12.3 ppm. Similar results were seen for **2Eu**, as can been seen in Fig. 1. These chemical shifts are characteristic for a monocapped square antiprism geometry in solution for such cyclen-based complexes [17]. This would indicate that the structures of these complexes in solution could give rise to bowl or cavity shaped conformations, where the 'dipeptide' would line the walls of such a cavity. In contrast, the ^{1}H NMR of the La(III) complexes **1La** and **2La**, showed some line broadening, but their resonances were not as shifted as their paramagnetic counterparts.

2.2. Crystallographic investigation

Eventhough most of the above complexes were obtained as hydroscopic salts, **2Tb** was obtained as a brown solid that was recrystallised from a mixture of MeOH/diethylether to give brown crystalline material that was suitable for crystallographic determination [18]. Fig. 2 shows the crystal structure of **2Tb**, showing the expected concave structure, with the Tb(III) ion in the centre. Selected bond lengths (Å) and angles (°) are given in Table 1. For **2Tb**, the Tb(III) ion is nine coordinated, with four of these coordination sites being taken up by the four amino moieties of the macrocycle, with average N···Tb bond length of 3.635 Å. A further four coordination sites are donated from the four amide carbonyl oxygen atoms of the glycine residues of the

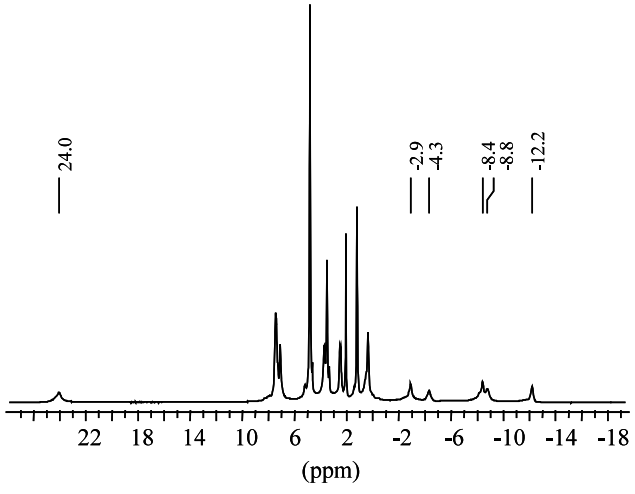


Fig. 1. The ^{1}H NMR (400 MHz, CD_3OD) of **2Eu** at room temperature.

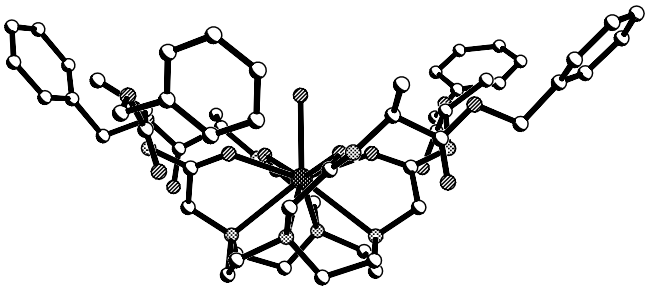


Fig. 2. Diagram of the **2Tb** complex showing how the four GlyAla residues form the walls of the cavity, with the Tb(III) ion placed in the centre. The ninth coordination side is occupied by a water molecule, giving the overall mono-capped square antiprismatic geometry. Hydrogen atoms have been omitted from the ligand for clarity.

pendant arms, with average O···Tb bond length of 2.423 Å (giving an octadentate coordination by **1** and **2**). The mean N–C–C–N and N–C–C–O torsion angles average out to −58.55° and 24.85° respectively [19]. From Fig. 2, it can be seen that the four dipeptide arms give rise to the wall of the cavity which has a water molecule coordinating to the central Tb(III) ion with O···Tb bond length of 2.383 Å, giving a nine overall coordination complex. Furthermore, a single diethylether molecule coordinating is hydrogen bonded, through the oxygen of the ether, to this water molecule (not shown in Fig. 2). A triflate anion is also found within this cavity. Fig. 3 gives the top view of the crystal structure. Here it can be seen that the structure has C_4 symmetry through the central cation, with a fairly regular monocapped square antiprism geometry [13,19]. In **2Tb** three independent elements of chirality are observed [17,19]. The first one of these is the presence of the chiral L-alanine moieties (that are assigned as *SSSS*, for the four chiral centres on Ala). The two latter are defined by their pedant arm N–C–C–O and N–C–C–N torsion angles (Table 1). The complexes can adopt two enantiomeric conformations, with respect to each of the five-ring chelates: δδδδ or λλλλ. In the case of **2Tb** the latter is observed. Secondly, the pendant arms may adopt either anticlockwise (Λ) or clockwise (Δ) enantiomeric conformations. Here only the Δ isomer is observed in the crystal structure. It has been shown by Parker and co-workers that interconversion between these latter two diastereoisomeric pairs may occur either via stepwise or synchronous arm rotation and ring inversion [19]. However, we have not detected such conformational change in our systems in solution at room temperature, but it has been shown that such interconversion is temperature dependent and that the introduction of extra chirality into the complexes can substantially reduce such interconversion, which might be the case for **1Ln** and **2Ln** [19]. We believe that in aqueous solution, **1Ln** and **2Ln** where Ln = Eu, Tb and Yb (for **1Ln**) all occupy such mono-capped square antiprism geometry, with a single metal bound water molecule

Table 1
Selected bond lengths (Å) and angles (°) for **2Tb**

Bond lengths			
Tb(1)–O(24C)	2.350(6)	Tb(1)–N(2)	2.617(7)
Tb(1)–O(24D)	2.358(5)	Tb(1)–N(4)	2.614(6)
Tb(1)–O(24B)	2.369(5)	Tb(1)–N(1)	2.642(7)
Tb(1)–O(1W)	2.383(4)	Tb(1)–N(3)	2.668(7)
Tb(1)–O(24A)	2.391(6)		
Bond angles			
O(24C)–Tb(1)–O(24D)	82.25(18)	O(1W)–Tb(1)–N(4)	126.50(19)
O(24C)–Tb(1)–O(24B)	87.90(18)	O(24A)–Tb(1)–N(4)	72.53(19)
O(24D)–Tb(1)–O(24B)	145.17(16)	N(2)–Tb(1)–N(4)	106.3(2)
O(24C)–Tb(1)–O(1W)	73.0(2)	O(24C)–Tb(1)–N(1)	141.2(2)
O(24D)–Tb(1)–O(1W)	72.37(16)	O(24D)–Tb(1)–N(1)	132.62(19)
O(24B)–Tb(1)–O(1W)	72.80(16)	O(24B)–Tb(1)–N(1)	71.41(19)
O(24C)–Tb(1)–O(24A)	144.50(16)	O(1W)–Tb(1)–N(1)	127.1(2)
O(24D)–Tb(1)–O(24A)	85.47(18)	O(24A)–Tb(1)–N(1)	66.9(2)
O(24B)–Tb(1)–O(24A)	83.47(17)	N(2)–Tb(1)–N(1)	68.5(2)
O(1W)–Tb(1)–O(24A)	71.5(2)	N(4)–Tb(1)–N(1)	68.7(2)
O(24C)–Tb(1)–N(2)	73.3(2)	O(24C)–Tb(1)–N(3)	65.66(19)
O(24D)–Tb(1)–N(2)	139.58(19)	O(24D)–Tb(1)–N(3)	72.80(18)
O(24B)–Tb(1)–N(2)	66.46(18)	O(24B)–Tb(1)–N(3)	132.16(19)
O(1W)–Tb(1)–N(2)	127.19(19)	O(1W)–Tb(1)–N(3)	128.5(2)
O(24A)–Tb(1)–N(2)	132.1(2)	O(24A)–Tb(1)–N(3)	140.4(2)
O(24C)–Tb(1)–N(4)	130.34(19)	N(2)–Tb(1)–N(3)	68.0(2)
O(24D)–Tb(1)–N(4)	66.53(18)	N(4)–Tb(1)–N(3)	68.6(2)
O(24B)–Tb(1)–N(4)	138.94(18)	N(1)–Tb(1)–N(3)	104.4(2)

sitting in the middle of the cavity. The presence of the water molecule in **2Tb** is essential to our design since, as previously explained, it can participate directly in the nucleophilic activation of the 2′-hydroxy group of HPNP, making it more nucleophilic. Furthermore, such metal-bound water molecules can be deprotonated, making them more nucleophilic. For the Ln(III) complexes it is possible that the ions have two such metal bound water molecules, due to larger coordination requirements. We have, however, been unable to verify their structure in the solid state to date.

2.3. Evaluation of the effectiveness of transesterfication of HPNP

The ability of **1** and **2** and their lanthanide ion complexes (0.17 mM) to promote phosphodiester hydrolysis of HPNP (0.17 mM) was monitored by observing the changes in the UV–Vis spectra of HPNP as a function of time at 37 °C and pH 7.4 (using 50 mM HEPES buffer). The hydrolysis of HPNP (which absorbs at 300 nm) yields two new products: *p*-nitrophenolate (absorbing at 400 nm) and a cyclic phosphate, Scheme 1. Each set of measurements was carried out over several half-lifetimes. The changes in the absorption as a function of time for **1Eu** at pH 7.4 and 37 °C are shown in Fig. 4.

The values for k, $\tau_{1/2}$ and k_{obs} for the hydrolysis of HPNP by **1Ln** and **2Ln** at pH 7.4 are listed in Table 2. The rate of hydrolysis (k) of HPNP in the absence of any 'catalyst' (k_{uncat}) at pH 7.4 was evaluated by Breslow and Huang and was determined as 0.00012 h^{-1}, with half-lifetime: $\tau_{1/2} = 5.78 \times 10^3$ h [20]. We use this value to determine k_{obs}, which is the ratio between k and k_{uncat} (we were not able to quantify this background hydrolysis over several days since it was too slow). For **1Ln** complexes the rate of hydrolysis of the phosphodiester bond of HPNP by **1La** was particularly slow at

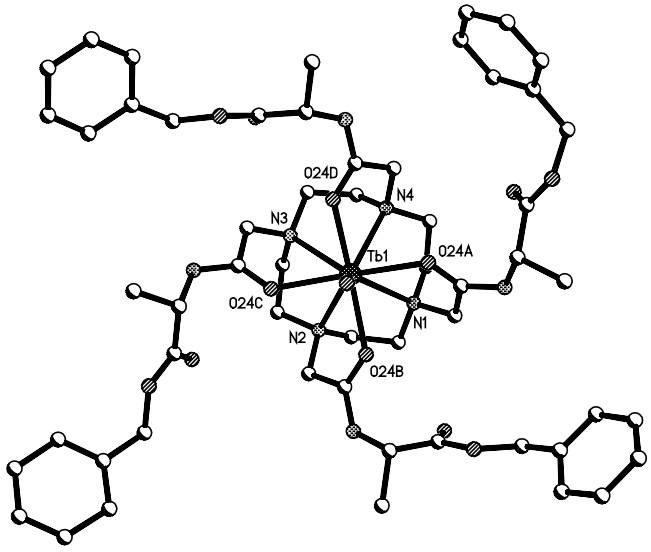


Fig. 3. Views down the C_4 axis of **2Tb**. Showing the four GlyAla pendent arms coordinating to the Tb(III) ion thorough the four nitrogens of the macrocycle and the oxygens of the carboxylic amide of the adjacent glycine residue.

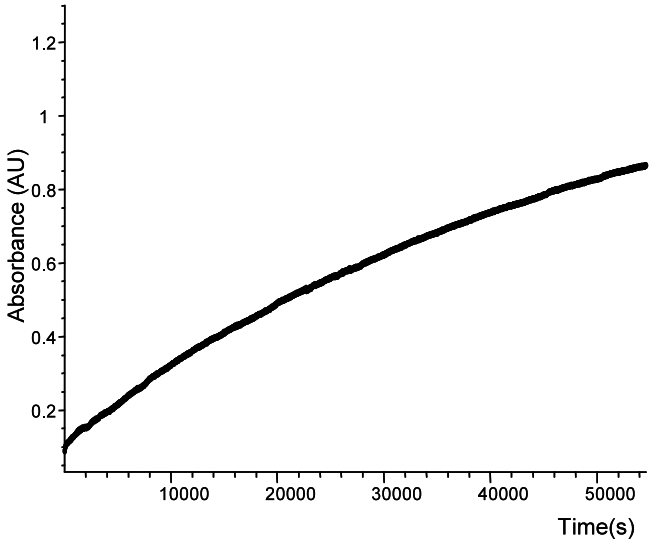


Fig. 4. The changes in the absorption spectra as a function of time (s) when monitoring the formation of *p*-nitrophenolate at 400 nm.

pH 7.4, with $\tau_{1/2} = 17.6$ h. Under the same experimental conditions, **1Eu** cleaved HPNP with $\tau_{1/2} = 7.7$ h, which is a rate enhancement of approximately 752, whereas **1Yb** gave $\tau_{1/2} = 12.0$ h. However, in this series, the **1Tb** complex was the most efficient in hydrolysing HPNP at pH 7.4 giving $\tau_{1/2} = 4.9$ h, and $k_{obs} = 1183$. We also evaluated the ability of **1Ln** and **2Ln** to cleave HPNP in the presence of 20% EDTA. In all cases the presence of EDTA affected the rate of hydrolysis slightly but we believe that this is due to binding of one or more of the EDTA carboxylic acids to the metal ion complex rather than metal extraction. We also evaluated the stability of several of these complexes to competitive Cu(II) (sulfate) exchange in water at pH 6.5. In all cases the complexes were stable to exchange over a period of 1 week (measured by UV–Vis), with the Tb(III) complexes being more stable than the Eu(III) complexes, but some dissociation was observed for the La(III) complexes over 24 h. We also measured the ability of **1** to promote HPNP hydrolysis. No significant hydrolysis was observed by **1** under the above experimental conditions over 24 h. This indicates that the presence of the lanthanide ion is necessary for HPNP hydrolysis to occur. In a related study, Morrow and co-workers developed both La(III) and Eu(III) complexes of tetra-amide functionalised cyclen ligands. Under similar experimental conditions, the corresponding La(III) complex hydrolysed HPNP with $k = 5.8 \times 10^{-2}$ h^{-1}, whereas the Eu(III) complex was found to be inactive [21]. Similar activity has also been demonstrated by Akkaya and Baykal [22]. In our own investigation using a GlyGly analogue of the **1Ln** series, we demonstrated that the La(III) was extremely efficient in hydrolysing the phosphodiester of HPNP with $k = 0.41$ h^{-1}. We also demonstrated that the Eu(III) GlyGly analogue was quite active with $k = 0.15$ h^{-1} [11].

We were thus quite interested in the 'lack of ability' of **1La** to promote hydrolysis of HPNP under these conditions in comparison with **1Eu**. We thus decided to evaluate the hydrolysis of HPNP at different pHs (all other experimental conditions remained the same). The k (h^{-1}) vs. pH profile for **1La** is given in Fig. 5. Each data point is the average value of two experimental runs within a 10% error. From this profile it can be seen that the hydrolytic cleavage by **1La** was extremely pH dependent; between pH 6 and 6.5 the hydrolysis was too slow for accurate k determinations. However, between pH 6.5 and 7.5 the rate of hydrolysis is especially pH dependent, and a 'bell shape' curve is observed [23]. Here **1La** is most active in promoting the phosphodiester hydrolysis at pH 7.0 with $\tau_{1/2} = 4.6$ h and $k_{obs} = 1225$. Between pH 7.2 and 7.5 the rate of hydrolysis reduced. However, beyond pH 7.5 the rate is greatly enhanced, increasing almost linearly with pH between 7.5 and 9.9. In highly alkaline solutions the rate is very fast, for instance at pH 9.9, HPNP is hydrolysed with $\tau_{1/2} = 1.7$ h and $k_{obs} = 3333$. This is almost three times the rate observed at pH 7.0. From these findings, it is obvious that **1La** is displaying dual pH dependence and that within the physiological pH range the complex is capable of hydrolysing HPNP within a very narrow window. To the best of our knowledge, **1La** is the first example of such a complex to exhibit such clear dual pH behaviour.

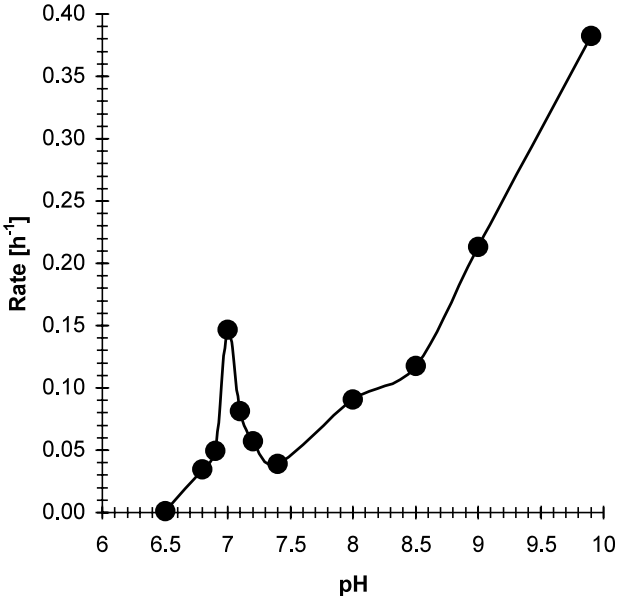


Fig. 5. The pH-rate profile (h^{-1}) of the hydrolytic cleavage of HPNP by **1La** when measured at 37 °C (1:1 equivalents). All measurements are the average values of two to three measurements, and all are within 10% error. Dual behaviour is observed; within the physiological pH the complexes shows large pH dependence with the formation of a bell-shaped curve, with the highest activity at pH 7.0.

The cleavage of HPNP by the **1Ln** series was in all cases much faster than we had anticipated, given the fact that both Gly and Ala lack the extra cooperative sites found in basic amino acids such as lysine and histidine which are found within the active site of ribonuclease. Moreover, since the Ala esters extend the size of the cavity, it could have been argued that this might hinder, or weaken the interactions of the HPNP with the Lewis acid centre. With the above in mind, we evaluated the ability of the **2Ln** series to promote hydrolysis of HPNP under identical conditions to those described for the **1Ln** complexes. The difference between the two systems, is in principle, that the **2Ln** might have larger or extended hydrophobic cavities in comparison to **1Ln**, e.g. will this structural difference affect the rate of hydrolysis of HPNP? As stated earlier, we have been unable to obtain suitable crystals for crystallographic determination of **1La**, however, we were able to resolve the crystal structure of the Eu(III) GlyGly analogue of **1Eu**. From this structure it was clear that the GlyGly did give rise to a hydrophobic cavity. However, in comparison to **2Tb**, the cavity did not have the 'bowl shape' appearance of **2Tb**. The hydrolysis of HPNP by **2La** was in comparison to **1La** much faster, with $\tau_{1/2} = 3.7$ h at pH 7.4, Table 2. For **2Eu** and **2Tb** the hydrolysis was slower than seen for **1Eu** and **1Tb** respectively with $\tau_{1/2} \approx 12$ h. Hence, only **2La** gives rise to any significant rate enhancement compared to the **1La** series. Because of this, we evaluated the pH dependence of HPNP hydrolysis by **2La** in the same way as for **1La**. The results of that investigation can be seen in Fig. 6. Here it can be seen that the rate of transesterification of HPNP is, as seen previously for **1La**, bell shaped. Below pH 7.0 the rate of hydrolysis is rather slow, but is then greatly enhanced, peaking at pH 7.4. Above pH 7.5, the rate again becomes slower, showing a small 'well' centered at pH 8. In more alkaline solution the rate is slightly enhanced, but then becomes almost linear as previously observed for **1La**. In comparison, the hydrolysis of HPNP between pH 8 and 9.5 was much slower, indicating that the rate enhancement below pH 10 was mainly due to the activation of **1La** and **2La**. From these findings it is obvious that the two La(III) complexes have very different pH dependence within the physiolo-

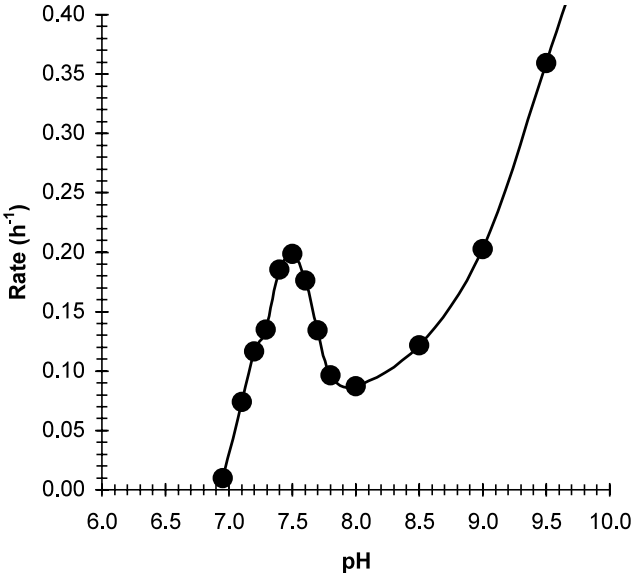


Fig. 6. The pH-rate profile (h^{-1}) of the hydrolytic cleavage of HPNP by **2La** (1:1 equivalents). A bell-shaped curve is observed as for **1La**. Here the highest activity within the physiological pH range is at pH 7.5.

Table 2
Results of the hydrolysis of HPNP using **1Ln** and **2Ln**

Complex	k (h^{-1})[a,b,c]	$\tau_{1/2}$ (h)	k_{obs}[d]
1La	0.0394	17.6	328
1Eu	0.0902	7.7	752
1Tb	0.1420	4.9	1183
1Yb	0.0577	12.0	480
2La	0.1855	3.7	1546
2Eu	0.0567	12.2	473
2Tb	0.0628	11.0	523

[a] Measured using *Agilent 8453* spectrophotometer fitted to circulating temperature controlled water bath, and water driven mechanical stirring, in 50 mM HEPES buffer, at pH 7.4 and at 37 °C.
[b] Average over three measurement and three half-lifetimes.
[c] k values were determined by fitting the data to first order rate kinetics using *Biochemical Analysis Software for Agilent ChemStation*. Errors are within ±10%.
[d] The ratio of the 'catalyst' vs. the 'uncatalysed' reaction using $k_{ucat} = 0.00012$ h^{-1}.

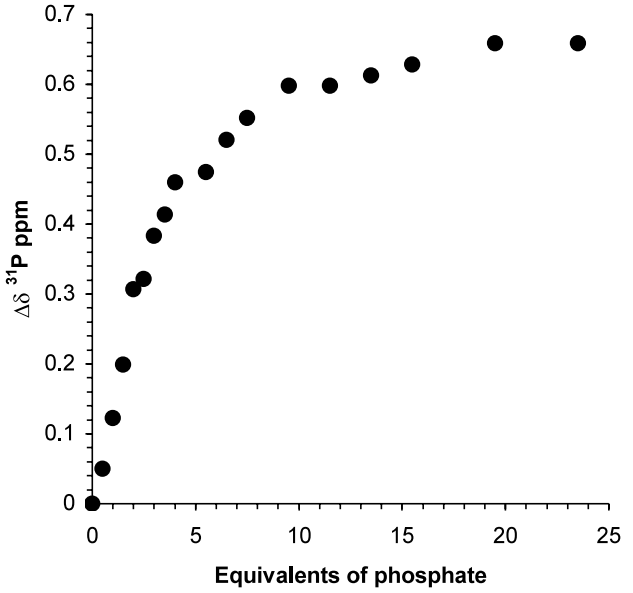


Fig. 7. The changes in the ^{31}P signal of HPNP upon binding to **2La**.

gical pH range, which could be assigned to their different structures.

2.4. Evaluating the binding of diethylphosphate to *1Ln*

With the aim of examining the possible binding of the phosphate diester to **1La**, **2La**, and **1Eu** and **2Eu** and correlating the binding affinity to the activity described above, we carried out ^{31}P NMR titrations of these complexes in water. Upon adding diethylphosphate (DEP) [$(CH_3CH_2O)_2PO_2^-$)] (which lacks the 2′-hydroxy group, and should thus be less susceptible to hydrolysis) to **1La** (7.1 mM) a shift of the ^{31}P signal was observed, indicating binding to **1La**. Fig. 7 shows these changes as $\Delta\delta^{31}P$ vs. equivalents of DEP. From these changes it can be seen that it requires about 12–13 equivalents of DEP to achieve saturation of the effect. However, it required only 1–3 equivalents to achieve saturation when these measurements were repeated using **1Eu**. We were, however, unable to fully assess the binding to **1Eu** because, upon introduction of DEP, precipitation occurred, which we assign to the formation of a ternary complex between **1Eu** and DEP. We can speculate, that since fewer equivalents of DEP were needed to achieve precipitation of the **1Eu** complex, the binding of DEP to **1Eu** is stronger than for **1La**. When similar measurements were attempted with **2La** and **2Eu**, the complexes proved to be insufficiently soluble in H_2O at the required concentration. However, in an analogous investigation using the GlyGly analogues of **1Ln** we showed that that only 3 equivalents were needed to obtain saturation in the ^{31}P signal for Eu(III) vs. approximately 30 for La(III) [11]. This indicates that the binding of the phosphodiester to the metal centre is much weaker for **1La** than for **1Eu**. We propose that similar binding preferences would be expected for HPNP.

2.5. Evaluating the cleavage of RNA by *1Eu* and *2Eu* and their free ligands

Our preliminary investigations have also shown that the **1Eu** and **2Eu** complexes efficiently cleave a 5′-^{32}P-end-labelled 23-mer-mRNA sequence (5′-AACC-CUUUAGAGACUAUGUAGAC-3′) from the HIV GAG gene (that has previously been used by Bashkin et al. [15] to demonstrate site-specific cleavage by ribozyme mimics), at pH 7.4 and 37 °C after 4 h of incubation using excess of the complexes. The occurrence of strand cleavage was evaluated by using polyacrylamide gel electrophoresis in conjunction with autoradiographic detection. Fig. 8 shows the densitometry profile for treatment with **1Eu**, indicating that the complex induces cleavage at every nucleotide residue. This cleavage was not quantified, and the complexes were not, under these experimental conditions, expected to induce site-specific or regio-specific cleavage. Similar results were observed for **2Eu**, with every nucleotide bond being cleaved. Upon incubation of the free ligand **2** with the 23-mer RNA under the same experimental conditions, no cleavage was observed, indicating the vital role of the Lewis acid centre in the hydrolytic process. We are currently modifying our systems with the aim of achieving site-specific cleavage of mRNA, by tethering them to suitably modified oligonucleotides.

3. Conclusion

The above results show that the families of **1Ln** and **2Ln** can induce cleavage of HPNP under physiological conditions. These results also indicate that the two families and different ions within these families do promote such hydrolysis differently. In all cases much faster hydrolysis was observed than anticipated, given the fact that the GlyAla walls of the complexes lack the

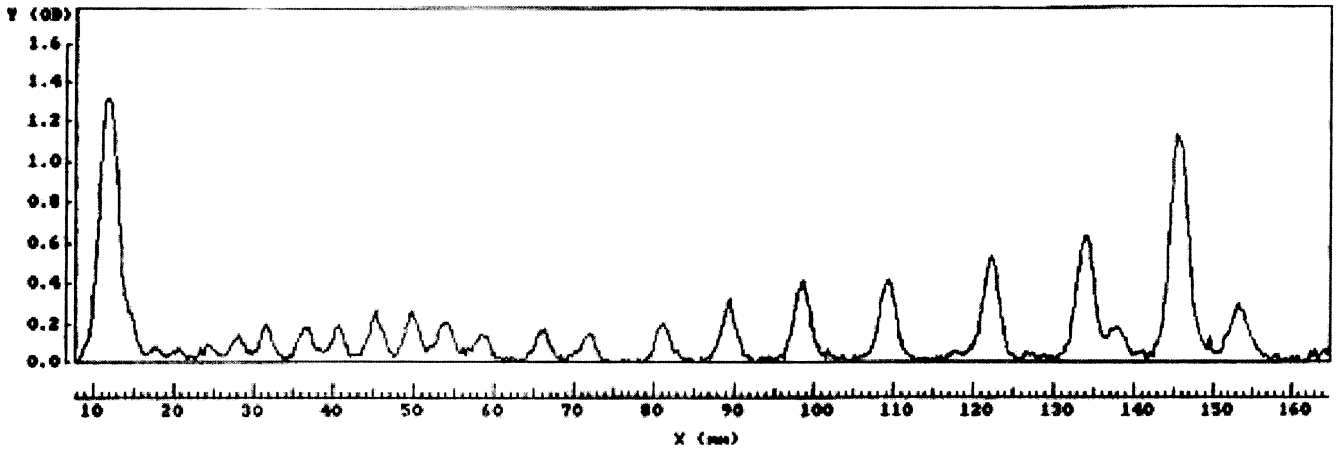
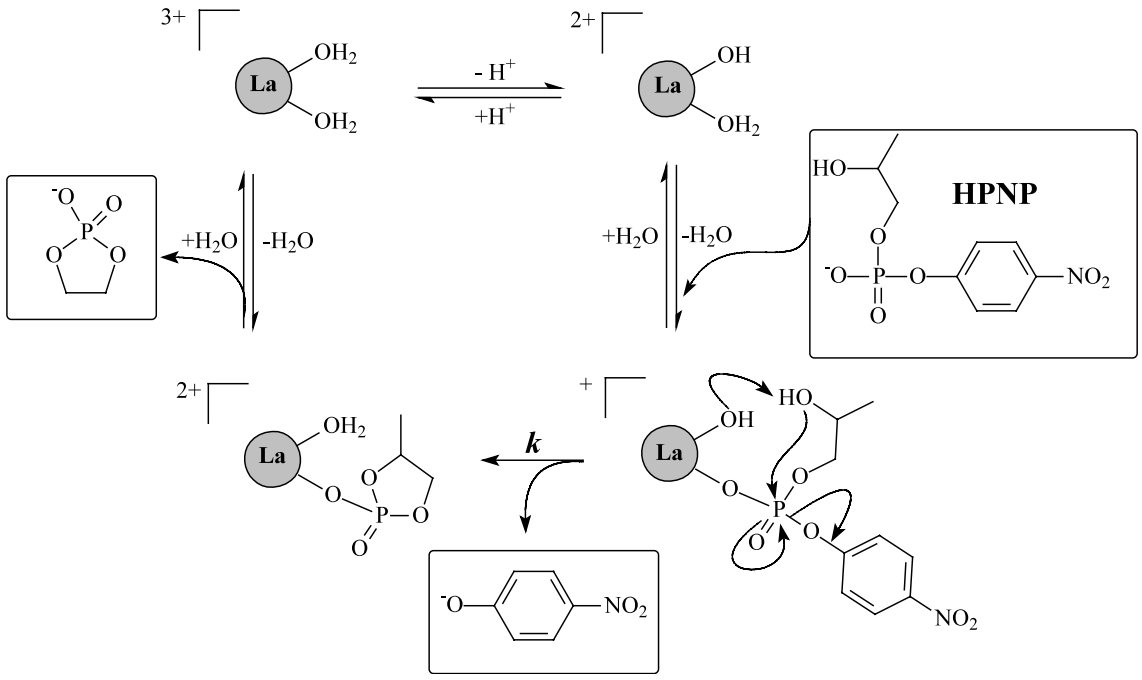


Fig. 8. The densitometry profile for treatment with **1Eu**, indicating that the complex induces cleavage at every nucleotide residue.

extra cooperative sites that could participate in the reaction—for instance through general acid base catalysis. It is also very important to note that both **1Eu** and **2Eu** promote the cleavage of RNA at every nucleotide in the sequence after only 4 h of incubation, whereas their free ligands do not. It is remarkable that these complexes show such a high rate enhancement considering the fact that the Lewis acid centre is more shielded from the solvent environment by the steric effect of the alanine methyl and benzyl esters, which greatly extend the size of the cavity, as we have clearly demonstrated by solid state crystallography. One could have predicted that this extension might inhibit the approach of the substrate in comparison to that seen by Morrow and co-workers [21] and by Akkaya and Baykal [22] who have used a 'shallower' version of the cyclen structure. In our examples, **2La** is most efficient in promoting phosphodiester hydrolysis at pH 7.4, whereas for **1La** the highest activity is observed at pH 7.0, when measured within the physiological pH range. It is our prediction that these rate enhancements are due to hydrophobic effects caused by the arrangement of the amino esters around the metal ion centre, giving rise to the formation of a hydrophobic pocket around the ion. This possibly favours the formation of stronger interactions between the ion and the phosphodiester. Such observations have been made previously, e.g. in Collman's 'picket fence' porphyrins [24], and by Walton and Boxwell in mimicking the active site of carbonic anhydrase [25], to name just a few. It is also particularly important to note that both the Eu(III) and the Tb(III) complexes show a significant rate enhancement, and that **1Eu** is more potent than **2Eu**; and that they both, along with **1Tb** and **2Tb**, possess a single metal bound water molecule in solution, as observed by evaluating their hydration number (q) (evaluated from their luminescent excited state lifetimes in H_2O and D_2O respectively by direct excitation of the Eu(III) ion at 397 nm) at pH 7–7.4. Furthermore, we investigated the effect of adding several equivalents of either EDTA or DEP to the solution of **1Eu** or **2Eu**. On both occasion the q value was determined to ~ 1, indicating that EDTA did not remove the ion from the cavity. Since La(III) has generally higher coordination requirements than Eu(III) or Tb(III) it can be expected that both **1La** and **2La** could have an extra coordination site available, and that if **1La** and **2La** are adopting a square antiprism geometry, i.e. a structure in solution like their Eu(III) and Tb(III) counterparts, they would have two water molecules associated with their structures (Morrow and co-workers showed this to be the case in their example [21]). We have been able to measure the pK_a of the water molecule of both **1Eu** and **2Eu** using potentiometric titration, these being 6.9($\pm$0.1) and 7.8 ($\pm$0.1) respectively. Our attempts to measure the pK_as of the water molecules for **1La** and **2La** have not been conclusive to date. However we have been able to measure two pK_as for the GlyGly analogue of **1La** [11]. It is thus our prediction that both **1La** and **2La** possess two water molecules that could give rise to more efficient phosphodiester hydrolysis since one of these water molecules could be used to bind to the phosphodiester, while the second one would be available to deprotonate the 2′ group on HPNP making it more nucleophilic. This would then be followed by the expulsion of the phosphorane from the cavity of the lanthanide ion complex. This hypothesis is shown schematically in Scheme 3 (drawn as if the La(III) complex was acting as a catalyst) and is similar to the mechanisms proposed for the catalysed hydrolysis of related phosphodiesters using Cu(II) and Co(III) complexes [26]. In the scenario that one of these metal bound water molecules was deprotonated, one could expect that the efficiency of the nucleophilic activation step would be greatly improved. However, at more alkaline pH both water molecules could possibly be deprotonated giving two metal bound hydroxy groups. These could give enhanced binding and could lead to increased rate of hydrolysis, which might explain the increased activity in the alkaline environment for **1La** and **2La**. On the other hand, if the phosphate is bound too strongly by the metal ion under these circumstances, it might be unable to leave the cavity of the complex and hence block the entry of a new substrate. Consequently, any further hydrolysis would be inhibited. This might be the case for **2La** but we have not yet been able to experimentally support this idea. In the case of those complexes that have only one water molecule present, the binding of the phosphodiester would be most likely through electrostatic interactions with the lanthanide ion, followed by deprotonation of the 2′-hydroxy group on HPNP, by the metal bonded water molecule (in a similar way as shown in Scheme 1) [29]. Alternatively, this water molecule can be displaced upon binding to HPNP, particularly if it is not deprotonated. This would only lead to Lewis acid activation of the phosphodiester by the lanthanide ion centre making the nucleophilic reaction step of the 2′-hydroxy group of HPNP slower in comparison to that shown for La(III). We are currently investigating these possible mechanisms.

So why, in general, are the larger ions more able to promote the HPNP hydrolysis than the smaller ions, which are stronger Lewis acids? One obvious explanation is the presence of the two water molecules for La(III) vs. the other ions. Secondly, it might be explained by the fact that the larger ions do not sit as deep within the modified cyclen cavity than the smaller ones because they can form stronger bonds with the amino moieties of the cyclen structure. Even though we have not been able to demonstrate this by crystallography, work by Parker and co-workers has shown that for related cyclen complexes the distances between



Scheme 3. A proposed mechanism for the cleavage of HPNP by **1La** and **2La**. This scheme is presented as a catalytic circle. However, we are not proposing that this is necessarily the case for these compounds. The first three steps in this circle have been evaluated (see text for clarification).

the nitrogen of the ring and the central Ln(III) is shortened in the order of La > Eu > Yb [27]. This could give rise to two possible effects; firstly the ions would be more shielded from the solvent environment making them less able to interact with the substrate, although ^{31}P NMR measurements indicate that this may in fact not be the case, and secondly, the substrate may have more room within the cavity to interact with the metal and the metal bonded water molecule. It is difficult to predict which of these possibilities is correct. However from our ^{31}P NMR investigation we can say that it is quite likely that the Eu(III) and Tb(III) complexes have a rather higher affinity for the substrate, and that at pH 7.4 these ions bind to the substrate in a manner that possibly reduces the release of the phosphorane product after hydrolysis. For La(III), these interactions are most likely weaker. We are currently investigating these features in greater detail with the aim of shedding some light on these possible interactions and their contributions to the overall phosphodiester hydrolytic process.

So why do the pH-rate profiles differ so much for **1La** and **2La**? Even though we do not have a clear explanation for this, one could predict that the pK_a of the water molecules is somewhat affected by the surrounding walls of the cavity and the volume they provide to the overall size of the cavity. Similar observations have been made by Coates et al. [28] and by Molenveld et al. [29]. However, we have not been able to demonstrate this by using X-ray crystallography. It can thus be concluded that **1La** and **2La** behave very differently, and that changing the ester from a methyl to benzyl dramatically affects the ability of these complexes to interact and promote the phosphodiester hydrolysis of HPNP. Hence it is thus quite likely that the cavity of **1La** is more hydrophobic than that of **1La** since it has a more wider structure, or is more 'bowl' shaped.

In summary, we have developed several new lanthanide ion complexes by incorporating peptide conjugates into the basic structure of cyclen giving **1** and **2** respectively. We have shown that these complexes can give rise to significant rate enhancement in the cleavage of HPNP, and that they can effectively promote the hydrolysis of a 23-mer RNA oligonucleotides under physiological conditions. To the best of our knowledge, **2La** displays one of the largest rate accelerations observed for HPNP hydrolysis by trivalent, non-redox active macrocyclic lanthanide complexes at pH 7.4. We believe that the rate enhancements shown here are due to the formation of a hydrophobic cavity within the structures of **1La** and **2La**, this being more of a 'bowl' like structure for **2La** as demonstrated by X-ray crystallography. Such ion induced structural changes, in combination with the metal bound water molecules, give rise to increased rates of hydrolysis of HPNP. We thus conclude that the lanthanide complexes of **1** and **2** can be used to mimic the hydrophobic nature of the active site of ribonucleases. We found that when using La(III) the rate of hydrolysis was highly pH dependent, showing dual pH behaviour for **1La** where within the

physiological pH range the greatest efficiency was achieved at pH 7.0, whereas for **2La** maximal activity was observed at pH 7.4. We predict that the difference in these two activities is due to the effect of the wall of the cavity and its volume, and its ability to accommodate the substrate within this cavity. In contrast both compounds showed increased activity in more alkaline conditions (below pH 9.5). We are currently investigating the effect of pH on the ability of several of the above complexes to cleave HPNP, as well as developing analogous compounds by incorporating other cofactors into the cyclen structure. When conjugated to modified oligonucleotides, these complexes may have potential as antisense agents for the therapeutic control of gene expression.

4. Experimental

4.1. Crystallography

Data were collected a Bruker-AXS SMART diffractometer using the SAINT-NT [18a] software with graphite monochromated Mo Kα radiation. A crystal was mounted on to the diffractometer at low temperature under nitrogen at approximately 120 K. The structure was solved using direct methods and refined with the SHELXTL version 5 [18b] and the non-hydrogen atoms were refined with anisotropic thermal parameters. An empirical absorption correction was applied using SADABS. The absolute configuration was determined using the Flack parameter (0.04). Hydrogen-atom positions were added at idealised positions with a riding model and fixed thermal parameters ($U_{ij} = 1.2U_{eq}$ for the atom to which they are bonded). The function minimised was $\Sigma[w(|F_o|^2 - |F_c|^2)]$ with reflection weights $w^{-1} = [\sigma^2|F_o|^2 + (g_1P)^2 + (g_2P)]$ where $P = [\max|F_o|^2 + 2|F_c|^2]/3$. Crystal data for $C_{63}H_{80}F_9N_8O_{23}S_3Tb$ (1): $M = 1743.45$, monoclinic, space group $P2_1$, $a = 11.847$ (3) Å, $b = 20.658$ (6) Å, $c = 16.778$ (5) Å, $\beta = 110.522$ (5)°, $U = 3845.3$ (19) Å^{-3}, $Z = 2$, $\mu = 1.099$ mm^{-1}, $R_{int} = 0.0773$, transmission range (max, min) = 0.928, 0.747. A total of 44 587 reflections were measured for the angle range $2.6° < 2\theta < 59.3°$ and 17 083 independent reflections were used in the refinement. The final parameters were $wR_2 = 0.1551$ and $R_1 = 0.0625$ [$I > 2\sigma I$].

4.2. Kinetic evaluation

All kinetic evaluations were carried out by using an *Agilent 8453* spectrophotometer fitted with a circulating temperature controlled water bath, and water driven mechanical stirring. The rate of hydrolysis (k) of the phosphodiester by above complexes were determined by fitting the data to first order rate kinetics using Biochemical Analysis Software for Agilent ChemStation. All reactions gave 'pseudo' first order kinetics. We estimate that the errors in these measurements are within ±10%.

4.3. Other spectroscopy

Melting points were determined using an Electrothermal 1A9000 melting point apparatus. Infrared spectra were recorded on a Mattson Genesis II FTIR spectrophotometer equipped with a Gateway 2000 4DX2-66 workstation; solid samples were dispersed in KBr and recorded as clear pressed discs. ^{1}H NMR spectra were recorded at 400 MHz using a Bruker Spectrospin DPX-400 instrument. Tetramethylsilane was used as an internal reference standard, with chemical shifts expressed in parts per million (ppm or δ) downfield from the standard. ^{13}C NMR spectra were recorded at 100 MHz using a Bruker Spectrospin DPX-400 instrument. Mass spectra were determined using electrospray ionisation on a Micromass LCT spectrometer, interfaced to a Waters HPLC. The whole system was controlled by MassLynx 3.5 on a Compaq Deskpro workstation.

Elemental analyses were determined at University College Dublin. Lanthanide excited state lifetimes (determination of q) were obtained using a Varian Cary Eclipse Fluorescence Spectrophotometer. Potentiometric measurements were carried out at 298 K using a Molspin 1 ml auto-titrator equipped with a calibrated micro-electrode to measure pH. Data were analyzed using Superquad.

4.4. Reagents and solvents

Reagents (obtained from Aldrich) and solvents were purified using standard techniques. Solvents were dried over drying agent before use: CH_3CN and $N(CH_2CH_3)_3$ over CaH_2, toluene over Na with benzophenone as an indicator. 1,4,7,11-Tetraazacyclododecane is commercially available from Strem Chemicals and was used as received.

4.5. Synthesis

4.5.1. Chloroacetylalanine benzyl ester (*4*)

L-Alanine benzyl ester hydrochloride (0.5 g, 2.32 mmol) was added to a solution of triethylamine (0.96 ml, 6.96 mmol) and toluene (20 ml) that was cooled in an acetone/liquid nitrogen bath to below −10 °C. A solution of chloroacetyl chloride (0.26 g, 2.32 mmol) in toluene (10 ml) was added dropwise over an hour, maintaining the temperature below 0 °C. The reaction was allowed to warm to room temperature overnight under argon. The reaction was filtered and the toluene removed under reduced pressure. The remaining brown

liquid was taken into chloroform and extracted several times with water and then once with 0.1 M HCl. The organic layer was dried over potassium carbonate and the solvent removed under reduced pressure to give brown oil that solidified upon standing. Yield: 0.602 g (98%); *Anal.* Calc. for $C_{12}H_{14}ClNO_3$: C, 56.37; H, 5.52; N, 5.48. Found: C, 55.90; H, 5.47; N, 5.37; δ_H ($CDCl_3$, 400 MHz): 7.36 (4.5H, m, Ar–*H*), 7.19 (1H, s, N–*H*), 5.23 (2H, dd, $J_1 = 12.0$ Hz, $J_2 = 8.0$ Hz, CH_2Ph), 4.68 (1H, $J = 8.0$ Hz, NH–C*H*–CH_3), 4.03 (2H, s, Cl–CH_2–CO–), 1.47 (3H, d, $J = 8.0$ Hz, NH–CH–*C*H_3); δ_C ($CDCl_3$, 100 MHz): 171.6, 165.7, 134.8, 128.2, 66.8, 48.1, 41.9, 17.6; *m/z*: 278 $(M+H_2O)^+$, 279 $(M+Na)^+$; IR ν_{max} (cm^{-1}): 3278, 3073, 2966, 2362, 2343, 1731, 1656, 1556, 1452, 1353, 1313, 1224, 1141, 948, 752, 698.

4.5.2. L-Alanine methyl ester ligand (*1*)

Cyclen (0.213 g, 1.24 mmol), **3** (1.000 g, 5.57 mmol), cesium carbonate (1.816 g, 5.57 mmol) and potassium iodide (0.920 g, 5.57 mmol) were added to dry acetonitrile (30 ml) and freeze–thawed three times before being heated to 80 °C for 48 h. The reaction mixture was left to cool to room temperature, then filtered and the solvent removed under reduced pressure. The remaining solid was dissolved in chloroform and washed three times with a saturated KCl solution. The organic extract was dried over potassium carbonate and the solvent removed under reduced pressure to give brown oil, which was dried under vacuum to give a pale brown hygroscopic solid. Yield: 0.456 g (49%); *Anal.* Calc. for C_{32} $H_{56}N_8O_{12}$: C, 51.60; H, 7.58; N, 15.04. Found: C, 51.52; H, 7.28; N, 14.74. Expected for $C_{32}H_{56}N_8O_{12}$: *m/z* 745.4096 $(M+H)^+$, Found: 745.4058; δ_H ($CDCl_3$, 400 MHz): 7.91 (1H, d, $J = 7.0$ Hz, N–H), 4.65 (1H, m, $J =$ 7.0 Hz and 7.5 Hz, C*H*$CH_3CO_2CH_3$), 3.73 (3H, s, CO_2CH_3), 3.23 (2H, dd, $J = 14$ and 17, CH_2CO–), 2.88 (4H, dd, $J = 10.0$ Hz, cyc-CH_2s), 1.4 (3H, d, $J = 7.0$, CHC$H_3CO_2CH_3$); δ_C ($CDCl_3$, 100 MHz): 173.5 (C=O), 170.6 (C=O), 58.4, 52.5 (CH_2), 51.9, 47.4 (CH_2), 17.0 (CH_2); *m/z*: 745 (M^+), 768 $(M+Na)^+$; IR ν_{max} (cm^{-1}): 3384, 3048, 2954, 2825, 1745, 1667, 1539, 1455, 1291, 1212, 1163, 1102, 1055, 1012, 951, 898, 850, 757, 605, 502.

4.5.3. L-Alanine benzyl ester ligand (*2*)

The procedure for **1** was used: cyclen (0.1 g, 0.57 mmol), **4** (0.602 g, 2.3 mmol), Cs_2CO_3 (0.75 g, 2.3 mmol) and KI (0.38 g, 5.96 mmol) were added to dry acetonitrile (30 ml) and freeze–thawed three times before being heated to 80 °C for 48 h. Yield: 0.445 g (74%); *Anal.* Calc. for $C_{56}H_{72}N_8O_{12}K(2H_2O)$: C, 59.82; H, 6.81; N, 9.97; Found: C, 60.13; H, 6.69; N, 9.98; Expected for $C_{56}H_{72}N_8O_{12}Na$: 1072.5246 $(MHNa^+)$, Found: 1072.5205; δ_H ($CDCl_3$, 400 MHz): 7.68 (1H, s N–*H*), 7.36 (5H, m, Ar–*H*), 5.19 (2H, dd, $J_1 = 12.0$ Hz, $J_2 = 8.0$ Hz, Ar–CH_2–), 4.71 (1H, t, $J = 7.0$ Hz, NH–C*H*–CH_3), 3.16 (2H, d, $J = 12$ Hz, Cl–CH_2–CO–), 2.82 (2H, dd, $J = 10.0$ Hz, cyc-CH_2), 2.65 (2H, d, $J = 10$ Hz, cyc-CH_2), 1.42 (3H, d, $J = 7.0$ Hz, NH–CH–*C*H_3); δ_C ($CDCl_3$, 100 MHz): 172.3, 170.4, 162, 134.9, 128.1, 66.6, 58.9, 52.4, 47.4, 17.3; *m/z*: 524 (M^{+2}) 1049 (M^+), 1072 $(M+Na)^+$; IR ν_{max} (cm^{-1}): 3421, 2925, 2854, 2364, 2345, 1735, 1654, 1542, 1508, 1457, 1261, 1155, 1099, 1027, 802, 736, 698, 669, 593, 472.

4.5.4. Synthesis of *1Ln* and *2Ln*

All lanthanide ion complexes of **1** and **2** were formed by refluxing either of these ligands with 1.1 equivalents of the appropriate lanthanide triflate in 5 ml dry acetonitrile with the exception of the La(III) complexes which was formed by refluxing **1** and **2** in 30 ml methanol or ethanol respectively. The resulting solution was lowed to cool to room temperature before pouring them onto 200 ml of diethylether. The resulting mixture was stirred for several hours before the isolation of the solid (complexes) by filtration. All the complexes with the exception of **2Tb** were obtained as highly hydroscopic waxy-oils.

1La: Yield: 53%; m.p. 262–265 °C; Expected for $C_{32}H_{56}N_8O_{12}La$: 883.3081 (M^+), Found: 883.3099; δ_H ($(CD_3)_2CO$, 400 MHz, broad spectra): 9.2 (1H), 4.6 (1H), 4.0 (1H), 3.7 (3H), 2.9 (5H), 1.5 (4H), 0.01 (2H); *m/z*: 294 (M^{+3}), 308 $(M+K)^{+3}$, 321 $(M+2K)^{+3}$, 515 $(M+Trif)^{+2}$, 1180 $(M+2Trif)^+$; IR ν_{max} (cm^{-1}): 3448, 2926, 2856, 1750, 1625, 1579, 1459, 1259, 1162, 1029, 956, 640, 577, 518.

1Eu: Yield: 18.5%; m.p. 270–273 °C; Expected for $C_{32}H_{56}N_8O_{12}Eu$: 897.3230 (M^+), Found: 897.3273; δ_H (CD_3OD, 400 MHz): 24.4 (1H, s, cyc), 2.5 (6H, s), 1.2 (3H, s), 0.5 (6H, s), −2.7 (2H, cyc-H), −4.3 (1H, cyc-H), −8.1 (2H, cyc-H), −8.9 (1H, cyc-H), −12.2 (1H, cyc-H); *m/z*: 299 (M^{+3}), 448 (M^{+2}), 523 $(M+Trif)^{+2}$; IR ν_{max} (cm^{-1}): 3466, 2926, 2360, 1736, 1626, 1473, 1260, 1168, 1084, 1031, 958, 647.

1Tb: Yield: 34%; m.p. 267–269 °C; Expected for $C_{32}H_{56}N_8O_{12}Tb$: 903.3271 (M^+), Found: 903.3276; δ_H ($(CD_3)_2CO$, 400 MHz): 24.2, 16.9, 13.9, 4.9, 4.7, 3.3, 2.6, 1.3; *m/z:* 301 (M^{+3}), 452 (M^{+2}), 526 $(M+Trif)^{+2}$, 1202 $(M+2Trif)^+$; IR ν_{max} (cm^{-1}): 3436, 3119, 2961, 2360, 1743, 1629, 1460, 1249, 1164, 1083, 1030, 959, 639, 576, 518.

1Yb: Yield: 29%; m.p. 272–275 °C; Expected for $C_{32}H_{56}N_8O_{12}Yb$: 918.3406 (M^+), Found: 918.3374; δ_H ($(CD_3)_2CO$, 400 MHz): 16.8, 13.9, 10.64, 3.7, 2.9, −0.96, −1.5, −10.2, −22.3, −27.4; *m/z*: 306 (M^{+3}), 459 (M^{+2}), 533 $(M+Trif)^{+2}$; IR ν_{max} (cm^{-1}): 3446, 2925, 2360, 1743, 1637, 1459, 1260, 1163, 1084, 1031, 960, 913, 761, 639.

2La: Yield: 28%; m.p. 120–123 °C; Expected for $C_{56}H_{72}N_8O_{12}La(CF_3SO_3)$: 1336.3854 $(M+Trif)^+$, Found: 1336.3857; δ_H ($(CD_3)_2CO$, 400 MHz): 9.32 (1H, bs, N–H), 7.42 (5H, s, Ar–H), 5.23, 4.64, 4.09,

3.79, 3.45, 1.40 (6H); IR ν_{max} (cm^{-1}): 3449, 2929, 1736, 1627, 1469, 1258, 1164, 1083, 1031, 957, 759, 639.

2Eu: Yield: 30%; m.p. 96–99 °C; Expected for $C_{56}H_{72}N_8O_{12}Eu$: 1201.4482 (M^+), Found: 1201.4486; δ_H (CD_3OD, 400 MHz): 24.03 (0.5H, cyc), 7.44 (5H, m, Ar–H), 5.1 (1H, s), 2.50 (2H, d, $J = 24$) 2.05 (3H, s), −2.91 (1H, cyc), −4.32 (0.5H, cyc), −8.42 (1.4H, cyc), −12.20 (0.5H, cyc); *m/z*: 600 (M^{+2}), 675 $(M+\text{Trif})^{+2}$, 1200 (M^+), 1350 ($M+\text{Trif}^+$), 1499 $(M+2\text{Trif})^+$; IR ν_{max} (cm^{-1}): 3421, 3289, 3122, 2923, 2854, 2362, 1743, 16 432, 1457, 1259, 1162, 1029, 958, 802, 759, 700, 638, 574, 516.

2Tb: Yield 36%; m.p. 210–212 °C; Expected for $C_{56}H_{72}N_8O_{12}Tb$: 1207.4523 (M^+), Found: 1207.4496; δ (CD_3CN, 400 MHz): 24.76, 25.06, 13.6, 10.45 (1H), 8.09 (2H), 7.5 (2H), 3.68 (2H), 1.9 (1H), 1.4 (3H); *m/z*: 603 (M^{+2}), 1206 (M^+), 1355 $(M+\text{Trif})^+$, 1505 $(M+2\text{Trif})^+$, 1676 $(M+3\text{Trif})^+$; IR ν_{max} (cm^{-1}): 3448, 3270, 3120, 2960, 2923, 2360, 2343, 1741, 1629, 1457, 1257, 1160, 1029, 960, 802.

5. Supplementary material

Additional crystallographic material is available from the Cambridge Crystallographic Data Centre comprises relevant tables of atomic coordinates, bond lengths and angles, and thermal parameters (CCDC = 19 5648). Copies of this data may be obtained free of charge om application to The Director, CCDC, 12 Union Road, Cambridge CB2 1EZ, UK (fax: +44-1223-336033; e-mail: deposit@cam.ac.uk or www: http://www.ccdc.cam.ac.uk).

Acknowledgements

We thank Kinerton Ltd, Enterprise Ireland (Basic Research Grant Scheme), Dublin Corporation (post-graduate scholarship to S.M.), and TCD for financial support. We particularly like to thank Dr Hazel M. Moncrieff for helpful discussion and Professor John M. Kelly (TCD) for his continuous support.

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