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Synthesis and Studies of Cyclen Based Lanthanide Complexes for the Detection of Ions and Molecules and the Hydrolysis of Phosphodiesters

By Andrew Harte,
January 2005

University of Dublin Trinity College

Based on research carried out under the direction of Prof. Thorfinnur Gunnaugsson

A thesis submitted to the Department of Chemistry, University of Dublin, Trinity College for the degree of Doctor of Philosophy.
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Declaration

This thesis is submitted for the degree of Doctor of Philosophy to the University of Dublin, Trinity College and has not been submitted for any degree or examination to this or any other University. Other than where acknowledged, all work described herein is original and carried out by the author.

Andrew Harte

Andrew Harte
DECLARATION

This thesis has not been submitted as an exercise for a degree at any other university. Except where stated, the work described therein was carried out by me alone.

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Signed: Andrew Harte
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“So I said to myself ‘What would God do?’”
“Itaque ipse quaesit quid facet Deus”

H.J. Simpson
Abstract

This thesis entitled "Cyclen Based Lanthanide Complexes for the Detection of Ions and Molecules and the Hydrolysis of Phosphodiesters" is divided into five chapters. Chapter 1, the introduction, is divided into two parts. The first half introduces the principles of luminescent sensing before illustrating some examples of fluorescent sensors. Then the advantages of lanthanide luminescent signalling systems are discussed followed by a description of the mechanism by which lanthanide luminescence is produced. Finally, a review of various lanthanide luminescent chemosensors is presented. The second half of the introduction deals with the area of phosphodiester hydrolysis. It shall begin by looking at natural ribonucleases capable of hydrolysing RNA, followed by a look at the mechanism of hydrolysis. The use of metal ions to promote phosphodiester hydrolysis is then discussed, followed by a discussion on the use of metal complexes. A review of the acceleration of phosphodiester hydrolysis by binuclear metal complexes is then presented with special attention paid to the rate acceleration provided by the cooperative action of two metal ions. This shall be followed by a review of binuclear lanthanide complexes capable of producing this cooperative action. Chapter 2 details the synthesis, characterisation and photophysical studies of a lanthanide luminescent sensor, designed for the detection of Cu(II). The synthesis involves the incorporation of a 1,10-phenanthroline unit into a tris-alkylated cyclen molecule, followed by complexation with a Eu(III) ion. The resulting complex exhibits the characteristic Eu(III) emission bands after excitation of the phenanthroline chromophore under physiological conditions. This complex signals the presence of Cu(II) under physiological conditions by means of a quenching of the Eu(III) luminescence. The complex displays good selectivity over other physiologically prevalent ions and operates over the desired concentration range. Chapter 3 describes the synthesis, characterisation and photophysical studies of a range of lanthanide luminescent sensors, designed for the detection of aromatic carboxylates. The chapter begins with the development of co-ordinately unsaturated mononuclear Tb(III) and Eu(III) cyclen complexes before moving on to an unsaturated binuclear Tb(III) cyclen complex. Luminescent studies demonstrated the ability of the Tb(III) complexes to bind aromatic carboxylates via the displacement of the metal bound water molecules, which occupy the vacant coordination sites around the lanthanide ion. Furthermore, this binding event is signalled by and increase in the previously "silent" Tb(III) emission. Chapter 4 is concerned with the area of phosphodiester hydrolysis and details the synthesis,
characterisation and kinetic studies of a range of binuclear lanthanide complexes, designed for the acceleration of phosphodiester hydrolysis. The incorporation of various pendant arms, as well as the effect of different spacer groups is investigated. A convenient method for the production of α-chloroamides in water is also presented. The ability of the binuclear lanthanide complexes to promote the hydrolysis of the HPNP phosphodiester, a model RNAcompound, shall be discussed with particular attention upon rate enhancement over corresponding mononuclear complexes. The trends that emerge from these studies shall be discussed, before a detailed investigation of the Tb.117 complex, which was found to be by far the most active binuclear complex, reducing the half-life of HPNP to 28 minutes from 240 days, a 12,333 fold rate increase. This is comparable to the largest rate enhancements detailed within the literature. A discussion of the possible reaction mechanism shall also be presented. Chapter 5 outlines the experimental procedures, and presents the synthesis and characterisation of the compounds discussed within the thesis.
Abbreviations

A  adenine
Abs  absorbance
AcOH  acetic acid
Ala  alanine
Arg  argentine
Asn  Asparagine
Asp  aspartic acid
a.u.  arbitrary units
B  binding constant
bipy  bipyridine
BNPP  bis(p-nitrophenyl)phosphate
BOC  tert-butoxycarbonyl
Br  broad
C  cytosine
CHCl₃  chloroform
CH₂Cl₂  dichloromethane
CH₃CN  acetonitrile
CTACl  cetyltrimethylammonium chloride
Cyclen  1,4,7,10-tetraazacyclododecane
Cyclam  1,4,8,11-tetraazacyclotetradecane
Cys  cysteine
d  doublet
DCM  dichloromethane
DMF  dimethyl formamide
DMSO  dimethyl sulfoxide
DNA  deoxyribonucleic acid
DTPA  diethylenetriamine pentaacetic acid
ε  molar extinction coefficient
EDTA  ethylenediamine tetraacetic acid
EPNP  ethyl-p-nitrophenyl phosphate
ES  electrospray
pH  \(-\log[H_3O^+]\)
pKa  \(-\log[K_a]\) where $K_a$ is the acidity constant
pM  \(-\log[M]\)
Phe  Phenylalanine
Phen  phenanthroline
ppm  parts per million
Pro  praline
RNA  ribonucleic acid
rRNA  ribosomal RNA
$S_0$  Singlet ground state
$S_1$  First excited singlet state
Ser  serine
T  thymine
$T_1$  First excited triplet state
THF  tetrahydrofuran
TMACl  tetramethyl ammonium chloride
TMAPCi  tetramethyl ammonium perchlorate
TMAOH  tetramethyl ammonium hydroxide
TNP  tris-p-nitrophenyl phosphate
TRIS  Tris(hydroxymethyl)aminomethane
Trif  triflate (trifluorosulfonate)
tRNA  transfer RNA
Thr  threonine
Trif  triflate
Trp  tryptophan
Tyr  tyrosine
U  uracil
UV  ultra violet
Val  valine
$\nu$  frequency
$\mu$  micro ($x 10^{-6}$)
$\lambda$  wavelength
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Chapter 1

Introduction
1.0 Prelude

The work within this thesis encompasses two different research areas; lanthanide luminescent sensing and phosphodiester hydrolysis. Each of these areas shall be introduced separately. As such, the first half of this introduction deals with luminescent sensing, detailing the advantages offered by lanthanide luminescent sensors over fluorescent devices, before examining how to go about provoking a lanthanide emission, followed by a look at some examples of lanthanide luminescent pH, cation and anion sensors. This will be followed by an introduction to the field of phosphodiester hydrolysis, focusing on the attainment of rate enhancements due to the cooperative action of two or more metal ions.

1.1.0 Introduction to Sensors

A sensor is a substance that provides information about a specific target within a specific matrix, for example, the concentration of a specific metal ion within blood or water, etc. It must perform two functions; sensing/recognition and signalling. Recognition must be selective, therefore, within its chosen environment, the sensor must selectively recognise one target species out of all the possible species present. Recognition usually occurs through reversible binding. Upon recognition the sensor must then be able to signal this event. A signalling event is usually heralded, after binding has occurred, by a modification of some property of the sensor such as a change in colour, a variation in the light emitting properties or an alteration in some other electronic, magnetic or thermal property (Scheme 1.1). The work within this thesis shall be confined to luminescent sensing, i.e. where the signal is observed as a variation in the light emitting properties of the sensor.

![Scheme 1.1. Representation of a luminescent sensor showing selective binding of a substrate (recognition), followed by production of a light indication (signalling).](image-url)
1.1.1 Fluorescent Sensors

Some of the classic examples of luminescent sensors utilise a fluorescent signalling moiety. The term fluorescence describes the emission of a photon of light from the first excited singlet state ($S_1$). When a molecule absorbs a photon of light an electron is excited from the singlet ground state ($S_0$) to the first excited singlet state ($S_1$). After relaxing to the lowest vibrational level within this state, the electron may then return to the ground state $S_0$, either by a non-radiative deactivation, or by emitting a photon of light with energy ($h\nu$) equal to the energy gap between the two states. The fluorescent emission of a photon from a $S_1$ state is an allowed process and is characterised by a very short lifetime, $10^{-9}$ seconds (ns).\(^2\) Alternatively, the electron can enter the triplet excited state ($T_1$) by means of intersystem crossing (ISC). The emission of a photon accompanying the transition of an electron to the ground state from the triplet state ($T_1 \rightarrow S_0$), is known as phosphorescence. However, this transition is spin forbidden and hence occurs over a longer timescale than fluorescence. These processes can be illustrated in a Jablonski diagram, which is a simplified depiction of the relative positions of the electronic energy levels of a molecule (Figure 1.1). Vibrational levels in a given electronic state lie above each other, but their relative horizontal locations bear no relation to the nuclear separation of the states.\(^2\)

Figure 1.1. Jablonski diagram illustrating energy levels and states associated with fluorescence and phosphorescence\(^2\)

There are numerous examples of sensors that employ organic molecules as fluorescent signalling moieties. One early such example developed by de Silva \textit{et al.} are the “off-on”
sensors for $H^+$, $1$, $Na^+$, $2a$ and $K^+$, $2b$. In these systems the anthracene moiety functions as the fluorophore, while the crown ether is the receptor, and they are attached by a methylene spacer. In the absence of any cations the fluorophore shows very little fluorescence as an electron transfer to the anthracene moiety, from nitrogen lone pair of the receptor, prevents any radiative decay (Scheme 1.2). However, upon binding $H^+$ in the case of $1$, $Na^+$ in the case of $2a$, or $K^+$ in the case of $2b$, the receptor is unable to participate in electron transfer due to the increased oxidation potential of the receptor’s HOMO. As a result, the anthracene fluorophore can now fluoresce and shed its excited energy by emitting a photon, returning to the ground state. This is illustrated in Scheme 1.3.

![Scheme 1.2. Free Receptor – an electron transfer (ET) from the receptor prevents the fluorophore from losing its excited energy by radiative decay](image)

![Scheme 1.3. Bound Receptor – the receptor is now lower in energy than the fluorophore and hence cannot participate in an electron transfer. Consequently the fluorescence is switched on as the fluorophore loses its energy by emitting a photon](image)
Another example of an early fluorescent sensor was developed by Tsien and co-workers who synthesised the Ca$^{2+}$ sensor. In its metal free state the nitrogen lone pairs are again involved in stabilizing the excited state of the chromophore giving rise to a long wavelength emission. Upon binding of a Ca$^{2+}$ ion, however, the emission shifts to shorter wavelengths as the nitrogen lone pairs are now involved in the binding.

The above molecules are all classed as cation sensors, their signalling systems are based on fluorescent organic molecules and their receptors are selective for cations. Of equal importance is the area of anion sensing, especially anions with biological relevance. Gunnlaugsson et al. have recently developed a range of “on-off” sensors which showed ideal PET behaviour in DMSO. In the absence of any ions the fluorescence is “switched on”. The fluorescent emission is then quenched by the presence of AcO$^-$, H$_2$PO$_4^-$, and F$^-$, selectively over Cl$^-$ and Br$^-$. These sensors operate in a manner similar to that described in Scheme 1.2, except that the unbound receptor is lower in energy and does not participate in electron transfer, while the bound receptor is higher in energy and does participate in electron transfer thereby quenching the anthracene fluorescence, i.e. the sensor is “switched on” in its free state and quenched, or “switched off”, after binding the analyte.

One of the major drawbacks of fluorescent sensors comes into play when working in vivo due to autofluorescence from biological systems. This is noise that is caused by short-lived background fluorescent emissions from the tissue itself, which can mask or drown out the fluorescent signal of the sensor. The relatively new field of metal based luminescent sensors overcomes this problem due to the long lived nature of the light emission. The most common metal based luminescence systems utilise transition metals such as Ru(II) and Os(II). However, the focus of this thesis shall be upon lanthanide luminescent systems, which are the subject of the following section.
1.1.2 Photophysics of Lanthanide Luminescence

As mentioned above, the final signalling step performed by a luminescent sensor can be problematic when dealing with biological samples, as any light signal must compete against autofluorescence and light scattering, short lived background radiation with a ns timescale, from the body tissue itself. Lanthanide luminescent sensors may be employed here in order to circumvent these problems.

The fifteen elements from lanthanum to lutetium with access to their 4f shell are known as the lanthanides.\(^\text{10}\) They are all hard, electropositive metals and the nature of their 4f shells imparts some unique properties to the lanthanides. The electronic shielding of the f electrons is quite weak and hence increasing atomic number or nuclear charge, causes a decrease in the radii of the atoms as one proceeds from La to Lu, which is known as the lanthanide contraction.\(^\text{10}\) The lanthanides are easily oxidised and favour the 3\(^+\) oxidation state with few exceptions.\(^\text{11}\) The f orbitals are buried within the atom and hence interact with ligand orbitals only very weakly. As a result, \(f\rightarrow f\) transitions, which occur in the visible region of the spectrum, give rise to very narrow bands and the lifetimes of their excited states are quite long lived (\(\mu\text{s-ms}\)).\(^\text{11}\)

Systems which utilise emissive lanthanide conjugates as signalling moieties have some significant advantages over analogous fluorescent systems allowing them to overcome the autofluorescence and light scattering of body tissue.\(^\text{12}\)

- Their long lived excited states (ms for Eu(III) and Tb(III)) allows the short-lived background fluorescence to disperse before the lanthanide emission occurs (Figure 1.2).\(^\text{15}\)
- The long wavelengths (500-750 nm for Eu(III)) at which lanthanides emit occur beyond the absorbance of body tissue, this is important for signal quality.
- Their line-like emission bands (10 nm) also give a better signal to noise ratio compared to fluorescent bands (several hundred nm).
Chapter 1 - Introduction

Longlived Lanthanide emission

Background Fluorescence

Time

Figure 1.2. Lanthanide ions possess a long lived exited state which continues to emit long after background fluorescence has dispersed.

The long-lived lanthanide excited state allows the short-lived background fluorescence to disperse before the lanthanide emission is measured thus giving a much improved signal to noise ratio. However, before a lanthanide emission can occur, the lanthanide ion must first be elevated into this excited state. Direct excitation of a lanthanide ion is not easily achieved, as it requires a Laporte forbidden f-f transition (low extinction coefficient $\varepsilon \sim 5-10 \text{ M}^{-1} \text{ cm}^{-1}$). As a result lanthanides are usually photophysically "silent" and have low molar absorbencies. However, this problem can be circumvented via indirect excitation by connecting a sensitising chromophore or "antenna" to the lanthanide ion (Figure 1.3). Once excited, the antenna can then pass its excited energy onto the lanthanide ion by an energy transfer process, resulting in a lanthanide emission (high extinction coefficient $\varepsilon \sim 40K \text{ M}^{-1} \text{ cm}^{-1}$).

Figure 1.3. Indirect lanthanide excitation, involving, excitation of the antenna, followed by energy transfer to the lanthanide ion resulting in a lanthanide emission.
This indirect excitation of a lanthanide ion can be illustrated in a Jablonski diagram, (Figure 1.4). The sensitising antenna, denoted Ar, absorbs a photon of energy hv, and is excited to its singlet excited state, \(^1\text{Ar}\). From here the energy can be passed into the antennas triplet excited state, \(^3\text{Ar}\), via intersystem crossing (ISC). The lanthanide’s excited state, \(^*\text{Ln}\), can now be populated from the antennas triplet state, by means of an intramolecular energy transfer (ET), provided that this triplet state is higher in energy than the lanthanide excited state.\(^{17}\) The excited energy now resides within the lanthanide ion, and can be lost by emission of light, resulting in the characteristic lanthanide emission spectra as each transition will correspond to an emission band.

![Jablonski Diagram](image)

**Figure 1.4. Jablonski Diagram illustrating sensitisation of a lanthanide ion. Other energy loss pathways have been omitted for clarity\(^{18}\)**

Singlet and triplet excited states share a common geometry where their potential energy curves intersect (Figure 1.5),\(^2\) but intersystem crossing from a \(S_1\) to \(T_1\) state is a spin forbidden process. Nonetheless, when a mechanism for unpairing electrons exists the excited molecule may undergo intersystem crossing ISC to the triplet state. The presence of a heavy atom such as a lanthanide ion in the molecule, gives rise to a significant amount of spin-orbit coupling and it is this which provides a mechanism for intersystem crossing from the \(S_1\) to \(T_1\) state.\(^2,19\)
Chapter 1 - Introduction

Figure 1.5. Potential energy wells showing vibrational levels. Note the overlapping common geometry of the $T_1$ and $S_1$ states. Intersystem crossing (ISC) from a singlet to a triplet excited state $S_1 \rightarrow T_1$ is made possible by spin-orbit coupling.\textsuperscript{2}

The mechanism for energy transfer from the $^3\text{Ar} \rightarrow \text{Ln}^*$ state is somewhat debated. One possible mechanism is the Förster energy transfer mechanism,\textsuperscript{20} which involves overlap of energy levels. In the Förster mechanism energy transfer occurs through space and has a $r^{-6}$ distance dependence, where $r$ is the distance between the metal ion and the excited chromophore. Consequently, the energy transfer process can be made more efficient by minimising the distance between the lanthanide ion and the chromophore or “antenna”.

The alternative mechanism for the energy transfer is known as the Dexter mechanism and involves an electron exchange between the excited chromophore and the metal ion in a through bond interaction and should display a $e^{-r}$ dependency.\textsuperscript{21, 22} Again the process can be made more efficient by minimising the distance $r$.

Of course, the pathway shown in Figure 1.4 is not the only means by which the excited energy can be lost. This pathway must compete with other mechanisms through which the energy can be discarded (Scheme 1.4).\textsuperscript{2} Energy can be lost from the singlet excited state $^1\text{Ar}$ through radiative decay \textit{i.e.} fluorescence, or non-radiative decay by collisions and vibration interactions with the surrounding molecules. When a photon is emitted from the triplet $^3\text{Ar}$ state, this is known as phosphorescence. Phosphorescence has a longer lifetime than fluorescence owing to the fact that it is a spin forbidden process.\textsuperscript{2} Triplet states may also be quenched by interactions with molecular oxygen.\textsuperscript{15} The lanthanide excited state $^\text{Ln}^*$ can also lose its energy through non-radiative decay by vibrational interactions with other molecules, such as water.\textsuperscript{23}
In order for the preferred pathway to operate i.e. population of the lanthanide excited state followed by a lanthanide emission, the design must minimise energy loss to other pathways. By maximising the efficiency of the intersystem crossing and energy transfer processes, it is possible to minimise the amount of time that the excited energy spends in the $^1\text{Ar}$ and $^3\text{Ar}$ states and therefore minimise the loss to deactivation pathways from these states. As stated before, ISC is made possible by spin-orbit coupling, which is increased by the presence of a heavy atom, so the very presence of a lanthanide ion itself will elegantly increase the efficiency of this process.\(^2\)

Once in the lanthanides orbitals, the energy is somewhat isolated from its environment.\(^11\) Back energy transfer is possible but minimised by having the $^3\text{Ar}$ state sufficiently higher above that of the $^*\text{Ln}$ state. Vibrational quenching by metal bound water molecules is now the main competing deactivation pathway.\(^23\) The use of a tightly binding chelating ligand will reduce the number of metal bound water molecules, and thus minimise non-radiative quenching by O-H oscillators.\(^15\)

So to summarise, in order to produce a lanthanide emission a conjugate must be designed such that an antenna with a triplet energy above that of the lanthanide excited state, is in close proximity to the lanthanide ion. Furthermore, the design requires the lanthanide ion to be tightly bound by a ligand to minimise the number of metal bound water molecules and maximise stability and solubility. The next section shall detail some examples where sensors have utilised lanthanide luminescence as a signalling moiety.
1.1.3 Lanthanide Luminescent Sensors

Numerous sensing systems have been designed that take advantage of luminescent lanthanide ions as signalling moieties. One such system that was reported by de Silva et al. is the pentadentate terpyridyl ligand 7. When complexed to a lanthanide ion, this conjugate is designed to function as a pH sensor with the amino groups acting as the proton receptors, the pyridyl groups acting as the sensitising antenna and the lanthanide ion acting as the signalling moiety. The sensor functions in water but was not stable and thus a 100 fold excess of lanthanide ion was used to ensure the desired complex was present. Upon protonation of the amine receptors the lanthanide luminescence was observed to increase with enhancements of up to 16 times the original intensity. This was attributed to the suppression of electron transfer to the singlet excited state of the ligand due to the protonation of the aminoalkyl group.

![Image of 7]

Another more stable example was reported by Gunnlaugsson et al., who developed the lanthanide conjugate 8, whose Eu(III) luminescence displayed a dependence upon pH, under highly acidic conditions. In this case the N-(3-quinolyl)ethanamide functions as both the antenna and the receptor, as the quinoline nitrogen behaves as a H⁺ acceptor. Under alkaline conditions (above pH 6), the Eu(III) luminescence of 8 was quenched, while between pH 6 and 3.5, the complex was found to be only weakly luminescent with the Eu(III) emission remaining almost constant over this pH range. However, between pH 3.5 and 1.5 and after excitation at 350 nm, the Eu(III) luminescence was “switched on” with an enhancement factor of ca. 300. This was attributed to the protonation of the quinoline moiety, which allowed the antenna to better transfer its excited energy to the lanthanide ion.
Woods and Sherry have also reported examples of lanthanide luminescent pH probes.\(^{27}\) The aryl substituted tetraamide complexes 9-11, take advantage of the \(\Delta J_2/\Delta J_1\) intensity ratio. The Eu(III) \(^5\!D_0\!\!\rightarrow\!^7\!F_1\) \((\Delta J_1)\) transition is known to be insensitive to ligand field effects, while the \(^5\!D_0\!\!\rightarrow^7\!F_2\) \((\Delta J_2)\) transition is considered to be hypersensitive to the coordination environment of the Eu(III) ion.\(^{28}\) After excitation \((via\; the\; chromophore)\), the Eu(III) complex 9, displays a Eu(III) emission, however, the \(\Delta J_2/\Delta J_1\) intensity ratio of this emission is dependant upon the pH of the solution, reaching a maximum between pH 6 and 8. This particular method of measurement may offer a unique advantage as the results are independent of the Eu(III) complex concentration. The authors found it was possible to alter the pH window of operation by varying the ligand, for instance 10 demonstrates changes between pH 7 and 9. The authors attributed the changes in luminescence to the deprotonation of the amide groups followed by increased electron transfer to the easily oxidized Eu(III) ion.\(^{14}\)
Parker et al. described the charge neutral, Eu(III) complex of 12, which employs a phenanthridine moiety as the sensitising antenna. The complex was found to display significant pH dependence. After excitation at 340 nm, a 500 fold luminescent enhancement of the Eu(III) emission could be observed upon protonation of the phenanthridine nitrogen ($pK_a = 4.45$). Meanwhile, the $N$-methylated analogue Eu.13, was found to have its luminescence “switched on”, independent of the pH. However, its luminescence was effectively quenched by the addition of $\text{Cl}^-$, $\text{Br}^-$ or $\text{I}^-$. Interestingly the luminescence was unaffected by the presence of the anions of phosphate, citrate, lactate or bicarbonate and as such may have applications as an intracellular $\text{Cl}^-$ sensor. Further examples of anion sensors shall be discussed in section 1.7.

As stated earlier, the Tb(III) $^5D_4$ excited state is of higher energy than the Eu(III) $^5D_0$ state (20500 and 17200 cm$^{-1}$ respectively), and this higher energy state conveys some interesting properties to the Tb(III) complexes of 12 and 13. The triplet state energies of the phenanthridine antennae were estimated to be 22000 and 21300 cm$^{-1}$ respectively. As the gap between the triplet states and the $^5D_4$ excited state is less than 1500 cm$^{-1}$, thermal back energy transfer from the lanthanide to the chromophore is possible and consequently, the luminescence becomes dependant upon the presence of molecular oxygen, which can effectively quench the chromophore triplet state. The complex Tb.13, exhibited a strong luminescence in degassed solution, which was independent of the pH between 2 and 9. However, the presence of dissolved oxygen in the solution efficiently quenched the luminescence of Tb.13, which effectively acts as an oxygen sensor.

Gunnlaugsson et al. have also developed luminescent sensors that act like pH probes as well as molecular logic gates. The Eu(III) complex Eu.14, is an example of a pH chemosensor. When Eu.14 was excited at 330 nm under ambient conditions in aqueous alkaline solution the Eu(III) emission was found to be “switched off”. Nevertheless, the
Eu(III) emission could be gradually enhanced by increasing the acidity of the solution with luminescent enhancement factors of up to 250 fold. This increase in luminescence was attributed to the protonation of the quinolyl nitrogen moiety, which greatly enhanced sensitisation of the lanthanide ion, due to increased ease of populating the $S_1$ state of the antenna. The Eu(III) emission was found to be "switched on" over the pH 6.5 – 4.5 region. In contrast, the acidification of an aqueous solution of the Tb(III) complex Tb.14, gave rise to much smaller luminescent enhancements of ca. 1.7. This was ascribed to a quenching of the antenna’s triplet state by the presence of molecular oxygen. Consequently, when the measurements were repeated in degassed acidic solution, the Tb(III) emission was "switched on" with a 50 fold luminescent enhancement factor. As the Tb(III) emission is governed by two molecular inputs, and is only “switched on” in the presence of $\text{H}^+$ and the absence of $\text{O}_2$, Tb.14 can be described as a NAND or “inhibit” logic gate (a combination of the NOT and AND logic functions).

From these examples it can be seen that lanthanide luminescent sensors have great potential as in vivo probes as they are non-invasive causing minimal disturbance to the system under study, yet emit at long wavelengths offering better signal quality over fluorescent probes. The next section shall detail the use of lanthanide luminescent probes as cation sensors.

1.1.4 Lanthanide Luminescent Probes as Cation Sensors

Lanthanide luminescent probes have been shown to be effective tools for pH sensing however, by altering their receptors, such probes can be utilised as cation sensors. One
such system that utilises such probes for the sensing of Zn(II) cations was developed by Gunnlaugsson et al. Their zinc sensor Eu.15 showed a 42% enhancement in luminescence upon 1:1 binding of Zn(II), while Tb.16 showed a 60% increase in luminescence. The increasing luminescence upon the coordination of Zn(II), was attributed to the suppression of photo-induced electron transfer from or to the intermediate singlet excited state, $S_1$. Both sensors were selective for Zn(II) over Ca$^{2+}$ and Mg$^{2+}$.

Nagano and Kikuchi reported another such sensor, the Eu(III) complex of a combination of diethylene-triaminepentaacetic acid (DTPA) and a quinoline containing TPEN ligand, 17. The quinoline antenna was chosen due to its longer absorption wavelength (>300 nm), which offers advantages for in vivo work. Upon addition of Zn(II) to an aqueous solution of Eu.17 (50 μM), at pH 7.4, a 8.5 fold increase in the luminescence was observed after excitation at 320 nm. Further investigation revealed a 1:1 stoichiometry, with an apparent dissociation constant $K_d$ of 59 nM for Zn(II). The presence of Na$^+$, K$^+$, Ca$^{2+}$ or Mg$^{2+}$ did not give rise to any enhancement in the luminescence and furthermore, the luminescence of the complex was found to be insensitive to pH between 3.6 and 8.8. Consequently, the Eu(III) complex Eu.17, can be considered as a useful probe for the biological detection of Zn(II).
Wong and co-workers described the Tb(III) complexes of 18 and 19. The luminescent lifetime of Tb.19 in H₂O was found to increase by 65% upon the addition of NaCl 0.13 mM, from 1.81 ms to 3.02 ms, this was accompanied by a decrease in the hydration state q from one to zero. Further investigation showed that this effect was independent of the choice of anion. The effect was ascribed to the interaction between the crown ether and the Tb(III) centre resulting in the displacement of a metal bound water molecule. Interestingly, the Tb(III) complex Tb.18, did not respond to the presence of the alkali metal ions.

The authors also found that the luminescent intensities of both 18 and 19 increased substantially upon the addition of p-chlorobenzoate 20, with luminescent enhancement factors of 49 and 5 respectively, after excitation at 246 nm in H₂O. Significantly, when the Na⁺ cation in 20 was replaced by a tetrabutylammonium cation the luminescent enhancement decreased from 49 to 7. These studies suggested that the crown ether effectively binds the Na cation which facilitates the close approach of the benzoate anion to the Tb(III) complex, permitting it to act as a sensitising antenna, providing efficient energy transfer to the Tb(III) metal centre. This ion pair sensor leads nicely to the next section, which discusses the use of luminescent lanthanide probes as anion sensors.

1.1.5 Lanthanide Luminescent Probes as Anion Sensors

The large positive charge of the lanthanide ions as well as the sensitivity of their emission spectra to their coordination environment, make lanthanide ions ideal sensors for the detection of anions. Charbonnière et al. developed lanthanide complexes of a bis-bipyridine-phosphine-oxide ligand 21, as luminescent anion sensors. The bipyridine (bipy) units were expected to coordinate to the lanthanide ion and function as sensitising antennae, while the strong coordinating ability of the P=O bond would increase the
stability of the complex, despite the fact that it would remain coordinately unsaturated, with two solvent molecules occupying the final coordination positions. It was found that in aqueous solution neither Eu.21 nor Tb.21 gave rise to any luminescence, indicating that neither of the bipy units were coordinated to the lanthanide ions under these conditions. In MeCN solution, after excitation of the bipy absorption bands (310 nm), the triflate salt complexes were found to display the typical lanthanide luminescence bands. No change was observed upon the addition of fluoride or acetate ions, however, upon addition of tetrabutylammonium nitrate Eu.21 and Tb.21, respectively, underwent an 11 and 7 fold increase in their luminescent intensities. The same trend was observed upon addition of chloride anions with a 5 and 2.5 fold increase in luminescent intensities, respectively. The reason for these dramatic changes in the luminescence spectra was ascribed to the coordination environment of the lanthanide ions. In MeCN the triflate counter anions do not bind directly to the lanthanide ion and hence two solvent molecules occupy the remaining open coordination positions. However, the addition of nitrate anions leads to an increased emission from the lanthanide ions as the nitrate ion displaces the metal bound solvent molecules and binds directly to the metal ion, thereby shielding the lanthanide from the solvent molecules and increasing the luminescence. The addition of a second equivalent of the nitrate anion displaces one of the bipy units. This leads to a further enhancement in the luminescence due to the resulting stronger electronic interaction between the lanthanide ion and the second bipy unit. Addition of a third nitrate ion to the metal ion decreases the luminescence as it displaces the final bipy unit and hence weakens the energy transfer mechanism.

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Other anion sensors have also been reported. Parker et al. developed a range of phosphate anion sensors 22 – 24. The acridone moiety of these complexes functions as the antenna, absorbing energy and passing it to the Eu(III) ion. Selectivity towards phosphates can be
tuned by varying the electrostatic gradient around the lanthanide ion, achieved in this case by varying R. The binding of selected phosphates, as well as phosphorylated amino acids such as Ser-OP and Tyr-OP, were investigated by observing the emission spectra of the complexes 22 – 24 (0.1 mM) at 295 K, pH 7.4 (0.1 M MOPS) in the presence of a ten fold excess of phosphate. Job plot analysis revealed the formation of 1:1 adducts, which displayed subtly different emission spectra, with Tyr-OP giving the lowest $\Delta I_2/\Delta I_1$ intensity ratio. As such phosphorylated tyrosine could be selectively detected by subtle changes in the Eu(III) luminescence.

Tsukube and co-workers reported the Eu(III) complex of tris(2-pyridylmethyl)amine 25, as a luminescent sensor for nitrate, NO$_3^-$, while the corresponding Tb(III) complex displayed Cl$^-$ selectivity. The Eu(III) complexes of 25 and 26 display 1:1 stoichiometry and are stable enough for use in solution. In this case, the pyridine units fulfil the role of the sensitising antenna, absorbing at 260 nm before passing their energy to the lanthanide ion and inducing a lanthanide emission. However, the complexes are coordinately unsaturated and hence the metal bound solvent molecules effectively quench the luminescence. Nevertheless, upon the addition of 3 equivalents of NO$_3^-$ to Eu.25 in MeCN, or Cl$^-$ in the case of Tb.25, the solvent molecules are displaced and the luminescence is increased by a factor of 4.9 and 5.4 respectively. Conversely, the addition of ions such as I$, Br^-$, F$, ClO_4^-$, SCN$^-$ and HSO$_4^-$ did not elicit a response. The achiral ligand 26 showed similar anion selectivity in MeCN, however, it displayed a lower sensitivity than the chiral analogue.

Michels and Reinhoudt described the Eu(III) and Tb(III) complexes of the EDTA-based $\beta$-cyclodextrin dimer 27, which operate in H$_2$O at pH 7.0 as luminescent sensors for aromatic carboxylates. As the ligand does not provide any sensitising antenna no luminescence
was observed. Nevertheless, upon the addition of \textbf{28}, the luminescence intensities of \textit{Eu.27} and \textit{Tb.27} increased by a factor of 350 and 310, respectively, after excitation of the aromatic chromophore at 250 nm. These large enhancements were ascribed to the hydrophobic binding contribution of the \(\beta\)-cyclodextrin units which can bind the aromatic unit thereby positioning the Ln(III) coordinating carboxyl group correctly to function as a sensitising antenna and allow efficient energy transfer to the lanthanide ion. The stoichiometry was reported to be 1:2 with a stability constant of \(K = 5.0 \times 10^4\) M\(^{-1}\). As expected, the addition of the benzyl alcohol \textbf{29}, led to much smaller enhancements of \textit{ca.} 2 fold. The authors explained this lower enhancement as owing to the lack of a carboxylate functionality through which the chromophore can bind to the lanthanide ion.

The interaction between \textbf{Eu.27} and bisadamantyl biphenyl sensitisers \textbf{30} and \textbf{31}, was also studied. Once again, upon excitation of the sensitisers at 285 nm, large increases in the Eu(III) luminescence was observed, \textit{ca.} 300 and 360 fold for \textbf{30} and \textbf{31} respectively, in D\(_2\)O. A 1:1 binding stoichiometry was observed with stability constants \(K\) of \(9.9 \times 10^6\) and \(1.0 \times 10^7\) M\(^{-1}\) for the \textbf{Eu.27.30} and \textbf{Eu.27.31} conjugates respectively. The very high association constants suggest that both \(\beta\)-cyclodextrin cavities of \textbf{27} are involved in the binding of the bisadamantyl biphenyl sensitisers \textbf{30} and \textbf{31}.

Georges and Arnaud reported a method of salicylic acid detection using a combination of Tb(III) and ethylenediaminetetraacetic acid (EDTA).\(^{44}\) The presence of the EDTA prevents
the formation of insoluble terbium hydroxides and also acts to exclude water molecules from the coordination sphere of the lanthanide ion, reducing quenching due to OH oscillators. A 1 x 10^-4 M solution of \textbf{Tb.EDTA}, could detect salicylic acid with a limit of detection (LOD) of 5 x 10^{-9} M in water at pH 12, the high pH was required in order for the ternary luminescent chelates between salicylic acid and Tb(III) to form. In this case the analyte itself acts as the sensitising antenna, forming a ternary complex with \textbf{Tb.EDTA} and then passing its excited energy to the Tb(III) centre, provoking a lanthanide emission. The sensitivity increased in the presence of cetyltrimethylammonium chloride (CTACl) (LOD = 4 x 10^{-10} M) and the authors attributed this to the cationic surfactant forming micelles in solution. The use of organised materials such as micelles and cyclodextrins can greatly increase the efficiency of the intramolecular energy transfer and hence the overall luminescence.\textsuperscript{24}

A similar method utilising ternary chelates of Tb(III) and trioctylphosphine oxide (TOPO) was reported by Pérez-Bendito and co-workers as a luminescent sensor for the determination of benzoic acid and saccharin in soft drinks.\textsuperscript{45} This group also reported a kinetic method of determining salicylic acid and diflunisal (a salicylic acid derivative) based on lanthanide luminescence.\textsuperscript{46} Coupled with the aid of a stopped-flow mixing technique, it again involved the formation of ternary chelates of salicylate (or diflunisal) and \textbf{Tb.EDTA}, this time in the presence of cetyltrimethylammonium bromide (CTAB), which acts as the cationic surfactant. They were able to determine between the two analytes due to the different rate of formation of the ion-association complex of CTAB and the anionic ternary complex. They used a similar method for the detection of bromadiolone (a coumarine derivative).\textsuperscript{47}

In 2000, Parker et al. reported the heptadentate complex \textbf{32}.\textsuperscript{48, 49} Lifetime studies revealed that the Eu(III) and Tb(III) complexes \textbf{Eu.32} and \textbf{Tb.32} each possessed two metal bound water molecules. The authors reported that in aqueous solution these water molecules could be displaced by anions such as phosphate lactate and carbonate, and that the formation of these 1:1 ternary complexes was signalled by changes in the lifetime of the Eu(III) and Tb(III) excited state.
So far we have focused on the luminescence of the Eu(III) and Tb(III) complexes. They are by far the most popular ions due to their easily accessible excited states, their long lived excited nature and intense long wavelength emissions in the visible region of the spectrum. Furthermore, these particular ions are not usually quenched by organic sensitiser molecules. However, other lanthanide ions of interest are the near infrared emitting lanthanides of Yb(III) and Nd(III). Compared to Eu(III) and Tb(III), these ions posses lower energy emission states and hence can utilise chromophores with longer absorption wavelengths, offering improved detection limits for *in vivo* applications. As these ions emit at wavelengths greater than 800 nm, specialised instruments are required for measuring them. The Faulkner group, and others, have detailed some elegant examples of sensitisation of Yb(III) and Nd(III) complexes by long wavelength absorbing chromophores. However, as yet these systems have not been utilised as sensors.

### 1.1.6 Conclusion

It has been shown how a photonic, or light, response can be obtained from a lanthanide luminescent system by way of a sensitising antenna, and how this signalling moiety can be utilised for luminescent sensing, offering advantages over conventional fluorescent systems. We are concerned with the development of new luminescent sensors that employ lanthanide ion as signalling systems. The aim of this project is to construct a novel lanthanide luminescent system for the detection of the biologically relevant Cu(II) cation. The design, synthesis, photophysical properties and analysis of this system shall be the subject of Chapter 2. We shall also utilise lanthanide luminescent systems as anion
detectors, and Chapter 3 shall detail the development of sensors for aromatic carboxylates, focusing on \(N,N\)-dimethyl benzoic acid, salicylic acid and terephthalic acid.

1.2.0 Phosphodiester Hydrolysis

In recent years, there has been a sustained effort to develop synthetic molecules that cleave phosphodiester bonds efficiently.\(^{54, 55, 56}\) Phosphodiester bonds perform an important task in nature, linking together the genetic code of the nucleic acids, deoxyribonucleic acid (DNA) and ribonucleic acid (RNA). In essence, the phosphodiester linking the nucleic acids together forms the backbone of both DNA and RNA. It was chosen by nature due to its incredible stability,\(^ {57}\) in order to preserve the genetic information. The negative charge on the phosphate drastically reduces the rate of hydrolysis and as a result, nucleic acids are very inert to cleavage under physiological conditions. It has been estimated that DNA has a half-life of 200 million years at pH 7 and 25 °C.\(^ {58}\) The major difference between DNA and RNA is the presence of the 2′-OH hydroxyl group on the ribose of RNA, which can act as an intramolecular nucleophile. As such, RNA can be hydrolysed with greater ease than DNA.\(^ {55}\) Despite this, RNA is still a very stable molecule, with a half-life thought to be of the order of hundreds of years, under physiological conditions.\(^ {59}\)

\[
\text{DNA} \quad \text{RNA}
\]

Figure 1.6. Illustrating the structural differences between the negatively charged phosphodiester backbone of DNA and RNA and nucleotide bases
The translation into proteins, of the genetic information encoded within DNA, is conducted by messenger RNA (mRNA). The ability to selectively cleave this RNA would provide control over the production of undesirable proteins. Many diseases, including bacterial, viral, fungal and even some forms of cancer, could be targeted since they operate by producing harmful proteins. The work within this thesis shall focus on RNA hydrolysis.

In nature, the hydrolysis of nucleic acids is carried out by a class of enzymes called hydrolases or ribozymes. These enzymes frequently contain metal cations such as Ca, Mg2+ and Zn(II), at their active sites. The hammerhead ribozyme, for instance, which effectively cleaves RNA, is known to possess multiple Mg2+ metal cations at its active site. The crystal structure of ribonuclease P1, which catalyses the hydrolysis of single stranded DNA and RNA, reveals the presence of three Zn(II) ions, at the active site (Figure 1.7).

![Figure 1.7. The active site of ribonuclease P1 possesses Zn(II) ions with bridging water molecules](image)

The mechanism for the hydrolysis of RNA has been studied in detail. It is thought to proceed via a two step mechanism, which displays a pH dependence and can be catalysed by both general and specific acid/base catalysis (Scheme 1.5). In the first step, transesterification, the 2'-OH group acts as a nucleophile and attacks the tetrahedral phosphodiester, giving a 5 coordinate phosphorane intermediate. The second step involves removal of the 5'-OH of the ribose from the phosphorous atom, i.e. hydrolysis of the
phosphorane. Nucleophilic attack on a negatively charged phosphodiester is unfavourable, the five coordinate, doubly charged phosphorane intermediate is also unstable, however, both can be stabilized by protons, metal cations and/or a Lewis acid. This has been cited as the reason why such a wide range of substances including protons, Cu(II), Zn(II) and lanthanide ions accelerate RNA cleavage. The following section shall show and discuss how metal ions can accelerate the rate of phosphodiester cleavage.

Scheme 1.5. Proposed mechanism for the hydrolysis of RNA

1.2.1 Acceleration of Phosphodiester Hydrolysis by Metal Cations

It has been shown in numerous accounts that highly charged cations, particularly lanthanides, can greatly accelerate phosphodiester hydrolysis. For instance, in 1993, Breslow et al. showed that metal cations accelerated the rate of hydrolysis of the RNA dinucleotide UpU (Table 1.1). The lanthanide cation Eu(III), was found to promote the hydrolysis 15 times faster than Zn(II). This was attributed to the higher charge density of Eu(III) compared to Zn(II), which makes the lanthanide ion a stronger Lewis acid. Lewis acids accelerate the cleavage of phosphodiesters by binding to and stabilising the negatively charged phosphate ion, hence activating the phosphate towards nucleophilic attack. This is known as Lewis acid activation. The authors found that a ten fold increase in Eu(III) concentration led to only a three fold rate increase, which suggests a kinetic saturation.
Table 1.1. Pseudo first order rate constants (k) and relative rate enhancements (k_{rel}) for the cleavage of UpU (3 mM) in 10 mM HEPES (pH 7.0) at 80 °C^70

<table>
<thead>
<tr>
<th>Catalyst</th>
<th>k x 10^3 hr^{-1}</th>
<th>k_{rel}</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>0.046 (± 0.004)</td>
<td>1</td>
</tr>
<tr>
<td>ZnCl_2 (1 mM)</td>
<td>1.46 (± 0.04)</td>
<td>32</td>
</tr>
<tr>
<td>EuCl_2 (1 mM)</td>
<td>22 (± 7)</td>
<td>475</td>
</tr>
<tr>
<td>EuCl_2 (10 mM)</td>
<td>67 (± 10)</td>
<td>1460</td>
</tr>
</tbody>
</table>

In addition to this, it was also shown that metal cations increased the rate of hydrolysis for simpler model phosphodiesters such as 2-hydroxy p-nitrophenylphosphate (HPNP) (Table 1.2). The phosphodiester HPNP, is often utilised as an RNA model compound due to the ease of kinetic analysis, which can be followed by absorbance spectroscopy. It will be employed throughout this thesis, and discussed in greater detail later.

Table 1.2. Pseudo first order rate constants (k) and relative rate enhancements (k_{rel}) for the cleavage of HPNP (0.18 mM) in 10 mM HEPES (pH 7.0) at 37 °C with various metal ions (0.5 mM)^70

<table>
<thead>
<tr>
<th>Catalyst</th>
<th>k x 10^2 hr^{-1}</th>
<th>k_{rel}</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>0.012 (± 0.001)</td>
<td>1</td>
</tr>
<tr>
<td>CaCl_2</td>
<td>0.032 (± 0.004)</td>
<td>3</td>
</tr>
<tr>
<td>MgCl_2</td>
<td>0.53 (± 0.20)</td>
<td>46</td>
</tr>
<tr>
<td>ZnCl_2</td>
<td>1.70 (± 0.12)</td>
<td>150</td>
</tr>
<tr>
<td>PbCl_2</td>
<td>32 (± 6)</td>
<td>2800</td>
</tr>
<tr>
<td>EuCl_3</td>
<td>88.9 (± 4.5)</td>
<td>7700</td>
</tr>
<tr>
<td>TbCl_3</td>
<td>110 (± 6)</td>
<td>9500</td>
</tr>
<tr>
<td>YbCl_3</td>
<td>109 (± 6)</td>
<td>9500</td>
</tr>
</tbody>
</table>

The results shown in Table 1.2, clearly illustrate that lanthanide ions are by far the most effective at accelerating the hydrolysis of phosphodiesters. The cleavage of HPNP by Eu(III) was found to be 50 fold more effective than cleavage promoted by Zn(II). The Tb(III) ions were found to be slightly more active than the Eu(III) ions, which can be explained by their smaller size and hence greater Lewis acidity. However, the similarity between Tb(III) and Yb(III), suggests that ionic radius is not the only determining factor for the activity of the free ions, as Yb(III) is considerably smaller. \[^70\]

Roigk and Schneider, investigating the effect of varying lanthanide concentrations upon hydrolysis of phosphate esters, found that the lanthanides displayed Michaelis-Menten saturation kinetics (i.e. the rate became independent of the concentration of metal ion at high concentrations). \[^69\] Furthermore, it was found that the efficiency of the heavier lanthanide ions such as Lu(III) and Yb(III), actually decreased at higher concentration (0.1
0.5 mM). This decrease in efficiency was attributed to the formation, at higher concentrations, of aggregates of these ions. Indeed the lanthanides with the greatest Lewis acidity were only the most effective when applied at low concentrations (0.05 mM). It was also noted that the presence of NaCl or MgCl₂ reduced the rate of cleavage of phosphodiesters due to competitive binding with the substrate.

Despite these factors, metal ions, and lanthanide ions in particular, remain excellent promoters for phosphodiester hydrolysis. The reason behind the large enhancements provided by the metal ions is that they are Lewis acids and can promote hydrolytic cleavage in a number of ways, such as:\(^{62,71}\)

- (a) Electrostatic activation of the substrate in the ground state
- (b) Stabilisation of the transition state
- (c) Stabilization of leaving group
- (d) Generation of nucleophile via proton abstraction (general base catalysis)
- (e) Stabilization of leaving group via proton donation (general acid catalysis)
- (f) Nucleophilic attack by metal coordinated hydroxide

Figure 1.8. Showing three direct and three indirect, possible modes of Lewis acid activation\(^ {62,71}\)
Chapter 1 - Introduction

The reason lanthanide ions are so successful at accelerating phosphodiester hydrolysis stems from their large positive $3^+$ charge combined with their relatively small ionic radius, which leads to a large charge density, thus making the lanthanide ions powerful Lewis acids. The next section shall detail some of the substrates often utilised as phosphodiester model compounds within the literature.

1.2.2 Phosphodiester Models

At this point it should be noted that instead of RNA, different researchers use various model compounds to test their cleaving agents. This is mainly for convenience and to avoid the difficulties associated with the handling of RNA. The model compounds usually contain a phosphodiester with a good leaving group, which upon hydrolysis releases a product that can be observed by absorption spectroscopy. For instance 2-hydroxy $p$-nitrophenylphosphate (HPNP) is a phosphodiester, which, like RNA, has a hydroxyl group that can act as an intramolecular nucleophile. However, HPNP has the advantage that its hydrolysis kinetics can be followed by absorption spectroscopy, as HPNP absorbs at 300 nm, while the product of its cleavage, 4-nitophenolate (34), absorbs at 400 nm (Scheme 1.6).

![Scheme 1.6. The hydrolysis of HPNP gives 34 ($p$-nitro phenolate) and a cyclic phosphate, 35, and can be monitored by absorption spectroscopy](image)

Other model compounds often cited in the literature are displayed in Table 1.3. They too release 34 upon hydrolysis and as such, their kinetics can be followed by absorption
spectroscopy. However, apart from the RNA dinucleotide UpU, they do not contain an internal hydroxyl nucleophile and as such they more closely resemble DNA.

It is important to remember that although these model compounds can provide valuable information about phosphodiester hydrolysis, they do not precisely depict RNA or DNA hydrolysis. Indeed it has been found in some cases that cleaving agents, which were found to accelerate the hydrolysis of model compounds were inactive towards RNA cleavage and vice versa. In general, however, they remain important indicators as to the activity of a cleaving agent. The next section will look at the development of metal complexes that can cleave model phosphodiesters, such as these as well as RNA and DNA.

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Name</th>
<th>Structure</th>
</tr>
</thead>
<tbody>
<tr>
<td>BNPP</td>
<td>Bis(p-nitrophenyl) phosphate</td>
<td><img src="image1" alt="Structure" /></td>
</tr>
<tr>
<td>NPP</td>
<td>p-nitrophenyl phosphate</td>
<td><img src="image2" alt="Structure" /></td>
</tr>
<tr>
<td>EPNP</td>
<td>Ethyl p-nitrophenyl phosphate</td>
<td><img src="image3" alt="Structure" /></td>
</tr>
<tr>
<td>TNP</td>
<td>Tris p-nitrophenyl phosphate</td>
<td><img src="image4" alt="Structure" /></td>
</tr>
<tr>
<td>UpU</td>
<td>Uracil dinucleotide</td>
<td><img src="image5" alt="Structure" /></td>
</tr>
</tbody>
</table>

Table 1.3. DNA and RNA model compounds

1.2.3 Phosphodiester Hydrolysis by Metal Complexes

As previously mentioned, large rate enhancements for phosphodiester hydrolysis were obtained by Breslow et al., using metal cations. The largest of these enhancements were obtained using lanthanide ions. In spite of this, the use of metal ions in vivo is undesirable, as they are often toxic and unselective, and can form hydroxides that precipitate out of solution. In order to harness the potential offered by metal cations, for in vivo
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applications, they must be incorporated into a stable coordination environment. There are many examples in the literature of metal complexes accelerating the rate of phosphodiester hydrolysis.

One such example is the Cu(II) complex 36, reported by Chin et al. A ten fold excess of complex 36 was found to cleave HPNP (0.2 mM) at pH 7.0 and 25 °C, with a pseudo first order rate constant of $5.74 \times 10^{-6}$ s$^{-1}$, corresponding to a rate enhancement, $k_{rel}$ of 172 over the uncatalysed reaction. Zinc complexes have also been found to hydrolyse phosphodiester bonds. The Zn(II) complex 37 (0.5 mM) was reported by Breslow et al. to accelerate the rate of HPNP (0.19 mM) hydrolysis with a pseudo first order rate constant of $7.4 \times 10^{-4}$ hr$^{-1}$ corresponding to a 7 fold rate enhancement over the uncatalysed reaction at pH 7.0 and 37 °C. Mirroring the trend set by the free metal ions themselves, lanthanide complexes have been found to be among the best at promoting phosphodiester hydrolysis. The La(III) complex 38 (0.18 mM) was reported by Morrow et al. to cleave HPNP (0.18mM) at pH 7.4 and 37 °C, with a pseudo first order rate constant of $5.8 \times 10^{-2}$ hr$^{-1}$, a rate enhancement, $k_{rel}$, of 483 over the uncatalysed reaction. Unfortunately, direct comparisons between these systems are not always possible as reported reaction conditions, such as temperature, pH, solvent and concentration often vary. The work conducted within this thesis shall employ the conditions utilised by Morrow et al. in order to allow comparison where possible.

To date, some of the greatest enhancements have been observed using lanthanide complexes. Gunnlaugsson et al. reported the complex 39, which utilised extended pseudo peptide arms to form a hydrophobic cavity. At pH 7.4 and 37 °C, the lanthanum complex La.39 (0.18 mM) was reported to cleave HPNP (0.18 mM) with a pseudo first order rate constant of $0.410 (\pm 0.026)$ hr$^{-1}$, corresponding to a half-life, $\tau_{1/2} = 1.70$ hours, and a relative rate enhancement, $k_{rel}$, of 3417.
Morrow reported the europium complex \textbf{Eu.40}, as the first example of a metal complex, which exhibited catalytic turnover in the hydrolysis of RNA at 37 °C neutral pH. \textbf{Eu.40} (490 \mu M) was found to cleave 41% of the dinucleotide ApUp (20 \mu M) after four hours.\textsuperscript{78} The complex \textbf{Eu.40} (200 \mu M) was also reported to cleave 81% of the RNA oligomer of adenylic acid (A\textsubscript{12} – A\textsubscript{18}) (190 \mu M) after four hours.

Previous work within the Gunnlaugsson laboratory has concentrated on mononuclear systems such as \textbf{39}, described above. The focus of this thesis, however, shall be upon the phosphodiester hydrolysis ability of binuclear systems. The following sections describe some of the binuclear systems already published within the literature.

\section*{1.2.4 Cooperative Action by Binuclear Transition Metal Ion Systems} X-ray crystallography, has shown that the natural enzymes which hydrolyse RNA often posses two or more metal ions at their active sites.\textsuperscript{79} These findings strongly indicated that a bimetallic system should lead to greater catalytic efficiency. The synthesis of many elegant binuclear transition metal ion complexes has been achieved,\textsuperscript{80, 81} and numerous accounts have now been reported, showing that the presence of a second metal ion, can lead to large rate enhancements of phosphodiester hydrolysis over similar mononuclear systems.\textsuperscript{82} One of the first reports of this greater efficiency, achieved by cooperating metal ions came from Breslow \textit{et al.} They synthesised a binuclear Zn(II) complex, \textbf{41}, which at pH 8.0 hydrolysed the neutral ester diphenyl \textit{p}-nitrophenyl phosphate (DPNP) 4.4 times faster than the corresponding mononuclear complex.\textsuperscript{83}
Since then, numerous examples of this increased efficiency by binuclear systems have been reported. Hamilton et al. reported the binuclear Cu(II) system 42, based on a covalently linked terpyridine and bipyridine ligand system. This binuclear complex exhibited a notable base selectivity, and 2.0 mM of 42, was found to hydrolyse the RNA dinucleotide ApA, (0.1 mM) 11 times faster than the similar mononuclear complex 36, with a first order rate constant of $1.52 \times 10^{-4}$ s$^{-1}$ at pH 7.5 and 25 °C. The selectivity towards adenine bases was attributed to strong π-π stacking interactions with the bipyridine unit. Komiyama reported the dinuclear Zn(II) complex 43. A solution containing 2.5 mM, of 43, at 50 °C, pH 7.0, was found to hydrolyse ApA (0.1 mM), with a pseudo first order rate of $7 \times 10^{-5}$ s$^{-1}$, whereas, the mononuclear complex, was found to be inactive towards ApA hydrolysis.

Chin et al. reported the dinuclear Cu(II) complex 44, 1 mM of which, was found to cleave HPNP (50 μM) at 25 °C and pH 7.0 with a pseudo first order rate constant of $2.1 \times 10^{-3}$ s$^{-1}$, a 52 fold increase over the rate obtained for the analogous mononuclear system.

Similar findings were reported by Morrow et al. who described the binuclear Cu(II) complexes 45 and 46 (0.5 mM), which were respectively found to cleave GpppG (30 μM),
a model of the 5'-cap mRNA structure, 46 and 32 times faster per metal centre, than the mononuclear Cu(II) system, with pseudo first order rate constants of $5.6 \times 10^{-5} \text{s}^{-1}$ and $3.9 \times 10^{-5} \text{s}^{-1}$, respectively, at pH 7.3 and 37°C.\textsuperscript{88}

The binuclear Zn(II) complex 47 was reported as one of the best dinuclear zinc catalysts for HPNP cleavage with the highest reported second order rate constant (0.25 M\textsuperscript{-1} s\textsuperscript{-1}) in 100% aqueous solution at pH 7.6, 25°C.\textsuperscript{89} This represented a 120 fold increase over the mononuclear system. The complex 47, was also reported to cleave 90% of a oligoribonucleotide containing six adenosines (A\textsubscript{6}), after 24 hours. Interestingly, the Cu(II) complex of 47 was reported to be inactive in the cleavage of HPNP. This was tentatively attributed to a decrease in the available coordination sites on the Cu(II) ion in comparison to the Zn(II) analogue.

Further examples of large rate enhancements due to the presence of a second metal ion were reported by Bianachi \textit{et al}.\textsuperscript{90} The dinuclear Zn(II) complex 48 was found to cleave BNPP 10 times faster than its mononuclear analogue. The binuclear Cu(II) complex of 49, was reported by Chin \textit{et al}. to cleave ApA 300 times faster than the mononuclear system at pH 6.0.\textsuperscript{91} This complex was reported to have a Cu – Cu distance of approximately 4.5 Å. Interestingly, this is similar to the distance between metal ion centres in natural enzymes, reported to be between 3 and 5 Å.\textsuperscript{92}
These large rate enhancements over the mononuclear systems are far greater than can be explained by a simple increase in metal ion concentration. It is widely accepted that the rate enhancements are due to a cooperative action between the two metal centres. Binuclear complexes can utilise a double Lewis acid activation mechanism Figure 1.9 whereby,

- One metal ion can bind and activate the incoming nucleophile (the 2'-OH)
- The second metal ion can bind to the leaving group (the 5'-OH),
- While both metals can bind to the phosphate activating it towards nucleophilic attack and stabilizing the developing negatively charged transition state.

![Figure 1.9](image_url)

**Figure 1.9.** (a) Proposed mechanism for the cooperative action of two metal centres in the hydrolysis of HPNP (b) Alternate mechanism involving metal bound water molecules

An alternate mechanism involving metal bound water molecules is also possible Figure 1.9 b, whereby a metal bound hydroxide functions as a general base catalyst deprotonating the 2'-OH, while a metal bound water molecule functions as a general acid catalyst for the 5'-OH leaving group.

From the examples detailed so far, it can be seen that there is a wide divergence between binuclear systems. Some are only moderately superior to their corresponding mononuclear complex; for example 41 was only 4.4 times faster. Others show more than an order of magnitude improvement upon their mononuclear systems, for instance 44 exhibited a 52 fold enhancement. The factors affecting the extent of the binuclear cooperation shall be the subject of the next section.
1.2.5 Pertinent Factors for Double Lewis Acid Activation

A crucial factor for efficient cooperative action between the metal ions is the spacer group employed. Investigating the effect of the spacer group, Breslow et al. reported a series of Zn(II) complexes $50 - 53$.\(^\text{96}\)

They found all the binuclear complexes to be more active than the corresponding mononuclear systems (Table 1.4). It was also shown that the shorter spacers of Zn.50 and Zn.51 were more active in the hydrolysis of monoesters, while the longer biphenyl spaced Zn.52 was the most active for phosphodiesters. The biphenyl spaced Zn.52, was shown to be 10 times more active than its corresponding mononuclear system. This highlights the importance of the spacing between metal centres as well as the ability to adopt a suitable geometry for a specific substrate. The authors reported the flexible pentamethylene spaced Zn.53 to be a poor promoter compared to the more rigid complexes. It has since been reported that the rate enhancement observed for the Zn.52 complex was not due to the cooperative action of the two metal ions but to an increased hydrophobic binding contribution from the biphenyl spacer.\(^\text{97}\)

<table>
<thead>
<tr>
<th>Complex</th>
<th>$k \times 10^5 \text{ s}^{-1}$</th>
<th>$k_{rel}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Zn.50</td>
<td>8.1</td>
<td>450</td>
</tr>
<tr>
<td>Zn.51</td>
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<td>556</td>
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<tr>
<td>Zn.52</td>
<td>19.3</td>
<td>1072</td>
</tr>
<tr>
<td>Zn.53</td>
<td>8.0</td>
<td>444</td>
</tr>
<tr>
<td>Zn.Mono$^a$</td>
<td>3.5</td>
<td>194</td>
</tr>
</tbody>
</table>

Table 1.4. Pseudo first order rate constants ($k$) and relative rate enhancements ($k_{rel}$) for the cleavage of HPNP (454 mM) in 50 mM TRIS (pH 8.4) at 30 °C with various Zn(II) complexes (227 mM). \(^a(454 \text{ mM})\)\(^\text{96}\)
The flexibility of the system is also an important feature. Czarnik reported the synthesis of both the very flexible and rigid binuclear Co(III) complexes, Co.54$^{98}$ and Co.55$^{99}$. The flexible binuclear complex Co.54, promoted the hydrolysis of BNPP 6.4 fold faster than the mononuclear analogue, with a rate constant of $7 \times 10^{-5}$ s$^{-1}$, at pH 7.0 and 25 °C. In contrast, the rigid binuclear Co.55 complex did not give rise to any cooperative rate enhancement for the hydrolysis of BNPP, as it could not accommodate the geometry required. This highlights the need for an element of flexibility within the design of the molecule.

In general, however, rigidly spaced molecules that are preorganised to interact with a substrate show greater activity than more flexible spaced analogues. For example, the same rigid complex Co.55 (1 mM) gave rise to a large cooperative rate enhancement for the hydrolysis of the phosphate monoester NPP (25 μM), which at pH 7.0 and 25 °C, was cleaved with pseudo first order rate constant of $1.33 \times 10^{-2}$ s$^{-1}$, corresponding to a 20 fold increase over that observed for the corresponding mononuclear analogue.

In summary, it can be concluded that:

- Binuclear systems with rigid spacer groups without a favourable geometry do not give rise to any cooperative rate enhancement
- Binuclear systems with flexible spacer groups exhibit weak cooperative enhancements of the order of 4 – 6 fold
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- Complexes with semi-rigid spacer groups with a preorganised geometry that accommodates the substrate, give the largest cooperative rate enhancements, of the order of $10 - 100$ fold

Canary et al. reported the binuclear ligands $56$ and $57$, whose ability to cleave BNPP (2.0 mM) was evaluated in a 30 % DMSO solution at 55 °C, with 0.4 mM Cu(ClO$_4$)$_2$ and 0.2 mM of the binuclear ligand, respectively. The first order rate constants were calculated by the initial rate method. The complex Cu$_2$56 was found to cleave BNPP, exhibiting a maximum activity at pH 8.4 with a pseudo first order rate constant $1.14 	imes 10^6$ s$^{-1}$ a $10^4$ fold rate enhancement over the uncatalysed reaction and a $\sim 10$ fold rate enhancement over the analogous mononuclear system. The hydrolysis by the complex exhibited a bell shaped pH dependence, which is common for binuclear systems. This bell shaped pH dependence was explained by the formation of the active species. Each Cu(II) ion possesses one metal bound water molecule, whose ionisation state is determined by the pH of the solution.

The active species is believed to be the [Cu$_2$56(OH$^\cdot$)(H$_2$O)]$^{3+}$ species, as this species provides a potent nucleophile in the form of the metal bound hydroxide, while also providing Lewis acid activation through the other metal ion, which can bind to the phosphodiester via displacement of a metal bound water molecule. In contrast, the di-aqua species cannot provide a nucleophile, while the di-hydroxide species is known to inhibit coordination to the phosphodiester due to the tightly bound hydroxide molecules.
Interestingly, the binuclear conjugate 57 gave no rate enhancement over the mononuclear system. One possible reason for this may be the shorter intermetal distance, which may prevent the two Cu(II) centres from adopting a favourable orientation for binuclear cooperativity.\textsuperscript{101}

To date, one of the greatest rate enhancements has been achieved by Reinhoudt \textit{et al.} who reported the synthesis of a calix[4]arene based, binuclear Zn(II) complex, 58.\textsuperscript{102} In a 50:50 MeCN:aqueous buffered solution at pH 7.0 and 25 °C, the complex 58 (0.48 mM), was reported to cleave HPNP (0.19 mM) with a pseudo first order rate constant of $6.3 \times 10^{-4} \text{ s}^{-1}$ corresponding to a half-life of 18 minutes, calculated by the initial rates method. This represents a remarkable 23,000 fold rate enhancement over the uncatalysed system. The complex also exhibited catalytic turnover and was reported to be 50 times more active than the corresponding mononuclear complex.

![58](image1.png)

Reinhoudt \textit{et al.} also reported the binuclear Cu(II) complex 59, which under similar conditions in a 35:75 EtOH:H$_2$O solution, buffered at pH 6.2, cleaved HPNP with a pseudo first order rate constant of $2.9 \times 10^{-4} \text{ s}^{-1}$ corresponding to a half-life of 40 minutes and a rate enhancement, $k_{rel}$, 10,000.\textsuperscript{103} The complex was found to be 22 times more active than its mononuclear system and also exhibited catalytic turnover cleaving a four fold excess of HPNP with no loss of activity. The authors attributed the activity to the cooperative action of the two preorganised but still dynamic metal cations, which are held in a somewhat rigid fashion by the calixarene moiety.
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The authors also noted a higher binding affinity for phosphate by the binuclear systems over the mononuclear analogue. This increase is often observed for binuclear systems, and reflects the stronger electrostatic stabilization offered by the highly cationic, dinuclear complex.\textsuperscript{104} The remarkable activity of the complexes was attributed to a moderate turnover combined with a strong binding affinity for the case of \textit{Zn}.\textsuperscript{58}, or a high turnover rate combined with a moderate binding affinity, in the case of \textit{Cu}.\textsuperscript{59}.

It appears that metal centres linked by very flexible scaffolds display a smaller cooperative activity compared to more rigid scaffolds.\textsuperscript{96} Nevertheless, molecules that are too rigid have also shown poor cooperative activity.\textsuperscript{105} A balance between preorganised rigidity and a certain degree of flexibility is required for optimum activity.\textsuperscript{56,103} The intermetal distance as well as the overall geometry also play important roles.

1.2.6 Trinuclear complexes

A number of natural enzymes, such as ribonuclease P1, possess three metal ions at their active site,\textsuperscript{64} and hence it was suggested that the incorporation of a third metal into a phosphodiester cleavage agent would further improve upon the rate of hydrolysis obtained. This hypothesis was further supported by the trinuclear Zn(II) complex \textit{60}, which was developed as a receptor for phosphate dianions, and showed a greater binding affinity (Log $K = 5.8$) for nitrophenylphosphate (NPP), than the corresponding dinuclear Zn(II) complex (Log $K = 4.0$).\textsuperscript{106} In 1997 Komiyama \textit{et al.} reported the trinuclear Zn(II) complex \textit{61}, which was found to hydrolyse ribonucleotide dimers (0.1 mM) at pH 6.9 at elevated temperature \textit{ca.} 50 °C.\textsuperscript{107} A concentration of 5 mM \textit{61} was found to hydrolyse CpA with a pseudo first order rate constant of $7.2 \times 10^{-4}$ s$^{-1}$, and ApA with a rate constant of $4.6 \times 10^{-4}$ s$^{-1}$. This corresponded to half-lives of 16 and 23 minutes, respectively. A similar dinuclear Zn(II) complex was found to cleave CpA and ApA with pseudo first order rate constants of 0.6 and $0.5 \times 10^{-4}$ s$^{-1}$, corresponding to half-lives of 183 and 250 minutes, respectively. Clearly, these trinuclear complexes are efficient and active phosphodiester cleavage agents.
In 2002 Komiyama et al. reported the trinuclear Cu(II) complex 62, which was found to hydrolyse ribonucleotide dimers (0.1 mM) at pH 7.0 and 50 °C. It was reported to be the first complex to distinguish between 2′-5' and 3′-5′ phosphodiester linkages. The hydrolysis of the 2′-5' UpU dimer was 56 fold more rapid than the 3′-5′ isomer (Table 1.5). Interestingly, the specificity was reversed in the case of ApA. The rigidified analogue 63, was found to have a lower activity than that of 62, showing that flexibility is essential for efficient trinuclear complexes.

<table>
<thead>
<tr>
<th>Ribonucleotide</th>
<th>2′-5′</th>
<th>3′-5′</th>
</tr>
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<tbody>
<tr>
<td>UpU</td>
<td>28 (0.6)</td>
<td>0.5 (0.7)</td>
</tr>
<tr>
<td>GpG</td>
<td>0.3 (0.2)</td>
<td>0.3 (0.4)</td>
</tr>
<tr>
<td>ApA</td>
<td>0.5 (2.0)</td>
<td>24 (6.0)</td>
</tr>
<tr>
<td>CpC</td>
<td>–</td>
<td>7.0 (2.0)</td>
</tr>
</tbody>
</table>

Table 1.5. Pseudo first order rate constants ($10^{-4}$ min$^{-1}$) for the hydrolysis of (2′-5′) and (3′-5′)-diribonucleotides (0.1 mM), by 62 (2 mM) at pH 7.0, 50 °C. Parentheses shows the values for the dinuclear complex (3 mM).

Molenveld and Reinhoudt reported the trinuclear Zinc complex 64. In 50 % MeCN aqueous solution buffered at pH 7.0 and 25 °C, 64 (0.48 mM) was found to cleave HPNP (0.19 mM) with a pseudo first order rate constant of $8.55 \times 10^{-4}$ s$^{-1}$, corresponding to a rate acceleration of 32,000 over the uncatalysed reaction, and a 1.4 fold rate enhancement over the dinuclear zinc complex 58, which was discussed in the previous section.
Furthermore, the complex exhibited catalytic turnover cleaving a four fold excess of HPNP with a 40 % loss of activity after each turnover. In a further study the trinuclear Zinc complex (0.9 mM) was found to efficiently hydrolyse RNA dinucleotides (3'→5' NpN) (0.09 mM) in a 35 % EtOH aqueous solution buffered at pH 8.0 and 50 °C (Table 1.6).109 The rate accelerations over the uncatalysed reaction are of the order of $10^4$–$10^5$, and once again the trinuclear complex exhibits catalytic turnover, cleaving a three fold excess of UpU. The trinuclear complex 64, is a factor of 10, 19, and 160 times more active than the dinuclear complex for the cleavage of CpC, UpU and GpG, respectively. The greater reactivity towards GpG hydrolysis was explained by an enhanced binding coupled with a higher conversion rate.

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<tr>
<td>64</td>
<td>72</td>
<td>8.5</td>
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<tr>
<td>58</td>
<td>0.45</td>
<td>0.45</td>
<td>0.58</td>
<td>N/A</td>
<td>N/A</td>
<td>0.28</td>
</tr>
</tbody>
</table>

Table 1.6. Observed pseudo first order rate constants ($10^{-5}$ s$^{-1}$) for the cleavage of RNA dinucleotides (0.09 mM) by Zn(II) complexes (0.9 mM), in 35 % EtOH aqueous solution buffered at pH 8.0, 50 °C

Until now, we have only discussed multinuclear complexes of transition metal ions, however, the focus of this thesis shall be on binuclear lanthanide complexes, and these will be the subject of the next section.
1.2.7 Cooperative Action by Binuclear Lanthanide Systems

The cooperative action and rate enhancements detailed above have also been observed in binuclear lanthanide systems. Chin et al. reported the formation of a La(III) dimer which self-assembles in solutions above pH 8.5 and rapidly cleaves RNA. From potentiometric titrations, it was proposed that the dimer was bridged by five hydroxide ions.

The La(III) dimer, was reported to cleave ApA with a half-life of 13 seconds at pH 8.5, 25 °C. This represents a 10^4 fold increase in activity over the mononuclear lanthanide ions. Chin et al. also reported the peroxide bridged La(III) dimer, which demonstrated a 10^9 fold rate acceleration for the hydrolysis of BNPP at pH 7.0, 25 °C. However, the use of peroxide in biological systems is undesirable, as oxidative cleavage of DNA and RNA produces unselective diffusible free radicals, which can cause damage to surrounding cells. Hydrolytic, rather than oxidative, cleavage is preferred for biological uses.

There are numerous examples of binuclear and even higher order lanthanide complexes within the literature, as many elegant multinuclear Gd(III) complexes have been developed for use as medicinal chemical contrast agents. However, few if any, of these complexes have demonstrated an ability to accelerate the rate of phosphodiester hydrolysis. Indeed, despite the large rate accelerations demonstrated by lanthanide ions, mononuclear lanthanide complexes and lanthanide ion dimers, there are relatively few examples of rate accelerations provided by cooperative binuclear lanthanide complexes. As explained earlier, metal ions must be incorporated into a stable ligand framework if they are to be of use for pharmaceutical applications. One possible reason for the lack of reported binuclear lanthanide complexes may stem from their large coordination number requirement, which necessitates the use of stable, multidentate ligands, often requiring complex synthesis.

Moving in this direction, Yatsimirsky et al. reported that a combination of BisTris Propane (BTP) (20 mM) and LaCl₃ (4 mM) cleaved BNPP (0.04 mM) with a pseudo first order rate
constant of approximately $6.8 \times 10^{-4} \text{ s}^{-1}$ at pH 9.0, 25 °C.\textsuperscript{115} From potentiometric titrations, they proposed the active species to be the binuclear La(III) structure, $M_2$(BTP)$_2$(OH)$_2^{4+}$ \textsuperscript{67}. This, however, is not a stable complex and mass spectral analysis revealed the presence of numerous other species in solution including the free metal ion, which itself is an active catalyst.

A similar hydroxy bridged complex utilising BisTris as a ligand, has also been reported by Park and co-workers.\textsuperscript{116} The active species in solution at pH 9.8 and above, was reported to be a BisTris La(III) dimer bridged by four hydroxy molecules $La_2$(BisTris)$_2$(OH)$_4^{2+}$. The activity of this compound was found to be similar to that of the La(III) dimer \textsuperscript{64}. A 1:1 mixture of LaCl$_3$ and BisTris (0.5 mM) with two equivalents of NaOH was found to cleave BNPP with a pseudo first order rate constant, $k$, of $7.3 \times 10^{-3} \text{ s}^{-1}$. These results indicate that lanthanide complexes can act in a cooperative fashion to provide large rate enhancements for phosphodiester hydrolysis. However, the high pHs required for the formation of these dimers, not to mention the low stability of the resulting complexes negates their use for medicinal purposes.

Schneider \textit{et al.} reported one of the relatively few examples of a cooperative binuclear lanthanide complex for phosphodiester hydrolysis in 1996.\textsuperscript{117} The 2:1 Eu(III) complex of 71 was found to be 5 times more active towards BNPP hydrolysis than the 1:1 complex. The complex \textbf{Eu.71} (1 mM) at pH 7.0 and 50 °C, in a 5 % DMSO solution, was reported to reduce the half-life of BNPP (33.5 μM) from 75 years to 8 minutes. Furthermore the Pr(III) complex \textbf{Pr.71} (1mM), was reported to cleave 77 % of double stranded, plasmid DNA (19.5 μM in base pairs) at pH 7.0 and 37 °C, after two hours. Nevertheless, the addition of EDTA was found to decrease the rate by approximately 70 % indicating that the complexes were not kinetically stable. Schneider \textit{et al.} also reported the binuclear complexes 68 – 70, however, these complexes were found to give only moderate rate enhancements at best. Their disappointing activity was attributed to the shorter metal to metal distance of the complexes, as mentioned earlier, efficient binuclear cooperativity requires a certain separation of the metal centres in order to facilitate Lewis acid stabilisation of a bound phosphate at one end, with concomitant activation of nucleophile at the other.\textsuperscript{118}
In 1999, Zhu et al. described the more kinetically stable complex 72. The 2:1 Er(III) complex, Er.72 (0.7 mM), was reported to cleave plasmid DNA (1 μg) to both nicked and linear forms, after one hour, in yields of 42 % and 11 % respectively, under neutral conditions at 37 °C.\textsuperscript{119} The authors reported the pseudo first order rate constant for the cleavage of the plasmid DNA to be 2.8 x 10\textsuperscript{-2} min\textsuperscript{-1}. The kinetic inertness was studied using excess Cu(II) as a trapping agent. The drawback to these systems, however, is the lack of a handle to further functionalise the molecule. This is important, as the incorporation of catalytically active groups has been shown to greatly increase the activity of ribozyme mimics. Some examples of this shall be detailed in section 1.10.

In 2000 Jurek and Martell, reported the synthesis of the binuclear La(III) complex of 73.\textsuperscript{120} Measurements with this complex were conducted in a 75:25 EtOH:H\textsubscript{2}O solution with 0.1 M KCl at 35 °C. The pH was kept constant by addition of dilute KOH and the rate constants were determined using the initial rates method. A species diagram showed the presence of multiple species, however, only [La\textsubscript{2}73(OH)]\textsuperscript{3+} and [La\textsubscript{2}73(OH)\textsubscript{2}]\textsuperscript{2+} are
believed to contribute significantly to the hydrolysis. The 2:1 La(III) complex of 73 (0.486 mM) reportedly cleaved BNPP (0.539 mM) at pH 8.25, with a rate constant of $7.3 \times 10^{-8}$ M$^{-1}$ s$^{-1}$. The cooperativity between the two La(III) ions in this binuclear complex was evidenced by the much lower activity displayed by the mononuclear 1:1 La(III) complex of 73 (0.487 mM), which was found to cleave BNPP (0.500 mM) at pH 8.00, with a rate constant of $8.0 \times 10^{-10}$ M$^{-1}$ s$^{-1}$, corresponding to a 90 fold decrease in activity. An apparent third order dependence upon concentration, observed in the case of the binuclear La(III) complex, was attributed to the mechanism for the hydrolysis of BNPP, which is believed to proceed via the aggregation of three binuclear complexes and one molecule of BNPP.

In 2001, one final example of a dinuclear lanthanide complex capable of hydrolysing DNA, was reported by Que et al.$^{121}$ The dicerium complexes of ligands 74 – 76, were found to promote the hydrolysis of double stranded DNA. Plasmid DNA (0.1 μg/μL) was incubated for at pH 8.0 and 37 °C, in the presence of the dicerium complex 75 (100 μM). After 12 hours the mixture contained 17 % supercoiled, 63 % nicked and 20 % linear
DNA. The dicerium complexes of 74 and 76 were found to be less active, as under the same conditions after 12 hours the mixtures contained, 55 % supercoiled, 42 % nicked, and 3 % linear DNA. Of the other lanthanide ions, only Yb(III) formed a dinuclear complex capable of hydrolysing DNA under these conditions, though its activity was found to be half that of the dicerium complex. The cleavage of DNA by free cerium ions is known to proceed through an oxidative pathway. In order to confirm that the mechanism operating here was hydrolytic rather than oxidative, the authors investigated the cleavage products, and found them to be predominantly due to hydrolytic cleavage. The dicerium complex of 75 (0.1 mM) was also found to cleave BNPP (1 mM) with a second order rate constant of 0.10 M⁻¹ s⁻¹ or 1.0 x 10⁻⁵ s⁻¹ at 37 °C.

1.2.8 Hydrolysis by Hetrodinuclear Complexes

Another class of complexes worth discussing are the hetrodinuclear complexes. Inspired by the work of Komiyama et al., who reported efficient cleavage of both BNPP and ApA, by the cooperation of free La(III) and Fe(III) ions, Manseki et al., detailed the hetrodinuclear complex 77, which cleaved NPP (0.5 mM) with a rate constant of 2 x 10⁻⁵ s⁻¹, representing a 2.8 fold enhancement over the mononuclear La(III) complex, which gave a rate constant of 7 x 10⁻⁶ s⁻¹. Significantly, neither the mononuclear nor the dinuclear Cu(II) complexes promoted the hydrolysis of NPP. All of these measurements were carried out in a 1:9 H₂O:DMF solution at pH 7.0 and 37 °C, and the rates were determined by the initial rates method.
Mixed transition metal systems have also been reported. In 1998 .Okawa et al., described the synthesis of the heterodinuclear Zn(II)Pb(II) complex of 78. This complex (1 mM) was found to cleave TNP (10 mM) in 100% DMSO solution. The rate constant was not reported, however, at 25 °C, the hydrolysis of TNP was found to be completed after 5 minutes.

Komiyama et al., reported the heterodinuclear Fe(III)Zn(II) complex of 79. The hydrolysis of ApA (5 mM) in 5% MeCN at pH 5.6, 50 °C, by various combinations of metals and ligands were reported and are shown in Table 1.7. A 1:1:1 mixture of Fe(III), Zn(II) and 79, was found to efficiently hydrolyse ApA, while in contrast, 2:1 mixtures of Fe(III) or Zn(II), with 79 showed only poor activity. The results were attributed to the bimetallic synergism between the Fe(III) and Zn(II). The mechanism is believed to involve base catalysis by a Zn(II)-bound hydroxide ion, which activates the 2'-OH of the ribose, and acid catalysis by the Fe(III), which can stabilize the intermediate and promote removal of the 5'-OH.

<table>
<thead>
<tr>
<th>[Fe(III)] M</th>
<th>[Zn(II)] M</th>
<th>[79] M</th>
<th>Rate Const (10^3 h⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.5</td>
<td>0.0</td>
<td>2.5</td>
<td>0.2</td>
</tr>
<tr>
<td>5.0</td>
<td>0.0</td>
<td>2.5</td>
<td>0.2</td>
</tr>
<tr>
<td>0.0</td>
<td>5.0</td>
<td>2.5</td>
<td>0.3</td>
</tr>
<tr>
<td>2.5</td>
<td>2.5</td>
<td>2.5</td>
<td>4.1</td>
</tr>
<tr>
<td>5.0</td>
<td>2.5</td>
<td>2.5</td>
<td>0.7</td>
</tr>
</tbody>
</table>

Table 1.7. Pseudo first order rate constants for the hydrolysis of ApA (5 mM) in 5% MeCN at pH 5.6, 50 °C
In 2003 Krämer et al. reported the hetro trinuclear complex $^{80}$. The Cu(II)/Co(III) complex ($7 \times 10^{-4}$ M) was reported to cleave HPNP ($5 \times 10^{-4}$ M) in buffered water/DMSO 3:1 at pH 7.0 and 20 °C with an initial rate of $ca. 6 \times 10^{-4}$ s$^{-1}$. In contrast a dinuclear Cu(II) complex of 4,6-di(2-pyridyl)pyridine was found to cleave HPNP under the same conditions with an initial rate of $8 \times 10^{-6}$ s$^{-1}$, a 62 fold decrease in activity.

1.2.9 Functional Organic Pendant Groups

Both general and specific acid/base catalysis is involved in the mechanism of phosphodiester hydrolysis.$^{65, 122, 129}$ It has been shown that introducing a pendant group, which can act as an acid or base, can promote the rate of phosphodiester hydrolysis.$^{75}$ Hamilton et al. have shown that the incorporation of pendant tertiary amine groups increases the activity of the complexes $^{81}$ and $^{82}$ by nearly 3 and 7 fold, respectively, over $^{36}$ (Table 1.8).$^{130}$ This increase in activity was ascribed to the tertiary amine providing general base catalysis. This demonstrates how functionalisation of metal complexes with organic, catalytic groups can lead to more efficient ribozyme mimics.$^{62}$

![Chemical structures](image)

<table>
<thead>
<tr>
<th>Complex</th>
<th>$k \times 10^5$ s$^{-1}$</th>
<th>$k_{rel}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>$^{36}$</td>
<td>0.58</td>
<td>174</td>
</tr>
<tr>
<td>$^{81}$</td>
<td>1.53</td>
<td>459</td>
</tr>
<tr>
<td>$^{82}$</td>
<td>3.89</td>
<td>1167</td>
</tr>
</tbody>
</table>

Table 1.8. Pseudo first order rate constants ($k$) and relative rate enhancements ($k_{rel}$) for the cleavage of HPNP (0.2 mM) in 50 mM HEPES (pH 7.0) at 25 °C with various Cu(II) complexes (2.0 mM).

Breslow et al. observed the same effect upon adding additional catalytic groups to the Zn(II) complex $^{37}$. The addition of thiophenol, $^{83}$, or imidazole, $^{84}$, groups to the
molecule led to a 9 and 20 fold rate enhancement, respectively, for the hydrolysis of HPNP, over the original system 37, under the same conditions, in 10 % DMSO aqueous solution with 10 mM HEPES, at pH 7.0 and 37 °C (Table 1.9).

<table>
<thead>
<tr>
<th>Complex</th>
<th>$k \times 10^2 \text{ h}^{-1}$</th>
<th>$k_{rel}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>37</td>
<td>0.074</td>
<td>1</td>
</tr>
<tr>
<td>83</td>
<td>0.690</td>
<td>9</td>
</tr>
<tr>
<td>84</td>
<td>1.50</td>
<td>20</td>
</tr>
</tbody>
</table>

Table 1.9. Pseudo first order rate constants ($k$) and relative rate enhancements ($k_{rel}$) for the cleavage of HPNP (0.19 mM) in 10 % DMSO in water with 10 mM HEPES (pH 7.0) at 37 °C with various Cu(II) complexes (0.5 mM).

1.2.10 Antisense Approach

In order to be of use as a potential ribozyme mimic, the metal complexes described above must be engineered to cleave RNA in a sequence specific manner. It has been proposed that this sequence specificity can be obtained by covalently connecting a catalytically active complex to a DNA oligonucleotide. The oligonucleotide would be specific for its complementary RNA sequence due to the Watson-Crick hydrogen bonding. This is known as the antisense approach and should serve to increase the overall efficiency of RNA cleavage by both increasing binding and specificity.

One of the first examples of this specificity was presented by Komiyama et al., who showed that a Lu(III) complex that had been covalently bound to a DNA oligomer could selectively hydrolyse RNA at the target site. The hydrolysis of the RNA (0.3 μM) was conducted at pH 8.0 and 37 °C, and under these conditions, a mixture of 1:1 DNA-IDA and LuCl₃ (10 μM), was found to cleave 17 % of the RNA outside the duplex, after 8 hours.
It is known that DNA-RNA hybrids are much more inert towards hydrolysis than single stranded RNA.\textsuperscript{135} Consequently, cleavage usually occurs outside the DNA-RNA duplex, as in the above case. However, the high affinity of complimentary strands hinders product release, thus impeding catalytic turnover.\textsuperscript{112} Positioning the cleaving moiety in the centre of the antisense oligomer leads to smaller product fragments, which are more easily released. Furthermore, the use of slightly non complementary antisense oligomers, leads to “bulges” of single stranded RNA within the DNA-RNA hybrid which have been shown by Komiyama and Kalesse, to be cleaved with far greater ease.\textsuperscript{136, 137}

Figure 1.10. Formation of single stranded RNA bulge

Hovinen \textit{et al.}, demonstrated that a 5 fold excess of 84, an oligonucleotide with a histamine conjugate tethered at 3'-terminus, promoted the hydrolysis of the complementary RNA 16-mer (0.5 $\mu$M) in the presence of Zn(II) (50 $\mu$M).\textsuperscript{138} The cleavage was predominantly observed at one site, and between 2 % and 5 % of the RNA was cleaved after 19 hours at room temperature in water buffered at pH 7.0.

An alternate strategy to cleaving the RNA strand itself, is to cleave or modify the 5'-cap structure of the mRNA. The 5'-cap structure is a N7 methylated guanosine residue that is
linked to the 5'-terminus of mRNA by a triphosphate linkage. It acts as a protein recognition element in several processes of mRNA metabolism. It is believed that its alteration will result in the inhibition of gene expression. Baker, Morrow and co-workers reported the 15-mer DNA tethered Eu(III) complex 86, where the cleaving agent was attached such that upon binding its complementary RNA fragment, it would be in proximity to the 5'-cap structure of the target mRNA. The antisense oligonucleotide 86, granted 50% inhibition of protein expression (IC$_{50}$), of its target RNA 43-mer, ICAM-1 (0.25 nM), at a concentration of 18 nM in water buffered at pH 7.4, in the presence of KCl (150 mM) and NaCl (10 mM). The antisense oligonucleotide without the Eu(III) complex had a much higher IC$_{50}$ of 64 nM. Investigation of the hydrolysis products confirmed that 86 had cleaved the 5' cap structure, however the reaction did not go to completion, due to dissociation of the Eu(III) ion from the tethered complex.

![Chemical structure of 86](image)

To date, the most active antisense oligonucleotide conjugate has been the dinuclear Cu(II) complex 87. It was found to cleave at only one nucleotide site, and offered a 130 fold rate enhancement over its mononuclear analogue. It cleaved RNA catalytically, with a rate constant of 1.8 x 10$^{-4}$ s$^{-1}$, corresponding to a 6.7 x 10$^{4}$ fold rate enhancement over the uncatalysed reaction. This result further highlights the importance of developing binuclear complexes for the hydrolysis of RNA.

![Chemical structure of 87](image)
1.2.11 Project Aim

Metal ions, in particular lanthanide ions, have been shown to be extremely successful at accelerating the hydrolysis of phosphodiester bonds including those of RNA. For pharmaceutical applications these ions must be complexed as they can be toxic and unselective. Two or more complexed metal ions have been shown to function in a cooperative manner providing rate accelerations many times above that of similar mononuclear systems. However, relatively few examples of cooperative rate enhancements by binuclear lanthanide complexes exist. With the ultimate aim of efficient and selective RNA hydrolysis for the reasons of disease control mentioned earlier, the aim of this project is to design and synthesise binuclear lanthanide complexes, which can cleave phosphodiesters by utilising the cooperative action between two metal ions. These complexes should show large rate enhancements over similar mononuclear systems and offer possibilities for further functionalisation.

As such the design shall be based on a bis-cyclen template 88, which allows for functionalisation with various R groups. It has been previously observed that modification of the cyclen pendant arms, can lead to large increases in phosphodiester hydrolysis activity. Therefore, a number of different pendant arms, including amide, glycine and pyridine pendants, shall be employed in order to allow comparison to similar mononuclear systems and investigate the effect of varying pendant groups for binuclear lanthanide complexes.

Particular attention shall be focused upon the choice of spacer group employed, as it will play a vital role in determining the intermetal distance, as well as the overall flexibility and geometry of the binuclear complexes, and thus it will have a large input into determining whether the two metal ions function in a cooperative manner or not. As already discussed, it appears that metal centres linked by very flexible scaffolds display a smaller cooperative activity compared to more rigid scaffolds. Nevertheless, molecules that are too rigid have also shown poor cooperative activity. Consequently, a range of rigid and flexible spacers, or bridging groups shall be investigated, and their phosphodiester cleaving ability will be compared. Comparisons shall also be drawn against similar mononuclear systems. This work shall be discussed in Chapter 4.
Chapter 1 - Introduction

\[ S = \text{Spacer} \]
Chapter 2

Lanthanide Luminescent Cu(II) Sensor
2.1 Introduction

Copper is a tough and ductile metal from group 11 of the periodic table. Loss of an electron leads to the diamagnetic Cu(I) ion, which has a $3d^{10}$ electronic configuration. Biologically however, the most important oxidised form of copper is Cu(II), which is paramagnetic due to its $3d^9$ electronic configuration, and characteristically prefers a distorted octahedral or square planar coordination environment. Copper plays a vital role in the human body. It is one of relatively few metals essential for human health and is a vital component of many enzymes including, ceruloplasmin, superoxide dimutase, cytochrome oxidase, tyrosinase, monoamine oxidase, lysyl oxidase and phenylalanine hydroxylase. Copper is found in all the organs and tissues of the human body, with the highest concentrations present in the liver, brain, heart and kidneys. The distribution of copper ranges from 1.4 – 2.1 mg/kg for the average human or 12 – 21 µM. In blood copper is bound in its Cu(II) oxidation state to albumin or transcuprin as well as other chelating peptides and amino acids. Copper is also the main oxidation catalyst for one electron reactions occurring outside the cell. While trace amounts are essential, excessive amounts are extremely toxic and can result in fatal liver damage. There are a number of genetic disorders that affect copper utilisation, the most serious of which are associated with Menkes syndrome and Wilson disease. Wilson disease involves a fault in copper transport throughout the body and results in accumulation of copper in the liver and brain of affected individuals. Menkes syndrome is a fatal copper transport disorder that kills infants before the age of three if not treated. A defect in the absorption of copper through the gut leads to a deficiency of copper in the body, and hence to both mental and physical deterioration.

The common methods for the quantification of copper are neutron activation analysis (NAA), atomic absorption spectroscopy (AAS) and inductively coupled plasma-mass spectrometry (ICP-MS), which are all based on expensive and sophisticated instruments. As such, a stable and selective Cu(II) sensor which functions under physiological conditions, without causing harm to the host is desirable. Chapter 1 highlighted various examples of luminescent sensors for the detection of cations such as Na(I), Ca(II) and Zn(II). The following section shall detail some examples of luminescent Cu(II) sensors.
2.1.1 Fluorescent Copper(II) Sensors

Recently, there have been a number of examples of fluorescent Cu(II) sensors detailed within the literature.\textsuperscript{150} One such example is the two component chemosensor developed by Fabbrizzi \textit{et al.}, which utilises a fluorescent anthracene chromophore as a signalling moiety and a tetrathia-macrocycle as the receptor.\textsuperscript{151} In anhydrous EtOH, after excitation at 368 nm, the compound 89 (1 \textmu M), exhibited a strong fluorescence band centred at 470 nm. Upon addition of Cu(II) however, the intensity of the emission gradually decreased and was fully quenched by one equivalent of Cu(II). The authors attributed this to the quenching of the charge transfer excited state of the anthracene by an electron transfer to the Cu(II) ion. The sensor was also reported to be selective for Cu(II) in the presence of the ions of Mn(II), Fe(II), Co(II) and Zn(II).

\begin{center}
\includegraphics[height=1.5in]{89}
\end{center}

\begin{center}
\includegraphics[height=1.5in]{90}
\end{center}

Zhou \textit{et al.} reported another example of a fluorescent Cu(II) sensor, the N-substituted 2,6-bis(benzimidazol-2-yl)pyridine 90.\textsuperscript{152} In DMF, after excitation at 320 nm, 90 (10 \textmu M), showed a broad fluorescence peak at 380 nm. This fluorescence was found to be quenched after the addition of one equivalent of Cu(II), while the presence of the divalent ions Co(II), Ni(II) and Zn(II) did not effect the fluorescence of 90.

The examples discussed above, operate in non aqueous media, however, as the main applications envisaged for successful copper sensors would be analysis of environmental or biological samples, the ability to operate in water at pH ~ 7 is vital.\textsuperscript{153} Gunnaugsson \textit{et al.} have developed a colorimetric Cu(II) sensor, which functions in water at pH 7.4.\textsuperscript{154} Under these conditions the azobenzene based chemosensor 91, has a deep red colour due to the internal charge transfer (ICT) from the electron donating amine to the electron withdrawing nitro group. Upon the binding of Cu(II), however, the amine can no longer participate in the ICT process and the colour of the solution is observed to change to
yellow. The process was found to be reversible as the addition of EDTA returned the solutions colour to a deep red. Group I and II metals such as Na⁺, Ca²⁺ and Mg²⁺ had no effect upon the sensor, while Cd(II) and Zn(II) were found to elicit the same response, albeit in a different concentration range. Interestingly, for compound 92, no response towards transition metals was observed, and this was attributed to the lack of the o-methoxy group.

There have also been examples of fluorescent Cu(II) sensors that operate under these aqueous conditions. Imperiali et al. reported the chemosensor 93, which utilises a Gly-Gly-Hys tripeptide as a binding receptor. In buffered water at pH 7.0 (150 mM NaCl), after excitation at 333 nm, the compound 93 (10 μM), was found to have a broad fluorescence band at ~ 550 nm. The addition of Mn(II), Co(II), Mg(II), Ca(II), Zn(II) or Cd(II) had no effect upon the fluorescence, nevertheless, the addition of one equivalent of Ni(II) was found to quench the fluorescence to 15 – 35 % of the original intensity, while one equivalent of Cu(II) quenched the fluorescence to 7 % of its initial value. The addition of EDTA reversed the quenching, reinstating the fluorescence.

Beltramello et al. reported another example, which was based on a triamino-trihydroxy-cyclohexane scaffold. The compound 94 (5 μM), in water at pH 7.0 displayed a broad fluorescence band at 425 nm after excitation at 330 nm. Addition of one equivalent of Cu(II) quenched the fluorescence to 15 % of its original value, while Ni(II), Co(II), Zn(II), Pb(II), Cd(II) and Mn(II) had no effect, although Fe(II) and Hg(II) did quench the fluorescence by ~ 20 %.

One other example is the anthracene based ligand 95, reported by Czarnik et al., which signals the presence of Cu(II) or Hg(II) in water. Under aqueous conditions at pH 7.0, the compound 95 (4 μM), after excitation at 368 nm, showed a broad fluorescent band which was unaffected by the presence of Ca(II), Cr(III), Ni(II), Co(II), Zn(II), Pb(II),
Cd(II), Mn(II) and Fe(III). Upon addition of Hg(II) or Cu(II), however, the fluorescence was found to be reduced by 18 fold and 4 fold respectively.

As mentioned in Chapter 1, the drawback to all of these fluorescent systems comes into play when working with biological samples, where the fluorescent signal has to compete against the autofluorescence of the biological sample itself. The aim of this project is to synthesise a lanthanide luminescent Cu(II) sensor, which can overcome the autofluorescence issue due to the long-lived nature of the lanthanide emission, as discussed in Chapter 1.

2.1.2 Requirements for Luminescent Sensor

If the sensor is to be used as a diagnostic tool for medicinal purposes there are a number of requirements that it must fulfil.

- The sensor should not be toxic as it may need to be administered directly to the patient, as such it should be kinetically and thermodynamically stable.
- It is essential that the sensor function under physiological conditions (i.e. aqueous environment, pH 7.4, high ionic strength).
- The sensor must also be selective for a specific target as it will be in the presence of numerous other ions.
- It should also be sensitive for a specific concentration range.
- Finally, the sensor must be able, after recognition of a specific target species, to somehow signal this presence.

As stated before the final signalling step is not a trivial problem when dealing with tissue samples due to the short lived background radiation (ns time scale) from the body tissue.
itself. Lanthanide luminescent sensors may be employed here in order to circumvent this problem.

As stated in Chapter 1, lanthanide luminescent systems have some significant advantages over analogous fluorescent systems.

- They are able to overcome the autofluorescence and light scattering of body tissue.\textsuperscript{158,186}
- Their long lived excited states (ms) allows the short-lived background fluorescence to disperse before the lanthanide emission occurs.
- The long wavelengths (500-750 nm for Eu(III)) at which lanthanides emit occur beyond the absorbance of body tissue, this is important for signal quality.
- Their line-like emission bands (10 nm) also give a better signal to noise ratio compared to fluorescent bands (several hundred nm).

With these requirements and conditions in mind we set about designing and synthesising a luminescent sensor for Cu(II).

2.2 Design of Lanthanide Luminescent Sensor

Free lanthanide ions are not very soluble in water and tend to form hydroxide gels at high concentration, which precipitate out of solution.\textsuperscript{159} They are also toxic due to their similarity in both charge and size to Mg(II) and Ca(II).\textsuperscript{160} In addition to this, protic solvents quench lanthanide luminescence by non-radiative vibrational decay through the O-H oscillators.\textsuperscript{161} In order to overcome these drawbacks the lanthanide ions must be complexed \textit{i.e.} incorporated into a ligand. This increases their stability,\textsuperscript{162} and provides shielding from water molecules hence increasing the luminescence. There are many such examples of this in the literature.\textsuperscript{163,180,181} The cyclen macrocycle, or 1,4,7,10-tetraazacyclododecane, is a good example of this, which when suitably functionalised virtually encapsulates the lanthanide ion forming complexes that are inert to dissociation in water at 37 °C, neutral pH.\textsuperscript{162,186,187} Europium (III) was selected as the lanthanide ion, because it emits in the red (700 nm) \textit{i.e.} beyond the absorbance of body tissue. Lanthanide ions have a high coordination number requirement, nine for Eu(III). This high coordination requirement will be fulfilled by the four donor nitrogen atoms from the cyclen macrocycle, and four donor oxygen atoms from the pendant amide arms. The remaining coordination site will be filled by a water molecule.
In order for a lanthanide to emit it must first be excited. As explained in Chapter 1, directly exciting a lanthanide ion is not feasible, as it requires a Laporte forbidden $f-f$ transition.\textsuperscript{164,176} This problem is solved by connecting a sensitising chromophore or “antenna” to the lanthanide ion, which can pass its excited energy onto the lanthanide ion by an energy transfer process, resulting in a lanthanide emission.

It is worth recalling the Jablonski diagram from Chapter 1, which illustrates this indirect excitation (Figure 2.1).\textsuperscript{165}

\begin{figure}[h]
\centering
\includegraphics[width=0.5\textwidth]{Jablonski_Diagram.png}
\caption{Jablonski Diagram illustrating sensitisation of a lanthanide ion. Other energy loss pathways have been omitted for clarity.\textsuperscript{166}}
\end{figure}

The sensitising antenna, denoted Ar, absorbs a photon, $h\nu$, and is excited to its singlet excited state, $^1$Ar. From here the energy can be passed into the antennas triplet excited state, $^3$Ar, via intersystem crossing (ISC). The lanthanide’s excited state, $^*\text{Ln}$, can now be populated from the antennas triplet state, by means of an intramolecular energy transfer (ET), provided that this triplet state is higher in energy than the lanthanide excited state. The excited energy now resides on the lanthanide ion, and can be lost by emission of light, resulting in the characteristic lanthanide emission spectra as each transition will correspond to an emission band.

As explained in Chapter 1, the pathway shown in Figure 2.1 is not the only path by which the excited energy can be lost. This pathway must compete with other mechanisms through which the energy can be discarded (Scheme 2.1).\textsuperscript{165} Energy can be lost through radiative
decay *i.e.* fluorescence and phosphorescence, or non-radiative decay by collisions and vibration interactions with the surrounding molecules. The lanthanide excited state \( {}^*\text{Ln} \) can also lose its energy through non-radiative decay by vibrational interactions with molecules such as water.\(^{167}\)

In order for our preferred pathway to operate *i.e.* population of the lanthanide excited state followed by a lanthanide emission, our design must minimise energy loss to other pathways. By maximising the efficiency of the intersystem crossing and energy transfer processes, it is possible to minimise losses to other deactivation pathways. As stated before, ISC is made possible by spin-orbit coupling, which is increased by the presence of a heavy atom, so the very presence of a lanthanide ion will increase the efficiency of this process.\(^{165}\)

Once in the lanthanides orbitals, the energy is somewhat isolated from its environment.\(^{11}\) Back energy transfer is possible but minimised by having the \( ^3\text{Ar} \) state sufficiently higher above that of the \( {}^*\text{Ln} \) state. Vibrational quenching by metal bound water molecules is now the main competing deactivation pathway. By minimising the number of these this non-radiative quenching can be minimised.

So to recap the design requires a lanthanide ion, Eu(III), tightly bound by a ligand to minimise metal bound water molecules and maximise stability and solubility. This will be accomplished using the cyclen macrocycle functionalised with amide arms. The design also requires a sensitising chromophore or antenna, which has a triplet state energy level higher than that of the Eu(III)'s \( ^5\text{D}_0 \) excited state. The distance between this antenna and the lanthanide ion must be minimised. Finally a suitable receptor to bind Cu(II) ions is a necessity.
Phenanthroline was chosen as the antenna (Figure 2.2). Phenanthroline has a triplet energy above that of Eu(III)'s $^5D_0$ excited state and is known to be able to populate this state and hence stimulate a lanthanide emission.\cite{168,169} The elegance of this system is that the antenna will also function as the receptor. Phenanthroline has two nitrogen atoms which can bind Cu(II).\cite{170} The binding of the Cu(II) will interfere with the energy transfer process by changing the redox potential of the antenna, lowering its triplet state below that of the lanthanides excited state, thereby quenching the luminescence. So this will be an "on-off" system.

![Figure 2.2. Copper Sensor showing various parts.](image)

### 2.3 Synthesis

The aim was to synthesise a lanthanide luminescent sensor for Cu(II), where europium would play the part of the signalling system. As mentioned before lanthanide ions tend to be toxic and have a high coordination number requirement and so to circumvent both of these problems a cyclen macrocycle functionalised with pendant amide arms was employed. The eight donor atoms would satisfy the high coordination requirement while also forming a stable complex.

#### 2.3.1 Synthesis of Tris Functionalised Cyclen

The cyclen macrocycle was functionalised with three pendant amide arms, according to the literature procedure\cite{171} by reacting three equivalents of the $\alpha$-chloroamide, 2-chloro-$N,N$-dimethyl-acetamide, 98a, with cyclen, 99, in the presence of NaHCO$_3$ in refluxing MeCN for three days (Scheme 2.2). After the inorganic salts were filtered off, the solvent was
removed and the residue purified by alumina column chromatography gradient elution 100 → 80:20 CH₂Cl₂:MeOH(NH₃). The desired product 2-(4,7-Bis-dimethylcarbamoylmethyl-1,4,7,10-tetraaza-cyclododec-1-yl)-N,N-dimethyl-acetamide, 100, was yielded as a white foam 8.63 g, in 35% yield.

\[ \text{Scheme 2.2. Synthesis of tris functionalised cyclen}^{171} \]

The spectral data for this product was in agreement with the published characterisation.\(^{171}\) The \(^1\)H NMR showed the presence of 1 NH proton at 9.99 ppm and also the peaks corresponding to the CH₂ protons from the pendant amide arms at 3.59 and 3.58 ppm (2 peaks due to C₂ symmetry).

The α-chloroamide arms were formed by adding a solution of chloro acetylchloride, 97, in CH₂Cl₂ to an aqueous solution of N, N dimethylamine, 96, and NaOH at -15 °C.\(^{172}\) The reaction was left to stir at room temperature overnight. The resulting solution was then filtered and the organic layer separated and washed repeatedly with 0.1M HCl. The organic layer was then dried over K₂CO₃ and the solvent was removed under reduced pressure to yield an oil in 46% yield. The spectral data for this product was in agreement with the published characterisation.\(^{172}\)

The pendant amide arms were chosen due to their stability and their ability to form strong complexes. Amides have the ability to form a resonance structure (Scheme 2.3). This resonance form hinders rotation about the N=C double bond, makes the amide molecule planar and very stable towards nucleophilic attack or hydrolysis.\(^{173}\) It also increases the donor ability of the oxygen towards Lewis acids.

\[ \text{Scheme 2.3. Resonance forms of α-chloroamide} \]
2.3.2 Synthesis of Eu.104

Phenanthroline was to play a dual role as both antenna and receptor. In order to link it to the cyclen macrocycle framework it was to be modified with a short α-chloroamide connector. This was synthesised from 5-nitro-[1,10]-phenanthroline, 101, which was reduced by refluxing with Pd/C 5 % and hydrazine hydrate in absolute ethanol to yield 5-amino-[1,10]-phenanthroline, 102. The resulting yellow solid was triturated with ether and dried under vacuum to give a yellow solid in 93% yield (Scheme 2.4). The spectral data for this product agreed well with the published characterisation. The 1H NMR showed the presence of a doublet at 9.18 ppm corresponding to the H$_2$ proton, while the H$_9$ proton was seen as a doublet at 8.95 ppm. The clearest indication of the formation of the product however, was the appearance in the spectrum of the two NH protons at 6.15 ppm.

Scheme 2.4. Synthesis of the phenanthroline antenna/receptor

The 5-amino-[1,10]-phenanthroline, 102, was next acylated by refluxing with chloroacetyl chloride, 97, in a solution of THF and triethylamine under argon for 18 hours. The product, 2-chloro-4,7-bis(dimethylcarbamoylmethyl)-1,4,7,10-tetraaza-cyclododec-1-yl]-A^,A^ dimethyl-acetamide, 103, a brown solid, was isolated in 59% yield. This was characterised by high resolution mass spectrometry and NMR. The 1H NMR spectrum of 103 showed the presence of a single amide NH proton at 8.91 ppm. The appearance of a new peak at 4.43 ppm corresponding to the two CH$_2$ protons was also observed. The product was also readily identifiable from the $^{13}$C NMR spectrum by the appearance of the carbonyl peak at 164.2 ppm as well as the CH$_2$ peak at 42.8 ppm.

This antenna was then connected to the macrocycle framework. 100 and 103 were reacted together in DMF in the presence of Cs$_2$CO$_3$ and refluxed for 12 hours under argon (Scheme 2.5). The resulting brown oil was purified by alumina column chromatography using gradient elution 100 → 90:10 CH$_2$Cl$_2$:MeOH, to give the desired product 2-[4,7-Bis(dimethylcarbamoylmethyl)-10-(5-yIcarbamoylmethyl)-1,4,7,10-tetraaza-cyclododec-1-yI]-N,N-dimethyl-acetamide, 104, as a pale solid in 38% yield. This
was characterised by conventional methods, $^1$H, $^{13}$C NMR, ESMS, IR and high resolution mass spectroscopy.

\[ 
\text{Scheme 2.5. Ligand Formation} 
\]

The $^1$H NMR spectrum of 104 (Figure 2.3) showed the presence of 1 NH proton at 11.35 ppm as well as the seven phenanthroline resonances. The cyclen and CH$_2$ resonances were observed as a broad signal at $\sim 2.57$ ppm due to the fast relaxing nature of the molecule. The $^{13}$C NMR spectrum showed three quaternary carbonyl peaks (due to the C$_2$ symmetry plane through the cyclen) and four quaternary aromatic peaks. The seven aromatic peaks of the phenanthroline are also visible as well as seven CH$_2$ and two CH$_3$ peaks.

\[ 
\text{Figure 2.3. 400 MHz $^1$H NMR of 104 in CDCl}_3 
\]
Finally the lanthanide complex $\text{Eu.104}$ was formed by refluxing the ligand with 1.1 equivalents of $\text{Eu(CF}_3\text{SO}_3)_3$ (Europium triflate) under argon in MeCN for 18 hours. The complex was precipitated from ether and then from $\text{CH}_2\text{Cl}_2$ before being collected by filtration and dried under vacuum to give a brown solid in 90 % yield. The characterisation of this sample will be discussed in the next section.

![Image of Eu.104 complex]

2.4 Characterisation of Eu.104

The complex was characterised by $^1\text{H}$ NMR, ESMS, IR, and high resolution mass spec. Lanthanides are shift reagents. Protons in close proximity to the paramagnetic lanthanide ion will experience an extra magnetic field and will be shifted in the NMR spectrum accordingly. In this case the axial and equatorial protons of the cyclen ring as well as the acetamide CH$_2$ protons will be shifted by the Eu(III) ion. Lanthanide complexes also give rise to fast relaxation times and broadens the peaks of the spectrum. This shall be discussed in greater depth in Chapter 4.

Normally an NMR spectrum is run from 0 to 20 ppm however, as europium is a NMR shift reagent, a much larger spectral width is employed from 50 to -50 ppm (Figure 2.4). Notice the peaks at 28, -3, -8, -12, and -15. These are europium shifted axial and equatorial CH$_2$. This is indicative of the geometry in solution; which is mono capped square antiprismatic. The broad spectrum is what we would expect for a europium complex due to its fast relaxation properties along with the presence of other geometries in solution due to stereochemical nonrigidity.
Figure 2.4. $^1$H NMR of Eu.104 showing the effect of the paramagnetic europium metal ion

The mass spectrum for the complex showed two peaks. One at 406.9 corresponding to the M+2H/2 species. The other at 481.8 corresponds to the M+Triflate/2 species. The distinctive isotope distribution pattern (Figure 2.5), is due to the presence of the two europium isotopes. High resolution mass spectrometry confirmed the characterisation of the molecule.

Figure 2.5. Mass spectrum of Eu.104 showing europium isotope pattern
As mentioned before lanthanides have a very high coordination number.\(^{176}\) When a ligand cannot totally accomplish this high coordination number, solvents, such as water molecules, will often fill the extra coordination positions. In this case one metal bound water molecule was expected to be present as europium has a coordination number of nine.\(^{178}\) In order to confirm this, the luminescent lifetimes (\(\tau\)) of the lanthanide-excited states were measured in D\(_2\)O and H\(_2\)O, by direct excitation of the Eu(III) ion at 395 nm. From these measurements the number of metal bound water molecules, or \(q\) value, was evaluated using the following equation.\(^{179, 180, 181}\)

\[
q_{Eu(III)}^{(i)} = 1.2 \left[ \frac{1}{\tau_{H_2O}} - \frac{1}{\tau_{D_2O}} \right] - 0.25 - 0.075x
\]

Here the prefix 1.2 is a proportionality constant that mirrors the sensitivity of the europium ion to quenching by metal bound water molecules. The correction term – 0.25 represents quenching by second sphere water molecules, while – 0.075x represents the quenching by N-H oscillators, where \(x\) is the number of such oscillators directly bound to the complex. From this the \(q\) value was indeed determined to be 1 (Table 2.1).

<table>
<thead>
<tr>
<th>Complex</th>
<th>(\tau_{H_2O}) (ms)</th>
<th>(k_{H_2O}) (1/ms)</th>
<th>(\tau_{D_2O}) (ms)</th>
<th>(k_{D_2O}) (1/ms)</th>
<th>(q) (±0.5)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Eu.104</td>
<td>0.328</td>
<td>3.050</td>
<td>0.528</td>
<td>1.892</td>
<td>1.00</td>
</tr>
</tbody>
</table>

Table 2.1. Determination of \(q\) value shows there is one metal bound water molecule

The following section will detail studies performed to evaluate the molecules ability to operate as a sensor for Cu(II). Of particular interest, will be the complexes lanthanide luminescence behaviour, both as a function of pH and of metal ion concentration.

### 2.5 Photophysical Properties

The design of the sensor envisaged indirect excitation of the europium ion by a covalently attached antenna, which could absorb a photon of light and then pass its excited energy to the lanthanide excited state \(via\) its own triplet state. To test whether the molecule functioned as it was designed, a series of measurement were carried out in water at pH 7.4 (buffered with 0.1 M HEPES) under ambient conditions. These conditions were selected due to their similarity to the physiological environment that the sensor should function in.
The 1, 10-phenanthroline molecule is known to have an absorption peak at \( \sim 265 \text{ nm} \). The absorption spectrum of Eu$_{104}$ showed two peak maxima at 266 nm (log \( \varepsilon = 3.66 \)) and 230 nm (log \( \varepsilon = 3.90 \)). After excitation at 266 nm the complex had a broad fluorescent band at 425 nm. This fluorescence band is due to the only fluorophore in the molecule, the phen antenna. This is important as it shows that the antenna’s singlet state \( S_1 \) is being populated. The question now was whether this energy would be transferred to the lanthanide excited state and result in a lanthanide emission (Scheme 2.6).

![Scheme 2.6](image.png)

Scheme 2.6. The antenna absorbs a photon of light. It then passes its excited energy to the lanthanide via a energy transfer mechanism. The europium ion losses this energy with a characteristic emission spectrum

### 2.5.1 Lanthanide Luminescence of Eu$_{104}$

After excitation of the antenna at 266 nm, the lanthanide luminescent spectrum of Eu$_{104}$, showed the characteristic Eu(III) emission (Figure 2.6). This shows that the antenna is indeed able to sensitise the lanthanide ion and “switch on” the luminescence. This was an important aspect of the design as the luminescence needed to be “switched on” in order to be able to signal the presence of Cu(II) ions by a quenching of the luminescence.

There are a number of things to note here. Firstly, the lifetime of this emission is in the ms time scale, important as this negates the autofluorescence problem. Secondly it emits in the red 700 nm, which is beyond the absorbance of body tissue. Thirdly the line like emission bands (ca. 10 nm) which improve the signal to noise ratio.
So far the molecule is functioning as desired. The phen moiety is acting as an antenna, absorbing energy and entering its singlet excited state. Some of this energy is being lost due to fluorescence, however, the luminescence spectra shows that the antenna is passing its energy, through its triplet excited state to the Eu(III) ion, where it is lost in the form of a photon or light emission.

It was next necessary, to determine the pH behaviour of the complex. It was foreseen that the nitrogen atoms of the phen moiety could be protonated, and that the amide nitrogen could possibly be deprotonated. A pH titration was carried out in order to ascertain whether these protonations would affect the spectral properties of the complex. The pH titrations were carried out in water with TMACl or TMAPCl (0.1 M) in order to maintain high ionic strength.

2.5.2 pH Dependence of Absorption Spectrum

At neutral pH the complex had two absorption bands with $\lambda_{\text{max}} = 266$ nm and 230 nm. As the pH was increased the absorption spectrum showed a bathochromic shift in the absorbance band at 266 nm, which was shifted to 278 nm. This bathochromic shift was again observed upon moving to alkaline pH's, along with a concomitant hypochromic effect (Figure 2.7). The shifts in absorbance correlate well with the pK$_a$'s for the protonation of the phen nitrogen at low pH, and the deprotonation of the amide nitrogen at high pH, which will be shown later.
Chapter 2 – Lanthanide Luminescent Copper(II) Sensor

Figure 2.7. Absorption spectrum of Eu.104 showing effect of varying pH on absorbance. Note there was no clear isosbestic point.

By plotting the change in pH against absorbance it is clear that the actual absorbance does not change significantly (Figure 2.8). As such the complex should function under physiological conditions or in the pH 4 – 9 region, and any major changes in luminescence cannot be ascribed to changes in the absorption spectrum.

Figure 2.8. Plot of pH Vs Absorbance of Eu.104 illustrates that only small changes were observed upon protonation and deprotonation.
2.5.3 pH Dependence of Fluorescence Spectrum

The changes in the fluorescence spectrum with respect to pH was also investigated in an identical manner. When the complex was irradiated at 266 nm (corresponding to the maximum absorbance of the phen antenna), a broad fluorescence emission band, centred at 425 nm, was observed. There was a small change in the fluorescence intensity over the pH scale $\sim 38\%$ (Figure 2.9). However, over the physiologically important pH 4 – 8 range, the change was only 15 %. The fluorescence shows that the singlet excited state of the antenna is still being populated at both acidic and alkaline pH’s.

![Figure 2.9](image)

**Figure 2.9.** Plot showing the change in fluorescence emission of Eu.104 at 423 nm as a function of pH. Excite at 266 nm. Inset shows fluorescence spectrum

The changes in fluorescence intensity mirror those of the absorption spectra at 266 nm. This may indicate that the lower intensity in emission, at a particular pH, is due to the lower intensity of absorbance at that pH. It is clear that the protonation and deprotonation events are affecting the spectral properties, however, the overall change in absorbance and emission are relatively small. The antenna is still absorbing energy and populating its singlet excited state. Whether this energy would continue to be transferred to the Eu(III) ion, *via* the triplet excited state, shall be investigated in the next section.
2.5.4 pH Dependence of Eu(III) Luminescence

The pH dependence of the Eu(III) luminescence was also investigated in an identical manner. It was found that the changes in the Eu(III) emission, with respect to pH, were much greater than observed for the fluorescence or absorbance spectra. Furthermore, unlike the examples discussed previously in Chapter 1, such as 8, reported by Gunnlaugsson et al. the lanthanide emission of Eu.104 was not a simple "off-on" pH switch. In order to clearly ascertain the effect of pH upon the luminescence the results shall be discussed in two parts, the effect of base, and the effect of acid. Figure 2.10 shows the effect on the luminescence of Eu.104 over the pH range 5.5 to 12.5. As the pH was increased a gradual decrease in the luminescence intensity was observed. This was attributed to the deprotonation of the amide moiety interfering with the energy transfer process (Scheme 2.7). Recall that the changes in absorbance and fluorescence over this pH range were relatively minor and that the singlet excited state of the antenna is still being populated. As such, there are two possible explanations for the deprotonation event quenching the Eu(III) emission. Firstly, when the amide is deprotonated this allows an electron transfer form the amide to the Eu(III) ion. The resulting Eu(II) ion is not emissive in the same area and hence the luminescence is quenched. Alternatively, the deprotonation of the amide moiety, changes the oxidation potential of the antennas excited state, moving the triplet state below the lanthanides excited state and hence prohibiting the population of the lanthanides excited state.

![Figure 2.10. Base titration of Eu.104 showing gradual quenching of the luminescence with increasing pH. Excitation at 266nm](image_url)
Scheme 2.7. Deprotanation of the amide interferes with the energy transfer process and hence quenches the luminescence of Eu.104

Figure 2.11 shows the effect on the luminescence of Eu.104 over the pH range 5.5 to 1.3. Upon moving from neutral to more acidic pHs, the luminescence was found to be gradually quenched. Once again this is a drastic change compared to the changes in absorption and fluorescence discussed earlier, which showed that the singlet excited state is still being populated under these conditions of pH.

Figure 2.11. Acid titration of Eu.104 showing gradual quenching of the luminescence with decreasing pH. Excitation at 266nm

The quenching of the Eu(III) luminescence here can be attributed to the protonation of the phen nitrogen interfering with the energy transfer process to the lanthanide ion (Scheme 2.8). The reason for this is that as the phen is protonated the oxidation potential of the phen is changed. This lowers the energy level of the triplet excited state below that of the lanthanide excited state and hence no energy transfer can occur (Scheme 2.9).
Scheme 2.8. Protonation of the phen interferes with the energy transfer process and hence quenches the luminescence of Eu.

Scheme 2.9. Protonation of the phen nitrogen lowers the triplet excited energy level, \( T_1 \), below that of the lanthanide excited state which, therefore, cannot be populated.

Combining the two titrations and plotting the luminescence intensity at 592 nm (\( J=1 \) transition), as a function of pH gives a bell shaped pH dependent graph reminiscent of many pH driven enzymes, such as those involved in phosphodiester hydrolysis, discussed in Chapter 1 (Figure 2.12).\(^{184}\) The luminescence is quenched under alkaline and acidic conditions. Crucially the luminescence is switched “on” within the physiological pH range 4 – 8. As such, this pH behaviour gives rise to an “off-on-off” system, which is switched “on” under physiological conditions, but switched “off” under acidic and alkaline conditions. This switching process was found to be fully reversible, and the same solution could be used to go from acid to base and back again. Furthermore the luminescence was found to be almost independent of pH between 5 and 7. This is important as small fluctuations within this pH window will not give rise to changes in the luminescence spectra and hence any changes that do occur, must be attributed to some other factor.
Figure 2.12. pH profile of Eu.104 showing fully reversible off-on-off pH dependence

It should be noted that the pH profile consists of two sigmoidal curves. From these it is possible to deduce two pK_a values; 4.1 (± 0.2) for the protonation of the phen nitrogen and 8.2 (± 0.2) for the deprotonation of the amide.

These pK_a values were confirmed by potentiometric titration of Eu.104 (21.48 μM) with NaOH (93.92 mM) (standardised against KHphthalate) (Figure 2.13). The measurements were carried out in triply distilled water purged with argon. The starting pH was adjusted to the desired value (e.g. pH 3.86) using conc. HCl.

Figure 2.13. Potentiometric plot for Eu.104 shows pK_a values of 4.3 (± 0.2) and 7.8 (± 0.2). The plot of dy/dx (insert) shows the equivalence points at the maxima

For potentiometric plots the pK_a's are found by plotting volume of base added against dy/dx, where y = pH, x = volume of base added. The equivalence points will be shown as
maxima here. The first pKₐ will be the pH at half the x value at the first equivalent point. Subsequent pKₐ's will be the pH at half the x value between equivalence points.¹⁸⁵ The complex was extensively studied to determine its stability in solution. It was found that when stored in buffered aqueous solution at pH 7.4, no discernable decrease in the luminescent intensity was observed over a six month period.

2.5.5 Determination of Metal Bound Water Molecules

Metal bound water molecules decrease the luminescence from lanthanide ions as they offer alternative pathways to losing excited energy through O-H vibrations instead of radiative decay. As a result increasing the number of metal bound water molecules on a lanthanide ion will lead to a decrease in luminescence, while decreasing the number of metal bound water molecules will lead to an increase in luminescence.¹⁶³,¹⁸⁶,¹⁸⁷ In order to rule out any possibility that the pH dependence displayed by Eu.₁₀⁴ was due to a change in the number of metal bound water molecules, it was decided to evaluate the q values of Eu.₁₀⁴ at varying pH. This was done by measuring the excited state lifetime τ, in D₂O and H₂O, at different pH's. The pH, or pD, was adjusted using HCl and NaOH in the case of H₂O, and DCl and NaOD in the case of D₂O. The pD was obtained by measuring the pH and applying the formula; pD = pH measured + 0.4.¹⁸⁸ In all cases the q value was found to be 1 (Table 2.2).

<table>
<thead>
<tr>
<th>pH</th>
<th>1/τ_H₂O (ms⁻¹)</th>
<th>1/τ_D₂O (ms⁻¹)</th>
<th>q (±0.5)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.4</td>
<td>2.320</td>
<td>1.270</td>
<td>0.87</td>
</tr>
<tr>
<td>3.0</td>
<td>2.443</td>
<td>1.133</td>
<td>1.18</td>
</tr>
<tr>
<td>4.0</td>
<td>2.643</td>
<td>1.554</td>
<td>0.92</td>
</tr>
<tr>
<td>5.3</td>
<td>2.559</td>
<td>1.160</td>
<td>1.29</td>
</tr>
<tr>
<td>7.1</td>
<td>3.050</td>
<td>1.892</td>
<td>1.00</td>
</tr>
<tr>
<td>7.9</td>
<td>3.359</td>
<td>1.930</td>
<td>1.32</td>
</tr>
<tr>
<td>10.1</td>
<td>3.709</td>
<td>2.695</td>
<td>0.83</td>
</tr>
</tbody>
</table>

Table 2.2. Determination of q values showed there to be one metal bound water molecule at all pHs.

In conclusion the Eu(III) luminescence is modulated by the pH to a much greater extent than either the fluorescence or absorbance spectra. The number of metal bound water
molecules remains constant over the pH range and as such, cannot be held responsible for any change in luminescence. The changes in luminescence correspond well with the pKᵦ values determined for the protonation and deprotonation of the phen and amide moieties respectively, indicating that these processes are responsible for a disruption of the energy transfer mechanism needed to produce a luminescent signal.

The luminescence is switched “on” in the physiological pH range and this is essential to the design of the sensor which envisages a quenching of the luminescence by the presence of Cu(II) ions under physiological conditions. Before testing whether the complex could detect and signal the presence of Cu(II), it was decided to investigate the properties and functions of a phen type model receptor in order to allow comparative analysis. The following sections will detail the properties and response to Cu(II) of this model receptor.

2.6 Comparison to Model Receptor

A model receptor, consisting of an acylated 1,10-phenanthroline unit was synthesised according to literature procedure. This model would have similar properties to the recognition, or “receptor” part, of the Eu.104 complex and as such, would allow comparative analysis to be drawn.

The receptor N-[1,10]phenanthroline-5-yl-acetamide, 106, was synthesised by refluxing a solution of 5-amino-[1,10]-phenanthroline, 102, in MeOH overnight, with acetic anhydride in the presence of NaHCO₃ (Scheme 2.10). The desired product was isolated as an off white powder in 64 % yield. The spectral data for the product agreed well with the published characterisation. The ¹H NMR showed the presence of a doublet at 8.63 ppm, corresponding to the H₄ proton, while the H₇ proton was seen as a doublet at 8.44 ppm. The clearest indication of the formation of the product however, was the appearance in the spectrum of the three CH₃ protons at 2.24 ppm.

![Scheme 2.10. Synthesis of model receptor 106](image-url)
The absorption spectrum of 106 showed two peaks with $\lambda_{\text{max}}$ at 231 nm ($\log \varepsilon = 4.51$) and 267 nm ($\log \varepsilon = 4.44$). Upon excitation at 267 nm the compound 106, gave a strong fluorescent band centred at 427 nm, similar to that seen for Eu.104. As in the case of Eu.104 the pH behaviour of the compound was investigated. The absorbance peak at 231 nm seemed to be more sensitive to the protonation of the amide than the absorbance at 267 nm, indicating that the peak at 231 nm is due to the $n \rightarrow \pi^*$ transition for C=O and the peak at 267 nm is due to the $\pi \rightarrow \pi^*$ transition for the phen. Recall the 1, 10 phenanthroline unit, is known to have an absorption peak at $\sim 265$ nm. From the changes in the absorption spectrum at 267 nm and 231 nm respectively, it was possible to assign two pK$_a$ values; 4.6 (± 0.2) for the protonation of the phen and 9.3 (± 0.2) for the protonation of the amide (the absorption changes for the band at 266 nm are shown in Figure 2.14, while the absorption changes for the band at 231 nm are shown in the appendix). The known literature pK$_a$ value for 1,10 phenanthroline is 4.6. The pK$_a$ value for the protonation of the phen nitrogen also compares well with the value calculated for Eu.104. However, the pK$_a$ value of the amide is vastly different. This is to be expected due to the vastly different environment of the respective amides. The amide of Eu.104 is coordinated to the positively charged Eu(III) cation. As such it is donating electron density towards the metal ion. This increases the $\delta^+$ nature of the amide nitrogen, which results in lowered affinity for the bound proton and hence, a lowering of the pK$_a$.

![Graph](image)

**Figure 2.14. Effect of pH on absorption of 106 at 267 nm, showing hypochromic shift upon protonation of phen nitrogen pK$_a$ 4.6.**

Protonation of phen moiety results in a bathochromic shift in the 267 nm band between pH 5.6 $\rightarrow$ 278 nm at pH 3.6. However, the same protonation of the phen moiety results in a
hypsochromic shift in the band centred at 231 nm $\rightarrow$ 222 nm. In contrast to Eu.104, protonation of the amide nitrogen does not shift the absorbance peaks of 106.

Figure 2.15. Changes in fluorescence of 106 with respect to pH

Analysing the changes in fluorescence intensity, as a function of pH revealed that the fluorescence was at its most intense when the phen moiety was protonated and protonation of the amide did not seem to affect the fluorescence (Figure 2.15).

In order to evaluate the model systems response to the presence of Cu(II) ions, measurements were carried out in water at pH 7.4 (buffered with 0.1 M HEPES) in order to resemble physiological conditions. CuCl$_2$ was gradually titrated into a 40.5 $\mu$M solution of 106. A clear change was recorded in the absorption spectrum, indicating that Cu(II) was indeed binding to the phenanthroline receptor (Figure 2.16).

Figure 2.16. Absorption spectrum of 106 showing changes upon addition of Cu(II)
Upon addition of Cu(II) to 106, the absorption band at 231 nm underwent a hypochromic shift. This hypochromic effect was also observed in the 267 nm band along with a concomitant bathochromic shift to 280 nm. Upon addition of excess Cu(II) a hyperchromic effect was observed in the 280 nm band, this was attributed to the absorbance by Cu(II) at 280 nm. A plot of $p_{[Cu(II)]}$ against absorbance, where $p$ is the negative Log of concentration, revealed that the hypochromic effect occurred over one log unit $p \ 5.5 \rightarrow 4.4$ (Figure 2.17).

![Figure 2.17. Changes in absorption of 106 as a function of $-\log[Cu(II)]$.](image)

**2.6.1 Fluorescence Response of 106 to Cu(II)**

The effect of increasing Cu(II) concentration upon fluorescence was also monitored. As the concentration of Cu(II) was increased a concomitant decrease in the fluorescence of 106 was observed. The fluorescence was quenched after 0.6 equivalents of Cu(II) had been added (Figure 2.18), which suggested that a 2:1 binding mode was in operation.

![Figure 2.18. Equivalents plot against fluorescence of 106 shows quenching after 0.6 equivalents of Cu(II). Inset shows changes in fluorescence. Excite at 266 nm](image)
A plot of $p[\text{Cu(II)}]$ against fluorescence revealed that the binding occurred over 1 log unit (Figure 2.19).

![Figure 2.19. $p[\text{Cu(II)}]$ Vs Fluorescence of 106 shows quenching over 1 log unit](image)

In order to confirm the binding mode mass spectrometry was employed. Three equivalents of 106 were dissolved in MeOH and one equivalent of $\text{Cu(ClO}_4)_2$ was added. The solution was left to stand and green crystals were obtained. The mass spectrum of the solution revealed peaks with the correct isotope distribution pattern corresponding to 268.4 [2M+Cu/2], 387.1 [3M+Cu/2], 537.0 [2M+Cu], 637.9 [2M+Cu+ClO$_4$]. It is clear that at least two binding methods are possible.

The studies on the model receptor revealed that it does indeed bind Cu(II) and that this binding event modulates the absorption spectrum as well as the fluorescence spectrum, with quenching of the fluorescence observed after 0.6 equivalents of Cu(II) had been added. The binding of Cu(II) clearly quenches the singlet excited state of the phenanthroline receptor. The spectroscopic studies suggest a to a 2:1 binding mode, however, physical studies employing mass spectrometry, revealed that a 3:1 binding mode is also possible. The $pK_a$ value for the protonation of the phen nitrogen was found to be similar to the value obtained for the Eu.104 complex. This might suggest that this “receptor” part of both molecules may operate in a similar fashion. This data will allow comparisons to be drawn when investigating the Eu.104 complexes response to the presence of Cu(II), which is the subject of the next section.
2.7 Luminescent Studies of the Effect of Copper (II) upon Eu.104

The design of the Eu.104 system factored that the phenanthroHne moiety would function like a sensitising antenna, absorbing light and passing on its excited energy onto the lanthanide ion, resulting in a lanthanide emission. As shown the antenna effect was achieved and the system functions like an "off-on-off" pH switch with its luminescence "switched on" over the physiological pH range. So far the system functions as designed and operates under the conditions desired. However, the design envisaged a dual role for the phen moiety with it also operating as the receptor. The phen ligand itself has been shown to bind Cu(II) before. The question now was whether the phen moiety within the complex would function as a receptor, bind to Cu(II) and whether this event would cause a change in the luminescent properties of the complex.

2.7.1 Absorption Spectrum

To investigate the systems response to the presence of Cu(II) ions, measurements were carried out in water at pH 7.4 (buffered with 0.1 M HEPES). CuCl₂ was gradually titrated into a 35.8 \( \mu \text{M} \) solution of Eu.104. The absorption spectrum showed that as the concentration of Cu(II) was increased a gradual hypochromic effect in the absorbance at 230 nm and 266 nm, as well as a slight bathochromic shift in the 266 nm band ensued (Figure 2.20). Note the isosbestic point at 280 nm.

![Absorption Spectrum](image)

Figure 2.20. Absorption spectrum of Eu.104 showing decrease in absorbance along with bathochromic shift upon gradual addition of Cu(II)
The shift to longer wavelengths in the absorption spectrum, upon addition of Cu(II), implies that a change in the oxidation potential of the phen is occurring.

2.7.2 Fluorescence Spectrum

After excitation at 266 nm, or at the isosbestic point at 280 nm, a fluorescent band was observed at 440 nm. The fluorescence spectrum showed a gradual decrease in the fluorescence of the compound as the concentration of Cu(II) was increased. By plotting $p[\text{Cu(II)}]$ against the relative intensity it is possible to compare the responses to Cu(II) of both the complex Eu.104 and the model receptor 106 (Figure 2.21). The results were in good agreement. It is immediately clear that the two systems operate in similar fashions quenching over 1 log unit in the same concentration range, $p \ 5.5 \rightarrow 4.5$.

![Figure 2.21. Quenching of fluorescence occurs over 1 log unit. Inset shows Eu.104 fluorescent spectra quenching upon addition of Cu(II). Excite at 266 nm](image)

The fluorescence spectrum gives an insight into what is happening in the singlet excited state of the antenna. If the singlet state is being quenched, then it will not be able to transfer its excited energy to the triplet excited state and hence should be unable to populate the lanthanide excited state and therefore a quenching of the Eu(III) luminescence is also expected to occur (Scheme 2.11). Note that binding of Cu(II) is also expected to lower the energy levels of the phen hence impeding energy transfer to the *Ln
2.7.3 Lanthanide Luminescence

As stated above, the binding of Cu(II) quenches the singlet excited state and is also expected to interfere with the energy transfer process and hence quench the Eu(III) luminescence (Scheme 2.12). This was indeed found to be the case. The Eu(III) luminescence spectrum showed a gradual decrease in the intensity as the concentration of Cu(II) was increased, until the luminescence was completely quenched at ~ 20 \( \mu \text{M} \) [Cu(II)] (Figure 2.22).
Once again any of the bands can be used to analyse the data as each gives the same result. A plot of the luminescent intensity at 620 nm ($J = 2$ band) clearly shows that the quenching of the luminescence occurs over one log unit (Figure 2.23). The response range here is significant, $p 5.8 \rightarrow 4.7$, which corresponds to a $2.5 \sim 20 \mu M$ Cu(II) concentration range. The normal range of Cu(II) in serum is $12.5 - 21 \mu M$ Cu(II).  

Figure 2.23. Quenching of Eu(III) luminescence of Eu.104 by Cu(II) occurs over one log unit
Cu(II) d⁰ is well known for its distorted square planer geometry due to the Jahn Teller effect of its single unpaired electron. However, for strong field ligands such as phen, Cu(II) often prefers a distorted octahedral geometry. As such a 2:1 or 3:1 binding mode was expected. The equivalents plot indicated that the binding mode operating in this case was 2:1, as the quenching of the luminescence occurs just after 0.5 equivalents of Cu(II) has been added (Figure 2.24).

![Plot of intensity versus equivalents of Cu(II)](image)

**Figure 2.24. Equivalent plot for Cu(II) showing quenching of luminescence of Eu.104 after 0.6 equivalents of Cu(II)**

An investigation of the mass spectrum, similar to that carried out for 106, did not reveal any useful information about the binding due to the extreme complexity of the spectrum. In order to further investigate the binding mode a Job plot analysis was carried out. To do this measurements were conducted as the mole fraction ([G]/[G]+[H]) was varied, while the overall concentration of [G]+[H] was kept constant at 5.6 µM. Where G denotes the guest, Cu(II), and H denotes the host, **Eu.104**. Under these conditions the absorption band at 267 nm exhibited its maximum, at a mole fraction of 0.33 (Figure 2.25). Furthermore, a minimum was observed in the Eu(III) luminescence intensity, again at a mole fraction of 0.33. This is shown in the appendix. This figure implies that the binding mode operating in this case, is indeed 2:1 as 0.33/(1-0.33) = 2.0, i.e. two **Eu.104** complexes binding to one Cu(II) ion.
Chapter 2 – Lanthanide Luminescent Copper(II) Sensor

Figure 2.25. Absorption Job plot where [G] is the concentration of guest i.e. Cu(II) and [H] the concentration of host

As stated before 0.5 equivalents of Cu(II) quenches the Eu(III) luminescence of Eu.104. However, upon addition of EDTA the luminescence was “switched” back “on”. This switching effect can be seen in the appendix and shows that the binding of Cu(II) by the phenanthroline receptor is reversible. This is an important property of any sensor.

In order to evaluate the systems ability to function at high ionic strength measurements were conducted in water at pH 7.4 (buffered with 0.1 M HEPES) in the presence of 0.1M TMACl. As previously, CuCl$_2$ was gradually titrated into a 35.8 µM solution of Eu.104.

Figure 2.26 Luminescence titration of Eu.104 with Cu(II) in pH 7.4 buffered solution at high ionic strength (0.1M TMACl). Excite 266 nm, 35.8 µM
Once again the system responded with a gradual decrease in the luminescence finally quenching after 0.5 equivalents of Cu(II) had been added (Figure 2.26). However, there were some interesting points to note here. For instance throughout the titration in high ionic strength the $J = 1$ and $J = 2$ bands are closer in intensity ($J_2/J_1 = 1.4$), while for the original titration the $J = 2$ band was significantly larger ($J_2/J_1 = 1.7$). It is thought that these changes are due, under the conditions of high Cl$^-$ ion concentration, to the displacement of the metal bound water molecule by a Cl$^-$ ion, as the $J = 2$ band is known to be sensitive to its coordination environment.$^{192}$

The systems response to the counter anion was also investigated. When Cu(OOCH$_3$)$_2$ was used instead of CuCl$_2$ the Eu.104 complex, responded in an identical manner to that described above, quenching after 0.5 equivalents of Cu(II) or ~ 20µM.

It can be seen that under the physiological conditions applied (i.e. aqueous solution pH 7.4, high salt concentration) the conjugate Eu.104, can detect and signal the presence of Cu(II) ions. The recognition occurs through reversible binding to the phen moiety, while signalling is accomplished by quenching of the long lived Eu(III) emission. The sensor is stable and sensitive to an important concentration range (2.5 – 20 µM). However, the systems selectivity towards Cu(II) over other ions must now be investigated.

2.8 Selectivity studies for other metal ions

Following the success in the detection of Cu(II), the systems response to various other biologically prevalent metal ions was investigated (Table 2.3).

<table>
<thead>
<tr>
<th>Metal</th>
<th>Extra cellular concentration</th>
<th>Test concentration</th>
<th>Response</th>
</tr>
</thead>
<tbody>
<tr>
<td>Na$^+$</td>
<td>150 mM</td>
<td>100 mM</td>
<td>No change</td>
</tr>
<tr>
<td>K$^+$</td>
<td>5 mM</td>
<td>10 mM</td>
<td>No change</td>
</tr>
<tr>
<td>Ca$^{2+}$</td>
<td>2 mM</td>
<td>10 mM</td>
<td>No change</td>
</tr>
<tr>
<td>Mg$^{2+}$</td>
<td>10 mM</td>
<td>10 mM</td>
<td>No change</td>
</tr>
<tr>
<td>Zn$^{2+}$</td>
<td>2 mM</td>
<td>10 mM</td>
<td>No change</td>
</tr>
</tbody>
</table>

Table 2.3. Response of Eu.104 to biologically prevalent metal ions

The system gave no response to Zn(II) or to the group I and II metals. However, a response was observed for the transition metal ions of Co(II), Fe(II) and Fe(III). To confirm the sensors insensitivity to group I/II ions, a titration was carried out in a simulated biological
buffer with 150 mM NaCl, 10 mM KCl, 5 mM CaCl$_2$ and 5 mM MgCl$_2$ at pH 7.4. As seen previously the Eu(III) emission was quenched upon the addition of 0.5 equivalents of Cu(II). The same pattern was observed if the sample was excited at its isosbestic point 280 nm (Figure 2.27). The luminescence could again be switched on by addition of EDTA.

![Figure 2.27. Luminescence titration of Eu.104 with Cu(II) in simulated biological background pH 7.4. Excited at the isosbestic point 280 nm](image)

Note that the luminescence intensity is lower when the sample is excited at the isosbestic point. This is due to the lower absorbance value at 280 nm (Figure 2.20).

Thus far the studies performed have shown the sensor is stable, functions under physiological conditions, detects and signals the presence of Cu(II) ions, deals with the autofluorescence problem and is sensitive to a specific concentration range. The final requirement which the sensor must fulfil, is to be selective for a specific target, in this case Cu(II). As shown, the sensor was not responsive to the presence of the group I and II metal ions. This is crucial as they are present in high concentrations in blood samples.

The sensor elicits no response for Zn(II) ions however, as mentioned, a response was observed for the transition metal ions of Co(II), Fe(II) and Fe(III). In nature these biologically important ions are usually taken up or complexed by porphyrins. However, a small amount of the free or uncomplexed ions may be present in the blood and as such may impede the selectivity and accuracy of the sensor. In order to elucidate the effect these ions would have, selectivity studies were performed to determine the response to these transition metal ions.
2.8.1 Studies for Fe(III)

Of the three metal ions Fe(III), 3d⁵, showed the lowest affinity for the sensor. Fe(III)Cl₃ was titrated into a 35.8 μM solution of Eu.104 in buffered water at pH 7.4. The luminescence spectrum shows that the addition of Fe(III) quenched the Eu(III) luminescence in a similar manner to that previously observed with Cu(II) (Figure 2.28).

![Luminescence spectra of Eu.104 in pH 7.4 buffered solution is quenched upon addition of Fe(III).](image)

Figure 2.28. Luminescence spectra of Eu.104 in pH 7.4 buffered solution is quenched upon addition of Fe(III). Excited at 266 nm

Nevertheless, the quenching of the luminescence by Fe(III) occurs over a vastly different concentration range (20 μM → 3 mM), compared to the quenching by Cu(II) (3 → 20 μM). This can be highlighted by plotting p[Fe(III)] against intensity (Figure 2.29). The quenching by Fe(III) occurs over 4 log units. Recall the binding of Cu(II) occurs over a narrow concentration range p[Cu(II)] 5.8 → 4.7, as such Cu(II) can be detected selectively over Fe(III).

![Quenching of Eu(III) luminescence of Eu.104 by Fe(III) over 4 log units](image)

Figure 2.29. Quenching of Eu(III) luminescence of Eu.104 by Fe(III) over 4 log units
2.8.2 Studies for Fe(II)

The response of the sensor **Eu.104**, towards Fe(II) (3d⁶), was also investigated. Fe(II)Cl₂ was titrated into a 35.8 μM aqueous solution of **Eu.104** buffered at pH 7.4. The luminescence was quenched upon addition of Fe(II) similar to the quenching of the luminescence by Fe(III) (Figure 2.28). However, the concentration range was again different *ca.* 3 → 600 μM. In this case, the binding of Fe(II) occurs over 3 log units p[Fe(II)] 6→ 3 (Figure 2.30). Once again Cu(II) can be detected selectively over Fe(II).

The luminescence spectra for the Fe(II) and Co(II) titrations can be found in the appendix.

![Graph showing the quenching of Eu(III) luminescence of Eu.104 by Fe(II) occurs over 3 log units](image)

**Figure 2.30.** Quenching of Eu(III) luminescence of Eu.104 by Fe(II) occurs over 3 log units.

2.8.3 Studies for Co(II)

Cobalt is another biologically relevant ion. Its concentration in the human body (20 mg/1000 Kg) is far less than that of Cu(II) (1000 mg/1000 Kg). It is usually taken up in porphyrins and the concentration of the free ion in the blood should be very low. Nevertheless, the effect of Co(II) (3d⁷), upon the luminescence of **Eu.104**, was investigated in an identical manner to that above. Co(II)Cl₂ was titrated into a 35.8 μM aqueous solution of **Eu.104** buffered at pH 7.4. The luminescence was found to be quenched over a similar concentration range to that of Fe(II) (Figure 2.31). As stated before this is much removed from the concentration range for Cu(II) and as such Cu(II) can be detected selectively over Co(II).
By plotting the relative intensities of the various metal ions a direct comparison of the sensor’s sensitivity to each metal ion is possible. The plot of the relative intensities clearly reveals that the sensor can selectively detect Cu(II) in the concentration range in which we are interested namely 2.5 → 20 μM (Figure 2.32).
At this point it can be seen that the sensor is fulfilling the requirements needed.

- It is stable in water at pH 7.4.
- It functions under physiological conditions (i.e. aqueous environment, pH 7.4, high ionic strength).
- It is selective for Cu(II).
- It senses in the desired concentration range.

One final series of measurements were required to confirm this ideal behaviour, under physiological conditions.

2.9 Effect of Ionic Strength

The sensor has been shown to be selective for Cu(II) in water at pH 7.4. However, as mentioned earlier the sensor must function under conditions of high ionic strength. As such, it was decided to investigate the effect of ionic strength upon the sensors sensitivity towards the other metal ions. The above titrations were repeated and each of the metal ions titrated into a 35.8 μM aqueous solution of Eu₁₀⁴ buffered at pH 7.4 with high ionic strength (0.1 M TMACl).

The high ionic strength seemed to have very little effect upon the Fe(III) titration. However, it clearly effected both the Fe(II) and Co(II) titration but in different ways. Under conditions of high ionic strength the sensitivity of the sensor towards Fe(II) was reduced \( p[Fe(II)] \to 3 \). Indeed it now resembled the Fe(III) titration more accurately. On the other hand the sensitivity towards Co(II) increased dramatically \( p[Co(II)] \to 4.5 \).

Once again by plotting the relative intensities against \( p[M] \), a direct comparison can be drawn (Figure 2.33). From the relative intensities plot it can clearly be seen how the sensitivity of Fe(II) now more closely resembles that of the Fe(III) titration. Unfortunately the increase in sensitivity towards Co(II) now means that it has a similar detection range as that of Cu(II) although its shape is still different as it quenches over three log units \( p[Co(II)] \to 4 \).
Chapter 2 – Lanthanide Luminescent Copper(II) Sensor

1.2

It should be noted how the profile for the quenching in high ionic strength (TMACl), mirrors that of the quenching in the simulated biological solution (Biol) for both Cu(II) and Co(II) titrations.

It is hoped that the Eu.104 conjugate, may still be useful as a diagnostic tool for the detection of Cu(II) in blood samples as the concentration of Co(II) is so much smaller than that of Cu(II), 20 ppb and 1000 ppb, respectively."

2.10 Conclusion

The purpose of this project was to develop a lanthanide luminescent system which could detect and signal for the presence of Cu(II) under physiological conditions in a concentration range similar to that expected in blood serum samples, but which could overcome the autofluorescence issue by time gating techniques. To this end the synthesis and characterisation of Eu.104 was accomplished. This is the first example of a lanthanide luminescent system which senses for the presence of Cu(II) cations.

Photophysical analysis of Eu.104 showed that it was stable in solution, and able to produce a Eu(III) emission after excitation of the phenanthroline chromophore, which acts like a sensitising antenna, absorbing energy and transferring it to the lanthanide ion. The luminescence of Eu.104, was also determined to have a strong bell shaped pH dependence where the luminescence was switched “on” within the physiological pH range 4 – 8, but

Figure 2.33. Plot of p[M] Vs Relative Intensities of Eu.104 showing change in sensitivity towards metal ions under conditions if high ionic strength
quenched under more alkaline or acidic conditions. As such, the luminescence gives rise to an “off-on-off” system, which is switched “on” under physiological conditions, but switched “off” under acidic and alkaline conditions. This switching process was found to be fully reversible and almost independent of pH between 5 and 7.

At pH 7.4, both the fluorescence and the Eu(III) luminescence of Eu.104, was found to be quenched by the presence of Cu(II) ions. The sensor detected Cu(II) within the 3 - 20 μM concentration range, which was comparable to the concentration range desired (12 – 21 μM). The binding mode was determined to be 2:1 Eu.104:Cu(II). This was inferred through Job plot analysis and equivalence plots as well as comparison to the model receptor Eu.106. The geometry around the Cu(II) ion is expected to be a distorted square planar geometry due to the Jahn Teller effect of the Cu(II), (3d⁹), single unpaired electron. However, there are two possible supramolecular isomers, the cis and trans isomers.

The sensor was selective for Cu(II) in the presence of group I and II metal ions, and was also selective over other biologically prevalent transition metal cations. The sensor's sensitivity did show a dependence upon ionic strength, however, and under conditions of high ionic strength its sensitivity towards Co(II) was similar to that of Cu(II). Despite this, the prevalence of Co(II) in the body is much smaller than that of Cu(II), 20 ppb and 1000 ppb, respectively. As such, it is envisaged that Eu.104 may still be useful as a diagnostic tool for the detection of Cu(II) within blood serum samples.

Figure 2.34. Showing cis and trans structural isomers for the 8⁺ supramolecular ternary complex
Chapter 3

Lanthanide Luminescent Sensing of Aromatic Carboxylates
3.1 Introduction

The previous chapter dealt with the sensing of Cu(II), a transition metal cation, however, the sensing of anions is also an active area of research. As mentioned in Chapter 1, metal complexes, including lanthanide complexes, have great potential as anion sensors as they can interact with anions through Coulombic attractions that are much stronger than the hydrogen bonding interactions, which are often exploited for anion recognition. Chapters 1 and 2 have already detailed the advantages of lanthanide luminescent systems as signalling moieties for sensors, as well as how to elicit a lanthanide emission via the use of a sensitising chromophore or antenna. Some examples of lanthanide luminescent probes as anion sensors were presented in section 1.7, and most of them function by the conventional method, discussed in Chapter 2, utilising a covalently attached sensitising antenna to populate the lanthanide excited state. However, an alternative method, which uses the analyte itself as the antenna has also been described. In this case, the analyte, usually an aromatic chromophore, coordinates to the sensor, either directly to the metal centre itself or in close proximity to it, and subsequently behaves as the sensitising antenna, populating the lanthanide excited state and inducing a lanthanide emission.

Some examples of this indirect excitation were detailed in section 1.7, including the sensitisation of a Tb(III) EDTA complex by salicylic acid in water at pH 12, described by Georges and Arnaud, and the aromatic carboxylate sensors Tb.27 and Eu.27, which were reported by Michels and Reinhoudt, at the same time as the work to be described in this Chapter.

The aim of this project was to design and develop a sensor for aromatic carboxylates, whereby the target analyte, i.e. the aromatic carboxylate would bind directly to a coordinately unsaturated lanthanide ion that was part of a kinetically stable cyclen complex, via the displacement of two metal bound water molecules by the carboxylate moiety. The lanthanide complex itself, does not contain a sensitising antenna and thus in the absence of the analyte, remains photophysically silent i.e. does not give rise to any luminescence. Once bound, however, and assuming a suitable triplet state energy level was available, the analyte would then function as a sensitising antenna, in an analogous manner to that discussed in Chapter 2, absorbing energy and transferring it to the lanthanide ion, thereby populating the lanthanides excited state and inducing a lanthanide emission (Scheme 3.1). Furthermore, it was desirable that this sensor function under physiological conditions, i.e. in water at pH 7.4 and high ionic strength, in order to allow for in vivo
applications. The work upon the mononuclear lanthanide systems, presented in this Chapter, was conducted in collaboration with Dr. Joseph Leonard.

Scheme 3.1. Proposed mechanism for aromatic sensing; analyte displaces the metal bound water molecules and functions as a sensitising antenna, absorbing light before passing it to the lanthanide ion by means of a energy transfer mechanism, resulting in a lanthanide emission

In order for the analyte to function as a sensitising antenna, it must possess a carboxyl group in order to displace the metal bound water molecules and hence bind to the lanthanide ion. Furthermore, the analyte must contain a chromophore and have access to an excited triplet state energy level above that of the lanthanides excited state (20,400 cm⁻¹ for Tb(III) and 17,200 cm⁻¹ for Eu(III)).

3.2 Synthesis of Heptadentate Tri-arm Cyclen

The aim of the project was to synthesise a lanthanide luminescent sensor for aromatic carboxylates, where the lanthanide would play the part of the signalling system. As mentioned before lanthanides tend to be toxic and have a high coordination number requirement and so to circumvent both of these problems a cyclen macrocycle, functionalised with three pendant amide arms, was employed. The large number of donor atoms would be capable of forming a stable complex,¹⁹⁵ but should leave the lanthanide complex co-ordinately unsaturated, which is important in order to allow for later binding of the analyte. Furthermore, the ligand does not contain a chromophore capable of sensitising a lanthanide ion and hence any lanthanide complex of the ligand should remain photophysically silent.
Chapter 3-Lanthanide luminescent sensing of aromatic carboxylates

Scheme 3.2. Synthesis of heptadentate 3 arm cyclen ligands

The synthesis was carried out according to the literature procedure, by reacting the α-chloroamides of \( N,N \)-dimethyl acetamide 98b and \( N \)-methyl acetamide 98a respectively with cyclen, 99, in dry \( CH_3CN \) (3:1 molar ratio) at \( 65^\circ C \) for three days in the presence of \( NaHCO_3 \), (Scheme 3.2). The synthesis of 100 was discussed in detail in Chapter 2. The compound 2-(4,7-bis-methylcarbamoylmethyl-1,4,7,10-tetraaza-cyclododec-1-yl)-N-methyl-acetamide, 107, was synthesised in a similar manner and purified by precipitation from dry diethyl ether and obtained in 58 % yield. The \(^1H\) NMR spectrum corresponded with the published characterisation showing the presence of 1 NH proton at 7.59 ppm and 2 NH protons at 7.30 ppm, due to the \( C_2 \) plane of symmetry through the molecule. Resonances corresponding to the \( CH_2 \) protons from the pendant amide arms appeared at 3.06 and 2.96 ppm. The mass spectrum contained two signals at \( m/z = 386.3 \) and 408.4 corresponding to the [M+H] and [M+Na] species, respectively. High resolution mass spectrometry confirmed the presence of the desired molecule.

These two ligands would be capable of forming stable lanthanide complexes and hence the next step was to insert the lanthanide metal ions.

3.3 Complexation of the Heptadentate Cyclen Ligands 100 and 107

The Eu(III) and Tb(III) complexes of 100 and 107, were synthesised by J. P. Leonard, by refluxing together an equivalent amount of 100 or 107 with either Eu(III) or Tb(III) triflate ((C\( _5F_3SO_3 \))\(_3 \)) in dry \( CH_3CN \), under inert atmosphere for 24 hours (Scheme 3.3). Upon cooling to room temperature the solution was poured into a stirring solution of dry diethyl ether, which resulted in oily residues that were isolated by decanting the organic layers and rinsing the resulting residues with \( CH_2Cl_2 \). The complexes were isolated after exhaustive drying under vacuum over \( P_2O_5 \).
Scheme 3.3. Complexation of heptadentate cyclen ligands

The complexes were characterised by conventional means $^1$H NMR spectroscopy, high resolution mass spectroscopy, ESMS, IR and elemental analysis. The $^1$H NMR spectrum showed that the complexes had formed, evidenced by the large shift in the positions of the peaks due to the paramagnetic nature of the metal ions. This will be discussed in greater detail in section 4.3.5. High resolution mass spectra were obtained for all of the complexes and each showed the correct characteristic isotope distribution pattern.

3.4 Determination of Metal Bound Water Molecules

As previously shown in Chapter 2, lanthanides have a very high coordination number requirement, usually nine for Eu(III) and Tb(III), which if left unfulfilled are usually occupied by metal bound water molecules. The heptadentate macrocyclic framework employed here leaves the lanthanide ion coordinatively unsaturated and therefore the complexes of 100 and 107 were expected to possess two metal bound water molecules (Figure 3.1). The metal bound water molecules are an essential part of the design, as they shall be displaced by the incoming analyte.
In order to confirm this, the luminescent lifetimes (τ) of the lanthanide-excited states were measured in D_2O and H_2O after direct excitation of the metal ion. Using these, the number of metal bound waters, i.e. q value, was evaluated from the following equations.\(^{199,200}\)

\[
(i) \quad q^{\text{Eu(III)}} = 1.2 \left[ \frac{1}{\tau_{\text{H}_2\text{O}}} - \frac{1}{\tau_{\text{D}_2\text{O}}} - 0.25 - 0.075x \right]
\]

\[
(ii) \quad q^{\text{Tb(III)}} = 5 \left[ \frac{1}{\tau_{\text{H}_2\text{O}}} - \frac{1}{\tau_{\text{D}_2\text{O}}} - 0.06 \right]
\]

Here the prefixes 1.2 and 5 are proportionality constants that mirror the sensitivity of the corresponding ions to quenching by metal bound water molecules. The correction terms –0.25 and –0.66 represent quenching by second sphere water molecules, whereas –0.075x in equation (i) represents the quenching by N-H oscillators, where x is the number of such oscillators directly bound to the complex. In all cases the q value was determined to be two (Table 4). In addition, X-ray crystal structures of three of the four complexes, namely Eu.100, Eu.107 and Tb.100 (Figure 3.2), were obtained.\(^{201}\) These clearly showed that there were indeed two water molecules attached to the metal centre.

<table>
<thead>
<tr>
<th>Complex</th>
<th>(\tau_{\text{H}_2\text{O}}) (ms)</th>
<th>(k_{\text{H}_2\text{O}}) (1/(\mu)s)</th>
<th>(\tau_{\text{D}_2\text{O}}) (ms)</th>
<th>(k_{\text{D}_2\text{O}}) (1/(\mu)s)</th>
<th>q(±0.5)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Eu.100</td>
<td>0.281</td>
<td>3.55</td>
<td>0.855</td>
<td>1.17</td>
<td>2.55</td>
</tr>
<tr>
<td>Eu.107</td>
<td>0.315</td>
<td>3.17</td>
<td>0.936</td>
<td>1.07</td>
<td>1.95</td>
</tr>
<tr>
<td>Tb.100</td>
<td>0.720</td>
<td>1.38</td>
<td>1.080</td>
<td>0.92</td>
<td>2.01</td>
</tr>
<tr>
<td>Tb.107</td>
<td>0.869</td>
<td>1.15</td>
<td>1.386</td>
<td>0.72</td>
<td>1.85</td>
</tr>
</tbody>
</table>

Table 3.1. Measured lifetimes and rate constants for Eu.100, Eu.107, Tb.100 and Tb.107 and the corresponding hydration number (q±0.5)

In each case, the geometry of the crystal structure was found to be a square antiprism with the lanthanide ion coordinating to the four macrocyclic ring nitrogens and the three pendant amide oxygens. As expected, two metal bound water molecules completed the high coordination environment. The average La(III)·N and La(III)·O distances for the complexes were found to be 2.64 Å and 2.37 Å, respectively (Table 3.2). The OW1·La(III)·OW2 angle was found to be 71.80°, 72.17° and 70.86° for Tb.100, Eu.100 and Eu.107, respectively. It is important that these bite angles are similar to the bite angles of the carboxylate anions that will displace them. Parker et al. and Dickins et al. recently reported similar three-arm cyclen complexes, which form adducts with various carboxylate anions with bite angles of between 54° and 69°.\(^{202,203}\)
Figure 3.2 View from the top of Tb.100 showing the binding of Tb(III) to 100 and the two water molecules. Anions and solvent molecules have been removed for clarity.

<table>
<thead>
<tr>
<th>Bond Label</th>
<th>Tb.100</th>
<th>Eu.100</th>
<th>Eu.107</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ln-O15 (Å)</td>
<td>2.348</td>
<td>2.342</td>
<td>2.378</td>
</tr>
<tr>
<td>Ln-O27 (Å)</td>
<td>2.325</td>
<td>2.364</td>
<td>2.394</td>
</tr>
<tr>
<td>Ln-O20 (Å)</td>
<td>2.368</td>
<td>2.378</td>
<td>2.399</td>
</tr>
<tr>
<td>Ln-O1W(water) (Å)</td>
<td>2.429</td>
<td>2.418</td>
<td>2.449</td>
</tr>
<tr>
<td>Ln-O2W(water) (Å)</td>
<td>2.441</td>
<td>2.421</td>
<td>2.485</td>
</tr>
<tr>
<td>Ln-N1 (Å)</td>
<td>2.601</td>
<td>2.605</td>
<td>2.673</td>
</tr>
<tr>
<td>Ln-N10 (Å)</td>
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<td>2.624</td>
<td>2.617</td>
</tr>
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<td>Ln-N7 (Å)</td>
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<td>2.625</td>
<td>2.67</td>
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<tr>
<td>OW1-Ln-OW2 (°)</td>
<td>71.80</td>
<td>72.17</td>
<td>70.86</td>
</tr>
</tbody>
</table>

Table 3.2. Selected lengths and angles for Tb.100 taken from Mercury 1.1 CIF file
3.5 Displacement of Metal Bound Water Molecules

No significant luminescence was observed from the complexes i.e. they were photophysically "silent". As explained before, this was due to the difficulty in directly exciting a lanthanide ion, combined with the lack of a sensitising antenna and the effective quenching mechanism provided by the two metal bound water molecules via their OH oscillations. The design of the sensor envisaged the metal bound water molecules being displaced by a suitable antenna. This would reduce the quenching of the lanthanide excited state by displacing the OH oscillators while offering an alternate indirect route to lanthanide sensitisation and as such, should lead to large enhancements in the luminescence. As discussed in Chapter 1, Parker et al. and Dickins et al. have shown related three-arm cyclen complexes that can form bidentate adducts with organic anions such as acetate, citrate and lactate by displacing these metal bound waters.

In considering a suitable antenna a number of factors needed to be addressed. Firstly, the antenna must posses a carboxyl group in order to displace the metal bound water molecules and hence bind to the lanthanide metal ion. Secondly, the antenna must have access to an excited triplet state and the energy of this state must be above that of the lanthanides excited singlet state (20,400 cm^{-1} for Eu(III) and 17,200 cm^{-1} for Tb(III)). Finally, the antenna must be able to transfer its energy to the lanthanide efficiently, as such the distance between the chromophore (in this case an aromatic group) and the metal must be short.

3.6 Luminescence Measurements

Dimethylaminobenzoic acid (DMABA), 108, was chosen as the antenna as it fulfilled the above requirements. It contains a carboxyl group through which it may bind to the lanthanide ion by displacing the metal bound water molecules. It also contains an aromatic ring, with a suitable triplet energy level, which can act as a sensitising chromophore. Also the distance between the two components is short, which should promote efficient energy transfer.

To investigate the complexes response to DMABA, measurements were carried out in water, at pH 7.4 (buffered with 0.1 M TRIS), at high ionic strength (0.1 M TMACl), in order to resemble physiological conditions. DMABA was gradually titrated into a 16 \mu M solution of the corresponding lanthanide complex. The complexes themselves did not absorb in the visible region, however, upon addition of DMABA the appearance of an absorption band at 288 nm was observed corresponding to the \lambda_{\text{triax}} of the chromophore.
Scheme 3.4. Upon binding the antenna the previously silent lanthanide complex is ‘switched on’ as its excited state is populated by an energy transfer from the antenna.

The luminescent spectrum was also investigated. As noted earlier, the lanthanide complexes are photophysically silent prior to addition of the antenna. It was found that when the antenna DMABA, was excited at its $\lambda_{\text{max}} = 288$ nm, in the presence of Tb.100, a Tb (III) emission was observed (Scheme 3.4). Furthermore the luminescence intensity increased with increasing concentration of DMABA, reaching a maximum at ~ 2 equivalents, with a luminescence enhancement factor of ca. 220 (Figure 3.3).

![Luminescence spectra of Tb.100](image)

Figure 3.3. Luminescence spectra of Tb.100, showing increase in Tb(III) emission with increasing [DMABA] (108). Tris buffer pH 7.4, 0.1M TMACl, excite at 288 nm

Unexpectedly, the luminescence began to decrease upon further addition of DMABA, and was quenched after about 60 equivalents had been added (Figure 3.4). The pH of the solution remained constant over these measurements and consequently the decrease in luminescence can be directly attributed to the increased concentration of DMABA. One
possible explanation for the observed quenching of the luminescence may be the inner filter effect, caused by the presence of such an excess of antenna, as only the small percentage of the analyte directly bound to the Tb(III) ion may function as a sensitiser.\(^{205}\)

![Graph showing changes in intensity of Tb.100](image)

**Figure 3.4.** The changes in the intensity of the \(J = 5\) transition of Tb.100 as a function of added equivalents of DMABA (108)

The appearance of the characteristic Tb(III) spectral bands can be explained by the use of a Jablonski diagram (Figure 3.5). Once bound to the lanthanide ion the analyte, denoted Ar, absorbs a photon of light and enters its singlet excited state, \(S_1\). From here the energy can be transferred to the analytes triplet excited state, \(T_1\), by means of intersystem crossing, ISC.

![Jablonski diagram](image)

**Figure 3.5.** Jablonski diagram illustrating indirect excitation of Tb(III) ion by the bound analyte, Ar, which functions as a sensitising chromophore. Each transition \(^5\!D_4 \rightarrow \!^7\!F_j\) will correspond to an emission band
As this state is higher in energy than the Tb(III) excited state, Ln, it is possible for the analyte to pass its energy onto the lanthanide ion by means of the energy transfer mechanism, which was discussed in the introduction. The excited energy now resides on the Tb(III) ion, and can be lost by emission of light, resulting in the characteristic Tb(III) emission spectra as each transition will correspond to an emission band, $J = 6$ etc. The Tb(III) emission differs from the Eu(III) emission due to the different nature of the corresponding energy levels. The Tb(III) $^5D_4$ excited state is at 20,400 cm$^{-1}$, while the Eu(III) $^5D_0$ excited state is at 17,200 cm$^{-1}$.

A plot of $p[108]$, where $p$ is the negative log of concentration, against intensity of any of the bands, in this case the $J = 5$ band, revealed that the luminescence was switched on over two log units, $p \approx 6.2 \to 4.2$, which is indicative of 1:1 binding (Figure 3.6).

![Plot of p[108] against Intensity reveals binding occurs over two log units, where p is the negative Log of concentration](image)

Figure 3.6. Plot of $p[108]$ against Intensity reveals binding occurs over two log units, where $p$ is the negative Log of concentration

The above measurements were repeated for Tb.107 and again positive sensitisation occurred, although the enhancement factor was somewhat smaller ca. 80. This was attributed to additional quenching of the Tb(III) excited state by the presence of additional N-H oscillators in Tb.107. These results indicate that for both Tb.100 and Tb.107 the carboxyl group was binding to the Tb(III) metal centre and successfully populating their excited states. Nevertheless, when these measurements were repeated for the europium compounds Eu.100 and Eu.107, no sensitisation was observed. Lifetime measurements were carried out, this time in the presence of four equivalents of DMABA (108), in an attempt to elucidate whether the analyte/antenna was displacing the metal bound water
molecules and binding to the metal ion as had been envisaged in the design (Table 5). From these measurements a $q$ value of zero for the terbium complexes, and a $q$ value of two for the europium complexes was determined. This inferred that 108 was not binding to the europium metal ion as neither of the metal bound water molecules were being displaced. The $q$ value of zero for the terbium complexes indicated that the water molecules had been successfully displaced by 108 and that the ligand was binding through both the oxygens of its carboxyl group. This agrees with previous findings by Dickins and Parker, who found that oxyanion ternary complexes of Tb(III) complexes in water, were an order of magnitude more stable than their Eu(III) analogues.\(^{207}\) This can be attributed to the enhanced Lewis acidity of Tb(III) over Eu(III).

<table>
<thead>
<tr>
<th>Complex</th>
<th>$\tau_{H2O}$ (ms)</th>
<th>$k_{H2O}$ (1/ms)</th>
<th>$\tau_{D2O}$ (ms)</th>
<th>$k_{D2O}$ (1/ms)</th>
<th>$q(\pm0.5)$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Eu.100</td>
<td>0.235</td>
<td>4.25</td>
<td>0.438</td>
<td>2.28</td>
<td>2.07</td>
</tr>
<tr>
<td>Eu.107</td>
<td>0.276</td>
<td>3.62</td>
<td>0.733</td>
<td>1.35</td>
<td>1.80</td>
</tr>
<tr>
<td>Tb.100</td>
<td>1.593</td>
<td>0.62</td>
<td>1.795</td>
<td>0.55</td>
<td>0.06</td>
</tr>
<tr>
<td>Tb.107</td>
<td>1.626</td>
<td>0.61</td>
<td>1.895</td>
<td>0.52</td>
<td>0.14</td>
</tr>
</tbody>
</table>

Table 3.3. Measured lifetimes and rate constants for Eu.100, Eu.107, Tb.100 and Tb.107 and the corresponding hydration number in the presence of four equivalents of $N,N$-dimethylaminobenzoic acid (108)

Further luminescence measurements were performed for the ester 109, and ketone 110, derivatives of DMABA. The excitation wavelength was chosen as the $\lambda_{max}$ of 109 and 110, respectively. However, neither one could sensitise the complexes. This seemed to confirm that both oxygens were required to successfully bind to the complex.

As remarked in Chapter 1, Georges and Arnaud have previously used salicylic acid as a sensitising chromophore for Tb(III) EDTA complexes in water at pH 12. It was decided to investigate whether the heptadentate complexes of 100 and 107 could also function as salicylic acid sensors under the physiological conditions employed above, i.e. in water at pH 7.4, and high ionic strength.

### 3.7 Sensitisation by Salicylic Acid

Aspirin® (112) is a well-known non-steroidal, anti-inflammatory drug that has many pharmacological actions. The active form of the drug is salicylic acid 111, which is formed in vivo after hydrolysis of the ester group by enzymes.\(^{208}\) Salicylic acid, 111, is often used as a painkiller but can cause gastric bleeding due the free phenolic group.\(^{208}\) This is
prevented by transforming the phenolic group to an ester to form the well known drug aspirin (112). Salicylic acid (111) is also an apt contender to be an antenna as it has a carboxylic group attached to an aromatic ring and is known to have a suitable triplet energy state above that of the Tb(III) excited state (Figure 3.9).

Luminescence studies were carried out using salicylic acid. As found previously, no sensitisation was observed for the europium complexes. However, upon addition of 111 to a 16 µM solution of Tb.100, after excitation at 296 nm, sensitisation was observed with enhancement factors of ca. 40 and 30 (in H2O and in Tris buffer, pH 7.4, respectively) (Figure 3.7). Again the intensity increased with increasing concentration of 111, reaching its maximum at 10 equivalents before being slowly quenched with further increases in concentration (Figure 3.8). The plot of p[111] against intensity revealed that the luminescence was switched on over two log units, p 5.5 → 3.5, indicative of 1:1 binding (Figure 3.8).

![Figure 3.7. Luminescence spectra showing changes in Tb.100 emission upon addition of salicylic acid (111) in Tris buffer pH 7.4, 0.1M TMACl, excite at 296 nm](image)

![Figure 3.8. Plot of p[111] against Intensity showing switching on of Tb(III) luminescence over two log units. Inset showing equivalents plot for 111](image)
Sensitisation was also observed for Tb.107, but the enhancement was much less than that of Tb.100 and hence was not investigated further as the increases in luminescence were too small to be of practical use. The smaller enhancement was once again attributed to additional quenching of the Tb(III) excited state by the presence of the extra N-H oscillators in Tb.107. 

Figure 3.9. Compounds evaluated for ability to sensitise lanthanide complexes and their enhancement factors (LE) for Tb.100

Subsequently, aspirin (112) was evaluated as a sensitiser. Measurements were conducted in an identical manner to that described above. Surprisingly though, 112 was found to be unable to sensitise the lanthanide complexes, and in all cases, the luminescence remained switched “off”. This was unexpected as the molecule contains a free carboxylic group attached to an aromatic ring and thus was expected to bind to the lanthanide centre and function as a sensitising antenna. Lifetime measurements were conducted and the $q$ value for Tb.100, in the presence of aspirin (112) was determined to be two. This result revealed that the metal bound water molecules had not been displaced and therefore, aspirin (112) was not binding to the metal centre and as a result, was unable to sensitise the Tb(III) ion. Furthermore, competition experiments showed that Tb.100 could selectively detect 111, in the presence of 112, i.e. when 111 was added to a photophysically silent solution of
Tb.100 and 112 (four equivalents), the luminescence was found to be “switched on” in an identical manner to that previously observed. The inability of 112 to bind to the lanthanide centre may have been due to steric factors from the increased bulk of the ester or it may point to a different binding mode (Figure 3.10). In order to investigate further, tests were carried out on the salicylic acid analogues 113, 114 and 115.

In each case, the excitation wavelength was chosen as the $\lambda_{\text{max}}$ of the antenna. As expected 115 did not sensitise any of the lanthanide complexes, as both of the hydroxyl binding sites are protected. The characteristic Tb(III) emission was observed for 114, with luminescence enhancements of ca. 270. Surprisingly 113, which does not have a free carboxylic acid group with which to bind, gave the greatest enhancements with luminescence increasing by a factor of ca. 520. This discrepancy points to the possibility of different binding modes, both in this case and in the case of salicylic acid 111. To sensitise the Tb.100 complex, 113 must bond to the metal either via its phenolic oxygen or through a 6 membered ring using the carboxyl oxygen as well. In order to establish which bonding mode was operating, lifetime measurements were carried out to determine the $q$ value. The $q$ values for Tb.100 in the presence of 113, 114 and 115 were all measured and found to be between zero and one ($q \pm 0.5$). This suggested that only one of the metal bound water molecules was being replaced upon the binding of these compounds. It is also possible that a mixture of these binding modes were operating. Nevertheless, whichever binding mode is in operation, we can conclude that Tb.100 can selectively bind and sense salicylic acid (111) over aspirin (112).

In order to be viable as an in vivo sensor, Tb.100 must function in the presence of other biological anions such as, phosphate, lactate and carbonate. As mentioned in the introduction, Parker et al. demonstrated that the similar heptadentate Eu(III) and
Tb(III) complexes Eu.100 and Tb.100, which each possessed two metal bound water molecules, form 1:1 ternary complexes with these anions. When measurements were repeated upon Tb.100 in the presence of 0.1 M carbonate (Na₂CO₃) or (NaHCO₃), the luminescence remained "switched off" even after the addition of four equivalents of 111. The preferential binding of carbonate by Tb.100, indicates that the sensor would be impractical for in vivo applications.

In summary, four novel unsaturated lanthanide complexes Eu.100, Eu.107, Tb.100 and Tb.107, have been prepared and each of them has been shown to possess two metal bound water molecules. In the case of the two Tb(III) complexes the addition of aromatic carboxylate anions such as DMABA (108) and salicylic acid (111) gave rise to significant enhancements in the luminescence of the complexes, due to the self assembly of ternary complexes in aqueous solution under ambient conditions, i.e. in water at pH 7.4 at high ionic strength (0.1M TMACl). The complexes themselves do not contain a chromophore and require the formation of the ternary complex, with the analyte, in order to allow indirect sensitisation by the analyte/antenna to lead to a lanthanide emission. For the Eu(III) complexes, no increase in luminescence was observed, as under these conditions they did not form ternary complexes with the analyte and hence could not be sensitised. This was confirmed by lifetime measurements in the presence of excess analyte.

In the case of the DMABA analyte, the binding is believed to proceed through both carboxyl oxygens via the formation of a four-membered bidentate ring, with both metal bound waters being displaced. The binding mode for 111 is less certain and may operate in a monodentate or bidentate fashion. The sensors did not function in the presence of 0.1 M carbonate, indicating that they may be unsuitable for in vivo applications.

3.8 Bis Terbium System

The success of Tb.100 as a sensor for aromatic carboxylates, encouraged us to pursue this work further. It was of interest whether the incorporation of a second metal ion into the design would impact upon the properties of the sensor. It was envisaged that a binuclear Tb(III) conjugate could bind bis-carboxylates and signal this event through a change in the luminescence. Therefore the aim of the project was to synthesise a bis-lanthanide luminescent sensor for aromatic carboxylates and investigate its luminescent response to antennae, with the goal of sensing aromatic bis-carboxylates. The synthesis and characterisation of this compound shall be the subject of the following section.
3.9 Synthesis

Building upon the previous design it was decided to link two Tb.100 complexes together by a short xylene bridge. The Tb.100 unit was chosen as it had given the best results to date. The synthesis was carried out as shown in Scheme 3.5. The ligand 100 was refluxed in MeOH with α,α-dichloro-p-xylene 116, in a 2:1 molar ratio, in the presence of Cs₂CO₃ and KI for four days. The solvent was then evaporated and the residue was suspended in CHCl₃ and filtered. The volume was then reduced and the solution was left to stand at 4 °C. The resulting crystals were filtered and washed with cold CHCl₃, yielding the product 2-{4,7-bis-dimethylcarbamoylmethyl-10-[4-(4,7,10-tris-dimethylcarbamoylmethyl)-1,4,7,10-tetra-aza-cyclododec-1-ylmethyl]-benzyl]-1,4,7,10-tetraaza-cyclododec-1-yl}N,N-dimethyl-acetamide, 117, in 10 % yield.

The bis-cyclen ligand 117, had not been previously reported and was fully characterised. The ¹H NMR spectrum in D₂O of 117 revealed the high degree of symmetry inherent in the molecule. The four aromatic protons resonanced at 7.30 ppm while the four benzyl protons were observed at 3.95 ppm (Figure 3.11).

The ¹³C NMR spectrum showed two carbonyl resonances at 172.5 and 172.1 ppm. The aromatic signals were visible at 136.5 (quaternary) and 129.8 ppm, while seven CH₂ resonances were visible at 57.7, 54.5, 53.8, 50.8, 50.3, 49.7 and 48.3 ppm respectively. Three CH₃ resonances were observed at 35.5, 35.1 and 34.8 ppm, due to a syn and anti relationship with the amide carbonyl group (Figure 3.12).
Figure 3.11. 400 MHz $^1$H NMR in D$_2$O of 117

Figure 3.12. The lack of rotation around the N=C double bond leads to the syn methyl group “experiencing” the electric field of the oxygen, to a greater extent than the anti methyl group.

The mass spectrum contained three signals at $m/z = 479.1, 490.0$ and 500.9, corresponding to the [M+H]/2, [M+Na]/2 and [M+K]/2 species respectively. Elemental analysis and high resolution mass spectrometry confirmed that the ligand had been synthesised. A crystal structure corresponding to the sodium complex of the ligand was also obtained (Figure 3.13). Sodium is known to have a similar coordination geometry to that of terbium. The sodium complex was seen to have two metal bound water molecules.
Chapter 3–Lanthanide luminescent sensing of aromatic carboxylates

Figure 3.13. Sodium complex of $\text{M}_{2}\text{Na}_2\text{H}_2\text{O}_8\text{CHCl}_3\text{.2I}$. Hydrogen, solvent and anions have been removed for clarity.

The ligand adopts a square antiprism geometry around the sodium ion, which coordinates to the four macrocyclic ring nitrogens as well as the three pendant amide oxygens with average $\text{Na}^-\text{N}$ and $\text{Na}^-\text{O}$ distances of 2.55 Å and 2.45 Å, respectively. This geometry is also common for octadentate lanthanide cyclen complexes. The similarity in geometry stems from the fact that, like the lanthanide ions, sodium prefers a high coordination environment. The crystal structure also shows the presence of two metal bound water molecules with a Na to water (O4) distance of 3.461 Å, while the Na–Na distance was found to be 8.688 Å.

Table 3.4. Selected lengths and angles taken from Mercury 1.2.1 CIF file

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<tr>
<th>Atom1</th>
<th>Atom2</th>
<th>Length (Å)</th>
<th>Angle Label</th>
<th>Angle (°)</th>
</tr>
</thead>
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<td>O1-Na1-N2</td>
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</tr>
<tr>
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<td>N2</td>
<td>2.43</td>
<td>O2-Na1-N4</td>
<td>66.52</td>
</tr>
<tr>
<td>Na1</td>
<td>N4</td>
<td>2.492</td>
<td>O3-Na1-N6</td>
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</tr>
<tr>
<td>Na1</td>
<td>N6</td>
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<td>O4-Na1-O3</td>
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</tr>
<tr>
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</tr>
<tr>
<td>Na1</td>
<td>O4</td>
<td>3.461</td>
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<td></td>
</tr>
</tbody>
</table>
The corresponding terbium complex was formed by refluxing 117 with two equivalents of Tb(CF$_3$SO$_3$)$_3$ in MeOH overnight (Scheme 3.6). The complex was precipitated from ether and washed with CH$_2$Cl$_2$ to give the desired product Tb.117, in 41 % yield. The $^1$H NMR spectrum revealed that the complex was present, evidenced by the large shift in the proton signals due to the paramagnetic metal ion (Figure 3.14). This effect shall be described in greater detail in section 4.3.5. The spectrum showed signals ranging from 120.25 to -122.88. The mass spectrum contained signals at $m/z = 573.5$ and 284.8 corresponding to the [M+3 triflate]/3 and [M+1 triflate]/5 species. The IR spectrum showed that the carbonyl stretching frequency had decreased from 1639 cm$^{-1}$ to 1624 cm$^{-1}$, again indicating that the complex had been formed. Elemental analysis confirmed the characterization of the complex.

![Scheme 3.6. Complexation of 117 proceeded in 41 % yield](diagram)

![Figure 3.14. 400MHz $^1$H NMR of Tb.117](graph)
Once again, the number of metal bound water molecules was deduced by measuring the luminescent lifetimes ($\tau$) of the Tb(III) excited state, after direct excitation, in both D$_2$O and H$_2$O. As in the case of the mononuclear system, the number of metal bound water molecules on each metal centre was found to be two (Table 3.5).

<table>
<thead>
<tr>
<th>Complex</th>
<th>$\tau_{\text{H}_2\text{O}}$ (ms)</th>
<th>$k_{\text{H}_2\text{O}}$ (1/ms)</th>
<th>$\tau_{\text{D}_2\text{O}}$ (ms)</th>
<th>$k_{\text{D}_2\text{O}}$ (1/ms)</th>
<th>$q$ ($\pm 0.5$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tb.117</td>
<td>0.730</td>
<td>1.35</td>
<td>1.071</td>
<td>0.93</td>
<td>1.93</td>
</tr>
</tbody>
</table>

Table 3.5. Measured lifetimes and rate constants for Tb.117 and the corresponding hydration number $q$

Interestingly the lifetime and rate constant values were very similar to the corresponding mononuclear complex, Tb.100. As such, the relative binding was expected to operate in a similar fashion. In contrast to the mononuclear system, however, Tb.117 now possesses its own xylene chromophore and thus is no longer photophysically silent.

### 3.10 Photophysical Studies of Tb.117

The complex, Tb.117, absorbed in the UV with $\lambda_{\text{max}}$ at 237 nm. When excited at 237 nm the characteristic Tb(III) emission was observed, showing that the xylene bridge can act as an antenna and populate the Tb(III) excited state. The systems response to the original antenna DMABA, 108, was investigated, in order to compare it to the mononuclear system Tb.100. Measurements were conducted in water at pH 7.4 (buffered with 0.1 M HEPES),
at high ionic strength (0.1 M TMACl). DMABA was gradually titrated into a 16 μM solution of the lanthanide complex, Tb.117.

Upon addition of DMABA the appearance of a band at 288 nm was observed. A fluorescent band was also observed at 356 nm after excitation at 288 nm (see appendix). Both the absorption and fluorescent transitions, were seen to increase in intensity with increasing concentration of DMABA and they can be directly attributed to the antenna. The luminescent spectrum of Tb.117 was also investigated as a function of DMABA concentration. When excited at 288 nm, prior to addition of DMABA, the complex, Tb.117, was found to have a very weak luminescence (20 a.u.). However, upon the addition of the antenna DMABA 108, and after excitation at 288 nm, a large increase in the Tb (III) emission was observed (Figure 3.16).

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{figure316}
\caption{Luminescence spectrum of Tb.117 (16 μM), showing increase in luminescent intensity upon addition of DMABA. Excite at 288 nm, pH 7.4, 0.1 M TMACl.}
\end{figure}

Furthermore the luminescence intensity increased with increasing concentration of DMABA 108, reaching a maximum at ~ 2 equivalents, with a luminescence enhancement factor of ca. 1000 (Figure 3.17). As noted for Tb.100, the luminescence began to decrease upon further addition of DMABA and was fully quenched after about 70 equivalents had been added (Figure 3.17). Once again, the pH of the solution remained constant over the course of these measurements and thus the quenching can be directly attributed to the high concentration of the free antenna compared to the relatively small percentage of bound antenna in the ternary complex.
By plotting $p[\text{DMABA}]$ against the relative intensity at 545 nm, a direct comparison to the mononuclear system $\text{Tb.100}$, is possible. The plot of $p[108]$ against intensity revealed that the luminescence was switched on over two log units, $p 5.9 \rightarrow 4.2$ (Figure 3.18).

It can clearly be seen that both titrations behave similarly over similar concentration ranges, clearly quenching over two log units $p 6.2 \rightarrow 4.2$. As they are both at 16 uM, $\text{Tb.117}$ has twice as many metal centres as $\text{Tb.100}$. This is evidenced by the much larger luminescent enhancement viewed for $\text{Tb.117} \sim 1000 \text{ ca. 220}$. It may have been expected that the $\text{Tb.117}$, having twice as many metal centres would behave differently. However,
the two metal centres seem to be acting independently of each other and as such, this can be viewed as a 1 antenna: 1 metal centre, binding motif (Figure 3.19), similar to the 1:1 binding motif of the mononuclear Tb.100 complex.

![Figure 3.19. Showing possible binding mode for 108 with Tb.117](image)

Luminescent lifetime measurements were carried out upon Tb.117 in the presence of four equivalents of DMABA. The lifetime values were similar to those observed for Tb.100 in the presence of DMABA, and again corresponded to a hydration value, $q$, of zero (Measured lifetimes and rate constants for Tb.117 and the corresponding hydration number in the presence of four equivalents of $N,N$-dimethylaminobenzoic acid (108)). This further supported the theory that the two metal bound water molecules on each Tb(III) ion were displaced by DMABA.

<table>
<thead>
<tr>
<th>Complex</th>
<th>$\tau_{H_2O}$ (ms)</th>
<th>$k_{H_2O}$ (1/ms)</th>
<th>$\tau_{D_2O}$ (ms)</th>
<th>$k_{D_2O}$ (1/ms)</th>
<th>$q$ (±0.5)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tb.117</td>
<td>1.315</td>
<td>0.761</td>
<td>1.504</td>
<td>0.665</td>
<td>0.18</td>
</tr>
<tr>
<td>Tb.100</td>
<td>1.593</td>
<td>0.62</td>
<td>1.795</td>
<td>0.55</td>
<td>0.06</td>
</tr>
</tbody>
</table>

Table 3.6. Measured lifetimes and rate constants for Tb.117 and the corresponding hydration number in the presence of four equivalents of $N,N$-dimethylaminobenzoic acid (108).

In summary, a novel binuclear Tb(III) complex, Tb.117, has been synthesised, which was found to possess two metal bound water molecules per Tb(III) ion. The addition of the aromatic carboxylate DMABA (108), to a solution of Tb.117, gave rise to large enhancements in the Tb(III) emission intensity, due to the self assembly of ternary complexes in aqueous solution under ambient conditions, i.e. in water at pH 7.4 at high
Ionic strength (0.1M TMACl), whereby the DMABA acts as a sensitising antenna for the Tb(III) ion.

Luminescent lifetime measurements in the presence of excess DMABA, revealed that both metal bound water molecules had been displaced, indicating that the binding of the DMABA anion is proceeding through both carboxyl oxygens via the formation of a four membered ring, giving rise to a one DMABA to one Tb(III) ion, or two DMABA to one Tb.117 ternary complex.

3.11 Sensitisation with dicarboxylates

Following the response to the DMABA carboxylate a series of measurements were conducted to determine whether the binuclear Tb(III) complex Tb.117, could be sensitised by aromatic dicarboxylates. Terephthalic acid, 118, was chosen as a sensitiser as it is a bis carboxylic acid, has a complementary geometry to that of Tb.117, and is known to have a suitable triplet energy state above that of the Tb(III) excited state.²⁰⁴

Luminescence studies were conducted in an identical manner to that described previously using 118. The analyte, 118, absorbs in the UV with a λ<sub>max</sub> at 240 nm. This is very close to the λ<sub>max</sub> of the Tb.117 complex itself (237 nm), and indeed upon excitation of Tb.117, at 240 nm, in the absence of any antenna, the characteristic terbium emission was observed.

Upon addition of 118 to a 16 μM solution of Tb.100, after excitation at 240 nm, sensitisation was observed with enhancement factors greater than 1000 (i.e. off the scale). As a result the fluorimeter settings were altered and the emission slit width was changed from 5 nm to 2.5 nm, all other settings remained unchanged. Under these settings luminescent enhancements of ca. 350 were obtained (Figure 3.20).

![Figure 3.20. Changes in luminescence of Tb.117 upon addition of 118 at pH 7.4, 0.1 M TMACl, excite at 240 nm. Note emission slit reduced to 2.5 nm](image-url)
As expected, the intensity increased with increasing concentration of 118, reaching its maximum at ~ 3 equivalents before being quenched with further increases in concentration (Figure 3.21).

![Figure 3.21. Plot of equivalents of 118 against Intensity at 545 nm for Tb.117 at pH 7.4, 0.1 M TMACl, excite at 240 nm](image)

The plot of p[118] against intensity revealed that the luminescence was switched on over two log units, p 5.9 → 4.0, indicative of 1:1 binding (Figure 3.22).

![Figure 3.22. Relative intensities plot showing the effect of 118 and DMABA upon Tb.117 at pH 7.4, 0.1 M TMACl](image)
Figure 3.23. Possible binding modes for Tb.117 and the sensitising antenna 118

As yet, it was unclear whether the Tb.117: 118 binding mode operating was A: a two Tb(III) ion to one 118, B: a one Tb(III) ion to one 118, or C: a one Tb.117 to two 118, interaction (Figure 3.23). In order to elucidate more clearly, lifetime measurements were once again conducted upon Tb.117 in the presence of four equivalents of 118. From the lifetime values obtained the hydration number was ascertained to be zero (Table 3.7). This immediately ruled out the 1:1 binding interaction B, as in that case, the hydration number would be higher as one of the Tb(III) ions would retain two metal bound water molecules. Whether both Tb(III) ions are binding to the same 118 unit, A, or are acting independently, C, is still unclear, and work is continuing within the Gunnlaugsson laboratory to solve this conundrum. Nevertheless, whichever binding mode is in operation, we can conclude that Tb.117 can bind, recognise and signal the presence of terephthalic acid (118).

<table>
<thead>
<tr>
<th>Complex</th>
<th>$\tau _{H2O}$ (ms)</th>
<th>$k_{H2O}$ (1/ms)</th>
<th>$\tau _{D2O}$ (ms)</th>
<th>$k_{D2O}$ (1/ms)</th>
<th>$q$ (±0.5)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tb.117</td>
<td>1.315</td>
<td>0.761</td>
<td>1.452</td>
<td>0.689</td>
<td>0.06</td>
</tr>
</tbody>
</table>

Table 3.7. Measured lifetimes and rate constants for Eu.100, Eu.107, Tb.100 and Tb.107 and the corresponding hydration number ($q$±0.5) in the presence of four equivalents of terephthalic acid (118)
As in the case of the mononuclear system, when the measurements were repeated upon Tb.117 in the presence of 0.1 M carbonate (Na₂CO₃), the luminescence remained "switched off" even after the addition of four equivalents of DMABA or 118. The preferential binding of carbonate at high concentrations, indicates that the sensor would be impractical for in vivo applications.

3.13 Conclusion
In summary, lanthanide complexes have been prepared that can sense for aromatic carboxylates by means of self-assembling ternary complexes. It has been shown both through x-ray crystallography and lifetime measurements that each lanthanide ion possesses two metal bound water molecules. These metal bound waters can be displaced by aromatic carboxylates, which can then act as 'antennae', passing their energy to the lanthanide excited state and thereby inducing a luminescent emission from the previously photophysically silent lanthanide complex. Of the four mononuclear complexes studied, Eu.100, Eu.107, Tb.100 and Tb.107, the Eu(III) complexes did not give rise to the formation of such self-assembling complexes. It was concluded that this was due to the lower affinity of europium complexes towards the carboxylate antennae. Other researchers have made similar observations. The effective binding of DMABA (108) to both Tb.100 and Tb.107 was observed with large increases in luminescence and proceeded via the formation of a four-membered bidentate ring, with both metal bound waters being displaced. The binding of salicylic acid (111) however, could operate through either a mono or bidentate fashion. This was shown by probing the bonding modes of 113, 114 and 115. All of these processes occur under ambient conditions i.e. in water at pH 7.4 at high ionic strength (0.1M TMACl). A novel binuclear Tb(III) complex, Tb.117, has also been synthesised. The crystal structure of the sodium complex of 117 indicated that metal bound water molecules would be present and lifetime measurement confirmed that each Tb(III) ion possesses two metal bound water molecules. This complex also exhibited large increases in its luminescence ca. 1000, after the formation of a ternary complex with the DMABA antenna, over an identical concentration range to that observed for Tb.100. Lifetime measurements in the presence of the DMABA antenna indicated that each of the Tb(III) ions was binding one carboxylate anion, as all the metal bound water molecules had been displaced, q = 0 (±0.5).
Furthermore the addition of the bis-carboxylate, terephthalic acid, gave rise to even stronger luminescent enhancements. The effective binding of 118, occurred over an identical region to that of DMABA. Lifetime measurements in the presence of 118 confirmed that all metal bound water molecules had been displaced, $q = 0 \pm 0.5$. Further work is necessary to determine the precise binding mode as whether it is 1:2 or 1:1 is still undetermined.

In the presence of 0.1 M Na$_2$CO$_3$, the luminescence of both the mononuclear and binuclear systems was quenched and the addition of increasing concentrations of antenna had no effect. This was attributed to the coordination of the carbonate anion by the Tb(III) ion, preventing binding of the analyte. In conclusion a new general class of sensor for aromatic carboxylates such as DMABA, salicylic acid, 111, and terephthalic acid, 118, have been successfully developed. They operate under ambient conditions through the formation of ternary complexes, which give rise to large increases in the Tb(III) luminescence.
Chapter 4

Lanthanide Complexes as Phosphodiester Cleaving Agents
4.1 Introduction

Phosphodiester bonds play an important role in nature. The very stable 3',5'-phosphodiester is used to link together the genetic code of the nucleic acids, DNA and RNA. The large stability of this molecule stems from the negative charge on the phosphate, which drastically reduces the rate of hydrolysis. As mentioned in Chapter 1, the ability to selectively cleave the RNA phosphodiester, could lead to the targeting and control of specific diseases, which operate by the production of harmful proteins. As such, efforts to develop ribozyme mimics that cleave phosphodiester bonds efficiently are currently underway.

Nature utilises a class of enzymes known as ribozymes, to efficiently cleave unwanted nucleic acids. As previously discussed in Chapter 1, these enzymes often contain metal ions as well as catalytically active cofactors like histidine, at their active sites. Drawing upon nature’s ideas, these components are being studied and utilised in the design of ribozyme mimics. Metal ions, and lanthanide ions in particular, have been shown to greatly accelerate the hydrolysis of phosphodiesters. However, as they can be both toxic and unselective, they must be incorporated into a ligand framework to be of use for in vivo applications. Chapter 1 detailed some examples of phosphodiester hydrolysis accelerated by coordinated metal ion complexes. To date, work in the Gunnlaugsson group has focused on the use of mononuclear lanthanide systems and their ability to promote phosphodiester hydrolysis.

The focus of this thesis, however, is the rate enhancement provided by the cooperative action of two metal ions. As discussed in Chapter 1, binuclear metal ion systems can promote phosphodiester cleavage many times more rapidly than similar mononuclear systems. The work herein concentrates solely on binuclear lanthanide complexes, which to date, despite large rate enhancements reported for mononuclear lanthanide systems, have been largely overlooked. With the goal of obtaining large rate enhancements above those of similar mononuclear systems, this chapter shall detail the synthesis of some binuclear lanthanide complexes. It will then discuss the phosphodiester cleavage ability of such compounds, comparing them to similar mononuclear systems from the literature and the Gunnlaugsson laboratory.
4.2 Design of Binuclear systems

The objective of this project was to design and synthesise binuclear lanthanide metal ion systems that would improve upon the rate of hydrolysis of RNA and RNA mimic compounds currently attained by mononuclear complexes. As previously stated, lanthanides are toxic to biological systems, because of their similarity to Ca(II) and Mg(II), and hence, for pharmaceutical applications, must to be incorporated into stable macrocyclic ligands. The overall charge on the ligand must remain neutral so as not to depress the hard Lewis acid character of the lanthanide metal ions. For example negatively charged ligands such as EDTA, which are known to strongly bind lanthanide ions, have been found to be unable to promote the cleavage of RNA. However, in order to form a stable complex the neutral ligand must go some way towards fulfilling the lanthanide ions high coordination number requirement, which commonly reach nine or ten and sometimes higher.

The cyclen macrocycle was chosen as the basic template for the ligand in order to allow comparison with mononuclear lanthanide systems previously reported, such as those described earlier, in Chapter 1. Cyclen offers a number of advantages for coordinating lanthanide ions, over other systems. It provides a handle for further functionalisation, and this allows organic catalytically active groups to be incorporated into the design. Cyclen also provides four donor nitrogen atoms, however, additional coordinating donor groups can be incorporated into the macrocycle which, when properly functionalised, is known to virtually encapsulate lanthanide ions forming lanthanide (III) complexes that are inert to dissociation at 37 °C in water at neutral pH.

In order to form a stable lanthanide complex the ligand must provide numerous donor atoms to satisfy the high coordination requirement of the lanthanide ions. Nevertheless, studies have shown that the best hydrolysis rates are achieved when the ion is left coordinatively unsaturated with one or more metal bound water molecules occupying the vacant sites. As such, it was decided to functionalise the cyclen macrocycle with four pendant amide arms. The carboxyl oxygen of the amide groups would help satisfy the high coordination requirement of the lanthanide ion, forming a stable complex. This would ensure that the overall charge of the metal complex would remain positive, thereby maintaining the strong Lewis acid character of the metal ion. In addition, the eight donor sites of the ligand will leave the lanthanide metal ion coordinatively unsaturated.
The desired molecule will be comprised of four parts (Figure 4.1):

- A spacer or linker to link the two metal binding sites
- The two cyclen macrocycles
- The neutral pendant amide arms
- Two lanthanide metal ions

![Figure 4.1. Showing component parts of the intended target molecule a cationic dinuclear complex with overall charge of 6+](image)

### 4.3 Synthesis of Binuclear Lanthanide Complexes

As discussed in Chapter 1, the choice of spacer group has been noted in the literature, as one of the critical factors in the development of cooperative binuclear complexes. Very flexible spacer groups have been shown to give only moderate rate enhancements, of the order of 3–6 fold, over mononuclear systems, as observed by Czarnik who reported the flexible binuclear Co(III) complex, which promoted the hydrolysis of BNPP 6.4 fold faster than the mononuclear analogue. Systems that employ more rigid spacer groups have been found to exhibit a larger cooperative rate enhancement. For example, Morrows xylyl bridged binuclear Cu(II) complex was found to cleave GpppG, a model of the 5’-cap mRNA structure, 46 times faster than the corresponding mononuclear Cu(II) system. However, if the spacer group is too rigid it may be unable to match the desired geometry required for a cooperative mechanism to take effect. For example Canarys binuclear Cu(II) complex, did not give rise to any cooperative enhancement over the corresponding mononuclear system. The spacer must possess enough flexibility to allow both metals to interact with the phosphodiester. The most effective examples to date have utilised rigid
scaffolds, such as calixarenes, that offer a degree of flexibility. It was decided to test a range of spacers, including flexible, alkyl chain spacers and more rigid, aromatic xylylene spacers. The following section will deal with the synthesis of these spacers.

4.3.1 Synthesis of α-Chloroacetamide Spacers

The spacers were synthesised from diamine precursors by reaction with two equivalents of chloroacetyl chloride. This was done to facilitate their incorporation into the macrocycle structure via a S_N2 reaction, where the chlorine atom would act as the leaving group. Initially, the acylation of p-xylylenediamine 119, to yield 1,4-bis-(2-chloro-acetylamino)-xylyene, 120, was carried out in CHCl_3 with Et_3N present as a base. However, as this method led to low yields (11 %) and involved a tedious work up, it was decided to try and optimise the reaction by use of an alternative method (Scheme 4.1).

![Scheme 4.1. Acylation of p-xylylenediamine to yield 1,4-bis (2-chloro-acetylamino) xylylene (120)](image)

Various solvents and conditions were examined, including CH_2Cl_2 and THF, however, no significant improvement was observed (Table 4.1). The problem seemed to stem from the insolubility of 119, which was only fully soluble in alcohols and water. Alcohols react with chloroacetyl chloride to produce esters and so an alternative was sought.

The formation of amides by the reaction of an amine and acid chloride results in the production of an equivalent of HCl, which must be neutralised. Under the classic Schotten-Baumann conditions this HCl is neutralised with NaOH by employing a two phase system consisting of water and CH_2Cl_2. The solvents chosen are immiscible so that the amine and acid chloride remain in the lower CH_2Cl_2 layer, while the NaOH remains in the upper aqueous layer. 

![Figure 4.2: Schotten-Baumann two phase system](image)
However, as previously stated, the diamine 119, is poorly soluble in CH$_2$Cl$_2$, and so in this case, it was decided to modify the reaction by carrying out the acylation under purely aqueous conditions. The diamine 119, along with four equivalents of NaOH were dissolved in H$_2$O and cooled in an acetone/ice bath. Four equivalents of chloroacetyl chloride were added dropwise over one hour. The solution was left to stir overnight and the desired product 120, precipitated out of solution and was isolated by filtration in yields of up to 75 % with no need for further purification.

<table>
<thead>
<tr>
<th>Solvent</th>
<th>Base</th>
<th>Temperature</th>
<th>Yield</th>
</tr>
</thead>
<tbody>
<tr>
<td>CH$_2$Cl$_2$</td>
<td>Et$_3$N</td>
<td>-5 – 20 °C</td>
<td>~5 &gt;13 %</td>
</tr>
<tr>
<td>THF</td>
<td>Et$_3$N</td>
<td>-5 – 20 °C</td>
<td>~5 &gt;13 %</td>
</tr>
<tr>
<td>BuOH</td>
<td>Et$_3$N</td>
<td>-5 – 20 °C</td>
<td>10 %</td>
</tr>
<tr>
<td>MeCN</td>
<td>Et$_3$N</td>
<td>-5 – 20 °C</td>
<td>0 %</td>
</tr>
<tr>
<td>EtOAc</td>
<td>Et$_3$N</td>
<td>-5 – 20 °C</td>
<td>10 %</td>
</tr>
<tr>
<td>EtOAc</td>
<td>DMAP</td>
<td>-5 – 20 °C</td>
<td>.1 %</td>
</tr>
<tr>
<td>Toluene</td>
<td>Et$_3$N</td>
<td>-5 – 20 °C</td>
<td>~10 %</td>
</tr>
<tr>
<td>H$_2$O</td>
<td>NaOH</td>
<td>-10 – 20 °C</td>
<td>75 %</td>
</tr>
</tbody>
</table>

Table 4.1. Effect of various reaction conditions for the synthesis of 120

The reaction was found to be temperature dependant as the yield dropped to 18 % when the reaction was carried out at room temperature. It is believed that this phenomena is due to the chloroacetyl chloride reacting with the H$_2$O at higher temperatures, thereby competing against the desired reaction (Scheme 4.2).

Scheme 4.2. The reaction of chloroacetyl chloride with H$_2$O leads to undesired side products
The $^1$H NMR (DMSO) of the compound displayed four resonances including the NH proton at 8.72 ppm, the four aromatic protons at 7.22 ppm, the four benzyl protons at 4.26 ppm and the four acetamide protons at 4.11 ppm (Figure 4.3). The $^{13}$C NMR (DMSO) revealed five peaks including the carbonyl signal at 165.9 ppm, the aromatic signals at 137.5 and 127.3ppm, as well as two CH$_2$ signals at 42.6 and 42.2 ppm respectively. High resolution mass spectrometry and elemental analysis confirmed that the desired compound had been successfully synthesised.

![Figure 4.3. 400 MHz $^1$H NMR of 120 in DMSO](image)

4.3.1.1 N-Acylation Reactions in Water

Due to the success and simplicity of the above reaction, it was decided to investigate its range and suitability for other N-acylation type reactions. Under the same conditions as employed for 120, the $\alpha$-chloroamides 121 – 124, were synthesised from their corresponding aromatic diamines (Figure 4.4).
Figure 4.4. Compounds 121 – 124 were synthesised from their corresponding aromatic diamines

At this point, it was decided to investigate whether the scope of this reaction could be extended beyond aromatic amines, towards aliphatic amines.

Scheme 4.3. Synthesis of alkyl α-chloroamides and yields after combined CH$_2$Cl$_2$ extraction

The compounds 125 – 128, were synthesised under the same conditions, previously described. Once again the desired products were found to precipitate out of solution however, the yields of these alkyl α-chloroamides were lower than the aromatic α-chloroamides. The alkyl α-chloroamides were found to be more soluble in H$_2$O than the aromatic analogues and hence, in order to increase the yield a CH$_2$Cl$_2$ extraction of the aqueous solution was carried out. Combining the extracted product with that obtained from filtration was found to increase the yield (Table 4.2).
Table 4.2. Showing % yields before (a) and after (b) combined extraction with CH₂Cl₂

The versatility of the reaction was investigated further, by introducing more functional groups into the starting materials to observe whether this would effect the overall reaction. Compounds 129 – 136 were synthesised from their corresponding aromatic amines, using the above general procedure. From Figure 4.5 it can be seen that despite introducing various functional groups onto the benzene ring the reactions proceeded satisfactorily and the overall procedure is a versatile and adaptable way of conducting N-acylation reactions. The advantage of the reaction is the ease of the work up, all the compounds in Figure 4.5 were isolated by simple filtration, with no further need for purification.

Figure 4.5. The products and yields using various functionalised aromatic amines
It should be noted that in general all the starting materials were soluble in aqueous solution, and that all the products precipitated out of solution. However, the starting materials for 131 and 134 were not soluble in aqueous solution. Despite this the reaction still proceeded and the desired products 131 and 134 were isolated, by filtration, in 64 % and 15 % yields respectively. For 131 the starting material, a suspended solid, was observed to undergo a colour change from yellow to white as the reaction proceeded. This highlights the versatile nature of the procedure, as it can even be utilised for insoluble starting materials.

This versatility was further highlighted by the synthesis of 137, 138 and 139. These compounds as well as their amine starting materials are hydrophobic (Figure 4.6). The aromatic amine precursors are liquids at room temperature, and are immiscible with H₂O. However, upon the addition of chloroacetyl chloride, the products precipitated out of the aqueous solution, as white solids, which were again isolated by filtration.

![Figure 4.6. Synthesised hydrophobic α-chloroamides and their yields](image)

In the case of the more hydrophilic pyridine α-chloroamides 140 and 141, the reaction was less useful. 140 and 141 were successfully synthesised, however, they did not precipitate from solution, as they were highly soluble in H₂O. They were isolated after a tedious work up that included the removal of solvent under reduced pressure, followed by suspension of the residue in EtOH before filtration to remove the salt. The EtOH was then evaporated and the residue suspended in acetone and filtered to remove the chloroacetyl chloride. The solid was then dissolved in EtOH and this solution was added slowly to swirling ether to further purify the compounds.

One of the major advantages of the reaction so far has been the ease of work up. The loss of this advantage coupled with the fact that the pyridine systems have an easier, known synthesis in acetone with higher yields,⁻²³¹ negates the practicality of this reaction, for these particular systems.
The reaction procedure was less successful when more extended ring systems were employed. The $N$-acylation reaction was attempted upon the amines of anthracene, phenanthroline and methyl-quinoline under the aqueous conditions. However, the reactions did not proceed to completion and the desired $\alpha$-chloroamide products could not be isolated.

For 144 and 98a, under the same general reaction conditions employed, no precipitate was observed. However, the desired products were isolated, in 42 % and 5 % yield respectively, after a CH$_2$Cl$_2$ extraction of the solution. It is notable that the yields tend to be linked to the solubility of the product in H$_2$O, with less soluble products giving higher yields.
In order to further test the flexibility of the reaction, an acylation with acetic anhydride was attempted under the similar aqueous conditions. In the presence of four equivalents of acetic anhydride and NaOH, \( p \)-amino phenol was found to undergo acylation at both the N and O position (Scheme 4.4). This is further evidence of the adaptability of this reaction.

\[
\begin{align*}
\text{H}_2\text{N} & \quad \text{OH} \\
\text{145} \\
\text{O} & \quad \text{P} \\
\text{H}_2\text{N} & \quad \text{OH} \\
\text{146} & \quad \text{O} \\
\text{H}_2\text{O} & \quad \text{NaOH} \\
\text{147} & \quad 48 \% \text{ yield}
\end{align*}
\]

Scheme 4.4. Acylation of \( p \)-amino phenol with acetic anhydride under aqueous conditions

### 4.3.1.2 The Effect of Changing the Base

An investigation was conducted in order to determine whether the choice of base employed had an effect upon the reaction. Four reactions from the above section, with a range of yields were chosen. From Table 4.3 it can clearly be seen that the choice of base does have an impact upon the yield for the reaction. While no immediate trend for the effect is visible it is clear that the reaction yields are dependant upon the choice of base. Recall that the temperature too can be used to optimise the reaction.

<table>
<thead>
<tr>
<th>Name</th>
<th>NaOH</th>
<th>NEt(_3)</th>
<th>NaHCO(_3)</th>
<th>No Base</th>
</tr>
</thead>
<tbody>
<tr>
<td>128</td>
<td>32 %</td>
<td>27 %</td>
<td>7 %</td>
<td>13 %</td>
</tr>
<tr>
<td>130</td>
<td>64 %</td>
<td>69 %</td>
<td>85 %</td>
<td>74 %</td>
</tr>
<tr>
<td>122</td>
<td>8 %</td>
<td>28 %</td>
<td>14 %</td>
<td>0 %</td>
</tr>
<tr>
<td>120</td>
<td>70 %</td>
<td>39 %</td>
<td>17 %</td>
<td>9 %</td>
</tr>
</tbody>
</table>

Table 4.3. Effect of changing base upon the yields for \( N \)-acylation

The reason for the large changes in yield is that there are, at least, two possible mechanisms at work (Scheme 4.5). Varying the temperature and base, can change which mechanism is favoured.

In order for the desired product to be formed in good yield, \( k_2 \) must proceed faster than \( k_1 \). Recall for 120 that the yield was found to be lower when the reaction was carried out at higher temperatures. This implies that, for 120, \( k_2 \) is thermodynamically favoured while \( k_1 \)
is kinetically favoured. While in depth temperature studies were not performed on the other compounds, it was noted that some compounds displayed a similar dependence. The strength and nature of the base will also influence whether \( k_2 \) or \( k_1 \) is the preferred pathway. Strong bases such as NaOH will increase the rate of \( k_1 \), however this may be offset by a larger increase in the rate of \( k_2 \), depending on the nature of the amine present. As such no simple trend for the effect of the base was observed.

![Scheme 4.5. Competing pathways within the reaction](image)

4.3.1.3 Conclusion

A versatile method for \( N \)-acylation of amines has been shown to function under purely aqueous conditions. It differs from the well known Schotten Bauman method, in that no organic solvent is required. Its usefulness stems from the fact that the solvent, \( \text{H}_2\text{O} \), is cheap and environmentally friendly and as such, would be favoured by industry. The simplicity of the reaction as well as the ease of work up are also major advantages. The reaction has been shown to be successful for a range of amines and diamines, both aromatic and aliphatic. It has also been shown that optimisation of the reaction can be achieved by tuning the temperature and the base employed for each reaction. Some of the bis \( \alpha \)-chloroamides synthesised here will be used as spacers in the next section.

4.3.2 Selective Protection of Cyclen

Having successfully synthesised the bridging moieties, the next step was to introduce them into the cyclen framework. Various methods were explored for the attachment of the cyclen macrocycles to the spacer. Directly reacting the cyclen with the spacer was unsatisfactory due to the formation of numerous possible side products and polymers. a
variety of methods for the mono alkylation of cyclen have been described in the literature, most proceed via the protection of three of the cyclen nitrogens, leaving the fourth available for alkylation. A number of these methods were attempted in this project including tert-butyl-carbonate (Boc) protection and formyl (CHO) protection methods (Scheme 4.6). 

The tris Boc protected cyclen, 1,4,7,10-tetraaza-cyclododecane-1,4,7-tricarboxylic acid tri-tert-butyl ester, 148, was synthesised by stirring cyclen with three equivalents of di-tert-butyl-dicarbonate (Boc₂O), in CHCl₃ in the presence of Et₃N. The desired product 148, was obtained in 57 % yield after purification by flash silica column chromatography using EtOAc:Hexane 5:1 as the eluent. The alternative tris protected compound, 1,4,7,10-tetraaza-cyclododecane-1,4,7-tricarbaldehyde, 149, was synthesised by stirring cyclen with six equivalents of chloral-hydrate in EtOH at 60 °C for three hours. The desired product, 149, was obtained in 87 % yield after purification by flash silica column chromatography using DCM:MeOH 9:1 as the eluent. The advantage of these protecting groups is that they are labile and can be easily removed, they are not air sensitive and hence, can be stored and used later. Normally, alkylation of 148 and 149 is carried out in MeCN, however, the spacer 120, was insoluble in MeCN, and so the reactions were carried out in DMF in the presence of Cs₂CO₃. Unfortunately, under these conditions the reaction did not proceed (Scheme 4.6).

Scheme 4.6. Synthesis of tris protected cyclen derivatives 148 and 149
An alternative method for the protection of cyclen using molybdenum hexacarbonyl\(^\text{236}\) was found to be the most consistently effective method, despite the drawback that the product \(150\), is known to be air sensitive. The protection was conducted according to the literature procedure.\(^\text{236}\) The cyclen and molybdenum hexacarbonyl were suspended in dry dibutyl ether and freeze-pump-thawed three times in order to remove any oxygen. The suspension was then refluxed for two hours under argon. The product, \(1,4,7\)-molybdenum tris carbonyl-(1,4,7,10-tetraaza-cyclododecane) \(150\), a bright yellow solid, was then isolated by filtration in 88\% yield using the special suction filtration apparatus shown in Figure 4.10. This reaction required scrupulously anhydrous conditions and all glassware joints had to be sealed with parafilm. The compound \(150\), was treated as a reaction intermediate due to its air sensitive nature and was not characterised, but reacted on immediately. The following section will discuss the bridging of two of these protected cyclen molecules by the spacer.

![Figure 4.10](image_url)

**Scheme 4.7. Protection of cyclen with molybdenum hexacarbonyl**

### 4.3.3 Synthesis of Bis-Cyclen Framework

Coupling the macrocycles to the spacer involved freeze-pump-thawing \(120\) with two equivalents of \(150\), two equivalents of \(\text{CS}_2\text{CO}_3\) and two equivalents of \(\text{KI}\) in DMF. The solution was then stirred at 85 °C for 40 hours and then filtered. The DMF was removed under reduced pressure and the resulting residue was left stirring overnight in an aqueous solution of HCl \((15\% \text{ v/v})\), in order to deprotect the cyclen (Scheme 4.8). The acidic solution was then filtered and washed with \(\text{CH}_2\text{Cl}_2\), before being basified (pH ~ 13) with
KOH and extracted with CH$_2$Cl$_2$. The CH$_2$Cl$_2$ was removed under reduced pressure and the desired product 2-(1,4,7,10-tetraaza-cyclododec-1-yl)-N-{4-[(2-1,4,7,10-tetraaza-cyclododec-1-yl-acetylamino)-methyl]-benzyl} -acetamide 152, was isolated as a yellow oil in 41 % yield. Once again the reaction required scrupulously anhydrous conditions and all glassware joints had to be sealed with parafilm. Even still, the reaction was problematic and very sensitive to the conditions employed and frequently the desired compound could not be isolated.

![Scheme 4.8. Synthesis of 152 proceeded with 41 % yield](image)

The inherent symmetry of 152 was evident in the $^1$H NMR (Figure 4.11) as well as the $^{13}$C NMR spectra. The aromatic protons were clearly visible as a singlet at 7.25 ppm, however, their position obscured the CDCl$_3$ signal. The four benzyl protons were observed at 4.38 ppm, while the four acetamide protons were observed at 3.19 ppm. The $^{13}$C NMR spectra showed the presence of one carbonyl group at 171.1 ppm, while the aromatic signals were observed at 137.2 and 127.7 ppm respectively. The mass spectrum presented signals at $m/z$ 561.4, 583.4, 599.4 and 281.2 corresponding to the [M+H], [M+Na], [M+K] and [M+H]/2 species respectively. A high resolution mass spectrum of 152 was obtained, confirming the synthesis of the product, although, the hygroscopic nature of the compound meant that elemental analysis could not be successfully obtained.
The hexane spacer (128) analogue of 152 was also synthesised under similar conditions by stirring 128 with two equivalents of 150 in DMF at 85 °C for 40 hours, in the presence of two equivalents of Cs₂CO₃ and two equivalents of KI. The procedure was similar to that of 152, however the desired product 2-(1,4,7,10tetraaza-cyclododec-1-yl)-N-[6-(2-1,4,7,10tetraaza-cyclododec-1-yl-acetylamino)-hexyl]-acetamide 153, was isolated as its HCl salt in 37 % yield after precipitation from a EtOH:conc. HCl 5:1 solution.

Again the ¹H NMR in D₂O was relatively simple due to the high degree of symmetry in the molecule. The four acetamide protons were observed at 3.31 ppm, while the cyclen protons resonated two signals at 3.06 ppm and 2.88 ppm respectively. The three sets of methylene protons from the alkyl spacer were observed at 2.88 ppm (overlapping with 8 cyclen protons), 1.39 ppm and 1.20 ppm respectively. The ¹³C NMR showed the presence of a carbonyl signal at 172.4 ppm along with eight methylene resonances. The mass spectrum contained two signals at m/z 541.6 and 271.4 corresponding to the [M+H] and [M+2H/2] species. Elemental analysis confirmed the presence of the six HCl molecules.

Two basic templates for a binuclear system had now been synthesised. One contained a rigid aromatic xyylene spacer, while the other contained a flexible alkyl spacer. However,
in their present form, they would not satisfy the high coordination requirement of the lanthanide ions. Consequently, further functionalisation was required, and that will be the subject of the next section.

4.3.4 Synthesis of Acetamide-Based Bis-Cyclen Ligands 154-157

In order to accommodate the lanthanide ions high coordination number requirement, 9 and 10 for Eu(III) and La(III) respectively, the incorporation of further donor atoms into the bis-cyclen ligands 152 and 128, was necessary. This was accomplished by functionalising the cyclen moieties with pendant amide arms via a $\text{Sn}_2$ alkylation reaction. Initially simple acetamide arms were investigated in order to probe the validity of the bis lanthanide systems as cleaving agents for phosphodiester hydrolysis as they would allow comparison with analogous mononuclear lanthanide systems previously reported by Morrow et al.²²³

![Scheme 4.9. General synthesis of acetamide-based bis-cyclen ligands 154, 155 and 156](image)

<table>
<thead>
<tr>
<th>Compound</th>
<th>$R$ group</th>
<th>Yield</th>
</tr>
</thead>
<tbody>
<tr>
<td>154</td>
<td>$R_1, R_2 = H$</td>
<td>57%</td>
</tr>
<tr>
<td>155</td>
<td>$R_1 = H, R_2 = \text{CH}_3$</td>
<td>30%</td>
</tr>
<tr>
<td>156</td>
<td>$R_1, R_2 = \text{CH}_3$</td>
<td>38%</td>
</tr>
</tbody>
</table>
The three pendant arms employed in this case were, 2-bromo-acetamide, 98c, 2-chloro-\(N,N\)-dimethyl-acetamide, 98a, and 2-chloro-\(N\)-methyl-acetamide, 98b. The bis-cyclen ligand 152, was only sparingly soluble in solvents such as MeCN, \(\text{CH}_2\text{Cl}_2\) and \(\text{CHCl}_3\) and using these solvents, the reaction did not proceed. Consequently, the reactions were carried out in refluxing EtOH in the presence of \(\text{Cs}_2\text{CO}_3\) and KI, as the above compounds were all soluble in this solvent.

The primary amide 2-{4,10-bis-carbamoylmethyl-7-[4-{2-(4,7,10-tris-carbamoylmethyl-1,4,7,10-tetraaza-cyclododec-1-yl)-acetylamino]-methyl}-benzylcarbamoyl}-methyl]-1,4,7,10-tetraaza-cyclododec-1-yl]-acetamide, 154, was isolated in 57% yield, after precipitation from \(\text{CH}_2\text{Cl}_2\), followed by recrystallisation from boiling EtOH. The secondary amide 2-{4,7-bis-methylcarbamoylmethyl-10-[4-{2-(4,7,10-tris-methylcarbamoylmethyl-1,4,7,10-tetraaza-cyclododec-1-yl)-acetylamino]-methyl}-benzylcarbamoyl}-methyl]-1,4,7,10-tetraaza-cyclododec-1-yl]-acetamide, 155, was isolated in a similar fashion in 30% yield. The tertiary amide 2-{4,10-bis-dimethylcarbamoylmethyl-7-[4-{2-(4,7,10-tris-dimethylcarbamoylmethyl-1,4,7,10-tetraaza-cyclododec-1-yl)-acetylamino]-methyl}-benzylcarbamoyl}-methyl]-1,4,7,10-tetraaza-cyclododec-1-yl]-\(N,N\)-dimethyl-acetamide, 156, was found to be soluble in \(\text{CH}_2\text{Cl}_2\) and so was isolated in 38% yield after washing with \(\text{H}_2\text{O}\) and 0.1 M HCl.

![Figure 4.12. 400 MHz \(^1\text{H}\) NMR of 154 in \(\text{D}_2\text{O}\)](image)
The $^1$H NMR in D$_2$O of 154 revealed the high degree of symmetry in the molecule. The four aromatic protons resonanced at 7.16 ppm while the four benzyl protons were observed at 4.26 ppm (Figure 4.12). The $^{13}$C NMR revealed the presence of three carboxyl signals at 173.8, 173.4 and 170.2 ppm respectively, while the aromatic carbon signals were observed at 136.6 and 127.1 ppm, respectively. Eight methylene signals were also observed at 58.1, 56.9, 56.7, 56.1, 55.5, 55.3, 50.2 and 42.2 ppm respectively. High resolution mass spectrometry and elemental analysis confirmed that the product had been formed. Characterisation of 155 and 156 yielded similar results.

The hexane linker analogue of 154, was also synthesised by refluxing 153 in EtOH, with seven equivalents of bromo acetamide in the presence of fourteen equivalents of Et$_3$N (Scheme 4.10). A large excess of Et$_3$N was needed, as 153 was present as a hexa-HCl salt. The desired product 2-[4,7-bis-carbamoylmethyl-10-\{6-[2-(4,7,10-tris-carbamoylmethyl-1,4,7,10-tetraaza-cyclododec-1-yl)-acetylamino]-hexylcarbamoyl\}-methyl]-1,4,7,10-tetraaza-cyclododec-1-yl]-acetamide 157, was isolated in 83% yield, after precipitation from CH$_2$Cl$_2$ followed by precipitation from i-propanol:EtOH 3:1.

![Scheme 4.10. Synthesis of hexyl spaced acetamide bis-cyclen ligand 157](image-url)

The $^1$H NMR (D$_2$O) revealed the high degree of symmetry inherent in the molecule (Figure 4.13), as four of the methylene resonances of the alkyl chain appeared at 1.38 and 1.19 ppm respectively. The $^{13}$C NMR revealed three carbonyl peaks at 174.2, 173.5 and 169.7 ppm as well as eight methylene resonances at 56.1, 55.6, 50.1, 49.8, 48.9, 38.9, 27.8 and 25.3 ppm respectively (only two cyclen signals, confirmed by CHCOSY). The mass
spectrum consisted of two peaks \( m/z \) 883.7 and 442.5 corresponding to the \([M+H]\) and \([M+2H/2]\) signals. Elemental analysis confirmed the characterisation of the molecule.

![Figure 4.13. 400 MHz ^1H NMR of 157 in D_2O](image)

In summary, four new ligands 154, 155, 156 and 157, incorporating three different pendant arms and two different spacer groups, have been made and characterised. The next step was to synthesise the corresponding lanthanide complexes from these ligands, in order to produce potential ribozyme mimics. This process is described in the next section.

### 4.3.5 Complexation of Acetamide-Based Bis-Cyclen Ligands

Europium and lanthanum were chosen as the lanthanide ions in order to allow comparison to similar mononuclear systems previously reported. The lanthanide complexes of the ligands 154, 155, 156 and 157 were prepared by refluxing each ligand with two molar equivalents of the relevant lanthanide triflate in MeOH for 16 hours (Scheme 4.11). The resulting solution was filtered and reduced in volume to \( \sim 5 \) mL. This solution was added dropwise to swirling ether and the resulting precipitate was collected by filtration. The complexes Eu.154, La.154, Eu.155, La.155, Eu.156 and La.157 were further purified by
precipitation from CH$_2$Cl$_2$, and isolated by suction filtration. This gave the complexes in pure form.

![Chemical structure](image)

<table>
<thead>
<tr>
<th>Compound</th>
<th>R group</th>
<th>Yield</th>
</tr>
</thead>
<tbody>
<tr>
<td>Eu.154</td>
<td>R$_1$, R$_2$ = H</td>
<td>80 %</td>
</tr>
<tr>
<td>La.154</td>
<td>R$_1$, R$_2$ = H</td>
<td>88 %</td>
</tr>
<tr>
<td>Eu.155</td>
<td>R$_1$ = H, R$_2$ = CH$_3$</td>
<td>81 %</td>
</tr>
<tr>
<td>La.155</td>
<td>R$_1$ = H, R$_2$ = CH$_3$</td>
<td>69 %</td>
</tr>
<tr>
<td>Eu.156</td>
<td>R$_1$, R$_2$ = CH$_3$</td>
<td>47 %</td>
</tr>
<tr>
<td>La.157 (CH$_2$)$_6$</td>
<td>R$_1$, R$_2$ = H</td>
<td>87 %</td>
</tr>
</tbody>
</table>

Scheme 4.11. Complexation of 154

Paramagnetic lanthanide ions, like Eu(III), are known to be NMR shift reagents. In this case the methylene resonances of the acetamide and the cyclen ring protons experience an extra magnetic field due to the proximity of the paramagnetic Eu(III) ion. This extra magnetic field causes a shift in the position of the affected protons signal, the extent of which is determined by the protons proximity to the metal centre. This can be seen from the Figure 4.14, as the axial protons, H$_{ax}$, will experience the magnetic field of the metal ion to a
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greater extent than the equatorial protons, $H_{eq}$. In the case of Eu.154 the shifted methylene protons came between 26.16 and $-13.56$ ppm. The $^1H$ NMR spectra of the complexes consisted of broad signals due to the fast relaxation times induced by the paramagnetic metal ion, which precluded full characterisation.

![Diagram of Eu.154](image)

**Figure 4.15.** 400 MHz $^1H$ NMR spectrum in D$_2$O of Eu.154. Shifted peaks show Eu(III) complexation has occurred

The $^1H$ NMR spectrum of Eu.154 (D$_2$O) (Figure 4.15) showed peaks at 26.16, $-2.66$, $-5.25$, $-9.09$, $-11.28$, and $-13.56$ ppm. These peaks are indicative of the Eu(III) shifted, axial and equatorial protons of the cyclen and acetamide CH$_2$’s. This was clear evidence that the europium had complexed to the cyclen ligand. The ESMS (Figure 4.16) also showed that complexation had occurred. It revealed a number of peaks all of which related to the desired complex, as seen by the characteristic europium isotope distribution pattern observed. The range of peaks seen were due to the method of ionisation and corresponded to the $m/z$ of the complex with differing numbers of triflate counter ions i.e. the signals at $m/z = 901.1$ and 376.5 corresponded to the [M+4Trif]/2 and [M+2Trif]/4 species respectively. Elemental analysis confirmed the characterisation of this complex.
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Figure 4.16. Europium isotope distribution pattern. [M+4triflates]/2

The europium complexes, Eu.155 and Eu.156, gave similar results. Their $^1$H NMR spectra in D$_2$O again showed the expected shifted cyclen resonances at ~ 30, −1, −5, −8, −12 and −16 ppm, clear evidence that complexation had occurred. The europium isotope distribution pattern could again be observed in the mass spectrum for peaks corresponding to the complex with numerous triflate counter ions.

Figure 4.17. $^1$H NMR of La.157 in D$_2$O showing broadened peaks
The lanthanum ion is not paramagnetic and as a result the shifted peaks observed in the $^1H$ NMRs of the Eu(III) complexes were not observed for the La(III) complexes. However, lanthanide ions are known for their fast NMR relaxation properties. Indeed, it is this property that is utilised for medicinal purposes in MRI contrast agents such as [Gd(DOTA)(H$_2$O)].$^{241}$ The strong relaxation properties of the La(III) ion result in broadened spectral peaks in the NMR of its complexes. This can be seen in the $^1H$ NMR of La$_{157}$ (Figure 4.17).

The $^1H$ NMR of the lanthanum complexes, La$_{154}$ and La$_{155}$ showed similar broadened peaks. The La(III) complexes were also characterised by other conventional methods, ESMS, IR and elemental analysis.

Infrared (IR) spectroscopy is a useful method in determining whether a complex has been formed as the vibrational bands associated with the bonds involving the coordinating atoms, usually move to lower frequency (i.e. lower energy), upon complexation of a metal ion.$^{242}$ In this case the ligands carbonyl bond is expected to donate electron density to the metal centre, resulting in an increase in the bond length and a concomitant decrease in the IR stretching frequency. This was indeed found to be the case for the ligands 154 – 157, as upon complexation of a metal ion, the IR stretching frequency of the carbonyl band was changed (Table 4.4).

<table>
<thead>
<tr>
<th>Compound</th>
<th>Ligand (cm$^{-1}$)</th>
<th>La Complex (cm$^{-1}$)</th>
<th>Eu Complex (cm$^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>154</td>
<td>1678</td>
<td>1634</td>
<td>1665</td>
</tr>
<tr>
<td>155</td>
<td>1657</td>
<td>1638</td>
<td>1638</td>
</tr>
<tr>
<td>156</td>
<td>1647</td>
<td>–</td>
<td>1621</td>
</tr>
<tr>
<td>157</td>
<td>1670</td>
<td>1635</td>
<td>–</td>
</tr>
</tbody>
</table>

Table 4.4. IR carbonyl stretching frequencies decrease upon complexation of metal ion

For the amide bis-cyclen Eu(III) complexes Eu$_{154}$, Eu$_{155}$ and Eu$_{156}$, the octadentate ligands were expected to leave one free coordination site, and as such, each Eu(III) metal centre was expected to possess one metal bound water molecule, in a similar manner to that previously observed for the analogous mononuclear systems.$^{223}$ In order to confirm this, the luminescent lifetimes ($\tau$) of the complexes excited states were measured in D$_2$O and
H₂O, after direct excitation of the Eu(III) ion at 395 nm. The lifetimes were used to
determine the number of metal bound water molecules, or q value, using equation (i),
previously discussed in Chapter 2:¹²³,²⁴⁴,²⁴⁵

\[ q^{\text{Eu(III)}} = 1.2 \left[ \frac{1}{\tau_{\text{H}_2\text{O}}} - \frac{1}{\tau_{\text{D}_2\text{O}}} \right] - 0.25 - 0.075x \]

As discussed in Chapter 2, the prefix 1.2, is a proportionality constant that reflects the
sensitivity of the europium ion to quenching by metal bound water molecules. The
correction term – 0.25 represents quenching by second sphere water molecules, while –
0.075x represents the quenching by N-H oscillators, where x is the number of such
oscillators bound to the complex. In the cases of Eu.154, Eu.155 and Eu.156, the
number of coordinated amide NH groups x, were seven, four and one respectively.
The results of these measurements are shown in Table 4.5. As expected, each of the Eu(III)
complexes were found to have one metal bound water molecule per Eu(III) ion.

<table>
<thead>
<tr>
<th>Complex</th>
<th>x</th>
<th>τ_{H₂O} (ms)</th>
<th>k_{H₂O} (1/ms)</th>
<th>τ_{D₂O} (ms)</th>
<th>k_{D₂O} (1/ms)</th>
<th>q (±0.5)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Eu.154</td>
<td>7</td>
<td>0.507</td>
<td>1.971</td>
<td>2.265</td>
<td>0.440</td>
<td>0.91</td>
</tr>
<tr>
<td>Eu.155</td>
<td>4</td>
<td>0.553</td>
<td>1.808</td>
<td>2.451</td>
<td>0.408</td>
<td>1.02</td>
</tr>
<tr>
<td>Eu.156</td>
<td>1</td>
<td>0.553</td>
<td>1.808</td>
<td>1.851</td>
<td>0.541</td>
<td>1.13</td>
</tr>
</tbody>
</table>

Table 4.5. Lifetime measurements revealed that each Eu(III) ion possessed one metal
bound water molecule

Unfortunately the q values for La(III) complexes can not be measured in the same way,
however, it is reasonable to assume that the coordination environment of the binuclear
La(III) systems is similar to the mononuclear systems, and that each La(III) ion possesses
two metal bound water molecules.²²³

In summary this section has described the synthesis of six novel binuclear lanthanide
complexes Eu.154, La.154, Eu.155, La.155, Eu.156 and La.157. Lifetime studies have
revealed that each of the Eu(III) complexes possesses one metal bound water molecule per
Eu(III) ion, while the La(III) complexes are expected to possesses two. The ability of these
complexes to promote the hydrolysis of phosphodieters, shall be investigated later in this
chapter. At this point it was of interest to see whether changing the pendant arm from the
4.3.6 Changing the Pendant Arm

The nature of the ligand itself is known to play a role in modulating the properties of the metal ion. For instance, the Lewis acidity of a metal ion will be modulated by the nature of the ligand and mirrored by a change in the $pK_a$ of any metal bound water molecules.$^{246}$ Previous investigations carried out using mononuclear lanthanide systems have highlighted the importance of the nature of the pendant arms.$^{220, 249}$ Natural ribozymes, such as RNaseA, are known to utilise groups such as histidine, which can provide acid-base catalysis to promote hydrolysis of RNA.$^{247}$ Introducing a pendant group, which can function as an acid or a base, into the ligand of a ribozyme mimic can also promote the rate of phosphodiester hydrolysis. This has been demonstrated by several researchers including Hamilton et al. who demonstrated that incorporating pendant tertiary amine groups increased the activity of $^{82}$ by providing general base catalysis.$^{248}$ The pyridine functional group can act as a general base catalyst and complexes incorporating pyridine pendant arms, such as Ln$^{158}$, have been shown to be highly successful cleaving agents by Gunnlaugsson et al.$^{249}$ The synthesis of binuclear systems incorporating these pendant arms was desired, in order to improve upon the accelerations already obtained by mononuclear systems.

Amino acids such as His and Arg, are utilised by natural ribozymes,$^{247}$ and indeed amino acids such as poly(Leu-Lys) have been shown to promote the cleavage of ApAp.$^{250}$ Gunnlaugsson et al. have shown that ribozyme mimics incorporating pseudo dipeptide...
pendant arms (Ala, Gly, Leu. etc.), increased the rate of hydrolysis of phosphodiesters. Of these systems, the 4-armed glycine methyl ester complex La.39, was found to give the greatest rate enhancement and this was attributed to the ability to form hydrophobic cavities. The following sections shall describe the synthesis of binuclear lanthanide systems incorporating glycine methyl ester and pyridine pendant arms.

4.3.7 Synthesis of Glycine and Pyridine Pendant Arms

The α-chloroamide arm, (2-chloro-acetylamino)-acetic acid methyl ester, 159, was synthesised according to the literature procedure by stirring the HCl salt of glycine methyl ester with one equivalent of chloroacetyl chloride, in an ice bath, overnight, in the presence of Et3N in CH2Cl2. The desired product was isolated as an oil in 60 % yield after aqueous work up. The 1H NMR of 159 agreed well with the published characterisation, showing four signals including the NH proton as a singlet at 7.11 ppm, and the “Gly” methylene resonances as a doublet \( J = 6.0 \text{ Hz} \) at 3.95 ppm. The three methoxy protons were observed at 3.64 ppm.

Of the three possible pyridine isomers, previous investigations in the Gunnlaugsson group had revealed the mononuclear 3-pyridine isomer was the most effective at accelerating the rate of phosphodiester hydrolysis of HPNP, and hence it was decided to utilise this isomer in the synthesis of the binuclear system. The α-chloroamide pyridine arm, 2-chloro-N-pyridin-3-yl-acetamide, 140, was also synthesised according to the literature procedure by stirring 3-aminopyridine with chloroacetyl chloride in acetone overnight at room temperature. The desired product precipitated out of solution as its HCl salt in 86 % yield. The characterisation agrees with the literature, M.P. 134 - 135.6 °C (Lit M.P. 134 - 135 °C).

The next section shall describe the introduction of these α-chloroamide arms into the cyclen ligand framework.

4.3.8 Synthesis of 3-Armed Cyclen Systems

Initially, an attempt was made to functionalise the bis-cyclen ligand, 152, with the pendant arms 159 and 140, as illustrated in Scheme 4.12, however, this proved unsuccessful, as the reactions did not proceed. Various solvent conditions were employed including EtOH, MeOH and DMF, however, no trace of the desired compound was detected. Solvent had
been previously noted as a key factor in the formation of the mononuclear 4-armed ligand 39, as the reaction only proceeded in MeCN.\textsuperscript{237} The bis-cyclen ligand 152, was however, insoluble in MeCN and hence, yet again the reaction did not proceed.

![Scheme 4.12. Attempted synthesis of Glycine and Pyridine bis-cyclen](image)

An alternative approach was attempted, involving the synthesis of the 3-armed cyclen ligand by stirring three equivalents of 159 in MeCN at 60 °C, with one equivalent of cyclen, in the presence of three equivalents of NaHCO\textsubscript{3} (Scheme 4.13). The reaction, had previously been described by Ann Marie Fanning,\textsuperscript{290} and was monitored by mass spectrometry, which showed the formation of three new species, the di, tri and tetra functionalised cyclen moieties. The desired 3-armed ligand, (2-{4,7-bis-[(methoxycarbonylmethyl-carbamoyl)-methyl]-1,4,7,10-tetraaza-cyclododec-1-yl}-acetylamino)-acetic acid methyl ester, 160, was isolated from this mixture by alumina column chromatography using CH\textsubscript{2}Cl\textsubscript{2}/MeOH(NH\textsubscript{3}) gradient elution, 0 → 10 %, to give a white foam in 27 % yield.

![Scheme 4.13. Formation of 3-armed glycine cyclen 160, proceeded with 27 % yield](image)

The mass spectrum of the compound 160, showed one peak at m/z 560.2 corresponding to the M+H species. The CDCl\textsubscript{3} \textsuperscript{1}H NMR (Figure 4.18) clearly showed the presence of a C\textsubscript{2} plane of symmetry running through the molecule with the amine proton visible as a broad singlet at 9.38 ppm, while two amide NH signals were observed at 8.69 and 8.38 ppm.
The $^{13}$C NMR showed four carbonyl peaks at 172.2, 171.1, 170.5 and 169.6 ppm, while the methoxy signals were observed at 51.7 and 51.2 ppm. Elemental analysis confirmed the characterisation of the product.

![Figure 4.18. 400 MHz $^1$H NMR in CDCl$_3$ of 160 exhibiting C$_2$ plane of symmetry](image)

The 3-armed pyridine system, 2-[4,7-bis-(pyridin-3-yl-carbamoylmethyl)-1,4,7,10-tetraaza-cyclododec-1-yl]-N-pyridin-3-yl-acetamide, 161 was synthesised in a similar fashion by refluxing cyclen with three equivalents of 140 in MeOH for 36 hours, with Cs$_2$CO$_3$ and KI. The desired compound was isolated by alumina column chromatography using CH$_2$Cl$_2$/MeOH(NH$_3$) gradient elution, 0 → 10 %, to give a white solid in 29 % yield. The mass spectrum showed two peaks at $m/z$ 597.3 and 575.3 corresponding to M+Na and M+H, respectively. Again the $^1$H NMR showed there was a C$_2$ plane of symmetry through the molecule as there were two signals for each pyridine position. The $^{13}$C NMR revealed two carbonyl signals at 171.4, and 170.9 ppm. Again there were two signals for each pyridine position and six methylene resonances were also observed. High resolution mass spectrometry confirmed the that the desired product had been obtained.

Once the two 3-armed cyclen systems incorporating the different pendant arms had been synthesised, the next step was to use them to make binuclear systems, by bridging two of the molecules with an appropriate spacer group. The following section will detail how this was achieved.

### 4.3.9 Synthesis of Bis-Pyridine and Bis-Glycine Ligands

<table>
<thead>
<tr>
<th>Compound</th>
<th>X</th>
<th>Yield</th>
</tr>
</thead>
<tbody>
<tr>
<td>162</td>
<td></td>
<td>41 %</td>
</tr>
<tr>
<td>163</td>
<td>(CH₂)₄</td>
<td>41 %</td>
</tr>
<tr>
<td>164</td>
<td>(CH₂)₆</td>
<td>52 %</td>
</tr>
</tbody>
</table>

Scheme 4.15. General synthesis of glycine-bis-cyclen systems
In order to form the desired binuclear systems, the 3-armed cyclen systems had to be linked together with a spacer group. Initially, the 3-armed glycine system 160, was bridged with the aromatic xylylene spacer 120. This was achieved by stirring 160 together with half an equivalent of 120, at 80 °C in DMF, in the presence of Cs2CO3 and KI, for five days. The desired product, \{2-[4,7-bis-[(methoxycarbonylmethyl-carbamoyl)-methyl]-10-(4-[2-{4,7,10-tris-[(methoxycarbonylmethyl-carbamoyl)-methyl]-1,4,7,10-tetraaza-cyclo dodec-1-yl]-acetylamino]-methyl]-benzylcarbamoyl]-methyl]-1,4,7,10-tetraaza-cyclo dodec-1-yl]-acetylamino\}-acetic acid methyl ester, 162, was isolated by precipitation from acetone in 41 % yield (Scheme 4.15).

The 1H NMR of 162 in D2O, exhibited quite broad peaks, however, once again the high degree of symmetry was evident (Figure 4.19). The four aromatic protons were observed at 7.16 ppm, while the four benzyl protons could be resonated at 4.28 ppm. The methoxy CH3's and “Gly” CH2's resonated between 3.64 and 3.56 ppm, while the cyclen protons were observed at 2.71 ppm. The 13C NMR showed three carbonyl signals at 175.3, 171.3 and 171.1 ppm. The aromatic signals were observed at 136.5 and 127.0 ppm and the two methoxy CH3's resonated at 52.3 and 52.2 ppm, while ten methylene resonances were also observed.

Figure 4.19. 400 MHz 1H NMR of 162 in D2O. Broad signals, due to fast relaxation.
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Both [2-(4,7-bis-[(methoxycarbonylmethyl-carbamoyl)-methyl]-10-{{4-(2-{4,7,10-tris-[(methoxycarbonylmethyl-carbamoyl)-methyl]-1,4,7,10-tetraaza-cyclododec-1-yl}-acetyl-amino)-butylcarbamoyl]-methyl}-1,4,7,10-tetraaza-cyclododec-1-yl]-acetylamino]-acetic acid methyl ester, 163 and [2-(4,7-bis-[(methoxycarbonylmethyl-carbamoyl)-methyl]-10-{{6-(2-{4,7,10-tris-[(methoxycarbonylmethyl-carbamoyl)-methyl]-1,4,7,10-tetraaza-cyclododec-1-yl}-acetylamino)-hexylcarbamoyl]-methyl}-1,4,7,10-tetraaza-cyclododec-1-yl]-acetylamino]-acetic acid methyl ester, 164, were synthesised, using the butane spacer 126, and hexane spacer 128, respectively, under the same reaction conditions, in 41 % and 52 % yield, respectively. Three new glycine bis-cyclen ligands 160, 163 and 164, with different spacer groups have now been synthesised.

In a similar manner the 3-armed pyridine cyclen 161 was coupled to the spacer 120 by heating in DMF at 85 °C, in a 2:1 molar ratio, in the presence of Cs$_2$CO$_3$ and KI for 5 days, after which, the solution was filtered and the DMF removed under reduced pressure (Scheme 4.16). The desired product, 2-[4,7,10-tris-(pyridin-3-ylcarbamoylmethyl)-1,4,7,10-tetraaza-cyclododec-1-yl]-N-{4-(2-[4,7,10-tris-(pyridin-3-ylcarbamoylmethyl)-1,4,7,10-tetraaza-cyclododec-1-yl]-acetylamino}-methyl]-benzyl]-acetamide, 165, was isolated in 41 % yield after precipitation from ether, to remove any DMF, followed by precipitation from acetone, to further purify the ligand.

Scheme 4.16. Synthesis of pyridine bis-cyclen 165, 41 % yield
The $^1$H NMR in CD$_3$OD revealed the symmetry of the molecule, as there were two sets of pyridine resonances (Figure 4.20). Two pyridine H2' protons resonated at 8.62 ppm, while the four pyridine H2 protons were observed at 8.47 ppm. The multiplet at 8.03 ppm corresponded to the twelve H4 and H6 pyridine protons. The four benzene protons were visible as a singlet at 7.12 ppm, while the six pyridine H5 protons were seen at 6.90 ppm as a double doublet ($J = 6.0$ Hz, $J' = 6.0$ Hz). The methylene resonances were observed as a broad multiplet integrating for 48H at 2.64 ppm. The $^{13}$C NMR revealed five CH$_2$ signals including one broad signal for the cyclen carbons at 50.1 ppm. Twelve aromatic signals along with three carbonyl signals were also observed. Elemental analysis confirmed the characterisation of the molecule. Unfortunately, due to time constraints, the more flexible analogues of this binuclear pyridine system were not synthesised.

This section has described the development of four new ligands 162, 163, 164 and 165, incorporating two different pendant arms and three different spacer groups. The next step was to introduce the lanthanide ions into these ligands to form the corresponding complexes, in order to produce potential ribozyme mimics. This process shall be detailed in the following section.

![Figure 4.20. 400 MHz 1H NMR of 165 in MeOD](image-url)
4.3.10 Complexation of Bis-Pyridine and Bis-Glycine Ligands

The lanthanum complex of 162 was formed by refluxing two equivalents of La(CF₃SO₃)₃ with 162 in MeOH overnight. The product La.162, was isolated in 90 % yield, after precipitation from ether followed by precipitation from CH₂Cl₂. Similarly, the lanthanum complexes of the bis-glycine cyclen ligands, 163, 164 and 165 were synthesised in 72 %, 85 % and 83 % yield, respectively. The europium complex of 164 was also synthesised, in 66 % yield. Again time constraints meant that only one of the ligands was complexed with europium. The resulting lanthanum complexes each showed the characteristic broadening in their ¹H NMR spectra, while the Eu.164 ¹H NMR showed the characteristic Eu(III) shifted peaks. The changes in the IR carbonyl stretching frequency were also notable, showing that complexation had occurred (Table 4.6). Unfortunately, the mass spectrum of these complexes could not be obtained. The hygroscopic nature of the glycine complexes precluded the obtainment of elemental analysis, nevertheless, elemental analysis was obtained for the pyridine complex La.165, and confirmed that the complex had been successfully synthesised.

<table>
<thead>
<tr>
<th>Compound</th>
<th>Ligand (cm⁻¹)</th>
<th>La Complex (cm⁻¹)</th>
<th>Eu Complex (cm⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>GBC 162</td>
<td>1663</td>
<td>1627</td>
<td>-</td>
</tr>
<tr>
<td>GBB 163</td>
<td>1655</td>
<td>1638</td>
<td>-</td>
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<tr>
<td>GBH 164</td>
<td>1662</td>
<td>1638</td>
<td>1638</td>
</tr>
<tr>
<td>PBC 165</td>
<td>1685</td>
<td>1654</td>
<td>-</td>
</tr>
</tbody>
</table>

Table 4.6. Showing changes in IR carbonyl stretching frequencies upon lanthanide complexation

This section has described the synthesis of five novel binuclear lanthanide complexes La.162, La.163, La.164, Eu.164 and La.165 incorporating two types of pendant arm, namely glycine methyl ester and pyridine arms. The general base catalytic ability of the pyridine cofactors, and the hydrophobic nature of the glycine cofactors may lead to greater acceleration of phosphodiester hydrolysis. Lifetime studies again revealed that the Eu(III) complex Eu.164, possesses one metal bound water molecule per Eu(III) ion, while the La(III) complexes are expected to possess two. The ability of these complexes to promote the hydrolysis of phosphodieters, shall be investigated later in this chapter. At this point, the synthesis of one final complex, which utilised an alternative bridge, was desired.
It was of interest to see whether the use of a shorter xylyl bridge, would impact upon the rate of phosphodiester hydrolysis attained by the complexes. The synthesis and characterisation of this system is the subject of the following section.

4.3.11 Synthesis of \( p \)-Xylyl Bridged Binuclear System

As mentioned earlier the coordination of metal bound water molecules plays an important role in the hydrolysis of phosphodiesters. The shorter xylylene bridge was chosen, in order to investigate the effect of leaving the lanthanide ion coordinatively unsaturated. As this spacer group lacks the acetamide functionality, the complex should gain one extra free coordination site per Eu(III) ion, which in turn should provide the complex with a better ability to bind and accelerate phosphodiester hydrolysis.\(^{225} \) \(^{254} \) The synthesis and characterisation of the ligand 2-\{4,7-bis-dimethylcarbamoylmethyl-10-[4-(4,7,10-trisdimethylcarbamoylmethyl-1,4,7,10-tetraaza-cyclododec-1-ylmethyl)-benzyl]-1,4,7,10-tetraaza-cyclododec-1-yl\}-\( N,N \)-dimethyl-acetamide, 117, was discussed in Chapter 3. The europium complex of 117 was produced by refluxing 117 and two equivalents of Eu(CF\(_3\)SO\(_3\))\(_3\) in MeOH for sixteen hours (Scheme 4.17). The complex Eu.117, was soluble in acetone however, precipitation from CH\(_2\)Cl\(_2\) yielded the desired product in 59 % yield. The \(^1\)H NMR (d-acetone) showed the characteristic, shifted axial and equatorial peaks at 36.02, 26.52, 19.14, 17.02, 12.88, 11.32, 8.30, 4.72, 3.70, 2.85, 1.24, 0.83, -0.18, -1.40, -2.69, -6.45, -8.68, -12.48, -14.39, -20.64, -22.99, -25.01 and -25.90 ppm (Figure 4.22). The IR spectrum revealed a shift in the carbonyl stretching frequency from 1639 cm\(^{-1}\) for 117 to 1626 cm\(^{-1}\) for Eu.117.

\[ \text{Scheme 4.17. Complexation of 117 proceeded with 59\% yield} \]
At this point it is worth recalling the crystal structure of the corresponding sodium complex discussed in Chapter 3 (Figure 3.10), as sodium is known to have a similar coordination geometry to that of europium. The sodium complex exhibited a square antiprism geometry around the sodium ion and was seen to have two metal bound water molecules.

Figure 4.21. Mass spectrum of Eu.117 and inset showing europium isotope distribution pattern and expected pattern above

Figure 4.22. 400 MHz $^1$H NMR in d-acetone of Eu.117
Previously, the Tb(III) complex \( \text{Tb.117} \), was found to possess two metal bound water molecules per Tb(III) ion. As Eu(III) occupies a similar coordination environment to Tb(III), \( \text{Eu.117} \) was also expected to possess two metal bound water molecules.

<table>
<thead>
<tr>
<th>Complex</th>
<th>( \tau_{\text{H2O}} ) (ms)</th>
<th>( k_{\text{H2O}} ) (1/ms)</th>
<th>( \tau_{\text{D2O}} ) (ms)</th>
<th>( k_{\text{D2O}} ) (1/ms)</th>
<th>( q ) (±0.5)</th>
</tr>
</thead>
<tbody>
<tr>
<td>( \text{Eu.117} )</td>
<td>0.443</td>
<td>2.258</td>
<td>1.356</td>
<td>0.737</td>
<td>1.52</td>
</tr>
</tbody>
</table>

Table 4.7. Measured lifetimes and rate constants for \( \text{Eu.117} \) and the corresponding hydration number \( q \)

The \( q \) value for \( \text{Eu.117} \) was found to be 1.52 ± 0.5, however, as \( \text{Tb.117} \) was found to possess two metal bound water molecules it is reasonable to assume that \( \text{Eu.117} \) will display a similar coordination environment.

4.3.12 Summary

This section has described the development of twelve new complexes, \( \text{Eu.154}, \text{La.154}, \text{Eu.155}, \text{La.155}, \text{Eu.156}, \text{La.157}, \text{La.162}, \text{La.163}, \text{La.164}, \text{Eu.164}, \text{La.165} \) and \( \text{Eu.117} \), which have been designed to accelerate the rate of phosphodiester cleavage as discussed in Chapter 1. These complexes utilise four different bridging groups and five different pendant arms. The selection of pendant arms will allow comparison to similar mononuclear lanthanide systems previously reported, while the various bridging groups will allow for comparison between the binuclear systems. Lifetime studies have indicated that the Eu(III) complexes are nine coordinate, where the ninth site is occupied by a single metal bound water molecule. In the case of the heptadentate binuclear ligand \( \text{Eu.117} \), the ion is expected to possess two metal bound water molecules. The ability of these complexes to cleave phosphodiesters shall be discussed in the following sections.

4.4 Phosphodiester Hydrolysis Studies

As previously discussed in Chapter 1, most research groups employ phosphodiester model compounds, instead of RNA, when evaluating potential ribozyme mimics, due to the convenience offered by such molecules, such as the ability to follow the hydrolysis kinetics by absorption spectroscopy. Throughout this chapter the model compound, \( \text{HPNP} \) (2-hydroxypropyl \( p \)-nitrophenylphosphate), 33, shall be utilised in order to assess the complexes’ ability to promote phosphodiester hydrolysis. Like RNA, \( \text{HPNP} \) is a phosphodiester, which has a hydroxyl group that can act as an intramolecular nucleophile,
Chapter 4 – Ln(III) Complexes as phosphodiester Cleaving Agents

in the 2' position. However, HPNP has the advantage that its hydrolysis can be followed by absorption spectroscopy. HPNP absorbs at 300 nm, while the product of its cleavage, 4-nitophenolate, 34, absorbs at 400 nm (Scheme 4.18). By following the rise in the peak at 400 nm, over the course of the hydrolysis, the rate constant \( k \) can be determined.

![Scheme 4.18: Hydrolysis of HPNP (33) (abs @ 300 nm) to give p-nitrophenolate (34) (abs @ 400 nm)](image)

As seen in Chapter 1, it can be difficult to directly compare different reported ribozyme mimics due to the differing conditions employed. In order to facilitate comparison between systems the conditions employed here were chosen to mimic those reported by Morrow et al.\(^{258}\) Consequently, the measurements were conducted using a 0.18 mM solution of HPNP (Abs = 1.22 at 300 nm), in buffered aqueous solution (50 mM HEPES) pH 7.4, at 37 °C. To this was added one equivalent of the complex. The kinetics were then followed by absorption spectroscopy over sixteen hours with constant stirring, by measuring the absorbance at 400 nm \( (\varepsilon = 6430 \text{ M}^{-1} \text{ cm}^{-1}) \).\(^{255}\) The rate constant \( k \) values were determined by fitting the data to first order rate kinetics\(^{256}\). The half-life was then calculated from the rate constant obtained \( (\tau_{1/2} = \ln 2/k) \). The relative rate enhancement, \( k_{rel} \), is the ratio between \( k \) and the rate constant of the 'uncatalysed' hydrolysis of HPNP, \( (k_{uncat}) \) which has been measured to be 0.00012 h\(^{-1} \), \( \tau_{1/2} = 5.78 \times 10^3 \) h, at pH 7.4.\(^{257}\) Every rate is an average of two to three measurements agreeing to within a 10 % error. Another reason for choosing these conditions was their similarity to physiological conditions, pH ~ 7.4, 37 °C. The following sections shall detail the results of these measurements beginning with the binuclear acetamide systems.
4.4.1 Hydrolysis of HPNP by Binuclear Acetamide Systems

The simple acetamide bis systems were tested first. The measurements were conducted as described above. The changes in the absorbance spectra of HPNP in the presence of Eu.154 can be seen in Figure 4.23. The peak at 300 nm, corresponding to [HPNP] was found to decrease over time, while a new peak at 400 nm appeared, indicating the formation of 34. By plotting the changes in absorbance as a function of time (Figure 4.24), it can be seen that the decrease in absorbance at 300 nm is smaller than the increase in absorbance at 400 nm. This is due to the absorbance at 300 nm by the products of cleavage. By fitting the change in absorbance at 400 nm to first order rate kinetics, the rate constant was determined. This can also be determined manually by plotting the natural log of the absorbance at 400 nm against time, an example of which is shown in the appendix. The complex Eu.154, was found to cleave HPNP with a ‘pseudo first order’ rate constant of 0.099 (±0.011) hr⁻¹, corresponding to a half-life of 7.08 hours. This is a rate enhancement (kcat) of ca. 822 over the uncatalysed reaction.

Significantly, under identical conditions, the analogous mononuclear 4-armed complex Eu.38, was reported not to accelerate the rate of hydrolysis at all. This inactivity was attributed to the reduced number of metal bound water molecules (one for Eu.38,
compared to two for \textbf{La.38}).\cite{258} As the binuclear system \textbf{Eu.154}, now provides two metal bound water molecules (one from each ion), this may no longer be such a limiting factor.

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{absorption_profile}
\caption{Kinetics profile for \textbf{Eu.154}. Top line corresponding to abs at 300 nm by [HPNP], Bottom line corresponding to abs at 400 nm by [34].}
\end{figure}

The \textbf{La(III)} analogue \textbf{La.154}, was next evaluated. Under identical conditions to those previously described, \textbf{La.154} was found to cleave \textbf{HPNP} with a ‘pseudo first order’ rate constant (k) of 0.088 (±0.012) hr\(^{-1}\), corresponding to a half-life of 7.85 hours. This is a rate enhancement (k\textsubscript{rel}) of \textit{ca.} 736 over the uncatalysed reaction.\cite{257} The rate constant for the analogous mononuclear 4-armed complex, \textbf{La.38}, was found to be 0.058 hr\(^{-1}\), \(\tau_{1/2} = 11.59\) hours, (k\textsubscript{rel}) of \textit{ca.} 483.\cite{258} The binuclear system does show an increase in activity at pH 7.4 over the mononuclear system, however, in this case it is less than a two fold increase. This type of increase is consistent with the relative increase in concentration of the metal ion\cite{259} and as such, can be viewed as a cumulative rather than cooperative effect. The rigid xylylene spacer may be inhibiting the cooperative action of the metals by preventing them from approaching one another.

Comparing the two binuclear systems \textbf{La.154} and \textbf{Eu.154}, it can be seen that the \textbf{Eu(III)} analogue is slightly faster than the \textbf{La(III)} one, although when the errors are taken account of the difference is quite small. Previous mononuclear systems reported by Morrow\cite{258} and Gunnlaugsson\cite{251,252} have typically found the \textbf{La(III)} complexes to be significantly faster than the \textbf{Eu(III)} analogues, due to lanthanum’s larger coordination environment, which facilitates the presence of an extra metal bound water molecule and thus an extra coordination site to bind the phosphodiester. In this case however, the binuclear system \textbf{Eu.154}, provides two metal bound water molecules (one from each ion), and hence the number of metal bound water molecules may no longer be the most significant factor.
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Having evaluated the complexes of 154, identical measurements were carried out upon the other acetamide binuclear complexes, Eu.155, La.155, Eu.156 and La.157. The results of these complexes are shown in Table 4.8. All of the complexes were found to accelerate the rate of phosphodiester hydrolysis to some extent. However, some interesting trends can be deduced from the results.

Comparing the three Eu(III) complexes of 154, 155, 156 it can be seen that the rate of phosphodiester hydrolysis seems to decrease upon addition of N methyl groups (Table 4.8). A similar trend is evident for the La(III) complexes of 154 and 155. This trend may be due to the hydrogen bonding ability of the amide ligand gradually being diminished as the number of methyl groups increases. Of the mononuclear systems only La.38 had been shown to accelerate HPNP hydrolysis by a measurable amount. The term cooperativity is often used when a mononuclear complex is inactive, but the binuclear complex displays activity. This is incorrect however, as cooperativity requires demonstration. As the rate accelerations produced by the binuclear complexes of 155 and 156 can not be directly compared to their mononuclear analogues no conclusion upon cooperative action can be drawn.

<table>
<thead>
<tr>
<th>Complex</th>
<th>R</th>
<th>Rate k (h⁻¹)</th>
<th>t₁/₂ (h)</th>
<th>k_rel</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td></td>
<td>0.0001</td>
<td>5730</td>
<td>1</td>
</tr>
<tr>
<td>Eu.38</td>
<td>R₁, R₂ = H</td>
<td>N/A</td>
<td>N/A</td>
<td>N/A</td>
</tr>
<tr>
<td>La.38</td>
<td>R₁, R₂ = H</td>
<td>0.058</td>
<td>11.59</td>
<td>483</td>
</tr>
<tr>
<td>Eu.154</td>
<td>R₁, R₂ = H</td>
<td>0.099 (+0.011)</td>
<td>7.08</td>
<td>822</td>
</tr>
<tr>
<td>La.154</td>
<td>R₁, R₂ = H</td>
<td>0.088 (+0.012)</td>
<td>7.85</td>
<td>736</td>
</tr>
<tr>
<td>Eu.155</td>
<td>R₁ = CH₃ R₂ = H</td>
<td>0.043 (+0.015)</td>
<td>17.72</td>
<td>432</td>
</tr>
<tr>
<td>La.155</td>
<td>R₁ = CH₃ R₂ = H</td>
<td>0.065 (+0.004)</td>
<td>10.66</td>
<td>545</td>
</tr>
<tr>
<td>Eu.156</td>
<td>R₁, R₂ = CH₃</td>
<td>0.027 (+0.012)</td>
<td>25.67</td>
<td>230</td>
</tr>
<tr>
<td>La.157</td>
<td>R₁, R₂ = H, X = (CH₂)₆</td>
<td>0.247 (+0.026)</td>
<td>2.80</td>
<td>2058</td>
</tr>
</tbody>
</table>

Table 4.8. Pseudo first order rate constants (k), half lives (τ) and relative rates (k_rel) for the cleavage of HPNP 0.18 mM in 50 mM Hepes (pH 7.4) at 37 °C with various complexes

Thus far, although the binuclear systems discussed have shown improved rate enhancements over their analogous mononuclear systems, no evidence of a cooperative enhancement has been observed. Consequently, it was decided to test a binuclear complex with a more flexible spacer i.e. La.157. Of the simple acetamide systems, La.157, the hexane linked analogue of 154, promoted the hydrolysis of HPNP to the greatest extent.
Comparing the kinetics profile of \textbf{La.157} (Figure 4.25) to that of \textbf{Eu.154}, highlights the extent of the rate acceleration.

![Figure 4.25. Kinetics profile for the hydrolysis of HPNP by La.157 at pH 7.4 37°C](image)

The complex \textbf{La.157} was found to cleave \textbf{HPNP} with a ‘pseudo first order’ rate constant (k) of 0.247 (±0.026) hr$^{-1}$, corresponding to a half-life of 2.80 hours. It was found to be almost three fold more active than the original binuclear system, \textbf{La.154}, and four fold more active than the analogous mononuclear system at pH 7.4. This increase is more than can be explained by a simple cumulative effect. The complex \textbf{La.157} is providing a cooperative rate enhancement, and the four fold rate increase over the mononuclear system is in line with the cooperative activity displayed by other binuclear systems with flexible spacer groups.\textsuperscript{227}

At this point it can be seen that the flexible alkyl bridged binuclear system \textbf{La.157} is more effective than the rigid xylylene bridged binuclear conjugates, although, all measurements so far have been conducted at one pH \textit{i.e.} pH 7.4. As mentioned in the introduction, however, the rate of hydrolysis by such compounds, is known exhibit a pH dependence. Consequently, the effect of pH upon the hydrolysis was investigated and shall be detailed in the next section.
4.4.2 pH Dependence of HPNP Hydrolysis by Binuclear Acetamide Systems

It has previously been observed that the rate of phosphodiester hydrolysis can be dependant upon the pH.\textsuperscript{220} Studies were therefore, carried out to investigate the effect of pH upon the rate of hydrolysis by these complexes. The measurements were conducted at various pHs, in an analogous manner to that already described. The results are presented here in two graphs, one showing the Eu(III) complexes and the other showing the La(III) complexes. The tertiary amide complex Eu.156 was the poorest of the acetamide systems and hence its pH dependence was not investigated. The pH profile of the Eu(III) complexes Eu.154 and Eu.155 are shown in Figure 4.26. The first item to note is that the maximum activity of the complexes is at pH 7.0 for Eu.154 and pH 7.2 for Eu.155, respectively. The second item of note is the “pseudo” bell shaped pH dependence displayed by both complexes.

![Graph showing pH profiles for the hydrolysis of 0.18 mM HPNP at 37 °C by acetamide based Eu(III) complexes](image)

Figure 4.26. pH profiles for the hydrolysis of 0.18 mM HPNP at 37 °C by acetamide based Eu(III) complexes

A similar pseudo bell shaped pH dependence was observed for the La(III) complexes, shown in Figure 4.27. It is also of interest that the La(III) complexes exhibit their maximum activity at a slightly higher pH than the Eu(III) complexes (pH 7.6 for both La.154 and La.157). The point of maximum activity is important, as if the complexes are to be utilised as ribozyme mimics they will be required to function under physiological...
conditions, pH ~ 7.4. The pH dependence of the complexes can be explained in terms of the protonation state of the metal bound water molecules.

Figure 4.27. pH profiles for the hydrolysis of 0.18 mM HPNP at 37 °C by acetamide based La(III) complexes

A bell shaped pH rate profile is usually taken as evidence that the reaction is catalysed by both acid and base, however, it can also indicate a change in the rate determining step. As discussed in the introduction, the hydrolysis of phosphodiesters proceeds via a two step mechanism. The first step, transesterification, is catalysed by base, while the second step, hydrolysis of the phosphorane intermediate, is catalysed by acid. The reaction is bifunctional since both acid and base catalysts are involved. However, each step involves only one catalytic species, so overall the reaction is pseudo first order no matter which step is rate limiting, even if the two steps are of comparable rate. With an excess of the base catalyst, the second step is rate limiting. With an excess of the acid catalyst, the first step is rate limiting. The maximum rate is obtained when both catalysts are present and both steps are accelerated, so the pH rate profile is bell shaped. This bell shaped pH dependence has previously been observed for mononuclear systems. Its presence is linked to the protonation state of the metal bound water molecules. Metal bound hydroxide is known to be a potent base catalyst, while metal bound water molecules can act as acid catalysts and also facilitates binding of the phosphodiester. The maximum rate is obtained when the balance between binding and cleaving is optimum. For mononuclear La(III) complexes the optimum active species is believed to be the M(H₂O)(OH°). It offers a potent hydroxyl base catalyst as well as the metal bound water
molecule, which can act as an acid catalyst and also be displaced in order to bind the phosphodiester. The M(OH)\(_2\) species is known to be less active, as the two tightly bound hydroxide molecules inhibit the coordination of the anionic phosphodiester.\(^{265}\) A proposed mechanism for the hydrolysis of HPNP by a mononuclear La(III) complex can be seen in Scheme 4.19.

![Scheme 4.19. Proposed mechanism for the hydrolysis of HPNP by mononuclear La(III) complex](image)

The situation is more complicated for binuclear systems, however the same principles apply. As yet the minimum number of metal bound water molecules or binding sites required for a cooperative binuclear system, has not been established.\(^{259}\) These requirements may differ from mononuclear complexes, as highlighted by Eu.154. However, mechanisms involving two, three or even four metal bound water molecules can be envisaged for cooperative binuclear systems. This shall be discussed further in section 4.6.3.

It is clear from the pH rate profiles that La.157, with its more flexible bridge, is by far the most effective system for the promotion of phosphodiester hydrolysis (Figure 4.27).
This seemed to confirm that the lack of flexibility afforded by the original linker group 120, was inhibiting the promotion of hydrolysis. The flexibility granted by the alkyl chain allows the two metal centres to act in a more cooperative manner, hence the larger rate enhancements in the hydrolysis of HPNP.

In general, all of the binuclear acetamide complexes accelerated the rate of HPNP hydrolysis to some extent, displayed a pseudo bell shaped pH dependence, and were found to be significantly more active than the corresponding mononuclear systems. Nevertheless, the rate acceleration provided by the very rigid xylylene spaced systems could be attributed to a simple cumulative effect. However, the most active complex, the more flexible alkyl linked La.157, produced a rate enhancement, which was larger than a relative metal ion concentration effect could explain. The four fold rate improvement can be tentatively assigned to the double Lewis acid activation provided by the cooperative action of two metals.269

4.4.3 Hydrolysis of HPNP by Coordinately Saturated Complexes

At this point it should be noted that the Cu(II) and Zn(II) complexes of 152 were also synthesised, and their hydrolytic ability tested against the HPNP phosphodiester. Neither Cu.152 nor Zn.152 were found to be active. It is believed that the fully saturated coordination spheres of the metal ions prohibits them from interacting with the phosphodiester and hence minimizes their cleavage ability. Similar observations have been made by Fry et al. who reported the four coordinate binuclear Cu(II) complex Cu.166, to be inactive.270 This highlights the important role played by metal bound water molecules and the need to provide coordinatively unsaturated ligand environments.
4.4.4 Hydrolysis of HPNP by Binuclear Glycine Systems

To date, one of the largest rate enhancements for the hydrolysis of HPNP, provided by a mononuclear lanthanide complex, was reported by Gunnlaugsson et al. Utilising the hydrophobic nature of the pseudo peptide, glycine methyl ester pendant arms, they reported the La(III) complex La.39, which was found to cleave HPNP at pH 7.4 37 °C, with a ‘pseudo first order’ rate constant of 0.410 (±0.026) hr⁻¹, corresponding to a half-life \( \tau \) of 1.70 hours, \( k_{rel} \) 3417. This enhancement is even greater than that achieved by the cooperative binuclear complex La.157, already discussed. The ability to provide these large rate enhancements was attributed to the capability of the complex to form a hydrophobic cavity.

The synthesis of a binuclear lanthanide complex incorporating these “glycine” cofactors was desired in order to improve upon the rate of hydrolysis already obtained by utilising the cooperative effect of two metal ions in conjunction with the glycine pendant arms, which would more closely mimic the hydrophobic nature at the active sites of natural ribonucleases. The synthesis of four new binuclear glycine complexes was discussed earlier in section 4.3.9. This section shall look at the ability of these complexes to promote the hydrolysis of HPNP, beginning with the rigid xylylene spaced conjugate La.162.

The “glycine” complexes ability to promote the hydrolysis of HPNP was evaluated in an identical manner to that already described. The kinetics rate profile for La.162 can be seen in Figure 4.28.
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Figure 4.28. Kinetics profile for the hydrolysis of HPNP (0.18 mM) by La.162 at pH 7.4 37°C

At pH 7.4, 37 °C, La.162 was found to cleave HPNP with a ‘pseudo first order’ rate constant of 0.157 (±0.012) hr⁻¹, corresponding to a half-life of 4.42 hours, giving a rate enhancement (k_rel) of ca. 1308 over the uncatalysed reaction. This increase in activity represents a large improvement over the xylylene spaced binuclear acetamide complexes Ln.154, Ln.155 and Ln.156, discussed earlier, showing that the effect of the pendant group is quite important. However, this rate acceleration was actually less than that provided by the analogous mononuclear 4-armed complex, La.39, reported to be 0.410 (±0.026) hr⁻¹, τ½ 1.70 hours, k_rel of 3417, at the same pH (7.4). A pH profile was carried out to determine whether the pH was a contributing factor.

Figure 4.29. pH profile comparing rate constant k for the hydrolysis of 0.18 mM HPNP by La.162 and La.39

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Comparing the pH profile of La.162 to the mononuclear complex it can be seen that in this case the mononuclear system is significantly faster than the binuclear system at all pHs (Figure 4.29). Just as in the case of the rigid xylylene binuclear acetamide systems, no evidence of a cooperative effect was observed for La.162.

Having evaluated the complex La.162, identical measurements were carried out on the other glycine binuclear complexes, La.163, La.164 and Eu.164. The results for these complexes are shown in Table 4.9. All of the complexes were found to promote the hydrolysis of HPNP and the kinetic profiles for these three complexes can be seen in the appendix section.

Section 4.11 has shown how changing the spacer moiety can drastically alter the rate of phosphodiester hydrolysis. In the case of the “glycine” systems, once again the more flexible alkyl linked binuclear systems were found to promote the cleavage of HPNP to a greater extent than the original, rigid, xylylene linked system (Table 4.9). Nevertheless, in the case of the La(III) complexes the changes were relatively small and not as dramatic as those observed for the binuclear acetamide complexes.

<table>
<thead>
<tr>
<th>Complex</th>
<th>Rate constant $k$ (h$^{-1}$)</th>
<th>$\tau_{1/2}$ (h)</th>
<th>$k_{rel}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Eu.39</td>
<td>0.153 (0.013)</td>
<td>4.53</td>
<td>1250</td>
</tr>
<tr>
<td>La.39</td>
<td>0.410 (±0.026)</td>
<td>1.70</td>
<td>3147</td>
</tr>
<tr>
<td>La.162</td>
<td>0.157 (±0.012)</td>
<td>4.42</td>
<td>1308</td>
</tr>
<tr>
<td>La.163</td>
<td>0.182 (±0.014)</td>
<td>3.82</td>
<td>1517</td>
</tr>
<tr>
<td>La.164</td>
<td>0.201 (±0.045)</td>
<td>3.53</td>
<td>1675</td>
</tr>
<tr>
<td>Eu.164</td>
<td>0.477 (±0.034)</td>
<td>1.45</td>
<td>3978</td>
</tr>
</tbody>
</table>

Table 4.9. Hydrolysis of 0.18 mM HPNP at pH 7.4, 37 °C by “glycine” complexes

The results from Table 4.9 show that the greatest increase in activity by the binuclear systems, was observed for Eu.164, which showed an almost three fold increase in activity over La.162 as well as a three fold increase over the corresponding mononuclear complex Eu.39 at pH 7.4. This three fold increase is right at the limit of what can be considered a cooperative effect. It confirms similar results in the literature, which found that very flexible spacers tend to generate only weak cooperative effect between metal ion centres. The results are somewhat atypical and hence, it was decided to investigate the pH dependence of all the binuclear complexes.
4.4.5 pH Dependence of HPNP Hydrolysis by Binuclear Glycine Systems

An investigation into pH behaviour of the complexes was conducted by measuring the rate of hydrolysis of HPNP at various pHs, in an analogous manner to that already described. The results are shown in Figure 4.30. The pH rate profile of La.163 is similar to that of La.164 and can be seen in the appendix. A full pH rate profile for the mononuclear Eu.39 system was not carried out, although the point at pH 7.4 was reported to be a local maximum.\(^{220}\) It is also of note that the Eu.164 complex displays its maximum at pH 7.2 – 7.4. This is important as a ribozyme mimic must function under physiological conditions and thus, it is desirable for the mimic to exhibit its maximum cleaving ability at pH 7.4 to grant the most efficient cleavage.

![Figure 4.30. pH profile for the hydrolysis of 0.18 mM HPNP at 37 °C by binuclear “Gly” ligands](image)

Once again, pseudo bell shaped pH rate profiles were obtained for each of the complexes, and the more flexible spacers were found to be more active than the rigid xylylene spaced system. Interestingly, the Eu(III) complex was found to be the most active of the binuclear systems. This is in contrast to previous results for the mononuclear systems, which found La(III) complexes to be more active. The larger size of the La(III) ion allows it one extra coordination site, usually occupied by a metal bound water molecule, which aids in the phosphodiester hydrolysis.\(^{220,258}\) As the binuclear systems now also provide two metal bound water molecules (one from each ion), the dominant factor may become the larger
Lewis acidity of the Eu(III) ion compared to La(III) ion. Yet, as this work is only in its infancy, the scope of it was not to investigate the effect of the metal ion, but to obtain a cooperative effect between the metals, which lead to large rate enhancements over mononuclear systems. Nonetheless, in this case, it was clear that the mononuclear La(III) system was still the most active (Figure 4.29).

A number of factors could be responsible for the decrease in activity. The large rate enhancement observed for the 4-armed \textit{La.39} system was attributed to the formation of a hydrophobic cavity. Such a cavity may be prevented from forming here by the steric environment forced upon the complex by the spacer. Also the increased steric bulk of the pendant arms, as well as the relative rigidity of the spacer, in the case of \textit{La.162}, may prevent the two metal centres from approaching one another, thus prohibiting the desired cooperative action and contributing to the lower than expected activity.

**4.4.6 Hydrolysis of HPNP by Binuclear Pyridine System**

As previously discussed, natural ribozymes are known to utilise groups, which can provide acid-base catalysis to promote the hydrolysis of RNA. The incorporation of pendant groups such as pyridine, which can provide base catalysis, into ribozyme mimics has been shown to improve the rate of phosphodiester hydrolysis. The synthesis of a binuclear lanthanide complex incorporating pyridine pendant arms was discussed in Chapter 4. The ability of this complex to promote the hydrolysis of HPNP was evaluated in an identical manner to that described earlier. The kinetics profile of \textit{La.165} can be seen in Figure 4.31.

![Kinetics profile for the hydrolysis of HPNP (0.18 mM) by La.165 at pH 7.4 37°C](image-url)
The pyridine moiety absorbs at 300 nm and hence the higher absorbance at this point ~ 1.8. At pH 7.4, 37 °C, the binuclear pyridine system La.165, was found to cleave HPNP with a ‘pseudo first order’ rate constant of 0.440 (± 0.029) hr⁻¹, corresponding to a half-life of 1.57 hours, giving a rate enhancement (k_rel) of ca. 3.666 over the uncatalysed reaction.\(^{257}\) This is almost a seven fold increase over the enhancement of La.155, again showing the importance of the pendant group. The rate constant for the analogous mononuclear 4-armed complex, La.158, was reported to be 0.189 (±0.003) hr⁻¹, \(\tau_{1/2}\) of 3.66 hours, \(k_{rel}\) of 1578.\(^{249}\) The hydrolysis of HPNP by La.165 was evaluated as a function of pH (Figure 4.32). It can be seen that La.165 has its maximum activity at pH 8.0, with a ‘pseudo first order’ rate constant of 0.611 (±0.005) hr⁻¹, \(\tau_{1/2}\) of 1.13 hours, \(k_{rel}\) of 5096. This is the largest rate enhancement provided by the binuclear conjugates described here, so far. Pyridine seemed to be an extremely effective pendant group for the hydrolysis of phosphodiesters, however, the two fold rate enhancement over the mononuclear system, while promising, seemed to be cumulative rather than cooperative. Unfortunately, time constraints prevented the synthesis and testing of the more flexible analogues of this system.

![Figure 4.32. pH profile for the hydrolysis of 0.18 mM HPNP by La.165](image)

### 4.5 Trends in Activity

At this point it is useful to review the activities of the binuclear complexes tested so far. Figure 4.33 compares the rate constant for the hydrolysis of HPNP at pH 7.4. It is immediately clear that the choice of pendant arm has a dramatic effect upon the activity of
the complex. It is also evident that larger rate enhancements are observed for the more flexible alkyl bridged complexes. Indeed, so far the rigid xylyene spaced binuclear systems have failed to demonstrate any cooperative effect. One likely explanation for this may be the rigid nature of this spacer, which may prevent the two metal centres from adopting a favourable orientation for binuclear cooperativity.\textsuperscript{271, 272}

\textbf{Figure 4.33. Hydrolysis of 0.18 mM HPNP at pH 7.4, 37 °C}

By comparing the hydrolysis as a function of pH it can be seen that La(III) and Eu(III) complexes display their maximum activity at different pHs (Figure 4.30). This is due to the differing pK\textsubscript{a}s of their metal bound water molecules. Although these values have not yet been determined, it is known that the lanthanide contraction causes the heavier lanthanides to have a smaller ionic radius. This results in an increased charge density, which makes the heavier lanthanides stronger Lewis acids. It also results in a decrease in the pK\textsubscript{a} of any metal bound water molecules.\textsuperscript{273} As such, \textbf{Eu.164} was found to peak at a lower pH (7.2) than the \textbf{La.164} complex (pH 7.8). Of course the nature of the ligand will also impact upon the Lewis acidity of the metal ion and hence the resulting pK\textsubscript{a} of any metal bound water molecules.
4.6 Changing the Spacer: Combining Rigid and Flexible Properties

Up until now, the complexes with the very rigid xylylene spacer 120, have shown no cooperative enhancement effects. Those with the very flexible alkyl spacers have shown weak cooperative effects with 3 – 4 fold rate enhancements. The spacers seem to be either too rigid or too flexible, to provide the desired cooperativity. A balance between the two extremes is required. Work by Morrow\textsuperscript{228} and Breslow,\textsuperscript{229} has shown that binuclear Cu(II) and Zn(II) complexes, which utilise a shorter $p$-xylyl spacer that lacks the acetamide functionality, can provide large cooperative rate enhancements over similar mononuclear systems. It was decided to utilise this shorter $p$-xylyl spacer in a binuclear lanthanide system. It would combine the rigid and flexible qualities desired and would also offer another advantage.

It had been noted in the Gunnlaugsson group that leaving the lanthanide ion coordinatively unsaturated was important for phosphodiester hydrolysis. Reducing the number of donor groups, by for example, removing one of the pendant amide arms, resulted in an increase in the number of metal bound water molecules, which in turn led to an increase in the rate of phosphodiester hydrolysis. This was explained by the presence of an extra metal bound water molecule, which in turn provided a better ability to bind and accelerate phosphodiester hydrolysis.\textsuperscript{225, 258, 274} As the shorter $p$-xylyl spacer lacks the acetamide functionality, the complex should gain one extra free coordination site.

4.6.1 Hydrolysis of HPNP by Eu.117

The binuclear complex Eu.117, was synthesised as described in section 4.3.11. It contained the same dimethyl acetamide pendant arms as the Eu.156 conjugate discussed in section 4.4.1, however, it differed from the original binuclear system in that it had a shorter bridge, and also left each metal ion with one extra open coordination site. Its ability to promote phosphodiester hydrolysis was tested against the phosphodiester HPNP in an analogous manner to that described earlier.

At pH 7.4 and 37 °C, Eu.117 was found to cleave HPNP with a ‘pseudo first order’ rate constant of 1.48 ($\pm$ 0.08) hr$^{-1}$, corresponding to a half-life of 0.47 hours or 28 minutes (Figure 4.34). This gives an unprecedented rate enhancement ($k_{rel}$) of ca. 12,333 over the uncatalysed reaction.\textsuperscript{257}
Chapter 4 – Ln(III) Complexes as phosphodiester Cleaving Agents

The octadentate mononuclear 4-armed Eu(III) complex was found not to accelerate the rate of hydrolysis to any measurable extent. However, as Eu.117 possesses one extra coordination site per metal ion than mononuclear 4-armed complex, a more reasonable comparison can be drawn with the heptadentate mononuclear 3-armed Eu(III) complex which was reported to give a moderate rate enhancement effect, with a rate constant \( k \) of 0.052 (± 0.021) hr\(^{-1}\), corresponding to a half-life of 13.32 hours, \( k_{rel} \) of ca. 243. Therefore, the acceleration provided by the binuclear Eu.117 complex, represents a 28.5 fold increase over the mononuclear 3-armed system. This is the largest rate enhancement over both the uncatalysed reaction as well as the mononuclear complex, which has yet been observed in the Gunnlaugsson laboratory.

Comparing Eu.117 to the similar binuclear dimethyl acetamide conjugate Eu.156, discussed in section 4.5.1, highlights the importance of the role of the spacer group. Recall that the Eu.156 conjugate, cleaved HPNP with a ‘pseudo first order’ rate constant of 0.027 (± 0.012) hr\(^{-1}\), corresponding to a half-life of 25.67 hours, \( k_{rel} \) of ca. 230. The shorter xylene bridged Eu.117 complex, shows a 75 fold rate increase over the similar Eu.156 system. The only major differences between the structures are the linking bridge and the increased number of metal bound water molecules. The xylene bridge of Eu.117 is shorter and more flexible than the very rigid linker 120. It is believed that this allows the two metal centres to approach each other to a greater extent and hence adopt a favourable orientation for the metal ions to act in a cooperative fashion allowing the double Lewis...
acid activation discussed earlier. The shorter spacer also lacks the amide functionality, which leaves an extra coordination site open to be occupied by a metal bound water molecule. As such, each Eu(III) ion was expected to have two metal bound water molecules. Extra metal bound water molecules have previously been shown to accelerate hydrolysis.\textsuperscript{274} Although the co-ordinately unsaturated nature of the metal ion undoubtedly plays a role in the enhancement, its effect should not be overestimated as the coordinatively unsaturated analogous 3-armed mononuclear system was reported to have only a moderate rate enhancement effect.

Thus far, all the complexes tested herein, have displayed a significant pH dependence. Consequently, the pH dependence of Eu.117, was investigated and shall be detailed in the next section.

### 4.6.2 pH Dependence of HPNP Hydrolysis by Eu.117

The pseudo first order rate constants for the hydrolysis of HPNP by Eu.117, at various pHs were evaluated in the manner previously described. The results are presented here in Figure 4.35. Comparing the complex to the binuclear methyl acetamide system Eu.155, highlights just how effective the Eu.117 system is at accelerating the rate of phosphodiester hydrolysis. Recall from section 4.4.1 that a pH profile for the dimethyl acetamide conjugate Eu.156, was not investigated as it displayed the poorest activity of the acetamide systems however, it would be expected to have a similar, if smaller, rate profile to that of Eu.155.

![Figure 4.35. pH profile for the hydrolysis of 0.18 mM HPNP by Eu.117](image)

**Figure 4.35. pH profile for the hydrolysis of 0.18 mM HPNP by Eu.117**
The binuclear complex **Eu.117**, was found to exhibit a pseudo bell shaped pH dependence and boasted its maximum rate enhancement at pH 7.8 with a ‘pseudo first order’ rate constant of 1.60 (± 0.40) hr⁻¹, corresponding to a half-life of 26 minutes, $k_{rel}$ of ca. 13,333. To the best of our knowledge, this is one of the largest reported rate enhancements for the hydrolysis of **HPNP** under aqueous conditions.

![Diagram](image)

**Scheme 4.20. Proposed mechanism for double Lewis acid activation provided by the cooperative action of a binuclear lanthanide system**

The reason for this huge increase over the mononuclear system can be attributed to the double Lewis acid activation provided by the cooperative action of the two metal centres. A possible mechanism for the hydrolysis of **HPNP** by a binuclear Ln(III) complex with two metal bound water molecules per ion, is shown in Scheme 4.20. The first step involves binding of **HPNP** to the metal ion by displacement of one of the metal bound water molecules. This activates the phosphodiester towards nucleophilic attack by neutralising the negative charge. A metal bound hydroxide can provide general base catalysis by abstracting a proton from the OH, which in turn acts as an intramolecular...
nucleophile and attacks the phosphorous. The resulting five coordinate, negatively charged phosphorane transition state can now be stabilised by double Lewis acid activation using both metal ions. A metal bound water molecule can now provide general acid catalysis, thereby stabilising the leaving group. The final step is the release of the cyclic phosphate with displacement by water. This step must proceed if the process is to be catalytic.

The effect of varying concentration of Eu.117, upon the first order rate constant k, is shown in Figure 4.36. A second order rate constant of 2.82 (± 0.22) M⁻¹ s⁻¹ for the hydrolysis of HPNP by Eu.117 was obtained by calculating the slope of the linear plot of rate constant k, against concentration, [Eu.117]. ²⁵⁹ This is significantly faster than the second order rate constant of 0.25 M⁻¹ s⁻¹ for the dinuclear Zn(II) complex ⁴⁷, which was reported by Morrow et al. to be one of the highest rate constants for the cleavage of HPNP. ²⁵⁹ Despite the considerably large second order rate constants, the cleavage of HPNP by Eu.117, was not catalytic. While, a 2.5 fold excess of HPNP was cleaved with a “pseudo first order” rate constant of 0.26 hr⁻¹ a four fold excess of HPNP could not be fully hydrolysed. The lack of catalytic turnover combined with the considerably large rate constants, points to a strong binding interaction. ²⁵⁹ It is believed that after the cleavage event, the resulting cyclic phosphate is not being released and hence is inhibiting catalytic turnover. In order to elucidate further, ³¹P NMR studies were conducted.

Figure 4.36. Dependence of pseudo first order rate constant for the hydrolysis of HPNP (0.18 mM) on concentration of Eu.117 at pH 7.4 37 °C

The ability of Eu.117 to bind diethyl phosphate was investigated by ³¹P NMR. Diethyl phosphate was chosen as it can interact with Eu.117 in a similar fashion as HPNP, but
cannot undergo cleavage itself. Increasing equivalents of diethyl phosphate were added to a 10 mM solution of Eu.117 in H₂O, and the resulting change in the ⁴¹P NMR signal was monitored. The results of this are depicted in Figure 4.37. It can be seen that after the addition of four equivalents of phosphate the plot began to plateau, indicating that binding was complete at this point. What is most striking however, is the drastic change in the ppm of the phosphate signal ca. 28.98 ppm. Similar studies upon mononuclear lanthanide complexes in the Gunnlaugsson group, observed the changes in the ⁴¹P NMR signal to occur over approximately one ppm. The dramatic changes observed in the case of Eu.117 indicate a demonstrably strong binding interaction with phosphates, and lends credence to the theory of poor product release, inhibiting catalytic turnover. A higher binding affinity for phosphate by the binuclear systems has previously been observed, and is believed to reflect the stronger electrostatic stabilisation offered by highly cationic, dinuclear complexes.²⁵⁹, ²⁷⁹

Figure 4.37. Changes in ⁴¹P NMR as a function of molar equivalents of diethyl phosphate added to Eu.117

The tetratdendate ligand, EDTA, is known to strongly bind lanthanide ions, however the resulting complexes have been found to be unable to promote the cleavage of phosphodiesters.²²³ In order to confirm that the large rate enhancements achieved by Eu.117, was not due to the presence of free lanthanide ions in solution, the hydrolysis measurements were repeated in the presence of EDTA. Under similar conditions at pH 7.4,
37 °C, with 0.22 equivalents of EDTA present, the reaction still went to completion, however, the rate constant fell to 0.634 hr⁻¹, corresponding to a half-life of 1.1 hours or 65.5 minutes. With one equivalent of EDTA present again the reaction went to completion but the rate constant was reduced to 0.280 hr⁻¹, giving a half-life of 2.5 hours or 148 minutes. Although the hydrolysis of HPNP by Eu.117 in the presence of 0.22 or 1 equivalent of EDTA is still being promoted by a large extent, the respective 2 fold and 5 fold reduction in activity may indicate that the complex is not stable to exchange with EDTA. However, as each molecule possesses four binding sites, it is possible that the EDTA could be coordinating to the complex itself, and thus reducing the activity by this manner. Further work is needed to confirm the stability of the complex.

The promising results obtained with the cooperative action of the binuclear lanthanide system Eu.117, emphasise the importance of this work and highlight the need for future development of the binuclear lanthanide complex area. This shall be further discussed in the next section.

4.7 Conclusion

In conclusion, new bimetallic lanthanide complexes for the cleavage of phosphodiesters have been synthesised, characterised and tested for phosphodiester hydrolysis ability. A wide variety of factors can be seen to effect the activity of the Ln(III) complex cleavage agents, including the choice of metal ion, pH, the choice of pendant arm and, crucially, the spacer group. Examining the choice of metal ion, it was observed that the Eu(III) complexes were at their most active at lower pHs (7.2 for Eu.164) then the corresponding La(III) complexes (pH 7.8 for La.164). This could be an important factor when tuning for the greatest activity under biological conditions.

The choice of pendant arm was found to also be an extremely important factor in obtaining the greatest rate enhancements. The simple acetamide pendant arms showed only moderate rate enhancements, while better enhancements were observed for the more complex arms, the greatest being observed for the pyridine pendant arms. This improvement can be assigned to the basic nature of the pyridine group allowing it to operate as a general base catalyst.

The choice of spacer group was found to be the central factor in the promotion of hydrolysis. The cooperative action sought between the two metals was prevented by the very rigid nature of the original spacer group 120. Employing more flexible alkyl chains as
bridging groups, did lead to a modest cooperative rate enhancement, in line with similar results using flexible bridged binuclear systems reported earlier.\(^{227}\) The alkyl bridged \textit{La.157} was found to be four fold more active than its analogous mononuclear complex.

The largest rate enhancement was provided by the \textit{Eu.117} system which cleaved \textit{HPNP} at pH 7.4 at 37 °C, with a pseudo first order rate constant of 1.48 hr\(^{-1}\), corresponding to a half-life of 0.47 hours and a rate enhancement \(k_{\text{rel}}\) of 12,333. Under these conditions, this is one of the largest rate enhancements reported in the literature. It is believed that this large rate enhancement is due to the cooperative action of the two metal centres working in unison. The coordinately unsaturated nature of the metal ion undoubtedly plays a part, nevertheless, it should not be overestimated as the coordinatively unsaturated analogous 3-armed mononuclear system was found to have only a moderate rate enhancement effect.

The \textit{Eu.117} complex did not exhibit catalytic turnover and this was attributed to the strong binding of the substrate resulting in poor product release. The exceptionally strong binding of phosphate by \textit{Eu.117} was demonstrated by the large change in the \(^{31}\text{P}\) NMR signal, \(ca. 29\) ppm, upon addition of diethyl phosphate.

In summary, \textit{Eu.117} cleaves \textit{HPNP} with a rate constant of 1.48 (± 0.08) hr\(^{-1}\), corresponding to a half-life of 0.47 hours or 28 minutes, an unprecedented rate enhancement \(k_{\text{rel}}\) of \(ca. 12,333\), while the corresponding mononuclear 3-armed system was reported to cleave \textit{HPNP} with a rate constant \(k\) of 0.052 (± 0.021) hr\(^{-1}\), corresponding to a half-life of 13.32 hours, \(k_{\text{rel}}\) of \(ca. 243\). Further optimisation is possible and shall be discussed as future work in the final section.

### 4.8 Future Work

It is clear that the acceleration of phosphodiester hydrolysis, by the binuclear compounds discussed throughout this thesis provided by both the nature of the pendant groups and the linker are significant in the acceleration of hydrolysis. In order for cooperative action between two metals to function a rigid spacer with some flexibility must be employed. Future work should concentrate on finding the correct combination of pendant arm and linker to further accelerate the hydrolysis. The significance of the linker cannot be overstated as the fastest rate enhancement was observed with simple amide arms that had previously given the worst result. The ability of similar type spacer groups, such as \(-\text{o-xylyl, m-xylyl and biphenyl groups},\) to provide large cooperative enhancements should be investigated. Whether this type of cooperative rate enhancement can be achieved using
more complicated pendant arms, such as the pyridine pendant arms, is also of interest. The pyridine pendant arms should be combined with the shorter p-xylyl spacer group. It is imperative that the ability of this compound to cleave the RNA phosphodiester is tested and established. The effect of the metal ion should also be investigated to determine whether preliminary results, which indicated that the Lewis acidity of the metal ion is now more important than the size, are accurate. Determination of $pK_a$ values should also be investigated.
Chapter 5

Experimental Procedures
5.1 General experimental details

Melting points were determined using an Electrothermal IA9000 digital melting point apparatus. Infrared spectra were recorded on a Mattson Genesis II FTIR spectrophotometer equipped with a Gateway 2000 4DX2-66 workstation. Oils were analysed using NaCl plates, 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 (TMS) 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 were recorded at 100 MHz using a Bruker Spectrospin DPX-400 instrument. Mass spectrometry was carried out using HPLC grade solvents. Mass spectra were determined by detection using Electrospray on a Micromass LCT spectrometer, using a Shimadzu HPLC or Water's 9360 to pump solvent. The whole system was controlled by MassLynx 3.5 on a Compaq Deskpro workstation. High resolution mass spectra were determined relative to a standard of leucine enkephaline. Elemental analysis was performed in the Microanalytical Laboratory, University College Dublin. Starting materials were obtained from Sigma Aldrich, Strem Chemicals and Fluka. Columns were run using Silica gel 60 (230-400 mesh ASTM) or Aluminum Oxide (activated, Neutral, Brockmann I STD grade 150 mesh). Solvents were used at GPR grade unless otherwise stated.

5.2 Synthesis

2-(4,7-bis-dimethylcarbamoylmethyl-1,4,7,10-tetraaza-cyclododec-1-yl)-N,N-dimethyl-acetamide (100) $^{171}$

1, 4, 7, 10-tetraazacyclododecane, 99, (10.00 g, 58.04 mmol) and NaHCO$_3$ (21.10 g, 174.13 mmol) were placed in a 150 mL RBF with MeCN (40 mL). The RBF was placed in a ice bath and allowed to cool. 98a (14.60 g, 174.13 mmol) in MeCN (10 mL) was added quickly and the solution was stirred at 0 °C for 5 minutes and then refluxed for 96 hours. The solution was filtered and the solvent was then removed. The residue was suspended in CH$_2$Cl$_2$, filtered and then purified by alumina column chromatography using gradient elution 100 → 80:20 CH$_2$Cl$_2$:MeOH(NH$_3$). This yielded a white foam, 8.63 g, 35 %. Calculated for C$_{20}$H$_{42}$N$_7$O$_3$ [M+H] m/z = 428.3344. Found m/z = 428.3349. δ$_{\text{H}}$ (400 MHz, CDCl$_3$) 9.99 (s, 1H, NH),
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3.59 (s, 2H, CH₂CO), 3.58 (s, 4H, CH₂CO), 3.09 (s, 8H, CH₂), 3.04 (s, 3H, CH₃), 2.97 (s, 6H, CH₃), 2.89 (s, 9H, CH₃), 2.84 (s, 8H, CH₂cycien). δ C (100 MHz, CDCl₃) 169.9, 169.8, 55.2, 53.3, 51.2, 50.5, 49.3, 45.7, 36.4, 36.0, 34.9. Mass Spectrum: (MeOH, ES+) m/z Expected: 427.3 Found: 428.3 [M+H], 450.4 [M+Na]. IR v max (cm⁻¹) 3434, 2927, 2852, 1637, 1508, 1475, 1402, 1338, 1261, 1103, 1064, 1022, 881, 806, 769, 667, 649, 574, 484.

2-(4,7-bis-methylcarbamoylmethyl-1,4,7,10-tetraaza-cyclododec-1-yl)-N-methyl-acetamide (107)

1, 4, 7, 10-tetraaza-cyclododecane (0.37 g, 2.13 mmol) and NaHCO₃ (0.54 g, 6.39 mmol) were placed in a 150 mL RBF with MeCN (15 mL). The RBF was placed in a ice bath and allowed to cool. 98b (0.71 g, 6.60 mmol) in MeCN (3 mL) was added quickly and the solution was stirred at 65 °C for 72 h. The solution was filtered through celite and the solvent was removed. The residue was suspended in EtOH, filtered and then precipitated from ether and the desired product was isolated as a cream foam, 0.48 g, 58 %. Calculated for C₁₇H₃₆N₇O₃ [M+H] m/z = 386.2880. Found m/z = 386.2874. δ H (400 MHz, CDCl₃) 7.59 (s, 1H, NH), 7.30 (s, 2H, NH), 3.06 (s, 4H, CH₂CO), 2.96 (s, 2H, CH₂CO), 2.75 (d, 6H, J = 4.5 Hz, CH₃), 2.76 (d, 3H, J = 4.5 Hz, CH₃), 2.60 (s, 4H, CH₂), 2.58 (s, 4H, CH₂), 2.53 (s, 4H, CH₂), 2.49 (s, 2H, CH₂). δ C (100 MHz, CDCl₃) 171.5, 171.4, 59.5, 57.5, 53.3, 52.6, 51.8, 46.1, 25.9, 25.7. Mass Spectrum: (MeOH, ES+) m/z Expected: 385.5 Found: 386.3 [M+H], 408.4 [M+Na]. IR v max (cm⁻¹) 3430, 2846, 2103, 1643, 1567, 1456, 1415, 1367, 1311, 1257, 1164, 1111, 989, 482.

Procedure 1: General procedure for the synthesis of compounds 98

The relevant amine was placed in a RBF with NaOH (1 equivalent) and CH₂Cl₂ (25 mL). The solution was cooled to -15 °C. Chloroacetyl chloride (1 equivalent) was dissolved in CH₂Cl₂ (20 mL) and added dropwise over 1 hour and the solution was left stirring at room temperature overnight. The organic and aqueous layers were then separated and the aqueous layer was washed with CH₂Cl₂ (2 x 15 mL). The organic extracts were combined and washed with 0.1 M HCl (3 x 10 mL). The organic layer was then dried over K₂CO₃ and the solvent was removed.
Chapter 5 – Experimental Procedures

2-chloro-\(N,N\)-dimethyl-acetamide (98a)\(^{172}\)

\[
\text{Cl} \quad \text{O} \quad \text{CH}_3 \\
\text{N} \quad \text{CH}_3 \\
\]

98a was synthesised by following Procedure 1, using \(N, N\)-dimethylamine 60% aqueous solution (6.30 g, 0.14 mol), along with NaOH (5.60 g, 0.14 mol), \(\text{CH}_2\text{Cl}_2\) (50 mL) and chloroacetyl chloride (15.82 g, 0.14 mol). The product was obtained as a clear oil, 7.88 g, 46%. \(\delta\) \((400 \text{ MHz, CDCl}_3) 4.02 \text{ (s, 2H, CH}_2\text{)}, 3.02 \text{ (s 3H, CH}_3\text{)}, 2.90 \text{ (s 3H, CH}_3\text{)}. \delta\) \(_\text{C} \text{ (100 MHz, CDCl}_3\) 166.0, 40.7, 37.0, 35.3.

2-chloro-\(N\)-methyl-acetamide (98b)\(^{172}\)

\[
\text{Cl} \quad \text{O} \quad \text{CH}_3 \\
\text{N} \quad \text{H} \\
\]

98b was produced according to Procedure 1, using \(N\)-methylamine 40% aqueous solution (10.00 g, 0.32 mol), NaOH (12.9 g, 0.32 mol), \(\text{CH}_2\text{Cl}_2\) (25 mL) and chloroacetyl chloride (36.4 g, 0.32 mol). 98b was isolated as a clear oil which solidified upon cooling, 10.60 g, 30%. \(\delta\) \((400 \text{ MHz, CDCl}_3) 6.67 \text{ (bs, 1H, NH), 4.03 \text{ (s, 2H, CH}_2\)}, 2.87 \text{ (s, 3H, CH}_3\text{)}. \delta\) \(_\text{C} \text{ (100 MHz, CDCl}_3\) 166.0, 42.1, 26.0.

5-amino-[1,10]-phenanthroline (102)\(^{174}\)

5-Nitro-[1,10]-phenanthroline (101) (0.50 g; 2.22 mmol) and Pd/C (5%; 0.12 g; 1.11 mmol) were placed in a tri necked round bottomed flask, dried under vacuum and put under argon. Absolute ethanol (150 mL) was added via syringe. Hydrazine mono hydrate (0.35 g; 11.1 mmol; 0.35 mL) was then added dropwise. The solution turned yellow and was refluxed for forty hours after which, it was filtered through celite to remove the palladium. The filtrate was then evaporated at reduced pressure. The resulting yellow solid was triturated with ether (3 x 15 mL) and dried under vacuum. 0.41 g; 93% yield. Melting point 250 °C (lit m.p. 245 – 246 °C). \(\delta\) \((400 \text{ MHz, CDCl}_3\) 9.18 \text{ (d, 1H, J = 4.0 Hz, H}_2\text{)}, 8.95 \text{ (d, 1H, J = 4.0 Hz, H}_9\text{)}, 8.29 \text{ (d, 1H, J = 8.0 Hz, H}_4\text{)}, 8.01 \text{ (d, 1H, J = 8.0 Hz, H}_7\text{)}, 7.68 \text{ (dd, 1H, J = 8.0 Hz, 4.0 Hz, H}_3\text{)}, 7.53 \text{ (dd, 1H, J = 8.0 Hz, 4.0 Hz, H}_8\text{)}, 6.87 \text{ (s, 1H, H}_8\text{)}, 6.15 \text{ (s, 2H, NH}_2\text{)}. \delta\) \(_\text{C} \text{ (100 MHz, CDCl}_3\) 149.3, 144.8, 142.6, 132.7, 130.8, 130.5, 123.2, 122.1, 121.8, 101.7.
2-chloro-N-[1,10]phenanthroline-5-yl-acetamide (103)

5-Amino-[1,10]-phenanthroline, 102, (0.30 g, 1.54 mmol) was placed in a round bottomed flask under argon. THF (dry, 55 mL) and triethylamine (0.16 g, 1.58 mmol, 0.22 mL) were added and the suspension was stirred for 30 mins. The mixture was cooled to 0 °C before chloroacetyl chloride (0.213 g, 1.89 mmol, 0.22 mL) in THF (5 mL) was added dropwise. The mixture was left to stir overnight at room temperature. The solution was washed with a 5% NaHCO₃ aqueous solution and the organic layer was collected, evaporated, and the residue washed with water and ether to yield a brown solid, 0.241 g, 59 %. M.P. 240 °C. Calculated for C₁₄H₁₀N₃OCl: C, 61.89; H, 3.71; N, 15.47. Found: C, 60.84; H, 3.71; N, 14.84. Calculated for C₁₄H₁₁N₄OCl: [M+H] m/z = 272.0579. Found: m/z = 272.0591. δH (400 MHz, CDCl₃) 9.27 (d, 1H, J = 4.0 Hz, H₂), 9.18 (d, 1H, J = 4.0 Hz, H5), 8.91 (s broad, 1H, NH), 8.39 (s, 1H, H₄), 8.33 (d, 1H, J = 8.0 Hz, H₇), 7.74 (dd, 1H, J = 8.0 Hz, J = 4.0 Hz, H₃), 7.67 (dd, 1H, J = 8.0 Hz, J = 4.0 Hz, H₈), 4.43 (s, 2H, CH₂). δC (100 MHz, CDCl₃) 164.2, 150, 149.8, 146.1, 144.2, 138.9, 137.2, 135.6, 128.5, 127.6, 123.2, 122.6, 119.2, 42.8. Mass Spectrum: (MeOH, ES+) m/z Expected: 212.0. Found: 272.0 [M+H]. IR νmax (cm⁻¹) 1687, 1541, 1422, 1318, 1250, 1153, 1130, 896, 804, 739, 653.

N-[1,10]phenanthroline-5-yl-2-(4,7,10-tris-dimethylcarbamoylmethyl-1,4,7,10 teraaza-cyclododec-1-yl)-acetamide (104)

2-(4,7-Bis-dimethylcarbamoylmethyl-1,4,7,10 tetraazacyclododec-1-yl)-N,N-dimethyl-acetamide, 100, (0.23 g, 0.53 mmol), 2-chloro-N-[1,10]phenanthroline-5-yl-acetamide, 103, (0.17 g, 0.64 mmol) and CS₂CO₃ (0.21 g, 0.64 mmol) were dissolved in DMF (10 mL) and refluxed overnight under argon. The mixture was allowed cool and then filtered through celite. The solvent was then evaporated under reduced pressure and the resulting brown oil purified by alumina column chromatography using gradient elution 100 → 95:5 CH₂Cl₂:MeOH. This successfully yielded a pale brown solid, 0.135 g, 38 %. Calculated for C₃₄H₅₁N₁₀O₄: [M+H] m/z = 663.4069. Found: m/z = 663.4095. δH (400 MHz, CDCl₃) 11.12 (s, 1H, NH),
9.09 (d, 1H, J = 8.2 Hz, H$_{2\text{phen}}$), 8.98 (m, 2H, H$_{4+9\text{phen}}$), 8.04 (m, 2H, H$_{6+7\text{phen}}$), 7.45 (m, 2H, H$_{3+8\text{phen}}$) 3.86 (broad s, 2H, CH$_2$), 3.02-2.05 (broad m, 4H, CH$_2$, CH$_3$). 5c (100 MHz, CDCl$_3$) 172.2, 170.5, 170.4, 149.1, 148.6, 145.7, 144.2, 135.0, 133.9, 132.6, 127.9, 122.3, 121.8, 119.2, 56.4, 54.4, 54.3, 52.9, 51.2, 50.5, 50.0, 35.5, 34.9. Mass Spectrum: (MeOH, ES+) $m/z$ Expected: 663.4. Found: 663.4 [M+H]. IR $\nu_{\text{max}}$ (cm$^{-1}$) 3413, 2968, 2825, 2361, 1647, 1536, 1507, 1456, 1408, 1347, 1299, 1262, 1192, 1151, 1104, 1062, 951, 901, 828, 742, 630.

Eu.104. (CF$_3$SO$_3$)$_3$.(H$_2$O)

104 (16.00 mg, 24.1 $\mu$mol) and Eu(CF$_3$SO$_3$)$_3$ (Europium triflate) (15.90 mg, 26.5 $\mu$mol) were dissolved in acetonitrile (5 mL). The solution was freeze-pump-thawed twice in order to remove any oxygen. The reaction was refluxed under argon overnight. The complex was precipitated from ether and then from CH$_2$Cl$_2$, before being collected by filtration and dried under vacuum. The isolated product was a light brown hydroscopic solid, 27.60 mg, 90 %. Calculated for C$_{35}$H$_{51}$N$_{10}$O$_7$F$_3$SeU: $m/z = 965.2876$. Found: $m/z = 965.2827$. [M+1Trif]. $\delta_{1H}(400$ MHz, D$_2$O) 28.3, 9.1, 8.1, 7.6, 3.21, 2.8, 1.0, 0.2, -3, -8, -12, -15. Mass Spectrum: (MeOH, ES+) $m/z$ Expected: 813.8 Found: 406.9 [M$^+$/2], 481.8 [M+Triflate/2]. IR $\nu_{\text{max}}$ (cm$^{-1}$) 3362, 2945, 2833, 1656, 1449, 1414, 1278, 1257, 1114, 1028.

$N$-[1,10]phenanthroline-5-yl-acetamide (106)$^{174}$

5-Amino-[1,10]-phenanthroline, 102, (0.10 g, 0.51 mmol) was placed in a round bottomed flask with acetic anhydride (0.11 g, 1.00 mmol), NaHCO$_3$ (0.05 g, 0.59 mmol) and MeOH (15 mL). The solution was refluxed overnight. The solvents were removed and the residue was suspended in CH$_2$Cl$_2$ (25 mL) and filtered. The CH$_2$Cl$_2$ was then washed with water (2 x 10 mL). The solvent was then removed yielding a white solid, 0.08 g, 64 %. M.P. 236 °C (lit M.P. 231 – 233 °C). $\delta_{1H}$ (400 MHz, DMSO) 10.17 (s,1H, NH), 9.11 (s, 1H, H$_2$), 9.02 (s,1H, H$_3$), 8.63 (d, 1H, $J = 8.0$ Hz, H$_4$), 8.44 (d, 1H, $J = 8.0$ Hz, H$_7$), 8.16 (s, 1H, H$_6$), 7.82 (dd, 1H, $J = 8.0$ Hz, 4.5
Hz, H\textsubscript{3}), 7.74 (dd, 1H, $J = 8.0$ Hz, 4.5 Hz, H\textsubscript{8}), 2.24 (s, 3H, CH\textsubscript{3}). Mass Spectrum: (MeOH, ES\textsuperscript{+}) $m/z$ Expected: 236.0 Found: 236.9 [M+H].

2-{4,7-bis-dimethylcarbamoylmethyl-10-[4-(4,7,10-tris-dimethylcarbamoylmethyl-1,4,7,10-tetraaza-cyclododec-1-ylmethyl)-benzyl]-1,4,7,10-tetraaza-cyclododec-1-yl}-N,N-dimethyl-acetamide (117)

Tris dimethylacetamide cyclen (100) (0.22 g, 0.51 mmol), $\alpha,\alpha$-dichloro-$p$-xylene (118) (0.04 g, 0.24 mmol) and Cs\textsubscript{2}CO\textsubscript{3} (0.12 g, 0.37 mmol) and KI (0.08 g, 0.49 mmol) were dissolved in MeOH (25 mL) and refluxed for 120 hours. The solvent was evaporated and the residue was then suspended in CHCl\textsubscript{3} and filtered. The volume was then reduced and the solution was left to stand. The resulting crystals were filtered and washed with cold CHCl\textsubscript{3}. The desired product was isolated as off white crystals, 0.04 g, 10\%. M.P. decomp above 118 °C. Calculated for C\textsubscript{48}H\textsubscript{88}N\textsubscript{40}O\textsubscript{6}Na\textsubscript{2}.(H\textsubscript{2}O)\textsubscript{2}.CHCl\textsubscript{3}: C, 50.79; H, 8.09; N, 16.92. Found: C, 50.82; H, 7.95; N, 17.13. Calculated for C\textsubscript{48}H\textsubscript{90}N\textsubscript{14}O\textsubscript{6} [M+2H] $m/z = 958.7168$. Found $m/z = 958.7117$ (-5.3 ppm). $\delta$\textsubscript{H} (400 MHz, D\textsubscript{2}O) 7.30 (s, 4H, Ar), 3.95 (s, 4H, Ar-CH\textsubscript{2}), 3.22 (s, 4H, CH\textsubscript{2}), 2.99 (s, 8H, CH\textsubscript{2}), 2.79 (bm, 68H, cyclen + CH\textsubscript{3}). $\delta$\textsubscript{C} (100 MHz, D\textsubscript{2}O) 172.5, 172.1, 136.5, 129.8, 57.7, 54.5, 53.8, 50.8, 50.3, 49.7, 48.3, 35.5, 35.1, 34.8. Mass Spectrum: (MeOH, ES\textsuperscript{+}) $m/z$ Expected: 956.7 Found: 479.1 [M+H]/2, 490.0 [M+Na]/2, 500.9 [M+K]/2. IR $\nu$\textsubscript{max} (cm\textsuperscript{-1}) 3442, 2937, 2829, 1639, 1507, 1453, 1404, 1348, 1262, 1102, 1006, 819, 631.

Tb.117.(CF\textsubscript{3}SO\textsubscript{3})\textsubscript{6}.(H\textsubscript{2}O)\textsubscript{4}

117 (26.00 mg, 27.2 $\mu$mol) and Tb(CF\textsubscript{3}SO\textsubscript{3})\textsubscript{3} (33.00 mg, 54.4 $\mu$mol) were placed in a 50 mL RBF. MeOH (15 mL) was added and the solution was refluxed at 75 °C overnight. The solution was filtered and the solvent was then removed. The residue was triturated with CH\textsubscript{2}Cl\textsubscript{2} (3 x 20 mL). The residue was then dissolved in MeOH (5 mL) and added dropwise to ether (150 mL). The resulting precipitate was filtered, allowing the collection of a pale
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yellow solid, 24.00 mg, 41%. M.P. decomp above 170 °C. Calculated for C_{47}H_{93}N_{14}O_{6}Tb_2.(CF_3SO_3)_6.(H_2O)_2.(CH_2Cl)_4 C, 27.06; H, 3.86; N, 7.75. Found: C, 26.68; H, 3.74; N, 7.59. δ_H (400 MHz, D_2O) 120.25, 85.41, 74.99, 72.99, 59.79, 34.95, 31.35, 19.33, 13.33, 7.48, 5.44, 3.54, 3.31, 3.12, 1.17, -14.75, -15.76, -20.76, -37.78, -46.99, -61.40, -98.04, -106.25, -122.88. Mass Spectrum: (MeOH, ES+) m/z Expected: 1274.5. Found: 573.5 [M+3 triflate]/3. IR v_{max} (cm^{-1}) 3460, 2939, 1624, 1508, 1458, 1409, 1259, 1165, 1082, 1031, 959, 826, 639, 574, 517, 468.

Eu.117.(CF_3SO_3)_6.(H_2O)_4

117 (50.00 mg, 52.30 μmol) and Eu(CF_3SO_3)_3 (65.80 mg, 109.83 μmol) were placed in a 50 mL RBF. MeOH (15 mL) was added and the solution was refluxed overnight. The solution was filtered and the solvent was removed. The residue was suspended in EtOH (2 mL) and added dropwise to swirling CH_2Cl_2 (75 mL). The resulting precipitate was filtered and triturated with CH_2Cl_2 (2 x 20 mL). The desired product was isolated as a pale yellow solid, 69.00 mg, 59%. Calculated for C_{50}H_{88}N_{14}O_{12}F_{6}S_{2}{^{152}}Eu{^{153}}Eu [M+2Triflate] m/z = 1558.4463. Found m/z = 1558.4404 (~3.8 ppm). δ_H (400 MHz, d-acetone) 36.02, 26.52, 19.14, 17.02, 12.88, 11.32, 8.30, 4.72, 3.70, 2.85, 1.24, 0.83, -0.18, -1.40, -2.69, -6.45, -8.68, -12.48, -14.39, -20.64, -22.99, -25.01, -25.90. Mass Spectrum: (MeOH, ES+) m/z Expected: 1262.5. Found: 569.14 [M+3 triflate]/3, 389.61 [M+2 triflate]/4. IR v_{max} (cm^{-1}) 3458, 2941, 2351, 1626, 1410, 1257, 1168, 1082, 1031, 959, 825, 762, 638, 575.
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Procedure 2: General experimental procedure for bis systems 120-124.
The relevant aromatic diamine was placed in a 100 mL RBF with NaOH (4 equivalents) and dissolved in H₂O. The solution was placed in an acetone/ice bath and allowed to cool. Chloroacetyl chloride (4 equivalents) was then added dropwise over 1 hour. The solution was left to stir overnight. The solution was then filtered and the precipitate washed with water and ether.

1,4-bis-(2-chloro-acetylamino)-xylyene (120)

120 was synthesised according to Procedure 2, using p-xylylenediamine (119) (0.50 g, 3.67 mmol), NaOH (0.59 g, 14.70 mmol), H₂O (10 mL) and chloroacetyl chloride (1.66 g, 14.70 mmol). The product was isolated as white solid, 0.79 g, 75% yield. M.P. 206 - 207 °C. Calculated for C₁₂H₁₄N₂O₂Cl₂: C, 49.85; H, 4.88; N, 9.69. Found: C, 49.64; H, 4.75; N, 9.55. Calculated for C₁₂H₁₄N₂O₂Cl₂Na [M+Na] m/z = 311.0330. Found m/z = 311.0339 (+ 2.8 ppm). δH (400 MHz, DMSO) 8.72 (b.s, 2H, N-H), 7.22 (s, 4H, Ar-H), 4.26 (d, 4H, J = 6.0 Hz, CH₂NH), 4.11 (s, 4H, CH₂Cl). δC (100 MHz, DMSO) 165.9, 137.5, 127.3, 42.6, 42.2. Mass Spectrum: (MeOH, ES+): m/z Expected: 288.0. Found: 311.0 [M+Na], 326.9 [M+K]. IR νmax (cm⁻¹) 3280, 3072, 2952, 1656, 1549, 1415, 1267, 1235, 1066, 1001, 831, 769, 680, 547, 416.

1,2-bis-(2-chloro-acetylamino)-benzene (121)

121 was produced according to Procedure 2, using o-diamino benzene (2.00 g, 18.50 mmol), NaOH (2.96 g, 74.00 mmol), H₂O (80 mL) and chloroacetyl chloride (12.50 g, 110.00 mmol) The desired product was found to be a grey solid, 2.87 g, 60% yield. M.P. = 198 °C. Calculated for C₁₀H₁₁N₂O₂Cl₂ [M+H] m/z = 261.0198. Found m/z = 261.0195 (− 0.9 ppm). δH (400 MHz, CDCl₃) 8.67 (s, 2H, N-H), 7.55 (s, 2H, Ar-H), 7.33 (s, 2H, Ar-H), 4.25 (s, 4H, CH₂Cl). δC (100 MHz, CDCl₃) 164.7, 129.2, 126.8, 125.1, 42.3. Mass Spectrum: (MeOH, ES+): m/z Expected: 260.0. Found: 260.9 [M+H], 282.9 [M+Na], 300.8 [M+K]. IR νmax (cm⁻¹) 3255, 1667, 1655, 1596, 1550, 1516, 1451, 1411, 1339, 1283, 1230, 1143, 766, 706, 536.
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2,5-bis-(2-chloro-acetylamino)-pyridine (122)

122 was synthesised following Procedure 2, using m-diamino pyridine (1.50 g, 13.70 mmol) with NaOH (2.20 g, 55.00 mmol), H₂O (100 mL) and chloroacetyl chloride (15.55 g, 138.00 mmol). This yielded the desired product as a grey solid, 0.29 g, 8% yield. M.P. 156 °C. Calculated for C₉H₁₀N₂O₂Cl₂ [M+H] m/z = 262.0150. Found m/z = 262.0160 (+ 3.9 ppm). 5h (400 MHz, DMSO) 8.66 (s, 2H, NH), 7.98 (d, 2H, J = 8.0 Hz, Ar-H), 7.81 (dd, 1H, J = 8.0 Hz, 8.0 Hz, Ar-H), 4.22 (s, 4H, CH₂Cl). 5c (100 MHz, DMSO) 163.9, 148.1, 140.7, 109.8, 42.3. Mass Spectrum: (MeOH, ES+): m/z Expected: 261.0. Found: 261.9 [M+H], 283.9 [M+Na], 299.9 [M+K]. IR ν max (cm⁻¹) 3336, 3010, 1715, 1648, 1452, 1164, 803.

1,3-bis-(2-chloro—acetylamino)-xylylene (123)

123 was synthesised according to Procedure 2, using m-xylylenediamine (1.00 g, 7.30 mmol), NaOH (0.88 g, 22.00 mmol), H₂O (40 mL) and chloroacetyl chloride (2.49 g, 22.03 mmol). The product was collected and was a white solid, 0.83 g, 40 %. M.P. 153 – 154 °C. Calculated for C₁₂H₁₄N₂O₂Cl₂Na [M+Na] m/z = 298.9756. Found m/z = 298.9756 (+ 2.2 ppm). δH (400 MHz, DMSO) 8.75 (s, 2H, NH), 7.27 (t, 1H, J = 8 Hz, Ar-H₃), 7.15 (s, 2H, Ar-H₄,6), 7.14 (s, 1H, Ar-H₅), 4.28 (d, 4H, J = 5 Hz, CH₂), 4.11 (s, 4H, CH₂Cl). δC (100 MHz, DMSO) 165.9, 138.9, 128.4, 126.3, 125.9, 42.6, 42.4. Mass Spectrum: (MeOH, ES+): m/z Expected: 288.0. Found: 311.0 [M+Na]. IR ν max (cm⁻¹) 3285, 3083, 3009, 2954, 2882, 2795, 2360, 1658, 1547, 1489, 1447, 1416, 1360, 1330, 1230, 1171, 1094, 1065, 1024, 922, 898, 780, 710, 599, 484.

1,4-bis–(2-chloro-acetylamino)-benzene (124)

124 was synthesised similarly, following Procedure 2, using p-diamino benzene (1.50 g, 13.90 mmol), NaOH (2.20 g, 55.00 mmol), H₂O (100 mL) and chloroacetyl chloride (9.40 g, 83.30 mmol). This successfully produced the desired product as a brown solid, 3.57 g, 98% yield. M.P. decomp above 320 °C. Calculated for C₁₀H₁₀N₂O₂Cl₂K [M+K] m/z = 298.9756. Found m/z = 298.9756 (− 0.1
Procedure 3: General experimental procedure for bis systems 125-128.
The relevant diamino alkane was placed in a 100 mL RBF with NaOH (three equivalents) and dissolved in H₂O. The solution was placed in an ice bath at 0 °C and allowed to cool. Chloroacetyl chloride (three equivalents) was then added dropwise over one hour. The solution was left to stir overnight. The solution was then filtered and the precipitate was washed with ether. The solution was then extracted with CH₂Cl₂ (4 x 20 mL) and the solvent was subsequently evaporated and the residue suspended in ether and filtered.

2-chloro-N-[3-(2-chloro-acetylamino)-propyl]-acetamide (125)

\[
\text{Cl} \quad \text{NH} \quad \text{CH}_2 \quad \text{Cl} \\
\text{O} \quad \text{O} \quad \text{Cl}
\]

*n* = 3, 125

*n* = 4, 126

*n* = 5, 127

*n* = 6, 128

125 was synthesised following Procedure 3, using 1,3 diamino propane (0.44 g, 6.00 mmol), with NaOH (0.72 g, 18 mmol) and H₂O (25 mL), and chloroacetyl chloride (1.65 g, 15.00 mmol). The product was isolated as a white solid, 0.28 g, 20 %. M.P. 128 – 129 °C. Calculated for C₇H₁₂N₂O₂Cl₂Na [M+Na] m/z = 249.0174. Found m/z = 249.0168 (− 2.2 ppm). δ_H (400 MHz, CDCl₃) 7.17 (s, 2H, NH), 4.09 (s, 4H, CH₂Cl), 3.38 (dt, 4H, J = 6 Hz, J = 6 Hz, CH₂NH), 1.73 (quintet, 2H, J = 6 Hz, CH₂). δ_C (100 MHz, CDCl₃) 166.3, 42.2, 35.9, 28.9. Mass Spectrum: (MeOH, ES+): m/z Expected: 226.0. Found: 249.0 [M+Na]. IR v_max (cm⁻¹) 3283, 3078, 3005, 2970, 2953, 2879, 2361, 1675, 1638, 1554, 1464, 1448, 1401, 1316, 1292, 1269, 1249, 1194, 1128, 923, 759, 626, 574, 469, 414.

2-chloro-N-[4-(2-chloro-acetylamino)-butyl]-acetamide (126)

126 was synthesised according to Procedure 3, using 1,4 diaminobutane (0.50 g, 5.68 mmol) NaOH (0.68 g, 17.00 mmol) H₂O (25 mL) and chloroacetyl chloride (2.25 g, 19.89 mmol). The product was isolated as a white solid, 0.58 g, 42 % yield. M.P. 129 – 131 °C (lit²⁸⁰ M.P. 133 °C). Calculated for C₈H₁₄N₂O₂Cl₂Na [M+Na] m/z = 263.0330. Found m/z = 263.0336 (+ 2.3 ppm). δ_H (400 MHz, DMSO) 8.22 (s, 2H, NH), 4.03 (s, 4H, CH₂Cl), 3.07 ppm). δ_C (100 MHz, DMSO) 164.4, 134.4, 119.8, 43.5. Mass Spectrum: (MeOH, ES+): m/z Expected: 260.0. Found: 298.9 [M+K]. IR v_max (cm⁻¹) 3261, 3176, 3095, 1891, 1754, 1667, 1585, 1515, 1406, 1345, 1292, 1242, 1201, 1127, 1017, 963, 922, 851, 831, 751, 684, 518.
2-chloro-N-[5-(2-chloro-acetylamino)-pentyl]-acetamide (127)

127 was similarly produced according to Procedure 3, using 1,5 diamino pentane (0.17 g, 1.74 mmol), NaHCO₃ (0.43 g, 5.13 mmol), H₂O (25 mL) and chloroacetyl chloride (0.58 g, 5.10 mmol). The product was isolated as a white solid, 0.17 g, 39 %. M.P. 117 – 119 °C. Calculated for C₉H₁₆N₂Cl₂Na [M+Na] m/z = 277.0487. Found m/z = 277.0492 (+ 2.0 ppm). δH (400 MHz, DMSO) 8.19 (s, 2H, NH), 4.03 (s, 4H, CH₂), 3.04 (dt, 4H, J = 7 Hz, J = 6 Hz, CH₂NH), 1.39 (t, 4H, J = 7 Hz, CH₂CH₂NH), 1.24 (t, J = 7 Hz, 2H, CH₂). δC (100 MHz, DMSO) 165.7, 42.7, 38.8, 28.4, 23.6. Mass Spectrum (MeOH ES+): m/z Expected: 254.0. Found: 277.0 [M+Na]. IR ν max (cm⁻¹) 3276, 3079, 3005, 2969, 2929, 2859, 2362, 1638, 1549, 1463, 1445, 1401, 1379, 1313, 1270, 1247, 1222, 1193, 1138, 1080, 1004, 940, 935, 769, 751, 569, 489.

2-chloro-N-[6-(2-chloro-acetylamino)-hexyl]-acetamide (128)

128 was synthesised following Procedure 3, using 1,6 diamino hexane (0.50 g, 4.30 mmol) NaOH (0.43 g, 10.80 mmol), H₂O (25 mL) and chloroacetyl chloride (1.46 g, 12.90 mmol). The desired product was isolated as a white solid, 0.37 g, 32 %. M.P. 129 – 131 °C. Calculated for C₁₀H₁₈N₂Cl₂Na [M+Na] m/z = 291.0643. Found m/z = 291.0641 (− 0.7 ppm). δH (400 MHz, CDCl₃) 8.11 (s, 2H, NH), 3.94 (s, 4H, CH₂Cl), 2.97 (dt, 4H, J = 6 Hz, J = 6 Hz, CH₂NH), 1.31 (t, 4H, J = 6 Hz, CH₂(2,5)), 1.16 (s, 4H, CH₂(3,4)). δC (100 MHz, CDCl₃) 165.7, 42.7, 38.8, 28.7, 25.9. Mass Spectrum (MeOH ES+) m/z Expected: 268.1. Found: 269.1 [M+H], 291.1 [M+Na]. IR ν max (cm⁻¹) 3270, 3083, 2936, 2856, 1663, 1560, 1462, 1403, 1338, 1240, 1055, 926, 780, 728, 588, 517, 419.

Procedure 4: General experimental procedure for the synthesis of compounds 129-136

The relevant functionalised aromatic amine was dissolved in H₂O (40 mL) along with NaOH (3 equivalents). The solution was placed in an ice bath at 0 °C and allowed to cool. Chloroacetyl chloride (3 equivalents) was then added dropwise over 1 hour. The solution
was left to stir overnight. The solution was then filtered and the resulting precipitate was washed with water and ether.

**4-(2-chloro-acetylamino)-benzoic acid (129)**

![Chemical structure of 4-(2-chloro-acetylamino)-benzoic acid (129)](structure)

129 was synthesised according to Procedure 4, using 4-amino-benzoic acid (0.50 g, 3.60 mmol), H₂O (50 mL), NaOH (0.29 g, 7.30 mmol) and chloroacetyl chloride (1.24 g, 10.90 mmol). The product was successfully isolated as a white solid, 0.69 g, 88 %. M.P. 263 – 265 °C (lit\(^{281}\) M.P. 259 °C). \(\delta_H\) (400 MHz, DMSO) 12.77 (s, 1H, OH), 10.61 (s, 1H, NH), 7.91 (d, 2H, \(J = 9\) Hz, Ar-H₂), 7.69 (d, 2H, \(J = 9\) Hz, Ar-H₃,₅), 4.30 (s, 2H, CH₂Cl). \(\delta_C\) (100 MHz, DMSO) 165.8, 165.1, 142.5, 130.5, 125.7, 118.6, 43.6.

**4-(2-chloro-acetylamino)-benzamide (130)**

![Chemical structure of 4-(2-chloro-acetylamino)-benzamide (130)](structure)

130 was produced according to Procedure 4, using 4-amino-benzamide (0.50 g, 3.67 mmol), H₂O (25 mL), NaOH (0.44 g, 11.00 mmol) and chloroacetyl chloride (1.25 g, 11.00 mmol). The product was successfully isolated as a white solid, 0.49 g, 64 %. M.P. 242 – 243 °C (lit\(^{282}\) M.P. 241 – 242 °C). \(\delta_H\) (400 MHz, DMSO) 10.51 (s, 1H, NH), 7.88 (s, 1H, NH₂), 7.84 (d, 2H, \(J = 7\) Hz, Ar-H₂,₆), 7.64 (d, 2H, \(J = 7\) Hz, Ar-H₃,₅), 7.28 (s, 1H, NH₂), 4.29 (s, 2H, CH₂Cl). \(\delta_C\) (100 MHz, DMSO) 167.2, 164.9, 141.0, 129.4, 128.4, 118.4, 43.6.

**2-chloro-N-(4-nitro-phenyl)-acetamide (131)**

![Chemical structure of 2-chloro-N-(4-nitro-phenyl)-acetamide (131)](structure)

131 was synthesised by Procedure 4, using 4-nitroaniline (0.50 g, 3.62 mmol), H₂O (40 mL), NaOH (0.29 g, 7.20 mmol) and chloroacetyl chloride (1.22 g, 10.80 mmol). The product was successfully isolated as a white solid, 0.51 g, 64 %. M.P. = 178 °C (lit\(^{283}\) M.P. 181 – 183 °C). \(\delta_H\) (400 MHz, DMSO) 10.91 (s, 1H, NH), 8.24 (d, 2H, \(J = 9\) Hz, Ar-H₃,₅), 7.83 (d, 2H, \(J = 9\) Hz, Ar-H₂,₆), 4.34 (s, 2H, CH₂Cl). \(\delta_C\) (100 MHz, DMSO) 165.0, 144.6, 142.6, 125.0, 119.0, 43.5.
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3-(2-chloro-acetylamino)-4-hydroxy benzoic acid (132)

132 was synthesised according to Procedure 4, using 4-amino-4-hydroxybenzoic acid (0.30 g, 1.96 mmol), H₂O (25 mL), NaOH (0.78 g, 19.50 mmol) and chloroacetyl chloride (0.24 g, 2.15 mmol). The product was successfully isolated as a white solid, 0.36 g, 79%. M.P. 275.5 °C. Calculated for C₉H₈NO₄Cl: C, 47.08; H, 3.51; N, 6.10. Found: C, 46.79; H, 3.46; N, 6.22.

Calculated for C₉H₈NO₄ClNa [M+Na] m/z = 252.0040. Found m/z = 252.0050 (+ 4.1 ppm). δH (400 MHz, DMSO) 10.95 (s, 1H, OH), 9.54 (s, 1H, NH), 8.54 (d, 1H, J = 2 Hz, Ar-H₂), 7.59 (dd, 1H, J = 8 Hz, J = 2 Hz, Ar-H₆), 6.94 (d, 1H, J = 8 Hz, Ar-H₃), 4.39 (s, 2H, CH₂Cl). δC (100 MHz, DMSO) 167.0, 164.9, 151.8, 126.9, 125.3, 123.0, 121.3, 114.7, 43.3. Mass Spectrum: (MeOH, ES+): m/z Expected: 288.0. Found: 311.0 [M+Na]. IR νmax (cm⁻¹) 3366, 3077, 2878, 2008, 1678, 1596, 1555, 1514, 1461, 1402, 1374, 1286, 1234, 1200, 1124, 996, 911, 837, 772, 699, 631, 578, 535, 444.

4-(2-chloro-acetylamino)-2-hydroxy-benzoic acid (133)

133 was produced following Procedure 4, using 4-amino salicylic acid (0.50 g, 3.26 mmol), H₂O (40 mL), NaOH (0.13 g, 3.20 mmol) and chloroacetyl chloride (1.80 g, 15.92 mmol). The product was successfully isolated as a white solid, 0.62 g, 82%. M.P. 236 °C (lit M.P. 235 - 236 °C). δH (400 MHz, DMSO) 10.58 (s, 1H, NH), 7.75 (d, 1H, J = 9 Hz, Ar-H₆), 7.33 (s, 1H, Ar-H₃), 7.06 (d, 1H, J = 9 Hz, Ar-H₃) 2.50 (s, 2H, CH₂Cl). δC (100 MHz, DMSO) 171.5, 165.3, 162.0, 144.7, 131,2, 110.3, 108.2, 106.2, 43.6.

N-(4-acetyl-phenyl)-2-chloro-acetamide (134)

134 was synthesised according to Procedure 4, using 4-amino-acetophenone (0.30 g, 2.22 mmol), H₂O (40 mL), NaOH (0.18 g, 4.44 mmol) and chloroacetyl chloride (0.42 g, 3.78 mmol). The product was successfully isolated as a white solid, 0.07 g, 15%. M.P. = 148 – 150 °C (lit M.P. 150 – 151 °C). δH (400 MHz, DMSO) 10.64 (s, 1H, NH), 7.95 (d, 2H, J = 8 Hz, Ar-H), 7.72 (d, 2H, J = 8 Hz, Ar-H), 4.31 (s, 2H, CH₂Cl) 2.54 (s, 3H, CH₃). δC (100 MHz, DMSO) 196.6, 165.2, 142.7, 132.2, 129.5, 118.7, 43.5, 26.4.
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2-chloro-N-p-tolyl-acetamide (135)

135 was produced according to Procedure 4, using p-toluidine (0.30 g, 2.80 mmol), H₂O (40 mL), NaOH (0.22 g, 5.60 mmol) and chloroacetyl chloride (0.63 g, 5.60 mmol). The product was successfully isolated as a white solid, 0.39 g, 77%. M.P. 163 °C (lit²⁶ M.P. 163 °C). δₜ (400 MHz, DMSO) 10.20 (s, 1H, NH), 7.44 (d, 2H, J = 8 Hz, Ar-H), 7.11 (d, 2H, J = 8 Hz, Ar-H), 4.21 (s, 2H, CH₂Cl) 2.24 (s, 3H, CH₃). δc (100 MHz, DMSO) 164.3, 135.9, 132.8, 129.2, 119.3, 43.5, 20.4.

2-chloro-N-(4-hydroxy-3-nitro-phenyl) acetamide (136)

136 was synthesised by Procedure 4, using 4-amino-2-nitrophenol (0.30 g, 1.95 mmol), H₂O (25 mL), NaOH (0.78 g, 19.50 mmol) and chloroacetyl chloride (0.44 g, 3.89 mmol). The product was successfully isolated as an off white solid, 0.35 g, 78%. M.P. 131 – 133 °C. Calculated for C₈H₇N₂O₄Cl: C, 41.67; H, 3.06; N, 12.15. Found: C, 41.72; H, 2.95; N, 12.09. δₜ (400 MHz, CDCl₃) 10.42 (s, 1H, NH), 8.37 (d, 1H, J = 2 Hz, Ar-H₂), 8.29 (s, 1H, OH), 7.79 (dd, 1H, J = 9 Hz, J = 2 Hz, Ar-H₃), 7.19 (d, 1H, J = 9 Hz, Ar-H₃), 4.24 (s, 2H, CH₂Cl). δc (100 MHz, CDCl₃) 163.6, 151.9, 129.9, 128.8, 124.4, 120.1, 115.7, 42.2. IR νₘᵦₓ (cm⁻¹) 3346, 3288, 3086, 1670, 1591, 1555, 1517, 1485, 1411, 1320, 1284, 1239, 1174, 1125, 943, 884, 853, 782, 761, 635, 545.

Procedure 5: General experimental procedure for compounds 137-139

The relevant aromatic amine and NaOH (2.5 equivalents) were placed in a RBF to which H₂O was added. The solution was cooled to 0 °C. Chloroacetyl chloride (2 equivalents) was then added dropwise over 1 hour. The solution was left to stir overnight, and the resulting precipitate was filtered and rinsed with H₂O, hexane and cold CHCl₃.

2-chloro-N-(4-trifluoromethyl-phenyl)-acetamide (137)

137 was synthesised according to Procedure 5, using trifluoromethyl aniline (0.64g, 3.98 mmol) with NaOH (0.79g, 9.95 mmol) H₂O (25 mL) and chloroacetyl chloride (0.90g, 7.97 mmol). The product was isolated as a white solid, 0.29 g, 31% yield. M.P.

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158 – 160 °C. Calculated for C₉H₇NOCIF₃: C, 45.49; H, 2.97; N, 5.89. Found: C, 45.50; H, 2.95; N, 5.80. δH (400 MHz, CDCl₃) 8.39 (s, 1H, NH), 7.73 (d, 2H, J = 8.5 Hz, Ar-H), 7.65 (d, 2H, J = 8.5 Hz, Ar-H), 4.24 (s, 2H, CH₂Cl). δC (100 MHz, CDCl₃) 163.6, 139.2, 126.4, 126.0, 125.95, 119.2, 42.3. IR νmax (cm⁻¹) 3281, 3212, 3141, 3093, 1687, 1614, 1556, 1520, 1413, 1320, 1259, 1195, 1155, 1103, 1066, 1023, 845, 729, 595.

2-chloro-N-phenyl-acetamide (138)

δH (400 MHz, CDCl₃) 8.26 (s, 1H, NH), 7.58 (d, 2H, J = 7.5 Hz, Ar-H₂), 7.40 (dd, 2H, J = 7.5 Hz, J = 7.5 Hz, Ar-H₃, 3, 4, 2), 4.21 (s, 2H, CH₂Cl). δC (100 MHz, CDCl₃) 163.3, 136.2, 128.7, 124.8, 119.6, 42.4.

N-benzyl-2-chloro-acetamide (139)

δH (400 MHz, CDCl₃) 6.91 (s, 1H, NH), 7.35 (m, 5H, Ar-H), 4.52 (d, 2H, J = 5.5 Hz, CH₂NH), 4.13 (s, 2H, CH₂Cl). δC (100 MHz, CDCl₃) 191.1, 154.0, 128.4, 127.4, 127.3, 43.4, 42.2.

Procedure 6: General experimental procedure for compounds 140-141

The relevant amino pyridine was placed in a 100 mL RBF with NaOH (2 equivalents) and dissolved in H₂O. Chloroacetyl chloride (2 equivalents) was then added dropwise over 1 hour. The solution was then stirred at room temperature overnight. The solvent was removed under reduced pressure and the residue suspended in EtOH and filtered. The EtOH was evaporated and the residue suspended in acetone and filtered. The solid was dissolved in EtOH (5 mL) and this solution was added slowly to swirling ether (100 mL). The resulting precipitate was collected by filtration.
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2-chloro-N-pyridin-3-yl-acetamide (140)\(^{231}\)

140 was synthesised according to Procedure 6, using 3-aminopyridine (0.50 g, 5.32 mmol) along with NaOH (0.43 g, 10.64 mmol), H\(_2\)O (10 mL), and chloroacetyl chloride (1.20 g, 10.64 mmol). The product was isolated as a white solid, 0.44 g, 48%.\(^{231}\)

\[\delta_H (400 \text{ MHz, } D_2O) 9.16 (d, J = 3 \text{ Hz, } 1\text{H, Ar-H}_2), 8.44 (d, 1\text{H, } J = 6 \text{ Hz, Ar-H}_6), 8.39 (d, 1\text{H, } J = 9 \text{ Hz, Ar-H}_4), 7.91 (dd, 1\text{H, } J = 9 \text{ Hz, } 6 \text{ Hz, Ar-H}_5) 4.26 (s, 2\text{H, CH}_2\text{Cl}). \delta_C (100 \text{ MHz, } D_2O) 168.0, 136.9, 136.5, 132.6, 127.1, 42.2.\]

2-chloro-N-pyridin-4-yl-acetamide (141)\(^{231}\)

141 was synthesised following Procedure 6, using 4-aminopyridine (0.35 g, 3.72 mmol) with NaOH (0.29 g, 7.40 mmol), H\(_2\)O (10 mL), and chloroacetyl chloride (0.84 g, 7.40 mmol). The desired product was successfully isolated as a white solid, 0.26 g, 40%.\(^{231}\)

\[\delta_H (400 \text{ MHz, } D_2O) 7.83 (d, J = 8 \text{ Hz, } 1\text{H, Ar-H}_2), 7.75 (d, 2\text{H, } J = 7 \text{ Hz, Ar-H}_6), 6.70 (d, 2\text{H, } J = 7 \text{ Hz, Ar-H}_4), 4.60 (s, 2\text{H, CH}_2\text{Cl}). \delta_C (100 \text{ MHz, } D_2O) 166.5, 150.3, 146.8, 113.9, 43.54.\]

Procedure 7: General experimental procedure for compounds 98a and 144

The relevant amine was placed in a 100 mL RBF with NaOH and dissolved in H\(_2\)O. The solution was placed in an ice bath at 0 °C and allowed to cool. Chloroacetyl chloride was then added dropwise over 1 hour. The solution was then stirred at room temperature overnight. The solution was then extracted with CH\(_2\)Cl\(_2\) (5 x 20 mL). The CH\(_2\)Cl\(_2\) was washed with 0.1M HCl (2 x 15 mL) and H\(_2\)O (2 x 15 mL). The organic layer was subsequently dried over K\(_2\)CO\(_3\) and filtered before the solvent was removed.

2-chloro \(N,N\)-dimethylacetamide (98a)

98a was synthesised according to Procedure 7, using \(N,N\)-dimethylacetamide (7.20 g, 0.16 mol) with NaOH (6.80 g, 0.17 mol), H\(_2\)O (25 mL) and chloroacetyl chloride (18.50 g, 0.16 mol). The product was isolated as a white solid, 0.89 g, 4.6%.\(^{231}\)

\[\delta_H (400 \text{ MHz, CDCl}_3) 4.01 (s, 2\text{H, CH}_2), 3.01 (s, 3\text{H, CH}_3), 2.89 (s, 3\text{H, CH}_3). \delta_C (100 \text{ MHz, CDCl}_3) 165.9, 40.8, 37.0, 35.4.\]
**N-butyl-2-chloro acetamide (144)**

144 was synthesised following Procedure 7, using butylamine (0.30 g, 4.10 mmol) along with NaOH (0.33 g, 8.25 mmol), H$_2$O (25 mL) and chloroacetyl chloride (1.16 g, 10.27 mmol). The product was found to be a clear oil, 0.26 g, 42 %. 6h (400 MHz, CDCl$_3$) 6.93 (s, 1H, NH), 3.91 (s, 2H, CH$_2$Cl), 3.14 (t, 2H, J = 6 Hz, CH$_2$NH), 1.37 (tt, 2H, J = 8 Hz, J = 6 Hz, CH$_2$), 1.21 (m, 2H, J = 8 Hz, CH$_2$CH$_3$), 0.79 (t, 3H, J = 8 Hz, CH$_3$). $\delta_C$ (100 MHz, CDCl$_3$) 165.6, 42.1, 39.0, 30.7, 19.4, 13.1.

**1-acetoxy-4-acetylamino-benzene (147)**

4-aminophenol (145) (0.50 g, 4.59 mmol) was placed in a 100 mL RBF with NaOH (1.83 g, 4.59 mmol) and dissolved in H$_2$O (40 mL). Acetic anhydride (146) (2.5 equiv, 1.17 g, 11.4 mmol, 2 mL) was then added dropwise over one hour. The solution was then stirred at room temperature for 2 hours. The reaction was filtered and the precipitate was washed with water and then ether, isolating the desired product as a white solid, 0.3 g, 33 %. M.P. 149 – 150 °C (lit$^\text{289}$ M.P. 148 - 151°C) $\delta_H$ (400 MHz, DMSO) 7.50 (d, 2H, J = 9 Hz, Ar-H), 7.28 (s, 1H, NH), 7.04 (d, 2H, J = 9 Hz, Ar-H$_2$,6), 2.31 (s, 3H, CH$_3$), 2.19 (s, 3H, CH$_3$), 2.19 (s, 3H, CH$_3$). $\delta_C$ (100 MHz, CDCl$_3$) 169.2, 167.7, 146.3, 135.0, 121.5, 120.3, 24.1, 20.6.

**1,4,7,10-tetraaza-cyclododecane-1,4,7-tricarboxylic acid tri-tert-butyl ester (148)**

Cyclen (99) (0.80 g, 4.65 mmol) and Et$_3$N (1.45 g, 14.42 mmol) were placed in a 100 mL RBF and dissolved in CHCl$_3$ (30 mL). Di-tert-butyl-dicarbonate (Boc$_2$O), (3.14 g, 14.42 mmol) in CHCl$_3$ was then added very slowly and the solution was left stirring overnight. The solvent was evaporated to give an oil, which was then purified by flash silica column chromatography using hexane:EtOAc 1:5, as eluent. The product was isolated as white foam, 1.24 g, 57 %. $\delta_H$ (400 MHz, CDCl$_3$) 3.57 (s, 4H, CH$_2$), 3.23 (s, 8H, CH$_2$), 2.79 (s, 4H, CH$_2$) 1.41 & 1.39 (2s, 27H, CH$_3$). $\delta_C$ (100 MHz, CDCl$_3$) 155.0, 154.9, 78.7, 50.5, 49.3,
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48.9, 48.3, 45.4, 44.4, 28.1, 27.9. Mass Spectrum: (MeOH, ES+) m/z Expected: 472.3 Found: 473.0 [M+H].

1,4,7,10-tetraaza-cyclododecane-1,4,7-tricarbaldehyde (149)\textsuperscript{235}

Cyclen (99) (0.50 g, 2.90 mmol) and chloral hydrate (2.87 g, 17.40 mmol) were placed in a 50 mL RBF. EtOH (10 mL) was added and the solution was heated to 60 °C and stirred for 3 hours. The solvent was removed to give a clear oil, which was then dissolved in CH\textsubscript{2}Cl\textsubscript{2}. The product was purified by flash silica column chromatography using CH\textsubscript{2}Cl\textsubscript{2}: MeOH, 9:1, as eluent, (R\textsubscript{f} = 0.7). This yielded a clear oil, 0.65 g, 87 %.\textsuperscript{5h} (400 MHz, CDCl\textsubscript{3}) 7.96 (m, 3H, CHO), 3.36 (m, 12H, CH\textsubscript{2}), 2.74 (m, 4H, CH\textsubscript{2}). Mass Spectrum: (MeOH, ES+) m/z Expected: 256.1 Found: 257.0 [M+H].

1,4,7, Molybdenum tris carbonyl-(1,4,7,10-tetraaza-cyclododecane) (150)\textsuperscript{236}

To a 100 mL RBF, cyclen (99) (1.75 g, 10 mmol) and molybdenum hexacarbonyl (2.74 g, 10 mmol) were added. The system was placed under argon. Dry dibutylether (65 mL) was added. The solution was freeze-pump-thawed twice and then refluxed for 2 hours under argon. The solution was filtered and the resulting pale yellow solid was collected and dried under vacuum, 3.10 g, 88 %.

2-(1,4,7,10 teraaza-cyclododec-1-yl)-N-{4-[(2-1,4,7,10-tetraaza-cyclododec-1-yl-acetylamino)-methyl]-benzyl}-acetamide (152)

Method 1

148 (0.74 g, 1.57 mmol), 120 (0.21 g, 0.60 mmol), Cs\textsubscript{2}CO\textsubscript{3} (0.61 g, 1.87 mmol) and KI (0.10 g, 0.60 mmol) were placed in a 100 mL RBF. DMF (50 mL) was added and the solution was then heated to 75 °C and left to stir for 120 hours under argon. The solution was filtered off and the DMF removed under reduced pressure. The residue was suspended in EtOAc and separated by flash silica column chromatography,
using EtOAc, 100 %, as eluent. However, the NMR revealed that the majority was 148 and that the reaction had not proceeded.

**Method 2**

149 (0.12 g, 0.46 mmol), 120 (0.06 g, 0.23 mmol), Cs2CO3 (0.07 g, 0.23 mmol) and KI (0.04 g, 0.23 mmol) were placed in a 100 mL RBF. DMF (50 mL) was added and the solution was then heated to 75 °C and left to stir for 48 hours under argon. The solution was filtered off and the DMF removed under reduced pressure. The residue was suspended in EtOAc and separated by flash silica column chromatography, using EtOAc, 100 %, as eluent. However, the NMR and mass spec again revealed that the reaction had not proceeded.

**Method 3**

150 (1.08 g, 3.07 mmol), 1,4-bis-(2-chloro-acetylamino)-xylyene (120) (0.44 g, 1.5 mmol), Cs2CO3 (1.00 g, 3.07 mmol) and KI (0.49 g, 3.07 mmol) were placed in a 100 mL RBF. DMF (50 mL) was added under vacuum and the solution was freeze-pump-thawed three times. The solution was then heated to 85 °C and left to stir for 40 hours under argon. The cream precipitate was filtered off and the DMF removed under reduced pressure. The residue was dissolved in HCl (15 % v/v; 25 mL) and left stirring overnight. This aqueous solution was filtered and washed with CH2Cl2 (3 x 20 mL). The aqueous layer was then basified to pH >13 with KOH pellets and was subsequently filtered and extracted with CH2Cl2 (5 x 20 mL). The organic extracts were combined and dried over K2CO3 and filtered. The solvent was removed to yield a yellow oil, 0.35 g, 41 %. Calculated for C28H53N3O2: [M+H] m/z = 561.4353. Found: m/z = 561.4340 (- 2.4 ppm). δH (400 MHz, CDCl3) 8.45 (s, 2H, NH), 7.25 (s, 4H, Ar-H), 4.39 (d, 4H, CH2NH), 3.18 (s, 4H, CH2CO), 2.61 (s, 24H, cyclen CH2), 2.50 (s, 8H, cyclen CH2). δC (100 MHz, CDCl3) 171.1, 137.2, 127.7, 58.6, 52.8, 46.6, 46.2, 45.1, 42.4. Mass spectrum (MeOH, ES+) m/z Expected: 560.4. Found: 561.4 [M+H], 583.4 (M+Na), 599.4 (M+K), 281.2 [M+H]/2. IR νmax (cm⁻¹) 3194, 2724, 2362, 1640, 1543, 1269, 1170, 1076, 1020, 972, 941, 722.

**Bis-copper 2-(1,4,7,10 teraaza-cyclododec-1-yl)-N-[4-{2-(1,4,7,10-tetraaza-cyclododec-1-yl-acetylamino)-methyl]-benzyl]-acetamide (Cu.152)**
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152 (0.11 g, 0.19 mmol) and copper perchlorate hexa hydrate (0.15 g, 0.41 mmol) were placed in a 50 mL RBF with MeOH (20 mL). The solution was refluxed overnight. The solution was filtered and the solvent removed under reduced pressure. The residue was suspended in EtOH and filtered to yield a blue solid, 0.11 g, 53 %. M.P. decomp above 121 °C. Calculated for C$_{28}$H$_{52}$N$_{10}$O$_{10}$Cl$_2$Cu$_2$: [M+2ClO$_4$] $m/z$ = 884.1837. Found: $m/z = 884.1862$ (+ 2.9 ppm). Mass spectrum (MeOH, ES+) $m/z$

Expected: 686.3 Found: 984.6 [M+ 3ClO$_4$], 442.8 [M+2ClO$_4$]/2. IR $\nu_{max}$ (cm$^{-1}$) 3279, 2941, 2350, 1847, 1651, 1568, 1361, 1293, 1089, 805, 625.

Bis-zinc 2-(1,4,7,10-tetraaza-cyclododec-1-yl)-N-{4-[(2-1,4,7,10-tetraaza-cyclododec-1-yl-acetylamino)-methyl]-benzyl}-acetamide (Zn.152)

152 (0.11 g, 0.20 mmol) and Zn(CF$_3$SO$_3$)$_2$ (0.16 g, 0.44 mmol) were placed in a 50 mL RBF and dissolved in MeOH (15 mL). The solution was refluxed overnight. The volume of MeOH was reduced (~5 mL) and added dropwise onto ether (100 mL). The resulting precipitate was filtered and dissolved in MeOH (5 mL). This was added dropwise onto CH$_2$Cl$_2$ (100 mL) and the resulting precipitate was then filtered and dried to yield a brown hygroscopic solid, 0.15 g, 59 %. M.P. decomp above 136 °C. $\delta_H$ (400 MHz, D$_2$O) 7.23 (s, 4H,ArH), 4.39 (s, 4H, CH$_2$N), 3.46 (s, 4H, CH$_2$CO), 2.73 (m, 32H, cyclen CH$_2$). $\delta_C$ (100 MHz, D$_2$O) 172.6, 136.0, 127.3, 54.4, 45.9, 44.4, 44.3, 42.8. IR $\nu_{max}$ (cm$^{-1}$) 3521, 3289, 3111, 2941, 2887, 2285, 1651, 1568, 1463, 1257, 1166, 1091, 1030, 960, 854, 814, 760, 638, 574, 518.

2-{4,10-bis-carbamoylmethyl-7-[4-{2-(4,7,10-tris-carbamoylmethyl-1,4,7,10-tetraaza-cyclododec-1-yl)-acetylamino]-methyl]-benzyl-carbamoyl}-methyl]- 1,4,7,10-tetraaza-cyclododec-1-yl]- acetamide (154)

152 (0.11 g, 0.19 mmol), bromo acetamide (0.19 g, 1.36 mmol) and Et$_3$N (0.19 g, 1.95 mmol) were placed in a 50 mL RBF. EtOH (20 mL) was added and the solution was refluxed at 85 °C for 40 hours. The solution was filtered and then reduced in volume (5 mL) before it was added dropwise to a solution of CH$_2$Cl$_2$ (150 mL). The resulting
precipitate was filtered, dissolved in hot EtOH (5 mL) and left to stand. The resulting precipitate was filtered, yielding a pale yellow solid, 0.10 g, 57%. M.P. decomp above 163 °C. Calculated for 

\[ C_{40}H_{70}N_{16}O_{8} \cdot (CH_2Cl_2) _2 \cdot (H_2O)_2 \cdot MeOH: \]

C, 45.26; H, 7.24; N, 19.64. Found: C, 45.19; H, 6.89; N, 19.51. Calculated for 

\[ C_{40}H_{72}N_{16}O_{8} \]: [M+2H] \( m/z = 904.5719 \). Found: \( m/z = 904.5726 (+0.8 \text{ ppm}) \). \( \delta_H \) (400 MHz, D_2O) 7.16 (s, 4H, Ar-H), 4.26 (s, 4H, CH_2NH), 3.46 (bs, 4H, CH_2CO), 3.27 (s, 4H, CH_2C=O), 3.19 (s, 6H, CH_2C=O), 3.12 (s, 2H, CH_2C=O), 2.75 (bs, 32H, cyclen CH_2). \( \delta_C \) (100 MHz, D_2O) 173.8, 173.4, 170.2, 136.6, 127.1, 58.1, 56.9, 56.7, 56.1, 55.5, 55.3, 50.2, 42.2. Mass Spectrum: (MeOH, ES+) \( m/z \) Expected: 902.6. Found: 902.7 (M+), 924.7 (M+Na), 451.9 (M+2H)/2, 301.7 (M+3H)/3. IR \( \nu_{\text{max}} \) (cm\(^{-1}\)) 3336, 2949, 2831, 2074, 1595, 1678, 1551, 1420, 1288, 1117, 971, 902, 806, 647, 469.

2-\{4,7-bis-methylcarbamoylmethyl-10-\}-(4-\{2-(4,7,10-tris-methylcarbamoylmethyl-1,4,7,10-tetraaza-cyclododec-1-yl)-acetylamino\}-methyl\}-benzylcarbamoyl)-methyl\}- 1,4,7,10-tetraaza-cyclododec-1-yl\}-acetamide (155)

2-\{4,7-bis-methylcarbamoylmethyl-10-\}-(4-\{2-(4,7,10-tris-methylcarbamoylmethyl-1,4,7,10-tetraaza-cyclododec-1-yl)-acetylamino\}-methyl\}-benzylcarbamoyl)-methyl\}- 1,4,7,10-tetraaza-cyclododec-1-yl\}-acetamide (155)

152 (0.28 g, 0.50 mmol), K_2CO_3 (0.25 g, 1.85 mmol) and 2-chloro-N-methyl-acetamide (0.35 g, 2.25 mmol) were placed in a 100 mL RBF. EtOH (30 mL) was added and the solution was refluxed at 85 °C for 40 hours. The solution was filtered to remove the cream precipitate and the EtOH was removed under reduced pressure. The residue was suspended in CH_2Cl_2 (30 mL) and filtered. The solid was dissolved in EtOH (5 mL) and added to CH_2Cl_2/Ether 25:7. The resulting precipitate was filtered and then dissolved in a small volume of EtOH (<10 mL) and left to sit overnight. The resulting precipitate was filtered and was then dissolved in DMF and filtered, before being precipitated from ether to yield a yellow solid, 0.15 g, 30%. M.P. decomp above 139 °C. Calculated for 

\[ C_{46}H_{82}N_{16}O_{8} \cdot CH_2Cl_2 \cdot H_2O \cdot EtOH: \]

C, 51.79; H, 8.16; N, 19.72. Found: C,
15.72; H, 7.48; N, 19.67. Calculated for $C_{46}H_{84}N_{16}O_{8}$ \([M+2H]\) \(m/z = 988.6658\). Found \(m/z = 988.6623\). (-3.6 ppm).

6h (400 MHz, $D_2O$) 7.12 (s, 4H, Ar-H), 4.22 (s, 4H, CH$_2$NH), 3.21 (s, 4H, CH$_2$CO), 2.86 (bs, 12H, CH$_2$CO), 2.57 (s, 8H, cyclen CH$_2$), 2.52 (s, 18H, CH$_3$), 2.43 (s, 24H, cyclen CH$_2$). 6c (100 MHz, $D_2O$) 173.6, 172.9, 136.7, 127.1, 62.8, 57.4, 51.3, 41.9, 25.0. Mass Spectrum: (MeOH, ES+): \(m/z\) Expected: 986.6. Found: 987.9 [M+H], 1024.9 [M+K], 531.9 [M+2K]/2, 512.9 [M+K]/2, 494.0 [M+2H]/2. IR \(\nu_{max}\) (cm$^{-1}$) 3453, 3276, 3080, 2943, 2820, 2067, 1657, 1549, 1449, 1412, 1371, 1308, 1245, 1152, 1116, 972, 870, 707, 587.

2-\{4,10-bis-dimethylcarbamoylmethyl-7-\[(4-{[2-(4,7,10-tris dimethyl-carbamoy l methyl-1,4,7,10-tetraaza-cyclododec-1-yl)-acetylaminol- methyl]-benzylcarbamo yl}-methyl]- 1,4,7,10-tetraaza-cyclododec-1-yl\}-N,N-dimethyl-acetamide (156)

152 (0.15 g, 0.26 mmol), Cs$_2$CO$_3$ (0.30 g, 0.92 mmol), 2-chloro-N,N-dimethyl-acetamide (0.21 g, 1.70 mmol) and KI (0.13 g, 0.92 mmol) were placed in a 100 mL RBF and dissolved in EtOH (20 mL). The solution was refluxed at 85 °C for four days. The solution was filtered to remove cream precipitate and the solvent removed under reduced pressure. The residue was dissolved in CH$_2$Cl$_2$ (30 mL) and filtered. The CH$_2$Cl$_2$ was extracted with 0.1 M HCl (4 x 20 mL), which was then washed with CH$_2$Cl$_2$ (4 x 15 mL). The pH was adjusted to 7 using K$_2$CO$_3$ (10 %) and the solution extracted with CH$_2$Cl$_2$ (4 x 15 mL). The solvent was then removed to give a clear oil which solidified after drying in vacuum desicator over P$_2$O$_5$, 0.11 g, 38 %. M.P. decomp above 124 °C. Calculated for $C_{52}H_{96}N_{16}O_{8}$ \([M+2H]\) \(m/z = 1072.7597\). Found \(m/z = 1072.7567\) (-2.8 ppm).

6h (400 MHz, CDCl$_3$) 7.17 (s, 4H, Ar-H), 4.31 (s, 4H, CH$_2$NH), 3.49 (s, 4H, CH$_2$CO), 3.09 (bm, 12H, CH$_2$CO), 2.89 (bm, 68H, cyclen and CH$_3$). 6c (100 MHz, CDCl$_3$) 170.8, 170.4, 169.4, 139.8, 126.7, 63.6, 59.8, 55.6, 50.3, 48.3, 42.1, 36.5, 35.7, 35.3, 34.9. Mass Spectrum: (MeOH, ES+) \(m/z\) Expected: 1070.7. Found: 1071.9 [M+H], 535.9 [M+2H]/2, 357.9 [M+3H]/3. IR \(\nu_{max}\) (cm$^{-1}$) 3448, 3279, 3055, 2933, 2817, 1647, 1542, 1508, 1450, 1403, 1348, 1301, 1262, 1102, 1062, 1006, 950, 900, 822.
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Procedure 8: General procedure for synthesis of lanthanide complexes

Lanthanide complexes were prepared by refluxing each ligand with the relevant lanthanide triflate (2.1 equivalents) in MeOH (15 mL) for 16 hours. The resulting solution was filtered and reduced in volume to ~ 5 mL. This solution was added dropwise to swirling ether (100 mL) and the resulting precipitate was collected by filtration. The complexes were further purified by precipitation from CH₂Cl₂ (75 mL), and isolated by filtration. The complexes were dried in a vacuum desicator over P₂O₅. The ¹H NMR spectra of the complexes consisted of broad signals due to fast relaxation times from the paramagnetic metal ion, which precluded full characterisation. The fast relaxation times also precluded the acquisition of ¹³C spectra. Spectral widths were set at 100 ppm \((i.e. \pm 50 \text{ ppm})\). Additional spectral widths were obtained by adjusting the central offset \((i.e. -150 \text{ to } -50 \text{ ppm, 0 to } +150 \text{ ppm})\). Note that the lanthanide ions were expected to have metal bound water molecules, one for each europium and two for each lanthanum ion.

**Eu.154.(CF₃SO₃)₆.(H₂O)₂**

Eu.154 was prepared according to Procedure 8, using 154 (0.13 g, 0.15 mmol) and Eu(CF₃SO₃)₃ (0.20 g, 0.33 mmol) yielding a pale yellow solid upon drying, 0.25 g, 80 %. M.P. decomp above 215 °C.

Calculated for C₄₀H₇₀N₁₆O₈.Eu₂.(CF₃SO₃)₆(H₂O)₂: C, 25.85; H, 3.49; N, 10.48. Found: C, 26.31; H, 3.48; N, 10.03. Calculated for C₄₂H₇₀N₁₆O₁₄F₆S₂¹⁵¹Eu¹⁵³Eu [M+2triflate] m/z = 1504.3014. Found m/z = 1504.3027 (+ 0.9 ppm). δ(H (400 MHz, D₂O) 27.10, 26.28, 7.33, 7.16, 5.87, 4.20, 3.61, 3.22, 2.87, 1.93, 1.75, 1.55, 1.14, 1.04, -2.65, -5.25, -8.22, -9.09, -11.28, -13.55. Mass Spectrum: (MeOH, ES+): m/z Expected: 1208.4. Found: 901.1 [M+4Trif]/2, 376.5 [M+2Trif]/4. IR \(ν_{max} \text{ (cm}^{-1})\) 3374, 1665, 1459, 1252, 1150, 1082, 1029, 989, 638.
**La.154.**(CF$_3$SO$_3$)$_6$.H$_2$O)$_4$

La.154 was synthesised according to Procedure 8, using 154 (41.0 mg, 45.40 µmol) and La(CF$_3$SO$_3$)$_3$ (53.2 mg, 90.80 µmol) giving a yellow oil which became a yellow solid upon drying in desiccator, 83 mg, 88 %.

M.P. decomp above 227 °C. Calculated for C$_{40}$H$_{70}$N$_{16}$O$_8$.La$_2$.La(CF$_3$SO$_3$)$_6$.H$_2$O)$_4$.H$_2$O: C, 26.39; H, 4.16; N, 9.85. Found: C, 26.26; H, 3.76; N, 9.51. Calculated for C$_{43}$H$_{79}$N$_{16}$O$_8$.La$_2$.La(CF$_3$SO$_3$)$_6$.H$_2$O)$_4$.H$_2$O: C, 25.75; H, 3.29; N, 8.47. Found: C, 25.87; H, 3.66; N, 8.47. Calculated for C$_{45}$H$_{79}$N$_{16}$O$_8$.Eu$_2$.Eu(CF$_3$SO$_3$)$_6$.H$_2$O)$_2$.H$_2$O$_2$.CH$_2$Cl$_2$)$_5$: C, 25.87; H, 3.29; N, 8.47. Found: C, 25.87; H, 3.29; N, 8.47. 5h (400 MHz, D$_2$O) 7.26, 4.36, 3.54, 3.52, 3.37, 3.22. Mass Spectrum: (MeOH, ESI+): $m/z$ Expected: 1292.5. Found: 944.3 [M+ 4Trif]/2, 580.0 [M+ 3Trif]/3. IR $\nu_{\text{max}}$ (cm$^{-1}$) 3504, 3324, 3134, 2951, 1638, 1419, 1256, 1168, 1083, 1029, 978, 639, 575, 517.

**Eu.155.**(CF$_3$SO$_3$)$_6$.H$_2$O)$_2$

Eu.155 was prepared by following Procedure 8, using 155 (36.40 mg, 36.88 µmol) and Eu(CF$_3$SO$_3$)$_3$ (46.40 mg, 77.45 µmol). This yielded a pale yellow solid 66.00 mg, 81 %. M.P. decomp above 215 °C. Calculated for C$_{45}$H$_{79}$N$_{16}$O$_8$.Eu$_2$.Eu(CF$_3$SO$_3$)$_6$.H$_2$O)$_2$.CH$_2$Cl$_2$)$_5$: C, 25.87; H, 3.29; N, 8.47. Found: C, 25.87; H, 3.29; N, 8.47. 5h (400 MHz, D$_2$O) 7.26, 4.36, 3.54, 3.52, 3.37, 3.22. Mass Spectrum: (MeOH, ESI+): $m/z$ Expected: 1292.5. Found: 944.3 [M+ 4Trif]/2, 580.0 [M+ 3Trif]/3. IR $\nu_{\text{max}}$ (cm$^{-1}$) 3504, 3324, 3134, 2951, 1638, 1419, 1256, 1168, 1083, 1029, 978, 639, 575, 517.

**La.155.**(CF$_3$SO$_3$)$_6$.H$_2$O)$_4$

La.155 was synthesised according to Procedure 8, using 155 (35.20 mg, 35.66 µmol) and La(CF$_3$SO$_3$)$_3$ (42.00 mg, 71.67 µmol). The resulting
yellow/brown precipitate was dried in a dessicator, 53 mg, 69 %. M.P. decomp above 215 °C. Calculated for C_{46}H_{82}La_{2}Ni_{6}O_{8} \cdot (CF_{3}SO_{3})_{6} \cdot (H_{2}O)_{4} \cdot (CH_{2}Cl_{2})_{5}: C, 25.77; H, 3.79; N, 8.44. Found: C, 26.17; H, 3.39; N, 8.35. δ_{H} (400 MHz, D_{2}O) 7.26, 4.37, 3.23, 2.74. Mass spectrum: (MeOH, ES+): m/z Expected: 1264.4 Found: 930.5 [M+ 4Trif]/2, 570.4 [M+ 3Trif]/3, 390.9 [M+ 2Trif]/4. IR υ_{max} (cm^{-1}) 3518, 3326, 3131, 2955, 1638, 1417, 1255, 1168, 1086, 1029, 761, 639, 575, 517.

Eu.156. (CF_{3}SO_{3})_{6} \cdot (H_{2}O)_{2}

Eu.156 was synthesised by following Procedure 8, using Eu(CF_{3}SO_{3})_{3} (90.00 mg, 120 μmol, 2.2 equivalents). The product was successfully isolated as a yellow solid, 58.00 mg, 47 %. M.P. decomp above 235 °C. Calculated for C_{52}H_{94}Ni_{6}O_{8}Eu_{2} \cdot (CF_{3}SO_{3})_{6} \cdot (H_{2}O)_{2} \cdot (CH_{2}Cl_{2})_{6}: C, 27.30; H, 3.94; N, 7.96.; Found: C, 27.02; H, 3.61; N, 8.33. δ_{H} (400 MHz, D_{2}O) 31.63, 9.90, 3.21, 2.81, 2.56, 1.51, 1.14, 1.00, 0.76, 0.24, −1.04, −5.89, −7.44, −7.94, −12.30, −13.22, −16.19. Mass spectrum: (MeOH, ES+) m/z Expected: 1376.6. Found: 985.1 [M+4trif]/2, 607.1 [M+3trif]/3, 557.1 [M+2trif]/3, 343.1. IR υ_{max} (cm^{-1}) 3465, 2872, 2350, 1621, 1463, 1253, 1163, 1081, 1029, 957, 911, 824, 758, 638, 574, 427.

2-(1,4,7,10-tetraaza-cyclododec-1-yl)-N-[6-(2-1,4,7,10-tetraaza-cyclododec-1-yl-acetylamino)-hexyl]-acetamide (151)

1,6-bis-(2-chloro-acetylamino)-hexane (128) (0.40 g, 1.49 mmol), molybdenum cyclen (150) (1.05 g, 2.97 mmol), Cs_{2}CO_{3} (1.45 g, 4.46 mmol) and KI (0.20 g, 1.22 mmol) were placed in a 100 mL RBF. DMF (35 mL) was added under vacuum and the suspension was freeze-pump-thawed three times. The reaction was heated to 85 °C and left to stir for 120 hours under argon. The solution was filtered and the DMF was removed under reduced pressure. The residue was suspended in HCl (15 %; 25 mL) and left stirring overnight. The solution
was filtered and then washed with CH₂Cl₂ (3 x 20 mL). The aqueous solution was basified with KOH and extracted with CH₂Cl₂ (4 x 20 mL). The CH₂Cl₂ was dried over K₂CO₃, filtered and the solvent removed. The residue was dissolved in EtOH (15 mL) and conc. HCl (3 mL) was added and the solution. The resulting precipitate was filtered and washed with EtOH to yield a brown solid, 0.42 g, 37 %. M.P. decomp above 178 °C. Calculated for C₂₆H₆₂N₁₀O₂Cl₆.MeOH: C, 40.97; H, 8.40; N, 17.69. Found: C, 41.14; H, 7.97; N, 17.77. δH (400 MHz, D₂O) 3.31 (s, 4H, CH₂CO), 3.06, (s, 24H, cyclen CH₂), 2.88 (bs, 12H, cyclen CH₂ & CH₂NCO), 1.39 (bs, 4H CH₂), 1.20 (s, 4H, CH₂). δC (100 MHz, D₂O) 172.4, 54.9, 49.2, 42.2, 41.8, 39.1, 27.6, 25.3. Mass Spectrum: (MeOH, ES+) m/z Expected: 540.5. Found: 541.6 [M+H], 271.4 [M+2H/2]. IR (cm⁻¹) 3427, 2935, 2648, 1647, 1554, 1444, 1375, 1271, 1175, 1075, 1009, 947, 728, 574, 489.

2-[4,7-bis-carbamoylmethyl-10-((6-[2-(4,7,10-tris-carbamoylmethyl-1,4,7,10-tetraaza-cyclododec-1-yl)-acetylamino]-hexylcarbamoyl}-methyl)-1,4,7,10-tetraaza-cyclododec-1-yl]-acetamide (157)

151 (0.10 g, 0.14 mmol), bromoacetamide (0.13 g, 0.97 mmol) and Et₃N (0.21 g, 2.07 mmol) were placed in a 100 mL RBF and dissolved in EtOH (35 ml). The solution was heated at 70 °C for 96 hours. The solvent was removed under reduced pressure and the residue was triturated with CH₂Cl₂. The residue was dissolved in EtOH (2 mL) and added dropwise to swirling CH₂Cl₂ (100 mL). The resulting precipitate was filtered. This was then dissolved in boiling i-propanol : EtOH 3:1 (5 mL) and left to stand. The resulting precipitate was filtered, isolating a pale yellow solid, 0.10 g, 83 %. M.P. decomp above 147 °C. Calculated for C₃₈H₇₄N₁₆O₈.(CH₂Cl₂)₃.MeOH: C, 43.12; H, 7.24; N, 19.16. Found: C, 43.12; H, 7.61; N, 19.55. δH (400 MHz, D₂O) 3.35 (m, 12H, CH₂acet), 3.12 (bm, 4H, CH₂CObridge) 3.08 (bm, 12H, 4 x CH₂cycalen + 2 x CH₂(1,6)), 2.87 (bs, 24H, CH₂cycalen), 1.38 (s, 4H, CH₂(2,5)), 1.19 (s, 4H, CH₂(3,4)). δC (100 MHz, D₂O) 174.2, 173.5, 169.7, 56.1, 55.6, 50.1, 49.8, 48.9, 38.9, 27.8, 25.3. Mass Spectrum: (MeOH, ES+) m/z Expected: 882.6. Found: 883.7 [M+H],
442.5 [M+2H/2]. IR ν\text{max} (cm\textsuperscript{-1}) 3388, 3187, 2935, 2851, 1670, 1551, 1407, 1288, 1161, 1118, 1088, 974, 888, 772, 592.

**La.157.**(CF\textsubscript{3}SO\textsubscript{3})\textsubscript{6}.(H\textsubscript{2}O)\textsubscript{4} was prepared according to Procedure 8, using **157** (65.00 mg, 74.06 \(\mu\text{mol}\)) La(CF\textsubscript{3}SO\textsubscript{3})\textsubscript{3} (91.00 mg, 155.53 \(\mu\text{mol}\)). The residue was triturated with acetone and dried under vacuum, yielding a yellow solid, 13.70 mg, 87 %. M.P. decomp above 127 °C. Calculated for C\textsubscript{38}H\textsubscript{74}N\textsubscript{16}O\textsubscript{8} La\textsubscript{2}.(CF\textsubscript{3}SO\textsubscript{3})\textsubscript{6}(H\textsubscript{2}O)\textsubscript{4}.(CH\textsubscript{2}Cl\textsubscript{2})\textsubscript{4}.(MeOH)\textsubscript{4}: C, 24.07; H, 4.12; N, 8.64. Found: C, 24.05; H, 4.22; N, 8.38. \(\delta\text{H} (400 \text{ MHz, D}_2\text{O})\) 4.03, 3.76, 3.43, 3.27, 2.89, 2.59, 1.45, 1.25. Mass Spectrum: (MeOH, ES+) \(m/z\) Expected: 1160.4. Found: 535.7 [M+3Trif]/3, 364.5 [M+2Trif]/4. IR ν\text{max} (cm\textsuperscript{-1}) 3377, 2975, 2868, 1669, 1635, 1466, 1270, 1169, 1085, 1030, 974, 913, 819, 762, 639, 576, 517, 434.

**2-chloro-acetylaminoo** acetic acid methyl ester (159)\textsuperscript{253} Glycine methyl ester.HCl (2.00 g, 15.94 mmol) and Et\textsubscript{3}N (3.22 g, 31.87 mmol) were placed in a 100 mL RBF and dissolved in CH\textsubscript{2}Cl\textsubscript{2} (15 mL). The solution was cooled to 0 °C, and chloroacetylchloride (1.80 g, 15.94 mmol) in CH\textsubscript{2}Cl\textsubscript{2} (20 mL) was added dropwise over one hour. The reaction was left stirring overnight. The solution was washed with 0.1M HCl (4 x 20 mL) and then with H\textsubscript{2}O. The solvent was removed to yield the product as a brown oil, 1.57 g, 60 %. \(\delta\text{H} (400 \text{ MHz, CDCl}_3)\) 7.11 (s, 1H, NH), 3.99 (s, 2H, CH\textsubscript{2}Cl), 3.95 (d, 2H, J = 6.0 Hz, CH\textsubscript{2}NH), 3.64 (s, 3H, OCH\textsubscript{3}). \(\delta\text{C} (100 \text{ MHz, CDCl}_3)\) 169.3, 166.2, 51.9, 41.8, 40.8. Mass Spectrum: (MeOH, ES+) \(m/z\) Expected: 165.5 Found: 166.4 [M+H].
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(2-{4,10-Bis-[{methoxycarbonylmethyl-carbamoyl}-methyl]-1,4,7,10-tetraaza-cyclododec-1-yl}-acetylamino)-acetic acid methyl ester (160)

Cyclen (99) (0.94 g, 5.45 mmol), and NaHCO₃ (1.60 g, 19.07 mmol) were placed in a 100 mL RBF to which was added MeCN (15 mL). 159 (2.84 g, 17.16 mmol) was dissolved in MeCN (35 mL) and added drop wise over 2 hours. The solution was left stirring overnight at room temperature and then heated to 60 °C and left for 96 hours. The solution was filtered and the solvent removed. The residue was dissolved in CH₂Cl₂ and purified by alumina column chromatography using gradient elution 100 → 80:20 CH₂Cl₂:MeOH(NH₃) to give a white foam, 0.84 g, 27 %. Calculated for C₂₃H₄₁N₇O₉·CH₂Cl₂: C, 44.72; H, 6.72; N, 15.21. Found: C, 45.08; H, 6.86; N, 15.63. δH (400 MHz, CDCl₃) 9.38 (bs, 1H, NH), 8.69, (s, 1H, NH), 8.38 (s, 2H, NH), 3.90 (s, 4H, NCH₂CO), 3.83 (d, 2H, 6 = 5.5 Hz, NHCH₂CO), 3.61 (s, 6H, OCH₃), 3.57 (s, 3H, OCH₃), 3.31 (s, 2H, CH₂CO), 3.21 (s, 4H, CH₂CO), 3.14 (s, 4H, cyclen CH₂), 2.88 (s, 4H, cyclen CH₂), 2.73 (s, 8H, cyclen CH₂). δC (100 MHz, CDCl₃) 172.2, 171.1, 170.5, 169.6, 60.6, 55.5, 54.8, 53.0, 52.7, 51.7, 51.2, 46.6, 40.3, 40.1. Mass Spectrum: (MeOH, ES+) m/z Expected: 559.3. Found: 560.2 [M+H]. IR νmax (cm⁻¹) 3431, 3066, 2840, 1744, 1659, 1540, 1437, 1370, 1216, 1121, 1034, 982, 708, 582.

Procedure 9: General procedure for synthesis Bis Glycine systems

The 3-armed glycine cyclen ligand 160, was placed in a 100 mL RBF with the relevant linker (0.5 equivalents), Cs₂CO₃ (1.5 equivalents) and KI (0.5 equivalents). DMF (30 mL) was added. The suspension was heated to 80 °C and left stirring for 120 hours. The solution was filtered and the DMF removed under reduced pressure. The residue was triturated with ether and acetone. The residue was precipitated from ether (100 mL) and, subsequently, from acetone (75 mL). The precipitate was then triturated with CH₂Cl₂ and dried under vacuum over P₂O₅.

{2-[4,7-bis-[{methoxycarbonylmethyl-carbamoyl}-methyl]-10-((4-[2-{4,7,10-tris-[{methoxycarbonylmethyl-carbamoyl}-methyl]-1,4,7,10-tetraaza-cyclododec-1-yl}-acetylamino)-methyl]-benzylcarbamoyl}]-
methyl)-1,4,7,10-tetraaza-cyclododec-1-yl]-acetylamino}-acetic acid methyl ester (162)  

162 was prepared according to Procedure 9, using 120 (65.00 mg, 0.24 mmol), 160 (265.00 mg, 0.12 mmol), Cs₂CO₃ (232.00 mg, 0.71 mmol) and KI (0.20 g, 0.12 mmol). The desired product was yielded as a brown solid, 0.13 g, 41 %. M.P. decomp above 107 °C. δₓ (400 MHz, D₂O) 7.16 (s, 4H, Ar-H), 4.28, (d, J = Hz, CH₂-Ar), 3.92 (bs, 4H, CH₂CO₂bnd), 3.64-3.56 (bm, 30H, OCH₃ + 6x CH₂gly), 3.20 (bs, 12H, CH₂acet), 2.71 (bs, 32H, CH₂cyclo). δₓ (100 MHz, D₂O) 175.7, 175.3, 171.8, 171.3, 171.1, 136.5, 127.0, 57.1, 56.5, 56.1, 55.1, 52.3, 52.2, 50.1, 49.9, 42.7, 42.6, 42.1, 40.67. IR νₓ (cm⁻¹) 3362, 3074, 2952, 2837, 1743, 1663, 1606, 1543, 1437, 1386, 1305, 1216, 1116, 1033, 974, 679, 569.

[2-(4,7-Bis-[(methoxycarbonylmethyl-carbamoyl)-methyl]-10-{[4-(2-[4,7,10-tris-[(methoxycarbonylmethyl-carbamoyl)-methyl]-1,4,7,10-tetraaza-cyclododec-1-yl]-acetylamino)-butylcarbamoyl]-methyl]-1,4,7,10-tetraaza-cyclododec-1-yl)-acetylamino]-acetic acid methyl ester (163)  

163 was synthesised according to Procedure 9, using 126 (64.00 mg, 0.26 mmol), 160 (0.30 g, 0.53 mmol), Cs₂CO₃ (0.26 g, 0.79 mmol) and KI (0.05 g, 0.30 mmol) the residue was then triturated with MeCN and dried under vacuum to give a brown solid, 0.14 g, 41 %. M.P. decomp above 108 °C. δₓ (400 MHz, D₂O) 3.94 (bm, 12H,
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CH\textsubscript{2gly}, 3.64 (s, 6H, OCH\textsubscript{3}), 3.61 (s, 12H, OCH\textsubscript{3}), 3.42 (bs, 8H, CH\textsubscript{2}CO), 3.14 (bm, 20H, CH\textsubscript{2}CO, CH\textsubscript{2cyclen}), 2.63 (bm, 24H, CH\textsubscript{2}). \(\delta\)C (100 MHz, D\textsubscript{2}O) 173.3, 171.1, 168.9, 165.6, 164.1, 60.4, 56.8, 52.3, 50.0, 49.2, 42.7, 40.6, 38.5, 25.4. IR \(\nu\)\textsubscript{max} (cm\textsuperscript{-1}) 3407, 3088, 2949, 2854, 2362, 1735, 1655, 1542, 1438, 1387, 1308, 1222, 977, 569.

[2-(4,7-bis-[(methoxycarbonylmethyl-carbamoyl)-methyl]-10-{{6-(2-{4,7,10-tris-[(methoxycarbonylmethyl-carbamoyl)-methyl]-1,4,7,10-tetraaza-cyclododec-1-yl}-acetylamino)-hexylcarbamoyl]-methyl}-1,4,7,10-tetraaza-cyclododec-1-yl}-acetylamino]-acetic acid methyl ester (164)

164 was prepared by following Procedure 9, using 128 (0.13 g, 0.48 mmol), 160 (0.57 g, 0.96 mmol), Cs\textsubscript{2}CO\textsubscript{3} (0.46 g, 1.43 mmol) and KI (0.05 g, 0.30 mmol), yielding a brown solid, 0.33 g, 52%.

M.P. decomp above 114 °C. \(\delta\)H (400 MHz, D\textsubscript{2}O) 3.97 (bs, 12H CH\textsubscript{2gly}), 3.66 (s, 6H, OCH\textsubscript{3}), 3.65 (s, 12H, OCH\textsubscript{3}), 3.50 (bm, 20H, CH\textsubscript{2}CO, CH\textsubscript{2NH}), 2.80 (bm, 32H, CH\textsubscript{2cycien}), 1.41 (s, 4H, NHCH\textsubscript{2}CH\textsubscript{2}), 1.21 (s, 4H, NHCH\textsubscript{2}CH\textsubscript{2}CH\textsubscript{2}). \(\delta\)C (100 MHz, D\textsubscript{2}O) 171.3, 171.1, 165.0, 164.8, 164.2, 60.4, 57.0, 56.8, 56.4, 52.3, 52.2, 49.6, 49.0, 42.8, 40.7, 40.6, 38.8, 27.8, 25.3. IR \(\nu\)\textsubscript{max} (cm\textsuperscript{-1}) 3406, 2941, 2859, 1943, 1739, 1662, 1542, 1439, 1385, 1307, 1221, 1119, 1033, 979, 904, 768, 670, 583.

La.162.(CF\textsubscript{3}SO\textsubscript{3})\textsubscript{6}.(H\textsubscript{2}O)\textsubscript{4}

La.162 was prepared according to Procedure 8, using 162 (72.00 mg, 54.35 \(\mu\)mol) and La(CF\textsubscript{3}SO\textsubscript{3})\textsubscript{3} (64.00 mg, 108.69 \(\mu\)mol) yielding a brown solid,
Chapter 5 – Experimental Procedures

0.13 g, 90 % yield. M.P. decomp above 239 °C. δ_H (400 MHz, D_2O) 7.20, 4.30, 4.03, 3.95, 3.64, 3.54, 2.81, 2.54. IR ν_max (cm⁻¹) 3423, 3126, 2956, 2925, 2326, 1742, 1627, 1439, 1420, 1260, 1167, 1080, 1031, 763, 642, 576, 519.

**La.163.(CF₃SO₃)₆(H₂O)₄**

La.163 was prepared by following Procedure 8, using 163 (77.00 mg, 59.87 μmol) and La(CF₃SO₃)₃ (71.00 mg, 119.75 μmol). The residue was then triturated with acetone to yield a brown solid, 0.13 g, 85 %. M.P. decomposition above 221 °C. δ_H (400 MHz, D_2O) 4.08, 4.02, 3.67, 3.64, 3.44, 3.23, 2.83, 2.74, 1.42. IR ν_max (cm⁻¹) 3405, 2958, 2870, 2569, 1742, 1638, 1443, 1412, 1259, 1170, 1081, 1031, 969, 897, 763, 641, 576, 517.

**La.164.(CF₃SO₃)₆(H₂O)₄**

La.164 was prepared according to Procedure 8, using 164 (92.00 mg, 70.01 μmol) and La(CF₃SO₃)₃ (82.00 mg, 140.03 μmol) yielding a brown solid, 0.13 g, 72 %. M.P. decomposition above 224 °C. δ_H (400 MHz, D_2O) 4.01, 3.94, 3.67, 3.63, 3.41, 2.99, 2.83, 2.74, 1.39, 1.21. IR ν_max (cm⁻¹) 3399, 2954, 2866, 2565, 1742, 1638, 1442, 1411, 1255, 1170, 1082, 1031, 969, 896, 763, 640, 576, 517.
Eu.164.(CF₃SO₃)₆.(H₂O)₂

Eu.164 was synthesised according to Procedure 8, using 164 (90 mg, 68.87 µmol), and Eu(CF₃SO₃)₃ (82.00 mg, 137.70 µmol). The residue was then triturated with acetone to give a brown solid, 0.12 g, 66%. M.P. decomp above 225 °C. δ₁H (400 MHz, D₂O) 25.74, 24.85, 23.61, 8.05, 4.27, 4.01, 3.74, 3.49, 2.92, 1.48, 1.29, 0.85, −2.09, −3.10, −3.87, −6.11, −6.89, −8.35, −9.13, −11.48, −12.03, −13.27. IR νₘ₉₉ (cm⁻¹) 3380, 3135, 2956, 2868, 2564, 1743, 1638, 1443, 1412, 1278, 1249, 1443, 1412, 1278, 1249, 1168, 1080, 1030, 761, 639, 574, 517.

2-chloro-N-pyridin-3-yl-acetamide (140)

3-aminopyridine (6.35 g, 67.55 mmol) was dissolved in acetone (70 mL). The solution was placed in an ice bath (0 °C). Chloroacetyl chloride (15.22 g, 135.10 mmol) was then added dropwise over 1 hour. The solution was left stirring overnight at room temperature. The white precipitate that formed was filtered and washed with acetone. It was then dried at 80 °C for 30 minutes to yield a white solid, 12.04 g, 86%. M.P. 134 - 135.6 °C (Lit M.P. 134 - 135 °C).[^3] δ₁H (400 MHz, D₂O) 9.16 (d, J = 3 Hz, 1H, H₂), 8.44 (d, J = 6 Hz, 1H, H₄), 8.39 (d, J = 9 Hz, 1H, H₆), 7.91 (dd, J = 3 Hz, J = 9 Hz, 1H, H₅), 4.26 (s, 4H, CH₂). δ₁C (100 MHz, D₂O) 168.0, 136.9, 136.5, 132.9, 132.6, 127.1, 42.2.

2-[4,7-bis-(pyridin-3-ylcarbamoylmethyl)-1,4,7,10tetraaza-cyclododec-1-yl]-N-pyridin-3-yl-acetamide (161)

Cyclen (99) (0.70 g, 4.09 mmol) 140 (2.54 g, 12.28 mmol) Cs₂CO₃ (4.00 g, 12.28 mmol) and KI (0.5 g, 3.00 mmol) were placed in a 100 mL RBF to which MeOH (35 mL) was added. The solution was refluxed for 36 hours. The orange solution was then filtered and
the volume reduced (~5 mL). CH₂Cl₂ (10 mL) was added. The solution was filtered and the compound purified by alumina column chromatography, using gradient elution 100 → 80:20 CH₂Cl₂:MeOH(NH₃), to give a white solid, 0.69 g, 29 %. Calculated for C₂₉H₃₇N₁₀O₃ [M+H] m/z = 573.307. Found m/z = 573.3050 (–3.7 ppm). δH (400 MHz, MeOH) 8.71 (s, 1H, H2’), 8.63 (s, 2H, H2), 8.26 (d, 1H, J = 5 Hz, H4’), 8.19 (d, 2H, J = 5 Hz, H4), 8.15 (s, 1H, H6’), 7.96 (s, 2H, H6), 7.29 (dd, 1H, J = 5 Hz, J = 3.5 Hz, H5’), 7.03 (dd, 2H, J = 5 Hz, J = 3.5 Hz, H5), 3.56 (s, 4H, CH₂CO), 3.41 (s, 2H, CH₂CO), 3.15 (s, 4H, CH₂cycien), 3.01 (s, 4H, CH₂cycien), 2.91 (s, 4H, CH₂cycien), 2.79 (s, 4H, CH₂cycien). δC (100 MHz, MeOH) 171.4, 170.9, 143.2, 143.1, 140.1, 139.6, 135.6, 135.0, 127.2, 126.9, 123.4, 123.3, 57.3, 56.1, 51.3, 48.1, 44.9, 44.6. Mass Spectrum: (MeOH, ES+) m/z Expected: 574.3. Found: 575.3 [M+H], 597.3 [M+Na].

heated at 85 °C for 120 hours. The solution was filtered and the DMF was evaporated at reduced pressure. The residue was dissolved in MeOH (5 mL) and added dropwise to swirling ether (100 mL). The resulting precipitate was filtered, dissolved in MeOH (5 mL) and dropped onto swirling acetone (80 mL). The resulting precipitate was filtered and triturated with CH₂Cl₂. This allowed the isolation of the desired product as a yellow solid, 0.33 g, 41 %. M.P. decomp above 143 °C. Calculated for
La.165 was prepared according to Procedure 8, using 165 (96.00 mg, 70.34 µmol) and La(CF₃SO₃)₃ (82.40 mg, 140.69 µmol). This yielded a brown solid upon drying, 0.15 g, 83 %.

M.P. decomp above 196 °C. Calculated for C₇₀H₈₈N₂₂O₈.(CF₃SO₃)₆.(H₂O).(CH₂Cl₂)₅.((CH₃)₂CO)₂: C, 24.07; H, 4.12; N, 8.64. Found: C, 24.05; H, 4.22; N, 8.38.δH (400 MHz, MeOD) 8.68, 8.33, 8.04, 7.23, 4.27, 4.09, 3.69, 3.32, 3.18, 2.81, 2.58. IR νmax (cm⁻¹) 3474, 3290, 3099, 2925, 2871, 2362, 1654, 1562, 1486, 1437, 1279, 1253, 1167, 1085, 1029, 963, 8872, 809, 705, 639, 574, 517.
10 % EtOH in acetone solution. A white precipitate was collected, 0.25 g, 15 %. δ_H (400 MHz, D_2O) 8.14 (d, 2H, J = 9 Hz, Ar-H), 7.23 (d, 2H, J = 9 Hz, Ar-H), 3.85 (d, 2H, J = 20 Hz, CH_2), 3.68 (m, 1H, CH), 3.51 (q, 3H, J = 7 Hz, CH_3). Mass Spectrum: (MeOH, ES+) m/z Expected: 276.0 Found: 299.0 [M+Na]. UV λ_{max} 299 nm.

5.3 Lanthanide Complexes – Photophysical Studies

All photophysical studies were carried out in aqueous solution or in aqueous solution buffered at pH 7.4 and high ionic strength (0.1 M TMACl or 0.1M TMAPCl) as indicated. Complex concentrations were at ~ 36 μM for Chapter 2 and 16 μM for Chapter 3. Luminescence measurements were made on a Varian Carey Eclipse Fluorescence spectrophotometer with the settings displayed below unless otherwise indicated. All scans were saved in CSV format and processed using Microsoft Excel to produce all graphs.

### Luminescence Settings

- **Mode**: Phosphorescence
- **Flash**: 1
- **Gate**: 10 ms
- **Excitation slit width**: 10 or 5 nm
- **Total Decay**: 0.02 ms
- **Delay**: 0.1 ms
- **PMT Voltage**: High
- **Emission slit width**: 5 nm

Lifetime studies were performed in H_2O and D_2O with ~ 3 mg in 2 mL of the relevant complex. The settings for the Varian Carey Eclipse Fluorescence spectrophotometer are displayed below.

### Lifetime Settings

- **Direct excitation**: Eu(III) – 395 nm, Tb(III) – 366 nm
- **Emission**: Eu(III) – 615 nm, Tb(III) – 545 nm
- **No. Cycles**: 1000
- **Flash**: 1
- **Gate**: 0.1 ms
- **Excitation slit width**: 5 nm
- **Total Decay**: 10 - 15 ms
- **Delay**: 0.1 ms
- **PMT Voltage**: High
- **Emission slit width**: 5 nm

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5.4 Phosphodiester Hydrolysis Studies

Kinetic measurements were carried out using an Agilent 8453 spectrophotometer or a Varian Carey Eclipse 100, fitted with a circulating temperature controlled water bath, and a mechanical stirrer. The rate constant $k$, for the hydrolysis of $\text{HPNP}$ was determined by fitting the data to first order rate kinetics.

The measurements were conducted in aqueous solution buffered at the relevant pH (50 mM HEPES). The concentration of $\text{HPNP}$ was 0.18 mM (Abs 1.22 at 300 nm). The solution (2.4 mL) was incubated at 37 °C for 10 minutes. One equivalent of the relevant lanthanide complex, prepared in 100 μL of methanol, was then added to the $\text{HPNP}$, and the reaction monitored by absorption spectroscopy over 16 hours.
Chapter 6

References
References

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References

References

References


References

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226
Spectral width was set at 100 ppm (i.e. ± 50 ppm). Additional spectral widths were obtained by adjusting the central offset (i.e. – 150 to – 50 ppm, 0 to + 150 ppm).


The rate constant (k) values were determined by fitting the data to first order rate kinetics using Biochemical Analysis Software for Agilent Chem. Station, or Varian Cary 100 Eclipse.

Breslow, R.; Huang, D. L. Proc. Natl. Acad. Sci. USA, 1991, 88, 4080. [krel is the ratio between k and the rate constant of the ‘uncatalysed’ reaction, (kuncat) which has been measured to be 0.00012 h⁻¹, τ₁/₂ = 5.78 x 10⁻³ h, at pH 7.4].


References


275 Unpublished work Gunnlaugsson, T.; Leonard J. P.


290 Ann Marie Fanning PhD Thesis Trinity College Dublin 2006
Appendix
Appendix

Appendices

Figure 1. Changes in absorbance at 230 nm with respect to pH for 106, showing hypochromic shift upon deprotonation the amide

Figure 2. Eu(III) Luminescence JOB Plot where [G] is the concentration of guest i.e. Cu(II) and [H] the concentration of host i.e. Eu.104
Figure 3. Luminescence spectra of Eu.104 in pH 7.4 buffered solution is quenched upon addition of Fe(II). Excited at 266 nm.

Figure 4. Luminescence spectra of Eu.104 in pH 7.4 buffered solution is quenched upon addition of Co(II). Excited at 266 nm. Slit widths at 10 nm resulted in broader peaks.

Figure 5. Luminescence spectra of Eu.104 in pH 7.4 buffered solution (0.1M TMACl) is quenched upon addition of Co(II). Excited at 266 nm. Note J2/J1 ratio.
Figure 6. Effect upon luminescence intensity of Eu.104 after additions 1 equivalent of Cu(II) followed by 1 equivalent of EDTA, pH7.4

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Figure 7. Absorption spectra for DMABA, Tb.117 titration, pH 7.4, 0.1 M TMACl

Figure 8. Changes in fluorescence spectra for DMABA Tb.117 titration pH 7.4, 0.1 M TMACl, excite at 288 nm. Note band at 320 nm due to Tb.117
Figure 9. 400 MHz $^1$H NMR of La.165 in D$_2$O

Figure 10. Manual plot of ln(Abs$_{400}$) Vs time for La.157 gave a rate constant of 4.16 x $10^{-3}$ min$^{-1}$ or 0.249 hr$^{-1}$
Figure 11. Kinetics profile for the hydrolysis of HPNP (0.18 mM) by La.163 at pH 7.4, 37°C

Figure 12. Kinetics profile for the hydrolysis of HPNP (0.18 mM) by La.164 at pH 7.4, 37°C

Figure 13. Kinetics profile for the hydrolysis of HPNP (0.18 mM) by Eu.164 at pH 7.4, 37°C
Figure 14. pH profile for the hydrolysis of 0.18 mM HPNP by La.163
Publications
Delayed lanthanide luminescence sensing of aromatic carboxylates using heptadentate triamide Tb(III) cyclen complexes: the recognition of salicylic acid in water†

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The coordinately unsaturated terbium complexes Tb.1 and Tb.2 possess two labile metal-bound water molecules that can be displaced upon metal chelation to aromatic carboxylic anions such as salicylic acid in water, which gives rise to large enhancements in the Tb(III) luminescence.

The use of fluorescence detection for the sensing and physiological monitoring of cations, anions and neutral molecules has been extensively investigated. For in vivo sensing, it has been shown to be essential to use fluorophores that absorb and emit at long wavelengths, or have long lived excited states. This is to prevent poor signal-to-noise ratio due to excitation and background emission. The use of fluorescence detection for the sensing and recognition of neutral molecules has been extensively investigated. For these receptor sites consequently modulates the energy transfer process causing the Eu(ni) or Tb(ni) emission to be switched on.6,7 Concurrently, Parker et al. have developed lanthanide luminescent sensors for anions such as bicarbonate using covalently coordinated unsaturated complexes with covalently bonded chiral antennae.8 In these, the oxy-anions displayed quenching of metal-bound water molecules, which caused the lanthanide ion emission to be switched on. Inspired by this work, we were interested in developing new types of luminescent chemosensors for aromatic carboxylic acids, such as N,N-dimethylaminobenzoic acid (3) and salicylic acid (6), with biological concentration of ca. 0.4 mM. Here, the sensing action would be due to enhanced sensitisation of the lanthanide ion excited state by the aromatic acid itself upon displacement of the labile metal-bound water molecules. The attractiveness of such complexes is that they do not have covalently attached antennae, and as such are photophysically inert (ε ~ 10 M~1 cm~1), we have covalently incorporated into these complexes antennae, or chromophores whose function is to populate the excited states of the lanthanide ions indirectly (εD0 and εD4 for Eu(ni) and Tb(ni), respectively) via energy transfer. Recognition at these receptor sites consequently modulates the energy transfer process causing the Eu(ni) or Tb(ni) emission to be switched on.6,7 The structure shows that the ion is coordinating to the four nitrogens of the cyclen structure and the three oxygens of the amides, with a 01W -Eu-02W bite angle of 90.0°. The presence of two water molecules was confirmed by X-ray crystallography for both Eu.1 and Eu.2, the latter being shown in Fig. 1. As far as we know, these are the first crystal structures of heptadentate tri-arm amide cyclen complexes showing two metal bound water molecules.6 The fact that the propiolic anions such as salicylic acid in water rise to large enhancements in the Tb(III) luminescence.10 The presence of two water molecules was confirmed by X-ray crystallography for both Eu.1 and Eu.2, the latter being shown in Fig. 1. As far as we know, these are the first crystal structures of heptadentate tri-arm amide cyclen complexes showing two metal bound water molecules.6 The presence of two water molecules was confirmed by X-ray crystallography for both Eu.1 and Eu.2, the latter being shown in Fig. 1. As far as we know, these are the first crystal structures of heptadentate tri-arm amide cyclen complexes showing two metal bound water molecules.6

Fig. 1 Diagram of Eu.2 showing the two coordinated water molecules with atomic displacement parameters at the 30% level. Hydrogen atoms have been omitted from the ligand for clarity.

The ability of Tb.1, Tb.2, Eu.1 and Eu.2 to bind and recognise the aromatic carboxylic acids 3, 6 and 7, the ester 4 and the ketone 5, was investigated in water, in both pH 7.4 TRIS and pH 7.4 HEPES buffers, and in the presence of 0.1 M of tetramethylammonium chloride to maintain constant ionic strength. In a typical experiment, 17 μM solutions of the complexes were titrated with several potential sensitisers (chromophores) 3-7 and the changes in the lanthanide emission spectra and in lanthanide excited state lifetimes monitored. All of these sensitisers have triplet state energies of ca. 22000–26000 cm~1 which is close to that found for Eu(ni) 3D0 (E = 17200 cm~1) and Tb(ni) 3D4 (E = 20500 cm~1), suggesting that energy transfer from their excited states to the column chromatography for 1 and 2, respectively. The corresponding cationic complexes Tb.1, Tb.2, Eu.1 and Eu.2 were made upon complexation to trilate salts of Tb(ni) and Eu(ni), respectively, in refluxing dry CH3CN (ESI†). Due to the high coordination requirements of the lanthanide ions (usually 9–10), the heptadentate lanthanide ion complexes have vacant coordination sites that are occupied by solvent molecules such as water.6,10

† Electronic supplementary information (ESI) available: experimental section, Fig. S1, Table S1. See http://www.rsc.org/suppdata/cc/b2/b204888d/
excited state of the lanthanide ions is feasible. As expected none of the above complexes were emissive in the absence of these chromophores indicating that the complexes were physically silent at low concentration (when excited at 300 nm). However, it was possible to determine the hydration number $q$ for all the complexes by measuring their excited state lifetimes in D$_2$O and D$_2$O at high concentration. All of these gave a $q$ value of $ca.$ 2, indicating that two water molecules were coordinating to the metal centres in solution.

Initially the ability of the complexes to recognise 3 was evaluated in water. Of the above four complexes, only Tb.1 and Tb.2 became emissive upon titrating with 3, when excited at 300 nm. For Tb.2 the emission at 491, 548, 587 and 622 nm was 'switched on' with luminescence enhancement factors of ca. 680, the largest changes being in the 548 nm band for the 3D$_{4s}$→F$_{5}$ transition. For Tb.1 the changes were somewhat smaller, ca. 220. When these measurements were carried out in buffered solutions and high ionic strength, large emission enhancements were also observed as shown in Fig. 2 for Tb.2, but the 3D$_{4s}$→F$_{5}$ transition was not observed at 622 nm. For Tb.2 the lifetimes of the excited state of Tb.2 in the presence of 0.40 mM of 3 was measured to be 1.79 and 1.59 ms in D$_2$O and H$_2$O, respectively, which gives $q = 0$ (using $q = 5/(1/\tau_{D_2O} - 1/\tau_{H_2O}) - 0.06^{[6]}$ See ESIf), i.e. both water molecules had been displaced upon coordination of the carboxylic acid to Tb(ni). By plotting the changes in the Tb(ni) emission at 548 nm as a function of $-\log[3]$ a bell-shaped curve$^{[6]}$ was observed (see insert in Fig. 2). Examination of this curve showed that the emission was 'switched on' from $-\log[3]$ ca. 5.8 to 4.4 (ca. 2 log units), which is an indication of 1:1 binding and simple equilibrium. An estimated log $\beta = 5 \pm 0.1$ can be deduced from these changes between $-\log[3] = 5.8-4.4$. However, between $-\log[3] = 4-3.4$ the emission was 'switched off'. No pH changes were observed upon titrating Tb.2 with 3 (in buffered solution) indicating that this was not due to protonation of 3 and concomitant dissociation of the ternary complex. We believe that this 'switching off' is rather due to self-quenching but we are currently investigating these features more closely. Upon titrating Tb.2 using 4 or 5, no Tb(ni) emission was observed. However, the addition of 0.4 mM of 3 to a solution of Tb.2 and 4 (3 mM) gave rise to large enhancements in the Tb(ni) emission, indicating the selective recognition and binding of 3 to the Tb(ni) ion and the formation of a ternary complex structure. These results suggest that efficient binding and population of the Tb(ni) 3D$_{4s}$ excited state is only possible if the metal bounded water molecules are removed upon binding.$^{8}$

The effect on the Tb(ni) emission upon binding of salicylic acid 6 ($\lambda_{ex} = 296$) and its acetate ester 7 (Aspirin$^\copyright$) under physiological conditions was also investigated. As described above, the emission from Tb.2 was highly dependent on the concentration of 6, with a larger order of magnitude enhancement in the Tb(ni) emission (see ESIf). The enhancement factor for Tb.1 was somewhat smaller. However, unlike that seen for 3, only two emission bands at 491 and 548 nm were observed. As for 3, the binding of 6 ($-\log[6]$) to Tb.2 gave a bell-shaped dependence from $-\log[6] = 2.6-4.5$ when measured at 548 nm ($\log \beta \sim 4.5$ in the range of 3.5-4.9, see ESIf). In contrast, the binding of 7 to Tb.2 was not observed, i.e. no Tb(ni) luminescence was observed. We propose that this lack of binding and hence sensitisation, is due to steric effects since 7 might be too large to approach the metal ion centre efficiently. Therefore Tb.2 is an excellent chemosensor for 6, the biologically active form of 7 under physiological conditions. We predict that upon recognition of 3 and 6, the oxygen atoms of the aromatic carboxylates bind to the Tb(ni) ion, forming a four-membered ring chelate, but a similar (five-membered) binding mode has been reported by Dickins et al. for lactate$^{[6]}$ (see also ref. 8) However, it is also possible that 6 binds to Tb.2 via a one of the carboxylate oxygens and the phenolic oxygen in a six-membered chelate. This is currently under investigation.

We thank National Pharmaceutical Biotechnology Centre, BioResearch Ireland, Enterprise Ireland, Kinerton Ltd, and TCD for financial support, Dr Hazel M. Moncrieff, Dr Angelo Taglietti and Dr Stephen Faulkner for helpful discussion and Dr John E. O'Brien for assisting with NMR.

Notes and references


**Fig. 2** The change in the Tb(ni) emission in Tb.2 upon addition of 3 in water when excited at 300 nm under physiological conditions. Insert: The changes in the Tb(ni) emission at 548 nm as a function of $-\log[3]$. 

---

\[\text{Chemical structure of 3 and 6.}\]

---

\[\text{Graph showing the decrease in emission intensity with increasing concentration of 3.}\]

---

\[\text{Table summarising the binding parameters for 3 and 6.}\]
The Formation of Luminescent Supramolecular Ternary Complexes in Water: Delayed Luminescence Sensing of Aromatic Carboxylates Using Coordinated Unsaturated Cationic Heptadentate Lanthanide Ion Complexes

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The synthesis of four lanthanide ion complexes Eu-1, Eu-2, Tb-1 and Tb-2, from the heptadentate tri-arm cyclen (1,4,7,10-tetraazacyclododecane) ligands 1 and 2 that were made in one-pot syntheses is described. These coordinatively unsaturated complexes have two labile metal-bound water molecules, as demonstrated by X-ray crystallography. This was also confirmed by evaluating their hydration state ($q \approx 2$) by measuring their lifetimes in D$_2$O and H$_2$O, respectively. The above complexes were all designed as being "photophysically silent" prior to the recognition of the anion, since they do not possess an antenna that can participate in sensitisation of the Eu(III) or the Tb(III) excited state. However, the two water molecules can be displaced upon anion binding by the appropriate aromatic carboxylates to give ternary complexes in water, through either four- or six-member ring chelates ($q \approx 0$), or possibly via a monodentate binding. In the case of Tb-1 and Tb-2, large luminescence enhancements were observed upon the formation of such ternary complexes with N,N-dimethylaminobenzoic acid at ambient pH. Such binding and luminescent enhancements were also observed for Tb-1 in aqueous solution.

The Future of Supramolecular Chemistry

There is no doubt that Supramolecular Chemistry is a fast growing field of research. As the field grows, and more complex targets and applications are addressed, we must ensure that we do not neglect the fundamental science needed to achieve many of our principle aims. Recognition and signalling using chemosensors have become an ever more important part of supramolecular chemistry, particularly from a medical diagnostic point of view, since it enables the use of non-invasive, real-time monitoring of physiological species in vivo, something that we all will benefit from. In this paper, we try to address some of these fundamental aims such as recognition and signalling by developing "simple" self-assembly lanthanide-based sensors.

Thorfinnur (Thorri) Gunnlaugsson was born in Iceland in 1967. He obtained his PhD with Professor A. P. de Silva at Queen’s University Belfast, working on luminescent switches and sensors. He then moved to Durham University, England, as a postdoctoral fellow with Professor David Parker, working on developing luminescent lanthanide sensors, an area which still features strongly in his work. In 1998, he was appointed as the Kinerton Lecture in Medicinal Chemistry at Trinity College Dublin, and in 2000 as a lecturer in Organic Chemistry. In 2002 he was elected as Fellow of Trinity College Dublin. His main research interests are in the areas of Supramolecular and Medicinal Chemistry, and particularly in the fields of recognition and targeting.

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the presence of salicylic acid. On all occasions, the anion recognition "switched" the emission "on" over two logarithmic units. At higher concentrations, the emission is reduced possibly due to quenching. In the case of aspirin, the binding was too weak to be measured, indicating that Tb-1 selectively detects salicylic acid, the active form of aspirin in water. In the case of Eu-1 and Eu-2, the affinity of these complexes towards such aromatic carboxylates was too weak for efficient ternary complex formation.

Keywords: Lanthanide luminescence; Lanthanide complexes; Sensing; Aspirin; Salicylic acid

INTRODUCTION

The use of luminescence detection for the sensing and monitoring of ions and molecules is of great current interest [1-13]. Whereas earlier work was mainly focused on the use of fluorescence as the mode of detection, which has excited state lifetimes in the nanosecond time range, the need for the development of luminescent chemical sensors that can be employed for online detection in vitro has led to the need to develop chemosensors that possess long-lived excited states [14-18]. The use of such delayed emission has great advantages over fluorescence, since it overcomes the poor signal-to-noise ratio that is caused by short-lived background emission (autofluorescence) and light scattering of the surrounding biological environment [19,20]. As in the case of fluorescence detection, the recognition event can be monitored by observing the changes in the various photophysical properties of the emitting moiety such as wavelength, intensity (or quantum yield of luminescence: \( \Phi_L \)) or lifetime [21-25].

We have been interested in the sensing of cations and anions by employing fluorescence and/or colorimetric detection [26-32]. Recently, we have developed both luminescence switches [33,34] and chemosensors [35-37] which utilise lanthanide luminescence, by preparing well-defined and stable octadentate Eu(III) and Tb(III) cyclen (1,4,7,10-tetraazacyclododecane) macrocyclic complexes [38-40]. Such complexes possess long-lived excited states (approximately in the millisecond range) and emit at long wavelengths (500-750 nm) with large Stokes shifts and characteristic line-like emission bands (10-30 nm bandwidth) under ambient conditions [41-44]. These are all qualities necessary for online in vitro monitoring. However, normally the lanthanide ions are photophysically inert due to low molar absorptivities, which is related to Laporte forbidden f–f transitions making direct excitation difficult [33-46]. Nevertheless, this drawback can be overcome by incorporating one or more antennae covalently into the lanthanide complexes, ensuring that the lanthanide-excited states [which are \( ^5D_0 \) and \( ^5D_4 \) for Eu(III) and Tb(III), respectively] are efficiently populated through sensitisation [33-47]. This is a well-established process that occurs via an energy-transfer mechanism from the singlet-excited states of the antenna to the lanthanide excited states via the antenna triplet state [33-48]. Such lanthanide sensitisation has also been demonstrated by using non-covalently linked antennae by employing \( \beta \)-diketones [45-51] and other related organic ligands [52-63], and also by using \( \beta \)-cyclodextrins (CD)-based lanthanide complexes, as recently demonstrated by several researchers [64-69].

In designing our lanthanide chemosensors, we took advantage of the use of the sensitising antennae by simply incorporating the recognition site into the antennae themselves [33-37]. Hence, any perturbation at this site, i.e. recognition, subsequently led to the modulation in the sensitisation process with concomitant changes in the lanthanide luminescence. However, the drawback to such chemosensors is the elaborate synthetic pathways required for the desired sensors, which is both time-consuming and often results in a low overall yield. Because of this, we have recently turned our attention to the development of kinetically stable heptadentate coordinative unsaturated cyclen complexes that do not have the receptor-antenna moieties as an integrated part of their structures [70]. Examples are the triarmamide complexes Eu-1, Eu-2, Tb-1 and Tb-2 [70]. It has recently been reported by Parker et al. [71,72] that in water at pH 7.4, such related coordinatively unsaturated complexes have two water molecules associated with their structures, since the lanthanide ions fulfil their high coordination requirements (typically 9-10) by binding two solvent molecules. Furthermore, it has also been shown that these are labile water molecules, which can be removed upon binding of anions such as bicarbonates, acetate, phosphates and halides [71,72].

Inspired by this work, our aim was to develop novel chemosensors where the two labile metal-bonded water molecules could be displaced upon metal chelation yielding self-assembly ternary complexes between Eu-1, Eu-2, Tb-1 and Tb-2 and several aromatic carboxylates [70]. Here, the idea was that instead of having the antenna covalently bound to

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{figure1.png}
\caption{Schematic diagram of the self-assembly process that leads to the formation of the luminescent ternary complex between the lanthanide complex and the carboxylate antenna.}
\end{figure}
the ligand, the aromatic carboxylate themselves would act as antennae. Hence, only upon coordination of the aromatic anion, through self-assembly, would the lanthanide ion emission be "switched on" (via excitation of the antennae). Such a recognition event is depicted in Fig. 1. Furthermore, because of the population of the lanthanide excited state by the coordinated anions, the above complexes could all be considered to be "photophysically silent" prior to the anion recognition. Moreover, as the binding of these antennae would be directly to the metal centre, the population of the lanthanide ions would be greatly improved due to the small distance between the antenna and the metal ion (assuming a Förster mechanism where efficiency is dependent on $\sim 1/r^6$) [73,74]. Furthermore, such binding would be expected to be in a 1:1 ratio. However, it would be expected that this binding would be highly dependent on the nature and the structure of the antenna, and their ability to chelate to the metal centre. Furthermore, the choice of aromatic carboxylates as sensitiser is somewhat limited by their triplet excited state energy, which needs to be greater than that of the excited states of Eu(III) and Tb(III) (20,400 and 17,200 cm$^{-1}$ for $^5D_0$ and $^5D_4$, respectively). With this in mind, we decided to use neutral pendent amide arms, as they would ensure that the complexes were cationic, and as such would maximise the binding interactions.

In this paper, we give a full account of the synthesis of Eu-1, Eu-2, Tb-1 and Tb-2 from the heptadentate tri-arm functionalised cyclen ligands 1 and 2, and the photophysical evaluations of these compounds in the presence of several aromatic carboxylic acid derivatives.

**DESIGN AND SYNTHESIS OF 1 AND 2 AND THEIR Eu(III) AND Tb(III) COMPLEXES**

We set out to develop simple lanthanide ion complexes that would allow for the monitoring of important aromatic carboxylic acids such as salicylic acid, the active form of the prodrug aspirin, at the same time of requiring only minimal synthetic efforts [75]. With this in mind, we set out to make the two ligands 1 and 2 in one-pot syntheses.

The synthesis of 1 and 2 was achieved by reacting the α-chloroamides of N-methylacetamide (3) and N,N-dimethylacetamide (4), respectively, with cyclen in dry CH$_3$CN (in 3:1 ratio of acetamide: cyclen) at 65°C for 3 days in the presence of NaHCO$_3$. For 1, the macrocycle was purified by alumina column chromatography using CH$_2$Cl$_2$, followed by gradient elution using NH$_3$-saturated MeOH (0 → 3%). For 2, the macrocycle was purified by tituration with diethyl ether. Both 1 and 2 were obtained in over 50% yield after purification. The ligands were characterised using standard methods. However, both were found to be hygroscopic, and elemental analyses were therefore not obtained. High-resolution accurate masses were obtained for both 1 and 2. The $^1$H NMR spectrum of both ligands showed the presence of C$_2$ symmetry that runs along an axis through the unalkylated amine in position 1 and the tertiary amine in position 7 of the cyclen ring. The $^1$H NMR spectrum of 2 is shown in Fig. 2 and shows the presence of two N-H resonances at 7.60 and 7.30 ppm, in a ratio of 1:2, for the two amide protons. The two α-protons for the pendant arms appear as singlets at 3.06 and 2.97 ppm (in a ratio of 4:2), respectively, whereas the N-C$_3$H$_7$ protons appeared as two doublets in a ratio of 6:3 at 2.75 and 2.67 ppm, respectively. The cyclen methyl protons appear as four broad resonances in the region of 2.7-2.4 ppm. The $^1$H NMR spectrum for 1 showed similar characteristics.

The Eu(III) and Tb(III) complexes of 1 and 2 were made by refluxing together an equivalent amount of 1 or 2 with either Eu(III) or Tb(III) triflate (SO$_3$CF$_3$)$_3$ in dry CH$_3$CN under an inert atmosphere for 24 h.
FIGURE 2. $^1$H NMR spectrum of the ligand 2 in CDCl$_3$ (400 MHz) showing the presence of the C$_2$ symmetry in 2.

Upon cooling to room temperature the solution was poured into a stirring solution of dry diethyl ether, which resulted in oily residues that were collected by decanting the organic layers and rinsing the resulting residues with either CH$_2$Cl$_2$ or CHCl$_3$. These complexes were characterised by elemental analysis, electrospray MS (ESMS), IR and by NMR. The $^1$H NMR spectrum of all the complexes showed the presence of the paramagnetic metal centres, as indicated by several broad resonances appearing over a large ppm range [76]. The ESMS for the four complexes showed the presence of two triflate counterions as the major peak with several other combinations of multiple charges, which had an identical isotopic pattern to the corresponding calculated spectrum. In the ESMS, the presence of the two expected metal-bonded water molecules was not observed. We were, however, able to obtain X-ray crystal structures of three of the four complexes, namely Eu-1, Eu-2 and Tb-1.

**X-RAY CRYSTALLOGRAPHIC INVESTIGATION OF Eu-1 AND Tb-1**

We were able to grow crystals of Eu-1, Eu-2 (not shown) and Tb-1 that were suitable for X-ray crystallographic determination [77,78]. The Eu(III) crystals were obtained by slow evaporation from a mixture of CH$_3$CN and CH$_2$Cl$_2$ (in the ratio of 1:1). All the crystal structures showed the presence of the two metal-bonded water molecules. The structure of Tb-1 is shown in Fig. 3, but we have previously published the structure of the Eu(III) analogue [70]. To the best of our knowledge, these are the first examples of such triamid lanthanide ion-based cyclen complexes [70,76,79]. From Fig. 3, it is evident that the Tb(III) centre is coordinating to the four nitrogen of the macrocyclic ring and to the three oxygens of the carboxylic amides on the pendent arms, with average Tb–N and Tb–O distances of 2.634(4) Å and 2.347(3) Å, respectively. In general, the lanthanide ions prefer a high-coordination environment, which is usually in the order of 9–10 [77,78]. For Tb-1, seven of these are provided by the ligand, with two further coordination sites being occupied by two metal-bound water molecules. The Tb–O1W and Tb–O2W distances are almost identical, being 2.429(3) Å and 2.441(3) Å, respectively. For the isostructural Eu-1 complex, these distances were measured to be 2.418(5) and 2.421(5), respectively. Table I lists selected bond angles and distances for the two complexes. From these, it can be seen that there are only minimal differences observed for the two complexes. Similar results were observed for Eu-2 (not shown here). From the crystal structure data of the complexes, it is apparent that they all adapt a general square antiprism geometry in the solid state. Such geometry is well known for the tetra-substituted octadentate lanthanide-based cyclen complexes. For such square antiprism geometry, two elements of chirality can be observed, which are associated with the sign of the torsion angles of the NCCO chelate (Δ or Λ) and the NCCN chelate rings (β or ε) helicity of the N-alkylated pendent arms [80,81]. For Tb-1 (as shown in Fig. 3), these were determined to be an average of 19.2° and −58.5°, respectively. However, for Tb-1, the complex crystallised in a centrosymmetric space group containing both Δ and Λ conformations. Similar results were observed for Eu-1.
FORMATION OF LUMINESCENT SUPRAMOLECULAR COMPLEXES IN WATER

TABLE I Selected bond lengths and angles for Eu-1 [70] and Tb-1

<table>
<thead>
<tr>
<th>Bond Type</th>
<th>Eu-1</th>
<th>Tb-1</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ln−O (Å)</td>
<td>2.342(5), 2.364(5), 2.378(5)</td>
<td>2.326(3), 2.348(3), 2.368(3)</td>
</tr>
<tr>
<td>Ln−O (water) (Å)</td>
<td>2.418(5), 2.421(5)</td>
<td>2.429(3), 2.441(3)</td>
</tr>
<tr>
<td>N−C−C−N (°)</td>
<td>58.9(9), 59.4(9), 62.2(9), 57.0(9)</td>
<td>58.9(9), 59.4(9), 62.2(9), 57.0(9)</td>
</tr>
<tr>
<td>N−C−C−O (°)</td>
<td>72.20(18)</td>
<td>71.80(11)</td>
</tr>
</tbody>
</table>

For our design, it is essential that all the complexes possess two water-bonded molecules, as seen for Tb-1 in Fig. 3. There are two main reasons for this. First, even though both ligands lack the covalently functionalised antennae, it is possible to generate an excited state in both Eu(III) and Tb(III) by direct excitation of the metal centres (see later). However, the water molecules can efficiently quench the excited state through OH vibrations (or by any other energy-matched oscillators), which further diminishes any lanthanide luminescence prior to the recognition event (the formation of the ternary complex). Secondly, the need for two “vacant” coordination sites at the metal centre would ensure that the binding of the carboxylate would be possible through a bidentate manner (for instance by both of the oxygens of the carboxylate). Subsequently, the water molecules would be expelled and no longer able to quench the lanthanide excited state. From the above crystal structures, it can be seen that this criterion is fulfilled. However, the angle at which these two water molecules bind to the ions is also of importance, since the nature of the binding mode would depend on the bite angle between the two water molecules. For Tb-1, the O1W−Tb−O2W bite angle was measured to be 71.80(11)° whereas for Eu-1, the O1W−Eu−O2W bite angle was measured to be 72.20(18)°, indicating that both complexes would be able to interact with the carboxylates via such a bidentate manner.

Recently, Parker et al. [71] and Dickins et al. [82] have shown that related three-arm cyclen complexes can form bidentate adducts with organic anions such as acetate, citrate, glycinate, and lactate through four- and five-member chelates. The bite angles for all of these complexes were between 54° and 69°. In the case of acetate, this binding occurred through both of the carboxylate oxygens. We thus proposed that the aromatic carboxylates would bind to Eu-1, Eu-2, Tb-1 and Tb-2 in a similar manner. However, to date, we have not been able to crystallise these complexes in the presence of any of the aromatic carboxylates to prove this theory.

LUMINESCENCE STUDIES

Determining the Hydration Numbers of Eu-1, Eu-2, Tb-1 and Tb-2 in the Absence and Presence of N,N-Dimethylaminobenzoic Acid (7) Antenna

As stated above, both the Eu(III) and the Tb(III) complexes were expected to possess two labile metal-bound water molecules. This was verified for three of these complexes by determining their solid-state structures. The number of metal-bound water molecules can also be evaluated by estimating the hydration state or number of the complexes using a luminescent method, where the rate constant for the radiative decay (k) of the 5D0 excited state of Eu and the 5D4 excited state of Tb are measured in H2O (kH2O) and D2O (kD2O), respectively [83]. These values can be obtained by direct excitation of the lanthanide ion complexes at high concentrations. The hydration number (q) can then be determined from Eqs. (1) and (2) for the Eu and Tb complexes, respectively.

\[ q_{\text{Eu(III)}} = 1.2 \left( \frac{1}{k_{\text{H2O}}} - 1 \right) - 0.25 - 0.075x \] (1)

\[ q_{\text{Tb(III)}} = 5 \left( \frac{1}{k_{\text{H2O}}} - 1 \right) - 0.06 \] (2)

Here, the prefixes 1.2 and 5 in Eqs. (1) and (2), respectively, are proportionality constants that mirror the sensitivity of the corresponding ions to quenching by metal-bound water molecules. The correction terms −0.25 and −0.66 represent quenching by second sphere water molecules, whereas −0.075x in Eq. (1) represents the quenching by N−H oscillators, where x is the number of such oscillators within a given complex [83–85].

Table II summarises the observed lifetimes (\( \tau \)) of the corresponding values and

<table>
<thead>
<tr>
<th>Complex</th>
<th>( \tau_{\text{H2O}} ) (ms)</th>
<th>( k_{\text{H2O}} ) (1/ms)</th>
<th>( \tau_{\text{D2O}} ) (ms)</th>
<th>( k_{\text{D2O}} ) (1/ms)</th>
<th>q</th>
</tr>
</thead>
<tbody>
<tr>
<td>Eu-1</td>
<td>0.281</td>
<td>3.55</td>
<td>0.855</td>
<td>1.17</td>
<td>2.55</td>
</tr>
<tr>
<td>Eu-2</td>
<td>0.315</td>
<td>3.17</td>
<td>0.936</td>
<td>1.07</td>
<td>1.95</td>
</tr>
<tr>
<td>Tb-1</td>
<td>0.720</td>
<td>1.38</td>
<td>1.080</td>
<td>0.92</td>
<td>2.01</td>
</tr>
<tr>
<td>Tb-2</td>
<td>0.869</td>
<td>1.15</td>
<td>1.386</td>
<td>0.72</td>
<td>1.85</td>
</tr>
</tbody>
</table>
the $q(\pm 0.5)$ values for the above complexes in the absence of any carboxylate antennae [hence, by direct excitation of the Ln(III) ions] [84,85]. From these data, it can be seen that all the complexes have two water molecules associated with their structure in solution. The above measurements were repeated by exciting the lanthanide ions at 300 nm, the same wavelength that most of the carboxylate antennae (Scheme 2) absorb at. However, in all cases, the emission was too weak for evaluating $q$ accurately.

As stated above, it was hoped that the formation of a ternary complex between the lanthanide complexes and the antenna would lead to large enhancements in the lanthanide luminescence of these complexes. Such ternary complexes can be formed only if both or one of the water molecules are displaced. To evaluate this, the $q$ values were measured in the presence of several antennae (Scheme 2). Here, the proof for the formation of such ternary complexes would be achieved if $q = 0$, with concomitant enhancements in the lanthanide luminescence.

Initially, the ability of the coumarin antennae 5 and 6 was investigated in water. However, neither of these gave rise to any enhancements in the Eu(III) or Tb(III) luminescence. This suggests that these antennae were unable to bind to the metal centre, because, according to their triplet state energies ($T_1$), the sensitisation by the Tb(III) excited state should be energetically favourable. Because of this, a series of structurally more simple carboxylates were tested, namely the benzoic acids and derivatives 7-16. All of these have triplet state energies of ca. 22,000-26,000 cm$^{-1}$, which is close to that found for $^5D_0$ and $^5D_4$ for Eu(III) and Tb(III), respectively [86]. This suggested that an effective energy transfer form these antennae to the excited state of the lanthanide ions would be feasible, provided that the antennae were able to form stable ternary complexes with Ln1 and Ln2. The first of these antennae to be investigated was the N,N-dimethylaminobenzoic acid 7. Upon addition of 7 to either Eu1 or Eu2 (ca. 17 mM) in water, no Eu(III) emission was observed when excited at 300 nm, indicting that 7 was either not

![Scheme 2 Various carboxylic-acid-based antennae tested.](image-url)
TABLE III Measured lifetimes and rate constants for Eu-1, Eu-2, Tb-1 and Tb-2 and the corresponding hydration number \((q \pm 0.5)\) in the presence of \(N,N\text{-dimethylaminobenzoic acid}\)

<table>
<thead>
<tr>
<th>Complex</th>
<th>(\tau_{H_2O}) (ms)</th>
<th>(k_{H_2O}) (1/(\text{ms}))</th>
<th>(\tau_{D_2O}) (ms)</th>
<th>(k_{D_2O}) (1/(\text{ms}))</th>
<th>(q)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Eu-1</td>
<td>0.235</td>
<td>4.25</td>
<td>0.438</td>
<td>2.28</td>
<td>1.79</td>
</tr>
<tr>
<td>Eu-2</td>
<td>0.276</td>
<td>3.62</td>
<td>0.733</td>
<td>1.35</td>
<td>2.15</td>
</tr>
<tr>
<td>Tb-1</td>
<td>1.593</td>
<td>0.62</td>
<td>1.795</td>
<td>0.55</td>
<td>0.06</td>
</tr>
<tr>
<td>Tb-2</td>
<td>1.626</td>
<td>0.61</td>
<td>1.895</td>
<td>0.52</td>
<td>0.14</td>
</tr>
</tbody>
</table>

binding to the Eu(III) centre or not able to populate the Eu(III) excited state efficiently. Furthermore, it is also known that the Eu(III) excited state can be quenched by an electron-transfer mechanism using similar antennae [72].

When these measurements were repeated using Tb-1 and Tb-2, a positive sensitisation occurred, with the Tb(III) emission being clearly visible. For instance, upon addition of 4 equivalents of 7, the emission of Tb-1 at 491, 548, 687 and 622 nm was "switched on" with luminescence enhancement factors of ca. 700 (the quantum yield was not determined). Here, the largest changes were seen in the \(^5D_4 \rightarrow ^7F_5\) transition at 548 nm, which is magnetic-dipole allowed. For Tb-1, these enhancements factors were somewhat smaller or ca. 220. These results indicate that for both Tb-1 and Tb-2, the carboxylate was binding to the Tb(III) centres and successfully populating their excited states (upon excitation of the antenna at 300 nm).

Having established that 7 was able to sensitisate the Tb(III) excited states, we measured the lifetimes of the excited states of Eu-1, Eu-2, Tb-1 and Tb-2, in the presence of 7 (0.68 mM, 4 equivalents) with the aim of determining the hydration numbers of the four complexes. These results are summarised in Table III. From these measurements it can be seen that for either Eu-1 or Eu-2 \(q\) was evaluated to be ca. 2, indicating that both have two water molecules associated with their structures and that neither are binding to 7. However, for Tb-1 and Tb-2, both give rise to similar excited state lifetimes in either \(H_2O\) or in \(D_2O\), which indicates that no inner-sphere solvent molecules were present, so \(q\) was evaluated to be ca. 0 for both of these complexes. This signifies that for both Tb-1 and Tb-2, a self-assembly process occurs between 7 and these complexes, giving rise to the formation of luminescent ternary complexes in solution. Furthermore, since both of the water molecules have been displaced upon coordination of the carboxylates to Tb(III), we conclude that such binding occurs through both of the oxygens of the carboxylate in a bidentate manner via a four-member ring chelates. This is depicted in Fig. 4, where the Tb(III) emission of Tb-2 is only "switched on" upon sensitisation by 7 after the formation of the ternary complex.

Luminescent Sensing of Aromatic Amino Carboxylates and Related Derivatives

Having observed the self-assembly process between the Tb(III) complexes and 7, which gives rise the formation of luminescent ternary complexes, we carried out a titration of all the complexes (typically in 17 \(\mu\text{M}\) solution) in the presence of 7-15 in \(H_2O\) and at pH 7.4 using either Tris or Hepes buffers containing 0.1 M of tetramethylammonium chloride to maintain a constant ionic strength. All measurements were carried out in aerated solutions. At such low concentrations, none of the complexes were particularly luminescent, and as such, the emission from these complexes is "switched off".

When Tb-1 was titrated in water using 7, the Tb(III) emission was switched on as previously described. When these measurements were repeated in buffered solution at pH 7.4, the emission was also "switched on". However, the enhancement factors

![FIGURE 4](https://via.placeholder.com/150)

Formation of the ternary complex between 7 and Tb-1 giving rise to large enhancements in the Tb(III) emission. The emission from Tb-1 is said to be "switched on" upon excitation at 300 nm.
were somewhat smaller or 480 for Tb-1. The changes in the Tb(III) emission as a function of increased concentration of 7, between pN 0 - 4.2 (where pN = -log[N], and N is the number of the compound in Scheme 2), can be seen in Fig. 5, where J represents the emission bands corresponding to the deactivation of the $^5D_4 \rightarrow ^7F_J$ transitions. Here, the largest changes occurred in the $J = 5$ and 6. However, to our surprise, upon increasing the concentration of the antenna, the emission was "switched off" again. This became apparent when plotting the changes in the intensity of the 548 nm transition as a function of p7 (Fig. 6). From these changes, a sigmoidal curve was observed between p7 = ca. 6.5 and 4.5, where the emission was "switched on". Since this occurs over ca. two pN units, it can be concluded that this represents a 1:1 binding and a simple equilibrium between the Tb(III) centre of Tb-1 and 7 [26-32,48]. From these changes, a binding constant, log $\beta$, of 4.9 ($\pm 0.2$) was determined [48]. However, between p7 = 4.2-2.9, the emission was "switched off", giving rise to the formation of a sigmoidal "On-Off-On-Off" switch.

To investigate this, we looked at the changes in the 548-nm transition as a function of (equivalents of) 7. These changes can be seen in Fig. 7, where the emission is highly dependent on the concentration of 7. From 0 to ca. 3 equivalents of 7, the Tb(III) is dramatically "switched on", with large order of magnitude enhancements (with ca. 80% of the emission being switched on after 1 equivalent). However, beyond these equivalents, the emission is quenched (see insert in Fig. 7 for 0 → 80 equivalents).

Furthermore, the pH of the solution upon addition of 7 did not change during these measurements (in buffered solution), indicating that this quenching was not due to the protonation of 7 with concomitant dissociation of the ternary complex, but rather was directly due to the increased concentration of the antenna. Consequently, we investigated the effect of increasing the concentration of the complexes in the presence of high concentration of the carboxylate (in ca. 20-fold excess prior to increasing the concentration of Tb-1). As expected, this gave rise to large enhancements in the Tb(III) emission, suggesting that the luminescent ternary complex was formed.\(^3\) Degassing the solution only led to minor additional increase in the luminescence, indicating that quenching of Tb by oxygen was only minor.

Similar results were observed when Tb-2 was titrated using 7, but the enhancement factors were somewhat smaller. This is possibly due to additional quenching of the Tb(III) excited state by N–H oscillators in Tb-2 which are absent in Tb-1. As before, the emission was highly dependent on the concentration of the antenna. Between p7 = 6.2 and 4.4, the emission was "switched on", with log $\beta$ = 5.0 ($\pm 0.2$), whereas between 3.5 and 4.5, the emission was "switched off".

The above measurements were repeated using other related derivatives of N,N-dimethylaminobenzoic acid, namely the ketone 8 and the ester 9. No changes were seen in the emission spectra of Eu-1, Eu-2, Tb-1 and Tb-2 upon titration with either 8 or 9, suggesting that it was necessary to have

\(^8\)There is also a possibility that at a very high concentration of 7 (OD ~ 0.6 at its max.), some inner filter effects are observed. However, 8 and 9 (see below), which absorb at similar wavelengths, did not give rise to any sensitisation when interacting with Tb-1. Furthermore, using 10, this quenching was much less pronounced (see Fig. 11) for the same concentration range (see later).
the carboxylate present as the part of the antenna for efficient binding to the metal ion and for sensitisation to occur. Hence, such binding would have to take place through the formation of at least a four-member ring chelate, involving the two oxygens of the carboxylate binding to the metal centre in a bidented manner. This is depicted in Fig. 8A. Furthermore, addition of 7 to a solution of Tb-1 and 8 or 9 gave rise to large enhancements in the Tb(III) emission, indicating that these potential antennae were not able to bind to the metal ion centre. We thus conclude that 7 can be selectively recognised in the presence of these potential antennae.

Over the last few months, two other research groups have reported the use of similar complexes to bind aromatic carboxylates. Faulkner et al. [87] have shown that using tetrathiafulvalene carboxylic acid, the sensitisation of neutral Yb(III)-based triarm carboxylate derived cyclen complexes is possible in the near IR, whereas Li and Wong [88] have shown that Tb(III)-based cyclam complexes functionalised with pendant crown ethers can be used to determining Na\(^+\) and K\(^+\) in the presence of p-chlorobenzoate. On both occasions, the \( q \) values were found to be reduced from ca. 2 to \( \sim 0 \) after binding to these carboxylates antennae to the metal centre for these complexes.

**Luminescent Sensing of Salicylic Acid and Related Derivatives**

The above luminescence studies were also repeated using salicylic acid (which has a biological concentration of ca. 0.4 mM), and several of its derivatives. Salicylic acid is the active form of aspirin, a well-known non-steroidal anti-inflammatory drug. Salicylic acid is also a good painkiller but causes gastric bleeding due to the free phenolic group [89]. Because of this, it is necessary to mask the phenolic group as an ester (aspirin), which is hydrolysed by esterases to yield the free active drug in circulating blood. Furthermore, aspirin has many other pharmacological actions, some of which have the salicylic acid as the active form. For instance, it has anti-thrombotic action via the suppression of plated COX-1 (cyclooxygenase) activity and is thought to have potential anti-tumour actions [90]. However, the cause of gastric bleeding is of major concern, since it can lead to fatal conditions, but currently it is estimated that up to 17,000 people die from this condition every year in USA. The fatal dose of salicylic acid is estimated to be 0.2–0.5 g/kg [91]. Toxic effects appear at varying plasma levels, depending on the duration of poisoning, but are uncommon below 30 mg/dL [91]. However, ingestion of 4 g of salicylic acid has been fatal to infants. It is thus valuable to be able to monitor salicylic acid levels in blood or in serum. With this in mind, we investigated the effect on the Eu(III) and Tb(III) emission of our complexes upon binding to 10 and 11.

As in the case of 7, no enhancements were observed in the Eu(III) emission using Eu-1 and Eu-2 in the presence of 10 or 11 when measured in either H\(_2\)O or in buffer solution at pH 7.4. However, for both Tb-1 and Tb-2, significant enhancements were seen in the Tb(III) emission upon addition of 10. For instance, upon addition of 10 equivalents of 10, the Tb(III) emission of Tb-1 was enhanced by a factor of ca. 40 and 30 in H\(_2\)O and in buffer at pH 7.4, respectively. The changes in the Tb(III) emission of

![FIGURE 8](image-url)
**FIGURE 9** Changes in the Tb(III) emission of Tb-1 upon addition of 10.

Tb-1 can be seen in Fig. 9 when recorded in 0.1 M Tris buffer and in the presence of 0.1 M TMACl. However, these are substantially smaller changes than that seen previously for 7, with the $j = 5$ transition showing the largest changes. For Tb-2, the relative enhancement was large, but the relative intensities were much smaller than that seen for Tb-1, and it was thus not investigated any further.

As before, the changes in the Tb(III) emission were highly concentration-dependent, though not to the same extent as that seen for 7. This is clear from Fig. 10, where the intensity at 548 nm is plotted vs. p10. Here, the luminescence is switched on over ca. 2 log units from 5.5 to 3.5, an indication of 1:1 binding and simple equilibrium. From these changes, a binding constant $\log \beta = 4.5 (\pm 0.2)$ was determined. The self-quenching by the anion was also less pronounced, as can be seen in Fig. 11. Here, the Tb(III) emission gradually increases until ca. 6 equivalents have been added. However, between 6 and ca. 15 equivalents of 10, the emission was not dependent on the concentration of 10. At higher concentrations, ca. 17 equivalents and above, the emission was however "switched off" (not shown).®

When these titrations were repeated using 11, no luminescence enhancements were observed in the Tb(III) emission for either Tb-1 or Tb-2. This indicates that aspirin was not able to bind to the metal centre strongly enough. It is possible that this is due to the size of the compounds (the presence of the ester), which hinders the access to the metal centre. This was confirmed by measuring the $q \sim 2$ for Tb-1 in the presence of 11. Hence, even though 11 has a free carboxylate, no binding was observed.

To investigate the discrimination of 10 over 11 by Tb-1, we carried out a series of titrations using other derivatives of salicylic acid, namely 12–14. As expected, no luminescence enhancements were observed when the mixed ether–ester derivative 14 was used, since both of its possible binding sites are protected. However, when either 12 or 13 was used, large enhancements were seen in the Tb(III) emission. For 12, these enhancements were in the order of ca. 80, whereas for 13, a factor of several hundred was observed. These are very interesting results, since they suggest that two possible binding modes can exist for the binding of these salicylic acid derivatives, as depicted in Fig. 8B and C, respectively. In the former, the binding to the Tb(III) centre occurs through a bidentate manner, where the binding occurs via either a four-member ring chelate which involves both of the oxygens of the carboxylates, whereas in the latter, or only one of these oxygens can participate in the binding, and the second coordination site is occupied by the phenolic oxygen, giving rise to a six-member chelate. Nevertheless, since 13 was also found to be a good sensitisier, this suggests that the binding can occur through the phenolic oxygen alone as depicted in Fig. 8 C.

With the aim of shedding some light on this puzzle, we evaluated the $q$ values for Tb-1 in the presence of 10, 12 and 13 (0.68 mM). The results of this investigation indicated that $q \sim 0–1$, so it is obvious that the removal of the two metal-bound water molecules is not as pronounced as in the case.

®In our earlier communication [70], we reported that we were unable to observe more than two emission bands (at 491 and 548 nm corresponding to $j = 6$ and 5, respectively) when measuring these antennae. Though we did not conclude why this was the case, we have since then recorded all the above measurements on a new fluorimeter. As can be seen from Fig. 9, only the $j = 2$ transition is not observed. Hence, our original measurements were carried out on a machine that was not sensitive to these long-wavelength emission bands.
FORMATION OF LUMINESCENT SUPRAMOLECULAR COMPLEXES IN WATER

FIGURE 11 Changes in the Tb(III) emission of Tb-1 at 548 nm as a function of equivalents of 10.

of 7. An estimated error of ±0.5 is generally acceptable for determining \( q \) by this method. It is thus quite likely that only one of the metal-bound water molecules is replaced upon the binding of these compounds. This is most likely the case for 13, which also might explain the relatively smaller luminescent enhancement factors observed for 13 in comparison with 12. However, we have not been able to fully determine to date whether one or both of these metal-bound water molecules are removed either concurrently or in a stepwise manner. It is also possible that a mixture of these binding modes is operating in the case of 10, 12 and 13. Nevertheless, from the above results, we can conclude that Tb-1 can selectively bind salicylic acid 10 over aspirin 11. We are currently studying these interactions in greater detail. The above measurements were also carried out in the presence of several other carboxylates, namely 15, 16 and 17. Of these, 17 (Diclofenac\(^6\)) is another well-known non-steroidal anti-inflammatory drug like aspirin. However, for all of these compounds, no enhancements were observed in the Tb(III) emission of either Tb-1 or Tb-2.

We are also interested in the potential application of these complexes in highly competitive environments. For instance, using the sodium salts of AMP, ADP or ATP, which can potentially bind to the Tb(III) complexes via their phosphate ester terminus, no luminescence enhancements were observed when measured at neutral pH. Concurrently, Parker et al. have shown that coordinately unsaturated Eu(III) and Tb(III) complexes with covalently bonded chiral antennae can be employed as lanthanide luminescent sensors for simple aliphatic carboxylates such as bicarbonate, carbonate and amino acids [71]. In these complexes, the acetate and carbonates were found to have a stronger affinity for Tb(III) complexes over that of Eu(III). Because of this, we were interested in investigating the effect of competitive binding of carbonate and other biologically active acids to our complexes in the presence of 7 or 10. We thus monitored the changes in the Tb(III) emission upon addition of a solution containing 30 mM of NaHCO\(_3\), 2.3 mM lactate (Na\(^+\) salt), 0.13 mM citric acid and 0.9 mM of Na\(_2\)H\(_2\)PO\(_4\). However, these additions led to a reduction in the Tb(III) emission, most likely indicating that HCO\(_3^-\) or the acids were binding to the Tb(III) centre in a competitive manner and causing the dissociation of the ternary luminescent complex within their physiological concentration ranges. We thus conclude that for the monitoring of 10 in serum, it would be necessary to at least degas the sample prior to its use, but we are currently developing new systems for overcoming these drawbacks.

CONCLUSION

We have shown that it is possible to develop simple sensory systems for aromatic carboxylates by employing self-assembly ternary complexes, where the analyte can function as an antenna, and give rise to the population of the lanthanide excited state in water or in buffered aqueous solution at pH 7.4. We have shown using X-ray crystallography that these complexes have two metal-bound water molecules that can be displaced upon binding to aromatic carboxylates such as 7 and 10. The presence of these water molecules was also confirmed by measuring the luminescent lifetimes of the lanthanide-excited states of these complexes. Of the four complexes Eu-1, Eu-2, Tb-1 and Tb-2, the Eu(III) complexes did not give rise to the formation of such self-assembly complexes. We conclude that this is due to a lower affinity of these complexes towards the carboxylates antennas. Similar observations have been made by other researchers [71,72]. We propose that the binding of 7 to either Tb-1 or Tb-2 is via the formation of four-member bidented ring chelate, whereas for the binding of 10 to Tb-1, this can be through either a mono- or bidentate manner. This binding was confirmed using 12 and 13. In retrospect, salicylic acid 10, the active form of 11, was found to bind to Tb-1 (and Tb-2) with concomitant enhancements in the Tb(III) luminescence. Related structures such as 17, another well-known non-steroidal anti-inflammatory drug like aspirin, were not detected. These complexes all gave bell-shaped dependent pN profiles, which were determined to be due to self-quenching of the anions. Proof for such quenching was obtained by gradually increasing the amount of the Tb(III) complexes in the presence.
of excess amount of 7 or 10. On both occasions, large enhancements were seen in the Tb(III) emission, indicating the formation of luminescent ternary complexes.

In summary, we have developed new types of chemosensors for anions such as aromatic carboxylates. We are currently working towards enhancing the selectivity and sensitivity of these complexes towards important drugs such as salicylic acid and polynucleotides, as shown by their formation in the presence of excess amount of 7 or 10. On both occasions, large enhancements were seen in the Tb(III) emission, indicating the formation of luminescent ternary complexes.

EXPERIMENTAL

General Procedures

Infrared spectra were recorded on a Mattson Genesis II FTIR spectrophotometer equipped with a Gateway 2000 4DX2-66 workstation. Oils were analysed using NaCl plates, and solid samples were dispersed in KBr and recorded as clear pressed discs. 1H NMR spectra were recorded at 400 MHz using a Bruker Spectrospin DPX-400 instrument. Tetramethylsilane (TMS) was used as an internal reference standard, with chemical shifts expressed in parts per million (ppm or δ) downfield from the standard. 13C NMR spectra were recorded at 100 MHz, and 19F NMR spectra were recorded at 376 MHz using a Bruker Spectrospin DPX-400 instrument. Mass spectroscopy was carried out using HPLC-grade solvents. Mass spectra were determined by detection using Electrospray on a Micromass LCT spectrometer, Waters 9360 to pump solvent. The whole system was controlled by MassLynx 3.5 on a Compaq Deskpro workstation. Starting materials were obtained from Sigma Aldrich, Strem Chemicals and Fluka. Columns were run using aluminium oxide (activated, Neutral, Brockmann I grade 150 mesh). Solvents were used at GPR grade unless otherwise stated.

X-ray Crystallography

Crystal data for C23H40F6N9O25S5Tb (Tb1): M = 1105.79, triclinic, space group P 1, a = 8.930 (2) Å, b = 13.321 (3) Å, c = 18.691 (4) Å, α = 95.615 (4), β = 97.542 (4), γ = 104.149 (4), V = 2117.6 (9) Å3, Z = 2, μ = 1.928 mm−1, Rint = 0.0275, transmission ratio (max, min) = 0.738. A total of 18,996 reflections were measured for the angle range 2 < 2θ < 57, and 9140 independent reflections were used in the refinement. The final parameters were wR2 = 0.1069 and R1 = 0.0406 (I > 2σ(I)).

Diffraction data were collected on a Bruker SMART diffractometer using the SAIINT-NT [77,78] software with graphite monochromated Mo-Kα radiation. A crystal was mounted on the diffractometer at room temperature (ca. 298 K) for Tb1. Lorentz and polarisation corrections were applied. Empirical absorption correction was applied using SADABS. The structure was solved using direct methods and refined with the program package [77,78], and the non-hydrogen atoms were refined with anisotropic thermal parameters. Hydrogen-atom positions were added at idealised positions, and a riding model with fixed thermal parameters [Uiso = 1.2Ueq for the atom to which they are bonded (1.5 for CH3)] was used for subsequent refinements. Hydrogen atoms could not be located for the water molecules. The function minimised was Σ[w(|Fo|2 - |Fc|2)] with reflection weights w−1 = [σ2Fo2 + (g1P)2 + (g2P)] where P = [max(|Fo|2) + 2|Fc|2]/3. Additional material available from the Cambridge Crystallographic Data Centre comprises relevant tables of atomic coordinates, bond lengths and angles, and thermal parameters (CCDC number 198706).

Luminescence Studies

All luminescence studies were carried out in aqueous solution or in aqueous solution with a buffer and high ionic strength at pH 7.4. Buffered solutions were made up in aqueous phase with 0.1 M TMACl, 0.1 M HEPES and or 0.1 M Tris buffer, at pH 7.4. Solution concentrations were ~ 16.7 μM. Test solutions were 5 mL or 10 mL Sensitising chroomophore solutions were made up in water 2.0 × 10−3 M and 5.0 × 10−4 M. Luminescence measurements were made on a Perkin Elmer LS 50B and repeated on a Varian Cary Eclipse.

2-(4,10-Bis(dimethylcarbamoylmethyl)-1,4,7,10-tetraaza-cyclododec-1-yl)-N,N-dimethylacetamide (1)

1,4,7,10-Tetraazacyclododecane (98%), (0.375 g, 2.13 mmol) was placed in a 50-mL single-necked RBF. To this were added NaHCO3 (0.537 g, 6.39 mmol) and 15 mL of dried acetonitrile. 2-Chloro-N,N-dimethylacetamide (0.804 g, 6.6 mmol) was added, and the mixture was stirred at 65°C for a further 72 h. The resulting solution was then cooled and passed through a celite filter. The pale yellow solution was eluted to 97:3 DCM:MeOH (NH3) mobile phase. 0.470 g (1.1 mmol), 52% yield, of 2-(4,10-bis(dimethyl- carbamoylmethyl)-1,4,7,10-tetraaza-cyclododec-1-yl)-N,N-dimethylacetamide was collected as a white foam after drying under vacuum, which returned to a residue state over time. Calculated for C20H43N5O5: M + H peak m/z = 428.3344, found: 428.3349; δH (CDCl3, 400 MHz) 10.00 (broad s, 1H, N-H), 3.61(s, 2H, CH2– acetamide), 3.58(s, 4H, CH2– acetamide),
3.09 (s, 8H), 3.04 (s, 3H), 2.96 (s, 6H), 2.90 (s, 10H), 2.84 (s, 7H): δC (CDCl₃, 100 MHz) 170.25, 170.15, 55.51, 53.80, 51.70, 50.57, 49.72, 46.70, 36.39, 35.27; Mass Spec (MeOH, ES +) m/z: Expected: 427.59. Found: 428.33 (M + H), 450.30 (M + Na), 472.30 (M + K); IR νmax (cm⁻¹) 3434, 2927, 2852, 1637, 1508, 1475, 1402, 1338, 1261, 1103, 1064, 1022, 881, 806, 769, 667, 649, 574, 484.

2-(4,7-Bis-methylcarbamoylmethyl-1,4,7,10-tetraaza-cyclododec-1-yl)-N-methylacetamide (2)

1,4,7,10-Tetraaza-cyclododecane (98%) (0.375 g, 2.13 mmol) was placed in a 50-mL single-necked RBF. To this was added NaHCO₃ (0.537 g, 6.39 mmol) and 15 mL of dried acetonitrile. To this were added NaHCO₃ (0.537 g, 6.39 mmol) and 15 mL of dried acetonitrile. RBF. To this were added NaHCO₃ (0.537 g, 6.39 mmol) and 15 mL of dried acetonitrile. 2-Chloro-N-methylacetamide, (0.706 g, 6.39 mmol) and 15 mL of dried acetonitrile. 1,4,7,10-Tetraazacyclododecane (98%) (0.375 g, 88.2 mg (0.23 mmol) of [Ln(SO₄)₂] or 0.26 mmol of Ln (III) Trifluoromethane sulphonate [Ln(SO₄)₂CF₃] was added to a 25-mL single-necked RBF which contained 10 mL of freshly dried acetonitrile. The solution was freeze-thawed three times, placed under an argon atmosphere and left stirring at 82°C for 24 h. The resulting solution was cooled to room temperature and then dropped slowly on to 100 mL of dry diethyl ether. The diethyl ether was poured off to leave 1Ln or 2Ln as oil that was washed with CH₂Cl₂ or CHCl₃ and dried under high vacuum. Yields were ca. 95% in all cases.

2-(4,7-Bis-methylcarbamoylmethyl-1,4,7,10-tetraaza-cyclododec-1-yl)-N,N-dimethylacetamide (2)

Calculated for C₂₃H₄₁N₇O₄F₃S₂ Eu(III) (Eu-2):

2-(4,7-Bis-methylcarbamoylmethyl-1,4,7,10-tetraaza-cyclododec-1-yl)-N,N-dimethylacetamide Eu(III) (Eu-1):
found: 688.1548. Calculated for CigHsgNyOgFeSaEu: 638, 576, 516. 
(M[Triflate] + H), 837.1 (M[Triflate]

[18x790]m/z =  837.1133, found: 837.1181; 1587, 1465, 1419, 1288, 1245, 1160, 1091, 1027, 725,
(3455, 3386, 3297, 3143, 3000, 2933, 2885,1639,
6H(MeOD, 400 MHz) 27.04, 14.96, 11.44,
We thank Kinerton Ltd, Enterprise Ireland (Post-
with running NMR.

References

The development of luminescent signaling devices is an active area of supramolecular chemistry.1 Those where the emission is modulated by single or several external sources (inputs) such as light, ions, and molecules are of particular interest.2 In this regard luminescent switches, sensors, and logic gate mimics have recently been reported.3,4 Many life processes, such as enzymes, operate within a very narrow pH window, where their function or activity can be described as being "switched on" or "switched off" as a function of pH.4 Attempts to mimic such "off—on—off" or "on—off—on" behavior by constructing luminescence devices that are modulated by a single input, e.g., pH, have recently been achieved by employing organic fluorophores.5 Lately, Pallavicini et al. have extended this kind of work and reported "on—off—on" fluorescent indicators for H+ using a tetraaza ligand, Cu(II) and Coumarin 343 as a fluorescent "chemosensing ensemble".6 We have been particularly interested in designing robust multifunctional lanthanide complexes from cyclen (1,4,7,10-tetraazacyclododecane) as kinetically and thermodynamically stable luminescent chemosensors, switches, and logic gate mimics, as well as ribonuclease mimics.7–9 Herein we present the synthesis and the photophysical evaluation of the cationic tetraamide 1,10-phenanthroline (phen)-based Eu(III) complex 1.Eu, which is the first example of a fully reversible pH controlled "off—on—off" signaling system that employs lanthanide luminescence rather than fluorescence, where the "on—off—on" process is due to pH modulation of the phen ligand.10 In addition to being purely metal-based emission, the Eu(III) emission changes occur in the physiological pH range, in a pH window between ca. 4—8. Moreover, the emission of 1.Eu is long-lived (ms range), emitting at long wavelengths (between 500 and 750 nm) with line-like emission bands under ambient conditions. As such, 1.Eu holds greater advantages over fluorescence systems that can be seriously affected by autofluorescence and light scattering, for instance from the physiological environment.

The synthesis of 1.Eu (ESI) was achieved as shown in Scheme 1. 1.Eu was purified by multiple precipitation; first from ether and then from CH2Cl2, giving the desired complex in 80% yield from 1.Eu, Cl2H3Ni(phen)Cl2Eu: 965.2876. Found: 965.2827). The ESMS showed peaks at 406.9 and 481.9 (m/z) for the M+ and (M + triflate)/2 respectively, whereas the 'H NMR (400 MHz, D2O) showed peaks at 406.9 and 481.9 (m/z) for the M+ and (M + triflate)/2 respectively, whereas the 'H NMR (400 MHz, D2O) indicated a typical mono-capped square antiprism geometry8 as a major isomer (ca. 95%) in solution, with resonances at 28.4, 0.28, —3.3, —8.8, —12.1, and —15.7 ppm for the equatorial and axial ring, and the methylene protons of the pendant arms.

The pH dependence of the Eu(III) emission was evaluated in H2O in the presence of 0.1 M tetramethylammonium perchlorate to maintain constant ionic strength (I).8 Due to the low molar absorptivities of Eu(III) the population of the Eu(III) excited state (3D0) is achieved indirectly by the phen antenna via sensitization.8,9,10 We predicted that the ability of the phen ligand to engage in such sensitization would be pH dependent since both the amide and the nitrogens of the phen ligand are sensitive to protonation. Indeed, this was found to be the case. In alkaline solution between pH 12—8.5 only a weak Eu(III) emission was observed at 581, 593, 615, 624, 654, 686, and 702 nm for the deactivation of the 3D0 to the ground states 3F1, 3F2, and 3F3, respectively (at pH 11), giving q ≈ 1, and as such an overall nine-coordinated complex.11 Upon further acidification, this sensitization process was greatly enhanced, and the Eu(III) emission was "switched on" between pH 8.5—5.5, with sharp luminescence enhancements as demonstrated in Figure 1; e.g. LE ≈ 20 for 3D0 → 3F1, with rEu(III) = 0.395 and 0.952 ms in H2O and D2O respectively, which gives q ≈ 1.3, again indicating that the complex was monohydrated. Further acidification gave rise to two effects; notably between pH 5—6.5 the Eu(III) emission was almost constant, whereas between pH 5—3 the emission was gradually "switched off", being ca. 70% quenched at pH 2.5 vs that at pH 6 (Supporting Information). Similarly, using I = 0.1 M
of tetramethylammonium chloride showed identical luminescence behavior, i.e. these changes were not anion dependent. By plotting the changes in the intensity of the $\Delta D_{0} \rightarrow F_{2}$ transitions as a function of pH gave, for all of these emission bands, a bell shaped curve, consisting of two sigmoidal slopes, between pH ca. 3—5 and 6.5—8.5, with maximum intensity being reached at pH 5.8—6.0. Figure 2 shows these changes for the $\Delta D_{0} \rightarrow F_{2}$ in blue for the titration of the alkaline solution with HCl, and the back-titration of this same solution in red, indicating that this pH dependence is fully reversible. It also clearly shows that the changes of these ‘off—on—off’ pH-dependent emissions clearly transpire over the physiological pH range, and, as such, mimic the pH dependences of many enzymatic processes. From these luminescence changes two $\mathrm{pK}_{a}$s were determined as 3.8 (±0.1) and 8.1 (±0.1), the latter being assigned to the deprotonation of the amine nitrogen moiety, the oxidation potential is $\nu = 1$. The concomitant changes in the fluorescence emission spectra between 300 and 550 nm ($\lambda_{\text{em}} = 266$ nm) were less drastic, being ca. 40% quenched upon acidification from pH 11 to 1.5 with an associated shift in $\lambda_{\text{em}}$ max from ca. 420 nm to 440 nm (Supporting Information). In tandem, the absorption spectrum of 1Eu showed hypochromic effects and evidence of some bell-shaped pH dependence, but these were much less drastic in comparison to the Eu(III) emission, as is evident from Figure 3. From these combined spectroscopic changes, it is obvious that only the lanthanide luminescence is substantially affected by pH. However, what determines the large observed bell-shaped pH dependence for the Eu(III) emission? We predict that in an established manner, the excitation of the antenna sensitizes the Eu(III) $\Delta D_{0}$ excited state via an energy-transfer mechanism, and that, in relatively alkaline solution, the Eu(III) ion can be reduced by an electron transfer to Eu(II) which is not emissive. Secondly, the deprotonation of the amide proton increases the reduction potential of the antenna which is reflected in its reduced ability to populate the excited state of the Eu(III) ion. Within a narrow pH window of ca. pH 5—6.5 and within experimental error, the emission is fully pH independent. In acidic solution upon proto-

![Figure 2](image-url)

**Figure 2.** pH-dependence in the luminescent profile for 1Eu, showing the ‘off—on—off’ pH dependent changes in the Eu(III) emission at 592 nm. This pH dependence was fully reversible: in blue the changes from pH 12 → 1.5, and in red the back-titration of the same sample.

![Figure 3](image-url)

**Figure 3.** pH dependence in the absorption spectra of 1Eu, at 266 nm blue circles, and at 278 nm in red.
Eu(III)-cyclen-phen conjugate as a luminescent copper sensor: the formation of mixed polymetallic macrocyclic complexes in water†

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The cationic cyclen based Eu(III)-phen conjugated 1-Eu was synthesised as a chemosensor for Cu(II), where the recognition in water at pH 7.4 gave rise to quenching of the Eu(III) luminescence and the formation of tetranuclear polymeric Cu(II)-Eu(III) macrocyclic complexes in solution where Cu(II) was bound by three 1-Eu conjugates.

The development of photochemically based supramolecular devices is a dynamic area of research, where many excellent examples of luminescent sensors, switches, logic gates and wires have been reported.1-5 To this end we have developed and studied several lanthanide luminescence Tb(III) and Eu(III) complexes as sensors, switches and logic gate mimics by employing ionic and molecular inputs.5 The benefits of such lanthanide devices lie in their long lived excited states (~ ms) and their emission at long wavelengths (500-750 nm) with line-like emission bands which confer an advantage over most fluorescent systems.* Herein, we discuss the development of 1-Eu, a kinetically stable cationic tri-amide cyclen (1,4,7,10-tetraazacyclododecane) based Eu(III) macrocycle conjugated to a 1,10-phenanthroline (phen) ligand. The role of the phen ligand is dual as it serves both as an antenna sensitizing chromophore6,7 for the indirect population (via S1 \to T1) of the Eu(III) excited state *(Do), as well as a receptor for either H+ or Cu(II). Both of these ionic inputs are expected to modulate the photophysical properties of the phen ligand upon ion recognition, and consequently the Eu(III) sensitisation process.6,7 Furthermore, the interaction of the Cu(II) with 1-Eu leads potentially to the formation of a mixed supramolecular macrocyclic complex in solution, containing three 1-Eu complexes coordinating to the Cu(II) ion. Such mixed polymeric transition-lanthanide ion arrays, using coordination macrocyclic systems, are rare.8 1-Eu is also the first example of a kinetically stable lanthanide luminescent sensor that shows good selectivity and sensitivity for Cu(II) over other competitive cations in pH 7.4 water.

The synthesis of 1-Eu was achieved in good yield by first reacting the α-chloroamide 2 with one equivalent of tri-substituted N,N-dimethylacetamide cyclen macrocycle at 80 °C in DMF and Cs2CO3 giving the ligand 1, followed by reaction with Eu(CF3SO3)3 in CH2CN and precipitation from diethyl ether.1 H NMR (400 MHz, D2O) studies showed that 1-Eu has a typical mono-capped square antiprism geometry in solution.

The pH dependence of the Eu(III) emission of 1-Eu was investigated in water in the presence of 0.1 M tetramethylammonium chloride (I = 0.1 M TMACl) to maintain constant ionic strength. At neutral pH, 1-Eu gave rise to typical Eu(III) emission bands (λex = 266 nm), at 581, 593, 615, 654, 686 and 702 nm due to the deactivation of the *D0 excited state to the ground states.5,7 By plotting the changes of the *D0 population of the Eu(III) emission bands as a function of pH, it is evident that the binding of Cu(II) occurs over a narrow concentration range (~log [Cu(II)] = 6-5, which overlaps with that of the physiological concentration of Cu(II)). This is not surprising, given the high coordination requirement of the Cu(II) and its affinity for phen type ligands.9 Importantly, the addition of EDTA (using the same concentration that gave rise to fully 'switched off' state by Cu(II)) to this fully quenched solution, switched the emission back on'. This signifies that the Cu(II) detection by 1-Eu is reversible. Similar titrations were carried out using other transition metal ions. From Fig. 2, it is evident that in water at pH 7.4 the Cu(II) is selectively detected over both Co(II) and Fe(II) (M = Co(II) and Fe(II)). For both the Eu(III) emission is quenched upon recognition but at significantly higher concentration than for Cu(II). We also evaluated the recognition of Cu(II) at high ionic strength, I = 0.1 M.
yixir is; (Cu/Cu+1Eu) when the Eu(iii) emission is fully quenched after full titration with Cu(ii) in both media, and upon addition of EDTA it was switched back on. However, under these conditions the Cu(ii) sensitivity was marginally increased. Similar results were observed for Co(ii), but in high ionic strength the Cu(ii) sensitivity was substantially modulated and became similar to that of Cu(ii) (ESI).

The stoichiometry of the above interactions between Cu(ii) and 1-Eu was investigated using various methods. By plotting the emission changes as a function of equivalents of Cu(ii), it became apparent that the emission was fully quenched after 1:3 Cu(ii) to Eu(iii) complexes. Cu(ii) is known to be able to coordinate to three 1-Eu complexes. Cu(ii) is known to be able to coordinate to phen in either 1:2 or 1:3 ratio, the latter being achieved by distorted octahedral geometry. However, the most reliable proof for the stoichiometric ratio between Cu(ii) and 1-Eu was obtained from Job method analysis.10

By plotting the changes in the Eu(iii) emission (Fig. 3) against molar fraction, and analysing these changes using the equation: Ratio = f_{Cu}/(1 - f_{Cu}) (where f_{Cu} is the mole fraction of Cu(ii)), we determined that the Ratio = 3. This suggests the formation of mixed 1:3 Cu(ii): Eu(iii) supramolecular complexes in solution, as many isomeric 1:3 complexes are possible due to the asymmetry of 1-Eu ligand. To the best of our knowledge, such mixed multinuclear macrocyclic transition-lanthanide ion complexes are not common, and have not previously been shown for cyclen based Cu(ii)-Eu(iii) macrocyclic phen ligands.8 Furthermore, this supramolecular complex is reversibly formed as the addition of EDTA switches the Eu(iii) emission back on, signifying its dissociation. In comparison, when the fluorescence emission spectra of Acm-phen12 was monitored upon addition of Cu(ii), the emission was ca. 90% quenched after ca. 0.5 equivalents of Cu(ii), suggesting 1:2 complex formation.

In summary we have developed a delayed lanthanide luminescence sensor for Cu(ii), which shows good selectivity for Cu(ii) over other ions such as Co(ii), Fe(ii) and Fe(iii) at pH 7.4. Job method analysis of the Cu(ii) recognition indicated the formation of a cationic (11+) tetranuclear mixed polymeric macrocyclic supramolecular complexes in solution where the Cu(ii) ion is coordinating to three 1-Eu complexes. For all the cases, these supramolecular complexes were reversibly formed as the Eu(iii) emission was reversibly ‘switched on’ upon addition of EDTA. We are currently investigating these systems in greater detail, as well as using other lanthanide ions.

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Notes and references

Fig. 2 The relative changes in the Eu(iii) emission of 1-Eu as function of various transition metal ions at J = 2 at 615 nm. All measured at pH 7.4: pink = Cu(ii); blue = Co(ii); black = Fe(ii); green = Fe(iii); red = Cu(ii) in the presence of 150 mM NaCl, 10 mM KCl, 5 mM MgCl2, and 5 mM CaCl2.

Fig. 3 Job plot analysis (intensity changes vs. molar fraction) for the changes in the Eu(iii) emission at 700 nm at pH 7.4. [1-Eu]_initial = 7.16 μM; [Cu(ii)]_initial = 0.89 μM; [Co(ii)]_initial = 0 μM; [Fe(ii)]_initial = 6.26 μM. Insert are the changes in the Eu(iii) emission when recorded at this low concentration.