The Development of Luminescent Lanthanide Chelates for
Cellular Based Assays

A Thesis Presented to the University of Surrey by

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Abstract

In order for luminescent lanthanide complexes to be used in fluoroimmunoassays, a number of criteria must be adhered to, such as high quantum yield, high stability in aqueous media and insensitivity to the surrounding environment. In this thesis, a number of new ligand systems, designed with regard to achieving novel sensitising ligands of europium and terbium for use in cellular assays are discussed.

A new ligand based upon a macrocyclic polyaminocarboxylate incorporating a single bipyridine chromophore and its complexes of europium and terbium are reported. Luminescence properties of both complexes are very good, with the terbium analogue displaying more complex behaviour, which suggest a back energy transfer mechanism from the emissive metal centre to the bipyridine triplet. The development of a different ligand incorporating the bipyridine chromophore as the binding groups based on a macrocyclic backbone are also reported.

Simple intramolecular europium and terbium complexes of salicylaldehyde and o-hydroxybenzophenone were prepared and their photophysical properties recorded. These chelates showed poor stability in solution which led to the investigation ofaza-crown appended version of these ligands with the aim to improve stability. Europium chelates of these sensitisers displayed poor emissive properties at room temperature postulated to be due to a ligand to metal charge transfer deactivation mechanism.

Finally, with the aim of developing charge neutral, fat-soluble luminescent lanthanide complexes capable of cell loading, donors of mixed pyrido-phenol sensitisers were developed. The luminescent properties were found to be poor for the europium complex due to ligand to metal charge transfer. Terbium complexes of these ligands showed sensitivity towards oxygen and temperature, indicating the presence of back energy transfer from the metal centre to the aryl triplet. Complexes of 8-hydroxyquinoline with europium are also reported.
'On the arid lands there will spring up industrial colonies without smoke and without smokestacks; forests of glass tubes will extend over the plains and glass buildings will rise everywhere; inside of these will take place the photochemical processes that hitherto have been the guarded secret of the plants, but that will have been mastered by human industry which will know how to make them bear even more abundant fruit than nature, for nature is not in a hurry and mankind is.'

I would like to thank Professor Peter Sammes for giving me the opportunity to work on this project, for his continual support and encouragement during this time. I would also like to thank Dr. Stephen Faulkner for discussions on the photophysical aspects of this project and for providing equipment for low temperature and degassing experiments.

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Finally, I would like to thank my family for their support and encouragement, and also taking an active interest in what I enjoy doing, which made my research more worthwhile. To my parents especially, who gave me the confidence to achieve my goals and succeed. To Sharon, for understanding and being there throughout the highs and lows over the past three years.
### Abbreviations

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
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<tbody>
<tr>
<td>$S_0$</td>
<td>ground singlet state</td>
</tr>
<tr>
<td>$S_1$</td>
<td>first excited singlet state</td>
</tr>
<tr>
<td>$T_1$</td>
<td>first excited triplet state</td>
</tr>
<tr>
<td>ISC</td>
<td>intersystem crossing</td>
</tr>
<tr>
<td>IC</td>
<td>internal conversion</td>
</tr>
<tr>
<td>ET</td>
<td>energy transfer</td>
</tr>
<tr>
<td>HOMO</td>
<td>highest occupied molecular orbital</td>
</tr>
<tr>
<td>LUMO</td>
<td>lowest unoccupied molecular orbital</td>
</tr>
<tr>
<td>$k_x$</td>
<td>rate constant for some process $x$</td>
</tr>
<tr>
<td>$\tau$</td>
<td>luminescence lifetime, reciprocal rate constant ($k^{-1}$)</td>
</tr>
<tr>
<td>$q_{Ln}$</td>
<td>solvation number of Ln(III) ion</td>
</tr>
<tr>
<td>$corr_{Ln}$</td>
<td>correction factor specific to Ln</td>
</tr>
<tr>
<td>Q</td>
<td>quencher</td>
</tr>
<tr>
<td>CT</td>
<td>charge transfer</td>
</tr>
<tr>
<td>LMCT</td>
<td>ligand to metal charge transfer</td>
</tr>
<tr>
<td>BET</td>
<td>back energy transfer</td>
</tr>
<tr>
<td>FIA</td>
<td>fluoroimmunoassay</td>
</tr>
<tr>
<td>TR-FIA</td>
<td>time-resolved fluoroimmunoassay</td>
</tr>
<tr>
<td>DELFIA</td>
<td>dissociation enhanced lanthanide fluoroimmunoassay</td>
</tr>
<tr>
<td>HTRF</td>
<td>homogenous time-resolved fluoroimmunoassay</td>
</tr>
<tr>
<td>FRET</td>
<td>fluorescence resonance energy transfer</td>
</tr>
<tr>
<td>DEFRET</td>
<td>delayed fluorescence resonance energy transfer</td>
</tr>
<tr>
<td>APC</td>
<td>allophycocyanin dye</td>
</tr>
<tr>
<td>EALL</td>
<td>enzyme amplified lanthanide luminescence</td>
</tr>
<tr>
<td>Cy-5</td>
<td>cyanin-5-dye</td>
</tr>
<tr>
<td>PDT</td>
<td>photodynamic therapy</td>
</tr>
<tr>
<td>Bipy</td>
<td>2, 2-bipyridyl</td>
</tr>
<tr>
<td>TACN</td>
<td>1, 4, 7-triazacyclononane ([9]aneN$_3$)</td>
</tr>
<tr>
<td>CYCLEN</td>
<td>1, 4, 7, 10-tetraazacyclododecane ([12]aneN$_4$)</td>
</tr>
<tr>
<td>Acronym</td>
<td>Full Name</td>
</tr>
<tr>
<td>----------</td>
<td>-----------------------------------------------------</td>
</tr>
<tr>
<td>EDTA</td>
<td>ethylenediamine tetraacetic acid</td>
</tr>
<tr>
<td>DTPA</td>
<td>diethylenetriamine pentaacetic acid</td>
</tr>
<tr>
<td>DOTA</td>
<td>1, 4, 7, 10-tetraazacyclododecane-1, 4, 7, 10-tetraacetate</td>
</tr>
<tr>
<td>DO3A</td>
<td>1, 4, 7, 10-tetraazacyclododecane-1, 4, 7-triacetate</td>
</tr>
<tr>
<td>DBU</td>
<td>1, 8-diazabicyclo-[5.4.0]-undec-7-ene</td>
</tr>
<tr>
<td>Hepes</td>
<td>4, 2-hydroxyethyl-1-piperazine ethane sulfonic acid</td>
</tr>
<tr>
<td>Hunig's base</td>
<td>N,N-diisopropylethylamine</td>
</tr>
<tr>
<td>TOPO</td>
<td>Tri-n-octylphosphine oxide</td>
</tr>
</tbody>
</table>
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General Introduction

Chapter 1
Chapter 1

Luminescent Lanthanide Chelates as Diagnostic Tools in Bioassays

In recent years, the use of luminescent lanthanide chelates as diagnostic tools has come to prominence. Immunoassays utilising radioisotopes are widely used as they are very sensitive but maintain many drawbacks associated with the handling of radioactive materials. As an alternative to radioisotopes, organic fluorophores have been employed as fluorescent labels but are often not as sensitive since many biological systems have high background fluorescent signals, and because of instrumental light scattering, self-quenching and oxygen quenching problems. Background noise will be increased by light being scattered by other components in the sample, such as proteins and colloidal particles found in serum, as well as any unbound fluorophore being used. Background fluorescence originating from serum exhibits a wide fluorescence range (300-600 nm), which overlaps extensively with the emission spectra of many organic fluorophores. Luminescent lanthanide chelates offer an alternative as they are non-toxic, exhibit large Stokes shift (>250 nm), very narrow band structured emissions and long luminescent lifetimes (typically between 200-2000 µs) when compared to common organic fluorophores (5-50 µs), allowing the use of time resolved measurement.

Most transition metals absorb UV/visible light but very few re-emit the absorbed energy in the form of UV/visible emissions due to the strong coupling of their d-electron excited states with their environment via the ligand field effect, that provides an efficient de-excitation mechanism. Trivalent lanthanide ions (apart from La and Lu) comprise of a partially filled 4fn electronic configuration, which are shielded from chemical reactions by the outer 5s and 5p shells, and are minimally involved in bonding. As a result, electronic transitions between the energy levels of the f-orbitals gives rise to sharp emission lines and show luminescence.

The energy levels of the 4fⁿ electronic configuration of a lanthanide ion are determined by a combination of electrostatic interaction, spin-orbit coupling and in a coordination
environment, the ligand field. The electrostatic interaction yields terms with separation
energies in the order of $10^4 \text{cm}^{-1}$. The spin-orbit coupling interaction splits these terms
into $J$ states, with typical splittings of $10^3 \text{cm}^{-1}$. The $J$ degeneracy of free ion states is
partially or fully removed in co-ordinated compounds by the ligand field, with the
splitting being in the order of $10^2 \text{cm}^{-1}$ in magnitude.

The principal lanthanide ions that exhibit luminescence in the chelated form are
samarium (Sm), europium (Eu), terbium (Tb) and dysprosium (Dy). This is due to there
being a large energy gap between the potentially emissive excited states of the metal ion
and the acceptor level within the ground state manifold (Figure 1).

![Figure 1: The principal emission lines of some emissive lanthanides. Units of energy
are stated in $10^3 \text{cm}^{-1}$.](image)

For europium and terbium, the relative energy gaps between the excited and ground
state of the metal permits emission in the "red" and "green" region, respectively. These
two lanthanide ions are the most important for use as luminescent probes when chelated
to a suitable ligand due to their large Stoke’s shift and longer luminescent lifetimes in
comparison to samarium and dysprosium. The latter have relatively short lifetimes in
comparison ($\mu$s).
For gadolinium, the large energy gap between the excited and ground states is equivalent to an emission in the UV-vis region, at around 310 nm, making this ion impractical for use as an emissive species in biological applications. Both Tb and Eu have energy gaps that allow emission in the visible region of the spectrum; the major transitions observed are listed in Table 1. The pattern of emission reflects the probability for each transition with some transitions being sensitive to the ligand environment. For Eu$^{3+}$, the main transitions occur within the $^5D_0\rightarrow^7F$ manifold with the strongest being transitions to $^7F_1$ and $^7F_2$. Transitions within the $^5D_0\rightarrow^7F_{0,3,5}$ manifold are strictly forbidden, and are relatively weak or unobservable.

<table>
<thead>
<tr>
<th>Transition</th>
<th>Spectral region (nm)</th>
<th>Relative intensities</th>
<th>Other characteristics</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Terbium</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$^{2}D_{4}\rightarrow^{7}F_{6}$</td>
<td>485-500</td>
<td>Medium-strong</td>
<td>Moderate sensitivity to ligand environment</td>
</tr>
<tr>
<td>$\rightarrow^{7}F_{5}$</td>
<td>540-555</td>
<td>Strongest</td>
<td>Exhibits some structuring under high resolution</td>
</tr>
<tr>
<td>$\rightarrow^{7}F_{4}$</td>
<td>580-595</td>
<td>Medium</td>
<td>Moderate sensitivity to ligand environment</td>
</tr>
<tr>
<td>$\rightarrow^{7}F_{3}$</td>
<td>615-625</td>
<td>Medium-weak</td>
<td>Some structuring under high resolution</td>
</tr>
<tr>
<td>$\rightarrow^{7}F_{2}$</td>
<td>645-655</td>
<td>Weak</td>
<td>Moderate sensitivity to ligand environment</td>
</tr>
<tr>
<td><strong>Europium</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$^{5}D_{0}\rightarrow^{7}F_{0}$</td>
<td>578-580</td>
<td>Weak</td>
<td>Non-degenerate transition; always appears as a single, sharp line</td>
</tr>
<tr>
<td>$\rightarrow^{7}F_{1}$</td>
<td>585-600</td>
<td>Strongest</td>
<td>Sharp and structured under high resolution</td>
</tr>
<tr>
<td>$\rightarrow^{7}F_{2}$</td>
<td>610-630</td>
<td>Strongest</td>
<td>Intensity exhibits hypersensitivity to ligand environment</td>
</tr>
<tr>
<td>$\rightarrow^{7}F_{3}$</td>
<td>645-660</td>
<td>Weak</td>
<td>Always weak</td>
</tr>
<tr>
<td>$\rightarrow^{7}F_{4}$</td>
<td>680-705</td>
<td>Weak</td>
<td>Intensity and structuring sensitive to ligand environment</td>
</tr>
</tbody>
</table>

*Table 1*: Major characteristics of Tb$^{3+}$ and Eu$^{3+}$ emission intensity for complexes in aqueous solution. Adapted from reference 1.
Transitions between states of $4f^n$ configurations are strictly parity forbidden and the probability of such transitions is low so that molar absorption coefficients ($\varepsilon$) are of the order of 1 and emission lifetimes in the millisecond time frame. In practice, measured lifetimes are substantially less than that of theoretical lifetimes. This indicates that mainly non-radiative processes govern the decay of the emitting state. The theory on non-radiative decay in lanthanide complexes\(^2\) states that non-radiative relaxation between the various J states may occur by interaction of the electronic levels with suitable vibrational modes of molecules within their environment. The efficiency of these non-radiative processes depends on the energy gap between the ground and excited states of the metal and the vibrational energy of the oscillators.\(^2,3\) Hydroxyl containing solvents that are co-ordinated to the metal centre can induce non-radiative deactivations via the vibronic coupling with the vibrational states of the O-H oscillators.\(^4\) By replacing O-H oscillators with lower frequency O-D oscillators, the vibronic deactivation becomes less efficient.

Following the work of Stein and Wurzberg, Horrocks and Sudnick\(^5,6\) developed a quantitative expression for the number of water molecules co-ordinated to the inner sphere of the lanthanide ions. From the linear relationship of the observed luminescence decay constants for a series of terbium and europium crystalline complexes with a known number of hydrated water molecules in the solid hydrates, Equation 1 was derived. Where $q$ is the number of water molecules in the inner sphere of the lanthanide ion; $A_{Ln}$ is a normalising factor (1.05 for Eu\(^{3+}\) and 4.2 for Tb\(^{3+}\)); and $k$ is the inverse of the radiative lifetimes in water and deuterium oxide in milliseconds. The range of uncertainty for Eu\(^{3+}\) and Tb\(^{3+}\) hydration numbers using Equation 1 is ±0.2 and 0.5 water molecules, respectively.

$$q = A_{Ln}(k_{H_2O} - k_{D_2O}) \quad \text{Equation 1}$$

The equation can be redefined based on the assumption that methanol behaves like half a water molecule to give Equation 2,\(^7\) where $B_{Ln}$ is 2.1 for Eu\(^{3+}\) and 8.4 for Tb\(^{3+}\).
As molar absorption coefficients of aqua lanthanide ions are of the order of 1 due to the transitions involved being forbidden processes, organic chromophores attached to ligands are employed to produce sensitised emission (the Antenna effect). The principle behind this is that the chromophore absorbs strongly at a suitable wavelength and transfers its excitation energy to the neighbouring metal ion. On accepting this energy, the ion becomes excited to the emissive state with an emission of a photon upon relaxation to its ground state (Figure 2). This process is favoured by the chromophore being within a short distance of the metal ion.

A Jablonski diagram (Figure 3) shows the sensitised emission of lanthanide chelates. The chromophore absorbs a photon of light resulting in the promotion of an electron from the ground state (S₀) to the first excited singlet state (S₁). The molecule rapidly undergoes internal conversion to lower vibrational levels of S₁. The excited singlet may either be deactivated by combining radiatively with the ground state (ligand fluorescence) or the molecule may undergo intersystem crossing (ISC) from the singlet to the triplet state, with the promoted electron undergoing spin inversion. Internal conversion allows the ligand to reach the lowest vibrational level in its excited electronic state. From this state, it can then combine with the ground state (S₀) by a spin forbidden transition (T₁-S₀) to give long lived molecular phosphorescence. The molecule alternatively undergoes a non-radiative transition from the triplet to a low lying resonance level of the lanthanide ion, resulting in the promotion of one of its

\[ q = B_{\text{La}}(k_{\text{CDOH}} - k_{\text{CDOD}}) \]  

\( \text{Equation 2} \)
electrons to the excited 4f state. After loss of excess energy via vibrational relaxation, the luminescent state can relax to the ground state with the emission of a photon specific to the lanthanide ion in use. To achieve optimal lanthanide luminescence, prompt ligand fluorescence, phosphorescence and non-radiative deactivations of the luminescent state should be minimised.

\[ \text{Figure 3: Jablonski diagram illustrating the mechanism of sensitised lanthanide emission.} \]

The efficiencies of energy transfer in lanthanide chelates can be expressed in terms of their radiative and non-radiative decay constants. Taking into account the effect of solvents, the overall decay rate constant \( k \) of the luminescent level can be expressed by Equation 3, where \( k_r \) is the temperature independent radiative decay constant and \( k_{nr} \) and \( k_{nr}(T) \) are the non-radiative temperature independent and dependent non-radiative decay constants.

\[
k_r = 1/\tau = k_r + k_{nr} + k_{nr}(T) \quad \text{Equation 3}
\]

Solvents containing O-H oscillators will contribute a deactivation mechanism via high energy vibronic oscillations, and is expressed as \( k_{nr}(OH) \). Oscillations arising from species other than O-H can be expressed as \( k_{nr}(\text{other vibrations}) \) in Equation 4.

\[
k = 1/\tau = k_r + k_{nr}(OH) + k_{nr}(T) + k_{nr}(\text{other vibrations}) \quad \text{Equation 4}
\]
The decay rate constants can be obtained by lifetime measurements in hydrogenated and deuterated solvents at 300K and 77K. Assuming that $k_{\text{nr}}$(other vibrations) is negligible, which is not always the case, the coupling with O-D oscillators is completely inefficient and at 77K, thermally activated decay plays no part in the non-radiative decay, $k_r$ can now be expressed as Equation 5.

$$k = \frac{1}{\tau_{\text{D}_2\text{O}}}^{77K}$$  \hspace{1cm} \text{Equation 5}

The non-radiative decay rate constants $k_{\text{nr}}(T)$ and $k_{\text{nr}}(\text{OH})$ at room temperature (300K) can be expressed as Equations 6 and 7.

$$k_{\text{nr}}(T) = \left(\frac{1}{\tau_{\text{D}_2\text{O}}}^{300K}\right) - \left(\frac{1}{\tau_{\text{D}_2\text{O}}}^{77K}\right)$$  \hspace{1cm} \text{Equation 6}

$$k_{\text{nr}}(\text{OH}) = \left(\frac{1}{\tau_{\text{H}_2\text{O}}}^{300K}\right) - \left(\frac{1}{\tau_{\text{D}_2\text{O}}}^{300K}\right)$$  \hspace{1cm} \text{Equation 7}

In addition to deactivation processes caused by O-H oscillators in solvents, efficient deactivation processes can arise from the use of aromatic antennae. It has been postulated that the position of the triplet should ideally lie around 1700 cm$^{-1}$ above that of the accepting lanthanide ion. If this energy gap is less than 1500 cm$^{-1}$, then thermally activated back energy transfer competes by repopulating the aromatic triplet state from the principal emissive species of the metal ion. This process is more pronounced for Tb$^{3+}$ than Eu$^{3+}$ due to the $^5\text{D}_4$ emissive state in Tb$^{3+}$ having an energy of 20500 cm$^{-1}$, which is of similar energy to many triplet levels of aromatic compounds (Figure 4a).

Vibrations often assist this process and the temperature dependence of a lifetime can be described by an Arrhenius type equation (Equation 8).

$$k(T) = \left(\frac{1}{\tau}\right) - \left(\frac{1}{\tau^*}\right) = A. \exp \left(\frac{E_A}{RT}\right)$$  \hspace{1cm} \text{Equation 8}
Where $\tau$ is the experimental lifetime and $\tau^0$ is the lifetime taken at low temperature where no temperature dependence is observed.\textsuperscript{10}

Typically, the difference in energy of the ligand triplet excited state and the europium $^5D_0$ emissive state (17200cm\textsuperscript{-1}) is greater, therefore thermal repopulation of the triplet level is unlikely. However, ligand to metal charge transfer (LMCT) processes can lead to non-radiative deactivations, and operates almost exclusively for Eu\textsuperscript{3+} as it is readily accepts an electron, reducing to the weakly emissive Eu\textsuperscript{2+} species. In this process, an electron in the excited state can feed into one of the empty orbitals of the metal and then back into the ground state of the ligand. This cycle allows energy loss without the emission of a photon (Figure 4b).

![Diagram](image)

Figure 4: Typical deactivation processes involving excited metal ions a) Tb\textsuperscript{3+} and b) Eu\textsuperscript{3+}; deactivation processes are shown in blue lines.

The efficiency of energy transfer process between the singlet excited state of the ligand and the emissive state, $\eta_{\text{en.tr}}$, cannot be measured directly but can be calculated from Equation 9, which is simply the ratio of quantum yield upon ligand excitation ($\phi$) and quantum yield upon metal excitation ($\phi_M$). When this quantity cannot be measured, it may be substituted as in Equation 10 for the efficiency of metal luminescence, $\phi_M$, obtained from Equation 9. Since it can be assumed that at 77K in deuterated solvents, decay of the metal emitting state will be purely radiative and Equation 11 can be used.

$$\eta_{\text{en.tr}} = \frac{\phi}{\phi_M}$$  \textit{Equation 9}

$$\phi_M = \eta_M = \frac{\eta}{[k_t + k_{nT}(T) + k_{nT}(\text{OH}) + k_{nT}(\text{other})]}$$  \textit{Equation 10}
However, use of Equation 11 is not a valid substitution for Equation 9 when there is an equilibrium involving the metal emitting state and other excited states, which decay to the ground state. Substituting $\tau_{\text{exp}}/(\tau_{\text{D2O}})^{77K}$ for $\phi_M$ will only yield a lower limiting value for $\eta_{\text{en.tr}}$ and not an absolute value.

Energy transfer to the emitting metal species will occur via the excited singlet state, which is populated upon ligand absorption, which deactivates to the ligand triplet excited states via intersystem crossing followed by energy transfer to the metal ion. In theory, both the excited singlet state and the triplet state can be involved in energy transfer to the emitting metal centre. In reality, as the lifetime of singlet states in aromatic compounds utilised as chromophores are of the order of $10^{-9}$s so that energy transfer from these states will have to be very fast indeed ($k>10^{-9}$s) in order to be effective. Triplet excited states of aromatic compounds have much longer lifetimes ($10^{-6}-10^{-2}$s) and are therefore expected to be more involved in energy transfer to the metal ion than the shorter lived excited singlet. It is therefore useful to know the triplet$\rightarrow$metal energy transfer efficiency ($\eta_{\text{en.tr}}$), as it will reflect ligand to metal interactions. The relationship of energy transfer can be expressed as

$$\eta_{\text{en.tr}} = \eta_{\text{singlet}}\rightarrow\text{triplet} \eta_{\text{triplet}}\rightarrow\text{metal}$$

where $\eta_{\text{singlet}}\rightarrow\text{triplet}$ is the efficiency of singlet to triplet conversion. In the absence of any decay process form the excited singlet to the triplet, $\eta_{\text{singlet}}\rightarrow\text{triplet}$ is at least as efficient as intersystem crossing in the free ligand and should approach unity. This should also be enhanced by the presence of the heavy atom. However, when the ligand is complexed it is possible for deactivation to occur, for example, LMCT from the excited singlet to CT bands in some europium chelates, resulting in the efficiency being lower than that of $\eta_{\text{ISC}}$ of the free ligand. As well as deactivations through O-H
vibrations, quenching through proximate C-H and N-H oscillations are also well established. Quenching by N-H oscillators ($v(N-H) \approx 3300 \text{cm}^{-1}$) from amine groups that are in close proximity to the metal are effective. For terbium, each N-H oscillator will contribute 0.09 ms$^{-1}$ to the overall deactivation rate constant, while the process is much more efficient for europium, with a value of 1.2 ms$^{-1}$ per N-H oscillator. Early reports on C-H quenching ($v(C-H) \approx 2900 \text{cm}^{-1}$) were made following a study of lanthanide salts in various non-deuterated and deuterated organic solvents. Luminescent lifetimes of europium tris-acetate complexes measured in the same solvent were shown to have an increased lifetime from 0.17 ms to 0.21 ms upon deuteration of the hydrogens of the acetate moieties. This corresponds to approximately to a quenching rate of 0.1 ms$^{-1}$ per C-H oscillator.

As emission is occurring through sensitised energy transfer via an organic chromophore, deactivations involving the aromatic antenna are also of importance. The most significant pathway is the quenching of the excited triplet states by molecular oxygen as its ground state is a triplet state that can interact with the triplet state of the aromatic antenna either by an energy transfer process, which results in the production of the low energy excited singlet state of molecular oxygen (singlet oxygen) or by transfer of an electron which generates the oxygen radical anion and the radical cation of the aromatic compound. Rates of quenching will depend on both the decay rate constant and the concentration of available oxygen. Under ambient conditions, atmospheric oxygen dissolves in water to give concentrations, [O$_2$], of $\sim 10^{-2}$ M. For a $k_q \sim 10^{10}$ M$^{-1}$s$^{-1}$, the rate of quenching would be $k_q[O_2] \sim 10^8$ s$^{-1}$. Thus, for triplet states with rates faster than $10^8$ s$^{-1}$, little quenching by the oxygen present will be observed, whilst for triplet states with decay $< 10^8$ s$^{-1}$, significant quenching will be observed. The behaviour of degassed solutions should also be considered. Under typical nitrogen purging or vacuum degassing conditions, [O$_2$] is $\sim 10^{-6}$ M. The quenching rate, $K_q[O_2]$ is then $\sim 10^4$ s$^{-1}$ and quenching will only be observed for those triplets sensitizers whose decay rates are slower than this, say $<10^{-3}$ s$^{-1}$.
Quenching of aromatic triplets by oxygen is often associated with terbium complexes where back energy transfer is present, which leads to inherently short luminescent lifetimes of the $^4D_4$ state.¹³

Mechanisms of energy transfer in lanthanide complexes

Electronic energy transfer can be thought of as the simultaneous relaxation of a molecule in the excited state (donor) and the excitation of a molecule or ion that is in a lower lying state (acceptor). This process requires electronic interaction between the two species and can occur via two types of interaction, termed the Förster mechanism and the Dexter mechanism.

- The Förster Mechanism relates to the donor and acceptor interacting through space and without any physical contact, by the induction of a dipole oscillation in the acceptor (metal ion) and the excited donor (sensitiser) (Figure 5).

![Figure 5: Energy transfer from the sensitiser to an acceptor due to coulombic interactions (Förster mechanism)](image)

- The Dexter mechanism involves the mutual electron exchange between the sensitiser and acceptor through their overlapping electron clouds, thus requiring physical contact between the two components (Figure 6). This mechanism represents a simultaneous electron exchange where an electron "jumps" from the excited sensitiser to the acceptor and an electron from the acceptor "jumps" to the donor excited state.
General Introduction

Chapter 1

HOMO –> LUMO

ground state

Sen* Acc Son Acc*

Figure 6: Energy transfer from the sensitiser to an acceptor via mutual electron exchange (Dexter mechanism).

Coordination properties of lanthanides

Lanthanide ions share common coordination properties. They can be classed as type “a” cations in the Ahrland, Chatt and Davies classification scheme\(^1\)\(^4\) and as “hard acids” in the Pearson classification scheme\(^1\)\(^5\). Therefore, lanthanides bind preferentially to O>N>S with Ln\(^{3+}\) ligand coordination occurring mainly through ionic bond interactions leading to a strong preference for negatively charged donor groups that are “hard bases”, such as carboxylates and hydroxide moieties. The ionic nature of Ln\(^{3+}\) and the relatively low charge to ionic radius ratio leads to very little directionality in the Ln\(^{3+}\)-ligand interactions. Therefore, the coordination properties are almost entirely governed by the nature of the ligand with respect to donor groups and conformational properties. In basic or neutral aqueous conditions, Ln(OH)\(_3\) is readily precipitated from solution and is only prevented when negatively charged donor groups are present. In aqueous solution, neutral oxygen and nitrogen donor groups are only able to bind when in multidentate ligands with one or more negative oxygens, resulting in highly labile complexes in aqueous solutions.

In any application where chelated lanthanide ions are to be used, it is essential to select sensitising ligands that form stable complexes with binding constants \((K_{ass})\) in the region of \(10^{12}\) and do not undergo exchange reactions with competing cations, such as Mg\(^{2+}\) and Ca\(^{2+}\). Often, ligands based upon polyamino carboxylates, such as EDTA and
DTPA, are used as they display high binding constants. The binding constant for EDTA with Eu$^{3+}$ at pH7 was found to be $10^{12.16}$.

**Scope of this thesis**

The work contained in this thesis aims to discuss and elaborate on many of the ideas presented in this introduction, with the view to develop novel luminescent lanthanide chelates for use as luminescent labels in time resolved homogenous applications.

In Chapter 2, we will expand on the use of lanthanide chelates and their application in biological assays with respect to a number of commercial based assays that are currently available. We will examine the applications of lanthanide probes in immunoassays and other biological applications.

The underlying aim of this project is centred around the development of charge neutral luminescent lanthanide chelates that have the potential of being membrane permeable and can subsequently be used in cellular applications. The theme of developing charge neutral chelates is carried on throughout all chapters in this thesis.

In developing a suitable reagent that may be capable for loading into mammalian cells, in Chapter 3, we will describe the synthesis and photophysical properties of charge neutral complexes of terbium and europium, utilising the bipyridine chromophore and carboxylate donor groups. Then, the development of charged complexes using bipyridine as the donor and sensitiser that incorporates a functional arm that can be derivatised with a suitable group for conjugation to a biomolecule.

In Chapter 4, we will re-examine earlier work$^8$ carried out using mixed carbonyl-phenol donor groups as potential sensitisers and assessing their photophysical properties as 3:1 complexes with the appropriate lanthanide. Then, 1:1 complexes using a triazacyclononane backbone will be investigated with the overall aim of achieving charge neutral complexes and to increase stability with respect to the luminescent species.
In Chapter 5, in our attempt to move away from carboxylate donor groups, we report a new sensitiser based on a coupled pyrido-phenol moiety with the aim of developing a charge neutral, fat-soluble complex that can be used in homogenous cellular based assays. In this chapter, we describe the investigation of 8-hydroxyquinoline as a potential sensitiser of europium and investigate the photophysical properties of this chromophore.
Review

Chapter 2
Chapter 2

Lanthanide Coordination Chemistry and its Role in Biology

Lanthanide complexes offer a robust alternative to organic fluorophores in biological assays. Their application in biology is not just restricted to luminescent labels in fluorosimmunoassays but also applications as structural probes for biological substrates, MRI contrast and imaging agents, tissue selective markers and imaging of tumour cells.

This review intends to highlight the importance of lanthanide ions and their complexes with reference to their use in various biological applications and their advantages over more traditional reagents and fluorophores. The intention is not to review the various types of luminescent complexes that have been synthesised but to examine the technologies behind the applications of such chelates in biology.

Traditional Immunoassays

Immunoassays have been developed in response to the need for more sensitive and specific means of detection of small quantities of biological materials such as antigens, antibodies and other analytes.

The radio-immunoassay (RIA) was introduced in the late 1950's. The principal for any immunoassay regardless of the "label" being used is, in essence, the same. In the case of RIA's, the analyte in a sample is measured by competition between the radio-labelled antigen, typically \((^{125}\text{I})\), and unlabelled antigen for binding to an antibody which in turn is bound onto a solid phase, such as a plastic tube. The unbound antigen, both free and labelled, is washed away using a separation step and the amount of radioactivity associated with the bound labelled antigen is measured using a radiometer such as a scintillation counter. Standard curves of analyte concentration can be obtained using a range of radiolabelled standards of this type for competitive binding assays. RIA competitive assays are generally applied to the detection of small molecules such as drugs and are not suited for the detection of large molecules such as proteins.
The use of radioisotope detection has a number of distinct drawbacks with its use despite its high sensitivity and independence of its environment. The utilisation of organic fluorophores in place of radioisotopes often present a suitable alternative but suffer from their own unique set of drawbacks. Fluorophores exhibit high quantum yields but suffer from photo bleaching, self-quenching and short fluorescent lifetimes. A very important limitation associated with fluorescent labels is that due to their short lifetimes, the fluorescent signal can be masked by the presence of biological autofluorescence that arises from the solution media. Also, the components present in serum will emit in the same wavelength region as many typical organic fluorophores, which makes the discrimination between "background signal" and the required fluorescent signal very difficult.

Europium and terbium complexes offer an alternative as their luminescent lifetimes are within the order of milliseconds and therefore can allow the use of time resolved measurement, where the emission of light is measured after a set delay time. During this delay time, the background fluorescence from sources such as endogenous fluorophores in reagents and samples, glass and plastic cuvettes, and scattered light from the source has dissipated. Measurement can be made of a selected part (gated) of the exponential decay curve of the luminescent lanthanide chelate (Figure 7).

![Excitation I = 340 nm 1000 cycles/s](image)

**Figure 7:** The principal of time resolved luminescence

This is achieved by gating the phototube, either by turning on the power after the decay of the prompt fluorescence background has dissipated, or by electric gating, where a portion of the decay curve is selected for integration. The result is an unmasked
emission signal that is measured against a dark background allowing high sensitivities to be achieved.

Leif$^7$ attempted to incorporate luminescent lanthanide chelates to antigens for use in immunoassays, namely phenanthroline and β-diketone derivatives. These chelates showed excellent sensitisation properties and long-lived luminescent lifetimes but the complexes investigated displayed very poor stability in aqueous solution. From this early work, a number of useful guidelines that have been extended towards the design of suitable lanthanide chelates for use in immunoassay applications:

- The sensitiser should have high absorption properties, with extinction coefficients ($e$) in excess of 10 000 dm$^3$mol$^{-1}$cm$^{-1}$.
- The sensitiser should absorb at wavelengths above 350nm to avoid absorption by common biomolecules, such as proteins and nucleic acids.
- Efficient sensitised energy transfer from initial irradiation to lanthanide luminescence should be optimised to maximise the overall quantum yield, and to minimise other pathways.
- The ligand should saturate the inner coordination sphere of the lanthanide ion so as to avoid non-radiative deactivation processes. The most common coordination numbers exhibited are between eight and nine for Eu$^{3+}$ and Tb$^{3+}$.
- High kinetic and thermodynamic stability of the 1:1, metal:ligand complex at a biological pH range, in biological media and towards competing reagents.
- Relatively long luminescent lifetimes so as to allow time resolved measurements and discrimination from biological auto-fluorescence and scattering from the light source.

Two of the most important lanthanide based time resolved fluoro-immunoassays (TR-FIA) that have been developed over the last 15 years are the DELFIA and CYBERFLUOR systems and were two of the first commercial systems to evolve.
DELFIA

The Dissociative Enhanced Lanthanide Fluoro-immunoassay (DELFIA) was developed by Soini and Hemmilä and was patented in the 1980's.18 It was one of the first commercially available heterogeneous TR-FIA. The principal of the system is shown in Figure 8 and in more detail in Figure 9. It involves an indirect measurement of bound europium released from a strong chelator after the immuno reaction has taken place. The measurement of the released europium is made in a micelle-protected form, which is highly luminescent.

\[
\text{Ab} + \text{Eu}^{3+} \xrightarrow{\text{Step 1}} \text{Ab} \xrightarrow{\text{Step 2}} \text{Ab} + \text{Eu}^{3+} \text{in enhancement solution}
\]

Ab = Antibody

*Figure 8: Principle of the DELFIA heterogeneous assay system*

The first stage of the assay system is the linkage of the weakly luminescent europium chelate, an isothiocyanate phenyl functionalised EDTA derivative [Eu(1)₄], to the immobilised binding site at pH 9-10, (e.g. by reacting with a lysine amino group in the substrate), followed by washing to remove any unbound chelate. Once the non-emissive chelate is bound to the immobilised solid support, the lanthanide is dissociated from the bound chelate by the addition of an "enhancement solution", which consists of an acidic buffer, β-NTA (2-naphtoyltrifluoroacetone), TOPO (tri-n-octylphosphine oxide) and triton X-100. The acidic buffer serves to dissociate the chelated europium whilst the β-NTA ligands form a highly luminescent species, Eu(NTA)₃(TOPO)₂, with the released europium, which is shown in Figure 9. As the assay conditions are aqueous, solvent quenching of the luminescent species is reduced by the use of TOPO, which reduces co-ordinated water and enhances radiative lifetimes. Triton X-100 is a
detergent that will solubilise the components and the resultant chelates. The europium chelated in the micelle form is measured in the time-resolved mode.

Figure 9: The labelling and dissociation process in the DELFIA system

The DELFIA system has been applied to a number of competitive and non-competitive immunometric assays. Siitari et al.,\textsuperscript{19} described an immunoassay for the detection of hepatitis B surface antigen. The immunoglobulin is first coated onto polystyrene tubes followed by incubation with the plasma specimen in the presence of the antibody labelled with [Eu(1)]. The tube is washed with saline and the europium fluorescence is developed in the same manner as a previously described. Rabbit IgG has been detected
with a related immunoassay system where the rabbit IgG is labelled with $\text{[Eu(1)]}$. After the immuno-reaction and washing steps, the lanthanide luminescence is measured in a buffer at pH 7-8 in the presence of the β-diketonate, TOPO and Tween 29. The luminescence can also be monitored by the dissociation of the solid phase antibody at low pH in the presence of the β-diketonate, TOPO and Triton X.

Quantification of steroids and cortisol has been achieved in a competitive assay.\(^{20}\) Testosterone is immobilised on a solid support as its testosterone-3-(carboxymethyloxine)-ovalbumin conjugate. The sample containing the steroid is loaded before the addition of $\text{[Eu(1)]}$ labelled antigen. The immobilised and free steroids will compete for the binding sites on the labelled antibody. After washing, dissociation and enhancement procedures, the europium luminescence is measured. The concentration of the steroid is directly inversely proportional to the luminescence intensity of the europium, the higher the signal the lower the concentration of the steroid present. The sensitivity of these competitive assays are at least equal or even greater that that of the corresponding RIA.

The strong interaction of biotin with streptavidin (up to 4 biotin molecules per streptavidin molecule) has been exploited in order to connect biomolecules and labels in fluoroimmunoassays. The labelling of the 13 lysine amino acid groups of streptavidin with the europium chelate does not affect its binding affinity to biotin or the protein, which is contrary to the binding specificity of antibodies. The recognition of biotinylated antibody by the europium labelled streptavidin has led to increased sensitivities of these types of assays.\(^{21}\)

The DELFIA system has been used for simultaneous multi-analyte determinations by taking into account that β-diketone chelates of Eu, Tb, Sm and Dy will have different emission wavelengths and excited state lifetimes. Again, in this system, an enhancement solution is employed which contains $\text{Y}^{3+}$, which acts as a "heavy atom" to catalyse the ISC from the $S_1$ to the $T_3$ when it is present with the emitting chelates in solution or in a micelle environment. $\text{Y}^{3+}$ was used as an enhancer as is does not...
contain any 4f or 4d levels that are below the energy of the excited triplet state of the sensitising β-diketonate that are typically used. It was observed that the greatest enhancement was observed when Y3+ is used in large excess with energy transfer from the non-emissive Y3+ chelates to the emissive Eu3+ and Sm3+ chelates. The use of such enhancement solutions that also contain synergistic ligands, such as derivatised phenanthrolines and poly-pyridines, TOPO and the detergent triton-X, allows the detection of Sm (III) and Dy(III) that have shorter lifetimes and relatively weak emission intensities in comparison to Eu3+ and Tb3+. Dual-labelled fluoro-immunoassays have been reported and the underlying principal is the same. An example is the dual labelling of luteinising hormone (LH) and follicle-stimulating hormone (FSH). Their respective monoclonal antibodies, anti-β-LH and anti-β-FSH, are labelled with Eu3+ and Sm3+ reagents. The micro-titre strip wells are coated with monoclonal antibodies against the α-sub units of LH and FSH, washed and labelled with the previously prepared Eu3+ anti-β-LH and Sm3+ anti-β-FSH. After the immuno-reaction, the strips are washed and then the lanthanides are dissociated by addition of the acidic dissociation enhancement solution. Owing to the different lifetimes and emission maxima of Sm3+ and Eu3+, both labels can be determined simultaneously (Figure 10). Dual labelling with Eu3+ and Tb3+ as well as quadruple labelling with chelates of Eu3+, Sm3+, Tb3+ and Dy3+ have also been reported.23, 24, 25
Figure 10: A dual labelled DELFIA type immunoassay of luteinising hormone (LH) and follicle stimulating hormone (FSH)

Cyberfluor (FIAgem™)

A different commercial methodology utilises a luminescent europium chelate as a label in heterogeneous assays, known as the FIAgem™ system, marketed by Cyberfluor. This system differs from the DELFIA system by the fact that the luminescence is measured directly from the surface of the solid support after the immunoreaction has taken place. The chelate employed is 4, 7-bis(chlorosulfonyl)-1,10-phenanthroline-2,9-dicarboxylic
acid (BCPDA) 2, and is stable and forms a highly luminescent species with europium.\textsuperscript{26} The sulfonyl chloride groups are conjugated to amino groups of the protein to be labelled under relatively mild conditions. Proteins that have been investigated using this procedure include streptavidin and avidin as well as monoclonal and polyclonal antibodies.\textsuperscript{27} This detection system has many advantages pertaining to the use of directly labelled luminescent labels. Unlike the dissociative enhanced method, it is insensitive to contamination by exogenous Eu\textsuperscript{3+} because the chelate and not the lanthanide, is detected.

A typical procedure for a Cyberfluor assay consists of the labelling of the immobilised specific binding reagent with the analyte, followed by washing and then labelling with the specific binding agent that is conjugated to the [Eu(2)]\textsuperscript{+} chelate. The microtitre plates are then washed and dried, which removes coordinated water from the chelate, prior to the metal centred emission being measured (Figure 11).

Diamandis and co-workers exploited the strong affinity of biotin with avidin (or streptavidin) to develop a universal detection system by labelling 2 with streptavidin. This proved to be a useful tool in the field of immunological and DNA hybridization assays, as biotin-streptavidin interactions are considerably stronger than antibody-antigen interactions. Biotin can be covalently bound to either an antibody or a DNA probe which can interact with avidin that is labelled with a suitable "marker" (luminescent label), the resulting biotin-avidin complex can be used to assess the biotinylated molecule. Labelling biomolecules this way is highly desirable due to the high affinity of biotin and streptavidin for each other. Biological activity of DNA sequences and antibodies are maintained upon conjugation to biotin and streptavidin does not tend to deactivate upon labelling.
DEFRET

Fürster or (fluorescence) resonance energy transfer (FRET) can be applied in biological assays as a means for determining distance relationships between biological molecules. In general terms, the excited fluorescence molecule (donor), transfers excited state energy via a non-radiative dipole-dipole interaction to an acceptor molecule, which can be fluorescent or non-fluorescent, when the two entities are in close proximity. Irradiation of the donor fluorophore with light of a suitable wavelength produces an oscillating dipole. This in turn will resonate with a dipole in the acceptor probe in the near field, and the resonating dipole-dipole interaction involves a transfer of energy from a donor fluorophore to an acceptor chromophore (Figure 12). This process is normally regarded as a non-radiative process and therefore not involving the emission and absorption of photons. For any given donor-acceptor pair, the energy lost by the donor is gained by the acceptor and this energy transfer can be effective over distances ranging from <10-100Å. In homogenous assays, the donor and acceptor are bound to biomolecules, such as antibodies and antigens, that can interact with each other and giving rise to the energy transfer process. The efficiency of energy transfer is defined by $R_0$, the distance of 50% of energy transfer, which is dependent on the inverse sixth
power of the distance between the two probes. Equations 12 and 13 were derived according to the Förster theory;

\[
E = \frac{1}{1 + \frac{R}{R_0}^6} \quad \text{Equation 12}
\]

where

\[
R_0 = (8.79 \times 10^{-5} \times \kappa^2 n^4 \phi_d J_{da})^{1/6} \ \text{Å} \quad \text{Equation 13}
\]

\( R \) is the distance between the donor and acceptor, \( R_0 \) (Å) is the distance where energy transfer is at 50% and \( E \) is the efficiency of energy transfer; \( \phi_d \) is the quantum efficiency of the donor probe attached to the biomolecule of interest; \( \kappa^2 \) is the Förster orientation factor; \( n \) is the refractive index of the solvent; and \( J_{da} \) is the overlap of the donor emission spectra with the absorption spectra of the acceptor. Providing the two tags are within such distances of each other (50-100 Å), energy transfer will occur and give rise to a new distinct signal that can be measured. Depending on the molecules used, this distance varies, but will always be shorter than 10nm (100 Å). A further criterion for energy transfer from the donor and emission of the donor is that the absorption spectra of the acceptor and the emission spectra of the donor must overlap.

\[\begin{array}{c}
\text{Figure 12: The principal of FRET using conventional organic fluorophores as a donor and an acceptor. Upon conjugation of the donor moiety with the acceptor, emission from the acceptor molecule becomes enhanced and emission from the donor becomes quenched due to the close proximity of the two entities resulting from energy transfer. The acceptor can still emit arising from its own absorption due to the often-small difference in excitation wavelengths for the two entities.}
\end{array}\]

FRET applications have been employed in structural biology, immunoassays and to study macromolecular complexes of DNA and RNA. Energy transfer is detected as a
decrease in the donor fluorescence intensity and the increase in fluorescence intensity of
the acceptor. Although measurement of the decrease in donor emission is sufficient to
calculate the efficiency of energy transfer, it is preferable to monitor the acceptor
emission as well as processes other than non-radiative energy transfer will contribute to
the quenching of the donor emission. The choice of FRET pairs is mainly governed by
the $R_0$ value, and should be equal to or larger than the distance to be measured. Organic
fluorophores are commonly used but display a number of serious flaws due to their
impracticability in the conditions required for DEFRET measurements. The maximum
distance that can be measured is less than optimal for many biological systems.
Another important point is that the fluorescent lifetimes of many common donor
fluorophores are short (ns) and are usually poly-exponential, making lifetime
measurements difficult and often of limited accuracy. Background signal noise arising
from the fluorescent molecules in the sample and also from the interfering fluorescence
from the donor and the direct excitation of the acceptor will also lead to reduced
sensitivity. Also, as the efficiency of energy transfer relies not only on the $R_0$ distance
but also on the relative orientation (defined by the $\kappa^2$ factor), precise distances are
difficult to determine and subject to a degree of uncertainty.

Luminescent lanthanide complexes have been employed as fluorescent donors in both
model and applied studies with a high degree of success, with conventional organic
fluorophores used as the acceptors. The long luminescent lifetimes of lanthanides allow
discrimination from background fluorescence by time resolution (DElayed FRET), and
allow the measurement of large distances ($R_0$ up to 100 Å). Background emission
arising from direct excitation is eliminated temporally because of the short lifetime of
the typical organic donor (ns) whereas sensitised emission from lanthanide complexes is
considerably longer (ms). Delayed sensitised emission arising from the organic
acceptor will only occur from donor-acceptor pairs, incomplete labelling (donor only or
acceptor only labelled molecules) will not contribute to the DEFRET signal and be
discriminated from the required signal (Figure 13).
An important example of a luminescent lanthanide donor was presented by Mathis\textsuperscript{28} in which \([\text{Eu}^3(3)]^{3+}\) was used as a donor and the acceptor is a modified allophycocyanin dye (a phycobiliprotein from red algae). The assay is marketed as a homogenous high throughput fluoro-immunoassay (HTRF). \([\text{Eu}^3(3)]^{3+}\) is modified in the 4, 4'-positions of one of the bipyridine moieties to allow conjugation to biomolecules via standard chemical reactions and is shown in Figure 14. Allophycocyanin (APC) was chosen as the acceptor due to its high molar absorptivity in the cryptate emission wavelength range and its specific emission at 665nm occurs at a spectral range where the \([\text{Eu}^3(3)]^{3+}\) emission is insignificant. In this specific example, energy transfer was detected between two antibodies, one labelled with the Eu chelate and the other labelled with the acceptor APC, bound to a single antigen (prolactin). The HTRF principal has been applied to a number of assays including immunoassays, protein-protein binding, receptor binding, enzyme assays and DNA hybridization. It also displays a number of advantages such as its use in a plate format for multiple detection, allowing high throughput detection. By the measurement of specific signals of both the donor and acceptor fluorophore as an internal control, the assay gives a ratiometric measurement that compensates for the presence of absorbing compounds in the assays and unknown concentration levels.
Cooper et al.,\textsuperscript{29} reported a similar system in which the terpyridyl, [Eu(4)]\textsuperscript{3+} (Figure 15), was used as the donor and APC as the acceptor. The high affinity of biotin and streptavidin was exploited and the donor and acceptor were bound to biotin and streptavidin, respectively. Excitation of [Eu(4)]\textsuperscript{3+} in solution showed no emission at 660nm when measured with a time delay of 0.1ms with excitation at 295nm, as did the excitation at the same wavelength of a solution of the labelled APC. Since APC exhibits a short luminescence lifetime, its directly excited luminescence will have decayed to zero after the time delay. Upon mixing of the two solutions and after an equilibration time, excitation at 295 nm yields emission at 660nm arising from energy transfer (DEFRET). The lifetime of the coupled donor when measuring the $^5{}$D$_0$$\rightarrow$$^7$F$_2$ transition (615nm) was considerably reduced when bound to the acceptor, from 1.12 ms to 0.62 ms, also confirming that energy transfer was taking place.
Selvin used DTPA-quinolinone functionalised terbium chelate Tb(5) donor to measure relatively long distances in myosin and to detect conformational changes in voltage ion channels. In the latter, Shaker potassium ion channel containing a unique Cys residue on each of the four identical sub-units were expressed in Xenopus oocytes. The channels were labelled with a mixture of donor and acceptor probes, with the donor in excess to ensure that most channels contained at most one acceptor (Figure 16). A donor therefore sees an acceptor on a contiguous sub unit (distance $R_{ac}$) or on a sub-unit across the pore (distance $R_{sa}$). The sensitised emission lifetime observed was bi-exponential, with the shorter lifetime corresponding to the greater transfer efficiency arising from the shorter distance between the acceptor and the donor. The two distances obtained ($R_{sa}$ and $R_{ac}$) are in good agreement with those derived from the pythagorean relationship ($R_{sa}^2 = 2R_{ac}^2$) based on the tetrameric symmetry of the channel. As the voltage across the membrane changes, the distance between the sub units change correspondingly, producing a model indicating that the so-called voltage sensing region of the ion channel likely undergoes a rotation rather than a large translation, as had been previously proposed. The longer lifetimes associated with sensitised lanthanide emission allows this type of measurement as opposed to the shorter lived organic fluorophores.
Figure 16: The tetrameric structure of "Shaker" potassium channels with four identical sub units labelled with Tb(5) as donors and fluorescein derivative 6 as acceptors. About 70% of the channels that generate sensitised emission have 3 terbium labelled sub-units. The S-protein represents the ion channel cysteine residue that attaches itself to the maleimide ring.

Several studies have taken advantage of the fact that long distances between biological molecules can be measured. Heyduk\textsuperscript{32} measured distances up to 100 Å in protein-DNA complexes that contained heterogeneous mixture of labelled biomolecules. The donor was a functionalised DTPA utilising a derivatised coumarin sensitising moiety, coupled to proteins via the thiol groups of cysteine residues. The acceptor was the cyanin Cy-5 fluorochrome attached to the opposite ends of 15-base pair chains of doubly stranded DNA.

Stenroos et al.,\textsuperscript{33} utilised DEFRET to study the receptor ligand interaction between the 15-kDa human lymphokine-interleukin 2 (hIL-2) and its 55-kDa sub unit receptor protein (hIL-2R\(\alpha\)). The former is a cytokine that stimulates both proliferation and differentiation of various cells of the immune system.\textsuperscript{34, 35} This hIL-2-hIL-2R\(\alpha\) interaction forms the low affinity IL-2 receptor, with a K\(d\) value ranging from 2 to 20 nM. The Eu chelate (no structure is given in the reference) is labelled with recombinant IL-2 and incubated with a Cy-5 labelled anti-hIL-2\(\alpha\) specific antibody, 7G7B6, and a preparation of the human IL-2Ra protein. The extent of complex formation or competition was evaluated by measurement of Cy-5 emission at 665 nm using time resolved fluorimetry.
Root\textsuperscript{36} utilised lanthanide DEFRET to measure the interaction between antibody labelled dystrophin and actin within a cell of rat skeletal muscle fibres (Figure 17). Dystrophin is the gene product of the Duchenne muscular dystrophy gene and its absence will lead to progressive atrophy of the muscle.\textsuperscript{37} The role of dystrophin with actin and its precise cellular functions are tightly linked, and it has been implied that dystrophin may aid in stabilising muscle fibres by linking actin filament networks to external connective elements, thus reducing atrophy.\textsuperscript{38, 39} The donor in this case was Tb(5) as used by Selvin,\textsuperscript{44} which allows attachment to lysine residues of the anti-dystrophin IgG. The fluorescence acceptor, tetramethylrhodamine, was labelled with phalloidin-actin conjugate. The association between actin and dystrophin was assessed by dual labelling frozen muscle tissue with the terbium chelate conjugated with anti-dystrophin monoclonal antibody and the actin specific tetramethylrhodamine phalloidin. The resonance energy transfer between donor labelled dystrophin and the acceptor labelled actin indicated that a close association between the two proteins occurred in situ. As it has been shown that purified dystrophin will bind to actin in solution\textsuperscript{40} and the association shown by these in situ measurements proves that there is a direct binding between actin and dystrophin in the muscle fibre. This and other findings go some way to explain why muscular dystrophy occurs in patients who lack dystrophin. A higher degree of resolution was obtained (10-100 times) in comparison with traditional immuno-fluorescence co-localisation methods.
DNA Hybridization Assays

Nucleic acid hybridization assays are based on the specific binding reaction between a labelled DNA or RNA fragment and its complementary nucleic acid sequence (the analyte). The fragment maybe either a short, synthetic oligonucleotide or a long nucleic acid sequence. Nucleic acid hybridization assays are usually semi-quantitative and are typically used for diagnosis of genetic, malignant or infectious diseases or for forensic identification. The DNA (or RNA) whose sequence is to be determined is initially cut by restriction enzymes into defined fragments, then the individual fragments of double stranded DNA are denatured by heating (split into single strands) which can hybridise with complementary labelled DNA sequence.

An ideal label for a DNA probe would have the following properties:

- Easy attachment to DNA
- Detectable at low concentrations

Figure 17: a) Sensitised resonance energy transfer between antibody labelled dystrophin and tetramethylrhodamine phalloidin-labelled actin. b) The protocol for tissue labelling.
• Stability at elevated temperatures during hybridization procedures
• "Switching on" the signal upon hybridization of the DNA-probe to its complementary DNA sequence.

The simplest approach uses lanthanide labels as direct replacements for radioactive labels. Both the immunological assay formats of FIAgen and DELFIA have been applied to DNA hybridization assays.

Signal generation upon hybridization allows the use of homogenous conditions when performing hybridization assays. Valet et al.,41 employed a pair of oligonucleotide probes with one linked to a salicylate group (pAS) as the sensitiser and the other probe attached to a terbium chelate (DTPA-Tb). Upon hybridization of the complementary sequences with the target DNA, a luminescent ternary complex is formed with the luminescence increasing with increasing concentrations of the DNA target (Figure 18). The detection limit for analyte DNA was found to be 1 nmol, which is insufficient for most applications.
Figure 18: Intermolecular energy transfer using a salicylic acid sensitizer and a terbium acceptor. Energy is transferred from the salicylic acid (sensitizer) to the terbium-DTPA conjugate (acceptor) upon hybridization, giving rise to metal centred emission.

Sammes et al., \textsuperscript{42} have employed a similar approach in which the 5'-end of the DNA probe is linked to a europium chelate, Eu-EDTA-DNA \textsuperscript{8}, which will hybridize with target DNA (Figure 19). After hybridization, 1, 10-phenanthroline-2, 9-dicarboxylic acid linked to the DNA intercalator phenanthridinium \textsuperscript{7} will form a 1:1:1 complex with the duplex and bind to the metal ion. Only the co-operative complex gives rise to a europium signal upon UV-irradiation. The binding constant of the sensitising ligand for the chelated europium is in the range of $10^6$-10$^7$ M\textsuperscript{-1} with little of the ternary complex forming at concentrations <10$^{-4}$M. In the presence of the target DNA with a complementary sequence to the probe, the sensitizer is brought near to the DNA hybrid as a result of the intercalation of the phenanthridinium to the duplex. The binding constant of the sensitizer to the chelated europium is increased up to 10$^6$M\textsuperscript{-1} in the presence of the complementary DNA. Only when the DNA probe is hybridized can the intercalation occur. A related system has been shown to detect single point mutations in DNA targets such as those in some mutations associated with cystic fibrosis. \textsuperscript{43}
The use of homogenous DNA hybridization assays is of great importance when a signal can be generated upon hybridization, and therefore attention has recently turned on the use of DEFRET in such applications. Selvin et al.,\(^4\) report the use of Tb(5) as the donor and tetramethylrhodamine as the acceptor, with both the donor and acceptor being labelled with complementary DNA strands. Upon hybridization of the target DNA with the probe, energy transfer occurs and emission from the rhodamine moiety will only occur upon hybridization and therefore there is no need to remove any unbound probe molecules. Jones \textit{at al.},\(^5\) report the use of a similar system but incorporate multiple donor labels with a single acceptor fluorophore. This was found to improve sensitivity, at least four times, at equivalent molar concentrations compared with singly labelled donors.
Enzyme Amplified Lanthanide Luminescence (EALL)

Enzyme amplified assays utilise enzymes as labelling groups that release a substrate that can be detected by means appropriate to the substrate in question. Typical assays are performed using colorimetric, chemiluminescent or fluorescence detection, in which a colourless or non-fluorescent substrate is converted to a coloured, chemiluminescent or a fluorescent product, respectively by the action of an enzyme. The product arising from the enzymatic reaction can be measured in solution or deposition on a solid support or membrane surface. Colorimetric detection has been utilised for a large number of enzymes but measurement of light absorption by the coloured product is an intrinsically insensitive method, resulting in only moderate enzyme detectability.\(^46\)

Improvements in detectability of the enzyme released substrate have been made by using fluorescent or chemiluminescent probes instead of coloured substrates. Chemiluminescent substrates of enzymes are very sensitive and have been used in a number of formats and have been for detection of horseradish peroxidase, alkaline phosphatase, β-galactosidase and luciferase.

Fluorescence based detection systems can be used for a variety of enzymes and can lead to improved sensitivities.\(^46\) Detection of the emissive fluorophore is often difficult to discriminate from background signal and autofluorescence biological systems. Enzyme amplified lanthanide luminescence (EALL), a combination of enzyme immunoassay and time resolved fluoroimmunoassays, was developed in the early 1990's by the groups of Diamandis and Evangelista, and showed detection limits for enzymes in the pico-molar concentration range.

Evangelista et al.,\(^47\) presented a number of assays where the signal generation is performed enzymatically, transforming a substrate into a product that can form a strongly luminescent complex with a lanthanide. In essence, the substrate is a masked sensitisier and upon action of the enzyme, the masking group is removed so that the luminescent complex is formed. The detection of xanthine oxidase was carried out by using the substrate salicylaldehyde 9 (Figure 20). The enzyme catalyses the oxidation
of the substrate into salicylic acid 10 which can bind strongly to Tb-EDTA at high pH, giving rise to a highly luminescent, ternary complex [Tb-EDTA(10)]³⁻. A common feature with the majority of EALL is that a ternary complex is formed with Tb-EDTA and a salicylic acid derivative, which has been shown to form a highly luminescent species at relatively high pH.⁴⁸

Figure 20: The basis for the oxidative estimation of xanthine oxidase. The resulting salicylic acid 10 liberated from the enzyme action on 9 goes on to form a ternary luminescent complex [Tb-EDTA(10)]³⁻.

Alkaline phosphatase (AP) was detected in a similar manner where the substrate molecule R-OPO₃H⁻ is hydrolysed by AP, with the hydrolysis product forming a luminescent complex with Tb-EDTA at high pH. In this case, the salicylic acid was a para-fluorinated derivative and the nature of this substitution was found to affect the magnitude of the luminescent signal obtained under constant measurement conditions. This is probably due to a combination of the alteration in the formation constant of the ternary complex by effectively reducing the pKa value of the phenol and the energy transfer efficiency.
The quantification of β-galactosidase was also achieved using the substrate salicyl-β-galactoside, which is also converted to salicylic acid that can be measured in the same way as previously stated.

It was found that 1,10-phenanthroline-2,9-dicarboxylic acid dihydrazide (PDCAdh) is converted to the corresponding acid (PDCA) by the action of H$_2$O$_2$ and light (Figure 27). The acid derivative is a strong chelator and sensitisier of europium forming 1:1 and 2:1 complexes. This process can be incorporated into an EALL by producing the H$_2$O$_2$ from the catalytic oxidation of the substrate β-D-glucose by the enzyme glucose oxidase (GOD). PDCAdh, Eu$^{3+}$ and β-D-GOD are irradiated with UV light with PDCA. Eu luminescence only being produced in the presence of GOD due to the production of H$_2$O$_2$. The results obtained for the detection of GOD over a 30 minute period were comparable to those obtained from chemiluminescent, colorimetric and electrochemical detection methods.

![Figure 21: The photo-oxidation PDCAdh to PDCA and its application in enzyme amplified GOD determination](image)

Investigations of suitable fluorogenic moieties other than salicylic acid were carried out by Diamandis. The compounds that were investigated were aromatic so as to allow the absorption of light and contained at least 1 carboxylate donor and a phenol moiety that could be converted to the phosphate ester or galactosidase for action by AP or β-galactosidase, respectively. It was interesting to note that the chelate 4-
methylumbelliferyl phosphate (4-MUP) formed a highly luminescent complex with Eu$^{3+}$ ions but upon the action of AP, metal centred luminescence is quenched. Detection methods for thyroid stimulating hormone and thyroxine in human serum were subsequently developed.

As shown by many examples in the literature, EALL procedures mostly rely on salicylic acid as substrates and excitation donors for Tb$^{3+}$, and therefore restricted to enzymes like AP, β-D-galactosidase, glucose oxidase and xanthine oxidase. The determination of horseradish peroxidase by EALL could not be achieved. Recently, adaptation to peroxidase enzymes was attempted using hemin and 4-hydroxybenzoic acid 11. Hemin is a naturally occurring iron-porphin complex that is an inexpensive byproduct from bovine blood and has been shown to catalyse the luminol chemiluminescence reaction as a substitute for HRP. Hemin was used as a substitute for HRP and the utilisation of lanthanide luminescence enhancement was used for determination of hemin as well as other substrates. It was found that the substrate 11 does not form a long lived luminescent complex with Tb$^{3+}$-EDTA, but can be oxidised by H$_2$O$_2$ in the presence of hemin to give the chelator, bis-2, 2'-(4-hydroxyethylbenzoic) acid 12. This compound forms a highly luminescent synergistic complex, [Tb-EDTA(12)]$^{3-}$, in the presence of Tb$^{3+}$-EDTA (Figure 22). It was found that the detection limit for hemin using this method was 8x10^{-10} mol dm$^{-3}$ and was used in the determination of the conjugate of bovine serum albumin (BSA)-hemin. The simple assay developed for determination of the BSA-hemin conjugate showed a detection limit of 2x10^{-10} mol dm$^{-3}$. 
Meyer et al.\textsuperscript{55} utilised HRP to perform the same dimerisation of 4-hydrophenylpropanoic acid 13 and followed by complexation with Tb\textsuperscript{3+}-EDTA (Figure 23). Measurement of the resulting ternary complex [Tb-EDTA(14)]\textsuperscript{3-} is enhanced by the addition of caesium chloride to the solution, acting as a "heavy atom" which enhances intersystem crossing in the fluorophore.\textsuperscript{56} Emission of the resulting ternary complex was mainly from the narrow band like emission from Tb\textsuperscript{3+} but the short lived fluorescence from the fluorophore was still observed. Contrary to Xu et al.\textsuperscript{53} several compounds could be used to sensitise Tb\textsuperscript{3+} emission after peroxidase catalysed coupling and it was postulated that binding was occurring through the phenolate and not the carboxylate donors. A variety of substituted p-hydroxy phenol compounds were tested and the only common structural feature between them all was the phenol groups. After
optimization of the method, the detection limit for HRP in aqueous solution was toward $2 \times 10^{-12}$ mol dm$^{-3}$.

Figure 23: The reaction sequence for the EALL detection of HRP

**Lanthanide ions as structural probes**

Lanthanide ions can form complexes with many biological substrates, such as amino acids, nucleotides, sugars and nucleosides, through the negatively charge groups on these substrates. Lanthanides can enter into biologically active compounds, replacing Ca$^{2+}$ as well as Zn$^{2+}$, Mg$^{2+}$, Mn$^{2+}$, Fe$^{2+}$ and Fe$^{3+}$, and because of their unique spectroscopic properties, they can act as probes for the structure and coordination environment of these substrates. Ca$^{2+}$ and Ln$^{3+}$ are particularly alike with respect to their size, nature of bond formation in biological systems, coordination geometry and the favouring of certain donor groups.
The two ions have coordination numbers higher than six and similar values of ligand exchange rate constants (ca., $10^8$ s$^{-1}$). Their ionic radii are very similar (0.1-0.118 nm for Ca(II) and 0.086-0.122 nm for Ln$^{3+}$. Lanthanides are hard acids, like Ca$^{2+}$ and display high affinity for oxygen donor atoms (O>N>S). Both Ca$^{2+}$ and Ln$^{3+}$ are characterised by very elastic coordination geometry and their bonds are mainly electrostatic in nature. Ln$^{3+}$ have a greater charge density than Ca$^{2+}$ resulting in the greater stability of the resulting complexes. The similarities described allow substitution of these metal ions in biologically active materials, which is mainly governed by size rather than overall charge of the ion. The use of trivalent lanthanide ions has been well established as Ca$^{2+}$ replacement probes for a variety of proteins and has been shown that isomorphous replacement of Ca$^{2+}$ by Ln$^{3+}$ is achievable as evidenced by protein X-ray crystallography. Several structures suggest that it is generally possible to replace Ca$^{2+}$ with Ln$^{3+}$ with minimal disruption of the binding site or the overall structure of the protein. It is expected that upon Ca$^{2+}$→Ln$^{3+}$ substitution, an increase of coordination number by 1 will follow, often by adding a water molecule of coordination. The fact that Ln$^{3+}$ ions can give rise to sensitised emission when irradiated with UV-vis light in the absorption band arising from coordination to aromatic amino acids proves to be a useful tool for structural investigations of biological molecules.

Information regarding the coordination environment of Ln$^{3+}$ ions in biomolecules can be obtained from their excitation/emission spectrum and luminescent excited state lifetimes, and information regarding the structural environment of the ion can be deduced from this. The emphasis is on the use of Eu$^{3+}$ owing to its advantages over Tb$^{3+}$ in many cases, such as its sensitivity towards its coordination environment. The $^{7}F_0\rightarrow^{5}D_0$ transition of Eu$^{3+}$ occurs between non-degenerate energy levels, neither of which can therefore be split by a ligand field, a single environment (a type of complex or class of metal binding site) will give rise to only a single such transition. If more than one Eu$^{3+}$ environment is present, in principle, each will have its own characteristic transition energy. Provided the various transitions occur at sufficiently different energies, these different environments will be revealed. Usually, the excited state
lifetimes of Eu$^{3+}$ ions in different environments are distinguishable. From the number of unique lifetimes observed upon laser excitation, the number of Eu$^{3+}$ binding sites can be deduced, as well as their relative affinities for Eu$^{3+}$ ions. The latter can be accomplished by plotting the luminescence amplitudes (intensities at the time of laser excitation) of the individual component exponential, obtained during the course of a titration, versus the [Eu$^{3+}$]/[biomolecule] ratios. By lifetime measurements of complexed Eu$^{3+}$ with biomolecules in D$_2$O and H$_2$O, the number of coordinated water molecules (q) can be determined for each class of binding site by the Horrock’s equation, as described in Chapter 1.

Analysis of the excitation and emission spectra, and luminescent lifetime of calmodulin, a multifunctional protein with 4 Ca$^{2+}$ binding sites, in the presence of europium and neodymium gives information on the binding of europium to the protein and the relative distances between the calcium binding site of the protein by using Förster type non-radiative energy transfer. Calmodulin is a ubiquitous intracellular protein that functions as a sensor for calcium concentrations that occurs upon cell stimulation. Calmodulin can bind up to 4 Ca$^{2+}$ ions in its helix-loop-helix domains, which appear in two pairs at the globular end of the protein (Figure 24). Starting at the N-terminus, the calcium binding domains are labelled I-IV, with sites I and II being high affinity sites and III and IV being of lower affinity for Ca$^{2+}$ and Ln$^{3+}$ ions. By looking at the excitation and emission spectra of Eu$^{3+}$/calmodulin at different concentration ratios it was possible to assess the preferential binding of Eu$^{3+}$ to the four Ca$^{2+}$ binding sites of calmodulin. Using energy transfer from Eu$^{3+}$ to Nd$^{3+}$ between the various calcium binding sites of calmodulin, it was possible to measure the distance between them through Förster type energy transfer by monitoring the emissive lifetimes of the two species by generated upon laser excitation. Lifetimes of the Eu$^{3+}$ ion were measured and the uncertainty in the calculated R values were primarily due to the estimates of the quantum yields rather that the orientation factor, in which case were assumed to be 2/3. The R$_0$ values for this donor acceptor pair at the four Ca$^{2+}$ sites were 10-11 Å and the distances between the sites were determined to be 11-12 Å. These results are in a good agreement with those obtained by X-ray structure, being 10.9-12.4 Å.
Europium luminescence was used as a probe for the metal binding sites of bovine-α-lactalbumin (BLA) in D$_2$O. BLA is a small protein containing 123 amino acids and has a molecular weight of 14177 g mol$^{-1}$, and is present in cow’s milk at a concentration of 1 g dm$^{-3}$. It is the modifier protein of the lactose synthetase complex. BLA associates with the enzyme galactotransferase to form a complex which catalyses the biosynthesis of lactose from UDP-galactose and glucose in the mammary gland. The A-conformer of apo-BLA binds strongly and reversibly to one Ca$^{2+}$ ion causing a conformational change into the N-conformer, leading to a reduction in solvent interaction of the hydrophobic region of the protein surface. BLA also binds to Zn$^{2+}$ as well as Ca$^{2+}$, and it is believed that both play a role in its conformation. Previous studies utilising non-luminescent techniques only demonstrated the similarity between the calcium binding sites of BLA and the calmodulin family of proteins. Upon addition of apo-BLA to a solution containing Eu$^{3+}$, the intrinsic fluorescence of the protein is quenched and Eu$^{3+}$ centred luminescence is enhanced. Luminescent titrations revealed that at least 2 different metal binding sites present in the apoprotein and three different coordination environments for the bound Eu$^{3+}$. Two of the sites contained four negatively charged groups and were related to Ca$^{2+}$ binding sites, while the third was a non specific binding site that became occupied upon saturation of the other two sites and postulated to be a Zn$^{2+}$ binding site.

DNA structure has been investigated by laser excited Eu$^{3+}$ luminescence, with metal complexes with guanine containing nucleotides and single stranded oligomers leading
to the identification of two classes of binding site. The two binding sites were observed in oligo(dG)10, oligo(dG)8, oligo(dG)4, and d-GMP. One class of sites binds Eu$^{3+}$ ions more strongly than the other, and shows that two water molecules are coordinated to the metal centre. The weaker Eu$^{3+}$ binding site involves the coordination of six or seven water molecules and therefore only coordinated by 1 or 2 atoms of the oligomer. The tight binding class of Eu$^{3+}$ binding site was attributed to an inter-strand association of Eu$^{3+}$ with oligomers forming dimeric or polymeric structures.

Certain lanthanides, particularly ytterbium and europium, have a potential application as redox probes in proteins owing to their spectroscopic and electrochemical properties. Long range electron transfer in proteins is an important area of research of bio-inorganic chemistry and is involved in a number of physiological processes such as photosynthesis, deoxyribonucleotide production for DNA and respiration. A common feature of these proteins is the presence of a metal binding site or a metal cluster, which can act as an electron sink since they can cycle between redox states. The ease at which metals can easily cycle between redox states is determined by the properties of the metal and the binding environments available within the protein. Studies into the mechanisms of electron transfer require a system that has an electron donor, an electron acceptor and a method of initiating the electron transfer process, such as absorption of light (photosynthetic proteins). Proteins can act as long range electron shuttles by accepting electrons from one protein and delivering them to another, such as cytochrome c, which can have ruthenium complexes ligated to the surface histidines of the proteins. Electron transfer can then be monitored by instantaneously reducing the ruthenium and observing the transfer of an electron from the reduced ruthenium to another moiety, such as intrinsic Fe$^{2+}$ heme.

Supokowski et al., showed that tryptophan (Trp) fluorescence in the calcium binding protein parvalbumin obtained from codfish is quenched by an electron transfer process when the lanthanide ions Eu$^{3+}$ or Yb$^{3+}$ are bound at the Ca$^{2+}$ binding site. The electron donor in this system is a Trp excited singlet state, which can then reduce the nearby bound Ln$^{3+}$ metal centre to its +2 oxidation state. In a single Trp containing calcium
binding protein parvalbumin from codfish, in which the two bound calcium ions have been replaced by Yb$^{3+}$ ion, a near infrared red peak is observed at 977nm upon excitation at 290nm. The Trp is situated equidistant between the two calcium binding sites with the nearest indole ring being 8-11 Å from the metal ion sites. The electron, in the case of Yb$^{3+}$, will return to the Trp radical cation and leave the Yb$^{3+}$ in its excited state. The proposed mechanism for this electron transfer (ET) in Eu$^{3+}$ and Yb$^{3+}$ substituted codfish parvalbumin is shown in Figure 25. The Yb$^{3+}$ ion, unlike Eu$^{3+}$ or Tb$^{3+}$ has no emissive levels of similar energy to that of ligand singlet or triplet states and the spectral overlap integral according to the Förster theory is zero. These two factors raise the question of how the energy is getting from the excited Trp to the Yb$^{3+}$, giving rise to emission from the $2F_{7/2}$ state. Both Eu$^{3+}$ and Yb$^{3+}$ are readily reduced to Ln$^{2+}$ but the ground state indole moiety of Trp cannot reduce either of these ions to their respective divalent states. However, the excited state can and it has been postulated by Abusaleh and co-workers for the quenching of indole and related fluorophores when tethered to EDTA chelates of Ln$^{3+}$. The initial reduction of Yb$^{3+}$ by tryptophan in its excited singlet state produces the Trp radical cation, Trp$^{+}$, and Yb$^{2+}$. The former is a strong oxidant and the latter a strong reducing agent, causing the electron to jump back, producing Yb$^{3+}$ and the ground state Trp. The driving force for this ET back reaction, $-\Delta G_{Ln} = E(\text{Trp}^{+}/\text{Trp}) - E(\text{Ln}^{3+}/\text{Ln}^{2+})$ for the Yb system, which has a value of 2.36 eV. This is higher in energy than the $^{2}F_{7/2}$ state (1.27 eV), thus the Yb$^{3+}$ is formed in either the ground or the excited state. The fraction of Yb$^{3+}$ formed gives rise to near-infrared luminescence. The postulated ET mechanism also explains why there is little sensitised emission from the $^{5}D_{0}$ emissive level of europium. The driving force for the Eu$^{3+}$ system is $-\Delta G_{Eu}^{0} = 2.24$ eV and that the back ET from Eu$^{2+}$ has a driving force of 1.66eV, which is of lower energy than the $^{5}D_{0}$ emissive level (2.14 eV). Therefore, Eu$^{3+}$, with a larger driving force than Yb$^{3+}$, efficiently quenches Trp$^{*}$ fluorescence but itself is not photosensitised by the ET process. These observations make Yb$^{3+}$ and Eu$^{3+}$ potential probes for long range electron transfer in calcium binding proteins.
Figure 25: The proposed electron transfer scheme from Trp* to Eu$^{3+}$ or Yb$^{3+}$ where $k_1^{Ln}$ and $k_0^{Ln}$ are the forward and back ET rate constants where the Ln$^{3+}$ ion is left in its ground state. $k_0^{Yb}$ is the back ET rate constant for the situation where Yb$^{3+}$ is left in its excited emissive electronic state ($^2F_{jg}$).

Lanthanide chelates as tissue and tumour specific labels

Tissue imaging and spectral analysis has undergone significant advances in recent years. Various research groups have employed a combination of spectral analysis and endoscopic probes to perform in situ investigations of tissue and have shown their potential for minimally invasive diagnosis. Multi-dimensional imaging has been widely used to study cervical tissue, evaluate human artery tissue and track photodynamic cancer therapy. In addition, fluorescence imaging coupled to existing endoscopy offers advantages for diagnostics. Gastrointestinal tissue diagnosis using laser induced fluorescence resulted in 100% efficiency and a 94% predictive value.67 Bronchoscope fluorescence imaging showed a 50% higher sensitivity than conventional white light microscopy.68 Such investigations show that fluorescence imaging of tissue in situ in this manner has promising value in diagnostics but improvements are necessary. For comprehensive in vivo diagnostics, the spectrometer must combine with image processing techniques to perform time independent physiological process imaging events in real time. Research has been hampered by the limited resolution inherent to fibre optic imaging systems, by low contrast between normal and abnormal
tissue and by the low signal to noise ratio produced by tissue auto-fluorescence background. Improved imaging contrast can be achieved by employing tissue site selective markers that can spectrally shift signal emission away from that of tissue auto-fluorescence. Hubbard et al.,\textsuperscript{69} used a known fluorescent tags based upon those developed by Kim and co-workers\textsuperscript{70} which are based upon polyazamacrocyclic chelates of terbium (Figure 26) as tissue selective markers in Sprague Dawley rats. Tb(15) and Tb(16) show long luminescent lifetimes, high quantum efficiency ($\phi$ 0.65) and excellent thermodynamic and kinetic stability as well a low toxicity, which is an important factor for \textit{in vivo} diagnostic imaging. Phosphonate groups are employed instead of carboxylate donors as it is known that phosphate containing complexes are effectively retained by bone and calcified tissue and are routinely used in nuclear medicine as bone imaging agents.\textsuperscript{71}

![Figure 26: Aza-crown phosphonates used for bone imaging](image)

The use of lanthanide complexes reviewed so far has been restricted to luminescent ions that emit in the visible region. The ability of porphyrins, namely haematoporphyrin derivatives (HPD), to accumulate in malignant tumours has been exploited in developing new luminescence, diagnostic and photodynamic therapy (PDT) methods. PDT consists of the administration of a light active drug (photosensitiser) or pro-drug, followed by illumination. Selective tumour destruction is obtained by fibre optic delivery of the light and in the case of some photosensitisation drugs, is improved by tumour retention of the drug. The process of tumour destruction relies on the drug being able to localise at or near the tumour site and when irradiated in the presence of oxygen serves to produce cytotoxic materials, such as singlet oxygen ($O_2(1\Delta_g)$), from
otherwise benign precursors such as \((O_2(^3\Sigma_g))\). Porphyrins are powerful photosensitiser owing to their high quantum yield of triplet state formation upon photoexcitation and effective excitation energy transfer to oxygen, which leads to \(^1\text{O}_2\) generation and is the basis of the PDT mechanism. Haematoporphyrin derivatives are characterised by the fluorescence in the red region (630-690 nm). The longer the wavelength of light possible, the greater the penetration of tissue so more light can reach deep tumours and produce more singlet oxygen at the site of action. There are two major disadvantages to the use of HPD in luminescence diagnostics, namely HPD phototoxicity and a low luminescence contrast of tumours that is caused by the masking effect of the background luminescence of endogenic substances, in particular, haematoporphyrins that are present in biological tissue. These disadvantages have been overcome by using fibre laser spectrofluorimetry and a Yb\(^{3+}\) porphyrin complex that is luminescent in the IR region (900-1050 nm), where the background luminescence of biological tissues is practically absent in comparison with free porphyrin radicals. The introduction of a heavy metal into the tetrapyrrrole results in a sharp decrease in the triplet output and thus the phototoxicity of the resulting complex is significantly reduced.

Texaphyrins are a novel class of aromatic expanded porphyrins that differ from traditional porphorins in a number of ways. They contain 5 nitrogens in their chelating core with the cavity available for metal chelation being 2.4 Å (as opposed to 2 Å in many porphyrins) and will allow formation of a 1:1 complex with many trivalent lanthanide ions, making them excellent chelators for lanthanide ions. Currently, 2 different water soluble Ln\(^{3+}\) texaphyrin complexes, namely the [Lu(17)]\(^{3+}\) and [Gd(17)]\(^{3+}\) derivatives (Figure 27), are undergoing clinical trials for a number of applications. The use of porphyrins in PDT is somewhat limited due to the wavelength of light that is used to activate the sensitiser (usually around 630nm). Most of the incipient energy used for photo treatment is dispersed or attenuated before reaching the centre of a deep-seated tumour. This in turn results in little of the initial light being available for singlet oxygen conversion. By contrast sensitisers that absorb strongly above the 700nm spectral region would allow far more effective PDT treatments of
deep seamed tumours as effective tissue penetration will increase 2 to 6 times.\textsuperscript{73} The using lutetium texaphyrins reduces this problem significantly as it absorbs at a range of 730-740nm. The applications for the texaphyrin complexes, $[\text{Lu}(17)]^{3+}$ and $[\text{Gd}(17)]^{3+}$, are PDT for the treatment of recurrent breast cancer, photoangioplasty reduction of atherosclerosis involving peripheral arteries, light based treatment of age related macular degeneration (a vision threatening disease of the retina) as well as a potential enhancer of radiation therapy for patients with metastatic cancers to the brain.\textsuperscript{74}

\begin{center}
\textbf{Figure 27:} Lutetium and Gadolinium texaphyrins.
\end{center}

\textbf{Intracellular Assays}

Cellular assays utilising fluorescent probes are very common in biology and have a number of applications. Interest mainly lies with the regulation of intracellular processes by changes in both the levels and distribution of a number of physiological important cations such as $\text{Ca}^{2+}$, $\text{H}^+$, $\text{Na}^+$, $\text{K}^+$ and $\text{Mg}^{2+}$. The ultimate aim in the use of ion sensitive fluorophores is to obtain a measurement of the average concentration or intracellular distribution of the ion in question, under conditions where the result obtained is independent of the methodology used and the presence of the fluorophore. General problems that are encountered in such assays are:
• cell damage or by-product liberation while introducing readily detectable levels of the fluorophore
• high intracellular levels of fluorophore which buffer the concentration of the ion in question
• behavioural changes in the properties of the fluorophore once localised in the cell.

Cell loading methods can be generally divided into two groups, bulk loading and single cell procedures. Bulk loading procedures are applicable to large populations of cells and include acetoxymethyl (AM) ester loading, acid loading, ATP-induced cell permeabilization, cationic liposome delivery, electroporation, hypo-osmotic shock and scrape loading. Single cell techniques such as micro-injection and patch pipette perfusion can only be carried out on one cell at a time. Ideally, the label to be loaded into the cell should be achieved without damage to the cell and maintain cell structure, otherwise cell viability may be compromised.

Among one of the most useful and widely used techniques of cell loading is using functionalised AM ester fluorophores, which are cleaved by intracellular enzymes upon crossing the cellular membrane. A very simple example is that of fluorescein diacetate (FDA), which is not strictly an AM ester but the principal remains the same. This molecule is non-fluorescent but is capable of crossing the cellular membrane into the cytosol where it acts as a substrate for esterases (Figure 28). When FDA enters the interior of viable cells it is hydrolysed by esterases and free fluorescein and acetic acid are liberated. Fluorescein is incapable of crossing intact plasma membranes and therefore is trapped inside the live cells. As long as the integrity of plasma membranes is not compromised, cells will continue to accumulate fluorescein and become fluorescent, whereas damaged cells will remain non-fluorescent.
An important consideration when designing dyes for cellular applications is that there is a change in optical properties once the label has localised in the cell, either by cleavage of masking groups, by cellular esterases, or by binding of the substrate with the analyte that will cause a change in excitation or emission wavelength. The resultant signal is proportional to analyte concentration and the signal obtained can be related to the concentration of the analyte present. This factor is one of the obstacles that limits the use of luminescent lanthanide chelates as cellular labels for biological events involving metals. Parker et al., 79 investigated a number of modified “London” type ligands 18 and 19 (Figure 29) as potential probes for Zn$^{2+}$ and other intracellular divalent ions. It should be noted that these system were only intended as models and cannot have direct applications as LMCT bands in the target complexes restrict excitation wavelength to below 320nm (for europium). In each case, the connecting amide group is bound the Ln$^{3+}$ ion bringing the aryl chromophore close to the Ln$^{3+}$ ion, allowing sensitisation of the Ln$^{3+}$ ion. It was evident that such conjugates would only be expected to function via changes in the delayed emission intensity, as no significant change in the lanthanide ion coordination environment on ion binding would be likely. For Ln(19), it was observed that a 42% and 26% increase in emission intensity at 700nm and 545nm for europium and terbium, respectively accompanied zinc binding in an extra-cellular environment.
The use of lanthanide chelates in cellular applications has mainly been restricted to use in assays for measuring cytotoxicity in natural killer (NK) cell activity. A broad variety of techniques have been successfully used to evaluate cell death and include microscopy, vital dye uptake or retention, release of cytoplasmic enzymes, measurement of DNA fragmentation or the release of radio-isotopic labels. Traditionally, $^{51}$Cr is the most commonly used as a marker for target cell lysis. Following the co-culture of effector cells with $^{51}$Cr$_2$O$_7^{2-}$ pre-labelled target cells, the cytolytic activity is determined by collecting the culture supernatants and measuring $^{51}$Cr releases on a counter device. However, this assay is simple to perform and highly sensitive but requires considerable time for processing large numbers of samples, disadvantages associated with the use of radioactive labels and the relatively few number of cells ($10^4$) that can be measured in any one incubation sample. This problem was addressed by a number of research groups who initially used fluorescent dyes to measure cell viability or cytotoxicity. The use of fluorescent dyes is advantageous in comparison to radioisotopes and these include safety in handling, high sample throughput and short processing time. Drawbacks with the use of fluorescence labels is that they tend to suffer from either high spontaneous release of the dye or excessively slow release which will decrease sensitivity and increase processing time. The use of fluorogenic esterase substrates (calcein-AM) has been reported and offers greater retention than previously reported dyes and improved sensitivity when compared to the standard $^{51}$Cr assay. Even greater sensitivity was sought with the use of lanthanide based assays. Among one of the first lanthanide NK cell activity was reported by
Blomberg et al.,\textsuperscript{82} where Eu-DTPA chelates were used to label tumour cells as targets for NK cells. Detection of the released europium marker upon cell lysis was accomplished in the presence of 2-naphthyl trifluoroacetone by time resolved measurement. The specific Eu-DTPA release was higher than that of the \textsuperscript{51}Cr release with more Eu-DPTA was taken up by the target cells upon incubation, allowing for shorter assay times. However, due the hydrophilic nature of Eu-DTPA, cell loading was not complete and needed loading by means other than incubation, such as electroporation and dextran-sulphate loading. Both of these methods could, in theory, compromise the integrity of the cell. Blomberg et al.,\textsuperscript{83} reported the use of a hydrophobic ligand bis(acetoxyethyl)-2, 2':6', 2''-terpyridine derivative 20 that readily passes through the cell membrane and into the cytosol. The AM ester functions of 20 are hydrolysed by the intracellular enzymes within the cell resulting in the accumulation of the membrane impermeable 2':6', 2''-terpyridine dicarboxylic acid 21 inside the target cells. After incubation of labelled K-562 cells with effector cells, 21 released from lysed cells into the supernatant is chelated with Eu\textsuperscript{3+}, and the natural killer cell activity is then quantified by measuring the resultant metal centred luminescence from the formed [Eu(21)]\textsuperscript{3+}. The mechanism for this action is shown in Figure 30. The principal of this assay could be applied to the assessment of leukocyte adherence \textit{in vitro.}\textsuperscript{84}
Figure 30: The proposed mechanism for labelling of target cells with 20. The AM ester derivative 20 passes through the cell membrane, where it is hydrolysed by esterases to yield the membrane impermeant 21. 21 is liberated from the supernatant from the lysed target cells and chelated with Eu$^{3+}$, forming the fluorescent chelate [Eu(21)]$^+$. However, these assays are not homogenous and are subject to a number of steps prior to measurement of the luminescent chelate, which can lead to contamination of the measured supernatant.

The use of lanthanide chelates in biology has become more widespread, with their use not merely restricted to the use as luminescent labels. Applications of lanthanides as structural and analytical probes has proved a useful tool in biology and their use in the relatively new field of photodynamic therapy offers greater sensitivity in comparison to existing methods.
Results and Discussion

Chapter 3
Chapter 3

Bipyridine as a Sensitiser of Europium and Terbium

2, 2'-Bipyridine is widely reported in the literature to be an efficient sensitiser of both europium (III) and terbium (III) ions. It has been incorporated into a number of macrocyclic, cryptate and acyclic structures and the resulting lanthanide chelates have been studied. A particular set of chelates that has received much attention in recent years are the \( \text{cbpy.bpy.bpy} \ [\text{Ln}(22)]^{3+} \) chelates of terbium (III) and europium (III) (Figure 31).13,28 These particular complexes display intense absorption bands (\( \varepsilon = 29000 \text{ M}^{-1}\text{cm}^{-1} \)) in the ultraviolet region arising from the \( \pi-\pi^* \) transitions within the bipyridine units. The photophysical properties of these two complexes are described in Table 2.

![Figure 31: The “Lehn” cryptate complexes, [Euc(22)]\(^{3+}\) and [Tb(22)]\(^{3+}\)](image)

<table>
<thead>
<tr>
<th></th>
<th>UV absorption ( \lambda_{\text{max}}(\text{nm}) ), ( \varepsilon_{\text{max}}(\text{M}^{-1}\text{cm}^{-1}) )</th>
<th>Metal luminescence(^a)</th>
<th>( \tau^{300K} )</th>
<th>( \tau^{77K} )</th>
<th>( q )</th>
<th>( \phi^{300K} )</th>
</tr>
</thead>
<tbody>
<tr>
<td>[Euc(22)](^{3+})</td>
<td>303, 28000</td>
<td>0.34 (1.7)</td>
<td>0.81 (1.7)</td>
<td>2.5</td>
<td>0.02 (0.10)</td>
<td></td>
</tr>
<tr>
<td>[Tb(22)](^{3+})</td>
<td>304, 29000</td>
<td>0.33 (0.43)</td>
<td>1.79 (3.8)</td>
<td>3</td>
<td>0.03 (0.03)</td>
<td></td>
</tr>
</tbody>
</table>

*Table 2: Photophysical properties of [Euc(22)]\(^{3+}\) and [Tb(22)]\(^{3+}\). \(^a\)Excitation in the ligand at the \( \lambda_{\text{max}} \) value. The lifetimes (ms) are measured in correspondence with the \( ^5D_0 \rightarrow ^7F_2 \) and \( ^5D_4 \rightarrow ^7F_5 \) emissions for Eu\(^{3+}\) and Tb\(^{3+}\), respectively. Values in brackets are lifetimes in D\(_2\)O.
Excitation in the $^1\pi\pi^*$ ligand level in aqueous solution was followed by the typical lanthanide luminescence in the visible region characteristic of the $^5D_4$ and $^5D_0$ emissive levels of the terbium and europium ions, respectively. Luminescent lifetimes of both chelates were strongly affected upon changing to D$_2$O and at 77K, indicating that there is a solvent and temperature dependency associated with these chelates.

For [Tbc(22)]$^{3+}$ upon excitation at 304 nm, energy transfer from the $^1\pi\pi^*$ to $^3\pi\pi^*$ is a very efficient process as is the energy transfer from $^3\pi\pi^*$ to $^5D_4$ emissive level of terbium. However, it was shown that at room temperature, the $^5D_4$ state can undergo back energy transfer to the $^3\pi\pi^*$ state. This process was attributed to the relative slowness of the combined radiative and non-radiative decay of the $^5D_4$ level and the small energy gap (ca. 1200 cm$^{-1}$) between the lowest bipyridine triplet and the $^5D_4$ level, allowing thermal repopulation of the triplet at 21600 cm$^{-1}$ in energy. Proximal -OH oscillations also play a significant role in the non-radiative deactivations as well as the quenching of the aryl triplet by oxygen. For these reasons, the overall quantum yield in aqueous solution at 300K upon ligand excitation ($^1\pi\pi^*$) is only 0.03.

For [Euc(22)]$^{3+}$, the population efficiency of the emissive $^5D_0$ level was found to be lower than that of the Tb analogue upon ligand excitation. This was attributed to the presence of charge transfer bands, allowing repopulation of the ground state without energy transfer to the $^5D_0$ level. However, back energy transfer is eliminated in this complex as the difference between $^3\pi\pi^*$ and the $^5D_0$ level is around 4000 cm$^{-1}$, permitting all excited $^5D_0$ molecules to undergo radiative or non-radiative decay to the ground state.

Although these chelates have relatively poor quantum yields, and on average, two water molecules associated with the complex, [Euc(22)]$^{3+}$ has been modified for use in time resolved fluoro-immunoassays as a fluorescent label.$^{28}$ The structure has been modified in the 4, 4'-positions to allowing conjugation to biological molecules. However, the
emission profile of [Eu(22)]^{3+} is considerably different to that reported previously in the literature. [Eu(22)]^{3+} when used in a commercial bioassay has to be measured in the presence of a 400 fold molar excess of fluoride, which will change the spectral profile, emission intensity and increase the luminescent lifetime from 0.35 ms to 1.1 ms, due to total shielding of the cryptate from water. Work carried out in this laboratory showed that [Eu(22)]^{3+} and [Tb(22)]^{3+} are both greatly affected by the presence of fluoride and phosphate anions in the solution media. The results are summarised in the following sections.

\[\text{[Eu(22)]}^{3+}\]

Titration of [Eu(22)]^{3+} at 10^{-6}M in aqueous solution with fluoride ranging from 1 molar equivalent to a 10^5 molar excess showed an increased emission intensity coupled with a change in emission profile. The principal emission peak for the "bare" cryptate lies at 616 nm corresponding to the \(5D_0\rightarrow^7F_2\) transition, which is surpassed in intensity in the fluoride saturated species by the peak at 590 nm \(5D_0\rightarrow^7F_1\). The lifetime also increases from 0.35 ms to 1.14 ms in the bare cryptate and the fluoride saturated species, respectively. Analogous experiments in D\(_2\)O showed that the water molecules associated with the complex are displaced at high fluoride concentrations, leading to longer luminescent lifetimes as the deactivation by -OH oscillators are reduced. The \(q\) values for the bare cryptate and the saturated fluoride species are 2.4 and 0.3, respectively. It was also noted that the quantum yield of the fluoride saturated species is increased 7-fold when compared to the bare cryptate.

\[\text{[Tb(22)]}^{3+}\]

Analogous titrations with fluoride showed a decrease in emission intensity with the fluoride appearing to quench the metal centered emission. This is contrary to the effect previously reported by Mathis \textit{at al.}, who implied that terbium centered emission will be enhanced by the presence of fluoride. Luminescence decay data obtained after each titration showed non-exponential properties possibly due to the heterogeneous nature of the terbium emitting species or the process of the quenching mechanism. It was
postulated that the presence of fluoride is lowering the energy of the triplet, allowing efficient back energy transfer. There was no change in the emission profile of the terbium chelate with excess of fluoride in comparison to the analogous europium chelate, as terbium only shows moderate sensitivity to its coordination environment.

Aims and Objectives
It was believed that the anion and solvent effects associated with the Lehn cryptate could be significantly reduced by the use of charge neutral chelates that are coordinatively saturated, so as to avoid interactions with anions and solvent molecules. Ligand 23 was chosen as it would, in theory, be nine coordinate as the bipyridyl group will bind through its nitrogen atoms and DO3A 24 is a powerful lanthanide 7 coordinate chelator (Figure 32). Macrocyclic polyaminocarboxylates such as 24, form lanthanide complexes with enhanced thermodynamic stability and kinetic inertness, in comparison to their acyclic analogues, such as DTPA 25. The overall thermodynamic stability constant (log $\beta_{101}$) for 24 is 21 when complexed with gadolinium. The enhanced stability arises from the relative chemical inertness of the macrocyclic structure and the large degree of preorganisation of the ligand. Functionalisation of the remaining nitrogen with an extra sensitising, coordinating group can only lead to an increased stability constant, as observed with the complex Gd(26) (log $\beta_{101} = 23.8$). Also, as the lanthanide complexes of 23 will have an overall net zero electrical charge it may be possible to load these complexes into mammalian cells for use in cellular based assays. Using octanol-water partitions as a model for cell membrane permeability could initially test this hypothesis.
Synthesis of Ligand 23 and its Eu\(^{3+}\), Gd\(^{3+}\) and Tb\(^{3+}\) complexes

The synthesis of the complexes can be divided into two stages, and are shown in Schemes 1 and 2.

\[
\text{Scheme 1: (i) CH}_3\text{Li, Diethyl ether; (ii) H}_2\text{O, KMnO}_4, \text{ acetone; (iii) NBS, CCl}_4, \text{ benzoyl peroxide (init).}
\]

The first step of the synthesis is the methylation of 2, 2'-bipyridine 27 with methyllithium in diethyl ether and, after re-aromatisation of the intermediate 28 with potassium permanganate in acetone yields the non-symmetrically substituted product 29. The bipyridyl 29, upon treatment under radical bromination conditions with N-bromosuccinimide in carbon tetrachloride with a catalytic amount of benzoyl peroxide as a radical initiator, yielded the mono-bromide 30 as the major product.
Scheme 2: (i) NaHCO₃, CH₃CN, BrCH₂CO₂'Bu; (ii) Na₂CO₃, CH₃CN, 30; (iii) TFA, CH₂Cl₂; (iv) Ln(CF₃SO₃)₃, CH₃CN, aq Hepes buffer at pH 7.5.

The second part of the synthesis is shown in Scheme 2. The tris-substituted tert-butyl ester DO3A 32 was synthesised from CYCLEN 31 and tert-butyl bromoacetate. This compound was synthesised under relatively mild conditions, and despite having four reactive nitrogens in 31, only three of the four sites could be alkylated selectively under these conditions. Therefore, 31 was suspended in acetonitrile under an argon atmosphere and stirred with 3.3 equivalents of sodium hydrogen carbonate and 3.3
equivalents of tert-butyl bromoacetate. The resulting mixture was stirred for 48 hours at room temperature, filtered to remove the inorganic material and the filtrate concentrated. Recrystallisation of the residue from toluene afforded 32 as the hydrobromide salt in a 39% yield. Condensation of the 32 with an excess of the monobromide bipyridyl 30 in acetonitrile with sodium carbonate as the base produced the bipyridyl ester 33 in near quantitative yield after purification by column chromatography. Cleavage of the ester groups with trifluoroacetic acid in dichloromethane yielded the free acid 23.

The Eu$^{3+}$, Gd$^{3+}$ and Tb$^{3+}$ complexes Eu(23), Gd(23) and Tb(23) were prepared by gently warming the ligand with a slight excess of the appropriate metal triflate salt in acetonitrile and aqueous Hepes buffer at pH 7.5 for 24 hours. The crude reaction mixture was passed through a Celite plug and washed with acetonitrile and water. After evaporation of the solvent, the residue was dissolved in the minimum amount of ethanol and the complexes obtained after slow diffusion of ether to yield the complexes in near quantitative yield as high melting, white micro-crystalline solids. All complexes obtained were characterised by high-resolution mass spectrometry.

Spectrophotometric protocol

Stock solutions of the isolated complexes were made up at $10^{-4}$M in water and deuterium oxide respectively and diluted accordingly. Solutions were measured immediately and again after 12 hours to ensure equilibrium. Excitation and emission spectra were measured with the following set-up; delay time, 0.1 ms; gate time, 1 ms; excitation and emission slit widths at 5 nm. Lifetime data was recorded using Lemmings software and hydration numbers ($q_{\text{corr}}$) were calculated using Parker's modified Horrock's equation (Equation 14):$^{11}$

\[
q_{\text{corr}} = A' L_n \left[ (k_{H_2O} - k_{D_2O}) + \text{corr}_{L_n} \right]
\]

Equation 14
where $q_{\text{corr}}$ is the inner sphere hydration number, $k$ is the rate constant for the depopulation of the lanthanide excited state in H$_2$O and D$_2$O, respectively; $A'_{\text{Eu}}=1.2\text{ms}$ and $corr_{\text{Eu}} = -0.25\text{ms}^{-1}$; $A'_{\text{Tb}} = 5\text{ms}$ and $corr_{\text{Tb}} = -0.06\text{ms}^{-1}$.

Degassing experiments were carried out with the sample contained in a degassing cell, using 5 freeze-pump-thaw cycles to deaerate. Low temperature studies were carried out at 77K using an Oxford Instruments Optical Cryostat coupled with an Edinburgh Instruments Intelligent Temperature Controller.

Results and Discussion

Absorption properties of 23, Eu(23), Gd(23) and Tb(23)

An aqueous solution of ligand 23 displayed absorption maximum of 241 and 288nm, with extinction coefficients of 4690 and 7420 dm$^3$mol$^{-1}$cm$^{-1}$, respectively. The longer wavelength band was red-shifted to 309nm upon complexation with the lanthanide ion (Eu$^{3+}$, Gd$^{3+}$ or Tb$^{3+}$) in the UV-vis absorption spectra, suggesting the bipyridyl is coordinated to the lanthanide ion as expected. The same red shift can be produced upon addition of an excess of lanthanide triflate salt to the aqueous solution of the ligand 23, giving rise to absorption spectra of similar intensity.

Photophysical properties of Eu(23)

When a $10^{-5}$M aqueous solution of Eu(23) was excited into the $1\pi\pi^*$ band at 309 nm, characteristic europium band emission was observed (Figure 33). The principal emission band corresponding to the $^5D_0\rightarrow^7F_2$ transition which lies at 614 nm, but other transitions are also observed. The photophysical properties of Eu(23) are quoted in Table 3.
Table 3: Photophysical properties of Eu(23). *Excitation in the ligand at the $\lambda_{\text{max}}$ value. The lifetimes are measured in correspondence with the $^5D_0 \rightarrow ^7F_2$ transition of Eu$^{3+}$. Values in brackets are lifetimes in D$_2$O.

The relative decay rate of Eu(23) in water was found to be relatively slow, with the ln I versus t plot showing a linear fit, indicating that only one luminescent species is present in solution. The lifetime value was found to be 1.15 ms and upon changing to D$_2$O, the lifetime was longer still ($\tau = 1.69$ ms). Substituting these values into Equation 14 lead to the calculation of the $q_{\text{corr}}$ value (hydration number) and was found to be 0.03, which can be approximated to 0. This indicates that the ligand is indeed nine coordinate as first predicted. Upon addition of a single crystal of potassium fluoride to the aqueous solution in the cuvette, no change was observed in the spectral profile, emission intensity or lifetime of the chelate. This provides further evidence that the complex is coordinatively saturated and a high degree of shielding of the metal ion is present. Also, as the complex is charge neutral, there will be no electrostatic interaction between negatively charged anions and the complex. Emission and lifetime measurements of the
complex in a 0.01M aqueous phosphate buffered saline solution (pH 7.46), which is a common component in many biological assays, also remained unchanged.

Low temperature measurements of Eu(23) in H\textsubscript{2}O and D\textsubscript{2}O at 77K showed very little changed in luminescent lifetime, indicating there is no temperature dependence associated with metal centered emission, such as back energy transfer. However, it was noted at low temperature, in both water and deuterium oxide, that decay was not a single exponential, indicating the presence of one or more luminescent species in solution. This is believed to arise from the slow exchange of water at low temperature.\textsuperscript{89} This process is kinetically fast process at room temperature and therefore, only mono-exponential decay is observed. A more detailed discussion of this effect will be dealt with later in this chapter.

The temperature dependence of Eu(23) was further investigated by elevating the temperature of the solution from 275K to 332K and measuring the emission intensity and lifetime. It was found that Eu(23) shows very little temperature dependence at higher temperatures with the lifetime reduced by 120 µs at 332 K from 1.15ms at 300K. This change in lifetime and emission intensity is not due to BET as the energy gap between the luminescent \textsuperscript{5}D\textsubscript{0} level and the lowest \textsuperscript{3}π\textsuperscript{*} ligand centred level is large, assumed to be around 4000 cm\textsuperscript{-1} for a typical bipyridine chromophore bound to a europium ion. Such a temperature effect can be possibly be associated with the presence of ligand to metal charge transfer bands that involve the electron pairs of the aliphatic nitrogens. Since the effect is relatively minor effect, further investigations were not carried out.

Using the equations discussed in Chapter 1, the radiative and non-radiative decay constants associated with Eu(23) can be calculated (\textit{Table 4}).
Table 4: Radiative and non-radiative rate constants associated with Eu(23)

<table>
<thead>
<tr>
<th></th>
<th>$k_r$ (s$^{-1}$)</th>
<th>$k_{nr}(\text{OH})$ (s$^{-1}$)</th>
<th>$k_{nr}(T)$ (s$^{-1}$)</th>
<th>$\Sigma k$ (s$^{-1}$)</th>
<th>$\eta_r$</th>
<th>$\eta_{\text{en.tr}}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Eu(23)</td>
<td>580</td>
<td>280</td>
<td>&lt;10</td>
<td>870</td>
<td>0.67</td>
<td>0.25</td>
</tr>
</tbody>
</table>

The efficiency of energy transfer from the $^1\pi\pi^*$ to the $^5\text{D}_0$ regardless of actual path is 0.25 and the luminescence efficiency of the $^5\text{D}_0$ level is 0.67. This means energy transfer efficiency is relatively high in comparison to the [Euc(22)]$^{3+}$ complex, which exhibits a modest quantum yield due to deactivations occurring through solvated water molecules. The only possible deactivation mechanisms that are likely to be in operation in Eu(23) are the presence of low lying ligand to metal charge transfer bands and low energy proximal C-H vibrations. For the europium complex, it is possible that deactivation of the $^1\pi\pi^*$ can occur via lower lying charge transfer levels (LMCT). In such a case, further deactivation can lead to the reduced population of the $^3\pi\pi^*$ ligand centred state and europium excited state.

It was previously described for [Euc(22)]$^{3+}$ that the $^1\pi\pi^*$ to $^3\pi\pi^*$ energy transfer efficiency is close to unity, as it was assumed that as the efficiency of energy transfer is close to 1 (98%) in free bipyridine, that the presence of a lanthanide heavy atom would enhance this efficiency. This assumption does not, however, take into account the presence of charge transfer bands that can lead to deactivation of the $^1\pi\pi^*$ state as well as other ligand excited states, reducing energy transfer to the $^5\text{D}_0$ level of europium.

Photophysical properties of Tb(23)

When a $10^{-6}$M aqueous solution of the terbium complex Tb(23) was excited into the chromophore absorption band at 300K, typical terbium metal centred emission was observed (Figure 34). The lifetime was found to be 0.59 ms. Upon moving to deuterated water, the emission intensity increased, with the lifetime extended to 0.64 ms. Using Equation 14, the $q_{\text{corr}}$ value was obtained an found to be 0.36 molecules of water associated with the complex, which can be approximated to 0. Again, the result implies that the chelate is coordinatively saturated with participation of the 3 pendant
carboxylate groups, the 4 aza-crown nitrogens and the 2 nitrogens on the bipyridine moiety. Addition of potassium fluoride to the aqueous solution or measurement in phosphate buffered saline produced no change in lifetime of spectral profile indicating that there is little anion dependency associated with metal centred emission. Photophysical properties of Tb(23) are shown in Table 5.

Degassing of the solution showed an increase in emission intensity as well as an increase in lifetime (0.70ms), showing there is efficient quenching by oxygen through the aromatic antennae. A lifetime measurement at 77K in a frozen matrix showed a significant increase in emission intensity and lifetime to 2.50 ms. Again, as with Eu

\[
\begin{array}{ccccccc}
\text{UV absorption} & \text{Metal luminescence} \\
\lambda_{\text{max}}(\text{nm}), \varepsilon_{\text{max}}(\text{M}^{-1}\text{cm}^{-1}) & \tau^{300K} & \tau^{77K} & \tau^{\text{dev}300K} & q_{\text{corr}} & \phi^{300K} \\
\text{Tb(23)} & 309, 6590 & 0.59 (0.64) & 2.50, (2.85) & 0.70 & 0.36 & 0.07 (0.13) \\
\end{array}
\]

Table 5: Photophysical properties of Tb(23). The lifetimes are measured in correspondence with $^5D_4\rightarrow^7F_5$ transition of Tb$^{3+}$. Values in brackets are lifetimes in D$_2$O.

**Figure 34:** Excitation and emission spectra of Tb(23) at $10^{-6}$M in water; $\lambda_{\text{ex}} = 309$nm; $\lambda_{\text{em}} = 544$nm; excitation and emission slits, 5nm.
(23), non-exponential decay was observed due to slow exchange of water at reduced temperature. But in D$_2$O at 77K, a single exponential decay was observed. Deactivation of terbium centred emission is less affected than europium by O-H and O-D oscillators and is believed that Tb(23) forms the same hydrated complex with H$_2$O but O-D oscillations are less efficient and only play a minor role in deactivation, resulting in mono-exponential decay in D$_2$O being observed.

The increased luminescent lifetime of Tb(23) at 77K shows that there is significant temperature dependence which can be attributed to the presence of BET. Measurement of the phosphorescence spectrum at 77K of Gd(23) shows that the triplet level can be placed at a maximum of 22600 cm$^{-1}$ in energy (Figure 35). This is significantly higher in energy than the emissive $^5$D$_4$ level of terbium at 20400 cm$^{-1}$ (a difference of 2200 cm$^{-1}$). This spectra can only be used to estimate the upper limit of the triplet level from the "onset" of emission as no resolved fine structure is observed in the phosphorescence spectrum.

![Figure 35](image)

*Figure 35:* Phosphorescence emission spectrum of Gd(23) at 77K; excitation and emission slits at 2.5 nm

To investigate the effect of temperature on Tb(23), the emission spectra and lifetimes in water were measured in a range from 274K to 336K (Figure 36). The terbium centred
emission was shown to be temperature dependent with emission intensity decreasing by 70% at 336K and the lifetime to 0.26 ms, with decay being a single exponential.

<table>
<thead>
<tr>
<th>T (K)</th>
<th>274.4</th>
<th>282.6</th>
<th>293.1</th>
<th>301.1</th>
<th>303.7</th>
<th>314.1</th>
<th>327.1</th>
<th>336</th>
</tr>
</thead>
<tbody>
<tr>
<td>( \tau ) (ms)</td>
<td>1.16</td>
<td>0.93</td>
<td>0.72</td>
<td>0.59</td>
<td>0.56</td>
<td>0.44</td>
<td>0.33</td>
<td>0.26</td>
</tr>
</tbody>
</table>

*Table 6: The effect of luminescent lifetime of the \( ^5D_4 \rightarrow ^7F_5 \) transition of Tb(23) in H\(_2\)O with varying temperature; \( \lambda_{ex} = 309\text{nm}; \lambda_{em} = 544\text{nm} \).*

*Figure 36: Emission spectra of an aqueous solution of Tb(23) at 10\(^{-6}\)M with varying temperature; \( \lambda_{ex} = 309\text{nm}; \) excitation and emission slits, 5nm.*

Using the values obtained at elevated temperatures, it is possible to obtain the temperature dependent activation energy. Lifetimes at lower temperatures were not used as bi-exponential decay was observed which would further complicate matters. Using *Equation 15*, where \( \tau \) is the experimental lifetime at a specific temperature, \( k \) is the overall rate constant and \( E_a \) is the activation energy required by the system for
thermal quenching. This value can also be viewed as the difference in energy between
the emissive $^5\text{D}_4$ level of terbium and the aryl triplet level. The rate constant for the
temperature dependence at each temperature from 274K to 336K can be calculated.

$$k(T) = \frac{1}{\tau} = A \cdot \exp\left(\frac{E_a}{RT}\right) \quad \text{Equation 15}$$

From the linear ln $1/\tau$ versus $1/T$ plot, the activation energy can be obtained from the
slope and was found to be 18.3 KJ mol$^{-1}$ or 1530 cm$^{-1}$. This shows that the overall
temperature dependence of this system regardless whether deactivation occurs through
vibrational, solvent or back energy transfer pathways is very effective. From this value,
a more accurate energy of the triplet can be estimated by adding the energy of the $^5\text{D}_4$
emissive level and the temperature dependent activation energy, and is approximately
21,900 cm$^{-1}$. Analogous experiments in D$_2$O showed the same trend as observed in
H$_2$O and the same calculation using lifetime values in D$_2$O and known temperatures
produces an activation energy of 21.8 KJ mol$^{-1}$ (1820 cm$^{-1}$). Lifetimes obtained at
varying temperature are shown in Table 7. This indicates that there is a temperature
solvent dependent deactivation pathway as the energy of the triplet should remain
unchanged regardless of solvent. Significant changes in triplet energies are only
observed when changing from polar to non-polar aprotic solvents. Vibrational
deactivations of both H$_2$O and D$_2$O will increase with increasing temperature regardless
of whether it is coordinated or as bulk solvent oscillations, leading to decreased
luminescent lifetimes.

<table>
<thead>
<tr>
<th>T (K)</th>
<th>277.1</th>
<th>282.7</th>
<th>292.9</th>
<th>296.5</th>
<th>302.5</th>
<th>312</th>
<th>321.7</th>
<th>332.1</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\tau$ (ms)</td>
<td>1.16</td>
<td>0.94</td>
<td>0.74</td>
<td>0.71</td>
<td>0.55</td>
<td>0.40</td>
<td>0.30</td>
<td>0.26</td>
</tr>
</tbody>
</table>

*Table 7: The effect of luminescent lifetime of the $^5\text{D}_4 \rightarrow {}^7\text{F}_3$ transition of Tb(23) in D$_2$O
with varying temperature; $\lambda_{\text{ex}} = 309$nm; $\lambda_{\text{em}} = 544$nm.*

Using this equation stated, it does not take into account the individual effect on back
energy transfer temperature dependence of the system. The effect of back energy
transfer can be calculated more accurately by using a limiting lifetime in conditions, as
in Equation 8. In our case, it is taken where no temperature dependant process is present, which can be taken as the lifetime in D$_2$O at 77K, as it is assumed that all O-D oscillations are inefficient and decay is purely radiative.

\[ k(T) = \frac{1}{\tau} - \frac{1}{\tau^0} = A \exp(-E_a/RT) \]  

Equation 8

It should be noted that Equation 8 was used in temperature studies where the analyte was in crystalline form and not in solution. The activation energy was calculated to be 1950 cm$^{-1}$ (Figure 37). This places the triplet of the bipyridine at 22350 cm$^{-1}$. However, the use of $\tau^0$ as a limiting factor may not be entirely correct, as deactivations will still occur through vibronic coupling of O-D oscillators at low temperatures. Solid state studies at lower temperatures (4.2K) would give a more accurate figure but due to the instrumental constraints within our laboratory, these experiments could not be carried out. Analogous calculations using the lifetimes obtained in D$_2$O with varying temperature afforded an activation energy of 27.6 KJmol$^{-1}$ or 2300cm$^{-1}$ (Figure 37).

![Figure 37: Plot to show ln[(1/\tau)-(1/\tau^0)] versus 1/T for Tb(23) in H$_2$O (blue trace) and D$_2$O (red trace) at varying temperature.](image)

Using the equations described in Chapter 1, the radiative and non-radiative decay constants can be calculated (Table 8).
Table 8: Radiative and non-radiative rate constants associated with Eu(23)

<table>
<thead>
<tr>
<th></th>
<th>$k_r$ (s$^{-1}$)</th>
<th>$k_{nr}(OH)$ (s$^{-1}$)</th>
<th>$k_{nr}(T)$ (s$^{-1}$)</th>
<th>$\Sigma k$</th>
<th>$\eta_r$</th>
<th>$\eta_{em.tr}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tb(23)</td>
<td>350</td>
<td>130</td>
<td>1210</td>
<td>1690</td>
<td>0.21</td>
<td>0.33</td>
</tr>
</tbody>
</table>

It must be noted that use of the substitution $\eta_m = k_r/(k_r+k_{nr})$ for use in calculating $\eta_{em.tr} (=\Phi/\eta_m)$ is not a valid substitution as there is an equilibrium involving the metal emitting state and other excited states which decay to the ground state (BET). This value can only be used to give a lower limiting value of $\eta_{em.tr}$ and as an overall efficiency of energy transfer regardless of path. The fluorescence quantum yield of 2, 2'-bipyridine is approximately 0.02.$$^91$$ This implies that most of the molecules of the $^1\pi\pi^*$ undergo intersystem crossing to the $^3\pi\pi^*$. This explains the relative weakness or absence of fluorescence observed in the $[\text{Ln}ð(22)]^{3+}$ chelates and that $^1\pi\pi^*$ to $^3\pi\pi^*$ is a very efficient process.$$^93$$ However, it cannot be assumed that energy transfer in bipyridine lanthanide complexes is analogous to non-chelated bipyridine as $^1\pi\pi^*$ can decay via upper lying metal centred levels. For terbium, this could occur via the $^5D_3$ level but this is not deemed feasible due to the relatively short lifetime of the $^1\pi\pi^*$ decay and the relatively small metal ligand interaction between the two states.

It has been shown that for the $[\text{Tb}ð(22)]^{3+}$ complex, that $\eta_{em.tr}$ is close to unity after taking into account the deactivation of $^5D_4$ Tb$^{3+}$ via back energy transfer process by using the kinetic system proposed by Alberty and Miller.$$^92$$ However, this study was undertaken using $[\text{Ln}ð(22)]^{3+}$ in the solid state, in which energy transfer processes will vary considerably in comparison to the transfer processes in solution. It is also noteworthy that phosphorescence spectra of $[\text{Na}ð(22)]^+$ and $[\text{La}ð(22)]^{3+}$ for 0-0 phonon transitions to determine the triplet state energy were determined at 4.2K (liquid helium temperature) in the solid state. At temperatures above 25K, the phosphorescence spectra loses its fine structure and the 0-0 phonon transition observed at 4.2K disappears.$$^{93}$

The participation of charge transfer or f-d levels can be excluded due to the relative stability of Tb$^{3+}$ towards reduction to Tb$^{2+}$.$$^94$$ For Tb(23) the excited state lifetime of
The $^3\pi\pi^*$ is strongly temperature dependent which means there is back energy transfer from the $^5D_4$ to $^3\pi\pi^*$. Further evidence that the $^5D_4$ state is in equilibrium with $^3\pi\pi^*$ ligand centered level is the quenching of luminescence by oxygen in aerated solutions.

**Quantum yield determinations of Tb(23) and Eu(23)**

Quantum yields for both Tb(23) and Eu(23) were determined relative to the respective $[\text{Lnc}(22)]^{3+}$ complexes whose quantum yields have been determined as $\phi_{\text{H2O}} = 0.03$ and 0.02 at 300K in aerated aqueous solutions, respectively. The respective cryptates were used as a reference as they display absorption at similar wavelengths (304 nm, $\varepsilon_{\text{max}} = 29000 \text{ M}^{-1}\text{cm}^{-1}$) and emission will be specific to the lanthanide in question, allowing for the same spectral window to be used. Solutions at 3 different absorbencies between 0.05 and 0.12 were used, to ensure that the same amount of light was absorbed by the reference and the unknown, at a common wavelength of 300nm. Solutions with absorbencies of 0.068, 0.079 and 0.114 for terbium and 0.066, 0.087 and 0.109 for europium at 300 nm were prepared. For each unknown reference/pair, the total integrated emission upon excitation at this wavelength was then determined using identical set-up on the spectrofluorimeter. The gate time was set so as to cover the equivalent of 6 lifetimes of the unknown so as to ensure that 99% of the light emitted was being measured. From the plot of total integrated emission (E) against absorbance (A), linear plots were obtained with a slope $E/A$. The quantum yields of the unknowns can be calculated using the relationship in Equation 16 as proposed by Haas and Stein.

\[
\phi_x = \phi_r \cdot \frac{\text{slope}_x}{\text{slope}_r} \cdot \left(\frac{n_x}{n_r}\right)^2
\]  
*Equation 16*

The terms r and x refer to the reference and unknown respectively, and n is the refractive index of each solution.

However, it was realised that the lifetimes of both Tb(23) and Eu(23) are considerably longer than that of the cryptate complexes and, quantum yield of a compound is directly related to the lifetime by Equation 17;

\[
\phi_{\text{em}} = \phi^0 \cdot \tau_{\text{obs}}
\]  
*Equation 17*
where \( k^0 \) is the sum of all the rate constants that deactivate the emitting state and \( \tau_{\text{obs}} \) is the observed lifetime at 300K. For example, measuring the total emission spectra of Eu(23) over six lifetimes (ca. 7ms) with a delay of 0.1 ms with of [Eu(22)]\(^{3+} \) as the reference, a considerable amount of the light emitted from [Eu(22)]\(^{3+} \) will have dissipated during the 0.1 ms delay time, as the lifetime is only 0.34 ms. It is therefore necessary to apply a correction factor (C) that will account for the light dissipated during the delay time and give the total integrated area from \( t = 0 \), which is given by:

\[
C = \exp\left(\frac{d}{\tau}\right)
\]  
*Equation 18*

where \( d \) is the delay time and \( \tau \) is the lifetime of the reference or the unknown. This can then be used to obtain the corrected slope \( E/A \) with respect to the total integrated area for the amount of light that is dissipated during the delay time;

\[
\text{(slope } E/A) \cdot C = \text{corrected slope } E/A
\]
*Equation 19*

The equation representing the quantum yield can now be written as

\[
\phi_x = \phi_r \cdot (C_x \cdot \text{slope}_x / C_r \cdot \text{slope}_r) \cdot (n_x/n_r)^2
\]
*Equation 20*

The derivation and proof of this relationship is shown in *Appendix 1*.

The values for the correction factor (C) for the unknowns and reference materials in both H\(_2\)O and D\(_2\)O are shown in *Table 9*. 
Bipyridine Sensitisers

<table>
<thead>
<tr>
<th>Eu(23)</th>
<th>Tb(23)</th>
<th>[Eu⊂(22)]^{3+}</th>
<th>[Tb⊂(22)]^{3+}</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.0908 (1.0606)</td>
<td>1.1847 (1.1536)</td>
<td>1.3419</td>
<td>1.3539</td>
</tr>
</tbody>
</table>

Table 9: Correction factors (C) determined from Equation 18 with a delay time of 0.1 ms. Values in brackets are in D2O. The lifetime for [Eu⊂(22)]^{3+} and [Tb⊂(22)]^{3+} are taken as 0.34 ms and 0.33 ms, respectively, and were measured prior to each quantum yield determination at 300K.

It was noted that a delay of 0.05 ms made no significant difference to the value of the quantum yield obtained.

With the relative quantum yields in H2O now known for both complexes in aqueous solutions, D2O solutions of the complexes were made up at the same absorbencies at 300nm as before. The calculated quantum yields increased by a factor of 2 for both Tb(23) and Eu(23) (Tables 3 and 5), showing that even though the complexes are coordinatively saturated, deactivations can occur through bulk solvent interactions.

**Octanol Water Partitions**

Both Tb(23) and Eu(23) could not be extracted into n-octanol and were found to be insoluble in fat like solvents such as dichloromethane and octanol. This is postulated to be due to the hydration of carboxylate groups in an aqueous environment, inducing good hydrophilicity and poor fat solubility. This property may inhibit the use of these chelates as reagents that can pass through the cell membrane.

**Observation of water exchange**

Dissociative water exchange at lanthanide centres has traditionally been considerably fast, with rates of oxygen exchange to be of the order 5x10^6 s^{-1} at 298 K for similar anionic complexes. This rate is said to be much slower for neutral and cationic complexes. Parker et al., showed that cationic chiral ytterbium and europium tetramide complexes [Ln(34)]^{3+} will undergo dissociation of water, with the rate of
exchange 500 times faster for that of the ytterbium complex, by using variable temperature NMR and luminescence.

Solution and solid state structures of $[\text{Eu}(34)]^{3+}$ showed a square anti-prismatic geometry about the metal centre, whilst the Yb analogue showed a twisted square anti-prismatic isomer.

It was proposed that the rate of water exchange was slow enough that the measurement of excited state lifetimes of the ytterbium and europium complexes would show a difference in radiative lifetime. The shorter lived lifetime arising from the nine coordinate ($\text{Ln}(\text{34}.\text{OH}_2)$) species could be differentiated from the longer lived eight coordinate, non-water bound complex $\text{Ln}(\text{34})$. It was shown that the radiative rate constant for the Yb complex in D$_2$O at 295K was $1.6 \times 10^5$ s$^{-1}$, with single exponential decay being observed. In H$_2$O, the decay constant observed was $1.4 \times 10^6$ s$^{-1}$ at 295K and single exponential decay was observed. At temperatures below 280K, bi-exponential decay was observed. This was attributed to the presence of mono-hydrated (9 coordinate species) which has a faster decay, and the non-water bound complex (8 coordinate) species having a slower decay. For the Eu(34), single exponential decay was observed in both acetonitrile and water, with the observed rate constants being
0.76x10^3 \text{ s}^{-1} \text{ and } 1.72x10^3 \text{ s}^{-1}, \text{ respectively. The observed mono-exponential decay rate in acetonitrile increased as a function of added water concentration. At lower temperatures between 233K and 372K, the rate of water exchange was significantly slow that independent (bi-exponential) decay was observed arising from the 8 and 9 coordinate species, under conditions where sufficient amounts of water had been added to allow each species to be significantly populated. The proportion of the shorter lived species, the 9 coordinate water bound species, in the bi-exponential decay increased with decreasing temperature.

The work by Parker and co-workers goes some way to explaining the effect of bound water on the radiative decay profiles of both Eu(23) and Tb(23) at reduced temperatures. Further studies, such as crystallography and variable temperature NMR would confirm this assumption. Also, it would be interesting to see if the water is bound to the metal ion and undergoes slow exchange at lower temperatures through inner sphere coordination or through an outer sphere interaction with the bulk solvent, where the carboxylate moieties would indeed be hydrated as initially assumed.

Concluding remarks
To summarise the achievements with respect to the aims and objectives set out in this chapter, we have shown that the target ligand is an efficient sensitiser of both terbium and europium, with the complexes Eu(23) and Tb(23) being stable in aqueous solutions over a period of months. With respect to Eu(23), we have shown that a coordinatively saturated complex is formed that is unaffected by temperature (k_{rr}(T)<10^3 \text{ s}^{-1}), presence of anions and solvent medium. The quantum yield in comparison to the \([Eu\subset(22)]^{3+}\) complex is 8.5 times greater due to the metal being coordinatively saturated with little energy being lost through solvent oscillations. Energy transfer from the \(1\pi\pi^*\) to \(^5\text{D}_0\), \(\eta_{\text{en,-rr}}\), is relatively low. This is likely to be due to the presence of LMCT bands arising from the lone pairs of the aza-crown nitrogens, which can except energy from the \(1\pi\pi^*\) level, \(^3\pi\pi^*\) level and the emissive \(^5\text{D}_0\) level. The efficiency of radiative metal centred emission, \(\eta_n\), from the \(^5\text{D}_0\) level is high in comparison to other bipyridine complexes. This arises from the fact that Eu(23) is nine coordinate and no bound water to the metal
centre as implied by the low $q_{\text{corr}}$ value. Deactivations through solvent interactions therefore only occur through an "outer sphere" participation from the bulk solvent, which are not as efficient as "inner sphere" deactivations.\textsuperscript{100}

For Tb(23), luminescent lifetime and spectral profile were unaffected by the presence of anions or solution media but displayed a strong temperature dependence that was postulated to be back energy transfer from the emissive $^5\text{D}_4$ level of terbium to the bipyridine triplet. The quantum yield relative to the [Tb<sup>22</sup>]\textsuperscript{3+} is of the same order of magnitude in comparison with Tb(23), 0.03 and 0.07 respectively. The slight increase in quantum yield can be attributed to the difference in energy of the $^3\pi\pi^*$ in the 2 complexes, with the $^3\pi\pi^*$ in [Tb<sup>22</sup>]\textsuperscript{3+} being at lower energy (21900 cm\textsuperscript{-1}) than in Tb(23) (22600 cm\textsuperscript{-1}) and also the hydration number ($q_{\text{corr}}$). The overall efficiency of metal centred emission, $\eta_r$, is low due to the presence of the thermally activated back energy transfer from the excited $^5\text{D}_4$ luminescent level to the ligand centred $^1\pi\pi^*$ level. Attempts to quantify the temperature dependence of Tb(23) using the luminescent lifetimes in H\textsubscript{2}O and D\textsubscript{2}O at varying temperatures yield different activation energies depending on the solvent. $E_a$ in D\textsubscript{2}O is higher because of more the more efficient O-H oscillations in H\textsubscript{2}O than the O-D oscillations in D\textsubscript{2}O (some 200 times more efficient). Therefore, the activation energy associated with the temperature dependence of Tb(23) will include BET as well as temperature dependent solvent oscillations, and therefore can only be used to estimate the relative position of the aryl triplet from the $^5\text{D}_4$ emissive level of terbium.

Triplet determinations using Gd(23) at 77K only provide a upper limit for the triplet level as estimated from the onset of emission. Phosphorescence emission spectra obtained at 4.2K may provide a more accurate determination of the 0-0 phonon transition as observed by Blasse and co-workers.\textsuperscript{93}

**Charged bipyridine complexes**

Moving away from the theme of charge neutral chelates, we decided to investigate charged species utilising bipyridine chromophores. The triazacyclononane ligand 35 and
its complexes with terbium and europium have been previously reported and were shown to have both excellent stability and luminescence properties in aqueous solution (Table 10).\textsuperscript{101}

\begin{tabular}{|c|c|c|c|c|c|c|}
\hline
Complex & $\tau_{D_{2}O^{77K}}$ & $\tau_{H_{2}O^{77K}}$ & $\tau_{D_{2}O^{300K}}$ & $\tau_{H_{2}O^{300K}}$ & $\phi_{D_{2}O^{300K}}$ & $\phi_{H_{2}O^{300K}}$ \\
\hline
[Tb(35)]$^{3+}$ & 1.5 & 1.4 & 1.5 & 1.5 & 0.38 & 0.37 \\
[Eu(35)]$^{3+}$ & 1.1 & 0.87 & 0.57 & 0.50 & 0.07 & 0.05 \\
\hline
\end{tabular}

\emph{Table 10:} Photophysical properties of [Tb(35)]$^{3+}$ and [Eu(35)]$^{3+}$ adapted from reference \textsuperscript{101}. Excitation at 309 nm. \textsuperscript{a}Measured in correspondence with the most intense emission band ($^5D_4\rightarrow^7F_5$ for Tb$^{3+}$ and $^5D_0\rightarrow^7F_2$ for Eu$^{3+}$). \textsuperscript{b}Experimental error $\pm$10\%. \textsuperscript{c}Experimental error $\pm$30\%.

In summary of the results found by Prodi et al., the triazacyclononane ligand \textsuperscript{35} formed stable complexes with both europium and terbium in aqueous solutions with intense absorption bands in the near UV region and almost completely shield the metal centred from solvent interactions. The europium complex displayed relatively weak luminescence properties ascribed to poor ligand to metal energy transfer efficiency and an activated radiationless decay of the $^5D_0$ luminescent level. This relative inefficiency of energy transfer was associated with the presence of lower lying LMCT bands, which
of energy transfer was associated with the presence of lower lying LMCT bands, which involve the aliphatic nitrogens of the aza-crown ring. For the terbium complex, low energy LMCT bands are not present resulting in a high quantum yield (0.37). The terbium chelate also displayed very little temperature dependence, with the luminescent lifetime at 77K being the same at room temperature. From the phosphorescence spectra of \([\text{Gd} (35)]^{3+}\), the triplet level was postulated to be at 22700cm\(^{-1}\) in energy, which was believed to be of high enough energy so as to prevent back energy transfer from the \(5D_4\) luminescent level of terbium to the \(3\pi^*\) ligand centred level. The luminescent lifetime of \([\text{Tb}(35)]^{3+}\) in \(D_2O\) at 77K was found to be shorter in comparison to that of analogous bipyridine terbium complexes, which was postulated to be due to a high radiative rate constant or more likely, to be radiationless deactivation via \(-\text{CH}_2\) oscillations on the aza-crown ring that lie in close proximity to the metal.

With the reported luminescent properties of lanthanide complexes with ligand 35, we decided to investigate the properties of an analogous ligand 36 and its complexes with terbium and europium. This ligand is based on CYCLEN and has potentially 10 coordination sites available for binding and, in theory, would form a more stable complex due to the greater flexibility of the CYCLEN backbone. CYCLEN offers a larger ring cavity and an extra aza-crown nitrogen which is available for binding. 36 an ester group that can be easily functionalised with biological molecule or a labile ester for use in bio-assays.
Synthesis of [Tb(36)]\(^{3+}\) and [Eu(36)]\(^{3+}\)

A number of synthetic strategies have been developed for synthesising mono-functionalised aza crown ethers. Early work utilised the use of protecting groups to effectively “block” 3 of the nitrogens, leaving the fourth position free to be alkylated. Protecting reagents that have been employed are molybdenum hexacarbonyl\(^{102}\) and tris(dimethylamino)-borane.\(^{103}\) The use of such reagents is problematic as both the reagents and intermediates involved are air and moisture sensitive, requiring the reaction to be carried out under reduced pressure and in an inert atmosphere. Alternatively, Anelli et al.,\(^{104}\) have reported the synthesis of mono-functionalised CYCLEN without the use of elaborate protecting groups but it requires the aza-crown ether to be in a 5-10 fold molar excess with respect to the alkylating agent. It has also been reported by Kruper et al.,\(^{105}\) that mono-alkylation of aza crown ethers can be achieved by using a slight molar excess of the aza-crown with respect to the alkylating agent in polar aprotic solvents such as dichloromethane or chloroform. These reaction conditions afforded mono-alkylation in high yields and with excellent selectivity, bis and tris-products are formed but in low yields, 9% and 2%, respectively. Thus, we decided to try this synthetic route as opposed to the others reported. Scheme 3 outlines the synthetic strategy for compound 36. The chloromethyl ester 38 prepared by reacting the corresponding acid 37 with thionyl chloride in ethanol, to obtain the esterified compound in a near quantitative yield. A 1.5 molar equivalent of 31 was reacted with the chloromethyl ester 38 in chloroform and after purification by column chromatography, the mono-alkylated compound 39 was isolated in a reasonable yield. The synthesis of the branched macrocycle 36 was initially carried out by reacting and excess of 30 with the mono-functionalised CYCLEN 39 in the presence of triethylamine. After aqueous work up and purification by column chromatography, the desired macrocycle 36 was obtained in a low yield (20%). It was believed that the triethylamine was reacting with the alkylating agent to form a quaternised salt. The required compound was also very difficult to separate from the mono and bis substituted bipyridine compounds by column chromatography. Using a more sterically hindered base such as Hunig’s base reduced this problem and increased the yield to up to 25 %,
but separation of the required compound was again problematic. Use of the non-
nucleophilic base, DBU (1, 8 diazabicyclo[5.4.0] und-7-ecene), as the external base
produced the best result with 36 being obtained in a 57% yield.

Synthesis of the complexes was achieved in near quantitative yields by reacting 36 with
a slight excess of the appropriate lanthanide triflate salt in dry acetonitrile to obtain the
salts [Eu(36)]^{3+} and [Tb(36)]^{3+}. Mass spectral analysis (FAB^+) of both the terbium and
europium complexes indicated that the complex had formed but were in very low
abundance as the major peak evident was that of the ligand. The peaks corresponding to
the complex were that of the [M]^+ -2CF_3SO_3 and -CF_3SO_3, indicating the complexes are
possibly unstable under mass spectral conditions. Melting points of the salts isolated
were higher than those of the parent ligand but not significantly high, as expected for an
inorganic salt.

Scheme 3: (i) SO_2Cl, C_2H_5OH; (ii) 31, CH_3Cl; (iii) 30, 3.3eq DBU, CH_3CN; (iv)
Ln(CF_3SO_3)_3, CH_3CN.
Results and discussion

UV-vis absorption spectroscopy of the ligand showed 2 main absorption peaks at 232 and 283 nm with extinction coefficients of 53910 and 41650 dm$^3$mol$^{-1}$ cm$^{-1}$. Absorption spectra of [Eu(36)]$^{3+}$ and [Tb(36)]$^{3+}$ in methanol at the same concentration showed a slight red shift in the absorption band at 283 nm, moving to 288 nm. Addition of an excess of the appropriate lanthanide triflate salt to the respective solutions induced little change in UV-vis absorption.

Photophysical properties of [Tb(36)]$^{3+}$

It was also noted that the isolated compound was only soluble in water at low concentrations but very soluble in acetonitrile and methanol. The main excitation peaks were found to be 309 and 319 nm when monitoring the emission peak at 545 nm. Luminescence in methanol at $10^{-5}$M displayed sensitised terbium emission when exciting at 309 nm or 319 nm. The decay was found to be bi-exponential indicating more than one species in solution, with the lifetimes being 0.8 ms and 1.2 ms. It was also noted that there was a considerable drop in emission intensity after the solution was allowed to stand for a matter of hours. Upon addition of an excess of terbium triflate to this solution, the emission intensity increased 4-fold after allowing the solution to stand for 20 minutes with the lifetime appearing to be mono-exponential and of the same value as the longer lived value previously stated (ca. 1.2 ms). A time drive experiment of a freshly prepared methanolic solution at $10^{-5}$M was carried out to assess the relative stability of the emissive species in solution when excited at 309 nm and monitoring the $^5D_4\rightarrow^7F_2$ emission peak at 545 nm over a 12 hour period. The trace obtained is shown in Figure 38 and displays some interesting features. After very fast initial dissipation of the luminescent peak at 545 nm over 10 minutes, the emission intensity began to rise quite rapidly over the remaining time period. This indicates that the complexes is undergoing rearrangement in solution to form a more efficient luminescent species. The lifetime was measured before and after the time drive experiment and was shown to be the same at both points in time with bi-exponential decay being observed. The UV absorption spectra of the solution after the time drive showed very little change from the
spectra recorded prior to the experiment, although the appeared to be a "shoulder" on the main absorption peak at 285 nm extending over 309 nm.

![Graph](image)

**Figure 38**: Time drive plot of \([\text{Tb(36)}]^{3+}\) in methanol at \(10^{-5}\text{M}\); \(\lambda_{ex} = 309\text{nm}; \lambda_{em} = 545\text{nm}\); excitation and emission slits, 5nm.

Measurement of the isolated complex in water showed very similar properties to the sample in methanol. Initially, the "complex" shows quite strong sensitised luminescence when excited at 309nm with a bi-exponential decay and the lifetimes estimated to be 0.9 ms and 1.2 ms, with the solution showing a UV-vis absorption band that corresponds to the free ligand. An analogous time drive experiment showed the same result as obtained in methanol. However, the lifetime, which was still bi-exponential, had decreased considerably to 0.70ms and 0.8 ms. Degassing of the solution after the time drive experiment by 4 freeze-pump-thaw cycles showed no overall change in emission intensity or lifetime. It is therefore postulated that the compound isolated is not the discrete 1:1 complex as first anticipated and may even be indeed a mixture of compounds, including the free ligand. The luminescent species in solution is also a species that is unaffected by oxygen, indicating that it is not coordinating through both bipyridine nitrogens. If this were not the case, then there would be a significant increase in emission intensity and lifetime in the absence of oxygen in solution as shown by a number of bipyridine containing complexes with terbium.
Photophysical properties of [Eu(36)]^{3+}

The analogous europium complex showed metal centred sensitised emission when excited at 309 nm in an methanolic solution at $10^{-5}$M although, as before, this peak is not evident in the UV-vis absorption spectra.

The main excitation peaks were found to be 309 and 319 nm when monitoring the emission peak at 616 nm. Excitation at either wavelength was found to be 0.8 ms and was of single exponential in decay. After allowing the solution to stand for a few hours lead to a bi-exponential decay and decreased emission intensity, which was of too low intensity to measure accurately. Addition of an excess of Eu(CF$_3$SO$_3$)$_3$ made no apparent change in the UV-vis absorption spectra but increased the luminescent intensity very slightly. A time drive experiment exciting a freshly made solution at 309 nm and monitoring the $^5$D$_0 \rightarrow ^7$F$_2$ transition at 620 nm revealed a similar trace to that of the Tb analogue (Figure 39). The trace shows that the complex is rapidly dissociating in solution.

![Figure 39: Time drive plot of [Eu(36)]^{3+} in methanol at $10^{-5}$M; $\lambda_{ex}$ = 309 nm; $\lambda_{em}$ = 620 nm; excitation and emission slits, 5 nm.](image)

Concluding remarks

The results obtained with [Eu(36)]^{3+} and [Tb(36)]^{3+} were disappointing considering the implied photophysical and stability properties of [Ln(35)]^{3+} as reported by Prodi, who
stated that the TACN derivative in aqueous solution to be stable over days. It is possible, due to the lack of hard donor atoms such as oxygen in these ligands, that complexes are dissociating in solution and are subject to conformational isomerism. This has been observed in terpyridine complexes with europium.\textsuperscript{106} The lack of stability displayed in complexes $[\text{Eu}(36)]^{3+}$ and $[\text{Tb}(36)]^{3+}$ could also arise from the metal not being completely shielded from solvent molecules in solution. The ligand is not symmetrical and part of the metal is left bare to solvent interactions, which could effectively compete for the available binding sites of the metal with the weakly coordinating bipyridyl moieties. The resulting metal centred emission is occurring through intermolecular energy transfer rather than intramolecular energy transfer, as indicated by the lack of correlation between the UV-vis absorption and excitation spectra of the complexes. These complexes displayed poor stability and luminescence properties in solution and no further work was carried out on them.

In both time drive experiments of $[\text{Eu}(36)]^{3+}$ and $[\text{Tb}(36)]^{3+}$, the results showed initial complex dissociation but with $[\text{Tb}(36)]^{3+}$, emission intensity rising after a short period of time. It is possible that the complex is decomposing in solution but the bipyridine moieties are rotating to form a transoidal complex, with only 1 pyridine of the bipyridine being bound to the metal centre. The net effect of this is that the triplet of the sensitiser is raised as the chromophore is perturbed less by the heavy metal centre, with energy transfer occurring both intra- and inter-molecularly from the free and bound bipyridine chromophores, respectively. The complex in solution that has been allowed to stand for over twelve hours is insensitive to oxygen, indicating that the triplet has been raised, precluding the effect of back energy transfer. $[\text{Eu}(36)]^{3+}$ is undergoing the same decomposition to form the transoidal complex but as the triplet is being raised, the overlap between the emissive level of europium ($^5D_0$) and the sensitiser becomes greater, leading to reduced Förster overlap and therefore significantly reducing energy transfer. For terbium, the $^5D_4$ emissive level is of higher energy (20400 cm$^{-1}$) and is able to maintain a reasonable degree of overlap leading to greater energy transfer efficiency, resulting in an increase in emission intensity.
Results and Discussion

Chapter 4
Chapter 4

The Investigation of Mixed Carbonyl-Phenol Donors as Sensitising Moieties

Aromatic carbonyl compounds are widely reported in the literature to act as sensitisers of certain lanthanide ions. For instance, Weissman investigated the fluorescence of some salicylaldehyde and \( \beta \)-diketonate chelates of europium.\(^8\) Using a quartz monochromator and the sun as a source of white light radiation, he introduced the concept of "sensitised" lanthanide emission where light is absorbed through an aromatic antenna and the energy is consequently transferred to the metal centre, leading to lanthanide emission. Weissman mainly discussed experiments with europium chelates but he also observed the same phenomena with samarium and terbium complexes.

\[
9 \quad 40 \quad 41
\]

A comparison made with crystalline \( \text{EuCl}_3\cdot6\text{H}_2\text{O} \) and a solid \( \text{Eu}(9) \) complex both showed characteristic red emission associated with europium when exciting between 300-400nm, with \( \text{EuCl}_3\cdot6\text{H}_2\text{O} \) exhibiting specific excitations at 320 nm and 392 nm. It was observed that with \( \text{Eu}(9) \) complex, that a continuous absorption band between 320 nm and 440 nm was present and that the characteristic europium emission was of virtually uniform intensity at all excitation wavelengths between the two values.

He also found that the efficiency was dependent on the temperature and the nature of the solvent being used. A solution of \( \text{Eu}(9) \) at \( 5\times10^{-4}\text{M} \) in benzene showed a 5-fold increase in emission intensity when cooled from 45°C to 6°C. By comparison,
europium nitrate only showed a change of a few percent under the same temperature changes. Upon cooling from 300K to 90K, the yield of fluorescence intensity of solid Eu(9) increased 150-fold whilst EuCl₃.6H₂O increases only by a factor of 2. He concluded that luminescence efficiency was enhanced at reduced temperatures.

Luminescence was also found to vary from solvent to solvent. The fluorescence at room temperature of a 10⁻⁴M alcoholic solution of Eu(40) when excited by radiation from a high pressure mercury arc lamp was found to be barely visible in the dark. Upon changing to benzene, the solution becomes visible even in a fully lit room.

Rohagti et al.,¹⁰⁷ synthesised a number of mixed Eu(9)₃ chelates containing neutral ligands, such as phenanthroline 42 and bipyridine 27, with the aim of changing the symmetry of the electric field around the central metal ion (Figure 40). Europium (III) has a ground state of ⁷F₀ with the lowest multiplet being ⁷F₁ and the highest ⁵D₁. The selection rules for electric dipole transitions are ΔJ=2, 4, 6, and are spin and parity forbidden. Magnetic dipole transitions are ΔJ=0, ±1 (except 0↔0) and are only spin forbidden. At room temperature, europium emission occurs mainly from the ⁵D₀ and in a centro-symmetric system, only the magnetically allowed ⁵D₀→⁷F₁ transition are observed. Any changes from the symmetric configuration increases the probability of the electric dipole allowed ⁵D₀→⁷F₂,4,6 transitions.

![Figure 40: Mixed salicylaldehyde and nitrogen donor complexes with europium.](image)

where $\text{N} \rightarrow \text{N}$.
It was found that the excitation spectrum of the Eu(9)₃ complex in benzene solution corresponded to the absorption spectra of the complex and possess an excitation maximum at 392 nm. It was found that the mixed europium salicylaldehyde chelates were soluble in benzene and that the red emission is intensified whereas the Eu(9)₃ is only sparingly soluble in benzene and is weakly luminescent. The authors suggested that since the neutral ligands did not absorb in the excitation region (392nm), their role in increasing emission intensity must be through distorting the symmetry around the central metal ion. Measurements in benzene and in the solid state allowed the authors to compare the relative emission intensities of the \(^5\)D₁₀→\(^7\)F₂ to \(^5\)D₀→\(^7\)F₁ transitions, the electric dipole and magnetic dipole transitions, respectively. The outcome being that there was considerable difference between the solid state and solution measurements, arising from partial dissociation of the mixed chelate in solution. For the Eu(9)₃(42) complex, the ratios of emission intensities in solution and in the solid state were similar. This was attributed to the bulky and rigid phenanthroline moiety preventing solvent interactions with the complex, and thus ensuring a high degree of stability in solution.

Crosby\(^{108}\) used o-hydroxybenzophenone 41 and various \(\beta\)-diketonates as a means of discussing intramolecular energy transfer and the role of ligand triplet states in rare earth chelates. A solution of Gd(41)₃ in 3-methylpentane at 77K exhibited an intense but diffuse phosphorescence band at 17400 cm\(^{-1}\). Upon moving to an EP (1 part diethyl ether: 1 part 3-methylpentane) glass, this band shifts to higher energy (shorter wavelengths) at 18200 cm\(^{-1}\). In a hydroxylic EPA (2 parts diethyl ether:2 parts 3-methylpentane: 1 part ethanol) glass, there are at least two phosphorescence bands observed; the first being between 500-700 nm and the second between 400-500 nm, the former being less intense. This change in luminescence properties and appearance of more than one molecular phosphorescence band observed when moving to more polar solvents suggested that there was considerable dissociation of the chelate through solvation by hydrogen bonding. Examination of the absorption spectra of the rare chelates in methyl pentane, EP and EPA all showed dissociation of the chelates, with absorption bands at 342nm and 385 nm, the former corresponding to 41 and the latter to intramolecular transitions within the fully chelated complex. This was confirmed by
examining the total emission spectra of 41, its sodium salt and its complexes with the
rare earth chelates under examination in EPA. It was found that, in the rare earth
chelates, the well defined 400-500 nm phosphorescence band was evident in both the
chelating agent and its sodium salt. The addition of trichloroacetic acid to the EPA
solution of Gd(41)₃, causing complete dissociation of the complex, results in only the
"blue" phosphorescence being observed. From this, it was concluded that the
dissociation product was giving rise to that particular phosphorescence band and was
the parent chelating agent. The lack of well-defined structure in this phosphorescence
band and its intensity change upon the addition of the metal chloride to the solutions
suggested that emission originates from more than one species. This equilibrium
between emissive species was further demonstrated by solutions of Sm(41)₃. In 3-
methylpentane, only weak emission lines are observed under ultraviolet radiation. This
emission is enhanced upon moving to EP and greater still in EPA. It was concluded that
since the addition of alcohol enhances dissociation of Sm(41)₃ and the formation of
Sm(41)₂⁺ and Sm(41)₁²⁺, which are the luminescent species and not Sm(41)₃.
Europium (III) differs from other trivalent lanthanide ions in the way that it can emit
from two resonance levels, the $^5D_1$ and the $^5D_0$ states, whose energies lie at 19020 cm⁻¹
and 17250 cm⁻¹, respectively. Studies on Eu(41)₃ in 3-methylpentane showed bright line
emission originating from only the lower resonance level of the ion (17250 cm⁻¹),
indicating that all coordinated species in solution had a triplet below 19020 cm⁻¹ and the
fully chelated species has a triplet between the two resonance levels of europium (III).
Problems arose when moving to more polar, alcoholic solutions where dissociation to
Eu(41)₂⁺ and Eu(41)₁²⁺ was evident. This way of "bracketing" triplet levels was
successful as the phosphorescence spectra of Gd(41)₃ in methyl pentane showed the
triplet to be at 17400 cm⁻¹. However, emission arising from the $^5D_1$ level of europium is
every rarely observed in solution and is of very weak intensity which casts some doubt
over this initial postulation by the authors.

In earlier work published by Crosby et al.,¹⁰⁹ it was shown that many rare earth
chelates, such as salicylaldehydes, amino-phenols, hydroxybenzophenones and
hydroxyquinolines, were unsuitable for such studies as they were subject to photodecomposition and exhibited poor stability in solution.

**Aims and objectives**

Our objective was to re-examine the photophysical properties of 3:1 complexes of salicylaldehyde 9 and o-hydroxybenzophenone 41 with europium and terbium in polar protic and aprotic solvents. Although Weissman and Crosby carried out studies on europium salicylaldehyde complexes, no evidence was presented for their composition and structure, nor was a triplet energy determined for the chromophore. As it has already been well established that these complexes are unstable in polar and non-polar solvents, it would be necessary to design a ligand that would be able to form a complex which exhibited kinetic stability and photostability on the timescale of the luminescence experiment. This could be achieved by incorporating the chromophoric group onto an aza-crown ether, for example, by use of the aromatic Mannich reaction to give ligands 45 and 46.

![Chemical structures](image)

As a point of interest, both europium salicylaldehyde and hydroxybenzophenone complexes of europium have been excited at longer wavelengths, ranging from 380-420nm.\(^8,108,109\) This is particularly useful for biological applications as excitation at shorter wavelengths, below 350 nm, can lead to light being absorbed by nucleic acids and aromatic amino acids, such as tryptophan and tyrosine as well as by reduced
pyridine nucleotides, such as NADH. Excitation at longer wavelengths can eliminate these problems and also avoid the use of expensive quartz optics.

**Synthesis of Ln(\(9\))_3**

Weissman\(^8\) originally studied the fluorescence properties of europium salicylaldehyde but no evidence was given to support the structure given as Eu(\(9\))_3. Brimm\(^1\) attempted the synthesis of Ln(\(9\))_3 through the reaction of 3 equivalents of the sodium salt of \(9\) with the lanthanide chloride salt. It was evident that the compound was isolated as the hydroxide complex, La(\(9\))(OH)_2. Alire\(^1\) reported the synthesis of a number of rare earth chelates derived from \(9\). This involved the reaction of the rare earth metal chloride with \(9\) in the presence of piperidine as a hydrogen acceptor. This procedure gave compounds that had metal analysis in good agreement with the 3:1 stoichiometry but problems arose when trying to remove the excess of piperidine that was co-ordinated to the metal centre, as it is easily retained in the product.

Synthesis of Eu(\(9\))_3, Tb(\(9\))_3 and Gd(\(9\))_3 were achieved according to the method employed by Charles\(^1\) which involved forming the sodium salt of \(9\) *in situ* followed by the slow addition of an aqueous solution of the rare earth chloride (Scheme 4). Eu(\(9\))_3 was synthesised in this manner to give an analytically pure compound in a 42% yield. The terbium and gadolinium analogues were synthesised in the same manner, but elemental analysis for both was found to be incorrect for the required composition. Charles found that for heavier rare earth metals, the materials isolated showed a significant degree of sodium, with an Ln:Na ratio of 0.7. It was believed that the sodium present in the material isolated cannot correlate to any simple structure and the isolated compounds were thought to be a mixture of Ln(\(9\))_3 with Ln(\(9\))_3. Na(\(9\)).
Absorption and photophysical properties of Eu(9)₃

Ultraviolet and luminescence spectra were recorded for 10⁻⁴ M solutions, concentrations were kept deliberately high in an attempt avoid dissociation of the complexes in solution. All complexes isolated were sparingly soluble in water and likely to dissociate rapidly. Methanol was therefore used to prepare solutions.

The Eu(9)₃ in methanol at 10⁻⁴M showed two main absorption bands arising from the chelated and the dissociated salicylaldehyde, at 379 nm (ε=10000 dm³mol⁻¹cm⁻¹) and 328 nm (ε=6040) respectively. This indicates that the chelate dissociates quickly in alcoholic solution. The complex displayed very weak metal centred emission when excited at 297nm and 392 nm, which were the main peaks arising in the excitation spectra when monitoring at 615 nm. It was found that the complex was only sparingly soluble in toluene, contrary to what has been stated in the literature,⁸ and formed a turbid suspension. UV–vis absorption of the solution showed that very little of the chelate was in solution by virtue of a low extinction coefficient, even after sonification and gentle heating. Excitation of the solution at 392 nm gave rise to a more intense europium emission peak centred at 614 nm, with the lifetime found to be less than 100µs, which could not be measured accurately due to instrumental constraints. Changing to acetonitrile, the solutions appeared to have a greater degree of stability than in methanol with little of the salicylaldehyde appearing in the absorption spectra after standing over a longer period of time. This solution showed a red glow when irradiated with UV-light at 365 nm or the solution place in bright sunlight. The excitation and emission spectra show the same spectral profile as in toluene. The lifetime of the
chelate in acetonitrile was again less than 100 µs. Upon cooling this solution to 77 K, the lifetime increased to 480 µs and fitted well to a single exponential decay. This significant change in lifetime indicates that there is strong temperature dependence associated with the sensitisation and emission processes. The emission intensity was also increased 3 orders of magnitude in comparison to the spectrum at 300K (Figure 41). The emission observed with Eu(9)₃ mainly originates from the electric dipole allowed $^5D_0\rightarrow^7F_2$ transition, indicating that the central metal ion is in a non-symmetric environment.

Degassing the sample by 4 freeze-pump-thaw cycles showed an increase in emission intensity but no change in lifetime (still <100µs). This implies that the relatively short lifetime associated with the sensitised emission is not due to quenching of the aryl triplet by dissolved oxygen and can be postulated that energy transfer from the triplet is fast with no thermal repopulation of the triplet from the metal centre. From the emission spectra of Gd(9)₃ exciting at 392 nm in acetonitrile at 77 K, the triplet was found to be at 19100 cm⁻¹, taken from the onset of emission. In reality, this is close enough in energy to the emissive $^5D_0$ level of Eu³⁺ (17250 cm⁻¹) to allow back energy

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**Figure 41**: Excitation and emission of Eu(9)₃ in CH₃CN at 77K; λₓₑ = 392; λₑₘ = 615 nm; excitation and emission slits, 2.5nm.
transfer, but it appears there is a more efficient deactivation mechanism in operation. The phosphorescence spectrum of Gd(9)₃ was also recorded in a KBr disc at 77K so as to avoid the possibility of the complex dissociating in solution. The onset of emission was at 520 nm (19200 cm⁻¹) which is in reasonable agreement for the value obtained in solution (Figure 42).

Figure 42: Phosphorescence spectrum of Gd(9)₃ in a KBr disc at 77K; excitation and emission slits at 2.5 nm; λₑₓ = 392 nm.

**Temperature dependence of Eu(9)₃ luminescence**

The lifetimes of the $^5D_0 \rightarrow ^7F_2$ transition of Eu(9)₃ at 10⁻⁴M in acetonitrile were recorded at temperatures ranging from 77K to 300K in order to assess the temperature effect, with the aim of obtaining an activation energy for the non-radiative decay process. At lower temperatures (77K to 140K) the decays fitted well to a single exponential function ($R^2 = 0.999$ from the ln $I$ versus $t$ plot) but at temperatures above 140K, the lifetimes displayed a degree of bi-exponential behaviour. At temperatures above 200K, the lifetime began to fall significantly and was less than 100μs at temperatures above 260K. To maintain consistency, all lifetime decays were fitted to *Equation 21*, which will yield two lifetime components, but allows the variation of the intensity for each $\tau$ component:

$$I(\lambda) = I_{01}(\lambda) \exp(-t/\tau_1) + I_{02}(\lambda) \exp(-t/\tau_2)$$

*Equation 21*
where $I(\lambda)$ represent the emission intensity at time $t$ monitored at a specific wavelength $\lambda$, after excitation; $I_{01}(\lambda)$ and $I_{02}(\lambda)$ represent the emission intensity at time $t=0$ at the same wavelength for each component of the bi-exponential decay, and $\tau$ is the observed lifetime for the luminescence decay.

The activation energy can be calculated by using Equation 8. The term $1/\tau^0$ is the rate constant where no temperature dependence is present, and in this case it is assumed to be the radiative lifetime at 77K.

$$k = \frac{1}{\tau} - \frac{1}{\tau^0} = A \cdot \exp\left(\frac{E_a}{RT}\right)$$  

Equation 8

$\ln\left[\left(\frac{1}{\tau} - \frac{1}{\tau^0}\right)\right]$ versus $1/T$ plots for both $\tau_1$ and $\tau_2$ showed very little linearity due to the absence of change in lifetime at temperatures below 180K and the very short lifetimes at temperatures above 260K (Figure 43). The data obtained at low temperature in acetonitrile is unlikely to be accurate as the complex may dissociate in solution over the time period that these low temperature measurements are obtained (some 6 hours). The data obtained shows little linearity and a considerable difference in activation energies calculated using Equation 8, 1700cm\(^{-1}\) and 1180cm\(^{-1}\), when using $\tau_1$ and $\tau_2$, respectively. However, it is believed that the deactivation leading the poor emissive properties of Eu(9)\(_3\) is due to the presence of ligand to metal charge transfer states.
Figure 43: Temperature dependence of the observed lifetimes of $^5D_0 \rightarrow ^7F_2$ transition in Eu(III) in acetonitrile at $10^{-4}$M. Experimental lifetimes $\tau_1$ (●) and $\tau_2$ (■) are plotted against $1/T$ according to the Equation 8.

Lifetime measurement of Eu(III) in the solid state (KBr mull) at room temperature and at 77K deviated little from the measurements made in acetonitrile, with the values being less than 100 µs and 490 µs, respectively. An activation energy was also obtained for the radiative decay of the complex in the solid state with the decay at all temperatures fitted well to a single exponential decay.\footnote{In I vs t displayed linear decay plots with R² values of >0.999 fit for all temperatures measured} The activation energy was found to be 1380 cm⁻¹ (Figure 44).
Figure 44: The temperature dependence of the \( ^{5}D_{0} \rightarrow ^{7}F_{2} \) transition of Eu(9)\(_{3}\) in a KBr mull; \( \lambda_{ex}=392 \) nm. Experimental lifetime \( \tau \) is plotted against \( 1/T \) according to Equation 8.

It is postulated that the temperature dependence associated with the metal centred emission of Eu(9)\(_{3}\) is due to ligand to metal charge transfer (LMCT) states quenching the metal centred luminescence. LMCT deactivations can occur through the excited singlet and triplet states, as well as the emissive \( ^{5}D_{0} \) level. It is also believed that sensitisation is occurring after absorption by the singlet state followed by intersystem crossing to the triplet \( ^{3}n,\pi^{*} \) localised around the C=O moiety and eventually energy transfer to the metal ion. As observed in the UV-vis spectra, the absorption band at 380 nm has intramolecular charge transfer (IMCT) character.\(^{107}\) When 9 is chelated to a metal, the electron donating ability of the phenolate ion is vastly increased in comparison to the free phenol, as is the electron accepting capability of the C=O bond due to its lone pairs being attracted to the metal by the formation of the coordinate bond. These factors cause the UV-vis absorption to be "red" shifted from the 335 nm absorption in the free salicylaldehyde to 380 nm in the complexed form. As Eu\(^{3+}\) is easily, in comparison to other lanthanides, reduced to Eu\(^{2+}\), and is stabilised by having a half filled shell (\(f^{7}\)). By contrast, Eu\(^{2+}\) species will emit light proceeding electronic transitions within its excited state manifold but as its excited state consist of high energy 4f\(^{6}\)5d states, greater excitation energy is needed in comparison to Eu\(^{3+}\) and is therefore rarely observed unless excited specifically.\(^{122}\) It is postulated that the metal centre is
being reduced by the process of intramolecular charge transfer that arises from exciting the salicylaldehyde bound to the metal centre. In essence, the Eu$^{3+}$ ion is acting as an electron sink for the charge transfer process arising from complexation upon excitation into the IMCT band of Eu(9)$_3$. At lower temperatures, the rate of this deactivation is reduced and emission from the $^5$D$_0$ is increased. The change in lifetime in both methanol and acetonitrile upon cooling to 77K is vastly increased approximately by a factor of 10 indicates that this process of energy transfer is temperature controlled. The photophysical properties of Eu(9)$_3$ are shown in Table 11.

<table>
<thead>
<tr>
<th>medium</th>
<th>$\lambda_{\text{max}}$(nm)</th>
<th>$\epsilon$/ dm$^3$mol$^{-1}$cm$^{-1}$</th>
<th>$\lambda_{\text{ex}}$/nm</th>
<th>$\tau_{300K}$</th>
<th>$\tau_{77K}$</th>
<th>$\tau_{\text{deox}}^{300K}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Eu(9)$_3$</td>
<td>CH$_3$OH</td>
<td>377 (10000)</td>
<td>390</td>
<td>-</td>
<td>345</td>
<td>&lt;100</td>
</tr>
<tr>
<td></td>
<td>CH$_3$CN</td>
<td>372 (11810)</td>
<td>392</td>
<td>&lt;100</td>
<td>480</td>
<td>&lt;100</td>
</tr>
<tr>
<td></td>
<td>toluene</td>
<td>391 (1980)</td>
<td>392</td>
<td>&lt;100</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>KBr</td>
<td>-</td>
<td>392</td>
<td>&lt;100</td>
<td>490</td>
<td>-</td>
</tr>
</tbody>
</table>

Table 11: Photophysical properties of Eu(9)$_3$. Lifetimes are for the $^5$D$_0$→$^7$F$_2$ transition; Lifetimes in brackets are $\tau_1$ and $\tau_2$ calculated from Equation 21.

To fully examine the effect of thermal quenching arising from LMCT states in Eu(9)$_3$, measurement of emission lifetimes at higher temperatures would be useful. However, due to the very short lifetimes at temperatures above 260K, this is practically impossible without the use of a laser light source and was not possible to make such measurements in our laboratory.

Absorption and photophysical properties of Tb(9)$_3$
UV-vis absorption spectra of the Tb(9)$_3$ chelate dissolved in methanol showed a main absorption band 379 nm ($\epsilon$=6750 $\epsilon$/ dm$^3$mol$^{-1}$cm$^{-1}$) although a shoulder could be observed at 325 nm arising from the dissociated salicylaldehyde. The chelated species appeared to be relatively stable in solution over a period of a few hours, with very little
change in the ratio of absorbance peak at 379 nm. Monitoring the peak at 545nm showed the major excitation to be at 298 nm, it was noted that there was no peak at excitation 392nm as in the europium analogue (Figure 45). Excitation at this wavelength is of insufficient energy to sensitise terbium.\textsuperscript{113} The lack of structure observed in the emission spectra indicates a high degree of symmetry within the complex.

![Excitation and emission spectra](image)

*Figure 45*: Excitation and emission spectra of Tb(9)_3 in CH\textsubscript{3}OH at 10^{-4}M at room temperature; \( \lambda_{\text{ex}} = 298\text{nm} \); \( \lambda_{\text{em}} = 545\text{nm} \); excitation and emission slits, 5nm.

It is proposed that sensitisation is occurring through the higher energy aryl triplet and not the carbonyl moiety as in the europium analogue. The lifetime of the chelate in solution was found to be 1.07 ms and was mono-exponential. Upon standing for longer periods, it was noted that the lifetime consisted of 2 components with lifetimes of 0.87 ms and 1.01 ms, arising from the dissociation of the chelate. The instability of the chelate could also be observed by the formation of free salicylaldehyde band in the absorption spectra after standing for several hours. The photophysical properties of Tb(9)_3 are summarised in Table 12.
Table 12: Photophysical properties of Tb(9)₃ at 10⁻⁴ M in methanol and are recorded immediately after preparation of the solution. Lifetimes in milliseconds and are for the emission from the ⁵D₄→⁷F₅ transition.

<table>
<thead>
<tr>
<th></th>
<th>λ_max(nm), ε/dm³ mol⁻¹ cm⁻¹</th>
<th>λ_ex/nm</th>
<th>τ&lt;sub&gt;300K&lt;/sub&gt;</th>
<th>τ&lt;sub&gt;77K&lt;/sub&gt;</th>
<th>τ&lt;sub&gt;deo,77K&lt;/sub&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tb(9)₃</td>
<td>255 (14700), 379 (6750)</td>
<td>298</td>
<td>1.07</td>
<td>1.10</td>
<td>1.10</td>
</tr>
</tbody>
</table>

The lifetime of a freshly prepared methanolic solution measured at 77K was found to be the same order of magnitude as the lifetime at room temperature, but with a considerable increase in emission intensity, implying that there is some temperature dependence associated with the emission process. However this is deemed to be insignificant as the lifetime remains unchanged.

A degassed solution of Tb(9)₃ at 10⁻⁴ M in methanol also showed an increase in emission intensity coupled with a slight increase in lifetime. This is explained by some quenching of the sensitising group by oxygen.

Upon closer examination of the phosphorescent emission spectra of the Gd(9)₃ complex in methanol and solid KBr disc at 77K, a 0-0 transition at about 416 nm (24000 cm⁻¹) was observed when excited at the principal excitation wavelength associated with Tb(9)₃ (298nm). This emission band is of relatively weak intensity and is only observable under low resolution, i.e., excitation and emission slits at 5 nm. It is unlikely that the peaks observed is delayed fluorescence arising from back transfer from the triplet to the singlet state as they are still observed with a longer delay time and the relative position of the band is possibly at too low energy to be originating from an excited singlet (Figure 46).
Upon moving to polar aprotic solvents such as acetonitrile and dichloromethane, the resultant solutions were found to display weak sensitised terbium emission. This was postulated to be the complex forming non-luminescent clusters in solution rather than discrete 3:1 complexes. This effect has also been observed in many tris fluorinated β-diketonate lanthanide complexes.\textsuperscript{115,116} Also, Voloshin et al.,\textsuperscript{117} have reported that the luminescence of some samarium and terbium tris β-diketonates complexes are weakly luminescent in toluene and acetonitrile but luminescence was enhanced by the addition of water to the solution. It was believed that in such solution the chelate exists as a dimer and the addition of water favours the formation of a monomeric structure, increasing the luminescence properties. Addition of methanol to the solution of Tb(9)\textsubscript{3} in dichloromethane or acetonitrile did enhance the metal centred emission of the terbium chelate. No further work was carried out on this observation.

Photophysical analysis of Tb(9)\textsubscript{3} and Gd(9)\textsubscript{3} do not give a clear picture of the spectroscopic properties of the salicylaldehyde when bound to a lanthanide as there is significant uncertainty with regards to the composition of these materials and the species that are present in solution. Spectral analysis of Tb(45) would help to clarify the evidence obtained in this section.

\textbf{Figure 46:} Phosphorescence spectra of Gd(9)\textsubscript{3} in CH\textsubscript{3}OH; \(\lambda_{ex} = 298\text{nm}\); excitation and emission slits, 5nm.
Synthesis of Ln(41)₃

Synthesis of the 3:1 o-hydroxybenzophenone chelates with europium and gadolinium were carried out according to the literature procedure,¹⁰⁸ by stirring a 3:1 mixture of 41 with the lanthanide chloride in ethanol (Scheme 5). Precipitation of the complexes by ammonia gas followed by the addition of water yielded the chelates in 88% and 87% yields respectively. Elemental analysis of both chelates showed the presence of nitrogen, up to 1%, from incorporated ammonia. It was found that the residual ammonia was difficult to remove even under high vacuum and elevated temperatures. Both complexes were high melting solids but did not exhibit a discrete melting point, instead decomposing through a range of 200-300°C. The terbium complex was not synthesised as Crosby¹⁰⁸ postulated the triplet energy of Gd(41)₃ was postulated to be 17400 cm⁻¹, which is significantly lower in energy than the emissive ⁵D₄ level of terbium (20400 cm⁻¹).

Absorption and photophysical properties of Eu(41)₃

As with the salicylaldehyde complexes, UV-vis absorption and luminescence spectra were recorded in solutions with a concentration at 10⁻⁴M in an attempt to avoid ligand dissociation.
Eu(41)₃ in methanol showed a similar UV-vis absorption spectrum to that of the salicylaldehyde complexes i.e., the absorbance at 335 nm arising from the free o-hydroxybenzophenone shifted to 385 nm upon complexation with the metal. Monitoring the peak at 615nm of the solution showed excitation peaks at 298 nm and 390 nm. Emission spectra exciting at 390 nm showed typical metal centred emission, albeit very weak. Upon cooling the methanolic solution to 77K, the lifetime increased to 500 µs and fitted well to a single exponential decay. The emission intensity also increased by 3 orders of magnitude. It was noted, however, that over a relatively short period of time (hours) at room temperature, that the complex dissociates in solution. This was shown by the absorption spectra with the disappearance of the band at 385 nm and the reappearance of the band at 335nm that corresponded to the free o-hydroxybenzophenone. With the dissociation of the 3:1 complexed species, the luminescent intensity increased when exciting at 390 nm (Figure 47). The lifetime measured for the solution measured after 2 hours of standing was estimated to be 0.5ms, but displayed a degree of non-exponential decay and was of relatively weak intensity.

![Emission spectra](image)

**Figure 47**: Emission spectra of a 10⁻⁴M methanolic solution of Eu(41)₃ at room temperature over a period of 2 hours; \( \lambda_{ex} = 390\)nm; \( \lambda_{em} = 615\)nm, excitation and emission slits, 15nm. Emission intensity is increasing over time.
Degassing of this solution gave rise to an increase in emission intensity but no change in lifetime. It is proposed that, as observed with the samarium chelate investigated by Crosby and co-workers,\textsuperscript{108} Eu(41)\textsubscript{3} is non or weakly luminescent at room temperature, and it is in fact the dissociation complexes, Eu(41)\textsubscript{2}\textsuperscript{1+} and Eu(41)\textsubscript{1}\textsuperscript{2+}, that are luminescent at room temperature. Due to the poor stability of the complex and poor emissive properties of Eu(41)\textsubscript{3} in solution, solid state measurements in a KBr disc were carried out. In a mull, the chelate showed very strong sensitised emission when excited at 390 nm. However, this type of experiment can only be used as a qualitative measure and will not be absolute as the concentration is not known. The lifetime was measured and found to be very short (<100\,\mu s). Upon cooling the disc to 77K, the emission intensity increased considerably to the point where an emission spectrum could only be obtained with very narrow slit widths, a short gate and a longer delay time. A lifetime measurement of the chelate in these conditions showed an extended lifetime to 590 \,\mu s that displayed mono-exponential behaviour. Lifetime measurements at various temperatures ranging from 77K to 285K allowed the calculation of an approximate activation energy for this temperature dependence. At temperatures above 180K, bi-exponential decay was observed but the radiative decay plot could be fitted to Equation 21 as previously with the Eu(9)\textsubscript{3} chelate. Both lifetimes (\(\tau_1\) and \(\tau_2\)) displayed temperature dependence. Again, as with the salicylaldehyde complex, at reduced temperatures up to 180K, very little temperature dependence was observed and lifetimes were used from a range of 220K–275K, where the lifetime changed significantly with increasing temperature.

A plot of \(\ln[(1/\tau)-(1/\tau^0)]\) versus \(1/T\) from a range of 220K to 275K gives a straight line plot for both \(\tau_1\) and \(\tau_2\) with an activation energy of approximately 1100cm\textsuperscript{-1}, obtained from the slope of the line (Figure 48).
Figure 48: Temperature dependence of the observed lifetimes of $^5D_0 \rightarrow ^7F_2$ transition in Eu(41)$_3$ in a KBr disc. Experimental lifetimes $\tau_1$ (○) and $\tau_2$ (■) are plotted against $1/T$ according to the Equation 8.

As Eu(41)$_3$ has been shown to have very poor stability in solution, the phosphorescence spectra of Gd(41)$_3$ was recorded at 77K in a KBr disc. Excitation of the solid sample at 392 nm at this temperature gave a very intense phosphorescence spectrum with the onset of emission at 500nm, which places the triplet at approximately 20000cm$^{-1}$. Phosphorescence spectra recorded in methanol under the same conditions showed considerably weaker phosphorescence emission but the onset of emission was at a similar wavelength and the triplet postulated to be of the same energy under these conditions. The triplet energy found for Gd(41)$_3$ is of too low an energy to be able to sensitise Tb$^{3+}$ as the $^5D_4$ emissive level of terbium lies at 20400cm$^{-1}$. The value obtained for the triplet is in contradiction to the value obtained by Crosby et al.,$^{108}$ who placed the triplet to be at 17400 cm$^{-1}$. Kleinerman postulated the triplet of 41 to be at 19500 cm$^{-1}$ from the phosphorescence spectra of its gadolinium chelate in ethanol at 77K.$^{10}$ No evidence is given as to where the 0-0 phonon transition in the phosphorescence emission is taken from in either case.
Triazacyclononane appended ligands

Having established that salicylaldehyde will sensitise both terbium and europium whilst o-hydroxybenzophenone will only sensitise europium, the problem of complex stability was addressed. It was believed that this could be solved by appending the chromophoric groups to an aza-crown ether backbone by the aromatic Männich reaction. 1, 4, 7-Triazacyclononane was chosen as the backbone as it will render the complex charge neutral and will contain 3 chromophoric groups as in the 3:1 complexes. For condensations involving aromatic compounds, its is necessary to use compounds with substituents that are para to the phenol moiety so as to prevent the formation of linkage isomers as by products. Therefore, 5-methyl salicylaldehyde 47 and 5-methyl-2-hydroxybenzophenone 48 were used in this reaction.

\[ \text{HO} \quad \text{HO} \]
\[ \text{47} \quad \text{48} \]

Synthesis of TACN

The synthesis of TACN was carried out according to the method by Atkins et al., which is shown in Scheme 6. N, N', N''-tris(p-tolylsulphonyl) diethylenetriamine-N, N''-disodium salt 51 was prepared from 50 in methanol by the addition of sodium metal under nitrogen. The tosylated macrocycle 54 was prepared from 51 and the tosylated ester of ethylene glycol 53 in DMF. 1, 4, 7-Triazacyclononane 55 was obtained by refluxing the tosylated macrocycle 54 with 98% sulphuric acid, followed by the slow addition of 48% hydrobromic acid and then treatment with aqueous sodium hydroxide. According to the literature method, the aza-crown ether was isolated from the aqueous layer by azeotrope with toluene to remove the water. This was deemed impractical as the free base 55 has a boiling point of 129°C and may be removed with the aqueous toluene azeotrope. Once the hydrobromide salt of 55 had been treated with saturated sodium hydroxide solution, the free base was extracted exhaustively from the aqueous
layer with chloroform, dried over sodium sulphate and the solvent removed under reduced pressure. The aza-crown ether 55 was isolated in a much higher yield than compared to azeotropic treatment.

\begin{align*}
\text{H}_2\text{N} & \quad \text{TsHN} \\
\text{HN} & \quad \text{TsN} \\
\text{H}_2\text{N} & \quad \text{TsHN}' \\
49 & \quad 50 (71\%) \\
\text{TsNaN} & \quad \text{TsN} \\
50 & \quad 51 (82\%) \\
\text{TsN} & \quad \text{TsNaN} \\
\text{Ts} & \quad 53 (54\%)
\end{align*}

\textbf{Scheme 6:} (i) CH\textsubscript{3}C\textsubscript{6}H\textsubscript{4}SO\textsubscript{2}Cl, (Et)\textsubscript{2}O, H\textsubscript{2}O; (ii) Na, C\textsubscript{2}H\textsubscript{5}OH; (iii) (C\textsubscript{2}H\textsubscript{5})\textsubscript{3}N, CH\textsubscript{3}C\textsubscript{6}H\textsubscript{4}SO\textsubscript{2}Cl, CH\textsubscript{2}Cl\textsubscript{2}; (iv) DMF, 110° C; (v) 98% H\textsubscript{2}SO\textsubscript{4}, 100° C, 48% HBr, satd. aq. NaOH.

The substituted salicylaldehyde 47 was synthesised from p-cresol 56, tin (IV) chloride, tri-N-butylamine and paraformaldehyde by refluxing in anhydrous toluene (\textit{Scheme 7}). The compound 47 was obtained after purification by steam distillation and column chromatography in a low yield. 5-Methyl-2-hydroxybenzophenone 48 was purchased from Sigma-Aldrich.
The aromatic Mannich reaction has been reported for a number of compounds using secondary amines, aqueous paraformaldehyde and the alkylating reagent by refluxing in methanol. However, the method that was most successful, for both the \( \text{o-hydroxybenzophenone} \) and salicylaldehyde was to reflux 55 with a large excess of paraformaldehyde and the alkylating agent in acetonitrile (Scheme 8). Molecular sieves in a Soxhlet funnel fitted onto the reaction flask were employed to remove any water from the reaction mixture.

Initial attempts to complex 45 with terbium by the method employed for the 3:1 complexes were unsuccessful and yielded mainly the unreacted ligand by mass spectral analysis. After further experimentation, it was apparent that 1:1 complex formation was an extremely slow process. Eventually, a concentrated solution containing the ligand in
methanol ($1 \times 10^{-2}$ mol dm$^{-3}$) and aqueous sodium hydroxide (3 molar equivalents) was stirred at room temperature followed by the addition of 1 molar equivalent of terbium chloride hexahydrate (Scheme 8). The resulting solution was refluxed whilst monitoring any changes in the absorption and luminescence properties. After 17 days at reflux, when equilibrium had been reached as observed with the luminescence of the solution, the solvent was removed in vacuo and the residue redissolved in ethanol and the complex precipitated out by the slow diffusion of ether. The isolated material was characterised by high-resolution mass spectrometry. Tb(45) was found to be sparingly soluble in water and therefore its luminescence properties were recorded in methanol.

**Absorption and photophysical properties of Tb(45)**

The UV-vis spectrum of Tb(45) showed the same absorption peaks as the 3:1 chelate in the way that a red shift was apparent, arising from the metal coordinated species. Tb(45) showed a greater degree of stability in solution when compared to Tb(9)$_3$ and was found to be stable over days as observed with the absorption spectra.

Excitation monitoring the terbium centred peak at 545 nm of a $10^{-5}$M solution in methanol showed two main peaks at 307 nm, extending to 332 nm. The emission spectra of this solution showed typical metal centred luminescence when exciting at either 307 nm or 332 nm (Figure 49).
Figure 49: Excitation and emission spectra of Tb(45) at 10⁻⁴M in methanol; λ_ex = 307nm; λ_em = 545nm; excitation and emission slits, 5nm.

A ln I versus t plot monitoring emission from the ⁵D₄→⁷F₅ transition exciting at either wavelength was found to show non-exponential decay, with the longer lived species having a lifetime of 0.51 ms. Upon changing to deuterated methanol, the lifetime increased to 0.68 ms, but was again shown to have a degree of non-exponential decay although it was not as pronounced as in methanol. Using Equation 2 (Chapter 1), the number of bound solvent molecules (q) was found to be 4.5, which is a unusually high value for a potentially 9 co-ordinate complex and due to the uncertainty of the measurements, this cannot give an accurate picture of the solvent interactions. From this we can assume that Tb(45) is not 9-coordinate and there is a high degree of solvent coordination when in solution. Degassing the methanol solution resulted in an increased emission intensity and lifetime, increasing to 1.01 ms and was a single component. The lifetime of the solution as a rigid matrix at 77K was found to be of a single component with a value of 1.01 ms, indicating there is some temperature dependence associated with this complex. The lifetime at reduced temperature is still very short in comparison to other terbium chelates, implying that energy transfer is not that efficient, even at reduced temperature. The photophysical properties of Tb(45) are summarised in Table 13.
Table 13: Photophysical properties of Tb(45) in CH$_3$OH at $10^{-5}$M. Lifetimes in milliseconds and are for the emission of the $^5D_4 \rightarrow ^7F_5$ transition. Lifetime in brackets were recorded in CD$_3$OD.

The quantum yield of Tb(45) in methanol relative to $[\text{Tb}(22)]^{3+}$ was determined as previously described in Chapter 3. Methanolic solutions of Tb(45) were prepared which had absorbencies of 0.068, 0.079 and 0.109 at 300nm as well as aqueous solutions of $[\text{Tb}(22)]^{3+}$ with the same absorbencies. A plot of the total integrated emission (E) against absorbance (A) gives linear plots whose slope (E/A) can be used in Equation 20 to calculate the relative quantum yield.

$$\phi_x = \phi_r \cdot \left( \frac{C_r \cdot \text{slope}_x}{C_z \cdot \text{slope}_r} \right) \cdot \left( \frac{n_r}{n_x} \right)^2 \quad \text{Equation 20}$$

The correction factor for Tb(45) was found to be 1.22 (Equation 18), calculated from its luminescent lifetime in methanol at 300K (0.51ms). The quantum yield of Tb(45) relative to $[\text{Tb}(22)]^{3+}$ was found to be 0.016.

Although Tb(45) was insoluble in water, it could be solubilised by the addition of a few drops of DMSO added to the stock solution. Measurements of Tb(45) in aqueous solutions at $10^{-5}$M prepared in this manner showed decreased lifetimes and emission intensity in comparison to measurements made in methanol. Upon standing of these aqueous solutions over a period of about 2 hours, the emission arising from the $^5D_4$ state almost completely disappeared, indicating the complex was not stable in aqueous solutions.
Tb(45) was found to be very soluble in n-octanol and other fat-like solvents such as dichloromethane and acetonitrile, but as it is inherently unstable in water, partitioning experiments were not carried out.

**Synthesis of Eu(45) and Eu(46)**

As it was noted that the Tb(45) complex appeared to form a luminescent species over a long period of time under reflux conditions, the europium complexes of 45 and 46 were synthesised in the same way, in 50% and 27% yields, respectively (Scheme 8). From initial experiments with Eu(9)₃ and Eu(41)₃, Eu(45) and Eu(46) complexes were not expected to be luminescent at room temperature. The complexes isolated were characterised by high resolution mass spectroscopy.

**Absorption and photophysical properties of Eu(45)**

At room temperature, this complex showed very weak sensitised emission in both methanol and acetonitrile at room temperature but showed strong emission properties at 77K when excited in the region of 390-400nm, with lifetimes of 0.38ms and 0.39ms, respectively.

Degassing of both solutions at room temperature made little difference to emission intensity or lifetime. A temperature dependent activation energy could not calculated for Eu(45) as the lifetimes at temperatures above 77K were found to be too short to measure accurately with the lifetime less than 100µs at 180K, giving a very narrow temperature range for the calculation of a temperature dependent activation energy. Initial experiments showed a high degree of error and lifetimes could not be determined accurately at temperatures above 77K due to non-exponential decay.

**Absorption and photophysical properties of Eu(46)**

As with Tb(45), emission intensity appeared to increase over a 3 week reaction period and levelled off after this time, but was still very weak. It was noted that the lifetime throughout the reaction period remained constant and was very short indeed (0.2ms).
When a solution of Eu(46) in methanol at $10^{-5}$M was excited at 392 nm, as for the 3:1 complex, showed very weak sensitised lanthanide emission resulted. Degassing of the solution showed no change in emission intensity. Upon cooling the solution to 77K, the emission intensity increased by 3 orders of magnitude, with the main excitation peak having a maximum at 390-405 nm but extends over 450 nm. The luminescent lifetime was found to be 0.77 ms and was a single exponential.

Upon moving to acetonitrile, the complex again was very weakly luminescent but cooling to 77K produced a marked increase in emission intensity, with the lifetime being 0.78 ms. This temperature dependence is consistent with the results obtained with the analogous 3:1 complex. The lifetime of Eu(46) in methanol appeared to be more dependent on temperature than the corresponding 3:1 complex, with the lifetime being below 100 μs at temperatures above 210K. In $I$ versus $t$ plots at reduced temperatures showed mono-exponential decay and fitted well to a single exponential decay ($R^2 > 0.99$) for all temperatures measured. The activation energy calculated from the slope of $\ln[(1/\tau)-(1/\tau^0)]$ versus $1/T$, according to Equation 8, was found to be 1060 cm$^{-1}$ (Figure 50).

![Figure 50: The temperature dependence of the $^5D_0 \rightarrow ^7F_2$ transition of Eu(46) in methanol at $10^{-5}$M; $\lambda_{ex}=392$ nm; $\lambda_{em}=615$ nm.](image)

Complexes based upon the aza-crown backbone i.e., Eu(45), Tb(45) and Eu(46), appeared to have a greater degree of stability in solution in comparison to their
analogous intramolecular chelates but took a long period of time to form discrete 1:1 complexes.

Discussion on metal centred emission of Eu and Tb chelates

The discussion of all mixed carbonyl/phenol sensitising ligands will be discussed in the same section as their photophysical properties were found to be very similar regarding the intra and inter molecular complexes but only differ in stability when in solution.

It is believed that the different excitation wavelength of europium and terbium salicylaldehyde complexes arises from the different chromophores that are present in salicylaldehyde. For the europium analogue, it is believed that sensitisation is occurring through the low energy carbonyl moiety (hence longer wavelength) and is of strong LMCT character. Carbonyl compounds typically exhibit efficient intersystem crossing, which approaches unity and consequently have a very low fluorescence quantum yield. Excitation of the terbium analogues, Tb(9)$_3$ and Tb(45), occurs at shorter wavelengths and higher energy (i.e. 298, 307 and 332nm) which is postulated to be sensitisation through an aryl triplet arising from the benzene ring. Further evidence for this can be shown in the phosphorescence emission spectra of the Gd(9)$_3$ when excited at the two wavelengths (298nm and 392nm), which yields two different triplet levels ($24000\text{cm}^{-1}$ and $19100-19200\text{cm}^{-1}$, respectively). Excitation at 298 nm in either acetonitrile, methanol or in solid state yields a phosphorescence spectra that is similar when the excitation wavelength is at 392 nm, but shows the onset of emission to be at shorter wavelengths when examined under lower resolution. This low intensity can be seen as the relative inefficiency of the benzene moiety to undergo intersystem crossing from the excited singlet state to the excited triplet state. The poor singlet-triplet conversion may explain the relatively low quantum yield associated with the Tb(45) complex. Energy transfer will occur in a molecule that possess two independently absorbing but formally conjugated chromophores and the lowest excited state of that molecule are determined by the chromophore moiety possessing the lowest excitation energy.$^{120}$ In other words, the excitation energy will be passed on from one excited state to another until the lowest singlet or triplet state is reached. The proposed energy transfer process involved in both
the terbium and europium complexes investigated in this chapter is shown in Figure 51. It is believed that in the salicylaldehyde complexes, light is absorbed by the chromophore and passes to the benzene triplet \( ^3\pi,\pi^* (T_2) \). From this state, the energy passes from the \( ^3\pi,\pi^* (T_2) \) of the benzene to the lower lying \( ^3n,\pi^* (T_1) \) triplet state of the C=O moiety, for which quantum efficiency approaches unity for carbonyl compounds. In the terbium complexes, energy transfer from the benzene \( ^3\pi,\pi^* (T_2) \) to the \( ^3n,\pi^* (T_1) \) of the carbonyl is not completely efficient. The \( ^5D_4 \) level of terbium (20400 cm\(^{-1}\)) lies between to that of the benzene \( ^3\pi,\pi^* (T_2) \) triplet and carbonyl \( ^3n,\pi^* \) triplet \( (T_1) \) and therefore is able to accept this energy, leading to sensitised emission.

![Figure 51: Proposed energy transfer in Eu(9)\(_3\) and Tb(9)\(_3\).](image)

**Discussion on the temperature dependence of europium centred emission**

This leads onto the emissive properties of both the \( \alpha \)-hydroxybenzophenone and salicylaldehyde complexes of europium being temperature dependent. As previously stated, the UV absorption spectra of both sets of complexes display IMCT character when bound to the metal in comparison with the free chelates, which shows the absorption band following a "red" shift. The lowest triplet levels of Gd(9)\(_3\) and Gd(41)\(_3\) have been estimated to be at 19100-19200 cm\(^{-1}\) and 20000cm\(^{-1}\) from their phosphorescence spectra at 77K, respectively. The relative positions of these energies
implies that there could be thermal repopulation of the aryl triplet by back energy transfer but no change in lifetime is observed when degassing of the chelates in solution, indicating that energy transfer from the triplet is fast. Upon chelation to the metal centre, with Tb or Eu, the electron donor capability of the phenol and the electron accepting ability of the carbonyl group are both increased. Excitation into this absorption band (370-398nm) leads to very weak metal centred emission at room temperature for europium and terbium. For the latter is it simply because the excitation wavelength is of too low in energy for efficient excitation to occur as the emissive level of Tb lies at approximately 20400 cm\(^{-1}\). Upon cooling down solution or solid samples of the europium complexes lead to a much longer lived lifetimes and increased emission intensity that is of 2-3 orders of magnitude greater. This indicates that at reduced temperature, the \(5D_0\) emissive level is becoming more populated and that any deactivation mechanisms are significantly reduced. Activation energies obtained in the solid state from Eu\((9)\)_3 and Eu\((41)\)_3 so as to avoid complex dissociation from the lifetimes at variable temperature shows that the activation energy for both complexes is approximately 1380 cm\(^{-1}\) and 1100 cm\(^{-1}\). Activation energies obtained for Eu\((45)\) and Eu\((46)\) were discarded due to the non-exponential decay, irreproducibility and shorter lifetime at temperatures above 77K in comparison to their analogous intra-molecular chelates. Assuming that we are exciting the complex into a ligand absorption band and that energy transfer to the emissive metal centre is occurring by the mechanism stated in Chapter 1, the emissive \(5D_0\) level of the europium ion is more populated at reduced temperatures than at room temperature. At room temperature, a quenching mechanism is efficiently deactivating metal centred luminescence from the \(5D_0\) emissive level of europium. The temperature dependence of emission is likely to be a combination of effects, such as thermal quenching of the \(5D_0\) state by the \(5D_1\) state,\(^{121}\) but the main effect is postulated to be the presence of ligand to metal charge transfer states.

Charge transfer states will occur in lanthanide compounds in addition to their discrete 4f levels by an electron from the surrounding anions being promoted to the 4f orbit of the central lanthanide ion giving rise to the so-called charge transfer state.\(^{122}\) The position of this energy band depends on the nature of the surrounding anion or donor group. The
factor that determines the energetically lowest band of a charge transfer state is that half-filled f shells are very stable. For example, Eu$^{3+}$ (4f$^6$) will readily accept an electron to become Eu$^{2+}$ (4f$^7$, half-filled) and therefore the charge transfer states will occur at relatively low energy. In comparison to Gd$^{3+}$ (4f$^7$, half-filled), the 4f shell will require high energy to release an electron and therefore Gd$^{3+}$ is very stable. LMCT states are very difficult to ascribe by direct means, such as absorption or emission spectroscopy, but have been detected and assigned for a few lanthanide complexes. Due to these difficulties in assignment of LMCT states, usually they are claimed to exist in order to explain low quantum yields or a strong temperature dependence of luminescence.

Rightly or wrongly, LMCT has been used to explain the lack of europium centred luminescence of a number of complexes that are based on phenol donors, such as calixarenes. Shinkai and co-workers$^{123}$ investigated a number of substituted calixarenes when it was observed that simple calixarenes complexed with Eu$^{3+}$ were found to be non-emissive. Upon tetra-substitution of phenacyl and piperinoyl substituents on the phenol oxygens, more efficient sensitisation of europium was observed. This was ascribed to the lower energy triplet of the substituted sensitising groups bypassing the charge transfer states. Energy transfer from the phenol triplet was in essence being quenched by the presence of the CT band at lower energy to the aryl triplet. An alternative explanation is that the position of the lowest lying triplet of the phenol was possibly too high in energy (ca 28000 cm$^{-1}$) to prevent reasonable overlap of the two levels, therefore making energy transfer a non-efficient process. No evidence was provided to the relative positions of the triplet states in the functionalised calixarenes under investigation by means of phosphorescence emission spectra.

The thermal deactivation of europium emission has not been addressed much in the literature with regards to sensitised emission but has been addressed for compounds such as Eu(NO)$_3$ and Eu(ClO$_4$)$_3$, where thermal quenching was attributed to the depopulation of the emitting $^5$D$_0$ excited state via the upper $^5$D$_1$ excited state.$^{121}$ The lifetime of the $^5$D$_1$ state is of the order $10^{-6}$s and the rate constant for the process
$^5D_1 \rightarrow ^5D_0$ is 100s$^{-1}$, thermal population of the $^5D_1$ from the $^5D_0$ produces a shortening of the lifetime of the latter.\textsuperscript{121} The $\Delta E$ values that were obtained range from 1700cm$^{-1}$ and 2500cm$^{-1}$ which are in reasonable agreement with the energy gap between the $^5D_1$ and the $^5D_0$ levels (1740cm$^{-1}$). When the ion is complexed by macrocyclic or polydentate ligands, the thermal quenching process appears to operate via a different mechanism. Many europium macrocycles have shown intramolecular quenching that has been attributed to the thermal population of ligand to metal charge transfer states from the emissive $^5D_0$ state.\textsuperscript{124,125} Little has been considered of the photochemical reduction of Eu$^{3+}$ being a thermally governed process, even though the charge-transfer process was said to be responsible for the thermal quenching of Eu(thd)$_3$ (thd = 2, 2, 6, 6-tetramethyl-3, 5-heptanedionate).\textsuperscript{126} It was observed that the metal centred luminescence of the complex Eu(thd)$_3$ was strongly quenched in the region of room temperature and that thermal quenching was occurring through the crossover from the $^5D_0$ to the ligand to metal charge transfer state. Activation energies calculated from an Arrhenius type equation yielded a value of 5700 cm$^{-1}$ and did appear to have any relation to the position of the sensitisier triplet to the $^5D_0$ emissive level. The triplet state lying at least 8000 cm$^{-1}$ above the $^5D_0$ level, thus precluding thermally activated back energy transfer.\textsuperscript{127}

In a similar study carried out by Capparelli et al.,\textsuperscript{128} the luminescent lifetimes of Eu(fod)$_3$ (1, 1, 2, 2, 3, 3-heptafluoro-7, 7-dimethyl-4, 6-octandionato) were measured in carbon tetrachloride, benzene and acetonitrile at temperatures between 5°C and 75°C. LMCT involving Eu$^{2+}$ formation upon 300nm steady state irradiation of these Eu(fod)$_3$ solutions, besides the dependence of the difference in energy of the emitting $^5D_0$ and the upper $^5D_1$ level, was identified as the main deactivation mechanism in the thermal quenching of Eu$^{3+}$ emission.

Ligand and LMCT absorptions are not usually observed in the Eu$^{3+}$ excitation spectra because of the efficient non-radiative processes arising from the mixing of the LMCT state and the $^7F_j$ (Eu) state configuration. Extinction coefficients of these absorptions are relatively low and to the order of $10^2$ because of the small overlap between the donor ligand orbitals and the 4f metal orbitals.\textsuperscript{129} However, if the LMCT states lie at
higher energy than that of the $^7F$ states, the mixing is considerably reduced which allows the relative position of the LMCT bands to be identified. It was shown that, with the europium complex of $p$-tert-butyl calix-[4]-areneteraamide 57, the LMCT band was appearing as a shoulder around 300 nm on the absorption spectra ($E = 29400 \text{ cm}^{-1}$).\textsuperscript{130} The low quantum yield upon excitation at 300 nm of $[\text{Eu}(57)]^{3+}$ ($\phi = 2\times 10^{-4}$) was attributed to the presence of LMCT states, but it is difficult to be confident of such an assignment due to the relatively low extinction coefficient of such bands ($\varepsilon = 100 \text{ dm}^3\text{mol}^{-1}\text{cm}^{-1}$) and the predominance of more intense ligand absorptions “masking” these bands. The triplet level of this ligand was determined from the gadolinium complex and found to be 27 000 cm$^{-1}$, from this it can be assumed that energy is being transferred from the $^1\pi\pi^*$, through the $^3\pi\pi^*$ and the $^5D_0$ emitting state, as they are likely to be the most favourable process. The emissive properties of this complex were recorded at 4.2K in the solid state under high resolution.\textsuperscript{131} It was observed in the excitation spectra that a broad band from 300-400 nm which was ascribed to be the charge transfer transition between the C=O groups and the central Eu$^{3+}$ ion. When compared to the cryptate $[\text{Euc}(58)]^{3+}$, which also displays low lying charge transfer bands into which the complex can be excited, it was found that the relative quantum yields an emission intensities were greater for $[\text{Eu}(57)]^{3+}$ upon excitation into the CT bands. The significant difference in spectral properties of the two complexes was ascribed to the varying CT offset in the two complexes, with the offset in cryptate $[\text{Euc}(58)]^{3+}$ being greater that the $[\text{Eu}(57)]^{3+}$. 

![Chemical Structures](image)
Concluding remarks

We have revisited the 3:1 complexes of salicylaldehyde and o-hydroxybenzophenone as investigated by Weissman and Crosby and established triplet energies for these sensitisers when complexed with gadolinium. The complexes obtained show poor stability in solution. We have shown that the luminescence of the europium complexes is subject to a temperature dependence and that the lack of metal centred emission at room temperature is insensitive to oxygen, indicating initially that thermally populated back energy transfer is not the primary source of deactivation. Back energy transfer from the emissive $^5D_0$ level to the triplet is, however, possible with the proposed triplet energies found in this study. The relatively short lifetimes found for the 3:1 complexes made full photophysical studies on these complexes difficult with regard to the instrumentation at our disposal and that luminescence lifetime could only be measured accurately at values above 100μs. Activation energies obtained for Eu(9)$_3$ in acetonitrile solution are subject to a degree of uncertainty as the complex may be dissociating in solution throughout the experiment time. Solid state measurements of Eu(9)$_3$ and Eu(41)$_3$ in KBr discs provide a useful guideline to the thermal dependence of the complexes and indicate that the overall process is close in energy to that of the $^5D_0$ emissive level, some 1100-1380cm$^{-1}$, as indicated by the calculated $E_a$ values. The quenching of metal centred luminescence is readily achieved in Eu(9)$_3$ and Eu(41)$_3$ as shown by the low activation energies. However, the precise mechanism of charge transfer or energy transfer cannot be determined with the experimental data obtained. The triplets are of suitable energy to populate the $^5D_0$ emissive level, which could occur through energy transfer from the $^5D_1$ state (ca 19000 cm$^{-1}$) and subsequent relaxation to the $^5D_0$ level, although $^5D_1\rightarrow^7F_0$ transition will be of weak intensity and was not observed in our case. Evidence that there is charge transfer involved is given by the UV-vis absorption spectra of all mixed carbonyl/phenol donor systems. Upon complexation to the lanthanide ion, the absorption band arising from the carbonyl is red shifted up to 380-390nm, and is caused by intra molecular charge transfer. Further studies such as cyclic voltammetry would support the statements regarding LMCT associated with the europium complexes investigated.
Results and Discussion

Chapter 5
Chapter 5

Mixed Pyridine-Phenol donors as Sensitisers of Europium and Terbium

With the overall aim of this project being to design charge neutral luminescent lanthanide complexes that are fat soluble and that can be loaded into cells, we have investigated the use of mixed pyridine-phenol donor sets in order to move away from traditional carboxylate donors. Carboxylate donors tend to hydrate in an aqueous media and therefore may hinder the transport of the complex across the cell membrane.

We intended to develop complexes utilising an analogue of compound 59 that has been previously reported as a sensitisier of terbium. 133

\[
\text{\[O\hspace{1em}N\hspace{1em}OH\hspace{1em}59\]}
\]

Ward and co-workers investigated the photophysical and structural properties of complexes of 59 with Gd and Tb. It was found that the reaction of the ligand with the appropriate lanthanide salt in aqueous solution with potassium hydroxide resulted in the slow formation of their complexes as microcrystalline solids. It was found that the complexes obtained had a formulation of K[Ln(59)_2].4H_2O, which was consistent with the obtained microanalysis and subsequently confirmed by crystallography.

Excitation of a methanolic solution of the terbium complex into a ligand centred $\pi-\pi^*$ transitions at 266 nm showed typical metal centred emission arising from the $^5D_4 \rightarrow ^7F_j$ transitions, with the lifetime of the $^5D_4 \rightarrow ^7F_5$ transition being 0.81 ms. Upon changing to CD_3OD, the lifetime was increased to 1.61 ms and using Horrock’s equation (Equation 2), the q value was calculated to be 4.9 ±0.5. Measurements in H_2O and D_2O
showed very little luminescence and were deemed unsuccessful due to the apparent instability of the complex in water.

The q value obtained was unexpectedly high and was attributed to two reasons. The first being the effect of solvent oscillations in the second coordination sphere arising from the anion $\text{Tb(59)(H}_2\text{O)}_2^-$ present in solution being able to hydrogen bond to methanol molecules via the non-coordinated carboxylate oxygen atoms. The second reason is the partial dissociation of the complex in solution through the phenolate anion.

Choice of sensitising ligand

Whilst utilising a known sensitiser 59, we aimed to develop a series of charge neutral, nonadentate europium and terbium chelates for potential use as luminescent markers in homogenous, time resolved fluorometric cellular assays. As stated previously, the sensitiser utilised will be a coupled pyrido-phenol moiety. The problem of stability that Ward encountered will be addressed by appending the chromophore to an aza-crown ether as previously investigated in Chapter 4. Initially, a TACN backbone will be utilised so as to establish the photophysical properties of the new sensitiser when complexed with the appropriate lanthanide ion.

Synthesis of ligand 60 and its lanthanide complexes

The synthesis of ligand 60 and its lanthanide complexes were achieved according to Schemes 9 and 10. Firstly, 2-amino picoline 61 was diazotized to give the 2-bromo-6-picoline 62, by treating the former with bromine, followed by sodium nitrite whilst
stirring in an aqueous hydrobromic acid solution. After neutralisation and then extraction of the crude material and purification by vacuum distillation produced 2-bromo-6-picoline 62 in a 50% yield. 2-(2-Methoxyphenyl)-6-methyl pyridine 66 was prepared by reacting 2-bromopicoline 62 with an excess of the Grignard reagent 64 prepared from 2-bromoanisole 63 in the presence of a cross coupling catalyst, [Ni(dppe)Cl₂] 65, where dppe is equivalent to 1, 2-bis(diphenylphosphino)ethane. After work up and purification of the crude material by column chromatography, the cross-coupled product was obtained in a 73% yield. Bromination of 66 to yield 67 using NBS in carbon tetrachloride with benzoyl peroxide as the free radical initiator did not proceed in a good yield (ca. 15%). This was due to a number of reasons, such as similar Rf values of the required mono-and bis-bromide compounds in comparison with the starting material, in all solvent systems used. Mono-bromination using NBS was also difficult to control. The alternative route taken was to synthesise the N-oxide 68 using aqueous hydrogen peroxide in glacial acetic acid followed by chlorination of the adjacent methyl. This could be achieved with a number of reagents such as phosphorus oxychloride and triethylamine as reported by Ash and co-workers on picoline N-oxide 69.134 This procedure requires the addition of both reagents to a solution containing the N-oxide in such a way that there is always an excess of the intermediate complex 70 (Figure 52). It was also reported that chlorination of the methyl group will only occur as a byproduct with the main product being 4-chloro-2-methyl picoline 73.

![Figure 52: Synthesis of 2-chloropicoline](image-url)
It has been reported that 2-picoline N-oxide 69 when reacted with p-toluenesulfonyl or benzenesulfonyl chloride in benzene at reflux will yield the chloro-methyl compound 71 in 90% and 72% yields, respectively.\textsuperscript{135,136} The reaction proceeds via initial association of the sulfonyl group with the N-oxide oxygen followed by nucleophilic attack of the chloride ion on the methylene carbon with expulsion of the aryl sulfonyl group. The mono-chloride 74 was synthesised by the reaction of p-toluenesulfonyl chloride with the N-oxide 68 in toluene. Although the overall yield was modest, this route proved more suitable than free radical bromination as it was easier to purify the chlorinated compound by column chromatography as the only compounds present in the reaction mixture are the polar N-oxide 68, tosyl chloride and product itself. All of the compounds have markedly different R\textsubscript{f} values making separation by column chromatography easier.

\begin{center}
\begin{tikzpicture}
\node (a) at (0,0) {61 \[\text{Br} \to \text{MeO}] 63 \[\text{NH}_2 \to \text{MeO}] 64 \[\text{MeO}] 62 (60\%) \[\text{MeO}] 66 (73\%) \[\text{MeO}] 68 (68\%) \[\text{MeO}] \]
\node (b) at (2,0) {\text{Br} \to \text{MeO} \[\text{MeO}] 63 \[\text{Br}] 64 \[\text{MeO}] 62 (60\%) \[\text{MeO}] 66 (73\%) \[\text{MeO}] 68 (68\%) \[\text{MeO}] \]
\end{tikzpicture}
\end{center}

\textit{Scheme 9}: (i) 48\% HBr (aq), Br\textsubscript{2}, NaNO\textsubscript{2}; (ii) Mg turnings, THF; (iii) 3\% [Ni(dppe)]Cl\textsubscript{2},THF, 0\degree C; (iv) NBS, CCl\textsubscript{4}, benzoyl peroxide (init), \Delta; (v) H\textsubscript{2}O\textsubscript{2}, glacial AcOH, \Delta; (vi) p-Toluenesulfonyl chloride, toluene, \Delta.

Alkylation of 1, 4, 7-triazacyclononane 55 with the mono-chloride 74 in the presence of triethylamine in acetonitrile afforded the substituted macrocycle 75 in near quantitative yield, with the reaction proceeding readily at room temperature. Deprotection of 75
with boron tribromide in anhydrous dichloromethane followed by treatment with aqueous tartaric acid to remove any complexed boron, yielded the phenol analogue 60.

Scheme 10: (i) 74, Et₃N, CH₃CN; (ii) BBr₃, DCM, -78°C, aqueous tartaric acid; (iii) Ln(CF₃SO₃)₃, MeOH, Δ.

Initially, solutions of ligand 60 and the appropriate lanthanide salt were made up in methanol at 10⁻⁶M from stock solutions of 10⁻⁴M and allowed to stand prior to measurement. It was noted that the ligand 60 strongly sensitised terbium, displayed a lifetime of 1.2 ms and was not a single exponential decay, indicating the presence of more than one emitting species in solution. The solution containing europium and the ligand at the same concentration did not show any sensitised emission.

The lanthanide complexes of ligand 60 (where Ln = Eu, Gd and Tb) were prepared by refluxing the ligand in methanol with a slight excess of the respective lanthanide (III)
triflate salts (Scheme 10). After evaporation of the solvent, the remaining residues were dissolved in the minimum amount of methanol and were left to crystallize from the solution by slow diffusion of diethyl ether. The complexes isolated were relatively high melting (with respect to the ligand), pale yellow solids. The salts were analyzed by FAB mass spectrometry and whilst [MH]$^+$ and [M+Na]$^+$ for both Eu(60) and Tb(60) were readily detected, Gd(60) showed a low abundance peak for [MH]$^+$ as well as a more intense cluster peak centred around m/z 1287. This peak corresponds to 60+Gd(CF3SO3)$_3$. Eu(60) and Tb(60) were also characterised by high resolution mass spectrometry.

**Photophysical properties of 60**

A solution of the ligand 60 in methanol showed absorption bands at 298 and 318 nm with extinction coefficients of 21540 and 24930 dm$^3$mol$^{-3}$cm$^{-1}$ respectively. Addition of a few drops of 2M aqueous sodium hydroxide to the solution made no apparent change in the absorption wavelength. The longer wavelength was not altered significantly in the UV-vis absorption spectra upon complexation to a lanthanide ion. Moreover, addition of a large excess of Ln salts did not induce a change in absorption properties.

The complexes isolated were found to be insoluble in water but soluble in solvents such as methanol, acetonitrile, chloroform and octanol.

**Photophysical properties of Eu(60)**

When a 10$^{-6}$M methanolic solution of Eu(60) was excited into the pyrido-phenol chromophore absorption band, no characteristic europium centred emission was observed. No metal centred emission was observed at higher concentration, low temperature or even degassed conditions. The absence of metal centred emission was postulated to be the presence of LMCT bands, as discussed in Chapter 4, which can facilitate the reduction of Eu$^{3+}$ to Eu$^{2+}$ without the emission of a photon. In this case, this deactivation mechanism appears to be unaffected by temperature.
Photophysical properties of Tb(60)

When a $10^{-6}$M methanolic solution of Tb(60) was excited into the chromophore absorption band, typical terbium metal centred emission was observed (Figure 53). Excitation of Tb(60) at 545nm revealed that the main excitation peak was at 337nm, some 20 nm red shifted from that of the complex absorption observed in the UV-vis absorption spectra. Excitation of the solution at 337nm gave a lifetime of 1.5 ms and was of single exponential decay. This indicates the presence of a single emitting species in solution and is in contrast to the value obtained in situ (1.2ms) at the same concentration. This indicates that there is some time dependency with regard to complex formation.

Upon moving to deuterated methanol, the lifetime is increased to 1.78ms, indicating that there is a degree of solvent interaction associated. Applying Equation 2 to these values, yields a $q$ value of 0.7, indicating the complex may not be 9 coordinate as first assumed. Interestingly, degassing the methanolic solution by 5 freeze/pump/thaw cycles increased emission intensity considerably and the lifetime increased to 2.1 ms, showing that the complex is subject to quenching by oxygen. Low temperature (77K) measurement of the complex in methanol and deuterated methanol lead to increased emission intensity.

*Figure 53*: Excitation and emission spectra of Tb(60) in methanol at $10^{-6}$M; $\lambda_{\text{ex}} = 337$nm; $\lambda_{\text{em}} = 545$nm; excitation and emission slits, 5nm.
and longer lifetimes, 2.1 and 2.3 ms, respectively (Table 14). The Tb(60) complex shows temperature dependence and sensitivity to oxygen implying that a thermally activated back energy transfer deactivation mechanism operates.

<table>
<thead>
<tr>
<th>Complex</th>
<th>$\tau_{300K}$</th>
<th>$\tau_{deox}$</th>
<th>$\tau_{77K}$</th>
<th>$\phi_{300K}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tb(60)</td>
<td>1.50 (1.78)</td>
<td>2.10</td>
<td>2.10 (2.3)</td>
<td>0.038(0.053)</td>
</tr>
</tbody>
</table>

Table 14: Spectroscopic properties of Tb(60) in CH$_3$OH at 10$^{-6}$M. $\lambda_{ex} = 337$nm. Lifetimes were recorded for the $^5$D$_4 \rightarrow ^7$F$_5$ transition (545nm). Values in brackets are recorded in CD$_3$OD. Quantum yields were determined relative to [Tbc(22)]$^{3+}$.

Measurement of the phosphorescence emission spectra of Gd(60) at 5x10$^{-5}$M in methanol (excitation at 337nm) showed the triplet to be at 23250cm$^{-1}$, taken from the onset of emission, which was at 430nm (Figure 54). This places the triplet at 2850cm$^{-1}$ above that of the emissive $^5$D$_4$ level, which is, in theory, high enough in energy so as to preclude the possibility of BET. The rate of energy transfer from the excited triplet to the $^5$D$_4$ emissive state of terbium must be slow so as to allow quenching by molecular oxygen. Determination of the triplet energy this way can only be used as an approximation as there is no fine structure appearing in the spectra and the 0-0 transition is taken as the onset of emission.
The quantum yield of Tb(60) in methanol and deuterated methanol was calculated relative to an aqueous solution of [Tbc(22)]^{2+} as previously described in Chapters 3 and 4 and using Equation 20, and have values of 0.038 and 0.053, respectively (Table 14). These values appear to be low but the presence of BET will reduce the energy transfer to the 5D_4 level from the triplet and C-H oscillations of the aza crown ring that will lie in close proximity will also provide an efficient deactivation mechanism. It was also noted that the fluorescence spectra Tb(60) shows emission that can be associated with the free ligand, implying that energy transfer to the triplet is not efficient. This could also contribute to a low comparative quantum yield.

Stability of Tb(60)

To assess the stability of Tb(60) under competition with another chelating agent, a solution of Tb (60) at 10^{-5}M in aqueous methanol was allowed to stand at room temperature with an equimolar amount of EDTA. The emission peak at 545 nm was monitored upon excitation at 337 nm in a time drive experiment over 12 hour period (Figure 55). A control of the complex in aqueous methanol without EDTA was ran in parallel. The plots in Figure 55 shows that the complex is not particularly stable towards EDTA at equimolar concentrations but is stable in aqueous methanolic solution.
Figure 55: Plot to show the emission intensity of the $^5{D_4} \rightarrow ^7{F_5}$ transition (545nm) of Tb(60) in aqueous methanol at $10^{-5}$M, $\lambda_{ex} = 337$nm. Blue trace corresponds to standard Tb(60) solution with no EDTA and the black trace corresponds to the Tb(60)-EDTA solution; excitation and emission slits, 3nm.

Fluorinated analogues of the pyrido-phenol sensitiser

It was believed that the use of fluorinated derivatives of the pyrido-phenol sensitiser would reduce the effect of LMCT and to improve europium centred luminescence. Fluorine substituents can have a number of effects, for example, the pKa of the phenol donor group will be significantly reduced in the presence of electron withdrawing groups, such as fluorine or trifluoromethyl, reducing the electron donor capability of the phenol and thus reducing LMCT deactivations. It has also been reported in the literature that the presence of covalently bound fluorine to a sensitiser when complexed with europium will raise the energy of the LMCT bands in comparison to its non-fluorinated analogue, making them less accessible and therefore enhance the luminescence properties.\textsuperscript{137}
The ligands 76 and 77 were proposed and their synthesis was approached in much the same manner as previously described for ligand 60.

The attempted synthesis of 76 is shown in Schemes 11 and 12. The anisole derivative for the cross coupling Grignard reaction is not commercially available, therefore $p$-trifluoromethylphenol 78 was ortho-brominated by the addition of bromine in acetic acid to give the phenol 79. The phenol 79 was methylated with methyl iodide in the presence of potassium carbonate in THF to yield the anisole 80. The anisole 80 was coupled to the pyridine moiety via a Grignard reaction with the cross coupling reaction as before, but with a larger excess of the Grignard reagent 81. It was found that the $R_f$ values for the cross-coupled compound was of a similar value to that of the bromopicoline 62 and were difficult to separate from each other by column chromatography. Initially, the compound 82 was brominated by refluxing with $N$-bromosuccinimide in carbon tetrachloride with benzoyl peroxide as the free radical initiator to give the mono-bromide 83 in a 42% yield, and was to be used in the subsequent alkylation of TACN 55.
Problems were encountered when reacting the bromide 83 with 55 in the presence of triethylamine, and compound 76 could only be obtained in a relatively low yield (38%). It was postulated that the alkylating agent 83 was reacting with the base, producing a quaternised salt but this compound could not be isolated from the reaction mixture. Using a more sterically hindered base such as Hunig's base or DBU, or a non-nucleophilic, inorganic base such as potassium carbonate did not improve the experimental yield. Upon further attempts of this simple alkylation with potassium carbonate as the base and adding the alkylating agent in equimolar portions over time, a single product by tlc was isolated from the reaction mixture and characterised by $^1$H-NMR. It is proposed, under these conditions, that the alkylating agent was reacting with itself to form the dimer 86.
It was found that alkylation of TACN to produce the methoxy protected ligand 87 could be achieved in higher yield by using the chloride derivative as previously used in the synthesis of ligand 60, and this reaction proceeded without quaternisation of the alkylating agent. Chlorides are typically poorer leaving groups with respect to nucleophilic attack and the use of the more reactive bromide may induce attack from weaker nucleophiles, such as the external base (triethylamine or Hunig’s base) or with the pyridine nitrogen of the alkylating agent, leading to a quaternised byproducts. The chloromethyl compound 85 was therefore prepared from the N-oxide 84. The chloromethyl compound 85 was reacted with TACN to produce the trialkylated derivative 87 in a much improved yield.

Demethylation of the methoxy protected TACN 87 was attempted several times using boron tribromide as previously described to prepare ligand 76. The phenolic compound could not be isolated from the crude reaction mixture after quenching with aqueous tartaric acid.
Synthesis of the fluorinated series (ligand 77) was achieved by the same methodology as ligand 60 and is outlined in Schemes 13 and 14.

\[ 62 + \text{BrMg} \rightarrow \text{MeO} \rightarrow \text{90} \rightarrow \text{91 (75%)} \rightarrow \text{92 (60%)} \]

\( (i) \text{NaH, MeI, THF}; (ii) \text{Mg turnings, THF}; (iii) [\text{Ni(dppe)}Cl_2], \text{THF, 0°C}; (iv) \text{H}_2\text{O}_2, \text{glacial AcOH, Δ}; (v) \text{p-Toluenesulfonyl chloride, toluene, Δ}. \)

Synthesis of the complexes is outlined in Scheme 14. All the complexes show peaks at their [MH]\(^+\) and [M+Na]\(^+\) m/z values but are of low abundance. It is also noted that clusters are observable at m/z values that correspond to 77+Ln(CF\(_3\)SO\(_3\))\(_3\), i.e., the
complex incorporating triflate anions. These clusters are more predominant for the Gd complex than in the Eu and Tb complexes. Attempted microanalysis for the isolated complexes show lower than expected C/N ratios indicating the presence of the triflate anion.

![Scheme 14](image)

**Scheme 14**: (i) $\text{Et}_3\text{N}$, CH$_3$CN; (ii) BBr$_3$, DCM, -78°C; (iii) Ln(CF$_3$SO$_3$)$_3$, MeOH, Δ.

**Absorption properties of 77**
A methanolic solution of all three of the complexes show a red shift in the longer wavelength absorption band from 319nm to 329nm in comparison the non-fluorinated derivative 60.

**Photophysical properties of Eu(77)**
Excitation of a $10^{-6}$M methanolic solution of Eu(77) into the ligand absorption band produced no metal centred emission. Excitation of the solution at low temperature (77K), degassing conditions or even at higher concentrations produced no significant change in emission intensity. It is postulated that the fluorine substituents will not have a significant effect on reducing the efficiency of LMCT.
Photophysical properties of Tb(77)
When a methanolic solution of Tb(77) was excited a typical terbium centred emission was observed. The main excitation maximum was found to be at 337nm when monitoring emission at 545nm (Figure 56). The solution of Tb(77) was found to be considerably weaker in emission intensity in comparison to a solution of Tb(60) at the same concentration. The lifetime was found to be 0.21ms. Upon moving to deuterated methanol, the lifetime increased to 0.23ms. This yields an exceptionally high $q$ value indicating that the complex is not 9 coordinate. However, significant errors will occur when dealing with such short lifetimes. Degassing the methanolic solution by 4 freeze/pump/thaw cycles also leads to a significant increase in emission intensity and in lifetime, to 0.38 ms. Measurement of the complex in a frozen methanolic glass at 77K lead to an increase in emission intensity and a lifetime of 1.75ms. These properties are summarised in Table 15.

Figure 56: Excitation and emission spectra of Tb(77) in methanol at $10^{-5}$M; $\lambda_{\text{ex}} = 337$nm; $\lambda_{\text{em}} = 545$nm; excitation and emission slits, 5nm.
The incorporation of fluorine in the ligand system appears to have reduced the triplet state in energy, allowing more efficient quenching by oxygen and thermal repopulation of the aryl triplet. Coupled with this explanation is the phosphorescence emission spectrum of Gd(77), which shows the onset of emission to be at longer wavelengths which places the triplet at lower energy (Figure 57). However, this can only be used as a rough guide due to the poor characterisation associated with the complexes Gd(77) and Tb(77). The lowering of the triplet energy upon fluorination has been reported with fluorinated β-diketonates. Increased europium centred emission was observed on replacing methyl groups with trifluoromethyl groups in the series acetylacetonate, trifluoroacetylacetonate and hexafluoroacetylacetonate. This trend was explained by the energy of the resultant triplet state being in lower energy along the series which decreases the gap between the triplet and the emissive level of the europium, allowing
more efficient ligand to metal energy transfer. In the analogous terbium series, the trifluoroacetylacetonate was found to be more emissive than the symmetrical hexafluoro analogue even though the triplet of the latter was in closer proximity than the former. This was explained to be the susceptibility of terbium to undergo back energy transfer from the $^5D_4$ level to the triplet, allowing the energy to dissipate non-radiatively.

**Functionalised CYCLEN derivatives**

Having investigated the pyrido-phenol chromophore and established that Tb(60) shows reasonable luminescence properties, it was decided that a backbone incorporating a handle that could be functionalised to possess bioactive group should be investigated.

Initially, ligand 95 was proposed based on the CYCLEN backbone as it is known that CYCLEN can be selectively mono-functionalised. Incorporating a CYCLEN backbone will allow one nitrogen to be functionalised with a bioactive group and the remaining 3 nitrogens functionalised with the chromophore. The bioactive functional group in this case would be a simple ester, such as an acetoxy methyl (AM) ester. AM esters can undergo hydrolysis on passing through the cell membrane by non-specific esterases, to produce the membrane impermeant carboxylates. It was decided that the functionalised compound should contain a benzyl group that can act as a spacer between the complex and the action of the biomolecule so as not to effect the overall charge or binding properties of the complex around the metal ion.
As previously described in Chapter 3, mono-functionalised aza-crown ethers can be achieved in a numbers of ways. A 1.5 molar equivalent of CYCLEN was reacted with \( p \)-nitrobenzyl bromide 96 in chloroform, and after purification by column chromatography, the mono-alkylated compound 97 was isolated in excellent yield. 97 was then treated with 3.3 equivalents of 74 in acetonitrile with triethylamine as the base to yield the tetra-substituted compound 98. Demethylation of 98 with an excess of boron tribromide in dichloromethane yielded the phenol 99. Attempts to reduce the nitro compound 99 to the amine 100 with stannous chloride were unsuccessful. It appeared upon mass spectral and \(^1\)H-NMR analysis of the material obtained, that 99 had undergone benzyl elimination upon treatment with stannous chloride to give compound 101 in near quantitative yield (Scheme 15). It was believed that using an ethyl linker would reduce the possibility of elimination during the reduction step.
The phenethyl derivative 103 was synthesised according to the literature, it was noted that p-nitrostyrene 104 was also produced as an elimination from the phenethyl bromide 102 (Scheme 16). The mono-functionalised CYCLEN 103 was reacted with an excess of 74 in acetonitrile with triethylamine as the external base. However, compound 105 could not be isolated from the reaction mixture after column chromatography and it was noted that the characteristic peaks of p-nitrostyrene 104 were observed in the $^1$H-NMR spectra of the crude material. It was confirmed by mass spectrometry that the main compound present in the material isolated was the debenzylated compound 106 and was probably formed by the competing elimination in the presence of the base with the ethyl
linker hydrogens. Use of a sterically hindered, non-nucleophilic base such as DBU would probably limit this process but was not investigated further.

Scheme 16: (i) CHCl₃; (ii) 74, Et₃N, CH₃CN, Δ.

It was eventually decided to change the target to a benzylic ester, such as compound 108 (Scheme 17), as this would reduce the number of steps in the synthesis and eliminate the need to reduce the nitro derivative. Alkylation of 39 with an excess of 74 in acetonitrile with triethylamine as the base yielded compound 107. Demethylation of 107 with boron tribromide in dichloromethane afforded compound 108 as its carboxylic acid derivative.
Analysis of the material isolated showed that there was still a significant amount of boron (0.43%) associated with the ligand by boron analysis. It was found that the material could be re-esterified by refluxing the crude material with thionyl chloride in ethanol. This procedure also appeared to dissociate the complexed boron from the ligand. Complexation of the ligand with terbium was achieved by refluxing the ligand in acetonitrile followed by the addition of terbium triflate in ethanol (Scheme 17).

A simple mono-functionalised benzyl ligand was also synthesised so as to act as a comparison to the ester functionalised complex (Scheme 18).
Scheme 18: (i) CHCl₃; (ii) 74, Et₃N, CH₃CN, Δ; (iii) BBr₃, DCM, -78°C; (iv) Tb(CF₃SO₃)₃, EtOH, CH₃CN, Δ.

Absorption and photophysical properties of Tb(108) and Tb(112)
A methanolic solution of Tb(108) and Tb(112) showed absorption maximum to be at 328 nm. Excitation of both complexes at this wavelength in a methanolic solution at 10⁻⁶ M produced very weak metal centred emission when in comparison to that of Tb(60). The emission properties of Tb(108) were of low in intensity to measure an accurate lifetime. This observation was deemed unusual considering the emissive properties of Tb(60). It was also noted that Tb(112) showed the same poor emissive properties as Tb(108) when excited in solution. In solution, no green colouration associated with terbium centred emission could be observed when irradiated with UV light, this
colouration is observed with both Tb(60) and Tb(77). Also, the same lack of emissive properties was observed when the complexes were made in situ.

The lack of terbium centred emission was indeed strange considering that the chromophore had been shown to be a reasonable sensitisier of terbium. The only difference between the two systems is the aza-crown backbone and the benzyl substituents.

Taking a solution of Tb(108) at 5x10^-6M and bubbling nitrogen gas through it for 2 minutes and running an emission spectra showed a significant increase in emission intensity and the green colouration associated with terbium centred emission could be observed when irradiated with UV light at 365 nm. The lifetime of the N2 purged solution was 0.29 ms and was of a single exponential decay. Purging the solution with N2 will displace the dissolved oxygen in solution and therefore it can be assumed that the sensitisation process involving the triplet is efficiently quenched by oxygen under aerated conditions. Degassing of the same solution by 4 freeze/pump/thaw cycles lead to increased emission intensity and to an increased lifetime of 0.32 ms. Measurement of Tb(108) in a rigid frozen matrix leads to an increased lifetime (1.78 ms) and emission intensity, implying that there is efficient thermally activated back energy transfer mechanism in operation. The same trend in photophysical properties were observed in Tb(112) and are summarised in Table 16.

<table>
<thead>
<tr>
<th>Complex</th>
<th>τ N2 purge CH3OH</th>
<th>τ deox</th>
<th>τ^77K CH3OH</th>
<th>τ^77K CD3OD</th>
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<tbody>
<tr>
<td>Tb(108)</td>
<td>0.29</td>
<td>0.32</td>
<td>1.78</td>
<td>1.90</td>
</tr>
<tr>
<td>Tb(112)</td>
<td>0.27</td>
<td>0.32</td>
<td>1.82</td>
<td>1.83</td>
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</table>

Table 16: Photophysical properties of Tb(108) and Tb(112). Lifetimes are in ms and are for the ^5D_4→^7F_5 transition (545 nm). λ_ex = 337nm.

The next stage was to establish the triplet state energy of the chromophore when bound to gadolinium in this ligand system, as there is an obvious difference in photophysical
properties compared to Tb(60). The benzyl derivative was used for the triplet determination, thus Gd(112) was taken in methanolic glass at 77K and the phosphorescence spectra recorded (Figure 58). The 0-0 transition was again taken to be the onset of emission and was found to be 22, 500 cm⁻¹, some 800 cm⁻¹ lower than that in Gd(60). The chromophore in the CYCLEN series appears to have a lower triplet state energy than that in the TACN derivative.

![Figure 58: The phosphorescence emission spectra of Gd(112) at 77K; excitation and emission slits ,2.5nm.](image)

To establish the role of the thermal deactivation and its extent within this system, a series of low temperature measurements ranging from 77K to 300K were carried out with Tb(112) in methanol at 5x10⁻⁶M and the emission lifetimes recorded. The activation energy required for this thermal quenching can be found by Equation 8.

\[
k(T) = \left(\frac{1}{\tau} - \frac{1}{\tau^0}\right) = A \exp(-E_a/RT) \tag{Equation 8}
\]

A plot of ln \(\left[\left(\frac{1}{\tau} - \frac{1}{\tau^0}\right)\right]\) versus 1/T will give the activation energy as the slope and is the required energy for the total thermal deactivation process. The sample was excited at 340nm with the emission lifetime monitored at 546nm for the \(^5\text{D}_4\rightarrow^7\text{F}_5\) transition of terbium. It was noted at temperatures below 250K that decay fitted well
to a exponential decay but at temperatures above this value, the radiative decay displayed a degree of poly-exponential decay but the decay plots still displayed a degree of linearity. The temperature range for the lifetime values used were between 160 and 265K as the lifetimes below 160K varied little and the lifetime values above 265K were too short to be measured accurately on the instruments available in our laboratory.

\[
\ln(\frac{\tau}{\tau_0}) = 1539.8 + 14.221T
\]

\[R^2 = 0.9668\]

**Figure 59**: Temperature dependence of Tb(112) in methanol at 5x10^{-6}M; \(\lambda_{ex} = 340\text{nm}\); lifetimes are of the \(^5D_4 \rightarrow ^7F_5\) transition at 546 nm. Experimental lifetime \(\tau\) was plotted against \(1/T\) according to *Equation 8*.

The activation energy for the temperature dependence of this system was calculated to be 1070cm\(^{-1}\) from the slope of the line (*Figure 59*), which places the triplet state of the chromophore at approximately 21470cm\(^{-1}\). This is assuming that the only thermal deactivation process is back energy transfer, but this is only an approximate figure as solvent and ligand oscillations, such as C-H, will also be governed partly by temperature, and therefore can only be used as a upper limit. Quenching of the complex by oxygen will not be eliminated from the sample under these conditions. Stern-Volmer quenching analysis\(^{119}\) would need to be obtained but requires the concentration of the quencher (i.e., oxygen) to be known. Such experiments are be possible but would be subject to a degree of error in measuring the concentration of dissolved oxygen in the solution.
Concluding remarks
The TACN derivative Tb(60) shows different photophysical properties in comparison to the CYCLEN based complexes Tb(108) and Tb(112) can be ascribed the greater flexibility of the CYCLEN ring and its larger cavity size. The triplet of the sensitiser when in the CYCLEN system appears to be lower than that in its TACN analogue, as indicated by its temperature and sensitivity to dissolved oxygen when in solution. Even though resultant complexes incorporating CYCLEN backbones display poor emissive properties, they have a potential use as oxygen sensors due to the luminescence signal effectively being “switched on” in the absence of oxygen when in solution. There is a lot of scope for future work with these complexes as further photophysical studies could not be completed fully due to time constraints. The problem of complex stability and aqueous solubility would need to be addressed.

Hydroxyquinoline as a sensitiser of europium
Crosby and co-workers have previously reported the photophysical properties of 3:1 complexes of 8-hydroxyquinoline 113 and 8-hydroxy-2-methylquinoline 114 with europium and gadolinium.\textsuperscript{109} It was found however that these complexes were only soluble at elevated temperatures in EP and EPA solvent systems, and this treatment was found to increase complex dissociation in solution. The phosphorescence spectra of both parent ligands was extremely weak but was enhanced upon chelation to the lanthanide ion. The triplet state of 113 and 114 was found to be 17760cm\textsuperscript{-1} and 18000cm\textsuperscript{-1} in energy when chelated to gadolinium, respectively. Kleinerman also investigated 114 as a sensitiser of europium and found the triplet energy to be at 18500 cm\textsuperscript{-1}, from the phosphorescence emission spectra of its gadolinium complex at 77K in ethanol.\textsuperscript{140}
In order to address the stability of the 3:1 complexes initially investigated by Crosby and later by Kleinerman, the chromophoric group will be attached to an aza-crown ether, namely TACN, so as to establish the photophysical properties (ligand 115). The synthetic strategy for this ligand is relatively simple and comprises of phenolic protection of 114, halogenation, alkylation of TACN 55 followed by deprotection to yield ligand 115.

Initially, the protecting group employed was the methoxy group as previously used in the pyrido-phenol sensitisier series, with 116 readily synthesised from 114 in the presence of sodium hydride in THF followed by the addition of methyl iodide. Bromination of 116 with N-bromosuccinimide failed to produce the required benzyl bromide 117 but produced compounds 118 and 119 (Scheme 19), with their structures were confirmed by $^1$H-NMR and mass spectroscopy.
Attempts to synthesise the N-oxide 120 using peracetic acid were relatively successful with the required molecular ion detected by mass spectroscopy. However, the material isolated was not single spot by tlc and could not be separated by column chromatography. Nevertheless, the crude material was taken and reacted with an excess of p-toluenesulfonyl chloride in toluene with the condenser fitted with a Dean and Stark apparatus so as to remove any water. No new spots were observed by tlc of the crude reaction mixture, even after a period of 4 days. Mass spectral analysis of the crude material after work-up showed none of the required compound present.

An alternative protecting group would be required that did not induce bromination of the aromatic ring as in the methoxy compound and was able to protect the phenol during the alkylation of 55. It was believed that a benzoate ester would not be stable in the presence of an amine nucleophile as the carbonyl moiety is not hindered enough. Initial
experiments showed that the carbonyl carbon of the benzoate ester was indeed susceptible to nucleophilic attack by primary amines. Adamantoate and 2, 4, 6-trichlorobenzoate esters are bulky enough to prevent nucleophilic attack at the carbonyl carbon and inert under free radical bromination conditions but the precursors are expensive and their use is not cost effective for the scales required. Pivaloate esters have been shown to be stable towards bromination with N-bromosuccinimide and non-aqueous attack by amines, and cleavage can be afforded by refluxing in aqueous potassium hydroxide in ethanol under a nitrogen atmosphere.

The pivaloate protected quinoline was synthesised by the slow addition of pivaloyl chloride to a solution of in dichloromethane in the presence of an excess of triethylamine. After extraction, was obtained in a near quantitative yield. Bromination of was achieved with N-bromosuccinimide in carbon tetrachloride with benzoyl peroxide as the free radical initiator to yield the mono-bromide . Alkylation of TACN in acetonitrile with triethylamine as the base afforded the tri-substituted aza crown . It was apparent that no side reactions were observed when using this protecting group as with the mono-bromide pyrido-phenol compounds, presumably because the pivaloate group is bulky enough to prevent the nitrogen of the quinoline to react with the benzylic bromide (Scheme 21).

Attempts to deprotect with aqueous potassium hydroxide were unsuccessful, with apparent decomposition of the compound under the reaction conditions. This left a slight technical problem, as the pivaloate group is so inert toward nucleophilic attack and seemingly unstable towards basic hydrolysis conditions. It was postulated that reductive elimination using a lithium aluminium hydride as a source of hydride could liberate the deprotected analogue. To test this hypothesis, was reacted with a molar equivalent of LiAlH₄ in anhydrous THF at room temperature, and after work up the hydroxyquinoline was isolated in near quantitative yield. was deprotected with LiAlH₄ in the same manner as but was treated with aqueous tartaric acid during the work-up procedure to remove any complexed aluminium. Attempts to form
Eu(115) and Gd(115) by refluxing 115 with a slight excess of the appropriate metal triflate salt were unsuccessful. Mass spectral analysis of the material isolated showed the presence of the required [MH]+ ion but was in very low abundance. Also, UV-vis absorption of the materials isolated complexes showed very low extinction coefficients.

\[ \text{OH} \]
\[ \text{N} \]

\[ \text{Br} \]

(i) \( (\text{CH}_3)_3\text{COCl}, \text{DCM, Et}_3\text{N} \); (ii) NBS, benzoyl peroxide (init), CCl\(_4\), \( \Delta \);

(iii) 55, Et\(_3\)N, CH\(_3\)CN; (iv) LiAlH\(_4\), THF.

**Scheme 21**: (i) \( (\text{CH}_3)_3\text{COCl}, \text{DCM, Et}_3\text{N} \); (ii) NBS, benzoyl peroxide (init), CCl\(_4\), \( \Delta \);

(iii) 55, Et\(_3\)N, CH\(_3\)CN; (iv) LiAlH\(_4\), THF.

**Photophysical properties of 115, Eu(115) and Gd(115)**

Complexes were prepared *in situ* at 10\(^{-4}\)M from mM stock solutions of the free ligand 115 in methanol and mM stock solution of the appropriate metal triflate salt in methanol. Solutions were measured after 2 hours of preparation and again after 12 hours to ensure consistency of results. A methanolic solution of 115 showed absorption maximum at 298 with a corresponding extinction coefficient of 7290 dm\(^3\)mol\(^{-1}\)cm\(^{-1}\). Upon complexation with a lanthanide (Eu or Gd) the longer wavelength absorption band shifted to 321nm nm, but extends beyond 380nm. Excitation of the solution into
the ligand absorption band showed very weak metal centred luminescence. Cooling the solution 77K produced a more intense metal centred emission but was again of low intensity, with the main excitation peak at 350nm when monitoring emission at 615nm (Figure 60). Degassing of the solution made no significant change to the spectral properties.

![Excitation and emission spectra of Eu(115) in methanol at 5x10^{-6} M at 77K; excitation and emission slits at 5nm](image)

*Figure 60: Excitation and emission spectra of Eu(115) in methanol at 5x10^{-6} M at 77K; excitation and emission slits at 5nm*

Attempts to obtain a phosphorescence spectra of Gd(115) at 77K were unsuccessful as the complex was very weakly emissive and emission could only be observed using wide emission slit widths (15nm), which will not lead to an accurate determination of the 0-0 transition.

The photophysical properties of 115 when complexed with either europium or gadolinium in solution were disappointing when considering the effort taken to synthesise the ligand. Problems were previously encountered and described earlier in this chapter when using triazacyclononane as a backbone and may also be born out in this chromophoric series as well.
In an attempt to gain some evidence as to why 8-hydroxyquinoline is such a poor sensitisier of europium, the 3:1 complexes of 8-hydroxyquinoline with europium and gadolinium were synthesised as reported by Crosby (Scheme 22). The materials isolated showed the presence of the metal by CHN analysis and were in good agreement for 3:1, ligand to metal, stoichiometry but are not entirely accurate. Mass spectral analysis was not carried out as it is likely that the complexes would dissociate even under relatively “soft” ionisation techniques.

\[
\begin{array}{c}
\text{113} \\
\text{OH} \\
\end{array}
\xrightarrow{(i), (ii)}
\left[
\begin{array}{c}
\text{N} \\
\text{O} \\
\end{array}
\right]
\begin{array}{c}
\text{Ln}^{3+} \\
3 \\
\end{array}
\]

\[\text{Ln} = \text{Eu(113)}_3(80\%)\]
\[\text{Gd(113)}_3(83\%)
\]

\text{Scheme 22: (i) LnCl}_3.6\text{H}_2\text{O}, \text{EtOH}; (ii)NH}_3(\text{g}), \text{H}_2\text{O}.

The complexes isolated displayed very poor stability in methanol by analysis with UV-vis absorption spectroscopy but the maximum absorption was found to be at 350nm. This peak had almost disappeared upon standing for over an hour at room temperature. No metal centred emission was observed from Eu(113)_3 at room temperature or in a frozen glass matrix when exciting in a range of 300-400 nm. The effect of LMCT cannot be discounted as a quenching process of metal centred luminescence.

Measurement of the phosphorescence emission spectrum of Gd(113)_3 at 77K in solution was not attempted due to the apparent instability of the europium complex in solution. However, it was believed that measurement of the complexes in a KBr disc would certainly eliminate the problem of complex dissociation but only could be used as a qualitative measurement. Excitation of Eu(113)_3 in a KBr mull in a range of 300-400nm showed very little metal centred emission at room temperature, or at low temperature.
Measurement of the phosphorescence spectrum of Gd(113)₃ in a KBr mull indicated that the triplet energy of the chromophore is at 17000 cm⁻¹, from the onset of emission at 588 nm. This is of lower energy than the ⁵D₀ level of europium (17250 cm⁻¹) and therefore precluding this chromophore as a suitable sensitiser of europium. Recently, Iwamuro et al.,¹⁴² have shown that 8-hydroxyquinoline is an efficient sensitiser of neodymium due to its triplet being of similar energy (17200 cm⁻¹) to that of the ²G₇/₂ and ⁴G₅/₂ energy accepting levels of neodymium (17100 cm⁻¹). Although 8-hydroxyquinoline is an unsuitable sensitiser of europium, it has a potential use as a sensitiser of neodymium as illustrated by Iwamuro.¹⁴² Probes utilising neodymium as emissive metal centres are particularly useful as its electronic transitions are of lower energy and subsequently emit at longer wavelengths. Longer wavelength, near infra-red emitters are useful as skin and blood are transparent to this light allowing their use in in vivo-diagnostics.
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General Details

Melting points (mp) were determined on a Kofler hot-stage apparatus and are uncorrected. Elemental analysis was carried out by MEDAC Ltd, Surrey and by Ms. Nicola Walker, University of Surrey. Ultraviolet/visible absorption spectra were obtained using a Unicam PU8700 Series UV/vis spectrophotometer using 10mm cells; absorption maxima ($\lambda_{\text{max}}$) and corresponding absorption coefficient ($\varepsilon_{\text{max}}$) are reported in nm and dm$^3$/mol$^1$cm$^{-1}$, respectively. Infrared absorption spectra were recorded on a Perkin-Elmer System 2000 FTIR spectrometer as thin films for liquids, and either Nujol mulls, KBr discs or chloroform solutions for solid samples; absorptions ($\nu_{\text{max}}$) are quoted in cm$^{-1}$. $^1$H and $^{13}$C NMR spectra were obtained on a Bruker AC300 spectrometer at 300 MHz and 75MHz, respectively. Spectra were referenced internally to either tetramethylsilane (TMS) where specified, or else to solvent residual proton resonances. Reported carbon signals in $^{13}$C-NMR do not directly correlate to the number of carbon atoms in the compound reported. Chemical shift values are quoted in ppm while observed coupling constants ($J$) are reported in Hz. Assignments in the $^1$H NMR spectra are abbreviated as follows: s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet and b = broad. Mass spectra (m/z) were recorded by the EPSRC National Mass Spectroscopy Service Centre, Swansea.

Solvents were dried according to literature methods. All starting materials obtained from an external source were checked by tlc and $^1$H NMR, where applicable, prior to use. Thin layer chromatography (tlc) was carried out on either Merck 0.25 mm pre-coated 60 F$254$ silica gel glass plates or Merck 0.25 mm 60 F$254$ neutral alumina plates; column chromatography was carried out on Fluka silica gel 60 (220-440 mesh) or Fisher Scientific Activation I Neutral Aluminium Oxide (100-250 mesh), accordingly. Solvent ratios refer to volumes prior to mixing.
1, 4, 7, 10-Tetraazacyclododecane (CYCLEN) was obtained from Strem Chemicals, USA.

Partition Experiments
Octanol-water partition experiments were carried out by stirring an aqueous solution of the complex in question at $10^{-4}$M with an equal volume of octanol at a rate that avoided emulsification for 18h. The mixture was allowed to stand overnight and the two layers separated. UV-vis absorption spectra were recorded for each layer and comparisons made with standard solutions at known concentrations. Water saturated octanol and octanol saturated water were used.

Photophysical Protocols
Lanthanide luminescence spectra were recorded on a Perkin Elmer LS50B luminescence spectrometer, using FLWinlab software. Time resolved measurements were obtained under the phosphorescence mode, after a delay time $t_d$ and over a fixed gate time $t_g$; typically, $t_d = 0.1$ ms and $t_g = 1$ms. Generally, stock solutions of ligands and complexes were made up as specified, in distilled water, deuterium oxide (Aldrich, 99.9%), methanol (Aldrich, 99.99%) or deuterated methanol (Aldrich, 99.9%). Solvents such as acetonitrile, dichloromethane and toluene were freshly distilled on the day of solution preparation and measurement. Samples to be measured were made up to volumes of 5cm$^3$ at $10^{-4}$M, and diluted to the required concentration in a 10mm quartz cuvette, using Rainin electronic pipettes. Luminescence measurements were made immediately after solution preparation and repeated after 12 h to ensure equilibration. During the course of a series of measurements, cuvettes were rinsed thoroughly with water then methanol, then dried with hot air. When not in use, cuvettes were soaked in a solution of Caro’s acid. Solid state measurements in KBr discs were carried out on a solid state attachment fitted to an Oxford Instruments optical cryostat. KBr discs were prepared using approximately 0.1 mg of lanthanide chelate in approximately 200mg of oven dried KBr salt and pressed.
**Lifetime measurements**

Excited state lifetimes for europium and terbium chelates were acquired using *Lemming* software (written by Dr. A. Beeby, University of Durham), then data were transferred into *Microsoft Excel* and manipulated to obtain the lifetime ($\tau$). Lifetimes were measured by excitation of the sample with a short pulse of light at a specific wavelength, followed by monitoring the integrated intensity of light at a specified emission peak, after a delay time $t_d$, during a fixed gate time, $t_g$. Typically, the time taken to measure emission intensity was at least 6 lifetimes of the chelate to ensure that >99% of the emission had dissipated. If a single light emitting species is present and there is no quenching of the emissive state by other excited state molecules, then mono-exponential decay of luminescence is expected; i.e. the intensity $I$ at time $t$ after excitation will be given by *Equation 22*,

$$I_t = I_0 \exp(k_{obs} t)$$

*Equation 22*

where $I_0$ is the intensity at $t = 0$. The decay curve is characterised by $k_{obs}$, the observed rate constant for deactivation of the emissive state which can be calculated from a plot of $\ln I_t$ versus $t$ showing a straight line fit with gradient $-k_{obs}$.

$$\ln I_t = k_{obs} t + \ln I_0$$

*Equation 23*

$k_{obs}$ has an inverse relationship with the lifetime such that $\tau = k_{obs}^{-1}$.

**Calculation of hydration numbers**

The number of coordinated hydroxyl solvent molecules around the lanthanide ions in their complexes were determined using either,

a) Parker's modified Horrock's equation (*Equation 14*) for cyclen-based complexes in *Chapter 3*;

$$q_{corr} = A_{\text{Ln}}[(k_{H2O} - k_{D2O}) + \text{corr}_{\text{Ln}}]$$

*Equation 14*
where $g_{\text{corr}}$ is the inner sphere hydration number, $k$ is the rate constant for the depopulation of the lanthanide excited state in D$_2$O and H$_2$O, respectively; $A'_{\text{Eu}} = 1.2\text{ms}^{-1}$ and $\text{corr}_{\text{Eu}} = 0.25\text{ms}^{-1}$; $A'_{\text{Tb}} = 5\text{ms}$ and $\text{corr}_{\text{Tb}} = 0.06\text{ms}^{-1}$.

or,

b) Horrock's equation (Equation 2) for the triazaacyclononane appended ligands in Chapters 4 and 5;

$$q = B_{\text{Ln}} (k_{\text{CH}_3\text{OH}} - k_{\text{CD}_3\text{OD}})$$

Equation 2

where $q$ is the number of coordinated methanol molecules, $k$ is the rate constant for the depopulation of the lanthanide excited state in CH$_3$OH and CD$_3$OD respectively, while $B_{\text{Eu}} = 2.1\text{ms}^{-1}$ and $B_{\text{Tb}} = 8.4\text{ms}^{-1}$.

Degassing experiments

Degassing of samples was carried out in a cell equipped with a 10mm path length square cuvette and a degassing bulb; degassing was achieved using typically four 'freeze-pump-thaw' cycles and samples were measured under vacuum (samples were measured air-equilibrated both before and after degassing, for comparison).

Low Temperature studies

Variable temperature luminescence spectra were recorded using an Oxford Instruments optical cryostat coupled to an Edinburgh Instruments Intelligent Temperature Controller. Samples were allowed to equilibrate at the set temperature for 30 minutes prior to lifetime measurement.

Low temperature phosphorescence spectra for determining triplet energies were carried out using an Oxford Instruments optical cryostat at 77K (liquid nitrogen temperature). Generally, the triplet was determined from the highest energy (shortest wavelength) phosphorescence band, corresponding to the 0-0 transition.
Quantum yield determinations- Chapters 3, 4 and 5

Quantum yield measurements in all chapters were recorded relative to \([\text{Lnc}(22)]^{2+}\) (kindly provided by Dr. Anjum Dadabhoy, University of Surrey) in water, for which the quantum yields for the europium and terbium cryptates are 0.02 and 0.03, respectively. Solutions with different absorbances between 0.05 and 0.12 were used to ensure that the same amount of light was absorbed by both the standard and sample at a common wavelength of 300nm. For each sample/standard pair, the total integrated emission upon excitation at this wavelength was measured, under identical conditions. A plot of total integrated emission (E) against absorbance (A) then gave a straight line with slope \(E/A\). The unknown quantum yield \(\phi_x\) can then be calculated from the following modified equation,\(^{95}\)

\[
\phi_x = \phi_r \cdot \left( \frac{C_x \cdot \text{slope}_x}{C_r \cdot \text{slope}_r} \right) \cdot \left( \frac{n_x}{n_0} \right)^2
\]

\text{Equation 20}

where \(r\) and \(x\) refer to the reference and the unknown, respectively, and \(n\) is the refractive index of the solution. \(C\) is the lifetime correction factor given by the equation

\[
C = \exp\left(\frac{d}{\tau}\right)
\]

\text{Equation 18}

where \(d\) is the delay time and \(\tau\) is the experimental lifetime at 300K.
6-Methyl-2,2'-bipyridine 29144

2, 2-Bipyridyl (10g, 0.064 mol) in freshly distilled diethyl ether (320 cm³) was stirred in an ice bath under an argon atmosphere. 1.4 M Methyl lithium in diethyl ether (48 cm³) was added dropwise over a 30 minute period. The solution was then refluxed for 3 h, allowed to cool and water (50 cm³) was added cautiously. The ether layer was extracted and then the aqueous layer extracted with diethyl ether (3x50 cm³). The combined organics were then dried over sodium sulphate, filtered and the solvent removed in vacuo. The residue that remained was dissolved in acetone and potassium permanganate in acetone was added until a violet colour persisted. The manganese dioxide was filtered off and the acetone removed in vacuo. The brown oil that remained was purified by column chromatography (neutral alumina) with 3:2, 40-60 petroleum ether and diethyl ether as the eluent to give the title compound as a pale yellow oil, which solidifies upon standing (4.48 g, 48%), mp 30-32°C; (Found C, 77.4; H, 5.7; N, 16.5. C₁₁H₁₀N₂ requires C, 77.6; H, 5.9; N, 16.5); vₚₚ (Film)/cm⁻¹ 3061, 2988, 1583, 1460, 1428, 1257, 1153, 1083, 1025, 983 and 777; δH (300 MHz; CDCl₃; Me₄Si) 2.63 (3H, s, -CH₃), 7.16 (1H, d, J 7, 5-H), 7.29 (1H, td, J8 and 2, 5'-H), 7.70 (1H, t, J 7.5, 4-H), 7.80 (1H, td, J 8 and 2, 4'-H), 8.16 (1H, d, J 7.5, 3-H), 8.41 (1H, d, J 7.5, 3'-H), 8.67 (1H, J 4 and 2, 6'-H); m/z (EI) 170 ([M]⁺, 100%), 171 ([MH]⁺, 11%).
6-Bromomethyl-2,2'-bipyridine 30

The bipyridyl 29 (4g, 0.023 mol), N-bromosuccinimide (4.45 g, 0.025 mol) and benzoyl peroxide (100 mg) was refluxed in carbon tetrachloride (75 cm³) for 14 h. The reaction mixture was allowed to cool and the succinimide was removed by filtration. The filtrate solvent was removed in vacuo and the remaining residue was purified by column chromatography (silica gel) with 2% methanol in dichloromethane as the eluent to yield the title compound as a white crystalline solid (1.88g, 32%), mp 67-68°C (from hexane) (lit., 68-68°C); (Found C, 53.35; H, 3.4; N, 11.0, C₁₁H₉N₂Br requires C, 53.0; H, 3.6; N, 11.25); νmax (Film)/cm⁻¹ 3018, 2974, 1583, 1458, 1428, 1257, 1153, 1047, 1025 and 777; δH (300 MHz; CDCl₃; Me₄Si) 4.60 (2H, s, -CH₂Br), 7.27 (1H, td, J₈ and 1, 5'-H), 7.40 (1H, d, J 7.5, 5-H), 7.76 (2H, t, J 8, 4-H and 4'-H), 8.29 (1H, d, J 8.5, 3-H), 8.42 (1H, d, J 8.5, 3'-H), 8.65 (1H, dd, J 4 and 1, 6'-H); m/z (FAB) 249 ([MH]+79Br, 100%), 251 ([MH]+81Br, 97%).

1, 4, 7-tris-(tert-Butoxycarbonylmethyl)-1, 4, 7, 10-tetrazacyclododecane hydrogen bromide 32
CYCLEN (1g, 5.8 mmol), tert-butylbromoacetate (3.73 g, 19.1 mmol), sodium hydrogen carbonate (1.61 g, 19.1 mmol) and dry 4Å molecular sieves was stirred in freshly distilled acetonitrile (50 cm³) for 48 h under dry nitrogen. After which time, the solids were filtered off, washed with cold acetonitrile and the filtrate solvent was removed in vacuo. The residue that remained was recrystallised from hot toluene to yield the title ester as a white solid (1.60 g, 54 %), mp 178-180°C; (Found C, 52.4; H, 8.7; N, 9.5. C₂₆H₅₀N₄O₆.HBr requires C, 52.4; H, 8.6; N, 9.4); νₘₚₓ (Nujol)/cm⁻¹ 3428 (br, N-H), 2865, 2736, 1730 (C=O), 1622, 1583, 1368, 1256, 1150 and 754; δₜₙ(300 MHz; CDCl₃; Me₄Si) 1.46 (27 H, s, tert-Bu), 2.89 (4H, s, 8,12-CH₂), 2.92 (8H, s, 2, 3, 5, 6-CH₂), 3.09 (4H, s, 9, 11-CH₂NH), 3.29 (2H, s, 4-CH₂CO₂`Bu), 3.36 (4H, s, 1, 7-CH₂CO₂'Bu), 9.92-10.12 (2H, s, NH.HBr); m/z (EI) 515 ([MH]+-HBr, 100%), 401 ([MH]+-CH₂CO₂'Bu, 48%).

1-[6-Methyl-2, 2'-bipyridinyl]-4, 7, 10-tris-(tert-butoxycarbonylmethyl)-1, 4, 7, 10-tetraazacyclododecane 33

The ester hydrobromide salt 32 (0.6g, 1 mmol) was dissolved in freshly distilled acetonitrile (25 ml) with sodium carbonate (0.27g, 2.5 mmol) and the bromomethyl bipyridyl 30 (0.27g, 1.1 mmol). The mixture was heated to reflux under an argon atmosphere for 6h. The mixture was allowed to cool, the solids filtered and washed
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with cold acetonitrile (2x5 cm³). The filtrate was evaporated to dryness, and the residue was taken in dichloromethane (1 cm³) and purified by column chromatography (neutral alumina) using dichloromethane with a methanol gradient (0-2%) as the eluent. The title compound was obtained as a cream coloured solid (673 mg, 89%), mp 147-149°C; (Found: C, 56.4; H, 7.7; N, 10.6. C₃₇H₅₈N₆O₆NaBr requires 56.55; H, 7.4; N, 10.7); λₘₐₓ (EtOH)/nm 237 (ε/dm³mol⁻¹cm⁻¹ 11930), 287 (14680); νₘₐₓ (Nujol)/cm⁻¹ 3362 (br, NH), 1723 (C=O), 2726, 1582, 1574, 1262, 1160, 1098 and 757; δH (300 MHz; CDCl₃; Me₄Si) 1.31 (18H, s, tert-Bu), 1.44 (9H, s, tert-Bu), 2.18-3.78 (24H, broad m, aza ring and bridgehead -CH₂), 7.28 (1H, td, J 5 and 1, 5'-H), 7.39 (1H, d, J 6, 5'-H), 7.75 (1H, td, J 7.5 and 1.5, 4'-H), 7.84 (1H, t, J 8, 4-H), 8.36 (1H, d, J 8, 3-H), 8.58 (1H, d, J 4, 3'-H), 8.74 (1H, d, J 8.5, 6'-H).

1-(6-Methyl-2, 2'-bipyridinyl)-4, 7, 10-tris-(carboxymethyl)-1, 4, 7, 10-tetraazacyclododecane 23

The ester 33 (500 mg, 0.64 mmol) was dissolved in dichloromethane (5 cm³) and to the solution was added trifluoroacetic acid (5 cm³). The solution was left stirring for 24 h and then the solvents were removed under reduced pressure. The residue was taken up in dichloromethane (2 x 10 cm³) and twice evaporated, followed by the addition and evaporation of methanol (2 x 10 cm³). The remaining residue was dissolved in the minimum amount of methanol and the title complex was isolated by the precipitation by the slow diffusion of ether as fluffy beige flakes (307 mg, 93%), νₘₐₓ (Nujol)/cm⁻¹ 3468
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(br, OH) 3072, 1680 (C=O), 1535, 1188, 1123 and 712; \( \lambda_{\text{max}} \) (H\( _2 \)O)/nm 241 (\( \varepsilon/\text{dm}^3\text{mol}^{-1}\text{cm}^{-1} \) 4690), 288 (7420); \( \delta_H \) (300 MHz; CD\(_3\)OD; Me\(_4\)Si) 3.19-3.50 (16H, aza-crown – CH\(_2\), 6H, -CH\(_2\)CO\(_2\)H), 4.01 (2H, s, NCH\(_2\)-bipy), 7.79 (2H, m, 5'-H and 5-H), 8.10 (1H, t, J 8.5, 4-H), 8.37 (2H, m, 4'-H and 3-H), 8.57 (1H, d, J 8.5, 3'-H), 8.81 (1H, d, J 5, 6'-H); \( \delta_C \) (75 MHz; D\(_2\)O) 49.5, 50.7, 53.9, 58.6, 115.5, 117.9, 123.5, 124.8, 127.9, 128.7, 141.3, 142.7, 147.6, 148.2, 163.1 and 163.4; m/z (FAB), 537 ([M+ Na]\(^+\), 100%), 515 ([MH]\(^+\), 44%).

1-(6-Methyl-2, 2'-bipyridinyl)-4, 7, 10-tris-(carboxymethyl)-1, 4, 7, 10-tetraazacyclododecane Lanthanide Ln(23)

The acid 23 (75 mg, 0.146 mmol) was dissolved in acetonitrile (1 cm\(^3\)) and stirred at 50°C. The lanthanide triflate salt (0.15mmol) in aqueous Hepes buffer at pH 7.5 (1cm\(^3\)) was added and stirred for a further 24 h. The solution was allowed to cool and passed through a celite plug, and washed with acetonitrile (5cm\(^3\)) and water (2cm\(^3\)). The filtrate was evaporated to dryness and the residue dissolved in the minimum amount of ethanol. The title complexes were crystallised by the slow diffusion of ether and the solids collected as white microcrystalline solids.
1-(6-Methyl-2',2'-bipyridinyl)-4, 7, 10-tris-(carboxymethyl)-1, 4, 7, 10-tetraazacyclododecane Europium Eu(23)

(84 mg, 86%), mp >300°C; $\lambda_{\max}$(H2O)/nm 245 (ε/dm$^3$ mol$^{-1}$ cm$^{-1}$ 4520), 309 (6490); $\nu_{\max}$(Nujol)/cm$^{-1}$ 3435 (br), 2727, 1625 (C=O), 1535, 1279, 1167, 1029 and 722; m/z (FAB) 663 ([M$^+$H$^+$. 90%, $^{151}$Eu), 665 ([MH$^+$$. 100%, $^{153}$Eu); HRMS (FAB$^+$) 663.1572, ([MH$^+$]. C$_{25}$H$_{32}$N$_6$O$_{6}$$^{151}$Eu requires 663.1582).

1-(6-Methyl-2',2'-bipyridinyl)-4, 7, 10-tris-(carboxymethyl)-1, 4, 7, 10-tetraazacyclododecane Terbium Tb(23)

(77 mg, 79%), mp >300°C; $\lambda_{\max}$(H2O)/nm 243 (ε/dm$^3$ mol$^{-1}$ cm$^{-1}$ 4550), 309 (6590); $\nu_{\max}$(Nujol)/cm$^{-1}$ 3429 (br), 1626 (C=O), 1535, 1279, 1167, 1029 and 723; m/z (FAB) 671 ([MH$^+$$. 100%), 693 ([M+Na$^+$$. 10%); HRMS (FAB) 671.1628, ([MH$^+$]. C$_{25}$H$_{32}$N$_6$O$_6$Tb requires 671.1637).

1-[6-Methyl-2',2'-bipyridinyl]-4, 7, 10-tris-(carboxylmethyl)-1, 4, 7, 10-tetraazacyclododecane Gadolinium Gd(23)

(71 mg, 72%), mp >300°C; $\lambda_{\max}$(H2O)/nm 244 (ε/dm$^3$ mol$^{-1}$ cm$^{-1}$ 4500), 308 (6580); $\nu_{\max}$(Nujol)/cm$^{-1}$ 3410 (br), 2725, 1626 (C=O), 1535, 1281, 1161, 1028 and 722; m/z (FAB) 670 ([MH$^+$$. 100%, $^{158}$Gd), 668 ([MH$^+$$. 80%, $^{156}$Gd); HRMS (FAB$^+$) 667.1623, ([MH$^+$]. C$_{25}$H$_{32}$N$_6$O$_6$$^{155}$Gd requires 667.1610).

Ethyl (4-chloromethyl)-benzoate 38

4-Chloromethyl benzoic acid (3.94g, 0.023 mol) in ethanol (35cm$^3$) was stirred at room temperature. Thionyl chloride (1 cm$^3$) was added cautiously and the solution refluxed for 6 h. The solution was allowed to cool and the solvent removed under reduced pressure. The residue was dissolved in dichloromethane (10cm$^3$) and filtered to yield
the title compound as a yellow oil (3.81 g, 83%), \( \nu_{\text{max}} \) (Film)/cm\(^{-1}\) 2983, 1717, 1446, 1368, 1305, 1178, 1105, 1022, 857 and 713; \( \delta_H \) (300MHz; CDCl\(_3\); Me\(_4\)Si) 1.38 (3H, t, \( J \) 7, \(-OCH_2CH_3\)), 4.38 (2H, q, \( J \) 7, \(-OCH_2CH_3\)), 4.54 (2H, s, \(-CH_2Cl\)), 7.36 (2H, d, \( J \) 8, 3-H and 5-H), 8.01 (2H, d, \( J \) 8, 2-H and 6-H); \( m/z \) (EI) 198 ([M]+ 35Cl, 22%), 200 ([M]+ 37Cl, 7%), 153/155 ([MH]+ -OC\(_2\)H\(_5\), 100%).

1, 4, 7, 10-Tetraaza-1-N-(4-carboethoxyphenylmethyl) cyclododecane 39

To a stirred solution of CYCLEN (3g, 17.4 mmol) in dry chloroform (25 cm\(^3\)) was added the benzyl chloride 38 (2.38g, 11mmol) in dry chloroform (10cm\(^3\)) over 5 min. The resulting slurry was stirred at room temperature under an argon atmosphere for 36 hours. After this period, the slurry was applied to a wide bore silica column with chloroform, methanol and ammonium hydroxide (12:4:1) as the eluent with the mono-substituted macrocycle 39 obtained as a white solid (1.35g, 34 %), mp 69-71°C (from chloroform and hexane); \( \nu_{\text{max}} \) (Nujol)/cm\(^{-1}\) 3194, 3196 br (N-H), 2726, 1713 (C=O), 1658, 1555, 1354, 1284, 1104, 939, 877 and 722; \( \delta_H \) (300MHz; CDCl\(_3\); Me\(_4\)Si) 1.38 (3H, t, \( J \) 7, \(-OCH_2CH_3\)), 2.59 (8H, m, 11-H, 3-H, 9-H and 5-H), 2.70 (4H, m,2-H and 12-H), 2.82 (4H, m, 6-H and 8-H), 3.66 (2H, s, benzylic -CH\(_2\)), 4.34 (2H, q, \( J \) 7, \(-OCH_2CH_3\)), 7.38 (2H, d, \( J \) 8, benzylic 2-H and 6-H), 7.98 (2H, d, \( J \) 8, benzylic 3-H and 5-H).
Experimental Section

1, 4, 7-tris-(6-Methyl-2,2'-bipyridinyl)-10-(4-carboethoxyphenylmethyl)-1, 4, 7, 10-tetraazacyclododecane 36

The mono-functionalised cyclen 36 (264 mg, 0.79 mmol) and 1, 8-diazabicyclo-[5.4.0]-undec-7-ene (0.4 cm³, 2.61 mmol) in freshly distilled acetonitrile (10 cm³) was stirred at room temperature under an argon atmosphere. The bromomethyl bipyridine 30 (650 mg, 2.61 mmol) in dry acetonitrile (5 cm³) was added dropwise over a 30 minute period and the resulting solution was heated at 50°C for 1 week. The solution was allowed to cool and the solvent was removed in vacuo. The remaining residue suspended in 10% aqueous sodium hydrogen carbonate (5 cm³) and extracted with dichloromethane (4x10 cm³). The combined organics were dried over sodium sulphate, filtered and the solvent removed in vacuo. The remaining residue was purified by column chromatography (neutral alumina) using a dichloromethane with a methanol gradient (0-2%) as the eluent. The title compound was obtained as a pale yellow flaky solid (379 mg, 57%), mp 45-47°C; (Found: C. 70.6; H, 6.65; N, 16.2. C₅₁H₅₄N₁₀O₂.1.5H₂O requires 70.7; H, 6.6; N, 16.2); λ_max (CH₃OH)/nm 232 (ε/dm³mol⁻¹cm⁻¹ 53910), 283 (41650); ν_max (Nujol)/cm⁻¹ 1711 br (C=O), 1669, 1561, 1460, 1377, 1273, 1150, 1098, 774 and 722; δH (300 MHz; CDCl₃; Me₄Si), 1.36 (3H, t, J 7, ethoxy CH₃), 2.89 (16H, s, aza ring-CH₂), 3.54 (2H, s, benzyl -CH₂), 3.77 (6H, s, bipy-CH₂), 4.34 (2H, q, J 7, ethoxy-CH₂), 7.26 (3H, J 6 and 1, 5'-H), 7.48 (2H, d, J 8, benzoate 2-H and 6-H), 7.60 (3H, t, J 7.5, 5-H), 7.74 (6H, m, 4'-H and 4-H), 7.89 (2H, d, J 8, benzoate 3-H and 5-H),
Experimental Section

8.18 (3H, d, J 8, 3-H), 8.33 (3H, d, J 8.5, 3'-H), 8.65 (3H, dd, J 6 and 1, 6'-H); δc (75 MHz; CDCl₃) 14.5, 53.5, 60.0, 61.0, 61.7, 119.4, 121.3, 123.4, 123.7, 129.6, 128.9, 137.0, 137.4, 149.3, 155.6, 156.5 and 166.7; m/z (FAB) 839 ([MH]⁺, 36%), 861 ([M+Na]⁺, 100%).

1, 4, 7-tris-(6-Methyl-2,2'-bipyridinyl)-10-(4-carboethoxyphenylmethyl)-1, 4, 7, 10-tetraazacyclododecane tri-triflate Lanthanide Ln(36)

The substituted CYCLEN 36 (100 mg, 0.12 mmol) and the appropriate lanthanide triflate (0.13 mmol) was dissolved in freshly distilled acetonitrile (2 cm³) and refluxed overnight. The solution was cooled, passed through a celite plug and washed with acetonitrile (2 cm³) and ethanol (2 cm³). The filtrate solvent was removed under reduced pressure and re-dissolved in ethanol (0.5 cm³). The title complexes where crystallised by the slow diffusion of ether and the solids collected as white microcrystalline solids.

1, 4, 7-tris-(6-Methyl-2,2'-bipyridinyl)-10-(4-carboethoxyphenylmethyl)-1, 4, 7, 10-tetraazacyclododecane tri-triflate Europium (III) Eu(36)

(154 mg, 89%), mp 138-144°C; (Found: C, 43.7; H, 3.9; N, 9.1. C₅₁H₅₄N₁₀O₂Eu.3(CF₃SO₃).3H₂O requires 43.5; H, 4.05; N, 9.4); λmax (MeOH)/nm 235 (ε/dm³mol⁻¹cm⁻¹ 63480), 287 (50280); νmax (Nujol)/cm⁻¹ 3298 (br), 2725, 1715 (C=O), 1582, 1279, 1224, 1156, 1106, 1029, 830, 772 and 722; m/z (FAB) 1140 (([M]-2(CF₃SO₃), 24%), 1288 (([M]-CF₃SO₃), 5%).
1, 4, 7-tris-(6-Methyl-2,2'-bipyridinyl)-10-(4-carboethoxyphenylmethyl)-1, 4, 7, 10-tetraazacyclododecane tri-triflate Terbium (III) (36)
(162 mg, 93%), mp 142-144 °C; (Found: C, 42.8; H, 3.95; N, 9.2. C\textsubscript{51}H\textsubscript{54}N\textsubscript{10}O\textsubscript{2}.Tb. 3(CF\textsubscript{3}SO\textsubscript{3}). 3H\textsubscript{2}O requires C, 43.3; H, 4.0; N, 9.3); \(\lambda_{\text{max}}\) (MeOH)/nm 235 (\(\varepsilon/\text{dm}^3\text{mol}^{-1}\text{cm}^{-1}\) 63230), 287 (50850); \(\nu_{\text{max}}\) (Nujol) 3298 (br), 2725, 1716 (C=O), 1582, 1279, 1224, 1156, 1106, 1028, 830, 772 and 722; \(m/z\) (FAB) 1146 ([M]+-2(CF\textsubscript{3}SO\textsubscript{3}), 15%), 1295 ([M]+-CF\textsubscript{3}SO\textsubscript{3}), 8%).

tris-(Salicyaldehydato) Lanthanide Ln(\textsubscript{9})\textsubscript{3}\textsuperscript{112}

Salicylaldehyde (200 mg, 1.64 mmol) was stirred at room temperature with sodium hydroxide (60 mg, 1.5 mmol) in water (6.5 cm\textsuperscript{3}) and ethanol (1.5 cm\textsuperscript{3}). After a complete solution was obtained, the appropriate lanthanide chloride hexahydrate (0.5 mmol) in water (1 cm\textsuperscript{3}) was added dropwise over 20 minutes. A yellow flocculent precipitate formed throughout the addition. The mixture was stirred for a further 30 minutes, after which the solids were collected and washed several times with water to yield the title complexes as pale yellow powders.

tris-(Salicyaldehydato) Europium Eu(\textsubscript{9})\textsubscript{3}
(108 mg, 42%); mp 200-300°C (decomp); (Found: C, 48.6; H, 2.8. C\textsubscript{21}H\textsubscript{15}O\textsubscript{6}.Eu requires C, 48.95; H, 2.8); \(\lambda_{\text{max}}\) (CH\textsubscript{3}OH)/nm 379 (\(\varepsilon/\text{dm}^3\text{mol}^{-1}\text{cm}^{-1}\) 10000), 328 (6040); \(\nu_{\text{max}}\) (Nujol)/cm\textsuperscript{1} 1626 (C=O), 1529, 1330, 1154, 898, 760 and 722.
tris-(Salicyaldehydato) Terbium Tb(9)₃
(150 mg, 58%); mp 220-270 °C (decomp); (Found: C, 46.9; H, 2.75. C₂₁H₁₅O₆Tb.Na requires C, 46.3 H, 2.8); λ_max (CH₃OH)/nm 379 (ε/dm³mol⁻¹cm⁻¹ 6750), 258 (15190, 220 (21120); ν_max (Nujol)/cm⁻¹ 2721, 1630 (C=O), 1528, 1405, 1329, 1147, 899, 760 and 722.

tris-(Salicyaldehydato) Gadolinium Gd(9)₃
(188 mg, 72%); mp 220-300°C (decomp); (Found: C, 45.45; H, 2.8. C₂₁H₁₅O₆Gd requires C, 48.45; H, 2.9); λ_max (CH₃CN)/nm 380 (ε/dm³mol⁻¹cm⁻¹ 10820); ν_max (Nujol)/cm⁻¹ 1631 (C=O), 1529, 1337 1404, 1148, 1028, 760 and 735.

tris-(2-Phenoxybenzophenone) Lanthanide Ln(41)₃

tris-(2-Phenoxybenzophenone) Europium Eu(41)₃
(164mg, 88%); mp 210-258 °C (decomp); (Found: C, 60.4; H, 4.0; N, 1.6. C₃₉H₂₇O₈Eu.NH₃.H₂O requires C, 60.2; H, 4.1; N, 1.8); λ_max (CH₃OH)/nm 387

_ clo-Hydroxybenzophenone (149mg, 0.75mmol) and the appropriate lanthanide chloride hexahydrate (0.25mmol) was dissolved in ethanol (3 cm³) whilst stirring at room temperature. Ammonia gas was then bubbled through the solution causing a flocculent yellow solid to precipitate out of solution. Distilled water (5cm³) was added to complete precipitation. The solids were filtered and dried under vacuum over P₂O₅ to yield the title complexes as bright yellow powders.

tris-(2-Phenoxybenzophenone) Europium Eu(41)₃ (164mg, 88%); mp 210-258 °C (decomp); (Found: C, 60.4; H, 4.0; N, 1.6. C₃₉H₂₇O₈Eu.NH₃.H₂O requires C, 60.2; H, 4.1; N, 1.8); λ_max (CH₃OH)/nm 387
Experimental Section

\((\varepsilon/\text{dm}^3\text{mol}^{-1}\text{cm}^{-1} \, 9040), 258 (30100); v\text{max (Nujol)}/\text{cm}^{-1} \, 1606 (\text{C}=\text{O}), 1573, 1524, 1237, 1329, 1179, 1141, 940, 760 \text{ and } 700.\)

**tris-(2-Phenoxybenzophenone) Gadolinium Gd(41)₃**

(148mg, 79%); mp 215-225 °C (decomp); (Found: C, 60.5; H, 3.6; N, 0.9. \(\text{C}_{39}\text{H}_{27}\text{O}_{5}\text{Eu.NH}_{3}.\text{H}_{2}\text{O}\) requires C, 59.75; H, 4.1; N, 1.8); \(\lambda\text{max (CH}_{3}\text{OH)}/\text{nm} \, 388 (\varepsilon/\text{dm}^3\text{mol}^{-1}\text{cm}^{-1} \, 10530), 258 (30100); v\text{max (Nujol)}/\text{cm}^{-1} \, 1607 (\text{C}=\text{O}), 1573, 1524, 1240, 1180, 1141, 1112, 1029, 940, 760 \text{ and } 699.\)

**N, N', N''-tris-(p-Tolylsulphonyl)diethylenetriamine 50¹⁴⁶**

\[
\begin{array}{c}
\text{N} \quad \text{S} \quad \text{O} \\
\text{S} \quad \text{O} \\
\text{NH} \\
\text{N} \\
\text{S} \quad \text{O} \\
\text{S} \quad \text{O} \\
\end{array}
\]

To a mixture of tosyl chloride (285 g, 1.5 mol) in diethyl ether (300 cm³) and water (300 cm³) in an ice bath, was added slowly a solution of sodium hydroxide (60g, 1.5 mol in 400 cm³ of water) and diethylenetriamine (51.6 g, 0.5 mol) whilst stirring. The mixture was left to stir for 1 h at 0°C. The mixture was bought up to room temperature, stirred for a further 6 h and acidified with dilute hydrochloric acid (50 cm³). The solids were collected by vacuum filtration and washed with water, methanol and diethyl ether to give the title compound as a white crystalline solid (200g, 71%), mp 171-173°C (lit.,¹¹⁸ 173-175°C); \(v\text{max (Nujol)}/\text{cm}^{-1} \, 3291, 3020, 1598 (O=S=O), 1323, 1215, 1155, 1092 \text{ and } 814; \delta_H (300 MHz; \text{CDCl}_3; \text{Me}_4\text{Si}) 2.43 (9H, s, 3x-CH₃), 3.17 (8H, m, 4x-CH₂), 7.30 (6H, d, \text{J} \, 8), 7.61(2H, d, \text{J} \, 8.5), 7.76 (4H, d, \text{J} \, 8.5).\)
N, N', N''-tris-\(p\)-Tolylsulphonyl)diethylenetriamine N, N''-disodium salt 51

\[
\begin{align*}
\text{N, N', N''-disodium salt} \\
\end{align*}
\]

50 (190g, 0.34 mol) in ethanol (500cm\(^3\)) under nitrogen was heated to reflux whilst stirring. The heat source was removed and 1.5 N sodium ethoxide solution (15.42g of sodium metal in 500 cm\(^3\) of ethanol) was added as rapidly. The solution was decanted from any undissolved residue. The disodium salt crystallised upon standing overnight and was collected vacuum filtration as a white crystalline solid (170.54g, 82%).

1,2-bis-\(p\)-Toluenesulphonate) ethane 53

\[
\begin{align*}
\text{1,2-bis-}p\text{-Toluenesulphonate) ethane} \\
\end{align*}
\]

Ethylene glycol (43.45g, 0.7 mol) was taken in dichloromethane (140 cm\(^3\)) and cooled to 0°C in an ice bath. Triethylamine (350 cm\(^3\)) was added followed by the slow addition of tosyl chloride (293.52g, 1.54 mol) over a 3 h (so as to maintain the temperature at 5°C), and the mixture was allowed to stir overnight at room temperature. The triethylamine hydrochloride that formed was filtered off and washed with cold dichloromethane (3x50cm\(^3\)). The filtrate was reduced in volume (ca. 100cm\(^3\)), washed 3 times with water and the combined organics dried over sodium sulphate. The solvent was removed under reduced pressure and the crude product recrystallised from
dichloromethane and methanol to yield the title compound as white needles (141.12g, 54%), mp 116-118°C; ν_{max} (Nujol)/cm^{-1} 3021, 1591 (O=S=O), 1323, 1216, 1190, 1177, 929 and 814; δ_Η (300 MHz; CDCl_3; Me_4Si); 2.46 (6H, s, 2x-CH_3), 4.19 (4H, s, 2x-CH_2), 7.33 (4H, d, J 8), 7.75 (4H, d, J 8).

1, 4, 7-Triazacyclononane N, N', N'' tri-p-tosylate 54

51 (170g, 0.28 mol) was taken in DMF (2000 cm^3) and stirred whilst heating at 100°C. 53 (103.7g, 0.28 mol) was added slowly over an hour period, the solution was brought up to 110°C and stirred for a further 12 h. After which period, the heat source was removed and the solution allowed to cool. The volume of DMF was reduced and poured over crushed ice (1kg). The tosylated macrocycle was collected by vacuum filtration, washed with cold diethyl ether and dried in a vacuum oven before recrystallisation from dichloromethane and methanol, to yield the title compound as a white crystalline solid (107.12g, 69%), mp 221.223 °C (lit., 146 222-223°C); ν_{max} (Nujol)/cm^{-1} 3020, 2925, 1599 (O=S=O), 1451, 1347, 1215, 1161 and 815; δ_Η (300 MHz; CDCl_3; Me_4Si); 2.43 (9H, s), 3.42 (12H, s), 7.32 (6H, d, J 8), 7.70 (6H,d, J 8); m/z (FAB) 592 ([MH]^+, 100%), 614 ([M+Na]^+, 54%).
Experimental Section

1,4,7-Triazacyclononane 55

98% Sulphuric acid (100 cm³) was heated to 50°C whilst stirring under nitrogen. The tosylated macrocycle 54 (56.71g, 0.096mol) was added cautiously over 1h. The solution was brought up to 100°C and stirred for a further 48h at this temperature, yielding a brown viscous solution. The solution was cooled in an ice bath followed by the slow addition of ethanol (400 cm³), which caused the precipitation of the polyhydrosulfate salt. The salt was filtered under nitrogen, washed with ethanol and dried under vacuum.

The polyhydrosulfate salt was dissolved in the minimum amount of water (75cm³) followed by the slow addition of 48 % hydrobromic acid (200cm³), which caused a white precipitate to form. Ethanol (200cm³) was added and stirred for a further hour. The hydrobromide salt was filtered under nitrogen and washed with diethyl ether.

The hydrobromide salt was dissolved in the minimum amount of water (30cm³) followed by the addition of sodium hydroxide (11g, 0.28mol) in water (10cm³). The solution was stirred for a further hour. The solution was exhaustively extracted with dichloromethane (8x50 cm³), dried over sodium sulphate and the solvent removed in vacuo to yield the title macrocycle as white crystals, which were dried under vacuum over P₂O₅ (6.19g, 50%). mp 42-44°C (lit., 43-45°C); ν max (Nujol)/cm⁻¹ 3316 (br), 2929, 1660, 1558, 1456 and 1357; δ H (300 MHz; CDCl₃; Me₄Si) 1.96 (3H, s), 2.77 (12H, s); MS (EI) m/z 130 ([MH]+, 100%).
**Experimental Section**

2-Hydroxy-5-methyl benzaldehyde 47

![Structural formula of 2-Hydroxy-5-methyl benzaldehyde](image)

$p$-Cresol (10.81g, 0.1 mol), tin (IV) tetrachloride (2.61, 0.01 mol) and tri-n-butylamine (5.4 g, 0.04 mol) in toluene (25 cm³) was stirred for 30 minutes under an argon atmosphere at room temperature. After which period, paraformaldehyde (6.6 g, 0.22 mol) was added and then the mixture heated at 100°C for 8 hours. After cooling, the reaction mixture was poured into water (250 cm³) and acidified to pH 1-2 with 3M hydrochloric acid and extracted with diethyl ether (4x75 cm³). The organic layer was washed with brine, dried over sodium sulphate and the solvent removed in vacuo to yield a brown oil. The crude material was purified by steam distillation followed by column chromatography (silica gel) with dichloromethane and methanol (98:2) as the eluent to yield a straw coloured oil, which crystallises upon refrigeration. (1.98 g, 15%); mp 54-56°C, mp (lit., 147 55-57°C); (Found: C, 70.3; H, 5.7. C₈H₇O₂ requires C, 70.6; H, 5.9); ν_max (Nujol)/cm⁻¹ 3402 br (OH), 1662 (C=O), 1285, 1240, 1207, 1150, 931 and 733; λ_max (C₂H₅OH)/nm 259 (ε/dm³mol⁻¹cm⁻¹ 11150), 337 (4000); δ_H(300 MHz; CDCl₃; Me₄Si), 2.34 (3H, s), 6.89 (1H, d, 3-H, J 9), 7.28-7.35 (2H, in, 4-H and 6-H), 9.95 (1H, s, - OH), 10.93 (1H, s, - CHO).
In a round bottom flask fitted with a Soxhlet funnel containing 4Å molecular sieves and a reflux condenser, 55 (127 mg, 0.98 mmol) and paraformaldehyde (118 mg, 3.92 mmol) was stirred in dry acetonitrile (10 ml) at room temperature under an argon atmosphere and bought up to reflux for 2 hours. 2-Hydroxy-5-methyl benzaldehyde 47 (440 mg, 3.23 mmol) in acetonitrile (5 ml) was added and refluxed for a further 24 h. The solution was allowed to cool, the solvent removed in vacuo and the residue was extracted into dichloromethane and washed with saturated sodium carbonate solution. The combined organics were dried over sodium sulphate and the solvent removed in vacuo to yield a brown viscous oil. The crude product was purified by column chromatography (silica gel) using dichloromethane with a methanol gradient (0-10%) to yield a dark orange solid (346 mg, 67%). mp 66-68°C; (Found: C, 68.5; H, 7.3; N, 7.4. C<sub>33</sub>H<sub>39</sub>N<sub>3</sub>O<sub>6</sub>0.5H<sub>2</sub>O requires C, 68.0; H, 6.9; N, 7.2); ν<sub>max</sub> (Nujol) cm<sup>-1</sup> 3450 br (OH), 1648 (C=O), 1611, 1309, 1260, 1151, 1067 and 943; λ<sub>max</sub> (C<sub>2</sub>H<sub>5</sub>OH) nm 259 (ε/dm<sup>3</sup>mol<sup>-1</sup>cm<sup>-1</sup> 21500), 348 (8890); δ<sub>h</sub>(300 MHz, CDCl<sub>3</sub>, Me<sub>4</sub>Si), 2.23 (9H, s, 3-CH₃), 2.79 (12H, s, aza-CH₂), 3.71 (6H, s, bridge-CH₂), 7.20 (3H, s), 7.26 (3H, s), 9.21-9.67 (3H, broad s, -OH), 10.08 (3H, s, -CHO); δ<sub>c</sub>(75 MHz; CDCl<sub>3</sub>; Me<sub>4</sub>Si) 20.49, 46.29, 55.36, 121.69, 126.07, 128.36, 130.30, 138.07, 159.33 and 194.80; m/z (FAB) 574 ([MH]<sup>+</sup>, 100%), 596 ([M+Na]<sup>+</sup>, 8%).
Experimental Section

1, 4, 7-tris-[(3-Formyl-2-phenoxy-5-methylphenylmethyl)]-1, 4, 7-
triazacyclononane Lanthanide Ln(45)

The substituted TACN 45 (100mg, 0.17 mmol) was dissolved in methanol (10cm³) whilst stirring under argon at room temperature. Aqueous sodium hydroxide (1 cm³ of a 5.3x10⁻³M solution) was added and the solution stirred for a further 4 h. The appropriate lanthanide chloride salt (0.19 mmol) in methanol (7.5cm³) was added dropwise and the resultant solution heated at reflux whilst monitoring with UV-vis absorption and luminescence spectroscopy. After a period of time when constant luminescence and absorption measurements were obtained, the solution was allowed to cool and the solvent removed under reduced pressure. The remaining residue was redissolved in the minimum amount of methanol and the complexes obtained as yellow solids from the slow diffusion of diethyl ether.

1, 4, 7-tris-[(3-Formyl-2-phenoxy-5-methylphenylmethyl)]-1, 4, 7-
triazacyclononane Europium Eu(45)

(42 mg, 29%); (mp>300°C); νmax (Nujol)/cm⁻¹ 3400 br (OH), 2725, 1640 (C=O), 1541, 1258, 1167, 1120, 817 and 721; λmax (CH₃OH)/nm 298 (ε/dm³mol⁻¹cm⁻¹ 8000), 329 (8070), 380 (10000); m/z (FAB) 724 ([MH]+, 90%, ¹⁵¹Eu), 746 ([M+Na]+, 100%, ¹⁵¹Eu); HRMS (FAB) 724.1893, ([MH]+. C₃₃H₃₇N₃O₆¹⁵¹Eu requires 724.1895).
1, 4, 7-tris-[(3-Formyl-2-phenoxy-5-methylphenylmethyl)]-1, 4, 7-triazyacyclononane Terbium Tb(45) (86 mg, 67%); (mp>300°C); \( \nu_{\text{max}} \) (Nujol)/cm\(^{-1} \) 3401 br (OH), 2723, 1640 (C=O), 1542, 1260, 1167, 1076 and 723; \( \lambda_{\text{max}} \) (CH\(_3\)OH)/nm 298 (\( \varepsilon/\text{dm}^3\text{mol}^{-1}\text{cm}^{-1} \) 10100), 330 (9900), 391 (11760); \( m/z \) (FAB) 730 ([MH])\(^+\), 100%), 752 ([M+Na])\(^+\), 25%); HRMS (FAB) 730.1935, ([MH])\(^+\). \( \text{C}_{33}\text{H}_{37}\text{N}_{3}\text{O}_{6}\text{Tb} \) requires 730.1936).

1, 4, 7-tris-[(3-Benzoyl-2-hydroxy-5-methylphenyl)methyl]-1, 4, 7-triazyacyclononane 46

In a round bottom flask fitted with a Soxhlet funnel with 4Å molecular sieves and a reflux condenser, 55 (369 mg, 2.85 mmol) and paraformaldehyde (500mg, 16.6 mmol) was stirred in dry acetonitrile (50cm\(^3\)) at room temperature under an argon atmosphere and bought up to reflux for 2 hours. 2-Hydroxy-5-methylbenzophenone (2g, 9.42 mmol) in acetonitrile (10 cm\(^3\)) was added and refluxed for a further 48 hours. The solution was allowed to cool, the solvent removed \textit{in vacuo} and the residue was extracted into dichloromethane and washed with saturated sodium carbonate solution. The combined organics were dried over sodium sulphate and the solvent removed \textit{in vacuo} to yield a brown viscous oil. The crude product was purified by column chromatography (silica gel) using dichloromethane with a methanol gradient (0-10%) to yield a dark orange solid (974 mg, 43%). mp 73-75°C; (Found; C, 74.0 ; H, 6.4; N, 5.8. \( \text{C}_{51}\text{H}_{51}\text{N}_{3}\text{O}_{6}.0.5\text{H}_{2}\text{O} \) requires C, 73.9, H, 6.3; N, 5.7); \( \nu_{\text{max}} \) (Nujol)/cm\(^{-1} \) 3394 br (OH),
Experimental Section

1660 (C=O), 1622, 1598, 1339, 1230, 1149, 943 and 755; $\lambda_{\text{max}}$ (C$_2$H$_5$OH)/nm 253 (e/dm$^3$mol$^{-1}$cm$^{-1}$ 32820), 352 (10980); $\delta_{1H}$(300 MHz; CDCl$_3$; Me$_4$Si), 2.19 (9H, s, 3-CH$_3$), 2.85 (12H, s, aza-CH$_2$), 3.72 (6H, s, bridge-CH$_2$), 7.18 (6H, s), 7.41 (6H, d, $J_8$ and 1, 3'-H and 5'-H), 7.48 (3H, m, 4'-H), 7.71 (6H, m, 2'-H and 6'-H), 9.15-9.45 (3H, broad s, -OH); $\delta_{13C}$(75MHz; CDCl$_3$; Me$_4$Si) 20.68, 55.09, 57.51, 121.81, 126.44, 127.46, 128.38, 129.59, 130.10, 131.27, 131.71, 132.24, 136.39, 138.42, 158.98 and 200.26; m/z (FAB) 803 ([MH]$^+$, 100%), 825 ([M+Na]$^+$, 12%).

1, 4, 7-tris-[(3-Benzoyl-2-phenoxy-5-methylphenyl)methyl]-1, 4, 7-triazacyclononane Europium Eu(46)

The substituted TACN 46 (80mg, 0.1 mmol) was dissolved in methanol (7 cm$^3$) whilst stirring under argon at room temperature. Aqueous sodium hydroxide (1 cm$^3$ of a 3x10$^{-3}$ M solution) was added and the solution stirred for a further 4 hours. Europium chloride hexahydrate (40mg, 0.11 mmol) in methanol (2cm$^3$) was added dropwise and the resultant solution heated at reflux for 3 weeks. After this period of time, the solution was allowed to cool and the solvent removed under reduced pressure. The remaining residue was redissolved in the minimum amount of methanol and the title complex obtained as a bright yellow solid from the slow diffusion of diethyl ether. (50mg, 53%). mp>300°C; $\nu_{\text{max}}$ (Nujol)/cm$^{-1}$ 3400 br (OH), 1638 (C=O), 1594, 1542, 1339, 1287, 1159, 943 and 698; $\lambda_{\text{max}}$ (CH$_3$OH)/nm 240 (e/dm$^3$mol$^{-1}$cm$^{-1}$ 37620), 261 (36480) 390 (11310); m/z (FAB) 952 ([MH]$^+$, 100%), 974 ([M+Na]$^+$, 25%); HRMS (FAB) 952.2836, ([MH]$^+$, C$_{51}$H$_{49}$N$_3$O$_6$Eu requires 952.2834).
Experimental Section

2-Bromo-6-methylpyridine \(62^{148}\)

![Chemical Structure](image)

To a mechanically stirred solution of 2-amino-6-picoline (54g, 0.5 mol) in 48% aqueous hydrobromic acid (190 cm\(^3\)) stirring at -10°C, was added bromine (75 cm\(^3\), 1.5 mol) dropwise followed by the slow addition of aqueous sodium nitrite (87 g, 1.5 mol in 125 cm\(^3\) of water). The temperature was maintained below 0°C throughout both of the additions. The mixture was then neutralised with aqueous sodium hydroxide (189 g, 4.7 mol in 200 cm\(^3\) of water) then extracted with dichloromethane (3x 500 cm\(^3\)), dried over sodium sulphate and the solvent removed in vacuo to yield a brown oil. Purification by vacuum distillation afforded the title compound as a straw coloured oil (50.6 g, 60%) bp 36-37°C (0.5 mm Hg) [lit.,\(^{148}\) 62-65 °C (4mm Hg)]; \(v_{\text{max}}\) (Film)/cm\(^{-1}\) 3062, 2925, 1585, 1557, 1440, 1163, and 1123; \(\delta_H\) (300 MHz; CDCl\(_3\); Me\(_4\)Si) 2.53 (3H, s, 6-CH\(_3\)), 7.08 (1H, d, J 8, 5-H), 7.25 (1H, d, J 8, 3-H), 7.40 (1H, t, J 8, 4-H); \(m/z\) (EI) 173 ([M]\(^+\), 81Br, 40%), 171 ([M]\(^+\), 79Br, 40%), 92 ([M]\(^+\)-Br, 100%).

Nickel (II)1, 2-bis(diphenylphosphino)ethane dichloride \(65^{149}\)

![Chemical Structure](image)

To a warmed solution of 1, 2-bis(diphenylphosphino)ethane (2.16g, 5.4 mmol) in isopropanol (200 cm\(^3\)), was added a solution of nickel (II) chloride (1.26g, 5.3 mmol) in 2:1 isopropanol: methanol (120 cm\(^3\)), resulting in the formation of feathery orange crystals. After cooling the suspension, the crystals were collected, washed with diethyl
ether (2x10 cm³) and dried under vacuum (2.44g, 91%). This compound was used in all Grignard coupling reactions.

2-(2'-Methoxyphenyl)-6-methylpyridine 66₁⁵⁰

\[
\text{H}_3\text{C} - \text{N} \begin{array}{c}
\text{OMe} \\
\end{array} \text{O}
\]

To an ice cold solution of [Ni(dppe)Cl₂] (2.39g, 3 mmol) and 62 (12.20g, 70.9 mmol) in dry tetrahydrofuran (80 cm³) under argon was added the Grignard reagent prepared from 2-bromoanisole (16g, 85.5 mmol) and magnesium turnings (2.39g, 98.8 mmol) in dry tetrahydrofuran (80 cm³), maintaining a temperature of below 0°C. After the addition was complete, the reaction was allowed to attain room temperature and stirred for a further 12 h. The reaction was quenched 10% aqueous ammonium chloride (80 cm³), acidified with 10% hydrochloric acid and the organic solvent removed in vacuo. The acidic residue was extracted with dichloromethane (3x10 cm³), back washing the organic extract with dilute acid (10 cm³). The acidic layers were combined and basified to pH 10 with 10% potassium hydroxide and extracted into dichloromethane (4x30 cm³). The combined organics were dried over sodium sulphate, filtered and the solvent removed in vacuo to yield a dark brown oil. Purification by chromatography on silica using dichloromethane as the eluent afforded the title compound as a pale yellow oil (10.35g, 73%); (Found C: 77.8; H, 6.5; N, 7.0. C₁₃H₁₃NO requires C, 78.4; H, 6.6; N, 7.0); \(\nu_{\text{max}}\) (Film)/cm⁻¹ 3050, 2923, (COCH₃), 1601, 1587, 1503, 1463, 1437, 1261, 1243, 1179, 1148, 1062 and 1027; \(\delta_H\) (300 MHz; CDCl₃; Me₄Si) 2.61 (3H, s, 6'-CH₃), 3.83 (3H, s, 2'-OCH₃), 6.78 (1H, d, J₈, 6'-H), 7.04-7.09 (2H, m, 4'-H and 5-H), 7.35 (1H, td, J7.5 and 1.5, 5'-H), 7.58 (2H, m), 7.75 (1H, dd, J 7.5 and 1.5, 3-H); \(m/z\) (EI) 199 ([M]⁺, 100%), 170 ([MH]⁺-2CH₃, 70%), 168 ([M]⁺-OCH₃, 100%).
2-(2'-Methoxyphenyl)-6-bromomethylpyridine 67

![Chemical Structure](image)

The cross coupled anisole 66 (1g, 5 mmol), N-bromosuccimide (2.9g, 16mmol) and benzoyl peroxide (20 mg) was refluxed in carbon tetrachloride (35 cm³) for 6 h. The mixture was allowed to cool and the succinimide filtered off and the filtrate solvent removed in vacuo. The remaining residue was extracted into chloroform (4x20 cm³), washing with saturated sodium bicarbonate solution (2x10cm³). The combined organics were dried over sodium sulphate, filtered and the solvent removed under reduced pressure to yield the crude reaction product. Purification by column chromatography (silica) with dichloromethane as the eluent afforded the mono-bromide 67 as a white crystalline solid (0.22 g, 15%). mp 57-59°C (hexane); νₓ (Film)/cm⁻¹ 3063, 2953; δₓ (300 MHz; CDCl₃; Me₄Si) 3.85 (3H, s, -OCH₃), 4.62 (2H, s, -CH₂Br), 6.97(1H, dd, J8 and 1, 6'-H), 7.06 (1H, td, J8 and 1, 4'-H), 7.33 (2H, m, 5-H and 5'-H), 7.69 (1H, m, 3'-H), 7.71 (1H, t, J 7.5, 4-H), 7.76 (1H, td, J2.5 3-H).

2-(2'-Methoxyphenyl)-6-methylpyridine-N-oxide 68

![Chemical Structure](image)

To a solution of 66 (9.56g, 48 mmol) in glacial acetic acid (30 cm³) was added 30% hydrogen peroxide (7.5 cm³). The solution was heated at 80°C for 3 h followed by the addition of a further portion of H₂O₂ (7.5 cm³). The solution was heated for a further 12 h, allowed to cool and the volume reduced (ca 5cm³). Water (10cm³) was added followed by solid potassium carbonate until the acid had been neutralised. The slurry
Experimental Section

was extracted with dichloromethane (4x20 cm³) with the organics combined, dried over sodium sulphate, filtered and the solvent removed in vacuo to yield a waxy solid. The solid was triturated in diethyl ether and filtered to yield the N-oxide as an off white powder (7.04g, 68%). mp 144-146°C (from diethyl ether); ν max (Nujol)/cm⁻¹ 3020, 2926 (COCH₃), 1581, 1504, 1462, 1377, 1250, 1238 (N⁺-O'), 1070, 1023 and 780; δ H (300 MHz; CDCl₃; Me₄Si) 2.62 (3H, s, 6-CH₃), 3.79 (3H, s, 2-OCH₃), 6.98-7.06 (2H, m, Ar-H), 7.12-7.26 (3H, m, Ar-H), 7.32-7.44 (2H, m, Ar-H); m/z (EI) 216 ([MH⁺], 30%), 200 ([MH⁺-O], 100%).

2-(2'-Methoxyphenyl)-6-chloromethyl pyridine 74

![Chemical structure](image)

The N-oxide 68 (6g, 27.9 mmol) was taken in freshly distilled toluene (40cm³) under argon and heated to 50°C. Once the N-oxide had gone into solution, p-toluenesulphonyl chloride (5.85g, 30.7mmol) in dry toluene (40 cm³) was added dropwise and the solution heated at reflux for 12 h. The solution was allowed to cool and the toluene removed in vacuo. The remaining residue was dissolved in dichloromethane (20cm³) and washed with 10% sodium carbonate (2x5 cm³). The organic fractions were combined, dried and the solvent removed in vacuo. The oil that remained was purified by chromatography on silica using dichloromethane as the eluant to yield the title compound as yellow crystals (2.99g, 46%) mp 51-53°C (from hexane); (Found C, 66.7; H, 5.1; N, 5.9; Cl, 15.4. C₁₃H₁₂NOC₁ requires C, 66.8; H, 5.1; N, 6.0; Cl, 15.2); ν max (Film)/cm⁻¹ 3018, 2988, 1582, 1494, 1465, 1449, 1216, 1075, 1027 and 784; δ H (300 MHz; CDCl₃; Me₄Si) 3.86 (3H, s, 2-OCH₃), 4.75 (2H, s, 6-CH₂Cl), 6.99 (1H, dd, J 8 and 1, 6'-H), 7.08 (1H, td, J 8 and 1, 4'-H), 7.37 (2H, m, 5-H and 5'-H), 7.71 (1H, m, 3'-H), 7.91 (1H, m, 3'-H).
Experimental Section

7.73 (1H, t, J 7.5, 4-H), 7.78 (1H, td, J 2.5 3-H); m/z (EI) 234 ([MH]+, 35 Cl, 15%), 236 ([MH]+, 37 Cl, 5%), 200 ([MH]+, -Cl+H, 100%).

1, 4, 7-tris-[2-(2'-Methoxyphenyl)-6-methylpyridine]-1, 4, 7-triazacyclononane 75

The mono-chloride 74 (2.5 g, 10.7 mmol), 1, 4, 7-triazacyclononane 55 (0.46 g, 3.6 mmol) and triethylamine (2.16 g, 21.4 mmol) was stirred in freshly distilled acetonitrile (10 cm³) at room temperature under argon for 24 h. The organic solvent was removed in vacuo and the remaining residue was dissolved in dichloromethane (15 cm³) and washed with 10% sodium hydrogen carbonate. The combined organics were dried over sodium sulphate, filtered and the solvent removed in vacuo to yield a brown gummy solid. Purification by passing the crude material through a neutral alumina plug with 5% methanol in dichloromethane as the eluant yielded the title compound as an off white crystalline solid (2.45 g, 93%) mp 58-60°C (from methanol); (Found C, 72.1; H, 7.0; N, 11.4. C₄₅H₄₈N₆O₃.H₂O requires C, 72.3; H, 6.9; N, 11.2); ν max (Film)/cm⁻¹ 2935, 2835, 1601, 1572, 1493, 1463, 1307, 1242, 1026 and 754; λ max (CH₃CN)/nm 221 (ε/dm³mol⁻¹cm⁻¹ 40660), 247 (22960), 292 (20870); δ H (300 MHz; CDCl₃; Me₄Si) 2.99 (12H, s, aza-CH₂), 3.86 (9H, s, 3x2-CH₃), 3.95 (6H, s, bridge CH₂), 6.98 (3H, d, J 8), 7.05 (3H, t, J 8), 7.33 (td, 3H, J 9), 7.47 (3H, t, J 9), 7.65 (6H, d, J 8.5), 7.74 (3H, d, J 8.5); m/z (FAB) 721 ([MH]+, 52%), 743 ([M+Na]+), 100%).
1, 4, 7-tris-[2-(2' -Hydroxyphenyl)-6-methylpyridine]-1, 4, 7-triazacyclononane 60

Boron tribromide (35.5 mmol, 3.35 cm³) was added to freshly distilled dichloromethane (15 cm³) at -78 °C under an argon atmosphere whilst stirring. To this solution was added a solution of the aryl ether 75 (2.13 g, 2.96 mmol) in dry dichloromethane (15 cm³) and stirred over 24 h, allowing to attain room temperature. The reaction was quenched with 1M aqueous tartaric acid (10 cm³) and left to stir overnight. The solvent was removed in vacuo and the remaining residue was neutralised to pH 7 with 1M aqueous sodium hydroxide. The aqueous fraction was extracted several times with dichloromethane. The combined organics were washed with phosphate buffer at pH 7.5 (10 cm³), dried over sodium sulphate, filtered and the solvent removed in vacuo. Purification by chromatography on neutral alumina, using 5% methanol in dichloromethane, yielded the title compound as a white solid (1.36 g, 68%). mp 71-73°C; (Found C: 72.45; H: 6.15; N: 11.2; C₄₂H₂₂N₆O₃H₂O requires C: 72.4; H: 6.4; N: 11.0); 𝜇 max (Film)/cm⁻¹ 3019, 2988, 1668, 1596, 1568, 1462, 1413, 1299, 1216, 1176, 1047, 928 and 816; 𝜆 max (CH₃CN)/nm 219 (ε/dm³mol⁻¹cm⁻¹ 35500), 255 (30810), 298 (21540), 318 (24930); δ H (300 MHz; CDCl₃; Me₄Si), 2.91 (12, s, aza-CH₂), 3.85 (6H, bridge-CH₂), 6.97 (3H, t, J 8), 7.06 (3H, d, J 8), 7.31-7.40 (6H, m), 7.72-7.79 (9H, m), 13.40-13.48 (3H, broad s, 3-OH); δc (75 MHz; CDCl₃) 56.1, 64.1, 117.5, 118.8, 118.9, 119.1, 121.4, 126.4, 130.1, 138.4, 157.3 and 160.3; m/z (EI) 679 ([MH]⁺, 100%).
To a suspension of the ligand 60 (100 mg, 0.147 mmol) in anhydrous methanol (5 cm³) was added the appropriate lanthanide triflate salt (1.1 mmol) in methanol (2.5 cm³) and the resultant solution was refluxed for 48 h under an argon atmosphere. The solution was cooled to room temperature and passed through a celite plug, washing with methanol (2 cm³) and acetonitrile (2 cm³). The solvent was removed under reduced pressure and the remaining residue redissolved in ethanol (1 cm³) and the complex was crystallised out by the slow diffusion of diethyl ether as pale yellow solids.

1, 4, 7-tris-[2-(2'-Phenoxyphenyl)-6-methylpyridine]-1, 4, 7-triazacyclononane

Europium (III) Eu(60)

(87 mg, 71%), mp 119-121°C; \( \lambda_{\text{max}} \) (CH₃OH)/ 255 (ε/dm³mol⁻¹cm⁻¹ 36150), 288 (23010) 321 (24730); \( \nu_{\text{max}} \) (Nujol) 2725(vw), 1596, 1566, 1256, 1415, 1155, 1029, 1002, 817, 757 and 722; \( m/z \) (FAB) 829 ([M]+, 50%), 851 ([M+Na]+, 70%); HRMS (FAB⁺) 829.2378, ([M]+. C₄₂H₄₀N₆O₃¹⁵¹Eu requires 829.2374).

1, 4, 7-tris-[2-(2'-Phenoxyphenyl)-6-methylpyridine]-1, 4, 7-triazacyclononane

Terbium (III) Tb(60)

(69 mg, 56%), mp 135-140°C; \( \lambda_{\text{max}} \) (CH₃OH)/ 257 (ε/dm³mol⁻¹cm⁻¹ 33380), 288 (22900) 321 (24320); \( \nu_{\text{max}} \) (Nujol) 2725(vw), 1596, 1566, 1256, 1415, 1155, 1029, 1002, 817, 757 and 722; \( m/z \) (FAB) 835 ([M]+, 44%), 857 ([M]+Na, 55%); HRMS (FAB⁺) 835.2414, ([M]+. C₄₂H₄₀N₆O₃¹⁵¹Eu requires 835.2415).
Experimental Section

1, 4, 7-tris-[2-(2'-Phenoxyphenyl)-6-methylpyridine]-1, 4, 7-triazacyclononane
Gadolinium (III) Gd(60)

(84 mg, 86%), mp 137-148°C; \( \lambda_{\text{max}} \) (CH\(_3\)OH)/\( \text{nm} \) 255 (\( \varepsilon/\text{dm}^3\text{mol}^{-1}\text{cm}^{-1} \) 25630), 294 (16660) 322 (18440); \( \nu_{\text{max}} \) (Nujol) 2726(\( \nu\text{w} \)), 1591, 1566, 1507, 1256, 1215, 1155, 1029, 1002, 817, 757 and 722; \( m/\varepsilon \) (FAB\(^+\)) 834 ([MH]\(^+\), 20%), 857 ([M+Na]\(^+\), 35%), 1287 cluster ([M\(^+\)]\(^+\), + (CF\(_3\)SO\(_3\))\(_3\), 15%)

2-Bromo-4-trifluoromethyl phenol 79

\[ \text{CF}_3 \]
\[ \begin{array}{c}
\text{Br} \\
\text{OH}
\end{array} \]

\( p \)-Trifluorophenol (20g, 0.12 mol) was dissolved in glacial acetic acid (40 cm\(^3\)) whilst stirring at room temperature. Bromine (22.4g, 0.14 mol) in glacial acetic acid (40 cm\(^3\)) was added dropwise over a 10 minute period, and the resulting solution was left to stir for 48 h. The acetic acid was removed under reduced pressure, the remaining residue was neutralised with aqueous sodium carbonate. The neutral aqueous emulsion was extracted with dichloromethane (5x50cm\(^3\)), washed with brine (2x10cm\(^3\)) and the combined organics dried over sodium sulphate. The solvent was removed under reduced pressure yielded the title compound as a colourless oil (28.92g, 92%); \( \nu_{\text{max}} \) (Film)/cm\(^{-1}\) 3514 s (OH), 2980, 1610, 1505, 1320, 1126, 829 and 620; \( \delta_H \) (300 MHz; CDCl\(_3\); Me\(_4\)Si), 5.82 (1H, s, OH), 7.07 (1H, d, \( J \) 8.5, 6-H), 7.48 (1H, d, \( J \) 7, 5-H), 7.74 (1H, s, 3-H); \( m/\varepsilon \) (EI) 240 ([M]\(^+\) 79Br, 100%), 242 ([M]\(^+\) 81Br, 99%).
Experimental Section

2-Bromo-4-trifluoromethyl anisole 80

\[
\begin{array}{c}
\text{CF}_3 \\
\text{Br} \\
\text{OMe}
\end{array}
\]

The phenol 79 (13g, 0.054 mol) and potassium carbonate (24g, 0.17 mol) was stirred in dry THF (75 cm³) at room temperature under argon for 2h. Methyl iodide (7.2 cm³, 0.116 mol) was added and the mixture stirred for 72 h. The potassium carbonate was filtered off and the filtrate solvent removed in vacuo, the residue extracted into dichloromethane and washed with brine. The combined organics were dried over sodium sulphate, filtered and the solvent removed in vacuo to yield the title compound as a pale yellow oil (13.43g, 98%). ν max (Film)/cm⁻¹ 2953, 2845, 1611, 1505, 1326, 1274, 1123, 894, 816 and 619; δ H (300 MHz; CDCl₃; Me₄Si) 3.93 (1H, s, -OCH₃), 6.95 (1H, d, J 8.5, 6-H), 7.55 (1H, d, J 8.5, 3-H); m/z (EI) 254 ([M]+ 79Br, 100%), 256 ([M]+ 81Br, 98%).

2-(2'-Methoxy-5'-trifluoromethylphenyl)-6-methylpyridine 82

\[
\begin{array}{c}
\text{H}_3\text{C} \\
\text{N} \\
\text{OMe} \\
\text{CF}_3
\end{array}
\]

To an ice cold solution of [Ni(dppe)Cl₂] (0.8g, 1.5 mmol) and 62 (6.76g, 39 mmol) in dry tetrahydrofuran (30 cm³) under argon was added the Grignard reagent prepared from 2-bromo-5-trifluoromethylanisole (16g, 63 mmol) and magnesium turnings (1.68g, 63 mmol) in dry tetrahydrofuran (30 cm³), maintaining a temperature of below 0°C. After the addition was complete, the reaction was allowed to attain room temperature and stirred for a further 16 h. The reaction was quenched 10% aqueous
ammonium chloride (50 cm³), acidified with 10% hydrochloric acid and the organic solvent removed in vacuo. The acidic residue was extracted with dichloromethane (3x10 cm³), back washing the organic extract with dilute acid (10 cm³). The acidic layers were combined and basified to pH 10 with 10% potassium hydroxide and extracted into dichloromethane (4x30 cm³). The combined organics were dried over sodium sulphate, filtered and the solvent removed in vacuo to yield a dark brown oil. Purification by chromatography on silica using dichloromethane: hexane (9:1) as the eluent afforded the title compound as a pale yellow oil (5.24g, 51%) (Found C: 62.4; H, 4.4; N, 5.15. C₁₄H₁₂F₃NO requires C, 62.9; H, 4.5; N, 5.2); νmax (Film)/cm⁻¹ 3063, 2953, 2842, 1617, 1589, 1576, 1463, 1335, 1256, 1097, 819, 751 and 621; δH (300 MHz; CDC1₃; Me₄Si) 2.62 (3H, s, 6-CH₃), 3.89 (3H, s, 2-OCH₃), 7.03 (1H, d, J 8.5, 3'-H), 7.11 (1H, d, J 6.5, 5-H), 7.59(3H, in, 3'-H, 4'-H, 4-H), 8.04 (1H, s, 6'-H); m/z (EI) 267 ([M]+, 100%).

2-(2'-Methoxy-5'-trifluoromethylphenyl)-6-bromomethylpyridine 83

![Chemical structure](image)

The cross-coupled anisole 82 (3.3g, 0.012 mmol), N-bromosuccimide (2.5g, 0.014mmol) and benzoyl peroxide (100 mg) was refluxed in carbon tetrachloride (35 cm³) for 13h. The mixture was allowed to cool and the succinimide filtered off and the filtrate solvent removed in vacuo. The remaining residue was extracted into chloroform (4x20 cm³), washing with saturated sodium bicarbonate solution (2x10cm³). The combined organics were dried over sodium sulphate, filtered and the solvent removed under reduced pressure to yield the crude reaction product. Purification by column chromatography (silica) with dichloromethane and hexane (9:1) as the eluent afforded the mono-bromide 83 as a white crystalline solid (1.73 g, 42%). mp 73-75°C (hexane); (Found C: 48.5; H, 3.0; N, 3.9. C₁₄H₁₁BrF₃NO requires C, 48.6; H, 3.2; N, 4.05); νmax
(Film)/cm⁻¹ 3063, 2953; δH (300 MHz; CDCl₃; Me₄Si) 3.92 (3H, s, -OCH₃), 4.63 (2H, s, -CH₂Br), 7.07 (1H, d, J 8, 3-H), 7.45 (1H, dd, J 9, 4'-H), 7.63 (1H, d, J 9, 3'-H), 7.75 (2H, m, 5-H, 4-H), 8.10 (1H, s, 6'-H).

2-(2'-Methoxy-5'-trifluoromethylphenyl)-6-methylpyridine-N-oxide 84

![Chemical structure of 2-(2'-Methoxy-5'-trifluoromethylphenyl)-6-methylpyridine-N-oxide 84]

To a solution of 82 (5.96g, 22 mmol) in glacial acetic acid (30 cm³) was added 30% hydrogen peroxide (5 cm³). The solution was heated at 80°C for 3 h followed by the addition of a further portion of H₂O₂ (5 cm³). The solution was heated for a further 12 h, allowed to cool and the volume reduced to ca 2 cm³. Water (5 cm³) was added followed by solid potassium carbonate until the acid had been neutralised. The slurry was extracted with dichloromethane (4x20 cm³) with the organics combined, dried over sodium sulphate, filtered and the solvent removed in vacuo to yield a waxy solid. The solid was triturated in hexane and filtered to yield the N-oxide as an pale yellow powder (3.67g, 59%), mp 115-117°C (from hexane); (Found C, 59.1; H, 4.2; N, 4.85. C₁₄H₁₂F₃NO₂ requires C, 59.4; H, 4.3; N, 4.95); νmax (Nujol)/cm⁻¹ 3064, 2726 (COCH₃), 2360, 1581, 1594, 1462, 1376, 1259, 1238 (N'-O'), 1070, 1023, 958 and 728; δH (300 MHz; CDCl₃; Me₄Si) 2.57 (3H, s, 6-CH₃), 3.85 (3H, s, 2-OCH₃), 7.03 (1H, d, 3-H, J 8), 7.21 (3H, m, 3'-H, 4-H, 4'-H), 7.62 (1H, s, 6'-H), 7.68 (1H, d, J 9, 5-H).

2-(2'-Methoxy-5'-trifluoromethylphenyl)-6-chloromethyl pyridines 85

![Chemical structure of 2-(2'-Methoxy-5'-trifluoromethylphenyl)-6-chloromethyl pyridines 85]
The N-oxide 84 (1.2, 5.1 mmol) was taken in freshly distilled toluene (20 cm³) under argon and heated to 50°C. Once the N-oxide had gone into solution, p-toulenesulphonyl chloride (1.07 g, 5.61 mmol) in dry toluene (20 cm³) was added dropwise and the solution heated at reflux for 12 h. The solution was allowed to cool and the toluene removed in vacuo. The remaining residue was dissolved in dichloromethane (20 cm³) and washed with 10% sodium carbonate (2x5 cm³). The organic fractions were combined, dried and the solvent removed in vacuo. The remaining oil was purified by chromatography on silica using dichloromethane as the eluent to yield the title compound as yellow crystals (1.18 g, 33%) mp 68-70°C (from hexane); νmax (Nujol)/cm⁻¹ 3585, 2724, 2360, 1846, 1585, 1573, 1497, 1258, 1179, 1191, 1026, 886, 821, 740 and 696; δH (300 MHz; CDCl₃; Me₄Si) 3.91 (3H, s, 2'-OCH₃), 4.75 (2H, s, 6-CH₂Cl), 7.04 (1H, m, 3-H), 7.42 (1H, d, J 8, 4'-H), 7.63 (1H, d, J 9, 3'-H), 7.75 (2H, m, 5-H and 4-H), 8.10 (1H, s, 6'-H).

1,4,7-tris-[2-(2'-methoxy-5'-trifluoromethylphenyl)-6-methylpyridine]-1,4,7-triazacyclononane 87

![Chemical Structure]

The mono-chloride 85 (500 mg, 1.66 mmol), 1, 4, 7-triazacyclononane 55 (68 mg, 0.52 mmol) and triethylamine (317 mg, 0.32 cm³, 2.08 mmol) was stirred in freshly distilled acetonitrile (5 cm³) at room temperature under argon for 24 h. The organic solvent was removed in vacuo and the remaining residue was dissolved in dichloromethane (10 cm³)
and washed with 10% sodium hydrogen carbonate. The combined organics were dried over sodium sulphate, filtered and the solvent removed in vacuo to yield a brown gummy solid. Purification by passing the crude material through a neutral alumina plug with dichloromethane and methanol (95:5) as the eluent yielded the title compound as an off white crystalline solid (455mg, 95%) mp 58-61°C (from methanol); (Found C, 61.1; H, 4.7; N, 8.8. C₄₈H₄₅F₉N₆O₃. H₂O requires C, 61.1; H, 5.0; N, 8.9; ν max (Film)/cm⁻¹ 3373, 3054, 2953, 2842, 1671, 1617, 1588, 1573, 1507, 1459, 1460, 1335, 1270, 1031, 821 and 740; δH (300 MHz; CDCl₃; Me₄Si), 3.01 (12H, s, aza-CH₂), 3.88 (9H, s, 3x2-OCH₃), 3.96 (6H, s, bridge CH₂), 7.03 (3H, d, J 8.5, 3-H), 7.53 (3H, m, 4-H), 7.40-7.59 (3H, dd, J 9 and 2.5 5-H), 7.69 (6H, m, 4'-H and 6-H), 8.08 (3H, s, 6'-H); m/z (FAB) 925 ([MH]⁺, 100%), 947 ([M+Na]⁺, 65%).

 Attempted synthesis of 1, 4, 7-tris-[2-(2'-hydroxy-5'-trifluoromethylphenyl)-6-methylpyridine]-1, 4, 7-triazacyclononane 76

Boron tribromide (2.88 mmol, 0.28cm³) was added to freshly distilled dichloromethane (5 cm³) at -78 °C under an argon atmosphere whilst stirring. To this solution was added a solution of aryl ether 87 (247mg, 0.27 mmol) in dry dichloromethane (5 cm³) and stirred over 24 h, allowing to attain room temperature. The reaction was quenched with 1M aqueous tartaric acid (5cm³) and left to stir overnight. The solvent was removed in vacuo and the remaining residue was neutralised to pH 7 with 1M aqueous sodium hydroxide. The aqueous fraction was extracted several times with dichloromethane. The combined organics were washed with phosphate buffer at pH 7.5 (10 cm³), dried
over sodium sulphate, filtered and the solvent removed in vacuo. No organic material could be isolated from the aqueous layer even with exhaustive extraction with chloroform and pH adjustment.

2-Bromo-4-fluoroanisole 89

![Molecule of 2-Bromo-4-fluoroanisole](image)

2-Bromo-4-fluorophenol (10g, 0.052 mol) was dissolved in dry THF (60 cm³) and stirred at room temperature under argon. Sodium hydride (1.49g, 0.062 mol) was added slowly over a 30-minute period and stirred for a further 2 h. Methyl iodide (3.9 cm³) was added via a syringe and the resultant solution stirred for 48 h, after which period, the solvent was removed in vacuo. The remaining residue was extracted with chloroform (3x50 cm³) and washed with 10% aqueous potassium hydroxide. The combined organics were dried over sodium sulphate, filtered and the solvent removed in vacuo to yield a pale yellow oil (9.55g, 90%); νₐ₅ (Film)/cm⁻¹ 2927, 2824, 1592, 1493, 1260, 880 and 567; δH (300 MHz; CDCl₃; Me₄Si) 3.84 (3H, s, O-CH₃), 6.81 (1H, m, 6-H), 6.96 (1H, td, J₈ and 3, 5-H), 7.28 (1H, dd, J 7.5 and 3, 3-H); m/z (EI) 204 ([MH]⁺, 100%, ⁷⁹Br), 206 ([MH]⁺, 97%, ⁸¹Br).

2-(2'-Methoxy-5'-fluorophenyl)-6-methylpyridine 91

![Molecule of 2-(2'-Methoxy-5'-fluorophenyl)-6-methylpyridine](image)
To an ice cold solution of [Ni(dppe)Cl₂] (0.56g, 1.01 mmol) and 62 (4.28g, 0.025 mol) in dry tetrahydrofuran (30 cm³) under argon was added the Grignard reagent prepared from 2-bromo-5-fluoroanisole 89 (6g, 0.03 mol) and magnesium turnings (0.85g, 0.035 mol) in dry tetrahydrofuran (30 cm³), maintaining a temperature of below 0°C. After the addition was complete, the reaction was allowed to attain room temperature and stirred for a further 12 h. The reaction was quenched 10% aqueous ammonium chloride (30 cm³), acidified with 10% hydrochloric acid and the organic solvent removed in vacuo. The acidic residue was extracted with dichloromethane (3x10 cm³), back washing the organic extract with dilute acid (10 cm³). The acidic layers were combined and basified to pH 10 with 10% potassium hydroxide and extracted into dichloromethane (4x30 cm³). The combined organics were dried over sodium sulphate, filtered and the solvent removed in vacuo to yield a dark brown oil. Purification by chromatography on silica using dichloromethane as the eluent afforded the title compound as a deep red oil (4.07g, 75%). (Found C: 71.7; H, 5.45; N, 6.9. C₁₃H₁₂FNO requires C, 71.9; H, 5.7; N, 6.45); v (Film)/cm⁻¹ 1584, 1496, 1461, 1261, 1029, 809, 750 and 694; δH (300 MHz; CDCl₃; Me₄Si) 2.60 (3H, s, 6-CH₃), 3.81 (3H, s, 2-OCH₃), 6.91 (1H, dd, J 9 and 4.5, 3-H), 7.03-7.09 (2H, m), 7.59 (3H, m); m/z (EI) 218 ([MH]+, 100%).

2-(2'-Methoxy-5'-fluorophenyl)-6-methylpyridine-N-oxide 92

To a solution of 91 (2.5g, 12 mmol) in glacial acetic acid (10 cm³) was added 30% hydrogen peroxide (1 cm³). The solution was heated at 80°C for 3 h followed by the addition of a further portion of H₂O₂ (1 cm³). The solution was heated for a further 12 h, allowed to cool and the volume reduced to ca 2 cm³. Water (5 cm³) was added followed by solid potassium carbonate until the acid had been neutralised. The slurry was
extracted with dichloromethane (4x20 cm$^3$) with the organics combined, dried over sodium sulphate, filtered and the solvent removed in vacuo to yield a waxy solid. The solid was triturated in hexane and filtered to yield the N-oxide as an pale yellow powder (1.68g, 60%), mp 113-115°C (from hexane); (Found C, 66.35; H, 5.0; N, 5.8. C$_{13}$H$_{12}$FNO$_2$ requires C, 66.9; H, 5.2; N, 6.0); $\nu_{\text{max}}$ (Nujol)/cm$^{-1}$ 3064, 2726 (COCH$_3$), 2360, 1581, 1594, 1462, 1376, 1259, 1238 (N$^+$-O$^-$), 1070, 1023, 958 and 728; $\delta_{\text{H}}$(300 MHz; CDCl$_3$; Me$_4$Si) 2.61 (3H, s, 6-CH$_3$), 3.78 (3H, s, 2-OCH$_3$), 6.92 (1H, dd, 3-H, $J$ 8.5 and 3.5), 7.07-7.28 (5H, m).

2-(2'Methoxy-5'-fluorophenyl)-6-chloromethyl pyridine 93

![Chemical Structure]

The N-oxide 92 (1.2g, 5.1 mmol) was taken in freshly distilled toluene (20cm$^3$) under argon and heated to 50°C. Once the N-oxide had gone into solution, p-toluenesulphonyl chloride (1.07g, 5.61mmol) in dry toluene (20 cm$^3$) was added dropwise and the solution heated at reflux for 12 h. The solution was allowed to cool and the toluene removed in vacuo. The remaining residue was dissolved in dichloromethane (20cm$^3$) and washed with 10% sodium carbonate (2x5 cm$^3$). The organic fractions were combined, dried and the solvent removed in vacuo. The oil that remained was purified by chromatography on silica using dichloromethane as the eluent to yield the title compound as yellow crystals (0.69g, 53%) mp 43-45°C (from hexane); (Found C, 61.65; H, 4.3; N, 5.3. C$_{13}$H$_{11}$NFOCl requires C, 62.0; H, 4.4; N, 5.6); $\nu_{\text{max}}$ (Nujol)/cm$^{-1}$ 3585, 2724, 2360, 1846, 1585, 1573, 1497, 1258, 1179, 1191, 1026, 886, 821, 740 and 696; $\delta_{\text{H}}$ (300 MHz; CDCl$_3$; Me$_4$Si) 3.83 (3H, s, 2'-OCH$_3$), 4.73 (2H, s, 6-CH$_2$Cl), 6.93 (1H, dd, $J$ 8.5 and 4., 3-H), 7.05 (1H, m, 4'-H), 7.42 (1H, d, $J$ 7.5, 3'-H), 7.71-7.80 (2H, m); m/z (EI) 252 ([MH]$^+$, $^{35}$Cl, 100%), 253 ([MH]$^+$, $^{37}$Cl, 33%).
Experimental Section

1, 4, 7-tris-[2-(2'-Methoxy-5'-fluorophenyl)-6-methylpyridine]-1, 4, 7-triazacyclononane 94

The mono-chloride 93 (500 mg, 1.98 mmol), 1, 4, 7-triazacyclononane 55 (86 mg, 0.66 mmol) and triethylamine (400 mg, 0.56 cm³, 3.96 mmol) was stirred in freshly distilled acetonitrile (5 cm³) at room temperature under argon for 24 h. The organic solvent was removed in vacuo and the remaining residue was dissolved in dichloromethane (10 cm³) and washed with 10% sodium hydrogen carbonate. The combined organics were dried over sodium sulphate, filtered and the solvent removed in vacuo to yield a brown gummy solid. Purification by passing the crude material through a neutral alumina plug with 5% methanol in dichloromethane as the eluent yielded the title compound as an off white crystalline solid (480 mg, 94%). mp 46-48°C (from methanol); (Found C; 67.4; H, 6.05; N, 10.5. C₄₅H₄₅F₃N₆O₃.1.5 H₂O requires C, 67.5; H, 6.0; N, 10.2); νmax (Film)/cm⁻¹ 3020, 2837, 1586, 1573, 1498, 1460, 1352, 1031, 812 and 754; δH (300 MHz; CDCl₃; Me₄Si), 2.99 (12H, s, aza-CH₂), 3.79 (9H, s, 3x2-OCH₃), 3.94 (6H, s, bridge CH₂), 6.90 (3H, m, 3-H), 7.00 (3H, td, J8 and 3,4-H), 7.40-7.55 (12H, m); m/z (FAB) 775 ([MH]⁺, 100%), 797([M+Na]⁺, 65%); HRMS (FAB⁺) 775.3585, ([MH]⁺. C₄₅H₄₆F₃N₆O₃ requires 775.3584).
Experimental Section

1, 4, 7-tris-[2-(2'-Hydroxy-5'-fluorophenyl)-6-methylpyridine]-1, 4, 7-triazacyclononane 77

1.0 M Boron tribromide (6.12 mmol, 6.1 cm³) in dichloromethane (15 cm³) was stirred at -78 °C under an argon. To this solution was added a solution of aryl ether 94 (400mg, 0.52 mmol) in dry dichloromethane (10 cm³) and stirred over 24 h, allowing to attain room temperature. The reaction was quenched with 1M aqueous tartaric acid (10cm³) and left to stir overnight. The solvent was removed in vacuo and the remaining residue was neutralised to pH 7 with 1M aqueous sodium hydroxide. The aqueous fraction was extracted several times with dichloromethane. The combined organics were washed with phosphate buffer at pH 7.5 (10 cm³), dried over sodium sulphate, filtered and the solvent removed in vacuo. Purification by chromatography on neutral alumina, using 5% methanol in dichloromethane, yielded the title compound as a white solid (194mg, 51%). mp 98-99°C; \( \nu (\text{Film})/\text{cm}^{-1} \) 3398, 3020, 2877, 1584, 1596, 1570, 1472, 1464, 1350, 1299 and 820; \( \lambda_{\text{max}} (\text{CH}_2\text{CN})/\text{nm} \) 219 (ε/dm³mol⁻¹cm⁻¹ 35500), 254 (31820), 299 (21690), 325 (24610); \( \delta_{\text{H}} (300 \text{ MHz}; \text{CDCl}_3; \text{Me}_4\text{Si}) \), 2.93 (12, s, aza-\( \text{CH}_2 \)), 3.88 (6H, bridge -\( \text{CH}_2 \)), 6.93 (3H, dd, J 9.5 and 3.5, 3-H), 7.00 (3H, m), 7.45 (6H, dd, J 9.5 and 3.5), 7.68 (3H, d, J 8.5), 7.81 (3H, t, J 7); \( \delta_{\text{C}} (75\text{MHz}; \text{CDCl}_3) \) 55.9, 63.9, 111.9, 112.3, 118.3, 118.7, 119.2, 119.5, 119.6, 122.1, 138.7, 156.4 and 157.6; \( m/z \) (EI) 733 ([M-H]⁺, 100%).
To a suspension of the ligand 77 (40 mg, 0.055 mmol) in anhydrous methanol (2 cm$^3$) was added the appropriate lanthanide triflate salt (0.061 mmol) in methanol (2 cm$^3$) and the resultant solution was refluxed for 48 h under an argon atmosphere. The solution was cooled to room temperature and passed through a celite plug, washing with methanol (2 cm$^3$) and acetonitrile (2 cm$^3$). The solvent was removed under reduced pressure and the remaining residue redissolved in ethanol (1 cm$^3$) and the complex was crystallised out by the slow diffusion of diethyl ether as pale yellow solids.

1, 4, \textit{7-tris-[2-(2'-Phenoxy-5'-fluorophenyl)-6-methylpyridine]-1, 4, 7-triazacyclononane Lanthanide (III) Ln(77)}

![Diagram of the ligand](image)

1, 4, \textit{7-tris-[2-(2'-Phenoxy-5'-fluorophenyl)-6-methylpyridine]-1, 4, 7-triazacyclononane Europium Eu(77)}

(39 mg, 80%), mp 168-172°C; $\lambda_{\text{max}}$ (CH$_3$OH)/ 255 (e/dm$^3$/mol$^{-1}$/cm$^{-1}$ 29460), 288 (17120) 328 (21400); $\nu_{\text{max}}$ (Nujol)/cm$^{-1}$ 3391, 2723($\nu$), 1598, 1568, 1415, 1282, 1255, 1160, 1031, 1030, 819, 757 and 723; m/z (FAB) 883 ([MH$^+$], 18%), 906 ([M+Na$^+$], 7%).

1, 4, \textit{7-tris-[2-(2'-Phenoxy-5'-fluorophenyl)-6-methylpyridine]-1, 4, 7-triazacyclononane Terbium (III) Tb(77)}

(35 mg, 72%), mp 169-171°C; $\lambda_{\text{max}}$ (CH$_3$OH)/ 255 (e/dm$^3$/mol$^{-1}$/cm$^{-1}$ 34320), 288 (20240) 327 (25590); $\nu_{\text{max}}$ (Nujol)/cm$^{-1}$ 3391, 2723($\nu$), 1659, 1598, 1566, 1285, 1256, 1415, 1158, 1029, 1002, 818, 757 and 722; m/z (FAB) 889 ([MH$^+$], 15%).
1, 4, 7-tris-[2-(2'-Phenoxy-5'-fluorophenyl)-6-methylpyridine]-1, 4, 7-triazacyclononane Gadolinium (III) Gd(77)

(32mg, 68%), mp 159-161°C; $\lambda_{\text{max}}$ (CH$_3$OH)/ 253 (ε/dm$^3$mol$^{-1}$cm$^{-1}$ 22670), 287 (13730) 328 (14860); $\nu_{\text{max}}$ (Nujol) 3390, 2719(vw), 1661, 1599, 1566, 1285, 1254, 14175, 1158, 1027, 1000, 819, 757 and 725; m/z (FAB) 888 ([MH]$^+$, 20%), 1340 cluster ([MH]$^+$+Gd(CF$_3$SO$_3$)$_3$, 35%).

General synthetic procedure for the synthesis of mono-benzyl functionalised CYCLEN compounds

To a stirred solution of CYCLEN (3g, 17.4 mmol) in dry chloroform (25 cm$^3$) was added the appropriate benzyl halide (11mmol) in dry chloroform (10cm$^3$) over 5 min. The resulting slurry was stirred at room temperature under an argon atmosphere for 36 h. After this period, the slurry was applied to a wide bore silica column with chloroform, methanol and ammonium hydroxide (12:4:1) as the eluent. The compounds obtained by column chromatography were further purified by recrystallisation from chloroform and hexane.

1, 4, 7, 10-Tetraaza-1-N-(4-nitrophenyl)methylcyclododecane 97

A pale yellow solid (2.27g, 64 %). mp 105-107°C (mp lit., 128-129°C); (Found C, 58.6; H, 8.3; N, 22.4 C$_{15}$H$_{25}$N$_5$O$_2$ requires C, 58.6; H, 8.2; N, 22.8); $\nu_{\text{max}}$ (Nujol)/cm$^{-1}$ 3359, 3196 br (N-H), 2727, 1651, 1599 (NO$_2$), 1343, 1062, 939, 811 and 722; $\delta_{\text{H}}$ (300MHz; CDCl$_3$; Me$_4$Si) 2.58 (10H, m, aza crown-CH$_2$), 2.74 (4H, m, aza-crown -CH$_2$), 2.83 (5H, m, aza-crown -CH$_2$), 3.71 (2H, s, benzylic -CH$_2$), 7.59 (2H, d, J 8.5, 2-
H and 6-H), 8.21 (2H, d, J 8.5, 3-H and 5-H); m/z (EI) 308 ([MH]⁺, 100%), 330 ([M+Na]⁺, 8%).

1, 4, 7, 10-Tetraaza-1-N-[4-nitrophenyl] ethyl)cyclododecane 103

An orange solid (1.61g, 43%). mp 144-146°C (mp lit., 105 146.5-148.5°C); (Found C, 56.4; H, 8.7; N, 20.7 C₁₆H₂₇N₅O₂.H₂O requires C, 58.6; H, 8.6; N, 20.6); νₘₚₜ (Nujol)/cm⁻¹ 3358, 3196 br (N-H), 2727, 1652, 1600 (NO₂), 1512, 1460, 1377, 1344, 1062, 939, 811 and 722; δH (300MHz; CDCl₃; Me₄Si) 2.50 (4H, t, J 5.5, 2-H and 2-H), 2.60 (7H, br s, 11-H, 3-H, 9-H and 5-H), 2.72 (4H, t, J 5, 8-H and 6-H), 2.77 (2H, t, J 6.5, -CH₂(C₁)), 2.91 (2H, t, J 6.5, -CH₂(C₂)) 7.39 (2H, d, J 8.5, 2-H and 6-H), 8.13 (2H, d, J 8.5, 3-H and 5-H); m/z (EI) 322 ([MH]⁺, 100).

1, 4, 7, 10-Tetraaza-1-N-[4-phenylmethyl]cycloctadecane 110

A fine white powder (2.08g, 69%). mp 79-81°C; νₘₚₜ (Nujol)/cm⁻¹ 3248 br (N-H), 1652, 1558, 1576 1276, 1029, 934, 734 and 700; δH (300MHz; CDCl₃; Me₄Si) 2.54 (10H, m, aza-crown -CH₂), 2.68 (4H, m, aza-crown -CH₂), 2.80 (5H, m, aza-crown -CH₂) 3.61 (2H, s, benzylic -CH₂) 7.29 (5H, m, benzylic H); m/z (FAB) 263 ([MH]⁺, 100%), 285 ([M+Na]⁺, 8%).
Experimental Section

1, 4, 7-tris-[2-(2'-Methoxyphenyl)-6-methylpyridine]-10-[p-nitrobenzyl]-1, 4, 7, 10 tetraazacyclododecane 98

\[ \text{Experimental Section} \]

The mono-functionalised CYCLEN 97 (478mg, 1.56 mmol), the mono-chloride 74 (1.2g, 5.14 mmol) and triethylamine (0.52g, 5.14mmol, 0.7cm³) was refluxed in dry acetonitrile (20cm³) under an argon atmosphere for 72 h, during which time, a further portion of triethylamine (0.7cm³) was added. The solution was allowed to cool and the solvent removed in vacuo. The residue was extracted into dichloromethane and washed with saturated sodium carbonate solution. The combined organics were dried over sodium sulphate, filtered and the solvent removed under reduced pressure. The remaining residue was purified by column chromatography (silica) with chloroform, methanol and iso-propylamine (20:1:1) as the eluent to yield the title compound as pale yellow crystalline solid (1.10g, 78%). mp 75-77°C; \( \nu_{\text{max}} \) (Nujol)/cm\(^{-1} \) 2727, 1598, 1579, 1239, 1160, 1021, 853, 752 and 723; \( \delta_H \) (300MHz; CDCl\(_3\); Me\(_4\)Si) 2.63-2.85 (16H, aza-CH\(_2\)), 3.54-3.87 (17H, m, 3x-OCH\(_3\), 3x-CH\(_2\) pyr-bridge, -CH\(_2\) benzylic bridge), 6.93 (3H, d, J 8), 7.01 (3H, t, J 8), 7.30 (6H, m), 7.48-7.73 (11H, m), 7.98 (2H, d, J 8.5, benzylic H); \( m/z \) (FAB) 899 ([MH]\(^+\), 100%), 921 ([M+Na]\(^+\), 25%).
1, 4, 7-tris-[2-(2'-Hydroxyphenyl)-6-methylpyridine]-10-[4-nitrobenzyl]-1, 4, 7, 10
tetraazaacyclododecane 99

The tetra substituted CYCLEN 98 (400mg, 0.44mmol) was dissolved in dry
dichloromethane (5cm³) and stirred at -78°C under an argon atmosphere. 1M Boron
tribromide (5.28 cm³, 12 molar equivalents) was added rapidly and the mixture stirred
for 48 h, allowing to attain room temperature over this time. The reaction was
quenched cautiously with 10% aqueous tartaric acid (5cm³) and stirred for a further 6 h.
The mixture was neutralised with aqueous sodium hydroxide and the solvents were
removed in vacuo. The remaining residue was extracted into dichloromethane (5x5cm³)
and washed with phosphate buffer at pH7.5. The combined organics were dried over
sodium sulphate, filtered and the solvent removed under reduced pressure to yield the
title compound as a light brown crystalline solid (289 mg, 77%). mp 84-86°C; νmax
(Nujol)/cm⁻¹ 3402 br (OH), 1668, 1594, 1566, 1516, 1271, 1154, 1092, 854 and 755; δH
(300MHz; CDCl₃; Me₄Si) 2.64-2.83 (16H, aza-CH₂), 3.53-3.81 (8H, m, 4x-CH₂ bridge),
6.93 (3H, m) 7.08 (3H, t, J 8), 7.33-7.40 (6H, m), 7.48-7.73 (11H, m), 8.02 (2H, d, J 8,
3-H and 5-H benzyl); m/z (FAB) 857 ([MH]+ 100%), 879 ([M]+Na 16%).
Attempted synthesis of 1, 4, 7-tris-[2-(2'-hydroxyphenyl)-6-methylpyridine]-10-[4-aminophenyl]-1, 4, 7, 10-tetraazacyclododecane 100

The tetra-substituted CYLEN 99 (200mg, 0.23 mmol) was refluxed in ethanol (15 cm$^3$) whilst stirring under an argon atmosphere. Stannous chloride dihydrate (259 mg, 1.15 mmol) was added and the solution was heated for a further hour. The solution was cooled and poured into crushed ice (50g) and 3M HCl (10cm$^3$). The slurry was basified to pH 10 with EDTA in aqueous saturated sodium carbonate (5g in 10cm$^3$). The mixture was extracted with dichloromethane (5x20cm$^3$), dried over sodium sulphate, filtered and the solvent removed under reduced pressure to yield a brown gum, which was triturated in ether to yield a pale yellow powder (93 mg). mp 84-86°C; $\nu_{\text{max}}$ (Film)/cm$^{-1}$ 3667, 3584, 3018, 2952, 2401, 1668, 1596, 1461, 1216, 1092 and 924; $\delta_H$ (300MHz; CDCl$_3$; Me$_4$Si) 2.64-2.97 (16H, aza-CH$_2$), 3.67-3.72 (6H, m), 6.85 (6H, m), 7.25-7.40 (9H, m), 7.65-7.80 (6H, m); m/z (FAB) 722 ([MH]$^+$ 100%); the above information, especially MS, suggested that the compound isolated was the de-benzylated substrate 101 (m/z 722, [MH]$^+$, 100%).
Attempted synthesis of 1, 4, 7-tris-[2-(2'-methoxyphenyl)-6-methyl pyridine]-10-(4-nitrophenethyl)-1, 4, 7, 10-tetraazacyclododecane 105

The mono-substituted CYCLEN 103 (501 mg, 1.56 mmol), the mono-chloride 74 (1.2 g, 5.14 mmol) and triethylamine (520 mg, 0.7 cm$^3$, 5.14 mmol) was refluxed in freshly distilled acetonitrile (20 cm$^3$) 72 h. The mixture was allowed to cool and the solvent removed in vacuo. The residue was extracted with dichloromethane (5 x 10 cm$^3$) and water (10 cm$^3$), and the combined organics dried over sodium sulphate, filtered and the solvent removed in vacuo. The remaining residue was purified by column chromatography (silica gel) with chloroform, methanol and iso-propylamine (20:1:1) and the eluent (1.35 g). mp 82-90°C; $\nu_{\text{max}}$ (Film)/cm$^{-1}$ 3382, 2726, 1667, 1599, 1516, 1343, 1239, 1160, 1021, 853, 724; $^1$H-NMR indicated that the material isolated was not a single compound and was a mixture; m/z (FAB) 764 (100%); The above analysis suggests that the de-benzylated compound 106 was the major product under these reaction conditions, there is also evidence of p-nitrostyrene in the $^1$H-NMR spectra.
Experimental Section

The mono-functionalised CYCLEN 39 (501 mg, 1.5 mmol), the mono-chloride 74 (1.2 g, 4.95 mmol) and triethylamine (1.38 cm³, 9.9 mmol) was refluxed in dry acetonitrile under an argon atmosphere for 72 h. The mixture was allowed to cool and the solvent removed under reduced pressure. The residue was extracted with dichloromethane (5 x 10 cm³) and water (10 cm³), the combined organics dried over sodium sulphate, filtered and the solvent removed in vacuo. The residue was purified by column chromatography (silica) with 15% methanol in chloroform as the eluent (1.06 g, 77%). mp 68-70 °C; ν max (Film) cm⁻¹: 3382, 2940, 2837, 1712 (C=O), 1602, 1499, 1464, 1278, 1047, 820 and 877; δH (300 MHz; CDCl₃; Me₄Si) 1.39 (3H, t, J 7.3 Hz, -OCH₂CH₃), 2.70 (4H, m), 2.93 (12H, m), 3.65-3.77 (11H, m, -CH₂ benzyl, 3x- OCH₃), 4.04 (6H, m, 3x-CH₂ pyr), 4.36 (2H, q, J 7 Hz, -OCH₂CH₃), 6.98 (9H, m), 7.16 (2H, d, J 8,
2-H and 6-H benzyl), 7.36 (3H, m), 7.59-7.72 (9H, m), 7.99 (2H, d, J 8, 3-H and 5-H benzyl); m/z (FAB) 926 ([MH]+ 100%).

1, 4, 7-tris-[2-(2'-Hydroxyphenyl)-6-methylpyridine]-10-[4-carboxethoxy phenyl methyl]-1, 4, 7, 10-tetraazacyclododecane 108

The substituted CYCLEN 107 (550mg, 0.59 mmol) was added to a solution of 1 M boron tribromide in dichloromethane (7.1 cm³) whilst stirring at -78°C under an argon atmosphere. The mixture was stirred for a further 24 h, allowing to attain room temperature over that period of time. The reaction was quenched with saturated tartaric acid solution (5cm³) and stirred for a further 12 h. The mixture was neutralised with aqueous sodium hydroxide, solvent was removed in vacuo and the residue extracted into dichloromethane (5x5 cm³) and washed with phosphate buffer at pH 7.5. The combined organics were dried over sodium sulphate, filtered and the solvent removed under reduced pressure to yield the carboxylate product. Re-esterification of the product was achieved by refluxing the crude material in ethanol (10cm³) with thionyl chloride (1cm³). The solution was cooled, and the solvent removed in vacuo. The remaining residue was extracted into dichloromethane and washed with phosphate buffer at pH 7.5, dried over sodium sulphate, filtered and the solvent removed under reduced pressure to yield the title compound (265 mg, 51%). mp 80-82°C; (Found C, 73.7; H, 6.5; N, 11.4. C₅₄H₆₇N₇O₅ requires C, 73.4; H, 6.50; N, 11.1); νₑₐₓ (Nujol)/cm⁻¹ 2725, 1712 (C=O), 1593, 1565, 1299, 1274, 1210, 1171, 1097, 1019, 874, 752 and 724; δₜ
Experimental Section

(300MHz; CDCl3; Me4Si) 1.38 (3H, t, J 7, -OCH2CH3), 2.81 (16H, br m, aza-crown-CH2), 4.04 (6H, s, 3x-CH2 bridge), 4.38 (2H, J 7, -CH2OEt), 6.90-7.0 (6H, m), 7.24 (2H, d, J 7, 2-H and 6-H benzyl), 7.28 (3H, m), 7.58 (9H, m), 7.83 (2H, J 7, 3-H and 5-H, benzyl); δc (75 MHz; CDCl3) 14.6, 53.0, 60.5, 61.1, 118.5, 118.8, 118.9, 126.4, 129.5, 129.6, 129.7, 131.6, 138.3, 138.4 and 159.8; m/z (FAB) 884 ([MH]+, 100%), 906 ([M+Na]+, 12%).

1, 4, 7-tris-[2-(2'-Phenoxyphenyl)-6-methylpyridine]-10-[4-carboethoxyphenyl methyl]-1, 4, 7, 10-tetraazacyclododecane Terbium Tb(108)

The functionalised Cyclen 108 (100mg, 0.11mmol) was dissolved in dry acetonitrile (2cm3) and stirred under an argon atmosphere. Terbium triflate (75 mg, 0.12 mmol) in ethanol (2cm3) was added and the solution refluxed for 72 h. The solution was allowed to cool and passed through a celite plug, washing with cold acetonitrile (2cm3) and ethanol (2cm3). The filtrate solvent was removed in vacuo and the residue was dissolved in the minimum amount of ethanol (1cm3) and the complex was obtained by the slow diffusion of ether as a pale yellow solid (88mg, 77%). mp 165-168°C; λmax (CH3OH)/nm 257(ε/dm3mol⁻¹cm⁻¹ 28350), 288 (28130), 325 (27740); νmax (Nujol)/cm⁻¹ 2725, 1712 (C=O), 1596, 1567, 1279, 1261, 1168, 1029, 854, 829, 756 and 722; m/z (FAB) 1040 ([MH]+, 100%); HRMS (FAB⁺) 1040.3518 ([MH]⁺ C54H55N7O5Tb requires 1040.3541.
The mono-functionalised CYCLEN 110 (169 mg, 0.65 mmol), the mono-chloride 74 (500 mg, 2.13 mmol) and triethylamine (0.33 cm$^3$, 2.34 mmol) was refluxed in dry acetonitrile under an argon atmosphere for 24 h. The mixture was allowed to cool and the solvent removed under reduced pressure. The residue was extracted with dichloromethane (5x5 cm$^3$) and water (5 m$^3$), the combined organics dried over sodium sulphate, filtered and the solvent removed in vacuo. The residue was purified by column chromatography (silica) with dichloromethane and methanol (85:15) as the eluent as an off white crystalline solid (341 mg, 61%). mp 68-70°C; $\nu_{max}$ (Film)/cm$^{-1}$ 3391, 2838, 1602, 1587, 149, 1465, 1278, 1027, 929, 757 and 668; $\delta_H$ (300 MHz; CDCl$_3$; Me$_4$Si) 3.03 (16H, in aza-CH$_2$), 3.74 (11H, m, -CH$_2$ benzyl, 3x-OCH$_3$), 4.05 (6H, in, 3x-CH$_2$ pyr), 6.89-6.99 (6H, m), 7.10 (3H, d, J 8), 7.19 (3H, m), 7.28-7.37 (5H, m, benzyl H) 7.40-7.52 (9H, m); m/z (FAB) 855 ([MH]$^+$ 100%).
The substituted CYCLEN 111 (320mg, 0.37 mmol) in dry dichloromethane (5cm³) was added to a solution of 1 M boron tribromide in dichloromethane (4.5 cm³) whilst stirring at -78°C under an argon atmosphere. The mixture was stirred for a further 24 h, allowing to attain room temperature over that period of time. The reaction was quenched with saturated tartaric acid solution (5cm³) and stirred for a further 12 h. The mixture was neutralised with aqueous sodium hydroxide, solvent was removed in vacuo and the residue extracted into dichloromethane (5x5 cm³) and washed with phosphate buffer at pH 7.5. The combined organics were direct over sodium sulphate, filtered and the solvent removed in vacuo. The remaining residue was purified by column chromatography (silica) with dichloromethane and methanol (85:15) as the eluent to give the title compound as a pale brown solid (176 mg, 58%). mp 78-80°C; (Found C, 75.55; H, 6.5; N, 11.7. C₅₁H₅₃N₇O₃ requires C, 75.4; H, 6.6; N, 12.1); λ_max (CH₃OH)/nm 260 (ε/dm³mol⁻¹cm⁻¹ 27140), 299 (20080), 319 (20960); ν_max (Film)/cm⁻¹ 3019 br (OH), 2811, 1591, 1568, 1461, 1413, 1216, 1094, 1019, 874, 750 and 669; δ_H (300MHz; CDCl₃; Me₄Si) 2.80 (16H, in, aza-CH₂), 3.40 (2H, s, -CH₂ benzyl), 3.66 (6H, s, 3x-CH₂ pyr), 6.87 (3H, t, J 8), 7.00 (3H, t, J 8), 7.10-7.38 (11H, m, 5H benzyl), 7.52-7.78 (9H, m); δ_C(75 MHz, CDCl₃) 51.4, 56.1, 61.3, 64.9, 109.8, 110.0, 111.4, 117.8, 118.1, 118.5, 120.8, 122.7, 127.2, 127.8, 127.9, 136.1, 136.5, 137.5, 138.4, 152.2, 157.1 and 158.5; m/z (FAB) 812 ([MH]+, 100%), 834 ([M+Na]+, 12%).
The functionalised CYCLEN 112 (60mg, 0.074mmol) was dissolve in dry acetonitrile (1cm³) and stirred under an argon atmosphere. The appropriate lanthanide triflate (0.081 mmol) in methanol (1cm³) was added and the solution refluxed for 72 h. The solution was allowed to cool and passed through a celite plug, washing with cold acetonitrile (2cm³) and methanol (2cm³). The filtrate solvent was removed in vacuo and the residue was dissolved in the minimum amount of ethanol (1cm³) and the complexes were obtained by the slow diffusion of ether as a pale yellow solid.

An off white solid (55 mg, 77%). mp 192-195°C; (Found C, 54.9; H, 4.7; N, 8.6. C₅₁H₅₀N₇O₃Tb.H₂O. CF₃SO₃ requires C, 55.0; H, 4.6; N, 8.6); λₘₐₓ (CH₃OH)/nm 257 (ε/dm³mol⁻¹cm⁻¹ 43900), 326 (32330); νₘₐₓ (Nujol)/cm⁻¹ 2723, 2811, 1595, 1277, 1157, 1072, 1030, 757 and 722; m/z (FAB) 968 ([MH]⁺, 100%); HRMS (FAB⁺) 968.3310 ([MH]⁺ C₅₁H₅₁N₇O₃Tb requires 968.3307.)
Experimental Section

1, 4, 7-tris-[2-(2'-Phenoxyphenyl)-6-methylpyridine]-10-[4-benzyl]-1, 4, 7, 10-tetraazacyclododecane Gadolinium Gd(112)

An off white solid (48 mg, 67%). mp 178-180°C; λ_{max} (CH_{3}OH)/nm 257 (ε/dm^3 mol^{-1} cm^{-1} 43900), 326 (32330); ν_{max} (Nujol)/cm^{-1} 2724, 2809, 1593, 1277, 1157, 1072, 1029, 757 and 721; m/z (FAB) 967 ([MH]^+, 100%); HRMS (FAB') 967.3294 ([MH]^+ C_{51}H_{51}N_{7}O_{3}Gd requires 967.3294.

8-Methoxy-2-methylquinoline 116

8-Hydroxy-2-methylquinoline (10 g, 0.065 mol) was dissolved in freshly distilled THF (50 cm^3) and stirred at room temperature under an argon atmosphere. To this, sodium hydride (2g, 0.085 mol) was added slowly over a period of 1 h. The mixture was left to stir at room temperature for 3 h. After which period, methyl iodide (3.1 g, 0.105 mol) was added via a syringe and the mixture was stirred for a further 14 h. The mixture was then poured into ice/2 M sodium hydroxide solution (100g/50 cm^3 respectively), stirred for 1 h, extracted into dichloromethane and washed several times with 2M sodium hydroxide. The combined organics were dried over sodium sulphate, filtered and the solvent removed in vacuo to yield a green crystalline solid. The solid was recrystallised from ethyl acetate and 40:60 petroleum ether to give the O-methylated compound 116 as a white crystalline solid (11.26g, 75%). mp 127-129°C; ν_{max} (Nujol)/cm^{-1} 3066, 1636, 1604, 1333, 1264, 1240, 1111, 836, 769, 761 and 719; δ_{H}(300 MHz; CDCl_{3}; Me_{4}Si) 2.80 (3H, s, -CH_{3}), 4.08 (3H, s, -OCH_{3}), 7.02 (1H, d, J 7, 3-H), 7.30-7.43 (3H, m, 5-H, 6-H and 7-H), 8.02 (1H, d, J 8, 4-H).
Attempted synthesis of 8-methoxy-2-bromomethylquinoline 117

8-Methoxy-2-methyl quinoline 116 (1g, 5.78 mmol), N-bromosuccinimide (3.08g, 17.34 mmol) and benzoyl peroxide (70mg) were refluxed in carbon tetrachloride (25 ml) for 8 hours. The reaction mixture was cooled to room temperature, the succinimide filtered off and the solvent removed in vacuo. The residue was purified by column chromatography (silica gel) with dichloromethane and methanol as the eluent (98:2) to yield 8-methoxy-5-bromo-2-methyl quinoline 118 (0.35g); $\delta_{\text{H}}$(300 MHz; CDCl$_3$; Me$_4$Si) 2.93 (3H, s), 4.08 (3H, s), 6.93 (1H, d, J 8, 3-H), 7.40 (1H, d, J 7, 6-H), 7.67 (1H, d, J 7, 7-H), 8.39 (1H, d, J 8, 4-H); $m/z$ (EI) 253/251 ([M]$^+$50:50%) and 8-methoxy-5-bromo-2-bromomethyl quinoline 119 (0.28g); $\delta_{\text{H}}$(300 MHz; CDCl$_3$; Me$_4$Si) 4.08 (3H, s, -OCH$_3$), 4.79 (2H, s, -CH$_2$Br), 6.96 (1H, d, J 8, 3-H), 7.73 (2H, m, 7-H and 6H), 8.53 (1H, d, J 8, 4-H); $m/z$ (EI) 333/331/329 ([M]$^+$50:90:50%) as the only reaction products along with a minimal amount of the starting material.

Attempted synthesis 8-methoxy-2-methyl quinoline N-oxide 120
8-Methoxyquinaldine 116 (3.5g, 0.02 mol) was dissolved in glacial acetic acid (15cm³) and 30% aqueous hydrogen peroxide (1cm³) was added. The solution was heated at 80°C for 24 hours with an additional portion of hydrogen peroxide (1cm³) added during this time. The solution was allowed to cool and the acetic was removed under reduced pressure. The remaining residue was suspended in water (10cm³) and the mixture neutralised cautiously with solid sodium carbonate. The aqueous suspension was extracted exhaustively with dichloromethane (7x10cm³), dried over sodium sulphate, filtered and the solvent removed in vacuo to yield a brown sticky gum (3.12g). $\delta_H$(300 MHz; CDC13; Me4Si) 2.47(3H, s, -CH3), 3.82 (3H, s, -OCH3), 7.08 (1H, d, J8 3-H), 7.25-7.53 (4H, m); $m/z$ (EI) 189 ($[M]^+$ 30%). The material isolated was not a single spot by tlc and could not be purified by column chromatography.

**Attempted synthesis of 8-methoxy-2-chloromethyl quinoline 121**

![Chemical structure of 8-methoxy-2-chloromethyl quinoline](image)

The crude 8-methoxy quinaldine N-oxide 120 (2g, 0.011 mol) was refluxed in toluene (30 cm³) under dry nitrogen with tosyl chloride (6.03g, 0.032 mol) for 96 h with Dean and Stark apparatus. The solution was allowed to cool to room temperature and the toluene removed in vacuo. Mass spectral analysis of the crude residual material showed none of the required compound present.

**8-[2, 2-Dimethylpropanoyloxy]-2-methyl quinoline 122**

![Chemical structure of 8-[2, 2-Dimethylpropanoyloxy]-2-methyl quinoline](image)
8-Hydroxyquinaldine (20 g, 0.13 mmol) was dissolved in dry dichloromethane (250 cm³) and triethylamine (20.94 g, 0.21 mol) and stirred under argon at 0°C. Pivaloyl chloride (16.7 g, 0.138 mol) was added dropwise over one hour and stirred for a further 72 h, allowing the mixture to attain room temperature. The solvent was removed in vacuo and the residue extracted with dichloromethane and water. The organics were then washed with 2M sodium hydroxide, dried over sodium sulphate, filtered and the solvent removed in vacuo. The brown oil that remained was triturated in hexane, cooled overnight and the solid that remained was collected by vacuum filtration to yield the protected quinaldine 122 as a white crystalline solid (26.54 g, 86%). mp 49-51°C; (Found C, 74.3; H, 7.1; N, 6.1. C₁₅H₁₇NO₂ requires C, 74.0; H, 7.0; N, 5.8); νₘₐₓ (Film)/cm⁻¹ 1753 (C=O), 1703, 1505, 1472, 1107 (C-O); δₓ (300 MHz; CDCl₃; Me₄Si) 1.51 (9H, s, 'Bu), 2.67 (3H, s, 2-CH₃), 7.24 (1H, d, J 8, 3-H), 7.36 (1H, dd, J 7 and 1, 5-H), 7.43 (1H, t, J 8 and 1, 7-H), 7.63 (1H, dd, J 8 and 1, 7-H), 8.01 (1H, d, J 8, 4-H); m/z (EI) 243 ([M⁺] 100%).

8-[2, 2-Dimethylpropanoyloxy]-2-bromomethyl quinoline 123

![](image)

The protected quinoline 122 (20 g, 0.082 mol), N-bromosuccinimide (16.56 g, 0.92 mol) and benzoyl peroxide (200 mg) in carbon tetrachloride (200 cm³) was refluxed for 24 h. After which period, the mixture was allowed to cool, the insoluble material filtered off and the solvent removed in vacuo. The residue that remained was split into two fractions and each was purified by column chromatography (silica) with dichloromethane as the eluent to yield the mono-bromide 123 as a white crystalline solid (4.11 g, 16%). mp 58-60°C (from hexane); νₘₐₓ (Nujol)/cm⁻¹ 2360, 1753 (C=O), 1703, 1598, 1568, 1275, 1107, 838, 762 and 721; δₓ (300 MHz; CDCl₃; Me₄Si) 1.52
Experimental Section

\[ \text{9H, s, -}^\text{t} \text{Bu}, 4.63 (2H, s, -CH}_2\text{Br), 7.42 (1H, d, J 9, 3-H), 7.55 (2H, m, 5-H and 6-H),} \]
\[ \text{7.67 (1H, dd, J 8.5 and 1, 7-H), 8.13 (1H, d, J 9, 4-H);} \]
\[ m/z \text{ (EI) 323/321 ([M]^+ 81Br, 79Br, 100%).} \]

1, 4, 7-tris-[8-(2,2-Dimethylpropanoyloxyquinoline)methyl]-1, 4, 7-triazacyclononane 124

1, 4, 7-Triazacyclononane 55 (376mg, 2.91 mmol), 123 (3g, 9.31 mmol) and triethylamine (1.62cm³, 11.64 mmol) was stirred in dry acetonitrile (20cm³) for 24 h. After which period, the solvent was removed in vacuo and the residue extracted with dichloromethane and 10% aqueous sodium carbonate. The combined organics were dried were dried over sodium sulphate, filtered and the solvent removed in vacuo to yield an off white flocculent solid. The crude material was purified by column chromatography (neutral silica) with dichloromethane and methanol (9:1) as the eluent to give the title compound as a yellow oil. The oil was triturated in hexane to give a fine pale yellow solid (1.31g, 53 %). mp 61-63°C; (Found C, 67.6; H, 7.3; N, 9.3.
\[ C_{51}H_{60}N_{6}O_3 \text{ requires C, 67.5; H, 7.3; N, 9.3);} \]
\[ \nu_{\text{max}} \text{(Film)/cm}^{-1} \text{ 3404, 1747 (C=O), 1704, 1600, 1571, 1507, 1472, 1216, 1123, 1027 841 and 667; 8H(300 MHz; CDCl}_3; \]
\[ \text{Me}_4\text{Si}) 1.46 (27 H, s, 3x-}^\text{t} \text{Bu), 2.87 (12 H, s, aza-CH}_2\text{), 3.90 (6H, s, 3x-CH}_2\text{ bridge),} \]
\[ 7.35 (3 H, d, J 7.5, 3-H), 7.46 (3H, m, 5-H), 7.64 (6H, m, 6-H and 7-H), 8.04 (3H, d, J 8);} \]
\[ m/z \text{ (FAB) 853 ([MH]^+ 100%).} \]
The pivaloate protected quinoline TACN 124 (800mg, 0.94 mmol) was stirred in freshly distilled THF (10cm³) under argon in an ice bath. Lithium aluminium hydride (95.2 mg, 2.51mmol) was added cautiously and stirred for a further hour. The reaction was quenched by the slow addition of water and the pH adjusted to 7 with dilute hydrochloric acid. The aqueous mixture was treated with aqueous tartaric acid and stirred for 6h. The mixture was neutralised and the THF was removed under reduced pressure. The residue was suspended in chloroform (10cm³), filtered and the filtrate solvent washed with brine and extracted several times with chloroform with the combined organics dried over sodium sulphate, filtered and the solvent removed under reduced pressure. The remaining residue was triturated in ether to give the title compound as a pale yellow solid (241 mg, 57%). mp 67-69°C; νmax (Nujol)/cm⁻¹ 3500-3300 br (OH), 1662, 1599, 1570, 1505, 1325, 1246, 1086, 837 and 751; λmax (CH₃OH)/nm 298 nm (ε/dm³mol⁻¹cm⁻¹ 7310); δH(300 MHz; CDCl₃; Me₄Si) 2.96 (12H, s, aza-CH₂), 3.96 (6H, s, 3x-CH₂ bridge), 7.14 (3H, d, J 8, 7-H), 7.28 (3H, d, J 8, 5-H), 7.41 (3H, t, J 8, 3-H), 5.58 (3H, d, J 8, 6-H), 8.03 (3H, d, J 8, 4-H); δδ(75 MHz; CDCl₃; Me₄Si) 53.3, 60.0, 117.1, 118.7, 120.9, 126.2, 128.2, 128.7, 131.3, 136.1 and 157.1; m/z (FAB) 601 ([MH]⁺, 100%), 623 ([M]⁺H, 5%); HRMS (FAB⁺) 601.2934,[MH]⁺.C₃₆H₃₆N₆O₃ requires.601.2927).
Attempted synthesis of 1, 4, 7-tris-(8-phenoxy-2-methylquinoline)-1, 4, 7-triazacyclononane lanthanide Ln(115)

The phenol 115 (45 mg, 0.075 mmol) and the appropriate lanthanide triflate (0.083 mmol) was refluxed in methanol (2 cm³) for 48 hours. The solution was allowed to cool and the solvent removed under reduced pressure. The remaining residue was dissolved in methanol (0.5 cm³) and diethyl ether was added dropwise until a turbid suspension was obtained. The solution was left in a ether atmosphere overnight to effect crystallisation. The precipitate was filtered and washed with a little ether.

Mass spectral analysis of the solids isolated for both the Gd and Eu analogues showed the molecular ion in very low abundance. Extinction coefficients for both complexes were considerably lower than expected for uv-vis absorption.
Experimental Section

*tris-(Phenoxy quinoline) Lanthanide Ln(113)*

![Chemical Structure]

The lanthanide chloride salt (168 mg, 0.46 mmol) was dissolved in ethanol (1 cm³) whilst stirring at room temperature. 8-Hydroxyquinoline (200 mg, 1.38 mmol) in ethanol (2 cm³) was added dropwise causing a yellow precipitate to form. The mixture was stirred for a further 30 minutes followed by ammonia gas being bubbled through the mixture to further induce precipitation. Water (5 cm³) was added to complete precipitation and the solids collected by vacuum filtration.

*tris-(Phenoxy quinoline) Europium (III) Eu(113)*

A pale yellow solid (215 mg, 68%). mp 202-230°C; (Found C, 55.6; H, 3.8; N, 7.0. \( \text{C}_{27}\text{H}_{18}\text{N}_{3}\text{O}_{3}\)Eu requires C, 55.5; H, 3.1; N, 7.2); \(\lambda_{max}\) (CH₃OH)/nm 241 (ε/dm³mol⁻¹cm⁻¹ 97200), 370 (8350); \(\nu_{max}\) (Nujol)/cm⁻¹ 3300-3500 br (OH), 1382 (C=N), 1156 (C-O).

*tris-(Phenoxy quinoline) Gadolinium (III) Gd(113)*

A pale yellow solid (225 mg, 83%). mp 208-224°C; (Found C, 55.1; H, 3.9; N, 7.0. \( \text{C}_{27}\text{H}_{18}\text{N}_{3}\text{O}_{3}\)Gd requires C, 54.5; H, 3.1; N, 7.1); \(\lambda_{max}\) (CH₃OH)/nm 241 (ε/dm³mol⁻¹cm⁻¹ 95420), 369 (8460); \(\nu_{max}\) (Nujol)/cm⁻¹ 3300-3500 br (OH), 1379 (C=N), 1155 (C-O).
Bibliographic References


Appendices
Appendix 1

For a plot of intensity $I$ versus time $t$ with delay time $d$ will yield a decay curve as shown below. The total area of the decay curve cannot be calculated as the delay time $d$ will divide the area into two sections, areas A and B, with area B being the one that is measured. It is therefore necessary to calculate area A which allows for the light that is dissipated during the period up until the delay time $d$.

\[
I(t) = A e^{-t/\tau}
\]

The total area under the decay curve is given by

\[
\text{Total area} = \int_{t=0}^{\infty} A e^{-t/\tau} dt = \frac{A}{-1/\tau} [e^{t/\tau}]_{0}^{\infty} = \frac{A}{-1/\tau} \{0 - e^{-d/\tau}\} = \frac{A}{-1/\tau} \left( -e^{-d/\tau} \right)
\]

Area B = integral

\[
\text{Area } B = \int_{t=d}^{\infty} A e^{-t/\tau} dt = \frac{A}{-1/\tau} \{e^{-d/\tau} - e^{-d/\tau}\}
\]

Area A + B =

\[
\frac{A}{-1/\tau} \{e^{-d/\tau} - e^{0/\tau}\} = \frac{A}{-1/\tau} \{0 - 1\} = \left( \frac{A}{1/\tau} \right)
\]

237
\[
\text{Ratio} \frac{\text{Area } A + B}{\text{Area } B} = \frac{A / \tau^{-1}}{(A / \tau^{-1}) (e^{-d/\tau})} = \frac{1}{e^{-\pi/\tau}} = e^{d/\tau}
\]

Therefore the total area is given by

\[
\text{total area} = (\text{Area } B). e^{d/\tau}
\]