Anionic Heptadentate Lanthanide Complexes as Building Blocks for Bimodal Imaging

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School of Chemistry
CONTENTS

List of figures, schemes, and tables 5
List of abbreviations 9
Abstract 12
Declaration 13
Copyright statement 14
Dedication 15
Acknowledgements 16

CHAPTER ONE – INTRODUCTION
1.1 Luminescent lanthanides 18
1.2 Sensitised luminescence 20
   1.2.1 Triplet mediated energy transfer 22
   1.2.2 Non-radiative processes 23
1.3 Lanthanide complexes 25
1.4 NMR Spectroscopy of lanthanide complexes 27
   1.4.1 $^1$H NMR of DOTA type complexes 29
1.5 Luminescence Imaging 32
1.6 Magnetic Resonance Imaging (MRI) 36
1.7 Contrast Agents 38
   1.7.1 Relaxivity 39
   1.7.2 Optimization of relaxivity 40
      1.7.2.1 Hydration number (q) 41
      1.7.2.2 Rotational correlation time; $\tau_r$ 42
      1.7.2.3 Mean residence lifetime; $\tau_m$ 43
      1.7.2.4 Electronic spin relaxation times, $T_1$ and $T_2$ 43
   1.7.3 First generation contrast agents 44
      1.7.3.1 Stability of contrast agents 45
         1.7.3.1.1 Thermodynamic Stability 45
         1.7.3.1.2 Kinetic stability 47
   1.7.4 Second generation contrast agents 48
      1.7.4.1 Responsive (Smart) contrast agents 48
         1.7.4.1.1 pH Responsive contrast agents 48
         1.7.4.1.2 $pO_2$ Responsive contrast agents 50
CHAPTER ONE

T 1.1 Emissive transitions and wavelengths of the lanthanides 20
F 1.1 Illustration of sensitized luminescence 21
F 1.2 Diagram showing deactivation pathways for an absorbed photon 21
T 1.2 A and B correction factors for calculation of q 24
F 1.3 Lanthanide energy level diagram with vibrational levels of O-H and O-D oscillator 25
F 1.4 Illustration of the parameters in the Bleaney equation 28
F 1.5 Four possible isomers of Lanthanide DOTA in solution 30
F 1.6 Diagram illustrating time-gated removal of autofluorescence 33
F 1.7 UV excitation of europium chelate followed by energy transfer to the cyanine-5 dye 34
F 1.8 Jablonski diagram showing how energy transfer takes place at longer excitation wavelengths when a chromophore with a small $S_1$-$T_1$ gap is used 35
F 1.9 MRI scan of a human brain 37
F 1.10 Illustration of the different parameters that require optimization for maximum relaxivity of gadolinium CAs 40
F1.11 Clinically approved contrast agents and their relaxivities at 37°C and 1.5 T 44
T 1.3 $K_{obs}$ and $T_{1/2}$ values (at 25°C in 0.1 N HCl) of several gadolinium complexes 47
S 1.1 Intermolecular anion binding leading to lowered relaxivity 49
S 1.2 Intramolecular anion binding leading to lower relaxivity 49
S1.3 GdDOPTA with relaxivity being modulated in the presence of calcium 52
S 1.4 Reversible binding of Zn(II) 52
S 1.5 Cleavage of the pyranose sugar leading to increased relaxivity 53
S 1.6 Cleavage of the lysine groups by TAFI increases HSA affinity 54
F 1.12 (A) White-light image of C6 glioma cells (B) Fluorescence image (C) Blank (D) MRI image of cocktail-dosed C6 glioma cells (E) MRI image of undosed cells 57
F 1.13 Lipid coated, water soluble paramagnetic QD 59
CHAPTER TWO
F 2.1 Heptadentate ligands studied by Aime et al 67
F 2.2 Dual imaging agent 68
F 2.3 HOPO complexes synthesized by the Raymond group 69
S 2.1 Synthesis of protected and deprotected heptadentate isophthalate complexes 70
F 2.4 $^1$H NMR spectrum of 23 71
F 2.5 Electrospray mass spectrum of 25 73
F 2.6 $^1$H NMR spectrum of 25 73
F 2.7 MALDI mass spectrum of 26Gd 74
F 2.8 $^1$H NMR spectrum of 26Yb 75
F 2.9 Electrospray mass spectrum of 27 76
F 2.10 $^1$H NMR spectrum of 27 77
F 2.11 Emission spectrum of Tb(III) showing luminescence from 25Tb following excitation at 260 nm. (Time gate = 10 ms, delay time = 0.1 ms and slit width = 10 mm) Peaks correspond to decay from $^5$D$_4$ excited state to the $^7$F$_6$, $^7$F$_5$, $^7$F$_4$, $^7$F$_3$ ground states respectively 78
F 2.12 Fitted single exponential decay for compound 25Tb 79
T 2.1 Measured lifetimes of the complexes in H$_2$O and D$_2$O 80
T 2.2 Measured lifetimes of the complexes in PBS solutions 81
F 2.13 Illustration of phosphate binding taking place 82
F 2.14 Comparison of HOPO to the triamide complex 83

CHAPTER THREE
S 3.1 Synthetic route to DO3P tri-ester 34Ln and tri-acid complexes 36Ln 88
S 3.2 Synthetic route to monoprotected cyclen 31 89
F 3.1 $^1$H NMR spectrum of 38 90
F 3.2 Positive electrospray spectrum of 31 91
S 3.3 Reaction mechanism 92
F 3.3 $^1$H NMR spectrum of 31 93
F 3.4 $^1$H NMR spectrum of 32 94
F 3.5 MALDI MS of 33Eu 95
F 3.6 MALDI MS of 35Tb 96
F 3.7 $^1$H NMR spectrum of 35Eu
F 3.8 $^{31}$Phosphorus NMR of 35Eu at (a) pH1 (b) pH4 (c) pH7 (d) pH10
F 3.9 $^1$H NMR spectrum of 35Tb
F 3.10 Proton decoupled $^{31}$P NMR spectrum of 35Tb
T 3.1 Luminescence lifetimes and $q$ values of DO3P complexes
T 3.2 Luminescence data measured in PBS
S 3.4 Synthetic route to binuclear complexes
F 3.11 Positive electrospray spectrum of 42
F 3.12 $^1$H NMR spectrum of 43
F 3.13 $^1$H NMR spectrum of 44
F 3.14 MALDI MS of 45Yb
F 3.15 $^1$H NMR spectrum of 45Eu
F 3.16 Proton decoupled $^{31}$P NMR spectrum of 45Eu
S 3.5 Synthetic route to rhenium bipyridine complex
S 3.6 Synthesis of the d-f hybrid
F 3.17 $^1$H NMR spectrum of 49Eu
F 3.18 $^1$H NMR spectrum of 49Yb
F 3.19 UV/Vis spectra of 49Eu (pale blue), 49Tb (red) and 49Yb (dark blue)
F 3.20 UV/Vis Spectrum of 45Yb (yellow), 49Yb (red) and 49Eu (blue)
F 3.21 Excitation and emission spectra of 49Tb
F 3.22 Excitation and emission spectra of 49Eu
T 3.3 Luminescence lifetimes and hydration number of the complexes
F 3.23 Emission spectra intensity of 45Eu decreasing with increasing phosphate anion concentration
T 3.4 Titration data
T 3.5 Rhenium luminescence lifetime
F 3.24 Visible fluorescence spectrum of 49Yb
F 3.25 Relaxivity of 49Gd in H$_2$O
F 3.26 Relaxivity of 49Gd in PBS
F 3.27 Relaxivity of 49Gd when measured in HA (a) supernatant (b) slurry
T 3.5 Luminescence measurements for terbium and europium d-f complexes
CHAPTER FOUR
F 4.1 f-f energy levels of the NIR emitting lanthanides 125
S 4.1 Synthetic route to bisDO3P complexes 128
S 4.2 Synthetic route to 50 129
F 4.2 $^1$H NMR spectrum of 58 130
F 4.3 $^1$H NMR spectrum of 50 131
F 4.4 $^1$H NMR spectrum of 51 132
F 4.5 $^1$H NMR spectrum of 52 133
F 4.6 $^1$H NMR spectrum of 54 134
F 4.7 $^1$H NMR spectrum of 55Lu 135
F 4.8 $^1$H NMR spectrum of 55Eu 136
F 4.9 $^1$H NMR spectrum of 55Yb 137
S 4.3 Rhenium complex synthesis 137
S 4.4 d-f Hybrid synthesis 138
T 4.1 Infrared shifts of the rhenium complex carbonyl groups 138
F 4.10 $^1$H NMR spectrum of 63Eu 139
F 4.11 $^1$H NMR spectrum of 63Yb 139
F 4.12 UV/Vis spectra of 54, 55Eu and 55Nd 140
F 4.13 Comparison of the UV/Vis spectra of 55Eu and 63Eu/Nd 141
F 4.14 Time resolved emission spectra (TRES) of 63Nd 142
T 4.2 Luminescence lifetimes and calculated q values for bisDO3P complexes 143
T 4.3 Luminescence lifetimes of the rhenium component 144
F 4.15 Plot of relaxivity of 63Gd in $H_2O$ 145
F 4.16 Plot of relaxivity of 63Gd in PBS 146
F 4.17 Plot of relaxivity of hydroxy apatite slurry after shaking for 24 hours with 63Gd 147
F 4.18 Relaxivity of supernatant decanted from HA after shaking for 24 hours 147

CHAPTER FIVE
S 5.1 Synthetic route towards tetraphosphinic acid cyclen derivative 153
S 5.2 Indirect route to tetraphosphinate cyclen 77 155
F 5.1 $^1$H NMR spectrum of 73 156
F 5.2 Electrospray mass spectrum of 74 157
S 5.3 Successful synthesis of 78 157
### LIST OF ABBREVIATIONS

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Full Form</th>
</tr>
</thead>
<tbody>
<tr>
<td>AAZTA</td>
<td>6-amino-6-methylperhydro-1,4-diazepine tetraacetic acid</td>
</tr>
<tr>
<td>Boc</td>
<td>tert–Butoxycarbonyl</td>
</tr>
<tr>
<td>BPAMD</td>
<td>((4-[[bis-(phosphonomethyl)carbamoyl]methyl]-7,10-bis(carboxymethyl)-1,4,7,10-tetraazacyclododec-1-yl)acetic acid</td>
</tr>
<tr>
<td>Cbz</td>
<td>Benzyloxy carbonyl</td>
</tr>
<tr>
<td>CEST</td>
<td>Chemical exchange saturation transfer</td>
</tr>
<tr>
<td>CA</td>
<td>Contrast agent</td>
</tr>
<tr>
<td>cm(^{-1})</td>
<td>wavenumbers</td>
</tr>
<tr>
<td>CT</td>
<td>Computed Tomography</td>
</tr>
<tr>
<td>Cyclen</td>
<td>1,4,7,10 tetraazacyclododecane</td>
</tr>
<tr>
<td>DCC</td>
<td>dicyclohexylcarbodiimide</td>
</tr>
<tr>
<td>DCM</td>
<td>Dichloromethane</td>
</tr>
<tr>
<td>DMSO</td>
<td>dimethyl sulfoxide</td>
</tr>
<tr>
<td>DO3A</td>
<td>1,4,7,10-tetraazacyclododecane-1,4,7-trisacetic acid</td>
</tr>
<tr>
<td>DO3P</td>
<td>1,4,7,10-tetraazacyclododecane-1,4,7-trisphosphonic acid</td>
</tr>
<tr>
<td>DOTA</td>
<td>1,4,7,10-tetraazacyclododecane-1,4,7-tetraacetic acid</td>
</tr>
<tr>
<td>DOTAMP</td>
<td>1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraacetamidomethylene phosphonic acid</td>
</tr>
<tr>
<td>DOTMP</td>
<td>1,4,7,10-tetraazacyclododecane-1,4,7-tetramethylene phosphonic acid</td>
</tr>
<tr>
<td>DOTP</td>
<td>1,4,7,10-tetraazacyclododecane-1,4,7-tetraphosphonic acid</td>
</tr>
<tr>
<td>DTPA</td>
<td>Diethylenetriamine pentaacetic acid</td>
</tr>
<tr>
<td>DTPP</td>
<td>Diethylenetriamine pentamethylene phosphonic acid</td>
</tr>
<tr>
<td>EDCI</td>
<td>1-ethyl-3-(3-dimethylaminopropyl) carbodiimide</td>
</tr>
<tr>
<td>ES</td>
<td>Electrospray</td>
</tr>
<tr>
<td>ET</td>
<td>Energy transfer</td>
</tr>
<tr>
<td>h</td>
<td>hours</td>
</tr>
<tr>
<td>HA</td>
<td>Hydroxy apatite</td>
</tr>
<tr>
<td>HEPES</td>
<td>4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid</td>
</tr>
<tr>
<td>HOPO</td>
<td>Hydroxypyridininone</td>
</tr>
<tr>
<td>Abbreviation</td>
<td>Description</td>
</tr>
<tr>
<td>--------------</td>
<td>--------------------------------------------------</td>
</tr>
<tr>
<td>HPDO3A</td>
<td>10-(2-hydroxypropyl)-1,4,7-tetraazacyclododecane-1,4,7-triacetic acid</td>
</tr>
<tr>
<td>HSA</td>
<td>Human serum albumin</td>
</tr>
<tr>
<td>IC</td>
<td>Internal conversion</td>
</tr>
<tr>
<td>IR</td>
<td>Infrared</td>
</tr>
<tr>
<td>IS</td>
<td>Inner sphere</td>
</tr>
<tr>
<td>ISC</td>
<td>Intersystem crossing</td>
</tr>
<tr>
<td>λ</td>
<td>Wavelength</td>
</tr>
<tr>
<td>λ&lt;sub&gt;em&lt;/sub&gt;</td>
<td>Emission wavelength</td>
</tr>
<tr>
<td>λ&lt;sub&gt;ex&lt;/sub&gt;</td>
<td>Excitation wavelength</td>
</tr>
<tr>
<td>LIS</td>
<td>Lanthanide Induced Shift</td>
</tr>
<tr>
<td>LMCT</td>
<td>Ligand to metal charge transfer</td>
</tr>
<tr>
<td>MALDI</td>
<td>Matrix Assisted Laser Desorption Ionisation</td>
</tr>
<tr>
<td>MeOH</td>
<td>Methanol</td>
</tr>
<tr>
<td>MLCT</td>
<td>Metal to ligand charge transfer</td>
</tr>
<tr>
<td>MRI</td>
<td>Magnetic Resonance Imaging</td>
</tr>
<tr>
<td>MS</td>
<td>Mass spectrum</td>
</tr>
<tr>
<td>ms</td>
<td>Milliseconds</td>
</tr>
<tr>
<td>mmol</td>
<td>Millimoles</td>
</tr>
<tr>
<td>mW</td>
<td>MilliWatts</td>
</tr>
<tr>
<td>m/z</td>
<td>Mass to charge ratio</td>
</tr>
<tr>
<td>NIR</td>
<td>Near infrared</td>
</tr>
<tr>
<td>nm</td>
<td>Nanometer</td>
</tr>
<tr>
<td>NMR</td>
<td>Nuclear magnetic resonance</td>
</tr>
<tr>
<td>NMRD</td>
<td>Nuclear magnetic dispersion profile</td>
</tr>
<tr>
<td>ns</td>
<td>Nanosecond</td>
</tr>
<tr>
<td>OS</td>
<td>Outer sphere</td>
</tr>
<tr>
<td>PBS</td>
<td>Phosphate buffered saline</td>
</tr>
<tr>
<td>PCTA</td>
<td>3,6,9,15-tetraazabicyclo[9.3.1]pentadeca(15),11,13-triene-3,6,9-triacetic acid</td>
</tr>
<tr>
<td>PCTP</td>
<td>3,6,9,15-tetraazabicyclo[9.3.1]pentadeca(15),11,13-triene-3,6,9-tris(methanephosphonic) acid</td>
</tr>
<tr>
<td>PEG</td>
<td>Polyethylene glycol</td>
</tr>
<tr>
<td>Abbreviation</td>
<td>Definition</td>
</tr>
<tr>
<td>--------------</td>
<td>------------</td>
</tr>
<tr>
<td>PET</td>
<td>Positron Emission Tomography</td>
</tr>
<tr>
<td>ppm</td>
<td>parts per million</td>
</tr>
<tr>
<td>q</td>
<td>Hydration number</td>
</tr>
<tr>
<td>QD</td>
<td>Quantum dot</td>
</tr>
<tr>
<td>RIME</td>
<td>Receptor-Induced Magnetisation Enhancement</td>
</tr>
<tr>
<td>SAP</td>
<td>Square anti-prism</td>
</tr>
<tr>
<td>SPECT</td>
<td>Single Photon Emission Computed Tomography</td>
</tr>
<tr>
<td>τ</td>
<td>Lifetime</td>
</tr>
<tr>
<td>T</td>
<td>Tesla</td>
</tr>
<tr>
<td>TAFI</td>
<td>Thrombin Activable Fibrinolysis Inhibitor</td>
</tr>
<tr>
<td>THF</td>
<td>Tetrahydrofuran</td>
</tr>
<tr>
<td>TLC</td>
<td>Thin layer chromatography</td>
</tr>
<tr>
<td>TPPS</td>
<td>tetraphenylporphin tetrasulfonate</td>
</tr>
<tr>
<td>TRES</td>
<td>Time resolved emission spectrum</td>
</tr>
<tr>
<td>TRITC</td>
<td>tetramethylrhodamine-5-isothiocyanate</td>
</tr>
<tr>
<td>Triflate</td>
<td>Trifluoromethylsulphonate</td>
</tr>
<tr>
<td>TSAP</td>
<td>Twisted square antiprism</td>
</tr>
<tr>
<td>UV</td>
<td>Ultraviolet</td>
</tr>
<tr>
<td>µm</td>
<td>micro metre</td>
</tr>
<tr>
<td>µs</td>
<td>microsecond</td>
</tr>
<tr>
<td>ν</td>
<td>frequency</td>
</tr>
</tbody>
</table>
ABSTRACT

Contrast agents are used in over 35% of MRI scans today and their use is increasing as they become ever more sophisticated. The challenge for researchers in this field is to synthesise smart imaging agents, which can be addressed using more than one imaging modality. The ultimate goal of these types of contrast agents is to be able to increase the contrast between normal and diseased tissue with smaller amounts of the agent being administered.

This thesis describes the synthesis of complexes that are designed to have increased hydration numbers and rapid solvent exchange. The key to designing clinically effective contrast agents of this type is to minimize the affinity the contrast agent for endogenous anions such as phosphate that can exclude water from the inner coordination sphere, inhibiting exchange and reducing the relaxivity.

A hexaanionic ligand was synthesized by tri-alkylation of cyclen with the dimethyl ester of N-isophthaloylchloroacetamide and subsequent hydrolysis. Lanthanide complexes of the ester and acid form of the ligand were synthesized and were shown to be luminescent. Relaxometric and time–resolved studies were used to establish that phosphate binding is not significantly inhibited in the anionic complex, suggesting that proximate negative charge is essential to inhibition of anion binding.

Lanthanide complexes of DO3P and a bisDO3P analogue containing a bipyridyl bridge were also prepared, and shown to be more effective at inhibiting phosphate binding, though their properties exhibit dependence on pH. The bipyridyl derivative was used to prepare rhenium containing d-f hybrid complexes. These exhibited luminescence from both the rhenium MLCT and the lanthanide, though sensitized emission from the ytterbium is inefficient as a consequence of poor spectral overlap.

Routes towards preparation of ligands bearing pyridylphosphinate pendent arms have also been explored.
DECLARATION

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For Mum, Dad, Arianna, Essy, Ella, Okey, Emeka, Ije, Ike, Obi, Uzo and Ngozi
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CHAPTER ONE
INTRODUCTION

The demand for non-invasive imaging techniques has seen research into the lanthanide ions explode in the last few decades. Their advantages are many and include intense line-like and long-lived luminescence encompassing a range of wavelengths spanning the visible and near IR regions, large Stoke’s shifts and time gated rejection of unwanted signals from short-lived autofluorescence from biomolecules.¹

In particular, the high-spin paramagnetism and long electronic relaxation time of Gd(III) has made it pre-eminent among contrast agents for MRI. However due to the toxicity of the free ion in vivo, the lanthanide has to be administered as a thermodynamically and kinetically stable complex.² ³

This introduction sets out to describe the synthesis of such complexes and to investigate the interaction of these complexes with phosphate, an endogenous anion. It is not intended as a comprehensive review of the topics within but as an overview to understanding the aims of the project.

1.1 Luminescent lanthanides
The lanthanides resemble each other quite closely in their chemical and physical properties, especially oxidation state. This can be explained by the electronic configuration of the atoms and their ions which exist in their trivalent state Ln(III) ([Xe]4fⁿ, n=0-14, table 1.1) in aqueous solutions, in view of the various degrees of stabilization experienced by the 4f, 5d, and 6s orbitals upon ionization. As a consequence of the small radial extension, the 4f orbitals are effectively shielded from the environment by the 5s²5p⁶ arrangement and are only minimally involved in bonding. This means firstly that ligand-field splittings are small (100-250 cm⁻¹), thus spectral bands arising from f-f transitions are very sharp and are similar to the free ion.⁴
Also, f-f transitions are electric-dipole forbidden by the Laporte selection rule but weak interactions with the ligand field or vibrational states in electronic states with different parity give rise to low intensity transitions with sharp absorption lines that have extinction coefficients typically between $1-10 \text{ M}^{-1}\text{cm}^{-1}$. This means that direct excitation of the metal centre is inefficient, requiring either a high concentration of the metal or a very intense energy source.

Due to the poor radial extension of the f-orbitals, there is a strong interaction between f-electrons, and the inter-electronic repulsion and spin-orbit effects dominate the relative ordering of the electronic states. However, evidence of some crystal field effect can be observed in electronic spectra in the form of a shifting to lower frequencies of the absorption of complexes compared to the free ion which has been attributed to a small degree of metal-ligand covalent bonding e.g. in Yb(III) complexes. Some lanthanides also show hypersensitivity in transitions that involve $\Delta J=2$: this is particularly commonplace for Eu$^{3+}$, where the $^5D_0-^7F_2$ transition is hypersensitive. The intensity of these transitions are found to be very sensitive to the ligand environment with the most dramatic effects being observed for low symmetry complexes and those with polarisable ligands.
### Table 1.1 Emissive transitions and wavelengths of the lanthanide (III) ions

<table>
<thead>
<tr>
<th>Ln</th>
<th>4f configuration</th>
<th>Emissive transition</th>
<th>Emission wavelength</th>
<th>Emission Colour</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pr$^{3+}$</td>
<td>4f$^2$</td>
<td>$^1G_4 \rightarrow ^3H_J$</td>
<td>1300, 890, 1060, 525-680</td>
<td>NIR, NIR, Orange</td>
</tr>
<tr>
<td>Nd$^{3+}$</td>
<td>4f$^3$</td>
<td>$^3F_{3/2} \rightarrow ^4I_J$</td>
<td>880, 1060, 1340</td>
<td>NIR</td>
</tr>
<tr>
<td>Sm$^{3+}$</td>
<td>4f$^4$</td>
<td>$^3G_{5/2} \rightarrow ^6H_J$</td>
<td>590</td>
<td>Orange</td>
</tr>
<tr>
<td>Eu$^{3+}$</td>
<td>4f$^5$</td>
<td>$^3D_0 \rightarrow ^5F_J$</td>
<td>615</td>
<td>Red</td>
</tr>
<tr>
<td>Gd$^{3+}$</td>
<td>4f$^6$</td>
<td>$^5P_{7/2} \rightarrow ^5S_{7/2}$</td>
<td>312</td>
<td>UV</td>
</tr>
<tr>
<td>Tb$^{3+}$</td>
<td>4f$^7$</td>
<td>$^5D_4 \rightarrow ^7F_J$</td>
<td>545</td>
<td>Green</td>
</tr>
<tr>
<td>Dy$^{3+}$</td>
<td>4f$^8$</td>
<td>$^3F_{9/2} \rightarrow ^5H_J$</td>
<td>570</td>
<td>Yellow-orange</td>
</tr>
<tr>
<td>Ho$^{3+}$</td>
<td>4f$^{10}$</td>
<td>$^5F_5 \rightarrow ^5I_J$</td>
<td>970, 1050, 540</td>
<td>Green</td>
</tr>
<tr>
<td>Er$^{3+}$</td>
<td>4f$^{11}$</td>
<td>$^3S_{3/2} \rightarrow ^4I_J$</td>
<td>1530</td>
<td>NIR</td>
</tr>
<tr>
<td>Yb$^{3+}$</td>
<td>4f$^{13}$</td>
<td>$^2F_{5/2} \rightarrow ^2F_{7/2}$</td>
<td>980</td>
<td>NIR</td>
</tr>
</tbody>
</table>

#### 1.2 Sensitised luminescence

To overcome the problem of inefficient direct excitation of the lanthanide, organic chromophores are often incorporated onto the ligand scaffold to facilitate efficient excitation of the metal via a Laporte allowed electronic transition (figure 1.1). Most paramagnetic Ln(III) ions are luminescent, but some are more emissive than others. This difference can be attributed to the energy gap between the lowest lying excited (emissive) state of the metal ion and the highest sublevel of its ground multiplet. Essentially, the bigger the energy gap, the less likely it is that the emissive state will be quenched by vibrational modes of the molecule or solvent, therefore making the excited state more luminescent. This means that the most emissive lanthanides are Eu(III) and Tb(III). Although Gd(III) has the largest energy gap, the emissive state is too high to be populated by aryl chromophores.
Using an organic chromophore takes advantage of its allowed \( \pi - \pi^* \) transition which mediates energy transfer from the excited state of the aromatic unit to the metal centre. However, this can only be effective if the ligand-centred donor excited state is higher in energy than the lanthanide acceptor state.

**Fig.1.1** Illustration of sensitized luminescence

**Fig.1.2** Diagram showing deactivation pathways for an absorbed photon\textsuperscript{108}
The Jablonski diagram above (figure 1.2) illustrates what happens when a molecule is excited by UV-visible light. On absorption of a photon, an electron is promoted from the singlet ground state to the first excited singlet state (S\textsubscript{0}-S\textsubscript{1}). After relaxation to the lowest-energy vibrational level within the S\textsubscript{1} state, energy can be given up in different ways. First of all, fluorescence can occur when a photon is emitted as a consequence of the spin-allowed transition back to the ground state (S\textsubscript{1}-S\textsubscript{0}). Phosphorescence occurs when intersystem crossing takes place to the triplet state (T\textsubscript{1}) and emission involves the T\textsubscript{1}-S\textsubscript{0} transition. This transition is formally spin-forbidden making the emission much longer lived than fluorescence. These two processes are in competition with other deactivation processes, which do not all result in the emission of light. Those which do not are called non-radiative processes and are discussed in section 1.2.2. The following section deals with the most common type of energy transfer observed in these systems. Energy transfer can also take place by other mechanisms, namely redox processes and by two photon excitation.

1.2.1 Triplet mediated energy transfer

In this case, the S\textsubscript{1} state of the chromophore is generated by absorption of a photon. Fast intersystem crossing to the T\textsubscript{1} state is then facilitated by the presence of a heavy atom (Ln\textsuperscript{3+}). In the presence of a lanthanide based emissive acceptor state lying at the correct energy, energy can be transferred from the chromophore to the lanthanide, resulting in emission from the lanthanide upon relaxation to the ground state. This can occur by one of the following two mechanisms.

\textit{Förster} exchange is classed as the transfer of electronic excitation energy between otherwise well separated atomic or molecular electronic systems. For this to occur there must be a non-zero overlap between the emission spectrum of the chromophore and the absorption spectrum of the energy acceptor. As this is a through space dipole-dipole interaction, energy transfer is dependent on the chromophore-metal separation (r\textsuperscript{6}) and can take place both intra- and intermolecularly.
The Dexter\textsuperscript{17} energy transfer mechanism involves a double electron transfer between donor and acceptor usually with a conjugated ligand. It requires an overlap of the donor and acceptor electron clouds therefore short distances are required and this type of mechanism is commonly observed intramolecularly.

1.2.2 Non-radiative processes
Deactivation of the emissive state of a lanthanide can occur through quenching by the vibrational harmonics of the O-H oscillators of a protic solvent e.g water and methanol. However, Kropp and Windsor\textsuperscript{18, 19} were able to demonstrate that luminescence of Eu(III) and Tb(III) complexes was more intense in D\textsubscript{2}O than in H\textsubscript{2}O and that lifetimes were longer in D\textsubscript{2}O. This has been ascribed to the poorer Franck-Condon overlap between the wavefunction of the O-D oscillator (figure 1.3) with that of the lanthanide excited state and energy transfer has been found to be 200 times slower than for O-H oscillators.\textsuperscript{4-6}

This phenomenon has been used by Horrocks et al to develop an equation to assess the hydration number (q) of the metal centre in aqueous solution.\textsuperscript{20}

\[ q = A_{\text{Ln}}(k_{\text{H}_2\text{O}} - k_{\text{D}_2\text{O}}) \]  

\( A_{\text{Ln}} \) is an experimentally determined coefficient, which is unique to each lanthanide and \( k_{\text{H}_2\text{O}} \), \( k_{\text{D}_2\text{O}} \) are luminescent rate constants of the lanthanide in water and deuterium oxide respectively.

Proximate X-H oscillators such as amide and amine NH and CH groups may also quench luminescence. The latter particularly affects lanthanide ions such as Pr\textsuperscript{3+}, Nd\textsuperscript{3+} and Er\textsuperscript{3+} due to their large number of low energy emissive states. These oscillators are often more distant from the Ln(III) centre in kinetically stable complexes therefore their quenching effect is less marked than the effect of OH oscillators.
In addition to inner sphere water, outer sphere water molecules have also been shown to quench lanthanide based luminescence. As a result, Parker et al.\textsuperscript{21} came up with a modified equation to take these effects into account,

\[ q = A'_{\text{Ln}} (k_{\text{H2O}} - k_{\text{D2O}} - B) \]  

where \( B \) is the outer sphere contribution.\textsuperscript{22} A and B factors are summarized in table 1.2.

<table>
<thead>
<tr>
<th>Ln</th>
<th>A</th>
<th>B</th>
</tr>
</thead>
<tbody>
<tr>
<td>Eu\textsuperscript{3+}</td>
<td>1.2 ms</td>
<td>((0.25 + 0.075x) \text{ ms}^{-1})</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(x = \text{no. of exchanging amide N-H oscillators})</td>
</tr>
<tr>
<td>Tb\textsuperscript{3+}</td>
<td>5 ms</td>
<td>0.6 ms\textsuperscript{-1}</td>
</tr>
<tr>
<td>Yb\textsuperscript{3+}</td>
<td>1.0 \text{µs}</td>
<td>0.1 \text{µs}\textsuperscript{-1}</td>
</tr>
<tr>
<td>Nd\textsuperscript{3+}</td>
<td>130 ns</td>
<td>0.4 ns\textsuperscript{-1}</td>
</tr>
</tbody>
</table>

\textbf{Table 1.2} A and B correction factors for calculation of \( q \)

Even though the equation 2 works well for most complexes, the calculated value of \( q \) is rarely an integer. This is thought to be due to the fact that water exchange can occur by both associative and dissociative mechanisms and also that \( q \) is an average of the hydration state of the different isomers of the complex in solution.\textsuperscript{21}
1.3 Lanthanide complexes

Lanthanide ions are hard cations and are good Lewis acids. They exhibit a wide range of coordination numbers; typically from 3-9.\textsuperscript{23} For the trivalent lanthanide ions in aqueous solution, the coordination numbers are usually nine, therefore multidentate complexing ligands are chosen that contain several hard donor atoms such as nitrogen, oxygen or a combination of both. There are a large variety of ligands - both cyclic and acyclic - which can be used to complex lanthanides. In the synthesis of contrast agents, two frameworks are predominantly used namely tetraazamacrocycles (1) and the acyclic DTPA (2).

The need for complexes which are kinetically and thermodynamically stable means that macrocycles are generally preferred over their acyclic counterparts.\textsuperscript{24}
A macrocycle is defined as a cyclic molecule with three or more potential donor atoms in a ring of at least nine atoms. There are several different types of macrocycles that can be divided into two simple groups. Firstly, single donor atom macrocycles such as crown ethers, tetraazamacrocycles, porphyrins etc and mixed donor atom macrocycles containing two or more different types of donor atoms e.g. DOTA.
Stability of macrocycles
As afore mentioned, macrocyclic ligands are preferred over acyclic ligands. This is due to the extra stability imparted by the following characteristics:

i) Chelate effect
The chelate effect states that complexes with polydentate ligands will be more stable than analogous monodentate ligands. The increased stability of these complexes arises from the combination of large entropic effects due to desolvation of the aqua ion and charge compensation occurring upon complexation.

ii) Macrocyclic effect
Macrocyclic complexes are much more stable than open-chain ligands. They are pre-organized cavities that bear several donor atoms that can coordinate to the metal ion. In this way, reorganization energy of the ligand upon complexation is minimized, making the macrocyclic ligand more thermodynamically stable than an acyclic ligand.

1.4 NMR Spectroscopy of lanthanide complexes
Due to the presence of unpaired f-electrons, most lanthanide complexes are paramagnetic. As La and Lu represent $4f^0$ and $4f^{14}$ electronic configurations their complexes are diamagnetic in nature. During the NMR experiment for a paramagnetic complex, the nucleus under investigation experiences a local magnetic field due to the paramagnetic ion as well as the external magnetic field due to the NMR spectrometer causing a much greater chemical shift and this is called the lanthanide induced shift (LIS).

There are three main contributions to the LIS ($\Delta$) for a nucleus of a ligand coordinated to a paramagnetic lanthanide ion namely the diamagnetic ($\Delta_d$), contact ($\Delta_c$), and pseudocontact ($\Delta_p$) shifts.\(^\text{26}\)

$$\Delta = \Delta_d + \Delta_c + \Delta_p \quad \text{(3)}$$
Since the diamagnetic shift is usually small it is often neglected and if need be can be determined directly or by analogy from shifts of La(III) and Lu(III) complexes. The contact shift involves a through bond transmission of the unpaired electron density to the nucleus of interest. This contribution is large for a nucleus directly bonded to the lanthanide ion but decreases rapidly with increasing distance.

As there is very little covalent contribution to metal-ligand bonding in lanthanide complexes there is no delocalization of unpaired f-electrons onto ligands, therefore the mechanism of interaction is almost entirely through-space or dipolar and is called the pseudocontact shift. The Bleaney equation\textsuperscript{27} was developed to calculate the pseudocontact shift for axially symmetric complexes but is also useful for non-symmetric complexes.

\[ \Delta_p = D(3\cos^2\theta - 1)/r^3 \]  

(4)

The constant $D$ depends on $1/T^2$ and on the magnetic properties of the metal on and may be positive or negative. $r$ is the distance between the nucleus and the lanthanide demonstrated by figure 1.4, and $\theta$ is the angle between $r$ and the principal symmetry axis of the complex.\textsuperscript{5}

Fig. 1.4 Illustration of the parameters in the Bleaney equation
The pseudocontact shift is very sensitive to geometry and depends on both $r$ and $\theta$, and can give a great deal of structural information. It has been successfully used to predict and interpret $^1$H and $^{13}$C spectra of Ln.DOTA compounds.$^{28}$

1.4.1 $^1$H NMR of DOTA type complexes

Some lanthanide complexes exhibit a number of isomers in solution due to the stereoisomerism associated with the lanthanide and this gives rise to complicated paramagnetic spectra. The ratio of isomers observed is dependent on the lanthanide ion, solvent, temperature, pressure, concentration and counterion.

In lanthanide DOTA complexes, the four ethylenediamine groups adopt gauche conformations giving rise to two macrocyclic ring conformations, ΛΛΛΛ and δδδδ. Two possible arrangements are possible for the acetate arms, Λ or Δ giving rise to four possible stereoisomers which exist in solution as two enantiomeric pairs (figure 1.5).

Stereoisomers with a twist angle of ~40° are said to have a capped square-antiprismatic (SAP) geometry whilst stereoisomers with a twist angle of ~30° have a twisted SAP geometry (TSAP). The isomers can interconvert from SAP to TSAP by ring inversion (ΛΛΛΛ to δδδδ) or arm rotation (Λ to Δ). When both processes are combined in succession or in concert, they result in exchange between enantiomeric pairs.

For the larger lanthanide DOTA complexes, La(III) – Nd(III), the hydrated TSAP geometry is favoured whereas the hydrated SAP geometry is favoured by the smaller lanthanides, Sm(III)-Er(III).$^{26}$
Fig. 1.5 Four possible isomers of Lanthanide DOTA in solution.\textsuperscript{28, 29}

Aime \textit{et al} have studied the low-temperature NMR of 12 LnDOTA complexes and were able to observe the presence of both major and minor isomers.\textsuperscript{28, 29}
Due to the high symmetry of the DOTA complex, the ensuing spectrum is relatively simple. There are 12 resonances in all—6 major and 6 minor attributed to the SAP and TSAP isomers respectively. The six peaks have been assigned to four of the peaks corresponding to each of the axial (‘up’ and ‘down’) and equatorial protons on the macrocyclic ring. As the temperature is increased, all 12 resonances of the major and minor isomers broaden, collapse and then merge. In contrast to this, lanthanide complexes of the phosphinate analogue of DOTA (DOTP) only shows six resonances in its spectrum. This is because it exists mostly as the unhydrated TSAP isomer.\(^{30}\)

Phosphinate complexes of ligands such as 4 and 5 can theoretically exist as six isomers as complexation creates a chiral centre at phosphorus. Each of these isomers can have two ring conformations and pendant arm helicities leading to 24 isomers (16 diastereoisomers) and 8 enantiomeric pairs. However due to the steric bulk, over 90% of one major species is observed in solution, existing as an enantiomeric pair in the TSAP geometry (\textit{RRRR}-\textit{Λ}(\ldots\ldots\ldots) and \textit{SSSS}-\textit{Δ}(\ldots\ldots\ldots). This of course means that only one set of peaks is observed on the spectrum as the enantiomers are indistinguishable.\(^{31}\)

Substituting one or more arms on the DOTA scaffold leads to a lowering of symmetry and many more peaks are observed as can be observed with the spectra obtained for complexes synthesized in this project. Technically, this results from the break in symmetry and the pseudo-contact shift is now defined by the polar coordinates of the proton relative to the lanthanide according to the equation:

\[
\delta_{pc} = A((B_o^2(3\cos^2\theta-1)r^2) + (B_2^2\sin^2\theta\cos2\phi/r^3)^*\sqrt{6})^{109}
\]  

(5)
1.5 Luminescence Imaging

Fluorescent probes based on organic chromophores have long been used to visualize cell biology at many levels, from molecules to complete organisms. The technique offers imaging on a sub-cellular level unlike MR imaging which is currently a whole body imaging technique with insufficient resolution for use at the sub-cellular level.\textsuperscript{32}

Tsien et al\textsuperscript{33} developed two ligands INDO-1 (6) and FURA-2 (7) which could be used to measure calcium concentration. The ligands incorporate EGTA-like binding sites, which are known to be selective for calcium ions, attached to conjugated chromophore that absorbs light in the UV. On coordination to calcium ions, the excitation and emission wavelength changes, allowing the calculation of the calcium concentration in the sample.

The main drawback to most organic fluorescent probes like INDO-1 is autofluorescence from the sample, arising from competitive excitation of biological chromophores.

The use of lanthanide complexes goes some way to solving this problem. Lanthanides exhibit long-lived luminescence due to spin-forbidden phosphorescence. By employing time-gated techniques (figure 1.6), the long luminescence lifetimes of the lanthanide can be separated from autofluorescence of biological chromophores.
As shown in the above diagram, the sample is irradiated with an energy source and then after a short delay $t_d$, the detector is switched on by which time autofluorescence has decayed away and the luminescence from the probe can be detected free of background interference from the sample.\(^6\)

Lanthanide complexes have been used in immunoassays that rely on a biochemical reaction between an antigen and a specific antibody which is labeled with a lanthanide chelate. For example, a europium complex with a derivatised tris(bipyridine) cryptand \(8\) was linked to a specific antibody while the acceptor was grafted onto a second specific antibody.\(^3\) When the two react together, energy transfer occurs upon UV excitation of the europium chelate to the phycobiliprotein, which shows a fluorescence band at 660 nm. As the 660 nm emission reflects the decay of the europium luminescence, time-gating can be applied to remove autofluorescence from the sample. Also, the energy is only transferred when both donor and acceptor are close to each other i.e when they both bind to the substrate, therefore there is no need to eliminate unreacted reagents from the solution before measurement.
Hurskainen proposed a similar method for detecting nucleic acid hybridization. A single stranded DNA was fitted at its 5’ end with a europium chelate derived from a carbostyril dye as the energy donor (figure 1.7). The energy acceptor, a cyanine-5 dye was linked to another single stranded DNA. Upon hybridization, the donor and acceptor are brought within adequate distance and energy is transferred when the europium chelate is excited by UV light, allowing detection and quantification of the hybridization process.\cite{35}

![Chemical structure of the europium chelate and cyanine-5 dye](image)

**Fig. 1.7** UV excitation of europium chelate followed by energy transfer to the cyanine-5 dye\cite{35}

Using visible emitting lanthanide complexes in optical imaging is not without its limitations. Excitation of lanthanide requires the use of UV light which compromises *in vivo* applications since biological molecules usually absorb heavily in this range meaning they can be modified or destroyed by the radiation.\cite{36} The generation of visible light inside a sample may also be absorbed therefore reducing the signal from the analyte. One way to circumvent this is to use longer wavelengths to excite the metal.
Sammes *et al* used acridone derivatives with linkers of different lengths incorporated onto a DO3A unit to sensitise europium emission at wavelengths longer than 400 nm. It was found that the most efficient energy transfer took place when the acridone group was held closest to the lanthanide ion and a Förster energy mechanism was proposed to explain this. Figure 1.8 shows the proposed scheme by which energy transfer takes place at longer wavelengths. There is a smaller singlet to triplet energy gap which allows irradiation of the chromophore at lower energies and the quantum yield depends on the efficiency of the triplet energy transfer versus other decay processes.\(^{37}\)

![Jablonski diagram](image)

**Fig. 1.8** Jablonski diagram showing how energy transfer takes place at longer excitation wavelengths when a chromophore with a small \(S_1-T_1\) gap is used

Long wavelength excitation can also take place by a process called upconversion where two or more photons are absorbed. However, this requires the use of considerable excitation power \((200-300 \text{ mW})\) and the use of a Ti:sapphire laser.\(^{38}\)
Another solution to the problem of using UV light to excite the metal is to use metal ions which are emissive in the NIR; biological tissues are relatively transparent in this spectral range, and a much wider range of sensitizing chromophores can be used.\textsuperscript{36, 39} There are a number of NIR emitting fluorophores available for optical imaging, but they still suffer from short lifetimes. NIR emitting lanthanides such as neodymium and ytterbium have longer lifetimes albeit a lot shorter than the visible emitting lanthanides. As the lanthanides emit in the NIR, chromophores that absorb visible light can be used to excite them. More recently, polypyridyl complexes of d-transition metals have been used to excite NIR emission and this is discussed in more detail in Chapter 4.1.

Whilst luminescence microscopy offers superb spatial resolution at the sub-cellular level, it is limited to samples that are a few millimeters thick. The next section (1.6) discusses the use of magnetic resonance imaging, which offers whole body imaging. Section 1.9 deals with further advances in imaging which combine the advantages of both luminescence microscopy and MR imaging whilst minimising their disadvantages.

1.6 Magnetic Resonance Imaging (MRI)

Over the last two decades, magnetic resonance imaging has emerged as the leading diagnostic technique for imaging in clinical medicine and biomedical research. It has gained increasing popularity over other imaging modalities because it is non-invasive, has high spatial resolution, good sample penetration, no radiochemicals are needed and there is little perturbation caused to the patient's system. It also offers fast scan times and the capacity to produce excellent quality and high-resolution images.\textsuperscript{40, 41}

Clinical magnetic resonance imaging is essentially an elaborate proton nuclear magnetic resonance (NMR) experiment that visualizes water molecules in the human body.\textsuperscript{42} The body is made up of over 70\% water and when the patient is placed within the main magnetic field of an MRI scanner, a small percentage of the water nuclei align their magnetic axes with it.
A short radiofrequency pulse is then applied which causes the aligned nuclei to be deflected (by 90°) from the main magnetic field. After the radiofrequency pulse is switched off, the nuclei realign themselves with the main magnetic field giving off energy in the process, which is detected by a radiofrequency receiver. Computer manipulations allow three-dimensional localization of the signal from the body tissue, which is then displayed as a sequence of two-dimensional “slices”.

Fig.1.9 T₁ weighted MRI scan of a human brain⁴³

Current MR images are acquired using magnetic field strengths from 1.5 to 7 Tesla in clinical applications and as high as 21 T for high resolution molecular imaging.⁴⁴ Due to the varying water concentration in different environments of the body an intrinsic contrast can be observed that can differentiate between healthy and diseased parts of the same tissue.⁴⁵, ⁴⁶

Unfortunately, MR imaging is not without its drawbacks. It suffers from low sensitivity compared to radiotracer methods such as PET⁴⁷, ⁴⁸ and SPECT⁴⁹-⁵¹ and often contrast agents are employed to combat this setback.
1.7 Contrast Agents

MRI contrast agents are chemical compounds which are able to markedly alter the relaxation times of water protons in tissues where they are distributed and in so doing enhance contrast between normal and diseased tissue, to show blood flow or physiological function for example.\textsuperscript{52}

Contrast agents are currently used in about 35\% of MR imaging experiments; this figure is set to increase as contrast agents become ever more sophisticated. Most contrast agents used today are chelates of the lanthanide gadolinium due to high paramagnetism arising from its seven unpaired f-electrons and relatively slow electron spin relaxation rate.\textsuperscript{53} The free Gd(III) ion \textit{in vivo} has a radial size which is approximately equal to that of Ca(II) and can therefore disrupt calcium mediated signalling, forming strong complexes that can accumulate within the body.\textsuperscript{54} It is therefore imperative to complex the ion with appropriate ligands to prevent transmetallation from occurring \textit{in vivo}.

These chelates must conform to very stringent requirements since they are to be administered into the body. These are:

1. high water solubility
2. lack of toxicity
3. rapid excretion
4. high relaxation effect
5. high thermodynamic and kinetic stabilities

Contrast agents can be divided into two groups depending on whether they cause changes in longitudinal relaxation ($T_1$ - the time taken for the protons to realign with the external magnetic field) or transverse relaxation ($T_2$ - the time taken for the protons to exchange energy with the other nuclei) and are known as positive or negative agents respectively.
1.7.1 Relaxivity

The efficacy of a contrast agent in relaxation enhancement is often expressed in terms of relaxivity ($r_1$), which is defined as the increase of the longitudinal water proton relaxation rate per millimolar concentration of Gd(III). Paramagnetic relaxation of water protons occurs through dipole-dipole interactions between the nuclear spins and the fluctuating local magnetic field caused by the unpaired electron spins. This magnetic field around the paramagnetic centre vanishes rapidly with distance therefore proximity of the water molecule(s) is important for the propagation of relaxation to the bulk solution.

The presence of a Gd(III) complex increases the longitudinal ($1/T_1$) and transverse ($1/T_2$) relaxation rates of solvent nuclei. The observed solvent relaxation rate, $1/T_{1,\text{obs}}$, is the sum of the diamagnetic (d) and paramagnetic (p) contributions given by the following equation:

$$1/T_{1,\text{obs}} = 1/T_{1,d} + 1/T_{1,p}$$

(6)

The diamagnetic term arises from the relaxation rate of water proton nuclei in the absence of a paramagnetic ion whilst the paramagnetic term can be expressed as the relaxation rate enhancement induced by the paramagnetic species, which is linearly proportional to its concentration.

$$1/T_{1,\text{obs}} = 1/T_1/d + r_1[Gd]$$

(7)

By plotting $1/T_1$ against Gd(III) complex concentration, relaxivity can be obtained as the gradient.

The total paramagnetic relaxation rate due to a paramagnetic species receives contributions from two mechanisms: inner sphere (IS), due to water molecules directly bound to the metal, and outer sphere (OS) which involves all the solvent molecules diffusing by the complex.

$$R_1^{\text{OBS}} = R_1^{\text{IS}} + R_1^{\text{OS}} + R_1^W$$

(8)
1.7.2 Optimization of relaxivity

Proton relaxivity is influenced by a number of parameters outlined below (figure 1.10) and is also dependent on magnetic field strength and temperature. Most contrast agents used in clinical applications today have low relaxivities in the 4-10 mM\(^{-1}\) s\(^{-1}\) range, far below the theoretical maximum of \(\sim 100\) mM\(^{-1}\) s\(^{-1}\) predicted by the Solomon-Bloemberg theory\(^{56}\).

Due to the relatively low relaxivity values of commercial CAs, they have to be administered in high doses. To maximize the potential of contrast agents, the four most important factors influencing relaxivity need to be optimized. These are:

1. The exchange rate \(k_{ex}\) of the water molecules directly bound to the metal
2. The hydration number, \(q\)
3. The rotational diffusion of the complex, described by a correlation time \(\tau_R\)
4. The electronic spin relaxation times, \(T_1\) and \(T_2\).

![Fig.1.10 Illustration of the different parameters that require optimization for maximum relaxivity of gadolinium CAs\(^{56}\)](image-url)
1.7.2.1 Hydration number (q)

The majority of commercially available contrast agents have only one water molecule attached which give rise to modest relaxivities that are a small percentage of that predicted by the Solomon-Bloemberg-Morgan theory.

An obvious way to increase relaxivity would be to increase the hydration number by making contrast agents from hexa- or heptacoordinate ligands. The first studied complex with two water molecules in the inner sphere was the Gd(DO3A) complex. The relaxivity was found to be double that of commercially available agents but was easily quenched by substitution of the water molecules with small ligands such as phosphate and carbonate. This has subsequently been found to be a major problem for contrast agents of this nature.

Aime et al. have synthesized a contrast agent with the heptadentate ligand AAZTA (6-amino-6-methylperhydro-1,4-diazepinetetraacetic acid) 57. Synthesis of the complex was easy and made from cheap and readily available chemicals. It had a relaxivity value of 7.1 mM\(^{-1}\) s\(^{-1}\) (20MHz, 298K) that did not change on titration with 200 equivalents of lactate or phosphate.

Raymond et al. have reported a group of hydroxypyridonate (HOPO) 58-66 based chelates which have two or three water molecules coordinated to the metal centre which show high relaxivities and more importantly, high stabilities.

Although these complexes show high relaxivities that are not adversely affected by endogenous anions and high thermodynamic stabilities, they suffer from low kinetic inertness in comparison with most octadentate ligands.

**Phosphate repulsion**

In nine coordinate complexes possessing one bound water molecule, the bound water is generally very difficult to substitute in aqueous media even though it may be undergoing very fast exchange with bulk water. This is a consequence of the high concentration of water compared to that of monodentate anions which might bind.
In the case of complexes with two or three bound waters, the molecules are usually readily displaced by addition of ligating anions. This is especially true for chelating species such as lactate, citrate, malonate, phosphate, or carbonate as it is more entropically favourable for one anion to be bound rather than two water molecules. These anions are found in considerable concentrations in human serum and binding by a contrast agent can lead to a number of detrimental consequences. Firstly, relaxivity of the contrast agent will be reduced by competition of the anions with bulk water for coordination to the gadolinium centre. Secondly, if the gadolinium complex is particularly unstable, phosphate can compete with the ligand, resulting in the precipitation of insoluble adducts in the bloodstream.

Raymond et al synthesized a series of heptadentate HOPO based Gd(III) complexes of varying charges which were found to have two or three bound water molecules. By studying the interactions between the gadolinium complexes and physiological anions by relaxometry, they observed that the affinity for these anions decreased with overall negative charge of the complex.

1.7.2.2 Rotational correlation time; $\tau_r$
This parameter is controlled by the rate of tumbling of the Gd complex. Essentially, the slower the complex tumbles the higher the relaxivity exhibited. A number of approaches have been used to lengthen $\tau_r$. The most common approach is to attach the Gd complex to a slowly tumbling macrocycle such as a dendrimer, polysaccharide or protein.

Aime et al, found that by trapping several molecules of the contrast agent GdHPDO3A in a slowly tumbling protein- namely iron-free apoferritin, a huge increase in the relaxivity per gadolinium complex was observed from 4.2 to 80 mM$^{-1}$s$^{-1}$. 
Rotational tumbling time can also be increased by simply making complexes with larger ligands. This was demonstrated by Wong et al. using a variation on the commonly used polyaminocarboxylate macrocycles. These complexes demonstrated relatively high relaxivity values that can be attributed to their larger size. However, the water exchange rate was found to be slow and needed to be optimized further before application.

1.7.2.3 Mean residence lifetime; $\tau_m$

The overall charge of the complex has a great effect on this parameter with negatively charged complexes exhibiting the fastest exchange times. Rate of exchange also depends on the hydrophobicity of the complex and also on the lanthanide ion. For DOTA-like ligands, the water molecule has been found to exchange 10-100 times faster in the TSAP than in the SAP isomer. It has been found that having one or more phosphinic or phosphonate groups on the DOTA scaffold can lead to an increase in the ratio of TSAP isomers hence increasing the water exchange lifetime.

A new class of contrast agents based on Eu and Yb complexes of DOTA-tetraamides has been pioneered by Ward and Balaban called Chemical Exchange Saturation Transfer (CEST) contrast agents. This technique relies on the application of a constant saturating irradiation pulse at an exchangeable proton site a few ppm from the resonance of water. The consequent decrease in the intensity of the free water proton signal is manifested as contrast in the resulting image.

1.7.2.4 Electronic spin relaxation times, $T_1$ and $T_2$.

Contrast agents can be divided into two groups depending on whether they cause changes in the time taken for the protons to realign with the external magnetic field ($T_1$- longitudinal relaxation) or the time taken for the protons to exchange energy with other nuclei ($T_2$- transverse relaxation). MR contrast agents are classified as either $T_1$ or $T_2$ agents, corresponding to either positive or negative enhancement.
1.7.3 First generation contrast agents

The first generation contrast agents currently in clinical use are low molecular weight chelates of gadolinium. As mentioned earlier, the Gd(III) is the ion of choice over other paramagnetic ions such as Fe(III) and Mn(II) because it combines a large magnetic moment with a long electronic spin relaxation time of $10^{-9}$ s at the magnetic field strengths used in MRI experiments.\(^{40}\)

Two main ligand types that are used to encapsulate the gadolinium ion are cyclen derivatives (DOTA) and acyclic triamines (DTPA). They both have eight donor atoms, which coordinate to the metal leaving the last site free to be occupied by a water molecule. The protons of this molecule in the first coordination sphere of Gd(III) rapidly relax and the fast exchange of this H\(_2\)O with the bulk water results in transfer of the paramagnetic effect to the surrounding water protons.\(^{46, 75}\) Figure 1.11 shows the contrast agents in clinical use today.

\[\text{Fig. 1.11 Clinically approved contrast agents and their relaxivities at } 37^\circ\text{C and } 1.5\text{ T}^{46}\]
These contrast agents are non-specific and distribute throughout plasma and interstitial spaces and are excreted rapidly via the kidneys or the liver.\textsuperscript{42}

1.7.3.1 Stability of contrast agents

The safety of contrast agents \textit{in vivo} is of paramount concern due to the toxicity of the free metal ion and ligand. Gd(III) has an ionic radius of 107.8 pm which is close to that of Ca(II) at 114 pm. It is an inorganic blocker of many types of voltage-gated calcium channels at nano- to micromolar concentrations and can as a result inhibit physiological processes such as cardiac muscle contraction which rely upon Ca(II) influx.\textsuperscript{3, 76} Furthermore, due to the affinity of lanthanides for hard anions such as phosphate and carbonate which are present \textit{in vivo}, formation of insoluble adducts can occur. Two parameters are used to assess the stability of the chelate \textit{in vivo}, namely thermodynamic and kinetic stability.

1.7.3.1.1 Thermodynamic Stability\textsuperscript{75, 76}

When the gadolinium ion is chelated, a thermodynamic equilibrium exists that is described by the following equation:

\[ [M] + [L] \rightleftharpoons [ML] \] \hspace{1cm} (9)

The toxicity of the complex then depends on its ability to release free Gd(III) ions. Two concepts have been proposed to explain the thermodynamic stability of the gadolinium chelate.

Firstly, the thermodynamic stability constants \( K_{\text{therm}} \) and \( K_{\text{cond}} \) which reflect the affinity of gadolinium for its ligand at high basic pH and 7.4 respectively. They are expressed as:

\[ K_{\text{therm}} = \frac{[ML]}{([M] \times [L])} \] \hspace{1cm} (10)

And

\[ K_{\text{cond}} = K_{\text{therm}} \times [L]/L_T \quad (L_T = \text{total concentration of unchelated ligand}) \] \hspace{1cm} (11)
The value of $\log K_{\text{therm}}$ is valid at high basic pH and the conditional thermodynamic stability constant $\log K_{\text{cond}}$ is calculated at pH 7.4 on the basis of the $\log K_{\text{therm}}$ values and protonation constants of the ligand and describes the position of the equilibrium at physiological pH. The higher the stability constant, the more stable is the complex and the less free gadolinium ion and ligand are present when given enough time to reach thermodynamic equilibrium.

In the design of contrast agents, three main structural features have been found to influence the thermodynamic stability of gadolinium chelates:

1. The basicity of the polyaza-carboxylate scaffold, which can be evaluated by calculating the sum of the protonation constants of each donor atom of the ligand.
2. The number of five-membered rings (N-Gd-N and N-Gd-O) formed by the chelate between the metal and the various donor atoms of the ligand as five-membered rings minimise the steric strain in the chelate.
3. The macrocyclic effect which is related to the cavity size of the chelate ring, preorganization, rigidity and conformation of the ligand. The extent of dissociation has been found to be larger for the complexes formed with the open-chain DTPA and its derivatives than with the macrocyclic DOTA and its derivatives.

The second concept that has been used to explain the thermodynamic stability of gadolinium chelates is the selectivity of the ligand for Gd(III) over other endogenous metal ions, particularly Zn(II). In the presence of these endogenous ions the following equilibrium can exist if the ligand is not selective enough for gadolinium:

$$\text{GdL} + [\text{M}]^{2+} \rightleftharpoons \text{Gd}^{3+} + [\text{ML}]^- \quad (12)$$

The selectivity constant is described by:

$$\log K_{\text{sel}} = \log K_{(\text{GdL})} - \log K_{(\text{ML})} \quad (13)$$

and corresponds to the difference in thermodynamic stability constants between the complex GdL and ML.
Even though commercially used contrast agents have been shown to have high thermodynamic stabilities, attempts to find a relationship between the stability constants and the toxicity of the contrast agents have failed indicating that though important, stability constants do not tell the full story.

1.7.1.1.2 Kinetic stability\textsuperscript{75, 76}

It is now widely established that kinetic inertness is much more important than thermodynamic stability when considering the toxicity of contrast agents. It is classically estimated through the half-life of dissociation of the complex in acidic media as dissociation is very slow at physiological pH.

In a very acidic medium, the complex dissociation is a pseudo-first order reaction, with

\[
\text{Dissociation rate} = k_{\text{obs}} [\text{GdL}]
\]

Tweedle \textit{et al}\textsuperscript{107} measured the rate of approach of equilibrium for several chelates in 0.1 M HCl (pH 1.0) and the results are in table 1.3. They found that half-lives dramatically differed and were clearly found to be longer for macrocyclic chelates than for linear chelates. The kinetic inertness of DOTA type complexes is attributable to the rigidity of the chelate ring structure.

<table>
<thead>
<tr>
<th>Gadolinium chelates</th>
<th>$k_{\text{obs}}$ (s\textsuperscript{-1})</th>
<th>$T_{1/2}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gd-DTPA-BMA</td>
<td>$2.0 \times 10^{-2}$</td>
<td>35 s</td>
</tr>
<tr>
<td>Gd-DO3A</td>
<td>$2.3 \times 10^{-3}$</td>
<td>5 min</td>
</tr>
<tr>
<td>Gd-DTPA</td>
<td>$1.2 \times 10^{-3}$</td>
<td>10 min</td>
</tr>
<tr>
<td>Gd- HP-DO3A</td>
<td>$6.3 \times 10^{-5}$</td>
<td>3 h</td>
</tr>
<tr>
<td>Gd-DOTA</td>
<td>$2.1 \times 10^{-5}$</td>
<td>&gt;1 month</td>
</tr>
</tbody>
</table>

\textbf{Table 1.3} $k_{\text{obs}}$ and $T_{1/2}$ values (at 25°C in 0.1 N HCl) of several gadolinium complexes\textsuperscript{107}
1.7.4 Second generation contrast agents
The first generation contrast agents are very useful but are not specific as they distribute between all extra-cellular spaces. The next generation of contrast agents are designed to be more specific and effective, offering higher relaxivity, thermodynamic stability and a more favorable rate of excretion.

1.7.4.1 Responsive (Smart) contrast agents
Responsive contrast agents are those that respond to changes in physiological environment such as pH, metal ion concentration, partial pressure of oxygen or enzyme activity.

1.7.4.1.1 pH Responsive contrast agents
Extra-cellular pH of healthy tissue is 7.4 but that of tumour tissue is more acidic at pH 6.8-6.9.\textsuperscript{42} This fact has led researchers to try and exploit this difference to create contrast agents that can respond to these pH differences to highlight or map tumours.

Parker \textit{et al} took advantage of the propensity of seven coordinate lanthanide complexes to form ternary compounds with endogenous anions in their design of pH responsive agents. Firstly they made a GdDO3ala complex as a means to studying HCO$_3^-$ concentrations in solution and pH in the ambient range via H$_2$CO$_3$/HCO$_3^-$ equilibrium (Scheme 1.1). The complex was studied under physiological conditions in a simulated clinical anion background and was found to bind the anion in a pH dependent manner. As pH increased so did the amount of carbonate that was bound with a subsequent decrease in the relaxivity as inner sphere waters were displaced.\textsuperscript{77, 78}
Scheme 1.1 Intermolecular anion binding leading to lowered relaxivity

Parker et al also made a smart pH contrast agent, which used reversible intramolecular binding of a sulfonamide (scheme 1.2). The sulfonamide has a pH dependent affinity for the gadolinium centre; in basic media, the sulfonamide is deprotonated and bound to the Gd$^{3+}$ ion therefore $q=0$ and the relaxivity is low. In more acidic media, the sulfonamide is protonated and so is not bound to Gd, $q=0$ and higher relaxivity is observed. This pH response can be brought into the physiological range by varying the para-substituent on the aryl ring to fine-tune the pKa.$^{79, 80}$

Scheme 1.2 Intramolecular anion binding leading to lower relaxivity
Sherry et al observed that relaxivity of tetraamide based ligands with extended phosphonate or carboxylate side chains had a pH dependence between pH 4-10.5. There was a ~2.6 fold increase in relaxivity catalysed by their GdDOTAMP complex over the pH range 6-9 that seemed to parallel protonation of the extended phosphonate groups. They concluded that hydrogen bonding network created by protonation of the phosphonates provided a catalytic pathway for the exchange of bound water protons with those of bulk water. However, on further investigation the ligand was found to form two different complexes with gadolinium depending on the pH the complexation took place. The desired Type II pH sensitive complex was obtained at basic pH >9. The presence of even small amounts of the Type 1 complex formed under more acidic conditions could affect the calibration curve needed for quantifying tissue pH and hence care needed to be taken when preparing samples of GdDOTAMP.

1.7.4.1.2 \( pO_2 \) Responsive contrast agents

Partial pressure plays an important role in cellular metabolic processes and anomalies in \( pO_2 \) indicate many pathologies such as strokes and tumours. Responsive MRI contrast agents in this area are designed to cause a change in relaxivity depending on the redox state of the metal, itself determined by partial pressure of oxygen in the biological environment.

Some research has focused on the Eu\(^{III/II}\) redox couple in an attempt to achieve this. Eu(II) is isoelectronic with Gd(III) therefore they have similar relaxivities whereas Eu(III) is a poor relaxing agent. The challenge is to control the redox and thermodynamic stability of Eu(II) complexes. DOTA and DTPA type complexes have very negative redox potentials favouring Eu(III) therefore work is being done on finding suitable ligands to stabilize Eu(II).
The most stable Eu(II) chelate studied so far is the ten coordinate Eu(2.2.2)Cryptate (2.2.2(4,7,13,16,21,24-hexaoxa-1,10-diazabicyclo[8.8.8]hexacosane)\textsuperscript{84} which has two free sites for water molecules. It was proposed as a redox on/off switch where the oxidation of Eu(II) to Eu(III) will lead to a large decrease in relaxivity. However the cavity of the cryptand although well suited to Eu(II) does not fit well for Eu(III) so will be unstable.

Aime \textit{et al} have used the MnII/III redox couple in a TPPS\textsuperscript{85} complex which have been found to have a high affinity for tumor cells, but the relaxivities of both complexes were too close to be exploited.

1.7.4.1.3 Metal ion responsive contrast agents

Many diseases have been linked with altered concentration of metal ions in the body for example copper and iron deficiency are linked to anemia. \textit{In vivo} determination of metal ion concentration is therefore a considerable concern. Ca(II) has very important roles in the body including controlling muscular contraction, neural cell communication and hormonal secretion. However its excess can lead to cell death.

Meade \textit{et al} reported the first contrast agent (GdDOPTA) whose relaxivity was modulated by calcium concentration (Scheme 1.3). In the absence of Ca(II), the aromatic iminoacetates of BAPTA interact with Gd(III) of the DO3A complex through ionic attractions rendering $q$ equal to zero and hence lowering relaxivity. In the presence of Ca(II) the aromatic iminoacetates rearrange to bind Ca(II), thereby allowing water to bind directly to Gd(III) and increasing relaxivity. The contrast agents was also found to be selective for Ca(II) over Mg(III) and also insensitive to $[H^+]$ concentration in the physiological pH range.\textsuperscript{86}
Nagano et al synthesized a GdDTPA bisamide complex as a Zn(II) sensitive MRI contrast agent (scheme 1.4). The complex which bears pendant pyridyl groups displayed a reduction in relaxivity of ~33% in the presence of Zn(II) when one equivalent of Zn is bound. On addition of a second equivalent the relaxivity increases. This concentration dependent change was attributed to a displacement of an inner-sphere water on forming a 1:1 GdL:Zn complex bound by all four pyridyls, followed by a change in coordination geometry on forming the 1:2 complex with a water molecule once more bound to Gd(III).

Scheme 1.4 Reversible binding of Zn(II)
1.7.4.1.4 Enzyme Responsive contrast agents

These contrast agents are designed to be MRI silent until activated by an enzyme. A classic example of this was by Meade et al who designed an agent called Egad (scheme 1.5). The complex had a galactopyranose unit blocking the ninth coordination site of Gd(III) which was cleaved in the presence of the common marker enzyme β-galactosidase allowing a water molecule to be bound to the metal centre and increasing relaxivity.\(^{88}\)

\[ \text{Enzyme} \rightarrow \text{Gd}^{3+} + \text{H}_2\text{O} \]

Scheme 1.5 Cleavage of the pyranose sugar leading to increased relaxivity

McMurray et al used the theory of RIME (receptor-induced magnetization enhancement) to produce an enzyme responsive agent. A GdDTPA complex with a trilysine masking group which had poor HSA affinity was synthesized. In the presence of human carboxypeptidase B, thrombin activable fibrinolysis inhibitor (TAFI) the masking group is cleaved allowing HSA to be bound (scheme 1.6). This increased relaxivity by increasing the rotational correlation time.\(^{89}\)
1.8 Bimodal imaging

There are now several imaging modalities available for clinical applications but no single one is perfect and sufficient to gain all the necessary information. For instance, optical fluorescence imaging is difficult to quantify; MRI has excellent resolution but low sensitivity and PET has very high sensitivity but poor resolution. It would seem that the obvious solution would be simply adding two different classes of probes together but this is only effective if they both have identical pharmacodynamic properties.  

Multimodal imaging is the synergistic combination of two or more detection techniques, made possible by multimodal probes, imaging agents and instrumentation. It ensures enhanced visualization of biological materials and better reliability of collected data. One major potential for multimodal imaging is in guided surgery applications where the fluorescence imaging can guide incisions in surgical procedures and MR imaging can be used to ensure that surgery is complete.
Although there are several different combinations of ways in which multimodal imaging can be achieved e.g. PET/Optical, PET/MRI, SPECT/Optical, only those that use MRI and luminescence will be discussed here as these are the focus of this thesis.

Current commercial contrast agents are simple low molecular weight chelates of gadolinium. These molecules cannot provide luminescence imaging as the gadolinium ion is not luminescent due to the triplet state being at a very high energy level making it difficult to populate by standard methods. It would also emit in the UV which is a where a lot of biological molecules absorb making it difficult to detect. An obvious approach to multimodal imaging would be to link a luminescent species to an MRI active agent. Several groups have conceived different approaches to achieve multimodal imaging some of which are discussed in the following section.

1.8.1 MRI and Optical imaging

The well established gadolinium DTPA and DOTA chelates used for MR imaging have been used as platforms to achieve dual imaging. Meade et al modified the DOTA ligand with a pendant p-aminobenzyl group linked to one of the methylene carbons which could be linked covalently to a fluorescent marker such as tetramethylrhodamine-5-isothiocyanate (TRITC). In vitro studies using Gd(Rhoda-DOTA) (10) showed strong fluorescent and MR signals with a relaxivity comparable to GdDOTA. However, when similar concentrations were injected into living embryos, the fluorescence signal remained but the MR signal could not be observed. This was attributed to the absorption of the hydrophobic molecule into fatty tissues where there is little or no water signal, which was detrimental to the MR signal. 

\[ \text{Gd(DOTA)} \]

\[ \text{Gd(Rhoda-DOTA)} \]
More recently, DO3A was functionalized with the fluorophore-ethylthioureafluorescein on the fourth nitrogen as a precursor for bimodal imaging (11). Relaxivity studies showed that $r_2$ was greater than $r_1$ making it more of a $T_2$ contrast agent. Fluorescence microscopy of living cells revealed uptake of the agent into the cells at concentrations greater than 50 µM indicating the potential of the molecule as an intracellular contrast agent.\textsuperscript{101}

In order to optimize cell entry, Miller \textit{et al} synthesised Gd.DOTA.DSA 12 a gadolinium-lipid contrast agent for liposomal cell labeling and tumor imaging. The CA had high relaxivity values comparable to Magnevist and high cellular uptake by HeLa cells as revealed by fluorescence microscopy. A charge neutral version of the agent was encapsulated in a biocompatible polymer outer coat comprising poly(ethylene glycol) (PEG) and administered to nude mice inducted with IGROV-1 (human ovarian cancer cells) xenografts to demonstrate the efficacy of the agent toward imaging of xenograft tumours \textit{in vivo}. A 60\% reduction in tumor $T_1$ values was observed compared to control liposomes and visible enhancement of tumor image brightness was observed. The finding was supported by fluorescence imaging of postmortem tumor tissue slices which demonstrated liposome accumulation in intravascular spaces and surrounding tumor tissue.\textsuperscript{102}

![Diagram of Gd.DOTA.DSA](image)

The overexpression of peripheral benzodiazepine (PBR) has been linked to a number of diseases such as Alzheimer and cancer. Bornhop \textit{et al} described the first PBR-targeted agent using a cocktail of Eu(III) and Gd(III) complexes of a Ln-PK11195 molecule to produce cellular scale bimodal imaging in C6 glioma cells.
The Gd-PK11195 molecule (13) was used to provide imaging comparable to Magnevist. Fluorescence microscopy was performed after 60-min incubation of the sample with a 40-60 cocktail of the Eu-PK11195 and Gd-PK11195 to give a cellular image.\textsuperscript{103}

![Chemical Structure](image)

**Fig.1.12** (A) White-light image of C6 glioma cells (B) Fluorescence image (C) Blank (D) MRI image of cocktail-dosed C6 glioma cells (E) MRI image of undosed cells.\textsuperscript{102}

A major advantage of this method is that the ratio of each chelate in the administered dose can be controlled. Fluorescence imaging is a more sensitive technique than MR imaging therefore smaller quantities are needed compared to the Gd chelate which has to be administered in a larger concentration.

Bimodal imaging in the Faulkner group has involved the use of transition metal complexes linked via a pyridine moiety to GdDO3A (14).\textsuperscript{104}
Transition metal complexes such as ruthenium(II) polypyridyls can function as photosensitisers as they have intense, low energy and readily tunable charge-transfer absorption bands. The use of transition metals as a luminescent species is also advantageous due to their emission wavelengths being in the NIR region. Eu(III) and Tb(III) both emit in the visible region and require sensitization by long-wavelength UV radiation which is the range strongly absorbed by biological molecules making their use for in vivo applications unlikely even though they have longer lived luminescence lifetimes.

In 14, the macrocycle is heptadentate, therefore the Gd$^{3+}$ centre can coordinate two water molecules which in turn should provide good relaxivity. However this molecule was found to bind phosphate as expected for heptadentate complexes and is a problem that needs to be addressed if these types of molecules are to be used for bimodal imaging in vivo.

Toth et al designed a pyridine based ligand 15 which was shown to have high relaxivity when complexed to Gd(III) due to the bishydration of the chelate and efficient NIR sensitization when complexed to Nd(III). Furthermore, the relaxivity of the gadolinium complex was unaffected in bovine serum and the emission spectra of the europium complex were identical in both pure water and in the presence of 600 equivalents of carbonate or phosphate (pH 7.4), indicating that inner sphere water molecules were not replaced by potential donor groups. The complexes were also shown to be stable towards Zn(II) or Cu(II) transmetallation and also acid-catalysed dissociation was slow due to the rigid pyridinic skeleton.\textsuperscript{105}
Quantum dots have also been considered for bimodal imaging. They are nanoparticulate clusters of semiconductor material that are gaining more attention in the field of biological imaging due to their bright fluorescence, photostability, and their narrow and size tunable emission spectrum.

Mulder et al. coated the QD with a micellar and paramagnetic coating in order to make them MR detectable (figure 1.13). Relaxivity was revealed to be in excess of 2000 mM$^{-1}$ s$^{-1}$. By conjugating the pQD with cyclic RGD peptides, they were able to successfully target human endothelial cells *in vitro.*

![Fig. 1.13 Lipid coated, water soluble paramagnetic QD](image)

### 1.9 Aims and objectives

The aims of this project were twofold. First of all, heptadentate complexes that could coordinate two water molecules were synthesized. These complexes were designed to have good kinetic and thermodynamic stabilities, as well as incorporating hydrolysable functional groups as a means to generating negatively charged complexes, which should in theory repel phosphate. The second aim of this project was to link heptadentate complexes to a d-transition complex in order to probe their uses as potential bimodal imaging agents.
Chapter Two describes how tri-isophthalate complexes were synthesized with hydrolysable ester groups as a means to varying the local charge. Chapters three to five describe the use of phosphonate and phosphinate pendant arms that could be hydrolysed to increase the overall negative charge on the resulting complexes.
1.10 References


CHAPTER TWO
TRIS-ISOPHTHALATE COMPLEXES

2.1 Introduction
One of the aims of the work described in this thesis is to synthesise complexes with increased relaxivity compared to commercially available contrast agents. Optimisation of relaxivity can be achieved by tuning one or more of the parameters discussed in section 1.8.1 of the introduction. The focus of this chapter is to probe the effect of hydration number (q) upon relaxivity, and to investigate processes that can compete with solvent exchange.

Aime et al conducted a detailed analysis of the $^1$H and $^{17}$O NMR relaxometric properties of GdDO3A, GdPCTA and GdPCTP (figure 2.1) and found that these heptadentate complexes which have two coordinated water molecules show improved relaxivity over their octadentate counterparts. In addition to this, they also display faster water exchange rates at the metal centre suggesting that a dissociative mechanism whose rate-determining step is the dissociation of one coordinated water molecule by a pathway analogous to that occurring in related complexes with octadentate ligands with one coordinated water molecule.

![Fig. 2.1 Heptadentate ligands studied by Aime et al](image)

Fig. 2.1 Heptadentate ligands studied by Aime et al
Complexes derived from heptadentate ligands will clearly have lower kinetic stability than DOTA derivatives which may make them unsuitable for *in vivo* applications, unless they can be rendered sufficiently rigid to make them kinetically inert. Aime *et al* have been able to show that these complexes can have the thermodynamic stability required plus higher relaxivity and faster exchange rates making them promising targets for contrast agents. To date the kinetic stability of only a few gadolinium chelates of heptadentate ligands have been studied eg, GdPCTA, GdAAZTA but they seem to indicate that it is possible to make contrast agents from heptadentate ligands which will be sufficiently stable *in vivo*.

Continuing in this direction, Koullorou *et al* synthesized a prototype bimodal imaging agent based on a Gd(DO3A) unit linked to a d-transition metal complex that displayed both MRI and luminescence imaging moieties (figure 2.2).

![Fig. 2.2 Dual imaging agent](image)

This d-f hybrid possesses a relaxivity of $8.6 \text{ mM}^{-1} \text{s}^{-1}$ in aqueous solution but this drops significantly to $3.9 \text{ mM}^{-1} \text{s}^{-1}$ when measured in phosphate buffered saline. This is a result of the bidentate phosphate anion displacing the water molecules at the metal centre leading to a reduction in inner sphere solvation.

This is a common problem encountered with heptadentate complexes, in that they are prone to attack by bidentate anions such as carbonate and phosphate which are found in abundance *in vivo*. This has in fact been exploited by a number of groups to produce luminescent anion sensors.
Parker et al. were able to demonstrate reversible anion binding by studying the interaction of a few lanthanide macrocyclic complexes with a series of bioactive anions such as carbonate, phosphate, lactate, citrate and malonate. They found that the affinity for these anions decreased as a function of the overall negative charge of the complex.\textsuperscript{3, 6}

These findings are largely corroborated by the research of the Raymond group on their hydroxypyridonate (HOPO) type complexes \textsuperscript{19-20}. The HOPO ligands have been designed to have two or three coordination sites available for the fast exchange of water molecules whilst maintaining high thermodynamic stability.\textsuperscript{7-11}

\begin{center}
\includegraphics[width=\textwidth]{Fig_2_3.png}
\end{center}

\textit{Fig. 2.3} HOPO complexes synthesized by the Raymond group\textsuperscript{7-11}

They studied the relaxivities of a positively charged complex and a negatively charged complex in the presence of selected endogenous anions. In the study, solutions of both Gd(III) complexes in the presence of a 200-fold molar excess of the respective anion were neutralized to pH7.4 and equilibrated for 2 weeks. The relaxivities of the complex in the presence of each anion were then compared to that measured in pure water. They found that affinity for the endogenous anions decreased with the overall negative charge of the complex.\textsuperscript{11} However these complexes are unlikely to be stable \textit{in vivo} as they are kinetically labile.
These findings have been used to aid the design of the complexes in this research. This chapter describes the synthesis of heptadentate triamide complexes based on cyclen that incorporate hydrolysable moieties, which can give rise to negatively charged species that should repel phosphate. The fourth position on cyclen will be left vacant for future reaction with a pyridyl linker.

### 2.2 Synthesis of heptadentate isophthalate complexes

Scheme 2.1 shows the route towards the protected and deprotected isophthalate complexes.

$\text{Scheme 2.1 Synthesis of protected and deprotected heptadentate isophthalate complexes}$
2.2.1 Formation of protected isophthalate complexes

Initially, the arm was synthesized by dissolving dimethyl 1,5-aminoisophthalate 21 in DCM followed by the addition of sodium hydrogen carbonate. Chloroacetyl chloride was added dropwise and the mixture stirred overnight after which the solids were isolated by filtration.

The proton NMR spectrum (figure 2.4) shows the singlet peak at 3.8 ppm which corresponds to the methyl ester protons. This peak integrates to six protons, as expected. The peak at 4.1 ppm corresponds to the two methylene protons situated next to the chlorine and the two peaks at 8.3 and 8.4 ppm correspond to the phenyl protons. Negative ion electrospray gave a peak at [284]⁺ corresponding to the expected mass minus a proton.
The next step involved the tri-alkylation of cyclen with the chloroacetamide prepared earlier. Sodium hydrogen carbonate and potassium iodide were added to cyclen in acetonitrile at 0°C. A solution of the chloroacetamide arm also in acetonitrile was then added dropwise to the mixture and this was stirred for five days. Mass spectrometry of the crude product showed presence of the triamide, however the bis- and tetraamides were also present. Initially purification was attempted using column chromatography on neutral alumina column, but this gave only a small amount of white solid which was insoluble in most organic solvents and only sparingly soluble in DMSO.

Therefore purification was attempted by precipitation of the product from a concentrated solution of chloroform using diethyl ether. This gave a higher degree of purity and improved yields but the proton NMR spectrum still showed traces of an impurity.

The pure product was finally obtained as a white solid by recrystallisation from acetonitrile that involved heating the crude product in acetonitrile, then cooling to 4°C.
The electrospray mass spectrum of 25 in figure 2.5 displays a peak corresponding to the protonated molecular ion. The proton NMR spectrum in figure 2.6 shows the anticipated peaks at 4.8 ppm corresponding to the 18 methyl protons. The broad peaks from 2.7-3.3 correspond to the CH₂ protons on the cyclen ring. The broadness of the peaks corresponding to the ring protons is typical of tri-alkylated cyclen compounds as the ligand is less rigid compared to tetra-alkylated analogues. This means that the ring is more fluxional in solution and close to the timescale of the NMR experiment hence broadening of the peaks is observed.
Complexation of the tri-alkylated compound was achieved by slow addition of one equivalent of lanthanide (Gd, Eu, Yb and Tb) triflate solution in dry acetonitrile to a solution of the triamide 25 also in anhydrous acetonitrile. The resulting solution was stirred for one hour at 60°C under nitrogen. After an hour, the reaction mixture was cooled to room temperature and the solvent removed. The residue was dissolved in the minimum volume of acetonitrile and a cream solid was precipitated from solution by slow addition to diethyl ether. This was isolated by filtration and dried under reduced pressure.

MALDI mass spectrometry demonstrated that the desired complexes had been formed with their respective characteristic isotopic patterns.

![MALDI mass spectrum of 26Gd (DMSO)](image)

**Fig. 2.7** MALDI mass spectrum of 26Gd (DMSO)

The proton NMR spectra were also recorded for the ytterbium, terbium and europium complexes (the proton NMR spectrum of the gadolinium complexes could not be obtained as the spectrum is dominated by line broadening). These spectra differ from normal proton spectra because the peaks are shifted over a much wider spectral range. This is due to the paramagnetic lanthanide ion, which causes an induced shift arising from its local magnetic field. The extent of this effect varies between lanthanides and their complexes display characteristic spectral widths, that when compared to that of the ligand, confirm that complexation has occurred.
The peaks are also broader than expected when compared to that of simple organic molecules. This is firstly due to lanthanide induced relaxation where the relaxation time of the spins of nearby protons is shortened making relaxation fast on the timescale of the NMR experiment. Secondly, line broadening can be attributed to intramolecular fluxional processes that are fast on the timescale of the NMR experiment.

Fig. 2.8 $^1$H NMR spectrum of 26Yb (D$_2$O)

19 peaks are observed outside the range of the solvent peak which can be attributed to the protons on the cyclen ring as well as the amide protons as they are closest to the paramagnetic ion and hence experience the greatest shift as predicted by the Bleaney equation.$^{12}$ The aromatic protons are too far away from the metal and therefore do not shift much from their original position on the spectrum.
The large number of peaks observed on the spectrum is due to the puckered nature of the cyclen ring, which means that for every carbon there is an axial and equatorial proton that are at differing distances from the lanthanide ion and hence experience different environments. This means that every proton has a different shift, which can be observed on the spectrum.

2.2.2 Deprotection of the isophthalate arms

Hydrolysis of the methyl esters was performed in a mixture of water and methanol, due to the low solubility of the triamide in water. After stirring overnight, the solvents were removed under reduced pressure to leave a glassy white solid. Recrystallisation from ethanol removed some of the excess sodium salts but elemental analysis showed that some sodium was still present. Some of these would be due to sodium acting as a counterion for the three negative charges on the product.

This compound was further characterised by MALDI mass spectrometry showing a peak at [836]⁺ for the parent anion and a peak at [859]⁺ for the mass plus sodium. The peak at [1057]⁺ corresponds to the mass of the compound plus alpha methanol which was the solvent used. The spectrum also shows an absence of the starting material at [920]⁺.
The proton NMR spectrum of 27 shows complete absence of the methyl protons at 4.8 ppm, confirming complete deprotection of the compound.

**Fig. 2.10** $^1$H NMR spectrum of 27 (H$_2$O)

**Complexation of deprotected compound**

To facilitate formation of the lanthanide complexes, the pH of the aqueous ligand solution was reduced from ~11 to pH ~ 6. Then a solution of lanthanide (Gd, Yb, Eu, Tb) triflate salt was added to this and stirred for 24 h at 60°C. The solvent was removed and the product left to dry under vacuum overnight to yield white solids.
2.3 Luminescence studies

2.3.1 Luminescence spectroscopy
To obtain the emission spectra of the complexes, it was necessary to determine the most appropriate wavelength to induce sensitised emission of the lanthanide centre. Therefore the absorption spectra were measured to obtain suitable information on the chromophore of the complex. All the complexes exhibited a $\pi-\pi^*$ absorption with a maximum at 260 nm.

Emission spectra from the terbium and europium complexes were obtained by excitation at 260 nm. These spectra confirm that sensitisation of the lanthanide is efficient, implying that the chromophore and the metal are in close proximity, and thus provide further conclusive evidence that complexation has occurred.

![Emission spectrum of Tb(III) showing luminescence from $^{25}$Tb following excitation at 260 nm. (Time gate = 10 ms, delay time = 0.1 ms and slit width = 10 mm) Peaks correspond to decay from $^5D_4$ excited state to the $^7F_6$, $^7F_5$, $^7F_4$, $^7F_3$ ground states respectively.](image-url)

Fig. 2.11
2.3.2 Luminescence lifetimes

Luminescence lifetime measurements were obtained for terbium and europium in D₂O and in H₂O. This was achieved through recording the decreasing intensity of the luminescence at regular intervals (0.1 ms). The data were fitted to a single exponential decay curve using a spreadsheet running in Microsoft Excel.

The graph is of first-order decay and obeys the following equation;

\[ I = I_0 e^{-kt} \] or \[ I = I_0 e^{-t/\tau} \] (1)

Where \( I \) = emission intensity at time \( t \)

\( I_0 \) = emission intensity at \( t = 0 \) ms

\( k \) = rate constant of the luminescence decay

\( \tau \) = luminescence lifetime

\( t \) = time
The gadolinium complexes exhibit no metal centred luminescence due to the Gd(III) emissive state being too high in energy to be probed by standard methods. However, since terbium and europium lie on either side of gadolinium in the Periodic Table, the behaviour of analogous complexes give good approximation of the solution state behaviour of gadolinium complex in terms of its inner hydration sphere.

<table>
<thead>
<tr>
<th>Complex</th>
<th>$\tau_{\text{D}_2\text{O}/\text{ms}}$</th>
<th>$\tau_{\text{H}_2\text{O}/\text{ms}}$</th>
<th>Correction factor for N-H oscillators</th>
<th>$q$</th>
</tr>
</thead>
<tbody>
<tr>
<td>26Eu</td>
<td>1.03</td>
<td>0.36</td>
<td>0.075</td>
<td>1.6</td>
</tr>
<tr>
<td>28Eu</td>
<td>1.38</td>
<td>0.29</td>
<td>0.075</td>
<td>2.7</td>
</tr>
<tr>
<td>26Tb</td>
<td>2.44</td>
<td>1.24</td>
<td>N/A</td>
<td>1.7</td>
</tr>
<tr>
<td>28Tb</td>
<td>2.15</td>
<td>1.04</td>
<td>N/A</td>
<td>2.2</td>
</tr>
</tbody>
</table>

Table 2.1 Measured lifetimes of the tri-isophthalate ester (26Ln) and acid (28Ln) complexes in H$_2$O and D$_2$O

As can be observed in table 2.1, the lifetimes in H$_2$O are shorter than lifetimes in D$_2$O, due to the stronger quenching effect of the O-H oscillations of the water molecule (*vide supra*). The effect has been related to the modified Horrocks equation$^{13}$ (*eqn 2*), which is used to calculate the number of bound water molecules. As expected, the hydration number for each protected complex is about two corresponding to the fulfillment of the coordination of the lanthanide in the heptadentate ligand.
2.3.3 Phosphate binding studies

As mentioned earlier, heptadentate complexes are more susceptible to the chelating effects of bidentate physiological anions. Raymond et al. observed that the affinity of HOPO-based heptadentate complexes for these anions decreased with overall negative charge of the complex.

With this in mind, phosphate binding studies were carried out on the protected and deprotected complexes. Phosphate buffer solutions were made up in both H$_2$O and D$_2$O, at physiological pH (7.4). The complex was then dissolved in each of these solutions and the luminescence lifetimes were obtained. The number of bound waters was calculated in the same manner as before.

<table>
<thead>
<tr>
<th>Complex</th>
<th>$\tau_{\text{D}_2\text{O}}$/ms</th>
<th>$\tau_{\text{H}_2\text{O}}$/ms</th>
<th>q</th>
</tr>
</thead>
<tbody>
<tr>
<td>26Eu</td>
<td>2.54</td>
<td>1.51</td>
<td>0</td>
</tr>
<tr>
<td>28Eu</td>
<td>0.88</td>
<td>0.42</td>
<td>1</td>
</tr>
<tr>
<td>26Tb</td>
<td>2.62</td>
<td>1.58</td>
<td>0.95</td>
</tr>
<tr>
<td>28Tb</td>
<td>2.66</td>
<td>1.51</td>
<td>1.1</td>
</tr>
</tbody>
</table>

*Table 2.2 Measured lifetimes of the complexes in PBS solutions.*

If phosphate binding occurs, then the number of bound water molecules is expected to decrease with a subsequent increase in luminescence lifetimes due to removal of O-H oscillators. Indeed, this is the case. The number of bound water molecules has decreased to one in most cases and there is an overall increase in luminescence lifetimes for all the complexes compared to lifetimes in the absence of phosphate.
This implies that there is an equilibrium between the bound and unbound species in solution which are in fast exchange on the luminescent timescale. It might also be the case that the phosphate binds in a monodentate fashion leaving the other site free for one water molecule resulting in $q$ equals one instead of two.

![Diagram](image)

**Fig. 2.13** Illustration of phosphate binding taking place

Clearly, the negative charge on the complexes has only reduced phosphate binding to a limited degree. Furthermore, the effect is more pronounced for the larger europium ion, implying that phosphate binding has more to do with the accessibility of the charged lanthanide centre. By comparison of the isophthalate ligand with the HOPO ligand, it is possible to draw some inferences as to why the HOPO ligand is more successful at repelling phosphate.
Fig. 2.14 Comparison of HOPO to the triamide complex

The HOPO ligand adopts a structure in which the local environment at the lanthanide centre approximates to a bicapped trigonal prism, where the water binding sites are not adjacent to one another. Phosphate cannot act as a bidentate ligand in this type of system without a complete change of structure, thus reducing the affinity of the complex for phosphate. Furthermore, all the negative charge is concentrated close to the metal centre, reducing the local positive charge, and hence reducing the affinity for anions further. By contrast, the isophthalate system adopts a monocapped SAP/TSAP structure in which the water binding sites are \textit{cis} to one another,\textsuperscript{14,15} and in which the negative charge is situated around the periphery of the molecule. This is likely to make the approach of a phosphate anion much easier.
2.4 Conclusions

Lanthanide triamide complexes bearing protected and deprotected aminoisophthalate moieties were successfully synthesised. Luminescence spectroscopy of these complexes showed long-lived luminescence lifetimes and application of the modified Horrocks equation found the number of bound waters to be approximately equal to two as expected. Phosphate binding studies on these systems demonstrated that binding was taking place at pH 7.4 with the phosphate replacing at least one of the bound water molecules.

These results indicate that it is possible to prepare suitable anionic complexes with heptadentate cyclen derivatives, and that their affinity for anions is more than might have been expected from the hypothesis put forward by Raymond et al. Binding in stable TSAP/SAP systems seems to be more likely than in the relatively unstable multi-capped trigonal prismatic systems previously mentioned, particularly when the negative charge on the molecule is spread around the periphery. Since kinetic stability is so desirable for in vivo applications, departing from macrocyclic systems is not an option, and alternatives are needed if the hypothesis is to be evaluated fully. The next chapter describes the study of DO3P- the triposphonic analogue of DO3A. Replacing the carboxylates with phosphonates doubles the local charge close to the lanthanide centre.
2.5 References


CHAPTER THREE
DO3P COMPLEXES

3.1 Introduction
Luminescence and phosphate binding studies on tris-isophthalate complexes (26Ln and 28Ln) in chapter two showed that charge was not the only factor necessary for inhibiting phosphate binding. Comparison of the complex structure with that of HOPO based systems from the Raymond group indicate that the proximity of the charge to the metal centre might also be a factor. To test this hypothesis, it was decided to synthesise the phosphonate analogue of DO3A, DO3P.

Phosphonates are natural bone seeking agents\(^1\), a characteristic that has been exploited in the treatment of bone cancers such as multiple myeloma, a malignant disease arising from the plasma cells in the bone marrow which can lead to bone destruction\(^1,2\). \(^{166}\)Ho DOTP has been investigated as an effective means of delivering radiotherapy to the bone where the phosphonates localise on the bone surface and the radiation from the \(\beta\)-emitting \(^{166}\)Ho is delivered where it is needed sparing extraskeletal normal tissue in the process. Alpha emitting \(^{212}\)Bi, \(^{213}\)Bi, \(^{212}\)Pb DOTP complexes\(^3\) have also been investigated for the same purpose.

\[
\text{H}_2\text{O}_3\text{P} - \text{N} - \text{N} - \text{PO}_3\text{H}_2
\]

\[
\text{H}_2\text{O}_3\text{P} - \text{N} - \text{N} - \text{PO}_3\text{H}_2
\]

\(\text{DOTP}\)

\(\text{29}\)

Phosphonate derivatives have also been investigated in the development of bone-targeted MRI contrast agents\(^4\). Lanthanide complexes of DOTP were shown to adsorb strongly to hydroxy apatite (HA) a model for bone surface. However, the relaxivity of the gadolinium complex was quenched upon adsorption to HA due to expulsion of water molecules from the second-sphere of the complex\(^5\).
Phosphonate analogues of DTPA complexes (DTPP) showed critical loss of stability due to extensive interaction with HA\textsuperscript{6} rendering them inapplicable as \textit{in vivo} imaging agents.

Lukes \textit{et al} synthesized\textsuperscript{7, 8} a number of lanthanide complexes of bis(phosphonate) monoamide analogues of DOTA to counteract the problem of relaxivity quenching when bound to HA. A bis(phosphonate) was attached via an amide moiety to the fourth position on DO3A to give BPAMD. The gadolinium complex of BPAMD showed high relaxivity that was not quenched upon adsorption on HA but rather enhancement of relaxivity was observed due to immobilization of the complex. \textit{In vivo} studies of the \textsuperscript{177}Lu complex showed that excretion from the body is mainly via the kidneys, but the clearance from the skeleton is too slow for MRI application\textsuperscript{8}.

Lanthanide complexes such as DyDOTP\textsuperscript{9} have been investigated as paramagnetic \textit{in vivo} shift reagents for cations such as \textsuperscript{23}Na. More success has been found with a HTmDOTP\textsuperscript{4-} complex\textsuperscript{10} used for resolving intra- and extracellular sodium resonances in cell suspensions, isolated perfused organs and intact animals by \textsuperscript{23}Na NMR.

This chapter focuses on the synthesis of DO3P lanthanide complexes to investigate the effect of proximity of charge to the metal centre on phosphate repulsion. DO3P d-f complexes were also synthesised in order to probe their ability to act as bimodal imaging agents.
3.2 Synthesis and characterisation of DO3P ligand

A previous synthesis\textsuperscript{11} of DO3P employed the use of a protecting group on one position of cyclen to ensure that the tetra-alkylated compound (DOTP) was not formed during the reaction as this is the dominant species isolated when direct alkylation is attempted.

To this effect, the following scheme was devised in order to synthesise tris-alkylated DO3P analogues cleanly.

\begin{center}
Scheme 3.1 Synthetic route to DO3P tri-ester 34Ln and tri-acid complexes 36Ln
\end{center}
Scheme 3.2 Synthetic route to monoprotected cyclen 31

This protecting group route (scheme 3.2) has been used successfully by Sherry et al.\textsuperscript{12}. The first step towards obtaining the mono-protected cyclen 31 was trialkylation using the protecting group di-tert-butyl dicarbonate (Boc) 37. This was dissolved in chloroform and added to a cooled solution of cyclen 24 and triethylamine in chloroform. After stirring for 18 hours at room temperature, the crude product was purified by silica column chromatography, eluting with diethyl ether only. The positive electrospray spectrum showed the molecular ion peak at 473 and 495 [M+Na]\textsuperscript{+}. The proton NMR spectrum contained peaks corresponding to the cyclen protons in the expected region of 2.8 – 3.6 ppm and a singlet at 1.4 ppm for the tert-butyl protons confirming that the product had been successfully synthesised.
Incorporation of the Cbz protecting group was achieved by the addition of benzyl chloroformate 39 to a cooled solution of the tri-Boc protected cyclen 38 and triethylamine in chloroform. After stirring for 24 hours at room temperature, the product was isolated as a white solid following purification by flash chromatography on silica. The proton NMR spectrum shows that the cyclen peaks have coalesced into a broad peak at 3.3 ppm compared to the 3 peaks observed for tris-Boc 38 at 2.8, 3.2 and 3.6 ppm, which is probably due to an increase in the rate of interconversion between isomers. There is a peak at 7.3 ppm for the phenyl protons of the Cbz group and the benzylic protons at 5.1 ppm. All characterisation data were consistent with that reported in the literature.

To generate the mono-Cbz protected cyclen, the Boc protecting groups were selectively cleaved by the slow addition of concentrated hydrochloric acid to a cooled solution of 40 and the reaction stirred overnight. The pure product was isolated as a white hydrochloride salt after recrystallisation from a mixture of THF and water.
The proton NMR spectrum shows the absence of the tert-butyl peaks at 1.4 ppm confirming the complete removal of the Boc protecting group. The cyclen CH₂ peaks have also split into two in this spectrum and are observed at 3.1 and 3.6 ppm. Positive electrospray shows the molecular ion at 307 amu.

The mono-protected cyclen 31 was dissolved in sodium hydroxide solution (1 M) to remove the hydrochloride salt and extracted into DCM. The salt free compound was dissolved in anhydrous THF and paraformaldehyde was then added. The reaction mixture was stirred for 3 hours to allow successive formation of the imminium ion as illustrated by the mechanism in scheme 3.3. Addition of triethylphosphite to the mixture allowed the imine to be trapped followed by an Arbuzov reaction to yield the tri-phosphonate ester.
Step 1: Formation of iminium ion

Step 2: Arbuzov reaction

Scheme 3.3 Reaction mechanism for the formation of 32
The proton NMR spectrum of 32 shows the methyl peaks of the phosphonate group as two overlapping triplets at 1.3 ppm, consistent with the inequivalence of the methyl groups at positions -4, -10 and -7 on the cyclen ring. The most downfield shifted peaks on the left are also half the height of those that resonate further upfield, which is consistent with two of the methyl groups being equivalent as they experience the same averaged environment whilst one is inequivalent. This pattern is reflected with the ethylene peaks, which are overlapping quartets. All other peaks were as expected for this compound which was also characterised by mass spectrometry and carbon NMR.

The Cbz protecting group was removed by catalytic hydrogenation in the presence of a palladium catalyst on activated carbon. Deprotection was confirmed by the absence of the signals arising from the phenyl protons at 7.39 ppm and the benzylic protons at 5.09 ppm. The electrospray mass spectrum shows the molecular ion at [623]+ and the absence of the peak corresponding to the Cbz protected compound at [723]+.
Subsequent synthesis of DO3P involved first of all tri-protecting with the Cbz group and then adding Boc to the fourth nitrogen. Whereafter phosphorylation both the Boc and the phosphonate ester groups could be cleaved simultaneously.

Fig. 3.4 $^1$H NMR spectrum of 33 (CDCl$_3$)

Hydrolysis of the ester groups was then addressed in order to obtain the free acid. This was achieved by dissolving the ligand in excess hydrochloric acid (20%) and heating to reflux temperature for 2 days; the reaction was monitored by proton NMR spectroscopy. Complete hydrolysis was confirmed by the absence of methyl and ethylene peaks at 1.3 and 4.1 ppm respectively. The product was used without further purification.
3.2.1 Synthesis and characterisation of DO3P complexes

Lanthanide(III) complexes of the phosphonate ester 33 and acid 35 were synthesised to allow a direct comparison of the effect on the number of bound waters and phosphate repulsion with the variation in charge.

Complexes of 33 were obtained by adding a solution of the lanthanide triflate in acetonitrile dropwise to a solution of the ligand also in acetonitrile and heating for 3 days. The complex was purified through dialysis to remove any excess free lanthanide and other inorganic salts. A variety of techniques demonstrated that complexation has taken place; such as the MALDI mass spectrum in figure 3.5 for the europium complex which shows a cluster of peaks for the molecular ion that is in good agreement with the calculated isotopic pattern (top spectrum).

Fig. 3.5 MALDI MS of 34Eu (α-MeOH)
To generate the tri-phosphonic acid complexes $36\text{Ln}$, the ligand was dissolved in water and the pH adjusted from ~2 to ~6. The lanthanide triflate was dissolved in water and added dropwise to the solution and immediately a white precipitate was formed. It is thought that this is due to the initial formation of out of cage lanthanide phosphonate salts. Heating for 4 days yielded the complexes, which were purified by dialysis.

![MALDI MS of TbDO3P](#)

**Fig. 3.6** MALDI MS of $36\text{Tb}$ (H$_2$O)

Figure 3.6 shows the MALDI mass spectrum of TbDO3P $36\text{Tb}$ with a peak at [632]$^+$ corresponding to the mass plus a sodium ion. Proton NMR spectroscopy also shows that the complexes have successfully formed due to the paramagnetic nature of the lanthanide ion. This causes a shift in the position of the resonances relative to those of the ligand. Also, the spectral width is much wider than would be expected for a normal proton and is characteristic for each paramagnetic lanthanide.
Figure 3.7 shows the proton NMR spectrum of $^{36}$Eu(D$_2$O), which has 3 sets of peaks of differing intensities, suggesting that there are at least 3 diastereoisomers in solution. In comparison EuDOTP has only six peaks in its spectrum, meaning only one isomer is present in solution\textsuperscript{14} whilst EuDOTA has 12 peaks corresponding to a mixture of major and minor isomers (TSAP and SAP)\textsuperscript{15}. This large number of diastereoisomers can be attributed to the chirality present at phosphorus when one of the oxygen atoms is bound to the lanthanide. This coupled with the protonation state of the phosphonates leads to the complicated spectrum observed making it difficult to fully assign the peaks.

The effect of protonation can be more easily observed in the phosphorus NMR spectrum, which was measured at pH 1, 4, 7, and 10 (figure 3.8). At pH 1 when the phosphonates are fully protonated there are only two peaks observed whilst at pH 7 where there is a mixture of protonated and deprotonated species, several peaks can be observed.
Fig. 3.8 Proton decoupled $^{31}$P NMR spectrum of $^{36}$Eu at (a) pH1 (b) pH4 (c) pH7 (d) pH10 ($D_2O$)
Fig. 3.9 $^1$H NMR spectrum of $36Tb$ (D$_2$O)

Fig. 3.10 Proton decoupled $^{31}$P NMR spectrum of $36Tb$ (D$_2$O)
By contrast with the europium complex, the proton and phosphorus NMR spectra of the terbium complex are much simpler. The peaks are all of the same height suggesting that one diastereoisomer dominates in solution or that the diastereoisomers are in exchange. The peaks are also quite broad, indicating that fluxional processes within the molecule are slower than the timescale of the experiment. The observed chemical shifts are between those expected for SAP and TSAP terbium complexes, implying that isomerisation is occurring on the timescale of the NMR experiment.\textsuperscript{23}

3.3 Luminescence studies
Luminescence measurements were carried out on the complexes by direct excitation of the metal ion as there are no chromophores on the ligand to facilitate sensitised emission. The complexes were also only sparingly soluble in water therefore concentrated solutions were needed to obtain reliable luminescence lifetime data. Despite this, the solutions were still optically dilute enough to avoid intermolecular quenching of the lanthanide based emission.

<table>
<thead>
<tr>
<th>Complex</th>
<th>$\tau_{H_2O}/\text{ms}$</th>
<th>$\tau_{D_2O}/\text{ms}$</th>
<th>q</th>
</tr>
</thead>
<tbody>
<tr>
<td>34Eu</td>
<td>0.28</td>
<td>2.4</td>
<td>3.2$^*$</td>
</tr>
<tr>
<td>36Eu</td>
<td>0.51</td>
<td>2.18</td>
<td>1.2</td>
</tr>
<tr>
<td>34Tb</td>
<td>0.65</td>
<td>0.79</td>
<td>1.1</td>
</tr>
<tr>
<td>36Tb</td>
<td>1.18</td>
<td>1.87</td>
<td>1.3</td>
</tr>
<tr>
<td>34Yb</td>
<td>1.91$\mu$s</td>
<td>3.6$\mu$s</td>
<td>0.6</td>
</tr>
<tr>
<td>36Yb</td>
<td>1.31$\mu$s</td>
<td>11.6$\mu$s</td>
<td>0.6</td>
</tr>
</tbody>
</table>

$^*$ Sample contaminated with uncomplexed europium

Table 3.1 Luminescence lifetimes and q values of DO3P complexes
From the luminescence lifetimes in H$_2$O and D$_2$O, the number of inner sphere bound water molecules were calculated using the modified Horrocks equation. There is only a slight increase in q value on going from the tri-ester to the tri-acid complexes implying that charge does not affect the number of inner sphere water molecules in this family of complexes. The only significant anomaly is the EuDO$_3$P ester, which appeared to be contaminated with free europium.

The tris-isophthalate complexes from chapter two displayed q values of ~2 as expected for heptadentate complexes. The tris-phosphonate complexes on the other hand have q values that are closer to one, which are attributed to the lipophilic nature of the phosphonates compared to the carboxylates. This effect means that relaxivity of the contrast agent will be reduced, but also decreases the prospects for phosphate binding.

However, studies on phosphonate and phosphinate-cyclical complexes reveal that there is a considerable contribution to relaxivity from second sphere water molecules$^{16-18}$. This is attributed to the high charge from the uncoordinated oxygen atoms on the phosphonate groups promoting the formation of strong hydrogen bond networks with the solvent molecules. The lifetime of solvent molecules in the second sphere is also considerably smaller leading to higher relaxivity$^{17}$. 
3.3.1 Phosphate binding studies

One of the aims of this project is to make a heptadentate contrast agent that does not bind to phosphate. The complexes were dissolved in H$_2$O or D$_2$O PBS solutions and the luminescence lifetimes were measured. These again were used to calculate q in order to observe changes due to the phosphate anion, to see if it was binding to the metal centre and forming a ternary complex.

<table>
<thead>
<tr>
<th>Complex</th>
<th>$\tau_{H2O}$/ms</th>
<th>$\tau_{D2O}$/ms</th>
<th>q</th>
</tr>
</thead>
<tbody>
<tr>
<td>36Eu</td>
<td>0.51</td>
<td>2.18</td>
<td>1.2</td>
</tr>
<tr>
<td>in PBS</td>
<td>0.47</td>
<td>1.46</td>
<td>1.2</td>
</tr>
<tr>
<td>36Tb</td>
<td>1.18</td>
<td>1.87</td>
<td>1.3</td>
</tr>
<tr>
<td>in PBS</td>
<td>0.95</td>
<td>1.44</td>
<td>1.5</td>
</tr>
<tr>
<td>36Yb</td>
<td>1.31(\mu)s</td>
<td>11.6(\mu)s</td>
<td>0.6</td>
</tr>
<tr>
<td>in PBS</td>
<td>1.20(\mu)s</td>
<td>13.5(\mu)s</td>
<td>0.7</td>
</tr>
</tbody>
</table>

Table 3.2 Luminescence data of LnDO3P complexes measured in PBS (pH 7)

Comparison of the lifetimes in water and PBS solutions show no increase as would be expected if phosphate binding were occurring. This is further quantified by the calculation of the number of bound waters, which in all cases has remained the same within error. This adds credence to the theory that high overall charge, as well as proximity of the charge to the metal centre are important factors for the repulsion of phosphate.
3.4 Towards the synthesis of d-f hybrids

The second aim of this project was to make a bimodal imaging agent by linking a d-transition metal to the lanthanide complex via a pyridyl moiety in a similar fashion to the dual imaging agent synthesised by Koullorou (14).

The following scheme illustrates the proposed synthetic route.

![Scheme 3.4 Synthetic route to binuclear complexes](image)

Mono-alkylation with the pyridyl linker was achieved by slow addition of 2-chloromethyl pyridine 41 to a mixture of excess cyclen and base in acetonitrile19. The reaction was monitored by thin layer chromatography until the starting material 41 could no longer be observed. The crude product was purified by column chromatography to yield a white solid.

![Fig. 3.11 Positive electrospray spectrum of 42 (MeOH)](image)
The electrospray spectrum in figure 3.11 shows a single peak for the parent ion at [264]+. The purity of this compound was confirmed by proton NMR and elemental analysis.

The next step was phosphonation using the same method as previously used to make the DO3P ligand; the product was obtained in good yield.

The proton NMR spectrum in figure 3.12 shows the resonances for the methyl and ethylene groups at 1.2 and 4 ppm respectively. As observed with the DO3P ester, they also resonate as overlapping triplets and quartets due to the inequivalence of the phosphonate groups. There are also a number of broad peaks of the ring CH₂ groups which can be observed between 2.5-2.9 ppm and aromatic peaks at 7.3 and 8.5 ppm corresponding to the protons of the pyridine ring.

The phosphonate ester groups were hydrolysed by heating the ligand in hydrochloric acid to reflux temperature for 48 hours. The product was isolated in 89 % yield.
The proton NMR spectrum shows the absence of the ethyl group at 1.25 and 3.97 ppm respectively confirming that successful deprotection of all the ester groups had occurred.

Complexation was carried out by heating in methanol to reflux temperature for 4 days. The lanthanide complexes were obtained as a series of off-white solids after purification by dialysis. Successful formation of the complexes was confirmed by MALDI mass spectrometry and proton NMR spectroscopy.
**Fig. 3.15** $^1$H NMR spectrum of $^{45}$Eu (D$_2$O)

**Fig. 3.16** Proton decoupled $^{31}$P NMR spectrum of $^{45}$Eu (D$_2$O)
There are much fewer peaks in the spectrum of the europium complex, which is attributable to the additional bulk of the pyridine ring restricting the motion of the complex. There are two sets of peaks in this case with a more prominent major isomer and a minor isomer, which can be observed close to the baseline of the spectrum. The phosphorus spectrum shows two very broad peaks at -26 and -68 ppm. The broadness could be due to dynamic exchange processes of the two isomers approaching the timescale of the NMR experiment.

3.5 Synthesis and characterisation of d-f hybrid

The transition metal component of this d-f hybrid is a rhenium bipyridine complex and was synthesised according to the scheme below.

Rhenium pentacarbonyl chloride and 2,2'-bipyridine 46 were heated together in toluene for 24 hours and the yellow product was isolated after recrystallisation from hot acetonitrile. The next step involved replacing the chloride with a more labile leaving group to facilitate the next step of the reaction. Silver triflate was added to a stirring solution of the rhenium tricarbonyl bipyridine complex and the mixture heated to reflux temperature overnight. The reaction mixture was filtered over celite to remove silver chloride formed during the reaction and the solvent removed under reduced pressure. The pure product 48 was obtained as yellow needles after recrystallisation at -18°C from a mixture of DCM and diethyl ether. The product at each stage was characterised by elemental analysis, mass spectrometry and infrared spectroscopy and all were in good agreement with that observed in the literature.\textsuperscript{20}
Scheme 3.6 Synthesis of the d-f hybrid

The lanthanide complexes $45\text{Ln}$ were heated to reflux temperature in methanol with a slight excess of the rhenium complex in methanol for 3 days. Methanol was removed under reduced pressure and the crude product washed with copious quantities of DCM to remove any unreacted rhenium starting materials. The d-f complexes $49\text{Ln}$ were obtained as pale orange solids after drying.

Mass spectrum of the europium complex $49\text{Eu}$ showed clusters of peaks matching the calculated isotope pattern of the combined lanthanide and rhenium complex. Infrared spectroscopy also showed a broad peak ranging from 1917-1923 cm$^{-1}$ corresponding to the carbonyl peak of the rhenium complex.

Fig. 3.17 $^1$H NMR spectrum of $49\text{Eu}$ (D$_2$O)
The proton NMR spectrum of $^{49}\text{Eu}$ (figure 3.17) shows fewer peaks than in the previous spectrum (figure 3.15) with most of the peaks now on the right side of the spectrum. Focusing on the left side of the spectrum which is much simpler, four peaks can be observed on the far left which can be assigned as axial protons as they experience the greatest shift due to the proximity of the lanthanide. The complex is clearly dominated by a SAP configuration at the metal centre, suggesting that the bulk of the rhenium bearing substituent locks the configuration.\textsuperscript{23}

The ytterbium spectrum (figure 3.18) on the other hand shows fewer peaks that are very broad suggesting that exchange is faster on the NMR timescale. Due to the lanthanide contraction, the ytterbium ion is much smaller than the europium ion, which allows a much greater conformational freedom for the ligand around it. The shift range implies that the dominant form of the complexes is TSAP.

Fig. 3.18 $^1\text{H}$ NMR spectrum of $^{49}\text{Yb}$ (D$_2$O)
3.6 Photophysical studies

3.6.1 UV/Vis spectra

Absorption spectra of the lanthanide complexes were obtained for characterisation purposes and to ascertain the absorption maxima, which will give a suitable excitation wavelength for sensitised emission.

The spectrum in figure 3.19 shows a prominent peak at 260 nm for all three complexes corresponding to the $\pi - \pi^*$ transition of the pyridine chromophore. There is also a peak just below 200 nm corresponding to the $\pi - \pi^*$ transition of the phosphoryl group.

Figure 3.20 shows a comparison between the the UV-Vis spectra of picolyIDO3P complex 45Ln and the d-f hybrids 49Ln. The peaks of the d-f complexes at ~260 nm are broader than that of its precursor. This is due to an overlap of the $\pi - \pi^*$ transitions of both the pyridine and the bipyridine moieties. There are also two broad shoulders observed above 300 nm for the d-f complexes corresponding to the MLCT of the rhenium bipyridine complex. The peaks just extend into the visible region of the spectrum, hence the orange colour observed for the complexes.
3.6.2 Luminescence spectroscopy and lifetimes

A Perkin-Elmer LS55 spectrometer was used to study the terbium and europium complexes. The spectrum in figure 3.21 shows the excitation and emission spectra for $^{49}\text{Tb}$ when excited indirectly at 260 nm. The emission spectrum is quite weak in intensity, suggesting that excitation is not occurring efficiently via a triplet mediated pathway. The low intensity of the transitions implies that this signal may arise from a very low concentration of $^{49}\text{Tb}$. This is unsurprising since the energy of the $^5\text{D}_4$ state of Tb$^{3+}$ is too low to populate via the $^3\text{MLCT}$ state on the rhenium chromophore.
The excitation spectrum of the europium d-f complex looks more like its absorption spectrum after excitation at 254 nm and is also a lot more intense (figure 3.22). The subsequent emission spectrum is also more intense proving that sensitised emission is occurring more efficiently in this case. Once again, the excitation spectrum of the europium complex reveals much about the nature of the processes involved – the small peak at 397 nm is comparable in intensity to the intensity of the ReMLCT transition despite having much weaker absorption. This implies that non-radiative quenching by LMCT to Eu may limit the effectiveness of sensitisation by the rhenium chromophore.
The luminescence decay of the europium complexes were measured using the LS55 in both water and D₂O whilst those for ytterbium were measured using a pulsed nitrogen laser. The lifetimes were then obtained by iterative fitting to an exponential decay function as discussed in chapter two. The single exponential function yielded a good fit with no further improvement observed when a second exponential term was added.

The luminescence lifetimes in H₂O and D₂O were used to analyse the number of lanthanide bound waters (q). The complexes all display long luminescence lifetimes with q values approximately equal to one. Even though the complexes are heptadentate and a q value of two would normally be expected to saturate the lanthanide coordination sphere, the presence of bulky and hydrophobic phosphonate and pyridyl groups only allow one water molecule to approach the lanthanide.
Table 3.3 Luminescence lifetimes and hydration number of the complexes

<table>
<thead>
<tr>
<th>Complex</th>
<th>$\tau_{D_2O}$/ms</th>
<th>$\tau_{H_2O}$/ms</th>
<th>$q_{H_2O}$</th>
<th>$\tau_{D_2O-PBS}$/ms</th>
<th>$\tau_{H_2O-PBS}$/ms</th>
<th>$q_{PBS}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>45Eu</td>
<td>1.69</td>
<td>0.62</td>
<td>1</td>
<td>1.73</td>
<td>0.71</td>
<td>0.7</td>
</tr>
<tr>
<td>49Eu</td>
<td>2.37</td>
<td>0.66</td>
<td>1</td>
<td>1.59</td>
<td>0.72</td>
<td>0.6</td>
</tr>
<tr>
<td>45Yb</td>
<td>10.97µs</td>
<td>1.19µs</td>
<td>0.7</td>
<td>14.11µs</td>
<td>1.30µs</td>
<td>0.6</td>
</tr>
<tr>
<td>49Yb</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

The lifetimes of the complexes were also measured in H$_2$O and D$_2$O solutions of PBS and the hydration number calculated. As can be observed, there is a definite drop in the $q$ value that was not seen for the simple DO3P complexes. The magnitude of the decrease seems to increase from DO3P to picolyIDO3P to the d-f hybrid. This is likely due to decrease in the overall negative charge of the complex due to the positively charged rhenium molecule.

For all complexes there does not appear to be a consistent increase in the measured lifetime of both H$_2$O and D$_2$O solutions as would be expected if phosphate is binding to the lanthanide. Therefore 45Eu was titrated in the presence of increasing concentration of phosphate to quantify this.
**Fig. 3.23** Emission spectra intensity of 45Eu decreases with increasing phosphate anion concentration (H₂O).

The emission spectra show a marked decrease in intensity of the \(^{5}D_0 - ^{7}F_2\) transition peak with a subsequent growth in the peak for the \(^{5}D_1 - ^{7}F_1\) transition. These peaks are hypersensitive and prove that the europium environment is changing as would be expected on replacement of a water molecule with phosphate.

<table>
<thead>
<tr>
<th>Phosphate concentration/mM</th>
<th>Luminescence lifetime/μs</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>550</td>
</tr>
<tr>
<td>2</td>
<td>580</td>
</tr>
<tr>
<td>4</td>
<td>610</td>
</tr>
<tr>
<td>6</td>
<td>610</td>
</tr>
<tr>
<td>8</td>
<td>610</td>
</tr>
<tr>
<td>10</td>
<td>600</td>
</tr>
<tr>
<td>20</td>
<td>600</td>
</tr>
<tr>
<td>40</td>
<td>600</td>
</tr>
<tr>
<td>60</td>
<td>600</td>
</tr>
<tr>
<td>80</td>
<td>610</td>
</tr>
<tr>
<td>100</td>
<td>620</td>
</tr>
</tbody>
</table>

*Table 3.4* Titration data
The titration was carried out by adding solid aliquots of sodium monohydrogen phosphate to a 20 nM solution of \( \text{45Eu} \) in 20 mM HEPES. There are two initial increases in lifetime of 30 \( \mu s \) each on addition of the phosphate and there are no further increases for 3 more additions. This may be due to the equilibrium initially shifting to favour the phosphate anion hence the increase in lifetime. As there is still a relatively large excess of water molecules, the equilibrium shifts back to favour the water and a drop in the lifetime is observed. As the concentration of the phosphate increases, the equilibrium again shifts in favour of the anion and a further increase in lifetime is observed from 80 mM concentration onwards. Overall, there is a 70 \( \mu s \) increase in lifetime from 0 to 100 mM concentration. Supkowski et al observed an overall increase in lifetime for EuDO3A of 120 \( \mu s \) in phosphate (0.45 M) and 170 \( \mu s \) in carbonate (0.45 M).\(^{21}\) It is also interesting to note that the highest increase in lifetime coincides with physiological concentration of phosphate (4 mM) in blood plasma.

### 3.6.3 Luminescence from rhenium complex

Rhenium compounds are also able to luminesce in solution. The \(^3\text{MLCT}\) band was excited at 405 nm using a pulsed pico-second diode laser coupled to a visible detector with an interference filter (550-650 nm) and emission at 600 nm was observed.

<table>
<thead>
<tr>
<th>Complex</th>
<th>( \tau_{\text{D2O}/ns} )</th>
<th>( \tau_{\text{H2O}/ns} )</th>
<th>( \tau_{\text{D2O-PBS}/ns} )</th>
<th>( \tau_{\text{H2O-PBS}/ns} )</th>
</tr>
</thead>
<tbody>
<tr>
<td>49Eu</td>
<td>180</td>
<td>113</td>
<td>150</td>
<td>109</td>
</tr>
<tr>
<td>49Yb</td>
<td>173</td>
<td>97</td>
<td>178</td>
<td>100</td>
</tr>
</tbody>
</table>

*Table 3.5 Rhenium luminescence lifetime data*
The rhenium complexes all display long luminescence lifetimes. The difference in lifetime between measurements in H$_2$O and D$_2$O imply that photon assistance of energy transfer is more efficient in H$_2$O than in D$_2$O. Figure 3.24 shows the fluorescence emission spectrum of 49Yb that has a characteristic rhenium $^3$MLCT band with a maximum at 550 nm.

Fig. 3.24 Visible fluorescence spectrum of 49Yb in D$_2$O
3.7 Relaxivity ($T_1$) measurements

Inversion recovery experiments were carried out on the gadolinium d-f complex to assess its efficacy as a potential contrast agent. This determines the longitudinal relaxation time and this is related to the concentration of the complex by the following relationship;

$$\frac{1}{T_1} = R_1[Gd] + \frac{1}{T_{1w}}$$

where $T_1$ and $T_{1w}$ are the measured relaxation times in the presence and absence of paramagnetic species respectively, $[Gd]$ is the concentration of the complex and $R_1$ is the relaxation enhancement ability of the contrast agent.

By plotting a graph of the inverse of the measured $T_1$ times against the concentration of the gadolinium complex, the relaxivity value can be obtained from the gradient.

**Fig. 3.25** Relaxivity of 49Gd in H$_2$O
The plot above shows that there is a linear relationship between the $T_1$ times and the concentration indicative of one species in solution. The relaxivity is 7.4 mM$^{-1} \text{s}^{-1}$ in this case, much higher than the commercially used Magnevist (3.4 mM$^{-1} \text{s}^{-1}$) and comparable to that of the carbonate analogue GdDO3A d-f (7.6 mM$^{-1} \text{s}^{-1}$). However, when the relaxivity was measured in phosphate buffered saline, there was a dramatic decrease to 1.1 mM$^{-1} \text{s}^{-1}$. This value is close to that of GdDOTP suggesting that there are no inner sphere water molecules and the observed relaxivity is due to the presence of outer sphere water molecules. The data points in this case are not linear indicating that there is more than one species in solution due to the equilibrium between the hydrated complex and the phosphate bound complex.

![Fig. 3.26 Relaxivity of 49Gd in PBS](image)

Phosphonates are known to have great affinity for bone therefore relaxivity was also measured in hydroxy apatite (HA) which is a model of bone surface. The plots show that relaxivity has been greatly reduced even compared with the value obtained in PBS. This suggests that the complex has bound to the hydroxy apatite and that the HA has expelled the inner sphere and most of the outer sphere water molecules.
Luminescence measurements were carried out in HA to see if the trend in relaxivity reflected the q values. As mentioned earlier, gadolinium does not have an emissive state that can be probed by standard methods. Europium and terbium are positioned on either side of gadolinium on the Periodic Table and give good approximations of the behaviour of the analogous gadolinium complex in solution. The lifetimes and q values are summarised in table 3.5.

<table>
<thead>
<tr>
<th>Complex</th>
<th>$\tau_{D2O}$/ms</th>
<th>$\tau_{H2O}$/ms</th>
<th>q</th>
</tr>
</thead>
<tbody>
<tr>
<td>49Eu in H$_2$O</td>
<td>2.37</td>
<td>0.66</td>
<td>1</td>
</tr>
<tr>
<td>In PBS</td>
<td>1.59</td>
<td>0.72</td>
<td>0.6</td>
</tr>
<tr>
<td>In HA</td>
<td>1.82</td>
<td>0.80</td>
<td>0.5</td>
</tr>
<tr>
<td>49Tb in H$_2$O</td>
<td>3.65</td>
<td>1.67</td>
<td>1.4</td>
</tr>
<tr>
<td>In PBS</td>
<td>3.07</td>
<td>1.94</td>
<td>0.6</td>
</tr>
<tr>
<td>In HA</td>
<td>2.49</td>
<td>1.91</td>
<td>0.3</td>
</tr>
</tbody>
</table>

Table 3.6 Luminescence measurements for terbium and europium d-f complexes
From the data obtained for the europium and terbium complexes it can be estimated that the gadolinium complex has a q value of approximately one. The relaxivity value of 7.4 mM$^{-1}$ s$^{-1}$ can then be said to be consistent with the q value but with a significant contribution from outer sphere waters. The q value of 0.6 in PBS suggests that there is some water in the vicinity of the lanthanide and is consistent with the measured relaxivity of 1.1 mM$^{-1}$ s$^{-1}$ which is attributed to purely outer sphere waters. Finally, the relaxivity value measured in hydroxyapatite (0.2 and 0.4 mM$^{-1}$ s$^{-1}$) suggest that most of the outer sphere waters have been expelled from the gadolinium complex which again agrees with an average q value of 0.4 in HA.
3.8 Conclusion

DO3P complexes were successfully synthesised and found to be monohydrated through luminescence measurement of lifetimes. These values stayed the same within error when measured in phosphate buffered saline and seemed to corroborate the initial hypothesis that high charge and its proximity to the lanthanide were the key factors for repelling phosphate.

These positive findings then initiated the synthesis of DO3P with a pyridyl arm, which was later linked to a rhenium bipyridine complex. Europium, terbium, ytterbium and gadolinium complexes were synthesised with the first three complexes displaying long luminescence lifetimes. However, q values in PBS and HA showed a significant decrease, indicating that phosphate binding was taking place.

Relaxivity of the gadolinium d-f complex was determined to be 7.4 mM$^{-1}$ s$^{-1}$ in water. Subsequent measurement of the relaxivity in PBS and hydroxyapatite saw a decrease to 1.1 and 0.4 mM$^{-1}$ s$^{-1}$ respectively, consistent with the q values measured for the terbium and europium complexes and consistent with the expulsion of water molecules from the inner and outer sphere.

This change in affinity of the complex for phosphate from the initial simple DO3P complexes to the d-f hybrid complexes may be due to a variation in the type of isomer adopted in solution. The DO3P complexes may adopt a TSAP conformation with increased steric crowding at the metal centre inhibiting the approach of the phosphate ion to the metal whereas the opposite may be true for the d-f complex.

Further work would involve trying to determine the crystal structure of these complexes to gain further insight into the behaviour of these systems.
3.9 References


4.1 Introduction
Lanthanide complexes that emit light in the near infrared region of the electromagnetic spectrum (NIR): Pr(III), Nd(III), Er(III) and Yb(III) are of technological interest in two main fields: in telecommunications optical networks and \textit{in vivo} imaging. This is because light of these wavelengths is in the window of transparency for silica, aiding optical signal amplification for the former and human tissue is more transparent enabling deeper imaging for the latter. However due to the low-lying emissive states of the lanthanides, the excited state is readily quenched non-radiatively by the vibrations of the ligand and or solvent molecules.

\textbf{Fig. 4.1} f-f energy levels of the NIR emitting lanthanides\textsuperscript{1}
There are two ways to sensitise emission from NIR emitting lanthanide complexes. First of all organic chromophores with fully allowed $\pi-\pi^*$ transitions can be used as sensitisers. There are plenty of organic chromophores known which absorb in the UV, but these are better suited to sensitisation of visible light emitting lanthanides such as Eu(III) and Tb(III). Due to the low lying emissive states of the NIR emitting lanthanides, chromophores which absorb at longer wavelengths *i.e.* visible region are required and fewer examples of these have been shown to sensitise lanthanide emission.$^{2-4}$

Alternatively, d-transition metal complexes can be used as chromophores and this is the focus of this chapter. The most commonly used d-transition metal complexes are polypyridine derivatives of Ru(II)$^{5-7}$, Os(II)$^5$ and Re(I)$^7-8$. Complexes of Ir$^9$, Pt$^{10,11}$ and Cr$^{12}$ have also been used.

The advantages of using transition metal complexes as sensitisers are as follows;

1. They have strong absorption maximum which can be selected over a wide range of wavelengths in the visible or NIR region allowing the opportunity of fine-tuning of the photophysical properties of the system.
2. They contain a heavy metal ion which will facilitate intersystem crossing leading to high triplet quantum yields.
3. Long-lived triplet excited states which will facilitate energy transfer to a covalently attached lanthanide.
4. They also have kinetic inertness and photochemical stability and hence will be suitable for use *in vivo*.

Owing to the recent surge in interest over transition metal chromophores, there is now a wide variety of examples of d-f complexes available$^{13-15}$. 
An example of a d-f complex produced by Faulkner et al is the trimetallic complex below.

The complex contains two Re^I tricarbonyl units linked to a DTPA binding site via 2,2'-bipyridyl ligands. Excitation of the Re^I complex at 337 nm gave a short-lived intense peak at 625 nm. On addition of the lanthanide to the DTPA binding site, quenching of the Re^I emission is observed, followed by lanthanide centred emission at the appropriate wavelength. It was concluded that the observed quenching was consistent with energy transfer from the Re^I^3MLCT states to the proximate lanthanides.

This chapter focuses on the preparation of some near-infrared emitting complexes based on the DO3P platform used in the previous chapter that can also be used as a bimodal imaging agent when gadolinium is incorporated as the lanthanide component.
4.2 Synthesis and characterisation of bis-DO3P
The following scheme illustrates the design and synthesis of some near IR emitting complexes. Two kinetically and thermodynamically stable macrocyclic lanthanide complexes are linked covalently to a d-transition metal bipyridyl complex.

Scheme 4.1 Synthetic route to bisDO3P complexes 55Ln
Before the bis-cyclen ligand could be synthesised, the dibromomethyl bipyridine starting material 50 had to be prepared. This was achieved by the following literature method.\textsuperscript{16, 17}

\begin{align*}
\text{56} & \xrightarrow{\text{NaCr}_2\text{O}_7, \text{Conc. } \text{H}_2\text{SO}_4} \text{57} \\
\text{59} & \xrightarrow{\text{NaBH}_4} \text{58} \\
\end{align*}

\textbf{Scheme 4.2} Synthetic route to 50

The commercially available starting material dimethyl bipyridine 56 was oxidised to the dicarboxy acid 57 using sodium dichromate in concentrated sulphuric acid. The reaction was quenched in cold water from which a yellow/green precipitate was obtained. The residue was recrystallised by dissolution in alkaline aqueous solution followed by slow acidification to pH ~2 with aqueous HCl solution to afford the product free of Cr\textsuperscript{3+} ions. The formation of the acid was confirmed by proton NMR spectroscopy, elemental analysis and infrared spectroscopy. The IR spectrum shows a broad peak at 3382 cm\textsuperscript{-1} for the OH group and a strong peak at 1701 cm\textsuperscript{-1} for the carbonyl.

Next, the acids were converted to ester groups by refluxing in ethanol in the presence of conc. sulphuric acid. The proton NMR spectrum shows the two additional sets of peaks for the ester group. The triplet at 1.36 ppm corresponds to the methyl protons and there is a quartet at 4.38 for the –CH\textsubscript{2}. The resonances at 7.85, 8.79 and 8.88 ppm correspond to the aryl protons. The IR spectrum shows a shift of the carbonyl peak from 1701 to 1728 cm\textsuperscript{-1} as expected on changing from an acid to an ester functional group.
The diester 58 was then reduced to the diol 59 with sodium borohydride. The diester was dissolved in ethanol, sodium borohydride added and refluxed for 3 hours. After cooling to room temperature, an ammonium chloride saturated water solution was added to decompose the excess borohydride. The product was then extracted with several washings of ethyl acetate. The organic phases were combined and the solvent removed to afford the desired compound.

The proton NMR spectrum shows that the peaks at 1.36 and 4.38 ppm for the methyl ester groups have disappeared and instead there is a singlet at 3.22 ppm for -CH$_2$OH. The IR spectrum shows a broad peak for the alcohol at 3198 cm$^{-1}$ and no carbonyl peak at 1728 cm$^{-1}$. 

Fig. 4.2 $^1$H NMR spectrum of 58 (CDCl$_3$)
Bromination of the alcohol moieties was then carried out by dissolving the compound in a mixture of 48% HBr with conc. sulphuric acid and refluxing for 6 hours. After cooling, water was added and the pH adjusted to neutral with saturated sodium hydroxide solution. The resulting precipitate was dried and then recrystallised from chloroform.

The product was characterised by proton NMR spectroscopy, elemental analysis and infrared spectroscopy.

Now that the bipyridine bridge 50 had been made, the next step was to react this with tri-Boc protected cyclen 38 in order to incorporate cyclen in preparation for phosphonation. This was done by refluxing tris-Boc, caesium carbonate and dibromobpy in acetonitrile for 4 days when the reaction mixture had turned brown. The mixture was filtered to remove the excess base and the residue was purified by flash chromatography on silica to afford the product as a white solid.
The proton NMR spectrum shows the peaks for the 54 t-butyl protons as a
sharp peak next to a broader one at 1.3-1.4 ppm. The CH₂ peaks of the cyclen
ring can be observed as a series of broad peaks between 2.5 and 3.5 ppm. The
benzyl protons of the bipyridine have shifted upfield from 4.4 ppm to 3.8 ppm
on removal of the electronegative bromine atoms. The resonance of the
aromatic protons can be observed at 7.2, 8.2 and 8.5 ppm.

Fig. 4.4 \(^1\)H NMR spectrum of 51 (CDCl₃)

The Boc protecting groups were removed by refluxing in concentrated
hydrochloric acid.
The proton NMR spectrum in figure 4.5 shows there are no peaks for the \( t \)-butyl protons of the Boc group at 1.4 ppm. Major and minor peaks can be observed at the aromatic region of the spectrum. This is likely due to the fluxional behaviour of the bipyridine ring relative to the macrocycle as the molecule can exist as \textit{cis} and \textit{trans} isomers.

Phosphonation of the cyclen backbone was carried out in the same manner as that employed in the synthesis of DO3P reported in chapter three. The bis-cyclen hydrochloride salt was dissolved in aqueous sodium hydroxide solution (1M) and washed with DCM. The organic phase was evaporated under reduced pressure and the resulting residue dissolved in dry THF. Paraformaldehyde was added and stirred for three hours to allow iminium ion formation and then a large excess of triethylphosphite was added before stirring for a further 3 days at room temperature. After filtration of the reaction mixture to remove unreacted paraformaldehyde, the crude product was put under high vacuum overnight to remove the excess triethylphosphite.
The product was used without further purification as decomposition took place whenever column chromatography was attempted.

Hydrolysis of the ester groups was achieved by heating the compound in hydrochloric acid (20%) under reflux for several days. After removal of the solvent, the product was isolated by dissolving the residue in acetone and precipitation in diethyl ether. The proton NMR spectrum shows the ring CH\textsubscript{2} and the CH\textsubscript{2} of the phosphonate group from 2.8 to 3.7 ppm. The benzylic protons can be observed at 4.4 ppm and the aromatic protons at 8.1, 8.8 and 8.9 ppm. This compound was also characterised fully by phosphorus and carbon NMR, mass spectrometry and elemental analysis.

\textbf{Fig. 4.6} \textsuperscript{1}H NMR spectrum of 54 (D\textsubscript{2}O)
Complexation was accomplished by dropwise addition of an aqueous solution of the lanthanide salt into a stirred solution of the ligand in water and heating for 48 hours. The pH of the reaction mixture was raised to ~10 to precipitate any free metal as a hydroxide and filtered. The pH was readjusted to neutral before removal of the solvent to afford the complexes.

MALDI mass spectra could not be obtained for these complexes therefore other forms of characterisation were used. The infrared spectrum shows the peak for the phosphoryl group at 1254 cm$^{-1}$ and does not change much for each lanthanide. Figure 4.7 shows the proton NMR spectrum for the lutetium complex. As the lanthanide in this case is diamagnetic the resonances are not shifted from that of the ligand but there is significant line broadening due to exchange of isomers on the timescale of the NMR experiment.

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{figure4.7.png}
\caption{$^1$H NMR spectrum of 55Lu (D$_2$O)}
\end{figure}
The proton NMR spectrum in figure 4.8 shows a spectral width from 40 to -30 which is indicative of complexation for a europium containing complex. The peaks are quite broad compared to those of other europium complexes indicating that interconversion of isomers is comparable to the timescale of the NMR experiment. The phosphorus NMR spectrum shows six peaks and is much simpler than that observed for EuDO3P at similar pH (7). The neodymium and ytterbium proton and phosphorus NMR spectra both share the same characteristics as that of the europium one, with fewer than expected peaks observed and also significant line broadening.
4.3 Synthesis and characterization of the d-f hybrid

Chloride in rhenium pentacarbonyl chloride was replaced with the more labile triflate anion by stirring the rhenium compound with silver triflate in the dark for two hours. After filtration over celite to remove the silver chloride byproduct, the solvent was removed under reduced pressure to yield the product.

The triflated rhenium complex was then reacted with pyridine in DCM for 48 hours and the pure product obtained by recrystallisation from a mixture of dichloromethane and diethyl ether. The product was characterised by elemental analysis, mass spectrometry and infrared spectroscopy.
The bis-DO3P complexes 55\textit{Ln} were reacted with the rhenium pentacarbonyl pyridine complexes in a mixture of methanol and water. The crude product was washed with plenty of DCM to remove any unreacted rhenium starting material and dried to give the complexes as bright orange solids.

The infrared red spectra shows that the rhenium complex has been successfully incorporated as the peaks for the carbonyl group can be observed at the positions detailed in table 4.1.

<table>
<thead>
<tr>
<th>Complex</th>
<th>Carbonyl peak/cm\textsuperscript{-1}</th>
</tr>
</thead>
<tbody>
<tr>
<td>Eu</td>
<td>2017, 1882</td>
</tr>
<tr>
<td>Yb</td>
<td>2026, 1882</td>
</tr>
<tr>
<td>Nd</td>
<td>2019, 1883</td>
</tr>
<tr>
<td>Gd</td>
<td>2026, 1866</td>
</tr>
</tbody>
</table>

Table 4.1 Infrared shifts of the rhenium complex carbonyl groups

Proton NMR spectrum of the europium complex shows extra peaks in the aromatic region due to the pyridine protons from the rhenium complex. These peaks are unshifted due to their distance from the lanthanide. The peaks are also sharper and fewer than observed in the starting material.
Proton NMR spectrum of the ytterbium complex is broad with very few peaks, indicating that interconversion of isomers is close to the timescale of the NMR experiment.
4.4 Photophysical studies

4.4.1 UV/Vis spectroscopy

The absorption spectrum of the bisDO3P ligand in figure 4.12 shows a peak for the $\pi - \pi^*$ transition of the phosphoryl group at 201 nm and another at 239 nm of the bipyridine moiety. The peak at 298 nm corresponds to the $n - \pi^*$ transition of the bipyridine ring. On complexation, a blue shift can be observed that is more pronounced for the $n - \pi^*$ transition indicating that the lone pairs of the bipyridine nitrogens are interacting with the lanthanide.

![UV/Vis spectra](image)

**Fig. 4.12** UV/Vis spectra in H$_2$O of 54, 55Eu and 55Nd

After reaction with the rhenium complex, the peaks have become much broader and are not as intense as with the starting material (figure 4.13). The peaks now extend into the visible region giving the complexes their characteristic orange colour.
Fig. 4.13 Comparison of the UV/Vis spectra of $^{55}\text{Eu}$ and $^{63}\text{Eu}$/Nd in H$_2$O
4.4.2 Luminescence spectroscopy

Time resolved emission spectra of neodymium and ytterbium d-f hybrids were measured in which the complex is excited indirectly at 337 nm and the emission measured at a series of wavelengths through time.

![Time resolved emission spectra (TRES) of 63Nd in H$_2$O](image)

**Fig. 4.14** Time resolved emission spectra (TRES) of 63Nd in H$_2$O

The spectra in figure 4.14 show the presence of both rhenium and neodymium components. This means the measured lifetime of the neodymium complex is convoluted with that of the rhenium complex but the signal is too weak to be separated.
4.4.3 Luminescence lifetimes

Luminescence lifetimes for the 55Eu and 63Eu binuclear complexes were obtained by excitation at 285 nm and emission at 617 nm followed by fitting of the intensities to an exponential decay function. For the ytterbium and neodymium complexes, a pulsed nitrogen laser was used to excite the chromophore at 337 nm and the emission at 980 and 1055 nm observed. Iterative deconvolution using an excel spreadsheet then gave the lifetimes. The modified Horrocks equation was used to calculate the hydration number.

<table>
<thead>
<tr>
<th>Complex</th>
<th>$\tau_{D_2O}/\mu s$</th>
<th>$\tau_{H_2O}/\mu s$</th>
<th>$q_{H_2O}$</th>
<th>$\tau_{D_2O-PBS}/\mu s$</th>
<th>$\tau_{H_2O-PBS}/\mu s$</th>
<th>$q_{PBS}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>55Eu</td>
<td>1820</td>
<td>580</td>
<td>1</td>
<td>1770</td>
<td>680</td>
<td>0.8</td>
</tr>
<tr>
<td>63Eu</td>
<td>1550</td>
<td>800</td>
<td>0.4</td>
<td>1740</td>
<td>730</td>
<td>0.7</td>
</tr>
<tr>
<td>55Nd</td>
<td>0.62</td>
<td>0.25</td>
<td>0</td>
<td>1.2</td>
<td>0.25</td>
<td>0</td>
</tr>
<tr>
<td>63Nd</td>
<td>0.52</td>
<td>0.17</td>
<td>0.1</td>
<td>0.57</td>
<td>0.17</td>
<td>0.1</td>
</tr>
<tr>
<td>55Yb</td>
<td>6.96</td>
<td>1.94</td>
<td>0.3</td>
<td>6.9</td>
<td>1.99</td>
<td>0.3</td>
</tr>
<tr>
<td>63Yb</td>
<td>5.94</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

Table 4.2 Luminescence lifetimes and calculated q values for bisDO3P complexes

The europium mononuclear complex displays long luminescence lifetimes both in water and D$_2$O and is monohydrated over the whole complex suggesting that each metal centre has on average half a water molecule associated with it. In comparison, EuDO3P had one water molecule associated with it leading to the expectation that the bisDO3P complex would have a q value of 2. This decrease in the expected q value can be explained by the hydrophobic nature of the phosphonates and the bipyridine ring, which also sterically hinders the metal centre.
The neodymium complex on the other hand has a $q$ value of zero when it is expected that it should have a similar or larger value to the europium complex due to the relatively large cation size. Neodymium is susceptible to quenching by C-H oscillators as well as N-H and O-H oscillators therefore the measured lifetimes have a large error associated with them and the calculated $q$ value is unreliable. The ytterbium cation is much smaller due to the lanthanide contraction and the $q$ value of 0.3 is as expected. Hydration values measured in PBS indicate that there is little or no phosphate binding taking place as the numbers remain the same within error.

On reaction with the rhenium complex, there is a reduction of the $q$ value of the europium complex with the number now being closer to 0 than 1. The complex can exist as TSAP isomers, which will have no inner sphere waters as it is more sterically hindered or as the SAP isomer which will be monohydrated. The non-integer $q$ value observed will then be an average between the two scenarios.

Luminescence lifetimes could not be obtained for the ytterbium d-f hybrid as the emission intensity was too weak.

### 4.4.4 Luminescence from rhenium complex

Luminescence measurements were taken from the mini-tau picosecond laser to assess the lifetimes from the rhenium moiety of the d-f hybrid as detailed in chapter 3.6.3.

<table>
<thead>
<tr>
<th>Complex</th>
<th>$\tau_{\text{D}2\text{O}/\text{ns}}$</th>
<th>$\tau_{\text{H}2\text{O}/\text{ns}}$</th>
<th>$\tau_{\text{D}2\text{O}-\text{PBS}/\text{ns}}$</th>
<th>$\tau_{\text{H}2\text{O}-\text{PBS}/\text{ns}}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>63Eu</td>
<td>170</td>
<td>51</td>
<td>147</td>
<td>51</td>
</tr>
<tr>
<td>63Nd</td>
<td>152</td>
<td>-</td>
<td>91</td>
<td>-</td>
</tr>
<tr>
<td>63Yb</td>
<td>355</td>
<td>286</td>
<td>371</td>
<td>144</td>
</tr>
</tbody>
</table>

*Table 4.3* Luminescence lifetimes of the rhenium component
All the complexes show relatively long luminescence lifetimes but are especially long for the ytterbium complex. This may be a consequence of charge transfer mediated sensitisation of ytterbium that can occur before formation of the $^3\text{MLCT}$ state.$^{18}$

**4.5 Relaxivity ($T_1$) studies**

Relaxivity measurements were carried out for 63Gd as detailed in chapter 3 (section 3.7) in order to assess its efficacy as a contrast agent. This complex has two gadolinium centres compared to the one of DO3P in addition to the larger molecular volume, which will reduce the tumbling rate in solution. It was anticipated that these properties would lead to even larger relaxivity values than was previously observed with the DO3P monomer.

![UNSF248/BISDO3P D-F IN H2O](image)

**Fig. 4.15** Plot of relaxivity of 63Gd in H$_2$O

Relaxivity was initially measured in water and gave a value of 6.3 mM$^{-1}$ s$^{-1}$. This value is slightly lower than that observed for DO3P (7.4 mM$^{-1}$ s$^{-1}$) and is even lower when considering that the relaxivity is only 3.1 mM$^{-1}$ s$^{-1}$ per gadolinium centre.
This lower relaxivity is probably due to the hydrophobic environment surrounding the metal centres from the phosphonates and the rhenium bipyridine complex repelling water molecules from the vicinity of the lanthanide. Calculated q values for analogous europium (0.4) and neodymium (0.1) complexes indicate that there is little or no water at the metal centre and the relaxivity is almost entirely due to outer sphere waters. In this context the relaxivity per gadolinium centre is actually quite high as the relaxivity for DOTP benzyl phosphinate a purely outer sphere contrast agent is only 1.9 mM\(^{-1}\) s\(^{-1}\).

As the complex is made up of two heptadentate units that are susceptible to replacement of the water molecules by phosphate, relaxivity measurements were carried out in PBS.

![Graph](image)

**Fig. 4.16** Plot of relaxivity of 63Gd in PBS

There is a decrease in relaxivity from 6.3 mM\(^{-1}\) s\(^{-1}\) to 4.5 mM\(^{-1}\) s\(^{-1}\) in PBS. Although phosphate binding appears to be taking place, it is not as dramatic as in the case of GdDO3P, which showed a decrease from 7.4 to 1.1 mM\(^{-1}\) s\(^{-1}\). In fact the decrease per gadolinium centre is only 0.9 mM\(^{-1}\) s\(^{-1}\) in this case. This is consistent with replacement of a few outer sphere waters, which does not have such a profound effect on the measured relaxivity.
Phosphonate complexes are known to have strong affinity for bone therefore the relaxivity was also measured in hydroxy apatite (a model of bone). HA was added to solutions of known concentration of the gadolinium complex and shaken for 24 hours. The samples were allowed to stand until the precipitates had settled at the bottom of the vial. $T_1$ measurements were then carried out on both the supernatant and the slurry.

![Graph](image1)

**Fig. 4.17** Plot of relaxivity of hydroxy apatite slurry after shaking for 24 hours with $^{63}$Gd

![Graph](image2)

**Fig. 4.18** Relaxivity of supernatant decanted from HA after shaking for 24 hours.
The relaxivity of the slurry and the supernatant are virtually the same but have decreased relative to that measured in PBS. These results are again comparable to those observed with the DO3P complex where the decrease in relaxivity was attributed to the expulsion of outer sphere water molecules by hydroxyapatite. The same seems to be happening for the bisDO3P complex but to a lesser extent.

4.6 Conclusion

A series of bisDO3P complexes that emit in the visible and NIR were successfully synthesised that showed long luminescence lifetimes. Luminescence lifetimes of the NIR emitting complexes did not improve on addition of the rhenium complex indicating that energy transfer was not efficient. This can be ascribed to the replacement of the chloride on the rhenium complex with the pyridine ring, which has the effect of lengthening the lifetime of the d-complex but making energy transfer to the lanthanide inefficient. Hydration values were lower than expected but this is due to the hydrophobic nature of the phosphonate arms as well as the rhenium bipyridine complex.

Relaxivity measurement on the gadolinium complex gave values of 3.1 mM$^{-1}$ s$^{-1}$ in water for each metal centre, which decreased slightly to 2.2 mM$^{-1}$ s$^{-1}$ in PBS. Phosphate binding is obviously taking place but to a lesser extent than observed with DO3P complexes.

Future development of the work described in this chapter would involve measuring the lifetime of the NIR emitting d-f complexes where the chloride anion is left intact to see if this has a positive effect on increasing the lifetime through more efficient energy transfer.
4.7 References

CHAPTER FIVE
PHOSPHINATE COMPLEXES

5.1 Introduction
The principal aim of this project was to make contrast agents with increased relaxivity. Chapters two to four have focused on achieving this by increasing the inner sphere hydration number (q) to two by lowering the ligand denticity to seven.

Equation 1 shows how there are two main contributors to relaxivity i.e inner sphere (IS), arising from bound water molecules in exchange and outer sphere (OS), which involves all the solvent molecules diffusing by the complex.\(^1\)

\[
R^{\text{obs}}_1 = R^{\text{IS}}_1 + R^{\text{OS}}_1 + R^{\text{W}}_1
\]  
(1)

\(R^{\text{obs}}_1\) = observed relaxation rate
\(R^{\text{W}}_1\) = solvent relaxation rate in absence of paramagnetic complex

It is estimated that outer sphere contribution accounts for about 40% of the relaxivity of monoaquo Gd\(^{3+}\) complexes at the imaging fields used and is considerably higher for q = 0 species. As well as the generalised outer sphere contribution, there is growing evidence of a significant contribution from water molecules in the second coordination shell of the paramagnetic ion arising from hydrogen bonding interactions with polar groups on the ligand. This information has sparked interest in making outer sphere contrast agents through the use of complexes bearing phosphinate or phosphonate pendant arms.\(^2-9\)

Aime \textit{et al} made a series of tetra-benzylphosphinate lanthanide complexes 64 which were shown to have no bound inner sphere water through luminescence studies and NMRD profiling but yet still possessed a relaxivity of 1.9 dm\(^3\) mmol\(^{-1}\) s\(^{-1}\). The complexes had a tendency to associate strongly with proteins leading to a pronounced relaxivity enhancement.
Furthermore, detailed biodistribution studies revealed avid biliary uptake at low doses and a well defined tendency for the complexes to be cleared more slowly from tumour tissue allowing tissue differentiation.\textsuperscript{3, 4} The value for the relaxivity of these complexes may also be significant as it approximates to the limiting value observed for DO3A in the presence of phosphate (for which q is also zero).

In order to benefit from both inner and outer sphere contributions, Parker \textit{et al.} substituted one of the phosphinate arms of a tetra(methylphosphinate) macrocyclic ligand 65 with a carboxamide group thus reducing the steric encumbrance of the four phosphinate groups in the parent complex and allowing the approach of a water molecule into the inner sphere. Relaxivity measurements (20 MHz, 25°C) afforded values of 3.09 and 3.08 dm\(^3\) mmol\(^{-1}\) s\(^{-1}\) which are significantly higher than analogous tetra(methylphosphinate) (2.44 dm\(^3\) mmol\(^{-1}\) s\(^{-1}\)) and tetra(benzylphosphinate) (1.85 dm\(^3\) mmol\(^{-1}\) s\(^{-1}\)) derivatives, supporting their original theory that decreasing steric hindrance would allow greater access of water to the paramagnetic metal ion.\textsuperscript{2}
Lukes et al. have found that replacing even just one of the carboxylate arms on DOTA with a phosphinic acid arm has a profound effect on the relaxivity. They synthesized a series of complexes of monophosphinic-bis(phosphinic) DOTA analogues which are all $q=1$ systems. Relaxivities were found to be between $6-8 \text{ dm}^3 \text{ mmol}^{-1} \text{ s}^{-1}$, greater than that of DOTA itself and any other monohydrated chelate of similar size. This increase was attributed to a significant contribution from the second hydration sphere. In addition to this they also found that the water residence lifetime was shorter due to the increase in the ratio of TSAP isomers.

These examples show that it is possible to increase the number of outer sphere water molecules thereby increasing relaxivity without having to reduce the number of donor atoms of the ligand and compromise the kinetic and thermodynamic stability of the resulting contrast agent.

Proceeding further in this direction, it was decided to synthesise a series of outer sphere contrast agents which can be used to quantify the relative contributions of inner and outer sphere mechanisms to relaxivity by comparison with other compounds. It will also remove all prospects for pH dependence in signal arising from acidity of the lanthanide bound water and reduce prospects for anion binding as even GdDOTA shows evidence for competition with chloride for the ninth coordination site of the lanthanide. In the absence of inner sphere water, there should be an increase in luminescence and quantum yields due to removal of O-H oscillator mediated non-radiative quenching pathways.
To achieve this, the synthesis of a phosphinic(pyridinyl) pendant arm that can be appended up to four times on a cyclen based core was investigated. The pyridine moiety, as well as serving as a chromophore for sensitised emission, can also serve as a linker to a rhenium bipyridine complex.

### 5.2 Synthetic Strategy

Scheme 5.1 shows the initial route towards tetra-phosphinate cyclen complexes, which has been used successfully by Aime et al in their synthesis of tetrabenzyl and tetramethoxy benzyl cyclen complexes.³

![Scheme 5.1 Synthetic route towards tetraphosphinic acid cyclen derivative](image)

Synthesis of the phosphinate arm 68 was initially attempted via a Grignard reaction; 4-bromomethyl pyridine was added to magnesium turnings in THF under a nitrogen atmosphere and stirred for an hour. Although the substrate was not soluble in THF, it was hoped that any small amount that dissolved would react with the magnesium and push the equilibrium forward. However, quenching of a small amount of the reaction mixture with D$_2$O followed by proton NMR analysis showed that the desired reaction had not occurred. The reaction was attempted again in diethyl ether but again yielded no product.
It is likely that the solubility of the starting material was inhibiting the formation of the product. 4-Bromomethyl pyridine 67 is only commercially available as its hydrobromide salt and is therefore is more soluble in polar solvents such as water, methanol and acetonitrile.

To dissolve the starting material in the solvents needed for the Grignard reaction (THF or diethyl ether), it was first dissolved in aqueous sodium hydroxide (1M) and extracted into DCM. On removal of the solvent the residue slowly turned black and proton NMR indicated the spectrum of several species, implying that the deprotonated species is very unstable towards polymerization.

Due to the solubility problems encountered with 4-bromomethyl pyridine, 4-methyl pyridine was used as a starting material. Following a literature procedure,\textsuperscript{11, 12} LDA was added dropwise to a solution of 4-methyl pyridine in THF and stirred for an hour. The now orange reaction mixture was added via cannula to diethyl chlorophosphite and stirred overnight. Again proton NMR showed that the reaction was unsuccessful.

Several more attempts were made to synthesise the pendant arm through the Grignard route and also lithiation under different conditions but all were unsuccessful.
5.3 Synthesis of the phosphinate arm

Attempts to synthesise the phosphinate arm by direct means proved unsuccessful therefore an alternative approach was considered. The following scheme shows a modification of that employed by Lukes et al.\textsuperscript{8}

Scheme 5.2 Indirect route to tetraphosphinate cyclen 77

Firstly, bis(trimethylsiloxy)phosphine was generated \textit{in situ} by heating a suspension of dry ammonium hypophosphite in hexamethyl disilazane overnight under nitrogen, giving a clear solution which was subsequently cooled to room temperature. A solution of vinyl pyridine was added followed by further stirring overnight. The reaction mixture was then added to ethanol to hydrolyse the silyl compounds. Mass spectrometry on the crude compound obtained after aqueous workup showed the product and also the bis-alkylated species. Due to the highly polar nature of the product it was decided to carry out the next step of the reaction without attempting purification by column chromatography.
A Mannich reaction was conducted using the acid, concentrated hydrochloric acid and paraformaldehyde. The reaction was successful and after purification by chromatography on silica gel the product was isolated in good yield and fully characterized.

![Fig. 5.1 $^1$H NMR spectrum of 73 (D$_2$O)](image)

The proton NMR spectrum of 73 shows five sets of peaks which all integrate to two each. At 1.98 and 2.9 ppm the ethylene peaks can be observed. The peaks are expected to be two triplets but have been further split due to the presence of the phosphorus atom. Splitting is also observed for the CH$_2$ in PCH$_2$OH which is a doublet. The aromatic peaks can be observed at 7.4 and 8.4 ppm.

Methylation of the phosphinic acid 73 was first of all attempted by the addition of DCC in several portions into a solution of the acid in ethanol. However, proton NMR and mass spectrometry of the crude mixture did not show any sign of the desired product. The reaction was repeated in methanol and using a different coupling agent EDCI but again the reaction was unsuccessful.
A more successful method involved the addition of trimethylsilyl diazomethane to a stirred solution of the phosphinic acid in methanol. Although the reaction was fast, the yield was quite poor at only 18%. Repeating the reaction several times gave the same yield consistently. This low yield and lengthy synthetic procedure made it difficult to complete the synthesis, and the route was reluctantly abandoned.

![Scheme 5.3 Successful synthesis of 78](image)

**Scheme 5.3 Successful synthesis of 78**

![Fig 5.2 Electrospray mass spectrum of 74 (H₂O)](image)

**Fig 5.2 Electrospray mass spectrum of 74 (H₂O)**
5.4 Conclusion

This exploration using phosphorus (III) chemistry to establish routes to pyridyl phosphinate ligands proved interesting but ultimately unsuccessful. Attempts at making the pendant arm directly using the Grignard reaction and lithiation proved unsuccessful. The indirect route outlined in scheme 5.2 although successful at the earlier stages met with a stumbling block at the methylation stage. The synthetic target remains an interesting one whose synthesis and potential applications could be further explored by other researchers in the field.
5.5 References


Heptadentate complexes with increased hydration number have been successfully synthesized and characterized using NMR and luminescence spectroscopy to establish the structure of the complexes in solution and to investigate the binding of phosphate at the metal centre.

Cationic and anionic complexes with isophthalate derived pendant arms were synthesized and were shown to have $q$ values of approximately 2 in aqueous solution. However, calculation of the hydration number from the luminescence lifetimes in H$_2$O and D$_2$O PBS solutions showed that phosphate binding was taking place for both complexes, implying that the difference in charge makes little (if any) difference to phosphate binding. This can be explained by the mode of binding of solvent by the complexes- the two water molecules occupy adjacent sites on the metal, which leaves a cis binding site that can easily be occupied by the bidentate phosphate anion. Furthermore, the negative charges, which were supposed to repel the anion are located on the periphery of the complex and have been deemed to be too far away from the metal centre.

DO3P complexes were synthesized which would bring this high negative charge closer to the metal centre. Phosphate binding studies were carried out which showed that hydration number stayed the same in both water and PBS. A series of rhenium containing d-f hybrid complexes was then prepared; the luminescent complexes showed increased phosphate binding relative to DO3A, conceivably as a consequence of the reduced overall charge on the system. This was further confirmed by relaxivity measurements in the presence and absence of hydroxyapatite; relaxivity was high in water (7.4 mM$^{-1}$ s$^{-1}$) but decreased significantly to 1.1 mM$^{-1}$ s$^{-1}$ in the presence of hydroxyapatite, suggesting surface binding inhibits water binding and exchange. Synthesis of a bipyridine bridged bis-DO3P complex seemed to decrease the extent of phosphate binding but did not increase the relaxivity of the overall complex as expected.
Near infrared emissive complexes of the DO3P and bis-DO3P ligands were studied by luminescence spectroscopy both with and without a complexed rhenium component. Sensitised lanthanide luminescence was observed in solution, and the luminescence properties reflect those of the simpler systems, as would be expected where the emissive state remains the same. Inefficient spectral overlap in the ytterbium complex leads to relatively inefficient energy transfer.

Considerable efforts have also been devoted to the synthesis of pyridyl appended phosphinate derivatives of cyclen. These proved to be synthetically challenging, and ultimately unsuccessful. The idea of using phosphinate derivatives remains appealing, and should be investigated further. Perhaps one approach to this goal would be the preparation of phosphinate complexes bearing reactive groups that can be functionalized further once complexation is complete. The routes investigated in this work could be applied to the preparation of hydroxyethyl or aminoethyl phosphinate derivatives of cyclen by using appropriate protecting groups to mask the hydroxyl and amine functionalities until after complexation is complete.

The future of \( q = 2 \) complexes in clinical imaging is more difficult to predict. While DO3P derivatives show considerable potential, the pH dependent protonation of the phosphinate arms means that anion binding will vary with pH. Raymond’s HOPO systems do not suffer the problem of phosphate binding but are not kinetically inert, meaning that there are serious concerns about their use in living subjects. Perhaps one way forward lies in the use of bridged derivatives of DO2A, which will combine the rigidity of a macrocyclic system with two, non-adjacent, water binding sites. Even with these, the advantage of the rapid associative water exchange mechanisms normally associated with DO3A systems may mean that the advantage over DO3P derivatives is not that great.
CHAPTER SEVEN
EXPERIMENTAL

7.1 General methods

7.1.1 Chromatography
Purification by column chromatography was performed using silica gel treated with dichloromethane or neutral alumina in ethyl acetate. The eluent was specified for the individual compounds that have been purified by this method.

7.1.2 Spectroscopy
Infra-red spectra were recorded as solids, nujol mulls or thin films on a Perkin Elmer FT-IR, Attenuated Total Reflectance (ATR) spectrometer.

Nuclear Magnetic Resonance (NMR) spectra were recorded using a Bruker Avance 400 spectrometer (400 MHz for proton nuclei, 161 MHz for $^{31}$P nuclei and 100 MHz for $^{13}$C Nuclei). Proton NMR of some paramagnetic lanthanide complexes were recorded using a Varian Unity 500 spectrometer, courtesy of the group of Dr. Alan Kenwright of the University of Durham (the frequency has has been indicated wherever this was the case). Inversion recovery measurements were recorded using a Varian Unity Inova 400 spectrometer. The solvent used in each case is specified. Unless otherwise stated, all spectra were measured at room temperature.

Elemental analysis was carried out in the microanalysis laboratory at the School of Chemistry, University of Manchester, on a Carlo ERBA CHNS-O EA1108 elemental analyser.

Mass spectrometry was carried out by the staff of the Mass Spectrometry Laboratory, School of Chemistry, University of Manchester. Electrospray mass spectra were obtained using a micromass platform spectrometer. MALDI-ToF mass spectra were obtained using a micromass ToF spec 2E spectrometer.
7.1.3 Photophysical methods
Luminescence spectra for terbium and europium complexes were measured using a Perkin-Elmer LS55 fluorimeter. Lifetimes were calculated from the decay of the emission intensity of molecules after initial excitation using a programme written by Ken Ball (School of Chemistry, University of Manchester), and a spreadsheet in Microsoft Excel. The solver utility in Excel was used to fit a curve to the decay by minimising the residual squared. All decays fitted well to a single exponential decay unless otherwise stated.

Luminescence spectra for ytterbium and neodymium complexes were measured using a pulsed nitrogen (PTI-3301-337nm) laser. Luminescence from the sample was collected at right angles to the incident beam and focused onto the slits of a monochromator (PTI-120). The growth and decay of the luminescence at selected wavelengths were detected using a germanium photodiode (Edinburgh Instruments, EI-P) and were recorded using a digital oscilloscope (Tektronix TDS220). Luminescence lifetimes were obtained by iterative reconvolution of the detector response (obtained by using a scatterer) with exponential components for growth and decay of the metal centred luminescence, using Microsoft Excel.

7.1.4 Dialysis
Lanthanide complexes were purified by using a Biodialyser system purchased from Sigma-Aldrich. Sample solutions in water were loaded into the microdialysis chamber (1 mL) and an ultrafiltration membrane with a cut-off limit of 500 or 1000 Da fitted over the well and screwed down with the threaded cap ring. The chamber was placed in a large beaker with a litre of water and stirred for 2 days. The remaining solvent was removed under reduced pressure and the resulting solid was dried to afford the lanthanide products.
7.2 Synthetic Procedures

1,3- Dimethyl-5-aminoisophthalate acetyl chloride\textsuperscript{1} (23)

Dimethyl 1,5-aminoisophthalate (5.1 g, 24.4 mmol) was dissolved in dichloromethane (250 mL) and sodium hydrogen carbonate (25 g, 298 mmol) added. Chloroacetyl chloride (2 mL) was added dropwise to the mixture then stirred at room temperature for 24 h. Further dichloromethane (200 mL) was added to the reaction mixture, then the inorganic impurities were removed by filtration. The solvent was removed under reduced pressure to leave a beige solid which was recrystallised from the minimum amount of hot dichloromethane to leave the product as a white solid (5.93 g, 85\%).

\textbf{MS ES}^+ m/z (MeCN) 286 (M + H)\textsuperscript{+}, 303 (M + NH}_4\textsuperscript{+}

\textbf{\textsuperscript{1}H NMR (CDCl}_3, 400 MHz) } \delta (ppm) 3.8 (s, 6H, 2CH}_3), 4.1 (s, 2H, CH}_2Cl), 8.3 (s, 2H, CHCNH), 8.35 (s, 1H, CHCCO}_2Me)

\textbf{IR(ATR): } \nu (C=O) 1720 cm\textsuperscript{-1}, (C=O) 1686 cm\textsuperscript{-1}, (N-H) 1547 cm\textsuperscript{-1}.

CHN expected for C\textsubscript{12}H\textsubscript{12}NO\textsubscript{5}Cl: C, 50.45; H, 4.23; N, 4.90; Cl, 12.41; found C, 50.41; H, 4.06; N, 4.86; Cl, 12.52.
1,4,7- Tris(acetyldimethyl-5-aminoisophthalate)- 1,4,7,10-tetraazacyclododecane\(^1\) (25)

![Chemical structure of 1,4,7- Tris(acetyldimethyl-5-aminoisophthalate)- 1,4,7,10-tetraazacyclododecane](image)

Cyclen (1 g, 5.8 mmol), sodium hydrogen carbonate (1.6 g, 19 mmol) and potassium iodide (3 g, 19 mmol) were dissolved in acetonitrile (150 mL) and cooled to 0°C. A solution of the chloroacetamide 23 (5.5 g, 19 mmol) in acetonitrile (150 mL) was then added dropwise. The mixture was allowed to reach room temperature and left to stir for 5 d. The solvent was removed under reduced pressure and the residue was extracted into dichloromethane (3 x 20 mL), washed with brine (20 mL) and dried over sodium sulfate. The solution was evaporated under reduced pressure to leave a green solid, which was recrystallised in hot acetonitrile to yield a white solid (2 g, 38%).

**MS ES\(^+\) m/z (DMSO) 920 {M + H}\]**

\(^1\)H NMR (DMSO, 400 MHz) \(\delta\)\(_H\) (ppm) 2.8, 2.9, 3.25 (broad m, 16H, ring-CH\(_2\)), 3.55 (s, 6H, NCH\(_2\)CO \(_\)\)), 3.85 (s, 18H, CH\(_3\)), 7.9, 7.97, 8.25 (s, 9H, Ar-H), 10.25 (s, 1H, ONH\(_\)\)), 10.32 (s, 2H, ONH)

CHN expected for C\(_{44}\)H\(_{53}\)N\(_7\)O\(_{15}\).NaCl: C, 56.09; H, 5.93; N, 9.15; found C, 55.84; H, 5.57; N, 9.36.
1,4,7-Tris(acetyldimethyl-5-aminoisophthalate)-1,4,7,10-tetraazacyclododecane lanthanide complexes\(^1\), Gd, Eu, Tb, Yb (26Ln)

A solution of lanthanide triflate (1 eq) in anhydrous acetonitrile (1 mL) was added to a solution of ligand 25 (0.05 g, 0.005 mmol) in hot acetonitrile (1 mL) and the solution was heated at 60 °C, under nitrogen for 1 h. The solvent was removed under reduced pressure and the residue taken up in the minimum volume of acetonitrile and added dropwise to a large volume of diethyl ether (100 mL). The resulting hygroscopic beige white precipitate was isolated using a filter stick under vacuum and dried to give the lanthanide complexes.

**26Eu**: 27 mg, 46 %
MALDI MS (DMSO) \(m/z\): 921 \{(M + H – Eu)\}\(^+\), 1071 \{M\}\(^+\), 1095 \{(M + Na)\}\(^+\); UV-Vis (H\(_2\)O) \(\lambda_{\text{max}}\) (π-π\(^*\)) = 260 nm; Luminescence: \(\lambda_{\text{em}} = 617\) nm, \(\lambda_{\text{ex}} = 260\) nm, \(\tau_{(\text{H2O})} = 0.36\) ms, \(\tau_{(\text{D2O})} = 1.03\) ms, \(q = 1.6\)

**26Tb**: 45 mg, 77 %
MALDI MS (DMSO) \(m/z\): 920 \{(M + H – Tb)\}\(^+\), 1076 \{M\}\(^+\), 1100 \{(M + Na)\}\(^+\); UV-Vis (H\(_2\)O) \(\lambda_{\text{max}}\) (π-π\(^*\)) = 260 nm; Luminescence: \(\lambda_{\text{em}} = 545\) nm, \(\lambda_{\text{ex}} = 260\) nm, \(\tau_{(\text{H2O})} = 1.24\) ms, \(\tau_{(\text{D2O})} = 2.44\) ms, \(q = 1.7\); \(^1\)H NMR (D\(_2\)O, 500M Hz) δ -110.6, -100.4, -94.9, -82.3, -78.6, -70.2, -66.3, -56.5, -43.9, -39.1, -29.9, -21.8, -12.1, -5.9, -4.9 -1.8, -0.5, 31.7, 42.4, 60.57, 68.9, 95.9 (only major resolved peaks outside the range 0 to 30 ppm reported)
**26Yb:** 32 mg, 54 %
MALDI MS (DMSO) *m/z*: 921 {M + H – Yb}+, 1092 {M+2H};

1H NMR (D2O, 500MHz) δH (ppm) -117.6, -60.8, -29.5, -25.5, -24.6, -22.7, -14.4, -11.1, -9.2, -7.5, -7.3, -3.7, -2.0, 8.9, 9.5, 9.9, 11.2, 11.4, 13.2, 21.7, 22.5, 24.4, 28.8, 29.5, 33.3, 39.2, 61.2, 67.2, 92.2 (only major resolved peaks outside the range -1.0 to 8 ppm reported)

**26Gd:** 39 mg, 67 %
MALDI MS (DMSO) *m/z*: 921 {M + H – Gd}+, 1077 {M}+, 1100 {M + Na}+

1,4,7,10-tetraazacyclododecane-1,4,7-tris(dicarboxylicacid-5-isophthalato acetamide)\(^1\) (27)

![Image of 27]

Sodium hydroxide (0.019 g, 0.5 mmol) in water (5 mL) was added to a solution of 25 (0.05 g, 0.05 mmol) in methanol (5 mL) and stirred at 60°C for 24 h. The solvent was removed under reduced pressure to yield the hexaacid 27 as a white crystalline salt (0.08 g)

MS ES\(^+\) *m/z* (H2O) 836 (M + H)\(^+\), 859 M + Na)\(^+\)

1H NMR (D2O, 400 MHz) δ 2.83, 2.95, 3.34 (broad s, 16H, ring-CH\(_2\)), 3.3 (s, 6H, CH\(_2\)CO), 7.9 (s, 6H, PhH\(_{\text{t}}\)), 8.05 (s, 3H, PhH\(_{\text{t}}\)), 8.13, 8.15 (2s, 3H, NH).
1,4,7,10-tetraazacyclododecane-1,4,7-tris(dicarboxylic acid-5-isophthalato acetamide) lanthanide complexes\(^1\), Eu, Tb (28Ln)

\[
\begin{array}{c}
\text{28Ln} \\
\end{array}
\]

27 (0.05 g, 0.006 mmol) was dissolved in distilled water (1 mL) and the pH adjusted from pH11 to ~pH6 by adding hydrochloric acid (1M). A solution of lanthanide triflate salt (1 eq) was dissolved in distilled water (1 mL) and added dropwise to the stirring mixture. The solution was then heated at 60°C for 24 h after which the solvent was evaporated under reduced pressure to yield lanthanide complexes as white solids.

**28Eu:** 63 mg
MALDI MS (DMSO) \(m/z: 836 \{\text{M + H – Eu}\}^+, 988 \{\text{M}\}^+\); UV-Vis (H\(_2\)O) \(\lambda_{\text{max}} (\pi-\pi^*) = 260\) nm; Luminescence: \(\lambda_{\text{em}} = 617\) nm, \(\lambda_{\text{ex}} = 260\) nm, \(\tau_{(H_2O)} = 0.29\) ms, \(\tau_{(D_2O)} = 1.38\) ms, \(q = 2.7\)

**28Tb:** 59 mg, 99 %
MALDI MS (DMSO) \(m/z: 836 \{\text{M + H – Tb}\}^+, 994 \{\text{M}\}^+\); UV-Vis (H\(_2\)O) \(\lambda_{\text{max}} (\pi-\pi^*) = 260\) nm; Luminescence: \(\lambda_{\text{em}} = 545\) nm, \(\lambda_{\text{ex}} = 260\) nm, \(\tau_{(H_2O)} = 1.04\) ms, \(\tau_{(D_2O)} = 2.15\) ms, \(q = 2.2\)
Concentrated HCl (1 M, 3 mL) was added dropwise to a cooled solution (0°C) of the protected cyclen 40 (2.69 g, 4.4 mmol) in methanol (5 mL). The resulting solution was allowed to reach room temperature and stirred for a further 18 h. The reaction mixture was concentrated under reduced pressure to ~2 mL and the precipitates removed by filtration. THF was then added to the residue to precipitate the desired product as a white solid that was isolated by filtration and dried under reduced pressure (1.28 g, 96%).

MS ES\textsuperscript{+} m/z (MeOH) 307 {\textsuperscript{[M + H]}\textsuperscript{+}}

\textsuperscript{1}H NMR (D\textsubscript{2}O, 300 MHz) \(\delta\) (ppm) 3.2 (m, 16H, ring-CH\textsubscript{2}), 5.1 (s, 2H, OCH\textsubscript{2}Ph), 7.38 (m, 5H, PhH)

\textsuperscript{13}C (CDCl\textsubscript{3}, 100 MHz) \(\delta\) (ppm) 45.5, 47.3, 47.9, 49.2 (ring CH\textsubscript{2}), 71.4 (OCH\textsubscript{2}Ph), 131.2, 131.5, 131.6, 138.4 (Ar-C), 161.2 (C=O).
1,4,7-Tris-tert-butoxycarbonyl-1,4,7,10-tetraazacyclododecane$^2$ (38)

A solution of di-tert-butyl dicarbonate (3.8 g, 17.4 mmol) in chloroform (20 mL) was added dropwise to a solution of cyclen (1 g, 5.81 mmol) and triethylamine (2.43 mL, 17.4 mmol) in chloroform (30 mL) at 0°C. The reaction mixture was allowed to warm to room temperature and stirred for 18 h then washed with water (2 x 20 mL) and dried over sodium sulfate. The solvents were removed under reduced pressure and the residue was purified by column chromatography over silica gel eluting with diethyl ether to afford the title compound as a white solid (6.63 g, 81%).

MS ES$^+$ m/z (MeOH) 473 {M+H}$^+$, 496 {M+Na}$^+$

$^1$H NMR (CDCl$_3$, 400 MHz) $\delta$ (ppm) 1.4 (s, 27H, $^1$Bu CH$_3$), 2.8, 3.3, 3.6 (broad s, 16H, ring-CH$_2$)

IR(ATR): $\nu$ (C=O) 1696 cm$^{-1}$ (N-H) 3312 cm$^{-1}$. 
1-Benzylxycarbonyl-4,7,10-tris-tert-butoxycarbonyl-1,4,7,10-tetraazacyclododecane\textsuperscript{2} (40)

![Chemical Structure](image)

38 (3.3 g, 7.0 mmol) and triethylamine (1.2 mL, 8.4 mmol) were dissolved in chloroform (75 mL) and benzyl chloroformate (1.4 g, 8.64 mmol) was added. The resulting solution was allowed to warm to room temperature and stirred for 18 h. The precipitates were removed by filtration and the solvents were removed under reduced pressure. The residue was purified by column chromatography over silica gel eluting with 30% ethylacetate in hexanes to afford the title compound as a white solid (2.69 g, 63%).

MS ES\textsuperscript{+} m/z (MeOH) 507 (M+H)\textsuperscript{+}

\textsuperscript{1}H NMR (CDCl\textsubscript{3}, 400 MHz) \( \delta \) (ppm) 1.4 (s, 27H, \( ^{\text{tBu}} \text{CH}_3 \)), 3.1-3.5 (broad m, 16H, ring-\( \text{CH}_2 \)), 5.07 (s, 2H, O\( \text{CH}_2 \text{Ph} \)), 7.3 (m, 5H, Ph\( \text{H} \)).
1,4,7-Tris(benzyloxycarbonyl)-1,4,7,10-tetraazacyclododecane

Cyclen (4g, 23.3 mmol) and triethylamine (13.2 mL, mmol) were dissolved in chloroform (240 mL) and stirred at 0°C then benzyl chloroformate dissolved in chloroform (160 mL) was added dropwise over an hour. The mixture was allowed to reach room temperature and stirred overnight. The solvent was removed under reduced pressure and the pure product was obtained as a white solid after flash chromatography on silica (ethylacetate/hexane 17:3) (6.7 g, 50 %).

MS ES\(^+\) m/z (MeOH) 574 {M+H}\(^+\), 597 {M+Na}\(^+\), 636 {M+Na+K}\(^+\)
\(^1\)H NMR (CDCl\(_3\), 400 MHz) \(\delta\) (ppm) 2.71-2.79 (4H, m, ring CH\(_2\)), 3.26-3.58 (12H, m, ring CH\(_2\)), 4.78 (2H, s, COCH\(_2\)), 4.97 (3H, s, COCH\(_2\)), 5.06 (1H, s, COCH\(_2\)), 7.17-7.22 (15H, m, Ph-H)
\(^13\)C NMR (CDCl\(_3\), 100 MHz) \(\delta\) (ppm) 45.57, 49.17, 51.04 (ring CH\(_2\)), 127.66, 127.80, 128.13, 128.37, 128.43, 136.64, 136.93 (Ar-C), 155.96 (C=O)
1,4,7,10-Tetraazacyclododecane-1-tertbutoxycarbonyl-4,7,10-
trisbenzyloxyxycarbonyl

Triethylamine (2 mL, 15.1 mmol) was added to stirred solution of tri-Cbz-
protected cyclen (6.67 g, 11.6 mmol) in chloroform (100 mL) and cooled to 0°C. 
Di-tert-butyl dicarbonate (3.4 g, 15.1 mmol) was dissolved in chloroform (170 
ml) and added dropwise to the stirring mixture. The reaction mixture was 
allowed to reach room temperature and stirred for a further 24 h. The solvent 
was removed and the residue was purified by flash chromatography on silica 
eluting with 30% ethylacetate in hexanes to yield a white solid (6.98 g, 89%).

**MS ES⁺ m/z** (MeOH) 697 {M+Na}⁺, 1371 {2M+Na}⁺

**¹H NMR (CDCl₃, 400 MHz) δH (ppm)** 1.28 (9H, s, CH₃), 3.21-3.35 (16H, broad 
m, ring CH₂), 4.96 & 4.99 (6H, s, COCH₂), 7.22 (15H, s, Ph-H)

**¹³C NMR (CDCl₃, 100 MHz) δC (ppm)** 25.99, 26.42, 26.61 (CH₃), 48.12, 48.36, 
58.43(ring CH₂), 65.29 (OCH₂PH), 78.18 (C(CH₃)₃), 126.04, 126.26, 126.39, 
126.57, 134.45 (Ar-C), 154.81, 169.14 (C=O)

**IR(ATR): ν 2927, 1690 (C=O), 1468, 1415, 1365, 1244, 1168, 1099, 773, 698 
cm⁻¹**
1,4,7,10-Tetraaza-Cyclododecane-1-tert-butoxycarbonyl

To a two-necked flask was added a solution of tri-Cbz+Boc protected cyclen (6.9 g, 10 mmol) in methanol and a catalytic amount of 10% wt Pd/C. The air was evacuated from the flask and 1 atm of hydrogen was introduced. The reaction mixture was stirred for 24 h. The solution was filtered through celite and the solvent removed to leave a pale yellow solid which was recrystallised from methanol and diethyl ether to yield a white solid (1.35 g, 49%).

MS ES$^+$ m/z (MeOH) 273 {M+H}$^+$, 545 {2M+H}$^+$

$^1$H NMR (D$_2$O, 400 MHz) δ$_H$ (ppm) 1.35 (9H, s, CH$_3$), 2.54-2.58 (2H, broad m), 2.62-2.66 (2H, broad m), 2.69-2.72 (2H, broad m), 2.85-2.87 (3H, broad m), 2.89-2.92 (1H, broad m), 3.28 (2H, broad m), 3.34 (4H, broad m) all ring CH$_2$

$^{13}$C NMR (D$_2$O, 100 MHz) δ$_C$ (ppm) 27.56, 27.62 (CH$_3$), 44.92, 47.77, 48.27, 48.57, 48.85, 49.09 (ring CH$_2$), 81.99, 82.30 (C(CH$_3$)$_3$), 157.23, 158.04, 163.64 (C=O)

General procedure for phosphonation

Paraformaldehyde (3.3 eq) was added to a stirring solution of mono-protected cyclen (1 eq) in dry THF under nitrogen. The reaction mixture was allowed to stir for 3 h, then triethyl phosphite (3.3 eq) was added and allowed to stir for a further 18 h. The reaction mixture was filtered to remove excess unreacted paraformaldehyde and concentrated under reduced pressure for several hours to remove volatile impurities. The crude mixture was purified by flash chromatography on silica eluting with diethyl ether/methanol (9:1) to yield the product as an oil.
**4,7,10-Tris-(diethoxy-phosphorylmethyl)-1,4,7,10-tetraaza-cyclododecane-1-carboxylic acid benzyl ester (32)**

Mono-Cbz protected cyclen hydrochloride salt (0.7 g, 2.29 mmol) was dissolved in a 10% sodium carbonate solution (20 mL) and extracted with dichloromethane (3 x 30 mL). The organic phase was dried with sodium sulfate and the solvent was removed to leave a colourless oil. The residue was dissolved in THF (20 mL) and paraformaldehyde (0.23 g, 7.55 mmol) was added. After 3 h triethyl phosphite (1.32 mL, 7.55 mmol) was added. The product was obtained as a yellow oil (0.9 g, 52%).

**MS** ES+ m/z 779 (MeOH) [M+Na]+, HRMS (ES): calculated for C_{31}H_{59}O_{11}N_{4}P_{3}Na (MNa)+, 779.3285; found 779.3296

^{1}H NMR (CD_{3}CN, 400 MHz) δ_H (ppm) 1.28 (18H, t, ^3J_{HH}=7.2 Hz, CH_{3}), 2.78 , 2.95, 3.52, 4.06 (12H, q, ^3J_{HH}=7.2 Hz, CH_{2}CH_{3}), 5.09 (2H, s, CH_{2}Ph), 7.39 (5H, m, Ph-H)

^{13}C NMR (CD_{3}CN, 100 MHz) δ_C (ppm) 14.30, 15.55, 15.61 (CH_{3}), 45.58, 47.93, 49.25, 49.42, 50.79 (ring CH_{2}), 52.45, 52.51, 53.15, 53.21, 53.65, 53.73 (NCH_{2}P), 60.94, 61.02, 61.09 (CH_{2}CH_{3}), 64.94, 65.95 (OCH_{2}PH) 116.98, 127.26, 127.40, 128.08, 137.25 (Ar-C), 155.66 (C=O)

^{31}P NMR (CDCl_{3}, 161 MHz) δ_P (ppm) 25.63, 26.26
4,7,10-Tris-(diethoxy-phosphorylmethyl)-1,4,7,10-tetraaza-cyclododecane-1-carboxylic acid tert-butyl ester

Using the general procedure outlined on page 174, the product was isolated as a viscous yellow oil (0.87 g, 60%).

MS ES+ m/z (MeOH) 723 {M+H}+, 745 {M+Na}+

\[ {^{1}}H \text{ NMR (CDCl}_3, 400 \text{ MHz)} \delta_H (ppm) 1.25 (18H, t, } {^{3}J_{HH}=7.2Hz, \text{ CH}_2\text{CH}_3), 1.38 (9H, s, } t\text{-butyl CH}_3), 2.75-3.35 (22H, m, ring CH}_2\text{ & CH}_2\text{P), 4.01-4.08 (12H, broad m, CH}_2\text{CH}_3) \]

\[ {^{31}}P \text{ NMR (CDCl}_3, 161 \text{ MHz) } \delta_P (ppm) 5.12, 9.4 \]

[4,7-Bis-(diethoxy-phosphorylmethyl)-1,4,7,10-tetraaza-cyclododec-1-ylmethyl]-phosphonic acid hexaethyl ester (33)

The Cbz protected compound 32 (0.89 g, 1.18 mmol) was dissolved in methanol and Pd/C (0.3 g) added to the stirring solution. The flask was sealed with a rubber septum and the air evacuated with a syringe. A hydrogen filled balloon was inserted via a needle and mixture stirred for 24 hrs. The reaction
mixture was filtered through celite and the solvent removed to yield the product as a yellow oil (0.71 g, 97%).

MS ES$^+$ m/z  623 (MeOH) $\{M+H\}^+$, HRMS (ES): calculated for C$_{23}$H$_{54}$O$_9$N$_4$P$_3$ (MH)$^+$, 623.3098; found 623.3096

$^1$H NMR (CDCl$_3$, 400 MHz) $\delta_H$ (ppm) 1.33 (18H, broad s, CH$_3$), 2.64-3.13 (16H, broad m, ring CH$_2$), 3.45 (6H, broad s, CH$_2$P), 4.13 (12H, broad s, CH$_2$CH$_3$)

$^{13}$C NMR (CDCl$_3$, 100 MHz) $\delta_C$ (ppm) 16.55, 16.61 (CH$_3$), 46.74, 50.49, 51.43 (ring CH$_2$), 52.65, 53.25 (NCH$_2$P), 61.42, 61.49, 61.57, 61.64 (CH$_2$CH$_3$)

$^{31}$P NMR (CDCl$_3$, 161 MHz) $\delta_P$ (ppm) 26.14, 26.91

IR (thin film, cm$^{-1}$): $\nu$ 3459, 2982, 1663, 1453, 1392, 1226 (P=O), 1163, 964

[4,7-Bis-(diethoxy-phosphorylmethyl)-1,4,7,10-tetraaza-cyclododec-1-ylmethyl]-phosphonic acid diethyl ester lanthanide complexes (34Ln)

![Diagram of the tris-phosphonate ester](image)

The tris-phosphonate ester (0.05 g, 0.08 mmol) was stirred in acetonitrile (1 mL). The lanthanide triflate (2 eq) dissolved in acetonitrile (1 mL) was added dropwise to the stirring solution which was then heated to 50°C for 3 days. The solvent was removed and the residue was dissolved in water and subsequently purified through dialysis. The solvent was evaporated and the product dried to give the desired lanthanide complex.
**34Gd:** 53 mg, 85 %
MALDI MS \(m/z\) (\(\alpha\)-MeOH) 624 \{M-Gd\}^+, 900 \{M+OTf\}^+, 923 \{M+OTf+Na\}^+, 939 \{M+OTf+K\}^+

**34Eu:** 50 mg, 81 %
MALDI MS \(m/z\) (\(\alpha\)-MeOH) 624 \{M-Eu\}^+, 924 \{M+OTf+Na\}^+
\(\lambda_{ex}= 390\) nm, \(\lambda_{em}= 617\) nm, \(\tau_{H2O} = 0.28\) ms, \(\tau_{D2O} = 2.4\) ms, \(q = 3.2\)
\(^1\)H NMR (MeOD, 400 MHz) \(\delta_H\) (ppm) -22.5, -20.9, -17.6, -17.1, -15.2, -14.7, -13.9, -12.1, -11.7, -11.1, -10.1, -8.5, -7.3, -6.0, -3.7, -3.3, -2.8, -2.1, -1.3, 10.1, 12.5, 13.0, 15.4, 19.8 (only major resolved peaks outside the range -1 to 10 reported)

**34Tb:** 49 mg, 77 %
MALDI MS \(m/z\) (\(\alpha\)-MeOH) 624 \{M-Tb\}^+, 901 \{M+OTf\}^+, 924 \{M+OTf+Na\}^+, 1079 \{M+2OTf\}^+, 1118 \{M+2OTf+K\}^+
\(\lambda_{ex}= 280\) nm, \(\lambda_{em}= 545\) nm, \(\tau_{H2O} = 0.65\) ms, \(\tau_{D2O} = 0.79\) ms, \(q = 1.1\)

**34Yb:** 63 mg, 99 %
MALDI MS \(m/z\) (\(\alpha\)-MeOH) 624 \{M-Yb\}^+, 916 \{M+OTf\}^+
\(\lambda_{ex}= 337\) nm, \(\lambda_{em}= 980\) nm, \(\tau_{H2O} = 1.9\) \(\mu\)s, \(\tau_{D2O} = 3.6\) \(\mu\)s, \(q = 0.6\)
\(^1\)H NMR (D\(_2\)O, 400 MHz) \(\delta_H\) (ppm) -30.9, -15.9, -9.7, -9.1, -4.9, -3.8, -1.7, 7.4, 8.1, 51.9 (only major resolved peaks outside the range -1 to 7 reported)
**General procedure for phosphonate ester hydrolysis**

The tris-phosphonate compound was dissolved in hydrochloric acid (20 %, 20 mL) and heated to reflux temperature for 48 h. The solvent was removed under vacuum to leave the product, which was used without further purification.

*(4,7-Bis-phosphonomethyl-1,4,7,10-tetraaza-cyclododec-1-ylmethyl)-phosphonic acid (35)*

![Chemical structure of 35](image)

Using the general procedure outlined above, the product was obtained as a beige solid (0.29 g, 89 %).

**MS ES⁺ m/z** (H₂O) 456 {M+H}⁺, 478 {M+Na}⁺

¹H NMR (D₂O) δH (ppm) 2.77, 2.86, 3.01, 3.17, 3.51, 3.62 (all broad m)

CHN expected for C₁₁H₃₁N₄O₉P₃·4H₂O: C, 25.10; H, 7.09; N, 10.64; P, 17.65; found C, 25.05; H, 6.99; N, 10.57; P, 17.82.
The tris-acid 35 (0.07 g, 0.15 mmol) was dissolved in water (1 mL) and the pH adjusted from ~2 to ~6 with sodium hydroxide (0.1 M). The lanthanide triflate was dissolved in water (1 mL) and added dropwise to the stirring solution, which was heated to 50°C for 4 days. After purification by dialysis, the solvent was removed and the products dried to afford the lanthanide complexes as a series of off-white powders.

**36Gd**: 53 mg, 56%
MALDI MS \( m/z \) (H\(_2\)O) 624 {M-Gd}\(^+\), 900 {M+OTf}\(^+\), 923 {M+OTf+Na}\(^+\), 939 {M+OTf+K}\(^+\)

**36Eu**: 67 mg, 71%
MALDI MS \( m/z \) (H\(_2\)O) 629 {M+Na}\(^+\)
\( \lambda_{\text{ex}} \) = 390 nm, \( \lambda_{\text{em}} \) = 617 nm, \( \tau_{\text{H2O}} \) = 0.51 ms, \( \tau_{\text{D2O}} \) = 2.18 ms, \( q \) = 1.2,

\(^1\)H NMR (D\(_2\)O, 400 MHz) \( \delta_H \) (ppm) -25.9, -25.4, -23.4, -21.6, -18.6, -18.4, -16.7, -16.0, -15.4, -15.1, -14.5, -13.9, -12.9, -12.7, -12.2, -11.5, -11.2, -10.7, -10.3, -10.0, -9.8, -9.4, -8.4, -7.7, -7.4, -6.9, -5.7, -4.7, -4.0, -3.1, -2.5, -2.1, -1.5, -1.2, -0.9, 5.6, 6.7, 7.0, 7.4, 8.3, 8.9, 9.5, 10.1, 11.5, 12.7, 13.4, 14.6, 15.4, 18.4, 19.5, 20.0, 21.6, 22.0, 24.2

\(^{31}\)P NMR (D\(_2\)O, 161 MHz, pH10) \( \delta_P \) (ppm) 29.9, 33.8, 54.0, 56.2, 63.8, 71.1
IR (ATR, cm\(^{-1}\)) \( \nu \) 1258, 1062, 982
UV-Vis (H\(_2\)O) \( \lambda_{\text{max}} \) (\( \pi-\pi^* \)) = 195 nm
**36Tb**: 69 mg, 73 %
MALDI MS m/z (H₂O) 632 {M+Na}⁺
λₑₓ = 280 nm, λₑₘ = 545 nm, τₕ₂₀ = 1.18 ms, τ₅₂₀ = 1.87 ms, q = 1.3

¹H NMR (D₂O, 400 MHz) δ (ppm) -326.6, -287.6, -255.6, -222.7, -111.9, -95.6, -74.1, -65.5, -51.3, -45.5, -26.1, 21.9, 27.0, 30.6, 45.1, 62.8, 113.2, 117.6, 131.6, 272.4, 324.7 (only major resolved peaks outside the range -20 to 20 reported)

³¹P NMR (D₂O, 161 MHz) δ (ppm) 6.4, 16.7

**36Yb**: 80 mg, 82 %
MALDI MS (H₂O) 624 {M-Yb}⁺, 916 {M+OTf}⁺
λₑₓ = 337 nm, λₑₘ = 980 nm, τₕ₂₀ = 1.31 μs, τ₅₂₀ = 11.6 μs, q = 0.6

¹H NMR (D₂O, 400 MHz) δ (ppm) -72.9, -50.3, -31.5, -6.5, -3.1, -2.3, 19.1, 27.0, 38.7, 57.4, 74.3, 92.3 (only major resolved peaks outside the range -2 to 18 reported)

³¹P NMR (D₂O, 161 MHz) δ (ppm) 1.2, 5.8, 12.2, 15.1, 20.0, 34.0

IR (solid, cm⁻¹) ν 1251, 1054, 982
UV-Vis (H₂O) λₘₐₓ (π-π*) = 197 nm
High mesh potassium carbonate (0.084 g, 0.6 mmol) was added to a stirring solution of cyclen (0.1 g, 0.6 mmol) in acetonitrile (15 mL). 4-Picolyl chloride (0.049 g, 0.3 mmol) was added slowly to the mixture and left to stir for a further 6 h. The reaction mixture was filtered through celite and the solvent removed to leave the crude product was purified by flash chromatography eluting with chloroform/methanol/NH\textsubscript{4}OH (20:4:1). The product was isolated as a white solid (0.04 g, 49 %).

MS ES\textsuperscript{+} m/z (MeOH) 264 {M+H}\textsuperscript{+}

\textsuperscript{1}H NMR (CDCl\textsubscript{3}, 400 MHz) \( \delta \) \( H \) (ppm) 2.22&2.47&2.71&2.76 (16H, 4s, ring CH\textsubscript{2}), 7.18 (2H, d, \( ^{3}J_{HH}=5.6 \) Hz), 8.35 (2H, d, \( ^{3}J_{HH}=5.6 \) Hz)

CHN expected for C\textsubscript{14}H\textsubscript{25}N\textsubscript{5}: C, 63.84; H, 9.57; N, 26.59; found C, 62.77; H, 9.26; N, 26.26
[4,7-Bis-(diethoxy-phosphorylmethyl)-10-pyrindin-4-ylmethyl-1,4,7,10-tetraaza-cyclododec-1-ylmethyl]-phosphonic acid diethyl ester (43)

Using the general procedure outlined on page 174, the product was isolated as an orange oil (1.55 g, 89 %).

MS ES$^+$ m/z (MeOH) 714 {M+H}$^+$, 736 {M+Na}$^+$, HRMS (ES): calculated for C$_{29}$H$_{59}$O$_9$N$_5$P$_3$ (MH)$^+$, 714.3520; found 714.3519

IR (thin film, cm$^{-1}$): $\nu$ 3452, 2982, 2935, 2904, 2822, 1599, 1447, 1416, 1390, 1370, 1227 (P=O) 1163, 1099, 1049, 1026, 962

$^1$H NMR (CDCl$_3$, 400MHz) $\delta_H$ (ppm) 1.21-1.29 (18H, 2t, $^3J_{HH}$=7.2 Hz, CH$_3$), 2.51-2.92 (22H, broad m, ring CH$_2$, CH$_2$P), 3.97-4.10 (12H, 2q, $^3J_{HH}$=7.2 Hz, CH$_2$CH$_3$), 5.23 (2H, s, Ph-CH$_2$), 7.30 (2H, broad s), 8.49 (2H, broad s)

$^{13}$C NMR (CDCl$_3$, 100 MHz) $\delta_C$ (ppm) 16.41, 16.46, 16.51 (CH$_3$), 49.81, 51.35, 51.94 (ring CH$_2$), 53.72 (NCH$_2$P), 61.59, 61.65 (CH$_2$CH$_3$), 76.87 (CH$_2$Py), 129.42, 149.62 (Ar-C)

$^{31}$P NMR (CDCl$_3$, 161 MHz) $\delta_P$ (ppm) 25.55, 25.81
(4,7-Bis-phosphonomethyl-10-pyridin-4-ylmethyl-1,4,7,10tetraaza-cyclododec-1-ylmethyl)-phosphonic acid (44)

Using the general procedure outlined on page 179, the product was obtained as a yellow solid (1 g, 85%).

MALDI MS $m/z$ (H$_2$O) 545 {M}, 546 {M+H}$^+$, 568 {M+Na}$^+$

$^1$H NMR (D$_2$O, 400 MHz) δ$_H$ (ppm) 2.83-3.44 (22H, broad m, ring CH$_2$, PCH$_2$), 4.10 (2H, s, Ph-CH$_2$), 7.99 (2H, s), 8.55 (2H, s)

$^{13}$C NMR (D$_2$O, 100 MHz) δ$_C$ (ppm) 30.16, 47.97, 49.05, 49.92, 50.55 (ring CH$_2$), 51.01, 51.37, 52.26(NCH$_2$P), 56.24 (NCH$_2$Py), 128.59, 141.32 (Ar-C)

$^{31}$P NMR (D$_2$O, 161 MHz) δ$_P$ (ppm) -0.01, 2.42, 11.08, 17.99

CHN expected for C$_{17}$H$_{34}$N$_5$O$_9$P$_3$.2HCl.2.5H$_2$O: C, 30.78; H, 6.24; N, 9.80; found C, 30.60; H, 6.23; N, 10.56

IR (ATR, cm$^{-1}$) ν 3323, 2824, 2330, 1603, 1147, 1037, 895
(4,7-Bis-phosphonomethyl-10-pyridin-4-ylmethyl-1,4,7,10-tetraaza-cyclododec-1-ylmethyl)-phosphonic acid lanthanide complexes (45Ln)

To a stirring solution of the picolyl tris-acid 44 (0.1 g, 0.19 mmol) in methanol (2 mL) was added dropwise a solution of the lanthanide triflate (0.28 mmol) in methanol (2 mL). The mixture was refluxed for 2 d. The solvent was removed and the residue dissolved in the minimum volume of water for dialysis. The solvent was removed and the product dried to give the lanthanide complexes as a series of off-white solids.

45Gd: 73 mg, 55 %
MALDI MS m/z (H₂O) 704 {M+H}⁺, 724 {M+Na}⁺, 743 {M+K}⁺

45Eu: 89 mg, 68 %
MALDI MS m/z (H₂O) 699 {M+H}⁺, 721 {M+Na}⁺
λ_<sub>ex</sub>= 258 nm, λ_<sub>em</sub>= 617 nm, τ<sub>H₂O</sub> = 0.68 ms, τ<sub>D₂O</sub> = 2.06 ms, q = 0.9
UV-Vis (H₂O) λ_<sub>max</sub> (π-π*) = 191 nm, 259 nm
¹H NMR (D₂O, 400 MHz) δ<sub>H</sub> (ppm) -29.1, -23.1, -20.8, -19.1, -17.1, -13.4, -12.2, -11.3, -10.6, -8.9, -8.6, -7.7, -6.7, -5.6, -4.9, -2.6, -0.9, -0.1, 8.43, 9.9, 10.4, 10.9, 12.5, 12.9, 15.4, 15.7, 16.4, 18.3, 20.0, 27.1, 27.8, 28.8, 33.4, 37.1 (only major resolved peaks outside the range 0 to 8 reported).
IR (ATR, cm⁻¹) ν 1242, 1183, 1031,
³¹P NMR (D₂O, 161 MHz) δ<sub>P</sub> (ppm) -68.1, -26.9
**45Tb**: 102 mg, 77 %

MALDI MS (H₂O) 705 \{M+H\}⁺, 725 \{M+Na\}⁺

\(\lambda_{ex}=257\) nm, \(\lambda_{em}=545\) nm, \(\tau_{H₂O} = 1.32\) ms, \(\tau_{D₂O} = 2.23\) ms, \(q = 1.3\)

IR (ATR, cm⁻¹) ν 1242, 1182, 1030

UV-Vis (H₂O) \(\lambda_{max}\) (π-π⁺) = 259 nm

\(^1\)H NMR (D₂O, 400 MHz) \(\delta_H\) (ppm) -275.9, -228.6, -141.9, -117.0, -97.6, -90.8, -77.9, 140.9, 310.7 (only major peaks outside the range -70 to 140 reported)

**45Yb**: 77 mg, 57 %

MALDI MS (H₂O) 720 \{M+H\}⁺, 739 \{M+Na\}⁺, 759 \{M+K\}⁺

\(\lambda_{ex}=337\) nm, \(\lambda_{em}=980\) nm

IR (ATR, cm⁻¹) ν 1256, 1171, 1031

UV-Vis (H₂O) \(\lambda_{max}\) (π-π⁺) = 194 nm, 260 nm

\(^1\)H NMR (D₂O, 400 MHz) \(\delta_H\) (ppm) -23.1, -22.0, -19.0, -18.1, -14.6, -10.1, -7.9, -5.1, -3.5, -2.4, -1.2, 7.3, 8.4, 11.9, 13.4, 18.2, 20.8, 22.6, 26.6, 28.4, 30.9, 33.9, 37.8 (only major peaks outside the range -1 to 7 reported)

\(^{31}\)P NMR (D₂O, 161 MHz) \(\delta_P\) (ppm) -45.7, -42.3, -34.8, -31.0, -3.0, 1.9, 2.9, 11.1, 16.2, 24.7, 26.4, 32.6, 44.9

fac-Chlorotricarbonyl(2,2’-bipyridine)rhenium⁶ (47)

![fac-Chlorotricarbonyl(2,2’-bipyridine)rhenium](image)

**47**

Re(CO)₅Cl (1 g, 2.76 mmol) and 2,2’-bipyridine (0.45 g, 2.9 mmol) were dissolved in toluene (150 mL) and stirred at 60°C for 24 h. The bright yellow solid was isolated by filtration and washed with copious amounts of toluene to remove any unreacted starting material. The crude yellow solid was then recrystallised from hot acetonitrile (0.62 g, 48%).
IR (ATR, cm\(^{-1}\)) \(\nu_{\text{c=o}} 1870\) cm\(^{-1}\)

CHN expected for \(\text{C}_{13}\text{H}_8\text{N}_2\text{O}_3\text{Cl}\)Re: C, 33.81; H, 1.75; N, 6.07; Cl, 7.68; Re, 40.32; found C, 33.49; H, 1.68; N, 5.90; Cl, 8.23; Re, 39.69

fac-(acetonitrile)tricarbonyl(2,2'-bipyridine)rhenium(I) triflate\(^6\) (48)

\[
\begin{array}{c}
\text{N} \\
\text{N} \\
\text{Re} \\
\text{OC} \\
\text{O} \\
\text{C} \\
\text{CO} \\
\text{OTf}
\end{array}
\]

48

To a stirring solution of \(\text{Re(bpy)}(\text{CO})_3\text{Cl}\) \(47\) (0.60 g, 1.3 mmol) in acetonitrile was added silver triflate (0.34 g, 1.34 mmol) dissolved in THF (5 mL). The reaction was heated to reflux temperature overnight, then allowed to cool to room temperature. This was then filtered through celite to remove AgCl and the solvent removed under reduced pressure. The product was recrystallised from DCM/\(\text{Et}_2\text{O}\) at -18°C to yield the title compound as yellow needles (0.69 g, 93%).

IR (ATR, cm\(^{-1}\)) \(\nu_{\text{c=o}} 1928, 1908\) cm\(^{-1}\)

CHN expected for \(\text{C}_{13}\text{H}_8\text{N}_2\text{Re.CF}_3\text{SO}_3.\text{CH}_3\text{CN}\): C, 31.17; H, 1.80; N, 6.82; S, 5.20; Re, 30.20; found C, 31.04; H, 1.68; N, 6.46; S, 4.94; Re, 29.84
fac-rhenium(I)tricarbonyl-2,2'-bipyridine-(4,7-Bis-phosphonomethyl-10-
pyridin-4-ylmethyl-1,4,7,10-tetraaza-cyclododec-1-ylmethyl)-phosphonic
acid lanthanide complexes (49Ln)

The lanthanide complex was dissolved in MeOH and 48 (1.1 eq) added. This
was then heated to reflux and stirred for 72 h after which the solvent was
removed under vacuum. The residue was washed with copious amounts of
DCM to remove any unreacted rhenium starting material then dried under
vacuum to afford the product.

49Gd: 58 mg, 36 %
IR (ATR, cm\(^{-1}\)) \(\nu\) 2031, 1924, 1604, 1257, 1233, 1171, 1036

49Eu: 85 mg, 50 %
\(\lambda_{\text{ex}}\) = 253 nm, \(\lambda_{\text{em}}\) = 617 nm, \(\tau_{\text{H}_2\text{O}}\) = 0.66 ms, \(\tau_{\text{D}_2\text{O}}\) = 2.37 ms, q = 1
UV-Vis (H\(_2\)O) \(\lambda_{\text{max}}\) (\(\pi-\pi^*\)) = 253 nm

\(^1\)H NMR (D\(_2\)O, 400 MHz) \(\delta\) (ppm) -22.5, -21.6, -20.8, -18.7, -17.8, -15.9, -15.4,
-14.8, -14.1, -12.0, -10.9, -10.2, -9.8, -9.1, -7.4, -6.7, -6.3, -5.6, -4.5, -4.0, -2.0,
1.3, 2.4, 2.5, 2.7, 2.9, 3.2, 3.5, 3.6, 4.0, 5.9, 7.1, 7.4, 7.7, 8.0, 8.1, 8.4, 8.8, 9.0,
9.2, 9.7, 9.8, 11.3, 11.7, 30.4, 31.1, 35.3, 36.4

\(^{31}\)P NMR (D\(_2\)O, 161 MHz) \(\delta\) (ppm) 16.9, 17.5, 18.1, 53.0, 54.2, 63.8, 79.9
IR (ATR, cm\(^{-1}\)) \(\nu\) 2035, 1919, 1605, 1257, 1232, 1170, 1035, 988
49Tb: 68 mg, 42 %
\( \lambda_{\text{ex}} = 253 \text{ nm}, \lambda_{\text{em}} = 545 \text{ nm}, \tau_{\text{H}_2\text{O}} = 1.67 \text{ ms}, \tau_{\text{D}_2\text{O}} = 3.65 \text{ ms}, q = 1.4 \)
IR (ATR, cm\(^{-1}\)) \( \nu = 2036, 1918, 1604, 1256, 1231, 1170, 1034 \)
UV-Vis (H\(_2\)O) \( \lambda_{\text{max}} (\pi-\pi^*) = 253 \text{ nm} \)

49Yb: 98 mg, 41 %
\( \lambda_{\text{ex}} = 337 \text{ nm}, \lambda_{\text{em}} = 980 \text{ nm} \)
IR (solid) \( \nu = 2034, 1916, 1605, 1256, 1232, 1170, 1034 \)
UV-Vis (H\(_2\)O) \( \lambda_{\text{max}} (\pi-\pi^*) = 260 \text{ nm} \)
\(^1\text{H} \) NMR (D\(_2\)O, 300 MHz) \( \delta_{\text{H}} (\text{ppm}) = -45.3, -38.9, -35.1, -32.2, -24.8, -1.14, 7.5, 8.5, 13.6, 27.2, 40.0, 62.4, 66.46, 75.5 \) (only major resolved peaks outside the range -1 to 7 reported).
\(^{31}\text{P} \) NMR (D\(_2\)O, 121 MHz, pD10) \( \delta_{\text{P}} (\text{ppm}) = -58.1, -10.1, 2.73 \)

4,4'-Bis(bromomethyl)-2,2'-bipyridine\(^4\) (50)

\[
\begin{align*}
\text{4,4'-Bis(hydroxymethyl)-2,2'-bipyridine } & \text{ was dissolved in a mixture of 48}\% \\
& \text{ HBr (10 mL) and concentrated sulfuric acid (3.5 mL) then heated under reflux for 6 h. The reaction mixture was allowed to cool to room temperature and water (20 mL) was added. The pH was adjusted to neutral with a saturated sodium hydroxide solution and the resulting precipitate filtered, washed with water and air-dried. The crude product was dissolved in chloroform (20 mL), filtered then dried over sodium sulfate and evaporated to dryness to yield a beige solid (0.3 g, 38 \%).}
\end{align*}
\]
\( ^1H \) NMR (CDCl\(_3\), 400 MHz) \( \delta_H \) (ppm) 4.42 (4H, s, CH\(_2\)Br), 7.29 (2H, dd, \( ^3J_{HH}=5.2 \) Hz, \( ^4J_{HH}=4.8 \) Hz, H\(_2\)+H\(^2\)), 8.36 (2H, d, \( ^4J_{HH}=1.2 \) Hz, H\(^6\)+H\(^6\)), 8.60 (2H, dd, \( ^3J_{HH}=5.2 \) Hz, \( ^4J_{HH}=4.8 \) Hz, H\(^3\)+H\(^3\))

CHN expected for C\(_{12}\)H\(_{10}\)N\(_2\)Br\(_2\): C, 42.14; H, 2.95; N, 8.19; Br, 46.72; found C, 41.36; H, 2.65; N, 8.08; Br, 46.64

4,4'-Dicarboxy-2,2'-bipyridine\(^3\) (57)

4,4'-Dimethyl 2,2'-bipyridine (0.8 g, 4.3 mmol) was added slowly to a solution of sodium dichromate (3.14 g, 9.7 mmol) in concentrated sulfuric acid. The resulting orange slurry was stirred for 30 min at room temperature when the solution had become dark green. The reaction mixture was poured into water (10 mL) forming a yellow/green precipitate, which was isolated by filtration and dried. The solid was then dissolved in a 10% sodium hydroxide solution and acidified to pH \( \sim 2 \) with a 10% hydrochloric acid solution. The precipitate was collected by filtration and dried to give a white powder (0.79 g, 75%).

\( ^1H \) NMR (DMSO, 400 MHz) \( \delta_H \) (ppm) 7.86 (2H, d, \( ^3J_{HH}=4.8 \) Hz, H\(^2\)+H\(^2\)), 8.79 (2H, s, H\(^6\)+H\(^6\)), 8.86 (2H, d, \( ^3J_{HH}=4.8 \) Hz, H\(^3\)+H\(^3\)), 13.77 (2H, broad s, OH).

CHN expected for C\(_{12}\)H\(_8\)N\(_2\)O\(_4\).HCl: C, 51.35; H, 3.23; N, 9.98; found C, 51.12; H, 2.77; N, 9.98
4,4’-Diethoxycarbonyl-2,2′-bipyridine\(^4\) (58)

To a suspension of 4,4′-dicarboxy-2,2′-bipyridine 57 (0.79 g, 3.24 mmol) in absolute ethanol, concentrated sulfuric acid (1 mL) was added. The mixture was heated to reflux temperature for 80 h to obtain a deep pink solution. This was cooled to room temperature and water (70 mL) added. Excess ethanol was removed under reduced pressure and the pH adjusted to neutral with a saturated sodium hydroxide solution. The resulting precipitate was isolated by filtration, washed with water and dried to give a pink powder. The solid was recrystallised from the minimum volume of hot CHCl\(_3\)/MeOH to give a white crystalline solid (0.85 g, 87%).

ES MS (MeOH) m/z 301 (M+H)\(^+\), 323 (M+Na)\(^+\)

\(^1\)H NMR (CDCl\(_3\), 400 MHz) \(\delta_H\) (ppm) 1.36 (6H, t, \(^3\)J\(_{HH}\) = 7.2 Hz, CH\(_3\)), 4.38 (4H, q, \(^3\)J\(_{HH}\) = 7.2 Hz, CH\(_2\)), 7.84 (2H, dd, \(^3\)J\(_{HH}\)=4.8 Hz, J=1.2 Hz, H\(_2\)+H\(_2′\)), 8.79 (2H, d, \(^3\)J\(_{HH}\)=4.8 Hz, H\(_3\)+H\(_3′\)), 8.88 (2H, s, H\(_6\)+H\(_6′\))

4,4′-Bis(hydroxymethyl)-2,2′-bipyridine\(^4\) (59)

Sodium borohydride (2.12 g, 56.1 mmol) was added in one portion to a suspension of 4,4′-diethoxycarbonyl-2,2′-bipyridine 58 (0.8 g, 2.67 mmol) in absolute ethanol and heated to reflux temperature for 3.5 hours. The reaction mixture was cooled to room temperature and a saturated ammonium chloride solution (70 mL) added. Excess ethanol was removed under reduced pressure.
and the white solid was isolated by filtration and dried. The solid was dissolved in water and extracted with several washings of ethyl acetate. The organic layer was dried over sodium sulfate, filtered and the solvent removed under reduced pressure to leave a white solid (0.51 g, 88%).

CHN expected for C₁₂H₁₂N₂O₂; C, 66.65; H, 5.49; N, 12.85; found C, 66.34; H, 5.49; N, 12.85

4,4’-Bis-(1,4,7-tristertbutoxycarbonyl-1,4,7,10-tetraazacyclododecane)-[2,2’]bipyridinyl (51)

Di-bromomethyl bipyridine 50 (1.5 g, 4.4 mmol) was added to a stirring mixture of tri-Boc protected cyclen 38 (4.6 g, 9.67 mmol), potassium iodide (1.66 g, 10 mmol) and caesium carbonate (5.86 g, 18 mmol) in acetonitrile (150 mL). The mixture was heated to reflux for 3 days at which point the reaction had changed from a white to a dark brown colour. The reaction was allowed to cool to room temperature and then filtered through celite to remove the excess base.
After removal of the solvent under reduced pressure, the residue was washed with water and brine to give the crude product which was purified on silica gel eluting initially with neat diethyl ether, later increasing the gradient to ether/MeOH (20:1). The product was isolated as a brown solid (3.4 g, 69%).

MALDI MS m/z (MeOH) 1126 \{M+2H\}^+, 1148 \{M+Na\}^+

$^1$H NMR (CDCl$_3$, 400MHz) $\delta$ (ppm) 1.34-1.36 (54H, 2s, CH$_3$), 2.62-3.59 (32H, broad m, ring CH$_2$), 3.77 (4H, broad m, CH$_2$), 7.19 (2H, broad s), 8.22 (2H, broad s), 8.5 (2H, d, $^3$J$_{HH}$=5.2 Hz)

$^{13}$C NMR (CDCl$_3$, 100MHz) $\delta$ (ppm) 12.55, 15.65, 22.85, 22.90, 26.23, 26.47 (CH$_3$), 45.66, 47.82, 48.42, 52.44, 53.47 (ring CH$_2$), 54.30 (NCH$_2$Bpy), 74.54, 77.44 (C(CH$_3$)$_3$), 120.06, 122.75, 144.96, 146.84, 147.00 (Ar-C), 153.58, 153.79, 154.02, 157.53 (C=O)

CHN expected for C$_{58}$H$_{96}$N$_{10}$: C, 61.90; H, 8.60; N, 12.45; found C, 61.59; H, 9.09; N, 12.31

4,4'-Bis-(1,4,7,10tetraaza-cyclododec-1-ylmethyl)-[2,2']bipyridinyl (52)

Hexa-Boc compound 51 (3.04 g, 2.7 mmol) was dissolved in methanol (10 mL) and cooled to 0°C. Conc. HCl (3 mL) was added dropwise and then the reaction mixture allowed to warm to room temperature and stirred overnight. The methanol was removed under reduced pressure and the residue dissolved in a sodium hydroxide solution (1 M, 10 mL). The product was extracted into chloroform and the solvent removed to afford the product as a beige solid in quantitative yield.
MALDI MS  

\[ m/z \text{ (H}_2\text{O}) \ 525 \{\text{M+H}\}^+ \], 549 \{\text{M+Na+2H}\}^+

$^1$H NMR (D$_2$O, 400 MHz) \( \delta \text{ (ppm)} \ 2.79-3.17 \) (32H, m, ring CH$_2$), 3.88 (4H, s, CH$_2$), 7.44 (2H, d, $^3$J$_{HH}$=5.6 Hz), 7.98+8.02 (2H, 2s), 8.53+8.54 (2H, 2d, $^3$J$_{HH}$=8.4 Hz)

$^{13}$C NMR (D$_2$O, 100MHz) \( \delta \text{ (ppm)} \ 29.55, 41.44, 41.99, 44.30, 47.57 \) (ring CH$_2$), 55.71, 57.38 (NCH$_2$Bpy), 125.07, 127.80, 146.14, 147.62, 152.39 (Ar-C)

CHN expected for C$_{28}$H$_{48}$N$_{10}$.DCM: C, 57.13; H, 8.27; N, 22.97; found C, 58.49; H, 8.87; N, 21.80

(4,7-Bis-(diethoxy-phosphorylmethyl)-10-(4’-[4,7,10-tris-(diethoxy-phosphorylmethyl)-1,4,7,10-tetraaza-cyclododec-1-ylmethyl]-[2,2’]bipyridinyl-4-ylmethyl)-1,4,7,10-tetraaza-cyclododec-1-ylmethyl)-phosphinic acid diethyl ester (53)

Bis-cyclen bipyridine 52 (1.3 g, 2.48 mmol) was dissolved in anhydrous THF (50 mL) under nitrogen. To this, paraformaldehyde (1.1 g, 37 mmol) was added and the mixture stirred at room temperature for 3 h. Triethyl phosphite (6.3 mL, 37 mmol) was then added and the mixture stirred at room temperature for a further 3 days. Excess paraformaldehyde was filtered off and the solvent removed under vacuum. The crude product was left under high vacuum for several hours to remove the excess volatiles and used without further purification as it is prone to decomposition. The crude product was isolated as an orange oil (6.5 g).

MALDI MS  

\[ m/z \text{ (MeOH)} \ 1419 \{\text{M-5H}\}^+ \], 1442 \{\text{M-5H+Na}\}^+

$^{31}$P NMR (CDCl$_3$, 161 MHz) \( \delta \text{ (ppm)} \ 24.15, 25.98, 26.10 \)
{4,7-Bis-phosphonomethyl-10-[4’-(4,7,10-tris-phosphonomethyl-1,4,7,10-tetraaza-cyclododec-1-ylmethyl)-[2,2’]bipyridinyl-4-ylmethyl]-1,4,7,10-tetraaza-cyclododec-1-ylmethyl]-phosphonic acid (54)

See section on general procedure for phosphonate ester hydrolysis (page 179).

The crude product was obtained as a dark brown oil. This was dissolved in methanol and diethyl ether added to precipitate a solid, which was isolated by filtration. The solid was dissolved in a minimum amount of water and added dropwise to a large excess of acetone to precipitate a solid, which was filtered and dried to afford the product as a pink solid (1.51 g, 56%).

MALDI MS \( m/z \) (H\(_2\)O) 1086 \{M-2H\}^+

\(^1\)H NMR (D\(_2\)O, 400 MHz) \( \delta \) (ppm) 3.17, 3.32, 3.5, 3.75, 4.38, 8.08, 8.82, 8.89

CHN expected for C\(_{34}\)H\(_{66}\)N\(_{10}\)O\(_{18}\)P\(_6\): C, 34.09; H, 6.65; N, 11.69; P, 15.51; found C, 34.48; H, 6.40; N, 11.60; P, 16.40

UV-Vis (H\(_2\)O) \( \lambda_{\text{max}} \) (π-π*) = 203 nm, 239 nm, (n-π*) = 298 nm
{4,7-Bis-phosphonomethyl-10-[4’-(4,7,10-tris-phosphonomethyl-1,4,7,10-tetraaza-cyclododec-1-ylmethyl)-[2,2’]bipyridinyl-4-ylmethyl]-1,4,7,10-tetraaza-cyclododec-1-ylmethyl}-phosphonic acid lanthanide complexes (Gd, Nd, Eu, Yb)(55Ln)

To a solution of the ligand 54 in water (4 mL) was added the lanthanide triflate or chloride (1.8 eq) and the reaction heated to 80°C for 48 h. The reaction was allowed to cool to room temperature and the pH increased to ~10 using dilute sodium hydroxide solution (0.5 M). This was filtered with a micro disc syringe filter (pore size = 0.45 µm) to remove uncomplexed lanthanide as an insoluble hydroxide and then the pH readjusted to neutral. The solvent was removed under reduced pressure to afford the complexes as pink solids.

55Gd: (Ligand 65 mg, 5.97 x 10⁻⁵ mol, Gd(OTf)₃, 65 mg, 1.08 x 10⁻⁴ mol) 69 mg, 82 %
IR (ATR, cm⁻¹) ν 1257, 1170, 1032

55Eu: (Ligand 85 mg, 7.81 x 10⁻⁵ mol, Gd(OTf)₃, 84 mg, 1.41 x 10⁻⁴ mol) 82 mg, 75 %
λₑₓ = 285 nm, λₑₘ = 617 nm, τᵢ₂ₒ = 0.58 ms, τᵢ₂ₒ = 1.82 ms, q = 1
UV-Vis (H₂O) λₚₐₓ (π-π*) = 237 nm, (n-π*) = 285 nm
¹H NMR (D₂O, 400 MHz) δ (ppm) -23.3, -22.6, -17.6, -16.9, -14.9, -13.7, -11.7, -11.1, -10.2, -9.0, -8.6, -7.5, -5.6, -4.8, -0.7, -0.4, 0.7, 1.3, 1.9, 3.1, 3.6, 7.3, 7.8, 8.2, 15.3, 17.3, 20.0, 23.0, 25.4, 27.9, 28.9, 30.9, 31.6, 36.9, 37.9 (only major resolved peaks outside the range 0 to 1 reported).
$^{31}$P NMR (D$_2$O, 161 MHz) $\delta_P$ (ppm)
IR (ATR, cm$^{-1}$) v 1252, 1228, 1164, 1033, 985, 963

$^{55}$Nd: (Ligand 100 mg, 9.19 x 10$^{-5}$ mol, Gd(OTf)$_3$, 98 mg, 1.65 x 10$^{-4}$ mol) 110 mg, 87 %
$\lambda_{ex}$= 337 nm, $\lambda_{em}$= 1055 nm, $\tau_{H_2O}$ = 252 ns, $\tau_{D_2O}$ = 619 ns, q = 0
IR (ATR, cm$^{-1}$) v 1256, 1231, 1171, 1035

UV-Vis $\lambda_{max}$ ($\pi$-$\pi^*$) = 237 nm, (n-$\pi^*$) = 287 nm
$^1$H NMR (D$_2$O, 300 MHz) $\delta_H$ (ppm) -20.2, -17.4, -16.0, -13.7, -11.2, -6.5, -2.7, -1.1, 0.2, 1.3, 7.8, 8.2, 8.8, 15.1, 17.9, 19.2, 21.9, 24.2 (only major resolved peaks outside the range 1 to 7 reported).

$^{31}$P NMR (D$_2$O, 121 MHz) $\delta_P$ (ppm) -45.6, -14.9, -4.1, 1.6, 12.9, 22.2, 59.9

$^{55}$Yb: (Ligand 100 mg, 9.19 x 10$^{-5}$mol, Gd(OTf)$_3$, 103 mg, 1.65 x 10$^{-4}$mol) 126 mg, 96 %
$\lambda_{ex}$= 337 nm, $\lambda_{em}$= 980 nm, $\tau_{H_2O}$ = 1.94 $\mu$s, $\tau_{D_2O}$ = 6.96 $\mu$s, q = 0.3
IR (ATR, cm$^{-1}$) v 1257, 1172, 1033, 993

$^1$H NMR (D$_2$O, 300 MHz, pD 9) $\delta_H$ (ppm) -64.0, -49.7, -46.2, -41.6, -33.9, -32.7, -26.5, -21.8, -15.5, -7.3, -2.3, -1.54, 10.6, 13.6, 13.9, 16.3, 28.2, 40.0, 64.2, 67.9, 78.7, 94.5 (only major resolved peaks outside the range -1 to 10 reported).

$^{31}$P NMR (D$_2$O, 121 MHz, pD 10) $\delta_P$ (ppm) -63.2, -57.3, -41.1, -8.3, 0.8, 7.3, 16.6

Rhenium(I) pentacarbonyl triflate$^7$($^6$1)
Re(CO)$_5$Cl (0.5 g, 1.38 mmol) was stirred in DCM (30 mL) for 15 mins at room temperature then silver triflate added slowly to give a white mixture which was then stirred in the dark for 2 h. The reaction was filtered to remove AgCl and the volume of the filtrate reduced to $\sim$20 mL. Petroleum ether was added and the solvents removed under vacuum to afford the product as a white solid (0.45 g, 68 %).
IR (ATR, cm$^{-1}$) $\nu_{e=0}$ 1990 cm$^{-1}$, CHN expected for C$_5$O$_5$Re.CF$_3$SO$_3$: C, 15.16; S, 6.75; Re, 39.17; found C, 14.52; S, 5.68; Re, 38.55

197
Rhenium(I) pentacarbonyl pyridine (62)

61 (0.42 g, 0.88 mmol) and pyridine (0.08 g, 0.97 mmol) were dissolved in DCM (50 mL) and stirred under nitrogen for 48 h. The solvent was removed under reduced pressure to afford the crude product, which was recrystallised from DCM/Et₂O to produce a white crystalline solid (0.26 g, 73%).

IR (ATR, cm⁻¹) ν_c=O 2000 cm⁻¹
MS ES⁺ m/z 406 [M+H]⁺
CHN expected for C₁₀H₅NO₅Re·CF₃SO₃: C, 23.83; H, 0.91; N, 2.53; Re, 33.59; found C, 23.43; H, 0.89; N, 2.47; Re, 33.15

fac-rhenium(I)tricarbonyl-pyridine-{4,7-Bis-phosphonomethyl-10-[4’-(4,7,10-tris-phosphonomethyl-1,4,7,10-tetraaza-cyclododec-1-ylmethyl)-[2,2’]bipyridinyl-4-ylmethyl]-1,4,7,10-tetraaza-cyclododec-1-ylmethyl}-phosphonic acid lanthanide complexes (63Ln)

The lanthanide complex was dissolved in a H₂O/MeOH mix (2:1) and 62 (1 eq) was added. This was then heated to reflux temperature and stirred for 72 h after which the solvent was removed under vacuum. The residue was washed with copious amounts of dichloromethane to remove any unreacted rhenium containing starting material then dried under vacuum to afford the product.
63Gd: 134 mg, 72%
IR (ATR, cm\(^{-1}\)) ν 2026, 1866, 1622, 1255, 1169, 1029, 988

63Eu: 84 mg, 67%
λ\(_{ex}\) = 288 nm, λ\(_{em}\) = 617 nm, τ\(_{H_2O}\) = 0.8 ms, τ\(_{D_2O}\) = 1.55 ms, q = 0.4
UV-Vis (H\(_2\)O) λ\(_{max}\) (π-π\(^*\)) = 247 nm, (n-π\(^*\)) = 288 nm
\(^1\)H NMR (D\(_2\)O, 400 MHz) δ\(_{H}\) (ppm) -22.2, -21.1, -18.1, -16.5, -15.6, -14.4, -12.2, -11.1, -10.4, -9.4, -6.5, -5.7, -4.4, -3.9, 6.2, 7.5, 7.8, 8.5, 8.8, 9.5, 9.8, 11.7, 30.9, 35.8 (only major resolved peaks outside the range -1 to 6 reported).
\(^{31}\)P NMR (D\(_2\)O, 161 MHz) δ\(_{P}\) (ppm) 15.9, 17.2, 17.5, 18.2, 52.1, 54.7, 64.1, 80.0
IR (ATR, cm\(^{-1}\)) ν 2018, 1882, 1606, 1257, 1231, 1171, 1033

63Nd: 198 mg, 79%
λ\(_{ex}\) = 337 nm, λ\(_{em}\) = 1054 nm, τ\(_{H_2O}\) = 165 ns, τ\(_{D_2O}\) = 519 ns, q = 0.1
IR (ATR, cm\(^{-1}\)) ν 2019, 1884, 1606, 1256, 1171, 1030, 985
UV-Vis (H\(_2\)O) λ\(_{max}\) (π-π\(^*\)) = 247 nm, (n-π\(^*\)) = 287 nm
\(^3\)P NMR (D\(_2\)O, 121 MHz, pD10) δ\(_{P}\) (ppm) -58.4, -51.9, -10.0, 5.9, 15.4
\(^1\)H NMR (D\(_2\)O, 300 MHz, pD10) δ\(_{H}\) (ppm) -20.4, -15.9, -14.3, -11.8, -11.6, -5.6, -4.8, -2.5, -1.4, 8.0, 8.35, 9.5, 10.3, 10.9, 14.9, 15.3, 17.2, 19.5, 19.6, 20.5, 21.5 (only major resolved peaks outside the range -1 to 8 reported).

63Yb: 159 mg, 64%
λ\(_{ex}\) = 337 nm, λ\(_{em}\) = 980 nm, τ\(_{H_2O}\) = 0.84 μs, τ\(_{D_2O}\) = 5.94 μs
IR (ATR, cm\(^{-1}\)) ν 2026, 1883, 1607, 1257, 1170, 1031, 997
UV-Vis (H\(_2\)O) λ\(_{max}\) (π-π\(^*\)) = 247 nm, (n-π\(^*\)) = 288 nm
\(^3\)P NMR (D\(_2\)O, 121 MHz, pD11) δ\(_{P}\) (ppm) -9.1, 15.3
\(^1\)H NMR (D\(_2\)O, 300 MHz, pD 11) δ\(_{H}\) (ppm) -53.7, -46.5, -37.5, -35.8, -29.3, -21.1, -17.0, -9.0, 12.6, 28.1, 43.1, 44.7, 69.1, 72.4, 82.1 (only major resolved peaks outside the range -8 to 12 reported).
Hexamethyldisilazane (5.5 mL) was added to ammonium hypophosphite (0.87 g, 0.01 mol) under nitrogen and stirred at 110°C overnight. The resulting solution was cooled to room temperature and anhydrous DCM (10 mL) added. Then vinyl pyridine (0.51 mL, 4.8 mmol) dissolved in anhydrous DCM (4 mL) was added dropwise and left to stir at room temperature for a further 24 h. The reaction mixture was added dropwise to stirring ethanol stirring at room temperature, then the solvent was removed under reduced pressure to give an opaque orange oil. The crude oil was dissolved in chloroform and extracted with water (3 x 10 mL). The solvent was removed under reduced pressure to afford the crude product as an orange oily solid, which was used without further purification (2.3 g).

ES MS⁻ (H₂O) m/z 170 [M-H]⁻

³¹P NMR (D₂O, 161 MHz) δP (ppm) 3.2, 8.81, 30.3
Phosphinic acid 72 (2.3 g), water (15 mL) and conc. HCl (8 mL) were heated to reflux temperature for 30 min. Paraformaldehyde (0.43 g, 14 mmol) was added and the mixture was stirred at 105°C for 16 h. A further portion of paraformaldehyde (0.43 g, 14 mmol) was added and the mixture stirred at 105°C for another 3 days. After evaporation and further co-evaporation (3x) with water under reduced pressure the crude product was purified by flash chromatography eluting with DCM/MeOH/NH₄OH (10:2:1). The product was isolated as an oily brown solid (0.69 g, 72 %)

**ES MS⁻ (H₂O) m/z 200 {M-H}⁻**

**³¹P NMR δP (ppm) 39.7**

**¹H NMR (D₂O, 400 MHz) δH (ppm) 1.95 (2H, m, PCH₂CH₂), 2.90 (2H, m, PCH₂CH₂), 3.63 (2H, d, J₁H₂P = 6 Hz, PCH₂OH), 7.41 (2H, d, J₂H₂H₂ = 6.4 Hz, Ar-CHCHN), 8.42 (2H, d, J₃H₂H₂ = 6 Hz, Ar-CHCHN)**

**¹³C (D₂O, 100MHz) δC (ppm) 27.49, 27.52, 27.63 (PyCH₂), 28.51, 48.86 (CH₂P), 60.21 (d, J₁CP = 108 Hz, CH₂OH), 124.24, 148.0, 153.54, 153.69 (Ar-C)**
Trimethylsilyl diazomethane (2M in Hexane, 0.7 mL) was added dropwise to compound 73 (0.1 g, 0.5 mmol) dissolved in methanol until there was no further evolution of gas and the yellow colour persisted, then left to stir for a further 30 min. The reaction was quenched with acetic acid and the solvent removed under reduced pressure to give the crude product which was purified by flash chromatography on silica gel eluting with DCM/MeOH (3:1). The product was isolated as a colourless oil (0.02 g, 18 %).

ES MS (H$_2$O) $m/z$ 216 {M+H}$^+$, 238 {M+Na}$^+$

$^{31}$P NMR (D$_2$O, 161 MHz) $\delta$ (ppm) 39.6
7.3 References