



Terms and Conditions of Use of Digitised Theses from Trinity College Library Dublin

Copyright statement

All material supplied by Trinity College Library is protected by copyright (under the Copyright and Related Rights Act, 2000 as amended) and other relevant Intellectual Property Rights. By accessing and using a Digitised Thesis from Trinity College Library you acknowledge that all Intellectual Property Rights in any Works supplied are the sole and exclusive property of the copyright and/or other IPR holder. Specific copyright holders may not be explicitly identified. Use of materials from other sources within a thesis should not be construed as a claim over them.

A non-exclusive, non-transferable licence is hereby granted to those using or reproducing, in whole or in part, the material for valid purposes, providing the copyright owners are acknowledged using the normal conventions. Where specific permission to use material is required, this is identified and such permission must be sought from the copyright holder or agency cited.

Liability statement

By using a Digitised Thesis, I accept that Trinity College Dublin bears no legal responsibility for the accuracy, legality or comprehensiveness of materials contained within the thesis, and that Trinity College Dublin accepts no liability for indirect, consequential, or incidental, damages or losses arising from use of the thesis for whatever reason. Information located in a thesis may be subject to specific use constraints, details of which may not be explicitly described. It is the responsibility of potential and actual users to be aware of such constraints and to abide by them. By making use of material from a digitised thesis, you accept these copyright and disclaimer provisions. Where it is brought to the attention of Trinity College Library that there may be a breach of copyright or other restraint, it is the policy to withdraw or take down access to a thesis while the issue is being resolved.

Access Agreement

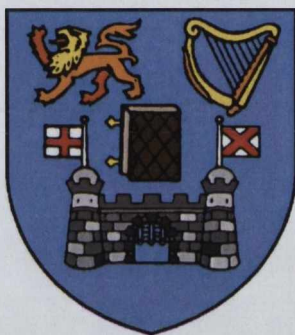
By using a Digitised Thesis from Trinity College Library you are bound by the following Terms & Conditions. Please read them carefully.

I have read and I understand the following statement: All material supplied via a Digitised Thesis from Trinity College Library is protected by copyright and other intellectual property rights, and duplication or sale of all or part of any of a thesis is not permitted, except that material may be duplicated by you for your research use or for educational purposes in electronic or print form providing the copyright owners are acknowledged using the normal conventions. You must obtain permission for any other use. Electronic or print copies may not be offered, whether for sale or otherwise to anyone. This copy has been supplied on the understanding that it is copyright material and that no quotation from the thesis may be published without proper acknowledgement.

Synthesis and Evaluation of Dinuclear Cyclen-Based Lanthanide Complexes for the Hydrolysis of Phosphodiester Bonds

Doireann Á. Moore, B.Sc. (NUI)

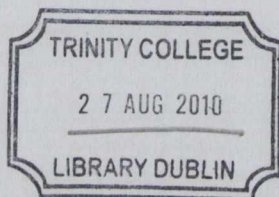
April 2010



**University of Dublin
Trinity College**

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

*A thesis submitted to the School of Chemistry,
University of Dublin, Trinity College, for the degree of
Doctor of Philosophy*



THS

8900

Declaration

This thesis is submitted for the degree of Doctor of Philosophy to the University of Dublin, Trinity College and has not been submitted before for any degree or examination to this or any other university. Other than where acknowledged, all work described herein is original and carried out by the author alone. Permission is granted so that the Library may lend or copy this thesis upon request. This permission covers only single copies made for study purposes, subject to normal conditions of acknowledgment.



For my family, and my friends.

Abstract

This thesis, entitled, “*Synthesis and Evaluation of Cyclen-based Lanthanide Complexes for the Hydrolysis of Phosphodiester Bonds*” is composed of five Chapters. In the first Chapter the structural importance of phosphodiester bonds in Nature and their cleavage by ribonucleases is introduced. The manipulation of the hydrolysis of such phosphodiester bonds using an artificial ribonuclease mimic for the development of a novel genetic therapy is outlined. The use of metal ions by ribonucleases, ribozymes, and artificial nucleases is examined and this is followed by a detailed discussion of recent advancements in the literature of metal based ribonuclease mimics, with a particular focus on the lanthanide metal ions. Finally, an overview of the work in this area by the Gunnlaugsson group is given and the work detailed in this thesis is briefly outlined.

Chapter Two gives a detailed description of the synthesis and characterisation of nine cyclen-based ligands, and their Eu(III) and La(III) complexes, including the attempted synthesis of a trinuclear complex. The dinuclear complexes were bridged by either a 2,5-dimethylpyridine, or a *para*- or *meta*-xylyl spacer moiety and all ligands were functionalised with either acetamide or alkylamine acetamide pendant donor arms. All novel compounds were fully analysed using NMR, HRMS, I.R., m.p. and elemental analysis, and the crystal structures of one ligand and two complexes are analysed.

In Chapter Three, the water soluble ligands synthesised in Chapter Two were potentiometrically titrated and their protonation constants were determined and assigned using HYPERQUAD. It was found that the pK_a values determined for the macrocyclic ring varied slightly as a function of the different pendant donor arms and the spacer moiety. It was attempted to determine the protonation constants of the pre-formed complexes using potentiometry, however, analysis of the data suggested that there were inherent problems in the titrations, such as precipitation and a possible dissociation of the complex, which prevented accurate pK_a values from being determined.

Chapter Four details an in depth kinetic analysis of the complexes synthesised in Chapter Two for the hydrolysis of the phosphodiester bond of the model RNA compound HPNP. All investigations were carried out in 100% H₂O, buffered with HEPES, at 37 °C. It was found that in general, Eu(III) complexes gave rise to higher rates of hydrolysis of the **HPNP** than their corresponding La(III) complexes. Comparison of the acetamide functionalised dinuclear Eu(III) complexes to their analogous mononuclear complexes revealed that they were most likely functioning in a cooperative manner. The potentiometric data suggested that the deprotonation of a metal bound water molecule on at

least one of the Eu(III) ions was essential for the hydrolysis of **HPNP**. Analysis of the alkylamine acetamide functionalised Eu(III) complexes, in particular the *para*-isomer, revealed both to be highly active towards the hydrolysis of **HPNP**. The *para* isomer was found to hydrolyse **HPNP** with a first order rate constant of $6.32 \times 10^{-3} \text{ s}^{-1}$ at pH 7.4, in buffered solution at 37 °C. Such a rate enhancement was the largest reported to date for a cyclen-based lanthanide complex in the physiological pH range. Furthermore, this was an 18-fold enhancement over the analogous mononuclear complex, a clear indication that the complex was functioning *via* a cooperative mechanism. Further analysis of this complex revealed it to be functioning catalytically and turnover of the complex was observed by ^{31}P NMR analysis. It was concluded that the alkylamine acetamide arms, in conjunction with the favourable structure of the complex allowed it to function catalytically, with the pendant donor arms contributing to general acid-base catalysis of the phosphodiester bond of **HPNP**. The analysis of both the acetamide and alkylamine acetamide functionalised complexes revealed that the orientation of the spacer moiety was crucial to the overall activity of the complexes, with the *para*-isomer giving rise to more efficient hydrolysis, in general. Finally, a detailed discussion of the possible mechanisms of action of these complexes for the hydrolysis of the phosphodiester **HPNP** will be presented.

All physical and synthetic procedures used in Chapters Two to Four are detailed in Chapter Five. References are given in Chapter Six, and the Appendices A.1 to A.3 detail crystallographic details, and experimental data pertaining to Chapters Two to Four.

Acknowledgements

To begin, I would like to thank my supervisor Thorri, for giving me a place in his group where I could become a researcher, and for his guidance and support over the years.

A huge thank you to Dr.'s John O'Brien, Manuel Reuther, Martin Feeny, Bernard Jean-Denis and Tom McCabe, for the many, many, NMRs, mass spec. and crystal structures. You've all gone out of your way to help me, and I am truly grateful. Thank you also to all of the departmental secretaries and the technical staff, in particular Fred Cowzer and Brendan Mulvaney, for all of their willing help over the years.

My most sincere thanks to everyone who proof read this thesis, it's truly appreciated.

To all of the members of the TG group, past and present, Andrew, Anne-Marie, Celine, Debbie, Eoin, Flo, Lin, Lisa and Sally-thanks for welcoming me into the group and showing me the ropes. To all the postdocs Julien, Aline, Cidalia, Emma, Steve and Oxana, and everyone else, here and gone, Gary, Danni, Niamh, Claire, Jen, Christophe, Sam, Brian, Rob, Laura, Elaine, Swagata and David; thanks for all of the help, the advice, cyclen-related discussions, cyclen-related woes, tarta-fletes, chocolate stashes, quick dips, scathing fashion assessments, soap opera lunchtimes, shared tea, shared wine and shared hangovers. I've loved (almost) every minute of it!

To all the members of the Rozas group who've worked with me down the back for the last two years, it's been fun-I will miss the Friday afternoon dance sessions!

To Celia "Bonnie" Bonnet, thank you so much so your patience, your guidance and your friendship over the last few years. Chapter Three might never have been without you.

Duker, what can I say....? It's been hard, but it's been fun. Thanks for being here with me for it all!

To Karen and Christina, my two Maynoothians in the big city with me, keeping me sane, one cup of tea at a time :) And to Anjuli and Rebecca who lived with me through the hardest year, thanks girls!

The biggest thank you to all my friends. You have all been so supportive and understanding for so long. I can't wait to get back out into the world with you all!

Last but not least, thank you to my family. To Aoife, for every email and every chat, you have helped more than you know. To the boys-Séamus, Conall and Paj-today has been a very good day at the races! Seamus-thank you for your patience, your humour, your support and all of those pep talks that I didn't want, but did need. And finally, thank you to my parents, Brendan and Anne-Marie for their never ending support and encouragement. I couldn't have done it without you, but I'm done now. I Promise.

Abbreviations

A	Adenine
AIDS	Acquired Immune Deficiency Virus
ApA	Adenyl-(3'-5')-adenosine phosphate
ApU	Adenyl-(3'-5')-uridine phosphate
BNPP	Bis(<i>p</i> -nitrophenyl) phosphate
BOC	<i>Tert</i> -butyloxycarbonyl
(BOC) ₂ O	Di- <i>tert</i> -butyldicarbonate
br	broad
C	Cytosine
CBz	Carboxybenzyl
CH ₂ Cl ₂	Dichloromethane
cCMP	Cytodine-3'5'-(cyclic)-monophosphate
CMV	Cytomegalovirus
Cyclen	1,4,7,10-Tetraazacyclododecane
DCM	Dichloromethane
DEP	Diethyl phosphate
DIPEA	<i>N,N</i> -Di- <i>iso</i> -propylethylamine
DMF	Dimethyl formamide
DMSO	Dimethyl sulfoxide
DNA	Deoxyribonucleic acid
DOTA	1,4,7,10-Tetraazacyclododecane- <i>N,N',N'',N'''</i> -tetraacetic acid
DOTAM	1,4,7,10-Tetrakis(carbamoylmethyl)-tetraazacyclododecane
<i>d</i>	doublet
EDDA	Ethylenediamine- <i>N,N</i> -diacetate
EDTA	Ethylenediaminetetraacetic acid
ENPP	Ethyl- <i>p</i> -nitrophenyl phosphate
ES	Enzyme-substrate
ESI	Electrospray Ionisation
ESMS	Electrospray Mass Spectrometry
Et ₃ N	Triethylamine
EtOAc	Ethyl acetate
EtOH	Ethanol
HSQC	Heteronuclear Singular Quantum Coherence
IR	Infrared

FT-IR	Fourier Transform Infrared Spectroscopy
G	Guanine
HDV	Hepatitis Delta Virus
HEDTA	Hydroxyethyl ethylenediamine triacetic acid
HPLC	High Performance Liquid Chromatography
HPNP	2-Hydroxypropyl- <i>p</i> -nitrophenyl phosphate
HRMS	High Resolution Mass Spectrometry
4-ICA	4-Imidazolecarboxylic acid
<i>i</i> -PrOH	<i>Isopropyl</i> alcohol
K2IDA	1,7-Diaza-4,10,13-trioxacyclopentadecane- <i>N,N'</i> -diacetic acid
TCMC	1,4,7,10-Tetrakis(carbamoylmethyl)-1,4,7,10-tetraazacyclododecane
MALDI-TOF	Matrix-Assisted Laser Desorption/Ionisation Time of Flight Mass Spectrometry
MeCN	Acetonitrile
MeOH	Methanol
<i>m</i>	Multiplet
MPP	Monophenyl phosphate
MRI	Magnetic Resonance Imaging
NMR	Nuclear Magnetic Resonance
NPDPP	<i>p</i> -Nitrophenyl diphenyl phosphate
<i>q</i>	Quartet
RNase A	Ribonuclease A
<i>s</i>	Singlet
T	Thymine
<i>t</i>	Triplet
TACN	Tri-aza-cyclononane
TEAP	Tetraethylammonium phosphate
TLC	Thin Layer Chromatography
TRIS	(Hydroxymethyl)aminomethane
U	Uracil
UpA	Uridyl-(3'-5')-adenosine
UpPNP	Uridine-3'-4-nitrophenyl phosphate
UpU	Uridyl-(3'-5')-uridine
UV-Vis	Ultraviolet-Visible
VT	Variable Temperature

CHAPTER 1: INTRODUCTION

1.1	Introduction	1
1.2	The Nucleic Acids and RNA	1
1.3	Phosphodiester bonds	3
1.4	Ribonucleases	6
1.4.1	Staphylococcal Nuclease	6
1.4.2	Purple Acid Phosphatase	7
1.5	Ribozymes	8
1.5.1	The Hepatitis Delta Virus Ribozyme	9
1.6	Role of Metal Ions in Phosphodiester Hydrolysis	10
1.6.1	Lewis Acid Activation (A)	10
1.6.2	Nucleophilic Activation (B)	11
1.6.3	Leaving Group Activation (C)	11
1.6.4	Double Lewis Acid Activation	11
1.6.5	Indirect Activation (D, E, F)	12
1.7	Use of RNA Model Compounds	12
1.8	Enzyme Kinetics	14
1.9	Chemical Kinetics	15
1.9.1	Zero Order Reaction	15
1.9.2	First Order Reaction	15
1.9.3	Second Order Reaction	16
1.9.4	Pseudo Order Kinetics	16
1.9.5	Half-life of a Reaction	16
1.10	Genetic Therapy	17
1.11	Design of Synthetic Ribonuclease Mimics	18
1.12	Transition Metal Based Ribonuclease Mimics	19
1.13	Properties of the Lanthanide Metal Ions	25
1.13.1	Lanthanide Metal-Based Ribonuclease Mimics	26
1.14	Advancements Within the Gunnlaugsson Group	32
1.15	Work Outlined in This Thesis	36
1.15.1	Objectives	36

CHAPTER 2: SYNTHESIS AND CHARACTERISATION

2.1	Introduction	39
2.2	Choice of Spacer Groups and Arms	40
2.3	Dinuclear Systems Incorporating Heteroatoms	41
2.3.1	Synthesis of Pyridine Spaced Bis-system	42
2.3.2	Lanthanide Ion Complexation of 39	45
2.3.3	Determination of q, the Number of Metal Bound Water Molecules	48
2.3.4	Summary	50
2.3.5	Synthesis of <i>para</i>-Cresol Spaced Bis-system	50

2.4	Introduction to Xylene Spaced Systems	55
2.4.1	Synthesis of <i>p</i> -Xylene Acetamide Bis-System	56
2.4.2	Lanthanide Ion Complexation of 41	57
2.4.3	Solid State Analysis of 41.La₂	60
2.4.4	Synthesis of <i>m</i> -Xylene Acetamide Bis-System	61
2.4.5	Lanthanide Ion Complexation of 43	65
2.4.6	Summary	67
2.5	Synthesis of Xylene Spaced Alkylamine Bis-Systems	67
2.5.1	Synthesis of Alkylamine Pendant Arm	68
2.5.2	Synthesis of <i>para</i> - and <i>meta</i> -Xylyl Spaced Alkylamine Bis-System	68
2.5.3	Lanthanide Ion Complexation of 42 and 44	70
2.5.4	Summary	72
2.6	Introduction to Trinuclear System	73
2.6.1	Synthesis of a Trinuclear System	73
2.6.2	Lanthanide Ion Complexation of 45	76
2.6.3	Summary	78
2.7	Introduction to Mononuclear Systems	78
2.7.1	Synthesis of Mononuclear Systems	78
2.7.2	Lanthanide Ion Complexation of 47	82
2.7.3	Lanthanide Ion Complexation of 48	84
2.7.4	Lanthanide Ion Complexation of 49	85
2.7.5	Solid State Analysis of 47	86
2.7.6	Solid State Analysis of 49.K	88
2.7.7	Summary	89
2.8	Overall Conclusion	89

CHAPTER 3: POTENTIOMETRIC MEASUREMENTS

3.1	Introduction	91
3.2	Determination of Protonation Constants	92
3.2.1	Determination of the Protonation Constants for the Pyridine Spaced Ligand 39	94
3.2.2	Determination of the Protonation Constants for the Xylyl Spaced Ligands 41 and 43	95
3.2.3	Determination of the Protonation Constants for the Xylyl Spaced Ligands 42 and 44	97
3.2.4	Determination of the Protonation Constants for the Ligands 47 and 48	99
3.3	Determination of Stability Constants	100
3.4	Investigation into the Protonation Constants of the Metal Bound Water Molecules of Cyclen-based Ln(III) Complexes	103
3.4.1	Investigation of the Protonation Constants of 47.Ln and 48.Ln	103

3.4.2	Investigation of the Protonation Constants of 39.Ln₂ , 41.Ln₂ , 43.Ln₂	107
3.4.3	Investigation of the Protonation Constants of 42.Ln₂ and 44.Ln₂	109
3.5	Conclusion	110

CHAPTER 4: HYDROLYSIS OF PHOSPHODIESTER BONDS

4.1	Introduction	113
4.2	Hydrolysis of Phosphodiester Bonds	113
4.2.1	Reaction Conditions for the Hydrolysis of HPNP	114
4.3	Development of an Efficient Artificial Ribonuclease Mimic	115
4.3.1	Metal Ion Size	115
4.3.2	Structure of the Pendant Arm and the Hydrophobic Cavity	116
4.3.3	Strength of Phosphate Binding	116
4.3.4	Number of Metal Bound Water Molecules	116
4.3.5	Structure of the Spacer	116
4.3.6	Design of an Optimum Complex for the Hydrolysis of HPNP	117
4.4	Kinetic Evaluation of Pyridine Spaced System, 39.Ln₂	118
4.4.1	The pH Dependence of the Hydrolysis of HPNP by 39.Ln₂	119
4.4.2	Conclusion	122
4.5	Phosphodiester Hydrolysis by Xylene-Spaced Complexes 41.Ln₂ and 43.Ln₂	122
4.5.1	Kinetic Evaluation of <i>para</i> -Xylene Spaced Acetamide Complexes	123
4.5.2	The pH Dependence of the Hydrolysis of HPNP by 41.Ln₂	125
4.5.3	Determination of a Second Order Rate Constant	127
4.5.4	Investigation of Binding of 41.Eu₂ to Phosphate	128
4.5.5	Summary	130
4.5.6	Kinetic Evaluation of <i>meta</i> -Xylene Spaced Acetamide System	130
4.5.7	The pH Dependence of the Hydrolysis of HPNP by 43.Eu₂	131
4.5.8	Conclusion	132
4.6	Evaluation of the Mechanism of Hydrolysis of HPNP by Xylene-Spaced Acetamide Systems	133
4.6.1	Conclusion	135
4.7	Phosphodiester Hydrolysis of HPNP by Xylene-Spaced Alkylamine Acetamide Complexes 42.Ln₂ and 44.Ln₂	137
4.7.1	Kinetic Evaluation of <i>para</i> -Xylene Spaced Alkylamine Acetamide System, 42.Ln₂	137
4.7.2	Kinetic Evaluation of <i>meta</i> -Xylene Spaced Alkylamine Acetamide System, 44.Ln₂	140

4.7.3	Evaluation of the Mechanism of Hydrolysis of HPNP by Xylene-Spaced Alkylamine Acetamide complex, 42.Eu₂	142
4.7.4	Investigation of Inhibition and Turnover of 42.Eu₂	143
4.7.5	Summary	145
4.8	Evaluation of Trinuclear Spaced 45.Eu₃	146
4.9	Predicting a Mechanism of Action for the Hydrolysis of HPNP	147
4.10	Conclusion	149

CHAPTER 5: EXPERIMENTAL DETAILS

5.1	General Experimental Details	151
5.2	Potentiometric Titrations	152
5.3	Experimental Data for Kinetic Measurements	153
5.4	UltraViolet-Visible Spectroscopy	154
5.5	Luminescence Measurements	154
5.6	Lifetime Determination for Ln(III) Complexes	154
5.7	General Synthetic Procedures	155
5.8	Synthesis and Characterisation of Compounds Detailed in Chapter 2	156

CHAPTER 6: REFERENCES **179**

APPENDICES

	Appendix A1	189
	Appendix A2	195
	Appendix A3	201
	Appendix A4	207

1.1 Introduction

Phosphodiester bonds are a major component of the nucleic acids, *deoxyribonucleic acid*, DNA and *ribonucleic acid*, RNA.^{1,2} The nucleic acids can be hydrolysed by ribonucleases and ribozymes, which are highly efficient enzymes within the body.³ Since the 1980's there has been a focus on the design and synthesis of a metal based complex capable of acting as a ribonuclease mimic for the purpose of artificially hydrolysing RNA, *i.e.* the synthesis of an artificial enzyme.³⁻⁷ Such an artificial enzyme could potentially prevent the production of protein within the body that would otherwise lead to the manifestation of disease.^{8,9} This chapter will introduce the concept of a ribonuclease mimic and will discuss the mechanism of the hydrolysis reaction. The use of metal ions to hydrolyse RNA will also be discussed. This will be followed by an overview of the recent literature in the area of transition metal based ribonuclease mimics and a more detailed discussion of lanthanide metal based ribonuclease mimics. Progress made in this field by the Gunnlaugsson group will also be discussed, as it forms the basis for later work in this thesis.

1.2 The Nucleic Acids and RNA

There are two different forms of nucleic acids, RNA and DNA. The genetic material that encodes for our bodies is stored in DNA.^{1,10} Both exist as polymeric chains linked together *via* phosphodiester bonds (see Section 1.3) connecting the 3' carbon of one ribose unit to the 5' carbon of the next, as shown in Figure 1.1.¹¹ This is referred to as the 'sugar-phosphate backbone' of DNA.¹⁰

As aforementioned, both DNA and RNA are polymeric chains, DNA exists as a double stranded helix (formed from two corresponding polymeric chains), with alternating large (major) and small (minor) grooves, whereas RNA exists as a single stranded chain. The other structural difference between the two polymers is the presence of a hydroxyl group in the 2' position on the ribose sugar of the RNA unit. This means that the RNA polymer is more susceptible to hydrolysis, which is the main objective of this thesis.¹²

The monomers that make up the DNA and RNA polymers are referred to as nucleotides, and are distinguished by the types of bases they contain. Those with two rings are purines and those with a single ring, pyrimidines.¹⁰ The purines include adenine (**A**) and guanine (**G**) and the pyrimidines include thymine (**T**), cytosine (**C**) and uracil (**U**). In RNA the nucleotide thymine is replaced by uracil (**U**). The structures of the bases are shown in Figure 1.2.

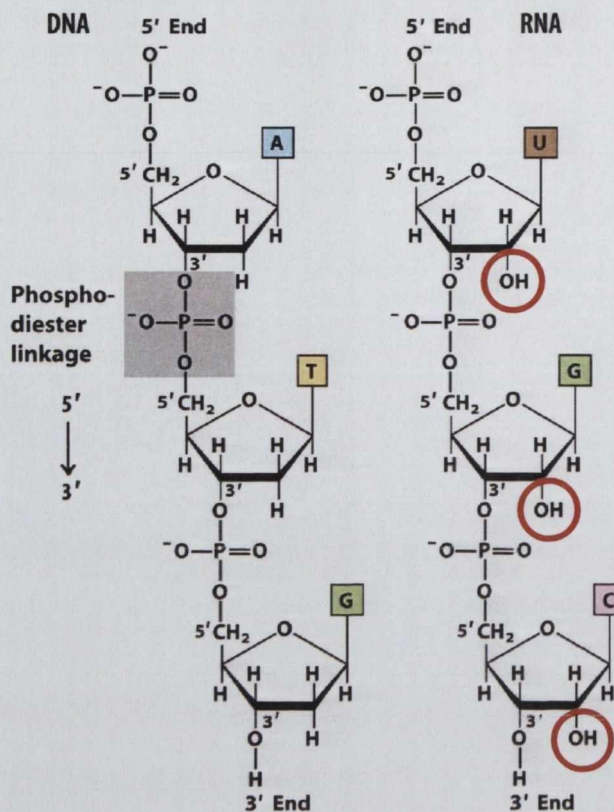


Figure 1.1: RNA and DNA. Note the presence of the 2'-hydroxy group on the ribose of RNA.¹¹

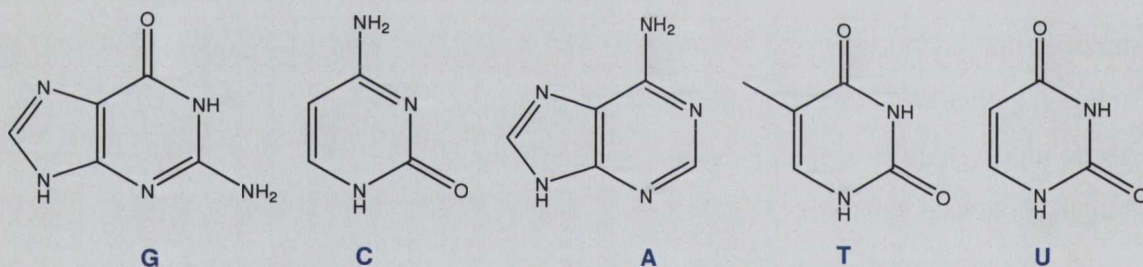


Figure 1.2: The nucleotides Guanine (G), Cytosine (C), Adenine (A), Thymine (T), and Uracil (U).

The sequence of the nucleotides is a code for the human body (of which A, G, C and T are the letters). It is in this nucleotide sequence that the genetic information is stored. The sequence of the different nucleotides encodes for different amino acids which in turn encode proteins, from which the human body is built. Proteins are synthesised from DNA in two steps; *transcription* and *translation*,¹⁰ see Figure 1.3. During transcription, the DNA is copied, and a complementary strand is synthesised, with uracil replacing thymine, as outlined above. When this new strand of base pairs is separated from the DNA, the strand becomes known as messenger RNA (mRNA) and transcription ends. During translation, the mRNA is incorporated into a structure known as a ribosome and is processed into proteins, as the sequence of the base pairs is “read” and “decoded”.

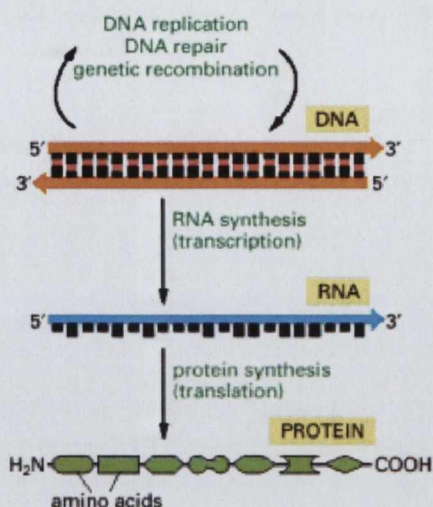


Figure 1.3: Simplified schematic of transcription and translation.¹

Knowledge of the replication process and an understanding of the genetic code, along with the completion of the mapping of the human genome, have presented the opportunity for genetic manipulation. The ability to selectively cleave mRNA allows for the control of abnormal production of harmful proteins. This has led to the development of a field of science interested in the development of novel therapeutics, capable of targeting the mRNA directly, with the aim of preventing the synthesis of proteins *via* the hydrolytic cleavage of the phosphodiester bonds of the mRNA.¹²

The structural integrity of the phosphodiester bonds, which constitutes the backbone of the nucleic acids and can facilitate their hydrolysis, is discussed in the next section.

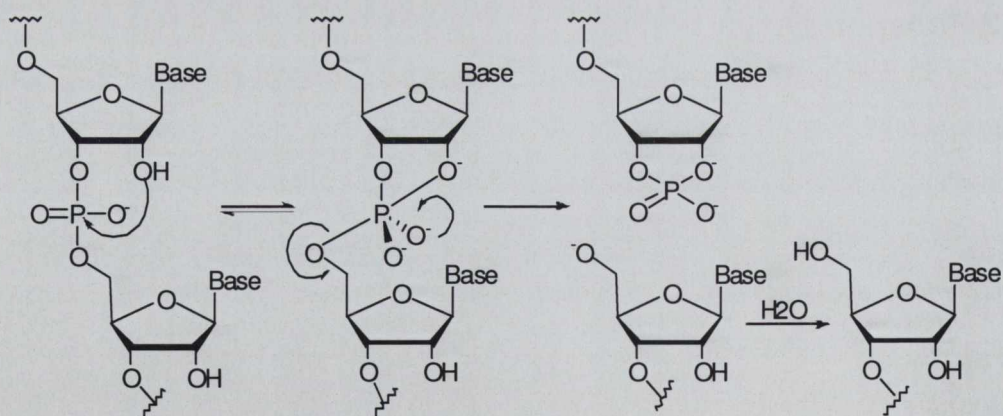
1.3 Phosphodiester bonds

In 1987, Westheimer published a paper in *Science* entitled, “*Why Nature Chose Phosphates*”.¹³ In this publication he stated the following, “*Synthetic organic chemists, however, preferentially use other groups for linking hydroxyl, carboxyl, and amino groups, and for activating them for reaction. Why were phosphates, and almost no other groups, selected by evolution for biochemical transformations?*”; which lead him to his original question, “*Why did nature choose phosphates?*”. He notes that although phosphates are extremely good leaving groups, it is more likely that chlorides, or tosylates would be used in a chemistry laboratory. Consequently, Westheimer proposed several prerequisites for the functional group that would be capable of linking the genetic code. The linker must be:

- **Trivalent**
- **Charged**
- **Negative**

He proposed that the linker must be at least divalent in order to connect one nucleotide to the next. However, studies have also shown that to retain the polymer within the cell membrane, the linkage must be ionised and hence, to facilitate this, the linker must be trivalent. Further to this, the linker must be charged, to activate it towards chemical reactions, and this charge must be negative. The linker was to be severed by hydrolysis, and so, the link must be negative to hinder nucleophilic attack, a positive charge would only serve to destabilise the genetic material. As such, nature utilised the phosphate diester, which undergoes exceedingly low rates of hydrolysis in the absence of a catalyst, and rapid hydrolysis in the presence of a catalyst. Because of this, the half life of DNA has been estimated to be approximately 200 million years, while the RNA, the information carrier, is not so stable, it's half life is estimated to be closer to 100 years. This anomaly arises from a structural difference between DNA and RNA, *i.e.* the presence of the 2'-hydroxy group on RNA, as discussed above, and thus results in a dramatically shorter half-life time.

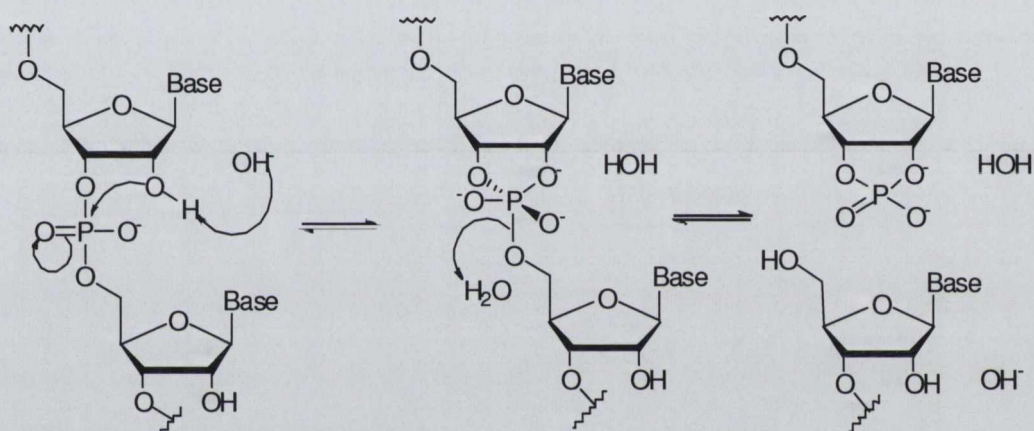
Generally it is thought that the RNA is hydrolysed in a two-step mechanism. The first step is a *cleavage-transesterification reaction*, in which the 2'-oxyanion nucleophilically attacks the phosphorous centre forming a marginally stable dianionic phosphorane intermediate (which has a pentacoordinate trigonal bipyramidal structure). The second step is a hydrolysis reaction, which results in the cleavage of the 5' P—O bond to give the 5' oxyanion leaving group and the product, a 2',3'-cyclic phosphodiester. This molecule can then undergo further hydrolysis to give either a 2' or a 3' nucleoside mono phosphate, as shown in Scheme 1.1.¹⁴



Scheme 1.1: Hydrolysis of RNA by transesterification.

The background autohydrolysis of RNA is quite slow, however, it is possible to accelerate this reaction using either acid or base catalysis, both of which proceed *via* an S_N2 mechanism, wherein the 2'-oxyanion attacks the phosphorous atom, Scheme 1.2.¹⁵⁻¹⁷ Of these, the alkaline conditions favour specific base catalysis. Under such conditions, the 2'-

hydroxyl group is deprotonated by hydroxide to form the nucleophilic 2'-oxyanion, which attacks the phosphorous atom to form the pentacoordinate intermediate and the reaction proceeds in a similar manner to that depicted in Scheme 1.1. However, under general acid-base catalysis conditions, prior or simultaneous protonation of the phosphodiester by a nucleophilic attack, along with the nucleophilic attack on the phosphate by the 2'-oxyanion results in a monoanionic phosphorane intermediate. The intermediate can then react further (by cleavage) to give the same product as shown in Scheme 1.1, or the compound can undergo pseudorotation of the phosphorous bond in the transition state, resulting in the cleavage of the 2' C—O bond, as opposed to the 5' P—O bond.¹⁸ This results in the migration of the phosphodiester from the 3' position to the 2' position, as is shown in Scheme 1.2.



Scheme 1.2: Base promoted hydrolysis of RNA.

An effective enzyme has the capability to manipulate the energy of the transition state, thus lowering the energy of the reaction. Enzymes do this by:

- **Bringing the reactants within close proximity to each other.**
- **Providing functional groups for acid-base catalysis.**
- **Inducing bond strain.**
- **Providing an attacking group.**

There are two different classes of enzymes capable of cleaving RNA. These are ribonucleases and ribozymes, which will be discussed in the next section. Both are capable of cleaving both DNA and RNA, however, this discussion will mostly focus on the hydrolysis of RNA, as the aim of this project is to prevent the production of proteins by the hydrolysis of the phosphodiester bonds of the mRNA, as discussed in Section 1.2.

1.4 Ribonucleases

Ribonucleases are enzymes which catalyse the hydrolysis of RNA by cleavage of the P—O bond attached to the 5' ribose carbon. They belong to a class of enzymes known as hydrolases.⁵ A wide variety of ribonucleases have been isolated and studied and some crystal structures of their active sites and mutated active sites have been solved.¹⁹ These systems have active sites at which the functional groups of the amino acids and/or the Lewis acidity of metal ions are used to facilitate the hydrolysis of the phosphodiester bonds.²⁰ A selection of ribonucleases that hydrolyse the ester through active site cooperation of metal ions and basic functional amino acid side chains are listed in Table 1.1.¹⁷ As an example of metal-ion mediated DNA and RNA hydrolysis by ribonucleases, staphylococcal nuclease and purple acid phosphatase are discussed in further detail in this section.

Table 1.1: *Hydrolases which utilise metal ions at their active site.*¹⁷

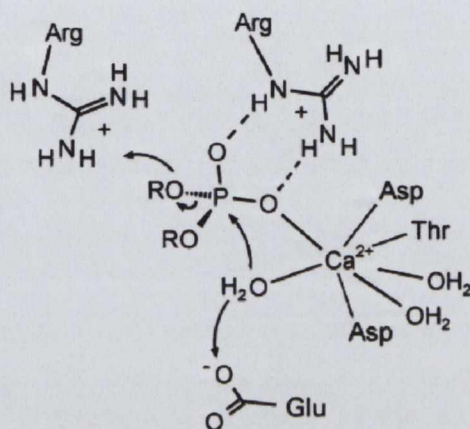
Enzyme	Metal	Functional group (role)
Staphylococcal Nuclease	Ca ²⁺	2 Arg-guanidinium (acid) Glu-carboxylate (base)
Nuclease S1	Zn ²⁺	Lys-ammonium (acid) Glu-carboxylate (nucleophile)
Urease	2 Ni ²⁺	Lys-ammonium (acid)
Alkaline Phosphatase	2 Zn ²⁺	Arg-guanidinium (acid) Ser-alcohol (nucleophile)
Purple Acid Phosphatase	Fe ³⁺ + Zn ²⁺	His-imidazolium (acid)
Protein Phosphatase	Fe ³⁺ + Zn ²⁺	His-imidazolium (acid)

1.4.1 Staphylococcal Nuclease

Staphylococcal nuclease is a digestive enzyme that is not sequence specific, *i.e.* it hydrolyses phosphodiester bonds at random. However, the enzyme catalyses the hydrolysis of phosphodiester bonds in DNA at the 5' phosphate bond to produce 3' phosphomononucleosides, giving rise to a 10¹⁶-fold acceleration of DNA hydrolysis.¹⁷

The structure of the active site has been determined by X-ray crystallography,²¹ which shows that the active site has a specific requirement for a single calcium ion. Replacement studies have shown that a wide variety of metal ions, with the exception of Sr(II), renders the ribonuclease inactive.¹⁹ Hence, the role of Ca(II) is essential to the activity of the enzyme. The mechanism is centred around the Ca(II) ion where the phosphodiester group is coordinated to the Ca(II) ion as well as being hydrogen bonded to

one of the Arg-guanidinium residues of the active site, which most likely activates the phosphodiester unit towards hydrolysis and is achieved by nucleophilic attack on the phosphorous atom, most likely by a metal bound hydroxide ion. The active site and reaction mechanism is shown in Scheme 1.3. The presence of a second Arg-guanidinium residue stabilises the resulting intermediate.



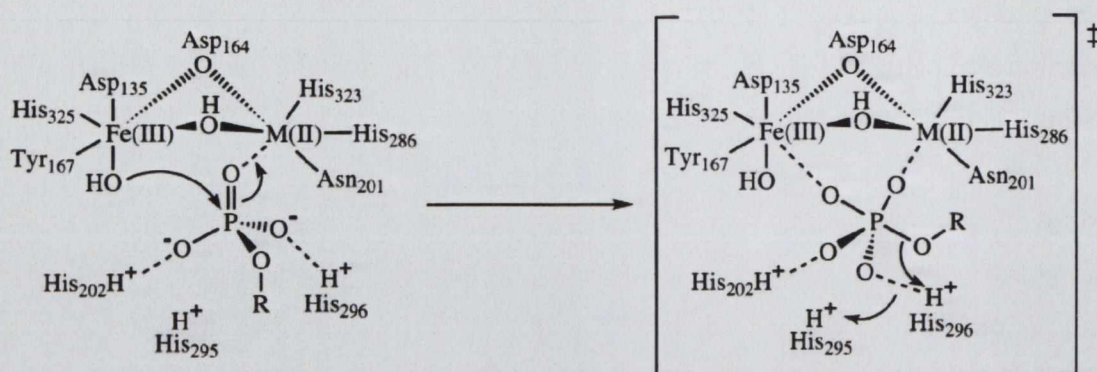
Scheme 1.3: Mechanism for the hydrolysis of a phosphodiester by Staphylococcal nuclease, promoted by Ca^{2+} .¹⁷

While staphylococcal nuclease is an efficient phosphatase, it only contains a single metal ion within its active site. However, many other enzymes have been identified for such purposes, where two metal ions, operating in a synergistic, or a cooperative manner, can carry out such hydrolysis. The next section deals with examples of such systems.

1.4.2 Purple Acid Phosphatase

The class of ribonucleases called Purple acid phosphatases are one of the best studied families of ribonucleases.^{20,22} These enzymes were originally isolated from porcine uterine fluid, however, they are now known to be present in a large variety of mammalian tissues. These enzymes catalyse the hydrolysis of phosphate monoesters, through the use of two metal ions at the active site, one which is always an Fe(III) ion, while the second is a divalent metal ion, commonly Zn(II) or Fe(II). The two metal ions are bridged by an oxygen atom from an aspartate residue and a hydroxide ion, while a single water molecule has been determined to be bound to each of the metal ions. As inferred from the name, this ribonuclease functions best at acidic pH, which is attributed to the deprotonation of an iron bound water molecule, forming the active nucleophile. During the reaction, the configuration at the phosphorous atom is inverted, suggesting that the phosphoryl group is transferred directly to water, giving credence to the idea that a metal bound hydroxide attacks the phosphate monoester to form a trigonal bi-pyramidal transition state. In contrast the divalent metal ion is thought to stabilise the transition state of the reaction, in

collaboration with basic residues such as His₂₀₂ and His₂₉₅.^{5,20} The reaction at the active site is depicted in Scheme 1.4.



Scheme 1.4: Mechanism for the hydrolysis of a phosphatemonoester by Purple Acid Phosphatase.⁵

The two enzymes discussed above employed metal ions at their active sites, however, ribonucleases are not the only enzymes capable of cleaving phosphodiester bonds. Ribozymes, which are catalytic RNA, are also capable of such hydrolysis,²³ and which can also utilise metal ions to facilitate cleavage, and these types of structures will be discussed in the next section.

1.5 Ribozymes

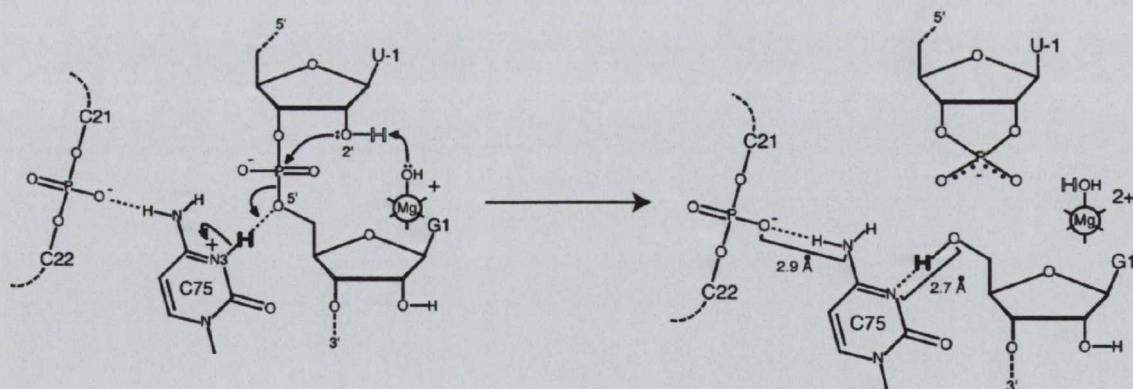
Ribozymes are strands of RNA which can catalyse the cleavage or ligation of the RNA backbone by transesterification or hydrolysis of the phosphate groups.²³ All enzymes were thought to be protein based until the 1980's when the catalytic nature of the RNA section of ribozymes was realised.²³ In 1989 Cech and Altman were jointly awarded the Nobel Prize in chemistry for their research into the activity of ribozymes.²⁴ Ribozymes were known to be composed of two parts, one protein and the other RNA in nature. Experiments which took each component alone and assessed the hydrolytic activity gave rise to the realisation that it was the RNA and not the protein that was catalytic.²⁵

Ribozymes are sequence specific, hence they only cleave RNA at specific locations which is achieved by employing base-pairing and tertiary interactions to align with the cleavage site. Several ribozymes for which the reaction mechanism is known, use divalent metal ions to promote the hydrolysis.^{14,26} These include the Hepatitis Delta Virus (HDV) ribozyme,²⁷ the hairpin ribozyme,²⁸ and the hammerhead ribozyme.²⁹ All three catalyse site-specific cleavage of RNA, using a variety of divalent metal ions including Mg(II), Mn(II) and Ca(II), to produce RNA strands with 2',3'-cyclic phosphate and 5'-hydroxyl termini.¹⁴ The HDV ribozyme will be discussed in the next section, as an example of a ribozyme which utilises metal ions to achieve phosphodiester hydrolysis.

1.5.1 The Hepatitis Delta Virus Ribozyme

The hepatitis delta virus is a small satellite RNA virus, 85 nucleotides in length, that infects Hepatitis B patients, which increases the severity of their symptoms and can lead to liver cirrhosis.³⁰ The HDV ribozyme is one of the fastest naturally occurring self-cleaving RNAs, with a first-order cleavage rate of $\sim 1 \text{ sec}^{-1}$.²⁵ This rate is approximately 100 times faster than that of other known ribozymes. While the divalent metal cation found in HDV is not necessary for the correct folding of the ribozyme, (as was proven through crystallisations in the presence of high concentrations of EDTA),²⁷ a direct correlation between the presence of the metal ions and the ribozyme activity has been established.

The cavity of the active site has a plasticity which renders it non-specific to metal ions. It functions equally well with a variety of divalent metal ions, including Mg(II), Ca(II), Mn(II), Sr(II), Ba(II) and Co(II).³¹⁻³⁴ It also functions in the presence of monovalent metal ions, such as Na(I) and K(I), however much higher concentrations are required ($\geq 4 \text{ M}$). Previous research has suggested that each metal activates the cytidine at position 75 (C75) which serves as a general base in the hydrolytic process, activating the 2'-hydroxyl nucleophile. Next, a hydrated divalent metal cation serves as a general acid, stabilizing the 5'-oxygen leaving group, as is shown in Scheme 1.5.³⁵ Mutation studies on cytidine 75, which is located close to the 5' hydroxyl leaving group, have shown that when this cytidine residue is knocked out, the ribozyme becomes inactive proving that the cytidine is essential to the activity of the ribozyme.^{27,36}



Scheme 1.5: Mechanism for the hydrolysis of RNA by the HDV ribozyme.³⁶

As discussed previously, crystal structures have revealed that many ribozymes and ribonucleases have one or more metal atoms at their active site which catalyse phosphoryl transfer reactions. By analysing their mechanisms of hydrolysis, along with those of synthetic analogues, roles for the metal ions have been established, and these will be discussed in the next section.

1.6 Role of Metal Ions in Phosphodiester Hydrolysis

Enzymes catalyse the hydrolysis of phosphodiester bonds in DNA and RNA on a time scale in the order of milliseconds and faster through the direct action of one or more metal ion centres.¹² It has been shown that the presence of metal ions can enhance the rate of hydrolysis by “activating” the phosphodiester bond,^{5,17,37} through two different modes of activation: a) by direct activation, also referred to as ‘inner-sphere’ and b) by indirect activation, also referred to as ‘outer-sphere’, depicted in Figure 1.4. Direct activation modes include **A) Lewis acid activation**, **B) Nucleophile activation** and **C) Leaving group activation**, while indirect activation modes include **D) Intramolecular general base catalysis**, **E) Intramolecular general acid catalysis** and **F) Electrostatic interactions**.

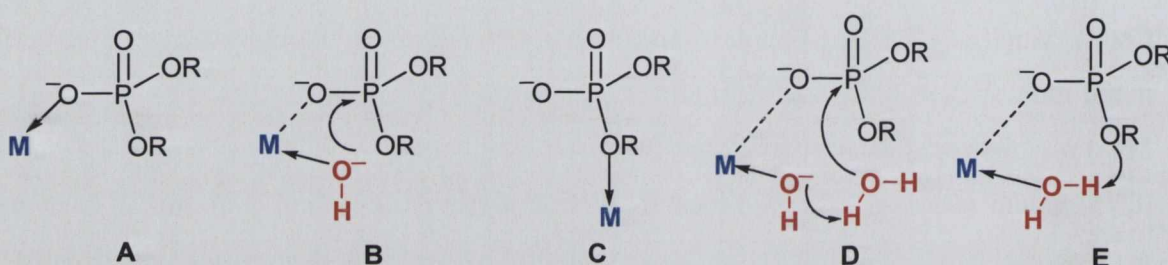


Figure 1.4: Direct (A. Lewis acid, B. nucleophile and C. Leaving Group activation) and Indirect (D. General base and E. General acid catalysis) modes of activation of phosphodiesters towards hydrolysis.^{38,39}

In order to achieve a rate of hydrolysis similar to that of naturally occurring enzymes, these modes of activation must be mimicked. The following three sections will discuss the direct modes of activation in detail.

1.6.1 Lewis Acid Activation (A)

Metal ions play an important role in Lewis acid activation, as their positive charge stabilises the phosphorous backbone of the phosphodiester allowing the 2' hydroxyl group to cleave the phosphodiester.³⁷ This occurs by the coordination of the metal ion to the phosphoryl oxygen, as depicted in Figure 1.4, (A). Such coordination leads to the electrostatic Lewis acid activation of the substrate which decreases the charge repulsion of the nucleophile. This increases the electrophilicity of the phosphorous group, resulting in intramolecular cleavage by the 2' hydroxyl group. The stronger the Lewis acid, the lower the pK_a of the metal bound water molecules, and as such, the rate of phosphodiester hydrolysis can be increased. Lewis acid activation often occurs in the presence of other mechanisms, and it can be difficult to distinguish one from the other.^{39,40}

1.6.2 Nucleophile Activation (B)

Nucleophile activation is generally achieved *via* the coordination of a hydroxide ion to a metal atom as shown in Figure 1.4, (B). Such coordination affects the pK_a of both the hydroxide and the metal, by increasing the Lewis acidity of the metal ion. The hydroxide ion can coordinate to one or more metal ions but it is more nucleophilic when coordinated to a single metal ion. However, the presence of a second metal ion can aid in the generation of a nucleophilic oxide by lowering the pK_a of the bridging hydroxide.⁴¹ The pK_a of a non-bound water molecule is 13.77 at 25 °C, in 0.1 M KCl.⁴² However, when it is bound to a metal, the pK_a is lowered due to the Lewis acid nature of the metal ion, and hence the water molecule is more easily deprotonated, forming a metal-hydroxide. Although a metal hydroxide is not as good a nucleophile as the hydroxide ion, it is a considerably better nucleophile than water. Metal-bound hydroxides have lower pK_a values than that of water. The pK_a of the metal bound water molecule varies with the metal ion, for example, a water molecule bound to a Ca(II) ion has a pK_a of 12.7, but when bound to Be(II) the pK_a of the water molecule falls to 5.7.⁴² This mechanism is usually observed for phosphodiesterases possessing good leaving groups, and it has been shown that nucleophile activation is more important for DNA hydrolysis, than for RNA hydrolysis, as the latter already possesses a very effective nucleophile, *i.e.* the terminal 2'-hydroxyl group. However, a metal activated nucleophile can also act as an intramolecular general base and hence assist in nucleophilically attacking the 2'-hydroxyl group activating it towards hydrolysis of the phosphodiester.

1.6.3 Leaving Group Activation (C)

Leaving group activation is the coordination of the oxygen of a leaving group to a metal ion, which is depicted in Figure 1.4 (C). This is not often the case, due to steric hindrances, which can make it difficult for such coordination to occur, however, it can greatly accelerate the rate of hydrolysis. The pK_a of the leaving group alcohol can greatly affect the rate of hydrolysis. Metal ion coordination will result in a change in the pK_a of the alcohol, and thus can result in a rate acceleration of up to six orders of magnitude. This mode of activation has been suggested to be partially responsible for the fast hydrolysis by alkaline phosphatase, where the Mg(II) metal ion at the active site coordinates to the leaving group oxygen.^{39,41}

1.6.4 Double Lewis Acid Activation

As mentioned in Section 1.6.1, it is often the case that more than one mode of activation can be employed to achieve enhanced rates of hydrolysis, for example the

combining of Lewis acid activation and nucleophile activation, *etc.* For the hydrolysis of phosphate diesters, Lewis acid activation alone has been shown to give a rate acceleration of up to 50-fold.⁴³ However, when combined with nucleophile activation, the rate of acceleration was significantly faster, and was increased to about 10^{10} fold over the background reaction at pH 7. This was an impressive enhancement, which clearly indicated a cooperative effect of the two modes of activation. Such a cooperative effect has also been observed by the employment of two metal ions. Double Lewis acid activation, shown in Figure 1.5, is often seen in nature where nucleases utilise two metal ions to facilitate hydrolysis.^{4,37,44} This is achieved by:

- One metal ion binding to the incoming nucleophile.
- A second metal ion binding to the leaving group.
- Both metal ions binding to the phosphate diester.

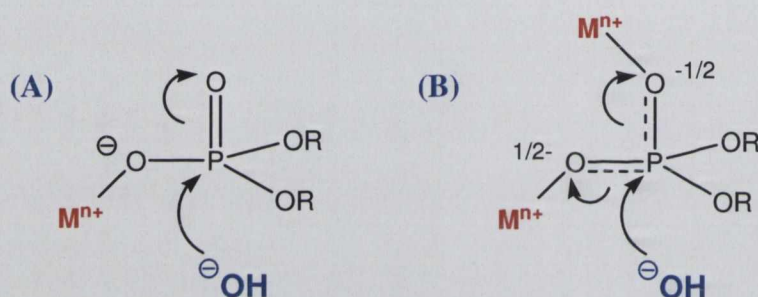


Figure 1.5: (A) Single Lewis Acid activation. (B) Double Lewis acid activation.

1.6.5 Indirect Activation (D, E, F)

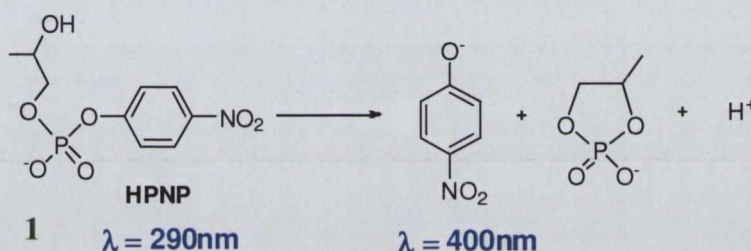
Indirect activation modes include both general acid and base catalysis as well as electrostatic interactions, as shown in Figure 1.4, (D, E). When enzymes do not use metal ions to promote hydrolysis, they will use amino acid residues as either acids or bases to achieve hydrolysis.⁴¹ Ribonuclease A (RNase A) catalyses the hydrolysis of phosphodiester bonds using two histidine residues, which act as an acid and a base and there are no metal ions involved.¹⁹ However, as the focus of this thesis is on metal based ribonuclease mimics, metal free activation modes will not be discussed any further.

This section has discussed the different modes of metal ion induced activation for phosphodiester hydrolysis. The following section will detail how the activity of potential ribonuclease mimics is measured.

1.7 Use of RNA Model Compounds

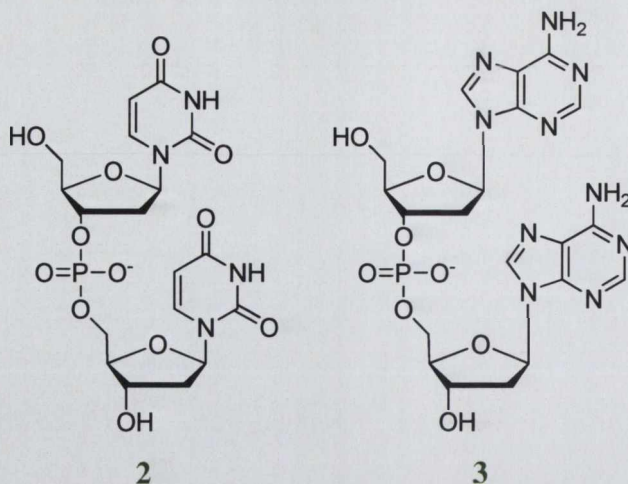
Potential ribonuclease mimics would ideally be tested on RNA to assess their hydrolytic efficiency. However, RNA is expensive, difficult to work with and it is difficult to accurately quantify the results. For these reasons, it is usual that potential compounds are initially tested on model RNA compounds, which closely resemble the structure of RNA.⁹

There are a wide variety of such model compounds. One of the most widely used is 2-hydroxy-*p*-nitrophenylphosphate (**HPNP**), **1**. It is a structurally simple phosphodiester that has a hydroxyl group similar to the 2'-hydroxyl group present in RNA. **HPNP** is a chiral, activated RNA analogue, meaning it is more reactive than RNA itself as it possesses a good leaving group, the *p*-nitrophenolate, as shown in Scheme 1.6. The major advantage of **HPNP** over other model RNA compounds is that the hydrolysis can be monitored *via* UV-Vis absorption spectroscopy, as **HPNP** absorbs at 290 nm while the leaving group, *p*-nitrophenolate, absorbs at 400 nm, Scheme 1.6. This monitoring allows for the quantification of the efficiency of the ribonuclease mimics through the determination of the rate of hydrolysis by fitting the absorption curve to first order kinetics, which will be discussed in further detail in Section 1.9.



Scheme 1.6: Hydrolysis of **HPNP** by transesterification, showing the absorption wavelengths of the starting material and the product.

Two dinucleotides which have also been used to evaluate the efficiency of potential ribonuclease mimics are uridylyl-(3'-5')-uridine **UpU**, **2**, and adenylyl-(3'-5')-adenosine phosphate (**ApA**), **3**.⁴⁵ Dinucleotides are structurally more closely related to RNA than **HPNP**, however, a drawback of using dinucleotides is that their hydrolysis can only be monitored by high pressure-liquid chromatography (HPLC). Other model compounds which can be used to evaluate phosphodiester hydrolysis lack a hydroxyl group and as such more closely resemble DNA in structure. These include bis(*p*-nitrophenyl) phosphate (**BNPP**), and ethyl-*p*-nitrophenyl phosphate (**ENPP**), amongst others.⁴⁶ In addition to a wide variety of substrates used to analyse the efficiency of ribonuclease mimics, different buffers, temperature and pH are also employed, and as such caution should be used when comparing data from different research groups for the hydrolysis of phosphodiester bonds.



This section has focused on the use of model compounds to assess the effectiveness of potential ribonuclease mimics. The following section will give a brief overview of the mathematics used to assess the speed and efficiency of such complexes.

1.8 Enzyme Kinetics

Enzyme kinetics refers to the study of reaction rates altered by the presence of a catalyst. A catalyst increases the rate of a reaction without being consumed by the reaction, *i.e.* it is regenerated.¹⁰ Almost every metabolic process in the human body is catalysed by an enzyme which enables the reaction to occur on a much smaller timescale than would be otherwise possible.¹⁰ Reactions catalysed by enzymes reach a saturation point when all the enzyme active sites are bound to a substrate, and the amount of enzyme-substrate ([ES]) complex is the same as the total amount of enzyme. As such, enzyme-catalysed reactions do not generate the same curves as can be measured by simpler chemical kinetics (which are discussed in detail in Section 1.9).

To accurately describe such reactions, Michaelis-Menten kinetics are used which were first proposed by Victor Henri in 1903.⁴⁷ However, a new interpretation was proposed in 1913 by Lenor Michaelis and Maud Menton.¹⁰ They proposed that the enzyme (catalyst) and substrate (reactant) are in fast equilibrium with their complex, which then dissociates to yield product and free enzyme. A reaction which obeys Michaelis-Menton kinetics is one in which the reaction proceeds rapidly initially, and then reaches saturation early, and displays a hyperbolic curve.⁴⁷

When hydrolysing phosphodiester bonds, ideally the reactions should obey Michaelis-Menton kinetics, similar to that observed using true enzymes, however this is not always the case. To enable the rate of the reaction to be determined, chemical kinetics are used, and these are discussed in the next section.

1.9 Chemical Kinetics^{10,47,48}

The rate of a reaction is dependant on various factors, the concentration of the reactants, temperature, pH, physical state of the reactants, and the presence or absence of a catalyst.¹⁰ The rate of change can be measured and expressed as a mathematical equation known as the rate law. There are two forms of the rate law for chemical kinetics;

- **Differential rate law:** which relates the rate of a reaction to the concentration of various species in solution.⁴⁸
- **Integrated rate law:** which is proportional to the rates of change in concentration of the reactants and products.⁴⁸

The progression of a reaction can be expressed as the ‘order’ of the reaction. The different order reactions can be expressed in different rate laws and in graphs.⁴⁸ It is common to refer to these parameters in publications detailing the development of potential ribonuclease mimics and as such they are outlined here. The next section details the different reaction orders and their rate laws commonly used.

1.9.1 Zero Order Reaction

A reaction that is zero order is one in which the rate of the reaction is independent of the concentration of the reactants. When all the reactants are used, the reaction will cease abruptly. The rate law for such a reaction is as follows:

$$r = k \quad \text{Equation 1.1}$$

Integration gives:

$$[A] = [A]_0 - kt \quad \text{Equation 1.2}$$

Hence, plotting $[A]$ vs time, t , will give a slope of $-k$.

1.9.2 First Order Reaction

A first order reaction is one in which the rate is directly proportional to the concentration of one of the reactants. The rate law is written as:

$$r = k[A] \quad \text{Equation 1.3}$$

And integrating gives:

$$[A] = [A]_0 e^{-kt} \quad \text{Equation 1.4}$$

Hence, plotting $\ln[A]$ vs t will give a slope $-k$ for a first order reaction.

1.9.3 Second Order Reaction

A second order rate constant is dependant upon the concentration of one second order reactant, or the concentration of two first order reactants (where $A = B$). The rate law is written as:

$$r = k[A]^2 \quad \text{Equation 1.5}$$

$$\text{or} \quad r = k[A][B] \quad \text{Equation 1.6}$$

The integrated rate law for $r = k[A]^2$ is written as:

$$[A] = \frac{[A]_0}{1 + kt[A]} \quad \text{Equation 1.7}$$

Hence, plotting $1/[A]$ vs t will give a positive slope, k , if the reaction is second order.

1.9.4 Pseudo Order Kinetics

Pseudo order kinetics are recorded when one reactant is in such an excess, that the concentration does not change appreciably during the course of the reaction. From this, the reaction rate dependence on the other reactant can be determined as the pseudo-order of the reaction.

1.9.5 Half life of a Reaction

The half life, $\tau_{1/2}$ of a substance is the time it takes for half of the reactant to be consumed. The rate of the reaction is proportional to the rate constant, thus the larger the rate constant, the shorter the half life. From Equation 7 the integrated rate law can be written as:

$$\ln \frac{[A]}{[A]_0} = -kt \quad \text{Equation 1.8}$$

$$\text{at } t = \tau_{1/2} \quad k\tau_{1/2} = \ln \frac{[A]}{1/2[A]_0} \quad \text{Equation 1.9}$$

$$\text{or} \quad k = \frac{\ln 2}{\tau_{1/2}} \quad \text{Equation 1.10}$$

Having detailed how phosphodiester bonds can be hydrolysed, and the use of metal ions in doing so, along with a brief discussion of the mathematics used to evaluate such complexes, the next section details the current state of such commercial products.

1.10 Genetic Therapy

As mentioned previously, the synthesis of a ribonuclease or a ribozyme mimic has great potential as a future genetic therapeutic agent. A ribonuclease mimic could specifically target mRNA for hydrolysis, preventing the translation of mRNA to proteins.^{8,37,39,49-51} As many genetic diseases arise from the presence of abnormal proteins in the body, this possible therapy is particularly appealing.^{4,39,51} Protein directed therapy has been used with some success, however, the development of drugs to target the active site on proteins is a lengthy process, and if the active site is unknown, it can be unreliable.³⁹ The completion of the mapping of the human genome has offered new targets for genetic therapy.⁵² By targeting the mRNA of a specific protein, for which the genetic code is known, the protein can be targeted in an easier manner.⁸ The development of anti-sense therapy⁵³ is instrumental to the success of this therapy, as it is more than likely through antisense genetics that a specific protein would be targeted.^{53,54}

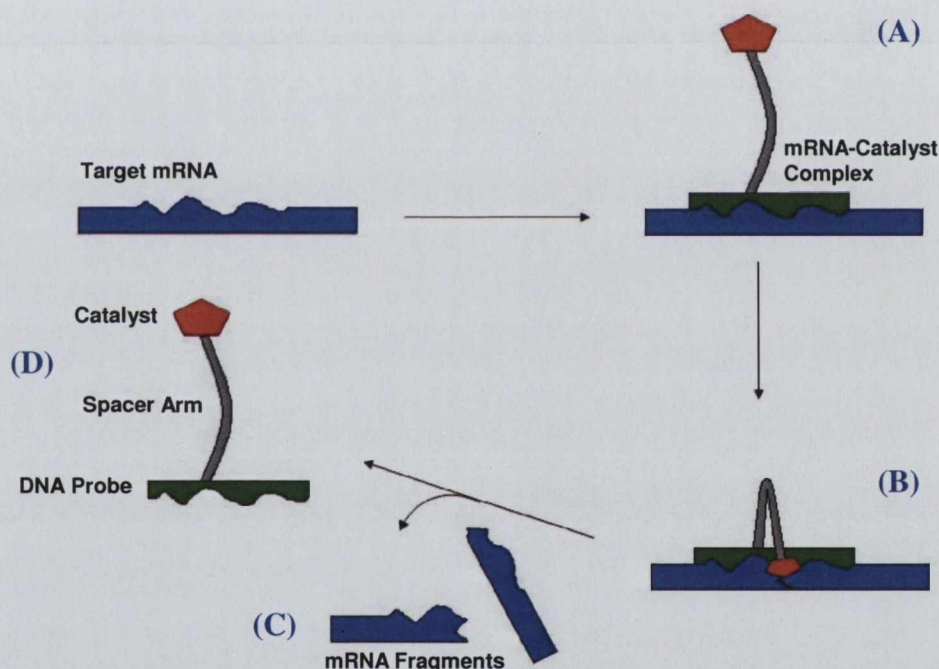


Figure 1.6: Outline of proposed catalytic cycle of a ribonuclease mimic.⁸ The first step involves the binding of the target mRNA with the catalyst (A). This is followed by the cleavage of the mRNA in a site-specific manner (B) and finally the fragments of the mRNA are released (C), and the ribonuclease mimic is ready for another catalytic cycle (D).

Synthetically this could be achieved by tethering an artificial ribonuclease with an antisense oligonucleotide, specific to the genetic sequence for the target protein. Upon formation of the antisense duplex, the artificial nuclease could hydrolyse the targeted mRNA, thus inhibiting translation to protein, as outlined in Figure 1.6,^{6,8,54-56} and discussed previously in Section 1.2. The artificial ribonuclease would then be released from the fragments of the mRNA, ready to begin a new catalytic cycle.

There is already one drug commercially available which makes use of such antisense technology to prevent the translation of mRNA. The drug is commercially known as Vitravene® and treats cytomegalovirus (CMV) in people with AIDS. It was developed by a company called ISIS® Pharmaceuticals, in conjunction with Novartis Ophthalmics. ISIS® Pharmaceuticals, in conjunction with a number of industry partners, have a range of other drugs utilising the same technology in various stages of development, including one in Phase three clinical trials and several in stage two.⁵⁷

The following section discusses the requirements for the design of a metal-based synthetic ribonuclease mimic.

1.11 Design of Synthetic Ribonuclease Mimics

The design of a compound that can efficiently catalyse biological reactions, with rate accelerations equalling those of natural enzymes, can be difficult to achieve. A fully functional ribonuclease mimic consists of two parts: the catalytic moiety and the antisense moiety.^{8,54,58} The catalytic moiety is responsible for the cleavage of the phosphodiester bond and the antisense moiety ensures that the phosphodiester bond cleavage is targeted. To date, a lot of research has primarily focused on the development of the catalytic moiety, with the intention of later tethering it to an antisense oligonucleotide to ensure specificity.

As outlined in Section 1.6, the rate of phosphodiester hydrolysis can be increased by the coordination of metal ions. Hence, the search for a synthetic ribonuclease mimic has largely been metal based.^{3,5,8,20,22,37} Although many mononuclear complexes have been developed and studied,⁵⁹⁻⁶³ there has been a large variety of multinuclear complexes synthesised.⁶²⁻⁶⁷ This is in an attempt to bring about double Lewis acid activation, as discussed in Section 1.6.4. Synthetic metal based ribonuclease mimics can be divided into two categories, **1) transition metal based complexes** and **2) lanthanide metal based complexes**.

As research into the development of an artificial ribonuclease continues, ligand design has shown itself to be increasingly important. It is not only the choice of metal ion, but the chelating ligand, that affects the rate of phosphodiester hydrolysis. Such properties include:

- **Metal ion centres.**
- **Basic co-factors.**
- **Metal bound water molecules.**
- **Hydrophobic binding pocket.**

When devising new systems as potential ribonuclease mimics, chemists take these factors into account, and ligand design is orientated to accommodate all potential co factors.⁶⁸⁻⁷⁰ The family of aza-crowns, which includes tri-aza-cyclononane (TACN) and tetraazacyclododecane (cyclen), have been widely used as the basis for the synthesis of such ligands.⁷¹⁻⁷⁵ Both are capable of encapsulating metal ions to form kinetically and thermodynamically stable complexes, and both can be functionalised with a wide variety of different chemical groups.^{76,77} As such, both structures have been investigated for their ability to hydrolyse phosphodiester bonds when coordinated to different metal ions and different co-factors.

The next section focuses on the development of metal based complexes based on transition metal ions, and will be followed by a more detailed discussion of the use of lanthanide metal ion based complexes.

1.12 Transition Metal-Based Ribonuclease Mimics

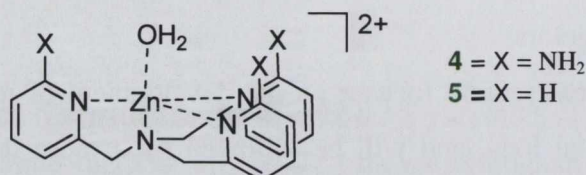
Transition metal based compounds for the hydrolysis of phosphodiester bonds have been synthesised for many years now.^{46,64,71,78,79} Transition metal ions are often used for synthetic ribonuclease mimics due to the fact that natural ribonucleases utilise them.¹⁹ The most commonly used metals are Zn(II), Co(II), Ni(II) and Cu(II). These metal ions have well established inorganic coordination chemistry properties which make them ideal candidates for use in ribonuclease mimics, including;⁸⁰

- **High natural abundance of the metals.**
- **Availability of appropriate hydration states.**
- **Fast ligand exchange rates.**
- **Redox inertness.**
- **High charge density.**

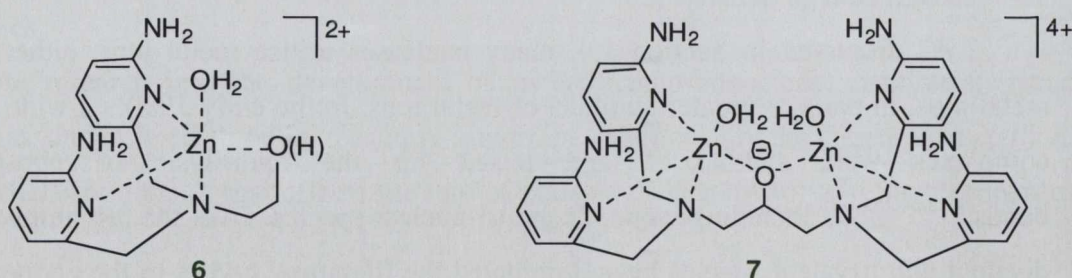
As discussed in Section 1.4, many nucleases utilise metal ions, either as single metal ions, in pairs or greater numbers of metal ions. In the early 1990's a wide variety of complexes were synthesised and tested for the hydrolysis of phosphodiester bonds,^{4,22,64,78,81-83} including mono, di and tri-nuclear species. Over the last number of years divalent and trivalent species have dominated the literature, owing to the cooperative rate enhancements achieved by using two or more metal ions.^{20,58,67,75,84-88} As aforementioned, a cooperative effect is one in which the presence of a second metal ion not only doubles the rate, but increases it manifold. The variety of metal ions being used has also changed considerably, Zn(II) is one of the main metal ions being investigated at present in the

literature, as Zn(II) has shown itself to give superior rate enhancements, as compared to other metal ions such as Ni(II) or Cu(II).

Williams and co-workers, in collaboration with the Rivas group have published a number of studies in the past few years assessing the ability of mono and dinuclear Zn(II) complexes of the pyridine based ligands, **4** and **5**.^{66,87,89,90} In 2005, a highly reactive mononuclear Zn(II) complex for phosphodiester cleavage was reported.⁸⁷ At the time, the second order rate constant of $7.9 \pm 0.1 \times 10^{-2} \text{ M}^{-1} \text{ s}^{-1}$, for $\text{X} = \text{NH}_2$, **4**, for the hydrolysis of **HPNP**, in buffer at 25 °C, was the highest known in the literature.

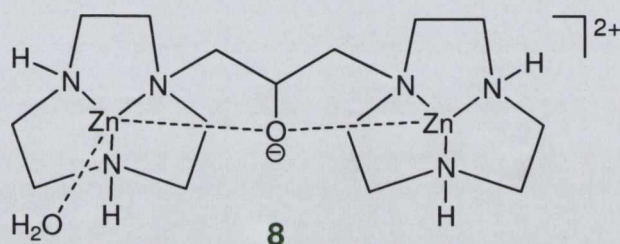


To further enhance the rate of phosphodiester hydrolysis, hydrogen bond donors were introduced to the ligand, by the addition of a hydroxyl group to the above mono system, in place of one of the pyridinyl groups, **6**.⁸⁹ This was based on research by the Rivas group which indicated that the strength of hydrogen bonding to metal-bound ligands could contribute to changes in the redox behaviour of metal centres,⁷⁰ which would in turn affect the rate of hydrolysis. A dinuclear system, based on the above mononuclear ligand, incorporating a bridging hydroxyl group was also synthesised, **7**. It was found that the presence of the hydrogen bond donor groups activated the mononuclear complex, which was more effective than the dinuclear complex, for the hydrolysis of the phosphodiester, **HPNP**. Further studies of the dinuclear complex, **7**, indicated that it was capable of hydrolyzing **HPNP** with a first order rate constant of $0.017 \pm 0.001 \text{ s}^{-1}$.⁹⁰ This remains one of the fastest known rate enhancements for a zinc complex known to date.

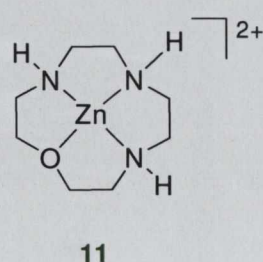
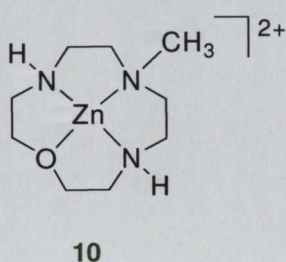
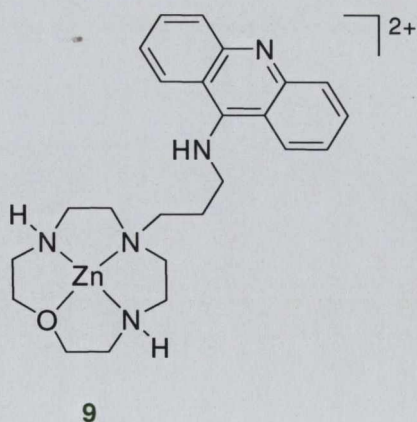


Morrow and co-workers have published several structurally different Zn(II) systems in recent times to achieve enhanced rates of phosphodiester hydrolysis.^{61,67,91} In 2006, a study of the TACN based dinuclear Zn(II) complex 1,3-bis(1,4,7-triazacyclonon-1-yl)-2-hydroxypropane, **8**, was published.⁶⁷ This complex had been previously studied in the

presence of **HPNP**, and had been found to be moderately active with a second order rate constant of $4.1 \times 10^{-3} \text{ M}^{-1} \text{ s}^{-1}$. This was attributed to the cooperative effect of the dinuclear species, as the second order rate constant was considerably higher than that of an analogous mononuclear complex. This new study analysed the dinuclear complex for its ability to hydrolysis uridine-3'-4-nitrophenyl phosphate (**UpPNP**) and **UpU**, **2**, and also to determine it's affinity for different substrates. They found a correlation between the number of uracil groups in the phosphate monoanion substrate and the binding affinity of the complex. Binding such as this has been observed previously for analogous Zn(II) complexes by Kimura, in which a Zn(II) complex was bound to the N-3 of the pyrimidine ring.⁶⁹



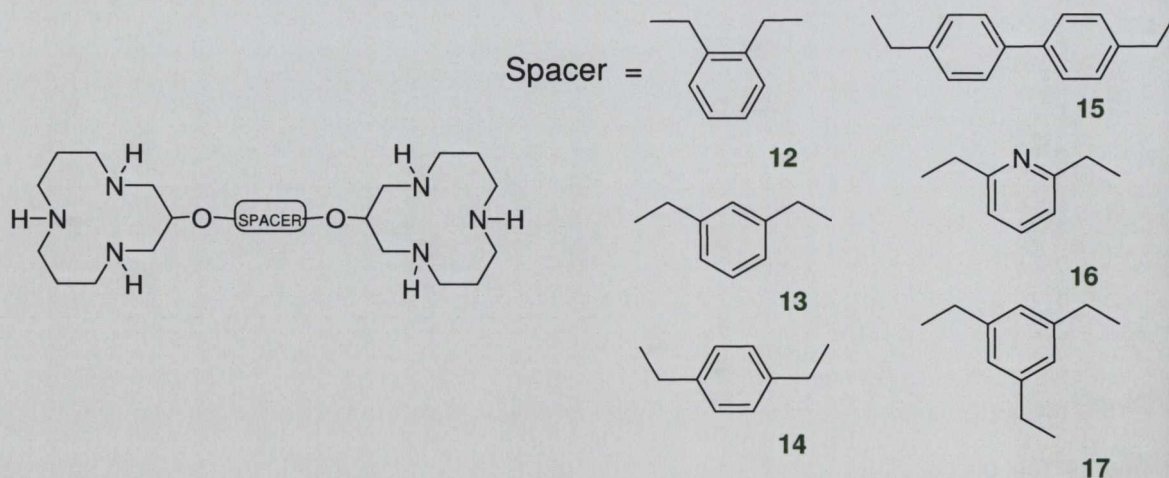
In 2007 Morrow and co-workers published a mononuclear macrocyclic Zn(II) complex, appended with either an acridine, **9**, or methyl group, **10**.⁶¹ The structure of the ligand was almost identical to that of cyclen, the polyaza crown which Morrow had used previously, however, a single nitrogen on the cyclen structure was replaced by an oxygen atom. Acridine was used as it is a known DNA intercalator. RNA intercalators, however, are mediated by bulged structures which open up the major groove enabling insertion between the base pairs.



Results showed that the catalytic properties of the two complexes were similar to that of the parent complex, **11**, on which they were modelled. All the experiments were carried out at 25 °C in buffered solution. Plotting the first order rate constants, for the cleavage of **HPNP** as a function of Zn(II) concentration, gave a linear plot indicating a first

order dependence on catalyst concentration. Compound **9**, which bears the acridine moiety, was found to hydrolyse the phosphodiester efficiently with a pseudo first order rate constant of $6.3 \times 10^{-6} \text{ s}^{-1}$. However, compound **10** was less reactive giving rise to a slightly reduced pseudo first order rate constant of $2.2 \times 10^{-6} \text{ s}^{-1}$. Similarly, the acridine bearing complex **9** gave rise to a much higher second order rate constant for the hydrolysis of **HPNP**, of $7.0 \times 10^{-3} \text{ M}^{-1} \text{ s}^{-1}$, as compared to **10**, which had a second order rate constant of $2.3 \times 10^{-3} \text{ M}^{-1} \text{ s}^{-1}$, at pH 7.0. This was a three fold increase in the rate constant, and was also higher than that of the parent complex, which has a second order rate constant of $3.3 \times 10^{-3} \text{ M}^{-1} \text{ s}^{-1}$. This enhanced rate constant was attributed to a stronger interaction of the species $\text{Zn}(\text{OH})\text{H}_2\text{O}$ with the anionic phosphorane transition state. This is possibly due to a higher positive charge on the complex, arising from protonation at the acridine amine, leading the authors to conclude that the presence of such groups served to enhance phosphodiester hydrolysis.⁶¹

Lönnerberg and co-workers have also investigated the ability of $\text{Zn}(\text{II})$ -TACN complexes for phosphodiester hydrolysis.⁹² A family of five dinuclear and one trinuclear complexes incorporating 1,5,9-triazacyclododecan-3-yloxy ligands, bridged by aromatic linkers, was recently synthesised, **12-17**.⁹² By functionalizing the macrocyclic frame at the carbon C-3, and not at a nitrogen atom, it was hoped to increase the binding of the azacrowns to nucleic acids. They were tested for their ability to effectively hydrolyse the dinucleosides **UpU**, **UpA**, **ApU** and **ApA**, using HPLC analysis, in the hope of giving a more detailed picture of the compounds' ability to hydrolyse RNA and also to determine if the compounds displayed any base selectivity for cleavage. As previously mentioned, $\text{Zn}(\text{II})$ chelates have been shown to bind tightly to the deprotonated N-3 atom of uracil and thymine bases.



Kinetic data was collected over a narrow pH range of 5.8 - 7.2, and at 90 °C. This temperature is very high as compared to other experimental conditions in the literature, which tend to monitor the hydrolysis at either 37 °C (body temperature) or at 25 °C (room temperature).^{5,93} There is also the possibility that at such a high temperature solvent could evaporate resulting in a reduction in the total volume of the experimental solution, causing a potential increase in the solution concentration. It was found that all complexes exhibited rates of hydrolysis two orders of magnitude greater than that observed for a parent mononuclear Zn(II)-TACN complex, when tested against **ApU** and **UpA**. No such acceleration was observed for **UpU** and **ApA**.

The trinuclear complex, **17**, showed accelerated rates of cleavage for all uracil containing substrates, however, cleavage of **ApA** remained low. The *ortho* linked complex, **12**, gave rise to the largest rate enhancements out of all the dinuclear complexes tested for all substrates, with the exception of **UpU**, in which case the *para* linked complex, **14**, gave rise to marginally faster rates of hydrolysis. Under the reaction conditions for this experiment, the mononuclear Zn(II) [12]aneN₃ hydrolysed **UpU** with a first order rate constant of $2.6 \times 10^{-5} \text{ s}^{-1}$. Only the observed rate constant recorded for the trinuclear ligand surpassed this effectively, giving rise to a rate constant of $1.58 \times 10^{-3} \text{ s}^{-1}$. Poor cleavage rates by the dinuclear complexes, and the mononuclear complex, as compared to the trinuclear complex was hypothesised to arise from the Zn(II)-aquo ion, which binds the catalytically active metal ion only to the scissile monoanionic phosphodiester linkage.

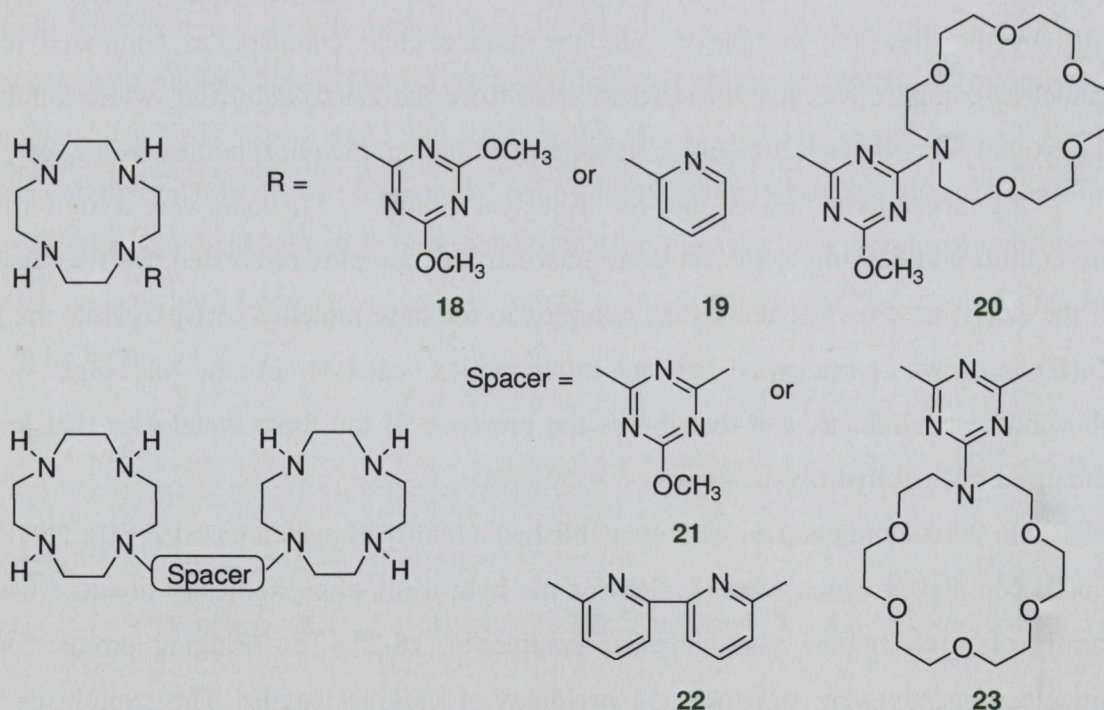
Lönnberg hypothesised that the trinuclear complex, **17**, underwent a high-affinity pre-equilibrium binding to the substrate in which the complex orientated itself so that two of the Zn(II)-azacrowns chelated the complex to the base moieties of **UpU** while the third Zn(II)-azacrown participated as an intracomplex catalyst in the cleavage of the phosphodiester linkage, and that it was the presence of the three metal ions that lead to enhanced rates of hydrolysis.⁹²

In 2008 König and co-workers published a family of cyclen based Zn(II), Ni(II) and Cu(II) complexes which were studied for the hydrolysis phosphodiester bonds,⁷² using a family of mixed mono- and dinuclear complexes, **18-23**. The bridging groups for the dinuclear ligands were triazines and pyridines of various lengths. The complexes were tested against bis-nitrophenyl phosphate (**BNPP**), a model compound more similar to DNA than RNA in structure, as discussed in Section 1.8.

The Cu(II) and Ni(II) complexes were found to be essentially inactive, having no significant effect on the rate of hydrolysis, however, the same was not true for the Zn(II) complexes. Rate constants obtained for the Zn(II) complexes were compared to those

obtained for a parent $\text{Zn}([12]\text{aneN}4)$ complex, and it was found that all the mononuclear complexes demonstrated higher rates of hydrolysis.⁷² Compound **18**, which incorporated a triazine based moiety, resulted in a rate increase of up to six-fold on the parent complex. However, overall the rate constants still fell in the same range as that of the parent complex, $10^{-5} \text{ M}^{-1} \text{ s}^{-1}$. The di- and trinuclear complexes all gave rise to enhanced rates of hydrolysis, as compared to the mononuclear complexes. They also displayed a dependence on spacer length, **22**, the complex with the largest spacer, was found to be the slowest of the dinuclear complexes tested, while the complexes possessing the smaller spacers gave rise to rate constants that were one to two orders of magnitude greater than those observed for **22**.

Potentiometric titrations of the trinuclear species determined that the monohydroxy species, $\text{Zn}_2\text{-L-(OH}_2\text{)(OH}^-)$ was the catalytically active species between the pH window of 7-8. It was also shown that **21**, the triazine spaced complex, behaved in a similar manner. However, complex **22**, which gave rise to the slowest rate of phosphodiester hydrolysis of all the multi-nuclear species, had both the mono and dihydroxy species present at pH 7 to 9 and this was most likely responsible for the slower rate of hydrolysis, due to the dihydroxy species inhibiting the hydrolysis.



In summary, over the last number of years there has been a variety of transition metal based complexes synthesised for the hydrolysis of phosphodiester bonds based on the azacrowns TACN and cyclen in particular. However, the majority of work has focused on the use of Zn(II) as the metal ion of choice, as previous publications have repeatedly

demonstrated that it is the most efficient transition metal ion for hydrolysis. Research has also shown, that in general di- and trinuclear complexes give rise to enhanced rates of hydrolysis as compared to analogous mononuclear complexes, as a result of the cooperative action of the metal ions.³⁷ However, the rate enhancement is also found to be dependant upon the spacer length and rigidity between the two coordinating ligands.

In addition to these reports based on transition metal ions, the use of lanthanide complexes for phosphodiester hydrolysis has been effective in the past and the next section will detail the properties of the lanthanide ions which make them suitable for phosphodiester hydrolysis, and will detail examples of such complexes from the recent literature.

1.13 Properties of the Lanthanide Metal Ions

Lanthanide ions have several physical properties which make them the ideal metal candidates for artificial ribonucleases.⁷⁶ The lanthanide metal ions:

- **Are hard Lewis acids.**
- **Have high charge densities.**
- **Have no accessible redox chemistry.**
- **Can adopt flexible geometries.**

The hard Lewis acid nature of the lanthanides is similar to that of Ca(II), Mg(II) and Zn(II),⁷⁷ and hence these can activate the P—O bond of the phosphodiester for cleavage, and can also activate the water nucleophile, as discussed in Section 1.6. The Lewis acidity of a metal ion is correlated to the metal's ionization potential. This can lower the pK_a of a metal bound water nucleophile resulting in a mechanism that enables the 'fine-tuning' of the pK_a values of the complex.⁴²

Despite their many properties which make lanthanide metal ions very useful in the design of potential drugs, they do have a few disadvantages.⁷⁷ Primarily, they are toxic to the human body. Free lanthanide ions can displace Ca(II) ions at the active sites of proteins, rendering them inactive. As such they must be complexed by a ligand to form both kinetically and thermodynamically stable complexes, which will prevent the dissociation of the complex and the dispersion of free lanthanide ions within the human body. When complexed as such, the lanthanide ions are safe for *in vivo* use. One such complex which has been commercialised is the cyclen based gadolinium complex, DOTarem®, an MRI contrast agent.^{94,95}

Due to the high coordination numbers of the lanthanide ions, of between eight and ten, the encapsulating ligand must have sufficient coordination sites to complex the metal

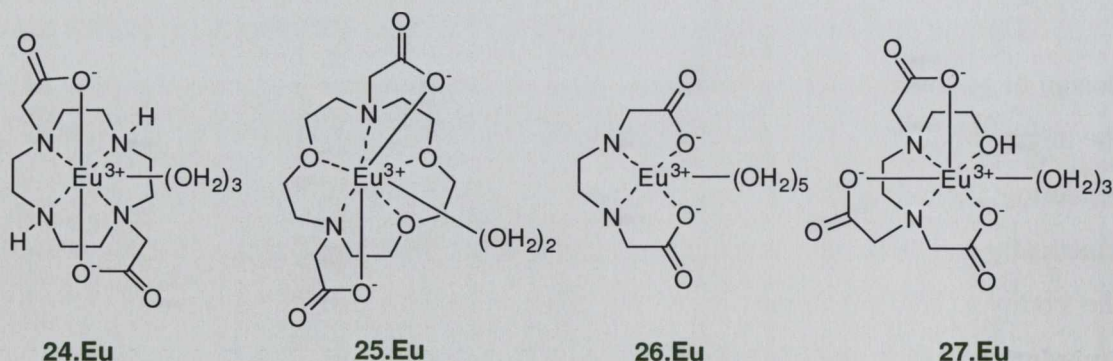
ion.⁷⁶ The synthesis of a ligand which can satisfy the high coordination environment of the lanthanide ion is not trivial. The ligand must coordinate to the lanthanide ion, rendering the complex stable, however, must not bind it too tightly, as this has been shown to inhibit the hydrolytic activity of the metal.⁵⁹

1.13.1 Lanthanide Metal-Based Ribonuclease Mimics

Although lanthanide ions were been reported to hydrolyse phosphate bonds rapidly,^{96,97} it was not until a study by Breslow and co-workers, in 1991,⁵⁰ which highlighted the hydrolytic ability of lanthanide ions, as compared to transition metal ions, that the idea of incorporating lanthanide ions into artificial ribonucleases came about.

In the early 1990's several groups had moderate success applying lanthanide metal ions to phosphodiester hydrolysis.^{59,98,99} This work has been reviewed in detail in the theses of Dr.'s Mulready,¹⁰⁰ Harte¹⁰¹ and Fanning,¹⁰² within the Gunnlaugsson group. As such, after a brief introduction to lanthanide mediated phosphodiester hydrolysis, this section will only focus on developments within the field since 2006.

A study by Chang and co-workers in 2005 investigated the effects of the variable factors of macrocyclic lanthanide complexes on phosphodiester hydrolysis.¹⁰³ Such variables included pH, metal ionic radii, number of coordinated water molecules, charge and concentration. The ligands investigated were chosen to vary the coordination environment of the lanthanide ions and included the cyclen based ligands, DO2A (six-coordinate), **24**, K21DA (seven coordinate), **25**, and the acyclic ligands, EDDA (four coordinate), **26**, and HEDTA (six coordinate), **27**. The lanthanide metal ions investigated were Yb(III), Eu(III) and La(III) and the phosphodiester substrate used was the DNA analogue **BNPP**.



Plots of the observed first order rate constants, k_{obs} , vs pH showed that as pH increases, so too did the rate of phosphodiester hydrolysis for all of the complexes. The largest rate enhancement was observed for the complex based on $\text{Eu}(\mathbf{24})^+$ and the curve displayed a jump in the rate of hydrolysis at pH 8.1 as the complex was converted to the

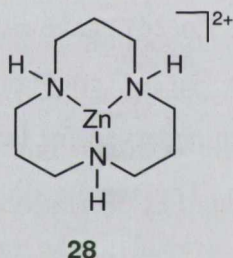
reactive hydroxo species, $\text{Eu}(\mathbf{24})(\text{H}_2\text{O})_2(\text{OH})$. The rate constants derived from hydrolysis by $\text{Eu}(\mathbf{24})^+$ were about 1.4-10 times greater than those of $\text{La}(\mathbf{24})^+$ in the pH range of 6.8-10.6 and this was attributed to the greater charge density of the Eu(III) complex. It had been expected that the Yb(III) complex would form a better nucleophile than the other, larger lanthanide complexes. However, when complexed to **24**, the hydrolysis rates of the Yb(III) complex were found to be the lowest. A similar trend was observed by Gunnlaugsson *et al.* when studying a variety of metal ions complexed to a ligand.⁶³ This may be as a result of fewer coordinated inner sphere water molecules, or possibly as a result of inactive dimer formation. The idea that a larger number of inner-sphere water molecules can lead to the promotion of phosphodiester hydrolysis was originally proposed by Morrow *et al.*⁵⁹ Results showed that $\text{La}(\text{TCMC})^{3+}$, which included two inner-sphere coordinated water molecules, promoted the rapid cleavage of RNA oligomers, however, similar complexes bearing only one inner-sphere coordinated water molecule did not.

The Eu(III) complexes for all of the ligands were analysed and at low pH the complexes showed a trend, where an increased rate of hydrolysis could be correlated to the increasing number of inner-sphere coordinated water molecules, hence: $\text{Eu}(\mathbf{26})^+ > \text{Eu}(\mathbf{24})^+ \gg \text{Eu}(\mathbf{25})^+$. At higher pH ranges, $\text{Eu}(\mathbf{24})^+$ gave rise to rate constants 1300 times greater than those of $\text{Eu}(\mathbf{25})^+$. The complex $\text{Eu}(\mathbf{26})^+$ could not be compared as it precipitated out of solution. Such precipitation is often observed for lanthanide complexes at high pH and as such this result is not uncommon. The results do strongly indicate that the number of coordinated water molecules was crucial to the rate of phosphodiester hydrolysis. The overall charge of the complex was investigated by comparison of the rate constants of the complexes $\text{Eu}(\mathbf{24})^+$ and $\text{Eu}(\mathbf{27})$, both of which have six donor ligands and three coordinated water molecules, however, $\text{Eu}(\mathbf{24})^+$ has a positive charge and $\text{Eu}(\mathbf{27})$ has a net charge of zero. $\text{Eu}(\mathbf{24})^+$ gives rise to faster rates of **BNPP** hydrolysis, this is possibly due to the positive charge of the complex effectively decreasing the negative charge of the phosphodiester, whereas the neutral $\text{Eu}(\mathbf{27})$ was not capable of this, and as such was not as active. To summarise this investigation, a high charge density, a high number of inner sphere coordinated water molecules and an overall positive charge on the complex were confirmed as ideal properties for lanthanide metal ion based complexes for accelerated rates of phosphodiester hydrolysis.

Significant effects on the rate of phosphodiester hydrolysis have also been attributed to the role of solvent effects, on which several studies have been published recently.¹⁰⁴⁻¹⁰⁶ Several Zn(II) complexes which were studied, and their hydrolysis rates determined in aqueous solution, have also been studied by Brown and co-workers in less

polar solvents such as MeOH and EtOH,¹⁰⁷ with the addition of methoxide ions, an active base. This alone has the potential to accelerate the hydrolysis of the phosphodiester bond.

It has been proposed in the literature that the cavity at the active site of proteins and ribonucleases has a lower water content, and thus a lower dielectric constant, than that outside of the cavity.^{108,109} As such, Brown hypothesised that testing potential ribonuclease mimics in a fully aqueous environment did not afford a true semblance to that of natural ribonucleases. Upon testing **28** in less polar solvents, extraordinary increases in the rate of hydrolysis were observed. When tested in MeOH, in the presence of methoxide ions by Brown and co-workers, the second order rate constant for the methanolysis of **HPNP** by **28** was found to be $18.9 \text{ M}^{-1} \text{ s}^{-1}$, which is a 1000-fold increase over the same reaction in buffered aqueous solution. The increase in the rate constants was attributed to a dramatic shift in the dielectric constant, D_r , of the active site, from 78 in H_2O to 31.5 in MeOH. This increases the potential energy of the attraction for oppositely charged ions by a factor of 2.5, thus increasing the binding of the complex to the phosphodiester substrate. It is postulated that the overall rate has the potential to be faster still, however, is limited by a prior process, possibly a change in the mode of the complexes' interaction with **HPNP**.



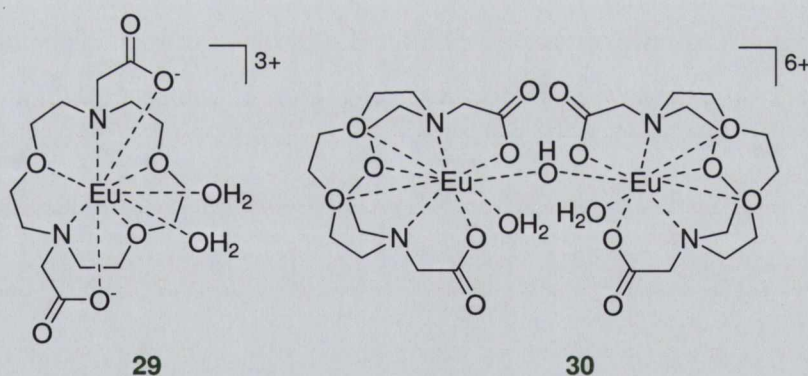
Yatsimirsky and co-workers studied the effect of solvent on lanthanide mediated phosphodiester hydrolysis.^{104,110} Previous studies had found that the hydrolytic activity of alkaline and alkaline earth metal ions was strongly increased in solvent systems containing greater than 80% v/v DMSO, and as such the study was extended to include the lanthanide metal ions.

The hydrolysis of **HPNP** and **BNPP** in 80% v/v DMSO was analysed and it was found that the rate of hydrolysis by lanthanide metals did indeed increase, giving a 10^2 fold increase on the rate of hydrolysis in buffered aqueous solution. In the DMSO solution, the lanthanide metal ions predominantly formed mononuclear complexes of the form $\text{M}(\text{OH})_n^{3-n}$ and a single dinuclear complex of the form $\text{M}_2(\text{OH})^{5+}$, where $\text{M} = \text{La(III)}, \text{Eu(III)}, \text{Nd(III)}$ and $n = 1, 2$ or 3 . Also studied was the effect of the addition of different concentrations of NaClO_4 to the hydrolysis reaction. The rate of hydrolysis decreased as salt concentration increased. A similar inhibitory effect was observed with the use of TRIS

buffer.¹¹⁰ These studies not only highlighted the difference that the solvent environment can induce in the rate of phosphodiester hydrolysis, but they also suggest that caution be used when comparing rates of hydrolysis of different complexes, in different conditions.

Morrow *et al.* has recently published a wide variety of lanthanide complexes for phosphodiester hydrolysis.¹¹¹⁻¹¹⁵ However, only a few of these examples have achieved rates similar to that observed for a dinuclear Zn(II) complex, **8**, which was published in 2003, which gave rise to a second order rate constant of $2.5 \times 10^{-3} \text{ M}^{-1} \text{ s}^{-1}$ for the hydrolysis of **HPNP**.⁹¹ Lanthanides ideally should hydrolyse phosphodiesteres more efficiently than Zn(II), as they are known to interact with the dianionic transition state more efficiently, leading to greater Lewis acid stabilization.^{50,58} However, to date this has not been achieved. Previous lanthanide complexes from the Morrow group were based on the polyazacrown, cyclen. It was hoped that by utilizing a ligand of greater structural integrity, but similar to the structure of cyclen, that hydrolysis would be promoted to a greater extent.¹¹⁵

Potentiometric titrations showed that the monomeric species, **29**, was formed at low pH, between 4 and 6. At higher pH, dimerisation proceeds by the ionization of a metal bound water molecule of $[\text{Eu}(\text{L})(\text{OH}_2)_2]^+$ (where L = ligand) to form $[\text{Eu}(\text{L})(\text{OH})(\text{OH}_2)]$, **30**, which combined with a second molecule of the species to form the dimer.

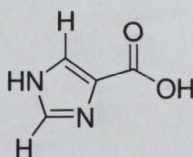


Kinetic studies show that the dimer **30** was the most active species in solution and that the activity of this system increased with concentration, and also with increasing pH. Over the pH series investigated, there was a 300-fold increase in the rate of hydrolysis going from pH 6.7 to pH 9.0. The apparent second order rate constants displayed a similar trend, for both the monomer **29** and the dimer **30**. The plot of the first order rate constant against pH curves upwards from $\sim$ pH 8, where the dimeric species begins to accumulate in solution.

To deduce some mechanistic information, third order rate constants were also calculated and from this, binding energies were derived. As compared to a previous monomeric zinc system, studied by the Morrow group, there was only a slight difference in

the stabilization of the transition state for specific-base-catalysed cleavage of **HPNP** by the monomeric Eu(III) system in question, **29**. A much larger difference was expected considering that Eu(III) systems tend to hydrolyse **HPNP** to a greater extent than Zn(II) systems. It was suggested that the reason for such poor stabilization was that the ligand itself interacted too strongly with the Eu(III) ion, reducing its ability to interact with the **HPNP**. The dimer did not, however, offer any great stabilization either. It was suggested that the reason for this was that the two Eu(III) ions were not ideally positioned to give rise to cooperative catalysis, and that perhaps the bridging alkoxide ion resulted in too large a degree of flexibility to the system, and hence the two Eu(III) ions were inaccessible to the phosphodiester substrate simultaneously. It was also noted that the ligand was septa coordinate, and that when the dimer was formed, the bridging alkoxide left only one unfilled coordination site free for interaction with the substrate and that this may have severely hampered its activity. Morrow *et al.* also concluded that although lanthanides were ideally much better metals for phosphodiester hydrolysis than current Zn(II) complexes, appropriate ligand synthesis remains a real challenge for the synthetic chemist.¹¹⁵

In the 1990's lanthanide hydroxo complexes were shown to effectively cleave phosphodiester bonds by Chin,⁹⁹ Komiyama,⁹⁸ Williams,⁴¹ and Hamilton.¹¹⁶ However, several problems were encountered, including inactivity at low and physiological pH and precipitation from solution. Yatsimirsky investigated the use of lanthanide hydroxo complexes. However, 4-imidazolecarboxylic acid (4-ICA), **31**, a stabilizing anion, was added to the solution with the aim of achieving hydrolysis within the physiological pH range, and preventing the precipitation of the hydroxo complexes from solution.¹¹⁷

**31**

The complexes were of the form $ML(OH)^+$ or $M_2L_n(OH)_{6-n}$, where $M = La(III)$, $n = 2$; $M = Pr(III)$, $n = 2, 3$; $M = Nd(III), Eu(III), Dy(III)$, $n = 1-3$ and potentiometric titrations revealed that the different lanthanide ions formed a variety of species in solution, of which the dominant species were $ML(OH)^+$, $M_2L_3(OH)_3$, $M_2L_2(OH)_4$ and $M_2L(OH)_5$. It was also observed that there was an increase in the stability of the hydroxo complexes going across the lanthanide series, from La(III) to Dy(III) which correlated with a decrease in the rate of **BNPP** hydrolysis.

Kinetic studies were carried out on all lanthanide complexes using three different phosphodiester substrates, **BNPP**, **HPNP** and **NPDPP** (for structures see Section 1.7). The first order rate constants for all three substrates, hydrolysed by different metal-hydroxo complexes are shown in Table 1.2. In the case of hydrolysis by free hydroxide, **HPNP** was 10^4 times more reactive than **BNPP**, however, **NPDPP** was more reactive than **HPNP**. The results for hydrolysis by the lanthanide metal-hydroxo species showed that the order of reactivity was entirely reversed.

The authors suggested that the reason behind this change in reactivity could be attributed to the complex-substrate interaction. They hypothesised that the terminal hydroxide of **HPNP** may actually interfere in substrate binding, which negated the need for the participation of the metal-bound hydroxide unlike **BNPP** hydrolysis, in which participation by the metal bound hydroxide was essential. The addition of inhibitory substrates, such as monophenyl phosphate (**MPP**) induced precipitation and diethyl phosphate (**DEP**) gave rise to strong inhibitory effects. It was proposed that the most likely mode of hydrolysis of **HPNP** was driven by the substitution of a bridging alkoxide group with the **NPDPP** alkoxide group, thus the bridging alkoxide was deprotonated and activated toward hydrolysis of the phosphodiester. These results demonstrated progress from previous lanthanide hydroxo complexes for phosphodiester hydrolysis, and warrant further investigation.

Table 1.2: Pseudo-first order rates of hydrolysis by lanthanide hydroxides recorded for different substrates ($M^{-1} s^{-1}$).¹¹⁷

Active Species	HPNP	BNPP	NPDPP
$La_2L_2(OH)_4$	41 ± 4	4.0 ± 0.2	0.76 ± 0.07
$Pr_2L_2(OH)_4$	n/a	2.19 ± 0.08	n/a
$Nd_2L_2(OH)_5$	4.9 ± 0.2	1.49 ± 0.06	0.27 ± 0.01
$Eu_2L_2(OH)_5$	n/a	0.84 ± 0.02	n/a
$Dy_2L_2(OH)_5$	3.7 ± 0.2	0.69 ± 0.02	0.45 ± 0.06

The hydrolysis of **BNPP** by lanthanide ions alone and in the presence of α -amino acids (aa) was studied by Kremer and co-workers.¹¹⁸ The amino acids stabilised lanthanide hydroxide species in solution, in a similar manner to the use of **ICA** by Yatsimirsky *et al.* It was found that the lanthanide metal ions combined with the amino acids to form the active monomeric cationic species, $[Ln(aa)_3(OH)]^{2+}$ or $[Ln(aa)_3(OH)_2]^+$. Hydrolysis of

phosphodiester bonds occurred above pH 7 and displayed Michaelis-Menten type saturation kinetics. Again, as found by Yatsimirsky *et al.*,¹¹⁷ at high pH lanthanide hydroxides precipitated out of solution.

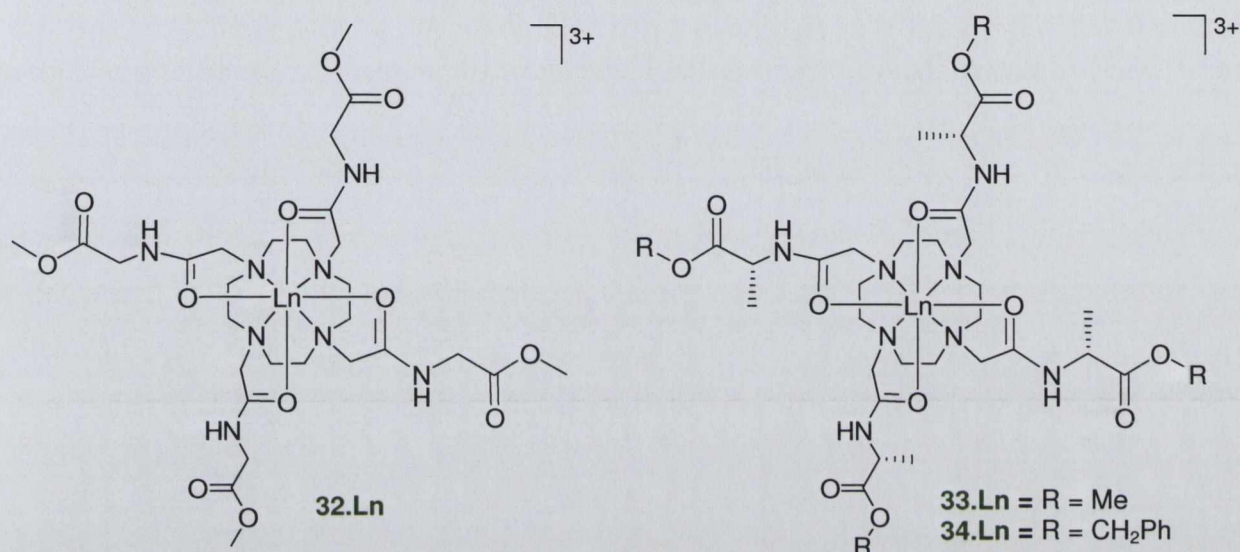
In summary, although the lanthanide metal ions have great potential as ribonuclease mimics, designing a ligand which will form a stable complex at physiological pH, and give rise to large rate enhancements in an aqueous environment remains a synthetic challenge. A general trend can be observed, in which dinuclear complexes tend to give rise to greater rates of hydrolysis than their analogous mononuclear complexes. The Gunnlaugsson group has published a large body of work on lanthanide based complexes which have shown very good rates of hydrolysis, and have progressed towards fine-tuning the ideal structure of the ligand to encapsulate the lanthanide ion. The next section will detail some of the group's results to date.

1.14 Advancements Within the Gunnlaugsson Group

The Gunnlaugsson laboratory has been developing compounds with the goal of phosphodiester hydrolysis over the last decade. The majority of the work has been based around the use of lanthanide ions, using cyclen as a scaffold for the ligand. It forms highly stable metal complexes and can be easily functionalised. Alkylation of the cyclen ring system affords complexes with a wide variety of chemical properties. The Gunnlaugsson group has synthesised and investigated a wide variety of functionalised cyclen ligands for the hydrolysis of phosphodiester, with considerable success.^{62,119-122} The Gunnlaugsson group has also utilised cyclen for the synthesis of lanthanide complexes with a variety of potential applications including anion sensing,^{123,124} supramolecular assemblies,^{125,126} and hydrogel development.¹²⁷ However, for the purpose of this introduction the focus will be on its use as a phosphodiester hydrolase alone. The expertise within the lab in the synthesis of such lanthanide-based cyclen complexes was applied to the synthesis of a family of complexes for the hydrolysis of phosphodiester bonds. Initially a wide variety of complexes with different coordinating arms were synthesised. As previously mentioned, the pendant arms could act as co-factors, similar to amino acids in the body, and enhance the rate of phosphodiester hydrolysis.

The first generation of complexes developed incorporated the amino acids, glycine, **32.Ln**,¹¹⁹ and a glycine-alanine dipeptide, **33.Ln** and **34.Ln**,^{63,120} into cyclen complexes. This resulted in a considerable enhancement in the rate of phosphodiester hydrolysis, as compared to the 'uncatalysed' reaction, and was significant in comparison to other results at the time.

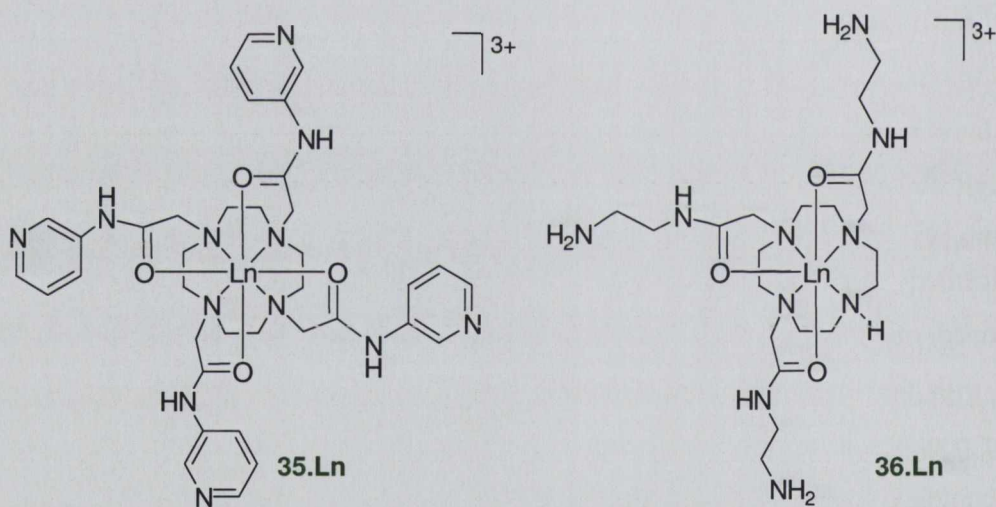
The glycine based ligand was complexed to a large number of lanthanide metal ions, such as Eu(III), La(III), Nd(III), Gd(III) and Yb(III) among others. The La(III) and Eu(III) complexes were the most efficient giving rate constants k_{obs} of 1.14×10^{-4} and $4.14 \times 10^{-5} \text{ s}^{-1}$ respectively. A trend was observed in which the larger lanthanide ions were more potent than smaller ones. This was attributed to the presence of a second metal bound water molecule on the larger lanthanide ions.



The optimum pH for hydrolysis for all complexes was found to be quite alkaline, pH 8.5 for La(III), at which k_{obs} increased to 0.807 h^{-1} , bringing the relative rate enhancement to 6725 over the background reaction. Two chiral GlyAla analogues were developed, one in which the arms terminated with a methoxy group, the other with a benzyl ester, with the intention of investigating the effect of extending the arms by creating a deeper pocket and a more hydrophobic environment around the metal centre. The benzyl ester complex was slower to hydrolyse **HPNP**, which was attributed to the binding pocket being too deep, and as such, preventing the metal from adequately interacting with the substrate. Neither complex gave any indication of chiral discrimination.

A family of complexes with various pyridine based arms were synthesised and investigated.⁶² The chloroacetamide-pyridine arm was synthesised with the pyridine nitrogen in the 2-, 3- and 4-position. Investigation of the hydrolysis by Eu(III) and La(III) complexes of the ligands revealed that only the 3-pyridine complex, **35**, was active towards the hydrolysis of **HPNP**. At pH 7.4 the La(III) complex was again more active than the Eu(III) for the hydrolysis of **HPNP**, with a rate constant k_{obs} of $5.28 \times 10^{-5} \text{ s}^{-1}$ and a k_{rel} of 1600. By comparison the Eu(III) complex gave a k_{rel} of 649. Again, at a more alkaline pH the rate of hydrolysis recorded by the La(III) complex increased to give a k_{rel} of 4800. From

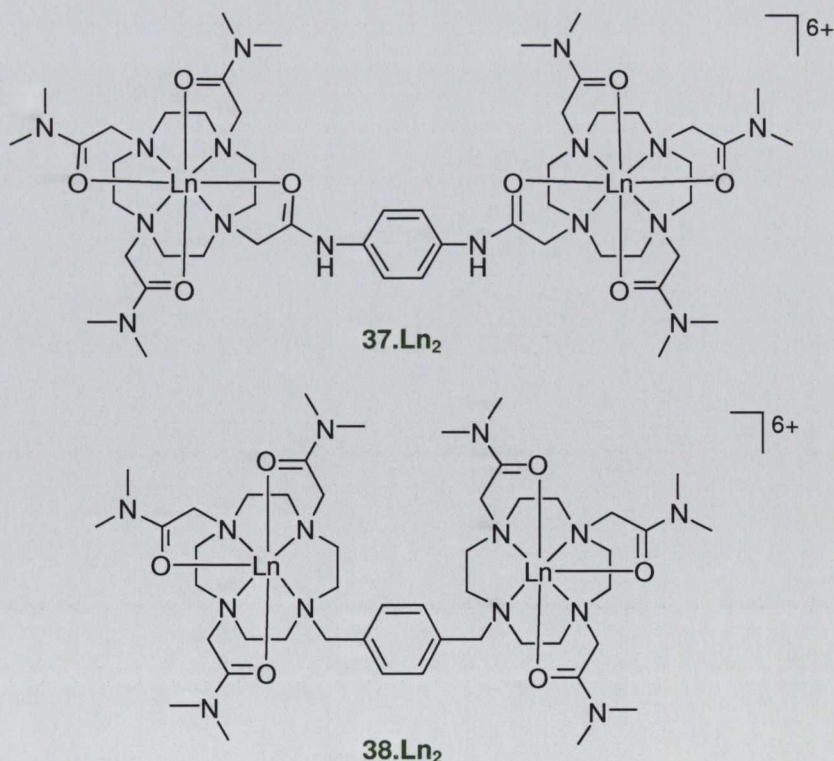
these results it was revealed how sensitive the efficiency of the complexes is to minor structural changes. These can affect the hydrolytic activity of the complex, possibly by modulating the complexes affinity for the substrate, it's ability to bind the substrate or by modifying any transition states and leaving groups. The degree of hydrogen bonding can also influence the hydrolysis, as can the presence of groups which promote acid/base hydrolysis. This information was very relevant for the rational design of future complexes. Such rational design was evident in a family of alkyl amine based ligands which were synthesised, with the alkyl chain varying from $n = 2$ to $n = 5$.¹²² Investigation of these systems showed that $n = 2$ was the optimum chain length for the most efficient hydrolysis of **HPNP**, **36.Ln**. A pH-rate profile revealed that the optimum pH for hydrolysis had been shifted to the physiological pH range, with the maximum rate of hydrolysis occurring at pH 7.4, with a rate constant k_{obs} of $2.06 \times 10^{-4} \text{ s}^{-1}$. This corresponded to a relative rate enhancement of 7775. Furthermore, detailed potentiometric titrations on the ligand and the complex showed that at pH 7.4, the terminal amines were the main species in solution, indicating that they were mainly responsible for the hydrolysis of **HPNP**.



Although the investigation focused on octadentate complexes, preliminary studies were carried out on the corresponding heptadentate Eu(III) and La(III) complexes, with an $n = 2$ chain length.¹⁰² The results were very promising, with the half life at pH 7.4 being reduced from $2.06 \times 10^{-4} \text{ s}^{-1}$ for the octadentate La(III) complex to $1.36 \times 10^{-4} \text{ s}^{-1}$. The Eu(III) complex showed a more pronounced change in the rate of hydrolysis, with the half life dropping from $5.25 \times 10^{-4} \text{ s}^{-1}$ for the octadentate complex, to $1.56 \times 10^{-4} \text{ s}^{-1}$ for the heptadentate complex. This dramatic increase in the rate of hydrolysis, especially concerning the Eu(III) complex, was attributed not only to the presence of the terminal amines, as found previously, but also to the presence of a second water bound molecule on

the Eu(III) metal ion, the hydroxyl group having been previously shown to play an active role in phosphodiester hydrolysis, as outlined in Section 1.6.

Whilst a family of mononuclear complexes were being developed, a family of dinuclear complexes were also synthesised with the aim of achieving enhanced rates of hydrolysis through the cooperative effect of two metal ions, as discussed in Section 1.6. Initially, for all of the dinuclear complexes synthesised, both metal ions were octacoordinate, where each metal centre possessed one or two metal bound water molecules, **37.Ln₂**.¹²⁸ These structures did not give rise to the anticipated fast rate of hydrolysis of **HPNP** and as a result of this work, a family of dinuclear complexes in which each metal ion was heptacoordinate, such as **38.Ln₂**, were synthesised and these were found to display extremely fast rates of hydrolysis of **HPNP**,¹⁰¹ the Eu(III) complex hydrolysed **HPNP**, at pH 7.4 with a rate constant, k_{obs} of $4.11 \times 10^{-4} \text{ s}^{-1}$. This corresponded to a half life of $1.31 \times 10^{-4} \text{ s}^{-1}$, which was one of the fastest rates of hydrolysis of **HPNP** by lanthanide ions at the time and displayed the anticipated cooperative effect of the two metal ions. The second order rate constant recorded for this complex was $2.82 \pm 0.22 \text{ M}^{-1} \text{ s}^{-1}$. This is compared to Morrows best system at the time, a dinuclear zinc TACN complex linked by an alkyl chain, which hydrolysed **HPNP** with a second order rate constant of $0.25 \text{ M}^{-1} \text{ s}^{-1}$. The high efficiency of **38** was attributed to the cooperative action of the two metal ions, and the presence of two metal bound water molecules per metal ion.



This section has outlined the progress made within Gunnlaugsson laboratory in the development of a potential artificial ribonuclease. The next and final section will detail the work that is contained within this thesis.

1.15 Work Outlined in this Thesis

The objective of this PhD thesis was the synthesis of novel, heptacoordinate, dinuclear, cyclen based lanthanide complexes for the hydrolysis of phosphodiester bonds. The previous study of these complexes has determined that several features of the complex are important for the efficient hydrolysis of phosphodiester bonds. These include:

- **The lanthanide metal ion:** In the past, larger lanthanide ions were thought to be more efficient for the hydrolysis of phosphodiester bonds. However, recent studies have shown that this is not always the case, and that lanthanide ions such as Eu(III) may in fact be a better choice than La(III).
- **The hydration number of the lanthanide metal ion:** Studies have shown that cyclen based lanthanide complexes are more efficient if the lanthanide ion possesses two metal bound water molecules.
- **Chemical nature of functional groups incorporated into the cyclen frame:** The chemical nature of the groups incorporated into the arms of the cyclen frame greatly influence the efficiency of hydrolysis, as discussed in Sections 1.13 and 1.14. Fine-tuning of these arms can result in general acid/base catalysis, improving the rate of phosphodiester hydrolysis.
- **Chemical nature of the spacer incorporated into the cyclen frame:** As discussed in Section 1.13, the structure of the spacer group used to connect the two cyclen moieties has a large influence on the rate of phosphodiester hydrolysis, and so fine-tuning of the spacer can result in dramatically different results.

The work detailed in this thesis focuses on the synthesis and in depth analysis of several families of dinuclear cyclen based lanthanide complexes, for which the above properties have been incorporated into the design strategy. The ability of these complexes to hydrolyse phosphodiester bonds is evaluated, and a mechanism of action for such complexes is proposed.

1.15.1 Objectives

Chapter two details the synthesis and characterisation of all ligands and complexes synthesised. A series of dinuclear complexes were synthesised, and can be grouped into several different families, Figure 1.7. Two dinuclear cyclen-based ligands were

synthesised, incorporating either a pyridine- or a phenol-based spacer, **39** and **40**, respectively. Unfortunately, despite many attempts, the synthesis of the phenol-spaced ligand, similar to that utilised by Morrow *et al.* as discussed in Section 1.12, was unsuccessful. The synthesis and difficulties associated with it will be discussed. The synthesis of the pyridine-spaced ligand was successful and the Eu(III) and La(III) complexes were synthesised. The next family synthesised is based on a xylene-spacer for the dinuclear complexes. The spacer was varied to give both the *para*- **41**, **42** and the *meta*- **43**, **44** substituted ligands. This family of complexes was also synthesised using both the 2-acetamide arms and the alkylamine acetamide arms, to determine the effect of the arm on the rate of hydrolysis.

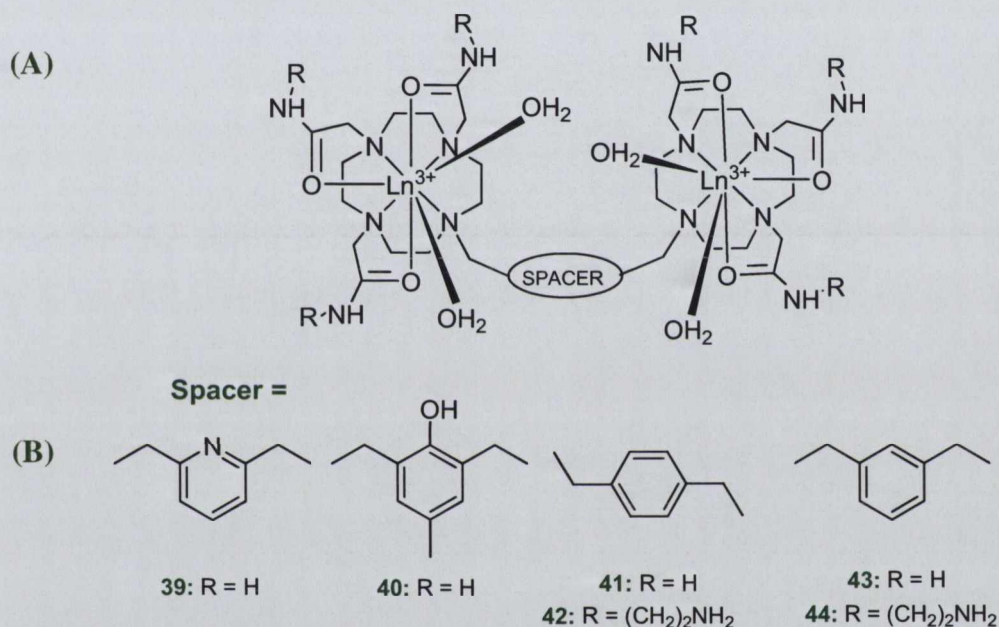


Figure 1.7: Proposed synthetic targets for the design of dinuclear cyclen-based systems. In (A), the generic structure for the dinuclear complexes is shown and (B) shows that spacers which were attempted to be incorporated into the ligand structure.

A trinuclear ligand linked by a 1,3,5-methyl-benzene group was also designed and synthesised, again utilising either the 2-acetamide arms, **45**, or the alkylamine acetamide arms, **46**, Figure 1.8. Although the complex was synthesised, it was quite unstable and as such only preliminary experiments were carried out on this structure.

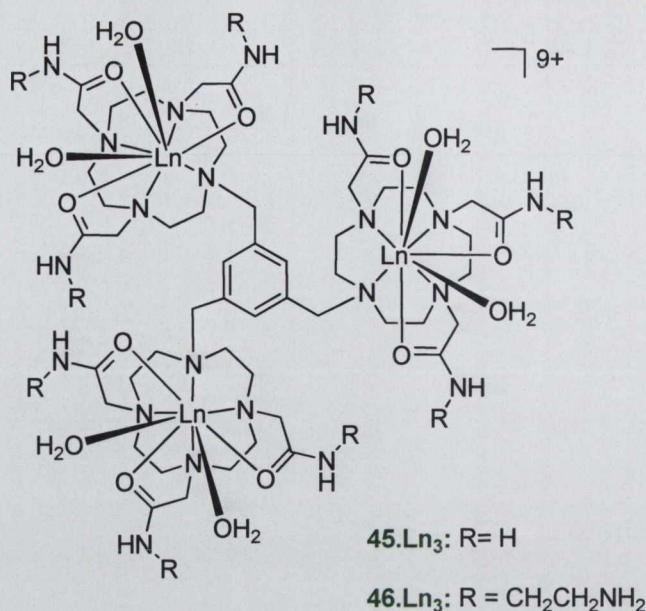


Figure 1.8: Proposed synthetic targets for the synthesis of trinuclear cyclen-based systems.

Finally, to facilitate some mechanistic studies, a family of analogous tri-arm-mononuclear complexes was synthesised, incorporating R-groups which corresponded to the spacers used in the dinuclear complexes, **47.Ln-49.Ln**, Figure 1.9. The synthesis and characterisation of these complexes is discussed.

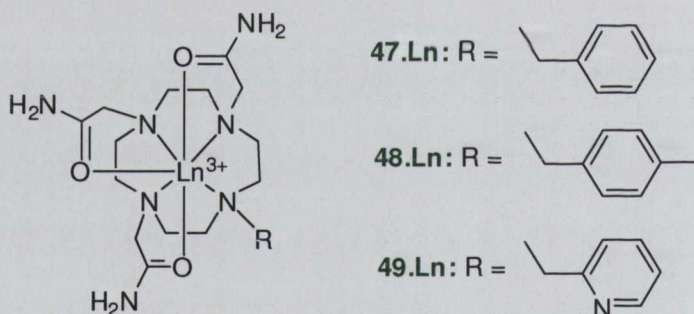


Figure 1.9: Proposed synthetic targets for the synthesis of mononuclear cyclen-based complexes.

Chapter three focuses on potentiometric experiments carried out on all ligands and complexes to determine their protonation constants. This information was used to create an understanding of the mechanism of action for the hydrolysis of phosphodiester by determining the species in solution at different pH.

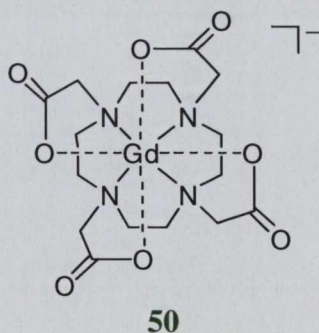
Chapter four details an evaluation of the hydrolytic ability of all complexes discussed in Chapter two. It also draws on results from Chapter three to propose a mechanism of action for the different complexes.

Finally, all experimental details are recorded in Chapter five.

2.1 Introduction

The objective of this thesis was to achieve the synthesis of several families of cyclen-based lanthanide complexes for the hydrolysis of phosphodiester bonds in competitive media, as outlined in Section 1.11. Dinuclear complexes have the potential to enhance the rate of phosphodiester hydrolysis through a cooperative action, as compared to mononuclear analogues.^{3,4,41,64,65,129} Consequently, the main focus of this project was the development of dinuclear spaced systems based on cyclen macrocycles. As noted in Section 1.13, the lanthanide metal ions can be toxic to the human body, unless they are complexed by a suitable ligand, such as a modified cyclen macrocycle.⁷⁶ A wide variety of such ligands exist, which have been shown to be capable of complexing lanthanide ions.^{94,130} As discussed in Chapter One, The ligand used must form kinetically and thermodynamically stable complexes which will not dissociate in aqueous solution, or other competitive media, at 37 °C at physiological pH.⁷⁷

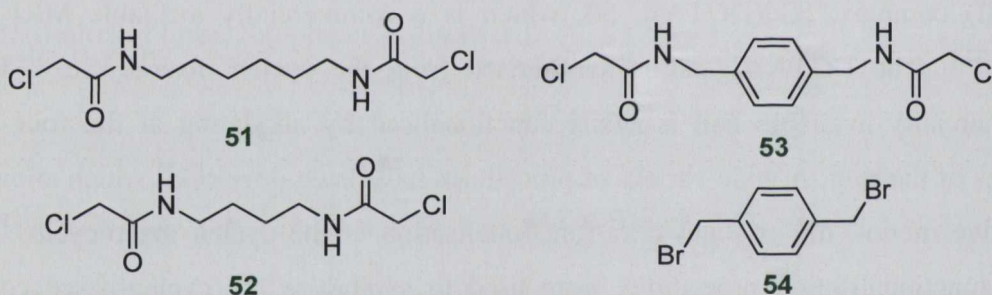
One such ligand that has been established for use within the human body is 1,4,7,10-tetraazacyclododecane-*N,N',N'',N'''*-tetraacetic acid (DOTA), and it's resulting GD(III) complex, [Gd(DOTA)], **50**, which is a commercially available MRI contrast agent.^{94,95} The DOTA ligand is synthesised from the cyclen macrocycle,¹³¹ which is commercially available and is easily functionalised by alkylating at the four nitrogen centres of the ring. A wide variety of procedures have been developed which allow for the selective mono-, di-, tri- and tetra-functionalisation of the cyclen macrocycle,¹³²⁻¹³⁷ and such functionalisation procedures were used to synthesise the cyclen-based complexes which are discussed in this Chapter.



The next Section discusses the design of the ligands synthesised in this chapter. The synthesis and characterisation of several families of dinuclear cyclen-based lanthanide complexes are detailed. This is followed by a discussion of the attempted synthesis of a trinuclear cyclen-based lanthanide complex. Finally, a family of mononuclear cyclen-based complexes, analogous to the family of dinuclear complexes, were synthesised and their characterisation discussed.

2.2 Choice of Spacer Groups and Arms

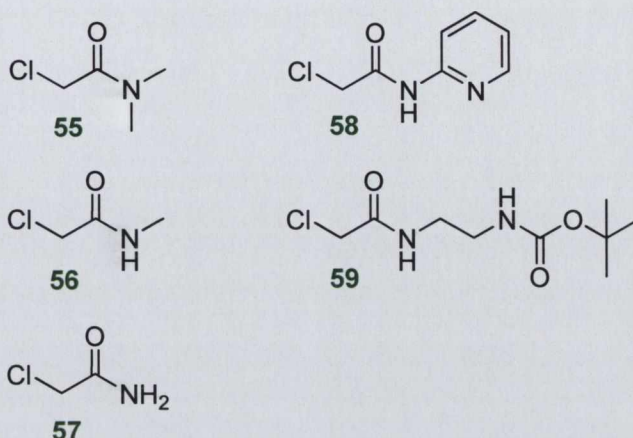
The design of the complexes discussed herein was largely based upon work previously carried out within the Gunnlaugsson group. As indicated in Section 1.13, a wide variety of dinuclear cyclen-based lanthanide complexes have previously been synthesised, using spacers **51-54**.¹⁰¹ These complexes all contained one or two metal bound water molecules per metal ion, which was established by crystal structure and luminescent measurements.¹⁰¹ Evaluation of these complexes for their ability to hydrolyse phosphodiester bonds revealed the importance of developing systems possessing more than one metal bound water molecule per metal ion, Section 1.13. To achieve this, the acetamide functionality was removed from the spacer, which reduced the available coordination environment. This resulted in a dramatic increase in the rate of hydrolysis for such dinuclear complexes.¹⁰¹ The chemical nature of the spacer was also found to be important. Although none of the complexes in which an acetamide functionality was incorporated into the spacer gave rise to fast hydrolysis, a trend based on the nature of the spacer group could be discerned.



From this investigation, it was concluded that a spacer which was neither too rigid, nor too flexible would give rise to the highest rate of phosphodiester hydrolysis. These characteristics were best observed in the xylyl based spacer, **54**, which lacked an acetamide functionality, thus allowing a second water molecule to coordinate to each of the lanthanide metal ions. As such, the majority of the systems investigated herein are based on a xylyl or similar spacer moiety.

The choice of arms was also important to the efficiency of the complex for phosphodiester hydrolysis. A wide variety of arms were investigated in the work carried out by Dr.s Mulready and Fanning.^{100,102,122} Different moieties were incorporated into these acetamide based arms to assess their influence on the rate of phosphodiester hydrolysis. The arms were intended to act as '*co-factors*', aiding hydrolysis and as such increasing the

rate of cleavage of the phosphodiester bonds. The results clearly indicated that basic arms such as **55-59**, resulted in an overall increase in the rate of hydrolysis.¹²²



The nature of the lanthanide metal ion for phosphodiester hydrolysis was also investigated by complexing a wide variety of these ions using a single ligand to enable easy comparison.¹²⁰ When complexed by a ligand which had eight coordination sites, the La(III) complex gave rise to the highest rates of phosphodiester hydrolysis of **HPNP**. However, when the ligand was modified to reduce the number of coordination sites to seven, it was found that Eu(III) now gave rise to the highest rates of hydrolysis, due to the presence of a second metal bound water molecule. As such, it was concluded that the most effective lanthanide metal ions for phosphodiester hydrolysis were Eu(III) and La(III).

In summary, the results indicated that among those cyclen based lanthanide complexes investigated within the Gunnlaugsson group, the most effective complex for the hydrolysis of the phosphodiester bond of **HPNP**, would be a dinuclear cyclen based lanthanide complex which was bridged by a xylyl spacer, and functionalised with either **57** or **59**, the acetamide-based arms, and metal ions such as Eu(III) or La (III).

Based on this, a family of dinuclear complexes was designed. Of these, one sub-family also incorporated heteroatoms into the spacer unit, as the effect of this had not been investigated previously. This is discussed in the next Section.

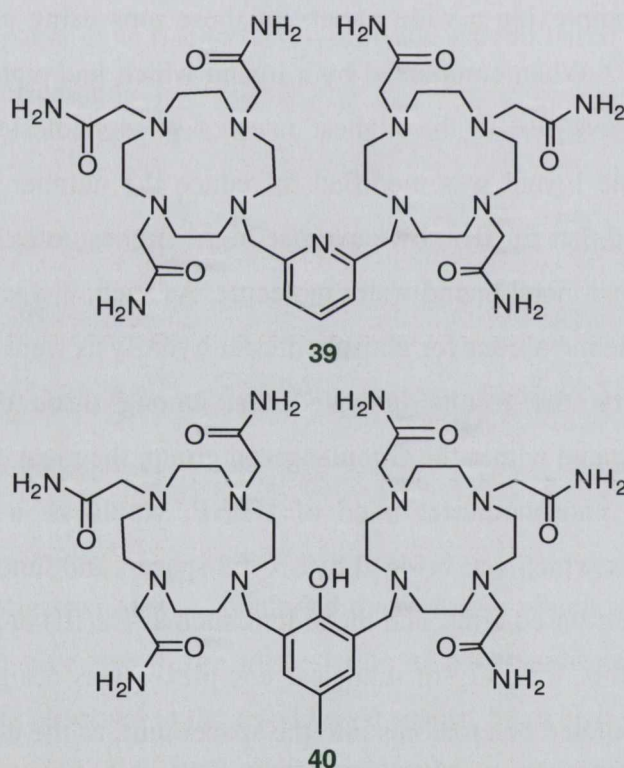
2.3 Dinuclear Systems Incorporating Heteroatoms

The two aromatic spacers to be incorporated were a pyridine and a cresol moiety. Considering the dramatic effect that changing the pendant arms can have on the rate of hydrolysis,^{62,122} it was anticipated that these two ligands, **39** and **40**, would enhance the rate of hydrolysis, possibly through acid/base catalysis.

Based on a study by Handel *et al.*, in which the binding of bis-cyclen ligands to phosphate anions was investigated, the pyridine moiety was selected as a potential

spacer.¹³⁸ Of the spacers studied, a 2,6-pyridinyl linker gave the best triphosphate recognition, and was shown to form strong complexes with all phosphate anions investigated, in a competitive environment. NMR titrations showed a large shift of the aromatic protons consistent with a H-bonding effect, possibly as a result of the protonated pyridine binding an oxygen atom of the phosphate centre. From this, it was postulated that a pyridine spaced bis-cyclen complex could potentially give rise to an enhanced rate of phosphodiester hydrolysis as a result of the pyridine moiety's phosphate binding potential.

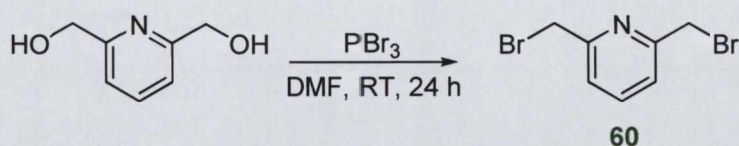
The cresol spacer was selected in order to investigate the effect of the presence of a hydroxyl group on the rate of phosphodiester hydrolysis. It is possible that the hydroxyl group could stabilise the transition state of the phosphodiester by donating a H-bonding motif, or that it could participate in general acid-base catalysis.^{91,139} The synthesis and characterisation of both ligands will be discussed in the next section.



2.3.1 Synthesis of Pyridine Spaced Bis-System

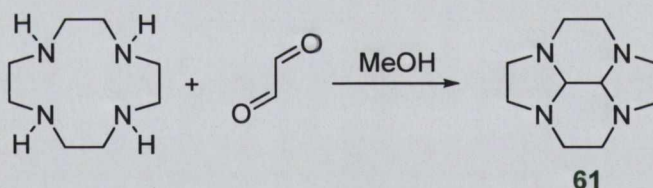
A dinuclear complex incorporating a *meta*-substituted pyridine moiety into the spacer was designed. The spacer, **60**, was synthesised *via* a bromination of 2,5-bis-(hydroxymethyl)pyridine using PBr_3 , according to a literature procedure described by Cram *et al.*, Scheme 2.1.¹⁴⁰ The desired product was isolated by extraction into CH_2Cl_2 , followed by washing with both 0.1 M HCl and 0.1 M NaOH. Although the crude yield of the reaction was high at approximately 90 %, the pure yield ranged from 47 to 65 %, as a

result of repeated washings and triturations which were necessary to remove all traces of DMF from the product.

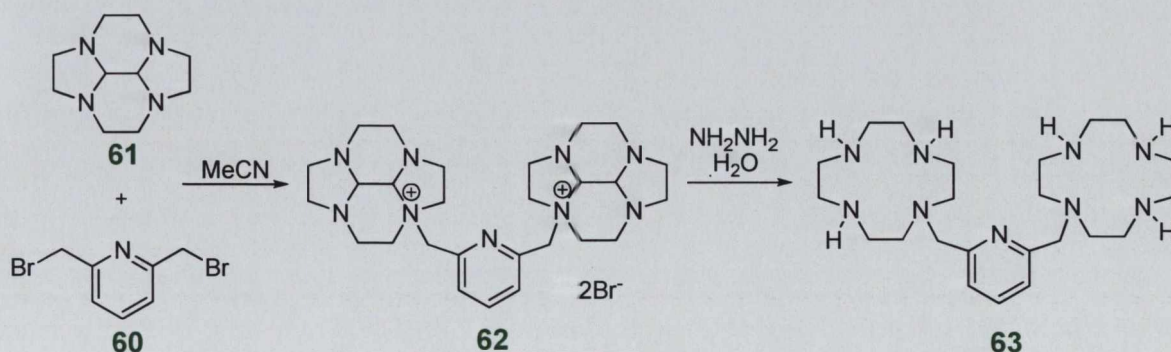


Scheme 2.1: Synthesis of pyridine spacer, **60**, by bromination of 2,5-(hydroxymethyl)-pyridine.

Due to the numerous available alkylation sites on the cyclen macrocycle, a direct alkylation to form the bis-cyclen frame was not feasible; hence the use of protection group chemistry was employed. As discussed in Section 2.1 and 2.2, a wide variety of synthetic techniques exist for the selective functionalisation of cyclen.^{137,141-143} A procedure that has been shown to give high yields and good purity for the formation of bis-cyclen frames is the formation of bis-aminals, developed by Handel and co-workers.¹³⁵ While this procedure has been shown to be efficient for the incorporation of a variety of spacers into the cyclen framework, the nature of the spacer can affect both the efficiency and the yield of the reaction. The starting material for this reaction was a glyoxal protected cyclen, **61**, which was synthesised following a literature procedure¹³⁵ and obtained as an off white solid in 90 % yield, Scheme 2.2.. The product was characterised by ^1H NMR, ^{13}C NMR, and ESMS.



Scheme 2.2: Glyoxal protection of cyclen.¹⁶



Scheme 2.3: Synthesis of **63** from the reaction of two equivalents of **61** with one equivalent of **60**. Compound **61** was treated as an intermediate and was not isolated.¹⁶

To form the pyridine spaced bis-cyclen frame, **63**, two equivalents of **61** were stirred with one equivalent of **60** in MeCN, Scheme 2.3.¹³⁵ The bis-aminal, **62**, was insoluble in MeCN and precipitated out of solution upon formation. The precipitate was dissolved in neat hydrazine monohydrate, and the resulting solution was refluxed for twelve hours at 120 °C and was then left to slowly cool to room temperature. Following the removal of the hydrazine, the residue was redissolved in a 30/70 v/v solution of acetone/EtOH and conc. HCl was added to precipitate the product as a HCl salt, which was collected by filtration. The desired product **63** was obtained as an orange solid in 79% yield.

The success of this reaction stems from the conformation of the glyoxal protected cyclen in solution; the aminal bridge causes the cyclen to fold over on itself, resulting in two of the nitrogens of the ring being inaccessible for alkylation. The short reaction time reduces the formation of polymers.¹³⁵

The pendant arms used for the alkylation of **63** were added *via* 2-bromoacetamide substitution. Previous dinuclear systems synthesised within the Gunnlaugsson group utilised both the *N,N'*-dimethyl- and *N*-methyl-acetamide arms,¹²⁸ **55** and **56**, respectively, and it was shown that these simple arms promoted hydrolysis to a larger extent than previously studied pendant arms such as the *GlyAla* dipeptide or the pyridine based acetamide arms, which were discussed in Section 1.13.^{62,120}

The bis-cyclen frame **63** was alkylated with 6 equivalents of 2-bromoacetamide, in the presence of 6 equivalents of triethylamine in MeOH. The solution was refluxed for four days and the reaction followed by ESMS. When the expected mass of the desired product was observed, with little evidence of other species such as the tri-substituted species in solution, the reaction was judged to be fully complete. To isolate the product, the solvent was reduced and the product precipitated by the addition of isopropanol (*i*-PrOH). The solid was collected with MeOH, the solvent was removed under reduced pressure and the residue dried under vacuum to give the product, **39**, as an orange solid in 65 % yield.

The C₂ symmetry of the ligand **39** was clearly observed in the ¹H NMR spectrum (400 MHz, D₂O), Figure 2.1. As expected, a doublet and a triplet, for the protons of the pyridine spacer, integrating to two and one protons, respectively, were observed in the aromatic region of the spectrum. Further upfield, a singlet was observed at 4.09 ppm which corresponded to the CH₂ protons of the spacer. Further to this, the resonances corresponding to the cyclen protons appeared as broad singlets or multiplets. The acetamide arms showed a similar symmetry and two sharp singlets were observed at 3.20 and 3.23 ppm, integrating to eight and four protons, respectively.

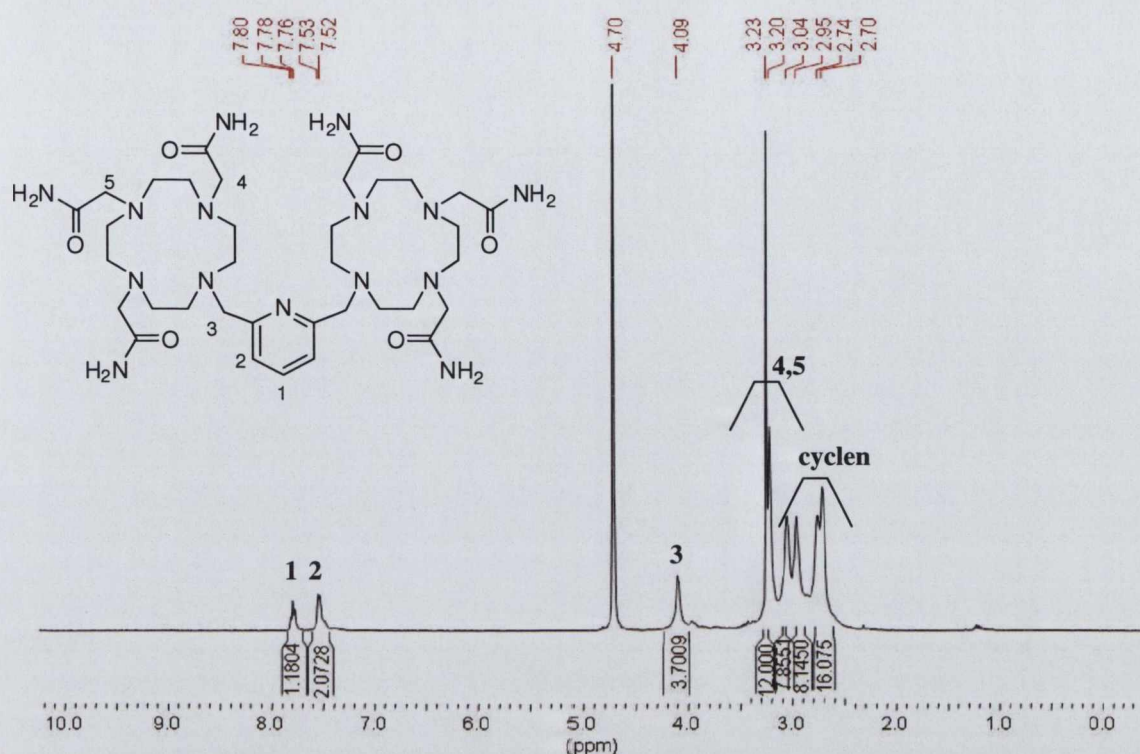
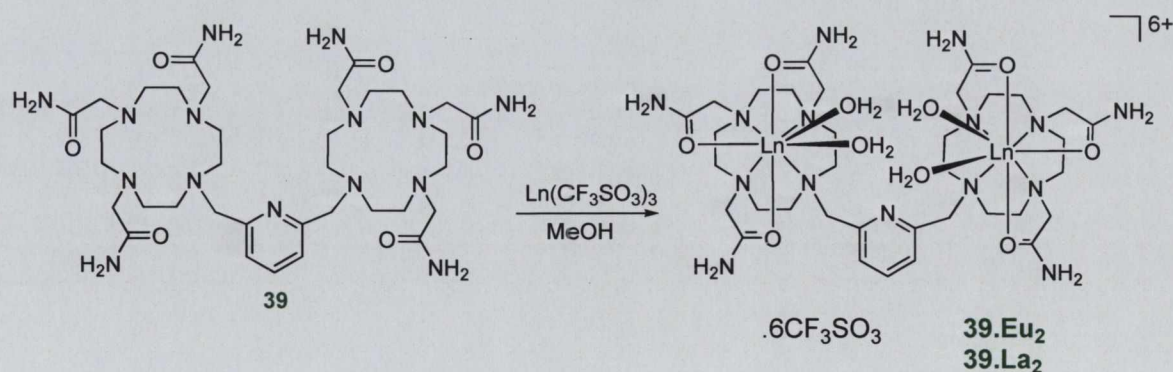


Figure 2.1: The ^1H NMR spectrum (400 MHz, D_2O) of ligand **39** showing the C_2 symmetry of the ligand.

2.3.2 Lanthanide Ion Complexation of **39**

The Eu(III) and La(III) complexes of **39**, **39.Eu₂** and **39.La₂**, respectively, were synthesised by refluxing one equivalent of **39**, with two equivalents of either $\text{Eu}(\text{CF}_3\text{SO}_3)_3$ or $\text{La}(\text{CF}_3\text{SO}_3)_3$ in MeOH overnight, Scheme 2.4. Both complexes were isolated by precipitation upon addition to a vigorously stirred solution of diethyl ether (150 mL). The resulting solids were collected and then dried under vacuum to yield the complexes as white solids in 76 and 73 % yield, respectively.



Scheme 2.4: Complexation of ligand **39** with $\text{Ln}(\text{CF}_3\text{SO}_3)_3$.

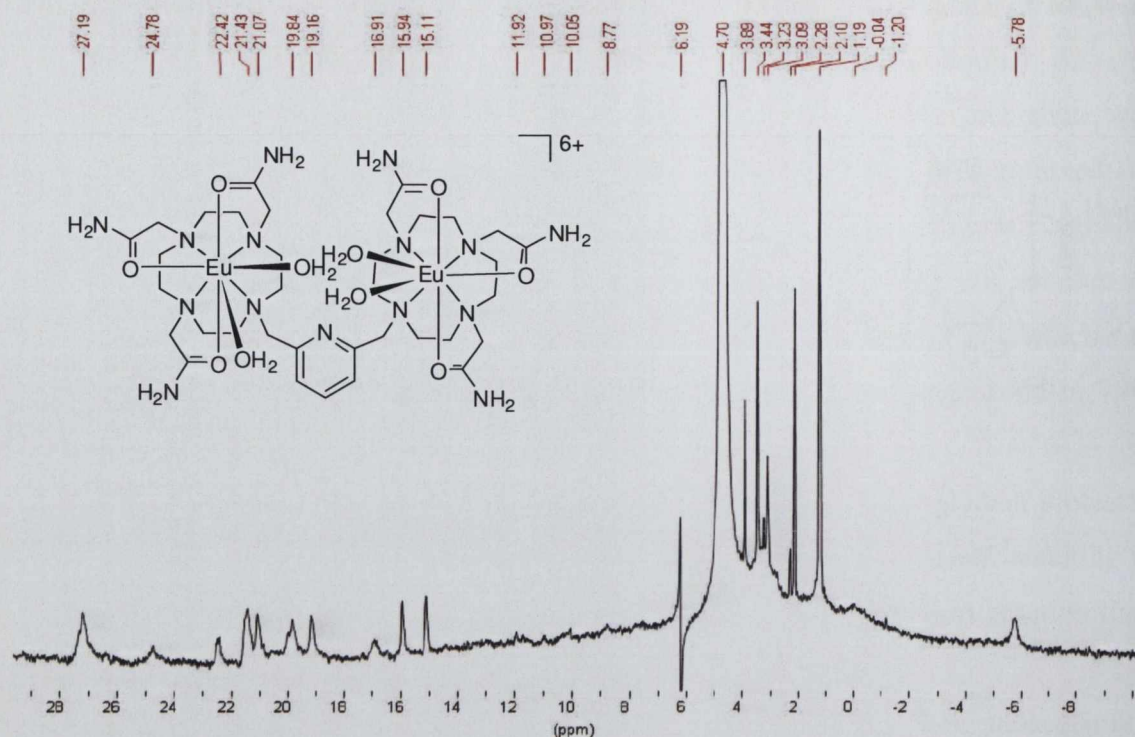


Figure 2.2: The ^1H NMR spectrum (400MHz, D_2O) of $39.\text{Eu}_2$ showing the shifted axial and equatorial protons of the cyclen ring.

The complexation of lanthanide ions by a ligand such as **39** can give rise to small structural rearrangements of the ligand, which can be observed using several different spectroscopic techniques. The Eu(III) ion is paramagnetic and is a well known NMR shift reagent. The proximity of the Eu(III) metal ion to the axial and equatorial cyclen and acetamide protons causes them to experience an enhanced magnetic field due to the presence of the unpaired f -orbital electrons of the ion. The protons closest to the metal ion experience this to a greater extent, which causes these resonances to shift in the ^1H NMR spectrum, as compared to that of the ligand alone.

The presence of the paramagnetic metal ion also gives rise to fast relaxation times which results in broadening of the signals. As a result, the ^1H NMR spectrum could not be fully characterised for $39.\text{Eu}_2$. The fast relaxation times also prevent a ^{13}C spectrum from being obtained. The ^1H NMR spectrum (400 MHz, D_2O) of $39.\text{Eu}_2$ is shown in Figure 2.2. It is clear that the axial and equatorial protons of the cyclen ring are shifted, as resonances are observed between 27.19 to -5.78 ppm.

In contrast, the ^1H NMR spectrum (400 MHz, D_2O) for $39.\text{La}_2$, Figure 2.3, was characteristically broad, and the resonances unshifted. La(III) is not paramagnetic and as such it does not induce shifts in the NMR spectrum in the same manner as Eu(III) . However, it does still result in fast relaxation times and so some resonances of the NMR spectrum can thus be very broad, Figure 2.3.

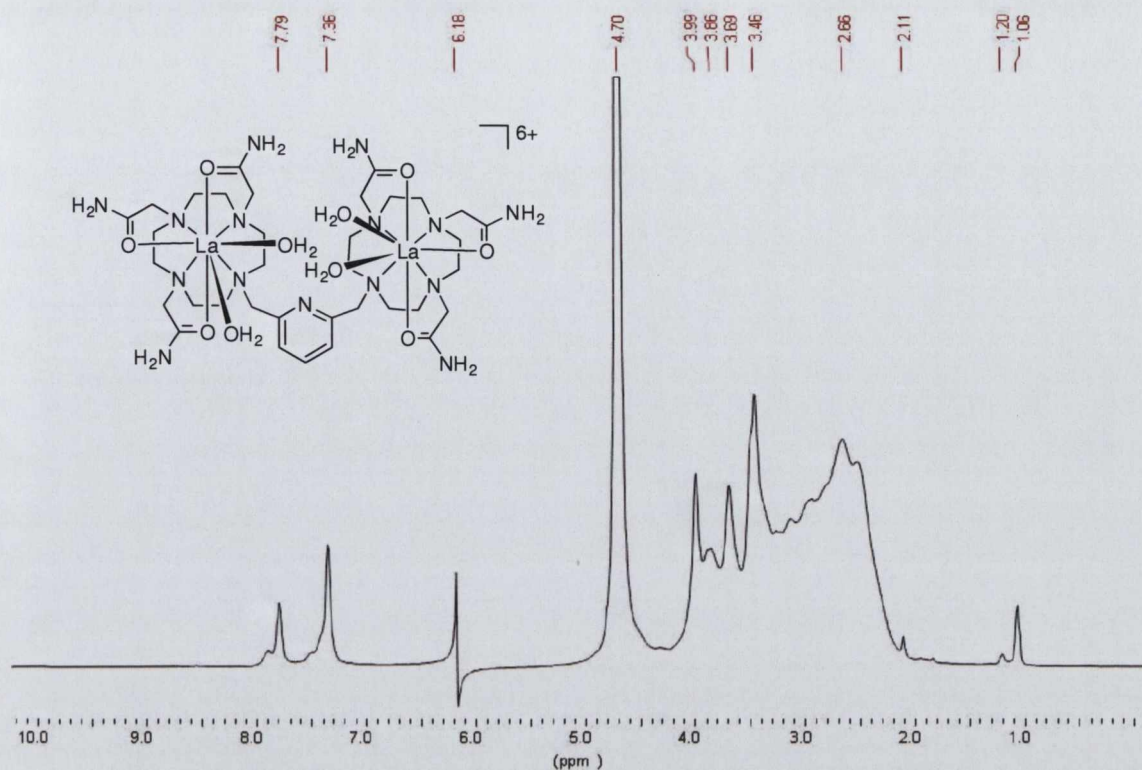


Figure 2.3: The ^1H NMR spectrum (400 MHz, D_2O) of **39.La₂** showing the characteristic broad resonances of the cyclen ring resulting from the presence of the La(III) ion in the complex.

The complexes were also characterised using infrared spectroscopy (IR). Complexation of a lanthanide *via* the carbonyls of the coordinated arms results in a decrease in the vibrational energy of the carbonyl bonds, which is observed in the IR spectrum. The carbonyl bond donates electron density into the metal centre, resulting in an increase in bond length, and thus, a decrease in the IR stretching frequency. The carbonyl stretching frequencies of ligand **39** and its corresponding Eu(III) and La(III) complexes are detailed in Table 2.1. The anticipated decrease in the C=O stretch was observed for both complexes, where the stretching decreased from 1621 cm^{-1} for **39**, to 1599 cm^{-1} and 1598 cm^{-1} , to **39.Eu₂** and **39.La₂**, respectively.

Table 2.1: Carbonyl stretching frequencies for ligand **39** and complexes **39.Eu₂** and **39.La₂**.

Compounds	C=O stretching freq. (cm^{-1})
39	1621
39.Eu₂	1599
39.La₂	1598

Using electrospray ionisation (ESI⁺), high resolution mass spectrometry (HRMS) was obtained for both **39.Eu₂** and **39.La₂**. The complex **39.Eu₂** was observed with $m/z =$

1389.2256, which corresponds to $[M + 2(\text{CF}_3\text{SO}_3)]^{4+}$. The La(III) complex, **39.La₂** was observed with $m/z = 1513.1695$, corresponding to $[M + 3(\text{CF}_3\text{SO}_3)]^{3+}$.

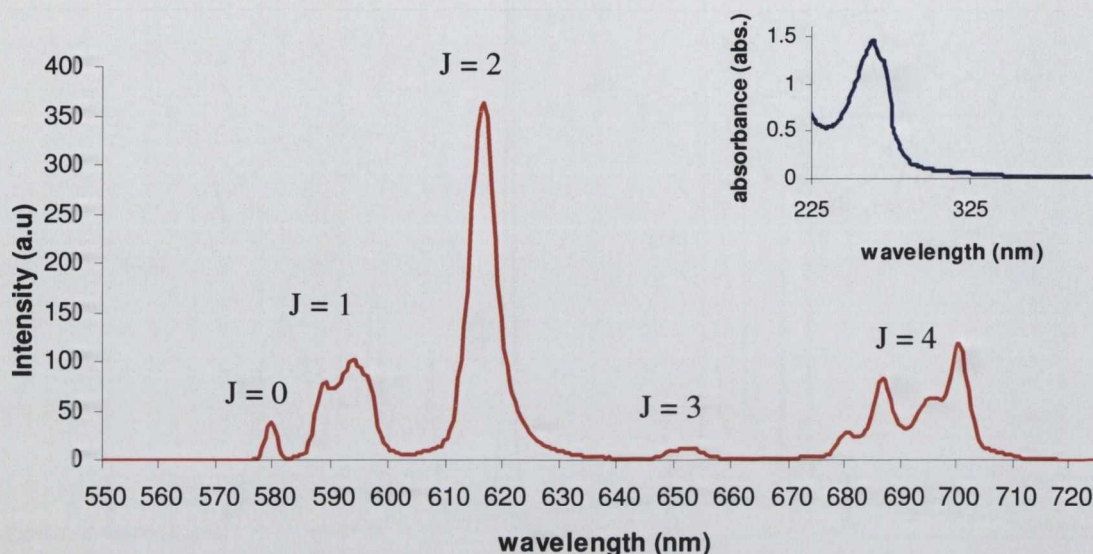


Figure 2.4: Phosphorescence spectrum of **39.Eu₂**. The spectrum was generated by excitation into the pyridinyl spacer at 266 nm, and emission was recorded between 550 and 725 nm. The characteristic Eu(III) phosphorescence spectrum is observed. The insert shows the UV-Vis absorption spectrum of **39.Eu₂** with $\lambda_{\text{max}} = 266$ nm.

Photophysical characterisation was also carried out on these complexes. The absorption spectrum was recorded and the λ_{max} was found at 266 nm for both complexes, which is attributed to the pyridine chromophore, insert, Figure 2.4. Excitation into the pyridinyl chromophore at 266 nm gave rise to an induced Eu(III) sensitisation, and resulted in the characteristic Eu(III) phosphorescence spectrum, which is shown for **39.Eu₂** in Figure 2.4.

2.3.3 Determination of q , the Number of Metal-Bound Water Molecules

The number of metal bound water molecules (q) was also determined for **39.Eu₂**. As discussed in Section 1.13, the lanthanide ions have a high coordination requirement of up to ten.⁷⁶ Those that remain unfulfilled are usually filled by solvent molecules such as H₂O. The number of coordinated water molecules can be determined in several ways; a crystal structure can show the number of coordinated water molecules, however, the solid state may not be an accurate representation of the solution state. For Gd(III) complexes the number of metal bound water molecules can be determined using ¹⁷O NMR experiments.¹⁴⁴ In addition, the number of metal bound water molecules can also be determined by measuring the luminescent decay of the complex.¹⁴⁵⁻¹⁴⁷

A method for determining the number of water molecules, bound to metal ions such as Eu(III) or Tb(III) has been developed by Horrocks and Sudnick,¹⁴⁵ and refined both by Parker and co-workers,¹⁴⁷ and later, again by Supkoski and Horrocks.¹⁴⁶ These methods

use the luminescent lifetimes of the lanthanide metal ions, measured in both H₂O and D₂O, to estimate the solution hydration state of the lanthanide ions. This is possible due to the fact that vibrational quenching of the lanthanide metal ion is much less in D₂O than in H₂O, which gives rise to a difference in the radiative rate constant in the two different solvents. As such, measurement of the observed radiative rate constants (k) of the ⁵D₀ excited states of Eu (III) and the ⁵D₄ excited states of Tb(III) in H₂O and D₂O allows an estimation of the solution hydration state. Tb(III) complexes have been shown to give rise to a lower rate of hydrolysis than Eu(III) and other metal ions and as such, the Tb(III) complexes of the ligands discussed herein have not been studied. As such, only the equation for determining the number of water molecules bound to Eu(III) will be discussed. The equations to which the rate constant is fitted are as follows:

$$q^{Eu(III)} = 1.2[(1/\tau_{H2O} - 1/\tau_{D2O}) - 0.25 - 0.075x] \pm 0.5 \quad \text{Equation 2.1}^{147}$$

$$q^{Eu(III)} = 1.11[(1/\tau_{H2O} - 1/\tau_{D2O}) - 0.31] \pm 0.3 \quad \text{Equation 2.2}^{146}$$

Equation 2.1 is a refinement of the original Horrocks and Sudnick equation by Parker and co-workers.¹⁴⁷ Equation 2.2 is a further refinement of that equation by Supkowski and Horrocks.¹⁴⁶ Although the error is smaller for Equation 2.2, it has been shown to be more accurate for complexes which have a higher number of metal bound water molecules, *e.g.* ≥ 5 . For this reason, Equation 2.1 was used throughout to determine the number of metal bound water molecules. In equation 2.1, the prefix 1.2 is a proportionality constant, determined over a large number of experiments,¹⁴⁷ while 0.25 is a correction factor to compensate for the quenching by second sphere water molecules, and 0.075x represents the quenching by *N-H* oscillators within the complex. The recorded lifetimes in H₂O (τ_{H2O}) and D₂O (τ_{D2O}) recorded, as well as the calculated q -value for **39.Eu₂** are shown in Table 2.2.

Table 2.2 Lifetime studies for Eu(III) complex **39.Eu₂**.

Complex	k (ms ⁻¹)	τ (ms)	k (ms ⁻¹)	τ (ms)	$q = (\pm 0.5)$
	H ₂ O	H ₂ O	D ₂ O	D ₂ O	
39.Eu₂	2.69	0.37	0.61	1.64	1.6

The resulting q -value of 1.6 (± 0.5), was somewhat lower than had been anticipated. Hence, it is possible that the lower q -value could be due to a partial

coordination of the pyridine nitrogen to one, or both, of the lanthanide ions, reducing the number of metal bound water molecules, as shown in Figure 2.5.

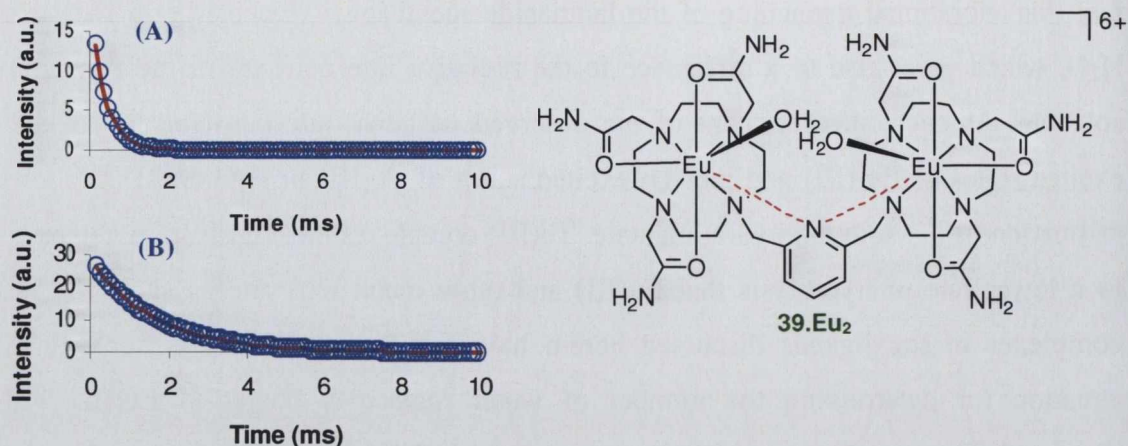


Figure 2.5: Luminescence decay profile for **39.Eu₂** in (A) H₂O and (B) D₂O. The red line shows the fit of a monoexponential decay to the rate profile. Structure of **39.Eu₂** showing the potential coordination of the pyridine spacer to the Eu(III) metal ion, giving rise to a decrease in the expected *q* value.

The decay profile was fit to both a mono and a biexponential decay to investigate the possibility that a single metal ion was binding to the pyridine moiety, however, it was observed that the lifetimes fit well to a monoexponential decay. Furthermore, having fit the decay profile to a biexponential decay, the populations were calculated and it was found that the biexponential species was only 5% populated, which indicated that the monoexponential fit was more accurate. With this in mind, along with the *q*-value of 1.6 (± 0.5), it is possible that the structure is fluctuating in solution, with neither metal ion coordinated to the nitrogen atom at all times.

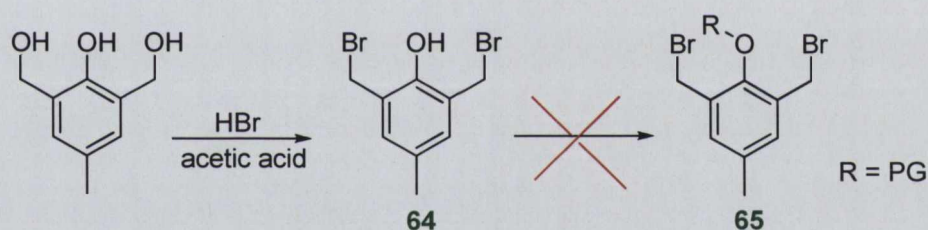
2.3.4 Summary

The synthesis of the pyridine ligand, **39**, was successful, resulting in two complexes, **39.Eu₂** and **39.La₂**. It is possible that the complexes will not have the desired effect of increasing the binding of the complex to the substrate, as the pyridine is possibly involved in the coordination of the lanthanide metal ion, which was concluded from the *q*-value. The following Section details the attempted synthesis of a *p*-cresol spaced system.

2.3.5. Synthesis of *para*-Cresol Spaced Bis-System

A spacer incorporating a hydroxyl group has been used in dinuclear zinc complexes developed and synthesised by both Williams *et al.*,⁸⁹ and Morrow *et al.*¹¹⁵ These were discussed in Section 1.12. These two complexes gave rise to very efficient hydrolysis, and as such a dinuclear lanthanide based complex possessing a structurally similar spacer was designed.

A literature procedure similar to that of the pyridine based ligand, **39**, was followed. To synthesise the *p*-cresol spacer, a literature procedure developed by Trost *et al.* was followed.¹⁴⁸ The spacer was synthesised *via* a bromination of the hydroxyl starting material, as shown in Scheme 2.6 however, repeated reactions failed to isolate the intended product. It was determined that the product was interacting with the K_2CO_3 drying agent and hence, changing the drying agent to $MgSO_4$ resulted in the isolation of the desired product, **64**. Due to the expected reactivity of the phenol group it was attempted to protect the hydroxyl group, Scheme 2.5. Several attempts to form the protected compound, **65**, were made, including protection with an acetal, an ether or a methyl group, however, none were successful. Very few examples can be found in the literature of the successful protection of an aromatic hydroxyl group that has two substituents *ortho* to the hydroxyl group, which presents the possibility that the position is too sterically hindered, or unreactive to protect.^{149,150} As such, it was attempted to protect the unsubstituted *p*-cresol, after which the arms could be added *ortho* to the hydroxyl group, however, this route also proved unsuccessful.

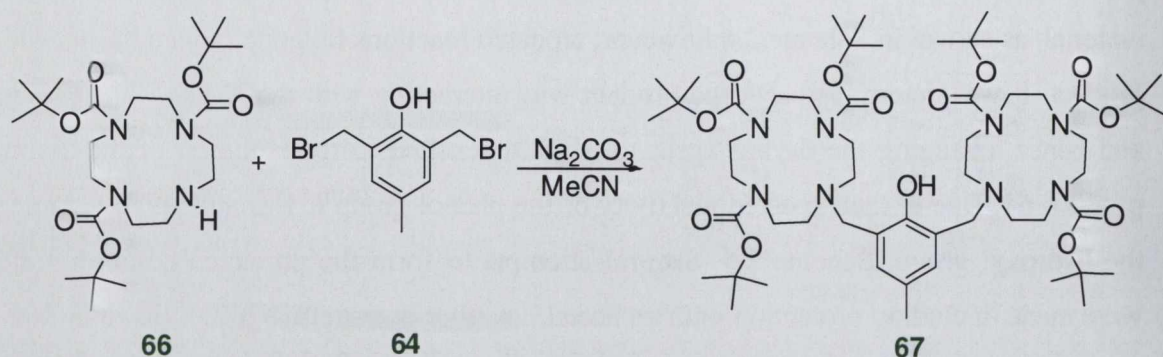


Scheme 2.5: Pathway for the synthesis of the protected *p*-cresol compound, **65**.

As such, it was decided to proceed to the next synthetic step with the hydroxyl group unprotected. However, the reaction of **64** with the glyoxal protected cyclen, **61**, was unsuccessful. It is possible that the electron withdrawing effect of the negatively charged hydroxyl group deactivated **64** towards alkylation, as there was no indication that any of the desired product was forming during the reaction.

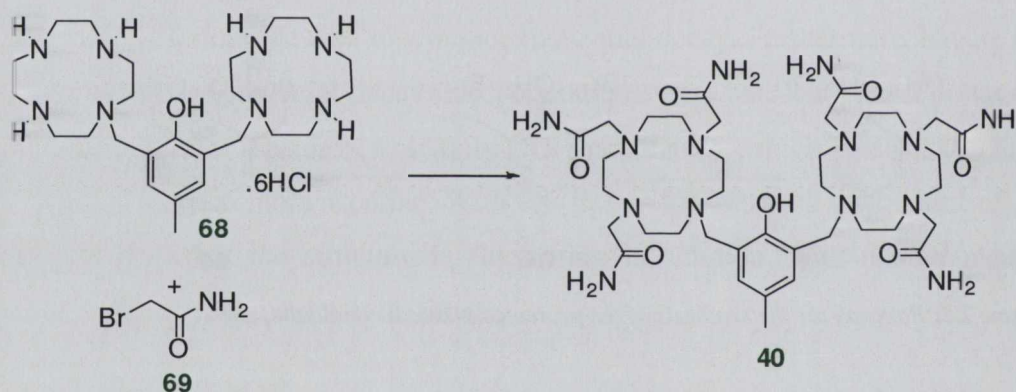
Following an alternative synthetic route, it was decided to protect the cyclen moiety in a different manner. Cyclen can be selectively BOC-protected at three of the four nitrogen atoms of the cyclen ring, by dropwise addition of three equivalents of di-*tert*-butyldicarbamate (BOC_2O) in the presence of triethylamine.⁶⁰ Subsequent purification by silica chromatography (EtOAc:Hexane 5:1 eluant) yielded the intended product, **66**, in 72% yield. To synthesise the cresol spaced bis-cyclen frame, **67**, two equivalents of the tri-BOC-protected cyclen, **66**, were stirred for 72 hours with one equivalent of the *p*-cresol

spacer, **64**, Scheme 2.6. Purification of the crude product by silica chromatography (EtOAc:hexane 5:1 eluant) gave the product, **67**, in 80% yield.



Scheme 2.6: Synthesis of the BOC-protected cresol-spaced bis system, **67**.

The BOC-deprotection of the bis-cyclen frame was achieved by stirring **67** overnight in a solution of 30% v/v conc. HCl/MeOH, from which a white solid precipitated. The solution was filtered, the solid collected and washed with EtOH. This gave the desired product, **68**, as the HCl salt in 72% yield.



Scheme 2.7: Planned synthetic pathway for the synthesis of the **40**.

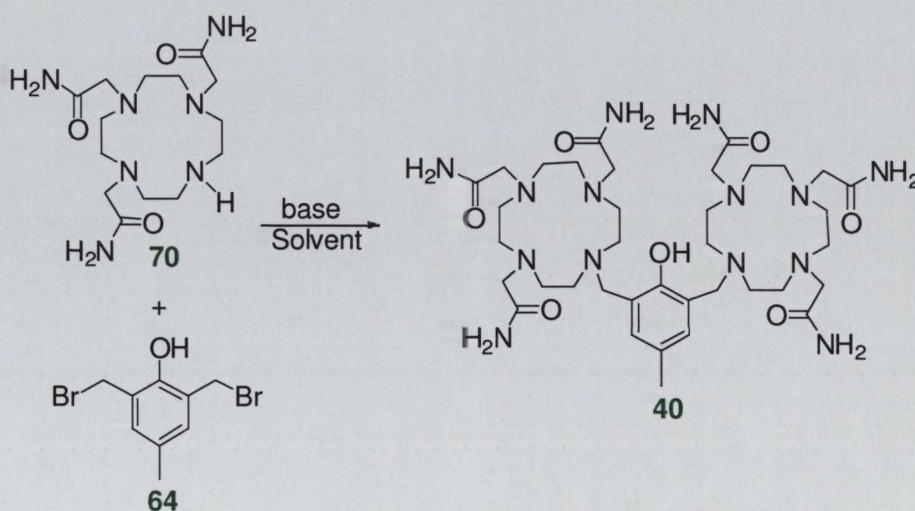
The next and final step in the formation of the ligand **40** was the alkylation of the bis-cyclen frame, **68**, Scheme 2.7. This step proved problematic, and the intended product, **40**, was never successfully isolated. Initially, the deprotected bis-cyclen frame was refluxed with 2-bromoacetamide, **69**, in the presence of Et₃N in EtOH. No product or expected side-products were detected by mass spectrometry or by ¹H NMR. The reaction was repeated and the solvent changed to MeOH. When this reaction also proved unsuccessful the base was changed, from Et₃N to K₂CO₃. This did not result in the formation of the desired product. Consequently the base was changed to di-*iso*-propylethylamine (DIPEA), which again did not result in the formation of **40**. The solvent was also changed, however, the reaction did not improve in either DMF or CHCl₃. The

different reaction conditions attempted for the synthesis of **40**, all of which were carried out at reflux temperature, are outlined in Table 2.3.

Table 2.3: Conditions for the attempted synthesis of **40**. None of the conditions lead to the formation of the desired product.

Attempt	Base	Solvent	Arm	Time
1	Et ₃ N	EtOH	69	1 wk
2	Et ₃ N	MeOH	69	1 wk
3	Et ₃ N	MeOH	69	2 wk
4	K ₂ CO ₃	MeOH	69	1 wk
5	DIPEA	EtOH	69	1 wk
6	DIPEA	DMF	69	1 wk
7	DIPEA	CHCl ₃	69	1 wk
8	DIPEA	MeCN	55	1 wk
9	DIPEA	MeOH	56	2 wk

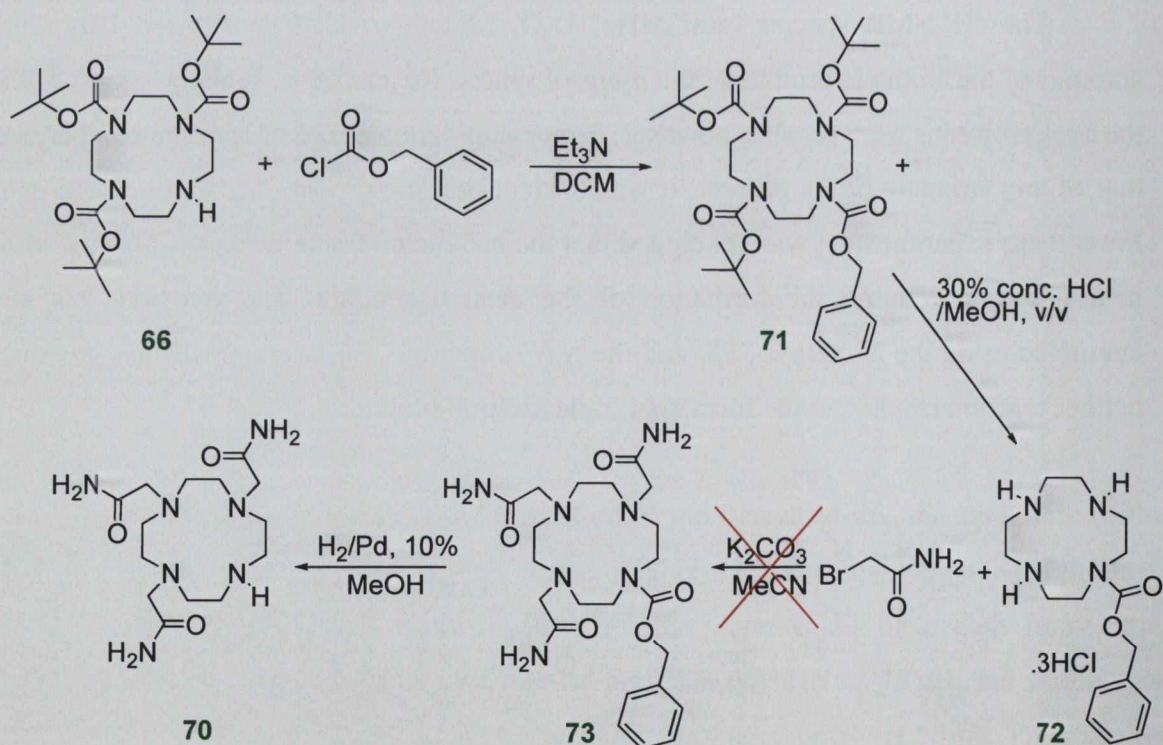
The ¹H NMR spectra (400 MHz, D₂O, MeOD or CDCl₃) showed only trace amounts of the aromatic protons of the *p*-cresol spacer. Resonances usually associated with the cyclen protons were present, however, from examination of the integration compared to that of any aromatic peaks present, it was evident that the product had not been formed. From these experiments it was concluded that the bis-cyclen frame was possibly degrading in solution, preventing the formation of the desired product. The reaction was also attempted using the *N*-methyl-, **55**, and the *N,N'*-dimethyl-, **56**, acetamide arms, however, neither reaction resulted in the formation of the desired product.



Scheme 2.8: Alternative strategy for the synthesis of **40**.

To circumvent the problems which had been encountered for the attempted alkylation reaction, a new synthetic strategy was devised in which the tri-acetamide cyclen, **70**, was reacted with the *p*-cresol spacer to yield the desired ligand, **40**, Scheme 2.8.

Gunnlaugsson *et al.* have developed a one-pot procedure for the successful synthesis and purification of tri-substituted cyclen using both **55** and **56**,¹⁴³ however, the purification of both ligands involved tedious column chromatography. The synthesis of cyclen functionalised with three acetamide arms, **70**, was even more difficult due to the nature of the pendant arms. In an attempt to purify the desired product, a wide variety of solvent systems were utilised as the mobile phase. However, none resulted in isolation of the product. While the tetra-alkylated side product was removed from the reaction mixture by recrystallisation from hot EtOH, isolation of the mono-, di- and tri-alkylated systems was not successful. All attempts to separate the tri-substituted system from the mixture resulted in an impure product. When it became apparent that this reaction was not as straightforward as perceived, it was decided to adopt another synthetic strategy. Both Kimura *et al.*,¹³² and Sherry *et al.*,¹³³ had published a five step synthetic strategy for the synthesis of compound **71**, as illustrated in Scheme 2.9.



Scheme 2.9: Synthetic pathway for the synthesis of tri-acetamide functionalised cyclen.

Following the procedure as outlined by Sherry and co-workers,¹³³ the free amine of tri-BOC-protected cyclen, **66**, was subsequently protected by a carboxybenzyl (CBz) protecting group to give compound **71**, in 83% yield. The ^1H NMR (400 MHz, CDCl_3)

displayed both the C_2 symmetry and the high purity of the compound. The BOC groups were then removed by stirring in a 30% v/v conc. HCl/MeOH solution to give the HCl salt of the mono-CBz-protected cyclen, **72**, in quantitative yield. Again, the C_2 symmetry of the compound was visible in the NMR spectrum, with the cyclen protons observed as two broad multiplets at 3.58 and 3.09 ppm. The ligand was also characterised by ^{13}C NMR and IR.

Prior to alkylation with the acetamide pendant arms, the HCl salt of **72** was neutralised by dissolving **72** in base followed by extraction into organic solvent. The desired product was then synthesised by refluxing **72** with 2-bromoacetamide in the presence of K_2CO_3 in MeCN to give **73**. However, this reaction failed to go to completion and a mixture of starting materials and products was obtained. It was attempted to isolate the product using flash silica chromatography, however, this was unsuccessful. The product was not recovered following column chromatography, and it was thought that it degraded on the silica. Morrow and co-workers published a dinuclear Eu(III) complex formed in solution by a bridging hydroxo group.¹¹⁵ They found that this compound did not give rise to the anticipated increase in the rate of phosphodiester hydrolysis. As such, it was decided to discontinue the attempted synthesis of compound **40** and our attention was focused on the synthesis of **41**, and other products.

2.4. Introduction to Xylene Spaced Systems

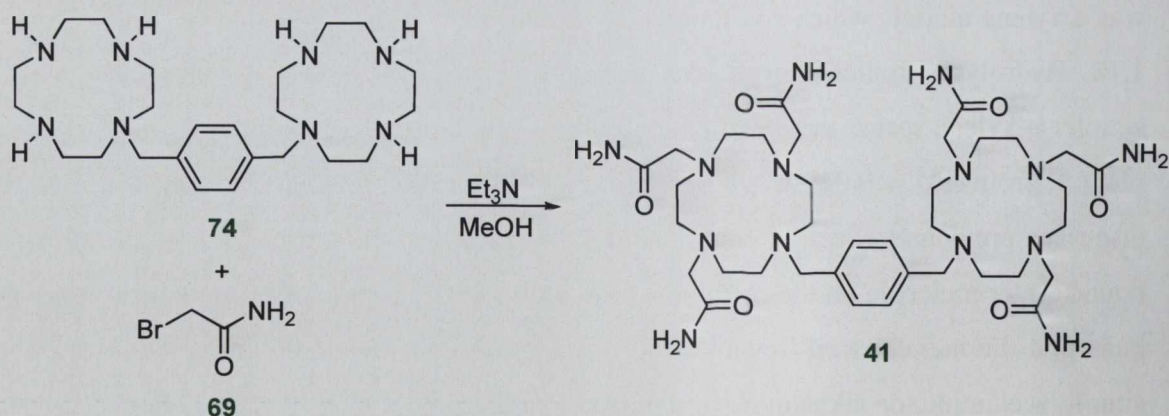
The PhD. research of Dr. Harte, in the Gunnlaugsson lab, investigated different combinations of spacers and arms in dinuclear systems for the effective hydrolysis of **HPNP**,¹⁰¹ as outlined in Section 1.14 and 2.2. It was found that the most effective spacer was a xylene moiety, which combined both flexibility and rigidity, as discussed in Section 1.12. Hydrolysis studies showed that replacing a bis-acetamide-*p*-xylene spacer with a simpler *p*-xylene spacer increased the rate of phosphodiester hydrolysis to $k_{\text{obs}} = 1.48 \text{ h}^{-1}$ at pH 7.4, from $k_{\text{obs}} = 0.027 \text{ h}^{-1}$, a dramatic 54-fold increase in the rate enhancement. As discussed previously (Section 1.14), this was attributed to the presence of a second metal bound water molecule on the metal ion. Continuing on this family of compounds, both the *para* and the *meta* spaced ligands were to be synthesised and functionalised with either simple acetamide, or alkylamine acetamide, **59**, arms. The *para*-spaced system had been previously synthesised by Dr. Harte, however it was necessary to re-synthesise the complex for the current study. The synthesis and characterisation of this family of complexes is discussed in the next section.

2.4.1. Synthesis of *p*-Xylene Acetamide Bis-System

As discussed in Section 2.1 and 2.2, there are a variety of synthetic methods for the successful formation of the bis-cyclen framework. One of these is the glyoxal protection method, developed by Handel and co-workers,¹³⁵ and detailed in Section 2.3.1. This method was utilised herein. The *p*-xylene bis-cyclen frame, **74**, was successfully synthesised in 85% overall yield as a white powder which precipitated from solution. The ¹H NMR spectrum (400 MHz, CDCl₃) displayed the expected C₂ symmetry of the compound with a single resonance at 7.23 ppm being observed for the four aromatic protons, while the four benzylic protons of the spacer were also observed as a singlet at 3.48 ppm. The 32 cyclen protons were observed as three broad singlets, one integrating for sixteen protons and the other two integrating for eight protons each. The C₂ symmetry was also evident from the ¹³C spectrum. The ESMS showed the product was present, and *m/z* = 447.3912 which corresponds to the (M+H)⁺ ion was observed.

The previously synthesised *N,N*-dimethyl functionalised analogue **38** (structure shown in Section 1.14) gave rise to the highest rate of phosphodiester hydrolysis recorded to that date by the Gunnlaugsson group with $k = 4.1 (\pm 0.2) \times 10^{-4}$, a rate enhancement, k_{rel} of 12,341.¹⁰¹ As such, the *N*-acetamide arm was utilised to further investigate the effect of the arm on the rate of phosphodiester hydrolysis.

Alkylation of **74** was carried out in MeOH, with 6.6 molar equivalents of 2-bromoacetamide in the presence of Et₃N, Scheme 2.10. A white powder precipitated out of solution, which was filtered and washed with DCM. This gave the desired product **41** with no need for further purification.



Scheme 2.10: Synthetic pathway for the preparation of **41**.

The ^1H NMR (400 MHz, $\text{DMSO-}d_6$) for the ligand is shown in Figure 2.6. The same C_2 symmetry as seen in the precursor was observed, with a single resonance, a singlet, occurring for the aromatic protons at 7.22 ppm and another singlet was observed corresponding to the benzylic protons, resonating at 3.47 ppm. The amide CH_2 were split into two signals, one resonating at 2.96 ppm, integrating for 4 protons, while a second signal occurred at 2.82 ppm, integrating for eight protons. The cyclen protons were verified by HSQC NMR experiment, as the solvent signal from the $\text{DMSO-}d_6$ partially obscured the cyclen signal. The presence of the product was again confirmed by ESMS where $m/z = 811.5019$, corresponding to $(\text{M}+\text{Na})^+$ was observed. The ligand was fully characterised by ^{13}C NMR, IR and elemental analysis.

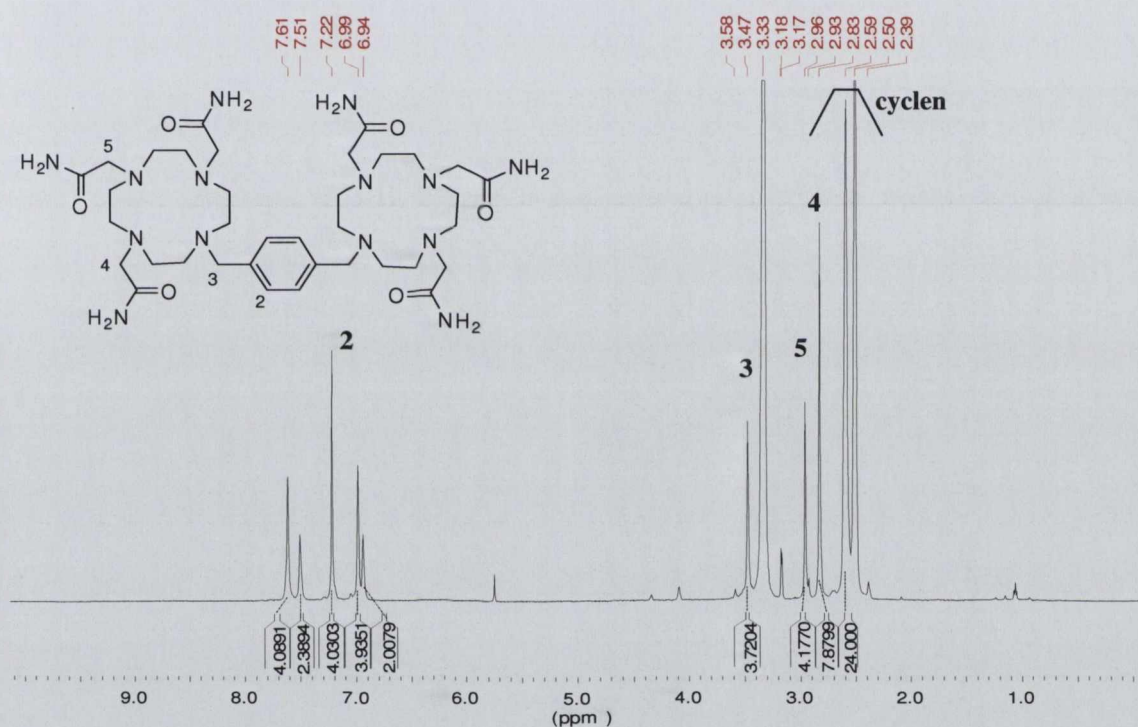
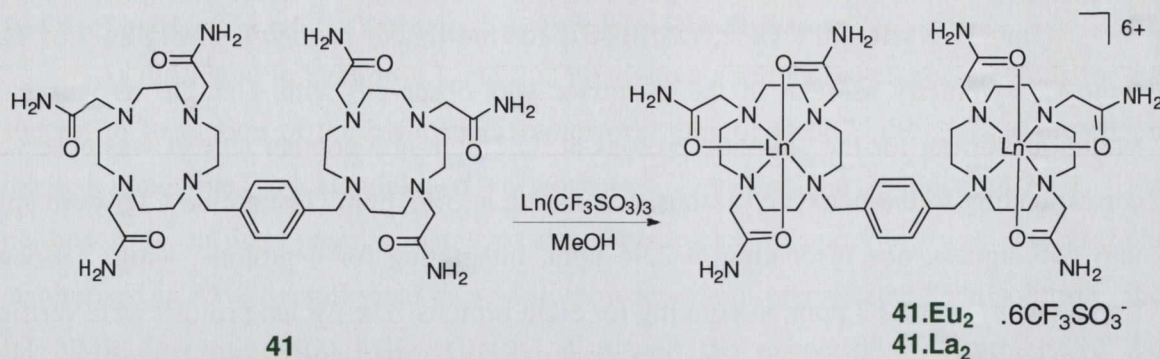
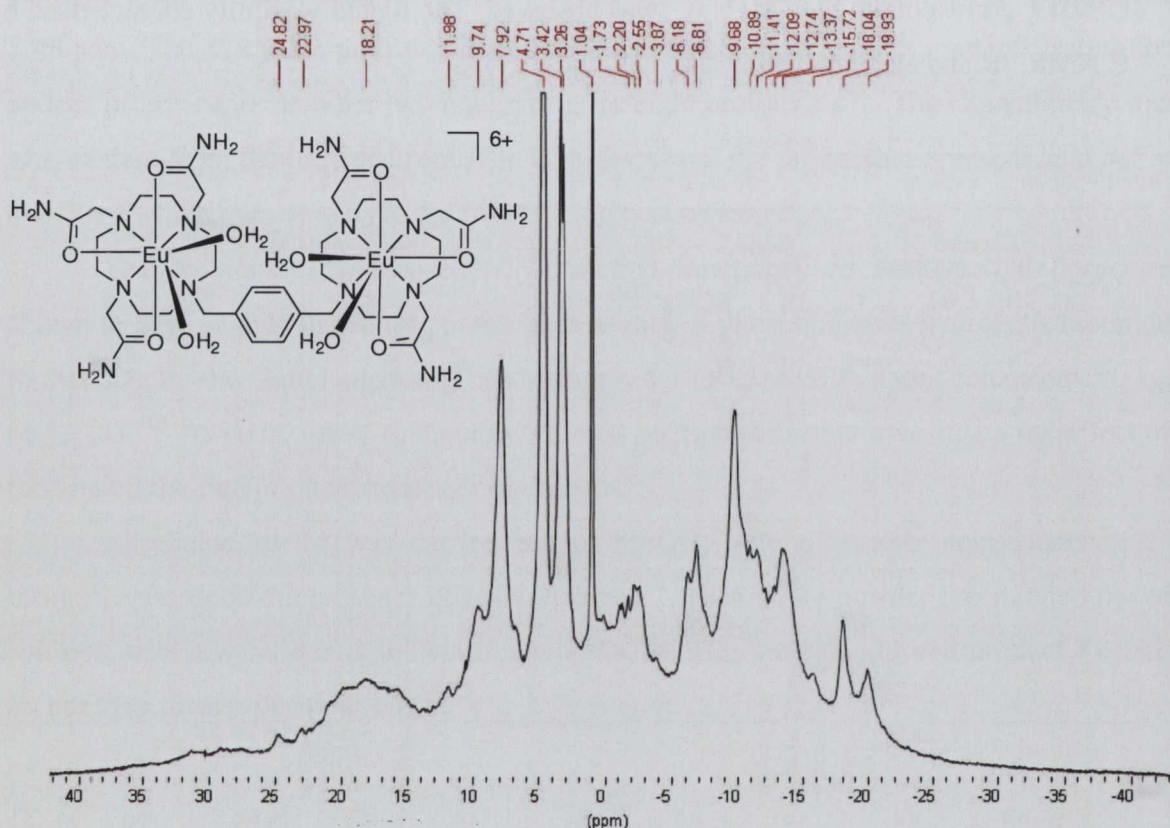


Figure 2.6: The ^1H NMR spectrum (400 MHz, D_2O) of ligand 41.

2.4.2 Lanthanide Ion Complexation of 41

The complexes **41.Eu₂** and **41.La₂** were synthesised by refluxing the ligand in MeOH, in the presence of two equivalents of the appropriate lanthanide triflate salt for approximately 16 hours, Scheme 2.11. The resulting complexes were soluble in MeOH and after cooling to room temperature, the solutions were reduced in volume under reduced pressure and added dropwise to vigorously stirred diethyl ether (150 mL). The resulting precipitates were isolated and dried under vacuum to give very hygroscopic white solids in 73 and 85% yield for **41.Eu₂** and **41.La₂**, respectively.

Scheme 2.11: Lanthanide ion complexation of **41**.Figure 2.7: The ^1H NMR spectrum (400 MHz, D_2O) of **41.Eu₂** showing characteristic broadening and shifting of the peaks.

The ^1H NMR spectrum (400 MHz, D_2O) of **41.Eu₂** is shown in Figure 2.7, and the characteristic broadening and shifting of peaks associated with the presence of the paramagnetic Eu(III) ion was observed. The spectrum included resonances ranging from 24.82 to -19.93 ppm. The ^1H NMR spectrum (400 MHz, D_2O) of **41.La₂** showed broad resonances in the cyclen region, which is significant of Ln(III) complexation.

The complexes were also characterised by several other spectroscopic techniques, including infrared spectroscopy which showed an anticipated decrease in the C=O stretching frequency of **41** from 1663 cm^{-1} to 1590 cm^{-1} for **41.Eu₂** and to 1598 cm^{-1} for **41.La₂**, Table 2.4. Mass spectrometry also verified the presence of the complexes. Using

ESI⁺, **41.La₂** was observed as $m/z = 831.01$, for the peak $[M+4(\text{CF}_3\text{SO}_3)]^{2+}$. The HRMS was obtained for the complex **41.Eu₂**, $m/z = 1392.2599$, $[M+2(\text{CF}_3\text{SO}_3)]^{3+}$ and for **41.La₂**, with $m/z = 1361.2115$, $[M+2(\text{CF}_3\text{SO}_3)_3-2\text{H}^+]^+$, and the isotopic pattern was shown to match the theoretical isotopic pattern well, Figure 2.8.

Table 2.4: Carbonyl stretching frequencies for ligand **41** and complexes **41.Eu₂** and **41.La₂**.

Compounds	C=O stretching freq. (cm ⁻¹)
41	1663
41.Eu₂	1590
41.La₂	1598

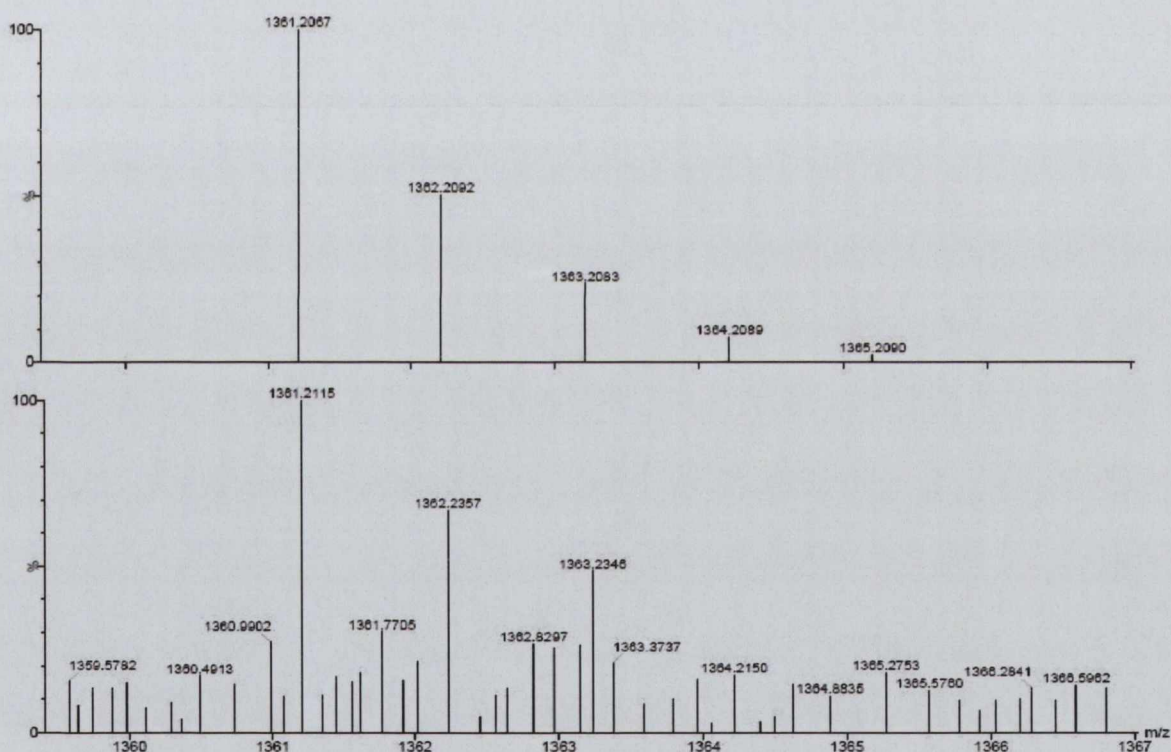


Figure 2.8: The isotope model (top) and observed spectrum (bottom) ESMS of **41.La₂** showing the La(III) isotope distribution pattern. $[M+2(\text{CF}_3\text{SO}_3)_3-2\text{H}^+]^+$

Luminescent measurements were used to determine the radiative rate constants for the Eu(III) complex, **41.Eu₂**, Table 2.5. A rate constant $k = 2.55 \text{ ms}^{-1}$ was recorded for the complex in H₂O, compared to a rate constant $k = 0.64 \text{ ms}^{-1}$ recorded in D₂O, which corresponded to lifetimes $\tau = 0.39 \text{ ms}$ and 1.6 ms , respectively. Shorter lifetimes are generally recorded for complexes in H₂O as a result of the quenching by OH oscillators bound to the metal ion. A q -value of 1.7 was determined, indicating that there were two

water molecules on each metal centre. The lifetimes were fit to a mono-exponential decay, indicating the presence of a single species in solution.

Table 2.5 Lifetime Studies for **41.Eu₂**.

Complex	k (ms ⁻¹)	τ (ms)	k (ms ⁻¹)	τ (ms)	$q = \pm 0.5$
	H ₂ O	H ₂ O	D ₂ O	D ₂ O	
41.Eu₂	2.55	0.394	0.64	1.57	1.7

2.4.3 Solid State Analysis of **41.La₂**

Single crystals of **41.La₂**, suitable for X-ray diffraction analysis, were grown by Dr. Harte by slow diffusion from a solution of the complex in MeCN. X-ray crystallographic analysis (Appendix A4.1) was carried out by Dr. Tom McCabe, in the School of Chemistry, TCD. The resulting crystal structure is shown and discussed herein, Figure 2.9, to shed light on the solid state structure of such dinuclear lanthanide complexes.

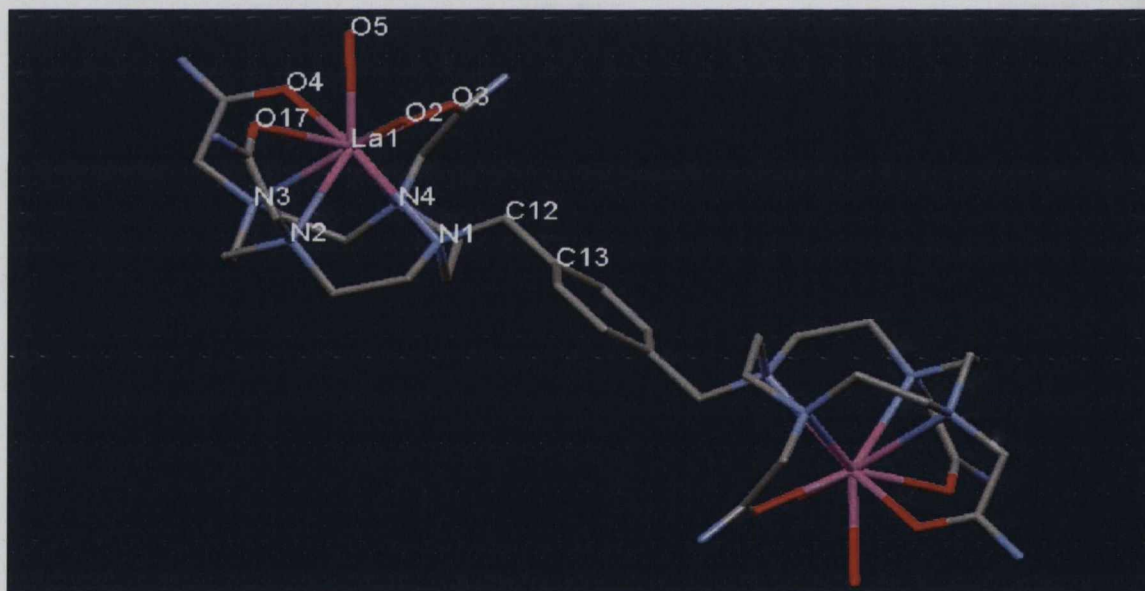


Figure 2.9: X-ray crystal structure of **41.La₂**. The hydrogen atoms, counter anions and solvent molecules have been removed for clarity.

The C₂ symmetry of the complex was observed in the solid state and the two cyclen rings were observed to be *anti* to each other, and that each of the La(III) ions was coordinated to the four nitrogens of the cyclen ring and to the three acetamide arms in a square antiprismatic geometry. Each of the La(III) ions was found to be nine-coordinate as each possessed two metal bound water molecules, giving an overall nine coordinate environment around each metal ion. This was unexpected as La(III) would normally be expected to be ten-coordinate in such a structure, due to its large ionic radius.⁷⁶ The crystal

structure also shows that the La(III) ion sits out of the cyclen frame, as the diameter of the metal ion is too large to be fully accommodated by the cyclen cavity. Despite many attempts, crystals of **41.Eu₂** suitable for X-ray diffraction analysis were not obtained. Selected bond lengths and angles for **41.La₂** are listed in Table 2.6.

Table 2.6: Selected bond lengths and bond angles for **41.La₂**.

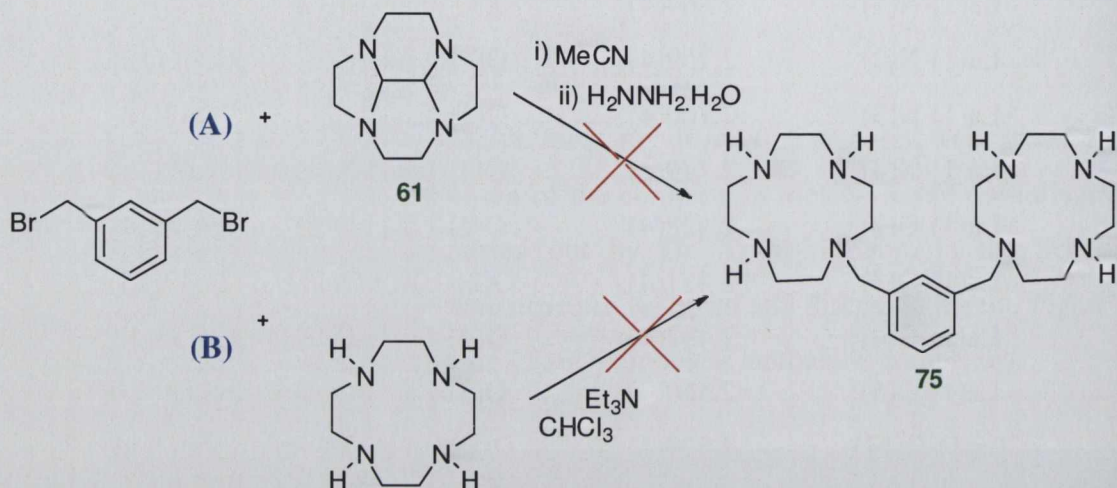
Bond Label	Length (Å)	Angle Label	Angle (°)
La(1)-N(1)	2.804(4)	O(17)-La(1)-N(2)	61.45(12)
La(1)-N(2)	2.759(4)	O(4)-La(1)-N(3)	60.76(12)
La(1)-N(3)	2.760(4)	O(2)-La(1)-N(1)	76.48(13)
La(1)-N(4)	2.779(4)	O(3)-La(1)-N(4)	61.62(13)
La(1)-O(2)	2.528(4)	O(2)-La(1)-O(5)	69.14(14)
La(1)-O(3)	2.471(4)	O(2)-La(1)-O(17)	74.49(13)
La(1)-O(4)	2.482(4)	O(4)-La(1)-O(2)	133.23(13)
La(1)-O(5)	2.560(4)	O(3)-La(1)-O(4)	84.84(13)
La(1)-O(17)	2.529(4)	O(3)-La(1)-O(17)	145.92(13)
C(12)-N(1)	1.503(7)	C(12)-N(1)-La(1)	108.9(3)
C(12)-C(13)	1.519(7)	N(1)-C(12)-C(13)	115.3(5)

2.4.4. Synthesis of *m*-Xylene Acetamide Bis-System

A similar synthetic pathway to that of the *para* system, **74**, was adopted for the synthesis of the *meta* ligand, Scheme 2.12 (A). Reaction of the glyoxal protected cyclen, **61**, with the commercially available dibromo-*m*-xylene was successful, and the bis-aminal intermediate precipitated from solution during the course of the reaction. However, the deprotection of the bis-aminal using hydrazine monohydrate was not successful. Despite various attempts, the deprotected *m*-xylyl bis-cyclen, **75**, was not isolated following this procedure.

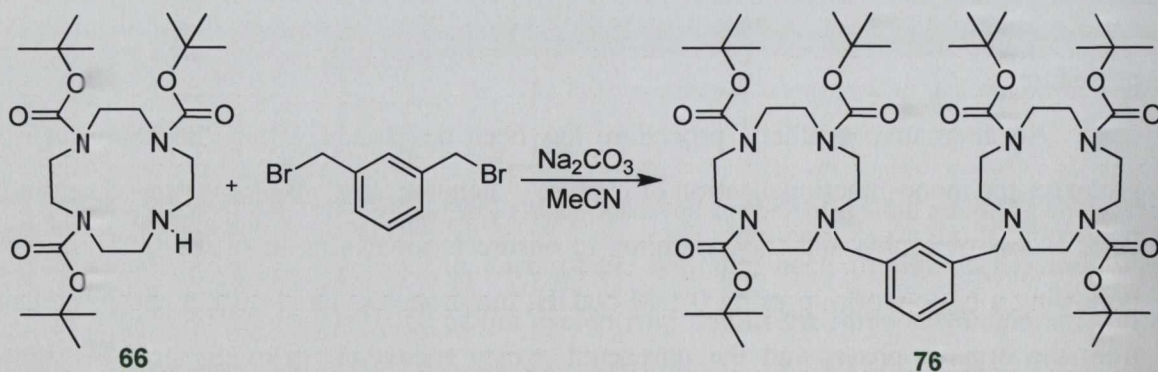
An alternative synthetic procedure has been developed within the Gunnlaugsson group for the mono-functionalisation of cyclen,¹³⁴ Scheme 2.12, (B). This procedure uses a large excess of cyclen and triethylamine, to ensure monoalkylation of the cyclen moiety. Following a basic work-up using 0.1 M NaOH, the monoalkylated cyclen can be isolated from the organic phase, and the unreacted cyclen recovered from the aqueous phase. Although this procedure has been used with great success to synthesise a wide variety of selectively functionalised cyclen-based compounds, the *m*-xylene based bis-cyclen frame, **77**, was never isolated as a pure compound using this procedure. Repeated washings failed

to remove any unreacted dibromo-*m*-xylene from solution and precipitations did not yield the pure product either. TLC analysis of the product confirmed that the product failed to move from the baseline of both silica and alumina TLC plates in a variety of solvents, including DCM/MeOH, up to 20%, hexane/EtOAc up to 100% or a solution of H₂O/MeCN/Na₂CO₃. It was also attempted to isolate the product by converting it into the corresponding HCl salt, however, this proved unsuccessful, and the impurities remained.



Scheme 2.12: Attempted synthesis of **75**, using two equivalents of glyoxal protected cyclen, **61** (**A**) or excess cyclen (**B**), both of which failed to give rise to the intended pure product.

As a result of the above problems, a new synthetic strategy based on BOC-protection, as outlined for the synthesis of **67** in Section 2.3.5, was adopted for the synthesis of **75**.¹³⁶ Two molar equivalents of tri BOC-protected cyclen, **66**, were reacted with one molar equivalent of 2,5-dibromo-*m*-xylene in the presence of excess Na₂CO₃, under reflux conditions, for 72 hours, in freshly distilled MeCN, Scheme 2.13.



Scheme 2.13: Synthetic pathway for the synthesis of **76** from two equivalents of tri-BOC protected cyclen, **66**, and 2,5-dibromo-*m*-xylene.¹³⁶

The ^1H NMR (400 MHz, CDCl_3) analysis of the crude product revealed a mixture of compounds, including unreacted di-bromo-*m*-xylene and tri-BOC protected cyclen, **66**, as well as a mono-cyclen derivative and the intended bis-cyclen product, **76**. Several attempts to isolate the desired compound, **76**, using column chromatography were carried out. Of these, the most successful separation was achieved using Hexane/EtOAc (3:2 - 2:3) as eluant which gave the desired product, **76**, in 42% yield. The ^1H NMR spectrum (400 MHz, CDCl_3) was very broad, however, this is not unusual for the spectra of BOC-protected compounds due to the tendency of the products to form as amorphous solids.

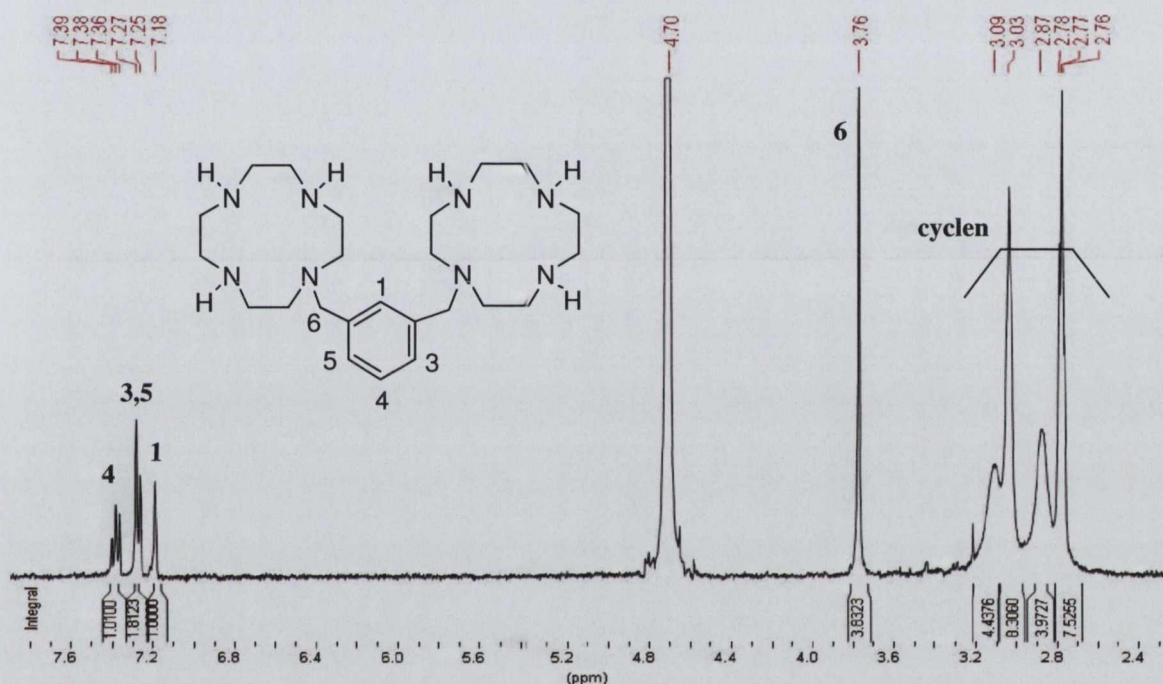
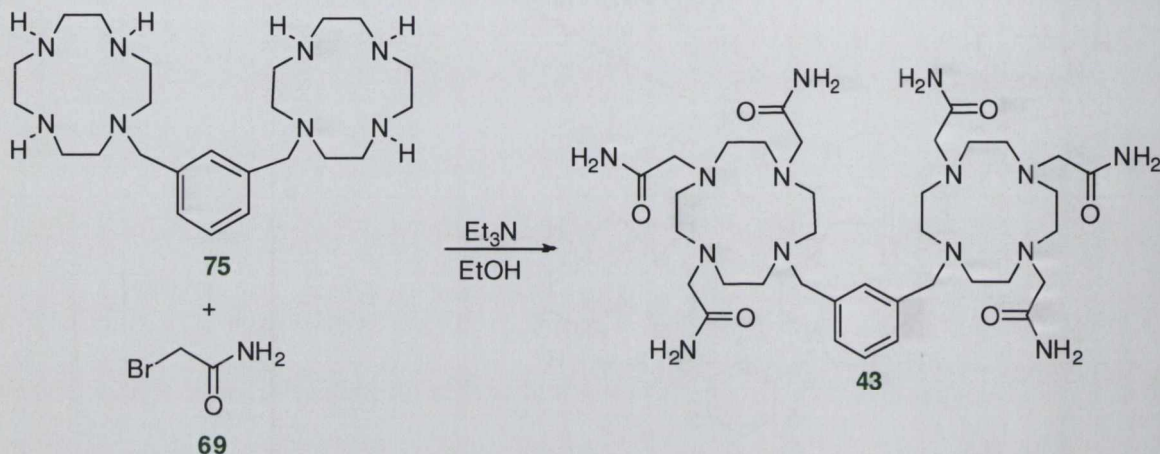


Figure 2.10: The ^1H NMR spectrum (400 MHz, D_2O) of **75** following BOC-deprotection.

The bis-system was deprotected by stirring **76** in a 30% v/v solution of conc. HCl/MeOH for three hours. The solvent was removed under reduced pressure and the residue was then redissolved in a small amount of MeOH, followed by the addition of EtOH. This led to the precipitation of the HCl salt, **77**, which was isolated *via* suction filtration as a pale creamy-yellow solid, in quantitative yield. The ^1H NMR spectrum (400 MHz, D_2O) shown in Figure 2.10, revealed the desired product to be of high purity. The C_2 symmetry of the compound was similar to that observed for the *p*-xylyl bis-cyclen frame, **75**.

The final step in the synthesis of the ligand was the alkylation of the bis-cyclen frame with 2-bromoacetamide, which was carried out in a similar manner to that of **41**, Scheme 2.14. The solution was refluxed for three days and after cooling to room

temperature, the solvent was concentrated to approx. 2 mL under reduced pressure, and added to vigorously stirred CH_2Cl_2 (150 mL). The resulting white precipitate was collected by suction filtration. The crude ^1H NMR spectrum (400 MHz, D_2O) showed the presence of triethylamine and 2-bromoacetamide, along with the intended product, and no evidence of other impurities. To remove the triethylamine, the product was dissolved in a minimum volume of MeOH and precipitated from toluene, after which a further precipitation from *i*-PrOH, to remove traces of 2-bromoacetamide, gave the pure product, **43**, as an off-white solid in 63 % yield.



Scheme 2.14: Synthetic pathway for the preparation of **43**, the acetamide functionalised *m*-xylyl spaced bis-system.

A variable temperature (VT) ^1H NMR spectrum (400 MHz, D_2O) of the ligand, **43**, at 20, 30, 40 and 60 °C is shown in Figure 2.11. The spectrum at 20 °C was very broad, with all of the cyclen methylene protons integrating as one large multiplet of 48 protons.

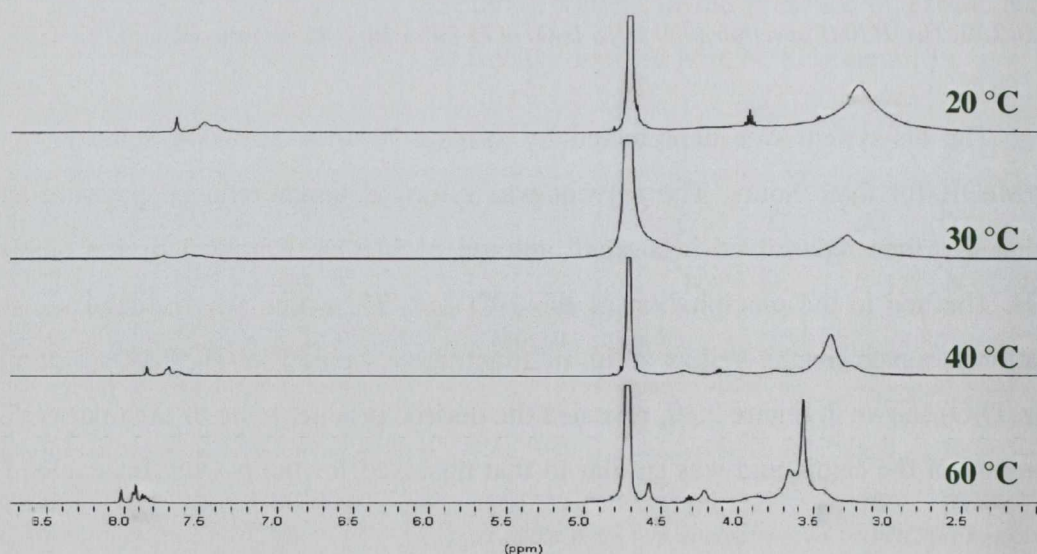
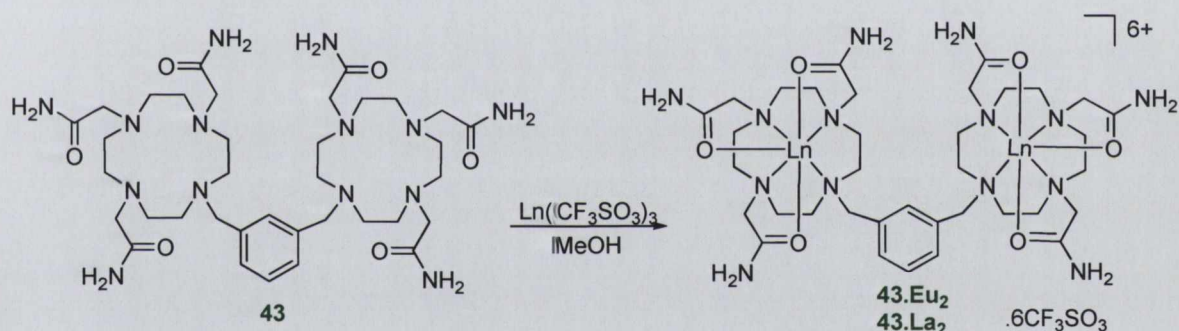


Figure 2.11: The VT ^1H NMR (400 MHz, D_2O) of the ligand **43**. Note that as the temperature increases, so too does the resolution of the ligand.

The VT NMR showed that the resolution of the spectrum improved with higher temperatures. As the fluctuation of the molecule in solution increased the number of possible conformers in solution decreased, resulting in the appearance of more resolved resonances. At 60 °C the resonance of the benzylic protons is clearly visible at 4.22 ppm, and the methylene protons of the cyclen ring and the acetamide arms were observed as separate resonances ranging from 3.89 to 3.57 ppm. A detailed ^1H NMR spectrum of **43** at 60 °C is shown in Appendix A1.2.

2.4.5 Lanthanide Ion Complexation of **43**

The lanthanide complexes **43.Eu₂** and **43.La₂** were synthesised and purified, as described for **43.Eu₂** and **43.La₂** in Section 2.4.2, by refluxing one molar equivalent of **43** in MeOH, in the presence of two molar equivalents of the appropriate Ln(III) triflate salt, Scheme 2.15. Following precipitation out of stirred diethyl ether (150 mL) the complexes were isolated in 29 % and 78 % yield for **43.Eu₂** and **43.La₂**, respectively.



Scheme 2.15: Lanthanide complexation of ligand **43**.

The Ln(III) complexes were characterised using several methods, including ^1H NMR, IR and HRMS. The ^1H NMR spectrum (400 MHz, D_2O) for **43.Eu₂** is shown in Figure 2.12, and the broadened and shifted resonances, characteristic of a Eu(III) complex are observed to range from 16.12 ppm to -17.45 ppm. The complex **43.La₂** gave rise to a broad ^1H NMR spectrum as a result of the complexation to the large La(III) metal ion, Appendix A1.3.

The complexes were also characterised using several other spectroscopic techniques, including infrared spectroscopy. The ligand gave rise to a strong band at 1665 cm^{-1} corresponding to the carbonyl group. Upon complexation to the Ln(III) metal ions, this band shifted to 1595 and 1598 cm^{-1} , for **43.Eu₂** and **43.La₂** respectively, indicating the successful formation of the complexes, Table 2.7.

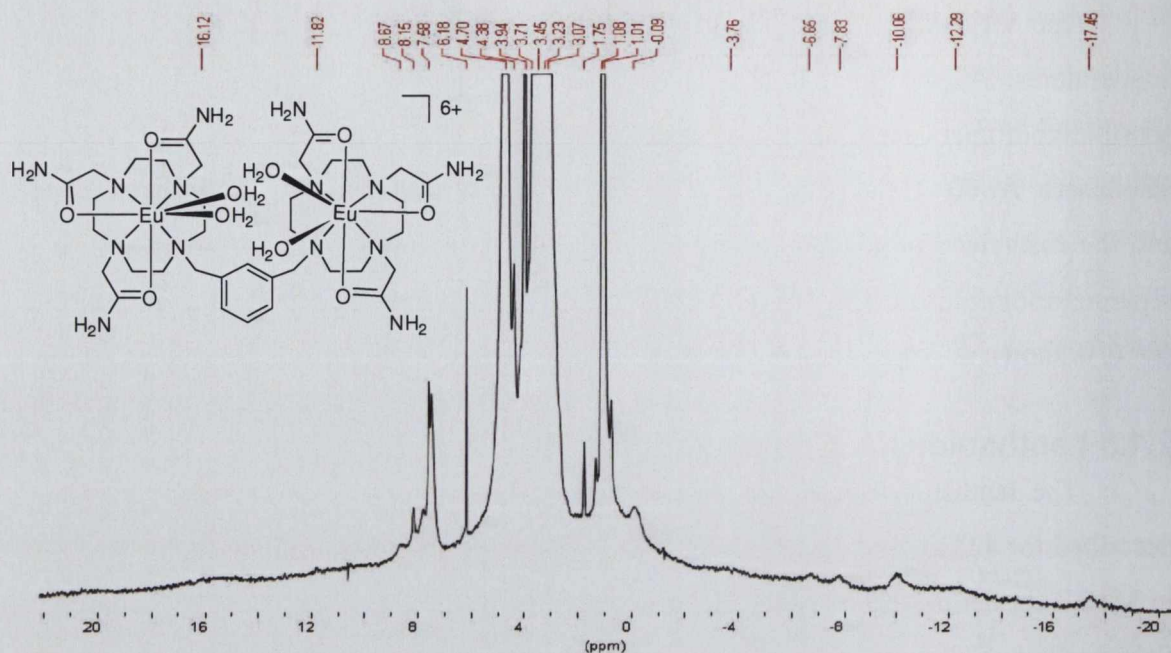


Figure 2.12: The ^1H NMR spectrum of **43.Eu₂** showing the characteristic shifted and broadened peaks arising from the presence of the Eu(III) metal ion.

Table 2.7: Carbonyl stretching frequencies for ligand **43** and complexes **43.Eu₂** and **43.La₂**.

Compounds	C=O stretching freq. (cm^{-1})
43	1665
43.Eu₂	1595
43.La₂	1598

The radiative rate constants were recorded for **43.Eu₂** and are detailed in Table 2.8. The lifetimes of the complex were determined to be 0.49 ms and 1.56 ms in H_2O and D_2O , respectively, and both were shown to fit well to a monoexponential decay, giving rise to an overall q -value of 1.9 (± 0.5). This indicated the presence of two water molecules on each metal centre.

Table 2.8 Lifetime Studies for **43.Eu₂**.

Complex	k (ms^{-1})	τ (ms)	k (ms^{-1})	τ (ms)	$q = \pm 0.5$
	H_2O	H_2O	D_2O	D_2O	
43.Eu₂	2.03	0.49	0.64	1.56	1.9

Mass spectrometry also verified the presence of the complexes. For both of the complexes, HRMS was obtained with $m/z = 1385.2323$, $[\text{M} + 2(\text{CF}_3\text{SO}_3)_3 - 3\text{H}^+]^+$ observed for **43.Eu₂**, and $m/z = 1511.1722$, $[\text{M} + 3(\text{CF}_3\text{SO}_3)_3 - 2\text{H}^+]^+$ observed for **43.La₂**. For both

complexes the isotopic distribution pattern was found to match that of the model well, and the spectrum for **43.Eu₂** is shown in Figure 2.13 below.

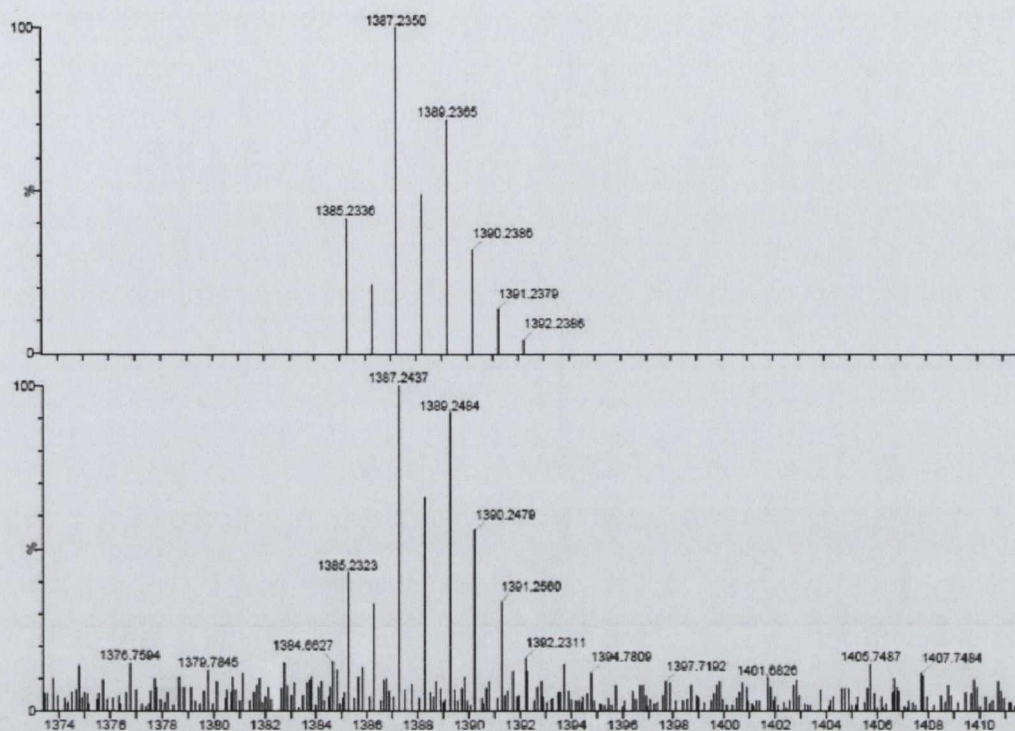


Figure 2.13: The isotope model (top) and observed spectrum (bottom) ESMS of **43.Eu₂** showing the Eu(III) isotope distribution pattern. $[M+3(CF_3SO_3)_3-2H^+]^+$.

2.4.6 Summary

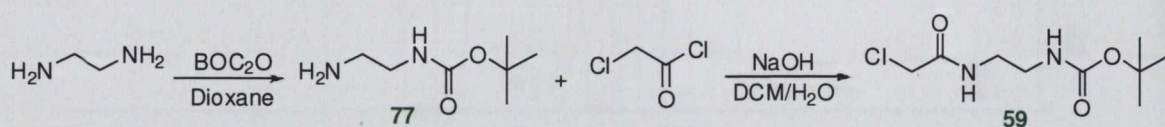
In summary, this Section has detailed the synthesis and characterisation of the acetamide functionalised ligands **41** and **43** and their corresponding Ln(III) complexes **41.Ln₂** and **43.Ln₂**. The following Section details the synthesis and characterisation of another similar family of complexes. However, these will be functionalised with a different pendant arm, which will allow further analysis as to how the structure of the complexes affects their ability to hydrolyse phosphodiester bonds.

2.5 Synthesis of Xylene Spaced Alkylamine Bis-Systems

A mononuclear ligand, functionalised with the alkylamine acetamide arm, **59**, had also proved very successful for the hydrolysis of phosphodiester bonds.^{102,122} The terminal amines of the pendant arm gave rise to a large enhancement in the rate of hydrolysis over other mononuclear complexes. Furthermore, the active pH range of the complex was shifted from basic pH to physiological pH, as outlined in Section 1.14, which was a very important development for such complexes. The *para*- and the *meta*-xylyl spaced systems were prepared with **59** as the pendant arm, and their synthesis and characterisation is detailed in the next Section.

2.5.1 Synthesis of Alkylamine Pendant Arm

The alkylamine acetamide pendant arm, **59**, was synthesised in two steps starting from ethylenediamine, Scheme 2.16. The mono-BOC-protected diamine, **77**, was prepared by reacting eight equivalents of ethylenediamine with BOC₂O in dioxane.¹⁵¹ The disubstituted product was insoluble in H₂O and so the product was purified by repeatedly washing the organic layer with H₂O. The desired compound, **77**, was isolated in 65 % yield as a pale yellow oil and was characterised by ¹H NMR, ¹³C NMR and ESMS.

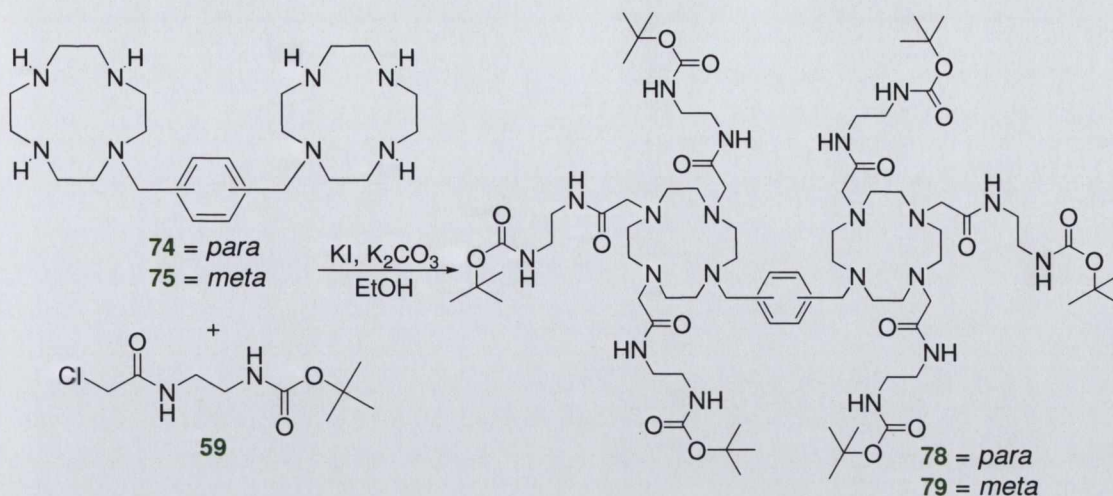


Scheme 2.16: Synthesis of BOC-protected alkylamine acetamide arm **59**.

The alkylamine chloroacetamide, **59**, was originally prepared using a modified Schotten-Baumann procedure by the reaction of the amine with chloroacetyl chloride in DCM, in the presence of Et₃N, to give the product as a brown solid in approximately 40% yield.¹²² In an effort to increase the yield of the reaction, a biphasic solution of DCM and H₂O was used, and the base was changed to NaOH, Scheme 2.16. Under these conditions the yield increased by an average of 10 % to 65 % and the product was obtained as a white solid.

2.5.2 Synthesis of *para*- and *meta*-Xylyl Spaced Alkylamine Bis-System

The bis-cyclen frames for both the *para*- and the *meta*-complexes were synthesised and characterised as described for **74** and **75**, respectively. The alkylation reaction was carried out using two similar methods, (a) and (b), Scheme 2.17. For both reactions, the bis-cyclen frame was reacted with an excess (6.6 molar equivalents) of the pendant arm **59**, in the presence of 7 equivalents of KI. For reaction (a), Cs₂CO₃ was used as base and the reaction was carried out in MeOH at reflux. For reaction (b), anhydrous K₂CO₃ was used as a base and EtOH as a solvent. All reactions were monitored by ESMS until completion. Reaction (a) took up to ten days to reach completion, however, reaction (b) reached completion within four days demonstrating that EtOH and K₂CO₃ are more efficient than MeOH and Cs₂CO₃ for this reaction. The synthesis of the *para*-system was carried out using both of the above methods, whereas, the *meta*-system was synthesised using the method, (b).



Scheme 2.17: Synthesis of **78** and **79**, the *para*- and the *meta*-spaced systems via alkylation with **59**.

The work up and purification of the alkylated *para*-, **78**, and *meta*-, **79**, systems was identical. To isolate the desired product, the reaction was cooled to room temperature, the solvent removed under reduced pressure and the resulting residue was redissolved in DCM, followed by filtration to remove any inorganic solids. Following an aqueous work-up, the products were isolated as pale yellow oils, in 74 and 60 % yield, for **78** and **79**, respectively, which solidified under high vacuum.

The ^1H NMR (600 MHz, CDCl_3) spectra of both **78** and **79** were quite broad. For compound **78**, the aromatic protons were observed as a singlet resonating at 7.19 ppm and the methylene protons of the BOC-protecting groups were observed as two singlets at 1.54 and 1.46 ppm. The ^1H NMR spectrum (600 MHz, D_2O) for the *meta*-spaced system, **79**, was broader than that of **78**. The aromatic protons of **79** did not give rise to three resonances as expected and instead were observed as two broad resonances, each integrating to two at 7.20 and 7.02 ppm. The methylene protons of the BOC-protecting groups were observed at 1.48 ppm, integrating to 54 protons and the alkyl and acetamide protons were observed as a broad multiplet at 3.41 ppm while the cyclen protons were observed as an extremely broad multiplet between 3.04 and 2.69 ppm.

The final step in the synthesis of the ligands was the removal of the BOC-protecting groups from the pendant arms. This was carried out by stirring **78** and **79** in a solution of 30 % v/v conc. HCl/MeOH for three hours. Subsequent removal of the solvent under reduced pressure gave pale yellow oils for both products. These residues were redissolved in a minimal amount of MeOH and EtOH was added to precipitate out the HCl salts. Both ligands were isolated by suction filtration to give **42** and **44** in quantitative yields, as a white and an orange solid, respectively.

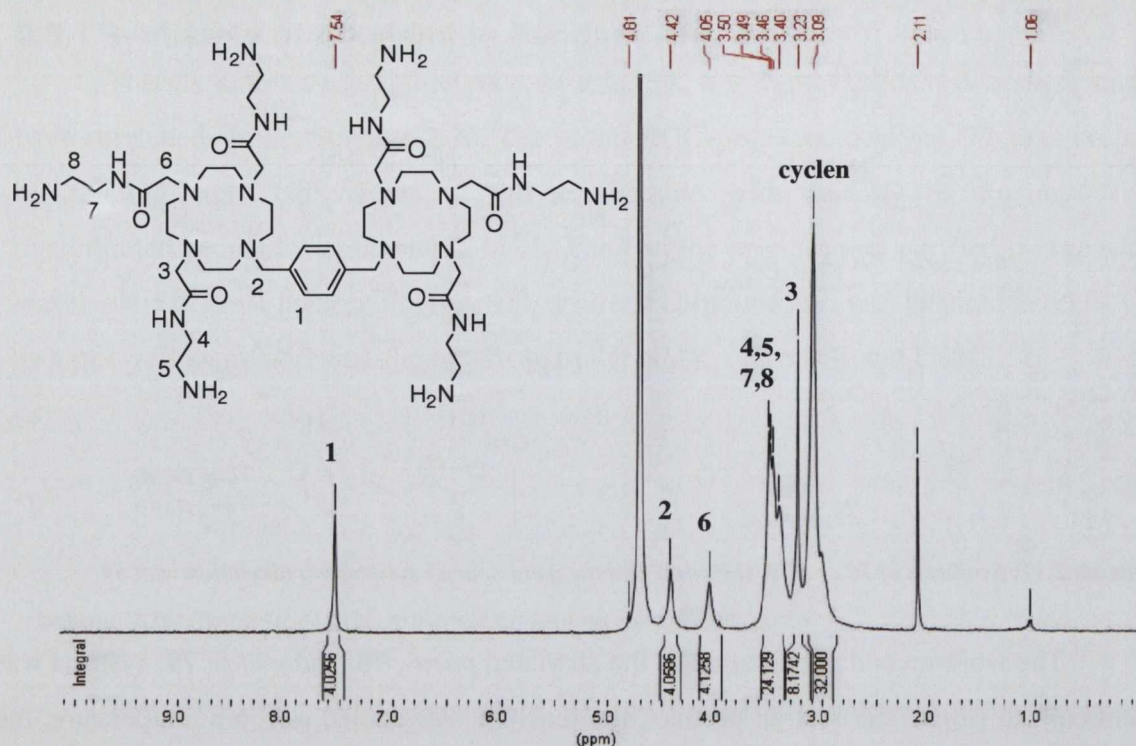


Figure 2.14: The ^1H NMR spectrum (400 MHz, D_2O) for ligand **44**. The C_2 symmetry of the compound can be seen, as well as the high purity of the compound.

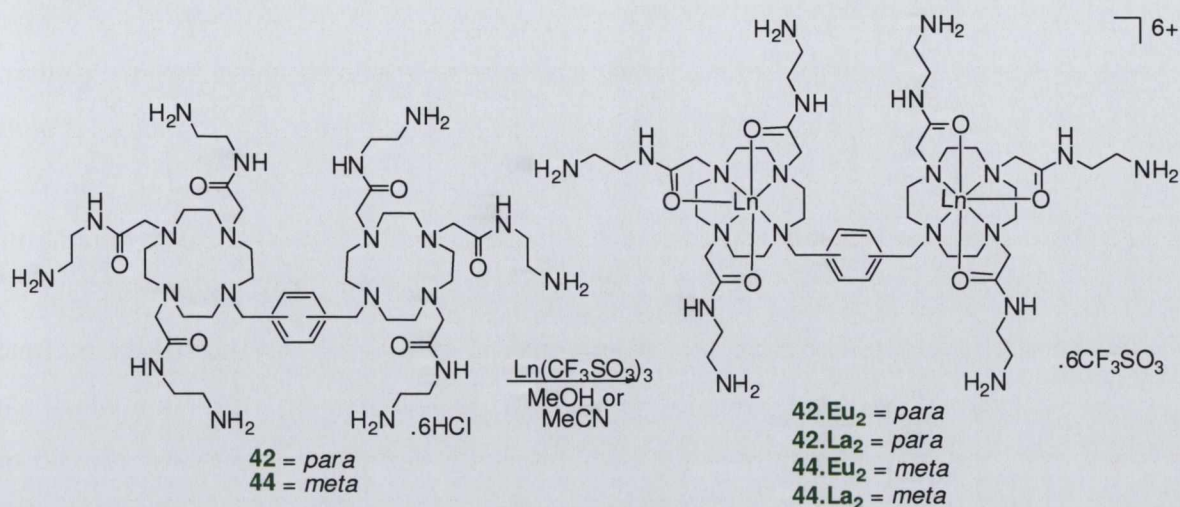
The ^1H NMR spectra (400 MHz, D_2O) for **42** is shown in Figure 2.14, and the spectra of both ligands showed the expected C_2 symmetry. The ^1H NMR spectrum (600 MHz, D_2O) of **44** gave rise to the anticipated doublet, singlet and triplet for the aromatic region at 7.60, 7.58 and 7.54 ppm, and all of the resonances were quite broad. A similar symmetry of the compound was observed in the ^{13}C NMR spectrum (150 MHz, D_2O).

The ^{13}C NMR spectrum (150 MHz, D_2O) of **44** revealed two carbonyl signals at 173.2 and 172.7 ppm, corresponding to the acetamide pendant arms. A single signal was also observed for the quaternary aromatic carbon, at 166.8, and a second resonance at 132.83 ppm which corresponded to the aromatic carbons. Both ligands were analysed by ESMS (ESI^+) and the HRMS of **42** gave $m/z = 1048.7752$, for the species $(\text{M}+2\text{H})^{2+}$, while the *meta*-ligand, **44**, gave $m/z = 1092.7460$ corresponding to $(\text{M}+2\text{Na})^{2+}$.

2.5.3 Lanthanide Ion Complexation of **42** and **44**

The complexes **42.Eu₂**, **42.La₂**, **44.Eu₂** and **44.La₂** were synthesised by refluxing the appropriate ligand in the presence of two molar equivalents of the lanthanide triflate salt for 16 h in MeOH or MeCN, Scheme 2.18. The cooled solutions were reduced in volume under reduced pressure, and added dropwise to vigorously stirred diethyl ether (150 mL), to precipitate the desired complexes. The products were isolated by suction filtration and dried under high vacuum. The complexes **42.Eu₂** and **42.La₂** were isolated as white solids in 69 and 57% yield, respectively, while the *meta*-complexes **44.Eu₂** and

44.La₂ were both isolated as hygroscopic pale orange solids in 92 and 82 % yields, respectively.



Scheme 2.18: Lanthanide complexation of **42** and **44** using lanthanide triflate salts.

The ¹H NMR (400 MHz, D₂O) spectrum of **42.Eu₂** is shown in Figure 2.15, and this effect of the metal ion can be seen by the appearance of the resonances between 19.58 and -17.34 ppm. The ¹H NMR (400 MHz, D₂O) analysis of both Eu(III) complexes of **42** and **44** showed the characteristic broadening and shifting of the axial and equatorial protons of the ligand

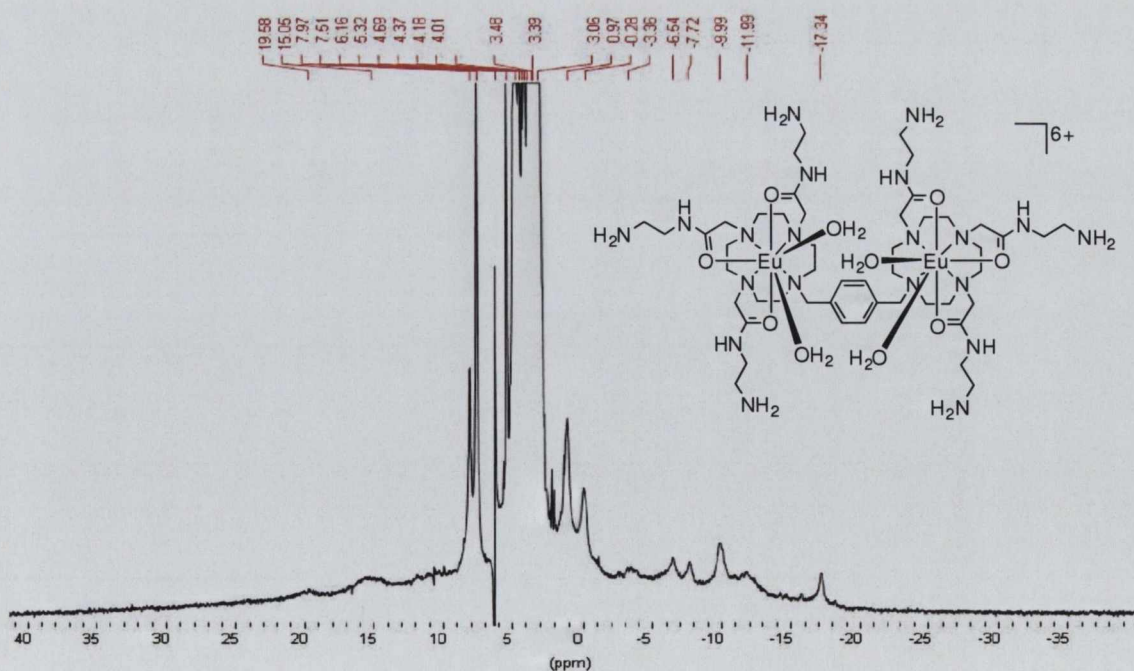


Figure 2.15: The ¹H NMR of **42.Eu₂** showing the shifted and broadened peaks of the Eu(III) complex.

The presence of the complexes was also verified using mass spectrometry. **42.Eu₂** was observed at $m/z = 1348.61$ corresponding to the $[M+H]^+$ species, and **42.La₂** was detected with $m/z = 1469.5060$, which corresponded to the $[M^{6+}+CF_3SO_3-4H^+]^+$ species and the observed isotopic pattern was found to match well to that of the calculated pattern.

Changes in the carbonyl stretching frequency of the IR spectrum indicated that the complexation of **42** to Ln(III) metal ions had been successful, Table 2.9. The number of metal bound water molecules, q , was also determined for **42.Eu₂** and **44.Eu₂** by measuring the luminescent lifetimes of the complex in H₂O and D₂O. The radiative rate constants, (k), corresponding lifetimes and the q -values are given in Table 2.10. For **42.Eu₂** the lifetimes were calculated to be 0.38 ms and 1.32 ms in H₂O and D₂O, respectively, from which a q -value of $1.7 (\pm 0.5)$ was determined. The lifetimes of **44.Eu₂** were measured as 0.65 ms and 2.04 ms in H₂O and D₂O, respectively, which gave rise to a q -value of $1.54 (\pm 0.5)$. The lifetimes for both complexes were fitted to a mono-exponential decay, indicating that there was a single species in solution. These results suggest the presence of two water molecules bound to each metal ion.

Table 2.9: Carbonyl Stretching frequencies for ligands **42** and **44** and their lanthanide complexes.

Compounds	C=O stretching freq. (cm ⁻¹)
42	1671
42.Eu₂	1627
42.La₂	1623
44	1654
44.Eu₂	1626
44.La₂	1627

Table 2.10: Lifetimes studies of complex **42.Eu₂** and **44.Eu₂**.

Complex	k (ms ⁻¹)	τ (ms)	k (ms ⁻¹)	τ (ms)	$q = \pm 0.5$
	H ₂ O	H ₂ O	D ₂ O	D ₂ O	
42Eu₂	2.61	0.38	0.74	1.32	1.70
44Eu₂	1.48	0.65	0.49	2.04	1.54

2.5.4 Summary

This Section has detailed the synthesis of a family of novel dinuclear cyclen-based lanthanide complexes, which were bridged by a xylyl based spacer and functionalised with alkylamine acetamide pendant arms. Using a variety of synthetic pathways all novel compounds were purified and fully characterised. Within this family of complexes, each

lanthanide metal ion centre was hepta-coordinated, and this left a minimum of two coordination sites free on each ion which were filled by solvent molecules, such as water. This was verified through the determination of q -values and was also confirmed by a crystal structure, in the case of **41.La₂**.

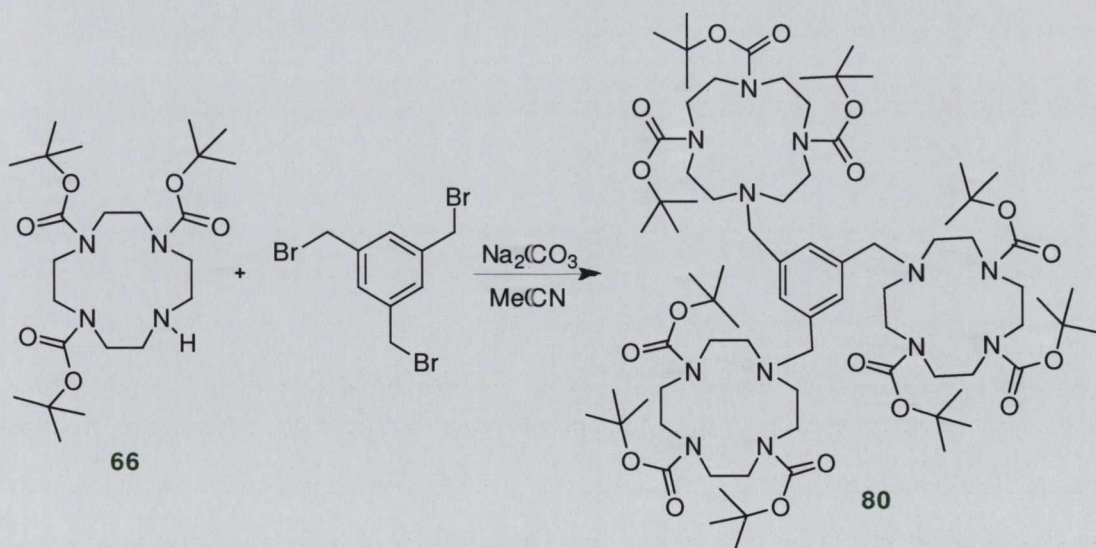
The following Section details the attempted synthesis of a trinuclear cyclen-based lanthanide complex.

2.6 Introduction to Trinuclear System

As discussed in Section 1.6.4, dinuclear complexes can give rise to highly efficient phosphodiester hydrolysis through a cooperative mechanism of double Lewis acid activation. Similarly, several trinuclear species utilising transition metal ions have been described, in which the presence of the third metal ion has resulted in a large rate enhancement over those generated by the corresponding dinuclear species.⁴⁴⁻⁴⁶ Examples include that of Komiyama *et al.* in which a trinuclear pyridinyl system gave rise to a significant rate enhancement over the analogous dinuclear system,⁷⁹ or the calixarene based trinuclear Zn(II) system by Reinhoudt *et al.*⁸⁶ As such, a trinuclear ligand, analogous to the xylyl-spaced dinuclear ligands outlined previously, was designed.

2.6.1 Synthesis of a Trinuclear System

The tri-cyclen frame was prepared according to a modification of a literature procedure by Kimura and co-workers,, using the commercially available 1,3,5-tribromomethyl benzene and tri-BOC-protected cyclen, **66**, Scheme 2.19.⁶⁰



Scheme 2.19: Synthesis of **80**, a BOC-protected tri-cyclen frame.

The literature procedure recommends the addition of benzylchloroformate to the reaction mixture after 72 hours in order to alkylate any unreacted tri-BOC-protected

cyclen, **66**. However, this product was found to have the same R_f as the desired product, **80**, in a variety of solvent systems, and consequently, the desired compound could not be isolated successfully. Instead, **80** was separated from any unreacted starting materials by column chromatography using C-18 silica, with eluant MeOH:DCM gradient elution (100→100%). This gave the desired product, **80**, as a colourless viscous oil in 75% yield. The ^1H NMR spectrum (600 MHz, CDCl_3) was quite broad and the C_3 symmetry of the product was observed, Figure 2.16. The aromatic region contained a single resonance at 7.0 ppm which integrated to the three protons of the aromatic ring. Another singlet was observed at 3.79 ppm for the benzylic protons which integrated to six protons. The methylene protons of the BOC-protecting groups were observed as two broad overlapping singlets which integrated to 81 protons at 1.49 ppm.

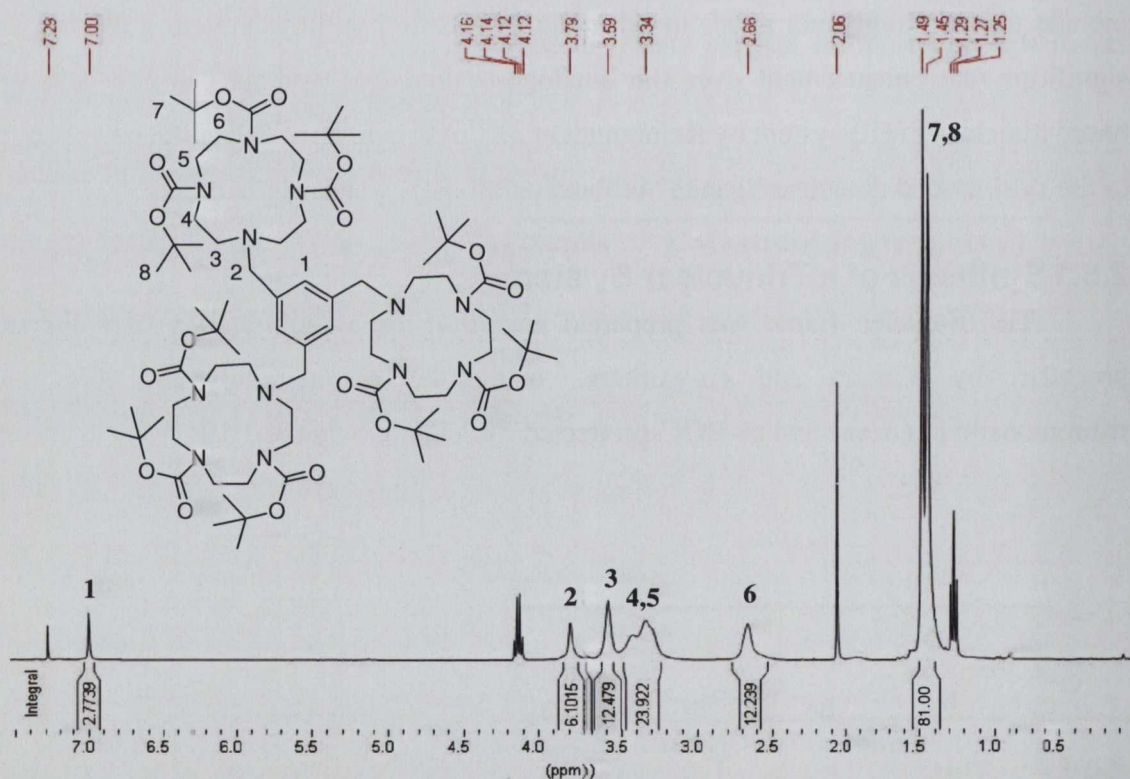


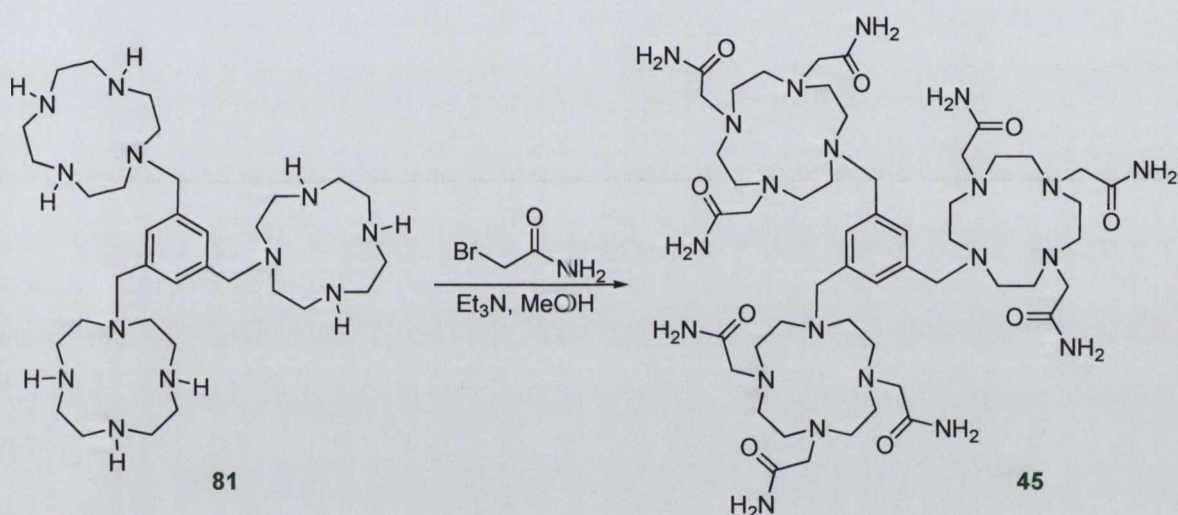
Figure 2.16: The ^1H NMR (600 MHz, CDCl_3) spectrum of BOC-protected tri-cyclen frame, **80**.

The BOC-deprotection of **80** was achieved by stirring in a solution of 30% v/v conc. HCl/MeOH, using the same procedure as detailed previously. The desired product **81** was precipitated from a MeOH solution *via* the addition of EtOH, and an orange solid was collected by suction filtration, in quantitative yield. The ^1H NMR was similar to that of the precursor, and again the C_3 symmetry of the compound was visible.

Alkylation of **81** was attempted using both the *N*-acetamide and the alkylamine acetamide arms to give **45** and **46**, respectively (Section 1.15.1). However, only the former

alkylation was successful. It is unclear why the reaction with the latter was unsuccessful, however, it is possible that the structure was too sterically hindered for the reaction to proceed.

Compound **45** was prepared using the same procedure as for the synthesis of **41**. The deprotected ligand was dissolved in MeOH with 9.5 molar equivalents of 2-bromoacetamide and 9 molar equivalents of triethylamine, Scheme 2.20, and the reaction was monitored using ESMS over five days until it was observed to reach completion. A white product was obtained by precipitation from DCM, which was then recrystallised from a 50/50 v/v solution of *i*-PrOH/MeOH, to yield the desired product, **45**, as a yellow solid in 79% yield.



Scheme 2.20: Preparation of **45** via alkylation of **81** in MeOH.

The ^1H NMR spectrum (600 MHz, D_2O) of **45** is shown in Figure 2.17. Where a single resonance had been anticipated in the aromatic region, corresponding to the three symmetrical protons of the aromatic ring, instead, a very broad resonance with a large shoulder was observed. This NMR was recorded at room temperature and it was possible that the shoulder was due to a “tumbling” effect. A VT ^1H NMR experiment carried out at 20, 40 and 60 $^\circ\text{C}$, revealed this to be most likely due to the presence of conformational isomers in solution, possibly as a result of a “two up, one down” conformation of the three cyclen moieties. As the temperature increased, the shoulder disappeared and the single resonance became sharper in appearance. The ^1H NMR of **45** at 40 $^\circ\text{C}$ is shown in Figure 2.17, despite drying at 40 $^\circ\text{C}$, under high vacuum, traces of solvents remained, most likely the solvents were trapped in the cyclen cavities.

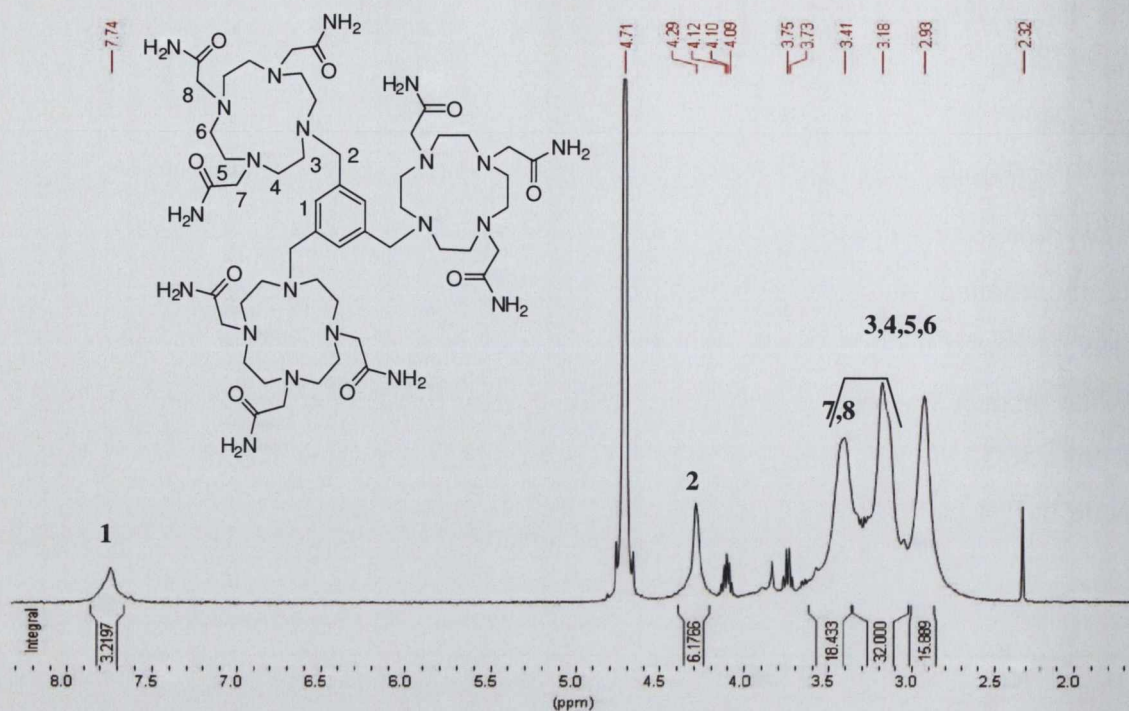
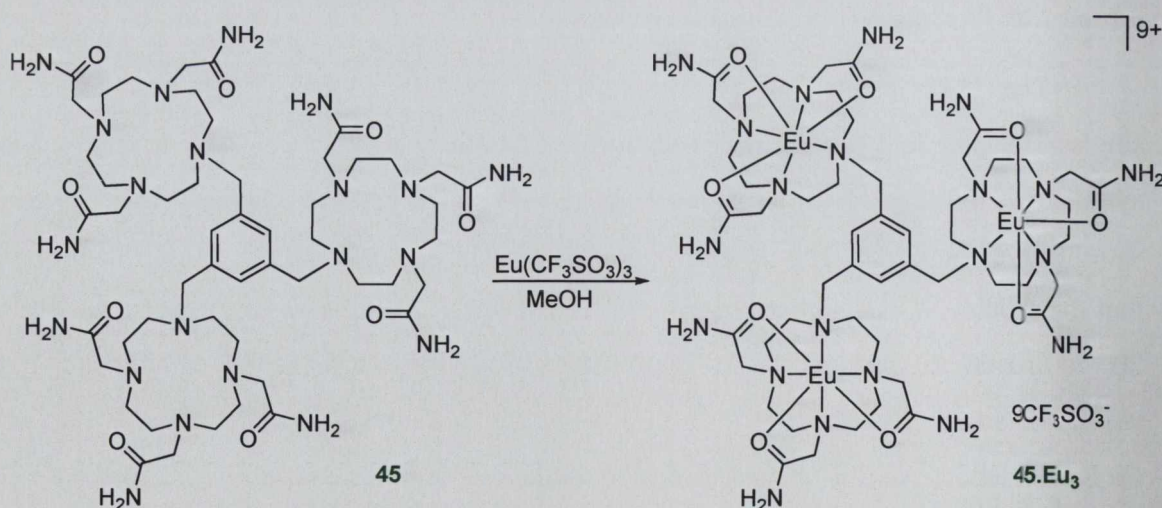


Figure 2.17: The ^1H NMR of **45** at 40 °C. The C_3 symmetry of the ligand can be seen in the NMR spectrum.

2.6.2 Lanthanide Ion Complexation of **45**

The Eu(III) complex of **45** was prepared by refluxing the ligand in the presence of 3 molar equivalents of $\text{Eu}(\text{CF}_3\text{SO}_3)_3$ in MeOH for sixteen hours, Scheme 2.21. The desired product, **45.Eu₃**, was isolated by precipitation from vigorously stirred diethyl ether (150 mL). The resulting white precipitate was isolated to give a white solid, **45.Eu₃**, in 73% yield.



Scheme 2.21: Preparation of **45.Eu₃** by overnight reflux in MeOH in the presence of three equivalents of $\text{Eu}(\text{CF}_3\text{SO}_3)_3$.

Despite drying the complex over P_2O_5 in a water bath at 40 °C, it remained saturated with MeOH. The 1H NMR spectrum (400 MHz, $D_2O/MeOH$) is shown in Figure 2.18, and was typical of that of a Eu(III) complex, with broad and shifted resonances, from 16.92 to -18.06 ppm, indicating that complexation had occurred. The product was not observed by mass spectrometry using either ESI⁺ or MALDI-TOF. Furthermore, the same NMR spectrum was not observed when the experiment was later repeated using the same sample. It was postulated that the complex was unstable and was possibly decomplexing in solution, or that the ligand was unstable and was decomposing, possibly at the benzyl position.

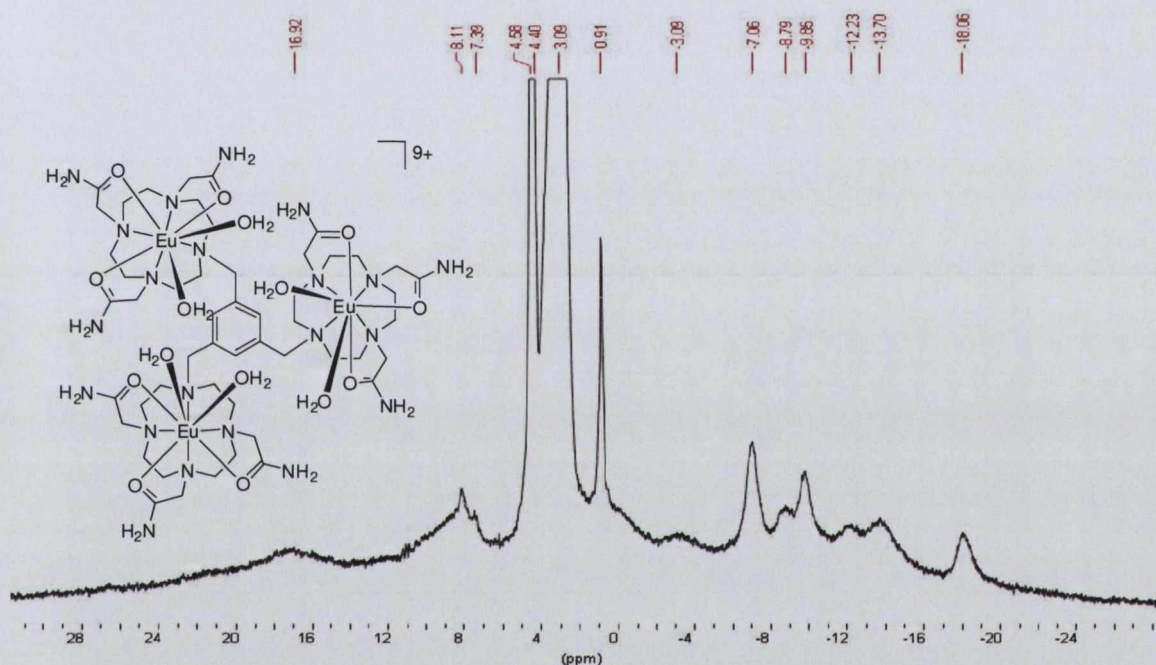


Figure 2.18: The 1H NMR spectrum (400 MHz, $H_2O/MeOH$) of $45.Eu_3$.

The phosphorescence of $45.Eu_3$ was measured in H_2O and is shown in Figure 2.19, the insert shows the absorption spectrum of $45.Eu_3$. To confirm that the complex had formed, the Eu(III) ions were indirectly excited through the spacer moiety at $\lambda_{max} = 266$ nm, which resulted in a typical Eu(III) phosphorescence spectrum, verifying the presence of the complex. However, this gave no information about the overall stability of the complex, only that the complex had formed.

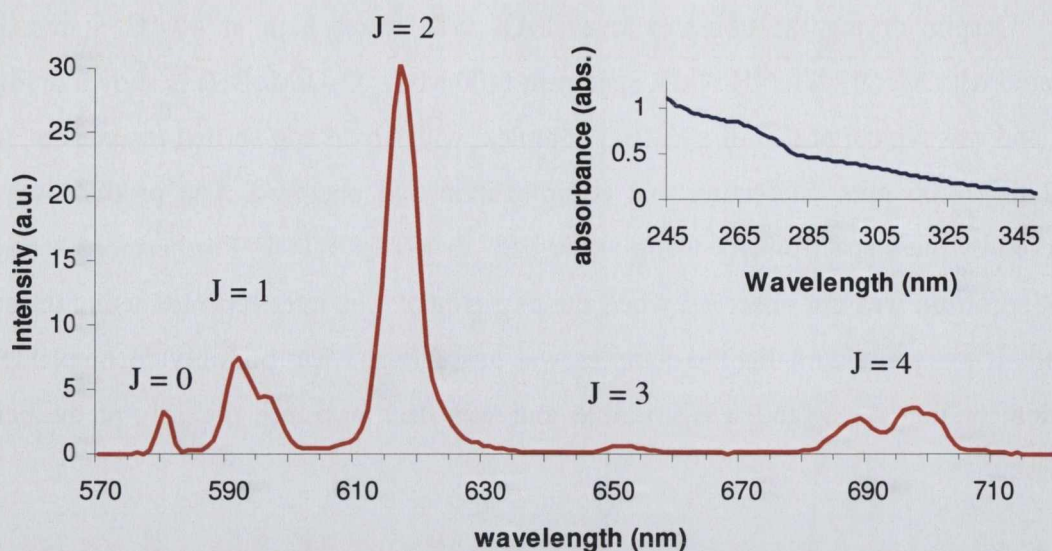


Figure 2.19: Phosphorescence spectrum of **45.Eu₃**. The spectrum was generated by excitation into the benzylic spacer at 266 nm, and emission was recorded between 570 and 720 nm. The characteristic Eu(III) phosphorescence spectrum is observed. The insert shows the UV-Vis absorption spectrum of **45.Eu₃** with $\lambda_{\text{max}} = 266$ nm.

2.6.3 Summary

This Section detailed the synthesis and characterisation of a trinuclear cyclen based lanthanide complex. Unfortunately, the final product, the complex **45.Eu₃** proved to be unstable in solution, and consequently, this complex was not subject to full characterisation. The next Section details the synthesis of a family of mononuclear complexes, which were designed as analogues of the dinuclear complexes synthesised in Sections 2.3-2.5.

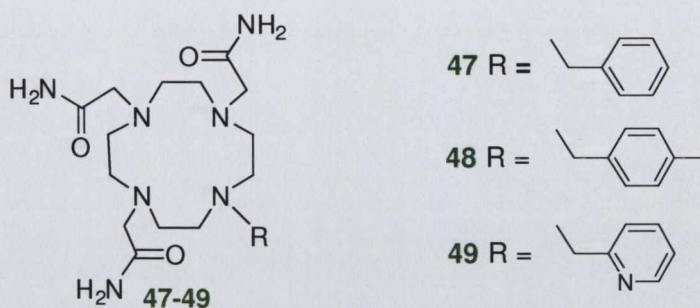
2.7 Introduction to Mononuclear Systems

The dinuclear cyclen based systems were designed with the aim of achieving the cooperative action of two metal ions for the efficient hydrolysis of phosphodiester bonds. To accurately define the mechanism as being cooperative,^{4,37} it was essential to compare these systems to their analogous mononuclear cyclen based complexes. Although a wide variety of such complexes had been synthesised previously within the Gunnlaugsson group,^{62,102,119,120,124} the majority of these were tetra-substituted cyclen-based complexes. While several tri-substituted cyclen-based lanthanide complexes have been synthesised and published,^{13,14} none were considered to be suitable analogues for the systems synthesised herein. As such, a family of mononuclear cyclen-based lanthanide complexes were synthesised and their hydrolytic ability evaluated.

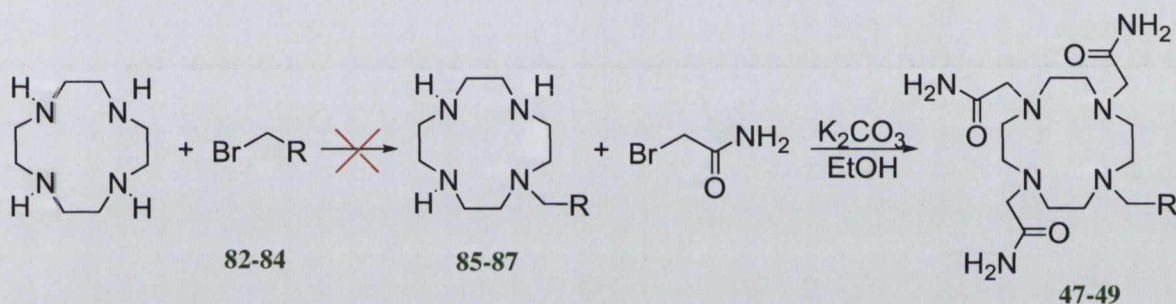
2.7.1 Synthesis of Mononuclear Systems

The R-groups chosen for the synthesis of the analogues included a pyridine, as an analogue of ligand **39**, a benzyl, as an analogue of ligands **41** and **75**, and a 4-methyl-

benzyl, as another analogue of **41**, incorporating both sides of the spacer. These are depicted below as compounds **47-49**.



The synthesis of the mono nuclear complexes was originally to be carried out *via* a monoalkylation with the appropriate R-group, followed by alkylation at the remaining three free amino moieties with the appropriate pendant arms, Scheme 2.22.



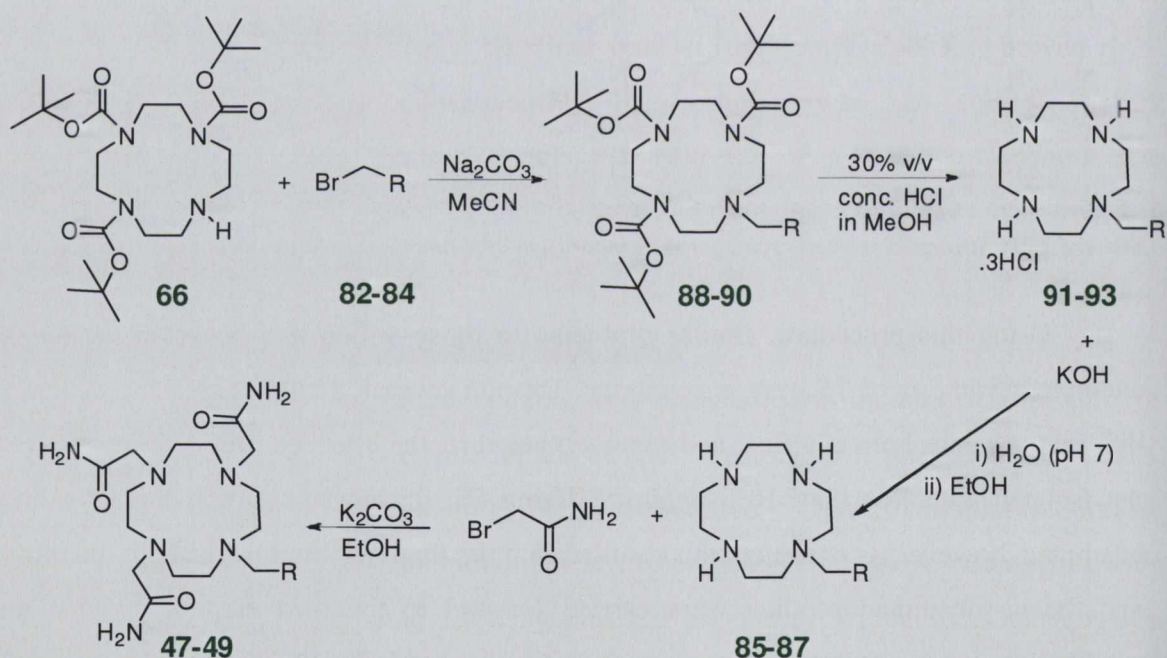
Scheme 2.22: Attempted synthetic route for the preparation of R-functionalised tri-acetamide cyclen ligands.

Using this procedure, similar problems to those which had occurred during the attempted synthesis of **75** were encountered. Despite several attempts at each reaction, in different solvents, both at reflux, and room temperature, the intended products, **85-87**, were not isolated with less than 10% impurity. Using **85**, the next step of the reaction was attempted, however, as expected, this resulted in more than one product, as both the mono- and the di-substituted product were carried forward to the next step. Separation and purification were attempted, however, the desired product, **47**, was not successfully isolated.

The glyoxal protection method which was discussed in Section 2.3.1, was also employed for the synthesis of **85**. This method has been used to synthesise monoalkylated cyclen compounds, as the monoalkylated cyclen can precipitate from solution upon formation. However, when this procedure was attempted for the synthesis of **85**, it resulted in the formation of polymers, which is usually a side product of this reaction.¹³⁵

Having attempted the synthesis of the mono alkylated cyclen systems using several different synthetic procedures, with no success, it was decided to utilise the BOC-protection method (Section, 2.3.5), Scheme 2.23. A modification of this procedure has been used successfully within the literature for the synthesis of similar ligands such as **70**, as shown previously in Scheme 2.9.¹³³

Ligand **88** was prepared by reacting one molar equivalent of tri-BOC protected cyclen, **66**, with one molar equivalent of bromobenzyl, **82**, in the presence of anhydrous Na_2CO_3 in MeCN at reflux for 72 h. After cooling to room temperature, the solvent was removed under reduced pressure and the residue re-dissolved in DCM and filtered through celite to remove inorganic solids. The solvent was removed under reduced pressure to give a residue which was purified by flash silica column chromatography, using eluant DCM:EtOAc (0-100%). This yielded the product, **88**, as a viscous oil which solidified to give an off-white solid in 95% yield. Preparation of **89** was identical to that of **88**, as was the purification method used. The product, **89**, was isolated as a viscous oil, which solidified to give an off white solid in 86 % yield.



Scheme 2.23: Synthesis of trisubstituted cyclen systems.

The synthesis of **90** was identical to that described above, however, the purification method was not. The product could not be separated from any unreacted starting materials by column chromatography, most likely due to the basic nature of the pyridine moiety. As such, the **90** was isolated by washing the organic layer with water, to give the product, **90**, as an orange solid in 87 % yield.

The three compounds, **88-90**, were subsequently deprotected by stirring the ligands in a 30 % v/v solution of conc. HCl/MeOH for three hours. During the reaction, compounds **91** and **92** precipitated out of solution as white solids. Filtration, followed by washing with DCM and diethyl ether resulted in the isolation of the pure HCl salts of **88** and **89** in quantitative yields without the need for further purification. Compound **93** did not precipitate from solution, and it was necessary to remove the solvent under reduced pressure. The residue was dissolved in minimal amount of MeOH, followed by the addition of EtOH to precipitate the product, **90**, as the HCl salt, as detailed in Section 2.4.6. Subsequent filtration, followed by washing with DCM and diethyl ether, gave the desired product, **90** in quantitative yield.

The next step in the synthesis of **47-49** was the alkylation of the cyclen with acetamide arms. To facilitate this, **91-93** were dissolved in H₂O and the solution neutralised by the addition of KOH. The solvent was removed under reduced pressure and the residue redissolved in EtOH to precipitate the KCl salt from solution. Following filtration to remove the salt, the ligand was reacted with 3.01 molar equivalents of 2-bromoacetamide in the presence of K₂CO₃ in EtOH at reflux for 72 h.

Again, the purification method used for **47** and **48** was identical, both reactions were cooled to room temperature and filtered to remove any inorganic solids. Any remaining K₂CO₃ in solution was removed as described previously. The solution was reduced in volume and added dropwise to vigorously stirred diethyl ether (150 mL), to precipitate a white solid for both ligands. These solids were isolated by suction filtration to give crude products, as verified by ¹H NMR. Recrystallisation from Millipore filtered H₂O gave the intended products in 56 and 66% yield for **47** and **48**, respectively.

The ligands, **47** and **48**, were fully characterised by ¹H NMR, ¹³C NMR, IR, ESMS and elemental analysis. The ¹H NMR spectrum (600 MHz, D₂O) of **48** is shown in Figure 2.20. The benzyl CH₂ gave rise to a broad singlet in the ¹H NMR spectrum for **47**, resonating at 3.64 ppm. The C₂ symmetry of the ligand was observed in the NMR spectrum, where the protons of the cyclen ring were split into three resonances integrating for four, four and eight protons. Such symmetry was also observed in the ¹³C NMR spectra for the ligands.

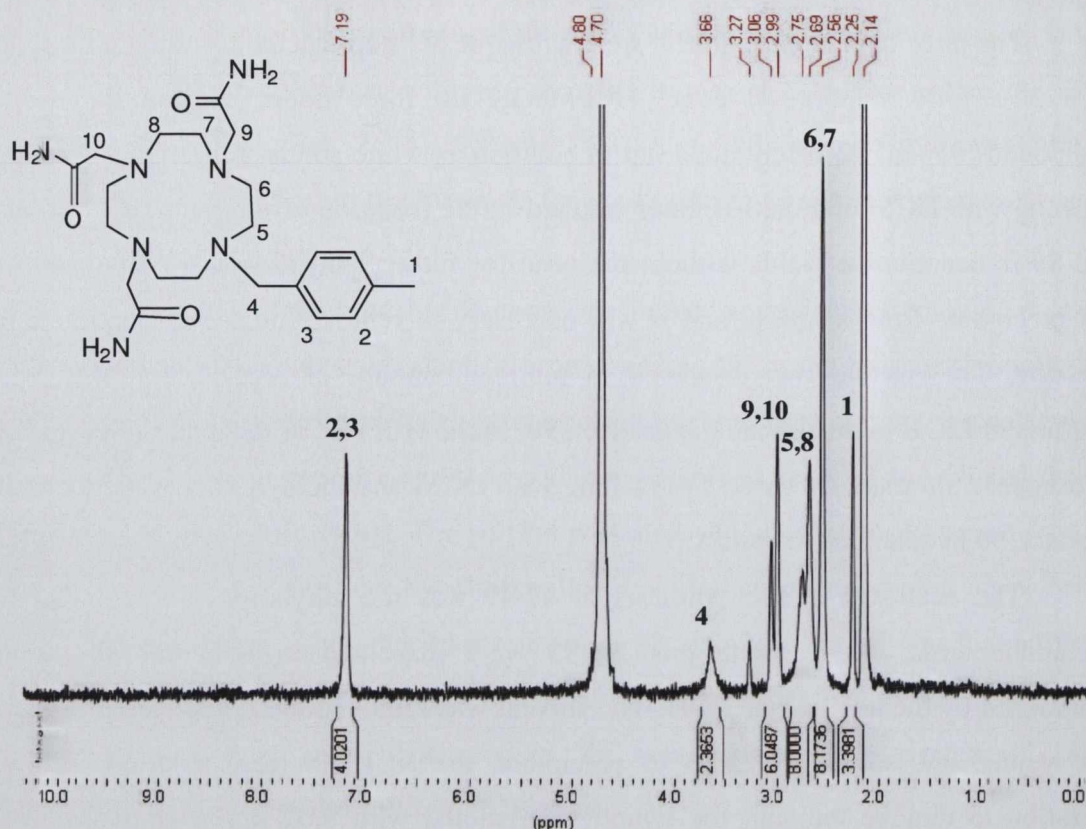


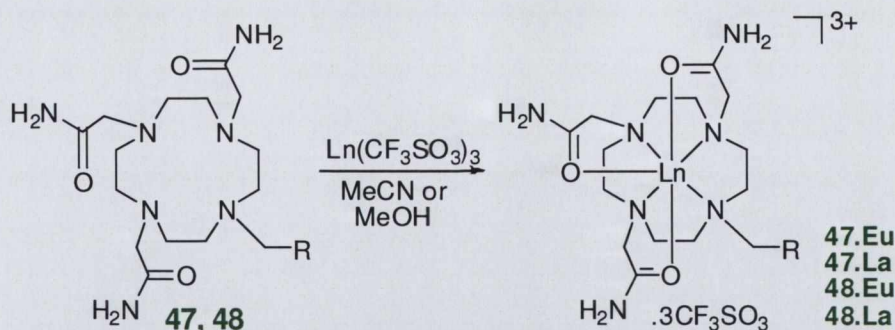
Figure 2.20: The ^1H NMR of **48** showing the C_2 symmetry of the ligand.

The ESMS gave product peaks for both compounds and HRMS was obtained for the ligand **47**, $m/z = 434.2880$, for $(\text{M}+\text{H})^+$, and also for the ligand **48**, $m/z = 448.3036$, again, corresponding to the $(\text{M}+\text{H})^+$ species.

The ligand **49** was water soluble, and was recrystallised from MeCN. The resulting crystals were filtered and washed with ice-cold CHCl_3 to yield the product in 40% yield as an orange solid. These crystals were of high quality and were suitable for analysis by X-ray crystallography, the results of which are discussed in Section 2.7.6. Using ESMS, the product was observed as $m/z = 457.26$, corresponding to the $(\text{M}+\text{Na})^+$ species.

2.7.2 Lanthanide Ion Complexation of **47**

To form the complexes **47.Eu** and **47.La**, the ligand was suspended in MeCN and one equivalent of the appropriate lanthanide triflate salt was added to the solution, Scheme 2.24. Following an overnight reflux (16 h), a precipitate was visible in the reaction vessel which was isolated by filtration and dried under high vacuum. The filtrate was reduced in volume and added dropwise to vigorously stirred diethyl ether (150 mL). The resulting white precipitates were isolated by suction filtration in yields of 87 and 85% for **47.Eu** and **47.La**, respectively. The ^1H NMR and ESMS analysis revealed both the precipitate and the filtered solids to be the desired lanthanide complexes, and so they were combined.



Scheme 2.24: Complexation of ligands **47-49** with $\text{Ln}(\text{CF}_3\text{SO}_3)_3$.

The ^1H NMR spectrum (400 MHz, D_2O) of **47.Eu** is shown in Figure 2.21. The characteristically shifted and broadened resonances of the axial and equatorial protons of the ligand were observed, with several resonances occurring between 18.16 and -18.18 ppm. Using ESI^+ mass spectrometry, the Eu(III) complex was observed as $m/z = 734.1456$, which corresponded to $[\text{M}+(\text{CF}_3\text{SO}_3)]^+$. The isotopic distribution pattern was found to match the calculated isotopic distribution pattern. The ^1H NMR spectrum (400MHz, D_2O) of **47.La** was unshifted, however, the resonances of the axial and equatorial protons of the cyclen ring were broadened, Appendix A1.7. Using MALDI-TOF MS, the complex **47.La** was observed at $m/z = 720.1282$, corresponding to the $[\text{M}+(\text{CF}_3\text{SO}_3)]^+$ species

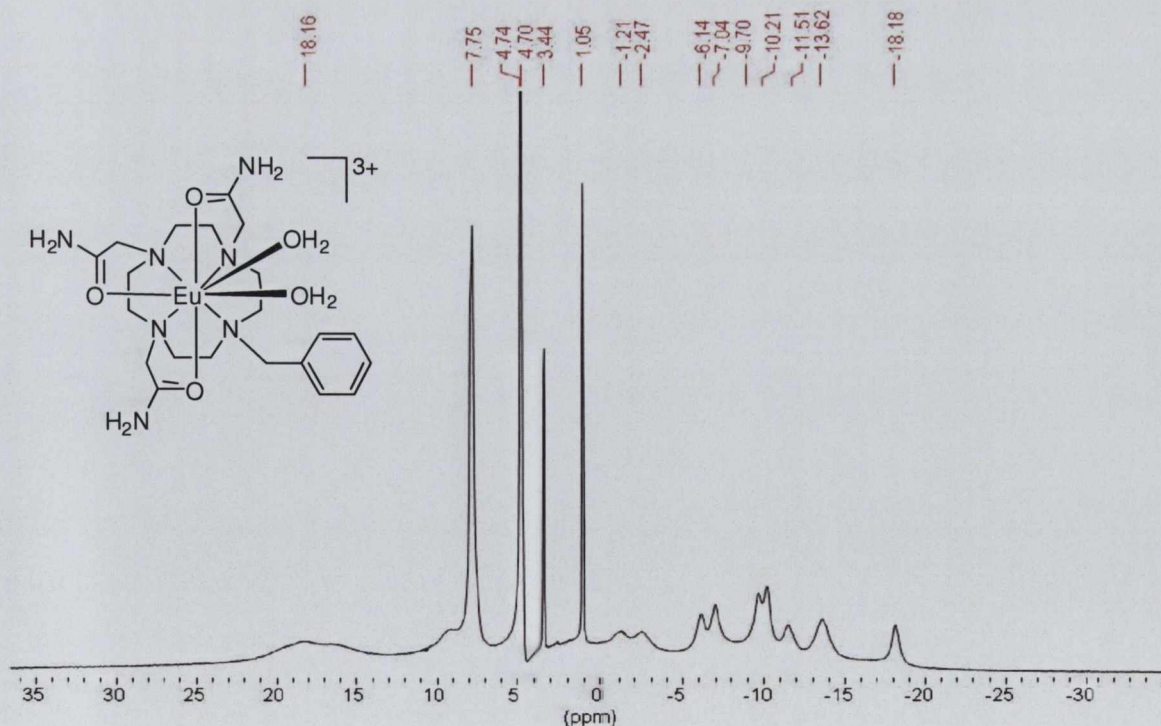


Figure 2.21: The ^1H NMR spectrum (400 MHz, D_2O) of **47.Eu**.

The IR spectra for the ligand **47**, and the complexes **47.Eu** and **47.La** were recorded and are detailed in Table 2.12 at the end of this section. Both complexes showed a

decrease in the carbonyl stretching frequency which was indicative of complex formation. The radiative rate constants (k) were recorded for **47.Eu** in both H₂O and D₂O and are given in Table 2.13, at the end of this section. The lifetimes were calculated to be 0.38 ms and 1.58 ms for the complex in H₂O and D₂O, respectively. This gave rise to a q -value of $2.3 (\pm 0.5)$, indicating that there was two water molecules present on the metal ion. All lifetimes recorded were fit to a monoexponential decay, signifying that there was only a single species present in solution.

2.7.3 Lanthanide Ion Complexation of **48**

The lanthanide complexation of **48** to Eu(III) and La(III) was identical to that of **47**, detailed above, Scheme 2.24. The desired product, **48.Eu** and **48.La** were isolated as white solids in 95 and 90% yields respectively.

The ¹H NMR spectrum (400 MHz, D₂O) of **48.Eu** gave rise to the broad and shifted resonances of a Eu(III) complex, which were observed between 18.36 and -13.15 ppm, Appendix A1.8. The ¹H NMR spectrum (400 MHz, D₂O) recorded for **48.La** is shown in Figure 2.22, and the broad resonances of the cyclen protons are observed.

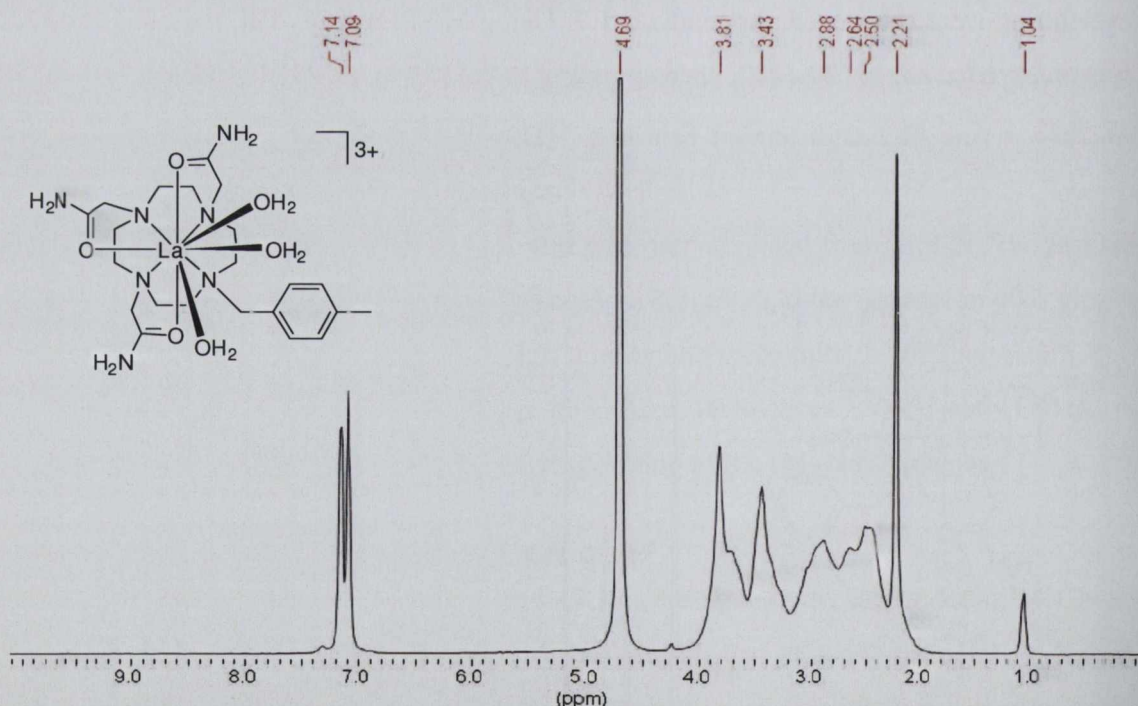


Figure 2.22: The ¹H NMR spectrum of **48.La**. Note the broad resonances of the cyclen ring as a result of complexation to a La(III) metal ion.

The IR spectrum showed a decrease in the carbonyl stretching frequency for both metal ion complexes, as compared to the ligand, verifying the formation of the complexes. The carbonyl stretches are detailed in Table 2.12. The q -value for the Eu(III) complex **48.Eu** was calculated from the measured lifetimes for the complex, which are given in

Table 2.13. A q -value of $1.7 (\pm 0.5)$ was determined, which indicated that two water molecules were bound to the Eu(III) ion.

2.7.4 Lanthanide Ion Complexation of **49**

The lanthanide ion complexation of **49** did not occur after refluxing in MeCN for 16 hours, and as such, the complexation reaction was carried out in MeOH, Scheme 2.24. After cooling the solution to room temperature, the solvent was reduced in volume and added dropwise to vigorously stirred diethyl ether (150 mL). The resulting yellow precipitate was isolated by suction filtration to give the desired product, **49.Eu**, in 83% yield.

The ^1H NMR spectrum (400 MHz, D_2O) of **49.Eu** showed the expected shifted and broadened peaks, characteristic of such Eu(III) complexes, with the resonances appearing between 25.5 to -16.4 ppm, Appendix A1.9. However, the complexity of the NMR spectrum indicated that there was several different isomers, or possibly more than one species, existing in solution. A VT ^1H NMR experiment, shown in Figure 2.23, suggested that these resonances were most likely as a result of the presence of several isomers existing in solution at room temperature. As the temperature was increased to 40 °C then to 60 °C, the number of resonances decreased. The final ^1H NMR spectrum at 60 °C was similar to that of the ligand, **49**. While it is likely that the many resonances arose as a result of a fast exchange rate between the different isomers in solution at room temperature, at higher temperature, the exchange rate was slower, giving rise to a lower number of resonances, which were broader in appearance.

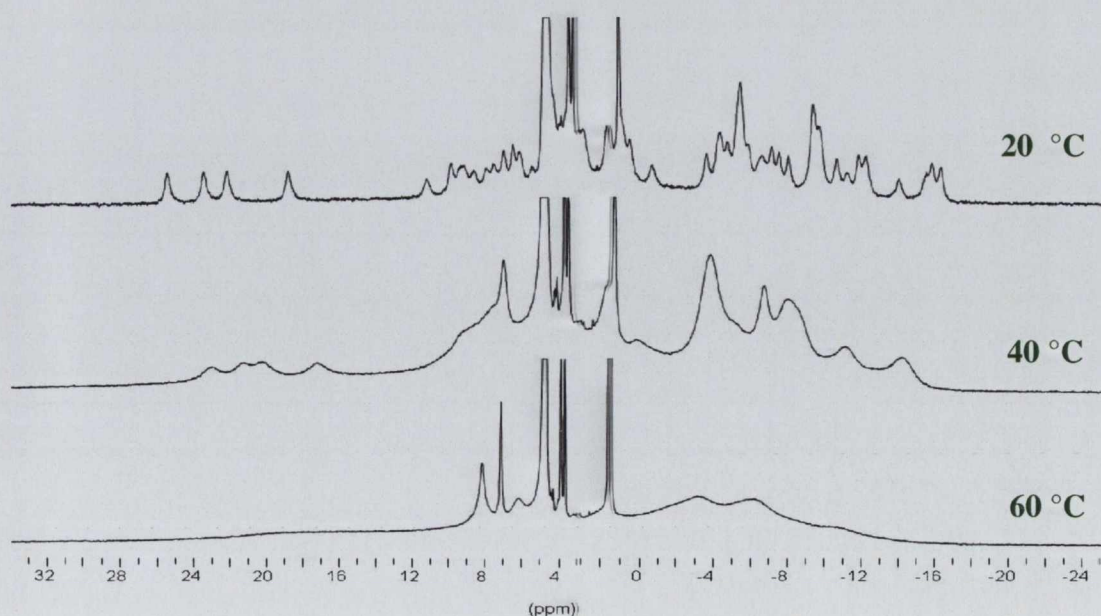


Figure 2.23: Variable temperature ^1H NMR analysis of **49.Eu**. As the temperature increases the number of peaks observed decreases, indicating the presence of diastereoisomers in solution.

Analysis by IR spectroscopy indicated the formation of the metal complex by the decrease in the carbonyl stretch from 1679 to 1600 cm^{-1} , Table 2.11. The number of metal bound water molecules, q , was calculated for **49.Eu**, Table 2.12. The luminescent lifetimes were calculated as 0.52 ms and 1.93 ms in H_2O and D_2O respectively, which gave rise to $q = 1.6 (\pm 0.5)$. This was lower than for either of the other two mononuclear Eu(III) complexes, **47.Eu** and **48.Eu** and is most likely due to the coordination of the pyridine moiety to the metal ion centre, reducing the number of vacant coordination sites available for solvent molecules such as H_2O . Such coordination was also observed for the dinuclear pyridine spaced complex **39.Eu₂**, (Section 2.3) and has been previously observed for similar pyridine functionalised complexes synthesised by the Gunnlaugsson group.²⁴

Table 2.11: Carbonyl stretching frequencies of mononuclear ligands and their corresponding lanthanide complexes.

Compound	C=O stretching freq. (cm^{-1})
47	1654
47.Eu	1600
47.La	1600
48	1660
48.Eu	1600
48.La	1599
49	1679
49.Eu	1600

Table 2.12: Lifetime determination of **47-49.Eu**.

Complex	$k (\text{ms}^{-1})$ H_2O	$\tau (\text{ms})$ H_2O	$k (\text{ms}^{-1})$ D_2O	$\tau (\text{ms})$ D_2O	$q = (\pm 0.5)$
47.Eu	2.62	0.38	0.63	1.58	2.3
48.Eu	2.07	0.48	0.63	1.58	1.7
49.Eu	1.90	0.52	0.52	1.93	1.6

2.7.5 Solid State Analysis of **47**

Single crystals of **47**, suitable for X-ray diffraction analysis, were grown by slow evaporation from H_2O at 40 °C. The crystals were obtained as colourless plates and X-Ray Crystallographic analysis (Appendix A4.2) was carried out in the School of Chemistry,

TCD, by Dr. McCabe. The resulting X-ray crystal structure is shown in Figure 2.24. The X-ray crystal structure clearly shows the puckered frame of the cyclen ring, the three acetamide arms and the benzyl group. Selected bond lengths are given in Table 2.13.

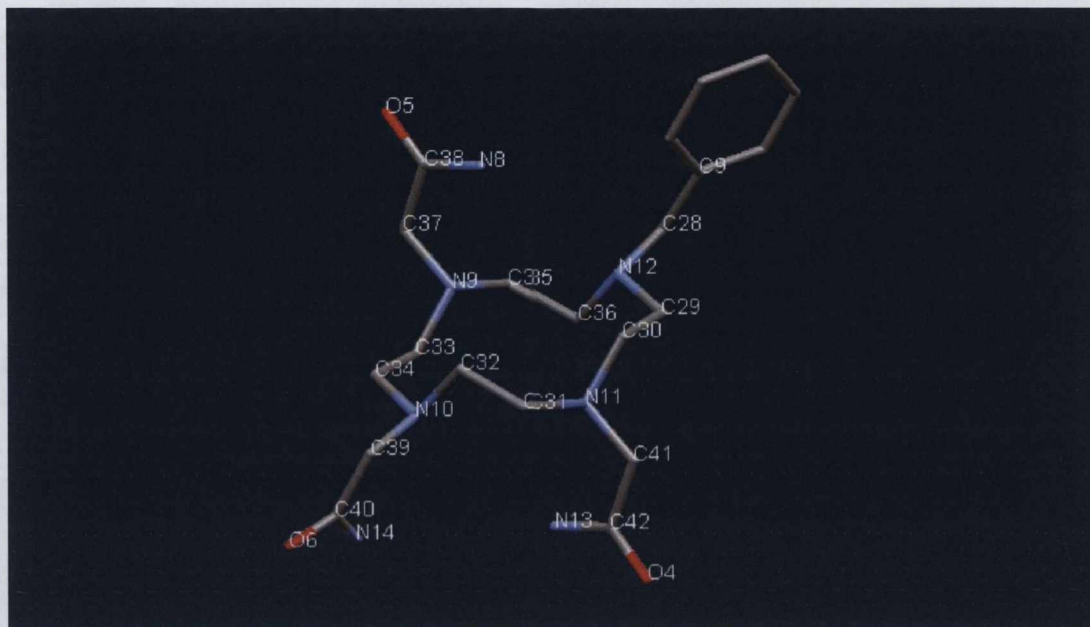


Figure 2.24: X-ray crystal structure of **47**, showing the puckered frame of the cyclen ring, the acetamide arms and the benzyl arm. H_2O molecules and the hydrogen atoms have been omitted for clarity.

Table 2.13: Selected bond lengths and bond angles for **47**.

Bond Label	Length (Å)	Angle Label	Angle (°)
O(4)-C(42)	1.241(3)	O(4)-C(42)-N(13)	123.7(3)
O(5)-C(38)	1.248(3)	O(5)-C(38)-N(8)	122.9(3)
O(6)-C(40)	1.248(3)	O(6)-C(40)-N(14)	122.1(3)
N(8)-C(38)	1.325(4)	C(33)-N(9)-C(35)	109.7(2)
N(13)-C(42)	1.329(4)	C(34)-N(10)-C(32)	111.3(2)
N(14)-C(40)	1.322(4)	C(31)-N(11)-C(30)	109.5(2)
N(12)-C(28)	1.471(4)	C(29)-N(12)-C(36)	112.0(2)
N(11)-C(41)	1.465(4)	C(28)-N(12)-C(29)	110.0(2)
N(10)-C(39)	1.468(4)	C(28)-N(12)-C(36)	107.9(2)
N(9)-C(37)	1.461(4)	N(12)-C(28)-C(9)	114.6(2)
C(9)-C(28)	1.508(4)	N(9)-C(37)-C(38)	113.8(2)
C(37)-C(38)	1.512(4)	N(10)-C(39)-C(40)	115.6(2)
C(39)-C(40)	1.511(4)	N(10)-C(41)-C(42)	115.1(3)
C(41)-C(42)	1.517(4)	N(12)-C(28)-C(9)	114.6(2)

2.7.6 Solid State Analysis of 49.K

During the purification of **49**, it was attempted to recrystallise the ligand from a solution of MeCN with a small amount of MeOH present, as detailed in Section 2.7.1. The solution was left to recrystallise at room temperature, and crystals were obtained as colourless plates which were suitable for X-ray diffraction analysis. X-ray Crystallography Analysis (Appendix A4.3) was carried out by Dr. T. McCabe in the School of Chemistry, TCD. The structure can be seen in Figure 2.25.

Analysis of the X-ray crystal revealed that the ligand was complexed to a potassium ion. This is not unexpected, as K_2CO_3 was present during the synthesis and workup of this ligand. The ionic radius of potassium is 152 pm, compared to 117 and 109 ppm for lanthanum and europium, respectively.^{48,76} In the crystal structure, the potassium ion is complexed by the four nitrogens of the cyclen ring, the three carbonyls of the acetamide arms and the pyridine pendant arm, which completes the coordination sphere. Potassium is an eight coordinate metal ion, and as such, there are no water or solvent molecules bound to the metal.

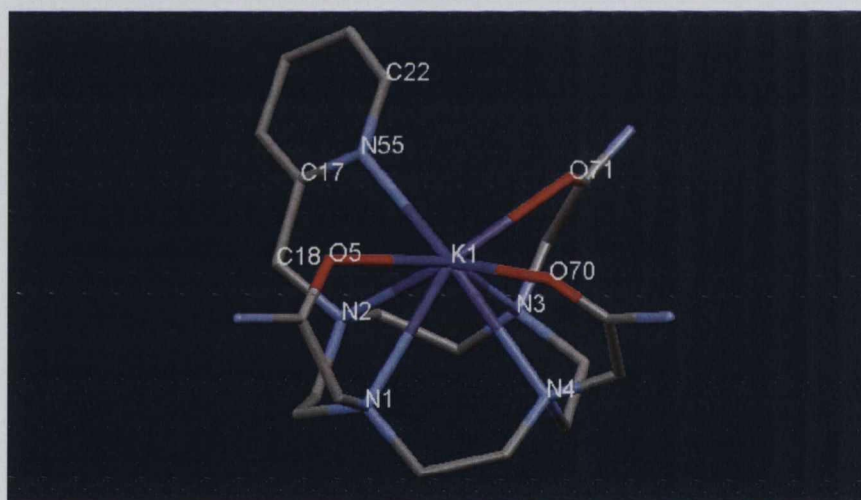


Figure 2.25: X-ray crystal structure of **49.K**, showing the potassium metal ion coordinated to the nitrogens of the cyclen, the acetamide arm and the pyridine moiety. Counterions and the hydrogens have been removed for clarity.

Studying the structure, it can be seen that the cyclen complexation of the potassium metal ion is similar to that of the La(III) metal ion, as discussed in Section 2.4.5. The potassium ion is too large to fit into the cyclen cavity and so it sits above it, with the cyclen ring and the pendant arms forming a concave structure which encapsulates the metal ion. The structure adopts a square antiprismatic geometry in the solid state, which is a common geometry for tetra substituted lanthanide complexes of cyclen.⁶² Selected bond lengths are given in Table 2.14.

Table 2.14: Selected bond lengths and bond angles for **49.K**.

Bond Label	Length (Å)	Angle Label	Angle (°)
K(1)-N(1)	2.932(4)	N(2)-K(1)-N(1)	63.21(10)
K(1)-N(2)	2.883(4)	N(3)-K(1)-N(1)	97.38(10)
K(1)-N(3)	2.929(3)	N(4)-K(1)-N(1)	64.98(10)
K(1)-N(4)	2.2.840(4)	N(55)-K(1)-N(1)	114.52(12)
K(1)-N(55)	2.797(4)	O(5)-K(1)-N(1)	60.09(9)
K(1)-O(5)	2.692(3)	O(70)-K(1)-N(1)	92.62(10)
K(1)-O(70)	2.712(3)	O(71)-K(1)-N(1)	153.82(10)
K(1)-O(71)	2.720(3)	O(5)-K(1)-O(70)	88.80(10)
K(1)-N(2)	2.883(4)	O(5)-K(1)-O(71)	145.69(9)
C(17)-C(18)	1.508(7)	C(17)-N(55)-K(1)	116.5(3)
N(55)-C(17)	1.332(6)	N(55)-C(17)-C(18)	117.3(4)
N(55)-C(22)	1.347(6)	C(22)-N(55)-K(1)	124.3(3)
N(2)-C(18)	1.474(6)	C(17)-N(55)-C(22)	117.8(4)

2.7.7 Summary

This Section has detailed the synthesis and characterisation of family of novel mononuclear cyclen based lanthanide complexes, **47-49**, analogous to the dinuclear complexes synthesised in Sections 2.3-2.5. Within this family, each ligand was functionalised such that the complexed lanthanide ion was hepta-coordinated, with a minimum of two vacant coordination sites for water molecules. Using a modification of a known synthetic pathway for the preparation of similar ligands, the three complexes were synthesised and all novel compounds were purified and fully characterised.

2.8 Overall Conclusion

In summary, this Chapter has detailed the synthesis and characterisation of several families of cyclen based lanthanide complexes. To begin, a pyridine spaced ligand, **39**, functionalised with acetamide arms was synthesised. The Eu(III) complex, **39.Eu₂**, and the La(III) complex, **39.La₂**, were both synthesised. The Eu(III) complex gave rise to a *q*-value of 1.6 (± 0.5), which was lower than what was anticipated, and it was proposed that the metal ions were possibly partially coordinated to the lanthanide metal ion.

The second family of dinuclear ligands synthesised was the acetamide functionalised *para*- and *meta*-xylyl spaced ligands **41** and **43**. Both were synthesised in good yields, however, the synthesis and purification of the *meta*-spaced bis cyclen frame

proved more tedious than that of the *para*-isomer. This is possibly as a result of steric influences in the *meta*-isomer. The Eu(III) and La(III) complexes of both ligands, **43.Ln** and **43.Ln**, were successfully synthesised and purified.

The final family of dinuclear ligands synthesised was the alkylamine acetamide functionalised *para*- and *meta*-xylyl spaced ligands, **42** and **44**, and again both the Eu(III) and the La(III) complexes of both ligands were synthesised and purified.

The synthesis of a trinuclear cyclen based lanthanide complex, **45.Eu₃**, was also attempted, and although evidence suggests that the complex was formed, it appeared to decompose in solution, and thus no further investigations were carried out on the ligand, **45**, or the complex, **45.Eu₃**.

Finally, a family of mononuclear cyclen based lanthanide complexes were synthesised which could be used as model compounds to aid the determination of the different mechanisms of action for the aforementioned dinuclear complexes. All of the mononuclear complexes were functionalised at three of the cyclen ring nitrogen atoms with acetamide pendant arms, and the final nitrogen atom was then functionalised with an appropriate R-group to represent the spacer moieties of the dinuclear complexes. As such, the three different R-groups used were a benzyl, a 4-methylbenzyl and a 2-pyridinyl group, **47**, **48** and **49**, respectively. The Eu(III) and La(III) complexes of all three ligands were synthesised and characterised.

Chapters Three and Four detail the potentiometric and kinetic evaluation of the eight ligands and fifteen complexes synthesised herein, to evaluate their ability to hydrolyse the phosphodiester **HPNP**, and to determine their mechanisms of hydrolysis.

3.1 Introduction

The metal bound water molecules of a complex can promote the hydrolysis of **HPNP**, as outlined in Section 1.6 and in such cases, the pK_a of such a metal bound water molecule can determine the pH at which a complex is most active.^{15,115,152} The pK_a of the metal bound water molecule is dependant on the Lewis acidity of the metal to which it is bound, which can be modulated by the ligand to which it is complexed.^{42,103} Previous studies within the Gunnlaugsson group determined that the nature of the pendant arms greatly affected the pK_a of the metal bound water molecules, through a “*fine tuning*” of the Lewis acidity of the metal ion.^{62,122} By the determination of the protonation constants of the complex, including those of the metal bound hydroxide molecules, the active species in solution at different pH values can be determined, and can be used to aid in the determination of the mechanism of action for each complex for the hydrolysis of **HPNP**.

Previous studies within the Gunnlaugsson group have focused on the development of a complex capable of efficiently hydrolysing **HPNP** by the refinement of the pendant arms and metal ion of choice, and also through the development of a dinuclear complex, as outlined in Section 1.14. The purpose of this thesis was to combine the most efficient pendant arms and metal ions into the most promising dinuclear system to achieve the development of a ribonuclease mimic that can operate under physiological pH conditions, *i.e.*, at pH 7.4, and 37 °C, in competitive media, Section 1.15. As alluded to above, the structure of the ligand can influence the pK_a of the metal bound hydroxide, thus affecting the pH range in which the complex will give rise to the maximum activity.^{63,114} As such, using potentiometric measurements, the influence of adjustments to the structure of the complexes, such as acetamide and alkylamine acetamide arms, and the orientation of the spacer moiety, will be investigated. The protonation constants for each of the ligands will be determined, followed by the determination of the protonation constants of the corresponding complexes.

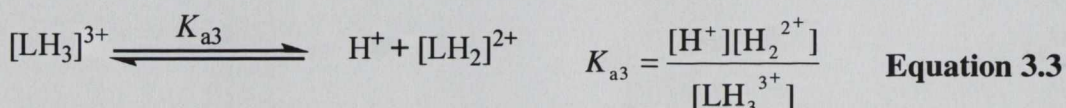
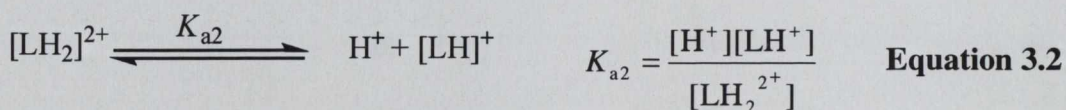
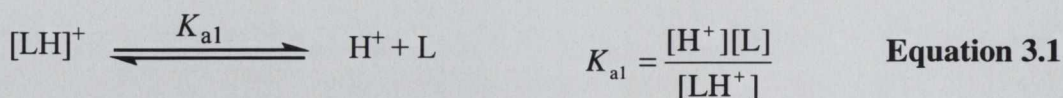
The first section will detail the determination and analysis of the protonation constants of the free ligands **39**, **41**, **43**, **42**, **44**, **47** and **48**, which were described in Chapter 2. This will be followed by a discussion on the determination of the stability constants for cyclen based lanthanide complexes, and finally the protonation constants for the complexes synthesised in Chapter 2, **39.Ln₂**, **41.Ln₂**, **43.Ln₂**, **42.Ln₂**, **44.Ln₂**, **47.Ln** and **48.Ln** (Ln = Eu(III) or La(III)), will be detailed.

3.2 Determination of Protonation Constants

The ligands synthesised in Chapter 2 are cyclen based, and their resulting lanthanide complexes were evaluated for their ability to hydrolyse **HPNP** in aqueous solution, the results of which will be discussed in Chapter 4. Macrocyclic ligands such as cyclen, exist in an equilibrium mixture of their free and protonated forms, in aqueous solution, as a result of their polybasic character.^{153,154} The protonation constants were determined for all of the ligand synthesised, all of which were found to be water soluble.

The protonation constants of all of the ligands were determined in aqueous solution, in the presence of high ionic strength, NEt_4ClO_4 , $I = 0.1 \text{ M}$. The tetraethylammonium salt was chosen to be used throughout for the potentiometric analysis due to the ability of cyclen to complex a wide variety of metal ions.¹⁵³ It has been shown that many polyaza cyclic ligands, and in particular cyclen, can easily complex a wide variety of transition metal ions, Na^+ and to a lesser extent, K^+ .¹¹⁻¹⁴ This meant that a wide variety of salts and counterions which would normally be used for potentiometric analysis, such as KCl and NaOH , could not be used. Sherry and co-workers carried out potentiometric titrations on a cyclen based ligand using both NEt_4ClO_4 , and KCl as ionic strength.¹⁵⁵ For the system that was titrated in the presence of KCl , a depression of the protonation constants was observed, which was most likely due to a competitive interaction of the K^+ with the cyclen ligand. As such, tetraethylammonium (NEt_4^+) was used as the cation for all solutions in this work.

To carry out the titrations, all ligand solutions were fully acidified with HClO_4 to ensure complete protonation of all nitrogen donor atoms. Using an automated potentiostat, Section 5.2, precise aliquots of NEt_4OH were titrated into the acidic solution, and the ligand was deprotonated. This results in the generation of a titration curve in which several inflections indicate the stepwise protonations of the ligand. As an example, the stepwise acid-base equations for a tri-basic system are expressed as follows:



The stepwise equilibrium constants are K_{a1} , K_{a2} and K_{a3} and L is the ligand that is being studied. The negative logarithm of K_a can be used to determine the pK_a of each nitrogen donor atom on the ligand. Each pK_a of an acid corresponds to the stepwise protonation constant of its conjugate base.

$$pK_{a1} = -\log K_{a1} \quad \text{Equation 3.4}$$

$$pK_{a2} = -\log K_{a2} \quad \text{Equation 3.5}$$

$$pK_{a3} = -\log K_{a3} \quad \text{Equation 3.6}$$

All of the ligands were titrated to determine their protonation constants and corresponding pK_a values. Not all pK_a values could be determined for each ligand, due to the limits of the glass electrode; pK_a values ≤ 2 , or ≥ 12 could not be accurately determined. Furthermore, several of the ligands formed precipitates in solution, and as such their pK_a values could not be measured. It was important to ensure that the temperature was maintained at 25 °C, the pK_a is temperature dependant, and as such, small fluctuations in temperature can affect the titration.¹⁵⁴ To ensure that the ligand had reached the thermodynamic equilibrium in solution after each addition of NEt_4OH , a back titration was performed with $HClO_4$. If the ligand was at equilibrium, the base (forward) and the acid (back) titrations should overlap.

To determine the pK_a values accurately, a fitting program, HYPERQUAD was used.¹⁵⁶ HYPERQUAD uses a non-linear least squares regression method to minimise the differences between the experimental data and the theoretical values for the proposed model.

In a system with multiple pK_a values, the value of each sequential pK_a will decrease as a result of the increasing charge, and also, as a result of a statistical effect. The protonation of a single nitrogen atom on a ligand gives an overall charge of +1, resulting in a large pK_a value. At the second protonation, the addition of a second proton causes repulsion between two protons, which can lower the pK_a value for the second protonation constant, pK_{a2} . For every subsequent protonation, the larger electrostatic repulsion between the charges results in larger decreases to the protonation constants, leading to smaller pK_a values.¹³⁸ Furthermore, the coordination of a proton to a single site decreases the probability with which the next proton will coordinate, resulting in a decrease in the pK_a value as a result of the aforementioned statistical effect.

The pK_a value can also be affected by intramolecular hydrogen bonding within the ligand between protonated and non-protonated amino groups.¹⁵⁴ A proton which is actively involved in hydrogen bonding is more difficult to abstract than an uncoordinated proton, thus leading to a difference in the measured pK_a value.

3.2.1 Determination of the Protonation Constants for the Pyridine Spaced Ligand **39**

The pK_a values were determined for the pyridine based ligand, **39**, and a typical titration curve of the protonated ligand against NEt_4OH is shown in Figure 3.1. The inflection points are clearly visible on the curve, and indicate that several deprotonations of the ligand occur.

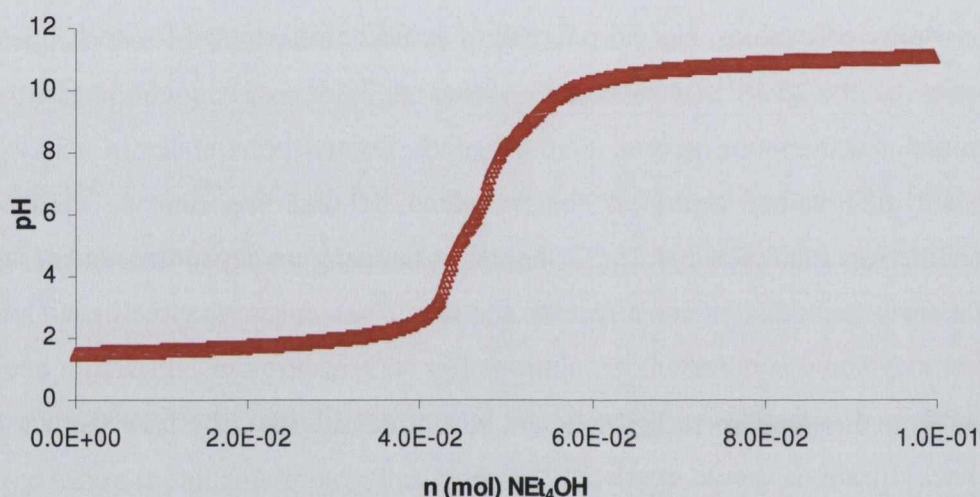


Figure 3.1: Typical titration curve of the protonated ligand **39** against NEt_4OH at 25 °C. $[39] = 1.0 \times 10^{-3} M$, $[H^+] = 4.45 \times 10^{-4} M$, $[NEt_4OH] = 0.103 M$, $I = 0.10 M$ (NEt_4ClO_4).

Following treatment of the data with HYPERQUAD, the logarithm of the stepwise protonation constants for **39** were determined, and these are represented in Table 3.1 below, along with the protonation constants of cyclen and DOTA. The conditions for the titration are listed below the table.

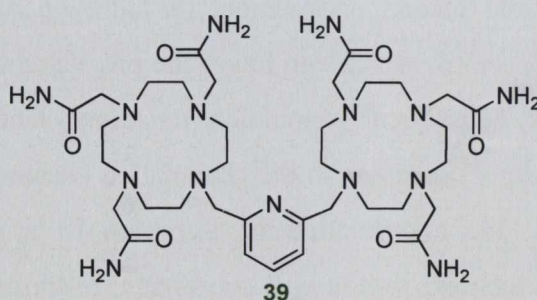


Table 3.1: The pK_a values of the protonated pyridine spaced ligand **39** and cyclen obtained at 25 °C with $I = 0.1\text{ M}$ (NEt_4ClO_4) in H_2O .

pK_a of protonated site	39 ^a	cyclen ^b	DOTA ^c
pK_{a1}	10.43 (± 0.03)	10.38	12.60
pK_{a2}	8.86 (± 0.05)	9.71	9.70
pK_{a3}	7.42 (± 0.09)	2.05	4.50
pK_{a4}	5.64 (± 0.09)		4.14
pK_{a5}	2.70 (± 0.09)		2.32

^a[**39**] = $1.0 \times 10^{-3}\text{ M}$, $[H^+] = 1.5 \times 10^{-2}\text{ M}$, $[NEt_4OH] = 0.103\text{ M}$, $I = 0.10\text{ M}$ (NEt_4ClO_4). [b] Ref.¹⁵³ aqueous solution, $I = 0.15\text{ M}$. [c] Ref.¹⁵⁷

There are up to fifteen possible protonation sites on the ligand **39**. However, it is very unlikely that all of these protonations will be observed as some will occur at a pH outside the limits of the glass electrode (≤ 2.0 , ≥ 12). The protonation sites can be tentatively assigned, by comparison to other potentiometric studies on cyclen-based macrocycles.^{138,158-160} It is likely that the first protonation, pK_{a1} , 10.43 (± 0.03), and the second protonation pK_{a2} , 8.86 (± 0.05) are the first protonation constants for each of the cyclen rings. The protonation constant pK_{a4} is possibly a second protonation of one of the cyclen rings, whereas pK_{a5} , 2.70 (± 0.09) is most likely a third protonation of the cyclen ring. The third protonation constant pK_{a3} , 7.42 (± 0.09) is slightly ambiguous and could be assigned either to a second deprotonation of one of the cyclen rings, or it could possibly be assigned to the pyridine of the spacer moiety. Although the pK_a of free pyridine is 5.21, the pK_a is known to be higher when substituted.¹⁶¹

3.2.2 Determination of the Protonation Constants for the Xylyl Spaced Ligands **41 and **43****

This section examines the protonation constants of the xylyl spaced ligands **41** and **43**. These were synthesised to determine the effect of changing the orientation of the spacer moiety on the cyclen frame. A typical titration curve of **41** is presented in Figure 3.2, and that of **43** is presented in Appendix A.2.1. Several inflection points are observed on the titration curve, indicating the presence of several deprotonations, in the same manner as seen for **39** above.

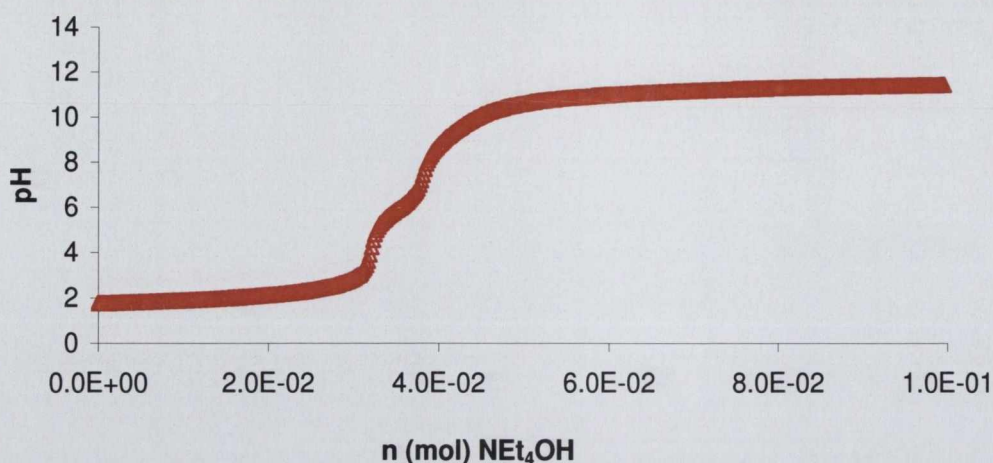
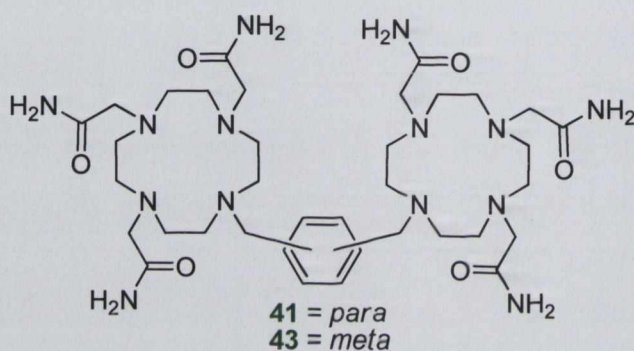


Figure 3.2: Typical titration curve of the protonated ligand **41** against NEt_4OH at 25 °C. $[\mathbf{41}] = 1.0 \times 10^{-3} \text{ M}$, $[\text{H}^+] = 1.4 \times 10^{-2} \text{ M}$, $[\text{NEt}_4\text{OH}] = 0.10 \text{ M}$, $I = 0.10 \text{ M}$ (NEt_4ClO_4).



The logarithm of the stepwise protonation constants for the studied ligands **41** and **43** are given in Table 3.2, and the conditions for the titrations are given below the table. Similar titration curves were observed for both ligands, which were similar to the titration curve observed for the ligand **39**.

Table 3.2: The pK_a values of the protonated pyridine spaced ligand **41** and **43** obtained at 25 °C with $I = 0.1 \text{ M}$ (NEt_4ClO_4) in H_2O .

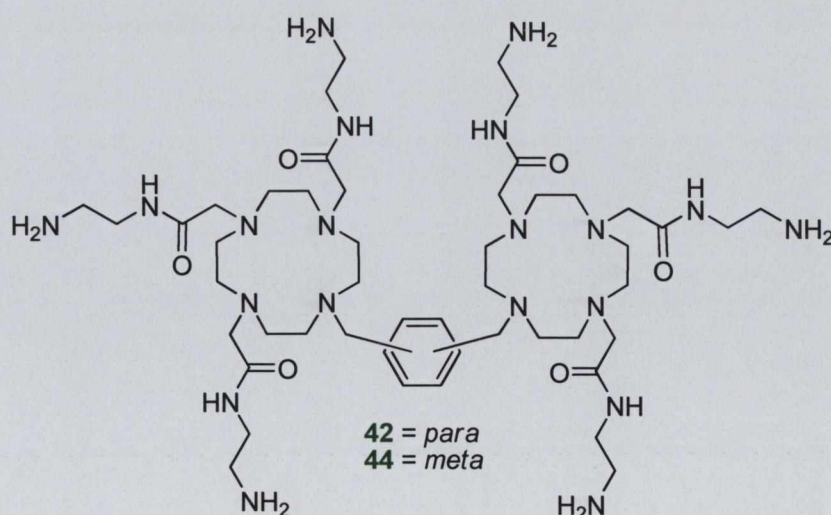
pK_a of protonated site	41 ^a	43 ^b
pK_{a1}	10.13 (± 0.07)	9.73 (± 0.05)
pK_{a2}	8.70 (± 0.07)	9.06 (± 0.04)
pK_{a3}	6.38 (± 0.10)	6.65 (± 0.05)
pK_{a4}	6.05 (± 0.09)	5.99 (± 0.05)

^a $[\mathbf{41}] = 1.0 \times 10^{-3} \text{ M}$, $[\text{H}^+] = 1.4 \times 10^{-2} \text{ M}$, $[\text{NEt}_4\text{OH}] = 0.10 \text{ M}$, $I = 0.10 \text{ M}$ (NEt_4ClO_4), ^b $[\mathbf{43}] = 6.4 \times 10^{-4} \text{ M}$, $[\text{H}^+] = 1.2 \times 10^{-2} \text{ M}$, $[\text{NEt}_4\text{OH}] = 0.09 \text{ M}$, $I = 0.10 \text{ M}$ (NEt_4ClO_4)

As was observed for **39**, although there were up to fourteen protonation sites on each ligand, only four protonation constants were determined for each due to the pH limits in which the protonations can be accurately measured. The two ligands, **41** (*para*) and **43** (*meta*) gave rise to similar protonation constants, suggesting that the orientation of the spacer does not have a large influence on the overall acidity of the ligand. For both ligands, all four protonation constants are most likely associated with the protonation of the macrocyclic ring, and are in good agreement with the values reported for the ligand DOTAM, a tetraamide substituted cyclen ring, where two protonations were observed for the cyclen ring at 9.08 (± 0.01) and 6.21 (± 0.01).¹⁵⁹ As was observed for ligand **39**, pK_{a1} and pK_{a2} are the first protonations of each of the macrocyclic rings of the dinculear system. The following two protonations are most likely the successive second protonations of the two macrocyclic rings. No protonations of the acetamide arms are observed due to the low pH range in which they occur.

3.2.3 Determination of the Protonation Constants for the Xylyl Spaced Ligands 42 and 44

The following section details the protonation constants of the xylyl spaced alkylamine acetamide functionalised ligands **42** and **44**. A typical titration curve for the ligand **42** is presented in Figure 3.3, and that of **44** is presented in Appendix A.2.2. Once again, the titration curve has several visible inflection points, indicating that there are several pK_a values associated with the ligand. A large number of pK_a values were expected for these ligands due to the presence of the terminal amines on the pendant donor arms.



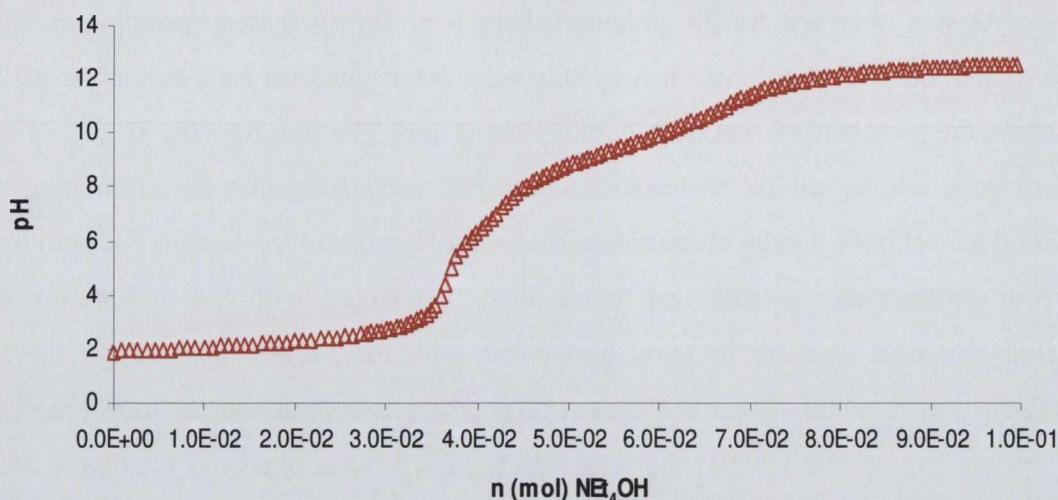


Figure 3.3: Typical titration curve of the protonated ligand **42** against NEt_4OH at 25 °C. $[\mathbf{42}] = 9.5 \times 10^{-4} \text{ M}$, $[\text{H}^+] = 2.1 \times 10^{-2} \text{ M}$, $[\text{NEt}_4\text{OH}] = 0.09 \text{ M}$, $I = 0.10 \text{ M}$ (NEt_4ClO_4).

The logarithm of the stepwise protonation constants for **41** and **43** are given in Table 3.3, and the conditions for the titrations are given below the table. The titration curves of both ligands were similar, and following treatment of the data with HYPERQUAD, a similar trend was determined for the protonation constants of both ligands.

Table 3.3: The pK_a values of the protonated xylyl- spaced ligand **42** and **44** obtained at 25 °C with $I = 0.1 \text{ M}$ (NEt_4ClO_4) in H_2O .

pK_a of protonation site	42^a	44^b
pK_{a1}	9.89 (± 0.02)	11.09 (± 0.06)
pK_{a2}	9.43 (± 0.02)	9.83 (± 0.06)
pK_{a3}	8.94 (± 0.02)	9.60 (± 0.09)
pK_{a4}	8.42 (± 0.02)	8.91 (± 0.09)
pK_{a5}	8.20 (± 0.02)	8.75 (± 0.09)
pK_{a6}	7.80 (± 0.17)	8.27 (± 0.06)
pK_{a7}	7.36 (± 0.02)	7.78 (± 0.06)
pK_{a8}	6.54 (± 0.02)	6.77 (± 0.06)
pK_{a9}	5.31 (± 0.02)	5.84 (± 0.06)

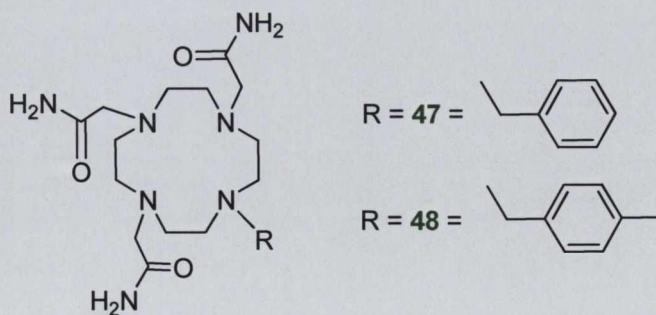
^a $[\mathbf{42}] = 9.5 \times 10^{-4} \text{ M}$, $[\text{H}^+] = 2.1 \times 10^{-2} \text{ M}$, $[\text{NEt}_4\text{OH}] = 0.09 \text{ M}$, $I = 0.10 \text{ M}$ (NEt_4ClO_4), ^b $[\mathbf{44}] = 1.0 \times 10^{-3} \text{ M}$, $[\text{H}^+] = 3.0 \times 10^{-2} \text{ M}$, $[\text{NEt}_4\text{OH}] = 0.10 \text{ M}$, $I = 0.10 \text{ M}$ (NEt_4ClO_4)

As before, the first two pK_a values for both ligands, pK_{a1} and pK_{a2} , were assumed to be associated with the first protonation of each of the cyclen rings. The first pK_a value determined for the ligand **44** was high compared to those of the other complexes studied

herein, however, this is possibly as a result of intramolecular hydrogen bonding. Due to the flexible nature of the alkyl amine pendant arms, there is the possibility that they are involved in hydrogen bonding within the ligand, which would make it more difficult to abstract a proton, contributing to the a higher pK_a value. Such intramolecular bonding was also proposed in Section 2.5.2, where the ^1H NMR spectrum of **44** was observed to be broader than that of the analogous **42**, and this was assumed to be as a result of intramolecular interactions of the ligand, including stacking and hydrogen bonding. The electron donating effects of the amines, which decrease the acidity of the ligands, thus increasing the pK_a values, can also contribute to overall higher protonation constants for the alkylamine acetamide functionalised ligands, as compared to the acetamide functionalised ligands. As such, it is also possible that pK_{a1} of **44** is due to the protonation of one of the terminal amines. The protonation constants pK_{a3} to pK_{a7} were tentatively assigned to the protonation of the alkyl amines. The near-simultaneous protonation of pK_{a4} and pK_{a5} in both ligands indicates that the two sites were separated by distance, and probably occur *trans* to each other. The final two protonations, pK_{a8} and pK_{a9} , were assigned to the two cyclen rings. Other pK_a values were not determined as they occurred at very acidic pH, and thus, were not measurable.

3.2.4 Determination of the Protonation Constants for the Ligands **47** and **48**

This section details the protonation constants of the xylyl spaced alkylamine acetamide functionalised ligands **47** and **48**. A typical titration curve for the ligand **48** is presented in Figure 3.4, and that of **47** is presented in Appendix A.2.3. The curve has several visible inflection points, indicating that there are several pK_a values associated with the ligand.



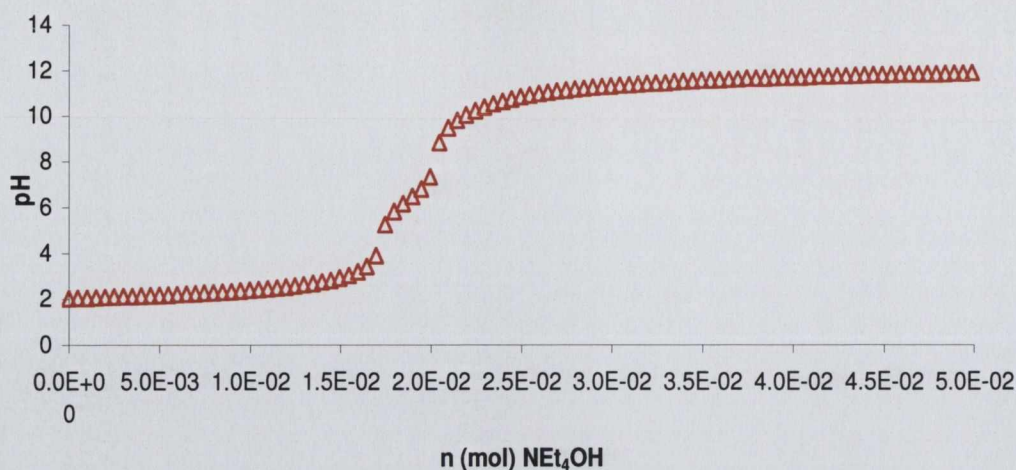


Figure 3.4: Typical titration curve of the protonated ligand **48** against NEt_4OH at 25 °C. $[\mathbf{48}] = 9.3 \times 10^{-4} \text{ M}$, $[\text{H}^+] = 8.6 \times 10^{-3} \text{ M}$, $[\text{NEt}_4\text{OH}] = 0.10 \text{ M}$, $I = 0.10 \text{ M}$ (NEt_4ClO_4).

The logarithm of the stepwise protonation constants for the studied ligands **47** and **48** are given in Table 3.4, and the conditions for the titrations are given below the table. Both ligands displayed similar titration curves, and gave rise to similar $\text{p}K_{\text{a}}$ values, indicating that the slight change to the fourth pendant arm did not have a large effect on the protonation constants of the ligand.

Table 3.4: The $\text{p}K_{\text{a}}$ values of the protonated pyridine spaced ligand **47** and **48** obtained at 25 °C with $I = 0.1 \text{ M}$ (NEt_4ClO_4) in H_2O .

$\text{p}K_{\text{a}}$ of protonation site	47^a	48^b
$\text{p}K_{\text{a}1}$	10.79 (± 0.08)	11.32 (± 0.09)
$\text{p}K_{\text{a}2}$	9.44 (± 0.07)	9.79 (± 0.08)
$\text{p}K_{\text{a}3}$	6.01 (± 0.07)	6.26 (± 0.09)

^a $[\mathbf{47}] = 9.3 \times 10^{-4} \text{ M}$, $[\text{H}^+] = 8.6 \times 10^{-3} \text{ M}$, $[\text{NEt}_4\text{OH}] = 0.10 \text{ M}$, $I = 0.10 \text{ M}$ (NEt_4ClO_4), ^b $[\mathbf{48}] = 1.0 \times 10^{-3} \text{ M}$, $[\text{H}^+] = 7.9 \times 10^{-3} \text{ M}$, $[\text{NEt}_4\text{OH}] = 0.10 \text{ M}$, $I = 0.10 \text{ M}$ (NEt_4ClO_4).

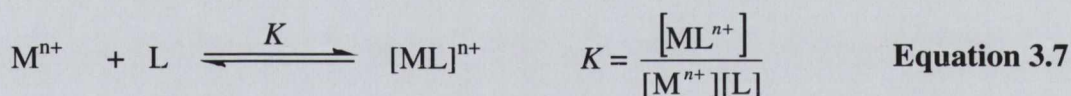
The first and second protonation constants of both ligands, $\text{p}K_{\text{a}1}$ and $\text{p}K_{\text{a}2}$, were determined to be 10.79 (± 0.08) and 9.44 (± 0.07), for **47**, and 11.32 (± 0.09) and 9.79 (± 0.08) for **48**, respectively. Both of these protonations, for both ligands, were assigned to the protonation of the amines of the macrocyclic ring. The final protonation, $\text{p}K_{\text{a}3}$, observed for each ligand was assigned to another protonation of the cyclen ring and again occurred at a similar $\text{p}K_{\text{a}}$ for both ligands.

3.3 Determination of Stability Constants

The stability, or formation constant of a complex gives a measure of the thermodynamic and kinetic stability of a metal complex.¹⁵⁴ Stability constants can be

determined using a variety of physical measurements, including UV-Vis absorption spectroscopy, luminescence titrations, NMR and potentiometric titrations, and are usually determined in aqueous solution, although mixtures of aqueous and non-aqueous solutions can also be used.

Both acyclic and cyclic ligands have been used to complex metal ions, however, owing to “the macrocycle effect”, cyclic ligands tend to give rise to higher stability constants, as a result of the preorganization of the ligand, a desolvation of the donor atoms within the confined structure of the cavity, a basicity effect as a result of the inductive effect of the ethylene bridges between donor atoms and finally, an enforced repulsion between the lone pairs of the donor atoms. Other factors which can influence the stability constant of a complex include the size of the metal ion (as compared to that of the cavity of the macrocycle) and the preference of the metal ion towards the donor atoms of the ligand *e.g.* metal ions such as the lanthanides prefer hard donor atoms such as nitrogen or oxygen.^{154,162} The concept of “size-match-selectivity” is of importance here, which is that a ligand will form the best complex with a metal ion that fits most closely to the size of the ligand cavity. It is of utmost importance that in the determination of stability constants of metal complexes that the thermodynamic equilibrium of the complex is reached.¹³⁰ The equilibrium equation for the complexation of a solvated metal ion (M^{n+}) by an uncomplexed ligand (L), to give a metal complex (ML^{n+}), is shown below, Equation 3.7.



Complexes between the polyazacrown cyclen and Ln(III) ions can have slow formation kinetics, and for this reason are generally formed by reflux, whereas stability constants are generally determined at room temperature.¹³⁰ Nevertheless, stability constants have been previously determined for cyclen-based lanthanide complexes, in aqueous solution at room temperature.^{19,24,25} During the course of our investigation into the stability constants of the cyclen-based lanthanide complexes described in Chapter 2, it was determined that the thermodynamic equilibrium of the complex was not being reached within a reasonable time frame for the titration (> 15 hours). To further investigate this, luminescence measurements were carried out on the ligand, **42**. A solution of $\text{Eu}(\text{CF}_3\text{SO}_3)_3$ (1.5×10^{-2} M) was titrated into a buffered solution (0.1 M TEAP) of the ligand (1.0×10^{-4} M), (at five minute intervals), and the phosphorescence and fluorescence of the metal ion (excited through the spacer chromophore of the ligand, 266 nm) was monitored. It was

expected that the luminescence would plateau after the addition of *ca.* two equivalents of the metal ion. Instead, the luminescence of the metal ion continued to rise, up to the addition of four equivalents of metal ion, as shown in Figure 3.5. Following this, the phosphorescence of the metal ion was monitored overnight, and the luminescence measured at 30 minute intervals, and it was observed that the luminescence did not plateau (Appendix A2.4). This led to the conclusion that septa-coordinated cyclen ligands, such as those synthesised in Chapter 2, have a slow kinetic formation at room temperature. Conversely, when formed by reflux, the resulting complexes are highly stable. This was demonstrated by monitoring the phosphorescence of the complex overnight, and then also in the presence of EDTA, at pH 7.4, and on both occasions, the luminescence remained unchanged, Appendix A2.5 and A2.6, respectively. However, this experiment meant that stability constants could not be accurately determined for the complexes detailed within.

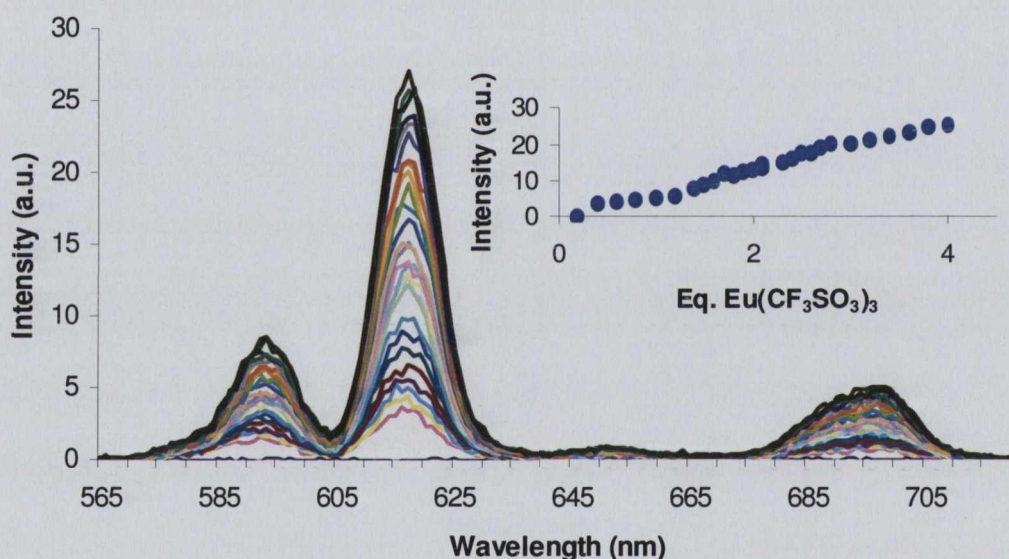
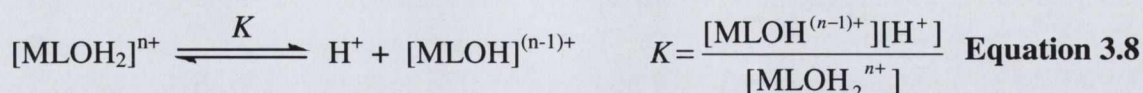


Figure 3.5: Luminescence spectra of **42** (1.0×10^{-4} M), showing the increase in Eu(III) emission upon increasing additions of $\text{Eu}(\text{CF}_3\text{SO}_3)_3$ (1.5×10^{-2} M). pH 7.4 (0.05 M HEPES), $I = 0.1$ M TEAP, excited at 266 nm. Insert shows the intensity as a function of equivalents of $\text{Eu}(\text{CF}_3\text{SO}_3)_3$ added.

However, it was possible to determine the protonation constants of the complex by potentiometric titration of the pre-formed complex in the same manner as that undertaken for the ligands discussed earlier. This enabled the determination of the $\text{p}K_a$ values for the metal bound water molecules of the Ln(III) ions. Given that the hydrolysis of cyclen-based lanthanide complexes has been shown to be highly pH dependant in the past,^{120,122} the determination of the $\text{p}K_a$ values for the metal bound water molecules was necessary for the investigation of the mechanisms of action of the complexes. The equilibrium equation for the deprotonation of a metal bound water molecule is given in Equation 3.8.



Potentiometric titrations were carried out on the Ln(III) complexes of the cyclen based ligands outlined previously to determine the protonation constants of the metal bound water molecules, and these investigations are detailed in the next Section.

3.4 Investigation into the Protonation Constants of the Metal Bound Water Molecules of Cyclen-Based Ln(III) Complexes

Potentiometric titrations were carried out on the complexes of the form $[\text{LM}_x(\text{OH}_2)_y]^{n+}$ (where M = La(III) or Eu(III), x = 1-2, y = 2-4 and n = 3, 6) of the ligands discussed in the previous section, to determine the protonation constants of the metal bound water molecules. Due to the slow formation kinetics of such cyclen-based Ln(III) complexes, the complexes were synthesised by refluxing the ligands in the presence of $\text{Ln}(\text{CF}_3\text{SO}_3)_3$ for 16 hours (as outlined in Chapter 2). These preformed complexes were then titrated to determine the protonation constants of the metal bound water molecules.

The titrations were carried out using the same technique as was used for the titration of the ligands, whereby following the titration with base, acid was titrated into the solution to give the back titration and to establish the equilibrium of the complex and the necessary time to leave between the addition of aliquots of base. Frequently, although the equilibrium had been investigated and established for the base titration, overlap of the base and acid titrations was not observed, indicating that the titration was not reversible. This was possibly due to several factors, the first being that at high pH, it was possible that the complex was precipitating out of solution, affecting the concentration and thus affecting the reversibility of the back titration.¹⁵⁴ It is also possible that at low pH there was a partial dissociation of the complex,¹⁵⁹ followed by a slow reassociation, which would give rise to irregularities in the titration and also ensure that overlap of the forward and back titration was difficult to obtain. The final possibility is that this lack of reversibility was due to an aggregation effect arising from the complex in solution. Such an effect has been observed before for neutral dinuclear cyclen-based Gd(III) complexes,²⁷ and although the complexes studied herein are positively charged, it remains a possibility that aggregation could occur.

3.4.1 Investigation of the Protonation Constants of 47.Ln and 48.Ln

The first complexes to be titrated were the mononuclear Eu(III) complexes, **47.Eu** and **48.Eu** and the titration curves for **47.Eu** are shown in Figure 3.6.. As was observed for the ligands **47** and **48**, both complexes gave rise to similar titration curves upon the

addition of NEt_4OH . The titration curves for **47.La** and **48.Ln** are shown in Appendix A2.7-A2.9.

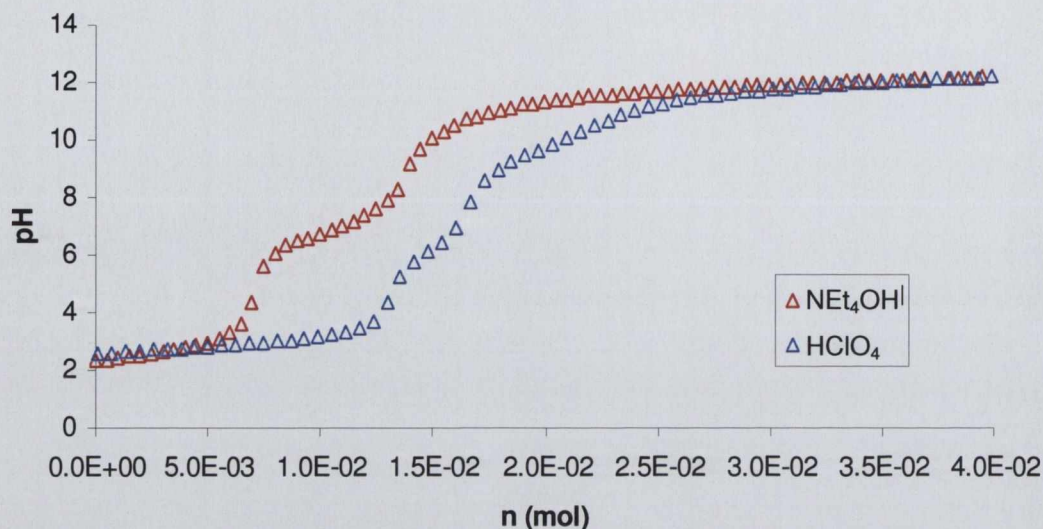


Figure 3.6: Titration curves for the complex **47.Eu** ($1.0 \times 10^{-3} \text{ M}$) against NEt_4OH (0.09 M), followed by the reverse titration against HClO_4 (0.10 M), in $I = 0.10 \text{ M NEt}_4\text{ClO}_4$.

The base was added to the complex solution with a delay time up to a maximum of 6 minutes per point, having carried out several titrations to ensure that the complex was at the thermodynamic equilibrium with such timing. From examination of the base titration curve, it can be determined that two equivalents of the complex are consumed between the two jumps in the titration curve, suggesting that the deprotonation of two metal bound water molecules possibly give rise to the changes in the curve. The possibility that the inflection point arises as a result of the deprotonation of an amide cannot be discounted entirely, however, amides are not easily deprotonated,¹⁶¹ and as such, despite the coordination of the amide carbonyl to the Ln(III) metal centre, this is unlikely to occur.

Another possible problem, as aforementioned, was the potential for the complex to dissociate at very acidic pH, of *ca.* 2. Given the slow formation kinetics of these cyclen-based Ln(III) complexes at pH 7.4 at room temperature, as discussed in Section 3.3, the possible dissociation of the complex at low pH, followed by a slow reformation may give rise to the presence of several species in solution, and an accurate fit for this data could not be determined.^{154,159} The poor reversibility of the two titration curves also suggested that the complex was possibly precipitating out of solution at high pH. To further investigate this possibility, the complex was titrated with base up to pH 9, and then with acid to observe if terminating the titration at a lower pH had any effect on the reversibility, Figure 3.7. Once again the acid titration curve did not overlap well with that observed for the base titration, and it was proposed that the complex was possibly not easily reprotonated during

the acid titration, or that the deprotonation of the metal bound water molecules was not reversible.

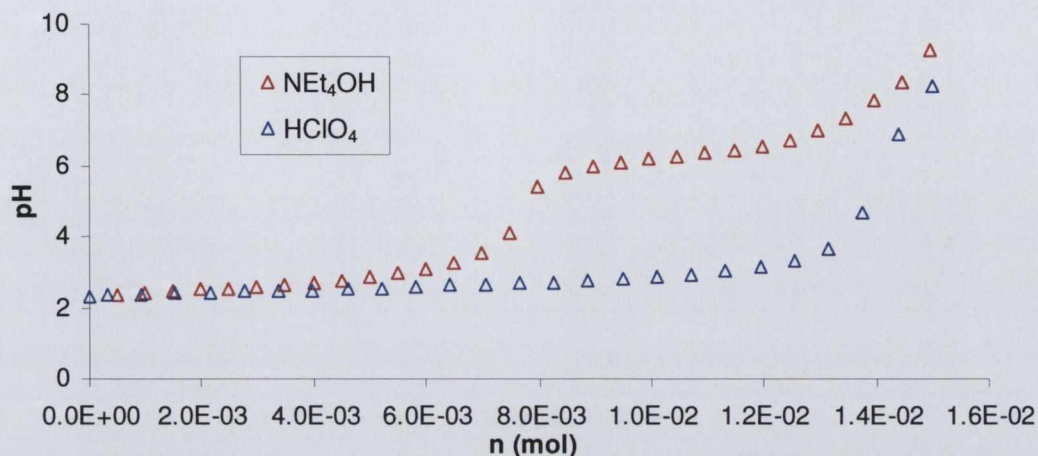


Figure 3.7: Titration curves for the complex **47.Eu** (1.0×10^{-3} M) against NEt_4OH (0.09 M), up to pH 9.25, followed by the reverse titration against HClO_4 (0.10 M) in $I = 0.10$ M NEt_4ClO_4 .

If the complex does indeed partly dissociate at acidic pH, it is possible that it may not have fully recomplexed at the point when the complex is being deprotonated during the titration, thus causing the jump in the curve to occur sooner than it would otherwise. The kinetics of such acid dissociation-reassociation have been evaluated for several cyclen-based Ln(III) complexes.^{19,28} However, the ligands studied have differed structurally from those outlined herein, both in the number of pendant arms and in the structural nature of the pendant arms. The formation and dissociation kinetics differ even between structurally similar complexes, and as such, the kinetics of one system cannot be accurately extrapolated from examination of a similar system.¹⁵⁹ Due to time constraints, a kinetic study of the complex's dissociation was not undertaken, and as such it cannot be assumed that the complex will have fully recomplexed by the stage in the titration at which the metal bound water molecules are deprotonated by the addition of base and as such it must be assumed that there is possibly a mixture of species in solution, and not just the complex in question.

It is also possible that there is a combination of these two processes at work in solution, giving rise to an irreproducible titration curve, along with irregular values for the protonation constants of the metal bound water molecules. The concentration of the complex in solution is calculated accurately and a small amount of precipitation can affect the concentration, altering the fit of the curve. Also, should there be even a small degree of dissociation of the complex at low pH, the concentrations in solution will again be affected as discussed above.

Despite the possibility that there was more than one species in solution, it was attempted to fit the base titration curves for the complexes using HYPERQUAD,¹⁵⁶ as for the ligands previously, and an example of the attempted fitting for **47.Eu** is shown in Figure 3.8. The HYPERQUAD program fits using a non-linear least squares regression method to minimise the differences between the experimental data and the theoretical values for the proposed model (Section 3.2),¹⁵⁶ however, the values determined for the pK_a of the two metal bound water molecules on the Eu(III) complex were 12.0 and 6.08, with an excessive error for each pK_a value. The pK_a value of 12.0 is unreliable in part due to the fact that the titration only goes as far as pH 12. The pK_a value of 6.08 is potentially more accurate as it falls within the pK_a region of the curve. However, due to the large error associated with the fit of the titration curve, neither of these values can be reported with confidence.

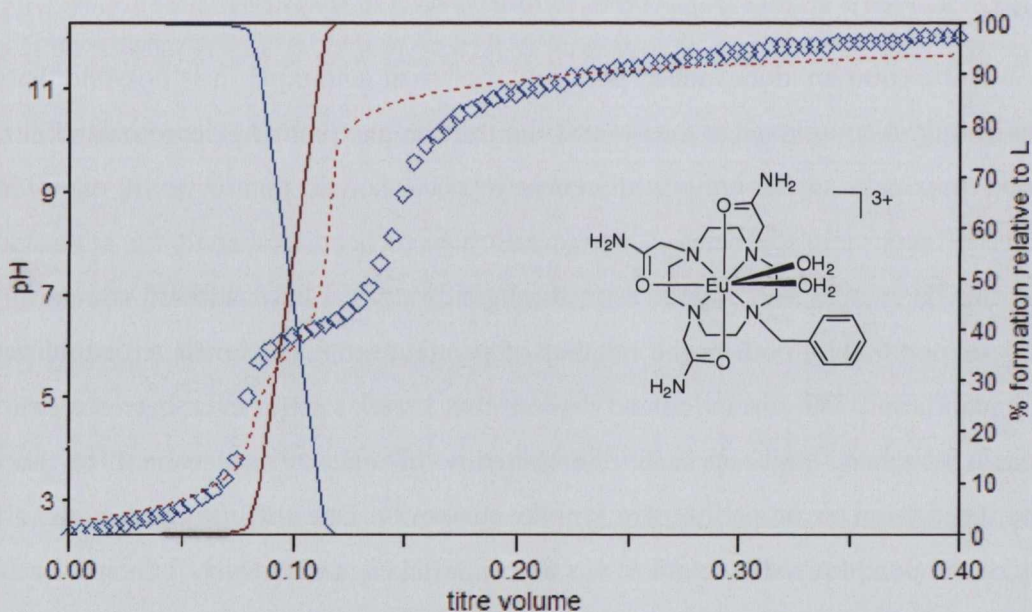


Figure 3.8: The attempted fit of **47.Eu** (1.0×10^{-3} M) titration against NEt_4OH (0.1 M) in $I = 0.10$ M NEt_4ClO_4 , using HYPERQUAD. The blue diamonds show the titration curve and the dashed red line the fit.

Following examination of the fit of the data for **47.Eu**, **47.La**, **48.Eu** and **48.La**, it was determined that there were similar problems with the titrations for the other complexes. Such irregularities led to an irreversibility of the titration, the result of which was that the titration curve could not be fitted to within any reasonable degree of error, and as such, the protonation constants of the metal bound water molecules could not be accurately determined.

3.4.2 Investigation of the Protonation Constants of $39.Ln_2$, $41.Ln_2$ and $43.Ln_2$

The complexes $39.Ln_2$, $41.Ln_2$ and $43.Ln_2$ were investigated in a similar manner as for the complexes outlined in the previous section, and again problems were encountered with the reversibility of the potentiometric titrations. Despite ensuring that the titrations were at the thermodynamic equilibrium, the shape of the curve for the back titrations did not overlay with those of the base titrations, nor did the curves overlap in terms of the equivalents of acid and base added. The titration curves for $41.Eu_2$ can be seen in Figure 3.9, and those of $39.Ln_2$ and $43.Eu_2$ are shown in Appendices A2.10 to A2.12, respectively. Again, several titrations were carried out with equilibration times of up to ten minutes between the addition of aliquots of base to establish the time needed to reach the thermodynamic equilibrium of the complex during the titration.¹⁵⁹ For the titration shown in Figure 3.9 an equilibrium time of six minutes per point was set. The acid titration shows very poor reversibility, with the shape of the curve differing drastically from that of the base titration. This suggests that this complex is suffering from the same potential problems regarding acid dissociation and precipitation as for $47.Ln$ and $48.Ln$. However, the extent to which the curve has changed indicates that the dinuclear complex is more complex than the analogous mononuclear complexes.

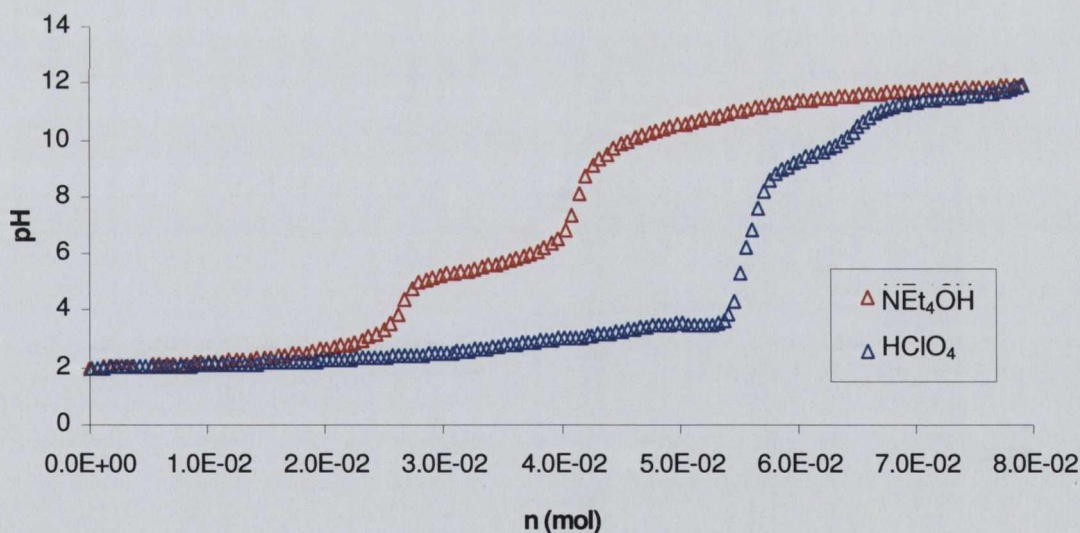


Figure 3.9: The titration of $41.Eu_2$ (1.0×10^{-3} M) with NEt_4OH (0.10 M) (red) followed by the reverse titration with $HClO_4$ (0.10 M) (blue), in $I = 0.10$ M NEt_4ClO_4 .

Five equivalents of the complex were consumed between the two major inflection points of the base titration curve shown in Figure 3.9, as opposed to the mononuclear complexes where only two equivalents of the complex were consumed between the two jumps in the curves. This indicated that the deprotonation of the second metal bound water molecule occurred at a lower pK_a than that of the first. It is also possible that the first jump

in the pH occurred sooner than would have been expected for the amount of acid in solution, and if there was a partial decomplexation of the complex in solution that the concentration of the acid in solution could be altered, thus distorting the fitting of the titration curve.

As for the titrations of the mononuclear complexes, it was attempted to fit the titration curve using HYPERQUAD,¹⁵⁶ and the resulting fit is shown in Figure 3.10. As anticipated, the values determined for the deprotonation of the metal bound water molecules had a large error associated with them, and the overall fit to the titration curve was poor, and gave rise to an unacceptable chi-squared value. The titration curve was observed to fit poorly both at low and high pH, which raised the possibility that there may be several species in solution giving rise to the poor titration curve. The titration curve was fitted for the deprotonation of four metal bound water molecules, however, this fit and all other combinations attempted gave rise to unacceptably high error values, indicating that the pK_a values determined were unreliable.

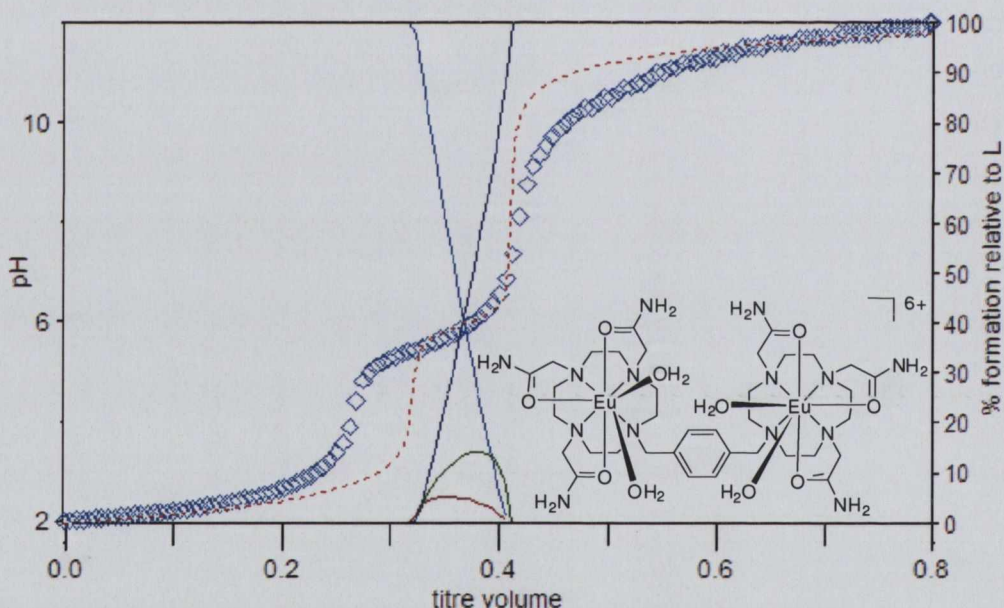


Figure 3.10: The attempted fit of the titration of **41**.Eu₂ (1.0×10^{-3} M) against NEt₄OH (0.1 M), in $I = 0.10$ M NEt₄ClO₄, using HYPERQUAD. The blue diamonds show the titration curve and the dashed red line the fit. This shows the fit for the deprotonation of four metal bound water molecules.

The ligand **41** was previously potentiometrically titrated in the presence of Eu(III), and it was attempted to fit the titration curve, again using HYPERQUAD, to determine the stability constants for the complex and to determine the pK_a values for the metal bound water molecules.¹⁵⁴ Although it was later determined that this data was unreliable due to the slow formation kinetics of the Ln(III) complexes of these septa-coordinated ligands, the pK_a values determined are reported herein for comparison.

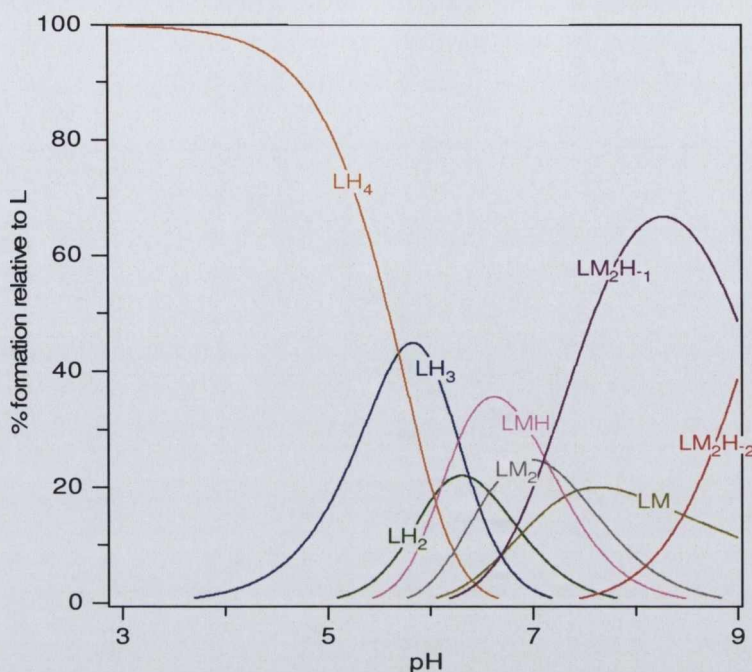


Figure 3.11: Speciation plot for $41.Eu_2$. $[41]_{total} = 7.5 \times 10^{-4} M$, $[Eu(III)]_{total} = 1.5 \times 10^{-3} M$, $I = 0.1 M$ NEt_4ClO_4 . Speciation is shown relative to the total concentration of 41 .

The speciation plot for $41.Eu_2$ is shown in Figure 3.11. The pK_a values of the metal bound water molecules were found to be $7.42 (\pm 0.03)$ and $9.28 (\pm 0.02)$, which are comparable to those reported for similar Eu(III) complexes.^{122,130,146} The pK_a value of 7.42 was relatively low for the deprotonation of the metal bound water molecule and suggested that the presence of the second Eu(III) ion possibly gave rise to a cooperative effect for the deprotonation of the metal bound water molecule, or perhaps a bridging hydroxide was formed, lowering the pK_a of the metal bound water molecule. Nevertheless, due to the *in situ* formation of the complex for this experiment, there was a large possibility that the complex was not at equilibrium, thus distorting the data and giving rise to unreliable protonation constants.

Investigation of the protonation constants of $41.Eu_2$ by the two methods outlined above gave rise to similar results, both of which were unreliable due to the problems outlined above, suggesting that the dinuclear complexes of the type investigated here are more complicated than previously investigated complexes

3.4.3 Investigation of the Protonation Constants of $42.Ln_2$ and $44.Ln_2$

As for the complexes discussed previously, the complexes $42.Ln_2$ and $44.Ln_2$ were potentiometrically titrated against NEt_4OH , and the reverse titrations were carried out against $HClO_4$. Again, the forward and reverse titration curves did not overlay, suggesting that there was a dissociation or a precipitation problem with the titration. The titration

curves for **42.Eu₂** are shown in Figure 3.12, and those of **42.La₂** and **44.Ln₂** are shown in Appendices A2.13-A2.15.

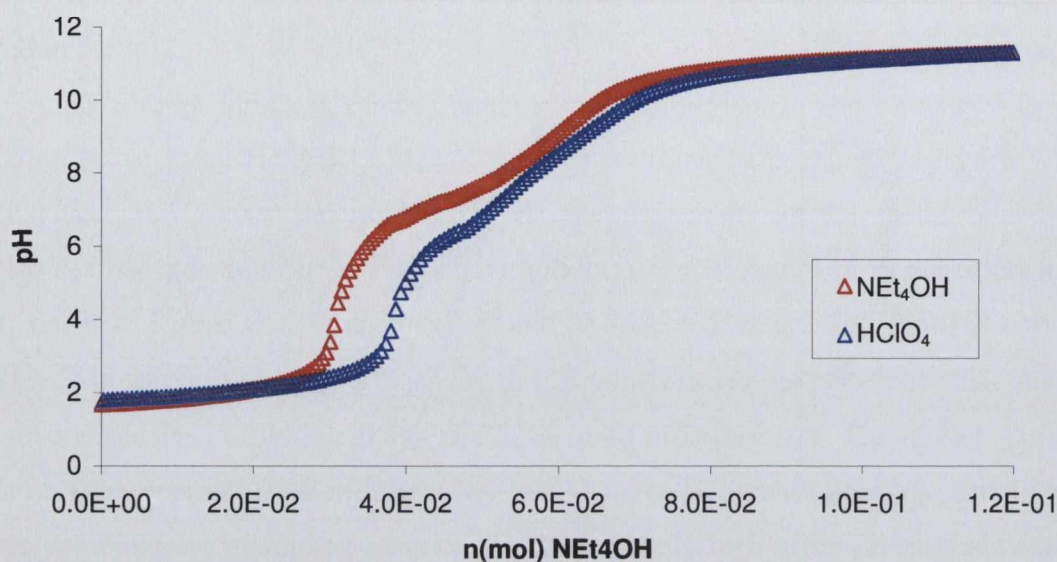


Figure 3.12: The titration of **42.Eu₂** (1.0×10^{-3} M) with NET_4OH (0.10 M) (red) followed by the reverse titration with HClO_4 (0.10 M) (blue).

Several inflection points can be seen on the curve between pH 6 and 8, indicating that there was numerous pK_a values associated with the complex in that pH range. Unlike the previous complexes studies, it was not possible to determine the number of equivalents of ligand consumed between the pK_a regions of the curve. The titration once again gave rise to a poor fit of the data using HYPERQUAD.¹⁵⁶ For the base titration of **42.Ln₂** and **44.Ln₂**, it was possible that alongside the deprotonation of the metal bound water molecules, the protonation of the terminal amines of the pendant arms would be observed. However, no reliable protonation constants for such species could be determined. Again, the inclusion of four possible deprotonations gave rise to the best fit of the curve, nevertheless, the error was unacceptably high, and thus the pK_a values were deemed unreliable.

The nature of the titration curve suggested that precipitation most likely occurred at more alkaline pH and so it was attempted to fit the data for the lower portion of the curve only, however this did not result in a better fit. Once again, it would appear that there is most likely a combination of partial dissociation and precipitation of the complex in solution rendering the titration data unreliable and uninformative.

3.5 Conclusion

For all the of the complexes investigated, the Ln(III) ions were septa-dentate, *via* coordination of the four cyclen amines and three coordinating pendant arms. This structure

was deliberately chosen to allow for the incorporation of a minimum of two metal bound water molecules onto each Eu(III) metal ion, with the aim of achieving enhanced rates of phosphodiester hydrolysis.^{61,110,163} It is possible that such coordination has reduced the overall stability of the complex, especially at acidic pH. Unfortunately, due to the slow kinetics of the system, it was not possible to determine the stability constants of the complexes potentiometrically or using luminescence measurements. There are various methods which can be used to accurately estimate the stability constants of complexes with such slow formation kinetics, including competition studies, however, due to time constraints, it was not possible to carry out such an experiment.

The results outlined herein do suggest that it may be possible to fit for the deprotonation of up to four metal bound water molecules for the dinuclear Ln(III) complexes. However, due to a combination of potential precipitation from solution and a possible partial decomplexation of the ligand, the results outlined were inconclusive.¹⁵⁶ It is possible that pH potentiometry could be used in the future for further investigations, however, to avoid any possible dissociation of the complex in solution, and as a result of the general poor fitting of the curve at low pH, it may prove beneficial to also study the kinetic stability of the complexes in acidic media over a range of pH values to determine the kinetic formation and dissociation values for the complexes. Another possibility would then be to start the potentiometric titrations at a higher pH, for example, at a pH above 4.0, and then titrate only up to pH 9.0. A titration such as this, in combination with kinetic stability studies may prove more accurate for the determination of the pK_a values of the metal bound water molecules for the complexes.

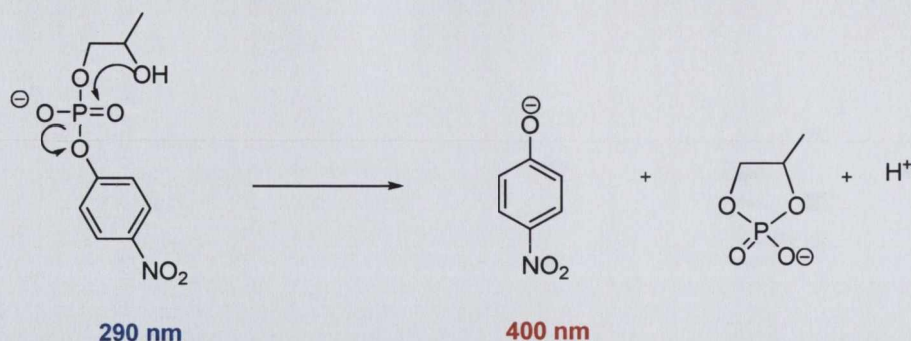
4.1 Introduction

As outlined in Chapter One, phosphodiesterases are important targets in medicinal chemistry.^{3,6} In particular, the hydrolysis of the phosphodiester bond of the mRNA, through the use of an artificial ribonuclease mimic, can be used to prevent protein production and therefore is of particular interest for the prevention of disease.^{4,8,39} As such, there is a growing interest in creating a new genetic therapeutic based on phosphodiester bond hydrolysis, *via* an artificial ribonuclease mimic.^{5,56,58,59}

This Chapter details the kinetic evaluation of the Ln(III) complexes presented in Chapter 2, for the ability of these metal based ribonuclease mimics to hydrolyse phosphodiester bonds. To begin, the effect of the pyridine spacer in complexes **39.Ln₂** (Ln = La(III), Eu(III)) on the rate of hydrolysis of **HPNP** will be examined. This will be followed by an examination of the ability of the xylene-spaced systems, **41.Ln₂**, **43.Ln₂**, **42.Ln₂** and **44.Ln₂**, to hydrolyse phosphodiester bonds of **HPNP**. The strength of the binding to phosphate by the most efficient complexes will be investigated, and inhibition studies will be carried out. The results of the dinuclear systems will then be compared to those of their mononuclear analogues, **47-49.Ln**. This will be followed by a discussion of the possible mechanisms of action of these complexes for achieving the hydrolysis of **HPNP**, based on the analysis of the results presented in this Chapter, along with those of Chapter 3.

4.2 Hydrolysis of Phosphodiester Bonds

As discussed in Section 1.7, the use of mRNA to evaluate the efficiency of compounds to hydrolyse phosphodiester bonds is impractical, both in terms of cost and in terms of ease of use within the laboratory, although it is done.⁹ As such, compounds are generally evaluated against RNA model compounds and in this Chapter, the model RNA compound **HPNP** will be employed. This compound is ideal to use as a model for RNA hydrolysis as it contains an intramolecular hydroxyl group, similar to the 2'-hydroxyl group found in RNA, which can be activated towards hydrolysis by a general acid or base.⁹ **HPNP** was synthesised as its barium salt, according to the literature procedure by Brown *et al.*¹⁶⁴ Hydrolysis of **HPNP** can be achieved by a transesterification reaction, as shown in Scheme 4.1, which can be followed spectroscopically by using UV-Vis absorption, where **HPNP** absorbs at 290 nm, and the product *p*-nitrophenolate at 400 nm. A typical spectrum observed for the hydrolysis of **HPNP**, by **39.Eu₂**, at pH 7.4 is shown in Figure 4.1.



Scheme 4.1: Transesterification of **HPNP** ($\lambda = 290 \text{ nm}$) to give *p*-nitrophenolate ($\lambda = 400 \text{ nm}$) and a cyclic phosphate.

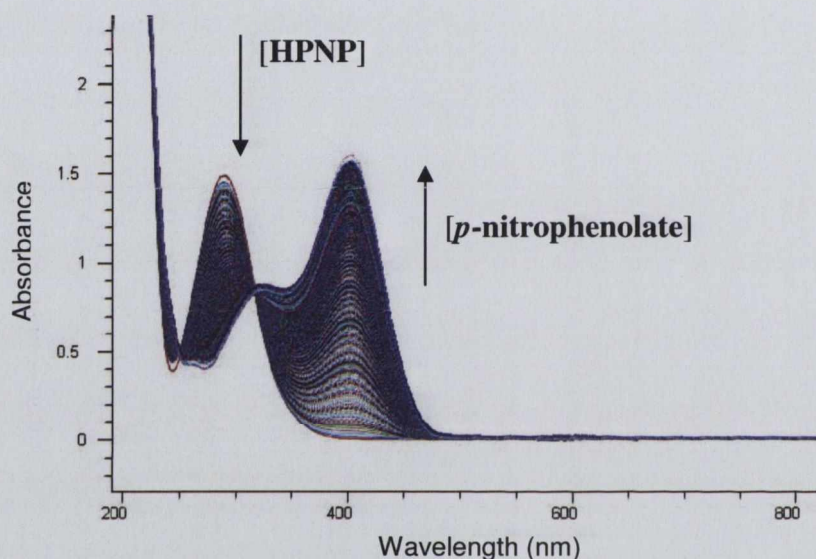


Figure 4.1: The UV-Vis absorption spectrum showing the decrease in the absorption of **HPNP** and the concomitant increase in the absorption of *p*-nitrophenolate, following the hydrolysis of **HPNP** (0.18 mM) by **39.Eu** (0.18 mM) at pH 7.4, in 50 mM HEPES buffer, 37 °C.

4.2.1 Reaction Conditions for the Hydrolysis of HPNP

The first order kinetic evaluations of the complexes were carried out in a 1:1 molar ratio of **HPNP** to complex. The extinction co-efficient of **HPNP** was determined, using the Beer-Lambert law, to be $\epsilon = 8390 \text{ M}^{-1} \text{ cm}^{-1}$ at 290 nm. The absorbance of **HPNP** was fixed at 1.5, resulting in a 0.18 mM solution. All complexes were made up to a concentration such that a 100 μL aliquot contained 4.32×10^{-7} moles of complex, a 0.18 mM solution.

For first order analysis, solutions of **HPNP** were made up in 50 mM HEPES buffer and the pH adjusted accordingly using a 1 M solution of either NaOH or HCl. A 2.4 mL aliquot of this **HPNP** solution was then incubated in a quartz UV-Vis cell at 37 °C for 10 min. All reactions were monitored over at least three half lives, or for a maximum of 16 h. All kinetic evaluations detailed in these studies were carried out using either an Agilent 8453 spectrophotometer or a Varian CARY 50 UV-Vis spectrophotometer. Both were

connected to circulating water baths to maintain a constant temperature at 37 °C and were stirred with a magnetic stirrer.

To determine the rate constant for each measurement, the changes in the absorption at 400 nm as a function of time were fit to a first order rate equation. The rate constant (k) can also be determined by plotting the logarithm of the change in the absorption spectrum as a function of time, as discussed in Section 1.9, an example of which is shown in Appendix A3.1, for **39.Eu₂** at pH 7.4. The background hydrolysis of **HPNP**, the hydrolysis of the ‘uncatalysed’ reaction, has been measured previously by Breslow and co-workers,⁵⁰ and has a first order rate constant of $k_{\text{uncat}} = 3.3 \times 10^{-8} \text{ s}^{-1}$ at pH 7.4, which corresponds to a half life of 5.78×10^3 hours. When evaluating the efficiency of the complexes developed in Chapter 2 for their ability to hydrolyse **HPNP**, a comparison, k_{rel} , will be used in reference to this rate constant, where k_{rel} is the ratio of the half life of the uncatalysed reaction over the half life of the measured reaction, ($\tau_{1/2\text{uncat.}}/\tau_{1/2\text{cat.}}$).

4.3 Development of an Efficient Artificial Ribonuclease Mimic

Previously within the Gunnlaugsson group, several families of cyclen based lanthanide complexes have been developed.¹³⁻¹⁷ Their ability to hydrolyse **HPNP** was investigated to establish the optimum structure for a complex to efficiently hydrolyse phosphodiester bonds, Section 1.13. Analysis of the kinetic investigations of these complexes led to the conclusion that several factors generally resulted in efficient phosphodiester hydrolysis of **HPNP**. In general, these were based upon:

- **Metal ion size.**
- **Structure of the pendant arm.**
- **Hydrophobic cavity.**
- **Strength of phosphate binding.**
- **Number of metal bound water molecules.**
- **Structure of the spacer.**

These factors, and their effect upon the efficiency of the hydrolysis of **HPNP** are detailed in the next Section.

4.3.1 Metal Ion Size

The size of the Ln(III) metal ion was found to greatly influence the rate of hydrolysis of **HPNP**.^{100,102} A general trend was observed in which the Ln(III) ions with larger atomic radii (e.g. La(III), Ce(III), Pr(III) and Nd(III)) gave rise to faster rates of hydrolysis than the smaller Ln(III) ions.¹⁰² Despite its size falling in the middle of the range of the Ln(III) metal ions, Eu(III) has often been found to give rise to good hydrolysis

of **HPNP** and as such, a large majority of research has focussed on the use of La(III), Ce(III) and Eu(III).^{6,16,20}

4.3.2 Structure of the Pendant Arm and the Hydrophobic Cavity

The effect of the chemical nature of the pendant arm has been shown to strongly influence the rate of hydrolysis of **HPNP**,^{101,165} as discussed in Sections 1.14 and 2.2. The pendant arms coordinate the Ln(III) metal ion, and it has been shown, by X-Ray crystal structure analysis, that they can form a hydrophobic cavity around the metal ion.¹²⁰ It is thought that such a hydrophobic binding pocket is ideal to encapsulate the substrate and promote the hydrolysis of **HPNP**.¹²⁰

4.3.3 Strength of Phosphate Binding

The strength of the binding of the complex to phosphates was also found to influence the hydrolysis of phosphodiester bonds.^{19,21} The interaction of cyclen based Ln(III) complexes with phosphate was studied using diethyl phosphate (**DEP**) ³¹P NMR titrations. A trend was observed in which those complexes that showed very slow rates of hydrolysis of **HPNP** also showed little to no interaction with the **DEP**, whereas the complexes which gave rise to good rates of hydrolysis of **HPNP** were shown to have a very strong interaction with the **DEP**.¹⁰² Such results suggest that the strength of the binding of the complexes to **DEP** corresponds to the rate of hydrolysis of **HPNP**.

4.3.4 Number of Metal Bound Water Molecules

As discussed previously in Chapters 1 and 2, the number of metal bound water molecules on the metal ion has been shown to have a strong influence on the rate of hydrolysis of **HPNP**.^{59,101,102} In the case of large metal ions, such as La(III), which generally has a minimum of two metal bound water molecules present, the introduction of a third vacant coordination site did not greatly effect the rate of hydrolysis of **HPNP**, however, when a second vacant coordination site was introduced to a Eu(III) complex, increasing the number of metal bound water molecules to two, the rate of hydrolysis of **HPNP** was observed to increase by a factor of 4-fold.¹⁰² This suggests that the number of metal bound water molecules on the complex is a very important factor for increasing the rate of hydrolysis of **HPNP**.

4.3.5 Structure of the Spacer

The structure of the bridging spacer in a dinuclear cyclen based Ln(III) complex has been shown to have a large influence on the rate of hydrolysis of **HPNP**, as discussed in Section 2.2.^{17,21,23,24} It was found that a spacer which incorporated a degree of flexibility

and rotation into the overall complex, and importantly, which did not coordinate to the Ln(III) metal ion, gave rise to the most efficient rate of **HPNP** hydrolysis for the family of complexes evaluated.¹⁰¹

4.3.6 Design of an Optimum Complex for the Hydrolysis of HPNP

Having studied a wide range of mono and dinuclear cyclen based lanthanide complexes within the Gunnlaugsson group, and established the characteristics that led to the most efficient hydrolysis of **HPNP**, the aim was to develop a complex which could incorporate all of the factors outlined above, and to synthesise such a complex. It was thought that this complex would result in a large increase in the rate of **HPNP** hydrolysis over that already observed for similar lanthanide complexes, and that this complex would exhibit catalytic turnover, functioning as a true ribonuclease mimic.

The structures of the mono and dinuclear complexes synthesised within the Gunnlaugsson group, which were shown to give rise to the fastest rates of hydrolysis of **HPNP** are shown below as in **94.Ln** and **38.Ln₂**, and their rates of hydrolysis both at physiological pH are detailed in Table 4.1.^{16,19,20}

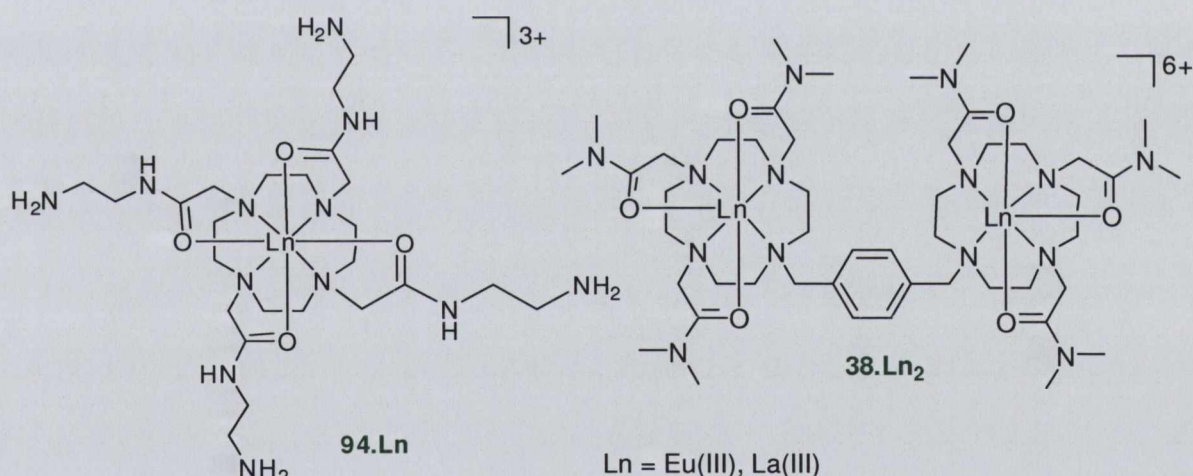


Table 4.1: Pseudo first order rate constants (k_{obs}) for the cleavage of **HPNP** (0.18 mM) in 50 mM HEPES at pH 7.4 and 37 °C by the most efficient compounds previously synthesised within the Gunnlaugsson group.

Complex	k_{obs} (s ⁻¹)	$\tau_{1/2}$ (h)	k_{rel}
94.Eu	$1.01 (\pm 0.05) \times 10^{-4}$	1.91	3032
94.La	$2.59 (\pm 0.09) \times 10^{-4}$	0.74	7775
38.Eu₂	$4.11 (\pm 0.22) \times 10^{-4}$	0.47	12338

As outlined in Section 1.13.3, **38.Ln₂** had given rise to the largest rate enhancements observed in the Gunnlaugsson group. It was hoped that by making small modifications to the spacer group and to the arms, further increases in the rate of **HPNP**

hydrolysis could be obtained. The use of the alkylamine acetamide pendant arms, shown in **94.Ln** had given rise to very large increases in the rate of **HPNP** hydrolysis for the mononuclear complexes.¹⁰² As such, it was intended to develop a dinuclear system which incorporated these arms, in order to yield a complex which would give rise to large increases in the rate of **HPNP** hydrolysis and function as a ribonuclease mimic.

The families of dinuclear complexes studied herein were designed with the factors outlined above in mind, and their influence on the rate of phosphodiester hydrolysis will be examined in this Chapter. The first Section details the kinetic evaluation of the pyridine spaced system, **39.Ln₂**.

4.4 Kinetic Evaluation of Pyridine Spaced System, **39.Ln₂**

The first family to be examined for their hydrolytic activity was the pyridine spaced complexes, **39.Eu₂** and **39.La₂**. The ligand **39** was designed based on research which showed that the pyridine spacer gave rise to strong phosphate binding,¹³⁸ as outlined in Section 2.3.1. By incorporating the pyridine moiety into the spacer of an unsaturated dinuclear complex, it was hoped to achieve a stronger binding to the substrate, which could in turn give rise to enhanced rates of phosphodiester hydrolysis of **HPNP**.¹⁶⁶

The Eu(III) complex, **39.Eu₂** was tested, and this complex was found to hydrolyse **HPNP**, at pH 7.4 and 37 °C, with a pseudo first order rate constant of $k_{\text{obs}} = 1.57 (\pm 2.3) \times 10^{-4} \text{ s}^{-1}$. The observed rate profiles for the absorption at 290 and 400 nm are shown in Figure 4.2.

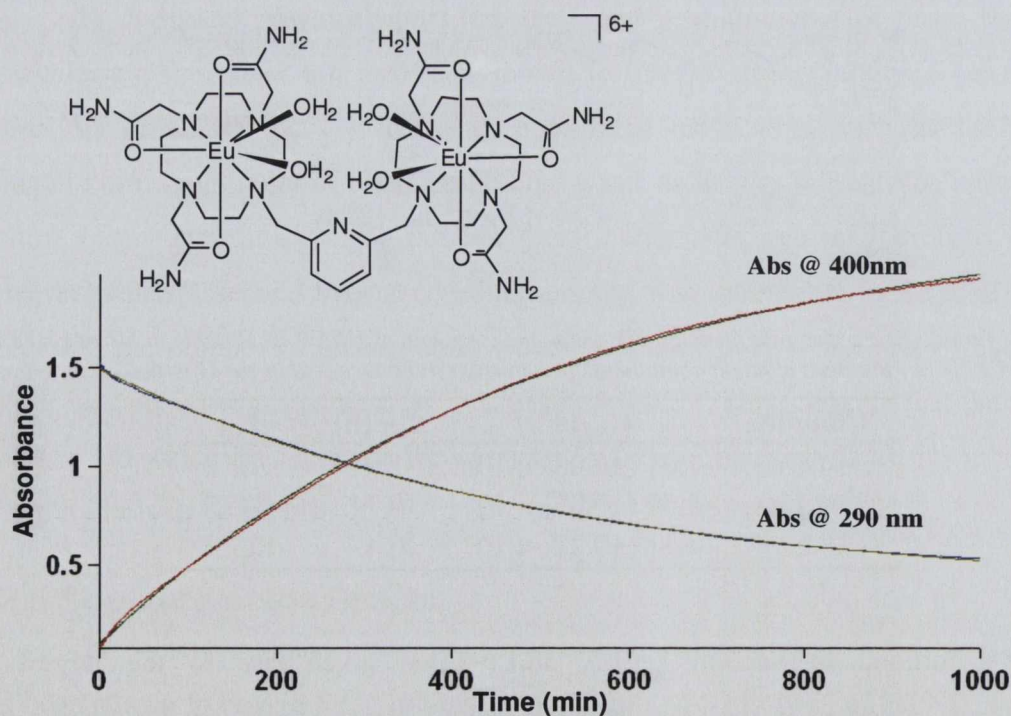


Figure 4.2: The kinetic profile for the hydrolysis of **HPNP** (0.18 mM) by **39.Eu₂** (0.18 mM) in 50 mM HEPES buffer at pH 7.4 and 37 °C. The increase of *p*-nitrophenolate (red line) as a function of time is observed and fitted to first order kinetics to determine the rate constant, k_{obs} for the reaction.

Interestingly, under the same conditions, the corresponding La(III) complex, **39.La₂** was found to hydrolyse **HPNP** at a much slower rate, with $k_{\text{obs}} = 7.07 \times 10^{-6} \text{ s}^{-1}$ (Appendix A3.2). This was an unexpected result, as in general the larger lanthanide ions, *e.g.* La(III), have given rise to higher k_{obs} values than the Eu(III) ion. However, **39.La₂** resulted in a rate enhancement, k_{rel} of only 213 in comparison to **39.Eu₂** which gave a k_{rel} of 4713.

Table 4.2: Pseudo-first order rate constants (k_{obs}) for the cleavage of **HPNP** (0.18 mM) in 50 mM HEPES buffer at pH 7.4 and at 37 °C, for **39.Eu₂**, **39.La₂** and **38.Eu₂**.

Complex	$k_{\text{obs}} (\text{s}^{-1})$	$\tau_{1/2} (\text{h})$	k_{rel}
38.Eu₂	$4.11 (\pm 0.22) \times 10^{-4}$	0.47	12298
39.Eu₂	$1.57 (\pm 0.23) \times 10^{-4}$	1.23	4699
39.La₂	$7.07 (\pm 0.15) \times 10^{-6}$	27.2	213

For comparison purposes, the rate constant observed for **38.Eu₂** has been included in Table 4.2, which shows that the rates for both **39.La₂** and **39.Eu₂** are smaller than that of **38.Eu₂**, despite being functionalised with six acetamide arms, which were anticipated to enhance the rate of hydrolysis of **HPNP**, as outlined in Section 4.3.2. It is possible that the pyridine spacer is binding the substrate very tightly, thus inhibiting, and not accelerating the hydrolysis of **HPNP**. To gain a further understanding for the mechanisms of action of the complexes **39.Ln₂**, the hydrolysis of **HPNP** was studied at various pHs, and this is detailed in the next Section.

4.4.1 The pH Dependence of the Hydrolysis of **HPNP** by **39.Ln₂**

As discussed in Chapter One, the hydrolysis of the phosphodiester bonds of **HPNP** by metal based complexes can be highly pH dependant. The objective of our design was to bring the active range of the complexes to within the boundaries of physiological pH, as many of the complexes developed within the Gunnlaugsson group have been shown to be very active for the hydrolysis of **HPNP**, however, in many the efficiency of the complexes was confined to the alkaline pH region. As such, following an initial analysis of the **39.Eu₂** and **39.La₂** at physiological pH 7.4, their efficiency was next analysed as a function of pH. Having shown a large k_{obs} at pH 7.4, the Eu(III) complex was first analysed, and the results are shown as a pH-rate profile, Figure 4.3. Each data point in the pH-rate profile is an average of 2-3 measurements in agreement to within 15% error, and the error bars are also determined from the average of 2-3 measurements.

The pH-rate profile observed in Figure 4.3 clearly demonstrates that the activity of **39.Eu₂** is highly pH-dependant. At acidic pH, *ca.* 6.5, the rate of hydrolysis was found to be extremely slow and a k_{obs} of $2.17 (\pm 0.02) \times 10^{-6} \text{ s}^{-1}$ was determined, which corresponded to a rate enhancement k_{rel} of 65.

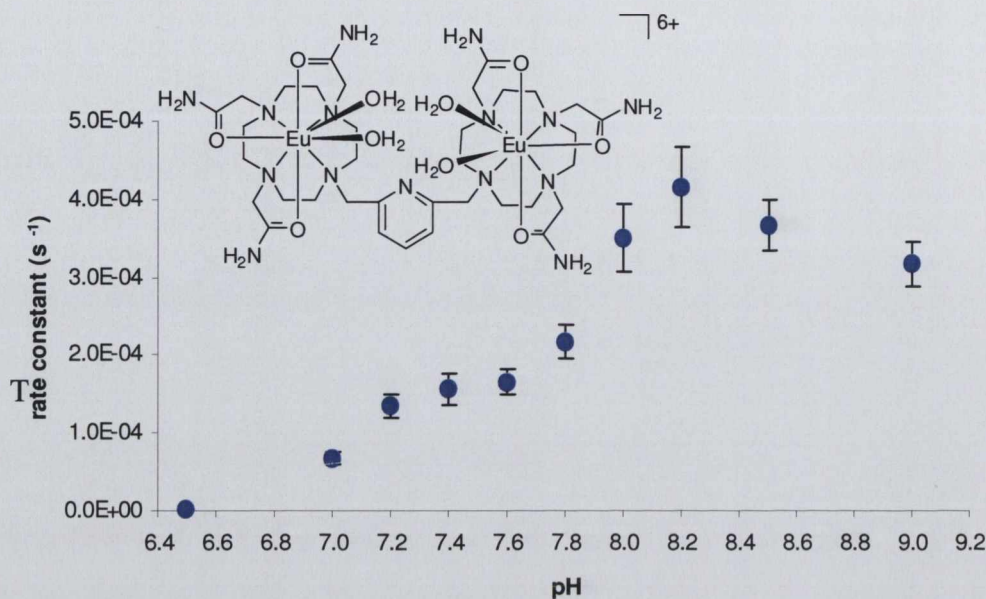


Figure 4.3: The pH-rate profile for the hydrolysis of **HPNP** (0.18 mM) by **39.Eu₂**, in 50 mM HEPES buffer at 37 °C. Each data point is an average of 2-3 measurements agreeing to within 15% error. The error bars are determined from the average of 2-3 measurements.

In contrast, the maximum rate of hydrolysis was observed at pH 8.2, where $k_{\text{obs}} = 4.16 (\pm 0.44) \times 10^{-4} \text{ s}^{-1}$ was determined, a rate enhancement k_{rel} of 12488 as compared to the uncatalysed reaction, which corresponds to a rate enhancement of 192-fold compared to that observed at pH 6.5. However, above pH 8.2, the rate of hydrolysis is slower, presumably as a result of the deprotonation of the second metal bound water molecule on the metal ion. Studies have shown that for the hydrolysis of **HPNP**, a metal bound hydroxide binds to the phosphorous atom, reducing the overall negative charge on the substrate. A metal bound water molecule can then activate the **HPNP** toward hydrolysis. However, if this metal bound water molecule is also deprotonated, it can no longer activate the substrate towards hydrolysis, thus reducing the rate of the hydrolysis of **HPNP**.^{5,46} As such, it is possible that this is occurring when the rate of **HPNP** hydrolysis by **39.Eu₂** begins to decrease in alkaline media, potentially due to the formation of the $\text{LM}_2(\text{OH})_4$ species.

The corresponding La(III) complex, **39.La₂**, was tested as a function of pH and the resulting pH-rate profile is shown in Figure 4.4. It is clear that **39.La₂** displays a very different pH-rate profile to that observed for **39.Eu₂**. Here, the maximum rate of hydrolysis

occurred at pH 7.6, however, the maximum rate remained well below that of **39.Eu₂**. Moreover, during the hydrolysis reaction, which was monitored over 16 hours, at pH 7.8 and above, the formation of a precipitate was observed. The precipitate could possibly be due to the formation of either the dihydroxo species which precipitates out of solution, as a result of poor solubility in H₂O, or an insoluble “enzyme-substrate”-type complex in alkaline solution. In both cases, this precipitate would explain the low rate of activity for the complex.

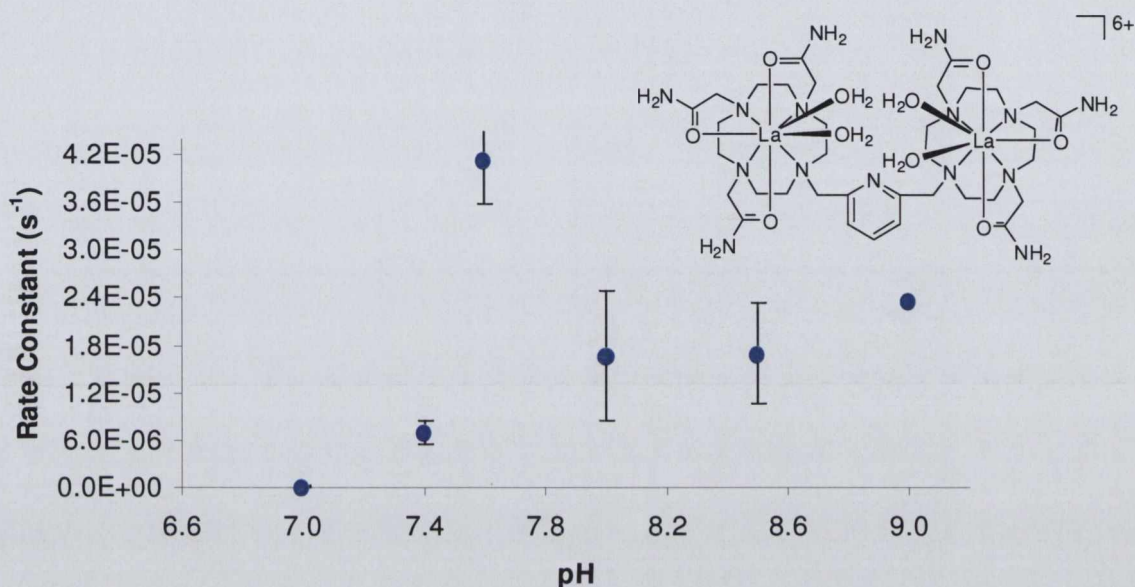


Figure 4.4: The pH-rate profile for the hydrolysis of **HPNP** (0.18 mM) by **39.La₂**, in 50 mM HEPES buffer at 37 °C. Each data point is an average of 2-3 measurements agreeing to within 15% error. The error bars are determined from the average of 2-3 measurements.

The analogous mononuclear pyridine complex, **49.Eu**, was synthesised to potentially give insight into the mechanism of the dinuclear complex. However, the complex was not fully soluble in H₂O, and displayed only low rates of phosphodiester hydrolysis when tested against **HPNP**, where at pH 7.4, a rate constant, k_{obs} of $6.6 (\pm 0.1) \times 10^{-6} \text{ s}^{-1}$ was determined. The complex **49.Eu** was found to give rise to a q -value of 1.6, and it is possible that there was only a single water molecule present on the metal ion in solution, thus inhibiting the rate of hydrolysis. Furthermore, comparison of the rate constants for **39.Eu₂** and **49.Eu** indicates that the dinuclear complex is more efficient than the corresponding mononuclear complex, suggesting that the dinuclear complex is perhaps working cooperatively towards the hydrolysis of **HPNP**. However, given the partial insolubility and slow rate of hydrolysis of **49.Eu**, no further investigations were undertaken on this complex.

A possible explanation as to why the activity of **39.Eu₂** and **39.La₂** was low, as compared to that of **38.Eu₂** is that both metal ions may not possess two metal bound water

molecules, as discussed in Section 2.3. The lifetime calculations for **39.Eu₂** gave rise to a q -value of 1.6 (± 0.5), when a value closer to two had been expected. The number of metal bound water molecules was possibly lowered by the partial coordination of one, or both, of the metal ions to the pyridine moiety. A decrease in the number of metal bound water molecules, as a result of such potential coordination, would also possibly result in a decrease in the rate of the phosphodiester hydrolysis.

4.4.2 Conclusion

In summary, it was found that **39.Eu₂** was more active than **39.La₂** over the pH range studied (6.5-9.0). As discussed in Section 1.14, La(III) complexes have previously given rise to higher rates of **HPNP** hydrolysis than their corresponding Eu(III) complexes. The reversal of this trend here suggests that when both La(III) and Eu(III) possess more than one metal bound water molecule, the rate of hydrolysis is less dependant on the overall size of the metal ion, and instead is dependant on the Lewis acidity of the metal ions, and that of their resulting metal bound water molecules. This potential trend will be investigated further for the rest of the complexes studied in this thesis.

Furthermore, the structure of the spacer could have an influence on the overall rate of phosphodiester hydrolysis. In this section the rate of hydrolysis of **HPNP** by **39.Ln₂** has been compared to that of **38.Eu₂**, which was bridged by a *para*-xylyl spacer group. The pyridine spacer of **39.Ln₂** was in the *meta*-orientation, and it is possible that this conformation does not promote the hydrolysis of **HPNP** to the same extent as the *para*-isomer. The influence of the conformation of the spacer will be discussed in greater detail in later sections of this Chapter.

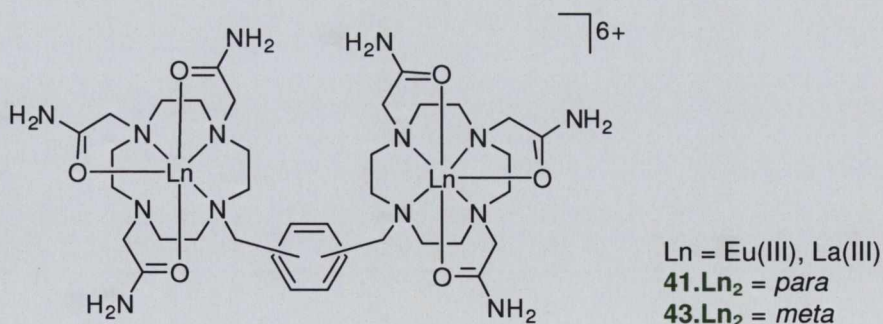
As none of the pyridine spaced complexes gave rise to any discernable increase in the rate of the hydrolysis of **HPNP** as compared to that of **38.Eu₂**, it was decided to instead focus on the xylyl spaced complexes. The rest of this Chapter investigates the ability of the xylyl-spaced complexes, discussed in Chapter 2, to hydrolyse the phosphodiester bond of **HPNP**.

4.5 Phosphodiester Hydrolysis by Xylene-Spaced Complexes **41.Ln₂** and **43.Ln₂**

The design of the cyclen-based xylene-spaced complexes was based upon that of the previously synthesised *para*-xylene spaced complex, **38.Eu₂**, as discussed in Sections 1.14 and 2.2. To date, one of the largest rate enhancements for the hydrolysis of **HPNP** recorded by the Gunnlaugsson group was achieved using **38.Eu₂**, which was found to cleave **HPNP** with a pseudo first order rate constant of $4.11(\pm 0.22) \times 10^{-4} \text{ s}^{-1}$ at pH 7.4

and 37 °C.¹⁰¹ The efficient hydrolytic ability of **38.Eu₂** was attributed to a combination of several factors including the simple aryl spacer which incorporated a degree of flexibility into the complex, most likely enabling the two metal centres to efficiently bind the phosphodiester substrate in a cooperative manner. Also, the spacer lacked any coordinating functionalities, thus rendering the Eu(III) metal ion coordinatively unsaturated, with two metal bound water molecules being determined for each of the metal ions. Finally, the large rate enhancement was indicative of a cooperative action by the two metal ions, possibly *via* a double Lewis acid activation (Section 1.6.4). This rate enhancement was a great advancement compared to previously synthesised complexes from the Gunnlaugsson group,^{62,119} and indeed, it was an enhancement over other lanthanide based complexes in the literature.^{117,167} However, it remained low in comparison to the rates achieved by natural ribonucleases,¹⁹ as discussed in Section 1.4.

To improve the activity of this type of complex, small changes in the structure of the ligands were made, such as changing the nature of the pendant arms and the substitution position of the spacer, as outlined in Section 2.2. In this section the complexes of two ligands, both substituted with acetamide arms, are investigated for their ability to hydrolyse **HPNP**. The complex **41.Ln₂** is bridged by a *para*-xylene spacer moiety, and the complex **43.Ln₂** is bridged by a *meta*-xylene spacer moiety. The effect of these changes on the rate of hydrolysis of phosphodiester bonds was investigated, and results are detailed in the following Section. It was thought that by studying the effect of such small changes on the rate of the hydrolysis of **HPNP** that a possible mechanism of action for the complexes could be deduced. To begin, the hydrolysis of **HPNP** by **41.Ln₂** is discussed in the next section.



4.5.1 Kinetic Evaluation of *para*-Xylene Spaced Acetamide Complexes

As before, the ability of **41.Ln₂** to hydrolyse **HPNP** was carried out at pH 7.4 and 37 °C and the results are summarised in Table 4.3, where the rate calculated for **38.Eu₂** is shown again for comparison. The rate profile for the hydrolysis of **HPNP** by **41.Eu₂** is shown in Figure 4.5, where it was found to hydrolyse **HPNP** with a pseudo first order rate

constant of $5.76 (\pm 0.47) \times 10^{-4} \text{ s}^{-1}$, which corresponds to a half-life of 0.41 hours (k_{rel} of 14098), while a half-life of 0.27 hours (k_{rel} of 21407) was found for the corresponding Eu(III) complex, **41.Eu₂**. The rate profile for **41.La₂** is shown in Appendix A3.3.

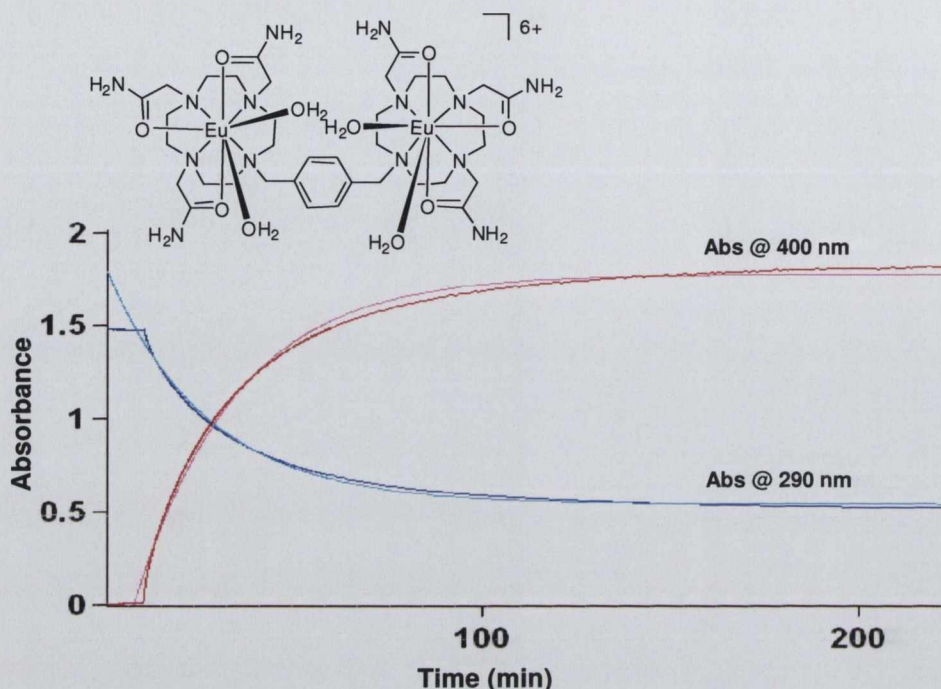


Figure 4.5: The kinetic profile for the hydrolysis of **HPNP** (0.18 mM) by **41.Eu** (0.18 mM) in 50 mM HEPES buffer at pH 7.4 and 37 °C. The increase of *p*-nitrophenolate (red line) as a function of time is observed and fitted to first order kinetics to determine the rate constant, k_{obs} for the reaction.

These results highlight the important influence that the nature of the pendant arms has on the efficiency of complexes, as **41.Eu₂** is nearly twice as active as **38.Eu₂** at pH 7.4. Structurally, the two complexes differ only in the nature of the pendant arms, where **38.Eu₂** is functionalised with *N,N'*-dimethylacetamide arms, and **41.Eu₂** with the corresponding acetamide arms. It is possible that the acetamide arms of **41.Eu₂** give rise to hydrogen bonding between the complex and the substrate, resulting in an increase in the rate of phosphodiester hydrolysis due to the favourable formation of an “enzyme-substrate”-type complex. Upon analysis of **41.La₂**, it was also clear that the Eu(III) complex gave rise to a greater rate of hydrolysis than the corresponding La(III) complex. Such a trend was also observed following the analysis of **39.Ln₂**. Moreover, the results confirm that the choice of the metal ion has a large influence upon the activity of the complex. Previously, it was thought that the Lewis acidity of the lanthanide metal ion had a great influence on the rate of phosphodiester hydrolysis,¹⁰⁰ however, these results suggest that conformation of the binding pocket could have an influence on the activity of the complex.

Table 4.3: Pseudo-first order rate constants (k_{obs}), half lives ($\tau_{1/2}$) and relative rate enhancements (k_{rel}) for the hydrolysis of **HPNP** (0.18 mM) in 50 mM HEPES at pH 7.4 and 37 °C by **38.Eu₂** and **41.Ln₂**.

Complex	k_{obs} (s ⁻¹)	$\tau_{1/2}$ (h)	k_{rel}
38.Eu₂	$4.11 (\pm 0.22) \times 10^{-4}$	0.47	12298
41.La₂	$5.76 (\pm 0.47) \times 10^{-4}$	0.41	14098
41.Eu₂	$7.06 (\pm 0.37) \times 10^{-4}$	0.27	21407

To further investigate the hydrolytic ability of the *para*-xylene spaced complexes, **41.Ln₂**, the rate constant was measured at various pH values, and the results for this investigation are detailed in the next Section.

4.5.2 The pH Dependence of the Hydrolysis of HPNP by **41.Ln₂**

An investigation into the pH dependence of **41.La₂** and **41.Eu₂** was undertaken by measuring the rate constant for the hydrolysis of **HPNP** at various pH values. Examination of the pH-rate profile for **41.La₂**, Figure 4.6, revealed that the complex was strongly pH-dependant. In acidic media, the rate of hydrolysis of **HPNP** was found to be negligible and the rate could not be accurately determined. An overall pseudo “bell-shaped” curve was observed for the pH-rate profile, which was similar to that which had been previously observed for many of the complexes tested for the hydrolysis of **HPNP** within the Gunnlaugsson group,^{62,120} and is a classic indicator of general acid-base catalysis.^{12,39,47}

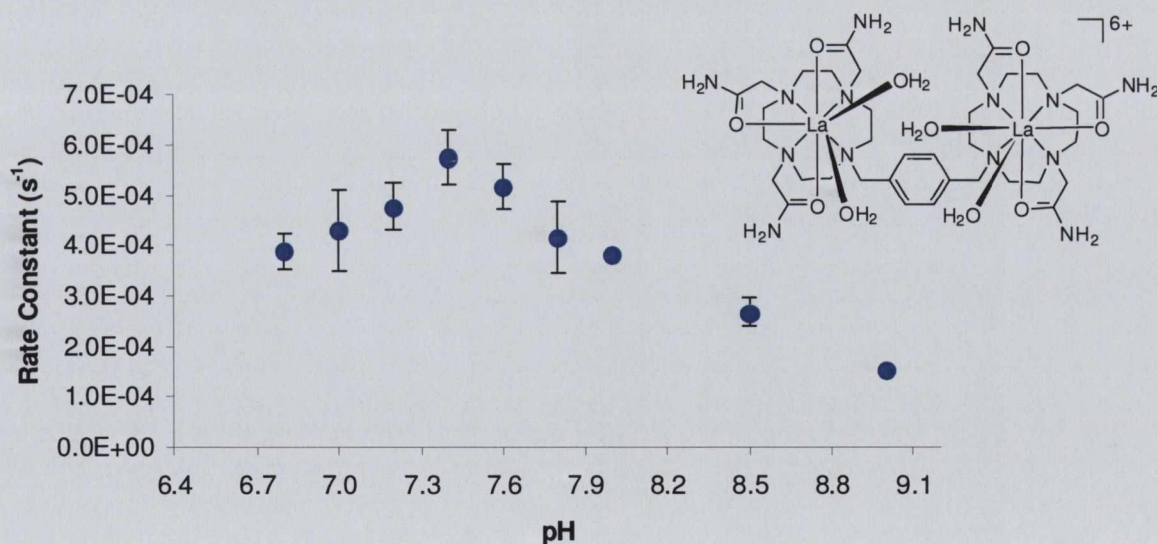


Figure 4.6: The pH-rate profile for the hydrolysis of **HPNP** (0.18 mM) by **41.La₂**, in 50 mM HEPES buffer at 37 °C. Each data point is an average of 2-3 measurements agreeing to within 15% error. The error bars are determined from the average of 2-3 measurements.

The La(III) complex, **41.La₂**, was found to be most active at *ca.* pH 7.4, with a $k_{\text{obs}} = 5.76 (\pm 0.5) \times 10^{-4} \text{ s}^{-1}$, Figure 4.6. Generally, it was observed that similar cyclen-based

La(III) complexes gave rise to their maximum activity in more alkaline conditions, however,^{3,14,32} this could also be an effect of the pK_a of the metal bound water molecules.^{15,168} It is possible that in the case of **41.La₂**, above pH 7.4, the second metal bound water molecule is deprotonated, which can give rise to the formation of a dihydroxy species, preventing the binding of the substrate to the metal complex, similar to that which was observed for **39.La₂**. It is likely that the hydrolysis of **HPNP** by **41.La₂** can be attributed to the Lewis-acid activation of the substrate and nucleophilic activation of the 2'-hydroxy group.

The effect of pH upon the activity of **41.Eu₂** was also investigated and the resulting pH-rate profile is shown in Figure 4.7. The complex **41.Eu₂** was found to be significantly more active towards the hydrolysis of **HPNP** than the corresponding **41.La₂** complex. However, the maximum rate of **HPNP** hydrolysis occurred at a more alkaline pH, around pH 8.0, as was observed for the hydrolysis of **HPNP** by **39.Eu₂**. At this pH, the rate of the reaction increased with a $k_{\text{obs}} = 1.62 (\pm 0.07) \times 10^{-3} \text{ s}^{-1}$, which corresponds to a half life, $\tau_{1/2}$, of 0.12 hours, or 7.1 minutes. This gives a relative rate enhancement, k_{rel} of 48167 which was larger than previously observed for lanthanide complexes developed within the Gunnlaugsson group,^{13-15,17} and raised the possibility that the complex was functioning *via* a cooperative action of the two metal ion centres. Moreover, the large rate enhancement, as compared to previously tested cyclen-based Eu(III) complexes, suggested that the complex was possibly functioning catalytically.

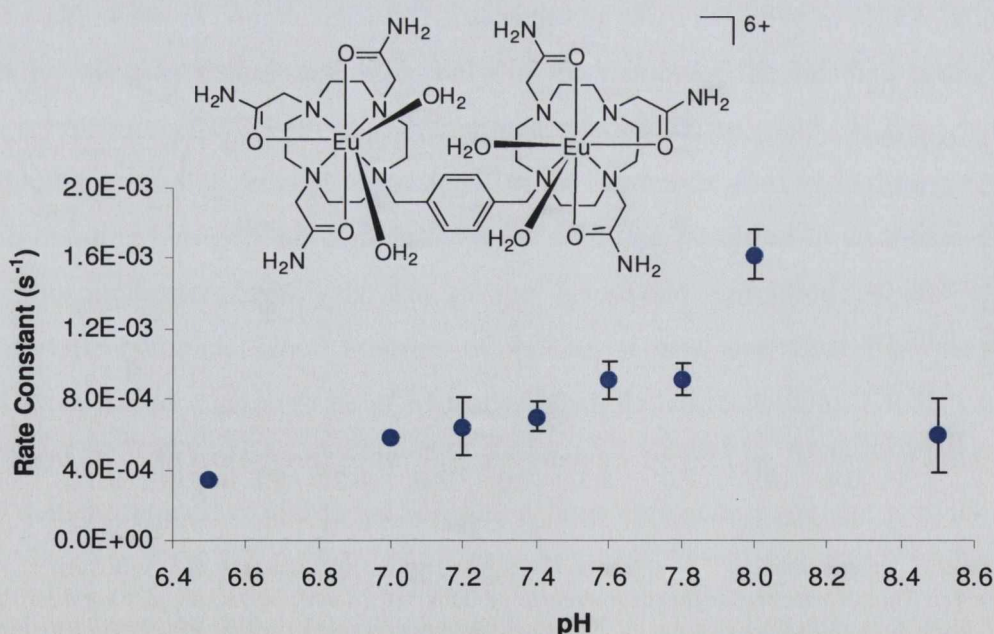


Figure 4.7: The pH-rate profile for the hydrolysis of **HPNP** (0.18 mM) by **41.Eu₂**, in 50 mM HEPES buffer at 37 °C. Each data point is an average of 2-3 measurements agreeing to within 15% error.

Considering the large rate enhancements observed for the Eu(III) complex, it is possible that the pK_a of the metal bound water molecule, as opposed to the size of the metal ion, may now be a critical parameter in obtaining a large rate of phosphodiester hydrolysis. Due to the lanthanide contraction, Eu(III) is smaller in size than La(III), and thus has a higher charge density, which affects the pK_a of the metal bound water molecule.⁷⁶ Having demonstrated that small changes in the structure of a complex, *e.g.* changing from *N,N*-dimethylacetamide arms to acetamide arms, can result in large changes to the activity of the complex, Section 4.5.1, it is also possible that a small change in the charge density of the metal ion could give rise to a more effective neutralisation of the negative charge on the phosphodiester, thus promoting hydrolysis to a greater extent than the corresponding La(III) complex. Although **41.La₂** hydrolysed **HPNP** more slowly than the corresponding **41.Eu₂**, this result is interesting, as the La(III) complex shifts the active region of the activity profile towards the physiological pH range, most likely as a result of a lowering of the pK_a of the metal bound water molecules.¹²²

To further investigate the activity of the complex for the hydrolysis of **HPNP**, potentiometric titrations were carried out on **41.Ln₂**, as discussed in Chapter three. Titrations of the pre-formed ligand of **41.Eu₂** revealed that it was most likely that all four metal bound water molecules were deprotonated over the course of the titration, however, due to problems associated with the stability of the complex throughout the titration, accurate pK_a values for the deprotonation of the metal bound water molecules could not be determined. It is likely that at pH 8.0 where the maximum rate of hydrolysis occurs that each of the two Eu(III) metal ions possesses one metal bound water molecule and one metal bound hydroxide. To gain a deeper understanding into the activity of the Eu(III) complex, further studies were carried out on **41.Eu₂**, and these are detailed in the following Sections.

4.5.3 Determination of a Second Order Rate Constant

A useful method for the evaluation of the hydrolytic activity of a complex is the determination of a second order rate constant. This can be found by varying the concentration of the complex upon the first order rate constant, k_{obs} . The second order rate constant is independent of the concentration of **HPNP**, or 'catalyst' and as such is a useful parameter for the comparison of different systems.⁹¹ The results of the analysis for **41.Eu₂** are shown in Figure 4.8, and are again the results of an average of 2-3 measurements for each concentration of **41.Eu₂**. A second order rate constant of $2.36 \text{ M}^{-1} \text{ s}^{-1}$ for the hydrolysis of **HPNP** was determined as the slope of the plot of k_{obs} against catalyst by fitting to Equation 4.1.

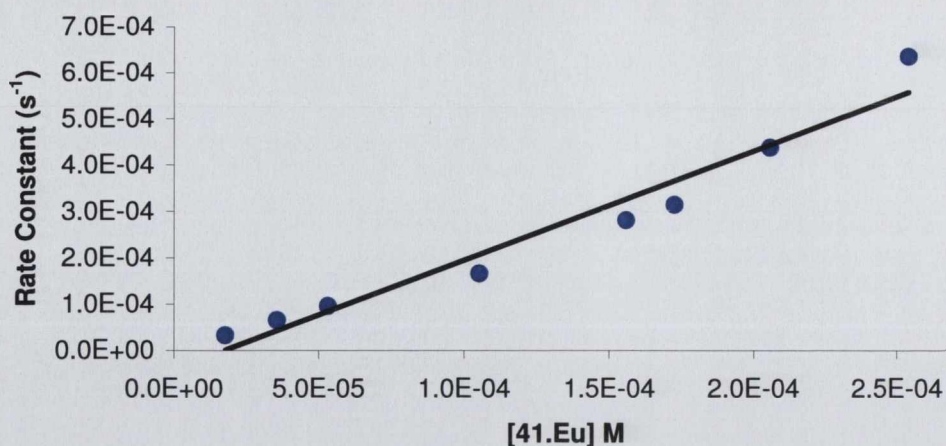


Figure 4.8: Dependence of the pseudo first order rate constant for the hydrolysis of **HPNP** (5.2×10^{-3} mM) on **[41.Eu₂]** in the concentration range 0.017 – 0.25 mM, at pH 7.4 and 37 °C. A second order rate constant was obtained by calculating the slope of the linear plot of the rate constant k against **[41.Eu₂]**.

$$k_{\text{obs}} = k_{\text{Eu}} [\text{complex}]$$

Equation 4.1

The second order rate constant determined was a significant increase upon that of previously synthesised mononuclear complexes, and over other dinuclear zinc complexes previously reported.⁹¹ This high second order rate constant indicated that perhaps the complex was functioning *via* a different mechanism to that of other complexes previously studied.

4.5.4 Investigation of Binding of 41.Eu₂ to Phosphate

To further investigate the mechanism of the hydrolysis of **HPNP** by **41.Eu₂**, the binding of the complex to phosphate was investigated by ³¹P NMR, using diethylphosphate (**DEP**) as a substrate. **DEP** is a phosphodiester which is similar in structure to **HPNP**, however, it cannot undergo cleavage in the same manner, as it lacks the 2'hydroxyl group which can activate the hydrolysis of **HPNP**. As such, **DEP** can be used to measure the interaction of the complex with a phosphodiester, whilst ensuring that the phosphodiester is not hydrolysed during the process. Previous studies have investigated phosphate binding using **DEP** and have positively correlated the strength of the binding to the ability of the complex to efficiently hydrolyse **HPNP**.¹⁶⁶ For this study, increasing equivalents of **DEP** were added to a solution of **41.Eu₂** in H₂O, and the resulting changes in the ³¹P NMR spectrum were monitored. Free **DEP** gives rise to a phosphorous signal at 1.8 ppm, and when this signal is observed in the titration it is assumed that all of the complex is fully bound to the **DEP**. The results of this titration are shown in Figure 4.9. A stack plot of several points of the ³¹P NMR titration is shown in Appendix A3.4.

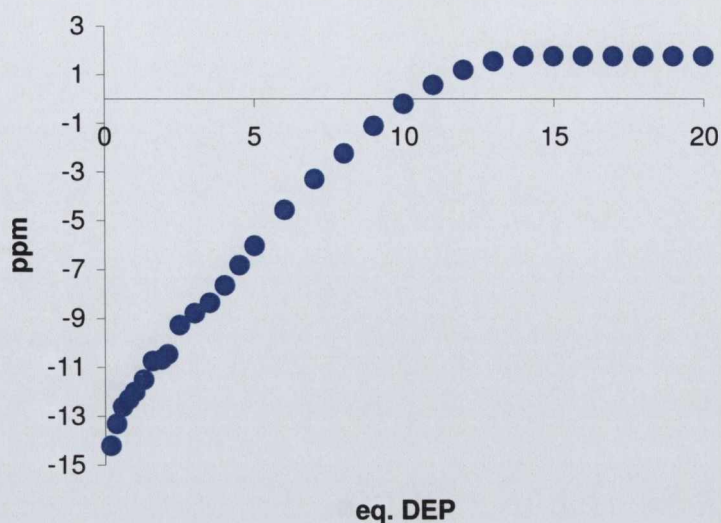


Figure 4.9: Changes in the ^{31}P NMR spectrum (162 MHz, H_2O) as a function of molar equivalents of **DEP** ($2.1 \times 10^{-1} \text{ M}$) added to **41.Eu₂** ($3.0 \times 10^{-3} \text{ M}$).

From Figure 4.9, it can be seen that the titration reached a plateau after the addition of twelve equivalents of **DEP**, indicating that the binding was complete. It was attempted to fit the data using both WINEqNMR¹⁶⁹ and NMRtit,¹⁷⁰ to determine a binding constant, however neither fit gave rise to a binding constant for the complex. Upon initial binding of the phosphodiester, the phosphorous signal was shifted from 1.8 ppm, for free **DEP**, to -14.2 ppm, which gives an overall shift in the phosphate signal of *ca.* 16 ppm. This indicated that **41.Eu₂**, which was the most active complex for the hydrolysis of **HPNP** studied to date, gave rise to good binding to the phosphodiester **DEP** as compared to those previously evaluated within the Gunnlaugsson group. These studies had determined that the mononuclear lanthanide complexes, which were moderately active for the hydrolysis of **HPNP**, bound to **DEP** strongly. However, the overall shift of the phosphorous signal from the **DEP** was significantly smaller, only about 3 ppm. Such an increase in the phosphate binding of **41.Eu₂** can be attributed to the presence of a second metal ion in the dinuclear complex, and also to the change in the nature of the pendant arms. The *N,N'*-dimethylacetamide functionalised dinuclear complex, **38.Eu₂**, gave rise to a very steep binding curve upon titration with **DEP**, in which the phosphorous signal shifted over *ca.* 28 ppm, which was indicative of a strong interaction. Such a strong interaction with the phosphate was hypothesised to occur as a result of the presence of two 3^+ charged Eu(III) metal ions in the complex, which gave rise to a strong electrostatic binding with the negatively charged **DEP**. The titration curve of **41.Eu₂** was not as sharp as that observed for **38.Eu₂**, suggesting that this is possibly the reason why a higher rate of hydrolysis is observed for **41.Eu₂**. It is possible that **38.Eu₂** binds too strongly to the phosphate, which

then prevents release of the cyclic phosphate, leading to inhibition of the complex. Given that **41.Eu₂** does not display such a strong affinity for phosphate, it is possible that the strength of phosphate binding by the complex has an effect on the overall activity of the complex.

4.5.5 Summary

In summary, the behaviour of **41.Eu₂** differed from that of **38.Eu₂**, including the pH at which the maximum activity was observed, second order rate constant and the strength of phosphate binding, and it was observed that **41.Eu₂**, despite giving rise to a weaker interaction with phosphate, hydrolysed **HPNP** more efficiently. To further investigate the hydrolysis of **HPNP** by **41.Eu₂**, the corresponding *meta*-spaced complexes, **43.Ln₂**, were synthesised and their ability to hydrolyse **HPNP** was evaluated, to determine the influence of the conformation of the spacer moiety on the rate of phosphodiester hydrolysis. The evaluation of the *meta*-complexes **41.Eu₂** and **43.La₂**, and a comparison to the activity of the *para*-complexes, **41.Ln₂**, are discussed in the next Section.

4.5.6 Kinetic Evaluation of *meta*-Xylene Spaced Acetamide System

The *meta*-xylene spaced analogue of **41** was synthesised to give **43.Eu₂** and **43.La₂**, as outlined in Chapter two, and their ability to hydrolyse **HPNP** was carried out in the same manner as described in previous sections. As for the previous complexes, the initial evaluation of the complex was carried out at pH 7.4 and 37 °C, the results of which are detailed in Table 4.4.

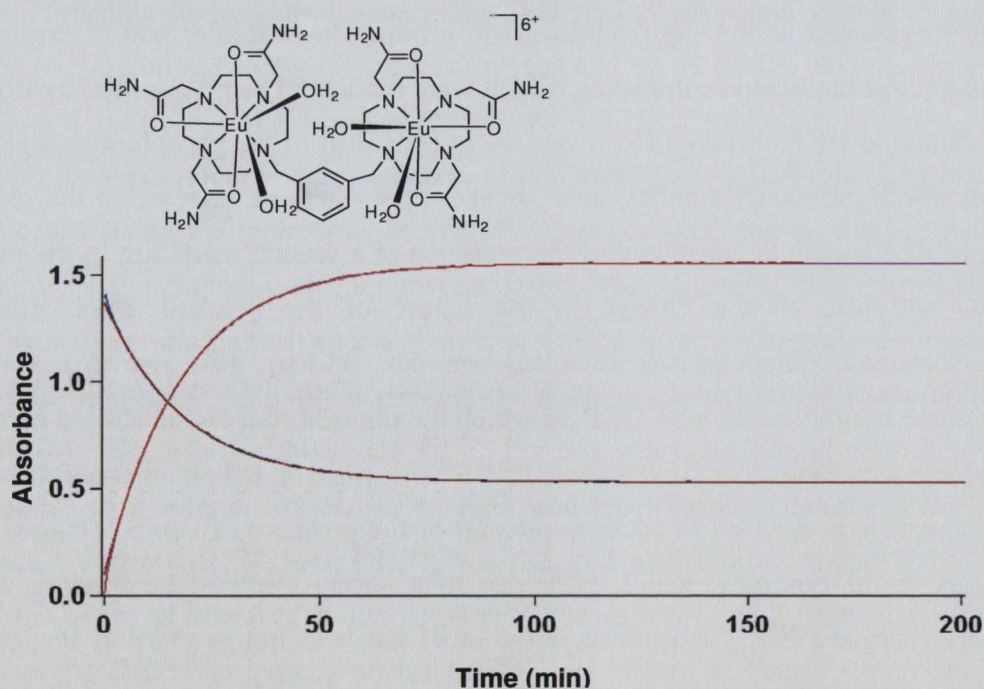


Figure 4.10: The kinetic profile for the hydrolysis of **HPNP** (0.18 mM) by **43.Eu₂** (0.18 mM) in 50 mM HEPES buffer at pH 7.4 and 37 °C. The increase of *p*-nitrophenolate (red line) as a function of time is observed and fitted to first order kinetics to determine the rate constant, k_{obs} for the reaction.

The *meta*-xylene spaced complex **43.Eu₂**, hydrolysed **HPNP** efficiently at pH 7.4, giving a rate constant of $k_{\text{obs}} = 9.9 (\pm 1.2) \times 10^{-4} \text{ s}^{-1}$, and the pH rate profile for the reaction is shown in Figure 4.10. This corresponds to a relative rate enhancement over the uncatalysed reaction of 30421 and a half life of $\tau_{1/2} = 0.19 \text{ h}$, or 11.4 minutes. In contrast, **41.La₂** did not give rise to any such significant hydrolysis, with a first order rate constant of $7.1 (\pm 0.9) \times 10^{-4} \text{ s}^{-1}$, which is a rate enhancement of 6004 over the uncatalysed reaction. The rate profile for **43.La₂** is shown in Appendix A3.5. The results are in good agreement with those observed for the *para*-spaced Ln(III) complexes of **41**, with the Eu(III) complex showing higher activity than the corresponding La(III) complex. Moreover, **43.Eu₂** gave a 1.5-fold rate enhancement over the analogous *para*-spaced Eu(III) complex **41.Eu₂** at pH 7.4, indicating that the conformation of the spacer did indeed affect the hydrolytic activity of the complex.

Table 4.4: Hydrolysis of **HPNP** (0.18 mM) by the complexes **41.Ln₂** and **43.Ln₂** (0.18 mM) in 50 mM HEPES buffer at pH 7.4, 37 °C.

Complex	$k_{\text{obs}} (\text{s}^{-1})$	$\tau_{1/2} (\text{h})$	k_{rel}
41.La₂	$5.76 (\pm 0.47) \times 10^{-4}$	0.41	14098
41.Eu₂	$7.1 (\pm 0.9) \times 10^{-4}$	0.27	21407
43.La₂	$2.0 (\pm 0.5) \times 10^{-4}$	0.96	6004
43.Eu₂	$9.9 (\pm 1.2) \times 10^{-4}$	0.19	30421

As for the previous complexes examined, an investigation into the pH-dependence of the complex **43.Eu₂** was carried out and the results are discussed in the following Section.

4.5.7 The pH Dependence of the Hydrolysis of HPNP by **43.Eu₂**

The pH-rate profile for **43.Eu₂** is shown in Figure 4.11, and upon examination, it can be seen that the rate of hydrolysis increased from pH 6.4 to 7.4. The maximum rate of hydrolysis was then found to occur at pH 7.4, with $k_{\text{obs}} = 9.9 (\pm 1.2) \times 10^{-4} \text{ s}^{-1}$, which corresponds to a rate enhancement k_{rel} of 30421. The hydrolytic activity of the complex then decreased as the pH increased. Interestingly, an anomaly was noted in the pH-rate profile at pH 7.6. After reaching its maximum rate of activity at pH 7.4, the complex was much less active at pH 7.6, only to increase in activity again slightly at pH 7.8, after which it declines as the pH increases. It is most likely that this is due to the active species in solution at pH 7.6, which perhaps does not promote hydrolysis of the phosphodiester bond.

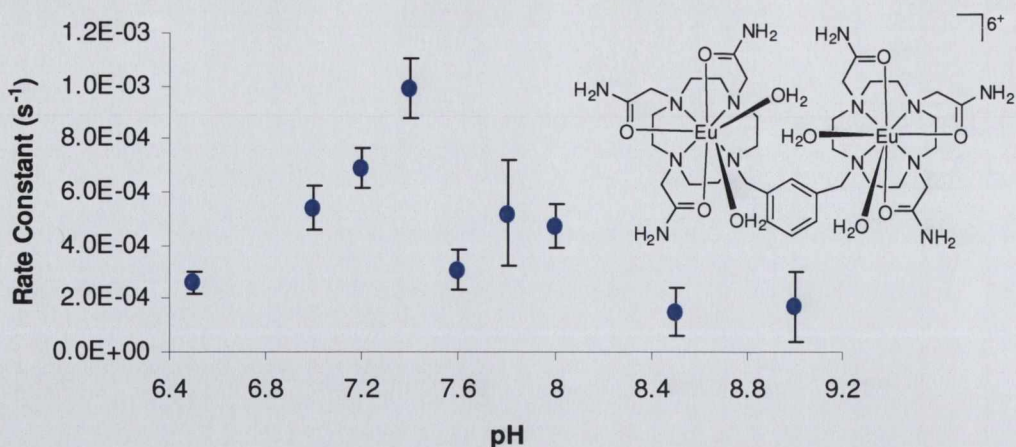


Figure 4.11: The pH-rate profile for the hydrolysis of **HPNP** (0.18 mM) by **43.Eu₂**, in 50 mM HEPES buffer at 37 °C. Each data point is an average of 2-3 measurements agreeing to within 15% error.

It is worth noting that **41.Eu₂** and **43.Eu₂** do not display their maximum activity at the same pH, which indicates that the nature of the spacer is very important to the activity of the complexes. At pH 8.0, where **41.Eu₂** is the most active, with a rate enhancement $k_{\text{rel}} = 48167$, the *meta*-isomer, **43.Eu₂**, hydrolysed **HPNP** with a rate constant of $k_{\text{obs}} = 4.7 (\pm 1.13) \times 10^{-4} \text{ s}^{-1}$, a rate enhancement, k_{rel} of 14109.

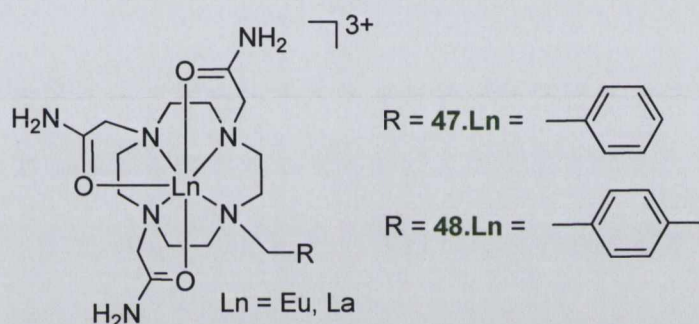
The comparison study of the *para*- and *meta*-isomers gave rise to interesting results, which clearly demonstrate the importance of the structure of the spacer to the hydrolytic activity of the complexes. It was thought that due to the similar structure of the two complexes, they would most likely display similar activity and pH-rate profiles. However, the evidence suggests that very small adjustments to the structure of the complex can have a large effect on the efficiency of the complex and its pH-rate profiles. It is possible that the hydrolysis of **HPNP** is more sensitive to the conformation of the spacer than was previously thought, and that it plays an important role in modulating the activity of the complex.

4.5.8 Conclusion

The results discussed herein suggest that the structure of the spacer may alter the nature of the binding pocket. It is possible that the *meta*-isomer cannot adopt a favourable conformation for the most efficient binding of **HPNP**, or that the distance between the two metal ions is too small to effectively bind the **HPNP** and give rise to a similar cooperative action to that which is observed for **41.Eu₂**. To further investigate this hydrolysis of **HPNP** by **41.Ln₂** and **43.Ln₂**, two mononuclear cyclen-based lanthanide complexes were synthesised, and their investigation, and comparison to the dinuclear families is detailed in the following Section.

4.6 Evaluation of the Mechanism of Hydrolysis of HPNP by Xylene-Spaced Acetamide Systems

The large rate enhancements observed for **41.Eu₂** were tentatively assigned to a cooperative action of the two metal ions. However, cooperativity cannot be accurately assigned without comparison to structurally similar mononuclear analogues, as outlined in Sections 1.6 and 2.7. As such, two mononuclear analogues, **47.Ln** and **48.Ln**, were synthesised, (Section 2.7), to shed light on the possible cooperative action of **41.Eu₂** and **73.Eu₂** for the hydrolysis of **HPNP**. Of these complexes, **47.Ln** possessed a benzyl moiety which was introduced to mimic the structure of the spacer, while at the same time, ensuring that the metal ion remained 7-coordinate, giving rise to the presence of two metal bound water molecules. The second mononuclear analogue, **48.Ln**, possessed a methylbenzyl moiety and was designed with a similar concept in mind.



The complexes **47.Ln** and **48.Ln** were initially evaluated at pH 7.4 and 37 °C, in a manner identical to that of their dinuclear analogues, and the resulting rate constants obtained for the hydrolysis of **HPNP** are detailed in Table 4.5. The rate profiles for **47.Ln** and **48.Ln** are shown in Appendices A3.6-A3.9. From this table it is clear that all of the complexes hydrolysed **HPNP**, however, none were as active as the corresponding dinuclear analogues. Whereas **41.Eu₂** hydrolysed **HPNP** with a first order rate constant of $7.1 (\pm 0.9) \times 10^{-4} \text{ s}^{-1}$, the analogous mononuclear complex, **47.Eu**, hydrolysed **HPNP** with a rate constant of $k_{\text{obs}} = 1.24 (\pm 0.3) \times 10^{-4} \text{ s}^{-1}$, and a similar trend was also observed for the La(III) complexes. As outlined in Table 4.5, **41.Eu₂** has a 5-fold rate enhancement over the corresponding mononuclear complexes. These results suggest that **41.Eu₂** is possibly behaving in a cooperative manner, with both metal ions functioning synergistically to give rise to efficient phosphodiester hydrolysis. However, further analysis of the mechanism of the hydrolysis of **HPNP** was required to confidently assign the rate enhancement to a cooperative effect.

Table 4.5: Pseudo first order rate constants (k_{obs}), half lives ($\tau_{1/2}$) and relative rate enhancements (k_{rel}) for the cleavage of **HPNP** (0.18 mM) in 50 mM HEPES buffer at pH 7.4 and 37°C by **41.Eu₂**, **43.Eu₂**, **47.Ln** and **48.Ln** (0.18 mM).

Complex	k_{obs} (s ⁻¹)	$\tau_{1/2}$ (h)	k_{rel}
41.Eu₂	$7.1 (\pm 0.9) \times 10^{-4}$	0.27	21407
43.Eu₂	$9.9 (\pm 1.2) \times 10^{-4}$	0.19	30421
47.Eu	$1.24 (\pm 0.3) \times 10^{-4}$	1.56	3705
47.La	$1.17 (\pm 0.4) \times 10^{-4}$	1.65	3512
48.Eu	$1.32 (\pm 0.1) \times 10^{-4}$	1.46	3959
48.La	$1.24 (\pm 0.3) \times 10^{-4}$	1.56	3722

Previously, octa-coordinate La(III) complexes investigated within the Gunnlaugsson group had been found to give rise to rate constants which surpassed those of the corresponding Eu(III) complexes, generally by a minimum of a 2-fold increase. Interestingly, the septa-coordinate mononuclear Eu(III) complexes investigated herein gave rise to rate enhancements that are higher than those of their corresponding La(III) mononuclear complexes. Evidently, the presence of a second water molecule on the Eu(III) metal ion results in a large increase in the rate of phosphodiester hydrolysis, unlike the addition of a third metal bound water molecule to the La(III) ion.

A comparison of the pH rate profile of **41.Eu₂** and **47.Eu** is shown in Figure 4.12, and clearly highlights the differences in the rates of the hydrolysis of **HPNP** by these two complexes as a function of pH. The pH rate profiles of **47.La**, **48.Eu** and **48.La** are shown in Appendices A3.10-A3.12. It is evident upon examination of these two profiles that **41.Eu₂** performs at a rate more than an order of magnitude greater than the mononuclear analogue. This could be taken as an indication of cooperative hydrolysis. In particular, at pH 8.0, where the maximum activity was observed for **41.Eu₂**, an 11-fold increase in the k_{obs} was observed. To conclude that a cooperative action is indeed at work, it must be demonstrated that the observed rate enhancements are more than just additive.⁴³ Cooperativity is usually indicated by an enhancement of *ca.* 10-fold, and as such, it can be concluded that where **41.Eu₂** is most active, the complex is most likely operating *via* a cooperative action of the two metal ions.

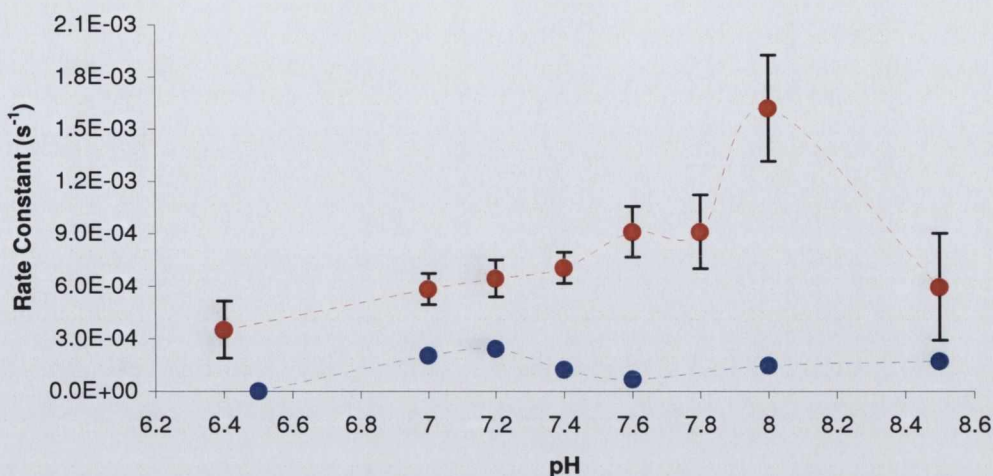


Figure 4.12: Comparison of the pH-rate profile for the hydrolysis of **HPNP** (0.18 mM) by the mononuclear **47.Eu** (blue) and the dinuclear **41.Eu₂** (red) in 50 mM HEPES buffer at 37 °C. Each data point is an average of 2-3 measurements agreeing to within 15% error. The error bars are determined from the average of 2-3 measurements (error bars for **47.Eu** are obscured by the data points).

By definition, a true catalyst should be capable of achieving catalytic turnover. Unfortunately, for the hydrolysis of **HPNP**, **41.Eu₂** did not display evidence of catalytic turnover. If the reaction is catalytic, the rate constant of the reaction should remain constant in the presence of an excess of **HPNP**.⁹ When one equivalent of **HPNP** was reacted with one equivalent of **41.Eu₂**, at pH 7.4 and at 37 °C, a rate constant of $k_{\text{obs}} = 7.06 (\pm 0.37) \times 10^{-4} \text{ s}^{-1}$ was determined. However, when one equivalent of **HPNP** was reacted with 0.1 equivalents of **41.Eu₂**, the rate constant dropped 25-fold to give a k_{obs} of $2.18 (\pm 0.68) \times 10^{-5} \text{ s}^{-1}$. As the rate constant decreases when there is more substrate than catalyst present, it indicates that this complex does not display catalytic turnover. It is possible that the cyclic phosphate produced in the hydrolysis of **HPNP** is binding to the Ln(III) complex, thus preventing the binding of an incoming substrate.

4.6.1 Conclusion

In summary, a family of xylyl spaced complexes **41.Ln₂** and **43.Ln₂** have been shown to give rise to some of the highest rates of phosphodiester hydrolysis of **HPNP** observed. It was determined that modifications to the structure of the ligand, such as changing the pendant arms, from *N,N'*-dimethylacetamide to acetamide, or changing the orientation of the bridging spacer from *para*- to *meta*-, gave rise to large effects on the overall activity of the Ln(III) complexes, both in terms of the rate constant for the hydrolysis of **HPNP**, and the pH at which the complex was most active towards hydrolysis. The introduction of the acetamide arms had most likely introduced an element

of hydrogen bonding into the binding pocket of the complex, which has given rise to a large increase in the rate of hydrolysis of **HPNP**.

The number of metal bound water molecules on the Eu(III) metal ion was also shown to have an important effect on the rate of the hydrolysis. Whereas La(III) metal ions of previous cyclen-based complexes already possessed two metal bound water molecules, in the past, most Eu(III) complexes only possessed a single water molecule.^{38,112,166} The introduction of a second empty coordination site on the metal ion saw the subsequent trend for the hydrolysis of **HPNP** being reversed, where Eu(III) complexes were now more efficient than their corresponding La(III) complexes for the hydrolysis of **HPNP**.

Following the investigation of **41.Ln₂** and **43.Ln₂** for their ability to hydrolyse **HPNP**, of which preliminary results were presented at the International Conference of Biological Inorganic Chemistry, Austria 2007,¹⁷¹ and during the course of the preparation of a manuscript for submission, a similar study on identical Eu(III) complexes was published by Morrow *et.al.*¹⁷² The work by the Morrow group focused on the *meta*-isomer, **43.Eu₂**, having found it gave rise to a higher second order rate constant than the corresponding **41.Eu₂** at pH 7.4 and 25 °C. Conversely, we chose to focus our efforts on the elucidation of a mechanism of action for the *para*-acetamide complex having investigated the activity of the two complexes across a range of pH values and found **41.Eu₂** to be the more active, at pH 8.0. While Morrow focused on luminescence spectroscopy to study the interreaction of the complexes with phosphates, we have investigated the hydrolysis of **HPNP** as a function of pH and attempted to determine the pK_a values of the metal bound water molecules using potentiometry, as outlined in Chapter Three. The results of both studies gave rise to similar rate of hydrolysis of **HPNP** and found both complexes to be functioning in a moderately cooperative action, giving rise to these enhanced rates of phosphodiester hydrolysis.

In summary, the importance of the choice of metal ion, along with the influence of the acetamide pendant arms, and the small modifications to the structure of the binding pocket induced by the structure of the spacer, resulted in large rate enhancements for the hydrolysis of **HPNP** over that recorded before for cyclen-based lanthanide metal complexes.

Previous studies in the Gunnlaugsson group by Dr. Fanning on a family of mononuclear complexes functionalised with alkylamine acetamide arms, **59**, had given promising results,^{102,122} as discussed in Section 1.1. This arm was also incorporated into the *para*- and the *meta*-spaced ligand to give the complexes **42.Ln₂** and **44.Ln₂** and the results of these investigations are detailed in the following Section.

4.7 Phosphodiester Hydrolysis of HPNP by Xylene-Spaced Alkylamine Acetamide Complexes **42.Ln₂** and **44.Ln₂**

As discussed above, a mononuclear cyclen based lanthanide complex was previously synthesised in which the cyclen frame was functionalised with alkylamine acetamide pendant arms. Both the tetra-substituted, **94.Ln**, and the tri-substituted, **36.Ln**, (shown in Section 1.14) complexes were synthesised and evaluated for their ability to hydrolyse **HPNP**.^{102,122} The results of this investigation are detailed in Table 4.6. The terminal amines of these pendant arms had the potential to act as nucleophilic activators which could promote hydrolysis by general acid-base catalysis. An important discovery from this investigation was that the maximum activity of the complex was shifted to within the physiological pH range. As outlined in Section 2.5.4, the dinuclear *para*- and *meta*-spaced complexes were functionalised with the alkylamine acetamide pendant arm, **59**, and the resulting complexes **42.Ln₂** and **44.Ln₂** were investigated for their ability to effectively hydrolyse the phosphodiester bond of **HPNP**.

4.7.1 Kinetic Evaluation of *para*-Xylene Spaced Alkylamine Acetamide System, **42.Ln₂**

The initial evaluation of the complexes **42.Eu₂** and **42.La₂** was carried out in the same manner as for previous complexes, **41.Ln₂** and others, at pH 7.4 and 37° C, and the results are detailed in Table 4.6. The dinuclear complex **42.Eu₂** was found to cleave **HPNP** with the unprecedented first order rate constant of $k_{\text{obs}} = 6.32 (\pm 0.6) \times 10^{-3} \text{ s}^{-1}$, which gave a half life, $\tau_{1/2}$, of 0.03 hours, or 1.86 minutes, Figure 4.13. This rate constant corresponded to the extremely high rate enhancement, k_{rel} , of *ca.* 189499 over the uncatalysed reaction, which, to our knowledge, is the highest rate constant reported for the hydrolysis of **HPNP** by a cyclen based lanthanide complex, at physiological pH, in a buffered solution. Indeed, such a rate enhancement is one of the largest known to date within the literature for the hydrolysis of **HPNP** by a metal based complex.³⁷⁻³⁹

Table 4.6: Pseudo first order rate constants (k_{obs}), half lives ($\tau_{1/2}$) and relative rate enhancements (k_{rel}) for the cleavage of **HPNP** (0.18 mM) in 50 mM HEPES buffer at pH 7.4 and 37° C by **94.La**, **36.Eu**, **42.La₂** and **42.Eu₂** (0.18 mM).

Complex	$k_{\text{obs}} (\text{s}^{-1})$	$\tau_{1/2} (\text{h})$	k_{rel}
94.La	$2.6 (\pm 0.5) \times 10^{-4}$	0.74	7775
36.Eu	$4.2 (\pm 1.9) \times 10^{-4}$	0.46	10216
42.La₂	$5.7 (\pm 1.7) \times 10^{-4}$	0.34	17111
42.Eu₂	$6.32 (\pm 0.6) \times 10^{-3}$	0.03	189499

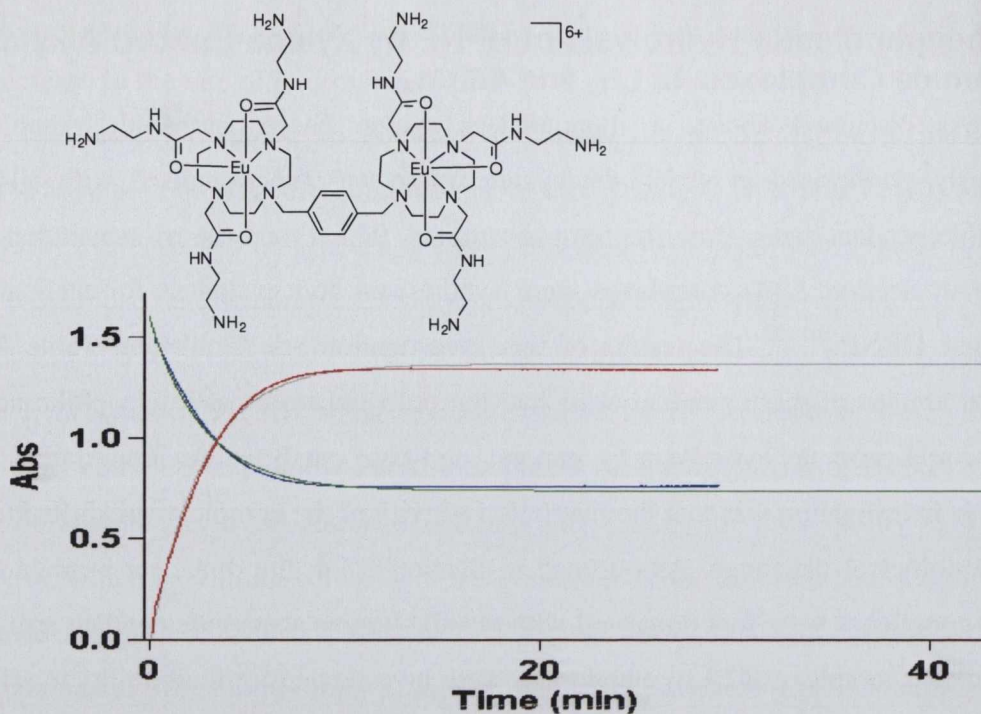


Figure 4.13: The kinetic profile for the hydrolysis of **HPNP** (0.18 mM) by **42.Eu₂** (0.18 mM) in 50 mM HEPES buffer at pH 7.4 and 37 °C. The increase of *p*-nitrophenolate (red line) as a function of time is observed and fitted to first order kinetics to determine the rate constant, k_{obs} , for the reaction.

The hydrolysis of **HPNP** by **42.Eu₂** at pH 7.4 was found to give rise to an 18-fold rate enhancement over the analogous mononuclear, tri-substituted Eu(III) complex, **36.Eu**. Such an enhancement clearly suggests that the complex operates *via* a cooperative action of the two metal ion centres. Comparing the activity of **42.Eu₂** to that of **41.Eu₂** at pH 7.4, it can be seen that **42.Eu₂** exhibits an almost 9-fold enhancement over the acetamide functionalised dinuclear complex, indicating the important role that the terminal amines play in the hydrolysis of **HPNP**.

The ability of the analogous dinuclear La(III) complex, **42.La₂**, to hydrolyse **HPNP** was also investigated. The rate profile for **42.La₂** is shown in Appendix A3.13. However, the rate constant determined was found to be an order of magnitude smaller than that observed for **42.Eu₂**. Nevertheless, this enhancement was more than double that observed for the analogous mononuclear complex, **94.La**. This indicated, once again, that the pK_a and the number of metal bound water molecules play a large role in determining the rate of the hydrolysis of **HPNP**.

As for previous complexes studied, the efficiency of the complex at various pH values was determined to investigate the pH-dependence of the complex, and the resulting pH-rate profile is shown in Figure 4.14.

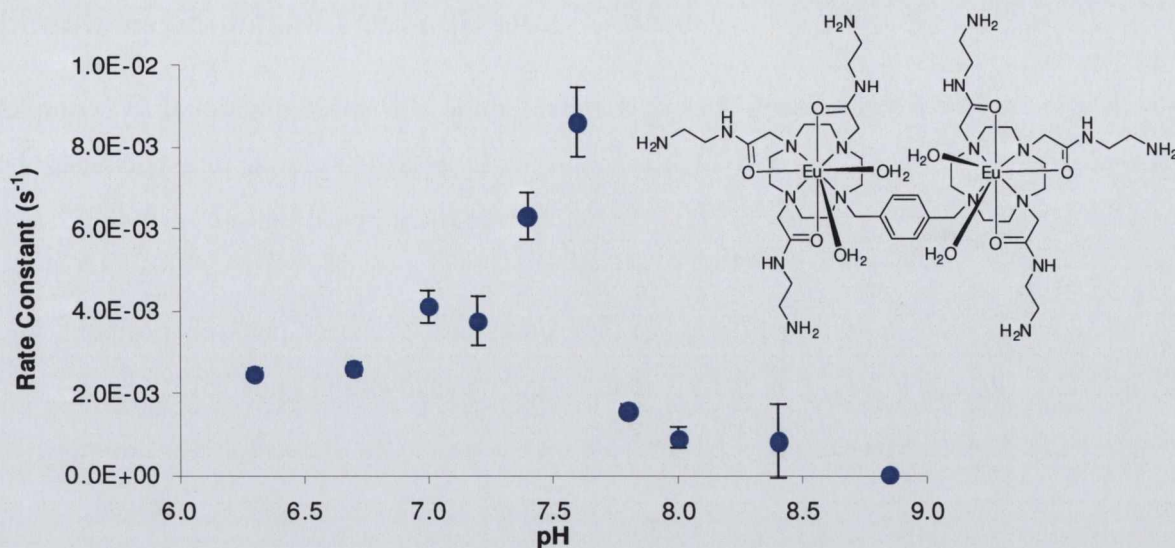


Figure 4.14: The pH-rate profile for the hydrolysis of **HPNP** (0.18 mM) by **42.Eu₂**, in 50 mM HEPES buffer at 37 °C. Each data point is an average of 2-3 measurements agreeing to within 15% error. The error bars are determined from the average of 2-3 measurements.

The evaluation of **42.Eu₂** over various pH values showed a different trend to that observed previously for dinuclear Eu(III) complexes, such as **41.Eu₂**. Here, k_{obs} increased from pH 6.0 up to pH 7.6, where it reached its maximum activity, giving rise to an even higher rate constant, $k_{\text{obs}} = 8.6 (\pm 0.6) \times 10^{-3} \text{ s}^{-1}$. This corresponded to a half life of $\tau_{1/2} = 0.02$ hours, or 1.34 minutes, which is a relative rate enhancement of 289000. To the best of our knowledge, this is the highest maximum rate enhancement ever recorded for the hydrolysis of **HPNP** by a lanthanide complex within the physiological pH range.^{104,117,118,167,173}

The La(III) complex, **42.La₂**, did not give rise to any such high rates of **HPNP** hydrolysis across the pH range investigated, with the maximum activity, $k_{\text{obs}} = 5.7 (\pm 1.7) \times 10^{-4} \text{ s}^{-1}$, observed at pH 7.4.

The extremely high rate of hydrolysis of **HPNP** by **42.Eu₂** has been attributed to a cooperative action of the two metal ions, as was observed for **41.Eu₂**. Without a doubt, the presence of the alkylamine acetamide pendant arms had a large influence, both on the rate of the hydrolysis of **HPNP**, and also on the pH at which the complex is most active. It is possible that the terminal amines give rise to general acid/base catalysis of the **HPNP**, which in combination with the increased number of metal bound water molecules on the Eu(III) metal ions, resulted in the shift of the active pH window to near-physiological pH. The activity of the complex drops dramatically above pH 7.6, suggesting the formation of the dihydroxo species which prevents the binding of the substrate to the metal ion. The next section details the kinetic evaluation of the *meta*-isomer, **44.Ln₂**, for the hydrolysis of **HPNP**.

4.7.2 Kinetic Evaluation of *meta*-Xylene Spaced Alkylamine Acetamide System, **44.Ln₂**

The *meta*-spaced analogues of the alkylamine acetamide functionalised complexes were also synthesised, and evaluated for their ability to hydrolyse the phosphodiester bond of **HPNP**, Table 4.7. At pH 7.4 and 37 °C, a large increase in the rate of hydrolysis was observed for **44.Eu₂**, where a rate constant of $k_{\text{obs}} = 3.4 (\pm 0.4) \times 10^{-3} \text{ s}^{-1}$ was determined. This corresponded to a half life, $\tau_{1/2}$ of 0.06 hours and a relative rate enhancement, k_{rel} , of 102066. The rate profile generated for this reaction is shown in Figure 4.15.

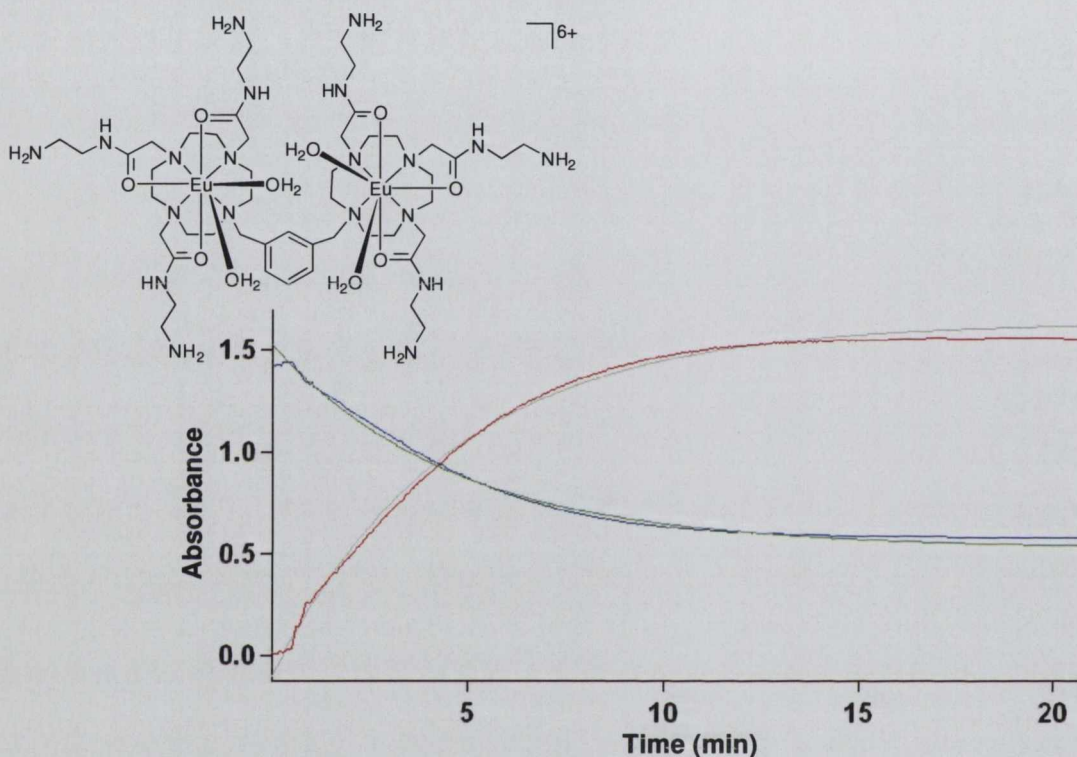


Figure 4.15: The kinetic profile for the hydrolysis of **HPNP** (0.18 mM) by **44.Eu₂** (0.18 mM) in 50 mM HEPES buffer at pH 7.4 and 37 °C. The increase of *p*-nitrophenolate (red line) as a function of time is observed and fitted to first order kinetics to determine the rate constant, k_{obs} , for the reaction.

Such an enhancement was a near 3.5-fold enhancement over the analogous acetamide functionalised **43.Eu₂** and was of the same order of magnitude as that observed for the *para*-isomer, **42.Eu₂**. The La(III) complex **44.La₂** displayed a similar activity to that of **42.La₂** and was an order of magnitude less active than its corresponding Eu(III) analogue. The rate profile for **44.La₂** is shown in Appendix A3.14.

The activity of **44.Eu₂** for the hydrolysis of **HPNP** was measured as a function of pH, and the resulting pH-rate profile is shown in Figure 4.16. This revealed that whereas for the *para*-isomer, **42.Eu₂**, the maximum activity was observed at pH 7.6, the maximum activity of the *meta*-isomer, **44.Eu₂**, occurred at pH 7.4, with a rate constant of $k_{\text{obs}} = 3.4 (\pm 0.4) \times 10^{-3} \text{ s}^{-1}$.

Table 4.7: Pseudo first order rate constants (k_{obs}), half lives ($\tau_{1/2}$) and relative rate enhancements (k_{rel}) for the cleavage of **HPNP** (0.18 mM) in 50 mM HEPES buffer at pH 7.4 and 37 °C by **43.Ln₂**, and **44.Ln₂** (0.18 mM).

Complex	k_{obs} (s ⁻¹)	$\tau_{1/2}$ (h)	k_{rel}
43.La₂	$2.0 (\pm 0.5) \times 10^{-4}$	0.96	60004
43.Eu₂	$9.9 (\pm 1.2) \times 10^{-4}$	0.19	30421
44.La₂	$3.9 (\pm 1.9) \times 10^{-4}$	0.49	11708
44.Eu₂	$3.4 (\pm 0.4) \times 10^{-3}$	0.06	102066

The La(III) complex, **44.La₂** did not display the same high rate enhancements for the hydrolysis of **HPNP** that were observed for the corresponding Eu(III) complex, **44.Eu₂**. This again, is most likely due to the relative Lewis acidity of the metal ions, and the influence that this has on the pK_{a} of the metal bound water molecules, as was previously observed for the analogues **42.La₂** and **43.La₂**.

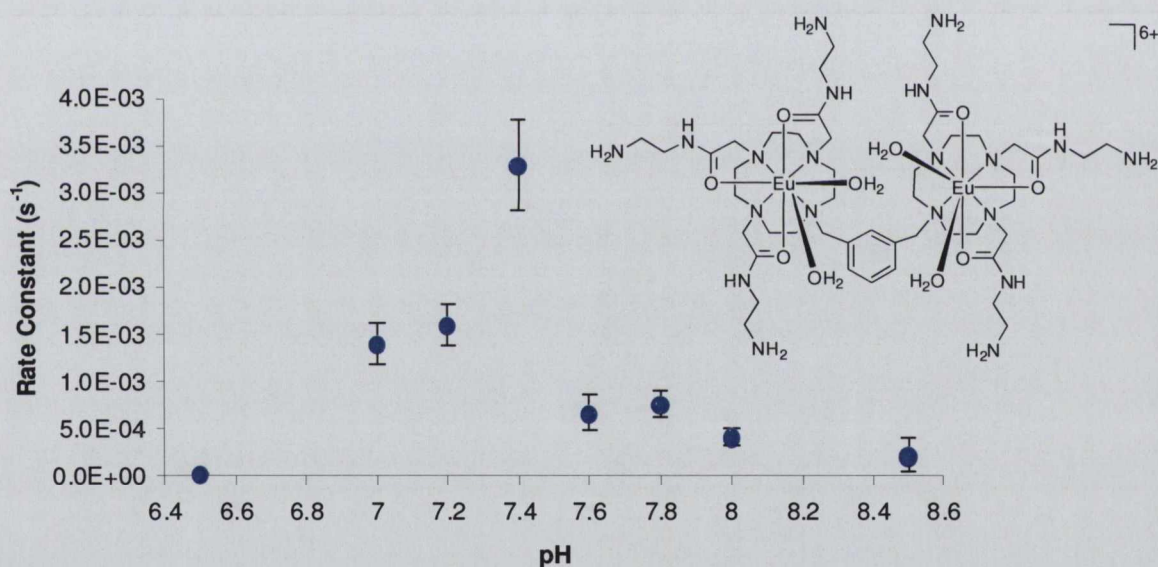


Figure 4.16: The pH-rate profile for the hydrolysis of **HPNP** (0.18 mM) by the meta-spaced dinuclear complex, **44.Eu₂**, in 50 mM HEPES buffer at 37 °C. Each data point is an average of 2-3 measurements within 15% error.

The results of this investigation again highlighted the important role that the structure and binding pocket of the ligand may play in modulating the activity of the complex towards the hydrolysis of **HPNP**. The crystal structure of **41.La₂** was detailed in Section 2.4.5, and it was noted that in the solid state, the two cyclen cavities adopted an *anti* conformation. It is possible that the *meta*-isomer introduces a degree of rigidity to the complex, arising perhaps from steric hindrances, which could potentially limit the freedom of rotation of the two cyclen macrocycles. For that reason, the *meta*-isomer may not be as

flexible as the *para*-isomer, and possibly adopts a conformation which is not entropically favoured for the binding and hydrolysis of **HPNP**.

4.7.3 Evaluation of the Mechanism of Hydrolysis of **HPNP** by Xylene-Spaced Alkylamine Acetamide Complex, **42.Eu₂**

To further elucidate a mechanism of action for the complex **42.Eu₂**, pseudo-first order kinetics were carried out, to generate a second order rate constant for comparison to other systems, and the results are shown in Figure 4.17. Whereas previous complexes had been fit to Equation 4.1 (Section 4.5.3), to obtain the second order rate constant, **42.Eu₂** could not be fit in such a manner. The data gave rise to a highly curved line, which indicated that the complex did not follow simple first order kinetics. The curve can be fitted to an exponential growth, which suggests that there may be two different concentration dependant kinetic processes at work here.

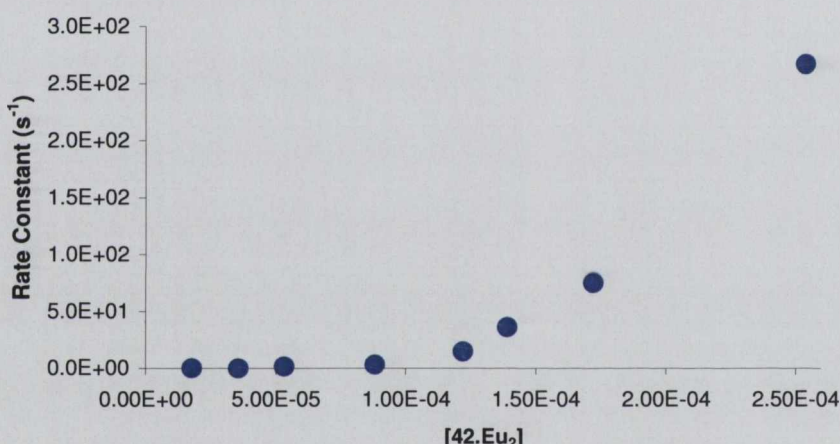


Figure 4.17: Dependence of the pseudo first order rate constant for the hydrolysis of **HPNP** (5.2×10^{-3} mM) on **[42.Eu₂]** in the concentration range 0.017 – 0.25 mM, at pH 7.4 and 37 °C.

The data at low concentration was fit to Equation 4.1, and gave rise to a second order rate constant of $48796 (\pm 1.1) \text{ M}^{-1} \text{ s}^{-1}$, Appendix A.3.15. Fitting to Equation 4.1 from $1.2 \times 10^{-4} \text{ M}$ to $2.5 \times 10^{-4} \text{ M}$, an unprecedented second order rate constant of $2.0 \times 10^{-6} (\pm 235)$ was determined (Appendix A3.16). However, at higher concentrations, upward curvature of the graph indicates that the pseudo-first order assumptions are possibly no longer valid and that a second order reaction is most likely occurring.

Further insight into the mechanism of **42.Eu₂** was gained from studying the interaction with **DEP**, as described in Section 4.4.3 for **41.Eu₂**. The titration was carried out in the same manner as that of **41.Eu₂**, where increasing molar equivalents of **DEP** were added to a solution of **42.Eu₂** in H_2O , and the changes in the ^{31}P NMR signal were

monitored. The resulting titration curve is shown in Figure 4.18 and a stack plot of several points in the titration is shown in Appendix A3.17.

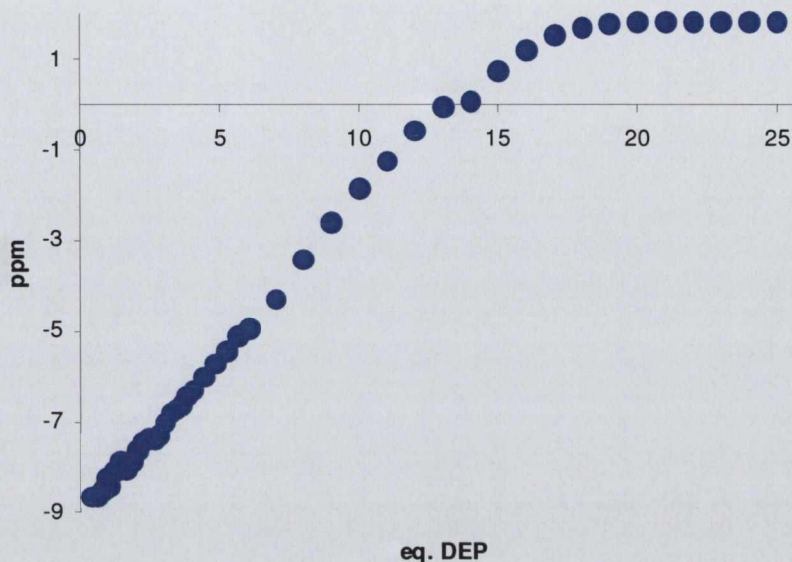


Figure 4.18: Changes in the ^{31}P NMR spectrum (162 MHz, H_2O) as a function of molar equivalents of DEP ($2.1 \times 10^{-1} \text{ M}$) added to **42.Eu₂** ($3.0 \times 10^{-3} \text{ M}$).

Again, it was attempted to fit the titration using WINEqNMR¹⁶⁹ and NMRtit,¹⁷⁰ however, a binding constant could not be determined for the titration. The binding curve indicates that the overall interaction of **42.Eu₂** with **DEP** was not as strong as that of **38.Eu₂**, or **41.Eu₂**, with **DEP**, which was discussed in Section 4.4.3. There was an overall shift of *ca.* 9 ppm in the phosphate signal, however, a plateau was not reached until approximately 16 equivalents of **DEP** have been added. Such a binding curve suggests that despite the 6 positive charges on the complex, **42.Eu₂** does not bind as strongly to phosphate as previous dinuclear complexes did, suggesting that the complex may not suffer from the same inhibition effect observed for previous complexes.^{14,22} A weaker interaction, such as that observed here suggests that it is possible that after hydrolysing the **HPNP**, **42.Eu₂** may be capable of dissociating from the cyclic phosphate thus giving rise to complex turnover. The following Section details an investigation into the turnover of the complex **42.Eu₂**.

4.7.4 Investigation of Inhibition and Turnover of **42.Eu₂**

The *para*-spaced, alkylamine acetamide functionalised complex **42.Eu₂**, was found to hydrolyse **HPNP** with a rate constant of $8.6 (\pm 0.6) \times 10^{-3} \text{ s}^{-1}$, at pH 7.4 and at 37 °C. This was the highest rate constant recorded to date to our knowledge, for a cyclen-based lanthanide complex at physiological pH, in buffered aqueous solution. To further

investigate the mechanism of hydrolysis of **HPNP**, and in particular the inhibition and turnover of **42.Eu₂**, several tests were carried out.

EDTA is a known metal ion chelator and is known to complex Eu(III) ions with a stability constant of $\log K = 17.32$.¹⁷⁴ In the presence of a Eu(III) complex, EDTA can behave in several different ways. Firstly, if the stability constant of the complex is low, EDTA will more than likely displace the ligand and bind directly to the Eu(III) ion. The EDTA can also bind to the Eu(III) complex, displacing the metal bound water molecules (which in the presence of **HPNP** will lead to a decrease or total inhibition of the rate of the reaction). Finally, EDTA is also capable of complexing free metal ions in solution, and if the observed hydrolysis is due, in part, to any metal ions in solution, as opposed to the intended complex, a decrease in the rate constant will be observed. The hydrolysis of **HPNP** by **42.Eu₂** was carried out in the presence of EDTA and no reduction in the rate of hydrolysis was observed, indicating that there were no free metal ions in solution contributing to the rate constant, and that the complex was stable in the presence of EDTA, which was in accordance with the kinetic analysis detailed in Section 3.3.

To determine the efficiency of **42.Eu₂** in a competitive environment, the hydrolysis of **HPNP** was carried out in the presence of one equivalent of **DEP** at pH 7.4 and 37 °C where it was observed that the reaction rate fell 3-fold to give a rate constant $k_{\text{obs}} = 1.9 (\pm 0.3) \times 10^{-4} \text{ s}^{-1}$, however, it was observed that the **HPNP** was fully hydrolysed at the end of the reaction. A similar reaction was observed in the presence of one equivalent of cCMP (Cytidine-3'5'-(cyclic)monophosphate), where the rate of the reaction was found to fall to $k_{\text{obs}} = 1.6 (\pm 0.2) \times 10^{-4} \text{ s}^{-1}$. The rate constants for the hydrolysis of **HPNP** in the presence of such competitors remains on a par with the rate constants determined for complexes such as **47.Eu**.

As outlined in Section 4.1, the hydrolysis of **HPNP** is generally monitored spectrophotometrically, however, it can also be monitored using ³¹P NMR spectroscopy. The ³¹P NMR spectrum of **HPNP** has been recorded and displays a single resonance at *ca.* 3.7 ppm. When hydrolysed, this resonance is shifted upfield to 19 ppm, indicating the formation of the cyclic phosphate resulting from the successful hydrolysis of **HPNP**. Moreover, the absence of any residual peak at -3.7 ppm ascertains that the reaction is complete. The hydrolysis of **HPNP** by **42.Eu₂** (1:1 molar equivalents) was monitored using ³¹P NMR and shows as expected a single phosphate resonance at 19 ppm, which indicated that the cyclic phosphate was formed during the reaction and that all **HPNP** had been hydrolysed.

To investigate if **42.Eu₂** was capable of acting as a true catalyst and exhibiting catalytic turnover, the hydrolysis reaction was carried out in an excess of **HPNP**. While the growth in the absorbance at 400 nm, arising from the production of *p*-nitrophenolate, indicated that **42.Eu₂** did indeed hydrolyse the excess **HPNP**, it was difficult to quantify the turnover of the catalyst. As such, the reaction was then monitored by ³¹P NMR. One equivalent of **HPNP** was added to a solution containing one molar equivalent of **42.Eu₂**, which was buffered at pH 7.6 (50 mM HEPES), where the maximum activity of the complex had been observed. The resonance of the phosphate was monitored and when it appeared that the **HPNP** had been fully hydrolysed, a second equivalent of phosphate was added and the phosphate signal again monitored for the hydrolysis of **HPNP**, Figure 4.19.

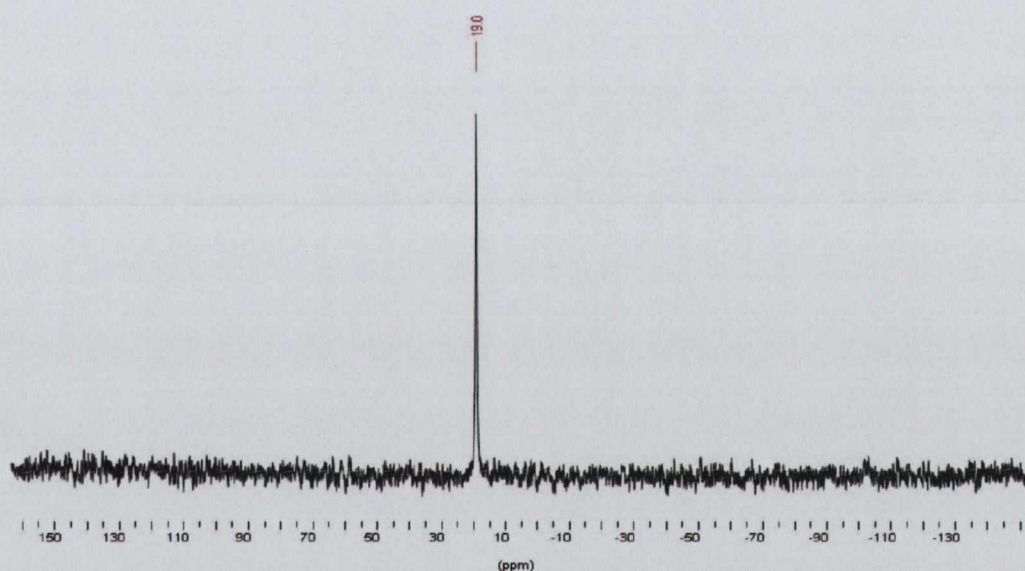


Figure 4.19: The ³¹P NMR spectrum (162 MHz, H₂O) following the hydrolysis of four molar equivalents of **HPNP** (2.1×10^{-1} M) by **42.Eu₂** (3×10^{-3} M).

Following the addition of 9 molar equivalents of **HPNP**, a small resonance was observed at approximately -4 ppm, indicating that turnover was slowing and that the latest addition of **HPNP** was not fully hydrolysed. This was the first example of turnover of a cyclen-based lanthanide complex for the hydrolysis of **HPNP** to date, carried out within the physiological pH range, in buffered aqueous solution.^{4,6,20,38,66}

4.7.5 Summary

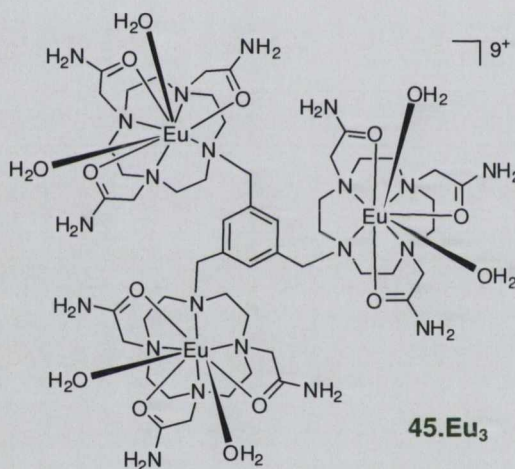
In summary, this Section has analysed the hydrolysis of **HPNP** by two families of cyclen based lanthanide complexes, **42.Ln₂** and **44.Ln₂**. The Eu(III) complex **42.Eu₂** was found to give rise to the highest rate recorded for the hydrolysis of **HPNP** by a cyclen based lanthanide complex at physiological pH, in buffered aqueous solution, and in the

presence of competitors such as DEP and cCMP. It has also displayed the first evidence of turnover of a lanthanide complex, and as such, can truly be called a ribonuclease mimic.

The following Section briefly discusses a preliminary analysis of the trinuclear complex, **45.Eu₃**, which is then followed by a discussion of the possible mechanisms for the hydrolysis of **HPNP** by dinuclear complexes.

4.8 Evaluation of Trinuclear Spaced **45.Eu₃**

The trinuclear complex **45.Eu₃** was synthesised with the aim of achieving a large rate enhancement for the hydrolysis of phosphodiester bonds. As outlined in Section 1.4, many ribonucleases utilise two or more metal ions at their active sites for the hydrolysis of phosphodiester bonds.^{19,175} The synthesis of **45.Eu₃** allowed for the incorporation of three lanthanide ions into the structure, and the overall 9⁺ charge on the complex could potentially neutralise the phosphodiester bond of **HPNP**, thus activating it towards hydrolysis.



The trinuclear Eu(III) complex, **45.Eu₃**, was tested for its ability to hydrolyse the phosphodiester **HPNP** at pH 7.4 in 50 mM HEPES buffer and at 37 °C, using a single batch of the complex (4.32×10^{-3} M). Initially, a rate constant of $k_{\text{obs}} = 3.37 (\pm 0.10) \times 10^{-3} \text{ s}^{-1}$ was determined. When repeated, this rate constant dropped to $k_{\text{obs}} = 7.61 (\pm 1.70) \times 10^{-4} \text{ s}^{-1}$ and when repeated for a third time, the rate dropped again, to give a rate constant of $k_{\text{obs}} = 3.45 (\pm 0.85) \times 10^{-4} \text{ s}^{-1}$, and these rate constants are outlined in Table 4.8. The large differences in the rate constants determined, suggested that the complex was unstable in solution, and subsequent investigations, detailed in Section 2.6, showed that this was most likely the case. Although **45.Eu₃** showed great potential to hydrolyse phosphodiester bonds very efficiently, its apparent instability hindered its investigation. It is possible that the complex dissociated in solution, as a result of steric hindrances, or possibly the binding

constant of the ligand to the Eu(III) metal ions was very low. As a result of this apparent instability, the investigation into the hydrolytic ability of **45.Eu₃** was abandoned.

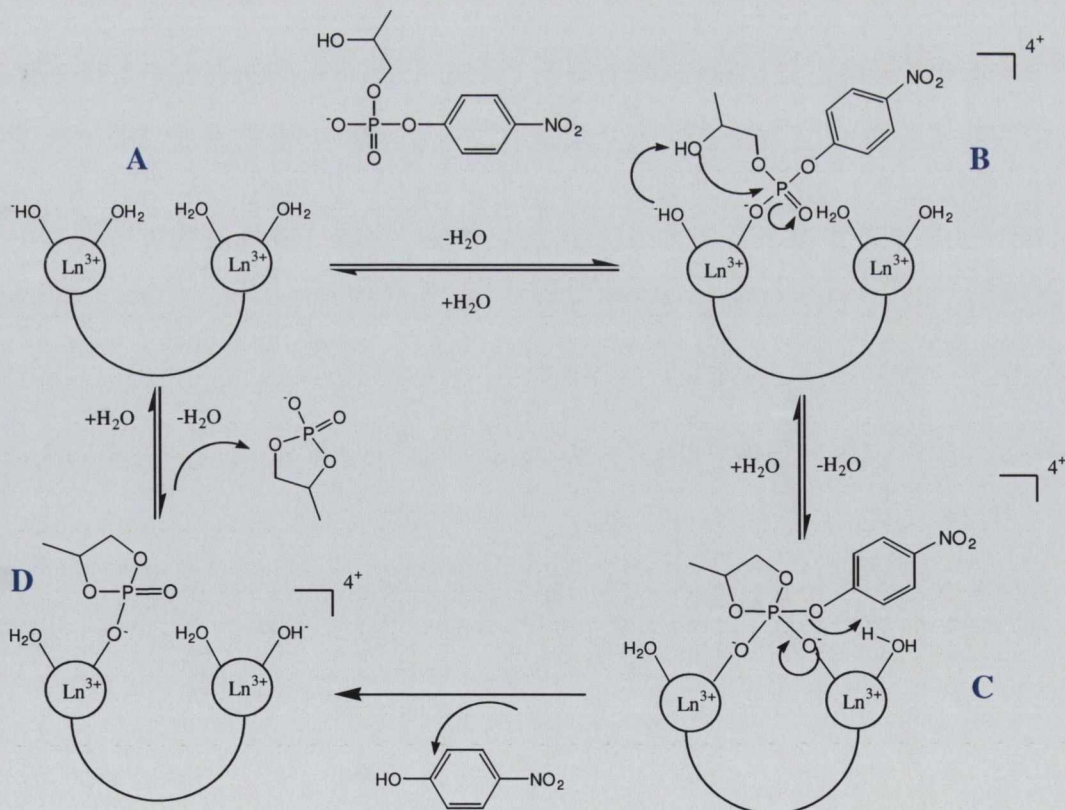
Table 4.8: Pseudo first order rate constants (k_{obs}), half lives ($\tau_{1/2}$) and relative rate enhancements (k_{rel}) for the cleavage of **HPNP** (0.18 mM) in 50 mM HEPES buffer at pH 7.4 and 37° C by **45.Eu₃** (0.18 mM).

Complex	k_{obs} (s ⁻¹)	$\tau_{1/2}$ (h)	k_{rel}
45.Eu₃	$3.37 (\pm 0.1) \times 10^{-3}$	0.06	96333
45.Eu₃	$7.61 (\pm 1.7) \times 10^{-4}$	0.25	23120
45.Eu₃	$3.45 (\pm 0.85) \times 10^{-4}$	0.56	10321

The next Section details the possible mechanisms of action for the hydrolysis of **HPNP** by dinuclear complexes, based on the studies and results detailed within this Chapter.

4.9 Predicting a Mechanism of Action for the Hydrolysis of HPNP

Examination of the results presented within this Chapter allows for the prediction of a possible mechanism of action for the hydrolysis of **HPNP** by a metal ion complex.^{9,37,166,176}



Scheme 4.2: Proposed mechanism of action for the proposed double Lewis acid activation, by a cooperative action of a dinuclear complex.

It is generally accepted that hydrolysis of **HPNP** occurs *via* a four step mechanism, whereby:

- A. A metal bound water molecule is deprotonated and the resulting hydroxyl group can then function as a nucleophile activator.¹⁷⁷
- B. The metal complex coordinates to the **HPNP** *via* the phosphate anion, and the 2'-hydroxypropyl group is deprotonated by the second metal bound water molecule.
- C. Having been activated, the 2'-hydroxy group attacks the metal coordinated phosphodiester to produce a five-coordinate phosphorane intermediate, which is stabilised by the metal ion. The transition state is then broken down into *p*-nitrophenolate and a cyclic phosphate.
- D. The complex releases the cyclic phosphate by ligand exchange with a water molecule.

The mechanism outlined above, in Scheme 4.2, is proposed for the complexes **39.Eu₂**, **39.La₂**, **41.Eu₂**, **41.La₂**, **43.Eu₂** and **43.La₂**. A similar mechanism is proposed for the complexes **42.Eu₂**, and **44.Eu₂**, and that is discussed later in this Section.

The first step in the reaction mechanism is the deprotonation of a metal bound water molecule on one of the metal ions. This step has previously been shown to be highly pH-dependant, and that was again seen in the studies herein. The pK_a of the metal bound water molecule is of great importance, as it will dictate the active pH of the metal complex. Moreover, it has been shown that the activity of the complex is directly correlated to the proportion of metal bound hydroxide formed in solution, and as such if there is no deprotonation of the metal bound water molecule, there will be little to no hydrolysis of the **HPNP**.^{5,43,46} However, if both metal bound water molecules on the metal ion become deprotonated, hydrolysis will be inhibited, as the substrate will not bind to the anionic phosphate centre.¹⁷⁸ In this situation, some hydrolysis will still be observed as a result of the substrate interacting with the complex *via* electrostatic interactions and hydrogen bonding, and also as a result of self-promotion of hydrolysis.

The second step is the binding and stabilisation of the substrate by the metal complex. This can occur either by the coordination of the metal ion directly to the phosphate, or by the coordination of metal bound water molecule to the substrate *via* Lewis acid activation. Following the binding of the substrate to the metal complex, a metal bound hydroxide can act as a general base and abstract a proton from the 2'-hydroxypropyl group, activating it towards nucleophilic attack on the phosphorous centre,¹⁶⁸ after which the two metal ions can then act in a cooperative manner to stabilise the pentacoordinate transition state of the **HPNP**.

This step is followed by the hydrolysis of the **HPNP** and it is thought that one of the metal bound hydroxides can act as a general acid promoting the expulsion of the *p*-nitrophenolate leaving group. The next and final step is the expulsion of the cyclic phosphate. It is presumed that this occurs by ligand exchange with water molecules, returning the complex to its initial state ready for a new catalytic cycle.¹⁵ Several of the complexes investigated showed a high rate of hydrolysis of **HPNP**, especially as compared to previously tested complexes, however, most of the complexes did not display any evidence of turnover. It is thought that the highly positively charged metal complexes bind very strongly to the phosphate, and this was confirmed by phosphate binding experiments using **DEP**.¹⁶⁶ Such strong binding can potentially inhibit the release of the cyclic phosphate thus prematurely terminating the catalytic cycle. It is at this step in the mechanism that **42.Eu₂** behaved differently to other complexes tested, as it did display evidence of turnover. The titration of **42.Eu₂** against **DEP** did not give rise to a titration curve as steep as those of **41.Eu₂** or **38.Eu₂** which were thought to bind quite strongly to phosphate. The weaker titration curve for the interaction of **42.Eu₂** with phosphate indicated that the complex was much less likely to be inhibited by its binding.

The mechanism for **HPNP** hydrolysis by the alkylamine functionalised ligands most likely deviates from that outlined above, as the terminal amines play a major role in the hydrolysis reaction, as evidenced by the large rate enhancements observed for the hydrolysis of **HPNP**. Although the potentiometric titrations of the complexes did not result in the determination of the pK_a values for the metal bound water molecules, it was observed that the titration curve for the alkylamine functionalised complexes, **42.Ln₂** and **44.Ln₂** was markedly different from those of the acetamide functionalised complexes. It is probable that the amines are participating in hydrolysis by a general acid-base catalysis contribution, and it is also possible that they are deprotonating the second sphere water molecules, causing them to become nucleophilic, thus enhancing the reaction.¹²²

4.10 Conclusion

The studies and results detailed within this Chapter have led to several conclusions as to the most successful design of the complex and the efficient hydrolysis of **HPNP**.

Most previous studies suggested that the size and Lewis acidity of the lanthanide ion, or the number of metal bound water molecules have a large influence on the rate of hydrolysis. However, the results detailed herein suggest that the size of the metal ion is not as important as previously thought, but that the pK_a of the metal bound water molecule quite likely has a great influence on the pH of the active region of the complex, as well as affecting the rate of hydrolysis. For all the complexes studied, the maximum rate of

hydrolysis for the La(III) complexes was found to occur at *ca.* pH 7.4, whereas for the Eu(III) metal ion complexes, it was more varied, with the maximum activity occurring between pH 7.4 and 8.0. There are several factors which could be influencing this trend. It is expected that the active region of the La(III) complexes is shifted towards physiological pH as a consequence of the pK_a of the metal bound water molecule being lowered by the stronger Lewis acidity of the La(III) ion versus that of the Eu(III) ion.

Secondly, a comparison of the *para*- and the *meta*-spacers for both families of complexes revealed the *para*- to be generally more active, suggesting that the importance of the structure of the spacer to the activity of the complex cannot be underestimated. Given that for the most part the *meta*-spaced complexes were less active than the corresponding *para*-complexes it is likely that the *meta*-complexes do not, or cannot adopt the most favourable orientation for successful hydrolysis of the **HPNP**. It is possible that the *meta*-orientation of the spacer hinders the freedom of rotation of the complex to a greater extent than the *para*-spacer does, and so it is more difficult to achieve a successful cooperative action of the two metal ions.

The strength of the binding was demonstrated to be a critically important factor for the catalytic hydrolysis of **HPNP**. If the complex binds to the **HPNP** too strongly, although it will still be hydrolysed, the complex is unlikely to be displaced from the resulting cyclic phosphate, and as such, the complex will be inhibited from carrying out any further reactions. Conversely, it has been shown that complexes which bind phosphates too weakly do not give rise to any measurable hydrolysis.^{100,166} The alkylamine functionalised complex **42.Eu₂** was shown to bind phosphate over a large number of equivalents with a moderate binding curve, and this may well be the reason that turnover was observed. It was thought that the 6⁺ positive charge on **38.Eu₂** and **41.Eu₂** was responsible for a strong binding of phosphate and the resulting inhibition of the complexes, however, **42.Eu₂** is also 6⁺ positively charged and behaves differently, which is most likely as a result of the presence of the terminal amines influencing the binding.

The results from the pH-studies, potentiometric titrations and ³¹P NMR studies allowed for a mechanism of action for the complexes to be postulated. Furthermore, these results as a whole indicate that through a fine tuning of all aspects of the structure of the complex including the spacer, the pendant arms, the number of metal bound water molecules and subsequent number of coordination sites on the complex, the nature and structure of the binding pocket can lead to greatly increased rates of phosphodiester hydrolysis, and the development of a true ribonuclease mimic such as **42.Eu₂**.

5.1 General Experimental Details

All chemicals were obtained from Sigma-Aldrich, Fluka, Strem, or Chematech and unless otherwise specified were used without further purification. Deuterated solvents for NMR analysis were purchased from Apollo Scientific. Dry solvents were prepared using standard procedures, according to Vogel,¹⁷⁹ with distillation prior to each use. Chromatographic columns were run manually using silica gel 60 (230-40 mesh ASTM) or aluminium oxide (activated, neutral, Brockman I STD grade, 150 mesh, or activated, basic, Brockman I STD grade, 150 mesh). Chromatographic columns were also run on a Teldyne Isco Combiflash Companion Automatic machine using prepacked silica or alumina columns. Solvents for synthetic purposes were used at GPR grade unless otherwise stated. Analytical TLC was performed using Merck Kieselgel 60 F₂₅₄ silica plates or Polygram Alox N/UV₂₅₄ Aluminium oxide plates. Visualisation was by UV light (254 nm), exposure to I₂ on silica or by immersion in aqueous alkaline KMnO₄ solution. NMR spectra were recorded at 400 MHz using a Bruker Spectrospin DPX-400 instrument that operates at 400.13 MHz for ¹H NMR and 100.3 MHz for ¹³C NMR. NMR spectra were also recorded at 600 MHz, using a Bruker advance II that operates at 600.13 MHz, for ¹H NMR and 150.9 MHz for ¹³C NMR. Shifts were referenced relevant to the internal solvent signals, with chemical shifts expressed in parts per million (ppm) downfield from the standard. Multiplicities are abbreviated as follows: singlet (s), doublet (d), triplet (t), quintet (q), multiplet (m) and broad (br). All NMR titrations were performed at 293 K. Mass Spectra were determined by detection using electrospray on a Mass Lynx NT V 3.4 on a Waters 600 Controller connected to a 996 photodiode array detector with HPLC-grade methanol, water or acetonitrile as carrier solvents. Accurate molecular weights were determined by a peak-matching method, using leucine enkephaline (H-Try-Gly-Gly-Phe-Leu-OH) as the standard internal reference (*m/z* = 556.2771). Samples were prepared as solutions in methanol or acetonitrile. Melting points were determined using an Electrothermal IA9000 digital melting point apparatus in an unsealed capillary tube. Where comparative literature melting points are not cited, none were recorded in the source literature. Infrared spectra were recorded on a Perkin Elmer Spectrum One FT-IR spectrometer fitted with a universal ATR sampling accessory. Elemental analysis was carried out at the microanalysis laboratory, School of Chemistry and Chemical Biology, University College Dublin. X-ray diffraction studies were carried out by Dr. Thomas McCabe, School of Chemistry, Trinity College Dublin.

The lanthanide metal salts Eu(CF₃SO₃)₃ and La(CF₃SO₃)₃ were purchased from Aldrich and were dried under high vacuum over P₂O₅ prior to use. The complexes were

prepared as described in Chapter 2 and they were dried over P_2O_5 under high vacuum prior to use.

5.2 Potentiometric Titrations

Water used in the preparation of all aqueous solutions was filtered through a Millipore filter prior to use.

Tetraethylammonium perchlorate (NEt_4ClO_4) was prepared by the addition of excess $HClO_4$ (1.0 M, 1.7 dm³) to NEt_4Br (300 g, 1.4 mol) in H_2O . The resulting NEt_4ClO_4 was recrystallised from perchloric acid (1.0 M, 100 mL) and EtOH (3 x 150 mL) until free of bromide and acid. The white crystalline NEt_4ClO_4 was then dried under high vacuum over P_2O_5 for 2 days. (**CAUTION:** Anhydrous perchlorate salts are potentially explosive and should be handled with caution).

Potentiometric titrations were performed using an automatic titrator system, Titrino, equipped with a glass electrode (Orion Ross SureFlow 8172 BN combination electrode) and connected to a Dell PC. The titrations were carried out at 298 K in a water-jacketed vessel. Argon was bubbled firstly through a 1 M KOH solution and then through a 0.1 M NEt_4ClO_4 solution, and the dry argon was then bubbled through the cell to exclude CO_2 and O_2 . All solutions were prepared using 100% water, with constant ionic strength $I = 0.1$ M NEt_4ClO_4 .

The glass electrode was calibrated daily by titrating 0.1 M NEt_4OH with 0.5 mL of 0.1 M $HClO_4$ in 5 cm³ 0.1 M NEt_4ClO_4 . The resulting data was fitted to the Nernst equation to determine E_0 and pK_w , Equation 5.1.

$$E = E_0 + \frac{RT}{F} \ln[H^+]$$

Where: E = observed potential (V)
 E_0 = standard electrode potential (V)
 R = gas constant (8.314 J mol⁻¹ K⁻¹)
 T = temperature (K)
 F = Faraday's constant (9.6487 x 10⁴ Coulombs mol⁻¹)

At 298.2 K, with E in millivolts and converting to logarithim base 10:

$$pH = \frac{(E_0 - E)}{59.15}$$

where: E = observed potential (mV)

$$pH = -\log[H^+]$$

and:

$$pK_w = \frac{(E_0 - E)}{59.15} + pOH$$

The Gran method was used to calculate the calibration parameters E_0 and pK_w (-13.78). For each system, two titrations were performed for accuracy. The protonation constants were determined by the titration of a 0.1 M NEt_4OH with a 3 cm³ solution of a ligand and $HClO_4$. The NEt_4OH was previously standardised against a 0.01 M solution of potassium hydrogen phthalate. The concentration of the ligand of interest and $HClO_4$ in solution varied for each system.

By means of the Titrino Autoburette, constant volume aliquots of the titrant were delivered during the titrations. For all titrations, the equilibrium was investigated and the adequate time allowed between aliquot additions for the ligand or complex to reach equilibrium.

The protonation and stability constants for each ligand and metal ion complex were determined by means of the Fortran programme HYPERQUAD,¹⁵⁶ a non-linear least squares regression fitting programme. The final constants represent an average from two calculated titrations where the chi-squared of each run was less than 12.6 at the 95% confidence level.

5.3 Experimental Data for Kinetic Measurements

All kinetic evaluations were carried out using either an Agilent 8453 spectrophotometer or a Varian CARY 50 spectrophotometer. The Agilent 8453 spectrophotometer was fitted with a circulating temperature controlled bath, and water driven mechanical stirring. The Varian CARY 50 spectrophotometer was fitted with a thermostatted block and a mechanical stirrer. The stirring rate of the mechanical stirrer in the Varian 50 CARY spectrophotometer was set to a similar rate to that of the Agilent 8453 spectrophotometer. The rate of hydrolysis (k) of the phosphodiester by the complexes prepared in Chapter 2 was determined by fitting the data to first order kinetics using *Biochemical Analysis Software* for the Agilent ChemStation and Software Version 3.00(182) for the CARY 50 spectrophotometer. All rate constants were calculated from an average of two or three experiments, with errors of $\pm 15\%$.

A 50 mM solution of HEPES buffer was prepared (1.19 g, 4.6 mmol) in Millipore filtered water (100 mL). The pH of the solution was adjusted using either 2 M NaOH or 2 M HCl solutions. Using this buffer, a 0.18 mM solution of **HPNP** was prepared and the concentration was adjusted such that the $Abs = 1.5$ ($\epsilon = 8392 \text{ M}^{-1} \text{ cm}^{-1}$). This **HPNP** solution ($4.2 \times 10^{-7} \text{ mol}$) (2.4 mL) was then incubated in a quartz UV cell at 37 °C for 10 min. A solution of the appropriate lanthanide complex was prepared in H_2O such that 100

μL contained 4.32×10^{-7} mol of the complex (0.18 mM). A 100 μL aliquot was then added to the **HPNP** solution at 37 °C and the reaction was monitored by UV over at least three half-lives, or for up to 16 hours. For very fast hydrolysis reactions, such as those that occurred in the presence of **42.Eu₂**, the reaction was monitored before the addition of the complex to the solution, to enable accurate rate determination. The pH was checked at the end of some of the reactions and was found to have changed by no more than ± 0.05 pH units.

5.4 UltraViolet-Visible Spectroscopy

UV-Visible absorption spectra were recorded with a Varian Cary 50 spectrophotometer. The solvents used were of HPLC grade. The wavelength range was 230–400 nm with a scan rate of 600 nm min^{-1} . The blank used was of the solvent system the titration was undertaken in. Baseline correction measurements were used in all spectra. The ligand concentration was 1×10^{-4} M for all of the UV-Vis titrations described. All stock solutions were freshly prepared before measurement.

5.5 Luminescence Measurements

Fluorescence and luminescence measurements were recorded using a Varian Carey Eclipse Fluorimeter with a 1.0 cm path length quartz cell. The solvents used were all of HPLC grade. The data in the luminescence titrations was obtained between 550 and 750 nm. The concentrations of the ligands and complexes examined were the same as those used for the UV-Vis absorption measurements (Section 5.3). The settings for the Fluorimeter for the luminescence measurements carried out in chapters 2, 3, and 4 are shown in Table 5.1.

Table 5.1: *Luminescence settings for Varian Carey Eclipse fluorimeter.*

Mode: Fluorescence	Total Decay: 0.02 ms
Flash: 1	Delay: 0.1 ms
Gate: 10 ms	PMT Voltage: High
Excitation slit width: 10 nm	Emission slit width: 10 nm

5.6 Lifetime Determination for Ln(III) Complexes

Luminescence lifetime measurements of all europium complexes were carried out in Millipore filtered water on a Varian Eclipse Fluorescence spectrophotometer. The settings for the Varian Carey Eclipse Fluorescence spectrophotometer are shown in Table 5.2.

Table 5.2: *General Settings for Lifetime studies.*

Direct Excitation Eu(III): 395 nm	Emission Eu(III): 615 nm
Delay time: 0.1 ms	Gate Time: 0.01 ms
Flash Count: 1	No. Cycles: 1000
Total Decay: 1-10 ms	PMT Voltage: High
Excitations slit width: 10 nm	Emission slit width: 10 nm

Several general procedures were used to synthesise some of the compounds detailed in Chapter 2, and these are outlined in the next section.

5.7 General Synthetic Procedures

Procedure 1: HCl deprotection of BOC-protected amines

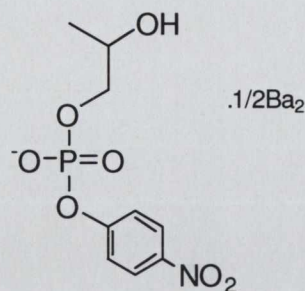
The desired ligand was dissolved in MeOH (2 mL) and a solution of 30% conc. HCl/MeOH, (v/v) was added. The solution was left to stir at RT for 3 hours. To this solution, CH₂Cl₂ (2 x 20 mL) was added, and the solvent was removed under reduced pressure. This was followed by the addition of Et₂O (2 x 20 mL), with removal under reduced pressure. If the compound remained an oil at this stage, it was redissolved in MeOH and EtOH added to precipitate the HCl salt, after which the solvent was removed under reduced pressure and the solid residue dried under vacuum. The product was stored in a dessicator.

Procedure 2: Synthesis of Ln(III) complexes using lanthanide triflate salts

Lanthanide complexes were prepared by heating the ligand at reflux, under an inert atmosphere, in the presence of either 1 or 2 molar equivalents of the appropriate lanthanide triflate salt in freshly distilled MeCN (15 mL) for 16 h. Following reduction of the solvent to *ca.* 2 mL, the complexes were isolated by precipitation from vigorously stirred ether or CH₂Cl₂ or CHCl₃ (150 mL). The ¹H NMR spectra of the lanthanide complexes consisted of very broad signals and therefore were not fully characterised, *i.e.* it was not possible to determine the integration. The same properties prevent ¹³C spectra from being obtained.

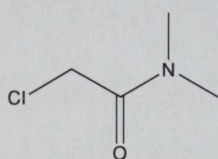
5.8 Synthesis and Characterisation of Compounds Detailed in Chapter 2

Hydroxy propyl nitrophenyl phosphate (HPNP) (1)



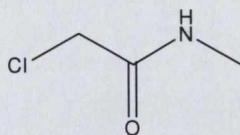
A solution of di-sodium *p*-nitrophenyl phosphate (1.32 g, 5.0 mmol) was dissolved in 10 mL of H₂O and passed through a column of amberlite IR-120 (+) ion exchange resin. It was rinsed through with air and H₂O. The acid effluent was adjusted to pH 8.0 using 10% aqueous ammonia. Propylene oxide (20 mL) was added slowly and the solution was stirred at 35 °C for 48 hours.

The solution was distilled under vacuum to remove any excess propylene oxide, then passed through amberlite IR-120 (+) ion exchange resin. The pH was adjusted to 6.98 (<7.0) with BaOH solution. The solvent was evaporated under reduced pressure and 10 mL of EtOH added. The precipitated starting material was removed by filtration and the solution reduced to approx. 2 mL. This was added dropwise to a stirred solution of 10% EtOH/Acetone and filtered to collect the precipitated product (0.38 g, 22%). m.p. decomposed above 40°C; δ_{H} (400 MHz, D₂O) 8.14 (d, 2H, $J = 9.0$ Hz, ArH), 7.23 (d, 2H, $J = 9.0$ Hz, ArH), 3.81 (m, 2H, OCH₂), 3.67 (m, 1H, CH₃CH), 1.03 (d, 3H, $J = 6.52$ Hz, CH₃); δ_{C} (100 MHz, D₂O) 157.4 (q), 143.4 (q), 125.8 (CH), 120.4 (CH), 71.0 (CH₂), 66.5 (CH), 17.6 (CH₃); δ_{P} (162 MHz, D₂O) -3.7; IR ν_{max} (neat sample)/cm⁻¹ 3367, 1591, 1511, 1491, 1348, 1257, 1092, 1053, 856, 815, 786, 741, 690; UV λ_{max} 290.1.

2-Chloro-*N,N*-dimethyl-acetamide (55)¹⁸⁰

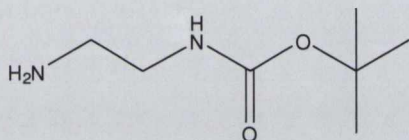
N,N-Dimethyl amine (6.3 g, 140 mmol) and NaOH (5.6 g, 140 mmol) was dissolved in 60 mL of CH₂Cl₂ in a 250 mL round bottom flask. The solution was cooled to -15 °C using an ice/acetone bath and a solution of chloroacetyl chloride (15.82 g, 140 mmol) in 50 mL CH₂Cl₂ was added

dropwise over 2 hours, after which the solution was brought to room temperature and stirred overnight. The organic/aqueous layers were separated and the aqueous layer washed with CH₂Cl₂ (3 × 15 mL). The organic layers were combined and washed with 0.1M HCl (3 × 10 mL) and H₂O (10 mL). The organic layer was dried over K₂CO₃, filtered and the solvent was removed under reduced pressure. The residue was dried under vacuum to give a yellow oil (7.88g, 46% yield). δ_{H} (400 MHz, CDCl₃) 3.97 (s, 2H, CH₂), 2.95 (s, 3H, NCH₃), 2.83 (s, 3H, NCH₃); δ_{C} (100 MHz, CDCl₃) 165.92 (C=O), 40.79 (CH₂), 36.98 (CH₃), 35.32 (CH₃); IR ν_{max} (neat sample)/cm⁻¹ 2940 1646, 1498, 1398, 1256, 1118, 1060, 978, 793.

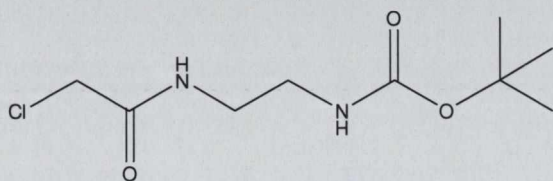
2-Chloro-*N*-methyl-acetamide (56)¹⁸⁰

N-Methyl amine (10 g, 320 mmol) and NaOH (12.8 g, 320 mmol) were dissolved in 60 mL of CH₂Cl₂ in a 250mL round bottom flask.

The solution was cooled to -15 °C using an ice/acetone bath and a solution of chloroacetyl chloride (15.82 g, 140 mmol) in 50 mL CH₂Cl₂ was added dropwise over 2 hours, after which the solution was brought to room temperature and stirred overnight. The product was extracted into CH₂Cl₂, (3 × 15 mL), washed with 0.1M HCl (3 × 10 mL) and H₂O (10 mL), dried over K₂CO₃, filtered, and the solvent was removed under reduced pressure. The resulting residue was dried under vacuum to give a yellow oil (6.22 g, 18% yield) which solidified upon cooling. δ_{H} (400 MHz, CDCl₃) 6.73 (s, br, 1H, NH), 4.05 (s, 2H, CH₂), 2.87 (d, 3H, *J* = 4.5 Hz, NCH₃). δ_{C} (100 MHz, CDCl₃) 166.1 (C=O), 42.2 (CH₂), 26.1 (CH₃); IR ν_{max} (neat sample)/cm⁻¹ 3318, 2960, 1634, 154, 1410, 1263, 1158, 1082, 929, 757.

(2-Aminoethyl)carbamic acid *tert*-butyl ester (77)¹⁵¹

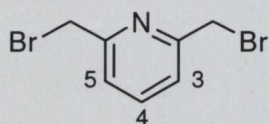
Ethane-1,2-diamine (10g, 16.6 mmol) was dissolved in 1,4-dioxane (20 mL) and cooled to 0 °C in an ice/salt bath after which a solution of di-*tert*-butoxycarbamate (4.52g, 2.07 mmol) in 1,4-dioxane (20 mL) was added dropwise over an hour, with stirring. The solution was brought to room temperature and stirred overnight. Following the removal of the solvent under reduced pressure, the resulting residue was dissolved in H₂O (30 mL) to precipitate the bis-protected product which was then removed by suction filtration. The filtrate was extracted with CH₂Cl₂ (4 × 40 mL), dried over K₂CO₃ and filtered. The solvent was removed under reduced pressure and the resulting residue dried under vacuum to yield a yellow oil that solidified slowly on air exposure, (0.43 g, 65%, yield). m.p. 110 °C (lit. 108-109 °C); δ_{H} (400 MHz, CDCl₃) 5.16 (s, br, 1H, NHBOC), 3.12 (d, 2H, *J* = 5.5 Hz, BOCNHCH₂), 2.73 (t, 2H, *J* = 6.0, NH₂CH₂), 1.99 (s, 2H, NH₂), 1.33 (s, 9H, C(CH₃)₃); δ_{C} (100 MHz, CDCl₃) 155.8 (C=O), 78.6(q), 42.9 (CH₂), 41.4 (CH₂), 27.9 (CH₃).

[2-(2-chloroacetyl-amino)-ethyl]-carbamic acid *tert*-butyl ester (59)¹⁰²

Compound **77** (1.60g, 9.99 mmol) was dissolved in CH₂Cl₂ (20 mL) and NaOH (0.10 g, 24.97 mmol) was dissolved in H₂O (20 mL). The two solutions were combined in a round bottom flask and chloroacetyl chloride (2.26 g, 19.97 mmol) was added to the biphasic solution which was then stirred at room temperature overnight. The organic layer

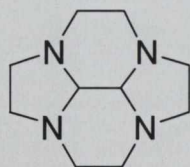
was isolated and was washed with 5% citric acid (4 × 30 mL), and H₂O (15 mL) and was then dried over MgSO₄ and filtered. The solvent was removed under reduced pressure and the residue dried under vacuum to yield a white solid (1.29 g, 55% yield). HRMS: Calculated for C₉H₁₇N₂O₃ClNa: m/z (ES⁺) = 259.0825 [M+Na]⁺, Found: 259.0833 (+2.9 ppm); δ_H (400 MHz, CDCl₃) 7.26 (s, 1H, ClCH₂CONH), 4.93 (s, 1H, NHBOC), 4.05 (s, 2H, ClCH₂), 3.42 (m, 2H, NHCH₂), 3.34 (m, 2H, BOCNHCH₂), 1.47 (s, 9H, OC(CH₃)₃); δ_C (100 MHz, CDCl₃) 166.4 (C=O), 156.4 (C=O), 79.5 (q), 42.0 (CH₂), 40.8 (CH₂), 39.3 (CH₂), 27.8 (CH₃).

2,6-bis(bromomethyl)-pyridine (60)¹⁴⁰



2,6-Bis(hydroxymethyl)-pyridine (0.827 g, 5.95 mmol) was dissolved in DMF (40 mL). To this solution, PBr₃ (3.54 g 13.09 mmol) was added carefully, and the solution was stirred at room temperature overnight. A pink solid formed and was removed by filtration through a celite plug. The filtrate was reduced under reduced pressure to approx. 5 mL. To this solution, H₂O (15 mL) was added and the product was extracted into CH₂Cl₂ (5 × 15 mL), washed with 0.5 M K₂CO₃ (2 × 15 mL), 2M HCl (15 mL) and H₂O (15 mL). The organic layer was dried over K₂CO₃, filtered and the solvent was removed under reduced pressure. This gave a pink solid which was triturated with H₂O to give a pale off-white solid (0.81 g, 51% yield). m.p. 78-82 °C (lit. 74-78 °C);¹⁴⁰ HRMS: Calculated for C₇H₈NBr₂: m/z (ES⁺) = 263.9023 [M+H]⁺, Found: 263.9013; δ_H (400 MHz, CDCl₃) 7.71 (t, 1H, J =8.0 Hz, ArH₄), 7.28 (d, 2H, J = 8.0 Hz, ArH_{3,5}), 4.56 (s, 4H, ArCH₂); δ_C (100 MHz, CDCl₃) 156.3 (q), 137.8 (CH), 122.4 (CH), 33.0 (CH₂); IR ν_{max} (neat sample)/cm⁻¹ 2980, 1590, 1572, 1451, 1204, 1162, 1084, 994, 818, 747.

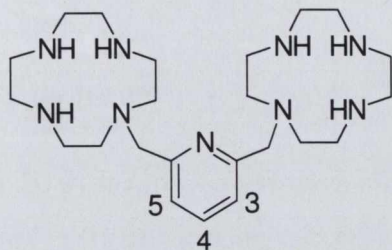
Perhydro-2a,4a,6a,8a-tetraazacyclopenta[f,g]acenaphthylene (61)¹³⁵



Cyclen (3.0 g, 17.44 mmol) was dissolved in MeOH (25 mL) and the solution was cooled to 0 °C in an ice/salt bath. A solution of glyoxal (1.998 mL, 17.44 mmol) in MeOH (25 mL) was added dropwise to the solution, which was then stirred at room temperature for three hours. The resulting clear solution was evaporated under reduced pressure and the residue solidified at room temperature. This solid was dissolved in diethyl ether (20 mL) and filtered through a celite plug to remove any polymers formed. The filtrate was then evaporated under reduced pressure and the residue dried under vacuum to yield a cream solid (3.3 g, 96% yield). m.p. 82 - 85 °C (lit. 94 °C);¹³⁵ HRMS: Calculated for C₁₀H₁₉N₄: m/z (ES⁺) = 195.1610, [M+H]⁺ Found: 195.1602 (-4.0 ppm); δ_H (400 MHz, CDCl₃) 3.07 (s, 2H, NCH), 2.92 (m, br, 8H,

NCH_2), 2.64 (s, br, 4H, NCH_2), 2.51 (s, 4H, NCH_2); δ_{C} (100 MHz, CDCl_3) 77.1 (CH_2), 50.7 (CH), 49.9 (CH); IR ν_{max} (neat sample)/ cm^{-1} 2933, 2885, 2789, 1443, 1389, 1269, 1177, 1060, 839.

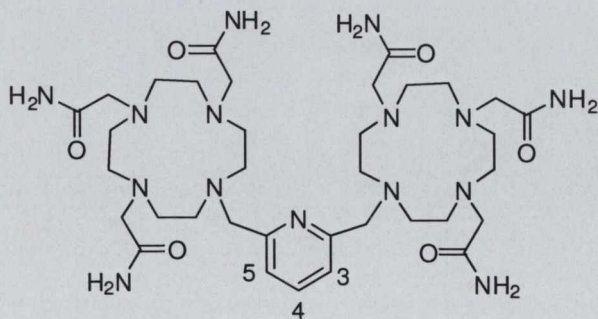
1-[3-(1,4,7,10-Tetraazacyclododecan-1-ylmethyl)pyridinyl]-1,4,7,10-tetraazacyclododecane hexahydrochloride (63)¹³⁵



Compound **61** (1.54 g 7.92 mmol) and **60** (1.0 g, 3.8 mmol) were dissolved in freshly distilled MeCN and stirred at room temperature for 16 hours. A cream solid precipitated from the solution and this was isolated by suction filtration.

The solid was then dissolved in neat hydrazine monohydrate (10 mL) and the solution refluxed overnight. The solvent was removed under reduced pressure and the resulting residue was dissolved in EtOH and then filtered. The filtrate was reduced in volume under reduced pressure and the resulting residue was dissolved in Acetone/EtOH, 2:1. To this solution, conc. HCl (4 mL) was added dropwise and the resulting precipitate was isolated by suction filtration and dried under high vacuum to yield **32** as a yellow solid (2.08 g, 79% yield). m.p. 78–85 °C; HRMS: Calculated for $\text{C}_{23}\text{H}_{46}\text{N}_9$ m/z (ES^+) = 448.3876 [$\text{M}+\text{H}$] $^+$ m/z (ES^+) = Found 448.3889 (+ 2.9 ppm); δ_{H} (400 MHz, D_2O) 7.68 (t, 1H, $J=8.0$ Hz, ArH_4), 7.33 (d, 2H, $J=8.0$ Hz, $\text{ArH}_{3,5}$), 3.61 (s, 4H, ArCH_2), 2.64 (m, 8H, NCH_2), 2.47 (m, br, 24H, NCH_2); δ_{C} (100 MHz, D_2O) 151.1 (q), 147.0 (CH), 127.7 (CH), 53.7 (CH_2), 47.5 (CH_2), 43.1 (CH_2), 41.7 (CH_2), 40.7 (CH_2); IR ν_{max} (neat sample)/ cm^{-1} 3400, 2825, 2562, 1436, 1357, 1290, 1254, 1137, 1082, 1039, 969, 762.

1,3-Bis[1-(4,7,10-tris(carbamoylmethyl)-1,4,7,10-tetraazacyclododecan-1-ylmethyl]-4-pyridinyl (39)



Compound **63** (0.4 g, 0.57 mmol) and *N*-bromoacetamide (0.51 g, 3.678 mmol) were dissolved in EtOH (30 mL, HPLC grade) and Et_3N (1.5 mL, 9.69 mmol) was added. The temperature was raised to 65 °C over a day then refluxed for five days, under argon. After cooling to room

temperature, the solvent was reduced to 5 mL and the product precipitated from solution by the addition of *i*-PrOH. This was filtered through a celite plug and the solid collected with MeOH and H_2O . This was repeated until the addition of *i*-Pr yielded no precipitate (0.29 g, 65% yield). m.p. 72 °C; HRMS: Calculated for $\text{C}_{35}\text{H}_{64}\text{N}_{15}\text{O}_6$ m/z (ES^+) = 790.5164

$[M+H]^+$, found: 790.5151 (-1.6 ppm); δ_H (400 MHz, $CDCl_3$) 7.81 (t, 1H, $J=7.5$ Hz, ArH_4), 7.52 (d, 2H, $J = 7.0$ Hz, $ArH_{3,5}$), 4.09 (s, 4H, $ArCH_2$), 3.20 (s, 12H, NCH_2CO), 3.04 (s, 8H, NCH_2), 2.95 (s, 8H, NCH_2), 2.69 (m, 16H, NCH_2); δ_C (100 MHz, $CDCl_3$) 173.7 (C=O), 165.6 (C=O), 139.0 (q), 125.3 (CH), 124.3 (CH), 57.8 (CH_2), 54.9 (CH_2), 53.9 (CH_2), 50.2 (CH_2), 49.5 (CH_2), 49.1 (CH_2), 46.2 (CH_2); IR ν_{max} (neat sample)/ cm^{-1} 3188, 2813, 1403, 1288, 1156, 1102, 1084, 995, 971.

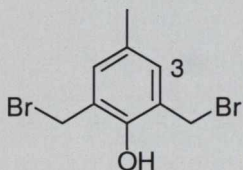
1,3-Bis[1-(4,7,10-tris(carbamoylmethyl)-1,4,7,10-tetraazacyclododecan-1-ylmethyl]-4-pyridinyl Eu.6CF₃SO₃ (39.Eu₂)

Complex **39.Eu₂** was synthesised according to **General Procedure 2** from **39** (0.05 g, 0.06 mmol) and $Eu(CF_3SO_3)_3$ (0.08g, 0.13 mmol). A white solid was obtained (0.10 g, 76% yield); Calculated for $C_{35}H_{63}Eu_2N_{15}O_6.6CF_3SO_3.3CHCl_3$: C 22.52, H 2.84, N 8.95% Found: C 22.10, H 2.95, N 8.99%; m.p. dec. 230 °C; HRMS: Calculated for $C_{37}H_{59}N_{15}O_{12}F_6S_2Eu_2$: m/z (ES^+) = 1389.2238 $[M+ 2(CF_3SO_3)]^{2+}$, found: 1389.2256 (+1.3 ppm); δ_H (400 MHz, D_2O) 25.07, 18.51, 13.13, 12.90, 10.10, 7.22, 5.62, 3.22, 1.18, -3.04, -5.34, -7.87, -9.55, -12.70, -17.99; IR ν_{max} (neat sample)/ cm^{-1} 3355, 1664, 1599, 1460, 1221, 1460, 121, 1161, 1079, 955, 829, 761.

1,3-Bis[1-(4,7,10-tris(carbamoylmethyl)-1,4,7,10-tetraazacyclododecan-1-ylmethyl]-4-pyridinyl.La.6CF₃SO₃ (39.La₂)

Complex **39.La₂** was synthesised according to **General Procedure 2** from **39** (0.05 g, 0.063 mmol) and $La(CF_3SO_3)_3$ (0.078g, 0.13 mmol). A white solid was obtained (0.10 g, 73% yield); Calculated for $C_{35}H_{63}La_2N_{15}O_6.6CF_3SO_3.4H_2O.2CH_2Cl_2$: C 23.43, H 3.43, N 9.53%, Found: C 23.69, H 3.22, N 9.82%; m.p. decomposed above 240 °C; HRMS: Calculated for $C_{38}H_{62}N_{15}O_{15}F_9S_3La_2$: m/z (ES^+) = 1513.1695 $[M+ 3(CF_3SO_3)]^{2+}$, found: 1513.1695 (+0.4 ppm); δ_H (400 MHz, D_2O) 7.79, 7.73, 6.18, 4.69, 3.98, 3.88, 3.86, 3.68, 3.44, 3.43, 3.22, 2.64, 2.49, 2.09, 1.18, 1.05; IR ν_{max} (neat sample)/ cm^{-1} 3354, 1663, 1598, 1458, 1221, 1162, 1080, 1025, 912, 882, 823, 761

2,6-Bis(bromomethyl)-4-methylphenol (64)¹⁴⁸

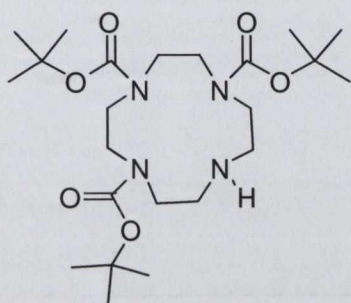


2,6-Bis(hydroxymethyl)-4-methylphenol (2.0 g, 11.88 mmol) was added to a 100 mL round bottom flask and 30 mL of glacial acetic acid was added. To this solution, HBr (3.36 mL, 37.82 mmol) was added and the resulting mixture was stirred at room temperature.

After four hours the solution was reduced to approx. 5 mL under reduced pressure and the resulting residue was treated with H_2O (20 mL). This gave a cream precipitate which was collected by suction filtration and then dissolved in a minimum amount of CH_2Cl_2 and

washed with water. The organic layer was dried over MgSO_4 , and the solvent was removed under reduced pressure and the resulting residue was dried under vacuum. The product was recrystallised from hexane to yield a white solid (1.94 g, 56% yield). m.p. 91-93 °C (lit. 85-93 °C); $^{148} \delta_{\text{H}}$ (400 MHz, CDCl_3) 7.28 (s, 2H, ArH_3), 5.45 (s, 1H, OH), 4.56 (s, 4H, ArCH_2), 2.28 (s, 3H, ArCH_3); δ_{C} (100 MHz, CDCl_3) 150.6 (q), 131.4 (q), 130.2 (q), 124.6 (CH), 29.1 (CH_2), 19.9 (CH_3); IR ν_{max} (neat sample)/ cm^{-1} 3519, 1603, 1484, 1324, 1258, 1209, 1114, 966, 878, 759.

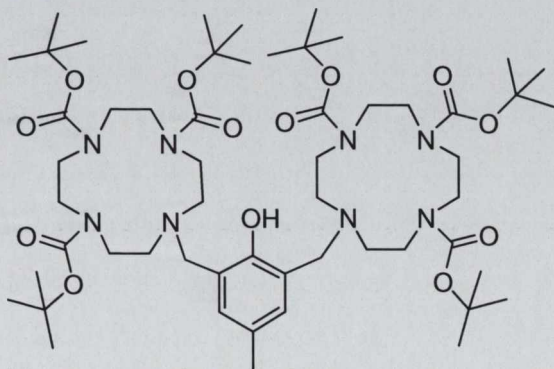
1,4,7-Tris-*tert*-butoxycarbonyl-1,4,7,10-tetraazacyclododecane (66)⁶⁰



Cyclen (3.0 g, 14.4 mmol) was dissolved in CH_2Cl_2 (15 mL) and Et_3N (7.27 mL, 52.32 mmol) was added. The resulting solution was cooled to 0 °C using an ice/salt water bath. Di-*tert*-butoxycabamate (11.40 g, 52.32 mmol) was dissolved in 15 mL of CH_2Cl_2 and added dropwise to this solution over 1 hour with stirring, followed by stirring at room temperature

overnight. The solvent was removed under reduced pressure and the resulting residue was purified using silica column chromatography (Eluent: $\text{EtOAc}:\text{Hexane}$ 5:1). After removal of the solvent under reduced pressure, a white solid was isolated (5.15 g, 62% yield). M.p. 70-72 °C (lit. M.p. 72-73.5 °C);⁶⁰ HRMS: Calculated for $\text{C}_{23}\text{H}_{45}\text{N}_4\text{O}_6$: m/z (ES^+) = 473.3339 $[\text{M}+\text{H}]^+$, Found: 473.3319 (-4.2 ppm); δ_{H} (400 MHz, CDCl_3) 3.61 (s, br, 4H, NCH_2), 3.26 (m, br, 8H, NCH_2), 2.83 (s, br, 4H, NCH_2), 1.45 (s, 9H, $\text{C}(\text{CH}_3)_3$), 1.42 (s, 18H, $\text{C}(\text{CH}_3)_3$); δ_{C} (100 MHz, CDCl_3) 155.2 (C=O), 154.9 (C=O), 78.9 (q), 78.7 (q), 50.7 (CH_2), 49.0 (CH_2), 45.4 (CH_2), 44.7 (CH_2), 28.2 (CH_3), 28.0 (CH_3); IR ν_{max} (neat sample)/ cm^{-1} 2974, 1674, 1459, 1409, 1363, 1245, 1149, 1088, 1046, 982, 942, 858, 772.

2,6-Bis[4,7,10-tris(*tert*-butyloxycarbonyl)-1,4,7,10-tetraazacyclododecylmethyl]-4-methylphenol (67)¹⁸¹

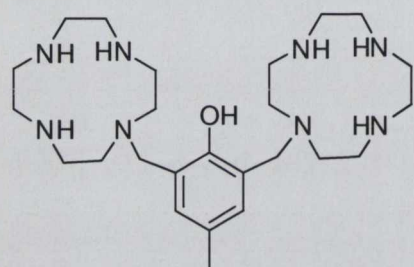


Compound **66** (1.5 g, 3.17 mmol) was dissolved in MeCN (15 mL) and anhydrous Na_2CO_3 (3 g, 28.3 mmol) was added. Compound **64** (0.44 g, 1.51 mmol), in MeCN (15 mL) was added dropwise to this solution with stirring and the resulting mixture was refluxed overnight. After cooling to room

temperature the solution was filtered through a celite plug and the filtrate evaporated to dryness under reduced pressure. The resulting residue was purified using silica column

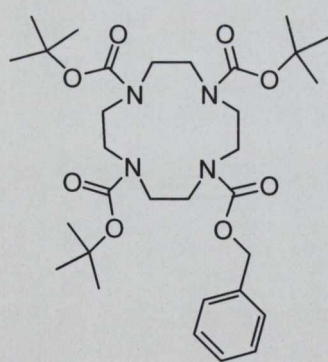
chromatography (Eluent: EtOAc:Hexane 5:1). The solvent was removed under reduced pressure to give a white solid (1.2 g, 87% yield). m.p. 121 °C; HRMS: Calculated for $C_{55}H_{97}N_8O_{13}$: m/z (ES^+) = 1077.7175 $[M+H]^+$, Found: 1077.7129 (-4.3 ppm); δ_H (400 MHz, $CDCl_3$) 6.80 (2H, s, ArH), 3.78 (s, 4H, ArCH₂), 3.56 (s, 8H, NCH₂), 3.40 (s, 16H, NCH₂), 2.72 (s, 8H, NCH₂), 2.21 (s, 3H, CH₃), 1.49 (s, 18H, C(CH₃)₃), 1.45 (s, 36H, C(CH₃)₃); δ_C (100 MHz, $CDCl_3$) 156.9 (C=O), 155.0 (C=O), 131.4 (q), 130.3 (q), 130.1 (q), 128.0 (CH), 81.0 (q), 80.4 (q), 62.0 (CH₂), 53.7 (CH₂), 49.9 (CH₂), 48.0 (CH₂), 44.8 (CH₂), 29.5 (CH₃), 29.2 (CH₃), 21.1 (CH₃); IR ν_{max} (neat sample)/cm⁻¹ 2974, 1686, 1458, 1412, 1364, 1246, 1151, 974, 860, 772.

2,6-Bis(1,4,7,10-tetraazacyclododecylmethyl)-4-methylphenol hexahydrochloride (68)¹⁸¹



Compound **68** was synthesised following **General Procedure 1** from compound **67** (0.350 g, 0.33 mmol). A white solid was isolated, (0.16 g, 72% yield). HRMS: Calculated for $C_{25}H_{49}N_8O$: m/z (ES^+) = 477.4029 $[M+H]^+$, found: 477.4012 (-3.6 ppm); δ_H (400 MHz, D_2O) 7.09 (s, 2H, ArH), 3.80 (s, 4H, ArCH₂), 3.08 (s, 8H, NCH₂), 3.00 (s, 8H, NCH₂), 2.89 (m, br, 16H, NCH₂), 2.15 (s, 3H, ArCH₃); δ_C (100 MHz, D_2O) 133.7 (q), 133.6 (q), 132.2 (q), 123.3 (CH), 52.8 (CH₂), 48.8 (CH₂), 43.6 (CH₂), 42.3 (CH₂), 42.2 (CH₂), 19.5 (CH₃); IR ν_{max} (neat sample)/cm⁻¹ 2619, 1576, 1493, 1428, 1220, 1004, 955, 926, 831, 765.

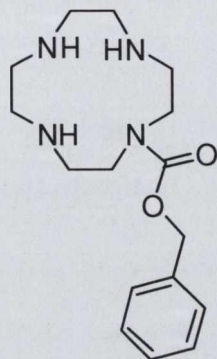
1-Benzyloxycarbonyl-4-7-10-tris-*tert*-butoxycarbonyl-1,4,7,10-tetrazacyclododecane (71)⁶⁰



Compound **66** (2.55 g, 5.39 mmol) and di-*isopropylethylamine* (1.13 mL, 6.47 mmol) were dissolved in $CHCl_3$. The solution was cooled to 0 °C in an ice/salt bath and benzyl chlorofomate (0.92 mL, 6.48 mmol) was added. The resulting solution was stirred overnight at room temperature after which the solvent was removed under reduced pressure and the residue purified using flash silica column chromatography (Eluent: EtOAc:Hexane 4:1). The compound **71** was isolated as a pale yellow amorphous solid (2.72 g, 83% yield), following the removal of the solvent under reduced pressure. m.p. 75-78 °C (lit. 76-77 °C);⁶⁰ HRMS: Calculated for $C_{31}H_{50}N_4O_8Na$: m/z (ES^+) = 629.3526 $[M+Na]$, found: 629.3535 (+1.4 ppm); δ_H (400 MHz, $CDCl_3$) 7.38 (s, br, 5H, ArH), 5.14 (s, br, 2H, OCH₂Ar), 3.46 (s, br, 16H, NCH₂), 1.47 (s, br, 27H, C(CH₃)₃); δ_C (100 MHz, $CDCl_3$) 155.8 (C=O), 155.6 (C=O), 155.5 (C=O), 135.8 (q), 127.7 (CH), 127.6 (CH), 127.4

(CH), 79.0 (q), 78.9 (q), 66.4 (CH₂), 49.7 (CH₂), 49.6 (CH₂), 49.5 (CH₂), 49.3 (CH₂), 27.7 (CH₃), 27.6 (CH₃); IR ν_{max} (neat sample)/cm⁻¹ 2974, 2932, 1686, 1465, 1409, 1364, 1244, 1159, 1100, 1051, 991, 946, 858, 774, 751, 697.

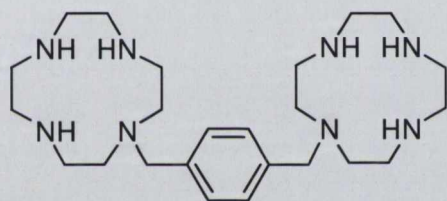
1-benzoyloxycarbonyl-1,4,7,10-tetraazacyclododecane trihydrochloride (**72**)⁶⁰



Compound **72** was synthesised according to **General Procedure 1** from compound **71** (2.7 g, 4.5 mmol). A white solid was isolated (1.14 g, 61% yield). m.p. 192 °C (lit. 192 °C);⁶⁰ δ_{H} (400 MHz, D₂O) 7.29 (m, br, 5H, ArH), 5.07 (s, 2H, OCH₂Ar), 3.58 (s, br, 4H, NCH₂), 3.09 (m, br, 12H, NCH₂); δ_{C} (100 MHz, D₂O) 157.9 (C=O), 135.1 (q), 128.3 (CH), 128.2 (CH), 127.9 (CH), 68.1 (CH₂), 45.9 (CH₂), 44.6 (CH₂), 43.7 (CH₂), 42.2 (CH₂); IR ν_{max} (neat sample)/cm⁻¹ 2493, 1705, 1574, 1490,

1425, 1385, 1339, 1259, 1160, 1060, 1025, 960, 936, 765, 697.

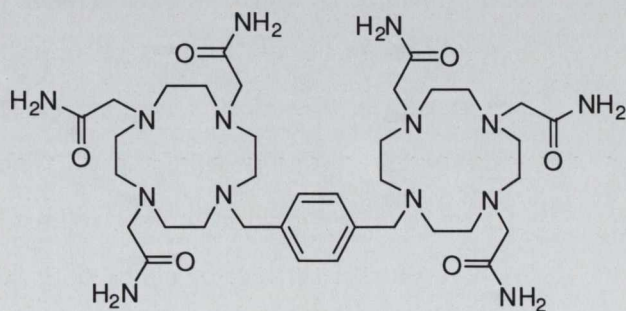
1-[4-(1,4,7,10-tetraazacyclododecan-1-ylmethyl)benzyl]-1,4,7,10-tetraazacyclododecane hexahydrochloride (**74**)¹³⁵



Compound **61** (1.0 g, 0.15 mmol) and dibromo-*p*-xylene (1.0 g, 3.8 mmol) were dissolved in freshly distilled MeCN (25 mL) and stirred at room temperature for 16 hours. The solution was suction

filtered to isolate a white precipitate, which was then dissolved in hydrazine monohydrate (10 mL). The solution was refluxed overnight at 120 °C. A solid precipitated out of solution and was collected by suction filtration to yield a yellow solid (1.0 g, 91% yield). HRMS: Calculated for C₂₄H₄₇N₈: m/z (ES⁺) = 447.3924 [M+H]⁺ Found: 447.3912 (-2.6 ppm); δ_{H} (400 MHz, D₂O) 7.23 (s, 4H, ArH), 3.48 (s, 4H, ArCH₂), 2.61 (m, 8H, NCH₂), 2.52 (m, 8H, NCH₂), 2.47 (m, 16H, NCH₂); δ_{C} (100 MHz, D₂O) 137.6 (q), 128.9 (CH), 57.2 (CH₂), 50.6 (CH₂), 49.7 (CH₂), 44.6 (CH₂), 43.7 (CH₂).

1,3-Bis[1-(4,7,10-tris(carbamoylmethyl)-1,4,7,10-tetraazacyclododecan-1-ylmethyl)-4-benzyl (**41**)



Compound **74** (0.2 g, 0.45 mmol) and 2-bromoacetamide (0.41 g, 2.95 mmol) were dissolved in MeOH (20 mL, HPLC grade). Et₃N (0.41 mL, 2.95 mmol) was added and the solution refluxed under argon for 48 hours. The resulting white precipitate was

filtered and washed with cold MeOH to yield a white solid (0.18 g, 49% yield). M.p. dec. 180 °C; Calculated for C₃₆H₆₄N₁₄O₆·H₂O: C 53.58, H 8.24, N 24.30%, Found: C 53.66, H

7.97, N 23.58%; HRMS: Calculated for $C_{36}H_{64}N_{14}NaO_6$: m/z (ES^+) = 811.5031, $[M+Na]^+$ Found: 811.5019 (-1.5 ppm); δ_H (400 MHz, $(CD_3)_2SO$) 7.61 (s, br, 4H, NH_2), 7.51 (s, br, 2H, NH_2), 7.22 (s, 4H, ArH), 6.99 (s, br, 4H, NH_2), 6.94 (s, br, 2H, NH_2), 3.47 (s, 4H, $ArCH_2$), 2.96 (s, 4H, NCH_2CO), 2.82 (s, 8H, NCH_2CO), 2.59 (s, br, 24H, NCH_2), 2.50 (s, 8H NCH_2); δ_C (100 MHz, $(CD_3)_2SO$) 173.3 (C=O), 173.0 (C=O), 137.3 (q), 129.1 (CH), 59.4 (CH_2), 58.5 (CH_2), 58.0 (CH_2), 53.9 (CH_2), 53.5 (CH_2), 53.4 (CH_2), 52.6 (CH_2); IR ν_{max} (neat sample)/ cm^{-1} 3300, 2945, 2808, 1663, 1543, 1295, 1083, 799.

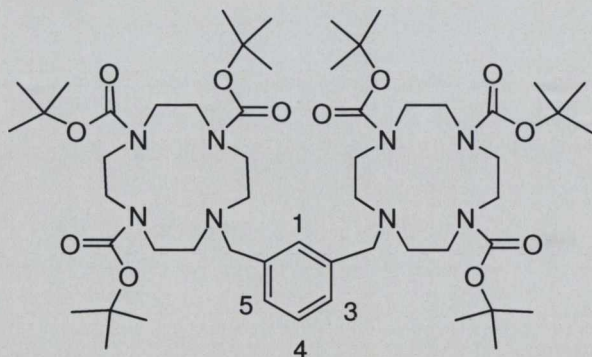
1,3-Bis[1-(4,7,10-tris(carbamoylmethyl)-1,4,7,10-tetraazacyclododecan-1-ylmethyl]-4-benzyl.Eu.6CF₃SO₃.4H₂O (41.Eu₂)

Following **General Procedure 2, 41** (0.05 g, 0.06 mmol) and $Eu(CF_3SO_3)_3$ (0.08 g, 0.13 mmol) were reacted to yield a white solid (0.07 g, 62%, yield). m.p. 129 °C; HRMS: Calculated for $C_{38}H_{64}N_{14}O_{12}F_6S_2Eu_2$: m/z (ES^+) = 1392.2599, $[M+2CF_3SO_3]^{3+}$ Found: 1392.2550 (-3.5 ppm); δ_H (400 MHz, D_2O) 24.78, 18.26, 11.93, 9.70, 7.92, 3.42, 3.26, 1.04, -1.25, -6.18, -8.81, -9.68, -13.37, -18.04, -19.89; IR ν_{max} (neat sample)/ cm^{-1} 3195, 2981, 1664, 1590, 1460, 1222, 1157, 1024, 832

1,3-Bis[1-(4,7,10-tris(carbamoylmethyl)-1,4,7,10-tetraazacyclododecan-1-ylmethyl]-4-benzyl.La.6CF₃SO₃.6H₂O (41.La₂)

Following **General Procedure 2, 41** (0.03 g, 0.04 mmol) and $La(CF_3SO_3)_3$ (0.05 g 0.09 mmol) were reacted to yield a white solid, (0.03 g, 34%). m.p. dec. 130 °C; HRMS: Calculated for $C_{38}H_{61}N_{14}O_{12}F_6S_2La_2$: m/z (MALDI) = 1361.2066, $[M+2CF_3SO_3-3H]^+$ Found: 1361.2115 (+3.6 ppm); δ_H (400 MHz, D_2O) 7.19, 3.89, 3.47, 2.88, 2.48, 2.19, 1.04; IR ν_{max} (neat sample)/ cm^{-1} 3166, 2981, 1655, 1595, 1459, 1395, 1240, 1156, 1079, 1027, 884.

2,6-Bis[4,7,10-tris(*tert*-butoxycarbonyl)-1,4,7,10-tetraazacyclododecylmethyl]-4-benzyl (76)¹³⁶

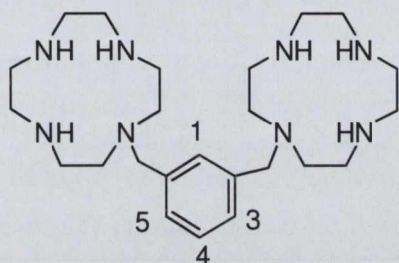


Compound **66** (0.75 g, 0.159 mmol) and dibromo-*m*-xylene (0.19 g, 0.72 mmol) were dissolved in MeCN (freshly distilled). Na_2CO_3 (3.18 g, 0.34 mmol) was added and the solution was stirred at reflux under argon for three days. The solvent was removed under reduced

pressure and the residue was dissolved in CH_2Cl_2 and filtered through a celite plug to remove any inorganic salts. The solvent was removed under reduced pressure and the residue purified by silica column chromatography (Hexane:EtOAc 3:2-2:3) to give a pale

yellow solid (0.20 g, 27%). HRMS: Calculated for $C_{54}H_{95}N_8O_{12}$: m/z (ES^+) = 1047.7069 $[M+H]^+$, Found: 1047.7109 (+3.8 ppm); δ_H (400 MHz, $CDCl_3$) 7.24 (1H, d, J = 7.52 Hz, ArH_4), 7.16 (2H, s, $ArH_{3,5}$), 7.02 (1H, s, ArH_1), 3.72 (s, 4H, $ArCH_2$), 3.56 (s, 8H, NCH_2), 3.23 (m, br, 16H, NCH_2), 2.63 (s, br, 8H, NCH_2), 1.45 (m, br, 54H, $C(CH_3)_3$); δ_C (100 MHz, $CDCl_3$) 156.0 (C=O), 155.6 (C=O), 136.3 (q), 130.0 (CH), 129.2 (CH), 128.1 (CH), 79.3 (q), 79.2 (q), 58.2 (CH_2), 56.8 (CH_2), 54.4 (CH_2), 49.7 (CH_2), 48.0 (CH_2), 28.6 (CH_3), 28.3 (CH_3).

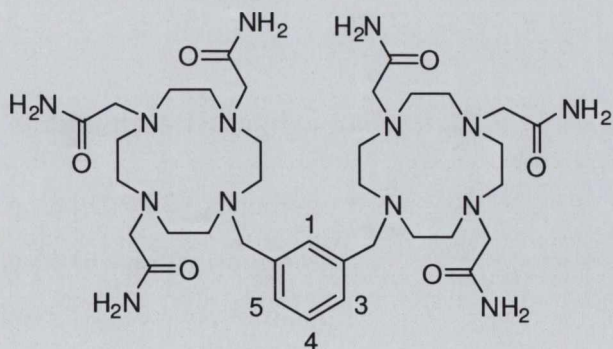
1-[-3-(1,4,7,10-tetraazacyclododecan-1-ylmethyl)benzyl]-1,4,7,10-tetraazacyclododecane hexahydrochloride (75)¹³⁶



Compound **75** was synthesised according to **General Procedure 1** from **76** (0.10 g, 0.10 mmol) to yield a yellow solid, (0.06 g, 87%). HRMS: Calculated for $C_{24}H_{47}N_8$: m/z (ES^+) = 447.3924 $[M+H]^+$, Found: 447.3920 (-0.8 ppm); δ_H (400 MHz, D_2O) 7.50 (1H, t, J = 5.1 Hz, ArH_4), 7.39 (2H, dd, J = 2.0 Hz, $ArH_{3,5}$),

7.32 (1H, s, ArH_1), 3.90 (4H, s, $ArCH_2$), 3.21 (8H, s, NCH_2), 3.16 (8H, m, NCH_2), 3.01 (8H, s, NCH_2), 2.92 (8H, m, NCH_2); δ_C (100 MHz, D_2O) 135.0 (q), 132.0 (CH), 129.7 (CH), 129.4 (CH), 56.3 (CH_2), 47.6 (CH_2), 44.1 (CH_2), 41.9 (CH_2), 41.7 (CH_2); IR ν_{max} (neat sample)/ cm^{-1} 3254, 2816, 1647, 1560, 1454, 1399, 1348, 1329, 1259, 1107, 1061, 970, 890.

1,3-Bis[1-(4,7,10-tris(carbamoylmethyl)-1,4,7,10-tetraazacyclododecan-1-ylmethyl)-3-benzyl (43)



Compound **75** (0.20 g, 0.45 mmol) was dissolved in EtOH (HPLC grade), *N*-bromoacetamide (0.40 g, 2.91 mmol) and Et_3N (0.29 g, 2.91 mmol) were added to the solution. The solution was made up to 25 mL with EtOH and refluxed for three days under argon. The solution was cooled to room temperature and the solvent was

removed under reduced pressure. The residue was dissolved in MeOH (1 mL) and added dropwise to vigorously stirred toluene (150 mL). A yellow oily precipitate was isolated, by decanting the toluene, and the residue was dissolved in MeOH (1 mL) and added dropwise to vigorously stirred *i*-PrOH (150 mL). The resulting white precipitate was collected by suction filtration and dried under vacuum to yield a white solid (0.11 g, 30%). m.p. dec.

122 °C; HRMS: Calculated for $C_{36}H_{64}N_{14}NaO_6$: m/z (ES^+) = 811.5031, $[M+Na]^+$ Found: 811.5012 (-2.3 ppm); δ_H (600 MHz, D_2O) 8.02 (1H, s, ArH₁), 7.93 (2H, m, ArH_{3,5}), 7.88 (1H, d, ArH₄), 4.22 (4H, s, ArCH₂), 3.89 (6H, m, br, NCH₂), 3.67 (12H, s, br, NCH₂), 3.57 (26H, s, br, NCH₂); δ_C (150 MHz, D_2O) 176.7 (C=O), 174.4 (C=O), 134.1 (q), 132.4 (CH), 130.1 (CH), 125.5 (CH), 59.4 (CH₂), 58.5 (CH₂), 58.0 (CH₂), 53.9 (CH₂), 53.5 (CH₂), 53.4 (CH₂), 52.6 (CH₂); IR ν_{max} (neat sample)/ cm^{-1} 3336, 3178, 1665, 1407, 1327, 1155, 1085, 968, 887.

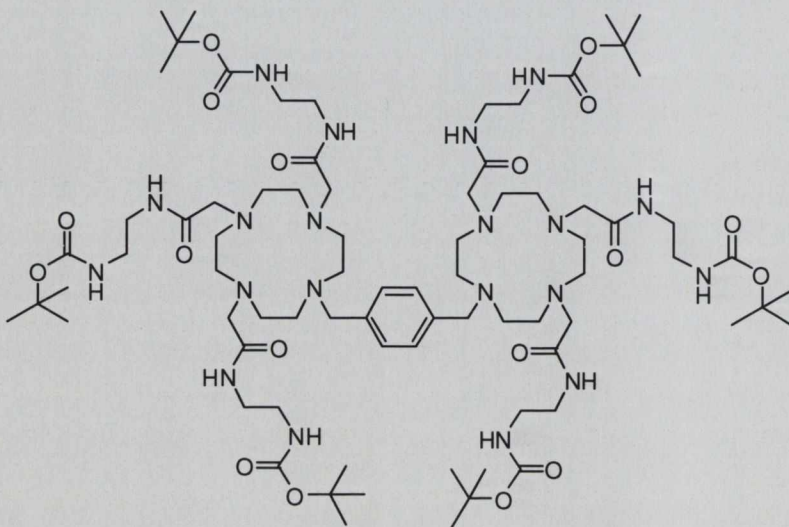
1,3-Bis[1-(4,7,10-tris(carbamoylmethyl)-1,4,7,10-tetraazacyclododecan-1-ylmethyl]-3-benzyl.Eu.6CF₃SO₃.4H₂O (43.Eu₂)

Complex **43.Eu₂** was synthesised according to **General Procedure 2** from **43** (0.10 g, 0.13 mmol) and Eu(CF₃SO₃)₃ (0.16 g, 0.27 mmol) to give a white solid, (0.75 g, 29%). m.p. dec. 224 °C; Calculated for $C_{36}H_{64}Eu_2N_{14}O_6.6CF_3SO_3.6H_2O$: C 24.07, H 3.66, N 9.36%, Found: C 23.73, H 3.53, N 9.55%; HRMS: Calculated for $C_{38}H_{61}N_{14}O_{12}F_6S_2Eu_2$: m/z (MALDI) = 1385.2336, $[M+2CF_3SO_3-3H]^+$ Found: 1385.2323 (-0.9 ppm); δ_H (400 MHz, D_2O) 20.96, 17.75, 7.83, 7.51, 5.52, 3.61, 1.35, 0.79, 0.18, -5.74, -9.99, -12.35, -17.91; IR ν_{max} (neat sample)/ cm^{-1} 3323, 3171, 1655, 1595, 1457, 1423, 1240, 1224, 1160, 1079, 1027, 916, 829.

1,3-Bis[1-(4,7,10-tris(carbamoylmethyl)-1,4,7,10-tetraazacyclododecan-1-ylmethyl]-3-benzyl.La.6CF₃SO₃.4H₂O (43.La₂)

Complex **43.La₂** was synthesised according to **General Procedure 2** from **43** (0.02 g, 0.02 mmol) and Eu(CF₃SO₃)₃ (0.02 g, 0.04 mmol) to give a yellow solid, (0.03 g, 78%). m.p. dec. 248 °C; HRMS: Calculated for $C_{39}H_{62}N_{14}O_{15}F_9S_3La_2$: m/z (MALDI) = 1511.1665, $[M+3CF_3SO_3-2H]^+$ Found: 1511.1722 (3.8 ppm); δ_H (400 MHz, D_2O) 7.63, 7.42, 7.28, 6.18, 4.19, 3.91, 3.71, 3.65, 3.22, 3.14, 2.65, 2.43, 2.10; IR ν_{max} (neat sample)/ cm^{-1} 3337, 3195, 1656, 1598, 1458, 1236, 1222, 1157, 1082, 1025, 912, 883.

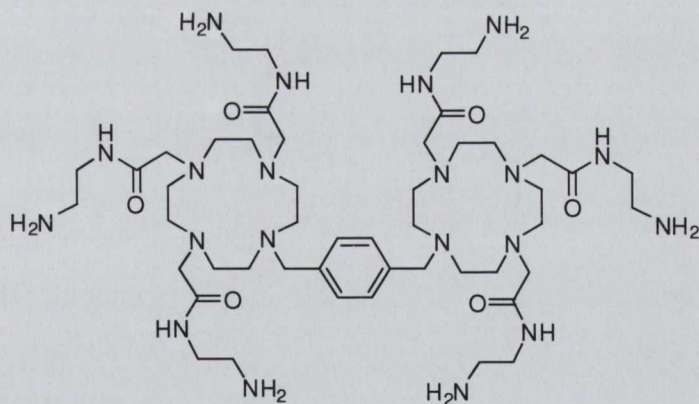
1,3-Bis{1-[4,7,10-tris(2-tert-butoxycarbonylamino-ethylcarbamoylmethyl)acetamide]-1,4,7,10-tetraazacyclododecan-1-ylmethyl}-4-benzyl (78)



Compounds **74** (0.10 g, 0.23 mmol) and **59** (0.35 g, 1.5 mmol), were dissolved in MeOH (30 mL, HPLC grade) and KI (0.20 g, 1.50 mmol) and Cs₂CO₃ (0.25 g, 1.50 mmol) were added. The resulting mixture was

refluxed at 65 °C, under argon, for 14 days. The solution was filtered through a celite plug to remove inorganic solids. The filtrate was removed under reduced pressure and the residue was dissolved in CH₂Cl₂ and then filtered to remove any insoluble impurities. The solution was washed with KCl (4 × 30 mL) and H₂O (15 mL) and the organic layer was dried over K₂CO₃ and filtered. The solvent was removed under reduced pressure and the residue dried under high vacuum to give an orange hygroscopic solid (0.28 g, 74% yield). Calculated for: HRMS: Calculated for C₇₈H₁₄₄N₂₀O₁₈: m/z (ES⁺) = 1649.0967 [M+2H]⁺, Found: 1649.0894 (-4.5 ppm); δ_H (400 MHz, CDCl₃) 7.98 (s, 4H, NH), 7.46 (s, 2H, NH), 7.16 (s, 4H, ArH), 5.57 (m, 6H, NH), 3.54 (s, 4H, ArCH₂), 3.34 (s, 12H, NCH₂), 3.22 (s, 12H, NCH₂), 3.12 (s, 4H, NCH₂CO), 3.01 (s, 8H, NCH₂CO), 2.60 (m, 32H, NCH₂), 1.40 (s, 54H, C(CH₃)₃); δ_C (100 MHz, CDCl₃) 171.9 (C=O), 171.5 (C=O), 159.7 (C=O), 156.3 (C=O), 134.5 (q), 129.1 (CH), 79.2 (q), 79.1 (q), 59.3 (CH₂), 58.9 (CH₂), 58.5 (CH₂), 53.8 (CH₂), 53.5 (CH₂), 53.3 (CH₂), 52.3 (CH₂), 40.8 (CH₂), 40.3 (CH₂), 39.6 (CH₂), 39.2 (CH₂), 28.4 (CH₃), 28.3 (CH₃); IR ν_{max} (neat sample)/cm⁻¹ 3333, 2976, 2935, 2821, 2575, 2365, 1695, 1529, 1453, 1391, 1366, 1274, 1170, 1116, 1001, 863, 758.

1,3-Bis{1-[4,7,10-tris(2-amino-ethylcarbamoylmethyl)acetamide]-1,4,7,10-tetraaza cyclododecan-1-ylmethyl}-4-benzyl (42)



Compound **42** was synthesised according to **General Procedure 1**, from **78** (0.25 g, 0.15 mmol) to give a white solid (0.185 g, 96% yield). m.p. 127-130 °C; HRMS: Calculated for C₄₈H₉₅N₂₀O₆: m/z (ES⁺) = 1047.7743 [M+H]⁺, Found: 1047.7704 (-3.8 ppm); δ_H

(400 MHz, D₂O) 7.53 (s, 4H, ArH), 4.04 (s, 4H, ArCH₂), 3.74 (s, 4H, NCH₂CO), 3.64 (s, 4H, NCH₂CO), 3.44 (m, 24H, NCH₂), 3.24 (s, 4H, NCH₂CO), 3.07 (m, 32H, NCH₂); δ_C (100 MHz, D₂O) 173.2 (C=O), 172.4 (C=O), 166.8 (q), 132.9 (CH), 56.5 (CH₂), 55.0 (CH₂), 54.6 (CH₂), 51.8 (CH₂), 48.8 (CH₂), 39.2 (CH₂), 38.6 (CH₂), 37.0 (CH₂), 36.7 (CH₂), 36.4 (CH₂), 30.2 (CH₂); IR ν_{max} (neat sample)/cm⁻¹ 3362, 2967, 1737, 1658, 1553, 1457, 1390, 1267, 1168, 1088, 925, 857.

1,3-Bis{1-[4,7,10-tris(2-amino-ethylcarbamoylmethyl)acetamide]-1,4,7,10-tetraaza cyclododecan-1-ylmethyl}.Eu.6CF₃SO₃.4H₂O (42.Eu₂)

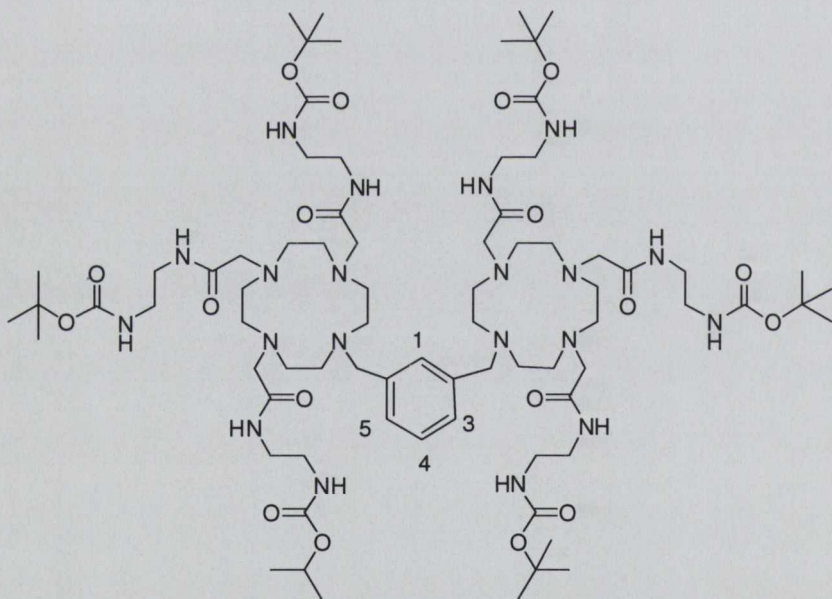
Complex **42.Eu₂** was prepared according to **General Procedure 2** from **42** (0.150 g, 0.12 mmol) and Eu(CF₃SO₃)₃ (0.15 g, 0.25 mmol) to yield an orange solid (0.21 g, 69% yield).

m.p. dec. 85 °C; m/z (ES^+) = 1348.61 $[M+H]^+$, δ_H (400 MHz, D_2O) 16.77, 15.56, 13.27, 11.86, 9.86, 7.97, 7.51, 6.18, 4.39, 4.24, 4.18, 3.46, 3.22, 3.06, 2.24, 1.94, 1.05, -1.18, -4.26, -6.2, -7.86, -10.16, -12.62, -17.42; MS (ES^+) m/z 750.2345 $[M+(CF_3SO_3)]^{2+}$; IR ν_{max} (neat sample)/ cm^{-1} 2981, 1627, 1457, 1240, 1162, 1087, 1026, 760.

1,3-Bis{1-[4,7,10-tris(2-amino-ethylcarbamoylmethyl)acetamide]-1,4,7,10-tetraazacyclododecan-1-ylmethyl}-4-benzyl.La.6CF₃SO₃.4H₂O (42.La₂)

Complex **42.La₂** was prepared according to **General Procedure 2** from **42** (0.150 g, 0.12 mmol) and Eu(CF₃SO₃)₃ (0.15 g, 0.25 mmol). A hygroscopic off white solid was isolated (0.21 g, 69% yield). HRMS: Calculated for C₄₉H₉₀N₂₀O₉F₃SLa₂: m/z (ES^+) = 1469.5000, $[M+CF_3SO_3-4H^+]^+$ Found: 1469.5060 (-4.1 ppm); δ_H (400 MHz, D_2O) 7.52, 6.17, 4.40, 4.03, 3.49, 3.22, 3.08; IR ν_{max} (neat sample)/ cm^{-1} 3171, 2981, 1655, 1595, 1458, 1322, 1240, 1160, 1079, 1027, 884.

1,3-Bis{1-[4,7,10-tris(2-*tert*-butoxycarbonylamino-ethylcarbamoylmethyl)acetamide]-1,4,7,10-tetraazacyclododecan-1-ylmethyl}-3-benzyl (79)

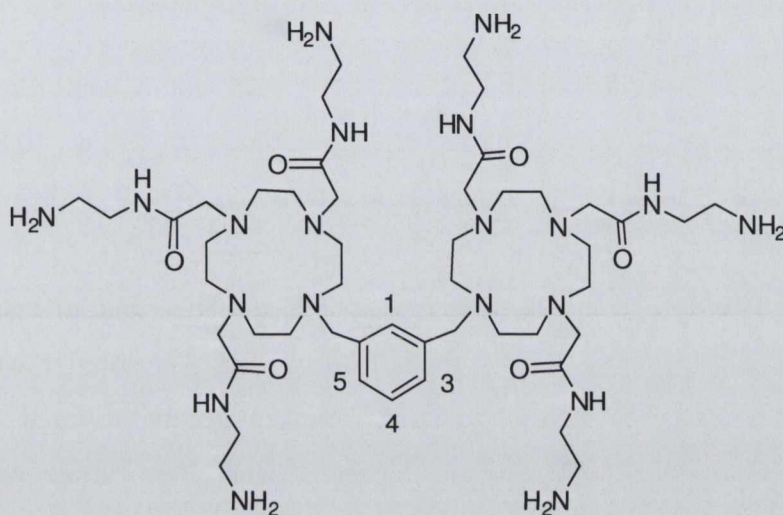


Compound **75** (0.05 g, 0.12 mmol) and **59** (0.17 g, 0.72 mmol) were dissolved in EtOH (HPLC grade), KI (0.10 g, 0.73 mmol) and K₂CO₃ (0.10 g, 0.73 mmol) were added to the solution. The solution was made up to 25 mL with EtOH and refluxed for five

days, under argon. The solution was cooled to room temperature and the solvent removed under reduced pressure. The residue was dissolved in CH₂Cl₂ and filtered through a celite plug to remove any inorganic salts. The filtrate was washed with a saturated solution of KCl (4 × 20 mL) and H₂O (1 × 20 mL). The organic phase was dried over MgSO₄ and filtered and the solvent was removed under reduced pressure. The residue was dried under vacuum to give a pale yellow hygroscopic solid (0.12 g, 63%). HRMS: Calculated for C₇₈H₁₄₄N₂₀O₁₈: m/z (ES^+) = 1649.0967 $[M+2H]^{2+}$, Found: 1649.0976 (+0.5 ppm); δ_H (600 MHz, D_2O) 7.92 (s, br, 4H, NH₂), 7.21 (s, br, 2H, ArH), 7.02 (s, br, 2H, ArH), 5.30 (s, br, 4H, NH₂), 5.03 (s, br, 4H, NH₂), 3.92 (s, 4H, ArCH₂), 3.41 (m, 36H, NCH₂), 2.70 (m, 32H,

NCH₂), 1.43 (m, 54H, C(CH₃)₃); δ_C (150 MHz, D₂O) 171.6 (C=O), 170.5 (C=O), 156.5 (C=O), 156.3 (C=O), 137.7 (q), 130.8 (CH), 128.7 (CH), 128.3 (CH), 79.3 (q), 79.0 (q), 69.7 (CH₂), 67.0 (CH₂), 59.4 (CH₂), 59.0 (CH₂), 58.4 (CH₂), 53.9 (CH₂), 53.2 (CH₂), 52.2 (CH₂), 41.6 (CH₂), 40.2 (CH₂), 39.4 (CH₂), 28.3 (CH₃), 28.2 (CH₃); IR $\nu_{\max}$ (neat sample)/cm⁻¹ 3317, 2976, 2933, 1690, 1654, 1520, 1451, 1365, 1270, 1248, 1165, 1115, 1040, 1001, 863.

1,3-Bis{1-[4,7,10-tris(2-amino-ethylcarbamoylmethyl)acetamide]-1,4,7,10-tetraazacyclododecan-1-ylmethyl}-3-benzyl (44)



Compound **44** was synthesised according to **General Procedure 1**, from **79** (100 mg, 0.06 mmol) to yield an orange solid (0.08 g, 100%); HRMS Calculated for C₄₈H₉₄N₂₀O₆Na₂ m/z (ES⁺) = 1092.7461, [M+2Na]²⁺, Found 1092.7460 (-0.1

ppm); δ_H (600 MHz, D₂O) 7.58 (d, 2H, J = 7.62 Hz, ArH_{3,5}), 7.54 (s, 1H, ArH₁), 7.43 (s, 1H, ArH₄), 4.02 (s, 4H, ArCH₂), 3.95 (m, 36H, NCH₂), 3.10 (m, 32H, NCH₂); δ_C (150 MHz, D₂O) 173.8 (C=O), 168.4 (C=O), 166.6 (q), 136.3 (CH), 133.0 (CH), 130.2 (CH), 68.7 (CH₂), 67.6 (CH₂), 57.3 (CH₂), 56.6 (CH₂), 54.9 (CH₂), 54.5 (CH₂), 48.8 (CH₂), 39.1 (CH₂), 38.6 (CH₂), 36.9 (CH₂), 36.3 (CH₂); IR $\nu_{\max}$ (neat sample)/cm⁻¹ 3213, 2855, 1656, 1607, 1547, 1442, 1390, 1368, 1265, 1170, 1087, 1027, 980, 912, 816.

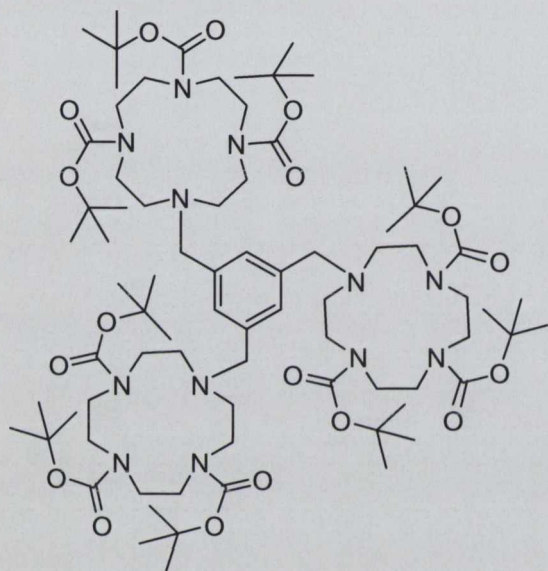
1,3-Bis{1-[4,7,10-tris(2-amino-ethylcarbamoylmethyl)acetamide]-1,4,7,10-tetraazacyclododecan-1-ylmethyl}-3-benzyl.Eu. 6CF₃SO₃.(44.Eu₂)

Complex **44.Eu₂** was synthesised according to **General Procedure 2** from **44** (0.03 g, 0.02 mmol) and Eu(CF₃SO₃)₃ (0.02 g, 0.03 mmol) to give a white hygroscopic solid, (0.04 g, 92%). Calculated for C₄₈H₉₄Eu₂N₂₀O₄.6CF₃SO₃.13H₂O.CHCl₃: C 25.41, H 4.69, N 10.78%, Found: C 25.44, H 5.05, N 10.79%; δ_H (400 MHz, D₂O) 20.53, 15.49, 8.70, 8.26, 7.58, 6.18, 4.38, 3.94, 3.71, 3.45, 3.23, 3.07, 1.75, 1.08, 0.78, 0.01, -6.61, -7.82, -10.0, -12.44, -17.66; IR $\nu_{\max}$ (neat sample)/cm⁻¹ 2973, 1626, 1590, 1458, 1368, 1239, 1224, 1164, 1089, 1026, 926, 815.

1,3-Bis{1-[4,7,10-tris(2-amino-ethylcarbamoylmethyl)acetamide]-1,4,7,10-tetraazacyclododecan-1-ylmethyl}-3-benzyl.La. 6CF₃SO₃.6H₂O (44.La₂)

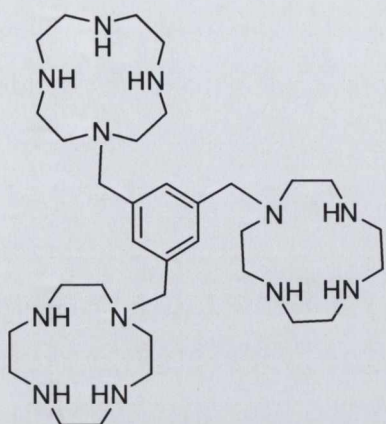
Complex **44.La₂** was synthesised according to **General Procedure 2** from **44** (0.02 g, 0.02 mmol) and Eu(CF₃SO₃)₃ (0.02 g, 0.03 mmol) to give a white solid, (0.03 g, 82%). Calculated for C₄₈H₉₄La₂N₂₀O₆.6CF₃SO₃.8H₂O.4CH₂Cl₂.MeOH C 25.90, H 4.50, N 10.24%, Found: C 25.82, H 4.45, N 9.93%; δ_{H} (400 MHz, D₂O) 7.59, 7.51, 7.44, 4.37, 3.95, 3.45, 3.24, 3.07; IR ν_{max} (neat sample)/cm⁻¹ 2968, 1628, 1560, 1457, 1369, 1238, 1224, 1163, 1089, 1025, 920, 814.

1,3,5-Tris[4,7,10-tris(*tert*-butyloxycarbonyl)-1,4,7,10-tetraazacyclododecan-1-ylmethyl]benzene (80)⁶⁰



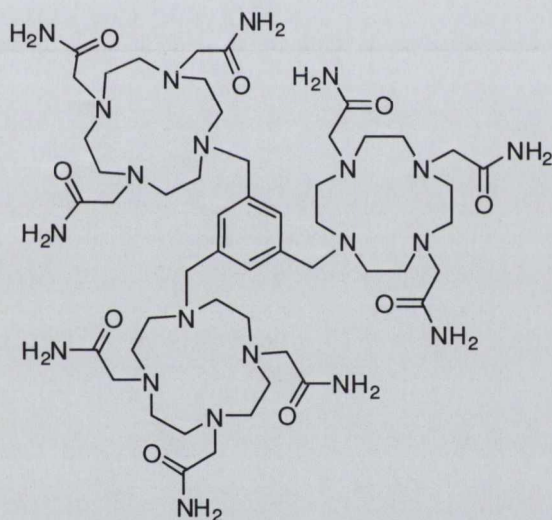
Compound **66** (1.3 g, 2.75 mmol) and 1,3,5-tris(bromomethyl)-benzene (0.29 g, 0.83 mmol) were dissolved in MeCN (40 mL, HPLC grade) and anhydrous Na₂CO₃ (0.57 g, 5.34 mmol) was added. The solution was stirred at reflux for four days. The solution was cooled to room temperature and filtered to remove any inorganic salts. The filtrate was concentrated under reduced pressure and the residue was purified by C-18 reverse phase flash silica chromatography (EtOAc:Hexane

100-0%). The solvent was removed under reduced pressure, and the residue dried under vacuum, to give an off white solid (1.4 g, 71%). HRMS: Calculated for C₇₈H₁₃₉N₁₂O₁₈: m/z (ES⁺) = 1532.0330 [M+H]⁺, Found: 1532.0338 (+0.5 ppm); δ_{H} (400 MHz, CDCl₃) 6.96 (s, 3H, ArH), 3.73 (s, 6H, ArCH₂), 3.52 (s, 12H, NCH₂), 3.28 (m, br, 24H, NCH₂), 2.61 (s, 12H, NCH₂), 1.39 (m, 81H, C(CH₃)₃); δ_{C} (100 MHz, CDCl₃) 155.3 (C=O), 154.8 (C=O), 135.1 (q), 131.2 (CH), 79.2 (q), 79.0 (q), 54.4 (CH₂), 53.0 (CH₂), 49.4 (CH₂), 47.2 (CH₂), 46.7 (CH₂), 28.2 (CH₃), 28.0 (CH₃).

1,3,5-Tris(1,4,7,10-tetraazacyclododecan-1-ylmethyl)benzene nonahydrochloride (81)⁶⁰


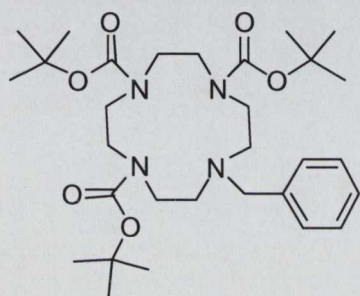
42.0 (CH₂), 41.7 (CH₂).

Compound **81** was synthesised according to **General Procedure 1** from **80** (0.07 g, 0.05 mmol) to yield a white solid (0.050 g, 100%). m.p. dec. 272 °C; HRMS: Calculated for C₃₃H₆₇N₁₂: m/z (ES⁺) = 631.5612 [M+H]⁺, Found: 631.5610 (- 0.3 ppm); δ_{H} (400 MHz, D₂O) 7.26 (s, 3H, ArH), 3.88 (s, 6H, ArCH₂), 3.08 (m, 24H, NCH₂), 2.93 (s, 12H, NCH₂), 2.82 (s, 12H, NCH₂); δ_{C} (100 MHz, CDCl₃) 134.7 (q), 132.1 (CH), 56.0 (CH₂), 47.3 (CH₂), 44.2 (CH₂),

1,3,5-Tris[1-(4,7,10-tris(carbamoylmethyl)-1,4,7,10-tetraazacyclododecan-1-ylmethyl)-benzene (45)


Compound **81** (0.13 g, 0.21 mmol) and *N*-bromoacetamide (0.29 g, 2.10 mmol) were dissolved in MeOH (25 mL, HPLC grade). Et₃N (0.21 g, 2.10 mmol) was added to the solution which was refluxed under argon for six days. The solution was concentrated in volume (to approx. 1 mL) and was added dropwise to a 50/50 v/v mixture of EtOH and *i*-PrOH, to yield a white hygroscopic solid which was isolated by centrifugation to yield (0.24 g, 79%). m.p. dec. 156 °C;

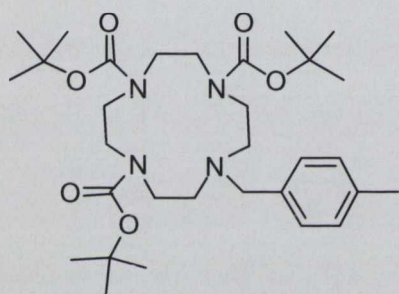
HRMS: Calculated for C₅₁H₉₄N₂₁O₉: m/z (ES⁺) = 1144.7543 [M+H]⁺, Found: 1144.7573 (+2.6 ppm); δ_{H} (600 MHz, D₂O) 7.74 (s, 3H, ArH), 4.29 (s, 6H, ArCH₂), 3.41 (s, br, 18H, NCH₂CO), 3.18 (s, br, 32H, NCH₂), 2.71 (s, br, 16H, NCH₂); δ_{C} (150 MHz, D₂O) 175.5(C=O), 174.4 (C=O), 140.36 (q), 134.38 (CH), 56.9 (CH₂), 55.8 (CH₂), 54.1 (CH₂), 50.9 (CH₂), 50.5 (CH₂), 50.0 (CH₂), 49.5 (CH₂); IR ν_{max} (neat sample)/cm⁻¹ 3307, 3173, 2833, 1655, 1457, 1392, 1326, 1282, 1158, 1121, 1085, 968, 887.

1-Benzyl-4,7,10-tris(*tert*-butyloxycarbonyl)-1,4,7,10-tetraazacyclododecane (88)¹⁸²


Compound **66** (0.35 g, 0.73 mmol) and benzyl bromide (0.12 g, 0.67 mmol) were dissolved in MeCN (freshly distilled). Na₂CO₃ (0.14 g, 1.34 mmol) was added and the solution was stirred at reflux under argon for three days. The solvent was

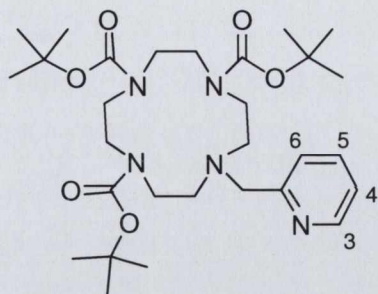
removed under reduced pressure and the residue redissolved in CH_2Cl_2 . The solution was filtered through a celite plug to remove any inorganic salts. The solvent was removed under reduced pressure and the residue was purified by flash silica chromatography (Eluent: CH_2Cl_2 :EtOAc 100-0%). The solvent was removed under reduced pressure and residue dried under vacuum to yield the product as a white solid, (0.36 g, 95%). m.p. 80-82 °C;¹⁸² HRMS: Calculated for $\text{C}_{30}\text{H}_{51}\text{N}_4\text{O}_6$: m/z (ES^+) = 563.3809 $[\text{M}+\text{H}]^+$, Found: 563.3799 (-1.7 ppm); δ_{H} (400 MHz, CDCl_3) 7.25 (m, 5H, ArH), 4.12 (s, br, 2H, ArCH₂), 3.75 (s, br, 4H, NCH₂), 3.26 (m, br, 8H, NCH₂), 2.68 (m, br, 4H, NCH₂), 1.45 (m, 27H, C(CH₃)₃); δ_{C} (100 MHz, CDCl_3) 156.2 (C=O), 155.8 (C=O), 136.7 (q), 130.4 (CH), 128.2 (CH), 127.3 (CH), 79.5 (q), 79.3 (q), 57.4 (CH₂), 56.0 (CH₂), 55.0 (CH₂), 50.0 (CH₂), 47.4 (CH₂), 28.7 (CH₃), 28.5 (CH₃).

1-(4-Methyl-benzyl)-4,7,10-tris(*tert*-butyloxycarbonyl)-1,4,7,10-tetraazacyclododecane (89)



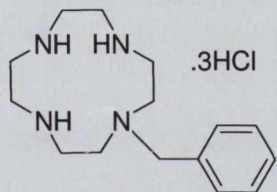
Compound **66** (0.350 g, 0.73 mmol) and benzyl bromide (0.12 g, 0.67 mmol) were dissolved in MeCN (freshly distilled). Na_2CO_3 (0.24 g, 2.22 mmol) was added and the solution stirred at reflux under argon for three days. The solvent was removed under reduced pressure and the residue redissolved in CH_2Cl_2 . The solution was filtered

through a celite plug to remove any inorganic salts. The solvent was removed under reduced pressure and the residue was purified by flash silica chromatography (Eluent: CH_2Cl_2 :EtOAc 100-0%). The solvent was removed under reduced pressure and residue dried under vacuum to yield the product as a white solid, (0.33 g, 86%). Calculated for: $\text{C}_{31}\text{H}_{52}\text{N}_4\text{O}_6 \cdot 0.25\text{H}_2\text{O}$: C 64.05, H 9.10, N 9.64%, Found: C 64.13, H 9.10, N 9.45%; m.p. 104-108 °C; HRMS: Calculated for $\text{C}_{31}\text{H}_{53}\text{N}_4\text{O}_6$: m/z (ES^+) = 577.3965 $[\text{M}+\text{H}]^+$, Found: 577.3973 (+1.4 ppm); δ_{H} (400 MHz, CDCl_3) 7.11 (s, 4H, ArH), 3.70 (s, 2H, ArCH₂), 3.59 (s, br, 4H, NCH₂), 3.26 (m, br, 8H, NCH₂), 2.65 (m, br, 4H, NCH₂), 2.35 (s, 3H, ArCH₃), 1.45 (m, 27H, C(CH₃)₃); δ_{C} (100 MHz, CDCl_3) 156.0 (C=O), 155.6 (C=O), 136.8 (q), 133.3 (q), 130.2 (CH), 128.7 (CH), 79.3 (q), 79.2 (q), 56.8 (CH₂), 55.8 (CH₂), 54.8 (CH₂), 49.9 (CH₂), 47.3 (CH₂), 28.7 (CH₃), 28.5 (CH₃), 21.1 (CH₃); IR ν_{max} (neat sample)/ cm^{-1} 2974, 1682, 1457, 1412, 1363, 1247, 1149, 1045, 978, 771.

1-(2-Pyridine-methyl)-4,7,10-tris(*tert*-butyloxycarbonyl)-1,4,7,10-tetraazacyclodecane (90)¹⁸²

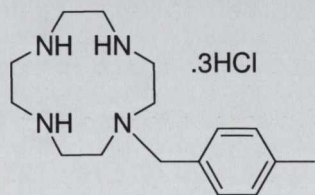
Compound **66** (0.50 g, 1.05 mmol) and 2-(bromomethyl)-pyridine hydrobromide (0.27 g, 1.06 mmol) were dissolved in MeCN (freshly distilled). Na₂CO₃ (0.45 g, 4.24 mmol) was added and the solution was stirred at reflux under argon for three days. The solvent was removed under reduced pressure and the residue was dissolved in CH₂Cl₂ and

filtered through a celite plug to remove any inorganic salts. The filtrate was washed with H₂O (3 x 20 mL) and the organic layer was dried over MgSO₄. The solution was filtered and the solvent removed under reduced pressure. The residue was dried under vacuum to yield the product as an orange solid (0.52 g, 87%). m.p. 112 - 114 °C; HRMS: Calculated for C₂₉H₅₀N₅O₆: m/z (ES⁺) = 564.3761 [M+H]⁺, Found: 564.3754 (-1.3 ppm); δ_H (400 MHz, CDCl₃) 8.57 (d, 1H, J = 3.5 Hz, ArH₃), 7.67 (s, br, 1H, ArH₅), 7.28 (s, br, 1H, ArH₆), 7.22 (t, 1H, J = 7.02, ArH₄), 3.91 (s, 2H, ArCH₂), 3.59 (s, br, 4H, NCH₂), 3.35 (m, br, 8H, NCH₂), 2.76 (m, br, 4H, NCH₂), 1.44 (m, 27H, C(CH₃)₃); δ_C (100 MHz, CDCl₃) 157.6 (C=O), 155.6 (C=O), 136.3 (q), 131.0 (CH), 128.9 (CH), 124.1 (CH), 122.4 (CH), 79.5 (q), 79.3 (q), 58.6 (CH₂), 55.4 (CH₂), 54.5 (CH₂), 49.5 (CH₂), 47.9 (CH₂), 28.7 (CH₃), 28.5 (CH₃).

1-Benzyl-1,4,7,10-tetraazacyclododecane trihydrochloride (91)¹⁸²

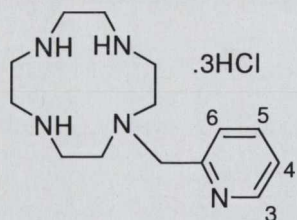
Compound **91** was synthesised according to **General Procedure 1** from **88** (0.34 g, 0.60 mmol) to yield a white solid (0.22 g, 98%). m.p. 249 - 250 °C; HRMS: Calculated for C₁₅H₂₇N₄: m/z (ES⁺) = 263.2236 [M+H]⁺, Found: 263.2237 (+0.5 ppm); δ_H (400 MHz,

D₂O) 7.27 (m, 5H, ArH), 3.75 (s, 2H, ArCH₂), 3.01 (m, 8H, NCH₂), 2.81 (m, 8H, NCH₂); δ_C (100 MHz, D₂O) 134.7 (q), 129.9 (CH), 129.0 (CH), 128.4 (CH), 56.8 (CH₂), 48.0 (CH₂), 44.0 (CH₂), 41.9 (CH₂), 41.8 (CH₂).

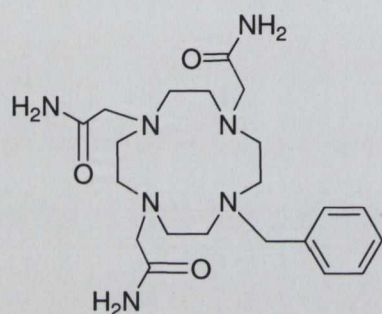
1-(4-Methyl-benzyl)-1,4,7,10-tetraazacyclododecane trihydrochloride (92)

Compound **92** was synthesised according to **General Procedure 1** from **89** (0.31 g, 0.54 mmol) to yield a white solid (0.20 g, 95%). m.p. 262-265 °C; HRMS: Calculated for C₁₅H₂₇N₄: m/z (ES⁺) = 263.2236 [M+H]⁺, Found: 263.2237 (+0.5 ppm); δ_H (400

MHz, D₂O) 7.17 (s, 4H, ArH), 3.78 (s, 2H, ArCH₂), 3.02 (m, 8H, NCH₂), 2.87 (m, 8H, NCH₂), 2.20 (s, 3H, ArCH₃); δ_C (100 MHz, D₂O) 150.7 (q), 138.7 (q), 129.8 (CH), 129.1 (CH), 56.5 (CH₂), 47.8 (CH₂), 43.4 (CH₂), 41.7 (CH₂), 41.5 (CH₂), 19.7 (CH₃).

1-(2-Pyridine-methyl)-1,4,7,10-tetraazacyclododecane trihydrochloride (93)¹⁸²

Compound **93** was synthesised according to **General Procedure 1** from **90** (0.52 g, 0.92 mmol) to yield an orange solid (0.33 g, 95%). HRMS: Calculated for $C_{14}H_{26}N_5$: m/z (ES^+) = 264.2188 $[M+H]^+$, Found: 264.2184 (-1.6 ppm); δ_H (400 MHz, $CDCl_3$) 8.67 (d, 1H, $J = 6.0$ Hz, ArH_3), 8.48 (t, 1H, $J = 8.0$ Hz, ArH_5), 7.98 (d, 1H, $J = 8.0$ Hz, ArH_6), 7.91 (t, 1H, $J = 6.8$ Hz ArH_4), 4.10 (s, 2H, $ArCH_2$), 3.19 (m, br, 4H, NCH_2), 3.08 (m, br, 4H, NCH_2), 2.85 (m, br, 8H, NCH_2); δ_C (100 MHz, $CDCl_3$) 150.0 (q), 147.4 (CH), 142.2 (CH), 128.5 (CH), 126.7 (CH), 54.2 (CH_2), 47.9 (CH_2), 44.3 (CH_2), 41.7 (CH_2), 41.4 (CH_2).

1-Benzyl-4,7,10-tris(carbamoylmethyl)-1,4,7,10-tetraazacyclododecane (47)

Compound **92** (0.15 g, 0.57 mmol) was dissolved in H_2O and neutralised with KOH and the solvent was removed under reduced pressure. The residue was dissolved in EtOH (3 mL, HPLC grade) and the solution was cooled in a freezer. The solution was filtered through a celite plug to remove any inorganic salts. 2-Bromoacetamide (0.237g, 1.72 mmol) and anhydrous K_2CO_3 (0.245 g, 1.77 mmol)

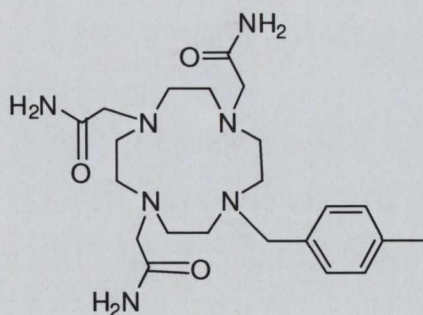
were added to the solution, and EtOH was added up to 20 mL. The solution was stirred at reflux for five days, under argon. After cooling to room temperature, the solution was filtered through a celite plug to remove any inorganic salts. The solvent was removed under reduced pressure and the residue redissolved in a minimum amount of EtOH. The solution was left in the freezer and filtered to remove remaining K_2CO_3 . The filtrate was reduced to a minimum volume and added dropwise to vigorously stirred Et_2O (150 mL) to yield a white precipitate which was collected *via* suction filtration. Following recrystallisation from water a white solid was isolated (0.16 g, 65%). The mother liquid was stored at approx. 40 °C, from which crystals suitable for X-ray diffraction were grown. Calculated for: $C_{21}H_{35}N_7O_3$: C 58.18, H 8.14, N 22.62%, Found: C 57.88, H 8.18, N 22.48%; m.p. 157 - 160 °C; HRMS: Calculated for $C_{21}H_{36}N_7O_3$: m/z (ES^+) = 434.2880 $[M+H]^+$, Found: 434.2888 (+1.9 ppm); δ_H (400 MHz, D_2O) 7.38 (m, br, 5H, ArH), 3.64 (s, br, 2H, $ArCH_2$), 3.11 (s, 2H, NCH_2CO), 3.05 (s, 4H, NCH_2CO), 2.71 (s, 8H, NCH_2), 2.61 (s, 8H, NCH_2); δ_C (100 MHz, D_2O) 177.7 (C=O), 177.3 (C=O), 136.2 (q), 130.1 (CH), 128.4 (CH), 127.7 (CH), 59.2 (CH_2), 57.2 (CH_2), 57.1 (CH_2), 56.9 (CH_2), 52.3 (CH_2), 52.2 (CH_2), 51.2 (CH_2); IR ν_{max} (neat sample)/ cm^{-1} 3325, 2811, 1654, 1455, 1401, 1343, 1298, 1129, 1083, 1015, 991, 926, 828.

1-Benzyl-4,7,10-tris(carbamoylmethyl)-1,4,7,10-tetraazacyclododecane.Eu.3CF₃SO₃.2H₂O (47.Eu)

Complex **47.Eu** was synthesised according to **General Procedure 2** from **47** (0.10 g, 0.23 mmol) and Eu(CF₃SO₃)₃ (0.14 g, 0.23 mmol). A white precipitate was isolated from the solution by filtration and was combined with the product to give a white solid, (0.17 g, 68%). m.p. 180-184 °C; Calculated for C₂₁H₃₅N₇O₃.3CF₃SO₃.2H₂O.0.5Et₂O: C 28.24, H 4.01, N 8.87%, Found: C 28.09, H 3.61, N 8.85%; M.P. 180-184 °C; HRMS: Calculated for C₂₂H₃₄N₇O₆SF₃Eu: *m/z* (ES⁺) = 734.1456 [M+CF₃SO₃]⁺, Found: 734.1443 (-1.7 ppm); δ_H (400 MHz, D₂O) 18.1, 15.79, 7.75, 3.44, 1.05, -1.21, -2.47, -6.14, -7.04, -9.70, -10.21, -11.51, -13.62, -18.18; IR ν_{max} (neat sample)/cm⁻¹ 3356, 3229, 1655, 1601, 1456, 1236, 1222, 1164, 1081, 1025, 886.

1-Benzyl-4,7,10-tris(carbamoylmethyl)-1,4,7,10-tetraazacyclododecane.La. 3CF₃SO₃.2H₂O (47.La)

Complex **47.La** was synthesised according to **General Procedure 2** from **47** (0.05 g, 0.12 mmol) and La(CF₃SO₃)₃ (0.07 g, 0.12 mmol). A white precipitate was isolated from the solution by filtration and was combined with the product to give a white solid, (0.10 g, 82%). Calculated for C₂₂H₃₇N₇O₃La.2H₂O.3CF₃SO₃: C 28.07, H 3.86, N 9.17%, Found: C 27.90, H 3.68, N 8.87%; m.p. dec. 130 °C; HRMS: Calculated for C₂₂H₃₄N₇O₆F₃SLa: *m/z* (MALDI) = 720.1307 [M+CF₃SO₃]⁺, Found: 720.1282 (-3.5 ppm); δ_H (400 MHz, D₂O) 7.33, 7.22, 3.85, 3.70, 3.43, 2.94, 2.63, 2.52, 2.46, 2.19; IR ν_{max} (neat sample)/cm⁻¹ 3354, 3228, 1662, 1599, 1457, 1236, 1216, 1159, 1082, 1025, 882.

1-(4-Methyl-benzyl)-4,7,10-tris(carbamoylmethyl)-1,4,7,10-tetraazacyclododecane (48)

Compound **90** (0.143 g, 0.52 mmol) was dissolved in H₂O and neutralized with KOH. The solvent was removed under reduced pressure and the residue dissolved in EtOH (3 mL, HPLC grade) and cooled in a freezer. The solution was filtered through a celite plug to remove any inorganic salts. 2-Bromoacetamide (0.217 g,

1.57 mmol) and anhydrous K₂CO₃ (0.223 g, 1.61 mmol) were added to the solution, and EtOH was added up to 20 mL. The solution was stirred at reflux for five days, under argon. After cooling to room temperature, the solution was filtered through a celite plug to remove any inorganic salts. The solvent was removed under reduced pressure and the residue redissolved in a minimum amount of EtOH. The solution was left in the freezer and filtered to remove remaining K₂CO₃. The filtrate was reduced to a minimum volume and added

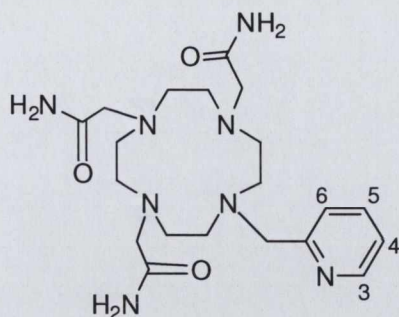
dropwise to vigorously stirred Et₂O (150 mL) to yield a white precipitate which was collected *via* suction filtration. Following recrystallisation from water a white solid was isolated (0.27 g, 66%). Calculated for C₂₂H₃₇N₇O₃: C 59.04, H 8.33, N 21.91%, Found: C 58.88, H 8.40, N 21.82%; m.p. 186-192 °C; HRMS: Calculated for C₂₂H₃₈N₇O₃: *m/z* (ES⁺) = 448.3036 [M+H]⁺, Found: 448.3052 (+3.5 ppm); δ_H (400 MHz, D₂O) 7.38 (d, 2H, *J* = 7.1 Hz, ArCH), 7.29 (d, 2H, *J* = 7.5 Hz, ArH), 4.17 (s, br, 2H, ArCH₂), 3.21 (s, br, 4H, NCH₂CO), 3.14 (s, 2H, NCH₂CO), 3.03 (s, 4H, NCH₂), 2.92 (s, 4H, NCH₂), 2.62 (s, 8H, NCH₂), 2.32 (s, 3H, ArCH₃); δ_C (100 MHz, D₂O) 176.8 (C=O), 175.7 (C=O), 161.8 (q), 160.7 (q), 130.9 (CH), 129.8 (CH), 57.6 (CH₂), 57.3 (CH₂), 56.7 (CH₂), 55.7 (CH₂), 51.4 (CH₂), 51.1 (CH₂), 50.5 (CH₂), 16.7 (CH₃); IR ν_{max} (neat sample)/cm⁻¹ 3288, 3168, 2807, 1660, 1444, 1403, 1347, 1296, 1149, 1082, 983, 801.

1-(4-Methyl-benzyl)-4,7,10-tris(carbamoylmethyl)-1,4,7,10-tetraazacyclododecane.Eu .3CF₃SO₃.2H₂O (48.Eu)

Complex **48.Eu** was synthesised according to **General Procedure 2** using **48** (0.11 g, 0.25 mmol) and Eu(CF₃SO₃)₃ (0.15 g, 0.25 mmol). A white precipitate was isolated from the solution by filtration and was combined with the product to give a white solid, (0.14 g, 95%). m.p. dec. 140 °C; HRMS: Calculated for C₂₂H₃₃N₇O₃Eu: *m/z* (MALDI) = 746.1456 [M+CF₃SO₃]⁺, Found: 746.1629 (+23.2 ppm); δ_H (400 MHz, D₂O); 18.38, 16.14, 9.32, 7.55, 6.16, 3.88, 3.21, 2.49, 1.04, 1.03, -6.02, -7.02, -9.68, -10.51, -11.38, -13.54, -18.15; IR ν_{max} (neat sample)/cm⁻¹ 3355, 3223, 2980, 1664, 1600, 1461, 1236, 1222, 1162, 1081, 1025, 943, 829.

1-(4-Methyl-benzyl)-4,7,10-tris(carbamoylmethyl)-1,4,7,10-tetraazacyclododecane.La .3CF₃SO₃.2H₂O (48.La)

Complex **48.La** was synthesised according to **General Procedure 2** from **48** (0.05 g, 0.12 mmol) and La(CF₃SO₃)₃ (0.07 g, 0.12 mmol). A white precipitate was isolated from the solution by filtration and was combined with the product to give a white solid, (0.09 g, 74%). m.p. 123 °C; Calculated for C₂₁H₃₅N₇O₃La.3CF₃SO₃.2H₂O: C 27.31, H 3.72, N 9.29%, Found: C 27.35, H 3.55, N 9.10%; HRMS: Calculated for C₂₄H₃₇N₇O₉F₆S₂La: *m/z* (ES⁺) = 884.1062 [M+2CF₃SO₃]⁺, Found: 884.1062 (+2.7 ppm); δ_H (400 MHz, D₂O) 7.15, 7.09, 4.25, 3.82, 3.44, 2.89, 2.64, 2.51, 2.22; IR ν_{max} (neat sample)/cm⁻¹ 3353, 3226, 2873, 1663, 1600, 1466, 1236, 1222, 1164, 1082, 1025, 945, 823.

1-(2-Pyridine-methyl)-4,7,10-tris(carbamoylmethyl)-1,4,7,10-tetraazacyclododecane (49)


Compound **91** (0.37 g, 0.99 mmol) was dissolved in H₂O and neutralised with KOH. The solvent was removed under reduced pressure and the residue dissolved in EtOH (3 mL HPLC grade) and cooled in a freezer. The solution was filtered through a celite plug to remove any inorganic solids. 2-Bromoacetamide (0.41 g, 2.98 mmol) and anhydrous K₂CO₃ (0.43 g, 3.08 mmol) was added to the solution, and EtOH was added up to 20 mL. The solution was stirred at reflux for five days, under argon. After cooling to room temperature, the solution was filtered through a celite plug to remove any inorganic solids and the solvent removed under reduced pressure. A yellow solid was isolated by precipitation from vigorously stirred diethyl ether (150 mL). The solid was recrystallised from MeCN with a minimum amount of MeOH added. This yielded crystals suitable for analysis by X-Ray diffraction (0.19 g, 44%). m.p. 200-210 °C; Calculated for C₂₀H₃₄N₈O₃·3K₂CO₃·MeCN·H₂O: C 32.74, H 4.45, N 14.10%, Found: C 33.06, H 4.33, N 13.85%; M.P.: 203 °C; HRMS: Calculated for C₂₀H₃₄N₈O₃Na: *m/z* (ES⁺) = 457.2652 [M+Na]⁺, Found: 457.2645 (+1.4 ppm); δ_H (600 MHz, D₂O) 8.35 (d, 1H, *J* = 3.9 Hz, ArH₃), 7.37 (t, 1H, *J* = 4.9 Hz, ArH₅), 7.36 (d, 1H, *J* = 6.84 Hz, ArH₆), 7.27 (t, 1H, *J* = 3.8 Hz, ArH₄), 3.69 (s, 2H, ArCH₂), 3.02 (s, 2H, NCH₂CO), 2.96 (s, 4H, NCH₂CO), 2.70 (m, br, 8H, NCH₂), 2.53 (m, br, 8H, NCH₂); δ_C (150 MHz, D₂O) 177.3 (C=O), 176.8 (C=O), 162.4 (q), 148.4 (CH), 137.9 (CH), 125.8 (CH), 123.3 (CH), 61.1 (CH₂), 60.5 (CH₂), 59.8 (CH₂), 56.9 (CH₂), 56.8 (CH₂), 52.1 (CH₂), 51.5 (CH₂); IR ν_{max} (neat sample)/cm⁻¹ 3302, 3170, 2806, 1680, 1618, 1429, 1404, 1328, 1281, 1259, 1102, 1007, 993, 904.

1-(2-Pyridine-methyl)-4,7,10-tris(carbamoylmethyl)-1,4,7,10-tetraazacyclododecane .Eu.3CF₃SO₃.2H₂O (49.Eu)

Complex **49.Eu** was synthesised according to **General Procedure 2** from **49** (0.10 g, 0.24 mmol) and Eu(CF₃SO₃)₃ (0.14 g, 0.24 mmol) to yield a yellow solid (0.12 g, 48%). m.p. 145 °C; HRMS: Calculated for C₂₂H₃₄N₈O₉F₆S₂Eu: *m/z* (ES⁺) = 885.1007 [M+Na]⁺, Found: 885.1086 (+3.3 ppm); δ_H (400 MHz, D₂O) 25.49, 23.47, 22.18, 18.81, 11.19, 9.89, 9.16, 8.61, 7.51, 6.99, 6.46, 6.11, 3.45, 3.23, 1.55, 1.06, 0.45, -0.74, -3.70, -4.45, -4.86, -5.54, -6.75, -7.24, -7.63, -8.14, -9.51, -10.76, -11.33, -11.95, -12.36, -14.16, -15.95, -16.44; IR ν_{max} (neat sample)/cm⁻¹ 3360, 3227, 1666, 1450, 1240, 1224, 1162, 1080, 1026, 915, 834.

- (1) Alberts, B.; Johnson, A.; Lewis, J.; Raff, M.; Roberts, K.; Walter, P. *Molecular Biology of the Cell*; Garland, 2002.
- (2) Lippard, S. J.; Berg, J. M. *Principles of bio-inorganic chemistry*; University Science Books: California, 1994.
- (3) Sigman, D. S.; Mazumder, A.; Perrin, D. M. *Chem. Rev.* **1993**, 93, 2295-2316.
- (4) Chin, J. *Curr. Opin. Chem. Biol.* **1997**, 1, 514-521.
- (5) Hegg, E.; Burstyn, J. *Coord. Chem. Rev.* **1998**, 173, 133-165.
- (6) Mancin, F.; Scrimin, P.; Tecilla, P.; Tonellato, U. *Chem. Commun.* **2005**, 2540-2548.
- (7) Morrow, J. R.; Iranzo, O. *Curr. Opin. Chem. Biol.* **2004**, 8, 192-200.
- (8) Bashkin, J. K. *Appl. Biochem. Biotech.* **1995**, 54, 43-56.
- (9) Trawick, B. N.; Daniher, A. T.; Bashkin, J. K. *Chem. Rev.* **1998**, 98, 939-960.
- (10) Zubay, G. *Biochemistry*; William C Brown Pub: New York, 1998.
- (11) Rana, T. M. *Nat. Rev. Mol. Cell Bio.* **2007**, 8, 23-36.
- (12) Berg, J. M.; Tymoczko, J. L.; Stryer, L. *Biochemistry*; W.H. Freeman & Co: New York, 2002.
- (13) Westheimer, F. H. *Science* **1987**, 235, 1173-1178.
- (14) Doherty, E. A.; Doudna, J. A. *Annu. Rev. Biochem.* **2000**, 69, 597-615.
- (15) Perreault, D. M.; Anslyn, E. V. *Angew. Chem. Int. Edit.* **1997**, 36, 432-450.
- (16) Li, Y.; Breaker, R. R. *J. Am. Chem. Soc.* **1999**, 121, 5364-5372.
- (17) Krämer, R. *Coord. Chem. Rev.* **1999**, 182, 243-261.
- (18) Westheimer, F. H. *Accounts Chem. Res.* **1968**, 1, 70-78.
- (19) Giuseppe D'Alessio; Riordan, J. F. *Ribonucleases: Structures and Functions*; Academic Press: New York and London, 1997.
- (20) Mitic, N.; Smith, S. J.; Neves, A.; Guddat, L. W.; Gahan, L. R.; Schenk, G. *Chem. Rev.* **2006**, 106, 3338-3363.
- (21) Cotton, F. A.; Hazen, E. E.; Legg, M. J. *P. Natl. Acad. Sci. USA* **1979**, 76, 2551-2555.
- (22) Wilcox, D. E. *Chem. Rev.* **1996**, 96, 2435-2458.
- (23) Altman, S. *Angew. Chem. Int. Edit.* **1990**, 29, 749-758.
- (24) http://nobelprize.org/nobel_prizes/chemistry/.
- (25) Gesteland, R. F.; Cech, T. R.; Atkins, J. F. *The RNA World*; Cold Spring Harbor Laboratory Press 1993.
- (26) Ke, A.; Doudna, J. A. In *The RNA World*; Cech, T., Atkins, Eds.; Cold Spring Harbor Laboratory Press: 2006, p 109-131.

- (27) Ferré-D'Amaré, A. R.; Doudna, J. A. *J. Mol. Biol.* **2000**, 295, 541-556.
- (28) Fedor, M. J. *J. Mol. Biol.* **2000**, 297, 269-291.
- (29) Scott, W. G.; Murray, J. B.; Arnold, J. R. P.; Stoddard, B. I.; Klug, A. *Science* **1996**, 274, 2065-2069.
- (30) Been, M. D.; Wickham, G. S. *Eur. J. Biochem.* **1997**, 247, 741-753.
- (31) Shih, I. H.; Been, M. D. *RNA* **1999**, 5, 1140-1148.
- (32) Suh, Y.-A.; Kumar, P. K. R.; Taira, K.; Nishikawa, S. *Nucl. Acids Res.* **1993**, 21, 3277-3280.
- (33) Nakano, S.-i.; Cerrone, A. L.; Bevilacqua, P. C. *Biochemistry* **2003**, 42, 2982-2994.
- (34) Wu, H. N.; Lin, Y. J.; Lin, F. P.; Makino, S.; Chang, M. F.; Lai, M. M. *P. Natl. Acad. Sci. USA* **1989**, 86, 1831-1835.
- (35) Nakano, S.-i.; Chadalavada, D. M.; Bevilacqua, P. C. *Science* **2000**, 287, 1493-1497.
- (36) Ferre-D'Amare, A. R.; Zhou, K. *Nature* **1998**, 395, 567-574.
- (37) Williams, N. H.; Takasaki, B.; Wall, M.; Chin, J. *Accounts Chem. Res.* **1999**, 32, 485-493.
- (38) Schneider, H. J.; Yatsimirsky, A. K. *Lanthanide-catalysed hydrolysis of Phosphate Esters and Nucleic Acids*; M. Dekker Inc.: New York and Basel, 2003; Vol. 40.
- (39) Breslow, R. *Artificial enzymes*; Wiley-Vch Verlag GmGH & Co: Weinham, 2005.
- (40) Browne, K. A.; Bruice, T. C. *J. Am. Chem. Soc.* **1992**, 114, 4951-4958.
- (41) Williams, N. H.; Chin, J. *Chem. Commun.* **1996**, 131-132.
- (42) Cech, T.; Bass, B. L. *Annu. Rev. Biochem.* **1986**, 55, 599-629.
- (43) Jones, D. R.; Lindoy, L. F.; Sargeson, A. M. *J. Am. Chem. Soc.* **1983**, 105, 7327-7336.
- (44) Leivers, M.; Breslow, R. *Bioorg. Chem.* **2001**, 29, 345-356.
- (45) Attila, J.; Satu, M.; Harri, L.; Kaspar, H.; Tamás, G. *Chem. Eur. J.* **2003**, 9, 5404-5415.
- (46) Molenveld, P.; Engbersen, J. F. J.; Reinhoudt, D. N. *Chem. Soc. Rev.* **2000**, 29, 75-86.
- (47) Fersht, A. *Enzyme structure and mechanism*; 2nd ed.; W.H. Freeman Co.: New York, 1985.
- (48) Silberberg, M. S. *Chemistry: The Molecular Nature of Matter and Change*; 2nd ed.; McGraw-Hill Higher Education Boston, 2000.
- (49) Komiyama, M.; Takeda, N.; Shigekawa, H. *Chem. Commun.* **1999**, 1443-1451.
- (50) Breslow, R.; Huang, D.-L. *P. Natl. Acad. Sci. USA* **1991**, 88, 4080-4083.

- (51) Bashkin, J. K.; Gard, J. K.; Modak, A. S. *J. Org. Chem.* **1990**, *55*, 5125-5132.
- (52) Human Genome Sequencing, C. I. *Nature* **2004**, *431*, 931-945.
- (53) Zamecnik, P. C.; Stephenson, M. L. *P. Natl. Acad. Sci. USA* **1978**, *75*, 280-284.
- (54) Baker, B. F.; Monia, B. P. *Biochim. Biophys. Acta.* **1999**, *1489*, 3-18.
- (55) Parker, R.; Song, H. *Nat. Struct. Mol. Biol.* **2004**, *11*, 121-127.
- (56) Komiyama, M.; Sumaoka, J.; Kuzuya, A.; Yamamoto, Y. In *Methods in Enzymology*; Academic Press: London, 2001; Vol. 341, p 455-468.
- (57) <http://www.isispharm.com/index.html>.
- (58) Nittymaki, T.; Lonnberg, H. *Org. Biomol. Chem.* **2006**, *4*, 15-25.
- (59) Amin, S.; Morrow, J. R.; Lake, C. H.; Churchill, M. R. *Angew. Chem. Int. Edit.* **1994**, *33*, 773-775.
- (60) Kimura, E.; Aoki, S.; Koike, T.; Shiro, M. *J. Am. Chem. Soc.* **1997**, *119*, 3068-3076.
- (61) Rossiter, C. S.; Mathews, R. A.; Morrow, J. R. *J. Inorg. Biochem.* **2007**, *101*, 925-934.
- (62) Gunnlaugsson, T.; Davies, R. J. H.; Kruger, P. E.; Jensen, P.; McCabe, T.; Mulready, S.; O'Brien, J. E.; Stevenson, C. S.; Fanning, A.-M. *Tetrahedron Lett.* **2005**, *46*, 3761-3766.
- (63) Gunnlaugsson, T.; O'Brien, J. E.; Mulready, S. *Tetrahedron Lett.* **2002**, *43*, 8493-8497.
- (64) Yashiro, M.; Ishikubo, A.; Komiyama, M. *J. Chem. Soc. Chem. Commun.* **1995**, 1793-1794.
- (65) Ragunathan, K. G.; Schneider, H.-J. *Angew. Chem. Int. Edit.* **1996**, *35*, 1219-1221.
- (66) Linjalahti, H.; Feng, G.; Mareque-Rivas, J. C.; Mikkola, S.; Williams, N. H. *J. Am. Chem. Soc.* **2008**, *130*, 4232-4233.
- (67) O'Donoghue, A.; Pyun, S. Y.; Yang, M.-Y.; Morrow, J. R.; Richard, J. P. *J. Am. Chem. Soc.* **2006**, *128*, 1615-1621.
- (68) Sreedhara, A.; Cowan, J. A. *J. Biol. Inorg. Chem.* **2001**, *6*, 337-347.
- (69) Aoki, S.; Iwaida, K.; Hanamoto, N.; Shiro, M.; Kimura, E. *J. Am. Chem. Soc.* **2002**, *124*, 5256-5257.
- (70) Mareque-Rivas, J. C.; Hinchley, S. L.; Metteau, L.; Parsons, S. *Dalton Trans.* **2006**, 2316-2322.
- (71) Delahenty, J. B.; Stuart, T. C.; Knight, D. A.; Goldman, E. R.; Thach, D. C.; Bongard, J. E.; Chang, E. L. *RNA* **2005**, *11*, 831-836.

- (72) Subat, M.; Woinaroschy, K.; Gerstl, C.; Sarkar, B.; Kaim, W.; Konig, B. *Inorg. Chem.* **2008**, *47*, 4661-4668.
- (73) Benniston, A. C.; Gunning, P.; Peacock, R. D. *J. Org. Chem.* **2005**, *70*, 115-123.
- (74) Fry, F. H.; Fischmann, A. J.; Belousoff, M. J.; Spiccia, L.; Brugger, J. *Inorg. Chem.* **2005**, *44*, 941-950.
- (75) Sheng, X.; Guo, X.; Lu, X.-M.; Lu, G.-Y.; Shao, Y.; Liu, F.; Xu, Q. *Bioconjugate Chem.* **2008**, *19*, 490-498.
- (76) Kaltsoyannis, N.; Scott, P. *The F elements*; Oxford University Press: New York, 1999; Vol. 76.
- (77) Fricker, S. P. *Chem. Soc. Rev.* **2006**, *35*, 524-533.
- (78) Wahnnon, D.; Hynes, R. C.; Chin, J. *J. Chem. Soc. Chem. Commun.* **1994**, 1441-1442.
- (79) Yashiro, M.; Ishikubo, A.; Komiyama, M. *Chem. Commun.* **1997**, 83-84.
- (80) Winter, M. J. *d-Block Chemistry*; Oxford University Press, 1995.
- (81) Kuo, L. Y.; Kuhn, S.; Ly, D. *Inorg. Chem.* **1995**, *34*, 5341-5345.
- (82) Lee, G.; Oka, M.; Takemura, H.; Miyahara, Y.; Shimizu, N.; Inazu, T. *J. Org. Chem.* **1996**, *61*, 8304-8306.
- (83) Pyle, A. M. *Interactions of metal ions with nucleotides, nucleic acids, and their constituents*; Marcel Dekker, Inc.: New York, 1996; Vol. 32.
- (84) Wang, Q.; Leino, E.; Jancsó, A.; Szilágyi, I.; Gajda, T.; Hietamäki, E.; Lönnberg, H. *ChemBioChem* **2008**, *9*, 1739-1748.
- (85) Takebayashi, S.; Shinkai, S.; Ikeda, M.; Takeuchi, M. *Org. Biomol. Chem.* **2008**, *6*, 493-499.
- (86) Cacciapaglia, R.; Casnati, A.; Mandolini, L.; Peracchi, A.; Reinhoudt, D. N.; Salvio, R.; Sartori, A.; Ungaro, R. *J. Am. Chem. Soc.* **2007**, *129*, 12512-12520.
- (87) Williams, N. H.; Feng, G.; Mareque-Rivas, J. C.; Rosales, R. T. M. d. *J. Am. Chem. Soc.* **2005**, *127*, 13470-13471.
- (88) Gupta, T.; Baranwal, B. P. *Transit. Metal Chem.* **2006**, *31*, 123-128.
- (89) Feng, G.; Mareque-Rivas, J. C.; Williams, N. H. *Chem. Commun.* **2006**, 1845-1847.
- (90) Feng, G.; Natale, D.; Prabaharn, R.; Mareque-Rivas, J. C.; Williams, N. H. *Angew. Chem. Int. Edit.* **2006**, *45*, 7056-7059.
- (91) Iranzo, O.; Elmer, T.; Richard, J. P.; Morrow, J. R. *Inorg. Chem.* **2003**, *42*, 7737-7746.
- (92) Wang, Q.; Lonnberg, H. *J. Am. Chem. Soc.* **2006**, *128*, 10716-10728.

- (93) Cowan, J. A. *Curr. Opin. Chem. Biol.* **2001**, 5, 634-642.
- (94) Lauffer, R. B. *Chem. Rev.* **1987**, 87, 901-927.
- (95) Caravan, P.; Ellison, J. J.; McMurry, T. J.; Lauffer, R. B. *Chem. Rev.* **1999**, 99, 2293-2352.
- (96) Eichhorn, G. L.; Butwoz, J. J. *Biopolymers* **1965**, 3, 79-54.
- (97) Ciesiolka, J.; Marciniak, T.; Krzyzosiak, W. J. *Eur. J. Biochem.* **1989**, 182, 445-450.
- (98) Komiyama, M.; Masumura, K.; Matsumoto, Y. *J. Chem. Soc. Chem. Commun.* **1992**, 640-641.
- (99) Hurst, P.; Takasani, B. K.; Chin, J. *J. Am. Chem. Soc.* **1996**, 118, 9982-9983.
- (100) Mulready, S. *PhD. Thesis*; University of Dublin, Trinity College, 2004.
- (101) Harte, A. *PhD. Thesis*; University of Dublin, Trinity College, 2005.
- (102) Fanning, A.-M. *PhD. Thesis*; University of Dublin, Trinity College, 2005.
- (103) Chang, C. A.; Wu, B. H.; Kuan, B. Y. *Inorg. Chem.* **2005**, 44, 6646-6654.
- (104) Sanchez-Lombardo, I.; Yatsimirsky, A. K. *Inorg. Chem.* **2008**, 47, 2514-2525.
- (105) Neverov, A. A.; Lu, Z. L.; Maxwell, C. I.; Mohamed, M. F.; White, C. J.; Tsang, J. S. W.; Brown, R. S. *J. Am. Chem. Soc.* **2006**, 128, 16398-16405.
- (106) Liu, C. T.; Neverov, A. A.; Brown, R. S. *Inorg. Chem.* **2007**, 46, 1778-1788.
- (107) Gibson, G. T. T.; Mohamed, M. F.; Neverov, A. A.; Brown, R. S. *Inorg. Chem.* **2006**, 45, 7891-7902.
- (108) Czerwinski, R. M.; Harris, T. K.; Massiah, M. A.; Mildvan, A. S.; Whitman, C. P. *Biochemistry* **2001**, 40, 1984-1995.
- (109) Cleland, W. W.; Frey, P. A.; Gerlt, J. A. *J. Biol. Chem.* **1998**, 273, 25529-25532.
- (110) Gomez-Tagle, P.; Vargas-Zuniga, I.; Taran, O.; Yatsimirsky, A. K. *J. Org. Chem.* **2006**, 71, 9713-9722.
- (111) Baker, B. F.; Lot, S. S.; Kringel, J.; Cheng-Flournoy, S.; Villiet, P.; Sasmor, H. M.; Siwkowski, A. M.; Chappell, L. L.; Morrow, J. R. *Nucleic Acids Res.* **1999**, 27, 1547-1551.
- (112) Epstein, D. M.; Chappell, L. L.; Khalili, H.; Supkowski, R. M.; Horrocks, W. D., Jr.; Morrow, J. R. *Inorg. Chem.* **2000**, 39, 2130-2134.
- (113) Huang, L.; Chappell, L. L.; Iranzo, O.; Baker, B. F.; Morrow, J. R. *J. Biol. Inorg. Chem.* **2000**, 5, 85-92.
- (114) Woods, M.; Woessner, D. E.; Zhao, P.; Pasha, A.; Yang, M. Y.; Huang, C. H.; Vasalitiy, O.; Morrow, J. R.; Sherry, A. D. *J. Am. Chem. Soc.* **2006**, 128, 10155-10162.

- (115) Farquhar, E. R.; Richard, J. P.; Morrow, J. R. *Inorg. Chem.* **2007**, *46*, 7169-7177.
- (116) Jubian, V.; Dixon, R. P.; Hamilton, A. D. *J. Am. Chem. Soc.* **1992**, *114*, 1120-1121.
- (117) Aguilar-Perez, F.; Gomez-Tagle, P.; Collado-Fregoso, E.; Yatsimirsky, A. K. *Inorg. Chem.* **2006**, *45*, 9502-9517.
- (118) Torres, J.; Brusoni, M.; Peluffo, F.; Kremer, C.; Domínguez, S.; Mederos, A.; Kremer, E. *Inorg. Chim. Acta* **2005**, *358*, 3320-3328.
- (119) Gunnlaugsson, T.; Davies, R. J. H.; Nieuwenhuyzen, M.; Stevenson, C. S.; Viguiier, R.; Mulready, S. *Chem. Commun.* **2002**, 2136-2137.
- (120) Gunnlaugsson, T.; Davies, R. J. H.; Nieuwenhuyzen, M.; O'Brien, J. E.; Stevenson, C. S.; Mulready, S. *Polyhedron* **2003**, *22*, 711-724.
- (121) Gunnlaugsson, T.; Nieuwenhuyzen, M.; Nolan, C. *Polyhedron* **2003**, *22*, 3231-3242.
- (122) Fanning, A.-M.; Plush, S. E.; Gunnlaugsson, T. *Chem. Commun.* **2006**, *36*, 3791-3793.
- (123) Leonard, J. P.; Santos, C. M. G. d.; Plush, S. E.; McCabe, T.; Gunnlaugsson, T. *Chem. Commun.* **2007**, 129-131.
- (124) Santos, C. M. G. d.; Fernandez, P. B.; Plush, S. E.; Leonard, J. P.; Gunnlaugsson, T. *Chem. Commun.* **2007**, 3389-3391.
- (125) Senechal-David, K.; Leonard, J. P.; Plush, S. E.; Gunnlaugsson, T. *Org. Lett.* **2006**, *8*, 2727-2730.
- (126) Harte, A. J.; Jensen, P.; Plush, S. E.; Kruger, P. E.; Gunnlaugsson, T. *Inorg. Chem.* **2006**, *45*, 9465-9474.
- (127) McCoy, C. P.; Stomeo, F.; Plush, S. E.; Gunnlaugsson, T. *Chem. Mater.* **2006**, *18*, 4336-4343.
- (128) Gunnlaugsson, T.; Harte, A. *Org. Biomol. Chem.* **2006**, *4*, 1572-1579.
- (129) Anda, C.; Bencini, A.; Berni, E.; Ciattini, S.; Chuburu, F.; Danesi, A.; Giorgi, C.; Handel, H.; Baccon, M. L.; Paoletti, P.; Tripier, R.; Turcry, V.; Valtancoli, B. *Eur. J. Inorg. Chem.* **2005**, *2005*, 2044-2053.
- (130) Hancock, R. D. *Chem. Rev.* **1989**, *89*, 1875-1914.
- (131) Knör, S.; Modlinger, A.; Poethko, T.; Schottelius, M.; Wester, H.-J.; Kessler, H. *Chem. Eur. J.* **2007**, *13*, 6082-6090.
- (132) Aoki, S.; Kawatani, H.; Goto, T.; Kimura, E.; Shiro, M. *J. Am. Chem. Soc.* **2001**, *123*, 1123-1132.

- (133) Woods, M.; Kiefer, G. E.; Bott, S.; Castillo-Muzquiz, A.; Eshelbrenner, C.; Michaudet, L.; McMillan, K.; Mudigunda, S. D. K.; Ogrin, D.; Tircso, G.; Zhang, S.; Zhao, P.; Sherry, A. D. *J. Am. Chem. Soc.* **2004**, *126*, 9248-9256.
- (134) Massue, J.; Bonnet, C. S.; Plush, S. E.; Moore, D. A.; Gunnlaugsson, T. *Tetrahedron Lett.* **2008**.
- (135) LeBaccon, M.; Chuburu, F.; Toupet, L.; Handel, H.; Soibinet, M.; Dechamps-Olivier, I.; Barbier, J.-P.; Alpincourt, M. *New. J. Chem.* **2001**, *25*, 1168-1174.
- (136) Brandes, S.; Gros, C.; Denat, F.; Pullumbi, P.; Guillard, R. B. *Soc. Chim. Fr.* **1996**, *133*, 65-73.
- (137) Li, C.; Wong, W.-T. *Tetrahedron* **2004**, *60*, 5595-5601.
- (138) Develay, S.; Tripier, R.; Le Baccon, M.; Patinec, V.; Serratrice, G.; Handel, H. *Dalton Trans.* **2005**, 3016-3024.
- (139) Mareque-Rivas, J. C.; Rosales, R. T. M. d.; Parsons, S. *Chem. Commun.* **2004**, 610-611.
- (140) Newcomb, M.; Gokel, G. W.; Cram, D. J. *J. Am. Chem. Soc.* **1974**, *96*, 6810-6810.
- (141) Yoo, J.; Reichert, D. E.; Welch, M. J. *Chem. Commun.* **2003**, 766-767.
- (142) Boldrini, V.; Giovenzana, G. B.; Pagliarin, R.; Palmisano, G.; Sisti, M. *Tetrahedron Lett.* **2000**, *41*, 6527-6530.
- (143) Gunnlaugsson, T.; Leonard, J. P.; Mulready, S.; Nieuwenhuyzen, M. *Tetrahedron Lett.* **2004**, *60*, 105-113.
- (144) Powell, D. H.; Dhubhghaill, O. M. N.; Pubanz, D.; Helm, L.; Lebedev, Y. S.; Schlaepfer, W.; Merbach, A. E. *J. Am. Chem. Soc.* **1996**, *118*, 9333-9346.
- (145) Horrocks, W. D.; Sudnick, J. D. S. *Accounts Chem. Res.* **1981**, *14*, 384-392.
- (146) Supkowski, R. M.; Horrocks, W. D. *Inorg. Chim. Acta* **2002**, *340*, 44-48.
- (147) Beeby, A.; Clarkson, I. M.; Dickins, R. S.; Faulkner, S.; Parker, D.; Royle, L.; Sousa, A. d.; Williams, J. A. G.; Woods, M. *J. Chem. Soc. Perk. T. 2* **1999**, 493-503.
- (148) Trost, B. M.; Yeh, V. S. C.; Ito, H.; Bremeyer, N. *Org. Lett.* **2002**, *4*, 2621-2623.
- (149) Greene, T. W.; Wuts, P. G. M. *Protective groups in organic synthesis*; 3rd ed.; John Wiley & Sons Inc.: New York, 1999.
- (150) Pearson, A. J.; Roush, W. J. *Activating Agents and Protecting Groups*; 1st ed.; John Wiley & Sons Ltd.: Chichester, 1999.
- (151) Krapcho, A. P.; Kuell, C. S. *Synthetic Commun.* **1990**, *20*, 2559-2564.
- (152) Katalin, S.; Michel, G.; Gábor, S.; Etelka, F.; Marius, R. *Eur. J. Inorg. Chem.* **2006**, *2006*, 1022-1031.

- (153) Bencinia, A.; Bianchi, A.; Garcia-España, E.; Micheloni, M.; Antonio Ramirez, J. *Coord. Chem. Rev.* **1999**, 188, 97-156.
- (154) Martell, A. E.; Hancock, R. D. *Metal complexes in aqueous solutions*; Plenum Press: New York, 1996.
- (155) Kalman, F. K.; Woods, M.; Caravan, P.; Jurek, P.; Spiller, M.; Tircso, G.; Kiraly, R.; Brucher, E.; Sherry, A. D. *Inorg. Chem.* **2007**, 46, 5260-5270.
- (156) Gans, P.; Sabatini, A.; Vacca, A. *Talanta* **1996**, 1739-1753.
- (157) Martell, A. E.; Smith, R. M.; Motekaitis, R. J. *Critically Selected Stability Constants of Metal Complexes Database Version 8.0*, 2004.
- (158) Kalman, F. K.; Woods, M.; Caravan, P.; Jurek, P.; Spiller, M.; Tircso, G.; Kiraly, R.; Brucher, E.; Sherry, A. D. *Inorg. Chem.* **2007**, 46, 5260-5270.
- (159) Azhar, P.; Gyula, T.; Tircsóné Benyó, E.; Brücher, E.; Sherry, A. D. *Eur. J. Inorg. Chem.* **2007**, 4340-4349.
- (160) Maumela, H.; Hancock, R. D.; Carlton, L.; Reibenspies, J. H.; Wainwright, K. P. *J. Am. Chem. Soc.* **1995**, 117, 6698-6707.
- (161) Clayden, J.; Greeves, N.; Warren, S.; Wothers, P. *Organic Chemistry*; Oxford Press: Oxford, 2000.
- (162) Lehn, J. M.; Sauvage, J. P. *J. Am. Chem. Soc.* **1975**, 97, 6700-6707.
- (163) Cassano, A. G.; Anderson, V. E.; Harris, M. E. *J. Am. Chem. Soc.* **2002**, 124, 10964-10965.
- (164) Brown, D. M.; Usher, D. A. *J. Chem. Soc.* **1965**, 6558.
- (165) Gunnlaugsson, T.; Harte, A. *Org. Biomol. Chem.* **2006**, 4, 1572-1579.
- (166) Amin, S.; Voss, D. A.; Horrocks, W. D.; Lake, C. H.; Churchill, M. R.; Morrow, J. R. *Inorg. Chem.* **1995**, 34, 3294-3300.
- (167) Richard, J. P.; Morrow, J. R.; O'Donoghue, A. C.; Pyun, S. Y. *B. Kor. Chem. Soc.* **2004**, 25, 403-406.
- (168) Amin, S.; Voss, D. A.; Horrocks, W. D.; Morrow, J. R. *Inorg. Chem.* **1996**, 35, 7466-7467.
- (169) Hynes, M. J. *J. Chem. Soc. Dalton Trans.* **1993**, 311-312.
- (170) Bisson, A. P.; Hunter, C. A.; Morales, J. C.; Young, K. *Chem. Eur. J.* **1998**, 4, 845-851.
- (171) Moore, D. A.; Harte, A. J.; Gunnlaugsson, T. In *J. Biol. Inorg. Chem.; 13th International Conference on Biological Inorganic Chemistry*; Vienna, Austria, 2007; Vol. 12(S:1), p S174 (P362).

- (172) Nwe, K.; Andolina, C. M.; Morrow, J. R. *J. Am. Chem. Soc.* **2008**, *130*, 14861-14871.
- (173) Schneider, H.-J.; Yatsimirsky, A. K. In *The lanthanides and their interrelations with biosystems*; Sigel, A., Sigel, H., Eds.; Marcel Dekker Inc.: New York, 2003; Vol. 40, p 394-475.
- (174) Martell, A. E.; Smith, R. M. *Critical Stability Constants*; Plenum Press: New York, 1974.
- (175) Breslow, R.; Huang, D. L.; Anslyn, E. P. *Natl. Acad. Sci. USA* **1989**, *86*, 1746-1750.
- (176) Oivanen, M.; Kuusela, S.; Lonnberg, H. *Chem. Rev.* **1998**, *98*, 961-990.
- (177) Koike, T.; Kimura, E. *J. Am. Chem. Soc.* **1991**, *113*, 8935-8941.
- (178) Zhu, L.; dos Santos, O.; Koo, C. W.; Rybstein, M.; Pape, L.; Canary, J. W. *Inorg. Chem.* **2003**, *42*, 7912-7920.
- (179) Vogel, A. I.; Tatchell, A. R.; Furnis, B. S.; Hannaford, A. J.; Smith, P. W. G. *Vogel's Textbook of Practical Organic Chemistry*; 5th ed.; Prentice Hall: New York, 1996.
- (180) Parker, D.; Williams, J. A. G. *J. Chem. Soc. Perk. T. 2* **1995**, 1305.
- (181) Xiang, Q. X.; J.Zhang; Liu, P. Y.; Xia, C. Q.; Zhou, Z. Y.; Xie, R. G.; Yu, X. Q. *J. Inorg. Biochem.* **2005**, *99*, 1661-1669.
- (182) Xiang, Q. X.; Yu, X. Q.; Wu, J.; Liu, P. Y.; Xia, C. Q.; Xie, R. G. *Chinese J. Chem.* **2003**, *21*, 910-916.

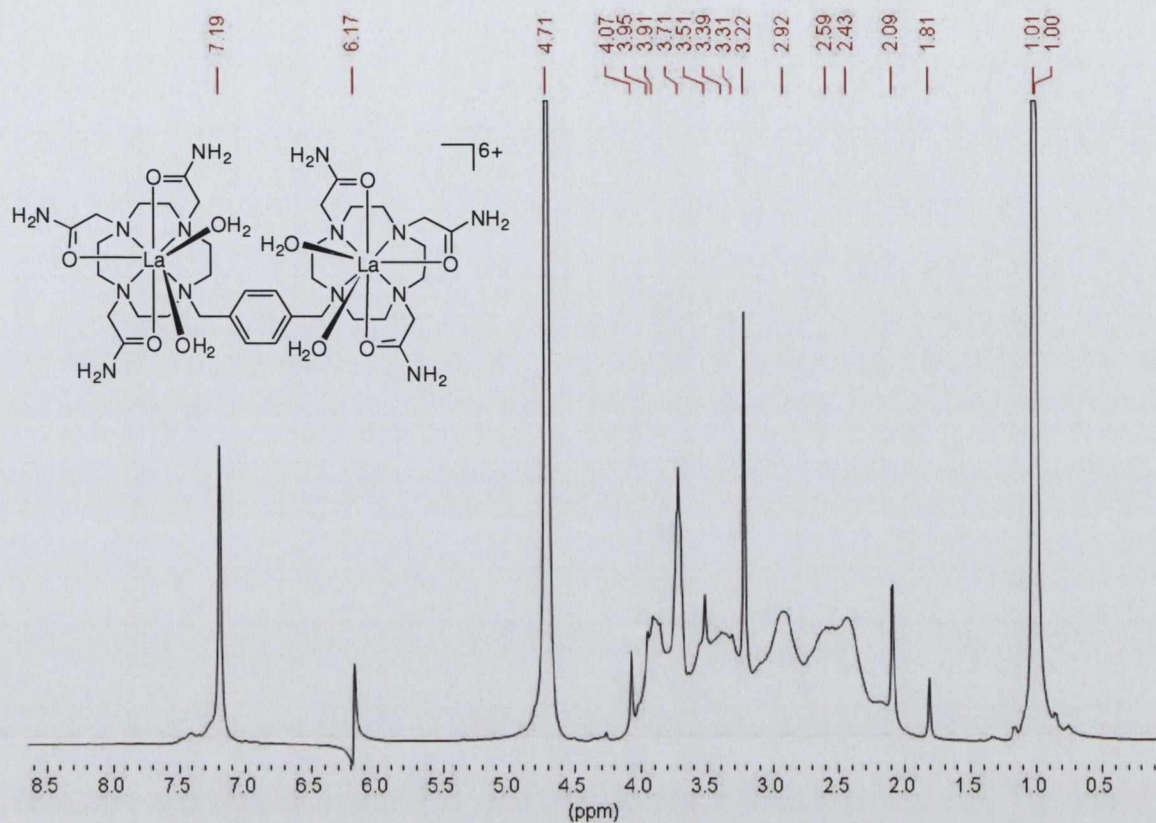


Figure A1.1: The ^1H NMR spectrum (400 MHz, D_2O) of **41.La₂**, showing the broadening of the resonances as a result of the complexation to the large La(III) ion.

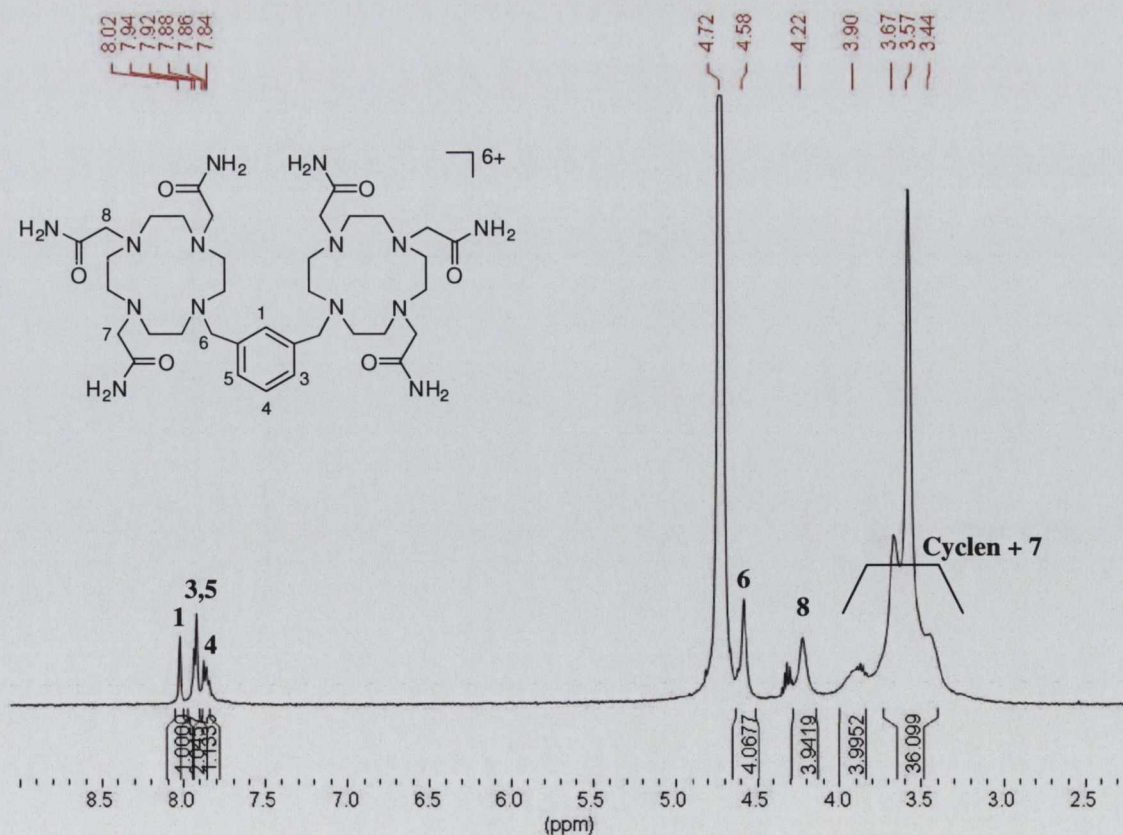


Figure A1.2: The ^1H NMR spectrum (400 MHz, D_2O) of **43** at 60 °C showing the integration of the ligand.

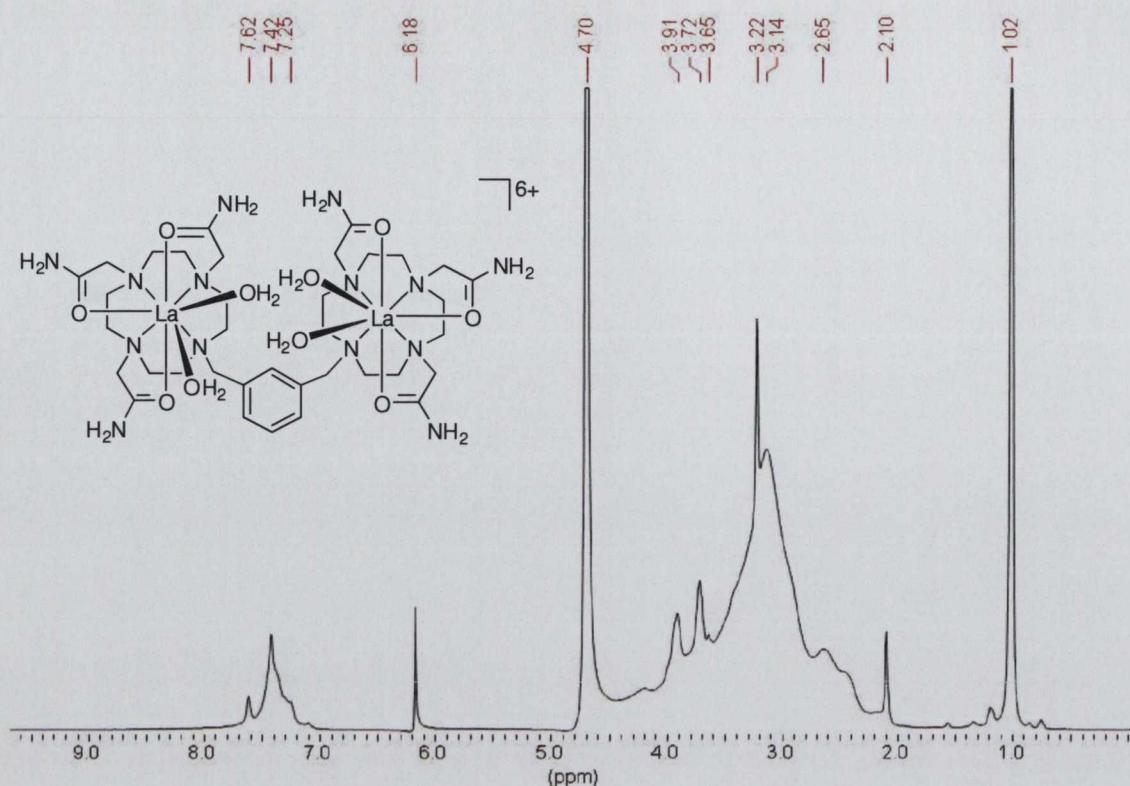


Figure A1.3: The 1H NMR spectrum (400 MHz, D_2O) of $43.La_2$, showing the broadening of the resonances as a result of the complexation of the ligand to the large $La(III)$ ion.

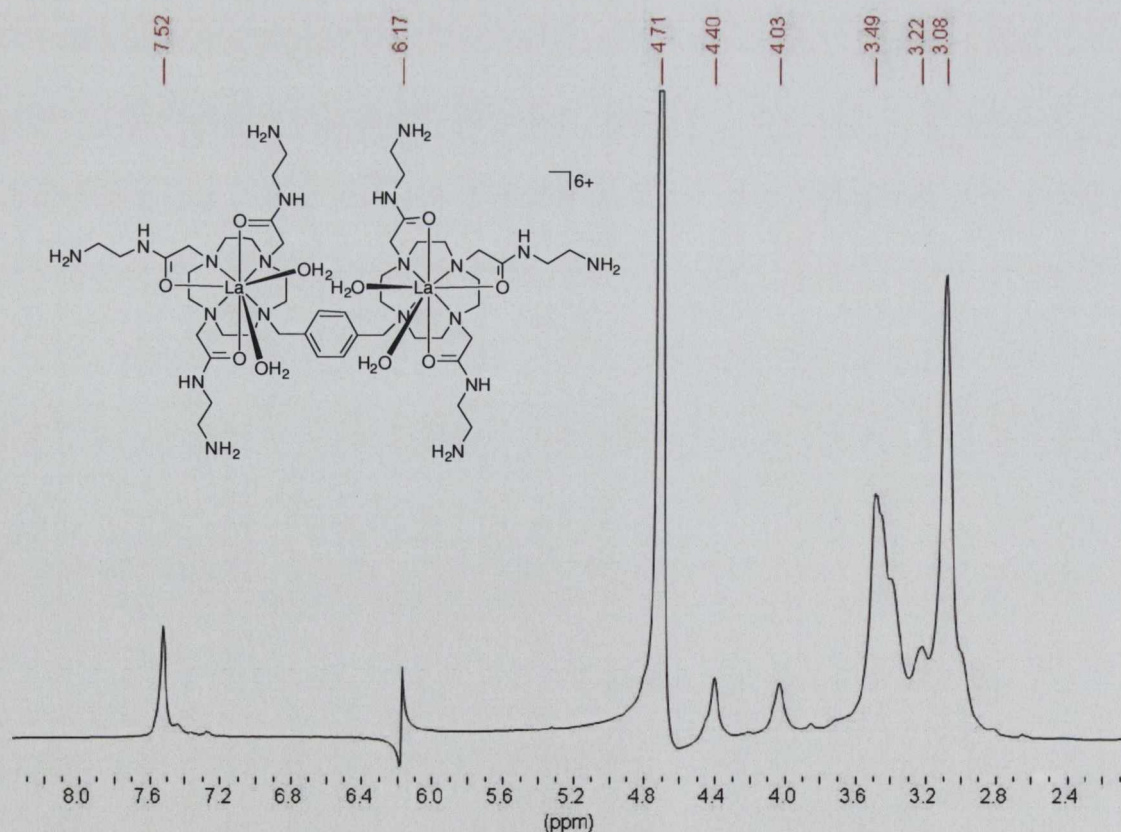


Figure A1.4: The 1H NMR spectrum (400 MHz, D_2O) of $42.La_2$, showing the broadening of the resonances as a result of the complexation of the ligand to the large $La(III)$ ion.

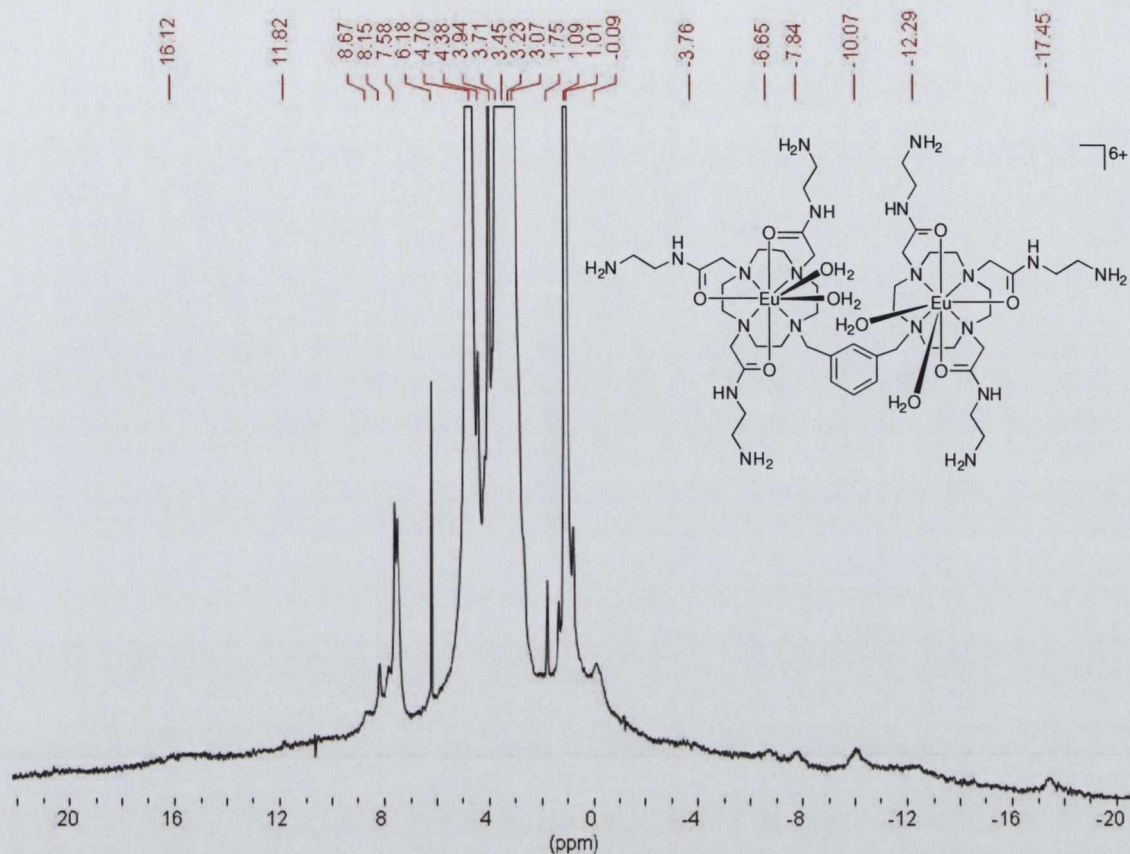


Figure A1.5: The ^1H NMR spectrum (400 MHz, D_2O) of $44.\text{Eu}_2$, showing the characteristic shifting and broadening of the resonances due to the complexation of the paramagnetic $\text{Eu}(\text{III})$ ion.

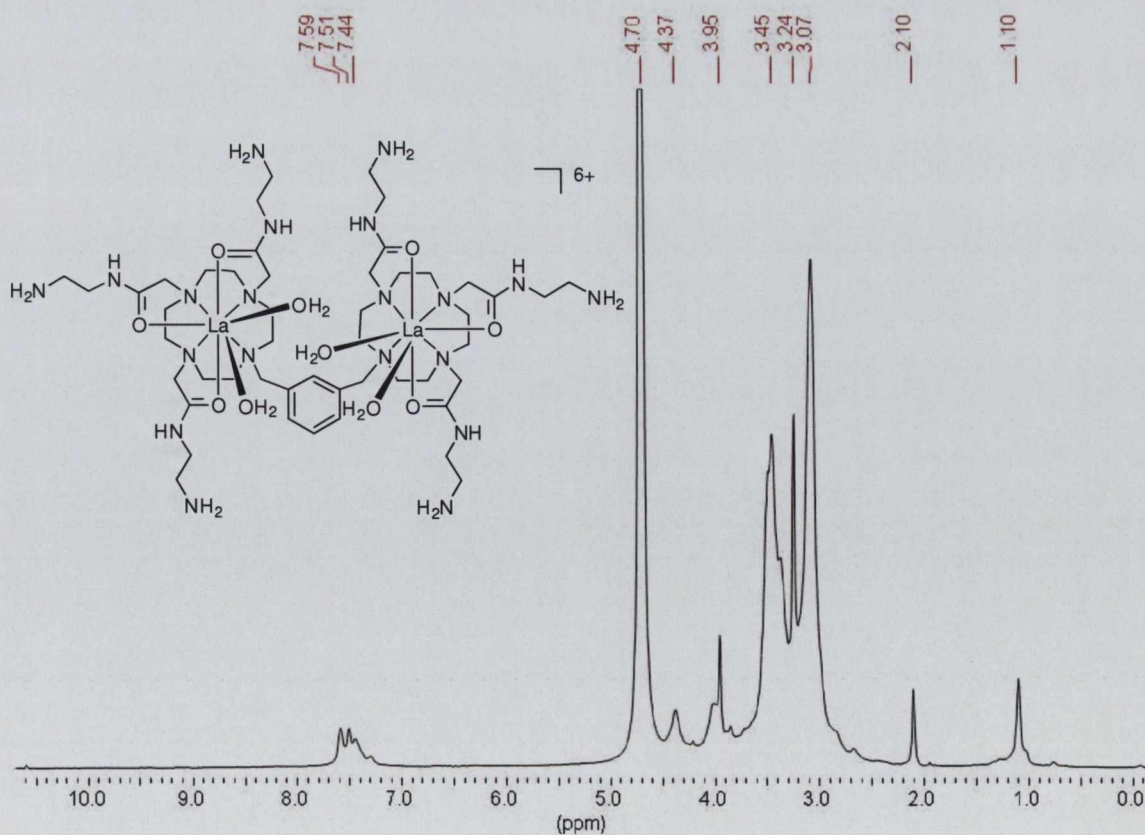


Figure A1.6: The ^1H NMR spectrum (400 MHz, D_2O) of $44.\text{La}_2$, showing the broadening of the resonances as a result of the complexation of the ligand to the large $\text{La}(\text{III})$ ion.

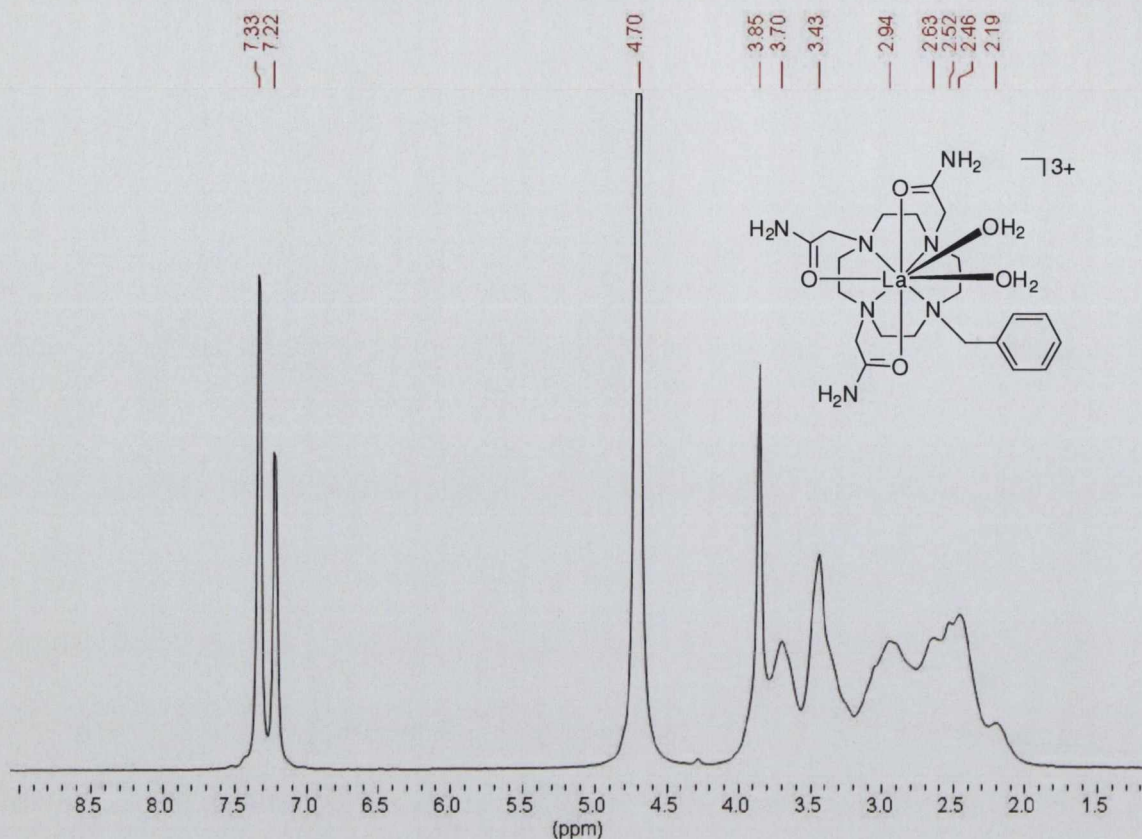


Figure A1.7: The 1H NMR spectrum (400 MHz, D_2O) of $47.La$, showing the broadening of the resonances as a result of the complexation of the ligand to the large $La(III)$ ion.

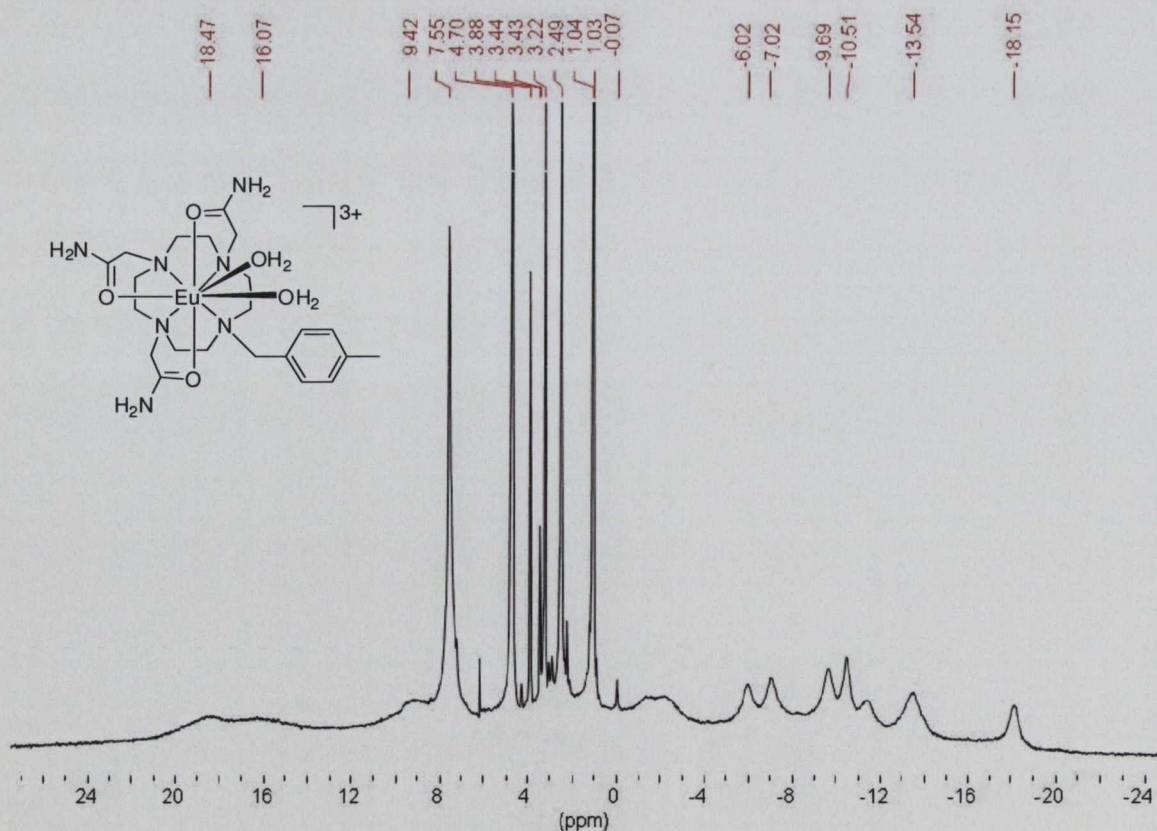


Figure A1.8: The 1H NMR spectrum (400 MHz, D_2O) of $48.Eu$, showing the characteristic shifting and broadening of the resonances due to the complexation of the paramagnetic $Eu(III)$ ion.

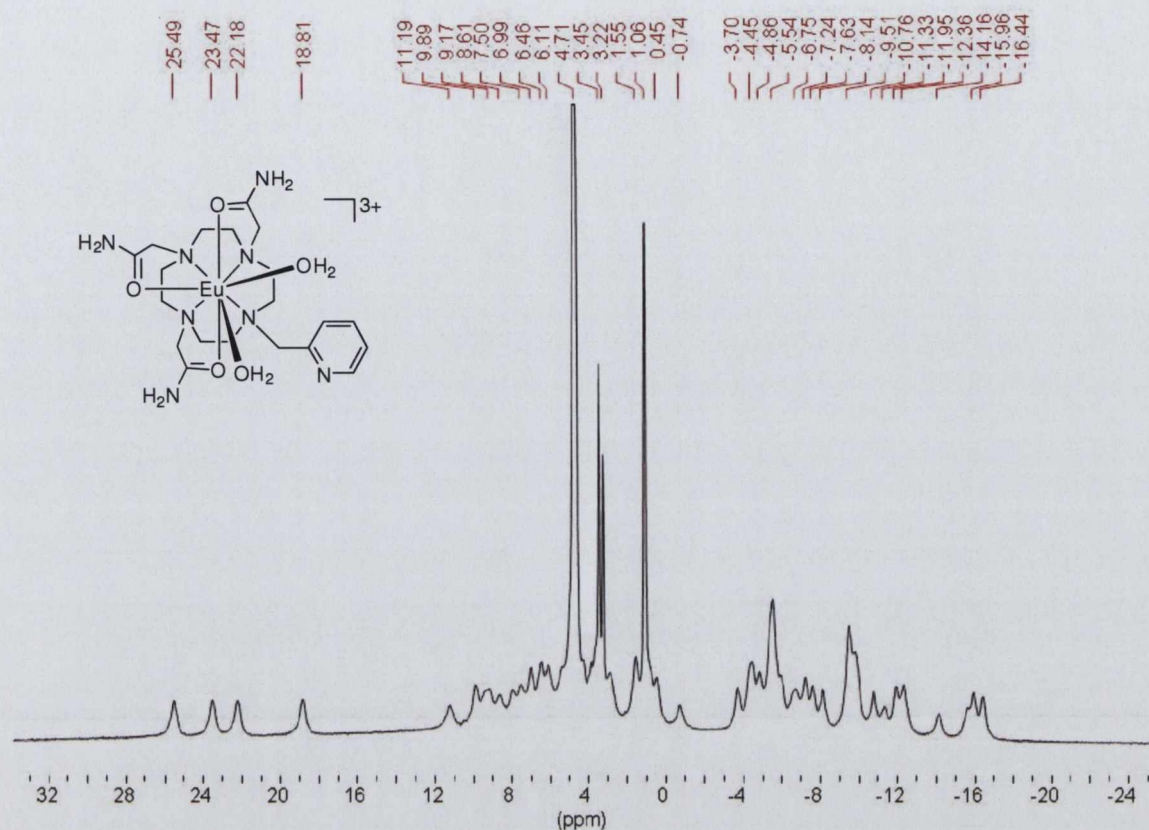


Figure A1.9: The ^1H NMR spectrum (400 MHz, D_2O) of **49.Eu**, showing the characteristic shifting and broadening of the resonances due to the complexation of the paramagnetic Eu(III) ion.

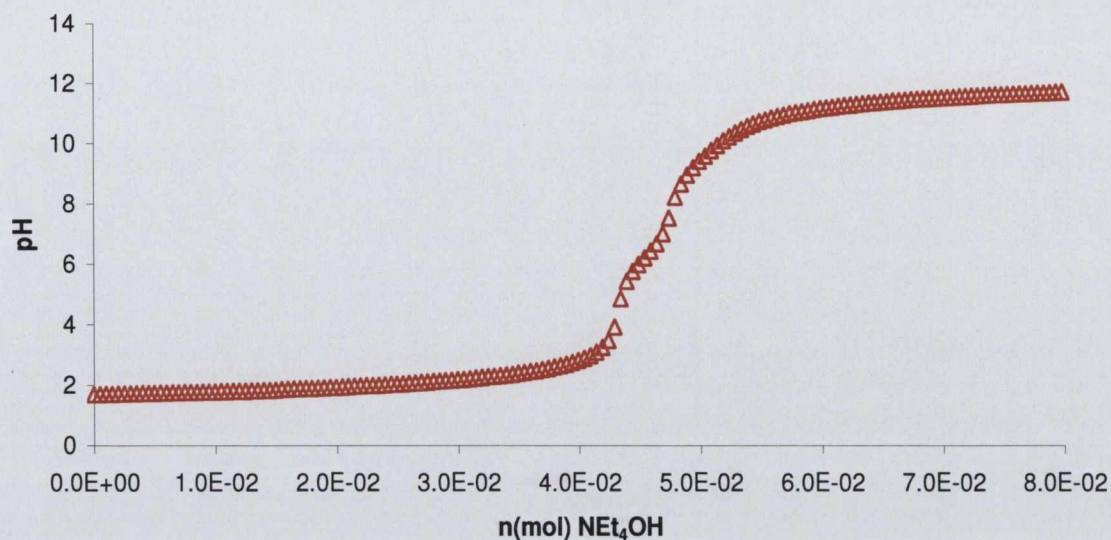


Figure A2.1: Typical titration curve of the protonated ligand **43** against NEt_4OH at 25 °C. $[\mathbf{43}] = 6.4 \times 10^{-4} \text{ M}$, $[\text{H}^+] = 1.2 \times 10^{-2} \text{ M}$, $[\text{NEt}_4\text{OH}] = 0.09 \text{ M}$, $I = 0.10 \text{ M}$ (NEt_4ClO_4).

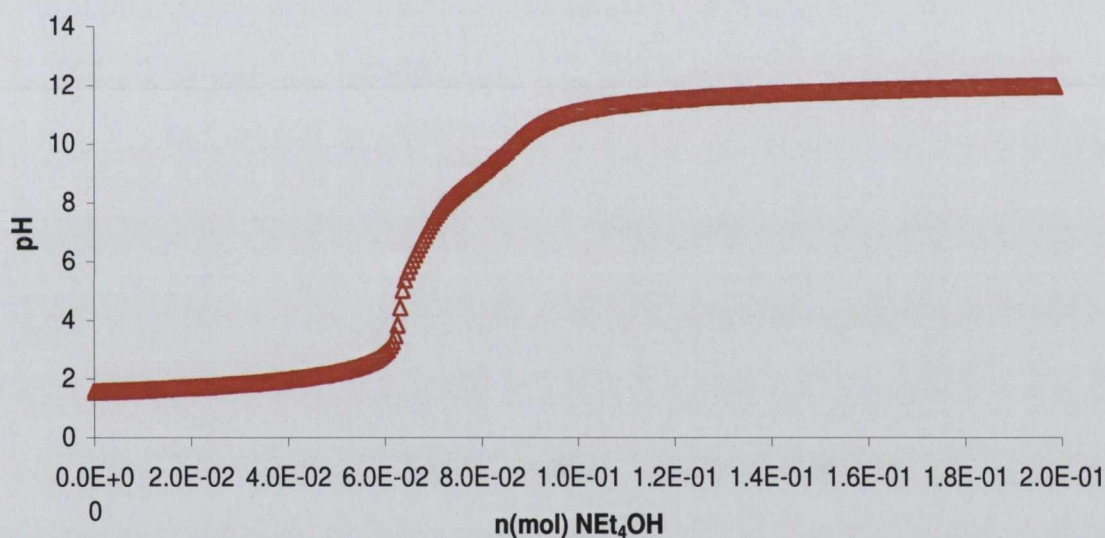


Figure A2.2: Typical titration curve of the protonated ligand **44** against NEt_4OH at 25 °C. $[\mathbf{44}] = 1.0 \times 10^{-3} \text{ M}$, $[\text{H}^+] = 3.0 \times 10^{-2} \text{ M}$, $[\text{NEt}_4\text{OH}] = 0.10 \text{ M}$, $I = 0.10 \text{ M}$ (NEt_4ClO_4).

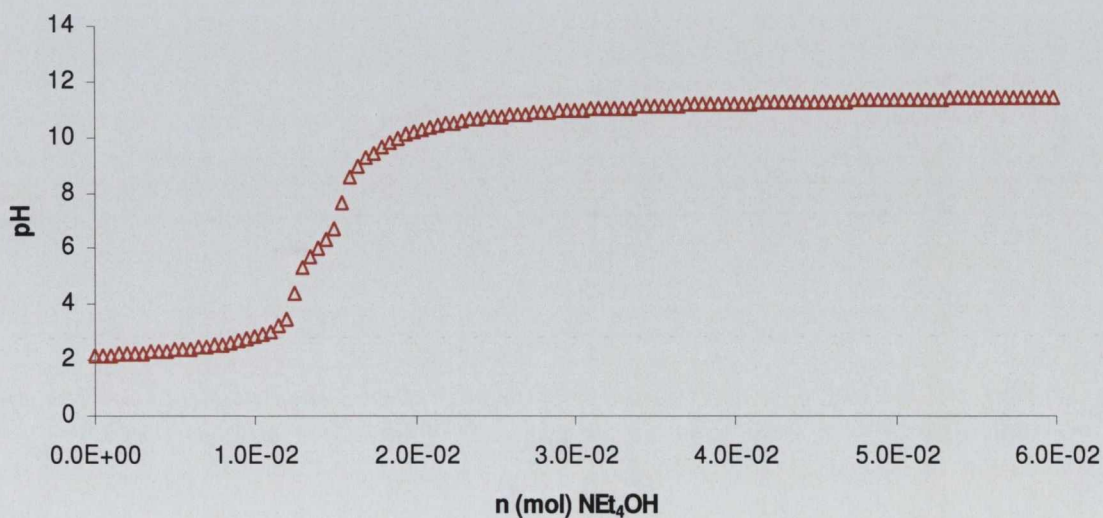


Figure A2.3: Typical titration curve of the protonated ligand **47** against NEt_4OH at 25 °C. $[\mathbf{47}] = 9.3 \times 10^{-4} \text{ M}$, $[\text{H}^+] = 7.9 \times 10^{-3} \text{ M}$, $[\text{NEt}_4\text{OH}] = 0.10 \text{ M}$, $I = 0.10 \text{ M}$ (NEt_4ClO_4).

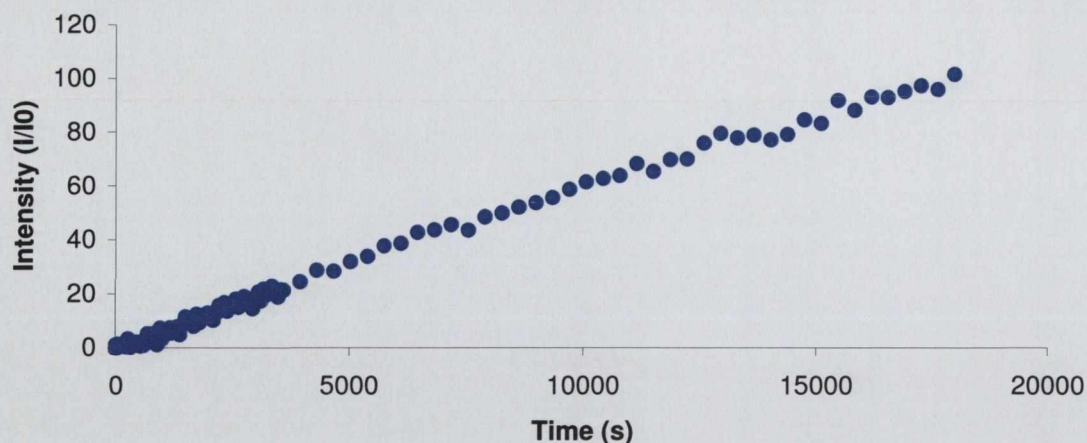


Figure A2.4: Kinetic monitoring of a solution of **42** ($1.0 \times 10^{-4} \text{ M}$) and in the presence of two equivalents of $\text{Eu}(\text{CF}_3\text{SO}_3)_3$ ($1.5 \times 10^{-2} \text{ M}$). Indirect excitation of $\text{Eu}(\text{III})$ at 266 nm (xylyl spacer of **42**).

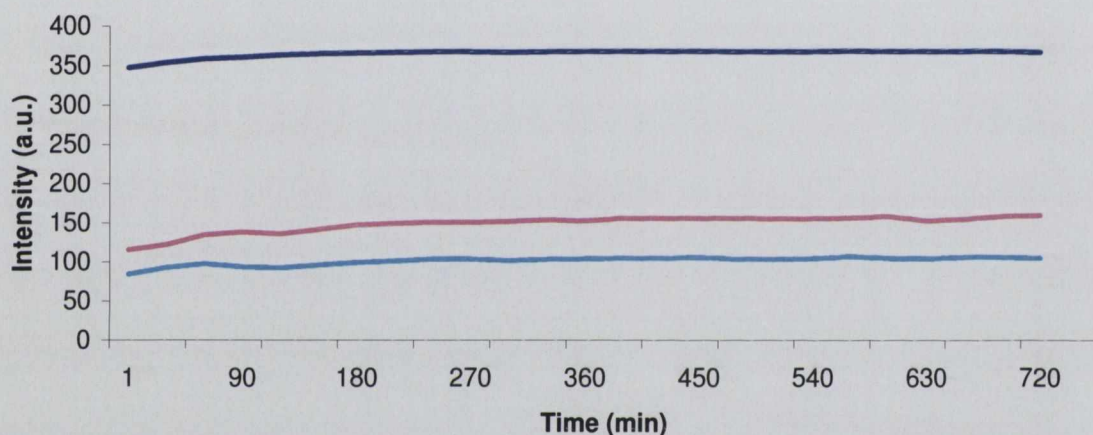


Figure A2.5: Kinetic monitoring of a solution of **42.Eu₂** ($1.2 \times 10^{-4} \text{ M}$) in 50 mM HEPES buffer, pH 7.4. Indirect excitation of $\text{Eu}(\text{III})$ at 266 nm (xylyl spacer of **42**).

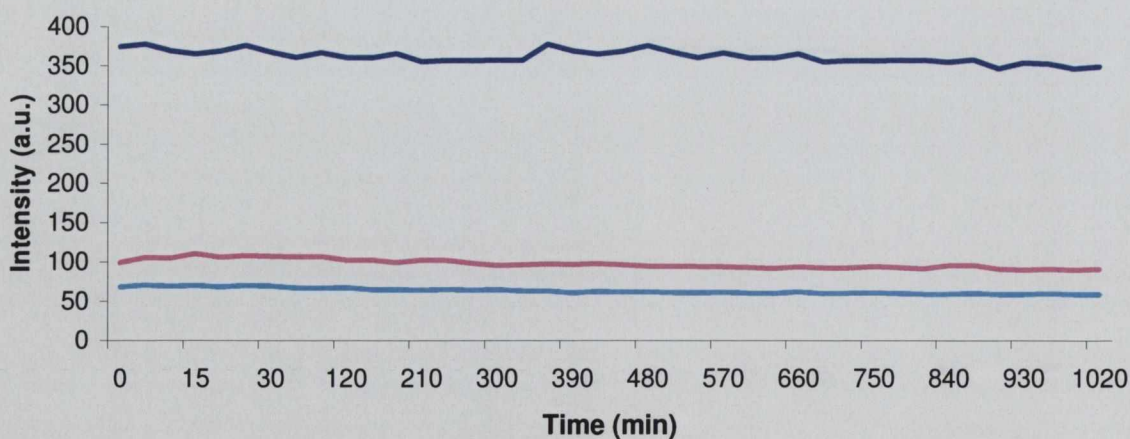


Figure A2.6: Kinetic monitoring of a solution of **42.Eu₂** ($1.2 \times 10^{-4} \text{ M}$) in the presence of EDTA ($1.5 \times 10^{-2} \text{ M}$), in 50 mM HEPES buffer, pH 7.4. Indirect excitation of $\text{Eu}(\text{III})$ at 266 nm (xylyl spacer of **42**).

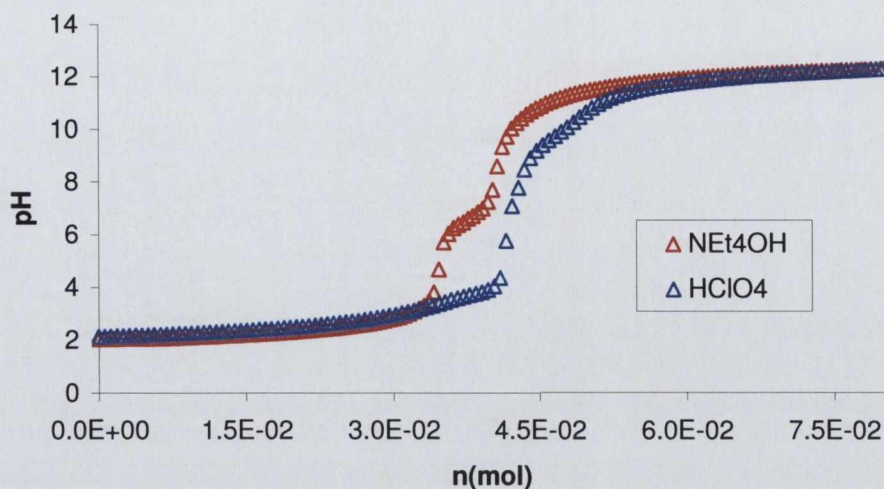


Figure A2.7: Titration curves for **47.La** (1×10^{-3} M) against NEt_4OH (0.10 M), followed by reverse titration against HClO_4 (0.10 M), in $I = 0.10$ M NEt_4OH .

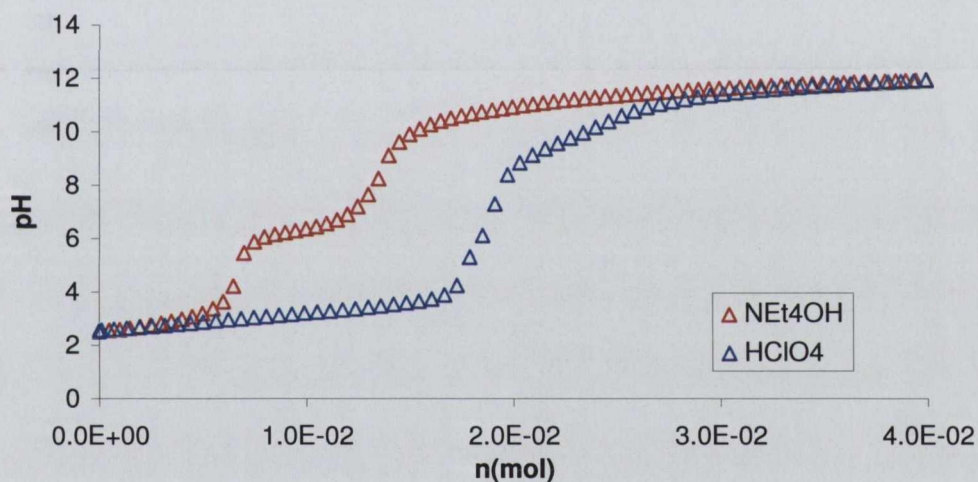


Figure A2.8: Titration curves for **48.Eu** (1×10^{-3} M) against NEt_4OH (0.09 M), followed by reverse titration against HClO_4 (0.10 M), in $I = 0.10$ M NEt_4OH .

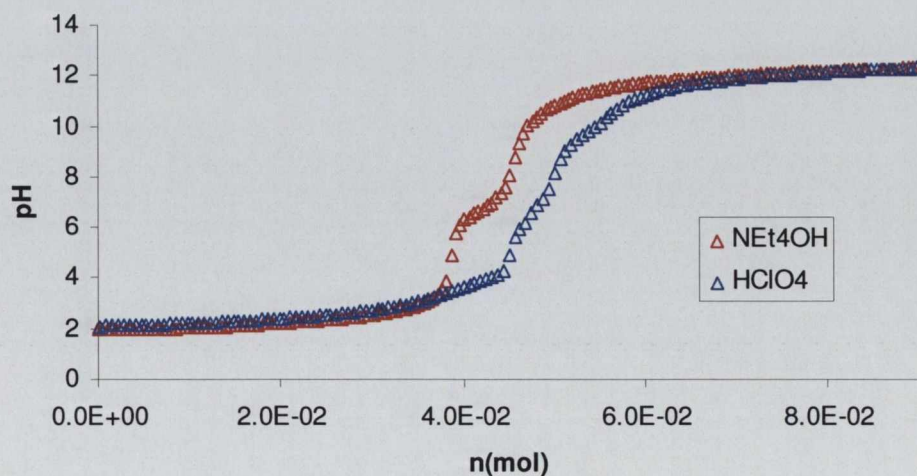


Figure A2.9: Titration curves for **48.La** (1×10^{-3} M) against NEt_4OH (0.10 M), followed by reverse titration against HClO_4 (0.10 M), in $I = 0.10$ M NEt_4OH .

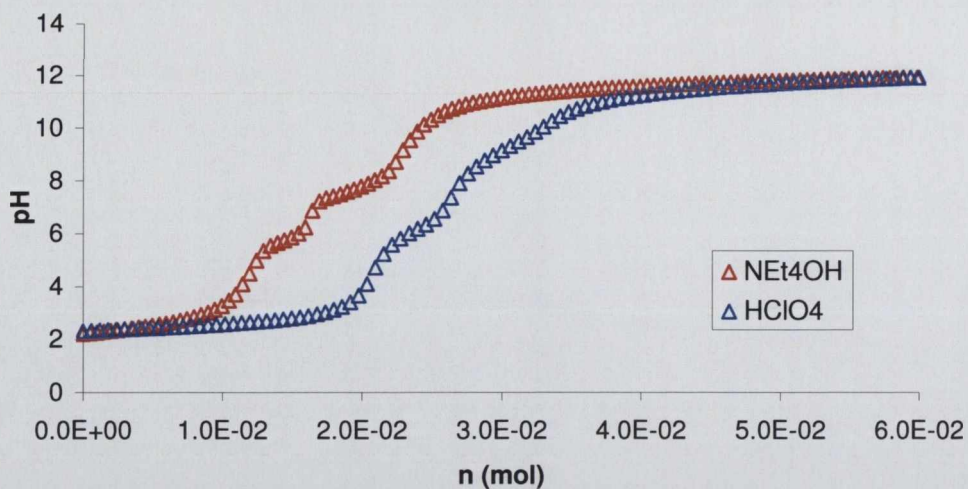


Figure A2.10: Titration curves for $39.Eu_2$ (1×10^{-3} M) against NEt_4OH (0.095 M), followed by reverse titration against $HClO_4$ (0.10 M), in $I = 0.10$ M NEt_4OH .

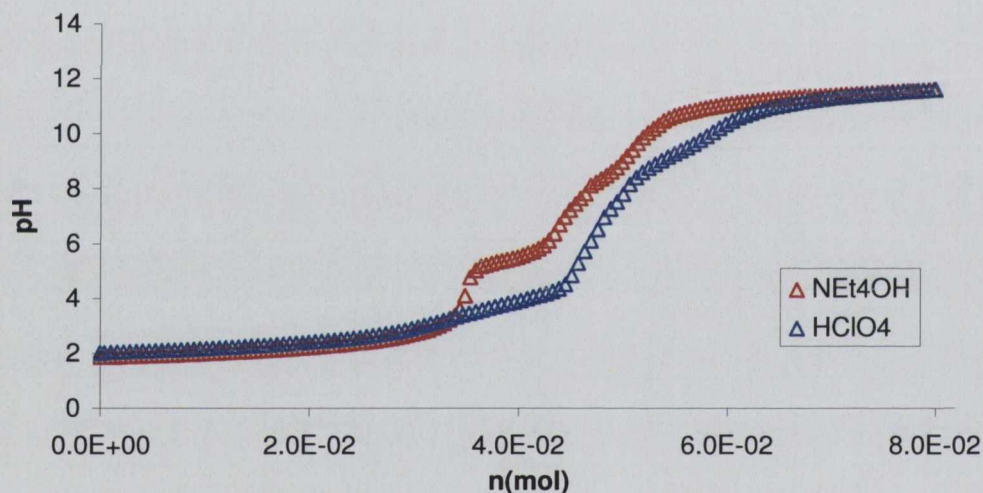


Figure A2.11: Titration curves for $39.La_2$ (1×10^{-3} M) against NEt_4OH (0.095 M), followed by reverse titration against $HClO_4$ (0.10 M), in $I = 0.10$ M NEt_4OH .

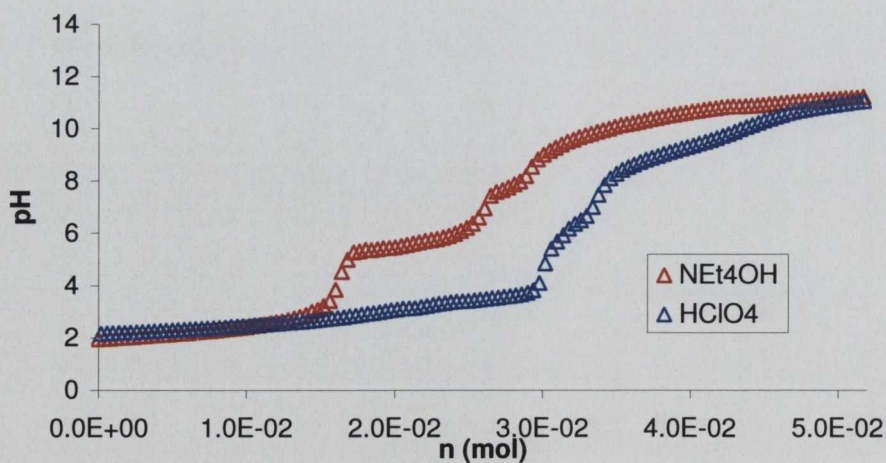


Figure A2.12: Titration curves for $43.Eu_2$ (1×10^{-3} M) against NEt_4OH (0.10 M), followed by reverse titration against $HClO_4$ (0.10 M), in $I = 0.10$ M NEt_4OH .

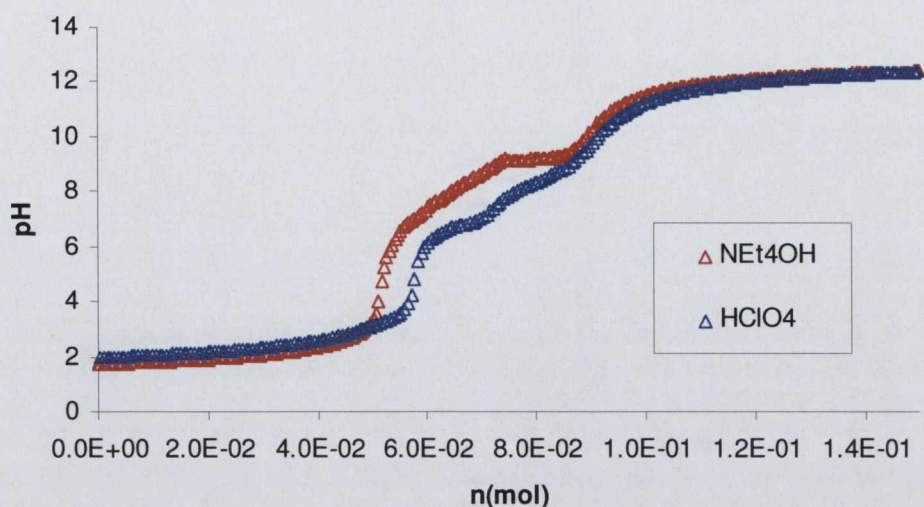


Figure A2.13: Titration curves for $42.La_2$ (1×10^{-3} M) against NEt_4OH (0.10 M), followed by reverse titration against $HClO_4$ (0.10 M), in $I = 0.10$ M NEt_4OH .

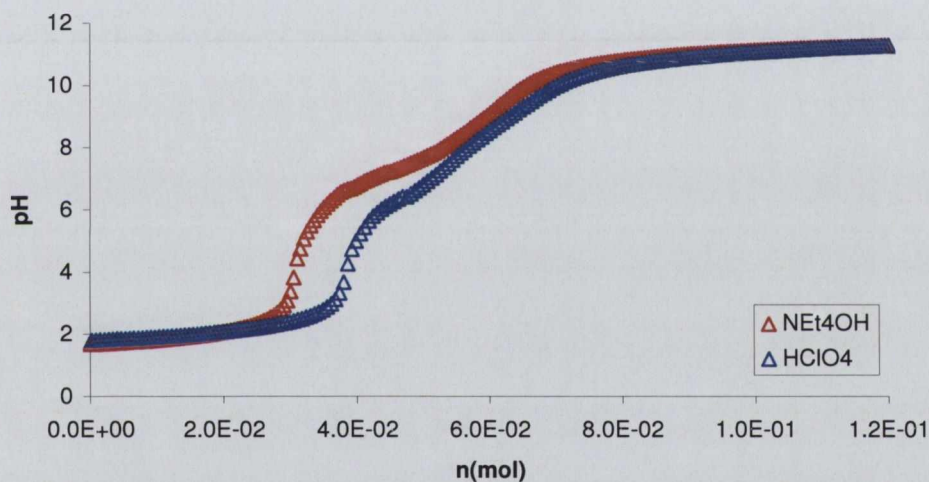


Figure A2.14: Titration curves for $44.Eu_2$ (1×10^{-3} M) against NEt_4OH (0.10 M), followed by reverse titration against $HClO_4$ (0.10 M), in $I = 0.10$ M NEt_4OH .

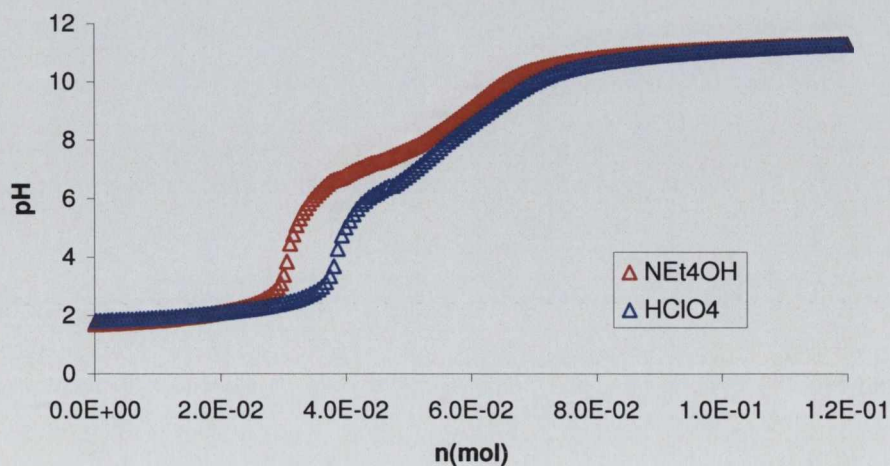


Figure A2.15: Titration curves for $44.La_2$ (1×10^{-3} M) against NEt_4OH (0.10 M), followed by reverse titration against $HClO_4$ (0.10 M), in $I = 0.10$ M NEt_4OH .

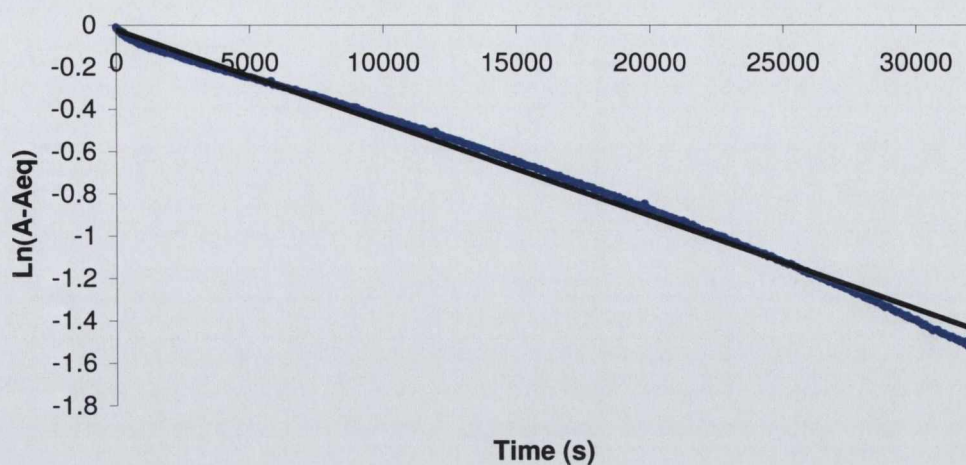


Figure A3.1: Manual determination of pseudo first order rate constant of 39.Eu₂ at pH 7.4, 37 °C. A rate constant $k_{obs} = 4.0 \times 10^{-5} \text{ s}^{-1}$.

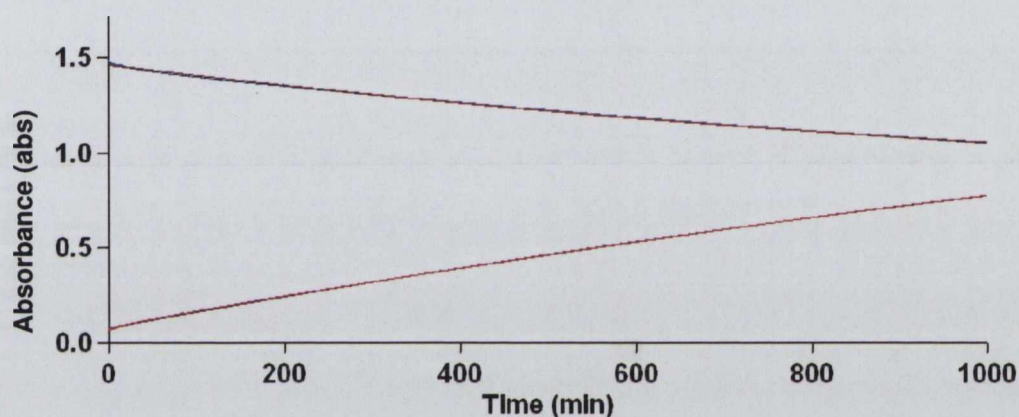


Figure A3.2: The kinetic profile for the hydrolysis of HPNP (0.18 mM) by 39.La₂ (0.18 mM) in 50 mM HEPES buffer at pH 7.4 and 37 °C. The increase in the absorption of p-nitrophenolate as a function of time was observed and fitted to first order kinetics to determine the rate constant, k_{obs} for the reaction.

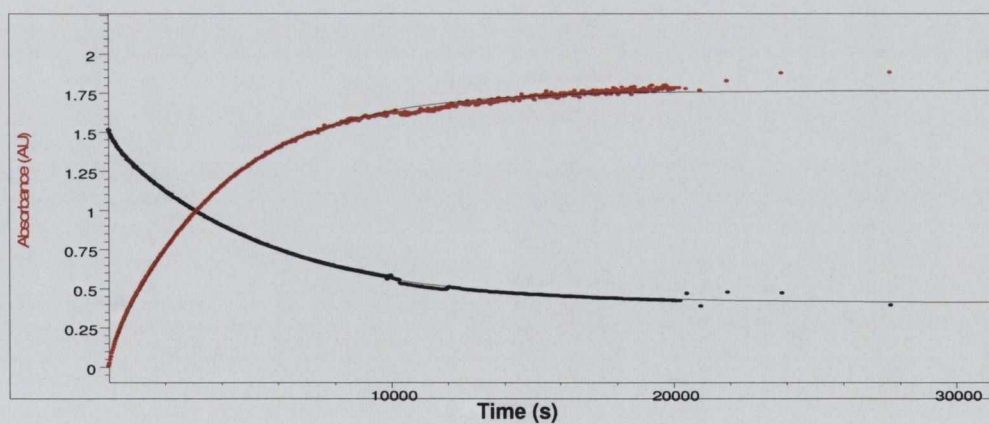


Figure A3.3: The kinetic profile for the hydrolysis of HPNP (0.18 mM) by 41.La₂ (0.18 mM) in 50 mM HEPES buffer at pH 7.4 and 37 °C. The increase in the absorption of p-nitrophenolate as a function of time was observed and fitted to first order kinetics to determine the rate constant, k_{obs} for the reaction.

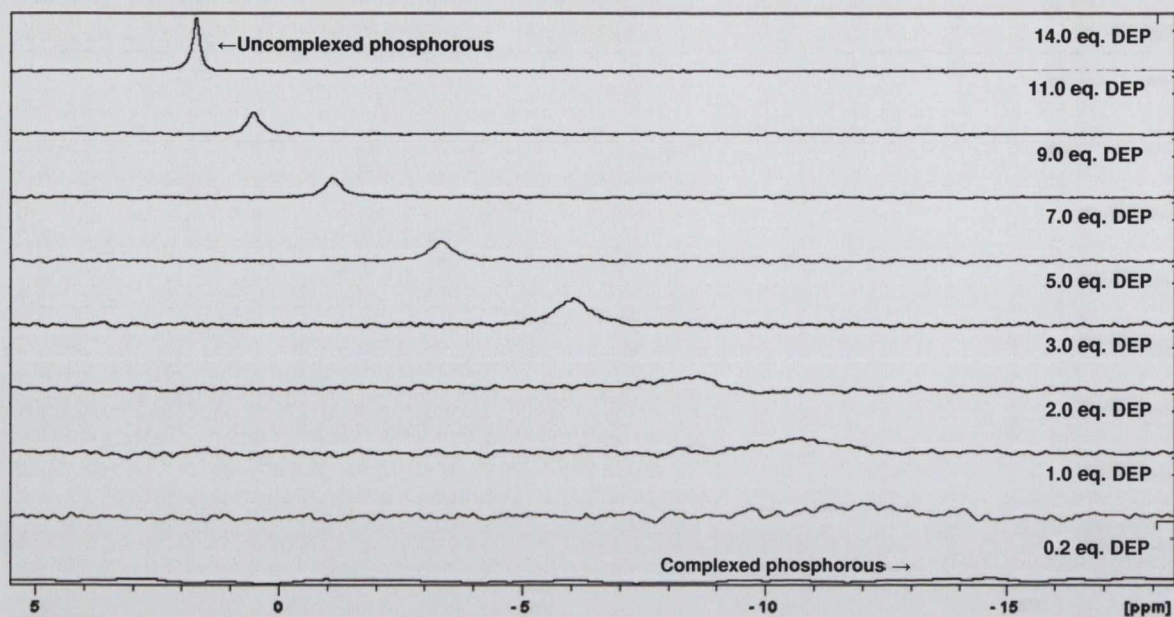


Figure A3.4: Stack plot of the ^{31}P NMR titration (162 MHz, H_2O) of $41.\text{Eu}_2$ ($3.0 \times 10^{-3} \text{ M}$) with increasing molar equivalents of DEP ($2.1 \times 10^{-1} \text{ M}$) up to 14 equivalents.

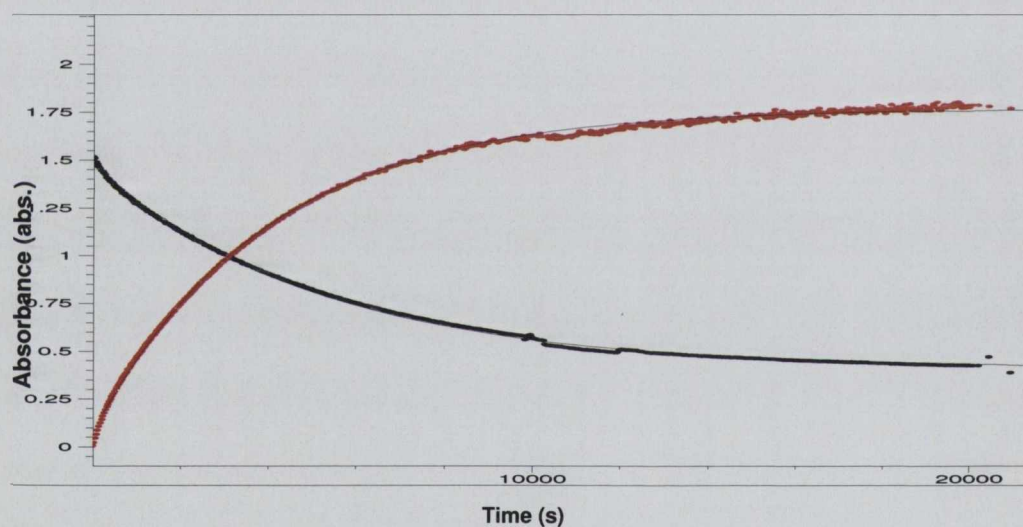


Figure A3.5: The kinetic profile for the hydrolysis of HPNP (0.18 mM) by $43.\text{La}_2$ (0.18 mM) in 50 mM HEPES buffer at pH 7.4 and 37°C . The increase in the absorption of p -nitrophenolate as a function of time was observed and fitted to first order kinetics to determine the rate constant, k_{obs} for the reaction.

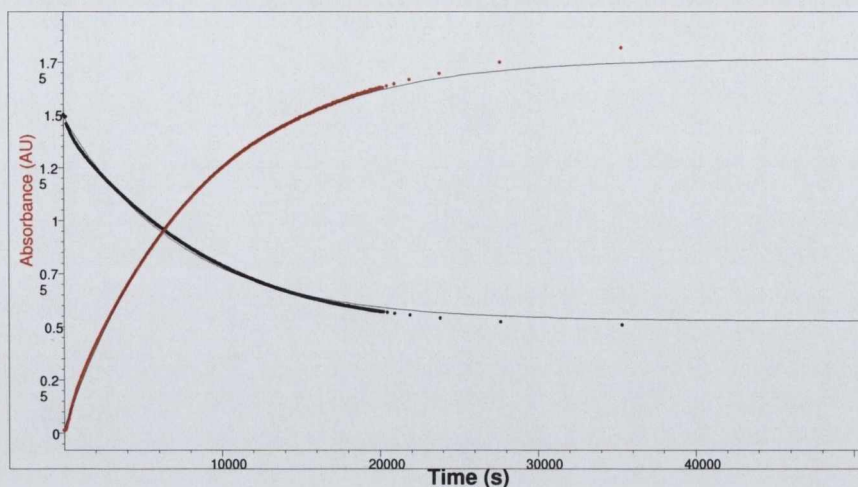


Figure A3.6: The kinetic profile for the hydrolysis of HPNP (0.18 mM) by **47.Eu** (0.18 mM) in 50 mM HEPES buffer at pH 7.4 and 37 °C. The increase in the absorption of *p*-nitrophenolate as a function of time was observed and fitted to first order kinetics to determine the rate constant, k_{obs} for the reaction.

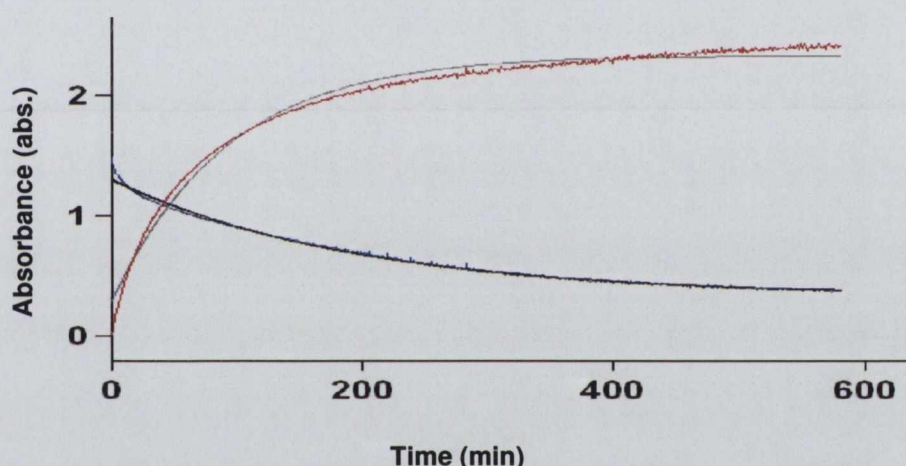


Figure A3.7: The kinetic profile for the hydrolysis of HPNP (0.18 mM) by **47.La** (0.18 mM) in 50 mM HEPES buffer at pH 7.4 and 37 °C. The increase in the absorption of *p*-nitrophenolate as a function of time was observed and fitted to first order kinetics to determine the rate constant, k_{obs} for the reaction.

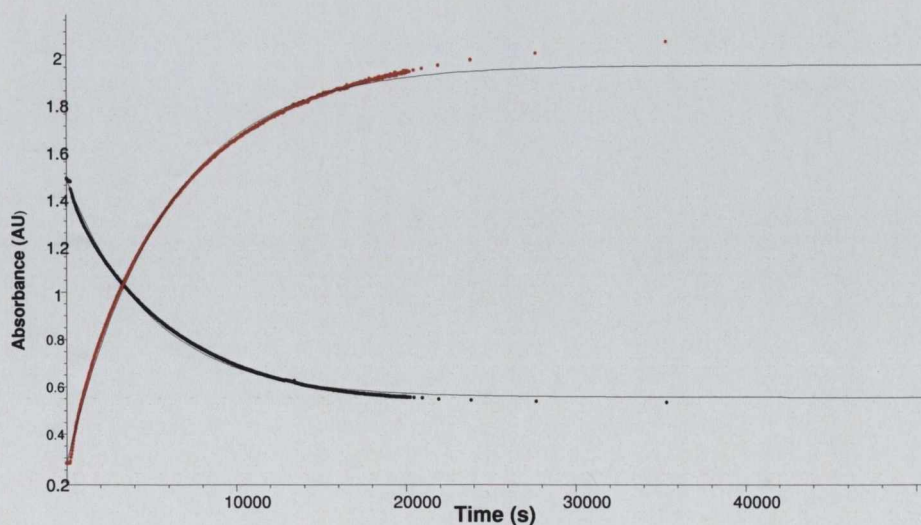


Figure A3.8: The kinetic profile for the hydrolysis of HPNP (0.18 mM) by **48.Eu** (0.18 mM) in 50 mM HEPES buffer at pH 7.4 and 37 °C. The increase in the absorption of *p*-nitrophenolate as a function of time was observed and fitted to first order kinetics to determine the rate constant, k_{obs} for the reaction.

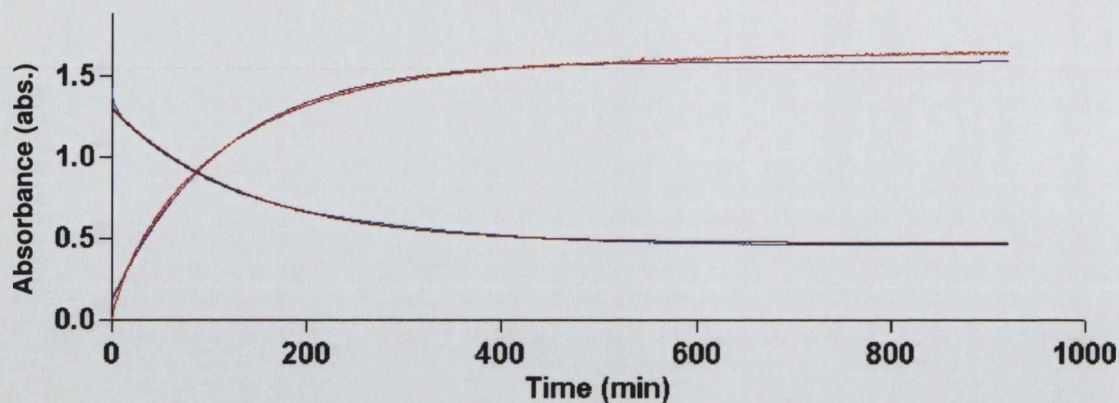


Figure A3.9: The kinetic profile for the hydrolysis of HPNP (0.18 mM) by 48.La (0.18 mM) in 50 mM HEPES buffer at pH 7.4 and 37 °C. The increase in the absorption of p-nitrophenolate as a function of time was observed and fitted to first order kinetics to determine the rate constant, k_{obs} for the reaction.

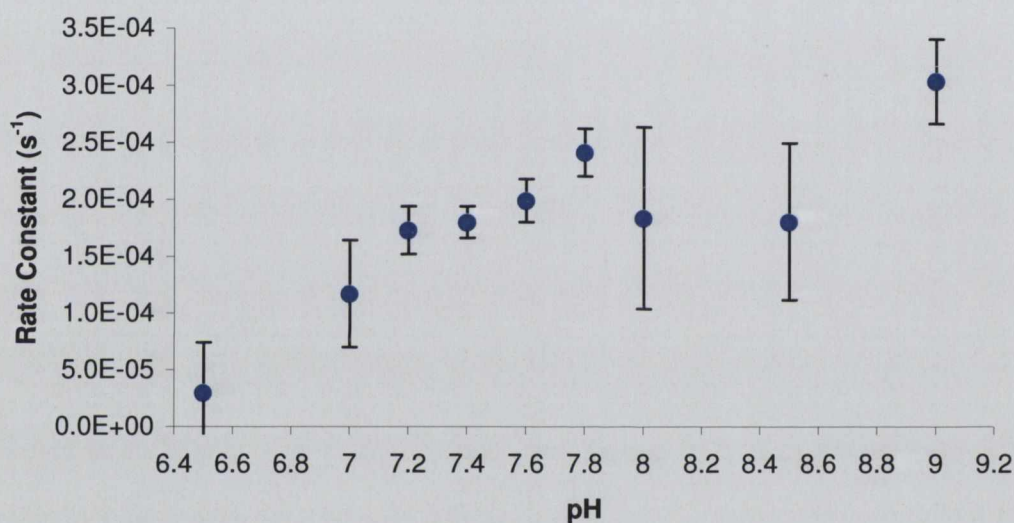


Figure A3.10: The pH-rate profile for the hydrolysis of HPNP (0.18 mM) by 47.La, in 50 mM HEPES buffer at 37 °C. Each data point is an average of 2-3 measurements agreeing to within 15% error.

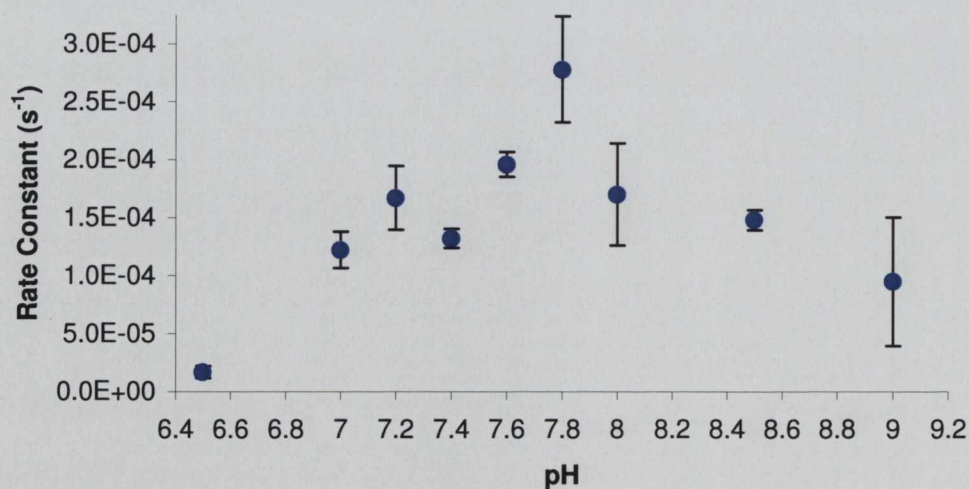


Figure A3.11: The pH-rate profile for the hydrolysis of HPNP (0.18 mM) by 48.Eu, in 50 mM HEPES buffer at 37 °C. Each data point is an average of 2-3 measurements agreeing to within 15% error.

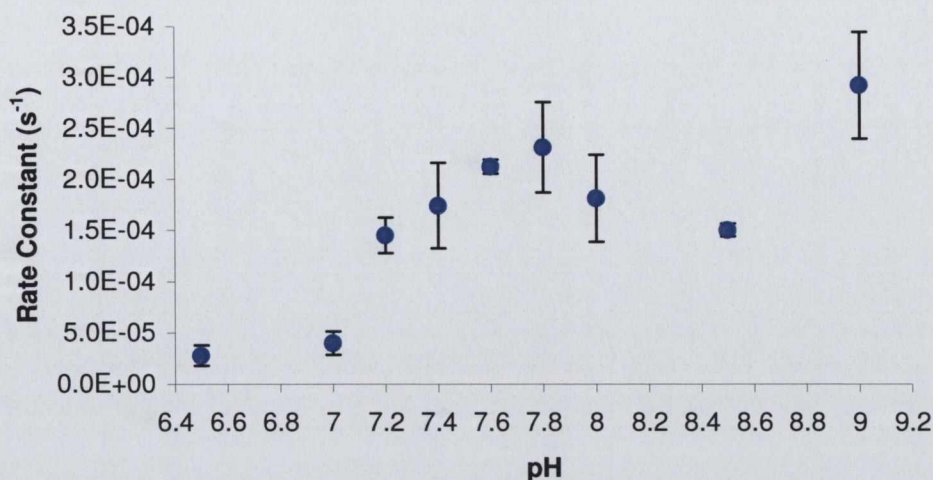


Figure A3.12: The pH-rate profile for the hydrolysis of HPNP (0.18 mM) by 48.La, in 50 mM HEPES buffer at 37 °C. Each data point is an average of 2-3 measurements agreeing to within 15% error.

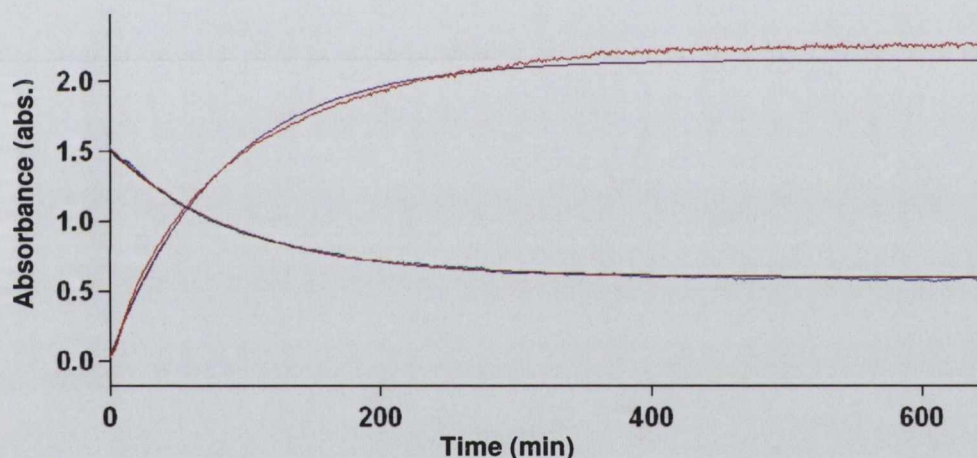


Figure A3.13: The kinetic profile for the hydrolysis of HPNP (0.18 mM) by 42.La₂ (0.18 mM) in 50 mM HEPES buffer at pH 7.4 and 37 °C. The increase in the absorption of p-nitrophenolate as a function of time was observed and fitted to first order kinetics to determine the rate constant, k_{obs} for the reaction.

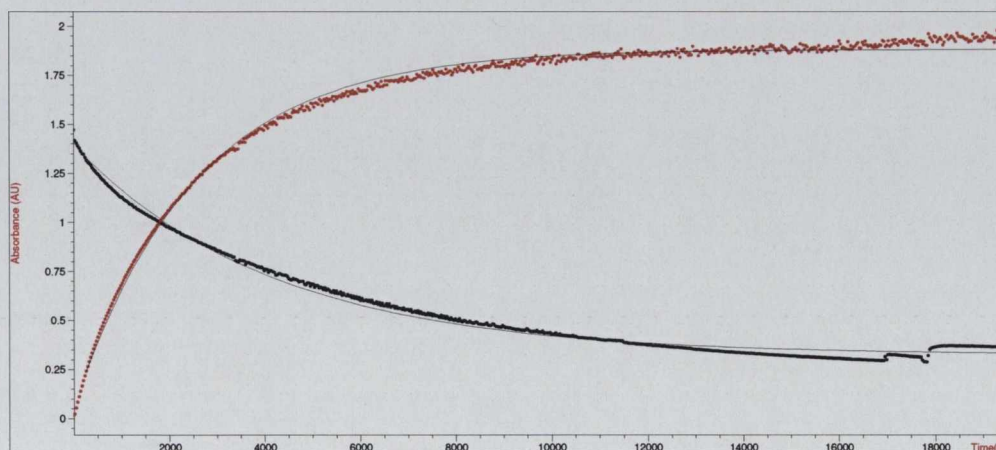


Figure A3.14: The kinetic profile for the hydrolysis of HPNP (0.18 mM) by 44.La₂ (0.18 mM) in 50 mM HEPES buffer at pH 7.4 and 37 °C. The increase in the absorption of p-nitrophenolate as a function of time was observed and fitted to first order kinetics to determine the rate constant, k_{obs} for the reaction.

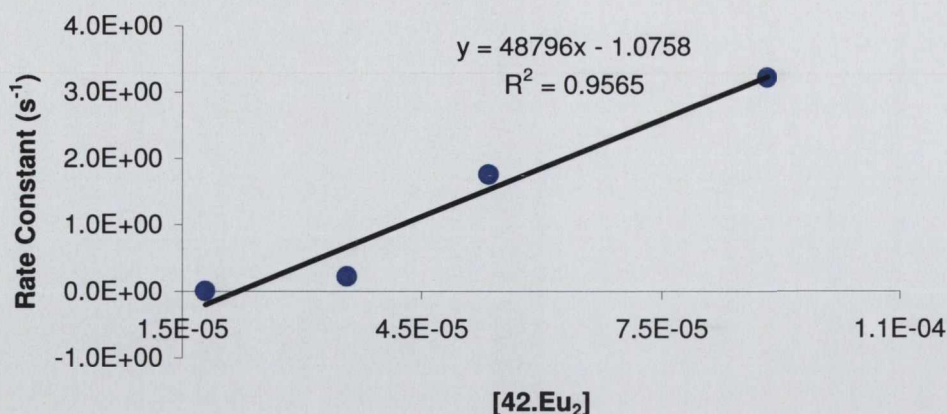


Figure A3.15: Dependence of the pseudo first order rate constant for the hydrolysis of **HPNP** (5.2×10^{-3} mM) on **[42.Eu₂]** in the concentration range 0.017 – 0.10 mM, at pH 7.4 and 37 °C (Low Conc.).

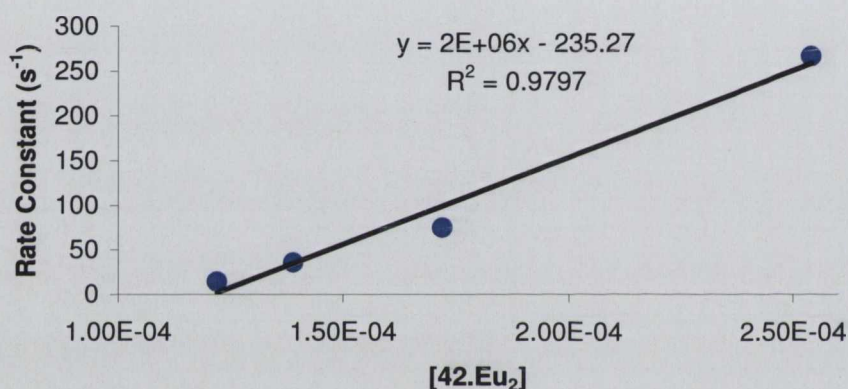


Figure A3.16: Dependence of the pseudo first order rate constant for the hydrolysis of **HPNP** (5.2×10^{-3} mM) on **[42.Eu₂]** in the concentration range 0.12 – 0.25 mM, at pH 7.4 and 37 °C (High Conc.).

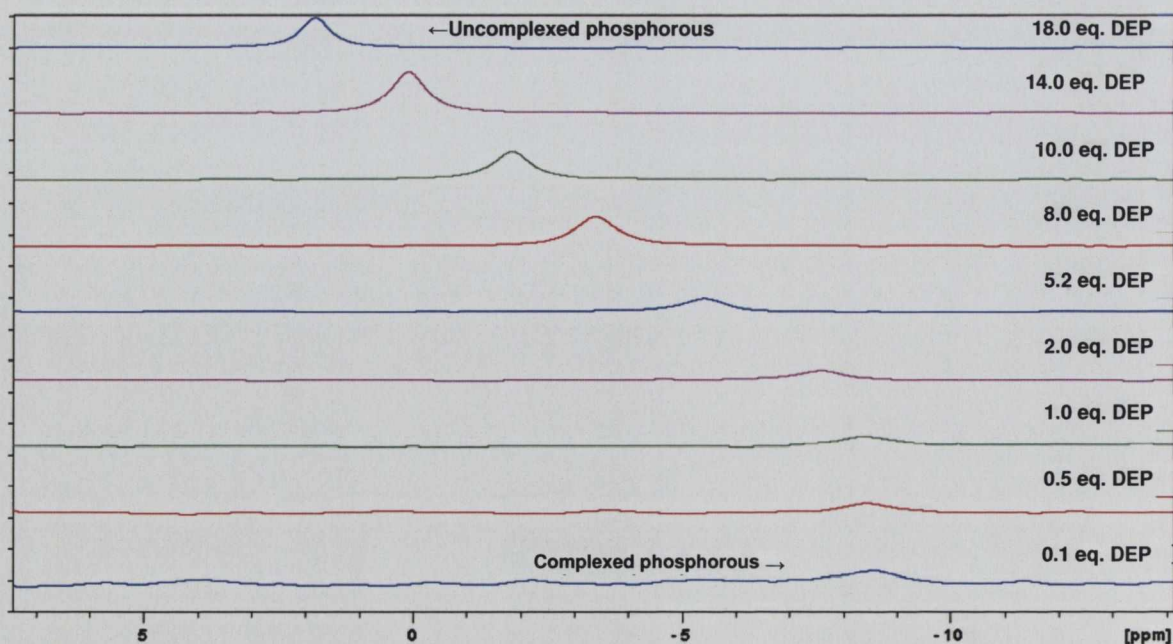


Figure A3.17: Stack plot of the ^{31}P NMR titration (162 MHz, H_2O) of **42.Eu₂** (3.0×10^{-3} M) with increasing molar equivalents of DEP (2.1×10^{-1} M) up to 20 equivalents.

A4. 1 : Crystallographic data for 41.La₂

Identification code	41.La₂	
Empirical formula	C ₄₈ H ₇₆ Cl ₀ F ₁₈ La ₂ N ₁₄ O ₃₁ S ₆	
Formula weight	2157.47	
Temperature	401(2) K	
Wavelength	0.71073 Å	
Crystal system	Triclinic	
Space group	P-1	
Unit cell dimensions	a = 11.9546(10) Å	α = 115.181(10)°.
	b = 12.8503(10) Å	β = 90.472(10)°.
	c = 14.9278(12) Å	γ = 90.090(10)°.
Volume	2075.2(3) Å ³	
Z	1	
Density (calculated)	1.723 Mg/m ³	
Absorption coefficient	1.291 mm ⁻¹	
F(000)	1078	
Crystal size	0.5 x 0.3 x 0.3 mm ³	
Theta range for data collection	1.70 to 25.00°.	
Index ranges	-14 ≤ h ≤ 14, -15 ≤ k ≤ 15, -17 ≤ l ≤ 17	
Reflections collected	16673	
Independent reflections	7312 [R(int) = 0.0170]	
Completeness to theta = 25.00°	99.9 %	
Absorption correction	Semi-empirical from equivalents	
Max. and min. transmission	1.00000 and 0.791891	
Refinement method	Full-matrix least-squares on F ²	
Data / restraints / parameters	7312 / 67 / 544	
Goodness-of-fit on F ²	1.053	
Final R indices [I > 2σ(I)]	R1 = 0.0512, wR2 = 0.1314	
R indices (all data)	R1 = 0.0530, wR2 = 0.1329	
Largest diff. peak and hole	2.154 and -2.761 e.Å ⁻³	

A4.2: Crystallographic data for 47

Identification code	47	
Empirical formula	C ₈₄ H ₁₅₆ Br ₀ N ₂₈ O ₂₀	
Formula weight	1878.37	
Temperature	123(2) K	
Wavelength	0.71075 Å	
Crystal system	Triclinic	
Space group	P-1	
Unit cell dimensions	a = 10.861(4) Å	α = 77.195(10)°.
	b = 11.252(4) Å	β = 81.122(11)°.
	c = 21.120(7) Å	γ = 88.982(13)°.
Volume	2486.3(15) Å ³	
Z	1	
Density (calculated)	1.255 Mg/m ³	
Absorption coefficient	0.091 mm ⁻¹	
F(000)	1016	
Crystal size	0.5 x 0.3 x 0.1 mm ³	
Theta range for data collection	1.90 to 25.00°.	
Index ranges	-12 ≤ h ≤ 12, -13 ≤ k ≤ 13, -25 ≤ l ≤ 25	
Reflections collected	38977	
Independent reflections	8732 [R(int) = 0.0643]	
Completeness to theta = 25.00°	99.7 %	
Refinement method	Full-matrix least-squares on F ²	
Data / restraints / parameters	8732 / 0 / 627	
Goodness-of-fit on F ²	1.357	
Final R indices [I > 2σ(I)]	R1 = 0.0851, wR2 = 0.1582	
R indices (all data)	R1 = 0.0921, wR2 = 0.1618	
Largest diff. peak and hole	0.222 and -0.200 e.Å ⁻³	

A4.3: Crystallographic data for 49.K

Identification code	49.K	
Empirical formula	$C_{172} H_{280} Br_8 K_8 N_{68} Na_0 O_{28}$	
Formula weight	4700.72	
Temperature	128(2) K	
Wavelength	0.71075 Å	
Crystal system	Monoclinic	
Space group	P2/c	
Unit cell dimensions	$a = 14.893(3)$ Å	$\alpha = 90^\circ$.
	$b = 12.327(2)$ Å	$\beta = 106.998(7)^\circ$.
	$c = 33.020(5)$ Å	$\gamma = 90^\circ$.
Volume	$5797.2(17)$ Å ³	
Z	1	
Density (calculated)	1.346 Mg/m ³	
Absorption coefficient	1.601 mm ⁻¹	
F(000)	2444	
Crystal size	0.2 x 0.2 x 0.15 mm ³	
Theta range for data collection	2.19 to 25.00°.	
Index ranges	-17<=h<=16, -14<=k<=14, -39<=l<=39	
Reflections collected	45193	
Independent reflections	10192 [R(int) = 0.0839]	
Completeness to theta = 25.00°	99.8 %	
Refinement method	Full-matrix least-squares on F ²	
Data / restraints / parameters	10192 / 0 / 675	
Goodness-of-fit on F ²	1.092	
Final R indices [I>2sigma(I)]	R1 = 0.0557, wR2 = 0.1559	
R indices (all data)	R1 = 0.0654, wR2 = 0.1643	
Largest diff. peak and hole	1.012 and -0.582 e.Å ⁻³	