University of Southampton

Synthesis and Metal Binding Properties of Novel C_2-Symmetric Tetraaza Ligand Systems

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Doctor of Philosophy

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For my Parents

UNIVERSITY OF SOUTHAMPTON

ABSTRACT

FACULTY OF SCIENCE

CHEMISTRY

Doctor of Philosophy

SYNTHESIS AND METAL BINDING PROPERTIES OF NOVEL C_2-SYMMETRIC TETRAAZA LIGAND SYSTEMS

by Susan Elizabeth Lawlor

This thesis describes the synthesis of novel C_2-symmetric tetramine ligands and an investigation of their metal binding properties via fluorimetry and mass spectrometry.

Chapter One provides an introduction to tetramine chemistry, discussing the synthesis and metal-binding properties of linear, cyclic and aromatic tetramines. It also highlights important applications of tetramines and their metal complexes in the areas of medicinal chemistry, catalysis of asymmetric synthesis and fluorescent sensing.

Chapter Two is concerned with synthetic routes to two series of novel C_2-symmetric tetramine ligands, based on naphthalene and pyridine. Optimisation of key steps in the synthetic route are discussed and opportunities to introduce chirality into the molecules are highlighted. Furthermore a two step route to tetramines, employing diamines and applicable to both series of ligands, is described. An efficient route to a series of tetramines, both achiral and chiral, is achieved in both the naphthalene and pyridine series.

The metal-binding properties of the tetramine ligands are detailed in the following two sections. The fluorescence studies outlined in **Chapter Three** reveal the effects of concentration, pH and the addition of metal ions on the fluorescence spectra of tetramines ligands in the naphthalene series. Mass spectrometry experiments are discussed in **Chapter Four**. This technique was shown to be suitable for observing the effects of metal ion addition in both series of tetramines and permitted a comparison of their metal binding affinities. In both series of tetramines the ability to selectively bind metal ions, particularly copper, nickel, and palladium, was shown.

In the concluding section, **Chapter Five**, the general experimental procedures and synthetic details are documented.

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Finally I would like to thank my parents and Stephen for all their love, support and encouragement over the last three years – I couldn't have done it without you!

Abbreviations Used in the Text

BINAP	2,2'-Bis(diphenylphosphino)-1,1'-binaphthyl
BINOL	1,1'-Bi-2-naphthol
DCM	Dichloromethane
DMF	*N,N*-Dimethylformamide
DMSO	Dimethylsulphoxide
DOTA	1,4,7,10-Tetraazacyclodecane-N,N,N'',N'''-tetraacetic acid
DOTETE	1,4,7,10-Tetrakis[2-methanylsulfanyl]ethyl-1,4,7,10-tetraazacyclododecane
DPEN	1,2-Diphenylethylenediamine
ee	enantiomeric excess
EI	Electron Ionisation
ES	Electrospray
ESI-MS	Electrospray Ionisation Mass Spectrometry
FAB	Fast Atom Bombardment
FAB-MS	Fast Atom Bombardment Mass Spectrometry
HRMS	High Resolution Mass Spectrometry
IR	Infra-red
ISC	Intersystem Crossing
LDA	Lithium diisopropylamide
LRMS	Low Resolution Mass Spectrometry
NBS	*N*-Bromosuccinimide
NMR	Nuclear Magnetic Resonance
PET	Photoinduced Electron Transfer
2,2,2-TET	Triethylenetetramine
2,3,2-TET	1,4,8,11-Tetraazaundecane
THEC	1,4,8,11-Tetrahydroxyethylcyclam
THF	Tetrahydrofuran
TLC	Thin Layer Chromatography
UV	Ultra-violet

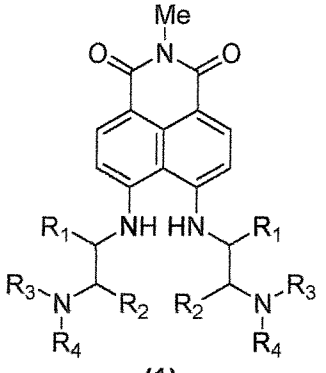
Chapter One

Introduction

Chapter One
Introduction

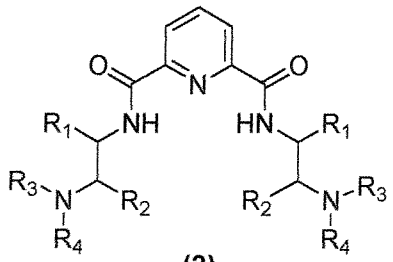
The origins of tetramine chemistry lie in Nature, with structures ranging from the simple linear tetramine, spermine, to the macrocyclic porphyrins. Although tetramines have been known for centuries, continued interest in their chemistry derives primarily from their ability to complex metal ions. This property has led to wide-ranging applications for tetramines and their metal ion complexes, particularly in the fields of medicine and catalysis. The synthesis of novel tetramine ligand systems and investigation of their metal binding properties are key elements in the development of this important area of organic chemistry.

It has previously been shown by Mellor *et al.*[1,2] that C_2-symmetric tetramine ligands can be prepared by varying the functionality of the naphthalimide unit **(1)** at the 4- and 5- positions of the aromatic ring. Chiral ligands in this series have shown promising results in their application as catalysts in asymmetric synthesis. The C_2-symmetry of the ligands simplifies their synthesis and is also an advantage in the catalysis of asymmetric synthesis since the reactive centres experience the same stereodiscriminating environment independent of which face of the ligand they approach. Furthermore, these ligand systems have been found to be fluorescent in nature and, as such, have a potential use as fluorescent sensors. Consequently, further investigation into the synthesis and metal binding properties of tetramine ligands based on this structure will assist the development of tetramine-based applications.



(1)

An analogous series of diamine/diamide ligands (called tetramines throughout the thesis for simplicity) to be based upon 2,6-disubstituted pyridine **(2)** is anticipated to exhibit similar metal binding properties. These ligands have the advantage of possessing an additional binding site, namely the nitrogen of the pyridine ring.



(2)

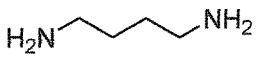
The synthesis and metal-binding properties of tetramines based on the two structures shown above forms the cornerstone of the research described in this thesis. To date, tetramines and their metal-ligand interactions have been studied extensively. This introductory chapter provides a review of the synthesis, metal binding properties and applications of linear, cyclic and unsaturated cyclic tetramines. It concludes with the specific aims of this piece of research, within the context of tetramine chemistry.

1.1 Linear Tetramines – Spermines

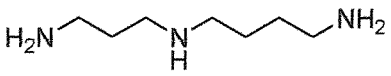
1.1.1 Background to linear tetramines

Linear polyamines such as the tetramine spermine are widely distributed in Nature and display a variety of important biological functions.[3,4,5,6,7,8,9] The first known naturally occurring polyamine was spermine which was discovered by Antoni Van Leeuwenhoek in 1678. Upon the cooling of human semen he observed that a crystalline substance formed.[10] Over 200 years later Rosenheim[11] and Wrede used organic synthesis to establish the identity of these crystals as the phosphate salt of spermine and also identified a related base, spermidine.

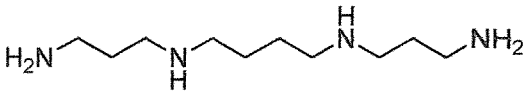
Spermine is the third most abundant of the naturally occurring linear polyamines after putrescine and spermidine.



Putrescine



Spermidine



Spermine

One or more of these compounds is present in every living cell. Studies by Rosenthal[12] and the Tabors[13] demonstrated that all eukaryotic cells synthesise putrescine, spermidine and spermine but that spermine rarely occurs in prokaryotic cells. The highest concentrations of spermine and spermidine in animal tissues are found in the prostrate, pancreas and human semen. The most common naturally occurring derivatives in humans are the *N*-acetyl polyamines. Many polyamine-containing compounds are also found in plants and insects. For example, aphelandrine is a spermine containing alkaloid from the flowering shrub *Aphelandra tetragona* and the venom of the funnel-web spider has been found to contain hydroxylamine derivatives of the polyamines.

1.1.2 Synthesis of linear tetramines

The polyamines are elegantly synthesised in Nature (Figure 1.1). The major pathway of polyamine biosynthesis utilises the amino acid L-ornithine and S-adenosylmethionine (SAM) in a stepwise transfer of aminopropyl groups to putrescine. Since the synthesis of spermidine and spermine are dependent on the availability of the aminopropyl donor, SAM is rate limiting in the polyamine biosynthesis.

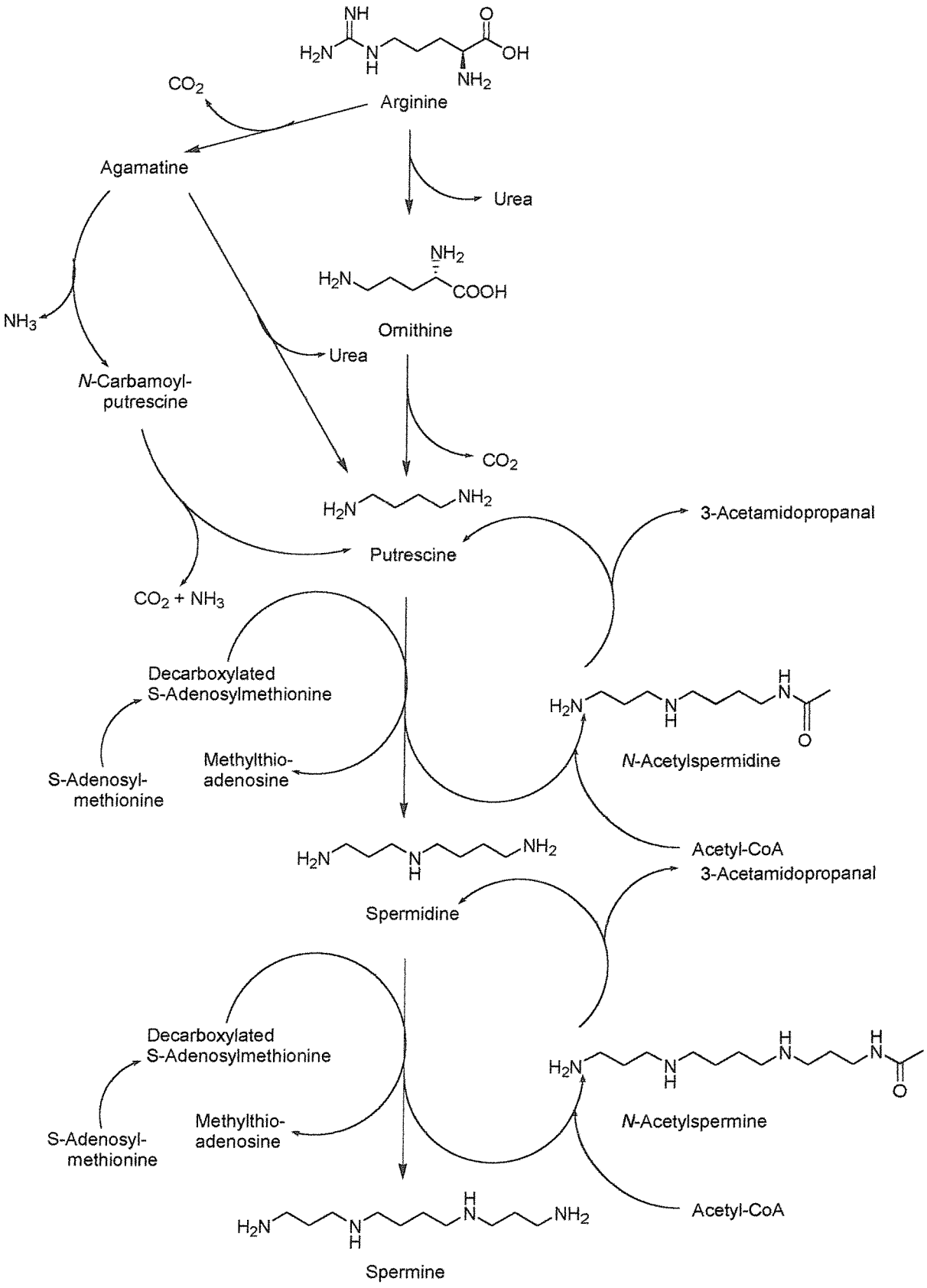


Figure 1.1 Synthesis of the polyamines in Nature

In the laboratory, many studies have been concerned with the synthesis of polyamines and their derivatives.[3,14,15,16,17,18,19] The original synthesis of spermine was carried out by Rosenheim[20] and involved the reaction of the diamine putrescine with excess $PhO(CH_2)_3Br$ to attach two γ-aminopropyl groups (Figure 1.2).

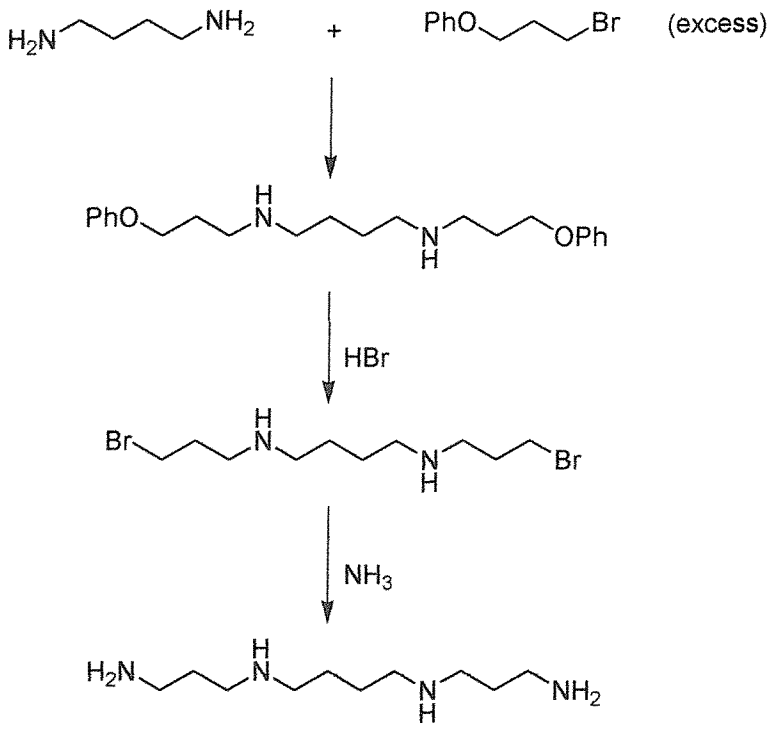


Figure 1.2 Rosenheim's synthesis of spermine

A second, more general synthesis[21] also involves the reaction of a diamine with one or two equivalents of CH_2=CHCN to prepare spermidine or spermine respectively (Figure 1.3).

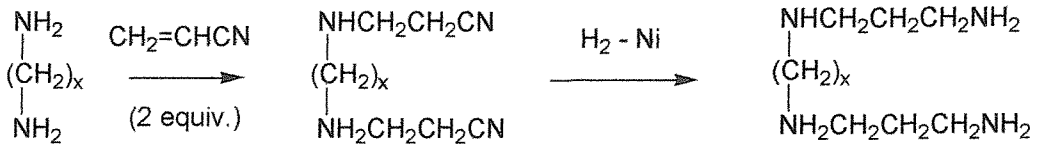


Figure 1.3 A general synthesis of spermidine or spermine

Two strategies can be employed in the preparation of polyamine derivatives, namely the synthesis of the polyamine *de novo* or a partial synthesis starting from putrescine, spermidine or spermine. However, one of the main synthetic problems remains

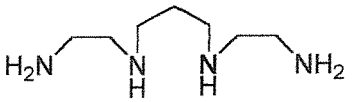
differentiation of the amino features of the polyamine to enable its conversion into a specific substituted derivative.

The role of polyamines in Nature remains obscure. It is known that polyamines are essential for normal growth processes. Among the most important physiological roles of polyamines are their effect on DNA replication and translation, stabilisation of specific DNA conformations and charge neutralisation of intracellular polyanions, for example in DNA and RNA.[22] It has been shown that the level of polyamines in dividing cells, for example cancer cells, are much higher than in resting cells. This prompted considerable interest in the use of polyamines as tumour markers, by measuring the concentration of polyamines in biological fluids. However, this has proved less successful than expected with the exception of a few well defined systems such as the use of measurements of urinary polyamines and their acetylated derivatives in heart transplant patients to predict rejection potential. Polyamine synthesis is also a potential target in tumour chemotherapy. Inhibitors of polyamines which can be taken up by the polyamine transport system and selectively interfere in polyamine metabolism have been used successfully in the treatment of some cancers.[23]

1.1.3 Metal binding properties of linear tetramines

Metal binding by linear tetramines results in the formation of chelate rings since more than one binding site is involved in the complex formation, that is to say they act as polydentate ligands. For example, the tetramine ligands triethylenetetramine (2,2,2-TET) and 1,4,8,11-tetraazaundecane (2,3,2-TET) form five- and six-membered chelate rings respectively. Complexes containing chelate rings have been found to have an enhanced stability as compared to complexes containing unidentate ligands, this is termed the 'chelate effect'.

2,2,2-TET



2,3,2-TET

The chelate effect is chiefly an entropy effect but additional stabilisation often arises from enthalpy changes. Two simplified ways of looking at the translational entropy are as follows:

(i) In complexes with unidentate ligands, such as ammonia, the dissociation of a ligand from the complex results in the ligand being quickly swept into solution and it is unlikely that it will return to its original site. However, for a bidentate ligand such as 2,2,2,-TET, if one of the amine groups dissociates from the complex it is retained by the end still attached to the ligand and the probability of the nitrogen atom reattaching to the metal is high. Thus, complexes with multidentate ligands are found to be more stable towards dissociation.

(ii) The replacement of a unidentate ligand with a multidentate ligand results in an increased number of molecules in solution and thus translational entropy favours the formation of chelate systems.

For the two nickel (II) complexes $[Ni(2,2,2\text{-TET})(H_2O)_2]^{2+}$ with a five-membered chelate ring and $[Ni(2,3,2\text{-TET})(H_2O)_2]^{2+}$ with a six-membered chelate ring, the latter is found to be more stable than the former. This is due to the release of steric strain in the 2,2,2,-TET complex, which is too short to span the Ni(II) ion effectively, on adding another methylene group to give 2,3,2-TET. Thus it would seem logical that increasing the size of the chelate ring would increase selectivity of large metal ions over small metal ions. However, precisely the opposite has been found to be true. An increase in the size of the chelate ring usually leads to a drop in the complex stability (Figure 1.4). This is generally believed to be associated with the increased difficulty in bringing together dipoles and charges on donor atoms as chelate ring size increases and to steric strain. Furthermore it has been shown that an increase in ring size leads to greater complex destabilisation for larger metals than for smaller metals.

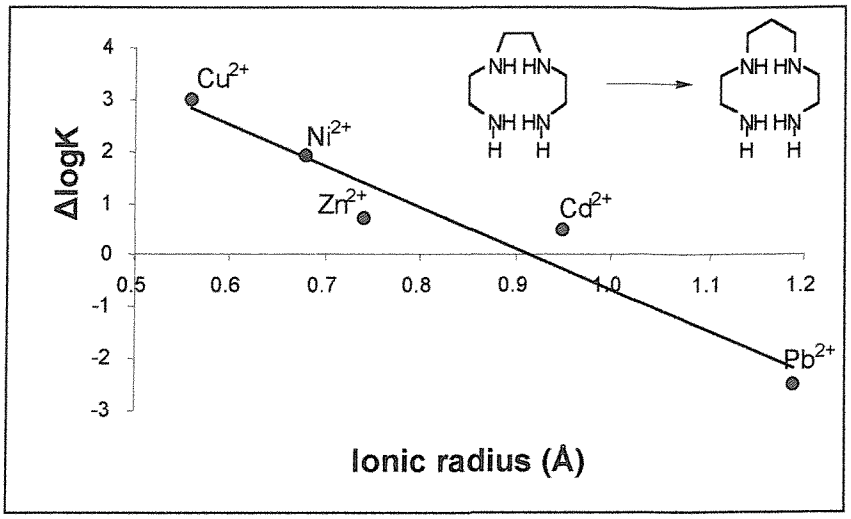


Figure 1.4 Effect of increasing chelate ring size on complex stability as a function of metal ion size

These observations can be explained as follows: generally as a metal ion increases in size, so its coordination number increases. As the coordination number increases, so the L-M-L bond angles within the chelate ring become smaller and the M-L bond lengths become longer. This is a favourable situation since strain energy is found to decrease as M-L bond length increases and M-L bond angle decreases. Since a cyclohexane ring represents minimum strain energy, any ring that can approximate to this arrangement will be of low strain energy. For a six-membered, nitrogen containing chelate ring it is possible to achieve N-M-N bond angles of 109.5°. For a five-membered, nitrogen containing chelate ring to come close to achieving strain-free geometry the organic part of the chelate ring adopts the same geometry as a cyclohexane ring. The metal atom is placed at the focus of the lone pairs in the nitrogen donor atoms, giving rise to long M-N bonds and a small M-N-M angle (Figure 1.5).

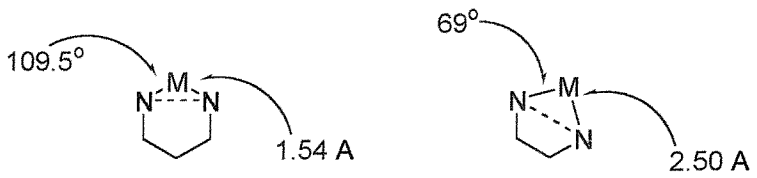


Figure 1.5 Bond angles and bond lengths in six and five-membered, nitrogen containing chelate rings

This accounts satisfactorily for the empirical observation that, in general:

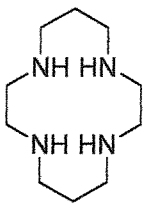
(i) Complexes of five-membered chelate rings are generally more stable than those of six-membered chelate rings

(ii) Large metals prefer five-membered chelate rings while smaller ions show a lesser aversion to six-membered rings than do large metal ions.

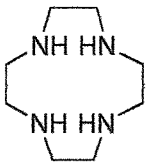
1.2 Cyclic Tetramines – Cyclams

1.2.1 Background to cyclams

The synthesis and complexing properties of tetraazamacrocycles and their derivatives have been extensively studied.[24,25,26,27] Saturated tetraazamacrocycles are known with ring sizes ranging from 12 to at least 44 atoms.[28] The most famous and most studied is the 14-membered ring 1,4,8,11-tetraazacyclotetradecane, known as cyclam, which was first synthesised in 1936. The second most studied tetraazamacrocycle is the smaller, 12-membered ring 1,4,7,10-tetraazacyclododecane, known as cyclen.



Cyclam



Cyclen

1.2.2 Synthesis of cyclams

There are two main synthetic routes to tetraazamacrocycles. The first method is that of Richman and Atkins[29] and its variant (Figure 1.6).[30,31] Cyclisation is effected, in good yield, between one precursor, which is the salt of a sulfonamide, and a second precursor containing sulfonate esters as leaving groups. The tosyl groups are subsequently removed *via* acid hydrolysis or reductive cleavage with HBr/acetic acid mixture.[32,33,34]

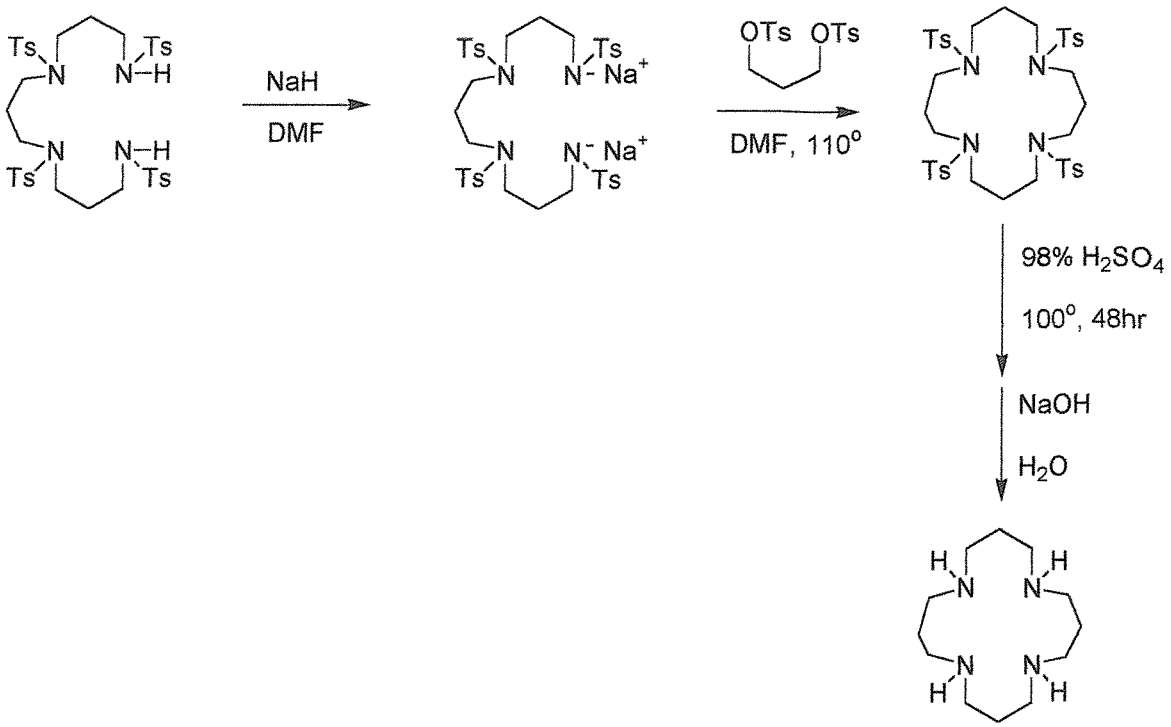


Figure 1.6 Synthetic route to tetraazamacrocycles

The second method of tetraazamacrocycle formation involves a condensation reaction in the presence of a metal ion, usually Ni(II) or Cu(II) (Figure 1.7). This is known as a 'template reaction'.[35] The metal ion can have two roles; firstly it can influence the steric course of the condensation such that formation of a cyclic product rather than a polymeric product is favoured (kinetic template effect) and secondly it can stabilise the macrocycle once formed (thermodynamic template effect). For example, the condensation of Ni(II) tetramine complexes with glyoxal yields unsaturated tetraazamacrocycles, which can be reduced with $NaBH_4$ or Ni/H_2. Treatment with alkaline cyanide solution gives the free macrocycle.

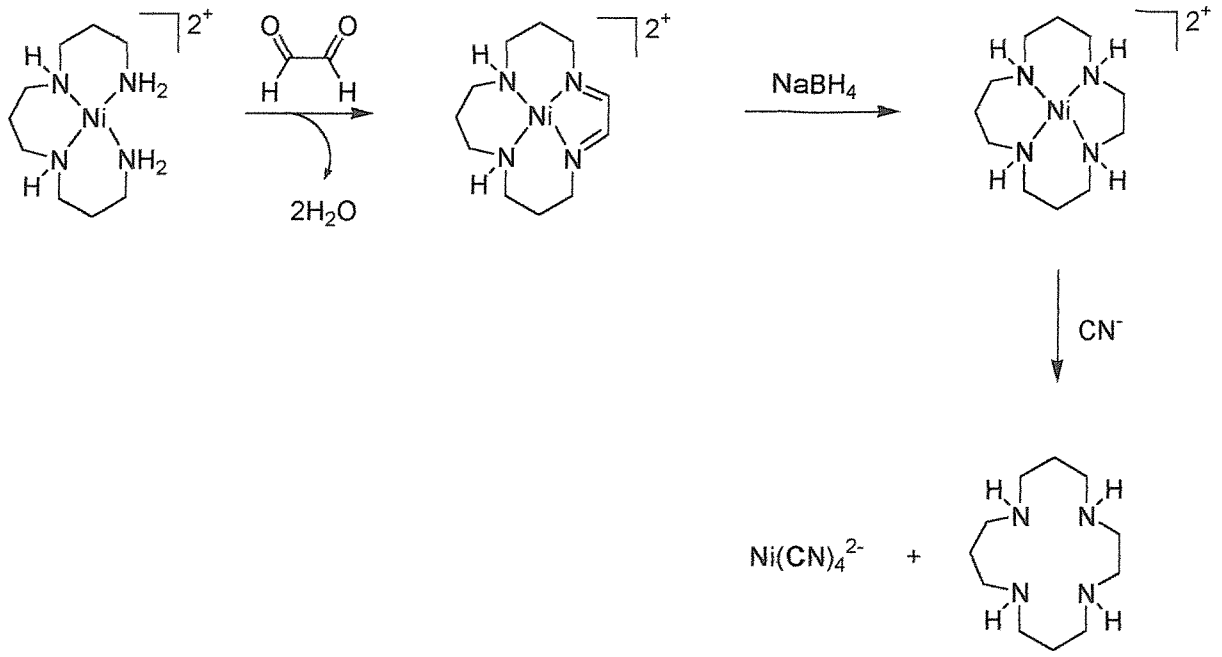


Figure 1.7 Synthesis of tetraazamacrocycles *via* a template reaction

However, owing to the high costs of the different tetraazamacrocycles commercially available, alternate routes to macrocycle synthesis continue to be investigated.[36,37]

1.2.3 Metal binding properties of cyclams

An important property of tetraazamacrocycles is their ability to complex a large range of metal cations.[25,27] Metal complexes of tetraazamacrocycles exhibit an enhanced stability as compared to their analogous open chain complexes - this is known as the 'macrocyclic effect'.[38] Furthermore tetraazamacrocycles can be designed for selective metal complexation of metal ions in solution.[39] Applications of such ligands include their use as therapeutic reagents for the treatment of metal intoxication, design of antibiotics whose mode of action depends upon specific metal complexation, imaging agents in the body,[40] chelate-ion exchange materials,[41] selective metal extractants in metallurgy and metal sequestering agents in detergents.

It could be imagined that the most stable metal-ion complexes would be formed between metal ions and tetraazamacrocycles where the match in size between the metal ion and the cavity in the ligand is closest – this is known as 'size-match selectivity'. However, the 'hole-sizes' for tetraazamacrocycles have been calculated and do not correlate with their metal selectivity patterns: this can be explained by the fact that complexes of these ligands can exist as different conformers and that metal-

ion preferences depend on the stability of the conformers formed. Furthermore, metal ions that are too large for the cavities of the tetraazamacrocycles are simply coordinated lying out of the plane of the donor atoms and this does not necessarily have an adverse strain effect. The macrocycles are too flexible to show genuine size-match selectivity and the factors controlling metal ion size selectivity are the same as those controlling selectivity in the open chain tetramine ligands, namely the size of the chelate rings formed upon metal complexation. Hence, contrary to expectations based on size-match selectivity, as the tetraazamacrocyclic cavity gets larger the affinity for large metal ions decreases and that for small ions increases (Figure 1.8).

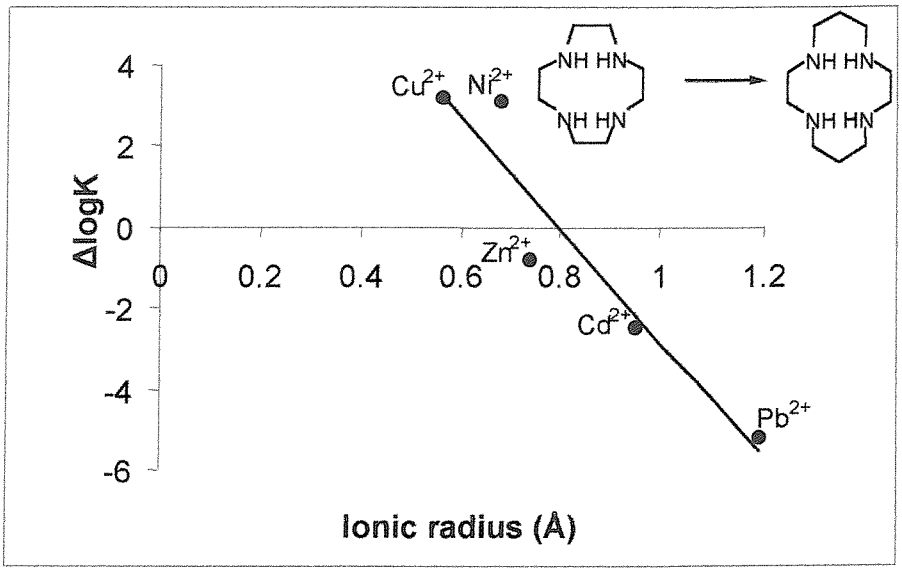
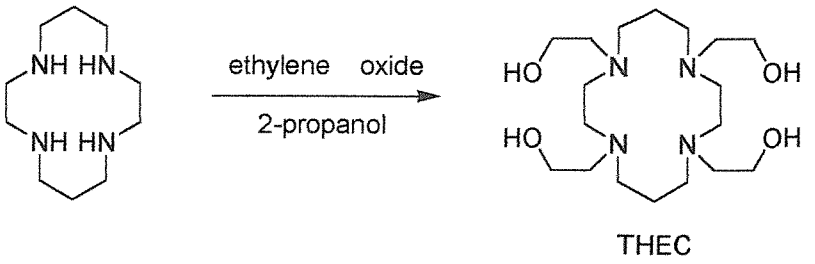


Figure 1.8 Effect of increasing the tetraazamacrocyclic cavity on complex stability as a function of metal ion size

In order to enhance the metal ion selectivity of the tetraazamacrocyclic ligands additional donor groups have been added to their periphery producing 'pendant donor' macrocycles.[42] In particular, *N*-functionalisation has been shown to be a remarkable tool for the synthesis of ligands possessing enhanced selectivity toward metal ion coordination.[39,43,27,44]

The synthesis of *N*-substituted macrocycles is relatively simple, quite general in applicability and well understood. For example, the synthesis of the tetrahydroxyethyl macrocycle derived from cyclam is achieved by simply adding a slight excess of ethylene oxide to cyclam in chilled 2-propanol and allowing it to stand overnight in a

refrigerator (Figure 1.9). This results in an approximately 100% yield of crystalline material.[45]



THEC

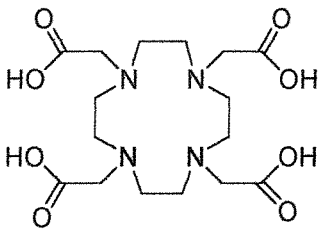
Figure 1.9 Synthesis of THEC

Synthetically the most straightforward elaboration of the macrocycle is the introduction of four pendant arms using an excess of alkylating agent. However, synthetic routes towards incompletely alkylated tetraazamacrocycles are more complicated. One relatively efficient method employs excess macrocycle in the presence of the alkylating agent with the excess unreacted parent macrocycle selectively extracted. Monosubstituted cyclam species containing, for example, carboxyalkyl and carboxyaryl side-chains have been successfully prepared via this method.[46] Routes to disubstituted cyclams and cyclens are also known.[47]

Of all the tetraazamacrocycles known the pendant donor derivatives of cyclam and cyclen have been most extensively studied. The introduction of four pendant arms onto cyclam generally leads to a geometry in which two arms project from one side of the macrocyclic plane and two from the other. Consequently the coordinating capability of the four pendant donors is unlikely to be fully utilised by a single metal ion. This leads to a tendency for bimetallic complexes to form where both metals are exocyclic and unable to derive the stabilisation associated with location within the tetraazamacrocyclic cavity.[48]

Cyclen, however, gives rise to complexes in which all the pendant arms project in the same direction. This allows the coordinating capability of the four pendant arms to be fully utilised by metal ions able to adopt a coordination number equal to or greater than eight.

The effect of *N*-functionalisation on metal selectivity can be effectively illustrated by the cyclen derivative with four added *N*-acetates known as DOTA (1,4,7,10-tetraazacyclodecane-N,N,N'',N'''-tetraacetic acid). The acetate ligands provide potential octadentate coordination, which allows for very strong complexation of large metal ions that can achieve the coordination number eight and thus full coordination of the donor atoms of the ligand. Consequently the calcium (II) complex of DOTA is the most stable known complex of calcium (II)[49,50] with a log *K* of approximately 16.5. The calcium (II) ion normally has little affinity for nitrogen donors or even acetate itself. However the stability of the complex is believed to reflect the ability of the very ionically bound calcium (II) ion to adapt to the coordination geometry required for complexing with DOTA. This is borne out by the fact that the lanthanides, which exhibit ionic bonding, also appear to coordinate well with DOTA.[51]

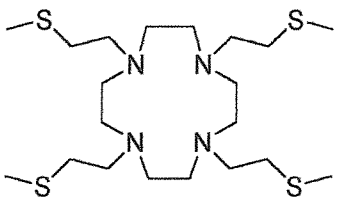


DOTA

1.2.4 Applications of cyclams

An important use of DOTA, due to its binding of lanthanides, is in radioimmunotherapy.[52] The development of monoclonal antibodies, which when injected bind firmly to tumour-associated compounds, is very significant in modern medical technology and can be used both for clinical diagnosis and therapy. The conjugation of metals, particularly radionuclides, to monoclonal antibodies results in agents for radioimmunotherapy. Derivatives of tetraazamacrocycles bearing a *C*-substituted functional group for attachment to the monoclonal antibody can be radiolabelled with ^{64}Cu, ^{111}In or ^{90}Y to form stable complexes. In particular, the *C*-substituted derivative of DOTA, containing a *p*-nitrobenzyl side chain rapidly forms a stable complex with $^{88}Y^{3+}$, which shows remarkable kinetic stability and thus has potential application in radioimmunotherapy.

A second example of the use of pendant donors to increase the selectivity of cyclen derivatives towards a particular metal is illustrated by the ligand 1,4,7,10-tetrakis[2-methanylsulfanyl]ethyl-1,4,7,10-tetraazacyclododecane, known as DOTETE.[53] The complexing of a silver (I) ion, which is hexacoordinated to the four nitrogen and two of the four possible sulfur donor atoms, results in the highest stability known for a silver (I) complex in solution: for a 1:1 mixture of methanol and water log *K* is 19.63. Furthermore, very high discrimination between silver (I) and lead (II) is achieved, with the former being favoured by more than a factor of 10^{10}. The DOTETE ligand also has a potential application in radioimmunotherapy owing to its strong binding of the β-ray-emitting radionuclide ^{111}Ag.



DOTETE

1.3 Unsaturated Cyclic Tetramines - Porphyrins

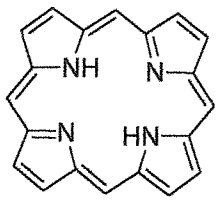
1.3.1 Background to porphyrins

The porphyrins are a class of naturally occurring macrocyclic ligands. The structure and chemistry of porphyrins, metalloporphyrins and related structures has been widely studied,[54,55] particularly in the first half of the last century with a large amount of work carried out by Hans Fischer.

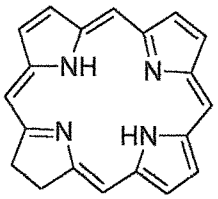
The porphyrin nucleus consists of four pyrrole rings linked via four methine bridges to give a macrocycle. Porphyrins are derived from porphin by substitution of all or some of the peripheral positions with a variety of side-chains. Structures related to porphyrins include:

(i) Reduced porphyrin macrocycles such as the chlorins and phlorins, which are dihydro-porphyrins, bacteriochlorin, which is a tetrahydroporphyrin and the hexahydroporphyrin known as porphyrinogen.

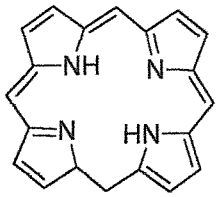
(ii) Oxidised porphyrin macrocycles such as the oxophlorins containing between one and four oxygen functions at the meso position.



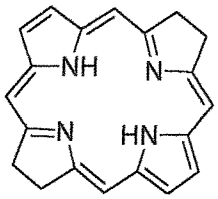
Porphin



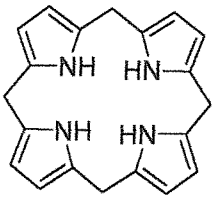
Chlorin



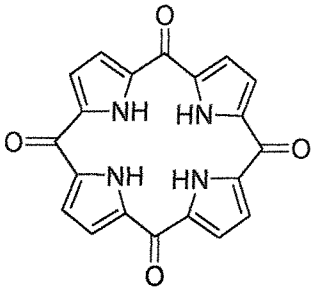
Phlorin



Bacteriochlorin



Porphyrinogen

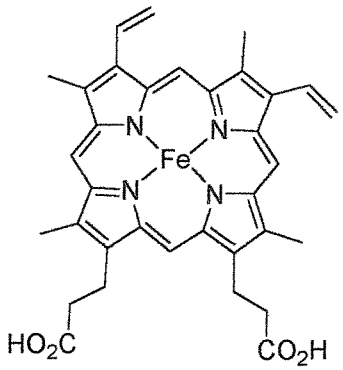


Oxophlorin

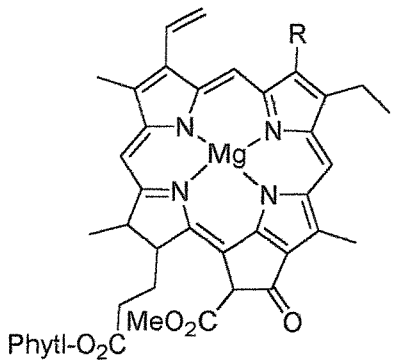
The porphyrin macrocycle is highly conjugated, possessing twenty two electrons, however only eighteen are included in any one delocalisation pathway, thus complying with Hückel's 4n+2 rule for aromaticity. The porphyrins are highly coloured and exhibit characteristic sharp and intense absorption bands in the visible region. The most intense absorption bands with very high extinction coefficients occur in the region from 400-500nm, known as the 'Soret band'. The porphyrin ring system is very stable, for example, exposure to concentrated sulfuric acid and neat trifluoroacetic acid has no effect. Acids such as chromic acid are required to destroy the ring system. Solutions of porphyrins, however, are unstable to light and undergo photo-oxidation.

Porphyrins rarely occur in Nature metal-free. Minute amounts are found in tissue fluids, urine and faeces. Metal-containing porphyrins, however, occur in abundance in Nature. Porphyrins are one example of pyrrole pigments, which constitute the most

abundant colouring matters in Nature. The iron (II) porphyrin, protoheme, is the prosthetic group in haemoglobin and myoglobin and is also contained in the cytochromes and the enzymes peroxidase and catalase. A variety of chlorophylls exist in Nature, most of which are chlorins. The most abundant chlorophylls are the magnesium (II) chlorins, chlorophyll-*a* and chlorophyll-*b*, which are the major photosynthetic pigments of the plant kingdom. Chlorophyll degradation products are often found in oil shales and crude petroleum oils as their nickel or vanadium complexes. The isolation and modification of these naturally occurring porphyrins has proved to be an abundant source of porphyrins.[56]



Protoheme



Chlorophyll-a R= Me
Chlorophyll-b R=CHO

1.3.2 Synthesis of porphyrins

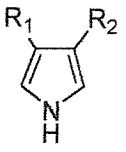
There are many synthetic routes to porphyrins, all of which involve pyrroles. The most common are summarised as follows:

(i) Polymerisation of monopyrroles – This approach works satisfactorily when the pyrrole has identical substituents at the 3 and 4 positions, otherwise polymerisation leads to a mixture of all 4 possible isomers.

(a) Polymerisation of 2,5-disubstituted pyrroles in the presence of agents that provide the meso methine groups of the product is usually carried out via Rothemund's method,[57,58] which involves the heating of a monopyrrole with aldehydes. This method usually results in yields of porphyrins in the region of 20%.

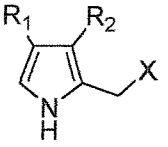
(b) Tetramerisation of pyrroles bearing two CH_2R substituents, **(3)** and **(4)**, the methylene carbon of which provides the meso carbon of the product,

followed by aerial oxidation affords good yields of symmetrical porphyrins.[59,60]



(3)

R1 = R2



(4)

R1 = R2

(ii) Condensation of dipyrrolic intermediates – This route is limited to the synthesis of porphyrins, which are centrosymmetrically substituted, or porphyrins that possess symmetry in one or both halves of the molecule.

(a) Porphyrin syntheses from pyrromethanes were developed almost exclusively by Fischer. Self-condensation of 5-bromo, 5'-methylpyrromethenes in organic acid melts or a mixture of two 5-bromo, 5'-methylpyrromethenes in formic acid give porphyrin yields as high as 50%.[61,62]

(b) MacDonald's method of porphyrin synthesis from pyrromethanes involves condensation of a 5,5'-diformylpyrromethane with a 5,5'-diunsubstituted pyrromethane in the presence of an acid catalyst leading to yields of porphyrins as high as 60% (Figure 1.10).[63]

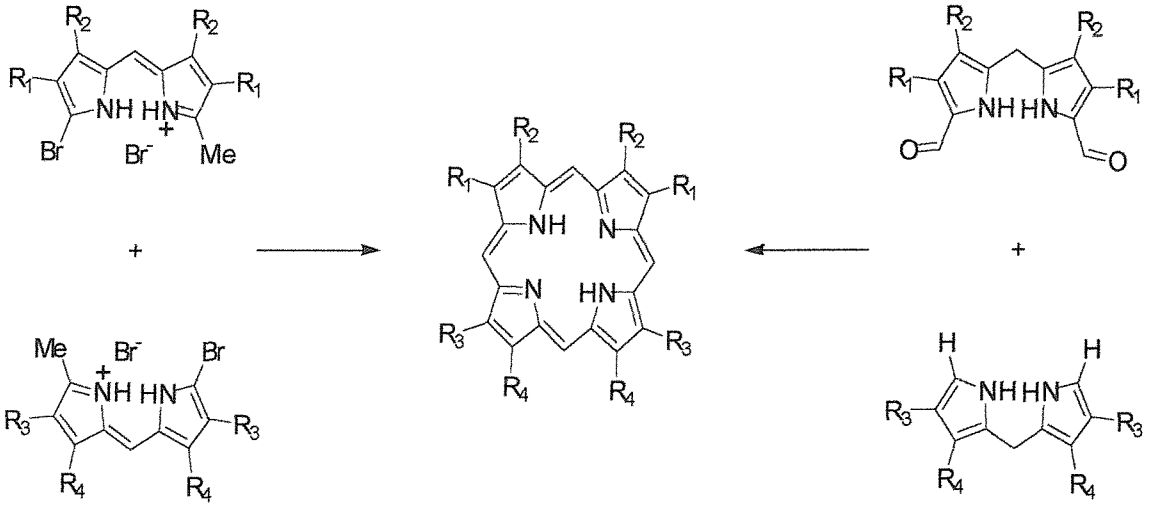
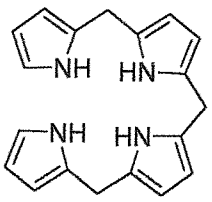
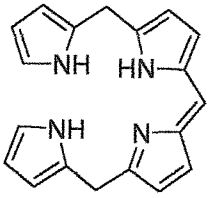


Figure 1.10 Fischer and MacDonald porphyrin syntheses

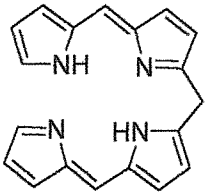
(iii) Cyclisation of open-chain tetrapyrrolic tetramines – The only general porphyrin syntheses are those which proceed in a stepwise manner via open-chain tetrapyrrolic intermediates. Cyclisation results in the formation of the desired porphyrin. A number of open-chain tetrapyrroles have been successfully used in this route to porphyrins, namely bilanes,[64] bilenes[65,66] and biladienes.[67,68]



Bilane



Bilene



Biladiene

1.3.3 Metal binding properties of porphyrins

The binding of metal ions to porphyrins is well documented.[69,70,71,72,73,74] Any porphyrin derivative in which at least one of the central nitrogen atoms forms a bond to a metal ion is called a metalloporphyrin. Usually when coordination occurs two protons are removed from the pyrrole nitrogen atoms leaving two negative charges. When divalent metal ions are chelated the resulting tetracoordinate chelate has no residual charge. Almost all metals and semimetals have been shown to combine with porphyrin ligands with several metals inserted by Nature, namely magnesium (chlorophylls), iron (hemes), nickel and vanadium (mineral oils), copper (Turacus indicus), manganese (blood), zinc (yeast mutants) and cobalt (vitamin B_{12}).

The simplest and most commonly occurring metalloporphyrins are 1:1 complexes. The metal ion sits in the 'equatorial plane' containing the four nitrogen atoms (Figure 1.11). For metal ions with a low affinity for additional ligands, such as copper (II) and nickel (II), this gives rise to complexes with a square-planar geometry.

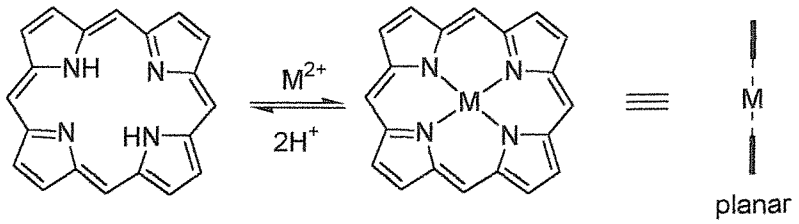


Figure 1.11 Representations of 1:1 metalloporphyrin complexes

However most metal ions take up additional donor ligands to complete their coordination sphere. Those metals that combine with one more ligand, magnesium (II), cadmium (II) and zinc (II), form square-pyramidal structures whereas those that combine with two extra ligand molecules, iron (II), cobalt (II), manganese (II), form distorted octahedral structures (Figure 1.12).

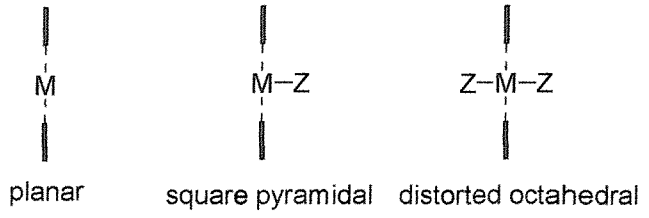


Figure 1.12 Representations of metalloporphyrin geometries

Many other porphyrin:metal ratios are known and the geometry of the complexes formed can be predicted, to some extent, by the size of the metal ion. The optimum ionic radius for a metal ion that is coplanar with the porphyrin lies between 0.60 and 0.69 Å.[75] Metals which are too large to fit into the porphyrin nucleus are displaced from the porphyrin plane and are said to be 'sitting atop' of the molecule. Smaller ions, such as sodium, potassium and lithium, form 2:1 complexes in which the metal atoms are incorporated above and below the porphyrin macrocycle plane (Figure 1.13).

Figure 1.13 Representations of metal ion positioning in metalloporphyrins

Furthermore, for metal ions such as molybdenum, ruthenium, osmium, tungsten and manganese, porphyrin complexes are formed in which the metal is in a stable but unusual oxidation state because its ionic radius gives the best fit for the porphyrin ligand system. However some cases cannot be explained by ionic radius alone. For some ions, for example silver (II), the radius of the ion appears too large and yet the

metal ion is found to be coplanar with the porphyrin. This has been attributed to metal-to-porphyrin back-bonding, which decreases the electron density at the metal and results in shrinkage of the metal ion.

Many preparative procedures are known for metalloporphyrins.[76] In general, the preparation of metalloporphyrins from free-base porphyrins involves reaction with a large excess of metal salt or a metal carbonyl in high boiling solvents. However the work-up usually requires chromatography and as such is not a particularly suitable method for large-scale preparations.

A second method is the metathesis reaction of alkali-metal porphyrins with metal halides affording metal porphyrin compounds cleanly and in high yields.[77,78] This method works well on a large scale owing to the use of low-boiling solvents, stoichiometric amounts of porphyrin and metal salts, and purification by recrystallisation or Soxhlet extraction.

A simple method for following the progress of a metallation reaction is to measure its absorption spectrum. Generally porphyrins show four absorption bands in the visible region in addition to the Soret band. Upon formation of a metalloporphyrin the four-banded spectrum collapses into a two-banded spectrum with some or no movement of the Soret band.

1.3.4 Applications of porphyrins

Porphyrin metal complexes are of major biological importance and have been extensively studied in order to understand the biosynthetic formation and biological activity of natural compounds. Porphyrins play a key role in essential biological processes such as photosynthesis and dioxygen transport and storage. Life itself relies on the unique redox and electron transfer abilities of the chlorophylls (magnesium containing chlorins) which are necessary for the conversion of light to chemical energy. The iron (II) porphyrin containing haem proteins are vital for dioxygen transport and storage in most vertebrates. The haem protein, haemoglobin, is found in blood and is concerned with the *in vivo* absorption of dioxygen from air or water and its transport via the blood to the muscle tissue. The haem protein myoglobin is found in the muscle and is involved in dioxygen storage until energy release is necessary. At

this time the dioxygen is transported to the mitochondria where energy is released by its reaction with glucose to produce ultimately carbon dioxide and water. Haemoglobin then has a second role, namely to bind the carbon dioxide produced and transport it back to the lungs.

In addition to their importance in Nature, porphyrins have important medical applications. Some porphyrin derivatives have been used in the diagnosis and therapy of tumours, some can even localise to tumour tissues.[79] Photoirradiation with a laser light leads to the fluorescence of tumour tissues containing porphyrin derivatives allowing detection of a cancer. Furthermore, some porphyrins produce singlet oxygen by photosensitization, which oxidises important parts of the cancer cells and deactivates them so destroying the cancer tissue.

Porphyrins also play an extensive role in analytical chemistry.[80] They have found application as spectrophotometric reagents for the determination of metal ions, they are employed in electroanalytical chemistry in, for example, potentiometry, voltammetry and as electrochemical biosensors, and have applications in HPLC[81] as complexing agents for the determination of transition metals.

1.4 Application of Diamine Based Ligands as Catalysts in Asymmetric Synthesis

1.4.1 Background to asymmetric synthesis

An important application of tetramine ligand systems, which has not been mentioned until now, is their use as catalysts for asymmetric synthesis. In particular, diamine based ligands from which tetradentate ligands can be synthesised, with heteroatoms such as nitrogen, phosphorus, sulphur and oxygen, are becoming increasingly popular in asymmetric synthesis.[82] The importance of chiral catalysis, in both academic and industrial communities, stems from the fact that efficient chiral catalysis can lead to large quantities of optically active compounds from relatively small amounts of enantiopure material. In addition, the most practical methods employ inexpensive and readily available chiral ligands and give high and predictable enantioselectivity across

a wide range of substrates.[83] Thus chiral catalysis has obvious advantages over the use of chiral reagents, such as BINOL modified lithium aluminium hydride,[84] and chiral auxiliaries, such as Evans's oxazolidinones,[85] which require one equivalent of chiral unit to synthesise one equivalent of chiral product (Figure 1.14).

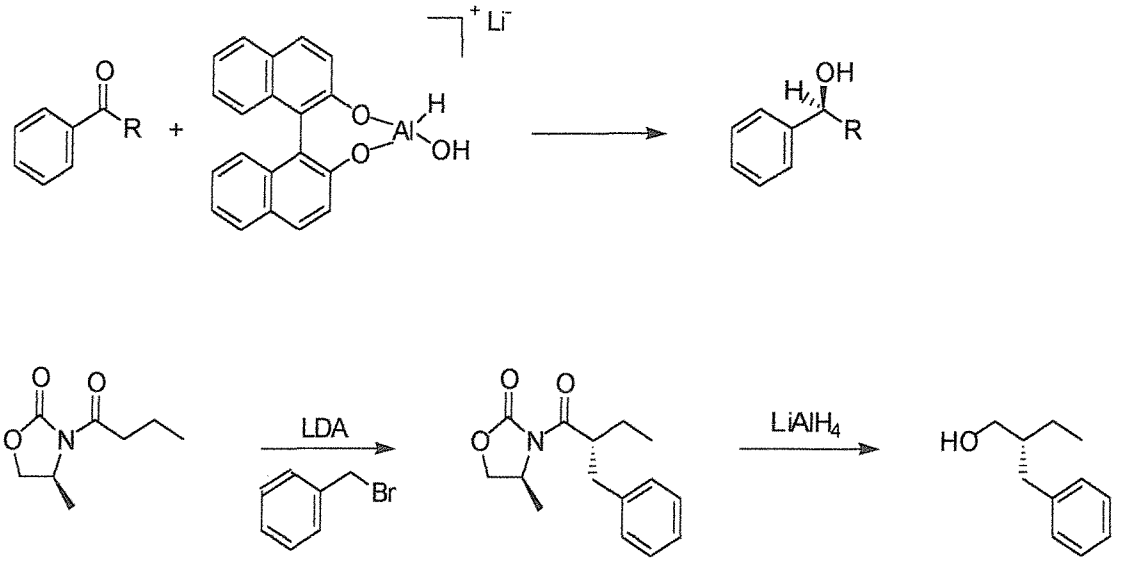


Figure 1.14 Asymmetric syntheses employing chiral reagents (top) and chiral auxiliaries (bottom)

1.4.2 Application of porphyrins as catalysts

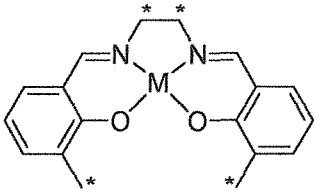
Of the tetramine systems discussed so far metalloporphyrins have been found to have the greatest application as catalysts. Metalloporphyrins have been found to be very versatile oxidation catalysts and have been used in olefin epoxidations and alkane hydroxylations. Furthermore chiral metalloporphyrins have been used in asymmetric synthesis.[86] The oxo-transfer enzyme cytochrome P-450, containing an iron porphyrin as its active site, is able to perform highly enantioselective olefin epoxidations and hydroxylations.[87] Groves *et al.* reported that simple iron porphyrins are good models for the reaction site of cytochrome P-450[88] and are readily oxidised to the active oxo species by treatment with iodosylbenzene. Consequently many metalloporphyrins were synthesised for use in various oxo transfers, with iron (II), manganese (II) and ruthenium (IV) porphyrins found to be the most efficient catalysts for the epoxidation of simple olefins and the oxidation of sulfides.[89]

In order to effect asymmetric oxo-transfer reactions optically active iron and manganese porphyrins have been synthesised. Optically active binaphthyls have been

extensively used in building chiral porphyrin ligands which give rise to relatively high enantiomeric excesses (ee's) (40 – 48%) in the iodosylbenzene epoxidation of styrene.[90] Naruta and Maruyama have designed a C_2-symmetric porphyrin ligand in which each face of the macrocycle is occupied by two binaphthyl units.[91] The advantages of C_2-symmetry in asymmetric synthesis are two-fold. Firstly, it simplifies the synthesis of the ligand system and secondly, the reactive centres experience the same stereodiscriminating environment independent of which face of the ligand they approach. An ee of 89 % was obtained with the C_2-symmetric porphyrin ligand in the iodosylbenzene epoxidation of 2-nitrostyrene.[92]

1.4.3 Application of optically active salen ligands as catalysts

There is a considerable limitation in the use of chiral metalloporphyrins, largely due to the difficulty in synthesising the ligands. This has been overcome by the use of Schiff base manganese complexes, which are more accessible from readily available chemicals. The use of metal complexes of the *N,N*-ethylenebis(salicyldeneaminato) ligand, known as 'salen complexes', as a catalyst for oxo-transfer reactions has been studied[93] since they have features in common with metalloporphyrins, namely their electronic structure and catalytic activity (Figure 1.15). Chiral manganese (III) complexes have been found to be the most efficient catalysts.[94]



* - stereogenic centre

Figure 1.15 Possible stereogenic centres in salen complexes

Although four stereogenic centres are possible (two on the diamine and two on the ortho-positions of the phenyl rings), Jacobsen *et al.* have shown that only the two chiral centres of the diamine are necessary to provide high ee's with PhIO[95] or NaOCl[96] as oxidants in epoxidation reactions.

1.4.4 Catalysts derived from trans-1,2-diaminocyclohexane

One of the commonest diamines from which salen complexes are synthesised is trans-1,2-diaminocyclohexane, which is commercially available. The C_2-symmetric diamine trans-1,2-diaminocyclohexane is easily resolved in aqueous medium using D- or L-tartrate to give either the (R,R) or the (S,S) enantiomer in enantiopure form. The enantiomers may then be derivatised to afford powerful stereodirecting ligands which have found application in a host of processes.[97] For example the chiral diphosphine rhodium (I) catalyst reported by Fujita and co-workers gives excellent yields and ee's up to 93% for the hydrogenation of α-acylaminoacrylic acids (Figure 1.16).[98]

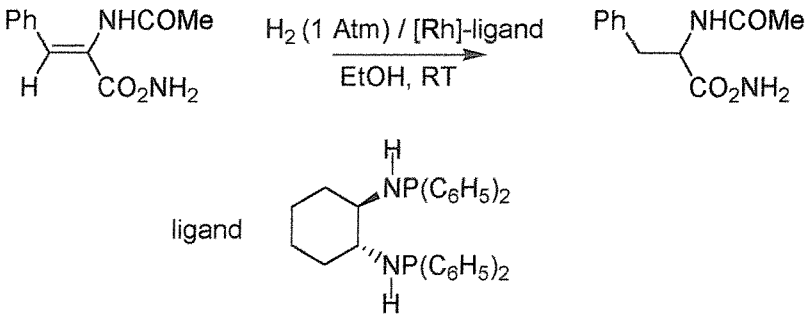


Figure 1.16 Asymmetric hydrogenation of α-acylaminoacrylic acids

Tetradentate ligand systems based upon trans-1,2-diaminocyclohexane have also been shown to be effective catalysts. Ruthenium complexes of Noyori's C_2-symmetric diphosphine/diamine ligand and diphosphine/diimine ligand have been shown to catalyse the reduction of acetophenone derivatives in high yields with up to 97% ee (Figure 1.17).[99,100]

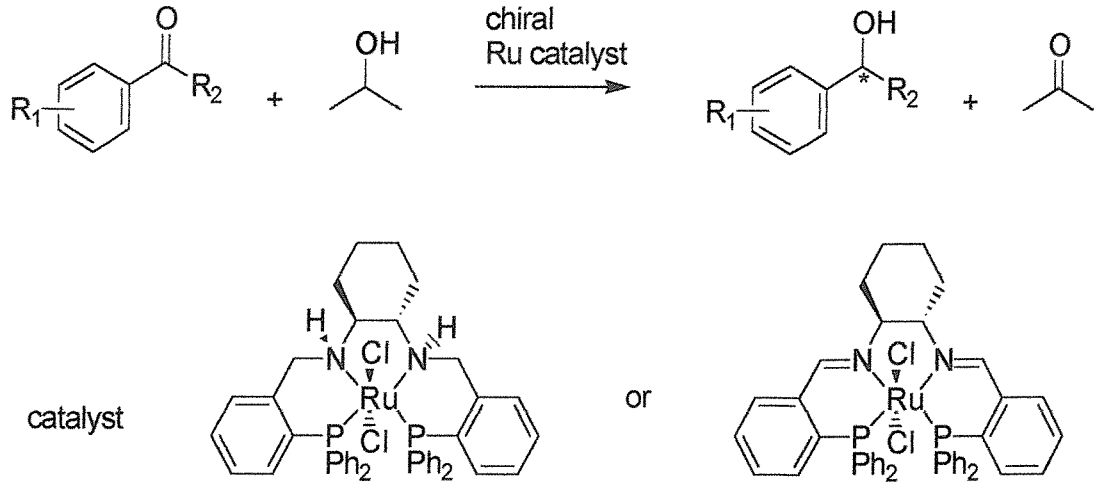


Figure 1.17 Asymmetric reduction of acetophenone derivatives

Palladium complexes of Trost's diphosphine/diamine ligands have been employed as catalysts for the asymmetric alkylation of ketones (Figure 1.18).[101,102]

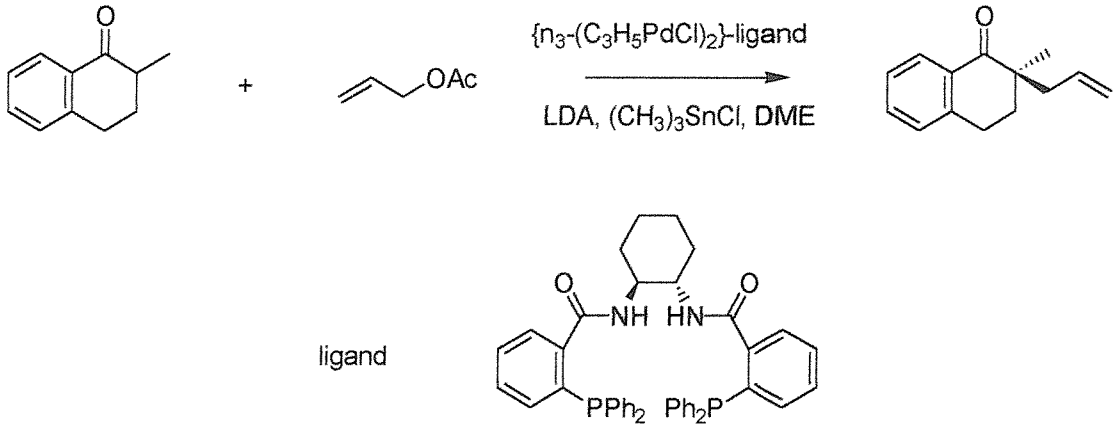


Figure 1.18 Asymmetric alkylation of substituted ketones

Enantioselective addition of diethyl zinc to benzaldehyde has been effected by chiral titanate tetradentate ligands based upon the trans-1,2-diaminocyclohexane motif (Figure 1.19).[103]

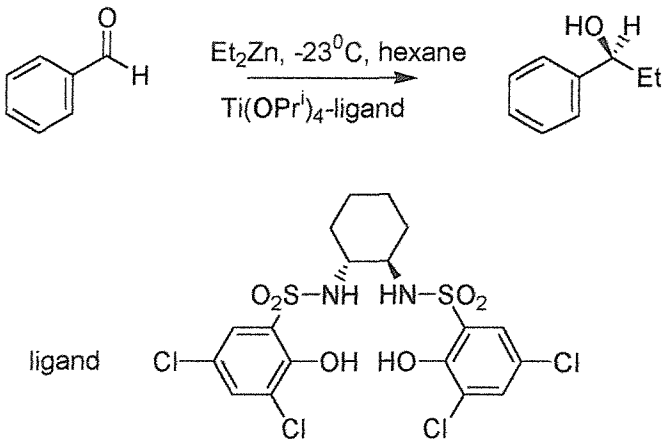


Figure 1.19 Enantioselective addition of diethyl zinc to benzaldehyde

1.4.5 Catalysts derived from (R,R)-diphenylethylenediamine

A second trans-diamine has been used as a motif for salen ligand design, namely (R,R)-diphenylethylenediamine. Furthermore, Noyori has shown that this C_2-symmetric chiral diamine acts as an efficient hydrogenation catalyst for unsaturated ketones (Figure 1.20).[104,105]

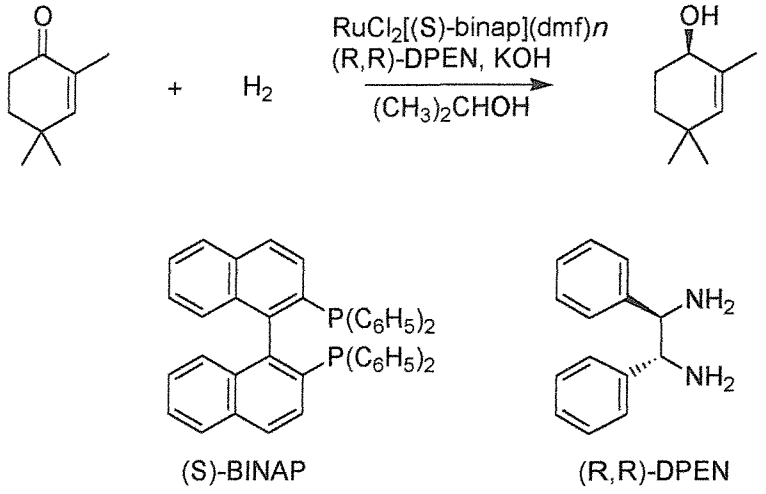


Figure 1.20 Asymmetric hydrogenation of unsaturated ketones

The asymmetric reduction of carbonyl compounds using a range of C_2-symmetric, *N*-substituted (R,R)-1,2-diaminocyclohexanes was investigated by Lemaire. It was found that the *N*-methyl derivative gave the highest yields and ee's (Figure 1.21 and Table 1.1).[106]

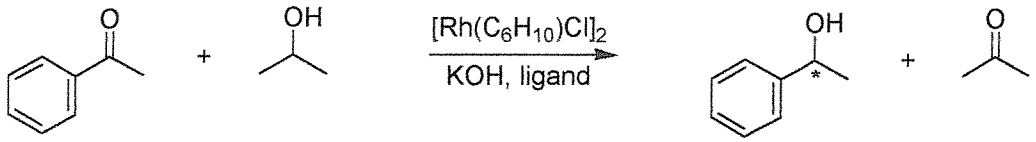


Figure 1.21 Asymmetric reduction of carbonyl compounds using *N*-substituted (R,R)-1,2-diaminocyclohexanes

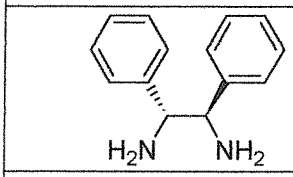
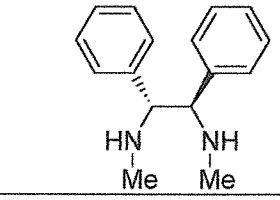
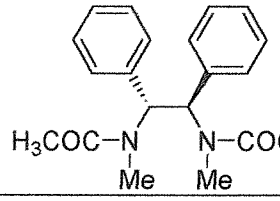
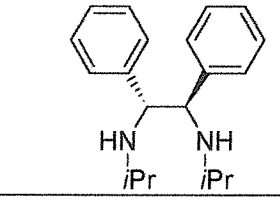
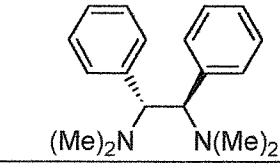
LIGAND	TIME (DAYS)	CONVERSION (%)	E.E. (%)
	8	94	17
	7	100	67
	8	90	4
	8	8	28
	4	93	6

Table 1.1 Variation in yield and ee observed with a range of *N*-substituted (R,R)-1,2-diaminocyclohexane ligands in the above reaction

1.4.6 Heterogeneous catalysis

One of the major disadvantages of catalytic asymmetric synthesis is that separation of the catalyst from the reaction mixture is often difficult, especially when large-scale applications are envisaged. For this reason the development of chiral heterogeneous catalysis is a field of great interest. One major aim is the synthesis of polymer supported catalysts which permit recovery of the catalyst by simple filtration and its reuse, thus minimising the cost of the synthesis. Two types of polymer supported

ligand are possible, firstly, non-covalently bound catalysts, on silica gel and alumina for example,[107] which can be easily removed from the polymeric support and secondly, permanently, covalently bound polymer supported catalysts have been shown to be effective in a variety of syntheses such as aldol reactions,[108] the preparation of Evans's oxazolidinones[109] and Diels-Alder reactions.[110] Often polymer supported chiral inductors demonstrate several advantages over their homogeneous analogues, such as higher chemical yields, reduced reaction times and higher ee's.

Efficient supported metalloporphyrin oxidation catalysts have been recently developed on supports such as ion-exchange resins, silica and zeolites. As mentioned above some supported metalloporphyrins are more effective than their homogeneous analogues, for example a manganese porphyrin has been prepared that is a more active catalyst in the NaOCl epoxidation of styrene when bound to colloids than alone in aqueous solution.[111]

1.5 Application of Tetramines as Fluorescent Sensors

1.5.1 Background to fluorescent sensors

A second application of tetramines is as fluorescent sensors.[112,113,114] Given any substrate (cation, anion or molecule), an appropriate receptor can be designed that possesses structural and chemical features suitable for substrate recognition. If a molecular subunit is attached to the receptor and is capable of signalling the occurrence of receptor-substrate interaction, then the whole assembly constitutes a 'sensor'.

The molecular subunit must display a well-defined and perceptible property which should change drastically upon receptor-substrate interaction. Fluorescence is an ideal property, displaying many useful features: in most cases it is visible to the naked eye, it is measurable in real time using instrumentation that is not too expensive, and it can be detected even in extremely dilute solutions.

The application of fluorescent sensors can be divided into four areas: fluorescent sensors for metal ions, fluorescent sensors for anions, molecular switches operating via a pH change, and redox switchable fluorescent systems.

1.5.2 Fluorescent sensors for metal ions

Many fluorescent sensors for metal ions contain anthracene as a fluorescent subunit, or fluorophore, owing to its strong and well-characterised emission and its chemical stability. De Silva has designed an anthracene-based sensor for the s-block metal ions (Figure 1.22).[115]

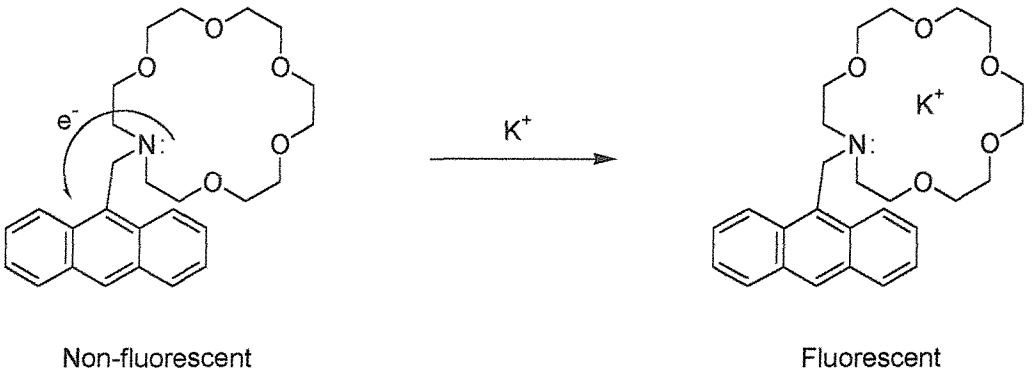
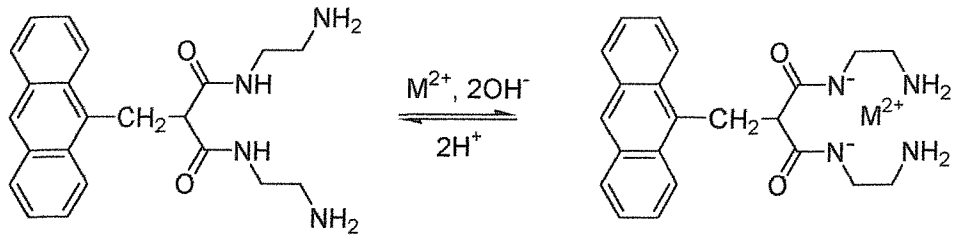


Figure 1.22 An anthracene-based sensor for potassium ions

The receptor is an 18-membered macrocycle analogous to the 18-crown-6 ligand and as such is suitable for a selective interaction with the potassium (I) ion. Prior to metal incorporation, the system is not fluorescent. The typical anthracene fluorescence is quenched via a photoinduced electron transfer (PET) from the lone pairs on the tertiary amine group of the macrocycle ring to the photoexcited fluorophore. Upon metal coordination the electron-transfer process is prevented since the lone pairs on the nitrogen are involved in coordination. Thus, the receptor-K^+ ion interaction is communicated by the appearance of the intense emission spectrum of anthracene.

A second fluorescent sensor based on anthracene, which works via a PET mechanism, has been developed by Fabrizzi (Figure 1.23).[116] Chelation of a copper (II) or a nickel (II) ion to the diamine-diamide tetradentate ligand, coupled with deprotonation of the two amide groups, results in a quenching of fluorescence. This is due to PET from the metal ion to the excited fluorophore.

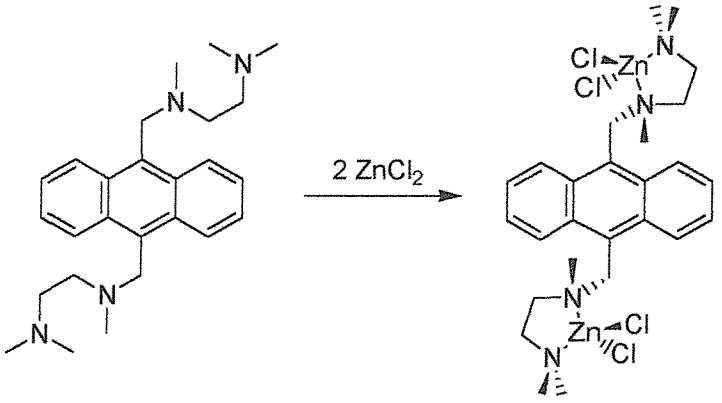


Fluorescent Non-fluorescent

Figure 1.23 An anthracene based sensor for copper (II) and nickel (II) ions

In addition, this sensor can discriminate between copper (II) and nickel (II) ions. Titration experiments have shown that the sensor binds copper (II) about 2 pH units before nickel (II), reflecting the generally observed greater solution stability of copper (II) complexes with polyaza ligands to those of nickel (II). Thus, at pH 7 the sensor recognises copper (II) but not nickel (II).

Czarnik has designed an anthracene-based fluorescent sensor which results in chelation enhanced fluorescence (CHEF) in the presence of zinc chloride (Figure 1.24).[117]



Non-Fluorescent Fluorescent

Figure 1.24 An anthracene-based sensor for zinc chloride

Imperiali has produced a fluorescent peptidyl sensor for divalent zinc (Figure 1.25).[118,119] The design of the sensor comprises a synthetic peptidyl template with a covalently attached fluorescent subunit, which is sensitive to metal-induced conformational changes in the polypeptide assembly.

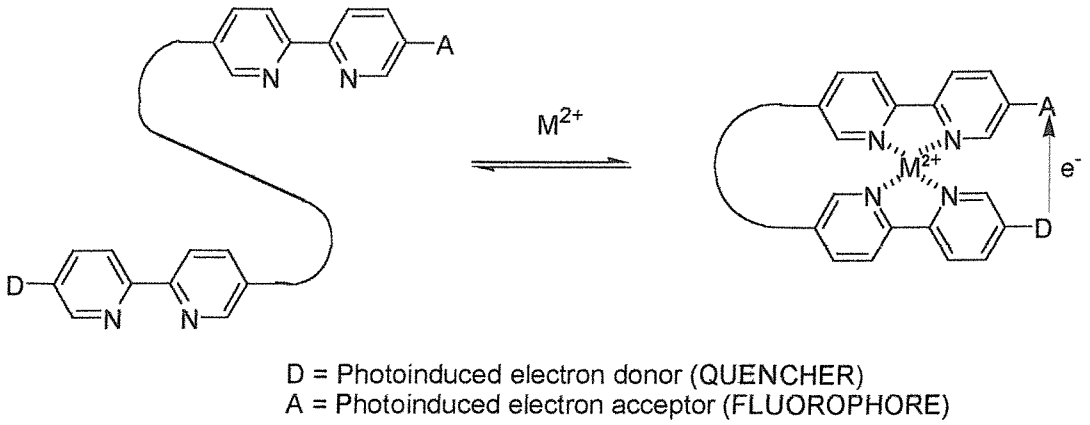


Figure 1.25 Fluorescent sensor based on metal-induced conformational changes

1.5.3 Fluorescent sensors for anions

Czarnik has reported several anthryl-polyamines that can be used as fluorescent sensors for anions such as phosphates, sulfates and acetates.[120,121,122] In each case the non-benzylic nitrogens are quaternary and consequently the sensor is insensitive to metal ions. Initially the sensors exhibit low fluorescence owing to PET from the lone pairs of the benzylic nitrogen. Upon complexation of the anionic phosphate oxygens the remaining hydroxyl of the phosphate group is placed in close proximity to the free amine. Intracomplex proton transfer leads to an enhancement in fluorescence owing to elimination of the intramolecular quenching (Figure 1.26).

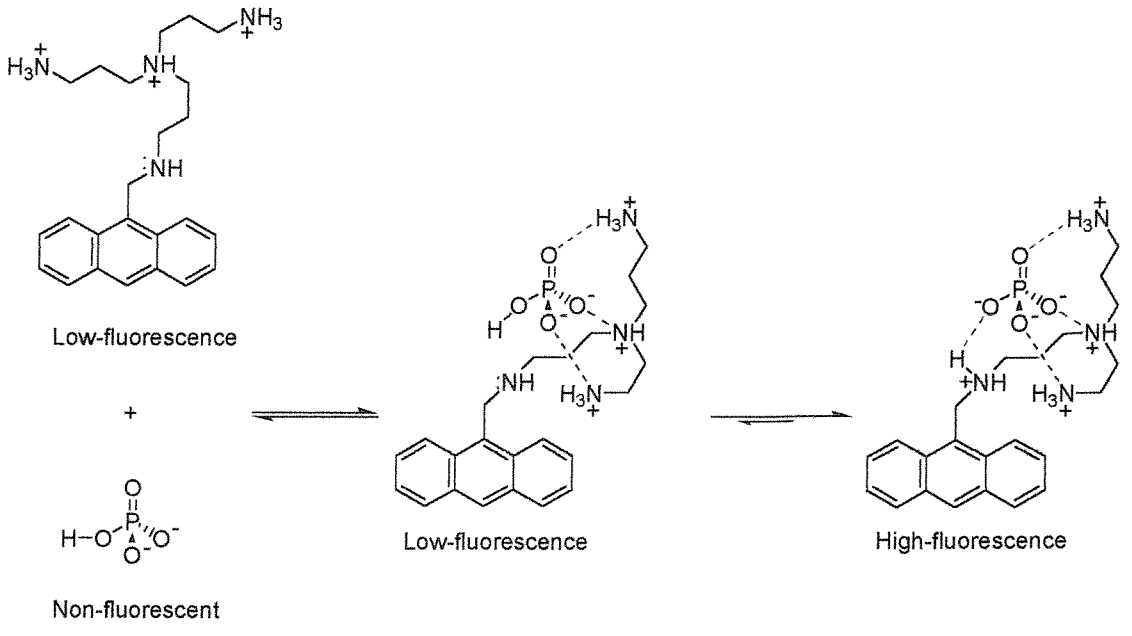
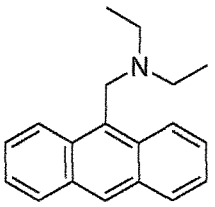


Figure 1.26 Enhancement in fluorescence arising from intracomplex proton transfer

1.5.4 Molecular switches operating via a pH change

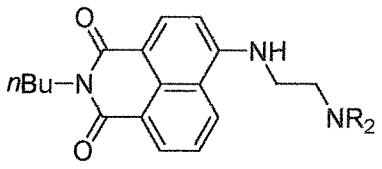
In the sensors described above the fluorescence is controlled by an external source, for example, metal ions. The 'control unit' however can be attached via a linker to the fluorophore. If the control unit can exist as two different states of comparable stability and reversible access to each state can be controlled through variation of an external parameter, then the control unit can be thought of as a 'switch'.[123,124] The change in fluorescence can be thought of as ON states and OFF states, or vice versa.

A molecular switch can be simply illustrated by varying the pH of a solution of **(5)**, which results in the switching on/off of the fluorescence of the anthracene fragment linked through a -CH_2- bridge to the tertiary amine group. The pH switchable fluorescent system can also be considered as a sensor for protons.

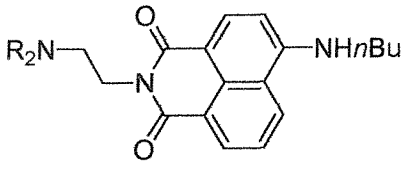


(5)

De Silva has shown that the design of the fluorescent pH sensor can have a pronounced effect on its ability to switch on and off.[125] The two compounds **(6)** and **(7)** are isomers. The fluorescence of **(6)** is strongly enhanced upon protonation due to quenching of the PET from the lone pairs of the nitrogen adjacent to the naphthalene ring. Compound **(7)** shows little change in fluorescence upon protonation. This is attributed to a lack of PET in the unprotonated form. Although PET is thermodynamically feasible it requires the electron to enter the fluorophore across the imide moiety with its repulsive electric field and thus it is not observed.



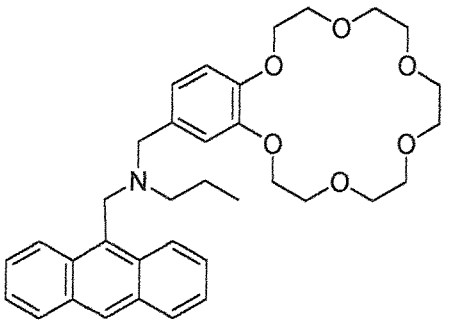
(6)



(7)

R = Et, Me, $(CH_2)_2O(CH_2)_2$

De Silva has also prepared systems in which fluorescence is observed only in the presence of both protons and metal ions.[126] In compound **(8)**, the presence of protons and sodium ions results in intense fluorescence since the amine is protonated and the crown is complexed. In the absence of both, or in the presence of just sodium ions or protons, the fluorescence is greatly reduced. Systems such as **(8)** are of interest since they display 'logical behaviour'; indeed the example shown can be described as a molecular AND logic gate.



(8)

1.5.5 Redox switchable fluorescent systems

A second parameter which can be varied to effect a switching behaviour is the redox potential. In order to modify the fluorescence through a change in redox potential the control unit must be redox-active and display comparable stability in both the reduced and oxidised states. This is commonly achieved by the control unit hosting a metal centre M which can change reversibly between $M^{(n+1)+}$ and M^{n+}. Ideally for a switching behaviour, one oxidation state quenches the fluorescence and the other does not.

Fabrizzi has studied a range of macrocycles linked to anthracene or naphthalene to determine their effectiveness as fluorescent molecular switches operating through a metal-centred redox couple - copper (I) / copper (II) or nickel (I) / nickel (II) (Table 1.2).[127]

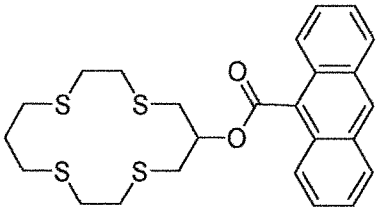
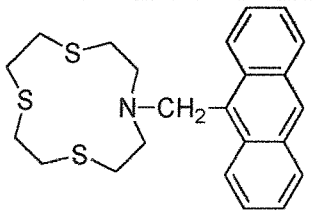
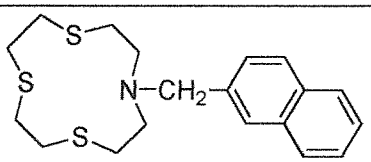
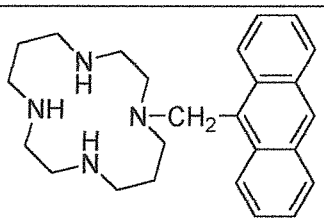
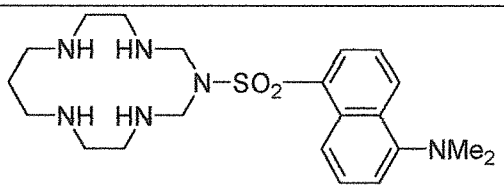
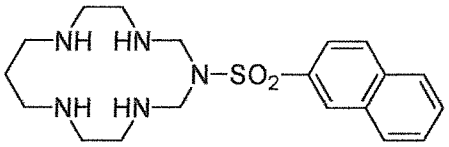
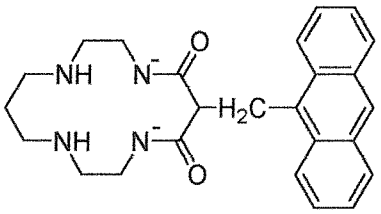
Molecule	Couple	Switching Behaviour
	Cu^{II} / Cu^{I}	OFF / ON
	Cu^{II} / Cu^{I}	OFF / ON
	Cu^{II} / Cu^{I}	OFF / ON
	Ni^{III} / Ni^{II}	OFF / OFF
	Ni^{III} / Ni^{II}	OFF / ON
	Ni^{III} / Ni^{II}	OFF / ON
	Ni^{III} / Ni^{II}	OFF / OFF

Table 1.2 Fluorescent switching behaviour of a range of macrocycles linked to anthracene or naphthalene in the presence of copper and nickel ions

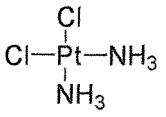
1.6 Application of Amines in Medicine

1.6.1 The role of the linear polyamines in biology

The polyamines – putrescine, spermidine and spermine – are of great significance in medicinal chemistry. It has been known for some time that polyamines play an important role in cell proliferation and differentiation. In more recent years, polyamines have been shown to be promoters of triplex DNA formation[128] and thus have potential important applications in gene therapy, which is being developed for the treatment of diseases such as cancer, AIDS and autoimmunity.

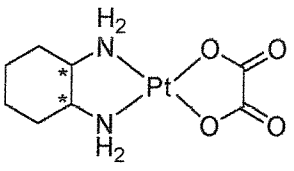
1.6.2 The use of cis-platin and its derivatives in the treatment of cancer

The most important anti-cancer drug of the last century was cis-$[PtCl_2(NH_3)_2]$, known as cis-platin, which was discovered in the 1960s.

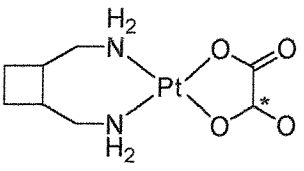


cis-platin

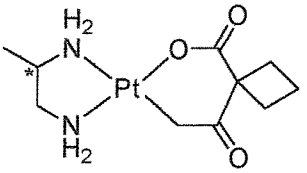
Although the mechanism of action of cis-platin is still not fully understood there is much evidence that DNA is its main target,[129] leading to platinum-induced inter and intra-strand crossing, as well as DNA-protein cross-links. The most probable sites for platinum binding being N_3-cytosine, N_7-guanine and N_1,N_7-adenine, following displacement of cis-platins' labile chlorines. Cis-platin has found application in the treatment of testicular and ovarian cancer and is increasingly used against cervical, bladder and head/neck tumours. However, owing to the toxic side effects of cis-platin, as well as the development of drug resistance in certain tumours, much research has been carried out into the development of new compounds, such as oxaliplatin, D-19466 and NK 121, that are based upon the cis-platin structure.[130]



Oxaliplatin

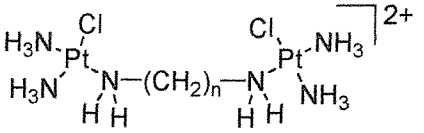


D-19466



NK 121

The recently reported dinuclear platinum-amine compounds, of general structure **(9)**,[131] appear to be able to bind to DNA at two different positions, thereby enhancing the antitumour effect, and have shown promising activity in cis-platin resistant cell lines.

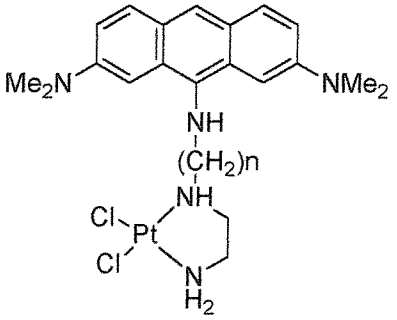


(9)

n=2-6

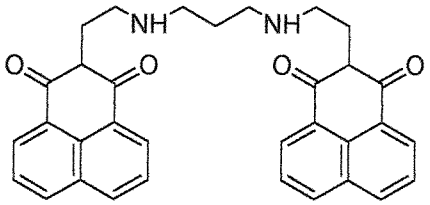
1.6.3 The use of naphthalimides and bis-naphthalimides in the treatment of cancer

In order to exploit the DNA intercalation, cis-platin has been attached to derivatives already known to intercalate with DNA, such as acridine-orange **(10)**.[132]



(10)

However, many DNA binders themselves have been found to have antitumour properties, of which one of the most important groups of compounds are those derived from aristolochic acid, which contains a β-nitronaphthalene moiety. Recognition of its anti-cancer properties led to the synthesis of a range of naphthalimide drugs.[133] Bis-naphthalimides, such as LU 79553, have exhibited even more encouraging biological activity. Several bis-naphthalimides bearing the 1,8-naphthalimide chromophore have been tested for cytotoxicity *in vitro* and found to be substantially more potent than the corresponding monomeric compounds.

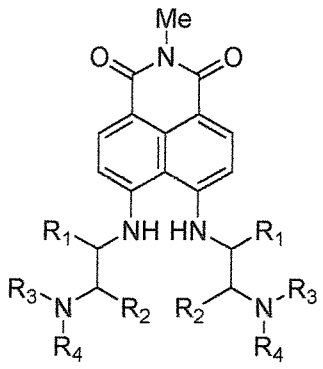


LU 79553

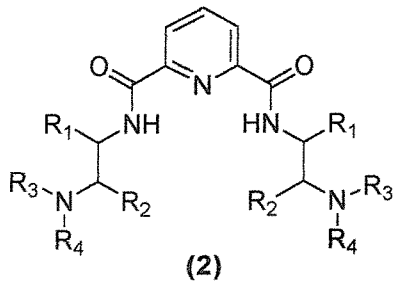
Much work has been carried out to elucidate the nature of the binding of bis-naphthalimides to DNA.[134] Unusually, the bis-naphthalimides have been found to intercalate bifunctionally into the double helix via its major groove, leaving the minor groove largely undisturbed. This mode of interaction is rare, being observed with only a few other compounds, and it has been speculated that it may account for the unique anti-tumour properties of the bis-naphthalimides.

1.7 Aims of this Research Project

The two principle aims of the research project described in this thesis are the synthesis of novel tetramines based upon the naphthalimide unit **(1)** and the 2,6-pyridine unit **(2)**, and the study of their metal binding properties.



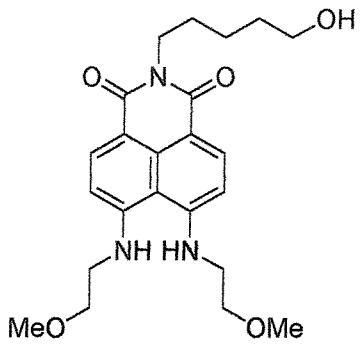
(1)



(2)

The results of this work will govern the direction of future developments within this area, however amongst the potential applications of the tetramines described herein are the following:

(i) Catalysts in asymmetric synthesis - Both series of tetramine ligands have a potential application as chiral catalysts in the field of asymmetric synthesis. Chirality can be introduced at positions R_1, R_2, R_3, or R_4 or a combination of these positions. By varying the steric nature of the groups at positions R_1, R_2, R_3, and R_4 a wide range of ligand directing effects can be explored. A short study of the use of chiral ligands, based upon the naphthalimide unit **(1)**, in asymmetric synthesis has already been conducted and yielded promising results.[2] Furthermore, it has been shown that naphthalimides synthesised from amines with remote functional groups, such as **(11)**, are suitable for binding to polymers and thus have a potential use as heterogeneous catalysts.

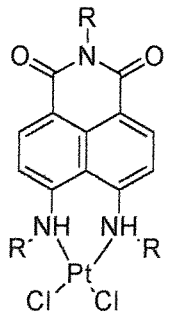


(11)

In the series based upon the 2,6-pyridine unit **(2)**, substitution at the 4-position of the pyridine ring also allows for incorporation onto a polymeric support. Thus these tetramine ligands have a potential application as both heterogeneous and homogeneous catalysts.

(ii) Fluorescent sensors - An interesting feature of the tetramine ligands based upon the naphthalimide unit **(1)** is their fluorescent nature. A short study to investigate the effect on fluorescence upon metal binding by these systems has been carried out and significant changes in fluorescence were observed upon both protonation and the addition of metal salts.[2] Hence these systems have potential as fluorescent sensors, with the selective binding of different metals achieved by varying the amino functionality of the metal binding pocket.

(iii) Biomedical applications - The 1,8-naphthalimides prepared in the course of this research have the potential to effectively bind DNA and as a result may exhibit anti-cancer properties. Furthermore, bis-naphthalimides, which have shown promising anti-cancer properties, could also be prepared via variation of the functionality of the imide side-chain. In addition, platinum complexes such as **(12)**, which is a precursor in the route to tetramines, have a structural similarity to cis-platin and may possess interesting biological properties.



(12)

The two principle aims of this thesis are discussed as follows:

Synthesis. Chapter 2 describes the improvement of established routes to tetramines within the series based upon naphthalimide unit **(1)** and the development of novel routes to tetramines in both series.

Metal Binding Properties. Chapters 3 and 4 describe the study of the metal binding properties of tetramines in both series through the use of fluorimetry and mass spectrometry.

Chapter Two

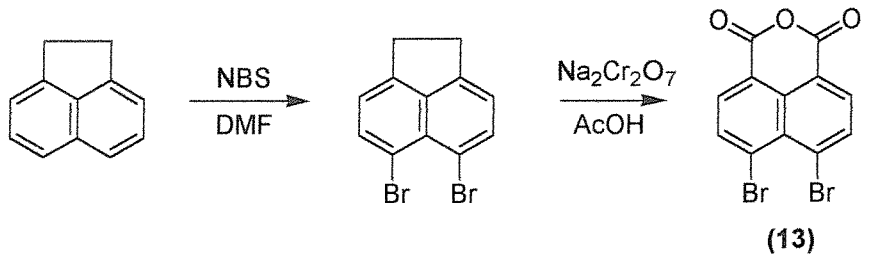
Synthesis

Chapter Two

Synthesis

2.1 Synthesis of Tetramines in the Naphthalene Series

The synthesis of 4,5-dibromo-1,8-naphthalic anhydride **(13)** from acenapthene, via bromination[135] and oxidation,[136,137] is well documented in the literature (Figure 2.1).



(13)

Figure 2.1 Synthesis of 4,5-dibromo-1,8-naphthalic anhydride **(13)**

According to the procedure of Morris,[2] imides **(14)** and **(15)** were synthesised from the anhydride **(13)**. A series of aminoalcohol derivatives of imide **(14)** were prepared and the subsequent reaction of the ethanolamine derivative **(16)** with thionyl chloride, followed by reaction with pyrrolidine led to tetramine **(20)** (Figure 2.2). All yields were in good agreement with those of Morris.

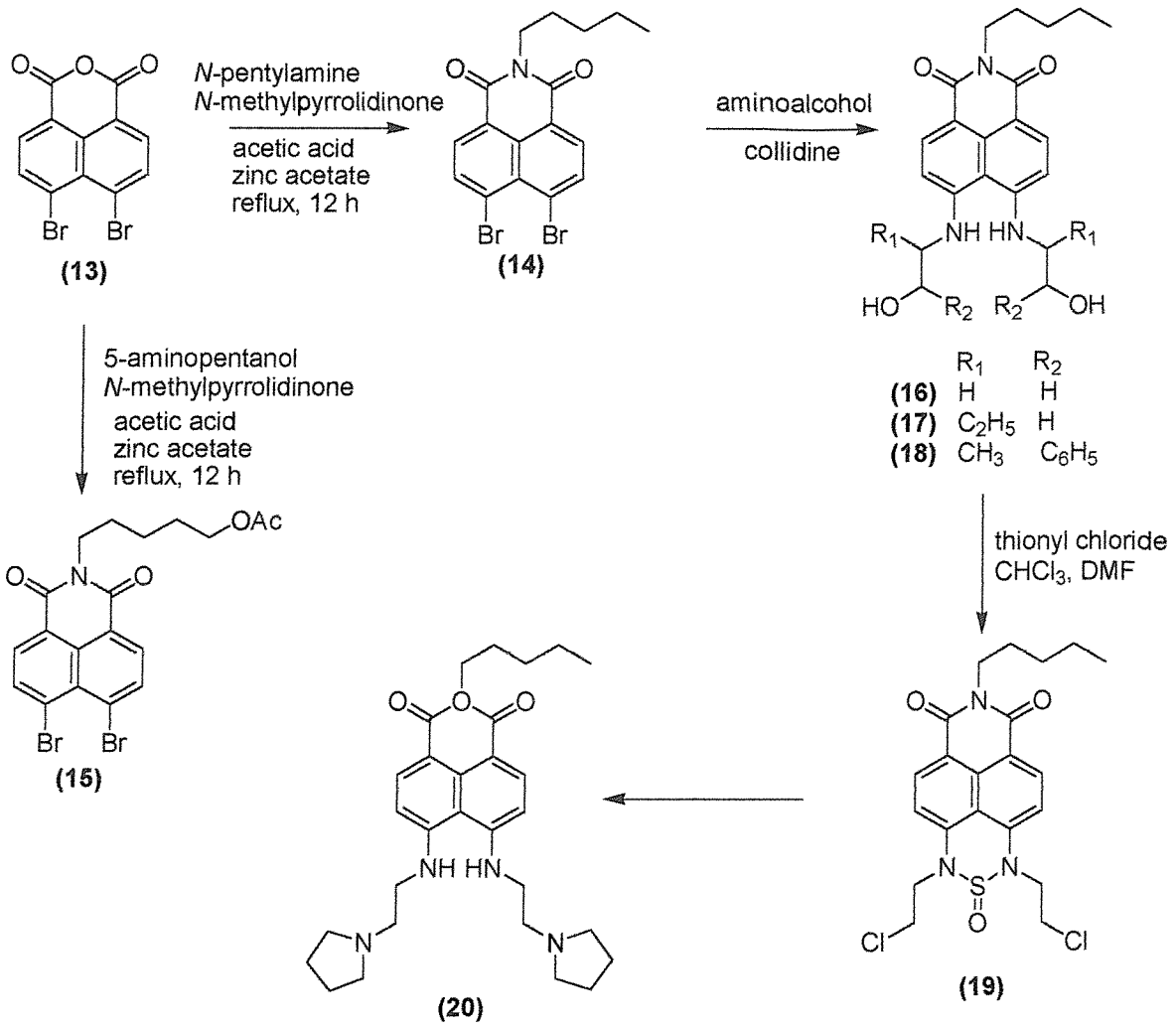


Figure 2.2 Synthetic route to tetramines starting from 4,5-dibromo-1,8-naphthalic anhydride **(13)**

2.1.1 Preparation of imides

A major obstacle in the synthetic route to tetramines was identified as the imide formation reaction, which proceeded in low yield. Imides **(14)** and **(15)** were synthesised in 35% and 12% yield respectively under the reaction conditions shown. Thus it was decided to investigate improvements to the conditions which would ideally lead to both a higher yield of the imide and a product which did not require purification by column chromatography.

Several procedures illustrating imide formation from 4,5-dihalo-1,8-naphthalic anhydrides have been reported in the literature.[138,139,140] Consequently an extensive investigation employing various reaction conditions was carried out (Table 2.1). In

contrast to the study of Morris, the amine used in each case was the simple alkyl amine, methylamine.

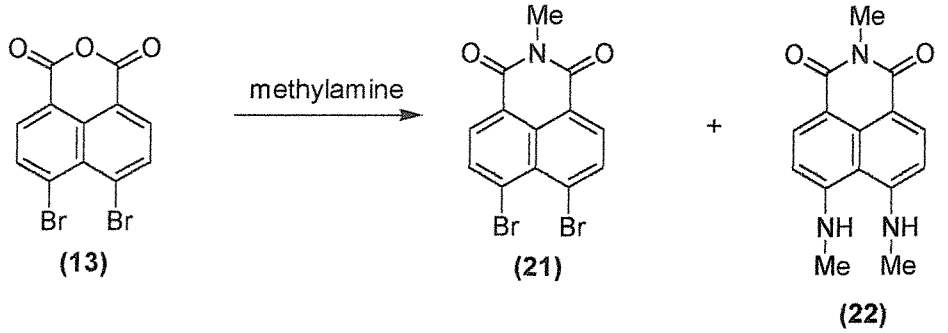


Figure 2.3 Products formed in the reaction of 4,5-dibromo-1,8-naphthalic anhydride **(13)** with methylamine

Reagent (equivalents)	Solvent	Conditions	Observations	Yield of (21)
3 % aq $MeNH_2$ (37)	Water	20 - 30°C for 1 h 80°C for 1 h	Colour change pink to orange Major product **(22)** by ^1H NMR	<5%
3 % aq $MeNH_2$ (37)	Water	20 - 30°C for 1 h 80°C for 5 h	Colour change pink to orange Major product **(22)** by ^1H NMR	<5%
3 % aq $MeNH_2$ (37)	Water	20 - 30°C for 1 h 80°C for 24 h	Colour change pink to orange ^1H NMR complicated	-
aq $MeNH_2$ (37)	Ethanol	20 – 30°C for 1 h 80°C for 1.5 h	Colour change pink to orange Major product **(22)** by ^1H NMR	<5%
aq $MeNH_2$ (37)	Ethanol	Room temperature for 3.5 h	Colour change pink to orange Minor product **(21)** by ^1H NMR	10%
aq $MeNH_2$ (37)	Ethanol	Room temperature for 20 h	Colour change pink to orange Major product **(21)** by ^1H NMR	50%
aq $MeNH_2$ (37)	Ethanol	Room temperature for 60 h	Colour change pink to orange Major product **(21)** by ^1H NMR	60%
aq $MeNH_2$ (19)	Ethanol	Room temperature for 60 h	Colour change pink to orange Major product **(21)** by ^1H NMR	67%
aq $MeNH_2$ (5)	Ethanol	Room temperature for 12 days	Colour change pink to orange Major product **(21)** by ^1H NMR	80%
aq $MeNH_2$ (37)	Ethanol / 10 % acetic acid	Room temperature for 20 h	Only product **(21)** observed by ^1H NMR	20%
aq $MeNH_2$ (37)	Ethanol / 10 % acetic acid	Room temperature for 60 h	Only product **(21)** observed by ^1H NMR	20%
aq $MeNH_2$ (37)	Ethanol / 10 % acetic acid	25°C for 1.5 h 40°C for 4 h	Only product **(21)** observed by ^1H NMR	15%
aq $MeNH_2$ (37)	Ethanol / 10 % acetic acid Zinc acetate	Room temperature for 20 h	Only product **(21)** observed by ^1H NMR	15%
aq $MeNH_2$ (37)	Ethanol / 10 % acetic acid Zinc acetate	25°C for 1.5 h 40°C for 4 h	Only product **(21)** observed by ^1H NMR	15%
aq $MeNH_2$ (37)	Acetic acid	25°C for 1 h 80°C for 24 h	No reaction observed	-

Table 2.1 Summary of the reaction conditions employed and observations made in the investigation of synthetic routes to methyl imide **(21)**

Owing to the low solubility of anhydride **(13)** in collidine, experiments were carried out in a range of different solvents, with ethanol giving the best results. Although formation of the methyl imide **(21)** was observed when the experiment was carried out using ethanol as the solvent, the formation of a second product **(22)**, in which both bromine atoms had been substituted by methylamine, was also observed (Figure 2.3). When ethanol and 10 % acetic acid were employed as the solvent system, only imide formation was observed, with no bromine substitution occurring. However the yield of such reactions was found to be low despite varying both the reaction times and the reaction temperature. The role of acid in the reaction was uncertain. The aforementioned reactions suggest that the acid may be responsible for (a) promotion of the imide formation reaction, (b) quenching of the bromine substitution reaction or (c) reduction of the number of equivalents of amine in solution, all of which would lead to the results observed. However it is difficult to explain the accompanying reduction in yield.

A series of reactions was attempted in which the ethanol / acetic acid ratio was varied to see if a correlation existed between the amount of acetic acid present and the yield of the reaction. However the amount of acetic acid required to completely quench the substitution reaction at the bromine atoms resulted in unsatisfactory yields of methyl imide **(21)**. Zinc acetate was then added to the reaction mixture in attempt to improve the yield since it has been shown to be effective in the formation of perylene imides.[141] Its mode of action is believed to be due to an improvement in the solubility of the anhydride.[142] However no significant improvement in yield was observed upon the addition of zinc acetate and thus it was decided to omit both acetic acid and zinc acetate from the reaction mixture.

In a further attempt to eliminate substitution at the bromine atoms without lowering the yield of methyl imide **(21)**, the reaction was carried out using fewer equivalents of methylamine compared to the amount of anhydride employed. Reducing the number of equivalents from 37 to just 5 equivalents of methylamine, coupled with a reduction in the amount of solvent used and an increase in the reaction time resulted in high yields of methyl imide **(21)** whilst minimising the amount of bromine substitution product **(22)** formed. The optimum reaction conditions were found to be stirring the

anhydride **(13)** in ethanol with 5 equivalents of methylamine at room temperature for 12 days, which resulted in an 80 % crude yield of methyl imide **(21)**.

Purification of methyl imide **(21)** was achieved by column chromatography in dichloromethane but a reduction in yield to 40 % was observed. Thin layer chromatography (TLC) of the crude reaction mixture showed a blue spot just below the solvent front corresponding to the methyl imide **(21)**, whereas the spot corresponding to the bromine substitution product **(22)** appeared barely above the baseline. Thus it was decided to attempt purification of the crude product simply by dissolving it in dichloromethane and stirring it with silica gel. As expected the bromine substitution product was absorbed onto the silica gel leaving pure methyl imide **(21)** in solution. Variation of the quantity of silica gel used, the volume of dichloromethane and the length of time the reaction mixture was stirred, resulted in an optimum yield of 60 % of pure methyl imide **(21)**. Purification via this method was successfully carried out on a 10g scale.

Although not attempted in the course of this work, it is believed that the imide synthesis described above is applicable to amines other than methylamine. A key property of imides with longer *N*-alkyl chains being increased solubility, as compared to the methyl imide **(21)**.

2.1.2 Preparation of aminoalcohol derivatives

The next step in the route to tetramines was the synthesis of aminoalcohol derivatives of methyl imide **(21)**. Employing the reaction conditions used by Morris to prepare aminoalcohol derivatives, namely refluxing in collidine under nitrogen, methyl imide **(21)** underwent reduction at one bromine atom and substitution at the other bromine atom to give the product **(23)**. A possible reason for this was that the methyl imide **(21)** was considerably less soluble in collidine than the imides previously prepared by Morris. The solubility problems encountered here and in other steps in the synthetic route to tetramines may have been avoided if an imide with a longer alkyl chain had been prepsred via the imporved route to tetramines were to be preopeder. Repetition of the reaction in ethanol, in which methyl imide **(21)** had been found to be more soluble, resulted in an 80 % yield of pure ethanolamine derivative **(24)**, with no column chromatography or crystallisation necessary (Figure 2.4).

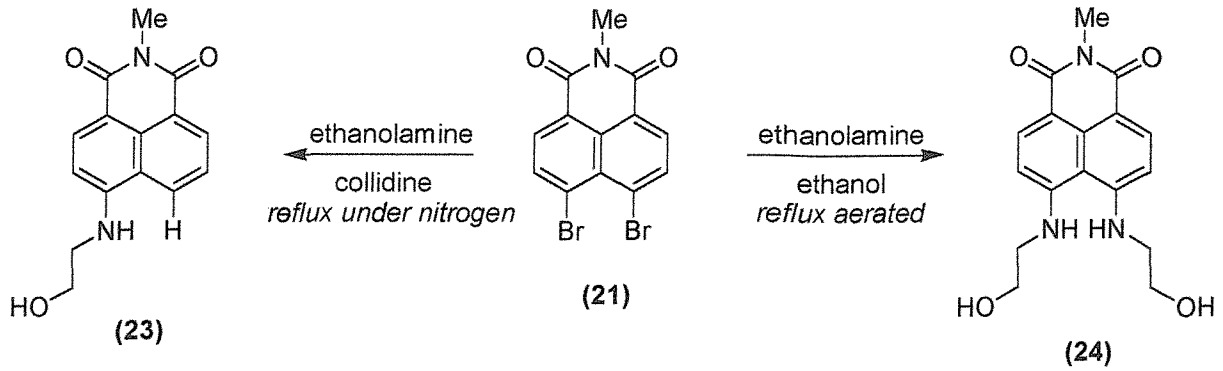


Figure 2.4 Products formed in the reaction of methyl imide **(21)** with ethanolamine in two different solvents

Upon scaling the reaction up to 1g, a reduction in yield to 30 % was observed, with the remaining product appearing as the reduction product **(23)**. When the reaction was carried out in the absence of nitrogen, however, an increase in the yield of ethanolamine product **(24)** to 70 % was observed. The mechanism of formation of the reduction product is unclear. A possible mechanism is shown below (Figure 2.5). Since the reduction product **(23)** is only formed in the presence of nitrogen, a radical process may be occurring in the presence of oxygen that inhibits the reduction pathway.

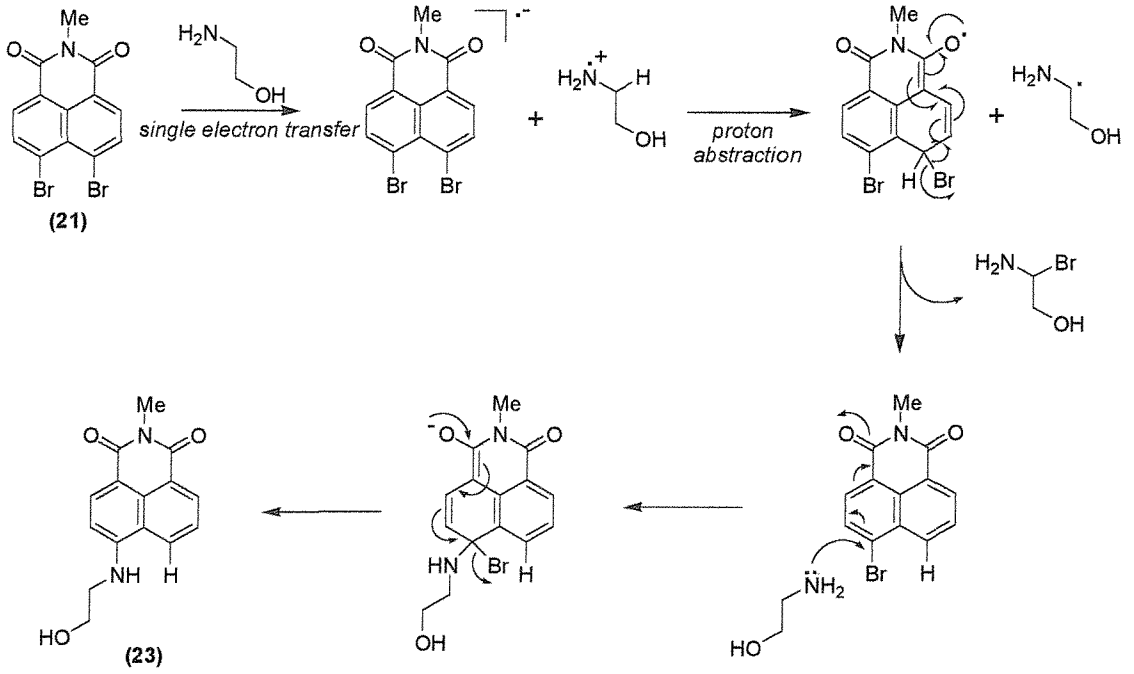


Figure 2.5 Possible mechanism of formation of the reduction product **(23)**

In order to introduce chirality, chiral aminoalcohols were employed. The reaction of methyl imide **(21)** with (R)-(-)-2-aminobutanol and (1R, 2S)-norephedrine, in ethanol, gave mainly the starting material after 24 hours at reflux. However, when collidine was employed as the solvent, reaction was complete after 24 hours at reflux in both cases. The norephedrine derivative **(25)** and the 2-aminobutanol derivative **(26)** were prepared in 62 % and 27 % yields respectively after purification by column chromatography (Figure 2.6).

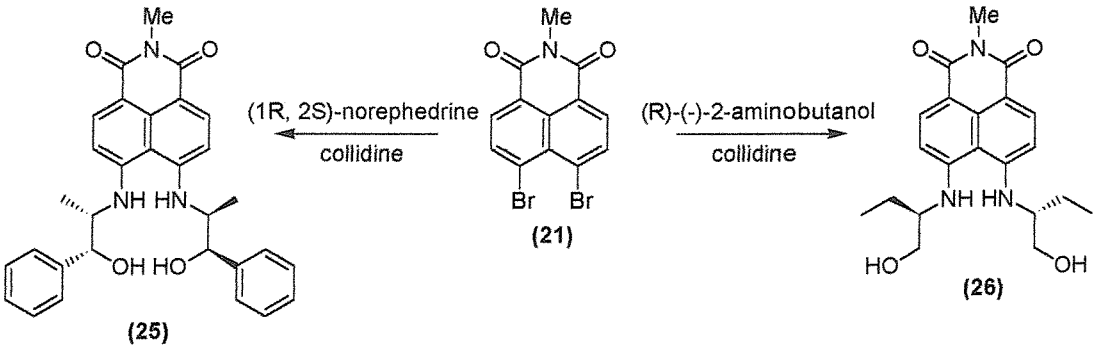


Figure 2.6 Synthesis of chiral aminoalcohol derivatives **(25)** and **(26)**

2.1.3 Preparation of dichloride derivatives

Substitution of the hydroxyl group of the ethanolamine derivative **(24)** by chlorine was effected by thionyl chloride under conditions of reflux giving dichloride **(27)** in 91 % yield after purification by column chromatography and crystallisation from dichloromethane (Figure 2.7). Scaling up to 1g resulted in precipitation of the pure product upon work-up thereby removing the need for column chromatography or crystallisation. Inclusion of the sulphoxide group, as evidenced by ^{1}H NMR and mass spectrometry, had been previously reported by Morris and did not pose a problem since it had been shown that subsequent reaction with an amine led to its removal.

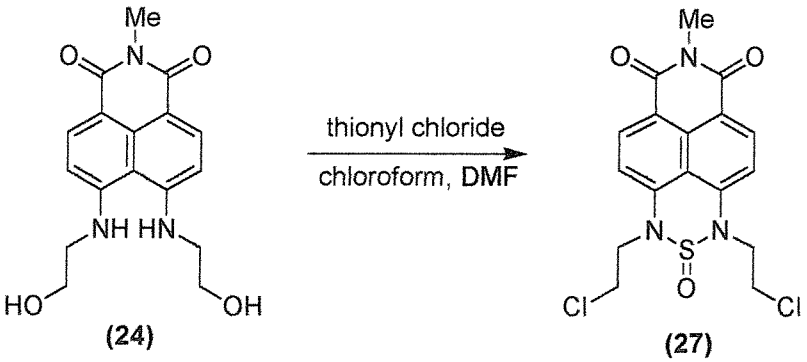


Figure 2.7 Synthesis of dichloride **(27)**

Reaction of the norephedrine derivative **(25)** with thionyl chloride resulted in a very low yield of dichloride **(28)**, once again possessing a sulphur bridge (Figure 2.8). The ^{1}H NMR of the dichloride **(28)** was complex owing to either formation of diastereoisomers or the presence of the sulphoxide group. In order to determine which effect was responsible a tetramine derivative was to be prepared to effect the loss of the sulphoxide group. However the tetramine derivative proved impossible to prepare owing to the small amount of dichloride **(28)** which was isolated. The aminobutanol derivative **(26)** gave rise to a complex mixture of products, upon reaction with thionyl chloride, which proved difficult to separate. Given the difficulty in synthesising these chiral chloride derivatives, it was decided to proceed with just dichloride **(27)** since it could be prepared both cleanly and efficiently.

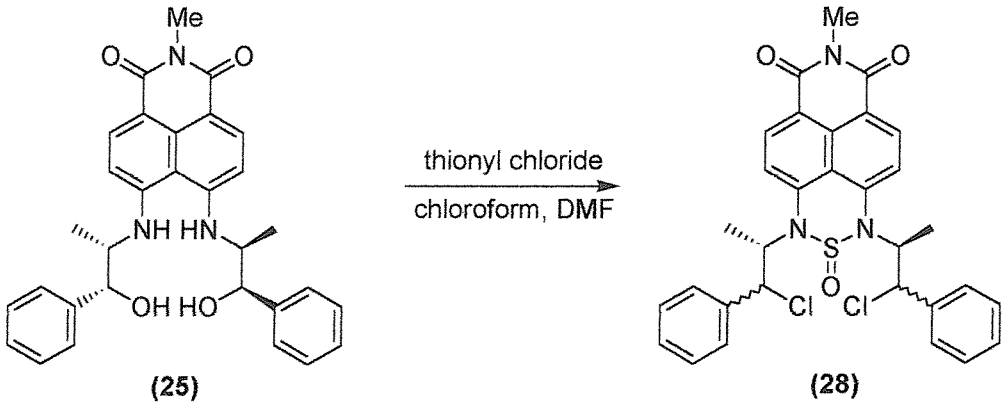


Figure 2.8 Synthesis of dichloride **(28)**

2.1.4 Tetramine synthesis

The preparation of tetramines was attempted employing amines as both the reagent and the solvent. This worked well for the amines butylamine, pyrrolidine and benzylamine, giving yields of 68 %, 72 % and 94 % respectively of tetramines **(29)**, **(30)** and **(31)** (Figure 2.9). Butylamine and benzylamine resulted in products that precipitated out of solution and thus no further purification was necessary.

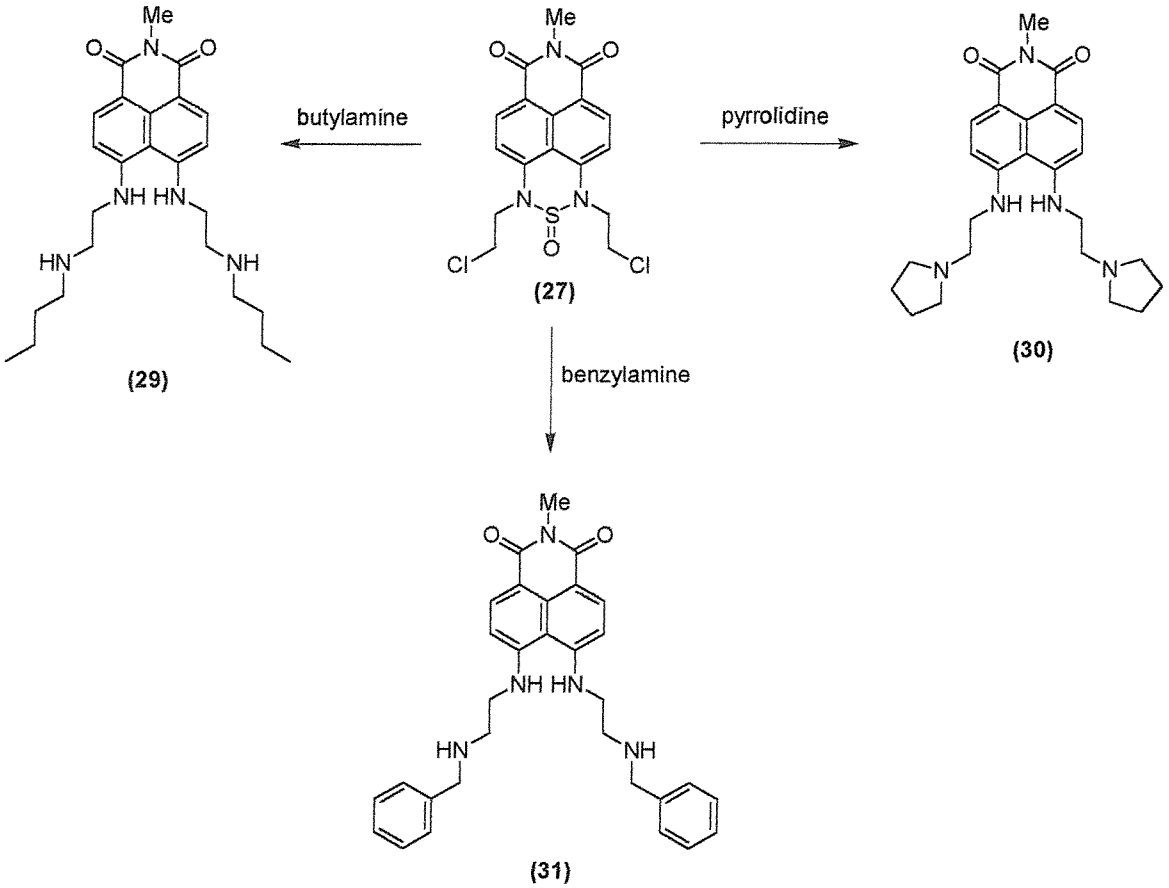


Figure 2.9 Synthesis of tetramines **(29)**, **(30)** and **(31)**

Although this method worked well for the amines shown, when more hindered aliphatic or aromatic amines were employed, such as (R)-(+)-α-methylbenzylamine and (S)-(+)-3-methyl-2-butylamine, no reaction was observed. This was as a result of the low solubility of the dichloride **(27)** in the amine. Furthermore the use of chiral amines as a solvent was undesirable owing to the cost and the need to remove the excess amine at the end of the reaction. Thus it was decided to investigate a suitable solvent for the tetramine formation reaction, allowing the amine to be employed as a reagent only.

Dichloride (**27**) was found to dissolve partially in dichloromethane and pyridine but no product formation was observed when butylamine was employed as the amine. In both cases some of the starting material (**27**) was recovered, in addition to some decomposed material. When dimethylformamide (DMF) was employed as the solvent and butylamine as the reagent, the reaction was heated to just 90°C to avoid excessive heating, which was believed to lead to decomposition of the dichloride (**27**). After 3 hours the reaction mixture was observed to be completely homogeneous and, after 7 hours, TLC indicated that all the dichloride (**27**) had disappeared. Following purification by column chromatography, tetramine (**29**) was isolated in 24 % yield. Repetition of the reaction in DMF with the amines (S)-(+)-prolinol and (R)-(+)-α-methyl benzylamine, resulted in the preparation of tetramines (**32**) and (**33**) in 80 % and 26 % yield respectively (Figure 2.10).

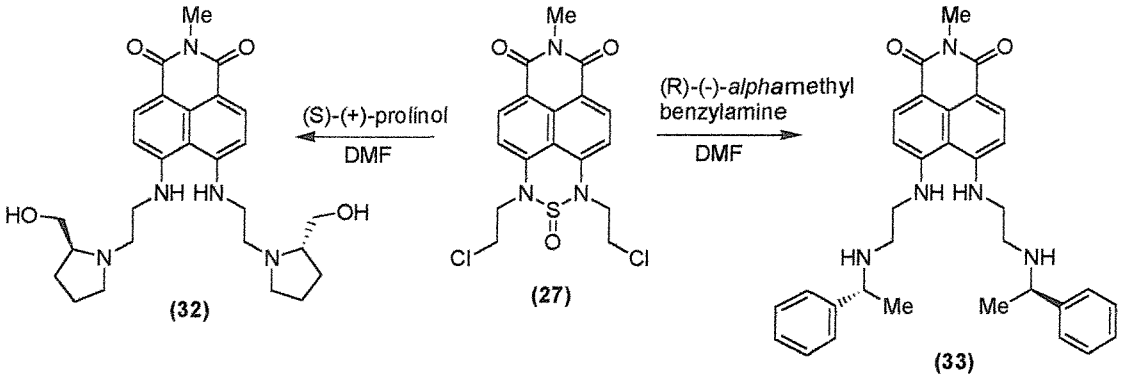
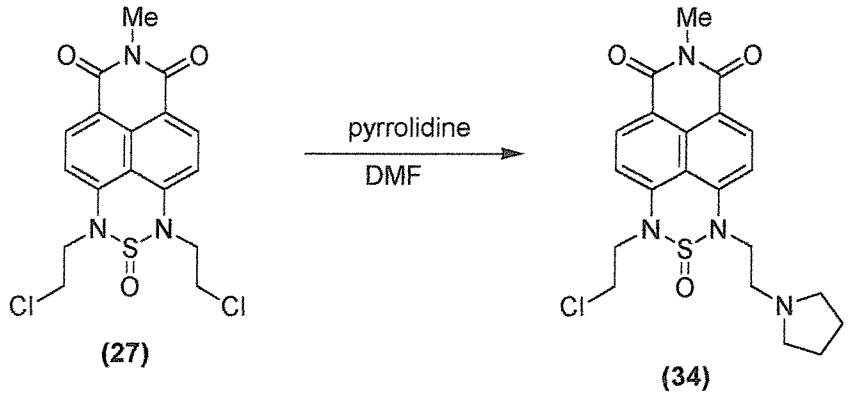


Figure 2.10 Synthesis of chiral tetramines (**32**) and (**33**)

Attempts were made to prepare a diiodide from dichloride (**27**) since the latter had exhibited low reactivity even when in solution, whereas diiodides have been shown to undergo substitution more readily than dichlorides. However attempts to prepare the diiodide failed owing to the insolubility of the dichloride in the reaction solvents employed, namely acetonitrile and acetone.[143,144]

It has been shown that reaction of the dichloride (**27**) with an amine leads to loss of the sulphoxide bridge. In an attempt to elucidate the nature of this reaction an experiment was carried out to determine whether substitution of the chlorines or displacement of the sulphoxide bridge occurred first. Two equivalents of pyrrolidine

were reacted with dichloride **(27)** in DMF, resulting in recovery of some starting material and compound **(34)**, which was isolated via column chromatography (Figure 2.11).



(27) **(34)**

Figure 2.11 Product formed in the reaction of dichloride **(27)** with 2 equivalents of pyrrolidine

The sulphoxide bridge was found to be still in place in compound **(34)** but one of the chlorines had been substituted by pyrrolidine. This suggests that substitution initially occurs at the chlorine atoms. Thus two equivalents of the desired amine could be added to a solution of dichloride **(27)** to effect substitution of the chlorine atoms, followed by addition of a cheaper amine to effect removal of the sulphoxide bridge.

2.1.5 Conclusions

Starting from the anhydride **(13)** an improved route for imide formation has been established. The synthesis of methyl imide **(21)** can be achieved in good yield, as compared to the previous yields obtained for imide formation. Furthermore methyl imide **(21)** can be easily purified by stirring with silica gel, thus removing the need for column chromatography. Subsequent reaction of the methyl imide **(21)** with ethanolamine, followed by thionyl chloride leads to the preparation of **(24)** and **(27)** both cleanly and efficiently. It has also been shown that chirality can be introduced into the compound at the level of the aminoalcohol derivative although further work is necessary in the preparation of chiral chloride derivatives. A range of achiral and chiral tetramines has been prepared from dichloride **(27)**, where either the amine or dimethylformamide are employed as the solvent, in moderate to good yield.

2.2 Synthesis of Tetramines in the Pyridine Series

In the pyridine series, a similar route to tetramine preparation was devised by elaboration of pyridine derivatives. This route involved preparation of an aminoalcohol derivative and its subsequent reaction with thionyl chloride, followed by reaction with an amine.

2.2.1 Synthesis of chloride and ester derivatives of 2,6-pyridinedicarboxylic acid (35)

Starting from 2,6-pyridinedicarboxylic acid **(35)**, the synthesis of 2,6-pyridinedicarbonyl dichloride **(36)** was achieved in 99 % yield following purification by Sohxlet extraction.[145] Subsequent reaction of 2,6-pyridinedicarbonyl dichloride **(36)** with dry methanol gave dimethyl 2,6-pyridinedicarboxylate **(37)** in quantitative yield (Figure 2.12).

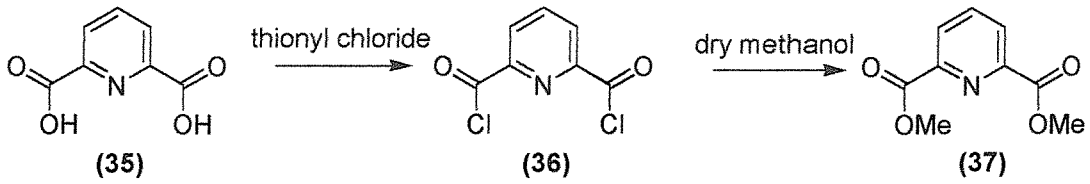


Figure 2.12 Synthesis of dimethyl 2,6-pyridinecarboxylate

2.2.2 Preparation of aminoalcohol and dichloride derivatives

Ethanolamine and (R)-(-)-2-aminobutanol derivatives, **(38)** and **(39)**, of dimethyl ester (37) were prepared according to the procedure of Kumar[146] in 80% and 46% yield respectively. Reaction of the aminoalcohol derivatives **(38)** and **(39)** with thionyl chloride[147] yielded 80 % and 53 % of the novel dichlorides **(40)** and **(41)** respectively (Figure 2.13). It is interesting to note that in contrast to the naphthalene series no sulphur bridge is formed upon reaction of the aminoalcohol derivatives **(38)** and **(39)** with thionyl chloride. This can be attributed to the fact that the amide nitrogen lone pairs are less readily available due to resonance with the carbonyl group.

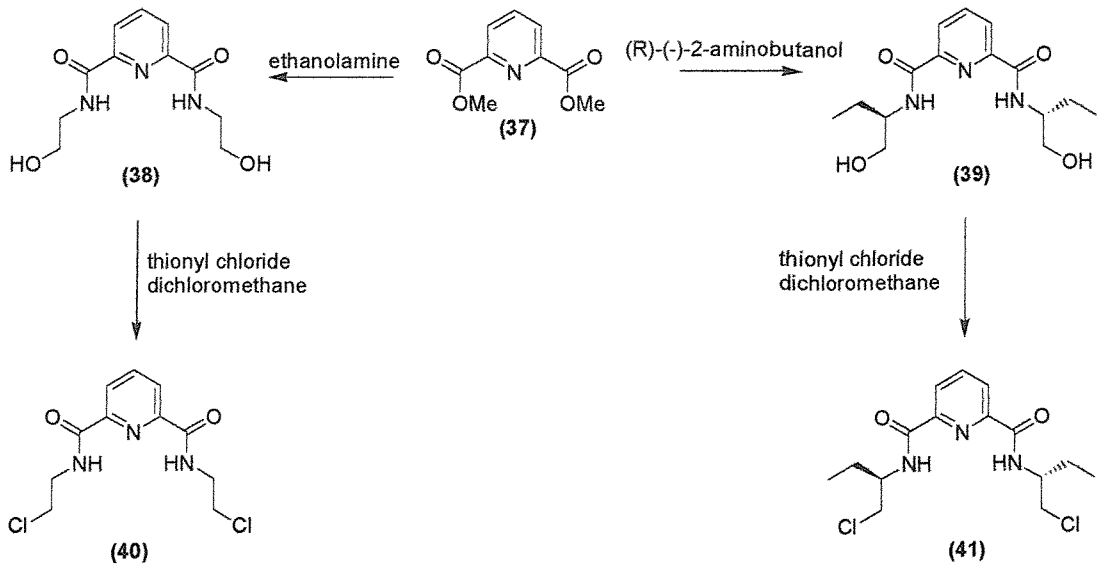


Figure 2.13 Synthetic route to dichlorides **(40)** and **(41)** from dimethyl 2,6-pyridinecarboxylate **(37)**, via aminoalcohol derivatives **(38)** and **(39)**

2.2.3 Preparation of chiral and achiral tetramines

A series of achiral and chiral tetramines were prepared from dichlorides **(40)** and **(41)** (Figure 2.14 and Figure 2.15), with the amine employed as both reagent and solvent, in moderate to good yield (Table 2.2).

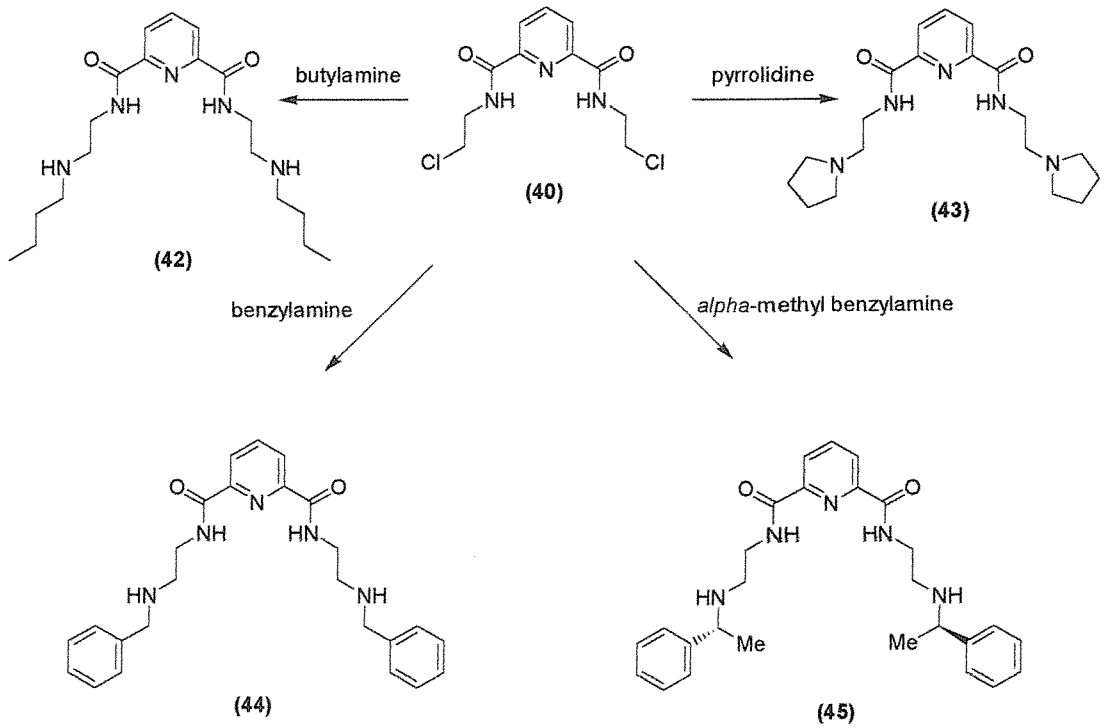


Figure 2.14 Synthesis of tetramines **(42)**, **(43)**, **(44)** and **(45)** from achiral dichloride **(40)**

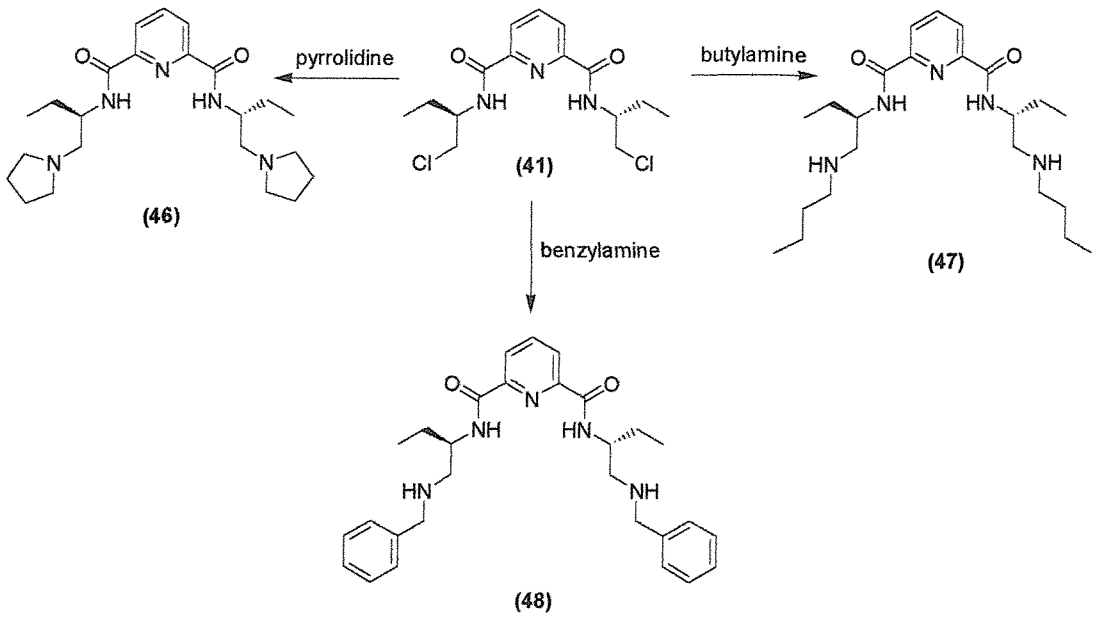


Figure 2.15 Synthesis of tetramines **(46)**, **(47)** and **(48)** from chiral dichloride **(41)**

Dichloride	Amine	Product	Yield (%)
40	Butylamine	**42**	60
40	Pyrrolidine	**43**	86
40	Benzylamine	**44**	97
40	α-Methyl benzylamine	**45**	62
41	Pyrrolidine	**46**	75
41	Butylamine	**47**	56
41	Benzylamine	**48**	50

Table 2.2 Yields obtained in the formation of tetramines from dichlorides **(40)** and **(41)**

In contrast to the naphthalene series, both dichlorides were found to dissolve in a range of amines, even those with increased steric hindrance. However, the disadvantages of using the amine as both the solvent and the reagent, namely the expense in the case of chiral amines and the difficulty in removing the excess amine at the end of the reaction, prompted an investigation into finding a more suitable reaction solvent.

The reaction of dichloride **(40)** with an amine was performed in a variety of solvents, namely dichloromethane, pyridine, collidine, tetrahydrofuran and dimethylformamide. In each case a homogeneous solution was observed, but reaction of the dichloride **(40)** was slow, resulting in the recovery of the majority of the starting material upon work-up. This was believed to be due to the slow formation of the aziridine intermediate **(49)**, via which the reaction of dichloride **(40)** with an

amine proceeded, thereby hindering the subsequent reaction with an amine to form the desired tetramine (Figure 2.16).

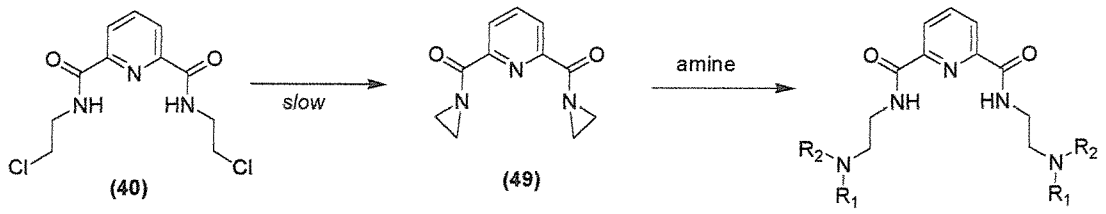


Figure 2.16 Synthetic route to tetramines from dichloride **(40)** via aziridine **(49)**

In an attempt to catalyse the reaction of dichloride **(40)** in THF with an amine, sodium iodide was added to the reaction mixture.[148] However this resulted in a complex mixture of products that proved difficult to separate by column chromatography. Given the increased reactivity of iodides as compared to chlorides it was thus decided to prepare and isolate the diiodide **(50)**. Dissolution of dichloride **(40)** in acetonitrile followed by the addition of sodium iodide resulted in the formation of pure diiodide **(50)** in 42 % yield (Figure 2.17).[144]

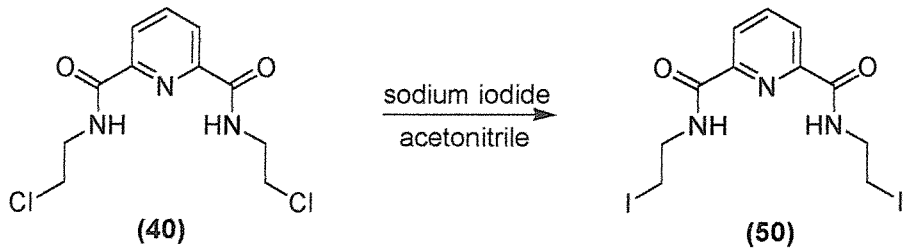
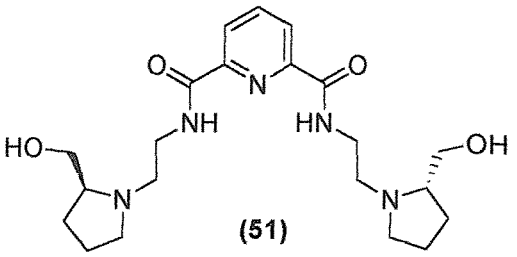


Figure 2.17 Synthesis of diiodide **(50)**

Subsequent reaction of diiodide **(50)** in THF with pyrrolidine resulted in pure tetramine **(43)** in 99 % yield. The preparation of several other tetramines was attempted via this method giving rise to tetramines **(42)**, **(45)** and **(51)** in good yield following purification by column chromatography (Table 2.3).

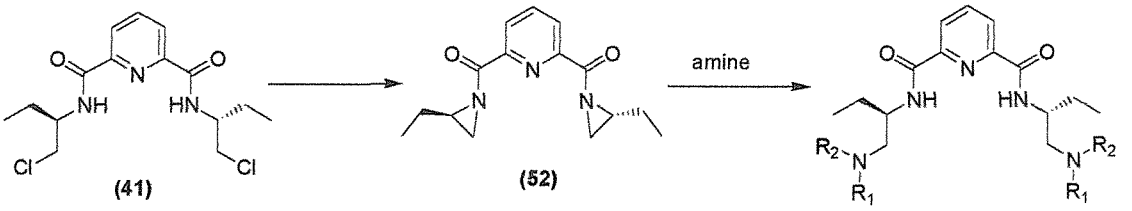


(51)

Amine	Product	Yield (%)
Butylamine	**42**	78
Pyrrolidine	**43**	99
α-Methyl benzylamine	**45**	80
(S)-(+)-Prolinol	**51**	91

Table 2.3 Yields obtained in the formation of tetramines from diiodide **(50)**

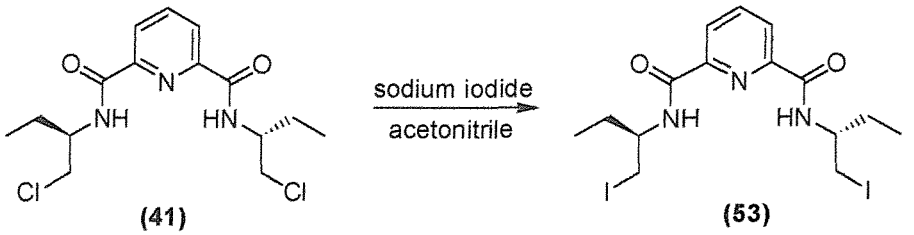
Reaction of the 2-aminobutanol derived dichloride **(41)** with pyrrolidine in THF resulted in a 58 % yield of the tetramine, following purification by column chromatography. In this case formation of the aziridine intermediate **(52)** was facilitated by conformational effects (Figure 2.18).



(41) (52)

Figure 2.18 Synthetic route to tetramines from dichloride **(41)** via aziridine **(52)**

The corresponding diiodide **(53)** was prepared, however, to compare its reactivity with that of dichloride **(41)** (Figure 2.19). The reaction of diiodide **(53)** with an amine in THF resulted in a complex mixture of products. This was believed to be due to the instability of the diiodide, which proved impossible to fully characterise.



(41) (53)

Figure 2.19 Synthesis of diiodide **(53)**

2.2.4 Conclusions

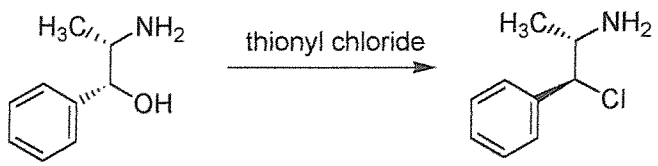
The preparation of aminoalcohol derivatives and dichlorides starting from dimethyl 2,6-pyridinedicarboxylate was achieved in high yield, with purification effected by crystallisation allowing preparation on a moderate scale (typically 5g). Subsequent reaction with amines gave good yields of both chiral and achiral tetramines. Reaction of dichloride **(41)** and diiodide **(50)** with amines in THF also proved to be an efficient route to tetramines and allowed the number of equivalents of amine used to be substantially reduced.

2.3 Alternative Route to Tetramines

The synthesis of tetramines in both the naphthalene and pyridine series, as described above, proceeded via several steps. At each stage purification of the intermediate product was required leading to an accumulative decrease in the overall yield. Consequently it was decided to investigate a shorter method of tetramine preparation, namely the direct reaction of *N*-methyl-4,5-dibromo-1,8-naphthalimide **(21)** and 2,6-pyridinedicarbonyl dichloride **(36)** with diamines.

2.3.1 Diamine preparation

A number of synthetic routes to vicinal diamines are known[149] and many vicinal diamines are commercially available. It was decided to investigate a method of preparing tetramines that would allow control of the steric nature and chirality of the end product. Starting with the chiral aminoalcohol **(54)**, chlorination was effected by the addition of thionyl chloride, resulting in product **(55)** in 80% yield (Figure 2.20).[150]



(54) **(55)**

Figure 2.20 Synthesis of chloride derivative **(55)**

The reaction proceeded with inversion of configuration, as evidenced by X-ray crystallography in Flores-Parra *et al.*[150] In the absence of base the reaction of an alcohol with thionyl chloride usually proceeds via an S_Ni (substitution nucleophilic, internal) reaction mechanism, leading to an overall retention in configuration.[151,152] However the presence of the amino group in compound **(54)** is equivalent to having a base present and thus the reaction proceeds via an S_N2 mechanism (substitution nucleophilic, bimolecular), thereby explaining the stereochemistry observed. Furthermore in the most stable conformation of the aminoalcohol, in which the ammonium group is anti to the sulphite resulting in only two gauche interactions, the ammonium protons give assistance to the chloride approach (Figure 2.21).

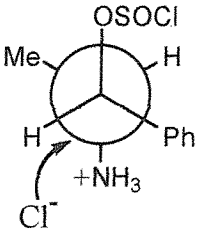
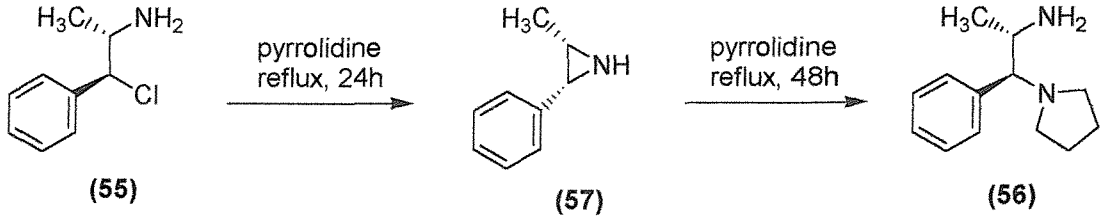


Figure 2.21 The most stable conformation of the aminoalcohol **(54)** during the reaction with thionyl chloride

Subsequent reaction of the chloride **(55)** with pyrrolidine at reflux for 3 days resulted in diamine **(56)**. The reaction proceeded *via* the aziridine **(57)**,[153] which was isolated upon work up of the reaction mixture after only 24 hours at reflux. Overall retention of stereochemistry was observed and only one regioisomer was isolated (Figure 2.22).



(55) **(57)** **(56)**

Figure 2.22 Synthesis of diamine **(56)** from chloride **(55)** via aziridine **(57)**

2.3.2 Tetramine preparation from 2,6-pyridinedicarbonyl dichloride (36)

The final step in the route to tetramines in the pyridine series was the direct reaction of diamine **(56)** and 2,6-pyridinedicarbonyl dichloride **(36)** (Figure 2.23). Owing to the reactive nature of acid chloride **(36)**, precautions were taken to minimise side-reactions. The reaction was carried out in dry solvent, just 2 equivalents of the diamine were used and the diamine **(56)** was added to 2,6-pyridinedicarbonyl dichloride **(36)** at 0°C, before being allowed to warm to room temperature. Tetramine **(58)** was isolated in 53% yield, following purification by column chromatography.

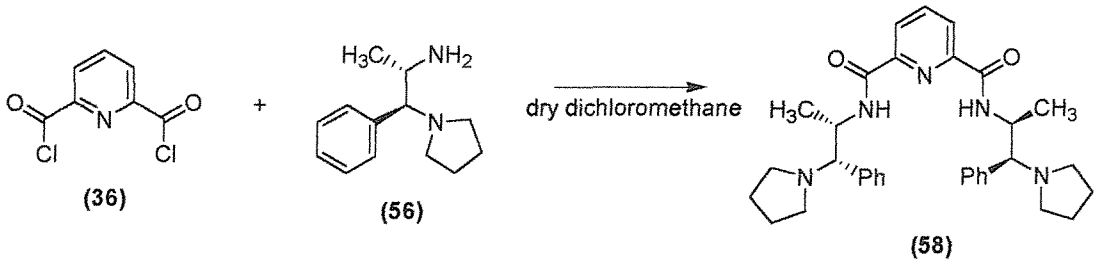


Figure 2.23 Synthesis of tetramine **(58)**

2.3.3 Tetramine preparation from *N*-methyl-4,5-dibromo-1,8-naphthalimide (21)

The reaction between diamine **(56)** and *N*-methyl-4,5-dibromo-1,8-naphthalimide **(21)** was complicated by the insolubility of methyl imide **(21)**. It was decided to employ the conditions of reflux in collidine. Methyl imide **(21)** was observed to have completely dissolved in the collidine after several hours and monitoring of the reaction mixture via TLC indicated that the methyl imide had undergone complete reaction after 24 hours. Several products were observed to have been formed by TLC of which two were isolated via column chromatography. ^{1}H and ^{13}C NMR suggested that both products were unsymmetrical. Product **(59)** had resulted from the reaction of methyl imide **(21)** with one equivalent of pyrrolidine. The pyrrolidine was believed to have come from diamine **(56)**, which was observed by ^{1}H NMR to contain free pyrrolidine even after several hours heating under vacuum. The second product **(60)** appeared to be the monosubstituted, unsymmetrical product resulting from reaction with just one equivalent of diamine **(56)** (Figure 2.24).

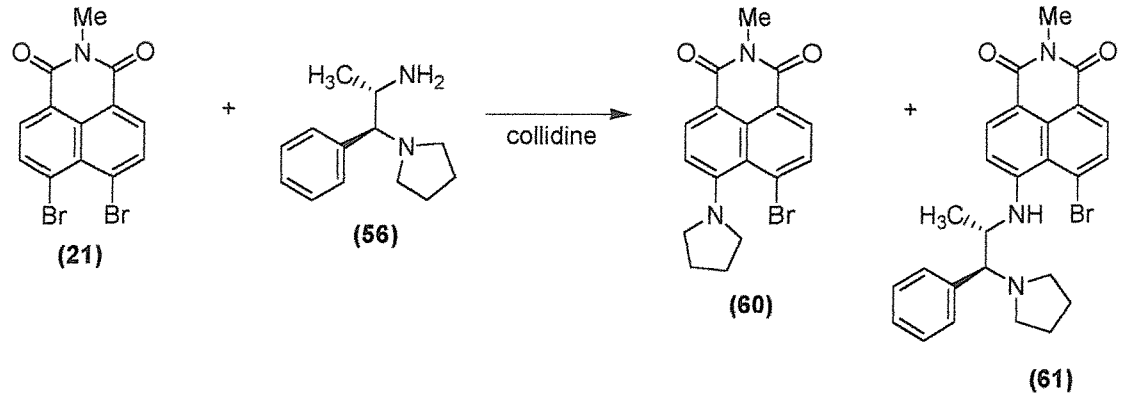
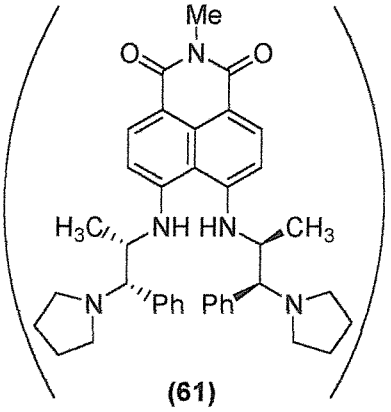


Figure 2.24 Products formed in the reaction of diamine **(56)** with methyl imide **(21)**

Extensive efforts were made to remove the excess pyridine from diamine **(56)** followed by repetition of the reaction for prolonged periods. However, tetramine **(61)** was not observed by TLC or isolated via column chromatography.



2.3.4 Conclusions

An efficient two-step route to diamines has been established, with purification simply effected at each stage by recrystallisation. Variation of the starting aminoalcohol and the amine in step 2 permits the introduction of chiral centres and allows the steric nature of the diamine to be determined. Consequently a series of chiral and achiral diamines could be prepared via this route.

In the pyridine series, tetramine **(58)** can be effectively prepared by the reaction of acid chloride **(36)** with diamine **(56)** in dry pyridine. In the naphthalene series, however, tetramine preparation is hindered by the insolubility of methyl imide **(21)**.

The mono-substituted product **(60)** has been observed *via* ^{1}H and ^{13}C NMR, which suggests that it should be possible to prepare tetramine **(61)** by further variation of the reaction conditions.

Chapter Three

Fluorimetry

Chapter Three
Fluorimetry

3.1 Introduction

Fluorescence can be defined as the light that is emitted when an electronically excited state relaxes to an electronic state of lower energy and possessing the same spin. The fluorescence associated with organic species in solution or in the solid state usually arises due to relaxation from the first excited singlet state to the ground state. However, there are several other processes by which the excited singlet state can relax (Figure 3.1).

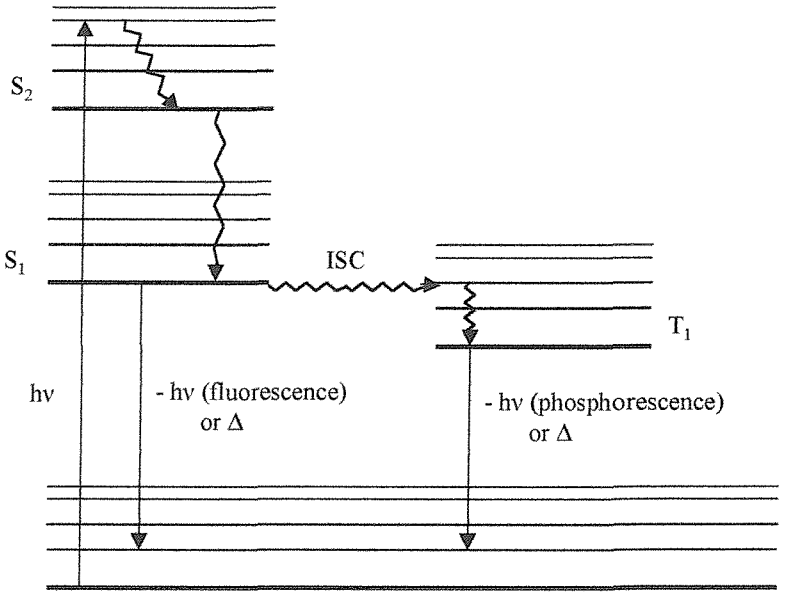


Figure 3.1 Jablonski diagram[154] showing absorption and the emission processes of fluorescence and phosphorescence (ISC – intersystem crossing).

Many compounds exhibit fluorescence but two features that mark highly fluorescent compounds are that they possess rigid structures and are aromatic. A rigid structure prevents the electronically excited state deactivating via intramolecular isomerisation. In aromatic systems, the longest absorption wavelength corresponds to a ($\pi \rightarrow \pi^*$) transition. Since intersystem crossing tends to be slow for ($\pi \rightarrow \pi^*$) states, the rate of

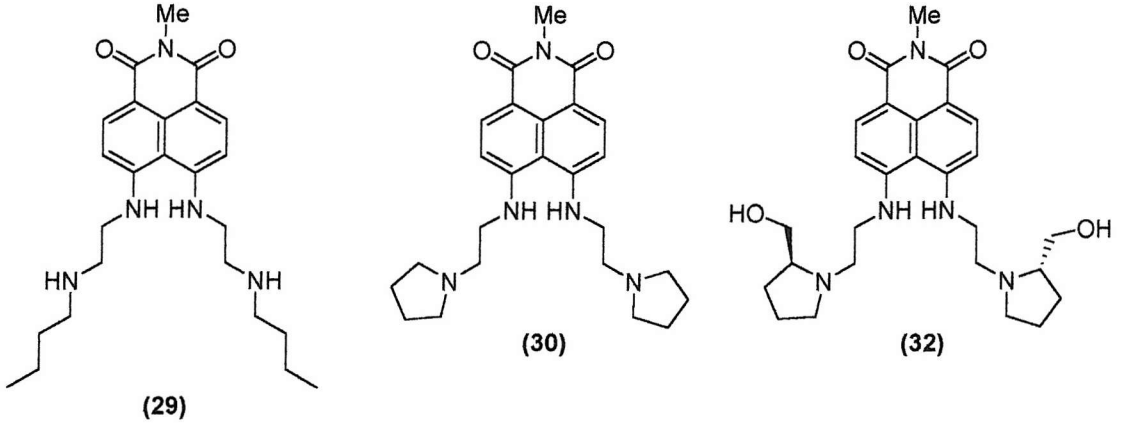
radiation is relatively fast compared to intermolecular energy transfer, thus increasing the probability of fluorescence. For example, the quantum yield for fluorescence of naphthalene in ethanolic solution ($\varphi_f = 0.80$) is high as compared to the quantum yield for intersystem crossing ($\varphi_{ISC} = 0.15$).[155] Furthermore increasing conjugation in aromatic systems shifts the ($\pi \rightarrow \pi^*$) absorption maximum towards longer wavelengths, thus further increasing the probability of fluorescence.

Structures based on a naphthalene nucleus are one class of aromatic system that display fluorescent properties. In particular naphthalimide derivatives are well known as brilliant green–yellow coloured dyes for synthetic–polymer fibres. In general they show high fluorescence quantum yields and the emission can be red-shifted by substitution at the 4-position. Consequently naphthalimide derivatives have found application in the area of both metal and pH fluorescence sensing.[156,157,158]

3.1.1 Measurement of excitation and emission

The tetramines prepared in the naphthalene series were, as expected, found to be fluorescent in nature since they are based upon a naphthalimide structure. Such compounds have been found to show substantial changes in fluorescence upon binding to a substrate and thus it was decided to probe their affinity for metal ions via fluorimetry. The tetramine derivatives in the pyridine series were not fluorescent and their metal binding properties were studied by ESI-MS, as described in Chapter 4.

The tetramines to be studied in the naphthalene series were butylamine tetramine **(29)** possessing a secondary amine moiety, pyrrolidine tetramine **(30)** possessing a tertiary amine moiety and the chiral prolinol tetramine **(32)**.



Firstly the excitation and the emission spectra of **(29)**, **(30)** and **(32)** were measured (Figures 3.2 – 3.4). For these and all other spectra in this chapter fluorescence intensity measurements are given in arbitrary units. All three tetramines were yellow in colour owing to the presence of an absorption band in the region of 450nm. Furthermore, a large difference was observed between the maxima of the absorption and emission curves (red shift) of each compound, which is important for sensory applications since it simplifies the distinction between excitation and emission light.[159] (The shoulder observed at 450nm in the emission spectra throughout this chapter is due to Rayleigh scattering.)

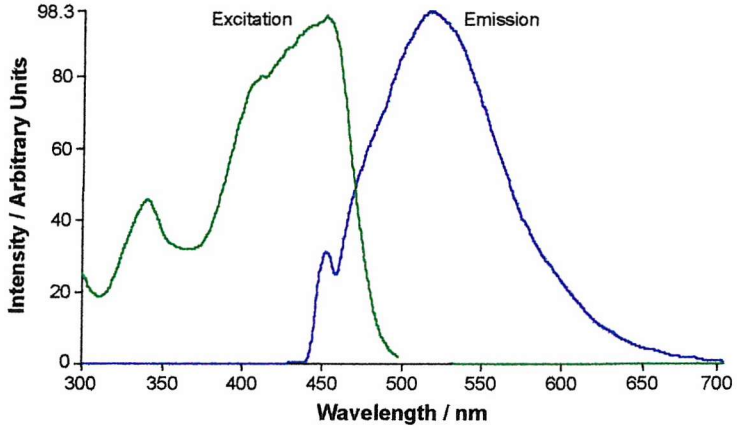


Figure 3.2 Excitation and emision spectra of tetramine **(29)** in methanol

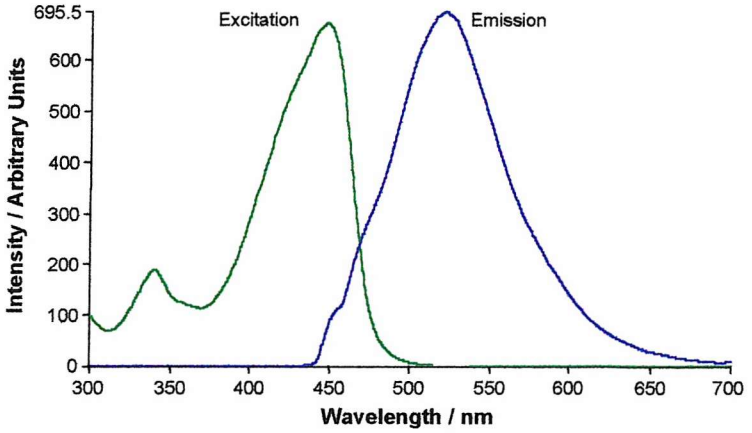


Figure 3.3 Excitation and emission spectra of tetramine **(30)** in methanol

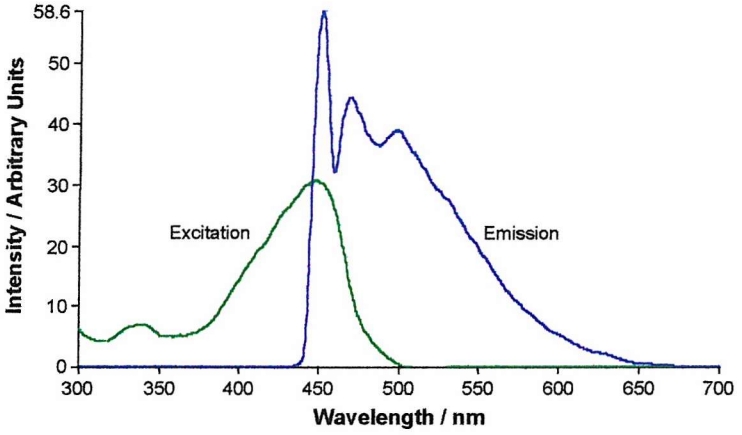


Figure 3.4 Excitation and emission spectra of tetramine **(32)** in methanol

Comparison of the emission spectra of all three tetramines at the same concentration ($5x10^{-5}M$) showed that tetramine **(29)** was the most fluorescent, with tetramines **(30)** and **(32)** displaying a similar degree of fluorescence to each other (Figure 3.5).

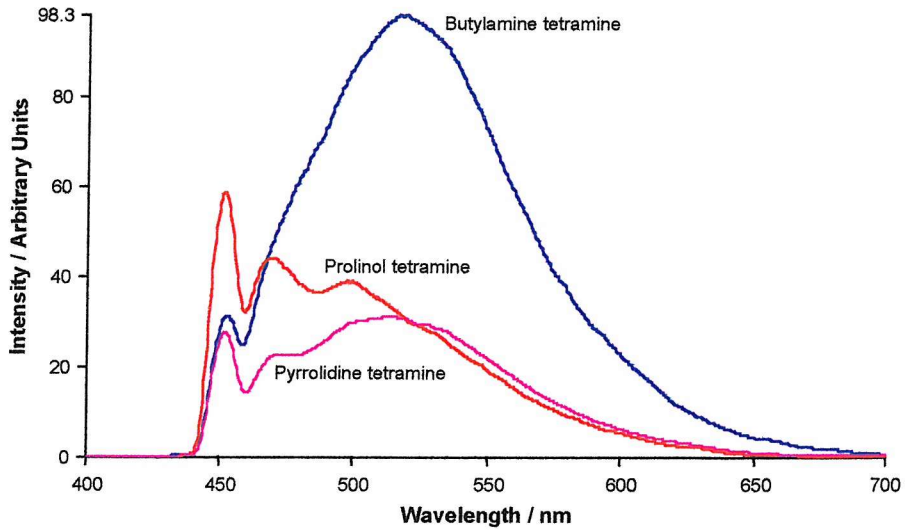


Figure 3.5 Comparision of the emission spectra of tetramines **(29)**, **(30)** and **(32)** in methanol

The absorption and fluorescence of amino substituted 1,8-naphthalimides has been studied by Brown.[160] The fluorescence of such compounds is notable with quantum yields in excess of 0.80 being observed. The absorption properties of such compounds tend to be fairly similar, however large differences in emission properties are often observed owing to changes in the substituent at a particular ring position and to alteration of the position of the substituent. Tetramines **(29)**, **(30)** and **(32)** were all substituted at the 4 and 5 positions of the naphthalene ring and thus the differences in emission spectra observed were due to the ring substituents themselves.

The reduced fluorescence intensity, and thus quantum yield, of the prolinol and the pyrrolidine tetramines **(30)** and **(32)** as compared to the butylamine tetramine **(29)** can be explained by the ionisation potentials of the butylamine, pyrrolidine and prolinol substituents. As will be shown in the next section, the fluorescence intensity of all three tetramines is largely controlled by photoinduced electron transfer (PET) from the nitrogen lone pairs of the butylamine, pyrrolidine and prolinol substituents. The greater the extent of PET, the lower the fluorescence intensity. Since the ionisation potential of butylamine is higher than that of pyrrolidine and prolinol, PET occurs to a lesser extent in butylamine tetramine **(29)** than in pyrrolidine and prolinol tetramines **(30)** and **(32)**. Consequently butylamine tetramine **(29)** exhibits the greatest fluorescence intensity.

3.1.2 Effects of concentration, acid and base

Taking tetramine **(29)** as an example, the effect of concentration and pH on fluorescence was investigated. Previous studies by Morris[2] of similar naphthalene compounds revealed a self-quenching of fluorescence at certain concentrations. Thus it was decided to investigate the optimum concentration of tetramine **(29)** at which to carry out further fluorescence studies, namely the effect of metal salt addition. With regard to similar studies by Morris, it was also decided to investigate the effect of photoelectron transfer on the fluorescence of tetramines **(29)**, **(30)** and **(32)**, by varying the pH of the tetramine solution.

An increase in fluorescence intensity was observed upon decreasing the concentration of tetramine **(29)** in methanol from ~$5x10^{-4}$M to ~$5x10^{-6}$M. Lowering the concentration further than ~$5x10^{-6}$M resulted in a decrease in fluorescence intensity. These results imply that at high concentrations, i.e. $x10^{-4}$M some degree of quenching of fluorescence by ground state molecules of the emitting species occur before fluorescence can take place. This process is known as self-quenching. Thus it was decided to perform all further experiments at a concentration of ~$5x10^{-5}$M since at this concentration self-quenching is negligible and the concentration is high enough to permit the addition of convenient volumes of metal salts.

The effect of pH on the fluorescence intensity was also measured via the addition of 0.5 – 2.0 equivalents of acid (HCl) to tetramine **(29)**, which resulted in an enhancement in fluorescence (Figure 3.6).

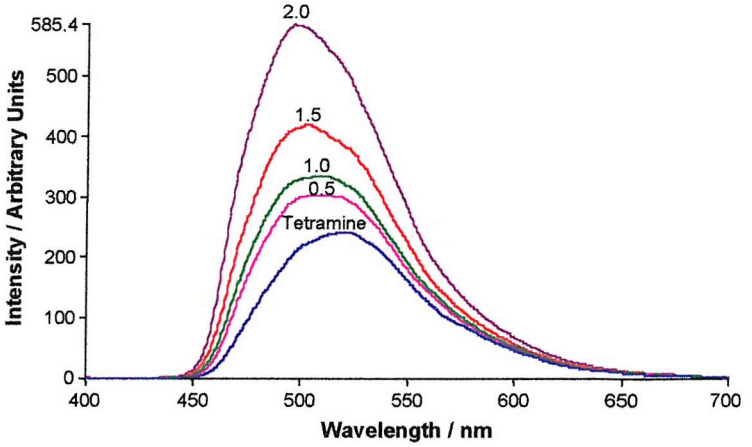


Figure 3.6 Effect of the addition of 0.5 – 2.0 equivalents of acid (HCl) to tetramine **(29)**

This was as expected since in the absence of protons the fluorescence is quenched by a photoinduced electron transfer (PET) originating from the nitrogen lone pair (Figure 3.7). When the amine is protonated the PET process is inhibited resulting in a recovery of fluorescence.[125]

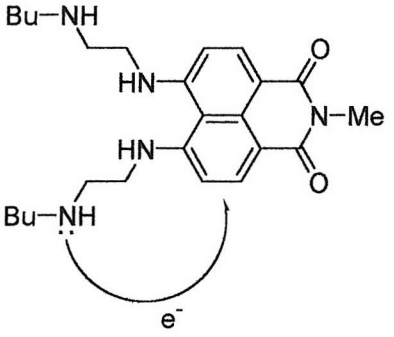


Figure 3.7 Photoinduced electron transfer (PET) originating from the nitrogen lone pair.

The addition of acid (HCl) to tetramines **(30)** and **(32)** resulted in a similar enhancement in fluorescence owing to quenching of the PET process (Figures 3.8 and 3.9).

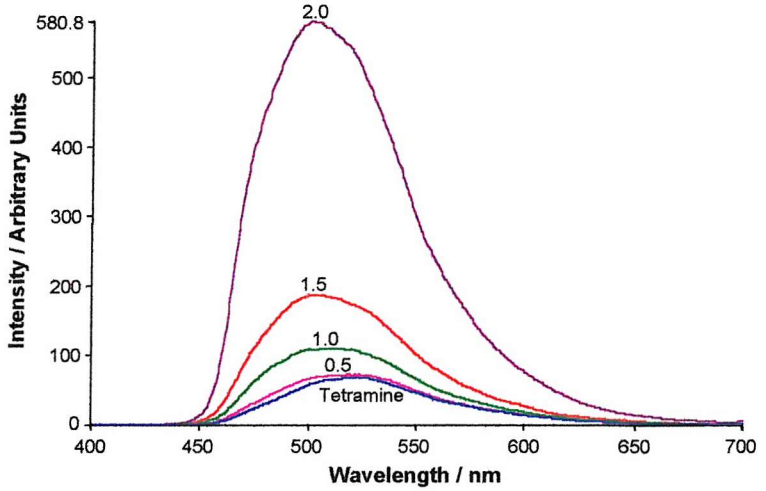


Figure 3.8 Effect of the addition of 0.5 – 2.0 equivalents of acid (HCl) to tetramine **(30)**

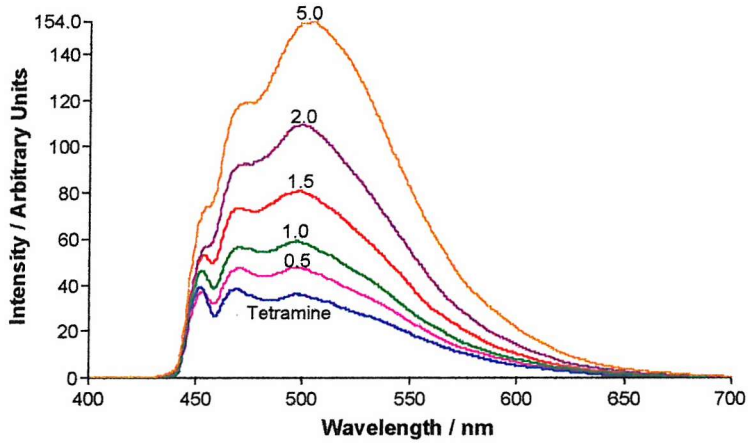


Figure 3.9 Effect of the addition of 0.5 – 5.0 equivalents of acid (HCl) to tetramine **(32)**

Owing to the large change in fluoresence observed upon protonation these tetramines have potential as fluorescent pH sensors.

3.2 Fluoresence Studies of Butylamine Tetramine (29)

In order to probe the metal affinities of tetramines **(29)**, **(30)** and **(32)**, 0.5 – 5.0 equivalents of aqueous metal salts were added in increments of 0.5 equivalents, to methanolic solutions of the three tetramines. Where possible the metal salts used were chlorides.

3.2.1 Metals with no effect

Addition of the following metal chlorides to tetramine **(29)** resulted in no significant change in the fluorescence intensity of tetramine **(29)**: Li (I) (Figure 3.10), Na (I), K (I), Ca (II), Mg (II), Mn (II) (Figure 3.11).

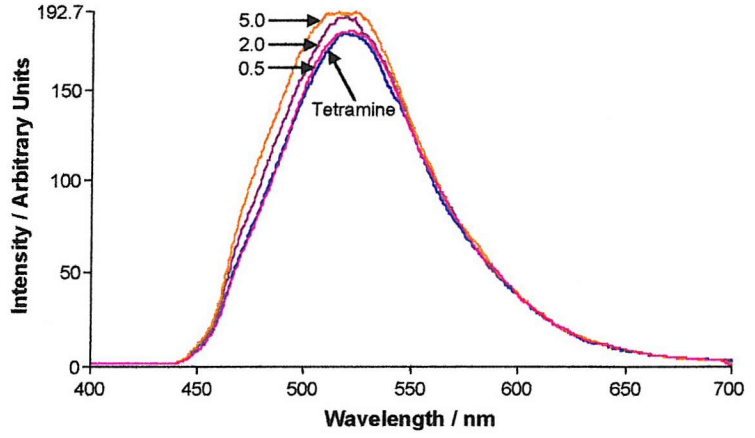


Figure 3.10 Effect of the addition of 0.5 – 5.0 equivalents of lithium (I) ions to tetramine **(29)**

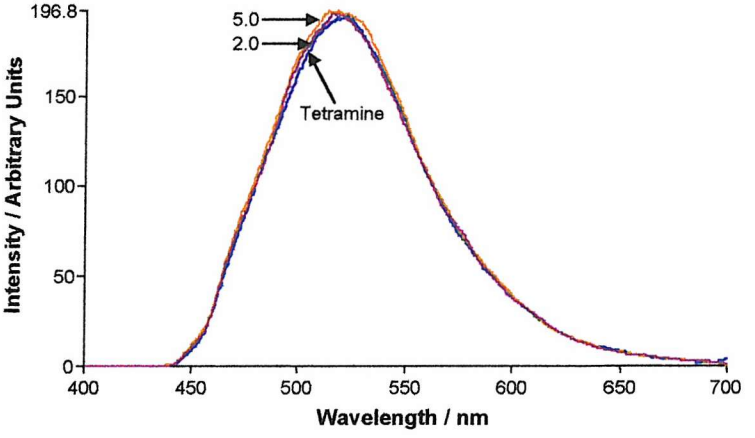


Figure 3.11 Effect of the addition of 0.5 – 5.0 equivalents of manganese (II) ions to tetramine **(29)** (results for less than 2.0 equivalents overlap with tetramine curve and are excluded for clarity)

Thus it was concluded that these metals did not give rise to any significant binding with tetramine **(29)**.

3.2.2 Quenching of fluorescence

The following metals gave rise to a significant decrease in the fluorescence intensity: Cu (II), Ni (II), Co (II), Pd (II), Ag (I).

Nickel (II) and palladium (II) chloride and silver (I) acetate – All three of these metal salts gave rise to a gradual, almost stepwise, decrease in fluorescence intensity (Figures 3.12 – 3.14).

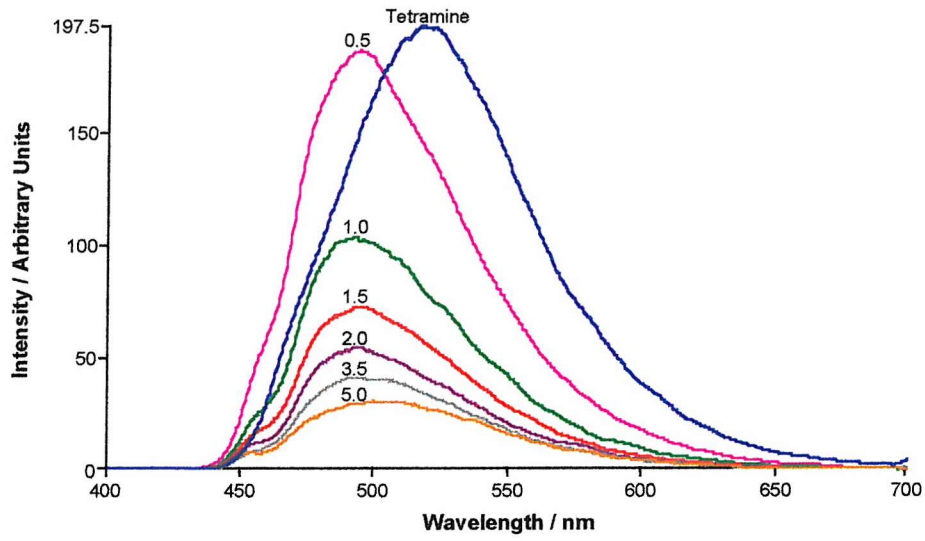


Figure 3.12 Effect of the addition of 0.5 – 5.0 equivalents of nickel (II) ions to tetramine **(29)**

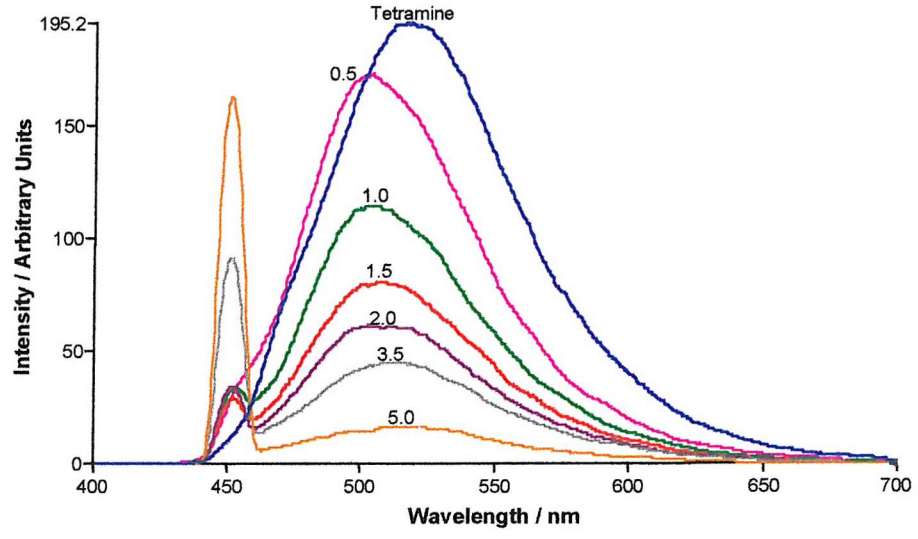


Figure 3.13 Effect of the addition of 0.5 – 5.0 equivalents of palladium (II) ions to tetramine **(29)**

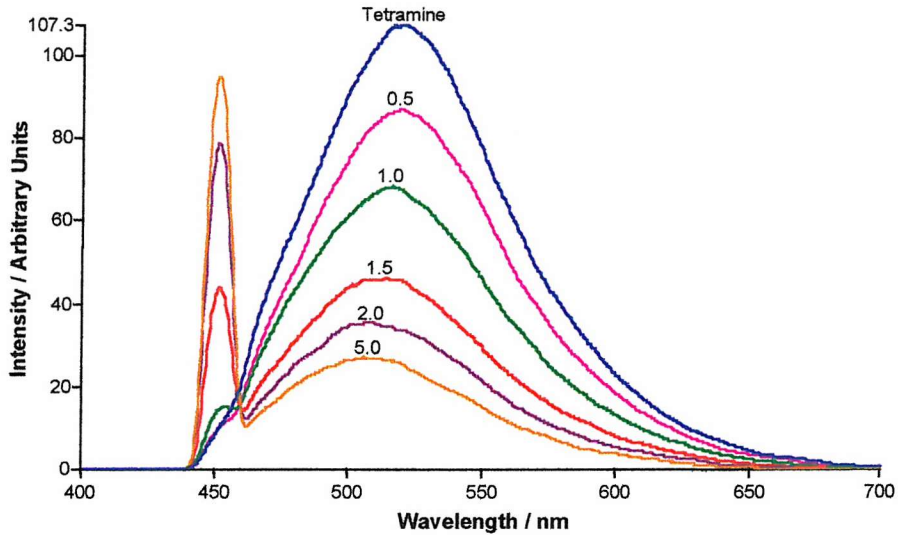


Figure 3.14 Effect of the addition of 0.5 – 5.0 equivalents of silver (I) ions to tetramine **(29)**

Copper (II) chloride – A significant decrease in fluorescence intensity was observed after the addition of 0.5 equivalents of copper (II) ions and no further quenching was observed following the addition of 1.5 equivalents of Cu (II) ions (Figure 3.15).

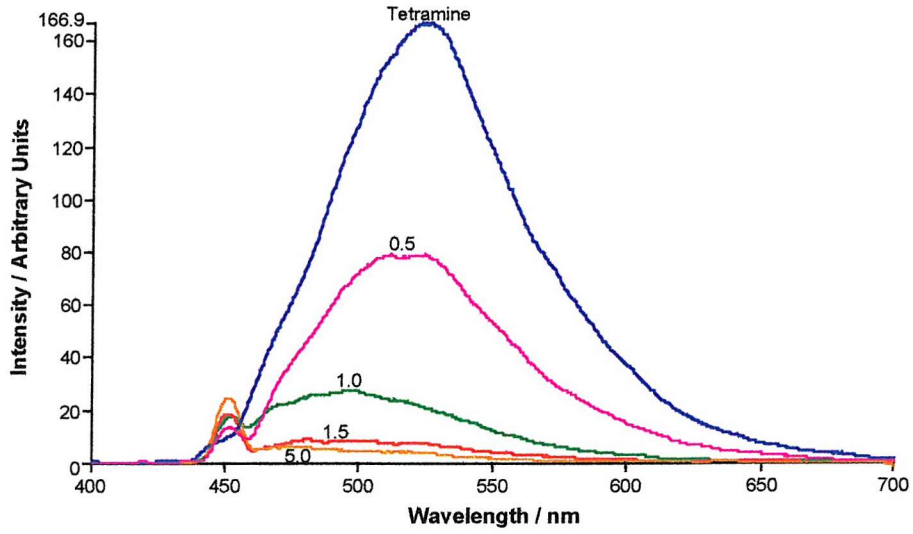


Figure 3.15 Effect of the addition of 0.5 – 5.0 equivalents of copper (II) ions to tetramine **(29)**

Cobalt (II) chloride – The addition of just 0.5 equivalents of cobalt (II) ions led to a dramatic quenching of fluorescence with no observed change upon the addition of further equivalents of Co (II) ions (Figure 3.16).

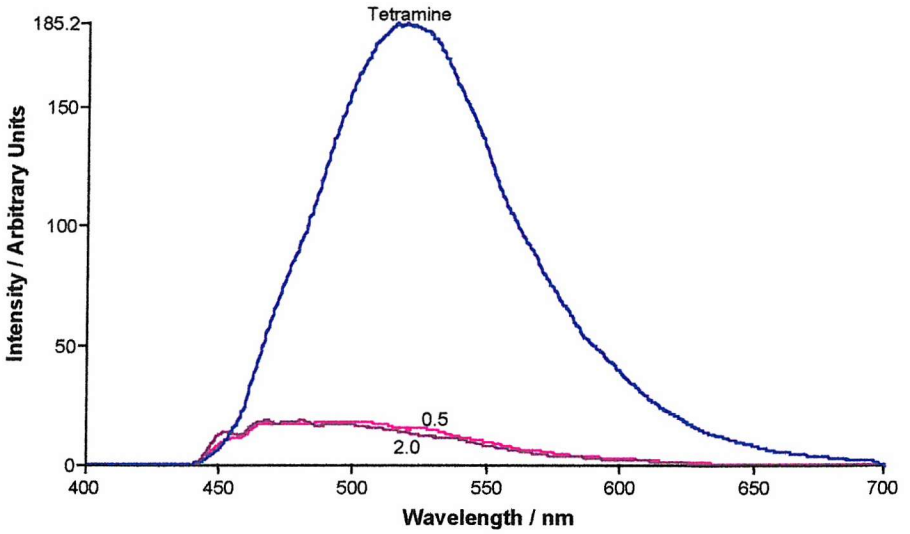


Figure 3.16 Effect of the addition of 0.5 – 2.0 equivalents of cobalt (II) ions to tetramine **(29)**

In each case a significant quenching of fluorescence is observed and thus it is concluded that complexation of Ni (II), Pd (II), Ag (I), Cu (II) and Co (II) is taking place. However a clear difference in the extent of fluorescence quenching is observed. This could simply be a reflection of the difference in affinity for each metal by tetramine **(29)** or may indicate the formation of complexes with varying stoichiometries. The stepwise decrease in fluorescence intensity observed with Ni (II), Pd (II) and Ag (I) may be as a result of a competition between the effect of the metal and the effect of acidity. This would indicate that either aqueous solutions of these metal salts are more acidic than those of copper and cobalt chloride or that cobalt and copper bind more effectively to tetramine **(29)** and thus the effects of acidity are negligible.

In order to compare the fluorescence quenching by each of these metals a Stern-Volmer plot[161] was obtained, as described by the following equation:

$$I^0/I = 1 + K_{SV}[Q] \quad \text{and} \quad K_{SV} = k_q\tau_0$$

Where I^0 and I are the fluorescence intensities in the absence and presence of [Q] moles of quencher Q, respectively. K_{SV} is the Stern-Volmer quenching constant, k_q the rate of

quenching and τ_0 the lifetime of the excited species. (See Appendix 1 for the derivation of the above equation.)

Stern-Volmer plots of the fluorescence quenching of tetramine **(29)** by the metals Ni (II), Pd (II), Ag (I), Cu (II) and Co (II) are shown in Figure 3.17.

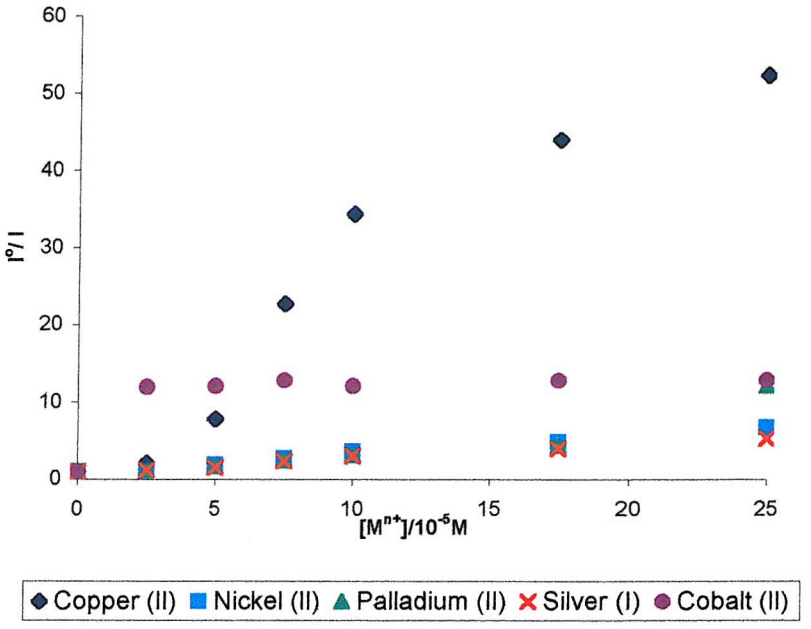


Figure 3.17 Stern-Volmer plot for the quenching of fluorescence by addition of 0.5 – 5.0 equivalents of M^{n+} to tetramine **(29)**

The pronounced effect of the addition of Cu(II) and Co(II) ions can be clearly observed from these plots. The steep initial gradients of these curves indicate that the rate of quenching is probably faster than the rate at which the fluorophore and the metal ion diffuse towards each other and that a pre-formed complex is formed in solution. This has been previously observed in such systems.[2] Furthermore deviation from a straight line can indicate the absorption of emitted light by the quencher[162] as solvated transition metal ions have been found to absorb in the UV spectrum. However measurement of the absorption coefficients of the solvated metal ions indicated that any absorption effect is negligible. Finally it should be noted that the Stern Volmer plot of fluorescence

quenching by Cu(II) gives rise to an upward curve. This effect is not unusual and has been noted in other systems.[163]

3.2.3 Enhancement of fluorescence

Addition of the following metal chlorides to tetramine **(29)** resulted in an enhancement of fluorescence: Zn (II), Fe (II), Sn (II), Al (III) (Figures 3.18 – 3.21).

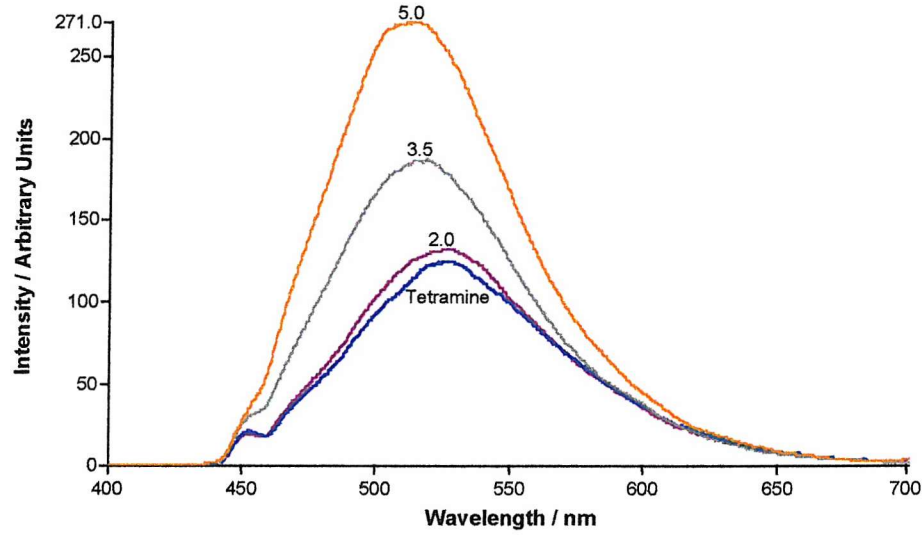


Figure 3.18 Effect of the addition of 0.5 – 5.0 equivalents of zinc (II) ions to tetramine **(29)** (results for less than 2.0 equivalents overlap with tetramine curve and are excluded for clarity)

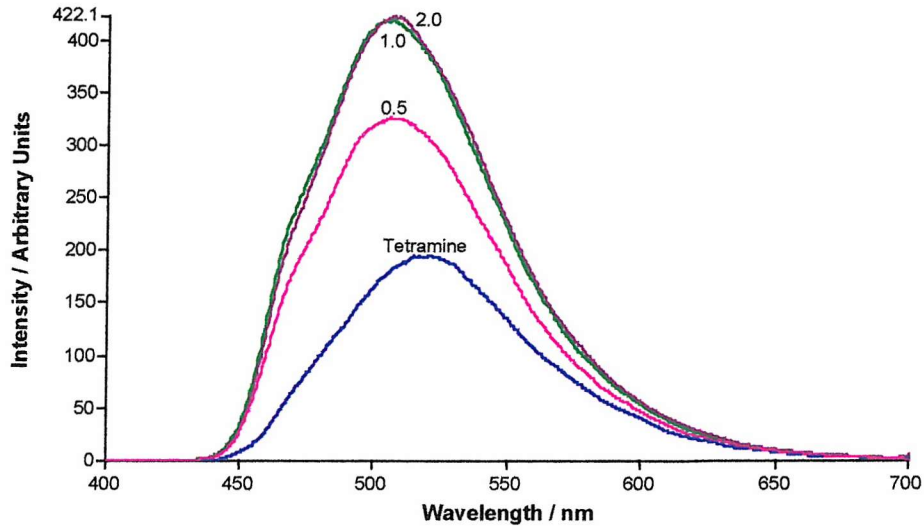


Figure 3.19 Effect of the addition of 0.5 – 2.0 equivalents of iron (II) ions to tetramine **(29)**

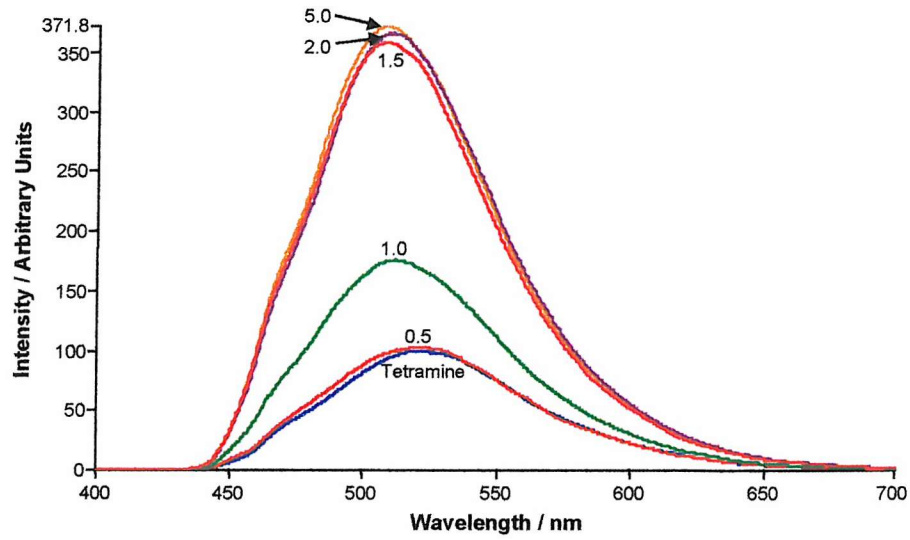


Figure 3.20 Effect of the addition of 0.5 – 5.0 equivalents of tin (II) ions to tetramine **(29)**

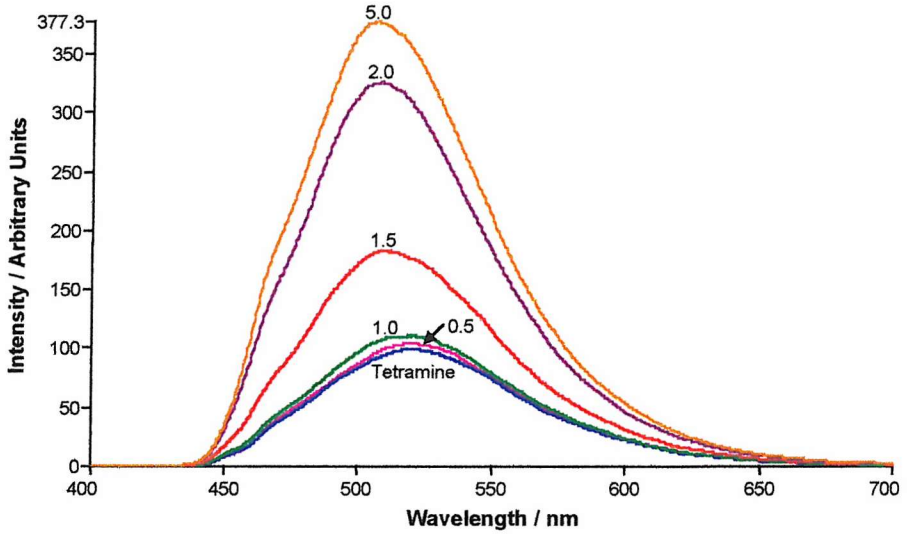


Figure 3.21 Effect of the addition of 0.5 – 5.0 equivalents of aluminium (III) ions to tetramine **(29)**

It was unclear whether these results were genuinely due to an enhancement in fluorescence or whether the effect was due to the acidity of the aqueous solutions. If the latter was indeed the case then an enhancement in fluorescence would be expected due to quenching of the PET upon protonation.

The acidity of an aqueous metal salt depends on both the cation and the anion. If the attraction of the metal cation for the negative end of the water molecule is strong enough the water molecule is itself affected. As the lone pairs on the oxygen of the water

molecule are pulled towards the metal ion the electrons in the O-H bond move towards oxygen to compensate. Consequently the hydrogen becomes more positively charged and may eventually dissociate completely to form a hydronium ion with the free water molecules. Thus the solution becomes acidic. Metal ion hydrolysis, as this process is known, can be represented by the following equation:

$$M^{z+}(aq) + H_2O \rightleftharpoons M(OH)^{(z-1)+}(aq) + H^+(aq)$$

$$\text{where } Ka = [M(OH)^{(z-1)+}][H^+] / [M^{z+}] \quad \text{and} \quad pKa = -\log Ka$$

Comparison of the pKa values is a useful way of determining the acidity of metal ions since the lower the value of pKa, the higher the degree of hydrolysis and thus the higher the acidity of the solution.

The extent of hydrolysis is also found to increase as the charge of the metal cation increases and its radius decreases. It is found that the higher the z^2/r value the more acidic the metal ion. Finally it is generally found that if the electronegativity of the metal ion is greater than 1.8 then the acidity is higher than that predicted via pKa and z^2/r values (Table 3.1).

Electronegativity < 1.5				Electronegativity > 1.5			
Ion	**Radius**	**Z^2/r**	**pKa**	**Ion**	**Radius**	**Z^2/r**	**pKa**
+ 1 Ions							
K	152	0.007	14.5	Ag	129	0.008	12.0
Na	116	0.009	14.2				
Li	90	0.011	13.6				
+ 2 Ions							
Ca	114	0.035	12.8	Sn	-	-	3.4
Mg	86	0.047	11.4	Mn	97	0.041	10.6
				Fe	92	0.043	9.5
				Co	88	0.045	9.6
				Ni	83	0.048	9.9
				Zn	88	0.045	9.0
+ 3 Ions							
				Al	67	0.134	5.0

Table 3.1 Z^2/r and pKa values for selected metal ions[164,165]

The nature of the anion is also important in determining the acidity of a metal salt in solution. If anions of a strong acid are used, such as chlorides, then the acidity of the aqueous metal salt will be increased.

The pH of the aqueous metal salts which resulted in an enhancement of fluorescence were measured (Table 3.2).

Metal Salt	pH
$SnCl_2$	2.5
$FeCl_2$	3.0
$AlCl_3$	3.9
$ZnCl_2$	5.8

Table 3.2 pH values for aqueous solutions of the metal salts that caused an enhancement in the fluorescence of tetramine **(29)**

Owing to the acidic nature of the aqueous metal salts the fluorescence experiments were repeated. This time the metal chlorides were added to a buffered solution of tetramine **(29)** or a metal acetate was employed, since acetate is the anion of a weak acid leading to a lower pH in solution (Figures 3.22 – 3.25)

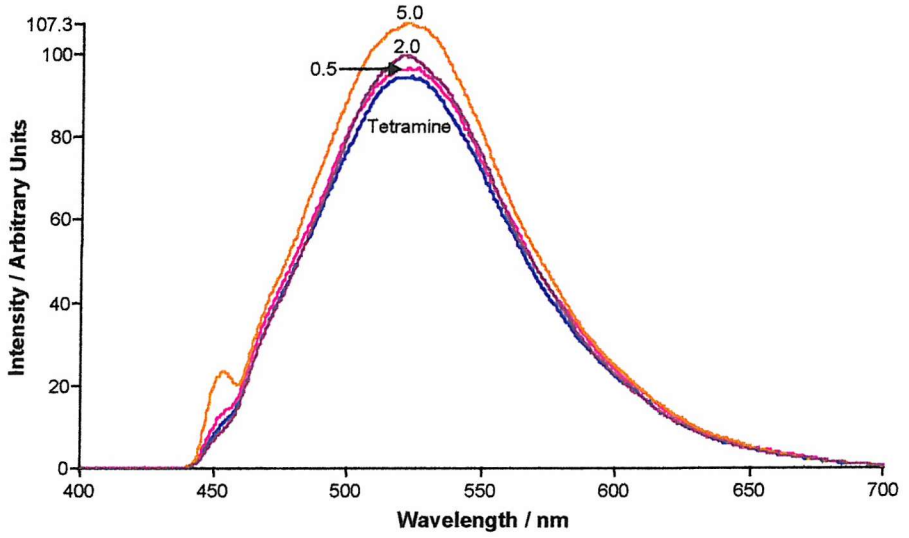


Figure 3.22 Effect of the addition of 0.5 – 5.0 equivalents of zinc (II) acetate to tetramine **(29)**

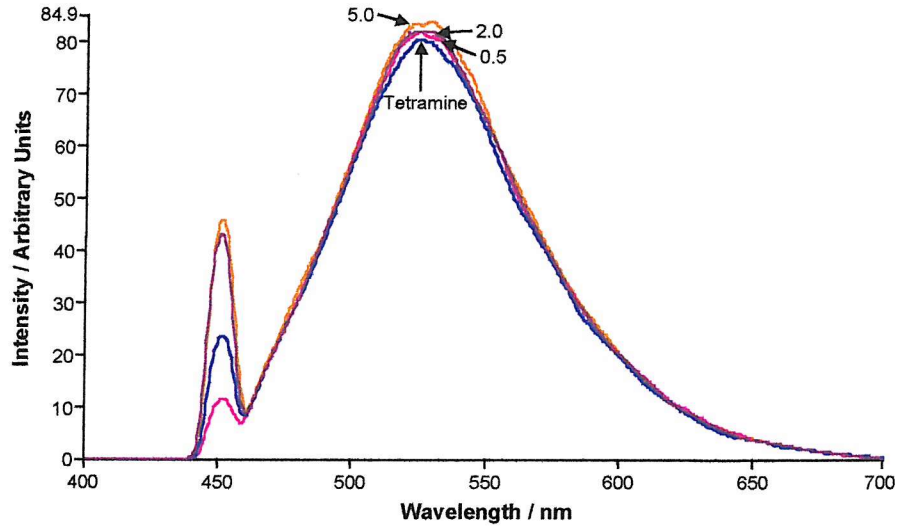


Figure 3.23 Effect of the addition of 0.5 – 5.0 equivalents of iron (II) chloride to tetramine **(29)** in the presence of buffer (pH 9.4)

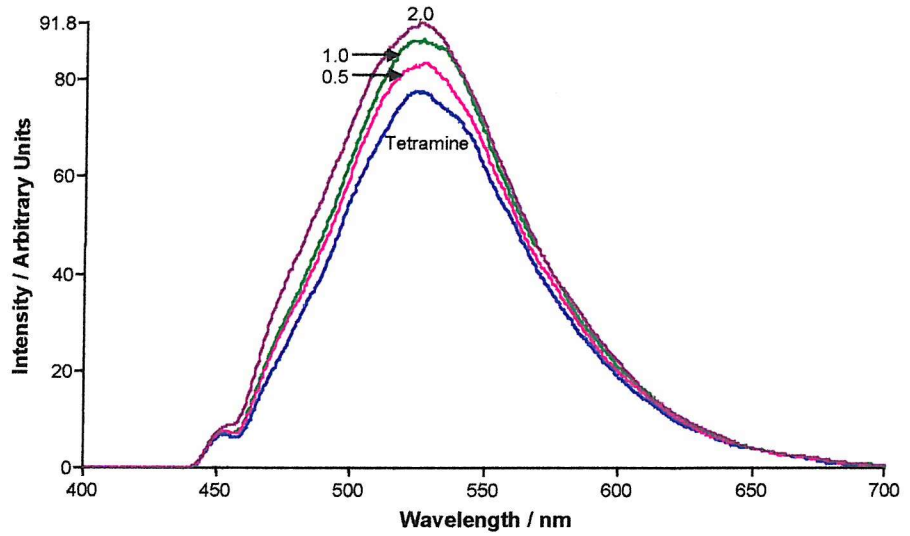


Figure 3.24 Effect of the addition of 0.5 – 2.0 equivalents of tin (II) chloride to tetramine **(29)** in the presence of buffer (pH 9.4)

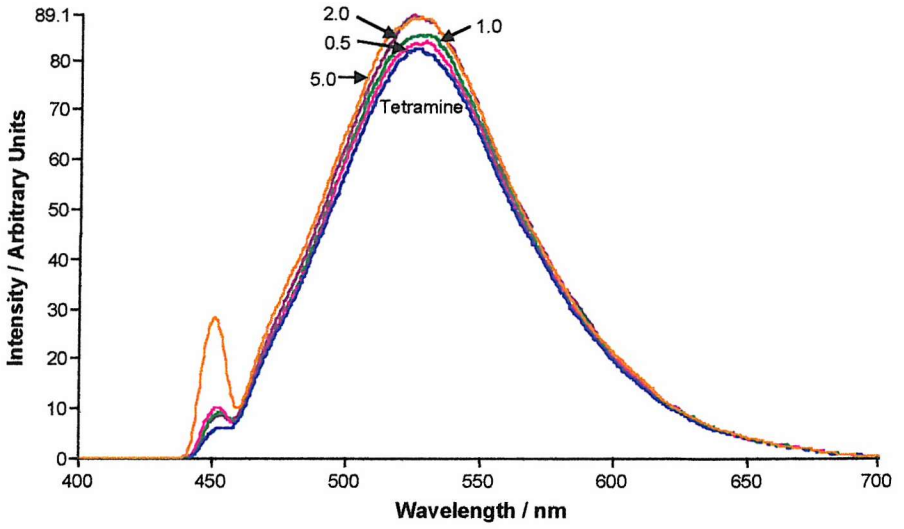


Figure 3.25 Effect of the addition of 0.5 –5.0 equivalents of aluminium (III) chloride to tetramine **(29)** in the presence of buffer (pH 9.4)

It can be seen that little change in fluorescence is observed in the presence of a buffer or when a metal acetate is employed. Thus it was concluded that the enhancement in fluorescence originally observed was simply an acid effect.

In summary, the following metal chlorides gave rise to an enhancement in fluorescence upon addition to tetramine **(29)**, as a result of the acidic nature of the metal ions solutions: $FeCl_2$, $SnCl_2$, $AlCl_3$, $ZnCl_2$. In contrast the following metal chlorides gave rise to a quenching of fluorescence and were unaffected by acid effects: $CuCl_2$, $NiCl_2$, $PdCl_2$, AgCl, $CoCl_2$.

3.3 Fluorescence Studies of Pyrrolidine Tetramine (30)

3.3.1 Metals with no effect

The following metal chlorides/acetates were added to buffered solutions of tetramine **(30)**: $FeCl_2$, $SnCl_2$ and $Zn(OAc)_2$. In each case, a negligible change in the fluorescence was observed and thus it was concluded that these metals did not show any significant binding to tetramine **(30)**.

3.3.2 Enhancement of fluorescence

The following metal chlorides gave rise to a significant enhancement in fluorescence, especially in the case of copper and palladium (Figures 3.26 and 3.27): $CuCl_2$, $PdCl_2$, $NiCl_2$, $CoCl_2$.

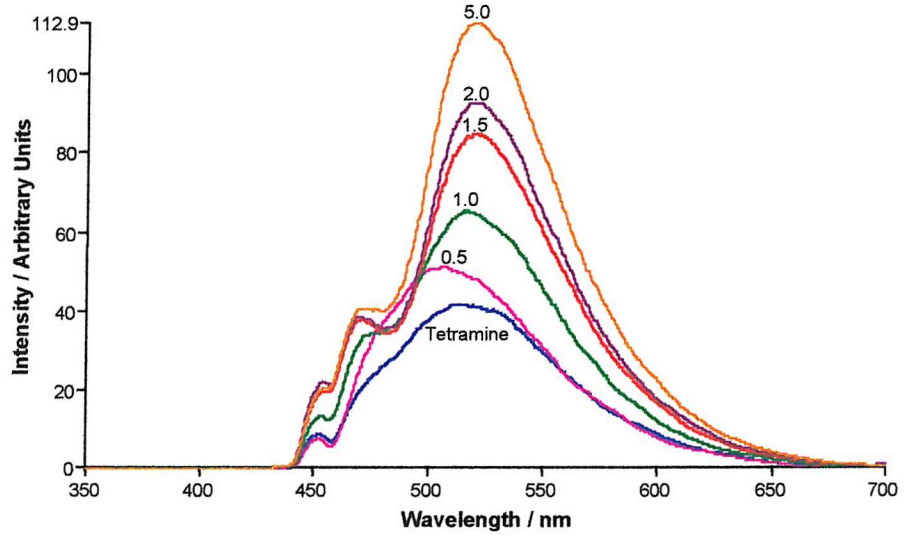


Figure 3.26 Effect of the addition of 0.5 – 5.0 equivalents of copper (II) ions to tetramine **(30)**

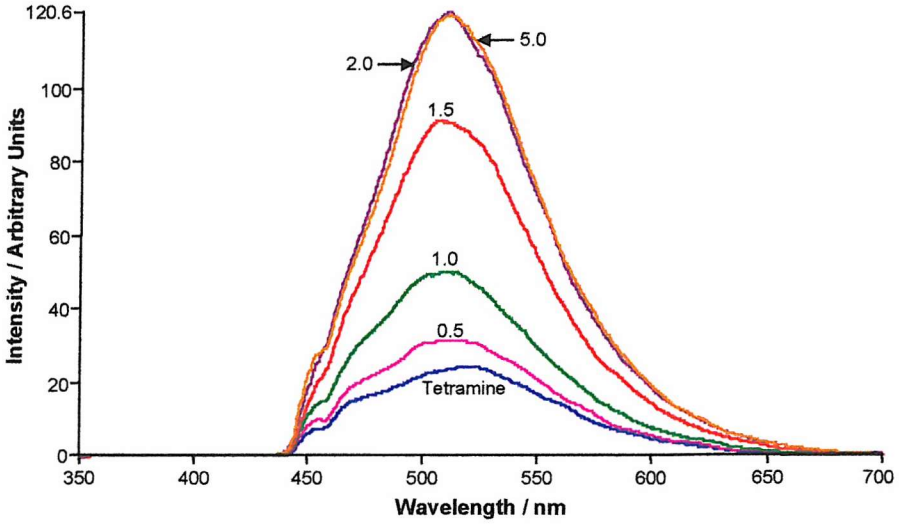


Figure 3.27 Effect of the addition of 0.5 – 5.0 equivalents of palladium (II) ions to tetramine **(30)**

Once again it was necessary to determine whether this was a genuine metal effect or an acid effect. Measurement of the pH of the aqueous metal salts showed them to be moderately strong to weak acids (Table 3.3).

Metal Salt	pH
$PdCl_3$	3.3
$CuCl_2$	5.5
$CoCl_2$	6.0
$NiCl_2$	6.9

Table 3.3 pH of aqueous metal salts

Since the greatest enhancement in fluorescence was observed with the two most acidic metal chlorides it seemed likely that this effect was indeed an acid effect. Consequently the metal ion additions were repeated using buffered solutions of tetramine **(30)**.

Copper (II) chloride – In the presence of buffer, pH 9.4, a significant quenching was observed after the addition of 0.5 equivalents of Cu (II) ions. The fluorescence intensity decreased again after the addition of a further 0.5 equivalents and remained at this level upon the addition of further equivalents of Cu (II) ions (Figure 3.28).

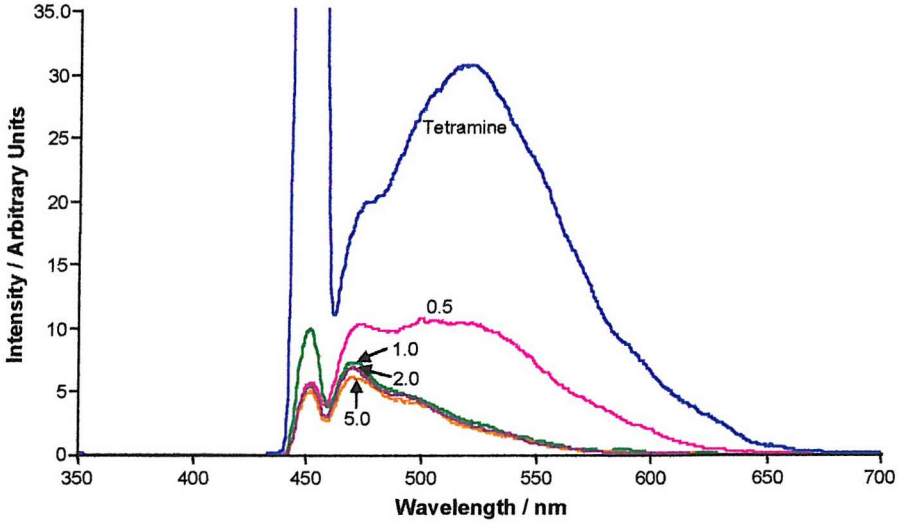


Figure 3.28 Effect of the addition of 0.5 – 5.0 equivalents of copper (II) ions to tetramine **(30)** in the presence of buffer (pH 9.4)

Nickel (II) and palladium (II) chloride – In the case of nickel chloride a small quenching in fluorescence was observed after the addition of 1 equivalent of metal salt to the buffered tetramine (Figure 3.29), whereas the quenching occurred after the addition of 2 equivalents of metal ions in the case of palladium chloride (Figure 3.30).

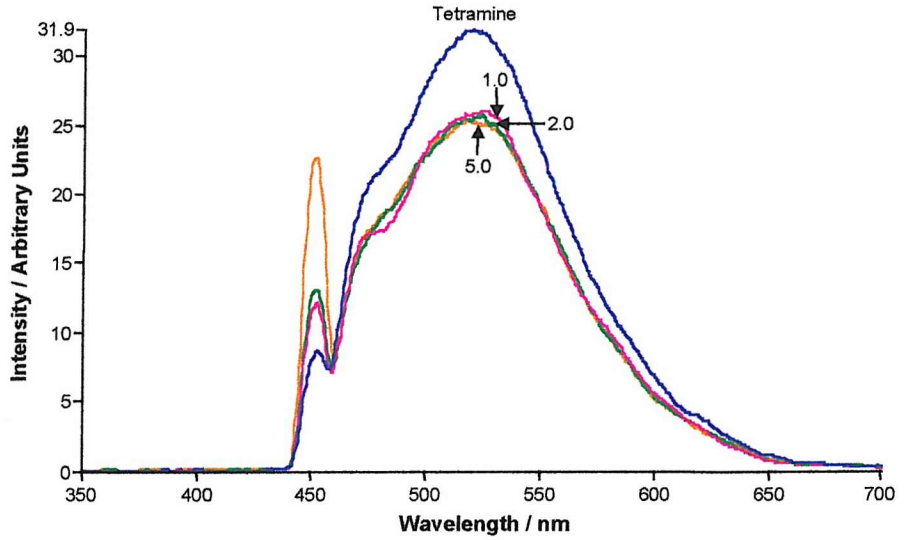


Figure 3.29 Effect of the addition of 1.0 – 5.0 equivalents of nickel (II) ions to tetramine **(30)** in the presence of buffer (pH 9.4)

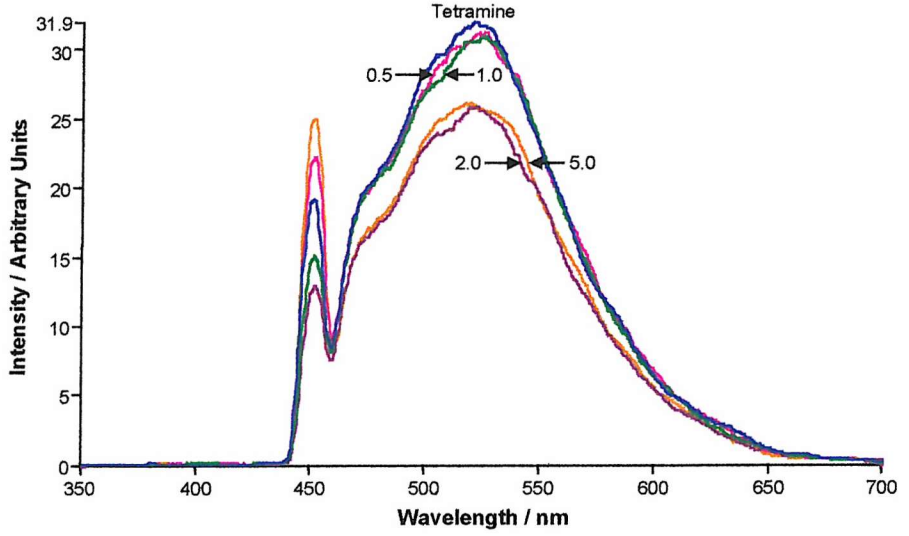


Figure 3.30 Effect of the addition of 0.5 – 5.0 equivalents of palladium (II) ions to tetramine **(30)** in the presence of buffer (pH 9.4)

Cobalt (II) chloride – In the presence of buffer (pH 9.4) no significant change in fluorescence was observed until the addition of 5 equivalents of Co (II) ions, which resulted in a small enhancement in fluorescence (Figure 3.31).

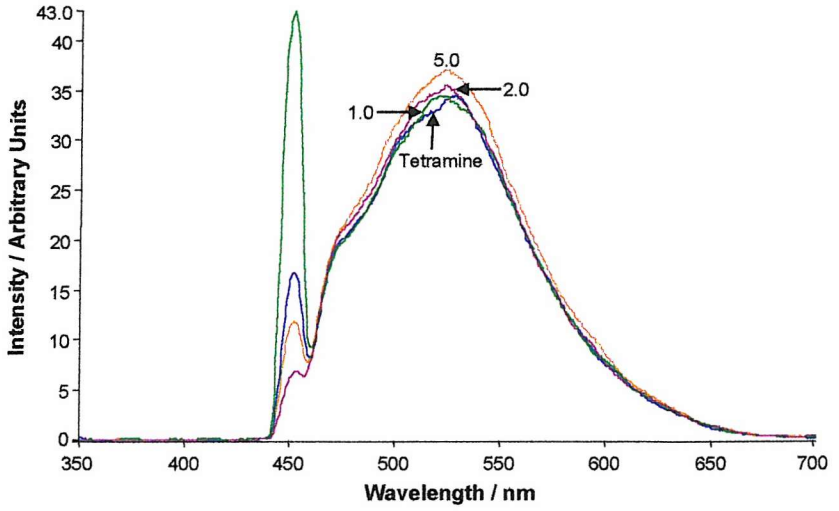


Figure 3.31 Effect of the addition of 0.5 – 5.0 equivalents of cobalt (II) ions to tetramine **(30)** in the presence of buffer (pH 9.4)

3.3.3 Quenching of fluorescence

An enhancement in fluorescence was initially observed upon the addition of silver (I) acetate to tetramine **(30)**, however quenching of fluorescence occurred following the addition of 1.5 equivalents of Ag (I) ions (Figure 3.32).

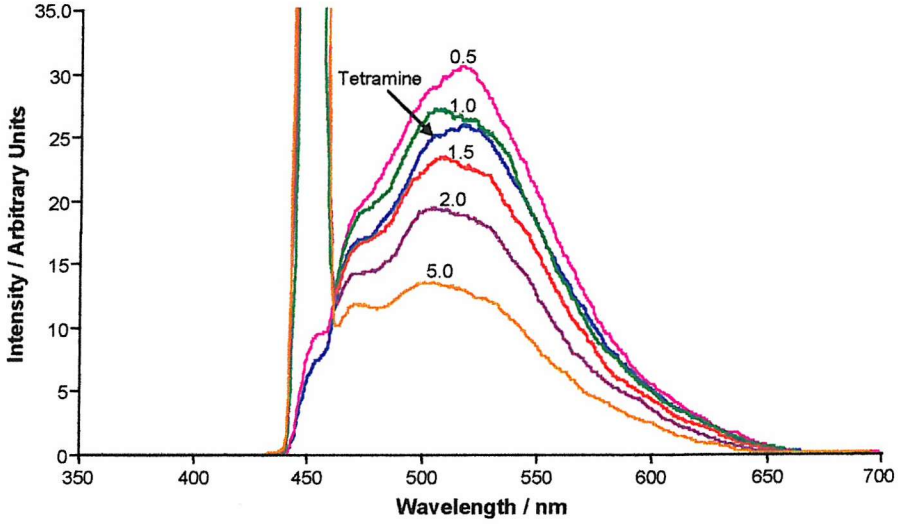


Figure 3.32 Effect of the addition of 0.5 – 5.0 equivalents of silver (I) ions to tetramine **(30)**

In order to compare the fluorescence quenching by each of the metals discussed above a Stern-Volmer plot was obtained (Figure 3.33). Significant binding appears to occur with

copper (II) ions as a significant quenching of fluorescence is observed, as clearly shown in the Stern-Volmer plot.

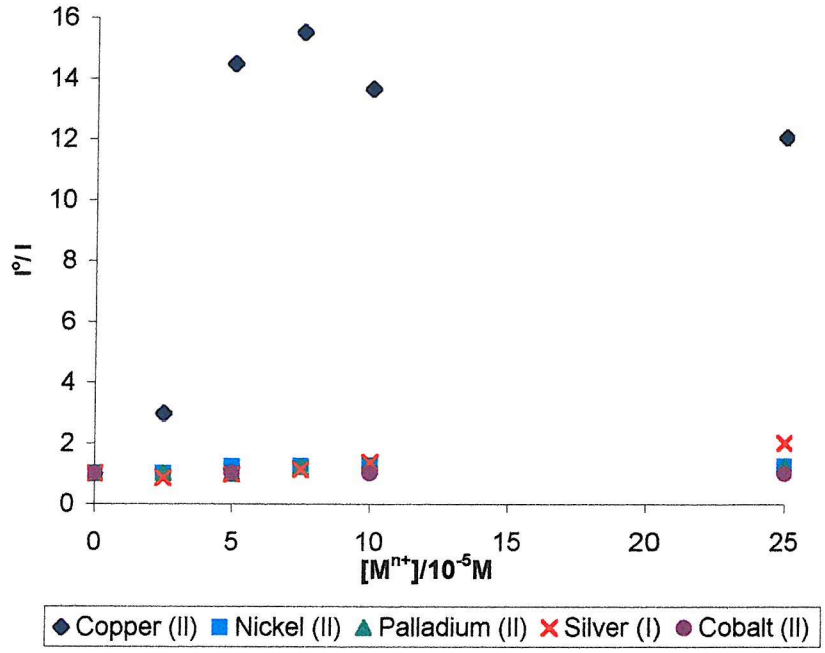


Figure 3.33 Stern-Volmer plot for the quenching of fluorescence by addition of 0.5 – 5.0 equivalents of M^{n+} to tetramine **(30)**

Silver (I), palladium (II) and nickel (II) give rise to a quenching of fluorescence but to a lesser extent, implying that they are not so readily bound by tetramine **(30)**. The other metals discussed here give rise to small changes in fluorescence, often showing both fluorescence enhancement and quenching making it difficult to judge if any metal binding is occurring. In comparison to the dramatic fluorescence quenching observed in the presence of some metals with tetramine **(29)**, tetramine **(30)** shows only small changes in fluorescence. This may reflect the lower affinity of tetramine **(30)** for metals or it may indicate that the nitrogens responsible for metal binding do not have a significant effect on the fluorescence of the compound. Steric effects may also be influencing the metal binding properties of tetramines **(30)**, as compared to tetramine **(29)**. Furthermore tetramine **(30)** seems to be far more sensitive to the effects of acid than tetramine **(29)**, requiring the increased use of buffers.

3.4 Fluorescence Studies of Prolinol Tetramine (32)

Initial experiments with prolinol tetramine **(32)** and metal chlorides resulted in an enhancement of fluorescence, which was shown to be an acid effect, and consequently all experiments were carried out in the presence of buffers or the metal acetate was used.

3.4.1 Quenching of fluorescence

Only palladium (II) and copper (II) ions gave rise to a quenching of fluorescence. A gradual quenching occurred upon the addition of palladium (II) ions (Figure 3.34).

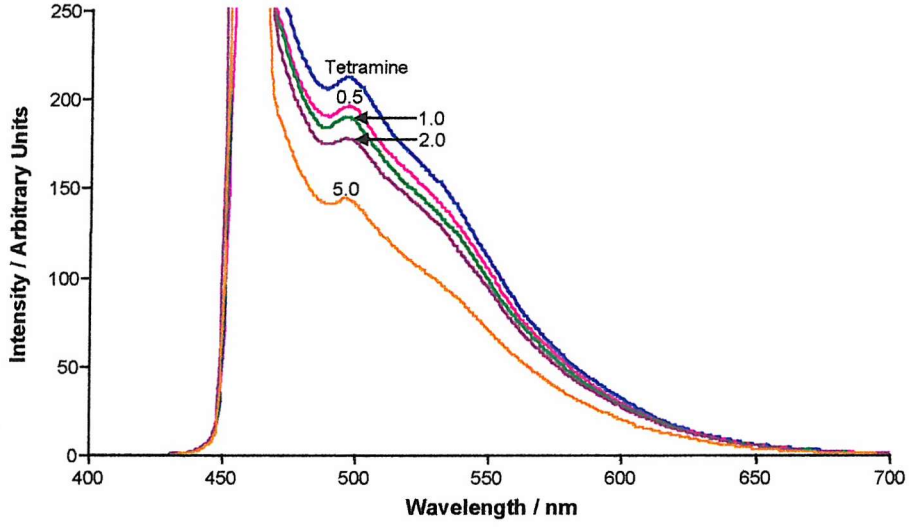


Figure 3.34 Effect of the addition of 0.5 – 5.0 equivalents of palladium (II) ions to tetramine **(32)** in the presence of buffer (pH 9.4)

Significant quenching occurred with the addition of 0.5 equivalents of copper (II) ions and no further major changes in fluorescence were observed upon the addition of up to 5 equivalents of copper (II) ions (Figure 3.35).

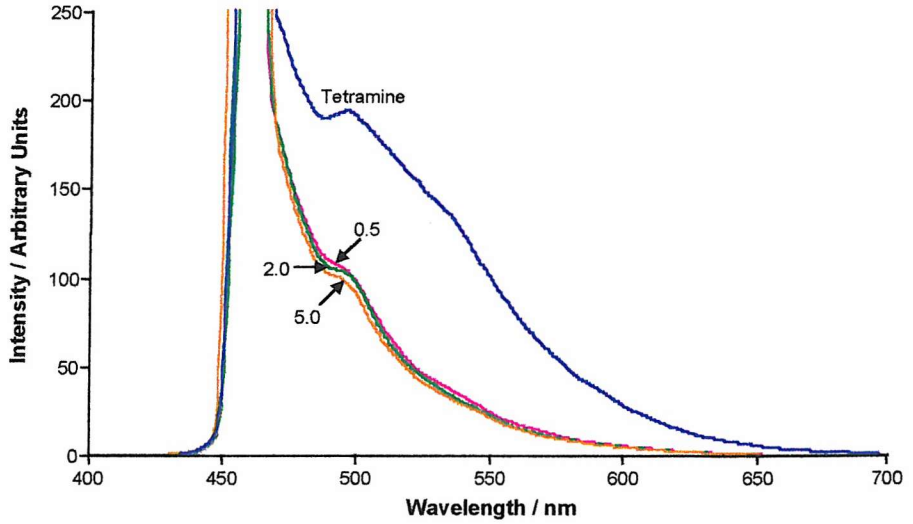


Figure 3.35 Effect of the addition of 0.5 – 5.0 equivalents of copper (II) ions to tetramine **(32)** in the presence of buffer (pH 9.4)

The following metal ions gave rise to only a very slight quenching of the fluorescence: Ni (II), Co (II), Ag (I) (Figures 3.36 – 3.38).

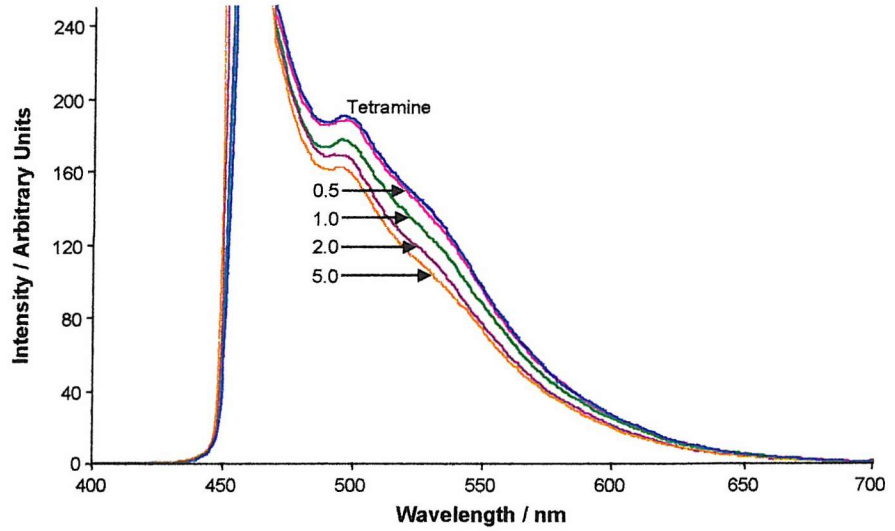


Figure 3.36 Effect of the addition of 0.5 – 5.0 equivalents of nickel (II) ions to tetramine **(32)** in the presence of buffer (pH 9.4)

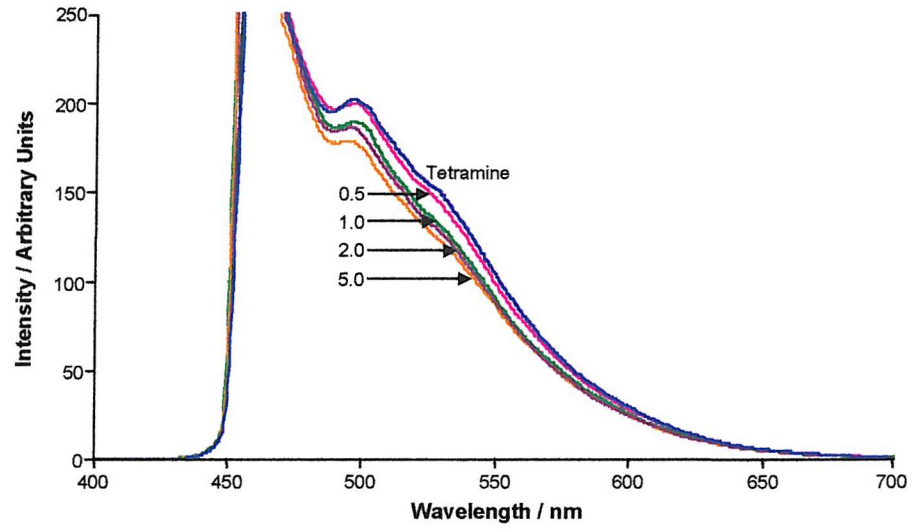


Figure 3.37 Effect of the addition of 0.5 – 5.0 equivalents of cobalt (II) ions to tetramine **(32)** in the presence of buffer (pH 9.4)

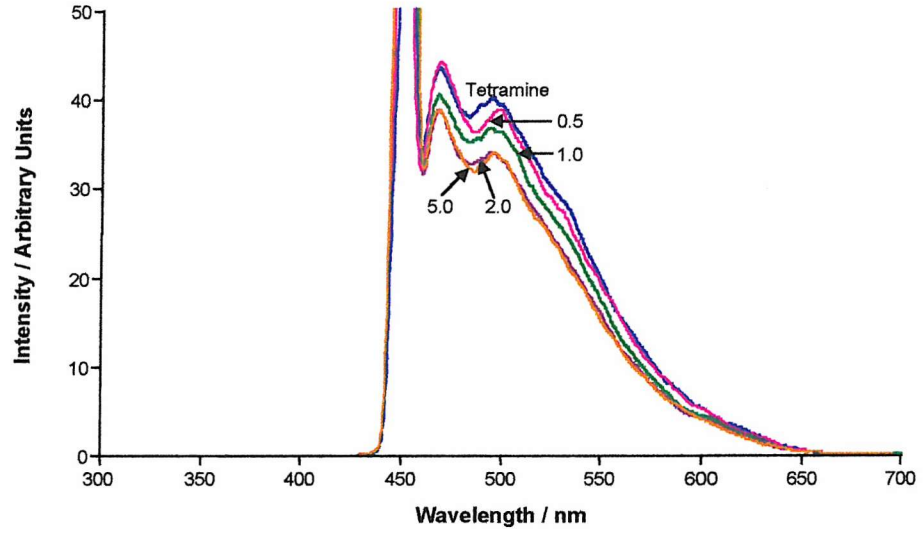


Figure 3.38 Effect of the addition of 0.5 – 5.0 equivalents of silver (I) acetate to tetramine **(32)**

Of all the metals that resulted in a quenching of fluorescence, copper (II) gave rise to the most pronounced change in the fluorescence intensity, indicating that significant metal binding occurs when Cu (II) ions are added to tetramine (32). This is clearly seen in the Stern-Volmer plot below (Figure 3.39).

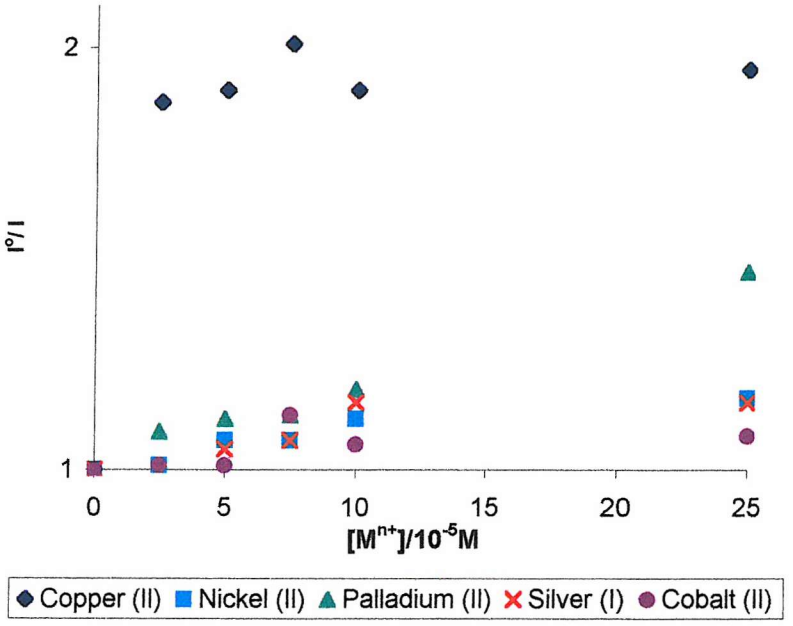


Figure 3.39 Stern-Volmer plot for the quenching of fluorescence by addition of 0.5 – 5.0 equivalents of M^{n+} to tetramine **(32)**

To a lesser extent palladium (II) ions are seen to effect fluorescence quenching. It appears that the other metal ions are resulting in only a negligible binding. These fluorescence spectra may reflect the metal affinity of tetramine **(32)** or they may reflect the nature of the metal complexes formed. In the case of palladium and copper complexing may be occurring at sites that have a pronounced effect on the fluorescence of the molecule whereas for the other metals this is not the case. The spectra may also be indicative of the stoichiometry of the complexes formed. It must be borne in mind that the oxygens of the prolinol group may also be involved in metal binding. Furthermore, it should be noted that tetramine **(32)**, like tetramine **(30)**, has been found to be more sensitive to the effects of acid than tetramine **(29)**.

3.5 Overall Conclusions

Variation of the pH of solutions of tetramines **(29)**, **(30)** and **(32)** resulted in an enhancement of fluorescence at low pH values, i.e. upon the addition of acid. This effect can be attributed to the quenching of PET, originating from the nitrogen lone pair, upon protonation, resulting in the recovery of fluorescence.

It has been shown that for each of the three tetramines, **(29)**, **(30)** and **(32)**, only some metals give rise to significant changes in fluorescence. Quenching of fluorescence appears to be a genuine metal effect resulting from the interaction between tetramine and metal ion, whereas enhancement of fluorescence has been shown to be due to an acid effect rather than a genuine metal effect. The fact that tetramines **(30)** and **(32)** are more susceptible to acid effects than tetramine **(29)** may be indicative of the nature of metal binding in each case.

The extent of the fluorescence quenching upon metal salt addition gives some indication as to the affinity of the tetramines for particular metals and the nature of the metal binding. In particular all three tetramines appear to bind copper (II) ions most readily.

In light of the results obtained via fluorimetry it was decided to employ mass spectrometry in order to identify the metal complexes formed and to determine their stoichiometries.

Chapter Four

Mass Spectrometry

Chapter Four

Mass Spectrometry

4.1 Electrospray Ionisation Mass Spectrometry

Electrospray ionisation mass spectrometry (ESI-MS) has provided a means for the analysis of a wide variety of host-guest complexes and other non-covalent complexes formed in solution.[166,167,168] ESI-MS works by producing gaseous ionised molecules from a liquid solution.[169] This is achieved by creating a fine spray of highly charged droplets in the presence of a strong electric field. ESI has many advantages over other ionisation techniques (Table 4.1) and a particularly important feature is a tendency to form multiply charged molecules. Since the mass spectrometer measures the mass / charge ratio, it permits the observation of large molecules with an instrument having a relatively small mass range.

Advantages	Disadvantages
• Practical mass range up to 70,000 Da • Good sensitivity. Femtomole to low picomole sensitivity is typical • Softest ionisation. Capability of observing biologically native non-covalent interactions • No matrix interference • Multiple charging, allowing for the analysis of high-mass ions with a relatively low m/z range instrument • Multiple charging, giving better mass accuracy through averaging	• Low salt tolerance • Difficulty in cleaning overly contaminated instrument due to high sensitivity for certain compounds • Low tolerance for mixtures. Simultaneous mixture analysis can be poor. The purity of the sample is important • Multiple charging, which can be confusing, especially with mixture analysis

Table 4.1 Advantages and Disadvantages of ESI-MS

In the literature ESI-MS has been employed in three different areas, all concerning the analysis of host-guest interactions. Firstly, it has been used to identify the formation of ligand-metal complexes and to observe their fragmentation patterns.[170,171,172]

Secondly, ESI-MS has been employed in the determination of binding affinities and selectivities.[173,174,175,176] Experiments of this nature typically involve the analysis of either solutions of a single host binding different guest ions or different hosts binding

the same guest ion. However a cautious approach is required when determining selectivities simply by comparing peak intensities. Langley et al. have observed that there are a number of experimental variables associated with the use of fast atom bombardment mass spectrometry (FAB-MS), which are equally applicable to ESI-MS, and must first be addressed.[177,178] It has frequently been assumed that if

$$I_{(Ligand+H)}^{+} = I_{(Ligand+Metal)}^{+} \text{ (where I = peak intensity)}$$

then these species are present in equimolar amounts. However this has been proved to be invalid. Firstly, the 'ionisation efficiencies' of the ligand itself and the various ligand-metal complexes vary widely. A second problem is accounting for background levels of metal ions; this effect is most significant at low concentrations. Sodium ions in particular are often present in solution since they are leached from glassware, thus the amount of free ligand available for binding is reduced. In conclusion measuring the ratio of peak intensities gives little meaningful information concerning relative extents of ion binding unless a calibration experiment has been carried out first.

The third application of ESI-MS found in the literature is the determination of binding constants involving the vancomycin group antibiotics. Williams[179] has shown that the formation of heterodimers in mixtures of glycopeptides can be detected and the dimerisation constants determined via ESI-MS, giving good agreement with those determined by NMR spectroscopy. Furthermore Heck[180] has shown that the solution binding constants for complexes between glycopeptides and bacterial wall peptide analogues can be determined by ESI-MS and are in good agreement with values obtained by standard spectroscopic titration techniques. In both cases measurements are based upon the relative ion intensities with the assumption that all ions in solution have similar ionisation efficiencies. Thus it appears that the binding constants of ligand-neutral species can be determined simply and effectively via ESI-MS, whereas the binding constants of ligand-cation species cannot, due to greater differences in ionisation efficiencies. Furthermore the hetero-dimerisation and the binding of peptide ligands to the vancomycin antibiotics involves the formation of several intermolecular hydrogen bonds between amide groups. This type of non-covalent binding is favourable in the gas phase and thus may account for the good agreement

observed between complex formation in solution and relative ion abundancies in the ESI-mass spectrum.

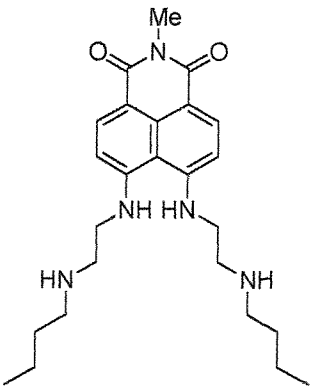
4.1.1 Overview of mass spectrometry studies

In the series of tetramines based upon naphthalene, fluorimetry provided an effective route by which to determine metal binding affinities. The extent of changes in fluorescence upon the addition of various metal salts indicated, to some degree, the relative extent of metal binding. However fluorimetry provided no means by which to determine the nature of the metal complexes formed. Thus ESI-MS was employed to examine further the metal selectivities of the tetramines based upon naphthalene and to determine the stoichiometry of the metal complexes formed. Comparison of the results obtained by fluorimetry and ESI-MS provided a means of validating the results of the mass spectrometry studies. As such ESI-MS was identified as an effective means of determining metal binding affinities and was subsequently used to determine metal selectivities and to identify complex stoichiometries in the series of tetramines based upon pyridine, given their lack of fluorescence precluded the use of fluorimetry.

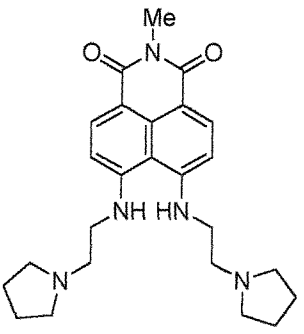
4.2 Mass Spectrometry Studies of the Naphthalene Series

4.2.1 Mass spectrometry in the absence of metal ions

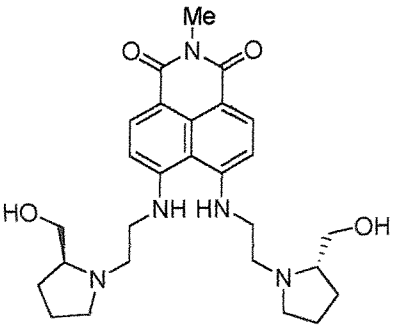
The three tetramines studied were **(29)**, **(30)** and **(32)**. First a mass spectrum was obtained for each tetramine in the mass range 120-1800 in the absence of metal ions.



(29)



(30)



(32)

Tetramine **(29)** gave rise to a peak at *m/z* 440 corresponding to the molecular ion $(M+H)^+$ and a peak at *m/z* 462 due to $(M+Na)^+$ (Figure 4.1). Since no metal ions had been added to tetramine **(29)** the sodium ions may have been leached from the glassware, in which a solution of tetramine **(29)** had been made up, or may already have been present in the spectrometer. A peak corresponding to the dimer $(2M+H)^+$ was observed at *m/z* 879 with a further peak at *m/z* 901 owing to $(2M+Na)^+$. The presence of dimers has previously been shown in similar 4,5-disubstituted naphthalimides, giving rise to peaks due to species such as $(2M+H)^+$, $(3M+H)^+$ and $(4M+H)^+$.[181,2]

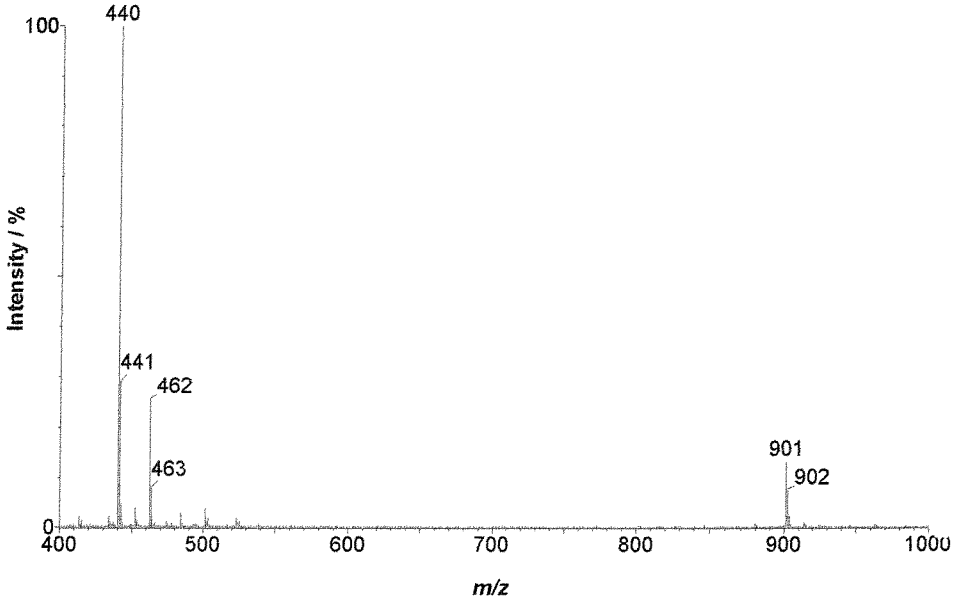


Figure 4.1 Mass spectrum of tetramine **(29)**

Tetramine **(30)** also gave rise to a dimer with peaks at *m/z* 436, 458 and 893 corresponding to $(M+H)^+$, $(M+Na)^+$ and $(2M+Na)^+$ (Figure 4.2).

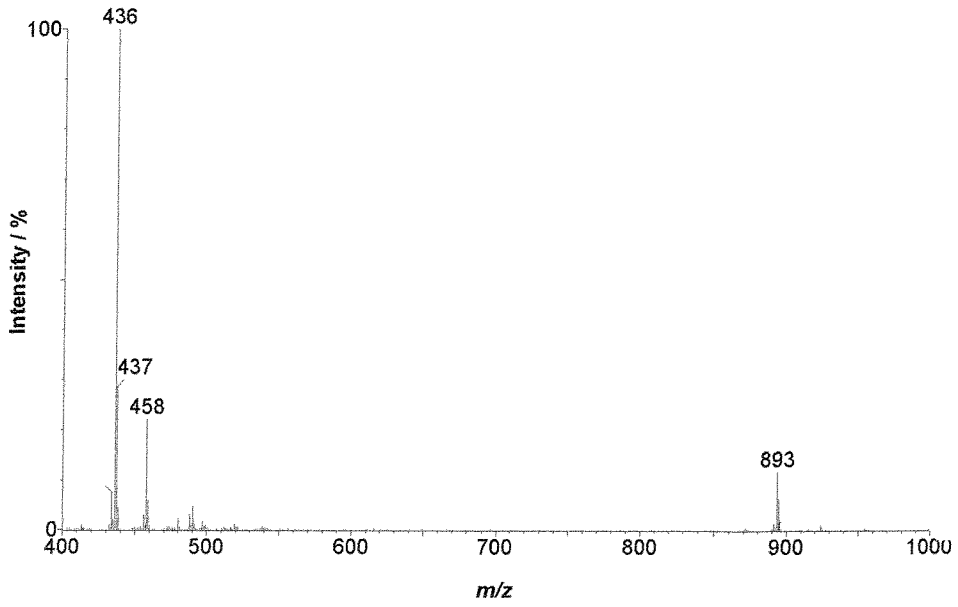


Figure 4.2 Mass spectrum of tetramine **(30)**

However, no dimerisation was observed in the case of tetramine **(32)** with peaks at *m/z* 496 and 518 only, due to $(M+H)^+$ and $(M+Na)^+$ respectively (Figure 4.3).

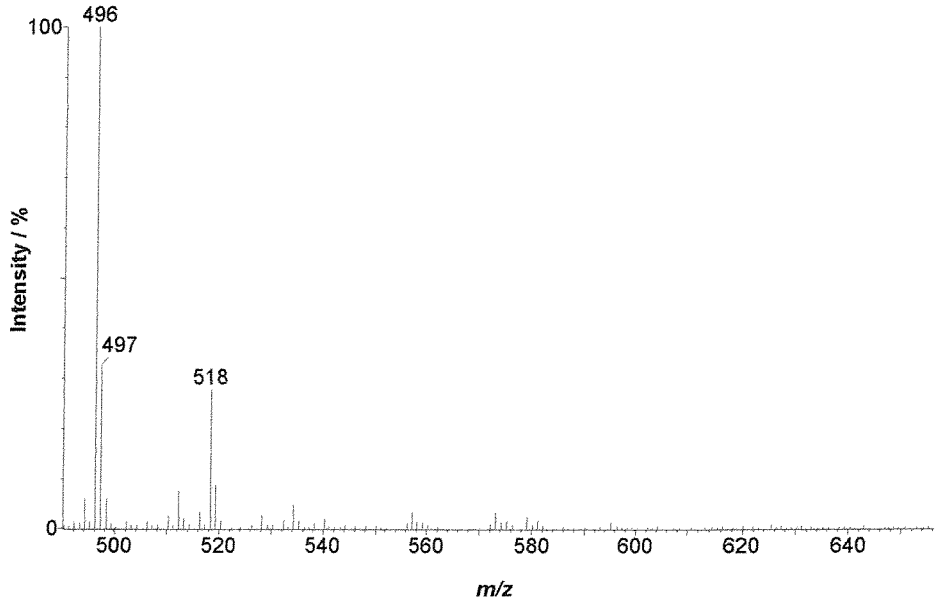


Figure 4.3 Mass spectrum of tetramine **(32)**

In order to determine which metals formed complexes with the tetramines **(29)**, **(30)** and **(32)** and the stoichiometry of such complexes, the effect on the mass spectrum of the addition of metal ions was observed. The general procedure involved the addition of 0.5 - 5.0 equivalents of an aqueous metal salt, in increments of 0.5, to a ~$5x10^{-6}$ M solution of the tetramine in methanol. The effect of the metal ions fell into two

distinct groups: those metals that gave rise to tetramine:metal complex peaks in the mass spectrum and those metals that resulted in no change in the mass spectrum.

4.2.2 Mass spectrometry in the presence of metals

4.2.2.1 Metals exhibiting no effect

The following metal ions led to no noticeable change in the mass spectrum of the tetramine to which they were added, even after the addition of 5 equivalents of the metal salt : Li(I), Na(I), Mg(II), Al(III), K(I), Ca(II), Mn(II), Fe(II), Zn(II), Sn(II). The molecular ion $(M+H)^+$ and the $(M+Na)^+$ peak remained unchanged and no new peaks corresponding to $(M+metal)^+$ were observed.

Although all three tetramines gave rise to a peak corresponding to $(M+Na)^+$, owing to the presence of background sodium ions, this peak was not observed to increase in size relative to the $(M+H)^+$ peak upon the addition of up to 5 equivalents of aqueous sodium chloride.

Thus it was concluded that the above metals do not form complexes with tetramines **(29)**, **(30)** and **(32)**, even in the presence of an excess of the metal salt. These metals also resulted in no noticeable change in the fluorescence spectrum of the tetramines **(29)**, **(30)**, and **(32)** and thus these two sets of results are in good agreement.

4.2.2.2 Metals exhibiting complex formation

For each of the following metals a peak due to metal complexation by at least one of the tetramines was observed: Co (II), Ni (II), Cu (II), Ag (I), Pd (II). The extent of metal complex formation varied between the tetramines **(29)**, **(30)** and **(32)** and the metal salt which was added.

Silver (I) nitrate – The addition of 1 equivalent of silver (I) nitrate to tetramine **(29)** resulted in the appearance of a distinctively shaped group of peaks corresponding to $(M+Ag)^+$ at *m/z* 546. The shape of the group of peaks reflected the relative abundance of the two isotopes of silver, namely Ag^{107} and Ag^{109}. (A list of the relative abundance of the isotopes of all the metals used in the mass spectrometry studies is given in Appendix 2). The peak remained small relative to the molecular ion until 10

equivalents of Ag (I) ions were added. At this point the peak due to $(M+Ag)^+$ became comparable in size to the molecular ion (Figure 4.4).

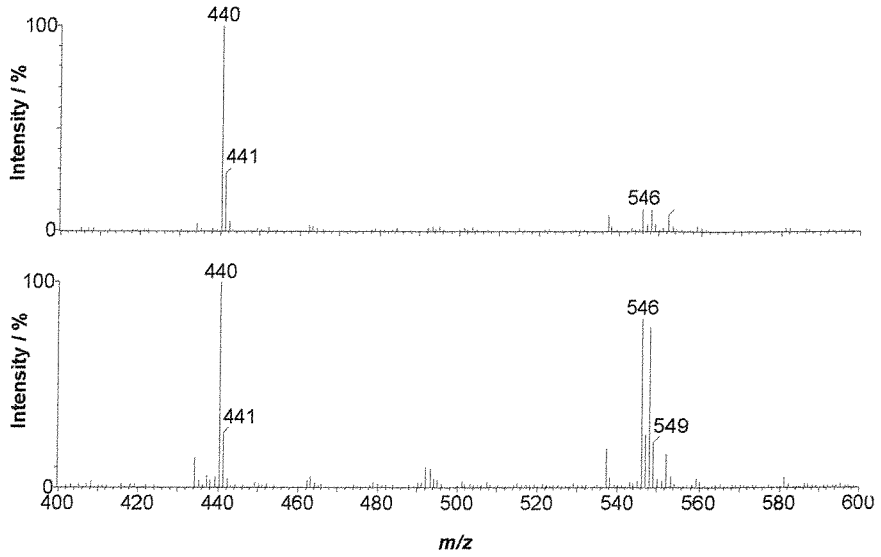


Figure 4.4 Top spectra: Tetramine **(29)** + 5 equivalents of Ag (I) ions
Bottom spectra: Tetramine **(29)** + 10 equivalents of Ag (I) ions

The addition of Ag(I) ions to tetramines **(30)** and **(32)** did not give rise to a $(M+Ag)^+$ peak even after the addition of 10 equivalents of the metal salt.

These results are broadly consistent with those observed via fluorimetry. The addition of silver (I) ions to each of the tetramines results in fluorescence quenching and the extent of the quenching effect is observed to be greater for tetramine **(29)** than either tetramine **(30)** or **(32)**. Similarly the effect of the addition of silver (I) ions, as observed via mass spectrometry, is most pronounced for tetramine **(29)** with the appearance of a peak corresponding to $(M+Ag)^+$. The fluorescence quenching observed upon the addition of silver (I) ions to tetramines **(30)** and **(32)** is less pronounced but still clearly visible and as such the absence of a $(M+Ag)^+$ peak in the mass spectrum of both tetramine **(30)** and **(32)** in the presence of silver (I) ions is slightly surprising. However this anomaly can be attributed to dilution effects, since the concentrations employed in the mass spectrometry experiments are only 1/10 of those used in the fluorescence experiments.

Cobalt (II) nitrate and Cobalt (II) chloride – The addition of 0.5 equivalents of cobalt (II) nitrate to tetramine **(29)** gave rise to a significant peak at *m/z* 496

corresponding to $(M+Co)^+$ and after the addition of 2 equivalents of cobalt (II) nitrate the molecular ion was found to have almost completely disappeared (Figure 4.5).

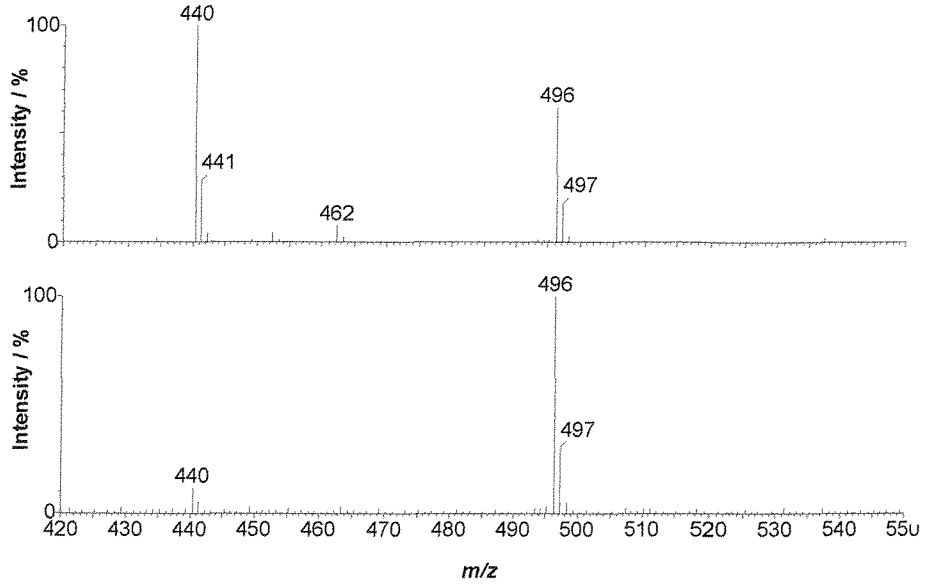
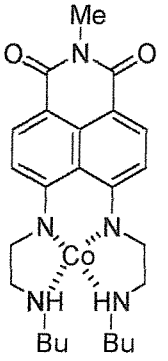


Figure 4.5 Top spectra: Tetramine **(29)** + 0.5 equivalents of Co (II) ions
Bottom spectra: Tetramine **(29)** + 2 equivalents of Co (II) ions

As shown below the binding of Co(II) results in the loss of two protons from tetramine **(29)**. Therefore the species observed via mass spectrometry is $[M\text{-}2H^+\text{+}Co^{2+}]H^+$, which corresponds to a *m/z* value of 497 (439-2+59+1).



(29)

The observed peak at *m/z* 496 had the correct isotope pattern for cobalt, but appeared 1Da (Da = Dalton, one mass unit) lower than the expected peak for the complexation of Co (II) by tetramine **(29)**. In fact it appeared that tetramine **(29)** was binding Co (III) and losing three protons to give the species $[M\text{-}3H^+\text{+}Co^{3+}]H^+$, which corresponds to an *m/z* value of 496 (439-3+59+1). The experiment was run again with a different cobalt (II) salt, namely cobalt (II) chloride. However this also gave rise to a peak at

m/z 496 indicating that the cobalt in solution was again in the +3 oxidation state (Figure 4.6).

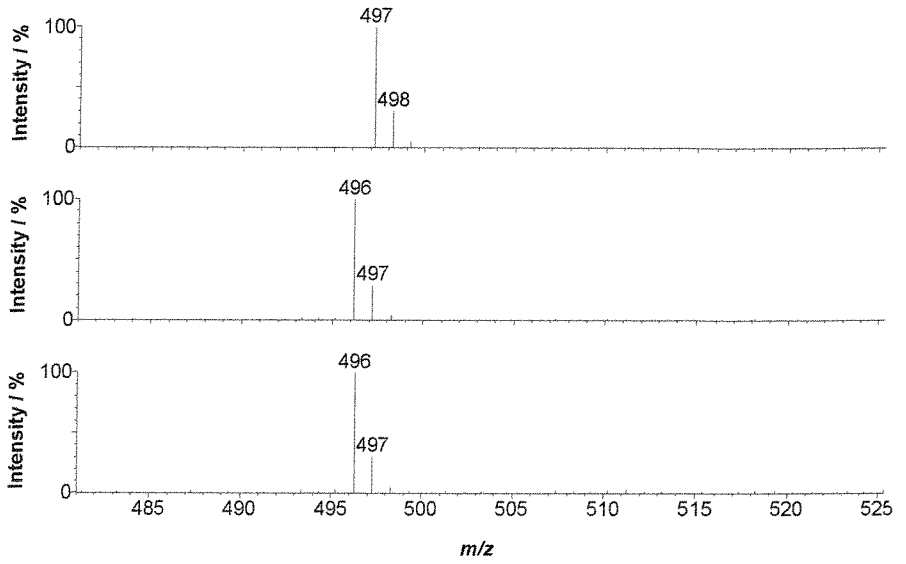


Figure 4.6 Top Spectra: Peak position expected for tetramine **(29)** + Co (II) ions
Middle Spectra: Tetramine **(29)** + $CoCl_2$
Bottom Spectra: Tetramine **(29)** + $Co(NO_3)_2$

Similarly the addition of cobalt (II) chloride to tetramine **(32)** gave rise to a significant peak at *m/z* 552 corresponding to $(M+Co)^+$ and after the addition of 1 equivalent of cobalt (II) chloride the molecular ion was found to have almost completely disappeared. As observed with tetramine **(29)**, the peak at *m/z* 552 had the correct isotope pattern but once again appeared 1 Da lower than expected, indicating the presence of Co (III) (Figure 4.7).

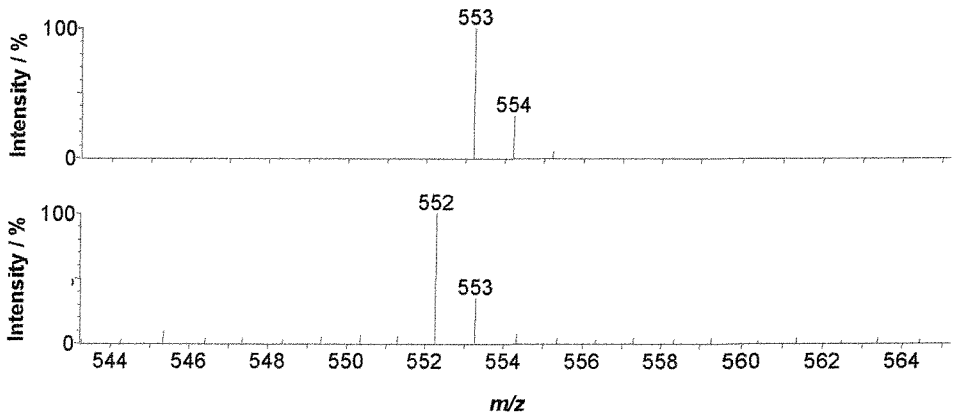


Figure 4.7 Top Spectra: Peak position expected for tetramine **(32)** + Co (II) ions
Bottom Spectra: Tetramine **(32)** + 1 equivalent of Co (II) ions

In order to determine the oxidation state of cobalt present the UV spectrum of the cobalt nitrate and cobalt chloride salts were obtained and compared with the UV spectra of cobalt (II) in the literature. From this it became apparent that although the oxidation state of cobalt in both salt samples was the same, the cobalt did not appear to be in the +2 state. Furthermore aqueous solutions of both salts gave a positive test with starch iodide paper indicating that the ion in solution was indeed Co (III).

The addition of cobalt ions to tetramine **(30)** did not give rise to a $(M+Co)^+$ peak, even after the addition of 10 equivalents of the metal salt.

The results of these experiments are as expected from the fluorescence studies previously conducted. Both tetramine **(29)** and **(32)** give rise to significant quenching upon the addition of cobalt ions, which is reflected in the rapid appearance and growth of a $(M+Co)^+$ peak in mass spectra of both tetramines **(29)** and **(32)** in the presence of cobalt ions. Similarly the negligible quenching in the fluorescence of tetramine **(30)** in the presence of cobalt (I) ions is reflected in the absence of any $(M+Co)^+$ peak in the mass spectrum of tetramine **(30)** in the presence of cobalt ions.

For simplicity the peaks arising from the binding of a metal ion by tetramine, M, will be referred to as $(M+metal)^+$ in all further sections of this chapter. However it should be noted that the actual species observed corresponds to $[M\text{-}xH^+ + (metal)^{x+}]H^+$.

Nickel (II) sulphate and Palladium (II) chloride – The addition of just 0.5 equivalents of nickel (II) sulphate to tetramine **(29)** resulted in the appearance of a small $(M+Ni)^+$ peak at *m/z* 496, which grew steadily, resulting in the almost complete disappearance of the molecular ion after the addition of five equivalents of the metal salt (Figure 4.8).

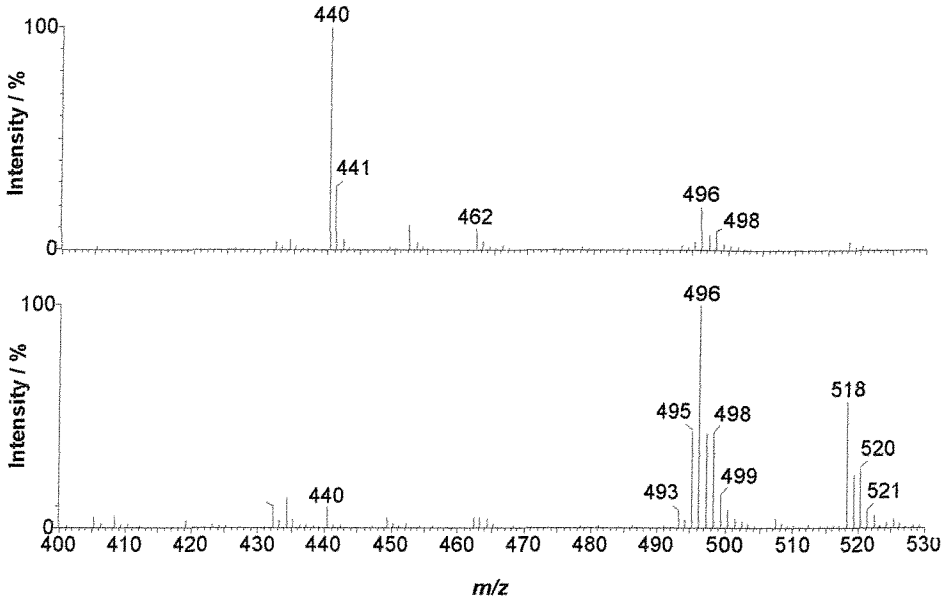


Figure 4.8 Top spectra: Tetramine **(29)** + 0.5 equivalents Ni (II) ions
Bottom spectra: Tetramine **(29)** + 5 equivalents of Ni (II) ions

Similarly the addition of 0.5 equivalents of nickel (II) ions to tetramine **(32)** resulted in a small peak at *m/z* 552 corresponding to $(M+Ni)^+$, which also grew steadily, but even after the addition of 5 equivalents of metal salt the molecular ion had not completely disappeared (Figure 4.9).

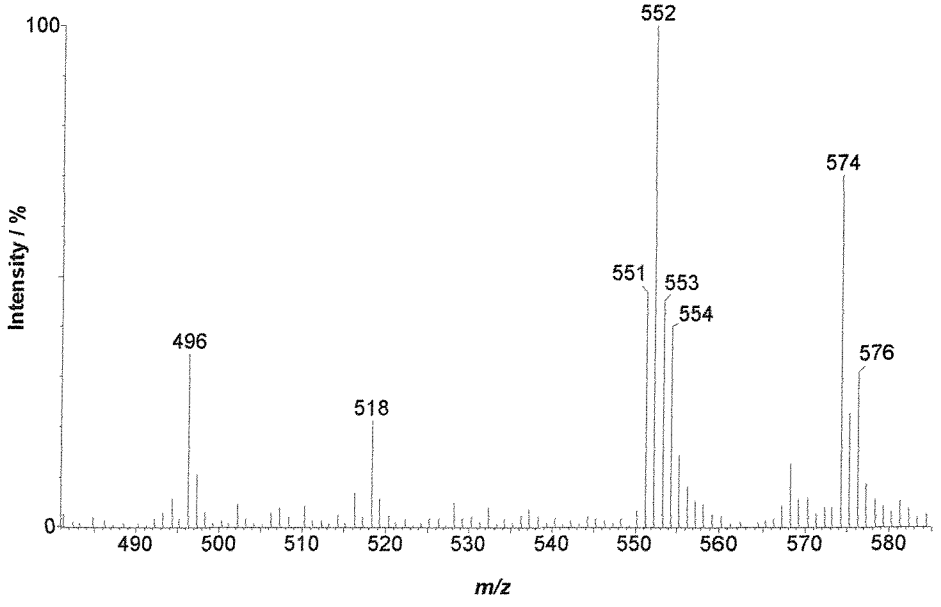


Figure 4.9 Tetramine **(32)** + 5 equivalents of Ni (II) ions

In the mass spectrum of tetramine **(30)** only a small peak at *m/z* 402 due to $(M+Ni)^+$ was observed after the addition of 5 equivalents of nickel (II) sulphate (Figure 4.10).

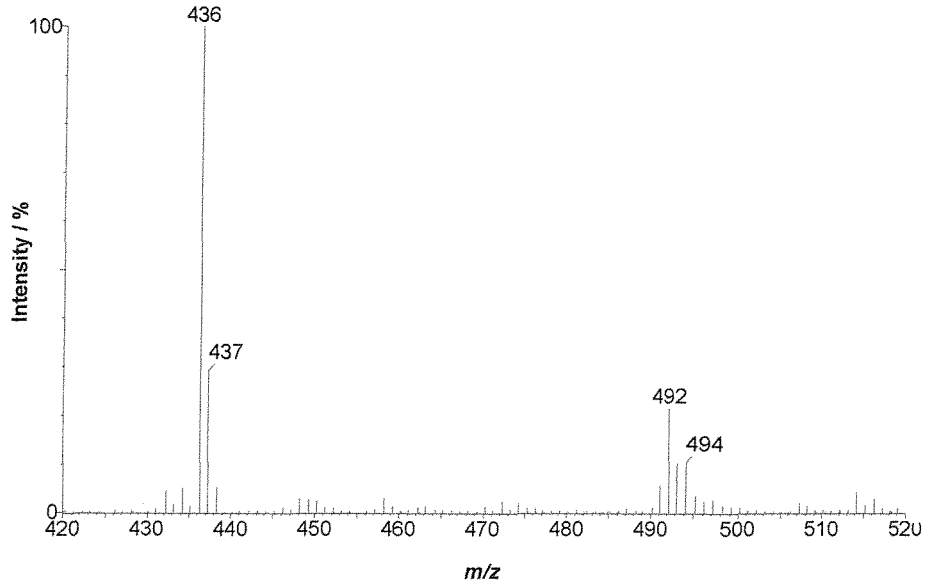


Figure 4.10 Tetramine **(30)** + 5 equivalents of Ni (II) ions

In all three cases the $(M+Ni)^+$ peak position was as expected, however the isotope pattern was slightly irregular. It appeared to correspond to an overlap in the isotope patterns of Ni (II) and Ni (III) (Figure 4.11). However the peaks due to $(M+Ni+Na)^+$ appeared at the correct position with the expected isotope pattern.

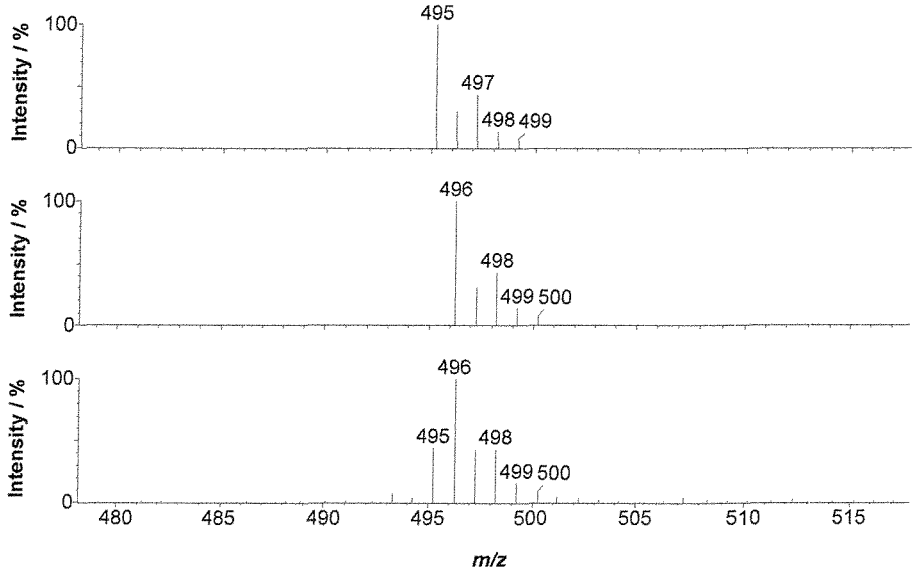


Figure 4.11 Top Spectra: Isotope pattern expected for tetramine **(29)** + Ni (III) ions
Middle Spectra: Isotope pattern expected for tetramine **(29)** + Ni (II) ions
Bottom Spectra: Observed isotope pattern for tetramine **(29)** + Ni (II) ions

The addition of 5 equivalents of palladium (II) chloride to tetramines **(29)** and **(32)** resulted in the complete disappearance of the molecular ion and appearance of a $(M+Pd)^+$ peak at *m/z* 543 and 599 respectively (Figures 4.12 and 4.13).

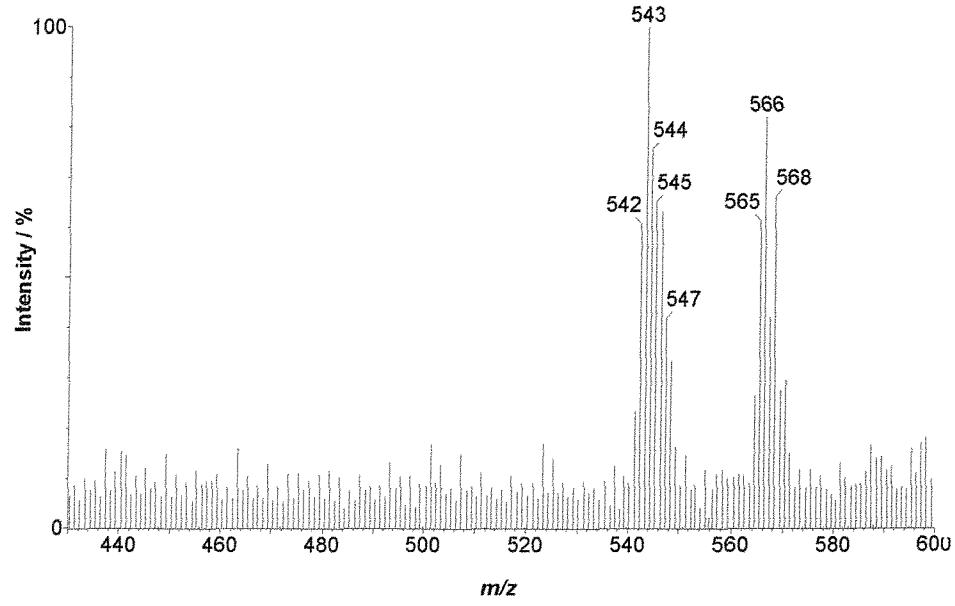


Figure 4.12 Tetramine **(29)** + 5 equivalents of Pd (II) ions

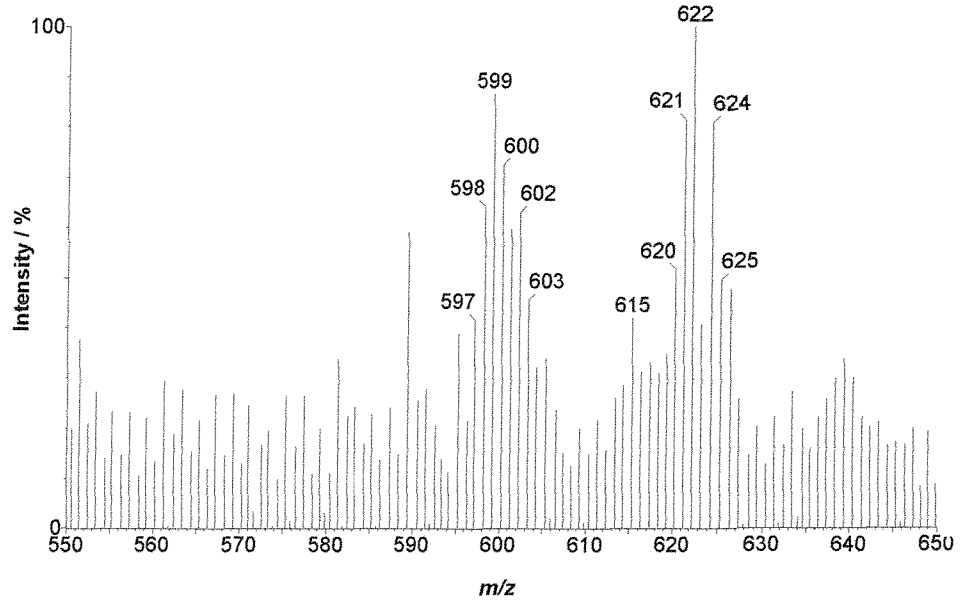


Figure 4.13 Tetramine **(32)** + 5 equivalents of Pd (II) ions

In the case of tetramine **(30)** the molecular ion was still apparent after the addition of 5 equivalents of palladium (II) chloride but a sizeable peak due to $(M+Pd)^+$ was also observed (Figure 4.14).

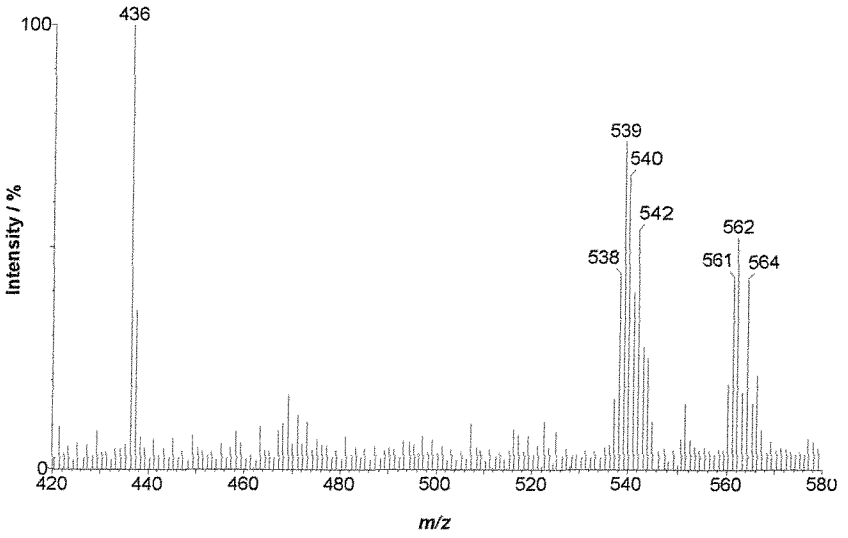


Figure 4.14 Tetramine **(30)** + 5 equivalents of Pd (II) ions

In all three cases, as observed in the addition of nickel sulphate, the $(M+Pd)^+$ peak position was as expected, however the isotope pattern was slightly irregular. It appeared to correspond to an overlap in the isotope patterns of Pd (II) and Pd (III) (Figure 4.15). However, once again, the peaks due to $(M+Pd+Na)^+$ appeared at the correct position with the expected isotope pattern.

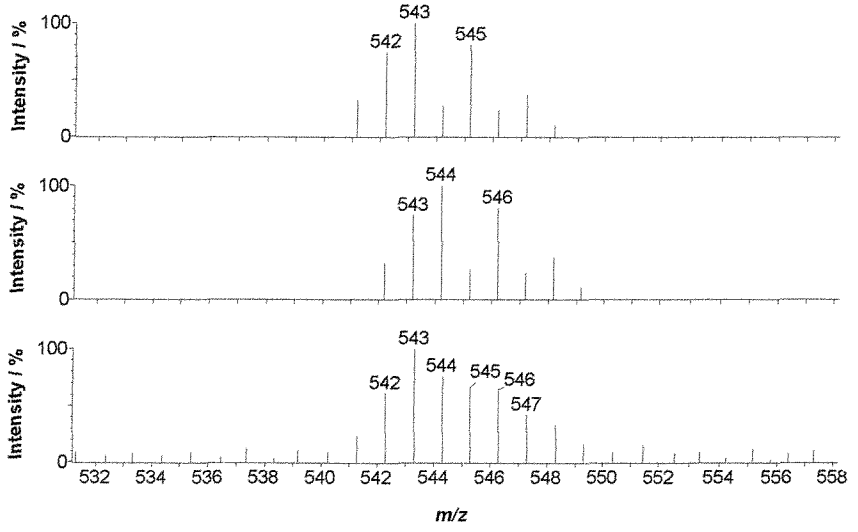


Figure 4.15 Top Spectra: Isotope pattern expected for tetramine **(29)** + Pd (III) ions
Middle Spectra: Isotope pattern expected for tetramine **(29)** + Pd (II) ions
Bottom Spectra: Observed isotope pattern for tetramine **(29)** + Pd (II) ions

In terms of a correlation between fluorimetry and mass spectrometry, the $(M+Ni)^+$ and $(M+Pd)^+$ peaks observed in the mass spectrum of each tetramine in the presence of nickel and palladium ions respectively is as expected, since fluorescence quenching is observed with all three tetramines in the presence of these metals. However the irregular isotope patterns are unexpected and not conclusive.

The possibility that the metal salts used contain a mixture of +2 and +3 metal ions can be discounted since nickel (II) and palladium (II), unlike cobalt (II), do not readily undergo aerial oxidation. A second possibility is that the tetramines themselves are effecting partial oxidation of the nickel (II) and palladium (II) salts upon binding. Many studies of the electrochemical properties of divalent metal complexes of tetramines, as well as other tetradentate nitrogen based ligand systems, have been reported in the literature.[182,183,184] Furthermore the factors influencing the stabilisation and destabilisation of nickel (III) and nickel (I) species in various co-ordination environments have received much attention and are of interest in both inorganic chemistry and biochemistry. Anionic ligands in particular have been found to influence the stability of Ni^{3+} relative to Ni^{2+}. Thus the phenomena observed in the mass spectra might be reflecting the redox properties of the nickel (II) and palladium (II) complexes formed with tetramines **(29)**, **(30)** and **(32)**.

Given the sensitivity of tetramines **(29)**, **(30)** and **(32)** to acid effects, as observed via fluorimetry, it was decided to repeat the addition of nickel (II) ions to tetramine **(32)** in the presence of base (aqueous ammonia). It is interesting to note that this resulted in a change in the isotope pattern of the $(M+Ni)^+$ peak so that it more closely resembled the predicted isotope pattern (Figure 4.16). This suggests that the irregular isotope patterns may be due to an acid effect.

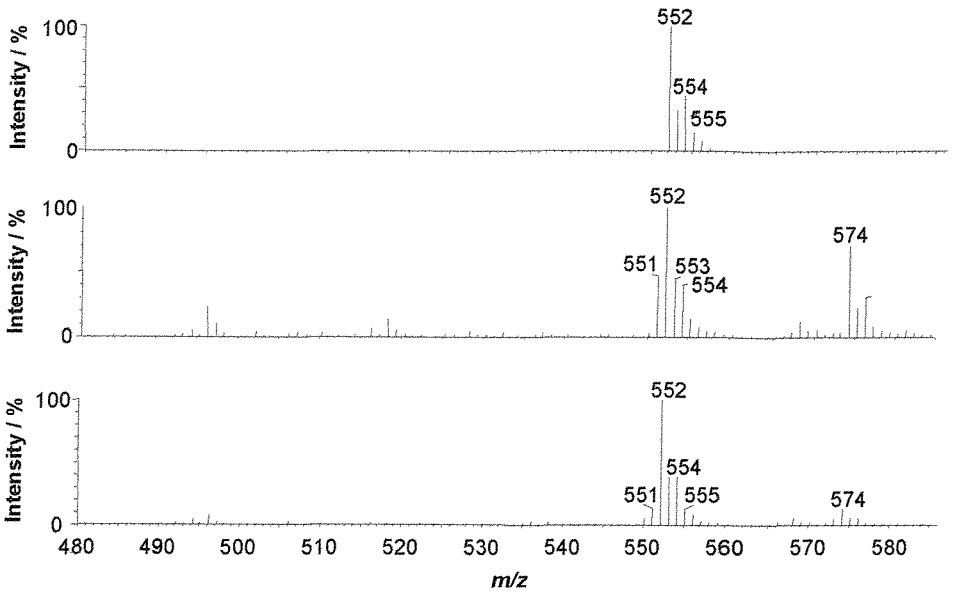


Figure 4.16 Top Spectra: Isotope pattern expected for tetramine **(32)** + Ni (II) ions
Middle Spectra: Observed isotope pattern for tetramine **(32)** + Ni (II) ions
Bottom Spectra: Observed isotope pattern for tetramine **(32)** + Ni (II) ions in the presence of base (aqueous ammonia)

Copper (II) sulphate – The addition of just 1 equivalent of copper sulphate to tetramines **(29)** and **(30)** led to the complete disappearance of the molecular ion and appearance of a $(M+Cu)^+$ peak at *m/z* 501 and 497 respectively (Figures 4.17 and 4.18).

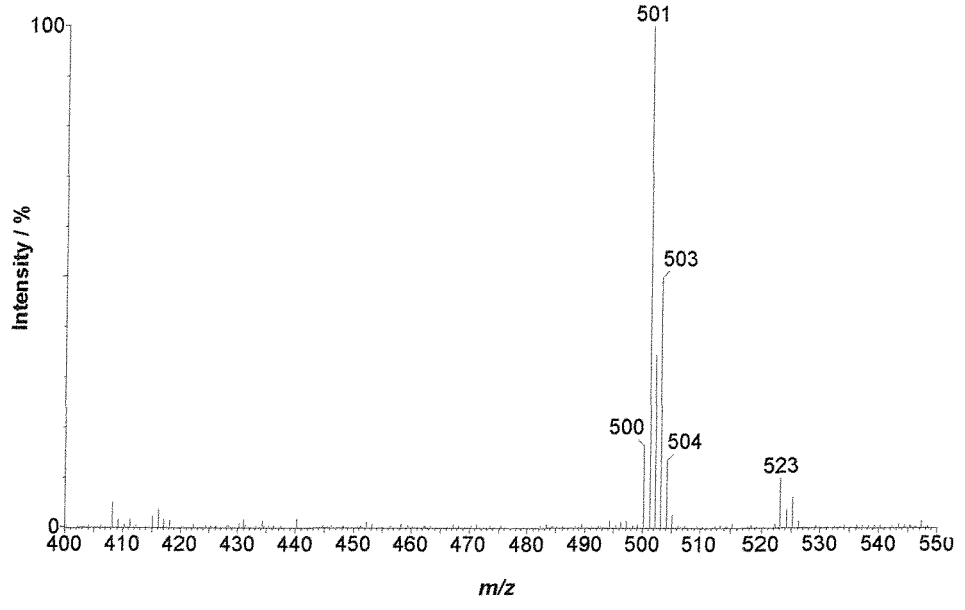


Figure 4.17 Tetramine **(29)** + 1 equivalent of Cu (II) ions

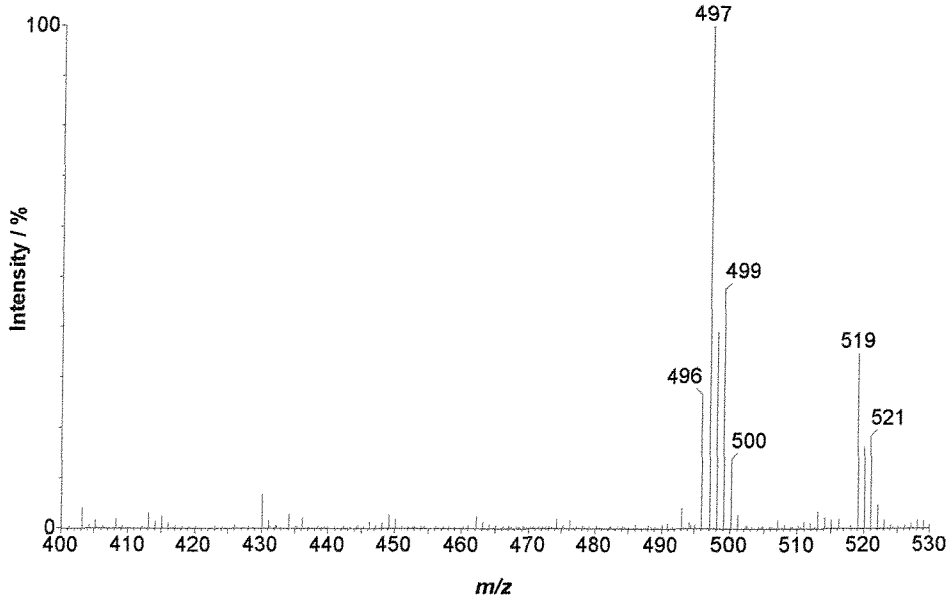


Figure 4.18 Tetramine **(30)** + 1 equivalent of Cu (II) ions

The molecular ion corresponding to tetramine **(32)** was observed to have completely disappeared after the addition of just 0.5 equivalents of copper (II) sulphate. However the peak observed due to the binding of copper (II) ions appeared at *m/z* 618 indicating that a simple 1:1 complex with copper had not been formed, but rather the species $(M+2Cu)^+$ (Figure 4.19).

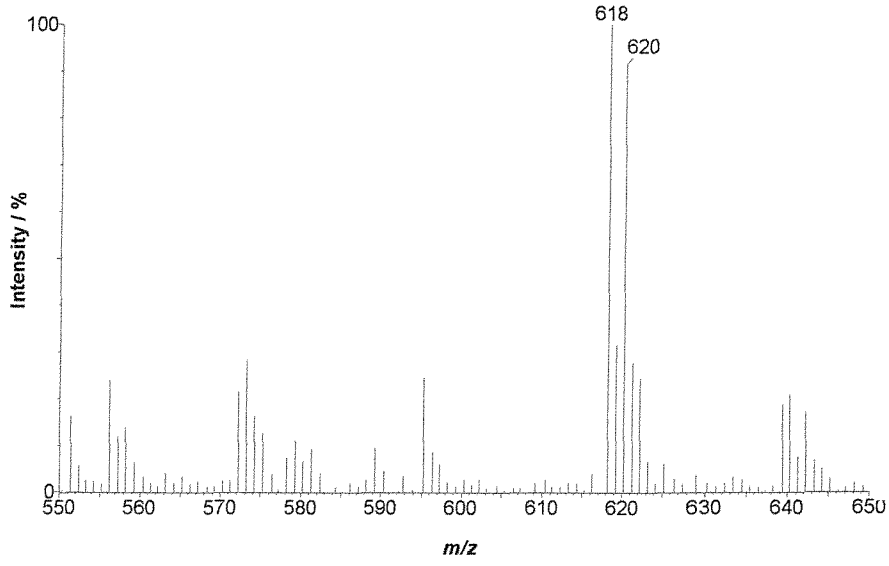


Figure 4.19 Tetramine **(32)** + 0.5 equivalent of Cu (II) ions

A subsequent experiment was carried out in which 0.01, 0.1, 0.25 and 0.5 equivalents of copper (II) ions were added to tetramine **(32)**. Upon the addition of 0.1 equivalents

of copper (II) ions a peak at *m/z* 557 was observed corresponding to $(M+Cu)^+$ (Figure 4.20). When 0.25 equivalents of copper (II) ions were added the peak due to $(M+2Cu)^+$ was also observed (Figure 4.21) and by the addition of 0.5 equivalents of copper (II) ions only the $(M+2Cu)^+$ peak was observed. Thus at very low concentrations of copper (II) ions the 1:1 complex is formed, however the addition of just 0.5 equivalents of copper (II) ions results in solely the 2:1 metal:tetramine complex.

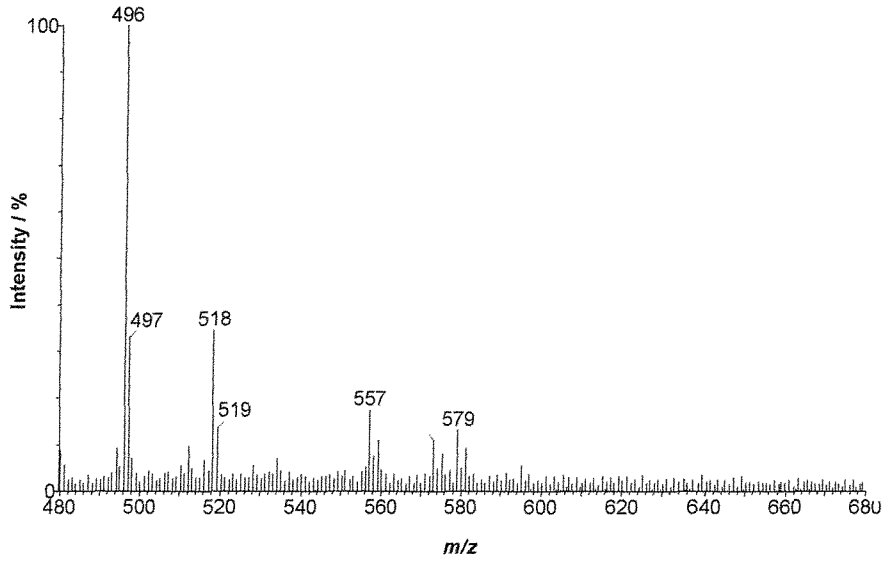


Figure 4.20 Tetramine **(32)** + 0.1 equivalents of copper (II) ions

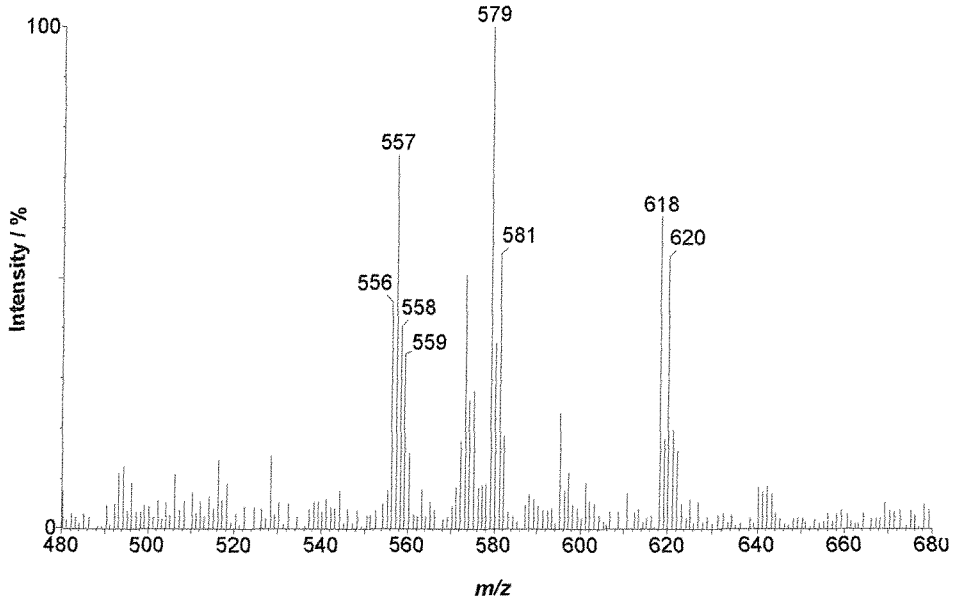


Figure 4.21 Tetramine **(32)** + 0.25 equivalents of copper (II) ions

It is interesting to note that although tetramines **(29)** and **(30)** do not appear to form the 2:1 complex mentioned above they did give rise to very small amounts of $(2M+Cu+Na)^+$ and $(2M+2Cu+Na)^+$ species at very low concentrations of copper (II) ions. In the presence of 0.25 equivalents of copper (II) ions, tetramine **(29)** gave rise to peaks at *m/z* 440, 501, 901, 962 and 1023 corresponding to $(M+H)^+$, $(M+Cu)^+$, $(2M+Na)^+$, $(2M+Cu+Na)^+$ and $(2M+2Cu+Na)^+$ respectively (Figure 4.22).

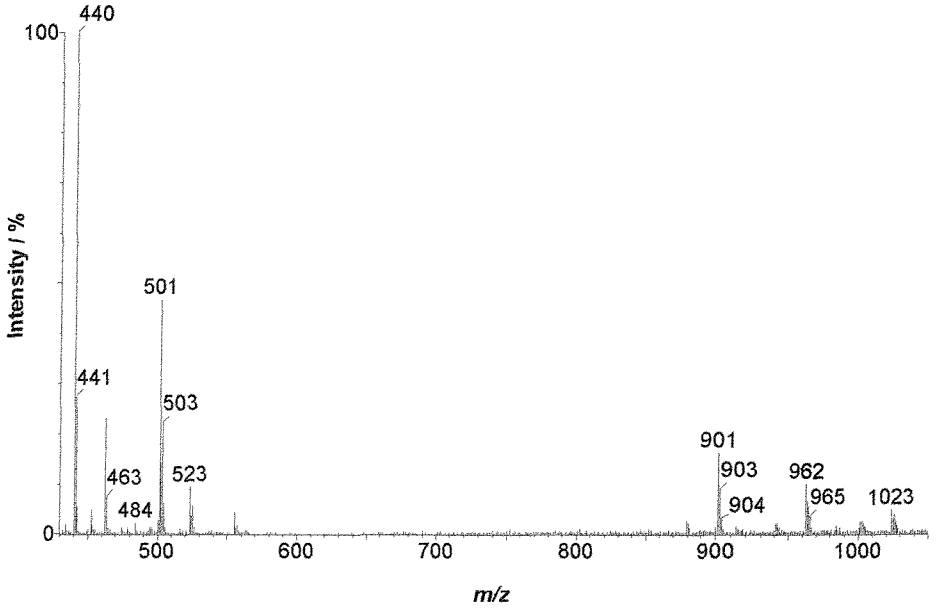


Figure 4.22 Tetramine **(29)** + 0.25 equivalents of copper (II) ions

In the presence of 0.25 equivalents of copper (II) ions, tetramine **(30)** gave rise to peaks at *m/z* 436, 497, 519, 893, 954 and 1015 corresponding to $(M+H)^+$, $(M+Cu)^+$, $(M+Cu+Na)^+$, $(2M+Na)^+$, $(2M+Cu+Na)^+$ and $(2M+2Cu+Na)^+$ respectively (Figure 4.23).

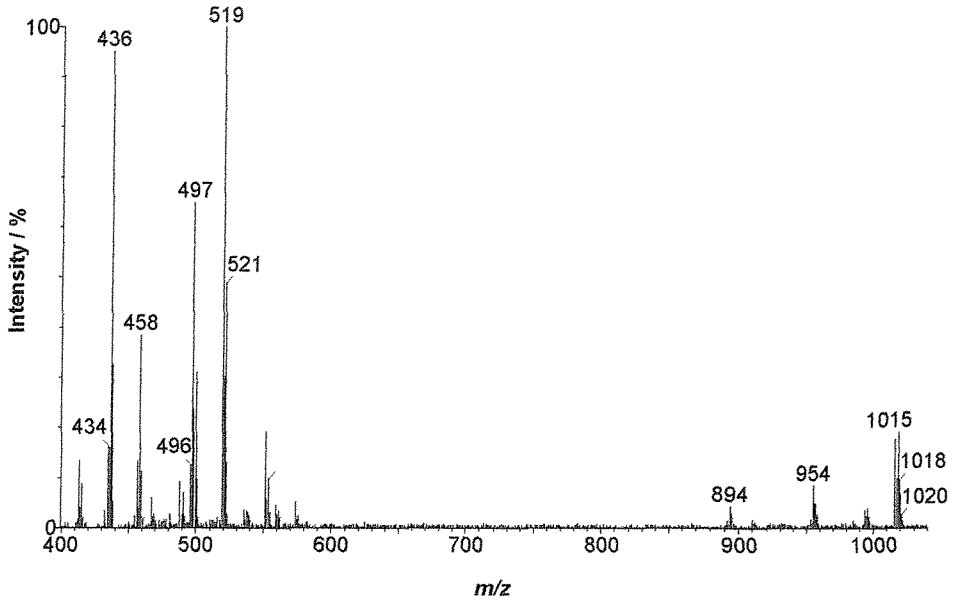


Figure 4.23 Tetramine **(30)** + 0.25 equivalents of copper (II) ions

The addition of copper (II) ions to each tetramine gives rise to the most rapid and the most pronounced fluorescence quenching, as compared to all other metals employed, indicating a high affinity for copper in each case. This is also observed via mass spectrometry with the addition of copper (II) ions giving rise to the most rapid appearance of a $(M+n\text{Cu})^+$ peak and the most rapid disappearance of the molecular ion, as compared to all other metals used.

4.2.3 Conclusions

The extent of complex formation of **(29)**, **(30)** and **(32)** observed via mass spectrometry are as shown in Table 4.2 below.

Tetramine	Extent of Complex Formation
Tetramine **(29)**	Ag < Ni ~ Pd < Co < Cu
Tetramine **(30)**	Pd < Ni < Cu
Tetramine **(32)**	Ni < Pd < Co < Cu

Table 4.2 Relative extent of tetramine complex formation

The extent of metal binding varies between each tetramine but in general all three tetramines bind palladium, nickel and copper, with a relatively high affinity for copper in each case. The butylamine tetramine **(29)** appears to complex metals most

readily, for example only tetramine **(29)** binds silver and the molecular ion tends to disappear more quickly than for the other tetramines. In contrast the pyrrolidine tetramine **(30)** appears to form complexes the least readily. The complexing power of the prolinol tetramine **(32)** appears to lie somewhere between that of the other two tetramines but tends to be more like the butylamine tetramine **(29)**, for example tetramine **(32)** also binds cobalt. Thus the results of mass spectrometry are broadly in line with those observed via fluorimetry.

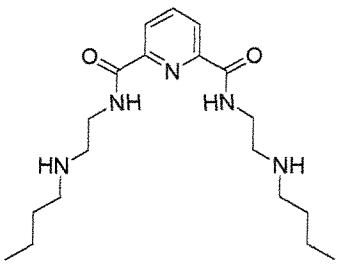
Additional information can be gained from a comparison of the two sets of results, particularly in the case of tetramine **(32)**. Whereas the addition of metals such as cobalt, nickel and palladium gives rise to only small changes in the fluorescence of tetramine **(32)**, the effect on the mass spectrum of tetramine **(32)** is much more pronounced. This suggests that the binding of these metals is significant, but that the site of binding is not largely involved in the fluorescence process. Secondly the fluorescence quenching of tetramine **(32)** by copper (II) ions is observed to be significantly greater than the quenching effect of the other metals employed, indicating that the nature of metal binding may be different in the case of copper. This is borne out by the fact that a 2:1 copper:tetramine complex is observed via mass spectrometry, whereas other metals give rise to 1:1 metal:tetramine complexes. Furthermore the fact that tetramine **(32)** is the only tetramine to give rise to complexes of the stoichiometry 2:1 metal:tetramine suggests that the alcohol functionality of the prolinol group may play a significant role in the binding of copper (II) ions by tetramine **(32)**.

4.3 Mass Spectrometry Studies of the Pyridine Series

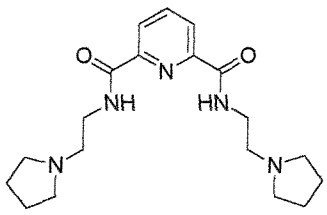
In the naphthalene series mass spectrometry proved to be a successfully technique for the identification and elucidation of metal complex formation, giving results broadly consistent with those obtained via fluorimetry. The tetramines based upon pyridine were found to be non-fluorescent and thus mass spectrometry was chosen as a method for investigating metal selectivities and complex stoichiometries in this series.

4.3.1 Mass spectrometry in the absence of metal ions

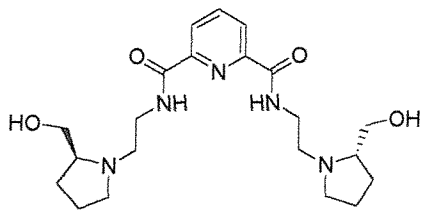
The three tetramines studied were **(42)**, **(43)** and **(51)**. First a mass spectrum was obtained for each tetramine in the mass range 120-1800 in the absence of metal ions.



(42)



(43)



(51)

Tetramine **(42)** gave rise to a peak at *m/z* 364 corresponding to the molecular ion $(M+H)^+$ and two further peaks at *m/z* 386 and 408 corresponding to $(M+Na)^+$ and $(M+2Na)^+$ (Figure 4.24), due to background levels of sodium ions.

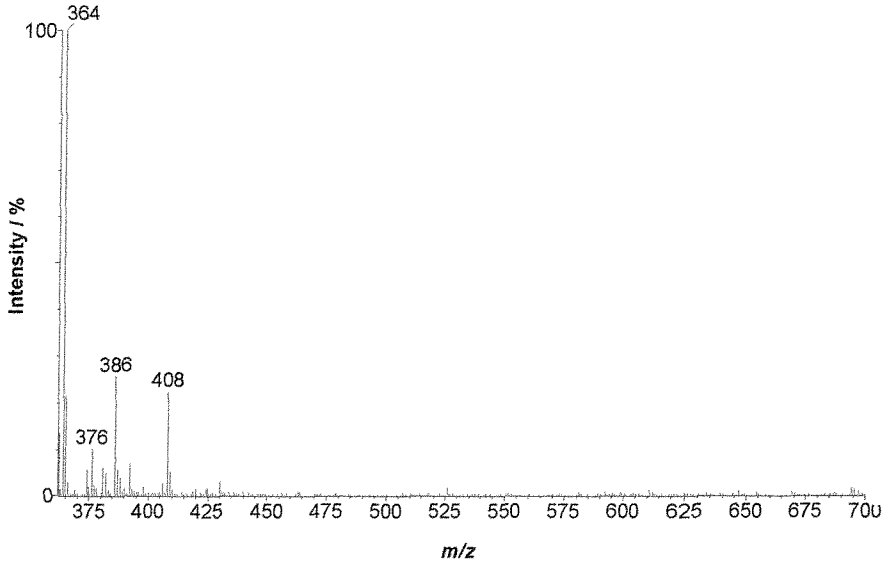


Figure 4.24 Mass spectrum of tetramine **(42)**

Tetramine **(43)** gave rise to two peaks at *m/z* 360 and 382 corresponding to $(M+H)^+$ and $(M+Na)^+$ respectively (Figure 4.25).

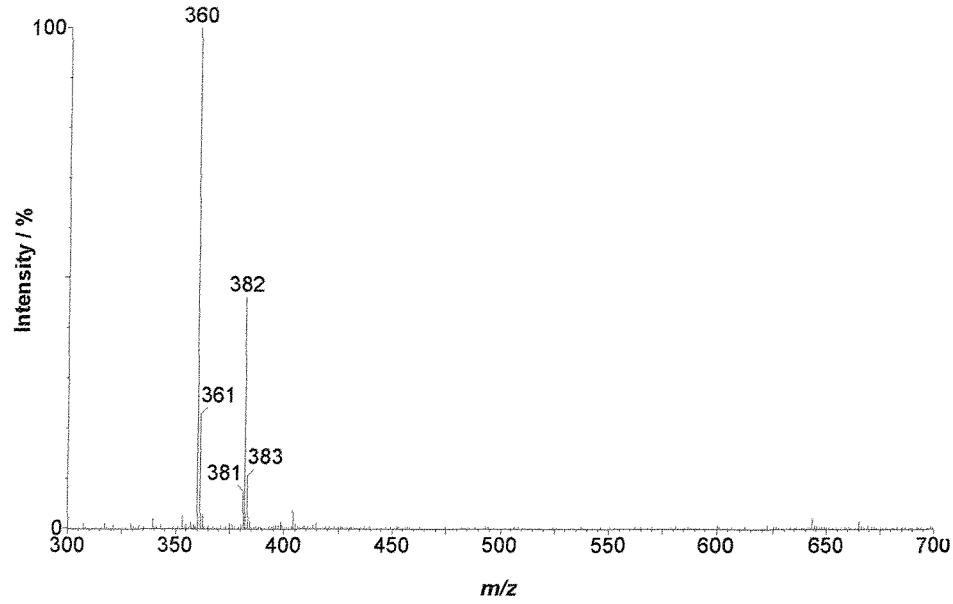


Figure 4.25 Mass spectrum of tetramine **(43)**

Similarly for tetramine **(51)**, peaks owing to $(M+H)^+$ and $(M+Na)^+$ appeared at *m/z* 420 and 442 respectively (Figure 4.26). In this series of tetramines no dimerisation was observed.

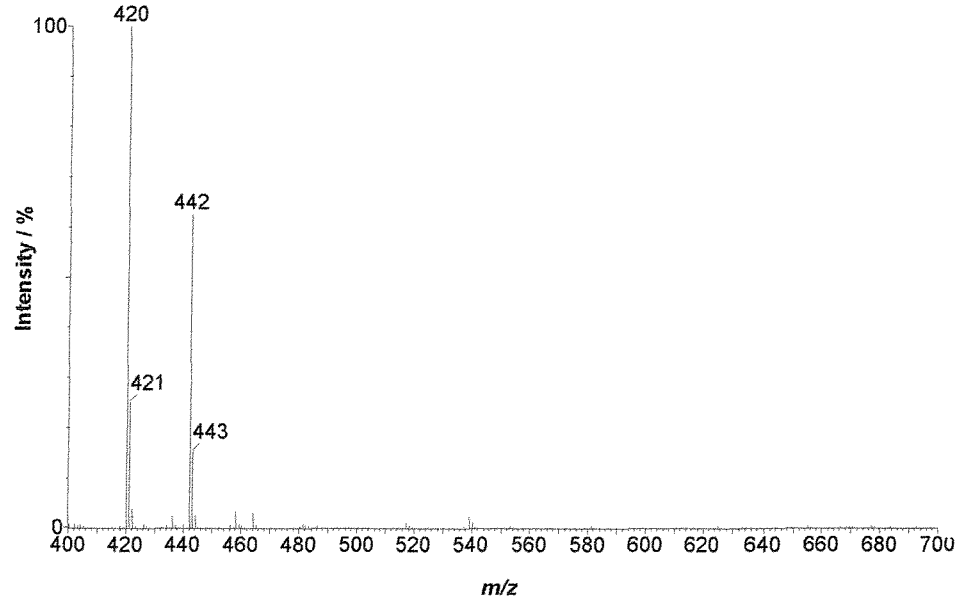


Figure 4.26 Mass spectrum of tetramine **(51)**

In order to determine which metals formed complexes with the tetramines **(42)**, **(43)** and **(51)** and the stoichiometry of these complexes, the effect on the mass spectrum of the addition of metal ions was observed. The general procedure used was the same as

in the naphthalene series. The effect of the metal ions fell into two distinct groups: those metals that gave rise to tetramine:metal complex peaks in the mass spectrum and those metals that resulted in no change in the mass spectrum.

4.3.2 Mass spectrometry in the presence of metals

4.3.2.1 Metals exhibiting no effect

The following metal ions led to no noticeable change in the mass spectrum of the tetramine to which they were added, even after the addition of 5 equivalents of the metal salt : Li(I), Na(I), Al(III), K(I), Ca(II), Fe(II), Co(II), Zn(II), Sn(II), Ag(I). The molecular ion $(M+H)^+$ and the peak due to $(M+Na)^+$ remained unchanged and no new peaks corresponding to $(M+metal)^+$ were observed.

As in the naphthalene series, although all three tetramines gave rise to a peak corresponding to $(M+Na)^+$, owing to the presence of background sodium ions, this peak was not observed to increase in size relative to the $(M+H)^+$ peak upon the addition of up to 5 equivalents of aqueous sodium chloride.

Thus it was concluded that these metals did not form complexes with the tetramines **(42)**, **(43)** and **(51)**, even in the presence of an excess of metal salt.

4.3.2.2 Metals exhibiting complex formation

For each of the following metals a peak due to metal complexation by the tetramine in question was observed: Ni (II), Cu (II), Pd (II). The extent of the effect of metal salt addition varied between the tetramines **(42)**, **(43)** and **(51)** and the metal salt which was added. Each metal will now be considered in turn.

Nickel (II) sulphate – The addition of just 0.5 equivalents of nickel (II) sulphate to tetramine **(42)** resulted in the appearance of a small $(M+Ni)^+$ peak at *m/z* 420, which grew steadily, resulting in an almost complete disappearance of the molecular ion after the addition of two equivalents of the metal salt (Figure 4.27).

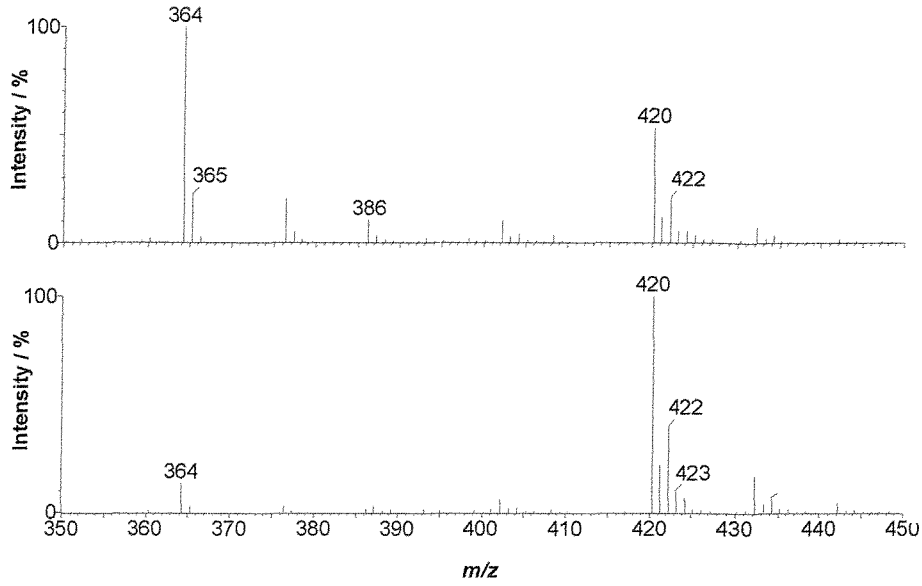


Figure 4.27 Top spectra: Tetramine **(42)** + 0.5 equivalents of Ni (II) ions
Bottom spectra: Tetramine **(42)** + 2 equivalents of Ni (II) ions

A peak corresponding to $(M+Ni)^+$ appeared after the addition of 1.5 equivalents of nickel (II) ions to tetramine **(43)** and the addition of 0.5 equivalents to tetramine **(51)**. Although in both cases the peaks, at *m/z* 416 and 476 respectively, grew in size upon the addition of further equivalents of nickel (II) ions, the molecular ion remained sizeable even after the addition of 5 equivalents of metal salt (Figures 4.28 and 4.29). In contrast to the naphthalene series, both the position and isotope pattern of the $(M+Ni)^+$ peaks were correct.

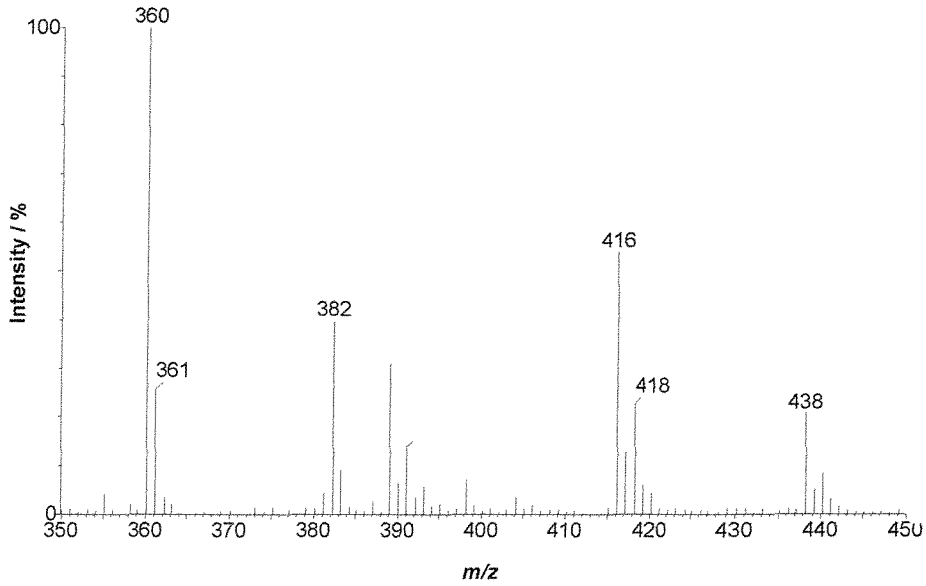


Figure 4.28 Tetramine **(43)** + 5 equivalents of Ni (II) ions

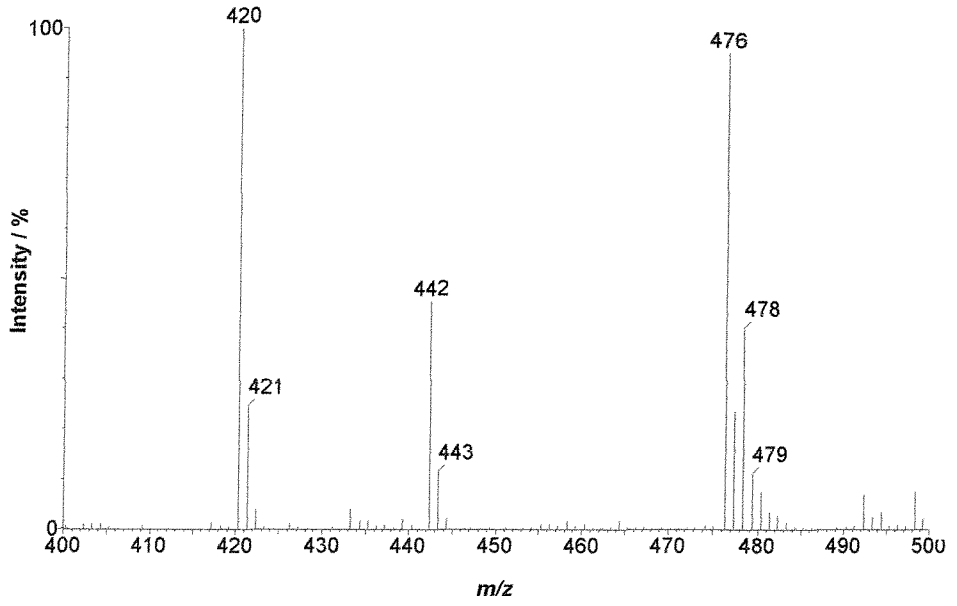


Figure 4.29 Tetramine **(51)** + 5 equivalents of Ni (II) ions

Palladium (II) chloride – The addition of 0.5 equivalents of palladium (II) chloride to tetramine **(42)** resulted in the appearance of a $(M+Pd)^+$ peak at *m/z* 468, with an almost complete disappearance of the molecular ion after the addition of 2 equivalents of metal salt (Figure 4.30).

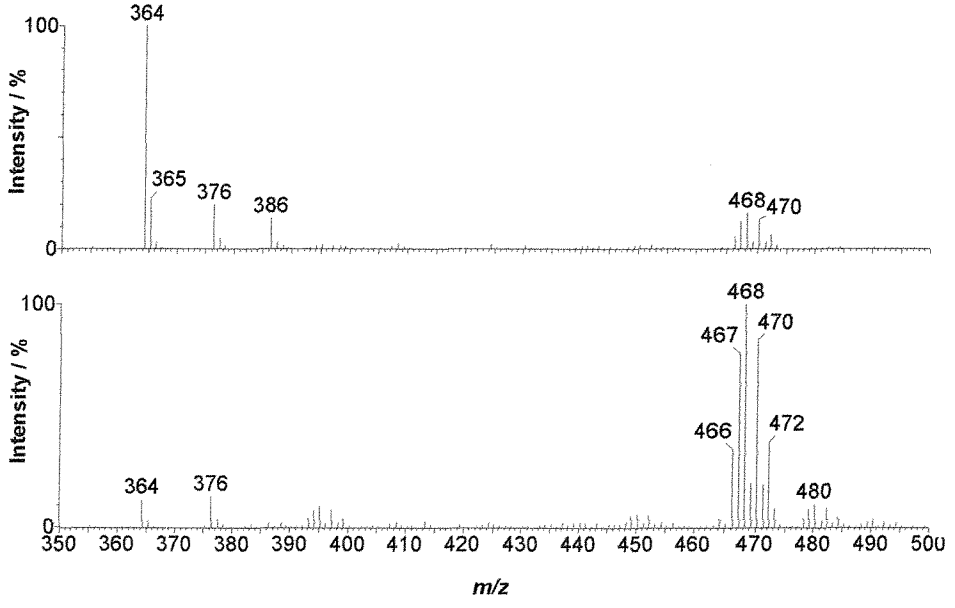


Figure 4.30 Top spectra: Tetramine **(42)** + 0.5 equivalents Pd (II) ions
Bottom spectra: Tetramine **(42)** +2 equivalents of Pd (II) ions

In the case of tetramines **(43)** and **(51)** small $(M+Pd)^+$ peaks were observed after the addition of 0.5 equivalents of palladium (II) ions, at *m/z* 464 and 424 respectively. The $(M+Pd)^+$ peak grew steadily upon the addition of further equivalents of palladium (II) ions but, in both cases, the molecular ions were still apparent after the addition of 5 equivalents of palladium (II) chloride (Figures 4.31 and 4.32).

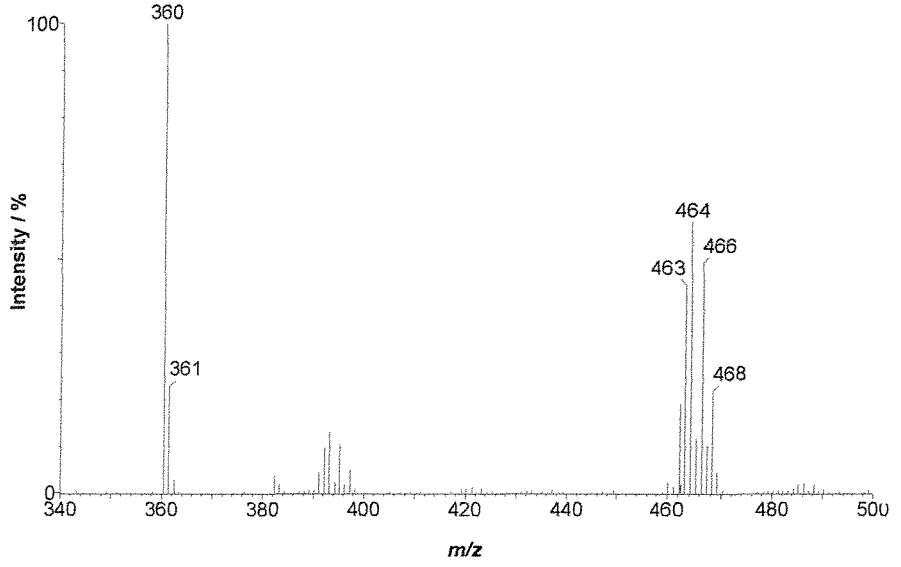


Figure 4.31 Tetramine **(43)** + 5 equivalents of Pd (II) ions

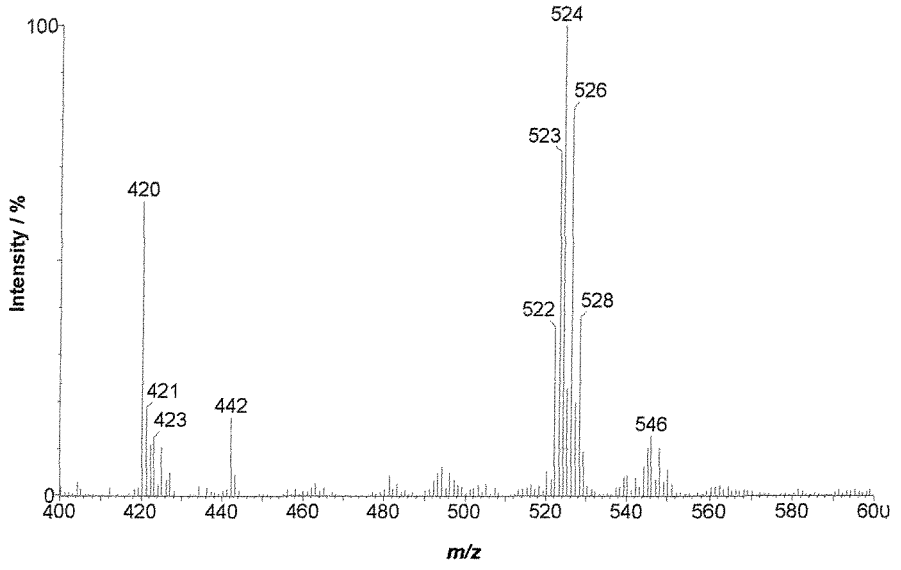


Figure 4.32 Tetramine **(51)** + 5 equivalents of Pd (II) ions

Copper (II) sulphate – The addition of just 0.5 equivalents of copper sulphate to all three tetramines **(42)**, **(43)** and **(51)** led to the appearance of an $(M+Cu)^+$ peak at *m/z*

425, 421 and 481 respectively. The molecular ion corresponding to tetramines **(42)** and **(51)** was observed to have almost completely disappeared after the addition of 1.5 equivalents of copper (II) sulphate (Figures 4.33 and 4.34).

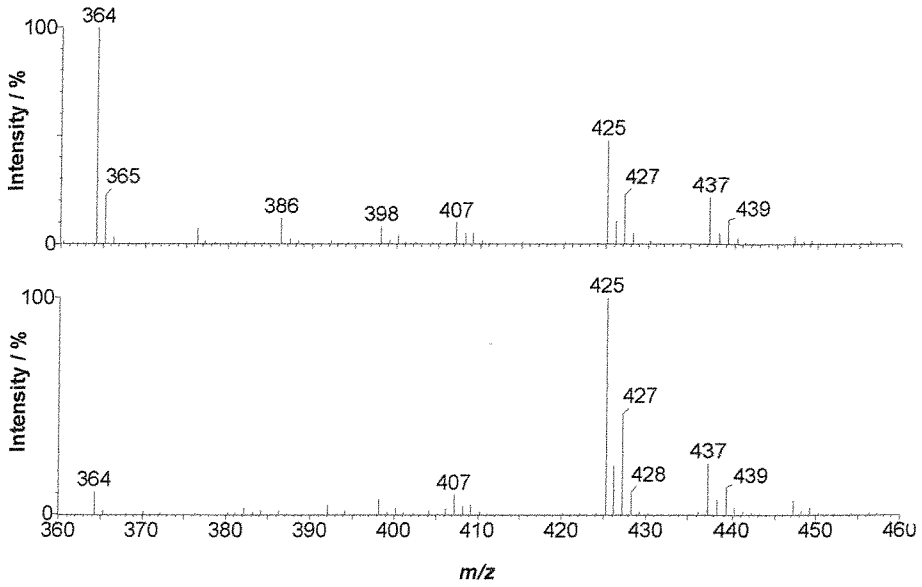


Figure 4.33 Top spectra: Tetramine **(42)** + 0.5 equivalents of Cu (II) ions
Bottom spectra: Tetramine **(42)** + 1.5 equivalents of Cu (II) ions

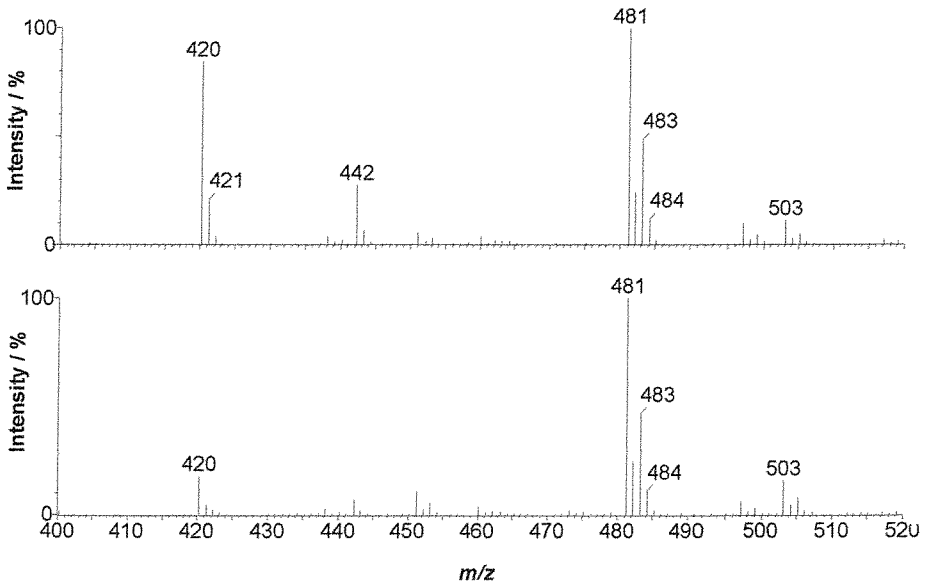


Figure 4.34 Top spectra: Tetramine **(51)** + 0.5 equivalents of Cu (II) ions
Bottom spectra: Tetramine **(51)** + 1.5 equivalents of Cu (II) ions

However the molecular ion due to tetramine **(43)** was still visible after the addition of 5 equivalents of copper (II) ions (Figure 4.35).

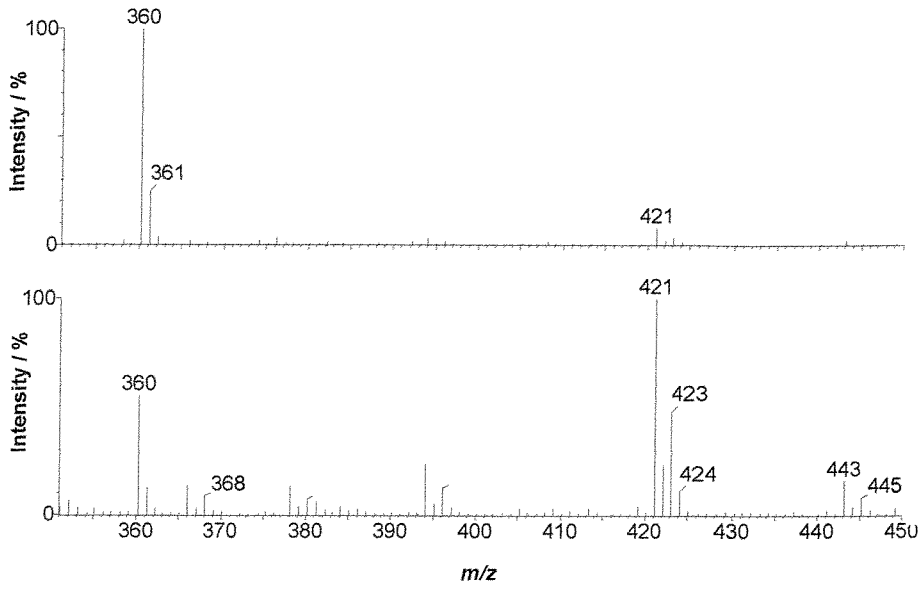


Figure 4.35 Top spectra: Tetramine **(43)** + 0.5 equivalents Cu (II) ions
Bottom spectra: Tetramine **(43)** + 1.5 equivalents of Cu (II) ions

A subsequent experiment was carried out in which 0.01, 0.1, 0.25 and 0.5 equivalents of copper (II) ions were added to each tetramine to determine whether 2:1 tetramine:metal complexes are formed at low metal ion concentrations. In each case no such complex was observed.

4.3.3 Conclusions

The extent of complex formation of **(42)**, **(43)** and **(51)** observed via mass spectrometry are as shown in Table 4.3 below.

Tetramine	Extent of Complex Formation
Tetramine **(42)**	Ni ~ Pd < Cu
Tetramine **(43)**	Ni ~ Pd < Cu
Tetramine **(51)**	Ni ~ Pd < Cu

Table 4.3 Relative extent of tetramine complex formation

All three tetramines are found to complex the same metals, namely palladium, nickel and copper, with a high affinity for copper in all cases. However the extent of metal binding varies between each tetramine. The butylamine tetramine **(42)** appears to complex metals most readily, with rapid disappearance of the molecular ion upon

metal binding relative to tetramines **(43)** and **(51)**. In contrast the pyrrolidine tetramine **(43)** appears to form complexes the least readily, the molecular ion remaining sizeable even after the addition of 5 equivalents of metal salt. The complexing power of the prolinol tetramine **(51)** appears to lie between that of the other two tetramines.

These results are very similar to the trends observed in the naphthalene series in terms of metal selectivities and the metal binding affinities of each tetramine. However metal complexing in the tetramines based upon pyridine appears unaffected by redox or acid effects with the isotope patterns of the $(M+Ni)^+$ and $(M+Pd)^+$ peaks appearing as expected. Furthermore only 1:1 metal:tetramine complexes are observed in the pyridine series and in general metal binding appears to occur to a lesser extent than in the naphthalene series. These differences in metal binding properties can be attributed to the differences between the size and shape of the binding pockets of the two series of tetramines.

4.4 Competitive Binding Studies

The butylamine tetramines in both the naphthalene series and the pyridine series, **(29)** and **(42)** respectively, had been found to have a relatively high affinity for both copper (II) and nickel (II) ions. Thus it was decided to use ESI-MS competitive binding experiments to determine whether tetramines **(29)** and **(42)** had a greater affinity for copper (II) or nickel(II) ions. Methanolic solutions of tetramines **(29)** and **(42)** were prepared and sufficient quantities of aqueous copper (II) sulphate and nickel (II) sulphate added to afford mixtures with a final tetramine:copper:nickel ratio of 1:50:50 in the case of tetramine **(29)** and 1:25:25 in the case of tetramine **(42)**. Each experiment was performed twice to allow the observation of the effect of adding copper ions first and then nickel ions, and *vice versa*. In both cases, the mixtures produced clean and intense spectra that contained only 1:1 tetramine:metal complexes. It is interesting to note that even at these high metal-to-tetramine ratios of 50:1 and 25:1, the formation of 1:2 tetramine:metal complexes were not observed,

indicating that the complexation of two metal ions by a single host does not occur with tetramines **(29)** and **(42)**.

The results of the competitive binding experiments are shown below (Figures 4.36 – 4.39). Note that the peak due to $(M+Cu)^+$ occurs at *m/z* 501 and 425 for tetramines **(29)** and **(42)** respectively and that the peak due to $(M+Ni)^+$ occurs at *m/z* 496 and 420 for tetramines **(29)** and **(42)** respectively.

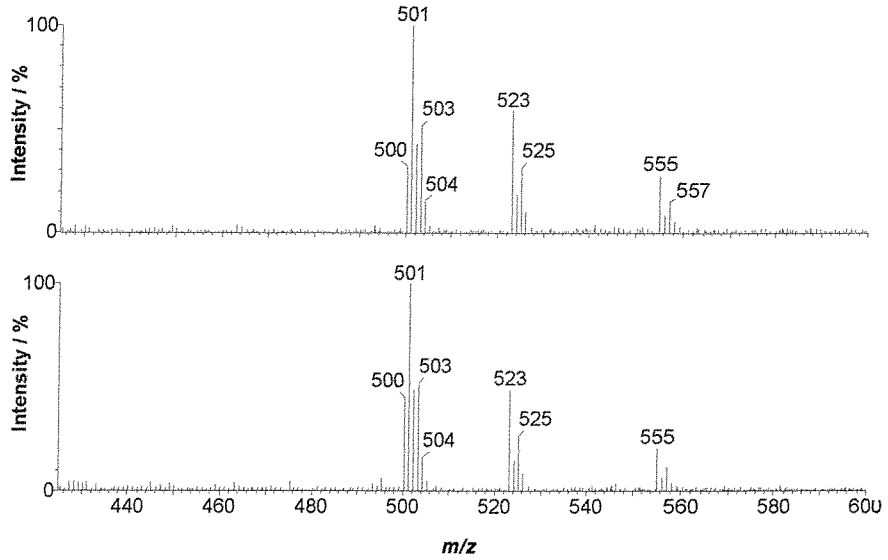


Figure 4.36 Top spectra: Addition of 50 equivalents of Cu (II) ions to tetramine **(29)** Bottom spectra: Subsequent addition of 50 equivalents of Ni (II) ions to tetramine **(29)**

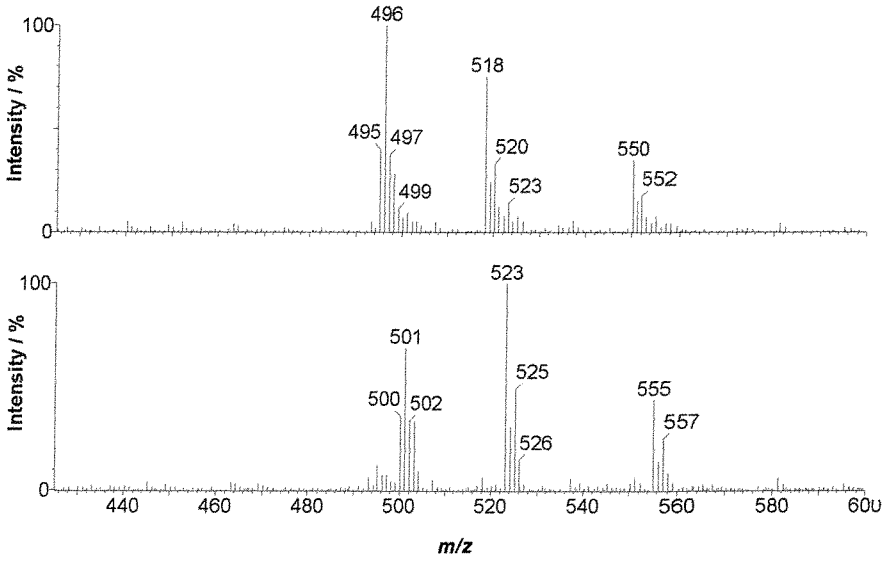


Figure 4.37 Top spectra: Addition of 50 equivalents of Ni (II) ions to tetramine **(29)** Bottom spectra: Subsequent addition of 50 equivalents of Cu (II) ions to tetramine **(29)**

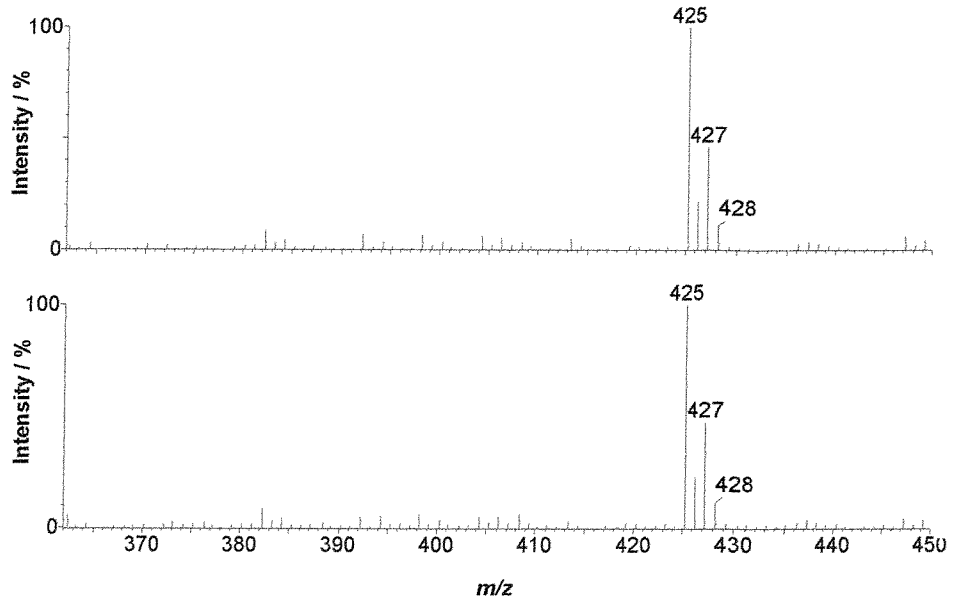


Figure 4.38 Top spectra: Addition of 25 equivalents of Cu (II) ions to tetramine **(42)**
Bottom spectra: Subsequent addition of 25 equivalents of Ni (II) ions to tetramine **(42)**

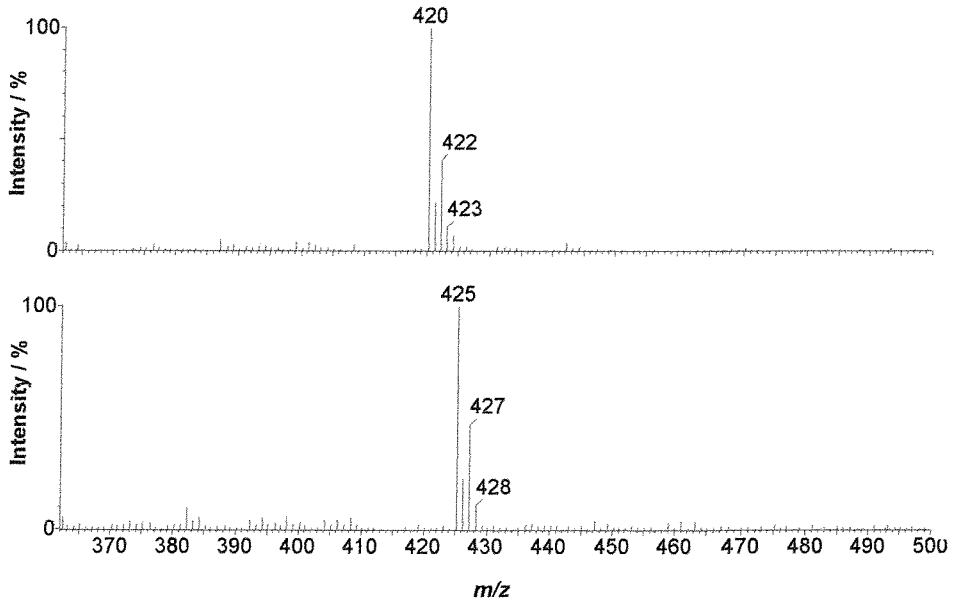


Figure 4.39 Top spectra: Addition of 25 equivalents of Ni (II) ions to tetramine **(42)**
Bottom spectra: Subsequent addition of 25 equivalents of Cu (II) ions to tetramine **(42)**

In both cases a significant preference for copper (II) ions is shown. The addition of nickel (II) ions, following the addition of copper (II) ions, resulted in no change in the mass spectra. However when nickel (II) ions were added first to tetramines **(29)** and

(42) a complete disappearance of the peak due to $(M+Ni)^+$ was observed, once copper (II) ions were added.

4.5 Crystallography

The most conclusive method available for determining the exact nature of metal complexes is X-ray crystallography. Preparation of crystals of the metal-free tetramines, in both the naphthalene and the pyridine series, proved difficult. The major problems encountered were the insolubility of the tetramines in a range of solvents and the difficulty in removing the excess of high boiling amines, used in the final step of the synthetic route to tetramines, resulting in oily solid products. Attempts to prepare tosyl derivatives of the tetramines, in order to encourage crystallisation, were also unsuccessful. Nevertheless, it was decided to prepare crystals of the copper and nickel complexes of the tetramines in both series. Several procedures were attempted employing different metal salts, including chlorides and acetates.[185,186] Although metal complexation appeared to have taken place, as observed by colour changes, it proved impossible to grow crystals of the metal complexes despite employing several different solvent systems as well as several different techniques for crystal growing.

4.6 Overall Conclusions

In both the naphthalene and pyridine series mass spectrometry has proved to be an effective means by which to examine metal binding by the tetramine derivatives. In the naphthalene series good agreement is observed between the results of fluorimetry and mass spectrometry. Furthermore mass spectrometry provides the additional benefit of permitting determination of the stoichiometries of the metal:tetramine complexes formed.

A shortcoming of both fluorimetry and mass spectrometry, however, is the inability to elucidate the exact nature of the bonds formed upon metal ion complexation. Only X-ray crystallography can provide the definitive answer to this question. Thus in both

series of tetramines the growth of crystals and the determination of crystal structures remains a key area to be investigated further.

Overall the results of the metal binding studies described within this chapter and in chapter 3 are most promising. A range of tetramines, in both the naphthalene and pyridine series, have shown the ability to bind metals and more importantly have exhibited a selectivity for certain metals over others, most notably all tetramines have shown a strong affinity for copper. Furthermore the metal binding affinities vary between the butylamine, pyrrolidine and prolinol tetramines in each series indicating that the metal binding properties of the tetramines can be determined by variation of the amine groups in the ligand. Further work is now required to determine the potential applications of these tetramines, based on their metal binding properties. Fluorescent sensing and catalysis of asymmetric synthesis stand out as the primary areas of focus.

Chapter Five

Experimental Details

Chapter 5
Experimental Details

5.1 General Procedures and Instrumentation

Thin Layer Chromatography

Thin layer chromatography was performed on Merck Aluminum TLC Sheets (Silica gel 60 F_{254}) and the compounds visualised by UV fluorescence.

Column Chromatography

Column chromatography was performed on Merck Silica gel 60 (0.040-0.063mm).

Melting Points

All melting points were determined in open capillary tubes using a Gallenkamp Electrothermal Melting Point Apparatus.

Elemental Combustion Analyses

Combustion analyses were performed at University College, London.

Ultra Violet/Visible Spectra

Ultra-Violet and Visible spectral measurements were made on a Perkin Elmer UV/VIS Lambda 2 Spectrometer. The solvent was blanked for any absorbance in the region of interest prior to investigation.

Infra-Red Spectra

Infra-red spectra were recorded using a Nicolet Impact 400 IR spectrometer fitted with a Spectratech Thunderdome Accessory. Samples were run neat (solids or oils) in the range 4000-600 cm^{-1}. Absorption maxima are quoted with the following notation: s-strong, m-medium, and w-weak.

Nuclear Magnetic Resonance Spectra

Nuclear magnetic resonance spectra were recorded on Brucker AC300 and Brucker AM300 spectrometers. The operating frequency is given in brackets. Chemical shifts are quoted as δ-values relative to tetramethylsilane and calibrated to residual $CHCl_3$, DMSO or TMS as an internal standard. The resonances are described as follows: s-singlet, d-doublet, t-triplet, q-quartet, br-broad, m-multiplet.
COSY and C-H correlation experiments were performed on Brucker AC 300 and Brucker AM 360 spectrometers.

Mass Spectrometry

Mass spectrometry experiments were performed on the following instruments:

(i) VG Platform single quadrupole mass spectrometer configured for open access operation (OAMS).
Ionisation techniques: Electrospray (ES), Atmospheric pressure Chemical Ionisation (APCI). HP1050 quaternary pump HPLC system for OAMS and on-line HPLC-MS.

(ii) Waters ZMD single quadrupole mass spectrometer configured for open access operation (OAMS).
Ionisation techniques: Electrospray (ES), Atmospheric pressure Chemical Ionisation (APCI). Waters 2700 autosampler, quaternary pump HPLC system for OAMS and on-line HPLC-MS.

(iii) VG 70-SE Normal geometry double focusing mass spectrometer
Ionisation techniques: Electron Ionisation(EI), Chemical Ionisation (CI) and Fast Atom Bombardment (FAB). Low and high resolution analyses. Direct insertion probe and capillary column GC-MS.

In the absence of microanalysis high resolution mass spectrometry was employed as the test of purity, all products being homogeneous in nature as checked by TLC.

Fluorimetry

Fluorescence experiments were performed on a Perkin Elmer LS50B Luminescence Spectrometer and are uncorrected (observation wavelength = 520nm, excitation wavelength = 450nm).

Solvent Purification

Dry pyridine and dichloromethane were distilled from calcium hydride.

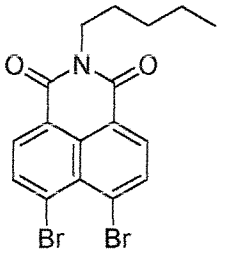
Dry tetrahydrofuran and diethyl ether were distilled from a mixture of sodium and benzophenone.

Dry methanol was distilled from magnesium turnings.

Dry N,N-dimethylformamide was stirred over calcium hydride for 2 hours and fractionally distilled.

5.2 Preparation of Compounds in the Naphthalene Series

6,7-Dibromo-2-pentyl-2,3-dihydro-1*H*-benzo[*de*]isoquinoline-1,3-dione (14)



(14)

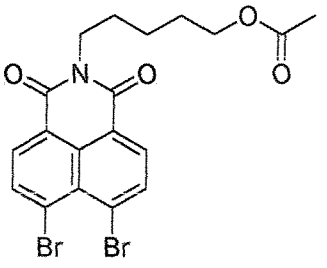
4,5-Dibromo-1,8-naphthalic anhydride **(13)** (2.00g, 5.61mmol) was suspended in *N*-methylpyrrolidinone (20mL) and acetic acid (20 mL). Zinc acetate (5g, 22mmol) and *N*-pentylamine (3g, 34mmol) were added and the reaction mixture was heated at 140°C for 12h. The mixture was poured into cold water (200mL) and the solid was separated by filtration. The solid was dissolved in dichloromethane, filtered and the solvent was evaporated *in vacuo*. The resulting solid was purified by column chromatography on silica gel [eluant petroleum ether / diethylether (9:1)]. Recrystallisation from ethanol afforded the title product as a beige, crystalline solid. Yield 0.84g (35%).

Mp 160-161 °C (lit. 162-163°C)[2]

δ_H (300 MHz ; $CDCl_3$) 0.91 (t, 3H, J 7.0, CH_3), 1.34-1.41 (m, 4H, CH_2), 1.65 (q, 2H, J 7.35, CH_2), 4.09 (t, 2H, J 7.5, CH_2N), 8.17 (d, 2H, J 8.1, ArH), 8.36 (d, 2H, J 8.1, ArH)

δ_C (75 MHz ; $CDCl_3$) 14.15 (CH_3), 22.54 (CH_2), 27.75 (CH_2), 29.34 (CH_2), 40.85 (CH_2N), 123.14 (ArC), 127.60 (ArC), 128.11 (ArC), 131.12 (ArC), 131.51 (ArCH), 136.20 (ArCH), 163.20 (CO)

6,7-Dibromo-2-(5-acetoxypentyl)-2,3-dihydro-1*H*-benzo[*de*]isoquinoline-1,3-dione (15)



(15)

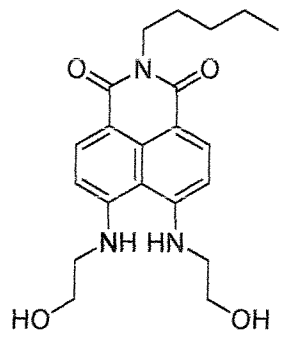
4,5-Dibromo-1,8-naphthalic anhydride **(13)** (2.00g, 5.61mmol) was suspended in *N*-methylpyrrolidinone (10mL) and acetic acid (20mL). 5-Aminopentanol (5g, 9.7mmol) and zinc acetate (5g, 22mmol) were added and the reaction mixture was heated at 140°C for 12h. The reaction mixture was poured into water (200mL) and the solid separated by filtration. The solid was dissolved in dichloromethane, filtered and the solvent removed *in vacuo*. The resulting solid was purified by column chromatography on silica gel [eluant petroleum ether / ethyl acetate (9:1)]. Recrystallisation from ethanol afforded the title product as a beige, crystalline solid. Yield 0.27g (12%).

Mp 130-133 °C (lit. 123-126°C)[2]

δ_H (300 MHz ; $CDCl_3$) 1.40-1.51 (m, 2H, CH_2), 1.64-1.79 (m, 4H, CH_2), 2.02 (s, 3H, CH_3CO), 4.05 (t, 2H, J 6.4, CH_2O), 4.13 (t, 2H, J 7.5, CH_2N), 8.18 (d, 2H, J 8.1, ArH), 8.37 (d, 2H, J 8.1, ArH)

δ_C (75 MHz ; $CDCl_3$) 21.18 (CH_3), 23.65 (CH_2), 27.71 (CH_2), 28.45 (CH_2), 40.56 (CH_2N), 64.45 (CH_2O), 123.10 (ArC), 127.74 (ArC), 128.30 (ArC), 131.21 (ArC), 131.60 (ArCH), 136.27 (ArCH), 163.23 (Imide CO), 171.35 (Acyl CO)

6,7-Di[(2-hydroxyethyl)amino]-2-pentyl-2,3-dihydro-1*H*-benzo[*de*]isoquinoline-1,3-dione (16)



(16)

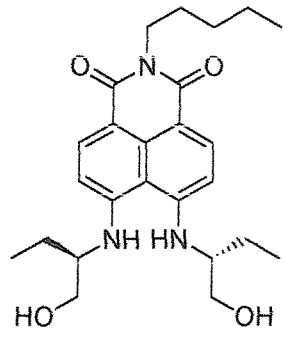
6,7-Dibromo-2-pentyl-2,3-dihydro-1*H*-benzo[*de*]isoquinoline-1,3-dione **(14)** (0.20g, 0.47mmol) was dissolved in collidine (2.5mL) and ethanolamine (0.54g, 8.8mmol) was added. The reaction mixture was heated under reflux, with stirring, under a nitrogen atmosphere for 6h. The solvent was removed as an azeotrope with water *in vacuo*. The resulting orange solid was purified by column chromatography on silica gel [eluant ethyl acetate / methanol (9.75:0.25)]. Recrystallisation from ethyl acetate afforded the title product as a yellow, crystalline solid. Yield 0.10g (55%).

Mp 196-198°C (lit. 203-204°C)[2]

δ_H (300 MHz ; DMSO) 0.97 (t, 3H, J 6.8, CH_3), 1.36-1.46 (m, 4H, CH_2), 1.69-1.73 (m, 2H, CH_2), 3.41-3.46 (q, 4H, CH_2N), 3.82-3.88 (q, 4H, CH_2O), 4.07 (t, 2H, J 7.5, CH_2N), 5.08 (t, 2H, J 5.7, OH), 6.93 (d, 2H, J 8.5, ArH), 7.36 (bs, 2H, NH), 8.29 (d, 2H, J 8.5, ArH)

δ_C (75 MHz ; DMSO) 13.97 (CH_3), 21.97 (CH_2), 27.47 (CH_2), 28.85 (CH_2), 38.95 (CH_2N), 46.31 (CH_2N), 58.99 (CH_2O), 106.64 (ArCH), 109.65 (ArC), 110.26 (ArC), 131.97 (ArC), 133.20 (ArCH), 153.08 (ArCN), 163.38 (CO)

6,7-Di{[(1R)-1-(hydroxymethyl)propyl]amino}-2-pentyl-2,3-dihydro-1*H*-benzo[*de*]isoquinoline-1,3-dione (17)



(17)

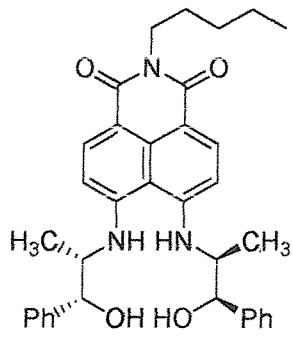
6,7-Dibromo-2-pentyl-2,3-dihydro-1*H*-benzo[*de*]isoquinoline-1,3-dione **(17)** (0.20g, 0.47mmol) was suspended in collidine (3mL) and (R)-(-)-2-aminobutanol (0.22g, 2.5mmol) was added. The reaction mixture was heated under reflux, with stirring, under a nitrogen atmosphere for 25h. The reaction mixture was poured into dilute hydrochloric acid (25mL) and extracted with ethyl acetate (3x25mL). The combined extracts were dried over magnesium sulphate and the solvent was evaporated *in vacuo*. The product was purified by column chromatography on silica gel [eluant ethyl acetate / petroleum ether (3:1)]. Recrystallisation from ethyl acetate afforded the title product as a yellow, crystalline solid. Yield 0.13g (63%).

Mp 166-168°C (lit. 168-170°C)[2]

δ_H (300 MHz ; DMSO) 0.97 (t, 3H, J 6.8, pentyl CH_3), 1.05 (t, 6H, J 7.0, butyl CH_3), 1.36-1.46 (m, 4H, CH_2), 1.64-1.84 (m, 6H, 2xbutyl CH_2 + pentyl CH_2), 3.50-3.56 (m, 2H, C<u>H</u>NH), 3.63-3.68 (m, 4H, CH_2O), 3.93 (t, 2H, J 7.9, CH_2N), 5.14 (t, 2H, J 5.0, OH), 7.00 (d, 2H, J 8.5, ArCH), 7.06 (2H, d, J 8.5, NH), 8.28 (d, 2H, J 8.5, ArCH)

δ_C (75 MHz ; DMSO) 10.53 (CH_3), 13.97 (CH_3), 22.01 (CH_2), 23.61 (CH_2), 27.52 (CH_2), 28.85 (CH_2), 56.30 (CH), 61.51 (CH_2N), 106.94 (ArCH), 109.15 (ArC), 109.96 (ArC), 132.38 (ArC), 133.09 (ArCH), 152.57 (ArCN), 163.35 (CO)

6,7-Di{[(1S,2R)-2-hydroxy-1-methyl-2-phenylethyl]amino}-2-pentyl-2,3-dihydro-1*H*-benzo[*de*]isoquinoline-1,3-dione (18)



(18)

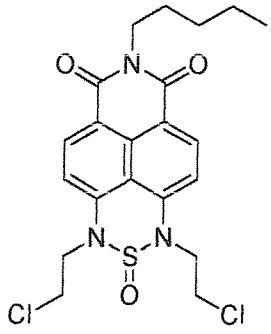
6,7-Dibromo-2-pentyl-2,3-dihydro-1*H*-benzo[*de*]isoquinoline-1,3-dione **(14)** (0.10g, 0.24mmol) and (1R,2S)-2-amino-1-phenylpropan-1-ol (0.40g, 2.6mmol) were suspended in collidine (3mL) and heated under reflux, with stirring, under a nitrogen atmosphere for 23h. The reaction mixture was poured into dilute hydrochloric acid (25mL) and extracted with ethyl acetate (3x25mL). The combined extracts were dried over magnesium sulphate and the solvent was evaporated *in vacuo*. Purification by column chromatography on silica gel [eluant petroleum ether / ethyl acetate (4:1)] afforded the title product as a dark orange solid. Yield 0.08g (59%).

Mp 104-106°C (lit. 108-112°C)[2]

δ_H (300 MHz ; $CDCl_3$) 0.80 (t, 3H, J 6.3, pentyl CH_3), 0.98 (d, 6H, J 6.6, ephedrine CH_3), 1.11-1.26 (m, 4H, CH_2), 1.51-1.58 (m, 2H, CH_2), 3.85-3.94 (m, 4H, CH_2N + 2xCHN), 5.24 (d, 2H, J 1.8, CHO), 6.60 (d, 2H, J 8.5, ArH), 6.70 (d, 2H, J 9.2, NH), 7.17 (t, 2H, J 7.2, *p*-Ar'H), 7.26 (t, 4H, J 7.4, *m*-Ar'H), 7.32 (d, 4H, J 7.0, *o*-Ar'H), 8.21 (d, 2H, J 8.5, ArH)

δ_C (75 MHz ; $CDCl_3$) 11.26 (pentyl CH_3), 14.20 (ephedrine CH_3), 22.63 (CH_2), 27.97 (CH_2), 29.44 (CH_2), 40.14 (CH_2N), 54.38 (CH), 75.48 (CH), 107.12 (ArCH), 111.15 (ArC), 125.75 (ArCH), 127.77 (ArCH), 128.59 (ArCH), 132.94 (ArC), 133.95 (ArCH), 141.32 (ArC), 151.52 (ArCN), 164.79 (CO)

1,3-Di(2-chloroethyl)-7-pentyl-1,2,3,6,7,8-hexahydro-2λ^4-[2,1,3]benzothiadiazino [6,5,4-*def*]isoquinoline-2,6,8-trione (19)



(19)

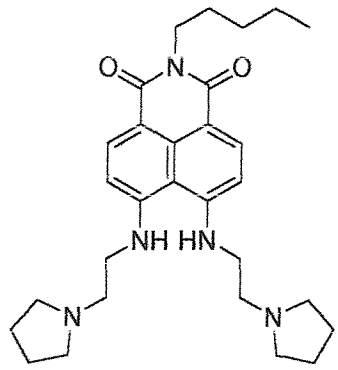
6,7-Di[(2-hydroxyethyl)amino]-2-pentyl-2,3-dihydro-1*H*-benzo[*de*]isoquinoline-1,3-dione **(16)** (0.50g, 0.13mmol) was suspended in chloroform (1mL) and DMF (1 drop) and thionyl chloride (1mL) was added dropwise with stirring. The reaction mixture was stirred overnight and then heated under reflux, with stirring, under a nitrogen atmosphere, for 3h. The reaction mixture was evaporated *in vacuo* and the resulting solid was dissolved in ethyl acetate (50mL) / water (50mL) and the aqueous solution was extracted with ethyl acetate (3x50mL). The combined extracts were dried over magnesium sulphate and the solvent was evaporated *in vacuo*. The product was purified by column chromatography on silica gel (eluant ethyl acetate / dichloromethane (19:1)). Recrystallisation from ethyl acetate afforded the title product as a yellow-green, crystalline solid. Yield 0.04g (66%).

Mp 218-220°C (lit. 214-216°C)[2]

δ_H (300 MHz ; $CDCl_3$) 0.89 (t, 3H, J 7.0, CH_3), 1.35-1.40 (m, 4H, CH_2), 1.65-1.77 (m, 2H, CH_2), 3.84 (dd, 4H, J 7.2, J 5.7, CH_2Cl), 4.06-4.20 (m, 4H, 2xCHN + CH_2N), 4.45 (dt, 2H, J 10.1, J 5.3, CH_2N), 7.01 (d, 2H, J 8.5, ArH), 8.58 (d, 2H, J 8.5, ArH)

δ_C (75 MHz ; $CDCl_3$) 14.18 (CH_3), 22.62 (CH_2), 27.92 (CH_2), 29.40 (CH_2), 40.09 (CH_2), 40.50 (CH_2), 50.70 (CH_2), 108.74 (ArCH), 113.86 (ArC), 116.38 (ArC), 130.70 (ArC), 133.79 (ArCH), 138.08 (ArCN), 163.74 (CO)

2-Pentyl-6,7-di[(2-tetrahydro-1*H*-1-pyrrolylethyl)amino]-2,3-dihydro-1*H*-benzo[*de*]isoquinoline-1,3-dione (20)



(20)

1,3-Di(2-chloroethyl)-7-pentyl-1,2,3,6,7,8-hexahydro-2λ^4-[2,1,3]benzothiadiazino [6,5,4-*def*]isoquinoline-2,6,8-trione **(19)** (0.04g, 0.09mmol) was dissolved in pyrrolidine (1.5mL, 17.3mmol) and refluxed, with stirring, under a nitrogen atmosphere, for 1h. The reaction mixture was evaporated *in vacuo* and the resulting solid was dissolved in dichloromethane (25mL). The organic layer was washed with potassium carbonate solution (1M, 25mL) and extracted with dichloromethane (3x25mL). The combined extracts were dried over magnesium sulphate and the solvent was evaporated *in vacuo*. The product was purification by column chromatography on silica gel [eluant ethyl acetate]. Recrystallisation from ethyl acetate afforded the title product as an orange solid. Yield 0.04g (90%).

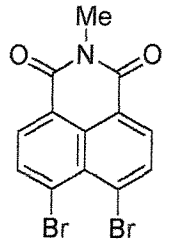
Mp 174-176°C (lit. 176-178°C)[2]

δ_H (300 MHz ; $CDCl_3$) 0.90 (t, 3H, J 7.0, CH_3), 1.35-1.43 (m, 4H, CH_2), 1.76-1.85 (8H, br s, 8H, CH_2), 2.58-2.69 (br s, 8H, CH_2), 2.90 (t, 4H, J 5.9, CH_2N), 3.34-3.40 (br s, 4H, $C\underline{H}_2NH$), 4.12 (t, 2H, J 7.5, CH_2N), 6.70 (d, 2H, J 8.5, ArH), 6.72-6.75 (br s, 2H, NH), 8.42 (d, 2H, J 8.5, ArH)

δ_C (75 MHz ; $CDCl_3$) 14.20 (CH_3), 22.69 (CH_2), 23.78 (CH_2), 28.80 (CH_2), 29.51 (CH_2), 40.12 (CH_2N), 43.07 (CH_2N), 53.97 (CH_2N), 54.24 (CH_2N), 106.57 (ArCH),

111.32 (ArC), 111.71 (ArC), 132.43 (ArC), 133.83 (ArCH), 153.01 (ArCN), 164.83 (CO)

6,7-Dibromo-2-methyl-2,3-dihydro-1*H*-benzo[*de*]isoquinoline-1,3-dione (21)



(21)

4,5-Dibromo-1,8-naphthalic anhydride **(13)** (5.0g, 14mmol) was suspended in ethanol (120mL). Methylamine (6.0mL, 70mmol) was added and the reaction mixture was stirred at room temperature for 11 days. The reaction mixture was then filtered, the residue was washed with dichloromethane and the solvent was evaporated *in vacuo*.

Purification 1 (for preparations on a 5g scale or lower)
The resulting solid was purified by column chromatography on silica gel [eluant dichloromethane]. Recrystallisation from ethyl acetate afforded the title product as a pale brown solid. Yield 1.8g (39%).

Purification 2 (for preparations over a 5g scale)
The resulting solid was dissolved in dichloromethane (500mL) and stirred over silica gel (25g) for 2h. The reaction mixture was filtered, the residue was washed with dichloromethane and the solvent evaporated *in vacuo*. Recrystallisation from ethyl acetate afforded the title product as pale brown solid. Yield 3.1g (60%).

Mp 248-250°C

δ_H (300 MHz ; $CDCl_3$) 3.52 (s, 3H, CH_3N), 8.17 (d, 2H, J 8.1, ArH), 8.36 (d, 2H, J 8.1, ArH)

δ_C (75 MHz ; $CDCl_3$) 27.29 (CH_3N), 123.14 (ArC), 128.39 (ArC), 131.25 (ArC), 131.62 (ArCH), 136.32 (ArCH), 163.55 (CO)

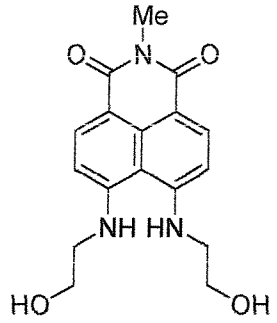
ν_{max} (solid) / cm^{-1} 1698.25 (C=O), 1654.73 (C=O), 1588.77 (s), 1551.92 (s), 1027.97 (s), 801.83 (s), 731.70 (s)

LRMS (APCI+): 370 (MH^+)

HRMS (EI+): 366.8840 ($C_{13}H_7Br_2NO_2$ (M^+) requires 366.8844)

Microanalysis: Found C=42.67, H=1.68, N=3.60% requires C=42.31, H=1.91, N=3.80%

6,7-Di[(2-hydroxyethyl)amino]-2-methyl-2,3-dihydro-1*H*-benzo[*de*]isoquinoline-1,3-dione (24)



(24)

6,7-Dibromo-2-methyl-2,3-dihydro-1*H*-benzo[*de*]isoquinoline-1,3-dione **(21)** (1.6g, 4.3mmol) was dissolved in ethanol (45mL) and ethanolamine (4.9g, 80mmol) was added. The reaction mixture was heated under reflux, with stirring, for 72h. The reaction mixture was filtered, the solid residue was washed with dichloromethane affording the title product as a yellow, crystalline solid. Yield 1.15g (81%).

Mp 286-288°C

δ_H (300 MHz ; DMSO) 3.43-3.50 (m, 4H, CH_2N), 3.50 (s, 3H, CH_3N), 3.73 (q, 4H, J 5.5, CH_2O), 5.10 (t, 2H, J 5.3, OH), 6.91 (d, 2H, J 8.1, ArH), 7.32 (br s, 2H, NH), 8.29 (d, 2H, J 8.1, ArH)

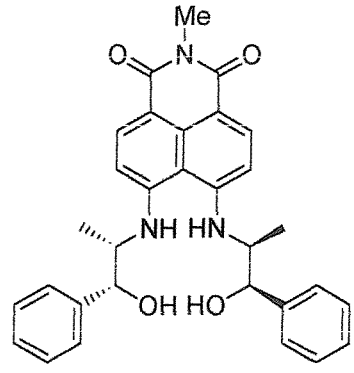
δ_C (75 MHz ; DMSO) 26.24 (CH_3N), 46.27 (CH_2N), 59.05 (CH_2O), 106.59 (ArCH), 109.69 (ArC), 110.28 (ArC), 131.83 (ArC), 133.13 (ArCH), 153.04 (ArCN), 163.67 (CO)

ν_{max} (solid) / cm^{-1} 3446.17 (NH), 3338.65 (NH), 2952.47 (CH), 1662.73 (C=O), 1609.63 (C=O), 1417.33 (m), 1360.21 (m), 1107.82 (m), 1056.20 (m), 809.32 (s), 746.12 (s)

LRMS (APCI+): 330 (MH^+)

HRMS (EI+): 329.1367 ($C_{17}H_{19}N_3O_4$ (M^+) requires 329.1376)

6,7-Di{[(1S,2R)-2-hydroxy-1-methyl-2-phenylethyl]amino}-2-methyl-2,3-dihydro-1*H*-benzo[*de*]isoquinoline-1,3-dione (25)



(25)

6,7-Dibromo-2-methyl-2,3-dihydro-1*H*-benzo[*de*]isoquinoline-1,3-dione **(21)** (0.10g, 0.27mmol) and (1R,2S)-2-amino-1-phenylpropan-1-ol (0.20g, 1.35mmol) were suspended in collidine (1.5mL) and heated under reflux, with stirring, under a nitrogen atmosphere for 24h. The reaction mixture was poured into dilute hydrochloric acid (25mL) and the aqueous layer was extracted with ethyl acetate

(3x25mL). The combined extracts were dried over magnesium sulphate and the solvent was evaporated *in vacuo*. Purification by column chromatography on silica gel [eluant dichloromethane] afforded the title product as a dark orange oil. Yield 0.08g (62%).

δ_H (300 MHz ; $CDCl_3$) 1.06 (d, 6H, J 6.6, ephedrine CH_3), 3.18 (s, 3H, CH_3N), 3.89-3.91 (m, 2H, C<u>H</u>NH), 4.65-4.68 (br s, 2H, OH), 5.21-5.24 (br s, 2H, C<u>H</u>OH), 6.54 (d, 2H, J 8.5, ArH), 6.74 (d, 2H, J 8.8, NH), 7.21 (t, 2H, J 7.2, *p*-Ar<u>H</u>), 7.31 (t, 4H, J 7.4, *m*-Ar<u>H</u>), 7.40 (d, 4H, J 7.0, *o*-Ar<u>H</u>), 8.05 (d, 2H, J 8.5, ArH)

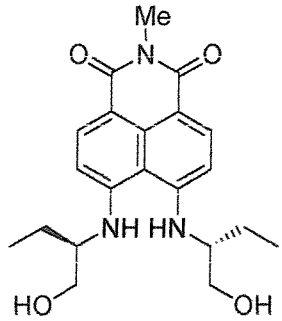
δ_C (75 MHz ; $CDCl_3$) 11.54 (CH_3), 26.67 (CH_3N), 54.39 (CHN), 75.20 (CHO), 107.04 (ArCH), 110.70 (ArC), 110.93 (ArC), 127.80 (ArCH), 128.63 (ArCH), 132.75 (ArC), 133.91 (ArCH), 141.40 (ArC), 151.60 (ArCN), 164.99 (CO)

ν_{max} (oil) / cm^{-1} 3460.17 (NH), 3342.82 (NH), 1616.10 (C=O), 1575.01 (C=O), 1408.12 (s), 1359.81 (m), 1292.71 (m), 744.60 (s)

LRMS (ES+): 510 (MH^+), 1019 ($2MH^+$)

HRMS (FAB+): 510.2389 ($C_{31}H_{32}N_3O_4$ (MH^+) requires 510.2393)

6,7-Di{[(1R)-1-(hydroxymethyl)propyl]amino}-2-methyl-2,3-dihydro-1*H*-benzo[*de*]isoquinoline-1,3-dione (26)



(26)

6,7-Dibromo-2-methyl-2,3-dihydro-1*H*-benzo[*de*]isoquinoline-1,3-dione **(21)** (0.10g, 0.3mmol) was suspended in collidine (3mL) and (R)-(-)-2-aminobutanol (0.12g, 1.3mmol) added. The reaction mixture was heated under reflux, with stirring, under a nitrogen atmosphere for 25h. The reaction mixture was poured into dilute hydrochloric acid (25mL) and the aqueous layer was extracted with ethyl acetate (3x25mL). The combined extracts were dried over magnesium sulphate and the solvent was evaporated *in vacuo*. The product was purified by column chromatography on silica gel [eluant dichloromethane]. Recrystallisation from ethyl acetate afforded the title product as a yellow, crystalline solid. Yield 0.03g (27%).

Mp 182-184°C

δ_H (300 MHz ; DMSO) 1.12 (t, 6H, J 6.8, butyl CH_3), 1.73-1.93 (m, 4H, CH_2), 3.52 (s, 3H, CH_3N), 3.74-3.78 (m, 2H, CHNH), 3.85-3.88 (m, 4H, CH_2O), 5.09-5.39 (br s, 2H, OH), 7.09 (d, 2H, J 8.8, ArCH), 7.17 (2H, d, J 7.4, NH), 8.38 (d, 2H, J 8.8, ArCH)

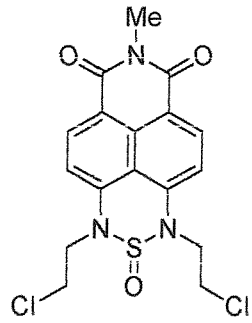
δ_C (75 MHz ; DMSO) 10.53 (CH_3), 23.61 (CH_2), 26.23 (CH_3N), 56.25 (CH), 61.47 (CH_2O), 106.95 (ArCH), 109.06 (ArC), 109.94 (ArC), 132.28 (ArC), 133.07 (ArCH), 152.62 (ArCN), 163.67 (CO)

ν_{max} (solid) / cm^{-1} 3364.38 (NH), 3336.92 (NH), 2960.56 (CH), 2939.12 (CH), 1620.28 (C=O), 1582.50 (C=O), 1410.80 (s), 1286.23 (m), 1048.95 (m), 809.77 (s), 747.79 (s)

LRMS (APCI+): 386 (MH^+)

HRMS (FAB+): 386.2064 ($C_{21}H_{28}N_3O_4$ (MH^+) requires 386.2080)

1,3-Di(2-chloroethyl)-7-methyl-1,2,3,6,7,8-hexahydro-2λ^4-[2,1,3]benzothiadiazino [6,5,4-*def*]isoquinoline-2,6,8-trione (27)



(27)

6,7-Di[(2-hydroxyethyl)amino]-2-methyl-2,3-dihydro-1*H*-benzo[*de*]isoquinoline-1,3-dione **(24)** (0.1g, 0.30mmol) was suspended in chloroform (2mL) and DMF (2 drops) and thionyl chloride (2mL, 28mmol) was added dropwise with stirring. The reaction mixture was stirred overnight and then heated under reflux, with stirring, under a nitrogen atmosphere, for 6h. The reaction mixture was evaporated *in vacuo* and the resulting solid was dissolved in ethyl acetate. The organic layer was washed with water (50mL) and the aqueous phase was extracted with ethyl acetate (3x50mL). The combined extracts were dried over magnesium sulphate and the solvent was evaporated *in vacuo*. Purification by column chromatography on silica gel [eluant dichloromethane], followed by recrystallisation from dichloromethane afforded the title product as a yellow, crystalline solid. Yield 113mg (91%).

Mp 258-260°C (dec.)

δ_H (300 MHz ; $CDCl_3$) 3.53 (s, 3H, CH_3N), 3.75-3.86 (m, 4H, CH_2Cl), 4.11-4.21 (m, 2H, CHN), 4.37 (dt, 2H, J 11.8, J 5.9, CH_2N), 7.01 (d, 2H, J 8.5, ArH), 8.59 (d, 2H, J 8.5, ArH)

δ_C (75 MHz ; DMSO) 26.93 (CH_3N), 39.89 (CH_2N), 50.60 (CH_2O), 108.60 (ArCH), 109.69 (ArC), 133.66 (ArCH), 116.51 (ArC), 138.04 (ArC), 164.01 (CO)

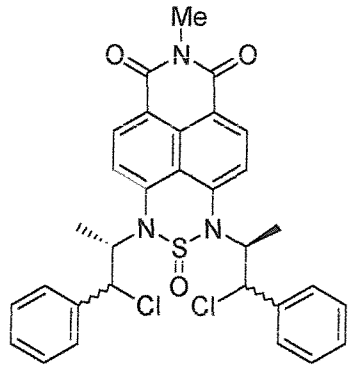
ν_{max} (solid) / cm^{-1} 1689.05 (C=O), 1646.51 (C=O), 1593.20 (s), 1573.59 (m), 1411.67 (m), 1384.56 (m), 1109.97 (s), 1077.90 (s), 810.33 (m), 746.33 (s)

LRMS (APCI+): 412 (M^+)

HRMS (EI+): 411.0190 ($C_{17}H_{15}Cl_2N_3O_3$ (M^+) requires 411.0211)

Microanalysis: Found C=49.70, H=3.45, N=9.98% requires C=49.52, H=3.67, N=10.19%

1,3-Di[(1S)-2-chloro-1-methyl-2-phenylethyl]-7-methyl-1,2,3,6,7,8-hexahydro-2λ^4-[2,1,3]benzothiadiazino[6,5,4-*def*]isoquinoline-2,6,8-trione (28)



(28)

6,7-Di{[(1S,2R)-2-hydroxy-1-methyl-2-phenylethyl]amino}-2-methyl-2,3-dihydro-1*H*-benzo[*de*]isoquinoline-1,3-dione **(25)** (0.10g, 0.2mmol) was suspended in dry dichloromethane (4mL) and thionyl chloride (0.15mL, 2mmol) added. The reaction mixture was stirred at room temperature, under a nitrogen atmosphere, for 24h. The reaction mixture was then poured into water and the aqueous phase was extracted with dichloromethane (3x25mL). The combined extracts were dried over sodium sulphate and the solvent evaporated *in vacuo*. The resulting oil was purified by column chromatography on silica gel [eluant ethyl acetate / dichloromethane (0.05 / 0.95)] and afforded the title product as a yellow wax. Yield 0.02g (13%).

δ_H (300 MHz ; $CDCl_3$) 1.75 (d, 6H, J 7.0, CH_3), 3.55 (s, 3H, CH_3N), 4.61 (dq, 2H, J 6.34, J 13.5, CHN), 5.21 (d, 1H, J 5.2, CHCl), 5.59 (d, 1H, J 4.8, CHCl), 6.94 (d, 1H, J 8.5, ArH), 7.14 (d, 1H, J 8.8, ArH), 7.31-7.56 (m, 10H, Ar'H), 8.53 (d, 1H, J 8.5, ArH), 8.61 (d, 1H, J 8.1, ArH)

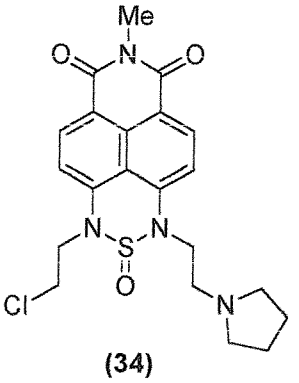
δ_C (75 MHz ; $CDCl_3$) 13.61 (CH_3), 16.29 (CH_3), 27.06 (CH_3N), 59.81 (CH), 62.73 (CH), 64.94 (CH), 65.07 (CH), 109.77 (ArCH), 110.25 (ArCH), 115.78 (ArC), 116.09 (ArC), 127.45 (ArCH), 127.53 (ArCH), 129.07 (ArCH), 129.20 (ArCH), 129.30 (ArCH), 133.58 (ArCH), 137.31 (ArC), 138.18 (ArC), 138.52 (ArC), 139.19 (ArC), 164.11 (CO)

ν_{max} (wax) / cm^{-1} 1691.97 (C=O), 1649.90 (C=O), 1589.06 (s), 1572.24 (m), 1409.69 (m), 1376.30 (m), 1288.13 (m), 1134.73 (m), 1078.58 (w), 746.87 (m)

LRMS (APCI+): 592 (M^+)

HRMS (FAB+): 592.1225 ($C_{31}H_{28}Cl_2N_3O_3S$ (MH^+) requires 592.1228)

1-(2-Chloroethyl)-7-methyl-3-(2-tetrahydro-1H-1-pyrrolylethyl)-1,23,4,5,6,7,8-hexahydro-2λ^4-[2,1,3]benzothiadiazino [6,5,4-*def*]isoquinoline-2,6,8-trione (34)



(34)

1,3-Di(2-chloroethyl)-7-methyl-1,2,3,6,7,8-hexahydro-2λ^4-[2,1,3]benzothiadiazino [6,5,4-*def*]isoquinoline-2,6,8-trione **(27)** (0.10g, 0.24mmol) was suspended in *N,N*-dimethylformamide (3mL) and pyrrolidine (41μL, 69.4mmol) added. The reaction

mixture was heated at 90°C, with stirring, under a nitrogen atmosphere, for 6h. The reaction mixture was then poured into water (25mL) and the aqueous layer was extracted with diethyl ether (3x25mL). The combined extracts were dried over sodium sulphate and the solvent evaporated *in vacuo*. Purification by column chromatography on silica gel [eluant dichloromethane] afforded the title product as a yellow oil. Yield 0.04g (37%).

δ_H (300 MHz ; $CDCl_3$) 1.79-1.85 (br s, 4H, CH_2), 2.67-2.74 (br s, 4H, CH_2NH), 3.01 (t, 2H, J 4.6, CH_2), 3.47 (s, 3H, CH_3N), 3.80 (t, 2H, J 5.9, CH_2), 4.09 (quintet, 3H, J 8.6, $CH+CH_2$), 4.38 (dt, 1H, J 11.5, J 5.7, $CH^{'}N$), 6.92 (d, 1H, J 8.5, ArH), 7.06 (d, 1H, J 8.5, ArH), 8.50 (d, 2H, J 8.5, ArH)

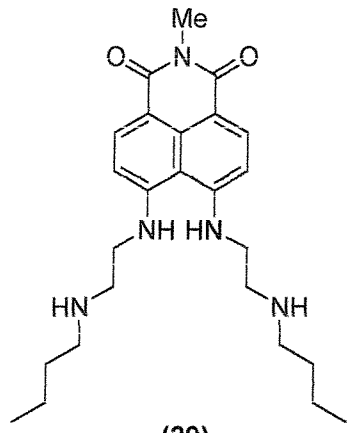
δ_C (75 MHz ; $CDCl_3$) 23.76 (CH_2), 27.07 (CH_3N), 40.32 (CH_2), 50.69 (CH_2), 52.97 (CH_2), 54.20 (CH_2), 108.27 (ArCH), 109.21 (ArCH), 115.53 (ArC), 133.71 (ArCH), 133.98 (ArCH), 164.14 (CO)

ν_{max} (oil) / cm^{-1} 2969.42 (CH), 1738.28 (C=O), 1688.20 (C=O), 1642.80 (s), 1591.16 (s), 1412.75 (m), 1365.52 (m), 1073.55 (m), 810.51 (s), 798.13 (m), 746.61 (s)

LRMS (APCI+): 447 (MH^+)

HRMS (FAB+): 447.1244 ($C_{21}H_{24}ClN_4O_3S$ (MH^+) requires 447.1258)

6,7-Di{[2-(butylamino)ethyl]amino}-2-methyl-2,3-dihydro-1*H*-benzo[*de*]isoquinoline-1,3-dione (29)



(29)

Method 1

1,3-Di(2-chloroethyl)-7-methyl-1,2,3,6,7,8-hexahydro-2λ^4-[2,1,3]benzothiadiazino [6,5,4-*def*]isoquinoline-2,6,8-trione **(27)** (0.15g, 0.36mmol) was suspended in butylamine (5.3g, 73mmol) and refluxed, with stirring, under a nitrogen atmosphere for 24h. The reaction mixture was then filtered. The residue was washed with hexane and dried, affording the title product as a yellow solid. Yield 0.10g (68%).

Method 2

1,3-Di(2-chloroethyl)-7-methyl-1,2,3,6,7,8-hexahydro-2λ^4-[2,1,3]benzothiadiazino [6,5,4-*def*]isoquinoline-2,6,8-trione **(27)** (0.15g, 0.36mmol) was suspended in dry *N,N'*-dimethylformamide (4mL) and butylamine (0.72mL, 7.27mmol) added. The reaction mixture was heated to 90°C, with stirring, under a nitrogen atmosphere for 7h. The reaction mixture was then poured into water (25mL) and the aqueous layer was extracted with dichloromethane (3x25mL). The combined extracts were dried over sodium sulphate and the solvent evaporated *in vacuo*. Recrystallisation from *N,N'*-dimethylformamide afforded the title product as a yellow, crystalline solid. Yield 0.04g (24%).

Mp 190-192°C

δ_H (300 MHz ; $CDCl_3$) 0.90 (t, 6H, J 7.4, CH_3), 1.31 (sextet, 4H, J 7.4, CH_3), 1.49 (quintet, 4H, J 7.3, CH_2), 2.67 (t, 4H, J 7.2, CH_2), 3.24 (t, 4H, J 5.7, CH_2N), 3.35 (t, 4H, J 8.5, CH_2N), 3.51 (s, 3H, CH_3N), 6.68 (d, 2H, J 8.5, ArH), 6.81-6.92 (br s, 2H, NH), 8.38 (d, 2H, J 8.5, ArH)

δ_C (75 MHz ; $CDCl_3$) 14.16 (CH_3), 20.59 (CH_2), 26.73 (CH_3N), 32.53 (CH_2), 43.74 (CH_2N), 47.96 (CH_2N), 49.36 (CH_2N), 106.47 (ArCH), 111.22 (ArC), 111.37 (ArC), 132.25 (ArC), 133.77 (ArCH), 153.03 (ArCN), 165.06 (CO)

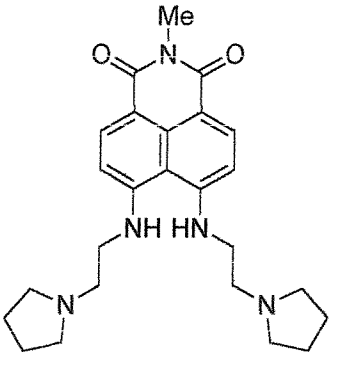
ν_{max} (solid) / cm^{-1} 3297.68 (NH), 2956.66 (CH), 2928.22 (CH), 1670.74 (C=O), 1617.82 (C=O), 1601.44 (s), 1451.07 (w), 1416.97 (m), 1361.84 (w), 1289.51 (w), 1274.83 (w), 1040.12 (m), 809.33 (m), 745.03 (m)

λ_{max} (EtOH) / nm 450 (ε 42192)

LRMS (ES+): 440 (MH^+)

HRMS (FAB+): 440.2990 ($C_{25}H_{38}N_5O_2$ (MH^+) requires 440.3026)

2-Methyl-6,7-di[(2-tetrahydro-1*H*-1-pyrrolylethyl)amino]-2,3-dihydro-1*H*-benzo[*de*]isoquinoline-1,3-dione (30)



(30)

1,3-Di(2-chloroethyl)-7-methyl-1,2,3,6,7,8-hexahydro-2λ^4-[2,1,3]benzothiadiazino [6,5,4-*def*]isoquinoline-2,6,8-trione **(27)** (0.14g, 0.35mmol) was dissolved in

pyrrolidine (5.8mL, 69.4mmol) and refluxed, with stirring, under a nitrogen atmosphere, for 1.5h. The reaction mixture was evaporated *in vacuo* and the resulting solid was dissolved in dichloromethane (25mL). The organic layer was washed with potassium carbonate solution (1M, 25mL) and the aqueous layer was extracted with dichloromethane (3x25mL). The combined extracts were dried over magnesium sulphate and the solvent was evaporated *in vacuo*. Purification by column chromatography on silica gel [eluant ethyl acetate] afforded the title product as an orange solid. Yield 0.11g (72%).

Mp 207-209°C

δ_H (300 MHz ; $CDCl_3$) 1.82-1.89 (br s, 8H, CH_2), 2.62-2.73 (br s, 8H, CH_2), 2.93-2.97 (br s, 4H, CH_2N), 3.44-3.50 (br s, 4H, $C\underline{H}_2NH$), 3.52 (s, 3H, CH_3N), 6.67 (d, 2H, J 8.5, ArH), 6.80-6.88 (br s, 2H, NH), 8.42 (d, 2H, J 8.5, ArH)

δ_C (75 MHz ; $CDCl_3$) 23.73 (CH_2), 26.70 (CH_3N), 42.92 (CH_2), 54.00 (CH_2), 54.25 (CH_2), 106.47 (ArCH), 111.20 (ArC), 111.30 (ArC), 132.26 (ArC), 133.75 (ArCH), 153.01 (ArCN), 165.01 (CO)

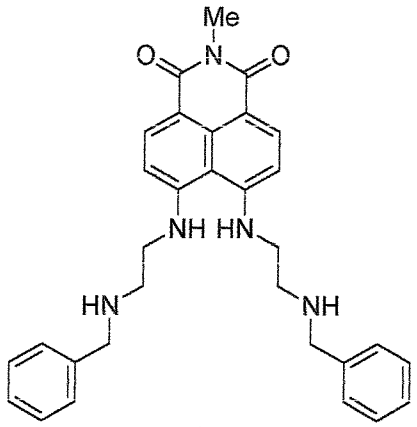
ν_{max} (solid) / cm^{-1} 3328.61 (NH), 2967.08 (CH), 2784.47 (CH), 1673.06 (C=O), 1629.51 (C=O), 1588.95 (s), 1412.89 (m), 1354.84 (m), 1286.05 (w), 1142.94 (w), 1043.62 (w), 743.94 (m)

λ_{max} (EtOH) / nm 448 (ε 24544)

LRMS (ES+): 436 (MH^+)

HRMS (FAB+): 436.2723 ($C_{25}H_{34}N_5O_2$ (MH^+) requires 436.2713)

6,7-Di{[2-(benzylamino)ethyl]amino}-2-methyl-2,3-dihydro-1*H*-benzo[*de*]isoquinoline-1,3-dione (31)



(31)

1,3-Di(2-chloroethyl)-7-methyl-1,2,3,6,7,8-hexahydro-2λ^4-[2,1,3]benzothiadiazino [6,5,4-*def*]isoquinoline-2,6,8-trione **(27)** (0.24g, 0.56mmol) was suspended in benzylamine (12.21g, 114mmol). The reaction mixture was heated at 90°C, with stirring, under a nitrogen atmosphere for 24h. The reaction mixture was then filtered. The residue was washed with hexane and dried, affording the title product as a yellow solid. Yield 0.29g (94%).

Mp 206-207°C (sub.)

δ_H (300 MHz ; $CDCl_3$) 2.99 (t, 4H, J 5.5, CH_2), 3.30-3.34 (br s, 4H, CH_2), 3.52 (s, 3H, CH_3N), 3.81 (s, 4H, CH_2Ph), 6.69 (d, 2H, J 8.5, ArH), 6.77-6.81 (br s, 2H, NH), 7.25-7.33 (m, 10H, Ar'H), 8.44 (d, 2H, J 8.5, ArH)

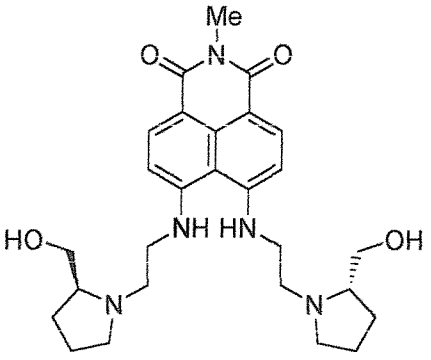
ν_{max} (solid) / cm^{-1} 3307.67 (NH), 2899.49 (CH), 2849.93 (CH), 1669.98 (C=O), 1616.39 (C=O), 1600.03 (s), 1494.58 (w), 1453.22 (w), 1416.63 (m), 1112.64 (m), 809.47 (m), 744.61 (s)

λ_{max} (EtOH) / nm 450 (ε 3442)

LRMS (ES+): 508 (MH^+)

HRMS (FAB+): 508.2714 ($C_{31}H_{34}N_5O_2$ (MH^+) requires 508.2713)

6,7-Di{2-[(2S)-2-(hydroxymethyl)tetrahydro-1*H*-1-pyrrolyl]ethyl}amino)-2-methyl-2,3-dihydro-1*H*-benzo[*de*]isoquinoline-1,3-dione (32)



(32)

1,3-Di(2-chloroethyl)-7-methyl-1,2,3,6,7,8-hexahydro-2λ^4-[2,1,3]benzothiadiazino [6,5,4-*def*]isoquinoline-2,6,8-trione **(27)** (0.60g, 1.46mmol) was suspended in dry *N,N*-dimethylformamide (10mL) and (S)-(+)-prolinol (2.9mL, 29.1mmol) added. The reaction mixture was refluxed, with stirring, under a nitrogen atmosphere for 4h. The reaction mixture was then poured into water (25mL) and the aqueous layer was extracted with diethyl ether (3x25mL). The combined extracts were dried over sodium sulphate and the solvent evaporated *in vacuo*. Purification by column chromatography on silica gel [eluant ethyl acetate / methanol (0.5 / 0.5)] afforded the title product as a yellow oil. Yield 0.58g (80%).

δ_H (300 MHz ; $CDCl_3$) 1.65-1.80 (m, 2H, CH_2), 2.56-2.63 (m, 2H, CH_2), 2.68-2.72 (m, 2H, CH_2), 3.22-3.29 (m, 2H, CH_2), 3.31-3.41 (m, 7H, CH_3+2xCH_2), 3.60-3.67 (dd, 2H, J 2.1, J 6.4, CH), 6.52-6.58 (br s, 2H, NH), 6.75 (d, 2H, J 8.5, ArH), 8.34 (d, 2H, J 8.5, ArH)

δ_C (75 MHz ; $CDCl_3$) 23.62 (CH_2), 26.79 (CH_3N), 27.37 (CH_2), 43.94 (CH_2), 53.07 (CH_2), 54.24 (CH_2), 62.98 (CH_2), 65.28 (CH), 107.24 (ArCH), 133.74 (ArCH), 153.80 (ArCN), 165.01 (CO)

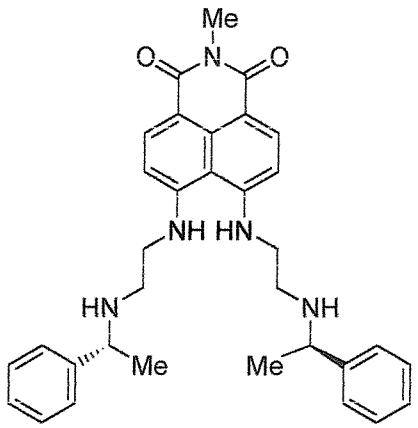
ν_{max} (oil) / cm^{-1} 3340.45 (NH), 2952.76 (CH), 2868.71 (CH), 1673.64 (C=O), 1632.47 (C=O), 1584.57 (s), 1411.61 (m), 1358.73 (m), 1289.77 (w), 1146.68 (w), 1041.55 (m), 810.22 (m), 746.63 (s)

λ_{max} (EtOH) / nm 450 (ε 9010)

LRMS (ES+): 496 (MH^+)

HRMS (FAB+): 496.2924 ($C_{27}H_{38}N_5O_4$ (MH^+) requires 496.2924)

2-Methyl-6,7-di[(2-{[(1R)-1-phenylethyl]amino}-ethyl)amino]-2,3-dihydro-1*H*-benzo[*de*]isoquinoline-1,3-dione (33)



(33)

1,3-Di(2-chloroethyl)-7-methyl-1,2,3,6,7,8-hexahydro-2λ^4-[2,1,3]benzothiadiazino [6,5,4-*def*]isoquinoline-2,6,8-trione **(27)** (0.10g, 0.24mmol) was suspended in dry *N,N*-dimethylformamide (3mL) and (R)-(+)-α-methylbenzylamine (0.62mL, 5mmol) added. The reaction mixture was refluxed, with stirring, under a nitrogen atmosphere for 3 days. The reaction mixture was then poured into water (25mL) and the aqueous layer was extracted with diethyl ether (3x25mL). The combined extracts were dried over sodium sulphate and the solvent evaporated *in vacuo*. Purification by column chromatography on silica gel [eluant ethyl acetate / methanol (0.5 / 0.5)] afforded the title product as an orange oil. Yield 0.03g (26%).

δ_H (300 MHz ; $CDCl_3$) 1.40 (d, 6H, J 6.6, CH_3), 2.78-2.95 (m, 4H, CH_2), 3.15-3.35 (m, 4H, CH_2), 3.50 (s, 3H, CH_3N), 3.73-3.82 (m, 2H, CH), 6.60 (d, 2H, J 8.5, ArH), 6.60-6.78 (br s, 2H, NH), 7.24-7.39 (m, 10H, Ar'H), 8.35 (d, 2H, J 8.5, ArH)

ν_{max} (solid) / cm^{-1} 3296.18 (NH), 2962.02 (CH), 2845.23 (CH), 1673.54 (C=O), 1638.36 (C=O), 1580.05 (s), 1411.52 (w), 1359.31 (w), 1260.14 (m), 1088.99 (m), 827.23 (s), 796.10 (s)

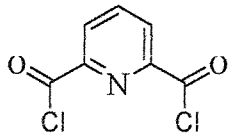
λ_{max} (EtOH) / nm 452 (ε 19554)

LRMS (ES+): 536 (MH^+)

HRMS (FAB+): 536.2980 ($C_{33}H_{38}N_5O_2$ (MH^+) requires 536.3026)

5.3 Preparation of Compounds in the Pyridine Series

2,6-Pyridinedicarbonyl dichloride (36)



(36)

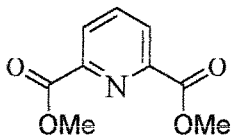
2,6-Pyridinedicarboxylic acid **(35)** (5.0g, 30mmol) was suspended in thionyl chloride (24.9g, 209mmol) and *N,N*-dimethylformamide (1 drop) was added. The reaction mixture was heated under reflux until gas evolution stopped. The excess thionyl chloride was evaporated *in vacuo* to give the crude product. Purification by Sohxlet (dry petroleum ether) afforded the title product as a white, crystalline solid. Yield 6.0g (99%).

Mp 59–60°C (lit. 56-58°C)[187]

δ_H (300 MHz ; $CDCl_3$) 8.15 (t, 1H, J 7.7, ArH), 8.37 (d, 2H, J 7.7, ArH)

ν_{max} / cm^{-1} 1755 (COCl)

Dimethyl 2,6-pyridinedicarboxylate (37)



(37)

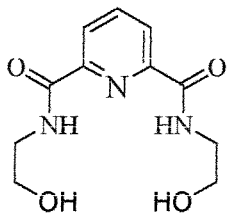
Methanol (40mL) was added dropwise to 2,6-pyridinedicarbonyl dichloride **(36)** (2.0g, 10mmol), in an ice bath, under nitrogen and the reaction mixture was stirred overnight. The reaction mixture was evaporated *in vacuo* and the resulting solid dissolved in dichloromethane (25mL). The organic layer was washed with water

(50mL) and the aqueous layer was extracted with dichloromethane (3x50mL). The combined extracts were dried over magnesium sulphate and the solvent was evaporated *in vacuo* to afford the title product as a white crystalline solid. Yield 1.9g (100%).

Mp 122-126°C (lit. 120-121°C)[188]

δ_H (300 MHz ; $CDCl_3$) 4.04 (s, 6H, CH_3), 8.01 (t, 1H, J 7.9, ArH), 8.01 (d, 2H, J 7.9, ArH)

_N_2,_N_6-Di(2-hydroxyethyl)-2,6-pyridinedicarboxamide (38)



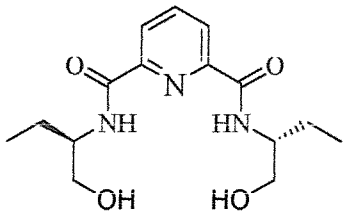
(38)

Dimethyl 2,6-pyridinedicarboxylate **(37)** (0.2g, 1mmol) was stirred with ethanolamine (0.13g, 2mmol) and heated at 90°C for 5 h. The methanol formed was evaporated *in vacuo* and the resulting white solid was washed with chloroform (5mL). Recrystallisation from ethanol afforded the title product as a white, crystalline solid. Yield 0.2g (80%).

Mp 183-185°C (lit. 162°C [EtOH])[146]

δ_H (300 MHz ; $CDCl_3$) 3.35 (q, 4H, J 6.0, CH_2), 3.53 (q, 4H, J 5.9, CH_2), 4.83 (t, 2H, J 5.3, OH), 8.12-8.21 (m, 3H, ArH), 9.36 (t, 2H, J 5.9, NH)

N2,N6-Di[(1R)-1-(hydroxymethyl)propyl]-2,6-pyridinedicarboxamide (39)



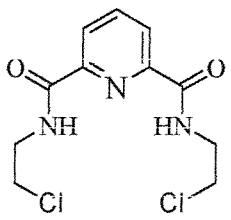
(39)

Dimethyl 2,6-pyridinedicarboxylate **(37)** (0.2g, 1mmol) was stirred with (R)-(-)-2-aminobutanol (0.18g, 2mmol) and heated at 90°C for 5 h. The methanol formed was evaporated *in vacuo* and the resulting white solid was washed with chloroform (5mL). Recrystallisation from ethanol afforded the title product as a white, crystalline solid. Yield 0.14g (46%).

Mp 182-184°C (lit.160°C [EtOH])[146]

δ_H (300 MHz ; DMSO) 0.88 (s, 6H, CH_3), 1.51-1.80 (m, 4H, CH_2), 3.48-3.60 (m, 4H, $C\underline{H}_2OH$), 3.82-3.93 (m, 2H, C<u>H</u>NH), 4.57- 5.00 (br s, 2H, OH), 8.13-8.22 (m, 3H, ArH), 8.62 (d, 2H, J 8.8, NH)

N2,N6-Di(2-chloroethyl)-2,6-pyridinedicarboximide (40)



(40)

*N*2,*N*6-Di(2-hydroxyethyl)-2,6-pyridinedicarboxamide **(38)** (0.09g, 0.4mmol) was suspended in dry dichloromethane (5mL) and thionyl chloride (2.3g, 19mmol) was added dropwise at room temperature. The reaction mixture was then heated at reflux

for 4h. The reaction mixture was evaporated *in vacuo* affording the title product as a white solid. Yield 0.08g (80%).

Mp 158-160°C

δ_H (300 MHz ; $CDCl_3$) 3.75 (t, 4H, J 5.3, CH_2), 3.84 (t, 4H, J 5.3, CH_2), 8.07 (t, 1H, J 7.7, ArH), 8.37 (d, 4H, J 7.4, ArH + NH)

δ_C (75 MHz ; $CDCl_3$) 41.36 (CH_2), 44.10 (CH_2), 125.49 (ArCH), 139.40 (ArCH), 148.62 (ArC), 163.68 (CO)

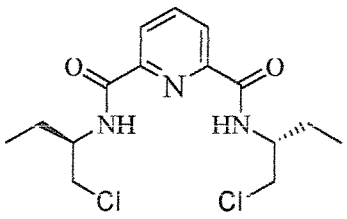
IR (solid): υ_{max}/cm^{-1} 3352.65 (NH), 3310.94 (NH), 1650.08 (C=O), 1522.00 (C=O), 1439.43 (m), 1363.15 (m), 1242.10 (w), 1198.17 (m), 843.49 (w), 735.10 (m)

LRMS (APCI+): 290 (M^+)

HRMS (EI+): 289.0378 ($C_{11}H_{13}Cl_2N_3O_2$ (M^+) requires 289.0385)

Microanalysis: Found C=52.35, H=6.04, N=12.07, Cl=20.28% requires C=52.03, H=6.11, N=12.14, Cl=20.48%

_N_2,_N_6-Di[(1R)-1-(chloromethyl)propyl]-2,6-pyridinedicarboxamide (41)



(41)

_N_2,_N_6-Di[(1R)-1-(hydroxymethyl)propyl]-2,6-pyridinedicarboxamide **(39)** (0.1g, 0.3mmol) was suspended in dry dichloromethane (5mL) and thionyl chloride (2.1g, 18mmol) was added dropwise at room temperature. The reaction mixture was then

heated at reflux for 4h. The reaction mixture was evaporated *in vacuo* and recrystallisation from ethanol afforded the title product as a white solid. Yield 0.08g (80%).

Mp 128-130°C

δ_H (300 MHz ; $CDCl_3$) 0.97 (t, 6H, J 7.4, CH_3), 1.72 (quintet, 4H, J 7.4, CH_2), 3.79 (d, 4H, J 3.3, CH_2Cl), 4.33 (br m, 2H, CHNH), 7.96 (d, 2H, J 10.1, NH), 8.03 (t, 1H, J 7.4, ArH), 8.34 (d, 2H, J 7.4, ArH)

δ_C (75 MHz ; $CDCl_3$) 10.53 (CH_3), 25.22 (CH_2), 40.09 (CH_2), 50.77 (CH), 125.27 (ArCH), 139.41 (ArCH), 148.61 (ArC), 162.99 (CO)

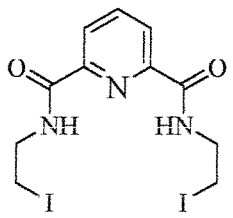
IR (solid): υ_{max}/cm^{-1} 3282.46 (NH), 3239.64 (NH), 2967.66 (CH), 1644.45 (C=O), 1540.26 (C=O), 1444.33 (w), 1349.97 (w), 1230.34 (w), 746.35 (s)

LRMS (APCI+): 346 (M^+)

HRMS (EI+): 345.1011 ($C_{15}H_{21}Cl_2N_3O_2$ (M^+) requires 345.1011)

Microanalysis: Found C=45.83, H=4.43, N=14.25, Cl=24.23% requires C=45.54, H=4.52, N=14.48, Cl=24.44%

_N_2,_N_6-Di(2-iodoethyl)-2,6-pyridinedicarboximide (50)



(50)

*N*2,*N*6-Di(2-chloroethyl)-2,6-pyridinedicarboximide **(40)** (0.15g, 0.52mmol) was suspended in acetonitrile (3mL) and sodium iodide (0.62g, 4.13mmol) added. The reaction mixture was refluxed, with stirring, under a nitrogen atmosphere, for 24h. The solid residue was removed by filtration. The filtrate was washed with water (25mL) and the aqueous layer was extracted with dichloromethane (3x25mL). The combined extracts were dried over sodium sulphate and the solvent evaporated *in vacuo* to afford the title product as a red/orange oil. Yield 0.09g (42%)

δ_H (300 MHz ; $CDCl_3$) 3.33 (t, 4H, J 6.6, CH_2), 3.80 (q, 4H, J 6.4, CH_2), 8.02 (t, 1H, J 8.1, ArH), 8.33 (d, 2H, J 8.1, ArH), 8.43 (t, 2H, J 6.1, NH)

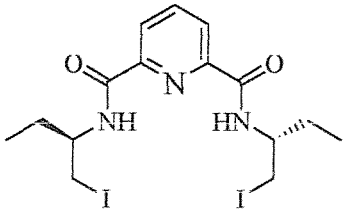
δ_C (75 MHz ; $CDCl_3$) 5.28 (CH_2), 41.89 (CH_2), 125.48 (ArCH), 139.50 (ArCH), 148.58 (ArC), 163.63 (CO)

IR (oil): υ_{max}/cm^{-1} 3475.06 (NH), 3268.08 (NH), 1653.34 (C=O), 1537.20 (C=O), 1443.94 (m), 1164.66 (s), 746.77 (s)

LRMS (APCI+): 474 (MH^+), 346 (MH^+-I)

HRMS (FAB+): 473.9195 ($C_{11}H_{14}I_2N_3O_2$ (MH^+) requires 473.9176)

_N_2,_N_6-Di[(1R)-1-(iodomethyl)propyl]-2,6-pyridinedicarboximide (53)



(53)

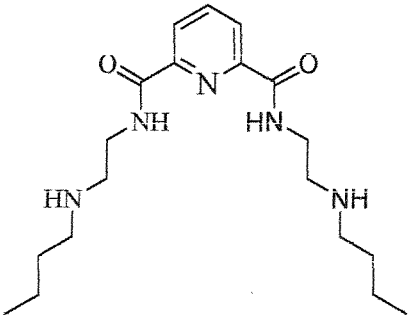
*N*2,*N*6-Di[(1R)-1-(chloromethyl)propyl]-2,6-pyridinedicarboxamide **(41)** (0.20g, 0.6mmol) was suspended in acetonitrile (5mL) and sodium iodide (0.70g, 4.6mmol) added. The reaction mixture was refluxed, with stirring, under a nitrogen atmosphere,

for 24h. The solid residue was removed by filtration. The filtrate was washed with water (25mL) and the aqueous layer was extracted with dichloromethane (3x25mL). The combined extracts were dried over sodium sulphate and the solvent evaporated *in vacuo* to afford the title product as a pale orange oil. Yield 0.04g (12%)

δ_H (300 MHz ; $CDCl_3$) 0.94 (t, 6H, J 7.4, CH_3), 1.58-1.72 (m, 4H, CH_2), 3.41 (dd, 2H, J 2.5, J 8.6, CHH I), 3.52 (dd, 2H, J 2.5, J 8.6, CHH I), 3.64-3.78 (br m, 2H, CHNH), 7.84 (d, 2H, J 10.1, NH), 7.99 (t, J 7.4, ArH), 8.26 (d, J 7.4, ArH)

LRMS (ES+): 552 (MNa^+)

N2,N6-Di[2-(butylamino)ethyl]-2,6-pyridinedicarboximide (42)



(42)

Method 1

N2,N6-Di(2-chloroethyl)-2,6-pyridinedicarboximide **(40)** (0.15g, 0.52mmol) was suspended in butylamine (7.6g, 104mmol) and refluxed, with stirring, under a nitrogen atmosphere, for 24h. The reaction mixture was evaporated *in vacuo* and purification of the resulting oil by column chromatography on silica gel [eluant methanol] afforded the title product as a light brown oil. Yield 0.12g (62%).

Method 2

N2,N6-Di(2-iodoethyl)-2,6-pyridinedicarboximide **(50)** (0.34g, 0.71mmol) was suspended in dry tetrahydrofuran (6mL) and butylamine (1.4mL, 14.2mmol) added. The reaction mixture was refluxed, with stirring, under a nitrogen atmosphere, for 20h. The reaction mixture was evaporated *in vacuo* and the resulting oil was

dissolved in dichloromethane (25mL). The organic layer was washed with potassium carbonate solution (1M, 25mL) and the aqueous layer was extracted with dichloromethane (3x25mL). The combined extracts were dried over magnesium sulphate and the solvent was evaporated *in vacuo* to afford the title product as a light brown oil. Yield 0.20g (78%).

δ_H (300 MHz ; $CDCl_3$) 0.84 (t, 6H, J 7.4, CH_3), 1.26 (sextet, 4H, J 7.3, CH_2), 1.70 (quintet, 4H, J 7.8, CH_2), 2.97 (t, 4H, J 7.5, CH_2N), 3.23-3.35 (br s, 4H, CH_2N), 3.81-3.92 (br s, 4H, CH_2N), 7.70 (t, 1H, J 7.9, ArH), 7.98 (d, 2H, J 7.9, ArH), 9.67 (t, 2H, J 5.9, NH)

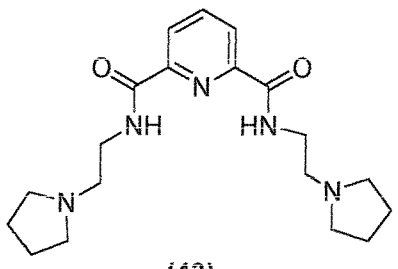
δ_C (75 MHz ; $CDCl_3$) 14.07 (CH_3), 20.53 (CH_2), 31.89 (CH_2), 39.39 (CH_2), 49.53 (CH_2), 49.65 (CH_2), 124.74 (ArCH), 138.76 (ArCH), 148.98 (ArC), 164.22 (CO)

IR (oil): υ_{max}/cm^{-1} 3291.86 (NH), 2957.48 (CH), 2931.47 (CH), 1661.30 (C=O), 1539.64 (C=O), 1446.42 (m), 1030.18 (s)

LRMS (ES+): 364 (MH^+)

HRMS (FAB+): 364.2713 ($C_{19}H_{34}N_5O_2$ (MH^+) requires 364.2701)

_N_2,_N_6-Di[2-tetrahydro-1_H_-pyrrolylethyl]-2,6-pyridinedicarboximide (43)



(43)

Method 1

_N_2,_N_6-Di(2-chloroethyl)-2,6-pyridinedicarboximide **(40)** (0.08g, 0.3mmol) was suspended in pyrrolidine (4.0g, 56mmol) and the reaction mixture was refluxed, with

stirring, under a nitrogen atmosphere, for 1.5h. The reaction mixture was evaporated *in vacuo* and the resulting oil was dissolved in dichloromethane (25mL). The organic layer was washed with potassium carbonate solution (1M, 25mL) and the aqueous layer was extracted with dichloromethane (3x25mL). The combined extracts were dried over magnesium sulphate and the solvent was evaporated *in vacuo*. Purification by column chromatography on silica gel (eluant methanol) afforded the title product as a yellow oil. Yield 0.09g (86%).

Method 2

*N*2,*N*6-Di(2-iodoethyl)-2,6-pyridinedicarboximide **(50)** (0.09g, 0.2mmol) was suspended in dry tetrahydrofuran (2mL) and pyrrolidine (0.33mL, 4mmol) added. The reaction mixture was refluxed, with stirring, under a nitrogen atmosphere, for 20h. The reaction mixture was evaporated *in vacuo* and the resulting oil was dissolved in dichloromethane (25mL). The organic layer was washed with potassium carbonate solution (1M, 25mL) and the aqueous layer was extracted with dichloromethane (3x25mL). The combined extracts were dried over magnesium sulphate and the solvent was evaporated *in vacuo* to afford the title product as a yellow oil. Yield 0.07g (99%).

δ_H (300 MHz ; $CDCl_3$) 1.76-1.82 (br m, 8H, CH_2), 2.64-3.00 (br m, 8H, CH_2), 2.79 (t, 4H, J 5.3, CH_2), 3.63 (q, 4H, J 5.4, $C\underline{H}_2NH$), 7.91 (t, 1H, J 7.7, ArH), 8.24 (d, 2H, J 7.7, ArH), 9.47 (t, 2H, J 5.2, NH)

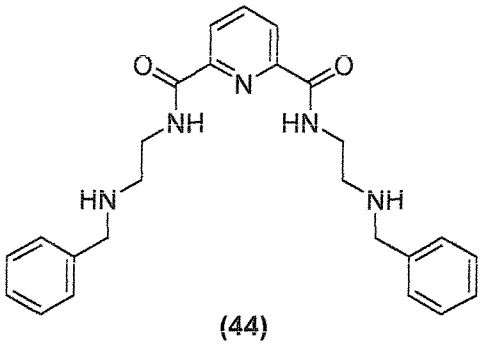
δ_C (75 MHz ; $CDCl_3$) 23.51 (CH_2), 37.95 (CH_2), 54.20 (CH_2), 56.17 (CH_2), 124.72 (ArCH), 138.68 (ArCH), 149.00 (ArC), 164.33 (CO)

IR (oil): υ_{max}/cm^{-1} 3370.39 (NH), 2966.47 (CH), 2819.17 (CH), 1659.74 (C=O), 1540.51 (C=O), 1447.21 (m), 1312.16 (w), 1143.90 (w)

LRMS (ES+): 360 (MH^+)

HRMS (FAB+): 359.2315 ($C_{23}H_{29}N_5O_2$ (MH^+) requires 359.2321)

N2,N6-Di[2-(benzylamino)ethyl]-2,6-pyridinedicarboximide (44)



(44)

Method 1

*N*2,*N*6-Di(2-chloroethyl)-2,6-pyridinedicarboximide **(40)** (0.10g, 0.3mmol) was suspended in benzylamine (7.4g, 69mmol). The reaction mixture was refluxed, with stirring, under a nitrogen atmosphere, for 2.5h. The reaction mixture was evaporated *in vacuo* and the resulting oil was purified by column chromatography on silica gel (eluant ethyl acetate) affording the title product as a brown oil. Yield 0.13g (97%).

Method 2

*N*2,*N*6-Di(2-iodoethyl)-2,6-pyridinedicarboximide **(50)** (0.20g, 0.4mmol) was suspended in dry tetrahydrofuran (5mL) and benzylamine (0.92mL, 8mmol) added. The reaction mixture was refluxed, with stirring, under a nitrogen atmosphere, for 20h. The reaction mixture was evaporated *in vacuo* and the resulting oil was purified by column chromatography on silica gel (eluant ethyl acetate) affording the title product as a light brown oil. Yield 0.14g (81%).

δ_H (300 MHz ; $CDCl_3$) 3.07-3.10 (br t, 4H, CH_2), 3.74-3.77 (br q, 4H, CH_2), 3.96-3.99 (br s, 4H, CH_2), 7.25-7.48 (m, 10H, ArH), 7.77 (t, 1H, J 7.9, ArH), 8.07 (d, 2H, J 7.9, ArH), 9.57 (t, 2H, J 5.9, NH)

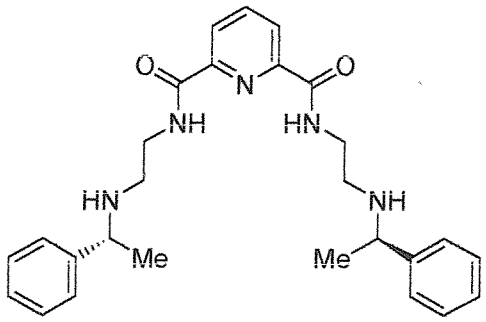
δ_C (75 MHz ; $CDCl_3$) 39.34 (CH_2), 48.14 (CH_2), 53.59 (CH_2), 124.82 (ArCH), 127.49 (ArCH), 127.88 (ArCH), 128.37 (ArCH), 138.92 (ArCH), 148.94 (ArC), 164.22 (CO)

IR (oil): υ_{max}/cm^{-1} 3309.29 (NH), 1662.30 (C=O), 1533.57 (C=O), 1451.70 (m), 744.58 (s)

LRMS (ES+): 432 (MH^+)

HRMS (FAB+): 432.2400 ($C_{25}H_{30}N_5O_2$ (MH^+) requires 432.2400)

_N_2,_N_6-Di{2-{[(1R)-1-phenylethyl]amino}ethyl)-2,6-pyridinedicarboximide (45)



(45)

Method 1

_N_2,_N_6-Di(2-chloroethyl)-2,6-pyridinedicarboximide **(40)** (0.20g, 0.69mmol) was suspended in (R)-(+)-α-methylbenzylamine (16.70g, 138mmol). The reaction mixture was heated at 90°C, with stirring, under a nitrogen atmosphere, for 3h. The reaction mixture was evaporated *in vacuo* and purification of the resulting oil by column chromatography on silica gel [eluant ethyl acetate] afforded the title product as a brown oil. Yield 0.30g (95%).

Method 2

_N_2,_N_6-Di(2-iodoethyl)-2,6-pyridinedicarboximide **(50)** (0.30g, 0.63mmol) was suspended in dry tetrahydrofuran (3mL) and (R)-(+)-α-methylbenzylamine (1.63mL, 12.7mmol) added. The reaction mixture was refluxed, with stirring, under a nitrogen atmosphere, for 20h. The reaction mixture was evaporated *in vacuo* and the resulting oil was dissolved in dichloromethane (25mL). The organic layer was washed with potassium carbonate solution (1M, 25mL) and the aqueous layer was extracted with dichloromethane (3x25mL). The combined extracts were dried over magnesium

sulphate and the solvent was evaporated *in vacuo* to afford the title product as a yellow oil. Yield 0.23g (80%).

δ_H (300 MHz ; $CDCl_3$) 1.69 (d, 6H, J 6.6, CH_3), 2.89-2.93 (br m, 2H, CHH'NH), 3.01-3.05 (br m, 2H, CHH'NH), 3.73-3.75 (br m, 4H, CH_2), 4.30 (q, 2H, J 6.1, CHPh), 7.21-7.24 (m, 10H, ArH), 7.75 (t, 1H, J 7.9, ArH), 7.97 (d, 2H, J 7.9, ArH), 9.60 (t, 2H, J 5.9, NH)

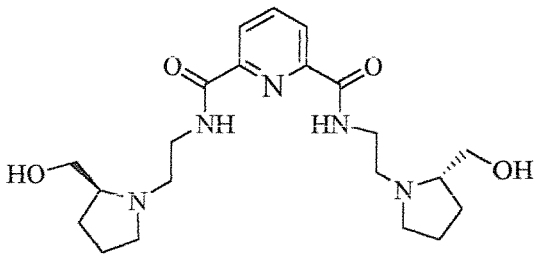
δ_C (75 MHz ; $CDCl_3$) 24.10 (CH_3), 39.69 (CH_2), 41.16 (CH_2), 58.36 (CH), 124.85 (ArCH), 126.65 (ArCH), 127.47 (ArCH), 128.80 (ArCH), 138.95 (ArCH), 144.46 (ArC), 148.93 (ArC), 164.10 (CO)

IR (oil): υ_{max}/cm^{-1} 3347.30 (NH), 2961.02 (CH), 2923.41 (CH), 2848.19 (CH), 1660.81 (C=O), 1538.50 (C=O), 1447.11 (m), 1260.40 (m), 1087.25 (m), 1019.59 (m)

LRMS (ES+): 460 (MH+)

HRMS (FAB+): 460.2707 ($C_{27}H_{34}N_5O_2$ (MH^+) requires 460.2713)

_N_2,N6-Di{2-[(2S)-2-(hydroxymethyl)tetrahydro-1_H_-1-pyrrolyl]ethyl}-2,6-pyridinedicarboximide (51)



(51)

*N*2,*N*6-Di(2-iodoethyl)-2,6-pyridinedicarboximide **(50)** (0.60g, 1.26mmol) was suspended in dry tetrahydrofuran (12mL) and (S)-(+)-prolinol (2.5mL, 25.4mmol) added. The reaction mixture was refluxed, with stirring, under a nitrogen atmosphere,

for 6h. The reaction mixture was evaporated *in vacuo* and the resulting oil was dissolved in dichloromethane (25mL). The organic layer was washed with potassium carbonate solution (1M, 25mL) and the aqueous layer was extracted with dichloromethane (3x25mL). The combined extracts were dried over magnesium sulphate and the solvent was evaporated *in vacuo* to afford the title product as an orange oil. Yield 0.48g (91%)

δ_H (300 MHz ; $CDCl_3$) 1.76-1.84 (m, 2H, CH_2), 2.29 (q, 2H, J 5.8, CH_2), 2.47 (dd, 2H, J 2.6, J 11.6, CH_2), 3.23-3.35 (m, 4H, CH_2), 3.66 (dd, 2H, J 3.9, J 8.0, CH), 7.91 (t, 1H, J 7.9, ArH), 8.13 (d, 2H, J 7.9, ArH), 8.58 (d, 2H, J 9.5, NH)

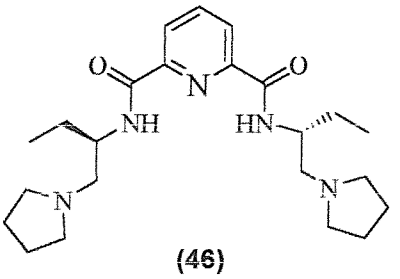
δ_C (75 MHz ; $CDCl_3$) 23.59 (CH_2), 27.46 (CH_2), 38.97 (CH_2), 54.58 (CH_2), 54.92 (CH_2), 63.28 (CH_2), 65.41 (CH), 124.74 (ArCH), 138.80 (ArCH), 149.03 (ArC), 164.20 (CO)

IR (oil): υ_{max}/cm^{-1} 3307.40 (NH), 2951.29 (CH), 2874.33 (CH), 2824.07 (CH), 1659.87 (C=O), 1539.52 (C=O), 1446.25 (m), 1038.84 (m)

LRMS (ES+): 420 (MH^+)

HRMS (FAB+): 420.2613 ($C_{21}H_{34}N_5O_4$ (MH^+) requires 420.2611)

_N_2,_N_6-Di[(1R)-1-(tetrahydro-1_H_-1-pyrrolylmethyl)propyl]-2,6-pyridinedicarboximide (46)



(46)

Method 1

*N*2,*N*6-Di[(1R)-1-(chloromethyl)propyl]-2,6-pyridinedicarboxamide **(41)** (0.06g, 0.17mmol) was suspended in pyrrolidine (2.42g, 34mmol) and refluxed, with stirring, under a nitrogen atmosphere, for 1.5h. The reaction mixture was evaporated *in vacuo* and the resulting oil was dissolved in dichloromethane (25mL). The organic layer was washed with potassium carbonate solution (1M, 25mL) and the aqueous layer was extracted with dichloromethane (3x25mL). The combined extracts were dried over magnesium sulphate and the solvent was evaporated *in vacuo*. Purification of the resulting oil by column chromatography on silica gel [eluant methanol] afforded the title product as a brown oil. Yield 0.03g (43%).

Method 2

*N*2,*N*6-Di[(1R)-1-(chloromethyl)propyl]-2,6-pyridinedicarboxamide **(41)** (0.10g, 0.29mmol) was suspended in dry tetrahydrofuran (2mL) and pyrrolidine (0.48mL, 5.8mmol) added. The reaction mixture was refluxed, with stirring, under a nitrogen atmosphere, for 24h. The reaction mixture was evaporated *in vacuo* and the resulting oil was dissolved in dichloromethane (25mL). The organic layer was washed with potassium carbonate solution (1M, 25mL) and the aqueous layer was extracted with dichloromethane (3x25mL). The combined extracts were dried over sodium sulphate and the solvent evaporated *in vacuo* to afford the title product as a yellow oil. Yield 0.07g (58%).

δ_H (300 MHz ; $CDCl_3$) 0.90 (t, 3H, J 7.5, CH_3), 1.69-1.75 (br m, 12H, CH_2), 2.65 (dd, 2H, J 3.9, J 11.2,CH_2N), 2.68-2.71 (br s, 8H, CH_2), 3.08 (t, 2H, J 11.0, CH_2N), 4.20-4.23 (br m, 2H, CHNH), 7.93 (t, 1H, J 7.9, ArH), 8.26 (d, 2H, J 7.9, ArH), 8.45 (d, 2H, J 8.5, NH)

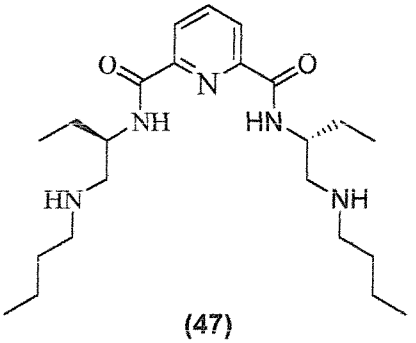
δ_C (75 MHz ; $CDCl_3$) 10.47 (CH_3), 23.50 (CH_2), 26.41 (CH_2), 49.96 (CH_2), 54.60 (CH_2), 58.81 (CH_2), 124.80 (ArCH), 138.60 (ArCH), 149.14 (ArC), 163.99 (CO)

IR (oil): υ_{max}/cm^{-1} 3276.28 (NH), 2964.62 (CH), 2876.14 (CH), 2809.68 (CH), 1661.61 (C=O), 1532.85 (C=O), 1458.79 (m)

LRMS (APCI+): 416 (MH^+)

HRMS (EI+): 415.2944 ($C_{23}H_{37}N_5O_2$ (M^+) requires 415.2947)

_N_2,_N_6-Di{(1R)-1-[(butylamino)methyl]propyl}-2,6-pyridinedicarboximide (47)



(47)

Method 1

_N_2,_N_6-Di[(1R)-1-(chloromethyl)propyl]-2,6-pyridinedicarboxamide **(41)** (0.06g, 0.2mmol) was suspended in butylamine (2.42g, 34mmol) and refluxed, with stirring, under a nitrogen atmosphere, for 6h. The reaction mixture was evaporated *in vacuo* and purification of the resulting oil by column chromatography on silica gel [eluant ethyl acetate / methanol (0.5 / 0.5)] afforded the title product as a light brown oil. Yield 0.05g (48%).

Method 2

_N_2,_N_6-Di[(1R)-1-(chloromethyl)propyl]-2,6-pyridinedicarboxamide **(41)** (0.15g, 0.4mmol) was suspended in dry tetrahydrofuran (3mL) and butylamine (0.63g, 9mmol) was added. The reaction mixture was refluxed, with stirring, under a nitrogen atmosphere, for 16h. The reaction mixture was evaporated *in vacuo* and purification of the resulting oil by column chromatography on silica gel [eluant ethyl acetate / methanol (0.5 / 0.5)] afforded the title product as a light brown oil. Yield 0.11g (65%).

δ_H (300 MHz ; $CDCl_3$) 0.88 (t, 6H, J 8.6, CH_3), 0.93 (t, 6H, J 7.5, CH_3), 1.34 (sextet, 4H, J 7.5, CH_2), 1.71-2.01 (br m, 8H, CH_2), 3.01-3.24 (br m, 6H, CH_2), 3.77 (t, 2H, J

11.0, CH_2), 4.48-4.67 (br s, 2H, CHNH), 7.33 (t, 1H, J 7.9, ArH), 7.78 (d, 2H, J 7.9, ArH), 9.68 (d, 2H, J 9.2, NH)

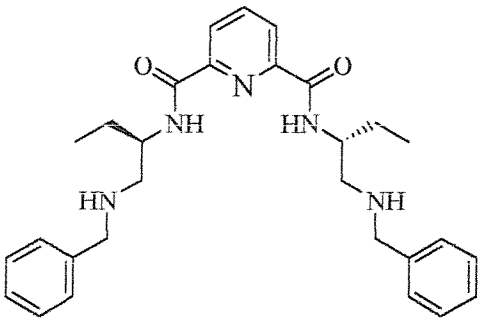
δ_C (75 MHz ; $CDCl_3$) 10.63 (CH_3), 13.68 (CH_3), 20.07 (CH_2), 26.00 (CH_2), 28.08 (CH_2), 48.81 (CH_2), 49.14 (CH), 50.81 (CH_2), 125.10 (ArCH), 137.83 (ArCH), 148.35 (ArC), 164.85 (CO)

IR (oil): υ_{max}/cm^{-1} 3291.91 (NH), 2961.44 (CH), 2931.90 (CH), 2873.70 (CH), 1658.92 (C=O), 1532.99 (C=O), 1458.16 (m)

LRMS (APCI+): 420 (MH^+)

HRMS (FAB+): 420.3339 ($C_{23}H_{42}N_5O_2$ (MH^+) requires 420.3333)

_N_2,_N_6-Di{(1R)-1-[(benzylamino)methyl]propyl}-2,6-pyridinedicarboximide (48)



(48)

_N_2,_N_6-Di[(1R)-1-(chloromethyl)propyl]-2,6-pyridinedicarboxamide **(41)** (0.15g, 0.43mmol) was suspended in benzylamine (9.3g, 87mmol) and refluxed, with stirring, under a nitrogen atmosphere, for 24h. The reaction mixture was evaporated *in vacuo* and purification of the resulting solid by column chromatography on silica gel [eluant methanol] afforded the title product as an oil. Yield 0.12g (56%).

δ_H (300 MHz ; $CDCl_3$) 0.89 (t, 6H, J 7.4, CH_3), 1.72 (dq, 4H, J 7.2, J 51.2, CH_2), 2.97 (dd, 2H, J 3.3, J 12.5, CHH'NH), 3.45-3.52 (m, 2H, CHH'NH), 4.06 (d, 2H, J 7.9,

CH_2Ph), 4.29-4.40 (br m, 2H, C<u>H</u>NH), 7.17 (m, 6H, ArH), 7.47-7.55 (m, 4H, ArH), 7.76 (t, 1H, J 7.9, ArH), 8.09 (d, 2H, J 7.9, ArH), 9.34 (d, 2H, J 8.8, NH)

δ_C (75 MHz ; $CDCl_3$) 10.87 (CH_3), 25.36 (CH_2), 48.26 (CH_2), 49.01 (CH), 51.26 (CH_2), 125.27 (ArCH), 129.01 (ArCH), 129.54 (ArCH), 130.47 (ArCH), 138.40 (ArCH), 148.44 (ArC), 164.72 (CO)

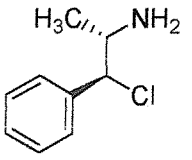
IR (oil): υ_{max}/cm^{-1} 3407.81 (NH), 2967.16 (CH), 1659.26 (C=O), 1533.33 (C=O), 1455.99 (m), 1026.38 (w), 749.25 (m)

LRMS (ES+): 488 (MH^+)

HRMS (FAB+): 488.3003 ($C_{29}H_{38}N_5O_2$ (MH^+) requires 488.3026)

5.4 Preparation of Tetramines via Diamine Coupling

(1S, 2S)-1-Chloro-1-phenylpropan-2-amine (55)



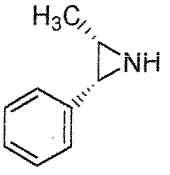
(55)

(1R,2S)-2-Amino-1-phenylpropan-1-ol **(54)** (1.0g, 6.6mmol) was suspended in thionyl chloride (1.43mL, 20.0mmol) and the reaction mixture was stirred at room temperature, under a nitrogen atmosphere, for 5h. The excess thionyl chloride was removed *in vacuo*. The resulting solid was washed with acetone (20mL), filtered and recrystallised from methanol to give the product as a white solid. Yield 0.90g (80%).

Mp 204-206°C (lit. 205-207°C)[150]

δ_H (300 MHz ; $CDCl_3$) 1.37 (d, 3H, J 10.3, CH_3), 3.82-3.97 (m, 1H, CH), 5.13 (d, 1H, J 6.6, CH), 7.41 (br s, 5H, ArH), 8.97 (br s, 2H, NH)

(2S,3R)-2-Methyl-3-phenylaziridine (57)



(57)

(1S,2S)-1-Chloro-1-phenylpropan-2-amine **(55)** (0.5g, 2.6mmol) was suspended in pyrrolidine (12.0mL, 147.3mmol) . The reaction mixture was refluxed, with stirring, under a nitrogen atmosphere, for 24h. The pyrrolidine was then removed *in vacuo*. The resulting solid was purified by column chromatography on silica gel [eluant ethyl

acetate / methanol (0.9 / 0.1)] and afforded the title product as an off-white solid. Yield 0.11g (29%).

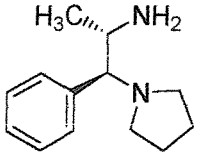
Mp 68-70°C (lit. 68°C)[153]

δ_H (300 MHz ; $CDCl_3$) 0.82 (d, 3H, J 6.6, CH_3), 1.16-1.20 (br s, 1H, NH), 2.33 (q, 1H, J 5.2, CH), 3.15 (d, 1H, J 5.9, CH), 7.22-7.28 (m, 5H, ArH)

δ_C (75 MHz ; $CDCl_3$) 13.78 (CH_3), 32.35 (CH), 37.28 (CH), 126.784(ArCH), 127.96 (ArCH), 128.06 (ArCH), 128.22 (ArC)

LRMS (ES+): 134 (MH^+)

(1S,2S)-1-Methyl-2-phenyl-2-tetrahydro–1*H*-1-pyrrolyl-ethylamine (56)



(56)

(1S,2S)-1-Chloro-1-phenylpropan-2-amine **(55)** (0.5g, 2.6mmol) was suspended in pyrrolidine (12.0mL, 147.3mmol) . The reaction mixture was refluxed, with stirring, under a nitrogen atmosphere, for 72h. The pyrrolidine was then evaporated *in vacuo*. The resulting brown solid was recrystallised from dichloromethane to give the product as a white solid. Yield 0.49g (82%).

Mp 229-231°C (lit. 220-222°C)[189]

δ_H (300 MHz ; $CDCl_3$) 1.28 (d, 3H, J 6.3, CH_3), 1.61-1.64 (br s, 4H, CH_2), 2.42-2.46 (br s, 2H, CH_2), 2.50-2.53 (br s, 2H, CH_2), 3.71-3.84 (m, 1H, CH), 3.87 (d, 1H, J 11.0, CH), 7.08 (d, 2H, J 7.7, ArH), 7.26-7.38 (m, 3H, ArH)

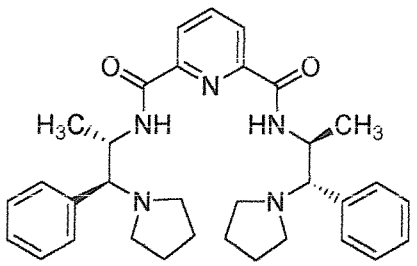
δ_C (75 MHz ; $CDCl_3$) 16.34 (CH_3), 22.79 (CH_2), 48.01 (CH_2), 48.36 (CH), 67.64 (CH), 128.30 (ArCH), 128.47 (ArCH), 129.74 (ArCH), 132.04 (ArC)

ν_{max}(solid) / cm^{-1} 3076.24 (NH), 1620.48 (m), 1538.26 (m), 1480.23 (m), 1228.73 (s), 1165.86 (s), 1117.50 (s), 1030.44 (s), 1001.42 (s), 812.80 (m), 759.60 (s)

LRMS (ES+): 205 (MH^+)

HRMS (FAB+): 205.1688 ($C_{13}H_{21}N_2$ (MH^+) requires 205.1705)

_N_2,_N_6-Di{(1S, 2R)-1-methyl-2-phenyl-2-tetrahydro-1_H_-1-pyrrolylethyl}-2,6-pyridinedicarboximide (58)



(58)

(1S,2S)-1-Methyl-2-phenyl-2-tetrahydro–1*H*-1-pyrrolyl-ethylamine **(56)** (0.17g, 0.83mmol) was dissolved in dry dichloromethane (5mL) and added dropwise to 2,6-pyridinedicarbonyl dichloride **(36)** (0.09g, 0.42mmol) at 0°C, under a nitrogen atmosphere. The reaction mixture was then allowed to heat to room temperature and was stirred for 16 hours. The reaction mixture was then evaporated *in vacuo* and the resulting oil was purified by column chromatography on silica gel (eluant ethyl acetate) affording the title product as a light brown oil. Yield 0.12g (53%).

δ_H (300 MHz ; $CDCl_3$) 1.11 (d, 6H, J 6.2, CH_3), 1.54-1.63 (br s, 8H, CH_2), 2.29-2.47 (br s, 8H, CH_2), 3.31-3.39 (br s, 2H, CH), 4.62 (q, 2H, J 4.8, CH), 7.10-7.15 (m, 4H, ArH), 7.17-7.23 (m, 6H, ArH), 7.37-7.43 (br s, 2H, NH), 7.97 (t, 1H, J 7.9, ArH), 8.29 (d, 2H, J 7.9, ArH)

δ_C (75 MHz ; $CDCl_3$) 15.86 (CH_3), 23.31 (CH_2), 49.51 (CH), 51.15 (CH_2), 71.66 (CH), 125.04 (ArCH), 127.88 (ArCH), 128.06 (ArCH), 129.63 (ArCH), 137.15 (ArC), 139.02 (ArCH), 148.96 (ArC), 162.96 (CO)

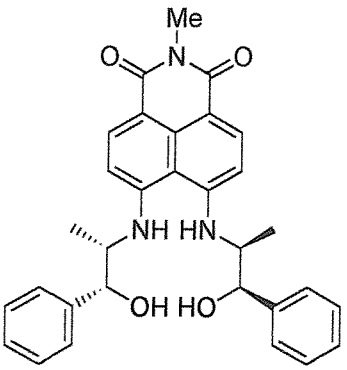
IR (oil): υ_{max}/cm^{-1} 3393.99 (NH), 2965.65 (CH), 2794.02 (CH), 1673.45 (C=O), 1519.46 (C=O), 1451.90 (m), 1261.07 (m), 1072.84 (m), 735.64 (s)

LRMS (ES+): 540 (MH^+)

HRMS (FAB+): 540.3339 ($C_{33}H_{42}N_5O_2$ (MH^+) requires 540.3339)

5.5 Example of 2-D Spectra

H-H and C-H correlation experiments were used in the assignment of NMR spectra for more complex compounds. Taking 6,7-Di{[(1S,2R)-2-hydroxy-1-methyl-2-phenylethyl]amino}-2-methyl-2,3-dihydro-1*H*-benzo[*de*]isoquinoline-1,3-dione **(25)** as an example, typical results obtained from these experiments are shown below.



(25)

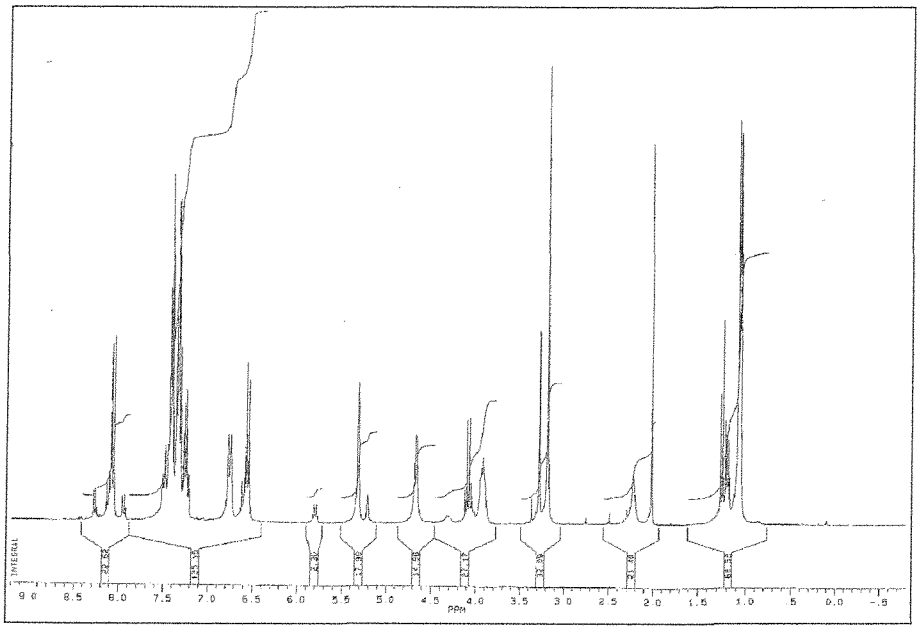


Figure 5.1 ^{1}H NMR spectrum of 6,7-Di{[(1S,2R)-2-hydroxy-1-methyl-2-phenylethyl]amino}-2-methyl-2,3-dihydro-1*H*-benzo[*de*]isoquinoline-1,3-dione **(25)**

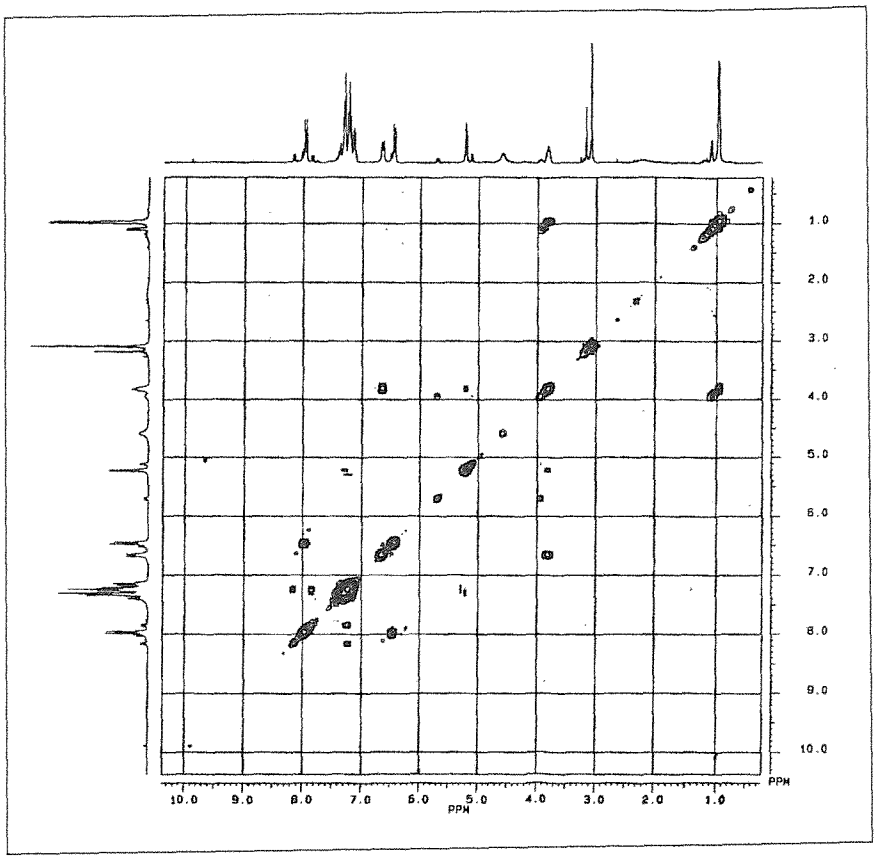


Figure 5.2 H-H correlation NMR spectrum of 6,7-Di{[(1S,2R)-2-hydroxy-1-methyl-2-phenylethyl]amino}-2-methyl-2,3-dihydro-1*H*-benzo[*de*]isoquinoline-1,3-dione **(25)**

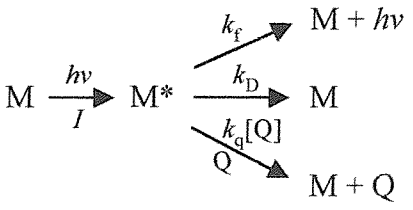
Chapter Six

Appendix

Appendix 1

The Sterm-Volmer Equation

Consider the situation where an excited species fluoresces, decays non-radioactively or is quenched, with rate constants k_f, k_D and k_q respectively:



Applying the steady-state approximation to the concentration of M* gives:

$$I = [M^*](k_f + k_D + k_q[Q])$$

$$\Phi_f = \frac{k_f[M^*]}{I} = \frac{k_f}{k_f + k_D + k_q[Q]}$$

The quantum yield of fluorescence in the absence of quencher is given by:

$$\Phi_f^0 = \frac{k_f}{k_f + k_D}$$

Hence

$$\frac{\Phi_f^0}{\Phi_f} = \frac{k_f + k_D + k_q[Q]}{k_f + k_D} = 1 + \frac{k_q[Q]}{k_f + k_D}$$

and

$$\frac{\Phi_f^0}{\Phi_f} = 1 + k_q\tau[Q]$$

where t is the lifetime in the absence of quencher.

Appendix 2

Relative Abundance of Isotopes of Selected Metals

The table below shows the abundance and relative abundance of the isotopes of the metals used in the mass spectrometry studies in Chapter 4.

Atomic Number	Element	Nominal Mass	Abundance (%)	Relative Abundance
27	Co	59	100.00	100.00
28	Ni	58	68.27	100.00
		60	26.10	38.23
		61	1.13	1.66
		62	3.54	5.26
		64	0.91	1.33
29	Cu	63	69.17	100.00
		65	30.83	44.57
46	Pd	102	1.02	3.73
		104	11.14	40.76
		105	22.33	81.71
		106	27.33	100.00
		108	26.46	96.82
		110	11.72	42.88
47	Ag	107	51.84	100.00
		109	48.16	94.90

Chapter Seven

References

Chapter Seven

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