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New Approaches to the Design of Redox Catalysts for use in Immunohistochemical Imaging

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A thesis submitted to the University of Wales in accordance with the requirements for the degree of Doctor of Philosophy in the Faculty of Science, Department of Chemistry, University of Wales, Cardiff.

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For Mum and Dad

Abstract

This thesis investigates potential classes of compound for use as immunohistochemical markers. A range of ligands and their corresponding metal complexes have been screened for their ability to catalytically reduce silver ions in a Timm's type reaction.

Chapter two focuses on the preparation and testing of bidentate tertiary phosphine metal complexes. The functionalised ligands, 1,2-bis-diphenylphosphino-4-methoxy-benzene, 1-diphenylphosphino-2[(diphenylphosphino)-methyl]-4-methoxybenzene and the corresponding platinum complexes have been prepared. Platinum complexes of 1,2-bis[diphenylphosphinoethane] were also synthesised. These platinum complexes were found to reduce the silver in times ranging from 5 minutes to in excess of 30 minutes.

Chapter three focuses on the preparation of marker systems based on platinum and palladium complexes of *meso*-tetraarylporphyrins. 5,10,15,20-Tetra(4-hydroxyphenyl)porphyrin and 5,10,15-tri-(4-tolyl)-20-(4-hydroxyphenyl)porphyrin have been prepared from the corresponding anisole and 3,4,5-trimethoxybenzoic acid phenyl ester derivatives. A number of symmetrical and unsymmetrical porphyrins bearing aryl phosphate esters derived from diethyl-4-formylphenyl phosphate and diethyl-4-formyl-2-methoxyphenyl phosphate have been prepared. The reaction of 5,10,15,20-tetra-(4-hydroxyphenyl)porphyrin or 5,10,15,20-tetra-(4-hydroxy-3-methoxyphenyl)porphyrin with chloro diethylphosphate in the presence of either sodium hydride or triethylamine failed to afford the corresponding tetra-phosphorylated products. The reaction between 5,10,15,20-tetra-(4-hydroxyphenyl)porphyrin palladium and chlorodiethylphosphate in the presence of triethylamine afforded a mixture of phosphorylated products. None of the prepared complexes displayed the ability to reduce silver ions in a Timm's type reaction.

Chapter four describes the design and synthesis of 4-phenyl-2,2':6',2"-terpyridines functionalised with a PEG chain terminating with a reactive group suitable for bio-conjugation. 4'-[(2-(2-[2-(1,3-Dioxo-1,3-dihydro-isoindol-2-yl)-ethoxy]-ethoxy)-ethoxy)-phenyl]- 2,2':6',2''-terpyridine was prepared using two methods starting from either 4-hydroxybenzaldehyde or (4-hydroxyphenyl)-2,2':6',2"-terpyridine. Base hydrolysis converted the phthalimde moiety to the free amine which was subsequently treated with 1,5-difluoro-2,4-dinitrobenzene. This ligand was not water soluble. The addition of two morpholinomethyl pendent groups *via* a Mannich reaction did not dramatically increase the solubility in water. A number of transition metal complexes were prepared and tested.

Chapter five presents a novel one-pot synthesis of 'mixed' *N*-arylated 1,4,7-triazacyclononane (tacn) ligands. Using this methodology tacn ligands functionalised with two nitrophenyl groups and either a benzaldehyde (L_{32A}), benzoic acid (L_{32BA}), benzyl ester ($L_{32Benzyl}$), alcohol (L_{32Alc}), tolyl (L_{32T}) or napthoquinone (L_{32Q}) groups have been prepared in yields ranging between 39 and 64 %. Hydrogenation of L_{32T} afforded L_{32TR}. Reaction with Ni(II) perchlorate afforded the air stable complex $[Ni^{II}(L_{32TR})][ClO_4]_2$, which was found to catalyse the reduction of aqueous silver ions *via* a Timm' type reaction in 3 minutes. Attempts to attach both a porphyrin and a terpyridine sub-unit are also presented.

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Contents

Abbreviations

{}	Decoupled
Å	Angstrom
AP	Alkaline phosphatase
APCI	Atmospheric pressure chemical ionisation
Ar	Aromatic
β	Equilibrium constant
br	Broad
^{13}C	Carbon 13 isotope
^{o}C	Degrees centigrade
cm^{-1}	Reciprocal centimetres/ wavenumber
$CDCl_3$	Deuterated chloroform
CD_3CN	Deuterated acetonitrile
COD	1,5-Cyclooctadiene
δ	NMR chemical shift
Δ	Crystal field splitting
d	Deuterium
d	Doublet
dd	Double doublet
DAB	Diaminobenzidine
DCM	Dichloromethane
DDQ	2,3-Dichloro-5,6-dicyano-1,4-benzoquinone
DFDNB	1,5-Difluoro-2,4-dinitrobenzene
DMAP	dimethylaminopyridine
DMF	*N,N*-dimethylformamide
DMSO	Dimethylsulfoxide
dppm	1,2-Bis-(diphenylphosphino)methane
dppe	1,2-Bis-(diphenylphosphino)ethane
D_2O	Deuterated water
en	Ethylene diamine
eq	Equivalent
ESI	Electron spray ionisation
Enz	Enzyme
Et	Ethyl
eV	Electron volt
^{19}F	Fluorine 19 isotope
g	Grams
h	hour
^{1}H	Proton
HOMO	Highest occupied molecular orbital
HRMS	High resolution mass spectrometry
HRP	Horseradish peroxidase
Hz	Hertz
Ig	Immunoglobulin
IR	Infrared
J	Coupling constant
kJ	kilojoule
L	Litre/ ligand
LUMO	Lowest unoccupied molecular orbital
m	Multiplet
M	Metal

M_r	Molecular weight
MALDI	Matrix assisted laser desorption ionisation
Me	Methyl
MeCN	Acetonitrile
Mes	Mesityl
mg	Milligram
MHz	Mega hertz
mL	Millilitre
mmol	Millimole
MS	Mass spectrometry/spectrum
m/z	Mass/charge ratio
NHS	*N*-hydroxysuccinimide
nm	Nanometre
NMP	*N*-methylpyrrolidinone
NMR	Nuclear magnetic resonance
Nuc	Nucleophile
O	Oxidant
^{31}P	Phosphorus 31 isotope
PEG	Polyethylene glycol
Ph	Phenyl
Por	Porphyrin
ppm	Parts per million
py	Pyridine
RT	Room temperature
s	Singlet
t	Triplet
tacn	1,4,7-Triazacyclononane
terpy	2,2':6',2"-Terpyridine
θ	angle
THF	Tetrahydrofuran
TMS	Trimethylsilane
Tol	Tolyl
TPP	Meso-tetraphenylporphyrin
Ts	Tosyl
UV	Ultra violet
Vis	Visible

Chapter 1

Introduction

1.1 Overview

Diagnosing disease is the first step in treatment. This can be achieved by the use of either invasive or non-invasive techniques. This work describes the preparation of markers suitable for use as immunohistochemical imaging agents. This technique is performed on tissue samples taken from the body.

1.2 Introduction

The environment that surrounds us contains a vast array of infectious agents such as viruses, fungi, protozoa and multicellular parasites. All of these can bring about disease and if go unchecked can ultimately result in the death of the host. However, most infections are only short lived owing to the immune system which has evolved to combat infectious agents. The human immune system is composed of approximately 10^{12} cells that protect against foreign invaders.

Substances that are foreign to the human body, for example bacteria, viruses and other infectious agents are known as antigens (derived from the term *anti*body *gen*erator) and are recognised by the white blood cells (leucocytes) as invaders. The immune response consists of two phases, firstly the recognition of an antigen and secondly a reaction to eradicate it. There are two classes of immune response being the innate or non-adaptive immune responses and adaptive immune responses. These immune responses are created by leucocytes. There are several different types of leucocytes, the most important being lymphocytes, which identify the foreign object and phagocytes, which destroy invaders (figure 1.1).

Type of Lymphocyte	*Role*
B lymphocyte or B-cells	Make antibodies which are then released into the bloodstream
T lymphocytes or T-cells	Make molecules similar to antibodies. These remain attached to the plasma membrane and are known as T-cell antigen receptors (TCRs).

Figure 1.1: Types of lymphocytes and their role.

The lymphocytes synthesise soluble glycoproteins, known as immunoglobulins (Ig) which can bind to antigens. Some immunoglobulins are carried on the surface of B-cells where they act as receptors for specific antigens (T-cell antigen receptors, TCRs) whilst others circulate in the blood and tissue fluids (antibodies, Ab). There are five classes of immunoglobulin molecules; IgG, IgA, IgM, IgD and IgE, differing in size, charge, amino acid composition and carbohydrate content (figure 1.2). Each B-cell only produces one type

of antibody and the immune system relies on there being so many different types so at least one will bind to the antigen. The human body can produce an estimated 100 million different antibodies enabling the recognition of all kinds of antigens.

Class of Ig	*%*	*Role*
IgG	76	The major serum Ig. Some can cross cell membranes (e.g. from mother to foetus through the placenta).
IgA	15	Antibody responsible for protecting the body surfaces (e.g. mucous membrane of the gut). Is also found in mother's milk
IgM	8	The first antibody that is produced in response to an infection
IgD	1	Thought to be involved in regulation of antibody synthesis
IgE	0.002	Maybe responsible for exaggerated immunological reaction to dust and pollen in allergy sufferers

Figure 1.2: The five classes of immunoglobulins.

The immunoglobulins are made up of four polypeptide chains which are held together by covalent and non-covalent interactions. All of the immunoglobulins consist of two identical heavy chains (molecular weight range of 50,000-75,000) and two identical light chains (molecular weight ~ 25,000). However, antibodies are not all the same. The first 100 amino acids at the *N*-terminus of the polypeptide chains varies from one antibody to another, this region is known as the hypervariable or variable region and generates binding regions of differing shape and charge (figure 1.3). Hence antibodies are highly specific and will only bind to one particular antigen.

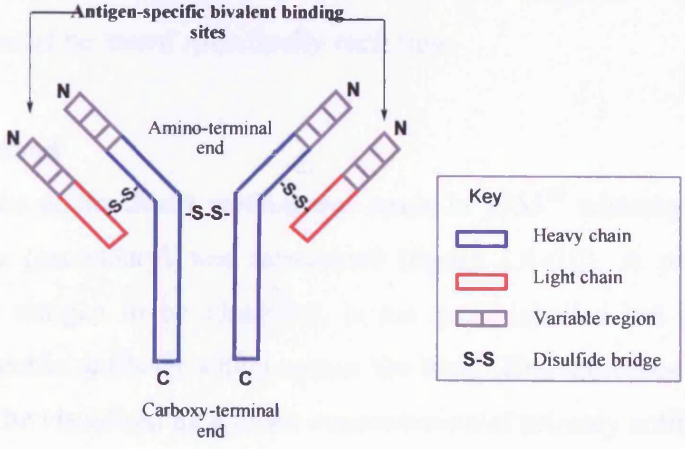

Figure 1.3: A schematic diagram of a typical Ig antibody.

Monoclonal antibodies are pure antibodies of a defined specificity and can be mass-produced in laboratories from a single clone and that recognises only one antigen. They are

typically made by fusing a normally short-lived, antibody producing B cell to a fast growing cell. The resulting hybrid cell, multiplies rapidly creating a clone that produces large quantities of the same antibody. The high specificity of monoclonal antibodies makes them powerful biological tools in medical diagnostics. However, to utilize them fully requires a means of detecting them at low concentration. This is usually achieved by labelling the antibody in a number of ways, such as enzyme labelling or biotin labelling. The technique of using labelled antibodies as specific probes for the localisation of tissue constituents (e.g. antigens) is known as *immunohistochemistry* or *immunocytochemistry.*[1]

The first attempts to label an antibody were made in 1934 using ordinary dyes.[2] However, this type of label could not be visualised under the microscope. In 1941 Coons and his co-workers[3] developed a method of locating tissue constituents by labelling a specific antibody with a fluorescent dye and visualising it using a fluorescence microscope.

1.3 Antibody Labelling

1.3.1 Direct Method

This is a one step reaction where a primary antibody is labelled directly with a marker (figure 1.4-(i)). It was first introduced in the 1941 when a primary antibody (specific to a particular antigen) was labelled with fluoroscein isocyanate.[3] Since then many other labels have been used to directly label antibodies, such as enzymes,[4-6] gold particles[7] along with other fluorochromes.[8,9] The direct labelling method is one-step and simple. However, it is limited since it provides no immunological amplification of the colour signal relative to the number of antigenic sites. It can also be an expensive technique since the primary antibody must be raised specifically each time.

1.3.2 Indirect Method

Modification of the direct method was made in 1955[10] whereby a second layer of antibody molecule (secondary) was introduced (figure 1.4-(ii)). A primary antibody is raised against the antigen to be identified, is not itself labelled but is conjugated to a secondary, non-specific antibody which carries the label. This increases the sensitivity and allows antigens to be visualised by a lower concentration of primary antibody.

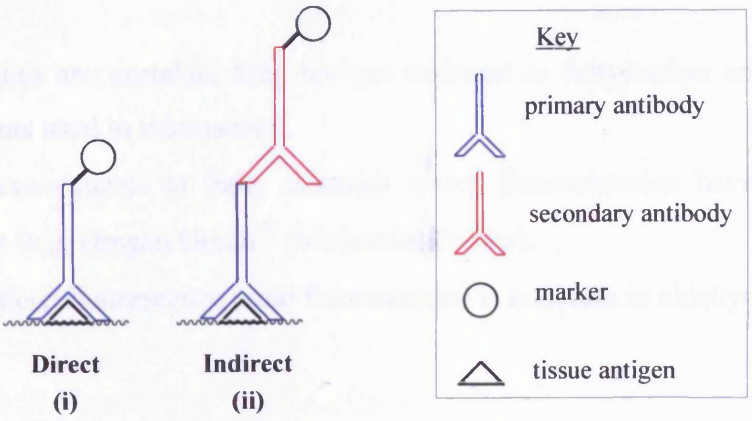

Figure 1.4: Schematic diagram illustrating the (i) direct and (ii) indirect antibody labelling methods.

1.4 Types of Label

1.4.1 Fluorochromes

The first fluorescent dye to be attached to an antibody was fluoroscein isocyanate[3,10] but this was later replaced by the more stable isothiocyanate derivative.[11] Fluoroscein isothiocyanate is still widely used today and emits a bright apple green colour when excited at a wavelength of 490 nm (figure 1.5). Other examples of fluorescent markers include the rhodamine derivatives which fluoresce red (figure 1.5). The orange to red luminescence of rhodamine derivatives is in stark contrast to the green of fluoroscein, hence these two types of fluorochrome are commonly used together in double immunofluoroscence staining.

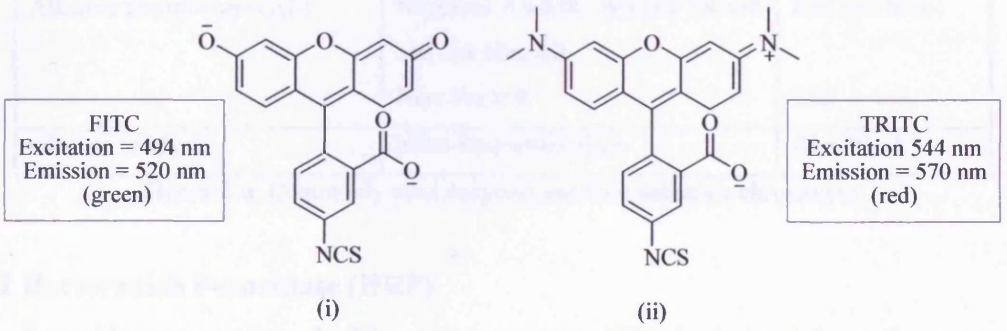

Figure 1.5: Labels used in immunofluoroscence: (i) fluoroscein-5- isothiocyanate (FITC) and (ii) tetramethylrhodamine-5-isothiocyanate.

Advantages:

- Provides an instantly visible label, with excellent contrast when used against dark, non-fluorescent backgrounds.
- Are available in a variety of colours so can be used in double labelling techniques.

Disadvantages:

- Conjugates are unstable; they are not resistant to dehydration and solvent-based mountants used in microscopy.
- Fluorescence tends to fade, although newer fluorochromes have overcome this problem (e.g. Oregan Green™ (Molecular Probes).
- Endogenous fluorescence (auto fluorescence) is common in aldehyde fixed tissue.

1.4.2 Enzymes

The instability of immunofluorescence led to the development of a more permanent type of preparation using enzymes. The catalytic activity of an enzyme can be used to convert substrate molecules into chromogenic or fluorescent products which can then be detected *via* microscopy (figure 1.6). Any endogenous enzyme must be inhibited so that it cannot react with the substrate. Immunoenzyme methods can be used on any type of sample preparation providing permanent results. Horseradish peroxidase (HRP) was the first enzyme to be conjugated to an antibody;[4,5] followed later by alkaline phosphatase (AP)[6] and glucose oxidase. Many antibodies are available from commercial suppliers already labelled with HRP and AP.

Enzyme Label	*Substrate (chromogen)*	*Colour reaction*
Horseradish peroxidase (HRP)	Diaminobenzidine (DAB)	Brown
	3-Amino-9-ethylcarbazole (AEC)	Reddish
Alkaline phosphatase (AP)	Naphthol AS-MX/ fast red TR salt and fast blue BB	Red and Blue
	New Fuchsin	Red
Glucose oxidase	Nitro-blue tetrazolium	Blue-black

Figure 1.6: Commonly used enzymes and their substrate/chromogen.

1.4.2.1 Horseradish Peroxidase (HRP)

Peroxidase is a stable, highly active enzyme. HRP is derived from the roots of the horseradish. It has a molecular weight of 40,000 and catalyses the reaction of its substrate, hydrogen peroxide with certain organic, electron-donating substrates to yield highly coloured products. Diaminobenzidine[12] is the most common chromogen used in the immunoperoxidase technique, it polymerises upon oxidation with H_2O_2 producing a brown colour. The DAB stain can be further enhanced by a variety of agents such as osmium.[13] It has the advantage that it is stable in organic solvents, however it has been shown to be

carcinogenic.[14] Non-carcinogenic alternatives such as 3-amino-9-ethylcarbazole[15] have been introduced but these provide less satisfactory results.

Advantages

- Polymerisation of DAB is rapid and the deposited coloured product is visible within a few minutes.
- Polymerised DAB adheres firmly at the site of the histochemical reaction.
- DAB can be amplified by either metals or physical developers.

Disadvantages

- Peroxidase is endogenous to many biological tissues.
- Many non-peroxidate enzymes and pseudoperoxidate factors (e.g. haem containing proteins) can catalyse the reaction.
- The reaction destroys the enzyme and both H_2O_2 and DAB are chemically unstable and toxic.
- The chemistry of DAB polymerisation and its reaction with metals is poorly understood.
- The quality of DAB varies between suppliers making reproducibility difficult.

1.4.2.2 Alkaline Phosphatase (AP)

There are two forms of alkaline phosphatase in mammals, one form present in a variety of tissues and the other form found only in the intestines. The AP isoenzymes have an optimal activity between pH 8 to 10 and cleave the associated phosphomonoester groups from a wide variety of substrates (figure 1.7). AP was first introduced for quantitative work with the enzyme-linked immunosorbent assay (ELISA) technique, providing a soluble, coloured end-product of reaction that could be measured with a spectrophotometer. The enzyme can also react with a chromogen (azo dye) to produce a coloured precipitate suitable for immunocytochemistry.[16] The isoenzyme most commonly used in immunocytochemistry is derived from calf intestine and has a molecular weight of 140,000. All of the isoenzymes except the intestinal one can be inhibited (blocked),[17] therefore methods depending on AP as a label are not suitable for immunostaining intestinal tissue.

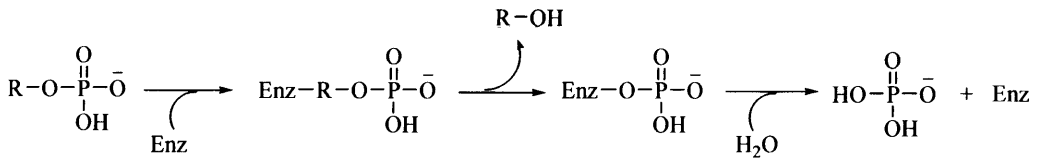

Figure 1.7: Enzymatic action of alkaline phosphatase.

Advantages

- AP displays a broad substrate specificity.
- Endogenous enzyme is easily inhibited.
- Highly coloured end-products can be viewed by light microscopy.
- AP is a useful alternative when specimens have a high endogenous peroxidase activity e.g. in bone marrow or the spleen.

Disadvantages

- Cannot be used in intestinal tissue.
- Coloured end products cannot be made electron dense or amplified with physical developers.
- Some end products are alcohol soluble therefore aqueous mountants are required.

1.4.3 Colloidal gold

The use of colloidal gold particles in immunocytochemistry was first introduced in 1971.[7] It was originally developed for use with an electron microscope[7] and remains the label of choice in this field since it is made in convenient well-defined sizes and is rendered immunoreactive by adsorbing antibodies. Colloidal gold can be seen under a light microscope as reddish particles but only when highly concentrated conjugates are applied. Such suspensions are unstable. Alternatively, a diluted conjugate concentration can be used and enhanced by silver amplification producing a black colouration.[18]

Advantages

- Colloidal gold is electron dense and can be easily prepared in different sizes (3-100 nm);[19] therefore multiple labelling of various binding sites can be performed.
- The amplification with silver produces one of the most sensitive immunocytochemical methods. None of the reagents are toxic and high dilutions of antibodies can be used thus increasing the sensitivity.

Disadvantages

- The particular nature of colloidal gold limits penetration into fixed tissues, although smaller gold particles (1-3 nm) have been developed e.g. Nanogold™ (1.4 nm).[20]
- Protein A, which is used to bind the gold particles to the antibody, has a high affinity for both binding sites of antibodies (F_{ab} and F_c),[21] this may lead to cross linking and a loss of specificity.

- The amplification with silver requires great expertise and the silver reduction is less pronounced than other amplified markers.

1.5 Silver Amplification

Silver has been used in photography since the early nineteenth century. A photographic emulsion contains silver halide crystals. On exposure to photons (light), a thin layer of metallic silver forms on the surface of these crystals. The development can be achieved by two different types of developer solutions, a chemical developer which is made up of a reducing agent and dissolves the silver halide crystals or a physical developer which contains a source of ionic silver in addition to the reducing agent. With both types of developing solutions the end-product is a black deposit of pure silver.

1.5.1 The Use of Silver in Biology

In 1911, Liesegang[22] and later in 1921, Liesegang and Rieder[23] applied the principles of photographic 'physical development' to histology. This technique known as 'physical development' is now often referred to as autometallography. The autometallographic technique (AMG) is based on the ability of small clusters of gold and/or silver atoms, mercury bismuth and zinc sulfide/selenide molecules to catalyse the reduction of silver ions from a developing solution into silver metal.[24] This technique was originally developed as a new way of increasing the staining intensity of silver impregnated tissue.[23] In 1935 Roberts[25] published a photochemical method for the *in situ* detection of trace amounts of gold deposited in gold-exposed animals. In his method, the tissue-bound gold was enlarged to microscopic dimensions by the deposition of silver.

1.5.2 Timm's Sulfide Silver Staining Method

In 1958, Timm[26] generalised a finding by the photographic scientist Zeiger[27] that silver sulfide accumulations in photographic emulsions could be silver-enhanced by physical development. Timm noted that the metal sulfides were always more catalytic, with respect to silver reduction, than their respective metals.[26] The method described by Timm launched a breakthrough for physical development in biology. The methodology has been since modified by Danscher[28] and has been used to visualise a variety of metals in brains and other tissues.[29] Among these are the trace metals essential for life, such as Zn, Cu, Fe, Co, and Ni, as well as toxic metals such as Hg, Cd, Pb, As, Bi, Au and Ag. The principal of the technique is based on sulfide-precipitation of metals in tissue, followed by a physical

development. During the latter stage the metal sulfides catalyse the reduction of silver ions by reducing agents.

1.5.3 Physical Developers

In biology, physical developers have been important in the detection and visualisation of both endogenous and exogenous sites of metal incorporation in biological tissue.[30] Endogenous metals such as mercury and silver can be visualised directly by physical developers[31,32] whereas many others are converted to insoluble sulfides which have been shown to rapidly deposit reduced silver from physical developers.[22,26,28,29,33-38] Exogenous metals which are introduced as markers of histochemical, immunohistochemical, *in situ* hybridisations and immunoblot reactions have also been identified using physical developers. Examples of these markers include colloidal gold[18,39,40] and polymerised DAB (a weak catalytic effect on physical developers[41-43]) although a greater amplification is observed in the presence of non-noble transitional elements.[44,45]

Physical developers contain a reducing agent and a soluble silver salt. The most basic physical developer, for example a solution of silver nitrate and hydroquinone, is very unstable and the deposition of metallic silver occurs almost immediately upon exposure to light. Today, physical developers contain buffers to control the pH. 'Protective colloids' such as gum arabic and gelatine are also added which inhibit the 'direct collision' of the silver ions and reducing molecules. Several formulations of physical developers have been developed to overcome the problems associated with self-nucleation upon exposure to light.

1.5.3.1 Danscher

Danscher[32,46] has shown that the availability of silver ions can be controlled by using organic silver salts e.g. silver lactate as opposed to inorganic salts. Such salts ionise less efficiently in solution and hence slow down the reaction. The developer solutions are at an acidic pH of 3-3.5. This has been found to slow down the development process and is controlled by buffers such as citric acid/citrate or boric acid/borate. Gum arabic is used as the sticky, protective colloid and slows down the collision rates between silver ions and reducer molecules.

Disadvantages: Whilst the inclusion of gum arabic lessens the need for total light exclusion, silver lactate is photolabile. Hence solutions should be protected from light, although this is often impractical in a histological setting. In addition a low pH may damage the tissue.

1.5.3.2 Gallyas

The physical developer formulated by Gallyas employs colloidal solutions of tungsten in order to protect the silver ions from both the light and coming into contact with the reducer molecules. This delays the blackening of the solutions for up to 30 minutes. The developer is weakly acidic (pH 5) which makes it suitable for many applications. Despite the relative light insensitivity of this solution, it does have disadvantages.

Disadvantages: Silver nitrate is used as the source of silver ions; it is not very robust and is poorly affected by contamination. The developer solution is not simple to make and is made up of three separate solutions. One of these needs to be freshly prepared prior to use.

1.5.3.3 Newman and Jasani

This is a neutrally-buffered, light insensitive physical developer.[47] It is made, just before use, by mixing together equal amounts of separate buffered silver and reducer solutions. Silver nitrate is used as the ionic source of silver ions. The silver ions and reducer molecules are kept apart by the introduction of silver ion chelators into both the silver stock and the reducer stock. When high molarity of tris[hydroxymethyl]aminomethane (Tris buffer) is combined with sodium sulfite and sodium thiosulfate, it prevents the unreduced silver ions being taken up by background tissue and controls the rate at which the reduced silver is deposited at the target site. Alcohol buffered pyrogallol is used as opposed to hydroquinone as the reducing agent. Pyrogallol is a very powerful reducing agent; it is soluble in alcohols and is chemically stable. In addition it has been shown that the addition of alcohols to a pyrogallol developer reduces self-nucleation and doubles the time it takes for light to affect the solution.[47] The solutions are found to be very stable with a bench life of up to two years, although adjustments in the pH are necessary. Upon exposure to light, the physical developer remains clear for at least 55 minutes at room temperature, much longer than previous formulations.

1.6 Design and Development of an Improved Marker System

The limitation of the current immunohistochemical techniques for the detection of antigens is the choice of chemical marker. Little investigation has been made in the development and design of alternative marker systems. Enzyme labels provide the greatest versatility of the immunohistochemical methods. Currently the enzyme labels horseradish peroxidase and alkaline phosphatase are detected by a chemical reaction which produces a

highly coloured end-product. AP is often the enzyme of choice as it displays a broad substrate specificity and the endogenous enzyme is easily inhibited.

1.7 Proposal

Our approach is based on an indirect antibody labelling method to identify the site of interest (figure 1.8). This technique involves both primary and secondary antibodies. Primary antibodies are specific and will only bind to their corresponding antigen, whereas secondary antibodies bind to the non-specific site of the primary antibody. The secondary antibody has an alkaline phosphatase enzyme label attached to it. Thus, the secondary antibody carrying this label may be localised to specific antigens expressed by cells of interest. When a marker substrate is added to the system it will undergo enzymatic cleavage causing it to precipitate onto the cell surface. Commonly, aqueous silver ions are added which are catalytically reduced by the marker and are deposited onto the surface of the cell. Since the secondary antibody is bound to alkaline phosphatase, cells that express the antigen of interest may be identified by this method.

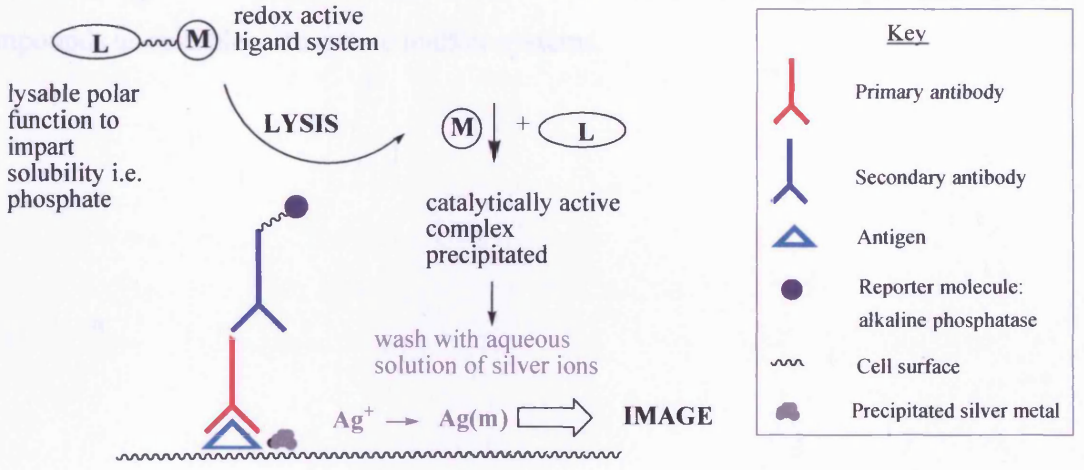

Figure 1.8: Schematic diagram of proposed system.

1.8 Aims

We intend to prepare new marker compounds which contain a lysable enzyme substrate and are catalytically active for the reduction of silver ions *i.e.* can be silver-enhanced. Incorporation of a functionalised ligand backbone will allow an initially water soluble metal complex to become water insoluble and precipitate on the cell surface upon enzymatic cleavage of the functional moiety, thus localising the redox catalyst at the site of interest. When the tissue section is washed with a physical developer, the redox-active

compound will reduce silver ions to silver metal which will be deposited locally at the site of interest.

The metal-based marker substrate should be:

- Water soluble to allow administration to tissue sample.
- Incorporate a lysable substrate of alkaline phosphatase *i.e.* phosphomonoester.
- Form kinetically inert complexes.
- Must not have an affinity for non-target substances.

The marker should be:

- Insoluble in histological preparations *i.e.* water at pH 6-9.5, ethanol, xylene.
- Strictly localised at the target site.
- Strongly adhere to the target site.
- Support redox catalysis *i.e.* be amplifiable with a physical developer to enable visualisation *via* light microscopy.

Little is known about the exact chemistry of silver reduction in a physical developer (a Timm's type reaction). The aim of this thesis is to investigate potential classes of compounds as suitable, alternative marker systems.

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Chapter 2

Markers based on bidentate phosphine ligands

Introduction

This chapter presents the preparation of platinum complexes of bidentate tertiary phosphines. The prepared compounds were screened to establish whether they were suitable catalysts for the reduction of silver in a Timm's type reaction.

2.1 The Chelate Effect

Chelating ligands are those with two or more donor atoms available to simultaneously attach to a metal ion. The stability constants of metal complexes containing chelating ligands are found to be higher than for their analogous non-chelate counterparts. An example is given in figure 2.1 (where **en** is the bidentate ligand ethylenediamine).

$$[Cu(OH_2)_6]^{2+} + 2\mathbf{NH_3} \rightleftharpoons [Cu(OH_2)_4(\mathbf{NH_3})_2]^{+} + 2H_2O \quad (1)$$

$\beta_2 = 10^{7.7}$; $\Delta H^o = -46$ kJ mol^{-1}; $\Delta S^o = -8.4$ J K^{-1} mol^{-1}; at 298 K $\Delta G^o = -46.47$ kJ mol^{-1}

$$[Cu(OH_2)_6]^{2+} + \mathbf{en} \rightleftharpoons [Cu(OH_2)_4(\mathbf{en})]^{2+} + 2H_2O \quad (2)$$

$\beta_1 = 10^{10.6}$; $\Delta H^o = -54$ kJ mol^{-1}; $\Delta S^o = +23$ J K^{-1} mol^{-1}; at 298 K, $\Delta G^o = -61.17$ kJ mol^{-1}

Figure 2.1: Illustration of the chelate effect.

The replacement of two coordinated water molecules by one ethylene diamine molecule forms a complex with a larger stability constant than the complex formed in the analogous reaction with two ammonia molecules. The difference in the stability constants can be seen in the enthalpy and entropy values for the two reactions. Both reactions have similar enthalpy values so the difference in stability constants is mostly attributed to the entropy value.

2.2 Hard-Soft Acid-Base Classification (HSAB)[1]

The stability constant of a metal complex also depends upon the nature of both the ligand and the metal ion. It has been found that certain types of ligand donor atoms form stronger complexes with certain metal ions *i.e.* have higher stability constants. This gives rise to a broad classification of metal ions according to the type of ligand they form the strongest complexes with. The smaller, highly charged metal ions are known as *hard acids* and form the strongest complexes with the smaller electronegative donors or *hard bases.* Whereas, the larger more polarizable metal ions are known as *soft acids* and form the

strongest complexes with the larger, less electronegative donor atoms or *soft bases*. For example, tertiary phosphines are classified as soft bases and will therefore form the strongest complexes with metal ions such as Pt^{2+}, Pd^{2+} and Rh^{+}. Other examples are illustrated in figure 2.2

(hard)	*Borderline*	*(soft)*
Metal ions	**Metal ions**	**Metal Ions**
Mn^{2+}, Sc^{3+}, Cr^{3+}, Fe^{3+}	Fe^{2+}, Co^{2+}, Ni^{2+}, Cu^{2+}	Ag^{+}, Pd^{2+}, Pt^{2+}, Rh^{+}
Ligands	**Ligands**	**Ligands**
R_2O, ROH, OH^{-}, SO_4^{2-}, Cl^{-}	Br^{-}, py	R_2S, $\mathbf{R_3P}$, CO, CN^{-}, I^{-}

Figure 2.2: Examples of hard and soft metal ions and ligands.

2.3 Square Planar Complexes

The energy level diagram for a square planar complex can be derived from that of an octahedral complex by removing two of the mutually *trans* ligands out to infinity. For d^8 ions a square planar geometry is favoured when the crystal field splitting (Δ) is greater than the pairing energy of placing two electrons in a single orbital. A square planar geometry is common to metals with a d^8 electron configuration (e.g. Pt^{2+}, Pd^{2+}) when bound to a strong (high) field ligand (figure 2.3).

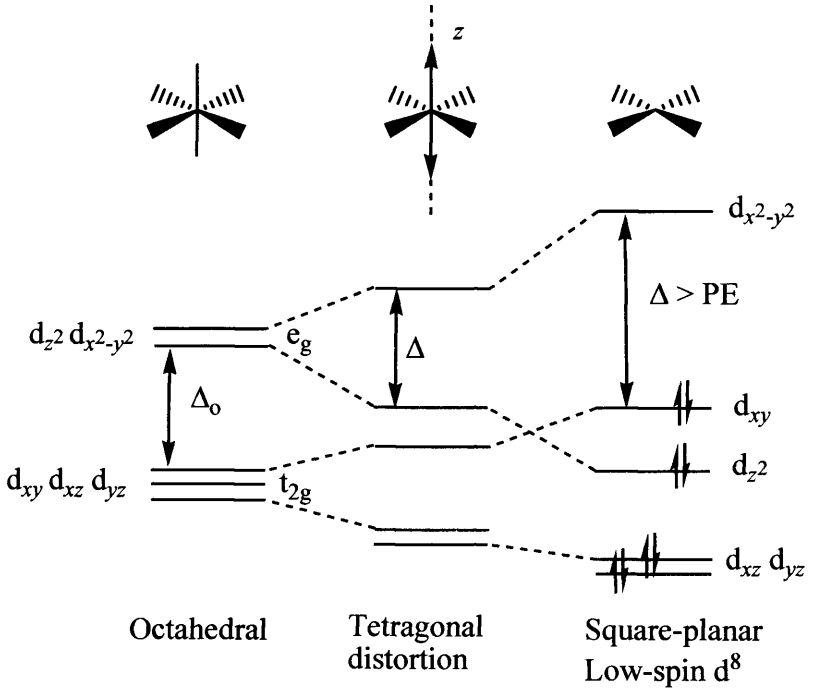


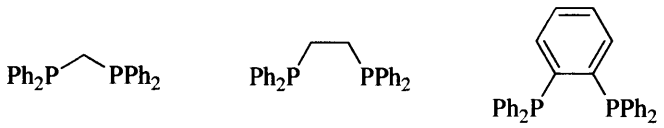
Figure 2.3: Energy diagram illustrating the effect of a square planar crystal field on the energies of d orbitals.

A strong field ligand is one which imposes a large crystal field splitting (Δ) whereas a weak (low) field ligand imposes a smaller crystal field splitting. Examples of strong field

ligands include CO, PR_3 and NO_2^-, these ligands are good π-acceptors. Halides and OH^- are examples of weak field ligands. The oxidation state and position of the metal in the periodic table also affect the crystal field splitting. A higher oxidation state and a higher atomic weight increase the crystal field split. For example, a phosphine-platinum complex will be square planar.

2.4 Phosphine Ligands

Tertiary phosphines (PR_3), in particular mono and diphosphines are some of the most extensively used ligands in both inorganic and organometallic chemistry.[2-5] The most widely reported diphosphine ligands are of the type $Ph_2P(CH_2)_nPPh_2$ where n = 1 (dppm) and n = 2 (dppe). These ligands are able to coordinate in a monodentate, chelating or bridging mode to a metal centre through the lone pair of electrons on the phosphorus atom. There are also reports of diphosphine ligands where n is greater than two[6-8] or where the phosphorus atoms are linked by an aromatic system[9,10] (figure 2.4).



dppm **dppe** **dppb**

Figure 2.4: Examples of diphosphine ligands; 1,2-bis(diphenylphosphino)methane (dppm), 1,2-bis(diphenylphosphino)ethane (dppe) and 1,2-bis(diphenylphosphino)benzene (dppb).

Metal-phosphine complexes are of immense industrial importance in the area of homogeneous catalysis. One of the most studied catalytic systems is the rhodium (I) complex $[Rh(PPh_3)_3Cl]$ (Wilkinson's catalyst[11]) which is used as a hydrogenation catalyst. Other metal-phosphine complexes, particularly those of Ru, Rh and Pd play a crucial role in homogeneous catalysis for a wide range of transformations.

2.5 Phosphine Properties

An important feature of phosphine ligands is the ability to alter their properties by systematically changing the nature of the R group substituents. This allows for the fine tuning of both the electronic and steric properties of the ligand.

2.5.1 Electronic Considerations

The phosphine metal bond is synergic in nature (figure 2.5) which enables the phosphine ligand to act as both a σ base and π acid, thus enabling them to stabilise metals in low oxidation states.

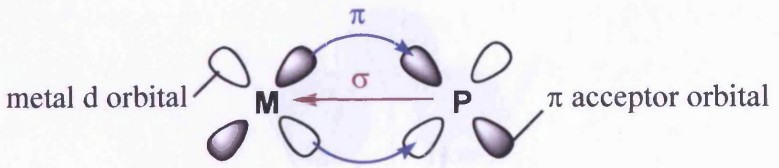


Figure 2.5: Schematic diagram illustrating the synergic nature of the M-P bond.

σ-Bonding: Donation of the lone pair of electrons on the phosphorus atom to the metal results in σ bonding. If the R group is electron releasing (e.g. in PBu^t_3) then the ligand acts predominantly as a σ type donor *i.e.* the lone pair of electrons on the phosphorus is the major component of the resulting P-M bond. The strength of the σ bond depends on the Lewis acidity of the metal where a more electropositive metal results in a stronger σ bond. The σ bonding ability can be equated to pK_a values, where a higher pK_a value indicates more dominant σ bonding (figure 2.6).

Tertiary phosphine	pK_a	Tertiary phosphine	pK_a
P^tBu_3	11.40	$P(4\text{-}MeC_6H_4)_3$	3.84
PCy_3	9.65	PPh_3	2.73
$P(4\text{-}MeOC_6H_4)_3$	4.75	$P(4\text{-}FC_6H_4)_3$	1.97

Figure 2.6: Basicities of selected tertiary phosphines[12]

π-bonding: As R becomes more electronegative the π-acceptor properties of the phosphine ligand increase. The π character arises from back-donation from the metal to the phosphine. The classical view is that the electron density is donated from the metal *d*-orbital into the vacant phosphorus *d*-orbital.[13] An alternative view however, is that the electron density is donated from the metal *d*-orbitals into the P-R σ^* (antibonding) orbitals.[14-17]

2.5.2 Steric Effects

Steric factors frequently dominate, particularly with bulky ligands. These effects contribute towards the stereochemistry, reactivity and structure of metal-phosphine complexes. The steric bulk of a coordinated phosphine can be measured using Tolman's cone angle.[4] This is defined as the angle (θ) at the metal atom of the cone swept out by Van der Waals radii of the groups attached to the phosphorus upon rotation of the M-P bond (figure 2.7).

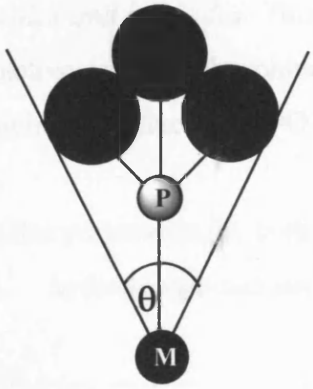


Figure 2.7: Tolman's cone angle (θ).

Values of θ are dependent on the interatomic distance between M and P. The cone angle can be used to explain the lability of phosphine complexes, where in general a larger cone angle results in a more labile phosphine ligand. Examples of the cone angles for tertiary phosphines are illustrated in figure 2.8.

Tertiary phosphine	Cone Angle	Tertiary phosphine	Cone Angle
$PMes_3$	212	PPh_3	145
$P(o\text{-}Tol)_3$	194	PPr_3	132
P^tBu_3	182	PCl_3	124
Pcy_3	170	PMe_3	118

Figure 2.8: Table of cone angles (θ) for selected tertiary phosphines.

2.6 Methods of Preparation

There are three main methods for the preparation of bi and polydentate tertiary phosphines (figure 2.9):[18]

i. *Reaction of organometallic reagents and halophosphines*: Grignard reagents are mainly used for the preparation of alkylphosphines and organolithium reagents are used for the preparation of arylphosphines. Organolithium reagents are more reactive than Grignard reagents and are therefore added to the halophosphine at low temperatures.

ii. *Nucleophilic substitutions using metal phosphides*: Alkali-metal diphenylphosphides ($MPPh_2$) can be prepared in a number of ways; by the cleavage of a phenyl group in triphenylphosphine by lithium, sodium or potassium, from chlorodiphenylphosphine and the alkali metal or from diphenylphosphine and butyllithium. The leaving group on the organic precursor is usually a halide, tosylate or mesylate.

iii. *Reduction of phosphine oxides and sulphides*: This presents a useful method for the preparation of optically active tertiary phosphines. Powerful reductants such as $LiAlH_4$ and $HSiCl_3$ are required to reduce the P=O bond owing to its strength.

More recent methods for the preparation of both symmetrical and unsymmetrical tertiary phosphines include hydrophosphinations[19,20] and metal catalysed phosphinations.[21,22]

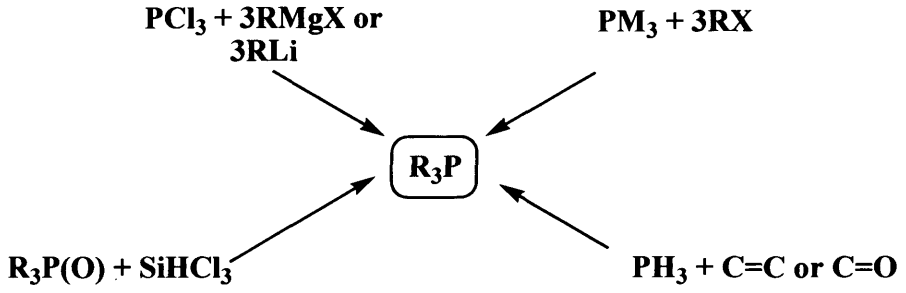
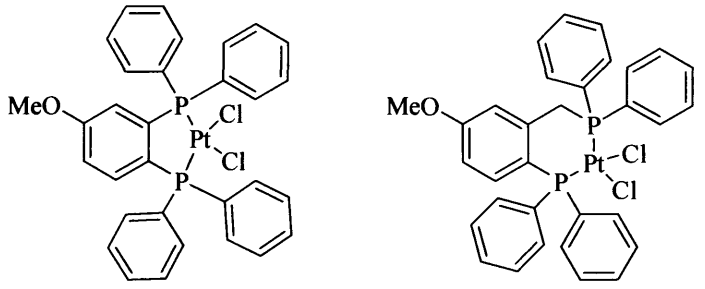


Figure 2.9: Common methods for the preparation of tertiary phosphines.

2.7 Aims

Previous work within our group has illustrated that bidentate phosphine complexes of platinum (II) show potential as catalysts for the reduction of silver in a Timm's type reaction. The aim of this work was to synthesise simple bidentate phosphine ligand complexes (figure 2.10) which are functionalised, such that they can be modified to an enzyme substrate (e.g. alkaline phosphatase). The activity of these complexes was compared with a series of platinum complexes based on bis(diphenylphosphino)ethane.



[Pt(2.4)] **[Pt(2.7)]**

Figure 2.10: Target Ligands

Results and Discussion

2.8 Synthesis of 1,2-Bis-diphenylphosphino-4-methoxy-benzene [2.4]

The diphosphine ligand **[2.4]** was synthesised in four steps (figure 2.11), from the commercially available nitro compound, 4-bromo-3-nitroanisole in an overall yield of 8 %.

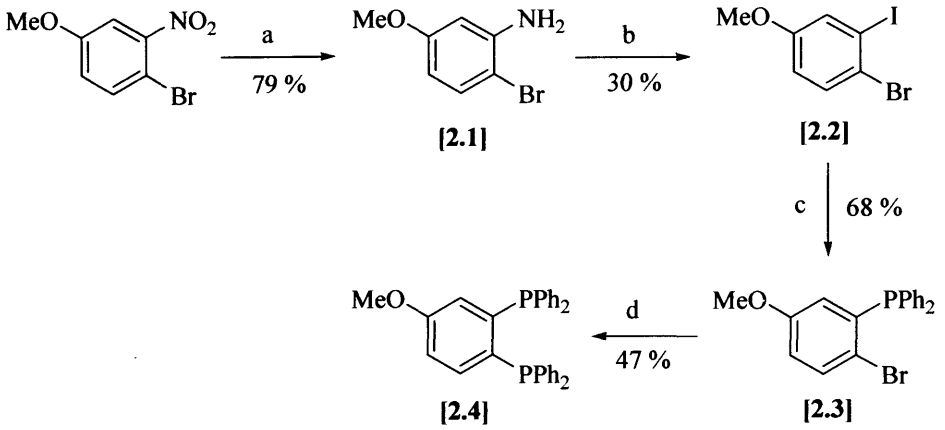


Figure 2.11: The synthesis of **[2.4]**. *Reagents and conditions*: (a) Fe, EtOH, HCl; (b) i) $NaNO_2$, HCl, ii) KI; (c) PPh_2H, $Pd(PPh_3)_4$, toluene, Et_3N; (d) i) *n*-BuLi, ii) PPh_2Cl.

Initial attempts to reduce the nitro function to the amine using an iron/acetic acid mixture[23] proved unsatisfactory. Although product **[2.1]** was formed, it was contaminated with unreacted starting material and unwanted by-products. Alternatively, a solution of 4-bromo-3-nitroanisole in ethanol, in the presence of iron and concentrated hydrochloric acid[24] afforded 2-bromo-5-methoxyaniline **[2.1]** in 79 % yield, without the need for further purification (figure 2.11). The appearance of two sharp peaks in the IR spectrum at 3502 and 3407 cm^{-1} confirm the presence of the primary amine. Other spectroscopic data is consistent with that in the literature.[24]

The amine **[2.1]** was converted to **[2.2]** through the nucleophilic attack of iodide on the aryl diazonium salt intermediate. Thus a solution of **[2.1]** in aqueous sodium nitrite and concentrated hydrochloric acid was added to a solution of potassium iodide, with stirring [25] to give 2-bromo-3-iodoanisole **[2.2]** in 30 % yield (figure 2.11). Experimental data was consistent with that previously reported.[25]

Since the early work of Heck[26] in the 1960's, palladium catalysed coupling reactions have become significant in the formation of carbon-carbon and carbon-heteroatom bonds.[27-29] Work by Stelzer has shown that bromo-phenylphosphine can be synthesised in high yields from *ortho*-bromoiodobenzene and diphenylphosphine by a Pd^0 catalysed P-C coupling.[30] Using $Pd(PPh_3)_4$ as the catalyst, (2-bromo-5-methoxy-phenyl)-

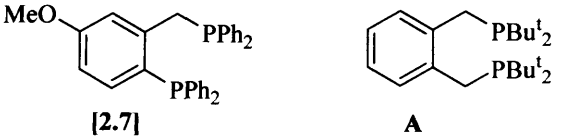
diphenyl-phosphine **[2.3]** was prepared in a 68 % isolated yield as a cream solid (figure 2.11). It is noteworthy that the presence of the anisole substituent has an influence on the efficiency of the cross-coupling reaction. The *ortho-para* activating nature of this group deactivates the *meta* position towards nucleophilic attack, which contributes to the lower yield relative to that of bromophenyl phosphine.[30]

During this work it was observed that under the conditions of Stelzer, poor yields of **[2.3]** were obtained with most of the Ph_2PH remaining unreacted. Product **[2.3]** was identified by a singlet in the $^{31}P\{^1H\}$ NMR spectrum (δ -3.6 ppm), which is consistent with that observed for (2-bromo-phenyl)diphenyl phosphine (δ -3.5 ppm) Addition of further catalyst increased the amount of product formed, thus suggesting the deactivation or poisoning of the catalyst during the course of the reaction. In order to avoid this reoccurring, an excess of $Pd(Ph_3P)_4$ was used, yielding only the desired product in a reasonable yield (68 %). The ^1H and ^{13}C NMR spectra are consistent with those reported for (2-bromo-phenyl)diphenyl phosphine.[30]

The diphosphine **[2.4]** was generated by the halogen-exchange of **[2.3]** with *n*-butyl lithium followed by quenching with chlorodiphenylphosphine (figure 2.11). Washing with petrol afforded the air sensitive ligand **[2.4]** as an oil (47 %).

The chemical shift difference between the two inequivalent phosphorus centres is small giving rise to a second order $^{31}P\{^1H\}$ NMR spectrum. This prevents the direct measurement of $^3J_{Pa\text{-}Pb}$. Using gNMR[31] the values have been calculated to be δ_A = -11.08, δ_B = -15.70 with $^3J_{AB}$ as 152 Hz and $\nu_{1/2}$ = 2 Hz (where $\nu_{1/2}$ = the line width at ½ height). These values are comparable to those reported in the literature for the two adjacent diphenyl phosphines in 1,2-bisdiphenylphosphine.[32] The ^1H NMR spectrum is consistent with the structure.

2.9 Synthesis of 1-Diphenylphosphino-2[(diphenylphosphino)-methyl]-4-methoxybenzene [2.7].



[2.7] **A**

Figure 2.12: Target ligand **[2.7]** and phosphine **A**.

It was initially proposed that the phosphine compound **[2.7]** could be prepared from the phosphonium salt of 4-bromo-3-(bromomethyl)-1-methoxybenzene, upon treatment with base (figure 2.13). The synthesis of the phosphonium salt was envisaged to be

achieved by refluxing the dibromide with diphenylphosphine in THF using a variation of the synthesis of **A** (figure 2.12).[33]

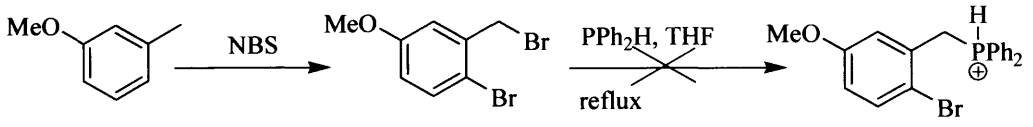


Figure 2.13: Attempted synthesis of phosphonium salt.

The reaction mixture was refluxed for a total of three weeks, and the course of the reaction monitored by ^{31}P NMR spectroscopy. Three signals were observed in the spectrum, a major peak at δ -40 ppm, (PPh_2H) and two minor resonances at δ 24 and -12 ppm. Phosphonium salts are typically observed in the region of 20-30 ppm thus confirming the presence of a phosphonium species, whilst the signal at -12 ppm can be assigned to a free triarylphosphine. Although it appeared that the desired species was formed, the slow conversion led us to devise an alternative methodology.

To this end, the diphosphine **[2.7]** was synthesised *via* a three step procedure from 4-bromo-3-methylanisole (figure 2.14) in an overall yield of 7 %.

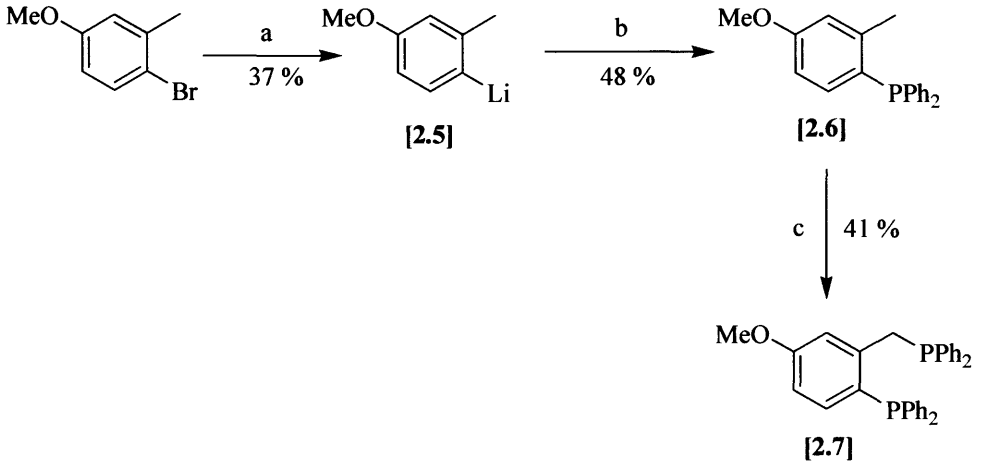


Figure 2.14: The synthesis of **[2.7]**. *Reagents and conditions*: (a) Li, ether; (b) $ClPPh_2$; (c) i) *n*-BuLi, ii) $ClPPh_2$.

4-Lithio-3-methylanisole **[2.5]** was prepared by the lithiation of 4-bromo-3-methylanisole[34] (figure 2.14). The organolithium species was subsequently used as an ether solution, the concentration of which was determined by a Gilman titration.[35]

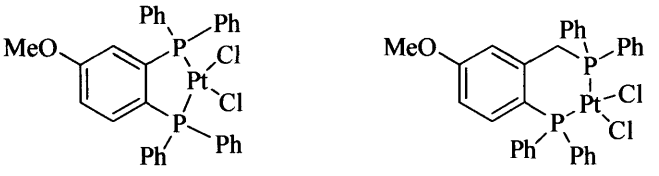
A solution of chlorodiphenylphosphine was added dropwise to the organolithium solution resulting in the precipitation of LiCl. The solution was filtered and dried *in vacuo* to give the crude product as a yellow oil. Recrystallisation from petrol afforded the air

sensitive compound **[2.6]** as a white crystalline material (48 %). The $^{31}P\{^{1}H\}$ NMR spectrum shows a singlet at δ -14.13 ppm indicating the formation of the mono phosphine. This resonance is observed upfield of product **[2.4]**. This can be accounted for by the presence of the anisole being *para* to the phosphine as opposed to *meta* (*cf.* *p*-MeO-$C_6H_4PPh_2$ δ (ppm) -12.03 [36] and *m*- MeO-$C_6H_4PPh_2$ δ (ppm) -3.81[37]).

Compound **[2.7]** was prepared in a two-step process, deprotonation of the terminal methyl group by *n*-butyllithium, followed by nucleophilic attack by the phosphine to afford the crude product as a viscous oil. Recrystallisation from hot methanol afforded the air sensitive compound **[2.7]** as a white solid in a 41 % yield. The first order $^{31}P\{^{1}H\}$ NMR spectra shows a pair of doublets exhibiting a roof effect at δ_A -12.31 and δ_B – 18.06 with $^{4}J_{AB}$ as 13.67 Hz.

2.10 Platinum complexes Pt[2.4] and Pt[2.7]

Addition of a 1: 1 ratio of the free ligands **[2.4]** or **[2.7]** to $Pt(COD)Cl_2$ in DCM afforded the corresponding platinum (II) complexes **Pt[2.4]** and **Pt[2.7]** as white air stable compounds in 60 and 51 % yields respectively (figure 2.15).



[Pt(2.4)] **[Pt(2.7)]**

Figure 2.15: The platinum complexes of **[2.4]** and **[2.7]**.

2.10.1 [Pt(2.4)]

The $^{31}P\{^{1}H\}$ NMR spectrum shows an AB pattern which is coupled to ^{195}Pt (30 %), with δ_a (40.54 ppm), δ_b (37.72 ppm), $^{3}J_{Pa\text{-}Pt}$ (3629 Hz) and $^{3}J_{Pb\text{-}Pt}$ (3584 Hz). The signals are shifted downfield with respect to the free ligand, indicative of coordination. Assignment of a *cis* or *trans* geometry at the platinum centre are based upon values of $^{1}J(Pt^{195}\text{-}^{31}P)$ of approximately 3500 or 2500 Hz respectively.[38,39,40] The coupling constants between the phosphorus and the metal nuclei is dependent on the electron density in the P-M bond. The observed coupling constants are therefore in agreement with a *cis* conformation. The ^{1}H NMR spectrum also shows a downfield shift of the aromatic proton peaks. In the mass spectrum a molecular ion peak is observed at 707.07 Da/e [-Cl] consistent with one coordinated ligand, there is also a minor peak at 507 corresponding to oxidised free ligand.

The IR spectrum confirms the presence of P-phenyl bonds with a stretching vibration observed at 1433 cm^{-1}.

2.10.2 Pt[2.7]

Due to the small quantity of product synthesised full characterisation of the complex was not possible. An ESI MS was obtained showing the molecular ion at 723.06 Da/e (-Cl). Again there is a minor peak at 522 corresponding to oxidised free ligand.

2.11 Platinum Complexes of Bis-Diphenylphosphinoethane.

A series of phosphine complexes with a range of coordinating ligands were prepared to establish whether there are any trends in catalytic behaviour towards the reduction of silver. Diphenylphosphinoethane (dppe) is a versatile ligand which forms complexes with a variety of metals in either a bridging or chelating manner.[41] The availability, along with the ease of preparation of air stable platinum complexes, made dppe a suitable choice for the constant bidentant ligand. Complexes **[2.8]-[2.14]** were synthesised by previous literature methods (figure 2.16).[42-46]

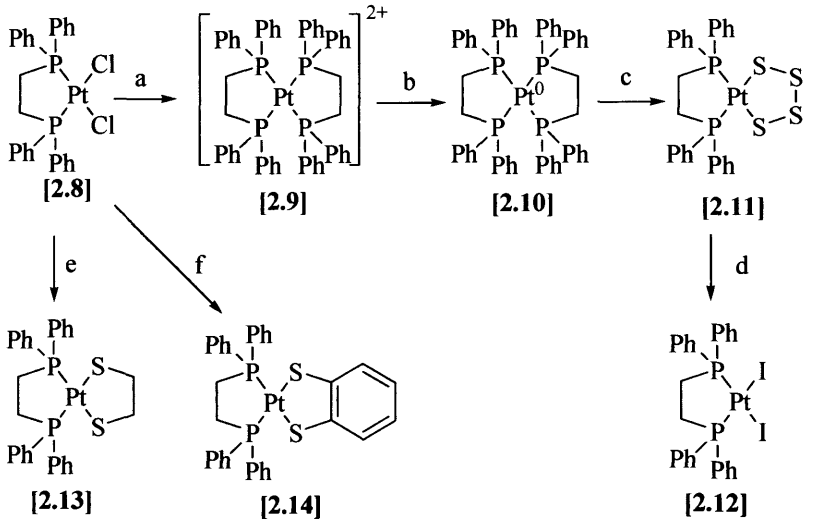


Figure 2.16: The preparation of the complexes **[2.8]** – **[2.14]**. *Reagents and conditions*: (a) dppe (1 eq), $EtOH/H_2O$, reflux; (b) $NaBH_4$; (c) sulfur, benzene; (d) MeI, DCM; (e) 1,2-ethanedithiol, DCM, Et_3N; (f) 1,2-benzenedithiol, DCM, Et_3N.

The physical reducer solution, which is added to aqueous silver ions, is composed of sulfur containing compounds.[47] This solution slowly reduces the silver ions to silver metal. Since the presence of the sulfur may aid the redox process it was thought that

phosphine sulfur compounds may also reduce silver. Dithiolates have been shown to act as electron transfer mediators [46] and hence were included in the investigation.

No	Complex	δ_P	$J_{P\text{-}Pt}$	reference
[2.8]	$Pt(dppe)Cl_2$	41.84	3620.44	[48]
[2.9]	$Pt(dppe)_2Cl_2$ [a]	49.50	2313.39	[48,49]
[2.10]	$Pt^0(dppe)_2$ [a]	31.07	3730.6	[50]
[2.11]	$Pt(dppe)S_4$	49.55	2810.6	[51]
[2.12]	$Pt(dppe)I_2$	46.6	3367.55	[52]
[2.13]	$Pt(dppe)SCH_2CH_2S$	46.34	2748.08	[46]
[2.14]	$Pt(dppe)SC_6H_4S$	45.53	2780.97	[46]

[a] recorded in D_2O

Figure 2.17: ^{31}P NMR data for the complexes **[2.8]-[2.14]**.

2.12 Laboratory Testing of Complexes.

To establish whether the synthesised compounds are suitable for our application a series of screen tests were performed in the lab.

2.12.1 Testing for Catalytic Activity.

A simple test using the physical developer developed by Newman and Jasani[47] was set up. This involved the addition of 1-5 mg of compound to 2 mL of the physical developer. The rate of deposition of silver metal, observed by the darkening of the solution was recorded.

2.13 Preparation of the Physical Developer

The physical developer is comprised of two stock solutions. For both solutions, glassware was cleaned with chromic acid, 0.1 M hydrochloric acid and distilled water. The silver and reducer solutions can be stored separately in the refrigerator for up to one year, although adjustments to the pH may be required.

2.13.1 Silver Stock Solution

To prepare the silver solution the chemicals (figure 2.18) are added sequentially to a stirred volume of water less than the final required volume (approx 150 mL for 200 mL), making sure each chemical has fully dissolved before adding the next. The pH is monitored using an accurately calibrated pH electrode. The solution is decanted into a volumetric flask and made up to the correct volume with distilled water.

Stock	*For 200 mL*	*pH*	*Final conc.*
tris[hydroxymethyl]aminomethane (Tris base)[a]	36.3 g	11.33	1.5 M
Silver nitrate	600 mg	11.18	0.3 %
Acetic acid (glacial)	11.4 mL (approx)	7.41	-
Distilled water	To 200 mL	7.4	-

[a] manufactured by Boehringer Mahnhein.

Figure 2.18: The silver stock solution

2.13.2 Reducer Stock Solution

This solution is initially prepared as two separate solutions (A and B, figures 2.19 and 2.20) which are later combined to give the final reducer stock. Solution A is prepared in the same manner as the silver stock solution.

Solution A

	For 200 mL	*pH*	*Final conc.*
Citric acid (H_2O)	1.05 g	2.35	0.025 M
Sodium Sulphite (anhydrous)	1.00 g	4.42	0.5 %
Sodium thiosulphate ($5.H_2O$)	0.25 g	4.34	0.125 %
Pyrogallol	3.00 g	4.39	1.5 %
Polyethylene glycol 1500	20.0 g	4.74	10 %
Sodium citrate ($2.H_2O$) approx	0.70 g	5.11	-
Distilled water (up to)	100 mL	5.00	50 %

Figure 2.19: Solution A of the reducer stock solution.

Solution B

	For 200 mL	*pH*	*Final conc.*
Glycerol	20 mL	-	10 %
Ethanol	80 mL	-	40 %
Final volume	200 mL	5.791	

Figure 2.20: Solution B of the reducer stock solution.

2.14 Complex Testing

Figure 2.21 summarises the screen tests performed.

Compound	*Colour*	*Solubility in the physical developer*	*First noticeable darkening*
$Pt(COD)Cl_2$	Cream	Insoluble	instant
$Pt(COD)I_2$	yellow	Insoluble	instant
Dppe	white	Insoluble	No reaction
[Pt(2.4)]	White	Insoluble	14 minutes
[Pt(2.7)]	White	Insoluble	15 minutes
[2.8]	white	Insoluble	20 minutes
[2.9]	white	Insoluble	No reaction
[2.10]	yellow	N/A	N/A
[2.11]	yellow	Insoluble	6 minutes
[2.12]	yellow	Insoluble	5 minutes
[2.13]	white	Insoluble	No reaction
[2.14]	white	Insoluble	No reaction

Figure 2.21: Results of laboratory testing of complexes.

2.15 Conclusion

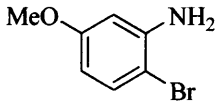
The ligands/complexes **[2.4]/ [Pt(2.4)]** and **[2.7]/ [Pt(2.7)]** were functionalised with an anisole moiety. In the future, demethylation to the phenol would allow the introduction of a phosphate group and hence render the compounds suitable substrates of alkaline phosphatase. The platinum (II) complexes **[Pt(2.4)]** and **[Pt(2.7)]** show potential as catalysts for the reduction of silver in a Timm's type reaction with noticeable darkening (precipitation of silver) of the physical developer solution occurring in 14 and 15 minutes respectively. Subsequent silver precipitation is gradual over a period of 30 minutes. The redox activity of various platinum dppe complexes **[2.8]** – **[2.14]** were also investigated. The complexes $[Pt(dppe)_2]^{2+}$ and the dithiolate complexes $[Pt(dppe)SCH_2CH_2S]$ and $[Pt(dppe)SC_6H_4S]$ showed no redox activity for the reduction of silver ions. The complexes $Pt(dppe)Cl_2$, $Pt(dppe)S_4$ and $Pt(dppe)I_2$ showed redox activity in times of 20, 6 and 5 minutes respectively. Since the bidentate phosphine remains constant we can postulate that the variable ligands (e.g. tetrasulfido, iodide, chloride etc.) play some role in the silver reduction mechanism. However, the chemistry for the reduction of silver ions in a Timm's type reaction is poorly understood.

Experimental

General Procedure

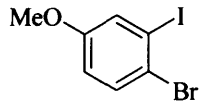
All experiments were carried out under an atmosphere of dry nitrogen using standard Schlenk line techniques. All solvents were dried and degassed by reflux over standard drying agents[53] under a nitrogen atmosphere. Dichloro(1,5-cyclooctadiene)platinum(II),[54] $Pt(dppe)Cl_2$,[45] $Pt^{II}(dppe)_2$,[48] $Pt^0(dppe)_2$,[43] $Pt(dppe)S_4$,[44] $Pt(dppe)I_2$,[42] $Pt(dppe)(SCH_2CH_2S)$[46] and $Pt(dppe)(SC_6H_4S)$[46] were prepared according to literature methods. Non-synthesised reagents were purchased from Aldrich, Avocado or Lancaster and were used as received. Where appropriate, chemicals were dried over molecular sieves and freeze-thaw degassed. The NMR spectra were recorded on a Brüker Avance AMX 400 instrument at 400 MHz (^1H) and 100 MHz (^{13}C), JEOL Lamda Eclipse 300 at 121.65 MHz (^{31}P); ^1H and ^{13}C chemical shifts are quoted in ppm relative to residual solvent peaks and ^{31}P chemical shifts are quoted in ppm relative to external 85 % H_3PO_4 (δ 0). Coupling constants are quoted in Hertz. Mass spectra were obtained in EI (electronic ionisation mode). IR spectra were obtained using a Jasco FTIR 110 series spectrometer.

[2.1] 2-Bromo-5-methoxyaniline



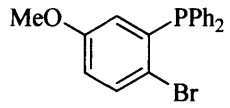
This was prepared according to a literature procedure by Tidwall.[24] 4-Bromo-3-nitroanisole (14.03 g, 60.5 mmol), iron powder (10.14 g, 181.6 mmol), ethanol (200 ml) and concentrated hydrochloric acid (28.9 ml of a 32 % solution) were added to a round bottomed flask. The reaction mixture was heated to reflux for 4 h. The solution was allowed to cool to room temperature and sodium carbonate was added until no more bubbling occurred. Ether (100 ml) was added and the reaction mixture was washed with water (3 x 100 ml), brine (100 ml) and dried over anhydrous magnesium sulfate. The solvent was evaporated *in vacuo* to give 2-bromo-5-methoxyaniline **[2.1]** as a red oil, without the need for further purification (9.61 g, 79 %); δ_H(400 MHz; $CDCl_3$) 7.38, (1H, d, *J* 8.0), 7.30 (d, 1H, *J* 2.93), 6.7 (dd, 1H, *J* 8.80, 2.93), 3.9 (br s, 2H, NH_2), 3.69 (s, 3H, OCH_3); δ_C(100 MHz; $CDCl_3$) 160.0, 144.5, 133.2, 105.3, 100.9, 100.0, 55.7; IR (NaCl, cm^{-1}) 3041, 2990, 2966, 3244, 1624, 1580, 1447, 1425, 1321, 1277, 1190, 1150, 1046, 1007, 948 and 843. *m/z* (APCI) 203 [M+1].

[2.2] 4-Bromo-3-iodoanisole



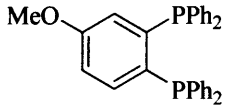
This was prepared according to a literature procedure by Tanida.[25] Into a mixture of **[2.1]** (9.61 g, 47.6 mmol), concentrated hydrochloric acid (22.3 ml) and cracked ice (14.7 g) was added a solution of sodium nitrite (3.66 g) in water (14.7 ml) at 0 °C. The reaction mixture was stirred for 25 min, the deep orange solution which resulted was filtered. This solution was slowly added at 5 °C, to a solution of potassium iodide (27.1 g, 163.6 mmol) in water (89 ml). The reaction mixture was left to stand overnight at room temperature. The violet oil which separated, was extracted with ether (3 x 100 ml), washed with 10 % aqueous sodium hydroxide (2 x 100 ml), water (2 x 100 ml) and 5 % aqueous sodium sulfite (100 ml) and dried over anhydrous sodium sulfate. The solvents were removed *in vacuo* to give the crude product as a dark red oil. The residue was distilled at 130 °C on a Kugelröhr apparatus to afford **[2.2]** as a yellow oil. (3.85 g, 30 %); δ_H(400 MHz; $CDCl_3$) 7.15 (d, 1H *J* 8.7), 6.2 (d, 1H, *J* 2.8), 6.1 (dd, 1H, *J* 2.8, 8.7), 3.65 (s, 3H, OCH_3); *m/z* (APCI) 314 [M+1]. Other data are consistent with the literature.[25]

[2.3] (2-bromo-5-methoxy-phenyl)-diphenyl-phosphine



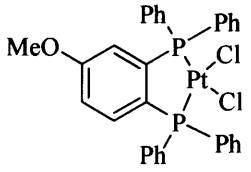
This was prepared in a method analogous to that of Stelzer,[30] where 4-bromo-3-iodoanisole **[2.2]** replaces 2-bromoiodobenzene. To a solution of **[2.2]** (3.81 g, 12.18 mmol) and diphenylphosphine (2.5 g, 13.43 mmol) in toluene (31 ml), triethylamine (1.5 g, 14.82 mmol) was added. After the addition of $Pd(PPh_3)_4$ (0.087 g, 0.07 mmol), the mixture was stirred at 90 °C for 4 h, resulting in a purple solution. The toluene was removed *in vacuo* to give a brick red solid which was dissolved in dichloromethane (30 ml), washed with water (30 ml) and dried over anhydrous magnesium sulfate. The solvents were removed *in vacuo* to give a pale orange solid, which was washed with hot methanol to afford **[2.3]** as a cream solid (3.23 g, 68 %); δ_H(400 MHz; $CDCl_3$) 7.37 (dd, 1H, *J* 8.72, 3.96), 7.21 (m, 10H), 6.62 (dd, 1H, *J* 8.66, 3.05), 6.2 (t, 1H, *J* 2.92), 3.43 (s, 3H); δ_C (100 MHz, $CDCl_3$) 158.8, 140.0 (d, J_{CP} 12.5), 135.7 (d, J_{CP} 10.7), 134.2 (t, J_{CP} 20.9), 133.7 (d, J_{CP} 22.8), 129.0 (d, J_{CP} 16), 128.7 (d, J_{CP} 7.1), 120.5, 120.1 (d, J_{CP} 29.9), 115.5, 55.2; $\delta_{P\{H\}}$ (121.65 MHz; $CDCl_3$) –3.33.

[2.4] 1,2-Bis-diphenylphosphino-4-methoxy-benzene)



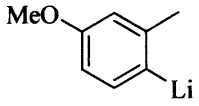
This was prepared in a method analogous to that of Tunney and Stille,[21] where (2-bromo-5-methoxy-phenyl)-diphenyl-phosphine replaces (2-bromophenyl)diphenylphosphine. To a cooled (-78 °C) solution of **[2.3]** (2.63 g, 7.08 mmol) in THF (77 ml) was added dropwise a solution of *n*-BuLi in petrol (4.4 ml of a 2.21 M solution) and was stirred for 30 min at -78 °C. To this chlorodiphenylphosphine (3.2 ml, mmol) was added dropwise, and the temperature was raised to room temperature then stirred overnight. Water (15 ml) was added to quench the solution, dichloromethane (20 ml) added, washed with saturated NaCl (30 ml) and dried over magnesium sulfate. The solvent was removed *in vacuo* to yield the crude product as an opaque yellow oil. Petrol (30 ml) was added, instantly forming a cream precipitate. The petrol solution was filtered off and cooled to -35 °C. The petrol solution was removed from the solid that had formed to afford **[2.4]** as an oil (1.58 g, 47 %); δ_H (400 MHz, $CDCl_3$) 7.31 (m, 1H), 7.20-7.25 (m, 20H), 6.75 (m, 1H), 6.45 (m, 1H), 3.50 (s, 3H); δ_C (100 MHz, $CDCl_3$) 160.2, 137.7 (d, J_{CP} 11.5), 137.0 (d, J_{CP} 11.5), 136.8, 136.1 (J_{CP} 6.9), 134.1-133.4, 132.7 (J_{CP} 18.1), 128.7-128.1, 119.5 (J_{CP} 8.4), 114.5, 54.9; $\delta_{P\{H\}}$ (121.65 MHz, $CDCl_3$) δ_A = -11.08, δ_B = -15.70 with $^3J_{AB}$ as 152 Hz .

Pt[2.4] 1,2-Bis-diphenylphosphino-4-methoxy-benzene Platinum (II)



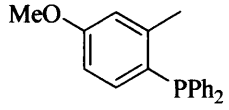
To a solution of (1,5 cyclooctadiene)dichloro platinum(II) (0.72 g, 1.92 mmol) in DCM (15 ml)was added to a solution of 1,2-bis-diphenylphosphino-4-methoxy-benzene **[2.4]** (0.97 g, 2.04 mmol) in DCM (20 ml) and stirred overnight. Ether (25 ml) was added dropwise to the solution to precipitate out the complex as a crude solid. Recrystallisation in hot dichloromethane (10 ml) yielded the pure complex **Pt[2.4]** as an off-white solid (0.90 g, 60 %); δ_H (400 MHz, $CDCl_3$) 7.7 (m, 10H, Ar-*H*) 7.4-7.5 (m, 11H, Ar-*H*), 7.1 (m, 1H, Ar-*H*), 6.95 (d, 1H, *J* 9.57, Ar-*H*), 3.7 (s, 3H, OCH_3); $\delta_{P\{H\}}$ (121.65 MHz, $CDCl_3$) δ_{Pa} 40.54 ($J_{Pa\text{-}Pt}$ 3629), δ_{Pb} 37.72 ($J_{Pb\text{-}Pt}$ 3584); IR (KBr, cm^{-1}) 3005, 2945, 2885, 2825, 1579, 1473, 1433, 1332, 1307, 1272, 1227, 1182, 1096, 1006, 830, 750, 695 *m/z* (ES) 707.07.

[2.5] 4-Lithio-3-methylanisole



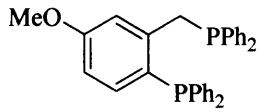
This was prepared according to a literature procedure by Mckinstry.[34] An oven dried, 250 mL, three-necked, round-bottomed flask was fitted with a pressure equalising dropping funnel, condenser and glass stopper and was purged with argon. Lithium powder alloyed with Na (2.86 g) was added and washed with petroleum ether (15 ml x 3), then ether (156 ml) was added. A solution of 4-bromo-3-methylanisole (11.2, 55.7 mmol) in ether (50 ml) was slowly added dropwise. The solution was heated with the aid of a heat gun, to bring it to reflux. The yellow solution was stirred at room temperature for an additional 2 h. Titration against standardised 0.1 M HCl confirmed that the reaction had been a success. (2.69 g, 37 %).

[2.6] (4-Methoxy-2-methyl-phenyl)-diphenyl-phosphine



Under argon, a solution of chlorodiphenyphosphine (1.229 g, 6.6 mmol) in ether (20 ml) was added dropwise to a cooled (-78 °C) solution of **[2.5]** (0.021 moles in ether (2.69 g, 21 mmol). The solution was allowed to stir at room temperature for 2.5 h to give a cream coloured precipitate in a pale yellow solution. The ether solution was filtered off and removed *in vacuo* to a give the crude material as a yellow opaque oil. Petrol (20 ml) was added to the oil, instantly forming a cream solid, the petrol was filtered off and was left overnight to yield the pure product **[2.6]** as small white crystals (3.08 g, 48 %); δ_H(400 MHz, D_2O) 7.05-7.20 (m, 10H), 6.65 (t, 1H, *J* 3.25), 6.60 (dd, 1H, *J* 8.33, 4.41), 6.50 (dd, 1H, *J* 8.46, 2.48), 3.60 (s, 3H, O*Me*), 2.20 (s, 3H, *Me*); δ_C (100 MHz, $CDCl_3$) 23.5 (d, J_{CP} 30.7, *C*H_3), 55.1 (O*C*H_3), 111.3 , 117.4, 128.0 (d, J_{CP} 6.6), 132.2 (d, J_{CP} 19.2), 138.5, 145.3 (d, J_{CP} 28), 161.5; $\delta_{P\{H\}}$ (300 MHz; D_2O) –14.13 .

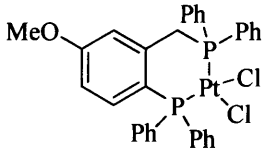
[2.7] 1-Diphenylphosphino-2[(diphenylphosphino)-methyl]-4-methoxybenzene



To a cooled solution (-78 °C) of **[2.6]** (0.42 g, 1.37 mmol) in THF (20 ml) was added *n*BuLi (0.63 ml of a 2.12 M solution in petrol) and was stirred for 45 min. Chlorodiphenylphosphine (0.3 ml) was added and the temperature was allowed to rise to

room temperature and the stirring continued overnight. The solution was quenched with water (10 ml) and extracted with DCM (10 ml). The organic phase was washed with saturated NaCl (20 ml) and dried over magnesium sulfate. The solvent was removed *in vacuo* to give a viscous liquid. The crude product was recrystallised in hot methanol twice to give the pure product **[2.7]** as a white solid (0.27 g,41 %); δ_H(300 MHz, $CDCl_3$) 3.3 (s, 3H, OCH_3), 3.7 (s, 2H, CH_2), 6.57 (d, 1H, *J* 8.58, Ar-*H*), 6.7 (dd, 2H, *J* 8.58, 4.62, Ar-*H*), 7.3 (m, 21H, Ar-*H*), $\delta_{P\{H\}}$ (121.65 MHz, $CDCl_3$) -12.311,– 18.062 ($^4J_{AB}$ 13.67 Hz).

Pt[2.7]



This was prepared in a similar manner to **[Pt(2.4)].** The applied reagents were **[2.7]** (0.1 g, 0.20 mmol) and $Pt(COD)Cl_2$ (0.08g, 0.20 mmol) to afford **[Pt(2.7)]** as a white solid (0.07g, 51 %); *m/z* (ES) 723.06 [-Cl].

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Chapter 3

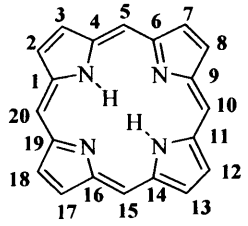
Markers based on porphyrin ligands

Introduction

This chapter presents the preparation of markers based on functionalised *meso*-tetra-arylporphyrins. Platinum and palladium complexes were prepared and their catalytic activity towards the reduction of silver in a Timm's type reaction was investigated.

3.1 Background

The porphin nucleus is comprised of four pyrrole rings joined by four methane bridges, giving rise to highly conjugated macrocycle. There are nominally 22π electrons but only 18 of these are included within the delocalisation therefore conforming to Hückel's 4n + 2 rule for aromaticity.



Porphin

Figure 3.1: The Basic Porphin Framework

Porphin is the parent compound of the substituted derivatives known as porphyrins. The two most common patterns of substitution are at the pyrrole (β substitution) and the methane (*meso* substitution) positions. All naturally occurring porphyrins are substituted at the pyrrole positions whilst the *meso* substituted porphyrins are purely synthetic.

3.2 Occurrence of Porphyrins

Porphyrins and the related compounds are of great importance in the natural world. They constitute the prosthetic group of hemes (found in hemeoglobins and myoglobins) which are responsible for oxygen transport and storage in tissues. Reduction of one of the pyrrole units in porphin gives rise to a class of derivatives known as chlorins. These are found in chlorophylls which are found abundantly in green plants and play an important role in photosynthesis. Porphyrins and their derivatives are highly coloured compounds exhibiting very high extinction coefficients, they are often found in abundance in natural pigments.

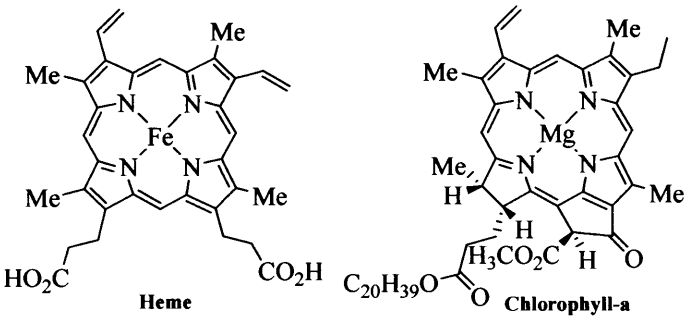


Figure 3.2: The structures of Heme (iron (II) protoporphyrin IX) and chlorophyll-a

Both metallated and metal-free forms widely occur in nature. For example, Fe (II) is the metal found in hemes[1] (e.g. iron (II) protoporphyrin IX) whilst Mg (II) is found in the chlorophylls.[2] Isomers of coproporphyrin (I and III type) and protoporphyrin IX are found in nature in their metal free states. They are found in small traces in tissue fluids and in larger quantities in certain pathological conditions.

3.3 Metal Derivatives

The metal complexes of porphyrins are of great importance in nature, often essential to the life of animal and plant organisms. The metal-porphyrin motif is found in many enzymes (e.g. cytochrome P450), vitamins (e.g. vitamin B_{12}), photosynthetic agents (e.g. chlorophyll) and oxygen transfer agents (e.g. haemoglobin). Synthetic metalloporphyrins are utilised in other areas such as medicine and in electronic devices.

Metalloporphyrins are porphyrin derivatives where the lone pair of electrons on the central nitrogen atom is shared with a metal, alongside this the two hydrogen atoms on the pyrrole NH are replaced, thus two more lone pairs are donated to the valence shell of the metal.[3] The donation of σ-electrons from the four nitrogen atoms to the central metal gives rise to complexes of high stability.

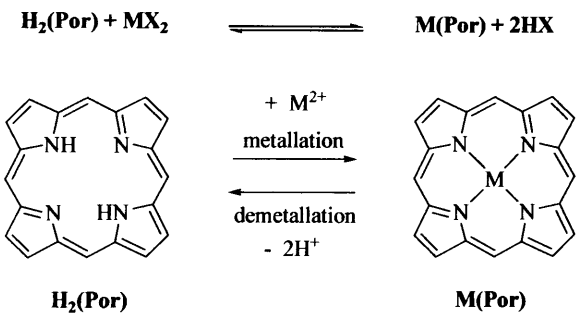


Figure 3.3: Formation of the equatorial coordination group of a monometallic porphyrin M(Por).

A wide variety of metal porphyrin derivatives are known by the reaction of the free base porphyrin with the appropriate metal salt in a high boiling solvent.[4,5] The most commonly found coordination types found in metalloporphyrins are square (4 coordinate), square pyramidal (5 coordinate) and octahedral (6 coordinate). In cases where the coordination number exceeds four, the coordination sphere is completed by the coordination of axial ligands. The size of the porphyrin cavity can accommodate metal ions of radii 0.60 – 0.65 Å.[4] Where the metal ion is too small, the porphyrin buckles (S_4 distortion) to accommodate the ion. For large ions, the metal sits either above or below the porphyrin equatorial plane.

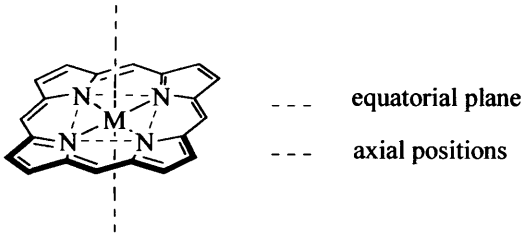


Figure 3.4: Illustration of the axial and equatorial planes in a porphyrin

3.4 Porphyrin Properties

3.4.1 UV/Vis Spectra

In 1883 an intense absorption band at 400 nm was observed in hemeoglobin by Soret.[6] This intense absorption ($\varepsilon \sim 400\ 000\ cm^{-1}$), now known as the Soret band, is exhibited in all fully conjugated tetrapyrroles and is characteristic of the macrocyclic conjugation. Porphyrins in their free-base form also exhibit four other bands (Q bands) in the region of 450-700 nm. The Soret band can be assigned to the a_{1u} (π) to e_g^* (π^*) transitions, whilst the Q band absorptions are ascribed to the two possible transitions from a_{2u} (π) to e_g^* (π^*). Incorporation and alteration of the peripheral porphyrin substituents may result in minor changes in the wavelength of the absorptions.

3.4.2 Metalloporphyrins

In simple square planar porphyrin complexes, an altered UV/Vis spectrum is observed, with the retention of the Soret band and the appearance of two visible bands labelled α and β.

The absorption spectra of metalloporphyrins can be placed into three categories:

i. Normal Spectrum

This is observed for all d^0 and d^{10} ions. The porphyrin π orbitals do not significantly interact with the metal π orbital (p_z or d_{xz}, d_{yz}). A bathochromic shift of the absorption bands is seen upon increasing atomic number. Complexes exhibiting a 'normal type' spectrum are purple in colour.

ii. Hypso Spectrum

This is found in d^6-d^9 metalloporphyrins. The metal d_{xz} and d_{yz} orbitals are of the correct symmetry to overlap with the porphyrin π^* levels, which results in backbonding from the metal to the porphyrin. Increasing the atomic number results in increased backbonding thus increasing the shift (e.g. Ni(II) < Pd(II) < Pt (II)). Thus the hypso spectrum is defined as 'a normal spectrum with hypsochromic shifts to all bands'.[5] The colour of these complexes is red to orange.

iii. Hyper Spectrum.

This is observed for d^1-d^5 ions. The spectrum consists of more-or-less shifted α, β and Soret bands and one or more additional bands.[5] Most of the metal ions in this class can realise lower oxidation states within the porphyrin which then causes normal light absorption. Common examples of this type are exhibited in Fe (III) and Mn (III) porphyrins. Complexes of this class are brown or green in solution.

Porphyrins are well known for their robust nature exhibiting stability to both strong acids and bases. The extended 18 π electron system affects the range of remarkable properties of these macrocycles.[7] This extended π system present in porphyrins generates a HOMO and LUMO that are separated by only 2 eV,[8] thus giving rise to materials with interesting photophysical and conductivity properties. Porphyrins and metalloporphyrins have similar energies of the singlet and triplet excited states, high intersystem crossing yield and long triplet lifetimes[3] making them of use as excellent photosensitisers.

3.5 Routes of Synthesis

The vast amount of research that has been carried out on porphyrins has led to numerous routes of preparation. These can be divided into two main categories; the transformation of naturally occurring porphyrins and the more widely used total synthesis. In the total synthesis the choice of method is dependent on the symmetry and the pattern of substitutions. Since the work undertaken has solely focused on *meso*-tetraaryl porphyrins, only their syntheses shall be considered.

Symmetrical tetra-aryl porphyrins can be prepared by the cyclocondensation of pyrrole and benzaldehyde. In many cases the porphyrin product is contaminated with the corresponding chlorin which can be either converted to the porphyrin using an oxidant (e.g. DDQ[9]) or separated by chromatography.

3.5.1 Rothemund and Adler-Longo Methods

Tetraphenylporphyrin was first synthesised in 1936 by Rothemund, by reacting benzaldehyde and pyrrole in pyridine in a sealed bomb at 150°C for 2 h.[10] The yields were low and the severe conditions that were used meant that few substituted benzaldehydes could be converted to the corresponding porphyrin. Since then, other methods of synthesising porphyrins have been developed, but the simple method of Adler and Longo whereby benzaldehyde and pyrrole are reacted in refluxing propionic acid[11] exposed to air, is one of the most widely used (figure 3.5). These conditions, which are milder than previous, allow for a wider range of substituted benzaldehydes to be used. This preparation enables large-scale synthesis in yields of up to 20 %. The main drawback of this synthesis is the formation of tarry by-products (polymerised pyrrole), chlorin contamination and its unsuitability when acid sensitive benzaldehydes are used.

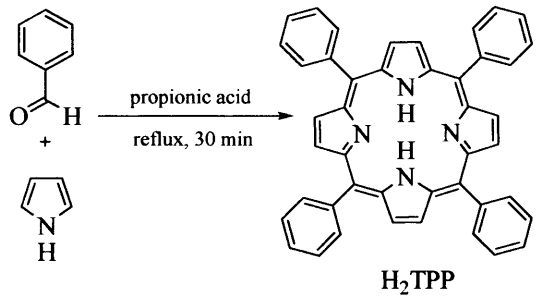


Figure 3.5: The Adler-Longo method, exemplified for *meso*-tetraphenylporphyrin [H_2(TPP)]

3.5.2 Lindsey Method

Lindsey[12] developed a methodology that allowed porphyrins from sensitive benzaldehydes to be prepared in higher yields than the previous methods (30-40 %[13]). This synthesis proceeds *via* the porphyrinogen by the reversible reaction of a benzaldehyde and pyrrole. The reaction is performed at room temperature under an inert atmosphere in dichloromethane in the presence of boron trfluoride etherate. Addition of a stoichiometric amount of oxidant, typically 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (DDQ), oxidises the porphyrinogen (A) to porphyrin (B) (figure 3.6).

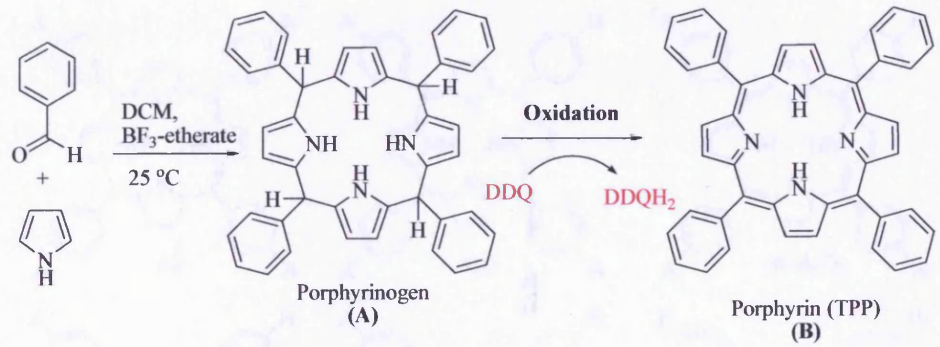


Figure 3.6: The Lindsey method, exemplified for *meso*-tetraphenylporphyrin

In order to obtain maximal yields, high dilutions are required[13] since the reaction has been found to be sensitive to concentration. This limits the reaction only being carried out on a small scale. In cases where the porphyrin product does not crystallise out (Adler method), the Lindsey method presents an attractive alternative since the production of tarry by-products is limited thus simplifying isolation and purification.

3.5.3 Unsymmetrical Tetra-aryl porphyrins

Unsymmetrical substituted aryl-porphyrins are often prepared with groups which are suitable for further modification. Simple porphyrins of type A_3B are useful precursors to more elaborate systems.

3.5.4 Mixed Aldehyde/ Statistical Approach

The preparation of porphyrins which have an A_3B substitution pattern can be achieved by the condensation of two different benzaldehydes with pyrrole. The condensation of a single benzaldehyde with pyrrole affords a symmetrical tetra substituted porphyrin. When two different aryl-aldehydes are reacted with pyrrole, up to six different porphyrins are formed (figure 3.7).[12]

The stoichiometry of the benzaldehydes used alters the ratio of the products, and by using the appropriate ratios, a mixture containing a majority of the A_4 and A_3B porphyrins can be achieved.[12,14] This method, although simple, often requires elaborate and tedious separation techniques; the ease of this is dependent on the difference in polarity of the differing *meso* substituents.[15] Despite this, a statistical approach is commonly used to prepare A_3B type porphyrins. When performed using the Adler conditions isolated yields of A_3B porphyrins are seldom greater than 5 %.

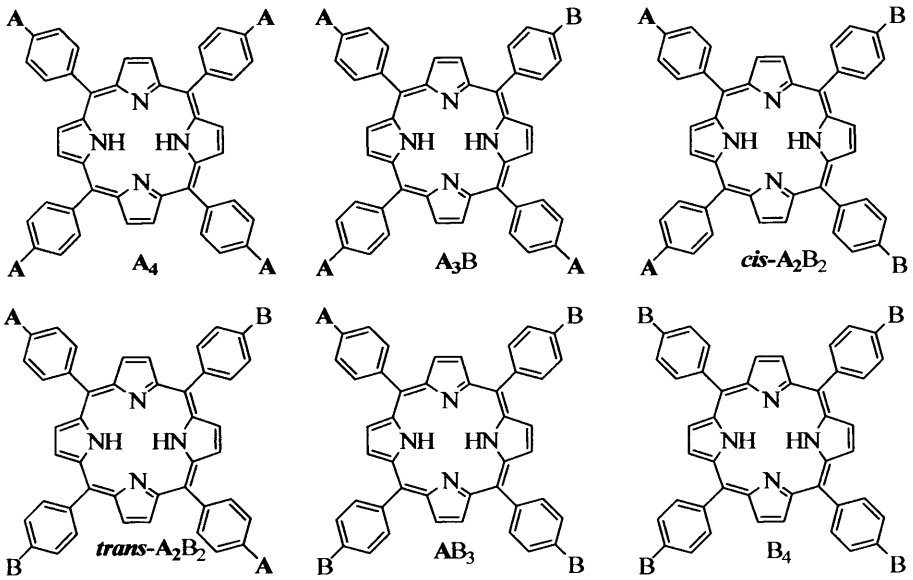


Figure 3.7: The six possible porphyrin products from a mixed aldehyde condensation.

3.5.5 Other Methodologies

In addition to the above, other routes of synthesis are available particularly for the preparation of highly unsymmetrical porphyrin products. Many of these routes include substituted dipyrromethanes as the building blocks.[16,17] These synthetic methods allow more control over the pattern of substitution. One of the most successful routes is known as the '2 + 2 synthesis' which was first devised by MacDonald[18] and involved the use of a dipyrromethane with two formyl group α to the pyrrolic nitrogens and an unsubstituted dipyrromethane. The introduction of substituents at both the *meso* and β positions can be achieved by varying the substituents on the dipyrromethanes and the pyrrole precursors.

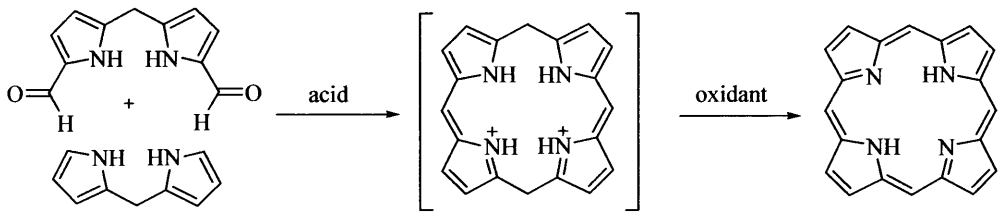


Figure 3.8: MacDonald 2+2 condensation.

3.6 Aims and Objectives

Research in Cardiff has previously shown that group ten metal complexes of tetra-aryl porphyrins demonstrate the ability to reduce silver ions *via* a Timm's type reaction.[19] We proposed to synthesise aryl porphyrin systems based on group ten metals which feature a lysable enzyme substrate *i.e.* a phosphate moiety. Literature procedures for the phosphorylation of a species focus on the addition of a phosphate group to a hydroxyl

containing compound[20-28] and in particular a phenol. We have devised two routes of preparation for phosphate containing porphyrins based on the relative ease of preparation of benzaldehyde precursors and the common practise of changing or introducing functionalities at the phenyl rings of a *meso*-tetra-arylporphyrin. This chapter outlines the attempted synthesis of both tetra and mono phosphorylated porphyrins from either the direct condensation of a benzaldehyde bearing a phosphate functionality with pyrrole or by the direct modification of a phenol porphyrin.

Results and Discussion

3.7 Synthesis of Phenol Substituted Porphyrins

3.7.1 Tetra Phenol Porphyrins (A_4)

Phenol derivatives of *meso* substituted porphyrins are often obtained from a protected species, most commonly the anisole. To this end, *meso*-5,10,15,20-tetra (4-methoxyphenyl)porphyrin **[3.1]** was synthesised by the direct condensation of 4-methoxybenzaldehyde and pyrrole in refluxing propionic acid[11] in a 20 % yield. Demethylation using boron tribromide at -80°C[29] afforded *meso*-5,10,15,20-tetra-(4-hydroxyphenyl)porphyrin[30,31] **[3.2]** as a purple solid in a 50 % yield (figure 3.9). Successful demethylation was indicated by the disappearance of the methoxyl protons at 4.0 ppm in the ^{1}H NMR spectrum whilst an OH stretching vibration at 3311 cm^{-1} appears in the IR spectrum.

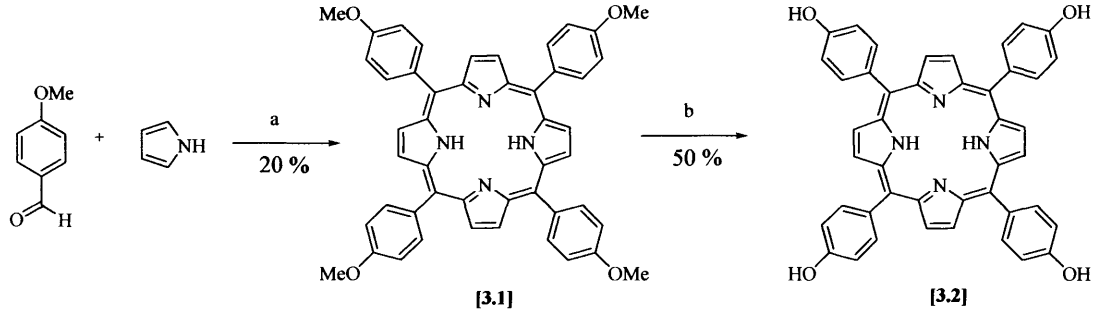


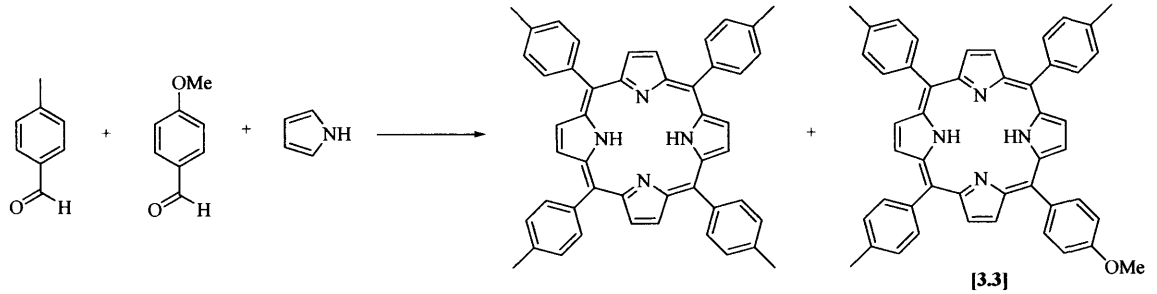
Figure 3.9: Reaction scheme for the synthesis of porphyrins **[3.1]** and **[3.2]**. *Reagents and conditions:* (a) propionic acid, reflux, 0.5 h; (b) BBr_3, -80 °C.

Both the methoxyphenyl and hydroxyphenyl porphyrins **[3.1]** and **[3.2]** were metallated using palladium (II) dichloride in refluxing benzonitrile[5] for 6 h to afford the corresponding Pd^{2+} complexes **[Pd(3.1)]** and **[Pd(3.2)]** as pink solids in 23 and 53 % yields respectively. Both complexes show the expected palladium isotope pattern in the MALDI

mass spectrum (838.25 and 782.2 Da/e). As anticipated, in the absorption spectra, subtle hypsochromic shifts to the bands was observed consistent with the insertion of palladium.

3.7.2 Mono-Phenol Tri-Aryl Porphyrins (A_3B)

5,10,15-Tri-(4-tolyl)-20-(4-methoxyphenyl)porphyrin **[3.3]** was synthesised by an adaptation of the method reported by Little[32] from a statistical mixture of 4-tolylbenzaldehyde and 4-methoxybenzaldehyde in a 20:1 ratio in a 11 % yield. The desired mono-substituted product **[3.3]** precipitates out of the reaction mixture along with 5,10,15,20-tetra-(4-tolyl)porphyrin (figure 3.10). The two porphyrins along with small amounts of poly-substituted tetra-arylporphyrins can be separated by column chromatography. On a small scale preparation purification is easily achieved,[33] however, when performed on a larger scale, separation of the fractions by chromatography has proved problematic. This can be attributed to a small difference in R_F values of the two products.

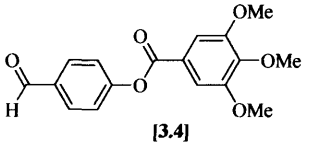


[3.3]
11%

Figure 3.10: Reaction scheme for the preparation of **[3.3]** using a 'mixed aldehyde' approach. *Reagents and conditions*: propionic acid, reflux, 0.5 h.

3.8 Use of an Alternative Protecting Group

To overcome the encountered problems associated with the separation of the two produced porphyrins a protecting group for the phenol with increased polarity was introduced, namely 3,4,5-trimethoxybenzoic acid phenyl ester **[3.4]** (figure 3.11).



[3.4]

Figure 3.11: Alternative protecting group.

The benzyl ester **[3.4]** was prepared from 3,4,5-trimethoxybenzoic acid *via* the acid chloride in 81 % yield. The IR spectrum confirms the presence of both ester and aldehyde

functional groups with C=O stretching frequencies observed at 1737 and 1699 cm^{-1} respectively. The mass spectrum shows the molecular ion for M^+ at 317 Da/e.

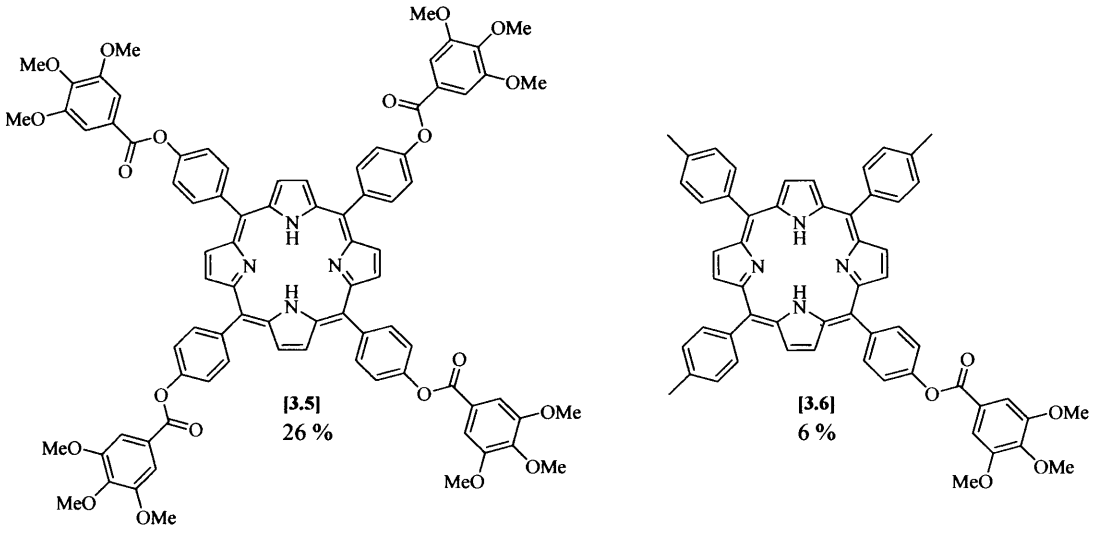


Figure 3.12: The symmetrical and unsymmetrical porphyrins **[3.5]** and **[3.6]**.

To investigate the feasibility of protecting the hydroxyl group with the benzyl ester, compounds **[3.5]** and **[3.6]** were prepared (figure 3.12). The symmetrical porphyrin **[3.5]** was prepared in a 26 % yield. Reaction with platinum (II) dichloride in benzonitrile afforded the platinum complex **[Pt(3.5)]** as an iridescent pink solid. In the ^1H NMR spectrum, an upfield shift is observed to both β-pyrrole and phenyl protons adjacent to the porphyrin ring and the disappearance of the internal NH protons is indicative of platinum binding. Consistent with the coordination of platinum, a hypsochromic shift of 20 nm is seen in the UV/Vis spectrum whilst the disappearance of the NH stretch at 3318 cm^{-1} is observed. The molecular ion is observed at 1648 Da/e in the mass spectrum.

Using a statistical mixture of 4-tolylbenzaldehyde and **[3.4]** (20:1) afforded the corresponding A_3B porphyrin **[3.6]** in 6 % yield (figure 3.12). Eluting with chloroform on a silica column removes the symmetrical product, and the now increased separation of R_F values (0.053 versus 0.55) allows **[3.6]** to be separated by simply increasing the polarity of the solvent system (10 % MeOH). The signals in the ^1H NMR spectrum attributed to the pyrrole protons are made up of more than one component and other signals in the aromatic region are observed in the correct ratio of 3:1, indicative of the unsymmetrical nature.

3.8.1 Removal of Ester Groups

Hydrolysis of the benzyl ester group in **[3.6]** was attempted on a small scale using potassium hydroxide in refluxing THF for 2 hours. To aid solubility 1,4-dioxane was added. It was found that the benzyl ester groups were not readily hydrolysed under these

conditions as confirmed by the mass spectrum, where only the ion for unreacted starting material **[3.6]** is seen.

Ester cleavage was alternatively performed using hydrazine monohydrate in ethanol (figure 3.13). The reaction of **[3.5]** was left for two days and the course of the reaction was monitored by thin layer chromatography. Filtration of the reaction mixture removes a large quantity of unreacted porphyrin; the phenol porphyrin being readily soluble in ethanol. Analysis of the mass spectrum indicates an incomplete reaction with observed ions corresponding to tetra-(4-hydroxyphenyl)porphyrin (MS M^+ = 679) and partially hydrolysed material (MS M^+ = 1356). Under analogous reaction conditions, analysis of the mass spectrum of the reaction of **[3.6]** shows ions corresponding to both unreacted and phenolic porphyrin materials, again indicating an incomplete reaction.

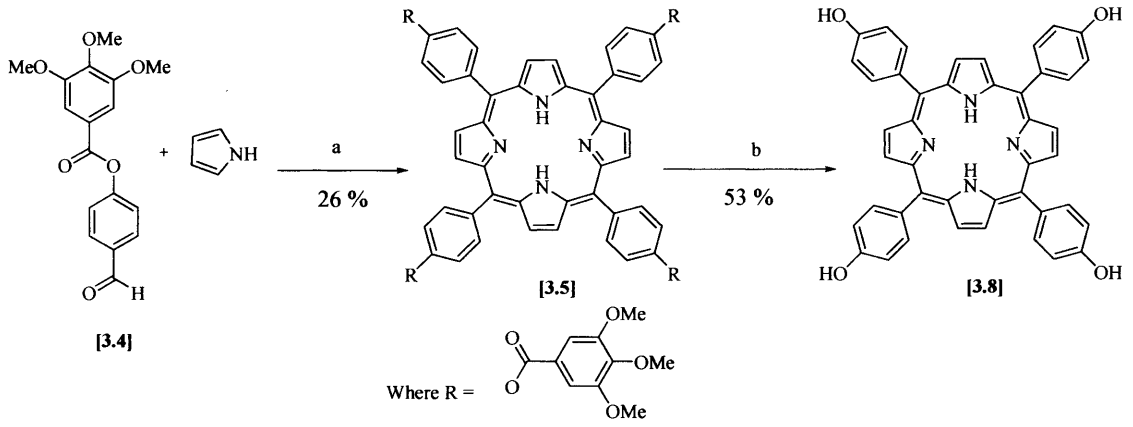


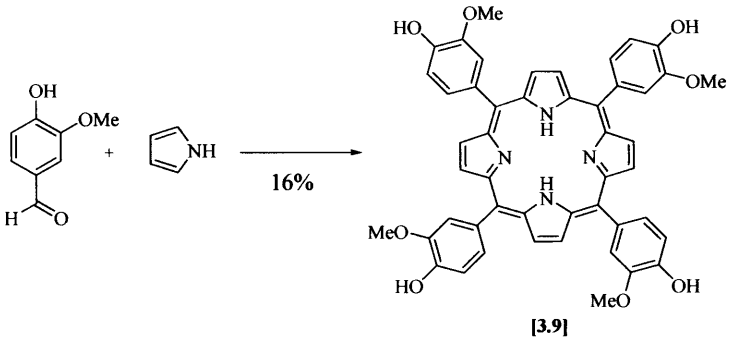
Figure 3.13: Reaction scheme illustrating the use of 3,4,5-trimethoxybenzyl ester as a protecting group for the phenol. *Reagents and conditions:* (a) propionic acid, reflux, 0.5 h; (b) hydrazine monohydrate, EtOH, reflux.

The conditions have not been optimised, never-the-less this method does illustrate potential as an alternative route for the synthesis of mono-substituted hydroxyphenyl porphyrins on a large scale. It is less suited to the tetra-substituted as the conditions do not give good yields when compared to current alternatives (pyridine hydrochloride[34] and BBr_3[35]). Preparation of tetra-substituted hydroxyphenyl porphyrins is still best achieved by demethylation using boron tribromide.

3.9 Alternative Route to Phenol Containing Porphyrins

Due to the problems encountered with the purification and the de-protection procedure described above, an alternative phenolic benzaldehyde was used, namely 4-hydroxy-3-methoxy benzaldehyde (vanillin). 5,10,15,20-Tetra-(3-methoxy-4-

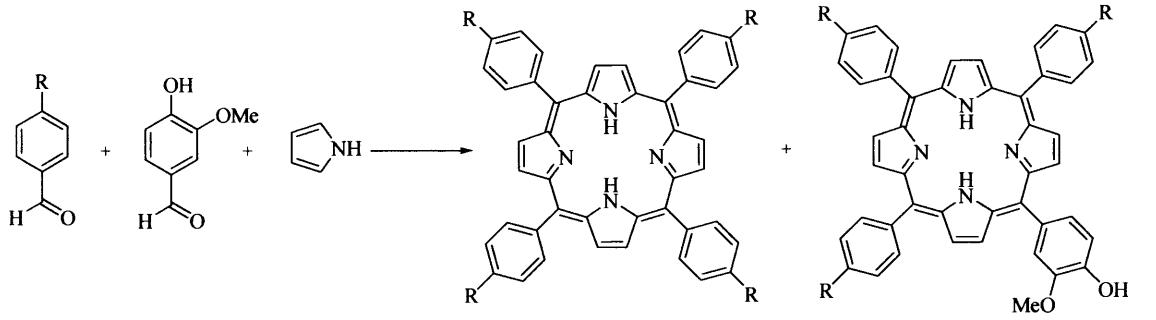
hydroxyphenyl)porphyrin[36,37] and the mono-substituted A_3B porphyrins (where B = phenyl[38] and B = tolyl[39]) have been previously reported.



[3.9]

Figure 3.14: Preparation of **[3.9]** *via* Adler methodology. *Reagents and conditions:* propionic acid, reflux, 0.5 h.

Porphyrins which contain vanillin moiety precipitate out of the propionic acid reaction mixtures thus eliminating the need for a protecting group. Using Adler methodology, **[3.9]** was prepared in 16 % yield (figure 3.14). Metallation was carried out using both platinum and palladium to afford **[Pt(3.9)]** and **[Pd(3.9)]**. In the UV/Vis spectra hypsochromic shifts were observed to the bands, an increased shift was observed in the platinum complex owing to increased metal-porphyrin backbonding.[5] Porphyrins of the A_3B type **[3.10]** and **[3.11]** were also prepared (figure 3.15); the obtained data was comparable to the literature.[38,39] The presence of both hydroxyl and methoxyl groups lowers the R_F values (R_F = 0.18) thus enabling ease of separation.



[3.10] where R= Me, R_F ($CHCl_3$) = 0.18 (4 %)

[3.11] where R= *t*-butyl, R_F ($CHCl_3$) = 0.21 (2 %)

Figure 3.15: Preparation of unsymmetrical, vanillin containing porphyrins **[3.10]** and **[3.11]** using the 'mixed aldehyde' approach. *Reagents and conditions*: propionic acid, reflux, 30 min.

3.10 Phosphorylation Methods

There are limited reports of porphyrins that incorporate a phosphate moiety in the literature. Porphyrins that have been functionalised at the *meso* position with a phosphate[40,41] have been used for the high sensitivity assay for alkaline phosphatase activity, enzyme immunoassay and trace metal ion quantification etc. These compounds have been synthesised by reacting a mono, di or tri hydroxybenzaldehyde with pyrrole to form 5,10,15,20-tetrakis-(hydroxyphenyl)porphyrin, which is then further reacted with pyrophosphoric acid and phosphorus pentoxide to give the corresponding phosphate derivative (i.e. 5,10,15,20-tetrakis(phosphonooxyphenyl)porphyrin[40]). The addition of a phosphate group is best achieved on compounds which contain a hydroxyl group, particularly a phenol.[20-28] With this in mind two routes of preparation for phosphate containing porphyrins was devised:

i. Incorporation of phosphate group into benzaldehyde.
ii. Incorporation of phosphate group directly to porphyrin.

3.11 Incorporation of phosphate group into benzaldehyde

Meso-tetrakis(4-phosphonatophenyl)-porphyrin has been previously prepared[42] by hydrolysis of the corresponding tetrakis diethyl ester (figure 3.16). Introduction of the phosphate group as the ester allows synthetic manipulations to be carried out in organic solvents, with the ethyl groups easily cleaved using trimethylsilyl iodide[43] or bromide[42] thus yielding a water soluble tetraphosphate.

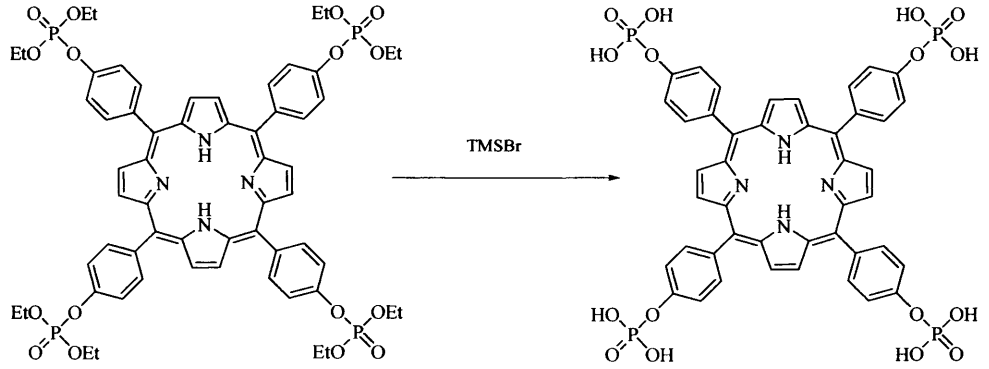
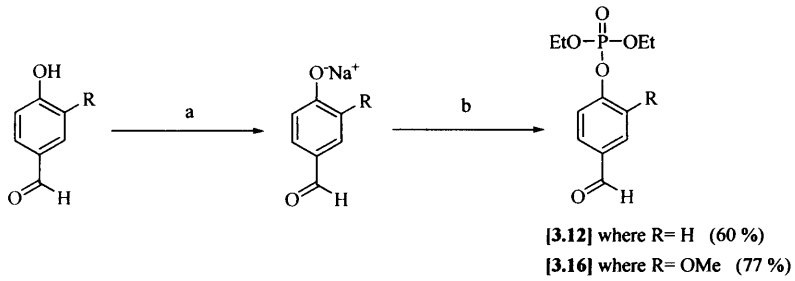


Figure 3.16: Preparation of *Meso*-tetrakis(4-phosphonatophenyl)-porphyrin as reported by De Napoli and workers.[42]

3.11.1 Synthesis of Benzaldehydes

Diethyl-4-formylphenyl phosphate **[3.12]** was prepared from chloro diethyl phosphate and the sodium salt of 4-hydroxybenzaldehyde (figure 3.17) as previously

reported[44] to afford **[3.12]** as a yellow oil (60 %). The $^{31}P\{^{1}H\}$ NMR spectrum shows a single signal at -6.48 ppm, which is consistent with values in the literature.[44] The phosphate ester of vanillin **[3.16]** was also synthesised in the same manner in a 77 % yield (figure 3.17). Again, the $^{31}P\{^{1}H\}$ NMR spectrum shows a signal at -6.09 ppm.[45]



[3.12] where R= H (60 %)
[3.16] where R= OMe (77 %)

Figure 3.17: Preparation of **[3.12]** and **[3.16]**. *Reagents and conditions:* (a) NaH, THF, inert atmosphere; (b) chloro diethylphosphate

As well as the mono-phosphorylated benzaldehydes, efforts were made to prepare benzaldehydes that incorporated two phosphate esters. By doing so, the effect of increasing the number of phosphate groups in a porphyrin has on water solubility could be investigated. Reaction of 2,4-hydroxybenzaldehyde with sodium hydride and chloro diethylphosphate (figure 3.18) did yield a phosphate ester (δ_P = -5.99 ppm). However, this may be a result of either mono or di substitution and the vast amount of unidentifiable phosphorus containing compounds (identified by $^{31}P\{^{1}H\}$NMR spectroscopy) was cause for concern since the cholinesterase inhibitor activity of chloro diethylphosphate poses a real threat.

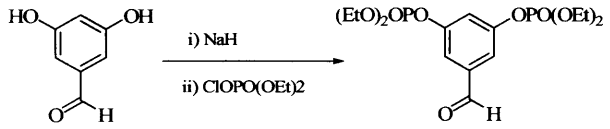


Figure 3.18: Attempted synthesis of a di-substituted phosphorylated benzaldehyde.

3.11.2 Synthesis of the Symmetrical Porphyrins [3.13] and [3.17]

The symmetrical tetra substituted porphyrin **[3.13]** was prepared (Adler methodology see figure 3.19), however the porphyrin material does not crystallise out of the propionic acid due to the increased polarity of the phosphate ester functions. Removal of the solvent and lengthy purification afforded the desired product in a low yield (3 %). The $^{31}P\{^{1}H\}$ NMR spectrum shows a signal at -4.60 ppm for the phosphate ester which is slightly downfield of the aldehyde **[3.12]** presumably due to incorporation into the porphyrin system. The HRMS is consistent with the formation of the tetra substituted product.

The symmetrical porphyrin **[3.17]** derived from diethyl 4-formyl-2-methoxyphenyl phosphate **[3.16]** was also synthesised (figure 3.19). The four methoxyl substituents modify the solubility in propionic acid which results in porphyrin precipitation. Purification on a silica column gave **[3.17]** in an 8 % yield. A singlet at -5.8 ppm is seen in the $^{31}P\{^{1}H\}$ NMR spectrum and multiplets at 1.40 and 3.80 ppm are observed in the ^{1}H NMR spectrum consistent with the phosphate ester. The molecular ion was confirmed by MALDI MS (1343.74).

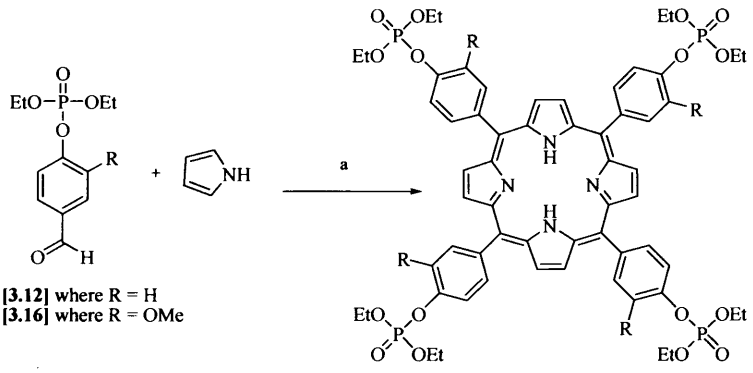


Figure 3.19: Preparation of porphyrins **[3.13]** and **[3.17]**. *Reagents and conditions:* propionic acid, reflux, 0.5 h.

3.11.3 Synthesis of the Mono-Substituted (A_3B) Porphyrins [3.14], [3.15] and [3.18]

Three unsymmetrical porphyrins **[3.14]**, **[3.15]** and **[3.18]** (A_3B type, where A = PhMe or PhCl) were prepared from the benzaldehydes **[3.12]** and **[3.16]** in 4, 2 and 0.5 % yields respectively (figure 3.20). The NMR spectral data is comparable to that of **[3.13]** and **[3.17]**, whilst the unsymmetrical nature was confirmed by the 3:1 ratio of the aromatic protons.

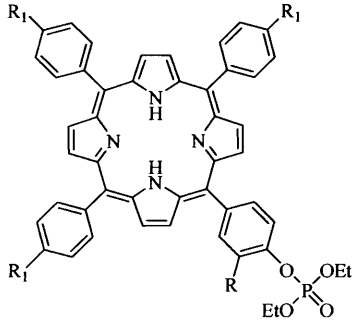


Figure 3.20: Mono-substituted porphyrins **[3.14]**, **[3.15]** and **[3.18]**.

The preparation of tetra-phenylporphyrins that incorporate a phosphate ester can be achieved by the reaction of a phosphate ester benzaldehyde with pyrrole in an Adler type methodology. Due to the nature and presence of four phosphate esters in **[3.13]**, synthesis *via* the Lindsey method may have been more appropriate. The prepared porphyrins are obtained as pure compounds. However the extensive separation and purification of the unsymmetrical porphyrins is reflected in the low yields.

Recent research within our group has shown that tetra-phenyl porphyrins that contain diethyl phosphate esters are unable to withstand the harsh conditions (MCl_2, PhCN, 200°C) which have been used to insert either platinum or palladium.[19] This discovery has led us to devise an alternative route for the preparation of metalled phosphate ester porphyrins.

3.12 Incorporation of phosphate group directly to porphyrin

3.12.1 Use of Sodium Hydride and Chloro Diethylphosphate

The phosphate containing porphyrin was envisaged to be prepared using the same method as previous; formation of the sodium salt followed by the treatment with chloro diethylphosphate.

3.12.1.1 Attempted Synthesis of 5, 10, 15, 20 Tetra ((4-diethyl phosphate) – 3 – methoxyphenyl) porphyrin [3.19]

Under an inert atmosphere, **[3.9]** was added to a suspension of four equivalents of sodium hydride in THF and stirred for 1.5 h. To this, four equivalents of chloro diethylphosphate were added and the mixture was stirred for a further 2 hours. The resultant NaCl was removed by filtration and the solvents were removed to give the crude product as a dark purple oily substance (figure 3.22). The $^{31}P\{^{1}H\}$ NMR spectrum shows a signal at -5.13 ppm which is consistent with that of **[3.17]** implying the formation of a phosphate containing porphyrin. Two further signals are also observed at 4.94 ($ClP(O)(OEt)_2$) and -12.55 ppm ($[(OEt)_2P(O)]_2O$).[46] Investigative experiments were performed on 4-hydroxybenzaldehyde using 1 molar equivalent and an excess of chloro diethylphosphate (figure 3.21). One equivalent of $ClOP(OEt)_2$ results in only production of the phosphate ester (δ_P = -5 ppm), whereas an excess produces the phosphate ester and pyrophosphate (δ_P = -12ppm).

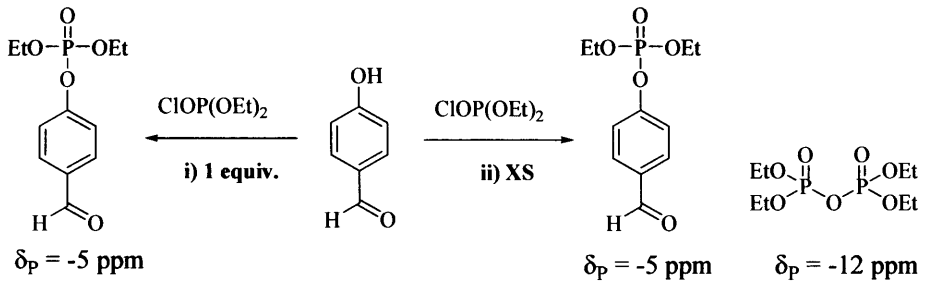
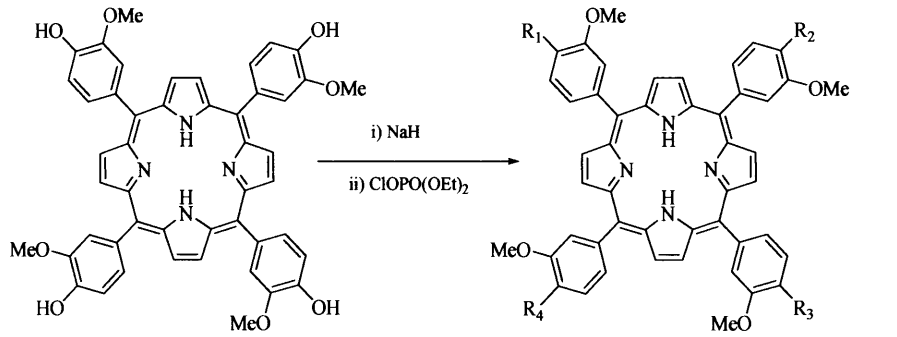


Figure 3.21: Experiments performed on 4-hydroxybenzaldehyde in the presence of either NaH or Et_3N and DMAP with either i) 1 equivalent or ii) an excess of chloro diethylphosphate.

Reactions performed on phenol porphyrins with chloro diethylphosphate were on a small scale so pyrophosphate formation may be a result of experimental error (*i.e.* an excess of reagent added). However, the hydrolysis of chloro diethylphosphate can also lead to the formation pyrophosphate and phosphoric acid.[47] In the MALDI MS the expected molecular ion for the tetra-phosphorylated porphyrin (Mr 1343.167) and twice-phosphorylated product (Mr 1071.31) are observed as weak intensity peaks. Peaks corresponding to unreacted porphyrin **[3.9]** and mono-phosphorylated product are seen at 766.35 and 935.38 Da/e respectively (figure 3.22). It can be deduced from the $^{31}P\{^1H\}$ NMR spectrum, that the chloro diethylphosphate is consumed in the formation of the tetra diethyl pyrophosphate resulting in the mono-phosphorylated product predominantly being formed (figure 3.22).



[3.9]

[3.19] where $R_1=R_2=R_3=R_4=$ OPO(OEt)$_2$

Figure 3.22: Attempted synthesis of **[3.19]** using NaH and ClOP(OEt)$_2$, where $R_1=R_2=R_3=R_4=$ OPO(OEt)$_2$. MS data shows the predominant presence of the product where $R_1=$ OPO(OEt)$_2$ and $R_2=R_3=R_4=$ OH (M_r = 935.38 Da/e).

3.12.1.2 Attempted Synthesis of Palladium Phosphorylated porphyrin

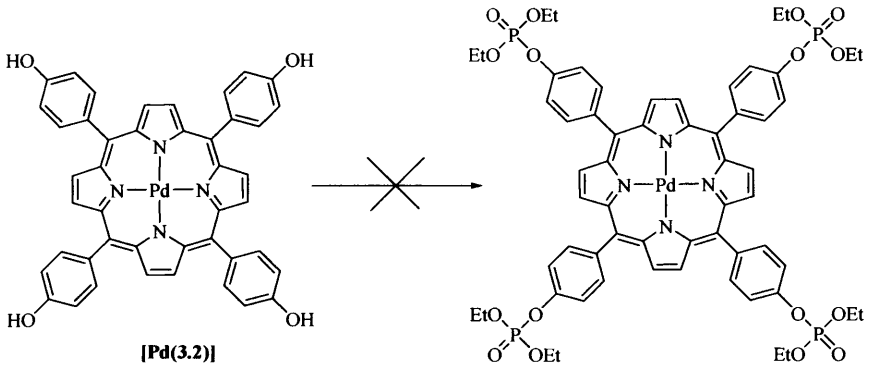


Figure 3.23: Attempted phosphorylation of **[Pd(3.2)]** using NaH and $ClOPO(OEt)_2$

Despite the incomplete reaction, the diethylphosphate addition to a palladium porphyrin was attempted (figure 3.23). To this end, **[Pd(3.2)]** was added to four equivalents of sodium hydride in THF and left to stir. This was followed by four equivalents of chloro diethylphosphate. The $^{31}P\{^1H\}$ NMR spectrum showed three signals at -12.94 (pyrophosphate[46]), 0.44 (diethyl phosphoric acid[48]) and 5.041 ppm ($ClP(O)(OEt)_2$), no signal is observed at -5 ppm. This implies that phosphate ester formation had not occurred and this was further reinforced by the ^1H NMR spectrum and the MALDI MS, both of which are consistent with that of **[3.2]**. Under these conditions, the coordinated palladium (+2) centre is reduced to free palladium metal, an occurrence that was visibly observed during the course of the reaction by the formation of a dark grey "mirror" on the inside of the Schlenk tube. The standard potential of the H_2/H^- couple is estimated to be -2.25 V[49], making H^- one of the most powerful reducing agents known. Despite employing NaH as a base, it appears that the formation of palladium hydride and the reduction to palladium metal is favoured over proton abstraction. Since the sodium salt of the phenol porphyrin is not produced, formation of the phosphate ester cannot proceed (figure 3.24).

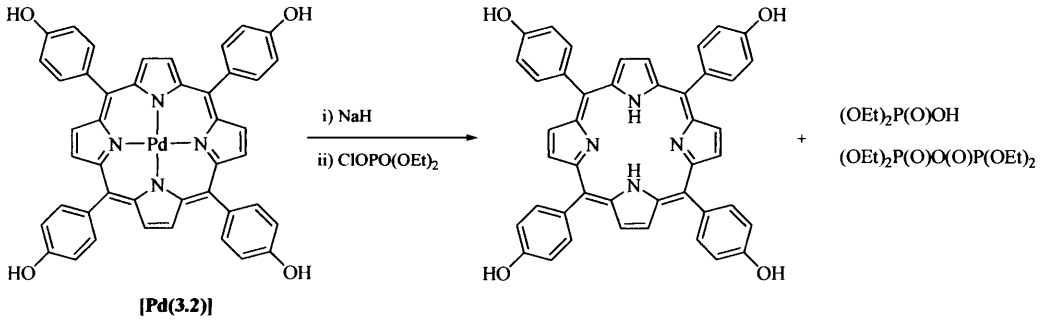
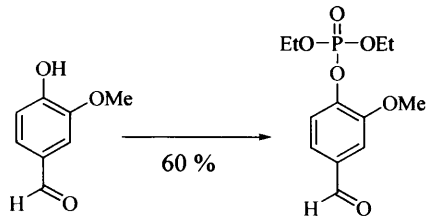


Figure 3.24: Reaction of **[Pd(3.2)]** with NaH and chloro diethyl phosphate.

3.12.2 Use of Triethylamine and Chloro Diethylphosphate

Phosphate esters can also be prepared by the reaction of either chloro diethylphosphate[50] or phosphite[45] in the presence of triethylamine. Reaction of chlorodiethyl phosphate in pyridine has also been reported.[51]

An alternative phosphorylation methodology was applied by a modification of the literature procedures using chloro diethylphosphate in the presence of triethylamine with the addition of a catalytic amount of dimethylaminopyridine (DMAP). 4 – (Diethyl phosphate) – 3 methoxybenzaldehyde **[3.16]** was prepared in this manner (60 %, figure 3.25) the spectroscopic data being consistent with that in the literature.[45]



[3.16]

Figure 3.25: *Reagents and conditions:* $ClOPO(OEt)_2$, DMAP (catalytic amount), Et_3N, THF.

3.12.2.1 Attempted Synthesis of 5, 10, 15, 20 Tetra ((4-diethyl phosphate) – 3 – methoxyphenyl) porphyrin [3.19]

Porphyrin **[3.9]** was reacted with four equivalents of triethylamine and chloro diethylphosphate in the presence of 0.1 equivalents of DMAP. Analysis of the $^{31}P\{^1H\}$ NMR spectrum indicates the presence of a phosphate ester with a singlet at -5.14 ppm. As with the reaction with NaH, signals are observed at -12.53 and 0.75 ppm which correspond to the pyrophosphate[46] and phosphoric acid.[48] Under these conditions all of the chloro diethylphosphate is consumed as it is not observed in the NMR spectrum. A small AB quartet is observed in the spectrum (δ -9.96 and -11.11 where $J_{P\text{-}P}$ 17.85 Hz), pyrophosphates typically exhibit geminal coupling constants in the region of 18-22 Hz.[52] Therefore, it is reasonable to presume that this phosphorus containing species is due to the formation of an unsymmetrical pyrophosphate. The unsymmetrical nature may have arisen by either trans-esterification or hydrolysis of the chloro diethylphosphate (figure 3.26).

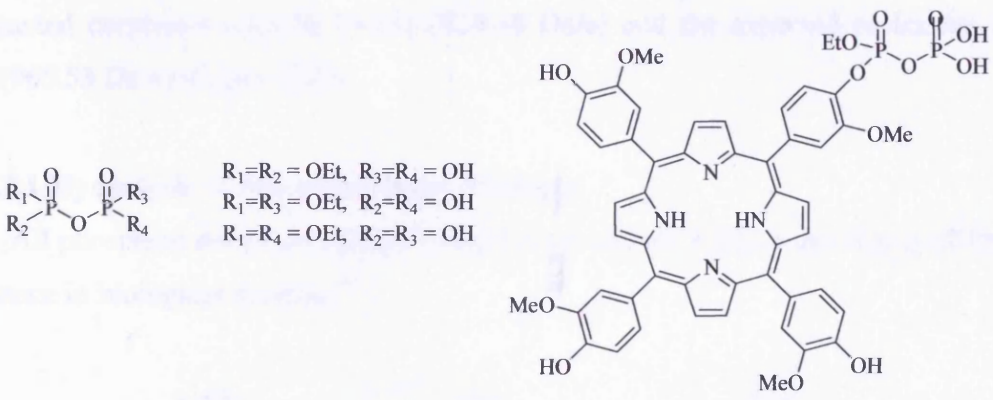


Figure 3.26: Possible structures of the unsymmetrical pyrophosphate formed during the attempted synthesis of **[3.19]**.

The MALDI mass spectrum does not show the expected molecular ion (M + 1) at 1343. A large peak is seen at 799.55 Da/e (peak **A**, figure 3.27) due to unreacted starting porphyrin **[3.9]** and as with the reaction with NaH, an ion corresponding to the mono phosphorylated species is seen at 935.38 Da/e. (peak **C**, figure 3.27) As anticipated, IR stretches at 1259 and 1036 cm^{-1} are observed attributed to the P=O bond.

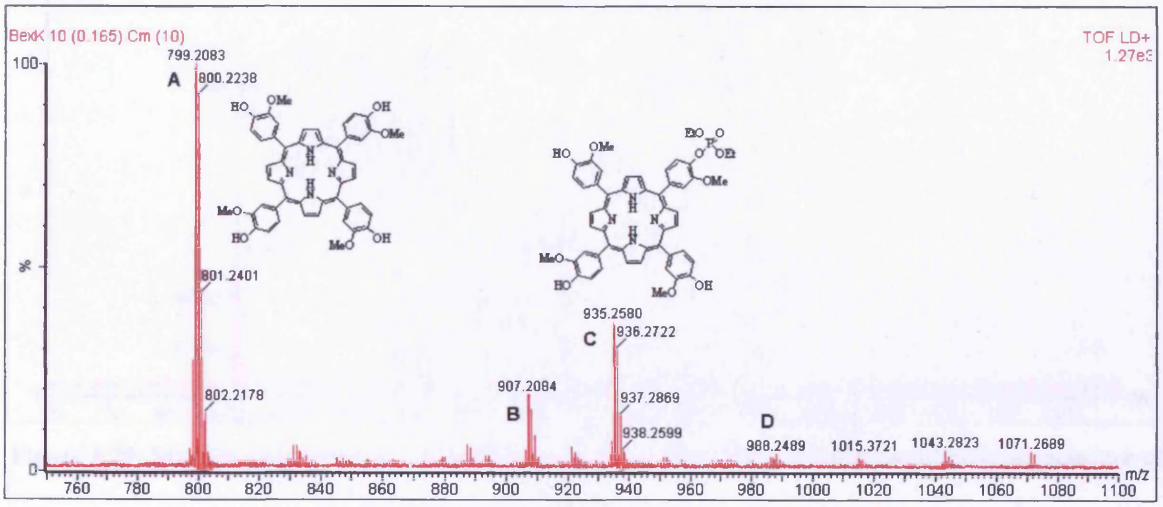


Figure 3.27: MALDI mass spectrum of the products obtained in the reaction of **[3.9]** and chloro diethylphosphate.

3.12.2.2 Synthesis of 5, 10, 15 Tri (4-*tert*-butylphenyl) 20 ((4-diethyl phosphate) – 3 – methoxyphenyl) porphyrin [3.20]

An analogous procedure was performed on porphyrin **[3.11]**. The presence of a phosphate ester was confirmed by a signal at -5.16 ppm in the $^{31}P\{^{1}H\}$ NMR spectrum. Signals ascribed to diethyl phosphoric acid, the symmetrical and unsymmetrical pyrophosphates are again observed. The MALDI mass spectrum shows ions corresponding

to unreacted porphyrin material **[3.11]** (829.48 Da/e) and the expected molecular ion for **[3.20]** (965.53 Da/e) (figure 3.29).

3.12.2.2.1 Hydrolysis of Phosphorylated Products

All phosphate esters are subject to hydrolysis (figure 3.28), a fact that is of immense importance in biological systems.[47]

$$(RO)_3PO \xrightarrow[-\,ROH]{+\,HOH} (RO)_2P(O)OH \xrightarrow[-\,ROH]{+\,HOH} (RO)P(O)(OH)_2 \xrightarrow[-\,ROH]{+\,HOH} P(O)(OH)_3$$

Figure 3.28: Hydrolysis of phosphate esters.

It is interesting to note, that in all the direct phosphorylation reactions molecular ions in the mass spectra are observed that correspond to hydrolysed product. For instance, in the reaction between **[3.11]** and chloro diethylphosphate in the presence of triethylamine, minor peaks are observed at 937.49 and 909.04 Da/e (peaks **B** and **C** in figure 3.29).

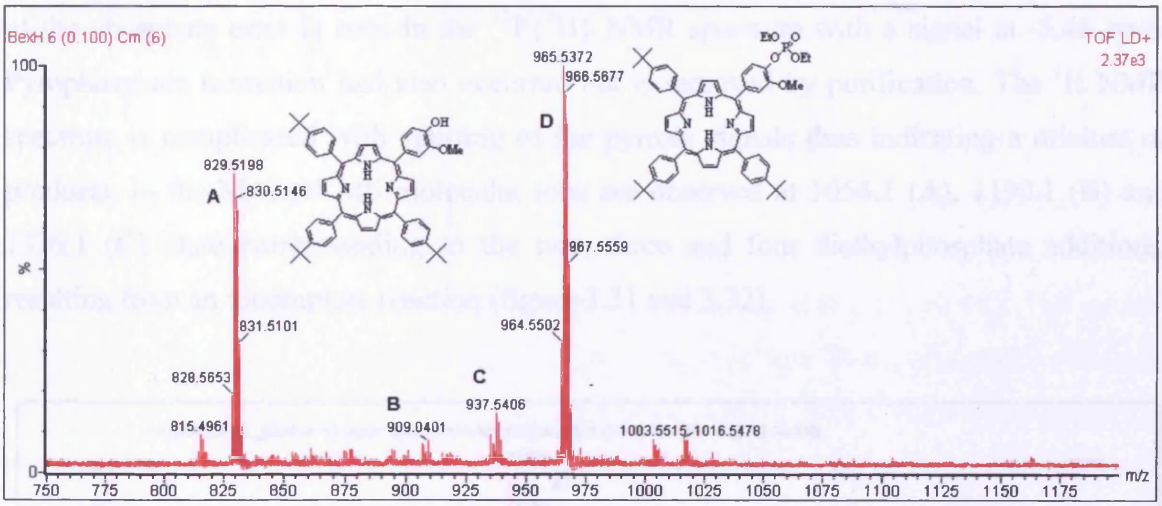


Figure 3.29: MALDI mass spectrum of the reaction of **[3.11]** with chloro diethylphosphate in the presence of Et_3N.

It is reasonable to presume that these ions (**B** and **C**) are a result of the hydrolysis of either one or both ester groups (figure 3.30). The presence of water, under these conditions may result in hydroxide formation which attacks the phosphate ester groups. This occurrence is also observed in attempts to phosphorylate tetra (4-hydroxy-3-methoxy porphyrin **[3.9]** with both NaH and triethylamine.

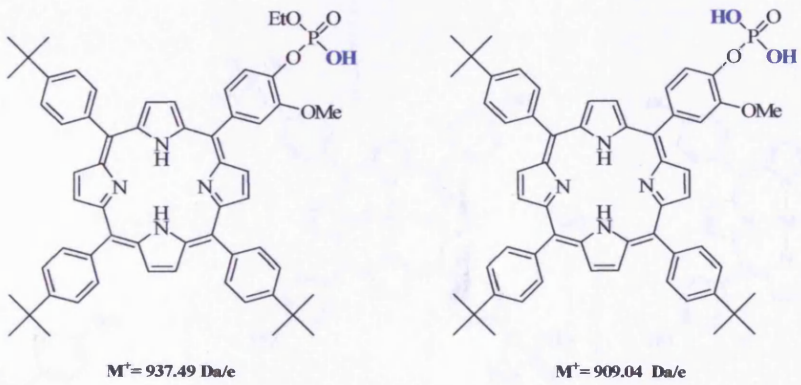


Figure 3.30: Possible structural assignments for MS ions at 937.49 and 909.04 Da/e shown in figure 3.29 as a result of the reaction of **[3.11]** with $ClOP(OEt)_2$.

3.12.2.3 Reaction of [Pd(3.2)] with Chloro Diethylphosphate

The palladium complex, **[Pd(3.2)]** was reacted with chloro diethylphosphate in the presence of triethylamine and DMAP under the same conditions as previous. No precipitation of palladium metal was observed during the course of this reaction. The product was purified by column chromatography followed by precipitation from chloroform/petroleum ether to give an orange solid **[Pd(3.21)]**. Evidence for the formation of the phosphate ester is seen in the $^{31}P\{^{1}H\}$ NMR spectrum with a signal at -5.48 ppm. Pyrophosphate formation had also occurred but is removed by purification. The ^{1}H NMR spectrum is complicated with splitting of the pyrrole signals thus indicating a mixture of products. In the MALDI MS molecular ions are observed at 1054.1 (**A**), 1190.1 (**B**) and 1326.1 (**C**) Da/e corresponding to the two, three and four diethylphosphate additions, resulting from an incomplete reaction (figure 3.31 and 3.32).

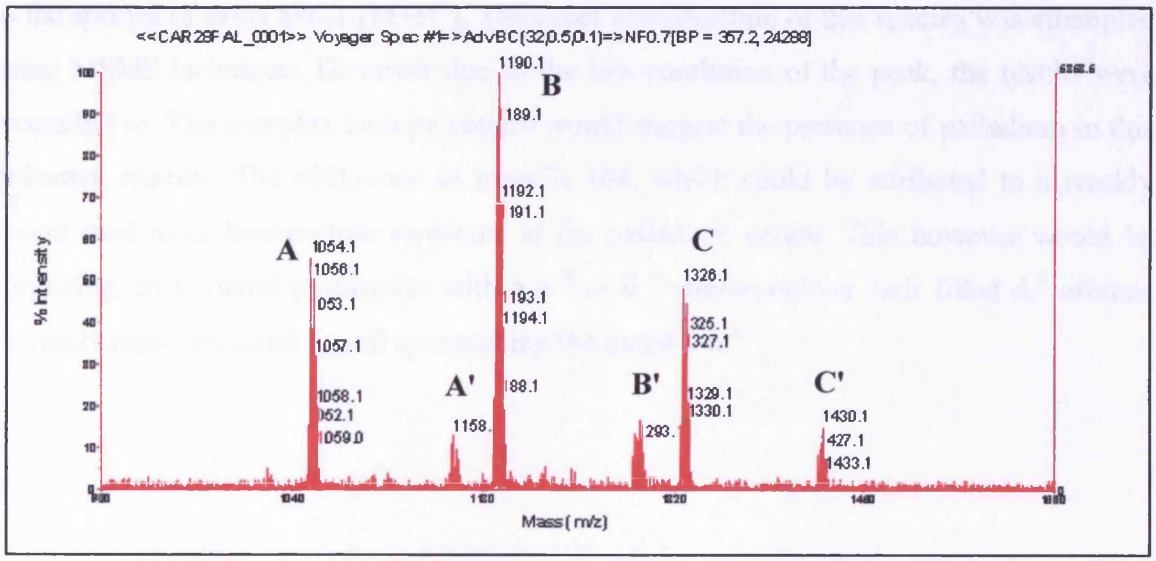


Figure 3.31: MALDI mass spectrum of the mixture of products obtained form the reaction between **[Pd(3.2)]** and chloro diethylphosphate.

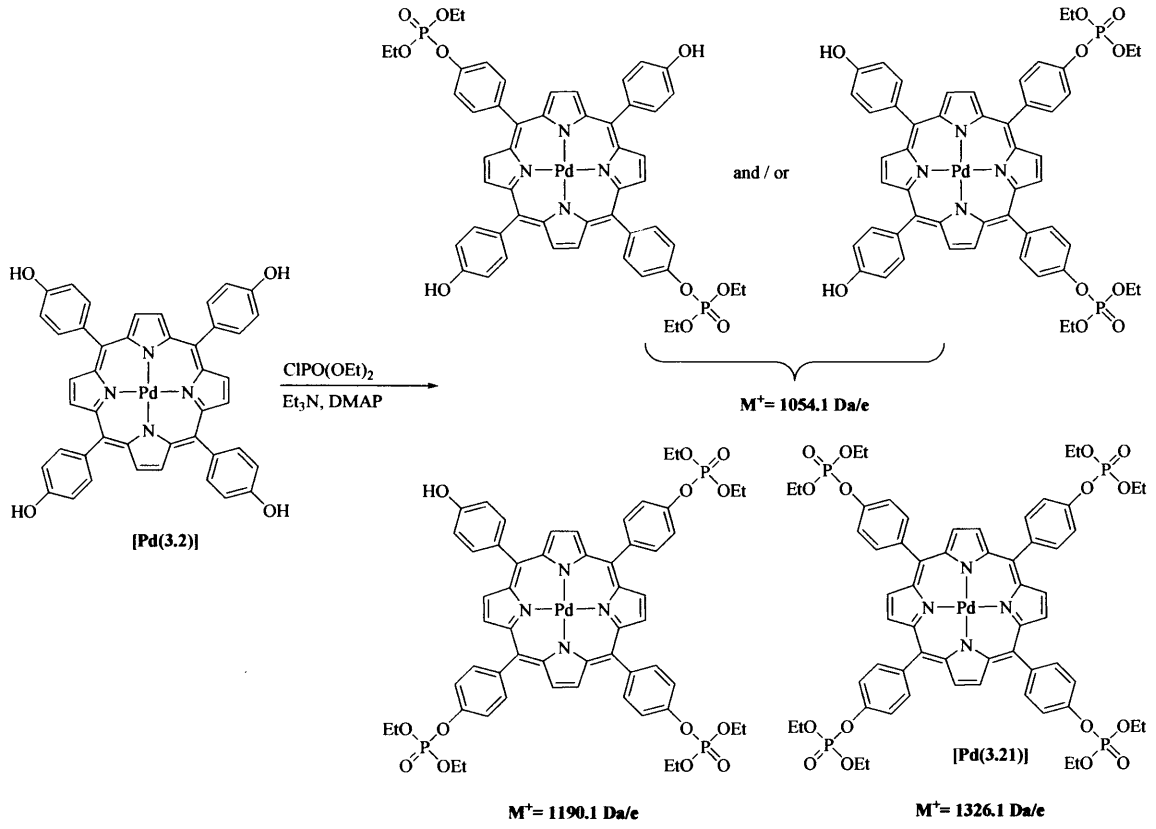


Figure 3.32: Mixture of products obtained in the reaction between **[Pd(3.2)]** and chloro diethylphosphate.

A second series of peaks are observed at 1158.1 (**A'**), 1294.1 (**B'**) and 1430.1 (**C'**) Da/e (figure 3.31). A possible explanation for this can be found in the mass spectrum of the starting porphyrin **[Pd(3.2)]** (figure 3.33). As well as the expected ion (782.1, **A**), a small, higher mass peak with a complex isotope pattern is observed at 886.1 Da/e (**B**). The second series of peaks appear to correspond to the two, three and four diethylphosphate additions to the species of mass 886.1 [$M+H^+$]. The exact identification of this species was attempted using MSMS technique. However due to the low resolution of the peak, the results were inconclusive. The complex isotope pattern would suggest the presence of palladium in this unknown species. The difference in mass is 104, which could be attributed to a weakly coordinated axial benzonitrile molecule at the palladium centre. This however would be surprising, as in metal porphyrins with a d^8 or d^9 configurations their filled d_z^2 orbitals normally repel any axial ligand approaching the metal ion.[5]

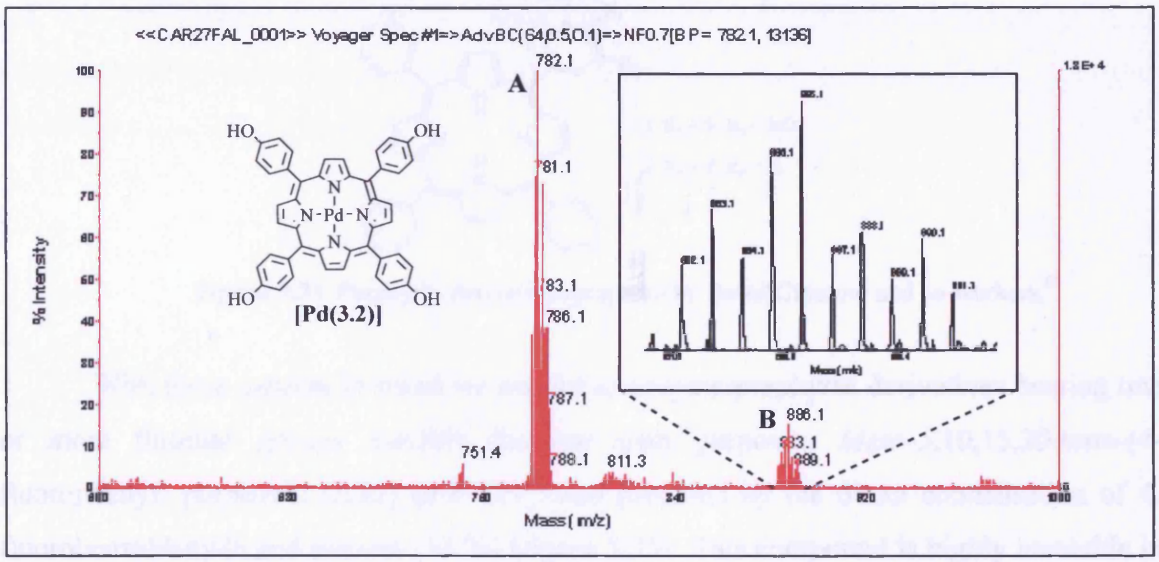


Figure 3.33: MALDI mass spectrum obtained for **[Pd(3.2)]**, with expansion of the higher mass peak at 886.1 Da/e.

3.13 Other work involving Porphyrin Systems

3.14 Fluorine Containing Porphyrins

In addition to porphyrin derivatives that contain an arylphosphate which is directly bound to the macrocyclic ring, investigations involving fluorinated porphyrins have also been carried out. We envisaged that the presence of fluoride atoms within the marker may form interactions with sites within a cell surface and hence enable the marker to more strongly adhere. The susceptibility of electron deficient arylfluorides to nucleophilic aromatic substitution can be utilised towards further modifications to the porphyrin structure.

Bedel-Cloutour and co-workers[53] have reported hydrosoluble and monofunctionalised *meso*-tripyridinylarylporphyrins which possess functional groups susceptible to covalent coupling with amino acid side chains present in both bovine serum albumin (BSA) and monoclonal antibodies (figure 3.34). It has been shown that the mesomeric effect of the nitro group in 3-nitro-4-fluorobenzaldehyde is sufficient enough to initiate fluoride lability.[54] Hence porphyrins bearing a fluoride in the *para* phenyl position, both with (***1***) and without (***2***) a nitro substituent were prepared.[53] Following the quaternization of the pyridyl rings and metallation with indium, the porphyrin derivatives ***1*** and ***2*** were coupled to bovine serum albumin in reasonable labelling efficiency (60 %, 75 %).[53]

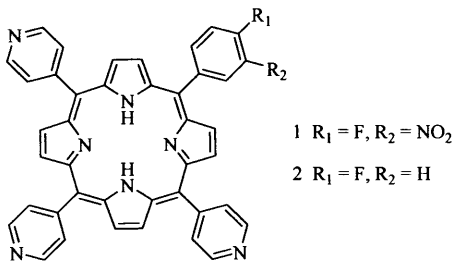
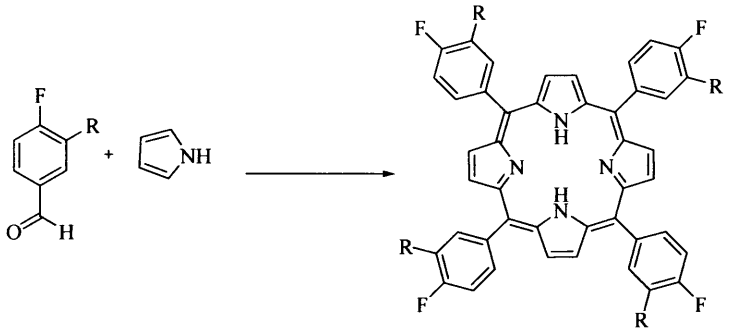


Figure 3.34 :Porphyrin derivatives prepared by Bedel-Cloutour and co-workers.[53]

With these aspects in mind we set out to prepare porphyrin derivatives bearing one or more fluoride groups suitable for our own purposes. *Meso*-5,10,15,20-tetra-(4-fluorophenyl) porphyrin **[3.22]** (*p*-F-TPP) was prepared by the direct condensation of 4-fluorobenzaldehyde and pyrrole (14 %) (figure 3.35). This compound is highly insoluble in both organic and aqueous mediums (e.g. the molar solubilities of *p*-F-TPP at 30°C in pyridine, benzene and chloroform are 7.1 x 10^{-5} M, 4.8 x 10^{-5} M and 4.9 x 10^{-5} M respectively).[55]



[3.22] where R= H (14 %)
[3.23] where R= NO_2 (21 %)

Figure 3.35: Preparation of fluorine containing porphyrins **[3.22]** and **[3.23]** *via* Adler method. *Reagents and conditions*: propionic acid, reflux, 0.5 h.

Meso-5,10,15,20-tetra-(4-fluoro-3-nitrophenyl)-porphyrin **[3.23]** (figure 3.35) was prepared from 4-fluoro-3-nitrobenzaldehyde **[5.1]** (synthesised as described in chapter 5.5.1), yielding a black material (21 %) which is extremely insoluble in both organic and aqueous solvents. The IR spectrum shows absorbencies at 3418 cm^{-1} (NH stretch), 1537 (antisymmetric N=O stretch) and 1348 cm^{-1} (symmetric N=O stretch) and 1266 cm^{-1} (CF). The water insolubility of both fluorinated porphyrins **[3.22]** and **[3.23]** could be used to our advantage since we seek to prepare compounds/markers that become water insoluble upon the cleavage of an enzyme substrate.

Attempts were made to displace the *para*-fluoride in **[3.22]** and **[3.23]** with a nucleophile in order to increase the solubility of these compounds. The reaction of **[3.22]**

and **[3.23]** with nucleophiles such as *tert*-butylanliline, *N*-methylglucamine, diethanolamine, benzylmercaptane and the disodium salt of 4-aminophenyl phosphate proved unsuccessful (figure 3.36).

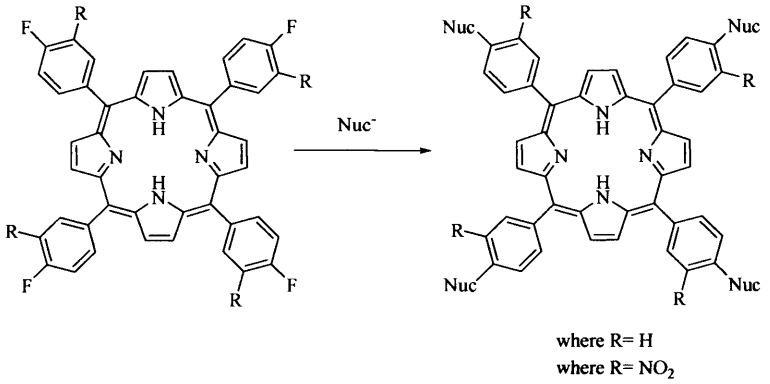


Figure 3.36: Attempted displacement of *para*-fluorides in **[3.22]** and **[3.23]** with a nucleophile (Nuc^-)

A series of asymmetric porphyrins were prepared by the method of Little *et al*[32] as illustrated in figure 3.37. Whilst the presence of some of these functions imparted an element of solubility in organic solvents such as chloroform, purification and manipulations still presented difficulties that could not be overcome.

R_1	R_2	R_3	R_4
4-F-Ph	4-F-Ph	4-F-Ph	4-OMe-Ph
4-F-Ph	4-F-Ph	4-F-Ph	4-OH-Ph
4-F-Ph	4-F-Ph	4-F-Ph	4-$OC_{12}H_{25}$-Ph
4-F-Ph	4-F-Ph	4-F-Ph	4-$OPO(OEt)_2$-Ph
Ph	Ph	Ph	4-F-Ph
Ph	Ph	Ph	4-F-3-NO_2-Ph

Figure 3.37: Table illustrating range of asymmetric porphyrins containing fluorine.

3.14.1 *Meso*-tetra-(pentafluorophenyl)porphyrin

The *para*-fluoride substituents in *meso*-tetra-(pentafluorophenyl)porphyrin can be selectively displaced by nucleophiles such as amines, thiols and phenolate anions.[56,57] McKeown[58] has shown that the reaction of *meso*-tetra-(pentafluorophenyl)porphyrin with 4 equivalents catechol results in the displacement of the *para*-fluoride followed by the rapid intramolecular substitution of the neighbouring *meta*-fluoride.

Using the conditions developed by Lindsey, pentafluorobenzaldehyde and pyrrole were reacted to give **[3.24]** as a red solid (20 %) (figure 3.38). In the ^{19}F NMR spectrum, three multiplets are observed corresponding to the *ortho* (-136.3 ppm, pair of doublets), *meta* (-161.1 ppm, multiplet) and *para* (-151.0, triplet) fluorides.

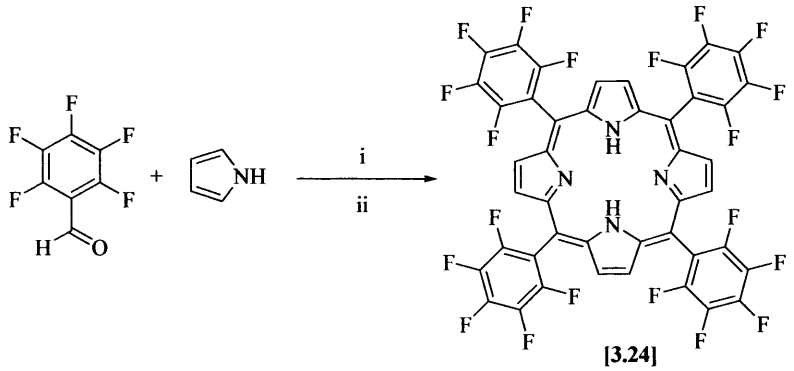


Figure 3.38: Preparation of **[3.24]** *via* the Lindsey method. *Reagents*: i) BF_3-etherate, DCM, N_2; ii) DDQ.

The introduction of an iodide can be utilised towards further modifications either *via* nucleophilic attack or a coupling reaction (e.g. Heck, Suzuki, Sonogashira). Hence, veratrole and iodine monochloride were reacted in a one molar ratio to give iodo-3,4-dimethoxybenzene **[3.25]** (62 %) which was subsequently treated with boron tribromide[29] generating iodo-3,4-dihydroxybenzene **[3.26]** (74 %) (figure 3.39).

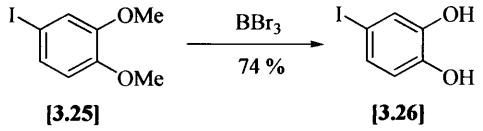


Figure 3.39: Preparation of **[3.26]** from **[3.25]**.

The reaction between **[3.24]** with four equivalents of **[3.26]** in the presence of potassium carbonate (8 eq) and N-methyl-2-pyrrolidinone at 100 °C was performed (figure 3.38).[58] The resulting precipitate of a brown material was analysed by ^{19}F NMR spectroscopy, giving rise to three multiplets at -139.9 ppm (doublet), -155.2 ppm and -164.1 ppm. A simplification of the fluoride spectrum is observed due to the displacement of the *para* and neighbouring fluoride atom. However, the low obtainable yields of both **[3.24]** and **[3.27]** and the elaborate nature of this synthesis restricted its use and further development.

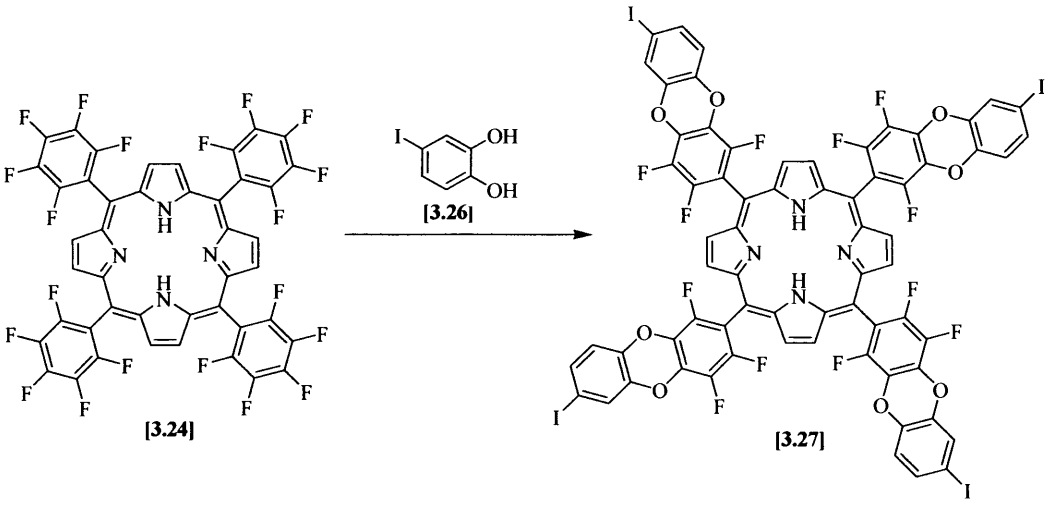


Figure 3.38: *Reagents and conditions*: K_2CO_3 (8 eq.), NMP, 100°C.

3.15 Azo Containing Porphyrins

Many of the porphyrins that have been prepared have been metallated with either platinum or palladium. The conditions employed are harsh (temperatures greater than 200 °C) and porphyrins that feature sensitive peripheral groups, namely phosphate do not withstand these conditions.[19] To overcome this problem, another metal source combined with milder conditions could be employed or alternatively another position of metallation incorporated. To date, the later has been explored by attempts to conjugate an azo group which incorporates a phosphate function. Compounds that contain an azo group are intensely coloured ranging from a deep red through to yellow. The incorporation of this functional group would enable markers to be visualised under a light microscope without the need for amplification with silver. An azo group attached to a phenyl ring can be metallated with palladium under mild conditions using palladium (II) bis(benzonitrile) dichloride, alleviating the need to metallate at the porphyrin centre.

A commercially available substrate of alkaline phosphatase, 4-nitrophenyl phosphate disodium salt was hydrogenated (H_2/Pd on carbon) to the water soluble amine **[3.28]**. Generation of the azo group was performed under aqueous conditions (figure 3.39).[59]

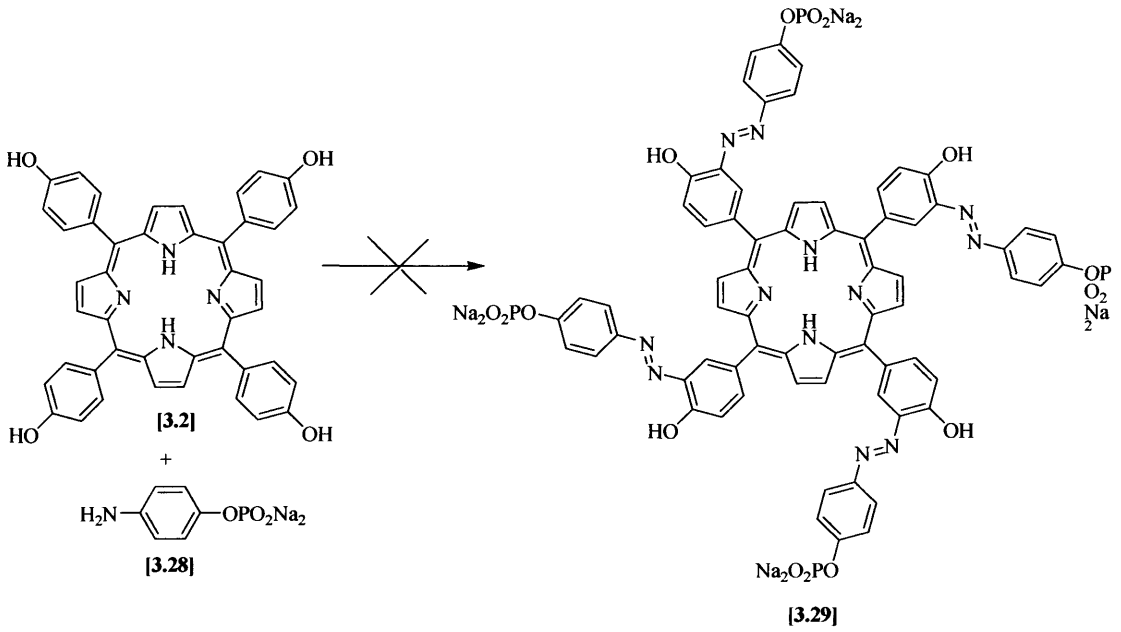


Figure 3.39: Attempted synthesis of **[3.29]**. *Reagents and conditions*: sodium carbonate, sodium nitrate.

Purification of the products was attempted using a cellulose dialysis membrane (see experimental section for details). The mass spectrum of the product contained a molecular ion at 782.2 Da/e and the ^{1}H NMR spectrum was identical to that of **[3.2]** thus indicating an unsuccessful reaction.

3.16 Laboratory Testing of Compounds

Previous studies[19] have demonstrated that platinum and palladium porphyrin derivatives showed good redox activity in the catalytic reduction of aqueous silver ions. In contrast, metal-free derivatives showed no activity. A series of screen tests, as described in chapter 2 (section 2.12), were carried out to establish whether the compounds synthesised are suitable for our application. Five complexes of 5,10,15,20-*meso*-tetraphenylporphyrin (TPP) were also included in the tests.

Results from the initial screen tests were in agreement with previous studies, with the metallated porphyrin derivatives catalysing the reduction of aqueous silver ions in times ranging from 5-30 minutes whilst the metal-free porphyrins showed no redox activity. The metallated porphyrin compounds were passed through a pad of Celite® and a short silica column to remove any traces of residual metal prior to a second screen testing. The results are presented in figure 3.40.

Compound	Solubility in Physical Developer	Redox Activity	Compound	Solubility in Physical Developer	Redox Activity
[3.1]H_2	insoluble	no activity	**[Pt(3.9)]**	insoluble	no activity
[Pd(3.1)]	insoluble	no activity	**[3.13]H_2**	insoluble	no activity
[3.2]H_2	insoluble	no activity	**[Pd(3.21)]**	insoluble	no activity
[Pd(3.2)]	insoluble	no activity	**[TPP]H_2**	insoluble	no activity
[Pt(3.2)]	insoluble	no activity	**[Pd(TPP)]**	insoluble	no activity
[3.5]H_2	insoluble	no activity	**[Pt(TPP)]**	insoluble	no activity
[Pt(3.5)]	insoluble	no activity	**[Ag(TPP)]**	insoluble	no activity
[3.9]H_2	insoluble	no activity	**[Zn(TPP)]**	insoluble	no activity
[Pd(3.9)]	insoluble	no activity	**[Mn(TPP)]**	insoluble	no activity

Figure 3.40: Summary of test results after trace metal removal. 'No activity' implies no notable change during a 30 minute period.

Following further purification the metallated porphyrin derivatives were found to show no noticeable redox activity for the reduction of aqueous silver ions (i.e. no darkening of the physical developer over a 30 minute period). From this it can be deduced that previous positive results may be due to traces of free metal or metal carrier in the compounds.

3.17 Conclusion

The objective of this work was to prepare porphyrin systems featuring a phosphate group that are redox-active for the reduction of silver. The introduction of the phosphate groups was achieved *via* the benzaldehyde and also by the reaction between a phenol-porphyrin and chloro diethylphosphate.

We have found that the separation of 5,10,15-tri-(4-methoxyphenyl)-20-(4-tolyl)porphyrin **[3.3]** from 5,10,15,20-tetra-(4-tolyl)porphyrin on a large scale problematic. We introduced a benzyl ester to protect the phenol, which enabled the successful separation of 5,10,15-tri-(3,4,5-trimethoxybenzoic acid phenyl ester)-20-(4-tolyl)porphyrin **[3.6]** from 5,10,15,20-tetra-(4-tolyl)porphyrin. The difference in R_F values of these two porphyrins is substantial enough to allow separation even on a large scale. The benzyl ester groups can be removed by treatment with hydrazine monohydrate to afford the corresponding phenol-porphyrin. Several symmetrical and unsymmetrical porphyrins bearing aryl phosphate esters have been prepared from the benzaldehydes **[3.12]** and **[3.16]** to afford the corresponding porphyrins in yields ranging between 0.5 and 8 %. The reaction of 5,10,15,20-tetra-(4-hydroxyphenyl)porphyrin, **[3.2]** and 5,10,15,20-tetra-(4-hydroxy-3-

methoxyphenyl)porphyrin, **[3.9]** with chloro diethylphosphate in the presence of either sodium hydride or triethylamine failed to afford the tetra-phosphorylated products. The reaction of 5,10,15-tri-(4-*tert*-butylphenyl)-20-(4-hydroxy-3-methoxyphenyl)porphyrin, **[3.11]** with chloro diethylphosphate afforded the product **[3.20]** along with unreacted **[3.11]**. Attempts were made to phosphorylate the metal complex **[Pd(3.2)]**. When using sodium hydride, phosphorylation did not occur and the coordinated palladium was reduced to palladium metal. The reaction using triethylamine generated a mixture of phosphorylated products that were unable to be separated.

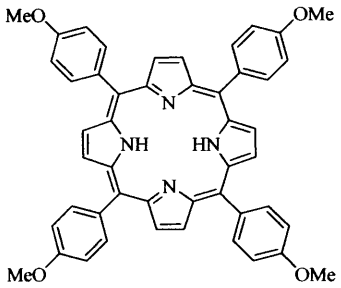
The metal complexes along with the corresponding free base analogues were tested to establish whether they were suitable catalysts for our application. Following purification the compounds were not found to show any activity for the reduction of silver in a Timm's type reaction. This is not in agreement with earlier findings and we conclude that previous positive results were attributed to impurities in the tested samples.

Experimental

General Procedure

Reagents were purchased from Aldrich, Avocado or Lancaster and were used as received. Solvents were purified by standard literature methods.[60] Tetra-arylporphyrins were synthesised using the Adler-Longo method[11] unless otherwise stated and the pyrrole used was distilled prior to use. The porphyrins $[TPP]H_2$,[11] [TPP]Pd, [TPP]Pt,[61] and [TPP]Ag, [TPP]Zn, [TPP]Mn[62,63] were prepared by literature methods. Column chromatography was performed using silica gel (60 Å). CAUTION: chloro diethylphosphate is highly toxic and care must be taken when using this reagent. Due to the possibility of accidental acetylcholinesterase synthesis, reactions must be carried out in a fluoride free environment. The NMR spectra were recorded on a Brüker Avance AMX 400 instrument at 400 MHz (^{1}H) and 100 MHz (^{13}C), JEOL Lamda Eclipse 300 at 121.65 MHz (^{31}P) and 282.78 MHz (^{19}F); ^{1}H and ^{13}C chemical shifts are quoted in ppm relative to residual solvent peaks and ^{31}P chemical shifts are quoted in ppm relative to external 85 % H_3PO_4 (δ 0). Coupling constants are quoted in Hertz. Mass spectra were obtained in APCI (atmospheric pressure chemical ionisation), EI (electronic ionisation) or MALDI modes. IR spectra were obtained as KBr or NaCl discs using a Jasco FTIR 110 series spectrometer. UV/Vis spectra were recorded on a Jasco V-750 UV/Vis/NIR spectrophotometer.

[3.1]H_2 *Meso*-5,10,15,20-tetra (4-methoxyphenyl) porphyrin

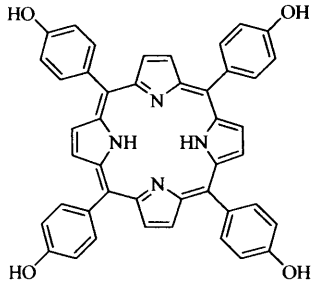


The general procedure is described for the preparation of *meso*-5,10,15,20-tetra (4-methoxyphenyl) porphyrin **[3.1]**. Standard reactions were performed in a round bottomed flask fitted with a condenser open to the air. 4-Methoxybenzaldehyde (20 g,0.146 mol) and freshly distilled pyrrole (9.19 g, 0.137 mol) were added to refluxing propionic acid (250 mL), reacted for 30 min, allowed to cool, filtered and washed with MeOH until the washings ran clear. The resulting purple solid was dissolved in chloroform and passed through with a short silica plug (approx 1g porphyrin to 20g silica gel). Eluting with chloroform and removal of solvents *in vacuo* affords the title compound **[3.1]** as a shiny purple solid (5.38 g, 20 %); δ_H (400 MHz, $CDCl_3$) -2.60 (s, 2H, N*H*$_2$), 4.0 (s, 12H, OC*H*$_3$), 7.20 (d, 8H, *J* 7.2, Ar *H*-3 and *H*-5), 8.1 (d, 8H, *J* 8.3, Ar *H*-2 and *H*-6), 8.8 (s, 8H, *H*-β-pyrrole); δ_C (100 MHz, $CDCl_3$) 28.0, 56.2, 113.2, 119.6, 134.1, 136.8, 160.1; IR (KBr, cm^{-1}) 3317 (NH stretch), 2930, 2840, 2365, 1745, 1609, 1510, 1470, 1438, 1351, 1281, 1248, 1179, 1106, 1054, 965, 840, 800; UV/Vis ($CHCl_3$, nm, log ε/dm^3 mol^{-1} cm^{-1}) 424 (5.10), 520, 556, 594, 652; *m/z* (MALDI) 735.2 [M+1].

[Pd(3.1)] *Meso*-5,10,15,20-tetra (4-methoxyphenyl) porphyrin palladium

Compound **[3.1]** (0.418 g, 0.56 mmol) and palladium (II) dichloride (100 mg, 5.6 x 10^{-4}) were heated in benzonitrile (15 mL) for 6 h at 200°C. The reaction mixture was allowed to cool, filtered to remove any excess palladium salts and the benzonitrile removed *in vacuo*. The crude product was taken up in a small amount of MeOH and passed through a short silica column eluting with chloroform to afford the pure metallated product **[Pd(3.1)]** as a pink solid (0.112 g, 23 %) δ_H (400 MHz, $CDCl_3$) 4.04 (s, 12H, OC*H*$_3$), 7.20 (d, 8H, *J* 8.2, Ar *H*-3 and *H*-5), 8.0 (d, 8H, *J* 8.4, Ar *H*-2 and *H*-6), 8.75 (s, 8H, *H*-β-pyrrole); δ_C (100 MHz, $CDCl_3$) 53.1, 109.4, 127.9, 132.1, 132.7, 139.2, 156.4; IR (KBr, cm^{-1}) 1605, 1540, 1457, 1352, 1286, 1247, 1173, 1008, 803; UV/Vis ($CHCl_3$, nm, log ε/dm^3 mol^{-1} cm^{-1}) 422 (4.95), 526, 560; *m/z* (MALDI) 838. 249 [calculated 839.25].

[3.2] *Meso*-5,10,15,20-tetra (4-hydroxyphenyl) porphyrin



Compound **[3.1]** (3.87 g, 5.27 mmol) was dissolved in degassed DCM (100 mL) and cooled to -80 C. To this, boron tribromide (2.26 mL) in DCM (10 mL) was added dropwise and stirred for 1 h at -80 °C. The solution was allowed to warm to room temperature and stirred overnight. Water was added to quench any unreacted boron tribromide and the solution was basified with triethylamine until a colour change from green to purple is observed. The precipitated solid was filtered, washed with water and was dried in a vacuum dessicator for 48 h to afford **[3.2]** as a purple solid (1.78 g, 50 %); δ_H (400 MHz, MeOD) 7.12 (d, 8H, *J* 8.3, Ar *H*-3 and *H*-5), 7.90 (d, 8H, *J* 8.34, Ar *H*-2 and *H*-6), 8.80 (br s, 8H, *H*-β-pyrrole); IR (KBr disc, cm^{-1}) 3311, 2954, 1610, 1524, 1401, 1286, 1260, 1177, 801, 769; UV/Vis (MeOH, nm, log ε/dm^3 mol^{-1} cm^{-1}) 422 (4.97), 518, 554, 594, 650; *m/z* (MALDI) 679.211 [calculated 678.745].

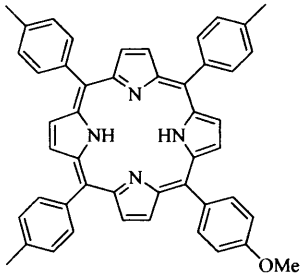
[Pd(3.2)] *Meso*-5,10,15,20-Tetra (4-hydroxyphenyl) porphyrin palladium

This compound was prepared in an analogous manner to that of **[Pd(3.1)]**. The applied reagents were **[3.2]** (0.1 g, 0.147 mmol) and palladium dichloride (200 mg) to afford **[Pd(3.2)]** as a pink solid (0.0611 g, 53 %); δ_H (400 MHz, MeOD) 7.05 (d, 8H, *J* 8.2, Ar *H*-3 and *H*-5), 7.73 (d, 8H, *J* 8.31, Ar *H*-2 and *H*-6), 8.65 (s, 8H, *H*-β-pyrrole); IR (KBr, cm^{-1}) 3426, 2959, 1724, 1608, 1506, 1457, 1351, 1270, 1123, 1073, 1012, 798; UV/Vis (MeOH, nm, log ε/dm^3 mol^{-1} cm^{-1}) 418 (5.00), 526; *m/z* (MALDI) 782.196 [calculated 783.14].

[Pt(3.2)] *Meso*-5,10,15,20-tetra (4-hydroxyphenyl) porphyrin platinum

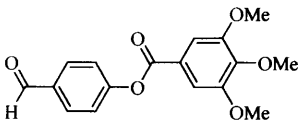
Compound **[3.2]** (0.2 g, 0.294 mmol) and platinum (II) dichloride (100 mg) were heated in benzonitrile (15 mL) for 6 h at 200°C. The reaction mixture was allowed to cool, filtered to remove any excess platinum salts and the solvent removed *in vacuo*. The crude product was passed through a short silica column eluting with THF to afford the pure product **[Pt(3.2)]** as an orange solid (0.15 g, 60%); δ_H (400 MHz, MeOD) 7.0 (d, 8H, *J* 8.3, Ar *H*-3 and *H*-5), 7.6 (d, 8H, *J* 8.27, Ar *H*-2 and *H*-6), 8.70 (s, 8H, *H*-β-pyrrole); UV/Vis (THF, nm, log ε/dm^3 mol^{-1} cm^{-1}) 408 (5.21), 512; *m/z* (MALDI) 872.142.

[3.3]H_2 *Meso*-5,10,15-Tri (4-tolyl)- 20-(4-methoxyphenyl) porphyrin



The general procedure is described for the preparation of *meso*-5,10,15-tri (4-tolyl)- 20-(4-methoxyphenyl) porphyrin **[3.3]**. Standard reactions were performed in a round bottomed flask fitted with a condenser open to the air. 4-Tolylbenzaldehyde (20 g, 0.16 mol), 4-methoxybenzaldehyde (1.13 g, 0.008 mol) and pyrrole (10.89 g, 0.1624 mol) were added to refluxing propionic acid (250 mL) and reacted for 30 minutes, Upon cooling the reaction mixture was filtered and washed with MeOH. The crude product was taken up in chloroform and passed through a short silica plug eluting with chloroform to remove tar-like impurities. The crude material was re-chromatographed on silica gel with hexane to elute *meso*-5,10,15,20-tetra(4-tolyl) porphyrin. The solvent system was changed gradually to a 3:5 ratio of hexane:chloroform to remove a second fraction (R_F 0.68) containing **[3.3]**. Removal of the solvents afforded **[3.3]** as a purple solid (0.62 g, 11 %); δ_H (400 MHz, $CDCl_3$) -2.62 (s, 2H, N*H*$_2$), 4.0 (s, 9H, OC*H*$_3$), 7.22 (d, 2H, *J* 7.1, Ar *H*-3' and *H*-5'), 7.45 (d, 6H, *J* 7.68, Ar *H*-3 and *H*-5), 8.05 (d, 6H, *J* 7.74, Ar *H*-2 and *H*-6), 8.1 (d, 2H, *J* 8.2, Ar *H*-2' and *H*-6'), 8.7 (s, 6H, *H*-β-pyrrole), 8.8 (s, 2H, *H*-β-pyrrole'); IR (KBr, cm^{-1}) 3329, 2968, 1571, 1510, 1473, 1408, 1384, 1347, 1208, 2000, 1156, 1072, 985; UV/Vis ($CHCl_3$, nm, log ε/dm^3 mol^{-1} cm^{-1}) 422 (4.93), 520, 556, 594, 652 *m/z* (MALDI) 687.325 [calculated 686.85] .

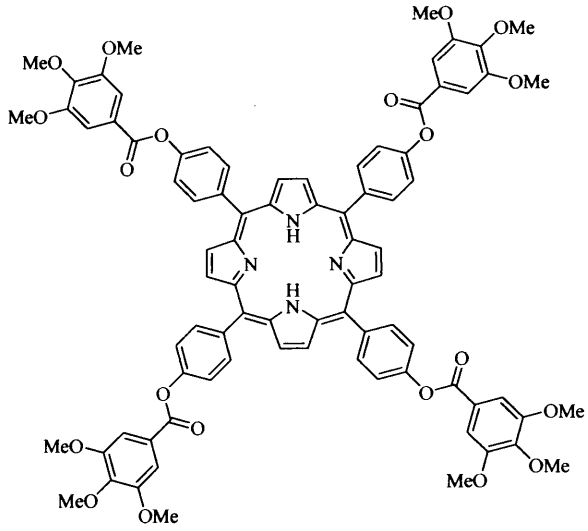
[3.4] 3,4,5-Trimethoxy benzoic acid Phenyl Ester



Thionyl chloride (6.2 mL, 47 mmol) and DMF (1 drop) were added to a of suspension of 3,4,5-trimethoxy benzoic acid (10 g, 47 mmol) in dry dichloromethane (200 mL) and stirred under an atmosphere of nitrogen for 1 h, until the solution had gone clear. The solvents were removed *in vacuo* to give 3,4,5-trimethoxyl benzyl chloride (11.28 g). 4-Hydroxybenzaldehyde (5.97 g, 48.9 mmol) was suspended in chloroform in a conical flask. To this, was added Et_3N (1 molar equivalent) and DMAP (~ 100 mg), and the solution was cooled in ice. 3,4,5-Trimethoxybenzyl chloride (11.28 g, 48.9 mmol) in chloroform was

cautiously added dropwise *via* a dropping funnel over a period of 1.5 h, after which the solution was allowed to stir for a further 1 h. The chloroform solution was washed with dilute HCl, aqueous $NaHCO_3$, water and dried over $MgSO_4$. The solvents were removed *in vacuo* to give the desired product **[3.4]** as a cream solid (14.3 g, 81 %); δ_H (400 MHz, $CDCl_3$) 3.9 (s, 9H, O*Me*), 7.35 (d, 2H, *J* 9.0, Ar *H*-3 and *H*-5), 7.4 (s, 2H, Ar *H*-8 and *H*-12), 7.95 (d, 2H, *J* 8.58, Ar *H*-2 and *H*-6), 10.0 (s, 1H, C*H*O), δ_C (100 MHz, $CDCl_3$) 191.0, 164.2, 155.7, 153.1, 143.1, 134.0, 131.3, 123.6, 122.6, 107.4, 56.3; IR (KBr, cm^{-1}) 2948, 2824, 2726, 1737 (ester C=O), 1699 (aldehyde C=O), 1589, 1504, 1406, 1417, 1338, 1233, 1157, 1123, 997, 935, 861,799, 751, 732, 704; *m/z* (APCI) 317.0.

[3.5]H_2 *Meso*-5,10,15,20-tetra-(3,4,5-trimethoxybenzoic acid phenyl ester) porphyrin



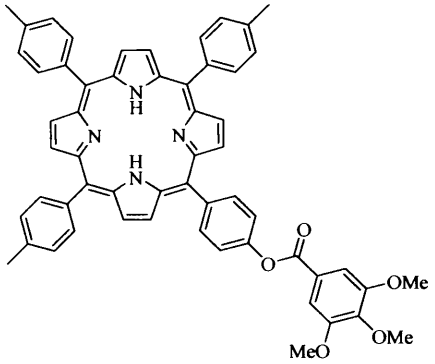
The procedure described for **[3.1]** was followed, the applied reagents being **[3.4]** (1.63 g, 5.3 mmol), pyrrole (2.33 g, 5.0 mmol) and propionic acid (30 mL). Chromatographic purification on silica gel eluting with THF afforded **[3.5]** as a shiny purple solid. (0.487 g, 26 %) δ_H (400 MHz, $CDCl_3$) -2.8 (s, 2H, N*H*) 3.95 (s, 36H, O*Me*), 7.55 (m,16H, Ar *H*-3, *H*-5, *H*-8 and *H*-12), 8.24 (d, 8H, *J* 8.35, Ar *H*-2 and *H*-6), 8.9 (s, 8H, β-pyrrole); IR (KBr, cm^{-1}) 3318 (NH), 2961, 1740 (ester C=O), 1563, 1502, 1474, 1397, 1361, 1266, 1222, 1194, 1106, 1023, 982, 968, 803; UV/Vis ($CHCl_3$, nm, log ε/dm^3 mol^{-1} cm^{-1}) 422 (5.00), 516, 552, 592, 648; *m/z* (MALDI)1455.4594 (calc 1455.4662) HRMS (TOF ES) *m/z* calc for $C_{84}H_{71}N_4O_{20}$ 1455.4594; found 1455.4662.

[Pt(3.5)]

This was prepared in the same manner **[Pt(3.2)]**, the applied reagents were **[3.5]** (0.1 g, 0.068 mmol) and platinum (II) dichloride (100 mg) to afford **[Pt(3.5)]** as a pink solid (0.074 g, 66%); δ_H (400 MHz, $CDCl_3$) 3.95 (s, 36H, OCH_3), 7.55 (m, 16H, Ar *H*-3, *H*-5,

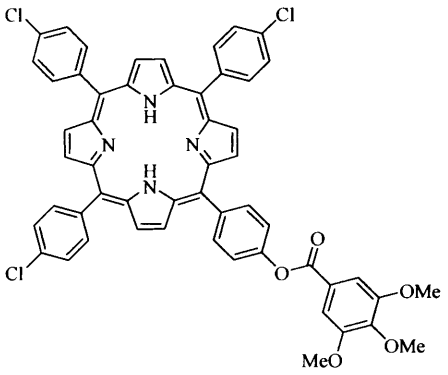
H-8 and *H*-12), 8.20 (d, 8H, *J* 8.35, Ar *H*-2 and *H*-6), 8.91 (s, 8H, β-pyrrole); IR (KBr, cm^{-1}) 2880, 2654, 1745, 1672, 1642, 1208, 1284, 1119, 1039; UV/Vis ($CHCl_3$, nm, log $\varepsilon/dm^3 mol^{-1} cm^{-1}$) 402 (5.01), 510; *m/z* (MALDI) 1648.702 [calculated 1648.55].

[3.6]H_2 *Meso*-5,10,15-tri-(4-tolyl)-20-mono-(3,4,5-trimethoxybenzoic acid phenyl ester) porphyrin



The procedure described for **[3.3]** was followed, the applied reagents being 4-tolylbenzaldehyde (5 g, 41.6 mmol), **[3.4]** (0.63 g. 1.99 mmol), pyrrole (2.7 g, 40.24 mmol) and propionic acid (40 mL) (1.173 g). Chromatographic separation on silica gel eluting with chloroform removes tetra-(4-tolylphenyl) porphyrin and changing to 10 % MeOH (R_F 0.053) affords **[3.6]** as a purple solid (0.01g, 6 %); δ_H (400 MHz, $CDCl_3$) -2.90 (s, 2H, N*H*), 2.65 (s, 9H, *Me*), 4.00 (3, 9H, *OMe*), 7.45 (d, 6H, *J* 7.68, Ar *H*-3 and *H*-5), 7.55 (m, 4H, Ar *H*-3', *H*-5', *H*-8' and *H*-12'), 8.05 (m, 6H, *J* 7.74, Ar *H*-2 and *H*-6), 8.25 (m, 2H, Ar *H*-2' and *H*-6'), 9.80 (s, 6H, β-pyrrole *H*), 9.85 (s, 2H, β pyrrole' *H*); IR (KBr, cm^{-1}) 3321, 2950, 1742, 1567, 1510, 1465, 1400, 1364, 1267, 1222, 1100, 958; HRMS (ES) *m/z* calc for $C_{57}H_{47}N_4O_5$ 867.3546; found 867.3503.

[3.7]H_2 *Meso*-5,10,15-tri-(4-chlorophenyl)-20-mono-(3,4,5-trimethoxybenzoic acid phenyl ester) porphyrin



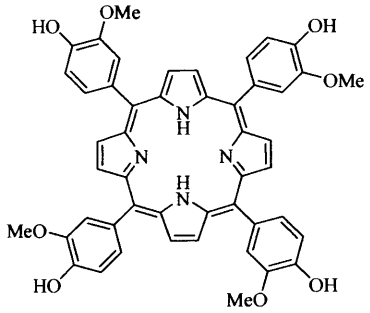
The procedure described for **[3.3]** was followed, the applied reagents being 4-chlorobenzaldehyde (10 g, 70 mmol), **[3.4]** (1.08 g, 3.41 mmol), pyrrole (4.67 g, 69 mmol)

and propionic acid (250 mL). Chromatographic separation as described above (R_F 0.09) afforded **[3.7]** as a purple solid (0.12 g, 4 %). δ_H (400 MHz, $CDCl_3$) 3.90 (s, 9H, O*Me*), 7.45 (d, 2H, *J* 8.35), 7.50 (m, 8H), 7.95 (m, 8H), 8.15 (d, 2H, *J* 8.30), 8.70 (s, 6H, β-pyrrole), 8.80 (s, 2H, β-pyrrole); IR (KBr, cm^{-1}) 3323, 2945, 1743, 1577, 1521, 1453, 1399, 1354, 1266, 1237, 1114, 958, 800; UV/Vis ($CHCl_3$, nm, log $\varepsilon/dm^3\ mol^{-1}\ cm^{-1}$) 422 (4.80), 516, 550, 590, 646; *m/z* (MALDI) 929.268 [calculated 928.268].

[3.8]H_2 *Meso*-5,10,15,20-tetra (4-hydroxyphenyl) porphyrin

Compound **[3.5]** (0.4 g, 1.78 x 10^{-4} mol) and hydrazine monohydrate (0.043 mL [d = 1.032]) were added to ethanol and refluxed for 48 h. Upon cooling the solution was filtered to remove unreacted started material and water was added to precipitate the phenol derivative and filtered. The crude material was recrystallised by dissolving in warm THF and then diluted with hot cyclohexane until crystallisation started to afford **[3.8]** as a purple solid (0.10 g, 53 %); The spectral data were analogous to that of **[3.2]**; *m/z* (MALDI) 679.139 [calculated 678.745].

[3.9]H_2 *Meso*-5,10,15,20-tetra-(3-methoxy-4-hydroxyphenyl) porphyrin



The procedure described for **[3.1]** was followed, the applied reagents being vanillin (12 g, 78.8 mmol), pyrrole (4.93 g, 73.6 mmol) and propionic acid (250 mL). Chromatographic purification on silica gel eluting with acetone (R_F 0.89) afforded **[3.9]** as a purple solid (2.565 g, 16 %); δ_H (400 MHz, d-acetone) -2.90 (s, 2H, N*H*), 3.90 (s, 12H, O*Me*), 7.15 (d, 4H, *J* 7.89), 7.55 (d, 4H, *J* 7.72), 7.70 (bs, 4H), 8.8 (s, 8H, β-pyrrole); IR (KBr, cm^{-1}) 3495, 3432, 2945, 1700, 1597, 1559, 1515, 1458, 1417, 1342, 1258, 1234, 1207, 1122, 802; UV/Vis (acetone, nm, log $\varepsilon/dm^3\ mol^{-1}\ cm^{-1}$) 424 (4.51), 518, 556, 594, 650; *m/z* (MALDI) 799.317 [calculated 798.84].

[Pd(3.9)]

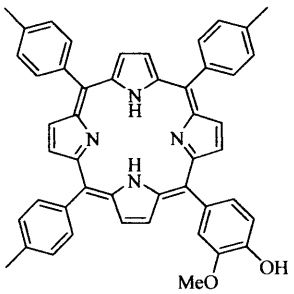
This compound was prepared in an analogous manner to that of **[Pd(3.1)]**. The applied reagents were **[3.9]** (0.5 g, 0.626 mmol) and palladium dichloride (200 mg) afforded

[Pd(3.9)] as a pink solid (0.34 g, 61 %); δ_H (400MHz, $CDCl_3$) 3.85 (s, 4H, OCH_3), 7.05 (d, 4H, *J* 8.0), 7.50 (d, 4H, *J* 7.8), 7.75 (s, 4H), 8.65 (s, 8H, *H*-β-pyrrole); UV/Vis ($CHCl_3$, nm, log ε/dm^3 mol^{-1} cm^{-1}) 422 (4.78), 526; *m/z* (MALDI) 904.82 [M + 1].

[Pt(3.9)]

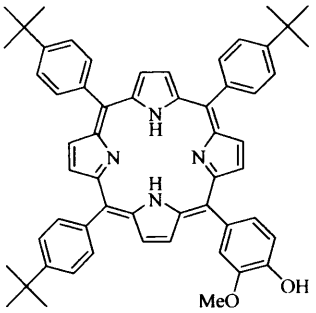
This compound was prepared in an analogous manner to that of **[Pt(3.2)]**. The applied reagents were **[3.2]** (0.2 g, 0.250 mmol) and platinum dichloride (100 mg) afforded **[Pt(3.9)]** as an orange solid (0.121 g, 49 %); δ_H (400MHz, $CDCl_3$) 3.80 (s, 4H, OCH_3), 7.0 (d, 4H), 7.45 (d, 4H), 7.75 (s, 4H), 8.70 (s, 8H, *H*-β-pyrrole); UV/Vis ($CHCl_3$, nm, log ε/dm^3 mol^{-1} cm^{-1}) 406 (4.81), 512; IR (KBr, cm^{-1}) 3430, 2923, 1597, 1508, 1446, 1416, 1353, 1314, 1262, 1198, 1165, 1118, 1020, 934, 869, 799; *m/z* (MALDI) 993.0 [M + 1].

[3.10]H_2 *Meso*-5,10,15-tri-(4-tolyl)-20-mono-(3-methoxy-4-hydroxyphenyl) porphyrin



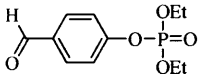
The procedure described for **[3.3]** was followed, the applied reagents being 4-tolylbenzaldehyde (10.6 g, 88 mmol), vanillin (0.67 g, 4.4 mmol), pyrrole (5.53 g, 82 mmol) and propionic acid (100 mL), (3.2 g). Chromatographic separation on silica gel eluting with chloroform afforded (R_F 0.18) **[3.10]** as a purple solid. (0.12 g, 4 %); δ_H (400 MHz, $CDCl_3$) -2.90 (s, 2H, N*H*), 2.6 (s, 9H, *Me*), 3.9 (s, 3H, O*Me*), 5.9 (bs, 1H, O*H*), 7.2 (d, 1H, *J* 8.50), 7.4 (m, 1H), 7.48 (d, 6H, *J* 7.70), 7.65 (s, 1H), 8.0 (d, 6H, *J* 7.79), 8.80 (s, 6H, β-pyrrole), 8.85 (s, 2H, β-pyrrole); IR (KBr, cm^{-1}) 3500, 1559, 1510, 1473, 1346, 1262, 1220, 1181, 1118, 1037, 967; UV/Vis ($CHCl_3$, nm, log ε/dm^3 mol^{-1} cm^{-1}) 424 (5.02), 518, 554, 594, 650; *m/z* (MALDI) 703.250 [calculated 702.85].

[3.11]H_2 *Meso*-5,10,15-tri (4-*tert*-butylphenyl)-20-(4-hydroxy-3-methoxyphenyl) porphyrin



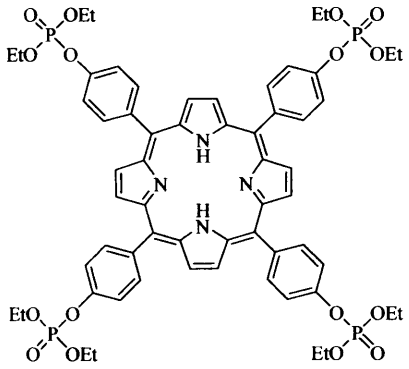
The procedure described for **[3.3]** was followed, the applied reagents being 4-*tert*-butylbenzaldehyde (20 g, 0.123 mol), vanillin (0.937 g, 0.0016 mol) pyrrole (8.08 g, 0.12 mol) and propionic acid (200 mL). Chromatographic separation on silica gel eluting with chloroform (R_F 0.21) afforded **[3.11]** as a purple solid (0.080 g, 2 %); δ_H (400 MHz, $CDCl_3$) -2.90 (s, 2H, N*H*), 1.52 (s, 27H, CH_3), 3.92 (s, 3H, OCH_3), 5.87 (br s, 1H, O*H*), 7.21; UV/Vis ($CHCl_3$, nm, log ε/dm^3 mol^{-1} cm^{-1}) 424 (5.15), 518, 554, 594, 650; *m/z* (MALDI) 829.54 [M + 1].

[3.12] Diethyl-4-formylphenyl Phosphate



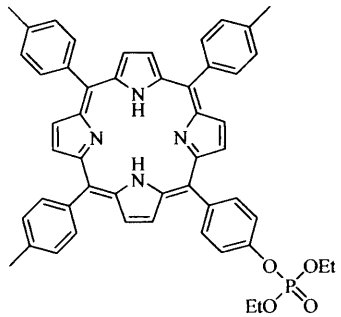
NaH (60 % mineral oil dispersion (1.67g) was added to a Schlenk tube, degassed and washed with petroleum ether (2 x 30 mL), to this THF (120 mL) was added and stirred. 4-Hydroxybenzaldehyde (5.08 g) was added to this suspension and was left to stir for 1.5 h. Chloro diethylphosphate (7.18 g, 6 mL, 0.0416 mmol) was added dropwise and was stirred at room temperature for 1h. The suspension was filtered and the solvent removed in *vacuo* to give the desired product **[3.12]** as a pale yellow oil (6.44 g, 60 %); δ_H (400 MHz, $CDCl_3$) 1.3 (m, 6H, CH_2CH_3), 4.2 (m, 4H, CH_2CH_3), 7.45 (d, 2H, *J* 8.22), 7.95 (d, 2H, *J* 8.26), 10.0 (s, 1H, β-pyrrole); δ_C (100 MHz, $CDCl_3$) 190.5, 130.2, 133.4, 120.0, 115.8, 65.0, 20.3 $\delta_{P\{H\}}$ (121.65 MHz, $CDCl_3$) -6.48; IR (NaCl, cm^{-1}) 3423, 2986, 2912, 2740, 1703 (C=O), 1600, 1504, 1479, 1444, 1422, 1393, 1370, 1279 (P=O), 1225, 1160, 1099, 1038 (P-O-Et), 965 (P-O-Ar), 840, 765, 710; *m/z* (APCI) 259 [M+H].

[3.13]H_2 *Meso*-5,10,15,20-tetra (Diethyl 4-(diethyl phosphate)phenyl) porphyrin



The procedure described for **[3.1]** was followed, the applied reagents being **[3.12]** (0.76 g, 52 mmol), pyrrole (0.3 g, 44 mmol) and propionic acid (20 mL). The propionic acid was removed *in vacuo*, and the remaining residue was passed through a short silica plug (6 x 7 cm) and eluting with MeOH. The MeOH was removed and the solution was absorbed onto silica. The silica was suspended in hot acetone, stirred and filtered, repeated twice. The resulting purple solution was reduced to half its original volume and hexane was added to precipitate any remaining polymeric material. The porphyrin was purified by column chromatography using ethyl acetate (98 %): MeOH (2 %) as the elutant, changing to MeOH (10%) to remove the desired purple band containing **[3.13]** (0.02 g, 3 %); δ_H (400 MHz, d^6-acetone) -2.90 (s, 2H, N*H*), 1.35 (m, 24H, CH_2CH_3), 4.30 (m, 16H, CH_2CH_3), 7.6 (d, 8H, *J* 8.28, Ar *H*-3 and *H*-5), 8.15 (d, 8H, *J* 8.27, Ar *H*-2 and *H*-6), 8.80 (s, 8H, β-pyrrole *H*); $\delta_{P\{H\}}$ (121.65 MHz, d^6-acetone) -4.60; IR (KBr, cm^{-1}) 3456, 2950, 1654, 1599, 1499, 1393, 1262 (P=O), 1213, 1157, 1096, 1056, 1029, 956, 801; *m/z* (MALDI) 1223.3695 (calc 1223.3503); UV/Vis ($CHCl_3$, nm, log ε/dm^3 mol^{-1} cm^{-1}) 421 (5.10), 446, 516, 551, 591, 649; HRMS (ES) *m/z* calc for $C_{60}H_{67}N_4O_{16}P_4$ (M) 1223.3503; found 1223.3695.

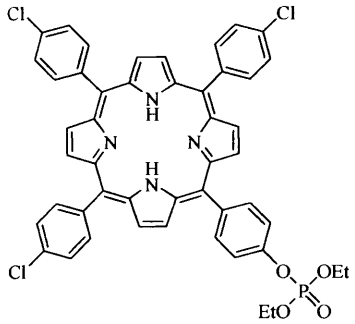
[3.14]H_2 *Meso*-5,10,15-tritolyl-20-mono-(4(diethyl phosphate) porphyrin



The procedure described for **[3.3]** was followed, the applied reagents being 4-tolylbenzaldehyde (10 g, 80 mmol), **[3.12]** (0.617 g, 40 mmol), pyrrole (5.25 g, 78.4 mmol) and propionic acid (100 mL). Chromatographic separation on silica gel eluting with chloroform removed *meso*-5,10,15,20-tetratolylporphyrin. Eluting with chloroform/MeOH

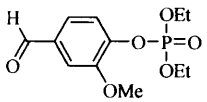
(98:2) removed the desired product (R_F 0.33). The material was re-chromatographed by preparative thin layer chromatography using the same solvent system as above to afford **[3.14]** as a purple solid (0.07 g, 4 %); δ_H (400 MHz, $CDCl_3$) -2.90 (s, 2H, N*H*), 1.4 (m, 6H, CH_2CH_3), 2.6 (s, 9H, CH_3), 4.30 (m, 4H, CH_2CH_3), 7.45 (d, 6H, *J* 7.45, Ar *H*-3 and *H*-5), 7.55 (d, 2H, *J* 7.70, Ar *H*-3' and *H*-5'), 8.05 (d, 6H, *J* 7.05, Ar *H*-2 and *H*-6), 8.1 (d, 2H, *J* 7.47, Ar *H*-2' and *H*-6'), 8.75 (s, 2H, β-pyrrole' *H*), 8.80 (s, 6H, β-pyrrole *H*); $\delta_{P\{H\}}$ (121.65 MHz, $CDCl_3$) -5.61; IR (KBr, cm^{-1}) 1699, 1603, 1501, 1471, 1398, 1348, 1272, 1215, 1181, 1021, 965, 799, 733; UV/Vis ($CHCl_3$, nm, log $\varepsilon/dm^3\ mol^{-1}\ cm^{-1}$) 422 (4.99), 518, 552, 592, 648; *m/z* (MALDI) 809.322 [calc 809.357]; HRMS (ES) *m/z* calc for $C_{51}H_{46}N_4O_4P$ 809.3257; found 809.3223.

[3.15]H_2 *Meso*-5,10,15-tri-(4-chlorophenyl)-20-mono-(4(diethyl phosphate) porphyrin



The procedure described for **[3.3]** was followed, the applied reagents being 4-chlorobenzaldehyde (5 g, 0.0355 mol), **[3.12]** (0.25 g, 0.0017 mol), pyrrole (2.31 g, 0.034 mol) and propionic acid (50 mL) (0.77 g). Chromatographic separation as described above affords **[3.15]** as a purple solid (0.025g, 3 %) δ_H (400 MHz, $CDCl_3$) -3.00 (s, 2H, N*H*) 1.45 (m, 6H, CH_2CH_3), 4.35 (m, 4H, CH_2CH_3), 7.55 (d, 2H, *J* 8.45, Ar *H*-3' and *H*-5'), 7.65 (d, 6H, *J* 8.27, Ar *H*-3 and *H*-5), 8.1 (d, 6H, *J* 8.3, Ar *H*-2 and *H*-6), 8.13 (d, 2H, *J* 8.35, Ar *H*-2' and *H*-6'), 8.75 (s, 8H, β-pyrrole); $\delta_{P\{H\}}$ (121.65 MHz, $CDCl_3$) -5.92; *m/z* (MALDI) 871.245 [calculated 870.08].

[3.16] Diethyl 4-Formyl-2-methoxyphenyl Phosphate

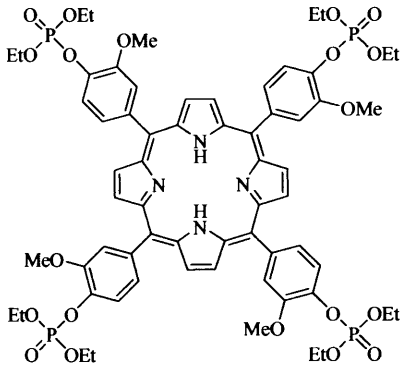


Method A: NaH (60 % mineral oil dispersion (2.61g) was added to a schlenk, degassed and washed with petroleum ether (x2), to this thf (150 mL) was added and stirred. Vanillin (10 g, 65.7 mmol) was added to this suspension and was left to stir for 1.5 h. Chloro diethylphosphate (11.3 g, 9.43 mL) was added dropwise and was stirred at room temperature for 1h. The suspension was filtered and the solvent removed in *vacuo* to give

the desired product **[3.16]** as a pale yellow oil (14.612 g, 77 %); δ_H (400 MHz, $CDCl_3$) 1.40 (m, 3H, C*H*$_3$), 4.30 (m, 2H, C*H*$_2$), 7.45 (m, 3H, Ar *H*-2, *H*-3 and *H*-6), 9.90 (s, 1H, C*H*O), δ_C (100 MHz, $CDCl_3$) 190.92, 151.83 (J_{CP} 10 Hz), 144.93 (J_{CP} 11 Hz), 133.95, 125.01, 121.55 (J_{CP} 4 Hz), 110.84, 64.93, 58.93, 15.97; $\delta_{P\{H\}}$ (121.65 MHz, $CDCl_3$) -6.09; IR (NaCl, cm^{-1}) 3483, 2984, 2940, 2839, 2736, 1701 (C=O), 1595, 1506, 1466, 1423, 1392, 1278 (P=O), 1216, 1151, 1124, 1032 (P-O-Et), 959 (P-O-Ar), 824, 780, 733; *m/z* (APCI) 289 [calc 288.43].

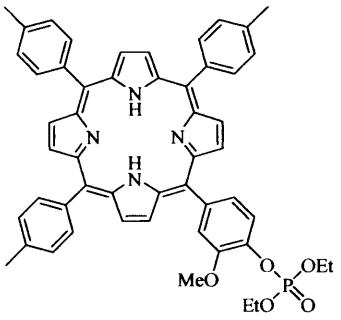
Method B: DMAP (0.079 g) and triethylamine (0.65 g) in dry THF (20 mL) were added to a 100 mL round bottomed flask and sealed with a rubber septum. To this was added chlorodiethyl phosphate (1.13 g, 0.94 mL) and vanillin (1 g, 6.5 mmol) and was left to stir for 2 h. The solvents were removed *in vacuo* to give the product **[3.16]** as a colourless oil (60 %) which has identical spectral properties as above.

[3.17]H_2 *Meso*-5,10,15,20-tetra ((4-diethyl phosphate) – 3 – methoxyphenyl) porphyrin



The procedure described for **[3.1]** was followed, the applied reagents being **[3.16]** (4 g, 13.8 mmol), pyrrole (0.86 g, 12.9 mmol) and propionic acid (15 mL). Chromatographic purification on silica gel eluting with chloroform/MeOH (90:10) affords **[3.17]** as a purple solid (0.37 g, 8 %); δ_H (400 MHz, $CDCl_3$) -2.82 (s, 2H, N*H*), 1.40 (m, 24H, CH_2CH_3), 3.80 (s, 12H, OCH_3), 4.40 (m, 16H, CH_2CH_3), 7.10 (d, 4H, *J* 8.0), 7.58 (d, 4H, *J* 7.8), 7.75 (s, 4H), 8.9 (s, 8H, β-pyrrole), $\delta_{P\{H\}}$ (121.65 MHz, $CDCl_3$) -5.48; IR (KBr, cm^{-1}) 3300 (NH *w*), 2976, 1646, 1591, 1522, 1488, 1394, 1260 (P=O, strong), 1167, 1038 (P-O, strong), 797, 612, 509; *m/z* (MALDI) 1343.742 [calc 1343.18].

[3.18]H_2 *Meso*-5,10,15-tri-(4-tolyl)-20-((4-diethyl phosphate) – 3 – methoxyphenyl) porphyrin



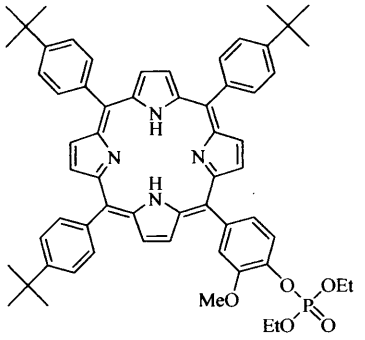
The procedure described for **[3.3]** was followed, the applied reagents being 4-tolylbenzaldehyde (4 g, 41.6 mmol), **[3.16]** (0.6 g, 2.08 mmol), pyrrole (2.73 g, 40.7 mmol) and propionic acid (40 mL), reacted for 30 min and allowed to cool and filtered. The resulting purple solid was dissolved in chloroform and absorbed onto silica. The crude mixture was separated on a silica column, eluting with chloroform removed the tetra chlorophenylporphyrin, changing the solvent system to chloroform: MeOH (99:1) to remove the desired porphyrin (R_F 0.51). The product was further purified using preparative thin-layer chromatography using $CHCl_3$:MeOH (99:1) to afford **[3.18]** as a purple solid (0.0086 g, 0.5 %); δ_H (400 MHz, $CDCl_3$) -2.7 (s, 2H, N*H*$_2$), 1.45 (m, 6H, CH_2CH_3), 2.62 (s, 9H, C*H*$_3$), 3.90 (s, 3H, OC*H*$_3$), 4.38 (m, 4H, CH_2CH_3), 7.50 (d, 6H, *J* 7.4, Ar *H*-3 and *H*-5), 7.60 (d, 1H, *J* 8.03, Ar *H*), 7.72 (d, 1H, *J* 8.06, Ar *H*), 7.76 (s, 1H, Ar *H*-6'), 8.05 (d, 6H, *J* 7.01, Ar *H*-2 and *H*-6), 8.80 (s, 8H, β pyrrole); $\delta_{P\{H\}}$ (121.65 MHz, $CDCl_3$) -4.99; IR (KBr, cm^{-1}) 3437, 2962, 1506, 1473, 1261, 1218, 1096, 1023, 969, 917 , 800 UV/Vis ($CHCl_3$, nm, log ε/dm^3 mol^{-1} cm^{-1}) 422 (5.06), 518/522, 552, 592, 648; *m/z* (MALDI) 839.336 [calculated 838.94].

[3.19] Attempted Synthesis of *meso*-5,10,15,20-tetra((4-diethyl phosphate) – 3 – methoxyphenyl) porphyrin

Method A: NaH (60 % mineral oil dispersion (0.076 g) was added to a schlenk, degassed and washed with petroleum ether (2 x 30 mL), to this THF (10 mL) was added and stirred. **[3.9]** (0.37 g, 4.81 x 10^{-4} mol) was added to this suspension and was left to stir for 1.5 h. Diethyl chlorophosphate (0.32 g, 0.26 mL, 1 mmol) was added dropwise and was stirred at room temperature for 2h. The suspension was filtered and the solvent removed in *vacuo* to give the desired product **[3.19]** as a mixture of products; $\delta_{P\{H\}}$ (121.65 MHz, $CDCl_3$) -5.04; IR (KBr, cm^{-1}) 3437, 2978, 1593, 1510, 1486, 1407, 1265, 1220, 1166, 1128, 1031, 961, 916, 800, 709; *m/z* (MALDI) calculated 1343.167 contains 799.208 and 935.258.

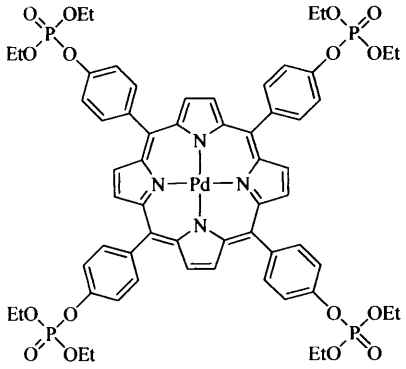
Method B: DMAP (0.0299 g) and triethylamine (0.248 g) in dry THF (20 mL) were added to a 100 mL round bottomed flask and sealed with a rubber septum. To this was added chlorodiethyl phosphate (0.423 g, 0.35 mL) and 5, 10, 15, 20 Tetra 4-hydroxy–3 – methoxyphenyl) porphyrin **[3.9]** (0.49 g) and was left to stir overnight. The solvents were removed *in vacuo* to give the product **[3.19]** as a mixture of products.

[3.20] *Meso*-5,10,15-tri-(4-*tert*-butylphenyl)-20-((4-diethyl phosphate) – 3 – methoxyphenyl) porphyrin



DMAP (0.002 g) and triethylamine (0.01 g) in dry THF (10 mL) were added to a 50 mL round bottomed flask and sealed with a rubber septum. To this was added chloro diethylphosphate (0.0166 g, 0.0139 mL) and **[3.11]** (0.08 g, 9.65×10^{-5} mol) and was left to stir for 2 hours. The solvents were removed *in vacuo* to give the product **[3.20]** as a purple solid; $\delta_{P\{H\}}$ (121.65 MHz, $CDCl_3$) -5.8; IR (KBr, cm^{-1}) 3406, 1648, 1564, 1445, 1400, 1261 (P=O, strong), 1217, 1055 (P-O, strong), 950, 800; *m/z* (MALDI) 965.537 [calculated 965.1751].

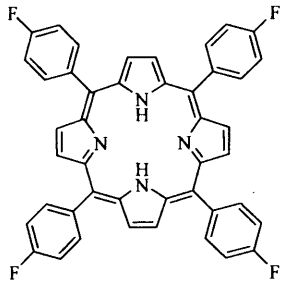
[Pd(3.21)] Attempted Synthesis of *meso*-5,10,15,20-tetra(4-(diethyl phosphate)phenyl) porphyrin palladium



DMAP (~100 mg, catalytic amount) and triethylamine (0.02g) in dry THF (20 mL) were added to a 50 mL round bottomed flask and sealed with a rubber septum. To this was added chlorodiethyl phosphate (0.75 mL) and **[Pd(3.2)]** (0.11 g, 1.43×10^{-4} mol) and was left to

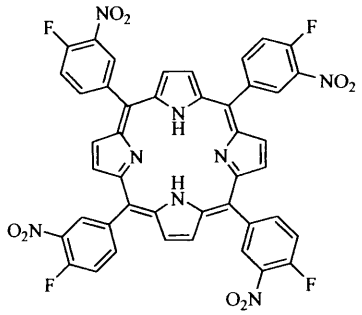
stir for 2 h. The solvents were removed *in vacuo* to give the product. The crude product was passed through a short silica column eluting with THF. The solvent was removed and taken up in chloroform, washed with water, dried over $MgSO_4$ and the solvent removed *in vacuo* to give the title compound **[Pd(3.21)]** as a mixture of products; $\delta_{P\{H\}}$ (121.65 MHz, $CDCl_3$) -5.45; IR (KBr, cm^{-1}) 2985, 2925, 1659, 1503, 1478, 1443, 1393, 1353, 1257, 1222, 1162, 1036, 976, 815; UV/Vis ($CHCl_3$, nm, log $\varepsilon/dm^3\,mol^{-1}\,cm^{-1}$) 418 (5.02), 421, 520, 525.

[3.22]]H_2 *Meso*-5,10,15,20-tetra(4-fluorophenyl)porphyrin



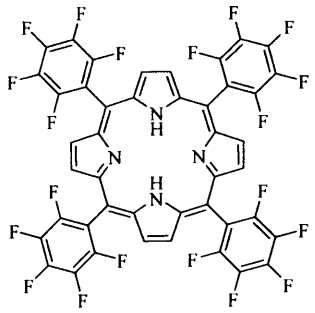
This was prepared in an analogous method to that of **[3.1]**. The applied reagents were 4-fluorobenzaldehyde (2g, 16.11 mmol) and pyrrole (1g, 15.04 mmol) to afford **[3.22]** as a purple solid (0.38g, 14 %). The spectroscopic data was consistent with the literature.[64]

[3.23]H_2 *Meso*-tetra(4-fluoro-3nitrophenyl) porphyrin



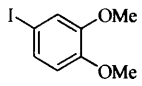
Pyrrole (0.08 g, 1.15 mmol) and 4-fluoro-3-nitrobenzaldehyde (0.209 g, 1.23 mmol) were added to refluxing propionic acid (20 ml). After 30 min the solution was left to cool overnight and filtered. The resulting black crystalline material was washed with methanol until the filtrate ran clear, and allowed to air dry to afford **[3.23]** as a black solid (0.056g, 21 %). Due to the insolubility of this product only characterisation by IR spectroscopy was possible. (IR KBr, cm^{-1}) 3418 (NH stretch), 1537, 1338 (N=O) and 1266 (C-F).

[3.24]]H_2 *Meso*-tetra(pentafluorophenyl)porphyrin



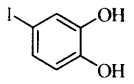
A 3-neck round bottomed flask was fitted a septum port, a reflux condenser and a gas inlet port. The flask was charged with distilled CH_2Cl_2 (122 mL), pentafluorobenzaldehyde (0.1 g, 0.5 mmol) and pyrrole (0.06 mL). The resulting solution was stirred at room temperature and purged with N_2 for 15 min. BF_3 etherate (4 ml of a 2.5 M solution in CH_2Cl_2.) was added *via* a syringe and stirred for 2 h under nitrogen. After addition of 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (0.28 g) the solution was stirred overnight, then passed through a short alumina column to remove the tar. The reaction mixture was separated by column chromatography on silica gel with CH_2Cl_2: *n*-hexane (1:4) to afford **[3.24]** as a dark red solid (0.024g, 20 %); δ_H (400 MHz, $CDCl_3$) -3.0 (s, 2H, N*H*), 8.9 (s, 4H, pyrrole-*H*); δ_F (282.78 MHz, $CDCl_3$) –161.1 (m), -151.1 (m), 136.3 (m); UV/Vis ($CHCl_3$, nm, log ε/dm^3 mol^{-1} cm^{-1}) 412 (4.99), 510, 580; *m/z* (MALDI) 975.64 [M+ 1].

[3.25] Iodo-3,4-dimethoxybenzene



Veratrole (0.5 g, 3.6 mmol) and iodine monochloride (0.58 g, 3.6 mmol) were added to acetic acid (10 mL) and was left to stir overnight, shielded from the light. The acetic acid was removed in *vacuo.* The residue was extracted with DCM (10 mL) and washed with sodium metabisulfate (2 x 10 mL), water (3 x 10 mL) and dried over $MgSO_4$. The solvents were removed in *vacuo* to afford **[3.25]** as a brown solid (0.6g, 62 %); δ_H (400 MHz, $CDCl_3$) 3.80 (s, 6H, O*CH*$_3$), 6.50 (d, 1H, Ar-*H*), 7.00 (d, 1H, Ar-*H*), 7.15 (dd, 1H, Ar-*H*); *m/z* (APCI) 265 [M + 1].

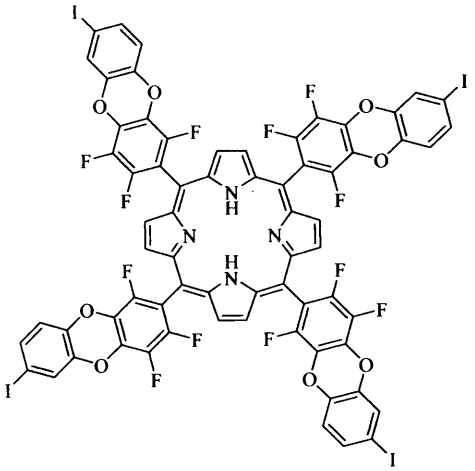
[3.26] Iodo-3,4-dihydroxybenzene



Compound **[3.25]** (0.15 g, 0.56 mmol) was dissolved in degassed DCM (25 mL) and cooled to -80 C. To this boron tribromide (0.48 g, 0.18 mL, 1.1 mmol) in DCM (2 mL) was added

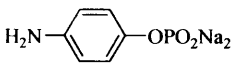
dropwise and stirred for 1 h at -80 °C. The solution was allowed to cool to room temperature and stirred overnight. Water was added to quench any unreacted boron tribromide and the product was extracted with diethyl ether (25 mL) and dried over $MgSO_4$. The solvent were removed in *vacuo* and recrystalistion from hot afforded **[3.26]** as a fawn solid (0.12g, 74 %) δ_H (400 MHz, $CDCl_3$) 5.7 (br s, 1H, O*H*), 6.6 (d, 1H, Ar-*H*), 7.0 (dd, 1H, Ar-*H*), 7.10 (d, 1H, Ar-*H*); *m/z* (APCI) 237 [M + 1].

[3.27]



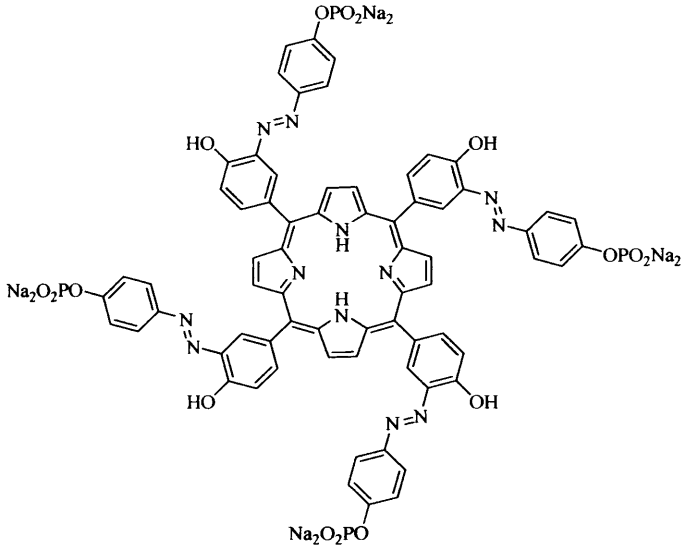
This was prepared using the method described by McKeown *et al.*[58] **[3.24]** (0.06g, 0.67 mmol), **[3.26]** (0.016g, 0.27 mmol) and potassium carbonate (0.009 g, 0.52 mmol) were added to *N*-methyl-2-pyrrolidinone (5 mL) and heated to reflux overnight. Upon cooling a small quantity of a brown precipitate formed which was collected by filtration; δ_F (282.78 MHz, D_2O) -139.9 (d), -155.2 (m), -164.1 (m); *m/z* (MALDI) 1758.7 [M+1].

[3.28] 4-Aminophenylphosphate disodium salt



To a solution of 4-nitrophenylphosphate disodium salt (5.00 g, 19 mmol) in a mixture of water (100 mL) and ethanol (4 mL) was added 10 % palladium on charcoal (~50 mg). The resulting black suspension was stirred at room temperature, under an atmosphere of hydrogen, for 72 hours. The suspension was then filtered through Celite® and the clear solution was evaporated to dryness under reduced pressure to yield **[3.28]** as a cream solid. The product was used without further purification (3.9g, 88 %); δ_H (400 MHz, D_2O) 6.60 (d, 2H, *J* 8.00), 7.05 (d, 2H, *J* 7.95); $\delta_{P\{H\}}$ (121.65 MHz, D_2O) -5.01; IR (KBr, cm^{-1}) 3441, 2615, 1512,m 1284, 1241, 1206, 1169, 1099, 969, 831.

Attempted synthesis of [3.29]



This was prepared using methodology described in the literature.[59] To a solution of sodium carbonate (0.11 g) and **[3.28]** (0.5 g) in water (10 mL) at 0 °C was added a solution of sodium nitrite (0.15 g) in water (5 mL) at 0 °C. The resulting solution was then added to a mixture of crushed ice and concentrated HCl (2 mL) with rapid stirring. The resulting ice cold solution was then added to a solution of 5,10,15,20-tetetra (4-hydroxyphenyl) porphyrin **[3.2]** (0.6 g) in 10 % NaOH (2 mL) at 0 °C for 1 h. The pH of the solution was adjusted to 7. A 50 cm strip of dialysis membrane was immersed in boiling water until soft. A knot was tied into one end and 25 mL of the porphyrin mixture was poured in, and the other end was tied. The bag was suspended by a piece of string into 1 L of distilled water; the water was changed after one week (orange colour) and was continued until the water remained colourless. The solid that had precipitated out inside the dialysis bag was analysed and was found to be 5,10,15,20-tetra (4-hydroxyphenyl) porphyrin.

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Chapter 4

Markers Based on 2,2':6',2"-Terpyridine

Introduction

This chapter presents preliminary work concerned with the attachment of a redox active compound directly to an antibody molecule.

4.1 Terpyridine background

Polypyridine compounds are an important class of nitrogen donor multidentate ligands, with examples including 2,2'-bipyridine, 2,2':6,2"-terpyridine and 1,10-phenanthroline. 2,2':6,2"-Terpyridine is constructed from three pyridine units bound by a single bond (figure 4.1) and was first discovered in 1932 by Morgan and Burstall[1] by the heating of pyridine and anhydrous iron (III) chloride at 340 °C and 50 atms for 36 hours.

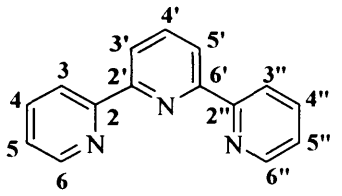
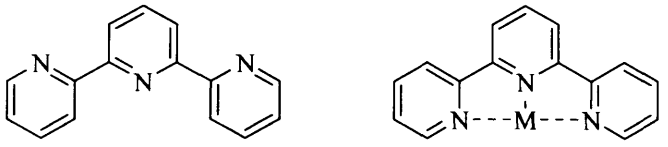


Figure 4.1: Unsubstituted 2,2':6,2"-terpyridine

In solution and the solid phase the terpyridine ligand adopts a planar configuration with the nitrogen atoms in adjacent rings aligning *trans* to each other, which minimises the repulsion between the nitrogen lone pairs and the aromatic protons (*H*-3, *H*-3' and *H*-5', *H*-3") (figure 4.2). This *trans* configuration in the solid state has been confirmed by crystallography e.g. for 2,2':6,2"-terpyridine[2] and 4-phenyl-2,2':6,2"-terpyridine.[3]



trans, trans conformation of free ligand

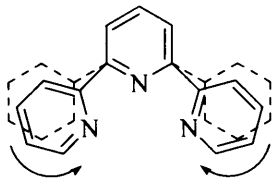
cis, cis conformation of chelated ligand

Figure 4.2: *Trans, trans* conformation of a free 2,2':6,2"-terpyridine ligand and *cis, cis* conformation of a chelated terpyridine

2,2':6,2"-Terpyridines can be functionalised at the central and terminal pyridine rings generating a wide variety of derivatives. The substituents can be incorporated into either the starting materials or introduced *via* a coupling procedure. The most common substitution is at the 4' position.

4.2 Coordination Chemistry and Metal Complexes

The coordination chemistry of 2,2':6,2"-terpyridine and the substituted analogues has been extensively studied and a wide variety of transition metal complexes are known. It coordinates as a planar tridentate ligand with a metal to form two five-membered chelate rings. Upon coordination the terpyridine ligand adopts a *cis-cis* conformation (figure 4.2) with changes to the dihedral angle between the terminal and central pyridyl rings (figure 4.3).



distortion in bond angles upon complexation

Figure 4.3: Changes in the dihedral angle upon coordination.

Terpyridine metal complexes display characteristic optical and electrochemical properties *i.e.* metal to ligand charge transfer (MLCT) in the visible light region, reversible reduction and oxidation processes and luminescence. The extent of these properties can be controlled or altered by the functional groups incorporated into the terpyridine ligand.

The majority of the resulting metal complexes exhibit either a 1:1 or 1:2 metal:ligand ratios. Many transition metal ions in low oxidation states form bis-complexes with two terpyridine ligands, including Cr(III), Cr(II), Fe(III), Fe(II), Ru(II), Ni(II), Cu(II) and Zn(II). The terpyridine ligands coordinate to the metal centre in a meridinal (*mer*) fashion. Complexes of this type are stabilised by the strong metal-ligand (d-π^*) back donation and a distorted octahedral geometry at the metal centre is displayed, as confirmed by crystal structural analysis.[4] Square planar geometries are also observed e.g. Pt(II) and Pd(II). In both cases the bond to the central pyridyl ring is shorter than the bonds to the terminal rings for instance, the central bond lengths in the Zn(II) octahedral complex[5] and Pt(II) square planar complex[6] are on average 0.1 and 0.15 Å respectively, shorter.

4.3 Applications

Terpyridine derivatives and their complexes have found use in a wide area of applications. The incorporation of electron withdrawing or releasing substituents can be used to fine tune the redox and photophysical properties of the terpyridine complexes, for example an electron withdrawing substituent in $[Ru(Xtpy)_2]^{2+}$ renders the complex luminescent at room temperature.[7] This electronic influence has led to the use of terpyridine

complexes in areas of photochemistry e.g. in the design of luminescent devices[8] and as sensitizers for light-to-electricity conversion.[9,10] The addition of iron (II) ions to a solution of a terpyridine compound gives rise to a purple colour indicating the formation of a metal complex, hence terpyridine compounds have been used as reagents for the colorimetric determination of metals.[11-13] Terpyridine derivatives have also found other potential uses in clinical and biochemical applications including DNA binding agents[14-16] and in anti-tumour research.[17-19] Chloro (terpyridine) platinum(II) (figure 4.4) has been used to non-invasively label amino acids, peptides and Cytochromes *c via* the displacement of the chloride by histidine[20,21] and guanidine[22,23] residues. Lanthanide complexes *i.e.* Eu (III) and Tb (III) of 2,2':6,2"-terpyridine ligands with pendent carboxylate groups (figure 4.4) are attracting interest as luminescent agents for protein labelling.[24,25]

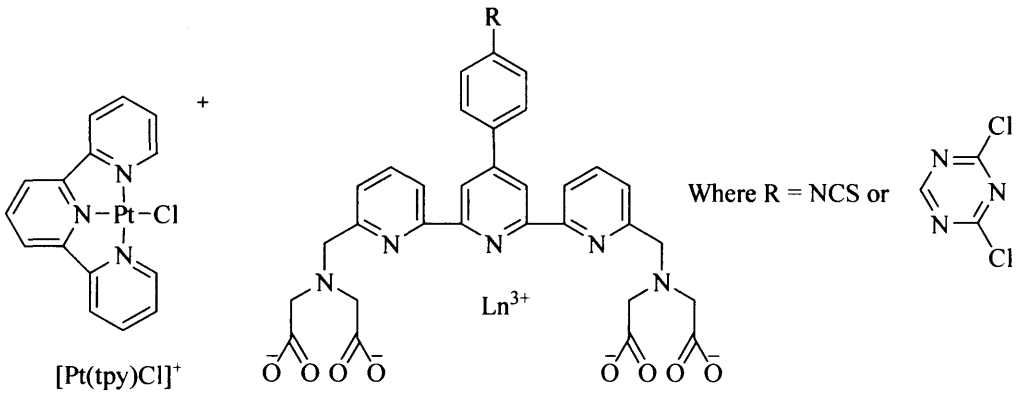


Figure 4.4: Examples of terpyridine systems used in biological applications.

4.4 Routes of Synthesis

4.4.1 The Kröhnke Method [13]

This is regarded as a useful method to synthesise 2,2':6',2'' aryl-substituted terpyridine ligands. It involves the reaction of a pyridinium salt **2** with an unsaturated ketone (chalcone) **3** *via* a Michael addition to form 1,5-diketone **4**. Treatment with ammonium acetate results in ring closure to generate the 4'-aryl substituted terpyridine **8**[13] (figure 4.5, route **A**). The pyridinium salt is obtained by the reaction of 2-halo-1-pyridin-2-ylethanone with the pyridine, and the chalcone from the reaction of a benzaldehyde with acetylpyridine. Despite the common use of this synthesis, the protocol is time consuming, products require purification and yields are generally moderate to low.[26-28]

4.4.2 Cave and Raston Method

Cave and Raston[29] reported a modified Kröhnke method where the solventless aldol condensation of benzaldehyde **5** with one equivalent of acetylpyridine **1** in the presence of

solid NaOH generates the unsaturated ketone product **6**. The reaction of a second equivalent of acetylpyridine results in **6** undergoing a Michael addition to generate the diketone **7**. This is followed by treatment with ammonium acetate to generate the terpyridine **8** in yields typically greater than 75%[29] (figure 4.5, route **B**). This versatile method allows access to a range of compounds that were previously unobtainable using conventional methods. The mild conditions utilised result in clean products.

4.4.3 Other Methods

Over the last decade interest in microwave-assisted synthesis has increased dramatically, with numerous reports that support the advantages and use of microwave irradiation to carry out organic synthesis.[30,31] As a result of using microwave irradiation, an increase in rates, yields and in the purities of the products have been observed.

More recently the preparation of 4'-aryl-2,2':6',2''-terpyridines by a one-pot reaction of 2-acetylpyridine with benzaldehyde in the presence of ammonium acetate under microwave irradiation has been reported.[32] This method provides a shorter, higher yielding route to terpyridines with an easier work-up, shorter reaction times and using more environmentally friendly conditions than conventional methods.

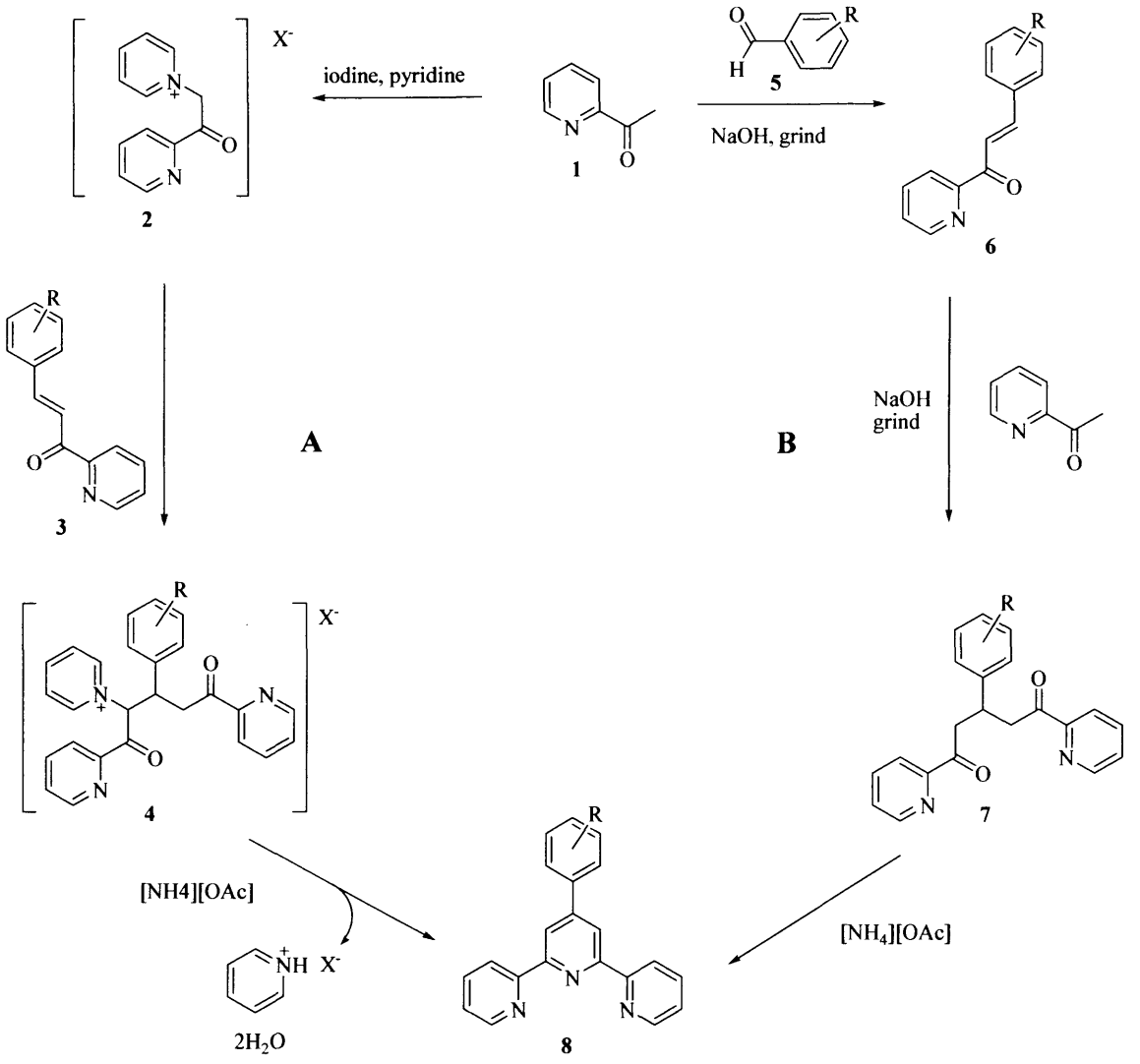


Figure 4.5: Scheme illustrating the Kröhnke synthesis (**A**) and Raston method (**B**) preparation of terpyridine **8.**

4.5 Bioconjugation Methods

The ability to chemically attach one biological molecule to another has generated a billion dollar industry which serves areas of research, diagnostics and the therapeutic markets. Modified or conjugated molecules have been used for the purification, detection or localisation of specific cellular components and in the treatment of disease. The reactive groups present in cross-linking reagents, tags and probes provide the means to specifically label certain target groups on proteins, peptides, carbohydrates, lipids, nucleic acids, oligonucleotides and synthetic polymers.

4.6 Reactive Groups

The conjugation process involves the reaction of a functional group on one molecule (e.g. a label) with a functional group on the desired target (*i.e.* an antibody),

resulting in the formation of a covalent bond. There are many reagent systems that are commercially available or that are described in the literature for coupling reactions and can be categorised into the following chemical reactions:

- Amine reactive
- Thiol reactive
- Carboxylate reactive
- Hydroxyl reactive
- Aldehyde and ketone reactive
- Active hydrogen reactive
- Photoreactive

Functional groups that are able to couple with amine-containing molecules are the most commonly used in bioconjugation. The wide use is since amine-coupling reactions can be used to conjugate with nearly all protein and peptide molecules. The reaction of the reactive group and an amine results in the formation of stable amide or secondary amine bonds. The thiol-reactive class are the second most commonly used whilst the others are more limited.

4.7 Examples of Reactive Groups

4.7.1 *N*-Hydroxysuccinimide (NHS) Ester

Many of the commercially available cross-linking or modification agents utilise NHS-esters. The NHS-esters can react with primary and secondary amines to form stable amide or imide bonds with the loss of a NHS leaving group (figure 4.6-(1)). Therefore they are most commonly used to couple proteins principally with the α-amines at the *N*-terminals and the ε-amines of lysine side chains. NHS-esters can also react with a sulfhydryl or hydroxyl groups to form thioester or ester bonds, however these bonds are not stable since they can be hydrolysed in an aqueous environment.

4.7.2 Isothiocyanates and Isocyanates

Isothiocyanates differ from isocyanates by the replacement of oxygen atom by sulfur. Isothiocyanates can react with amines, sulfhydryls and the phenolate ion of tyrosine side chains.[33] Only stable bonds are formed with primary amines to give an isothiourea bond (figure 4.6-(2)) so isothiocyanates are selective for coupling to ε- and *N*-terminal amines in proteins. Isocyanates also couple to amines to give isourea bonds (figure 4.6-(3)) and additionally to hydroxyl-containing molecules. However, the reactivity of isocyanates

is greater than that of isothiocyantes which results in problems associated with their stability. In fact many commercial suppliers do not offer them since they are readily decomposed by moisture.

4.7.3 Arylating Agents

Aryl halides can react with amines resulting in a stable arylamine covalent bond. They can also react with thiols, imidazolyls and the phenolate groups of amino acid side chains.[34] Fluorobenzene derivatives, such as 1,5-difluoro-2,4-dinitrobenzene (DFDNB) are utilised as homobifunctional cross-linker molecules (figure 4.6-(4)). Homobifunctional cross linkers are like a "molecular rope" that can 'tie' one protein to another by reaction of two common groups. The main disadvantage of using homobifunctional reagents is the potential for generating poorly defined conjugates, despite this these types of reagents are prevalent choices in conjugation applications. Difluorobenzene reagents have been used for a number of purposes including the cross-linking of phospholipids in human erythrocyte membranes[35] and conjugation of small peptides to the carrier protein albumin.[36]

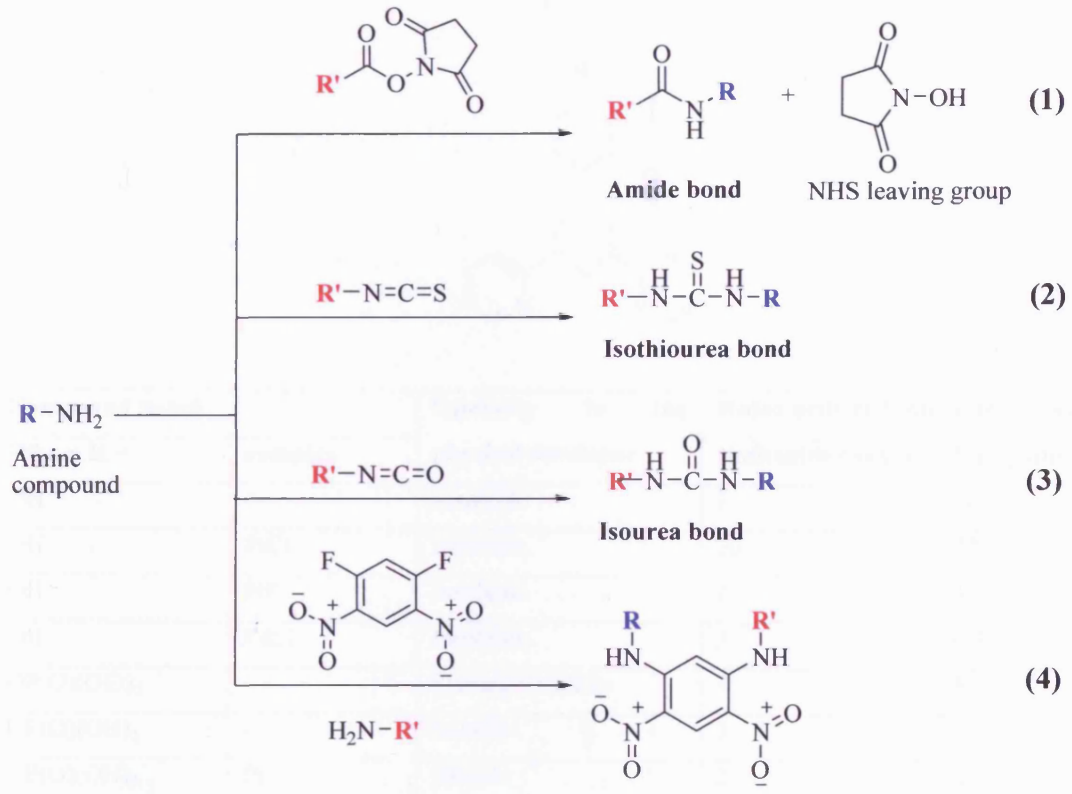
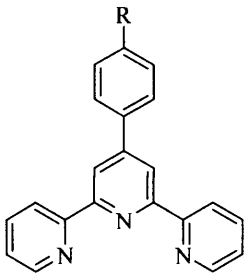


Figure 4.6: Bioconjugation methods. The reaction of an amine compound , RNH_2 with (1) NHS-ester, (2) isothiocyante, (3) isocyanate and (4) DFDNB.

4.8 Aims and Objectives

Our group has previously demonstrated the ability of systems based on 2,2':6,2"-terpyridine and 2,2'-bipyridyl ligands as positive catalysts for the reduction of silver in a Timm's type reaction (figure 4.7).[37]



Compound tested		**Solubility in the physical developer**	**Redox activity (time in minutes)**	
Where R =	**complex**		**Noticeable change**	**End point**
OH	-	Insoluble	3	10
OH	PtCl	Insoluble	20	40
OH	PtI	Insoluble	2	4
OH	PdCl	Insoluble	3	6.5
$OP(O)(OEt)_2$	-	Sparingly soluble	3	8
$OP(O)(OH)_2$	-	Soluble	3	6
$OP(O)(OH)_2$	PtI	Soluble	2	4

Figure 4.7: Summary of previous results based on 4'-phenyl-2,2':6',2''-terpyridine.

It was shown that the phosphate derivatives of the above systems were suitable substrates for the enzyme alkaline phosphatase. Following enzymatic phosphate hydrolysis the corresponding phenol derivatives were found to deposit at the site of action. Despite displaying redox activity in a Timm's style reaction, the deposited compounds did not adhere to tissue sections and were washed away. This could lead to a potential mis-diagonosis.

Building on from these encouraging results we opted to incorporate a means of attaching a similar system directly to an antibody (figure 4.8-(a)). The direct attachment of a marker to a secondary antibody would eradicate the need to incorporate an enzyme substrate and would not rely on the marker adhering to the cell surface. The marker system would be required to be water soluble, redox active for the reduction of silver and to contain a reactive group suitable for conjugation to an antibody.

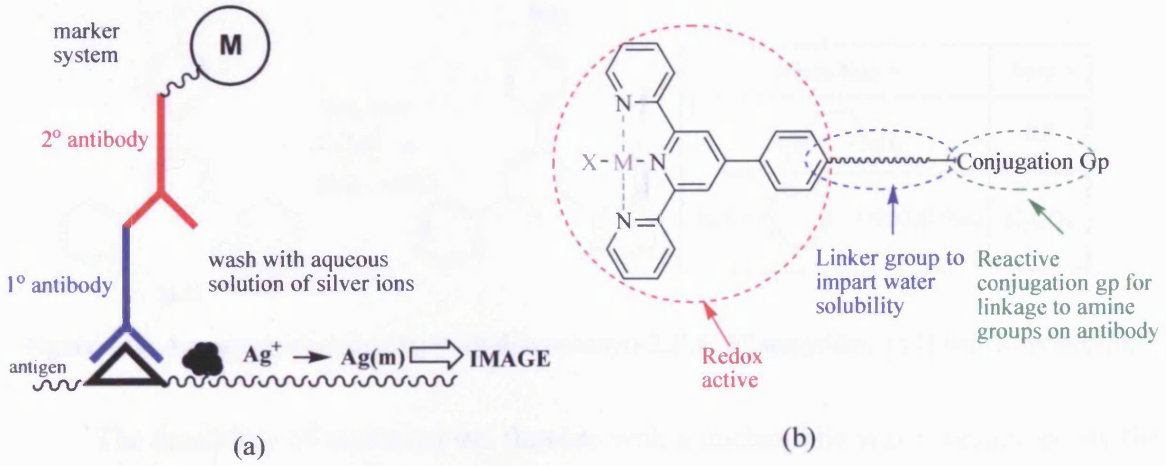


Figure 4.8: (a) Schematic diagram of proposed system and (b) Proposed marker compound.

This chapter outlines the work undertook in the design and synthesis of redox active systems suitable for antibody labelling based on 4'-phenyl-2,2':6,2"-terpyridine. This preliminary work has centred on systems that feature a short polyethylene glycol (PEG) chain to act as a linker to decouple the marker from an antibody and also to aid water solubility (figure 4.8-(b)).

Results and Discussion

4.9 Reactions Involving 4'-(4-Fluorophenyl)-2,2':6',2''-terpyridine

Our first approach was to synthesise a terpyridine derivative that was susceptible to nucleophilic attack, in order to incorporate a linker chain. An amine terminated short PEG chain was selected as the linker (figure 4.9), which could undergo further modification for bioconjugation purposes.

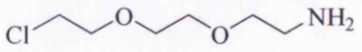
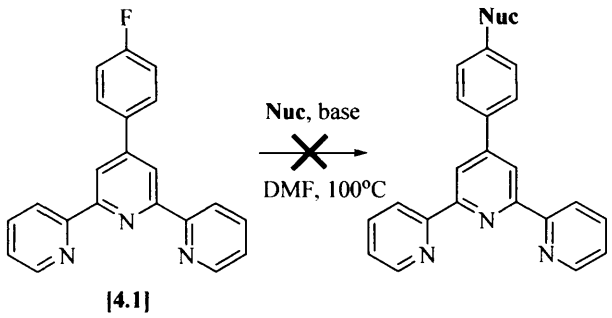


Figure 4.9: Polyethylene glycol chain.

4'-(4-Fluorophenyl)-2,2':6',2''-terpyridine **[4.1]** was synthesised using the method developed by Raston,[29] whereby the diketone intermediate is formed in the absence of a solvent. Subsequent cyclisation with ammonium acetate and ethanol afforded **[4.1]** as a cream solid. The spectroscopic data was consistent with that described elsewhere.[32]



Where **Nuc** =	base =
H_2N NH_2	KF
H_2N—⟨⟩—$OP(O)(ONa)_2$	K_2CO_3

Figure 4.10: Attempts to functionalise 4'-(4-fluorophenyl)-2,2':6',2''-terpyridine **[4.1]** with a nucleophile

The feasibility of replacing the fluoride with a nucleophile was investigated by the reaction of both ethylene diamine and 4-aminophenylphosphate disodium salt with **[4.1]** and base in anhydrous DMF for 24 hours (figure 4.10). Reaction with ethylene diamine in the presence of potassium fluoride afforded a yellow oil. Spectroscopic data confirmed this to be a mixture. Thin-layer chromatography revealed the mixture to be of unreacted terpyridine **[4.1]** and amine substituted product. Attempts to separate the mixture have proved unfruitful. The analogous reaction of 4-aminophenylphosphate disodium salt with potassium carbonate failed to yield any desired product. The limited success of these reactions prompted us to reconsider our synthetic strategy.

4.10 Reactions Involving Phenol Derivatives

Alternatively, the reaction of an electrophilic alkyl halide and 4'-(4-hydroxyphenyl)-2,2':6',2''-terpyridine was utilised to access the target ligand illustrated in figure 4.11.

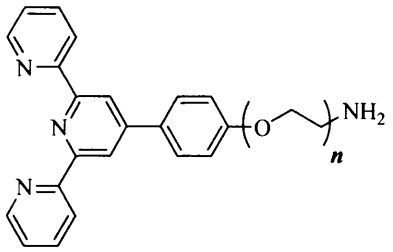


Figure 4.11: Alternative target ligand, where $n = 3$.

4'-(4-Methoxyphenyl)-2,2':6',2''-terpyridine **[4.2]** (figure 4.12) was prepared by grinding 4-methoxybenzaldehyde and 2-acetylpyridine in a pestle and mortar with sodium hydroxide to yield the corresponding diketone. Subsequent cyclisation with ammonium hydroxide in ethanol afforded **[4.2]** as a cream solid (67 %). Of the methods commonly used for demethylation, molten pyridinium hydrochloride was chosen since high yields are obtained and its use is economical for large scale preparations.[38-40] To this end, **[4.2]** was

converted to 4'-(4-hydroxyphenyl)-2,2':6',2''-terpyridine **[4.3]** in 87 % yield with the spectroscopic data for both compounds being consistent with the literature.[41,42]

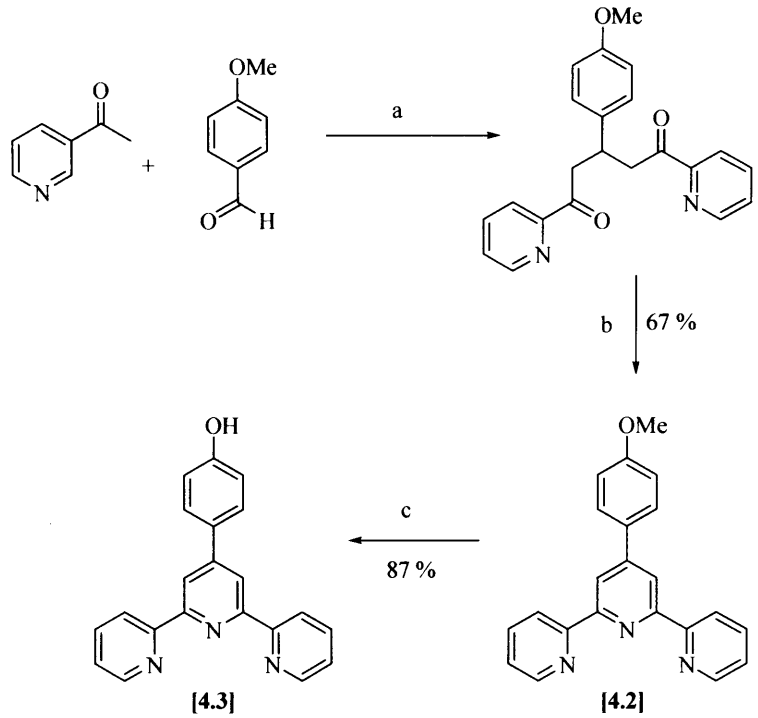
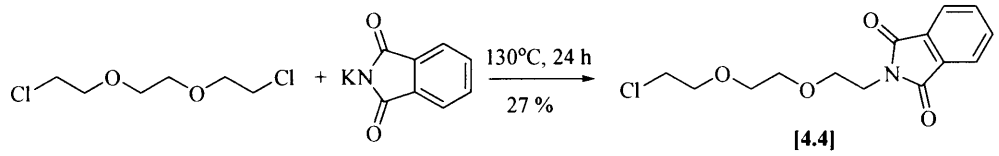


Figure 4.12: Preparation of **[4.2]** and **[4.3]** . *Reagents and conditions*: (a) NaOH, grind; (b) ammonium hydroxide, EtOH, reflux; (c) pyridinium hydrochloride, 210 °C.

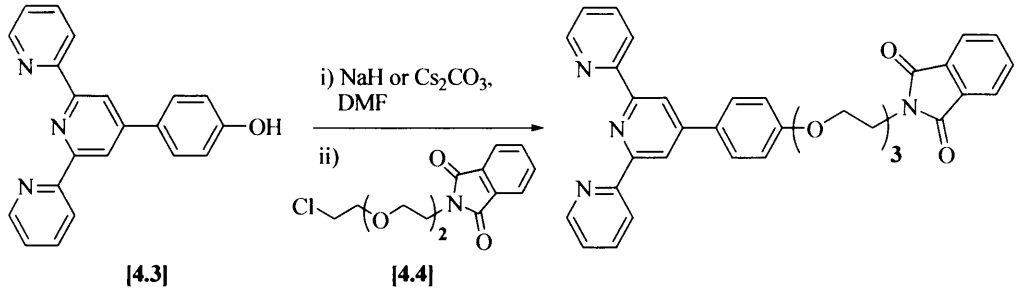
The Gabriel synthesis can be used to prepare primary amines by the reaction of potassium phthalimide and an alkyl halide to give an *N*-alkylphthalimide, followed by hydrolysis to generate the amine. A short PEG chain terminating with a halide and a phthalimide **[4.4]** was therefore prepared by reacting an excess amount of 1,2-bis(2-chloroethoxy)ethane with potassium phthalimide (figure 4.13-(1)).[43] The excess dichloride was removed by distillation and the resultant residue purified *via* a soxhlet extraction to give **[4.4]** as an oil in 27 % yield. The spectroscopic data was comparable to that previously reported.[43]

Initial attempts to react **[4.3]** and **[4.4]** *via* the sodium salt in presence of tetra-butyl ammonium iodide proved unsatisfactory (figure 4.13-(2)). Although the desired product appeared to have been formed, it was contaminated with many impurities.

The reaction between 4'-(tetrafluoro-4-hydroxyphenyl)-2,2':6',2"-terpyridine and chloroethanol using cesium carbonate to give 4'-(tetrafluoro-4-(2-hydroxyethoxy) phenyl)-2,2':6',2"-terpyridine has been reported.[44] An adaptation of this synthesis was used for our purposes, with the addition of sodium iodide (*in situ* Finkelstein reaction– figure 4.13-(2)).



(1)



(2)

Figure 4.13: (1) Synthesis of **[4.4]** of a protected amine via the Gabriel synthesis and (2) reaction of **[4.3]** and **[4.4]** with either i) NaH or ii) Cs_2CO_3 in DMF and 2-{2-[2-(2-chloroethoxy)ethoxy]ethyl}isoindole-1,3-dione.

During the reaction work-up, a significant amount of material was precipitated, confirmed by ^{1}H NMR spectroscopy to be unreacted **[4.3]**. The ^{1}H NMR spectrum showed other contamination, but the formation of the desired product was verified by a parent ion at 587.3 Da/e in the ESI mass spectrum. This indicates that our synthetic strategy may be successful. However, the low obtainable yields of **[4.4]** limit this reaction to small scales and the unclean products are undesirable.

The scheme below (figure 4.14) illustrates the successful routes undertaken for the preparation of amine ligand **[4.9]**. Addition of the short chain PEG was undertaken both prior to and following terpyridine assembly.

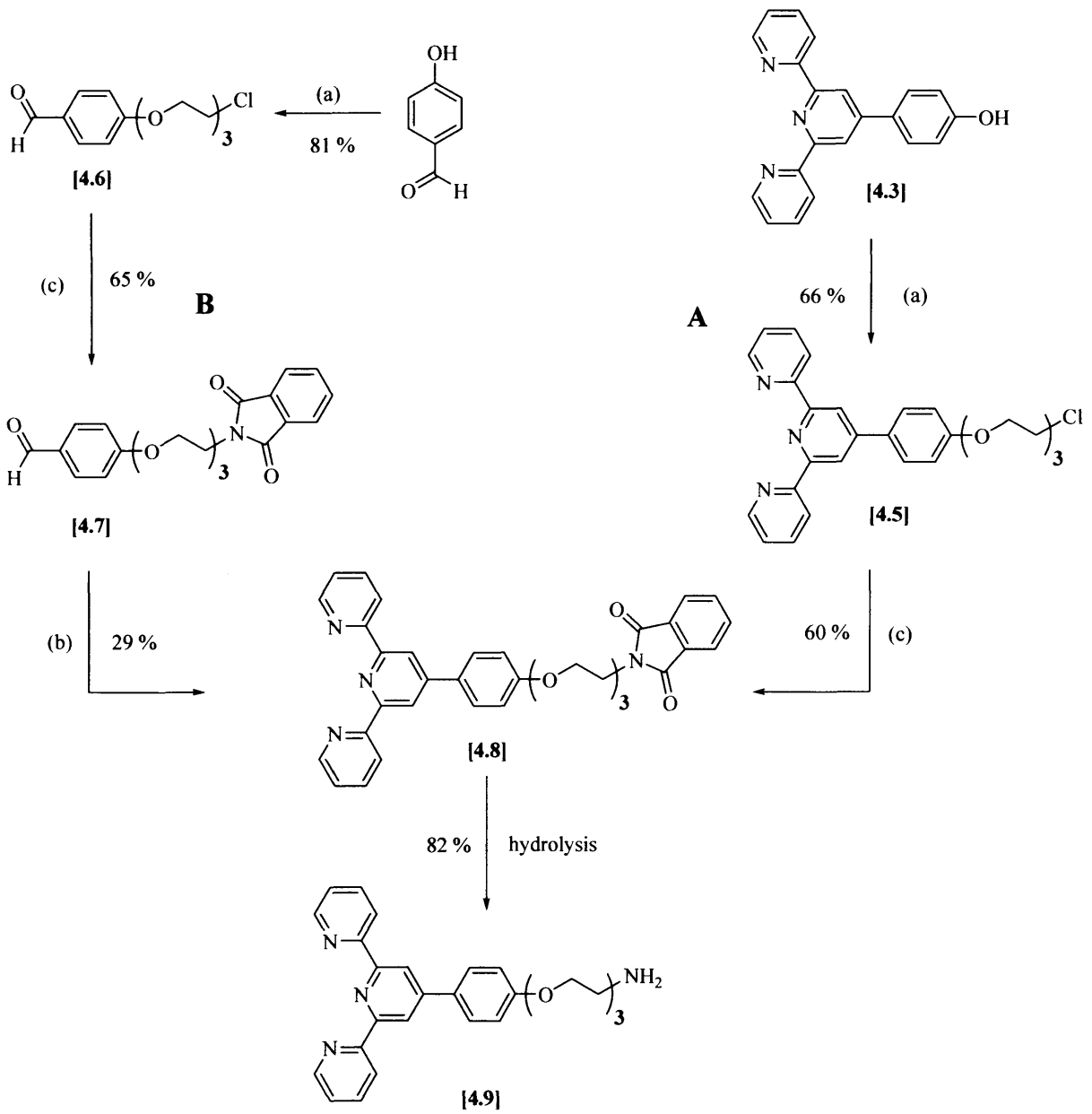


Figure 4.14: *Reagents and conditions*: (a) 1,2-bis(2-chloroethoxy)ethane, caesium carbonate, NaI, DMF, 65°C, 24 h; (b) i. 2-acetyl pyridine, NaOH, ii. Ammonium acetate, EtOH; (c) potassium phthalimide, NaI, DMF, 100°C, 24 h;

4.10.1 From 4'-(4-Hydroxyphenyl)-2,2':6',2''-Terpyridine (Method A, figure 4.14)

The reaction of **[4.3]** with cesium carbonate and a ten-fold excess of 1,2-bis(2-chloroethoxyethane) in DMF, cleanly afforded **[4.5]** as a pale brown solid (66 %). An excess of the dichloride was used to minimise the substitution at both ends of the chain. Removal of the excess dichloride was achieved *via* a Kugelröhr distillation and residual cesium salts were removed by treatment with water. A parent ion at 476.17 Da/e in the ESI-MS can be assigned to **[4.5]** and a minor ion at 340.14 Da/e corresponds to unreacted **[4.3]**. The aromatic resonances in the proton spectrum attributed to the three pyridyl and phenyl rings show little deviance from phenol derivative **[4.3]**. 1,2-Bis(2-chloroethoxyethane) is a

symmetrical compound as is seen in the ^{1}H / ^{13}C NMR spectra; the successful addition of the PEG chain is observed by the loss of this symmetry giving rise to four proton multiplets and six carbon resonances in the regions of 3.55-4.10 ppm and 71.4 - 42.8 ppm respectively. The IR spectrum shows the loss of the OH stretch and the appearance of an intense absorption at 1132 cm^{-1} in the region for the C-O of ethers.

Conversion of the alkyl chloride to a primary amine was achieved *via* a Gabriel procedure. The reaction of **[4.5]** with potassium phthalimide and trace amounts of sodium iodide in anhydrous DMF afforded **[4.8]** as a low melting solid (60 %). An excess of potassium phthalimide was used to ensure the complete conversion of chloride to phthalimide, unreacted material can be removed upon cooling by filtration. The ^{1}H NMR spectrum revealed the crude product to be of reasonable purity, chromatography on neutral alumina (ethyl acetate) removed any minor impurities. The ESMS confirmed the product **[4.8]** with the parent molecular ion at 587.1 Da/e. A strong IR stretching absorption at 1717 cm^{-1} (C=O amide) and a multiplet between 7.7-7.8 ppm in the proton spectrum are consistent with the presence of the phthalimide.

4.10.2 From 4-Hydroxybenzaldehyde (Method B, figure 4.14)

As illustrated in figure 4.14, terpyridine **[4.8]** was also synthesised from the benzaldehyde. Compound **[4.6]** was prepared in the same manner as **[4.5]**, where 4-hydroxybenzaldehyde replaces **[4.3]** to cleanly afford **[4.6]** as a brown oil (81 %). The parent ion was identified at 273.1 Da/e in the mass spectrum. Peaks assigned to the ether chain are comparative to those observed in both the ^{1}H and ^{13}C NMR spectrum of **[4.5]**. Reaction of **[4.6]** with potassium phthalimide in DMF afforded the phthalimide functionalised benzaldehyde **[4.7]** as a yellow solid (65 %).

Compound **[4.7]** was reacted with 2-acetylpyridine and NaOH using the method developed by Raston to afford the corresponding diketone as an orange, hygroscopic solid (figure 4.15). The NMR data for the diketone was more complicated than expected and recrystalisation attempts (from hot toluene and ethanol) were not wholly successful. Regardless, cyclisation was carried out using ammonium acetate in ethanol. Due to the nature of the diketone the proton NMR spectrum of the product was contaminated with some impurities. The cyclisation to the terpyridine **[4.8]** was confirmed by a molecular ion at 587.2 Da/e. The impurities can be removed by column chromatography on neutral alumina eluting with ethyl acetate to give the pure product (29 %) with identical spectral properties as previous.

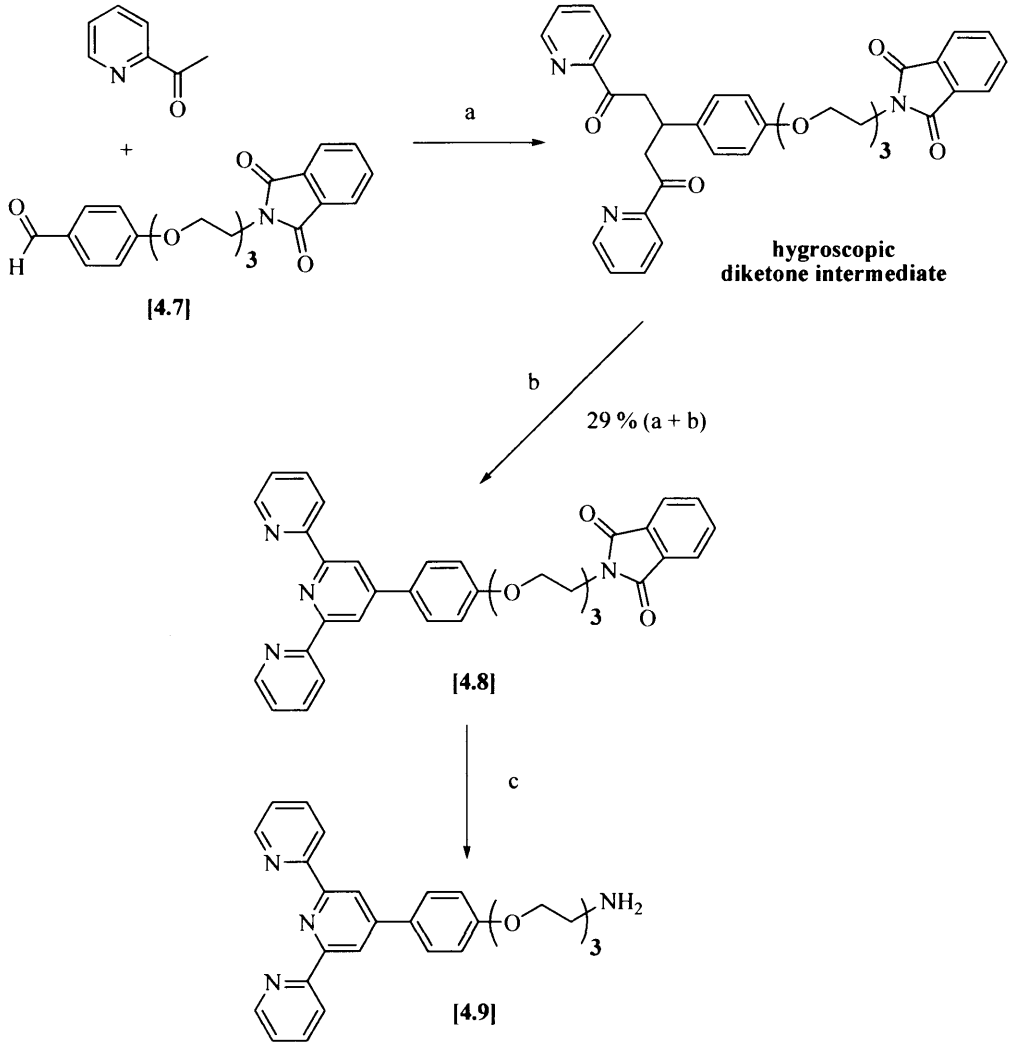


Figure 4.15: *Reagents and conditions*: (a) NaOH, grind; (b) ammonium acetate, ethanol, reflux; (c) i) HCl, H_2O, ii) NaOH, H_2O.

The ligand **[4.8]** has been prepared by the two routes **A** and **B** illustrated in figure 4.14, in overall yields of 23 and 15 % respectively. Introduction of the functionalised PEG chain to an aryl-terpyridine (route **A**) provides a four step synthesis of **[4.8]**. All four synthetic steps, including the preparation of **[4.2]** and **[4.3]**, were obtained in good yields (65-87 %) with few impurities present in the products. All of the synthetic steps are suitable for large scale preparations. The preparation of **[4.8]** from 4-hydroxybenzaldehyde (route **B**) is advantageous as it involves one less step, with the functionalised benzaldehydes **[4.6]** and **[4.7]** being prepared in 81 and 65 % yields respectively. However, the final terpyridine assembly using the Raston methodology was low yielding (29 %) attributed to the hygroscopic nature of the diketone intermediate. This problem may be overcome by the use of an alternative methodology that does not isolate the diketone intermediate. Examples of this are *via* the classical Kröhnke method[13] whereby the chalcone intermediate is isolated or

via a one-pot method[42,45] in which a benzaldehyde is reacted 2-acetylpyridine and potassium *t*-butoxide in THF followed by cyclisation with ammonium acetate.

4.11 Conversion to the Amine

De-protection of the amine was achieved by refluxing **[4.8]** in hydrochloric acid overnight (figure 4.15). The precipitated phthalic acid was filtered off upon cooling and the acid removed to give the salt as a yellow solid. The amine was generated by subsequent base treatment. The conversion of the phthalimide **[4.8]** to the amine **[4.9]** was confirmed by the observation of the parent ion at 457.2 Da/e in the ESI MS, however this conversion was not complete as an ion corresponding to **[4.8]** (587.1 Da/e) is also seen. The amine compound **[4.9]** was treated as hygroscopic and was kept refrigerated as a toluene solution. This compound is not readily water soluble.

4.12 Introduction of a Reactive Group for Bioconjugation

In order to be suitable for conjugation to a biomolecule, the compound must feature a reactive group. The primary amine in **[4.9]** can be modified for this purpose. We initially chose to react **[4.9]** with 1,5-difluoro-2,4-dinitrobenzene (DFDNB) giving rise to a compound which contains a reactive fluoride (figure 4.16).

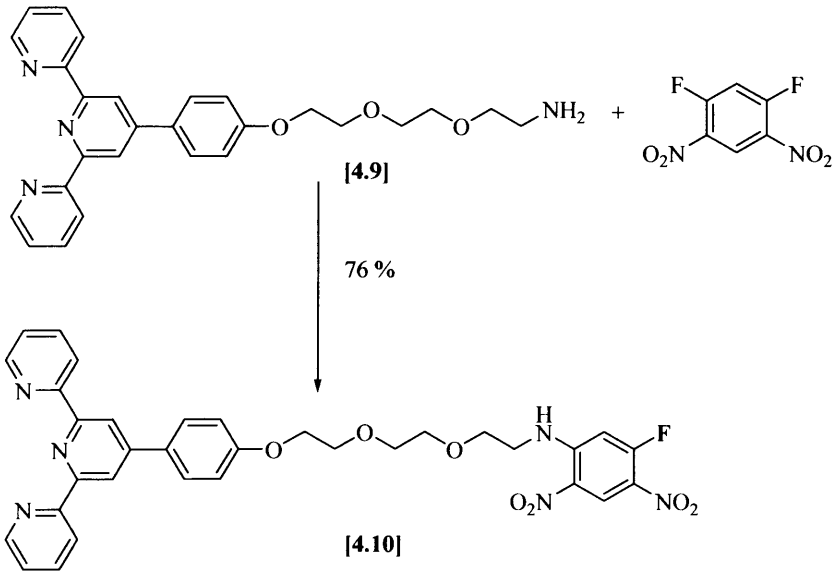


Figure 4.16: Introduction of reactive group. *Reagents and conditions*: K_2CO_3 (1 eq), MeCN, -15°C.

One molar equivalent of potassium carbonate was added to a cooled (-15 °C) solution of 1,5-difluoro-2,4-dinitrobenzene in acetonitrile. To this was added a solution of **[4.9]** in acetonitrile and an instant change of colour to yellow was observed. Removal of the solvent afforded **[4.10]** as a yellow oily substance. These reaction conditions were tested out prior with decylamine and 1,5-difluoro-2,4-dinitrobenzene. Selected NMR data is

presented in figure 4.17. A downfield shift of 4.2 ppm in the $^{19}F\{^{1}H\}$ NMR spectra is observed upon the displacement of one of the fluoride atoms. A disappearance of some of the fluorine coupling and a significant upfield shift of the aryl H_6 resonance is observed due to the displacement of a fluoride with a less electronegative substituent. This compound does not possess the required water solubility; hence attempts were made to resolve this.

	δ_F	δ_H	
		H_3	H_6
DFDNB	-104.6	8.9 (m, J_{H-H} 7.56, $J_{(H-F)}$ 15.1)	7.3 ($J_{(H-H)}$ 9.75, $J_{(H-F)}$ 19.5)
Decylamine + DFDNB	-100.4	9.05 (d, $J_{(H-H)}$ 8.02)	6.55 (d, $J_{(H-F)}$ 13.5)
[4.10]	-100.4	9.00 (d, $J_{(H-H)}$ 8.0)	6.55 (d, $J_{(H-F)}$ 13.4)

Figure 4.17: Selected NMR data

4.13 Modification to Impart Water Solubility

It has been shown that the addition of a morpholinomethyl group to salen ligands imparts water solubility.[46-49] The morpholinomethyl group can be introduced *via* a Mannich reaction[50] under non-aqueous aprotic conditions. We therefore decided to adopt this as an initial means of imparting water solubility to our ligands.

The Mannich base ethoxy-*N*-morpholinomethane **[4.11]** was prepared from morpholine and paraformaldehyde in ethanol with potassium carbonate.[50] Distillation using a Kugelröhr apparatus afforded the product as a colourless oil (44 %), the spectroscopic data was in agreement with the literature.[50]

The pendant morpholino groups were introduced using an adapted method of Fenton and co-workers[50] for the preparation of 4-bromo-2-formyl-6-(morpholin-4-ylmethyl)phenol. We chose to incorporate two pendent morpholino group to our system, the reaction conditions were tested using 4-*tert*-butylphenol with 2.2 equivalents of ethoxy-*N*-morpholinomethane **[4.11]** under an inert atmosphere in dry acetonitrile using potassium carbonate as the base (figure 4.18-(1)). The successful introduction of two pendent groups *ortho* to the phenol prompted an analogous reaction using (4-hydroxyphenyl)-2,2':6,2"-terpyridine **[4.3]** to be carried out (figure 4.18-(2)).

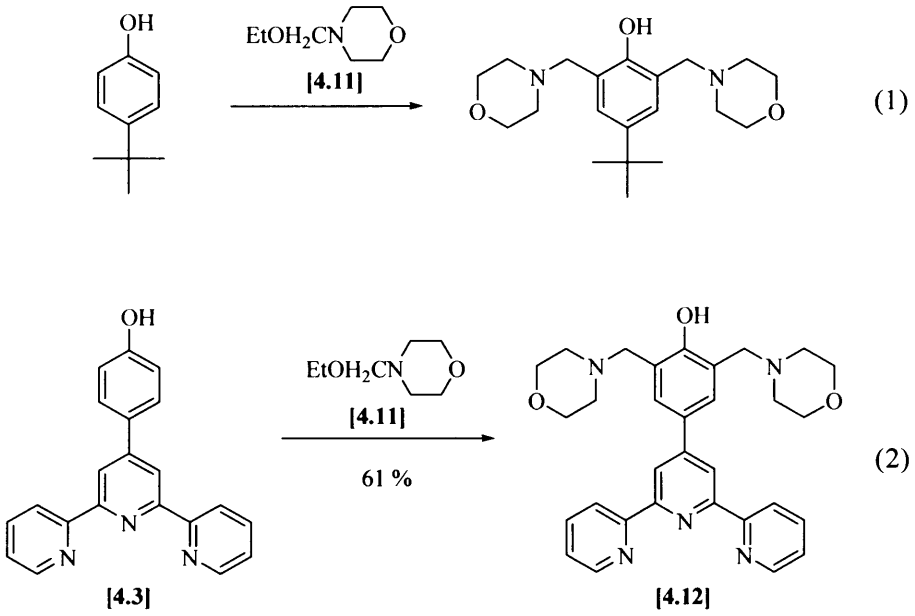


Figure 4.18: *Reagents and conditions*: (1) **[4.11]** (2.2 eq), MeCN, K_2CO_3, 3 days; (2) **[4.11]** (2.2 eq), DMF, K_2CO_3, 3 days.

It was observed that under these conditions poor yields of **[4.12]** were obtained, despite the reaction time being increased to 96 hours. Although the desired product was formed, identified by a molecular ion at 524.2 Da/e, it was contaminated with partially mono functionalised material (425.2 Da/e) and unwanted by-products. The low yield may be attributed to the poor solubility of **[4.3]** in refluxing acetonitrile. Alternatively, the reaction was repeated employing anhydrous DMF as the solvent and potassium carbonate as the base over a period of 72 hours. Removal of the DMF and purification by chromatography (alumina, ethyl acetate) gave the desired product as a brown solid (61 %). Mass spectral data (ESI) showed the expected molecular ion at 524 Da/e. Notable changes are observed in the ^{1}H NMR spectrum of **[4.12]**. The pair of doublets (δ 7.80 and 6.95 ppm) assigned to the four aromatic phenol protons are no longer seen as a result of the introduction of the morpholino pendent groups. Due to this substitution, a single signal (δ 7.60 ppm) corresponding to the two protons at the 2 and 6 positions is observed.

An amine terminated PEG chain was introduced at the 4 position, in an analogous method to that already described (figure 4.19). Each ligand **[4.13]**, **[4.14]**, and **[4.15]** has been characterised by ^{1}H NMR spectroscopy and ES mass spectrometry.

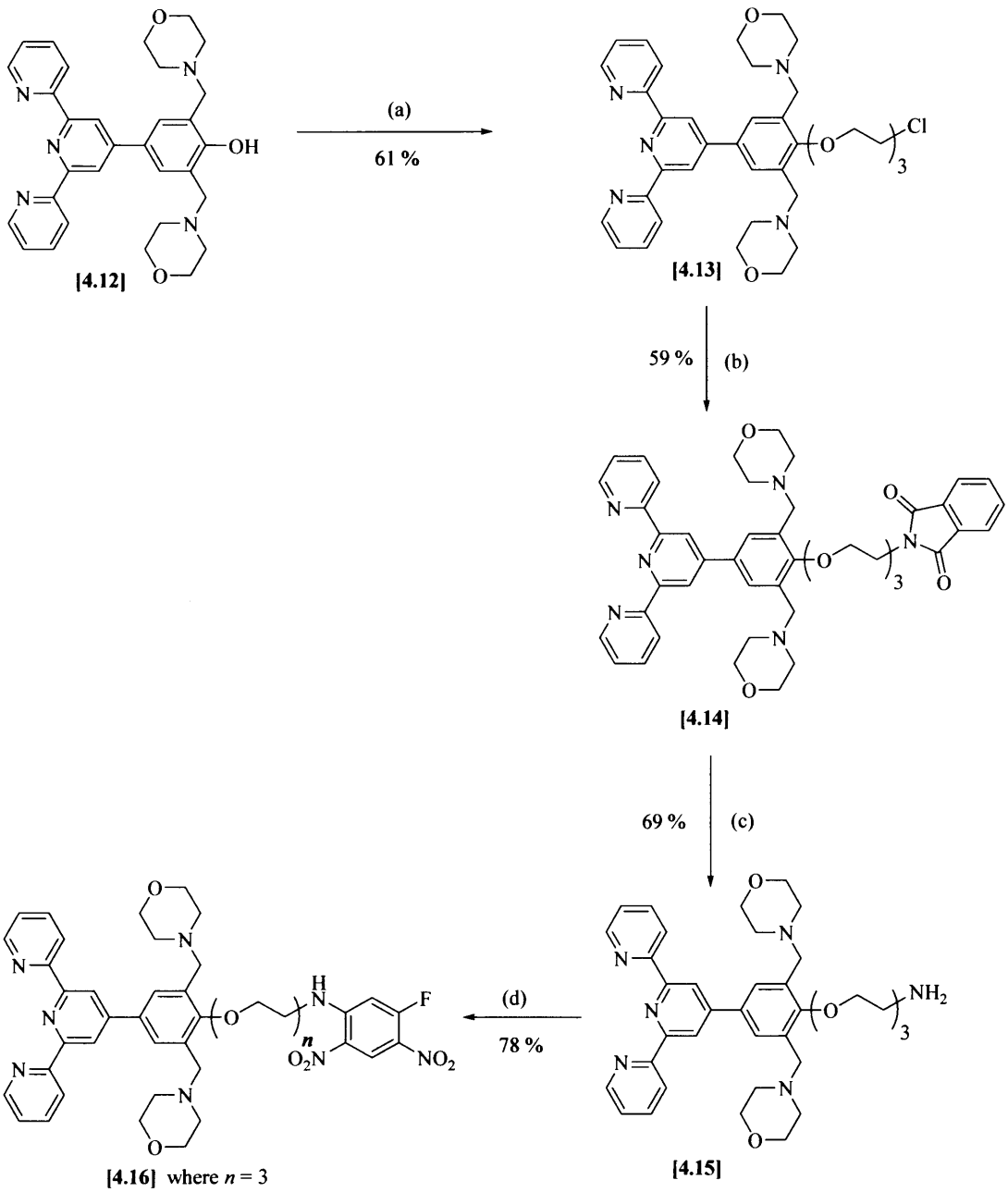


Figure 4.19: *Reagents and conditions:* (a) 1,2-bis(2-chloroethoxy)ethane, caesium carbonate, NaI, DMF, 65°C, 24 h; (b) potassium phthalimide, NaI, DMF, 100°C, 24 h; (c) i) HCl, H_2O, ii) NaOH, H_2O; (d) 1,5-difluoro-2,4-dinitrobenzene, K_2CO_3, MeCN, -15°C.

1,5-Difluoro-2,4-dinitrobenzene was used to modify the ligand **[4.15]** suitable for bioconjugation (figure 4.19). Hence **[4.15]** was added to a cooled solution of 1,5-difluoro-2,4-dinitrobenzene and potassium carbonate in acetonitrile[51] and a colour change of orange was observed. Removal of the solvents *in vacuo* afforded **[4.16]** as a light orange solid. The ^{19}F NMR spectrum shows a single resonance at -100.9 ppm and due to the displacement of one of the fluoride atoms, a loss of fluorine coupling is also seen in the ^{1}H NMR spectrum, both of which are comparable with that of **[4.10]**.

Despite the introduction of two morpholine pendent groups the ligands **[4.15]** and **[4.16]** remain water insoluble. The water solubility of these compounds may be increased by either the use of a longer polyethyleneglycol chain (e.g. where n = 10) or the use of an alternative amine pendent group such as diethanoldiamine or diglycine.

4.14 Testing of Metal Complexes

A number of octahedral transition metal complexes along with platinum (II) complexes were prepared on a small scale. (figure 4.20, details in experimental section) in order to test for redox activity and solubility. The complexes were characterised by ESI mass spectrometry.

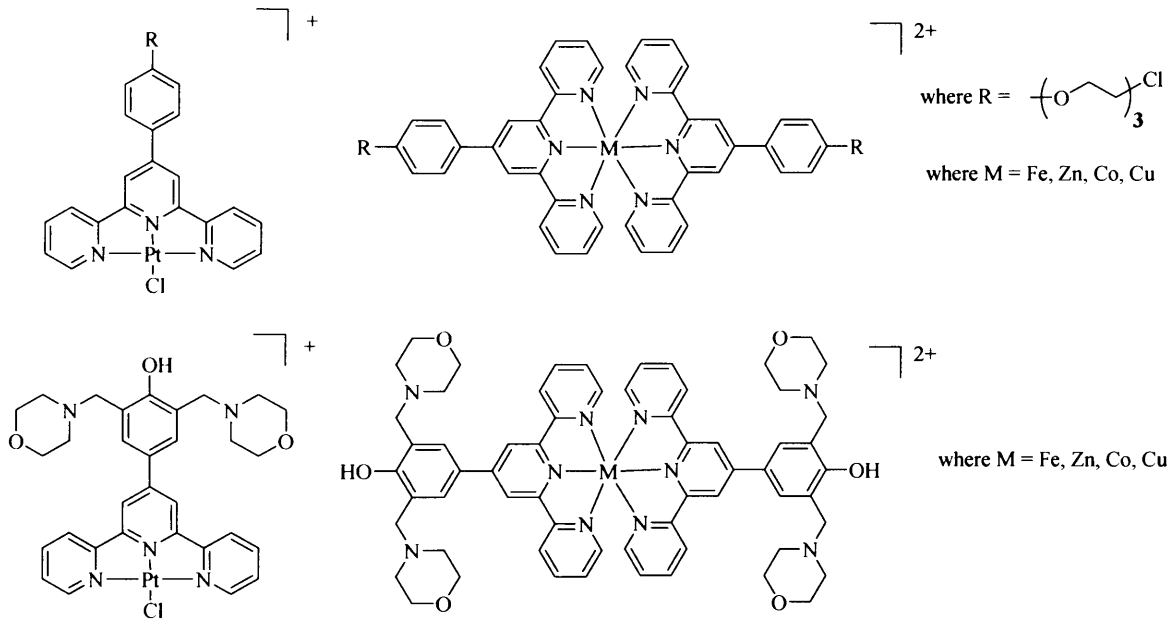


Figure 4.20: Metal complexes.

Testing of compounds was carried out in an analogous manner to that described in chapter 2 (section 2.12), compounds which demonstrated redox activity (*i.e.* initial darkening of the developer solution within 30 minutes) were filtered through celite®. The results obtained (figure 4.21) were in agreement with earlier studies[37] with the first row transition metal octahedral complexes showing no activity whereas the square planar complexes of platinum are able to catalytically reduce silver ions to silver metal *via* a Timm's type reaction. The complexes **[Pt(4.5)Cl]$^+$** and **[Pt(4.12)Cl]$^+$** show good redox activity (5 minutes in both the solid state and in a DMSO solution). After filtration through celite®, both complexes exhibited less redox activity (20 and 24 minutes) and a more gradual darkening of the physical developer. This indicates that traces of free metal may be

responsible for the rapid darkening of the solution. The complexes showed either no or little solubility in the physical developer solution.

Compound	Solubility in physical developer	Redox activity
[4.3]	Insoluble	No activity
[4.5]	Insoluble	No activity
$[Pt(4.5)Cl]^+$	insoluble	**20 minutes**
$[Fe^{II}(4.5)_2][ClO_4]_2$	Insoluble	No activity
$[Zn^{II}(4.5)_2][ClO_4]_2$	Insoluble	No activity
$[Co^{II}(4.5)_2][ClO_4]_2$	Insoluble	No activity
$[Cu^{II}(4.5)_2][ClO_4]_2$	Insoluble	No activity
[4.12]	Insoluble	No activity
$[Pt(4.12)Cl]^+$	Insoluble	**24 minutes**
$[Fe^{II}(4.12)_2][ClO_4]_2$	Sparingly soluble	No activity
$[Zn^{II}(4.12)_2][ClO_4]_2$	Sparingly soluble	No activity
$[Co^{II}(4.12)_2][ClO_4]_2$	Insoluble	No activity
$[Cu^{II}(4.12)_2][ClO_4]_2$	Sparingly soluble	No activity

Figure 4.21: Summary of test results after trace metal removal. 'No activity' implies no notable change during a 30 minute period.

4.15 Conclusion

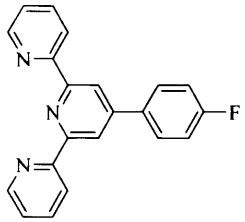
A series of functionalised 4'-aryl-2,2':6',2"-terpyridine ligands have been prepared. A short PEG chain was incorporated to impart water solubility and act as a spacer group between the terpyridine sub-unit and a reactive group suitable for bioconjugation. These ligands were found not to be water soluble. Unfortunately the addition of two morpholinomethyl pendant groups into our ligands did not dramatically increase the solubility of these ligands in water. Previous work had shown that metal terpyridine complexes and in particular complexes of platinum were able to catalyse the reduction the silver ions in a Timm's type reaction.[37] We have found that the time taken to darken the physical developer solution decreases following further purification of the complexes. It was also shown that 4'-(4-hydroxyphenyl)-2,2':6',2''-terpyridine was found to rapidly darkened the physical developer solution (3 minutes).[37] During the course of this work several batches of 4'-(4-hydroxyphenyl)-2,2':6',2''-terpyridine were prepared. Following recrystallisation from ethanol several times, each batch was tested by adding a small sample to the physical developer solutions. None of the samples exhibited any redox activity for the reduction of silver in a Timm's type reaction. From this we can conclude that earlier samples may have contained trace (unknown) impurities that were able to rapidly reduce silver ions to silver metal.

Experimental

General Procedure

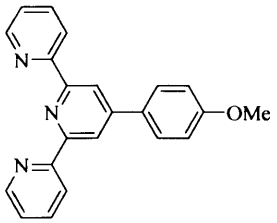
Non-synthesised reagents were purchased from Aldrich, Avocado or Lancaster and were used as received. Solvents were purified by standard literature methods.[52] The NMR spectra were recorded on a Brüker Avance 400 instrument at 400 MHz (^{1}H) and 100 MHz (^{13}C), Jeol Lamda Eclipse 300 at 282.78 MHz (^{19}F); ^{1}H and ^{13}C chemical shifts are quoted in ppm relative to residual solvent peaks and. Coupling constants are quoted in Hertz. Mass spectra were obtained in either APCI (atmospheric pressure chemical ionisation), EI (electronic ionisation). IR spectra were obtained as KBr or NaCl discs using a Jasco FTIR 110 series spectrometer.

[4.1] 4'-(4-Fluorophenyl)-2,2':6',2''-terpyridine



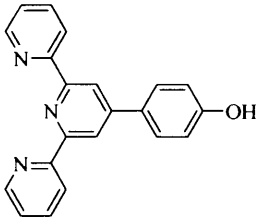
This was prepared in an analogous method to that of Raston *et al.*[29] 4-Fluorobenzaldehyde (7.068 g, 0.056 mol), 2-acetyl pyridine (13.79g, 0.110 mol) and sodium hydroxide (4.5 g, 0.112 mol) were ground in a pestle and mortar until a yellow powder was formed. This powder was then added to a solution of ammonium acetate (21.7 g, 0.281 mol) in ethanol (450 mL) and refluxed for 24 h. the volume of the reaction mixture was reduced *in vacuo* by one third, cooled and the crude product was collected by filtration. The product was recrystallised from hot EtOH to give the pure product **[4.1]** as a yellow solid (7.64 g, 41 %) δ_H (400 MHz, $CDCl_3$) 8.8 (d, 2H, *J* 3.89), 8.7 (m, 4H), 7.85 (m, 4H), 7.30 (m, 2H), 7.15 (t, 2H, *J* 8.59, Ar *H*-m); δ_F (282.78 MHz, $CDCl_3$) -112.7025; *m/z* (APCI) 328 [M + 1].

[4.2] 4'-(4-Methoxyphenyl)-2,2':6',2''-terpyridine



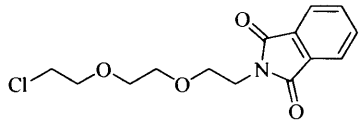
This was prepared in an analogous method to that of **[4.1]**, the applied reagents were 4-methoxybenzaldehyde (17.0 g, 0.125 mol), 2-acetyl pyridine (30.2 g, 0.250 mol) and sodium hydroxide (10 g, 0.250 mol) to afford **[4.2]** as a cream solid (28.3 g, 67 %); δ_H (400 MHz, $CDCl_3$) 8.65 (d, 2H, *J* 4.4, Ar *H*-6), 8.60 (s, 2H, Ar *H*-3'), 8.55 (d, 2H, *J* 7.95, Ar *H*-3), 7.80 (m, 4H, Ar *H*-4 and *H*-o), 7.25 (t, 2H, *J* , Ar *H*-5), 6.95 (d, 2H, *J* 8.62, Ar *H*-m), 3.8 (s, 3H, CH_3); δ_C (100 MHz, $CDCl_3$) 161.2, 157.4, 156.0, 150.3, 149.7, 137.6, 131.6, 129.0, 124.4, 122.8, 119.2, 115.0 and 55.5; IR (KBr disc, cm^{-1}) 3084, 3006, 2835, 1600, 1584, 1468, 1388, 1284, 1260, 1187, 1100, 1042, 888, 830 and 730; *m/z* (APCI) 340.1 (339.1).

[4.3] 4'-(4-Hydroxyphenyl)-2,2':6',2''-terpyridine



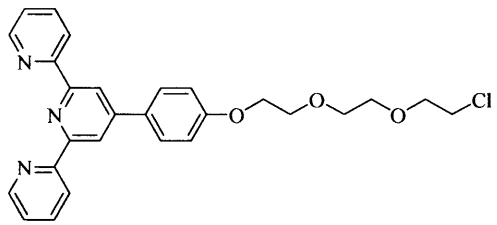
This was carried out in a similar manner to a standard preparative manner.[38] 4'-(4-Methoxyphenyl)-2,2':6',2''-terpyridine **[4.2]** (6.0 g, 17.69 mmol) was added was added to molten pyridinium hydrochloride (40 mL) at 210 °C and allowed to stir for 4 h. The reaction mixture was allowed to cool and warm water (50 mL) was added. The pH was adjusted to 7-8 with sodium hydrogen carbonate and the precipitate was removed by filtration. The crude product was then washed with water (3 x 15 mL), dichloromethane (3 x 15 mL) and dried *in vacuo*. Recrystalisation from hot ethanol afforded the pure product **[4.3]** as a fawn solid (5.061 g, 87 %); δ_H (400 MHz, d^6-dmso) 9.95 (br s, 1H, O*H*), 8.80 (d, 2H, *J* 4.12, Ar *H*-6), 8.65 (m, 4H, Ar *H*-3 and *H*-3'), 8.05 (t, 2H, *J* 7.67, Ar *H*-5), 7.80 (d, 2H, *J* 8.62, Ar *H*-o (2,6)), 7.50 (t, 2H, *J* 6.7, Ar *H*-4), 6.95 (d, 2H, *J* 8.57, Ar *H*-m(3,5)); δ_C (100 MHz, d^6-dmso) 159.0, 156.4, 155.2, 150.1, 138.0, 129.7, 128.5, 125.5, 121.3, 118.0, 117.0 and 116.1; IR (KBr disc, cm^{-1}) 3690, 3387, 3030, 1594, 1532, 1418, 1354, 1297, 1035, 832, 776 and 738; *m/z* (APCI) 326.

[4.4] 2-{2-[2-(2-Chloroethoxy)ethoxy]ethyl}isoindole-1,3-dione[43]



A mixture of 1,2-bis(2-chloroethoxy)ethane (59 g, 0.315 mol) and potassium phthalimide (5.8 g 0.031 mol) was stirred at 130 °C for 24 h. After concentration *in vacuo,* the residue was treated with toluene (30 mL) and the solid material was removed by filtration. The toluene was removed *in vacuo* yielding a brown oily residue. The residue was combined with sand and extracted with petroleum ether using a soxhlet extractor for 3 days. The solution was evaporated to give **[4.4]** as a yellow oil (2.559 g, 27 %); δ_H (400 MHz, $CDCl_3$) 7.90 (m, 2H), 7.65 (m, 2H), 3.85(m, 2H), 3.5-3.7 (m, 8 H), 3.45 (m, 2H); δ_C (100 MHz, $CDCl_3$) 168.2, 133.9, 132.1, 123.2, 71.4, 70.6, 70.1, 68.0, 42.7, 37.2; IR (NaCl, cm^{-1}) 3477, 2872, 1774, 1716, 1394, 1290, 1115, 956, 797 and 720; *m/z* (APCI) 298.

[4.5]



Cesium carbonate (14g, 42.9 mmol, 1.5 equiv) which had been ground in a hot pestle and mortar was added to a Schlenk tube containing dry, degassed DMF (50 mL). **[4.3]** (10 g, 30.67 mmol), 1,2-bis(2-chlorothoxy)ethane (57.49 g, 307 mmol) and a few crystals of sodium iodide in DMF (20 mL) were added to a pressure equalising dropping funnel which was attached to the Schlenk tube. The DMF-Cs_2CO_3 suspension was heated to 65 °C and the terpyridine-dichloride mixture was added slowly drop wise. After the addition stirring and heating was continued for 24 h. Upon cooling the reaction mixture was filtered and the DMF was removed *in vacuo*. The residue was treated with water to dissolve solid cesium salts and was extracted with chloroform (3 x 40 mL), dried over $MgSO_4$ and solvent removed *in vacuo* to give **[4.5]** as a pale brown solid (9.601 g, 66 %); δ_H (400 MHz, $CDCl_3$) 8.68 (m, 4H, Ar *H*-6 and *H*-3'), 8.55 (d, 2H, *J* 7.91, Ar *H*-3), 7.80 (m, 4H, Ar *H*-4 and *H*-o), 7.25 (m, 2H, Ar *H*-5), 6.95 (d, 2H, *J* 8.7, Ar *H*-m), 4.10 (m, 2H, CH_2), 3.82 (m, 2H, CH_2) 3.65 -3.70 (m, 6H, CH_2), 3.55 (m, 2H, CH_2); δ_C (100 MHz, $CDCl_3$) 159.7, 156.3, 155.8, 149.7, 136.9, 130.9, 128.5, 123.8, 121.4, 118.2, 115.0, 114.3, 71.4,

70.9, 70.6, 69.8, 67.5, 42.8; IR (KBr, cm^{-1}) 3051, 2964, 2893, 1609, 1585, 1543, 1491, 1468, 1457, 1389, 1260, 1185, 1132, 1094, 1022, 825 and 790; *m/z* (ES Q-TOF) 476.17

$[Pt^{II}(4.5)Cl]^+$

To a solution of **[4.5]** (0.04 g, 0.08 mmol), in ethanol (10 mL) was added a solution of $[Pt(COD)Cl_2]$ (0.034 g, 0.08 mmol) in ethanol (5 mL). The resulting yellow solution was refluxed for 12 hours. The solid product was removed by filtration, washed with ice cold ethanol and dried in *vacuo* to give **$[Pt(4.5)Cl]^+$** as a yellow solid (0.048 g, 82 %); δ_H (400 MHz, *d*-dmso) 8.82 (m, 4H,), 8.65 (d, 2H, *J* 5.13), 8.40 (t, 2H, *J* 7.87), 8.25 (d, 2H, 8.75, Ar *H*-o) 7.80 (t, 2H, *J* 6.67), 7.25 (d, 2H, *J* 8.77, Ar-*H*-m), 4.30 (m, 2H, CH_2), 4.0 (m, 2H, CH_2), 3.75-3.85 (m, 6H, CH_2), 3.70 (m, 2H, CH_2); *m/z* (APCI) 706.

$[Fe^{II}(4.5)_2][ClO_4]_2$

To an ethanolic solution of **[4.5]** (0.01 g, 0.021 mmol) was added a solution of iron(II) perchlorate hexahydrate (0.002 g, 0.011 mmol) in ethanol and stirred for 12 hours. Upon the addition of the iron(II) perchlorate the solution turned dark purple and a precipitate was formed. Diethyl ether was added and the purple solid filtered and dried in *vacuo* to afford **$[Fe^{II}(4.5)_2][ClO_4]_2$** (0.011 mg, 90 %); δ_H (400 MHz, CD_3CN) 9.05 (m, 8H), 8.50 (d, 4H, *J* 7.45), 8.25 (d, 4H, *J* 8.23), 7.82 (m, 4H), 7.25 (d, 4H, *J* 8.48), 7.1 (m, 4H), 4.0 (m, 4H), 3.8 (m, 4H), 3.6 (m, 4H), 3.3 (m, 4H; *m/z* (ES) calc. 1206.7 for $C_{54}H_{52}N_6O_{14}Cl_4Fe$ found 1107.8 (- ClO_4^-).

$[Zn^{II}(4.5)_2][ClO_4]_2$

This was prepared in a similar manner to **$[Fe^{II}(4.5)_2][ClO_4]_2$**, replacing zinc(II) perchlorate hexahydrate to afford **$[Zn^{II}(4.5)_2][ClO_4]_2$** as a yellow solid (0.016 g, 89 %); δ_H (400 MHz, CD_3CN) 9.20 (s, 4H), 9.0 (d, 4H, *J* 8.1), 8.4-8.5 (m, 8H), 8.10 (d, 4H, *J* 4.87), 7.70 (m, 4H), 7.60 (d, 4H, *J* 8.83), 4.60 (m, 4H, CH_2), 4.10 (m, 4H, CH_2), 3.99-4.05 (m, 16H, CH_2). *m/z* (ES) calc. 1216.2 for $C_{54}H_{52}N_6O_{14}Cl_4Zn$ found 1017.9 (-2ClO_4^-).

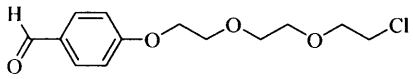
$[Cu^{II}(4.5)_2][ClO_4]_2$

This was prepared in a similar manner to **$[Fe^{II}(4.5)_2][ClO_4]_2$**, replacing copper(II) perchlorate hexahydrate to afford **$[Cu^{II}(4.5)_2][ClO_4]_2$** as a pale green solid (0.011 g, 81 %); *m/z* (ES) calc. 1214.4 for $C_{54}H_{52}N_6O_{14}Cl_4Cu$ found 1016.2 (-2ClO_4^-).

$[Co^{II}(4.5)_2][ClO_4]_2$

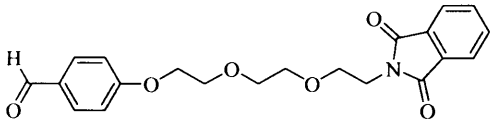
This was prepared in a similar manner to **$[Fe^{II}(4.5)_2][ClO_4]_2$**, replacing cobalt(II) perchlorate hexahydrate to afford **$[Co^{II}(4.5)_2][ClO_4]_2$** as a brick-red solid (0.009 g, 87 %); *m/z* (ES) calc. 1209.7 for $C_{54}H_{52}N_6O_{14}Cl_4Co$ found 1011.3 ($-2ClO_4^-$).

[4.6]



A mixture of cesium carbonate (11.9 g, 36.52 mmol, excess) and 4-hydroxybenzaldehyde (3 g, 24.5 mmol) was stirred in dry DMF (20 mL) for 45 min at 80°C, after which 1,2-bis(2-chlorothoxy)ethane (38.4 mL, 45.95 g, 240 mmol) and a trace of NaI were added and the heating continued overnight. After cooling the solution was filtered and the DMF removed *in vacuo*, followed by removal of the excess dichloride via a Kugelröhr distillation. The resulting residue was treated with water and extracted with chloroform (3 x 30 mL). The chloroform solution was dried over $MgSO_4$ and the solvent removed *in vacuo* to give **[4.6]** as a brown oil (5.41 g, 81 %); δ_H (400 MHz, $CDCl_3$) 9.80 (s, 1H, C*H*O), 7.75 (d, 2H, *J* 9.46, Ar *H*-2 and *H*-6), 6.90 (d, 2H, *J* 9.48, Ar *H*-3 and *H*-5), 4.15 (m, 2H, CH_2), 3.85 (m, 2H, CH_2), 3.65 (m, 6H, CH_2), 3.55 (m, 2H, CH_2); δ_C (100 MHz, $CDCl_3$) 190.7, 163.8, 131.9, 130.0, 114.9, 71.4, 70.8, 70.6, 69.5, 67.8, 42.8; IR (NaCl, cm^{-1}) 3011, 2872, 2741, 1684, 1600, 1577, 1509, 1453, 1427, 1393, 1311, 1258, 1219, 1161, 1114, 1055, 925, 835 and 755; *m/z* (APCI) 273.1 [calc 272.1].

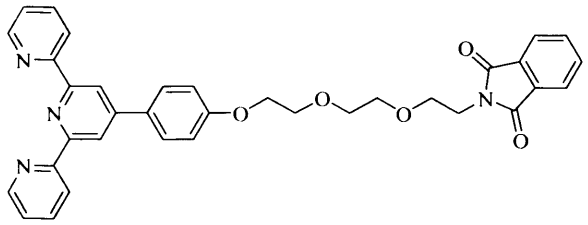
[4.7]



To a suspension of potassium phthalimide (27.16 g, 146.6 mmol, 10 fold excess) in dry DMF (20 mL) heated to 100 °C under nitrogen, was added **[4.6]** (4.0 g, 14.66 mmol) and the heating was continued overnight. The reaction mixture was cooled, poured onto crushed ice and allowed to stand for 1 h. The mixture was filtered; the residue was taken up in chloroform. The organic phase was washed with $NaOH_{(aq)}$ (0.5 M), water, dried over $MgSO_4$ and the solvent removed *in vacuo* to give **[4.7]** as a yellow soild (3.66 g, 65 %); δ_H (400 MHz, $CDCl_3$) 9.82 (s, 1H, C*H*O), 7.75 (m, 4H, Ar *H*-2, *H*-6 and *H*-phthalimide), 7.65 (m, 2H, Ar *H*-phthalimide), 6.95 (d, 2H, *J* 6.84, Ar *H*-3 and *H*-5), 4.08 (t, 2H, *J* 4.7, CH_2), 3.80 (t, 2H, *J* 5.7, CH_2), 3.75 (m, 2H, CH_2), 3.70 (m, 2H, CH_2), 3.62 (s, 4H, CH_2); δ_C (100 MHz, $CDCl_3$) 190.8, 168.3, 163.8, 134.0, 132.1, 131.9, 130.0, 123.2, 114.9,

70.8, 70.2, 69.5, 68.0, 67.7, 37.3; IR (KBr, cm^{-1}) 2873, 1771, 1715, 1600, 1576, 1509, 1467, 1394, 1312, 1257, 1161, 1111, 1027, 835 and 721; *m/z* (APCI) 384.1 [calc 383.4]

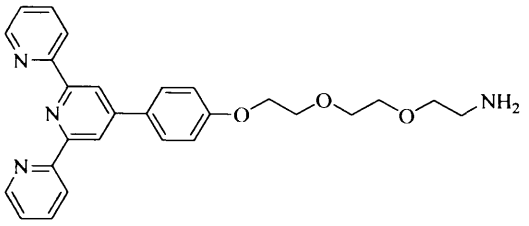
[4.8]



Method A: This was prepared in an analogous manner to **[4.7]**, the applied reagents were potassium phthalimide (15 g, 80.98 mmol 10 fold excess), **[4.5]** (7.914 g, 16.6 mmol) and DMF (20 mL). The crude product was taken up in the minimum amount of chloroform and passed through a short alumina (neutral) column, eluting with ethyl acetate to give **[4.8]** as a brown solid (5.85 g, 60 %);. δ_H (400 MHz, $CDCl_3$) 8.62-8.65 (m, 4H, Ar *H*-6 and *H*-3'), 8.58 (d, 2H, *J* 8.12, Ar *H*-3), 7.7-7.8 (m, 6H, Ar *H*-o and *H*-phthalimide), 7.6 (m, 2H, Ar *H*-4), 7.3 (m, 2H, Ar *H*-5), 6.9 (d, 2H, *J* 7.09, Ar *H*-m), 4.05 (m, 2H, CH_2), 3.85 (m, 2H, CH_2), 3.75 (m, 2H, CH_2), 3.68 (m, 2H, CH_2), 3.60 (s, 4H, CH_2); δ_C (100 MHz, $CDCl_3$) 168.2, 159.7, 156.3, 155.7, 149.6, 149.0, 136.8, 133.9, 132.0, 128.4, 123.8, 123.2, 121.3, 118.2, 114.9, 70.7, 70.2, 69.7, 68.0, 67.4, 40.6; IR (KBr, cm^{-1}) 3821, 2962, 1717, 1654, 1583, 1560, 1541, 1508, 1466, 1388, 1261, 1091, 1024 and 799; *m/z* (APCI) 587.1

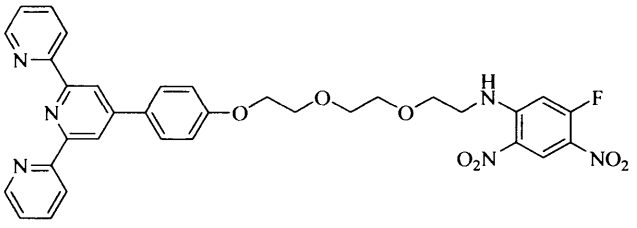
Method B: Compound **[4.7]** (2 g, 5.21 mmol), 2-acetyl pyridine (1.26 g, 10.43 mmol) and sodium hydroxide (0.41 g, 10.43 mmol) were ground in a pestle and mortar until a orange powder was formed. This powder was then added to a solution of ammonium acetate (2 g) in ethanol (200 mL) and was heated under reflux for 24 h. Upon cooling the reaction mixture was concentrated by approximately one third and the dark brown precipitate was collected by suction filtration. The crude product was purified by column chromatography on alumina (neutral) and eluting with ethyl acetate gave the title compound **[4.8]** as a brown solid (0.88 g, 29 %) with identical spectral properties as above.

[4.9]



Compound **[4.8]** (0.6 g, 1.02 mmol) was taken up in hydrochloric acid and heated to reflux for 24 h at 110 C. The solution was allowed to cool in ice and the precipitated solid was collected by suction filtration and washed with water. The filtrate was reduced *in vacuo* affording the hydrochloride salt as a pale yellow solid. The solid was taken up in the minimum amount of water; to this sodium hydroxide was added (pH 14) and 10 mL of toluene. A Dean-Stark apparatus was attached and the collection arm was filled with toluene. The mixture was heated to 120 °C overnight to remove the water and to afford the amine **[4.9]** as a toluene solution. Removal of the solvents *in vacuo* afforded **[4.9]** as a cream, moisture sensitive solid (0.38 g, 82 %); δ_H (400 MHz, $CDCl_3$) 8.62 (m, 4H, Ar *H*-6 and *H*-3'), 8.58 (d, 2H, *J* 7.98, Ar *H*-3), 7.75 (m, 4H, Ar *H*-4 and *H*-o), 7.25 (m, 2H, Ar *H*-5), 6.95 (d, 2H, *J* 8.83, Ar *H*-m), 4.1 (t, 2H, *J* 4.94, CH_2), 3.8 (m, 2H, CH_2), 3.7 (m, 2H, CH_2), 3.6 (m, 2H, CH_2), 3.45 (t, 2H, *J* 5.18, CH_2), 2.78 (t, 2H, J 9.16, CH_2); δ_C (100 MHz, $CDCl_3$) 159.78, 156.32, 155.84, 149.11, 137.83, 136.81, 128.49, 125.38, 123.79, 121.33, 118.22, 115.01, [73.6, 70.85, 70.35, 69.72, 67.50, 21.51; *m/z* (ES Q-TOF) 457.2

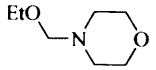
[4.10]



1,5-Difluoro-2,4-dinitrobenzene (0.13g, 0.66 mmol), freshly ground potassium carbonate (0.1g, 0.66 mmol) and dry acetonitrile (10 mL) were added to pressure tube and cooled to – 15 C. To this stirred solution, was added **[4.9]** (0.3g, 0.66 mmol) in acetonitrile (10 mL) and an instant colour change of colourless to intense yellow was observed. The solution was warmed to room temperature and stirred for a further 2 h. The reaction mixture was diluted with DCM, filtered and the solvent was removed *in vacuo* to give the product **[4.10]** as a yellow oil (0.32 g, 76 %), which solidified upon cooling. δ_H (400 MHz, $CDCl_3$) 9.05 (d, 1H, *J* 8.5, Ar *H*), 8.55 (m, 6H, Ar *H*-3, *H*-6 and *H*-3'), 7.8 (m, 4H Ar *H*-4 and *H*-o), 7.3 (m, 2H, Ar *H*-5), 6.98 (d, 2H, *J* 8.78, Ar *H*-m), 6.5 (d, 1H, J_{CF} 13.39, Ar *H*), 4.15 (m,

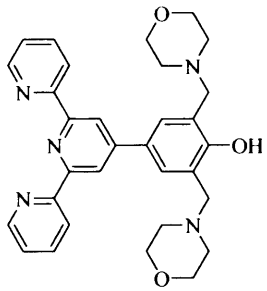
2H), 3.85 (m, 2H), 3.65-3.75 (m, 6H), 3.40 (m, 2H); δ_F (282.78 MHz, $CDCl_3$) -100.402; IR (KBr, cm^{-1}) 3838, 3751, 3625, 3447, 3102, 2962, 1624, 1596, 1559, 1521, 1423, 1326, 1297, 1261, 1189, 1097, 1054, 917, 800 and 740; *m/z* (ES) 641.2

[4.11] Ethoxy-N-morpholinylmethane[50]



Morpholine (20 g, 0.23 mol) was added dropwise to suspension of paraformaldehyde (8.61 g, 0.29 mol) and anhydrous potassium carbonate (63 g, 0.45 mol) in ethanol (125 mL) with external cooling in ice. The mixture was vigourously stirred for 48 h allowing the temperation to reach ambient gradually. The solid was then filtered off and washed with diethyl ether. The filtrate was concentrated *in vacuo* to give a yellow oil which was distilled on a Kugelröhr apparatus to give **[4.11]** as a colourless oil (14.633 g, 44 %); δ_H (400 MHz, $CDCl_3$) 3.95 (s, 2H, NCH_2O), 3.55-3.65 (m, 4H, CH_2-3 and CH_2-5), 3.40 (q, 2H, *J* 6.87, OCH_2CH_3), 2.5-2.6 (m, 4H, CH_2-2 and CH_2-6), 1.10 (t, 3H, *J* 6.94, CH_3); δ_C (100 MHz, $CDCl_3$) 15.2, 50.1, 65.2, 66.4, 88.7; *m/z* (APCI) 146.2.

[4.12]



A mixture of **[4.3]** (1.5 g, 4.61 mmol) and **[4.11]** (1.48 g, 140.14 mmol) in dry DMF (40 mL) were heated under reflux in a nitrogen atmosphere for 3 days . After cooling to room temperature the solvent was removed *in vacuo* to give the product **[4.12]** as a brown glassy solid (1.47 g, 61%); δ_H (400 MHz, $CDCl_3$) 8.65 (d, 2H, *J* 4.21, Ar *H*-6), 8.60 (m, 4H, Ar *H*-3 and *H*-3'), 7.80 (m, 2H, Ar *H*-5), 7.6 (s, 2H, Ar *H*-o (2,6)), 7.3 (m, 2H, Ar *H*-4), 3.65-3.75 (m, 12H), 2.55 (m, 8H); IR (KBr, cm^{-1}) 3425 (br, OH), 2811, 1671, 1598, 1581 (strong), 1562, 1485, 1437, 1392, 1347, 1298, 1262, 1178, 1115 (strong, C-O-C), 1070, 861, 794; *m/z* (ES) 523.3.

[Pt(4.12)]Cl^+

This was prepared in an analogous method to $[Pt^{II}(4.5)Cl]^+$, to afford $[Pt(4.12)Cl]^+$ as a yellow solid (0.008g, 74 %); *m/z* (ES) calc 754.16 for $C_{62}H_{33}N_5O_3PtCl$ found 755.2.

$[Fe^{II}(4.12)_2][ClO_4]_2$

This was prepared in a similar manner to **$[Fe^{II}(4.5)_2][ClO_4]_2$** to afford **$[Fe^{II}(4.12)_2][ClO_4]_2$** as a purple solid (0.010g, 87 %); δ_H (400 MHz, CD_3CN) 9.20 (s, 4H), 8.70 (d, 4H, *J* 7.47), 8.25 (s, 4H), 8.0 (t, 4H, *J* 7.24), 7.30 (m, 4H), 7.10 (m, 4H), 3.9 (m, 24H), 2.8 (m, 16H); *m/z* (ES) calc. 1302.0 for $C_{62}H_{66}N_{10}O_{14}Cl_2Fe$ found 1202.6 (- ClO_4).

$[Zn^{II}(4.12)_2][ClO_4]_2$

This was prepared in an analogous method to **$[Zn^{II}(4.5)_2][ClO_4]_2$** to afford **$[Zn^{II}(4.12)_2][ClO_4]_2$** as a yellow solid (0.015g, 81%); δ_H (400MHz, CD_3CN) 8.85 (s, 4H), 8.6 (d, 4H, *J* 7.96), 8.0 (m, 4H), 7.95 (s, 4H), 7.6 (m, 4H), 7.2 (m,4H), 3.6 (m, 24H), 2.5 (m, 16H); *m/z* (ES) calc. 1311.54 for $C_{62}H_{66}N_{10}O_{14}Cl_2Zn$ found 1112.8 (- $2ClO_4$).

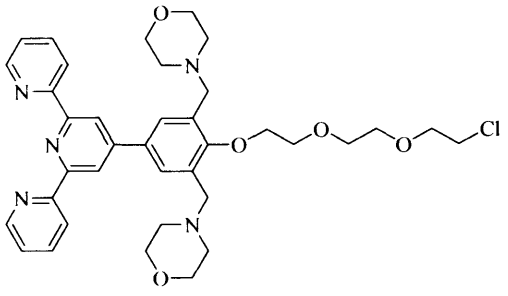
$[Cu^{II}(4.12)_2][ClO_4]_2$

This was prepared in an analogous method to **$[Cu^{II}(4.5)_2][ClO_4]_2$** to afford **$[Cu^{II}(4.12)_2][ClO_4]_2$** as a pale green solid (0.008g, 79 %); *m/z* (ES) calc. 1309.7 for $C_{62}H_{66}N_{10}O_{14}Cl_2Cu$ found 1110.9 (- $2ClO_4$).

$[Co^{II}(4.12)][ClO_4]_2$

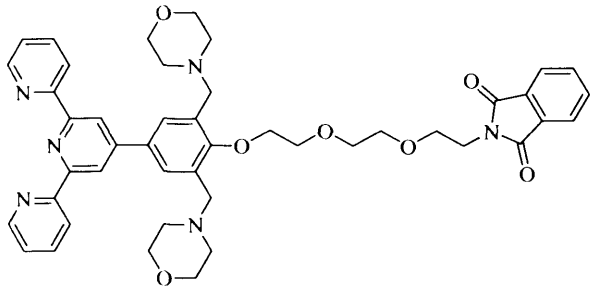
This was prepared in an analogous method to that of **$[Co^{II}(4.5)_2][ClO_4]_2$** to afford **$[Co^{II}(4.12)_2][2ClO_4]$** as a dark orange solid (0.01 g, 86 %); *m/z* (ES) calc. 1305.1 for $C_{62}H_{66}N_{10}O_{14}Cl_2Co$ found 1122.6 (- $2ClO_4$).

[4.13]



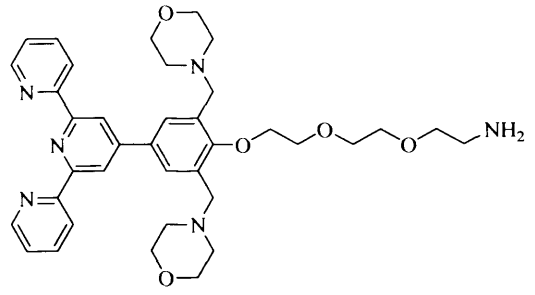
This was prepared in an analogous method to that of **[4.5]** substituting **[4.12]** (0.25 g, 0.477 mmol) to afford **[4.13]** as a pale brown solid (0.19 g, 61 %); δ_H (400 MHz, $CDCl_3$) 8.7 (d, 2H, *J* 4.15, Ar *H*-6), 8.65 (m, 4H, Ar-*H*-3 and *H*-3'), 7.80 (m, 2H, Ar-*H*-5), 7.75 (s, 2H, Ar *H*-o (2,6)), 7.30 (m, 2H, Ar *H*-4), 4.15 (m, 2H, CH_2), 3.8 (m, 2H, CH_2), 3.55-3.75 (m, 20H), 2.4 (s, 8H, NCH_2O); *m/z* (ES) 674.2 [M + 1].

[4.14]



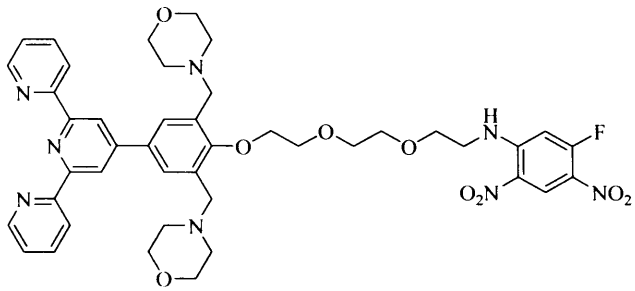
This was prepared in an analogous method to **[4.8]** *via* method **A**, substituting **[4.13]** (0.18 g, 0.34 mmol) to afford **[4.14]** as a brown solid (0.121 g, 59 %); δ_H (400 MHz, $CDCl_3$) 8.70 (d, 2H, *J* 4.17, Ar *H*-6), 8.63 (m, 4H, Ar *H*-3 and *H*-3'), 7.80 (m, 4H, Ar *H*-phthalimide), 7.70 (s, 2H, Ar *H*-o), 7.65 (m, 2H, Ar *H*-4) 7.25 (m, 2H, Ar *H*-5), 4.1 (m, 2H, CH_2), 3.90 (m, 2H, CH_2), 3.80 (m, 2H, CH_2), 3.6-3.7 (m, 14H), 3.5 (s, 4H, CH_2), 2.4 (s, 8H, NCH_2O); IR (KBr, cm^{-1}) 2856, 1772, 1712, 1583, 1567, 1467, 1393, 1350, 1295, 1115, 1027, 884; *m/z* (ES) 785.8 [M + 1].

[4.15]



This was prepared in an analogous method to **[4.9]** the applied reagents were **[4.14]** (0.1g, 0.127 mmol) to afford **[4.15]** as an off-white solid (0.06g, 69%); δ_H (400 MHz, $CDCl_3$) 8.68 (d, 2H, *J* 3.35, Ar *H*-6), 8.60 (m, 4H, Ar *H*-3 and *H*-3'), 7.85 (m, 2H, Ar *H*-4), 7.75 (s, 2H, Ar *H*-o), 7.30 (m, 2H, Ar *H*-5), 4.20 (m, 2H, CH_2), 3.80 (m, 2H, CH_2), 3.70 (m, 2H, CH_2), 3.55-3.65 (m, 14H), 3.50 (m, 2H, CH_2), 2.80 (m, 2H, CH_2), 2.45 (s, 8H, NCH_2O); *m/z* (ES) 655.9 [M + 1].

[4.16]



This was prepared in analogous method to **[4.10]** the applied reagents were **[4.15]** (0.05g, 0.076 mmol) and 1,5-difluoro-2,4-dinitrobenzene (0.02g, 0.076mmol) to afford **[4.16]** as a dark yellow solid (0.03g, 78 %); δ_H (400 MHz, $CDCl_3$) 8.85 (d, 1H, *J* 7.94, Ar *H*), 8.6 (m, 6H, Ar *H*-3, *H*-3' and *H*-6), 7.78 (m, 4H, Ar *H*-4 and *H*-o), 7.3 (m, 2H, Ar *H*-5), 6.70 (d, 1H, J_{CF} 11.95, Ar *H*), 4.20 (m, 2H, CH_2), 3.80 (m, 2H, CH_2), 3.55-3.70 (m, 18H), 3.20 (m, 2H, CH_2), 2.50 (m, 8H, NCH_2O); δ_F (282.78 MHz, $CDCl_3$) -101.0; *m/z* (ES) 839.9 [M + 1].

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Chapter 5

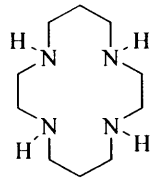
Markers Based on 1,4,7-Triazacyclononane

Introduction

This chapter investigates the potential of markers based on *N*-functionalised 1,4,7-triazacyclononane.

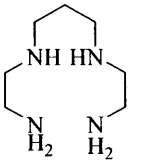
5.1 Azamacrocycles

The azamacrocycles are a class of ligands which contain varying numbers of nitrogen donor atoms within rings of various sizes. In 1972, a non-templated, low dilution method for the preparation of azamacrocycles was reported.[1] Modifications in 1974[2] provided an effective method of synthesis for ring sizes of 9-24 atoms containing 3-8 nitrogen atoms. These cyclic, multidentate ligands form more stable metal complexes than their corresponding open chain analogues. This is due to the macrocyclic effect which arises due to both entropic and enthalpic effects (figure 5.1).



1,4,8,11-Tetraazatetracyclodecane
(Cyclam)

1



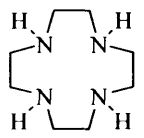
N^1,N^3-bis(2-aminoethyl)propane-1,3-diamine

2

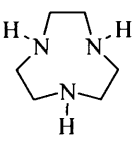
	Low Spin		High spin	
	ΔH (kJ mol^{-1})	$T\Delta S$ (kJ mol^{-1})	ΔH (kJ mol^{-1})	$T\Delta S$ (kJ mol^{-1})
1	-78.2	49.3	-100.8	24.3
2	-66.1	21.7	-80.3	10.9

Figure 5.1: Thermodynamic data for the formation of Ni(II) complexes with macrocyclic ligand **1** and open chain analogue **2** at 298 K.[3]

The most common and most studied azamacrocycles contain four nitrogen donor atoms known as tetraazamacrocycles. Examples include 1,4,8,11-tetraazatetracyclodecane (cyclam, figure 5.1) and 1,4,7,10-tetraazacyclododecane (cyclen, figure 5.2). Rings containing greater or smaller number of donor atoms are generally of less importance with the exception of 1,4,7-triazacyclononane (tacn, figure 5.2) which exhibits interesting coordination chemistry.



1,4,7,10-Tetraazacyclododecane
(Cyclen)



1,4,7-Triazacyclononane
(Tacn)

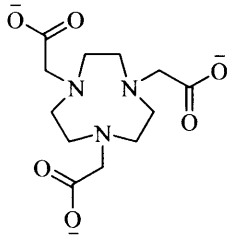
Figure 5.2: The tetra- and tri-azamacrocycles cyclen and tacn.

5.2 1,4,7-Triazacyclononane

The coordination chemistry of 1,4,7-triazacyclononane (tacn) has been extensively studied and complexes with most of the transition metals have been reported.[4] When coordinated as a tridentate ligand, it exhibits a strong ligand-field *i.e.* forms low spin complexes. Owing to the small ring size and consequently relatively small hole size, 1,4,7-triazacyclononane is unable to form equatorial complexes with transition metal ions, instead coordinating to the metal centre in facially capping manner. In the presence of six donors the resulting complexes exhibit either octahedral or distorted octahedral geometries. Vacant coordination sites can be occupied by anions such as Cl^-, Br^- or OH^-. Occupation by neutral donors such as CO *e.g.* in $[Mo(tacn)(CO)_3]^+$ exhibit a piano-stool geometry[5] whilst coordination to a second 1,4,7-triazacyclononane molecule affords a sandwich type structure e.g. $[Ni(tacn)_2]^{2+}$.[6]

5.3 *N*-Functionalisation of 1,4,7-Triazacyclononane

The introduction of one, two or three pendent arm donors at the nitrogen positions allows the formation of tetra, penta or hexadentate complexes. The first derivative to be extensively studied was 1,4,7-trisacetate-1,4,7-triazacyclononane (TCTA, figure 5.3).[7] This is an example of a potential hexadentate ligand with a N_3O_3 donor set. It forms mononuclear complexes with all of the first row transition metal in a pseudo octahedral environment[8] with the exception of titanium. In addition it also forms stable complexes with alkaline earth metals and some lanthanide metals.[9]



(TCTA)

Figure 5.3: 1,4,7-Trisacetate-1,4,7-triazacyclononae (TCTA).

The tridentate 1,4,7-triazacyclonone is a strong-field ligand, whereas the hexadentate TCTA ligand imposes a much weaker ligand field. For example, the Fe^{II} and Fe^{III} bis(tacn) complexes are low spin,[10] whereas the Fe^{II} and Fe^{III} complexes of TCTA complexes are high spin.[8] Therefore by altering the nature of the pendant arms the ligand environment around the metal centre can be controlled. Various pendant arms have since been investigated including hard donors such as alcohols,[11-14] amines,[15] and phosphonates[16] and softer donors such as alkenes[17] and sulfides.[18,19]

Much work has been carried out on the introduction of three pendant arms. However, less investigation on the selective *N*-functionalisation with either one or two arms has been carried out partly due to the associated synthetic difficulties. Protecting groups and high dilution methods are commonly employed in order to selectively functionalise 1,4,7-triazacyclononane.

5.3.1 Use of Protecting groups

Mono-substituted 1,4,7-triazacyclononane derivatives can be prepared from the orthoamine 1,4,7-triazatricyclo[5.2.1.$0^{4,10}$]decane. This method has been used to prepare several mono-substituted derivatives *e.g.* *N*-(2-hydroxybenzyl)-1,4,7-triazacyclononane.[20] Further functionalisation at the remaining two positions can be carried out if desired whilst treatment with either acid or base removes the formyl group (figure 5.4).

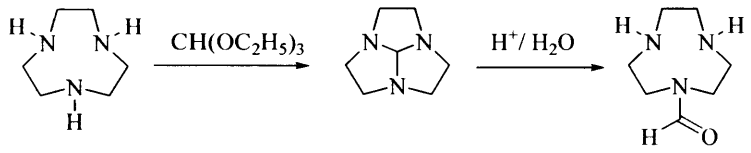


Figure 5.4 : The synthesis of mono-*N*-formyl Tacn *via* 1,4,7-triazatricyclo[5.2.1.$0^{4,10}$]decane.

Mono-protection can also be achieved by the treatment of 1,4,7-triazacyclononane (10 equivalents) with tosyl chloride (1 equivalent) or from 1,4,7-tris-tosyl-1,4,7-triazacyclononane[21] (figure 5.5).

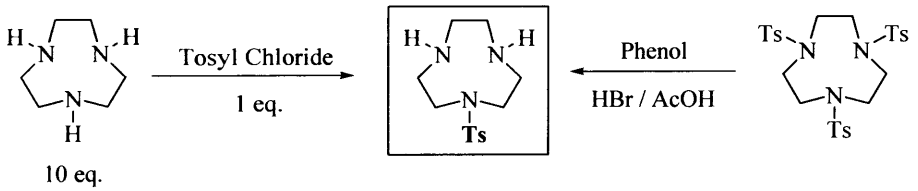


Figure 5.5: Synthesis of *N*-tosyl-1,4,7-triazacyclononne.

The tosyl group can also be used to protect two of the nitrogen positions in 1,4,7-triazacyclononane by either the addition of one equivalent of tosyl chloride to *N*-tosyl-1,4,7-triazacyclononane[21] of by the careful addition of two equivalents of tosyl chloride to the hydrobromide salt of 1,4,7-triazacyclononane[22] (figure 5.6).

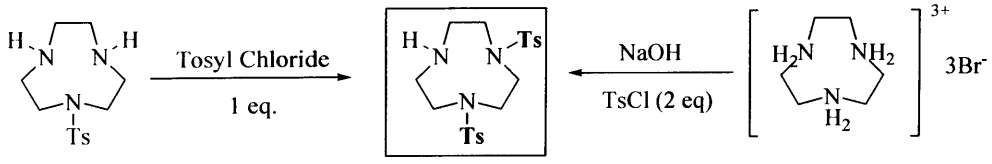


Figure 5.6: Synthesis of bis-protected tacn.

5.3.2 *N*-Nitroarylation of 1,4,7-Triazacyclononane

The straightforward synthesis of the ligand 1,4,7-tris-(2-aminophenyl)-1,4,7-triazacyclononane (L_{33R}) *via* the intermediate 1,4,7-tris-(2-nitrophenyl)-1,4,7-triazazcyclononane (L_{33}) has been previously reported[23] (figure 5.7). This ligand is a rigid pendant-arm macrocycle and forms complexes with the formula $[M^{II}(L_{33R})][ClO_4]_2$ (where M = Fe^{II}, Ni^{II}, Cu^{II}, or Zn^{II}) upon the reaction with divalent first row transition metal perchlorate salts. Crystallographic data shows that the complexes of Fe^{II} and Ni^{II} possess a distorted pseudo-octahedral geometry at the metal centre, coordinating with the three macrocyclic *N*-donors and the three anilino donors.

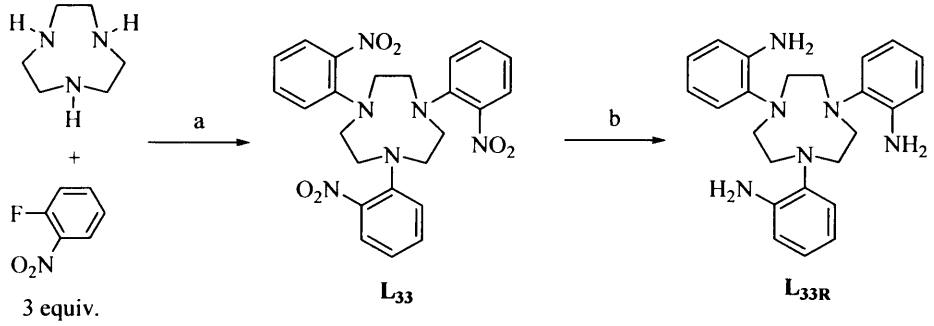


Figure 5.7: Synthesis of 1,4,7-tris-(2-aminophenyl)-1,4,7-triazacyclononane. *Reagents and conditions*: (a) K_2CO_3, MeCN, reflux overnight; (b) 10% Pd/C, H_2, THF-EtOH (20:1), 20 h.

It was shown, by electrochemical analysis that the nickel(II) and copper(II) perchlorate complexes exhibit irreversible oxidation and reduction processes. The iron(II) complex displayed two reversible oxidation process at +0.181 and +0.475 V and a weaker reversible process at +0.695 V (figure 5.8). In addition three irreversible oxidation processes at -1.03, -1.585 and -1.900 V were also observed. The oxidation process at + 0.181 V can be assigned to the metal based Fe^{II}/Fe^{III} process whilst the oxidation process at + 0.475 V can be tentatively assigned to either a ligand centred process or a second metal based process (Fe^{III}/Fe^{IV}).

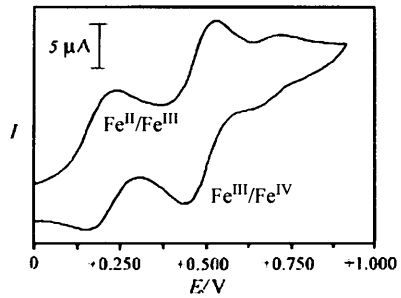


Figure 5.8: Cyclic voltammogram of $[Fe^{II}(L_{33R})][ClO_4]_2$ in 0.1M $[n\text{-}Bu_4N]PF_6$ in acetonitrile *vs.* $Fc\text{-}Fc^+$.[23]

5.3.2.1 Selective *N*-Nitroarylation of 1,4,7-Triazacyclononane

Our group has previously demonstrated that the addition of either one or two mole equivalents of 2-chloronitrobenzene to 1,4,7-triazacyclononane in the presence of potassium carbonate, affords almost exclusively the mono and di-substituted derivatives in 68 and 73 % yields respectively.[24] This straightforward route for the preparation of mono and di-nitroaryl substituted derivatives allows for subsequent introduction of another, different pendant arm to afford the "mixed" ligand 1,4-(2-nitrophenyl)-7-(4-nitrophenyl)-1,4,7-triazacyclononane, L_{mix}[25] as illustrated in figure 5.9.

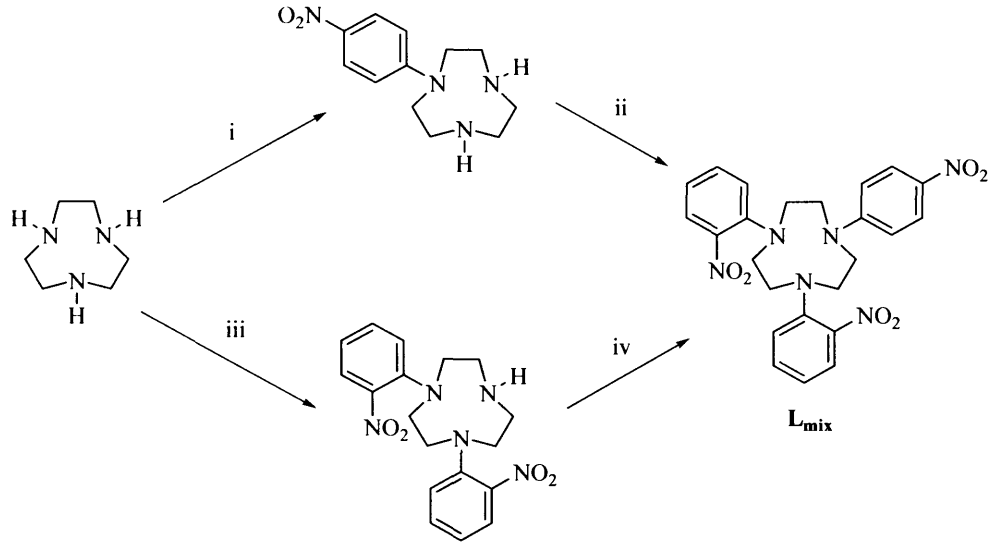


Figure 5.9: Preparation of the "mixed" ligand, L_{mix}. *Reagents and conditions*: (i) 4-chloronitrobenzene (1 eq), K_2CO_3, MeCN, reflux 8 h; (ii) 2-fluoronitrobenzene (2 eq), K_2CO_3, MeCN, reflux 12 h; (iii) 2-chloronitrobenzene (2 eq), K_2CO_3, MeCN, reflux 18 h; (iv) 4-chloronitrobenzene, K_2CO_3, MeCN, reflux 4 h.

5.4 Aims and Objectives

To date, metal complexes of 1,4,7-triazacyclononane have not been investigated as catalysts for the reduction of aqueous silver ions in a Timm's type reaction. The ability to selectively *N*-functionalise 1,4,7-triazacyclononane and depending on the nature of the pendant arms, controlling the ligand environment around the metal centre may afford compounds that exhibit the desired redox activity. Encouraged by the interesting redox properties of the compound $[Fe^{II}(L_{33R})][ClO_4]_2$ we set out to synthesise similar ligands/complexes for our own purposes.

Here we outline the preparation of 1,4,7-triazacyclononane derivatives containing nitrophenyl pendant arms. We have developed a one-pot methodology suitable for the synthesis of 1,4,7-triazacyclononane ligands that contain a mixture of pendant donor

groups. Attempts to attach porphyrins and terpyridines to the azamacrocycle ring are also presented.

Results and Discussion

5.5 Nitro-Benzaldehyde 1,4,7-Triazacyclononane Derivatives

We felt it would be interesting to introduce aniline pendent groups which incorporate a benzaldehyde. This would allow the attachment to other multidentate ligands such as porphyrins and terpyridines, but could also provide a means of conjugation to a biomolecule.

5.5.1 Synthesis of 4-Fluoro-3-nitrobenzaldehyde [5.1]

4-Fluoro-3-nitrobenzaldehyde **[5.1]** was prepared using an adapted procedure for the synthesis of 1,5-dichloro-2,4-dinitrobenzene by the nitration *m*-dichlorobenzene using a mixture of potassium nitrate and sulfuric acid (figure 5.10).[26] To this end, 4-fluorobenzaldeyde was cautiously added to a solution of potassium nitrate (1 mole equivalent) and concentrated sulfuric acid. The reagents were added at room temperature and the temperature gradually increased to 130 °C over a period of 1.5 hours. Pouring the reaction mixture onto crushed ice, results in the precipitation of the crude product. Extraction with dichloromethane and recrystallisation from diethyl ether afforded **[5.1]** as a cream solid (72 %). The spectroscopic data is consistent with that reported elsewhere in the literature.[27] Under these conditions only the mono-nitrated product is formed and any unreacted 4-fluorobenzaldehyde can be removed *via* a Kugelröhr distillation. This procedure has been extended to the synthesis of 4-fluoro-3-nitrobenzoic acid **[5.5]**.

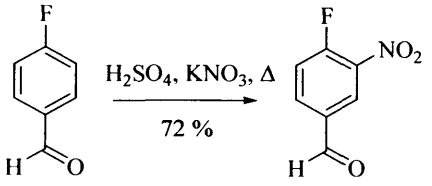


Figure 5.10: Preparation of **[5.1]**.

5.5.2 Preparation of 1,4,7-tris-(2-nitro-4-benzaldehyde) 1,4,7-triazacyclononane [5.2], (L_{33A})

3 Mole equivalents of 4-fluoro-3-nitrobenzaldehyde **[5.1]** and 1,4,7-triazacyclononane were reacted in refluxing acetonitrile in the presence of anhydrous potassium carbonate (3 mole equivalents) to afford a bright yellow solid (figure 5.11). A complicated ^{1}H NMR spectrum was obtained for the product, indicative of a mixture of

substituted derivatives. Repetition of the reaction using DMF as a solvent produced similar results. However, the reaction in acetonitrile employing potassium fluoride as the base afforded only a single product. The generation of the HF_2^- anion during the course of the reaction may thermodynamically drive this reaction to completion. Recrystallisation from hot toluene afforded **[5.2]** in 42 % yield. This compound in its pure form is sparingly soluble in solvents such as chloroform, but is soluble in DMSO. The ^1H NMR spectrum is comparable to that of the ligand $\mathbf{L_{33}}$ with a single resonance at δ 3.70 ppm attributed to the six equivalent methine groups of the macrocycle. The HRMS was consistent with the formation of the tris-substituted product.

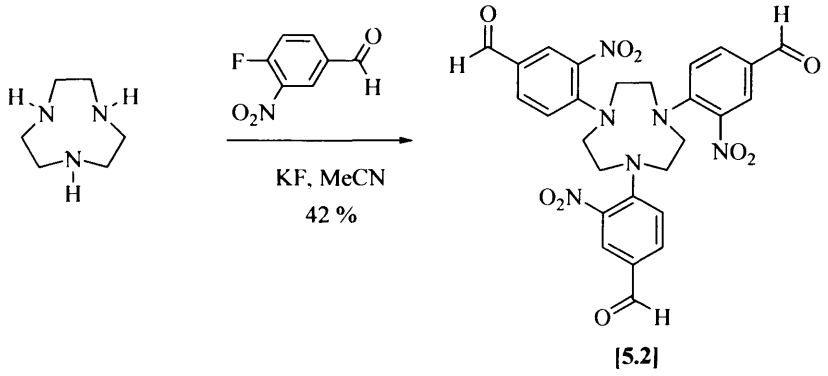


Figure 5.11: Preparation of the ligand L_{33A}, **[5.2]**.

5.6 Selective *N*-Nitroarylation

1,4,7-Triazacyclononane can be selectively *N*-nitroarylated by altering the stoichiometry of the reagents used. The ability to do this allows for "mixed" ligands, involving one or more different pendent groups to be prepared. 1,4-Bis-(2-nitrophenyl)-1,4,7-triazacyclononane, $[\mathbf{L_{32}}]$ was synthesised as previously.[25] Whereby two mole equivalents of 2-fluoronitrobenzene and potassium carbonate were added to a solution of 1,4,7-triazacyclononane in acetonitrile and heated to reflux overnight (figure 5.12). Recrystallisation from hot ethanol afforded **[5.3]** as an orange crystalline material (31 %). Under these conditions, the tris-nitroaryl substituted product is also produced contributing to the low isolated yield of **[5.3]**; the desired substituted derivative can be removed as the hydrochloride salt following an acidic workup.

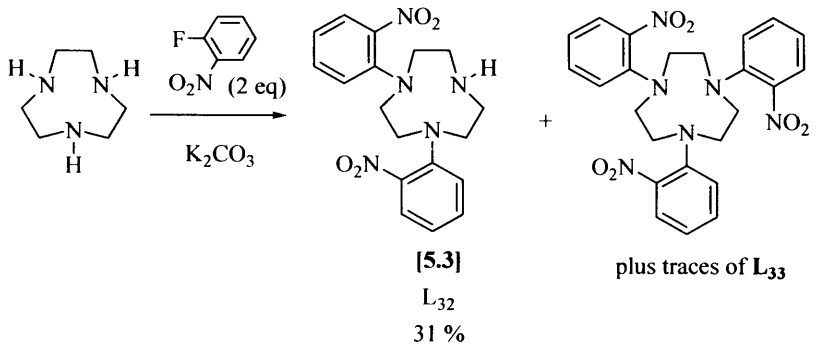


Figure 5.12: Preparation of **[5.3]** (L_{32}).

5.7 *N*-Nitroarylation using 4-Fluoro-3-Nitrobenzaldehyde [5.1]

Reaction of **[5.3]** (L_{32}) with 1 mole equivalent of 4-fluoro-3-nitrobenzaldehyde **[5.1]** and 1.1 mole equivalent of anhydrous potassium carbonate in refluxing acetonitrile overnight yielded the crude product as an oily residue (method **A**, figure 5.13). Recrystallisation from hot acetonitrile afforded the novel mixed ligand **[5.4]** (L_{32A}) in 53 % yield. The introduction of a third, different pendent group was substantiated by the splitting of the macrocyclic methine protons in the ^1H NMR spectrum. The ES mass spectrum showed two molecular ions at 521.2 and 559.1 Da/e corresponding to [M + 1] and [M + K^+] respectively.

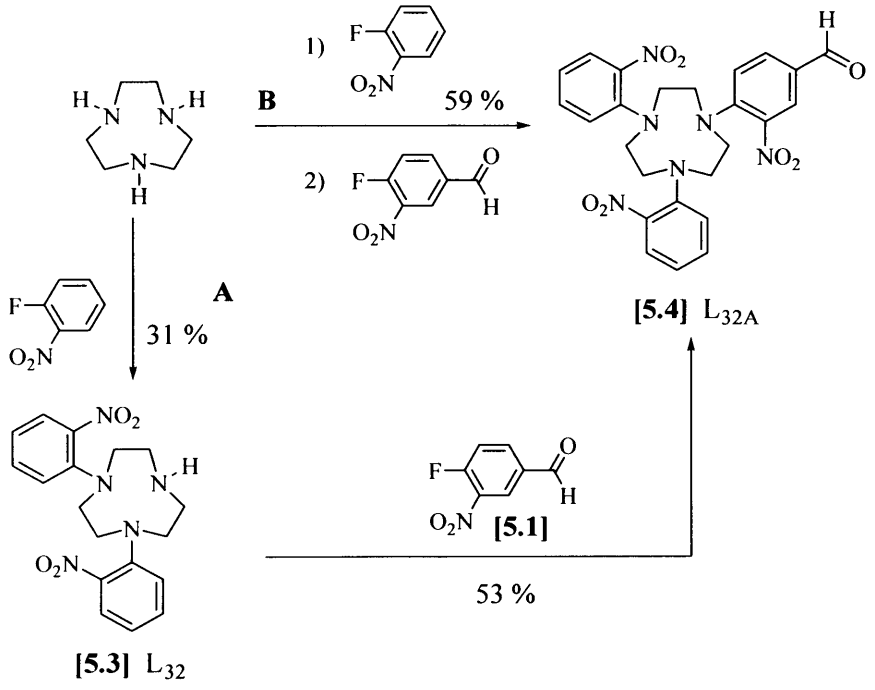


Figure 5.13: Synthesis of **[5.4]** (L_{32A}) by the two-step method A and the one step method B.

5.7.1 One-Pot Synthesis of L_{32A}

The ligand **[5.4]** (L_{32A}) was also prepared in a two-step, one-pot reaction directly from 1,4,7-triazacyclononane (figure 5.13, method **B**). The first step involved the *in situ* preparation of L_{32} by the reaction of 1 mole equivalent of 1,4,7-triazacyclononane with 2 mole equivalents of 2-fluoronitrobenzene and potassium carbonate. During the previous preparation of L_{32}, the tris-substituted nitroaryl product (L_{33}) was also formed. Therefore, in order to limit the production of L_{33} we decided to perform the reaction for 2 hours at room temperature and then increase the temperature to 80 °C for a further 2 hours. Upon cooling 1.1 equivalents of 4-fluoro-3-nitrobenzaldehyde **[5.1]** and a further portion of potassium carbonate (1.1 equivalents) were added and the heating at 80 °C was continued overnight. Removal of the solvent and recrystallisation from hot acetonitrile afforded **[5.4]** as a yellow solid (59 %). This compound has identical spectral properties to that produced by method **A**. Proton NMR spectral analysis of the crude material showed a small resonance at δ 3.45 ppm verified to be L_{33} in the ratio of 20:1 (L_{32A}:L_{33}).

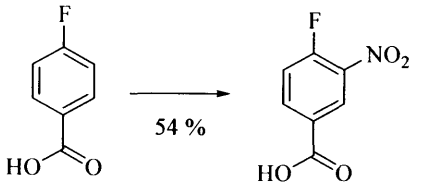
The two-step method **A** affords **[5.4]** (L_{32A}) in an overall yield of 16 % compared to the 59 % obtained by method **B**. The isolated yield of L_{32} is lower than previously reported (31 % *cf.* 73 %).[24] Taking this into account a hypothetical overall yield of 38 % was calculated, which is still considerably lower than the one-pot synthesis. Therefore, this one-pot method presents a novel and efficient method for the preparation of "mixed" 1,4,7-triazacyclononane ligand systems.

5.7.2 Reduction of the Benzaldehyde

In order to render the ligands **[5.2]** (L_{33A}) and **[5.4]** (L_{32A}) suitable for metal complexation with divalent first row transition metal salts, the nitro group must be reduced to the amine. This can be achieved by hydrogenation (10% Pd/C or Pt/C, THF-EtOH) as reported for L_{33R}.[25] Work-up of the reduced ligand must be carried out anaerobically since solutions of L_{33R} were found to darken rapidly upon exposure to air. Since our new ligand possesses an aldehyde function which can be subject to reduction under these types of conditions, investigations were performed to test their stability. To this end, hydrogenation of 4-fluoro-3-nitrobenzaldehyde **[5.1]** was carried out (10% Pt/C, THF-EtOH). Under these conditions the aldehyde appears to be partially reduced. In the IR spectrum of the product, a strong absorption at 3410 cm^{-1} (NH) and a weaker absorption at 1697 cm^{-1} (C=O) are observed. The ^1H NMR spectrum is complicated suggesting a mixture of products.

5.8 Benzoic Acid 1,4,7-Triazacyclononane Derivatives

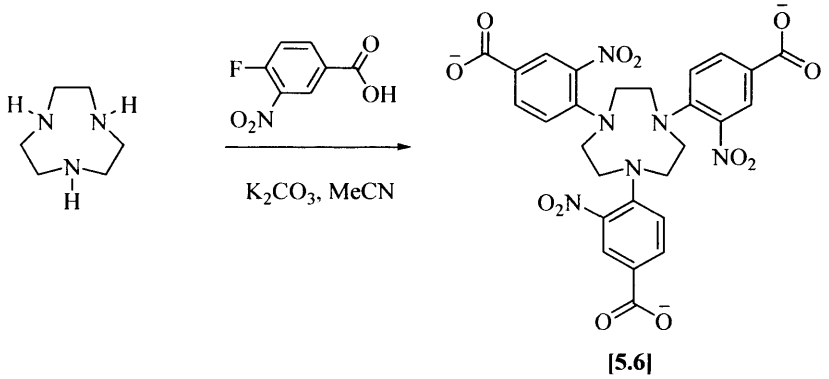
Due to the possible difficulties of reducing the nitro groups by hydrogenation in the presence of a benzaldehyde, attempts were made to prepare 1,4,7-triazacyclononane compounds that incorporate benzoic acid pendent groups. 4-Fluoro-3-nitrobenzoic acid **[5.5]** (figure 5.14) was synthesised in the same manner as 4-fluoro-3-nitrobenzaldehyde **[5.1]** (54 %).



[5.5]

Figure 5.14: *Reagents and conditions*: potassium nitrate, concentrated sulphuric acid, 130 °C

The reaction between 1,4,7-triazacyclononane and 4-fluoro-3-nitrobenzoic acid **[5.5]** (3.3 mole equivalents) with potassium carbonate in acetonitrile (figure 5.15) resulted in the precipitation of a yellow solid which was insoluble in both acetonitrile and dichloromethane. Proton NMR analysis of this material showed it to be a mixture of unreacted 4-fluoro-3-nitrobenzoic acid **[5.5]** and the trisubstituted product **[5.6]** in the ratio of 4:1. Hydrochloric acid was added to protonate the product and thus enable separation from the potassium carbonate. The ^{1}H NMR spectrum of the precipitated hydrochloride salt contained several peaks centred around 3.5 ppm possibly due to the presence of varying degrees of protonation. The IR spectrum supports this, with two strong absorbencies appearing at 1705 cm^{-1} (C=O stretch) and 1612 cm^{-1} (antisymmetric stretch of CO_2^-).



[5.6]

Figure 5.15: Preparation of **[5.6]** from 1,4,7-triazacyclononane and 3.3 equivalents of 4-fluoro-3-nitrobenzoic acid

Using our one-pot method, **[5.7]** was prepared from 1,4,7-triazacyclonone, 2-fluoronitrobenzene and 4-fluoro-3-nitrobenzoic acid **[5.5]** in the presence of potassium carbonate (figure 5.16). Upon cooling the reaction mixture was filtered to remove a large quantity of solid material, removal of the solvent *in vacuo* afforded an orange oil. The ^{1}H NMR spectrum identified this substance to be solely L_{32}. The solid material was washed with water to remove the potassium carbonate. The ^{1}H NMR spectrum of the material was found to consist of the product **[5.7]** and unreacted 4-fluoro-3-nitrobenzoic acid.

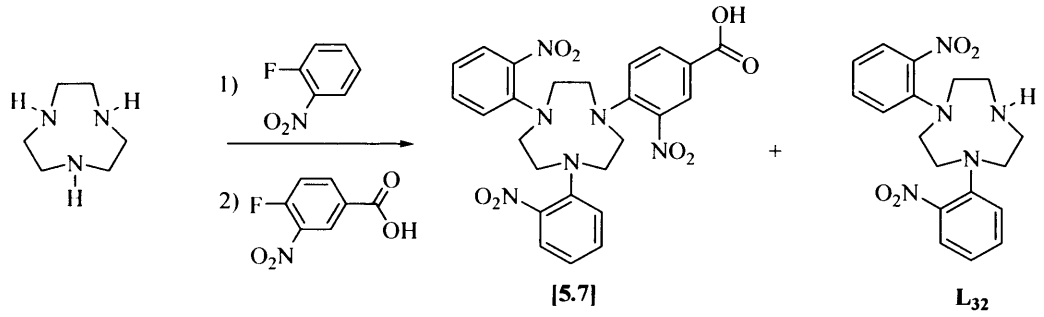


Figure 5.16: Preparation of **[5.7]** using the one-pot method gave a mixture of **[5.7]** and L_{32}. *Reagents and conditions*: (1) 2-fluoronitrobenzene (2 eq), K_2CO_3 (2 eq), MeCN, 2 h (25 °C), 2h (90 °C); (2) 4-fluoro-3-nitrobenzoic acid (1.1 eq), K_2CO_3, 90 °C overnight.

5.9 Benzyl Ester 1,4,7-Triazacyclononane Derivatives

The problems encountered in the reactions involving 4-fluoro-3-nitrobenzoic acid, prompted the protection of the benzoic acid as the benzyl ester. We envisaged that the ester could be removed by hydrogenation to generate the corresponding acid. Benzyl-4-fluoro-3-nitrobenzoate **[5.8]** was prepared as illustrated in figure 5.17.

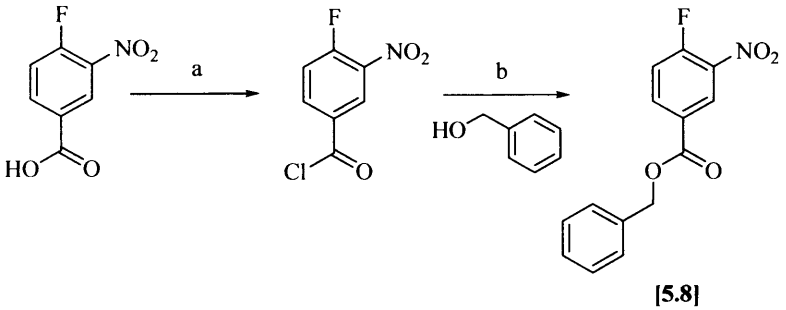
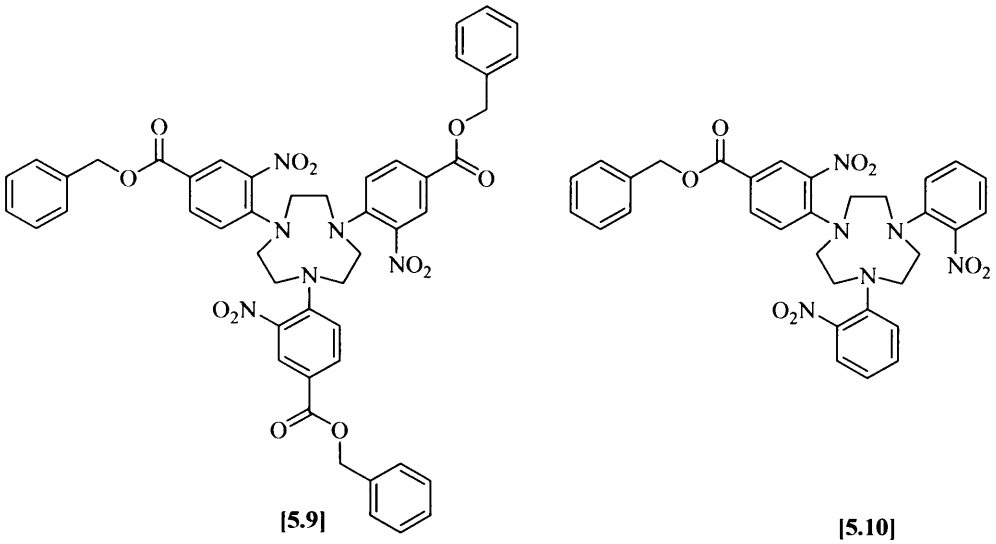


Figure 5.17: *Reagents and conditions*: (a) $SOCl_2$, DMF, reflux 4 h; (b) DMAP (catalytic amount), Et_3N, DCM

The reaction of **[5.8]** with 1,4,7-triazacyclononane in the presence of potassium fluoride and acetonitrile afforded **[5.9]** ($L_{33Benzyl}$) as a dark yellow solid (56 %) (figure 5.18). The mass spectrum (APCI) showed ions corresponding to both the two-on (640.2

Da/e) and three-on (895.3 Da/e) additions. Separation of these two products was achieved chromatographically on silica gel eluting with dichloromethane. The mono-benzyl derivative **[5.10]** (figure 5.18) was also prepared ($L_{32Benzyl}$) using the one pot synthesis. However this reaction does not proceed cleanly and the product is contaminated with both L_{33} and L_{32} amongst other unidentifiable trace impurities.



[5.9] **[5.10]**

Figure 5.18: The ligands **[5.9]** and **[5.10]**.

Attempts were made to reduce the nitro group and cleave the benzyl ester simultaneously using palladium on carbon under an atmosphere of hydrogen (figure 5.19). The reaction mixture was left to stir under a hydrogen atmosphere for 2 days, during which the yellow solution turned colourless indicating the successful reduction of the nitro moieties. However, we have encountered difficulties regarding this hydrogenation step. The product obtained is highly contaminated and further investigation is required to optimise and improve the reaction conditions.

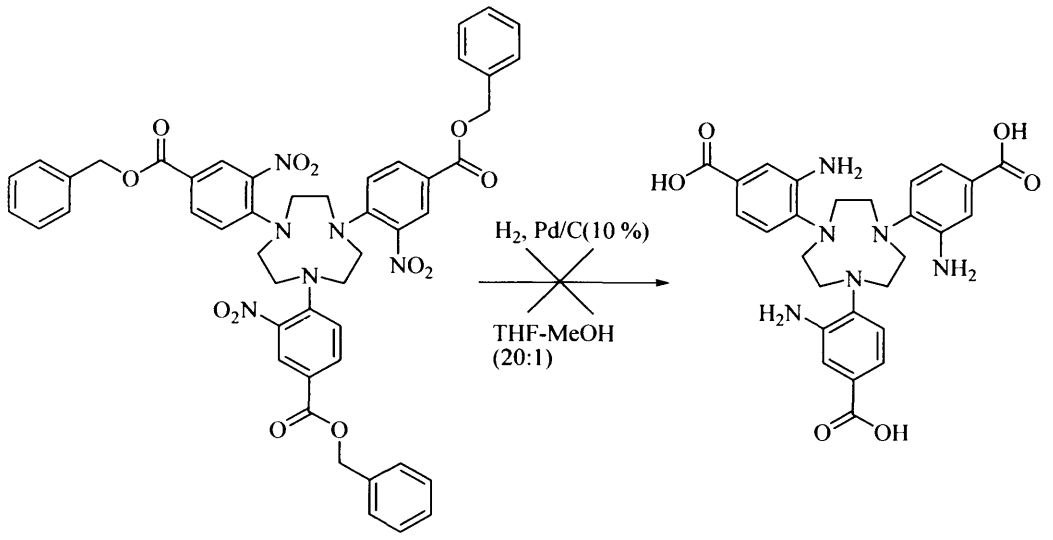


Figure 5.19: Attempts to simultaneously reduce the nitro groups and cleave the benzyl ester groups proved problematic.

5.10 Other Ligands

The use of the one-pot synthesis was further exemplified by the successful preparation of the ligands L_{32T}, L_{32Alc}, L_{32Q} as illustrated in figure 5.20.

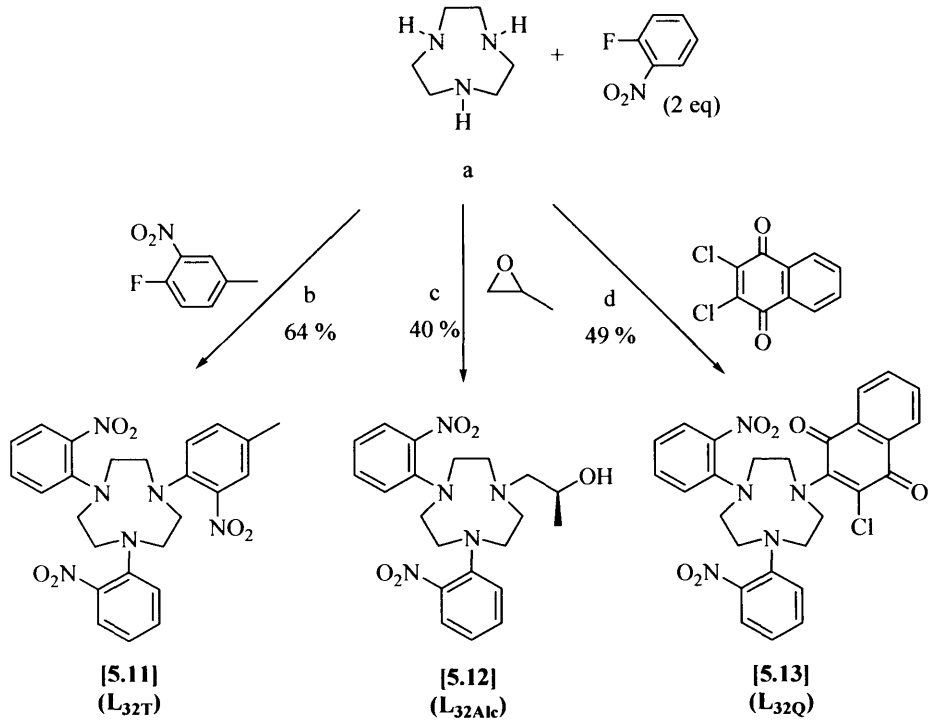


Figure 5.20: One-pot synthesis of L_{32T}, L_{32Alc} and L_{32Q}. *Reagents and conditions*: (i) 2-fluoronitrobenzene (2 eq), K_2CO_3 (2 eq), MeCN, 2h at 25°C, 2h at 90°C; (ii) 4-fluoro-3-nitrotolune (1.1 eq), K_2CO_3 (1.1 eq), 90°C overnight; (iii) racemic propylene oxide (excess), THF/EtOH, 5 days at 25°C; (iv) 2,3-dichloro-1,4-napthoquinone (1.1 eq), K_2CO_3 (1.1 eq), 90°C overnight.

5.10.1 Ligand [5.11] - L_{32T}

This was prepared in the same manner as **[5.4]** (L_{32A}) to afford the crude product as an orange oil (figure 5.20). Recrystallisation from hot ethanol gave **[5.11]** (L_{32T}) as an orange crystalline solid (64 %). Hydrogenation in toluene-MeOH (20:1) in the presence of Pt/C afforded **[5.11R]** (L_{32TR}) as an off-white solid (68 %) (figure 5.21). Solutions of this compound were found to rapidly decompose in the presence of air. An ethanolic solution of nickel (II) perchlorate hexahydrate was added to **[5.11R]** (L_{32TR}) instantly producing a pink solution. Removal of the supernatant and subsequent drying in *vacuo* afforded **$[Ni^{II}(5.11R)][ClO_4]_2$** as an air stable pale pink solid (84 %) (figure 5.21). Unfortunately recrystallisation by vapour diffusion did not produce crystals suitable for X-ray analysis. The obtained complex was washed several times with ethanol and diethyl ether to remove any traces of metal perchlorate. The compound was tested using the procedure described in chapter 2 (section 2.12). It was found that after a period of 3 minutes, solid particles of the complex darken in colour (brown-black) followed by a gradual darkening of the developer solution (10 minutes).

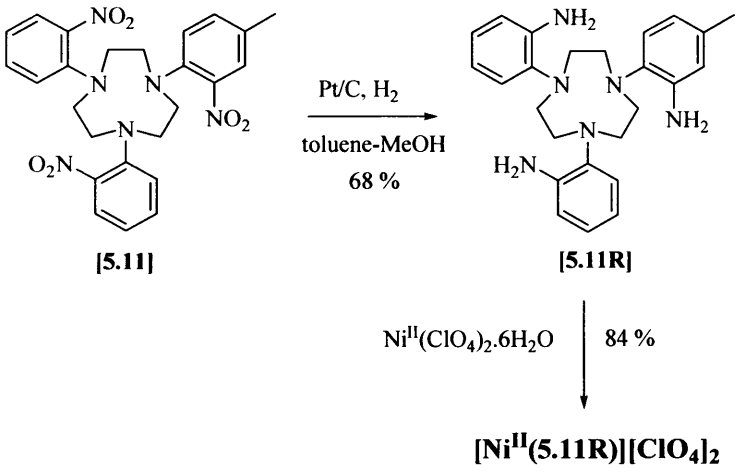


Figure 5.21: Preparation of the complex **$[Ni^{II}(5.11R)][ClO_4]_2$**.

5.10.2 Ligand [5.12] - L_{32Alc}

The hexadentate ligand 1,4,7-tris-[(2S)-2-hydroxypropyl]-1,4,7-triazacyclonone has been previously prepared by the regioselective ring opening of (S)(-) propylene oxide with 1,4,7-triazacylcononane, at the epoxide centre[11,14,28] (figure 5.22). This ligand has the potential to coordinate in less than a hexadentate fashion. But also has the potential to demonstrate protonation-deprotonation equilibria at the alcoholic OH groups.

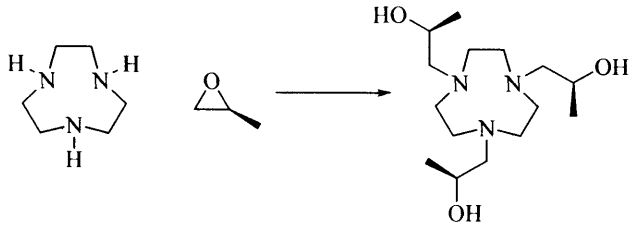
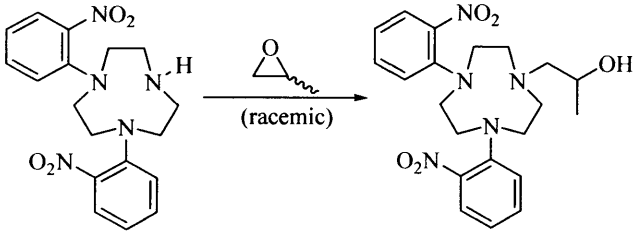


Figure 5.22: Synthesis of 1,4,7-tris-[(2S)-2-hydroxypropyl]-1,4,7-triazacyclononane as reported by Peacock *et al.*

We have prepared a mixed pendant arm ligand which incorporates two nitro/anilino-phenyl groups alongside the harder donor group (2S)-$CH_2CH(Me)OH$. Hence, compound **[5.12]** (L_{32Alc}) was prepared using both the one-pot method (figure 5.20) and also by the addition of propylene oxide to L_{32} (figure 5.23) to afford **[5.12]** in 40 % and 50 % yields respectively. The latter reaction provided a cleaner route of synthesis whilst the one-pot method generated a mixture of products.



[5.12]

Figure 5.23: Preparation of L_{32Alc} from L_{32}. *Reagents and conditions*: racemic propylene oxide (excess), THF-EtOH (1:1), 25°C, 5 days.

The *in situ* preparation of the di-substituted L_{32} may also generate traces of the mono-substituted product (mono-(2-nitrophenyl)-1,4,7-triazacyclononane, L_{31}) which will also react with propylene oxide. Since the racemic mixture was used, there is no control over the stereoselectivity and hence a number of possible products can be formed, contaminating the desired product (figure 5.24).

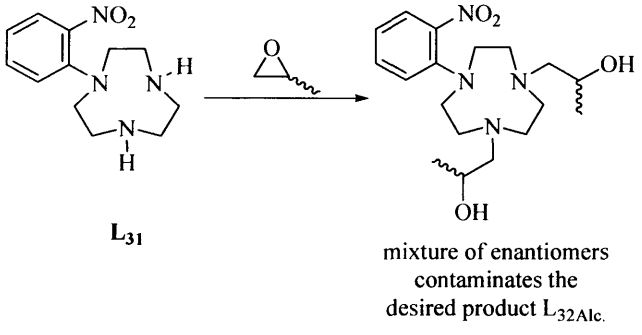


Figure 5.24 : Traces of mono-substituted L_{31} generated during the *in situ* preparation of L_{32} can react with propylene oxide (racemic) to give a mixture of enantiomers.

The nitro groups in L_{32AIc} were reduced using Pd/C as the hydrogenation catalyst in toluene-MeOH (30:1). The hydrogenation step is very much dependent on the purity of the aryl-nitro ligand. An unsuccessful hydrogenation can be attributed to trace impurities present in the sample which may poison the catalyst. The reduced ligand, L_{32AIcR} was found to be very air sensitive, with solutions readily discolouring in the presence of air, attempts to obtain a well resolved proton NMR spectrum failed. Several efforts were made to reduce the nitro groups but none were successful. Consequently attempts to prepare metal complexes (Ni(II) and Fe(II)) were also unsuccessful.

5.10.3 Ligand [5.13] -L_{32Q}

Quinones can act as single-electron carriers to assist oxidation-reduction reactions. Addition of one proton and one electron to a quinone affords a stable radical called a semiquinone, and addition of a second electron plus proton affords a hydroquinone (figure 5.25). The quinone/hydroquinone redox couple can interconvert reversibly in protic media. This redox couple is involved in various biological electron transfer systems e.g. the substituted *p*-benzoquinone, plastoquinone is known to be involved in electron transfer from photosystem II to photosystem I in green plants.[29] Quinones have been shown to be good electron acceptors whilst in contrast hydroquinones are good electron donors. Hydroquinone (the reduced form of quinone) and its derivatives are water soluble and are used as developing agents in black and white photography, lithography and x-ray films. In the presence of the compound Metol (*N*-methyl-*p*-aminophenolsulfate), hydroquinone reduces silver halides to silver metal to give a visible silver image. This process is similar to the Timm's reaction therefore quinone/hydroquinone compounds were worth investigating.

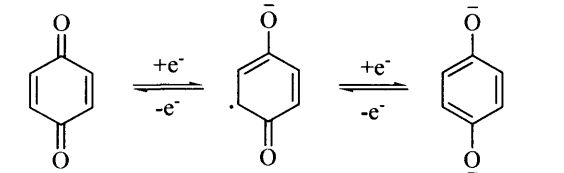
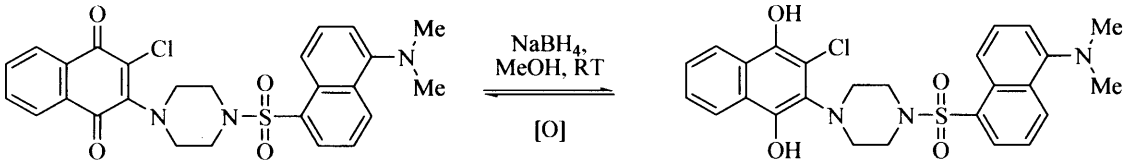


Figure 5.25: The quinone/hydroquinone redox equilibrium between *p*-quinone and *p*-hydroquinone.

The introduction of a napthoquinone moiety to 1,4,7-triazacyclononane was achieved using our one-pot methodology (figure 5.20). To the best of our knowledge, this is the first example of 1,4,7-triazacyclononane directly functionalised with a napthoquinone. Following the *in situ* generation of L_{32}, 1.1 mole equivalents of 2,3-dichloro-1,4-napthoquinone and potassium carbonate were added accompanied by a change of colour to

bright red. Following work-up, the crude product was purified by flash chromatography to remove unreacted 2,3-dichloro-1,4-napthoquinone. Removal of the solvent *in vacuo* afforded **[5.13]** as a red solid (49 %). It was found that solutions of this compound were light sensitive and darkened in colour on prolonged exposure to light. In the IR spectrum two typical strong quinonic absorptions are observed at 1676 and 1640 cm^{-1}. In the mass spectrum the parent ion is observed at 562.1 Da/e.

Previous work[30] has shown that when sodium borohydride is added to a solution of a napthoquinone compound **A** (as illustrated in figure 5.26) a colour change of red to colourless is observed due to the spontaneous reduction of the quinone to hydroquinone. This process is reversible unless kept under nitrogen.

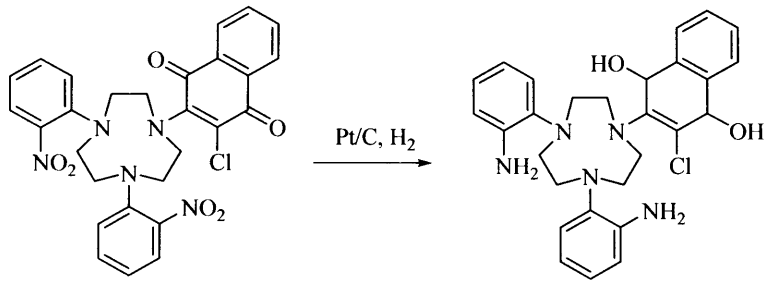


quinone compound, **A** - red

hydroquinone compound, **B** - colourless

Figure 5.26: Reversible oxidation/reduction of quinone **A** to hydroquinone **B** as reported by Bittner *et al.*[30]

A solution of **[5.13]** (L_{32Q}) in THF-MeOH was hydrogenated in the presence of Pt/C (figure 5.27). After 24 hours the solution became colourless, consistent with the reduction of both nitro and quinone functions. On exposure to air, the solution undergoes a colour change of colourless to red upon the oxidation of the hydroquinone to quinone. The solution quickly became dark in colour due to the instability of the aryl amines in air. A small sample of the colourless solution was added to the silver developer/reducer solutions. As anticipated the hydroquinone was rapidly oxidised to the quinone accompanied by the reduction of Ag^{+} to $Ag_{(m)}$. Unfortunately attempts to characterise the reduced ligand and prepared metal complexes have been unsuccessful due to the rapid decomposition.



[5.13] L_{32Q}
(red)

[5.13R]
(colourless)

Figure 5.27: Hydrogenation of **[5.13]** to **[5.13R]**.

5.11 Attachment of a porphyrin and terpyridine to 1,4,7-triazacyclononane

The ligands **[5.2]** and **[5.4]** both contain a benzaldehyde function. This would allow other systems *i.e* porphyrin or terpyridine to be incorporated. This section presents the early attempts to achieve this.

5.11.1 Attachment of Porphyrin

There are few reports of 1,4,7-triazazcyclononane-porphyrin conjugates in the literature. Collman *et al*[31,32] have reported 1,4,7-triazacyclononane-capped porphyrins, designed as an active site analogue of myoglobin. Such systems have been prepared by the addition of 1,4,7-triazacyclononane to a Michael acceptor porphyrin (figure 5.28)

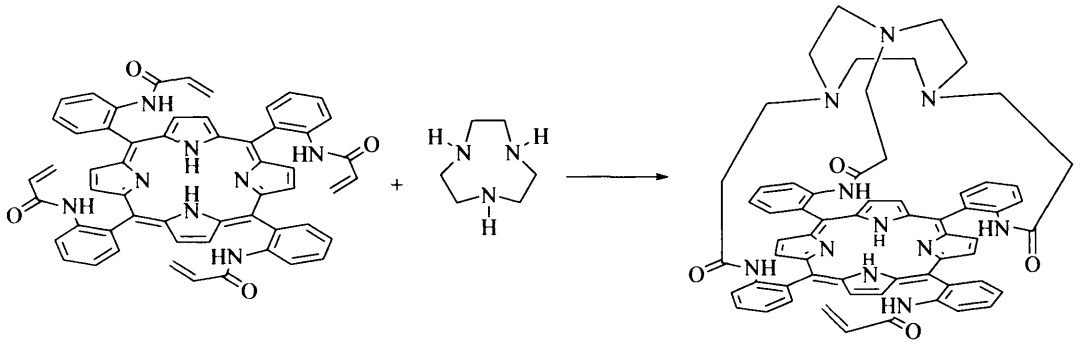
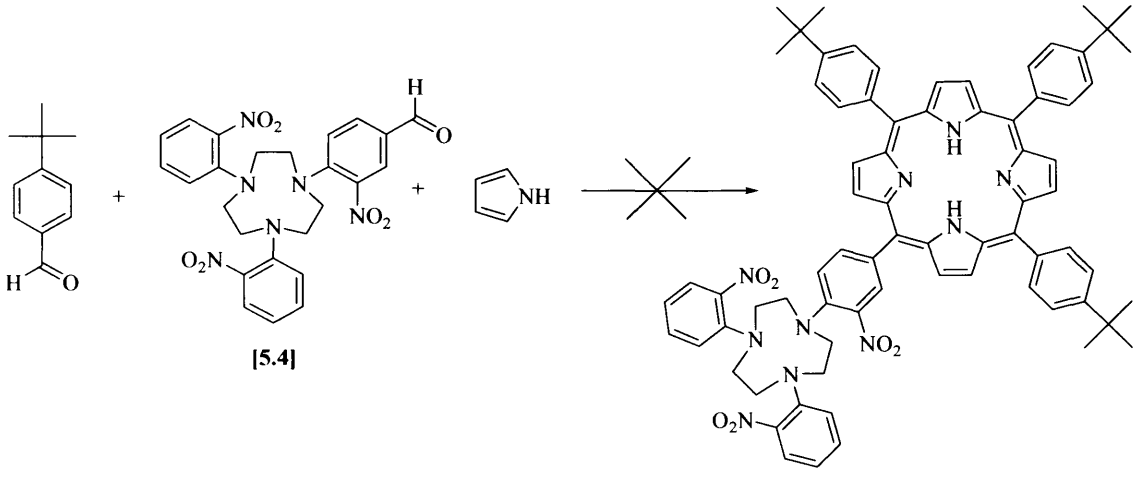


Figure 5.28: Example of a Tacn-capped porphyrin as reported by Collman *et al.*

We have attempted to build a porphyrin framework directly onto 1,4,7-triazacyclonone. To this end, a statistical mixture of **[5.4]** (L_{32A}), 4-*tert*-butylbenzaldehyde and pyrrole were added refluxing propionic acid (figure 5.29). Upon cooling the resultant precipitate was filtered and washed with MeOH.



[5.4]

Figure 5.29: Attempted synthesis of porphyrin-tacn system using Adler methodology. *Reagents and conditions*: propionic acid, 30 min, reflux.

The MALDI mass spectrum of the crude product mixture did not contain the expected molecular ion at 1197 Da/e, only a strong peak corresponding to 5,10,15,20-tetra(4-*t*-butylphenyl) porphyrin was observed. Attempts to react **[5.2]** with 4-*t*-butylbenzaldehyde and pyrrole in a similar reaction also appeared to be unsuccessful. The Adler methodology is not suitable for the preparation of all substituted porphyrins therefore an alterative methodology may be more successful e.g. Lindsey or MacDonald 2+2 condensation (described in chapter 3, section 3.5).

5.11.2 Attachment of a Terpyridine.

There has been a report of 1,4,7-triazacyclononane containing a phenyl-terpyridyl pendant arm,[33] whereby the terpyridylbenzyl pendant arm is introduced into the macrocycle by reaction with the tricyclic orthoamide 1,4,7-triazatricyclo[5.2.1.$0^{4,10}$]decane (figure 5.30). This ligand contains both a meridionally coordinating sub-unit (terpy) and a facially coordinating triazamacrocycle (tacn).

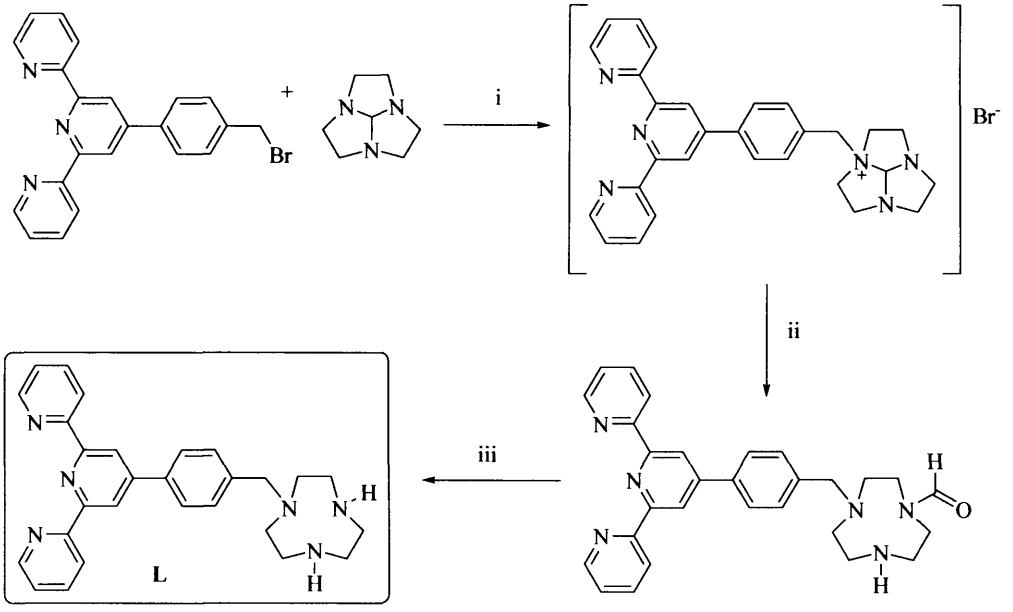


Figure 5.30 : Synthesis of a terpyridyl pendant-arm macrocycle, 4'-(*p*-1,4,7-triazacyclonon-1-ylmethylphenyl)-2,2':6',2"-terpyridine (**L**) as reported by Moore *et al.*[33] *Reagents and conditions*: (i) THF, r.t. 24 h; (ii) water, 100 °C, 3.5 h; (iii) KOH, EtOH:water (3:1), 100 °C, 48 h.

We set about to attach three terpyridyl sub-unit to our tris-nitrobenzaldehyde-1,4,7-triazacyclononane derivative **[5.2]**. Initial attempts to assemble the terpyridyl units using the Raston methodology were unsuccessful. Alternatively, a one pot synthetic method in which the benzaldehyde is reacted with 2 mole equivalents of 2-acetylpyridine and

potassium *t*-butoxide in THF followed by cyclisation with ammonium acetate was employed (figure 5.31).

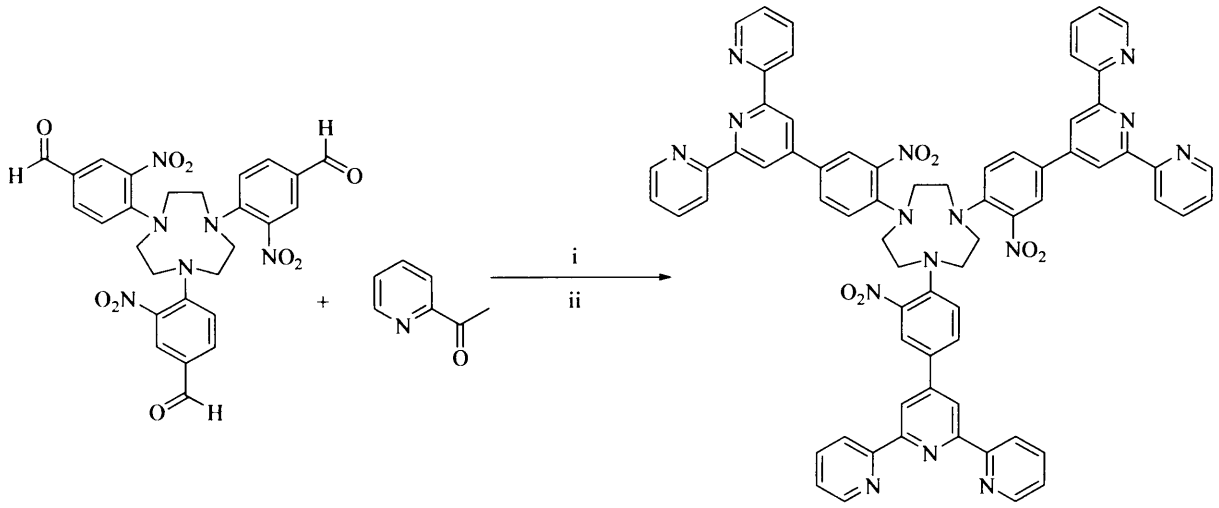


Figure 5.31: *Reagents and conditions*: (i) N_2, potassium *t*-butoxide, THF; (ii) anhydrous ammonium acetate, glacial acetic acid-ethanol (1:1).

Using this method, a yellow-brown solid was obtained; initial attempts to recrystallise this product have not been successful and hence the ^1H NMR spectrum was not well resolved. Upon the addition of an ethanolic solution of ammonium ferrous sulfate an instant colour change of purple was observed. Octahedral iron(II) complexes of terpyridine with a metal:ligand ratio of 1:2 are purple in colour. The observed colour change may be attributed to the presence of a terpyridine sub-unit. The low resolution MS of the product does not show the expected molecular ion at 1187 Da/e. The spectrum contains a lot of background noise, but a substantial peak is observed at 780.5 Da/e, a possible assignment is illustrated in figure 5.312.

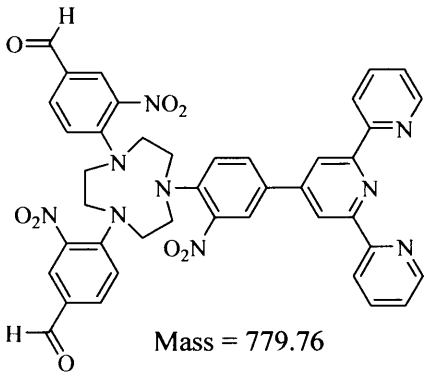


Figure 5.32: Possible structural for the peak observed at 780.5 Da/e.

5.12 Conclusion

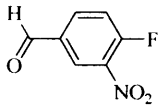
We have developed a novel one-pot synthesis for the preparation of "mixed" nitro-aryl containing 1,4,7-triazacyclononane ligands in reasonable yields. This synthesis has been extended to the preparation of 1,4,7-triazacyclononane derivatives containing alcohol and naphthoquinone pendant groups. The coordination chemistry and subsequent electrochemistry of the prepared ligands has not been fully explored owing to difficulties encountered during the hydrogenation steps. The complex **Ni[5.11R][ClO$_4$]$_2$** catalyses the reduction of silver ions in a Timm's type reaction in 3 minutes. This encouraging result shows the potential of hexadentate metal complexes of functionalised 1,4,7-triazacyclononane ligands as catalysts of silver ions to silver metal in Timm's type reaction. The ability to selectively *N*-functionalise 1,4,7-triazacyclononane would allow the introduction of groups suitable for bioconjugation.

Experimental

General Procedure

Non-synthesised reagents were purchased from Aldrich, Avocado or Lancaster and were used as received. Where appropriate solvents were dried and degassed by reflux over standard drying agents[34] under a nitrogen atmosphere. The NMR spectra were recorded on a Brüker Avance 400 instrument at 400 MHz (^{1}H) and 100 MHz (^{13}C), Jeol Lamda Eclipse at 282.78 MHz (^{19}F); ^{1}H and ^{13}C chemical shifts are quoted in ppm relative to residual solvent peaks and. Coupling constants are quoted in Hertz. Mass spectra were obtained in either APCI (atmospheric pressure chemical ionisation), EI (electronic ionisation). IR spectra were obtained as KBr or NaCl discs using a Jasco FTIR 110 series spectrometer.

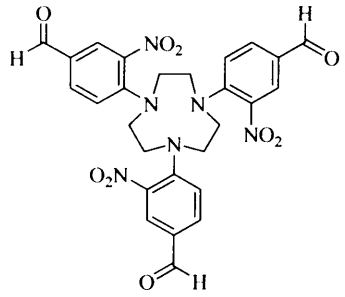
[5.1] 4-Fluoro-3-nitrobenzaldehyde



Sulfuric acid (75 mL) and potassium nitrate (19.69g, 0.194 mol) were added to an unsealed pressure tube and stirred well until dissolved. 4-Fluorobenzaldehyde (24g, 0.193 mol) was slowly added with stirring and the temperature was gradually raised to 130 °C over a period of 45 minutes, and stirred for an additional 1 h. The yellow mixture was allowed to cool slightly and poured over crushed ice and was vigorously stirred for 1 h. This aqueous mixture was extracted with dichloromethane, dried over $MgSO_4$ and the solvent remove *in vacuo* to give the crude product as an orange oil. Further purification on a Kugelröhr

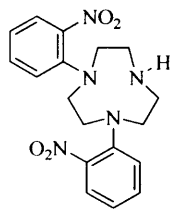
apparatus and recrystallisation from diethyl ether yielded the product as a yellow solid (23.54g, 72 %); δ_H (400 MHz, $CDCl_3$) 9.98 (1H, s, C*H*O), 8.55 (1H, m, Ar *H*), 8.15 (1H, m, Ar *H*), 7.45 (1H, m, Ar *H*), IR (KBr, cm^{-1}) 3360, 2867, 1716, 1698, 1614, 1531, 1493, 1422, 1355, 1257, 1203, 1135, 1078, 974, 927, 903 and 844; *m/z* (APCI) 170.0 [M + 1].

[5.2] L_{33A}



To a solution of 1,4,7-triazacyclononane (0.147 g, 1.13 mmol) in dry acetonitrile (10 mL) in a pressure tube, were added 4-fluoro-3-nitrobenzaldehyde **[5.1]** (0.638 g, 3.77 mmol) and anhydrous potassium fluoride (0.219 g, 3.3 equiv). The reaction mixture was heated to 80 ^oC overnight. Upon cooling the reaction mixture was diluted with dichloromethane (20 mL), filtered and the solvent removed *in vacuo* to give the crude product as a yellow solid. Recrystallisation from hot toluene gave the title compound as a yellow solid (0.275g, 42 %); δ_H (400 MHz, *d*-DMSO) 9.85 (3H, s), 8.25 (3H, d, *J* 1.98), 7.95 (3H, dd, *J* 8.87, 1.91), 7.40 (3H, d, *J* 8.92), 3.70 (12 H, s); δ_C (100 MHz, *d*-DMSO) 188.6, 145.9, 137.3, 131.3, 128.4, 125.4, 118.3, 51.3; IR (KBr disc, cm^{-1}) 1689.6 (strong, C=O stretch), 1602.8, 1524.9 (strong, N=O antisymmetric stretch), 1423.6, 1359.4, 1196.3, 1166.3, 1097.9, 990.2 and 813.8; *m/z* (ES) 575.4242 [M + 1]; HRMS (ES Q-TOF) *m/z* calc for $C_{27}H_{24}N_6O_9Na$ 599.1502, found 599.1497.

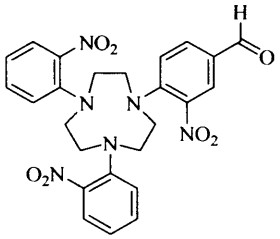
[5.3] L_{32}



To a solution of 1,4,7-triazacyclononane (0.5 g, 3.86 mmol) in dry acetonitrile (20 mL) in a pressure tube, were added 2-fluoronitrobenzene (1.09 g, 7.7 mmol) and finely ground potassium carbonate (1.06g, 7.66 mmol). The reaction mixture was heated to 90°C and stirred overnight. Upon cooling, the mixture was diluted with DCM (20 mL), filtered and the solvents were removed in *vacuo*. The residue was dissolved in $CHCl_3$ (50 mL) and

extracted with HCl (4 M, 2 x 50 mL). The aqueous layer was basified with aqueous NaOH to pH 12 and then extracted with $CHCl_3$ (100 mL). The organic phase was dried over $MgSO_4$ and the solvents removed in *vacuo*. Recrystallisation from hot ethanol afforded the title compound as an orange crystalline material (0.444 g, 31 %) δ_H (400 MHz, $CDCl_3$) 7.54 (dd, 2H, *J* 8.06, 1.44, Ar *H*), 7.32 (t of d, 2H, *J* 7.43, 1.56, Ar *H*)), 7.00 (d, 2H, *J* 8.47, Ar *H*), 6.80 (t, 2H, *J* 8.13, Ar *H*), 3.60 (s, 4H, CH_2 macrocycle), 3.35 (m, 4H, CH_2 macrocycle), 2.88 (m, 4H, CH_2 macrocycle); δ_C (100 MHz, $CDCl_3$) 143.9, 141.8, 132.9, 126.0, 120.1, 119.1, 56.0, 54.1, 48.9; *m/z* (ES) 372.5 [M + 1].

[5.4] L_{32A}

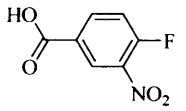


Method 1: To a solution of 1,4,7-triazacyclononane (0.2 g, 15.4 mmol) in dry acetonitrile (10 mL) in a pressure tube, was added 2-fluoronitrobenzene (0.437g, 30.9 mmol) and finely ground potassium carbonate (0.6017 g, 2.2 equiv). The reaction mixture was stirred at room temperature for 2 h, the temperature was raised to 80 ^{o} C and stirred for a further 2 h. Upon cooling 4-fluoro-3-nitrobenzaldehyde (0.288g, 17.0 mmol) was added and was stirred at 80 ^{o}C overnight. The reaction mixture was diluted with dichloromethane (30 mL), filtered and the solvent removed *in vacuo* to give the crude product as an orange oily solid. Recrystallisation from hot acetonitrile gave the title compound as a bright yellow solid (0.47 g, 59 %). Yield: δ_H (400 MHz, $CDCl_3$) 9.75 (s, 1H, CHO), 8.08 (d, 1H, *J* 2.04, Ar *H*), 7.80 (dd, 1H, *J* 8.95, 2.0, Ar *H'*), 7.55 (dd, 2H, *J* 8.05, 1.55, Ar *H*), 7.40 (t, 2H, *J* 8.6, Ar *H*), 7.15 (d, 2H, *J* 8.4, Ar *H*), 6.98 (m, 3H, Ar *H* and Ar *H'*), 3.65 (d, 4H, *J* 4.54, CH_2 macrocycle), 3.60 (d, 4H, *J* 4.39, CH_2 macrocycle), 3.30 (s, 4H, CH_2 macrocycle); δ_C (100 MHz, $CDCl_3$) 188.7, 147.7, 145.1, 144.3, 139.0, 133.7, 133.3, 130.5, 126.5, 126.1, 123.4, 122.6, 118.0, 56.4, 54.6, 54.1; IR (KBr disc, cm^{-1}) 1683.9 (strong, C=O stretch), 1601.6 (strong), 1511.8 (strong, N=O stretch), 1391.0, 1346.0, 1299.4, 1263.6, 1201.2, 1174.2 and 993.4; *m/z* (ES) 521.2 [M + 1], 559.1 [M + K]

Method 2: To a solution of 1,4-di(2-nitrophenyl)-1,4,7-triazacyclononane, L^{32} (0.444 g, 1.19 mmol) in dry acetonitrile (20 mL) in a pressure tube, was added 4-fluoro-3-nitrobenzaldehyde (0.2 g, 1.19 mmol) and finely ground potassium carbonate (0.18 g, 1.30 mmol) and was heated to 90 ^{o}C overnight. Upon cooling the reaction mixture diluted with

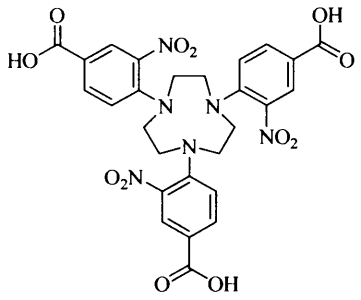
dichloromethane, filtered and dried *in vacuo* to give the crude product as an orange oily solid. Recrystallisation from hot acetonitrile gave the title compound as a bright yellow solid (0.319 g, 52 %) with identical spectral properties as above.

[5.5] 4-fluoro-3-nitrobenzoic acid



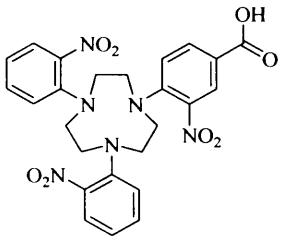
This was prepared in an analogous method to that of **[5.1]**, the applied reagents were 4-fluorobenzoic acid (15g, 107.1 mmol) and potassium nitrate (10.8g, 106.82 mmol). Recrystallisation from hot toluene afforded **[5.5]** as a cream needles (10.62g, 54 %); δ_H (400 MHz, $CDCl_3$) 12.60 (br s, 1H, O*H*), 8.90 (m, 1H, Ar *H*), 8.50 (m, 1H, Ar *H*), 7.45 (m, 1H, Ar *H*); δ_C (100 MHz, $CDCl_3$) 169.1, 159.7, 157.6, 137.5, 137.1 (J_{CF} 8 Hz), 128.6, 126.3, 119.1 (J_{CF} 17 Hz); IR (KBr, cm^{-1}) 3078 (OH stretch), 2830, 2674, 2559, 1710 (C=O stretch), 1618, 1539 (N=O antisymmetric vibration), 1427, 1351 (N=O symmetric stretch), 1286, 1267, 1159, 1124, 1077, 918, 849, 820, 770; *m/z* (APCI) 186.2 [M + 1].

[5.6] L_{33BA}



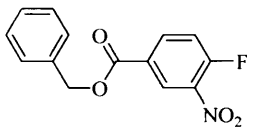
To a solution of 1,4,7-triazacyclononane (0.147 g, 1.13 mmol) in dry acetonitrile (10 mL) in a pressure tube, was added 4-fluoro-3-nitrobenzoic acid (0.638 g, 3.77 mmol) and dry potassium carbonate (0.219 g, 3.3 equiv). The reaction mixture was heated to 80 °C overnight. Upon cooling the reaction mixture was diluted with dichloromethane (20 mL) and filtered to remove the precipitated yellow solid. The solid was dissolved in water to remove the potassium carbonate and hydrochloric acid was added to precipitate the product as the hydrochloric salt. Attempts to isolate the desired product from unreacted starting materials were unsuccessful. δ_H (400 MHz, D_2O) 7.98 (d, 3H, *J* 2.15), 7.65 (dd, 3H, *J* 8.88, 2.15), 6.95 (d, 3H, *J* 8.91), 3.30 (s, 12H, C*H*$_2$) IR (KBr, cm^{-1}) 3427, 2963, 1705 (s, C=O stretch), 1612 (antisymmetric CO_2^- stretch), 1530 (N=O antisymmetric stretch), 1457, 1414, 1354 (N=O symmetric stretch), 1302, 1259, 1215 , 1171, 1152, 1125, 1073, 989, 917, 846; *m/z* (APCI) 625.6 [M + 1].

[5.7] L_{32BA}



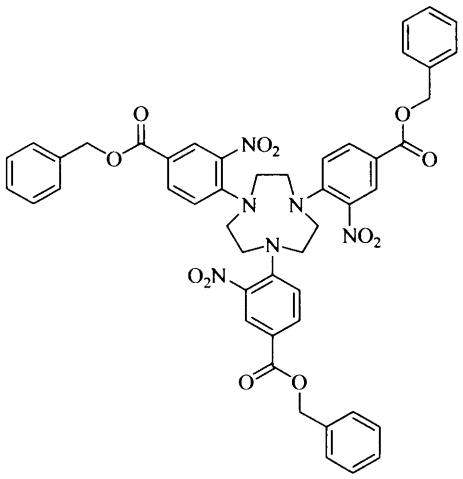
This was prepared in an analogous method to that of **[5.4] method 1**. the applied reagents were 1,4,7-triazacyclononane (0.634g, 4.90 mmol), 2-fluoronitrobenzene (1.38g, 9.78 mmol), potassium carbonate (1.35g, 9.76 mmol) and 4-fluoro-3-nitrobenzoic acid (1g, 5.40 mmol) and potassium carbonate (0.96 g, excess). Upon cooling the reaction mixture was filtered and the solvents removed *in vacuo* to afford an orange oil verified to be L_{32} by proton NMR spectroscopy. The filtered solid was washed with water to afford the crude product. Recrystallisation from hot ethanol yielded **[5.7]** as a yellow solid (1.02g, 39 %); δ_H (400 MHz, *d*-DMSO) 8.0 (d, 1H, *J* 1.86), 7.95 (dd, 1H, *J* 8.67, 1.89), 7.62 (dd, 2H, *J* 8.06, 1.47), 7.45 (m, 2H), 7.25 (d, 2H, *J* 8.64), 7.1 (d, 1H, *J* 8.64), 6.95 (t, 2H, *J* 7.4), 3.45 (m, 4H, CH_2), 3.35 (m, 4H, CH_2), 3.15 (s, 4H, CH_2); *m/z* (APCI) 537.6 [M + 1].

[5.8] Benzyl 4-fluoro-3-nitrobenzoate



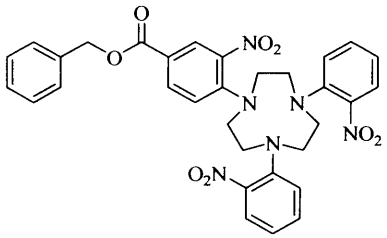
4-Fluoro-3-nitrobenzoic acid (5.00 g, 27 mmol), DMF (3 drops) and thionyl chloride (20 mL, excess) were refluxed for 4 h under a nitrogen atmosphere. The solvents were removed *in vacuo* to give the corresponding acid chloride (4.2 g). 4-Fluoro-3-nitro acid chloride (4.2 g) was dissolved in dry DCM (20 mL) and was cautiously added dropwise to a solution of benzyl alcohol (2.4 g, 22 mmol), triethylamine (2.29 g, 1.1 molar equiv), DMAP (~ 100 mg) in DCM. The combined solution was stirred for 2 h. The DCM solution was washed with dilute HCl, $NaHCO_{3(aq)}$ and water, dried over $MgSO_4$. The solvents were removed *in vacuo* to give the title compound as an oil which solidified upon cooling (4.76g, 64 %); δ_H (400 MHz, $CDCl_3$) 8.75 (dd, 1H, *J* 7.14, 2.06, Ar-*H*), 8.30 (m, 1H, Ar-*H*), 7.2-7.4 (m, 6H, Ar-*H*), 5.30 (s, 2H, CH_2); IR (KBr, cm^{-1}) 3034, 2929, 2851, 1731 (strong, C=O stretch), 1619, 1538 (N=O antisymmetric stretch), 1497, 1443, 1410, 1352 (N=O symmetric stretch), 1316, 1281, 1227, 1151, 1126, 1077, 993, 917, 843, 817; *m/z* (APCI) 276 [M + 1].

[5.9] $L_{33Benzyl}$



To a solution of 1,4,7-triazacyclononane (0.2g, 1.54 mmol) in dry acetonitrile (20 mL) in a pressure tube, was added benzyl 4-fluoro-3-nitrobenzoate (1.40g, 5.08 mmol) and dry potassium fluoride (0.269 g, 5.1 mmol). The reaction mixture was heated to 80 °C overnight. Upon cooling the reaction mixture was diluted with dichloromethane (20 mL), filtered and the solvents were removed *in vacuo*. The crude product was purified by flash chromatography on silica gel eluting with dichloromethane and subsequent recrystallisation from acetonitrile afforded **[5.9]** as a dark yellow solid (0.77g, 56 %); δ_H (400 MHz, $CDCl_3$) 8.25 (d, 3H, *J* 1.87, Ar-*H*), 7.95 (dd, 3H, *J* 8.81, 1.80, Ar-*H*), 7.3-7.4 (m, 15H, Ar-*H*), 6.95 (d, 2H, *J* 8.85), 5.30 (s, 6H, CH_2), 3.5 (s, 12H, CH_2 macrocycle); δ_C (100 MHz, $CDCl_3$) 164.7, 146.9, 140.7, 135.9, 134.6, 129.0-128.7, 121.8, 119.2, 67.4, 54.0; IR (KBr, cm^{-1}) 3465, 2961, 1717 (s), 1609 (s), 1526 (s), 1455 (w), 1361 (m), 1279, 1242 (s), 1171, 1119 (m), 753 (m); *m/z* (APCI) 895.3 [M + 1].

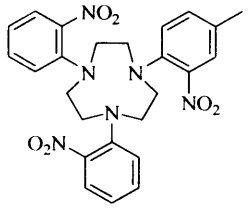
[5.10] $L_{32Benzyl}$



This was prepared in the same manner as **[5.4]** using **method 1**. The applied reagents were 1,4,7-triazacyclononane (0.469g, 3.36 mmol), 2-fluoronitrobenzene (1.02g, 7.26 mmol), potassium carbonate (1.10g, 7.9 mmol) and benzyl 4-fluoro-3-nitrobenzoate (1.01g, 3.7 mmol) and potassium carbonate (0.51g, 3.7 mmol). Recrystallisation attempts from various hot solvents were unable to separate the desired product from impurities. δ_H (400 MHz, $CDCl_3$) 8.30 (d, 1H, *J* 2.14), 7.95 (dd, 1H, *J* 8.93 ,2.19), 7.55 (dd, 2H, *J* 8.08, 1.68), 7.25-

7.40 (m, 7H), 7.10 (d, 2H, *J* 8.38), 6.95 (m, 2H), 5.30 (s, 2H, C*H*$_2$), 3.6 (d, 4H, *J* 5.33), 3.55 (d, 4H, *J* 4.37), 3.30 (s, 4H); *m/z* (APCI) 628.

[5.11] L_{32T}

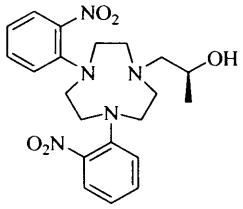


This was prepared in an analogous manner to that of **[5.4]** (**method 1**). The applied reagents were 1,4,7-triazacyclononane (0.833g, 6.45 mmol), 2-fluoronitrobenzene (1.81g, 12.8 mmol) potassium carbonate (1.95g, 14.1 mmol) and 4-fluoronitrotoluene (1g, 6.4 mmol) and potassium carbonate (0.88g, 6.4 mmol). Recrystallisation from hot ethanol afforded **[5.11]** as an orange crystalline material (2.09g, 64 %); δ_H (400 MHz, $CDCl_3$) 7.53 (dd, 2H, *J* 8.04, 1.34, Ar *H*), 7.32 (m, 3H, Ar *H* and Ar *H'*), 7.14 (dd, 1H, *J* 8.44, 1.75, Ar *H'*), 7.05 (m, 3H, , Ar *H* and Ar *H'*), 6.85 (m, 2H, Ar *H*), 3.52 (s, 4H, C*H*$_2$ macrocycle), 3.41 (d, 4H, *J* 3.34, C*H*$_2$ macrocycle), 3.37 (d, 4H, *J* 3.30, C*H*$_2$ macrocycle), 2.20 (s, 3H, C*H*$_3$), δ_C (100 MHz, $CDCl_3$) 144.7, 144.5, 143.8, 143.0, 142.3, 134.0, 133.2, 132.1, 126.0, 123.6, 121.3, 120.2, 55.3, 54.9, 54.5, 20.5; IR (KBr disc, cm^{-1}) 2890 (CH stretch), 1603, 1561, 1519 (strong, N=O antisymmetric, stretch), 1437, 1340 (strong, symmetric N=O stretch), 1294, 1256, 1204, 1169, 1071, 1049, 989, 907, 801; *m/z* (ES) 507.2 [M + 1]

Ni[5.11] $[Ni^{II}(L_{32TR})][ClO_4]_2$

To a solution of **[5.11]** (200 mg, 0.394 mmol) in toluene-methanol (20:1, 20 mL) was added platinum on carbon (10 %, 50 mg). Hydrogen was bubbled through the reaction mixture for 20 hours. Under an inert atmosphere, the reaction mixture was filtered through glass fibre filter paper and the solvent removed *in vacuo* to give the reduced ligand **[5.11R]** as an off-white solid (110 mg, 68 %). An excess of $Ni(ClO_4)_2.6H_2O$ was added to a suspension of **[5.11R]** (110 mg) in degassed ethanol and the reaction mixture was left to stir overnight. Removal of the supernatant by cannula and subsequent drying *in vacuo* afforded the desired complex as a pale pink crystalline powder (270 mg, 84 %); IR (KBr, cm^{-1}) 3414 (strong), 2360, 2341, 1653, 1616, 1497, 1457, 1364, 1279, 1261, 1143, 1109, 1089 (strong), 809.

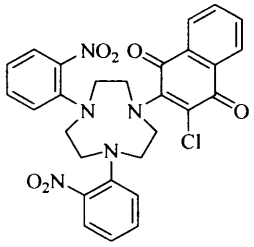
[5.12] L_{32Alc}



Method 1: To a solution of 1,4,7-triazacyclononane (0.2 g, 15.4 mmol) in dry acetonitrile (10 mL) in a pressure tube, was added 2-fluoronitrobenzene (0.437 g, 30.9 mmol) and finely ground potassium carbonate (0.6017 g, 2.2 equiv). The reaction mixture was stirred at room temperature for 2 h, the temperature was raised to 80 ° C and stirred for a further 2 h. Upon cooling, racemic propylene oxide (excess) in a mixture of THF (1 mL) and EtOH (1 mL) was added and the pressure tube was tightly sealed. The reaction mixture was stirred for 5 days at ambient temperature. The reaction mixture was filtered and the solvents removed *in vacuo* to give the product as an oily orange residue. The minimum amount of hot toluene was added and hexane was added until precipitation occurred to yield the title compound as a bright orange solid (0.265g, 40 %); δ_H (400 MHz, $CDCl_3$) 7.55 (dd, 2H, *J* 8.06, 1.47, Ar *H*), 7.35 (m, 2H, Ar *H*), 7.0 (d, 2H, *J* 8.24, Ar *H*), 6.80 (t, 2H, *J* 7.93, Ar *H*), 3.55 (s, 4H, C*H*$_2$ macrocycle), 3.50 (m, 1H, C*H*), 3.45 (m, 4H, C*H*$_2$ macrocycle), 2.75 (m, 4H, C*H*$_2$ macrocycle), 2.50 (dd, 1H, *J* 12.68, 2.37, C*H* of CH_2), 2.2 (m, 1H, C*H* of CH_2), 0.8 (d, 3H, C*H*$_3$); δ_C (100 MHz, $CDCl_3$) 144.1, 141.7, 133.1, 126.3, 119.3, 119.1, 68.0, 64.5, 56.1, 55.2, 55.0, 19.93; *m/z* (APCI) 430.2 [calc 429.4]; IR (KBr, cm^{-1}) 3568 (sharp, OH stretch), 2964, 1602, 1563, 1516 (strong, antisymmetric N=O stretch), 1444, 1398, 1354 (strong, symmetric N=O stretch) (medium, C-N stretch, 1301, 1261, 1227, 1167, 1131, 1093, 1046, 998, 870, 771 and 741.

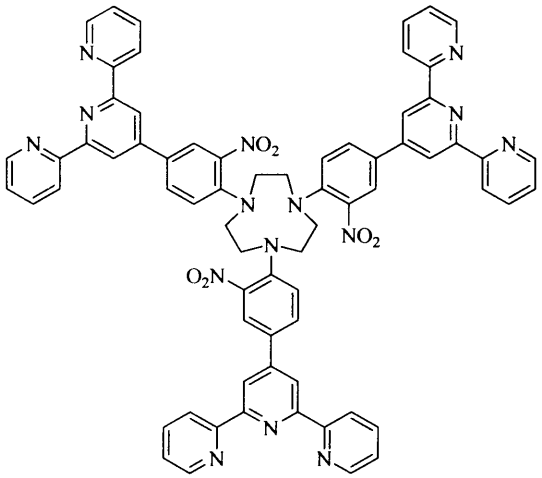
Method 2: 1,4-Di(2-nitrophenyl)-1,4,7-triazacyclononane, L^{32} (200 mg), propylene oxide (~ 400 mg, excess), ethanol (5 mL) and THF (5 mL) were placed in a tightly sealed pressure tube and stirred for 5 days at ambient temperature. The solvents were removed *in vacuo* to give the product as an oily orange residue. The minimum amount of hot toluene was added and hexane was added until precipitation occurred to yield the title compound as a bright orange solid (0.115g, 50 %) with identical spectral properties as above

[5.13] L_{32Q}



This was prepared in the same manner as **[5.4]** (**method 1**). The reagents applied were 1,4,7-triazacyclononane (1g, 7.73 mmol), 2-fluoronitrobenzene (2.18 g, 15.4 mmol), potassium carbonate (2.12g, 15.4 mmol) and 2,3-dichloro-1,4-dinapthoquinone (1.74g, 7.66 mmol) and potassium carbonate (1.05g, 7.66 mmol). The crude product was purified by flash chromatography on silica eluting with chloroform. Removal of the solvent in *vacuo* afforded **[5.13]** as a red solid (2.12g, 49 %); δ_H (400 MHz, $CDCl_3$) 8.05 (d, 1H, *J* 7.41, Ar $H_{quinone}$), 8.00 (d, 1H, *J* 7.24, Ar $H_{quinone}$), 7.62 (m, 2H, Ar $H_{quinone}$), 7.48 (dd, 2H, *J* 8.04, 1.48, Ar *H*), 7.35, (m, 2H, Ar *H*), 7.08 (d, 2H, *J* 8.19, Ar *H*), 6.90 (t, 2H, *J* 7.62, Ar *H*), 3.98 (m, 4H, CH_2 macrocycle), 3.60 (m, 4H, CH_2 macrocycle), 3.42 (s, 4H, CH_2 macrocycle); IR (KBr, cm^{-1}) 3467, 2962, 1676 (m), 1640, 1609 (strong), 1560, 1545 (N=O antisymmetric stretch, strong), 1515, 1487, 1456, 1435, 1354, 1342 (medium, N=O symmetric stretch) 1303, 1283, 1251, 1205, 1173, 1122, 1078, 1043, 993, 840, 803, 771 and 742; *m/z* (ES Q-TOF) 562.1 [M+1], 584.1 [M+Na] and 600.1 [M+K]; HRMS (ES Q-TOF) *m/z* calc for $C_{28}H_{24}N_5O_6Cl$ 561.973, found 562.1493.

Attempted synthesis of terpyridine-1,4,7-triazacyclononane system



2-Acetylpyridine (0.151g, 1.248 mmol) was added to a solution of potassium *t*-butoxide (0.307g, 2.74 mmol) in freshly distilled THF (20 mL) and was stirred for 10 min. The resultant yellow suspension was treated with a solution of **[5.2]** (0.12g, 0.208 mmol) in THF (5 mL) and the mixture was stirred overnight at room temperature under an

atmosphere of N_2. After this period, a solution of dry ammonium acetate (excess) in ethanol:glacial acetic acid (1:1) was added and was heated to reflux overnight. The reaction mixture was cooled and was treated with ice and water. The resultant yellow-brown solid was collected by filtration. IR (KBr, cm^{-1}) 1602, 1564, 1516, 1437, 1388, 1354, 1295, 1261, 1221,1172, 1090, 1048, 997, 830, 744; *m/z* (ESI) 780.5.

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Concluding Remarks

Conclusions

The objective of this research was to design and prepare compounds that possess properties that render them suitable immunohistochemical marker/marker substrates. The marker substrate compound must incorporate a phosphomonoester, a substrate of alkaline phosphatase. It must be water soluble to allow administration to the tissue sample. Following enzymatic cleavage of the phosphomonoester the marker must become insoluble in histological preparations e.g. water at pH 6-9.5, ethanol and xylene. The marker must be designed such that it is able to form interactions and thus adhere to the target site. Most importantly the marker must support redox catalysis to enable amplification with a physical developer. Since the silver ions present in the physical developer are slowly catalysed by light (approximately 55 minutes), marker compounds must reduce the silver ions in times ideally less than 20 minutes to obtain clear results. A successful marker/marker substrate system must fulfil all of the above criteria.

Previous research in this area identified the potential of platinum complexes of bidentate phosphines and platinum and palladium complexes of both *meso*-tetraarylporphyrins and 4'-aryl-2,2':6',2"-terpyridines as suitable markers. The Pt and Pd complexes of functionalised tetraphenyl porphyrin were all shown to rapidly reduce silver ions in a Timm's type reaction. Our findings initially appeared to be in agreement with this. However, it was found that following extensive purification the metal complexes no longer displayed any redox activity. This suggests that the previously tested samples contained trace impurities and it was these that catalysed the reduction of silver. The metal salts ($PtCl_2$, $PdCl_2$) rapidly reduce the silver ions in the physical developer forming an immediate black colouration.

4'-(4-Hydroxyphenyl)-2,2':6',2"-terpyridine, 4'-(4-diethylphosphatophenyl)-2,2':6',2"-terpyridine and 4'-(4-phosphatophenyl)- 2,2':6',2"-terpyridine and the platinum iodide and palladium chloride square planar complexes were previously shown to catalyse the reduction of silver ions in 2-3 minutes. Whereas, the platinum chloride complexes reduce the silver ions more slowly (20 minutes). The free ligands displayed surprising catalytic activity. The proposed explanation for this was that the ligand may form a silver complex with the silver ions present in the physical developer. This silver complex may then catalyse the reduction of silver. The platinum complexes **[Pt(4.5)Cl]$^+$** and **[Pt(4.12)Cl]$^+$** catalysed the reduction of silver in 20 and 24 minutes respectively which is in agreement with previous findings. None of the free terpyridine ligands prepared displayed any redox activity. This suggests that this earlier proposal was incorrect and the reduction of silver was caused by an unknown substance. This highlights the need for the marker

compounds to be rigorously purified to ensure it is indeed the marker and not some other entity catalysing the reduction of silver ions.

During the course of this research many problems associated with solubility were encountered. We therefore turned our attention to preparing water soluble marker compounds bearing a reactive group to allow conjugation directly to a secondary antibody. Unfortunately the work undertook was unsuccessful to this end, never-the-less this appears to be the most logical direction for future research in this project area.

A major downfall of this project is the reliability of the physical developer solution. The performance of the solutions varies depending on the supplier of the Tris base (a component of the silver stock solution). We were informed that optimal performances of the developer solution are obtained when using Tris base purchased in 1991! This will have an effect on obtaining reproducible results. From our own experience we have found that minor changes in pH and temperature of the physical developer solution alters the rate of silver reduction. The age of the solutions also has a similar effect. Ideally the developer solution should be freshly prepared before use although this is time consuming and the stock solutions are best prepared on a larger scale.

To conclude, since very little is known about the silver reduction mechanism it has proved difficult to design markers that catalytically reduce silver ions present in the developer solution. We have shown that altering the coordinated ligands (e.g in the compounds $[Pt(dppe)Cl_2]$, $[Pt(dppe)I_2]$, $[Pt(dppe)S_4]$ etc) alters the redox activity towards the reduction of silver in a Timm's type reaction. Although at present we do not understand why. We have also shown that the markers need to be of high purity as any impurity may be responsible for the reduction of silver.