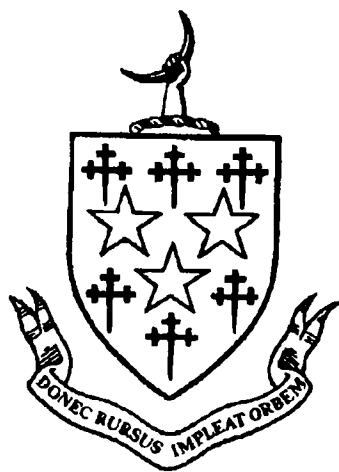


An Investigation of Anion Binding by Acyclic Metal-Centred Receptors

Andrew R. Graydon, MA

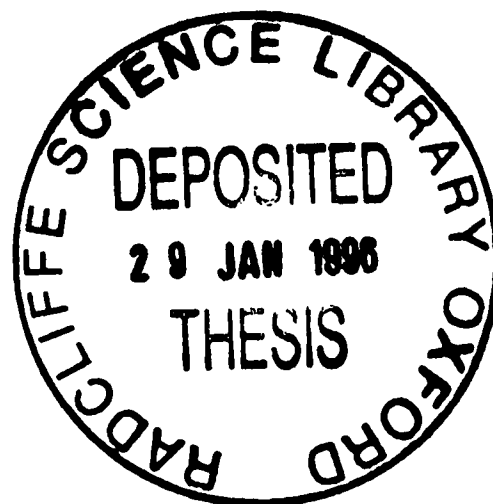
*Somerville College
Oxford*



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ABSTRACT

This thesis reflects two main aims. Firstly, the synthesis and characterisation of a number of potential anion receptors was undertaken and their anion binding properties were assessed. In so doing, a second aim was fulfilled, namely a comparison of the various methods of detecting the bound anion, and quantifying the binding strength. Four techniques appear in this thesis; ^1H nuclear magnetic resonance, UV-visible spectroscopy, electrochemistry and luminescent emission. Quantitative titrations were performed and, where possible, stability constants estimated.

Chapter One provides an introduction to some of the themes of molecular recognition and provides a brief overview of the literature associated with anion recognition. A Prologue describes the design of the receptors studied; they all incorporate a metal centre and appended amide groups which provide sources of hydrogen bonding. The molecules are mostly cationic and a combination of positive charge and hydrogen bonding constitutes the binding interaction.

Chapter Two is concerned with receptors based on cobalticinium, $[\text{Cp}_2\text{Co}]^+$. A number of receptors are presented and are found to bind anions with stability constants typically in the range of $500\text{--}1000\text{ dm}^3\text{mol}^{-1}$. Receptors involving more than one cobalticinium centre are found to bind much more strongly and, furthermore, variations in functional groups appended close to the proposed coordination site impart selectivity; dihydrogen phosphate is bound more strongly than chloride. It is also found that different techniques give different stability constants and comment is made on this phenomenon.

Chapter Three examines the role of positive charge in anion binding and describes the synthesis and coordination properties of several neutral receptors. These molecules retain hydrogen bonding sites, and it is found that this is sufficient to bind anions, but the strength of the interaction is greatly reduced.

Chapter Four introduces another system, based on $\text{RuL}(\text{bpy})_2^{2+}$, where L is a 4,4'-amide disubstituted bpy. The strength of binding is an order of magnitude greater than the cobalticinium systems as detected by several methods including emission studies, which are very sensitive. Comparison with a neutral, rhenium-based receptor is made. A dihydrogen phosphate-selective luminescent sensor is also presented.

The Epilogue identifies areas for future research. Specialised introductions and summaries are found at the beginning and end of each chapter.

I declare that the work presented in this thesis is entirely my own, except where I have acknowledged either help from a named person or a reference is given to a published source or a thesis. Text taken from another source is enclosed in quotation marks and a reference is given.

Dated this fifteenth day of September in the year nineteen hundred and ninety five.

signature removed

Andrew R. Graydon

Acknowledgements

The problem with the acknowledgements is keeping the thing shorter than the thesis. One doesn't wish to offend by omission, but the other extreme is to mention everyone from the local landlord to the Pope. I shall be ruthless and only mention those who have contributed to the completion of this learned discourse. Well, fairly ruthless...

Dr Paul Beer heads the list, of course. An endless stream of ideas that actually work, unflagging optimism, interest in his group and, latterly, erudite criticism of my writing have all helped to make this happen. Thank you, it's been very good.

Next comes Dave Smith. Someone I hold in great (and genuine) esteem, despite an unlikely fetish for foodstuffs. For three years now, he has helped me, cheered me up, and contributed his very considerable intellect to our discussions. All the best, Dave — I promise I'll come to the Nobel Lecture.

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If you looked for your name and didn't find it — I'm sorry. You weren't forgotten; I could type on and on. As it is, its dawn, and time to stop.

Oh, yes — and thank you to His Holiness.

Oxford
September 1995

*This thesis is dedicated to my mother, father, grandma and Jane,
and in memory of those grandparents who are not here to read it.*

*Dixeris egregie notum si callida verbum
Reddiderit iunctura novum.*

Horace, Ars Poetica

You will have written exceptionally well if, by a careful arrangement of your words, you have made an ordinary one seem original.

Abbreviations

Ar	aryl
bpy	2,2'-bipyridine
Bu	butyl
Cp	cyclopentadienyl
CV	cyclic voltammogram
dm	decimetre
DCCI	dicyclohexylcarbodiimide
DMF	N,N-dimethyl formamide
DMSO	dimethylsulphoxide
E	potential
EI-MS	electron impact mass spectrometry
Et	ethyl
FAB-MS	fast atom bombardment mass spectrometry
h	hour
IR	infra-red
m	multiplet (nmr)
μ A	microampere
Me	methyl
min	minute
ml	millilitre
mol	mole
nmr	nuclear magnetic resonance
ppm	parts per million
py	pyridine
s	second
s	singlet (nmr)
SWV	square wave voltammogram
t	triplet (nmr)
TBA	tetrabutyl ammonium
TLC	thin layer chromatography
UV-vis	ultra-violet-visible
V	volts

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CHAPTER One

The Ties that Bind:

Origins of Molecular Recognition

1.0 Introduction

The origins of some areas of chemistry are more remarkable than others. For our purposes, a significant milestone was reached in 1704 by a dye maker called Diesbach when he heated a mixture of equal parts cream of tartar, saltpetre and oxblood (the latter may be substituted with animal flesh). Aqueous work up with green vitriol, alum and hydrochloric acid gave a blue pigment which was christened *Prussian Blue*; it is notable as the first isolated coordination compound. Many years later, this substance was characterised as $\text{Fe}_4[\text{Fe}(\text{CN})_6]_3$; in the meantime many thousands of coordination compounds had been prepared. They display myriad fascinating properties and numerous fruitful applications have been developed. It is from such origins that the work contained in this treatise emerges — one of countless theses submitted within the wide scope of coordination chemistry.

Purists will decry a scientific concept which lacks a precise definition, but there appears to be none which can describe a coordination compound (or “complex”) adequately. All complexes have some features in common and in particular are aggregates of two or more ions or molecules which are familiar as distinct species in their own right. The difficulty lies, however, in determining what manner of force is responsible for holding these species together, for the bonds are diverse in strength and nature. In general terms, a complex is held together by forces which are weaker than a covalent bond but stronger than the van der

Waals' forces which can account for non-specific aggregation of molecules (a complex has a defined structure). But the range of bond strengths is vast. In some cases, the bonds are almost trivial in strength and the complex is highly labile; in contrast, complexes such as copper phthalocyanine are capable of sublimation at 800 °C.¹

A further common link is the fact that the bond between two species within a complex is always dative, that is the electrons are supplied by one of the species alone. Coordination chemistry is therefore a world populated by Lewis acids and Lewis bases. The Lewis acid is frequently a transition metal cation and the Lewis base is termed a *ligand*. The field was rationalised in the late 19th and early 20th centuries by the work of Mads Sophus Jørgensen and by Alfred Werner who laid the foundations of *ligand field theory*, which later elucidated the molecular orbital basis of interaction and gave coherence to the range of spectroscopic and magnetic properties that result from the perturbation of transition metal d-orbitals.

A concurrent strand of thought also emerged in the late 19th century from the work of Emil Fischer. He studied biological systems and observed the manner in which an enzyme was able to identify and act upon a particular substrate, the target molecule which was destined to undergo enzyme mediated transformation. It became apparent that there was a remarkable selectivity of enzyme for substrate and Fischer dubbed this a “lock and key” relationship.² It is the notion that a system can be created with a specific substrate in mind and will bind that chosen guest in preference to other, competing species; the two components sharing the same relationship as the key which opens its specific lock. They are said to display *complementarity*, which is achieved by addressing two principal criteria.

1. Steric complementarity: The binding site will be of an appropriate size to accommodate the target substrate. It will also reflect consideration of the guest's shape.
2. Binding site complementarity: It is known that some donor atoms display an affinity for a certain class of guest, e.g. sulphur donor atoms bind particularly strongly with mercury. To achieve an optimum interaction, the type and distribution of donor groups within the binding site will match the requirements of the substrate.

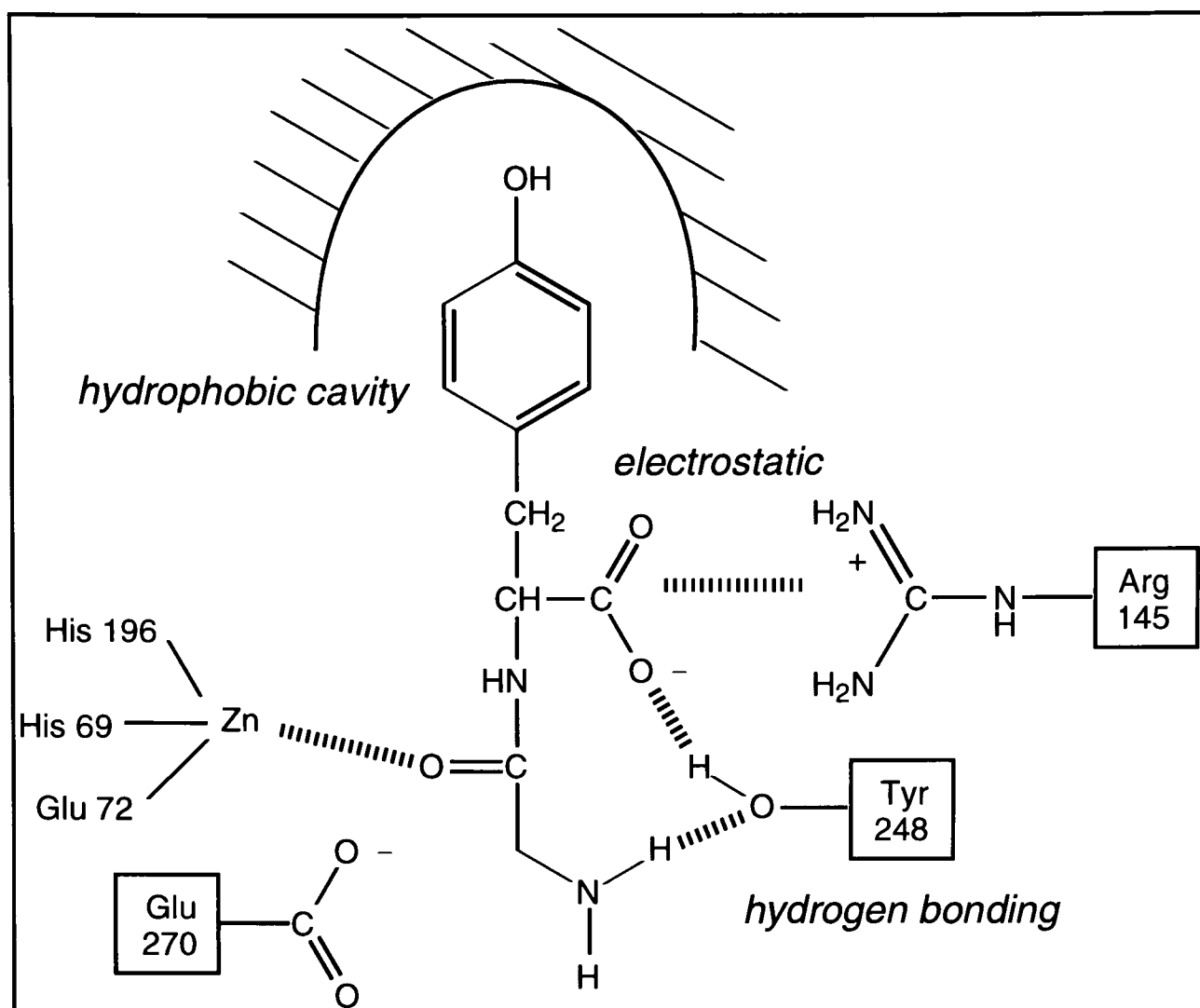


Figure 1.1: Schematic diagram of the active site of carboxypeptidase A showing the inclusion of glycyltyrosine (after L. Stryer, *Biochemistry* 3e, W. H. Freeman, New York, 1988).

Enzyme active sites frequently display elegant examples of such considerations; carboxypeptidase A is a case in point, an enzyme which hydrolyses the carboxy-terminal peptide bond of a polypeptide chain. The active site is shown schematically in figure 1.1 and it is notable that zinc is bound by O and N donors which are appropriate for the binding of such a metal ion. A hydrophobic cavity provides an attractive site for a bulky aliphatic or aromatic side chain and a combination of electrostatic and hydrogen bonding interactions completes the binding assembly. These forces will be discussed in more detail presently.

The concepts of coordination and selectivity were elegantly brought together in 1967 with Pedersen's discovery of the so-called crown ethers.^{3,4} These cyclic polyethers were found to be remarkable systems in two respects: firstly, they are capable of binding with alkali metal cations — ions not known for their propensity towards ligand environments — and secondly, they display selectivity for the bound ion based on an optimum correspondence between the metal ionic radius and the diameter of the cyclic cavity. The stability constants in water for the complex between 18-crown-6, [1] ("18" refers to the number of atoms in the ring and "6"

specifies the number of donor atoms present, figure 1.2a), and Group 1 metal cations⁵ are shown in figure 1.2c, along with the crystal structure of its potassium complex (figure 1.2b).

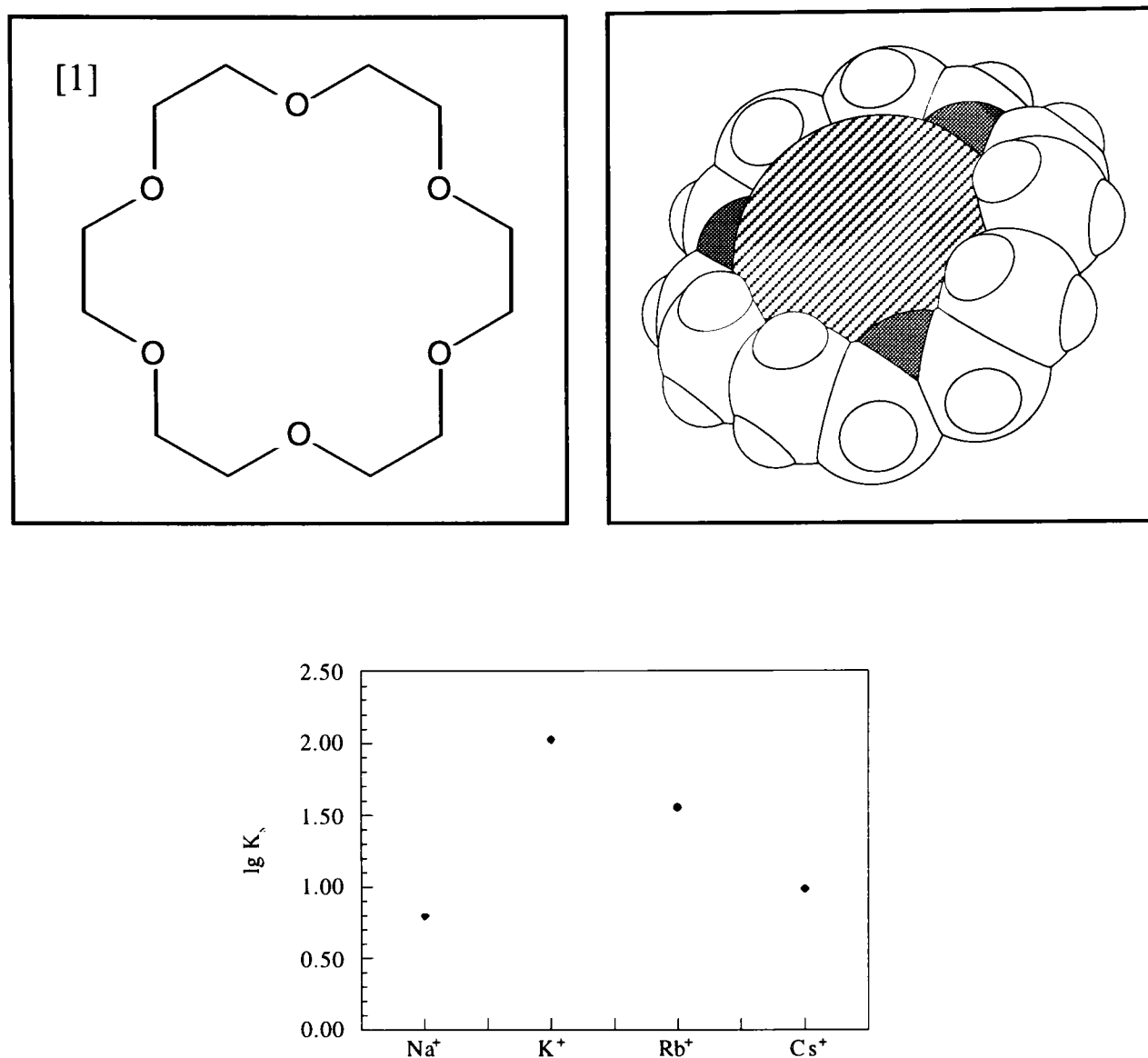


Figure 1.2: Crown ethers: (a) the structure of 18-crown-6; (b) the crystal structure of K⁺·18-crown-6; (c) stability constants and metal selectivity.

The two properties mentioned above both had important consequences for the coordination chemist who would grasp the precedents they revealed. In the first instance, it became apparent that it is possible to construct a ligand for some very unlikely species if one provides enough suitable binding interactions; crown ethers bind alkali metal cations by the concerted action of several donor atoms which are placed in close proximity by the constraints of the cyclic backbone. Moreover, crown ethers fostered the notion that synthetic ligands could be designed and created with a unique partner in mind; one which would be selected in preference to all others. Thus came the dawn of molecular recognition. Pedersen's discoveries heralded a new approach to coordination chemistry in which attention became focused on the synthetic construction of rings, cages and cavities incorporating a number of donor and acceptor groups. The intention of this combination was to provide a ligand able to bind strongly and specifically a molecule it had been designed to recognise, and a great many

successful examples are offered in the literature. The field is still expanding in complexity and elegance as its exponents continue to exploit the success of the design rationale embodied in the crown ethers. Specific examples will be presented during the discussion of interactions, later in this chapter.

Each new field carries an attendant new vocabulary and at this point a shift in terminology is introduced. The ligand is now known as a receptor or “host” molecule and is designed to bind a substrate or “guest”. It is these terms that will be adopted henceforth.

Molecular recognition straddles many boundaries of chemistry. Its origins lie in coordination chemistry, traditionally a province of the inorganic chemist, yet contemporary work employs advanced organic syntheses and calls upon analytical and physical techniques to quantify its results. Moreover, it draws much inspiration from biochemistry and often looks for applications in the biochemical arena. In pursuit of a receptor for a chosen substrate, the complementarity mentioned previously is considered and a favourable distribution of suitable binding sites must be incorporated (shown schematically in figure 1.3). The generation of such structures frequently calls upon several disciplines.

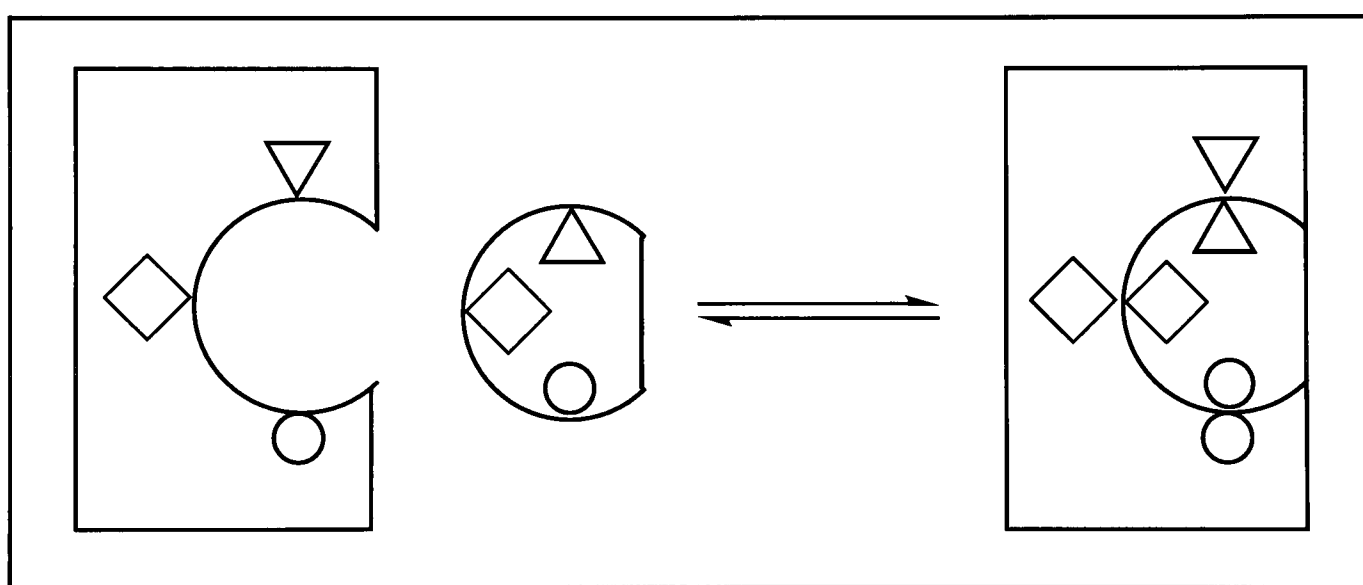
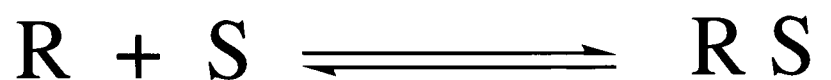


Figure 1.3: Complementary binding.

The types of interaction responsible for binding are discussed presently; in the main they rely on an electrostatic basis for their action. There are further ways of enhancing binding, however, which look beyond the mere presence of an interactive group. For example, the inclusion of more than one binding site within the same receptor is known to improve performance by virtue of the so-called “chelate effect”.⁶ This reflects an enthalpic⁷ and entropic advantage in having a number of binding groups close together, and a positive ΔS as one receptor displaces a higher number of other molecules, usually solvent.

You will have written exceptionally well if, by a careful arrangement of your words, you have made an ordinary one seem original.

Such effects can be placed on a quantitative basis by thermodynamic study. The quantity of primary concern is the stability (or binding) constant which expresses the strength of association between receptor, R, and substrate, S. Consider a simple equilibrium between the two:



The stability constant, K, for this process is merely the equilibrium constant and so a large stability constant implies a binding process which lies predominantly on the right, bound side of the equilibrium. The equilibrium constant, in turn, supplies information about the thermodynamics of the system through the relationship $\Delta G = -RT \ln K$.^{*} This confirms the intuitive expectation that the strongest binding arises from the most thermodynamically favourable binding process. Consider the enthalpy of binding. Exothermic contributions arise from the formation of a stable complex and its solvation. However, there are a number of enthalpic hurdles to be overcome, principal among which is the desolvation of both R and S. Clearly, then, a receptor which is not strongly solvated in the unbound state is advantageous. Such is the case for macrocyclic systems which tend to be poorly solvated and are known to form complexes in a manner superior to their open chain analogues — the “macrocyclic effect”.^{8,9} Another endothermic contributor is the energy required to adopt the binding conformation. Receptors are usually conformationally mobile and must assume a particular conformation to facilitate binding, a process which requires energy to overcome internal rotational barriers. This is enshrined in the concept of preorganisation; receptors which maintain the same conformation prior to binding and afterwards are notably efficient.¹⁰

Entropic effects are also significant. The substrate and receptor usually have an organised solvation sphere which is released on binding and constitutes the primary source of positive entropy change. This is countered by the loss of degrees of conformation freedom as the receptor adopts a limited number of binding conformations as mentioned above. Here is another principle reflected in macrocyclic and preorganised receptors: such systems do not suffer a large negative internal entropy change on binding.

^{*} In fact, K here incorporates concentrations divided by the standard state, 1 mol dm⁻³ to give a dimensionless constant which can be expressed as a logarithm. Stability constants *per se* are given appropriate units.

The stability constant itself can be determined by a number of methods, some of which are presented later. Accurate values, however, are difficult to determine, sensitive to temperature and suffer from the fundamental problem of equilibria which are expressed in concentrations rather than activities. These are some of the factors affecting the stability constant; the next section will examine some of them more closely.

1.1 Factors affecting the Stability Constant Magnitude

All binding interactions contain a significant electrostatic contribution, be it a Coulomb potential between two fully charged species, or the electrostatic attraction between a charged ion and a permanent dipole such as that contributing to a hydrogen bond. It can therefore be seen that factors which impinge on the strength of the Coulombic attraction will affect the stability constants of the host-guest complex.

1.1i Debye-Hückel Theory

The theory of Debye and Hückel (1923) considers the properties of strong electrolytes in solution and comments on two features relating to stability constants: ionic atmosphere and activity coefficients. There are detailed works¹¹ on this theory; this discussion however, will be confined to some of the key results.

The Ionic Atmosphere

The pure Coulomb potential, ϕ_i , between two charged species in a vacuum is given by the following expression:

$$\phi_i = (z_i e / 4\pi\epsilon_0)(1/r),$$

where z_i is the charge on the ions and r is their separation. ϵ_0 is the vacuum permittivity and in solution this is replaced by ϵ , given by $\epsilon = \epsilon_0\epsilon_r$ (ϵ_r is the *relative permittivity* or *dielectric constant* of the solvent). The solvent-solute interactions give rise to a departure from ideal behaviour and a corresponding reduction in potential. This is, of course, why solids dissolve in solvents; the introduction of solvent-ion interactions and the diminished ion-ion interactions overcome the lattice energy. For a host-guest complex, a solvent with high dielectric constant will reduce the strength of the binding forces and the stability constant for

the complex will be accordingly weaker. This agrees with an intuitive approach which sees solvents with a high dielectric constant as those with polar molecules whose dipoles interfere with the attraction between charges.

The solution phase, moreover, introduces a further reduction in potential. Each ion tends to be surrounded by a cloud of counter-ions which is known as the ionic atmosphere. The effect of this is to shield the charge on the ion, with a correspondingly more rapid decrease in Coulomb potential. This is quantified by the *Debye length* (or *shielding length*), r_D , which is an expression of the depth of the ionic atmosphere; the shorter the Debye length, the stronger the ionic atmosphere and, again, this serves to reduce the potential. Introducing these modifications into the previous expression leads to a new equation for the Coulomb potential, given by

$$\phi_i = (z_i e / 4\pi\epsilon r) e^{-r/r_D}.$$

These effects are illustrated in figure 1.4a, which shows the variation of ϕ_i with distance for a strong electrolyte, AX, in three cases.

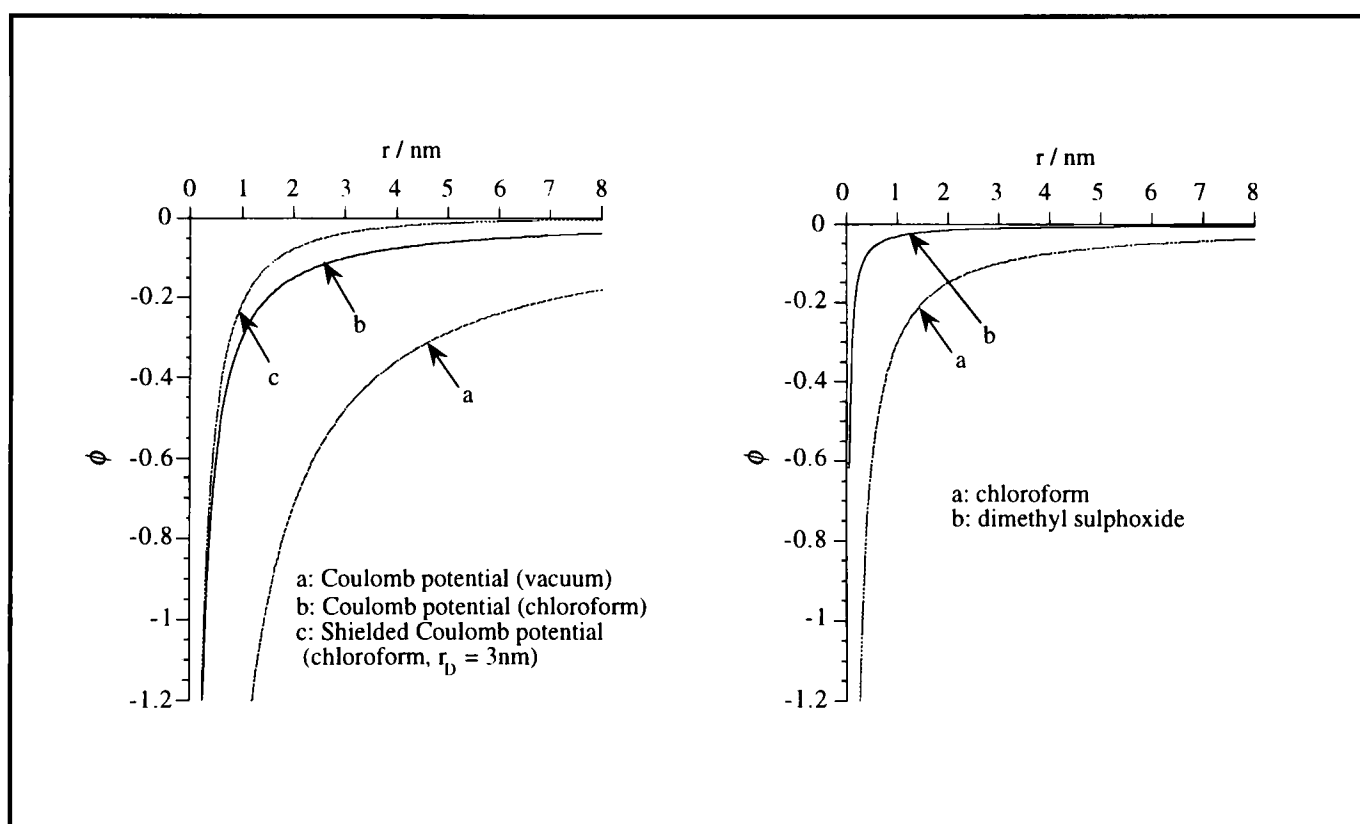


Figure 1.4: The variation of Coulomb potential with distance:

(a) effect of dielectric constant and ionic atmosphere; (b) effect of varying solvent.

It can be seen that there is a marked reduction in the potential from the vacuum potential, (a), to solution in chloroform, (b), and with the introduction of an ionic atmosphere

corresponding to a Debye length of 3 nm, (c). The difference between chloroform ($\epsilon_r = 4.8$) and dimethyl sulphoxide ($\epsilon_r = 46.7$) is also notable (figure 1.4b).

From these data, it can be inferred that the strength of interaction between host and guest will be altered by different solvents and different values of the Debye length. This is supported by the experimental observation that stability constants are often strongly solvent dependent.

The Ionic Strength

In cases where the receptor and substrate carry a formal charge, the binding is influenced by the concentration of ions in solution. The ionic strength, I , of a solution is defined by the following expression:

$$I = \frac{1}{2} \sum_j (m_j / m^\ominus) z_j^2,$$

where m_j is the molality of species j , z_j is its charge and $m^\ominus$ is the standard molality, 1 mol kg^{-1} . This quantity is of concern as a contributor to the difference between the thermodynamic or “true” stability constant, K_T , and the concentration stability constant, K_C , which is that obtained by experiment. The difference between these is again linked to departures from ideal solution conditions with K_T being the stability constant at ideality, i.e. expressed in terms of activities, a , rather than concentrations, c . Activity may not be measured directly but instead is related to the concentration by the *activity coefficient*, γ , in the expression $a = \gamma c$.

It can be shown that the following applies (for singly charged ions and simple 1:1 stoichiometry):

$$\lg K_T = \lg K_C + 2 \lg \gamma,$$

and reference to the Debye-Hückel limiting law allows the relation of activity coefficient to ionic strength to be expressed *under dilute conditions*. Stability constant measurements are made at suitably low concentration and so the following applies :

$$\lg \gamma = -A |z_+ z_-| \sqrt{I}; \quad A = 1.862 \times 10^6 / (\epsilon T)^{3/2}.$$

It should be noted that the ionic strength (and indeed the dielectric constant) contribute to the value of the activity coefficient and therefore, for charged species, one would not expect the stability constants measured at different concentrations to correlate (this is indeed the case; it

will be seen that constants determined from UV-vis measurements are consistently larger than those determined from nmr experiments which are conducted at higher concentrations).

It is important to recall, however, that among the assumptions inherent in Debye-Hückel theory is the consideration of all ions as point charges with symmetrical Coulombic fields and the failure to consider any interactions between ions other than those of an electrostatic nature between ions. Clearly, the systems under consideration in this work do not conform to either of these assumptions and so, while one might expect a qualitative agreement between theory and experiment, care should be taken in the pursuit of quantitative correlation.

1.2 Modes of Recognition and Binding

An early question which emerges is “by what means does a receptor bind to its chosen substrate?” In answer, there are several interactions which can lead to attraction between species, and a successful receptor frequently employs a combination to achieve its purpose.^{12,13} Some attention will now be paid to each one along with examples which demonstrate their significance.

1.2i Orbital Overlap

Overlap of atomic orbitals is the basis for the formation of covalent bonds and therefore one would expect that a coordination complex displaying appreciable orbital overlap between host and guest would be endowed with high stability. This is in fact the case and may account for several trends in stability such as the Irving-Williams series. Consider an octahedral complex such as $[\text{Fe}(\text{phen})_3]^{2+}$ where phen is 1,10-phenanthroline. The ligand here acts as a σ -base and a π -acid; a molecular orbital treatment yields an MO scheme with a full, low lying t_{2g} set of orbitals and a high energy e_g set which are antibonding and empty, hence the stability of this complex. Moreover, well defined orbital overlap imparts a fixed geometry to the complex. In this example, a six coordinate geometry is preferred because it gives maximum stability — lower coordination numbers give poorer overlap and steric constraints forbid a higher coordination.

A similar situation exists for organometallic sandwich compounds such as ferrocene. The combination of the metal ion and the two cyclopentadienide ligands gives a very stable complex due to many favourable orbital interactions. The molecular orbital scheme is shown in figure 1.5.

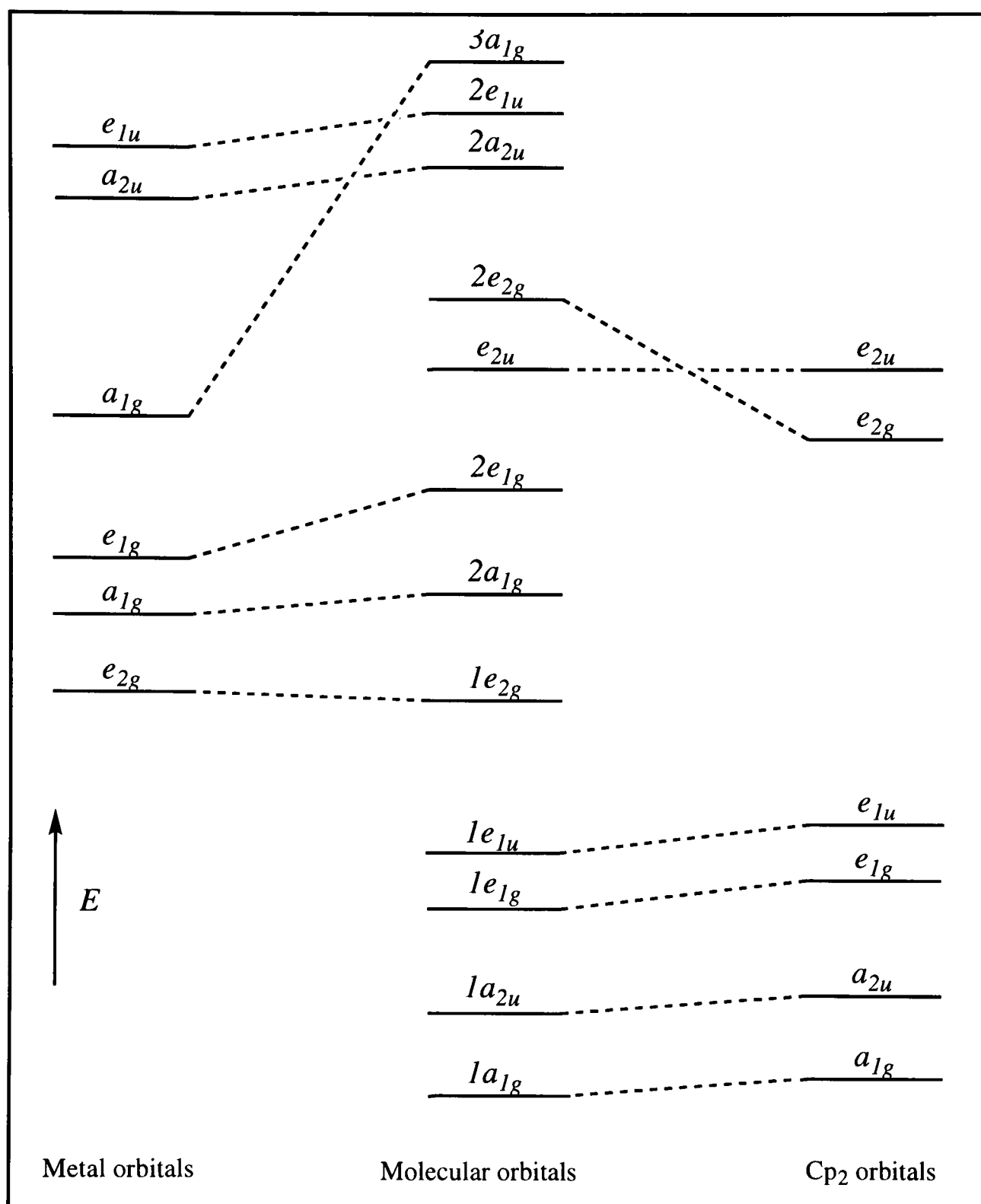


Figure 1.5: Molecular orbital diagram for metallocene complexes (after Douglas, McDaniel & Alexander, *Concepts and Models of Inorganic Chemistry* 2e, John Wiley & Sons, New York, 1983).

The number of metal and ligand electrons totals eighteen which fully occupy the bonding orbitals and hence give the molecule its stability. This is also the origin of the celebrated “eighteen electron rule” for other organometallic species which have molecular orbitals of sufficiently strong bonding/antibonding character to promote the full occupancy of bonding molecular orbitals.

Ferrocene is a particularly helpful example since a number of receptors discussed in later chapters are based on metallocene fragments.

1.2ii Coulombic Forces

These are synonymous with the ionic bond and are responsible for the aggregation of ions into a crystal lattice. The force has a magnitude that varies as $1/r^2$ and is associated with a potential of $1/r$. These are long range forces and are heavily dependent on the ionic atmosphere around the ions and the dielectric constant of any solvent concerned. Since a great number of hosts and guests are charged, a significant proportion of their binding interaction must arise from Coulombic forces, which have been discussed in section 1.1i.

The application of such forces to recognition may be seen in the quaternised systems of Schmidtchen ([2], figure 1.6) which were designed to bind anions. The structure of the receptor positions positively charged groups at the apices of a tetrahedron which then harness Coulombic attraction as the basis for inclusion of an anion such as chloride.

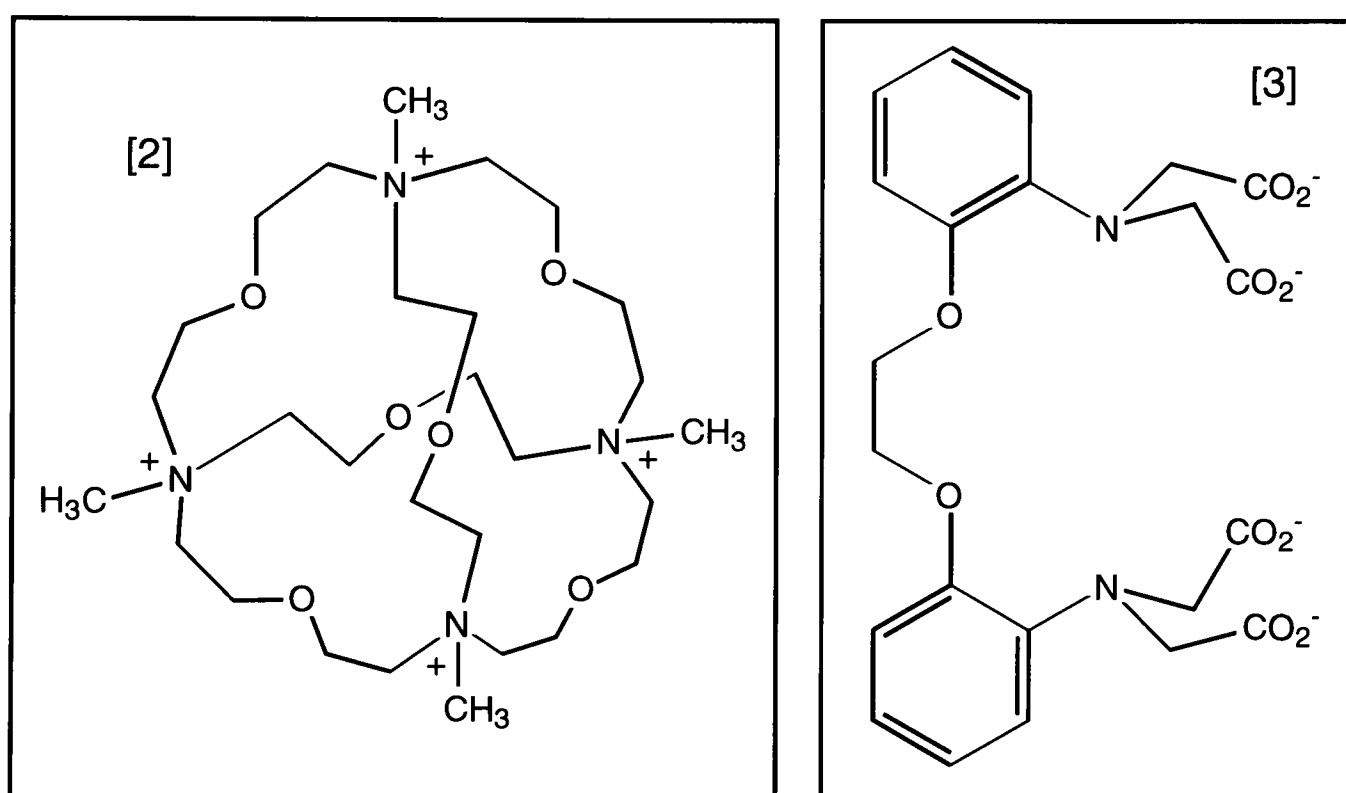


Figure 1.6: Coulombic receptors.

A further example is furnished by the work of Tsien, who published¹⁴ the receptor [3]. The binding of Ca^{2+} is accomplished with the formidable stability constant of 10^7 in aqueous solution (ionic strength of 0.1) and a selectivity over Mg^{2+} of 10^5 . This example brings together several illustrative points into a harmonious whole. The basis of the interaction is Coulombic and the concerted action of four carboxylate units adds to the strength of binding. Here is the advantage of multiple binding sites; the lone pairs of nitrogen and the ether

oxygen atoms serve to assist the receptor in its purpose. The structure of the receptor is relatively rigid which removes thermodynamic barriers, and the inclusion of the cation reduces repulsion between neighbouring carboxylates. This repulsion is also claimed to be the basis for selectivity; binding the smaller Mg^{2+} would bring the negatively charged groups too close together. Modification of the receptor has provided the foundation of a fluorescent sensor¹⁵ for Ca^{2+} (see section 4.2).

1.2iii Van der Waals' Forces

There are a number of long range attractive forces which contribute to the potential between two species and it is possible to partition the potential into the following: electrostatic, inductive and dispersion forces.

Electrostatic Forces

These are attractions arising from molecules which have non-symmetrical charge distribution and are sometimes known as dipole-dipole interactions. Many molecules possess a dipole or higher multipole as shown in figure 1.7 and interactions between them are widely encountered. The magnitude of such forces depends strongly on both the separation and orientation of the molecules in question and is described by the following equation:

$$U(r) = -2\mu_a\mu_b/(4\pi\epsilon_0)r^3,$$

where μ_a and μ_b are the dipole moments of the respective molecules.

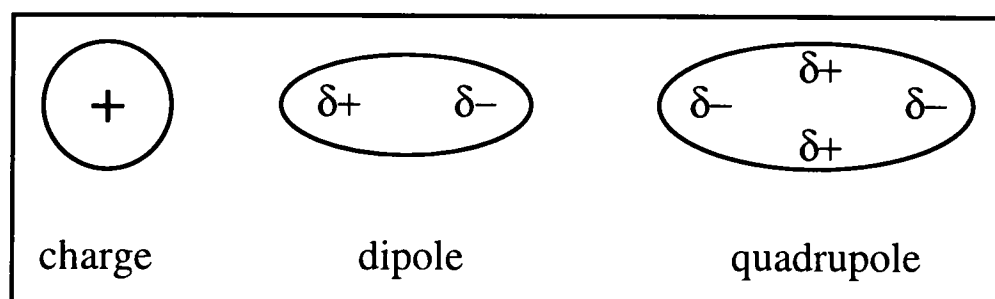


Figure 1.7: Schematic diagram of multipoles.

The interaction between a crown ether and ammonium is characteristic of a dipole-dipole binding interaction. It is known that 18-crown-6 associates strongly with ammonium cations based on an interaction between the positive dipole of the N-H bond and the negative charge density carried by the oxygen atoms within the crown. In this case, an interaction is only

formed between alternate oxygen donors because, of course, ammonium can only offer three acceptors.¹⁶

Induction Forces

Sometimes known as dipole-induced dipole interactions, these are the attractive forces which arise as a result of a dipole that has been induced by an electric field. Consider a molecule which is subjected to a field, F . A dipole may arise as shown in figure 1.8, the moment being denoted μ_{ind} .

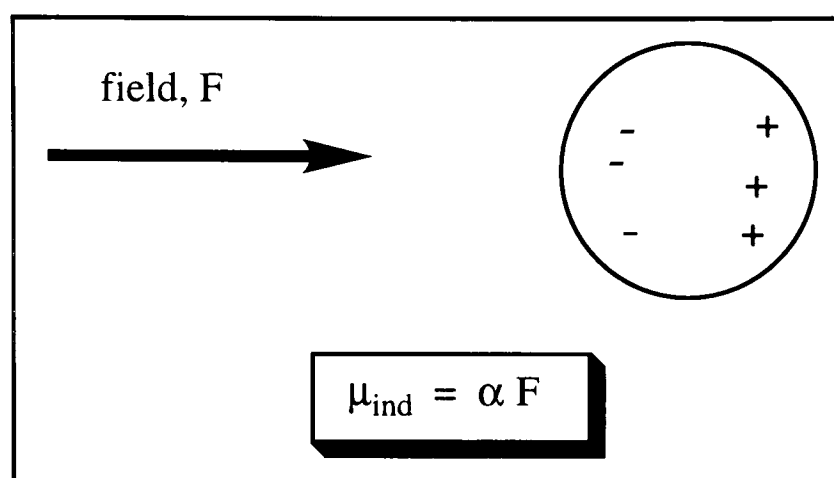


Figure 1.8: Induction of a dipole.

α is known as the polarisability of the molecule and the resultant dipole gives rise to an attractive force which varies as r^{-6} . It is unusual for a receptor to operate on the basis of these forces alone, as they are rather weak. They are well known as the principal interaction between the molecules of solid iodine, which demonstrates the weakness of such forces by ready sublimation.

Dispersion Forces

These are rather surprising forces and are the most important source of long range attractive energy. They are non-classical and were identified in 1930 by London in a “triumph of quantum mechanics”. Without painting too classical a picture, they may be envisaged as arising from attraction between the fleeting dipoles which are induced as two molecules pass close by and disturb the electron density of each other, hence the alternative name induced dipole-induced dipole interactions. Again, the magnitude of the forces varies as r^{-6} .

It is difficult to assign specific examples to such interactions due to their nebulous nature, but they are likely to play an important role in the binding of xenon atoms within a system published by Rebek. The molecule, [4], shown in figure 1.9 can dimerise due to

complementary hydrogen bonding interactions; the analogy is drawn with the two halves of a tennis ball. This process is observed to be induced by the presence of a guest such as methane or xenon^{17,18} which resides within the centre of the newly formed dimer as detected by several nmr studies in both deuteriated DMF and chloroform.

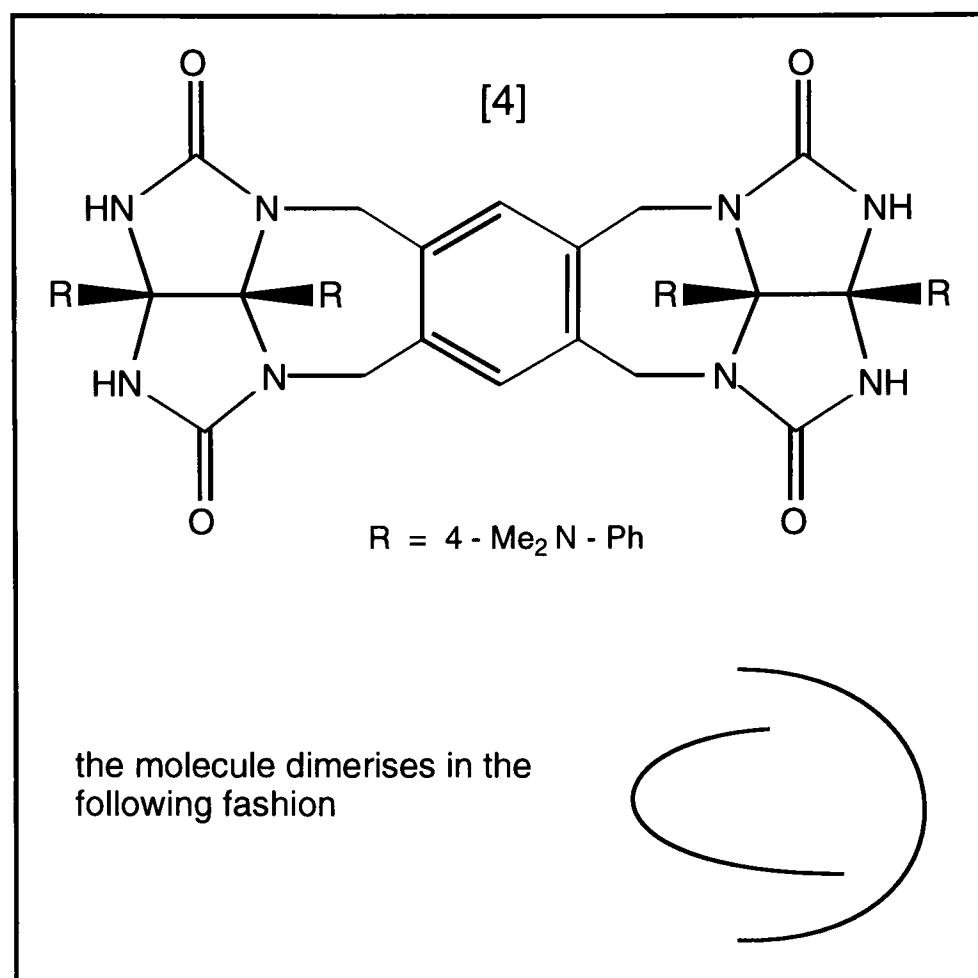


Figure 1.9: The Rebek “tennis ball” molecule.

Van der Waals’ and Coulombic forces are very commonly encountered in coordination chemistry and, by extension, in molecular recognition. For example, the crystal field theory of transition metal complexes is based entirely on the concept of electrostatic attraction between ligand and metal, for a ligand is either a charged ion or possessed of a strong dipole. Another example is 18-crown-6 (figure 1.2), which binds a potassium cation on the basis of a dipolar, electrostatic interaction between the metal ion and the lone pairs on oxygen.

Discussion of these forces may be found in standard texts.¹⁹

1.2iv Hydrogen Bonding

The importance of hydrogen bonding in molecular recognition cannot be overestimated. Employed frequently in natural systems, it determines secondary protein structure, assists in substrate binding and is responsible for the celebrated base pairing in DNA. Likewise, it

plays a key role in artificial systems, particularly in anion binding. All the receptors presented in subsequent chapters rely, at least in part, on hydrogen bonds and some discussion will now be devoted to their strength and origin.

Hydrogen bonds were first proposed²⁰ in 1912 and their significance was noted by Latimer and Rodebush in their comments on the structure of water.²¹ The hydrogen bond is found to occur when a hydrogen atom is associated with two other atoms in a $A-H\cdots B$ sense. The strongest interaction is observed when A and B are electronegative elements and early authors thought that hydrogen bonds were confined to cases involving the most electronegative elements. However, this has been challenged. Pimentel and McClellan²² comment: "It seems that the older view that the atoms A and B must be highly electronegative is undesirably restrictive," and advocate the possibility of hydrogen bonds to aromatic C-H and to electron-deficient boron. A more recent neutron diffraction study of interactions of water with C-H hydrogen concluded that these were hydrogen bonds and supports the view that such hydrogen bonds are possible.²³

The nature of the hydrogen bond is a little imprecise. In general, $A-H$ resembles a normal covalent bond, and $H\cdots B$ is weaker and longer, which is consistent with the view that a hydrogen bond is essentially electrostatic, as affirmed by Pauling.²⁴ He noted that the single $1s$ orbital on hydrogen precludes the formation of more than one covalent bond, and therefore any further interaction must be charge based. This theory further explains why the strongest hydrogen bonds are formed with the more electronegative elements. The role of the hydrogen atom is also explained. Being very small and having no inner electrons, it allows the binding partners to approach closely and maximise the electrostatic attraction without experiencing repulsion from an inner electron cloud. However, the hydrogen bond is not *simply* electrostatic. Coulson presents a number of objections,²⁵ most notably a lack of correlation between bond strength and the dipole moment. There are in fact a number of ways in which a covalent contribution to the bond may arise and the system is best viewed as essentially electrostatic with a small covalent contribution.²⁶

This type of bonding is frequently employed in molecular assemblies due to its relatively high strength and directional nature. A good example is seen in figure 1.10 where the position of two carboxylic acid groups in the host, [5], provide a binding site for 9-ethyl

adenine as guest. The stability constant is $4.5 \times 10^4 \text{ dm}^3\text{mol}^{-1}$ in chloroform.²⁷ The molecule is also illustrative of *preorganisation*; the bicyclic system holds the binding groups in a conformation close to that needed for binding. This removes the need for conformational rearrangement prior to binding, an unfavourable process in terms of both entropy and enthalpy.

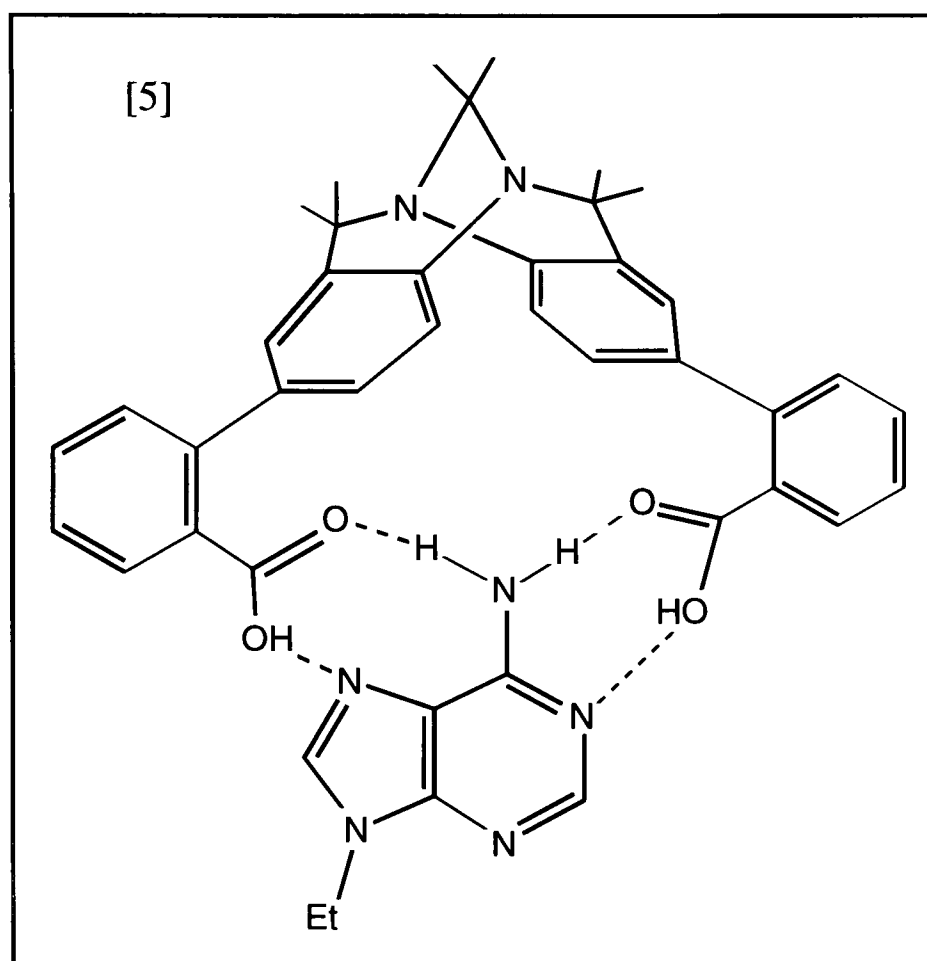
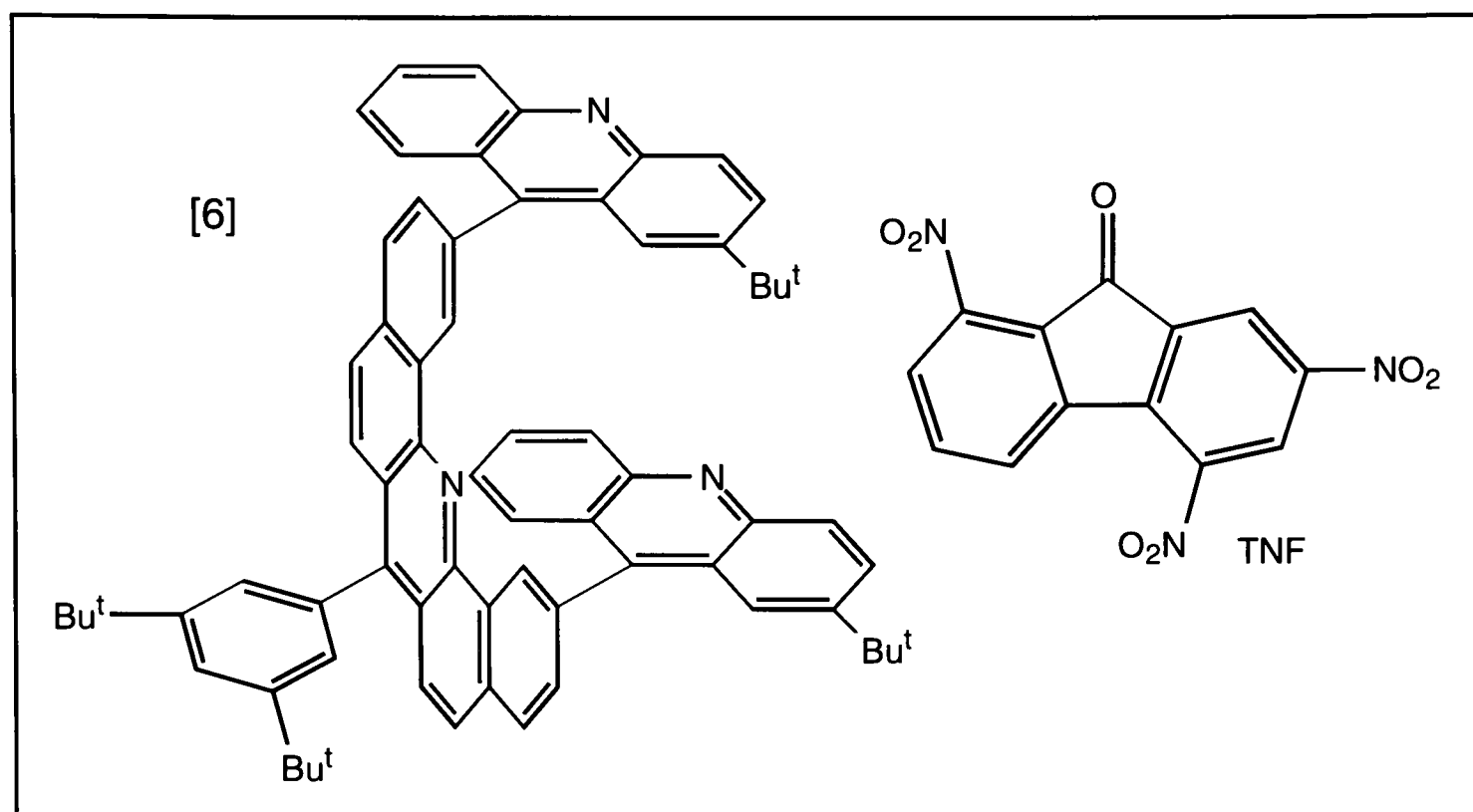


Figure 1.10: Hydrogen bonding to 9-ethyl adenine.

1.2v π - π Interactions

It is well known and documented in the literature that two aromatic systems have an attraction for each other, both in a face-face and edge-face deployment (the latter is particularly true in solid packing). The origins of this interaction are unclear and may arise from charge transfer, electron donor-acceptor properties or from electrostatic interactions due to fluctuations in the out-of-plane electron system. Recent work favours a theory dominated by electrostatics; a review by Hunter cites experimental evidence from several sources.²⁸ The receptor shown in figure 1.11 is a rigid construction which offers two extended π systems which are held parallel to each other.²⁹ The guest 2,4,7-trinitrofluorenone (TNF) is bound between the two with a stability constant of $197 \text{ dm}^3\text{mol}^{-1}$ in chloroform; the association is attributed to π -stacking forces.³⁰

Figure 1.11: Receptor based on π - π interactions.

1.2vi Hydrophobic Interactions

Hydrophobic interactions are an oft quoted contributor to the complexation process which merit some comment. There is much controversy in the literature concerning these effects which relate to the affinity of lipophilic groups to each other in aqueous media. It is clear that there is a notable increase in entropy when two such groups interact, but questions arise as to whether this is a discrete effect. An early paper³¹ suggested that the introduction of a non-electrolyte promoted solvent ordering and that this was broken on aggregation. Tanford, in his much cited book, also advocates an entropic basis for this effect along similar lines.³² However, a recent study³³ of the binding between pyrene and a cyclophane-based host noted a linear relationship between the free energy of binding and an empirical measure³⁴ of solvent polarity, $E_T(30)$, for a wide range of media. This suggests that the interaction which has frequently been dubbed “hydrophobic” is simply an expression of the cohesive strength and polarisability of the solvent. Water is such that it encourages aggregation of non-polar solutes. Hildebrand also denies the existence of the hydrophobic effect; on the subject of alkane aggregation in water, he comments:

“In conclusion, there is no hydrophobia between water and alkanes; there only is not enough hydrophilia to pry apart the hydrogen bonds of water so that the alkanes can go into solution without assistance from attached polar groups.”³⁵

The situation is far from clear. What is clear is that the hydrophobic effect is too commonly invoked as a binding force with little thought to its origins. The name itself is misleading; non-electrolytes do not fear water, rather water tends to love itself. Moreover, there are likely to be considerable dispersion forces at work which are largely ignored. It can be noted that there will be a positive ΔS when solvents are displaced from a “hydrophobic cavity” and that a lipophilic nature will mean that polar solvent molecules are less strongly held. However, a claim that a receptor is operating via the hydrophobic effect should be subjected to some scrutiny, for one may simply be observing effects that are attributable solely to the physical properties of the solvent.

1.3 Anion Coordination

Not content with the binding of cationic substrates, coordination chemistry has also sought to provide receptors for neutral and anionic guests. The binding of the latter has in fact commanded increasing attention within host-guest research. However, in an age when research must increasingly be justified, one may enquire about the purpose behind anion recognition. The first and defiant answer must be “Because few people have attempted it.” It is of academic interest *per se* and of course, such study often yields insight into the topic in general and thence to unforeseen application. In addition, anions have an interesting impact on life and their coordination presents potential sources of utility. In the biochemical arena, they are no strangers and natural systems exhibit many anion receptors. A brief literature search displayed over 150 citations related to study of such biological receptors in the last five years. Enzyme substrates are most commonly anionic³⁶ and one sees much activity in phosphate cycles illustrated by such molecules as ATP. Cystic fibrosis is the most common fatal genetic disease in the USA and arises due to a transport defect in chloride channels expressed as an impermeability to chloride in certain membranes.³⁷ Hence, a physiological interest, along with the constant preoccupation of the medical world with the polyanion DNA.

In terms of environmental impacts, the leaching of phosphates from fertilisers into the water table gives rise to eutrophication of fresh water, and the concern about pollution by chlorine-based anions from bleaching agents has prompted a widespread move to active oxygen systems instead. Nitrates are known to be physiologically harmful and their presence at high levels is a problem in drinking water; Thames Water is unable to use millions of gallons a year due to high nitrate levels.

These are just a few notable incidences which illustrate potential links between anion recognition and relevant commercial and physiological issues.

Anion coordination, with which this thesis is primarily concerned, employs many of the concepts already presented. However, in a reversal of the familiar protocol, the receptor needs to present electron deficient binding groups with positive dipoles. Traditionally, the following considerations specific to anions need to be weighed in receptor design because they strongly influence the process of anion binding.

1. **Size:** Anions are substantially larger than cations as demonstrated by a comparison of the ionic radii of F^- and K^+ . The respective ionic radii (Pauling²⁴) are 1.36 Å and 1.33 Å which demonstrates that a “small” anion such as fluoride corresponds to a sizeable cation. Later chapters present several examples of receptors for chloride which, at 1.81 Å, is even larger than the caesium cation (1.69 Å). The first consequence for binding is that larger assemblies are required to accommodate the guest ion with attendant synthetic challenges. The second is the fundamental observation that the binding will tend to be weaker because the charge density of an anion is lower than of a cation.
2. **Geometry:** Cations are generally confined to metal ions which are spherical. Anions, however, are found in a variety of geometries (sulphate, for example, is tetrahedral). This means that the position of the binding sites within a receptor must be selected to complement the guest, which may be seen as yet another synthetic demand. An advantage arises, however, because one is able to discriminate between different anions based not only on size but also on shape.

Despite relatively modest progress in the early stages of anion coordination, the literature now affords a wide selection of successful examples. In attempting to highlight some of the principal features of such receptors it will be necessary to depart from a chronological survey, thus allowing some simple, but later, examples to be discussed initially.

1.3i Quaternised Nitrogen

Anions are negatively charged and therefore many receptors rely on a high density of positive charge to operate; they act largely on a Coulombic basis. One of the easiest methods of creating a stable positive charge is to quaternise a nitrogen atom and consequently, this is the feature of a number of very simple anion receptors.

A fundamental example is seen in the work of Schmidtchen (mentioned previously) in the form of quaternised nitrogen systems³⁸ such as those shown in figure 1.12.

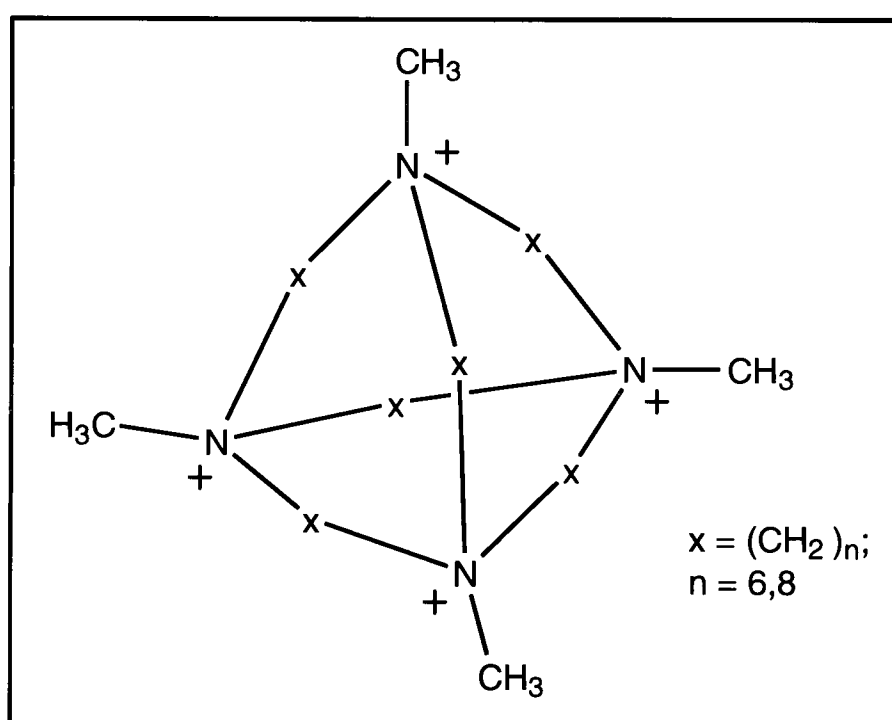
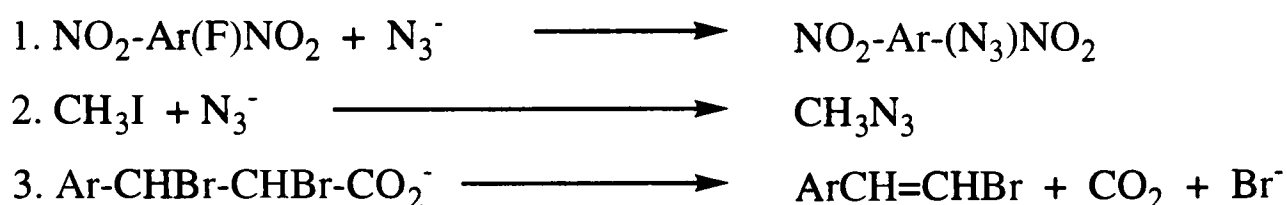


Figure 1.12: Quaternised ammonium receptors.

The positive charges ranged about a fixed cavity provide a binding site for anions and the forces at work were deemed to be electrostatic and hydrophobic.³⁹ Halides are bound with a stability constant in the order of 10^2 – $10^3 \text{ dm}^3\text{mol}^{-1}$ in water⁴⁰ and the crystal structure of the iodide complex has further confirmed that the binding site is situated within the centre of the cavity.⁴¹ A degree of selectivity is also observed, based on size discrimination.

In search of application of such receptors, the catalysis of a number of reactions involving anionic intermediates was studied and it was found that modest acceleration of the reactions shown below occurred on addition of the anion receptor.⁴²



It was felt that the presence of counter anions might impede binding of a guest anion and therefore, the $^+\text{N-CH}_3$ unit was replaced by overall neutral $^+\text{N-BH}_3^-$. The result however, was a considerable drop in the binding strength, presumably due to neutral resonance structures.⁴³

Some acyclic analogues were published by Beer,⁴⁴ based on quaternised bipyridine (figure 1.13).

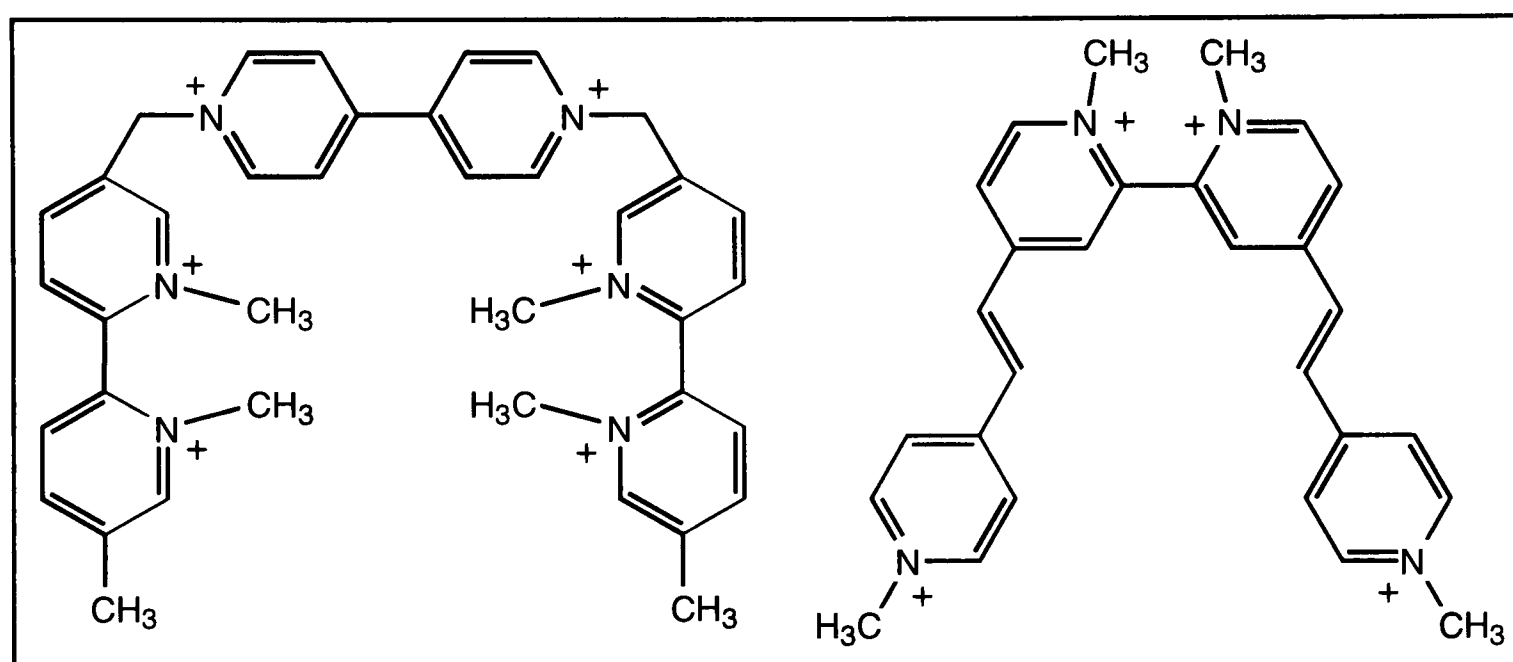


Figure 1.13: Quaternised bipyridine receptors.

The binding ability thus demonstrated is not outstanding and these results show that the binding of anions is not simply a matter of Coulombic attraction; in this respect, an analogy with cation binding is drawn. More successful anion receptors, then, draw on a combination of positive charge and other binding opportunities, particularly hydrogen bonding, and it is to such hosts that attention now moves.

1.3ii Protonated Nitrogen

The most potent and prolific class of anion receptor reported is that based on the protonated nitrogen atom. Such receptors combine a positive charge with opportunities for hydrogen bonding to the ammonium hydrogen; it was this combination which was responsible for binding of chloride in the first anion receptor to be explicitly published as such.⁴⁵ The receptor is shown in figure 1.14 and has a conformation where the hydrogen atoms are directed into the cavity. This conformer was found to include chloride, held within the cavity by the combination of Coulombic and hydrogen bonding interactions. An X-ray crystal structure confirmed the binding location.⁴⁶

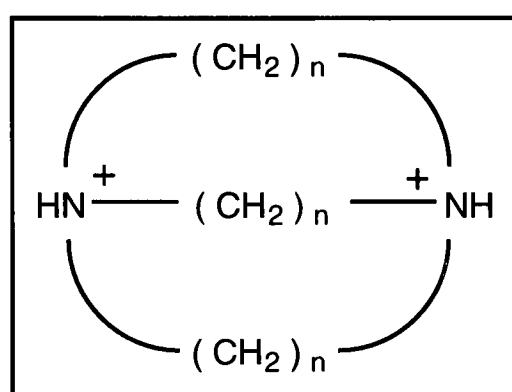


Figure 1.14: The first anion receptor.

The same principle was embraced in a series of protonated polyammonium macrocycles which sought to concentrate a large density of positive charge within the cavity.⁴⁷ There are a number of studies of polyaza macrocycles which are found to be polyprotonated at neutral or slightly acidic pH.^{48,49} The binding of anions to macrocycles such as those shown in figure 1.15 was therefore studied.⁵⁰⁻⁵²

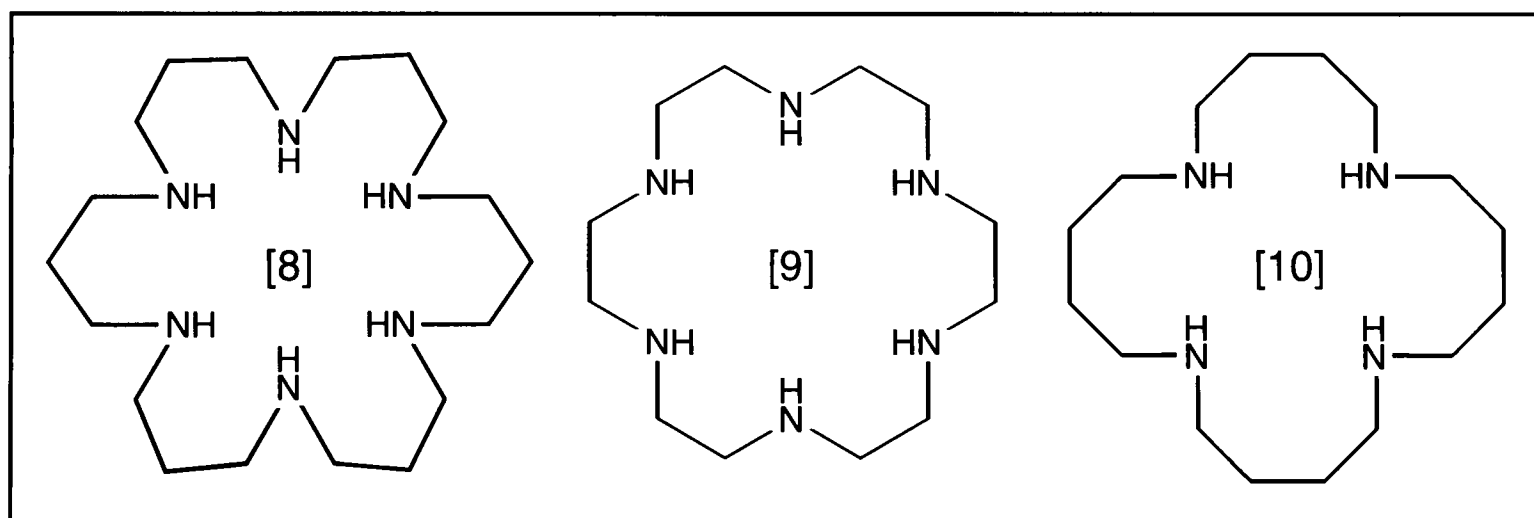


Figure 1.15: Polyaza macrocycles.

These receptors are soluble in water and show binding interaction with a wide variety of oxo-anions, notably carboxylates and phosphates. The range of stability constants is very wide,

spanning five orders of magnitude from 10^3 – 10^8 dm³mol⁻¹. Notable is the binding of ATP, ADP and AMP, which associate with such polyaza systems with very considerable stability constants.⁵³

The nature of the interaction is of some interest and has attracted much comment. There is no doubt that the binding is stronger than quaternised systems. This hints at additional hydrogen bonding contributions. It is also seen that the binding is enhanced by a greater density of positive charge. Many of the receptors are at least partially protonated at neutral pH and full protonation can be encouraged by lowering the pH. This is trivial to achieve but has a problematic side effect. The receptors frequently bind oxo-anions which are themselves protonated at low pH, at which point they cease to be anions. The *raison d'être* of the receptor is then somewhat incidental. Effort was therefore expended in producing receptors which would be more easily protonated. In the first instance, this was achieved by increasing the distance between the nitrogen atoms, thereby decreasing the distance between adjacent charged sites; this is seen to be effective in the case of [10] which is fully protonated at pH7 and acts as a receptor for fluoride.

In addition to increased hydrocarbon spacers, the distance between nitrogen atoms can be augmented with ethyl ethoxy linkers; the resultant macrocycle behaves much as those already seen.⁵⁰ A particularly fine example of molecular recognition is based on such a macrocycle and shown in figure 1.16. Addition of acridine (π - π opportunities) provides a receptor, [11], for ATP which binds well.⁵⁴ The receptor is not fully protonated, however, and presents a free amine group in close proximity to the guest which is thought to be responsible for its ability to catalyse hydrolysis of the bound ATP.

The electrostatic basis for binding has been supported by the observation that more highly charged receptors bind in the strongest fashion; the dependence on protonation is very strong. It is also noted that the more highly charged anions form stronger complexes.⁴⁹ A different view emerges, however, from the work of Zompa who studied the interaction of anions with hexacyclen, [8].⁵⁵ This receptor is tetraprotonated at neutral pH and forms complexes with a wide variety of anions including Cl⁻, NO₃⁻, CF₃CO₂⁻, ClO₄⁻ and IO₄⁻. However, X-ray structures showed that neither Cl⁻ nor NO₃⁻ were encapsulated by the ring, contrary to expectations.⁵⁶ Furthermore, thermodynamic study showed that complexation of Cl⁻ and CF₃CO₂⁻ is an endothermic process and therefore entropy driven. The important point here is

that the receptor has an extensive and highly ordered solvation sphere which is released on binding to an anion with a corresponding positive ΔS . This process, however, does not necessarily require inclusion within the ring, nor even a direct link; the two species may simply form an ion pair.

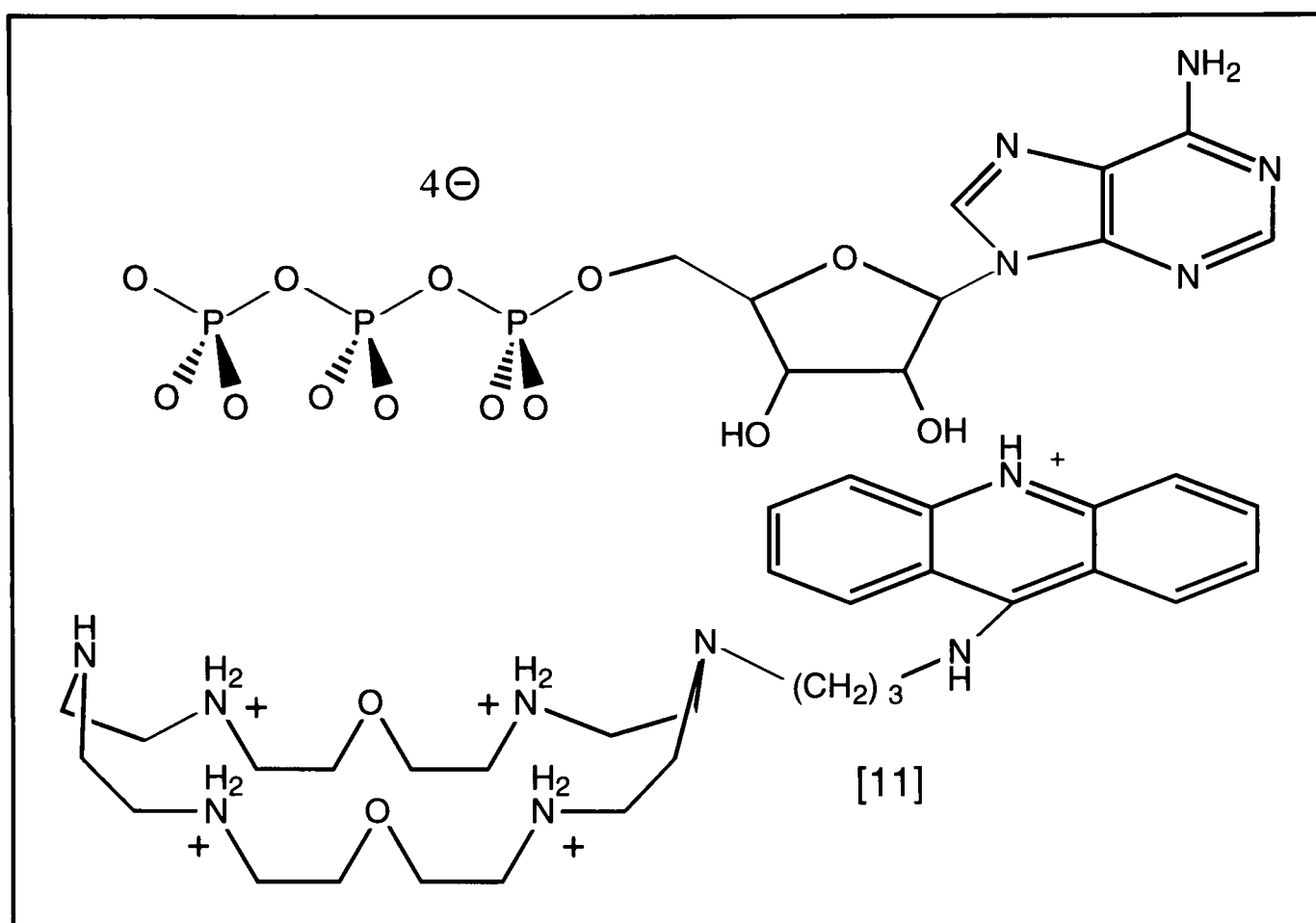
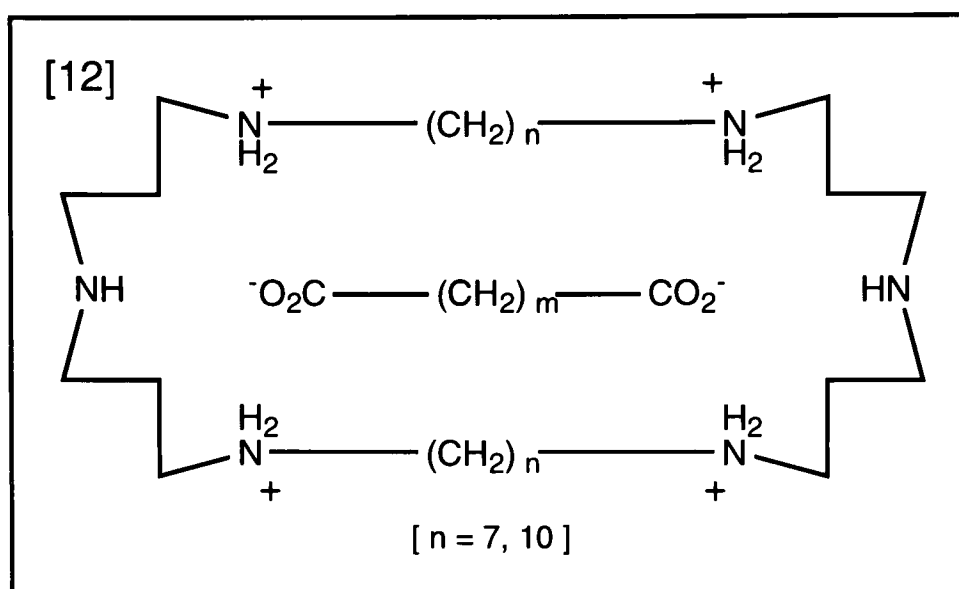


Figure 1.16: Binding and hydrolysis of ATP.

A further and extensive study of the binding between sulphate and hexacyclen again found an entropy driven process and sulphate located outside the ring, which lead to the unexpected conclusion that in this case, electrostatic receptor-anion interactions were not contributors to the binding process.

In summary, the perceived interaction between anions and protonated ammonia receptors is a combination of hydrogen bonding and electrostatics. However, there is sometimes a significant entropy contribution arising from solvent effects and this should not be neglected.

A development of the protonated ammonia principle is shown by [12] which exhibits two distinct binding sites and might therefore be expected to form an association with dianions such as dicarboxylates.^{57,58} The hypothesis is borne out by the existence of complexes with a range of dicarboxylates, formed in aqueous solution with stability constants of the order 10^3 – 10^4 dm³mol⁻¹. Moreover, there is a degree of steric complementarity according to the respective lengths, *m* and *n*, of anion and receptor.



The receptor, [13], shown in figure 1.17 is similar in style but is macrobicyclic and has a better defined cavity. Again there are two distinct recognition sites and this is reflected in the ability to form dinuclear complexes with Cu⁺ and Ag⁺ cations.⁵⁹ When protonated (pH6), the now familiar anion binding properties are in evidence in the binding of dianions such as terephthalate. In this specific case, an X-ray structure⁶⁰ reveals inclusion of the ion within the cavity and confirms the assistance of π - π interactions. The stability constant in water is $2.5 \times 10^4 \text{ dm}^3 \text{ mol}^{-1}$. Attempts were made to refine the binding further by extending the aromatic system to improve the π - π contribution as shown by [14].⁶¹ The receptor is tetraprotonated at pH6 and justified the design rationale by a stability constant for terephthalate which was an order of magnitude larger than observed with [13]. Thus we see another example of a knowledge of potential attractive forces being harnessed to yield a superior receptor.

A number of other cryptand molecules have been protonated and, in doing so, shown anion recognition properties. The “soccer ball” molecule ([15], figure 1.18) was an early example of a cryptand for inclusion of cations. Again, it is possessed of nitrogen atoms which might be protonated and thus provide a tetrahedral array of positive charge within the cavity.^{62,63} This proved an attractive location for halides; X-ray analysis showed chloride centrally situated within the cavity.⁶⁴ The receptor is also capable of some size selectivity and binds chloride three orders of magnitude more strongly than bromide, which is a little too large to be accommodated without inducing steric strain. The stability constant of [15] with chloride is over 1000 times greater than that of [16] demonstrating the higher affinity of the macrotricyclic for its guest.

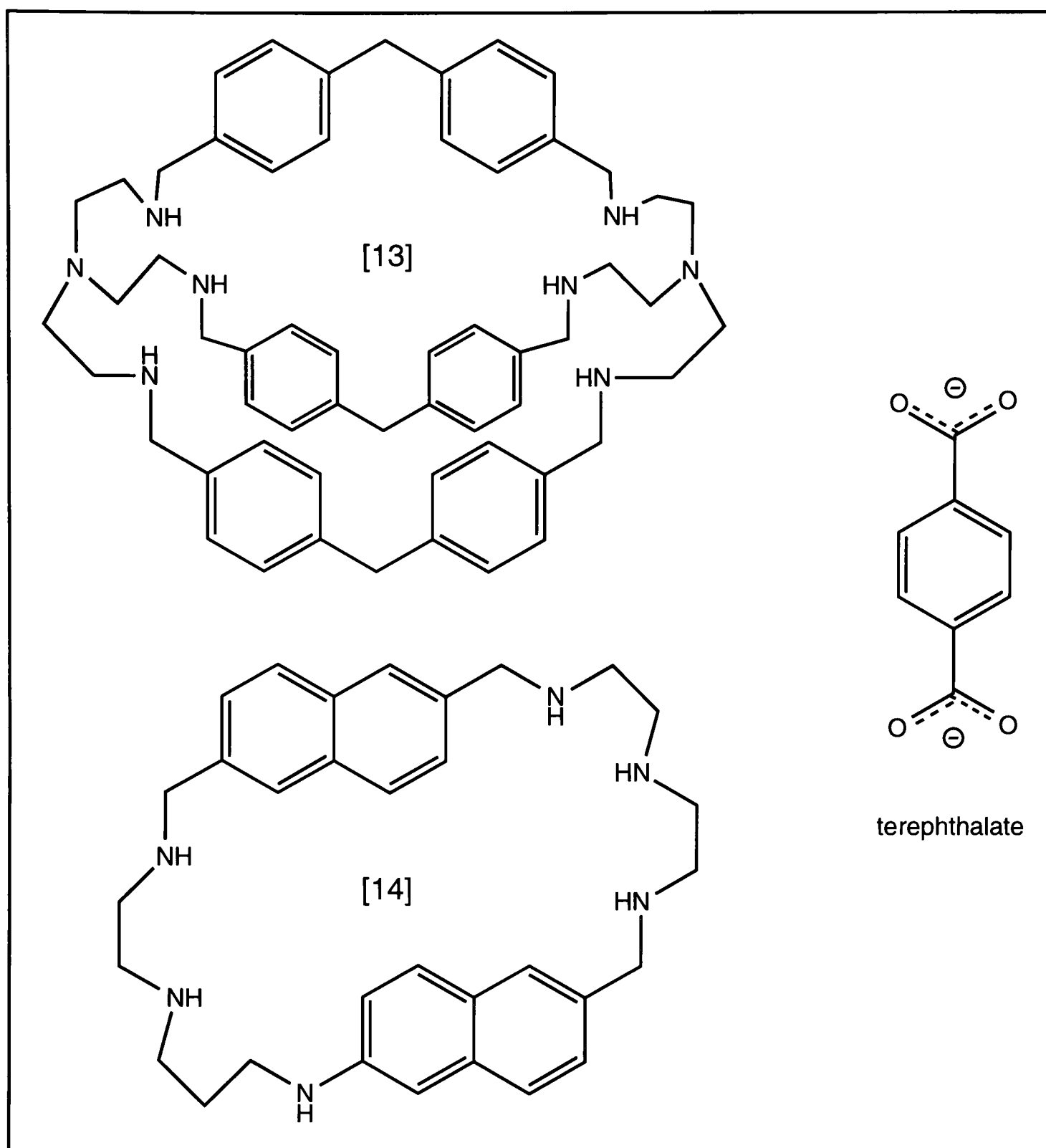


Figure 1.17: Binding of terephthalate.

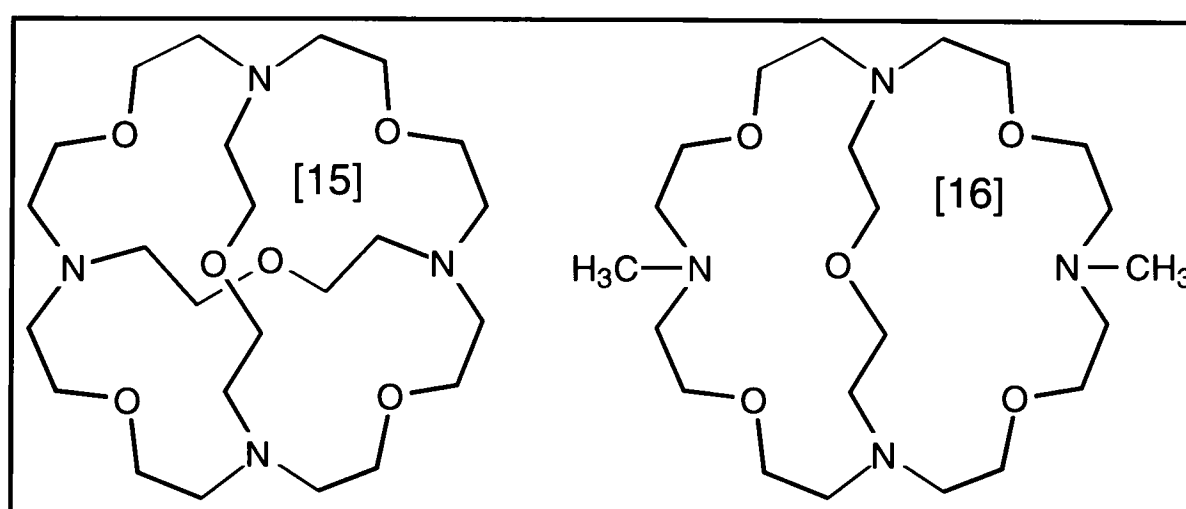
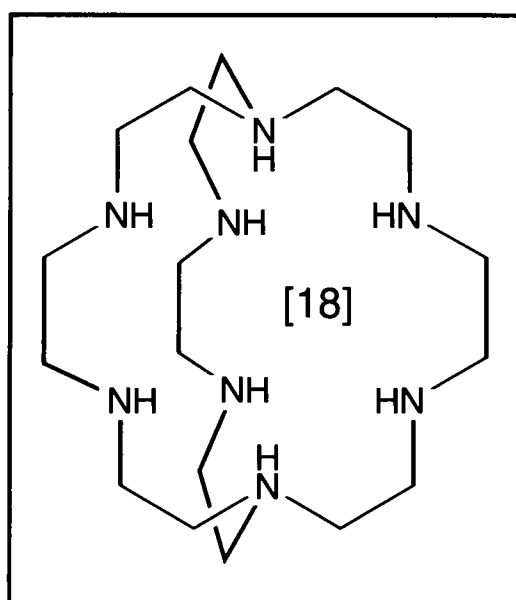
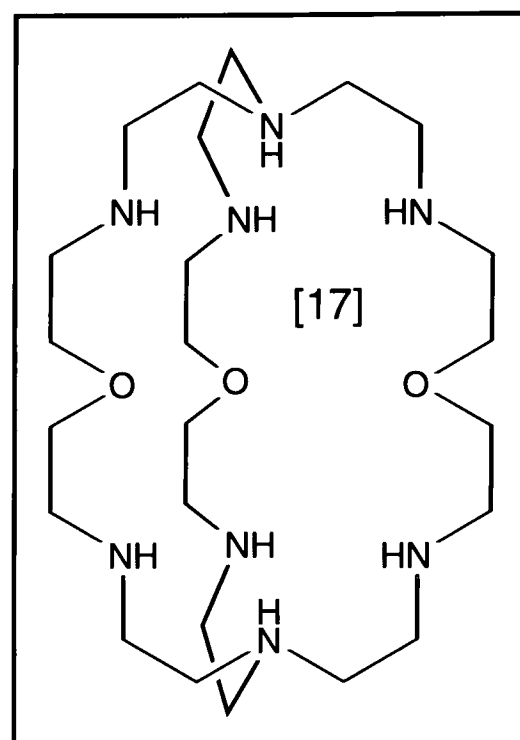


Figure 1.18: Cryptate receptors.

The molecule called bis-tren, [17], was well known to the early recognition chemists. With proven capabilities as a cryptand for cations,⁶⁵ it was hexaprotonated and found to include halides and the azide anion.^{66,67} The mode of binding differed, depending upon the size and geometry of the ion. All binding modes were confirmed by X-ray crystallography. Fluoride, for example, is relatively small and was found to reside in a tetrahedral site at one end of the cavity. This contrasts with chloride and bromide which were bound centrally in a geometry which is approximately octahedral. The receptor was particularly appropriate for the

inclusion of azide which has a length which complements that of the cavity; the termini of the anion were also found in close proximity to the binding sites with the result that the protonated receptor was strongly selective for azide over other anions.⁶⁸ The hexaprotonated form was compared with the pentaprotonated receptor; the stability constant for azide binding was greater in respect of the former, underlining the importance of the electrostatic basis of action.

Recalling that the bis-tren receptor was a little large for the inclusion of fluoride, it is to be expected that a smaller version would prove more suitable for this anion. In the reported structure of [18], it is therefore pleasing to note that fluoride is centrally bound within the cavity.



1.3iii Cascade Binding

Bis-tren, in its unprotonated, cation binding incarnation forms dinuclear complexes with transition metal cations. It was noted that the resultant complex has two centres of positive charge with a cavity between them; this forms a putative anion receptor which does indeed bind the ionic substrates chloride or hydroxide between the cations.^{69,70} Such a system is termed a *cascade complex* in that binding leads to further binding.⁷¹ It has not been reported that the receptor [12] and those in figure 1.17 behave in an analogous manner, although one would suppose that they might. A few other molecules have been published as cascade anion receptors; two are shown in figure 1.19. Both of them embrace two copper (II) ions and bind azide.^{72,73} The sulphur based system, [20], is found to include two azide ions which bridge the metal ions in an out-of-plane distribution.

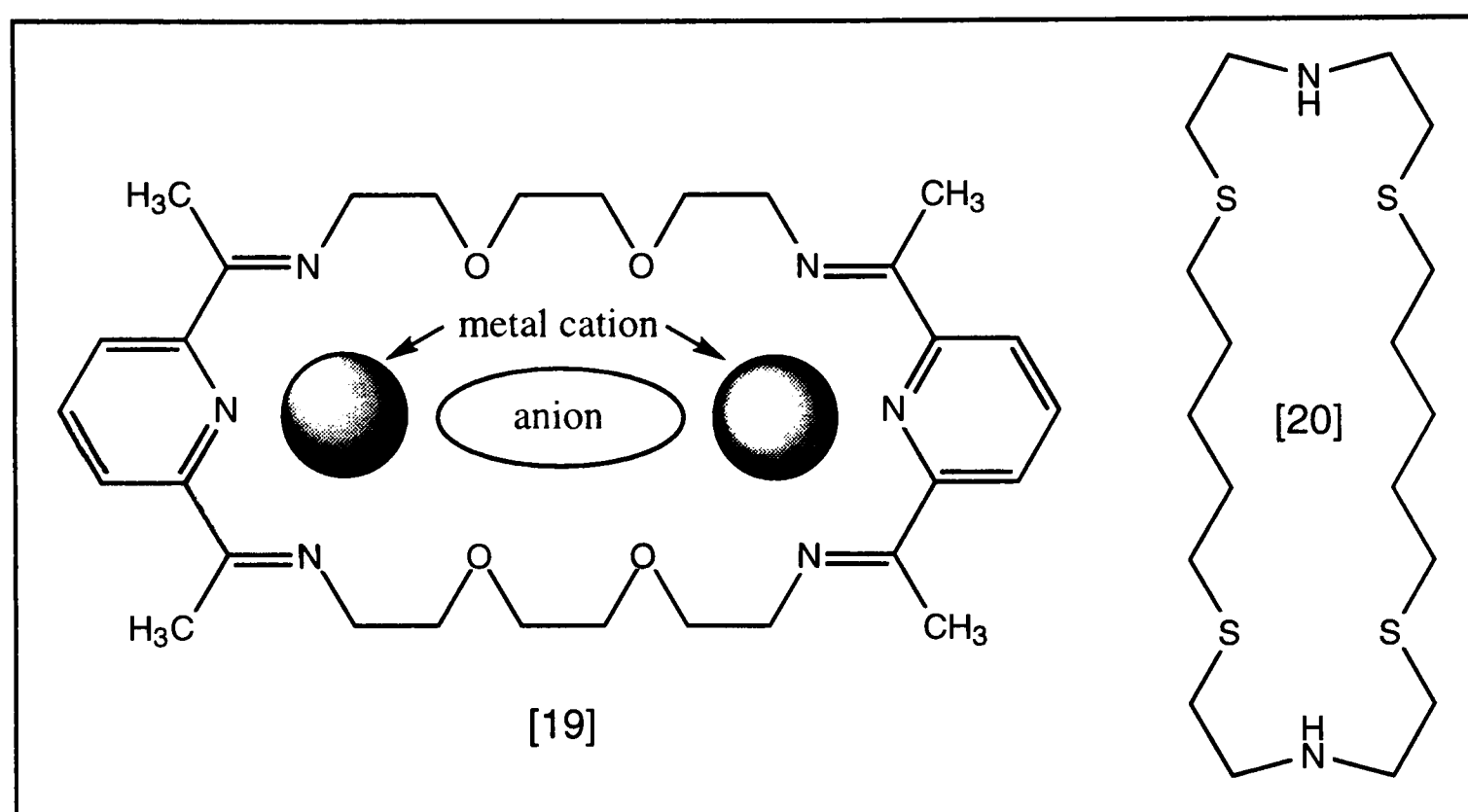
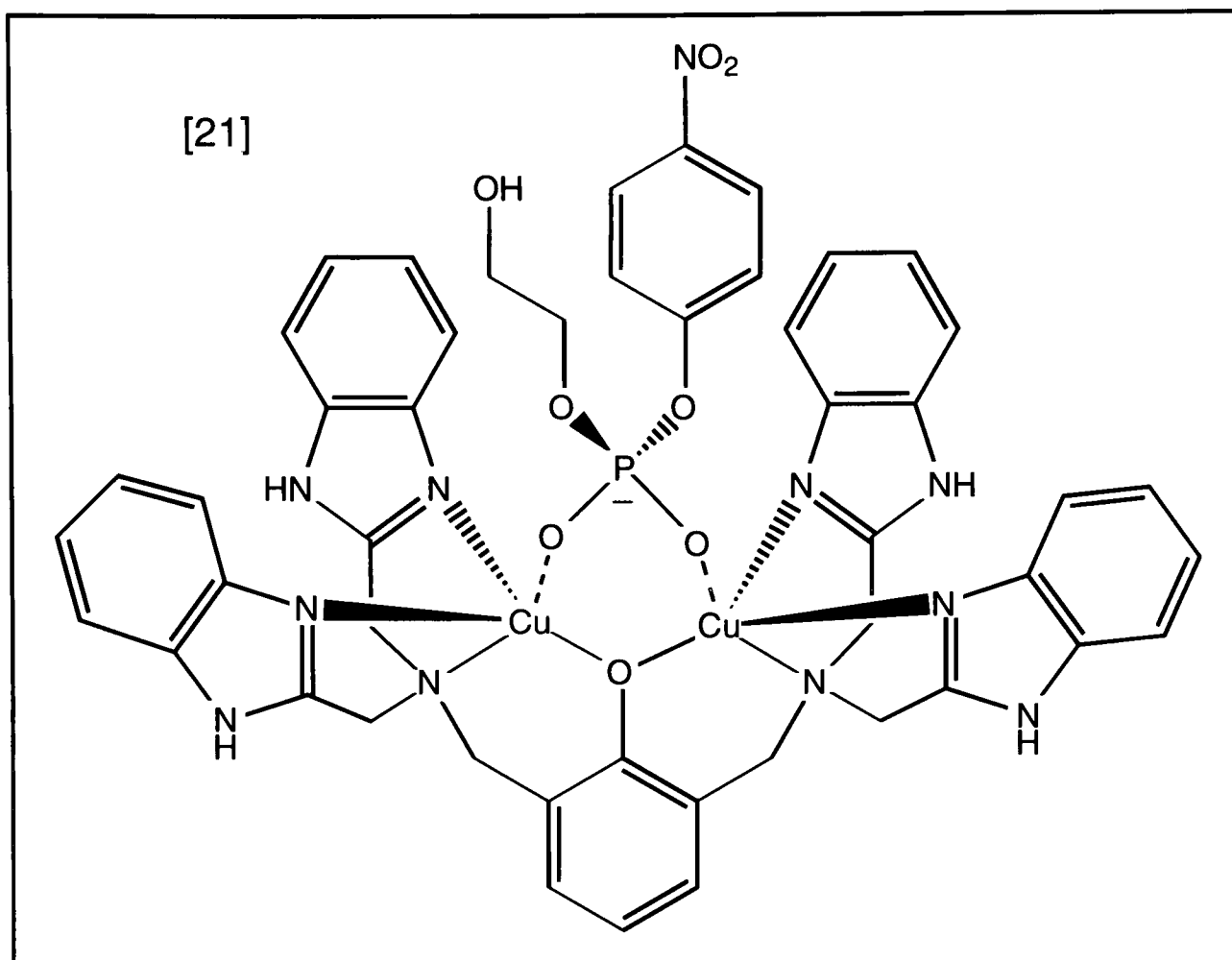


Figure 1.19: Potential cascade complexes. Binding is shown schematically in one case.

The notion of cascade binding is not new, of course. Many enzymes are known to bind metal co-factors as a prelude to receiving the substrate. Phospholipase C, to name but one, has two bound zinc (II) ions at the active site which are bridged by hydroxide and aspartate. The hydroxide anion is bound and the active site is also capable of accommodating phospholipids.⁷⁴ Such a situation was mimicked by the receptor [21] which binds phosphate between two copper (II) ions with a resultant increase in the rate of hydrolytic cleavage of the phosphate itself.⁷⁵



Another precedent is found in organometallic chemistry where metal cluster compounds frequently have anionic ligands in a bridging position. A number of complexes have been synthesised based on diphenylphosphine and either copper or silver. Crystal structures show perchlorate and nitrate bound bridging the two metal ions.⁷⁶ Other such systems have been published with carboxylates, phosphates and hydroxide and represent a small fraction of the examples on offer.

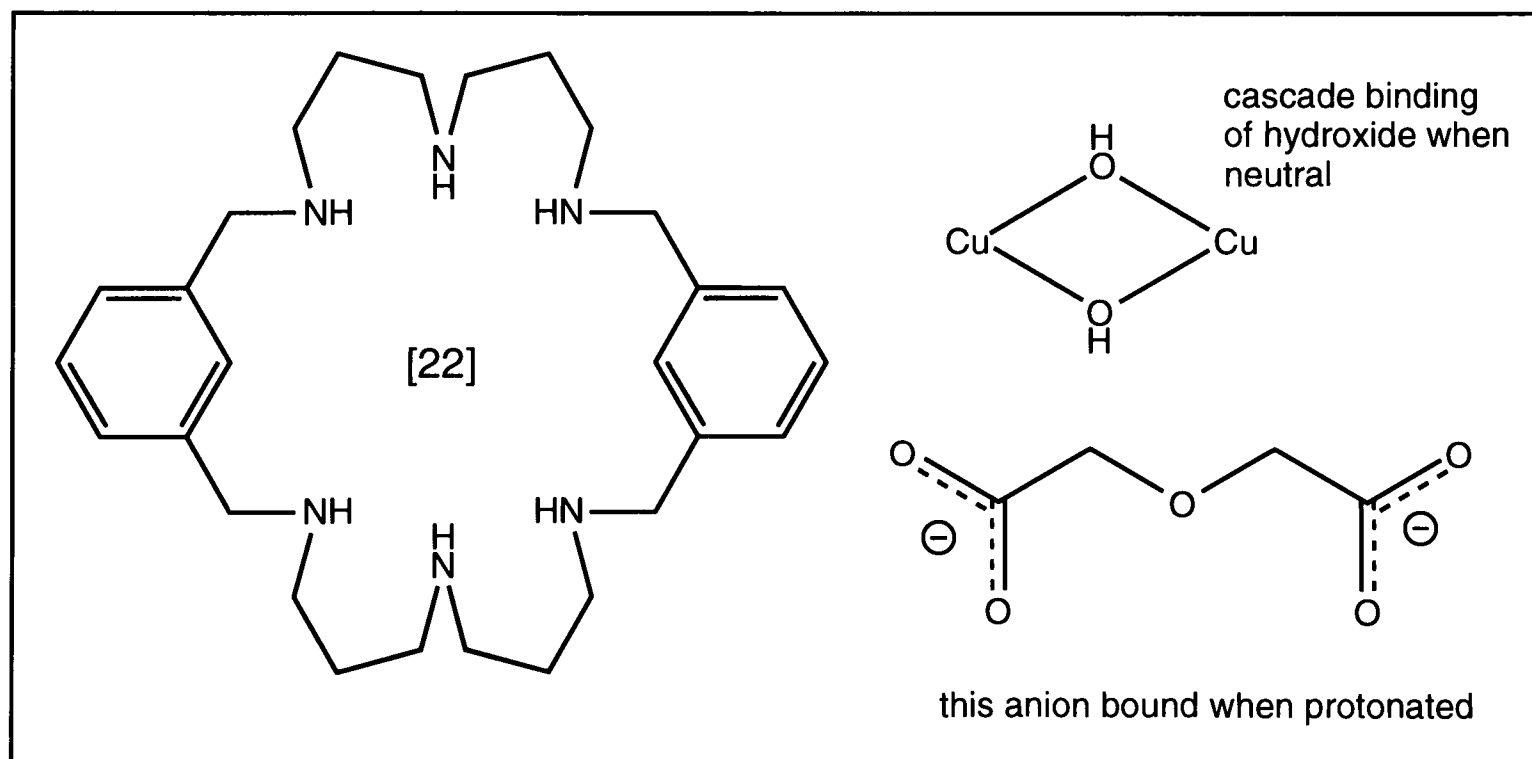


Figure 1.20: Bi-functional anion recognition.

A recent publication blends both binding by protonation with cascade binding. The receptor [22], shown in figure 1.20, is a polyaza macrocycle which, when protonated, is shown to include oxydiacetate. In the neutral state, two copper (II) ions are bound which are linked by bridging hydroxide as shown; in either case the exponents of anion recognition will be pleased to note the inclusion of a negatively charged guest. Thus two modes of action are available, dependent on pH.

1.3iv Expanded Porphyrin

One class of protonated macrocycle remains: a specialised example, developed by Sessler. The recognition of anions was initiated by work on sapphyrin, [23], an expanded version of porphyrin (figure 1.21). A crystal structure of the diprotonated form displayed a bound atom, which was eventually identified as fluoride.⁷⁷ Further study established stability constants in methanol: $\approx 10^5 \text{ dm}^3\text{mol}^{-1}$ for fluoride and $\approx 10^2 \text{ dm}^3\text{mol}^{-1}$ for chloride and bromide. Structural investigation also showed that chloride is bound above and below the ring and therefore does not experience hydrogen bonding as strongly as fluoride which finds itself in the plane of the ring: hence the lower binding constant.⁷⁸ Sapphyrin itself has been attached to a silica solid support and proves a good receptor for phosphate. An expanded version of the sapphyrin, [24], has a larger cavity which is now capable of embracing chloride when protonated and acts as a phase transfer agent. X-ray crystallography confirms the location of the anion.

These provide examples in which a macrocycle is binding within its ring, much like a crown ether but in this case with the added requirement of protonation to give the reduced charge density. Some ability to discriminate between guests based on a good size correspondence is observed, as might be expected for such a system.

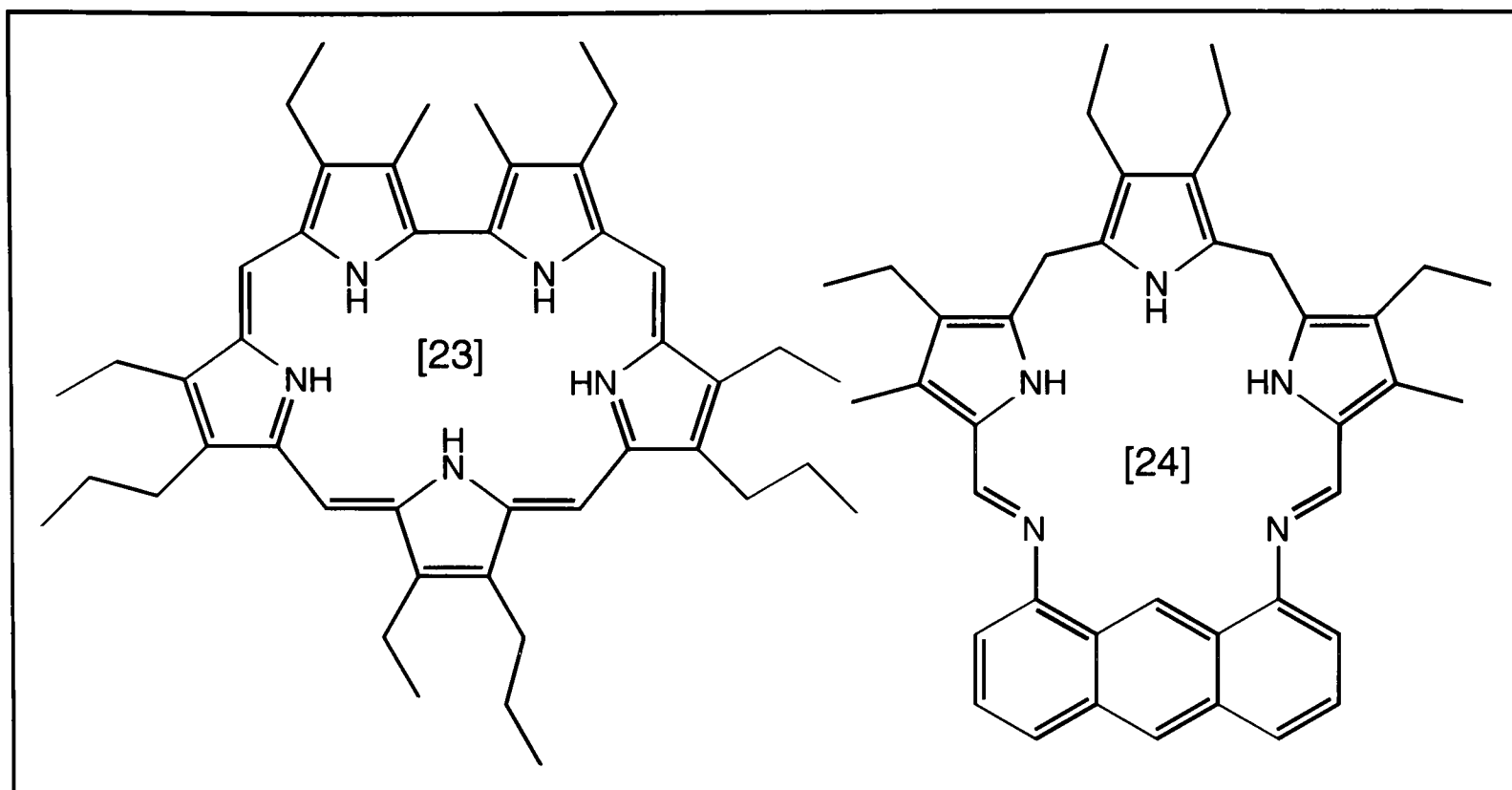


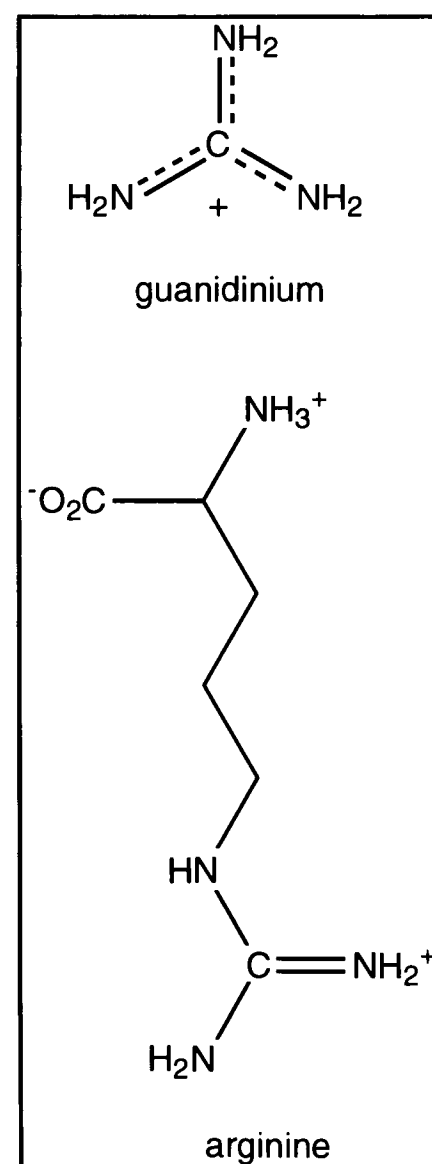
Figure 1.21: Sapphyrin based receptors.

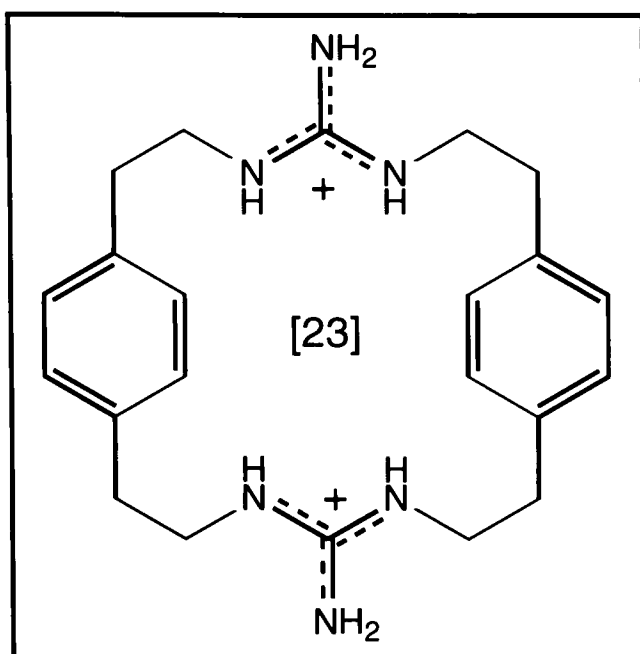
1.3v Guanidinium

A different approach to anion binding is displayed in the guanidinium based receptors. These grew out of a desire to employ a positively charged function which would not rely on low pH for protonation. Guanidinium was a good candidate as it was known to be involved in anion coordination at a number of enzyme active sites via the arginine residue which incorporates a guanidinium unit. An example is staphylococcal nuclease which has two arginine residues at the active site. Site-specific mutagenesis reactions have shown that they are closely involved in the enzyme action via charge neutralisation and proton transfer.

79,80

The utility of guanidinium lies in resonance stabilisation of the protonated form, which makes it a very strong base ($pK_a = 13.54$) and ensures that the system bears a positive charge over a wide pH range;⁸¹ hence the potential application in anion receptors. A precedent can also be found in published crystal structures which display a close association between methylguanidinium and dihydrogenphosphate anion.⁸²





Receptors such as [23] took advantage of the favourable properties mentioned above; these exist as positively charged macrocycles which were expected to bind anions by virtue of a combination of Coulombic, hydrophobic and hydrogen bonding interactions. Their potential was fulfilled in the binding of phosphate but with rather low binding constants.

Lehn conducted studies⁸³ on a number of similar macrocyclic systems with phosphate based anions such as PO_4^{3-} , HPO_4^{2-} , $\text{P}_2\text{O}_7^{4-}$ and confirmed the conclusion that anion binding is possible and yields stability constants in water (ionic strength, 0.1; pH 7) in the range 10^2 – $10^4 \text{ dm}^3\text{mol}^{-1}$.

In general, the binding strength of such receptors is weaker than protonated ammonium versions; a fact which can be attributed to the more delocalised nature of the charge lowering the charge density at the binding site. However, some very satisfactory results have been published. The receptor [24] is able to extract quantitatively p-nitro benzoate from water into chloroform⁸⁴ and the complex is found to have a stability constant of $1600 \text{ dm}^3\text{mol}^{-1}$ (figure 1.22). The chiral nature of the molecule also imparts the ability to effect enantioselective recognition.⁸⁵

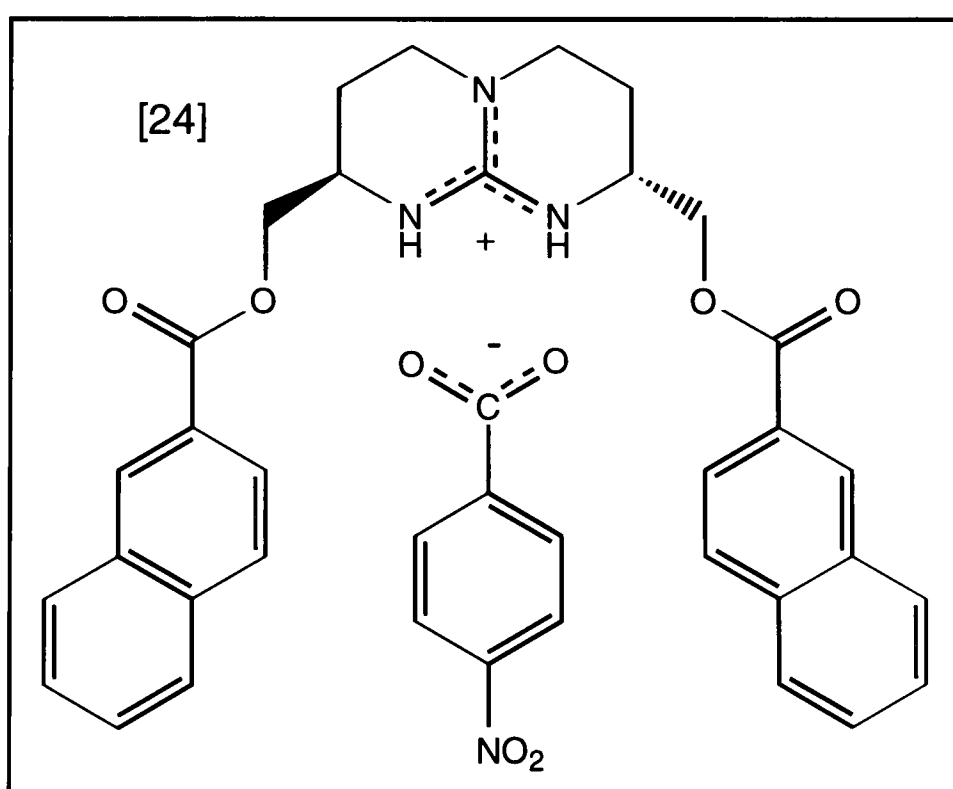


Figure 1.22: Extraction of p-nitro benzoate.

The superior performance exhibited here is attributable to the presence of several binding features acting in concert. Guanidinium provides Coulombic and hydrogen bonding interactions while the side arms also supply a hydrophobic cavity and opportunities for π - π stacking.

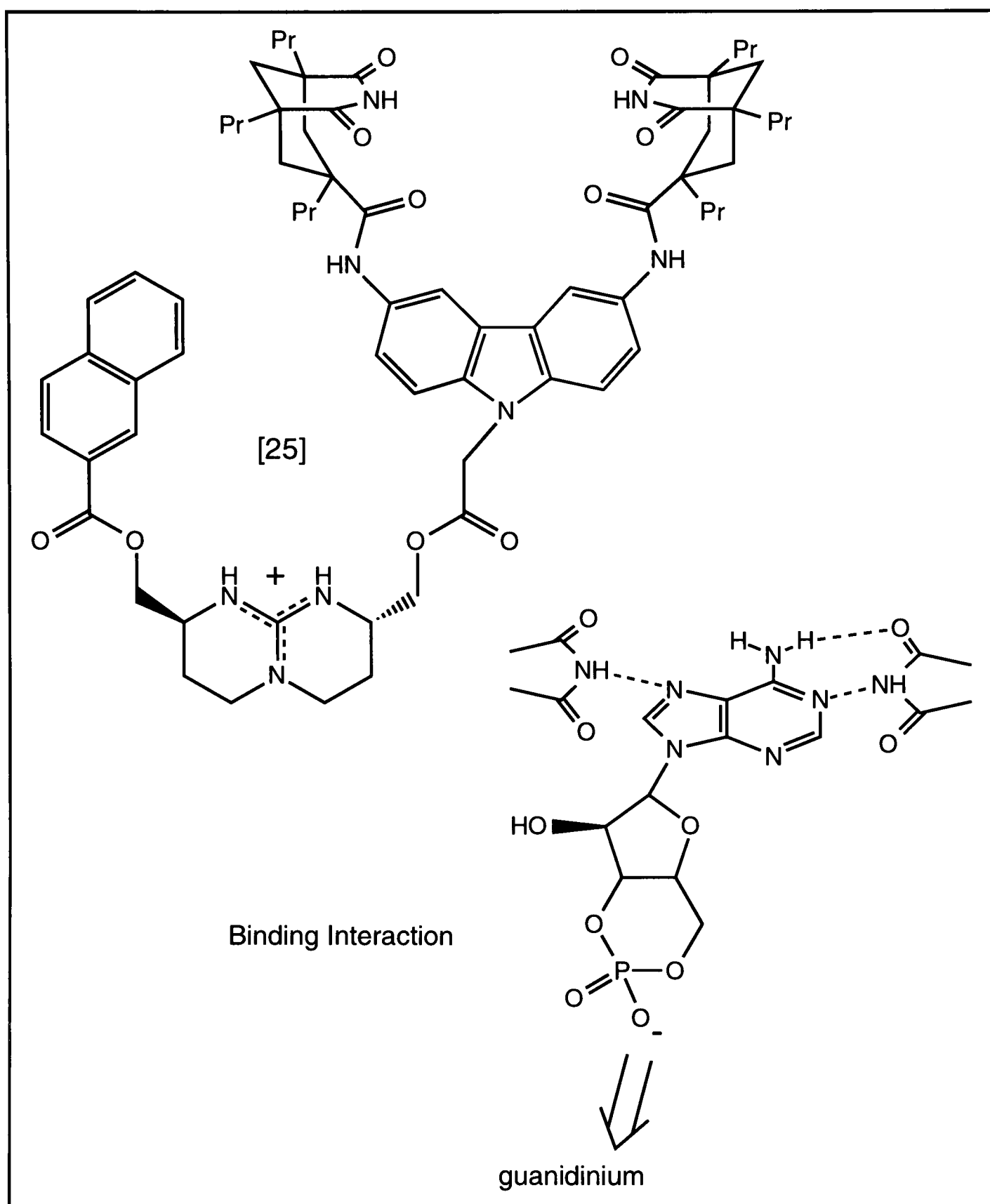
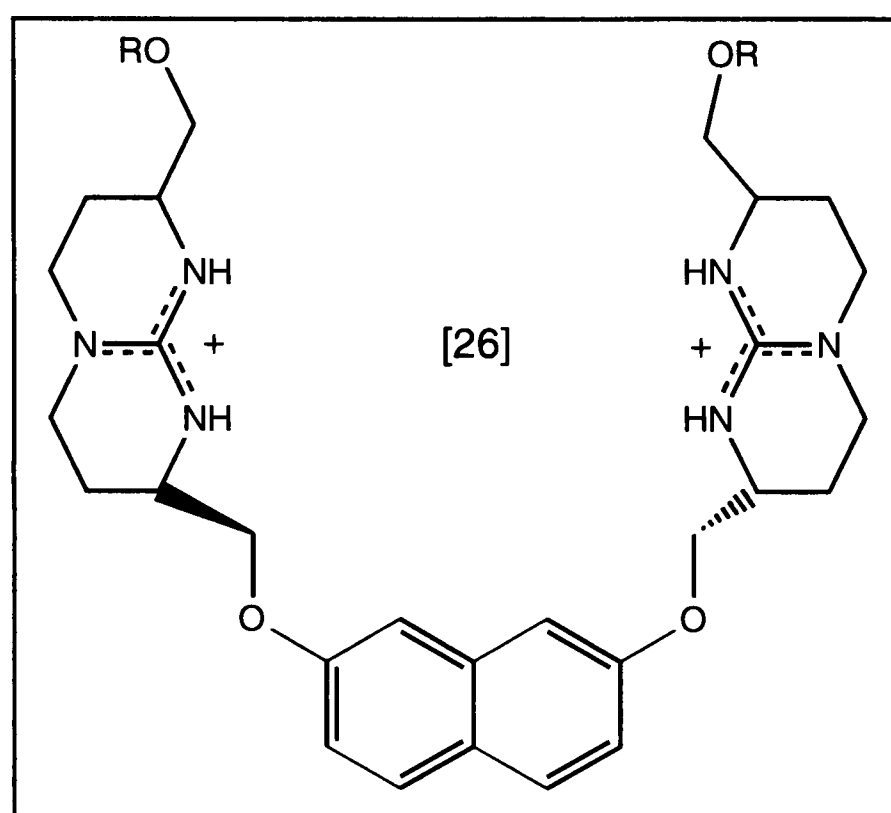


Figure 1.23: Extraction agent for 3',5'-cAMP.

The bicyclic motif has appeared in a number of published receptors. An analogue of [24] is functionalised with a crown ether in place of one anthracene, and the combination of both cation and anion binding sites allows binding of the zwitterionic amino acids. The remaining anthracene unit participates in π - π interactions and particularly promotes the inclusion of

phenylalanine and tryptophan.⁸⁶ Similarly, the system shown in figure 1.23 is a good extraction agent for 3',5'-cAMP by virtue of the interaction between phosphate and guanidinium, and hydrogen bonding to adenosine.⁸⁷

The same motif further appears in a number of receptors which incorporate two guanidinium groups. The molecule [26], with $R = -\text{SiPh}_2^t\text{Bu}$, acts as an extraction agent for dicarboxylates and biologically related phosphates. Some binding studies have been performed in water, but with very poor results.⁸⁸ Further work⁸⁹ quantified the binding of a number of dicarboxylates in methanol and identified stability constants in the range 10^3 – 10^4 $\text{dm}^3\text{mol}^{-1}$. In an attempt to improve water solubility, R was replaced with H and the resultant receptor was found to bind phosphates with stability constants of the order of 10^3 $\text{dm}^3\text{mol}^{-1}$. Binding with some biologically significant phosphates (e.g. 5'-AMP) was also observed.⁹⁰



The ability of guanidinium systems to bind phosphates and also take part in proton transfer reactions has led to the discovery that phosphate ester cleavage can be accelerated. The receptors shown in figure 1.24 both demonstrate such behaviour; [27] catalyses the cleavage of phosphodiester, ^{91,92} along with a close analogue with appended tertiary amines,⁹³ and [28] is seen to facilitate mRNA cleavage.^{94,95}

It has also been noted that guanidinium has a particular affinity for the hydrogensulphite anion and the receptor [29] has been incorporated into an ion selective electrode by virtue of its solubility in a number of plasticisers.⁹⁶

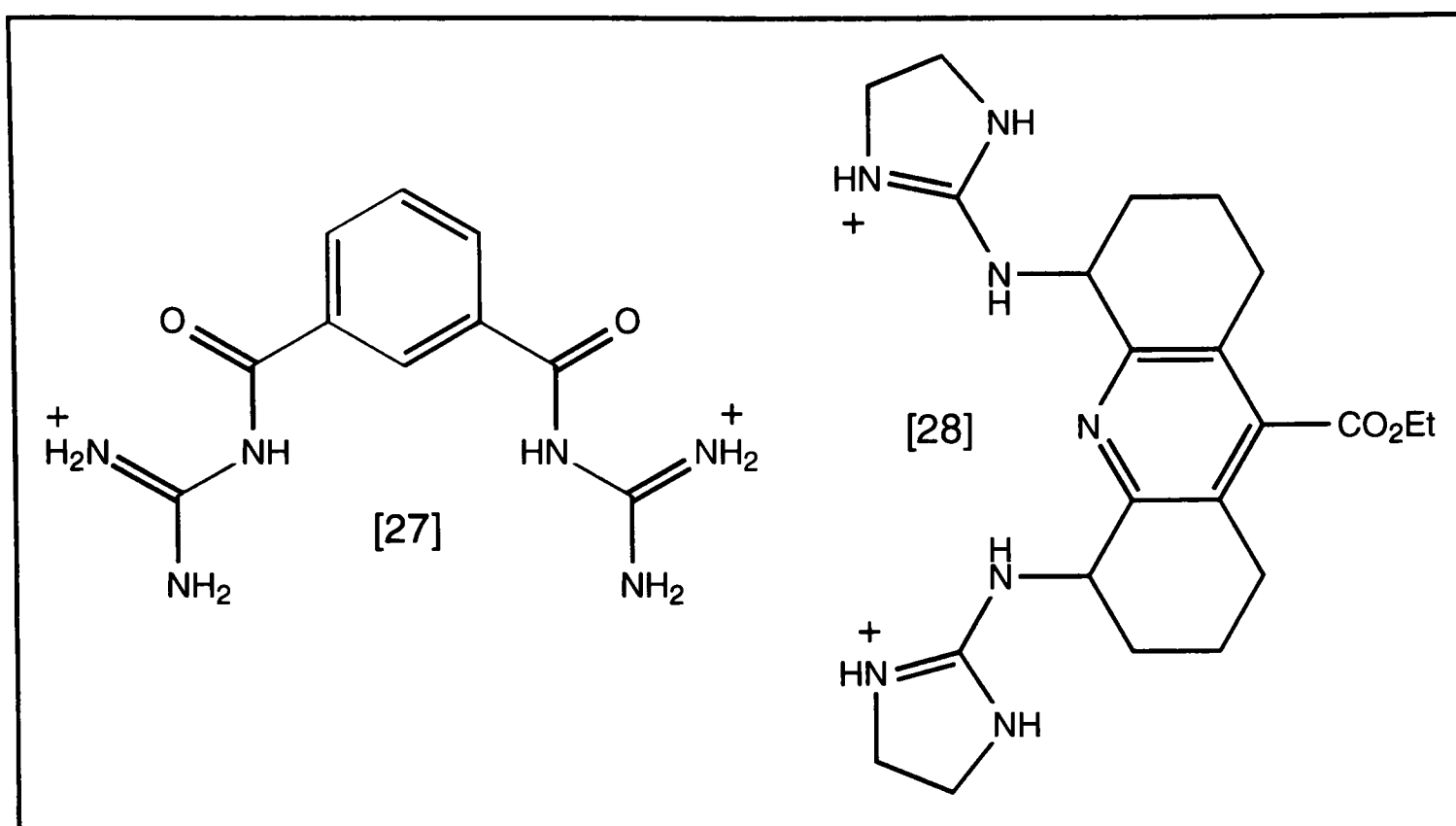
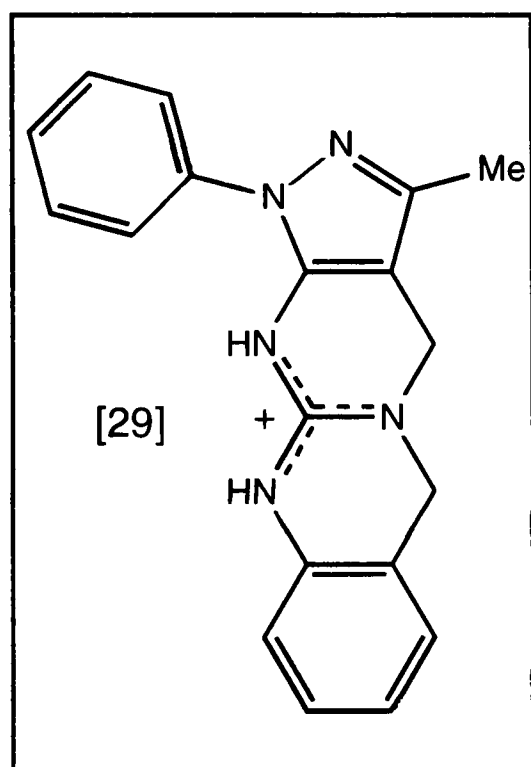


Figure 1.24: Catalysis of phosphate ester cleavage.

Guanidinium has in the past been perceived to be less effective in the binding of anions than protonated ammonium systems, the reduced efficacy being attributed to the distribution of

charge density over a number of atoms in the delocalised cation system. However, an increasing number of papers attest to its entirely satisfactory performance, particularly in the coordination of phosphates and carboxylates. Here, then, is a demonstration of the potency of hydrogen bonding coupled with a region of positive charge. Noteworthy also is the directional nature of the hydrogen bonding; the species bound most strongly are those that form two linear hydrogen bonds with the guanidinium as demonstrated in figure 1.22.



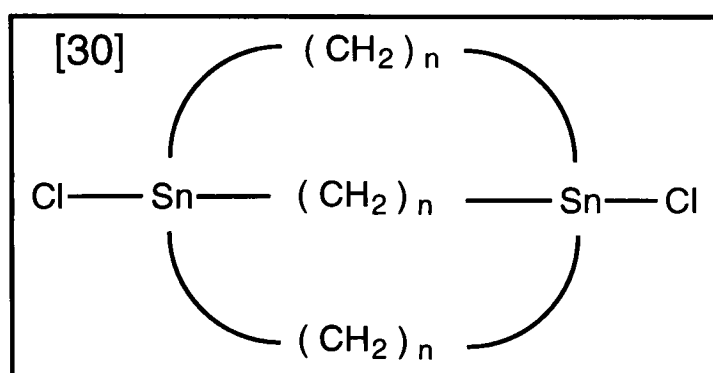
1.3vi Lewis Acid-Based Receptors

Some anion receptor design has turned aside from the notion that a positive charge is required and instead fulfils the need for low electron density by incorporating a series of

Lewis acid sites which are able to accept electron density from the anion. Several different approaches have been developed within this category.

Tin

Binding with organotin compounds^{97,98} showed some early promise; it was incorporated into the macrobicycle [30]. Analogues with $n = 6, 7, 8$ and 10 have been synthesised and display some size selectivity. When $n = 6$, interaction is only seen with fluoride⁹⁹ and chloride is bound when $n > 6$. X-ray crystal structures have confirmed that the anion resides within the cavity.¹⁰⁰



Solution studies, however, are less exciting since stability constants are all below 50 in chloroform and < 1 in a number of cases, a situation which can hardly be described as satisfactory.¹⁰¹ Comparison can be made with the similar structure of Simmons and Park's first anion

receptor and suggests that the Lewis acid approach is much inferior to protonated amines. Slightly better are the macrocycles shown in figure 1.25 which bind chloride and bromide and transport them across an organic phase.¹⁰²

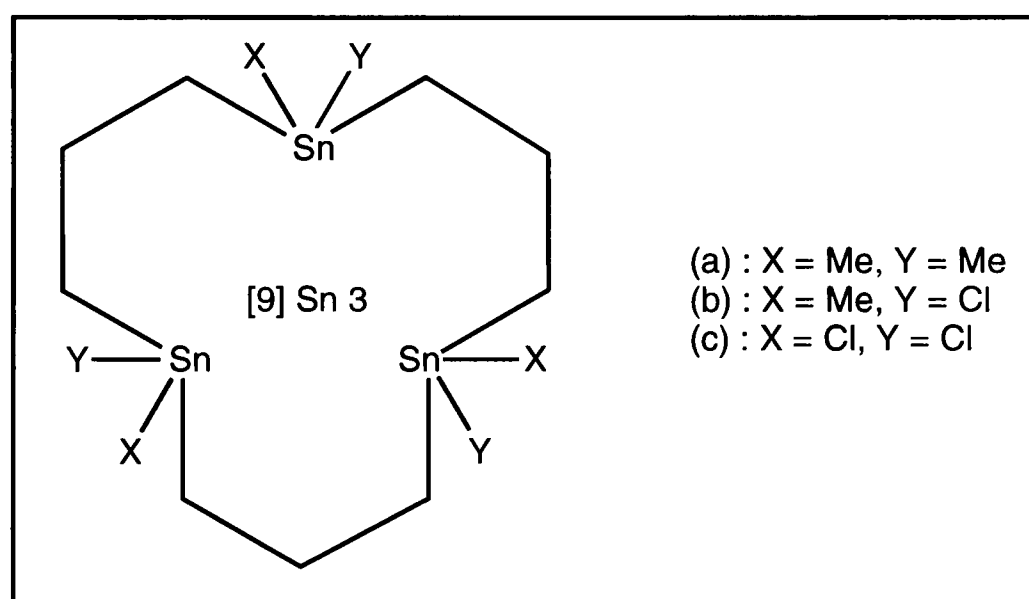


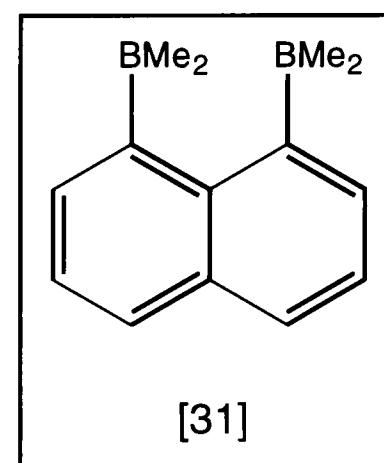
Figure 1.25: Tin macrocycles for anion binding.

Receptor (c) has been investigated with respect to chloride binding and appears to hold the anion in a bridging position between just two of the tin atoms.¹⁰³ No quantitative data are available for the complexes.

Boron

Boron is a well known Lewis acid and has accordingly appeared in the guise of an anion receptor. The very simple system, [31], described as a “hydride sponge”, is implied as a host for the hydride anion.¹⁰⁴

Furthermore, nmr evidence points to interaction with fluoride and hydroxide which presumably bridge the two boron atoms.¹⁰⁵ However, two points of concern can be raised. Firstly, the compound is found to be very moisture sensitive which severely limits its operation and secondly, the hydride “binding” is irreversible which begs the question about distinguishing between a binding process and a reaction. The analogue in which methyl is replaced with chlorine is found to bind chloride again bridging the boron atoms. X-ray analysis confirms the structure.¹⁰⁶



Reetz produced a different receptor, [32], in which the boron atom was included within a crown ether type structure shown in figure 1.26. It is found to bind salts such as KF, with the cation bound within the crown, and fluoride, bound by a combination of attraction to the Lewis acid boron and the Coulombic interaction with the cation held by the crown.¹⁰⁷

Mercury

The inclusion of mercury within anion receptors has led to the remarkable so-called mercuracarborands,¹⁰⁸ also shown in figure 1.26. Analogy is drawn with 12-crown-4, but here, the donor oxygen atoms are replaced by an acceptor, mercury. Hydrocarbon spacers are replaced with icosahedral C₂B₁₀H₁₂ units. The rationale is successful, and chloride is found to bind, an interaction which is attributed to p-orbital overlap.¹⁰⁹ X-ray structures also show both one and two ions of iodide included within the cavity.^{110,111} A smaller analogue, [9] mercuracarborand-3, has been synthesised and there is evidence of its interaction with chloride.¹¹² Recent work has also suggested that the anion can act as a template which governs the stereochemistry and size of the macrocycle during synthesis.¹¹³ It is notable in this work that a great deal of solid state evidence is produced and, given the known insolubility of such compounds, the absence of solution work is telling. This, then, may well be a receptor protocol confined to the solid state.

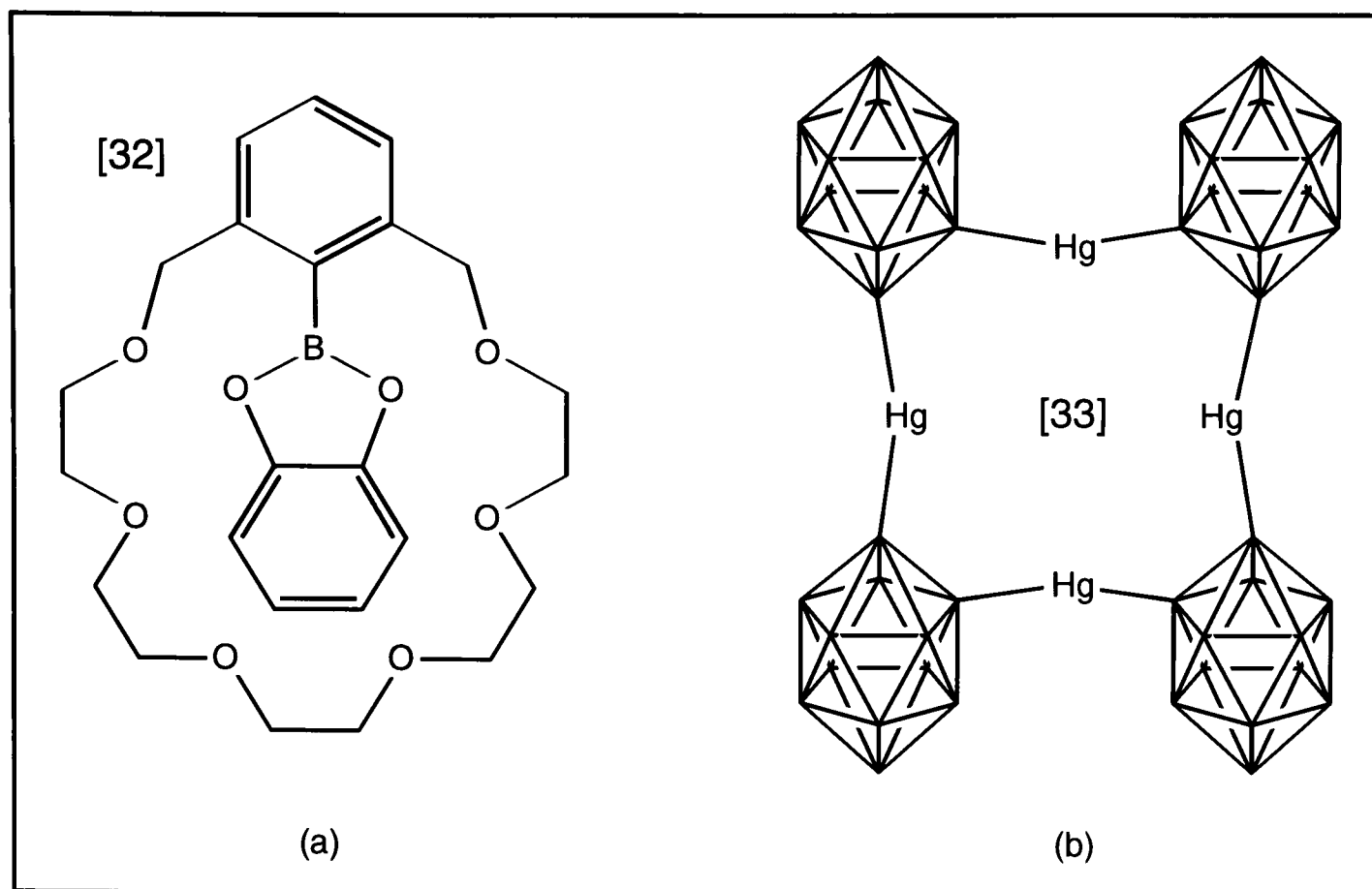


Figure 1.26: Lewis acid based receptors: (a) boron / crown ether; (b) [12] mercuracarborand-4.

Silicon

Just one example of silicon is notable in the field of anion recognition and comprises the now familiar strategy of including the Lewis acid site within a macrocycle. Three Me_2Si units are present within a 12 membered ring which is then seen to transport chloride and bromide across an organic phase.¹¹⁴

Uranyl Salenes

Reinhoudt reported receptors¹¹⁵ which bind the UO_2^+ group in close proximity to amide groups, thereby combining a Lewis acid with hydrogen bonding opportunities; an example is shown in figure 1.27. This is one of a series of similar molecules which proved that the binding strategy was a potent combination; anions, particularly dihydrogen phosphate, are bound in polar organic solvents with stability constants as high as $10^4 \text{ dm}^3\text{mol}^{-1}$.

A further modification saw the introduction of crown ethers at the end of the side arms which imparted the ability to bind both anions and cations simultaneously.¹¹⁶

It is not in doubt that the concept of anion binding by Lewis acids is successful, but it is important to gauge how well it works in comparison with other approaches. There is a lack of

numerical data on the subject, but the little that is offered seems to indicate that, alone, a Lewis acid is a poor receptor of anions. Therefore, whilst it may be helpful to include a Lewis acid within a suite of binding groups, designs of preparing a receptor which relies on such groups alone seem limited in potential.

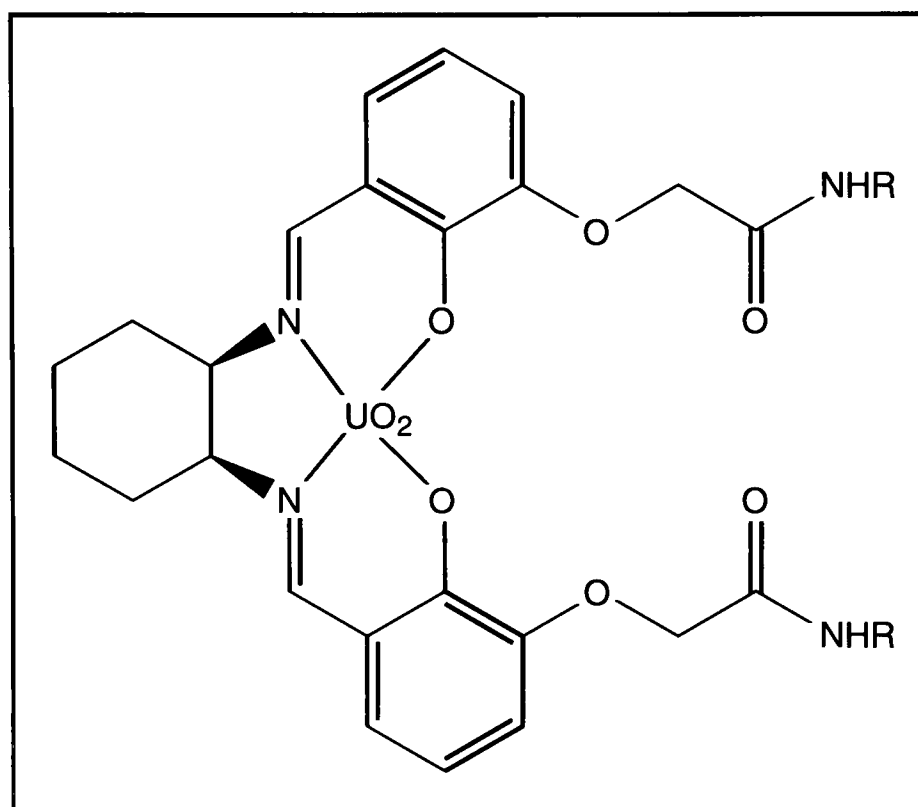


Figure 1.27: A uranium-based receptor.

1.4 Conclusion

The previous section has presented a brief review of the literature. It is not comprehensive, and efforts have been made to select particular examples which best illustrate the designs and concepts of those who have sought to create a host which appears welcoming to the anion guest. Further reading is provided by a number of review articles^{117,118} and introductory remarks specific to the areas directly connected with this thesis will appear at the beginning of the respective chapters.

1.5 Prologue to Subsequent Chapters

The aims of this project were to investigate a new approach to anion recognition. The protocol employed by Beer and co-workers is typified by certain common features which are included in the receptor design and illustrated schematically in figure 1.28. Precedents revealed by the literature led to the conviction that hydrogen bonding was an extremely important component of binding. However, it was also felt that the presence of a formal positive charge should be maintained because of its considerable assistance to the binding process. Some investigation of neutral receptors is reported and confirms this hypothesis. The functional bases of the hosts were therefore twofold:

1. The amide group: Primary and secondary amide groups contain a hydrogen atom bonded to nitrogen which is able to undergo hydrogen bonding. This group is largely inert to acids and bases and will therefore avoid complications caused by protonation. Furthermore, secondary amides allow the inclusion of further functional groups at both termini which can be used to probe interactions at the binding site.
2. The metal ion: Transition metal ions are a particularly suitable choice because they are readily enclosed within a ligand sphere which fixes them within the anion receptor. Present in a positive oxidation state, they are able to provide the source of positive charge if a judicious choice of ligand is exercised. Similarly, a knowledge of elementary ligand field theory allows the metal to be held in a position of considerable thermodynamic stability and kinetic inertness. A well chosen metal will also provide a number of means of detecting the arrival of the guest anion; the receptor then becomes a chemical *sensor*.

1.5i Chemical Sensors

The concept of a sensor is simple: binding of a guest mediates a measurable change in the physical properties of the receptor. This can then be monitored by an observer and frequently enables a quantitative assessment of binding to be made. There is also the potential for a commercial sensing device, but in this case the criteria are rather more demanding and include the following:

- a significant and linear response at the concentration levels of interest
- a high degree of selectivity over competing analytes
- accurate and repeatable performance
- inexpensive manufacture and operation.

The metal ions in the receptors to be discussed did show a change in physical property; indeed in several properties. Most notable were perturbations in absorptions in the UV-vis spectrum and in the redox potentials between accessible oxidation states. In both these respects the choice of a transition metal was especially expedient. Some of the receptors were also luminescent, a property which provides yet another mode of sensing.

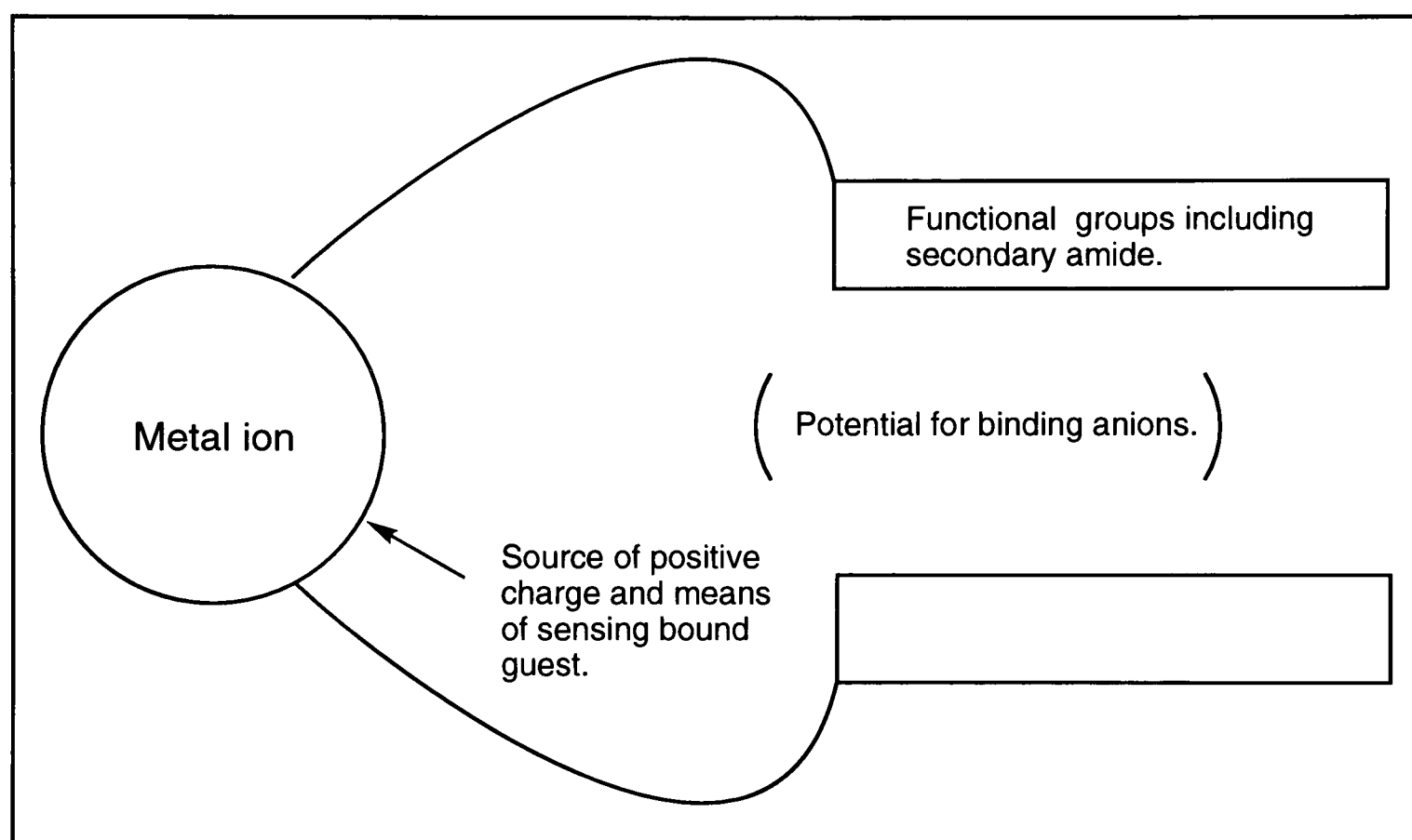


Figure 1.28: Protocol for design of an anion receptor.

Two classes of receptor are presented and exhibit different interpretations of the protocol outlined. The first is metallocene-based and centred on cobalticinium, a very stable sandwich compound with a positive charge. An investigation of a small number of ferrocene systems allowed comparison of charged and neutral receptors; Chapters Two and Three give details of synthesis and coordination properties of these hosts. The second class, metal tris (2,2'-bipyridyl) receptors, acted on a similar principle and are detailed in Chapter Four. More

specialised introductions will appear at the beginning of the respective chapters. In all cases, the primary aim was to investigate the origins and strength of binding by variation of nearby functional groups. Wherever possible a quantitative study was conducted and this goal in itself lead to an appreciation of the difficulties of stability constant calculation. In this way, the success of the protocol itself could be evaluated, but at the same time a more fundamental investigation of the process of anion binding could be made, and the results compared with the ever growing body of literature on this most interesting of topics.

CHAPTER TWO

Cobalticinium:

Acyclic Receptors incorporating an Amide Group

2.0 Introduction to Cobalticinium

This chapter is concerned with a number of receptors based on the organometallic fragment cobalticinium, $[\text{Cp}_2\text{Co}]^+$. Cobalticinium itself was first synthesised in 1952 by Wilkinson,¹¹⁹ one of a series of sandwich compounds discovered at that time. It was isolated as the picrate salt in 95% yield from the reaction of cobaltic acetylacetonate, $[\text{Co}(\text{C}_5\text{H}_7\text{O}_2)_3]$, with cyclopentadienyl magnesium bromide in benzene, and occurs as air-stable, yellow crystals, although the specific physical properties of the salts depend on the counter-ion present. Despite being isoelectronic with ferrocene, cobalticinium differs in its reactivity in several important respects. The presence of the formal positive charge renders the molecule inert to electrophilic substitution and also resistant to oxidation; aqueous solutions of cobalticinium are stable to such extreme conditions as treatment with boiling aqua regia, concentrated sulphuric acid and ozone. The molecule also has high thermal stability. In the behaviour of its salts, in fact, cobalticinium may be likened to a large alkali metal cation such as Rb^+ or Cs^+ .

Relatively little synthetic work has been reported and it is not a frequently used reagent. However, the reaction of cobaltocene with perfluorodecalin gives cobalticinium fluoride which has been used as a source of “naked” fluoride. This has found synthetic utility in metathesis reactions to give organofluorine compounds.¹²⁰

Instead of synthesis, work on cobalticinium has largely concentrated on its redox properties. A Japanese group has fabricated a mixed valence diode from neighbouring layers of poly-ferrocene and poly-cobalticinium,¹²¹ and its properties as an electrochemical reference have been probed.¹²² Similarly, it has been used as a probe in immunoassays where it was detected by square wave voltammetry and used to assay analytes at a nanomolar level.¹²³

2.1 Redox Chemistry of Cobalticinium

The redox chemistry of cobalticinium is relatively straightforward. Reduction to cobaltocene in aqueous solution is difficult to achieve chemically, a fact which may be rationalised by reference to its electrode potential; in 0.1 M perchlorate solution at pH 6.2, the reduction half wave potential is -1.16 V (measured at a dropping mercury electrode vs. the saturated calomel electrode).¹²⁴ However, electrochemical reduction can be carried out in the presence of an electrophile, leading to the exo-substituted product which subsequently collapses to the corresponding substituted cobalticinium salt.¹²⁵ The reduction product, cobaltocene itself, may be prepared and isolated by conventional air sensitive techniques. It is a dark purple solid which can be made to undergo reaction with mild electrophiles, although care must be taken to avoid oxidation reactions, to which it is prone.

The first stage of electrochemical reduction is reversible in a wide variety of solvents and occurs at a potential which is dependent on the solvent used, presumably due to the extent to which the cobalticinium ion is solvated. There is then a further reduction wave at strongly negative potentials which is confined to certain solvents. It occurs at around -1.9 V in acetonitrile and, in protic solvents, is strongly pH dependent, unlike the first wave. This is due to potential protonation of η^5 -cyclopentadiene to yield the η^4 ligand (figure 2.1).

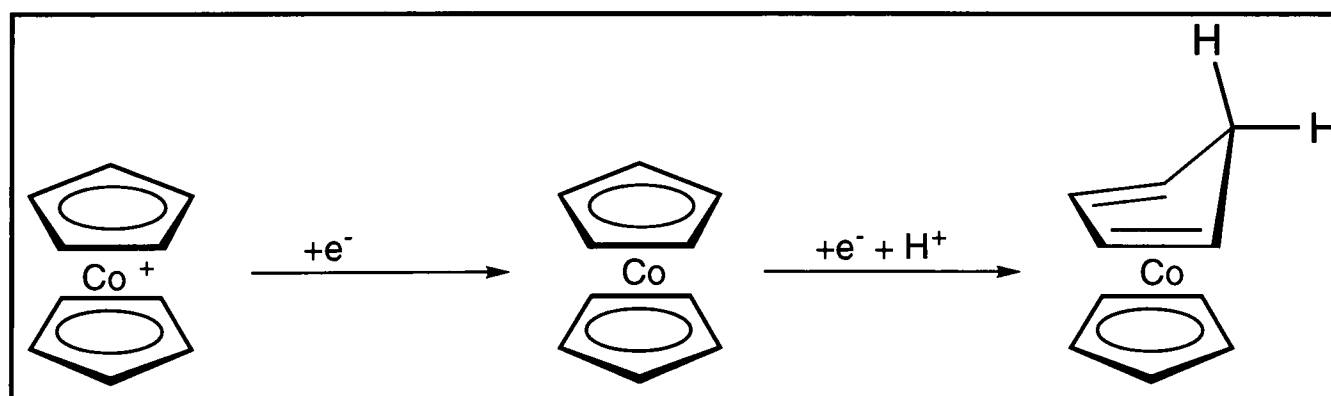


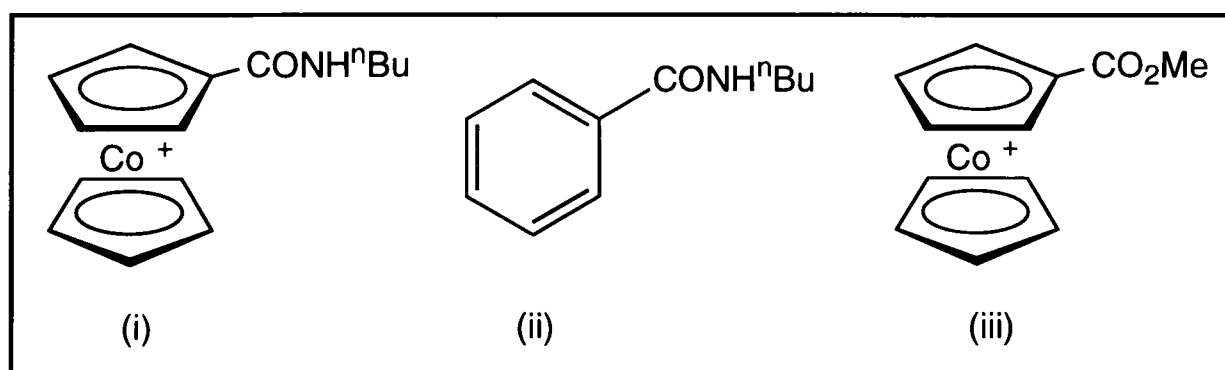
Figure 2.1: Electrochemical reduction of cobalticinium.

2.2 Cobalticinium as an Anion Receptor

The remarkable stability of cobalticinium and the presence of a formal positive charge make it a particularly suitable choice for the basis of an anion receptor. Moreover, the cyclopentadienyl rings are susceptible to functional group manipulation, which allows for the creation of a particular binding site in close proximity to the positive charge. However, additional factors were weighed. The ability to communicate information about binding processes is important and there were several properties of cobalticinium which fulfilled this requirement.

1. The proposed binding site lies in the vicinity of several key protons which allows ^1H nmr investigation of binding by examination of perturbations in chemical shifts.
2. The molecule is coloured and therefore has a UV-vis active chromophore. This absorption is likely to be sensitive to the presence of a bound anion.
3. Cobalticinium is redox active, with a readily determined half-wave potential between cobalticinium and cobaltocene. This will also be affected by the presence of a closely bound anion, allowing electrochemical binding studies to be performed.

Preliminary investigation showed that cobalticinium was indeed capable of functioning as an anion receptor and was followed by more detailed studies which confirmed amide-substituted cobalticinium as an efficient anion receptor and sensor.^{126,127} Control experiments highlighted the importance of both positive charge and, in particular, the amide group (capable of hydrogen bonding to the anion); in the series below, (i) binds chloride reasonably well in DMSO, (ii) binds much more weakly and (iii) shows little sign of interaction with the anion.¹²⁸



Some selectivity in binding could be induced by ditopic receptors which incorporated two cobalticinium units separated by a rigid spacer. In addition to binding two equivalents of simple anions, dicarboxylates such as adipate were bound in acetone with 1:1 stoichiometry. The titration profiles suggest that the interaction is one of some considerable strength.^{126,129}

Following publication of these results, details of a chiral receptor based on cobalticinium (figure 2.2) were reported. The receptor was found to bind tosylate and bound (+)-camphor-16-sulphonate with modest enantioselectivity.¹³⁰

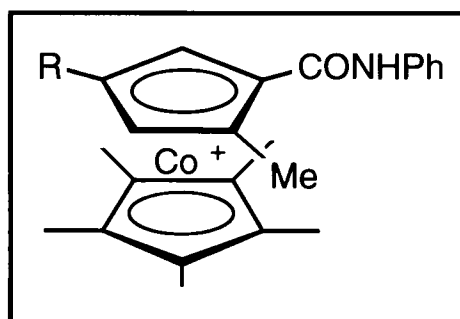


Figure 2.2: A chiral cobalticinium receptor.

The receptors discussed herein are mostly based upon mono-substituted cobalticinium derivatives. These are more synthetically accessible than the corresponding bis-substituted derivatives and studies have suggested that the binding potential of mono- and bis- receptors is similar.¹²⁷ Previous work also indicated that the interaction could be tuned by varying the substituent; for example, in the case of aryl substituents, there is a correlation between binding and the Hammett parameter of the functional group appended to the aryl ring.¹³¹ A series of molecules was therefore prepared with a variety of substituents. An early target was the extended type of receptor containing three cobalticinium units as shown in figure 2.8 (p. 54) and to this end, a number of precursors were synthesised which incorporated a free amine function (figure 2.7, p. 52). It was found at an early stage that these molecules exhibited interesting coordination properties in their own right; in particular, the effects of varying the substituent were of interest. The ability to interact selectively with either chloride or dihydrogen phosphate was encountered in a number of cases and these trends were noted. There was also a real possibility of sensing behaviour and, coupled with promising binding potential, this made an attractive area of research.

2.3 Synthetic Approaches

Since the first reports of cobalticinium, several synthetic approaches to cobalticinium have been published. Successful routes included reaction of sodium cyclopentadienide¹³² with $[\text{Co}(\text{NH}_3)_6]\text{Cl}_2$ and of CpTi with cobaltous chloride.¹³³ The reaction of fulvenes¹³⁴ with cobalt salts yields a variety cobalticinium species, and 1,1'-disubstituted derivatives may be prepared according to the method shown in figure 2.3.¹³⁵

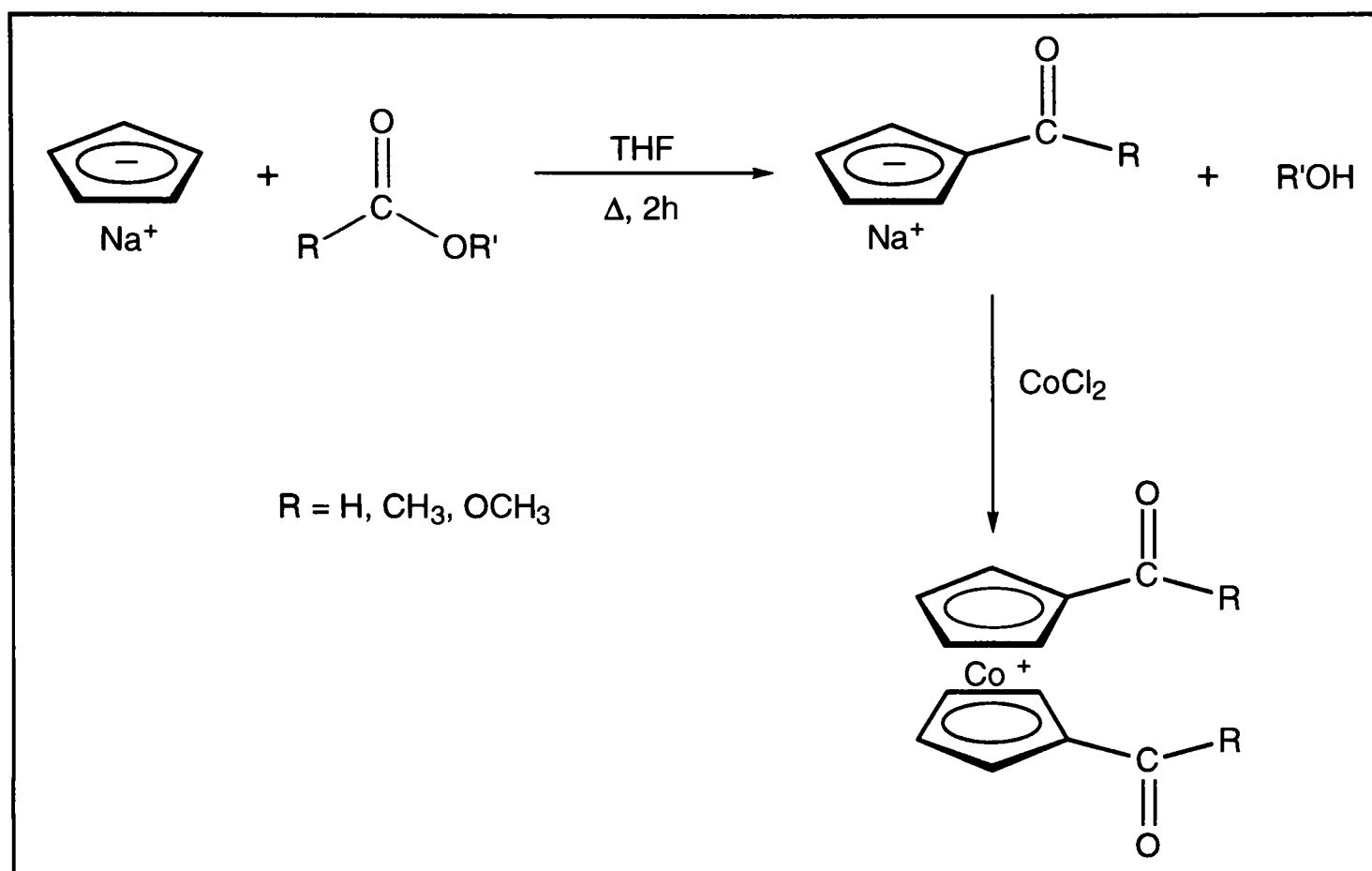


Figure 2.3 : Synthesis of 1,1'-disubstituted cobalticinium.

The synthesis of mono-substituted species is achieved either by attack of alkyl lithium on cobalticinium, followed by the abstraction of an endo-hydrogen by triphenylmethyl tetrafluoroborate (figure 2.4)¹³⁶ or by reaction of a mixture of methylcyclopentadiene and cyclopentadiene with cobalt (II) bromide in the presence of base.¹³⁷

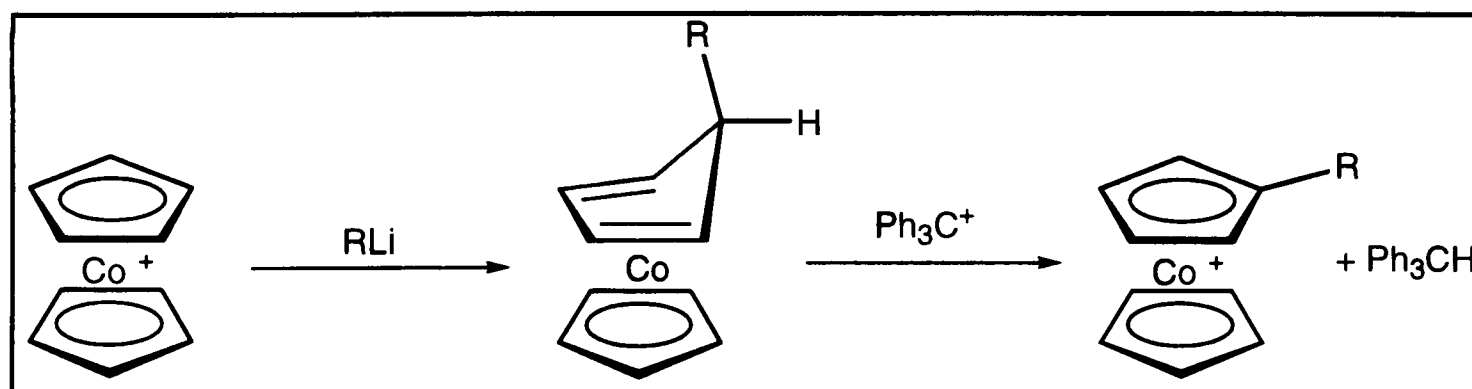


Figure 2.4 : Synthesis of mono-substituted cobalticinium.

In all reported cases, synthetic strategies for the preparation of substituted cobalticinium derivatives are confined to nucleophilic attack on cobalticinium or condensation of cobalt with pre-substituted cyclopentadiene. The resistance to electrophilic attack precludes other routes.

2.4 Synthetic Studies

In the light of previous work, a strategy was formed for the attempted receptor syntheses which drew on a common starting material in the form of mono(carboxy) cobalticinium hexafluorophosphate. This was prepared according to the method of Sheats and Rausch¹³⁷ as shown (figure 2.5). Cyclopentadiene dimer and its methyl substituted derivative were made to undergo a retro-Diels-Alder reaction by refluxing at 180°C for a number of hours. The monomer thus produced was collected by fractional distillation and persisted for some time when cooled by application of an ice bath. A mixture of cyclopentadiene and methylcyclopentadiene was then condensed with cobaltous bromide in the presence of a base (in this case, pyrrolidine) to give a statistical mixture of cobaltocene products. Aerial oxidation gave the respective cobalticinium salts which were isolated as the hexafluorophosphate salts. This mixture was oxidised by alkaline permanganate, at which point unsubstituted cobalticinium could be isolated. Acidification yielded the mono- and bis-carboxylic acids which were conveniently separated by extraction with acetone.

Having achieved the precursor in satisfactory purity, the synthesis of the cobalticinium receptors was largely conducted according to the synthetic pathway outlined in figure 2.6. Reaction was induced by addition of a slight excess of the peptide coupling agent dicyclohexylcarbo-diimide (DCCI) to a stirred solution of an amine and mono(carboxy) cobalticinium at room temperature. Acid and amine were present in equimolar concentrations. The solvent of choice was acetonitrile; it is sufficiently polar to dissolve all the reagents and was readily dried. Moreover, it is chemically inert to nucleophilic reactions. The use of dry solvent and an inert nitrogen atmosphere prevented competing reactions with water.

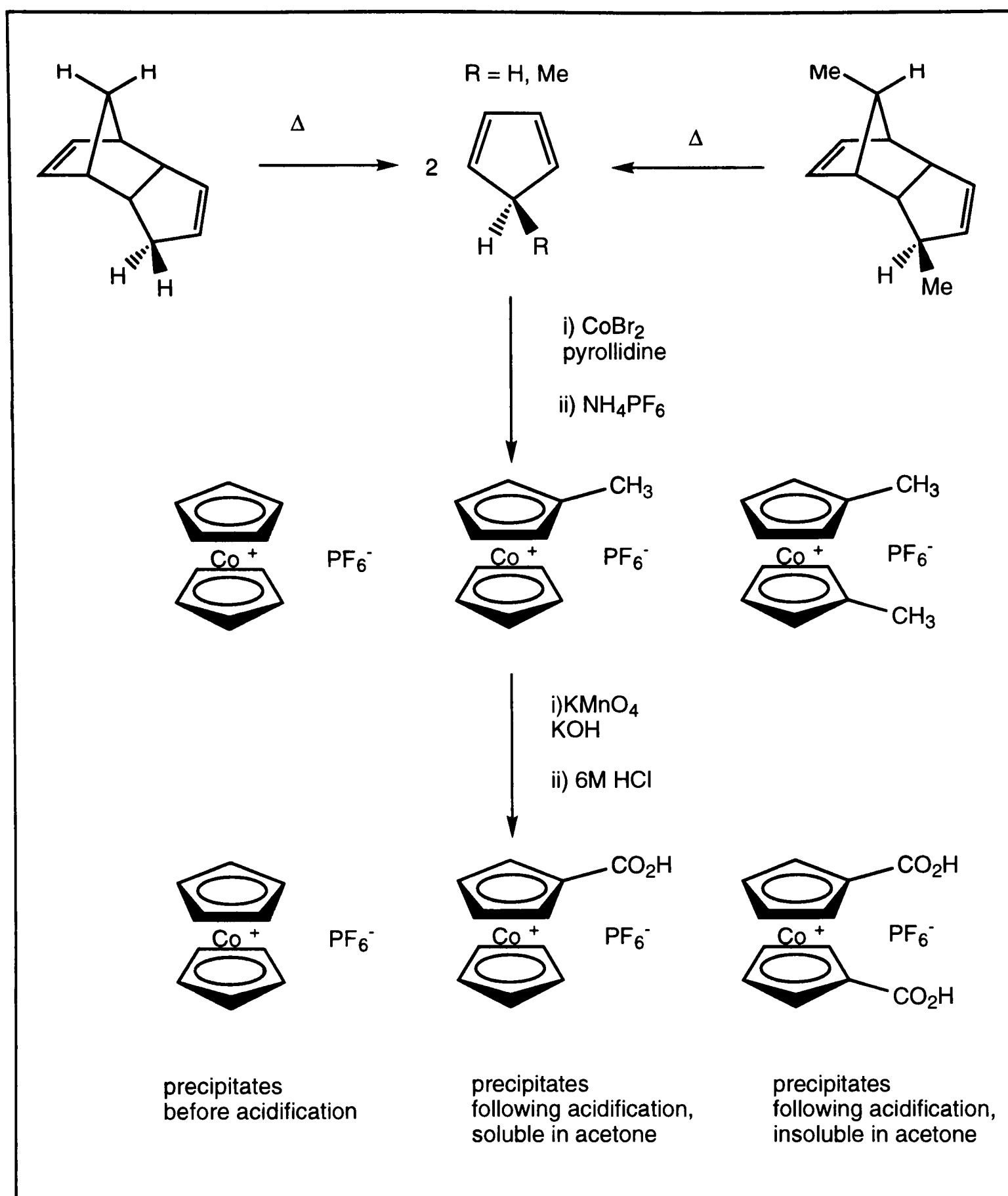


Figure 2.5: Synthesis of mono(carboxy) cobalticinium.

A mechanism for reaction is as follows. The amine is protonated by the acid and the resultant carboxylate attacks DCCI to give an activated intermediate which is in turn attacked by the amine to yield an amide and dicyclohexylurea. The latter, insoluble in most common solvents, forms as a heavy white precipitate within minutes. It was occasionally found that this did not occur and the absence of reaction was then attributed to a failure of the acid to protonate the amine, in which case preferential attack by the amine on DCCI occurs; nitrogen

is a stronger nucleophile than oxygen. The result of this attack is a stable guanidinium system which undergoes no further reaction.

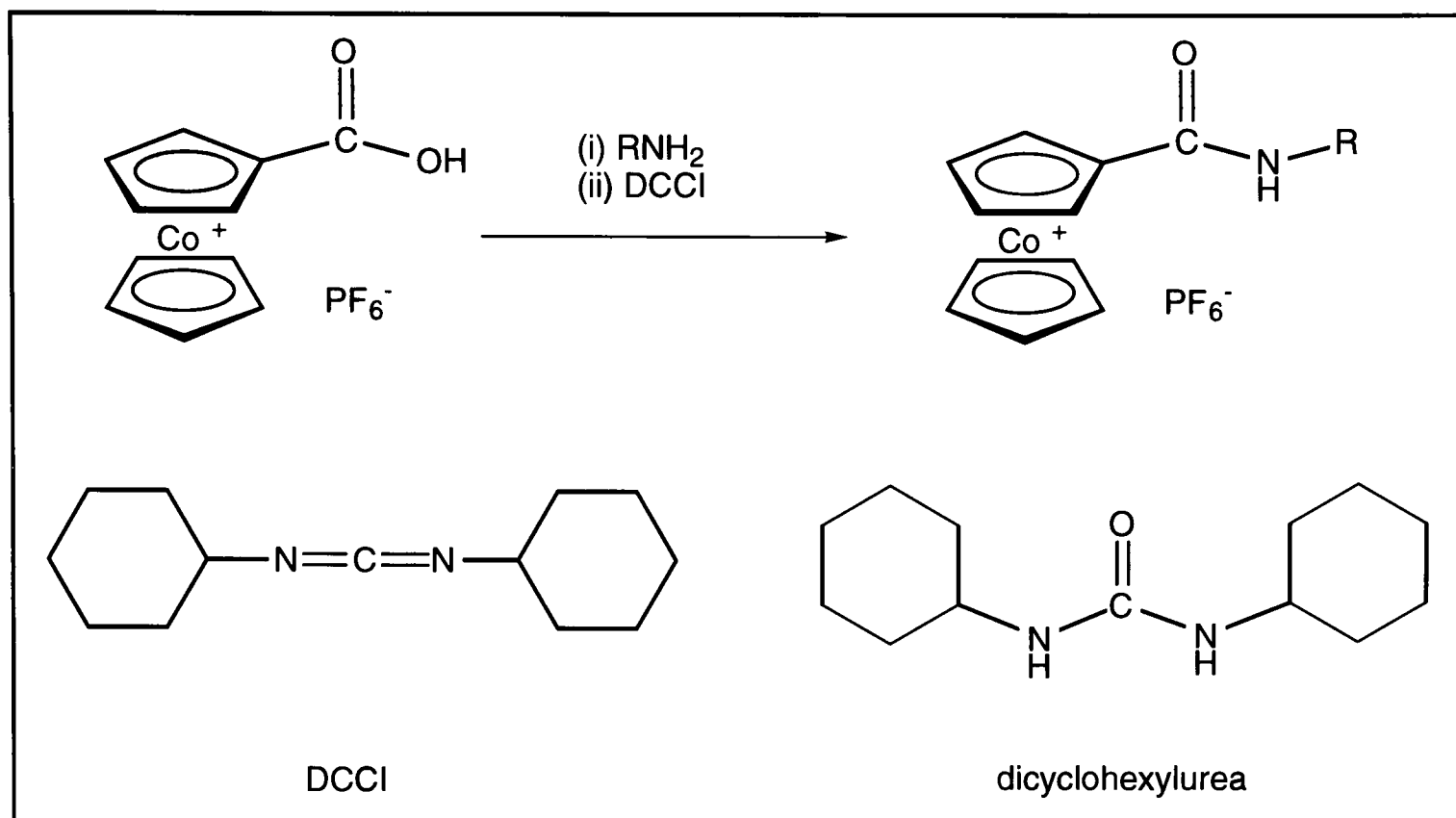


Figure 2.6: Synthesis of aminocarboxy-cobalticinium via direct coupling.

In most cases, the white precipitate was observed at an early stage but stirring was continued for some hours to ensure complete reaction. The solution was then filtered and the filtrate worked-up to give the product typically in 30–50% yield. The structures of the receptors isolated are shown in figure 2.7; in appearance the compounds were solids of a red, yellow or orange colour.

It was found that reactions of cobalticinium rarely proceeded cleanly and some form of purification was invariably required. At this point, certain difficulties arose. Attempts at recrystallisation were unsuccessful and the products were found to adsorb strongly to silica and alumina in chromatography, even with such polar eluents as methanol:ammonia. The presence of several inorganic anions in these chromatography media also added to difficulties by anion exchange with the product. The most satisfactory purification was achieved with Sephadex® LH-20-100, a gravimetric filtration gel suitable for use with organic solvents. The column was eluted with a mixture of methanol and acetonitrile as better performance was observed with polar solvent systems. Even so, the differences in molecular weight

between components of the reaction mixture was on the limit for resolution by Sephadex and more than one column was required in many cases, with a corresponding reduction in yield.

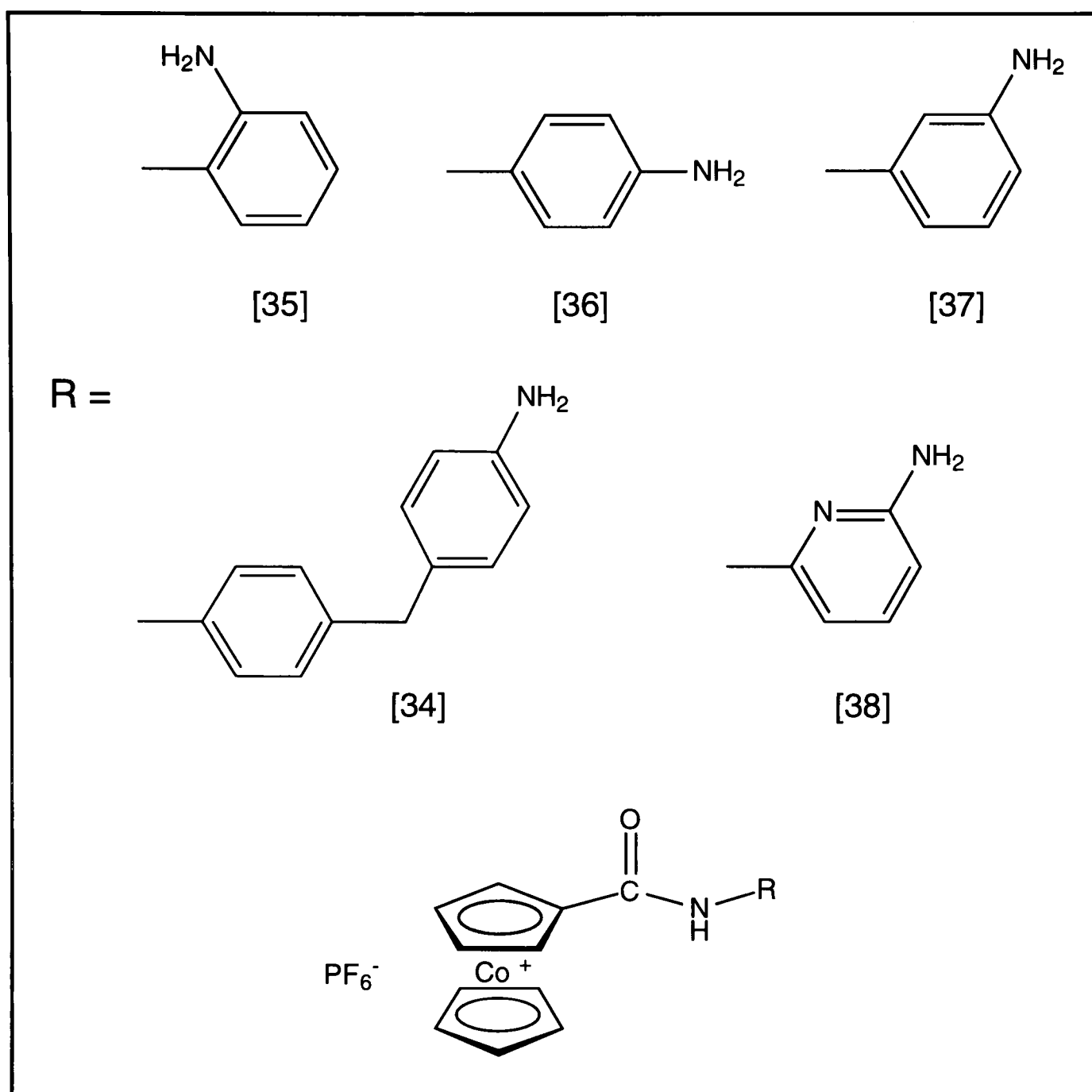


Figure 2.7: The amide-amine receptors.

Alternative synthetic routes were attempted, including using activated esters¹³⁸ (via N-hydroxy succinimide), acid chlorides and acid anhydrides as the activated intermediate. In many cases, these were found to be less satisfactory in terms of either yield or ease of purification. The activated ester, in particular, reacts poorly with aromatic amines, although success usually results in reactions involving primary aliphatic amines.¹³¹

Activated esters were formed from the reaction between mono(carboxy) cobalticinium and N-hydroxy succinimide; the reagents were coupled by addition of DCCI (figure 2.8). The product has a good leaving group which departs readily following attack of an amine. The method was successfully employed in the synthesis of receptors [40] and [44].

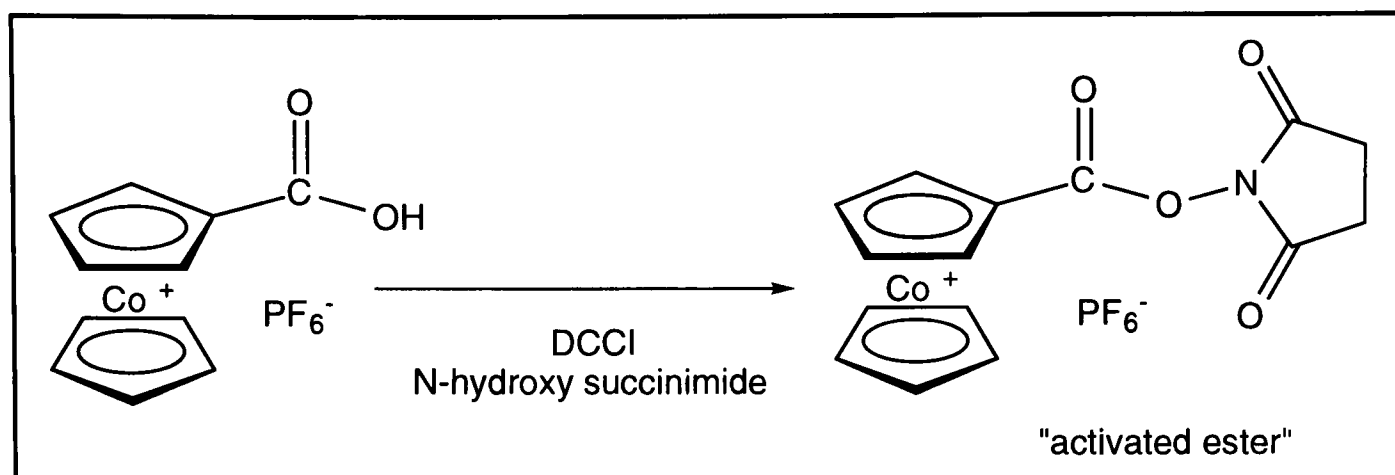


Figure 2.8: Formation of an activated ester.

The alternative synthesis via the production of an acid anhydride proved successful. An acetonitrile solution of mono(carboxy) cobalticinium was treated with 0.5 equivalents DCCl which, in the absence of any other nucleophile, promoted a self-condensation of the acid to the acid anhydride. This was, again, an activated species which reacted readily with an amine. For the purposes of mono-substitution of a diamine, a solution of the activated species was slowly added dropwise to a more concentrated solution of the amine. This ensured an effective excess of amine during the reaction which promoted the desired mono-substitution. The drawback of this method was the waste of starting material; half the carboxylic acid was regenerated in the course of the reaction. While it was possible to recover this by chromatography of the by-products with silica gel, the procedure added significantly to work-up and was considered a disadvantage of the method in comparison to alternative syntheses.

The most active derivative of a carboxylic acid is the acid chloride which, in the case of cobalticinium, was prepared by reflux in neat thionyl chloride for 48 hours followed by distillation of the excess reagent. While generally a satisfactory method for the preparation of an amide, in these particular reactions the acid chloride proved too reactive; a significant proportion of the crude product mixture was found to be the result of bis-substitution of the amine, which proved difficult to separate from the desired, mono-substituted product.

Bis-substituted receptors of the type shown in figure 2.9 were prepared by the condensation of an amine with bis(chlorocarbonyl) cobalticinium. The scope for condensations between amines and bis(carboxy) cobalticinium was limited by the very poor solubility of the latter in most organic solvents: there were very few solvent media in which a condensation reaction could be conducted. The acid was therefore suspended in thionyl chloride, which was taken

to reflux until the starting material had dissolved (usually a period of 48 hours); the corresponding bis-acid chloride was found to be soluble in a range of solvents including thionyl chloride.

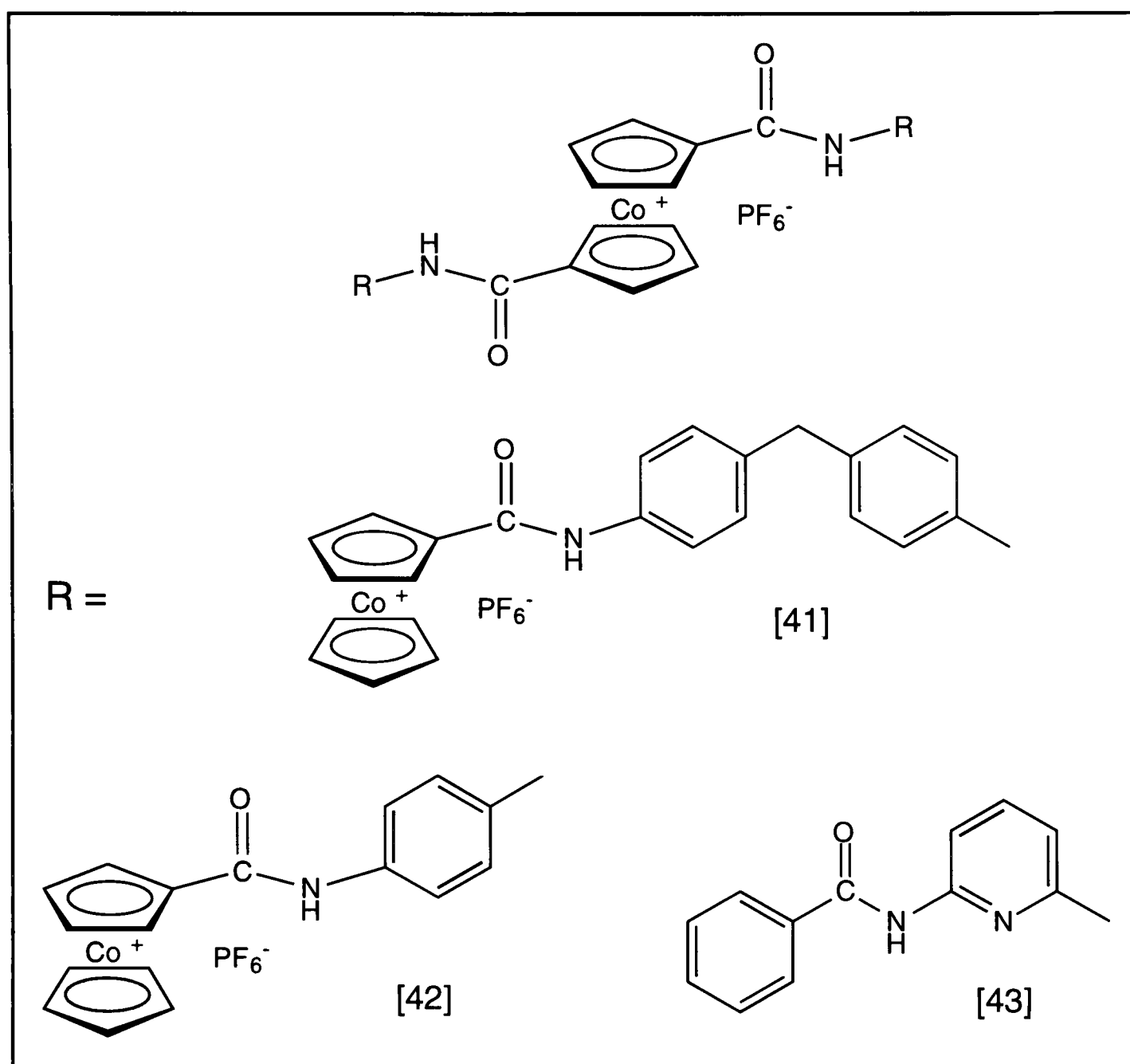


Figure 2.9: Bis-substituted cobalticinium.

The amide was subsequently prepared by reaction with an appropriate amine in acetonitrile under an inert atmosphere to prevent attack by atmospheric moisture. The crude product was seen to precipitate as a chloride salt and was isolated by filtration. Chloride salts of cobalticinium were generally found to be soluble in water and could then be converted to the hexafluorophosphate salt by addition of ammonium hexafluorophosphate which caused precipitation of the crude product. Final purification by Sephadex chromatography allowed the product to be isolated.

2.5 Characterisation

The new products were characterised by the usual techniques and exhibited a number of typical features.

2.5i Nuclear Magnetic Resonance

^1H nmr spectra display two sets of resonances in the 5.5–6.5 ppm range due to the two different cyclopentadienyl rings (see figure 2.10a). The lower ring has five equivalent protons, H_a , which appear as an intense singlet. Slightly downfield, the resonances from the upper ring protons H_b and H_c appear as two virtual triplets. In fact the spin coupling pattern is an $\text{AA}'\text{XX}'$ system and the central line in the “triplets” is a coincidence of four peaks. Spin coupling systems such as this have been termed “deceptively simple” and papers have provided spectral analysis.^{139,140} The amide proton H_d is observed as a broad singlet with chemical shift between 7.5 and 9.5 ppm; its position is strongly solvent dependent. The resonances from the substituents were as expected. The spectrum of a simple mono-substituted cobalticinium hexafluorophosphate salt (deuteriated acetonitrile) is shown in figure 2.10b.

^{13}C nmr spectra gave five signals characteristic of the mono-substituted cobalticinium core. The carbonyl carbon atom and the quaternary cyclopentadienide carbon gave weak signals at around 160 and 97 ppm respectively. The remaining two distinct carbon atoms on the upper ring and those on the lower ring gave rise to three very closely spaced resonances in the 85 ppm region.

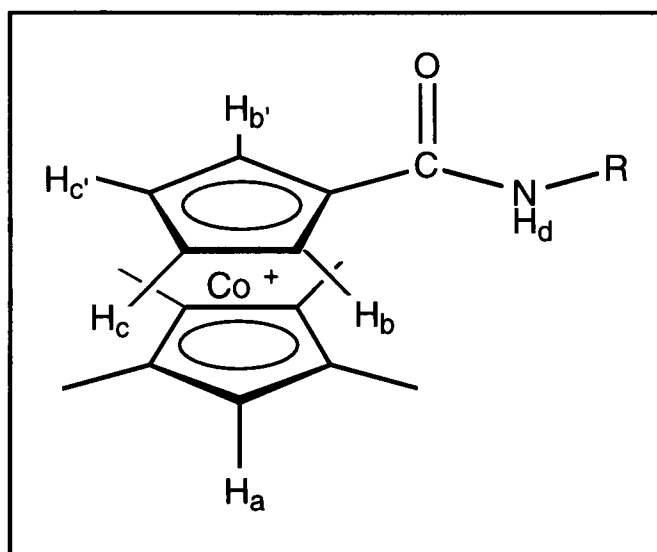


Figure 2.10a: Assignment of protons.

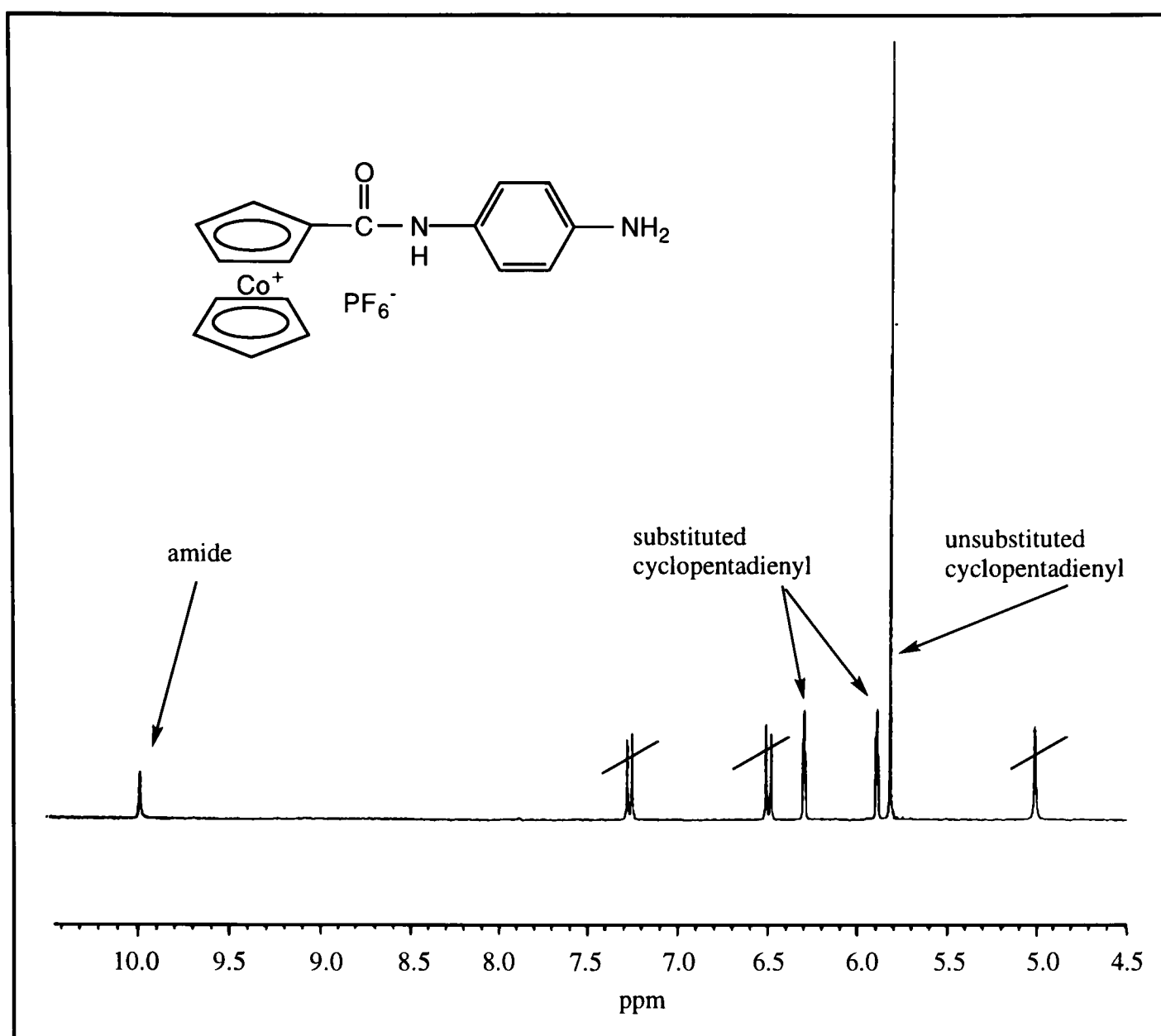


Figure 2.10b: ^1H nmr spectrum of mono-substituted cobalticinium. Substituent resonances are crossed.

2.5ii Mass spectrometry

Mass spectra of the ligands were obtained by fast atom bombardment (FAB-MS); a typical example is shown in figure 2.11. The molecular ion was infrequently observed but instead, the PF_6^- counter ion was lost and the most abundant fragment was invariably $[\text{M}-\text{PF}_6]^+$. In the few cases when the molecular ion appeared, it was found as a very weak cluster assigned as MH^+ . The example shown is a particularly clear example of the predominance of $[\text{M}-\text{PF}_6]^+$. Further fragmentation was relatively unimportant, and was manifest as weak clusters of low molecular weight.

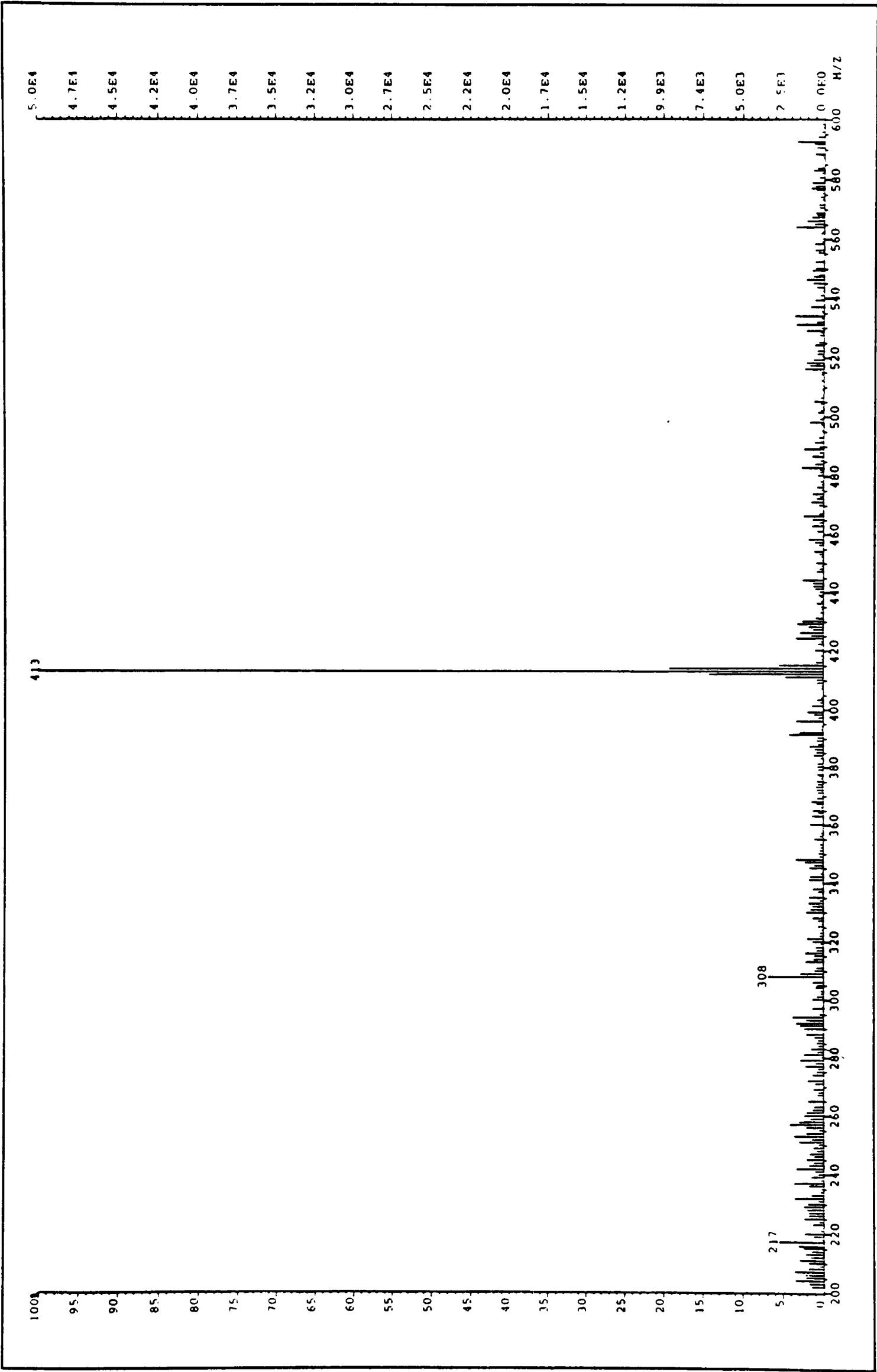


Figure 2.11: The FAB mass spectrum of a cobalticinium based receptor. The molecular weight is 558.

2.5iii Infra-red spectroscopy

The solid state infra-red spectrum of [34] is shown in figure 2.12 (KBr disk). The common features are linked to the presence of cobalticinium and the secondary amide group. The latter is known to exhibit two sharp and reasonably intense bands in the 3400–3460 cm^{-1} region due to an N-H stretch. A further, weaker band is expected at 3070–3100 cm^{-1} . The carbonyl stretch is found (in the solid state) at 1630–1680 cm^{-1} (amide I) and 1515–1570 cm^{-1} (amide II).

Cobalticinium itself contributes some notable bands.¹⁴¹ The C-H stretch appears at around 3120 cm^{-1} and a C-C stretch at around 1417 cm^{-1} is often visible but is occasionally obscured by nearby bands. Other cobalticinium-based bands are very weak and easily confused by similar bands from the organic substituents.

The appearance of the PF_6^- stretch in the fingerprint region close to 840 cm^{-1} was a feature common to the spectra.

2.5iv UV-vis spectroscopy

The characteristic yellow colour of cobalticinium may be attributed to a combination of two spin-allowed transitions which appear close together. These are the $^1\text{A}_{1g} \rightarrow ^1\text{E}_{1g}$ (335 nm) and $^1\text{A}_{1g} \rightarrow ^1\text{E}_{2g}$ (407 nm) transitions. They emerge from a strong ligand-to-metal charge transfer band ($^1\text{A}_{1g} \rightarrow ^1\text{A}_{1u}$) centred at 263 nm.

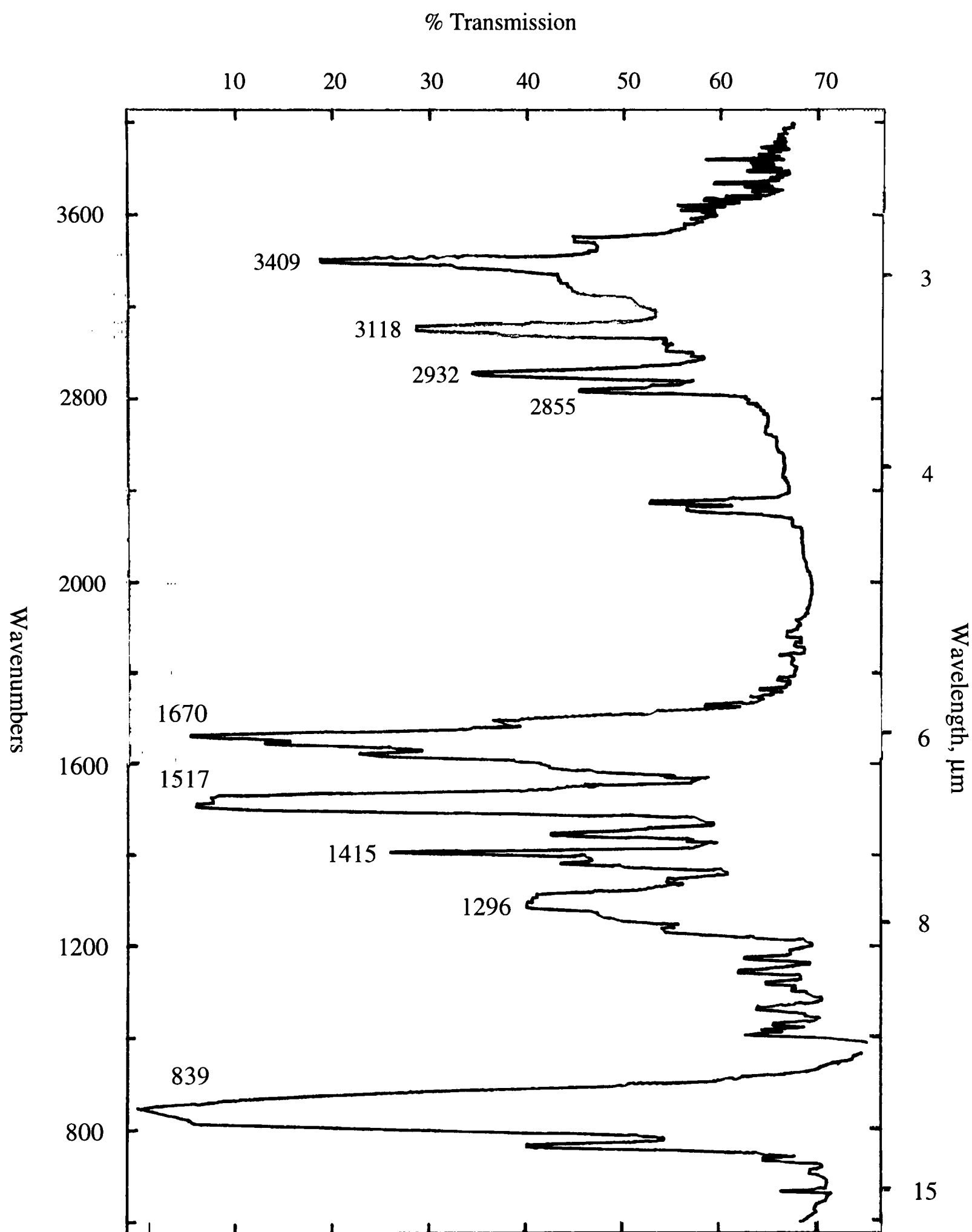


Figure 2.12: Infra-red spectrum of [34].

Assignment: 3409 — N-H stretch; 3118 — C-H stretch; 2932, 2855 — secondary amide bands; 1670 amide I carbonyl; 1517 amide II carbonyl; 1415 — C-C stretch; 839 — hexafluorophosphate.

2.6 Anion Coordination Studies

The binding properties of the receptors were investigated by a number of techniques. It has become increasingly possible to make a quantitative assessment of binding with the advent of several computer packages which will extract stability constants from spectroscopic titration data and, where possible, stability constants were estimated. At the outset, the presence of the amide group was perceived as a fundamental contributor to the binding interaction by virtue of hydrogen bonding to the anion. Studies by previous workers in the field lent credence to this theory by synthesising analogous molecules in which the amide proton was absent. These are shown in figure 2.13 and all failed to display anion recognition by nmr study.^{142,143}

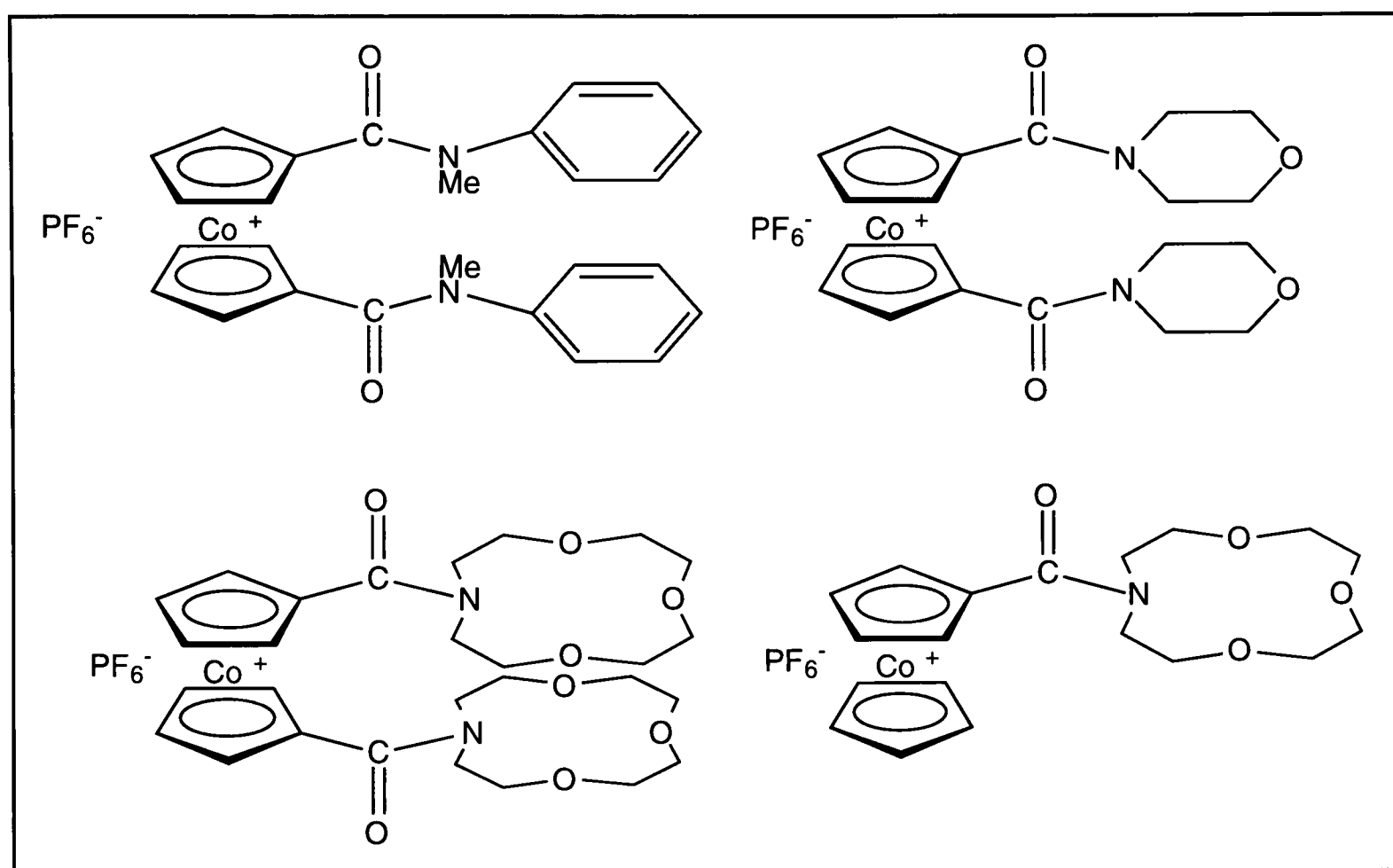


Figure 2.13: Tertiary amides of cobalticinium: failed anion receptors.

The inclusion of a formal positive charge was also an accepted convention of anion receptor design and therefore the coordination studies sought to address the following points:

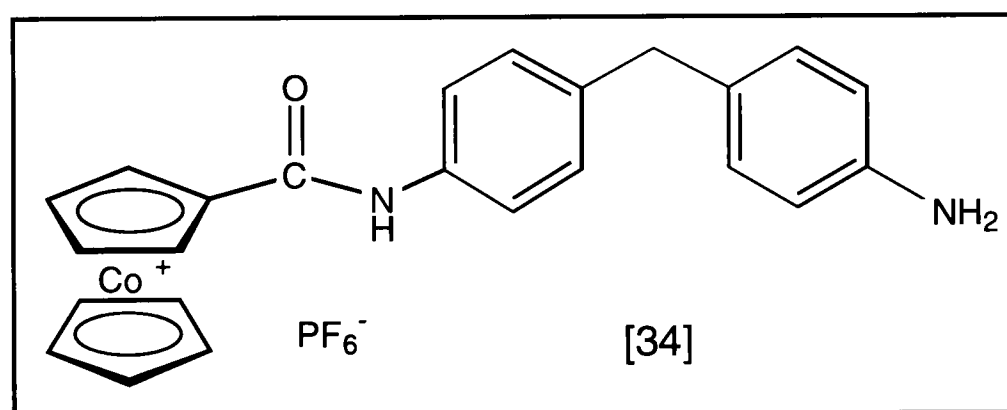
1. The ability of the receptor to bind anions
2. The strength of the binding interaction
3. The location of the binding site

4. The relative importance of the positive charge and hydrogen bonding via the amide
5. The potential for selectivity between anions
6. The sensing ability of the cobalticinium sub-unit; in particular, electrochemical sensing.

In the first instance, tetrabutylammonium chloride was chosen as the anion for study; it has the advantage of simplicity, for several reasons. It is small in size (in anion terms) and is spherical, therefore placing few geometric demands on the receptor. Moreover, it is the conjugate base of a strong acid and therefore does not introduce problems associated with protonation which exist with anions such as hydrogen sulphate, which is strongly acidic. In some cases, further study with other anions, notably dihydrogen phosphate, was conducted to gain more insight into the binding properties of the receptor.

2.7 Studies on Receptor [34]

The receptor [34] was synthesised and binding studies were performed. It was found to exhibit behaviour characteristic of many of the other receptors studied and thus provides a good introduction to the complexation properties of cobalticinium systems. The results of investigation conducted with the addition of tetrabutylammonium chloride (TBA chloride) are presented.



2.7i ^1H nmr titrations

The addition of chloride resulted in marked downfield displacements in the resonance of several key protons. Figure 2.14 shows the ^1H nmr spectrum (deuteriated acetonitrile) of the free receptor and upon addition of 0.25 mole equivalents of chloride. It can be seen that even

such small quantities can disturb the spectrum markedly, particularly with respect to the amide and cyclopentadienyl resonances.

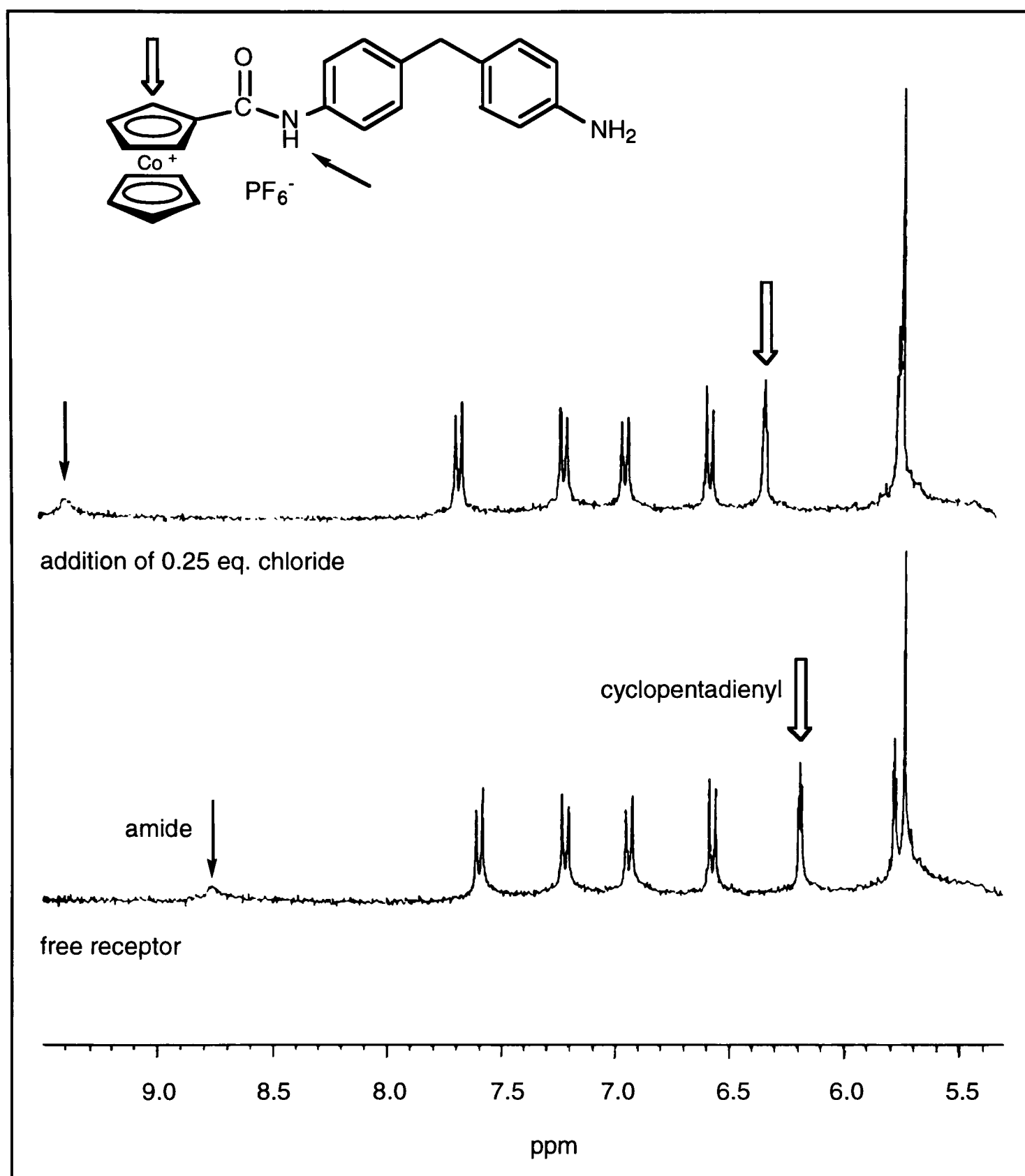


Figure 2.14: ^1H nmr spectra from titration of [34] with Cl^- in deuteriated acetonitrile.

The graph shown in figure 2.15 plots the displacement of the amide and cyclopentadienyl (proton H_b , figure 2.10a) protons' chemical shift for [34] as a function of added anion (in all such graphs, a downfield displacement is taken to be positive). The curves exhibit several

characteristic features. Firstly, the displacement of the Cp proton is smaller than that of the amide; the former is less easily perturbed. Small changes were also seen in the shifts of protons of the lower Cp ring but the other Cp proton resonances (H_c , figure 2.10a) remained unmoved. The amide proton resonance broadened and was not visible during the early part of the titration. This phenomenon was observed on a number of occasions, usually in titrations which ultimately bore evidence of strong binding.

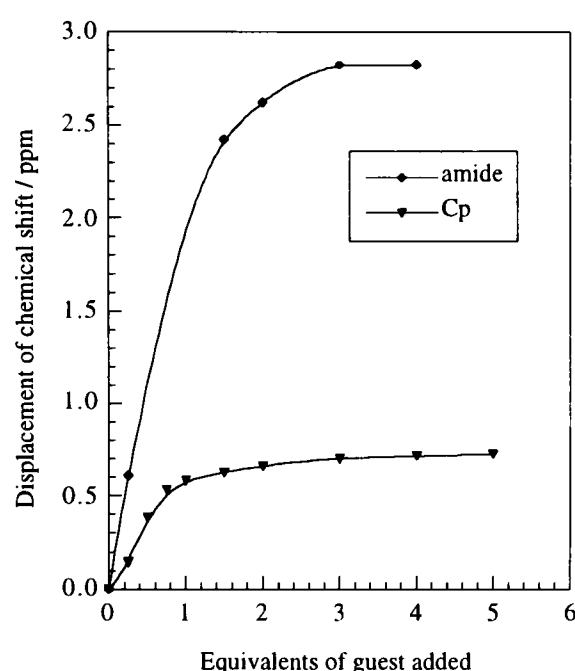


Figure 2.15: ^1H nmr titration profile for addition of chloride to [34] in deuteriated acetonitrile.

The ^1H nmr titration itself serves three purposes, and is a very useful preliminary assessor of binding properties. Firstly, it indicates whether an interaction between host and guest is occurring and, by inspection of which protons are affected, allows a tentative assignment of the binding site.

In the second instance, it may enable the binding stoichiometry to be assessed; tangents drawn through the plateau and the early part of the curve intersect at the binding stoichiometry. This of course requires binding of sufficient strength for a plateau region to be apparent. Alternatively, the stoichiometry may be assessed by Job's method of continuous variation, which will be discussed presently.

The final application of the nmr titration is the estimation of stability constant. Although the magnitude of the displacement of chemical shift yields no information about binding strength *per se*, the shape of the curve and the onset of the important plateau region are determined by the strength of binding and therefore a qualitative assessment is immediately possible by inspection. A potent receptor will show a steady displacement as a function of added guest

and upon saturation at one equivalent (for 1:1 binding) will give a plateau; the receptor is oblivious to further addition of guest. A weak receptor, where the binding equilibrium lies in favour of the unbound form, will display a titration profile with no plateau.

A more quantitative analysis is also possible. Assuming that the observed chemical shift is a weighted average of the extremes of bound and unbound forms, the equilibrium constant may be estimated. Further discussion of the theory is contained within Appendix One; at this point, it is sufficient to say that computer routines are available for fitting of curves, and EQNMR¹⁴⁴ was routinely used in analysis of nmr titrations presented herein. In such analyses, the pattern of the residuals (difference between experimental and calculated points) was always examined. A random distribution was required to ensure that there was no other systematic component to the data which had not been accounted for in the fitting. The residuals were indeed random in all cases which gave further confidence in the assumption of a 1:1 stoichiometry of binding.

Unfortunately, in the case of [34], precipitation of the complex during the latter stages of the titration prevented accurate determination, although the stability constant is thought to lie in the range 500–1000 dm³mol⁻¹ by comparison with similar titration curves. A further experiment was conducted with TBA dihydrogen phosphate, an anion with a defined tetrahedral structure. However, no displacement of chemical shift was observed indicating an absence of interaction. Further comment can be found in section 2.10.

The stoichiometry was also studied explicitly in the form of a Job analysis by continuous variations. Details of the theory are contained in Appendix One; the experiment involves mixing equimolar solutions of host and guest in differing mole ratios to produce a series of solutions of constant volume. A property characteristic of the complex is then measured for each solution. Because the concentration of a 1:1 complex will reach a maximum when the amounts of host and guest are equal, one expects the plot to reach a maximum when the mole fraction, X , is 0.5. The Job plot for addition of chloride to [34] in acetonitrile is presented in figure 2.16 and shows that this is indeed the case. The receptor binds one equivalent of chloride.

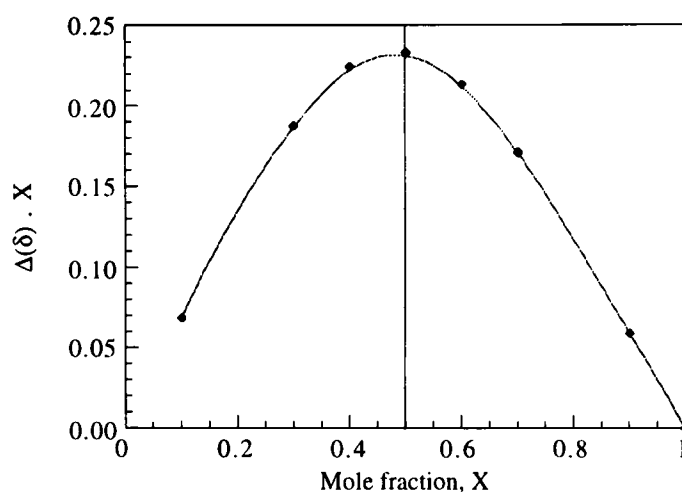


Figure 2.16: Job plot of chloride addition to [34].

2.7ii UV-visible spectroscopy

The UV-vis chromophore was found to be sensitive to the addition of anions, fulfilling expectations in the receptor design. Addition of successive aliquots of chloride elicited a shift in the intensity and position of the d-d absorption. Figure 2.17 shows the progressive change in the spectrum, along with a dissection at 328 nm, i.e. the absorbance at a particular wavelength as a function of added chloride.

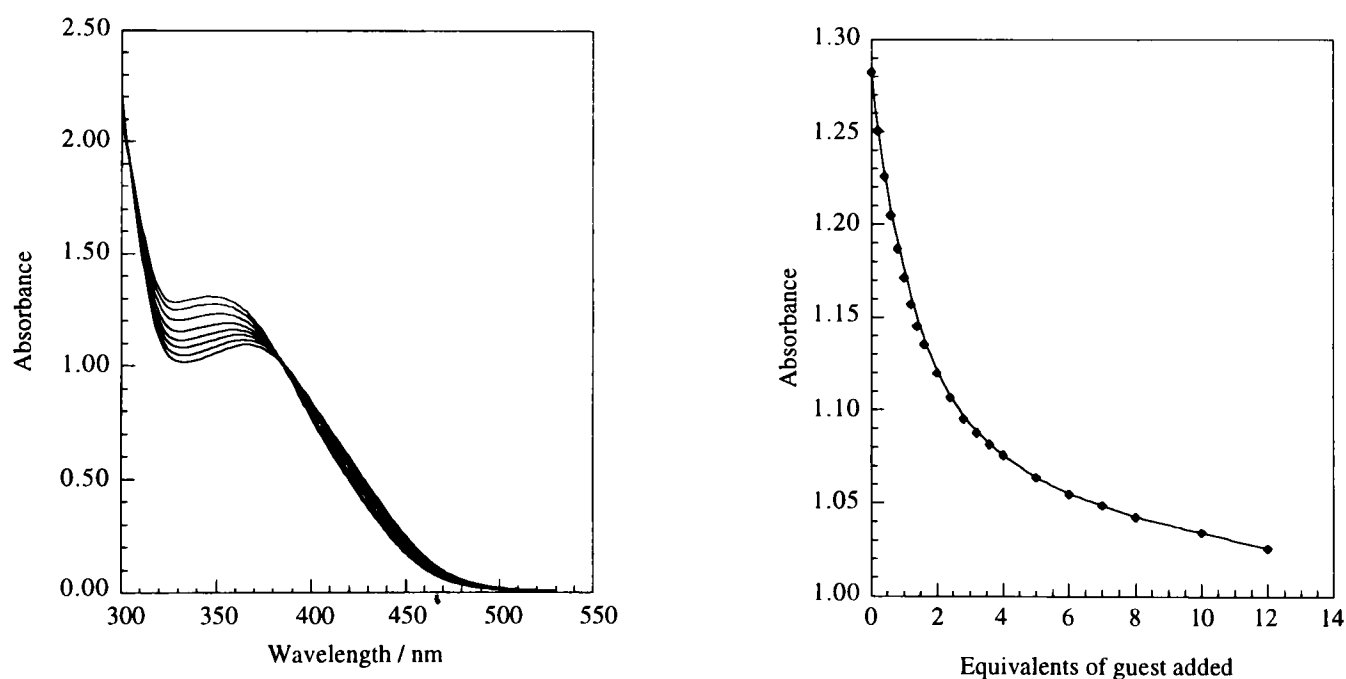


Figure 2.17: UV-vis titration of [34] with TBA chloride in acetonitrile:

(a) full spectra; (b) dissection at 328 nm.

The curve begins to plateau, a fact which cannot be accounted for by dilution effects alone — the inference is that binding disturbs the molecular energy levels and therefore the energy of

absorption. The effect of the binding on absorbance appears not to be discussed in the literature. However, since intensity is a function of the “allowedness” of the transition, the results suggest that this is also affected. The transition is Laporte- and parity-forbidden, but spin-allowed. It does not seem likely that any of these selection rules will be *fundamentally* changed by binding, but modifications to the point group may affect the parity selection rule and, moreover, changes to the vibrational states are likely, due to an enforced binding conformation. This may impinge on the so-called “vibronic coupling” by which the molecule overcomes the constraints of the Laporte rule.

Quantitative analysis of the spectra is possible, again via computer routines which fit a calculated curve to the experimental data and estimate the stability constant. The program used is a commercial product, Specfit[©] (see Appendix One). The calculated constant for the curve shown is $2800 \text{ dm}^3\text{mol}^{-1}$. Differences between stability constants from nmr and UV-vis data are discussed in the next section.

The fitting also generates other data which can be used to examine the reliability of the result. The magnitude of the error and the distribution of residuals was checked to ensure that the former was small and the latter, random. The calculated absorption spectra of all the coloured species were also examined and compared with known spectra to verify that “sensible” spectra were produced; some exceedingly unlikely absorptivities were occasionally suggested as the computer transgressed fundamental laws of spectroscopy in an effort to fit functions to experimental data!

Two further investigations added confidence to the calculated result. Firstly, the sum of squares, Σ (which is minimised), was plotted as a function of stability constant (figure 2.18a). The aim here was to ensure that a significant minimum had been found and that the answer was not simply based on a local minimum. In some cases this plot was seen to plateau, whereupon a limit for the stability constant was the best which could be derived. Finally, a manual check was made, in this case, by deriving a Benesi-Hildebrand^{145,146} plot from the data. The theory is given in Appendix One; it is sufficient to note that the plot should be linear with a stability constant given by the ratio of intercept to gradient. Such a plot is given in figure 2.18b. The equation of the line is shown and gives a stability constant of $2800 \text{ dm}^3\text{mol}^{-1}$.

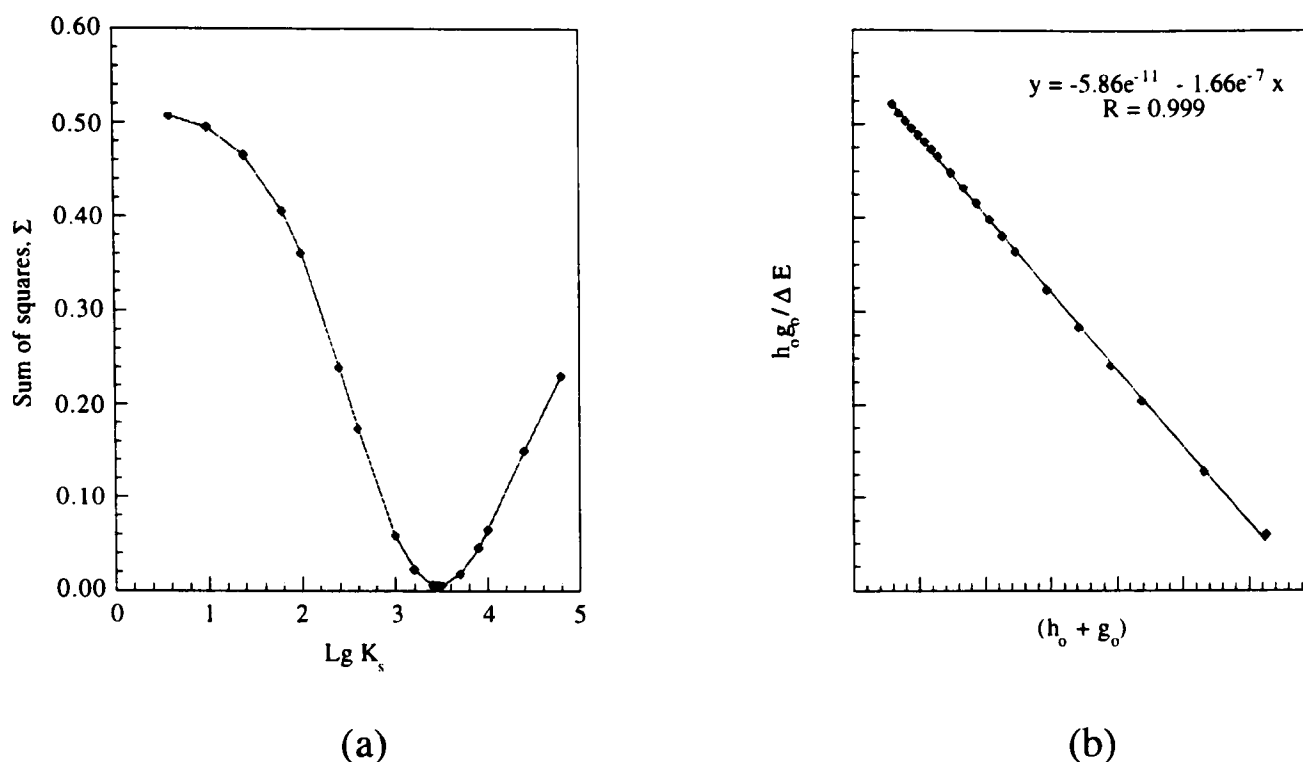


Figure 2.18: (a) the plot of Σ against K_s ; (b) the Benesi-Hildebrand plot.

2.7iii Electrochemistry*

The electrochemistry of the cobalticinium receptors is straightforward. Reduction of the receptor to the corresponding cobaltocene occurs as a reversible, one electron process at around -0.95 V (acetonitrile; vs. Ag/Ag^+ reference). This is slightly lower than values quoted for cobalticinium and reflects the electron withdrawing nature of the amide group. The reversibility of the process is defined by certain criteria, in particular that the mean current of a cyclic voltammogram $[(i_{pa} + i_{pc})/2]$ is proportional to the square root of the scan rate. This is shown for [34] in figure 2.19. A reversible redox centre should also display a CV with $i_{pa} \approx i_{pc}$ and inspection confirms that this is the case.

Another indication of reversibility is that the separation of peak potentials should be $59/n$ mV. It is observed that the separation in the cases studied is somewhat in excess of this figure, a feature thought to be due to uncompensated solution resistance.¹⁴⁷

* See Appendix Two for further discussion.

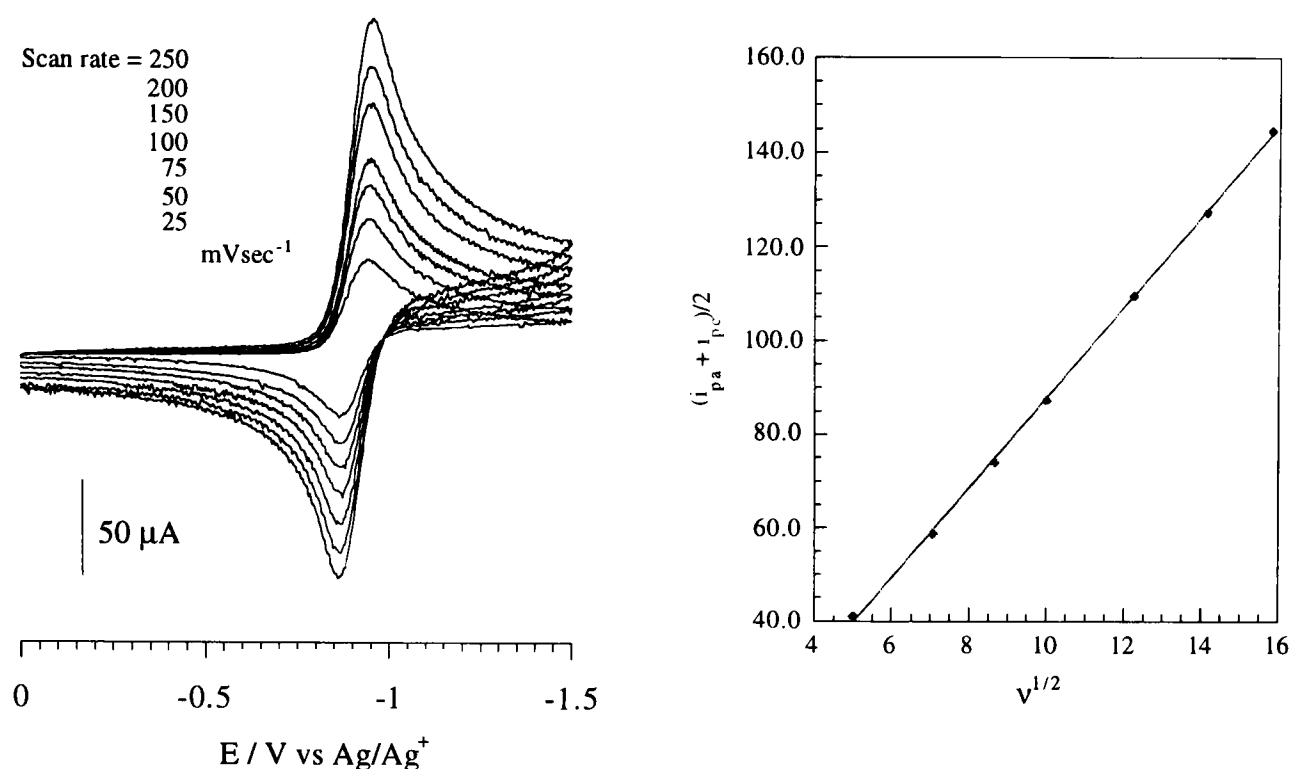


Figure 2.19: Reversibility of [34] by variation of scan rate.

Upon addition of chloride to electrochemical solutions of the receptors in acetonitrile, a reversible, one-electron process was maintained, but the potential was found to shift in a cathodic fashion. This is to be expected: the binding of a negative charge will increase the effective electron density on the redox centre and therefore make reduction more difficult. Figure 2.20 shows cyclic voltammograms of [34] in the free state and in the presence of 10 equivalents of chloride along with the half-wave potential as a function of added anion.

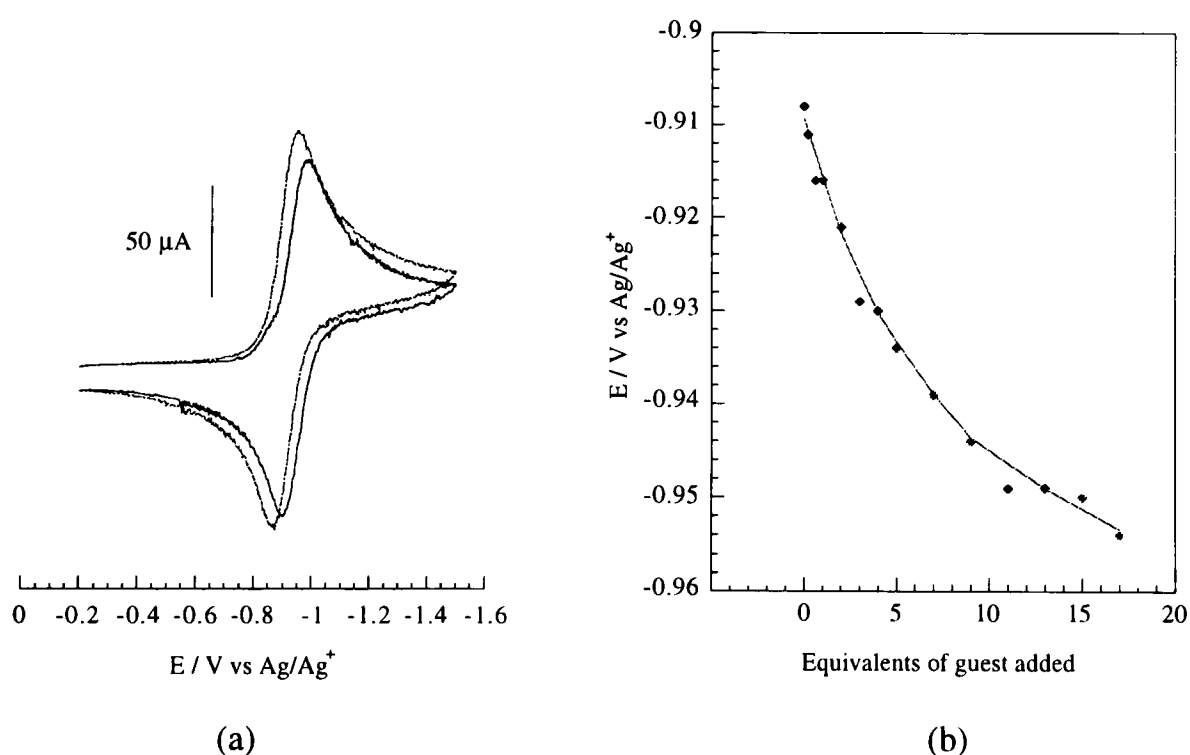


Figure 2.20: Electrochemical response of [34] to chloride: (a) Cyclic voltammograms before and after addition of 10 equivalents chloride; (b) Half-wave potential as a function of added chloride.

Attempts were made to fit a function to the plot shown in figure 2.20b with a view to calculating a stability constant, but this was unsuccessful. The applied function was derived on the basis of fully Nernstian behaviour throughout the titration; it is thought that this assumption is not valid. In qualitative terms, the magnitude of the shift is *not* related to the binding constant, but is reflective of the *binding enhancement* provided by the positive charge on the receptor over the neutral, reduced form. Consider the scheme of squares in figure 2.21.

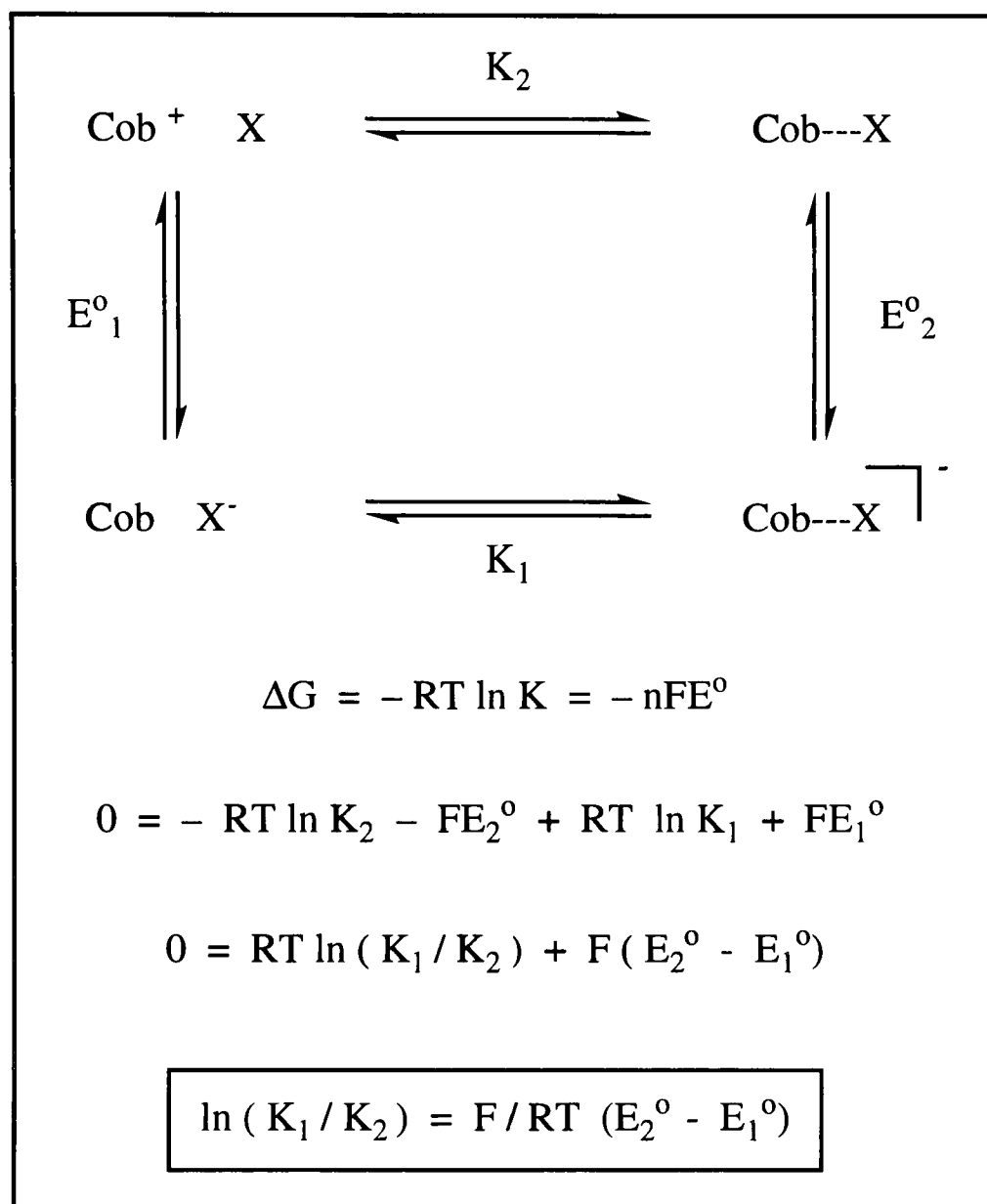


Figure 2.21: Scheme of squares for binding enhancement.

The sum of the free energies for a clockwise circuit of the scheme must be zero and from this basis, the relationship is derived which links a difference in the half-wave potentials with the ratio of K_1 / K_2 . In this case, the two stability constants represent binding by the charged and uncharged form of the receptor and it is found that a shift of 60 mV corresponds to binding enhancement in the charged form by an order of magnitude. Here, then, is the first indication that the positive charge is a real contributor to the overall binding process. The question of

the contribution of the positive charge is further investigated in the next chapter, where the binding of neutral receptors is studied.

Several conclusions were drawn from the binding studies. It is clear that a binding interaction is occurring between the receptor and chloride and that the stability constant is appreciable in anion binding terms. The proposed binding site, based on ^1H nmr evidence, is in the vicinity of the amide proton, which is known to be a key contributor to the overall binding process. Subsequent results were found to be consistent with these hypotheses. Furthermore, tangible evidence was provided by the single crystal X-ray structure of the chloride complex, two views of which are shown in figure 2.22.

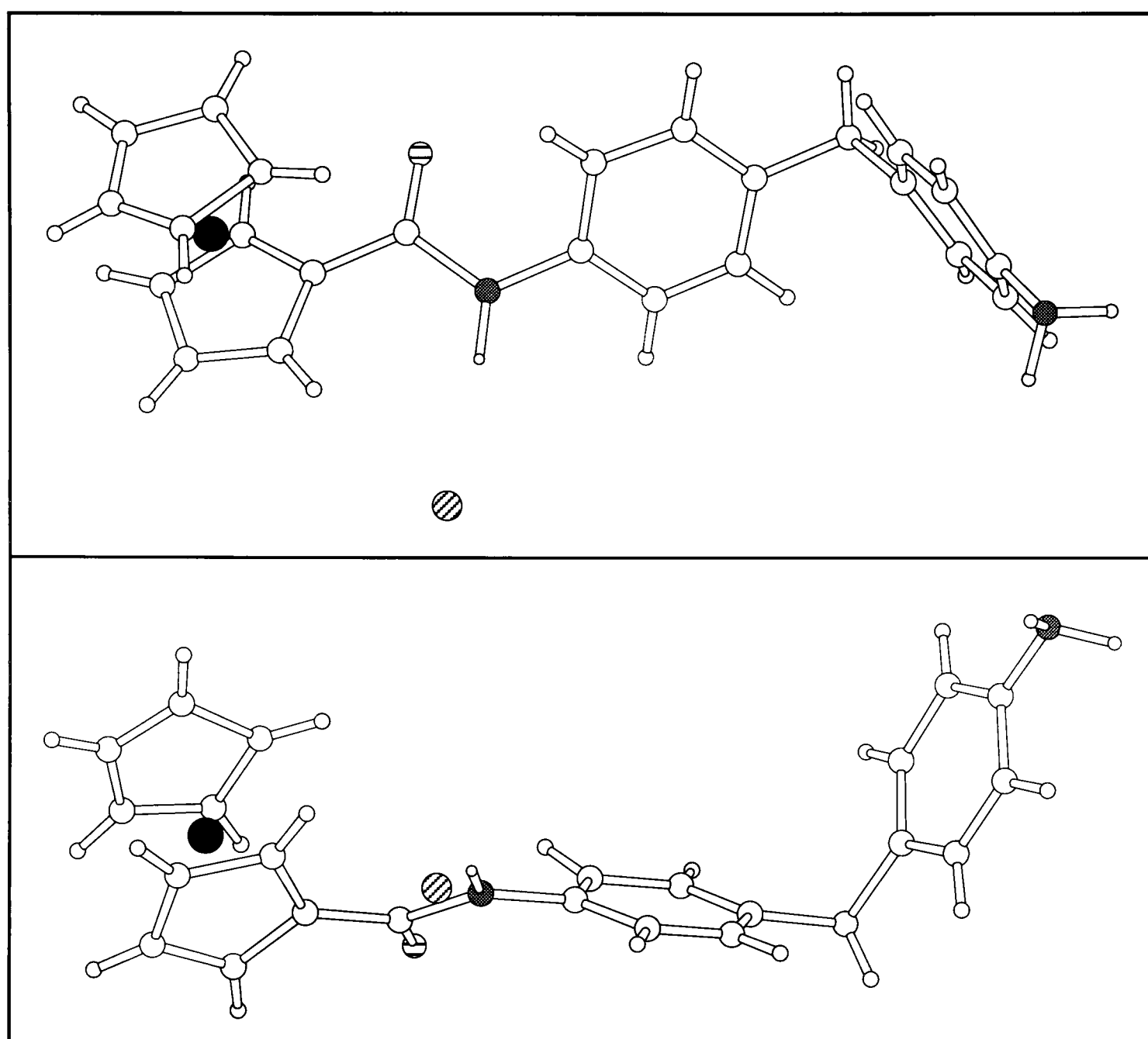


Figure 2.22: X-ray crystal structure of the chloride complex of [34].

The chloride ion (diagonal shading) is seen to be in the region of the proposed binding site. The relevant distances are $\text{CpH} \cdots \text{Cl}^-$ 2.95 Å and $\text{CONH} \cdots \text{Cl}^-$ 2.33 Å. These are within hydrogen bonding range, although they are rather long, possibly due to the non-linearity of the hydrogen bonds.

The solid state structure shows the chloride ion closely associated with the receptor amide proton and within hydrogen bonding distance of a cyclopentadienyl proton. The chloride-amide distance is 2.33 Å and the chloride-cyclopentadienyl proton distance is 2.95 Å (further data are contained in Appendix Three). While a crystal structure does not necessarily represent the solution structure, that shown in figure 2.22 is consistent with the results of solution studies and entirely in agreement with the proposed binding site.

2.8 The Effect of Varying Substituent Position

The next series of compounds to be studied were the receptors shown in figure 2.23. The presence of the amino substituent was expected to have two opposing effects on binding. In the first instance, it was known that the binding would be weakened due to the electron donating nature of the amino group. This reduces the acidity of the amide proton, which will reduce the strength of the hydrogen bonding; previous work has demonstrated a correlation between the Hammett parameter and binding.¹⁴⁸ However, the amino group is also capable of forming hydrogen bonds and, in conjunction with the amide group, might be expected to contribute towards binding.

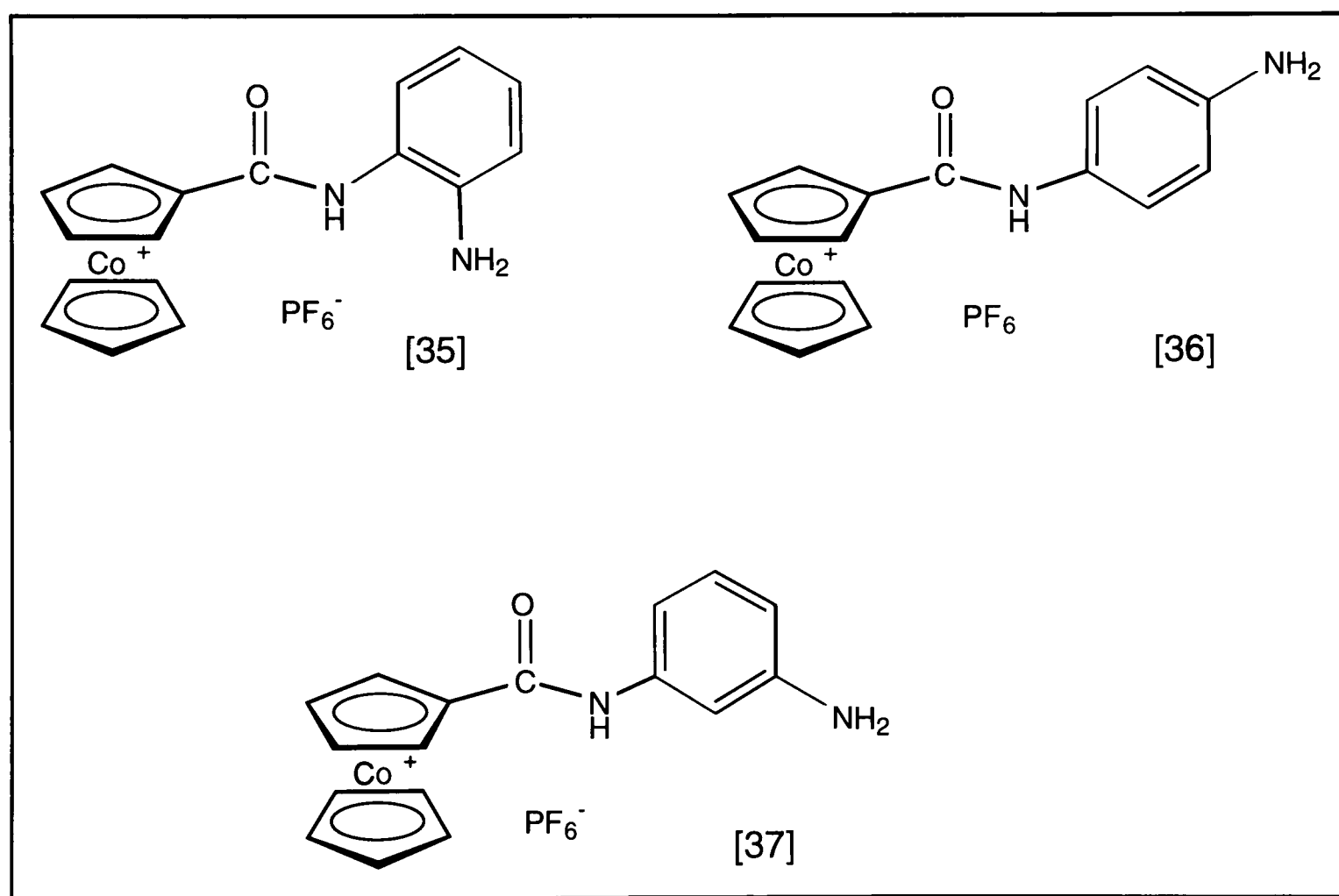


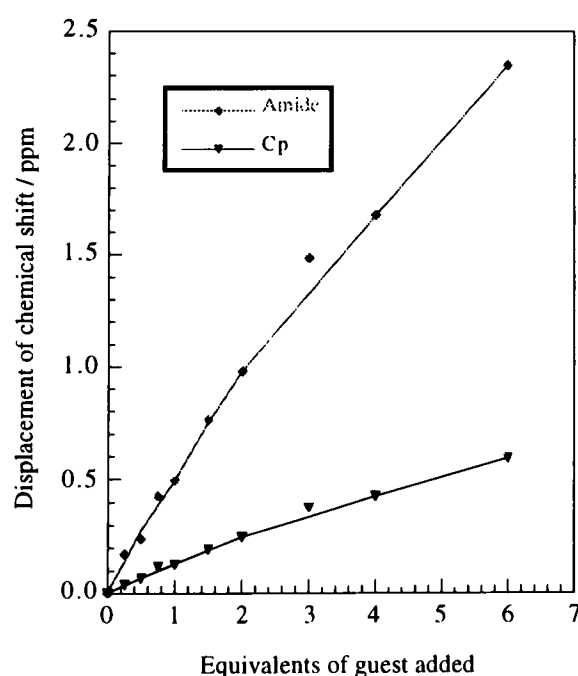
Figure 2.23: ortho-, para- and meta-substituted receptors.

2.8i ^1H nmr titration.

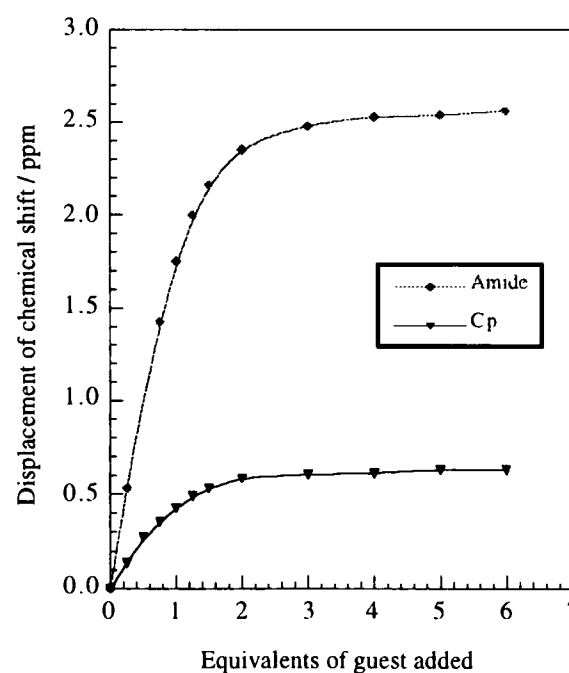
^1H nmr titrations in deuteriated acetonitrile yielded the curves shown in figure 2.24. Section 2.7i commented on the different profiles expected for strong and weak binding and both examples are seen here; (a) is characteristic of very weak binding whereas (b) and (c) are indicative of a superior binding interaction. These results support the proposed involvement of the amine; in both [35] and [37], interaction of both the amide and amine is sterically possible but this is not the case for [36] which binds much less strongly.

The stability constants were calculated for [35], [36] and [37] and were found to be $630 (\pm 50)$, $24 (\pm 14)$ and $660 (\pm 60) \text{ dm}^3\text{mol}^{-1}$ respectively. This is consistent with the qualitative analysis presented in the previous paragraph. Errors were routinely in the order of 10%, their high magnitude being due to the relatively few data points in the titrations. In all cases, the distribution of residuals was found to be random.

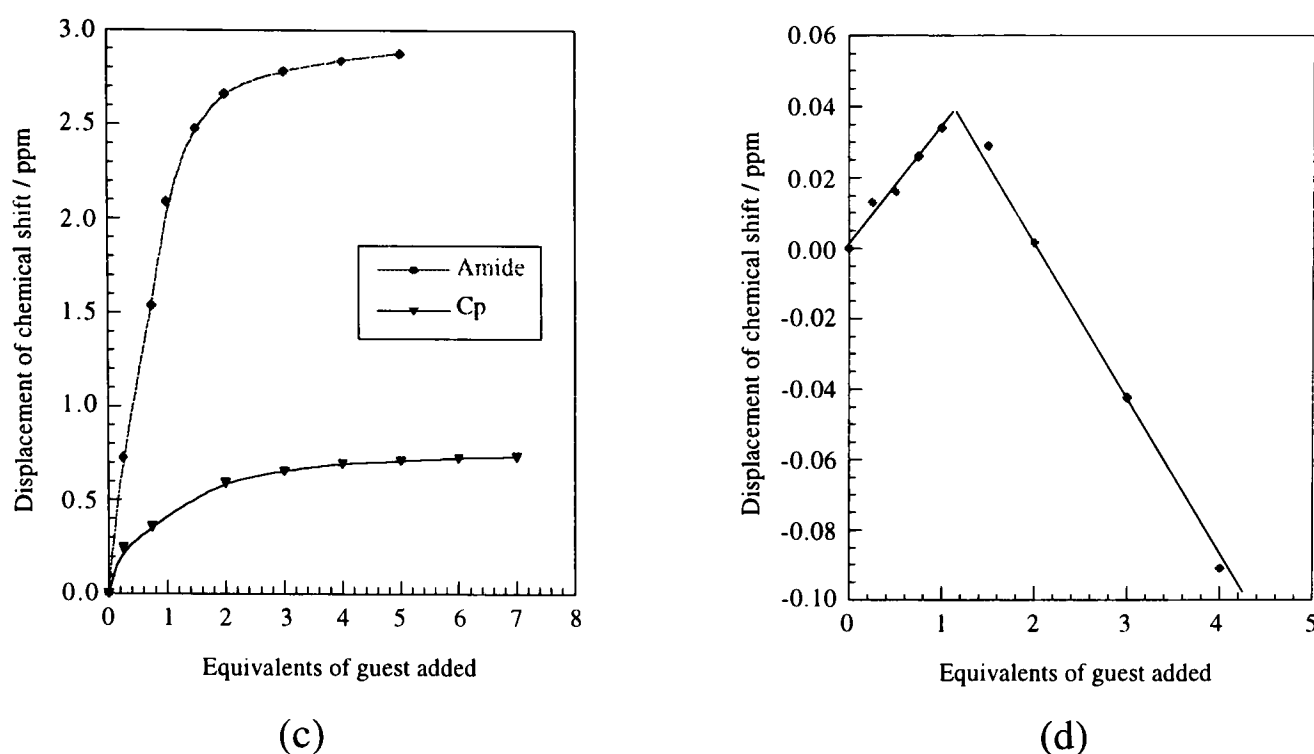
With regard to stoichiometry, the intercepts of tangents for [35] and [37] lay close to one equivalent which supported an assumption of 1:1 binding. Further evidence was furnished by the titration profile of the amine proton of [37] (figure 2.24d). This shows a sharp change close to one equivalent — an indication of a pre- and post-saturation states. The same resonance was perturbed in the titration of [35], but decayed rather than moved. However, in the case of [36], the amine resonance was unaffected by chloride addition; another indication that this group does not participate in binding.



(a)



(b)

Figure 2.24: ^1H nmr titration profiles:

(a) receptor [36]; (b) receptor [35]; (c) receptor [37]; (d) receptor [37], amine resonance.

2.8ii UV-visible spectroscopy

A similar study was conducted via UV-vis spectroscopy. Addition of aliquots of chloride to an acetonitrile solution of the receptors elicited a change in the intensity and position of the d-d absorption band. Titration profiles for all three cases were examined and the stability constants are reported in table 2.2 (found at the end of this chapter). In this experiment, the stability constant for [36] was unexpectedly high; a result which was peculiar to this experiment.

It is interesting to note that the stability constants calculated from these titrations are significantly larger than those from nmr studies. This is thought to arise, at least in part, from the different ionic strengths involved. The theory has been discussed in section 1.1i and calculations were carried out to investigate. The activity coefficients were calculated using the Debye-Hückel limiting law and found to be 0.85 and 0.94 for nmr and UV-vis titrations respectively. True thermodynamic stability constants could then be estimated and were found to be much closer in magnitude, although exact correlation was not observed. It was also thought that some degree of self-association of the receptors might occur, and this was

investigated by measurement of the nmr spectra of the receptors at high and low concentrations. However, no difference was observed in the spectra, which negated this theory. The fact that the molecule carries a positive charge would also militate against such a notion.

The effect of ionic strength was further investigated by repeating the UV-vis experiment in the presence of a background electrolyte (tetrabutylammonium tetrafluoroborate in this case). There was a very significant fall in stability constant, but the relative magnitudes were not at all affected, thereby confirming that the ionic strength was an important consideration. It also meant that direct comparison between techniques was not possible, nor was it attempted.

Two points arise. Firstly, future studies should be executed in the presence of a constant and excess concentration of base electrolyte in order to eliminate this problem. Secondly, it is notable that the literature frequently reports work on charged systems with no comment on the salt concentrations involved. One should be mindful, therefore, of the fact that a reported stability constant can be made to vary by over an order of magnitude simply by adjusting the experimental conditions.

2.8iii Electrochemistry

All three receptors produced a single and reversible one-electron reduction wave in cyclic voltammetry experiments. The electrochemical parameters are given in table 2.3, found at the end of this chapter, and show that all exhibited a cathodic shift in potential on addition of chloride in acetonitrile.

2.8iv X-ray crystallography

The crystal structure of [35] is shown in figure 2.25. Data were collected with Mo-K α radiation using the MAR research image plate system. Analysis was carried out with the XDS program¹⁴⁹ and the structure was solved using direct methods with SHELX86.¹⁵⁰ The non-hydrogen atoms were refined with anisotropic thermal parameters. The structure was refined using SHELXL. Calculations were carried out using a Silicon Graphics R4000 workstation by Dr M. G. B. Drew at the University of Reading.

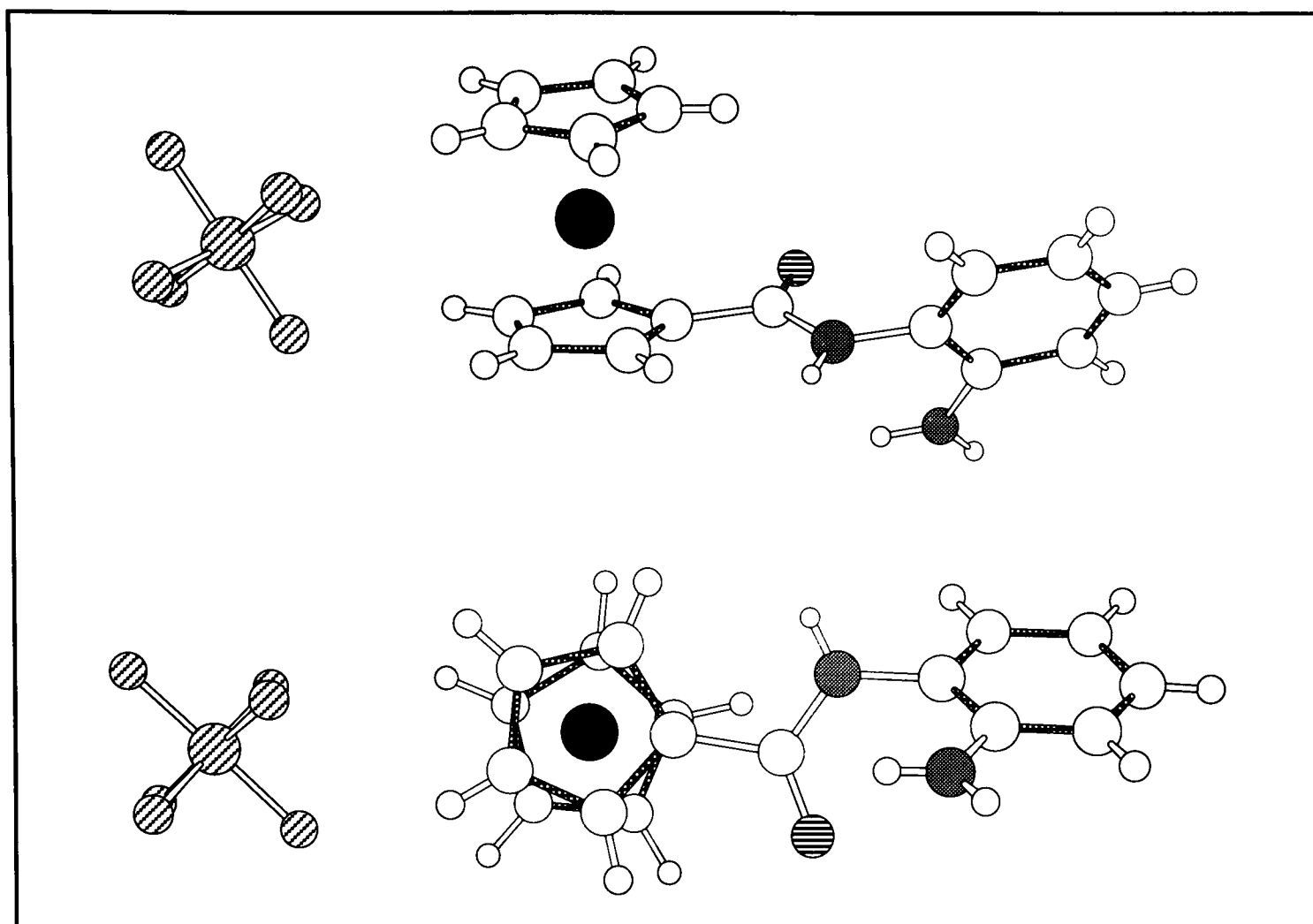


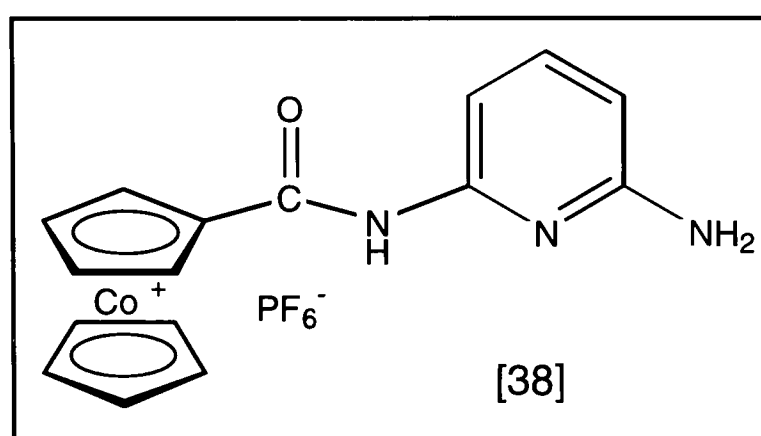
Figure 2.24: The X-ray crystal structure of [36] (two views).

Further data may be found in Appendix Three.

2.9 Varying the Nature of the Binding Site

2.9i Chloride binding

The synthesis of receptor [38] provided an interesting contrast to [37], possessing a nitrogen atom in the proximity of the proposed binding site. The presence of the nitrogen lone pair was thought to furnish a repulsive interaction to the approaching anion and the prediction was that binding of chloride would be greatly reduced.



Subsequent binding studies by the usual methods gave credence to the predictions and results are presented below. Figure 2.26 shows the ^1H nmr and UV-vis titration profiles against added chloride in acetonitrile.

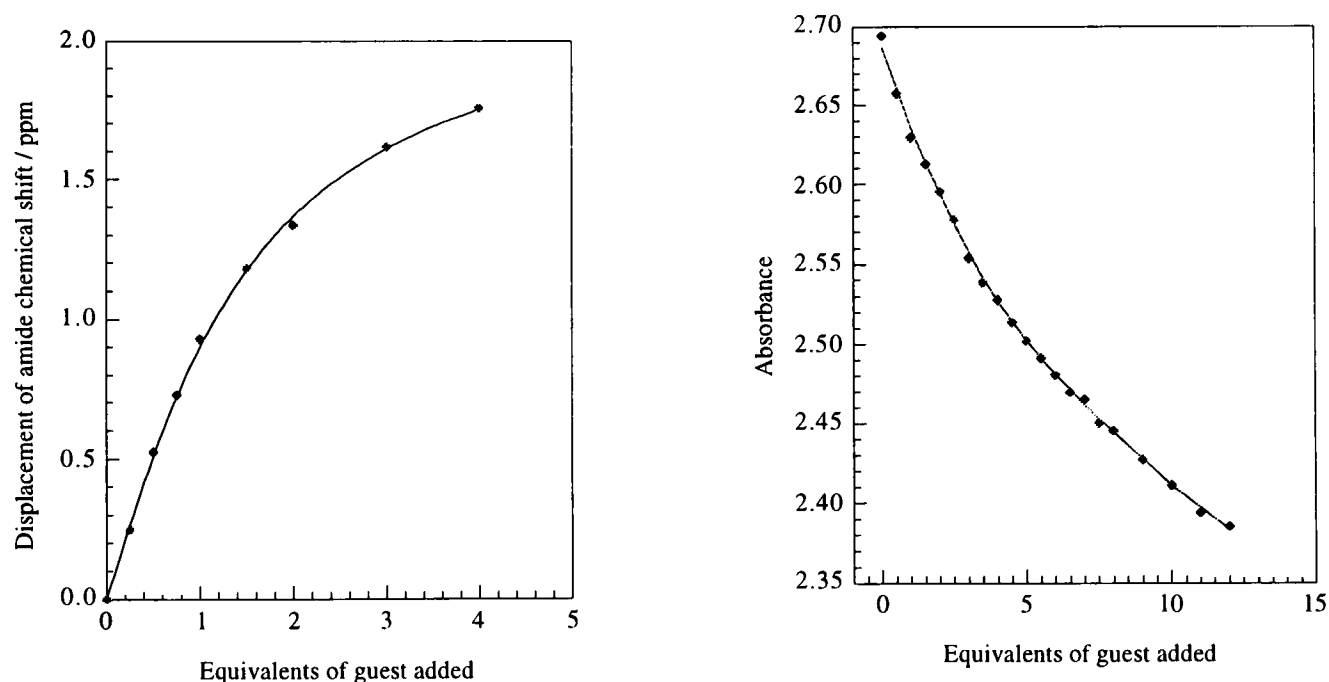


Figure 2.26: Titration profiles for addition of chloride to [38] in acetonitrile:

(a) ^1H nmr; (b) UV-vis at 370 nm.

The stability constants calculated by both methods are an order of magnitude lower than those for [37], as expected (tables 2.2 and 2.3, found at the end of the chapter). The UV-vis

titration was repeated in the presence of base electrolyte and the stability constant thus calculated ($\approx 50 \text{ dm}^3\text{mol}^{-1}$) was in good agreement with the nmr value of $60 (\pm 7) \text{ dm}^3\text{mol}^{-1}$.

Electrochemical studies were conducted in the presence of up to 10 equivalents of chloride. A shift in the redox wave of less than 10 mV was observed, indicating weak perturbation of the redox centre by added chloride. Some signs of adsorption on to the electrode surface were noted in the form of a falling current in the oxidation wave. This was frequently seen in cobalticinium systems and the electrode surface was therefore routinely polished between additions of anion.

2.10 Titrations with Dihydrogen phosphate

It can be seen that [38] binds chloride very weakly according to complexation studies. An interesting contrast, therefore, is furnished by the nmr titration with TBA dihydrogen phosphate. For solubility reasons, the titration was conducted in deuteriated DMSO and as this is a more polar solvent than acetonitrile, one would expect stability constants to be much lower. The titration profile is shown in figure 2.27a, and the fitted curve yields a stability constant of $250 \text{ dm}^3\text{mol}^{-1}$ with 10% error. Residuals showed a random distribution (figure 2.27b). The stability constant is clearly in excess of the value for chloride binding and may be attributed to the fact that the dihydrogen phosphate anion can form interactions with both the amide (or amine) group and the pyridine nitrogen atom. Here, then, is a case where the shape of the anion allows binding to occur in preference to chloride. The reverse behaviour is observed for [37] and it can be seen that the simple substitution of an atom at the binding site can significantly alter the binding properties of the receptor.

Investigations were conducted into the binding of dihydrogen phosphate by the other receptors mentioned previously but very little evidence of interaction could be found. The ^1H nmr experiments, which were known to be sensitive to binding interactions, exhibited negligible movement of either the amide or the Cp proton resonance. Here were the first signs of selectivity in the recognition of anions by the molecules synthesised and the origin of this discrimination is of interest. Dihydrogen phosphate is a tetrahedral anion with both donor and acceptor groups and therefore the best host will present groups which will complement both. Such is the case in the example just discussed. In contrast, the earlier receptors only

present one amide group, which can therefore only make one positive contact with an anion possessing four potential sites of interaction. It may be, therefore, that this one interaction is insufficient to tempt the anion from its solvated state into a union with the receptor.

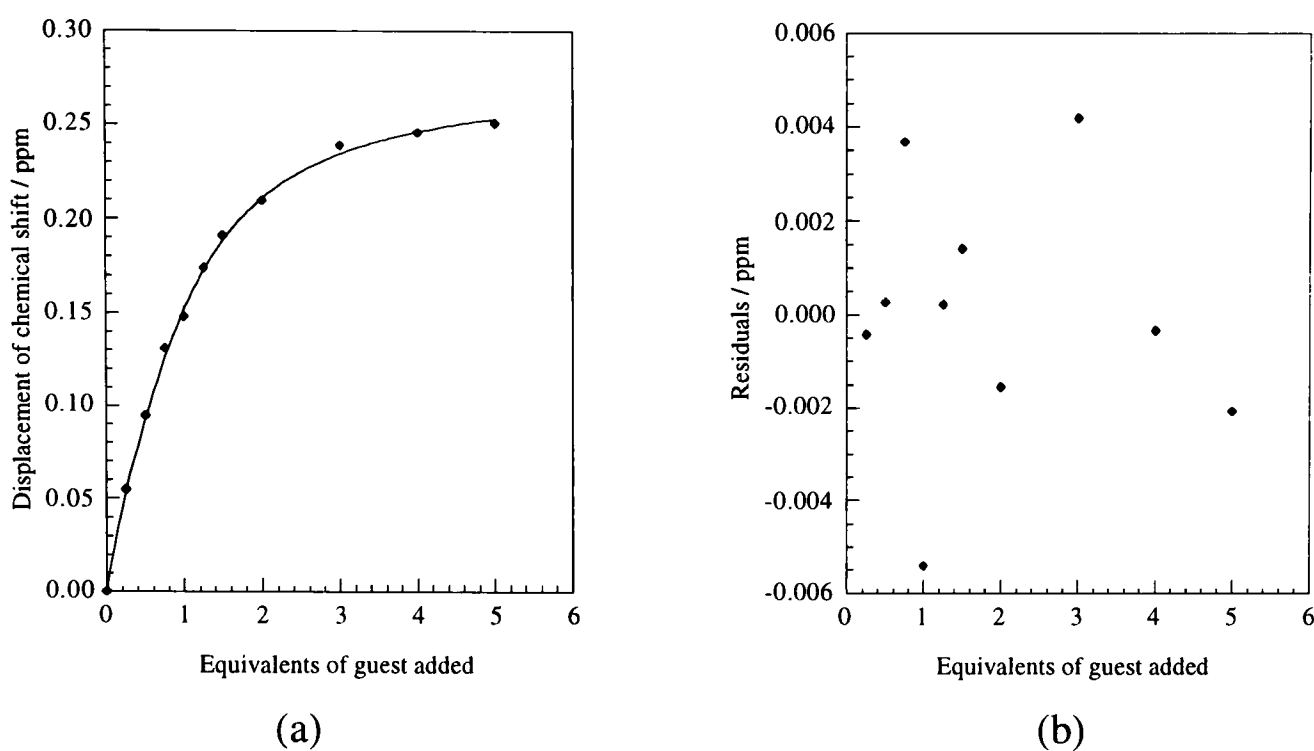
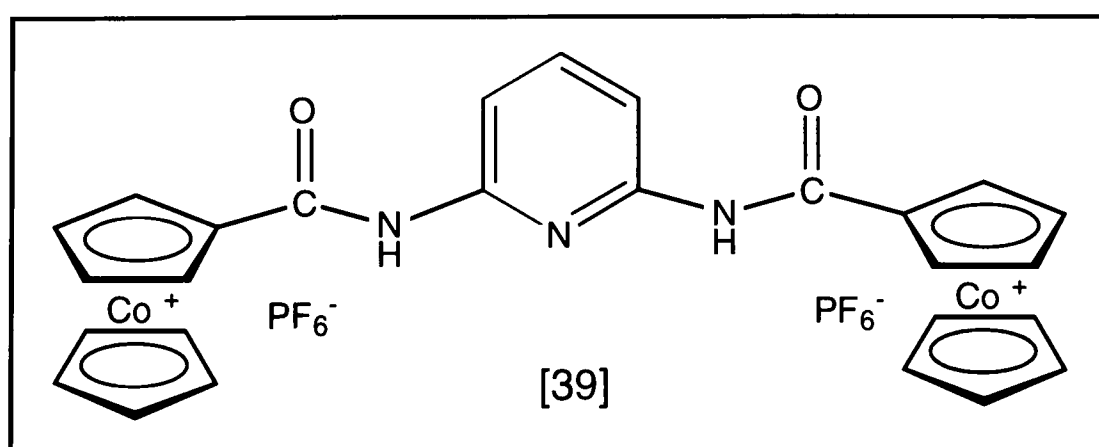


Figure 2.27: ^1H nmr titration of [38] with TBA dihydrogenphosphate in d_6 -DMSO: (a) titration profile; (b) residuals.

Further studies were attempted with other molecules, presented later in this chapter. However, the problems of solubility hampered progress; even in DMSO, the addition of dihydrogen phosphate could result in precipitation of the new salt, in which case it was difficult to differentiate between anion binding and salt exchange.

2.11 Varying the Number of Cobalticinium Units

An analogue of [38], [39] contains two cobalticinium moieties, and therefore has a better defined binding cleft and a higher overall charge which give it potential as a better receptor. Binding of chloride and dihydrogen phosphate was investigated and is reported.



2.11i ^1H nmr titrations

The titration with chloride was performed in acetonitrile in the first instance. Difficulties arose due to precipitation which precluded the quantitative analysis of the data. However, the experiment was repeated in DMSO, which alleviated the solubility problem but made direct comparison with the previous receptor impossible. The profiles in the two solvents are shown in figure 2.28. In acetonitrile, there is a slightly sigmoidal shape to the curve, which may be due to the precipitation problems that were encountered. Accordingly the quantitative analysis of the DMSO curves was conducted, based on a 1:1 and 2:1 combined model. The profiles in this solvent are very shallow and consequently errors were high, but the estimated magnitudes were $25\text{--}30\text{ dm}^3\text{mol}^{-1}$ for the first stability constant and $< 5\text{ dm}^3\text{mol}^{-1}$ for the second. Further comment is made in the UV-vis study.

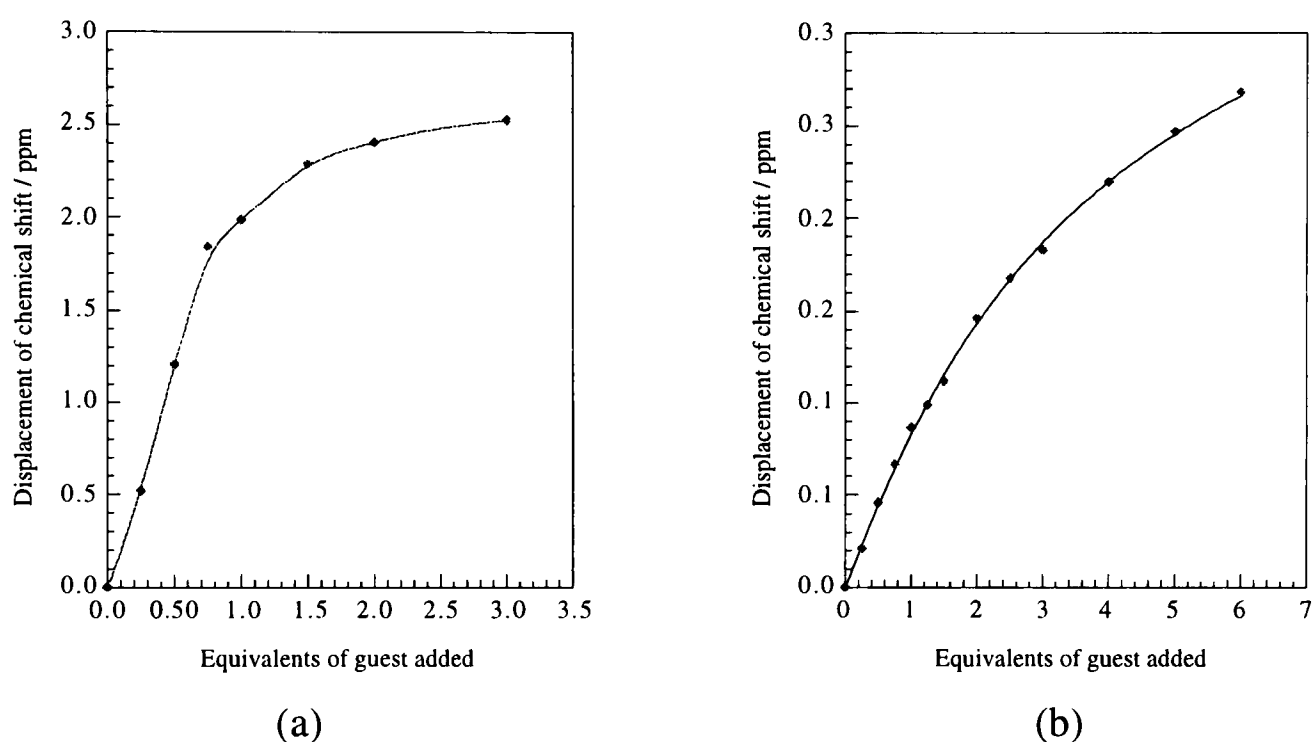


Figure 2.28: ^1H titration profile for [39]; (a) acetonitrile; (b) DMSO.

Titration with dihydrogen phosphate was attempted but immediate precipitation, even in DMSO, was observed and no conclusions about binding could be drawn.

2.11ii UV-visible spectroscopy

The UV-vis spectrum has a strong charge transfer band with a shoulder tailing off into the visible region. A titration was performed with chloride in acetonitrile and the data were analysed by the Specfit program, which detected three coloured components in the titration

profile; they were assigned to the free receptor and 1:1 and 2:1 complexes with chloride. The titration profile is shown in figure 2.29a and the concentrations of the relative components in figure 2.29b. Fitting of the curves gave estimated stability constants of $6500 \text{ dm}^3\text{mol}^{-1}$ and $160 \text{ dm}^3\text{mol}^{-1}$ for the first and second stability constants respectively. Their relative magnitudes are in reasonable agreement with the results of the nmr titration.

There are two points of note. Firstly, the first stability constant is over two orders of magnitude larger than that of [38] as can be expected for a system with a higher charge. Clearly, the combination of two centres of positive charge in close proximity has a profound influence on the binding properties of the receptor. This agrees with the conclusions of numerous workers in the field who have introduced multiple charged centres into their anion receptors.

The second point of note is that the second stability constant is very much smaller than the first, a phenomenon which is well documented. It has its origins both in statistical considerations and in the fact that the presence of the first anion will deter binding of a second if the two binding sites are close together. This is indeed the situation here and the results support this intuitive view.

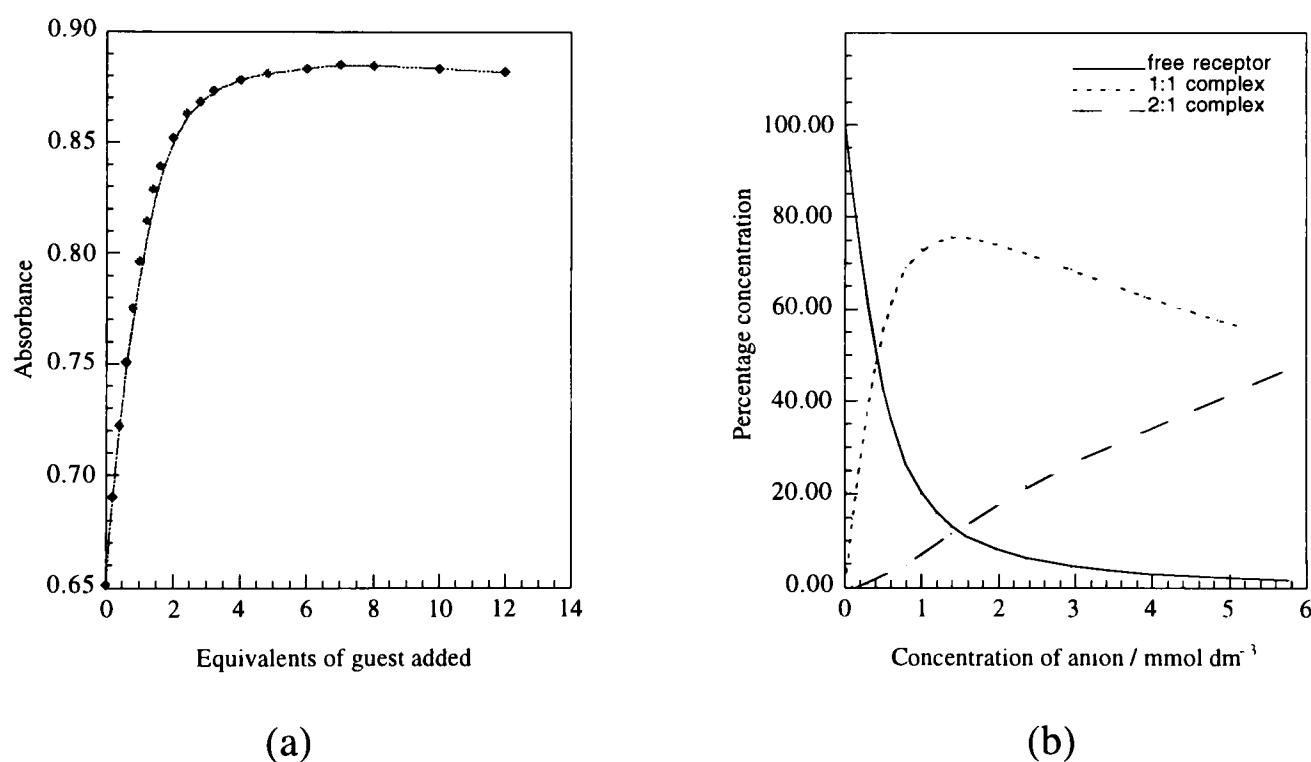


Figure 2.29: UV-visible titration of chloride to [39] in acetonitrile:

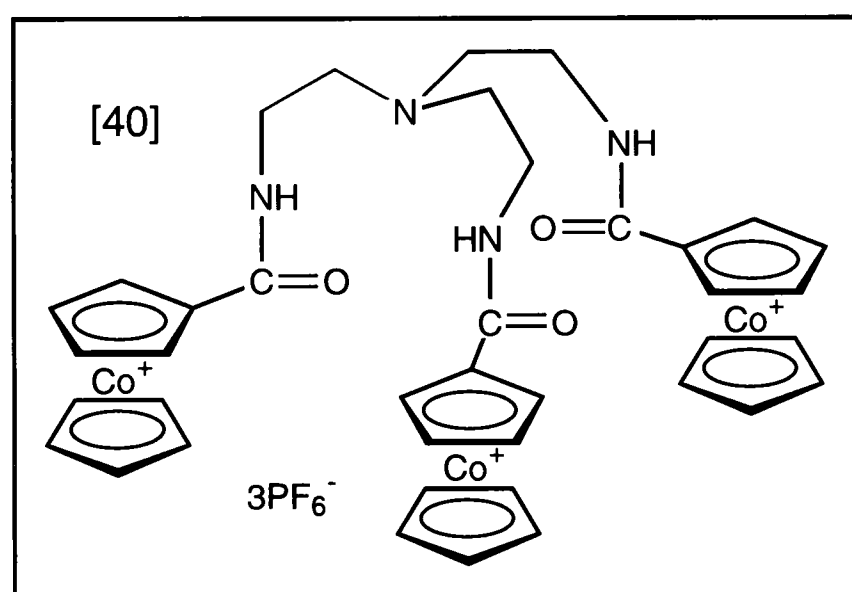
(a) titration profile; (b) concentration profile.

2.11iii Electrochemistry

The receptor [39] has a single reversible cyclic voltammogram, which implies that the two cobalticinium centres are electrochemically equivalent. Notably, the addition of chloride in acetonitrile elicited a cathodic shift in the half-wave potential but the single wave remained, which suggests that binding of an anion (which will surely introduce asymmetry into the molecule) is fast on the electrochemical timescale. A shift of the order of 60 mV was observed.

2.12 A Tripodal Receptor

From a receptor with two cobalticinium units, attention is turned to one with three. [40] is a tripodal molecule based on condensation of cobalticinium with tris(2-aminoethyl) amine (known as “tren”). This was a molecule of great interest in two main respects. Firstly, the inclusion of three cobalticinium units provided a high density of positive charge and therefore the potential for potent interaction with the anion. Secondly, it can adopt a C_3 geometry which might impart selectivity in its choice of preferred guest. A ferrocene analogue has been reported; it shows some selectivity for dihydrogen phosphate in electrochemical studies.¹⁴²



2.12i 1H nmr titration

Preliminary investigation had been carried out by Dr Hodačová who discovered an interaction with chloride. A first titration with no sub-equivalent additions showed reasonably strong binding with a stoichiometry of 2:1. Dr Hodačová's plot of displacement of

amide resonance on addition of chloride in DMSO is shown in figure 2.30a. Accordingly, the receptor was re-synthesised and a more detailed investigation executed. The preparation proceeded via an activated ester, as discussed in section 2.4.

A titration profile analogous to that of figure 2.30a is shown in figure 2.30b; the curve is the calculated function. Fitting of the curve with a model including both 1:1 and 2:1 components was difficult and errors were in excess of 50%. However, in terms of magnitude, the first stability constant was of the order of $10^3 \text{ dm}^3\text{mol}^{-1}$ and the second, $<50 \text{ dm}^3\text{mol}^{-1}$. It will be seen that these figures are very well supported by the UV-vis study. In qualitative terms, the three positive units combine to bind chloride with by far the strongest stability constant yet encountered (the solvent is DMSO, recall) with a small but detectable component from the 2:1 complex.

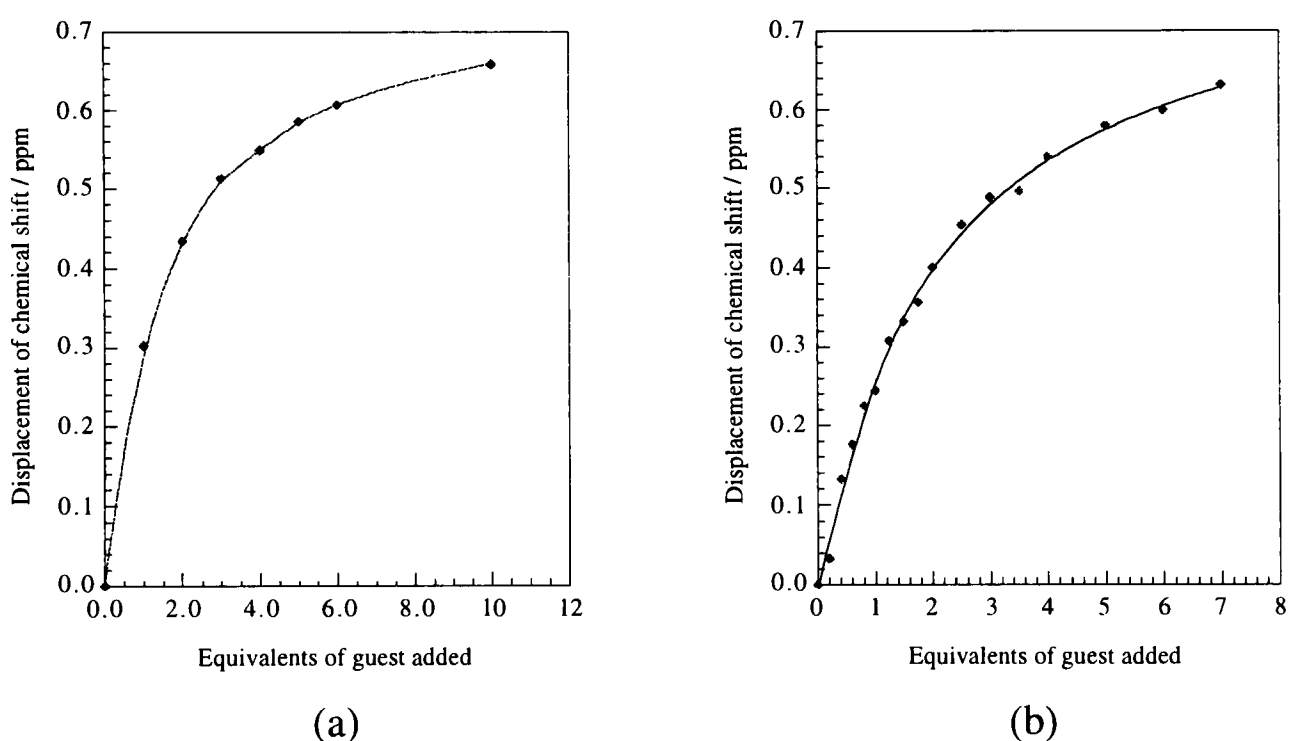


Figure 2.30: ^1H nmr titration profiles of addition of chloride to [40] in DMSO:

(a) preliminary study (J. Hodačová); (b) further study.

2.12ii UV-visible spectroscopy

A UV-vis study in acetonitrile agreed well. The titration profile (400 nm), at first sight, was very unusual with a sharp change in direction at one equivalent (figure 2.31a). A qualitative interpretation is suggested as follows. The change at one equivalent speaks of a 1:1 complex of very high strength. However, the absorbance then increases despite dilution effects

suggesting further interaction, i.e. the formation of the 2:1 complex. No plateau is observed here which implies that the binding is not as strong, in accordance with accepted theory. Fortunately, fitting was possible, albeit with errors of $\approx 25\%$ — on the limits of Specfit's capability. The stability constants were 2.5×10^5 and $700 \text{ dm}^3 \text{ mol}^{-1}$ for the respective constants. The ratio of the two was almost exactly the same as that of the values generated by EQNMR. Of course, the absolute magnitudes are much larger, as one has now come to expect. Specfit also detected yet another component which was assigned as the 3:1 complex with a stability constant of 30. Thus we see extremely strong binding of chloride with weak binding of a further equivalent and very weak binding of a third. The relative concentrations of the four species are presented in figure 2.31b, as determined by the fitting process.

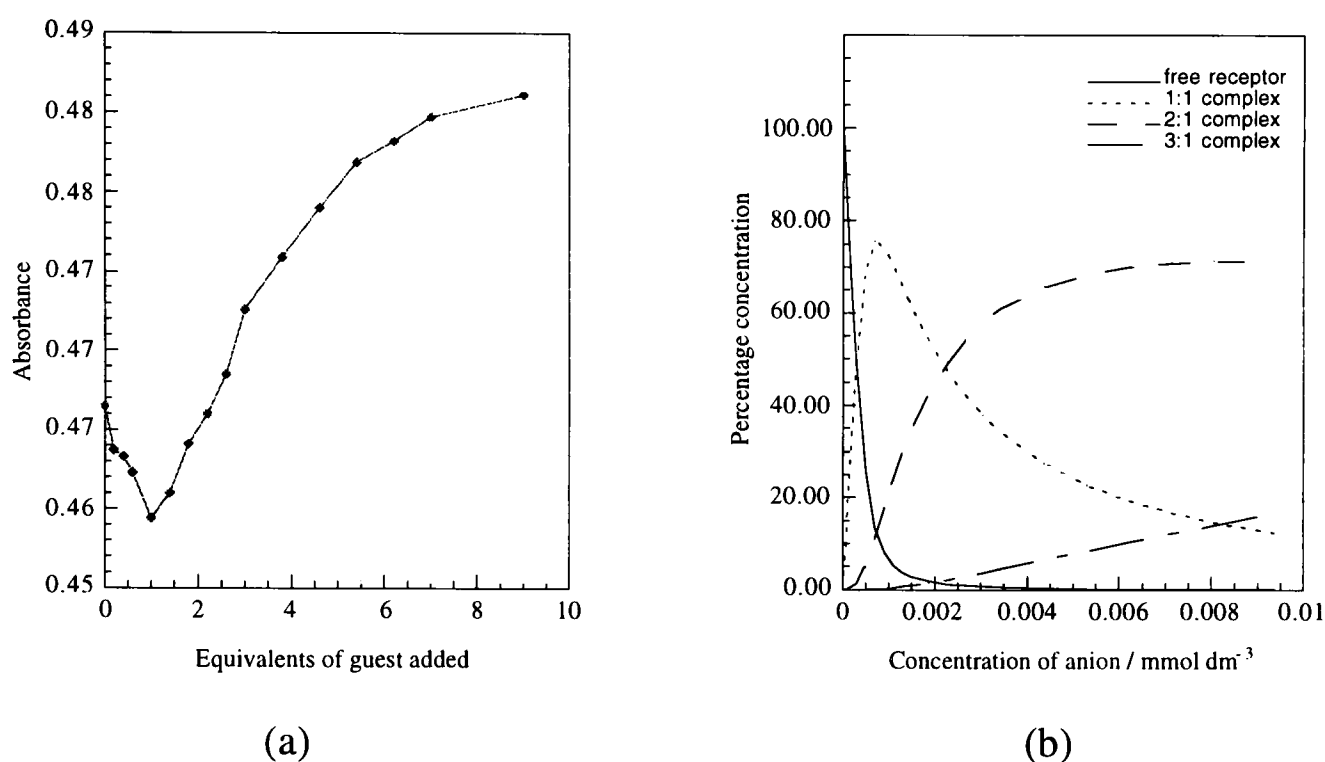


Figure 2.31: UV-visible titration of [40] with chloride in acetonitrile:

(a) profile at 400 nm; (b) concentration distribution.

A ^1H nmr titration was conducted with dihydrogen phosphate in DMSO and a titration curve was produced. Examination of the curve suggested a 1:1 stoichiometry and the profile suggested good binding, but heavy precipitation was observed during the experiment which precluded any quantitative analysis. The profile is shown in figure 2.32 for the amide resonance.

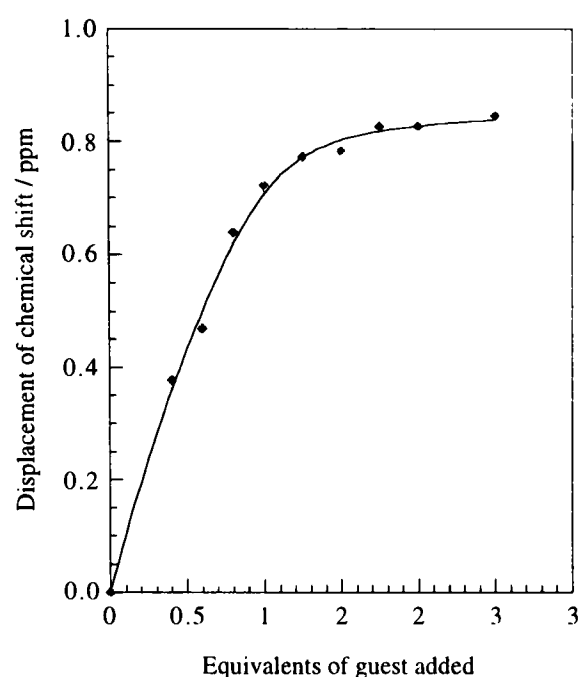


Figure 2.32: ^1H nmr titration profile for addition of dihydrogen phosphate to [40] in deuteriated DMSO.

2.13 Extended Tris-Cobalticinium Receptors

The extended receptors shown in figure 2.33 also have three cobalticinium units but in a different spatial arrangement. They were synthesised with two different spacer groups and it was thought that an extended structure might allow conformations in which a binding cavity could be formed as the receptor enveloped an anion.

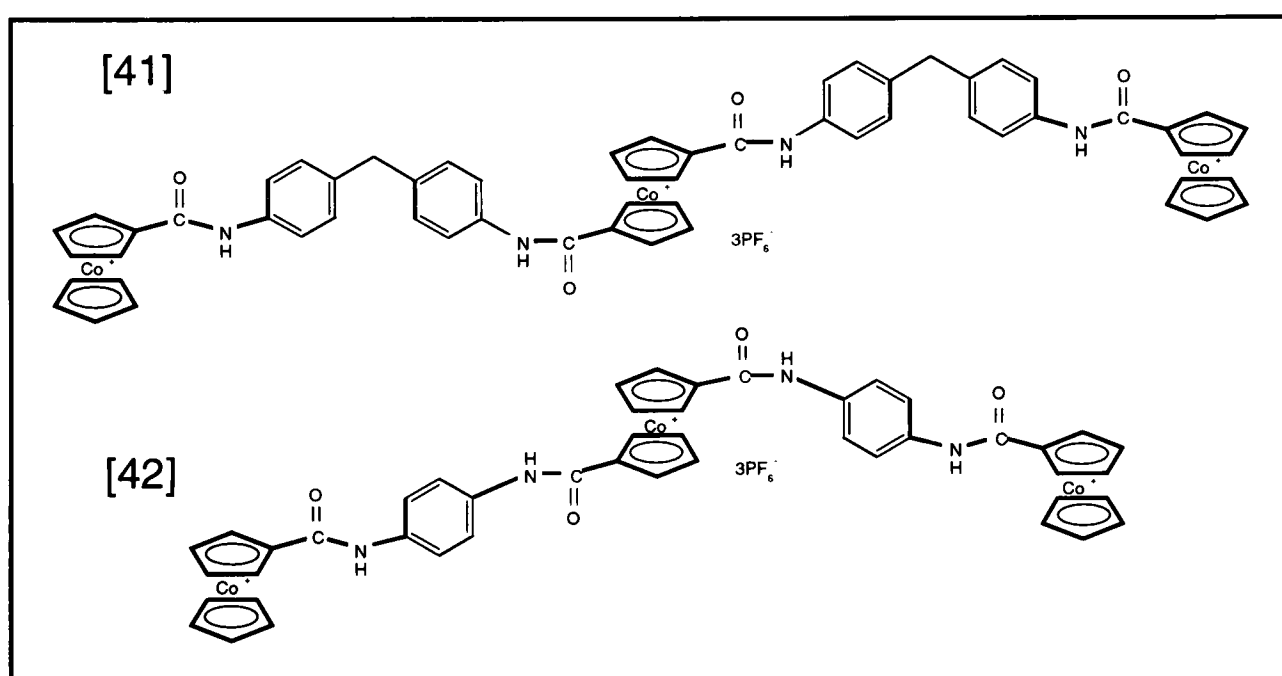


Figure 2.33: Extended tris-cobalticinium receptors.

2.13i ^1H nmr titrations

The titration profiles with TBA chloride for [41] are shown in figure 2.34. The receptor has two amide resonances and both protons appear to be involved in binding. Likewise, there are four types of substituted cyclopentadienyl resonance. However, only two of these are displaced by the addition of chloride as explained in the studies of [34]. Thus, interaction is observed at all of the potential binding sites. The overall process was complicated, however, and disappointingly no judgement could be made on either the stoichiometry or the stability constants for the titration.

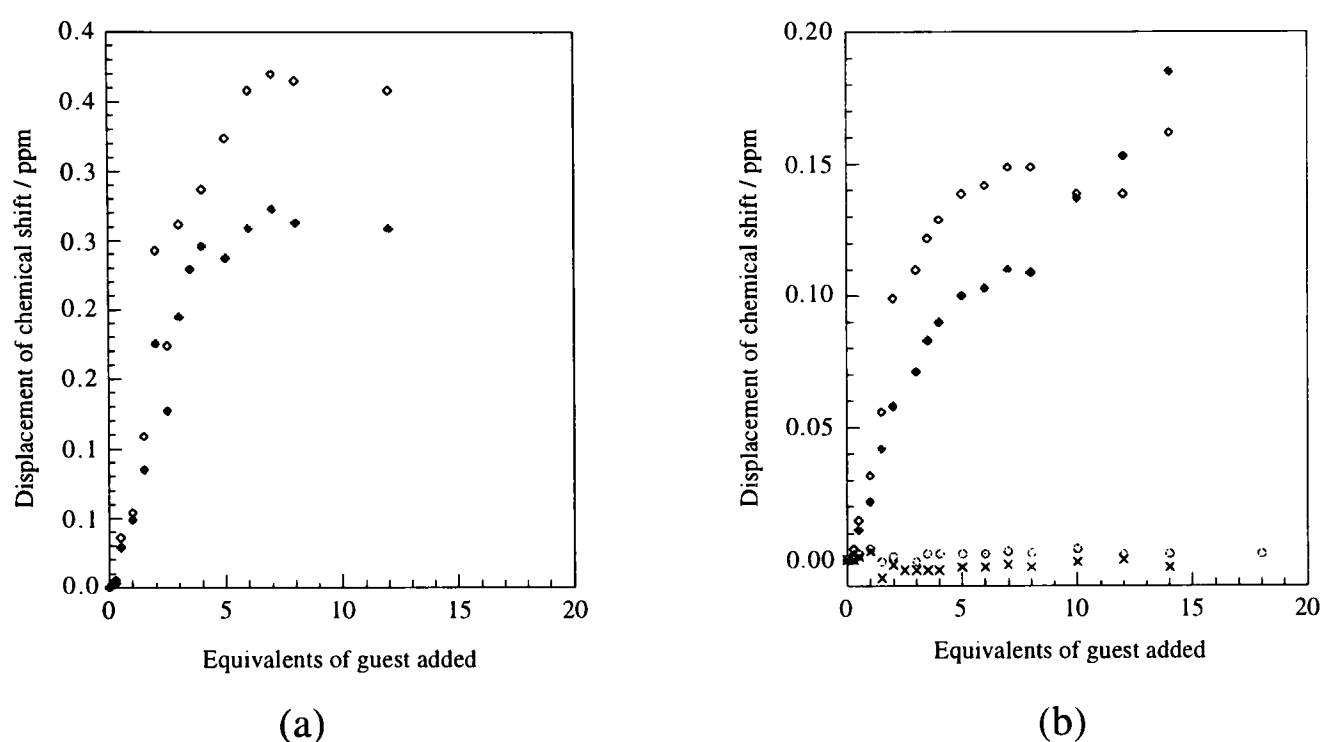


Figure 2.34: ^1H nmr titration profiles for addition of chloride to [41]: displacement of (a) amide proton; (b) cyclopentadienyl proton.

Slightly different results were obtained from [42]. The original ^1H nmr spectrum showed two amide resonances, four for the substituted Cp rings and a singlet for the aromatic spacer. On addition of chloride in deuteriated DMSO (the choice of solvent was dictated by solubility problems encountered in acetonitrile), the two amide resonances and two of the Cp signals were displaced; the titration profiles are shown in figure 2.35. Interestingly, the singlet due to the equivalent aromatic protons split into a four signal multiplet. This is presumably because the binding of an anion in close proximity lifts the magnetic equivalence of the protons.

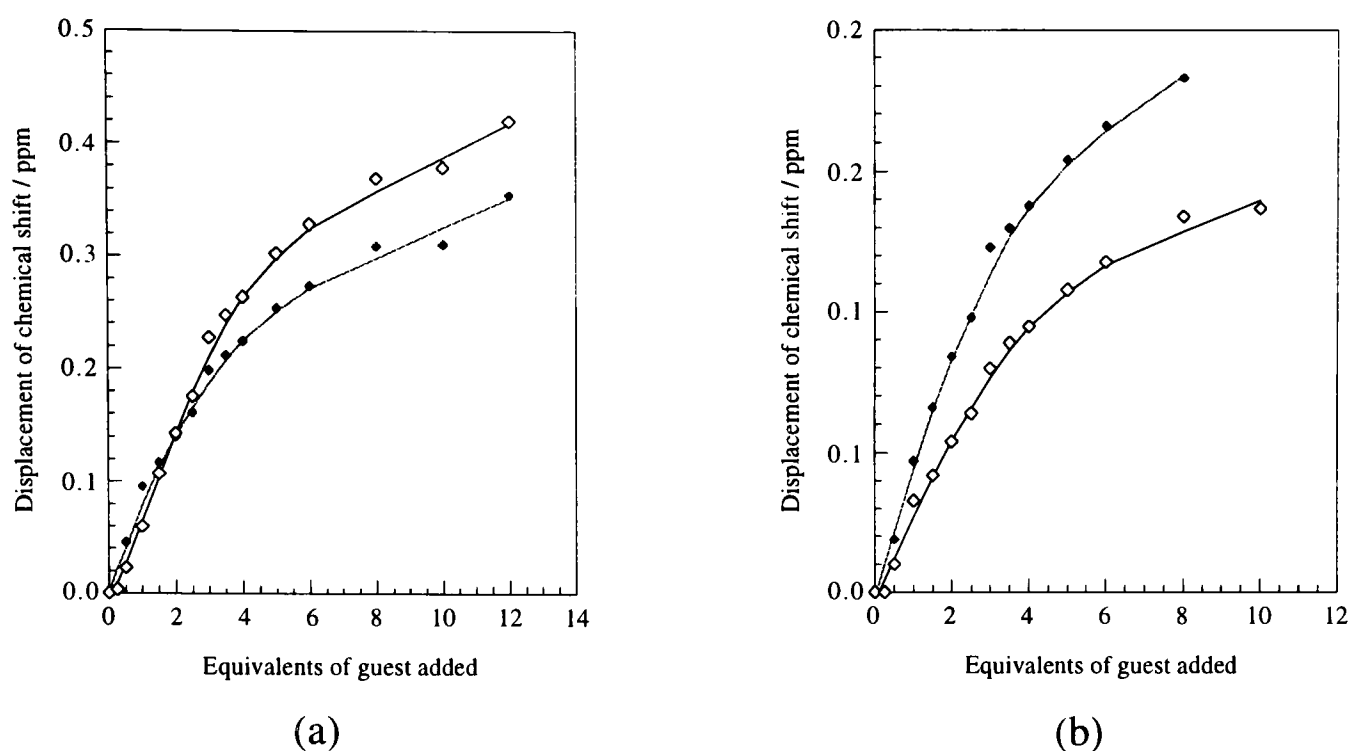


Figure 2.35: ^1H nmr titration profiles for addition of chloride to [42]:
displacement of (a) amide proton; (b) cyclopentadienyl proton.

It was not possible to determine stability constants from these data, but a qualitative observation can be made. The stoichiometry is clearly not 1:1 and this is hardly surprising for a receptor with a total of four possible binding sites. By inspection of tangents, a stoichiometry of approximately 3:1 occurs. This, of course, is the point at which the charge on the receptor is neutralised.

Titration of [42] with dihydrogen phosphate revealed no perturbation. The molecule cannot, in fact, wrap around the anions; it is actually quite rigid and therefore only one interaction can be formed with the anion, and this appears to be insufficient to elicit binding. It is probable that solvation of dihydrogen phosphate is too strong. This agrees with earlier comments which noted the weak interaction between this particular anion and a number of receptors.

2.13ii Electrochemistry

The cyclic voltammogram of [41] is shown in figure 2.36 and voltammetric data for both [41] and [42] are presented in table 2.3. Two redox couples are clearly visible for the two different cobalticinium units, one having a much higher peak current than the other. The two couples are more clearly resolved in the square wave voltammogram (recorded in

acetonitrile, figure 2.37) and the expectation that the ratio of peak heights should be 2:1 is fulfilled. The actual ratio is 1.8:1 but there is some peak overlap which may account for the shortfall. Titrations were performed but, despite the observation of perturbations in nmr experiments, no significant shifts (i.e. > 5 mV) in the half wave potentials were seen upon addition of chloride in DMSO.

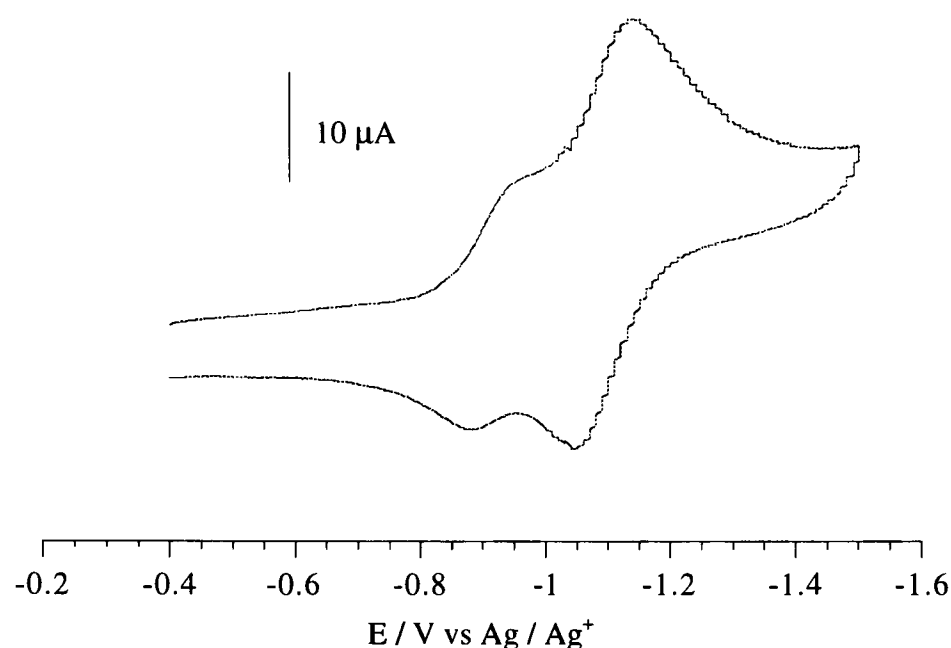


Figure 2.36: Cyclic voltammogram of [41] in DMSO.

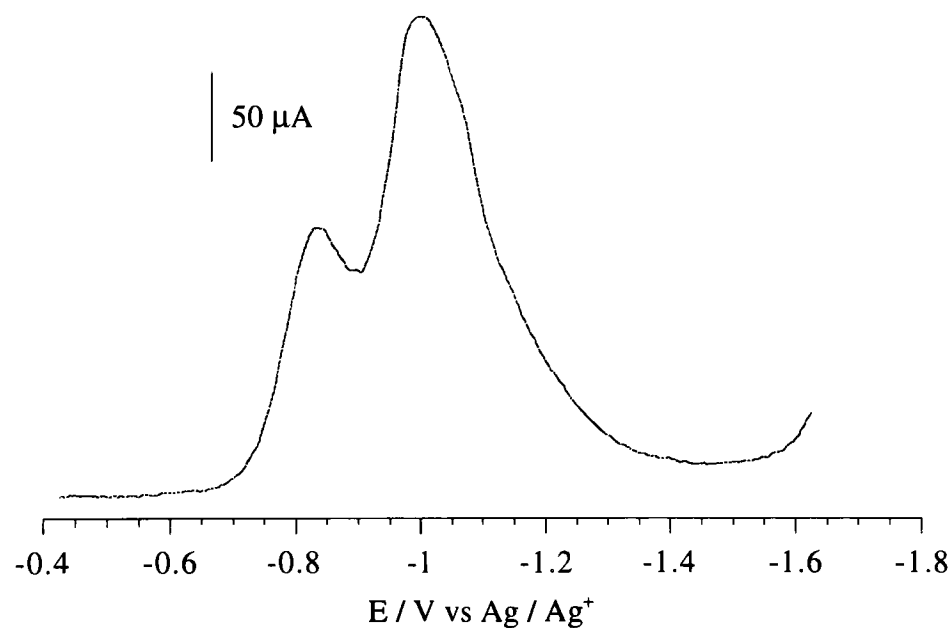


Figure 2.37: Square wave voltammogram of [41] in acetonitrile.

The cyclic voltammogram of [42] in acetonitrile was rather unexpected and displayed a very large current density on the first return wave (figure 2.38). This suggests some adsorption

process for the fully reduced species leading to a very high concentration of electroactive species at the electrode surface when the oxidation wave commences. The phenomenon is known as a “stripping peak” and is characteristic of electrode adsorption.¹⁵¹ It will be encountered again in the following chapter. Addition of DMSO removed this feature and restored the expected voltammetric profile which closely resembles that shown in figure 2.36, although the two couples are slightly further apart and, hence, better resolved.

Again, addition of chloride in DMSO induced no notable changes in the half wave potentials for this receptor. This behaviour is illustrative of an important point which was observed in several cases. It is found that the cobalticinium receptors are not particularly sensitive redox sensors for the presence of anions and although binding is clearly occurring, and with an appreciable strength, this is not always communicated to the redox centre. This is in contrast with the nmr studies which invariably detect a binding interaction and provide a much better diagnostic for anion recognition.

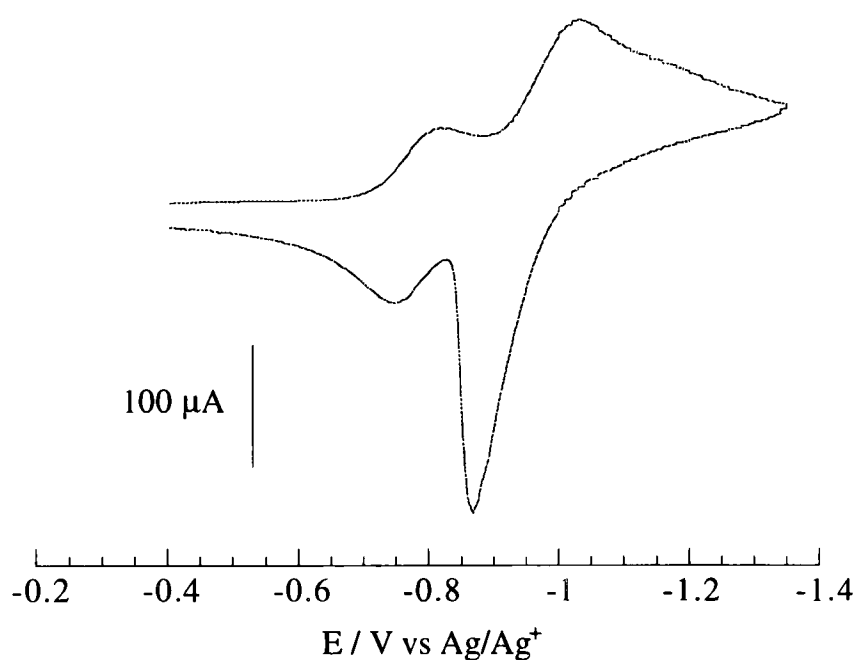
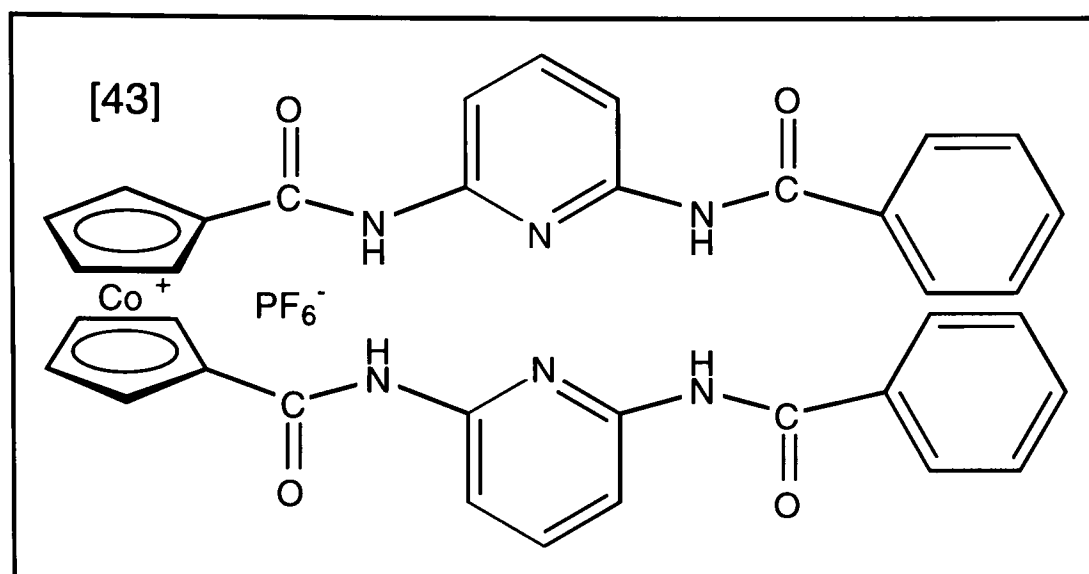


Figure 2.38: Cyclic voltammogram of [42] in acetonitrile. Note the stripping peak.

2.14 Dihydrogen phosphate Specific Receptors

Receptor [43] was synthesised using a synthon presented in the next chapter and displays some very interesting results. The synthesis proceeded via the bis(chlorocarbonyl)

cobalticinium as detailed in the synthetic section, 2.4. Characterisation by the usual methods confirmed that there were two products; that shown below being the minor one.



There are a number of amide groups present which were expected to provide good binding opportunities but which also limited the solubility of the compound; studies were confined to dilute solutions in DMSO. Those performed, however, displayed strong selectivity for dihydrogen phosphate, with negligible response to chloride, nitrate or hydrogen sulphate. The displacement of the cyclopentadienyl resonance upon addition of TBA dihydrogen phosphate in DMSO is shown in figure 2.41a (p. 93). The amide resonances proved too broad to follow precisely. There is also some noise in the data due to the relatively small magnitude of the shifts.

The synthesis of the receptor also produced an unexpected analogue, [44], as the major product. This was presumably due to steric hindrance preventing attack of the second equivalent of amine and, instead, hydrolysis occurring during work up. The molecule was characterised by the usual methods. Mass spectrometry showed a clear peak for the molecular ion (figure 2.39) and nmr was satisfactory. Furthermore, a broad band due to the O-H stretch was observed in the infra red spectrum but significant deviations were observed in microanalysis. This is thought to be due to partial deprotonation of the carboxylate which will affect the counter ion present. Certainly, the analysis was found to be high in all respects which is consistent with the loss of the inorganic anion. The mass spectra of both [43] and [44] are shown in figures 2.39 and 2.40 respectively.

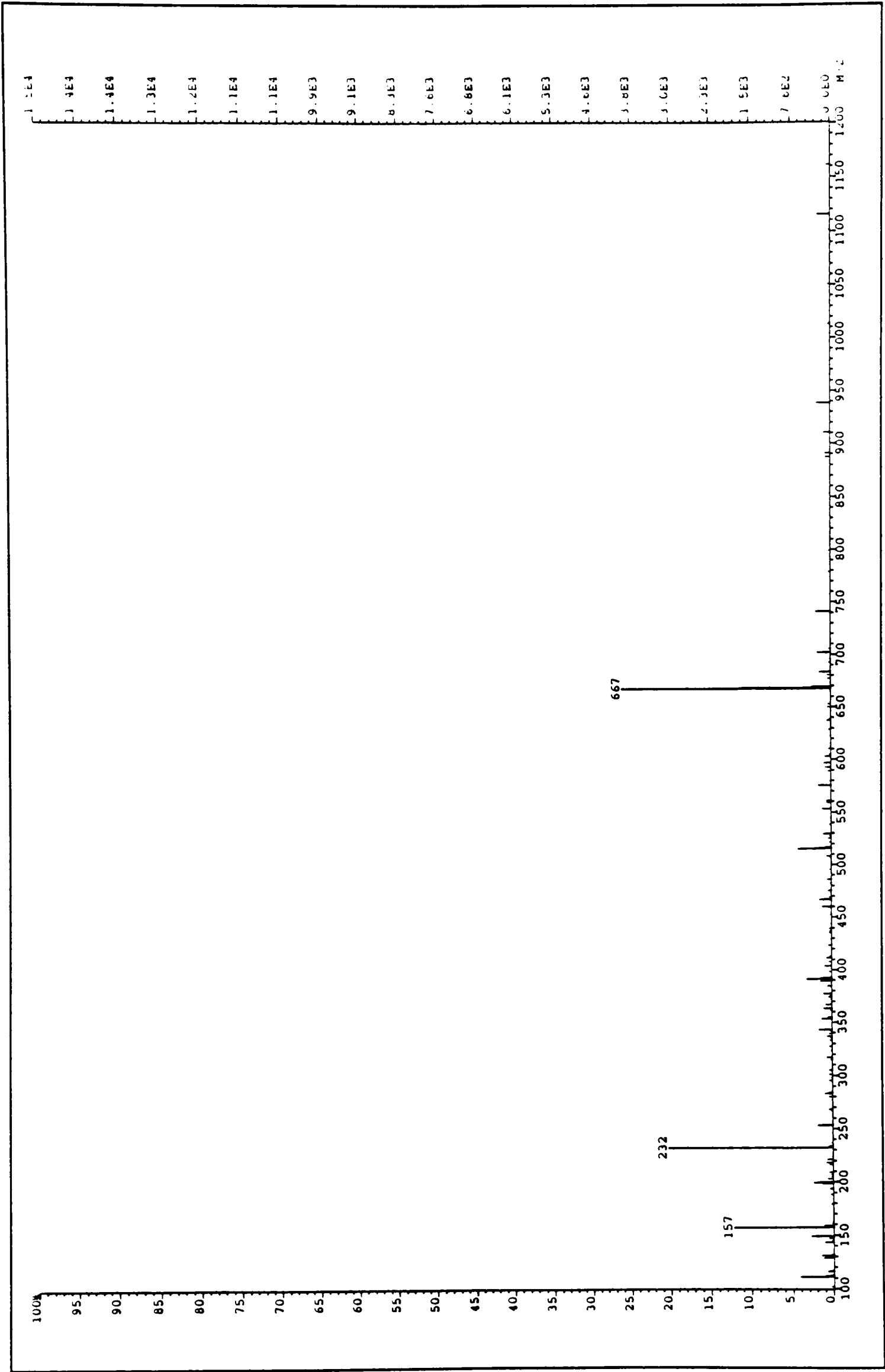


Figure 2.39: The FAB mass spectrum of [43].

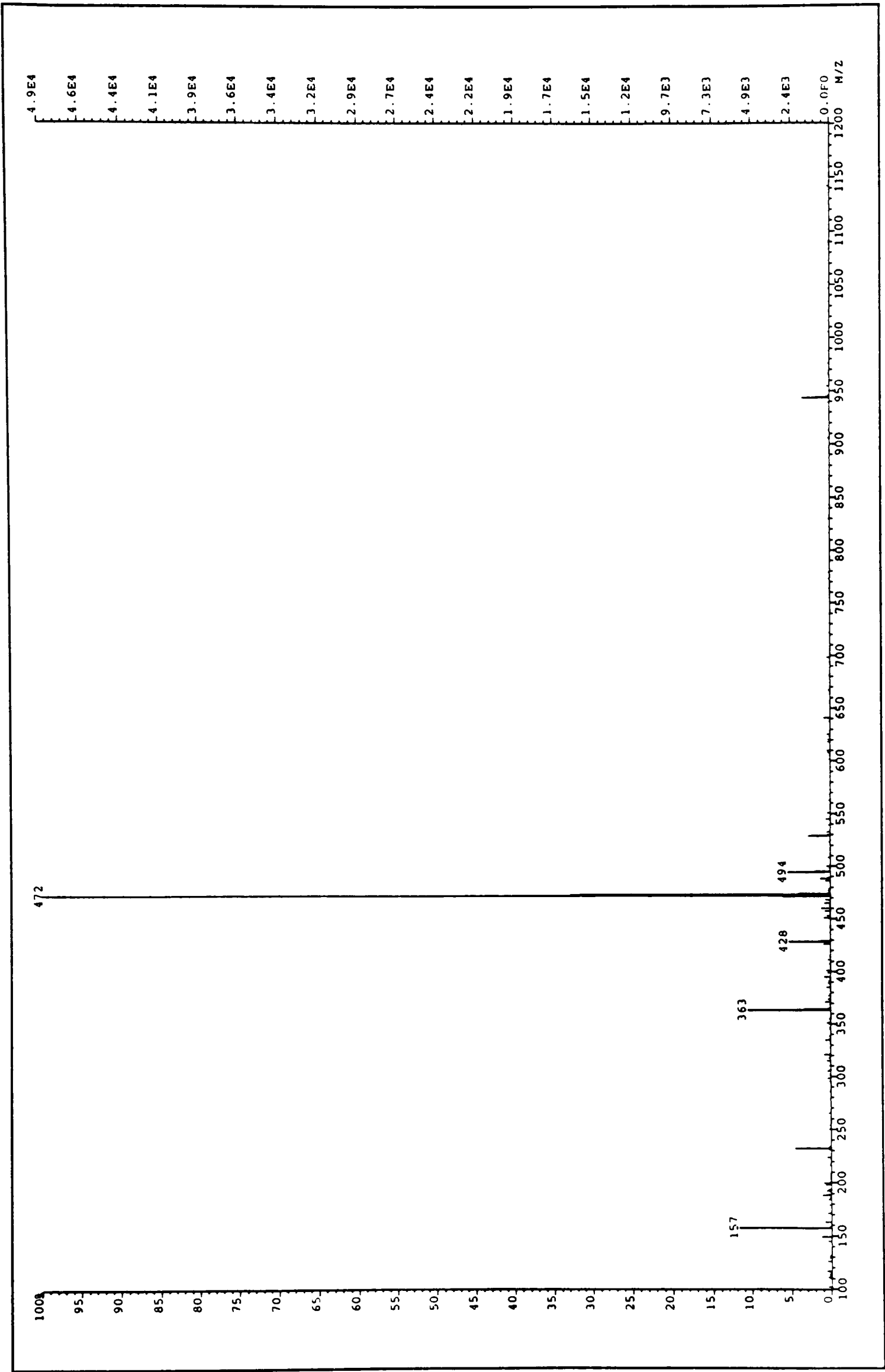
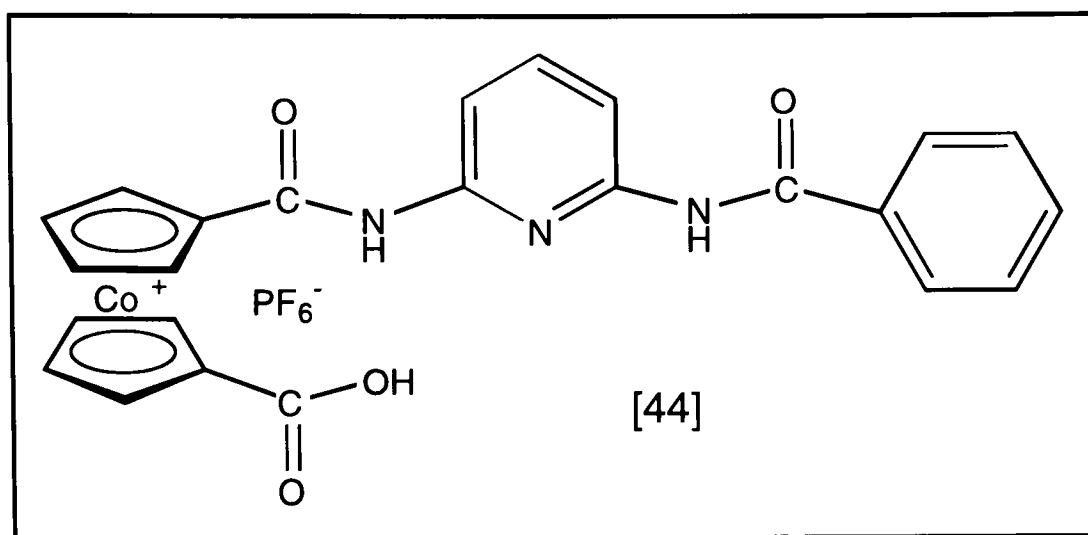


Figure 2.40: The FAB mass spectrum of [44].



Nmr investigation of the binding properties of [44] gave an analogous result in that only dihydrogen phosphate perturbed the system. The titration profile in DMSO is shown in figure 2.41b for three of the cyclopentadienyl protons. The fourth displayed erratic behaviour. Surprisingly, there was an upfield shift on binding — the only case in which this was observed.

The selectivity of binding was further investigated by an nmr competition experiment. A solution of [44] in DMSO was subjected to 10 equivalents of chloride followed by 10 equivalents of hydrogen sulphate with very little resultant displacement of the resonances. A titration with dihydrogen phosphate was then performed and found to correlate closely with the results presented in figure 2.41b.

In terms of binding stoichiometry and binding strength, the former appears to be a little in excess of 1:1 perhaps due to very weak binding of a second equivalent via hydrogen bonding to unattached amide groups and the first anion. The stability constant could not be calculated from the observed data, but qualitative inspection, particularly of the sharp end point, suggests that the binding is strong, even in the polar solvent DMSO.

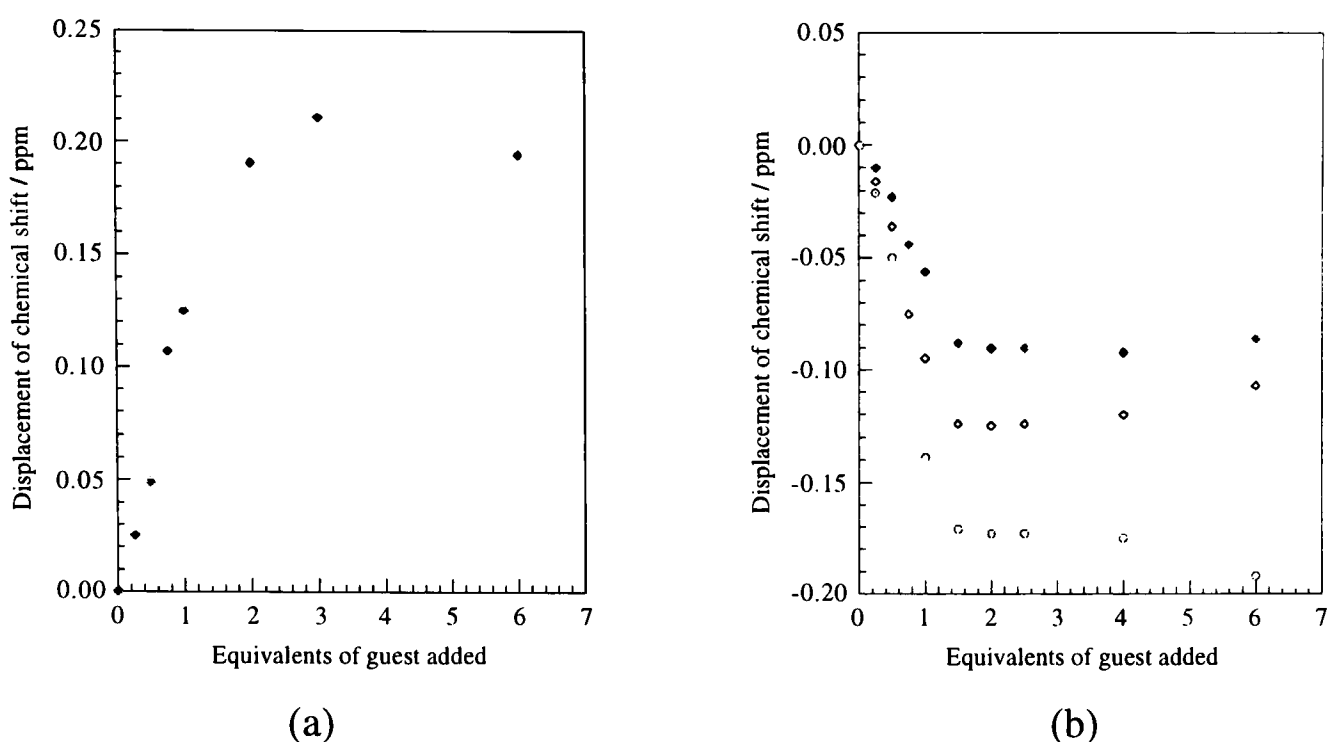


Figure 2.41: ^1H nmr titration with dihydrogen phosphate in DMSO; (a) [43]; (b) [44].

The origin of the binding is perhaps due to the arrangement of donor and acceptor sites within the molecule. An interaction with the upper ring substituents is possible as shown in figure 2.42 leaving opportunity for further binding to those on the lower ring. The donor sites on the receptor at the same time will discourage anions with too few donor sites such as chloride and hydrogensulphate.

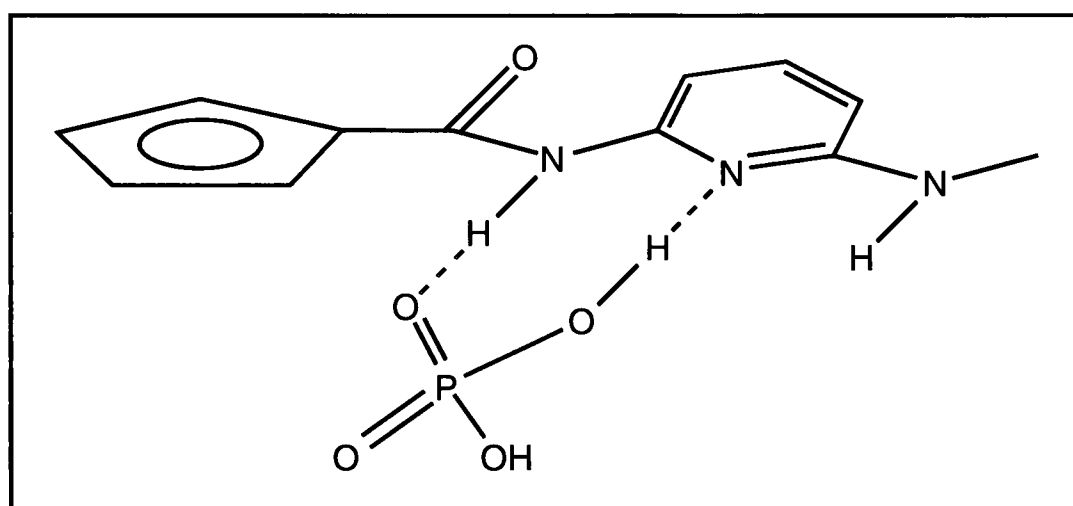


Figure 2.42: Schematic representation of binding to dihydrogen phosphate.

Mr David Smith, of this group, has observed protonation by acidic anions such as dihydrogen phosphate which assists binding in analogous, but neutral, hosts.¹⁵² This is addressed briefly in the next chapter but in the present case, protonation is not thought to be a significant contributor, probably due to the cationic nature of the receptor.

2.15 In Summary

The chapter has sought to introduce both anion binding by new mono- and poly-cobalticinium derivatives and the means by which this interaction was investigated. Coordination studies have been performed and the results are listed in tables 2.1–2.3 which present data from nmr, UV-visible spectroscopy and electrochemistry.

2.15i The Binding Site

The receptors were designed and synthesised with the aim of coordinating with an anion and it was proposed that the combination of the positive charge and hydrogen bonding would be sufficient for this purpose. Experiments in the binding of chloride were therefore conducted.

Hydrogen bonds, an important constituent of the binding interaction, were provided by the amide proton and a proton of the substituted cyclopentadienyl ring as shown by ^1H nmr studies and it was suggested that the binding site lay in close proximity to these functions. Tangible evidence in support of this hypothesis was provided by the crystal structure shown in figure 2.21 which exhibits an anion within hydrogen bonding distances of the two protons specified. They are known to be rather acidic and therefore are good acceptors of electron density.

Further evidence was furnished by a series of molecules in which the position of a hydrogen bonding amine group was varied, [35], [36] and [37]. Those isomers in which the amine lay close to the proposed binding site were stronger receptors for chloride as shown in ^1H nmr titration experiments.

2.15ii The Positive Charge

The intuitive expectation that a positive charge is instrumental in attracting an anion was fulfilled by examination of [38], [39] and [40]. These receptors have one, two and three cobalticinium units respectively and it was found that the binding of chloride increased exponentially in strength across the series. Here, then, is a clear indication that the presence of a positive charge is a major contributor to the association between host and guest. This discovery, of course, parallels many others mentioned in the previous chapter. The magnitude of the binding force between chloride and [40] is very substantial; the largest stability constant determined for any cobalticinium-anion complex. In absolute terms, the binding is also very

strong and few chloride receptors have been known to better its performance. The next chapter, which considers a number of neutral receptors, will allow further comparisons to be made.

The difference between [40], in which the cobalticinium units are tripodally arranged, and [41] and [42], with their linear distribution, is striking. In the linear receptors, the binding is suspected of being much lower which is probably due to two factors. Firstly, the relative inflexibility of the linking groups prevents the receptors from wrapping around the anion in a poly-cationic embrace, and secondly, repulsion between the positive charges will favour an extended conformation. Both factors would translate into an impaired binding performance.

RECEPTOR	ANION	SOLVENT (deuteriated)	INITIAL IONIC STRENGTH	STABILITY CONSTANT / dm ³ mol ⁻¹
[34]	chloride	acetonitrile	0.0127	(?) 500–1000 (a)
[35]	chloride	““	0.0127	630
[36]	chloride	““	0.0127	≈ 25 (b)
	dihydrogen phosphate	DMSO	0.0127	no perturbation
[37]	chloride	acetonitrile	0.0127	660
	dihydrogen phosphate	DMSO	0.0127	no perturbation
[38]	chloride	acetonitrile	0.0127	60
	dihydrogen phosphate	DMSO	0.0127	250
[39]	chloride	acetonitrile	0.0381	1:1 25 - 30
				2:1 < 5 (b)
	dihydrogen phosphate	DMSO	0.0381	precipitation
[40]	chloride	““	0.0763	1:1 ≈ 6000
				2:1 ≈ 50 (c)
	dihydrogen phosphate	““	0.0763	precipitation

NOTE: Errors are < 20 %. (a) Partial precipitation prevented accurate determination. (b) Errors were > 30 % due to shallow titration profile. (c) Errors > 30 %.

Table 2.1: ¹H nmr titration data.

2.15iii Anion Binding vs. Ion Exchange

It is important to establish that the responses observed on addition of anions are due to a binding interaction between host and guest, and not simply ion exchange. The electrochemical experiments help to discriminate because they are conducted in the presence of a large excess of base electrolyte. In these conditions, the receptor is in the presence of one equivalent of hexafluorophosphate and around 100 equivalents of tetrafluoroborate and yet it still responds to substoichiometric quantities of chloride. This implies that ion exchange is *not* the source of the interaction.

RECEPTOR	ANION	SOLVENT	INITIAL IONIC STRENGTH	STABILITY CONSTANT / dm ³ mol ⁻¹
[34]	chloride	acetonitrile	6.36 x 10 ⁻⁴	2800
[35]	chloride	""	6.36 x 10 ⁻⁴	2900
	""	""	0.123	950
	dihydrogen phosphate	""	6.36 x 10 ⁻⁴	precipitation
[36]	chloride	""	3.18 x 10 ⁻⁴	1700
	""	""	0.128	<400†
[37]	chloride	""	6.36 x 10 ⁻⁴	1750
	""	""	0.128	440
[38]	chloride	""	1.27 x 10 ⁻³	174
	""	""	0.125	<50†
	dihydrogen phosphate	""	1.27 x 10 ⁻³	precipitation
[39]	chloride	""	1.91 x 10 ⁻³	1:1 6500
				2:1 160
[40]	chloride	""	3.82 x 10 ⁻³	1:1 2.7 x 10 ⁵
				2:1 700

NOTE: Errors are all < 15 %. The variation of sum of squares with stability constant was checked and a true minimum was found except in cases marked by † in which a plateau region was observed.

Table 2.2: UV-visible titration data.

The fact that different anions elicit different levels of response is a further indication of binding. A number of the receptors did not show signs of significant interaction with dihydrogen phosphate and yet were efficient hosts for chloride, a fact attributed to an insufficient number of contacts between dihydrogen phosphate and the receptor. Those in which both acceptor and donor sites were available, e.g. [38], showed the reverse behaviour: attraction for dihydrogen phosphate but not chloride. In the extreme cases, [43] and [44], dihydrogen phosphate was the only acceptable guest to the host — the receptors displayed *specificity* in binding to dihydrogen phosphate. Again, these findings deny suggestions that there is a general, non-specific interaction with the anion.

2.15iv Receptors and Sensors

It was possible to quantify the binding by a number of analytical techniques but, having established the position and strength of binding, the question of potential sensing arose. Whether or not binding would be accompanied by a physical change in the receptor was of great concern, for in such changes lies the difference between receptor and sensor. Of particular interest were optical and electrochemical perturbations, and both were observed.

In terms of spectroscopy, the receptors *do* respond to coordination by a change in the UV-vis spectrum and titration allowed stability constants to be estimated. For various reasons, not least the change in ionic strength, the figures were an order of magnitude greater than those calculated by nmr and this became a common feature of the studies. It demonstrated that the term “stability constant”, which is commonly held as an absolute measure of the potency of a receptor, is in fact a relative term. The magnitude of the values is significant in anion terms and is superior to a great many published in the literature. When added to the stability and pH independence, cobalticinium is a very attractive system for anion recognition.

But this is not all. In addition to very satisfactory binding properties, there is a degree of sensing behaviour. While this is admittedly not of a standard suitable for commercial application, nevertheless there remains the fact that the receptors are sensitive to the presence of anion at the 0.1 equivalent level and respond by a change in several physical properties of a magnitude that is very easily detected in virtually every case. Table 2.3 illustrates that there is a redox response in most cases and, while it is unfortunate that a stability constant could

not be calculated, this remains one of the very, very few systems to show electrochemical sensing of anions.

The electrochemical response was promising. A cathodic shift in the half-wave potential was frequently observed, although the response was non-linear. The experiments confirmed that the positive charge was enhancing the binding; this is the meaning of the shift.

RECEPTOR	SOLVENT	$E_{pc}/$ V	$E_{pa}/$ V	$\Delta E_{ac}/$ mV	$E_{1/2}/$ V	i_{pc}/i_{pa}	ANION/ Equivs	$\Delta E_{1/2}$ /mV
[34]	acetonitrile	-0.958	-0.864	94	-0.91	1.23	Cl- / 14	45
[35]	""	-1.07	-0.99	80	-1.03	1.19	Cl- / 20	60
[36]	""	-1.14	-1.05	90	-1.09	1.17	Cl- / 22	45
[37]	""	-1.12	-1.04	80	-1.08	1.26	Cl- / 22	40
[38]	""	-0.71	-0.63	80	-0.67	1.15	Cl- / 10	< 5
[39]	""	-1.02	-0.89	130	-0.93	1.21	Cl- / 25	85
[43]	DMSO	(i) ?	-0.88	?	?		Cl- / 20	< 5
		(ii) -1.14	-1.05	90	-1.10	(?)1.86		
[44]	DMSO / acetonitrile	(i) -0.86	-0.96	70	-0.83	1.35	Cl- / 20	< 5
		(ii) -1.07	-0.79	110	-1.02	(?)1.42		

E_{pc} and E_{pa} are cathodic and anodic peak potentials; $\Delta E_{ac} = (E_{pa} - E_{pc})$; $E_{1/2} = (E_{pa} + E_{pc})/2$; i_{pa} and i_{pc} are oxidation and reduction wave currents. The scan rate was 100 mVs⁻¹ and the reference electrode was Ag / Ag⁺.

Table 2.3: Electrochemical parameters.

The overall prospects are good. If the current synthetic limitations of cobalticinium can be overcome, there is scope for success in creating better and better anion sensors. One approach might be to exercise synthetic manipulations on *cobaltocene* using the very considerable air-sensitive expertise within the department. There is some literature precedent, as mentioned in the introduction, and this might allow more sophisticated binding sites to be synthesised. In particular, if mono- and bis-methylamino cobalticinium could be produced there would be great scope for macrocyclisation reactions. Now this would be very interesting...

CHAPTER Three

Held Without Charge:

Binding by Neutral Receptors

3.0 Introduction

From the earliest days of anion recognition, receptor design reflected the fundamental assumption that centres of positive charge were absolutely necessary in order to achieve binding of the desired guest. This was first called into question by the publication of Lewis acid-based receptors, but even here, the charge was simply replaced by a group of low electron density. Currently, positive charge, one or preferably several, is still a very common feature of published work in the field. However, the assumption that anion receptors must be cationic was challenged, although only recently, in a paper by Reinhoudt who published details¹⁵³ of the receptor shown in figure 3.1.

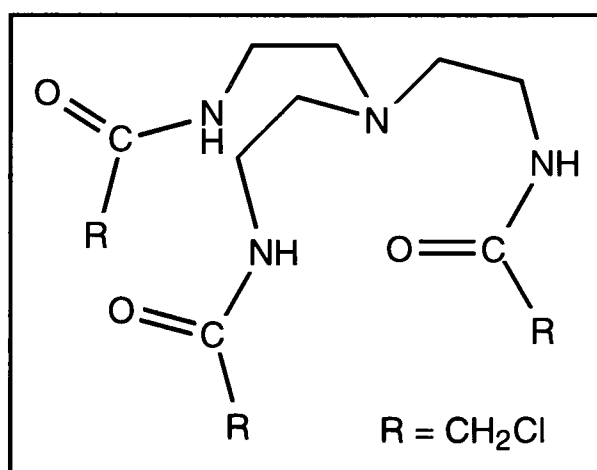


Figure 3.1: Neutral anion receptor.

The molecule shown is one of a series that selectively bound dihydrogen phosphate by a combination of hydrogen bonding and geometric complementarity; the stability constant for

that shown was $6100 \text{ dm}^3\text{mol}^{-1}$ in acetonitrile, which is very high for such a system. Inclusion of sulphonyl groups, which are electron withdrawing, doubled the binding strength. Such reports strongly suggest that, given a suitable combination of interactions, an anion can be bound by a neutral host. A recent paper has also shown selective fluoride recognition by ferrocene boronic acid, which responds electrochemically to the anion.¹⁵⁴ Other papers have shown binding of anions by a neutral molecule, but have not presented the findings as anion recognition.¹⁵⁵

However, these are in fact a re-discovery of a concept published in a number of papers from the early 1960s when research into hydrogen bonding was very active. It was noted that there was interaction with anions, but this was not hailed as such because perspective was focused on hydrogen bonding — it occurred several years before molecular recognition emerged as a field of research interest. A review article from 1968, for example, discusses “homoconjugate anions” in which the conjugate base of an acid hydrogen bonds to a molecule of undissociated acid, i.e. $\text{AH}\cdots\text{A}^-$.¹⁵⁶ Even at this stage, the author comments that “Experimental evidence...is much too extensive for complete coverage.” Accordingly, many documented examples of hydrogen bonding of anions exist.

Of course, the work presented so far has conformed to the assumption mentioned by deliberately including a cationic centre. At an early stage, however, a control experiment presented in section 2.6 demonstrated that the amide group was an essential component of anion binding, and a natural extension of this is to question the role of the positive charge in the properties displayed; an issue which is addressed in this chapter. Is a positive charge essential in this design? The bulk of the discussion will therefore concentrate on neutral molecules which still incorporate the amide group. In a number of cases, cobalticinium was replaced by ferrocene which resulted in a close analogue, but without a formal charge. The coordination properties of these molecules were investigated and in this way it was hoped that the Coulombic component of the binding interaction could be assessed. The inclusion of ferrocene as a reporter group has an extensive precedent in cation binding and its ability to communicate information via spectroscopy and electrochemistry is well-known.¹⁵⁷ The quest was therefore to see if these molecules would function as anion receptors and sensors, and to what extent they would reflect the success reported in Chapter Two.

3.1 Synthesis

3.1i Synthesis of receptors [48]–[50].

With the exception of the first receptor, [45], the synthesis of all the molecules was carried out via the acid chloride. Condensation with an amine in the presence of triethylamine, which removed HCl generated in the reaction, gave good yields of the amide product, typically around 60%. In some cases, chromatography was required and was performed with silica gel as the supporting medium and dichloromethane as the primary eluent. The polarity of the solvent was adjusted by addition of methanol. It was found that mono(chlorocarbonyl) ferrocene and benzoyl chloride were much less reactive than the cobalticinium analogue — presumably the lack of a positive charge makes them much less electrophilic. This allowed greater control over the reaction, for example in mono-substitution of a diamine the addition of acid chloride to amine gave the desired product whereas addition of amine to acid chloride gave the bis-amide. Thus the order of addition influenced the product; such control was not possible with mono(chlorocarbonyl) cobalticinium. Benzoyl chloride was commercially available whereas mono(chlorocarbonyl) ferrocene was generated from the corresponding carboxylic acid by the action of oxalyl chloride.

The compounds were isolated as crystalline powders and characterised in the usual manner. Further details are available in Chapter Six; the structures of the receptors are shown in figure 3.2.

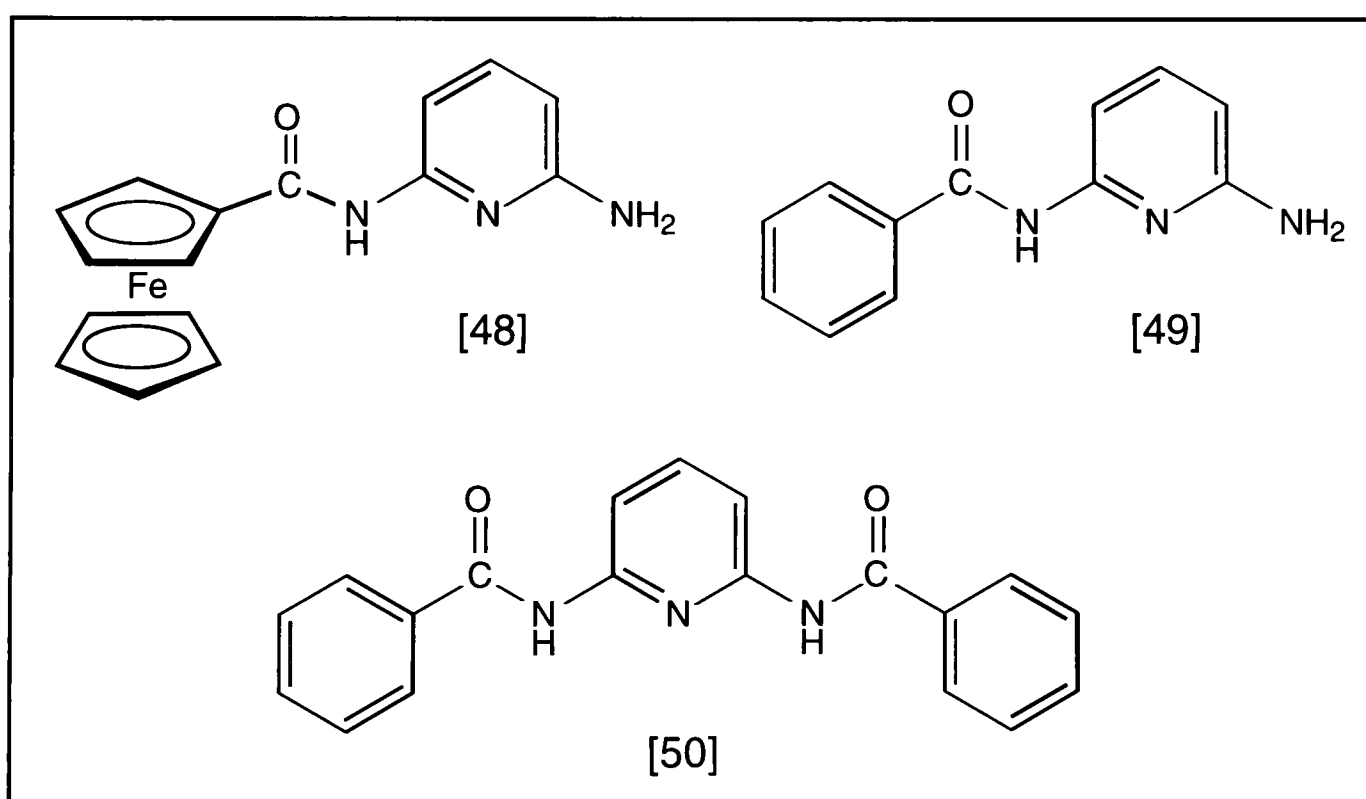


Figure 3.2: Neutral receptors synthesised via acid chloride.

Recrystallisation of [48] from methanol:hexane gave large single crystals, the structure of which was solved by Dr Drew. It is shown in figure 3.3.

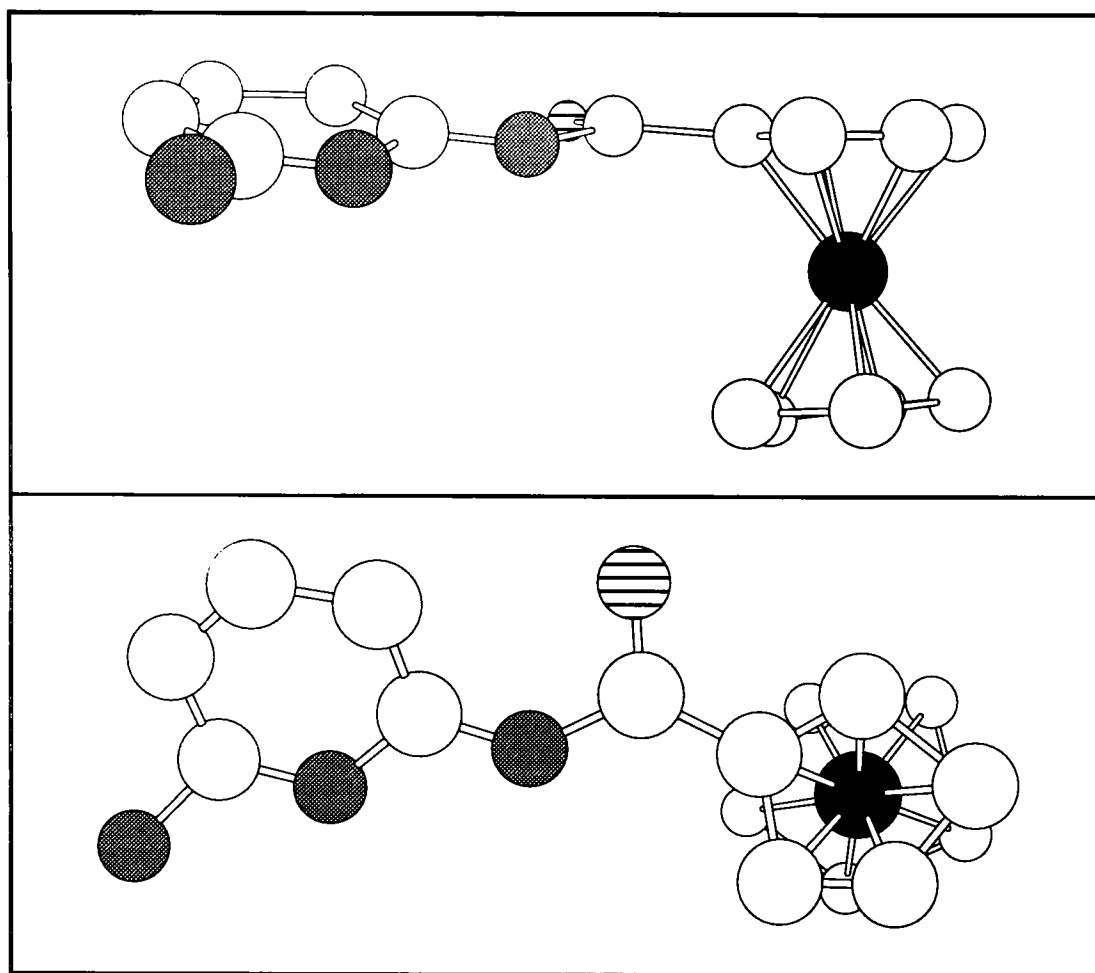


Figure 3.3: X-ray crystal structure of [48].

3.1ii Synthesis of receptor [45]

Receptor [45], discussed in the next section, was synthesised via an activated ester route, as discussed in section 2.4 (p. 52). The activated intermediate was formed within an hour, accompanied by precipitation of dicyclohexylurea; the solution was filtered and used immediately in the next stage (figure 3.4). In this case, the amine added was methylamino ferrocene which was prepared according to published methods.¹⁵⁸ The crude product was purified by chromatography using Sephadex.

Slow evaporation of the product from acetonitrile:methanol resulted in the formation of small crystals which were suitable for X-ray analysis; the structure is shown in figure 3.5.

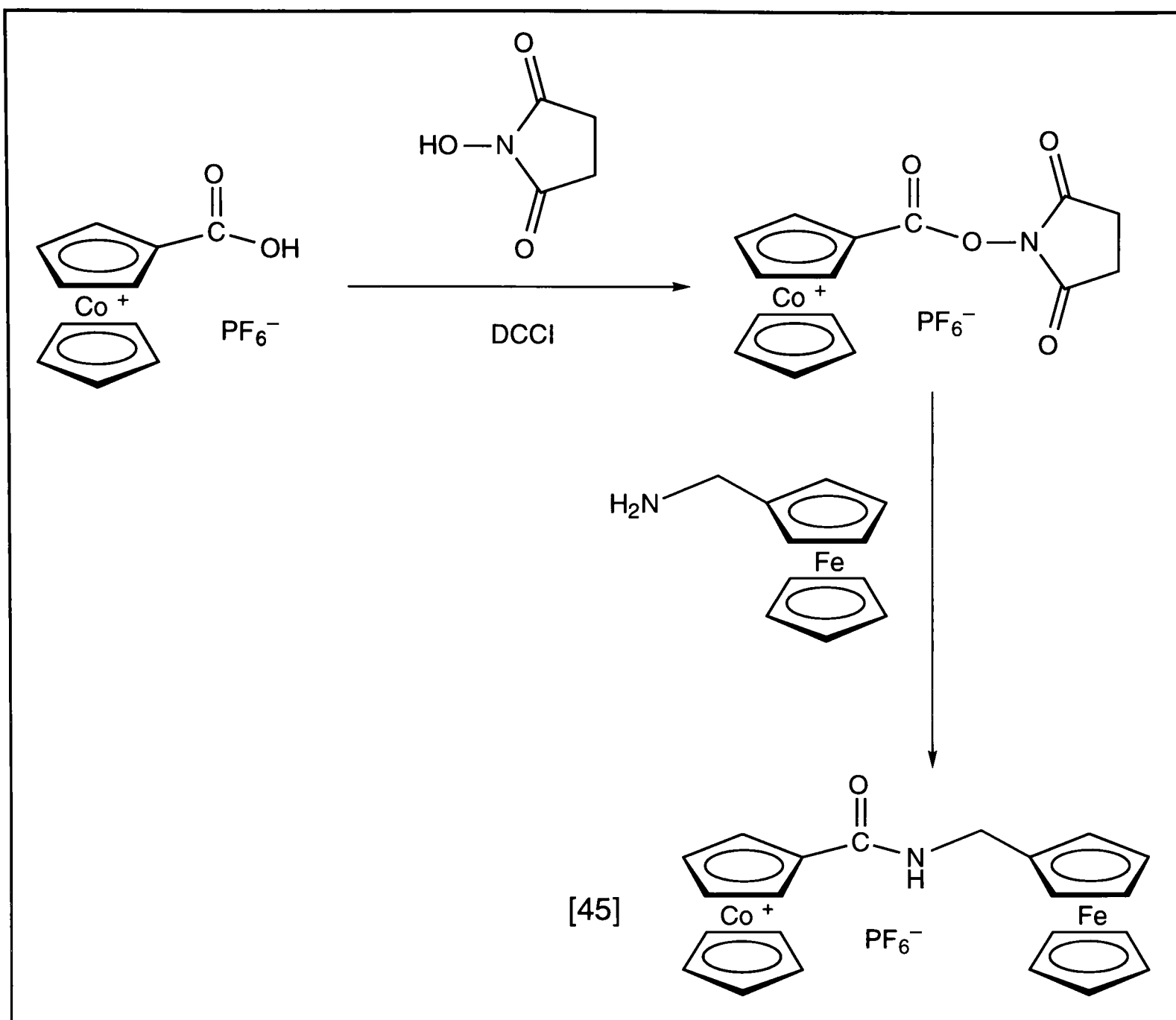


Figure 3.4: Synthesis of receptor [45].

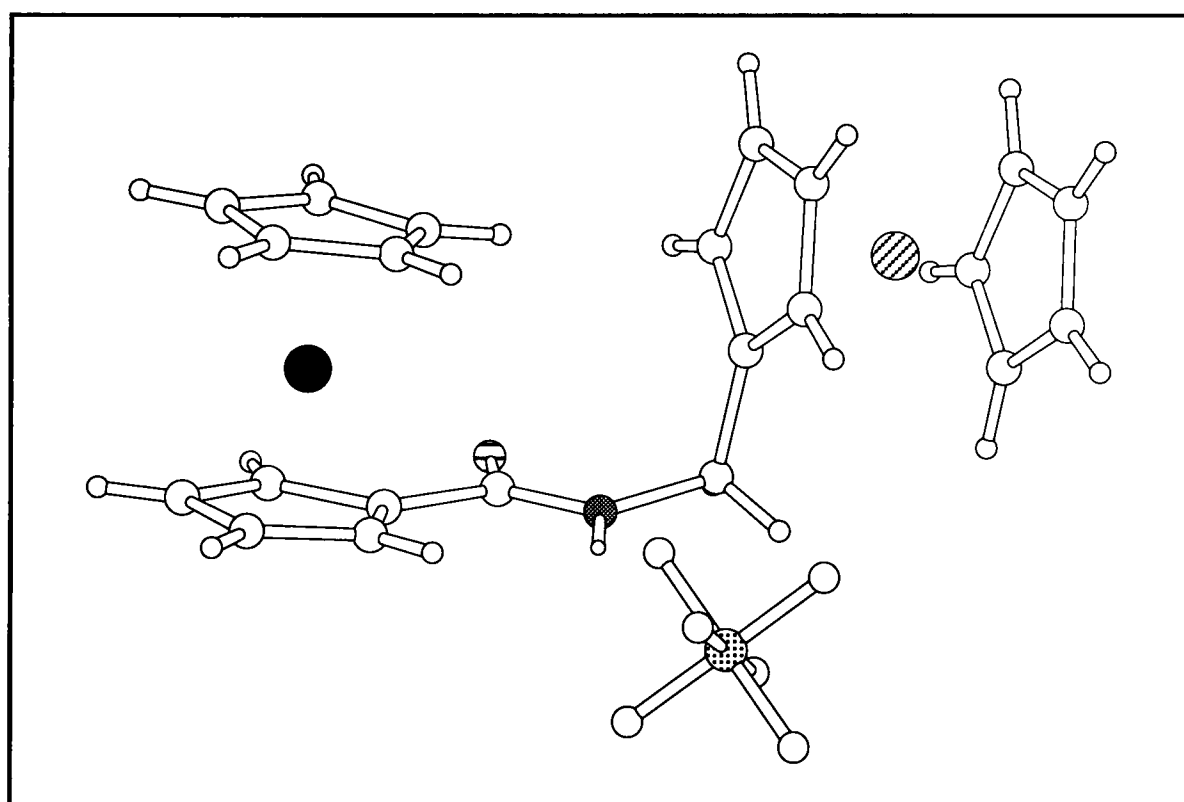


Figure 3.5: X-ray crystal structure of [45].

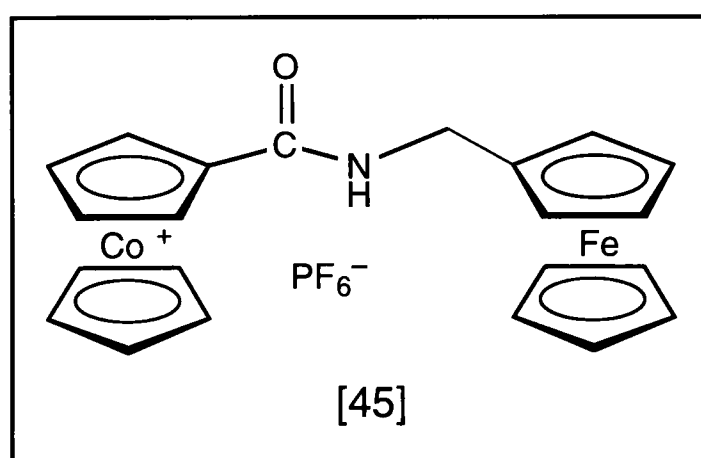
3.2 Introduction to Anion Coordination Studies

The receptors were investigated by the methods introduced in the previous chapter, where appropriate. The main anions studied were chloride and dihydrogen phosphate, although sections describing binding of hydrogen sulphate and 1-butyl thymine are also included. The binding was expected to be weaker and therefore a solvent of low polarity was often chosen for study. In nmr studies, the solvent of choice was deuteriated chloroform mindful, however, of possible contamination by chloride anion. In order to avoid this, old solvent was not used and the residuals generated by curve fitting analysis were carefully examined; a background concentration of chloride would introduce a systematic error in a chloride titration which would be reflected by a regular pattern in the residual data points. UV-vis experiments were conducted in a range of solvents whereas electrochemical investigation was exclusively confined to acetonitrile.

The first receptor, [45], is slightly anomalous because it carries a charge! However, it provides a useful link with the previous chapter and a source of reference for the comparisons made in this one.

3.3 A Mixed Cobalticinium-Ferrocene Receptor

The molecule [45], shown below, includes a cobalticinium unit and an amide group but has little else to promote association with an anion. It was felt that the inclusion of ferrocene would be interesting, particularly in observing what differences would emerge in the response of the respective organometallic fragments to the guest.



3.3i ^1H nmr titration

^1H nmr titration with chloride in deuteriated acetonitrile immediately showed evidence of anion binding, with an estimated stability constant of $860 (\pm 110) \text{ dm}^3\text{mol}^{-1}$. The titration profile of the cobalticinium Cp proton is shown in figure 3.6 and the very large magnitude of the displacement is noteworthy.

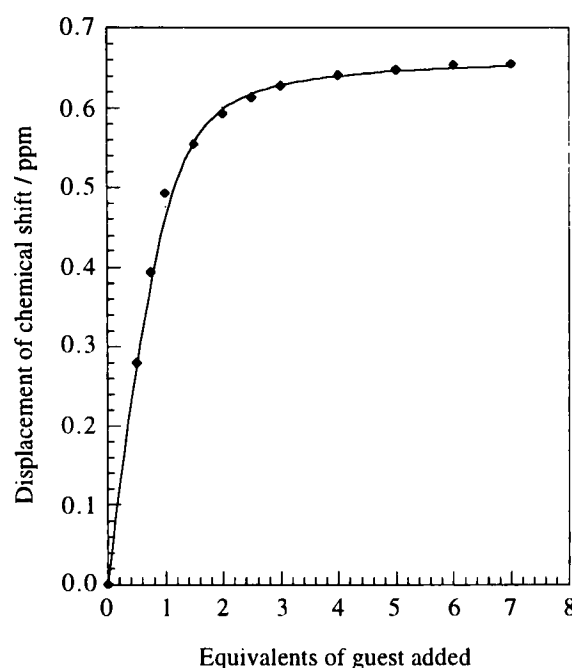


Figure 3.6: ^1H nmr titration profile for addition of chloride to [45].

There was also some perturbation of the ferrocene peaks; the substituted Cp signals moved, but interestingly, the other signal was very little affected. A through-bond perturbation is unlikely due to the intervening CH_2 link, which is saturated. Moreover, the lower ring of ferrocene does not appear to be close enough to the binding site to be greatly affected by a through-space interaction with the guest. In short, the ferrocene group can interact with the bound anion but not in a way which affects the lower ring. The spectra of the free receptor and that after addition of 7 equivalents of chloride are shown in figure 3.7.

The titration with dihydrogen phosphate painted a very different picture. To maintain solubility, the experiment was conducted in deuteriated DMSO. There was little apparent movement in the peaks, but considerable broadening of the cobalticinium resonances was observed, and every ferrocene peak was split into a signal of higher multiplicity. In this event, it was not possible to apply any curve fitting routine and, beyond evidence of strong interaction, few conclusions could be drawn from the data.

3.3ii UV-visible spectroscopy

The binding of chloride was also investigated by UV-vis spectroscopy. The absorption spectrum was not affected by the addition of chloride in acetonitrile to any appreciable extent; the absorbance was monitored at three different wavelengths and changed erratically within a margin of $< 1\%$. The reason for this is thought to be that any change in the cobalticinium absorbances are masked by ferrocene, which absorbs more intensely in the visible region and is not affected by the anion.

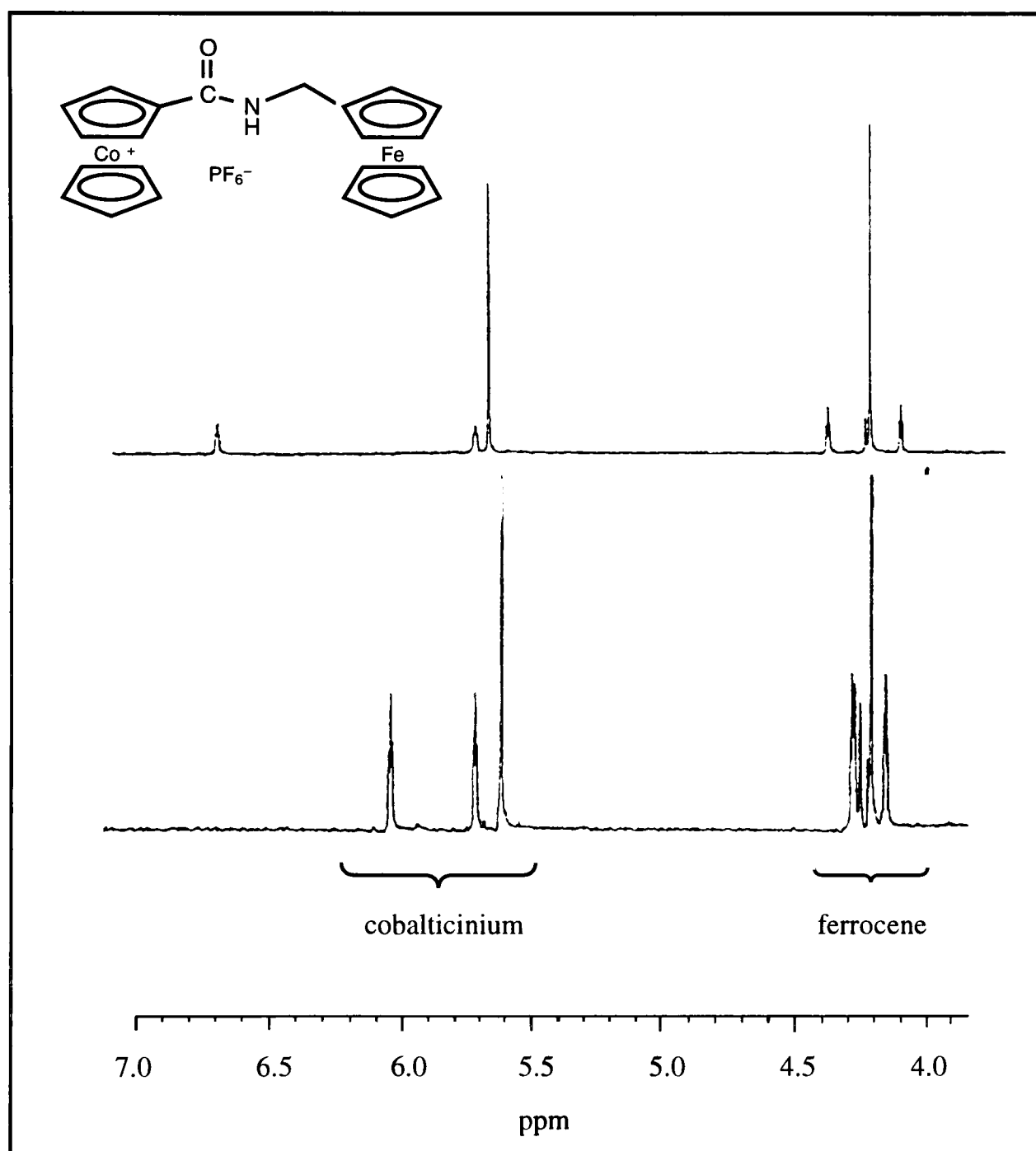


Figure 3.7: ^1H nmr spectra of [45] before (bottom) and after (top) addition of 7 equivalents of chloride.

3.3iii Electrochemistry

It was therefore thought that electrochemistry might be better able to distinguish between the two redox centres; the CV shows two well-resolved waves in acetonitrile (figure 3.8). The

cobalticinium wave was found to be reversible, although this was not the case for the ferrocene wave — the plot of $\sqrt{v}$ against i_p was slightly curved. A chloride titration was performed and it was seen that the half wave potential for both redox centres shifted cathodically on addition of 10 equivalents chloride, by 25 mV (cobalticinium) and 35 mV (ferrocene). In neither case was the reversibility affected.

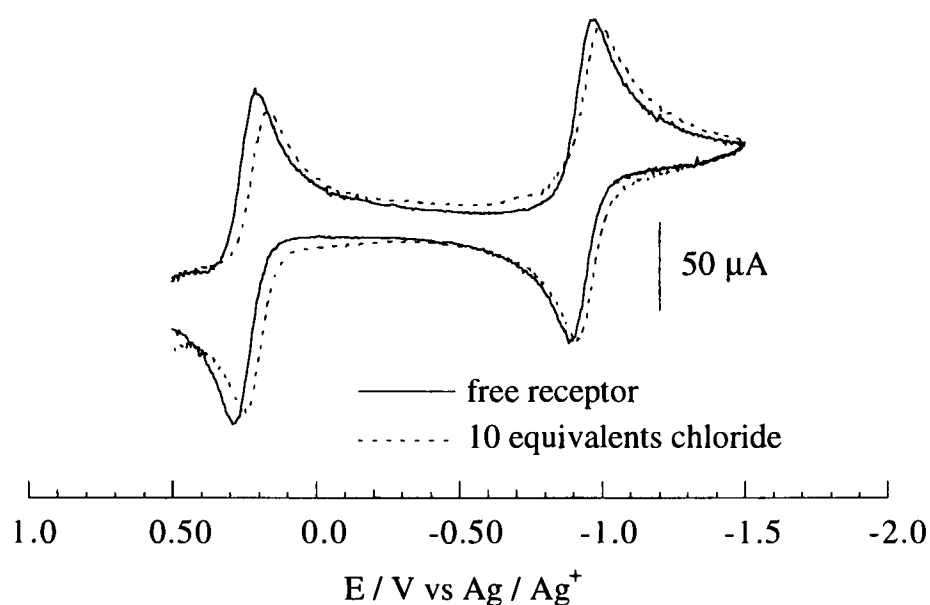


Figure 3.8: Effect of chloride on the CV of [45].

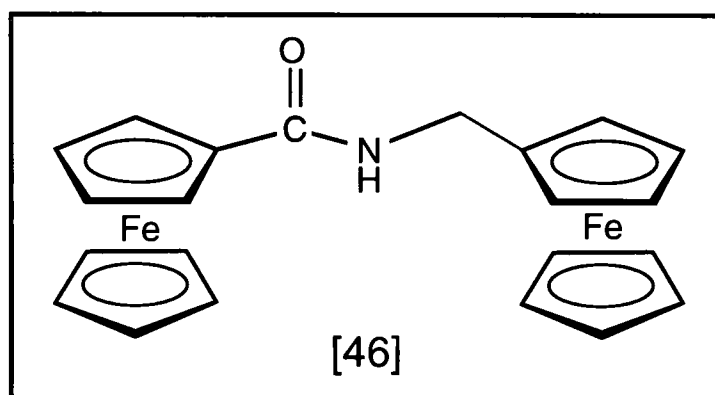
In this experiment, ferrocene and cobalticinium were perturbed to a similar extent. This is not altogether surprising; electrochemical oxidation of ferrocene will produce a cationic moiety in the vicinity of the binding site and interaction of with the anion is likely. The shift in potential indicates that ferricinium interacts more strongly with chloride than ferrocene (the *binding enhancement* introduced in section 2.7iii) and this is entirely reasonable. One would expect the binding enhancement for ferrocene and cobalticinium to be similar.

In summary, this receptor was able to bind and detect anions by a combination of interactions discussed in the previous chapter. Of the two redox centres, cobalticinium was perturbed more readily than ferrocene in nmr, to approximately the same extent in electrochemistry and the UV-vis results were inconclusive.

3.4 A Neutral Analogue

The question of the contribution of the positive charge to binding of anions by [45] can be addressed by considering the analogue [46], in which cobalticinium is replaced by ferrocene.

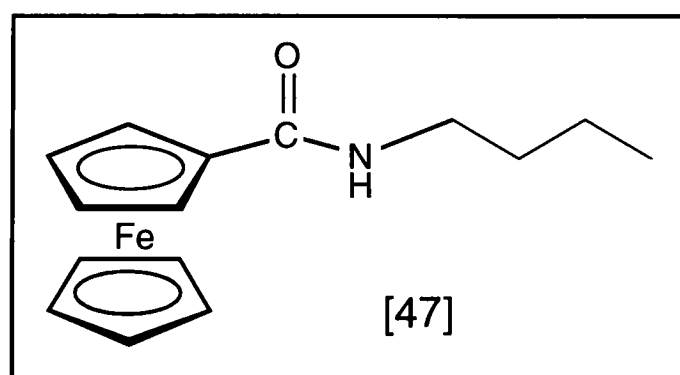
This compound was synthesised and studied by Miss Catherine Halliwell under the supervision of the author: the results presented below can be found in an earlier work by Miss Halliwell.¹⁵⁹



The most significant finding was that the molecule *did* act as an anion receptor, with the implication that the amide group alone is enough to induce an association between neutral receptor and anion. The contribution of the positive charge can therefore be examined by comparison of the stability constants for the receptors [45] and [46]. The binding of chloride by [46] was measured by ^1H nmr in deuteriated chloroform and found to occur with a stability constant of $10\text{ dm}^3\text{mol}^{-1}$; this is very weak, given the solvent. Dihydrogen phosphate was also bound; the interaction was rather stronger, with a stability constant of $100\text{ dm}^3\text{mol}^{-1}$ in the same solvent. However, in comparison with the stability constant found for [45], these values are trivial and suggest that the positive charge contributes a very significant proportion of the binding interaction.

3.5 Anion Binding by a Neutral Amide-Substituted Ferrocene

The anion binding potential of amide-substituted ferrocene was demonstrated by receptor [46], in the previous section, and the results found are were augmented by consideration of [47] (below), a very simple amide. The receptor was kindly provided by Dr Jana Hodačová, who conducted the synthesis by condensation of mono(chlorocarbonyl) ferrocene with n-butylamine.



3.5i ^1H nmr titration

The ^1H nmr studies in deuteriated chloroform gave similar results in titrations with both chloride and dihydrogen phosphate: very weak interaction. A displacement of the amide and Cp resonances resulted from the presence of anion, but no signs of a plateau region were observed and curve fitting analysis generated stability constants of $18 (\pm 10) \text{ dm}^3\text{mol}^{-1}$ and $5 (\pm 3) \text{ dm}^3\text{mol}^{-1}$ for chloride and dihydrogen phosphate respectively. At these levels of binding, it can be seen that the errors are extremely large and the receptor really cannot distinguish between the anions. The profiles themselves are very similar and are shown in figure 3.9. In fact, the curve drawn in both cases is a straight line, which is a better fit (in terms of lower Σ) than those generated by EQNMR, and it is likely that the values quoted are over-estimates.

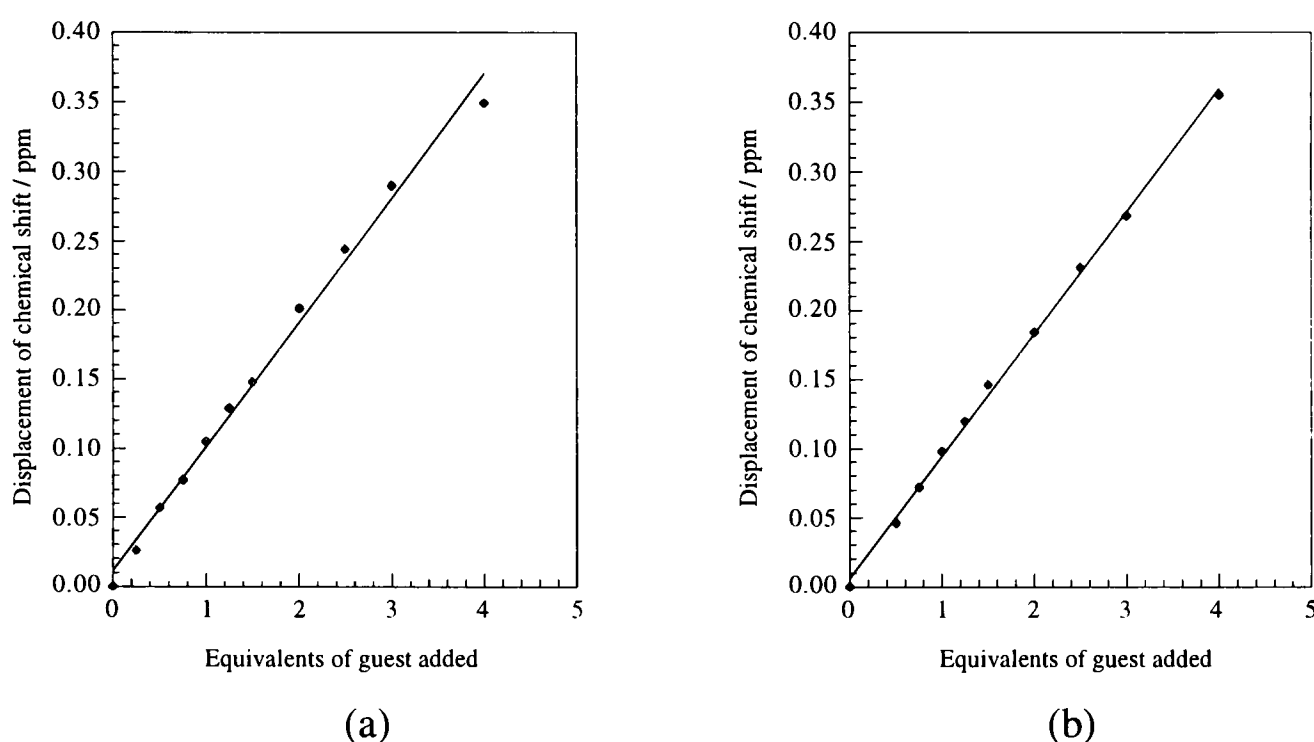


Figure 3.9: ^1H nmr titration profiles (amide proton) for addition of anion to [47]:

(a) chloride; (b) dihydrogen phosphate.

3.5ii Electrochemistry

In short, this molecule is not a good example of an anion receptor. However, it is a very good example of the way in which ferrocene can respond electrochemically to an anion. Electrochemical studies were conducted in acetonitrile, under which conditions the CV did not respond strongly to the addition of chloride; the position of the wave was unaffected and there was a steady decrease in i_{pc} as the couple became more irreversible.

However, when the potential was scanned to -0.8 V and then cycled, a new quasi-reversible peak emerged, which was attributed to the host-guest pair. The voltammograms in figure 3.10 illustrate this phenomenon; in the cases shown, the potential was held at the anodic limit for 2 minutes to generate appreciable amounts of ferricinium chloride near the electrode surface.

A new redox wave appeared at around -0.5 V and, at slow scan rates, was irreversible, indicating an EC mechanism.¹⁶⁰ This is the term for a case where the Electrochemical process is followed by a Chemical reaction. Here, reduction is followed by dissociation of the ion pair. However, at faster scan rates, the ion pair was seen to persist, and another oxidation wave could be seen. In effect, there are two redox couples, one for the free receptor and one for the host-guest pair. This behaviour was not seen for ferrocene itself and the conclusion therefore is that the inclusion of an amide group promotes this electrochemical response to chloride. A similar, but less pronounced, effect was seen on addition of dihydrogen phosphate.

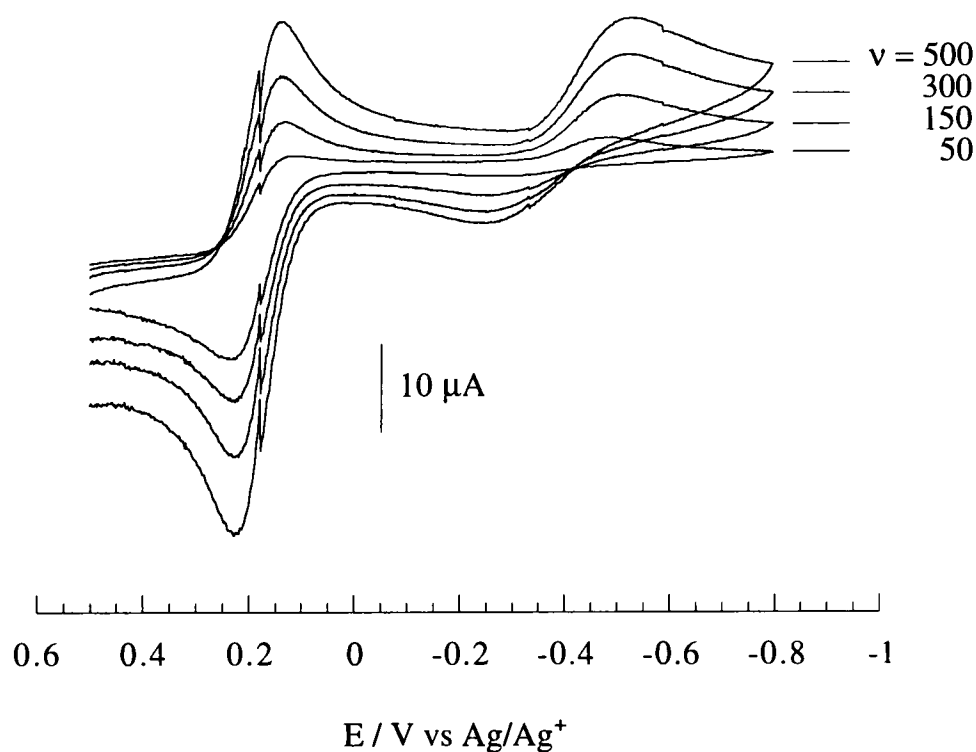


Figure 3.10: Effect of chloride upon CV of [47]. The voltammograms are shown for a series of scan rates (shown in mVs^{-1}).

This shows a case where electrochemistry can give an indication of quite subtle interactions between host and guest. The inclusion of ferrocene was the main reason for this; it readily undergoes EC processes. In contrast, the CVs of cobalticinium were almost always well

behaved and reversible and, beyond a shift in the half-wave potential, did not usually provide any additional information.

It is interesting to note that unsubstituted ferrocene itself is sensitive to the presence of an anion, although in a slightly different manner. The generation of a cationic oxidation product was often followed by a chemical reaction (the EC mechanism) which affected the reverse wave. An extreme example is the addition of dihydrogen phosphate to a solution of simple ferrocene in acetonitrile. Ferrocene cannot easily be protonated and has no means of binding with the anion and one would therefore expect the CV to be unaffected by the addition. However, it was found that the reduction wave was eroded by addition of dihydrogen phosphate and a sharp, intense peak appeared instead. Figure 3.11 shows the CVs for increasing amounts of dihydrogen phosphate to an upper limit of 3 equivalents. This feature is known as a *stripping peak* and is caused by adsorption of the product on to the electrode surface (cf. figure 2.38). It appears that ferricinium dihydrogen phosphate is insoluble in acetonitrile and is deposited on the electrode. This provides a very large concentration of electroactive species for the reduction wave, hence the large current.

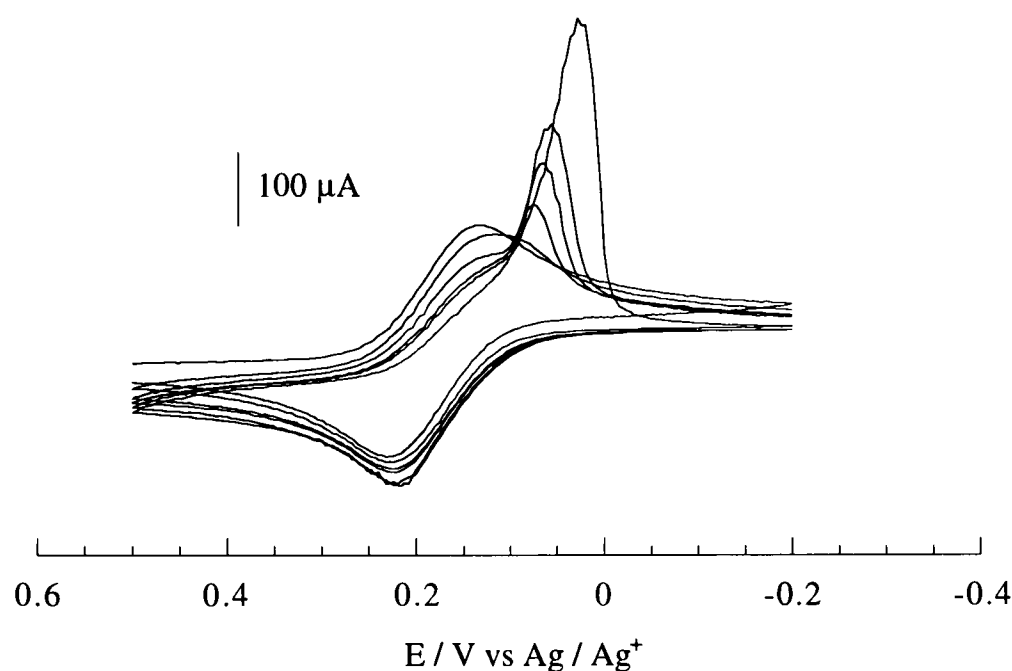
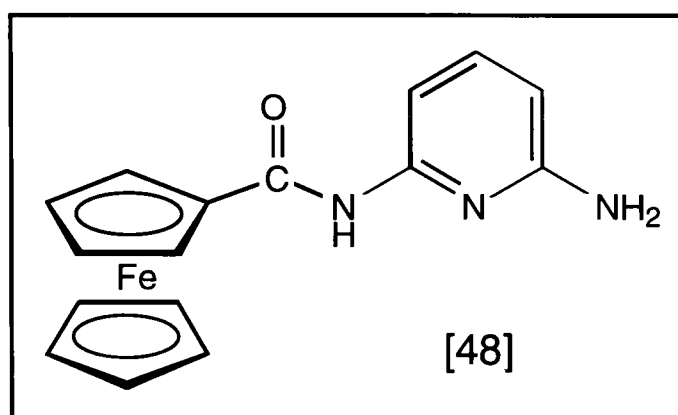


Figure 3.11: Effect of dihydrogen phosphate on CVs of ferrocene.

3.6 Anion Binding By Receptor [48]

Despite the weak binding displayed by [47], it was interesting to see if ferrocene-based receptors could respond to variation in the binding site, as in the previous chapter. To this end, the receptor [48], shown below was synthesised — a direct ferrocene analogue of one encountered in the previous chapter (receptor [38]).



3.6i ^1H nmr titration

The usual ^1H nmr study revealed that there was no appreciable displacement of the resonances on addition of chloride in deuteriated chloroform, suggesting that there is a negligible affinity of receptor for anion. The titration with dihydrogen phosphate, however, bore evidence of a weak interaction; the titration profile for the Cp proton is shown in figure 3.12 and gave rise to a stability constant of $48 (\pm 5) \text{ dm}^3\text{mol}^{-1}$.

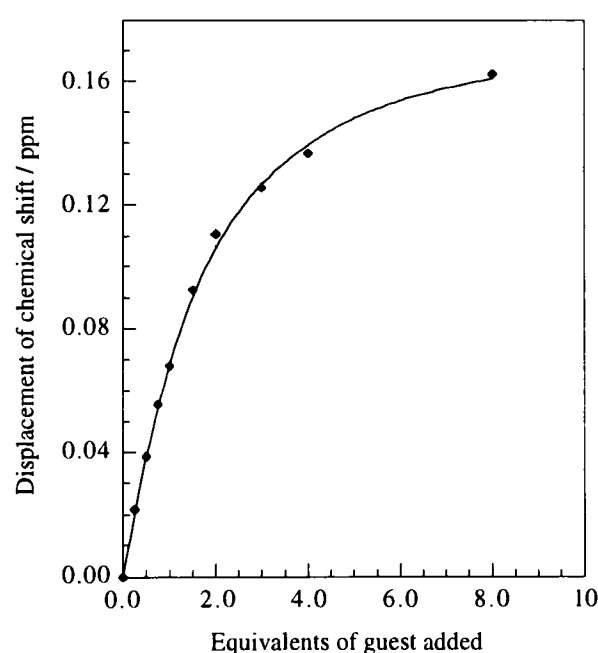


Figure 3.12: ^1H nmr titration profile for addition of dihydrogen phosphate to [48].

This mirrors the results of [38] (section 2.9) well; the binding of dihydrogen phosphate is very much stronger than that of chloride, which has been attributed to the presence of the lone pair close to the binding site. Comparison with [47] also reveals that the binding has been improved by an order of magnitude by the modification of the appended group. However, an absolute comparison with the stability constant of [38] ($250 \text{ dm}^3\text{mol}^{-1}$ in DMSO) bears witness to the vast difference which can be made by inclusion of a positive charge in the receptor design.

3.6ii UV-visible spectroscopy

The results of the UV-vis titration with dihydrogen phosphate in acetonitrile are shown in figure 3.13 for the absorbance at 430 nm. It can be seen that the profile indicates weak binding and that the change in absorbance was very small (i.e. $\approx 2\%$). At other wavelengths, the profile was very erratic and quantitative analysis of the spectra was not possible.

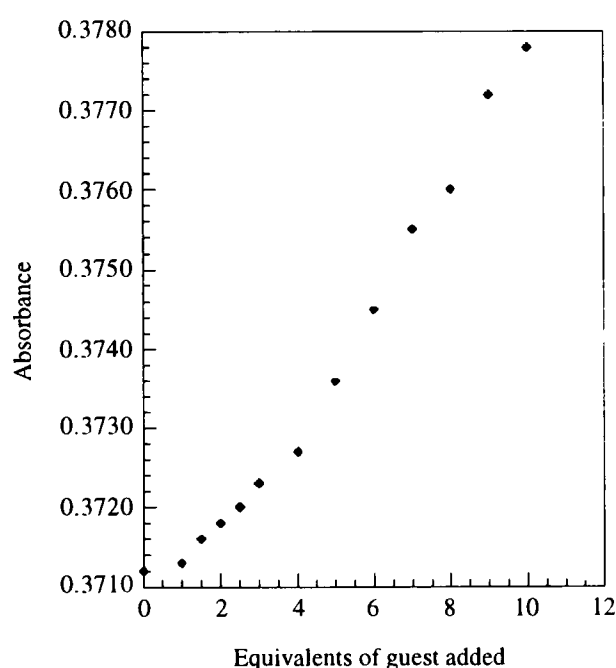


Figure 3.13: UV-vis titration profile (430 nm) for addition of dihydrogen phosphate to [48].

3.6iii Electrochemistry

Once again, however, the electrochemistry was very interesting. The CV of [48] in acetonitrile is shown in figure 3.14 for two different scan limits. The wave at around 0.20 V is characteristic of the reversible, one electron oxidation of ferrocene. At higher potentials, another wave is seen which is attributed to the oxidation of the amine function; the corresponding reduction is seen as a shoulder on the ferrocene reduction wave.

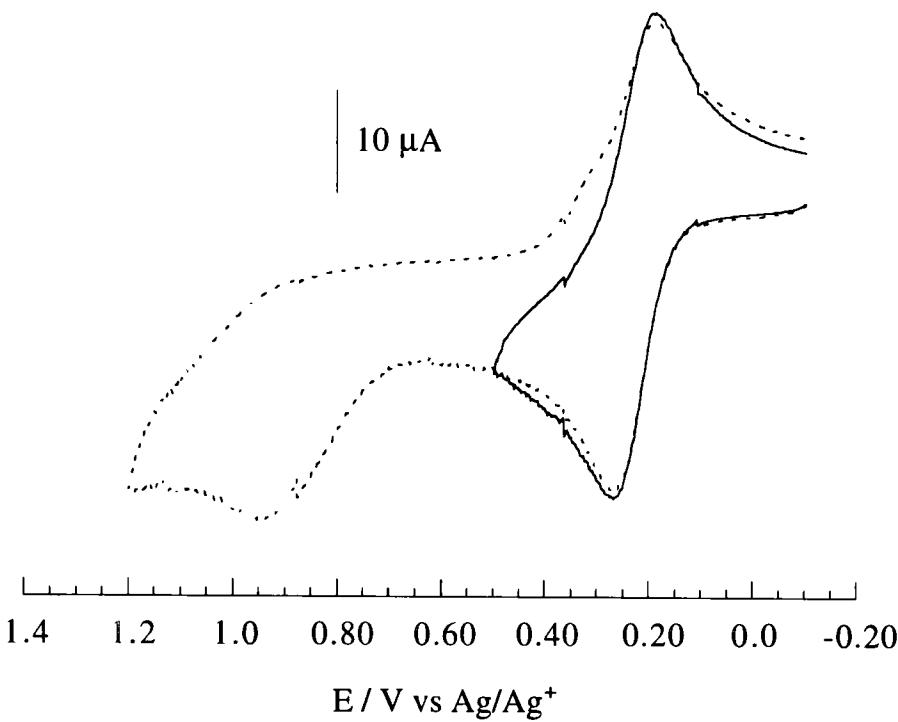


Figure 3.14: Cyclic voltammogram of [48].

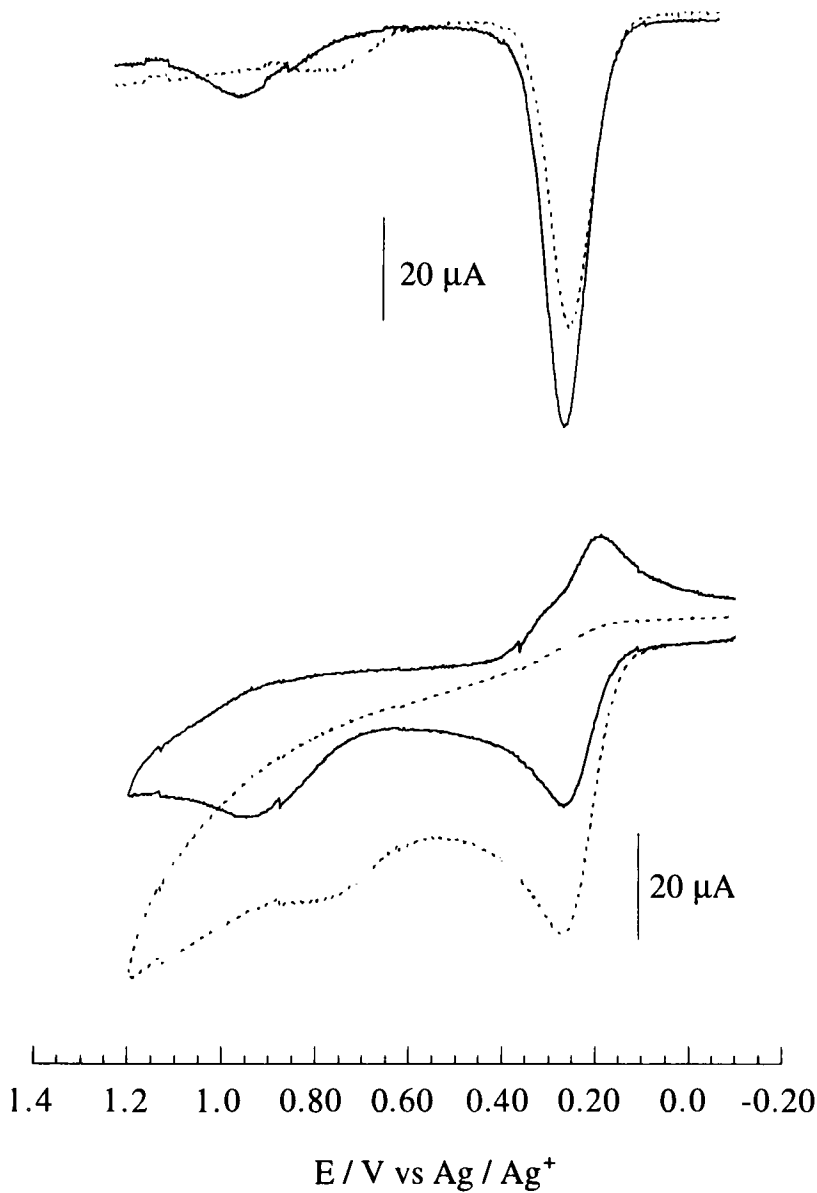


Figure 3.15: Voltammograms of [48] before (solid) and after (dashed) addition of 5 equivalents of chloride.

The addition of chloride gave the response shown in figure 3.15. The voltammograms are shown of the free receptor and after addition of 5 equivalents of chloride in acetonitrile. It can be seen that the CV becomes irreversible due to the EC mechanism as described earlier, but there is little shift in its position (≈ 10 mV), as shown in the SWV. The amine wave, however, is seen to move cathodically by 190 mV.

Extending the cathodic limit and cycling the potential results in an effect similar to that observed with [47], although it is less pronounced, presumably due to weaker interaction.

Binding of dihydrogen phosphate

The addition of dihydrogen phosphate provided an interesting contrast. In this experiment, the anion did not give rise to a shift in the half-wave potential, but instead induced a separate pre-peak at a potential 120 mV nearer the cathodic limit. This peak was seen to develop as the original wave decayed and is illustrated particularly well by the SWV. Both CV and SWV for increasing amounts of added dihydrogen phosphate are shown in figure 3.16.

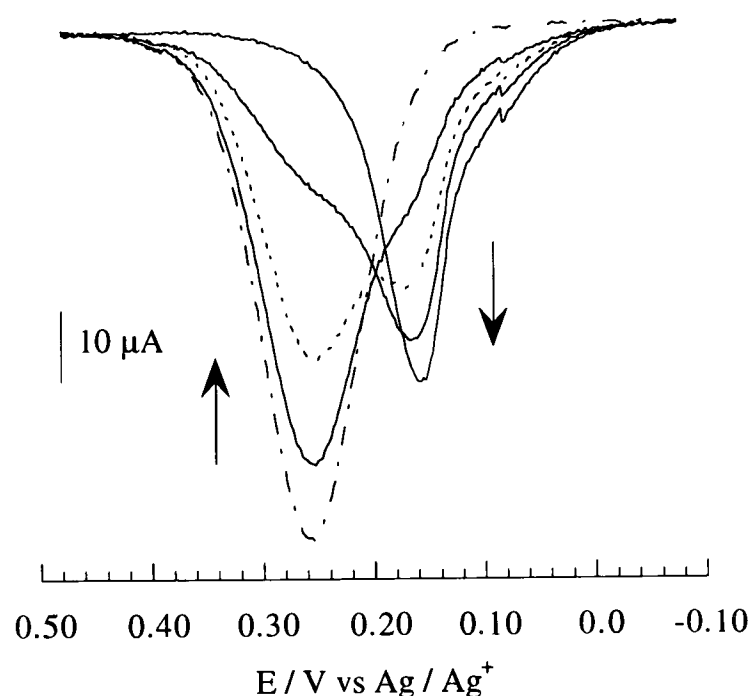


Figure 3.16a: Change in SWV on addition of dihydrogen phosphate to [48]
(shown for 0, 0.5, 1, 1.5, 2 equivalents anion).

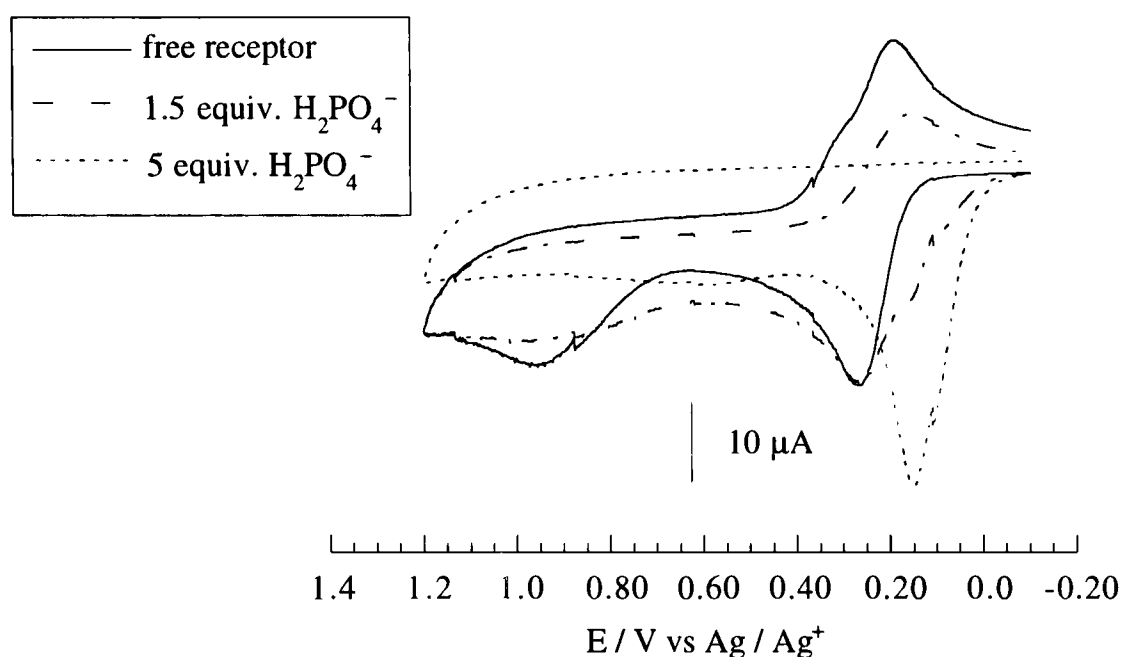


Figure 3.16b: Change in CV on addition of dihydrogen phosphate to [48].

The remarkable response to dihydrogen phosphate was further investigated by a competition experiment in which the CV of [48] was recorded in the presence of both chloride and dihydrogen phosphate and compared with the CVs in the presence of one anion alone. It was found that when one equivalent of dihydrogen phosphate was added, the results were very similar to those seen in figure 3.16, even in the presence of a ten-fold excess of chloride. This molecule therefore shows electrochemical selectivity for dihydrogen phosphate; a result which compares well with the behaviour of its cobalticinium analogue, mentioned in section 2.9.

Binding of hydrogen sulphate

The binding of one further anion was studied in this case, namely hydrogen sulphate. It is a tetrahedral anion with both donor and acceptor groups but is different in that it is acidic. This was a complicating factor which discouraged its routine use in studies. In the case of [48], there was an amine group which might be protonated, followed by probable binding of the anion conjugate base, sulphate; this mode of binding has been proposed following studies of similar ferrocene-based receptors.¹⁵² The interaction between [48] and hydrogen sulphate was therefore studied by ¹H nmr and it was apparent that the interaction was almost an order of magnitude stronger. The stability constant was estimated as 350 (± 50) dm³mol⁻¹ in deuteriated chloroform based on EQNMR analysis of the data shown in figure 3.17 (amide proton).

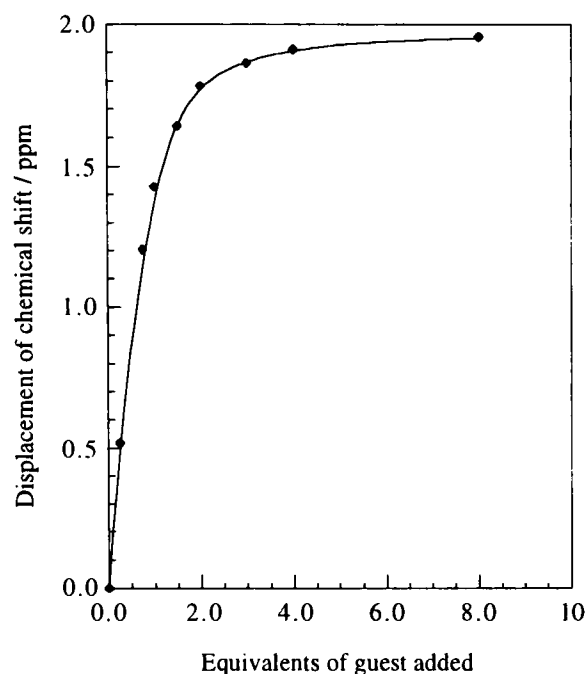


Figure 3.17: ^1H nmr titration profile for addition of hydrogen sulphate to [48].

The amine resonance was strongly influenced by the presence of hydrogen sulphate; it became very broad and was displaced downfield by over 3 ppm. This implies that the amine group is closely involved in the binding of the anion.

In electrochemical experiments the addition of hydrogen sulphate caused a shift in the amine wave and disappearance of the ferrocene reduction wave. The latter was replaced by a stripping peak at a lower potential indicating adsorption of the reduced product (figure 3.18).

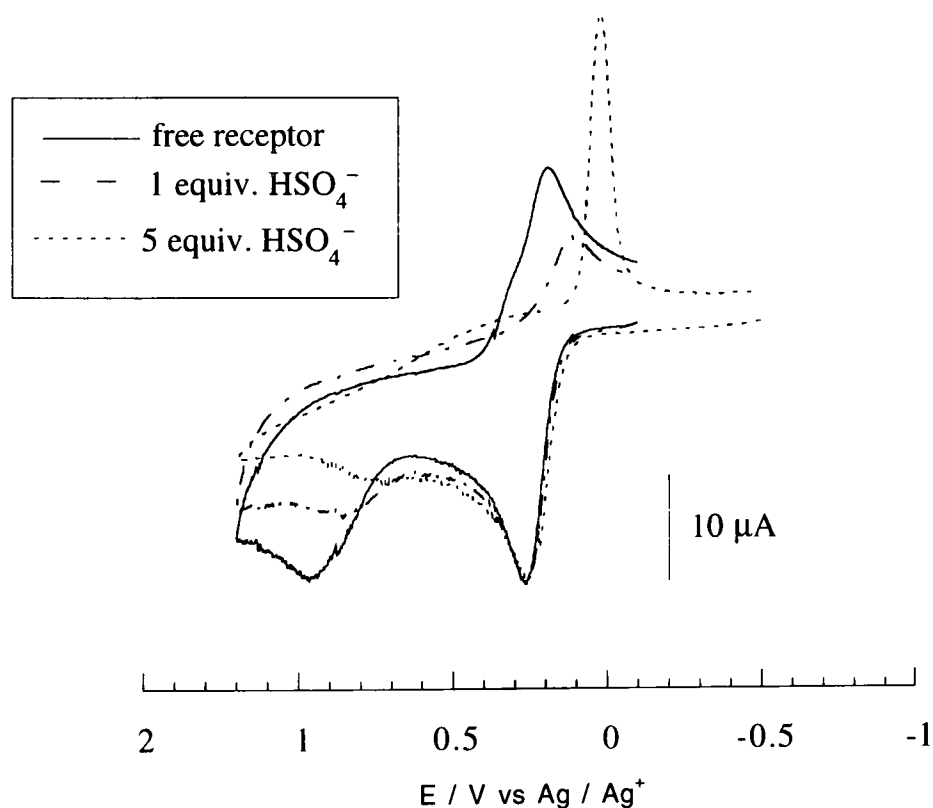


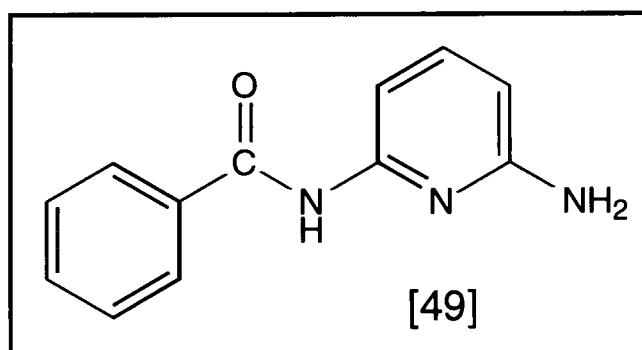
Figure 3.18: Change in CV on addition of hydrogen sulphate to [48].

The studies of this molecule have highlighted several points. Nmr studies showed that the binding of anions is generally much weaker than with the charge-carrying cobalticinium derivative. They also demonstrate that selectivity for dihydrogen phosphate can be introduced by a combination of donor and acceptor sites in the binding region. Electrochemical investigations allowed the effects of oxidation to ferricinium to be monitored. It was seen that the charged form could interact very strongly with the anion and, in doing so, modify the shape of the voltammograms to a large degree. Competition studies showed that dihydrogen phosphate selectively induced the most marked response.

It is suggested that protonation of the amine group can occur, a process which will enhance the binding of an acidic anion. This was seen for hydrogen sulphate in nmr studies; in fact the order of stability constants appeared to be correlated with the acidity of the anions.

3.7 Anion Binding by Receptors [49] and [50]

A further analogue is found in the form of [49]. Here, there is no metal centre — it is the sensor group, not the binding site, that has been varied. The receptor, in common with [48], is neutral and one might expect their binding properties to be very similar. Any variation will arise from the different electron donating properties of the phenyl and ferrocenyl groups, which will affect the acidity of the amide unit.



Unfortunately, the two receptors were difficult to compare. The ^1H nmr titration in deuteriated chloroform with dihydrogen phosphate resulted in very considerable broadening of the amide and amine resonances, which made their position very difficult to measure precisely. After addition of two equivalents, it became impossible to locate the signals at all. The profile for the amide proton is shown in figure 3.19; EQNMR was applied to the data, but produced values with errors in excess of 50%. An estimate of the stability constant based on EQNMR values and by comparison with other curves is $50\text{--}100\text{ dm}^3\text{mol}^{-1}$. A UV-vis

titration was also performed — the molecule has a conjugated chromophore which absorbs in the UV region of the spectrum. Specfit was used to analyse the spectra and the calculated stability constant (dichloromethane) was found to be $\approx 25 \text{ dm}^3\text{mol}^{-1}$. The change in absorbance, however, was very small and an accurate value proved difficult to determine. The general agreement between the two values is good, though (dichloromethane is slightly more polar than chloroform), and here we see the same order of magnitude from both UV-vis and nmr studies. This, of course, is because the receptor is neutral and so there is no difference in ionic strength between the two experiments.

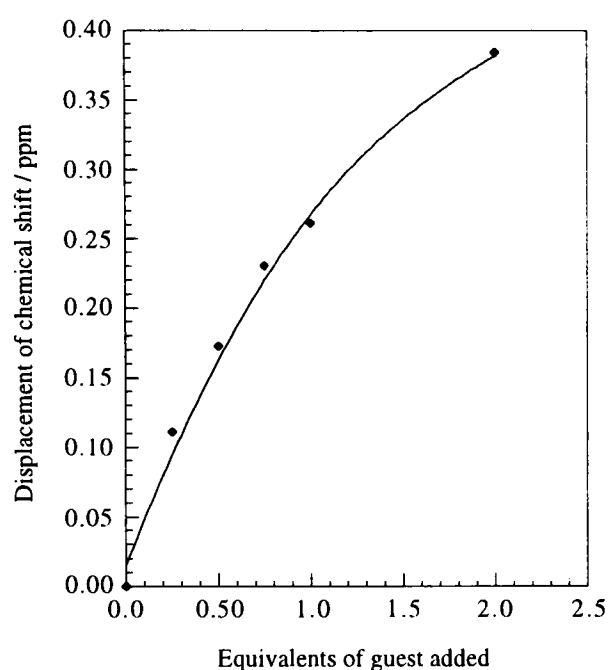
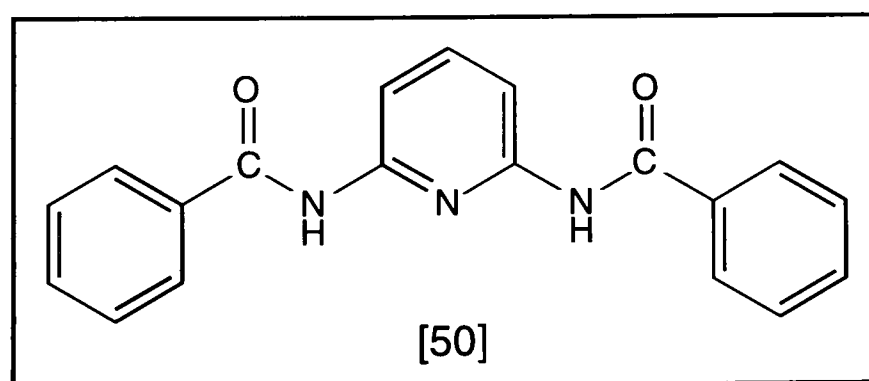


Figure 3.19: ^1H nmr titration profile for addition of dihydrogen phosphate to [49].

Comparison of the curves in figure 3.12 and 3.19 suggests that the binding of dihydrogen phosphate by [49] is slightly stronger than by [48]; this can be explained in terms of the phenyl group being slightly less electron donating than ferrocene.

A more dramatic response was seen in the UV-vis titration with receptor [50]. Now, the molecule is symmetrical and the conjugation is extended yet further.



Addition of dihydrogen phosphate gave rise to a new absorption band; this is shown in figure 3.20a along with a dissection at 420 nm (figure 3.20b). The binding profile is strong and fitting analysis suggests a stability constant in excess of $400 \text{ dm}^3 \text{ mol}^{-1}$. However, the low levels of absorbance meant that data were subject to significant noise and, again, accurate fitting was difficult.

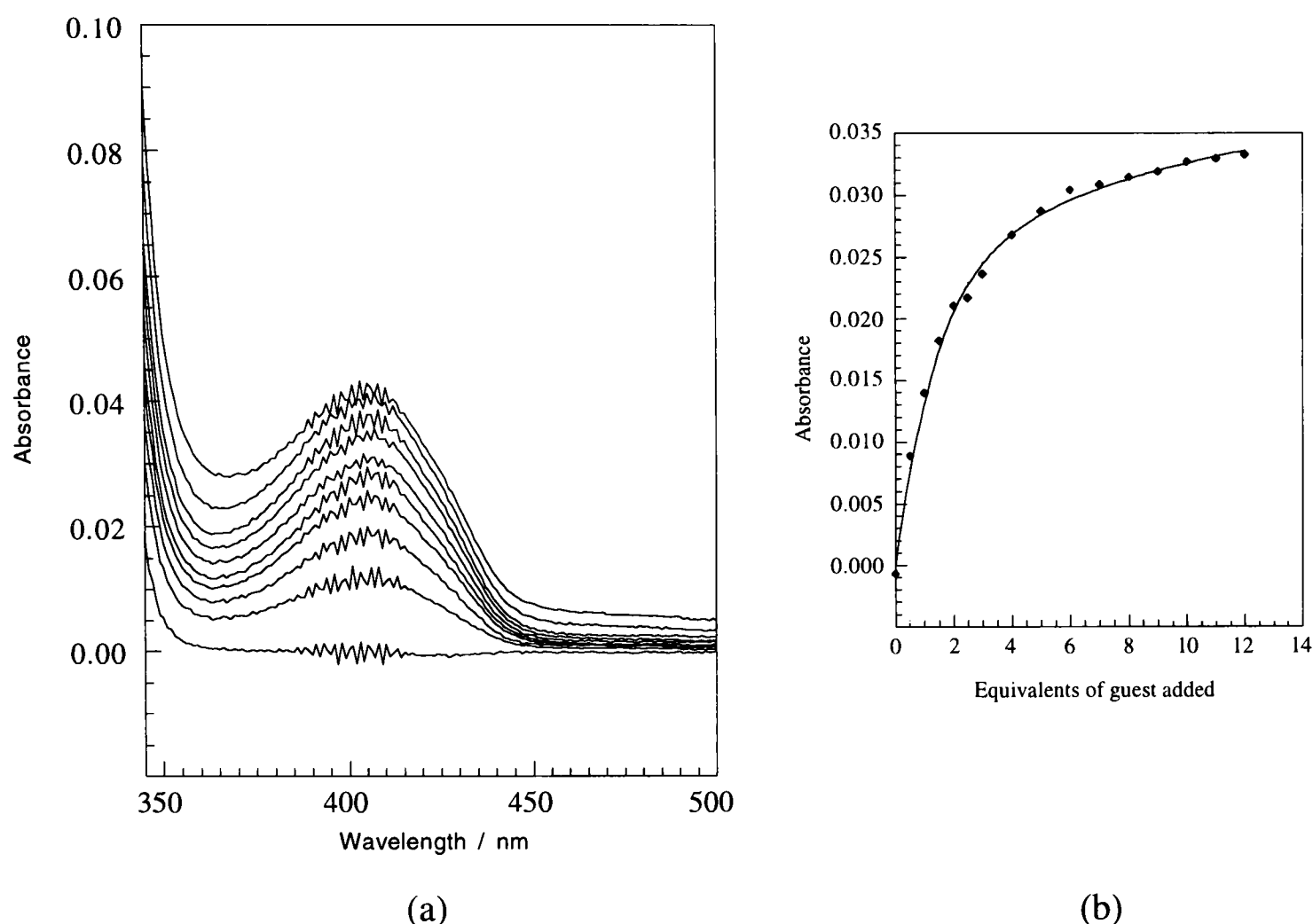


Figure 3.20: UV-vis titration of [50] with dihydrogen phosphate:
(a) change in spectra; (b) dissection at 420 nm.

The origins of the new band were not clear. However, a possible explanation is proposed as follows. In the unbound state, the molecule may not be planar; inspection of the crystal structure of [48] (figure 3.3) shows a slight torsion between the Cp and the pyridine ring systems. In this case, the binding of dihydrogen phosphate, which can interact with both the amide group and the pyridine nitrogen atom, may enforce a more planar conformation, thereby increasing the conjugation. The modified chromophore then gives rise to the absorption seen at 405 nm. A strong dependence between absorbance and conformation may also account for the noise in the spectrum, for the molecule will retain some conformational freedom, even in the bound state.

3.8 Binding of 1-Butyl thymine

In a brief departure from anion binding, the interaction of [48], [49] and [50] with 1-butyl thymine was considered. The host and guest are able to form three sets of complementary hydrogen bonds as shown in figure 3.21 and this investigation revealed the ability of the receptor to bind a guest on the basis of hydrogen bonds alone.

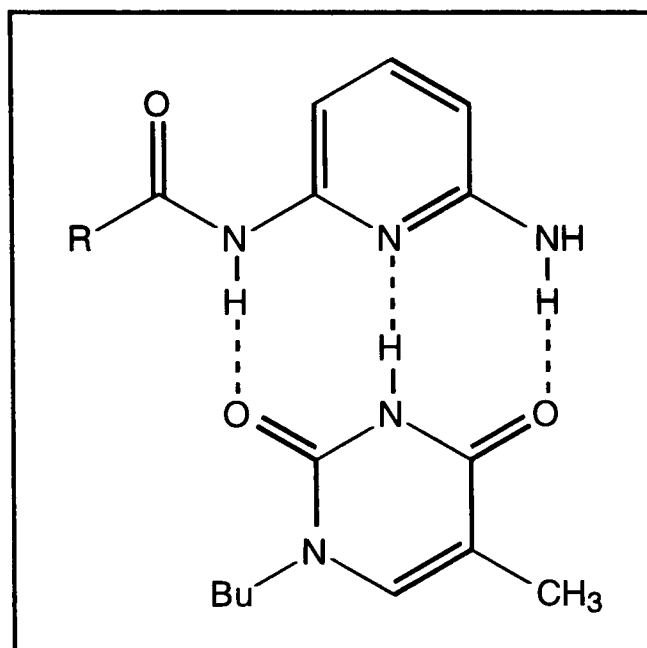


Figure 3.21: Hydrogen bond complementarity with 1-butyl thymine.

The binding was briefly studied by ^1H nmr experiments in deuteriated chloroform and illustrated weak binding by all three receptors, [48], [49] and [50]. The stability constants ($\text{dm}^3\text{mol}^{-1}$) were $24 (\pm 10)$, $45 (\pm 8)$ and $28 (\pm 3)$ respectively. In addition, [48] failed to show any electrochemical response to 1-butyl thymine.

3.9 In Summary

The values for binding of 1-butyl thymine are of the same order of magnitude as those for dihydrogen phosphate, which calls into question the use of the label “anion receptor” for these neutral systems. Perhaps the term “source of hydrogen bonds” is a better one because it is apparent that there is no special affinity for anions. More insight is gained if one changes the frame of reference: instead of thinking in terms of receptor seeking an anion, consider an anion examining the interactions available to it. In the absence of a positive charge, an anion is able to form hydrogen bonds and, in this way, associate with another molecule. The fact that it bears a charge is almost incidental in this process; it has been shown that dihydrogen phosphate behaves in much the same way as a neutral thymine derivative.

The most important message of this chapter has been the need for inclusion of a positive charge if simple systems, such as the ones presented in this thesis, are to be anion receptors.* This is a fundamental requirement. However, in the previous chapter, it was stated that a hydrogen bonding amide was also essential — is there a contradiction? In essence, the answer is “no”. Both are needed, but their roles are slightly different. The positive charge provides the majority of the attractive force, but the hydrogen bonds are the means of defining a binding site and fine tuning the interaction. Hydrogen bonds are also a source of selectivity. A definite binding location and some ability to discriminate between the anions present are the two main features that allow one to distinguish between [45], which is an anion receptor, and a lithium cation, which is not. The comparisons between this chapter and the previous one have helped to emphasise this point.

The ferrocene molecules have also shown some very interesting electrochemical properties, in particular the EC mechanism whereby electrochemical oxidation “switches on” an anion binding interaction which then interferes with the reduction wave. In some cases, oxidation simply led to deposition on to the electrode surface but in others, as shown in figures 3.10 and 3.16, a redox wave could be detected due to the ferricinium-anion pair, which was found to persist on the electrochemical timescale. The selectivity of [48] for dihydrogen phosphate is noteworthy.

The final point underlined in this chapter is the advantage of including a sensing group within the molecule. The trends observed were found via a number of analytical techniques made possible by the presence of a redox active, coloured unit in the design. This feature of the molecules is carried on in the next chapter where, despite a different approach to the problem of anion binding, the receptors still maintain an ability to convey information about their binding action, on a microscopic scale, to the outside observer with a macroscopic spectrometer.

* It should be noted that the neutral anion receptors presented in sections 1.3vi and 3.0 include either a Lewis acid or multiple hydrogen bonding sites acting in concert.

CHAPTER Four

Ruthenium:

Tris (2,2'-bipyridyl) Receptors

4.0 Introduction

The 2,2'-bipyridine molecule (bpy) has long been known as an effective ligand for a wide variety of transition metals and lies high in the spectrochemical series for a number of reasons. The nitrogen donor atoms are good σ -bases, and the π -system is able to accept electron density from a transition metal d-orbital and therefore the ligand is a good π -acid. The net result is pronounced splitting of the metal d-orbitals and consequent ligand field stabilisation of the complex. 2,2'-Bipyridine is also a bidentate ligand and complexes therefore benefit from additional stabilisation via the chelate effect.⁶ From an anion binding viewpoint there are several appealing features. The ligand itself can be subject to functional manipulation, allowing groups similar to those seen in previous chapters to be appended in order to attract an anion. Again, the amide group was seen as an essential element. Bpy ligands can then be attached to a metal which supplies both positive charge and spectroscopic and electrochemical means of investigating the binding process. It was therefore thought that a number of possible anion sensors could be formed by a combination of functionalised bpy and a suitable transition metal. This is shown schematically in figure 4.1.

The metal primarily investigated was ruthenium. It has a stable +II oxidation state with a d_6 electron configuration and, as a second row transition metal, it will adopt a low spin configuration. Furthermore, bpy complexes of ruthenium are well-known and their properties

have been thoroughly studied, revealing a number of interesting properties from a sensing viewpoint. These factors made ruthenium an attractive choice. Brief comparison was also made with rhenium but it is to ruthenium that attention shall be predominantly directed.

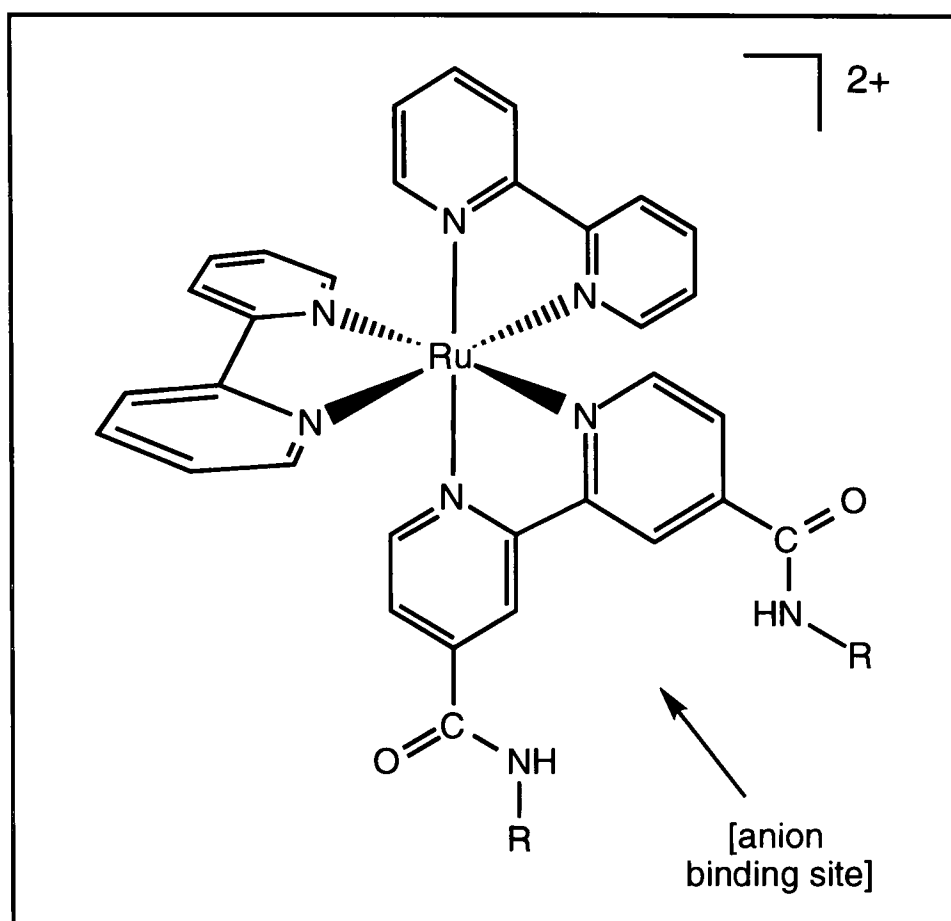


Figure 4.1: Basis of $\text{Ru}(\text{bpy})_3^{2+}$ anion receptors.

4.1 Tris (2,2'-bipyridyl) Ruthenium (II) as an Anion Receptor

A series of ligands was therefore prepared, based on the fragment shown in figure 4.1. The 4 and 4' positions of bpy were functionalised to provide hydrogen bond donor sites and to define a binding region for the anion, as indicated. It can be seen that, although superficially very different, the receptor bears all the design features which were mentioned in the prologue of Chapter One and displayed to such good effect in Chapter Two. The choice of ruthenium (II) as the metal ion imparted a formal charge of +2 to the receptor which would act in conjunction with the amide to bind anions. Stability was important and the low spin d_6 configuration (maximum ligand field stabilisation) rendered the complex inert to ligand substitution — solutions of the receptors have been found to be stable on a timescale of months in the presence of competing ligands.^{161,162} In terms of potential sensing, the amide and other proximal protons were expected to lend themselves to ^1H nmr study and the deep

colour of the molecule invited UV-visible investigation. Both the metal and the bpy ligand are electrochemically active and in addition, there was known to be one more, very important, physical property of interest — luminescent emission.

The sensing properties, in comparison to cobalticinium, were investigated with some interest because, although the receptor was potentially more effective (due to a higher charge and multiple amide groups), the proposed binding site was further away from the metal centre which was to communicate information about the guest. To what extent this would impair the ability to sense anions was a fundamental question.

4.2 Emission Properties

The tris (diimine) complexes of ruthenium (II) constitute a very significant part of ruthenium chemistry.¹⁶³ Typified by tris (2,2'-bipyridyl) ruthenium (II), these complexes have extremely interesting photophysical properties which have inspired considerable study.¹⁶⁴⁻¹⁶⁶ Irradiation of the complex near 470 nm results in a very strong emission around 605 nm, a striking red-orange colour. This was reported by Paris and Brandt¹⁶⁷ who made the first of numerous attempts to establish the origin of this property. The mechanism of luminescence has been a source of debate for many years, part of the confusion arising from an inability to assign a multiplicity to the excited state. It was noted that the emission lifetime is an order of magnitude too long for a spin-allowed transition and an order of magnitude too short for a spin-forbidden transition (observed lifetime 10^{-5} – 10^{-6} s at 77 K).¹⁶⁸ Following a great deal of study (notably by Crosby), the currently accepted theory is as follows: absorption of light populates excited singlet states which are largely delocalised in nature. Internal conversion is followed by inter-system crossing (with unit quantum efficiency¹⁶⁹) to populate several closely spaced localised states which are predominantly triplet in nature. The emitting state is a metal-to-ligand charge transfer state but the spin state cannot be properly defined due to spin-orbit coupling in the excited state.¹⁷⁰ The literature is reviewed in a number of general articles^{163,164,171} on the chemistry of $\text{Ru}(\text{bpy})_3^{2+}$.

The flurry of research in this direction was fuelled by the suggestion that the complex could be used for the photodecomposition of water to generate oxygen and hydrogen.¹⁷² The potential as a light absorption photosensitiser has been extensively explored but is limited by

difficulties such as fast radiationless decay from the excited state, ligand photodissociation and suppression of emission by modification of bpy.

In this work, however, the use of the $\text{Ru}(\text{bpy})_3^{2+}$ unit as a *sensor* is of most concern and in this field, there are numerous successful examples. Part of the attraction lies in the great sensitivity of luminescence as a means of signal transduction; very subtle perturbation of the molecule can affect the emission properties. Furthermore, the sensor operates on a visual basis and therein lies the possibility of non-intrusive communication via fibre-optics — a particularly useful property for *in vivo* applications. Finally, there are many mechanisms by which luminescence can be disturbed, which offers a wide scope for experiment.^{173,174}

The last of these points is underlined by the number and variety of fluorescent sensors which are reported in the literature; a selection is shown in figure 4.2. One class simply employs $\text{Ru}(\text{bpy})_3^{2+}$ which is electrochemically oxidised¹⁷⁵ to $\text{Ru}(\text{bpy})_3^{3+}$. The presence of a number of analytes reduces the complex, but to an excited state, $\text{Ru}(\text{bpy})_3^{2+*}$, which emits in decaying to the ground state. The intensity of the luminescence is then an indication of the concentration of analyte. In this way sensors have been developed for glucose (via production of NADH by the action of glucose dehydrogenase), oxalate, alkylamines and NADH at micro- and nanomolar levels.¹⁷⁶⁻¹⁷⁸ In addition, the ability of oxygen to quench luminescence has been harnessed to produce oxygen sensors.^{179,180}

Other workers have linked fluorescent groups to known receptors, with the intention of using the fluorophore as an antenna for the action of the receptor. The BAPTA molecule (encountered in section 1.2ii) has been functionalised with a fluorophore¹⁸¹ to give a calcium sensor [51] and several authors have linked a fluorescent group to crown ethers with the aim of detecting alkali metal binding.¹⁸²⁻¹⁸⁵ A recent paper by Fabbrizzi has demonstrated that [53] can switch between fluorescent and quenched states according to the oxidation state of a bound copper ion.¹⁸⁶

A neutral molecule, barbitol, is detected by perturbation of pyrene fluorescence following binding in the hydrogen bonding site of receptor [52], another example of the binding site–fluorophore approach.¹⁸⁷

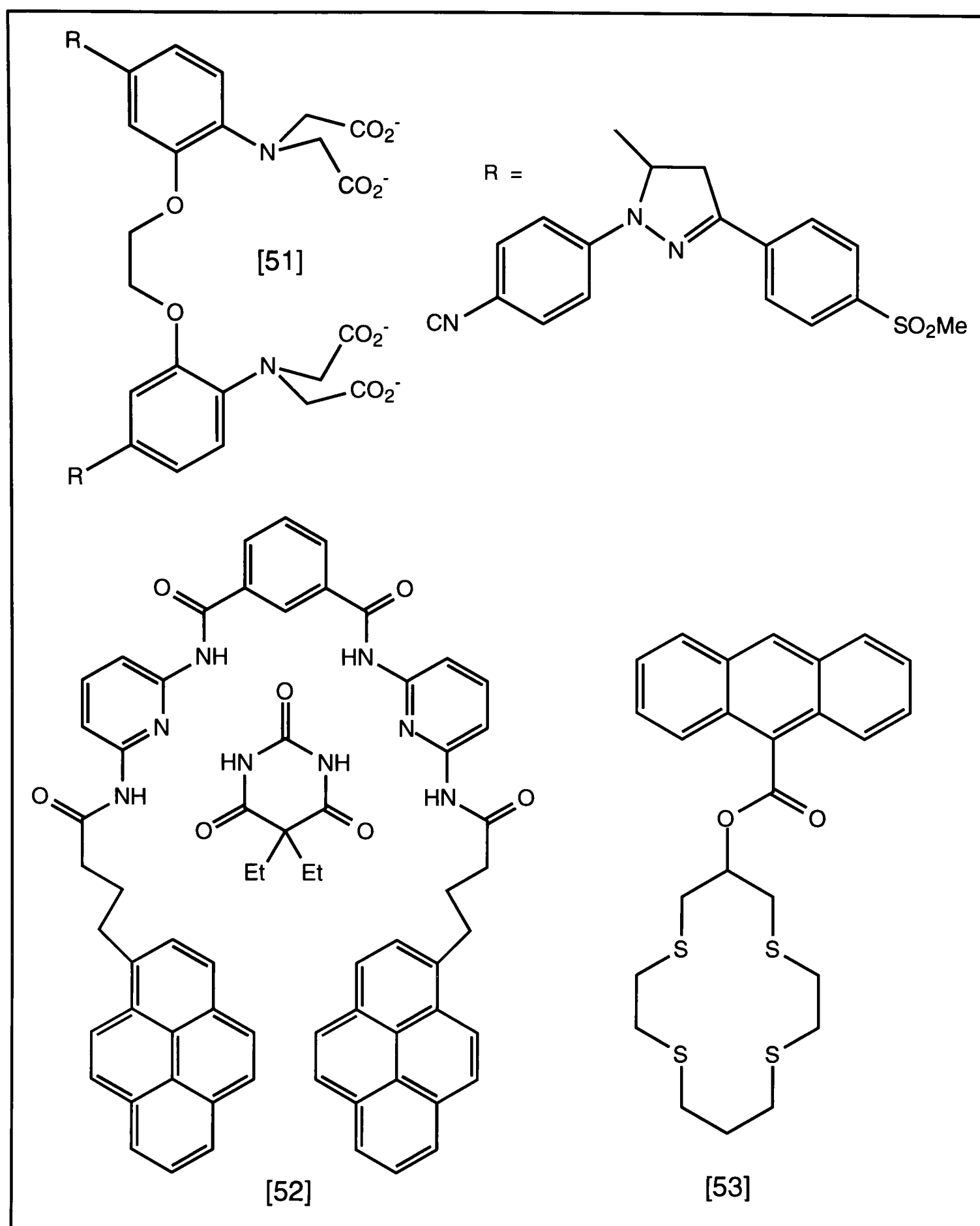


Figure 4.2: Fluorescent sensors.

These examples represent only a very small fraction of the published work in this area but serve to demonstrate that the principle of using fluorophores in sensor design is well established.

The prospect of using the $\text{Ru}(\text{bpy})_3^{2+}$ frame as the basis of a luminescent sensor in addition to the methods routinely employed in the study of anion recognition was therefore an appealing addition to the design of sensors presented in this chapter.

4.3 Synthesis

The choice of 4,4'-disubstituted-2,2'-bipyridine as a starting material is a good one. In the first instance, the angle between the functions was particularly suitable for creation of a binding site in that their relative orientation and distance allowed them to accommodate a guest. Secondly, the 4,4'-dimethyl precursor was commercially available. It was subsequently found to be more easily manipulated than other analogues. Previous work in the field supported this view; receptors based on 5,5'-substitution were found to be synthetically very challenging and the resulting receptors did not exhibit good anion binding.¹⁸⁸ The synthetic scheme adopted is shown in figure 4.3.

Jones oxidation of 4,4'-dimethyl-2,2'-bipyridine furnished the corresponding 4,4'-bis(carboxylic acid) in very good yield. This was found to be insoluble in most organic solvents and the only readily accessible route to the amide was via an acid chloride which was prepared by refluxing in thionyl chloride. The suspended acid dissolved during the course of two days' reflux after which time the solution was filtered and the solvent removed by vacuum distillation to yield the acid chloride as an off-white residue. This was dried *in vacuo* and used without delay in subsequent condensations.

The reaction between acid chloride and amine was executed in dry acetonitrile under a nitrogen atmosphere and invariably resulted in the precipitation of the bis-amide as a white solid in good yield. This could then simply be isolated by filtration, washed and dried. However, this insolubility, an advantage in synthesis, extended to most solvents and became an impediment to further reaction.

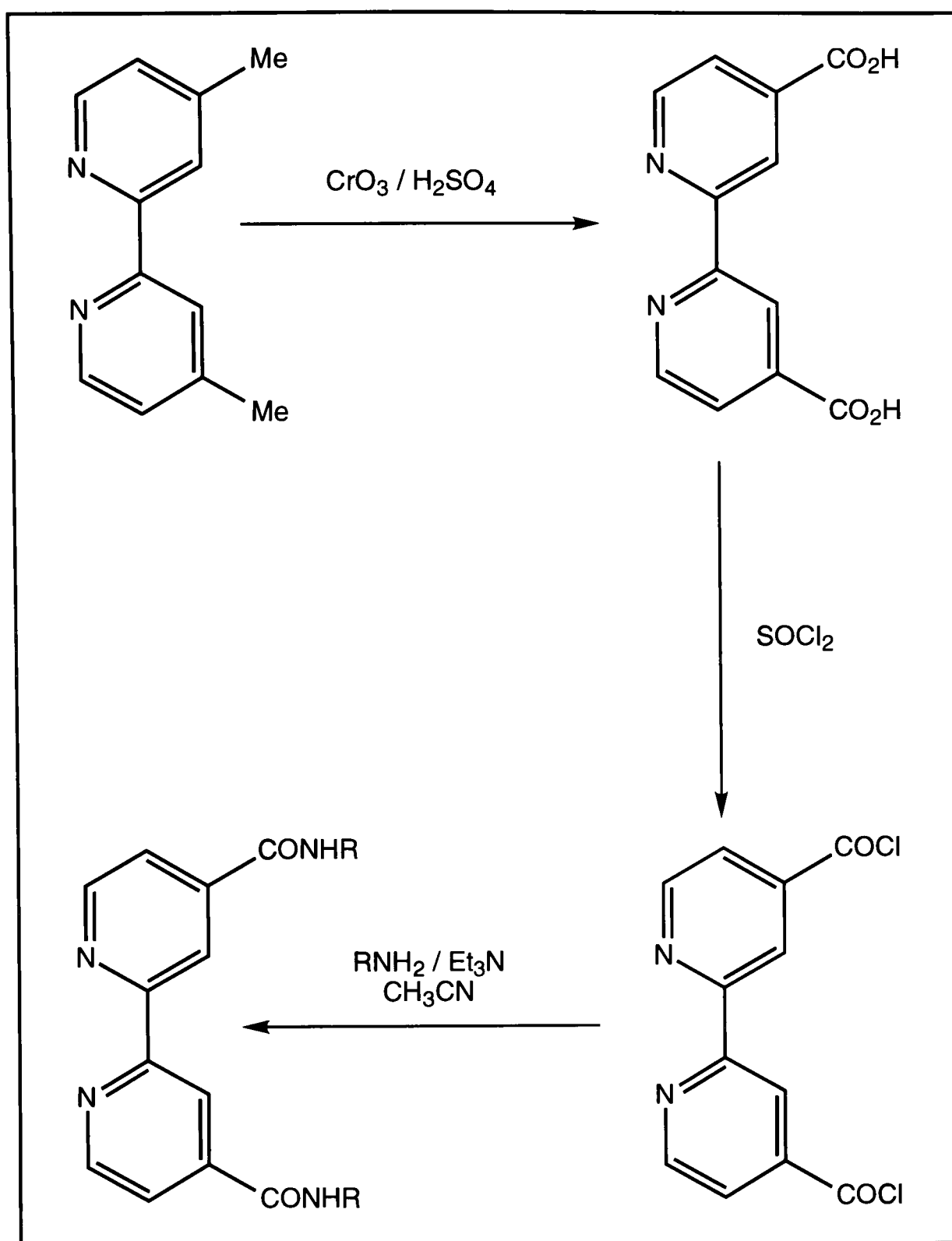


Figure 4.3: Scheme for synthesis of 4,4'-bis substituted-2,2'-bipyridine.

The principle of the receptor synthesis was simple; reaction between $\text{Ru}(\text{bpy})_2\text{Cl}_2$ (prepared from RuCl_3 and 2,2'-bipyridine by a literature procedure¹⁸⁹) and the substituted bipyridine would yield the product, fuelled by a thermodynamically favourable chelate, low spin- d_6 product. Several solvent mixtures were used in attempts at this reaction, the most frequently employed being either ethylene glycol or 1:1:1 water:ethanol:glacial acetic acid. Stirring at 100°C usually promoted complete reaction (evidenced by TLC) within a matter of hours, and the crude product was then converted to a hexafluorophosphate salt and purified with Sephadex in a manner analogous to that described in Chapter Two. Care was taken to avoid over-reaction which occasionally caused oxidation to ruthenium (III), indicated by the

appearance of a green spot in TLC. The presence of this compound usually led to decomposition of the rest of the reaction mixture.

It was clear that forcing reaction conditions were required to dissolve the amide-substituted bipyridine and some even harsher approaches are used by others in the field with success. In particular, workers within this group employ a 1:1 ethylene glycol:glacial acetic acid mixture at 200°C or periodically reflux the mixture in ethylene glycol for five minutes in a microwave oven on a high setting. All of these methods testify to the robust nature of all the species involved.

In all, five receptors were isolated in yields of typically 40% — their structures are shown in figure 4.4.

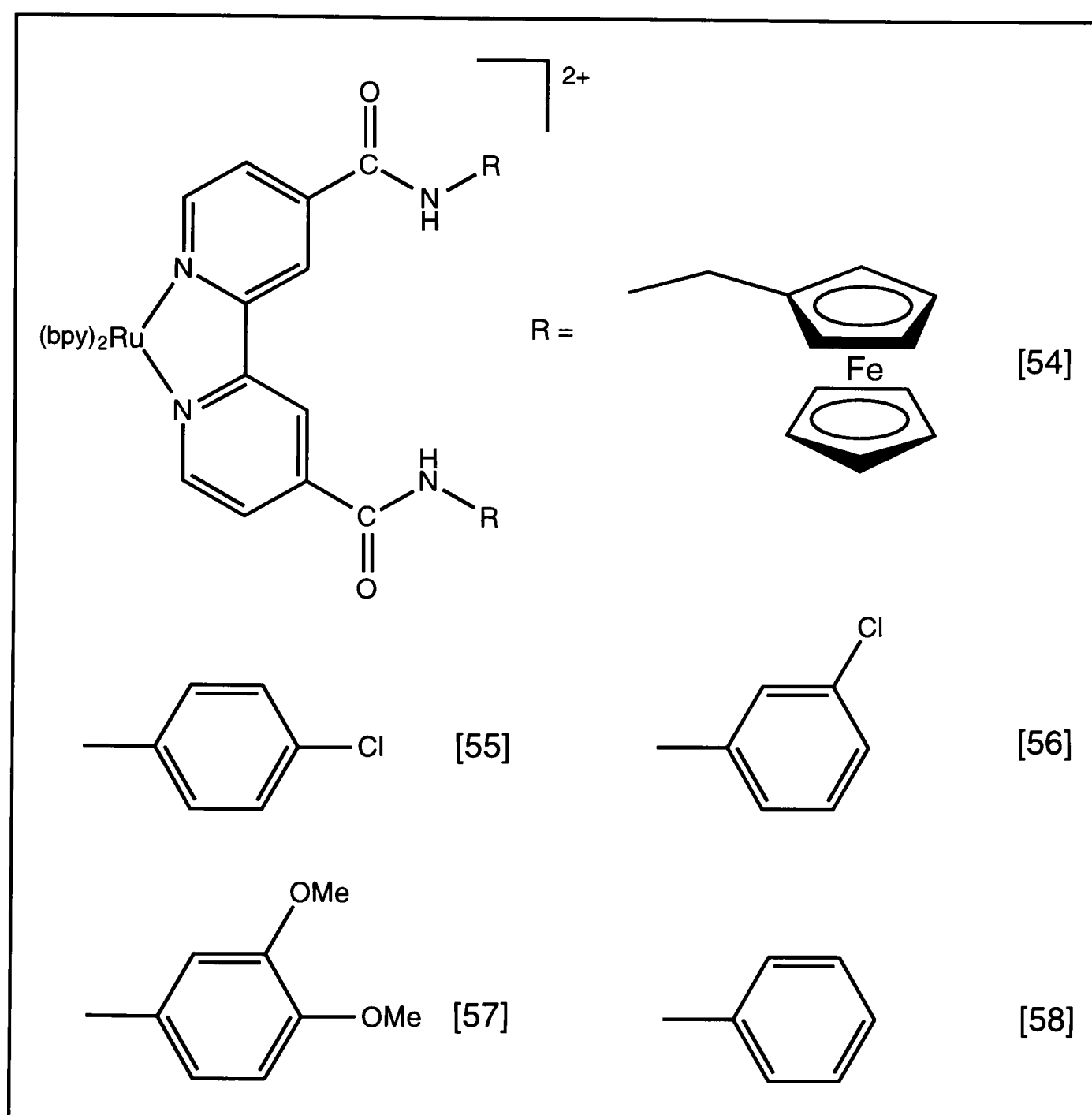


Figure 4.4: The anion receptors of Chapter Four. All molecules shown were isolated as the hexafluorophosphate salts.

4.4 Characterisation

4.4i ^1H nuclear magnetic resonance

The ^1H nmr spectrum of a simple receptor is shown in figure 4.6a. The pattern of resonances in the bpy region is characteristic and may be assigned by reference to known derivatives and the literature.^{190,191} The spectrum of $\text{Ru}(\text{bpy})_3^{2+}$ itself (figure 4.5) consists of two triplets and two doublets and is assigned as shown. The downfield position of the H_3 doublet is attributed to van der Waals' deshielding as a result of its close proximity to H_3' and the chemical shift of H_5 and H_6 is said to have undergone an upfield shift due to an isotropic interaction with neighbouring rings. Substitution of one bpy ligand at the 4 position introduces a singlet for the H_3 proton in place of a doublet. The H_5 and H_6 resonances would be expected to appear as a doublet of doublets but are partially obscured by other signals. A proton-proton correlation experiment shows the coupling of the various proton resonances within the spectrum of receptor [55] (figure 4.6a) and is shown in figure 4.6b.

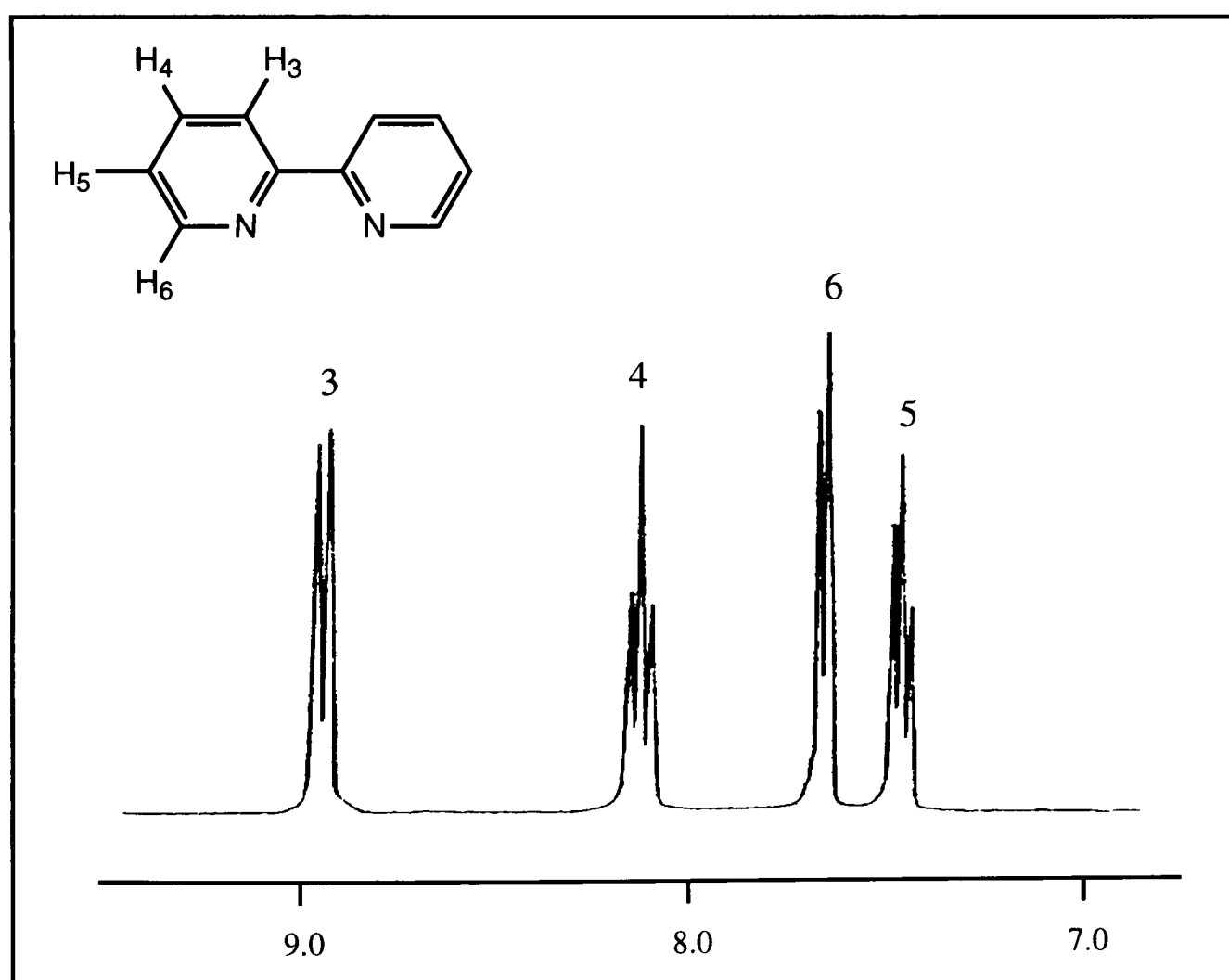
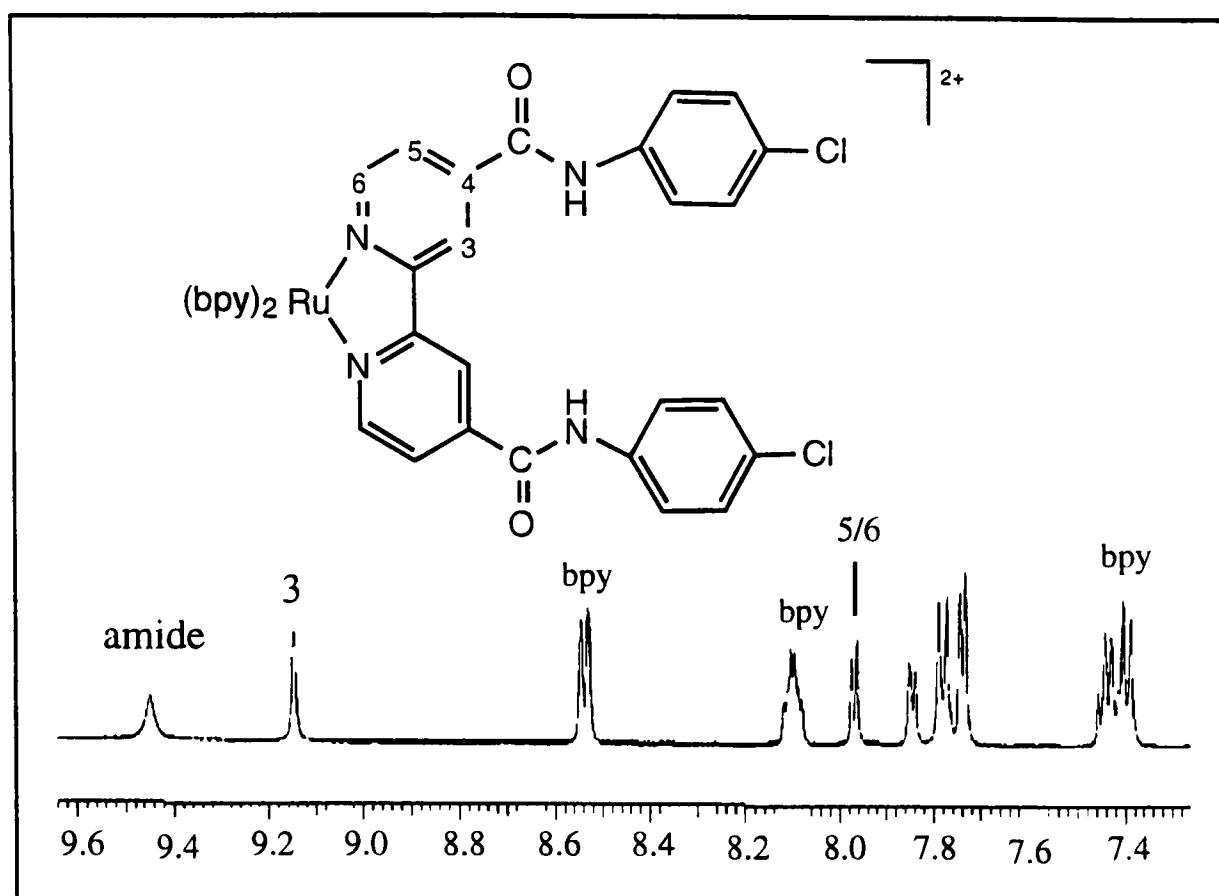
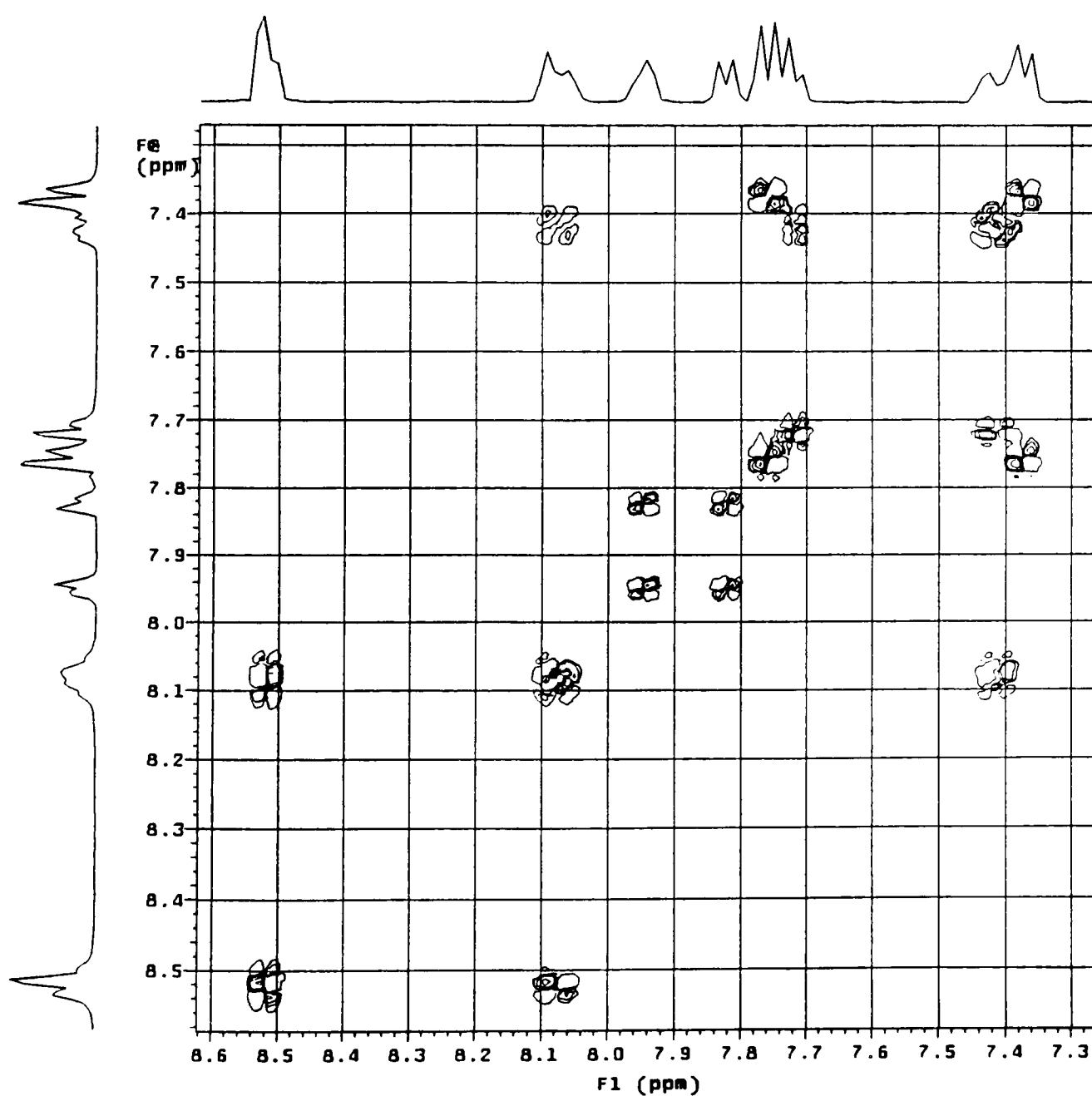


Figure 4.5: ^1H nmr spectrum of $\text{Ru}(\text{bpy})_3^{2+}$ in $\text{d}_6\text{-DMSO}$

Figure 4.6a: ^1H nmr spectrum of [55] in deuteriated acetonitrile.Figure 4.6b: ^1H - ^1H correlated spectrum of [55] in deuteriated acetonitrile

4.4ii ^{13}C nuclear magnetic resonance

The ^{13}C nmr spectrum is complicated by the large number of carbon atoms in magnetically similar environments. Some clarification is given by proton-carbon correlation experiments but, in general, it was not a useful analytical method and rather more emphasis was placed upon other techniques, particularly proton nmr and mass spectrometry.

4.4iii UV-visible spectroscopy

The absorption spectrum of the receptors is rich in transitions.¹⁷¹ In the UV region, fully allowed ligand centred (LC) transitions ($\pi \rightarrow \pi^*$) dominate the spectrum, assigned by reference to the absorbance of protonated bipyridine.¹⁹² The shoulder on this peak, along with the band at 360 nm are assigned as metal centred (MC) d-d transitions. The intensity of these bands is rather high, but the presence of so many nearby allowed transitions will allow the bands to absorb more strongly due to so-called “intensity stealing”, the mixing of the forbidden excited term with an allowed level.¹⁹³

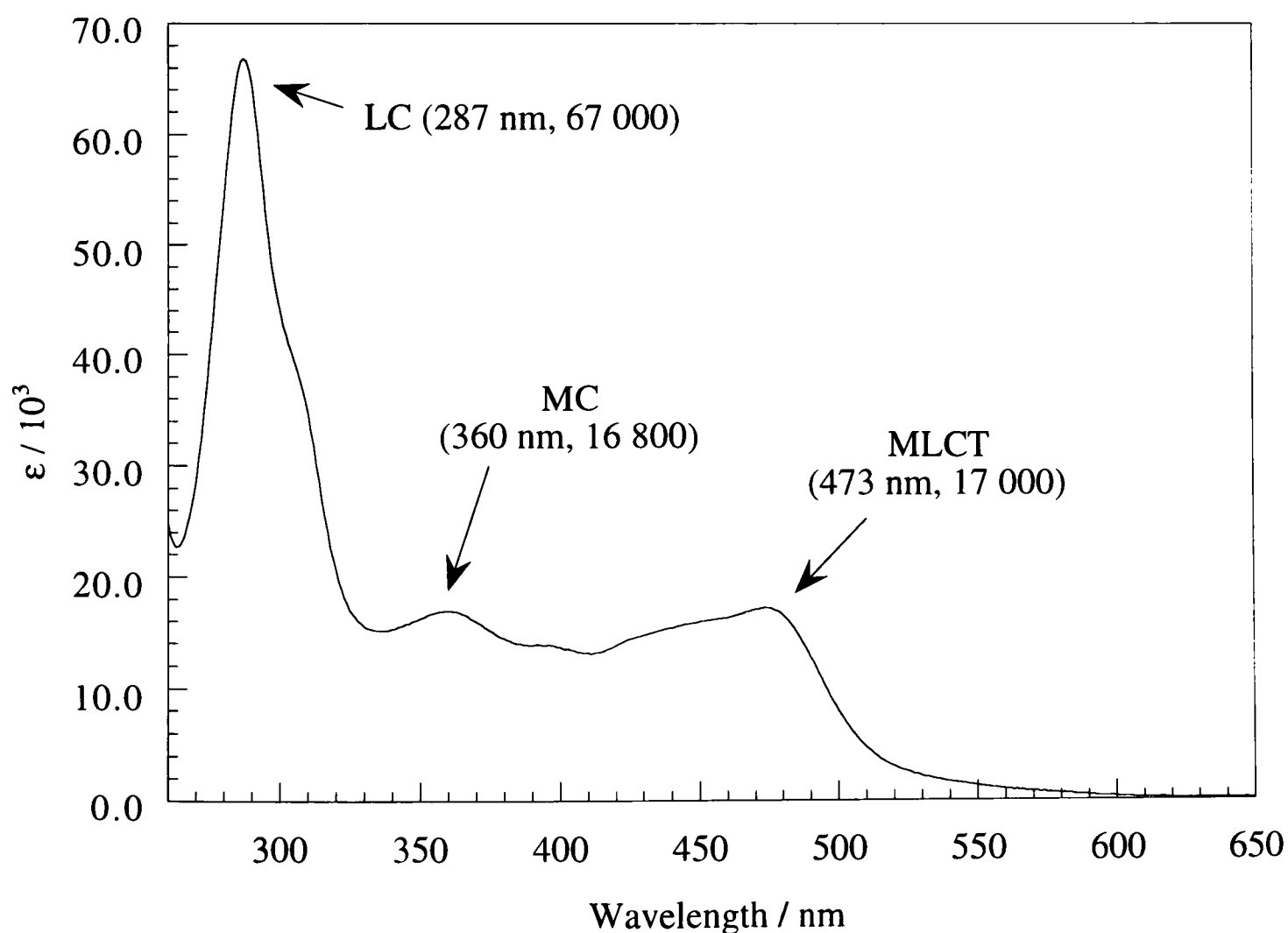


Figure 4.7: The absorption spectrum of [57] ($10^{-5} \text{ mol dm}^{-3}$) in acetonitrile.

The band with a maximum at around 470 nm is due to metal-to-ligand charge transfer ($d \rightarrow \pi^*$) and is found to be red-shifted with respect to an unsubstituted analogue. This is caused by the electron withdrawing amide groups which increase the electron affinity of the ligand. The long tail above 500 nm is a matter of controversy. It seems most probably to be a spin-forbidden MLCT band, and is likely to be the source of the emission.¹⁷⁰

4.5 Anion Coordination Studies

The receptors were studied by the usual range of investigative techniques. Of those studied, [54] was particularly remarkable in its behaviour whereas the remaining four, although differing in some respects, were more closely related. The coordination properties of [54] will therefore be considered as a separate section at the end of the chapter. Prior to this, the binding properties of the other receptors will be detailed by sub-division by anion rather than by receptor, as in previous chapters.

4.6 Binding of Chloride

4.6i ^1H nmr titration

As mentioned previously, the ^1H nmr studies sought to address the question of the orientation and strength of binding between host and guest. To this end, a series of nmr spectra were recorded for solutions containing receptor and anion in differing ratios and were examined for signs of binding. It was quickly established that the most prominent displacements due to the anion were those of the amide and 3H proton resonances, an observation common to all titrations with receptors of this type and also seen in the studies of four other workers in the field. A binding site was therefore proposed,¹⁹⁴ as shown in figure 4.8 and confirmed by a recent crystal structure from the work of Dr Alan Grieve which shows a chloride ion bound in the plane of the ring system, close to both amide and 3H protons (figure 4.9).¹⁹⁵ Comparison can be made with crystal structures of $\text{Ru}(\text{bpy})_3^{2+}$, which have a similar basic shape, i.e. functionalisation does not alter coordination about the metal centre.^{196,197} The

structures are also notable in that a short Ru-N bond length confirms the presence of extensive $d\pi \rightarrow p\pi$ ($t_{2g}^6 \rightarrow \pi^*$ bpy) bonding in the complex. Crystals of the receptors proved difficult to grow — the compounds had a tendency to form glasses, the presence of two optical isomers presumably making long range ordering difficult to achieve.

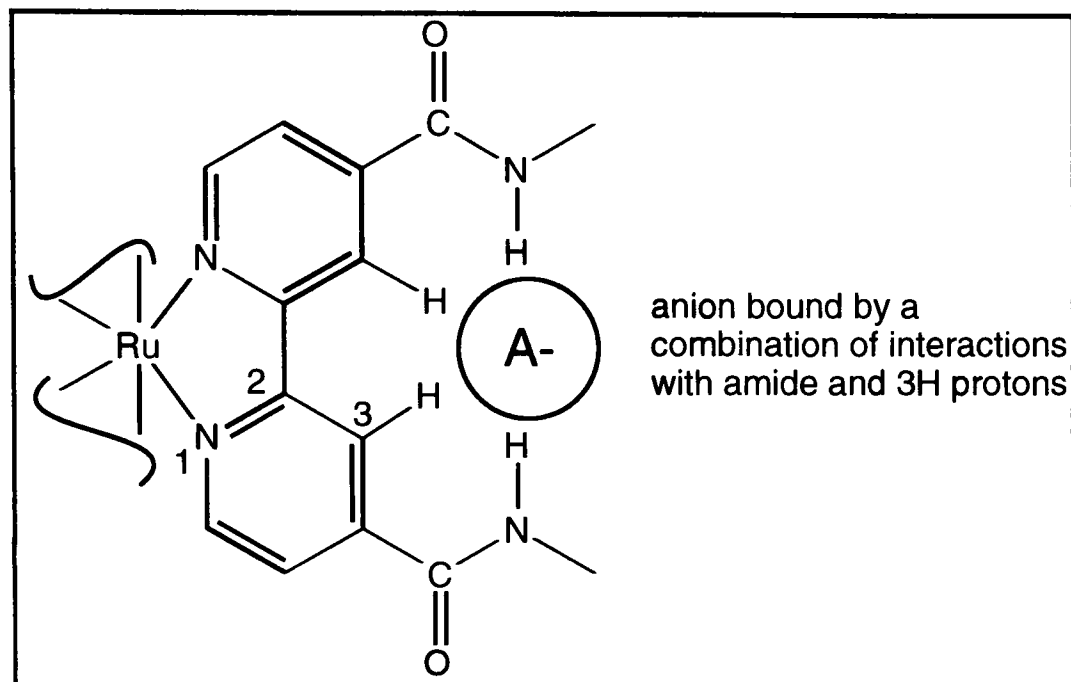


Figure 4.8: Proposed binding site.

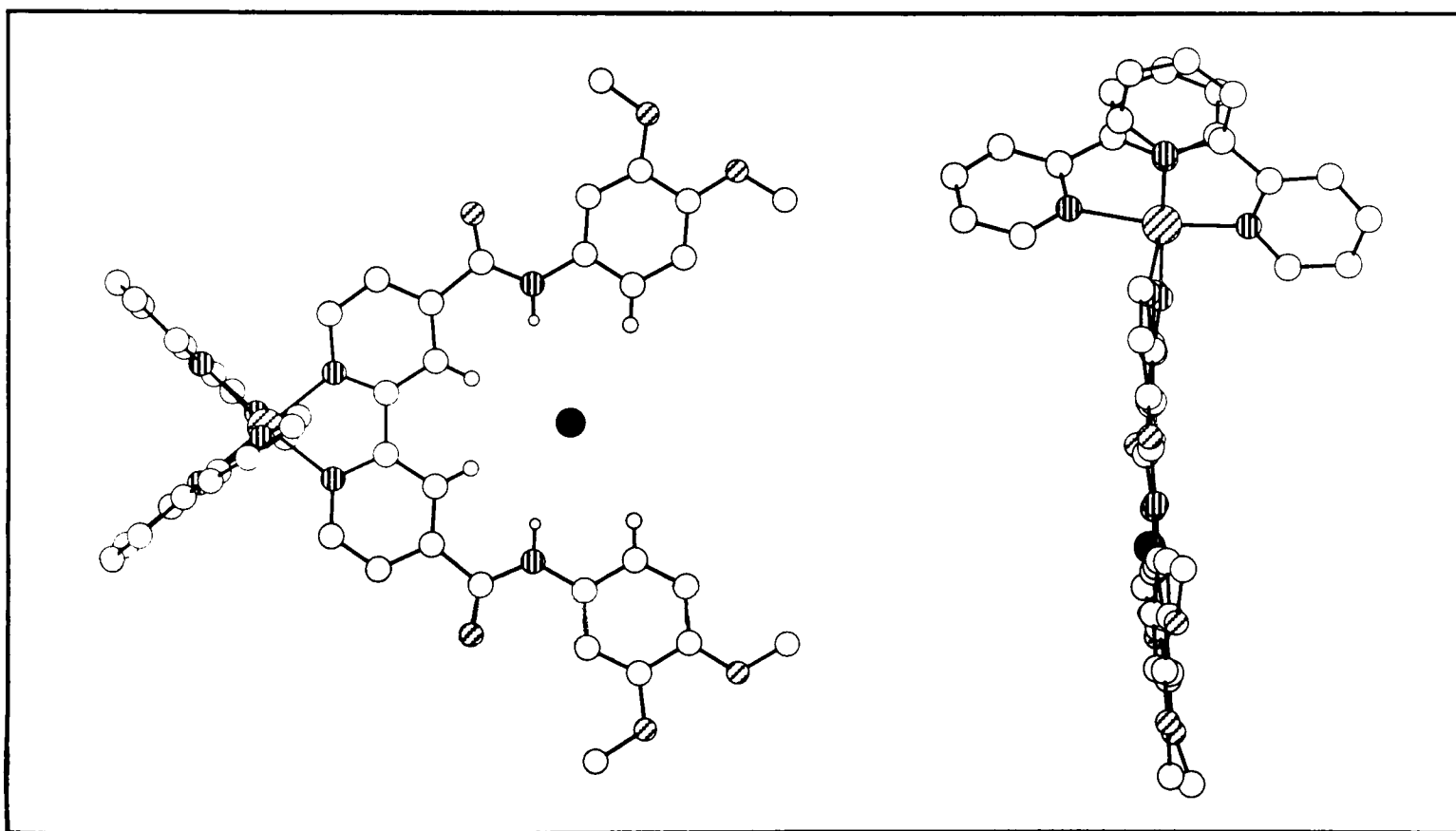


Figure 4.9: The X-ray crystal structure (two views) of the chloride complex of [57] (after A. Grieve, D.Phil. thesis, University of Oxford, 1994).

The position of the bound anion comes as no surprise; the contribution of the amide proton has already been discussed and, furthermore, a paper by Constable and Seddon has shown the

acidic nature of the 3H proton, due to an enforced close proximity to its neighbour at the 3'H position.¹⁹⁸ Here, then, is yet another example of the fundamental role played by electron deficient hydrogen atoms in anion recognition.

The titration profiles of the receptors [55] and [56] are shown in figure 4.10. The two receptors are very similar, differing only in the position of the chloride substituent — since this is unlikely to participate directly in binding, the receptors were expected to show similar binding properties. In the titration with [56], there was some broadening of the resonances which made precise determination of their position difficult, and this introduced the noise which is apparent in figure 4.10b. The relative magnitudes of displacement are such that the 3H resonances move much further than those of the amide. Again, this was a common feature of the nmr titrations and can be rationalised in terms of the greater acidity of the 3H proton which renders it more susceptible to perturbation by the binding process. The stoichiometry was taken to be 1:1, which agrees well with the curves shown.

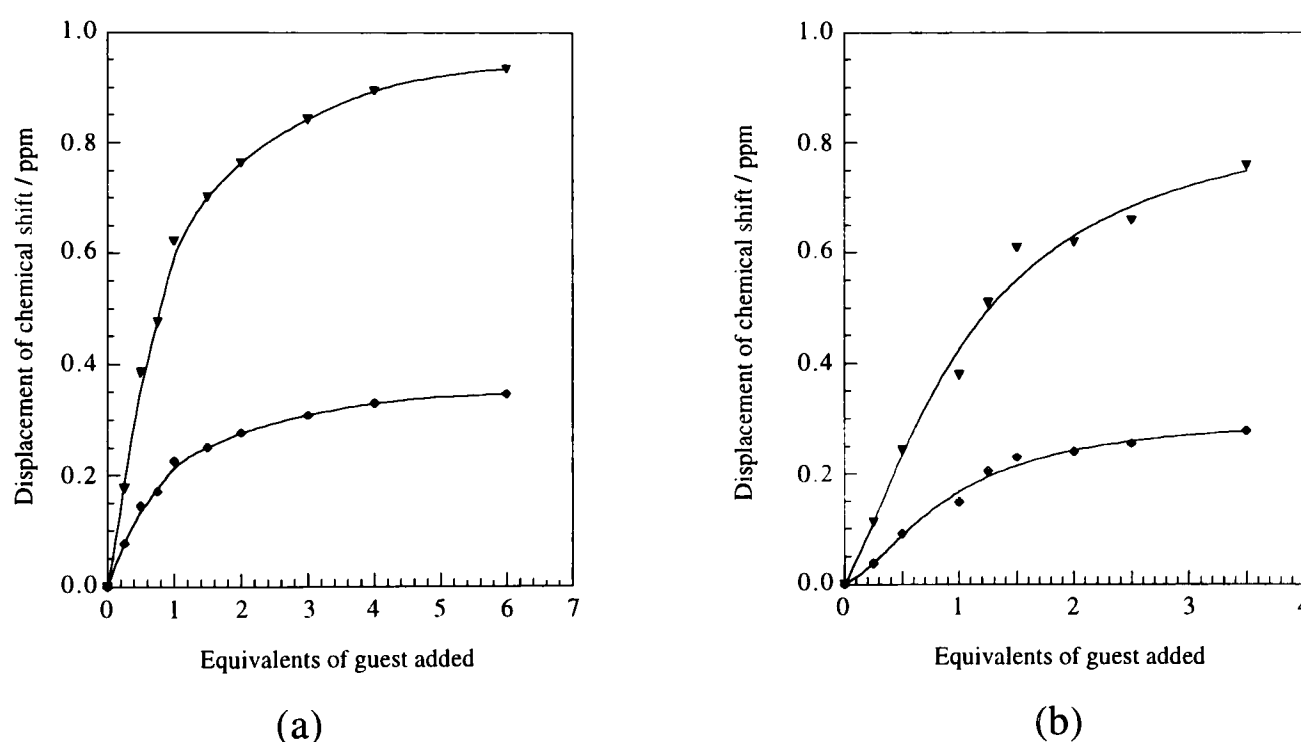


Figure 4.10: ^1H nmr titration profiles for addition of chloride in d_6 -DMSO: (a) [55]; (b) [56].

The upper curve represents the 3H proton, and the lower curve the amide proton.

It was possible to apply EQNMR to the data from the amide proton and stability constants were calculated. As predicted, they were very close in magnitude, although the errors were high, due to noisy data. The values were $260 (\pm 50)$ and $300 (\pm 120) \text{ dm}^3\text{mol}^{-1}$ for [55] and [56] respectively. Taking the solvent, DMSO, into consideration, such values are far in

excess of the binding strength for cobalticinium receptors and indicate a very satisfactory level of binding. A comparison between these molecules and a cobalticinium receptor such as [34] reveals two opposing factors. Firstly, $\text{RuL}(\text{bpy})_2^{2+}$ (where L is an amide substituted bpy) receptors are bidentate and are therefore able to form a greater number of interactions with the guest. The acidic amide and 3H protons provide a total of four hydrogen bonding interactions as opposed to the one available in [34] (neglecting $\text{C-H}\cdots\text{A}^-$ hydrogen bonds). Secondly, the magnitude of the charge is greater for a $\text{RuL}(\text{bpy})_2^{2+}$ receptor although the centre of charge is further removed from the binding site and, moreover, the electron density is delocalised over the bipyridyl ring system and is less concentrated. Nevertheless, it would appear that the better defined binding cavity and the increased number of acidic protons available for interaction with an anionic guest combine to produce an attractive site for an anion.

The titration curves for [57] and [58] are displayed in figure 4.11. These two profiles show a strong contrast: [57] gives a titration curve representing an estimated stability constant of $400\text{--}500\text{ dm}^3\text{mol}^{-1}$ (large errors prevent a more accurate estimate), whereas the profile for [58] (3H proton resonance) is much weaker, with a stability constant of $50 (\pm 7)\text{ dm}^3\text{mol}^{-1}$. However, the origins of this impaired performance are not fully apparent.

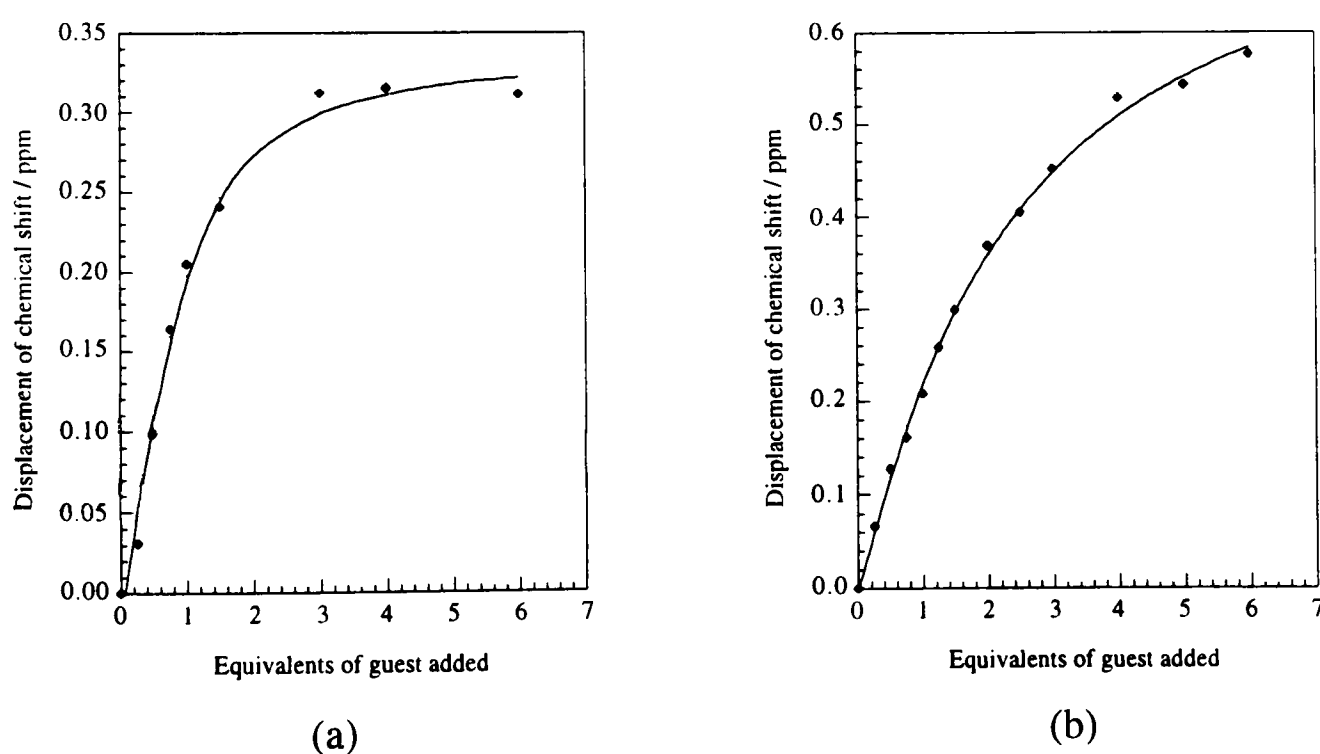


Figure 4.11: ^1H nmr titration profiles for addition of chloride in $\text{d}_6\text{-DMSO}$: (a) [57]; (b) [58].

Further studies were performed on an analogue of [58] synthesised with ^{15}N enriched aniline which allowed a ^{15}N nmr titration to be executed (the spectra were recorded in deuteriated DMSO courtesy of Kodak Limited using a Varian Unity Plus spectrometer at 40.56 MHz). The amide nitrogen atom appeared as a sharp singlet at -244.6 ppm and, upon addition of chloride, moved as shown in figure 4.15. This method had the advantage of producing a very sharp, clear signal with a chemical shift which could be precisely measured. The resonance was seen to move upfield but, for reasons which are not clear, then began to move downfield once more. No calculations were performed on the data, but the tangents drawn on figure 4.15 illustrate the 1:1 binding stoichiometry.

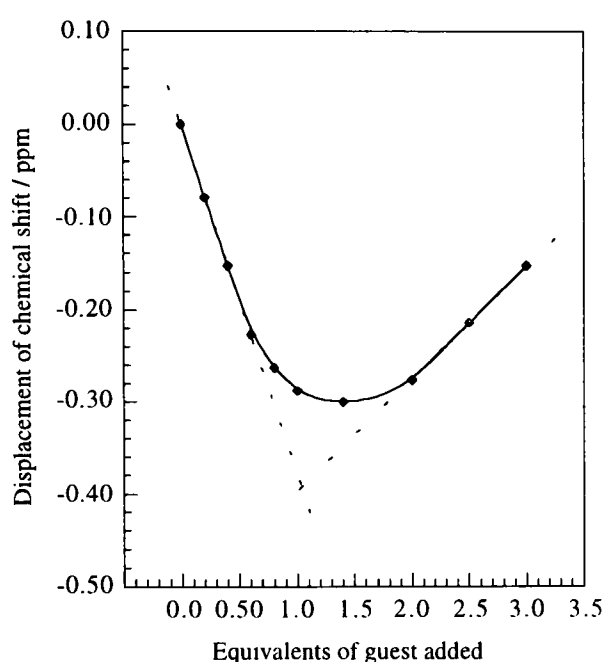


Figure 4.12: ^{15}N titration profile for addition of chloride to ^{15}N -[58].

4.6ii UV-visible spectroscopy

UV-vis titrations were performed on [55] and [57] with addition of chloride in acetonitrile. The concentration of receptor was much weaker ($2 \times 10^{-5} \text{ mol dm}^{-3}$) with the expectation of higher stability constants than revealed by nmr experiments, as explained in sections 1.1i and 2.8i. Changes in the absorption were found to be related to the quantity of anion present — figure 4.13 shows the UV-vis spectrum for [57] at a series of concentrations of added chloride. There is a notable decrease in absorbance in the ligand centred band at 287 nm and an increase in the metal-to-ligand charge transfer band at around 470 nm. An isosbestic point lies between the two at 348 nm. The perturbation of the metal centred absorption band is less pronounced and manifests itself as an increase in absorbance. It is interesting to note that the

bands most strongly affected are those which directly involve bpy ligand orbitals. Transitions only involving the more remote metal are much less sensitive to the guest ion.

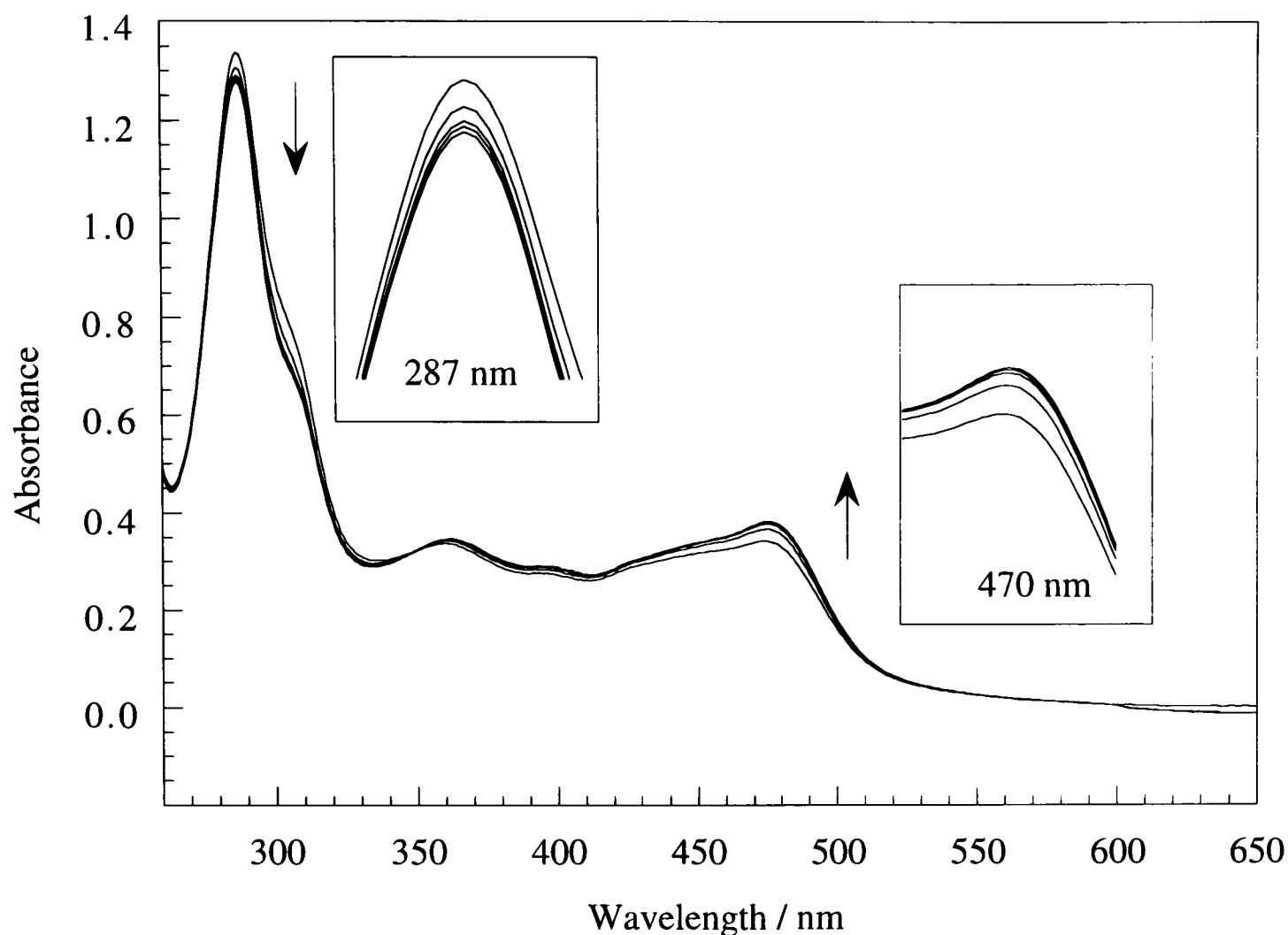


Figure 4.13: Effect of added chloride on absorption spectrum of [57].

In terms of the profile at a given wavelength, the dissections show very clear trends with a well defined 1:1 stoichiometry. Figure 4.14 shows the titration profile for [57] at the two wavelengths mentioned specifically in the figure above. However, the results of quantitative analysis were not clear mainly due to the small magnitude of perturbations and the inability of Specfit to match experimental and calculated curves.

Factor analysis of the spectra indicated that there were only two significant contributing species which were taken to be the free receptor and the complex. A *very* weak third component was seen, presumably a 2:1 complex of low binding strength, but the 1:1 component was thought to be dominant.

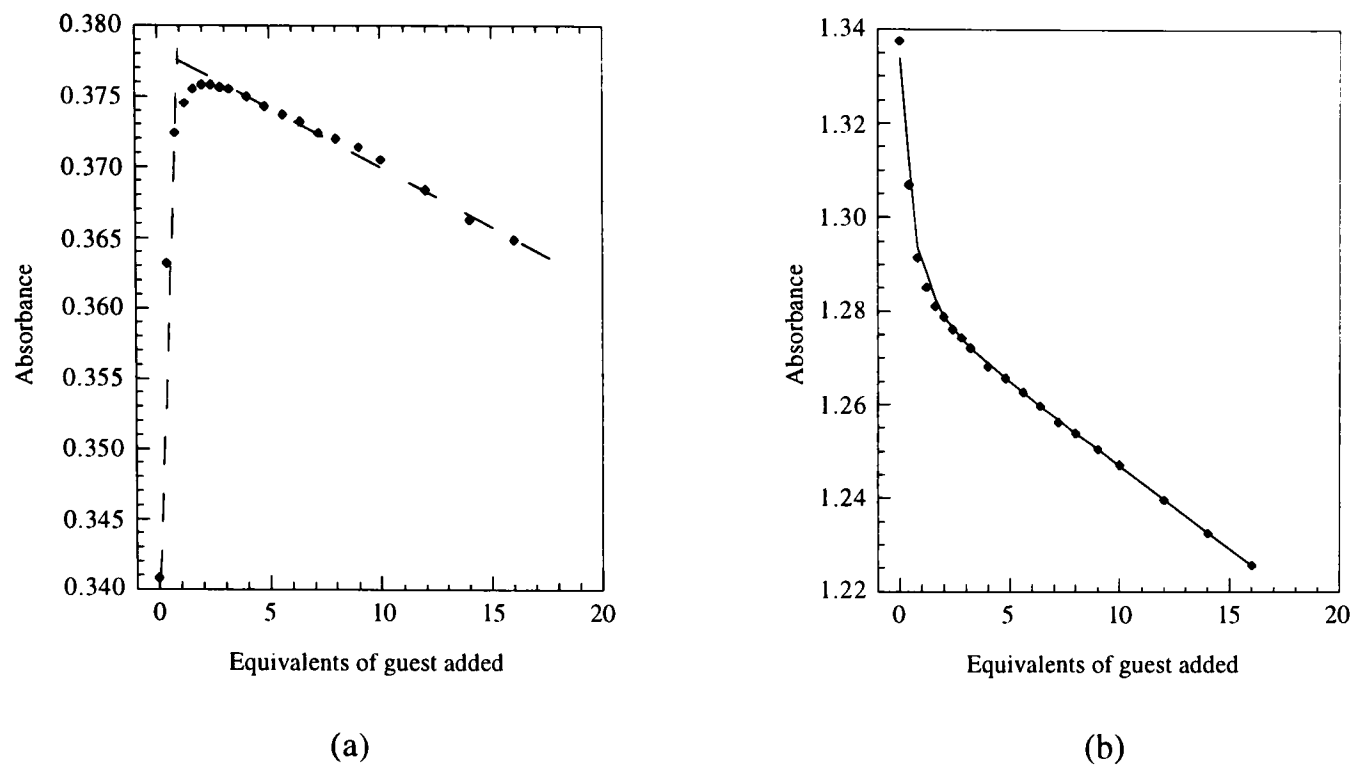


Figure 4.14: UV-vis titration profiles for addition of chloride to [57] in acetonitrile; dissection at (a) 470 nm; (b) 287 nm.

Unfortunately, a satisfactory fit could not be obtained, a fact which was explained by plotting the sum of squares as a function of the stability constant (here a 1:1 model was used to generate the data). This is the parameter which the program attempts to minimise, but it can be seen that, in figure 4.15, a shallow plateau region emerges and therefore only local minima are located. In this situation, only a lower limit may be deduced for the stability constant; here it is in the region of $10^6 \text{ dm}^3 \text{ mol}^{-1}$. The choice of solvent will influence the magnitude; acetonitrile is less polar than DMSO, but nevertheless, this is a very high value – the largest yet encountered in this work.

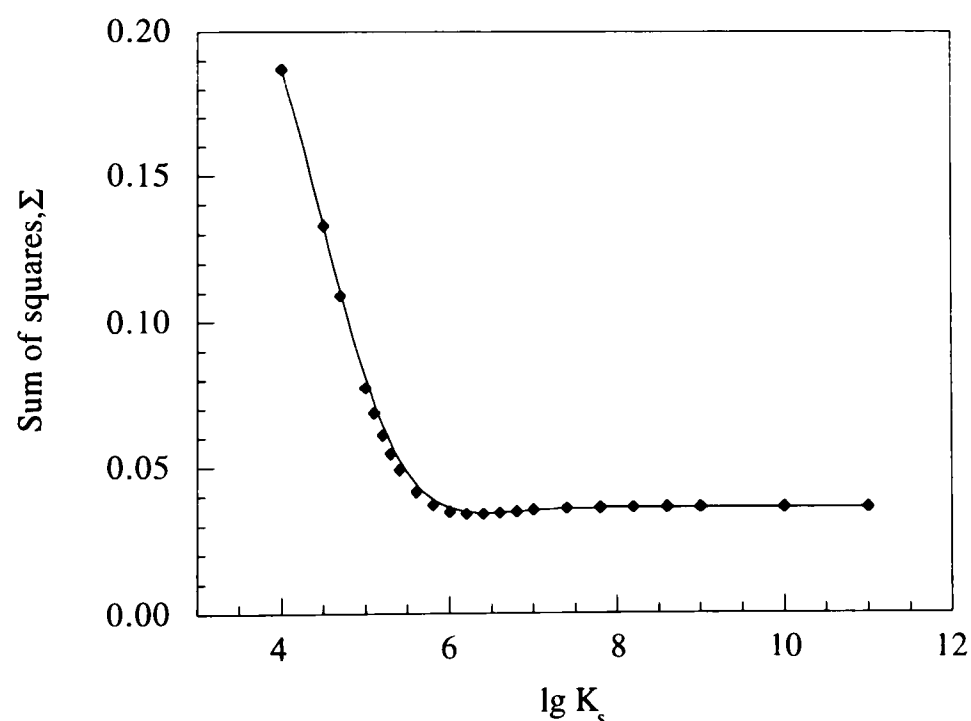


Figure 4.15: The plot of Σ against K_s for addition of chloride to [57] in acetonitrile.

A very similar picture emerges in the analogous experiment between [55] and chloride (figure 4.16).

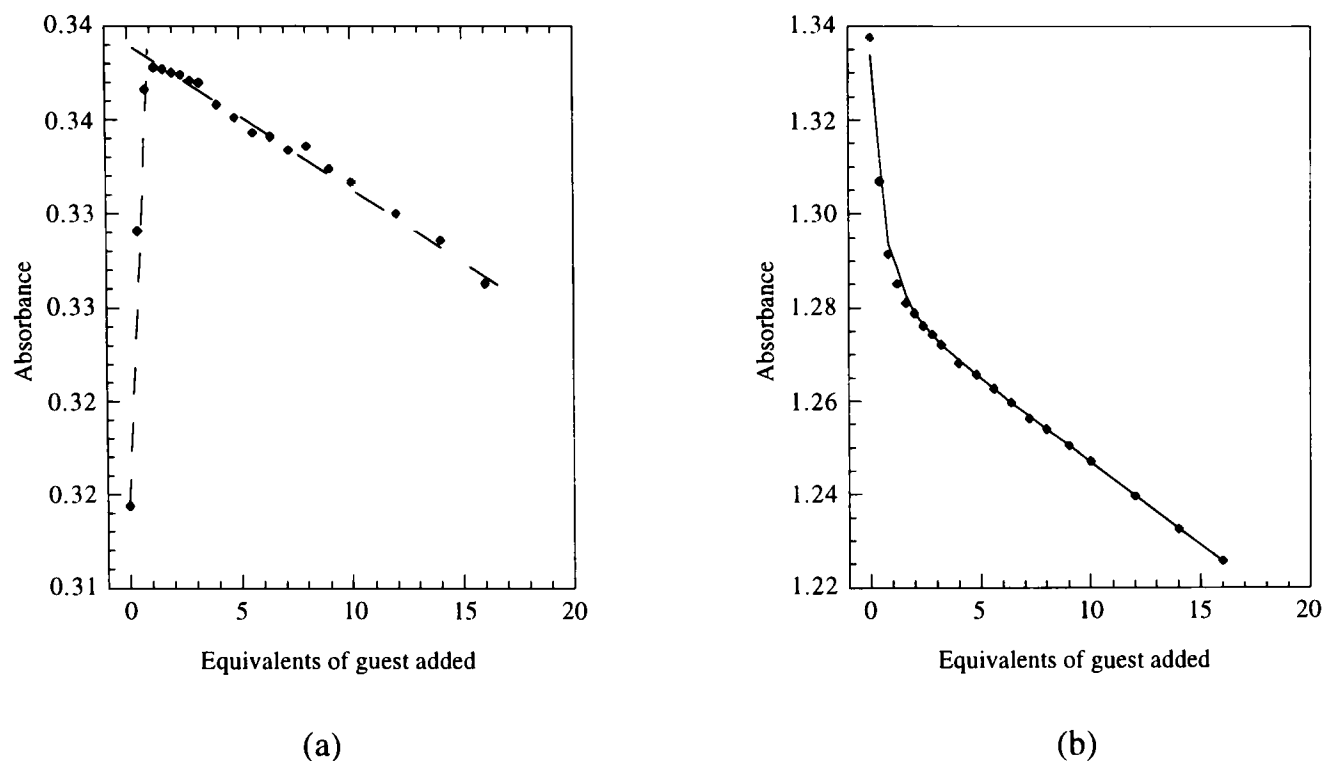


Figure 4.16: UV-vis titration profiles for addition of chloride to [55] in acetonitrile; dissection at (a) 470 nm; (b) 287 nm.

The same response is seen in terms of changes in the absorption spectrum and, again, the stoichiometry is visible by inspection of the profiles. Once more, no fitting was available and this was traced to the same origin; the analogous plot is seen in figure 4.17.

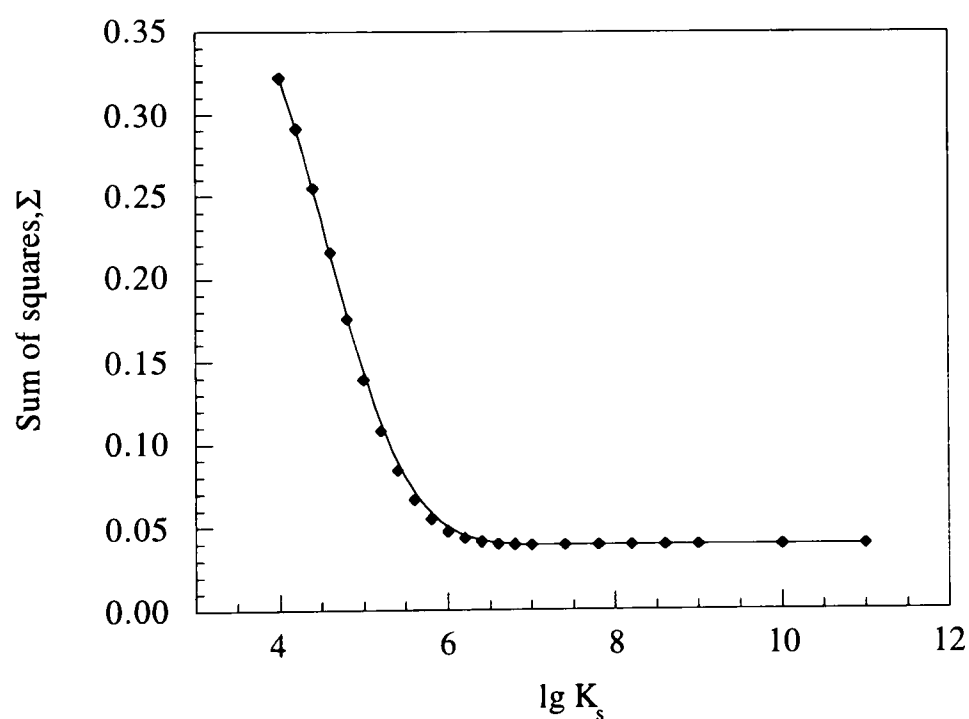


Figure 4.17: The plot of Σ against K_s for addition of chloride to [55] in acetonitrile.

The UV-vis experiments were therefore informative in the following respects. The assumed stoichiometry was further confirmed by the titration profile taken from a dissection in the

LMCT region of the spectrum which showed a strong change following addition of one mole equivalent of anion. However, the perturbations were not as great as those seen with other systems, which addresses one of the speculative points raised at the beginning of this chapter. The fact that the metal atom is further removed from the actual binding site lowers its sensitivity to the host-guest encounter.

The strength of the interaction was estimated by these studies — although an exact figure could not be assessed, the lower limit was around $10^6 \text{ dm}^3\text{mol}^{-1}$; a very substantial figure. In mitigation, it is true that the solvent was less competitive than DMSO and experimental conditions involved one of the lowest concentrations yet encountered (ruthenium based receptors are very intensely coloured due to charge transfer bands); both of these factors will serve to augment the magnitude of the constant. In absolute terms, though, the binding is strong.

4.6iii Luminescence

Luminescence studies were conducted on a Perkin-Elmer LS50B fluorimeter by kind permission of Dr Sheena Radford of the Oxford Centre for Molecular Sciences.

The receptors, when excited at 470 nm, produced a single broad emission centred around 650 nm — this is the intense orange-red colour seen under a long wavelength ultra-violet lamp. The addition of chloride induced the same response in each case, namely an increase in emission intensity. The value of λ_{max} was slightly affected, usually in the form of a hypsochromic shift of 5–10 nm. These effects were very well demonstrated by the titration between [57] and chloride; the spectra are shown in figure 4.18.

Titration were performed on 3 ml of solution containing the receptor at a concentration of $10^{-5} \text{ mol dm}^{-3}$ in a quartz, stoppered cuvette. Anion was added in solution with one mole equivalent in 10 μl and the solution was then manually stirred. The cuvette was not disturbed during the course of the titration.

Solutions were not degassed and the experiments were conducted on the open bench, and so the question of quenching by dioxygen was of concern. However, satisfactory levels of

emission were observed in the presence of dissolved oxygen and control experiments showed that emission intensity was stable within 1–2% under experimental conditions.

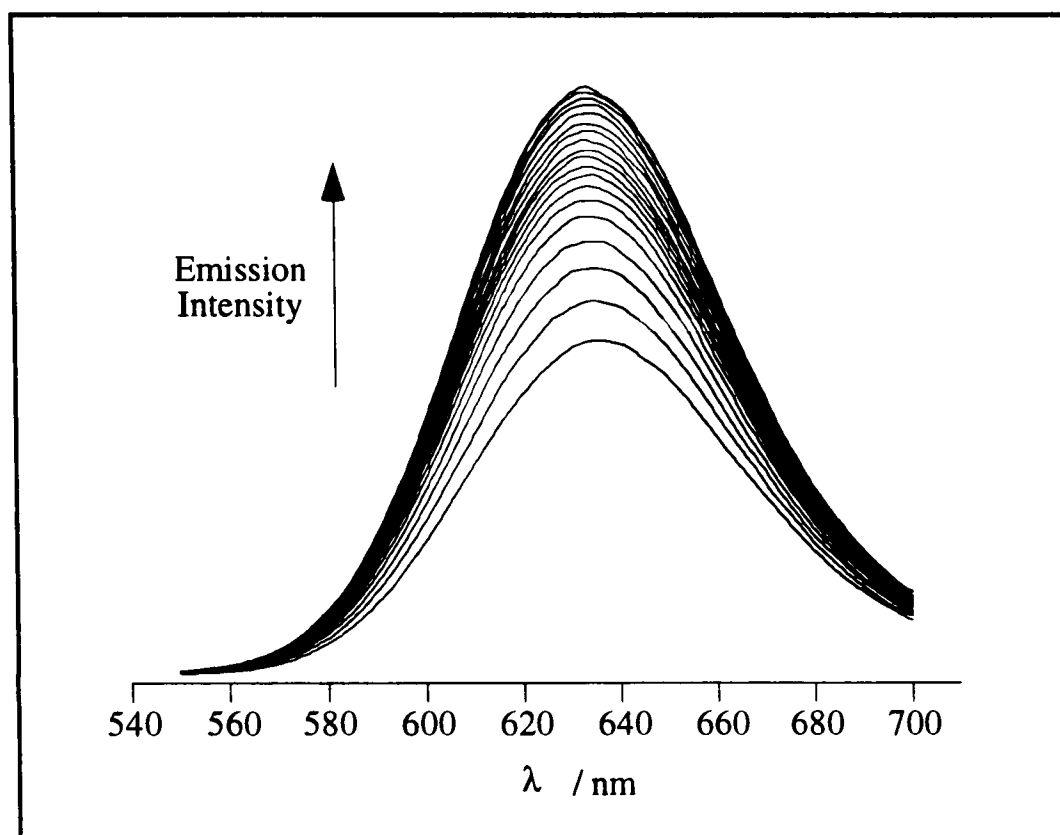


Figure 4.18: Effect of chloride on emission spectrum of [57].

The titration profiles show the variation of the emission maximum intensity as a function of equivalents of added chloride in acetonitrile. All are very similar in nature with a steady increase in intensity followed by an extended plateau region. A slight decrease in intensity is seen in this region as a result of dilution.

Figure 4.19 displays the profiles for [55] and [56] which are typical of the data from these experiments. Several approaches were used to analyse the data. The literature affords examples of functions which can be applied to such data with a view to calculating a stability constant; in particular, the methods of Valeur¹⁹⁹ and de Silva¹⁸⁴ were investigated. Fundamentally these two approaches have the same origin, but manipulation of the equations produce slightly different functions. The analyses were mostly inconclusive. The graphs plotted were non-linear, probably due to the assumption that the receptor concentration is unchanged (in fact, it is reduced by approximately 5%) and a failure to conform to mass action. Moreover, there was a very strong dependence on the maximum emission intensity. This had to be extrapolated from the graph; a process which introduced very large error.

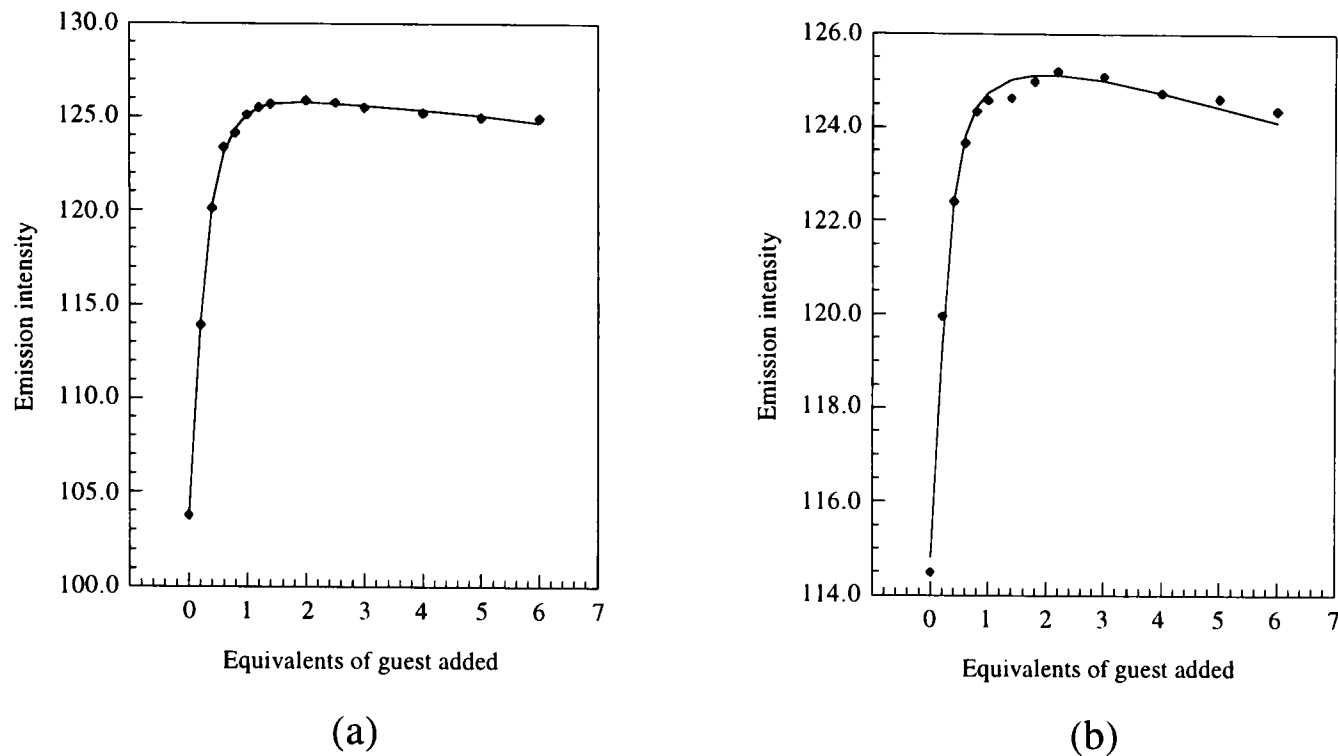


Figure 4.19: Luminescence titration profiles for addition of chloride;
(a) [55]; (b) [56].

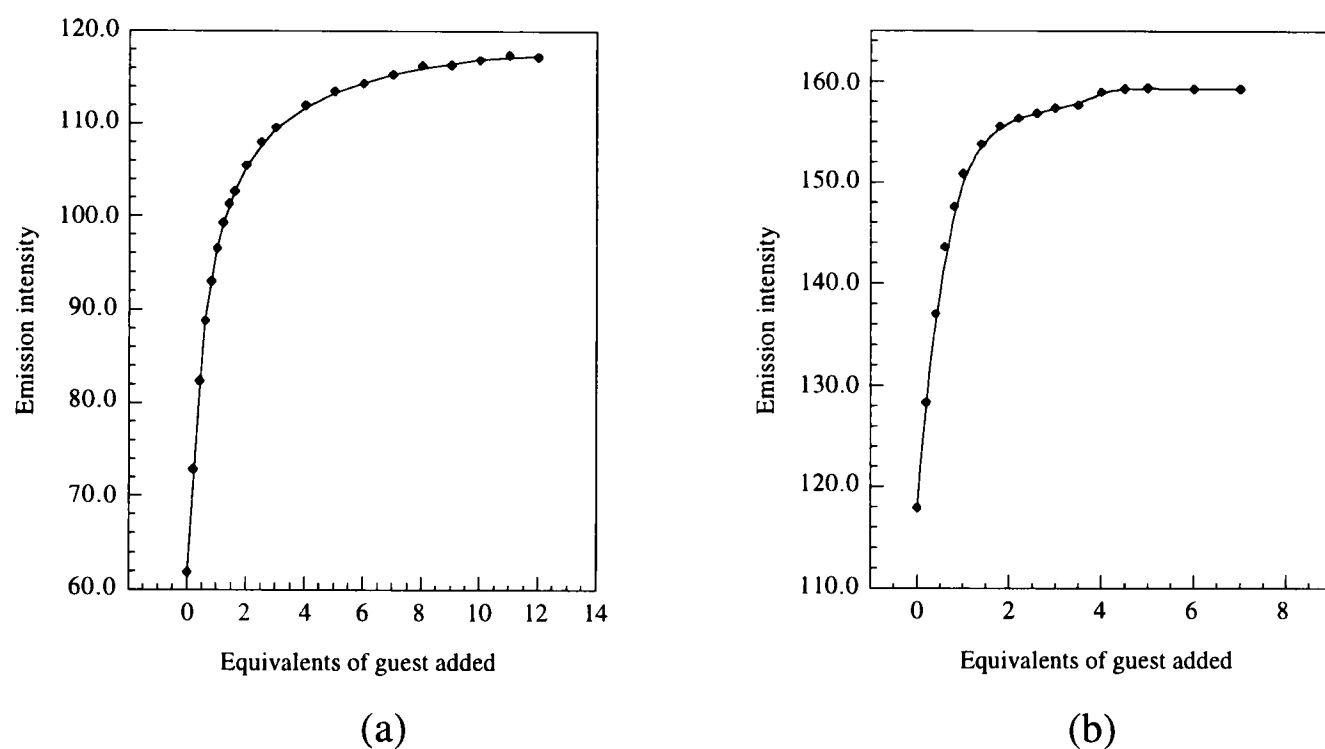


Figure 4.20: Luminescence titration profiles for addition of chloride;
(a) [57]; (b) [58].

Further analysis was conducted with Specfit which was able to import luminescence data. Again, the fitting was not entirely satisfactory but stability constants of the order of $10^5 \text{ dm}^3\text{mol}^{-1}$ were observed. In the case of graphical methods, the values extracted from the linear regions of the plots agreed well with Specfit. However, in the absence of complete fitting, these figures must remain mere estimates. It is interesting to note, however, that the

value for [58] was an order of magnitude smaller than the rest which compares favourably with the nmr studies.

Why does the emission intensity increase on addition of chloride? This is an important question and concerns the non-radiative pathways by which the excited state relaxes. The emission of the receptors is much less intense than that of $\text{Ru}(\text{bpy})_3^{2+}$ itself and therefore, these pathways must be accessible to the excited electron. One possible answer to the question is found in the locking of conformational modes by the bound chloride. The presence of the anion will rigidify the molecule, particularly in the region of the binding site. In this way, vibrational and rotational states will be restricted, intramolecular collisions will be reduced, particularly between the substituent groups, and the excited state will relax less efficiently with an attendant increase in the quantum yield for luminescence.

The profiles were found to be strongly solvent dependent; attempts to repeat the experiment in DMSO resulted in a steady fall of emission intensity with each aliquot of anion, to produce curves with high levels of noise. It is suspected that experimental conditions were the cause of these difficulties, in particular the difficulty of obtaining dry solvent — water will not only compete with binding, but also interfere with emission by hydrogen bonding to $\text{C}=\text{O}$ which becomes slightly charged in the charge transfer state.

The results described above are the outcome of preliminary investigations and represent the first emission studies conducted within the group. They are very encouraging, with regular functions seen for each titration and estimated stability constants (based on the combined results of all analyses) in the 10^5 – $10^6 \text{ dm}^3\text{mol}^{-1}$ range. They also clearly invite further study under more rigorous conditions and with the capability to analyse quantum yields and emission lifetimes under an inert atmosphere. At the time of writing, a collaboration with Prof. Vincenzo Balzani is being established and it is hoped that his group's considerable skill and experience can be brought to bear on these receptors with success.

4.6iv Electrochemistry

Four redox waves are apparent in the cyclic voltammograms of the receptors; they are due to one electron oxidation of ruthenium (II) and one electron reductions of the three bipyridyl ligands. This is typical redox behaviour for systems based on $\text{Ru}(\text{bpy})_3$ and is well documented.^{200,201} In fact, it has been shown that the bpy ligands are each capable of

undergoing a further reduction step if the potential is swept far enough. However, low temperatures were required for this study.²⁰² Figure 4.21a is the cyclic voltammogram of the oxidation region and displays a single oxidation wave for the $\text{Ru}^{2+}/\text{Ru}^{3+}$ couple. The corresponding square wave voltammogram is also shown.

The CV of the bpy region of [56] in acetonitrile is shown in figure 4.21b where it can be seen that the bpy reduction waves are very close together and poorly resolved. It is in such cases that the strengths of square wave voltammetry become apparent as illustrated in the same figure. The SWV is included and clearly shows the three waves.

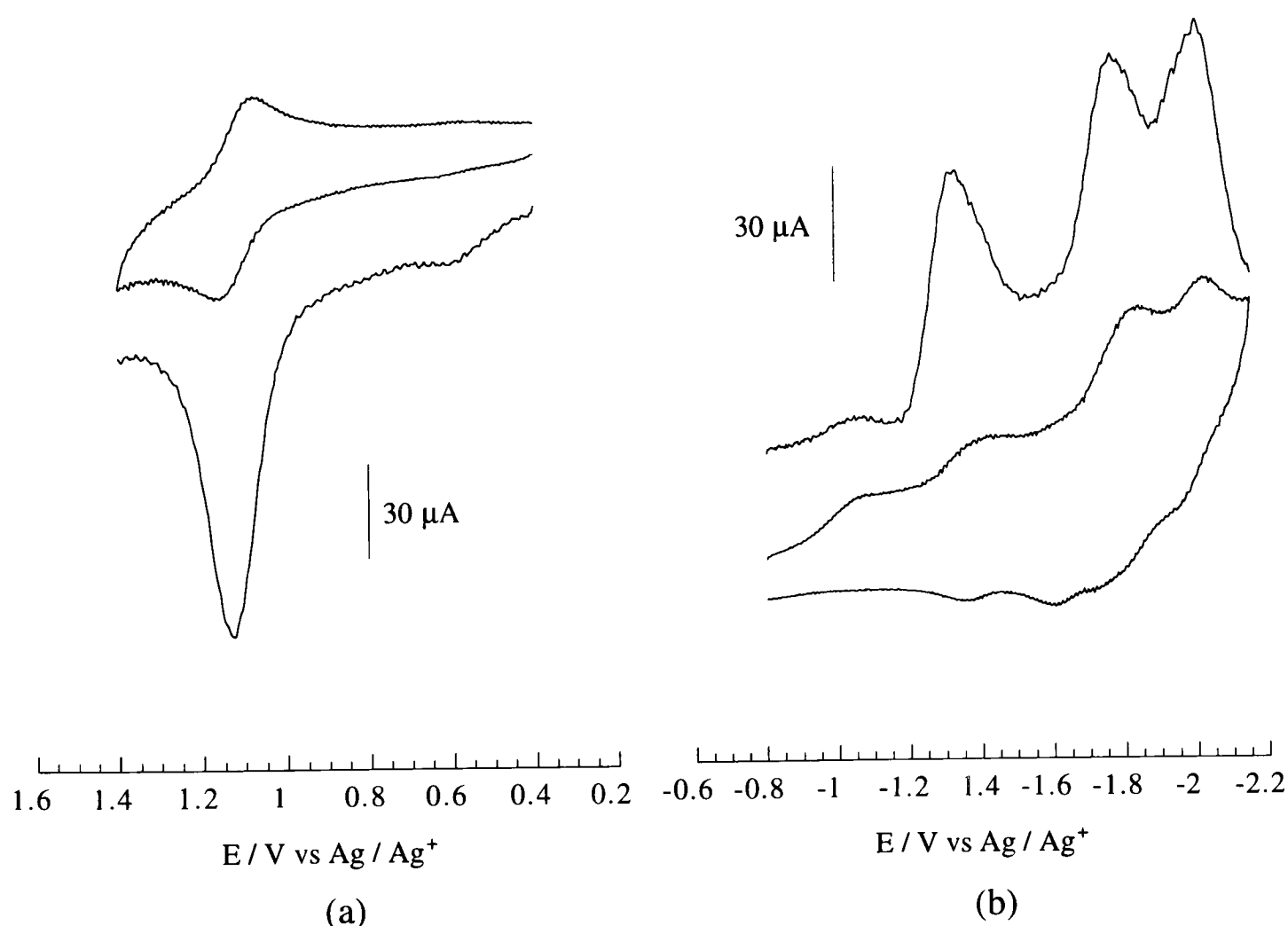


Figure 4.21: Cyclic and square wave voltammetry of [56] in acetonitrile;

(a) oxidation of Ru (II); (b) reduction of bpy.

The impact of chloride upon the voltammograms was modest but could be observed. In no case, however, was the observed cathodic shift greater than 15 mV and, in several experiments, there was broadening of the peaks. Of those shifts observed, the largest in magnitude was the first bpy wave which represents the substituted ligand, since the electron withdrawing amide will make reduction easier. This agrees with the proposed binding site based upon nmr and crystallographic evidence. It can be seen that the first wave stands apart from the other two which are much more closely spaced and which displayed a negligible

shift. There was a small effect on the ruthenium oxidation wave which became irreversible as the chloride levels increased.

It became clear that, electrochemically, the receptors are much less responsive to the guest than might be expected from comparison with other investigative techniques. Although a shift in the first bpy wave could be induced (after five equivalents), its size was rather small and it became difficult to differentiate between the performances of the respective receptors. In conclusion, the prospects of developing an anion sensor incorporating $\text{Ru}(\text{bpy})_3^{2+}$ will perhaps lie in arenas other than the electrochemical cell.

4.7 Binding of dihydrogen phosphate

4.7i ^1H nmr titration

Titration with dihydrogen phosphate in deuteriated DMSO gave rise to series of profiles with sharp plateaux which led to the preliminary conclusion that binding was stronger than that observed in the case of chloride. The profiles shown indicate the displacement of the H_3 proton resonance; this was nearly almost always the proton followed due to the rapid broadening and disappearance of the amide resonance. There was also considerable broadening and splitting of the H_3 resonance which added greatly to the difficulty of analysing the results.

The profiles shown in figure 4.22 are those of [55] and [56]. The very sharp onset of the plateau region confounded attempts to extract stability constants from the data; the binding is too strong for EQNMR fitting.

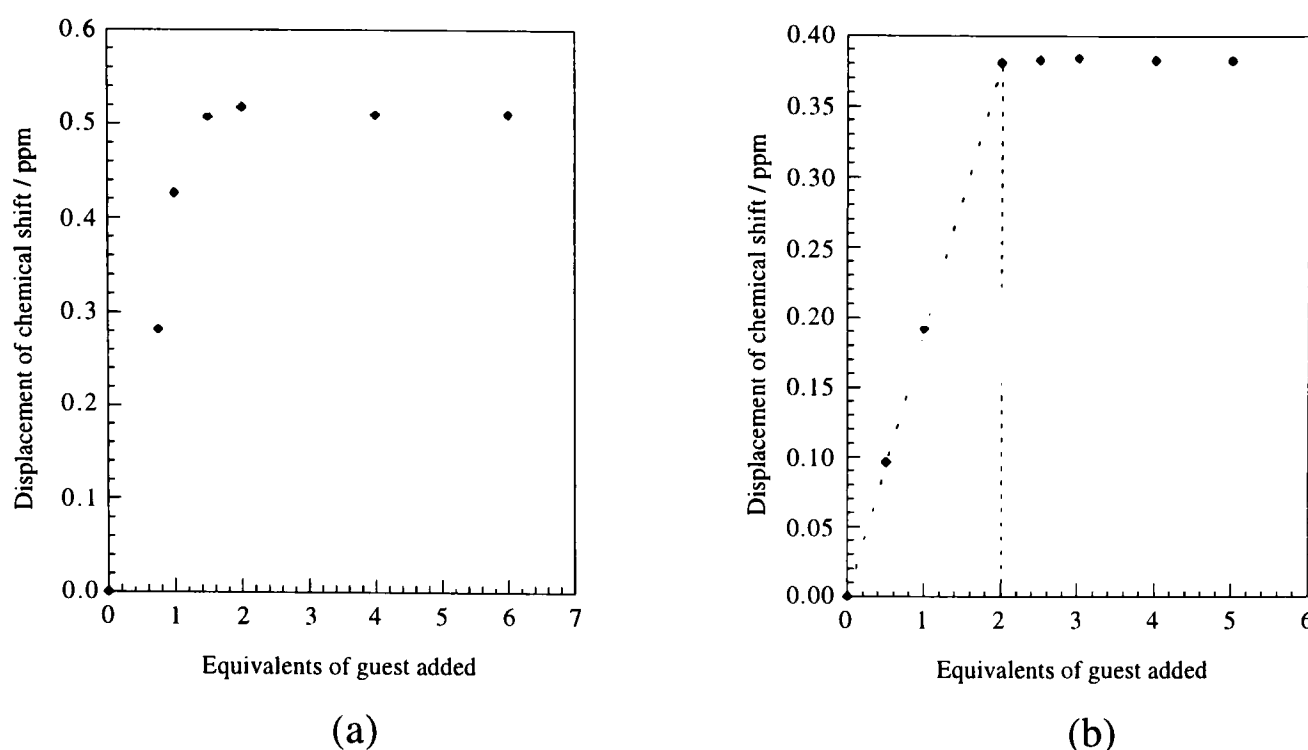


Figure 4.22: ^1H nmr titration profiles for addition of dihydrogen phosphate:

(a) [55]; (b) [56].

In addition, the stoichiometry of [56] is rather puzzling; it seems to indicate a strong 2:1 complex, although the other profile, and those in figure 4.23, all are consistent with a 1:1 model. There appears to be no obvious difference in [56] to explain the preference for 2:1 binding, although it will be seen that [54] behaves in a very similar manner. The binding of dihydrogen phosphate to the receptors is, as before, associated with the amide and 3H proton but evidence from the literature and other workers in the group suggests that it occurs in an out of plane fashion. A crystal structure from the work of Dr Fridrich Szemes, of this group, displays dihydrogen phosphate bound in such a manner,²⁰³ and a report by Sessler includes crystal structures of phosphate anions similarly bound to sapphyrin.²⁰⁴ This would allow one to propose two possible ways of forming a 2:1 complex. The first would involve an anion situated on either side of the plane of substituted bpy — a possible structure given the total of four binding points and the dipositive charge on the receptor. The second explanation is the possible dimerisation of dihydrogen phosphate which has been observed in crystal structures.¹¹⁵ In effect, this would be the 1:1 binding of a phosphate based dimer which carries a 2- charge.

The true origin of this stoichiometry and the reasons for its appearance in isolated cases remain an intriguing mystery which is unlikely to be solved until more data (preferably crystallographic) become available.

Addition of the same anion to [57] and [58] gives smoother profiles, as seen in figure 4.23. The stoichiometry, as mentioned above, is 1:1 and this model was used to determine the stability constants which were $750 (\pm 95)$ and $1200 (\pm 300) \text{ dm}^3\text{mol}^{-1}$ respectively.

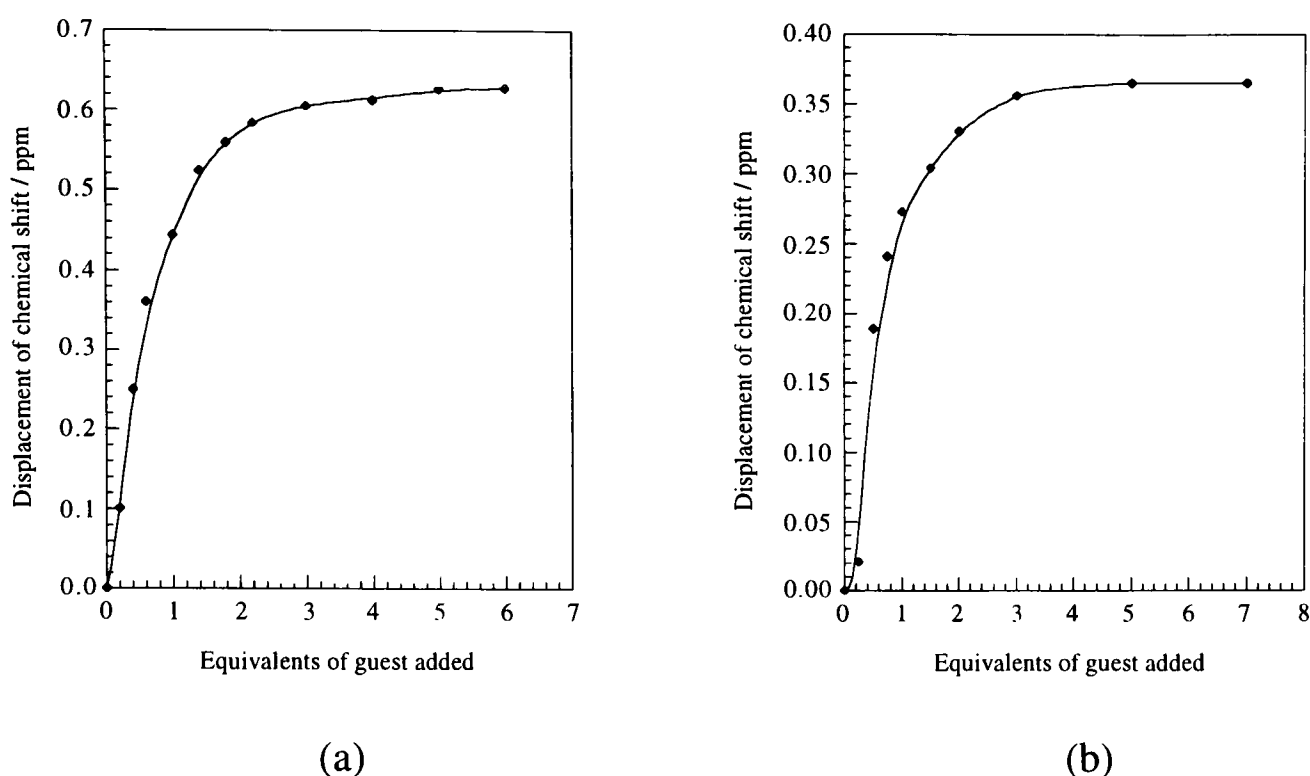


Figure 4.23: ^1H nmr titration profiles for addition of dihydrogen phosphate;

(a) [57]; (b) [58].

In general, the profiles suggest that binding of dihydrogen phosphate is very appreciable after consideration of the solvent; DMSO is a very competitive medium. The strength of interaction is rather stronger than that of chloride and is perhaps due to the more concentrated nature of the charge. The electron cloud of chloride is very diffuse whereas dihydrogen phosphate carries its charge on one of two oxygen atoms, a more confined domain. Furthermore, the specific shape of the latter enables it to form two well defined interactions between its oxygen atom donors and acceptor sites in the receptor. It may also be able to act as an acceptor itself by virtue of $-\text{OH}$ protons. This combination makes dihydrogen phosphate a versatile guest.

4.7ii Luminescence

The luminescence studies concerning addition of dihydrogen phosphate to receptors [55]–[58] in acetonitrile produced a selection of the most unusual titration profiles contained in this thesis. They are displayed in figures 4.24 and 4.25 and defied most attempts at

interpretation. All the profiles show a marked change in direction at some point, usually in the vicinity of two equivalents' addition. This would imply at least two different binding processes at work and the dramatic changes suggest that they are very distinct. However, beyond this, there is only one clear message; namely that, in this solvent at these concentrations (10^{-5} mol dm $^{-3}$), there is not simply 1:1 binding.

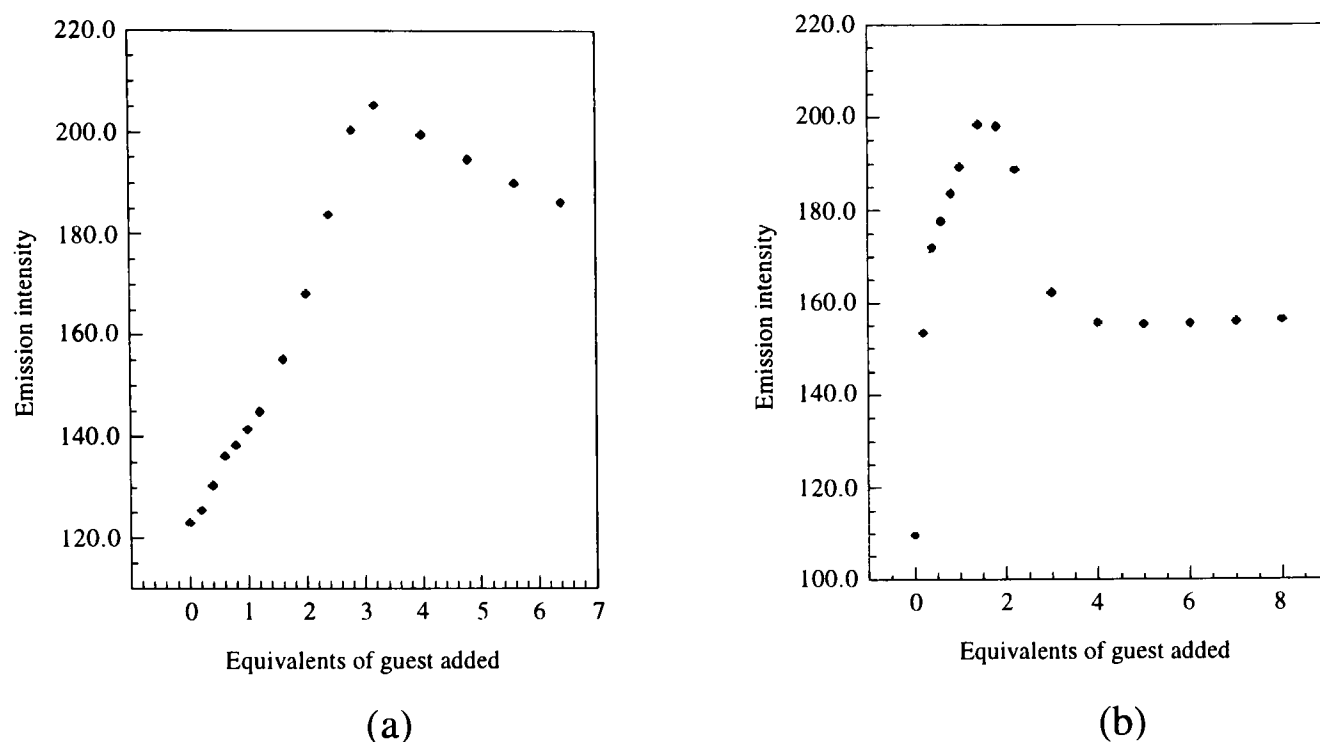


Figure 4.24: Luminescence titration profiles for addition of dihydrogen phosphate in acetonitrile:

(a) [55]; (b) [56].

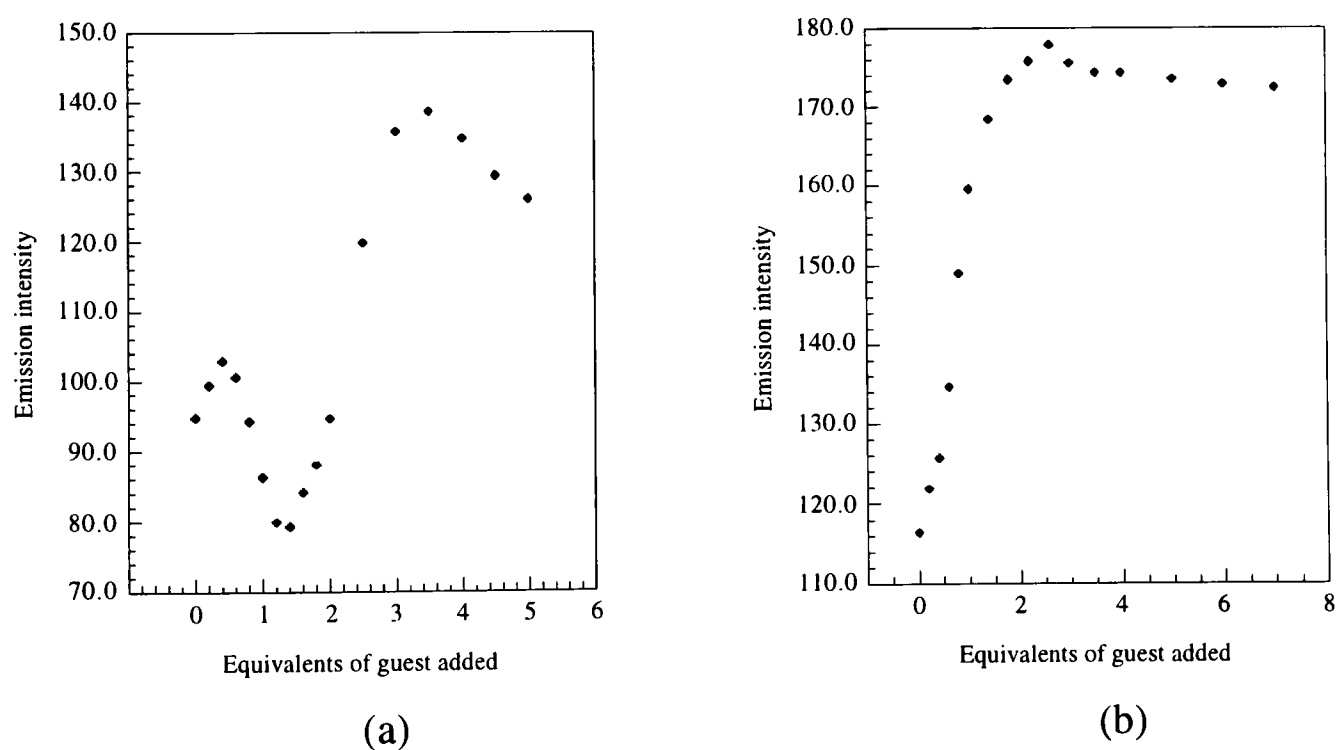


Figure 4.25: Luminescence titration profiles for addition of dihydrogen phosphate in acetonitrile:

(a) [57]; (b) [58].

The anion is not fluorescent in its own right, nor does it absorb light in the excitation region of the receptors. However, the possibility of quenching of fluorescence by lone pairs has been documented; Czarnik has showed that amine lone pairs quenched the fluorescence of anthracene, a process which was suppressed on binding of hydrogen phosphate.²⁰⁵ Ultimately, though, the source of this very odd behaviour must, at this time, remain a source of speculation.

4.8 Binding by Receptor [54]

The work described in this section was performed in collaboration with Mr. Liam Sutton.²⁰⁶ Receptor [54] was designed with the intention of producing a luminescent switch which would give an “off / on” response to the presence of anions, as shown schematically in figure 4.26. Here, emission from the metal centre, M, is quenched by an appended group, Q, until an anion, A, is located in the binding site. This then blocks the quenching interaction with the result that emission is induced.

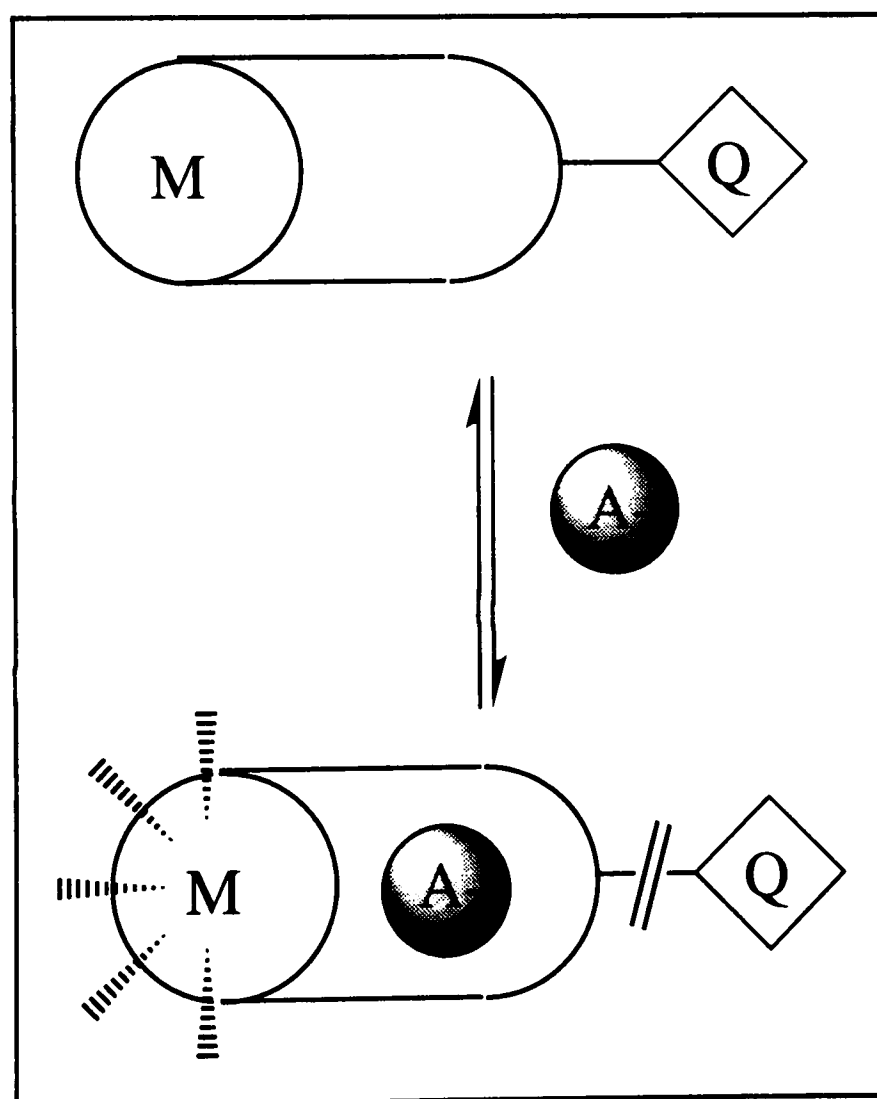


Figure 4.26: Schematic representation of “off / on” luminescent sensor.

Ferrocene was known to act as a quenching group and was appended to the $\text{RuL}(\text{bpy})_2^{2+}$ core. The synthesis of the receptor was conducted by Mr. Sutton who prepared methylaminoferrocene by a synthetic route developed within the group.¹⁵⁸ Reaction with $\text{Ru}(\text{bpy})_2\text{Cl}_2$ in ethylene glycol then afforded the receptor in 55% yield. Emission studies confirmed that the initial goal was successful, for there was negligible recorded luminescence. The investigation of binding via the usual methods was therefore undertaken.

4.8i ^1H nmr titration

The titration of [54] with both chloride and dihydrogen phosphate in deuteriated DMSO did not give conclusive results. The amide and 3H proton signals began to shift but then soon broadened on addition of further aliquots of anion, at which point their precise position could not be determined. This remained the case for chloride addition but with dihydrogen phosphate, sharp peaks re-emerged at a late stage in the titration, which was encouraging. However, precipitation of the anion complex prevented the assessment of a stability constant.

4.8ii UV-visible spectroscopy

The effect of added anion upon the UV-vis spectrum of the receptor was monitored and the results are presented in figure 4.27. In titration with both chloride and dihydrogen phosphate, the spectra are modified by the presence of an anion guest. In the case of the former, however, the changes observed in intensity were small in magnitude and consequently, there was some noise in the data. Nevertheless, a steady change followed by a plateau region is observed and this is indicative of appreciable binding interaction. The results do not contradict the expected stoichiometry of 1:1, but it is difficult to draw tangents which could confidently be used in support of this hypothesis.

Inspection of figure 4.27b provides a notable contrast to the comments of the previous paragraph. Not only is the change in absorbance an order of magnitude larger, but the shape of the curves are very different. Profiles are shown for two different wavelengths in the spectrum; the upper one representing ligand centred absorption and the lower, metal centred. Their shape is quite unusual for a simple binding process and appears to indicate a 2:1 binding stoichiometry by virtue of the pronounced change in the spectra at 2 equivalents of anion. Further conclusions, in particular concerning the strength of interaction, could not be drawn. Specfit analysis indicated a large number of contributing species to the titration

spectra and it is suspected that the presence of the ferrocene absorption band, which is likely to be perturbed by the anion but to a different extent, is a complicating component. Reference to figure 4.28, which shows the spectra from the early part of the titration, supports this suspicion; the metal-centred region of the spectrum is strongly affected, in marked contrast to the results presented earlier, in section 4.6ii (cf. figure 4.13).

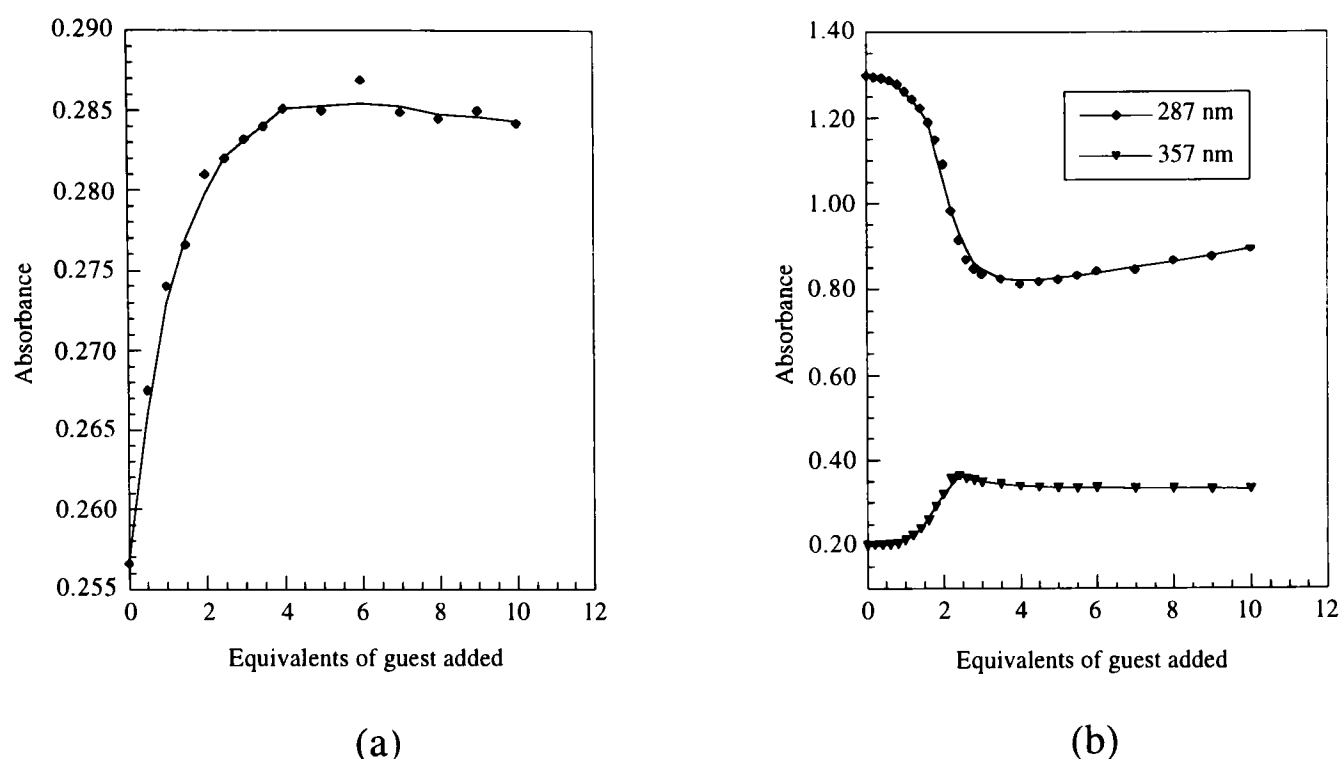


Figure 4.27: UV-visible titration profiles for anion addition to [54] in acetonitrile:
(a) chloride (470 nm); (b) dihydrogen phosphate.

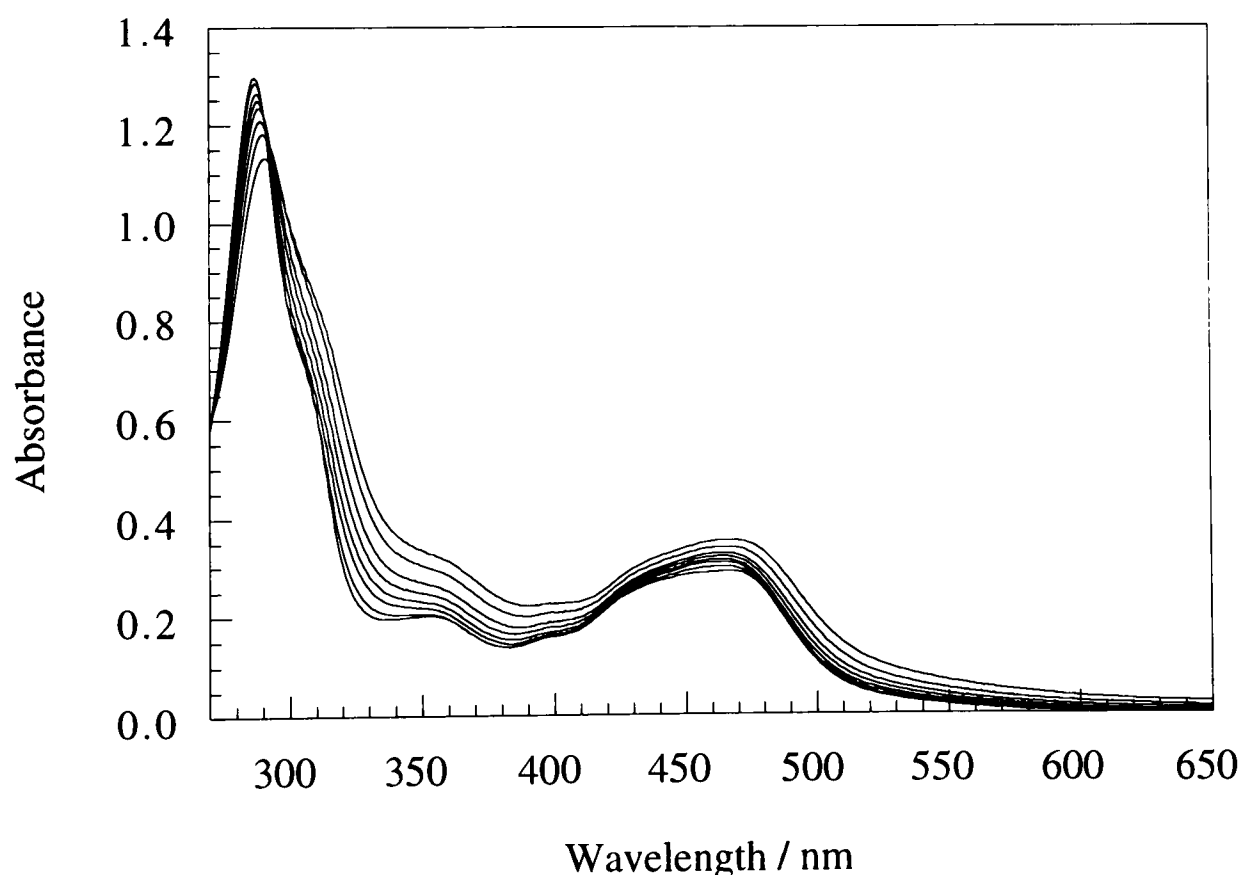


Figure 4.28: Effect of dihydrogen phosphate on the UV-visible spectrum of [54].

4.8iii Luminescence

The free receptor had an exceedingly weak emission spectrum. In comparison with $\text{Ru}(\text{bpy})_3^{2+}$, the modifications made to one bipyridyl ligand resulted in almost complete quenching of the celebrated emission properties. The origin of the quenching is not known, although a previous report has proposed that electronic energy from the excited state is relocated on to ferrocene, thereby populating an excited vibrational state which decays non-radiatively.²⁰⁷ The fact that an intramolecular mechanism operates was shown by mixing $\text{Ru}(\text{bpy})_3^{2+}$ with two equivalents of free ferrocene in solution; the levels of quenching did not exceed 10%. This suggests that the high effective concentration of the covalently linked ferrocene is instrumental in the quenching process.

The addition of chloride or hydrogen sulphate in acetonitrile had little impact on the emission, or lack thereof. However, the introduction of dihydrogen phosphate had rather more dramatic consequences, as shown in figure 4.29.

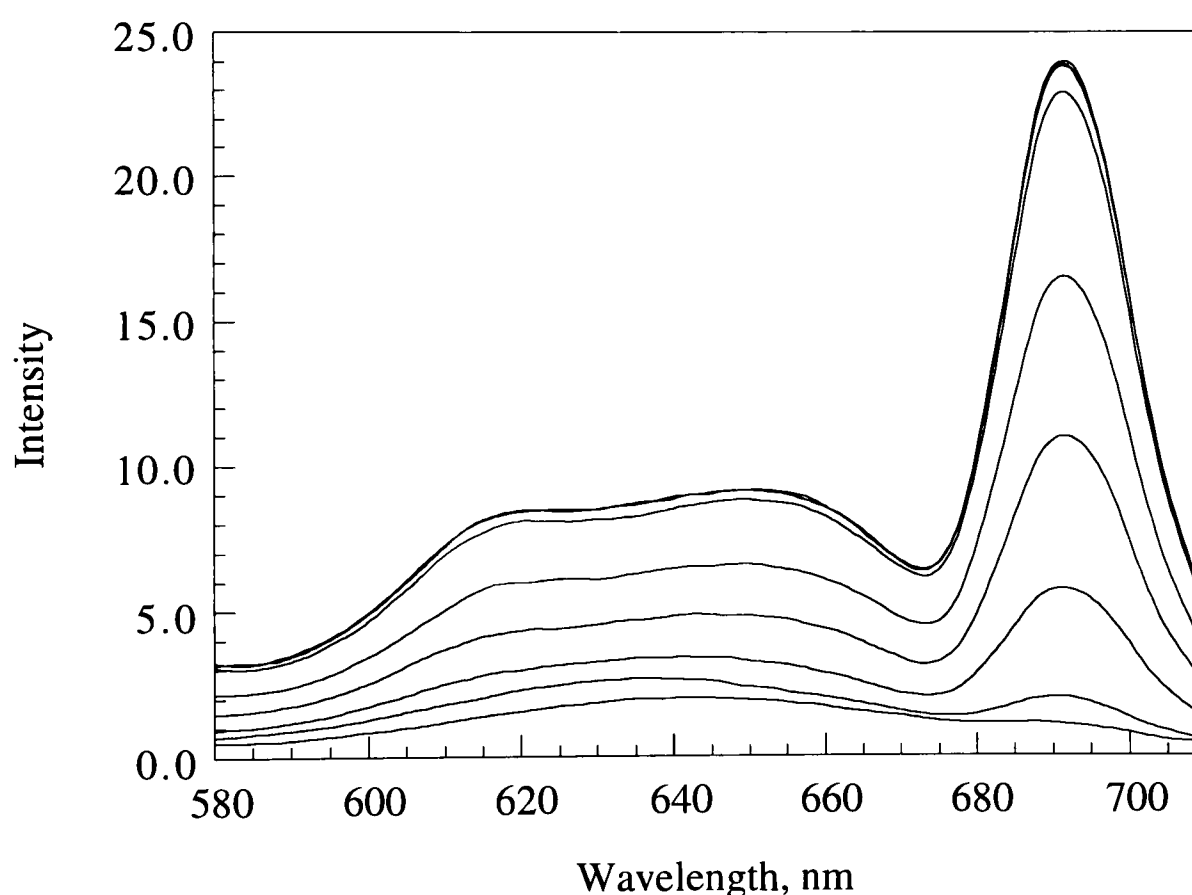


Figure 4.29: Effect of dihydrogen phosphate on the emission spectrum of [54].

Emission was induced at 620, 650 and most strongly at 690 nm. The latter is thought to be due to the formation of an exciplex which then emits at the lower frequency. The reason for this phenomenon is most probably conformational locking as a result of anion binding.

Dihydrogen phosphate is thought to bind out of the plane of the bpy system (as mentioned earlier) and this binding may hinder a conformation in which the most effective quenching occurs. It should be noted that, in spite of a 20-fold increase in intensity at 690 nm, the luminescence is still largely quenched with respect to the other receptors mentioned in this chapter. However, the binding and associated emission is a pleasing and unusual result. The titration profiles are shown in figure 4.30, and are noteworthy in that they mirror the 2:1 binding stoichiometry which was inferred from the UV-visible data.

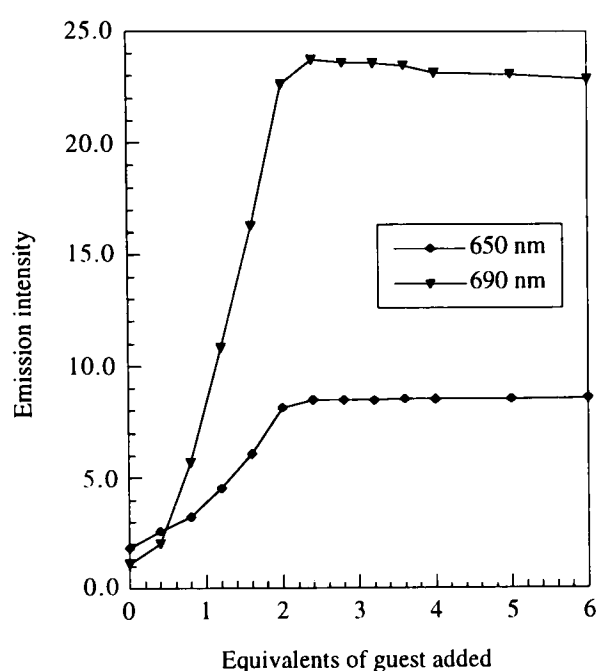


Figure 4.30: Luminescence titration profiles for addition of dihydrogen phosphate to [54].

The final experiment conducted was a competition experiment to see if the selectivity for dihydrogen phosphate was observed in the presence of other anions. Approximately five equivalents of both chloride and hydrogen sulphate were added to a solution of [54] in acetonitrile. The titration with dihydrogen phosphate was then repeated with very similar results. This confirms the very interesting finding that this induced luminescence is a phenomenon associated specifically with dihydrogen phosphate, which allows [54] to be described as a selective luminescent sensor for this particular anion.

4.9 The Role of the Positive Charge: A Rhenium Analogue

It has been the custom in previous chapters to question the role of the positive charge in the binding process. In this case, a rhenium analogue of [56] was synthesised by addition of a substituted 2,2'-bipyridine to rhenium pentacarbonyl bromide, and is shown in figure 4.31. The compound is also an eighteen electron compound and therefore shares the stability of the ruthenium receptors but the metal lies in the +I oxidation state, and the presence of bromide in the coordination sphere means that the complex is neutral overall. Even so, the same binding site exists and therefore the receptor provided an opportunity of examining the potential of the functionalised bpy as an anion receptor in isolation. The comparison was not strictly “fair” because the carbonyl ligands are strongly electron withdrawing (in a π -acid sense) and will therefore promote anion binding due to lowered electron density on the metal. However, the binding properties were studied with interest and are as follows.

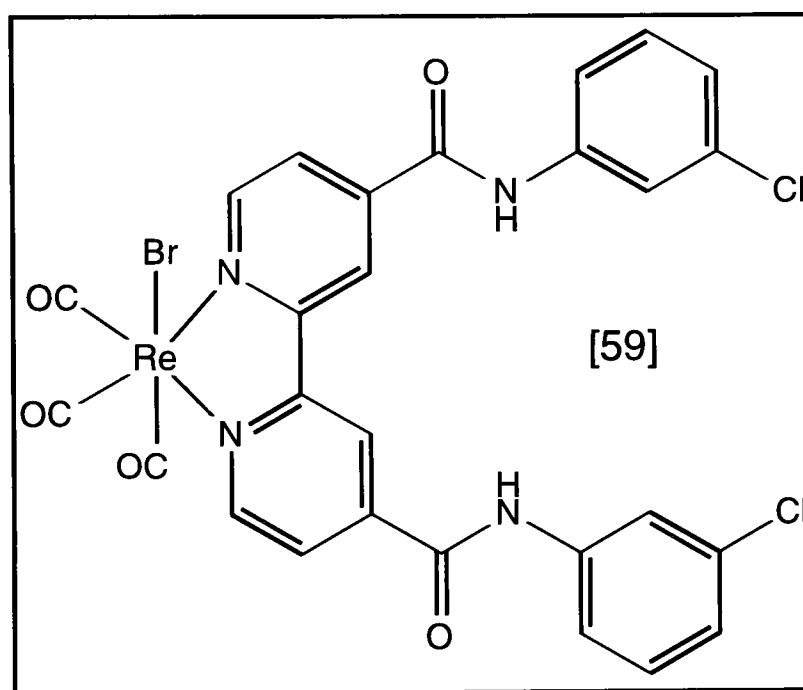


Figure 4.31: Rhenium based receptor.

The ^1H nmr titration with chloride yielded no results. In deuteriated DMSO (the only solvent in which the receptor would dissolve) negligible shifts were observed in the early part of the titration followed by collapse of the peaks which did not reappear. However, the addition of dihydrogen phosphate produced a smoother titration profile which is shown, for the 3H proton, in figure 4.32.

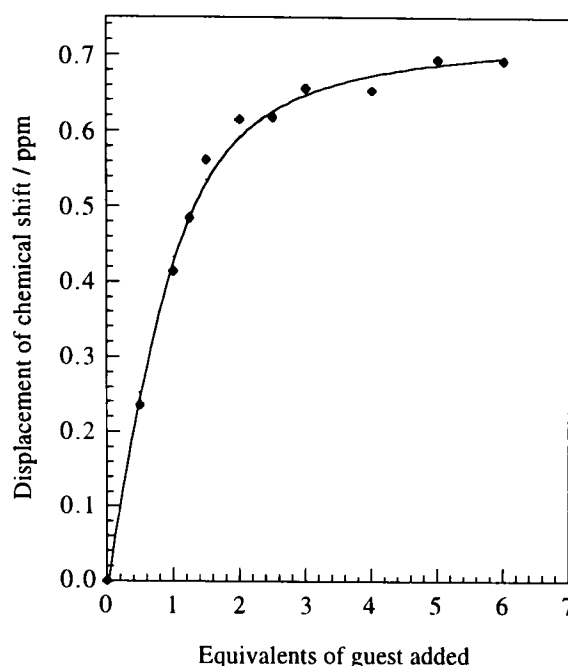


Figure 4.32: ^1H nmr titration profile for addition of dihydrogen phosphate to [59].

A calculated curve was fitted and the stability constant found to be $360 (\pm 60) \text{ dm}^3\text{mol}^{-1}$. This is a high value, given the solvent used, although not as high as the values displayed by the ruthenium receptors, which are estimated to be of the order of $10^3 \text{ dm}^3\text{mol}^{-1}$. However, it serves to demonstrate that the array of the acidic protons in the region of the bpy binding site alone is sufficient to attract an anionic guest. The contribution of the carbonyl ligands remains an unknown quantity and it would be interesting to examine the bpy ligand alone. Such a study, of course, is not possible due to the preferred anti orientation of the nitrogen groups in the absence of a metal acceptor, which locks the conformation of the ligand. This raises another advantage of these systems — the fact that they are preorganised to a large degree. Cram has commented at length on the advantage of forming a receptor which has very similar free and bound conformations,¹⁰ thereby avoiding the enthalpic and entropic price which must be paid as the receptor assumes a suitable arrangement for binding. There is very little freedom in the bpy receptors, and therefore the binding performance will benefit from a pre-formed site for the guest. The result also contrasts with examples in the previous chapter, where very little binding occurred, and underlines the fact that a successful neutral receptor has several binding interactions acting in concert and preferably includes a centre of low electron density.

Luminescence studies were also conducted in DMSO with excitation at 360 nm and monitoring of the emission at 570 – 700 nm. However, addition of dihydrogen phosphate

produced an entirely random response with the intensity varying by 40% of the initial value. The experiments therefore yielded no additional information.

4.10 In Summary

The performance of the ruthenium tris (2,2'-bipyridyl) receptors can be likened to a great philosopher with a severe stutter. It is clear that they perform their function with considerable efficiency but they suffer from an inability to communicate clearly information about their operation. In these respects, they are both better and worse than cobalticinium. The aim of producing a sensor was frustrated slightly by difficulties experienced with nmr, UV-vis spectroscopy and electrochemistry, the usual analytical methods. Of the three, nmr titrations allowed some stability constants to be determined and these are listed in table 4.1, but this was the only technique to produce data of a sufficiently high quality for a stability constant to be estimated with some confidence. In addition, it supplied useful information about the binding location. UV-vis spectroscopy did not permit any quantitative assessment to be made, rather a lower limit of the stability constant was all that could be determined. However, it did give clear information about the stoichiometry by virtue of sharp changes in the titration profiles. Electrochemical investigation, on the other hand, revealed rather small shifts in the redox waves of the ruthenium and substituted bipyridyl couples. Again, this technique provided information about the site of the binding interaction; the redox waves of the groups associated with binding shifted to a greater extent. Overall, in comparison with a cobalticinium receptor such as [35], it was found that the latter produced results which were more easy to interpret and data which were comparatively free of noise. This enabled quantitative conclusions to be reached more easily.

With regard to binding, there are two features of interest — strength and selectivity, and the $\text{RuL}(\text{bpy})_2^{2+}$ receptors displayed both. The binding of anions is the strongest by far in this thesis and is a great deal stronger than many examples in the literature. The reasons for this have been discussed and support the design criteria which were introduced in Chapter One. Positive charge, stability, multiple hydrogen bonding opportunities — all are present in this system and together are a potent force for anion recognition. Compared with cobalticinium, the stability constants appear to be over an order of magnitude higher.

In terms of selectivity, both systems are capable of displaying a binding preference. The ruthenium based receptors exhibit selective binding of dihydrogen phosphate over chloride, whereas the selectivity of cobalticinium receptors depends on the particular structure. This illustrates the fact that different receptors have different binding behaviour and also emphasises the potential for anion selectivity in both their designs.

RECEPTOR	ANION	SOLVENT (deuteriated)	INITIAL IONIC STRENGTH	STABILITY CONSTANT / $\text{dm}^3\text{mol}^{-1}$
[55]	chloride	DMSO	0.0384	260
	dihydrogen phosphate	""	""	(b)
[56]	chloride	""	""	300
	dihydrogen phosphate	""	""	(b)
[57]	chloride	""	""	400–500 (a)
	dihydrogen phosphate	""	""	750
[58]	chloride	""	""	50
	dihydrogen phosphate	""	""	1200

NOTE: (a) Errors > 30% due to noisy data. (b) Stability constant too large for calculation.

Table 4.1: ¹H nmr titration data.

For the receptors presented in this chapter, the sensitivity of emission was a saving grace. The preliminary studies conducted showed that the fluorophore was indeed very sensitive to the addition of a guest anion and, in the case of chloride, behaved in a regular fashion. The sensitivity was also evident in the experiments with dihydrogen phosphate but the regularity was somewhat lacking. The portents are good however and it is quite possible that an optical sensor for anions will be the result of the continuing work that other members of this group have undertaken. These were the first reported set of luminescent studies in the group and gave the opportunity to compare yet another analytical technique with the ones which were routinely applied in previous chapters. Luminescence titrations have the advantage of

producing marked and easily recorded responses to the anion, and moreover, require very small quantities of the receptor. The sensitivity is very good — no titration failed to produce a response of some variety and, in several cases, data suitable for quantitative analysis were collected. The method is therefore useful for sensing and quantifying interactions, although it offers no information on the location of binding.

The aim of this chapter was to assess the potential of the design and this has been successfully achieved. A second aim, namely to investigate the most suitable technique for studying binding was also met. This has been a goal throughout the thesis and more comment will be made in the Epilogue. It was hoped that the results could identify directions for future work, and they have highlighted emission properties as the area on which to concentrate. The collaboration with Prof. Balzani is therefore particularly exciting. It is also encouraging that this project is being pursued by a number of workers within the group and it is hoped that their efforts will shed light on some of the questions raised by the work presented herein.

CHAPTER Five

Epilogue

The mind of the molecular recognition chemist is preoccupied with three main problems: binding strength, binding selectivity and binding detection (assuming problem-free synthesis — a large assumption!). The work of this thesis has touched on all three issues and allows some conclusions to be drawn, which in turn highlights areas for future work. It is proposed to deal with them individually.

5.1 Binding Strength

By definition, a receptor must display an affinity with the guest it receives and therefore, the stability constant has to exceed a threshold value before the term “receptor” is justified. The receptor systems based on cobalticinium and $\text{RuL}(\text{bpy})_2^{2+}$ have both displayed acceptable levels of binding in polar, organic solvent. It is the author’s opinion that relatively little improvement is required before one can be content with the binding strength. Such development should be concentrated in the areas of $\text{RuL}(\text{bpy})_2^{2+}$ and poly-cobalticinium systems, for these are the receptors which have displayed very large stability constants already. A change of solvent is also required, in the direction of aqueous solution — this is a very common medium in biology and industry, and potential applications will frequently

require operation in water. While this was not possible three years ago, work within the group has progressed such that a move to water might now be attempted. The matter of solubility is addressed simply by changing the counter ion in both cases; it is already known that the chloride salts are soluble in aqueous solution and other anions may have the same effect. Here is another demonstration of the versatility of the design.

In terms of improving the binding strength, several points have come to light. In the case of cobalticinium, the inclusion of more than one positive charge is important, while maintaining the presence of hydrogen bonding groups. The formation of macrocycles would be very desirable, but is beyond the scope of current synthetic methods. Instead, reference to Chapter One highlights guanidinium as a very versatile system and one wonders what performance would emerge from a receptor that included both guanidinium and cobalticinium.

Ruthenium systems have great potential in terms of binding strength and, indeed, macrocycles have been prepared already and the results submitted for publication. Their binding is the strongest encountered in this work and it seems that improving binding is a less demanding goal for $\text{RuL}(\text{bpy})_2^{2+}$ in comparison with cobalticinium. Again, the inclusion of multiple hydrogen bonding sites is an obvious way to proceed.

Two further points need to be noted. Firstly, while it is important to assess binding performance in water, the receptor need not be ultimately soluble in this solvent. Sensors usually operate in the solid state, either attached to a solid support, or immobilised in a polymer matrix and, therefore, solubility is not a critical requirement. Secondly, it was stated that relatively little improvement in binding was needed and this reflects an important point. In terms of application, there is an optimum binding range, and larger stability constants are not always better. If one considers a receptor with an infinitely high stability constant, it is soon seen that such a molecule can never be regenerated as the free host, once exposed to a guest. This would make it useless as an ion transport agent and would mean that, as a sensor, it could only be used once. The path that leads to better and better binding is thus not always the one most wisely trod.

5.2 Binding Selectivity

The essence of a good receptor is in its selectivity for the chosen guest in the presence of competing species. Several such cases have been encountered within this work, although this aspect of binding has not been subject to systematic study. The two anions studied have been chloride and dihydrogen phosphate; both having been bound selectively. The former can be discouraged by insertion of an electron donor group close to the binding site — this, however, encourages the binding of dihydrogen phosphate which has electron acceptor groups and can therefore form multiple interactions. The donor-acceptor match seems to be the key to dihydrogen phosphate recognition. Geometric complementarity is also a useful tool which has not been harnessed in this work. However, future receptors might include larger assemblies designed to match the shape of the guest. With regard to featureless anions such as the halides, the size of the anion will be the means of providing discrimination. To this end, the construction of macrocycles is an obvious target.

There is much work to be done in tuning the recognition properties between host and guest and, with a clearer picture of the basic properties of the receptors, the goal of selective binding should be central to new research.

5.3 Binding Detection

A significant advantage of the type of receptor presented was the large range of methods by which the host-guest interaction could be studied. The previous three chapters have allowed a comparison of the various investigative techniques to be made, and some common themes emerge.

5.3i Nuclear Magnetic Resonance

Nuclear magnetic resonance remains the most useful general way of probing binding of the anion, and continues to be one of the first titrations executed. Of course, nmr could never provide the basis of a commercial sensor, but the information it yields is very important. As outlined in section 2.7i, the binding site, the stoichiometry and the stability constant can all be determined from a successful nmr titration, making it the most informative of all the

techniques. It is also very sensitive to the presence of a guest and, in the systems studied here, an absence of any change in the nmr spectrum was always indicative of negligible interaction.

The proton was the only nucleus probed, but with recent improvements in the nmr facility of the department, other nuclei could be harnessed for binding studies. ^{13}C may supplement the ^1H titrations, although it may be less sensitive. However, it is less likely to be subject to the broadening and distortions which often plagued ^1H experiments. For study of dihydrogen phosphate, ^{31}P nmr is potentially very useful. In particular, aqueous studies could proceed by titration of the receptor into a solution of the anion. It is found that the dihydrogen phosphate complex is often water-soluble and, therefore, this method might be a way of avoiding difficulties in dissolving the free receptor.

5.3ii Optical spectroscopy

Optical spectroscopy was employed with some success, although the level of response is not yet suitable for commercial application. Again, it was found to be a sensitive technique and had the advantage of lower noise levels than the nmr experiments. Furthermore, the concentration of receptor required was over an order of magnitude lower. Although this method did not supply any information about where the anion was bound, it was often able to comment on the stoichiometry and stability constant. The Specfit package was found to be very powerful and, with careful use, yielded much information. However, the temptation to “fiddle” with the model in attempts to gain a good fit of the data was strong — but must be avoided! Given enough degrees of freedom, the program can give a good fit to almost any set of experimental data, but this is no substitute for common sense about the nature of the binding process. The number of coloured components found by factor analysis should be carefully inspected and an appropriate model formed with this in mind. As with all methods, the residuals and the calculated profiles need to be examined and compared with known data. Particularly in the case of cobalticinium, it is felt that UV-vis titrations should be a central part of binding investigations; in particular, ways of increasing the degree of response and the linearity of the titration curve at sub-stoichiometric levels are of great interest. The improvement of commercial sensing properties lies in this direction.

Luminescence titrations were also available with $\text{RuL}(\text{bpy})_2^{2+}$ receptors and were the most sensitive and informative method for such systems. Many of the comments made above

apply to this method, especially regarding Specfit, which has been modified by its authors to enable emission data to be studied. As mentioned in Chapter Four, this is the most promising avenue for sensing binding by such systems.

In the light of results presented here, the need for a background electrolyte is strong, thereby allowing comparison of the results from different methods. At the outset, it will be advantageous to set a standard ionic strength and maintain this in all experiments. This was a fact that emerged at a fairly advanced stage of this thesis, and experiments showed that addition of an electrolyte gave much better correlation between the different titrations. Care should be taken, however, to ensure that levels of electrolyte are not so high that quenching becomes a problem in emission titrations.

5.3iii Electrochemistry

All of the systems were known to be redox active and many displayed a shift in the half-wave potential on addition of the anion. This is notable in itself in that very few redox-active anion sensors exist. However, in comparison with the other methods employed, it was the least informative in that neither binding site, stoichiometry nor stability constant emerged from the studies in any experiment. The obvious question is therefore “why persist?” In answer, electrochemistry is a very simple and cheap way of basing a sensor design. For these purposes, a linear and reproducible response is all that is required, and this does not seem beyond the realms of possibility. Therefore, means of improving communication between the binding site and the redox centre should be pursued. One way springs to mind quickly. Consider the synthetic disconnection of the receptor into acid and amine. Currently, the redox centre is always located in the acid — a reversal of this approach would place the redox centre in the amine portion and would bring the binding site closer. It might also alleviate many of the synthetic problems encountered with cobalticinium.

5.4 In Summary

This chapter has not been a summary of summaries; nor was it intended to be. Instead, it has indicated a few directions for future research based on the experience of the last three years. This has been the first thesis within the group to focus on comparisons of the methods of detecting binding and one message clearly emerges. The binding of anions is not difficult to

achieve with the systems currently employed. Instead the challenge will be to find the best way of imparting selectivity to the receptors and, furthermore, in deciding which of the sensing properties to harness and develop with the aim of faithfully detecting levels of the anion analyte. Herein lies enough exciting work to fill a number of future theses.

CHAPTER Six

Experimental

6.1 Instrumental

Elemental analyses were performed by the microanalytical service of the Inorganic Chemistry Laboratory at the University of Oxford.

Nuclear resonance spectra were measured using a Brüker AM300 spectrometer at 300 MHz for ^1H and 75.5 MHz for ^{13}C . Tetramethylsilane was used as an internal reference.

Infra-red spectra were measured using a Mattson "Polaris" FT spectrometer model IR 10410E. Spectra were recorded from 4000 - 600 cm^{-1} from samples prepared as a KBr disk.

Cyclic voltammetric studies were performed on a Princeton Applied Research potentiostat/galvanostat model 273. Reference electrode and acquisition parameters are quoted in individual cases.

Mass spectrometry was undertaken by the EPSRC mass spectrometry service of University College, Swansea.

6.2 Solvent and Reagent Pre-treatment

When necessary, solvents were distilled from a suitable desiccant and stored under nitrogen. DMF was dried over phosphorous pentoxide and distilled under reduced pressure in the absence of light. It was subsequently stored under nitrogen in the dark. THF was distilled under nitrogen from sodium using benzophenone as an indicator; acetonitrile and dichloromethane were distilled under nitrogen from calcium hydride. Triethylamine was dried over potassium hydroxide and distilled under nitrogen. It was stored over potassium hydroxide pellets. Thionyl chloride was treated with triphenyl phosphite and distilled under nitrogen.

Unless stated to the contrary, commercial grade reagents were used without further purification.

6.3 Syntheses

*Mono- and bis(methyl) cobalticinium hexafluorophosphate*¹³⁷

Dicyclopentadiene was cracked to cyclopentadiene monomer by heating to reflux for 2 h (180–200°C). A 40 cm Vigreux column was applied and the monomer was collected as a colourless liquid at 0°C. Methylcyclopentadiene monomer was obtained from methylcyclopentadiene dimer by an analogous method.

Pyrrolidine (350 ml, previously dried over magnesium sulphate for 48 h and distilled under nitrogen) was cooled to 0°C; methylcyclopentadiene (60.7 g, 0.75 mol) and cyclopentadiene (41.6 g, 0.63 mol) were added, and the mixture stirred vigorously under nitrogen. Cobalt dibromide (60.4 g, 0.28 mol) was added in small portions over a period of 1 h during which time the solution assumed a deep purple colour. The temperature of the mixture was maintained below 5°C during addition. When addition was complete, the mixture was

allowed to attain room temperature and stirring was continued for 18 h. After this time, the solvent was removed by distillation under reduced pressure and the residue was taken up in 1.75 l hot water (60–70°C) and filtered to give a green solid and a golden orange solution. The aqueous solution was allowed to cool and extracted with diethyl ether (4 x 200 ml portions) to remove excess cyclopentadienes. The aqueous portion was then refluxed briefly (30 min) with activated charcoal. During the initial period of reflux, dissolved ether and pyrrolidine were removed by distillation. The solution was filtered while hot and then allowed to cool, giving a deep yellow solution. An aqueous solution of ammonium hexafluorophosphate (≈ 30 g in 90 ml water) was added dropwise to give a curdy yellow precipitate which was collected and dried. The volume of solvent was reduced and a second crop recovered to give an overall yield of 22.5 g (mixture of products). Further purification and analysis was not attempted until after oxidation.

*1,1'-Bis(methyl) cobalticinium hexafluorophosphate*¹⁵⁵

Methylcyclopentadiene monomer was prepared according to the method in the previous section. Dry THF (150 ml) was cooled to 0°C and stirred under nitrogen. Methylcyclopentadiene (23.0 g, 0.29 mol) was added and to the stirred mixture was added sodium hydride (7.1 g, 0.24 mol) in small portions to give a red, cloudy solution. During addition, the temperature was maintained below 5°C. When addition was complete, the mixture was stirred at 5°C for 4 h then heated to 30–35°C for a further hour. The mixture was then cooled once more to 5°C and cobalt dichloride (10.7 g, 0.082 mol) was added in small portions with stirring. The resultant deep brown solution was allowed to attain room temperature and then stirred for 30 h under nitrogen. After this time, ≈ 20 ml methanol was cautiously added to destroy unreacted sodium hydride and the solvent removed by rotary evaporation. The residue was dissolved in 1 l hot water (60–70 °C), filtered and allowed to cool then extracted with diethyl ether (5 x 100 ml). The aqueous solution was clarified by refluxing briefly with activated charcoal and then filtered while still hot. The solution was allowed to cool and aqueous ammonium hexafluorophosphate was added to precipitate the

product as a dirty yellow solid. This was recrystallised from methanol to give a golden yellow flaky solid (yield: 5.86 g, 19.7%).

Elemental analysis: found C, 39.7; H, 3.85. $C_{12}H_{14}PF_6Co$ requires C, 39.8; H, 3.90%.

1H nmr (d_6 -DMSO): δ 5.62 (4H, s, CpH); 5.60 (4H, s, CpH); 2.05 (6H, s, CH_3).

^{13}C nmr (d_6 -DMSO): δ 103.3 ($\underline{C}$ - CH_3); 84.6 (Cp); 83.6 (Cp); 12.7 (CH_3).

Preparation of mono(carboxy) cobalticinium hexafluorophosphate: oxidation of methyl analogues

A mixture of cobalticinium, (mono)methyl cobalticinium and 1,1'-bis(methyl) cobalticinium from (prepared in an earlier section) was suspended in water (400 ml). Potassium permanganate (32.3 g, 0.204 mol) and sodium hydroxide (4.8 g, 0.12 mol) were added and the mixture stirred at a temperature of 95°C for 3 h. Towards the end of this time, a small quantity of activated charcoal was added to clarify the solution. At the end of this time, the mixture was filtered while hot to give a golden orange solution. As the solution cooled, cobalticinium was seen to precipitate and was removed by filtration. 6 M hydrochloric acid was then added dropwise to precipitate a mixture of mono and bis carboxylic acids of cobalticinium. This mixture was then isolated and refluxed in 1.5 l acetone during which time the mono acid dissolved. Bis acid was isolated by filtration and mono acid was isolated by rotary evaporation of the filtrate. Both appeared as fine yellow powders (yields: bis acid 1.46 g, mono acid 9.42 g; overall yield: 11.7%).

1,1'-Bis(carboxy) cobalticinium hexafluorophosphate

Elemental analysis: found C, 41.2; H, 2.66. $C_{12}H_{10}O_4CoPF_6$ requires C, 34.1; H, 2.39%.

1H nmr (D_2O): δ 5.99 (4H, m, CpH); 5.73 (4H, m, CpH).

IR ν_{max}/cm^{-1} : 3133, s, C-H str; 2790, s, O-H str; 1745, s, C=O str; 799, m, PF_6^- .

Mono(carboxy) cobalticinium

Elemental analysis: found C, 35.1; H, 2.66. $C_{11}H_{10}O_2CoPF_6$ requires C, 34.9; H, 2.67%.

1H nmr (CD_3CN): δ 6.10 (2H, m, CpH); 5.80 (2H, m, CpH); 5.76 (5H, s, CpH).

IR ν_{max}/cm^{-1} : 3123, s, C-H str; 2850, s, O-H str; 1712, s, C=O str; 812, s, PF_6^- .

Mono(chlorocarbonyl) cobalticinium hexafluorophosphate / chloride

Mono(carboxy) cobalticinium hexafluorophosphate (0.4 g, 1.06 mmol) was suspended in thionyl chloride (50 ml) and the mixture brought to reflux under nitrogen for 48 h. After this time, the excess thionyl chloride was removed by distillation and the residue dried in vacuo. In normal circumstances, no attempt was made to isolate the acid chloride and the pale green residue was used without delay in subsequent acylation reactions.

Receptor [34]

Mono(carboxy)cobalticinium (0.3 g, 0.78 mmol) and 4,4'-methylenedianiline (0.057 g, 0.29 mmol) were dissolved in acetonitrile (20 ml) and the solution stirred under nitrogen. DCCI (0.064 g, 0.31 mmol) was added and a white precipitate was seen to form. The mixture was stirred for 18 h after which time it was filtered and the solvent removed. The residue was washed with a small quantity of dichloromethane and purified by column chromatography (Sephadex, eluent 60:40 MeCN:MeOH). The product was dissolved in a minimum quantity of acetonitrile and then precipitated by dropwise addition of water as an orange powder (yield: 220 mg, 50%).

Elemental analysis: found C, 51.6; H, 3.99; N, 4.99. $C_{24}H_{22}N_2OCoPF_6$ requires C, 51.6; H, 3.97; N, 5.02%

1H nmr (CD_3CN): δ 8.75 (1H, s(br), CONH); 7.60 (2H, d($J=8.5$ Hz), ArH); 7.22 (2H, d($J=8.6$ Hz), ArH); 6.94 (2H, d($J=8.2$ Hz), ArH); 6.57 (2H, d($J=8.5$ Hz), ArH); 6.19 (2H, m, CpH); 5.78 (2H, m, CpH); 5.73 (5H, s, CpH); 4.01 (2H, s(br), NH_2); 3.62 (2H, s, CH_2).

^{13}C nmr (CD_3CN): δ 160.5 (C=O); 147.9 (C- NH_2); 140.6 (CONH-C); 136.5 (CH_2 -C); 130.9 (CH_2 -C); 130.5 (Ar- NH_2); 129.9 (Ar- NH_2); 121.7 (CONH-Ar); 116.3 (CONH-Ar); 96.2 (Cp); 87.1 (Cp); 86.1 (Cp); 85.0 (Cp); 40.9 (Ar-CH $_2$ -Ar).

IR ν_{max}/cm^{-1} : 3414, m, N-H str (i); 3351, m, N-H str (ii); 3121, m, C-H str; 1677, s, C=O str (amide I); 1515, s, C=O str (amide II); 1414, m, C-C str; 839, s, PF_6^- .

FAB MS: m/z 413 ($M-PF_6$) $^+$.

Receptor [35]

Mono(carboxy) cobalticinium (0.4 g, 1.06 mmol) and 1,2-phenylene diamine (0.12 g, 1.11 mmol) were dissolved in dry acetonitrile (30 ml) under nitrogen with stirring. DCCI (0.24 g, 1.16 mmol) was added to the solution which assumed an orange colour. A white precipitate was also seen to form. The mixture was stirred for 18 h then filtered and the filtrate evaporated to dryness. Preliminary purification of the residue was carried out by column chromatography (Sephadex: eluent 60:40 MeOH:MeCN). The compound was then taken up in boiling methanol and $\approx 5\%$ water was added. On standing, the product formed as large yellow crystals, suitable for X ray analysis (yield: 0.2 g, 40%).

Elemental analysis: found C, 43.7; H, 3.37; N, 5.79. $C_{17}H_{16}N_2OCoPF_6$ requires C, 43.6; H, 3.44; N, 5.98%.

1H nmr (CD_3CN): δ 8.70 (1H, s(br), CONH); 7.22 (1H, d, ArH); 7.12 (1H, t, ArH); 6.87 (1H, d, ArH); 6.77 (1H, t, ArH); 6.22 (2H, m, CpH); 5.81 (2H, m, CpH); 5.77 (5H, s, CpH); 4.30 (2H, s(br), NH_2).

IR ν_{max}/cm^{-1} : 3426, m, N-H str (i); 3354, m, N-H str (ii); 3127, m, C-H str; 1657, m, C=O str (amide I); 1537, C=O str (amide II); 1417, m, C-C str; 834, s, PF_6^- .

FAB MS: m/z 323 ($M-PF_6^-$)

Receptor [36]

Mono(carboxy) cobalticinium (0.40 g, 1.06 mmol) and 1,4-phenylenediamine (0.12 g, 1.11 mmol) were dissolved in acetonitrile (30 ml) under nitrogen with stirring. DCCI (0.24 g, 1.16 mmol) was added to the stirred solution which immediately assumed a deep red colour. A

precipitate was also seen to form. The mixture was stirred for 24 h after which time it was filtered and the solvent removed by rotary evaporation. The residue was washed with $\approx$ 15 ml dichloromethane and then purified by column chromatography (Sephadex: eluent MeCN) to give the product as a brick red powder (yield: 310 mg, 63%).

Elemental analysis: found C, 43.6; H, 4.00; N, 6.94. $C_{17}H_{16}N_2OCoPF_6$. 0.6 DMF requires C, 44.1; H, 3.98; N, 7.11% [Ratio of product:DMF estimated from 1H nmr integration].

1H nmr (CD_3CN): δ 8.60 (1H, s(br), CONH); 7.39 (2H, d($J=8.7$ Hz), ArH); 6.66 (2H, d($J=8.7$ Hz), ArH); 6.17 (2H, m, CpH); 5.77 (2H, m, CpH); 5.73 (5H, s, CpH); 4.20 (2H, s(br), NH_2) [7.90 (s); 2.90 (s); 2.78 (s) - DMF].

^{13}C nmr (CD_3CN): δ 160.0 (C=O); 146.6 (C- NH_2); 128.7 (CONHC); 123.5 (Ar); 115.2 (Ar); 96.6 (Cp); 87.1 (Cp); 86.6 (Cp); 84.6 (Cp) [32, 36, 162 - DMF].

IR ν_{max}/cm^{-1} : 3423, m, N-H str; 3122, m, C-H str; 1668, m, C=O str (amide I); 1543, m, C=O str (amide II); 834, s, PF_6^- .

FAB MS: m/z 469 (M) $^+$, 323 (M- PF_6) $^+$.

Receptor [37]

Mono(carboxy) cobalticinium (0.4 g, 1.06 mmol) and 1,3-phenylene diamine (0.12 g, 1.11 mmol) were dissolved in acetonitrile (30 ml) to give a yellow solution. DCCI (0.24 g, 1.16 mmol) was added; the solution assumed an orange colour and a heavy, white precipitate formed. The mixture was stirred for 48 h and then was filtered. The filtrate was evaporated to dryness under reduced pressure and the residue was purified by column chromatography (Sephadex: eluent 60:40 MeCN:MeOH). Purification was carried out under nitrogen to

prevent aerial decomposition of residual starting amine. The product was isolated as a yellow powder (yield: 0.22 g, 45%).

Elemental analysis: found C, 43.4; H, 3.44; N, 5.79. $C_{17}H_{16}N_2OCoPF_6$ requires C, 43.6; H, 3.44; N, 5.98%.

1H nmr (CD_3CN): δ 8.57 (1H, s(br), CONH); 7.13 - 7.07 (2H, m, ArH); 6.90 (1H, m, ArH); 6.50 (1H, m, ArH); 6.19 (2H, m, CpH); 5.78 (2H, m, CpH); 5.73 (5H, s, CpH); 4.28 (2H, s(br), NH_2).

^{13}C nmr (CD_3CN): δ 172.0 (C=O); 149.6 (C-N); 112.2 ($\underline{C}$ -C-N); 110.4 ($\underline{C}$ -C-N); 107.4 ($\underline{C}$ -C-N); 87.1 (Cp); 86.7 (Cp); 85.1 (Cp) [two quaternary resonances remained unresolved].

IR ν_{max}/cm^{-1} : 3401, m, N-H str (i); 3250, m, N-H str (ii); 3121, m, C-H str; 1668, m, C=O str (amide I); 1415, m, C-C str; 840, s, PF_6^- .

FAB MS: m/z 323 ($M-PF_6$) $^+$.

Receptor [38]

Mono(carboxy) cobalticinium (0.50 g, 1.30 mmol) was dissolved in acetonitrile (40 ml). The mixture was stirred under nitrogen and dicyclohexyl carbodiimide (DCCI) (0.14 g, 0.65 mmol) was added and the mixture stirred for 30 min. A heavy white precipitate was seen to form (dicyclohexyl urea) and was removed by filtration. The filtrate was then added dropwise under nitrogen to a stirred solution of 2,6-diamino pyridine (0.22 g, 2.0 mmol) in acetonitrile. The mixture was stirred for 4 h during which time, it assumed an orange colour. The solvent was then removed by rotary evaporation and the residue washed first with water and then a small quantity of dichloromethane. The residue was then purified by column chromatography

(Sephadex, eluent 60:40 MeCN:MeOH) to give the product as a green - yellow powder (yield: 100 mg, 33%).

Elemental analysis: found C, 41.8; H, 8.89; N, 3.31. $C_{16}H_{15}N_3OCo PF_6$ requires C, 41.0; H, 8.96; N, 3.22%.

1H nmr (CD_3CN): δ 8.76 (1H, s(br), CONH); 7.51 (2H, m, ArH); 6.43 (1H, d of d, ArH); 6.22 (2H, m, CpH); 5.78 (2H, m, CpH); 5.74 (5H, s, CpH); 4.90 (2H, s(br), NH_2).

^{13}C nmr (CD_3CN): δ 165.3 (C=O); 163.7 (CONH- $\underline{C}$ -N); 155.0 (N- $\underline{C}$ - NH_2); 110.2, 107.9 (N- $\underline{C}$ -CH); 100.1 ($\underline{C}$ -C=O); 92.2 (Cp); 91.0 (Cp); 89.7 (Cp).

IR ν_{max}/cm^{-1} : 3437, m, N-H str (i); 3355, m, N-H str (ii); 3117, m, C-H str; 1680, s, C=O str (amide I); 1540, m, C=O str (amide II); 834, s, PF_6^- .

FAB MS: m/z 324 ($M-PF_6$) $^+$.

Receptor [39]

2,6-diamino pyridine (0.055 g, 0.5 mmol) and triethylamine (0.1 g, 1.0 mmol) were dissolved in dry acetonitrile (30 ml) and added dropwise under nitrogen to a solution of acid chloride (1.06 mmol) in acetonitrile with stirring. The mixture was stirred at room temperature for 18 hours during which time, a green precipitate appeared over a dirty yellow solution. The precipitate was removed by filtration and washed with acetonitrile. It was then dissolved in a small quantity of water and aqueous ammonium hexafluorophosphate was added dropwise producing an olive green precipitate which was washed with water to give product (yield: 0.27 g, 31%).

Elemental analysis: found C, 39.0; H, 3.13; N, 4.54. $C_{27}H_{23}N_3O_2Co_2 (PF_6)_2$ requires C, 39.1; H, 2.80; N, 5.07%.

1H nmr (CD_3CN): δ 9.12 (2H, s (br), $NHCO$); 8.05 - 7.95 (3H, m, ArH); 6.24 (4H, m, CpH); 5.62 (4H, m, CpH); 5.77 (10H, s, CpH).

^{13}C nmr (CD_3CN): δ 161.0 (C=O); 150.1 (CONH-C-N); 142.0 (N-C-CH-CH); 112.2 (N-C-CH); 95.8 (C-C=O); 87.1 (Cp); 86.9 (Cp); 85.0 (Cp).

IR ν_{max}/cm^{-1} : 3637, m, N-H str (i); 3403, m, N-H str (ii); 3127, s, C-H str; 1683, s, C=O str (amide I); 1529, s, C=O str (amide II); 836 PF_6^- .

FAB MS: m/z 684 ($M-PF_6$) $^+$, 539 ($M-2PF_6$) $^+$.

Receptor [40]

N-hydroxysuccinimide (0.18 g, 1.5 mmol) was powdered, dried *in vacuo* and dissolved in dry acetonitrile (30 ml). To this was added under nitrogen mono(carboxy) cobalticinium hexafluorophosphate (0.57 g, 1.5 mmol) and the mixture was stirred. DCCI (0.33 g, 1.6 mmol) was added and a heavy white precipitate was observed to form. This was removed by filtration; the filtrate was stirred under nitrogen and a solution of tris(2-aminoethyl) amine (0.07 g, 0.5 mmol) and triethylamine (0.15 g, 1.5 mmol) in acetonitrile was added dropwise. The mixture was stirred for 18 h after which time it was evaporated to dryness and purified by column chromatography (Sephadex: eluent 50:50 MeCN:MeOH) to give the product as a yellow powder (yield: 340 mg, 56%).

Elemental analysis: found C, 37.9; H, 3.45; N, 4.42. $C_{39}H_{42}N_4O_3Co_3 P_3F_{18}$ requires C, 38.2; H, 3.45; N, 4.57%.

1H nmr (CD_3CN): δ 7.50 (3H, s(br), CONH); 6.03 (6H, m, CpH); 5.72 (6H, m, CpH); 5.68 (15H, s, CpH); 3.42 (6H, m, NCH_2CH_2); 2.73 (6H, t, NCH_2).

^{13}C nmr (CD_3CN): δ 162.7 (C=O); 95.2 (Cp); 87.1 (Cp); 86.6 (Cp); 84.6 (Cp); 54.6 (CH_2); 39.1 (CH_2).

IR $\nu_{\text{max}}/\text{cm}^{-1}$: 3429, m, N-H str; 3126, m, C-H str; 1657, s, C=O str (amide I); 1551, m, C=O str (amide II); 834, s, PF_6^- .

FAB MS: m/z 1081 (M-PF_6) $^+$, 936 (M-2PF_6) $^+$, 791 (M-3PF_6) $^+$.

Receptor [41]

1,1'-Bis(chlorocarbonyl) cobalticinium (0.08 g, 0.19 mmol) was dissolved in acetonitrile (30 ml) and stirred under nitrogen. To this solution was added a solution of [34] (0.2 g, 0.36 mmol) and triethylamine (0.04 g, 0.4 mmol) in acetonitrile. The mixture was stirred for 40 h during which time a dark green precipitate appeared. This was removed by filtration, taken up in water and the product precipitated as a yellow powder by dropwise addition of aqueous ammonium hexafluorophosphate (yield: 80 mg, 28%).

Elemental analysis: found C, 47.4; H, 3.44; N, 3.45. $\text{C}_{60}\text{H}_{50}\text{N}_4\text{O}_4\text{Co}_3 (\text{PF}_6)_3$ requires C, 48.0; H, 3.35; N, 3.73%.

^1H nmr (CD_3CN): δ 9.26 (2H, s (br), CONH); 8.88 (2H, s (br), CONH'); 7.64 (4H, d ($J=9.3$), ArH); 7.58 (4H, d ($J=9.6$), ArH); 7.25 (4H, d ($J=9.4$), ArH); 7.18 (4H, d ($J=9.3$), ArH); 6.24 (4H, m, CpH); 6.20 (4H, m, CpH); 5.86 (4H, m, CpH); 5.78 (4H, m, CpH); 5.72 (10H, s, CpH); 3.94 (2H, s, CH_2).

^{13}C nmr (CD_3CN): δ 160.7 (C=O); 160.1 (C=O); 139.4 (CONHC); 136.6 (CONHC); 130.1 (ArC-H) (two very close resonances); 121.7 (ArC-H) (two very close resonances); 97.1 (CC=O); 96.0 (CC=O); 87.7 (CpC-H); 87.1 (CpC-H); 86.8 (CpC-H); 86.6 (CpC-H); 85.1 (CpC-H); 41.2 (CH_2).

IR $\nu_{\max}/\text{cm}^{-1}$: 3402, m, N-H str; 3120, m, C-H str; 1674, m, C=O str (amide I); 1601, m, C=O str (amide II); 1413, m, C-C str; 840, s, PF_6^- .

FAB MS: m/z 1357 (M-PF_6)⁺, 1212 (M-2PF_6)⁺, 1067 (M-3PF_6)⁺.

Receptor [42]

[36] (280 mg, 0.6 mmol) was powdered and dried in vacuo. It was then taken up in acetonitrile (30 ml) to give a deep red solution which was stirred under nitrogen. A solution of 1,1'-bis(chlorocarbonyl) cobalticinium in acetonitrile was added dropwise and the mixture stirred for 18 h during which time a pale brown precipitate appeared. This was removed by filtration and washed with acetonitrile. It was then taken up in water and the product precipitated by dropwise addition of aqueous ammonium hexafluorophosphate as an orange-brown powder (yield: 165 mg, 21%).

Elemental analysis: found C, 41.0; H, 2.91; N, 4.11. $\text{C}_{46}\text{H}_{38}\text{N}_4\text{O}_4\text{Co}_3 (\text{PF}_6)_3$ requires C, 41.8; H, 2.90; N, 4.11%.

^1H nmr (CD_3CN): δ 9.16 (2H, s(br), CONH); 8.87 (2H, s(br), CONH'); 7.71 (8H, s, ArH); 6.25 (4H, m, CpH); 6.19 (4H, m, CpH); 5.92 (4H, m, CpH); 5.79 (4H, m, CpH); 5.75 (10H, s, CpH).

^{13}C nmr (CD_3CN): δ 160.7 (C=O); 160.2 (C=O); 135.6 (N-C); 122.1 (N-C-C); 121.9 (N-C-C); [96.9, 95.8, 87.6, 87.2, 86.9, 86.6] (Cp).

IR $\nu_{\max}/\text{cm}^{-1}$: 3404, m, N-H str; 3126, m, C-H str; 1666, m, C=O str (amide I); 1573, m, C=O str (amide II); 839, s, PF_6^- .

FAB MS: m/z 1177 (M-PF_6)⁺, 1032 (M-2PF_6)⁺, 887 (M-3PF_6)⁺.

Receptors [43] and [44]

Receptor [49] (0.2 g, 0.94 mmol) was powdered and dried *in vacuo* and then dissolved in acetonitrile (20 ml). This solution was added dropwise under nitrogen to a solution of 1,1'-bis(chlorocarbonyl) cobalticinium (0.21 g, 0.5 mmol) in acetonitrile with stirring. The solution was stirred for 24 hours during which time, a green precipitate appeared over a yellow solution. This was removed by filtration, taken up into water and the resultant yellow solution was filtered to give a yellow filtrate and a small quantity of orange solid. Aqueous ammonium hexafluorophosphate was added dropwise to the solution and a bright yellow curdy precipitate formed. This was isolated and washed with water to give product [44] (yield: 0.14 g, 47%). The orange solid was washed with water and isolated as [43] (yield: 0.03 g, 7%).

Receptor [44]

Elemental analysis: found C, 51.4; H, 3.54; N, 7.90. $C_{24}H_{19}N_3O_4CoPF_6$ requires C, 46.7; H, 3.10; N, 6.81%

1H nmr (d_6 -DMSO): δ 11.4 (1H, s(br), CONH); 10.5 (1H, s(br), CONH'); 8.01 - 7.51 (8H, m, ArH); 6.40 (2H, m, CpH); 6.09 (2H, m, CpH); 6.00 (2H, m, CpH); 5.93 (2H, m, CpH).

^{13}C nmr (d_6 -DMSO): δ 166.2 (CpC=O); 164.4 (CpC'=O); 160.8 (ArC=O); 150.8 (N-C); 150.0 (N-C); 140.5 (ArC-H); 134.3 (pyC-H); 132.3 (C=OArC); 128.8 (ArC-H); 128.0 (ArC-H); 111.2 (pyC-H); 110.9 (pyC-H); 95.6 (C-CO₂H); 94.4 (C-CONH); 87.0 (Cp); 86.7 (Cp); 86.6 (Cp); 85.7 (Cp).

IR ν_{max}/cm^{-1} : 3396, m, N-H str; 3311, m, O-H str; 3112, s, C-H str; 1679, s, C=O str (amide I); 1585, s, C=O str (amide II), 844, m, PF_6^- .

FAB MS: m/z 472 ($M-PF_6$)⁺.

Receptor [43]

Elemental analysis: found C, 54.6; H, 4.45; N, 10.4. $C_{36}H_{28}N_6O_4CoPF_6$ requires C, 53.2; H, 3.47; N, 10.3%.

1H nmr (d_6 -DMSO): δ 9.76 (2H, s (br), CONH); 7.43 - 6.90 (16H, m, pyH/ArH); 5.95 (4H, m, CpH); 5.46 (4H, m, CpH).

IR ν_{max}/cm^{-1} : 3400, m, N-H str; 3105, m, C-H str; 1680, m, C=O str (amide I); 1534, m, C=O str (amide II); 795, m, PF_6^- .

FAB MS: m/z 667 ($M-PF_6$) $^+$.

Receptor [45]

Mono(carboxy)cobalticinium (0.30 g, 0.8 mmol) and N-hydroxy succinimide (0.09 g, 0.8 mmol) were dissolved in acetonitrile (30 ml) and stirred under nitrogen. DCCI (0.19 g, 0.9 mmol) was added; a white precipitate was seen to form. The mixture was stirred for 2 h after which time, the precipitate was removed by filtration. A solution of aminoethyl ferrocene (0.17 g, 0.8 mmol, supplied by Mr. D. K. Smith) in acetonitrile was added dropwise under nitrogen. The solution assumed an orange colour and was stirred for 24h. After this time a deep brown solution resulted which was filtered then evaporated to dryness. The residue was purified by column chromatography (Sephadex: eluent 50:50 MeOH:MeCN) to give the product as a dark brown solid (yield: 270 mg, 59%).

Elemental analysis: found C, 45.1; H, 3.87; N, 2.83. $C_{22}H_{21}NOCofePF_6$ requires C, 45.9; H, 3.68; N, 2.44 %.

^1H nmr (CDCl_3): δ 7.39 (1H, s (br), CONH); 6.04 (2H, m, CpH); 5.71 (2H, m, CpH); 5.60 (5H, s, CpH); 4.26 (2H, m, CpH'); 4.23 (2H, s, CH_2); 4.19 (5H, s, CpH'); 4.14 (2H, m, CpH').

^{13}C nmr (CD_3CN): δ 161.9 (C=O); 87.6, 87.0, 86.7, 86.3, 85.4, 84.8 (Cp); 69.4, 69.0 (Fc-Cp); 40.0 (CH_2).

IR $\nu_{\text{max}}/\text{cm}^{-1}$: 3431, m, N-H str; 3126, m, C-H str; 1666, s, C=O str (amide I); 1537, m, C=O str (amide II); 836, s, PF_6^- .

FAB MS: m/z 575 (M) $^+$ [weak], 430 (M- PF_6) $^+$.

Receptor [48]

Oxalyl chloride (5 ml) was dissolved in dichloromethane (10 ml) and added dropwise under nitrogen to a solution of mono(carboxy) ferrocene (0.25 g, 1.09 mmol) dissolved in dichloromethane (10 ml) with stirring. The mixture was stirred for 18 h and then taken to reflux for 90 min. The solution was allowed to cool to room temperature, the solvent removed by rotary evaporation and the residue dried *in vacuo*. It was then transferred to a Soxhlet extraction apparatus and continuously extracted with hexane under nitrogen for 2 h. The orange solution was evaporated to dryness under reduced pressure and the residue dried *in vacuo* to give mono(chlorocarbonyl) ferrocene as a red crystalline solid. This was not isolated but immediately used in the subsequent acylation reaction.

Mono(chlorocarbonyl) ferrocene was taken up in acetonitrile and added dropwise under nitrogen to a stirred solution of 2,6-diaminopyridine (0.11 g, 1.0 mmol), triethylamine (0.1 g, 1.0 mmol) and a small quantity of DMAP in acetonitrile. The mixture was stirred for 18 h at room temperature and then taken to reflux for a further 2 h. The resultant red/brown solution was filtered and the filtrate purified by column chromatography with silica gel. The initial

eluent was 60:40 hexane:CHCl₃; the polarity was steadily increased as successive fractions were eluted, the final eluent being 5:95 MeOH:CHCl₃. The product containing fractions were combined and the solvent removed. The product was recrystallised from MeOH:hexane to give a deep red crystalline solid (yield 190 mg, 60%).

Elemental analysis: found C, 59.6; H, 4.63; N, 12.4. C₁₆H₁₅N₃OFe requires C, 59.8; H, 4.71; N, 13.1%.

¹H nmr (CD₃CN): δ 7.7 (1H, s(br), CONH); 7.65 (1H, d(J=7.6), ArH); 7.48 (1H, dd, ArH); 6.26 (1H, d(J=7.8), ArH); 4.80 (2H, m, CpH); 4.31 (2H, m, CpH); 4.33 (2H, s(br), NH₂); 4.26 (5H, s, CpH).

¹³C nmr (CD₃CN): δ 169 (C=O); 157 (C-N); 150 (C-N); 140 (N-C-C-C); 104 (N-C-C); 103 (N-C-C); 76 (Cp); 71 (Cp); 70 (Cp); 69 (Cp).

IR ν_{max}/cm⁻¹: 3455, m, N-H str (i); 3417, m, N-H str (ii); 3336, m, N-H str (iii); 1650, s, C=O str (amide I); 1616, s, C=O (amide II).

EI MS: m/z 321 (M)⁺, 256 (M-Cp)⁺, 229 (FeCp₂CONH₂)⁺, 164 (229-Cp)⁺.

M.pt. 190–195°C (dec.).

Receptor [49]

2,6-diamino pyridine (0.40 g, 3.8 mmol) and triethylamine (0.38 g, 3.8 mmol) were dissolved in acetonitrile (20 ml). To this solution was added a solution of benzoyl chloride (0.47 g, 3.34 mmol) in acetonitrile under nitrogen with stirring. The mixture was stirred for 18 hours after which time, the solvent was removed by rotary evaporation and the residue purified by column chromatography (silica gel, eluent 94:6 CH₂Cl₂:MeOH) to give the crude product as

a white powder. This was recrystallised from 10% ethanol in water to give the product as fine white crystals (yield: 310 mg, 44%).

Elemental analysis: found C, 66.4; H, 5.09; N, 18.6. $C_{12}H_{11}N_3O$ requires C, 67.6; H, 5.20; N, 19.7 %.

1H nmr ($CDCl_3$): δ 8.34 (1H, s(br), CONH); 7.88 (2H, m, ArH); 7.70 (1H, d($J=8.5$ Hz), ArH); 7.57 - 7.43 (4H, m, ArH'); 6.28 (1H, d($J=7.6$ Hz), ArH); 4.38 (2H, s(br), NH_2).

^{13}C nmr ($CDCl_3$): δ 165.5 (C=O); 157.2 (CONH-C); 150.0 (C- NH_2); 140.2 (pyC-H); 134.5 (C-C=O); 132.0 (ArC-H); 128.8 (ArC-H); 127.1 (ArC-H); 104.5 (NC-CH); 103.5 (NC-CH).

IR ν_{max}/cm^{-1} : 3843, m, N-H str; 3433, m, N-H str; 3284, m, N-H str; 1631, s, C=O str (amide I); 1534, m, C=O str (amide II).

Receptor [50]

Benzoyl chloride (0.72 g, 5.12 mmol) was dissolved in acetonitrile (20 ml) and stirred under nitrogen. To this solution was added dropwise a solution of 2,6-diamino pyridine (0.28 g, 2.56 mmol) and triethylamine (0.52 g, 5.12 mmol) in acetonitrile. The mixture was stirred for 24 hours during which time the product precipitated from solution. This was isolated by filtration and washed with acetonitrile to give the product as a white crystalline solid (yield: 490 mg, 60%).

Elemental analysis: found C, 70.1; H, 4.59; N, 13.18. C₁₉H₁₅N₃O₂ requires C, 71.9; H, 4.76; N, 13.24%.

¹H nmr (CDCl₃): δ 8.32 (2H, s(br), CONH); 8.13 (2H, d(J=7.9), N-C-CH); 7.91 (4H, d(J=8.0), CH-C-C=O); 7.83 (1H, t(J=8.0), N-C-CH-CH); 7.61 - 7.49 (6H, m, ArH).

¹³C nmr (CDCl₃): δ 165.4 (C=O); 149.7 (C-N); 141.0 (pyC-H); 134.2 (C-C=O); 132.3 (ArC-H); 128.9 (ArC-H); 127.1 (ArC-H); 109.9 (NC-CH).

IR ν_{max}/cm⁻¹: 3341, s, N-H str; 1651, s, C=O str (amide I); 1528, m, C=O str (amide II).

4,4'-Bis(carboxy)-2,2'-bipyridine

Sulphuric acid (50 ml) was cooled in a water bath and 4,4'-dimethyl-2,2'-bipyridine (4.0 g, 22 mmol) was added in small portions with stirring. The temperature of the mixture was maintained below 35°C during the addition. The mixture was stirred until the bipyridine had completely dissolved at which point, chromium trioxide (13.0 g, 130 mmol) was added in small portions over a period of 40 min. The mixture was then heated to 75°C and maintained at this temperature for 4 h; it assumed a blue-green colour. The mixture was allowed to cool to room temperature and stirred for a further 18 h. The viscous mixture was poured very slowly into vigorously stirred ice/water (500 ml); a fine pale green precipitate was seen to form. Filtration at this stage proved difficult and so the mixture was allowed to stand for 18 h during which time the precipitate settled. The solution was decanted as far as possible and water (300 ml) was added, along with approximately 10 g Celite. The mixture was then filtered under reduced pressure and the precipitate washed with water and dried. It was then suspended in water (300 ml) and 10% aqueous potassium hydroxide was added until the pH reached 8-9. The solution was heated to 50°C and then filtered to give a pale blue powder and a yellow solution. Dropwise addition of 3M hydrochloric acid precipitated the product as a fine white precipitate (vigorous stirring was required). Care was taken not to allow the pH to

fall below 3. The product was isolated by filtration, washed with water, ethanol and dried at elevated temperature (50°C) (yield: 4.7 g, 89%). Characterisation of the product was limited due to very poor solubility.

Elemental analysis: found C, 58.4; H, 3.30; N, 11.4. C₁₂H₈N₂O₄ requires C, 59.0; H, 3.30; N, 11.5%.

EI MS: m/z 244 (M)⁺, 200 (M-CO₂)⁺.

4,4'-Bis(chlorocarbonyl)-2,2'-bipyridine

4,4'-Bis(carboxy)-2,2'-bipyridine (0.4 g, 1.8 mmol) was suspended in thionyl chloride (50 ml) and refluxed under nitrogen for 48 h. After this time the solution was filtered under reduced pressure and the solid material washed with thionyl chloride. The filtrate was evaporated to dryness by distillation under reduced pressure to give a creamy-white residue which was dried *in vacuo*. The product was not characterised but used immediately in subsequent reactions.

4,4'-Bis-(4-chloro-phenylaminocarboxy)-2,2'-bipyridine

4-Chloroaniline (1.4 g, 11.0 mmol) and pyridine (3.8 ml, previously distilled under nitrogen) were dissolved in dry dichloromethane (35 ml) and stirred under nitrogen. 4,4'-Bis(chlorocarbonyl)-2,2'-bipyridine (90% yield assumed from previous reaction) was dissolved in dry dichloromethane and rapidly added to the stirred solution of the amines. A cream coloured precipitate was seen to form immediately. The mixture was taken to reflux for 3 h after which time it was allowed to cool to room temperature and stirred for a further

18 h. Water (20 ml) was added and the mixture stirred for 1 h after which time it was filtered and the precipitate washed with dichloromethane, water, dilute ammonium hydroxide and finally water (until the washings ceased to be alkaline). The product was dried and isolated as a white powder (yield: 1.83 g, 79%).

Elemental analysis: found C, 62.6; H, 3.42; N, 12.2. $C_{24}H_{16}N_4O_2$ requires C, 62.2; H, 3.48; N, 12.1%.

1H nmr (d_6 -DMSO): δ 10.85 (2H, s, CONH); 8.96 (2H, d ($J=5.5$), pyH), 8.91 (2H, s, pyH); 7.98 (2H, d ($J=5.0$), pyH); 7.86 (4H, d ($J=7.5$), ArH); 7.46 (4H, d ($J=8.8$), ArH).

IR ν_{max}/cm^{-1} : 3270, s, N-H str; 1651, s, C=O str; 1591, s, bpy; 1525, s, bpy.

4,4'-Bis-(3-chloro-phenylaminocarboxy)-2,2'-bipyridine

4,4'-Bis(chlorocarbonyl)-2,2'-bipyridine (0.4 g, 1.8 mmol) was dissolved in dry acetonitrile (30 ml) and added dropwise under nitrogen to a stirred solution of 3-chloroaniline (0.55 g, 4.3 mmol) and pyridine (1.5 ml). Fuming was observed, along with the precipitation of a white solid. The mixture was taken to reflux for 3 h and then allowed to cool to room temperature and stirred for 18 h. The mixture was then filtered and the precipitate was washed with acetonitrile, dilute ammonium hydroxide water, acetone and diethyl ether to give the product as a white powder following drying (yield: 0.62 g, 74%).

Elemental analysis: found C, 61.9; H, 3.41; N, 12.2. $C_{24}H_{16}N_4O_2$ requires C, 62.2; H, 3.48; N, 12.1%.

^1H nmr (d_6 -DMSO): δ 10.88 (2H, s, CONH); 8.97 (2H, d ($J = 5.6$), pyH); 8.91 (2H, s, pyH); 7.98 (4H, m, pyH / ArH); 7.74 (2H, d ($J = 8.2$), ArH); 7.45 (2H, t ($J = 8.2$), ArH); 7.23 (2H, t ($J = 7.8$), ArH).

IR $\nu_{\text{max}}/\text{cm}^{-1}$: 3300, s, N-H str; 1657, s, C=O str; 1594, m, bpy; 1520, s, bpy.

Bis-(2,2'-bipyridyl)-ruthenium (II) dichloride

This molecule was prepared according to literature procedures¹⁸⁹ with one modification to improve purity. The crude product was suspended in methanol (100 ml) and taken to reflux briefly to dissolve soluble impurity, mainly ruthenium tris(2,2'-bipyridyl) dichloride. The solution was then filtered to give the product as a very dark, almost black powder on drying (yield: 8.1 g, 81%).

Elemental analysis: found C, 47.0; H, 3.90; N, 10.31; Cl 12.5. $\text{C}_{20}\text{H}_{16}\text{N}_4\text{Cl}_2\text{Ru}$ requires C, 46.2; H, 3.87; N, 10.77; Cl 13.63%.

^1H nmr (d_6 -DMSO): δ 9.98 (4H, d ($J=5.3$), 6'-pyH); 8.63 (4H, d ($J=8.0$), 3/3'-pyH); 8.48 (4H, d ($J=8.0$), 3/3'-pyH); 8.06 (4H, t ($J=7.8$), 4'-pyH); 7.79 (4H, t ($J=6.1$), 4/5'-pyH); 7.67 (4H, t ($J=8.2$), 4/5'-pyH); 7.50 (4H, d ($J=5.4$), 6-pyH); 7.10 (4H, t ($J=6.1$), 5-pyH).

4,4'-Bis-(3,4-dimethoxy-phenylaminocarboxy)-2,2'-bipyridine

4,4'-Bis(chlorocarbonyl)-2,2'-bipyridine (0.4 g, 1.8 mmol) was dissolved in dry acetonitrile (20 ml) and added dropwise under nitrogen to a stirred solution of 4-aminoveratrole (0.46 g, 3.0 mmol) and triethylamine (0.30 g, 3.0 mmol) in dry acetonitrile (30 ml). A white precipitate was observed to form. Stirring was continued for 48 h after which time the precipitate was isolated by filtration and washed with acetonitrile, ammonium hydroxide, water, ethanol and diethyl ether to give the product as an off-white powder (yield: 350 mg, 38%).

Elemental analysis: found C, 63.7; H, 4.86; N, 10.65. $C_{28}H_{26}N_4O_6$ requires C, 65.4; H, 4.86; N, 10.89%.

1H nmr (d_6 -DMSO): δ 10.58 (2H, s, CONH); 8.95 (2H, d ($J=5.10$), pyH); 8.92 (2H, s, 3-pyH); 7.98 (2H, d ($J=5.1$), pyH); 7.48 (2H, s, ArH); 7.40 (2H, d ($J=8.6$), ArH); 6.97 (2H, d ($J=8.8$), ArH); 3.78 (6H, s, CH_3); 3.76 (6H, s, CH_3).

IR ν_{max}/cm^{-1} : 3273, m, N-H str; 1650, s, C=O str; 1604, m, bpy; 1516, s, bpy.

4,4'-Bis-(phenylaminocarboxy)-2,2'-bipyridine

4,4'-Bis(chlorocarbonyl)-2,2'-bipyridine (0.4 g, 1.8 mmol) was dissolved in dry acetonitrile (30 ml) and added dropwise under nitrogen to a stirred solution of aniline (0.4 g, 4.3 mmol) and triethylamine (0.4 g, 4.0 mmol). Fuming was observed, along with the precipitation of a white solid. The mixture was taken to reflux for 3 h and then allowed to cool to room temperature and stirred for 18 h. The mixture was then filtered and the precipitate was

washed with acetonitrile, dilute ammonium hydroxide and water, then dried to give the product as a white powder (yield: 0.34 g, 48%).

Elemental analysis: found C, 72.2; H, 4.48; N, 14.33. $C_{24}H_{18}N_4O_2$ requires C, 73.1; H, 4.60; N, 14.2%.

1H nmr (d_6 -DMSO): δ 10.73 (2H, s, CONH); 8.96 (2H, d (J = 4.9), pyH); 8.91 (2H, s, pyH); 7.98 (2H, d (J = 5.1), pyH); 7.82 (4H, d (J = 8.3), ArH); 7.40 (4H, d (J = 7.5), ArH); 7.16 (2H, t (J = 7.3), ArH).

IR ν_{max}/cm^{-1} : 3300, s, N-H str; 1651, s, C=O str; 1600, m, bpy; 1532, s, bpy.

4,4'-Bis-(^{15}N -phenylaminocarboxy)-2,2'-bipyridine

^{15}N aniline (0.30g, 3.6 mmol) was added to 4,4'-bis(chlorocarbonyl)-2,2'-bipyridine in a manner analogous to the previous section to yield the product as an off-white powder (yield: 370 mg, 52%).

Elemental analysis: found C, 72.1; H, 4.53; N, 14.4. $C_{24}H_{18}N_4O_2$ requires C, 73.1; H, 4.60; N, 14.2%.

1H nmr ($CDCl_3$): δ 10.86/10.56 (2H, d (J =90), CONH); 8.96 (2H, d (J =5.1), pyH); 8.91 (2H, s, 3-pyH); 7.99 (4H, d (J =3.4), pyH); 7.81 (4H, d (J =8.5), ArH); 7.40 (4H, t (J =8.0), ArH), 7.16 (2H, t (J =7.3), ArH).

FAB MS: m/z 1073 ($M-PF_6$) $^+$, 928 ($M-2PF_6$) $^+$ [clusters].

Receptor [55]

4,4'-Bis-(4-chloro-phenylaminocarboxy)-2,2'-bipyridine (0.23 g, 0.5 mmol) and Ru(bpy)₂Cl₂ (0.26 g, 0.55 mmol) were stirred in ethanol:water:glacial acetic acid (40 ml, 2:2:1 v/v) and the mixture taken to reflux for 24 h. After this time, the solution was allowed to cool and filtered. The filtrate was extracted with dichloromethane (5 x 25 ml) and the extracts evaporated to dryness. The residue was taken up in water and the crude product precipitated by addition of ammonium hexafluorophosphate. This was isolated by filtration and purified by column chromatography (Sephadex: eluent 50:50 MeOH:MeCN) to give the product as a deep red powder (yield 130 mg, 25%).

Elemental analysis: found C, 45.4; H, 2.80; N, 10.02. C₄₄H₃₂N₈O₂Cl₂RuP₂F₁₂ requires C, 45.3; H, 2.76; N, 9.60 %.

¹H nmr (d₆-DMSO): δ 9.38 (2H, s (br), CONH); 9.18 (2H, s, 3-pyH); 8.55 (4H, d, pyH); 8.11 (4H, m, pyH); 7.95 (4H, d, ArH); 7.86-7.73 (12H, m, pyH); 7.45-7.36 (8H, m, ArH/pyH).

IR ν_{max}/cm⁻¹: 3400, m, N-H str; 1676, m, C=O str; 1599, m, bpy; 1532, m, bpy; 843, s, PF₆⁻.

FAB MS: m/z 1021 (M-PF₆)⁺, 878 (M-2PF₆)⁺.

Receptor [56]

4,4'-Bis-(3-chloro-phenylaminocarboxy)-2,2'-bipyridine (0.23 g, 0.5 mmol) and Ru(bpy)₂Cl₂ (0.26 g, 0.55 mmol) were added in a manner analogous to receptor [55] to give the product as a red crystalline powder (yield: 161 mg, 31%).

Elemental analysis: found C, 45.9; H, 2.53; N, 10.60. $C_{44}H_{32}N_8O_2Cl_2RuP_2F_{12}$ requires C, 45.3; H, 2.76; N, 9.60 %.

1H nmr (d_6 -DMSO): δ 9.38 (2H, s (br), CONH); 9.18 (2H, s, 3-pyH); 8.55 (4H, d, pyH); 8.11 (4H, m, pyH); 7.95 (4H, d, ArH); 7.86-7.73 (12H, m, pyH); 7.45-7.36 (8H, m, ArH/pyH).

IR ν_{max}/cm^{-1} : 3392, m, N-H str; 1681, s, C=O str (amide I); 1573, m, C=O str (amide II); 839, s, PF_6^- .

FAB MS: m/z 1021 ($M-PF_6$) $^+$, 878 ($M-2PF_6$) $^+$.

Receptor [57]

4,4'-Bis-(3,4-dimethoxy-phenylaminocarboxy)-2,2'-bipyridine (0.23 g, 0.43 mmol) and $Ru(bpy)_2Cl_2$ (0.26 g, 0.55 mmol) were dissolved in water:ethanol:glacial acetic acid (25 ml, 1:1:1 v/v) and heated at 95°C for 2 h. After this time the reaction mixture was allowed to cool and extracted with dichloromethane. The aqueous layer was evaporated to dryness and the residue taken up in deionised water. Dropwise addition of an aqueous solution of ammonium hexafluorophosphate precipitated the crude product which was isolated by filtration and purified by column chromatography (Sephadex: eluent 50:50 MeCN:MeOH) to give the product as a deep red glassy solid (yield: 50 mg, 10 %).

Elemental analysis: found C, 48.0; H, 3.62; N, 9.57. $C_{48}H_{42}N_8O_6RuP_2F_{12}$ requires C, 47.3; H, 3.78; N, 9.20%.

1H nmr ($CDCl_3$): δ 9.36 (2H, s (br), CONH); 9.17 (2H, s, 3-pyH); 8.53 (4H, d ($J=8.1$), pyH); 8.11 (4H, m, pyH); 7.96 (2H, d ($J=5.9$), pyH); 7.85 (2H, d ($J=5.9$), pyH); 7.75 (4H, m, pyH); 7.46 (6H, m, ArH/pyH); 7.31 (2H, d ($J=8.7$), ArH); 6.90 (2H, d ($J=8.7$), ArH), 3.82 (6H, s, CH_3); 3.79 (6H, s, CH_3).

FAB MS: m/z 1073 ($M\text{-PF}_6$)⁺, 928 ($M\text{-2PF}_6$)⁺ [clusters].

Receptor [58]

4,4'-Bis-(phenylaminocarboxy)-2,2'-bipyridine (100 mg, 0.25 mmol) and Ru(bpy)₂Cl₂ (160 mg, 0.30 mmol) were dissolved in ethylene glycol (20 ml) and the mixture was stirred at 100°C for 8 h. After this time, the mixture was allowed to cool to room temperature and water (15 ml) was added. Dropwise addition of aqueous ammonium hexafluorophosphate yielded crude product as a red-brown solid which was purified by column chromatography (Sephadex: eluent 50:50 MeCN: MeOH) to give the product as a deep red solid (140 mg, 51%).

Elemental analysis: found C, 47.9; H, 3.20; N, 10.0. C₄₄H₃₄N₈O₂RuP₂F₁₂ requires C, 48.1; H, 3.12; N, 10.2%.

¹H nmr (CD₃CN): δ 9.76 (2H, s (br), CONH); 9.56 (2H, s, 3-pyH); 8.53 (4H, d (J=8.3), pyH); 8.13-8.07 (4H, m, pyH); 7.95 (2H, d (J=5.6), pyH); 7.91-7.86 (6H, pyH/ArH); 7.75 (4H, m, pyH); 7.46-7.38 (10H, m, pyH/ArH); 7.20 (2H, t (J=7.4), ArH).

IR $\nu_{\text{max}}/\text{cm}^{-1}$: 3436, m, N-H str; 1673, m, C=O str; 1602, m, bpy; 1537, bpy; 843, s, PF₆⁻.

FAB MS: m/z 953 ($M\text{-PF}_6$)⁺, 808 ($M\text{-2PF}_6$)⁺.

Receptor ^{15}N -[58]

4,4'-Bis-(phenyl-[^{15}N]-aminocarboxy)-2,2'-bipyridine (100 mg, 0.25 mmol) and $\text{Ru}(\text{bpy})_2\text{Cl}_2$ (160 mg, 30 mmol) were added in a manner analogous to receptor [58] to give the product as a red solid (yield: 120 mg, 44%).

Elemental analysis: found C, 47.5; H, 3.25; N, 10.2. $\text{C}_{44}\text{H}_{34}^{15}\text{N}_2\text{N}_6\text{O}_2\text{RuP}_2\text{F}_{12}$ requires C, 48.1; H, 3.12; N, 10.4 %.

^1H nmr (CD_3CN): δ 9.72/9.41 (2H, d ($J=9.1$), CONH); 9.35 (2H, s, 3-pyH); 8.53 (4H, d ($J=8.2$), pyH); 8.08 (4H, m, pyH); 7.96 (2H, d ($J=5.9$), pyH); 7.87 (2H, d ($J=5.9$), pyH); 7.82–7.74 (8H, m, ArH/pyH); 7.47–7.35 (8H, m, ArH, pyH); 7.17 (2H, t ($J=7.4$), ArH).

Receptor [59]

Rhenium pentacarbonyl bromide (0.10 g, 0.25 mmol) was suspended in dry toluene (30 ml) and heated to 60°C with stirring under nitrogen. 4,4'-Bis-(3-chloro-phenylaminocarboxy)-2,2'-bipyridine (0.10 g, 0.20 mmol) was added in small portions over 10 min. The mixture was then taken to reflux and maintained thus for 24 h. After this time, an orange solid had formed over a yellow solution. This was removed and washed with toluene followed by ether and then dried to give the product as a bright orange powder (yield: 0.10 g, 62%).

Elemental analysis: found C, 38.7; H, 1.78; N, 6.36. $\text{C}_{27}\text{H}_{16}\text{N}_4\text{O}_5\text{ReBrCl}_2$ requires C, 40.0; H, 1.98; N, 6.89%.

^1H nmr (CD_3CN): δ 9.41 (2H, s (br), CONH); 9.24 (4H, d ($J=6.1$), pyH); 9.08 (2H, s, 3-pyH); 8.06 (2H, d ($J=5.7$), pyH); 7.96 (2H, m, ArH); 7.68 (2H, m, ArH); 7.40 (2H, t ($J=8.1$), ArH); 7.23 (2H, m, ArH).

IR $\nu_{\text{max}}/\text{cm}^{-1}$: 3427, w, N-H str; 2027, s, C=O str; 1924, s, C=O str; 1679, m, C=O str; 1595, m, bpy.

FAB MS: m/z 813 (M) $^+$ [weak cluster], 733 (M-Br) $^+$ [cluster].

APPENDIX One

Stability Constants:

Experimental Methods and Calculation

A1.0 Determination of Stability Constant

Stability constants were determined by titration of receptor with anion. The principles of the experiment were the same for both nmr and UV-vis methods. In both cases, known equivalents of anion were added to a solution of receptor which perturbed some quantifiable physical property. Successive aliquots were added to generated a titration profile which would yield information about the binding process. The nmr experiment will be taken as an example.

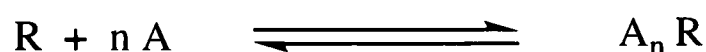
A1.1 ^1H nmr Titration

A solution of the receptor (500 μl volume) was prepared at a concentration typically of the order of 0.01 mol dm^{-3} . The initial spectrum was recorded and aliquots of anion were added by gas-tight syringe from a solution made such that one mole equivalent was added in 20 μl . After each addition and mixing, the spectrum was recorded again and changes in the chemical shift of certain protons were noted. The particular protons observed for the various systems have been discussed. The result of the experiment was a plot of displacement in chemical shift as a function of the amount of added anion, and this could then be subjected to

analysis by curve fitting since the shape of the curve is indicative of the stability constant for the complex. The computer program EQNMR¹ was used which requires the concentration of each component and the observed chemical shift (or its displacement) for each data point. A non-linear least squares analysis was then conducted as described below.

A1.1i The fitted function

The function fitted to the experimental plot was model independent; the model itself was suggested by the user in an input data file. Consider the general equilibrium for addition of a singly charged anion, A, to a receptor, R. It can be expressed as follows:



A particular nucleus common to both R and $A_n R$ is followed, one which will have a different chemical shift in each case. In a titration situation, a mixture of the two species will co-exist and the observed chemical shift will be a weighted average of the two extremes; the weighting factor being the concentration. In this case, the average chemical shift, δ_{obs} , will be expressed by:

$$\delta_{\text{obs}} = \sum_{n=0}^{n=i} \frac{\delta_{n:1} [A_n R]}{[R]_0} .$$

$\delta_{n:1}$ is the chemical shift of the various complexes in the equilibrium mixture and i is the maximum value of n. The denominator in the expression is the sum of all the weighting factors which is of, course, the sum of the concentrations of each component. This is known; it is the initial concentration of R.

The term $[A_n R]$ may be substituted by reference to the equation below which allows introduction of the respective stability constants, $K_{n:1}$, into the expression

$$[A_n R] = K_{n:1} [R] [A]^n .$$

The final expression is therefore

$$\delta_{\text{obs}} = \sum_{n=0}^{n=i} \frac{\delta_{n:1} K_{n:1} [R] [A]^n}{[R]_0} .$$

A1.1ii The least squares algorithm

Once in possession of experimental data and a theoretical function to describe them, the object is to optimise the values of $\delta_{n:1}$ and $K_{n:1}$ such that the best agreement between theoretical and experimental curves is reached. This is achieved by generating the theoretical curve for a range of values of the variables and examining the residuals (difference between real and generated data). These are squared and summed to give the sum of squares, Σ , and the computer then maps a multi-dimensional surface based on Σ as a function of the variables. The minimum point on this surface represents the situation in which there is the greatest probability of the data being representative of the curve.

The algorithm used in both EQNMR (and Specfit, which will be discussed shortly) is the Levenberg-Marquardt algorithm and defines a process of minimising Σ . Estimates of the variables are input by the user which defines a starting point on the surface and thence, there are two methods for locating the minimum. The first takes incremental steps down the surface in the direction of steepest descent, the size of which is determined by the gradient, and this approach will ultimately locate the minimum. However, the Taylor expansion shows that, to a first approximation, the surface close to the minimum is parabolic and therefore, once such a surface is detected, the minimum can be located with great speed. The Levenberg-Marquardt algorithm uses a combination of both of these, depending upon which it judges most suitable and reports the values of $K_{n:1}$ and $\delta_{n:1}$ for the minimum, along with an error estimate. The error relates to the curvature of the surface near the minimum point and therefore, the precision of its location. This, in turn, is related to uncertainty inherent in the input data.

The description above is a brief and very qualitative one; the interested (and brave, in the author's opinion) reader is directed to references in the bibliography.

A1.2 Determination of stoichiometry by Job's method

The method of continuous variation (Job's method) is a widely employed method of determining complex stoichiometry. The nmr adaptation employed, reported by Newcomb,² is derived as follows.

Consider two equimolar solutions of receptor, R, and substrate, S, with respective concentrations [R] and [S] (dilute solutions were used and concentrations are substituted for activities). A series of solutions are prepared of equal volume but different ratios of R:S — however, the total concentration [R] + [S] is unchanged. For a 1:1 complex, RS, the concentration [RS] will reach a maximum for the case where [R] = [S]. Therefore, a method which determines [RS] as a function of the mole fraction of R will yield the stoichiometry. It is usual to measure the absorbance of [RS] by spectrophotometric means; however, an nmr experiment is capable of delivering the same result.

Assume that the kinetics of exchange are fast on the nmr timescale and it is known that self association does not occur to any appreciable extent. In such a case, proton resonances arise from either R or RS. The observed chemical shift δ_{obs} is given by:

$$\delta_{\text{obs}} = \frac{\delta_{\text{R}} [\text{R}] + \delta_{\text{RS}} [\text{R}]}{[\text{R}]_0}$$

where R_0 is the original concentration of receptor. It is also known that

$$[\text{R}] = [\text{R}]_0 - [\text{RS}]$$

$$\therefore \delta_{\text{obs}} = \frac{\delta_{\text{R}} [\text{R}]_0 - \delta_{\text{R}} [\text{RS}] + \delta_{\text{RS}} [\text{RS}]}{[\text{R}]_0}$$

Rearranging gives:

$$[\text{RS}] = \frac{(\delta_{\text{obs}} - \delta_{\text{R}}) [\text{R}]_0}{(\delta_{\text{RS}} - \delta_{\text{R}})}$$

The term in the denominator is constant which means that [RS] is proportional to the product of the displacement in chemical shift, $\Delta\delta$, and the initial concentration of the receptor. Here, then, is an expression of [RS] in terms of a measurable quantity which can be plotted against the mole fraction of receptor. The plot in chapter two, however, plots $[\delta\Delta \times (\text{mole fraction})]$ as a function of (mole fraction), a valid replacement since the total concentration remains unchanged.

A1.2 Spectroscopic Titration

The determination of stability constant by spectroscopic means is similar to the approach taken in the previous section. The receptor solution was prepared, the concentration of which varied and is listed at appropriate points in the previous chapters. It was placed in a 3ml quartz cuvette and the emission / absorbance spectrum was recorded. Anion was added by syringe in solution such that one mole equivalent was contained in 10 μ l. The solution was manually stirred and the spectrum recorded again. This process was repeated until a titration data set was recorded.

The instruments used were as follows: UV-vis — Perkin-Elmer Lambda 6 UV/vis spectrophotometer; luminescence — Perkin-Elmer LS50B. Both instruments were able to output spectra as a digitised data set which enabled a global analysis of the spectra. This was achieved using a commercial package, Specfit. The qualitative operation of the program is as follows.

A1.2i Computer analysis

The spectral data are imported as a file and *factor analysis*^{3,4} allows estimation of the number of species which contribute to the data. Because the total absorbance of a solution (in the UV-vis case) is simply the weighted sum of the individual components, we effectively have a matrix of spectral data which contains a linear sum of independent factors, the number of which is known as the *rank* of the matrix. This can be found by factor analysis which initially decomposes the matrix into a product of three matrices — this process is known as singular value decomposition. Two of these are composed of orthogonal eigenvectors; the third is a diagonal matrix from which the number of contributing species is found. The eigenvectors do not have any direct chemical significance themselves — they are abstract solutions to the problem, but they *do* also assist in determining how many species contribute to the spectrum. The program suggests which of the elements of the diagonal matrix correspond to data and which to noise (based on their relative magnitude), and at this point, the user can suggest a model. Of course, in these experiments, he/she has a shrewd idea of how many species there are anyway, but the analysis helps to confirm this. The model is then fitted to the experimental data, again using the Levenberg-Marquardt algorithm as mentioned above.

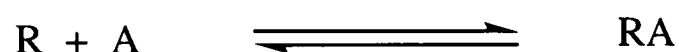
The output is a table of stability constants and associated errors, and a series of calculated plots. These include the simulated titration profile and residuals, the absorptivities of the individual species and the calculated concentration profile. All of these may be examined and compared with known data to ensure that sensible answers have been calculated.

The advantage of this powerful analysis is that the entire spectrum is fitted; it is a global analysis.^{5,6} Whereas in an nmr experiment one might calculate a fit for the shift of two or three protons, Specfit examines literally hundreds of profiles across the spectra and when a good fit is obtained for all of these, a certain confidence in the result is justified.

Specfit is copyright of the authors, R. A. Binstead and A. D. Zuberbühler, and of Spectrum Software Associates. Robert Binstead is very gratefully acknowledged for continued assistance and regular updates of the software.

A1.2ii *The Method of Benesi and Hildebrand*

This treatment was first⁷ proposed in 1949 and has since become widely used, in an adapted form, for the determination of stability constants.⁸ Let us return to the familiar equilibrium and its associated constant.



The absorbance measured at a given point will correspond to the sum of the contributions of the two coloured species (here is the assumption of a 1:1 complex and a colourless anion). The absorbance, α , will therefore be given by:

$$\alpha = [R] \epsilon_R + [RA] \epsilon_{RA} = [R]_0 \epsilon_R - [RA] \epsilon_R + [RA] \epsilon_{RA}.$$

The difference, $\Delta\alpha$, in absorbance will in turn be given by:

$$\Delta\alpha = \alpha - \alpha_0 = \alpha - [R]_0 \epsilon_R,$$

(α_0 and $[R]_0$ are the values at the outset of the titration, before anion is added) and since the concentration of R does not vary to a large degree, this simplifies to:

$$\Delta\alpha = [RA] \Delta\epsilon \quad ; \quad \Delta\epsilon = \epsilon_{RA} - \epsilon_R. \quad (1)$$

The stability constant may be written in the following way:

$$K = \frac{[RA]}{([R] - [RA])([A] - [RA])},$$

which rearranges to :

$$\frac{1}{K} = \frac{[R][A]}{[RA]} + [RA] - ([R] + [A]) \quad (2)$$

Substitution of (1) into (2) (to eliminate [RA]) and rearranging gives:

$$\frac{1}{K \Delta \epsilon} = \frac{[R][A]}{\Delta \alpha} + \frac{\Delta \alpha}{\Delta \epsilon^2} - \frac{[R] + [A]}{\Delta \epsilon}$$

The final assumption is that, since $\Delta \epsilon \gg \Delta \alpha$, then $\Delta \alpha / \Delta \epsilon^2$ is equal to zero to a first approximation.

The final expression is:

$$\frac{[R][A]}{\Delta \alpha} = \frac{1}{K \Delta \epsilon} + \frac{[R] + [A]}{\Delta \epsilon},$$

and therefore an appropriate plot is $[R][A] / \Delta \alpha$ against $[R] + [A]$.⁹ We then have the Benesi-Hildebrand plot. Care must be taken; this is not a method that can be universally applied. The assumptions inherent in the derivation place strict boundary conditions on its use.¹⁰ In particular, one component should be present in large excess and there are also restrictions of the ratio of concentration:stability constant. These conditions were not compatible with the usual titration technique and for this reason, the method was not routinely used.

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APPENDIX TWO

Electrochemistry

A2.1 Experimental Procedures

Electrochemical investigation was a routine analysis of the receptors synthesised. In all cases the same essential procedure was followed and will now be discussed.

The experiments were performed on a solution containing typically 10^{-5} mole of receptor in 5 ml volume of solvent contained in a 25 ml open beaker. These levels were found to give a satisfactory current response with little IR drop. The supporting electrolyte was tetrabutyl ammonium tetrafluoroborate which was synthesised in the laboratory. A neutralisation reaction between tetrabutyl ammonium hydroxide and tetrafluoroboric acid gave the crude product which was precipitated by addition of ether to a concentrated aqueous solution. The pure base electrolyte was isolated by filtration, washed and dried *in vacuo*. It was used at levels of 0.1 mol dm^{-3} .

The solvent for electrochemical experiments was dried and oxygen was removed by prolonged bubbling with dry argon. Both water and dioxygen have redox potentials within the range under investigation and were a serious impediment unless excluded. The argon gas was pre-saturated with solvent by bubbling through a reservoir before the final outlet, thereby minimising solvent evaporation from the electrochemical solution.

Anions were added in solution via syringe with one mole equivalent contained in a volume of 100 μl . After each addition, the surface of the working electrode was polished with 6 micron Kelmet diamond compound spray followed by washing with water and then the appropriate solvent. The solution was purged with argon to prevent introduction of oxygen and as a means of mixing.

A2.2 Electrodes

Cyclic and square wave voltammetry usually require three electrodes; reference, working and counter. The reference electrode was a Ag/Ag^+ half cell shown schematically in figure A2.1. The compartments were separated by scintered glass frits and the electrode was found to have a potential of 330 (± 10) mV against the saturated calomel electrode in acetonitrile. This potential was stable under the experimental conditions.

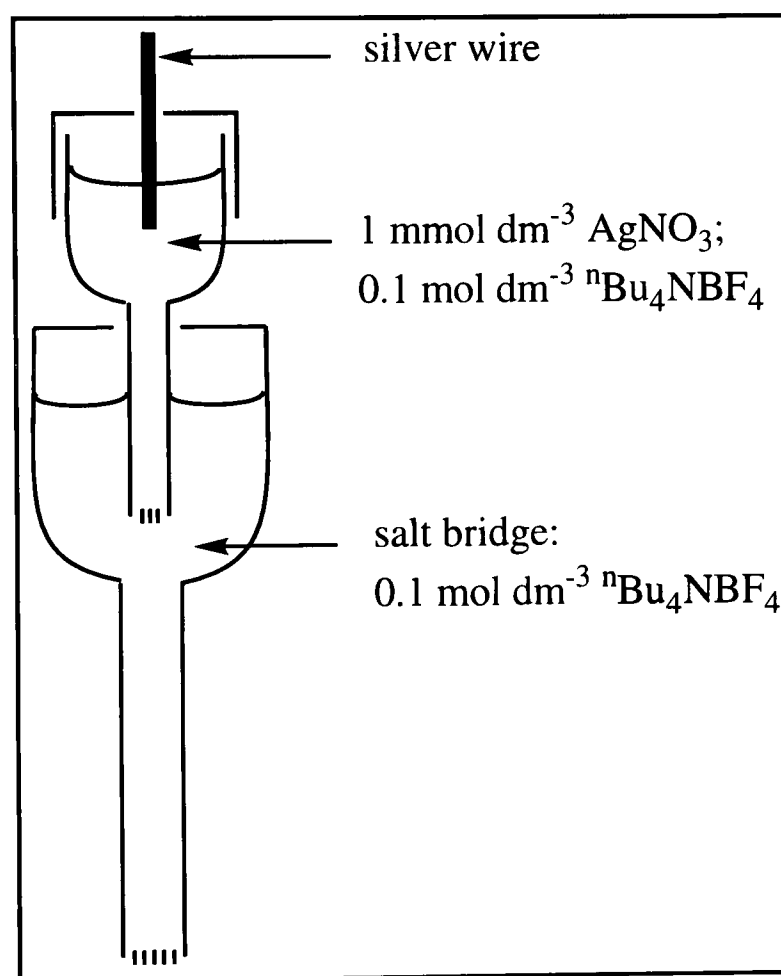


Figure A2.1: Reference electrode.

The working electrode was a glassy carbon disc with a diameter of 0.3 cm, and the counter electrode a piece of coiled platinum wire (length 10 cm). The latter was cleaned in a hot flame prior to use.

All voltammetry was controlled by a EG&G Princeton Applied Research Potentiostat / Galvanostat Model 273 which was driven by the EG&G m270 software. The voltammograms were digitised with a typical sampling of 2000 points each.

A2.3 Cyclic Voltammetry*

The technique of cyclic voltammetry was used almost exclusively in the electrochemical investigation.¹ A very popular potential sweep experiment, its essential theory is as follows.

A cyclic voltammogram (CV) of an electroactive species in solution is recorded by measuring current flow as a function of a changing potential. The latter is swept in a linear scan with triangular waveform from the starting potential E_1 , towards and past the transfer potential of the redox centre to another limit, E_2 , and then back again to E_1 (figure A2.2).

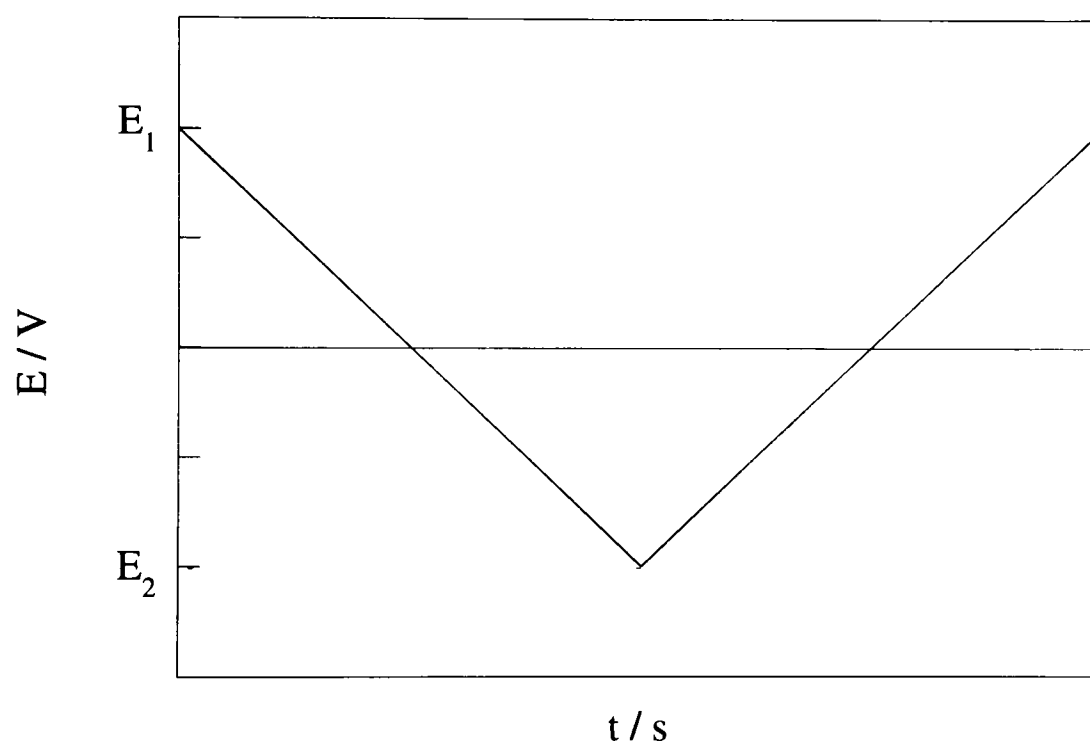


Figure A2.2: Potential sweep profile in cyclic voltammetry.

The magnitude of the derivative of this function is the scan rate, v , and is usually expressed in mVs^{-1} . A value of 100 mVs^{-1} was routinely employed.

* Elements of this discussion have appeared in a previous work: A. R. Graydon, Part II thesis, University of Oxford, 1992.

The application of this potential sweep affects the electroactive species in a manner which may be qualitatively described as follows. For the purposes of this discussion, a reduction process will be considered; it is assumed that only the oxidised species is present initially and that both oxidised and reduced forms are soluble and stable with respect to decomposition and further reaction. At the onset of the sweep, reduction occurs when an appropriate potential is reached and a current flows due to the electron transfer. The rate of transfer increases as the potential approaches $E_{1/2}$, the half-wave potential and therefore, the current increases.

The reduction process consumes the species near the electrode surface. A concentration gradient is established and therefore diffusion occurs along the gradient. At this stage, the magnitude of the current is proportional to the concentration gradient, and both increase with the advance of the potential. However, a limiting value is eventually reached as the concentration of the electroactive species at the electrode surface is effectively reduced to zero; the current peaks. Thereafter, the gradient relaxes due to diffusion from bulk solution and the current falls.

At a selected value, E_2 , the sweep is reversed and a point is reached where the reduced species begins to be reoxidised and a current flows in the opposite direction; the same scenario is repeated in reverse. The measured current is a little smaller, however, because some of the reduced species has diffused into bulk solution and cannot be recaptured.

In essence, the technique generates the reduced species on the forward scan and examines its fate on the reverse sweep. A typical cyclic voltammogram for a reversible, one electron reduction process is shown in figure A2.3.

The key variables are the cathodic and anodic peak potentials, E_{pc} and E_{pa} and the corresponding peak currents are i_{pc} and i_{pa} . The formal half-wave potential $E_{1/2}$ is given by $E_{1/2} = (E_{pc} + E_{pa}) / 2$.

In such considerations it is important that Nernstian behaviour applies which requires that the processes are diffusion controlled, i.e. electron transfer is fast compared to mass transfer of the bulk species. Furthermore, the system should ideally be reversible in which case the following conditions are observed:

1. $i_{pc} \approx i_{pa}$

2. $E_{pc} - E_{pa} = 59 / n$ where n is the number of electrons transferred
3. $(i_{pa} + i_{pc}) / 2 \propto \nu^{1/2}$ for given scan limits
4. E_{pc} and E_{pa} are not functions of ν .

In practice, the third of these conditions was employed to graphically check for reversibility.

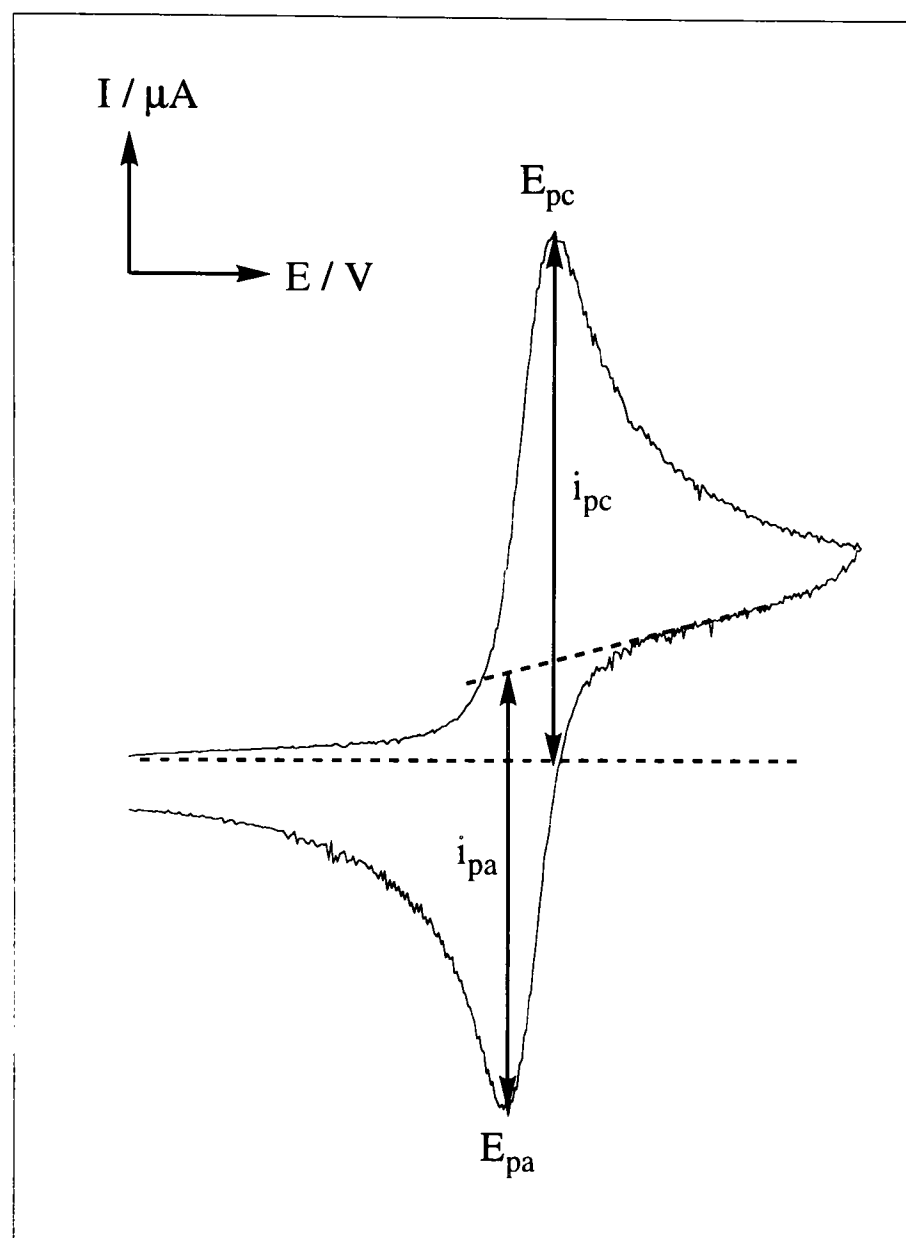


Figure A2.3: A cyclic voltammogram.

A2.4 Square Wave Voltammetry

This is an alternative to the above method which was employed when the peaks in a CV were not particularly well resolved. The method has the advantage of very frequently producing clear and simple voltammograms from which essential data can be easily extracted. Furthermore, it allows the elimination of the so-called “charging current” in the electrode double layer, which can be a problem in cyclic voltammetry, particularly at high scan rates or with low concentration systems. The result is that accurate values of $E_{1/2}$ and i_p can be determined; they are read directly from the voltammogram.

Square wave voltammetry is a pulse technique¹ with a potential sweep waveform as shown in figure A2.4.

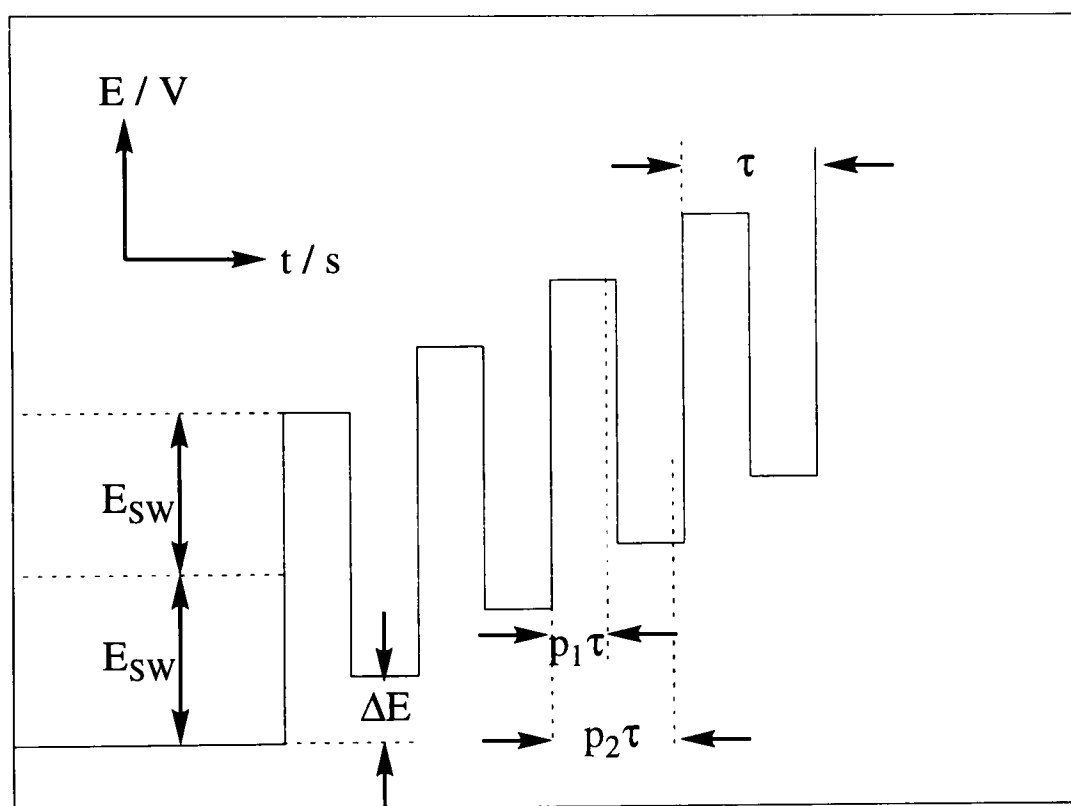


Figure A2.4: Potential - time profile for square wave voltammetry.

The variables associated with this waveform are as follows:

E_{sw} = square wave amplitude; ΔE = staircase step height; τ = staircase period.

During each period of the square wave the current is sampled twice, once in the forward pulse and again in the backward one. The times at which sampling occurs are designated $p_1\tau$ and $p_2\tau$ in the figure, and fall late in the pulse. This has the advantage of measuring the Faradaic current alone. The component of the current due to charging in the Nernst diffusion layer decays rapidly and at the time of sampling is very small. The voltammograms produced

therefore represent the forward and reverse current, and in addition the difference voltammogram is calculated; this is the square wave voltammogram (SWV) usually reported.

An example of a square wave voltammogram is given in figure A2.5 for a one electron, reversible oxidation. The half wave potential is simply the position of the difference peak and the peak currents are measured from the Y axis. The symmetrical shape of the difference peak testifies to the reversibility of the system.

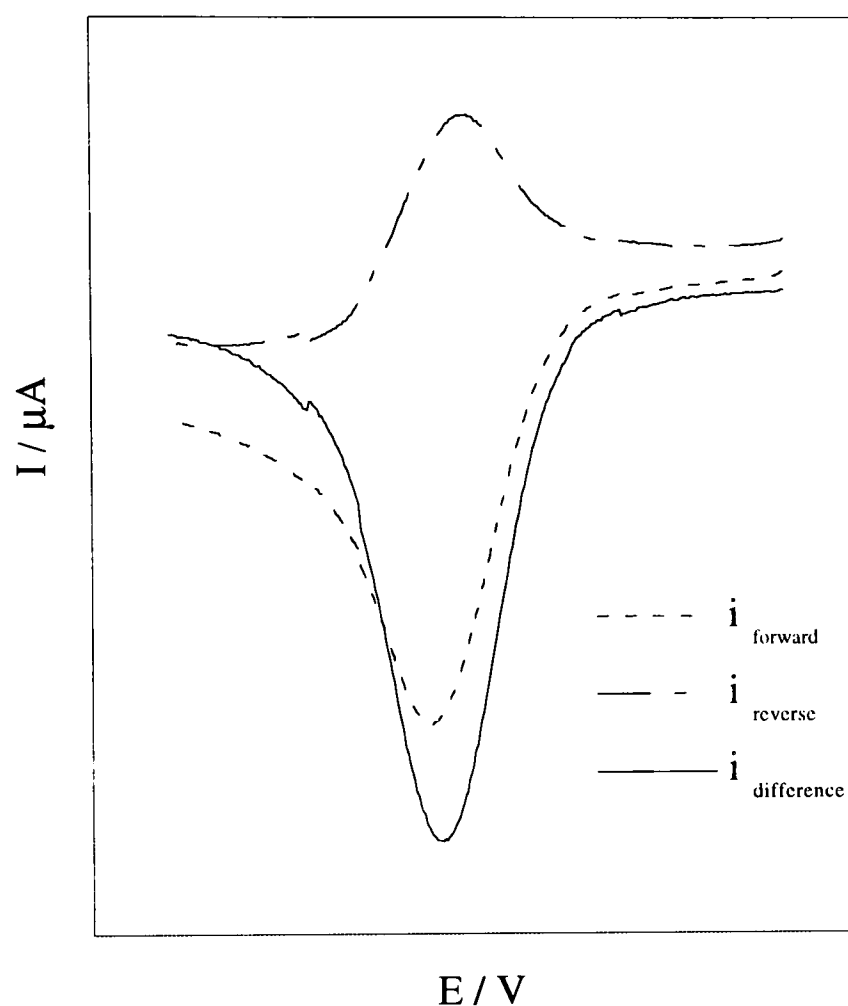


Figure A2.5: A square wave voltammogram.

The precise details of the experiment are reported by Osteryoung, after whom this particular type of square wave is named.²⁻⁴

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APPENDIX Three

Crystallographic Data

Experimental

Crystal Data are given in the following tables, together with refinement details. Data were collected with MoK α radiation using the MAR research Image Plate System. The crystal was positioned at 75 mm from the Image Plate. 95 frames were measured at 2° intervals with a counting time of 2 mins. Data analysis was carried out with the XDS program.[1] The structure was solved using direct methods with the Shelx86 program [2]. The non-hydrogen atoms were refined with anisotropic thermal parameters. The hydrogen atoms were included in geometric positions. The structure was then refined using Shelxl.[3]. All calculations were carried out on a Silicon Graphics R4000 Workstation at the University of Reading.

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Table A3.1i: X-ray crystal data for the chloride complex of receptor [34].

Empirical formula	C ₂₅ H ₂₂ ClCoNO
Formula weight	446.82
Temperature	293(2) K
Wavelength	71070 Å
Crystal system	orthorhombic
Space group	P2 ₁ 2 ₁ 2 ₁
Unit cell dimensions	a = 9.446(7) Å b = 11.859(7) Å c = 18.232(9) Å
Volume	2042(2) Å ³
Z	4
Density (calculated)	1.453 Mg/m ³
Absorption coefficient	0.987 mm ⁻¹
F(000)	924
Crystal size	0.20 x 0.14 x 0.32 mm
θ range for data collection	2.05 to 25.17°.
Index ranges	0 ≤ h ≤ 10, -14 ≤ k ≤ 14, -21 ≤ l ≤ 21
Reflections collected	6250
Independent reflections	3419 [R(int) = 0.0888]
Refinement method	Full-matrix least-squares on F ²
Data / parameters	3419 / 263
Goodness-of-fit on F ²	0.428
Final R indices [I > 2σ(I)]	R1 = 0.0691, wR2 = 0.1830
R indices (all data)	R1 = 0.1180, wR2 = 0.2451
Largest diff. peak and hole	0.659 and -0.814 e.Å ⁻³

Table A3.1ii: Atomic coordinates ($\times 10^4$) and equivalent isotropic displacement parameters ($\text{\AA}^2 \times 10^3$) for [34]. U(eq) is defined as one third of the trace of the orthogonalized U_{ij} tensor.

	x	y	z	U(eq)
Co(1)	2280(1)	9604(1)	9193(1)	35(1)
Cl(1)	-4793(3)	-469(3)	11680(2)	60(1)
C(11)	1399(11)	8233(8)	8706(5)	36(2)
C(12)	1700(14)	9075(9)	8183(5)	47(3)
C(13)	3178(14)	9335(8)	8212(6)	50(3)
C(14)	3776(12)	8636(8)	8746(7)	44(3)
C(15)	2717(11)	7957(8)	9063(5)	38(2)
C(16)	2997(11)	147(8)	9654(6)	43(2)
O(16)	4007(11)	7298(10)	10067(7)	87(4)
N(17)	2123(10)	6287(7)	9712(4)	38(2)
C(18)	2176(9)	5430(8)	10244(5)	32(2)
C(19)	1859(11)	4340(9)	10065(5)	38(2)
C(20)	1874(12)	3526(8)	10587(6)	40(2)
C(21)	2232(12)	3740(9)	11307(5)	40(2)
C(22)	2547(12)	4831(9)	11489(5)	41(2)
C(23)	2529(10)	5701(8)	10977(5)	36(2)
C(24)	2191(13)	2825(11)	11881(7)	55(3)
C(25)	642(13)	2579(9)	12079(6)	45(3)
C(26)	-97(14)	3251(9)	12558(7)	53(3)
C(27)	-1516(15)	3140(11)	12712(8)	59(3)
C(28)	-2282(15)	2267(11)	12346(7)	60(3)
C(29)	-1557(14)	1578(11)	11890(6)	55(3)
C(30)	-111(15)	1728(10)	11747(7)	56(3)
N(31)	-3706(13)	2157(12)	12443(9)	64(4)
C(41)	2978(20)	11110(14)	9571(14)	93(7)
C(42)	2991(19)	10343(18)	10089(12)	95(6)
C(43)	1610(26)	9892(14)	10214(9)	04(8)
C(44)	797(15)	10544(15)	9690(8)	73(4)
C(45)	1684(20)	11212(11)	9330(9)	71(4)

Table A3.1iii: Hydrogen coordinates ($\times 10^4$) and isotropic displacement parameters ($\text{\AA}^2 \times 10^3$) for [34].

	x	y	z	U(eq)
H(11)	518(11)	7916(8)	8802(5)	43
H(12)	1044(14)	9408(9)	7870(5)	56
H(13)	3651(14)	9868(8)	7929(6)	60
H(14)	4730(12)	8622(8)	8872(7)	53
H(17)	1454(10)	6245(7)	9393(4)	46
H(19)	1632(11)	4156(9)	9584(5)	45
H(20)	1633(12)	2793(8)	10454(6)	48
H(22)	2781(12)	4998(9)	11972(5)	49
H(23)	2743(10)	6438(8)	11112(5)	43
H(24A)	2703(13)	3066(11)	12315(7)	66
H(24B)	2638(13)	2147(11)	11694(7)	66
H(26)	397(14)	3821(9)	12797(7)	63
H(27)	-1963(15)	3619(11)	13043(8)	71
H(29)	-2033(14)	985(11)	11665(6)	66
H(30)	346(15)	1245(10)	11422(7)	67
H(31A)	-4200(13)	1598(12)	12194(9)	77
H(31B)	-4183(13)	2641(12)	12759(9)	77
H(41)	3759(20)	11513(14)	9404(14)	112
H(42)	3797(19)	10121(18)	10344(12)	114
H(43)	1316(26)	9335(14)	10539(9)	125
H(44)	-177(15)	10503(15)	9619(8)	87
H(45)	1416(20)	11693(11)	8953(9)	85

Table A3.2i: X-ray crystal data for receptor [35].

Empirical formula	C ₁₇ H ₁₆ CoF ₆ N ₂ OP
Formula weight	468.22
Temperature	293(2) K
Wavelength	0.71070 Å
Crystal system	orthorhombic
Space group	P _{bca}
Unit cell dimensions	a = 9.613(5) Å b = 18.700(10)Å c = 19.758(10)Å
Volume	3552(5) Å ³
Z	8
Density (calculated)	1.751 Mg/m ³
Absorption coefficient	1.128 mm ⁻¹
F(000)	1888
Crystal size	0.2 x 0.2 x 0.2 mm
θ range for data collection	2.06 to 23.85°
Index ranges	0<=h<=10, -20<=k<=21, -22<=l<=22
Reflections collected	8372
Independent reflections	2641 [R(int) = 0.0273]
Refinement method	Full-matrix least-squares on F ²
Data / restraints / parameters	2638 / 0 / 257
Goodness-of-fit on F ²	0.862
Final R indices [I>2σ(I)]	R1 = 0.0502, wR2 = 0.1392
R indices (all data)	R1 = 0.0639, wR2 = 0.1591
Largest diff. peak and hole	0.525 and -0.469 e.Å ⁻³

Table A3.2ii: Atomic coordinates ($\times 10^4$) and equivalent isotropic displacement parameters ($\text{\AA}^2 \times 10^3$) for [35]. $U(\text{eq})$ is defined as one third of the trace of the orthogonalized U_{ij} tensor.

	x	y	z	U(eq)
Co	434(1)	1258(1)	8705(1)	27(1)
P(1)	969(1)	2245(1)	11066(1)	49(1)
F(1)	2091(4)	2177(3)	11628(2)	112(2)
F(2)	-176(4)	2185(3)	11622(2)	100(1)
F(3)	941(7)	3070(2)	11181(2)	122(2)
F(4)	931(8)	1429(3)	10960(3)	151(2)
F(5)	-167(4)	2330(2)	10506(2)	91(1)
F(6)	2103(5)	2349(4)	10527(2)	158(3)
C(11)	-1359(5)	759(2)	8916(2)	41(1)
C(12)	-608(6)	888(3)	9506(2)	49(1)
C(13)	664(6)	553(3)	9453(2)	49(1)
C(14)	738(5)	208(2)	8824(2)	38(1)
C(15)	-528(4)	331(2)	8489(2)	31(1)
C(21)	1683(6)	2081(3)	8900(3)	57(2)
C(22)	2180(5)	1688(3)	8346(3)	57(1)
C(23)	372(6)	2322(3)	8746(3)	52(2)
C(24)	44(7)	2099(3)	8109(3)	61(2)
C(25)	1168(7)	1708(3)	7863(3)	58(2)
C(16)	-980(4)	109(2)	7805(2)	30(1)
O(17)	-2046(3)	346(2)	7569(2)	46(1)
N(18)	-190(4)	-370(2)	7495(2)	33(1)
N(31)	-2549(4)	-1187(2)	7134(2)	40(1)
C(31)	-430(4)	-587(2)	6820(2)	32(1)
C(32)	525(5)	-412(3)	6337(3)	52(1)
C(33)	320(6)	-615(3)	5677(3)	63(2)
C(34)	-841(6)	-975(3)	5507(3)	56(1)
C(35)	-1781(5)	-1157(2)	5980(2)	40(1)
C(36)	-1586(4)	-986(2)	6657(2)	29(1)

Table A3.2iii: Hydrogen coordinates ($\times 10^4$) and isotropic displacement parameters ($\text{\AA}^2 \times 10^3$) for [35].

	x	y	z	U(eq)
H(11)	-2249(5)	925(2)	8820(2)	64(5)
H(12)	-914(6)	1155(3)	9874(2)	64(5)
H(13)	1359(6)	555(3)	9780(2)	64(5)
H(14)	1488(5)	-53(2)	8659(2)	64(5)
H(21)	2160(6)	2164(3)	9302(3)	64(5)
H(22)	3035(5)	1458(3)	8314(3)	64(5)
H(23)	-197(6)	2592(3)	9029(3)	64(5)
H(24)	-781(7)	2192(3)	7879(3)	64(5)
H(25)	1220(7)	1495(3)	7438(3)	64(5)
H(18)	495(4)	-556(2)	7712(2)	64(5)
H(31A)	-2440(4)	-1064(2)	7550(2)	92(16)
H(31B)	-3261(4)	-1436(2)	7017(2)	92(16)
H(32)	1317(5)	-155(3)	6455(3)	64(5)
H(33)	981(6)	-505(3)	5349(3)	64(5)
H(34)	-995(6)	-1098(3)	5057(3)	64(5)
H(35)	-2579(5)	-1403(2)	5852(2)	64(5)

Table A3.3i: X-ray crystal data for receptor [45].

Empirical formula	C ₂₂ H ₂₁ CoF ₆ FeNOP
Formula weight	575.15
Temperature	293(2) K
Wavelength	0.71071 Å
Crystal system	triclinic
Space group	P1
Unit cell dimensions	a = 7.323 Å α = 81.52° b = 10.572 Å β = 79.82° c = 14.999 Å γ = 71.65°
Volume	1079.5 Å ³
Z	2
Density (calculated)	1.769 Mg/m ³
Absorption coefficient	1.582 mm ⁻¹
F(000)	580
θ range for data collection	3.60 to 24.96°
Index ranges	0 ≤ h ≤ 8, -11 ≤ k ≤ 12, -17 ≤ l ≤ 17
Reflections collected	3307
Refinement method	Full-matrix least-squares on F ²
Data / restraints / parameters	3307 / 0 / 299
Goodness-of-fit on F ²	1.025
Final R indices [I > 2σ(I)]	R1 = 0.0765, wR2 = 0.2005
R indices (all data)	R1 = 0.1019, wR2 = 0.2210
Largest diff. peak and hole	1.194 and -0.722 e.Å ⁻³

Table A3.3ii: Atomic coordinates ($\times 10^4$) and equivalent isotropic displacement parameters ($\text{\AA}^2 \times 10^3$) for [45]. U(eq) is defined as one third of the trace of the orthogonalized U_{ij} tensor.

	x	y	z	U(eq)
Co(1)	4146(1)	3006(1)	8461(1)	52(1)
Fe(2)	7691(1)	7522(1)	5466(1)	49(1)
C(11)	5218(11)	4122(7)	9074(5)	55(2)
C(12)	3158(13)	4507(9)	9254(6)	73(2)
C(13)	2646(16)	3399(11)	9706(7)	95(3)
C(14)	4325(17)	2317(10)	9792(6)	90(3)
C(15)	5924(13)	2765(8)	9403(5)	66(2)
C(16)	6526(11)	4888(8)	8537(5)	58(2)
O(17)	8235(9)	4346(7)	8404(5)	92(2)
N(18)	5651(10)	6148(6)	8248(4)	58(2)
C(19)	6693(13)	6924(8)	7586(5)	63(2)
C(20)	6581(9)	6688(6)	6648(4)	47(2)
C(21)	5088(9)	7370(7)	6109(5)	50(2)
C(22)	5498(10)	6830(8)	5284(5)	58(2)
C(23)	7236(11)	5787(8)	5296(5)	61(2)
C(24)	7898(10)	5690(7)	6142(5)	54(2)
C(31)	5705(14)	2085(11)	365(6)	83(3)
C(32)	4789(19)	3406(13)	7125(6)	91(3)
C(33)	2804(17)	3629(12)	7338(8)	95(3)
C(34)	2530(18)	2446(17)	7751(9)	113(4)
C(35)	4297(21)	1464(12)	7763(7)	100(3)
C(41)	8959(18)	8800(12)	5783(7)	89(3)
C(42)	7567(12)	9492(8)	5222(6)	68(2)
C(43)	8050(16)	8923(9)	4436(6)	77(2)
C(44)	9747(19)	922(11)	4474(10)	105(4)
C(45)	10311(15)	7822(12)	5315(12)	109(5)
P(1)	460(3)	8952(2)	8493(1)	61(1)
F(11)	-1572(10)	8946(8)	8391(7)	142(3)
F(12)	704(22)	9624(14)	7558(6)	221(6)
F(13)	2556(11)	8850(9)	8603(8)	169(4)
F(14)	-169(14)	10286(10)	8817(9)	202(6)
F(15)	490(20)	8087(14)	9394(7)	226(7)
F(16)	1114(17)	7550(10)	8098(11)	211(6)

Table A3.3iii: Hydrogen coordinates ($\times 10^4$) and isotropic displacement parameters ($\text{\AA}^2 \times 10^3$) for [45].

	x	y	z	U(eq)
H(12)	2313(13)	5344(9)	9099(6)	88
H(13)	1382(16)	3377(11)	9918(7)	114
H(14)	4371(17)	1458(10)	10059(6)	108
H(15)	7222(13)	2257(8)	9368(5)	79
H(18)	4454(10)	6511(6)	8455(4)	70
H(19A)	8042(13)	6664(8)	7683(5)	76
H(19B)	6127(13)	7869(8)	7664(5)	76
H(21)	4001(9)	8072(7)	6284(5)	60
H(22)	4754(10)	7109(8)	4810(5)	70
H(23)	7852(11)	5248(8)	4828(5)	74
H(24)	9013(10)	5070(7)	6329(5)	65
H(31)	7039(14)	1672(11)	7276(6)	100
H(32)	5401(19)	4047(13)	6863(6)	109
H(33)	1841(17)	4428(12)	7223(8)	114
H(34)	1330(18)	2322(17)	7989(9)	136
H(35)	4504(21)	571(12)	7991(7)	120
H(41)	8967(18)	8972(12)	6372(7)	107
H(42)	6493(12)	10220(8)	5365(6)	82
H(43)	7342(16)	9173(9)	3948(6)	93
H(44)	10417(19)	7392(11)	4008(10)	126
H(45)	11404(15)	7207(12)	5525(12)	131

Table A3.4i: X-ray crystal data for receptor [48].

Data were collected on a Stoe-Stadi2 diffractometer. The structure was solved by direct methods. There were two molecules in the asymmetric unit.

Empirical formula	C ₁₆ H ₁₅ FeN ₃ O
Formula weight	321.16
Temperature	293(2) K
Wavelength	0.71070 Å
Crystal system	monoclinic
Space group	P2 ₁ /n
Unit cell dimensions	a = 12.407(13) Å b = 10.133(12) Å c = 22.990(25) Å b = 93.0(1)°
Volume	2886.3 Å ³
Z	8
Density (calculated)	1.478 Mg/m ³
Absorption coefficient	1.046 mm ⁻¹
F(000)	1328
θ range for data collection	<25°
Reflections collected	3204
Data / parameters	2054 / 170
Goodness-of-fit on F	1.569
Final R indices [<i>I</i> >2σ(<i>I</i>)]	R1 = 0.1130
R indices (all data)	R1 = 0.1419
Largest diff. peak and hole	0.666 and -1.070 e.Å ⁻³

Table A3.4ii: Atomic coordinates ($\times 10^4$) and equivalent isotropic displacement parameters ($\text{\AA}^2 \times 10^3$) for [48]. U(eq) is defined as one third of the trace of the orthogonalized U_{ij} tensor.

	x	y	z	U(eq)
Fe(1)	2457(2)	4719(2)	4746(4)	45(3)
C(11)	1603(4)	5469(4)	5447(8)	73(6)
C(12)	2059(4)	6417(4)	5063(9)	69(6)
C(13)	3032(5)	6573(4)	5009(9)	71(7)
C(14)	3557(5)	5652(5)	5348(8)	83(7)
C(15)	2568(4)	4953(5)	5549(8)	72(6)
C(16)	2886(5)	4531(6)	3901(8)	66(6)
C(17)	3403(6)	3483(5)	4227(9)	59(6)
C(18)	2706(5)	2795(6)	4476(9)	66(7)
C(19)	1495(5)	3346(5)	4400(9)	77(8)
C(110)	1920(6)	4378(5)	3943(9)	82(8)
C(111)	2572(4)	1641(5)	4918(8)	74(7)
O(112)	1845(4)	0965(4)	5049(8)	66(7)
N(113)	3615(4)	1541(4)	5137(8)	73(7)
C(114)	4009(4)	0649(5)	5574(8)	68(7)
C(115)	3334(4)	-0422(5)	5757(9)	73(7)
C(116)	3851(4)	-1198(5)	6201(10)	77(8)
C(117)	4811(4)	-0995(5)	6371(9)	66(8)
C(118)	5419(5)	0013(5)	6098(9)	73(7)
N(119)	5018(5)	0786(5)	5689(10)	66(7)
N(120)	6468(5)	0241(5)	6244(8)	72(6)
Fe(2)	-0486(2)	1704(2)	2575(4)	45(7)
C(21)	0529(4)	1640(4)	3256(8)	54(7)
C(22)	0969(4)	2358(4)	2827(8)	62(7)
C(23)	0265(4)	3514(4)	2664(8)	69(6)
C(24)	-0532(4)	3332(4)	3017(8)	72(7)
C(25)	-0635(4)	2282(4)	3453(8)	72(7)
C(26)	-1180(4)	-0144(4)	2483(8)	72(7)
C(27)	-0426(4)	0053(4)	2048(8)	83(7)
C(28)	-0425(4)	1164(4)	1685(8)	77(7)
C(29)	-1433(4)	1722(4)	1867(8)	81(7)
C(210)	-1966(4)	1081(4)	2375(8)	75(8)
C(211)	2085(4)	2079(5)	2598(8)	66(8)
O(212)	2651(4)	1070(4)	2795(8)	73(8)
N(213)	2531(4)	2665(4)	2088(7)	69(8)
C(214)	3488(5)	2618(4)	1849(7)	73(7)
N(219)	3616(5)	3237(4)	1309(7)	64(7)
C(218)	4549(5)	3258(4)	1058(7)	73(7)
C(217)	5485(5)	2619(5)	1224(9)	77(6)
C(216)	5376(4)	1953(4)	1696(9)	82(5)
C(215)	4417(4)	1901(4)	2043(8)	74(7)
N(220)	4642(4)	4073(4)	0557(9)	82(6)

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